



Bio FSP 2020

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Dartmouth Studies in Tropical Ecology, Vol. 30 (2020)

Dartmouth College runs an annual 9-10 week ecological field research program in Costa Rica and the Caribbean. Manuscripts from the research projects in this program have been published in the annual volume “Dartmouth Studies in Tropical Ecology” since 1989. Copies are available online and are hardcopies are held in the Life Sciences Center at Dartmouth College and in the field stations that we visit.

Dartmouth faculty from the Department of Biological Sciences, along with two Ph.D. students from Dartmouth’s Ecology and Evolutionary Biology graduate program, advise ca. 15 advanced undergraduate students on this program.

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

We thank the Costa Rican Ministry of the Environment and Energy (MINAE) for permission to conduct research in Costa Rica’s extraordinary national parks. The Organization for Tropical Studies (OTS/OET) has provided essential support for our program for over 40 years, taking care of most of our logistical needs in Costa Rica, always to high standards of quality and reliability. We thank OTS staff at the Palo Verde and La Selva Biological Stations, and at the Wilson Botanical Garden at Las Cruces, for all their services rendered efficiently, politely and in good spirit. Staff at the Santa Rosa and Corcovado National Parks have also been gracious in accommodating and assisting us. We thank Carlos Solano at the Cuerici Biological Station for his depth of knowledge and inspiration. We are grateful to the staff of the Monteverde Biological Station for access to their facilities, and for making us so comfortable when we arrive late, dirty, hungry and tired from Santa Rosa.

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

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

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

Matt Ayres
Hanover NH, USA
28 Dec 2020



La Universidad de Dartmouth: Ecología tropical 2020

Inicio: 06/01/2020 Final: 17/02/2020

Como parte de los esfuerzos de la Universidad de Dartmouth, ubicada en Nuevo Hampshire (EEUU), por brindar una educación amplia en el campo de las ciencias biológicas, el departamento de Ciencias Biológicas diseñó el curso de Biología Tropical. Este curso se ha ofrecido y llevado a cabo en Costa Rica cada año desde 1977 auspiciado por la Organización para Estudios Tropicales (OET). El curso es dirigido a estudiantes de la universidad (estudiantes no graduados) y enfatiza la aplicación del método científico, probando hipótesis que los estudiantes deben generar. Asimismo, el curso expone a los estudiantes a los retos que enfrentan los ecosistemas tropicales como consecuencia de actividades antropogénicas.

El curso de biología tropical de la Universidad de Dartmouth toma como modelo los programas a nivel graduado ofrecidos por la OET (Biología tropical: un enfoque ecológico) ofreciendo una introducción a los principales temas de la biología tropical. El curso introduce a los estudiantes a los sistemas ecológicos tropicales y les enseña los métodos usados para llevar a cabo investigaciones ecológicas. Durante el programa, los estudiantes visitan los principales sistemas tropicales incluyendo el bosque tropical seco, nuboso, y húmedo, además de humedales y ecosistemas de manglar. Entre los objetivos del curso es que los estudiantes aprendan las principales características de estos ecosistemas, así como la ecología, biología e historia natural de los organismos encontrados en ellos.

Adicionalmente, los estudiantes llevan a cabo una serie de proyectos cortos (de dos o tres días). Basándose principalmente en sus propias observaciones los estudiantes plantean preguntas, diseñan estudios de campo para tratar de contestar sus preguntas y recogen los datos. Este proceso es seguido por un análisis de datos y la preparación de un informe con formato de artículo científico sobre el proyecto. Los estudiantes deberán además realizar una presentación oral ante sus compañeros y profesores.

En cinco de las localidades visitadas (Barro Colorado, Estación Biológica La Selva, Estación Biológica Monteverde, Estación Biológica Palo Verde, y El Campanario en la Península de Osa), bajo la supervisión de un(a) profesor(a) de Dartmouth y dos asistentes, los estudiantes diseñan y llevan a cabo un proyecto de investigación corto (de 2-4 días). El proyecto se diseña cuando estén en Costa Rica en base a las observaciones y el interés del estudiante. Todos los proyectos de investigación deben incluir hipótesis informadas, lógicas e interesantes y metodologías sólidas para testar la hipótesis. Ningún proyecto puede comprometer la integridad de las comunidades bióticas o ecosistemas. Todos los proyectos son consultados con la autoridad local competente (ej. personal de la OET) para asegurarnos de que no existen impactos biológicos. La mayoría de los proyectos se basan en observaciones no intrusivas de la conducta, abundancia y distribución por lo que no implican coger o manipular organismos. Algunos proyectos podrían implicar coleccionar material biológico tal y como se describió en el documento principal.

A continuación, están el programa del curso, los participantes, y los títulos y abstractos de los proyectos llevados a cabo durante el invierno de 2020.

El programa 2020

Lugar	Inicio	Final
La isla de Barro Colorado, Panamá	6/01/2020	14/01/2020
San José, Costa Rica	14/01/2020	16/01/2020
La Estación Biológica La Selva	16/01/2020	22/01/2020
La Estación Biológica Monteverde	22/01/2020	28/01/2020
Play Naranjo en el Parque Nacional Santa Rosa	28/01/2020	30/01/2020
Estación Biológica Palo Verde	30/01/2020	05/02/2020
El Campanario en la Península de Osa	05/02/2020	12/02/2020
La Estación Biológica Las Cruces	12/02/2020	17/02/2020

Los participantes 2020

Nombre	Profesión
Matthew Ayres	Profesor
Celia Chen	Profesor
Mark Laidre	Profesor
Melissa DeSiervo	Asistente al profesor(a)
Clare Doherty	Asistente al profesor(a)
Robert Alter	Estudiante
Katherine Axel	Estudiante
Maxwell Bond	Estudiante
Nathan Giffard	Estudiante
Colton Hayse	Estudiante
Reyn Hutton	Estudiante
Sarah Jennewein	Estudiante
Elijah Laird	Estudiante
Ashlyn Morris	Estudiante
Alejandra Prevost-Reilly	Estudiante
Shannon Sartain	Estudiante
Benjamin Schelling	Estudiante
Quintin Tyree	Estudiante
Benjamin Zdasiuk	Estudiante

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CENTRAL AMERICA 2020 SCHEDULE

Bio FSP 2020. Detailed schedule for Panama and Costa Rica.					2-Jan-19
Date	Day	Location	Morning	Afternoon	Evening
6-Jan	Mon	To Panama City	Travel	Travel	Arrive in evening
7-Jan		To BCI	Travel. Bus 0900. Boat 1330.	Orientation. Tropical ecology research (ML). Lec: Local katydid research (Hth, CK).	Nightwalk
8-Jan		On BCI	Orientation (guided hike)	Research tactics: good questions, strong inference, and field notebooks (ML)	ORP-1 project development and proposals
9-Jan		On BCI	ORP-1 research	Basic stats intro (ML, CD, and MD)	Lec: Evol of cooperation & social insects (ML). Student paper: Living bridges in army ants (AP-R)
10-Jan		On BCI	ORP-1 research	Writing lab (ML)	ORP-1 research
11-Jan		On BCI	ORP-1 research	Plant lab (MD). ORP-1 research	Lec: Avian cooperative breeding (CD). Student paper: Cooperation and parasitism in anis (BZ)
12-Jan	Sun	On BCI	Data handling. ORP-1 analyses.	ORP-1 analyses and context	ORP-1 symposium
13-Jan		On BCI	ORP-1 writing	ORP-1 v1 due.	Evening on Porch
14-Jan		To San Jose	Travel	Travel	Exploration
15-Jan		In San Jose	OTS, Program overview	Museum. San Jose exploration	Group dinner in SJ
16-Jan		To La Selva	Travel	Orientation	Nightwalk
17-Jan		At La Selva	Orientation (guided hike)	ORP-2 project development and proposals	Lec: Primate cognition (ML). Student paper: Triadic awareness in capuchins (EL)
18-Jan		At La Selva	ORP-2 research	ORP-2 research	Lec: Diversity and coexistence (MD). Student paper: birds and seeds (RH)
19-Jan	Sun	At La Selva	ORP-2 research	Vert practicum (CD). ORP-2 research	Lec: Animal communication (CD). Student paper: Mating signals in túngara frogs (BS)
20-Jan		At La Selva	ORP-2 research	ORP-2 research	ORP-2 research
21-Jan		At La Selva	ORP-2 research	ORP-2 analyses, context, and draft ms	ORP-2 symposium. ORP-1 final due
22-Jan		To Monteverde	Travel	Orientation. Vertebrates of cloud forest (ML)	Nightwalk. ORP-2 v1 due
23-Jan		At Monteverde	ORP-3 project development	ORP-2 project proposals	Lec: Conservation biology 1 (MD). Student paper: Upgrading protected areas (HT)
24-Jan		At Monteverde	ORP-3 research	ORP-3 research. Plants and arthropods of cloud forest (MD)	ORP-3 research
25-Jan		At Monteverde	ORP-3 research	ORP-3 research	Lec: Evol of behavior (ML).
26-Jan	Sun	At Monteverde	ORP-3 research	ORP-3 research	Discussion: What and wherefore science I
27-Jan		At Monteverde	ORP-3 research	ORP-3 analyses and context	ORP-3 symposium. ORP-2 final due
28-Jan		To Santa Rosa	Travel	Hike to Playa Naranjo. Orientation.	Hermit crab lab (CD)
29-Jan		At Santa Rosa	Mangrove lab (MD)	Exploration	Turtle lab (MA)
30-Jan		To Palo Verde	Travel	Orientation	Lec: Neotropical ecology (MA)
31-Jan		At Palo Verde	Orientation	ORP 4 development	Student paper: Neotropical anachronisms (MB)
1-Feb		At Palo Verde	Arthropod lab (CD)	ORP-4 research	Student paper: Climate, hosts, and pathogens (SJ)
2-Feb	Sun	At Palo Verde	ORP-4 research	ORP-4 research	Student paper: Mountain passes in the tropics (RA)
3-Feb		At Palo Verde	ORP-4 research	ORP-4 research	ORP-4 research
4-Feb		At Palo Verde	Riverboat excursion	ORP-4 analyses and context	ORP-4 symposium. ORP-3 final due.
5-Feb		To El Campanario	Travel to Sierpe	Boat, Sierpe to Campanario. Orientation	Nightwalk
6-Feb		At El Campanario	Orientation	Lec: Social insects 2 (MA). ORP-5 development.	Student paper: Lekking birds (SS). Writing
7-Feb		At El Campanario	ORP-5 research	Lec: Plant-herbivore interactions (MA). ORP-5 research	Student paper: Leafcutter ants (CH). Writing
8-Feb		At El Campanario	ORP-5 research	ORP-5 research	Student paper: Army ants (AM). Writing
9-Feb	Sun	At El Campanario	ORP-5 research	ORP-5 research	Nightwalk
10-Feb		At El Campanario	ORP-5 research	ORP-5 research	ORP-5 analyses
11-Feb		At El Campanario	ORP-5 research	ORP-5 analyses and context	ORP-5 symposium.
12-Feb		To Las Cruces	Travel	Orientation. Writing and botany	Lec: Conservation biology 2 (MA).
13-Feb		At Las Cruces	Orientation	Botany and writing	Student paper: Plant-pollination systems (NG)
14-Feb		At Las Cruces	Writing and botany	Botany and writing	Student paper: Plant-herbivore interactions (KA)
15-Feb		At Las Cruces	Writing and botany	ORP-4 final due. ORP-5 final due. Botany practicum	Discussion: What and wherefore science II
16-Feb	Sun	To San Jose	Travel to San Jose.	Swap gear at OTS.	Group dinner. Prepare for Caribbean
17-Feb		To Grand Cayman	Early departure from hotel. Fly to Miami	Fly to Grand Cayman	Overnight in Grand Cayman
18-Feb		To Little Cayman	Fly to Little Cayman	Arrive Little Cayman Research Center	Orientation
10-Mar	Tue	To Miami	Fly to Grand Cayman	Fly to Miami. End of program.	
ORP = Original Research Project					

LITTLE CAYMAN 2019 SCHEDULE

Date		Morning	Afternoon	Evening
18 Feb Tue	Group Arrives at 3:05 and 5:45 pm from Grand Cayman		Pick up students Unpack, get settled, and have dinner	Introduction to LCRC (Maisy Fuller) Lecture: Grouper Moon (Bryce Semmens) 6pm Discussion: schedule (tentative) and expectations for LC segment.
19 Feb Wed	Orientation and General natural history	Snorkel and diving safety talks (Giac, Miriam) Snorkeling in back reef Lecture: Coral reefs in the Caymans and past projects (CC)	Queen Conch Project Dive Gear assigning (Greg Locher) <i>SCUBA and Snorkeling</i> Shore dive/snorkel at Cumber's (1:30) Snorkeling South Hole	Lecture: Introduction to Queen Conch (CC)
20 Feb Thur	Queen Conch project	Queen Conch Project Data collection design Snorkeling in South Hole	Lecture: Miriam Pierotti Corals (1:00pm)	Creature Feature Celia's Mercury Talk at Southern Cross (6pm)
21 Feb Fri	Queen Conch project	Queen Conch Project Data analysis and findings	Queen Conch Project Algae sampling and identification	Lecture: Introduction to Caribbean geology, biogeography, and history (CC) <i>Film: Secret sex life of fish</i>
22 Feb Sat	Queen Conch project	<i>SCUBA and Snorkeling</i>	Queen Conch Project Visit other project sites (Salt Rock, Greg's house)	Queen Conch Project Presentation Creature Feature
23 Feb Sun	Project 1 begins	Project 1 Brainstorm and idea discussion	Project 1 Proposal Development	Critique: Invertebrates (Shannon Sartain) Lecture: Invertebrates (Melissa Desiervo)
24 Feb Mon	Project 1	Project 1 data collection	Project 1 data collection	Critique: Herbivory (Max Bond) Lecture: Reef Fish (CC)
25 Feb Tue	Project 1	Project 1 Proposal due Project 1 data collection	Project 1 data collection	Critique: Coral-algae competition (Hutch Tyree) Critique: Sponges (Colton Hayes) <i>Film: Sponges: Oldest Creatures</i>
Date		Morning	Afternoon	Evening
26 Feb Wed	Project 1	<i>SCUBA and Snorkeling</i>	Project 1 data collection	Critique: Zooplankton (Ben Schelling) Critique: Fish Biology (Ali Prevost-Reilly)
27 Feb Thur	Project 1	<i>SCUBA and Snorkeling</i>	Project 1 data collection	Indiana University Skype Critique: Damselfish and Lionfish (Sarah Jennewein) Critique: Fish Ecology (Katy Axel)

28 Feb Fri	Project 1 completion	Project 1 – data analysis, write methods **Project 1 PRESENTATIONS**	Write up Project 1	Lecture: Animal sensory and movement (Clare Doherty) <i>R&R: Karaoke 8pm</i>
29 Feb Sat	Dia libre (OFF)		Beach Cleanup and snorkeling at Point of Sand (cookout)	R&R
1 Mar Sun	Project 2 begins	Write up Project 1	Visit Owen Island Project 2 Brainstorm and exploration Project 1 1st draft	Critique: Fish Biology (Ashlyn Morris) Lecture: Marine plastics (CC)
2 Mar Mon	Project 2	Finalize project 2 idea, design, and group members Project 2 data collection	Coral Restoration and outplanting (Dagny) 1pm Project 2 proposal DUE Project 2 data collection	Critique: Fish Behavior (Reyn Hutten) Critique: Coral Calcification (Elijah Laird)
3 Mar Tues	Project 2	**Project 1 final draft Due** Project 2 – data collection	Lecture: LC Sea Turtles (Maisy Fuller) Project 2–data collection Project 2 data collection	Night SCUBA and Snorkeling
4 Mar Wed	Project 2 (Reefs Go Live)	Project 2 – data collection	Lecture: Coral Visit Iguanas with Tanja/ Project 2 – data collection	Lecture: Climate Change and coral reefs (CC) Critique: Reef Conservation (Nathan Giffard)
5 Mar Thur	Project 2	Project 2 – data collection	Lionfish lecture and dissection (Maisy) 1pm Project 2 –data analysis	Student Talk at Southern Cross (6pm) Lecture: Tanja Laaser Iguana Conservation 5-6:30 (Beach Resort) Film: “Chasing Coral”
6 Mar Fri	Finish Project 2	**Project 2 PRESENTATION**	Project 2 - writing Visit Iguanas with Tanja/ Joe Plopys (Bird Talk at Trust) 4pm-5:30	Critique: Mangroves (Benjamin Zdasiuk) Critique: Seagrass ecology (Robert Alter) <i>R&R: Karaoke 8pm</i>
7 Mar Sat	Finish Queen Conch write-up	SCUBA and Snorkeling?	**Project 2 1st draft DUE** Project 2 refine drafts	Film: Maug’s Caldera Discussion: Graduate School/Career
8 Mar Sun	Finish all project editing	**Project 2 final draft DUE** Self Evaluations	Revisions and copy editing of all LC projects Self Evaluations and Individual Meetings	Revisions & copy editing of all LC projects Individual meetings
9 Mar Mon	Clean up and pack	Clean up science equipment/field sites Individual meetings	Clean up & pack Individual meetings Faculty/TA evaluations/FSP survey	Dinner at Beach Resort
10 Mar Tues	Depart LC 7:50 and 10:20 am			

CENTRAL AMERICA 2020: PAPERS FOR STUDENT PRESENTATIONS

Bio FSP 2020: Papers for student presentations in Central America (in order of occurrence).					
#	Site*	Student	Student Paper		Staff
01	BCI	AP-R	Reid et al. 2015. Army ants dynamically adjust living bridges in response to a cost-benefit trade-off. <i>PNAS</i> 112:15113-15118.		ML
02	BCI	BZ	Riehl and Strong. 2019. Social parasitism as an alternative reproductive tactic in a cooperatively breeding cuckoo. <i>Nature</i> 567: 96-99.		CD
03	LS	EL	Perry et al. 2004. White-faced capuchin monkeys show triadic awareness in their choice of allies. <i>Animal Behaviour</i> 67: 165-170. And Boinske, S. 1988. Use of a club by wild white-faced capuchin to a attack a venomous snake (<i>Bothrops asper</i>). <i>Am. J. Primatology</i> 14: 177-179.		ML
04	LS	BS	Akre and Ryan. 2011. Female tungara frogs elicit more complex mating signals from males. <i>Behavioral Ecology</i> 22: 846-853.		CD
05	MV	RH	Galetti et al. 2013. Functional extinction of birds drives rapid evolutionary changes in seed size. <i>Science</i> 340:1086-1090. (See also: Palacio and Ordano. 2018. The Strength and drivers of bird-mediated selection on fruit crop size: a meta-analysis. <i>Frontiers in Ecology and Evolution</i> 6)		MDS
06	MV	HT	Pringle. 2017. Upgrading protected areas to conserve wild biodiversity. <i>Nature</i> 546: 91-99.		MDS
07	PV	MB	Janzen and Martin. 1981. Neotropical anachronisms: the fruits the gomphotheres ate. <i>Science</i> 215:19-27. [See also: Sanchez et al. 2003. Feeding ecology, dispersal, and extinction of South American Pleistocene gomphotheres (Gomphotheriidae, Proboscidea). <i>Paleobiology</i> 30: 146-161.]		MA
08	PV	SJ	Nowakowski et al. 2016. Infection risk decreases with increasing mismatch in host and pathogen environmental tolerances. <i>Ecology Letters</i> 19:1051-1061. (See also Pound et al. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. <i>Nature</i> 439:161-167.)		MA
09	PV	RA	McCain et al. 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. Sheldon et al. 2018. Fifty Years of Mountain Passes: A Perspective on Dan Janzen's Classic Article. <i>American Naturalist</i> 191:553-565.)		MA
10	Camp.	SS	Vanderbilt et al. 2015. Variation in the performance of cross-contextual displays suggests selection on dual-male phenotypes in a lekking bird. <i>Anim Behav</i> 107:213-219. (See also: McDonald and Potts. 1994. Cooperative display and relatedness among males in a lek-mating bird. <i>Science</i> 266:1030-1032.)		MA
11	Camp.	CH	Little et al. 2006. Defending against parasites: fungus-growing ants combine specialized behaviours and microbial symbionts to protect their fungus gardens. <i>Biology Letters</i> 2:12-16. (See also: Schultz and Brady. 2008. Major evolutionary transitions in ant agriculture. <i>PNAS</i> 105:5435-5440. Currie et al. 1999. Fungus-growing ants use antibiotic-producing bacteria to control garden parasites. <i>Nature</i> 398:701-704.)		MA
12	Camp.	AM	Brady, S.G. 2003. Evolution of the army ant syndrome: The origin and long-term evolutionary stasis of a complex of behavioral and reproductive adaptations. <i>PNAS</i> 100: 6575-6579. (See also: Rettenmeyer et al. 2011. The largest animal association centered on one species: the army ant <i>Eciton burckellii</i> and its more than 300 associates. <i>Insectes Sociaux</i> 58:281-292. Chadab and Rettenmeyer. 1975. Mass recruitment by army ants. <i>Science</i> 188:1124-1125. Schneirla, T. C. 1934. Raiding and other outstanding phenomena in the behavior of army ants. <i>PNAS</i> 20:316-321.)		MA
13	LC	NG	Ramirez et al. 2011. Asynchronous diversification in a specialized plant-pollinator mutualism. <i>Science</i> 333:1742-1746.		MA
14	LC	KA	Endara et al. 2017. Coevolutionary arms race versus host defense chase in a tropical herbivore-plant system. <i>PNAS</i> 114:E7499-E7505. (See also: Ehrlich, P. R., and P. H. Raven. 1964. Butterflies and plants: a study in coevolution. <i>Evolution</i> 18:586-608.)		MA

* Sites in order: Barro Colorado Island, La Selva, Monteverde, Santa Rosa, Palo Verde, El Campanario, Las Cruces

LITTLE CAYMAN 2020: PAPERS FOR STUDENT PRESENTATIONS

Student	Lecture	Paper
Ali Prevost-Reilly	Fish biology	Miller, G.M., S. Watson, S., J.M. Donelson, M.I. McCormick, P.L. Munday. 2012. Parental environment mediates impacts of increased carbon dioxide on a coral reef fish. <i>Nature Climate Change</i> 2: 858-861.
Ashlyn Morris	Fish biology	Gerlach, G., J. Atema, M. J. Kingsford, K. P. Black, and V. Miller-Sims. 2007. Smelling home can prevent dispersal of reef fish larvae. <i>Proceedings of the National Academy of Sciences</i> 104:858-863.
Benjamin Schelling	Zooplankton	Smith, J.M., G. De'ath, C. Richter, A. Cornils, J.M. Hall-Spencer, K.E. Fabricius. 2016. Ocean acidification reduces demersal zooplankton that reside in tropical coral reefs. <i>Nature Climate Change</i> 6: 1124-1129.
Benjamin Zdasiuk	Mangroves	Lovelock, C.E., D.R. Cahoon, D.A. Friess, G.R. Guntenspergen, K.W. Krauss, R. Reef, K. Rogers, M.S. Saunders, F. Sidik, A. Swales, N. Saintilan, L.X. Thuyen, T. Triet. 2015. The vulnerability of Indo-Pacific mangrove forests to sea-level rise. <i>Nature</i> 526: 559-562.
Shannon Sartain	Invertebrates	Carpenter, R. C. and P. J. Edmunds. 2006. Local and regional scale recovery of <i>Diadema</i> promotes recruitment of scleractinian corals. <i>Ecology Letters</i> 9:268-277.
Colton Hayse	Sponges	De Goeij, J.M., D. vab Oevelen, M.J.A. Verimeij, R. Osinga, J.J. Middelburg, A.F.P.M. de Goeij, W. Admiral. 2013. Surviving in a marine desert: the sponge loop retains resources within coral reefs. <i>Science</i> 342: 108-110.
Elijah Laird	Coral calcification	Albright, R., Y. Takeshita, D.A. Koweeck, A. Ninodawa, K. Wolfe, T. Rivlin, Y. Nebuchina, J. Young, K. Caldeira. 2018. Carbon dioxide addition to coral reef waters suppresses net community calcification. <i>Nature</i> 555: 516-519.
Katy Axel	Fish ecology	McMahon, K.W., M.L. Berumen, and S.R. Thorrold. 2012. Linking habitat mosaics and connectivity in a coral reef seascape. <i>Proceedings of the National Academy of Sciences</i> 109: 15372-15376.
Max Bond	Herbivory	Dixon, D. L. and M. E. Hay. 2012. Corals Chemically Cue Mutualistic Fishes to Remove Competing Seaweeds. <i>Science</i> 338:804-807.
Nathan Giffard	Reef conservation	Cinner, J.E., C. Huchery, M.A. MacNeil, N.A.J. Graham, T.R. McClanahan et al. 2016. Bright spots among the world's coral reefs. <i>Nature</i> 535: 416-419.
Hutch Tyree	Coral-algae competition	Rasher, D.B. and M.E. Hay. 2010. Chemically rich seaweeds poison corals when not controlled by herbivores. <i>Proceedings of the National Academy of Sciences</i> 107: 9683-9688
Reyn Hutten	Fish behavior	Welch, M.J., S. Watson, J.Q. Welsh, M.I. McCormick, P.L. Munday. 2014. Effects of elevated CO2 on fish behavior undiminished by transgenerational acclimation. <i>Nature Climate Change</i> 4: 1086-1089.
Robert Alter	Seagrass ecology	Lamb, J.B., J.A.J.M. van de Water, D.G. Bourne, C. Altier, M.Y. Hein, E.A. Florenza, N. Abu, J. Jompa, C.D. Harvell. 2017. Seagrass ecosystems reduce exposures to bacterial pathogens, of humans, fishes, and invertebrates. <i>Science</i> 355: 731-733
Sarah Jennewein	Damselfish and Lionfish	Davis, A.C.D. 2018. Differential effects o. native vs. invasive predators on a common Caribbean reef fish. <i>Environ. Biol. Fish</i> 101:1537-1548

MAPS: COSTA RICA AND LITTLE CAYMAN ISLAND



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



<https://www.southerncrossclub.com/>

Dartmouth Studies in Tropical Ecology
Curso de Biología Tropical, Universidad de Dartmouth
Research in the Tropics I and II: 7 January – 18 February 2020

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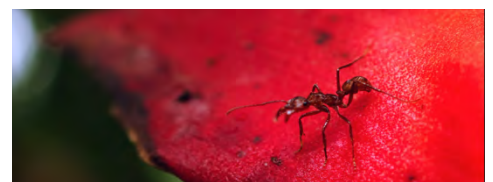
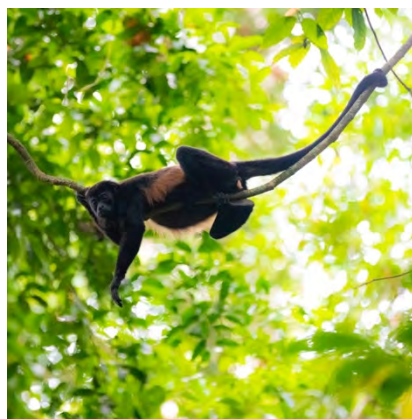
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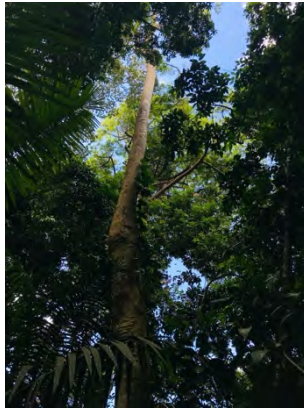
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Barro Colorado Island





HOW ALTITUDE AFFECTS BIODIVERSITY IN A LOWLAND TROPICAL RAINFOREST

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TA Editors: Melissa DeSiervo, Clare Doherty
Faculty Editor: Mark Laidre

Abstract: Tropical rainforests are the most biodiverse ecosystems on the planet. Effective conservation of the numerous medical and industrial resources contained within the ecosystem is contingent on a solid understanding of the causes of this biodiversity. Past studies indicate that biodiversity tends to be inversely related to elevation. In order to see if this pattern applies to lowland tropical rainforests, we collected palm tree and bird diversity data from 25 10 x 10 m plots across an elevation range of 32 to 125 m on Barro Colorado Island, Panama. We found no relationship between elevation and palm tree or bird diversity. In addition, elevation was found to strongly correlate with distance from shore. Future iterations of this experiment require larger plot sizes to accurately capture the ecological dispersal patterns of birds and palm trees. As climate change accelerates and increasing numbers of species go extinct, understanding how abiotic factors, including elevation, impact biodiversity will be important in ensuring effective conservation.

Key words: biodiversity, elevation, palm tree diversity, bird diversity, Barro Colorado Island

INTRODUCTION

Tropical rainforests are the most biodiverse ecosystem on the planet. From pharmaceutical drugs, including treatments for malaria and leukemia, to products like fruit and rubber, this biome provides us with a host of resources that many communities rely on for income. For example, Tobias (1991) valued the Monteverde Cloud Forest Biological Reserve in Costa Rica at 12.5 million USD based on ecotourism alone (Tobias and Mendelsohn 1991).

Multiple theories have been proposed to explain the high levels of diversity found in tropical rainforests. Niche theory postulates that selection pressures drive individuals to specialize on certain resources with the aim of increasing fitness, thereby occupying a distinct niche in the environment. According to the competitive exclusion principle, two species in an environment cannot coexist indefinitely off of the same limiting resource, and so the weaker competitor on a given resource will either be driven to extinction or will specialize further in order to allow the two to coexist. The existence of many distinct strategies for survival allows for a large number of species to persist in the same environment (Grinnell 1917).

A competing theory is neutral theory, which postulates that some of the observed biodiversity within a given trophic level is caused by random births, deaths, immigrations, emigration, and speciation events rather than just by selection

pressures (Hubbell 2001). While niche theory argues that biodiversity is high within a rainforest due to organisms specializing on narrow niches, neutral theory argues that it is chance events, including floods and historical dispersal, and not just selection pressures, that explain the observed distribution of organisms.

One implication of niche theory that does not follow from neutral theory is that alpha diversity should change as abiotic factors change. Past research has investigated the relationship between diversity and one abiotic factor: elevation. Homeier found that elevation has a negative relationship with flora diversity in an Ecuadorian montane rain forest (2008). Rahbek found that bird diversity in the tropics increases with elevation up until a point before decreasing again (2006). In this study, we investigate the applicability of these findings to lowland rainforests by the coast, specifically to rainforests on the easily accessible and intensively studied Barro Colorado Island (BCI).

We focused our study on one class of animals and on one family of plants to account for the possibility that patterns in biodiversity might vary based on choice of taxa. Specifically, birds (*Aves*) and palm trees (*Arecaceae*) were selected as indicators for broader biodiversity on the island. While we were confident in being able to differentiate between species within the entire class of birds, we chose to focus our

discrimination on a smaller, more practical family of trees to ensure high accuracy within our data.

A consideration of niche theory led to our hypothesis that palm tree biodiversity on BCI decreases with increasing elevation. Due to a lower concentration of nutrients in the soil at higher elevations, we predicted that palm tree diversity would be lower there. On the other hand, we predicted that bird diversity would not vary with elevation under niche theory since BCI spans such a small elevation range and birds can readily move up and down across this entire range (Figure 1). Specifically, we predicted a change in alpha diversity for trees, and not for birds, because trees are tethered to a given location while birds can forage across the whole island within a given day. On the other hand, we considered that elevation might have no effect on the diversity of palm trees or birds. Perhaps organisms within tropical rainforests do not organize primarily to occupy a narrow niche and instead distribute themselves largely randomly. This hypothesis, which is consistent with neutral theory, led us to the prediction that the number of palm trees and bird species recorded would remain constant or vary randomly as elevation increased (Fig. 1). Testing these predictions and exploring the relationships between abiotic factors, like elevation, and species diversity, will ultimately allow for a better understanding of how the tropics have become such a hotspot for biodiversity. A clear understanding of the causes of this diversity is necessary to effectively conserve the biome and to preserve the numerous resources that so many communities rely on.

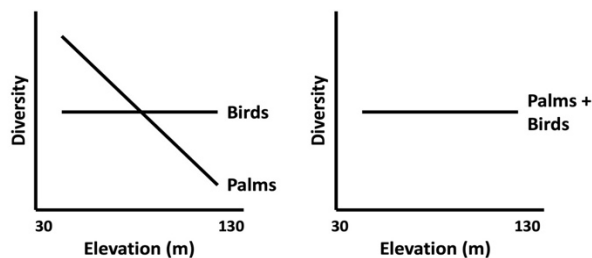


Figure 1: Hypothesized relationships between elevation and birds and palm species alpha diversity under our niche theory hypotheses (left). Hypothesized relationships between elevation and birds and palm species alpha diversity under our neutral theory hypotheses (right).

METHODS

We collected data from 25 10 x 10-meter plots along a gradient of 32 to 125 m in elevation on Barro Colorado Island, Panama. These sites were distributed across six trails on the north side of the island (Fig. 2) and were surveyed during the dry season between 9 January and 11 January 2020. To prevent bias towards picking sparsely vegetated plots, we randomly chose to sample from either the right or left side of a given trail first. We took 200 steps in between each plot and sampled on alternating sides of the trail to further avoid influencing results.

In the center of each plot, we recorded the latitude, longitude, and elevation. The error in the elevation measurements was always less than or equal to 10 meters. We used Google Earth to determine the distance from the center of each plot to the closest spot on the shore of the island. To measure resource availability within the soil, we qualitatively assigned the soil in the center of each plot a value that corresponded to the perceived amount of moisture in its organic layer (results not reported here). Using this scale, a score of 1 would be assigned to extremely dry soil, while a score of 5 would be assigned to soil with a very high moisture content. To determine tree density within each plot, we counted all trees that had a diameter of 4 or more centimeters at hip height. To assess palm diversity, we recorded the number of palm trees and the number of distinct palm species in each plot.

To measure bird diversity within each plot, we conducted a bird point-count using the methods from Hutto et al. (1986). During this point-count, a surveyor rotated their field of view for 10 minutes and recorded each distinct bird species once in the count if heard or seen. If a species was both seen and heard, we included it once in the total species count. The dense vegetation surrounding the trails created a natural visual radius of 10 meters within which birds could be seen. Because birds soaring above the canopy were located outside of this natural visual radius, we did not include them in our count. To avoid the noise created while collecting other data from the plots, we conducted each point-count 10 meters away from the plot.

We completed all statistical analyses with JMP[®] Pro 14.2.0.

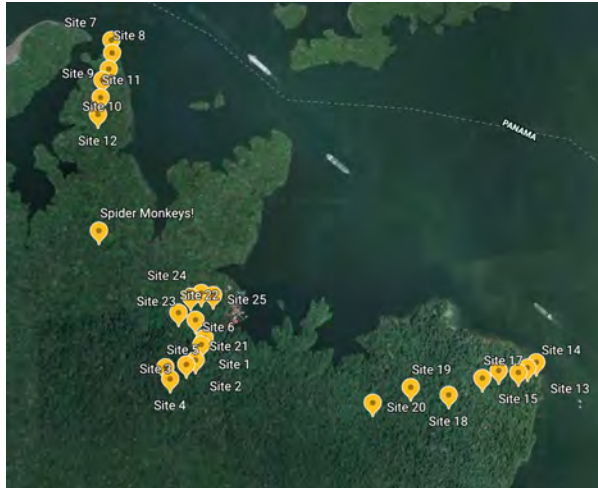


Figure 2: Map of field sites on Barro Colorado Island, Panama. Link to Google Earth map: <https://drive.google.com/open?id=10mTbq58mYYkmvH3RM5J5UtgnuC9Uw5GF&usp=sharing>

RESULTS

We found no significant effect of elevation on either the number of palm tree species ($F = 2.35$, $DF = 1$, $P = 0.14$; Fig. 3) or on the number of bird species ($F = 0.10$, $DF = 1$, $P = 0.75$; Fig. 4). However, elevation was significantly correlated with distance from the shore ($F = 65.41$, $DF = 1$, $P < 0.0001$; Fig. 5).

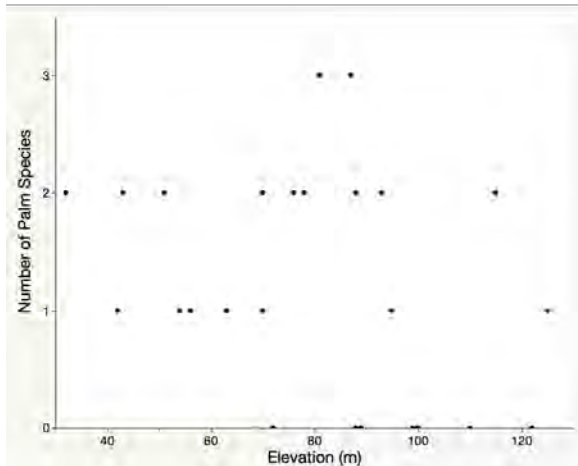


Figure 3. Number of palm species at each site plotted against elevation.

DISCUSSION

We found no significant relationships between elevation and alpha diversity for either palm trees or birds. Our palm tree alpha diversity results are

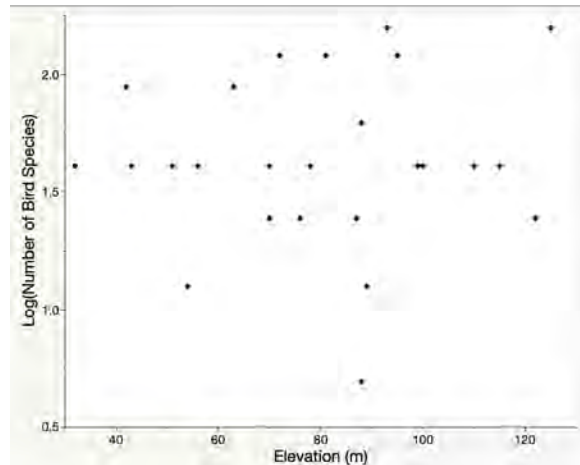


Figure 4. Logarithmic transformation of number of bird species at each site plotted against elevation.

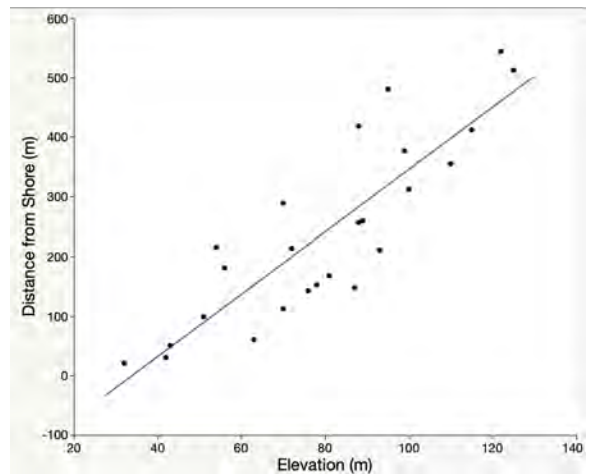


Figure 5. Distance from shore regressed against elevation on Barro Colorado Island.

consistent with our prediction stemming from neutral theory. Because our bird alpha diversity results are consistent with both our prediction stemming from niche theory and our prediction stemming from neutral theory, we cannot say whether our bird diversity results support one theory over the other.

Of note, we found a strong positive correlation between elevation and distance from the shore. The strength of this correlation is understandable given the geography of the island, which was a mountain before the flooding of the Panama Canal. At the same time, however, this relationship limited the inference we could make with our data, as we could not separate elevation and distance from the shore as independent variables.

One explanation for our finding that neither palm tree nor bird alpha diversity varied with elevation is that the island does not have a large enough elevation gradient to capture any biodiversity differences in these taxa. Past studies have found that elevation does affect tree and bird species diversity; unlike our results, theirs are consistent with niche theory (Rahbek 2006, Homeier et al. 2010 and Dallimer et al. 2012). However, these studies sampled a larger range of elevations, which correspond to marked differences in nutrient availability and distinct habitats for different organisms.

Our plots ranged in elevation from 32 to 125 m. In addition, while BCI is 15 km², each of our 25 plots was only 100 m² (STRI). In total, the sites we sampled comprise 0.016% of the island. This limited scale, in both plot size and elevation range, made it difficult to analyze changes in alpha diversity of birds and palm trees. The observer always imposes a level of bias by choosing a certain size lens with which to view the natural world (Levin 1992). Choosing a plot size and elevation range for the study that was more compatible with analyzing the diversity of our chosen taxa would have minimized this bias. More specifically, the elevation range of BCI is not large enough to capture differences in alpha diversity in birds, particularly given their ability to readily disperse and fly above and between our sample points. The island also does not appear to have a large enough difference in elevation or climate to see any differences in palm tree diversity.

Because of this, our results were consistent with our prediction stemming from neutral theory. These findings have implications within conservation, specifically that conservation methods could try incorporating population dynamic models invoking neutral theory. These population models would need to better incorporate stochasticity. Though at the same time, the value of niche theory in conservation efforts should not be underestimated.

Potential for future research still exists to better understand whether niche or neutral theory, or potentially a combination of both, is driving biodiversity in the tropics. Repeating our experiment with larger plots across a greater elevation range could lead to a more definitive understanding of how biodiversity changes with elevation. In addition, other factors, including

topography and the influence of non-arboreal vegetation, could be more precisely analyzed in relation to biodiversity. Finally, measuring beta diversity in addition to alpha diversity would allow for a better understanding of how species distributions change across a range of elevations. Supplemental Figure 1 displays how we predict alpha and beta diversity would change over a range of elevations, given these two competing theories.

Understanding how abiotic factors affect biodiversity in the tropics and around the world becomes increasingly important as climate change accelerates. Given the high percentage of the world's biodiversity that is concentrated in rainforests, conservation efforts will become more effective as the reasons underlying high diversity are elucidated.

ACKNOWLEDGEMENTS

We would like to thank the Smithsonian Tropical Research Institute (STRI) on Barro Colorado Island for their hospitality and generosity in allowing our research projects to proceed. Additionally, we would like to thank the Teaching Assistants for their help in structuring our experimental design.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this work.

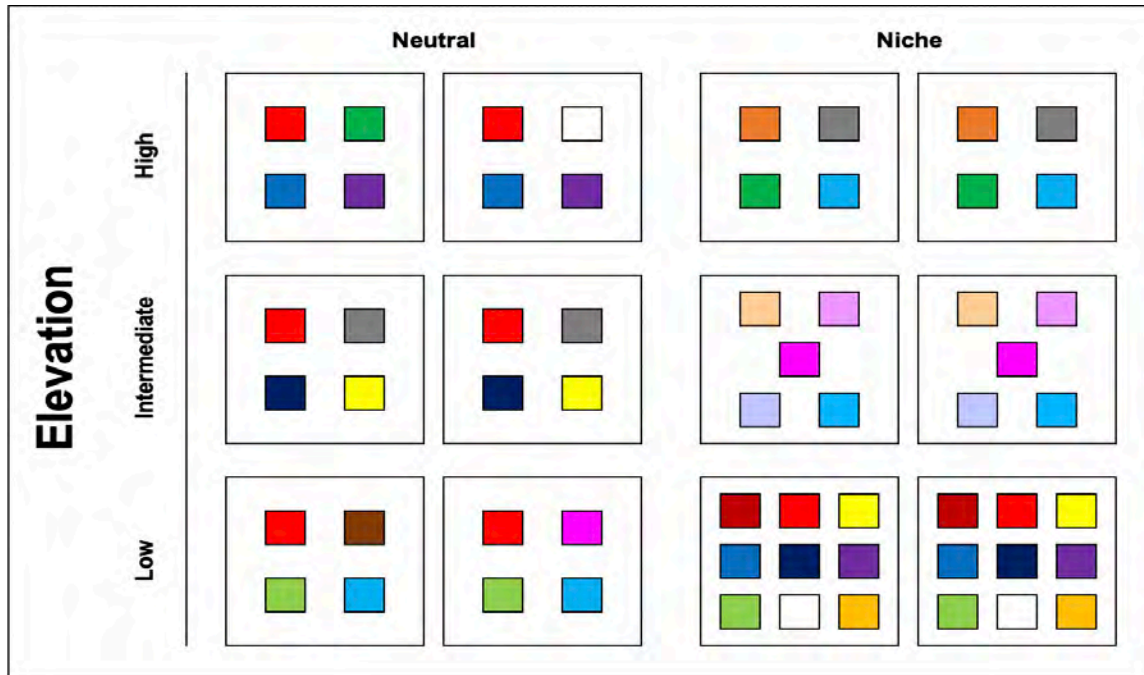
LITERATURE CITED

- Dallimer, M., J. R. Rouquette, et. al. 2012. Contrasting patterns in species richness of birds, butterflies and plants along riparian corridors in an urban landscape. *Diversity and Distributions* 18: 742-753.
- Grinnell, J. 1917. The Niche-Relationships of the California Thrasher. *The Auk*, 34(4), 427-433.
- Homeier, J., S. Breckle, S. Günter, R. T. Rollenbeck, and C. Leuschner. 2010. Tree Diversity, Forest Structure and Productivity along Altitudinal and Topographical Gradients in a Species-Rich Ecuadorian Montane Rain Forest. *Biotropica* 42(2): 140-148.
- Hubbell, Stephen P. 2001. *The Unified Neutral Theory of Biodiversity and Biogeography*. Princeton University Press, Princeton, NJ.
- Hutto, R. L., S. M. Pletschet, and P. Hendricks. 1986. A Fixed-Radius Point Count Method for Nonbreeding and Breeding Season Use. *The Auk* 103: 593-602.
- Janzen, D. H. 1970. Herbivores and the number of tree species in tropical forests. *Am. Nat.* 104: 501– 528.

- Rahbek, C. 2006. The Elevational Gradient of Species Richness: A Uniform Pattern? *Ecography* 18(2): 200-205.
- Rahbek, C. 1997. The Relationship Among Area, Elevation, and Regional Species Richness in Neotropical Birds. *The American Naturalist* 149(5): 875-902.
- Simon, Levin A. 1992. The Problem of Pattern and Scale in Ecology: The Robert H. MacArthur Award Lecture. *Ecology* 73(6).

- Stevens, G. C. 1992. The Elevational Gradient in Altitudinal Range: An Extension of Rapoport's Latitudinal Rule to Altitude. *The American Naturalist* 140(6): 893-911.
- Svenning, J., D. Harley, M. M. Sørensen, and H. Balslev. 2009. Topographic and spatial controls of palm species distributions in a montane rain forest, southern Ecuador. *Biodiversity and Conservation* 18: 219-228.

APPENDIX



Supplemental Figure 1. Hypothetical figure showing how alpha and beta diversity vary under our three hypotheses. Large boxes indicate plots at given elevations. Smaller colored boxes indicate different species within these plots. Under our neutral theory hypothesis, neither alpha nor beta diversity changes with elevation; however, beta diversity will be non-zero. Under our first hypothesis stemming from niche theory, alpha diversity decreases with elevation while beta diversity remains zero across all elevations. Under our second hypothesis that stems from niche theory, but takes the effect of disturbance into account, alpha diversity is highest at some intermediate elevation, but beta diversity remains zero across all elevations.

CÓMO LA ALTITUD AFECTA LA BIODIVERSIDAD EN UN BOSQUE TROPICAL DE TIERRAS BAJAS

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Editoras asistentes Melissa DeSiervo, Clare Doherty
Editor: Mark Laidre

Abstracto: Las selvas tropicales son los ecosistemas más biodiversos del planeta. La conservación efectiva de los numerosos recursos médicos e industriales contenidos en el ecosistema está supeditada a una comprensión sólida de las causas de esta biodiversidad. Estudios anteriores indican que la biodiversidad tiende a estar inversamente relacionada con la elevación. Para ver si este patrón se aplica a las selvas tropicales de tierras bajas, recopilamos datos de diversidad de palmeras y aves de 25 parcelas de 10 x 10 m a través de un rango de elevación de 32 a 125 m en la isla de Barro Colorado, Panamá. No encontramos ninguna relación entre la elevación y la diversidad de palmeras o aves. A medida que el cambio climático se acelera y un número cada vez mayor de especies se extinguen, entender cómo los factores abióticos, incluida la elevación, impactan la biodiversidad para garantizar una conservación eficaz.

Palabras claves: biodiversidad, elevación, diversidad de palmeras, diversidad de aves, Isla Barro Colorado

INDIVIDUAL SENSITIVITY TO FORAGING COSTS: EXPERIMENTAL MANIPULATION OF LEAF-CUTTER ANT LOADS

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TA Editors: Clare Doherty and Melissa DeSiervo
Faculty Editor: Mark Laidre

Abstract: Optimal foraging theory asserts that organisms seek to maximize nutrient intake while minimizing costs and this theory is critical for understanding foraging behaviors of social animals. The leaf-cutter ant *Atta colombica* offers a model system for studying the implementation of optimal foraging theory in complex social groups. We tested whether *A. colombica* maximize the rate of leaf transport on an individual level (based on the load each individual carries) or instead distribute individuals evenly along a foraging path irrespective of their loads (so that the colony can defend against external disturbances). We first measured ant-to-load-size proportions and determined that load size was not related proportionally to ant size. We then followed this with an experimental manipulation of the leaf load carried by each ant, recording the changes in speed for each individual ant that was manipulated. Leaf load represents the primary proximal cost for *A. colombica* workers, offering no immediate benefit. We predicted that alterations to leaf load would result in ant speed changes that were linked to the proximal cost of carrying the load. We found that *A. colombica* individuals sped up with decreased weight and slowed down with increased weight, indicating that each individual of a colony maximizes its speed in relation to leaf load. Our findings in this system are consistent with optimal foraging theory and suggest that cost-benefit decisions on the individual level may maximize efficiency at the colony level.

Key words: efficiency, eusociality, herbivory, Leaf-Cutter Ants (*Atta colombica*), Optimal Foraging Theory

INTRODUCTION

Daily survival decisions are anchored in a cost/benefit analysis. Resource acquisition is one of the primary tradeoffs that species evaluate through this lens. An individual must assess the potential benefits of a foraging expedition, like nutrient gain, in relation to the potential costs, such as expended energy. Optimal foraging theory offers a framework for this phenomenon. The theory focuses on the cost-benefit tradeoff individuals face when deciding how far and long to travel to forage, as well as what benefits they acquire from nutrient acquisition and choosing particular patches over others (Pyke 1977). One of the cornerstones of this theory is that individuals adjust naturally to local conditions under selection pressures (Shepherd 1985). While collective responses in the context of optimal foraging are frequently studied, few studies have examined the responses of individuals that are part of a larger collective.

The leaf-cutter ant *Atta colombica* offers a useful model system to study optimal foraging theory from the perspective of individual and collective behavior. As one of the primary herbivores in the neo-tropics, *A. colombica* are

capable of removing 1.7% of total leaf production annually, and as high as 15% on forest edges (Farias 2018). This foraging behavior is directed by the maintenance of a mutualistic relationship with the basidiomycete fungus, *Leucoagaricus gongylophorus* (Farias 2018, Garrett 2016). As the sectioning and subsequent transportation of leaves is the most energetically costly tasks leaf-cutter ants perform (Garrett 2016), the relationship necessitates consistent and efficient foraging. Therefore, the speed at which *A. colombica* transports leaves is critical for collective colony efficiency. While a number of studies have been performed analyzing the biomass accumulated by the ants and their overall foraging efficiency (Herz 2017, Lugo 1973, Costa 2008), few have explored the efficiency of their individual locomotion as a component of overall foraging activity. In the case of leaf-cutter ants, whose foraging techniques are a necessary intermediary for their individual nutrient gain, it is relevant to investigate the locomotive speed ants maintain when carrying their leaf loads back to the nest.

Leaf-cutter ants may react instantaneously to changes in potential costs during their foraging

journeys. Alternatively, they may travel at a uniform speed, regardless of load size. Previous research has posited that ants continue to perform at maximum efficiency when presented with obstacles challenging their normal behavior (Denny 2001). Therefore, one hypothesis posits that ants are consistently traveling at maximum locomotive efficiency and thus change their walking speed as an effect of leaf manipulation. An alternative hypothesis is that uniform speed along the foraging pathway may ensure an even distribution of individuals, thus enabling the colony to best respond to local disturbances (Middleton and Latty 2016). The resulting prediction is that an individual ant's walking speed may not change when the load is altered with either addition or subtraction of the leaf's surface area. To test these competing hypotheses, we experimentally manipulated individual leaf load and examined the resulting change in walking speed.

METHODS

Part 1: Leaf to Ant Size

We studied an *A. colombica* colony on Barro Colorado Island in Panama and measured the length and head width of 50 randomly sampled individuals and the surface area of the leaf they were carrying when captured. The surface area of each leaf fragment was calculated using ImageJ software, and the length and head width measurements were taken using a bee squeezer and calipers.

Part 2: Locomotion experiment

We used the same colony to measure the speed at which the foragers walked back to their nest when original leaf load was increased, decreased, or unchanged. We placed two 40 cm strips of tape on a tree with an established line of foraging ants. We placed two consecutive pieces of tape adjacent to the established line with a small gap between the two.

For each trial, an ant carrying a leaf-load was randomly selected as it traveled down the tree, and the speed of the ant was calculated. In the space between the two strips of tape, we modified the ant's leaf load and calculated the resulting speed. To increase the load weight, we added a piece of tape (approximately 0.50 cm x 2.0 cm) using tweezers. To decrease the weight of the load, we cut the original leaf in half. For

the control, we held the leaf of each ant for 2 seconds after the baseline speed recording without modifying the weight of the leaf. We randomized treatment order. We performed the experiment twice — once in the morning, and once in the afternoon. This accounted for the beginning and peak of the foraging day. Each group (addition, subtraction, and control) contained 25 individuals for a total sample size of $n=150$ individuals.

Statistical Analyses and Modeling

We used linear regression to determine the relationship between ant length and leaf surface area as well as the relationship between ant head width and leaf surface area. A one-way ANOVA was used to test the relationship between the change in speed of the individual ants after manipulation and the treatment type. We used a t-test to test the relationship between the baseline speed and the time of day, as well as the relationship between the change in speed and the time of day. This analysis was done using JMP 15 software.

RESULTS

Part 1: Leaf to Ant Size

There was no relationship between ant length or head width and leaf surface area (Linear regression for ant length: $F = 0.51$, $df = 46$, $p = 0.48$; Linear regression for head width: $F = 1.55$, $df = 46$, $p = 0.22$; Fig. 1).

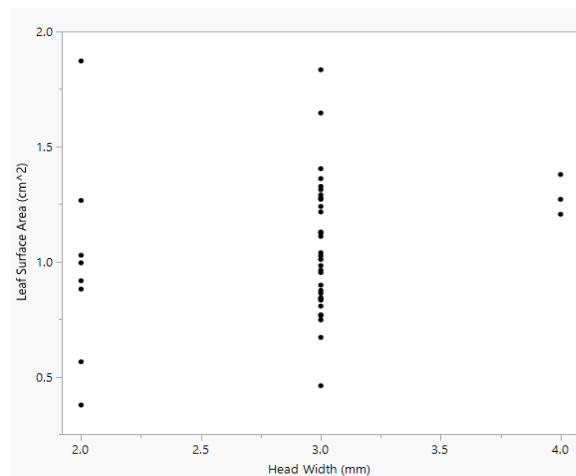


Figure 1. Surface area (cm²) of leaf carried vs. ant head width (mm). Each point represents one ant-leaf pair.

Part 2: Locomotion

The change in speed of ants before and after manipulation of load size differed from control speed changes when weight was added or subtracted from their initial load ($F = 12.77$, $df = 148$, $p < 0.001$; Fig. 2). There was no difference in baseline speed between morning and afternoon trials (t-test: $t = -0.30$, $df = 145$, $p = 0.76$). There was no relationship between change in speed after manipulation and time of day (t-test: $t = -0.57$, $df = 135$, $p = 0.57$).

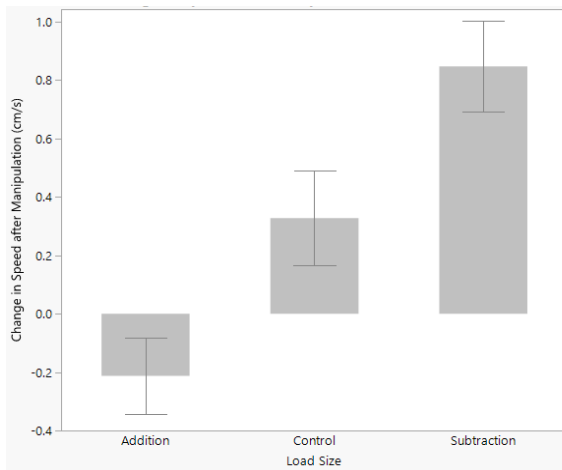


Figure 2. Mean change in speed after manipulation (cm/s) vs. load size. Mean \pm SE.

DISCUSSION

We did not find a relationship between ant head width or body length and leaf surface area, suggesting that *A. colombica* do not select leaves proportional to their body sizes. However, *A. colombica* significantly varied their transport speeds when leaf load was experimentally altered. Consistent with hypothesis one, ants sped up when their loads were reduced, and slowed down when their loads were increased. Control ants increased speeds slightly following manipulation, but speed changes of both addition and subtraction groups differed significantly from the control group. These results suggest that *A. colombica* does not maintain a consistent, colony-wide speed to protect the group from external disturbances. Instead, individual ants appear to transport leaves at a speed proportional to their load size, maximizing efficiency at an individual level by

adjusting their speeds according to current conditions.

The increase in speed in the control indicates that ants do respond to disturbances to leaf transport. This may be attributed to a need to compensate for lost foraging time, with each individual attempting to maintain a speed that avoids congestion in traffic flow. Alternatively, this response might be a fear or stress response, under which the ant intends solely to escape the source of the disturbance. This speed increase warrants further study into individual's sensitivity to disturbance.

Equipment sensitive to the slight masses of ants and leaf cuttings was unavailable on-site, so surface area was used instead as a proxy for leaf mass, and ant length was used as a proxy for ant mass. These proxies may have generated Type II error in our data, as leaf area and ant length are not necessarily related to respective masses. However, leaves collected seemed to originate from the same tree, which implies that density variations among leaves are likely insignificant to our results.

In conjunction with existing research describing the biomass accumulated by the ants and their overall foraging efficiency, our findings provide a framework for understanding two particular facets of foraging behavior: ant load size and walking speed (Herz 2017, Lugo 1973, Costa 2008). Employing the model of optimal foraging, the cost of foraging is directly manipulated through alteration of an ant's leaf load. With an altered cost, the ant alters its behavior, accordingly, walking more slowly after the addition of mass to a leaf or walking faster after the subtraction of mass from a leaf. Our findings imply that leaf cutter ants are maximizing colony success based on an individual-level cost-benefit analysis, which optimizes movement speed and efficiency.

Ants demonstrate tremendous evolutionary success based on their ability to actively regulate their colonies through simple, individual decision rules that enable rapid responses to changing environmental changes. While this experiment was not directly concerned with the social organization of individuals within the colony, our findings are indeed consistent with previous research demonstrating that individual ants make simple decisions based on local

information (Middleton 2016). This phenomenon has broad implications for all foraging systems, where critical assessments and re-assessments of cost-benefit tradeoffs are necessary as local conditions change.

ACKNOWLEDGEMENTS

We would like to thank the Smithsonian Tropical Research Institute (STRI) on Barro Colorado Island for their hospitality and generosity in allowing our research projects to proceed. Additionally, we would like to thank the Teaching Assistants for their help in structuring our experimental design.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this work.

LITERATURE CITED

- Costa et al. 2008. Do herbivores exert top-down effects in Neotropical savannas? Estimates of biomass consumption by leaf-cutter ants. *Journal of Vegetation Science* 19:6.
- Denny et al. 2001. Foraging efficiency in the wood ant, *Formica rufa*: is time of the essence in trail following? *Animal Behavior* 62: 1.
- Farias et al. 2018. Selective fern herbivory by leaf-cutter ants of *Atta cephalotes* (L.) in Brazil. *Brazilian Journal of Botany* 41: 4.
- Garrett et al. 2016. Leaf processing behavior in *Atta* leaf cutter ants: 90% of leaf cutting takes place inside the nest, and ants select pieces that require less cutting. *The Royal Society Open Science* 3:1.
- Herz et al. 2007. Assessing herbivory rates of leaf-cutting ant (*Atta colombica*) colonies through short-term refuse deposition counts. *Biotropica* 39: 4.
- Lugo et al. 1973. The impact of the leaf cutter ant *Atta Colombica* on the energy flow of a tropical wet forest. *Ecology* 54: 6.
- Middleton, E. and T. Latty. 2016. Resilience in social insect infrastructure systems. *Journal of the Royal Society Interface* 13: 116.
- Pyke, G. Et al. 1977. Optimal Foraging: A Selective Review of Theory and Tests. *The Quarterly Review of Biology* 52: 2.
- Shepherd, J. 1985. Adjusting foraging effort to resources in adjacent colonies of the leaf-cutter ant, *Atta colombica*. *Biotropica* 17: 3.

SENSIBILIDAD INDIVIDUAL A LOS COSTES DE FORRAJE: LA MANIPULACIÓN
EXPERIMENTAL DE CARGAS DE HORMIGAS CORTADORAS DE HOJAS

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Editor: Mark Laidre

Abstracto: La teoría óptima del forrajeo afirma que los organismos buscan maximizar la ingesta de nutrientes mientras minimizan los costos y esta teoría es crítica para entender los comportamientos de forrajeo de los animales sociales. La hormiga cortadora de hojas, *Atta colombica* ofrece un sistema modelo para estudiar la implementación de la teoría óptima del forrajeo en grupos sociales complejos. Hemos probado si *A. colombica* maximiza la tasa de transporte de hojas a nivel individual (basado en la carga que lleva cada individuo) o en su lugar distribuimos individuos uniformemente a lo largo de un camino de forrajeo independientemente de sus cargas (para que la colonia pueda defenderse de perturbaciones externas). Primero medimos las proporciones de tamaño de hormiga a carga y determinamos que el tamaño de la carga no estaba relacionado proporcionalmente con el tamaño de la hormiga. Luego seguimos esto con una manipulación experimental de la carga de la hoja llevada por cada hormiga, registrando los cambios en la velocidad de cada hormiga individual que fue manipulada. La carga de hoja representa el costo proximal primario para los trabajadores de *A. colombica*, sin ningún beneficio inmediato. Predijimos que las alteraciones en la carga de la hoja darían lugar a cambios en la velocidad de la hormiga que estaban vinculados al costo proximal de transportar la carga. Encontramos que los individuos de *A. colombica* aceleraron con una disminución de peso y disminuyeron con mayor peso, lo que indica que cada individuo de una colonia maximiza su velocidad en relación con la carga de la hoja. Nuestros hallazgos en este sistema son consistentes con la teoría óptima del forrajeo y sugieren que las decisiones costo-beneficio a nivel individual pueden maximizar la eficiencia a nivel de colonia.

Palabras claves: eficiencia, eusocialidad, herbívoro, hormigas cortadoras de hojas (*Atta colombica*), teoría óptima de forrajeo

BUTTRESSES ON TROPICAL TREES: BIOTIC AND ABIOTIC FACTORS INFLUENCING ARCHITECTURE AND INVESTMENT

MAXWELL C. BOND, BENJAMIN J. ZDASIUK

TA Editors: Melissa DeSiervo, Clare Doherty
Faculty Editor: Mark Laidre

Abstract: All organisms face difficult investment decisions between growth and security. Trees face this dilemma, and it has been suggested that conical buttress structures on tropical trees increase their stability. In this study, thirty-two trees with buttresses were sampled in the tropical rainforest of Barro Colorado Island, Panama. To investigate if buttresses are produced in response to environmental stressors, we calculated total buttress surface area for each tree and compared it to the aspect and angle of the tree's slope, as well as the nutrient soil depth, crown class, and circumference. Buttress surface area: circumference ratio was used as a measure of buttress investment by the tree. No significant relationship was found between buttress investment and slope angle, slope aspect, crown-class, or nutrient soil depth. Further studies could investigate the role of competition in buttress formation, specific buttress geometry, and how changing climate could further affect buttress development and overall investment patterns.

Key words: buttress, competition, investment, slope

INTRODUCTION

Since the early stages of construction and engineering, humans have found inspiration in nature's striking methods for solving structural dilemmas. Much as a human engineer must balance the cost of materials with the need for architectural support, all species make similar investment decisions throughout their development. In situations where individuals are subject to several stresses, tradeoffs necessarily arise, and these investment decisions become complex and consequential. Whether it is predation, intraspecies competition, or abiotic environmental stressors, an organism's adaptation to stress severely impacts its fitness. Over time, evolution forces optimize investment decisions; for example, a male elk that can balance the structural development of antlers while still surviving in a harsh environment is rewarded with higher reproductive success. This theme is repeated across all of life.

Plants in particular exhibit developmental plasticity, allowing them to adapt to changing stress conditions throughout their growth. Trees must balance the resource costs of building support structures, while facing competition on several levels: competition to grow tall and capture light, and to secure nutrient resources underground. Furthermore, as trees grow taller,

some environmental stressors may be amplified, e.g. greater risk of being blown over by wind.

One solution certain tropical trees exhibit is to build buttress structures around the base of their trunks. Fundamentally, building support structures like buttresses requires allocation of resources away from above ground growth. But critically, aboveground growth is needed to overcome competitors for light acquisition. Chapman et. al argues that trees build buttresses mainly to account for asymmetric stresses (i.e. wind force), and that emerging trees (those that breach the canopy), have relatively larger buttresses than canopy trees (1998). Newbery et. al, however, argues that buttresses may act as a mechanical support mechanism, but their main purpose is nutrient acquisition (2008). To resolve this conflict, we measured above ground stressors (e.g., slope angle, wind exposure, crown class) and a below ground stressor (nutrient availability) to compare buttress investment across varying asymmetries of stress.

Several environmental factors apply asymmetric stress on trees and investigating these could enlighten our understanding of buttress development. One such stress is the rotational force experienced by a tree on an angled slope. The greater the angle of the slope, the more rotational force the tree is experiencing, increasing its propensity to topple over. Thus, we would

expect to see greater buttress investment in trees on steeper slopes. Exposure to wind applies a similar asymmetric stress on a tree; different slope aspects can have a significant effect on the amount of wind a tree is exposed to (Chapman et. al 1998). Given prevailing wind patterns, trees on windward slopes should have greater buttress investment. Crown class can also affect wind exposure: as a tree starts to emerge into the canopy, it is experiencing more wind than it had been exposed to previously (Horn 1971, Chapman et. al 1998). If extreme changes in weather exposure are experienced when a tree emerges from the canopy, we expect to see the greatest buttress investment in trees that breach the canopy. Additionally, nutrient availability is commonly cited as an important factor influencing buttress development; in lower nutrient depth soils, buttresses allow a tree to spread its roots over greater horizontal distance and fix more nutrients (Newbery et. al 2008). If buttress ontogeny is influenced by nutrient soil depth, lower nutrient soil depth should relate with greater buttress investment.

Resource investments in buttress forming trees are of great scientific interest, as the relationship of buttresses and biomass can be used to improve carbon models of tropical forests (Cushman et. al 2014), important for climate change modelling. The hypotheses listed above were tested on trees in Barro Colorado Island (BCI) Research Reserve, a tropical Central American rainforest, thereby exploring the investment decisions made by tropical tree species.

METHODS

Sampling Method

To test which environmental factors were influencing buttress investment, we measured certain physical traits of a random sample of buttress-forming trees and their localized environment. To ensure diversity of species and environment in our samples, we assigned three different zones for sampling on Barro Colorado Island, Panama. We chose zones to occupy different geographical areas on the island with diverse topography including 1) a flatter, shallow angle forest, 2) a steep forest, and 3) a ridgeline forest. All zones were at elevations between 60 and 150 meters. At each zone we designated a random 2 km section of trail for sampling. We hiked for 5 minutes at a consistent pace, and at the

end of the 5 minutes would sample the 3 nearest buttress-forming trees that exhibited a circumference over 1 m and were at least 5 m away from any previously measured tree. A total of 32 buttress-forming trees and 117 buttresses were measured during sampling over a 3-day period.

To measure resource investment in buttresses, we used total buttress surface area divided by circumference as a size-normalized buttress investment metric. This assumes circumference as a proxy for tree growth, and thus, the greater the ratio of total buttress surface area: circumference, the more the tree has invested in buttresses throughout its growth. To test asymmetric stress hypothesis, we examined slope angle and aspects. To test the nutrient availability hypothesis, we measured soil depth (a proxy for nutrient availability) in a tree's localized environment. Finally, crown class was recorded to evaluate the exposure of a measured tree's canopy relative to the canopy of neighboring trees.

Measurements

For each sample, we recorded the measurement outlined in Supplemental Table 1.

Statistical Analyses

All data was analyzed using JMP-Pro 14. For each tree, we calculated 'total buttress surface area' by summing all the surface area of that tree's buttresses. To test the relationship between buttress investment and slope angle, we used a generalized linear mixed model with random effects to account for any variation from zone. We also used a generalized linear mixed model to test the relationship between buttress investment and nutrient soil depth. To test whether trees on north-facing slopes had higher buttress investment, we used a t-test to compare the buttress investment on north facing and non-north facing aspects. To test if crown maturity influenced buttress investment, we conducted an ANOVA between buttress investment and crown class. To test if the most downhill buttress typically had larger surface area than the most uphill buttress, we conducted a paired t-test of number of buttresses uphill versus downhill on the same tree. To test if there was a relationship between buttress angle and the steepness of the slope, we performed a linear regression with slope and buttress angle. Finally,

to test if the most downhill buttress typically had a steeper angle than the most uphill buttress, we performed a paired t-test of buttress angle uphill versus downhill.

RESULTS

Measured slope angles varied from 3 to 40 degrees with an average slope angle of 16.33 degrees. Slope angle did not have a significant relationship with buttress surface area to circumference ratio, and zone accounted for 0% of the variation in the sample (generalized linear mixed model: $F = 0.04$, $df = 30$, $p = 0.84$; Fig. 1).

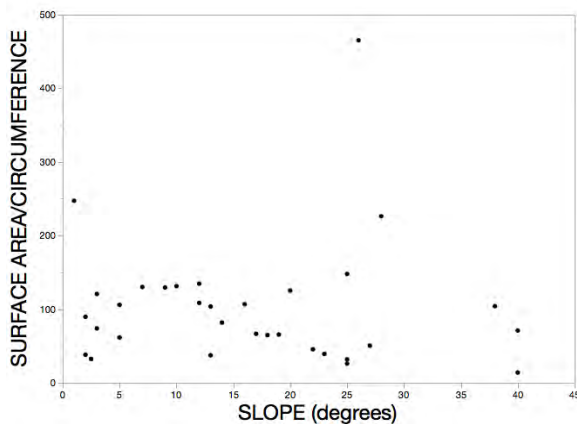


Figure 1. Buttress investment, measured by using total buttress surface area/circumference for each tree, changing with slope angle.

Additionally, no significant difference was found between the surface area of the most downhill and most uphill buttress (paired t: $t = 0.10$, $df = 31$, $p = 0.92$). However, one significant relationship was observed, between the average buttress angle (of all buttresses on a given tree) and slope angle (linear regression: $F = 4.73$, $df = 31$, slope -0.34 ± 0.16 , $p = 0.04$, $r^2 = 0.14$; Fig. 2).

Trees were assigned as being on a windward slope if the slope aspect fell between 315 and 45 degrees as prevailing winds come from the north on Barro Colorado Island. By this definition, 14 trees were on windward slopes and 18 trees were on leeward slopes. There was no significant difference between buttress investment on a windward slope and investment on a leeward slope (paired t-test: $t = -0.55$, $df = 27.2$, $p = 0.59$).

Nutrient soil depth ranged from 7 cm to 14.5 cm with an average depth of 9.5 cm. Nutrient soil

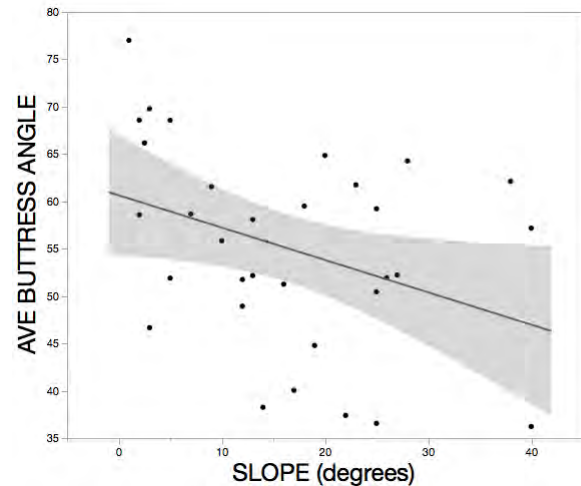


Figure 2. Average buttress angle decreasing with slope angle. Shaded area represents the 95% confidence interval.

depth also did not have a significant relationship with surface area to circumference ratio (generalized linear mixed model: $F = 1.819$, $df = 30.0$, $p = 0.19$).

Of the 32 trees measured, 8 were dominant (DO), 21 were co-dominant (CO), and 3 were intermediate (IN) crown classes. There was no significant difference in surface area to circumference ratio in any of the crown classes (ANOVA: $F_{2, 29} = 0.50$, $p = 0.61$; Fig. 3).

Finally, there was a difference between the number of non-buttress-forming neighbor trees and the number of buttress-forming neighbor trees, with significantly more non-buttress-forming

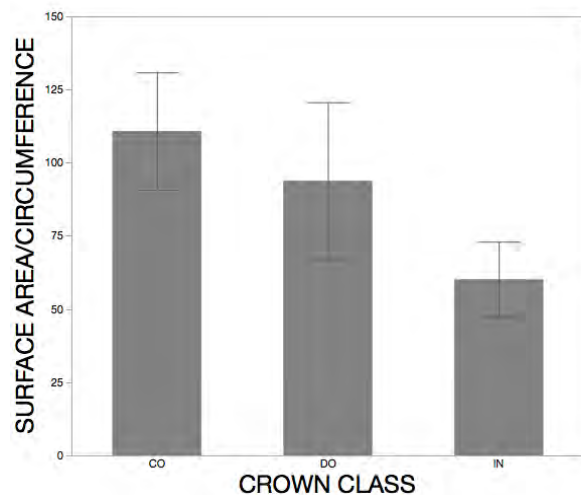


Figure 3. Bar graph for mean buttress investment, by crown class. Mean \pm SE.

neighbors (paired t-test = 9.989, df = 31, $p < 0.0001$; Fig. 4).

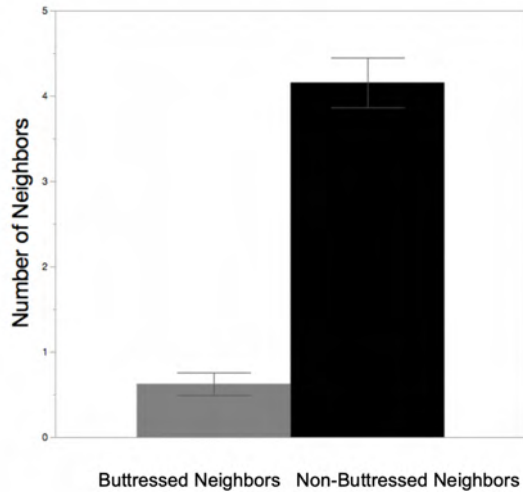


Figure 4: Neighbors of the sampled tree that had and did not have buttresses. Mean \pm SE.

DISCUSSION

All hypotheses in this study had no support: neither slope angle, wind exposure, nutrient soil depth, or crown class had significant relationships with the surface area to circumference ratio, the proxy variable for buttress investment. For the slope angle hypothesis, while the surface area to circumference ratio did not reveal a significant relationship, average buttress angle (which was measured to determine the total buttress surface area) did significantly decrease with an increase in slope angle. Hence, the steeper the slope, the less steep the buttress angle.

The slope aspect proxy for wind exposure based on prevailing wind direction was backed by theory, but perhaps too great an assumption. Regional measurements show general meteorological patterns across the island, but wind patterns can be greatly affected by localized topography (Windsor 1990). Future studies interested in wind exposure should account for these localized wind effects. It is also worth mentioning that surface area to circumference ratio may not be an ideal metric. The surface area to circumference ratio was a practical proxy for this study, but accurately determining the volume of tree trunk and buttress or root structure could be incredibly valuable for measuring buttress investment, metabolism, and carbon sequestration

of an individual tree. Perhaps ground-penetrating radar could be utilized in future studies to study the underground architecture of buttresses. Additionally, a more empirical study of soil chemistry and key nutrient density could be a valuable comparison.

In retrospect, an interesting observation that was not considered initially was the sheer number of non-buttress-forming trees found near each measured tree. Almost every neighboring tree was non-buttress-forming. This highlights the fact that, while we viewed buttresses as a novel solution to issues of nutrient soil depth and asymmetric stresses, other tree species have clearly found a way to survive and grow without buttresses. The high-frequency of non-buttress-forming trees in the same environment raises the question whether buttresses are essential. Perhaps buttresses only represent one of many different investment strategies, each with variable costs and benefits, and with the costly investment of the buttress's strategy yielding equal lifetime fitness with other strategies.

Extrapolating from the results of this study, resource investment in buttresses may be trivial compared to the resource demands of competition. In an extremely competitive environment such as BCI, there could be increased pressure to invest in above-ground competition as opposed to structural security. In a less competitive environment, perhaps investment in security, i.e. buttress growth might be greater and less random. However, buttresses do exemplify the plasticity of plants to make an impactful investment decision in constructing structures that are not directly related to vertical growth. For plants and animals, understanding the complex abiotic and biotic conditions that lead to investment decisions is critical both for understanding development of organisms and how conservation can be leveraged to protect organisms at critical developmental stages. In a globally unstable climate, all species will be facing new and novel pressures. Life will be forced to adapt and change investment patterns. Already, species succession is noticeably changing (Condit et. al 2017). As the world continues to change, so will evolutionary pressures, and thus, the investment decisions made by organisms.

ACKNOWLEDGEMENTS

Thank you to Melissa, Clare, and Mark for contributing massive advice on our sampling method, hypotheses, and overall scope of our project. Appreciations to Natalie, our excellent STRI Naturalist Guide. Love to Felipe, who lent us his trowels for digging. Nods to Ben Kopania for discussing buttresses with us over dinner and leading us to some excellent literature on the subject. Shout-outs to Sarah for bringing us succulents such as bananas and coffee. Lastly, knucks to Reyn for giving Max an excellent haircut which took 2 hours to complete, due to a faulty shaving device.

AUTHOR CONTRIBUTIONS

All authors contributed equally to data collection, analysis, and writing. Ben Zdasiuk specialized in nutrient soil depth measurements, while Max Bond specialized in buttress angle and aspect measurements. By the end of the project, Max Bond and Ben Zdasiuk could competently carry out telepathic communications.

LITERATURE CITED

- Chapman, C. A., Kaufman, L., and Chapman, L. J. 1998. Buttress formation and directional stress experienced during critical phases of tree development. *Journal of Tropical Ecology*, 14(3), 341-349.
- Condit, R., Pérez, R., Lao, S., Aguilar, S., and Hubbell, S. P. 2017. Demographic trends and climate over 35 years in the Barro Colorado 50 ha plot. *Forest Ecosystems*, 4(1), 17.
- Cushman, K. C., Muller-Landau, H. C., Condit, R. S., and Hubbell, S. P. 2014. Improving estimates of biomass change in buttressed trees using tree taper models. *Methods in Ecology and Evolution*, 5(6), 573-582.
- Horn, H. S. 1971. *The adaptive geometry of trees* (No. 3). Princeton University Press.
- Newbery, D. M., Schwan, S., Chuyong, G. B., and van der Burgt, X. M. 2009. Buttress form of the central African rain forest tree *Microberlinia bisulcata*, and its possible role in nutrient acquisition. *Trees*, 23(2), 219.
- Windsor, D. M. 1990. *Climate and moisture variability in a tropical forest: long-term records from Barro Colorado Island, Panama*. Smithsonian contributions to the earth sciences.

APPENDIX

Supplemental Table 1: Architectural and abiotic measurements of tree buttress and local area.

Measurement	Description
Time, Date	24h time, YYYYMMDD format
Latitude, longitude, and altitude	Recorded in degrees and feet, respectively
Slope aspect	Cardinal direction in degrees
Slope angle	Recorded in degrees using an inclinometer
Number of buttresses	Small buttresses which branches off a main buttress, larger buttresses not counted
Tree Circumference	Measured just above tallest buttress
Uphill nutrient soil depth	Determined by digging a vertical trench 2.5 m uphill of the tree trunk and measuring the depth of the nutrient soil in cm. Nutrient soil was defined as topsoil layer until a visible change to a redder, more compact, rockier soil indicative of clay
Downhill nutrient soil depth	Same method as uphill except 2.5 m downhill
Number of neighboring trees	Measured from a 5 m radius from the tree, counted all neighboring trees except those which were understory by crown class
Crown class	Define by the US Forest Service. CO = co-dominant trees, DO = dominant trees, IN = intermediate trees
Zone number	See sampling method, zone = 1, 2, or 3
Aspect of buttress	Aspect of each buttress
Length of buttress	Length in cm of each buttress
Buttress angles	Recorded from 1) top of buttress, 2) middle of buttress, 3) bottom of buttress
Surface area	$SA = (\text{length})^2 * \cos(\text{angle}) * \sin(\text{angle})$

LOS CONTRAFUERTE EN LOS ÁRBOLES TROPICALES: FACTORES BIÓTICOS Y ABIÓTICOS QUE INFLUYEN LA ARQUITECTURA Y LA INVERSIÓN

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Editor: Mark Laidre

Abstracto: Todos los organismos se enfrentan a decisiones de inversión difíciles entre el crecimiento y la seguridad. Los árboles se enfrentan a este dilema, y se ha sugerido que las estructuras de contrafuerte cónico en los árboles tropicales aumentan su estabilidad. En este estudio, treinta y dos árboles con contrafuertes fueron muestreados en la selva tropical de la isla Barro Colorado, Panamá. Para investigar si se producen contrafuertes en respuesta a los factores estresantes ambientales, calculamos la superficie total del contrafuerte para cada árbol y lo examinamos con respecto al aspecto y el ángulo de la pendiente que era el árbol, así como la profundidad del suelo de nutrientes, la clase de corona y la circunferencia de cada árbol. La relación de superficie del contrafuerte:circunferencia se utilizó como medida de la inversión de contrafuerte por el árbol. No se encontró ninguna relación significativa entre la inversión del contrafuerte y el ángulo de inclinación, el aspecto de la pendiente, la clase de corona o la profundidad del suelo nutritivo. Otros estudios podrían investigar el papel de la competencia en la formación de contrafuertes, la geometría específica del contrafuerte y cómo el cambio climático podría afectar aún más el desarrollo de los contrafuertes y los patrones generales de inversión.

Palabras claves: contrafuerte, competencia, inversión, pendiente

LIFE IS A HIGHWAY: OBSTRUCTION SIZE AND SPEED OF PATH CLEARING BY LEAF CUTTER ANTS

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TA Editors: Melissa DeSiervo and Clare Doherty
Faculty Editor: Mark Laidre

Abstract: Many animals invest energy into maintaining structures, such as nests, dams, and burrows. Some social animals even do so cooperatively, working together as a group to maintaining their home base and the areas around it. For example, leaf cutter ant colonies perform trail maintenance as a superorganism, deploying specific individuals to build and maintain an extensive trail network, which is used to transport materials integral to colony survival. However, little is known about how trail maintenance operates in response to variable obstacles. Here, we tested how leaf size and senescence influence the time it takes leaf cutter ant colonies to remove leaf obstacles. We found a significant increase in removal time with increased leaf mass, but no significant difference between the sizes of leaves that were cut or the percentage of green vs. brown leaves that were cut. This examination of trail maintenance by eusocial insects provides insight into the importance of complex structures to the livelihood of many social species.

Key words: ant trail maintenance, *Atta colombica*, Barro Colorado Island, eusociality, leaf cutter ants, super-organism

INTRODUCTION

Many animals act as architects, building and maintaining structures often larger than themselves (Gould 2012). These structures can serve a purpose beyond simple physical shelter—they can be homes with multiple functions, such as social centers or food storage. The amount of energy that species invest in maintaining these structures reflects their importance to fitness.

However physically complex these structures may be, the social complexity needed to maintain them is even more impressive. Extreme examples of structure building are found in species that exhibit eusociality, the highest level of cooperation among animals. In these species, non-reproducing individuals work together to solve problems to benefit the entire colony. By employing thousands of highly specialized-yet-coordinated individuals to create and maintain a centralized nest, eusocial insects invoke the likes of a “superorganism.” Such superorganisms effectively function with the efficiency of a single being, with the individuals that comprise them titrating their efforts across defense, home maintenance, and foraging.

Leaf cutter ants are a unique example of a eusocial species. They maintain an underground

nest with trails radiating out to trees from which they gather leaves. They maintain their nest and trails through exceptionally effective coordination of thousands of ants, each acting on locally obtained information. Specifically, ants whose role is to maintain the trail network constantly remove debris from their paths, cutting obstacles into smaller pieces or dragging them off entirely (Bochynek 2017). These paths serve as “highways,” which are constantly being repaired to enable transport of foraged material. This maintenance process is essential to the survival of the entire colony because of the unique way in which Leaf cutter ants sustain themselves: a mutualistic relationship where acquired leaves are used to grow a fungus, which represents the ants’ primary food source. It is estimated that leaf cutter ants remove an energy equivalent of 8,000 leaves for one year (Howard 2001).

To determine how leaf-cutter ants deal with obstacles on their trails, we measured response times of obstacle removal for one *Atta colombica* colony. We placed a leaf as an obstacle and measured the time it took the ants to remove it, considering the trail and leaf characteristics, removal strategy, and the number of ants involved in clearing the leaf. Previous

research has suggested that leaf color is an indicator of the health and nutrient content of a tree (Carter 2001 and Murakami 2005). Additionally, green leaves have softer tissue (Nichols-Orians and Schultz 1989). For these reasons, and because the ants are traveling far distances for green leaves to supply to the fungus, we hypothesized that the ants would be more likely to utilize a green leaf in their trail as a food source. Consequently, we predicted that green leaves would be cut more often than brown leaves. Furthermore, because larger leaves should be more challenging to remove, we hypothesized that removal time would increase with mass of the leaf obstacle. Finally, because ants often cut leaves that are too large to remove, we hypothesized that larger leaves will be cut more often than not.

METHODS

Experimental Design

We performed our experiment on four different highways emanating from one *Atta colombica* colony nest on Barro Colorado Island in Panama. We chose two different test spots on each path to not disturb the same spot each trial. We chose sites without steep path banks because we did not want extraneous obstacles such as slope and large roots to impact removal times. For each of the eight sites, we measured three highway widths at representative cross sections within 0.9 m and averaged them to obtain an approximate site width. At each site, we performed five trials, amounting to 40 trials total. For each trial we used a different leaf of varying size and color. We chose 20 green leaves and 20 brown leaves, using color as a proxy for senescence and nutrient level. For each leaf, we measured the length of the long and short axes and the mass of each. We multiplied the axes to get an approximate area measurement.

Because relative traffic on the highways could influence removal time, we measured ants per length of the highway. We placed our 19.1 cm field notebooks next to the trail and took three photos every 20 seconds. We counted the total number of ants within this length and the number that were carrying material. We used these numbers to estimate average total ants,

average percentage of ants carrying materials, and average ants available to carry material.

For each trial, we randomized the order that we introduced green and brown leaves as obstacles, and the angle at which we placed them. We began our trial when we dropped the leaf and recorded two timestamps: time of first lateral movement and time of path clearance. We recorded the first lateral movement as the time when the leaf was actively being moved away from traffic. We recorded time cleared when the leaf was either completely off the path or when it was out of the way of the main line of traffic and still for two minutes, in the latter case recording the initial time of movement cessation. In some cases, the ants cut the leaf before or while removing it from the path. For these cases, we recorded time of first cut when the cut was completed and number of cuts before successful removal. We also recorded the number of ants involved in the leaf removal: 0 (if it was not removed), 1, 2, or more than 2. We chose these categories because each involved a unique strategy. Specifically, when there were more than 2 ants, they were usually employing multiple strategies such as both cutting and dragging the leaf.

If no movement occurred for ten minutes (for example, ants were walking over the leaf without issue or the leaf was placed in such a way they could walk under it), we stopped our timer and did not consider a removal time for that trial. This was the case for 10 out of 40 (25%) cases. We did not include these trials in any time-related analyses.

Statistical Analyses

To examine which aspects of leaf size impacted removal time or strategy, we compared the relationship between both leaf mass and leaf area versus removal time. We found that leaf mass had a stronger correlation with removal time. For this reason, leaf mass was used as a proxy for leaf size for all subsequent analyses. “Leaf removal time” was calculated by subtracting the time of first lateral movement of the leaf from the time the leaf was cleared from the path. These data were log transformed to achieve a normal distribution. Analyses were performed on the transformed data, and the data

were plotted in their original form but with axes set to a log scale.

Using JMP 15, we conducted a multiple linear regression to examine the relationship of leaf removal time as a function of leaf mass and senescence category with average number of available ants as a covariate in the model. We conducted a multiple logistic regression to test if leaves were cut based on leaf color and mass.

RESULTS

A multiple linear regression of leaf mass, color, and average number of available ants vs. leaf removal time was significant (Multiple linear regression: $F = 4.81$, $df = 3$, $p = 0.0085$, $R^2 = 0.36$). Of leaf mass, color, and average number of available ants, only leaf mass had an individual effect on leaf removal time. Heavier leaves took longer to remove ($p = 0.0011$; Fig. 1, Supplemental Table 1). On average, removal took 2.6 mins when one ant was involved, 3.9 mins when two ants were involved, and 11.7 mins when more than two ants were involved. Leaves were carried off path by two or fewer ants 34% of the time and were rarely (exact numbers not recorded here) carried by more than five. There was one distinct outlier in the leaf mass vs. leaf removal time data; one leaf was 2.6 grams while all others in the sample were under 1 gram. The results did not change when we omitted this outlier. The ten trials in which the leaf was not successfully cleared from the path were not included in analyses involving leaf removal time, but they were included in all other analyses. A multiple logistic regression of leaf color and leaf mass vs. whether the leaf was cut or not was not significant (Chi-squared = 1.21, $df = 2$, $p = 0.55$, Fig. 2, Supplemental Table 2).

DISCUSSION

As hypothesized, removal time increased with respect to leaf mass. In some cases, larger leaves were more likely to get caught in obstructions on the fringe of the path such as overhanging sticks or previously cleared leaves. Large leaves also required more ants to move them, which often led to the utilization of differing strategies by different ants. While some ants dragged the leaf, another would simultaneously cut it.

Additionally, sometimes two ants would pull the leaf in opposite directions, making little

directional progress. This lack of coordination increased the time taken to come to a directional

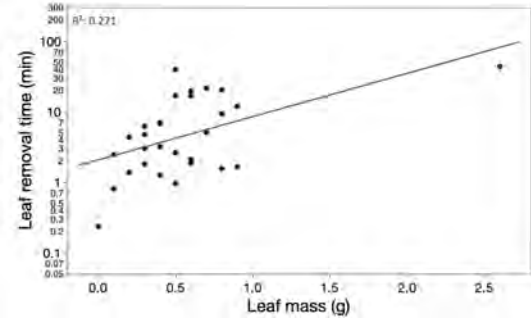


Figure 1. Leaf removal time (min) increases as leaf mass (g) increases. Each dot represents one leaf. Only trials in which the ants successfully removed the leaves are shown ($n = 30$). The outlier of (a leaf weighing 2.6 g) did not drive the overall relationship.

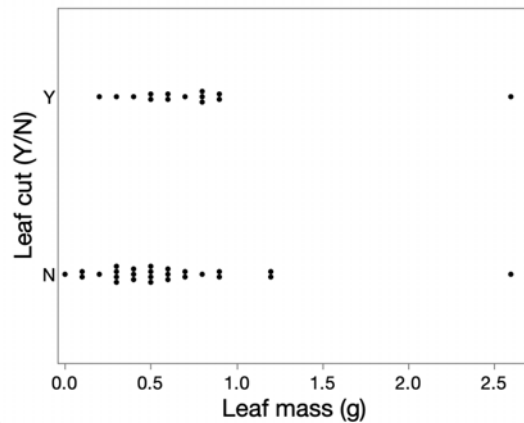


Figure 2. Whether a leaf was cut or not (Yes/No) based on the leaf mass in grams ($n=40$). Each dot represents one leaf.

consensus and move the leaf from the path. How this coordination problem is resolved is a fascinating area for future research. It is possible that the ants don't intentionally coordinate in removing obstacles, instead acting by conformity bias with incoming coordinators following the majority.

There was no significant relationship between the average number of available ants on a trail and the removal time of a leaf from that trail. This may be because not all ants we termed "available" were ants whose role was allocated to path maintenance. (Boychnek et al. 2016). To distill this, a future test could use ant characteristics to determine their functional

group, so only the obstacle-clearing ants would be considered in determining removal time.

Despite green leaves' soft tissue and role as a primary forage source, the relationship between leaf color and proportion of leaves cut was not significant. When ants cut a green leaf placed as an obstacle, they often would discard the pieces at the side of the trail. This may be because our randomly selected leaves were not within the specialized set of leaves that ants cut to provide to their mutualistic fungus. Indeed, if ants introduce incorrect or contaminated leaves to the fungus, they may jeopardize the health of their primary food source and therefore their entire livelihood. Hence, the strategy of the ants may be to only collect leaf fragments from the original source tree, rather than exercising opportunism along the way.

There was also no significant relationship found between the mass of a leaf and whether or not it was cut. It is possible that removal strategies change based on leaf length, with leaves over a certain size being cut rather than dragged. Critical to this decision is the ants' ability to travel around or beneath some of the larger obstacles we placed, which decreases their impact of these 'obstacles' on traffic and may even entirely eliminate the need to cut them. The difference in maintenance behavior based on ease of passage around an obstacle gives insight into how the ants are able to spontaneously adjust to a frequently changing forest floor.

Our study provides foundational insight into the relationship between path clearing and leaf selection in a highly cooperative eusocial species. This study also has implications for other social insects that exhibit role specialization and face tradeoffs between convenience and multitasking at the colony level. Perhaps most fascinatingly, our study

offers insight into the complex structures developed by animals and how animals can adaptively modify the environment to fit their needs.

ACKNOWLEDGEMENTS

We would like to thank the Smithsonian Tropical Research Institute (STRI) on Barro Colorado Island for their hospitality and generosity in allowing our research projects to proceed. Additionally, we would like to thank the Teaching Assistants for their help in structuring our experimental design.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this work.

LITERATURE CITED

- Bochynek, T., Meyer, B. and Burd, M. 2017. Energetics of trail clearing in the leaf-cutter ant *Atta*. *Behavioral Ecology Sociobiology* 71, 14.
- Carter, G.A., and Knapp, A.K. 2001. Leaf optical properties in higher plants: linking spectral characteristics to stress and chlorophyll concentration. *American Journal of Botany*.
- Gould, Carol Grant., and James L. Gould. 2012. *Animal Architects: Building and the Evolution of Intelligence*. Basic Books.
- Howard, J. 2001. Costs of Trail Construction and Maintenance in the Leaf-Cutting Ant *Atta colombica*. *Behavioral Ecology and Sociobiology*, 49(5), 348-356.
- Nichols-Orians, C. M., and Schultz, J. C. 1989. Leaf toughness affects leaf harvesting by the leaf cutter ant, *Atta cephalotes* (L.) (Hymenoptera: Formicidae). *Biotropica*, 80-83.
- Murakami, M., Yoshida, K. Hara, H., Toda, M.J. 2005. Spatio-temporal variation in Lepidopteran larval assemblages associated with oak, *Quercus crispula*: the importance of leaf quality. *Ecological Entomology*.

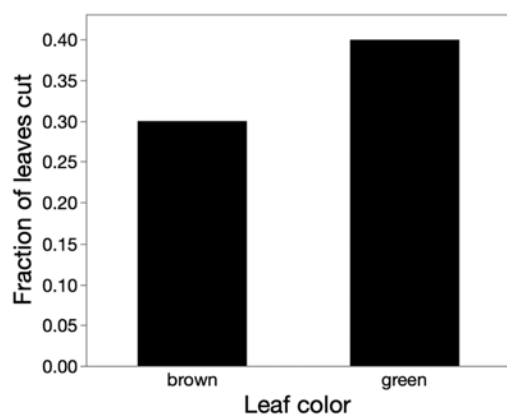
APPENDIX

Table 1: Parameter estimates from a multiple linear regression of leaf removal time vs leaf color, average number of available ants, and leaf mass.

Variable	Estimate	Standard Error	T Ratio	Prob. > t
Leaf color (brown)	0.15	0.086	1.78	0.087
Average available ants	-0.016	0.023	-0.68	0.5
Leaf Mass	0.71	0.19	3.68	0.0011

Table 2: Parameter estimates from a nominal logistic fit of whether a leaf was cut or not vs. leaf mass and leaf color.

Variable	Estimate	Standard Error	Chi Square	Prob. ChiSq
Leaf mass	-0.55	0.63	0.75	0.39
Leaf color (brown)	0.22	0.34	0.42	0.52

Supplemental Figure 1: Proportion of green vs. brown leaves cut by ants. The same number of green and brown leaves were tested ($n = 20$ for each).

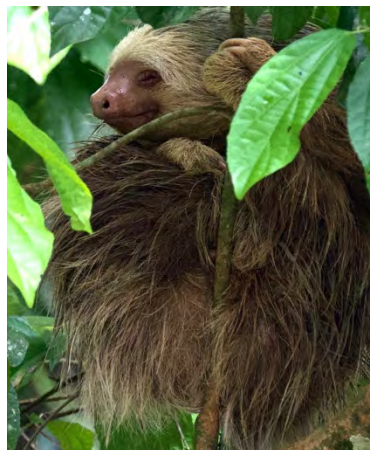
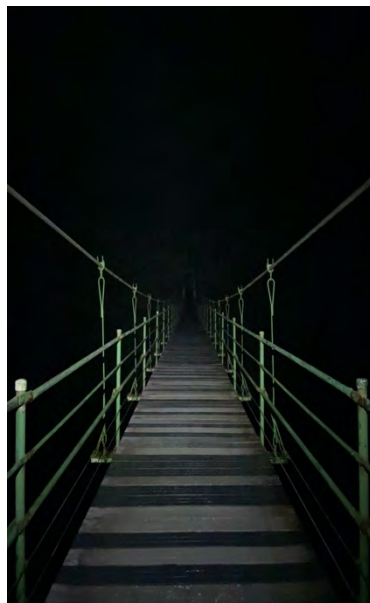
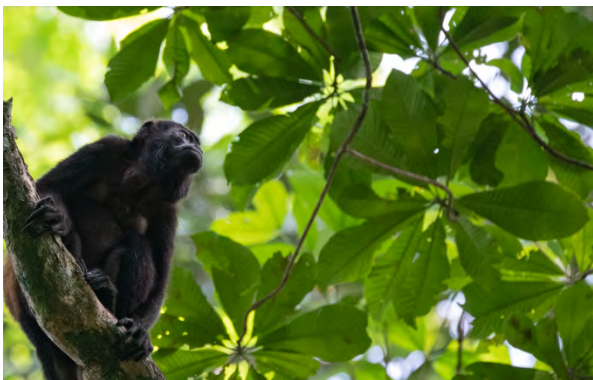
LA VIDA ES UNA CARRETERA: TAMAÑO DE LA OBSTRUCCIÓN Y VELOCIDAD DE DESPEJE DE LA TRAYECTORIA POR LAS HORMIGAS CORTADORAS DE HOJAS

SHANNON L. SARTAIN, ALIEJANDRA C. PREVOST-REILLY, QUINTIN H. TYREE, REYN A. HUTTEN

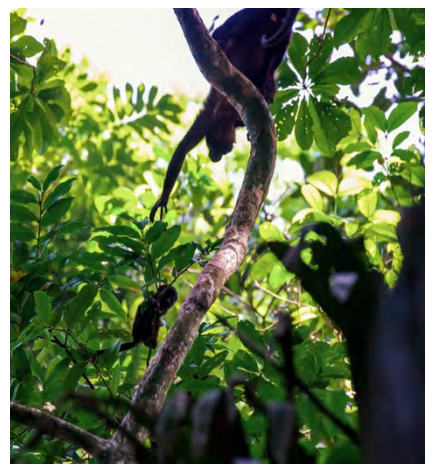
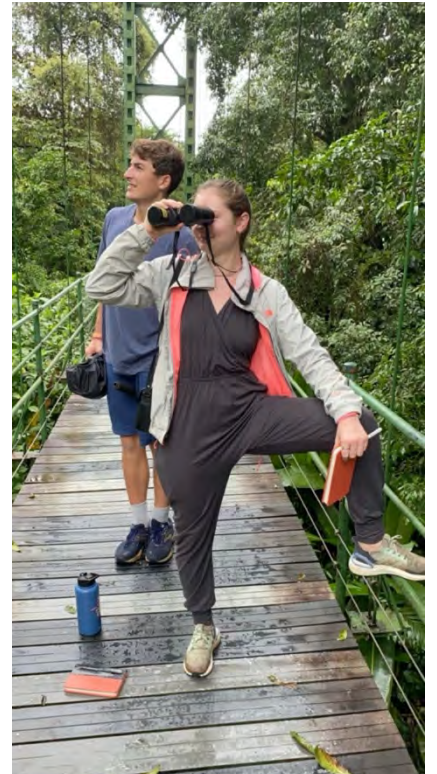
Editoras asistentes: Melissa DeSiervo, Clare Doherty
Editor: Mark Laidre

Abstracto: Muchos animales invierten su energía en el mantenimiento de estructuras, como nidos, presas y madrigueras. Algunos animales sociales incluso lo hacen de manera cooperativa, trabajando juntos como grupo para mantener su base y las áreas a su alrededor. Por ejemplo, las colonias de hormigas cortadoras de hojas realizan el mantenimiento de senderos como un súper-organismo, desplegando individuos específicos para construir y mantener una extensa red de senderos, que se utiliza para transportar materiales integrales a la supervivencia de las colonias. Poco se sabe sin embargo acerca de cómo funciona el mantenimiento de senderos en respuesta a los obstáculos variables. Aquí, probamos cómo el tamaño de la hoja y la senescencia influyen en el tiempo que tardan las colonias de hormigas cortadoras de hojas para eliminar los obstáculos de las hojas. Encontramos un aumento significativo en el tiempo de eliminación con el aumento de la masa de la hoja, pero no hay diferencia significativa entre los tamaños de las hojas que se cortaron o el porcentaje de hojas verdes frente a las marrones que se cortaron. Este examen del mantenimiento de senderos por insectos eusociales proporciona una visión de la importancia de estructuras complejas para el sustento de muchas especies sociales.

Palabras claves: mantenimiento de senderos de hormigas, *Atta colombica*, isla de Barro Colorado, eusocialidad, hormigas cortadoras de hojas, superorganismo



La Selva



TERRITORIAL SIGNALS: ABIOTIC OR SOCIAL DRIVERS OF GREEN IGUANA HEAD BOB DISPLAYS

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TA editors: Melissa DeSiervo and Clare Doherty
Faculty Editor: Mark Laidre

Abstract: Animal signals are striking but require careful observation to understand the functions they serve. We investigated the green iguana (*Iguana iguana*) head bob, a display proposed to serve several functions. At La Selva Research Station in Costa Rica, we observed four male green iguanas and surrounding females, noting the times at which each executed a head bob. We also recorded air temperature and rainfall rate. Using these data, we compared head bob frequency to air temperature and rainfall rate and tested the distribution of the data to determine if there was temporal clustering of head bob displays. We found a significant positive relationship between head bob frequency and air temperature. There was no significant clustering among head bobs in time, suggesting that iguanas are not producing head bob displays as immediate responses to conspecific head bobs. Our findings deepen our understanding of the dynamics between abiotic factors, energy conservation, and signaling displays in ectotherms, all of which are vital for territory maintenance.

Key words: animal signaling, iguana iguana, head bob displays

INTRODUCTION

Animal communication has evolved through natural selection. In these systems, a sender uses an act or structure to convey information. A receiver picks up on this signal and, in turn, modifies their behavior. This response can change the fitness of both the sender and the receiver. Over evolutionary time, the signals involved in animal communication have developed into intricate communication structures which represent some of the “most striking features of the natural world”. However, communication signals have yet to be fully understood (Laidre et. al 2013).

There are many proposed functions of animal communication across species. Perhaps the most challenging aspect of studying these systems is that no one function necessarily excludes another. For example, one proposed function of conspecific and heterospecific animal communication is advertising territorial ownership. In these instances, communication is used by individuals to demarcate territories and prevent others from encroaching on a set boundary, potentially by indicating a willingness and ability to defend the territory. This type of display goes hand-in-hand with another function of animal communication: mate attraction. The successful defense of a territory means a successful monopoly over resources, which

increases the likelihood that potential mates might choose a territory owner for reproduction.

The head bob displays of green iguanas (*Iguana iguana*) are an example of animal communication in the context of territoriality. These displays may be driven by multiple factors. For example, male green iguanas communicate threats, court females, and display territories through head bob displays. However, it is still unclear whether abiotic or social factors differentially influence the frequency of these displays (Dugan 1982, Pratt et al. 1992, 1994 and Phillips 1995). The metabolic rate of an ectotherm, such as a green iguana, is related to abiotic factors such as air temperature. Prior knowledge indicates a strong relationship between the metabolic rates of ectotherms and temperature. Rainfall may also contribute to the temperature of the iguanas. Given that evaporation of water from a surface transfers heat through conduction, a simple relationship (e.g., linear or exponential) can be used to model the metabolic rate of green iguanas and rainfall. As head bob displays are energetically costly to an individual, and the iguanas rely heavily on these abiotic factors to maintain homeostasis, we hypothesized that the frequency of displays would have a direct relationship with temperature and rainfall. Over wider temperature ranges, this relationship might be

sigmoidal, but we predicted over the narrow temperature range we studied that it would be linear.

The second objective of our observational study centered around the use of the displays of a male iguana as communication to other, conspecific, male iguanas. The definition of communication necessitates a change of behavior by the receiver. Some changes in behavior may be prolonged (e.g., keeping others at bay for extended periods), while other changes may be immediate (e.g. eliciting a head bob). Because the head bob display is used to indicate territory boundaries and multiple territory owners are often visible to one another, we predicted that head bob displays would elicit further head bob displays from neighbors, effectively clustering in time.

If temperature and rain affect body temperature, and body temperature affects head bob frequency, then we expected to see increased head bob frequency with increased air temperature. Conversely, with increased rain, we expected to see decreased head bob displays. Finally, if head bobs are a direct response to the displays of conspecific males, then we expected to see multiple head bob displays from unique individuals happening over short intervals in time. To test these different predictions, we observed four male green iguanas over the course of two days at La Selva Biological Research Center.

METHODS

Study site and species

We observed four male *Iguana iguana* that were adjacent territorial owners at the La Selva Biological Research Station in Costa Rica. We monitored nearby females for interactions with any of the four males. We recorded cloud cover, precipitation, and temperature for each trial, and for each individual head bob, we recorded the individual male performing the display and the time of day (to the second).

Observation area selection

This cluster of iguanas was chosen for its males' tendency to remain within established territories, their close proximity to each other, and visibility for observation. Our observation site was the La Selva stone bridge, giving full vantage of both

sides of the Rio Viejo River. This served as an excellent vantage point, as iguanas prefer forest edge habitats near water bodies, especially during the mating season (for Costa Rica, November-January) when they focus their energy on maintaining territory (Janzen 1983 and Dugan 1982).

Observation technique

The four male iguanas observed were assigned codes to identify each: CM, TQ, JW, and WT. Each observer focused on the iguanas on one tree to ensure every male iguana would be constantly monitored during the trial. Proximal females were also watched by each observer but were more mobile and were watched primarily for their interactions with males. We conducted 8 different trials of 15 minutes each. When an iguana began a head bob display, iguana name and time of day to the second were recorded by the designated data collector.

For each trial, rain and cloud cover were recorded using guidelines outlined by the USFS (American Avalanche Association 2016). Whenever possible, trials were conducted during periods of uniform weather conditions. When conditions varied within a trial, a representative average was taken. Temperature was measured using a HOBO temperature logger affixed to the bridge and covered with a white funnel to avoid water and solar interference; the air temperature for each trial was averaged across the 15-minute trial.

Data analysis

Using JMP Pro 14 we tested the effect of air temperature on head bob frequency, by regressing total number of head bob displays per 15-minute trial onto average trial temperature. We also tested for a relationship between rain and head bob frequency by using a t-test to compare trials with versus without rain.

To test if head bob displays clustered in time more often than would occur by chance, we calculated inter-display time (the elapsed time since the previous head bob display) for both our observational data and a random uniform distribution. $N = 200$ random uniform distributions were generated for each of the 8 trials using the exact sample size of head bobs within that trial. These 1,600 sets of inter-

display times were then compiled into one distribution and compared to the observational distribution of inter-display times across all trials using a Kolmogorov–Smirnov test.

RESULTS

Temperature positively correlated with display frequency (linear regression: $F = df = 7$, $p = 0.027$, $r^2 = 0.59$; Fig. 1).

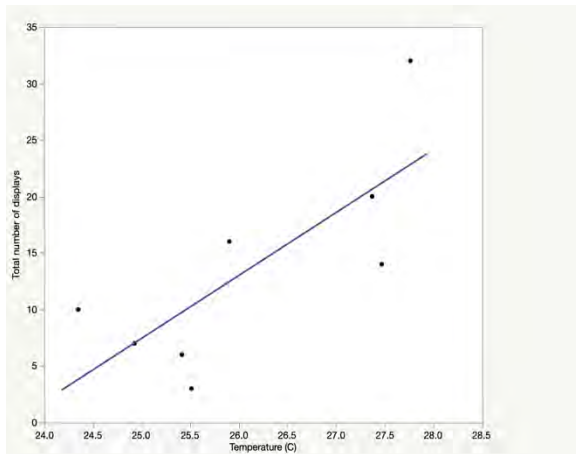


Figure 1: Number of head bob displays vs. air temperature ($^{\circ}\text{C}$).

Precipitation had no significant effect on display frequency (t-test: $t = 0.085$, $df = 2.29$, $p = 0.94$, Fig. 2).

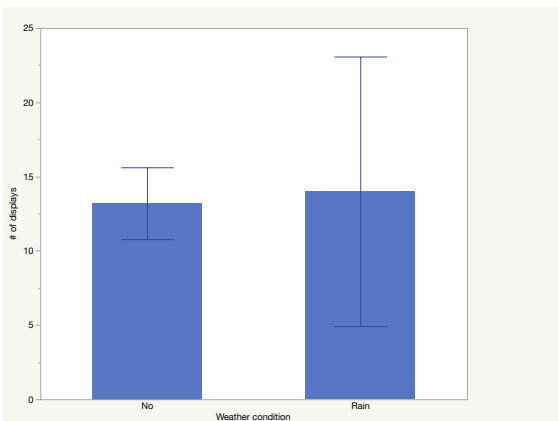


Figure 2: Number of head bob displays in trials with no rain versus rain.

No significant difference existed between the theoretical random uniform distribution for inter-display time and our observational

distribution for inter-display time (Kolmogorov–Smirnov Test: $D = 0.071$, $p = 0.70$, Fig. 3).

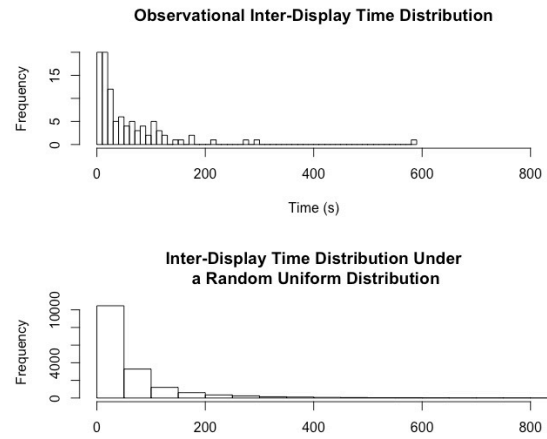


Figure 3: (A) Frequency distribution of inter-display interval (in seconds) for head bobs from all trials. (B) Frequency distribution from a randomly generated, uniformly distribution.

No significant difference existed between the log transformed observational inter-display times and the corresponding log transformed random uniform distribution inter-display times (Paired t-test; $df = 7$, $t = 1.10$, $p = 0.31$), Fig. 4)

DISCUSSION

Head bob frequency was strongly related to air temperature. This finding highlights the energy investment decisions made by green iguanas during the mating season. Our results suggest that as temperature decreases male iguanas have less energy available and must allocate their energy to maintain basic survival functions rather than protecting their territory or attracting a mate. As temperature increases, iguanas will have more energy available to spend on these less-essential behaviors. Considering the integral importance of territory defense to an iguana's reproductive success, one may infer that iguanas prioritize executing displays. That male iguanas display less frequently in colder conditions suggests that they are critically constrained by temperature and must maintain reserve energy for behaviors deemed more essential, such as physically defending the territory in the event of direct challenge with an outsider. A future study could investigate this further by observing the relationship between the timing of male-male

territory eviction events with changes in air temperature. These evictions may occur more often at high temperatures when the attacker has the highest energy available or, alternatively, at low temperatures when the defender is most vulnerable.

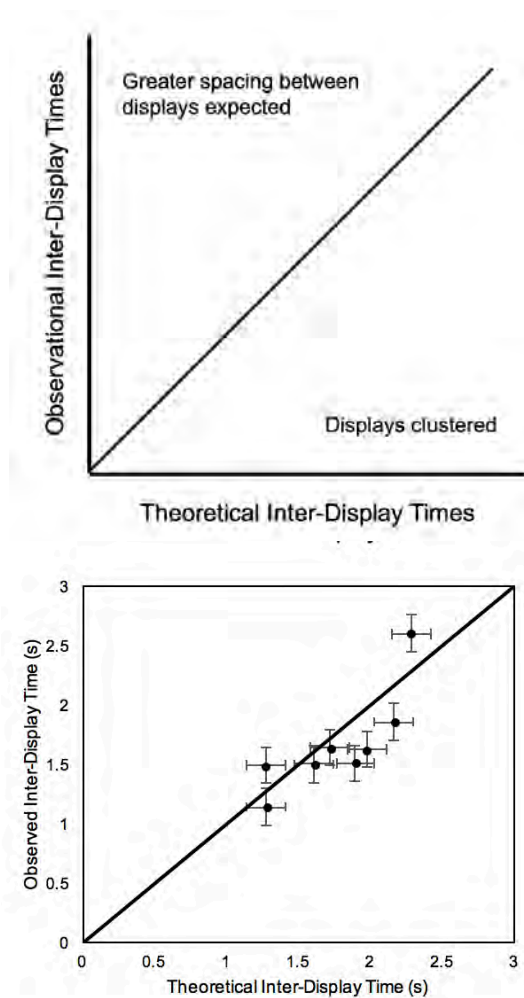


Figure 4: (A) Panel A shows how results interpretation relates to plotting method. The solid line represents a 1:1 relationship between the observed and theoretical data. If our data falls along this line, we would say it is randomly uniformly distributed. Above the line corresponds to greater spacing between the displays than expected under the random uniform distribution model. Below the line would indicate that inter-display time is less than expected – indicative of clustering. (B) Average log transformed inter-display time for each trial plotted against the average log transformed inter-display time for the corresponding random uniform distribution. Solid line represents a 1:1 relationship between the theoretical and observed values.

Rainfall had no effect on head bob frequency. However, we believe this could be due to the lack of variety in rainfall conditions during our observations. We conducted only three trials under rainfall conditions, within which there was high temperature variation which could have obscured an interaction between head bobs and rainfall. Future studies relating precipitation to iguana head bob displays should aim to collect more data in a wider variety of conditions.

Iguana displays did not occur in distinguishable clusters of immediate responses, implying that head bobs function more as unidirectional signaling than dialogue between conspecifics. However, the four iguanas we observed may not have been the only individuals involved in these interactions. Indeed, signals from other iguanas may have influenced our study system without our detection.

The situations precipitating green iguana head bobs and the particular decision rules governing this behavior remain uncertain. To determine these decision rules, and to further test the sensitivity of this communication system to clustering and contagion, it may be useful to design a study which introduces a false robotic male into an established population. This male could be set to follow simple decision rules, such as “follow each conspecific male head bob with a head bob,” consistent with direct communication, or “head bob every 3-5 minutes,” consistent with periodic territorial establishment. Iguana responses to the false male could be measured to test the decision rules and flexibility used by live iguanas.

Green iguana head bob displays are just one example of the many animal signals that can be observed in the natural world. Furthermore, displays such as the iguana head bob present an excellent opportunity to study animal signals, which are critical to their reproductive success. Much as iguana communication is affected by biotic and abiotic factors, every species experiences its own set of factors which greatly affect their signaling methods, frequency, and capability. Studies such as ours could be performed on any signaling phenomenon, generating a more complete knowledge of the languages of the animal kingdom. While human communication is still a puzzling concept to

grasp, nonhuman signaling is perhaps an even greater mystery, awaiting further inquiry.

ACKNOWLEDGEMENTS

Many thanks to Mark, Melissa, and Clare for their contributions in the observational design, analysis, and writing for this project. Appreciation to all of the excellent staff that make La Selva such an excellent place to conduct biological research. Lastly, love to all of the tourists who came up to us and said, “have you guys seen the iguana?” Yes, we have seen the iguana.

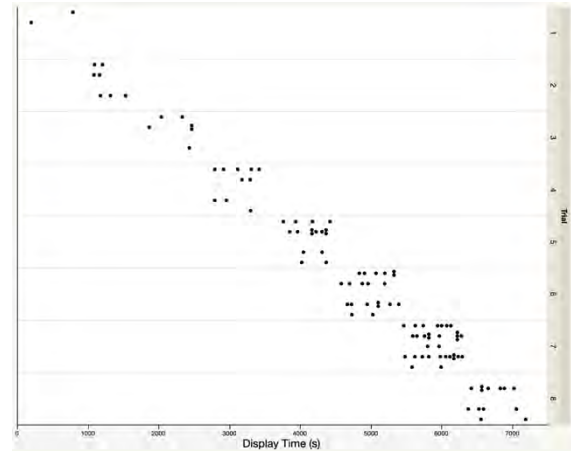
AUTHOR CONTRIBUTIONS

All authors contributed equally to this project. Max, Colton, and Reyn focused on the initial experimental design and execution. Robert and Nate completed the data analysis.

LITERATURE CITED

- American Avalanche Association 2016. Snow, Weather and Avalanches: Observation Guidelines for Avalanche Programs in the United States (3rd ed.). Victor, ID.
- Dugan, B. 1982. A field study of the headbob displays of male green iguanas (*Iguana Iguana*): Variation in form and context. *Animal Behavior* 30: 327-338.
- Janzen, D. H. 1983. Costa Rican natural history. Chicago: University of Chicago Press.
- Laidre, M. E., & Johnstone, R. A. 2013. Animal signals. *Current Biology* 23(18).
- Phillips, J. 1995. Does cadence of iguana iguana displays facilitate individual recognition. *Behavioral Ecology and Sociobiology* 37(5): 337-342.
- Pratt N. C., et. al. 1992. Behavioral, physiological, and morphological components of dominance and mate attraction in male green iguanas. *Zoo Biology* 11: 153-163.
- Pratt N. C., et. al. 1994. Functional versus physiological puberty: an analysis of sexual bimaturism in the green iguana, *Iguana iguana*. *Animal Behavior* 47: 1101-1114.

APPENDIX



Supplemental Figure 1: Display time (seconds) vs. trial number. Each dot represents one head bob display by any of the iguanas.

SEÑALES TERRITORIALES: CONDUCTORES ABIÓTICOS O SOCIALES DEL MOVIMIENTO ARRIBA Y ABAJO DE LA CABEZA DE LAS IGUANAS VERDES

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Editoras asistentes: Melissa DeSiervo and Clare Doherty
Editor: Mark Laidre

Abstracto: Las señales de los animales son llamativas, pero requieren una observación cuidadosa para entender las funciones que sirven. Investigamos el movimiento arriba y abajo de la cabeza de iguana verde (*Iguana iguana*), una pantalla propuesta para servir a varias funciones. En la Estación de Investigación La Selva en Costa Rica, observamos cuatro iguanas verdes masculinas y hembras circundantes, señalando los tiempos en que cada uno ejecutó un movimiento de cabeza. También registramos la temperatura del aire y la tasa de lluvia. Usando estos datos, comparamos la frecuencia del movimiento de la cabeza con la temperatura del aire y la tasa de lluvia, y probamos la distribución de los datos para determinar si había agrupación temporal de movimientos de cabeza. Encontramos una relación positiva significativa entre la frecuencia del movimiento de la cabeza y la temperatura del aire. No hubo agrupación significativa entre los movimientos de cabeza a tiempo, lo que sugiere que las iguanas no están produciendo movimientos de cabeza como respuestas inmediatas a los movimientos de cabeza de los conspecíficos. Nuestros hallazgos profundizan nuestra comprensión de la dinámica entre los factores abióticos, la conservación de la energía y las pantallas de señalización en los ectotermos, todos los cuales son vitales para el mantenimiento del territorio.

Palabras claves: señalización animal, *Iguana iguana*, movimientos de la cabeza

INTRA- VERSUS INTER-SPECIFIC TERRITORIALITY: PLAYBACK EXPERIMENTS OF HOWLER MONKEY CALLS

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TAs: Melissa DeSiervo, Clare Doherty
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Abstract: Niche partitioning defines the neo-tropics, which supports many diverse species. Primates offer an interesting study model because their diet-defined niches may have given rise to different social adaptations, including communication. Howler monkeys (*Alouatta*), for example, possess the loudest sound of any terrestrial animal, which could function in either intra- or inter-specific territoriality interactions. Here, we tested the link between this communication signal and territorial interactions, both within howler monkeys and between howler monkeys and sympatric primate species. Based on the greater threat posed by other groups of the same species, we hypothesized that howler monkeys, but not other monkeys, would display heightened territoriality behaviors in response to howl playbacks. We conducted playback experiments in La Selva using howler calls as the experimental stimulus and crested guan calls as a control. Among howler monkeys, we found an increase in scratching behavior after the howler playback but not following the guan playback, suggesting that proximity of a competing conspecific group is stressful. We also found a significant change in howler vigilance after exposure to both guan and howler playback calls, suggesting loud sounds in general can elicit increased alertness. Our findings provide insight into the utility of howling vocal signals in mediating territorial disputes between conspecific groups.

Key words: howler monkey, intraspecific competition, niche partitioning, territoriality

INTRODUCTION

All species occupy a distinct niche within an ecosystem. Further, investigation of a species' ecological niche centers around competition. Competition leads to niche partitioning, the process by which natural selection drives different species into different patterns of resource use, effectively reducing competition and allowing coexistence of species (Vacher 2016).

The tropics offer an interesting model for investigating niche partitioning because of the resource abundance and diversity. While primate are often generalists, some primate species have evolved to occupy a specialized niche in the tropics due to competitive overlap with other species. Critically, when multiple primate species overlaps in a given area, they must become each become more specialized, utilizing a narrower range of resources that is distinct from what other species use. The differences between sympatric primate species must be substantial enough to co-exist with limited competition and not drive one each another to local extinction. Additionally, because primates are complex social beings, their adaptations can manifest in the evolution of complex communication signals, such as characteristic vocalizations. Further, their sociality

adds to the challenge of navigating resource acquisition within and among groups. For these reasons, primates are a unique model for studying niche partitioning as well as inter- and intra-specific territoriality.

La Selva offered the opportunity to study niche partitioning in primates because three species overlap in space: the howler monkey (*Alouatta palliata*), the white-faced capuchin monkey (*Cebus capucinus*), and the spider monkey (*Ateles geoffroyi*). These species have evolved separate foraging strategies, including unique adaptations to succeed. All are opportunistic foragers, but their main food source is specialized. Howler monkeys are folivores, capuchin monkeys are omnivores, and spider monkeys are frugivorous. An interesting dichotomy arises because both spider monkeys and capuchins consume higher energy-yielding resources, as opposed to howler monkeys whose diet consists of lower energy-yielding leaves. Spider monkeys and capuchins, with a higher energy reserve, may be able to invest more into energy taxing movement, including escalated conflict-interactions. Howler monkeys, however, have much less energy to devote to such conflicts. Furthermore, howler monkeys are highly selective

about what leaves they consume, so they still require large territories. As a result of this foraging niche, howlers have developed an extraordinary auditory form of communication: an enlarged hyoid bone that creates a resonator and produces loud calls with little energetic cost. This signal appears to have evolved to allow troops to communicate their presence, thus, avoiding physical confrontations over resources (including territories). Whether this signal functions both within and species had not been addressed.

Here we tested the utility of howler calls in territoriality during intraspecific versus interspecific interactions. Despite the overlap in space of the three monkey species in La Selva, each species has a different niche, with limited overlap in resources they consume. As such, heterospecifics supposedly pose less of a threat than conspecifics. We therefore hypothesized that howler monkeys would be more likely to display heightened territorial responses (i.e. vigilance, scratching, and howls) when confronted with a threat from conspecifics rather than heterospecifics. We predicted howler monkeys would display an increase of howls, vigilance, and/or scratching in response to howler calls relative to control calls, and that furthermore other monkey species would be relatively unresponsive to howl calls.

METHODS

To test intraspecific reactions of primates to howler monkey calls we carried out playback experiments opportunistically to howler monkeys, white-faced capuchin monkeys, and spider monkey troops and individuals. Playbacks were performed along the trail system at the Organization for Tropical Studies at the La Selva. As this study was carried out on monkeys in the wild, it required us to move carefully and quietly through the forest to better find and identify these primates in the canopy.

Once we spotted a troop, we would begin our observations, each of us focusing on a separate randomly selected individual. To measure changes in behavior in response to calls we observed individuals for one-minute pre playback, gathering baseline behavioral data. After this minute, we randomly played either a howler monkeys call or control call, for 30 seconds through a speaker. We used guans calls for the control, since guans are a

common, non-threatening bird in the area. We then recorded behavioral data for the same individuals for 1 minute post playback. We waited for four minutes to allow for the subjects to return to their baseline behavior before playing the other call, again observing response behavior for one-minute post playback.

This same protocol was used for playing back howler calls to other primate species (spider monkeys and capuchin monkeys). Sample sizes for these playbacks are broken down in Supplemental Table 1, with a total sample size of $N = 18$ for howler monkeys, $N = 5$ for capuchin monkeys, and $N = 2$ for spider monkeys.

To identify behavioral changes in response to calls before vs after playbacks we measured scratching, a common stress signal in primates (Maréchal 2016); vigilance, which we defined as stopping behavior and gazing towards the sound or generally checking the environment around them at a swift pace, and howling, typically performed by males only. These behaviors were recorded as a binary, either as having happened or not; this was due to the inability to accurately measure times or counts spent performing response behaviors, as view of the subjects could become obscured by trees. In addition to these set variables we recorded qualitative observations of the troops of individuals we encountered.

Furthermore, we noted, the experiment start time, what trail we were on, and any characteristics we could distinguish about our subjects regarding their age and sex (not reported here).

The data was analyzed using JMP® Pro 14.1.0. To test if there was a significant difference in baseline scratching and vigilance before and after playbacks (howler or guan calls) we used paired t-tests.

RESULTS

Quantitative Results

Howler monkeys showed a significant change in scratching behavior from before to after the howler call playback (Paired t-test: $t = -2.35$, $DF = 11$, $p = 0.0194$, Fig. 1a) but not from before to after the guan call playback (Paired t-test: $t = 0.00$, $DF = 11$, $p = 0.0000$, Fig. 1b).

Howler monkeys significantly increased vigilance from before to after both the howl playback (Paired t-test: $t = -3.92$, $DF = 11$, $p = 0.0012$; Fig. 2a) and the guan playback (Paired t-test: $t = -2.57$, $DF = 11$, $p = 0.0261$, Fig. 2b).

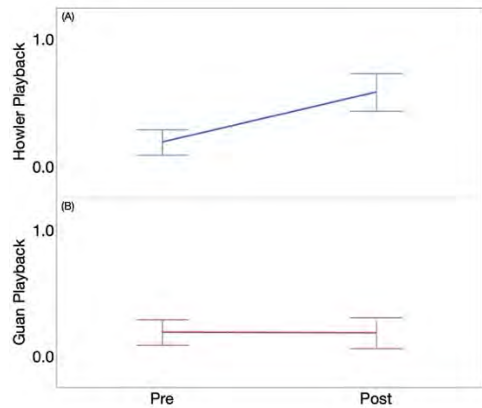


Figure 1. Howler scratching from before to after playbacks of (A) howler calls and (B) Crested Guan calls.

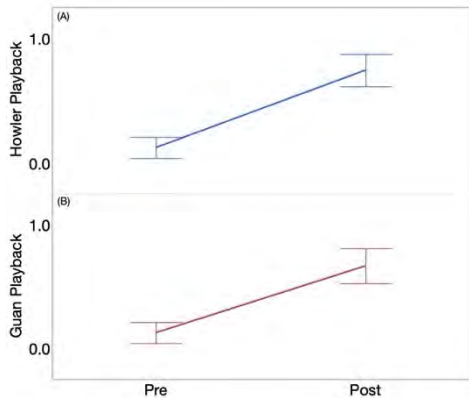


Figure 2. Vigilance of howler monkeys from before to after playbacks of (A) howler monkey calls and (B) crested Guan calls.

Qualitative Observations

Howler Exceptions: Two of our trials were conducted on lone male howler monkeys. The first lone male we observed was moving amongst a troop of capuchins. His initial response to our playback appeared to be a retreat into dense foliage, however our vision was obstructed. After a few minutes, he re-emerged into view and continued foraging activity. Neither of the lone males produced a call-in response to hearing the playback of a howl, but both became vigilant. In

all of our trials, two male howler monkeys within a troop howled back with the onset of the howler playback.

Capuchins and spiders: Experimental playback of howler calls to both spider monkeys and capuchins yielded an immediate vigilance response, but a quick return to their normal foraging behavior within the designated observation minute following the playback. In one instance, we played a howler call to the visible portion of a capuchin troop. Soon after, we observed the other capuchins move towards the playback, following their troop.

DISCUSSION

Howler monkeys displayed a heightened scratching response after the howler playback, but not for the control. Scratching in primates has been well studied and shown to be an indicator of stress (Maréchal 2016). This result is consistent with the assumption that conspecific groups represent the greatest threat. Howlers became more vigilant following both the crested guan and howler call, generally increasing their attentiveness towards the localized sound. Vigilance doesn't necessarily correlate with stress and territoriality, but rather, it could indicate curiosity, surprise, or any other reaction to an abrupt noise, which makes sense given the loudness of both the howl and guan playback.

It is interesting to ask why a howler playback does not always elicit a howl response from individuals that can howl. Notably, two lone males lacked any howl response whatsoever. Such a lack of response may make sense, given that territorial interactions can potentially escalate to physical conflict and such conflict would be riskier for lone males than those that are part of a larger group. Interestingly, once subordinate male howler monkeys reach maturity, they are kicked out of their troop and forced to be on their own until they can challenge another alpha male (Kitchen et al. 2004). Given these lone males lack a territory and a troop to protect and defend, it may be risky for them to howl back in response to another male call, which could cause the troop to attack them.

In addition to our quantitative findings, our qualitative observations can also be related to the natural history of primates. In La Selva, each species has evolved to utilize different resources. We found that howler calls did not affect the

normal foraging behaviors of capuchin and spider monkeys. This lack of response suggest that howler monkeys do not pose a substantial threat, and that howls serve mostly for intraspecific rather than interspecific communication. If howler monkey calls are typically reserved for intraspecific communication, they would be of less biological relevance to the capuchin and spider monkeys. It therefore makes sense that they have evolved to respond to a much lesser degree. Indeed, in one instance, a troop of capuchins continued following others in their group despite our experimental howler playback interrupting their foraging path. This implies that monkeys of other species do not change their foraging behavior in response to howler calls.

Howler monkey calls are a very distinct, honest means of auditory communication. This extreme adaptation can serve many functions (Oliveriera 1999). The purpose of intraspecific communication could include announcing the presence of a predator (Zuberbühler 1999), defending a space or food source (Oliveira 1999), signaling troop size (Kitchen 2004), or advertising their occupancy (Byrne & Chuna 2006). With assessment of howler's natural history, all of these possibilities could be reason for the howl to be selected for and persist into future generations. However, based on our findings and the scope of our study, we posit that intraspecific territoriality has the most direct relationship with howling.

Compared with the other sympatric primates at La Selva, howlers are highly selective foragers. Due to the low nutritional value of leaves compared to fruit and meat, howlers must preferentially select leaves with the highest nutritional benefit. As is the case with all folivores, howlers then must expend a lot of energy on digestion, compromising time spent moving. This lifestyle focused around a low-energy diet of leaves may have been the evolutionary starting point that caused howlers to develop resonating vocalizations, an energetically conservative way to communicate their claim to space and local resources. These howl signals may provide a solution to the biggest threat that howlers face: a competing conspecific troop. In the same vein, these calls can act as a method of assessment, enabling conflict avoidance, which benefits both parties. Therefore, this adaptation

acts as a mediator between different groups, each competing over the same space and resources.

Despite the differences in diet between the three species of primates, few studies have used these primates as model for niche partitioning. The differentiation in foraging strategies, which would have reduced competition, may have been the first step towards different niches. By studying the responses of sympatric species to playbacks we can ultimately better understand the function of vocalizations for both intra- and inter-specific communication of territoriality. Ultimately, such studies may shed light on how differences in responses may have evolved as result of niche partitioning.

ACKNOWLEDGEMENTS

We would like to thank La Selva for allowing us to perform playback experiments in their forest. Additionally, we would like to thank the TA's and Mark for urging us to study a system that we are passionate about and patiently helping us through the difficult endeavor. We would also like to credit this project for lighting the spark between Katy and Hutch. They are happily dating.

AUTHOR CONTRIBUTIONS

All authors contributed equally.

LITERATURE CITED

- Byrne, R. W., and Cunha, R. G. T. D. 2006. Roars of black howler monkeys (*Alouatta caraya*): evidence for a function in inter-group spacing. *Behavior*, 143(10), 1169–1199.
- Kitchen, Dawn M., Horwich, R., and Roxie A. James. 2004. Subordinate male black howler monkey (*Alouatta pigra*) responses to loud calls: experimental evidence for the effects of intra-group male relationships and age. *Behavior*, 141(6), 703–723.
- Maréchal, Laëtitia et al. 2016. "Primates' behavioral responses to tourists: evidence for a trade-off between potential risks and benefits." *Scientific reports* vol. 6 32465.
- Oliveira, D.A.G 1997. Vocalizacoes de longo alcance de bugio (*Alouatta fusca clamitans*) na area do Parque Estadual de Cantareira. Universidade de Sao Paulo.
- Vacher, C., and Bohan, D. 2016. Ecosystem Services: From Biodiversity to Society, Part 2. *Advances in Ecological Research*.

Zuberbühler, K., Jenny, D., and Bshary, R. 1999. The Predator Deterrence Function of Primate Alarm Calls. *Ethology*, 105(6), 477–490.

APPENDIX

Table 1: Total sample of playback experiments of howl call.

Howler			
<u>Sex</u>	<u>Status</u>	<u>Call</u>	<u>Sample</u>
Male	Lone	Howler, Guan	2
Male	Group	Howler, Guan	2
Female	Group	Howler, Guan	3
Unknown	Group	Howler, Guan	9
Unknown	Group	Howler, Guan, Spider Monkey	3
Capuchin			
<u>Sex</u>	<u>Status</u>	<u>Call</u>	<u>Sample</u>
Unknown	Group	Howler	5
Spider			
<u>Sex</u>	<u>Status</u>	<u>Call</u>	<u>Sample</u>
Unknown	Group	Howler	2

LA TERRITORIALIDAD INTRA Y INTERESPECÍFICA: LOS EXPERIMENTOS USANDO LAS LLAMADAS DE MONOS AULLANTES

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Editor: Mark Laidre

Abstracto: La partición de nicho define los neotrópicos, que soporta muchas especies diversas. Los primates ofrecen un modelo de estudio interesante porque sus nichos definidos por la dieta pueden haber dado lugar a diferentes adaptaciones sociales, incluida la comunicación. Los monos aulladores (*Alouatta*), por ejemplo, poseen el sonido más fuerte de cualquier animal terrestre, que podría funcionar en interacciones de territorialidad intra o interespecíficas. Aquí, probamos el vínculo entre esta señal de comunicación y las interacciones territoriales, tanto dentro de los monos aulladores como entre los monos aulladores y las especies de primates simpátricos. Basándonos en la mayor amenaza que representaban otros grupos de la misma especie, plantearíamos como hipótesis que los monos aulladores, pero no otros monos, mostrarían mayores comportamientos de territorialidad en respuesta a las grabaciones de aullidos. Realizamos experimentos de grabaciones en La Selva utilizando llamadas aulladoras como estímulo experimental y llamadas de la pava crestada como el control. Entre los monos aulladores, encontramos un aumento en el comportamiento de rascar después de la grabación del aullador, pero no después de la grabación de pava, lo que sugiere que la proximidad de un grupo conspecífico competidor es estresante. También encontramos un cambio significativo en la vigilancia del aullador después de la exposición a las grabaciones de la pava y el aullador, lo que sugiere que los sonidos fuertes en general pueden obtener un estado de alerta fallecido. Nuestros hallazgos proporcionan información sobre la utilidad de las señales vocales aulladoras en la mediación de disputas territoriales entre grupos específicos.

Palabras claves: Monos aulladores, competición intraespecífica, partición de nicho, territorialidad

LIVE STREAMS: ALTERED FORESTS CHANGE BIOTIC AND ABIOTIC CHARACTERISTICS OF NEOTROPICAL STREAMS

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Faculty Editor: Mark Laidre

Abstract: Human impacts on forested watersheds are widespread and increasing. In the tropics, where deforestation is common, these impacts can be substantial. The overall health of watersheds in response to this disturbance can be inferred from the health of the organisms living in them. To understand the impact of deforestation on neotropical streams, we measured the abundance of aquatic organisms and abiotic characteristics including temperature, pH, and sediment load in low-order streams in old and secondary growth forests at La Selva Biological Research Station in Costa Rica. Due to high sediment runoff in secondary forests, we hypothesized that streams in those forests would have lower macroinvertebrate and fish abundances. Alternatively, because secondary forests could release more nutrients into the surrounding streams, we hypothesized there could be higher macroinvertebrate and fish abundance. Our results indicated that visibility was higher in streams of secondary growth forests compared to those in old growth forests. Overall, we found very few aquatic invertebrates, and no difference in aquatic invertebrates or fish abundance between forest types. Notably, our sampling period spanned a large rain event, increasing the stage of streams sampled over the two-day period and demonstrating the impact of weather events on hydrologic characteristics. By understanding how deforestation and stochastic weather events impact watersheds, we can devise relevant strategies for mitigating further stream damage.

Key words: Neotropical streams, watersheds, deforestation, aquatic organisms, fish abundance, benthic invertebrates, forest succession

INTRODUCTION

Humans have profound effects on the natural environment, and their negative impacts on river systems are no exception. In tropical rainforests, where rivers are strongly regulated by seasonal and variable precipitation cycles (Latrubesse et al. 2005), effects of human disturbances such as deforestation can propagate from entire basins down to first-order streams. Deforestation often occurs to create land for agriculture, and it is generally associated with increased runoff (Gentry and Lopez-Parodi 1980; Sioli 1985), increased nutrient loading (Sioli 1985), and disruption of annual sedimentary loads and cycles (Restrepo et al. 2015). These effects can have negative biological, hydrologic, and even economic implications.

Deforestation impacts nutrient influx to streams. However, the way this occurs and subsequent impacts biota, including fish and invertebrates, remain poorly understood. Previous research suggests that old growth forests have more developed root systems and grow more slowly, therefore retaining fewer nutrients than secondary growth forests and releasing more into the surrounding stream systems (Sioli 1985;

Vitousek 1977). However, some research also suggests that old growth forests decrease nutrient runoff into the stream systems as they age (Goodale et al. 2003). These conflicting results suggest that the empirical connections between deforestation and river systems are imperfectly understood.

Phytoplankton production and leaf-litter degradation represent the two main carbon influxes to stream environments (Silvia-Junior 2014). All higher trophic levels are dependent on these two carbon sources, and generally exhibit adaptation to a source in a specific environment (Campbell Reece 2005). For example, certain benthic invertebrates such as shredders consume leaf litter (allochthonous carbon), while others, such as scrapers, primarily consume algae (autochthonous carbon). In more turbid environments, there is less productivity from phytoplankton, and more reliance on particulate organic matter from degraded leaves as a food source for consumers. Reduced abundance of phytoplankton due to decreased light should also decrease the abundance of macroinvertebrates that depend on phytoplankton production. This would make a community more dependent on particulate

organic matter inputs and on the benthic invertebrates that degrade leaf-litter. Furthermore, since tropical forests have poor nutrient soil content, nitrogen and phosphorus inputs from surrounding forests are likely a limiting factor for primary productivity of tropical stream ecosystems (Ricklefs 257). Fish are an effective indicator of overall stream health because they consume both phytoplankton-dependent species and leaf-litter-dependent species and are sensitive to fluctuation in abiotic conditions (Rieman et al. 2000).

Here we studied the impacts of deforestation on low-order, tropical streams at La Selva Biological Research Station in Costa Rica. We utilized historical land use data for this site (Clark and Clark 2012; Figure 1) and measured river responses to deforestation by sampling streams in old growth and secondary growth forests. We focused on how physical responses to deforestation, such as changes in turbidity, temperature, and pH, impact ecological stream characteristics, such as fish and benthic macroinvertebrate abundance.

Due to the less developed root structure of secondary forests, we hypothesized that more sediment would be released into streams in these systems. We predicted that this increased sediment runoff would increase stream turbidity, decreasing the light available to aquatic organisms. Because riverine species are adapted to pre-deforestation light availability (Davies-Colley & Smith, 2001), we hypothesized that this change in turbidity would ultimately decrease invertebrate abundance, and the abundance of the fish that eat them. We hypothesized that an increased nutrient runoff from old growth forests would stimulate autotroph growth and increase their abundance. Given this hypothesis, we predicted that an increased nutrient load in secondary growth forest streams would promote higher macroinvertebrate and fish abundance.

METHODS

Sampling Method

We selected six streams, three in old growth and three in secondary growth forests (Fig. 1). Two of the secondary growth forest sites experienced 21 to 37 years of succession, and the other was selectively logged. To minimize the amount of upstream effects from streams in different land types, we chose streams of the lowest possible

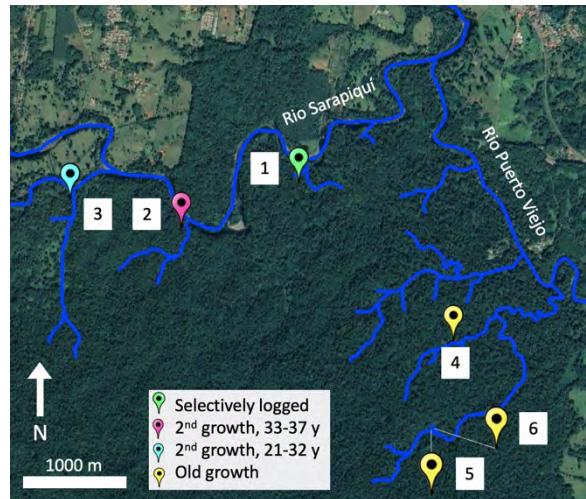


Figure 1. Map of study sites at La Selva Biological Research Station.

order. At each site, we collected a one-liter water sample. We used a YSI sensor to measure the pH and temperature. We also used a HOBO logger to record average air temperature over the sampling days. We measured the latitude, longitude, and elevation at each site via GPS, and measured the width of each stream at the access point, perpendicular to flow. Finally, we measured visibility as a proxy for turbidity, using fluorescent yellow tape on a meter stick and recording when we could no longer see the tape.

To determine how aquatic organisms varied across forest types, we collected information on the abundance of fish and benthic macroinvertebrates. To assess the abundance of fish in the stream, we performed a one-minute fish count. We selected a passage point in a high flow zone to prevent repeated fish passage over the same point and viewed fish from a bridge. We measured the abundance of benthic invertebrates by selecting three evenly spaced locations as close to the center of the stream as possible. We disturbed the bottom of the stream in front of the D-net and allowed water to flow through it for 20 seconds at each location. We then analyzed the material in the net on trays, separating components with a 2000-micron and a 250-micron sieve, counted the number of individuals and number of species in each stream. In our data analysis, we combined the abundance of invertebrates from the three D-net samples from each site.

Statistical Methods

All statistical tests were performed with JMP 14. To compare mean visibility and to compare mean fish abundance and pH between forest types, we used t-tests. We used a Wilcoxon non-parametric test to compare the benthic invertebrate abundance between forest types, since these data were not normally distributed. To decouple the effects of visibility and forest type on water temperature we used multiple regression.

RESULTS

Visibility was higher for secondary growth forests (t-test: $t = -3.79$, $df = 3.97$, $p = 0.0098$; Fig. 2).

Visibility had no relationship with fish abundance (linear regression: $F = 1.81$, $df = 1$, $p = 0.25$). In total, we found 11 macro-invertebrate individuals. Invertebrate abundance did not differ with respect to forest type (Wilcoxon non-parametric test: $Z = 1.34$, $df = 1$, $p = 0.25$; Fig. 3).

Similarly, fish abundance did not differ with respect to forest type (t-test: $t = 1.03$, $df = 3.20$, $p = 0.377$, Fig. 4).

Using a multiple regression, we found that temperature was significantly higher in secondary growth forest, but temperature had no relationship with visibility (multiple regression; forest type: $F = 29.87$, $df = 1$, $p = 0.012$; visibility: $F = 0.129$, $df = 1$, $p = 0.7429$). The average air temperature was 25.5 degrees Celsius on the old growth sampling day and 25.1 degrees Celsius on the secondary growth sampling day. pH did not differ with respect to forest type (t-test: $t = 1.15$, $df = 2.59$, $p = 0.34$).

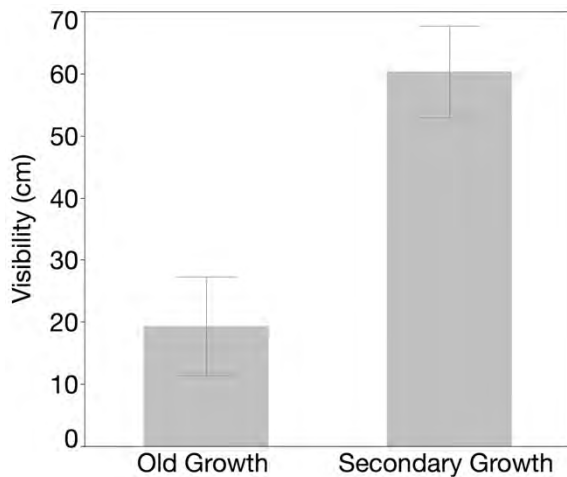


Figure 2: Visibility for streams in old and secondary growth forests (Mean \pm SE).

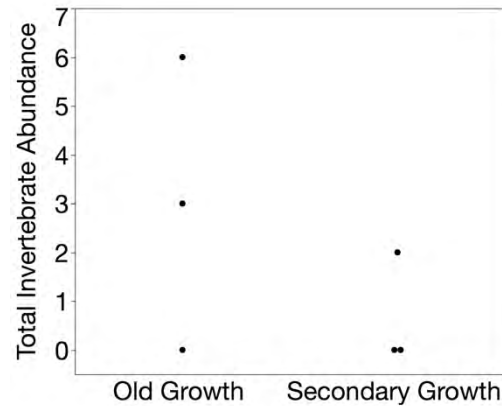


Figure 3: Total invertebrate abundance per three D-net samples per site for streams in old and secondary growth forests.

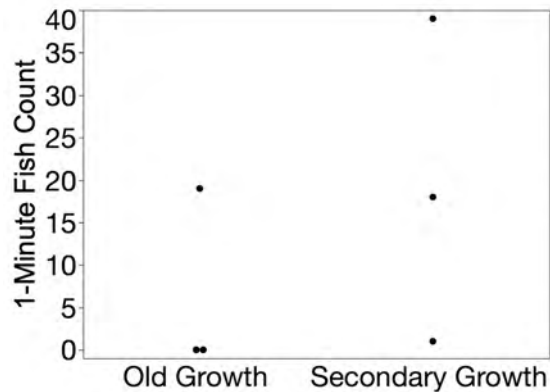


Figure 4: Number of fish counted in one minute for streams in old and secondary growth forests.

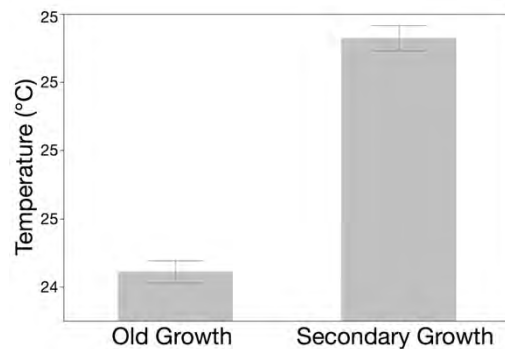


Figure 5: Water temperature for streams in old and secondary growth forests (Mean \pm SE).

DISCUSSION

We found that visibility in streams in secondary growth forests was higher, indicating a lower sediment load. This result contradicts a key aspect

of our hypotheses, that younger forests, with reduced root structures, would release more sediment into the stream system. Increasing turbidity decreases light availability, reducing autotroph abundance and creating a cascade effect on the stream food web. However, despite the reduced load of sediment in the secondary growth forests, abundance of aquatic organisms did not vary significantly with forest type. Furthermore, we did not find any relationship between turbidity and fish or benthic invertebrate abundance. Our prediction that reduced turbidity would increase abundance of fish and benthic invertebrates was not supported.

An alternate explanation for lower turbidity in streams in secondary growth forest relates specifically to tropical forest succession. In tropical forests, root structures occupy shallower soil depths and exhibit different root geometries than those in temperate forests (Newberry et al 2009). For example, unique structures such as buttresses, which provide structural support and increase nutrient acquisition (Bond and Zdziusik 2020), might reduce sediment runoff. Besides the ecological consequences of primary versus secondary forest types, other factors, such as where loggers decided to clear cut forests, may have contributed to unexpectedly high turbidity in streams in secondary growth forests. For example, forested areas with flat topography and proximity to roads were likely preferentially cleared. Watersheds in these areas would have lower relief, be of higher order, and contain streams with lower velocities. These factors that would inherently decrease turbidity and might conflate our findings.

We found that stream temperatures were significantly warmer in secondary growth forests. Reduced canopy cover in less-shaded secondary growth forests likely increases thermal heating of streams. This suggests that forest type may affect environmental conditions of streams, which can have important implications for both fish and invertebrate health (Rieman et al., 2000; Silva-Junior et al. 2014). However, we did not find any difference in fish or invertebrates, perhaps because enough time has passed that the secondary growth forests may have regenerated, and conditions are now similar to those in old growth forests.

Notably, our sampling period spanned an intense rain event in which the stage of the mainstem Rio Puerto Viejo rose about three

meters over the course of a few days (Supplemental Fig. 1). We suggest that this event substantially influenced our results and was potentially responsible for the high overall turbidity, low aquatic organism abundance, and high fish abundance. For example, this increase in stage caused abnormal flow in some of the streams in the watershed and we observed opposite flow directions due to backflow from the main river in some streams over two days. Also, the banks of ephemeral streams that we sampled for benthic invertebrates were newly underwater and likely contained fewer individuals, because these organisms usually inhabit the centers and deepest parts of streams. The increased flow in ephemeral streams may have improved both the amount and quality of fish habitat. When a flood event occurs, the increase in stage makes more habitat available in low-flow streams; organisms seek out this new habitat to shelter from increased flows and take advantage of new food sources (Grosholz & Gallo, 2006). Clearly, dynamic weather events in the tropics can dramatically change river conditions on short time scales. The high frequency of intense rain events in the dry season likely influences the entire riparian ecosystem and selects for species that can tolerate flood events. Investigating changes in available habitat would be a valuable continuation of this study given the likelihood of more frequent flood events due to climate change.

It is paramount that we continue to investigate anthropogenic impacts on river systems. The long-term effects of deforestation on streams, particularly between those in old growth and secondary growth forests, are still not fully understood. Connecting the abiotic conditions following deforestation to changes in biotic conditions in a stream is a critical step in understanding the recovery of watersheds following anthropogenic disruption. By discerning the physical and ecological consequences of historical land use change, we can better predict how human activity will influence watersheds in the future.

ACKNOWLEDGEMENTS

Thank you to Kenneth, a guide at La Selva, for lending us boots for sampling and advise on where to sample, Dr. Chip Small for his background knowledge of streams in La Selva, and David and

Deborah Clark for their intensive and detailed historical land use map.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this work.

LITERATURE CITED

- Bond, M. C & Zdasiuk, B. J 2020. Buttresses on tropical trees: Clashing biotic and abiotic factors influencing architecture and investment. Dartmouth Biology FSP 2020
- Campbell, N. A. and J. B. Reece. 2005. Biology. Pearson Education, Inc., San Francisco, CA.
- Davies-Colley, R. J., & Smith, D. G. 2001. Turbidity Suspended Sediment, and Water Clarity: A Review1. JAWRA Journal of the American Water Resources Association, 37(5), 1085–1101.
- Gentry, A. H., & Lopez-Parodi, J. 1980. Deforestation and Increased Flooding of the Upper Amazon. Science, 210(4476), 1354–1356.
- Grosholz, E., & Gallo, E. 2006. The influence of flood cycle and fish predation on invertebrate production on a restored California floodplain. Hydrobiologia, 568(1), 91–109.
- Latrubesse, E. M., Stevaux, J. C., & Sinha, R. 2005. Tropical rivers. Geomorphology, 70(3), 187–206.
- Newbery, D. M., Schwan, S., Chuyong, G. B., & van der Burgt, X. M. 2009. Buttress form of the central African rain forest tree *Microberlinia bisulcata*, and its possible role in nutrient acquisition. Trees, 23(2), 219.
- Restrepo, J. D., Kettner, A. J., & Syvitski, J. P. M. 2015. Recent deforestation causes rapid increase in river sediment load in the Colombian Andes. Anthropocene, 10, 13–28.
- Ricklefs, R. E. 1990. Ecology. W. H. Freeman and Company, NY.
- Rieman, B. E., Lee, D. C., Thurow, R. F., Hessburg, P. F., & Sedell, J. R. 2000. Toward an integrated classification of ecosystems: Defining opportunities for managing fish and forest health. Environmental Management. 25(4): 425-444., 25(4), 425–444.
- Silva-Junior, E. F., Moulton, T. P., Boëchat, I. G., & Gücker, B. 2014. Leaf decomposition and ecosystem metabolism as functional indicators of land use impacts on tropical streams. Ecological Indicators, 36.
- Sioli, H. (1985). The Effects of Deforestation in Amazonia. The Geographical Journal, 151(2), 197–203.
- Vitousek, P. M. 1977. The Regulation of Element Concentrations in Mountain Streams in the Northeastern United States. Ecological Monographs, 47(1), 65–87.

APPENDIX



Supplemental Figure 1: Increase in stage and decrease in visibility of the Rio Puerto Viejo after two days of rain.

LA CORRIENTE VIVA: LOS BOSQUES ALTERADOS CAMBIAN LAS CARACTERÍSTICAS BIÓTICAS Y ABIÓTICAS DE LAS CORRIENTES NEOTROPICALES

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Abstracto: Los impactos humanos en las cuencas hidrográficas forestales están muy extendidos y aumentando. En los trópicos, donde la deforestación es común, estos impactos pueden ser sustanciales. La salud general de las cuencas hidrográficas en respuesta a esta perturbación puede deducirse de la salud de los organismos que viven en ellas. Para entender el impacto de la deforestación en los arroyos neotropicales, medimos la abundancia de organismos acuáticos y características abióticas, incluyendo temperatura, pH y carga de sedimentos en corrientes de bajo orden en bosques de crecimiento antiguo y secundario en la Estación de Investigación Biológica La Selva en Costa Rica. Debido al alto escurrimiento de sedimento en los bosques secundarios, hipotetizamos que los arroyos en esos bosques tendrían menores macroinvertebrados y abundancias de peces. Alternativamente, debido a que los bosques secundarios podrían liberar más nutrientes en los arroyos circundantes, hipotetizamos que podría haber un mayor macroinvertebrado y abundancia de peces. Nuestros resultados indicaron que la visibilidad era mayor en los flujos de bosques de crecimiento secundario en comparación con los de los bosques de crecimiento antiguo. En general, encontramos muy pocos invertebrados acuáticos, y no hay diferencia en los invertebrados acuáticos o la abundancia de peces entre los tipos de bosques. En particular, nuestro período de muestreo abarcó un gran evento de lluvia, aumentando la etapa de los arroyos muestreados durante el período de dos días y demostrando el impacto de los eventos meteorológicos en las características hidrológicas. Al comprender cómo la deforestación y los eventos meteorológicos estocásticos afectan a las cuencas hidrográficas, podemos diseñar estrategias relevantes para mitigar más daños en las corrientes.

Palabras claves: corrientes neotropicales, cuencas hidrográficas, deforestación, organismos acuáticos, abundancia de peces, invertebrados bentónicos, sucesión forestal

QUEEN OF LA SELVA: DESPOTIC GROOMING BEHAVIOR IN COLLARED PECCARY HERDS

ASHLYN J. MORRIS, ALEJANDRA C. PREVOST-REILLY

TAs: Melissa DeSiervo, Clare Doherty
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Abstract: Social animals form societies that range from egalitarian to despotic. However, few studies have used grooming, and the frequency of the behavior being reciprocated, as a metric to study despotism within animal societies. Here, we recorded time and demographic information about grooming interactions within a collared peccary herd at La Selva in Costa Rica to address this question. Most of the observed grooming interactions were not reciprocated, suggesting the presence of a dominance structure within the herd. However, larger peccaries groomed smaller peccaries as often as smaller peccaries groomed larger ones, suggesting that size was not the key factor determining dominance. Grooming interactions varied by the sex of the peccaries involved. Little reciprocal grooming was observed between females, but males reciprocated when groomed by a female more often than not. This suggests that grooming may be a commodity traded by males within a biological market in return for opportunities to mate with females. Examination of the despotic behavior within peccary herds, and how this is further complicated by inter-sex relations, reveals insights into the deeper structures of animal societies.

Key words: biological market, collared peccary, despotism, egalitarianism, La Selva

INTRODUCTION

The social dynamics within groups of animals vary widely from egalitarian to despotic. Within despotic societies, certain individuals within a group are able to monopolize resources or fitness in their favor (Vehrencamp 1983). According to the prior attributes hypothesis, it is individual differences in certain traits, like body size or fighting ability, that allow one animal to become dominant over another (Franz 2015). Further, the ‘winner and loser effects’ hypothesis posits that linear dominance hierarchies arise because winners of a given conflict are more likely to win in future ones. Within egalitarian societies, on the other hand, animals do not exist within a formal dominance hierarchy. Conflict between organisms is less frequent, and individuals show higher rates of reconciliation following conflicts that do occur (Matsumura 1999). Further, animals within these societies affiliate with each other at high rates and frequently reciprocate altruistic behaviors.

Reciprocity rates of grooming are an underutilized tool in studying where a given animal society falls on the spectrum between egalitarian and despotic. Allogrooming, or behaviors that animals engage in to clean or maintain the appearance of conspecifics, likely evolved originally for hygienic reasons. However, a study of grooming across 44 species

of primates suggests that grooming is performed more frequently and with more intimacy than is necessary for hygienic purposes (Dunbar 1991). Terry (1970) argues that grooming instead serves an important social function within groups and is used to reduce stress and to promote bonding among individuals. It follows that studying whether these grooming interactions are one-directional or reciprocated can give us insight into the social dynamics within groups.

In this study, we investigated grooming within a herd of collared peccaries (*Pecari tajacu*) that are habituated to humans at La Selva, Costa Rica. Although hog-like in appearance, these medium-sized mammals belong to the same family, *Muridae*, as rodents. Collared peccaries are highly social and forage in groups of up to 30 individuals (Janzen 1983). Friendly individuals often groom each other, during which they rub their head on the large scent gland on the other’s back. If conflict over mating, which occurs year-round, or resources arises, they bark loudly, chatter their teeth, or snap at each other. The acclimation of the collared peccaries at La Selva to humans and the frequency of their grooming interactions provided the opportunity to gain insight into herd social structure.

If peccary herds are egalitarian in nature, we would expect to see high rates of reciprocated grooming behavior. Individuals of a group would exchange grooming in a mutual, balanced fashion, and no significant differences in grooming behavior based on size would be observed. Alternatively, peccary herds may be despotic in nature. If this is the case, they will exhibit imbalances in their grooming behavior, with grooming interactions frequently being unreciprocated. Furthermore, in cases where grooming is reciprocated, the peccary which initiated the grooming interaction will spend a higher proportion of time grooming its partner than being groomed by them. In addition to exploring the extent to which peccaries exhibited egalitarian versus despotic grooming behavior, we also tested whether two key variables—sex and size—could modify this behavior. Grooming can often be precursor to mating (Bissonette 1982), so we hypothesized that same-sex grooming interactions would be one-directional, while grooming between peccaries of opposite sexes would be reciprocated. Similarly, since size is often an indicator of dominance within animal social groups, we predicted that the smaller of the two peccaries in a grooming pair would groom the larger more frequently than the larger would groom the smaller. We tested all of these predictions by collecting detailed observational data on grooming behavior.

METHODS

Experimental Design

We collected data from 64 grooming interactions, or bouts, between two peccaries from 18 January to 20 January 2020. Grooming bouts were defined as any instance in which one peccary rubbed its head and snout on another peccary, or when two peccaries rubbed each other simultaneously. For each bout, we recorded the time from when one peccary first made physical contact with the other until the two ceased contact. If grooming was reciprocated, we recorded both the total length of the interaction and the time that each peccary was doing the grooming within the bout. To analyze the effect of age on grooming, we recorded the developmental state, adult or juvenile, of both the initiator and receiver of the

grooming. To examine the effect of peccary size on grooming, we recorded whether the initiator of each grooming session was smaller, larger, or equal in size to the receiver of the grooming.

We recorded whether each grooming interaction was completely one-sided or whether it was reciprocated. We further differentiated between two-way bouts in which both peccaries groomed each other simultaneously and two-way bouts in which one peccary finished grooming the other before that other peccary reciprocated by grooming the first peccary. Because distinguishing between individual peccaries over extended periods of time was not possible, reciprocal bouts only encompassed scenarios in which the reciprocation of grooming occurred as the next interaction between the same two peccaries.

To assess the effects of group dynamics on grooming interactions, both the total number and the number of juveniles in the group at the time when the grooming bout occurred were documented (results not reported here). Finally, to assess differences between grooming sessions that occurred between females and males and those that occurred between two peccaries of the same sex, the sex of both the initiator and recipient of the grooming was noted whenever possible.

Statistical Analyses

We completed all statistical analyses with JMP® Pro 15.0. To test the extent to which grooming bouts were one-way versus reciprocal we used chi-square. When examining the relationship between the nature of a bout and the sex of the peccaries involved in the interaction, we only included the data points for which we had information on the peccaries' sex ($n = 14$).

When comparing the number of occurrences of a bigger peccary grooming a smaller peccary to those where a smaller peccary groomed a bigger peccary, we excluded grooming observations for which we considered the peccaries to be equal in size. A label of equal size stemmed from our inability to accurately determine their relative sizes, rather than them being truly equal in size.

RESULTS

Grooming bouts were significantly skewed toward being one-way, rather than reciprocal (Pearson's Chi-Squared: $X^2 = 19.12$, $df = 1$, $p = <0.0001$; Fig. 1).

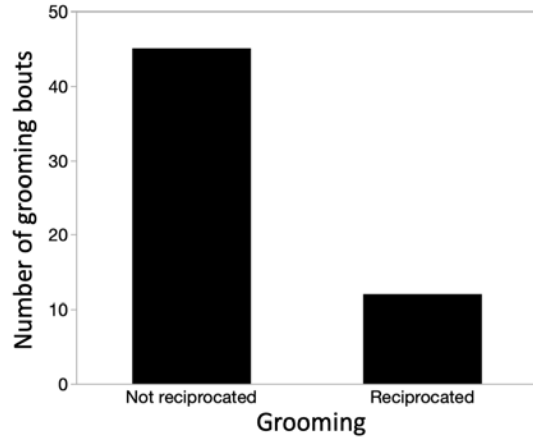


Figure 1. The number of one-directional vs. reciprocated grooming bouts observed between peccaries ($n = 57$ bouts). Reciprocated interactions include both sequential and simultaneous mutual grooming events.

If grooming was reciprocated, the peccary that initiated the grooming interaction spent more time grooming than subsequently being groomed in all cases but one (Fig. 2). Mutual grooming occurred simultaneously between two peccaries only 10% of the time.

The sex of the peccaries influenced the type of grooming bout (Fig. 3). Female-on-female grooming was rarely reciprocated, while female-on-male grooming was reciprocated by the male

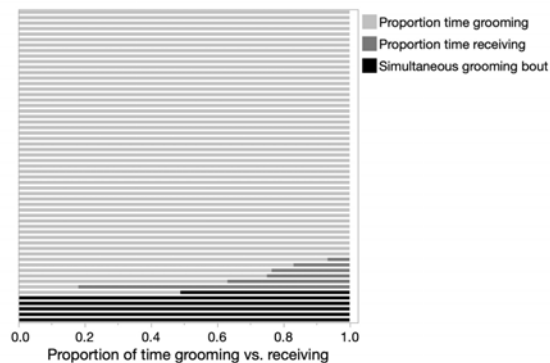


Figure 2. For all observed grooming bouts ($N = 57$) the proportion of time a peccary spent grooming a partner (light grey) vs. subsequently being groomed by that same partner (dark grey). Grooming bouts in which two peccaries groomed each other simultaneously are labeled “simultaneous grooming bout” (black).

more often than not. Male-on-female grooming was never reciprocated by the female, and male-on-male grooming was never observed. Grooming was performed by the male before all instances of mating observed.

Finally, the relative size of the two peccaries did not significantly influence who groomed who in a grooming bout (Pearson's Chi-Squared: $X^2 = 0.16$, $df = 1$, $p = 0.69$; Fig. 4).

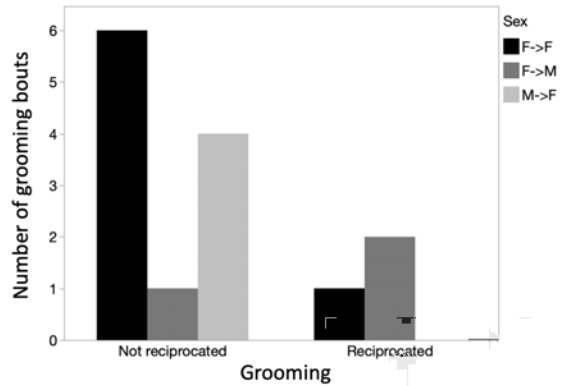


Figure 3. The number of grooming bouts that were one-directional vs. reciprocated, based on the sex of the peccaries involved in the interaction ($n = 14$ bouts). F->F implies a female grooming a female (black). F->M implies a female grooming a male (dark grey). M->F implies a male grooming a female (light grey).

DISCUSSION

Few grooming interactions within the peccary herd were reciprocal. On the face, these results suggest a highly despotic grooming structure within peccary societies.

However, the key limitation of our study involved the fact that the peccaries alternated between spending time in an open field, where they could easily be observed, and in the surrounding forest. Due to the thick foliage within the forest, it was not possible to keep track of individuals within the forest or to collect data on grooming interactions that occurred within it. It is possible that some grooming interactions that we assumed were one-directional were later reciprocated within the forest.

Our results indicate that there is no difference between the frequency that small peccaries groom large ones and the frequency

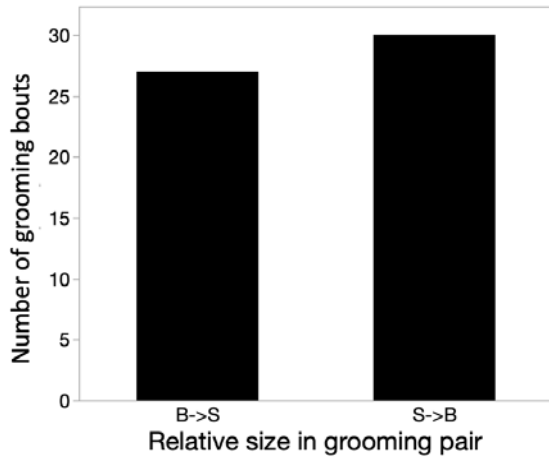


Figure 4. The number of times a bigger peccary was observed grooming a smaller peccary (B->S) vs. a smaller peccary was observed grooming a bigger peccary (S->B) ($n = 57$). Grooming bouts for which we could not accurately determine relative size were excluded.

that large peccaries groom smaller ones. If we accept the idea that unreciprocated grooming is done by subordinate individuals to curry favor with a dominant one, then this suggests that size is not a strong indicator of dominance within a peccary herd. Other factors, including fighting ability and kin relationships between peccaries, need to be taken into account to fully understand the structure of the dominance hierarchy. For example, mothers and immature offspring, despite being of different size, may both groom one another and receive grooming.

Although the overall count of one-way grooming interactions was greater than that of reciprocated interactions, the patterns become more complicated when the sex of the peccaries is taken into account. Female-on-female grooming was rarely reciprocated, and male-on-female grooming was never reciprocated. However, female-on-male grooming was reciprocated by the male more often than not. Our qualitative observations of the peccaries indicated that females were the sex determining whether or not a copulation would occur. In a number of cases, a female rejected a male that was attempting to mate with her by baring her teeth or snapping at him. Additionally, males were often observed following females around; females were never observed seeking out males with whom they desired to mate. Given these high rates of rejection, males are likely to

reciprocate when females groom them as a possible attempt to strengthen the bond between the two and increase the probability that the two of them will later successfully mate.

Past research supports the idea that within collared peccary herds, grooming is an important form of courtship between males and females (Bissonette 1982). In observations of collared peccaries within Big Bend National Park, 81 percent of courtship encounters were not exclusively sexual in nature and instead were hypothesized to be a way for two peccaries to bond before mating in a later encounter (Bissonette 1982). This kind of exchange of services is common between two social animals when one is searching for a partner. The trading of a resource for mating privilege can be construed as a 'biological market' (Noe 1995) where commodities are exchanged for mutual benefit. In La Selva peccaries, it appears that males are effectively trading grooming behavior for a chance at mating with a chosen female.

Future research should elucidate further reasons underlying allogrooming within collared peccaries. In this study, it was assumed that grooming is a purely social behavior used to improve the strength of relationships between peccaries. However, past experiments have indicated that grooming in other animals sometimes occurs simply as a way to remove ectoparasites from conspecifics and should not be considered a purely social interaction. For example, when Madden (2009) reduced parasite loads within groups of meerkats, he observed a significant decrease in the amount of grooming that occurred in the following days. However, he did see an increase in the frequency that meerkats expressed signs of stress as the frequency of grooming decreased. This suggested that, although highly correlated to levels of parasites on their bodies, grooming interactions still had important implications for social dynamics within the group. Applying the experimental design that Madden utilized to a herd of collared peccaries would clarify the causes of allogrooming within this species.

Human and nonhuman animals alike are unwilling to put up with an indefinite amount of despotism. Indeed, despots face tradeoffs between reaping the benefits of more monopolized resources versus causing

subordinates within the group to revolt. A better understanding of how despots achieve and maintain their power will greatly inform our understanding of animal behavior.

ACKNOWLEDGEMENTS

Thank you to the La Selva research station for their hospitality and to the TA's and Mark for pushing us to do a study on the wonderfully cute peccaries. We wish we could have sexed them.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this work.

LITERATURE CITED

- Barki, A. 1992. Effects of size and morphotype on dominance hierarchies and resource competition in the freshwater prawn *Macrobrachium rosenbergii*. *Animal Behaviour* 44: 547-555.
- Barrette, C. and Vandal D. 1986. Social Rank, Dominance, Antler Size, and Access to Food in Snow-Bound Wild Woodland Caribou. *Behaviour* 97: 118-146.
- Bissonnette, J. A. 1982. Ecology and Social Behavior of the Collared Peccary in Big Bend National Park, Texas. U. S. Department of the Interior, Washington, D. C.
- Dunbar, R. I. M. 1991. Functional Significance of Social Grooming in Primates. *Folia Primatologica* 57: 121-131.
- Franz, M., E. McLean, J. Tung, J. Altmann, and S. C. Alberts. 2015. Self-organizing dominance hierarchies in a wild primate population. *Proceedings of the Royal Society B: Biological Sciences* 282: 1-9.
- Janzen, D. H. 1983. Costa Rican Natural History. Chicago, Illinois: The University of Chicago Press.
- Madden, J. R. and T. H. Clutton-Brock. 2009. Manipulating grooming by decreasing ectoparasite load causes unpredicted changes in antagonism. *Proceedings of the Royal Society B: Biological Sciences* 276: 1263-1268.
- Matsumura, S. 1999. The Evolution of "Egalitarian" and "Despotic" Social Systems Among Macaques. *Primates* 40(1): 23-31.
- Noe, R. and P. Hammerstein. 1995. Biological Markets. *Trends in Ecology and Evolution* 10: 336-339.
- Terry, R. L. 1970. Primate Grooming as a Tension Reduction Mechanism. *The Journal of Psychology* 76: 129-136.
- Vehrencamp, S. L. 1983. A model for the evolution of despotic versus egalitarian societies. *Animal Behavior* 31(3): 667-682.

LA REINA DE LA SELVA: COMPORTAMIENTO DE ASEO DESPÓTICO EN LAS MANADAS DE LOS PECARÍS DE COLLAR

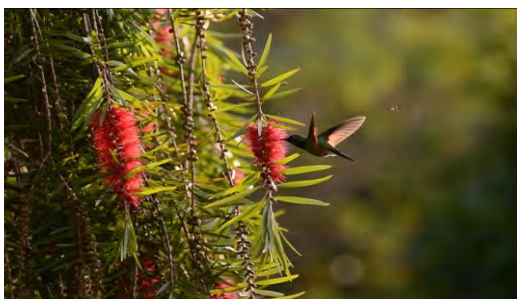
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Abstracto: Los animales sociales forman sociedades que van desde igualitarias hasta despóticas. Sin embargo, pocos estudios han utilizado el aseo personal, y la frecuencia del comportamiento que se corresponde, como una métrica para estudiar el despotismo dentro de las sociedades animales. Aquí, registramos información demográfica y de tiempo sobre las interacciones de aseo dentro de un rebaño de pecarís en La Selva en Costa Rica para abordar esta pregunta. La mayoría de las interacciones de aseo observadas no fueron correspondidas, lo que sugiere la presencia de una estructura de dominio dentro del rebaño. Sin embargo, los pecarís más grandes acicalaron pecarís más pequeños tan a menudo como pecarís más pequeños acicalaron los más grandes, lo que sugiere que el tamaño no era el factor clave que determinaba el dominio. Las interacciones de aseo variaron por el sexo de los pecarís involucrados. Se observó poco acicalamiento recíproco entre las hembras, pero los machos correspondieron cuando son acicalados por una hembra más a menudo que no. Esto sugiere que el aseo puede ser un producto comercializado por los hombres dentro de un mercado biológico a cambio de oportunidades para aparearse con las hembras. El examen del comportamiento despótico dentro de los rebaños de pecarís, y cómo esto se complica aún más por las relaciones entre sexos, revela ideas sobre las estructuras más profundas de las sociedades animales.

Palabras claves: Mercado biological, pecarí de collar, despotismo, igualitarismo, La Selva

MONTVERDE





THE OPPORTUNITY COST OF ANTI-PREDATOR VIGILANCE OF FORAGING IN COATIS

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Abstract: When foraging, animals face an important tradeoff between dedicating energy to resource acquisition and dedicating energy to being vigilant against predators. As solitary foragers, male white-nosed coatis, *Nasua narica*, engage with this tradeoff in an attempt to maximize their food obtained while staying alive. To test how the presence of a predator affects foraging behavior in coatis, we put food inside of a puzzle box and timed how long it took coatis to retrieve the food when a snake stimulus was present or absent. We found that there was no difference in the proportion of coatis that solved the puzzle box when a predator stimulus was present or absent; however, the coatis took significantly longer to solve the puzzle box when a predator stimulus was provided. We infer that the increased average time in solving the box when a predator was present was due to increased vigilance by the coatis and less attention directed towards solving the box. In addition, the coatis solved the puzzle boxes more quickly as trials progressed; notably, the change in puzzle solving speed occurred at a greater rate when predator cues were present compared to when predator cues were absent. This change in puzzle solving speed can be attributed to a combination of learning about how to open the box and habituation to the predator cues when those were present. Cost-benefit analyses regarding resource allocation must be taken into account by species across the animal kingdom to optimize fitness. Further, the ability to integrate new information into these analyses, which our experiment demonstrates coatis have the ability to do, can greatly impact the resilience of a population.

Key Words: foraging, vigilance, tradeoffs, white-nosed coatis, Monteverde, fission-fusion

INTRODUCTION

How an animal chooses to allocate its resources can be the difference between life and death. Across the animal kingdom, countless examples exist of organisms who weigh the opportunity costs associated with differing resource allocation to optimize their fitness. For example, green iguanas face a tradeoff between spending their energy on attracting mates and on territorial displays and conserving their energy for defense in the face of an attempted eviction from their territory (Alter et al. 2020). Squirrels foraging for food in the fall must also weigh the costs and benefits associated with eating foraged meals immediately or saving them for the upcoming winter months. Little is known, however, about how and why animals make these split-second decisions of where they want to allocate their resources.

For animals who face predation, the tradeoff between allocating their attention to obtaining food and allocating their attention to antipredator vigilance has high stakes. Though necessary for survival, vigilance induces direct foraging costs that are felt by animals across several taxa (Fortin et al. 2004). These prey animals have been shown to minimize the direct

foraging costs that they face. Past research indicates that the time spent being vigilant decreases as the marginal energy value of food an animal is foraging for increases (Periquet et al. 2012). On the other hand, due to the high costs to fitness associated with predation, prey put a higher emphasis on vigilance when in environments where they face a greater risk of predation. While in an area known to be frequented by a predator or whenever a potential predation cue is observed, a greater percentage of the cognitive load of the prey animals will be dedicated towards vigilance (Beauchamp and Ruxton 2016). In fact, sensory cues associated with particular predators have been experimentally shown to induce vigilance-associated neurochemical and behavioral changes within prey animals (Osada et al. 2015; Monclus et al. 2006).

Male white-nosed coatis, *Nasua narica*, are opportunistic and creative foragers. They consume anything they can find, from tarantulas and birds to fruits and garbage. From pulling with their hands and mouth to pushing with their nose, coatis employ several methods for finding and extracting food (Olive et al., 2018). In addition, coatis display high levels of curiosity

and problem solving while foraging. For example, wild coatis in the Cloudbridge Nature Reserve have been observed breaking into outdoor pantries, removing heavy stones on top of garbage bin lids, and stealing food from bird feeders. It is this innovation in their foraging behavior that makes coatis a useful system for studying how predation pressures affect foraging ability.

To analyze the effect of the presence of a potential predator on the abilities of white-nosed coatis at the Monteverde Biological Research Station in Costa Rica to problem solve while foraging, we assessed the length of time that it took the coatis to solve a puzzle box for a food reward. Because snakes are a known predator of coatis, we hypothesized that coatis would focus more on vigilance in trials in which we simulated the presence of a moving snake around the box. This would cause the coatis to take a longer amount of time to solve the puzzle box. Additionally, we predicted that, for some coatis, the cost of an attack, which could range from injury to death, would outweigh the benefits of a single meal; these coatis would choose to leave the box before they solved it to avoid such risks. As a result, we would observe a lower percentage of coatis solving the box in the presence of predator cues. Alternatively, the presence of a predator might make the coati more motivated to solve the puzzle box due to an increased risk associated with staying in the area for a long period of time. If this was the case, the coatis might actually solve the puzzle box more quickly in the presence of a predator.

METHODS

Sampling Methods

Trials without a snake mimic were conducted on white-nosed coatis from 24 January to 27 January 2020, while trials with a snake mimic were conducted from 25 January to 26 January 2020. Trials were conducted primarily at two sites located by the Monteverde Biological Research Station in Costa Rica where coatis are known to frequent due to a history of humans leaving out compost for them. At these two locations, we left out a puzzle box, a box with dimensions of 28 cm x 21 cm x 10 cm that had a hinged lid, for coatis to solve to gain access to a food reward. Approximately two handfuls of

food were placed into the box for each trial and holes were cut in the box so that animals would smell the food and investigate. To increase the coatis' interest in the puzzle boxes, we limited the compost left out at the Monteverde Biological Station to only that found in the boxes.

We began timing a trial whenever a coati entered the 0.30 m radius centered at the puzzle box. We recorded the time when the coati solved the puzzle box, which was defined as when it had opened the lid and gained access to the food inside the box. In cases where the coati failed to solve the puzzle box, we recorded the time when it exited the same 0.30 m radius.

To assess the impact of vigilance on problem solving ability in coatis, we placed a handmade red, yellow, and black-striped stick, mimicking a snake, 1.50 m from the box in half of the trials conducted. The snake was attached to a piece of string that could be pulled to simulate a snake moving. When the coati first entered the 0.30 m radius surrounding the box, the homemade snake was moved by pulling the string. In these trials, the same data assessing the time taken to open the box was recorded.

To keep each coati from becoming satiated and thus being unmotivated to participate in any trials later that day, we only allowed it to eat for 20 seconds after it solved the puzzle box. At that point, the puzzle box and food were removed. To keep our data as independent as possible, we waited 10 minutes after the end of a given trial before putting the puzzle box back out. Over the span of 10 minutes, the coati who had participated in the last trial tended to lose interest in waiting for more food and would move on.

Statistical Analyses

We completed all statistical analyses with JMP® Pro 15.0. We used a 2 x 2 Pearson's Chi-Squared analysis to test for the effects of the snake mimic on the proportion of coatis who solved the puzzle box. We tested for the effects of the snake mimic on solving time in coatis with a General Linear Model that included the presence or absence of a snake mimic as a categorical variable, trial order as a continuous variable, and their interaction.

RESULTS

76% of coatis solved the puzzle box across all trials conducted ($N = 33$). The time between each trial was dependent on a coati coming across the puzzle box and ranged from 22 minutes to just over 4 hours. Though a lower proportion of coatis solved the puzzle box when the predator was present compared to when it was absent, this result was not statistically significant (Pearson's Chi-Squared: $\chi^2 = 2.33$, $df = 1$, $p = 0.13$, Table 1).

The mean solving time of the puzzle box was significantly longer in trials when the predator was present (Main Effect of Snake Mimic: $F = 53.93$, $df = 1$, $p < 0.0001$, Fig. 1).

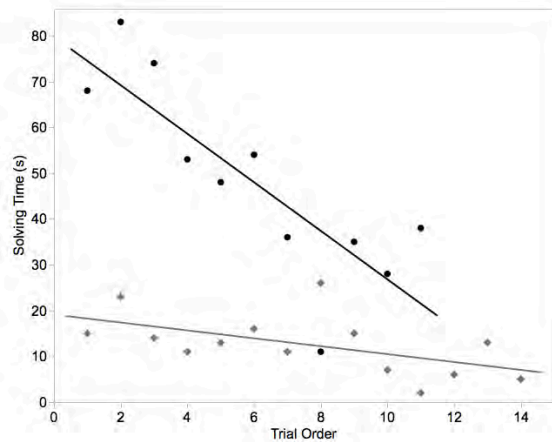


Figure 1. The solving time of the puzzle box plotted in chronological trial order in the presence of predators (circle data markers, black trend line) vs. the absence of predators (diamond data markers, grey trend line). Each of the data markers represents the time that it took one coati to solve the puzzle box.

Trials in which the puzzle box was unsolved were not included in calculations of mean solving time. As subsequent trials were conducted, the coatis solved the puzzle boxes faster (Main Effect of Trial Order: $F = 22.33$, $df = 1$, $p = 0.0001$, Fig. 1). The rate of change in solving time of the puzzle box was greater over subsequent trials in which the predator was present when compared to trials in which the predator was absent (Interaction Between Snake Mimic and Trial Order: $F = 5.04$, $df = 1$, $p = 0.036$, Fig. 1).

Table 1. The number of coatis who failed to solve the box vs. the number of coatis who solved the box based on whether or not the predator was present.

		<u>Solved</u>		<u>Total</u>
		<u>No</u>	<u>Yes</u>	
<u>Predator</u>	<u>Absent</u>	2	14	16
	<u>Present</u>	6	11	17
	<u>Total</u>	8	25	33

DISCUSSION

Coatis took more time on average to solve the puzzle box in the presence of predators than they did in their absence. The relationship between perceived predation risk and time spent solving the box suggests that coatis display heightened vigilance in the presence of predator cues. Coati reactions to the moving model snake included quick jolts away from the stimulus, staring at the stimulus, and temporary disregard for the puzzle box and food within it. In other words, vigilance towards the perceived predator exerted a direct cost on coati foraging abilities.

Despite the observed fear response and the increased time taken to solve the box in the presence of predators, the presence of a predator cue did not significantly decrease the percentage of the coatis who successfully solved the puzzle box. That these results weren't statistically significant suggest that, in the presence of a predator, coatis alternate between vigilance and foraging behavior; this has the effect of increasing the time necessary for foraging without inhibiting foraging ability completely. At the same time, though, a negative relationship was observed between the presence of predator cues and puzzle solving ability. While only 12.5% of coatis were unable to solve the box in the absence of predator cues, 35.3% of coatis were unable to solve the box when predator cues were present. Though these findings lack statistical power due to our low sample size,

they do hint at higher rates of puzzle abandonment in the presence of predator cues.

As subsequent trials were conducted, coatis began opening the puzzle boxes faster. This increase in puzzle solving speed occurred at a greater rate when predator cues were present than when they were absent. In both predator present and predator absent trials, some of the change in puzzle solving speed can be accounted for by coatis learning how to open the puzzle box. In the trials employing the snake mimic, habituation to the predator cue also may have played a role in the decreasing puzzle box solving time over trials. The coatis' belief in the model snake as a legitimate potential harm to them decreased over trials, and so the time that the coatis spent being vigilant towards it, and incurring the foraging costs of doing so, decreased. Further, it is possible that the effects of learning and habituation compound on one another to result in the observed change in solving speed when predator cues were present. Based on our experimental design, it is not possible to quantify the differing contributions of habituation to the predator cue and learning how to open the box to the observed change in puzzle box solving speed.

However, future studies can be designed to separate the effects that habituation and learning have on the change in puzzle box solving time. To selectively observe the effect of habituation on foraging ability, a set of trials could be conducted for which the effect of the coatis learning how to open the box was controlled. This could be accomplished by having the coatis solve different puzzle boxes on each trial while the same predator stimulus was provided and observing the decrease in puzzle solving ability over trials that resulted. To selectively observe the effect of learning on foraging ability, a set of trials could next be conducted in which the effect of the coatis becoming habituated to a given predator cue was controlled for. This could be accomplished by having the coatis solve the same puzzle box while being exposed to a different predator cue on each subsequent trial and observing the change in puzzle solving ability over trials that resulted. Finally, whether or not the effects of habituation and learning compound on one another could be determined by comparing the change in puzzle box solving

time in trials with a constant puzzle box and constant predator cue to the results of the above two sets of trials.

Coatis offer a useful system for studying the tradeoff between attention to antipredator vigilance and foraging because of their problem-solving capabilities. Our experimental design would not have been effective had we used insects, or another species that lacks the ability to solve puzzle boxes, instead of coatis. Past studies have indicated that the problem-solving ability of coatis actually surpasses that observed through the solving of our single-step puzzle box. Over the four-month period of a study conducted in the Cloudbridge Nature Reserve in Costa Rica, one male coati was shown to remember location, movement, position, and event within puzzle solving tasks. When given puzzles that required multiple steps, the individual was able to remember movements he had performed the previous day, typically resulting in faster completion times (Olive et al. 2018). The use of a single-step puzzle within our experiment, however, allowed our focus to be on the effect of the presence or absence of predator cues on puzzle solving ability. Further, the use of a puzzle box that was not physically demanding allowed us to isolate time and attention as the sole resources that the coati was dedicating to foraging or vigilance. By ensuring that the ability to open the lid of the box was not dependent on coati size or strength, we were able to remove a variable that could have confounded our results.

Though our experiment gives a clear example of an animal altering its vigilance behavior as it learns about the predation risk associated with its environment, it is still poorly understood how animals conceptualize fear and how they make the decision to alter their vigilance behavior. Laundre (2010) developed the landscape of fear, a theoretical model in which hypothetical peaks and valleys represent the level of predation risk in different parts of an individual's area of use, to explain why prey animals express higher rates of vigilance in certain areas. An underlying assumption of this model is that prey animals are able to alter their landscape of fear in accordance with changing predation risk. When wolves were reintroduced into Yellowstone National Park, for example,

female elk and bison began showing significantly higher vigilance levels (Laundre 2001). However, while some animals like elk and bison are able to vary their behavior according to changing predation risks, other animals are less effective in doing so. Populations of marsh rabbits in the Greater Everglades Ecosystem, for example, have declined greatly as invasive Burmese pythons spread throughout their area (McCleary 2015). In this case, a failure of individuals within a population to update their landscape of fear in accordance with changing predation risk led to a large decrease in population size.

Organisms across the animal kingdom face conflicting resource allocation pressures. Cost-benefit analyses regarding resource allocation must be taken into account by these species to optimize fitness. Further, the ability to integrate new information into these analyses can have a large effect on the resilience of a population. Recent human technological developments in the anthropocene have dramatically altered the environment within which organisms live. Failure by an animal to adapt its landscape of fear to fit the changing risks associated with urban development may result in inability to thrive in the new environment. Further analysis of how animals make the decision to behave in a certain way will allow for a deeper understanding of the complex rules that drive resource allocation within the animal kingdom.

ACKNOWLEDGEMENTS

Thank you to the wonderful Monteverde Research Station for allowing us to do this groundbreaking animal behavior study. Thanks also to Clare for her passion for Coatis.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this work.

LITERATURE CITED

- Alter, R., M. Bond, N. Giffard, C. Hayse, and R. Hutten. 2020. Territorial Signals: Abiotic or Social Drivers of Green Iguana Head Bob Displays. *Dartmouth Studies in Tropical Ecology* 30.
- Beauchamp, G. and G. D. Ruxton. 2016. Modeling scan and interscan durations in antipredator vigilance. *Journal of Theoretical Biology* 390: 86-96.
- Fortin, D., M. S. Boyce, E. H. Merrill, J. M. Fryxell. 2004. Foraging costs of vigilance in large mammalian herbivores. *Oikos* 107: 172-180.
- Laundre, J. W., L. Hernandez, and K. B. Altendorf. 2001. Wolves, elk, and bison: reestablishing the "landscape of fear" in Yellowstone National Park, U. S. A. *Canadian Journal of Zoology* 79(8): 1401-1409.
- Laundre, J. W., L. Hernandez, and W. J. Ripple. 2010. The Landscape of Fear: Ecological Implications of Being Afraid. *The Open Ecology Journal* 3: 1-7.
- McCleary, R. A., A. Sovie, R. N. Reed, M. W. Cunningham, M. E. Hunter, and K. M. Hart. 2015. Marsh rabbit mortalities tie pythons to the precipitous decline of mammals in the Everglades. *Proceedings of the Royal Society* 282: 1-7.
- Monclus, R., H. G. Rodel, and D. von Holst. 2006. Fox Odour Increases Vigilance in European Rabbits: A Study under Semi-Natural Conditions. *Ethology* 112: 1186-1193.
- Osada, K., S. Miyazono, and M. Kashiwayanagi. 2015. The scent of wolves: pyrazine analogs induce avoidance and vigilance behaviors in prey. *Frontiers in Neuroscience* 9: 1-11.
- Periquet, S., L. Todd-Jones, M. Valeix, B. Stapelkamp, N. Elliot, M. Wijers, O. Pays, D. Fortin, H. Madzikanda, H.
- Fritz, D. W. Macdonald, A. J. Loveridge. 2012. Influence of immediate predation risk by lions on the vigilance of prey of different body size. *Behavioral Ecology* 23(5): 970-976.

EL COSTO DE OPORTUNIDAD DE LA VIGILANCIA ANTI-PREDADOR EN EL FORRAJEJO DE LOS COATÍS

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Abstracto: Cuando se alimentan, los animales se enfrentan a un importante equilibrio entre dedicar energía a la adquisición de recursos y dedicar energía a estar vigilantes contra los depredadores. Como forrajecedores solitarios, los coatís de nariz blanca macho, *Nasua narica*, se involucran con este equilibrio en un intento de maximizar su comida obtenida mientras permanecen con vida. Para probar cómo la presencia de un depredador afecta el comportamiento de forrajeo en coatís, ponemos comida dentro de una caja de rompecabezas y cronometramos cuánto tiempo tardó coatís en recuperar la comida cuando un estímulo de serpiente estaba presente o ausente. Encontramos que no había diferencia en la proporción de coatís que resolvió la caja del rompecabezas cuando un estímulo de depredador estaba presente o ausente; sin embargo, el coatís tardó mucho más en resolver la caja del rompecabezas cuando se proporcionó un estímulo de depredador. Deducimos que el tiempo promedio aumentado en la resolución de la caja cuando un depredador estaba presente se debió a una mayor vigilancia por parte de los coatís y menos atención dirigida a resolver la caja. Además, el coatís resolvió las cajas del rompecabezas más rápidamente a medida que avanzaban las pruebas; en particular, el cambio en la velocidad de resolución de rompecabezas ocurrió a un ritmo mayor cuando las señales de depredadores estaban presentes en comparación con cuando las señales de depredadores estaban ausentes. Este cambio en la velocidad de resolución de rompecabezas se puede atribuir a una combinación de aprendizaje sobre cómo abrir la caja y la habituación a las señales de los depredadores cuando estaban presentes. Las especies de todo el reino animal deben tener en cuenta los análisis costo-beneficio relativos a la asignación de recursos para optimizar la aptitud física. Además, la capacidad de integrar nueva información en estos análisis, que nuestro experimento demuestra que los coatís tienen la capacidad de hacer, puede afectar en gran medida la resiliencia de una población.

Palabras claves: forrajeo, vigilancia, compensaciones, coatís de nariz blanca, Monteverde

SPECIALIZATION AND SOCIAL CUES DRIVE ORCHID BEE VOLATILE ACQUISITION

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Abstract: Resource acquisition varies widely across the animal kingdom. Some animals have evolved to gather certain resources to prove their worthiness as a mate, resulting in a strong sexual pressure to locate those resources. It is well known that orchid bees (Hymenoptera: *Euglossini*) utilize volatiles for mating, although their strategies for volatile acquisition are not well understood. We aimed to address this gap by examining *Euglossini* in the Monteverde neo-tropical cloud forest in Costa Rica. We offered orchid bees nine novel volatiles, positing that the bees would either be drawn to a specialized subset of volatiles or be enticed by the novelty of the volatiles provided. Our results show that the bees preferred eucalyptus, suggesting that odor specialization is a driver of resource acquisition. Secondly, to examine whether bees follow an ideal free distribution (IFD) or take advantage of social cues to follow their conspecifics, we examined bee distribution by placing multiple eucalyptus packets on a single tree and observing their aggregation tendencies. This experiment was inconclusive due to a small sample size. Further, we placed two eucalyptus packets at a foraging site, with a deceased orchid bee on one of the bags. We found that bees are significantly influenced by the presence of another bee at a volatile site, providing support for the use of social cues in orchid bee volatile acquisition. Together, a coevolved specialization and utilization of social cues shape orchid bee volatile acquisition, a process influenced by sexual selection. The specialization resulting from this unique mutualism allows for some of the great biodiversity observed in the neo-tropics.

Keywords: ideal free distribution, resource acquisition, orchid bees

INTRODUCTION

Resource acquisition in animals is driven by different possible motivations and performed using a variety of strategies. In addition to food, animals may collect resources for nest building, tool use, and mating. In some cases, sexual selection favors individuals (typically males) that demonstrate their worth as a mate by demonstrating that they have acquired rare items or compounds from the environment. For example, male Bowerbirds build large, colorful, and decorate display sites to attract female mates (Borgia 1985).

There may also be different strategies for *how* individuals collect resources. One model of resource acquisition is the Ideal Free Distribution (IDF), where individuals forage where their acquisition rate will be the highest considering the density of conspecifics (Fretwell 1969). In this case, the presence of conspecifics at a resource patch tends to decrease the quality of the patch for a prospective new arrival. Alternatively, the presence of conspecifics might indicate a good patch in that it is more likely to contain a rare resource. For instance, bumblebees are known to select flowers with conspecifics already present on them (Baracchi 2017). Such copying is

taxonomically widespread (Baracchi 2017), as are systems that better conform to the IDF (Tregenza 1995).

Orchid bees are a taxon where foraging behavior of males is heavily influenced by sexual selection. Male orchid bees collect fragrances from orchid flowers and their mating success is dependent on the cocktail of fragrances that they display to females (Roubik 2004). The collection of fragrances by male orchid bees is central to the mutualism between orchid plants and orchid bees (Moore 2009).

In this study, we examined the resource acquisition methods of orchid bees, chiefly *Euglossini*, in the Monteverde neo-tropical cloud forest in Costa Rica. It has been hypothesized that females favor males that display a complex bouquet of novel volatiles because it indicates male ability to fly far and forage successfully (Roubik 2004). This is thought to be an example of female choice based on good genes. If females are attracted to novel volatiles, we would expect to see male bees collecting volatiles based on their novelty. Alternatively, it may be that females favor few specific volatiles. In this case, male orchid bees exposed to a diversity of volatiles

would be expected to consistently prefer a few specific volatiles.

We also studied how orchid bees use social cues for the gathering of fragrances. Copying the foraging behavior of conspecifics might decrease their searching time, but also necessitates sharing. If orchid bees use social cues to locate resource patches, they would more frequently forage from a source that already has another orchid bee present. Alternatively, orchid bees may better match the IDF, in which case they would avoid resources that already have a conspecific present and would more frequently go to an otherwise equivalent volatile source that is unoccupied.

METHODS

To test orchid bee specialization in volatile acquisition, we compared their reaction to nine different volatile options (Supplemental Table 1) in Monteverde, Costa Rica. We conducted three experiments (Fig. 1). Volatiles were eluted by wetting cotton balls with one or more essential oils and hanging the cotton balls within 1m of flowers. We used a consistent amount of essential oil by measuring it in the bottle cap.

Experiment 1

In experiment 1, the nine volatile options were divided into three blocks separated by 15 m (Fig. 1, left), each consisting of two individual volatiles and one combination of those two volatiles. We recorded the number of orchid bees on each volatile bag every two minutes for an hour. A wind reading was also taken in two-minute intervals. This experiment was completed twice.

Experiment 2

Experiment 2 (Fig. 1, middle) utilized the most attractive volatile from experiment 1, eucalyptus, to test the dispersion of foraging bees across six sources separated by only 40 cm. We placed six identical eucalyptus scented mesh bags in a circle around a tree at one of our prior experimental sites. We recorded the number of orchid bees on each volatile bag every minute for an hour. A wind reading was also taken in one-minute intervals. This experiment was completed twice.

Experiment 3

To further test whether bees use social cues in their foraging, we pinned a deceased orchid bee to the exterior of one eucalyptus scented bag and attached it to a tree in our experimental location. The control, an identical bag lacking the bee, was

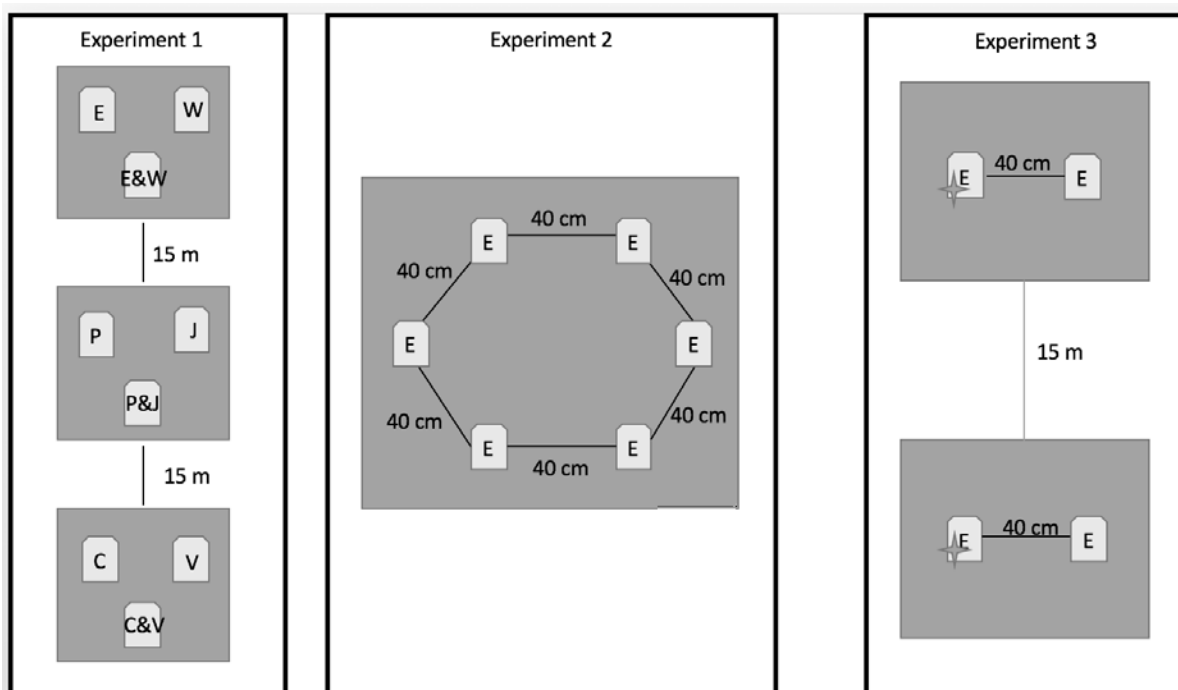


Figure 1: Schematic of methods. Left, middle, and right panels illustrate experiments 1, 2, and 3, respectively. E = eucalyptus, W = wintergreen, P = peppermint, J = jasmine, C = cinnamon, V = vanilla.

placed 40 cm away. We recorded the number of orchid bees on each volatile bag every minute for an hour. A wind reading was also taken in one-minute intervals. This experiment was replicated during the same time frame at an additional location 15 m away (Fig. 1, right).

Supplemental Methods

We cross referenced a list of chemical compounds found in orchids that are known attractants to orchid bees with the known chemical ingredients in the essential oils, in an attempt to identify the appeal of eucalyptus over the other essential oils (Table 2).

Statistical Analysis

We analyzed our data with JMP 14.1.0.

RESULTS

Experiment 1

The maximum number of bees present on a volatile bag at any time was highest for eucalyptus (4 bees) and second highest for the bag with eucalyptus and wintergreen combined (3 bees) (Fig. 2). Bees spent the greatest proportion of time on bags containing eucalyptus throughout trials, for a total of 53 minutes (Fig. 3).

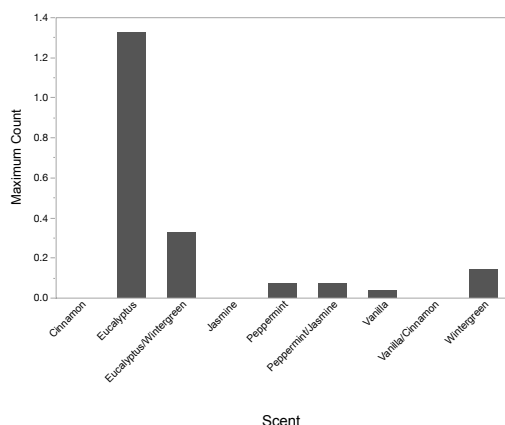


Figure 2: Maximum number of bees present at any one time vs. volatile (Experiment 1).

Experiment 2

When six bags containing identical amounts of eucalyptus volatile were spread evenly throughout a shrub to test if bees tend to aggregate or disperse uniformly when collecting volatiles, we observed a total five bee visits. Each visit went to a different

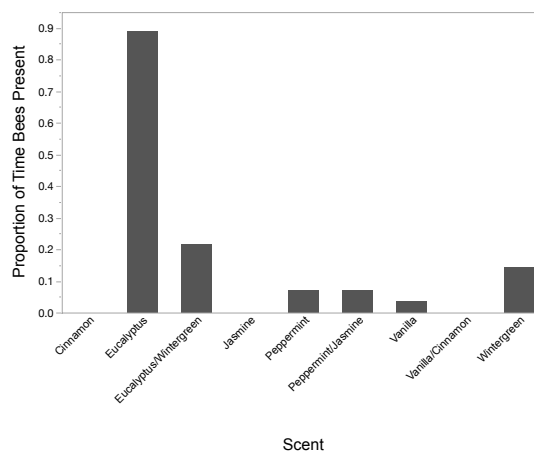


Figure 3: The proportion of time at least one bee was present at each volatile over an hour trial (Experiment 1).

bag, but there was never more than one bee at a time.

Experiment 3

In two replicates of our third experiment, bees spent 10% and 15% of their time on bags with another bee and 0% to 2% of their time on bags without another bee. There was no difference in maximum number of orchid bees drawn to bags with or without another bee present (maximum values ranged from 0 to 2).

Wind measurements ranged from 0 m / s – 8.2 m / s throughout our experiment without conspicuous differences between days or sample bouts.

DISCUSSION

During our studies, the eucalyptus oil attracted more visits by orchid bees than other oils or combinations of oils. Combining eucalyptus with a less attractive volatile, like wintergreen, decreased its attractiveness. These results suggest that sexual pressures do not select for volatile novelty, but instead specialization for specific volatiles. Known attractive compounds found in orchids are found in all 6 of the volatiles we used, but the bees were still overwhelmingly drawn to the eucalyptus volatile. Additionally, while eucalyptus oil does contain 1,8 cineole, the best-known chemical attractant, it also contains two supposed repellents and fewer moderate to good attractors than other volatiles tested (Supplemental Table 2).

Peppermint oil also contains 1,8 cineole and was less attractive to bees in our trials (Supplemental Table 2). This suggests that bees may disregard volatiles containing attractive compounds in favor of other specific volatiles. Further, this choice may be mediated by more complex chemical cues, but this evidence supports the notion that bees are specialized in their volatile acquisition choices.

Analyzing our experiments in tandem, if bees were selecting volatiles based on the sexual selection pressure of novel odors, we may also expect the bees to exhibit an ideal free distribution in our second two experiments. If a bee stood to benefit from acquiring a different volatile than their conspecific, it would make sense that they utilize different resource patches when possible. But, a low sample size in the second part of our experiment made it difficult to discern the difference between our two experimental distributions patterns, IFD compared to social cue reliance. The third experiment suggested that orchid bees tend to join other bees rather than avoid them as expected under an ideal free distribution. By following other bees, resources can be located with less individual effort. The tendency to copy preferences of other bees further opposes the possibility that novelty is driving volatile acquisition. Instead, the results from experiment 3 supports the idea that particular volatiles are favored.

Orchid bees are a classic example of a coevolved mutualism. Their relationship with orchid flowers is of the utmost importance in the neo-tropics where around 200 species of orchid bees are the exclusive pollinators of around 700 species of orchids (Pemberton 2006). What make this relationship unusual among mutualisms is the strong influence of sexual selection. Over time, sexual pressure has led to the narrowing of

volatiles orchid bees prefer and in turn, the specialization of volatiles orchid flowers produce. The increased specialization resulting from this mutualism has contributed to the great biodiversity observed in the neo-tropics.

ACKNOWLEDGMENTS

Thank you to the TA's for supporting us during the ideation and preparation for this study. Thank you also to Clare for supporting our love of the fancy hotel.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this work.

LITERATURE CITED

- Baracchi, David et al. 2018. Foraging Bumblebees Use Social Cues More When the Task Is Difficult. *Behavioral Ecology* 29:186–92.
- Borgia, Gerald. 1985. Bower quality, number of decorations and mating success of male satin bowerbirds (*Ptilonorhynchus violaceus*): an experimental analysis. *Animal Behaviour* 33: 266-271.
- Fretwell, S. and Henry Lucas Jr. 1969. On territorial behavior and other factors influencing habitat distribution in birds. *Acta Biotheoretica* 19: 16-36.
- Moore, Lucille. 2009. Orchid Fragrance Complexity as a Mechanism for Euglossine Bee Pollinator Specialization. Writing Excellence Award Winners.
- Pemberton, R. and Wheeler, G. 2006. Orchid bees don't need orchids: Evidence from the naturalization of an orchid bee in Florida. *Ecology*. 87. 1995-2001.
- Roubik, D. and Paul Hanson. 2004. Orchid Bees of Tropical America. National Institute of Biodiversity, Costa Rica.
- Tregenza, Tom. 1995. Building on the ideal free distribution. *Advances in Ecological Research* 26: 253-302.

LA ESPECIALIZACIÓN Y LAS SEÑALES SOCIALES IMPULSAN LA ADQUISICIÓN DE LAS VOLÁTILES PARA ABEJAS ORQUÍDEAS

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Abstracto: La adquisición de recursos varía ampliamente en todo el reino animal. Algunos animales han evolucionado para reunir ciertos recursos para demostrar su dignidad como pareja, lo que resulta en una fuerte presión sexual para localizar esos recursos. Es bien sabido que las abejas orquídeas (*Hymenoptera: Euglossini*) utilizan volátiles para el apareamiento, aunque sus estrategias para la adquisición volátil no se entienden bien. Nuestro objetivo era abordar esta brecha examinando *Euglossini* en el bosque nuboso neotropical de Monteverde en Costa Rica. Ofrecimos a las abejas orquídeas nueve nuevos volátiles, planteando que las abejas serían atraídas a un subconjunto especializado de volátiles o se sentirían seducidas por la novedad de los volátiles proporcionados. Nuestros resultados muestran que las abejas preferían el eucalipto, lo que sugiere que la especialización de olores es un impulsor de la adquisición de recursos. En segundo lugar, para examinar si las abejas siguen una distribución libre ideal o aprovechan las señales sociales para seguir sus conspecíficos, examinamos la distribución de abejas colocando varios paquetes de eucalipto en un solo árbol y observando sus tendencias de agregación. Este experimento no fue concluyente debido a un pequeño tamaño de muestra. Además, colocamos dos paquetes de eucalipto en un sitio de forrajeo, con una abeja orquídea fallecida en una de las bolsas. Encontramos que las abejas están significativamente influenciadas por la presencia de otra abeja en un sitio volátil, proporcionando apoyo para el uso de señales sociales en la adquisición volátil de abejas orquídeas. Juntos, una especialización y utilización co-evolucionada de las señales sociales dan forma a la adquisición volátil de abejas orquídeas, un proceso influenciado por la selección sexual. La especialización resultante de este mutualismo único permite parte de la gran biodiversidad observada en los neotrópicos.

Palabras claves: distribución libre ideal, adquisición de recursos, abejas orquídeas

LIFE AFTER DEATH: COMPETITION AND NICHE-FINDING IN NEOTROPICAL CRYPTOGRAM ASSEMBLAGES

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Abstract: Niches among wood-inhabiting cryptogams (defined here as bryophytes, lichens, and fungi) are mediated by competition. Cryptogams compete for similar spatial, mineral nutrient, energy, and water resources, and in many systems, play a vital role in decomposition and succession. In temperate forests, cryptogam niches form around specific abiotic conditions, however, less is known about mediating factors of this system in neotropical cloud forests; here we investigate this relationship. Based on temperate system dynamics, we hypothesized that cloud forest cryptogam diversity would differ between temporal, spatial, and competitive niches. Our results show higher fungi diversity in logs and higher lichen diversity in live trees. They support the existence of some spatial niches on fallen logs and competitive advantages of lichens at earlier decay stages and of fungi at later stages. These results highlight the competition mediated succession of cryptogams. In a changing climate, it is vital to understand these relationships to advise management and document change in forest succession processes.

Key words: competition, decomposition, forest succession, fission-fusion, niche theory, telepathy, wood-inhabiting cryptogams

INTRODUCTION

Competition is a critical component of natural selection. All species experience competition for resources, space, and opportunities to reproduce. Under niche theory, competition can yield competitive exclusion and species sorting in ecological time or sometimes character displacement in evolutionary time. Furthermore, some species modify their environment in ways that affect future competition. Studying competition amongst species has improved our understanding of evolution, niche theory, and species distributions.

Environments can change rapidly, creating transient niches that force succession of species. In general, competition tends to favor species that can maintain their populations at the lowest levels of limiting depletable resources for which species compete (R^* after Tilman 1982). In forests, trees are often foundational species that modify the environment in ways that affect many other species, even on the most localized level. On the surface of a tree, assemblages of bryophytes, lichens, and fungi all depend on the water, energy, and mineral nutrient resources that they can acquire from the bark surface. When that tree dies and falls, conditions begin to change for the inhabitants of the bark surface, which may lead to a succession of changes in the community

composition. Species already present may have advantages due to their initial presence and relatively high abundance (priority effect).

Tree-surface assemblages are dominated by three clades, bryophytes, lichens, and fungi, which are collectively referred to as cryptogams. Each group has slightly different resource requirements yet often co-occur within centimeters, making these communities model systems for the study of competition, niches, and coexistence. Bryophytes, which include mosses, liverworts, and hornworts, are photosynthetic and have relatively high moisture requirements (Sales et al. 2016). Bryophytes do not extract energy resources from trees (Lepp 2008). Lichens, an association of algae and decomposing fungi, are also autotrophs (obtain energy via photosynthesis) that obtain water and mineral nutrients (but not energy) from their substrate (Lichens: Life History & Ecology). Fungal cryptogams, in contrast, are saprotrophs that obtain all energy and mineral nutrients from their substrate, usually dead organic matter, and are not dependent on light for photosynthesis (Clegg and Mackean 2006). Zonation of these wood-inhabiting cryptogam groups has been the focus of previous research for some time: species of tree, humidity, bark character, and trunk circumference have all been found to play a role in structuring cryptogam communities on live trees

(Sales et al 2016), while age at death, diameter, and decomposition status are of importance on dead and decomposing logs (Kruys et al 1999). Studies in Europe and Scandinavia have reported a somewhat predictable succession in cryptogam communities on fallen dead trees (Söderström 1988, Nascimbene et al 2008, Kruys et al 1999), but less is known for cloud forest communities. Cloud forests are a globally notable feature of ecological reserves in Costa Rica but may be at risk from changes in weather and climate. Study of the verdant cryptogam communities of cloud forests may be helpful in understanding the processes that give cloud forests their character.

Due to the different environments on live, standing trees vs. dead and downed logs, which include light, water, mineral nutrients, and decomposable dead wood, we hypothesized that cryptogam assemblages would differ between trees and logs. The different resource demands of lichens and fungi could be expected to result in a greater diversity of lichens on trees and greater diversity of fungi on logs. Alternatively, community changes could be minimal if priority effects buffer effects from the changing environment. If microhabitat conditions are important, as expected under niche theory, we expected that the top, side, and bottom of logs, will be dominated by different cryptogam types. For example, fungi, which are non-photosynthetic, could be expected to outcompete bryophytes in low light micro-habitats, while bryophytes and lichens could be most abundant on the relatively well-lit top and sides of logs. Finally, we hypothesized that the increased availability of carbon-rich dead organic matter in decaying logs would favor increasing prevalence of fungi over autotrophs as log decomposition proceeds.

METHODS

Sampling Method

We studied cryptogam communities on 9 live standing trees and 9 dead fallen trees near Monteverde Biological Station during 22-28 January 2020. The abundance of bryophytes, lichen, and fungi were measured as percent cover and biomass in subplots on trees and logs. We also recorded decay status of logs and light availability.

We collected data at 9 randomly chosen sites on trails above the field station. At each site, we selected one tree and one log with diameter >40

cm, avoiding those with evidence of chainsaw use or across the trail and likely subject to human disturbance. We measured the percent open canopy as a proxy for light availability using a spherical densitometer. Next, we sampled from the first log we encountered that fit our criteria and one nearby tree of similar diameter. We placed three equally spaced 10-cm² quadrats along the log or live tree. For logs, we located one plot each on the top, side, and bottom of the log (Supplemental Fig. 1). For trees, the compass aspect of each sample site was randomized, facing either 0°, 120°, or 240°, one of each aspect per tree. We classified decomposition status of each log following Woodall 2005; (Table 1). We recorded three force penetrometer readings at each quadrat to measure log hardness. We counted the number of bryophytes, lichen, and fungi morphospecies present within each quadrat (Supplemental Fig. 2). Vascular plant species were also counted but were rare. We took pictures of each quadrat for later analyses of percent cover. Then we collected each cryptogam group within each quadrat into individual, pre-weighed plastic Ziploc bags and recorded the wet weights in the field using a portable scale. Biomass was estimated after desiccation in the lab for 13 hours.

Statistical Analyses

ImageJ software was used to calculate percent cover for each cryptogam group within each quadrat. We used JMP Pro 14 to conduct all statistical tests. Percent cover measurements were arcsine-square-root transformed to improve normality.

The relationship between penetration force and decay class was analyzed with a linear regression. We compared the abundance and species richness of cryptogam groups on trees and logs with an ANOVA that included whether the tree was dead or alive (as a fixed effect), log nested within dead or alive as a random effect, and plots within logs as a random effect.

To test for predictable spatial patterning on logs, we used the dead, fallen logs only in an ANOVA to compare the percent coverage of bryophytes, lichens, and fungi among top, side, and bottom of logs.

To test for changing abundances of cryptogam groups across decay stages, we used an ANCOVA that included cryptogam group as a categorical

variable, penetrometer measurements as a continuous variable, and their interaction.

RESULTS

The square root of penetrometer force was well related to decay class (linear regression: slope = 0.22, $p < 0.0001$, $r^2 = 0.41$; Fig. 1).

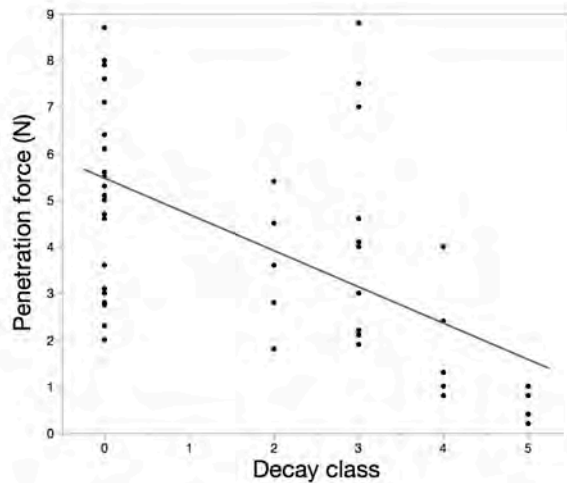


Figure 1: Force required to penetrate logs versus decay class following Table 1. Live trees were represented as decay class 0.

There were significantly more bryophyte and lichen species on trees and more fungi species on logs (ANOVA; cryptogam type x live or dead: $F = 8.79$, $df = 3, 16$, $p = 0.0001$; Fig. 2).

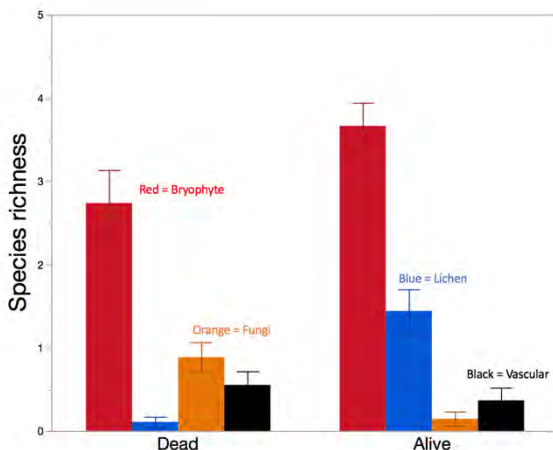


Figure 2: Species richness by cryptogam group on dead and alive trees. Mean \pm SE.

Section and tree explained less than 1.5% of the variation in the model when analyzed for random effects. Neither bryophytes, lichens, nor fungi exhibited significant differences in percent cover between live trees and fallen logs (placement x type: $F = 1.38$, $df = 3, 16$, $p = 0.25$; Fig. 3). Random effects of section and tree accounted for 0% of the variation in this model.

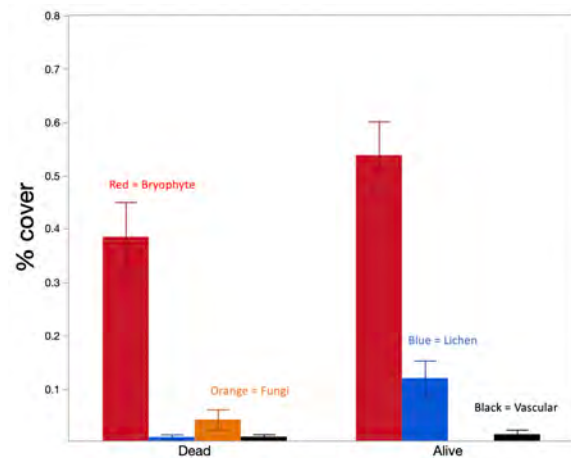


Figure 3: Percent cover by cryptogam group type on dead and alive trees. Mean \pm SE.

Bryophytes had significantly more coverage on tops or sides of logs than on the bottoms of logs; fungi exhibited significantly more coverage on samples located on the bottom of logs than on the tops or sides; and lichen coverage did not differ between sample locations on the top, side, or bottom (type x location: $F = 10.55$, $df = 6, 8$, $p = 0.0001$; Fig. 4). Tree accounted for less than 4% of random variation in the model. There was a relationship between percent water content and percent cover between decay classes; mostly because cryptogam tissue on dead trees had higher water contents. Bryophyte and lichen coverage were higher on live trees and less decomposed logs, while fungi had higher coverages on more decomposed logs (general linear mixed model; penetrative force x type: $F = 5.91$, $df = 3, 16$, $p = 0.0007$; Fig. 5). However, there was no significant relationship between biomass and penetrative force for any type of cryptogam (general linear mixed model; penetrative force x type: $F = 0.97$, $df = 16$, $p = 0.41$).

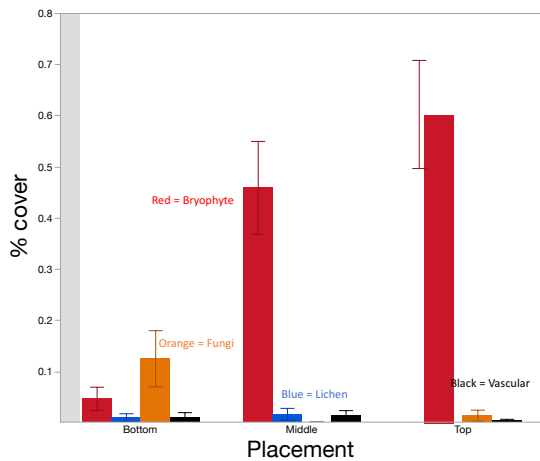


Figure 4: Cryptogam coverage by placement on log for decay class 1-5. B=bottom of log, S=side of log, T=top of log. Mean \pm SE.

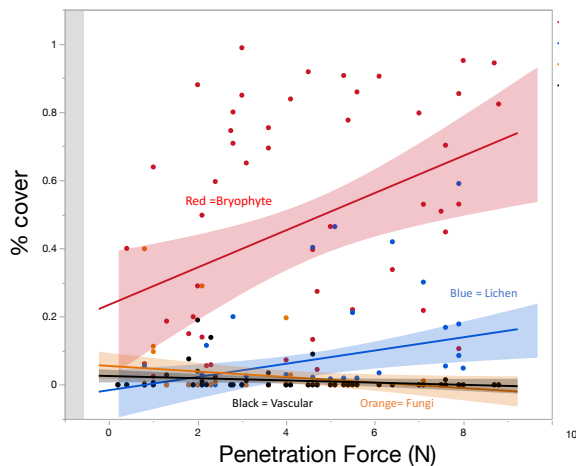


Figure 5: Percent cover of each cryptogam group type versus force required to penetrate the log beneath them.

DISCUSSION

We found support for differences in cryptogam assemblages between live trees and fallen logs, among top, side, and bottom of logs, and across log decomposition stage.

Cryptogam communities were different between trees and logs, with species richness of lichen and bryophyte being higher on live trees, and that of fungi being higher on fallen logs. This seems related to environmental differences between trees and logs and the differing requirements of autotrophs and saprotrophs. More species richness within a cryptogam group may indicate a more resilient community, which has

implications to survival in the face of human disturbance and changing climate.

The progression of decay in logs appear to favor different groups at different decay stages. As predicted, lichens were more abundant in earlier decay classes. Fungi became progressively more abundant by coverage and biomass with increased decay class. It is likely that fungi are able to dominate more with increased time since tree death due to the collapse of tree defenses and increasing availability of decomposing wood. This implies a positive feedback as increasing decay leads to increasing availability of additional wood for decay. Bryophytes, on the other hand, did not change much from decay. Bryophytes were the only cryptogam we surveyed for which percent cover and biomass did not change with decay class (Fig. 2), suggesting that they possess the broadest niche.

Cryptogam assemblages are apparently subject to strong competition. These micro-forests are a nice system for studying niche-formation, competition, and species succession. Furthermore, as key primary producers and decomposers in this extremely sensitive ecosystem, understanding the succession of cryptogams on trees and logs enriches our understanding of the ecosystem as a whole.

Understanding community dynamics is relevant to the conservation and management of a sensitive ecosystem. The Monteverde Cloud Forest Reserve receives over a quarter-million visitors per year and contributes to the ecotourism industry within Costa Rica. Management and maintenance of this ecosystem is of economic importance to the town of Monteverde and Costa Rica. Our studies add to evidence that fallen dead trees are a foundation resource for many other species in the cloud forest. These cryptogam communities may also have practical value as sensitive, easily measured indicators of potential changes to cloud forests from changes in weather and climate (Foster 2001, Hannah 2015).

ACKNOWLEDGEMENTS

Thank you to our TA Melissa DeSiervo for extensive experimental and statistical advising throughout the research and analysis process. Also, thank you to the lovely cocineras at the Estación Biológica de Monteverde who packed us

delicious bagged lunches so we could complete our extensive fieldwork.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this study.

LITERATURE CITED

- Clegg, C. J.; Mackean, D. G. 2006. *Advanced Biology: Principles and Applications* (2nd ed.). Hodder Publishing.
- Foster, P. 2001. The potential negative impacts of global climate change on tropical montane cloud forests. *Earth-Science Reviews*, 55(1-2), 73–106.
- Hannah, L. 2015. Ecosystem Change. *Climate Change Biology*, 103–133.
- Kruys, N., Fries, C., Jonsson, B. G., Lämås, T., and Ståhl, G. 1999. Wood-inhabiting cryptogams on dead Norway spruce (*Picea abies*) trees in managed Swedish boreal forests. *Canadian Journal of Forest Research*, 29(2), 178–186.
- Lepp, H. 2008. Habitats. *Lichens: Life History & Ecology*.
- Nascimbene, J., Marini, L., Caniglia, G. et al. 2008. Lichen diversity on stumps in relation to wood decay in subalpine forests of Northern Italy. *Biodiversity Conservation* 17, 2661–2670.
- Söderström, L. 1988. Sequence of bryophytes and lichens in relation to substrate variables of decaying coniferous wood in Northern Sweden. *Nordic Journal of Botany*, 8(1), 89–97.
- Tilman, David 1982. *Resource competition and community structure*. Princeton: Princeton University Press.
- Woodall, C. 2005. 3.0 Phase 3 Field Guide--Down Woody Materials.

APPENDIX

Table 1: Decay class characteristics as defined by the USFS (taken from Woodall 2005).

Decay Class	Structural Integrity	Texture of Rotten Portions	Color of Wood	Invading Roots	Branches and Twigs
1	Sound, freshly fallen, intact logs	Intact, no rot; conks of stem decay absent	Original color	Absent	If branches are present, fine twigs are still attached and have light bark.
2	Sound	Mostly intact; sapwood partly soft (starting to decay) but can't be pulled apart by hand	Original color	Absent	If branches are present, many fine twigs are gone and remaining fine twigs have peeling bark.
3	Heartwood sound; piece supports its own weight	Hard, large pieces; sapwood can be pulled apart by hand or sapwood absent	Reddish-brown or original color	Sapwood only	Branch stubs will not pull out.
4	Heartwood rotten; piece does not support its own weight, but maintains its shape	Soft, small blocky pieces; a metal pin can be pushed into heartwood	Reddish or light brown	Throughout	Branch stubs pull out.
5	None, piece no longer maintains its shape, it spreads out on ground	Soft; powdery when dry	Red brown to dark brown	Throughout	Branch stubs and pitch pockets have usually rotten down.



Supplemental Figure 1. Authors Reyn Hutten (front) and Ben Zdasiuk (back) taking samples at two of three sections on the log, dispersed evenly throughout the length of the log (photo: Melissa DeSiervo).



Supplemental Figure 2. A 10-cm² quadrat; for each we 1) counted number of bryophyte, lichen, and fungi species, 2) recorded penetrometer measurements, 3) took a photograph to estimate percent cover of each cryptogam type, and 4)

VIDA DESPUÉS DE LA MUERTE: COMPETENCIA Y BÚSQUEDA DE NICHOS EN CONJUNTOS DE CRIPTÓGAMA NEOTROPICAL

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Editoras asistentes: Melissa DeSiervo and Clare Doherty

Editor: Matt Ayres

Abstracto: Los nichos entre las criptógamas que habitan la madera (definidas aquí como briofita, líquenes y hongos) están mediados por la competencia. Las criptógamas compiten por recursos espaciales, minerales, energéticos y hídricos similares, y en muchos sistemas, desempeñan un papel vital en la descomposición y la sucesión. En los bosques templados, los nichos de criptógama se forman alrededor de condiciones abióticas específicas, sin embargo, se sabe menos sobre los factores mediadores de este sistema en los bosques de nubes neotropicales; aquí investigamos esta relación. Basándonos en la dinámica templada del sistema, postulamos que la diversidad de criptógamas de bosques en la nube diferiría entre nichos temporales, espaciales y competitivos. Nuestros resultados muestran una mayor diversidad de hongos en los troncos y una mayor diversidad de líquenes en árboles vivos. Apoyan la existencia de algunos nichos espaciales en troncos caídos y ventajas competitivas de líquenes en etapas de descomposición anteriores y de hongos en etapas posteriores. Estos resultados ponen de relieve la sucesión mediada por la competencia de criptógamas. En un clima cambiante, es vital entender estas relaciones para asesorar a la gestión y documentar el cambio en los procesos de sucesión forestal.

Palabras claves: competencia, descomposición, sucesión forestal, fisión-fusión, teoría de nicho, criptógamas habitantes en madera

FORAGING IN THE ULTRAVIOLET: MONTEVERDE HUMMINGBIRDS AVOID UV ALTERATIONS

NATHAN G. GIFFARD, ELIJAH W. LAIRD, SHANNON L. SARTAIN, BENJAMIN M. SCHELLING

TA Editors: Melissa DeSiervo and Clare Doherty
Faculty Editor: Matt Ayres

Abstract: Coevolution has created specialized relationships between species, such as the specialized relationship that exists between angiosperms and pollinators. Some flowers, for example, reflect ultraviolet (UV) light, which is visible to some pollinators. We investigated the foraging relationship between hummingbirds of the genus *Elvira* and two angiosperms, *Stachytarpheta jamaicensis*, native to the Neotropics, and *Callistemon citrinus*, native to Australia, at the Monteverde Cloud Forest Biological Preserve in Costa Rica. To understand if birds use UV reflectance for foraging, we added reflectance powder to inflorescences for each species. We found that flower visits were lower for flowers with added UV reflectance and that the number of flower visitations decreased with temperature. The change in behavior of hummingbirds in response to UV powder shows that *Elvira* spp. use UV for foraging. Further study of this system could investigate the specific mechanisms involving UV reflectance that hummingbirds use in their foraging.

Key words: Coevolution, *Elvira*, hummingbirds, Monteverde, foraging cues, ultraviolet, fission-fusion

INTRODUCTION

Coevolution, when two species evolve in tandem, can drive relationships between pairs or groups of species. The relationship between angiosperms and pollinators is a clear example. Pollination of a flower is fundamental to individual fitness; without moving pollen from one flower to another, most angiosperms cannot reproduce. While some flowers employ pollination syndromes reliant on abiotic factors such as wind or water, over 80% of flowering plants recruit animal pollinators to transport pollen (Rae and Vamosi 2013). These plant-animal interactions create coevolutionary relationships that influence the fitness of both participants.

Many examples of plant-pollinator coevolution involve angiosperms and Hymenoptera. In the simple, and apparently ancestral version of this mutualism, the flower is pollinated, allowing it to reproduce, and the Hymenopteran receives a sugar-rich nectar. Over evolutionary time, the morphology and physiology of the flower can evolve to cater to specific Hymenoptera species, and vice versa, resulting in a structured community of angiosperms and pollinators. In some cases, relations between flowering plants and Hymenoptera depend upon the capacity of Hymenoptera to see into the ultraviolet light

(UV) spectrum. This is evident, for example, in floral patterns (nectar guides) that direct insect visitors to nectaries but are only visible under UV light (Thorp et al. 1975).

Similar to certain Hymenoptera species, hummingbirds can see into the ultraviolet (UV) spectrum (George 1980; Chen et al. 1984). While it is known that Hymenoptera use UV reflectance specifically for foraging (Thorp et al. 1975), the same is not known for hummingbirds. Two hummingbird species in Costa Rica, White-tailed Emerald Hummingbird (*Elvira chionura*) and Coppery-headed Emerald Hummingbird (*Elvira cupreiceps*), forage on plant species that do and do not naturally reflect UV. The ornamental red bottlebrush (*Callistemon citrinus*, Myrtaceae), native to Australia, which naturally reflects UV, and the ornamental blue porterweed (*Stachytarpheta jamaicensis*, Verbenaceae), native to the Neotropics, which does not naturally reflect UV plant species visited by *Elvira* spp.

To better understand foraging behavior of *Elvira* spp., we tested for responses to experimentally manipulated reflectance in flowers *C. citrinus* and *S. jamaicensis*. If *Elvira* spp. use UV reflectance in foraging for flowers, changes in UV reflectance could be expected to influence flower visitation rates. Given that *S. jamaicensis* does not naturally exhibit UV

reflectance, an increase in reflectance may not have the same effect for both species. Additionally, the metabolic rate of birds, and hence their need to forage, are temperature dependent (Supplemental Figure 1). Their foraging responses to manipulated flowers may depend upon temperature.

METHODS

Experimental design

To test how a change in ultraviolet reflectance influences hummingbird foraging choices, we identified two plants (*C. citrinus* and *S. jamaicensis*) where White-Tailed Emerald Hummingbirds (*Elvira chionura*) and Copper-Headed Emerald Hummingbirds (*Elvira cupreiceps*) foraged at Monteverde Cloud Forest Biological Preserve in Costa Rica. *C. citrinus* showed natural UV reflectance under black light while *S. jamaicensis* did not. It was not possible for us to record the identity of each hummingbird visit, so we report visits by *Elvira* spp. There were at least 5 different individuals of *Elvira* spp. in the area of our observation.

On the *C. citrinus* tree, we identified six pairs of inflorescences that were of similar size and within 20 centimeters of each other. We then isolated each pair from other inflorescences by pruning other inflorescences within 50 centimeters of the pair. We chose a yellow ultraviolet powder which matched the inflorescence's natural coloration under UV light and added it to one inflorescence in each pair. We mixed the powder with water and evenly spread it on the tips of the inflorescence so that, under blacklight, the treated inflorescence exhibited enhanced UV reflectance (Supplemental Figure 1). We observed pairs of inflorescences for 20-minute intervals. We recorded how many times a bird visited each inflorescence and how many flowers within each inflorescence they probed with their bill.

We similarly altered one bush of *S. jamaicensis* that was flowering. We treated half of the inflorescences spread throughout the plant the same UV powder and left the other half untreated. Again, we made subsequent observations during 20-minute intervals and recorded air temperature. During these observations, we watched the entire bush,

counting the total number of flower visits (bill insertions) to treated and untreated inflorescences in each 20-minute period.

Statistical Analysis

We used a simple regression to test for a relationship between flower visits to *S. jamaicensis* and temperature. We tested for effects of UV treatment on the number of flower visits to *C. citrinus* with a generalized linear model Poisson distribution and log-link function that included treatment, temperature, and their interaction. We also used a paired t-test to test the relationship between inflorescence visits to *S. jamaicensis* and of UV treatment. Statistical tests were run using JMP® Pro 14.2.0

RESULTS

The number of flower visits to *C. citrinus* inflorescences by *Elvira* spp. and temperature were negatively related (Generalized Linear Model: chi-square = 79.31, $P < 0.0001$, $df = 1$, Fig. 1).

We also found a higher number of flower visits on untreated inflorescences of *C. citrinus* than UV-treated inflorescences (Generalized Linear Model: chi-square = 32.95, $P < 0.0001$, $df = 1$, Fig. 1). Hummingbirds visited untreated flowers more frequently than UV-treated flowers, especially at higher temperatures (Fig. 1).

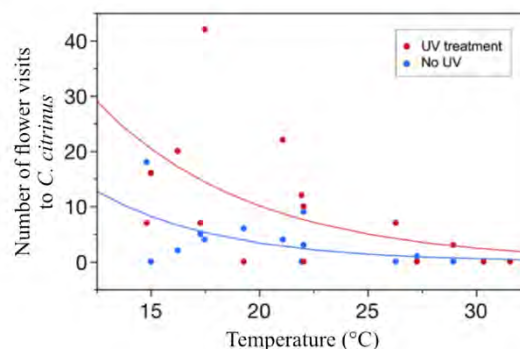


Figure 1. Number of hummingbird visits to *C. citrinus* flowers with and without addition of powder that fluoresces under UV light. Each 20-minute observation period is represented by a pair of points (UV treatment and control) that is plotted with respect to shaded air temperature at the start of the observation period.

There was no relationship between temperature and the number of flower visits on *C. citrinus* inflorescences by *Elvira* (Generalized

Linear Model: Chi-square = 79.31, $P = 0.38$, $df = 1$, Fig. 2). There was no relationship between total hummingbird visits to *S. jamaicensis* and temperature (Linear Regression: $F = 3.22$, $P =$

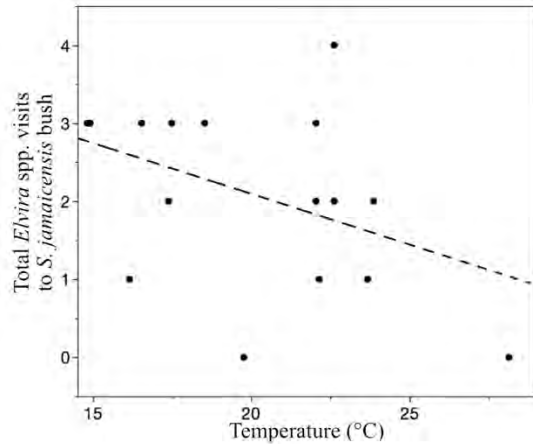


Figure 2. Total visits per 20-minute trial to *S. jamaicensis* vs. air temperature.

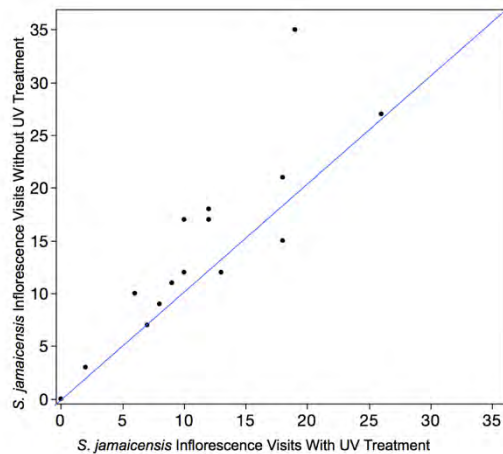


Figure 3. Total inflorescence visits to *S. jamaicensis* for natural and UV-treated inflorescences that were observed simultaneously. Diagonal indicates the line of equality.

0.09, $df = 16$, Fig. 2). Visits to untreated inflorescences were higher than UV-treated inflorescences on *S. jamaicensis* (Paired t-test: $t = 2.67$, $df = 16$, $P = 0.017$, Fig. 3).

DISCUSSION

Flower visits were reduced in both the *C. citrinus* and *S. jamaicensis* by the addition of UV powder. For the *C. citrinus*, which naturally reflects UV, the higher visitation rate to untreated inflorescences indicates a preference by *Elvira* spp. to the natural appearance of

untreated inflorescences. For the *S. jamaicensis*, which does not naturally reflect UV, the decrease in visits to the treated flowers could be because the addition of UV makes the flower look different, and therefore less desirable. In some species, the pattern of UV reflectance and absorbance also influence flower choice (Papiorek et al. 2016). These results support our hypothesis that hummingbirds do use UV for foraging. UV reflectance in general may have evolved specifically to attract clades other than hummingbirds, such as Hymenoptera. We qualitatively observed visitations by Hymenoptera to inflorescences with added UV reflectance during our trials, which may suggest a separate coevolutionary relationship between angiosperms and these pollinators. Though coevolutionary relationships may explain our results, the addition of the UV power without a true control limits our ability to make this conclusion. In a future study, we suggest using similar powder without UV reflectance as a control.

Our results displayed a negative relationship between air temperature and the number of visits to *C. citrinus* inflorescences, but not to *S. jamaicensis* inflorescences. Hummingbirds have an optimal temperature at which they function, and thus their behavior will change daily as temperatures fluctuate. Our experiment was performed only on two days with similar temperature ranges, and thus does not represent a complete temperature range, which may explain these findings (Supplemental Figure 2). It is worth noting that time of day and temperature are often highly correlated and hard to decouple. Generally, birds forage after waking up and before not eating overnight, so their activity is decreased mid-day. Mid-day is also when temperatures are highest, so it is difficult to discern whether hunger or temperature determines their foraging schedule.

The difference in visitation rates to each plant species with respect to temperature can likely be attributed to relative levels of sunlight exposure for each plant. Each experienced direct sunlight, however, the area around the *S. jamaicensis* was more shaded than that around the *C. citrinus*. Hummingbirds need to maintain a narrow body temperature. Also, increased exposure to direct sunlight during hotter points

in the day is energetically costly to an individual. Therefore, we saw the rate of hummingbird visitation to the shaded *S. jamaicensis* site to be higher than the visitation rate to the sunnier *C. citrinus* site with increased temperatures.

Visits to *C. citrinus* showed an interaction between temperature and UV reflectance. At lower temperatures, *Elvira* spp. preferred untreated *C. citrinus* inflorescences. As temperatures increased, however, this preference decreased in magnitude. Given the fact that they preferred untreated flowers overall, this change suggests that at lower temperatures, when hummingbirds are using less energy to regulate their metabolism, they can expend energy on making better foraging decisions.

Overall, our results can also be interpreted in a broader coevolutionary context involving other taxa. It is possible that *C. citrinus*, which is native to Australia, coevolved with Australian honeyeaters (Meliphagidae), which are known to forage on *C. citrinus* (Desta et al. 2016). Hummingbirds and honeyeaters have similar phenotypes; both are similar in size and have long bills for sipping nectar. There may, however, be a difference in how honeyeaters use and respond to UV reflectance while foraging when compared to *Elvira* spp. A more pointed study could perform similar alterations, but to plant species that are known to exhibit UV reflectance to attract bird foragers to understand what role UV plays in hummingbird foraging.

ACKNOWLEDGMENTS

Thank you to Matt, Mark, Clare and Melissa for their contributions in the design, analysis and writing of this project. Thank you to all of the staff at Monteverde Cloud Forest Biological Preserve. Lastly, thank you to the hummingbirds for drinking nectar.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this project.

LITERATURE CITED

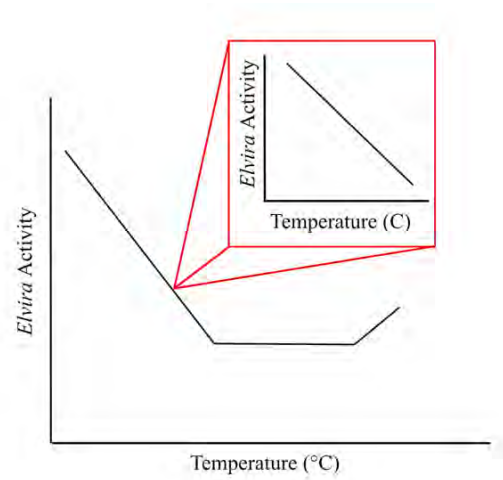
Chen, D., Collins, J., and Goldsmith, T. 1984. The ultraviolet receptor of bird retinas. *Science*, 225: 337-340.

- Cronk Q., Ojedra, I. 2008. Bird-pollinated flowers in an evolutionary and molecular context. *Journal of Experimental Botany*, 59:715-727.
- Desta, H.T., Dzakpasu, P.N., and Lyonga, N.M. 2016. Patterns of Sunbird (Family: Nectariniidae) Visitation to Four Sympatric Plant Species in Kibale National Park, Uganda, *International Journal of Molecular Evolution and Biodiversity*, 6(3): 1-8.
- George, M. 1980. Hummingbird Foraging Behavior at *Malvaviscus arboreus* Var. *Drummondii*. *Ornithological Advances*, 97:790-794.
- Goldsmith, T. 1980. Hummingbirds see near ultraviolet light. *Science*, 207:786- 788.
- Papiorek S., Junker, R., Alves-dos-Santos, I., Melo G., Amaral-Neto L., Sazima M., WolowskiM., Freitas L., Lunau K. 2015. Bees, birds and yellow flowers: pollinator-dependent convergent evolution of UV patterns. *Plant Biology*, 18:46-55.
- Rae, J., Vamosi, J. 2013. Ultraviolet reflectance mediates pollinator visitation in *Mimulus guttatus*. *Plant Species Biology*, 28(3): 177-184.
- Telles, F., Lind, O., Henze, M. 2014. Out of the blue: the spectral sensitivity of hummingbird hawkmoths. *Journal of Comparative Physiology*, 200(6): 537–546.
- Thorp R., Briggs, D., Estes, E., Erickson, E. 1975. Nectar Fluorescence under Ultraviolet Irradiation. *Science*, 189: 476-478.

APPENDIX



Supplemental Figure 1: A pair of red bottlebrush inflorescences under blacklight (A) and in natural light (B). The inflorescence with added UV powder is on the left of the pair in each photo.



Supplemental Figure 2. *Elvira* activity vs. Temperature (°C). Red box represents the temperature range under which the experiment was conducted.

EL FORRAJE O EN EL ULTRAVIOLETA: LOS COLIBRÍES MONTEVERDE EVITAN ALTERACIONES UV

NATHAN G. GIFFARD, ELIJAH W. LAIRD, SHANNON L. SARTAIN, BENJAMIN M. SCHELLING

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La coevolución ha creado relaciones especializadas entre especies, como la relación especializada que existe entre angiospermas y polinizadores. Algunas flores, por ejemplo, reflejan la luz ultravioleta (UV), que es visible para algunos polinizadores. Investigamos la relación de forrajeo entre los colibríes del género *Elvira* y dos angiospermas, *Stachytarpheta jamaicensis*, nativo de los neotrópicos, y *Callistemon citrinus*, nativo de Australia, en la reserva biológica de Monteverde, Costa Rica. Para entender si las aves utilizan reflectante UV para el forrajeo, añadimos polvo reflectante a las inflorescencias para cada especie. Encontramos que las visitas a las flores eran más bajas para las flores con reflectante UV añadida y que el número de visitas a las flores disminuyó con la temperatura. El cambio en el comportamiento de los colibríes en respuesta al polvo UV muestra que *Elvira* spp. Un estudio más profundo de este sistema podría investigar los mecanismos específicos que implican la reflectante UV que los colibríes utilizan en su forrajeo.

Palabras clave: Coevolución, *Elvira*, colibríes, Monteverde, señales de forrajeo, ultravioleta

Palo Verde





SPATIAL DISPERSION OF BIRD SPECIES AMONG MICROHABITATS IN A TROPIC MARSH

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TA Editors: Melissa DeSiervo and Clare Doherty

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Abstract: Abundance and species richness are two metrics often considered when estimating the value and conservation of an ecosystem. However, at times it may be impossible to maximize both of these elements through the same conservation strategy. We studied the abundance, diversity, and spatial distribution of birds in the Palo Verde biological reserve across four different marsh microhabitats. We found that while the water fern (*Salvinia minima*) microhabitat supported the greatest abundance of individuals proportional to the area it occupied in our study area, all four microhabitats (water fern, mud, open water, and graminoid) played an important role in supporting the community of birds in the marsh. Additionally, we saw that the frequently observed Northern Jacana tended to be clumped within the microhabitats that they occupied rather than distributing themselves randomly. Thus, less land may be required for conservation of some species (e.g. Northern Jacana) than for others (e.g. territorial species). While one microhabitat may support the largest number of individuals, the collection of different microhabitats promoted overall diversity of the marsh ecosystem.

Key words: birds, conservation, dispersion, marshland, microhabitats, Palo Verde

INTRODUCTION

Informed management and resource allocation are crucial for conservation. Resources are limited and decisions need to be made about where funds should go and what lands should be protected. Conservation decisions are frequently misaligned with optimal practices, and resources are then wasted. Only a nuanced understanding of the system in question can result in successful conservation practices.

The heterogeneous nature of many ecosystems adds to the complexity of land management and must be accounted for in conservation strategy. The ecological theory of patch dynamics recognizes the fragmentation and uneven distribution of organisms and resources within a larger ecosystem (Pickett 2013). Within one habitat, there often exists a diverse collection of smaller microhabitats. These microhabitats, each with their own community dynamics, are the basis of complex ecosystems. One such ecosystem is the tropical rainforest, composed of a massive range of microhabitats, differentiated by elevation, canopy level, rivers and pools, or any number of other distinguishing features. A nuanced understanding of the mosaic of patches in an ecosystem is required to illustrate its functionality and the interaction of species within that ecosystem.

A combination of ideal free distribution and niche theory may be helpful for understanding patch dynamics in the tropics. Under ideal free distribution, animals distribute themselves among patches of differing resource quality optimally. As a result, patches with higher resource abundance and quality harbor a greater number of animals than a patch with low resource quality. Niche theory proposes that organisms have been selected to fill specialized niches in their environment. As a result, dispersion of animals across patches of different microhabitats might be as much a function of their specialization as it is a function of resource quality and availability.

In the Palo Verde wetlands, marshlands are deliberately maintained to attract a wide range of bird species. In the maintained marshlands, there are various, clearly fragmented, microhabitats distinguishable by the observed fauna. With ever present conservation constraints, it is essential to understand the variation across microhabitats and their relative importance. By understanding which microhabitats are of use to which birds in the Palo Verde biological reserve, we can create a more informed land management plan for the wetland.

It may be that particular microhabitats contain the highest quality resources and are preferred by all bird species in the wetlands at Palo Verde regardless of specialization. If this were the case, we would see the highest species richness and bird

abundance in one or a few of the microhabitats of the marsh, while other microhabitats are relatively unused by the birds. Alternatively, it may be that the birds are specialized to occupy specific niches within the marsh, resulting in complete utilization of the microhabitats, but by different bird species. In this case, we would expect similar species richness and bird abundance within each microhabitat, but the beta diversity among habitats would be high, with different species found in different microhabitats. Lastly, it could be that birds distribute themselves randomly across the marsh, regardless of microhabitat type.

If one microhabitat type is used preferentially by many birds in the marsh, it may be best to focus on the maintenance of that habitat type, or even the creation of more of that habitat. Alternatively, it may be that maintaining a diverse array of microhabitats within the marsh is crucial for sustaining the diverse community of bird species that makes Palo Verde unique and spectacular.

METHODS

To determine the area of each microhabitat in the Palo Verde marsh, we used two methods. First, we physically mapped a 30 x 50 m area of the marsh by walking with a GPS along the edges of the different microhabitats. We differentiated four microhabitats, water fern (*Salvinia minima*), open water, mud, and graminoids, due to the differences in fauna observed among them. From these data, we produced a map of the study area, showing the location and size of each microhabitat. We then used ImageJ to determine the surface area in m² of each microhabitat. To cross-reference these results using another method, we surveyed 100 random points within the same 30 x 50 m area. For each point, we recorded the microhabitat type. We used these points to estimate the percent of total surface area each microhabitat occupied.

To record the location of all the birds observed in the same 30 x 50 mapped area, we laid out a 30-m transect tape running north to south, analogous to an “x-axis.” To get a y-coordinate for each bird, we used a Nikon laser distance rangefinder to determine the perpendicular distance of each bird from the x-axis—up to 50 meters away to the east. For each bird observed within the mapping area we recorded the time of the trial, the x and y coordinates of the bird, the species of the bird, and the microhabitat the bird was on. We performed a

trial every 30 minutes between the hours of 7:00 and 17:30, amounting to 21 trials over two days, during which we recorded a total of 267 observations of 7 different species. The maximum time between two trials was 15 hours.

To analyze the distribution of the Northern Jacanas we divided the plot into 5 x 5-meter sections to create a 6 x 10 grid. Having 5 x 5-meter bins allowed us to test for aggregation on a biologically relevant scale; larger bins would have shown clumping even if the birds were further from each other, and too small of bins would not have shown clumping even if birds were in direct proximity of one another. We calculated the average count and variance of Northern Jacana abundance across the 60 bins. The ratio of variance to mean was used to calculate lambda—a measure of dispersion. We compared lambda to a distribution of lambdas based on 1000 random Poisson distributions, each with a sample size equivalent to the number of observed Northern Jacanas.

RESULTS

Our two methods of microhabitat mapping, by GPS and random sampling, provided almost identical surface area determinations for the four micro-habitats. In our study area, we recorded 142 Black-bellied Whistling Duck (*Dendrocygna autumnalis*), 110 (90 mature, 20 immature) Northern Jacana (*Jacana spinosa*), 8 Glossy Ibis (*Plegadis falcinellus*), 4 Double-striped Thick-knee (*Burhinus bistriatus*), 1 Cattle Egret (*Bubulcus ibis*), 1 Green Heron (*Butorides virescens*), and 1 Snowy Egret (*Egretta thula*) (Table 1).

Table 1: Surface area by microhabitat as estimated from GPS polygons (middle column) and random sampling (right column).

Microhabitat	Area (m ²) Based on GPS	Percent of Surface Area Based on GPS	Percent of Surface Area Based on Random Sampling
Water fern	1324	0.57	0.56
Open water	702	0.30	0.21
Graminoids	126	0.05	0.1
Mud	178	0.08	0.13

Of the four micro-habitats, open water and graminoids had bird abundance counts proportional to surface area. Water ferns had a disproportionately high abundance while mud had disproportionately low abundance. Alpha diversity was similar across microhabitats with an increase in water ferns (Table 2).

Table 2: Breakdown of bird counts across the four microhabitats, both overall and by species.

	Mud	Open Water	Water Fern	Graminoid	Total
Percent Coverage	13	21	56	10	100
Total Birds	4	54	190	19	267
Total Species	3	3	6	3	8
By Species					
Black-bellied Whistling Duck		50	92		142
Northern Jacana	2	3	73	12	90
Northern Jacana (Immature)	1		18	1	20
Glossy Ibis			2	6	8
Double-striped Thick-knee			4		4
Cattle Egret	1				1
Green Heron			1		1
Snowy Egret		1			1

The Black-bellied Whistling Duck was the most abundant species observed, followed by the Northern Jacana (Table 2). As the Black-bellied Whistling Duck was obviously aggregated, we analyzed the dispersion of Northern Jacanas,

which foraged solitarily, and found them to be highly, mostly within water fern ($\lambda = 6.64$, $p < 0.0001$). Black-bellied Whistling Duck was mostly found in water fern and open water. Larger birds, including the Glossy Ibis and Cattle Egret, were almost exclusively found in graminoids or mud (Fig. 1).

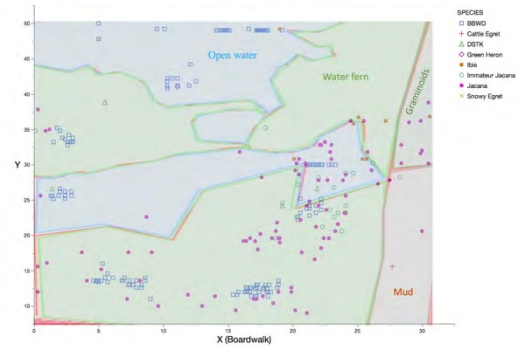


Figure 1: Spatial patterning of birds overlaid onto GPS map of the plot.

DISCUSSION

The open water and graminoid microhabitats had bird abundances proportional to surface area, while the water fern microhabitat had proportionally greater abundances and the mud microhabitat had proportionally less. This suggests that water fern is the most important microhabitat if the goal is to maximize the sheer number of birds in the marsh,

Species richness did not vary much among microhabitats, but the water fern microhabitat did have a slightly higher richness than the others. Different species were observed in different microhabitats. This suggests the specialization of species to utilize specific microhabitats within the marsh. However, the Black-bellied Whistling Duck was found in both the water fern and open water environments, suggesting that some more generalist species take advantage of diverse microhabitats for different needs. While some species, like the Northern Jacana, were primarily found in one microhabitat, they were recorded in all microhabitats in the course of our survey; therefore, access to all microhabitat types may be necessary for their continued presence. In contrast to our conclusion based on bird abundance, this implies that no one microhabitat is the most important for fostering biodiversity. If this is the

focus of conservation efforts, then preserving a range of distinct microhabitats should be prioritized.

Our study found that bird species tended to group spatially within the microhabitats that they occupied rather than distributing themselves randomly. This may be explained by the ideal free distribution. Under this explanation, birds are distributing themselves in these patterns according to differential resource availability. For example, perhaps Northern Jacana, which eats invertebrates found among assemblages of aquatic plants, were finding clusters of prey, and aggregating there. Alternatively, there could be social dynamics at play. For example, Northern Jacana have been shown to stick near their young and defend them (Stephens 1984). Better understanding of the features that underlie this clumping of individuals are yet another consideration that can inform conservation practices.

In our investigation, we analyzed the Northern Jacana (juveniles and adults) because it was conspicuous, abundant, and clearly foraging. Given that Northern Jacanas tended to spatially cluster, less land may be required for conservation of this species relative to others. For more territorial species, larger areas would be needed to support a population. Additionally, many species that are non-territorial would show high overlap at certain times of the year when they do become territorial. Understanding spatial patterning of the biological system is crucial for informing conservation efforts, especially when considering the size of the area to conserve.

Our findings suggest different land management practices are optimal for achieving different conservation goals. If maximizing bird abundance is the goal, then certain microhabitats may be more important than others to conserve.

On the contrary, if rich biodiversity is the goal, then each microhabitat is important, as different species specialize in different microhabitats. The spatial distributions of species within a microhabitat type is also important to consider, as this gives a more accurate estimate of how many individuals and species an area of land can support. Finally, it is helpful to view these systems through the lenses of ideal free distribution, patch dynamics, and social dynamics. By studying how the mosaic of microhabitats and biology of the organisms inhabiting them interact we can implement better informed conservation practices to meet the goals desired.

ACKNOWLEDGEMENTS

Thank you to the employees at the Organization for Tropical Studies in Palo Verde Biological Reserve for the muy rica comida and the great accommodations throughout this project. Huge shout out to Jose, our naturalist guide, for his inspiration and guidance throughout this project. Muchas gracias to the TAs and Matt for their help throughout the entire process of this project.

AUTHOR CONTRIBUTIONS

All authors contributed equally. RNA specialized in the analysis of data. EWL and ACPR (the marsh-walkers) manually created a map of the marsh. MCB brought tremendous energy and was crucial in the formation of our surface area estimates.

LITERATURE CITED

Stephens, M. L. 1984. Interspecific aggressive behavior of the polyandrous northern jacana (*Jacana spinosa*). *The Auk*, 101(3), 508-518.

DISPERSIÓN ESPACIAL DE ESPECIES DE AVES ENTRE MICROHÁBITATS EN UN PANTANO TRÓPICO

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Abstracto: La abundancia y la riqueza de especies son dos métricas que a menudo se consideran al estimar el valor y la conservación de un ecosistema. Sin embargo, a veces puede ser imposible maximizar ambos elementos a través de la misma estrategia de conservación. Estudiamos la abundancia, diversidad y distribución espacial de las aves en la reserva biológica de Palo Verde a través de cuatro microhábitats pantanosos diferentes. Encontramos que mientras que el microhábitat del helecho de agua (*Salvinia minima*) sostenía la mayor abundancia de individuos proporcionales a la zona que ocupaba en nuestra área de estudio, los cuatro microhábitats (helecho de agua, barro, aguas abiertas y gramínoideas) desempeñaban un papel importante en el apoyo a la comunidad de aves en el pantano. Además, vimos que los Jacanas del Norte observados con frecuencia tendían a ser agrupados dentro de los microhábitats que ocupaban en lugar de distribuirse aleatoriamente. Por lo tanto, puede ser necesaria menos tierra para la conservación de algunas especies (por ejemplo, Jacana del Norte) que para otras (por ejemplo, especies territoriales). Mientras que un microhábitat puede apoyar el mayor número de individuos, la recolección de diferentes microhábitats promovió la diversidad general del ecosistema de las marismas.

Palabras claves: aves, conservación, dispersión, marismas, microhábitats, Palo Verde

CONTEXT-SPECIFIC INTERACTIONS OF AQUATIC PLANTS IN A TROPICAL MARSH ECOSYSTEM

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Faculty Editor: Matt Ayres

Abstract: Wetland environments support biodiversity and play an important role in global carbon sequestration. Wetland environments also support many niches due to diverse abiotic and biotic conditions. Invasive plant species are of concern in these environments because they can come to dominate and disrupt the ecosystem. This is exemplified in the Palo Verde wetland environment, which is subject to a yearly cycle of filling and drying. We examined two aquatic plant species which are notably invasive in other parts of the world, common water hyacinth (*Eichhornia crassipes*) and common salvinia (*Salvinia minima*). However, salvinia is native to the Palo Verde area, while water hyacinth is non-native. This interplay is interesting given the two species evolved under different pressures and therefore have varied competitive adaptations. We tested how water depth and relative abundance of water hyacinth, is related to salvinia biomass. Salvinia biomass was consistently lower in the areas of high abundance of water hyacinth. This difference was greater in deeper water. The interplay between these two aquatic plants may change dramatically in response to varying abiotic factors throughout the year. Studying the context-specific interaction between these invasive species can uncover the threats they may pose to native species and ecosystems.

Keywords: Common water hyacinth, common salvinia, hydroperiod, invasive species, non-native species, wetlands

INTRODUCTION

The breakdown of biogeographic barriers with globalization is homogenizing plant communities (Hobbs 2000). Ecosystems are increasingly becoming dominated by a few plant species that are good colonizers, aggressive competitors, and resistant to herbivores and pathogens. This is evident in tropical wetlands, which play an important role in supporting biodiversity (Wantzen and Junk 2000) and sequestering atmospheric carbon (Mitsch et al 2009) but are particularly vulnerable to invasion by non-native plants. It is reported that 24% of the world's 33 most invasive plants are wetland species (Zedler and Kercher 2010). The term invasive has a wide range of definitions depending on context, but here we define it as a species that is native to elsewhere. Sometimes disturbances such as salt influx and drying cycles create opportunities used by potentially non-native species. (Zedler and Kercher 2010).

Two species that impact wetlands globally are common water hyacinth, *Eichhornia crassipes* (Pontederiaceae), and common salvinia, *Salvinia minima* (Salviniaceae). Water hyacinth is a free-floating vascular plant native to South America and is one of the world's most

prevalent invasive aquatic plants (Villamanga and Murphy 2010). It reproduces asexually with stolons and sexually with seeds, which can remain viable for 20 years. This makes them very difficult to control (Center et al. 1999). Water hyacinth can outcompete most other species growing in an area by accelerating evaporation from water bodies and rapidly absorbing nutrients and oxygen (Bhattacharya 2010). These conditions may lead to a decrease in biodiversity in ecosystems where the plant is newly present.

Salvinia is a small, free-floating fern native to South America and Mesoamerica, but is invasive elsewhere (Jacono et al 2001). It moves easily in water, aiding its dispersal when located among larger aquatic plants. Small, stiff hairs on the surface of the leaf slow desiccation of the plant, making it relatively robust in arid conditions (Mikulyuk and Nault 2008). Sporocarp structures allow the fern to reproduce asexually (Mikulyuk and Nault 2008) and continuous branching and fragmentation of rhizomes allow for rapid reproduction throughout the growing season. Additionally, buds embedded in the rhizome may lie dormant during drier periods (Global Invasive Species

Database 2020), allowing the plant to be successful in seasonal wetlands.

Multiple factors may determine the relative abundance of these two aquatic plants. They are both strong competitors. Where both exist, competition for resources may heavily influence their respective abundance. Water hyacinths grow larger and produce copious flowers and seeds, and therefore require more sunlight and nutrients than does the salvinia. This potential asymmetry in resource requirements could lead the water hyacinth to outcompete the salvinia. However, the water hyacinth's rooting system may also benefit salvinia by providing structural support. Further, tropical wetlands' annual flood pulses create many different temporal niches (Murray-Hudson et al. 2014). Annual cycling of abiotic and biotic factors in wetlands of the seasonally dry tropics promote coexistence may allow each species to thrive at different times of year.

We investigated the relationship between water hyacinth and salvinia in a tropical wetland inside Palo Verde National Park in Costa Rica. Here, where salvinia is native but water hyacinth is invasive, the interaction may be complex. We studied how the relative abundance of water hyacinths influenced salvinia biomass, and how this relationship changed with depth. Water hyacinths are invasive, and therefore equipped with competitive adaptations to easily exploit resources in diverse environments. Thus, salvinia would likely be less abundant in areas with high abundance of water hyacinths. Alternatively, water hyacinths could also facilitate salvinia abundance. A second possibility is that the relationship changes with water depth. If salvinia use the water hyacinths as a supportive substrate in deep water, where water is typically moving faster, the negative effects of water hyacinths on salvinia might be ameliorated in deeper water. Alternatively, if salvinia are better adapted to shallower water than are water hyacinth, we predict the negative effects of water hyacinth on salvinia to be ameliorated in shallow water.

METHODS

To test how water hyacinth abundance impacted salvinia in the Palo Verde marsh environment, we measured salvinia biomass with respect to

the abundance of water hyacinth. We used stratified random sampling that recognized zones (strata) of low and high abundances of water hyacinth crossed with deep and shallow water (Fig. 1). High abundance strata had between 80 - 100% percent cover of water hyacinth, while low abundance strata had about 10 - 30% percent cover. Within zones of high and low hyacinth abundance, we recognized zones of deep or shallow water: average (range) = 53 (38 - 62) and 23 (12 - 37) cm, respectively. Within each of these four strata, we collected measurements in 14 - 15 randomly selected quadrats (N = 59), of 22 x 22 cm.

Within each quadrat we removed all of the salvinia and weighed the biomass wet weight with a Chatillon force gauge (10N). Lastly, we measured water temperature once each (in the same afternoon) in three areas: open water, high water hyacinth abundance, and high salvinia abundance.



Figure 1. Areas of relatively high and low abundance of water hyacinth, *Eichhornia crassipes* (Pontederiaceae).

Statistical Analyses

We analyzed salvinia biomass with a two-way ANOVA that included water depth, water hyacinth abundance, and their interaction (all as categorical fixed effects).

RESULTS

Salvinia biomass was generally greater in plots with low abundance of water hyacinth than in plots with high abundance of water hyacinth (main effect of water hyacinth abundance: $F_{1,55} = 42.01$, $P < 0.0001$; Fig. 2). There was no main effect of water depth on salvinia biomass (main effect of water depth: $F_{1,55} = 0.98$, $P = 0.32$), but the negative effects of increased water hyacinth abundance were greater in deep water than in shallow water (water depth x water hyacinth abundance interaction: $F_{1,55} = 20.83$, $P < 0.0001$).

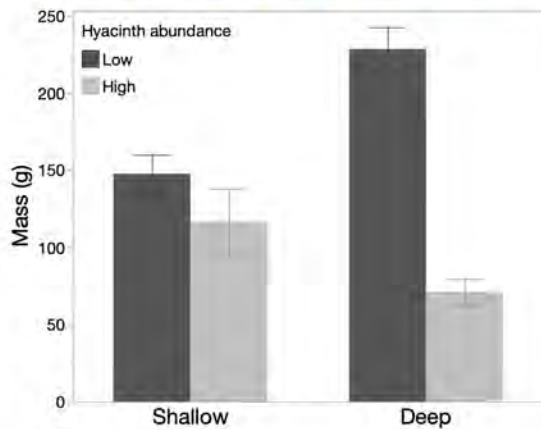


Figure 2: Biomass of salvinia in areas of low and high abundances of water hyacinth in shallow (<37cm) and deep (>37cm) water. Mean \pm SE.

Qualitative Results

In some areas of low hyacinth abundance, we observed large clusters of salvinia, held together by their own root system. In areas of high hyacinth abundance, we observed smaller groups of individual salvinia nested within the stems and roots of other plants, especially hyacinth. We did not see evidence of herbivory on either species. While wading among our plots, it was evident that water temperatures were cooler in areas of high hyacinth abundance. This was supported by our measurements. The measured temperature was about the same in an area of low water hyacinth abundance (28.4 °C) and open water (28.3 °C), but it was lower in an area of high-water hyacinth abundance (25.6 °C).

DISCUSSION

Salvinia biomass was reduced in areas of high abundance of water hyacinth. This supports the hypothesis that water hyacinth competes with salvinia for space, nutrients, light and water. The negative impacts of water hyacinth on salvinia increase with water depth. Thus, we reject the hypothesis that water hyacinth facilitates salvinia at depth by providing structural support. During our studies, we noted that salvinia by itself already clumps together to form mats that likely accomplish what water hyacinth might do (providing structural support but without the competition). We can identify a few hypotheses for the stronger effect of hyacinth in deep water. Water hyacinth facilitates salvinia more in shallow water than deep water. The difference in temperature might explain the change of the impact at depth. In areas of higher abundance of water hyacinth, we observed and measured lower temperatures than we did in areas of low abundance. Water hyacinth, and its large, net-like root structures, might significantly reduce the amount of solar radiation reaching the water. This difference could be more important at depth, where solar radiation per surface area is already distributed over a larger volume of water than it is in shallower water.

We hypothesized that the annual drying cycle of the marsh is important to interactions between Salvinia and water hyacinth. The reproductive strategies of all aquatic plants in this system must be adapted to this annual cycle. A majority of aquatic plants in the Palo Verde wetland drop seeds or resting propagules which lay dormant until the following wet season. It would be informative to study the annual cycle and in relative abundance and distribution of water hyacinth and Salvinia. To fully understand the interactions between water hyacinth and Salvinia, we would also need to observe their annual distribution across wet and dry seasons. We were only able to observe for a short period during a transitional portion of the cycle, and therefore cannot easily infer the nature of their year-round interactions. Perhaps conducting this experiment a month earlier or later could have produced different results. Finally, though the presence of both species indicates general success of each, the small timescale of our project also means that we do not know how the

relative success of each species is changing on multi-year cycles. Perhaps if we came back in 10 or 50 years, we would find that the water hyacinth had slowly outcompeted the *Salvinia* in the Palo Verde marsh. Quantifying context-specific population dynamics among these known invasive species may expose the possible threats these species could have on native species and ecosystems.

ACKNOWLEDGEMENTS

Special thanks to Matt for urging us to get our feet dirty and walk around the marsh. Thanks also to the Palo Verde research station for the wonderful accommodations, food, and boardwalk.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this work.

LITERATURE CITED

- Bhattacharya, A. and P. Kumar 2010. Water Hyacinth as a Potential Biofuel Crop. *Electronic Journal of Environmental, Agricultural and Food Chemistry*. 9:1 112-122.
- Center, T.D., Dray, Allen., Jubinsky, G.P., and Grodowitz, M. 1999. Biological Control of Water Hyacinth Under Conditions of Maintenance Management: Can Herbicides and Insects Be Integrated? *Environmental Management*: 23. 241-256.
- Global Invasive Species Database 2020. Species profile: *Salvinia minima*.
- Hobbs, Richard J. 2000. *Invasive Species in a Changing World*. Island Press.
- Jacono, C.C. and Davern, T.R. and Center, T.D. 2001. The adventive status of *Salvinia minima* and *S. molesta* in the Southern United States and the related distribution of the weevil *Cyrtobagous salviniae*. *Castanea*. 66. 214-226.
- Junk, W.J., Wantzen, Karl. 2004. The Flood Pulse Concept: New Aspects, Approaches and Applications—An Update. *Proceedings of the Second International Symposium on the Management of Large Rivers for Fisheries*. Vol. 2.
- Mitsch, W.J., A. Nahlik, P. Wolski, B. Bernal, L. Zhang, and L. Rhamberg. Tropical wetlands: seasonal hydrologic pulsing, carbon sequestration, and methane emissions. *Wetlands Ecology and Management*: 18 573-586
- Murray-Hudson, M., et. al 2014. Remote Sensing-derived hydroperiod as a predictor of floodplain vegetation composition. *Wetlands Ecology and Management*. 23. 1-14.
- Villamagna, A. and B. R. Murphy. 2010. Ecological and socio-economic impacts of invasive water hyacinth (*Eichhornia crassipes*): a review. *Freshwater Biology*. 5:2 282-298.
- Zedler, Joy B., Kercher, Suzanne. 2005. Wetland Resources: Status, Trends, Ecosystem Services, and Restorability. *Annual Review of Environment and Resources*. 30 39-74.

INTERACCIONES ESPECÍFICAS DEL CONTEXTO DE PLANTAS ACUÁTICAS EN ECOSISTEMAS PANTANOSOS TROPICALES

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Abstracto: Los humedales apoyan la biodiversidad y desempeñan un papel importante en el secuestro mundial de carbono. Los humedales también soportan muchos nichos debido a diversas condiciones abióticas y bióticas. Las especies vegetales invasoras son preocupantes en estos ambientes porque pueden llegar a dominar y perturbar el ecosistema. Esto se ejemplifica en el entorno del humedal de Palo Verde, que está sujeto a un ciclo anual de llenado y secado. Examinamos dos especies de plantas acuáticas que son notablemente invasoras en otras partes del mundo, el Jacinto de agua común (*Eichhornia crassipes*) y la Salvinia común (*Salvinia minima*). Sin embargo, la Salvinia es nativa de la zona de Palo Verde, mientras que el Jacinto de agua no es nativo. Esta interacción es interesante dado que las dos especies evolucionaron bajo diferentes presiones y por lo tanto tienen diversas adaptaciones competitivas. Hemos probado cómo la profundidad del agua y la abundancia relativa de Jacinto de agua está relacionada con la biomasa de Salvinia. La biomasa de Salvinia fue consistentemente menor en las áreas de alta abundancia de Jacinto de agua. Esta diferencia fue mayor en aguas más profundas. La interacción entre estas dos plantas acuáticas puede cambiar drásticamente en respuesta a diversos factores abióticos a lo largo del año. El estudio de la interacción específica del contexto entre estas especies invasoras puede descubrir las amenazas que pueden representar para las especies y ecosistemas nativos.

Palabras clave: Jacinto de agua común, salvinia común, especies invasoras, especies no nativas, humedales

CONDITIONAL INTERACTION: THE PLASTICITY OF INVESTMENT BETWEEN ACACIA TREES AND PSEUDOMYRMEX SPP. ANTS

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“Suppose that you and I were sitting in a quiet room overlooking [an acacia] garden, chatting and sipping at our cups of green tea while we talked about something that happened a long while ago, and I said to you, “That afternoon when I [measured those 24 trees] ... was the very best afternoon of my life, and also the very worst afternoon.” - Arthur Golden

Abstract: High rates of herbivory in dry Neotropical forests prompt many neotropical species to invest resources in defenses against herbivores. One example of plants investing in defense is the mutualistic relationship between *Vachellia* (= *Acacia*) *collinsii* (hereafter referred to as *Acacia*) and the following ant species: *Pseudomyrmex spinicola* and *Pseudomyrmex flavicornis*. While the tree provides sugar excreted from extrafloral nectaries (EFNs) and shelters resident ants through domatia (thorns), the ants provide physical defense against herbivores and clear the base of the tree of competition. To quantify the mutualism between *Acacias* and *Pseudomyrmex* spp., we measured ant and plant investment through metrics of ant defense activity and tree habitat investment in 24 *Acacia* trees at Palo Verde National Park in Costa Rica. Given the higher energetic demands of the more active *P. spinicola*, a relationship could exist between ant type and density of thorns and EFNs. Given that *P. spinicola* are also more aggressive, there could also be a relationship between ant type and tree health, amount of base clearing, and herbivory-response activity. Conversely, thorn density, mean EFNs per leaf, amount of base clearing, and defense activity levels may be completely random, which would demonstrate that *Acacias* with certain morphologies do not have higher fitness with certain ant species inhabitants. Trees inhabited by *P. flavicornis* contained a greater density of thorns and EFNs per leaf than trees inhabited by *P. spinicola*. Additionally, we determined that *P. spinicola* cleared the base of their host trees significantly more than *P. flavicornis*. Tree health did not vary by ant species, but we found significantly more trees with *P. spinicola* in our sample area. These results highlight the plasticity of investment in the mutualism between *Acacias* and *Pseudomyrmex* spp. based on ant species, suggesting that this relationship is a conditional interaction. In addition, these results demonstrate that different species of ants may colonize trees with distinct levels of defense investment. Conditional interactions between organisms like *Acacias* and *Pseudomyrmex* spp. are complex, and this work aims to better understand how investment in mutualist defense relationships by each species can impact herbivory, competition, and overall fitness of an individual.

Key Words: mutualism, *Acacia*, ant, *P. flavicornis*, *P. spinicola*, extrafloral nectaries, herbivory, defense, Palo Verde

INTRODUCTION

Mutually beneficial relationships between species are common in nature. Some can be thought of as supporting biological mercenaries: e.g. pilot fish cleaning sharks in exchange for protection, and bacteria defending neotropical frogs from toxic fungi in exchange for a place to live. Mutualisms can be sustained in evolutionary time when the fitness of both participants is enhanced by the association. There is often plasticity of investment in such relationships. Natural selection will favor individuals who maximize benefits relative to costs. In some cases, depending on environmental conditions and resource

availability, mutualisms can even become parasitisms. For example, there are small annelid worms that graze on organisms on the gills of crayfish, cleaning them (Skelton et al. 2016). This generally benefits both species involved. However, when worms are very abundant and there is not enough biofilm available on the gills, the worms become parasites and feed on the gills themselves. Such associations are called conditional interactions. While there are many examples of conditional interactions in nature, the capacity of organisms to adaptively manage their investment in associations is not well understood.

For plants, herbivory can dramatically reduce fitness. Thus, defense against herbivory can be highly adaptive in some environments. This is particularly true in tropical dry forests, where rates of herbivory are higher when compared to rates in tropical wet forests (Coley and Barone 1996). Within tropical dry forests, most of the damage from herbivory occurs when trees are young; even in older trees, damage occurs at higher rates in the understory. To prevent herbivory during critical understory maturation, many forms of plant defense exist, including leaf toughness, trichomes, and the production of secondary metabolites (Malishev and Sanson 2015; Levin 1973; Hay and Fernical 1988; Rosenthal and Berenbaum 1992). Mutualistic relationships can also serve as a form of herbivory defense.

A key example of a mutualistic relationship that decreases rates of herbivory is the relationship between *Vachellia* (= *Acacia*) *collinsii* (hereafter referred to as *Acacia*), which is native to Palo Verde in Costa Rica, and the following ant species: *Pseudomyrmex spinicola* and *Pseudomyrmex flavicornis* (Janzen 1966). In this relationship, *Acacias* provide nectar from extrafloral nectaries (EFNs), produce protein-rich beltian bodies on the ends of leaflets, and house the resident ants in domatia (hereafter referred to as thorns) of the plant. In return, the ants provide physical protection against herbivores and decrease competition around the host by killing neighboring plants. Previous studies suggest that individual *Acacias* typically only harbor one of three species of mutualistic ants: *P. spinicola*, *P. flavicornis*, or *P. nigrocinctus* (New 2017). *P. spinicola* has also been reported to be more vigorous in defense than *P. flavicornis* (Hansell 2000). However, uncertainty remains regarding differences in host tree defense among different species of *Pseudomyrmex*, and their relationship to the morphology, growth and vigor of trees.

P. spinicola is known to be more aggressive than *P. flavicornis*, and, therefore, may provide better protection for their trees. If so, one could expect a greater relationship between ant species and ant activity after simulated herbivory and greater clearing of other plants around the host tree. There could also be relationships between

the resident ant species, density of EFNs and thorns.

If *P. spinicola* better protect their trees against herbivores and competitors, and the better defenses confer higher fitness, then trees hosting *P. spinicola* would be healthier overall compared to trees hosting *P. flavicornis*. We tested these hypotheses by measuring a suite of tree and ant traits in *Acacias* occupied by either *P. spinicola* or *P. flavicornis*.

METHODS

Sampling Methods

To determine the abundance of trees inhabited by each ant species, we counted the number of *Acacias* along the road within half a mile of the Palo Verde Biological Research Station in Costa Rica and recorded whether each tree was inhabited by *P. spinicola* or *P. flavicornis*. Five *Acacia* trees with parasitic ants, *Crematogaster scutellaris*, were excluded from this count. We then selected a subset of 24 trees for further study. Study trees were restricted to heights of 0.91 to 2.1 m for ease of measurement. Twelve of the trees were inhabited by *P. flavicornis*, and 12 were inhabited by *P. spinicola*. We measured the height of each tree from the base to the crown. To assess ant efficacy in clearing competing plants at the base of the host, we counted the number of live plants within a 50-cm radius around each *Acacia* trunk. As a proxy for tree health, we counted the number of living vs. dead branches on each tree. We also counted EFNs and thorns on three randomly selected branches at mid height (between the base and crown) of each tree. We recorded the number of EFNs on each of the terminal three leaves of each branch. We also counted the total number on each branch and the length of the branch. To record the activity of the ants after simulated herbivory, we forcefully tapped the base of the tree five times with a stick of about 50 cm length and 3 cm diameter. After 15 seconds, we counted the number of ants crossing an imaginary line encircling the middle of a branch previously measured for EFNs and thorns. This was compared with a baseline measurement of ants crossing the same point prior to simulated disturbance.

Statistical Analyses

All statistical tests were performed with JMP 14. We employed a 2 x 2 Pearson's Chi-Squared analysis to test if the number of trees with each ant species differed. We used Pearson t-tests to compare trees occupied by *P. spinicola* vs. *P. flavicornis* with respect to tree height and proportion of live branches. We evaluated EFNs per leaf and thorns per meter of branch with an ANOVA that included ant species as a fixed effect, tree nested within ant species as a random effect, and branches within trees as replicates. We also calculated a correlation matrix of all tree-specific measurements. All variables were evaluated for normality prior to statistical analysis.

RESULTS

Our survey of 64 Acacia trees showed 73% to be inhabited by *P. spinicola* and 27% by *P. flavicornis* (Pearson's Chi-Squared: $\chi^2 = 14.06$, $df = 1$, $p = 0.0002$). Acacias housing *P. flavicornis* were significantly taller (Pearson's t-test: $t = 3.68$, $df = 17.87$, $p = 0.0010$). However, there was no difference in proportion of live to total branches between trees housing *P. flavicornis* and those housing *P. spinicola* (t-test: $t = 0.01$, $df = 21.82$, $t = 0.51$; Fig. 1).

Ant activity in response to simulated herbivory did not differ between *P. flavicornis* and *P. spinicola* (Pearson's t-test: $t = 0.51$, $df = 20.30$, $p = 0.615$; Fig. 2a). However, there were significantly more non-Acacia neighbors around the base of trees inhabited by *P. flavicornis* (Pearson's t-test: $t = 3.46$, $df = 20.65$, $p = 0.0024$; Fig. 2b).

Trees that housed *P. spinicola* had somewhat fewer EFNs per leaf (ANOVA: $F = 2.94$, $df = 1, 22$, $p = 0.066$; Fig. 3a) and markedly fewer thorns per meter (ANOVA: $F = 11.94$, $df = 1, 22$, $p = 0.0023$; Fig. 3b). There was notable variation among trees within ant species in EFNs/leaf (34% of the random variance) but less so in thorns/m (16% of random variance).

There was a strong correlation between the number of leaves and the number of EFNs on a branch ($r = 0.85$; Table 1). Also, taller trees were correlated with a lower proportion of live to total branches, indicating reduced health ($r = -0.45$; Table 1). Increased thorn density was also

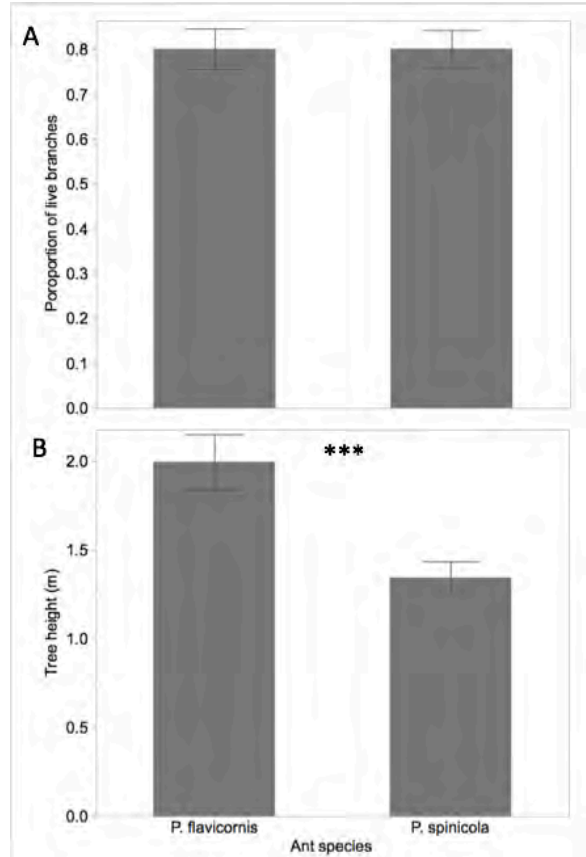


Figure 1: Proportion of live branches (upper) and tree height (lower) inhabited by *P. flavicornis* vs. *P. spinicola*.

correlated with an increased number of leaves ($r = 0.51$; Table 1). Increased tree height was correlated with increased average number of EFNs per leaf ($r = 0.42$; table 1). Number of EFNs was correlated with both the average number of EFNs per leaf and thorn density ($r = 0.48$, $r = 0.41$; Fig. 2). Finally, there were no significant correlations between number of leaves, number of EFNS, thorn density, and height ($r < |0.404|$).

DISCUSSION

Our findings begin to distinguish how the mutualistic relationship between Acacias and *Pseudomyrmex* spp. shifts depending on the species inhabiting the host. This is suggestive of a conditional interaction. Consistent with this, Acacias inhabited by *P. flavicornis* had significantly more non-Acacia neighbors than those inhabited by *P. spinicola*. However, the changes in activity level in response to simulated herbivory were not different between the two ant

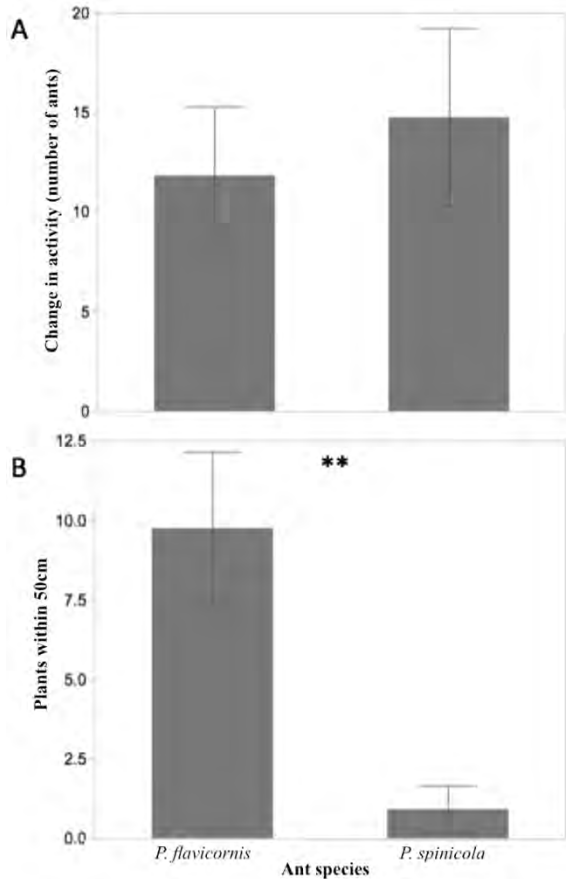


Figure 2: Ant activity in response to simulated herbivory (upper) and plants within 50cm (lower) of Acacias inhabited by *P. flavicornis* and *P. spinicola*.

species. Apparently, *P. spinicola* decreases competition more effectively around the base of the tree, but the two species of ants provide similar levels of defense against herbivory in the tree crown. The health of Acacias did not appear to differ with ant species. These relationships are likely not confounded by tree morphology because we did not find any relationship between thorns or leaves and tree height. Trees inhabited by *P. flavicornis* had somewhat more EFNs per leaf, suggesting greater nectar supply to *P. flavicornis*, but we could not measure secretion rates per EFN. The density of thorns was markedly higher in trees occupied by *P. flavicornis*, which might reflect greater investment in ant domatia by trees with less aggressive ant species. Thorn number and ant activity have been found to be correlated by previous studies, suggesting that trees containing *P. flavicornis* may host more ants than trees containing *P. spinicola* (Campbell and Cook

2013). A larger population of less active ants may have similar activity levels to those of a smaller population of more active ants. This could explain the similarity in ant activity following simulated herbivory (Fig. 1A).

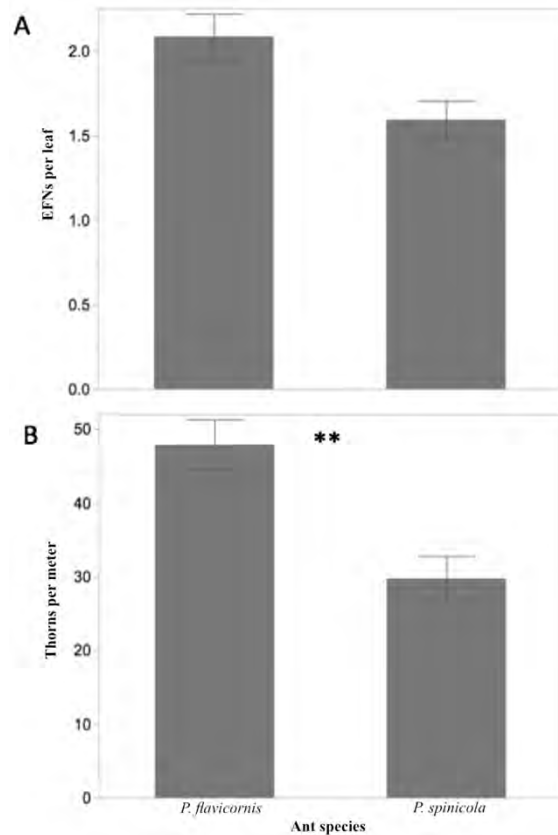


Figure 3: Extrafloral nectaries per leaf (upper) and thorns per meter of branch (lower) in Acacia trees occupied by *P. flavicornis* or *P. spinicola*.

These results are relevant when we consider Acacia morphology as it relates to its evolutionary history. While Acacia trees today are dependent on ants for survival, thorns alone may have previously provided sufficient protection from predators. Acacias are native to Central America and would have experienced strong selection pressures to develop herbivory defense against megafauna like giant ground sloths when they were more prevalent in the Americas. However, after the large-scale extinction of megafauna, it is likely that the main herbivores of Acacias switched from large mammals to birds, insects, and other parasites that are less deterred by the spines on Acacia trunks. If this were the case, Acacias would need

additional defense from herbivory, creating a niche for *Pseudomyrmex* spp. Given the quick generational time of Acacias, it is likely that selection pressures shifted to favor mutualism between Acacias and *Pseudomyrmex* spp. that conserve the thorn morphology observed today. Future studies could further explore this mutualism on a larger time scale, studying the differences in tree morphology of Acacias over several generations and how morphology has influenced the relationship between the tree and the *Pseudomyrmex* spp.

The greater number of trees inhabited by *P. spinicola* in our study area may indicate higher fitness of trees with *P. spinicola*. It is possible that more effective base clearing in trees inhabited by *P. spinicola* increases the overall fitness of these trees. However, if this were the case, it seems that selection would exclude *P. flavicornis* over time. Yet, *P. flavicornis* populations have appeared to be stable in our study area for the past 50 years (Matthew Ayres, pers. comm.). It is also possible that *P. flavicornis* are better at initially colonizing trees and that the more aggressive *P. spinicola* displace the less aggressive ants afterwards. But, *P. flavicornis* were not found to be present in smaller, and presumably younger, trees in our study. Alternatively, increased base clearing in *P. spinicola* inhabited trees and increased thorn density in *P. flavicornis* inhabited trees may result in similar overall fitness in Acacias. This would result in constant proportions of trees inhabited by each ant species over time.

Trees with *P. flavicornis* were significantly taller than trees with *P. spinicola*. Thus, it is possible that there may be more herbivory than competition, which would cause base clearing to be less important in Palo Verde than in other environments. The reduced herbivory due to greater thorn density on trees inhabited by *P. flavicornis* could explain the significantly taller trees inhabited by *P. flavicornis*. However, we only sampled trees between 0.91 m and 2.1 m in this study, and our resulting trees sampled may not be representative of Acacias in general.

To test the generality of the proportion of trees of each species observed in our study area, we could examine the independently developed ant and Acacia relationship in Africa. If there were only one species of ant in African systems,

it is possible that trees with more aggressive ants had better fitness and were able to outcompete other species of ants. This would support the idea that the more aggressive species of ant in Palo Verde have higher fitness. If this were the case, then over time it is possible that *P. spinicola* will outcompete *P. flavicornis*, driving them to extinction. However, if there are multiple species of ants in African systems, there may be a generalized trend across systems that there are differing but stable proportions of trees hosting each species.

This study furthers our understanding of the conditional interaction between Acacia trees and *Pseudomyrmex* spp. and suggests that mutualistic relationships are more complicated than they appear. As future studies continue to investigate these interactions, we continue to refine our definition of mutualism. By analyzing such complex relationships, we can establish a framework with which to better understand symbiotic interactions and the cost-benefit analyses of the species involved.

ACKNOWLEDGMENTS

Thank you to Matt, Clare and Melissa for their contributions in the design, analysis and writing of this project. Thank you to all of the staff at Palo Verde, especially José. A special thank you to Arthur Golden.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this project.

LITERATURE CITED

- Campbell, H. and Cook, J. 2013. Arboreal thorn-dwelling ants coexisting on the savannah ant-plant, *Vachellia erioloba*, use domatia morphology to select nest sites. *Insectes Sociaux*, 60(3): 373-382.
- Coley, P. D. and Barone, J. A. 1996. Herbivory and Plant Defenses in Tropical Forests. *Annual Review of Ecology and Systematics*, 27(1): 305-355.
- Hansell, M. 2000. Bird Nests and Construction Behavior. Cambridge University Press.
- Hay, M. and Fenical, W. 1988. Marine plant-herbivore interactions: The ecology of chemical defense. *Annual Review of Ecological Systematics*, 19: 111-145.

- Janzen, D. 1966. Coevolution of Mutualism Between Ants and Acacias in Central America. *Evolution*, 20(3): 249-275.
- Levin, D. A. 1973. The role of trichomes in plant defense. *Quarterly Review of Biology*, 48: 3-15.
- Malishev, M. and Sanson, G. 2015. Leach mechanics and herbivory defense: How tough tissue along the leaf body deters growing insect herbivores. *Ecological Society of Australia*, 40(3).
- New, T. 2017. *Mutualisms and Insect Conservation*. Springer International Publishing.
- Rosenthal, G. and Berenbaum, M. 1992. Herbivores: their interactions with secondary plant metabolites. *Physiological Entomology*, 17(3).
- Skelton, J., Doak, S., Leonard, M. Creed, R.P., and Brown, B.L. The rules for symbiont community assembly change along a mutualism–parasitism continuum. *Journal of Animal Ecology* 83(3).

APPENDIX

Table 1. Correlations among tree attributes for 24 Acacia trees.

Field	Tree height	Live branches/ total branches	Leaves per branch	EFNs per branch	EFNs per leaf	Thorns per meter
Height	1					
Live branches/ total branches	-0.45	1				
Leaves per branch	0.2	0.16	1			
EFNs per branch	0.31	0.05	0.85	1		
EFNs per leaf	0.42	0.08	0.18	0.48	1	
Thorns per meter	0.16	0.12	0.51	0.41	0.12	1

LA INTERACCIÓN CONDICIONAL: LA PLASTICIDAD DE LA INVERSIÓN ENTRE LOS ARBOLES ACACIA Y *PSEUDOMYRMEX* SPP.

NATHAN GIFFARD, SARAH L. JENNEWEIN, ASHLYN J. MORRIS, BENJAMIN J ZDASIUK

Editoras asistentes: Melissa DeSiervo, Clare Doherty

Editor: Matthew Ayres

“Supongamos que tú y yo estábamos sentados en una habitación tranquila con vistas al jardín [de acacia], charlando y bebiendo nuestras tazas de té verde mientras hablábamos de algo que sucedió hace mucho tiempo, y les dije: “Esa tarde cuando [medí esos 24 árboles]... fue la mejor tarde de mi vida, y también la peor tarde”

-Arthur Golden

Las altas tasas de herbívoro en los bosques neotropicales secos incitan a muchas especies neotropicales a invertir recursos en defensas contra los herbívoros. Un ejemplo de las plantas que invierten en defensa es la relación mutualista entre *Vachellia* (*Acacia*) *collinsii* (en adelante acacia) y las siguientes especies de hormigas: *Pseudomyrmex spinicola* y *Pseudomyrmex flavicornis*. Mientras que el árbol proporciona azúcar excretada de nectarios extra florales (NEF) y refugios de hormigas residentes a través domacios (espinas), las hormigas proporcionan defensa física contra los herbívoros y limpian la base del árbol de la competencia. Para cuantificar el mutualismo entre Acacias y *Pseudomyrmex* spp., medimos la inversión en hormigas y plantas a través de métricas de actividad de defensa de hormigas e inversión en hábitats de árboles en 24 árboles de Acacia en el Parque Nacional Palo Verde en Costa Rica. Dadas las mayores demandas energéticas de la *P. spinicola* más activa, podría existir una relación entre el tipo de hormiga y la densidad de espinas y NEF. Dado que *P. spinicola* también son más agresivos, también podría haber una relación entre el tipo de hormiga y la salud de los árboles, la cantidad de limpieza de la base y la actividad de respuesta herbívoro. Por el contrario, la densidad de espinas, los NEF. medios por hoja, la cantidad de limpieza de la base y los niveles de actividad de defensa pueden ser completamente aleatorios, lo que demostraría que las Acacias con ciertas morfologías no tienen una mayor aptitud con ciertos habitantes de especies de hormigas. Los árboles habitados por *P. flavicornis* contenían una mayor densidad de espinas y NEF. por hoja que los árboles habitados por *P. spinicola*. Además, determinamos que *P. spinicola* despejó la base de sus árboles anfitriones significativamente más que *P. flavicornis*. La salud de los árboles no varió según las especies de hormigas, pero encontramos significativamente más árboles con *P. spinicola* en nuestra área de muestra. Estos resultados ponen de relieve la plasticidad de la inversión en el mutualismo entre Acacias y *Pseudomyrmex* spp. basado en especies de hormigas, lo que sugiere que esta relación es una interacción condicional. Además, estos resultados demuestran que diferentes especies de hormigas pueden colonizar árboles con distintos niveles de inversión en defensa. Las interacciones condicionales entre organismos como Acacias y *Pseudomyrmex* spp. son complejas, y este trabajo tiene como objetivo entender mejor cómo la inversión en relaciones de defensa mutualistas por cada especie puede afectar el herbívoro, la competencia y la aptitud general de un individuo.

Palabras claves: mutualismo, Acacia, hormiga, *P. flavicornis*, *P. spinicola*, nectarios extra florales, herbívoro, defensa, Palo Verde

METABOLIC RATE EFFECTS FORAGING BEHAVIOR IN NEOTROPICAL MARSH BIRDS

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TA Editors: Melissa DeSiervo and Clare Doherty
Faculty Editor: Matt Ayres

Abstract: Smaller endothermic animals have higher mass-specific metabolic rates, and thus must consume more energy for their body size. Selection has favored different means meeting energy needs. We compared foraging behavior of Northern Jacanas (*Jacana spinosae*) and Cattle Egrets (*Bubulcus ibis*) in Palo Verde National Park, Costa Rica. Since the Northern Jacana has a higher basal metabolic rate than the Cattle Egret due to its comparatively small size, it must compensate for this difference by consuming more food per unit body mass than the Cattle Egret. We hypothesized that Northern Jacana's compensatory foraging strategy would depend on the abundance of its prey, the Jacana exhibiting a heightened foraging time to forage for sparse prey or a heightened attack rate to forage for abundant prey when compared with Cattle Egrets. Northern Jacanas foraged constantly throughout the day, perhaps because of their need to meet high metabolic demands, while Cattle Egrets decrease activity midday. Northern Jacanas exhibited a higher attack rate compared to Cattle Egrets, but while Northern Jacanas decreased attack rates midday, the Cattle Egrets did not. This behavior by Cattle Egrets is consistent with the sparseness of its prey, its ability to sustain longer fasting periods, and its foraging strategy.

Key words: *Bubulcus ibis*, basal metabolic rate, foraging behavior, mass-specific metabolic rate, *Jacana spinosae*

INTRODUCTION

Endothermic homeotherms (chiefly birds and mammals) dedicate a high amount of energy to maintaining a stable internal body temperature, resulting in a higher basal metabolic rate (BMR) when compared with poikilotherms and endotherms. Small endotherms exhibit proportionally higher BMRs ($\text{J}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$) than large endotherms (Dodds 2001, West 1997, Klieber 1947), including that smaller endotherms must also have proportionally higher resource demands. Different species manage mass dependent resource demands using a variety of tactics, but all must meet their energy demands to survive.

The effect of size on metabolic rate puts evolutionary selection pressure on energy acquisition behaviors in all endothermic homeotherms. For birds, the primary behaviors subject to metabolism-dependent selective pressure are foraging technique, intradiurnal foraging modulation, and prey selection. Foraging behavior may also vary with diet, competitors, predators, and abiotic environmental factors. The prey of different bird species may vary in size, caloric density, dispersal, movement, and abundance, any of which could influence the spatiotemporal

concentration of an endotherm's foraging efforts. Presence of competitors or predators may encourage birds to relocate or increase vigilance. Abiotic environmental factors such as air temperature, solar radiation, and wind speed may alter a bird's ability to forage or the energy it must allocate to temperature regulation. These factors may also affect its prey, predators, or competitors. Finally, birds may be more motivated to forage at certain times of day, perhaps following a night without food, or in preparation for the next night (Grue et. al. 1981). Any of these factors may interact with size and metabolic rate to influence the pressures favoring one diurnal foraging strategy over another.

Wetland birds of Guanacaste, Costa Rica provide a model to study foraging behavior. The Northern Jacana (*Jacana spinosae*) and Cattle Egret (*Bubulcus ibis*), both feed on wetland insects (Stiles and Skutch 1989) but forage within different distinct habitats (Authors' observation 2020). Northern Jacanas and Cattle Egrets are morphologically similar, but Cattle Egrets have twice the mass of Northern Jacanas, implying that Northern Jacanas have a higher mass specific BMR, but lower energy demands per bird per day (Jenni and Collier 1972, Cornell

Lab of Ornithology 2020). To account for its higher BMR, the Northern Jacana must consume more food per gram body mass than the Cattle Egret, though the Cattle Egret must consume more food in total. The tactics used by Northern Jacana to compensate for its higher mass-specific metabolism are inadequately understood.

To compensate for their high mass-dependent metabolism compared with Cattle Egrets, Northern Jacanas must: 1) spend a greater portion of the day foraging, 2) have a higher attack rate, or 3) target proportionally larger prey. Given that Northern Jacanas have a smaller gape size than Cattle Egrets, we discounted the possibility that Northern Jacanas target proportionally larger prey. Assuming a similar caloric density for prey across preferred foraging territories, we predicted that prey invertebrates in Northern Jacana-preferred foraging habitat will be smaller or equal in size to those of the Cattle Egret. We investigated how prey abundance or sparseness in the Northern Jacana and Cattle Egret foraging habitats may favor one tactic over another.

We hypothesized two possible syndromes of compensatory energy acquisition behaviors of the Northern Jacana. First, if the Northern Jacana's preferred foraging habitat has a higher prey abundance than that of the Cattle Egret, the two species could spend the same amount of time foraging but the Northern Jacana would have a higher attack frequency when foraging (Fig. 1a). Alternatively, if the prey abundances in both preferred foraging habitats were similar, the Northern Jacana would spend more time foraging than the Cattle Egret, but the two species would not differ in their attack rates when foraging (Fig. 1b).

METHODS

To quantify bird foraging behaviors and prey dynamics, we conducted two observational studies at the wetland boardwalk in Palo Verde National Park, Costa Rica. We quantified foraging metrics of Northern Jacanas and Cattle Egrets in bouts from sunrise to sunset. We also surveyed dispersion, average mass, and abundance of insect prey in the preferred foraging habitat of each species.

Bird Surveying Methods

To quantify the proportion of foraging individuals, we conducted point counts of Northern Jacanas and Cattle Egrets within a 160-m radius from the boardwalk in ten-minute intervals. In one-point count, we observed the number of each species present and the proportion that were foraging. Point count methodology was derived from Hutto et al. (1986).

To measure the frequency of prey consumption while foraging, we conducted focal studies of Northern Jacana and Cattle Egret individuals. We randomly selected one Northern Jacana and one Cattle Egret and counted the number of attacks performed by each within 5 minutes. Focal counts were performed at ten-minute intervals.

To evaluate the potential effect of abiotic factors on bird foraging behavior, we retrieved air temperature, wind speed and solar radiance data from the Palo Verde weather station.

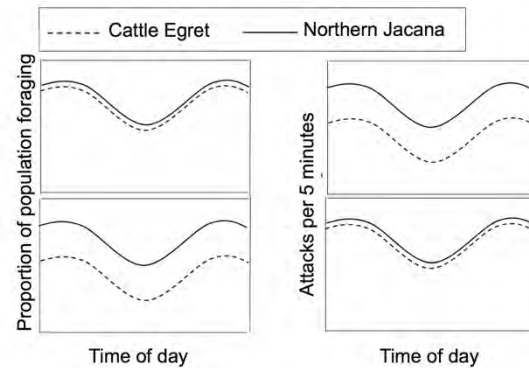


Figure 1: The predicted diurnal foraging habits for the Cattle Egret (dotted line) and Northern Jacana (solid line). The panels on the left show predicted proportion of the population foraging as it relates to the time of day. The panels on the right show predicted number of attacks. The top panels illustrate the hypothesis that both species will spend the same amount of time foraging but the Northern Jacanas will have a higher attack rate than Cattle Egrets. The bottom panels illustrate the hypothesis that Northern Jacanas will spend more time foraging but have the same attack rate as Cattle Egrets.

Prey Sampling Methods

To quantify the abundance and average size of Northern Jacana and Cattle Egret prey, we established six plots (15x20cm) in the preferred habitat of each species. Cattle Egrets preferred

areas with tall graminoids, while Northern Jacanas preferred floating water fern patches (Authors' observations 2020). Within each plot, we searched by hand and eye and counted the total number of invertebrates >1mm in length. To calculate the abundance of invertebrates per plot, we divided the total number of invertebrates >1mm by the plot area. We pooled the invertebrates from each plot and measured fresh mass to the nearest milligram. We then divided this mass by the number of the invertebrates massed.

Statistical Methods

To compare the attack rates between Northern Jacanas and Cattle Egrets, we conducted a paired t-test on the attacks per 5-minute focal observation between the two species. We estimated the proportion of time spent foraging by comparing the slopes of regressions forced through the origin of number of foraging birds for each species vs. total number of birds for each species.

To test the influence air temperature, wind speed and solar radiance had on foraging behavior, we ran a linear regression between each species' attack frequency against all three abiotic factors.

To compare the mean insect masses between the two foraging habitats, we compared log

transformed mean total insect masses per plot with a t-test. To compare the insect abundances between the foraging habitats, we conducted a t-test with log transformed abundances. To measure insect dispersion between foraging habitats, we qualitatively compared the standard deviations of the insect abundances for the respective foraging habitats.

All statistical analyses were performed using JMP 14.0.

RESULTS

Northern Jacanas had a higher attack rate than Cattle Egrets (Paired t-test: $t = 7.62$, $p < 0.0001$, $df = 48$, Fig. 3). Northern Jacanas decreased their attack rates midday while Cattle Egrets had a consistent attack rate throughout the day (Fig. 2). Northern Jacanas spent a greater proportion of their time foraging (0.98 ± 0.001) than Cattle Egrets (0.935 ± 0.013). Cattle Egrets appeared to decrease the time spent foraging in the middle of the day while Northern Jacanas foraged consistently (Fig. 2). Neither Cattle Egrets nor Northern Jacanas were ever observed foraging before sunrise or after sunset.

The average insect size between foraging habitats were not different (t-test: $t = 1.52$, $p = 0.18$, $df = 5.47$, Fig. 4). The abundance of prey in the preferred foraging habitat of the Northern Jacana was higher than in that of the Cattle Egret

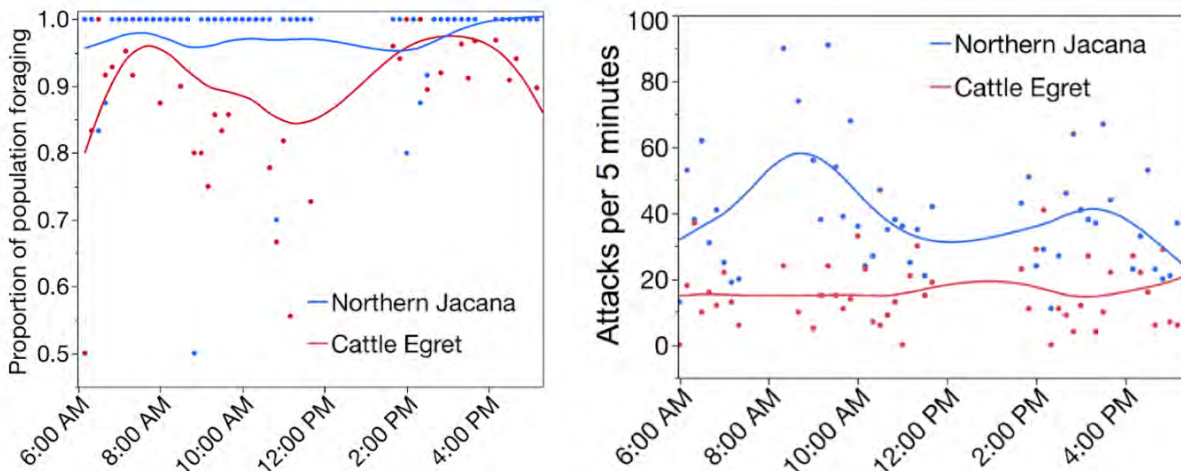


Figure 2: Left: The proportion of population foraging (y-axis). Right: the number of attacks per 5-minute observation vs time of day. The observations are separated by the species; Northern Jacana (Blue) and Cattle Egret (Red). Lines are constructed in JMP Graph Builder under the smoother function with a lambda of 0.155.

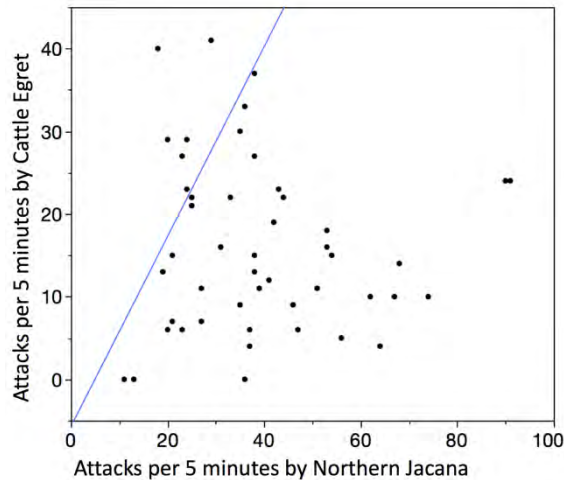


Figure 3: The number of attacks per 5-min. observation of the Northern Jacana (x-axis) against the number of attacks per 5-minute observation by the Cattle Egret (y-axis). Each point represents two simultaneous observation periods. The line of equality is indicated in blue.

(t-test: $t = 5.58$, $p = 0.0002$, $df = 9.91$, Fig. 4). The prey observed in the water fern habitat were several species of water beetle, small fish, leeches, unidentified insect nymphs, and mosquito larvae, while graminoid habitat contained leafhoppers, small grasshoppers, spiders, caterpillars, and flies. The majority of these were flightless and presumably remained in the same habitats.

There was no relationship between Northern Jacana attack frequency and air temperature, wind speed nor solar radiance ($p > 0.50$). There was also no relationship between Cattle Egret attack frequency and air temperature, wind speed, nor solar radiance ($p > 0.30$). We did not observe any influence of predator presence on Cattle Egret or Northern Jacana behavior.

DISCUSSION

Northern Jacanas foraged more often and with a higher attack frequency throughout the day than Cattle Egrets. The degree of heightened foraging behavior indicates that more time *and* intensity of foraging are required for a Northern Jacana to compensate for its high metabolic demands. The magnitude of this difference in both time and intensity conflates both predictions of increased Northern Jacana foraging. It also appears, however, that both Northern Jacanas and Cattle

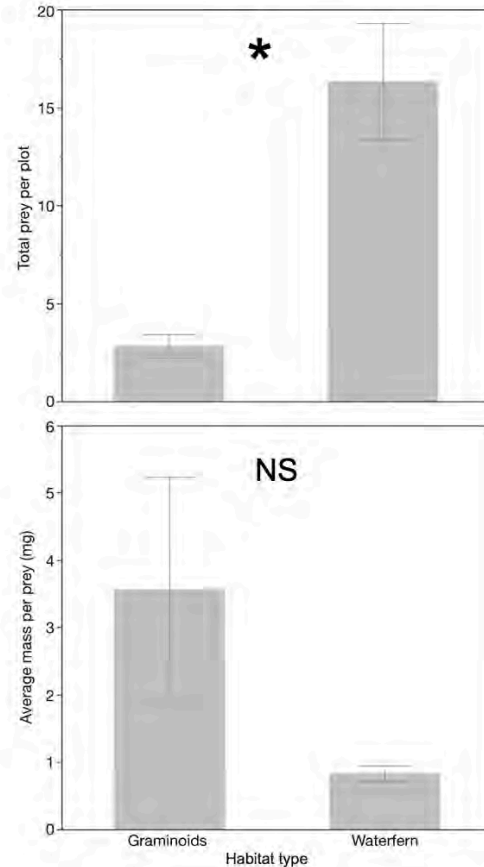


Figure 4: The mean number of insects per plot (15x20 cm) (top) and average mass per prey item (bottom) separated by habitat type; graminoids, preferred by Cattle Egrets, and water fern, preferred by Northern Jacanas. Mean \pm SE.

Egrets are actively foraging throughout a vast majority of their waking hours. Accordingly, the Northern Jacana's higher intensity of foraging compared with that of the Cattle Egret perhaps represents the Northern Jacana's high metabolic demands more directly than its heightened foraging time. However, pairing of this higher foraging time with higher intensity exposes the extent of food intake necessary for Jacanas, and the syndrome of foraging behaviors accompanying that need.

Unexpectedly, Northern Jacanas and Cattle Egrets demonstrated different patterns of intradiurnal cycling in their respective foraging times and attack frequencies. Northern Jacanas appeared to maximize foraging time constantly, while Cattle Egret modulated foraging time as the day proceeded, with peaks in the morning and evening. This may be a product of the Northern Jacana's high BMR; it must constantly

forage to survive, while the larger Cattle Egret can afford to decrease foraging and fast between morning and evening feeding. However, the Northern Jacana's attack frequency decreased midday, while the Cattle Egret maintained a consistent attack frequency throughout the day, perhaps because Cattle Egret prey were less abundant than Northern Jacana prey. Apparently, the foraging strategies differ for Cattle Egrets and Northern Jacanas.

The Cattle Egret always had a lower attack frequency than that of the Northern Jacana. The high frequency of Northern Jacana's attacks in the morning and evening may indicate that Northern Jacanas forage heavily in the morning for their abundant prey after a metabolically costly night but can then afford to decrease intensity of foraging after satiation. Success rates of attack were not possible to determine for all birds studied, as successfully caught invertebrates were visually undetectable. If these success rates are substantially unequal, our results regarding heightened Jacana attack frequency may not accurately indicate higher prey intake. Difference in prey size could also affect the relationship between Jacana foraging intensity and prey abundance. Cattle Egret prey tended to be larger than Northern Jacana prey, though this result was statistically insignificant. If this trend held true given a larger sample size, and Jacanas and Egrets hunt prey proportional to their own body masses, an effect of this magnitude would reinforce the supposition that Jacanas vary high-intensity and low-intensity foraging periods directly due to their prey's vast abundance.

Despite the Jacana's ability to modulate food intake by varying intensity, the majority of Jacanas were foraging at any given time of day, apparently because they must still satisfy their high metabolic demands consistently. Meanwhile, Cattle Egrets hunt less abundant prey, and maintained a slower but more constant attack frequency throughout the day. Perhaps Cattle Egrets can afford this strategy because their prey are larger, and their low BMR allows them to fast for longer than Jacanas are able.

It appears that the higher foraging time and attack rate observed in the Northern Jacana compared to the Cattle Egret, along with the Northern Jacana's lack of diurnal modulation of

foraging time, are all consistent with the requirements of the Jacana's high mass-specific metabolism. In contrast, the Cattle Egret's lack of diurnal modulation of attack frequency seems to relate to the sparseness of its prey and the deliberate foraging tactic it employs. We had expected heightened foraging near dawn and dusk but did not find this to be true. Perhaps Jacanas forage constantly throughout the day not only because of their high BMR, but also simply because they *can*. Future research could improve present models of foraging behavior by better accounting for BMR, foraging strategies, and prey characteristics.

ACKNOWLEDGEMENTS

A huge thank you to Jose at Palo Verde OTS who answered questions about long term observations of bird behavior. Thank you to Matt and the TAs for investing many hours not only in improving this paper but in encouraging us to be better science writers and ecologists.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this study.

LITERATURE CITED

- Dodds, P.S., Rothman, D.H. & Weitz, J.S. 2001. Re-examination of the '3/4-law' of metabolism. *Journal of Theoretical Biology* 209, 9–27.
- West GB, Brown JH, Enquist BJ 1997. "A general model for the origin of allometric scaling laws in biology". *Science*. 276 (5309): 122–6.
- Hutto, R. L., S. M. Pletschet, and P. Hendricks. 1986. A Fixed-Radius Point Count Method for Nonbreeding and Breeding Season Use. *The Auk* 103: 593-602.
- Stiles, F. G., & Skutch, A. F. 1989. Guide to the birds of Costa Rica. Comistock.
- Skead, C. J. 1966. A study of the Cattle Egret, *Ardeola ibis*, Linnaeus. *Ostrich*, 37(sup1), 109-139.
- Grue E. C. 1981. "Diurnal Activity Patterns and Population Estimates of Breeding Birds Within a Distribution and Undisturbed Desert-scrub Community". *Studies in Avian Biology* No. 6: 287-291
- Jenni A. Donald and Collier, Gerald. 1972. Polyandry in the American Jacana (*Jacana Spinosa*). *The Auk* 89: 743-765.
- Cornell Lab of Ornithology. 2020. "All About Birds." Allaboutbirds.org.

EFFECTOS METABÓLICOS DE LA TASA DE COMPORTAMIENTO EN AVES DE PANTANO NEOTROPICALES

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Editoras asistentes: Melissa DeSiervo and Clare Doherty
Editor: Matt Ayres

Abstracto: Los animales endotérmicos más pequeños tienen tasas metabólicas específicas de la masa más altas, y por lo tanto deben consumir más energía para su tamaño corporal. La selección ha favorecido diferentes medios para satisfacer las necesidades energéticas. Comparamos el comportamiento de forrajeo de Jacanas del Norte (*Jacana spinosae*) y las Garcetas Bovinas (*Bubulcus ibis*) en el Parque Nacional Palo Verde, Costa Rica. Dado que el Jacana septentrional tiene una tasa metabólica basal más alta que la Garceta Bovina debido a su tamaño comparativamente pequeño, debe compensar esta diferencia consumiendo más alimentos por unidad de masa corporal que la Garceta Bovina. Hemos planteado la hipótesis de que la estrategia de forrajeo compensatorio del norte de Jacana dependería de la abundancia de su presa, la Jacana exhibiera un mayor tiempo de forrajeo para la escasa presa o una mayor tasa de ataque a forraje para presas abundantes en comparación con la Garceta Bovina. El norte de Jacanas se alimentaba constantemente durante todo el día, tal vez debido a su necesidad de satisfacer las altas demandas metabólicas, mientras que las Garcetas Bovinas disminuyen la actividad al mediodía. Las Jacanas del Norte mostraron una mayor tasa de ataque en comparación con las Garcetas Bovinas, pero mientras que Jacanas del Norte disminuyó las tasas de ataque al mediodía, las Garcetas Bovinas no. Este comportamiento de garza bovina es consistente con la escasez de su presa, su capacidad para sostener períodos de ayuno más largos y su estrategia de forrajeo.

Palabras claves: *Bubulcus ibis*, tasa metabólica basal, comportamiento de forrajeo, tasa metabólica específica de la masa, *Jacana spinosae*



CAMPANERIO





RELATIVE BOLDNESS IN TERRESTRIAL HERMIT CRABS ACROSS SOCIAL SETTINGS

ROBERT N. ALTER, NATHAN G. GIFFARD, SARAH L. JENNEWEIN, ASHLYN J. MORRIS

TA Editors: Melissa DeSiervo, Clare Doherty
Faculty Editor: Matt Ayres

Abstract: There are benefits and drawbacks to being bold in the animal kingdom. Further, whether an individual is alone or in a group can affect the boldness it displays. It has been reported that hermit crab's boldness varies among individuals. However, the complexities of this behavioral trait are not well understood. We examined the boldness of *Coenobita compressus* in the lab and on the beach near Campanario Biological Reserve in the Osa Peninsula of Costa Rica. We flipped hermit crabs onto the back of their shell and measured their latency time to flip back over, interpreting shorter latency time as a proxy for increased boldness. We verified that boldness was repeatable for individuals and found that boldness was independent of crab size, fit within its shell, sex, and whether females were gravid. We assessed boldness in solitary and group settings in the lab and examined whether levels of boldness were retained in groups of different sizes on the beach. Boldness could be a static trait in hermit crabs that is retained in solitary and group settings or may be a plastic trait that changes with contexts, e.g. when alone or in groups. We found that boldness increased in a group setting in the lab, possibly due to increased eviction risk when an individual is on its back, but that relative boldness levels were stable for individuals. Hermit crabs did not significantly alter their boldness to match that of others in their group. On the beach, hermit crab group size did not affect average group response time, perhaps due to an inability of individuals to perceive the size of the group as trials were done at night. Our findings suggest the existence of personalities in hermit crabs, but further research is necessary to generalize from the single behavior studied here.

Key Words: hermit crab, boldness, animal behavior, group dynamics, personality, Campanario

INTRODUCTION

Boldness can be advantageous to animals in certain situations. To impress females and to increase their chances of mating, solitary male sticklebacks must be bold enough to get close to predators. Similarly, bold male howler monkeys that engage in inter-troop confrontations and win are more likely to move up in the dominance hierarchies of their own troops and gain access to mates. However, in other situations, being bold can be detrimental to the survival of an individual. If a stickleback gets too close to a predator, for example, he may be consumed. Within the losing group of an inter-troop howler monkey confrontation, the less bold monkeys that did not engage in the confrontation are more likely to survive unharmed.

Whether an individual is alone or in a group can also have an effect on the level of boldness that it displays. In large groups, male sticklebacks may display higher levels of boldness due to the higher levels of competition from other males also trying to impress females. In addition, the males may be emboldened by decreased risks of predation due to predator swamping. In other cases, animals may avoid

engaging in bold behavior in groups as it can increase the risk of conflict with other conspecifics or heterospecifics.

The genetic underpinnings of boldness have been found to vary among species. The behavioral trait may be heritable and linked to a certain gene, as in zebrafish (Norton et al. 2011), or it may be plastic and influenced by the aggregation of life experiences of an individual, as in dumpling squid (Sinn, D.L. et al. 2008). It has been reported that hermit crabs in many populations display varying levels of boldness and that the boldness of individual hermit crabs holds across time (Mowles et al. 2012). However, the plasticity of boldness in hermit crabs between solitary and group settings is not well understood.

We investigated the boldness of hermit crabs in a population of *Coenobita compressus* at Campanario Biological Reserve on the Osa Peninsula of Costa Rica. We also investigated the consistency of boldness (time to right themselves when crabs are flipped onto their backs) in solitary and group settings, and among groups of varying sizes. There is fierce competition among hermit crabs to be in

increasingly large shells, and individuals are at higher risk of losing their shells to conspecifics when flipped on their backs. Therefore, a difference in the boldness of hermit crabs might be observed between solitary crabs and those in groups. Alternatively, boldness might not be a plastic trait in which case no relationship would be expected between boldness and social context. Additionally, if group size isn't perceived by an individual, then no relationship will be observed between boldness and the size of a group.

If boldness is a static behavior in hermit crabs, then the time it takes a hermit crab to right itself after being flipped over will not differ significantly between group and solitary settings. In this case, it is also possible that the time it takes a hermit crab to right itself will be consistent in groups of varying sizes. Alternatively, if boldness differs by context, individuals may right themselves more quickly in groups to avoid eviction by other hermit crabs while still retaining their relative levels of boldness when compared to each other. Conversely, instead of retaining relative boldness levels, individuals may conform to the average boldness of the group. If this is the case, individual hermit crab may right themselves when other members do so. It could also be in that hermit crabs in larger groups right themselves more quickly due to greater eviction risk when more conspecifics present.

The findings of this study may have broader implications in the field of animal behavior. For many animals, there are some environments in which being bolder might increase fitness and others where being bolder might decrease fitness. Boldness may be an asset in contexts where there are few predators or where boldness is impressive to females. In other contexts, shyness may result in greater fitness by allowing individuals to avoid predation or conflict with other group members. It is therefore possible that increasing polarization in how shy or bold individuals are will result within species over time. By understanding the impact of group dynamics on hermit crab behavior, we can better understand the population biology of social animals.

METHODS

Experiment 1: verification of a boldness spectrum

To assess the boldness of individual hermit crabs, we flipped them onto the back of its shell and measured latency time to flip back over so that their legs were in contact with the ground. To test if boldness was repeatable, we repeated this measure once every hour for three hours ($N = 19$ crabs). Before determining the boldness of an individual, we placed it in the bucket where the assessment would take place and allowed it to habituate to its environment for three minutes.

We determined if boldness of individual hermit crabs related to the size of the crab, its fit within its shell, its sex, and, if female, whether it was gravid (i.e., carrying eggs). To do this, we first ensured the existence of a relationship between claw width, mass, and thorax length of hermit crabs ($N = 25$) (see supplemental figure 1A). All measurements were taken using calipers and a hanging Newton scale. Next, we labeled the shells of 60 individuals, measured the claw size and shell aperture of the individuals (see supplemental figure 1B), and determined sex. To assess the fit of a crab to its shell, we divided each individual's shell aperture by their claw width. We then determined the boldness of these individuals when they were alone.

Experiment 2: effect of group on boldness

To determine the effect that being in a group had on the boldness of hermit crabs, we measured the latency time to flip back over when in groups of four using the crabs from Experiment 1 ($N = 60$). Before determining the boldness of a group, we placed all four in the same bucket where the assessment would take place and habituated them for three minutes. The order of individual measure or group measure as the primary treatment was randomized.

Experiment 3: effect of group size on boldness

We tested for a relationship between the size of a group of hermit crabs and the boldness of its members. We walked randomly chosen transects along the beach at El Campanario in the evening and found naturally occurring groups of hermit crabs that contained between two and seven members. We immediately flipped all members of a group onto their back in unison and

measured the latency of each crab to flip itself back over ($N = 32$).

Statistical Analyses

All hermit crab response times were transformed with natural logarithms to improve normality. We assessed the repeatability of latency to flip with an ANOVA model that included trial ($N = 3$) as a fixed effect and crab as a random effect ($N = 19$). We assessed effects on boldness of sex and gravidity using student's *t*-tests. We assessed effects on boldness of shell fit and crab size with linear regressions.

We evaluated patterns in latency to flip, both individually and by group, with an ANOVA that grouped-or-solo as a fixed effect, group ($N = 15$) as a random effect, grouped-or-solo \times group as a random effect, and crab nested within the group as a random effect.

To assess alterations in an individual's boldness to match others in a group, we compared variance in each group's response times when alone to the variance in response times in a group setting with a paired *t*-test. To assess the effects on boldness of group size, we used a linear regression.

RESULTS

The boldness of individual hermit crabs, as measured by the time it took an individual to right itself after being flipped over, was found to hold across repeated trials ($r = 0.95$, -0.98 among trial pairs; Fig. 1). Within these trials, individual crabs accounted for 84% percent of the random variation ($p < 0.01$) while the effect of trial was not significant (ANOVA: $F = 1.33$, $df = 2,36$, $p = 0.28$).

Hermit crab claw size was well correlated with mass ($r = 0.55$, $p < 0.0047$; Fig. 2). Neither fit to their shell, claw size, sex, or gravidity were related to the boldness of individual hermit crabs ($p > 0.01$). Of the 60 crabs, we found 23 gravid females, 15 females that were not gravid, and 15 males. Seven crabs were not sexed due to inability to remove them from their shells.

The latency to flip was an average of 16.3 s shorter when crabs were in groups than when they were alone ($F = 13.72$, $df = 1,14$, $p = 0.0024$; Fig. 3). There was modest variation among groups (18.7 percent of the total random variance, $p = 0.21$; Fig. 3). There was no

evidence for an interaction between grouped-or-solo \times group (6.9 percent of the total random variance, $p = 0.17$; Fig. 3). There was high variance among crabs within groups (51 percent of the total random variance, $p = 0.0002$; Figure 3).

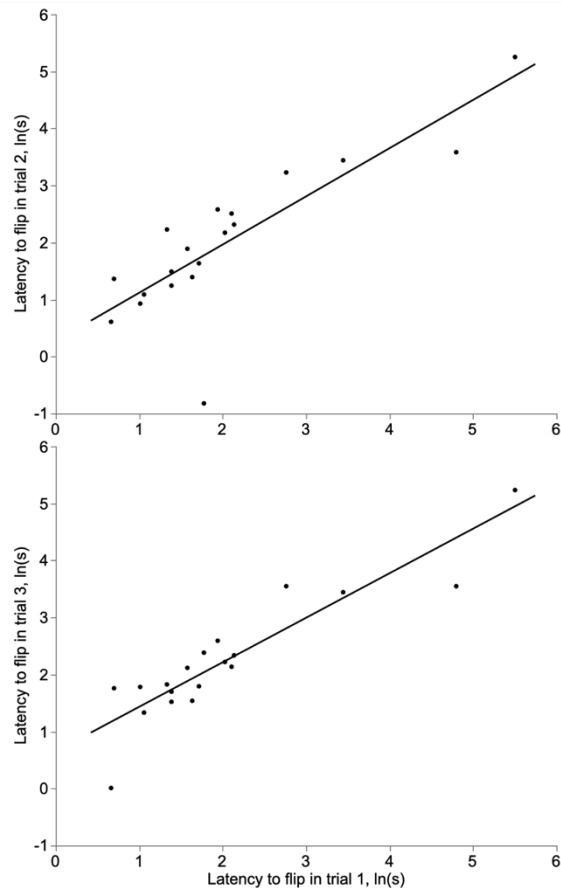


Figure 1. The natural logarithm of the latency to flip of each individual hermit crab in Trial 2 vs. the natural logarithm of the latency to flip of each individual hermit crab in Trial 1 (top panel). $R = 0.95$. The natural logarithm of the latency to flip of each individual hermit crab in Trial 3 vs. the natural logarithm of the latency to flip of each individual hermit crab in Trial 1 (bottom panel). $R = 0.94$. Based on lab trials.

There was a tendency for individuals to alter their latency time to match the group, however this was not statistically significant (Paired *t*-test: $t = 1.90$, $df = 14$, $p = 0.078$; Fig. 4).

On the beach, average response time remained constant at about 11 seconds irrespective of group size (Linear regression: $F = 0.29$, $df = 1,138$, $p = 0.59$; Fig. 5).

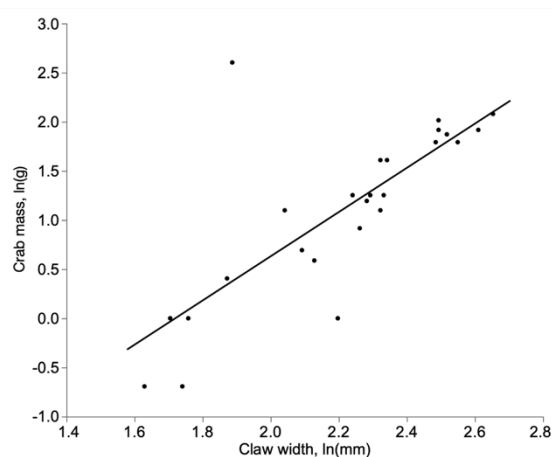


Figure 2. The natural logarithm of the mass of individual hermit crabs (g) vs. the natural logarithm of the length of their claws (mm). Based on lab trials. $r = 0.55$

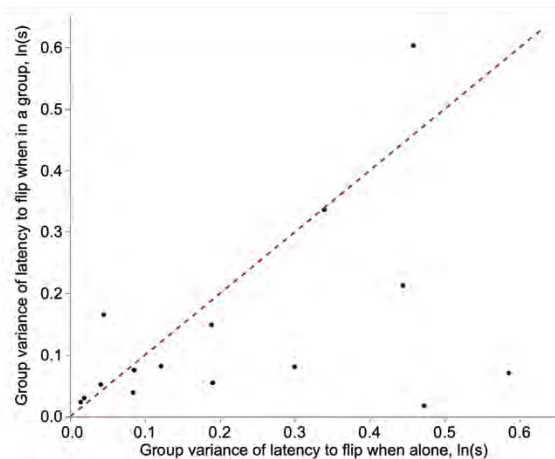


Figure 4. The variance among the response times of the same four individuals when in a group setting vs. when alone. The dashed line represents a 1:1 relationship. Based on lab trials.

DISCUSSION

We found that boldness was an inherent behavior of individual hermit crabs that was quite stable over time. However, while hermit crabs retained their relative level of boldness in groups, overall boldness levels increased in a group setting. While the effects of predator swamping may lead to greater security and therefore higher boldness levels in some crab populations, there are few natural predators that threaten the population of terrestrial hermit crabs

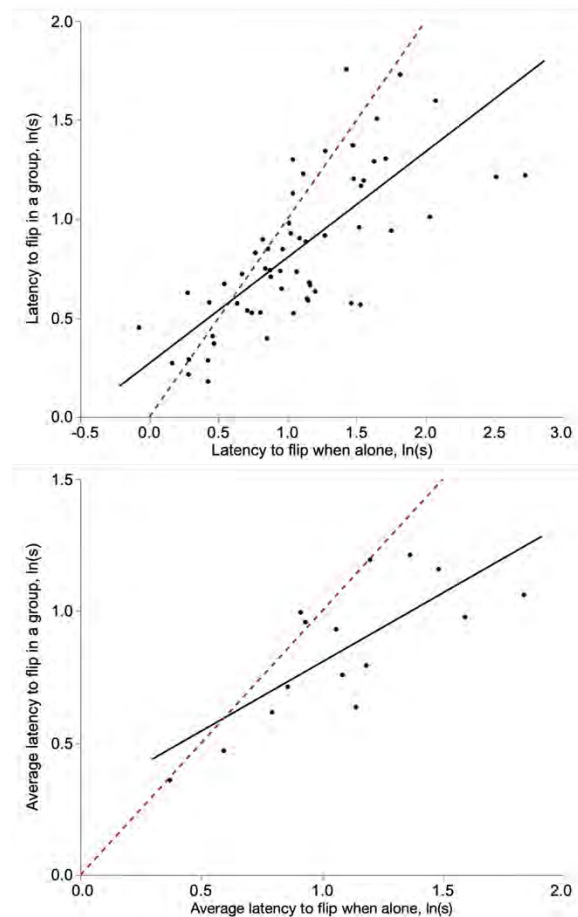


Figure 3. The natural logarithm of the latency to flip of hermit crabs when in groups of four vs. alone. Upper panel shows individual crabs. $r^2 = 0.53$. Lower panel shows group averages. In both panels, the dashed line represents a 1:1 relationship. $r^2 = 0.58$. Based on lab trials.

in our study area. As a result, it is unlikely that predator swamping plays a significant role in informing the behavior of this population. However, shell competition may play a key role in explaining the overall increased boldness observed in groups. Individuals may right themselves more quickly when in groups because they are more vulnerable to losing their shell to conspecifics when they are on their backs.

Boldness was not found to correlate with claw size, sex, fit to shell, and whether or not an individual female was gravid, suggesting that boldness is independent of these characteristics

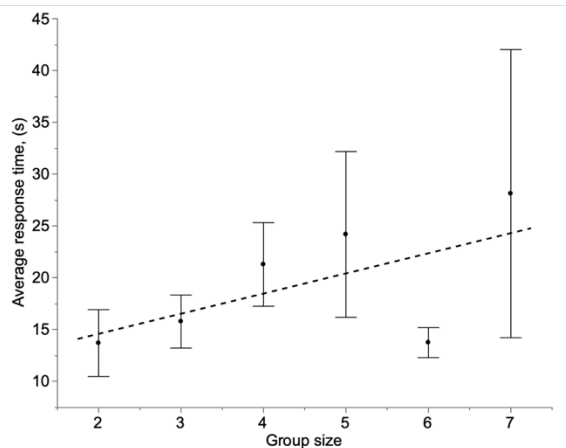


Figure 5. The average of the natural logarithms of the latencies to flip of each individual hermit crab vs. the size of the group. Based on beach trials.

that can change over an individual's lifespan. The finding that the boldness of individuals is unaffected by shell fit is especially interesting and supports the idea that boldness is an inherent behavior of an individual. Our finding that sex and gravidity did not play a role in determining the boldness of an individual is also notable because this study was performed on the full moon when gravid females lay their eggs (Skov et al. 2005). If gravidity caused females to act more conservatively and display lower levels of boldness to protect their eggs, then this effect would have been most pronounced on the full moon.

Boldness was also unaffected by the size of the group that the individual was in. While these findings do not invalidate our finding that group setting makes crabs bolder, a level of complexity is added to the relationship. Hypothetically, there is an increased threat of an individual losing their shell to a conspecific when they are in larger groups. However, our results suggest that hermit crabs don't perceive an increased risk of eviction with increased group size, as it didn't influence their flip time. Additionally, the hermit crabs we measured in the lab tended to be bolder than those measured on the beach. While groups of four hermit crabs had an average flip time of 12.0 s in the lab, hermit crabs on the beach had an average flip time of 20.4 s. A possible explanation for these findings is that groups of hermit crabs were found on the beach when it was dark and that individuals were not

able to perceive the size of the group effectively. Another possibility is that hermit crabs display different levels of boldness in natural settings and in the lab.

Within the field of animal behavior, an individual is determined to have a behavioral syndrome, or personality, when a behavioral trait is shown to hold over time when measured with different metrics (Dall et al. 2004). Our experiment contributes evidence for the existence of behavioral syndromes within hermit crabs. We determined that the boldness of an individual hermit crab holds across time and is not merely dependent on the immediate context preceding the assessment. Further, although hermit crabs generally become bolder when in a group setting, we also demonstrated that the relative level of boldness of individuals is retained between solitary and group settings. However, future studies of boldness within hermit crabs could look at boldness in other contexts, for example, when competing for resources. One could place two hermit crabs without shells in a bucket containing one available shell to determine which crab acquired the shell first and the time that it took the individual to do so. Bolder hermit crabs would be the first individuals to obtain the shell in the least time. One could then determine if there was a relationship between individual boldness as measured by both this new metric and that used in this experiment (latency to flip). If levels of boldness were similar across metrics, this would make a strong case for the existence of hermit crab personalities.

It is not currently understood whether or not the boldness displayed by individual hermit crabs is due to their genetics or due to the aggregation of the life experiences of an individual. However, recent studies suggest that the boldness and aggressiveness displayed by zebrafish have a genetic basis that can be experimentally manipulated by altering individuals' DNA (Norton et al. 2011). Although boldness has been shown to have a heritable component in tropical poeciliids as well, experimenters have been able to change boldness levels in individuals by repeatedly exposing them to aggravating stimuli over extended periods of time (Brown et al. 2007). This suggests that boldness levels in poeciliids

are not a static inherited trait but instead change based on the life experiences of an individual. Further experiments on hermit crabs are necessary to determine the relative importance of genetics and life experiences to the boldness displayed by individuals.

Our findings, which suggest relative boldness in hermit crabs holds between solitary and group settings, provide a framework for understanding the behavioral and social tendencies of other animals. In species where boldness is a behavior that doesn't differ by setting, it is possible that bolder individuals will have greater fitness in certain environments and therefore those populations will trend towards greater boldness overall. In animals where boldness is plastic in group settings, it is also likely that bold groups will be more successful in some environments and notably less successful in others. This may result in very bold populations in some places and very reserved populations in others. A greater understanding of the plasticity of behavioral traits such as boldness would allow us to better quantify and predict trends, such as polarization, in populations of social animals.

ACKNOWLEDGEMENTS

Thanks to Mark for getting us excited about Hermit crabs and Clare for supporting our passions.

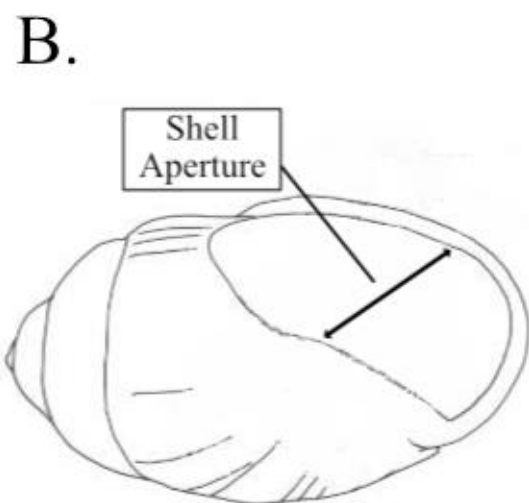
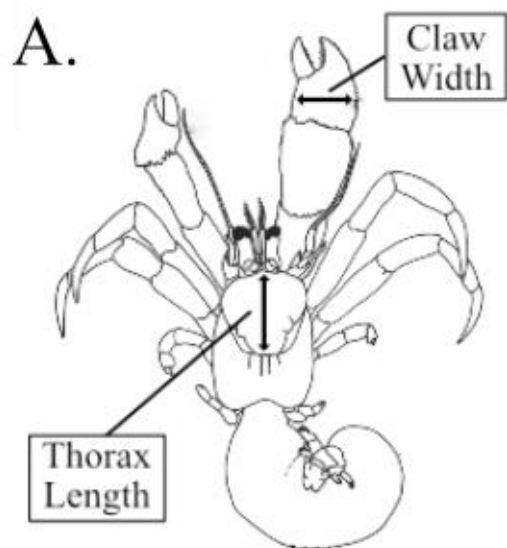
AUTHOR CONTRIBUTIONS

All authors contributed equally to this project.

LITERATURE CITED

- Brown, C., F. Burgess, and V. Braithwaite. 2007. Heritable and Experiential Effects of Boldness in a Tropical Poeciliid. *Behavioral Ecology and Sociobiology* 62: 237-243.
- Dall, S. R. X., A. I. Houston, and J. M. McNamara. 2004. The behavioural ecology of personality: consistent individual differences from an adaptive perspective. *Ecology Letters* 7(8): 734-739.
- Jolles, J. W., B. A. Taylor, and A. Manica. 2016. Recent social conditions affect boldness repeatability in individual sticklebacks. *Animal Behaviour* 112: 139-145.
- Skov, M. W., R. G. Hartnoll, R. K. Ruw, J. P. Shunula, M. Vannini, and S. Cannici. 2005. Marching to a different drummer: crabs synchronize reproduction to a 14-month lunar-tidal cycle. *Ecology* 86(5) 1164-1171.
- Mowles, S. L., P. A. Cotton, and M. Briffa. 2012. Consistent crustaceans: the identification of stable behavioural syndromes in hermit crabs. *Behavioral Ecology and Sociobiology* 66: 1087-1094.
- Norton, W. H. J., K. Stumpfenhorst, T. Faus-Kessler, A. Folchert, N. Rohner, M. P. Harris, J. Callebert, and L. Bally-Cuif. 2011. Modulation of *Fgfr1a* Signaling in Zebrafish Reveals a Genetic Basis for the Aggression-Boldness Syndrome. *The Journal of Neuroscience* 31: 13796-13807.
- Starling, M. J., N. Branson, P. C. Thomson, and P. D. McGreevy. 2013. Age, sex and reproductive status affect boldness in dogs. *The Veterinary Journal* 197: 868-872.
- Sinn, D. L., Gosling, S.D., and Moltschaniwskyj, N.A. 2008. Development of shy/bold behaviour in squid: context-specific phenotypes associated with developmental plasticity. *Animal Behaviour* 75(2): 433-442.

APPENDIX



Supplemental Figure 1. Claw width and thorax measurements (panel A). Shell aperture measurement (panel B.)

LA AUDACIA RELATIVA DE LOS CANGREJOS ERMITAÑOS TERRESTRES A TRAVÉS DE ENTORNOS SOCIALES

ROBERT N. ALTER, NATHAN G. GIFFARD, SARAH L. JENNEWEIN, ASHLYN J. MORRIS

Editoras asistentes: Melissa DeSiervo, Clare Doherty

Editor: Matt Ayres

Abstracto: Hay beneficios e inconvenientes de ser audaz en el reino animal. Además, si un individuo está solo o en un grupo puede afectar a la audacia que muestra. Se ha informado que la audacia de los cangrejos ermitaños varía entre los individuos. Sin embargo, las complejidades de este rasgo conductual no se entienden bien. Examinamos la audacia del *coenobita compressus* en el laboratorio y en la playa cerca de la Reserva Biológica Campanario en la Península de Osa de Costa Rica. Volteamos cangrejos ermitaños en la parte posterior de su concha y medimos su tiempo de latencia para voltear hacia atrás, interpretando un tiempo de latencia más corto como un proxy para una mayor audacia. Verificamos que la audacia era repetible para los individuos y descubrimos que la audacia era independiente del tamaño del cangrejo, encajaba dentro de su caparazón, sexo y si las hembras eran gravitadas. Evaluamos la audacia en entornos solitarios y grupales en el laboratorio y examinamos si los niveles de audacia se mantuvieron en grupos de diferentes tamaños en la playa. La audacia podría ser un rasgo estático en los cangrejos ermitaños que se retiene en entornos solitarios y grupales, o puede ser un rasgo plástico que cambia con contextos, por ejemplo, cuando están solos o en grupos. Encontramos que la audacia aumentaba en un entorno de grupo en el laboratorio, posiblemente debido al mayor riesgo de desalojo cuando un individuo está de espaldas, pero que los niveles de audacia relativa eran estables para los individuos. Los cangrejos ermitaños no alteraron significativamente su audacia para igualar a la de otros miembros de su grupo. En la playa, el tamaño del grupo de cangrejo no afectó el tiempo promedio de respuesta grupal, tal vez debido a la incapacidad de las personas para percibir el tamaño del grupo como pruebas se hicieron por la noche. Nuestros hallazgos sugieren la existencia de personalidades en cangrejos ermitaños, pero se necesita más investigación para generalizar desde el comportamiento único estudiado aquí.

Palabras claves: cangrejo ermitaño, audacia, comportamiento animal, dinámica de grupo, personalidad, Campanario

LIMPETS TO DISTRIBUTION: LIMITATIONS TO THE LOCAL DISTRIBUTION OF TWO INTERTIDAL MOLLUSKS

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Abstract: All species are subject to a range of factors that influence their distribution in a given environment. These commonly include abiotic factors, predator presence, resource availability, and dispersal limitations. The distribution of intertidal species can be influenced by these factors. We studied the distributions of two substrate-bound organisms, periwinkle snails (*Litorinimorpha*) and true limpets (*Patellogastropoda*), in an intertidal zone on the Osa Peninsula of Costa Rica. Using both observations and manipulations, we assessed which factors influence the upper and lower limits of each organism's vertical distribution. We also studied the distribution of limpets and snails with respect to distance from the shore and examined the association between the two species. Our multiple tests and observations allowed us to confirm that abiotic factors such as water exposure and splash force influence the distributions of limpets and snails in intertidal zones. Snails and limpets were not limited by their dispersal abilities in our study system. Additionally, snails showed variation in their horizontal distribution. Future studies should address how the distribution of algae, the main food source of these species, influences their distribution. By testing and falsifying multiple factors that possibly limit the distributions of these species, this study highlights the complexity of what defines the limits to species' distribution.

Keywords: abiotic, dispersal, distribution, intertidal, littorinimorpha, Osa Peninsula, patellogastropoda, periwinkle snails, predation, prey, true limpets

INTRODUCTION

A defining question in ecology is: why are species where they are? What controls the boundaries defining the presence of an organism in a given environment? These questions can be addressed by determining the abiotic factors, predator presence, prey availability, and dispersal ability of a given species, each of which limits an organisms' distribution. Abiotic factors include environmental conditions such as temperature, water availability, and exposure to solar radiation. Predators can mediate species' distribution patterns by keeping certain species out of a location or necessitating they find a habitat that offers some level of safety. Additionally, species are limited by the distribution of available resources, especially food. Lastly, species can be limited by their physical ability to move among and between environments. The complex combination of these factors at play makes determining the distribution of a species a difficult, yet interesting puzzle to solve.

Intertidal zones are unique environments for observing distinct distribution patterns across small areas. Shifting ocean tides subject intertidal zones to environmental changes on a short

timescale. In such a variable environment, a small change in location often means a drastic change in conditions. Organisms are often found in distinct bands across intertidal rocks, separated by the specific factors which influence their distributions. The Stephenson's scheme for intertidal distribution describes these common banded patterns of habitat partitioning observed in marine organisms across intertidal ecosystems (Raffaelli and Hawkins 1999).

In this study, we examined two marine organisms, true limpets (*Patellogastropoda*) and periwinkle snails (*Litorinimorpha*), located in an intertidal zone on the Osa peninsula in Costa Rica. Snails are found in the supralittoral zone and tend to clump in groups to minimize water loss (Raffaelli and Hawkins 1999). Snails have a preference for green algae and are effective grazers (Wilhelmsen 1994). At high densities, periwinkle snails are able to prevent the establishment of green algae (Wilhelmsen 1994). Limpets also consume algae and their grazing habits can even structure populations of

macroalgae (Marzinelli 2012). They have a streamlined profile to avoid being lifted and dragged by high-impact wave action (Ellem 2002). Additionally, though they have a high tolerance for water loss, limpets curate a ‘home’ on a rock in which they fit perfectly, to minimize water loss (Ellem 2002).

Though limpets and snails both eat algae and are generally found in similar areas, potential differences in their individual distributions within intertidal zones can be discerned through detailed observations and intentional testing. Factors that consistently limit their distributions define both their upper and lower bounds. Therefore, we created a conceptual framework to determine the upper and lower bounds of each species’ distributions on the rock and what factors drive these boundaries.

In intertidal zones, abiotic factors commonly determine the upper limit and predation commonly determines the lower limit of species’ distributions because marine organisms must simultaneously avoid desiccation and aquatic predators. If these principles apply to our study system, abiotic factors would determine the upper bound for both the limpets and snails, and predation would determine the lower bound for both species. Alternatively, factors of only one category (abiotic, predation, prey, dispersal) may determine both the upper and lower bounds of both snails and limpets. In this case, the predominant factor limiting both species’ upper and lower bounds would be saltwater exposure. Lastly, factors of different categories could determine the upper and lower bounds for the two different species.

METHODS

We performed a series of manipulations and observational studies in an effort to narrow the list of possibility factors limiting the upper and lower distribution of snails and limpets in the intertidal zone. This research structure is illustrated in Figure 1.

Distribution

Over a three-day period, we measured the abundance of snails and limpets that were visible on two different rocks in the intertidal zone. Both rocks were about 30 meters long and 4 meters tall. The low tide line was defined by the bottom of the rock where it meets the sand, mid tide was 1.5

	Abiotic	Predators	Resources	Dispersal
Snail upper bound	Water dependence	Terrestrial predators	Algae distribution	Locomotive ability on rock
Snail lower bound	Water tolerance ----- Splash force	Aquatic predators	Algae distribution	Locomotive ability on rock
Limpet upper bound	Water dependence ----- Substrate	Terrestrial predators	Algae distribution	Locomotive ability on rock
Limpet lower bound	Water tolerance ----- Substrate	Aquatic predators	Algae distribution	Locomotive ability on rock

Figure 1: Matrix of hypothesized possibilities for limits to each species’ distributions.

meters up and high tide was 3 meters above the low tide line. We counted limpets and snails at low tide, mid-tide, and high tide when different water levels exposed more or less of the rock. We also measured the horizontal distribution of snails and limpets across these two rocks by measuring the number of individuals in three different zones on each rock at low tide over two days (Fig. 2). Zone 1 was ~10 m of the rock farthest from the ocean and closest to the shore, zone 3 was the part of the rock farthest from the shore, and zone 2 was in between. It should be noted that high water levels during high tide prevented us from accessing the rocks to perform our counts. During these instances, we performed our counts using binoculars.

To test for an association between limpets and snails we examined their overlap in distribution. We placed a 0.25 square meter quadrat at random heights twice in each zone and counted the number of individuals of limpets and snails in each.

To test for the presence of aggregation patterns in both species, we used the data from the association study to look at variance/mean. Ratios



Figure 2: Schematic of bird-eye view of experimental zones on intertidal rock during low tide. Two adjacent rocks were used for trials and both followed this zonation model. The vertical grey bar represents where the water level hits the rock during low tide; water reaches the bottom of the rock to the left of the bar and rock to the right is dry.

under one would suggest they were not aggregated, and over one would suggest they were aggregated, with higher values indicating a higher level of aggregation.

Abiotic Factors

To study limpets' and snails' physiological dependence on, or intolerance to, seawater, we took an experimental approach. We brought limpets and snails back to the laboratory and exposed them to three treatments: three of each species were held in completely dry enclosures, enclosures where they were completely submerged in water, and enclosures where they had access to both wet and dry surfaces to act as a control. We recorded how long the limpets and snails were able to survive in each of the three treatments.

To test if the lower limit of snails' distribution was limited by the force of splashing waves, we moved 20 snails onto a part of the rock experiencing high wave action and frequent submersion. We then watched to see if they moved out of the area of high wave action to a calmer location. Because limpets are often found in areas of high wave action at multiple tidal levels and they possess a more streamlined morphology compared to that of snails, we gathered that splashing does not limit their distribution and thus did not perform this same relocation test on the limpets.

To test if limpet distribution was limited by substrate, possibly due to impaired ability to secure themselves on certain substrates, we moved nine marked limpets onto dense algae found below their typical distribution. As a control, we moved another nine marked limpets onto a nearby rock which matched the type of surface they were originally on. After ensuring all limpets successfully secured themselves on the rock at low tide, we left them for a whole tidal cycle and came back at the next low tide and counted how many of each treatment were still there.

Predators

To determine whether terrestrial predation limited the upper bounds of limpet and snail distribution, we watched for predation events. To determine whether aquatic predation limited the lower bounds of their distribution, we moved four marked limpets and five marked snails to a very low spot on the rock that would remain

underwater, and thus accessible to marine predators such as sea stars, for a period of time longer than usual. As a control, we moved four other marked limpets and five other marked snails to a new place on the rock, about three meters away from their original location, but at their original height. After one tidal cycle, we returned and counted how many marked limpets and snails of each treatment were still present. Because we were unable to watch the limpets and snails throughout the tidal cycle, it is possible that limpets or snails fell off the rock and washed away without experiencing predation.

Resources

Because snails and limpets both rely on algae for food, we qualitatively observed the distribution of algae across two rocks and compared it to the distribution of snails and limpets.

Dispersal

To examine the dispersal ability of both organisms, we marked six groups of between 20 and 40 limpets or snails within an area, photographed their positions, and qualitatively examined their movement over the course of the three-day study.

Statistical Methods

To analyze the association between limpets and snails, we square-root transformed the data and completed a regression between the number of limpets and snails. To analyze the effect of zones and species on the number of individuals, we performed an ANOVA followed by a Tukey HSD test. We applied a square root transformation to the dependent variable. To understand the difference between all six interactions in both ANOVAs, we performed a Tukey HSD test.

RESULTS

Distribution

Overall, there were more limpets than snails on the intertidal rocks (ANOVA: $F = 35.27$, $df = 1$, $P < 0.0001$, Fig. 3). When we counted the number of limpets and snails visible on the rock at low, mid-, and high tide, we found that 0.7% of the limpets on the rock were located above the water line at high tide. Below this line, limpets were highly concentrated in a distinct band, with 89.3% of limpets between the mid and high tide line. Only 10.0% of limpets were below the mid-tide line. While the snails were also predominantly located between the mid-and high tide line (47.5 %), their distribution was less concentrated than the limpets; 25.3% of snails were above the high tide line, and 27.2% of snails were between the low and mid-tide line.

When we divided up the rock into zones based on distance from the shore, we found that the zone closest to the shore, zone 1, had a higher number of individuals than zones 2 and 3 (ANOVA: $F = 5.31$, $df = 2, 18$, $P = 0.0154$, Fig. 3). There was no significant interaction between species and zone (ANOVA: $F = 0.3798$, $df = 2, 18$, $P = 0.69$, Fig. 3).

Based on these results, we can conclude that the distribution of each is dependent on both distance above the ocean and distance from the

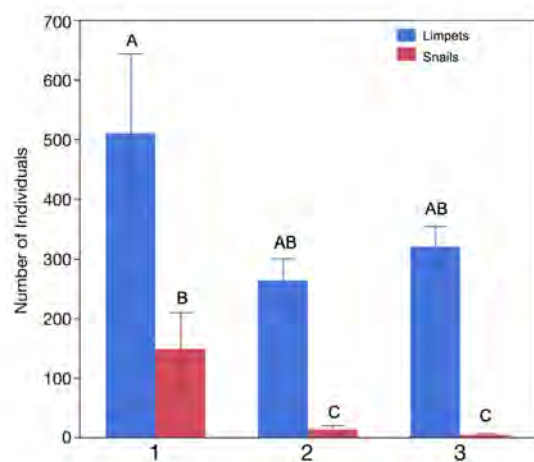


Figure 3. Number of limpets and snails found in each zone. Zone 1 was the farthest from the ocean, zone 3 was the closest to the ocean. Each zone was 10 meters in length. Letters indicate groups distinguished by Tukey's HSD tests, statistically similar to each other. A, B, and C are statistically different from each other, as determined from post-hoc tests. Bars labeled "AB" are not statistically different from A or B, but are statistically different than bars labeled C. Mean \pm 1 SE.

shore. However, they differ in their respective distributions at high tide when compared to low tide. It is likely that we were not able to see all individuals observed at low tide, especially snails, which we observed clumping in cracks. There was no association between limpets and snails (Linear Regression: Slope = -0.12 ± 0.22 , $P = 0.62$, $df = 12$, Fig. 4), indicating the presence of one does not influence the presence of the other. In testing limpets and snail's aggregation, the variance to mean ratio was 32.87 for snails and 13.50 for limpets (Table 2), indicating both are highly aggregated, but snails are more aggregated than limpets.

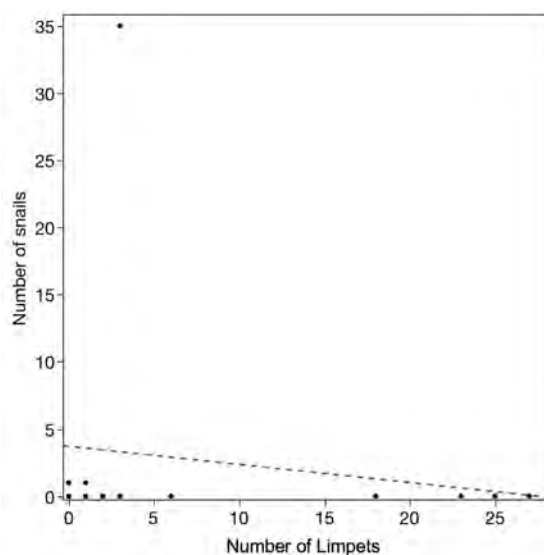


Figure 4: Linear regression between number of limpets and number of snails.

Table 2: Results from limpet and snail aggregation study in zone 1. Rows show the number of limpets and snails found in each 0.50 x 0.50 m quadrat. The mean, variance, and variance to mean ratios appear at bottom.

Interaction	Number of Limpets	Number of Snails
Both	3	35
Only Limpets	18	0
Only Snails	0	1
Both	1	1
Only Limpets	2	0
Only Limpets	23	0
Only Limpets	6	0
Only Limpets	27	0
Only Limpets	2	0
Only Limpets	1	0

Neither	0	0
Only Limpets	3	0
Neither	0	0
Only Limpets	25	0
Mean	7.93	2.64
Variance	106.99	86.86
Variance to Mean Ratio	13.49	32.97

Abiotic factors

We tested the limpets' and snails' tolerance to and dependence on water. In both our control and submerged treatments, two of the three limpets died after 20 hours. All three of the limpets in the completely dry enclosure were still alive 30 hours after the start of the experiment. However, all nine snails survived for 30 hours. Wave action caused on average, 47.5% of the snails to move up out of the high wave action zone, 37.5% stayed in place and 15% fell off (Table 1).

Table 1: Results from both wave action trials. Total snails represent the number of snails moved into the "splash zone." The 'moved-up' column describes snails that moved out of the splash zone.

Trial	Total Snails	Moved Up	Stayed in Place	Fell Off
1	20	9	10	1
2	20	10	5	5
Percentage	100%	48%	37%	15%

From the substrate test, eight of the nine limpets relocated to the dense algae at low tide fell off of the rock by the next low tide. The last limpet moved from the algae back onto its normal rock substrate during that time. Conversely, only two of the nine control limpets that were relocated to a rock surface matching their original substrate, were not found after one whole tidal cycle.

Overall, we can conclude that the survival of snails is likely not dependent on their need to be either submerged or out of water during an entire tidal cycle. However, it is likely controlled by the inability to remain on the rock in the area of high wave action. As for limpets, they are likely limited by the fact that they cannot be submerged for more than a tidal cycle, and that they do not attach well to an abnormal substrate.

Predators

We did not observe terrestrial predation during our study. However, in our test of aquatic predation on limpets and snails, all of the limpets and snails that were moved to a lower location at low tide, and thus vulnerable to aquatic predators for a longer than normal period of time, were gone from the rock by the next low tide. Three out of the four limpets and three out of the five snails that were relocated to normal height as control were still present on the rock at the next low tide. It is possible that the snails not present were still on the rock somewhere but moved to a place where we could not find them.

Terrestrial predation likely does not control the upper limit of the distribution of limpets or snails. It is possible that the lower limit to the distribution of limpets is controlled by aquatic predation, but we did not directly observe this. We cannot conclude whether aquatic predation controls the lower distribution of snails.

	Abiotic	Predators	Resources	Dispersal
Snail upper bound	Water dependence	Terrestrial predators	Algae distribution	Locomotive ability on rock
Snail lower bound	Water tolerance Splash force	Aquatic predators	Algae distribution	Locomotive ability on rock
Limpet upper bound	Water dependence Substrate	Terrestrial predators	Algae distribution	Locomotive ability on rock
Limpet lower bound	Water tolerance Substrate	Aquatic predators	Algae distribution	Locomotive ability on rock

Figure 5: Results of research. X's indicate hypotheses that were rejected. Box indicates hypotheses for which there was support. Others remain unknown.

Resources

There was little visible algae on most of the rock, located exclusively at the margin of the sandy shore and the rock. We do not know the specific algae that the limpets and snails prefer. Therefore, it is inconclusive if there is an association between the two species in their resource acquisition.

Dispersal

Limpets and snails are not limited by their locomotive ability in movement within the rock. Both were observed moving, although snails moved farther and faster than limpets. Over three

days, the painted limpets remained in a similar location, while the painted snails gradually dispersed. For these reasons, we can conclude that dispersal does not influence their distributions for this study.

DISCUSSION

The upper and lower bounds of limpets and snails were controlled by different factors, many of which are abiotic. The upper bound of snails was restricted by possible low prey abundance above the high tide line, and their lower bound at low tide was restricted by their sensitivity to wave action and possibly the presence of aquatic predators. The upper bound of limpets was restricted by possible low algae abundance above their current distribution, and their lower bound was restricted by their intolerance to submergence for periods longer than a tidal cycle, inability to stick to the substrate found below their distribution, and possibly the presence of aquatic predators.

The distribution of snails also changed across three horizontal zones of increasing distance from the shore. The abundance of limpets did not change with zone. Snail abundance was higher in zone one than it was in zones two and three. This may be explained by the differing tolerance to wave action between limpets and snails. Limpets are more resistant to wave action than are snails, potentially due to their more streamlined morphology. This may explain why snails were found farther away from the waves and limpets were found on all parts of the rock.

Upon noticing the obvious band of limpets on the rock, we assumed that the upper and lower boundaries of this band were controlled by the water level at high and low tide, respectively. If this is true, then a majority of limpets would have been visible at low tide, but not at high tide. Though this is true, we found that more specifically, a majority of limpets were located between the mid and high tide line, supported by our results that they die when they are submerged for too long. We also noticed a distinct difference in texture of substrate between low and mid-tide. We addressed this unexpected pattern by testing for differences in the ability of limpets to attach to the rock across substrate, confirming that a factor aside from tidal levels is controlling their distribution. To further support this finding,

another test could confirm if their distribution is also controlled by the presence of algae; maybe they cannot permanently exist on algae but need to be near it for food.

In the future, a study on the algae limpets and snails consume can strengthen our understanding of how their food influences their distribution. The limpets or snails may consume the algae soon after it grows, which may explain why we were not able to identify it during our data collection. It would also inform whether limpets and snails compete for resources. Another possible result is that each species eats different algae. This finding would support our argument that there is no competition between limpets and snails. Additionally, since we did not observe terrestrial predation, and could not confirm the existence of aquatic predation, for either species, a study on the predators of limpet and snails is necessary. A study that successfully defines their predators would further inform how predators limit the distributions of each animal.

Despite the proximity and shared environment of these two species, different factors are at play in determining their respective distributions. The complex web of interactions between species and abiotic factors, predation, prey, and dispersal ability make possible the range of distributions seen in nature. This level of complexity can be applied to many other distribution systems in different biomes. In general, distributions are not controlled by one simple factor, rather, they are controlled by many—often conflicting—factors, stressing the importance of considering all possible factors to conclude a result.

ACKNOWLEDGEMENTS

Thank you endlessly to Nancy, the most incredible woman in science and adventure supporter. We hope to return to catch more bats in the future (without the rabies scare). Thanks also to Matt for talking to us for hours at end about our project.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this work.

LITERATURE CITED

Ellem. G., Furst, J., and Zimmerman, K. 2002. Shell clamping behaviour in the limpet *Cellana tramoserica*. *Journal of Experimental Biology* 205: 539-547.

- Hawkins S. J., and Hartnoll R. G. 1983. Grazing of intertidal algae by marine invertebrates. *Oceanography and Marine Biology: An Annual Review* 21: 195–282.
- Marzinelli, E. M., Burrows, M. T., Jackson, A. C., and Mayer-Pinto, M. 2012. Positive and negative effects of habitat-forming algae on survival, growth and intra-specific competition of limpets. *PloS one* 7(12).
- Wilhelmsen, U., and Reise, K. 1994. Grazing on green algae by the periwinkle *Littorina littorea* in the Wadden Sea. *Helgolander Meeresunters* 48: 233–242.

LAPAS A DISTRIBUCIÓN: LIMITACIONES A LA DISTRIBUCIÓN LOCAL DE DOS MOLUSCOS INTERMAREALES

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Abstracto: Todas las especies están sujetas a una serie de factores que influyen en su distribución en un entorno determinado. Estos comúnmente incluyen factores abióticos, presencia de depredadores, disponibilidad de recursos y limitaciones de dispersión. La distribución de especies intermareales puede verse influenciada por estos factores. Estudiamos las distribuciones de dos organismos ligados a sustratos, bígaros (*Litorinimorpha*) y lapas verdaderas (*Patellogastropoda*), en una zona intermareal en la Península de Osa de Costa Rica. Utilizando observaciones y manipulaciones, evaluamos qué factores influyen en los límites superior e inferior de la distribución vertical de cada organismo. También estudiamos la distribución de lapas y caracoles con respecto a la distancia de la costa y examinamos la asociación entre las dos especies. Nuestras múltiples pruebas y observaciones nos permitieron confirmar que factores abióticos como la exposición al agua y la fuerza de salpicaduras influyen en las distribuciones de lapas y caracoles en zonas intermareales. Los caracoles y las lapas no estaban limitados por sus habilidades de dispersión en nuestro sistema de estudio. Además, los caracoles mostraron variación en su distribución horizontal. Los estudios futuros deben abordar cómo la distribución de las algas, la principal fuente de alimento de estas especies influye en su distribución. Al probar y falsificar múltiples factores que posiblemente limitan las distribuciones de estas especies, este estudio destaca la complejidad de lo que define los límites a la distribución de las especies

Palabras claves: abiótico, dispersión, distribución, intermareal, *Litorinimorpha*, península de Osa, *Patellogastropoda*, caracoles bígaro, depredación, presa, lapas verdaderas

MACROBRACHIUM MYSTERY:
EVALUATING TROPHIC MEDIATION IN NEOTROPICAL FRESHWATER SHRIMP

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Abstract: Trophic structure defines energy movement through food webs. The strength of top-down vs. bottom-up forces can vary among communities. *Macrobrachium*, a genus of amphidromous Neotropical shrimp, provided a model study system for analyzing trophic structure as they were subject to constraints in bottom-up allochthonous input and top-down predation pressures. We quantified *Macrobrachium* in three streams on the Osa Peninsula, Costa Rica. To evaluate top-down and bottom-up effects, and behavioral impacts of predation on the *Macrobrachium* population, we conducted abundance surveys and a predation-behavioral experiment. Our results indicated some *Macrobrachium* morphotypes may be impacted by top-down effects and that *Macrobrachium* behavior reflects strong impacts from predation. We found no support for bottom-up constraints of this system. These findings highlight how interactions within a single trophic level, such as intraguild predation, can shape the structure of an entire trophic system.

Key words: freshwater shrimp, fission fusion, *Macrobrachium*, riparian communities, trophic cascades

INTRODUCTION

In ecological communities, energy can travel from the smallest organisms in the food web to the dominant predators by means of a trophic cascade. These systems are mediated by limitations at certain trophic levels, effects that can ripple throughout the ecosystem. From the perspective of a single population within the cascade, the limiting factor on population growth rate and abundance can come from predation or parasitism (top-down effects), or from limited food resources (bottom-up effects) (Knight et. al. 2005).

Decapods, (an order of crustaceans that includes lobsters, shrimp, crabs, and prawn) in Neotropical freshwater streams are model study organisms to study trophic interactions because they often have large predators, such as herons, and prey upon smaller invertebrates that eat decayed organic matter. Freshwater shrimp that live in the streams of the Osa Peninsula are interesting, yet poorly studied Decapods that are adept at finding and inhabiting all riparian pools, regardless of size, obstacles, or flow (authors observations 2020). We studied members of the genus *Macrobrachium*, which are found in many freshwater and estuarine environments in the tropical and subtropical zones (Lara 2009). Virtually nothing is known about *Macrobrachium* within neo-tropical freshwater stream pools,

including trophic level interactions, intra-family interactions, and individual behavior. Observations of intraguild predation, cathemeral (day and night) activity, and dramatic variation in morphotypes and sizes of shrimps near our field station on the Osa Peninsula suggested that these communities may differ from known systems.

The most defining feature of *Macrobrachium* spp. are the extreme enlargement of their second pereiopods (Supplemental Fig. 1 and 2). These pereiopods are transformed to have chelae (claws) which might have evolved for foraging, predation or methods of intraspecific communication. In addition, *Macrobrachium* have varying life cycles within species. Many species in this genus are amphidromous, relying on saltwater to complete larval development, while others can reach maturity independent of estuaries. For example, *Macrobrachium ohione* is an amphidromous species which can be found thriving in the Ohio River System, approximately 1500 km away from the nearest marine body (Bauer and Delahoussaye 2008) It is possible that some *Macrobrachium* spp. In the Osa Peninsula are also able to inhabit ponds isolated by large barriers, such as waterfalls, which may explain their unique distribution.

We studied two distinct morphotypes of shrimp: those with claws (chelate), and those without claws (non-chelate) in streams near

Campanario Biological Research Station on the Osa Peninsula. Shrimp with claws were generally larger than shrimp without claws and could be positively identified as belonging to the genus *Macrobrachium*. Shrimp without claws could not be identified definitively as juvenile *Macrobrachium* or as belonging to different taxa. For example, shrimp of the family Atyidae are often found occupying the same streams as *Macrobrachium*, and closely match the observed phenotypes of the smaller shrimp. Hereafter, we refer to *Macrobrachium* to include the chelate and non-chelate morphotypes. The taxonomic identity of the studied individuals, however, is not relevant to the focus of our study because predation on shrimp seemed to be dependent on size differential, not species type. We investigated the relationship between shrimp morphotypes and predator avoidance behavior, diurnal v. nocturnal daily rhythms, and spatial dispersion.

Macrobrachium populations in our study system could be mediated by top down or bottom up effects from predators or allochthonous input. If communities are mediated by top-down predation pressures, we expected decreased shrimp abundance and increased flightiness with increased predator rating. We also expected increased nocturnal activity to avoid predation by diurnal herons. If *Macrobrachium* populations are mediated by bottom-up food resources, we expected shrimp abundance to be affected by allochthonous input, but not time of day or predator presence. A third hypothesis posits that morphotype of shrimp influences trophic mediation within the system. We could observe small, non-chelate shrimp with a different activity pattern syndrome than large, chelate shrimp in response to predator presence and allochthonous input.

METHODS

Abundance Surveys

To quantify the abundance of *Macrobrachium* at different times of day, we surveyed three streams and three pools per stream, every four hours continuously for 48 hours in Feb 2020 for a total of 12 trials per pool. At each trial, we placed a 16x16cm quadrat in the pool, randomizing quadrat position during each trial. After a 5-minute habituation time to mediate the disturbance of introducing the quadrat marker, we counted the

number of *Macrobrachium* that entered the quadrat during a 2-minute period. We counted small, non-chelate *Macrobrachium* and large, chelate *Macrobrachium* separately to analyze differences in behaviors between these two morphotypes.

At each of our survey pools, we measured surface area, allochthonous input (terrestrially derived organic material), and predator abundance. To measure allochthonous input, we measured the surface area and depth of the leaf litter found in the bottom of the pool. To estimate predator abundance, we qualitatively rated each stream on a 3-point-scale based on the amount of bird scat present (1 = least, 2 = middle, 3 = most bird scat observed). These bird-scat measurements were supported by qualitative field observations of bird predator presence.

Trapping and Flight-Distance In-Situ Experiments

To measure the flight-distance of *Macrobrachium*, we placed a baited minnow trap in each stream, at a separate pool from our abundance study pools. At 4-hour intervals, we collected all *Macrobrachium* in the trap and completed flight-distance experiments in-situ, using a plastic bin filled with water from the same stream with a ruler taped to the bottom for distance reference. We placed an individual *Macrobrachium* into the bin, allowing time for the shrimp to stop moving. We then moved a gloved hand along the bottom of the bin at a standardized speed towards the shrimp head-on from 60 cm away to simulate the approach of a predator. We recorded this on video to calculate the flight-distance (the distance from glove to shrimp at which the *Macrobrachium* moved away from the glove) and the length of the shrimp from rostrum to tail. We also recorded the color of the shrimp's body and joints, its sex, whether or not it had dimorphic claws, and whether it was missing any claws or other appendages.

Statistical Methods

We used JMP® Pro 14 to conduct all statistical tests. Chelate and non-chelate abundance, flight distance, and shrimp length were logarithmically transformed to improve normality. We tested for correlations of environmental metrics collected at the stream level which included riffle crest, proportion of allochthonous input, depth of

allochthonous input, and pool surface area. Because of the distribution and strong correlation between metrics of allochthonous input we treated allochthonous input as a categorical variable with three levels (low, medium, and high). To test if fear response was related to *Macrobrachium* size and predator score, we evaluated a general linear model that included size as a continuous variable, predator score as a categorical variable, and their interaction. To test if each morphotype abundance varied with predator score, light level, or allochthonous input, we evaluated an ANOVA that predator score, light level, allochthonous input, and their interaction. We included pool nested within stream as a random effect in both models.

RESULTS

Allochthonous input depth and proportion of pool covered by allochthonous input were the only significantly correlated environmental metrics ($r = 0.97$). All the other correlations between riffle crest, pool surface area, proportion allochthonous surface area, and allochthonous depth were not significant ($r < |0.67|$).

There was no significant difference in flight distance when compared by predator score (general linear model; predator score: $F = 0.45$, $df = 2, 54$, $p = 0.873$). However, larger shrimp had significantly shorter flight distances (general linear model; shrimp length: $F = 9.90$, $df = 1, 54$, $p = 0.0028$), and the interaction effect of shrimp size

and predator score was not significant (general linear model; predator score x shrimp length: $F = 2.07$, $df = 2, 108$, $p = 0.14$; Fig. 1).

Chelates were significantly more abundant at night (general linear model: light level: $F = 8.53$, $df = 1, 98$, $p = 0.0043$; Fig. 2), but predator abundance did not affect chelate abundance (general linear model: predator score: $F = 0.26$, $df = 2, 4$, $p = 0.78$; Fig. 3). There was variation in chelate abundance between allochthonous input scores, but there was no significant trend (general linear model: allochthonous category: $F = 6.61$, $df = 2, 4$, $p = 0.0539$). The random effect of individual pools accounted for 0.00% of the variation of chelate variance in the sample. Non-chelates were significantly more abundant during

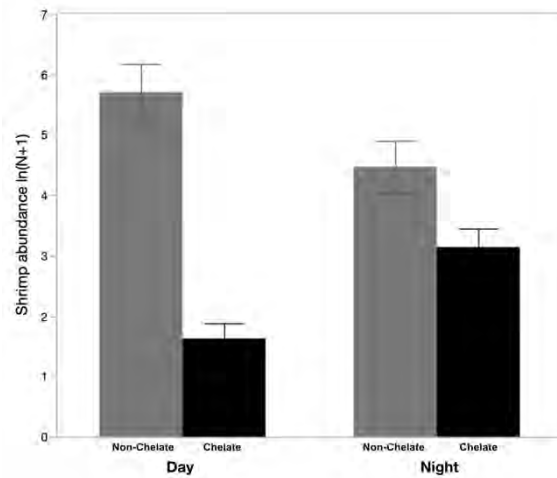


Figure 2: Shrimp abundance by morphotype during day and night.

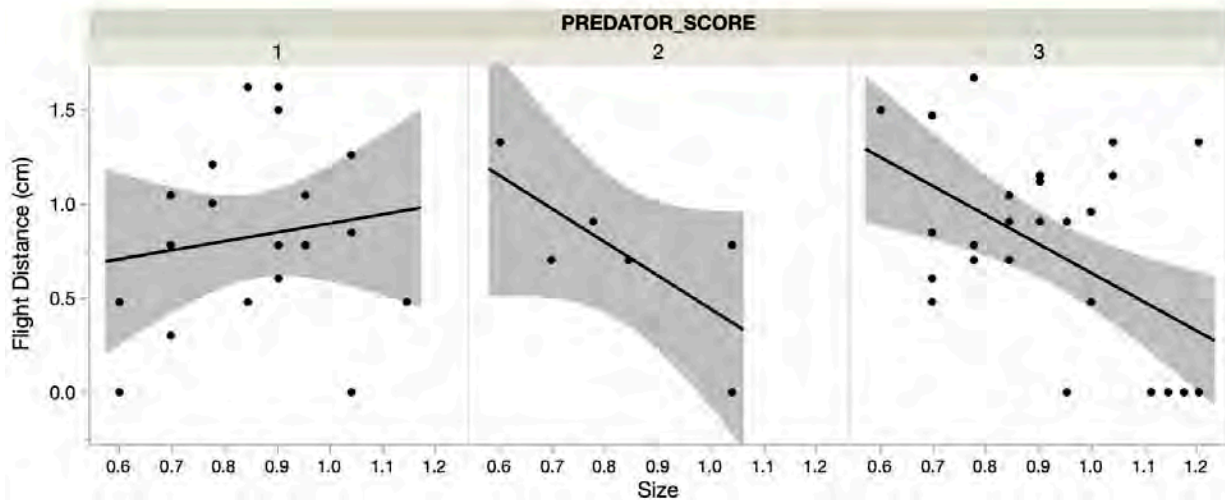


Figure 1: Flight distance vs *Macrobrachium* size in pools with low to high predator scores. Each point equals a shrimp.

the day (general linear model: light level: $F = 8.43$, $df = 1, 98$, $p = 0.0046$; Fig. 2) and non-chelates to be more abundant at lower predator scores however this was not statistically significant (general linear model; predator score: $F = 6.24$, $df = 2, 4$, $p = 0.0590$; Fig. 3). Allochthonous input category had no significant effect on non-chelate abundance (general linear model; allochthonous category: $F = 0.12$, $df = 2, 4$, $p = 0.89$).

During this study, we also observed a female *Macrobrachium* with dimorphic claw and eggs (Supplemental Figure 1) and several blue shrimp (*Macrobrachium hancocki*) (Supplemental Figure 2), only seen active at night.

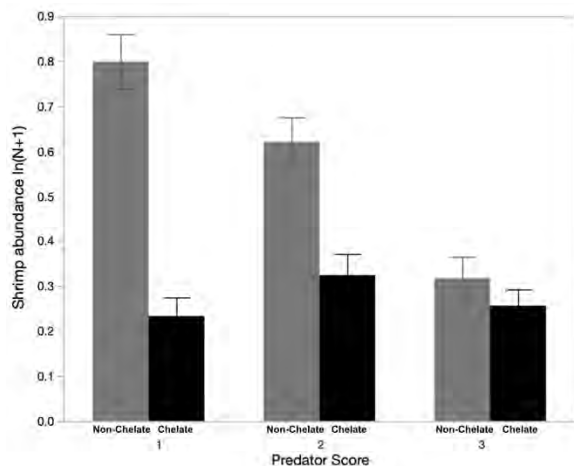


Figure 3: Shrimp abundance by morphotype in ponds with low to high predator scores.

DISCUSSION

Pools with fewer predators present exhibited higher numbers of active non-chelate shrimp, though this was not statistically significant. Non-chelate shrimp were much more active during the day than at night, while those of the chelate morphotype were more active at night. Meanwhile, no shrimp varied in abundance with allochthonous input. As shrimp appeared unaffected by this input, *Macrobrachium* populations appear not to be bottom-up limited. Rather, our data indicate a top-down limitation on non-chelate shrimp dispersion and may imply a particular set of selective pressures and a related compensatory behavioral syndrome for non-chelate shrimp.

Regarding temporal patterns in activity, small non-chelate shrimp were most active in the day while avian predators are hunting, and thus appear

not to utilize nocturnal foraging to avoid these predators. However, chelate shrimp are most active at night, and may avoid avian predators by foraging nocturnally. Chelate shrimp may directly exclude non-chelate shrimp from a temporal territory, inhibiting non-chelate shrimp from avoiding avian predators by night. On several occasions, chelate shrimp exhibited severe aggression at night towards non-chelate shrimp, pulling them towards their mandibles and forcing rapid escape measures. Therefore, chelate shrimp may avoid avian predators temporally, while non-chelate shrimp must balance pressures both from larger nocturnal shrimp and from diurnal avian predators, possibly until reaching a competitive size that allows nighttime foraging. Further research may illuminate the means by which chelate shrimp do or do not exclude non-chelate shrimp from nighttime foraging.

Avian predators may affect *Macrobrachium* populations by various means: 1) predators eat shrimp and reduce abundance directly; 2) predators motivate shrimp to migrate to safer pools; or 3) predators motivate shrimp to spend more time in hiding. Our observations indicate that avian predators forage along sections of streams, hunting several adjacent pools. Meanwhile, non-chelate shrimp were more active during the day, despite the fact that Tiger Herons, their primary observed predators, are diurnal foragers.

Therefore, non-chelate shrimp do not avoid predators by either intra-stream migration or nocturnal foraging, and it appears that avian predators influence non-chelate shrimp by feeding on them and directly reducing population sizes. If this trend is real, then predators mediate the population dynamics of these shrimp, while bottom-up effects appear obsolete.

Flight distance, normalized for shrimp length, was not influenced by predator presence or allochthonous input. However, in all streams, flight distance significantly decreased as size increased. This indicates that large shrimp exhibit less predator-related fear than small shrimp. This may occur because larger shrimp perceive themselves as more easily defensible, perhaps after surviving predation events. Alternatively, large shrimp may simply be better able to compete with conspecifics for safer territories, and selection may favor boldness accompanying this competitive advantage. Future studies may

determine which selection pressures favor boldness in large shrimp.

The detection of top-down predation pressures, temporal territory defense, and intraguild predation in these shrimps adds a great deal of detail to the current scientific knowledge surrounding these shrimps. Much remains unknown about the genus *Macrobrachium*; their life history, developmental biology, and behavioral patterns are poorly studied. Our findings discern the existence of predatory limitation on these shrimps from within the same trophic level, a pressure which heavily influences the emergent behaviors and foraging periods of smaller *Macrobrachium* individuals.

While trophic cascades have been studied for many systems, the neotropical shrimp observed in this study demonstrate how trophic pressures can vary between morphologically similar organisms. This phenomenon may be more prevalent in systems exhibiting intraguild predation. As inferred in our study, intraguild predation may select for varying behaviors within predators at the same trophic level; this variation has been observed in species serving several other trophic roles, such as sharks and spiders (Hagler and Blackmer 2015; Meneses 2016). Further investigation may reveal the cascading effects of interactions within a single trophic level on the structure of entire trophic systems.

ACKNOWLEDGEMENTS

Heaviest shout-out of all time to Nancy, the absolute coolest cat this side of the Tempisque, and all of the staff at Campanario Biological Research Station for our incredible accommodations and post-project refreshments. Big knucks to Matt, Melissa, and Clare for all of their help in the research process. Apologies to the rest of the 2020 Bio FSP group for our constant “weeeooo’s,” radio chatter, and positive energy, as these seemed surprisingly unappreciated. Finally, we would like to pour one out for Robby, who never believed in us.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this project, offering blood, sweat, tears, constant risk of death by fer-de-lance, and a *lot* of sleep deprivation.

LITERATURE CITED

- Hagler, J. R., & Blackmer, F. 2015. Evidence of intraguild predation on a key member of the cotton predator complex. *Food Webs*. 4, 8-13.
- Knight, T., McCoy, M., Chase, J. et al. 2005. Trophic cascades across ecosystems. *Nature* 437, 880–883.
- Meneses, C. I. M., Rojas, Y. E. T., Magaña, F. G., García, S. A., & Carrillo, L. D. T. 2016. Trophic overlap between blue sharks (*Prionace glauca*) and shortfin makos (*Isurus oxyrinchus*): trophic linkages between two shark species in the Eastern Pacific Ocean food web. *Food Webs*. 7, 13-19.
- Pace, M. L., Cole, J. J., Carpenter, S. R., & Kitchell, J. F. 1999. Trophic cascades revealed in diverse ecosystems. *Trends in Ecology & Evolution*, 14(12), 483–488.

APPENDIX

Supplemental Table 1: Correlation matrix of riffle crest, surface area of the pond, proportion of allochthonous coverage, and average allochthonous depth. Each point equals one pool. Significant correlation value of $r = |0.67|$

Field	Riffle Crest	Surface Area	Allochthonous Proportion	Average Allochthonous Input
Riffle Crest	1			
Surface Area	-0.45	1		
Allochthonous Proportion	0.38	0.43	1	
Average Allochthonous Input Depth	0.02	0.01	0.03	1



Supplemental Figure 1: Female *Macrobrachium* with dimorphic claws and eggs caught in minnow trap at Bat Cave stream site.



Supplemental Figure 2: Blue freshwater shrimp (*Macrobrachium hancocki*). Observed in preliminary observation.

MISTERIO DE *MACROBRACHIUM*: EVALUACIÓN DE LA MEDIACIÓN TRÓFICA EN
CAMARONES DE AGUA DULCE NEOTROPICALES

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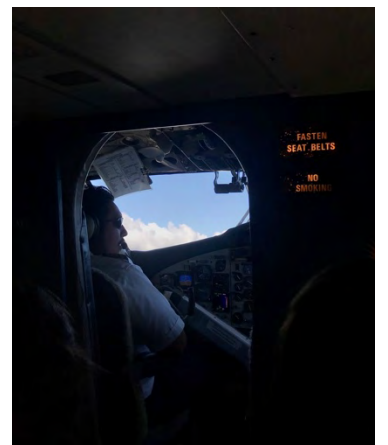
Editor: Matthew Ayres

Abstracto: La estructura trófica define el movimiento energético a través de las redes de alimentos. La fuerza de las fuerzas de arriba hacia abajo puede variar entre las comunidades. *Macrobrachium*, un género de camarones neotropicales anfítrons, proporcionó un sistema de estudio modelo para analizar la estructura trófica, ya que estaban sujetos a limitaciones en la entrada alóctona de abajo hacia arriba y presiones de depredación de arriba hacia abajo. Cuantificamos *Macrobrachium* en tres arroyos en la Península de Osa, Costa Rica. Para evaluar los efectos de arriba hacia abajo y de abajo hacia arriba, y los impactos conductuales de la depredación en la población de *Macrobrachium*, realizamos encuestas de abundancia y un experimento de comportamiento de depredación. Nuestros resultados indicaron que algunos morfotipos de *Macrobrachium* pueden verse afectados por efectos de arriba hacia abajo y que el comportamiento de *Macrobrachium* refleja fuertes impactos de la depredación. No encontramos soporte para las restricciones ascendentes de este sistema. Estos hallazgos resaltan cómo las interacciones dentro de un solo nivel trófico, como la depredación intraguilda, pueden dar forma a la estructura de todo un sistema trófico.

Palabras claves: camarón de agua dulce, *Macrobrachium*, comunidades ribereñas, cascadas tróficas



Little Cayman





THE EFFECT OF ABIOTIC FACTORS ON GASTROPOD AND HERMIT CRAB DENSITY IN TIDE POOLS

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TA Editors: Melissa DeSiervo, Clare Doherty

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Abstract: Abiotic factors limit the distribution of many species. However, the ability of individual organisms to move towards hospitable conditions varies by species. Tide pools are a model system for studying how abiotic factors limit the distribution of species because they contain a range of abiotic conditions in a relatively small area. To determine the effect of pool size and ocean connectivity on the density of snails and hermit crabs found in tide pools, we surveyed 30 tide pools at Salt Rock on Little Cayman that possess easy to define boundaries. Likely due to low turnover of water and high rates of evaporation, we observed that tide pools that weren't connected to the ocean had higher temperatures and salinities on average when compared to connected tide pools. Despite these harsh abiotic conditions and wider variations in temperature, we found that unconnected tide pools had significantly higher densities of both gastropods and hermit crabs when compared to connected pools. These results may be explained by the higher turbulence in connected pools from consistent wave action. As pool volume increased, gastropod density in connected pools remained constant. Hermit crabs were not found in connected pools. However, as pool volume increased in unconnected pools, both gastropod and hermit crab density increased. The facts that daily variation in temperature decreases significantly with increasing pool size and that higher peak temperatures are found in smaller unconnected pools may explain these findings. Despite hermit crabs' increased mobility when compared to snails, no difference was observed in any of the trends between the two species. This may be because the conditions at Salt Rock have held constant for a great enough length of time for both species to move towards a more preferred, optimal environment.

Key Words: gastropods, hermit crabs, tide pools, abiotic conditions, harsh environment, Salt Rock, Little Cayman

INTRODUCTION

Species do not distribute themselves uniformly across an environment. Both biotic and abiotic factors can be used to explain the spatial heterogeneity of a species' distribution. For example, the two primary factors limiting the distribution of poeciliid fish of the genus *Limia* within Hispaniola are the salinity and temperature tolerances of the fish (Haney and Walsh 2003). Similarly, though much more tolerant to increased temperatures when compared to that of other Antarctic marine invertebrates, the sea star *Odontaster validus* stops exhibiting any signs of activity when in temperatures greater than 9°C, possibly explaining why populations are localized to certain parts of the Southern Ocean that do not reach this threshold temperature (Peck et al. 2008).

The ability of an organism to move towards optimal abiotic conditions varies significantly by species. For example, while dolphins, which can attain swimming speeds of over 20 miles per

hour, are easily able to move towards hospitable conditions, sponges are sessile and don't possess the ability to disperse away from harsh conditions. The mobility of a species can have significant implications for its conservation. Species that can move more quickly away from a disturbance or unfavorable change in abiotic conditions may have an increased chance at persisting.

Tide pools are an ideal system for studying how abiotic factors limit the distribution of species because they contain a range of abiotic conditions in a relatively small area. These shallow pools of water that form on rocky shores in the intertidal zone are home to a diverse array of organisms, including sea stars, chitin, and sea urchins. Due to large differences in abiotic conditions across an environment containing tide pools, these organisms do not distribute themselves uniformly across the habitat. Further, of the species that inhabit tide pools, some can move more readily than others.

Tide pools found at Salt Rock on Little Cayman Island are dominated by large numbers of gastropods, as well as blue-legged hermit crabs (*Clibanarius tricolor*) that inhabit the shells of dead snails. The dominant species of gastropods observed in these pools are prickly periwinkles (*Nodilittorina tuberculata*), West Indian top shells (*Cittarium pica*), and an unidentified gastropod species that was significantly larger in size when compared to the other two. Of note, the blue-legged hermit crabs are more capable of fast dispersal when compared to all three observed gastropod species. In order to test how connectivity to the ocean and volume of these tide pools affect the physical conditions and distribution of the gastropods and hermit crabs found in them, 30 tide pools at Salt Rock were surveyed.

Tide pools that are connected to the ocean receive a constant influx of new water via waves. On the other hand, tide pools that are farther from the ocean contain a given amount of water that experiences little turnover over relatively long periods of time. As these unconnected tide pools are subjected to heat from the sun, evaporation occurs, and the temperature and salinity of these pools rise. Because connected tide pools have smaller ranges in average temperatures and salinities when compared to unconnected ones, we may observe higher densities of gastropods and hermit crabs in connected tide pools. This trend may be more pronounced in hermit crabs because hermit crabs are more mobile and can move away from harsh environments more quickly than gastropods. However, because connected tide pools face a constant onslaught of waves, connected pools may actually have harsher conditions than unconnected ones. If this turbulent environment discourages snails and hermit crabs from living in connected tide pools, then we may observe lower densities of gastropods and hermit crabs in connected tide pools. Because snails are better able to hold onto the rocks than the more mobile hermit crabs, an even lower density of hermit crabs than gastropods might be observed in the connected tide pools. Finally, it is possible that the differences in temperature, salinity, and turbulence from waves between connected and unconnected tide pools are not large enough to

be biologically significant to gastropods or hermit crabs. If this is the case, then we may observe no difference in gastropod or hermit crab density between connected and unconnected tide pools of the same size.

Because it takes more energy to heat a larger volume of water, larger tide pools experience less variation in temperature and salinity over the course of the day. Because the relatively larger variation in abiotic factors over the course of the day in smaller pools may represent harsher conditions for gastropods and hermit crabs, we may observe higher gastropod and hermit crab densities in pools of a larger volume. This trend may be more pronounced in hermit crabs, which can move more quickly away from harsh environments. Alternatively, any observed differences in temperature and salinity variation between pools of different sizes may not be large enough to be biologically significant to gastropods or hermit crabs. In fact, in connected pools, it is possible that no variation in temperature or salinity occurs over the course of the day due to the constant replacement of the water in the pools by the waves. If this is the case, then we may observe no difference in gastropod or hermit crab density between pools of varying sizes.

METHODS

Sampling Methods

To determine the effect of pool size and ocean connectivity on the density of snails and hermit crabs found in tide pools, we surveyed 30 tide pools at Salt Rock on Little Cayman that possess easy to define boundaries. 15 of the pools were connected to the ocean and receive a constant influx of new water from the ocean via waves. The other 15 pools, which were not directly connected to the ocean, were found at a distance of 15 +/- 5 m from the ocean. To determine how different abiotic factors, affect the density of snails and hermit crabs found in the tide pools, YSIs (Model 63-10FT and Model Pro 10102030, Yellow Springs Instruments) were used to measure values for abiotic conditions in each of the pools. The Model 63-10FT was used to determine the temperature and salinity in the center of each pool. Similarly, the Model Pro 10102030 was used to determine the dissolved oxygen content in the center of each pool. All of

these point measurements were taken between 3 and 5 pm.

To determine how the temperature of the tide pools varied throughout the day, we placed Hobo Pendant Loggers (onset), which took temperature measurements every hour, in the center of the first eight pools surveyed in our study for 24 hours. This turned out to be 6 unconnected tide pools of varying volumes and 2 connected tide pools. The second Hobo Pendant Logger placed into a connected tide pool was lost due to the turbulence of the pool in which it was placed. To find an approximate volume for each pool, we used a transect tape to measure the greatest length of the pool. The width of each pool was then measured at the widest point of the pool at a 90° angle to the plane in which the length was measured. Finally, the depth of each pool was measured at its deepest point using a meter stick. Rough approximations for pool volume were calculated by multiplying together these width, length, and depth measurements.

After laying out transect tape along the greatest length of the pool, we performed a count of both the hermit crabs and snails found within 0.125 m on either side of the tape measure. To determine the proportion of these individuals that were snails, we took a subsample of the population (one individual of every 20) that we counted and kept a separate tally of how many were hermit crabs or snails. To determine the density of snails found within each pool, we multiplied the total number of shelled individuals in the transect by the proportion of shelled individuals in the transect that were snails, and then divided this value by the area of the transect, to get the number of snails per m². The area of the transect changed for each pool and was found by multiplying 0.25 m by the length of that pool.

Statistical Analyses

We utilized student's t-tests to analyze the effect of connectivity on pool salinity, temperature, and dissolved oxygen. We tested the relationship of pool volume and connectivity to gastropod and hermit crab density using a generalized linear model with a Poisson distribution. We tested for the effect of connectivity on gastropod density with a generalized linear model, with a

Poisson distribution, that included ocean connectivity as a categorical variable, volume as a continuous variable, and their interaction. We also tested for the effects of connectivity on hermit crab density with a generalized linear model, with a Poisson distribution, that included ocean connectivity as a categorical variable, volume as a continuous variable, and their interaction.

RESULTS

When compared to unconnected tide pools, connected tide pools had lower salinity (Student's t-test: $t = 3.80$, $df = 22.44$, $p = 0.0010$; Fig. 1), lower temperature (Student's t-test: $t = 8.15$, $df = 15.61$, $p < 0.0001$; Fig. 1), and lower dissolved oxygen (Student's t-test: $t = 7.81$, $df = 19.87$, $p < 0.0001$; Fig. 1). Connected tide pools had a mean temperature of 27 °C, while unconnected tide pools had a mean temperature of 32.1 °C. Connected tide pools had a mean salinity of 41.9 ppt, while unconnected tide pools had a mean salinity of 44.2 ppt. Finally, connected tide pools had a mean dissolved oxygen content of approximately 6 mg/L, while unconnected tide pools had a mean dissolved oxygen content of approximately 9 mg/L.

Across the 30 tide pools surveyed, a total of 6656 gastropods and 3253 hermit crab were counted. The mean number of gastropods counted per pool was 222, while the mean number of hermit crabs counted per pool was 108. Pools varied in volume between 0.040 m³ and 27.42 m³.

Connected tide pools had significantly lower gastropod densities than unconnected pools (Generalized linear model, main effect of connectivity: $\chi^2 = 4,836.65$, $df = 1$, $p < 0.0001$; Fig. 2). As pool volume increased, gastropod density in connected pools remained constant while gastropod density in unconnected pools increased (Generalized linear model, interaction effect between connectivity and volume: $\chi^2 = 258.74$, $df = 1$, $p < 0.0001$; Fig. 2). Similarly, hermit crab densities were higher in unconnected pools when compared to that in connected pools; in fact, hermit crabs were entirely absent from connected pools (Generalized linear model, main effect of

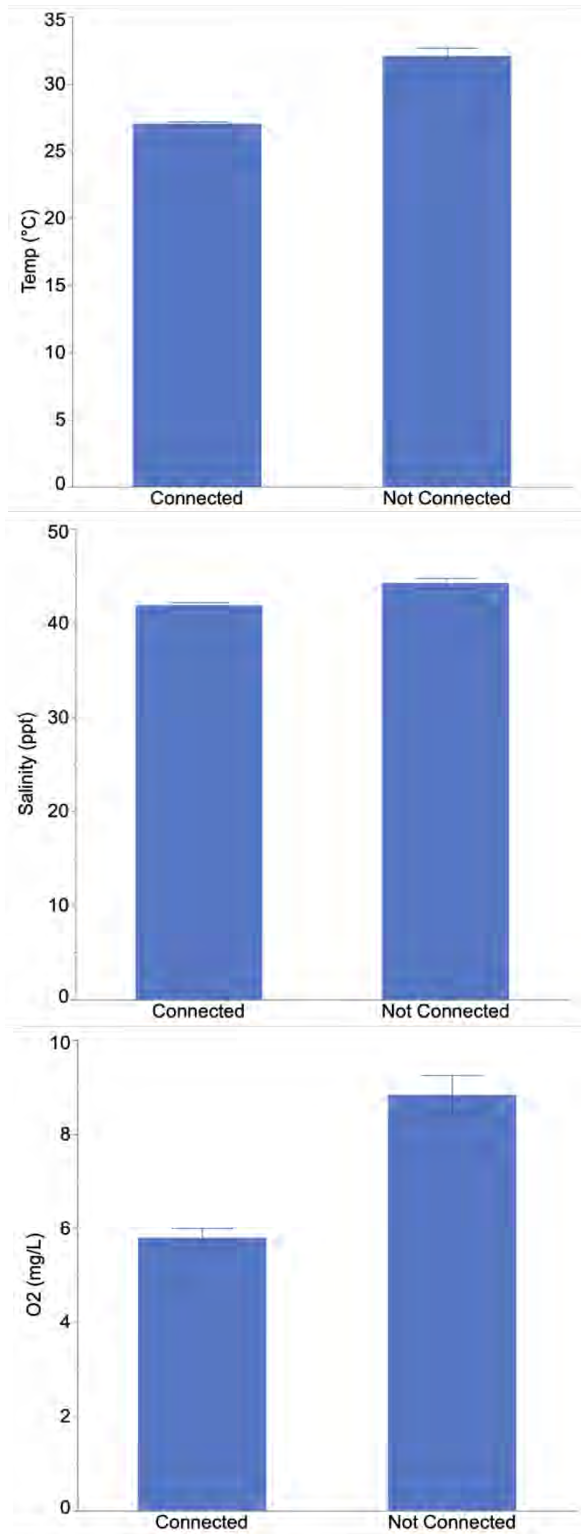


Figure 1: Mean temperature (top), salinity (middle), and dissolved oxygen (bottom) for connect vs. not connected pools. Error bars constructed using one standard error from the mean.

connectivity: $\chi^2 = 1,562.93$, $df = 1$, $p < 0.0001$; Fig. 2). As pool volume increased, hermit crab density in connected pools remained zero while snail density in unconnected pools increased (Generalized linear model, interaction effect between connectivity and volume: $\chi^2 = 4.31$, $df = 1$, $p < 0.0378$; Fig. 2).

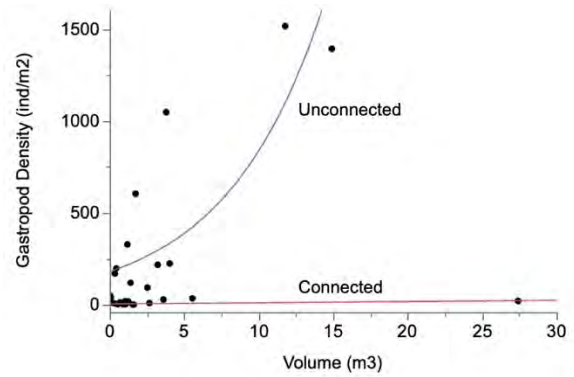


Figure 2: Gastropod density as a function of pool volume. Trend lines based on a generalized linear model based on a Poisson distribution. Exponential function represents unconnected pools, linear function represents connected pools.

Unconnected pools appeared to have more daily variation in temperature than connected pools, with lower volume unconnected pools having greater variation than larger volume unconnected pools (Fig. 3). Daily variation in temperature decreased significantly with increasing pool size in unconnected tide pools (Linear regression: $F = 15.88$, $df = 1,4$, $p = 0.016$; Fig. 4).

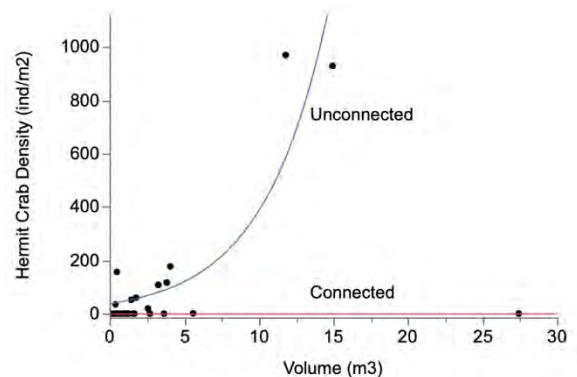


Figure 3: Hermit crab density as a function of pool volume. Trend lines based on a generalized linear model based on a Poisson distribution. Exponential function represents unconnected pools, linear function represents connected pools.

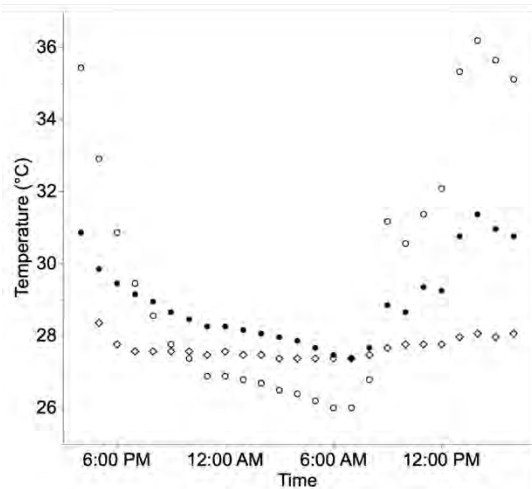


Figure 4: Temperature data comparison for three select tide pools of varying size and connectivity. Open circles represent a small, unconnected pool. Closed circles represent a large, unconnected pool. Open diamonds represent a large, connected pool.

DISCUSSION

Despite having lower temperatures and salinities than the unconnected pools, we found that connected tide pools had significantly lower densities of both gastropods and hermit crabs. These disparities in density are likely a result of the higher turbulence in connected pools from consistent wave action. Observationally, only a much larger, unidentified, gastropod species (shells had a diameter of ~ 13 cm) was found in the connected tide pools, potentially due to their increased ability to maintain a grip on the pool bottom. No hermit crabs were found in connected pools, potentially due to their inability to maintain a stable hold in the turbulent environment.

Gastropod and hermit crab densities exhibit a significant, positive, exponential relationship with unconnected pool size. These results may be explained by the differences in daily temperature variation experienced by pools of different sizes. Larger pools have significantly less variation in temperature and therefore might be more hospitable to gastropods and hermit crabs. Over time, both gastropods and hermit crabs may move away from the harsher environment of the smaller tide pools and congregate in larger densities in the larger ones. Although not measured in our study, variation in

other abiotic factors over the course of the day, including salinity, is also impacted by pool size and may have a similar impact on gastropod density (Daniel 1975). After significant evaporation occurs in pools during hot afternoons, the salinity in these pools is likely to be very high. At other times, rainwater entering unconnected pools has the potential to decrease the salinity of unconnected tide pools. This effect would be most stark in smaller pools. The large variations in salinity found in small, unconnected pools may also explain the lower observed densities of gastropods and hermit crabs within them.

Pool volume did not affect gastropod density in connected pools. With a constant exchange of water between the tide pool and ocean, these pools experience a much smaller variation in temperature regardless of pool size. As a result, temperature fluctuations are less likely to present as a limiting factor in gastropod density in these pools. It is likely that other factors, including salinity and dissolved oxygen, also follow the trend of varying little in connected pools.

Given the physiology of hermit crabs and their increased ability to move quickly towards tide pools with hospitable conditions when compared that of snails, we expected hermit crabs to be found in lower densities in pools with harsher conditions. However, we observed similar patterns of distribution between gastropods and hermit crabs. A potential explanation for these findings is that the general conditions experienced in the tide pools had been consistent for an extended duration prior to our study in which density measurements were taken. This would give even the less mobile snails time to move towards the more hospitable tide pools. Over the course of our study, there was no rainfall or large storms. Had rainfall significantly altered the temperature or salinity of the pools or had storms washed new snails and hermit crabs into the tide pools, this period of stability may have been interrupted. Had we taken measurements immediately following some stress to the system or had we artificially altered equilibrium values for snail and hermit crab densities in each of the pools, then we may have been able to observe the differences in mobility between the different species.

Despite the differences in their mobility, both gastropods and hermit crabs were found to follow the same trends in relation to the abiotic factors measured. Although they were both still found in the smaller unconnected pools that have much greater temperature fluctuations, both were found at higher densities in larger pools. Because the communities in these pools have developed over a long period of time, it is likely that the gastropods and hermit crabs are both physiologically capable of enduring pools that contain either high or low variations in temperature and salinity. This thought process is in line with Janzen's seasonality hypothesis, which postulates that organisms which experience greater variability in their environment are more suited for variation, and that this, in time, may lead to adaptation (Janzen 1967). Having observed both species in large and small unconnected pools, our study supports this conclusion about variation and tolerance.

Both gastropods and hermit crabs were found in greater densities in larger unconnected pools. One explanation for these findings is that, though they can survive in both environments, the preferred environment for organisms of both species is pools where the abiotic conditions vary little. If this is the case, gastropods and hermit crabs may follow a version of the ideal free distribution theory wherein they choose to inhabit larger pools in greater numbers until the point at which favorability due to low variation in abiotic conditions is outweighed by the cost of overcrowding and resource sharing (Fretwell 1969). In order to test this theory, one could experimentally manipulate the densities of gastropods and hermit crabs across tide pools and track the movement of manipulated and control individuals towards a stable distribution across a series of days. When combined with a laboratory experiment testing for the physiological stress of abiotic factors, such as temperature and salinity, on gastropods and hermit crabs, you could see if field distribution patterns match what would be predicted by lab stress levels.

Similar to other marine and terrestrial systems, abiotic factors were found to be critical in determining the distribution of species in tide pools. However, it cannot be assumed that general principles used to explain distributions of species within other systems with lower rates of variation can be used to describe tide pools. Rather, as communities that exist in an environment of regular, dramatic changes in abiotic conditions, they must be studied separately and extensively in order to understand how ecological theories apply, and how their effects may be confounded by the mobility capabilities of individual organisms.

ACKNOWLEDGEMENTS

Thank you for Ben for leaving our group and thank you to Katy for standing up to Max and Lij when they were being annoying.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this work.

LITERATURE CITED

- Daniel, M. J. 1975. Diurnal variations in physiochemical conditions within intertidal rockpools. *Field Studies* 4: 161-176.
- Forcada, J., and P. N. Trathan. 2009. Penguin responses to climate change in the Southern Ocean. *Global Change Biology* 15: 1618-1630.
- Fretwell, Haney, D. C., and S. J. Walsh. 2003. Influence of Salinity and Temperature on the Physiology of *Limia melanonotata* (Cyprinodontiformes: Poeciliidae): A Search for Abiotic Factors Limiting Insular Distribution in Hispaniola. *Caribbean Journal of Science* 39: 327-337.
- Stephen Fretwell. 1969. On territorial behavior and other factors influencing habitat distribution in birds. *Acta Biotheoretica* 19: 45-52.
- Janzen, D. H. 1967. Why Mountain Passes are Higher in the Tropics. *The American Naturalist* 101: 233-249.
- Peck, L. S., K. E. Webb, A. Miller, M. S. Clark, and T. Hill. 2008. Temperature limits to activity, feeding and metabolism in the Antarctic starfish *Odontaster validus*. *Marine Ecology Progress Series* 358: 181-189.

ARE OCTOPUSES PICKY EATERS?

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Abstract: Specialized generalists are species' whose foraging behaviors fall in between the classifications of generalist and specialist. They are opportunistic generalist feeders that have specialized diets at the individual level. This individual level distinction is rare in comparison to the generalist/specialist distinction in foraging patterns observed at the species' level. While the common octopus (*Octopus vulgaris*) is a known generalist feeder, research suggests that they may be specialized generalists. To further test this hypothesis, we observed the composition of discarded carcasses, called middens, outside 18 common octopus dens on Little Cayman Island. We found a density of 1.5 common octopus dens / 100 m², an unexpectedly high density compared to previous studies. Of the 18 middens measured, we found a total of 11 prey types and classified 13 octopus as specialists for crustaceans, bivalves or gastropods. Using a niche-breadth index measurement, we concluded that the population we sampled are indeed specialized generalists. The octopuses' different specialized diets may allow for them to partition the available prey in such a small area. Though midden composition may not disclose the full extent of an individual octopus's foraging patterns and preferences, the observed variance among prey composition outside of dens highlights a unique behavior, which may have more complex social implications for the enigmatic species.

Keywords: common octopus (*Octopus vulgaris*), middens, prey abundance, specialized generalist

INTRODUCTION

All animals require food resources to survive; however, there is considerable variation in resource use from very narrow to very wide across the animal kingdom. Generalists are opportunistic foragers who have a wide range of prey types, and therefore have the freedom to adapt to varying biotic conditions. Raccoons are a well-known example of generalist foragers, as they can utilize any food source. Conversely, specialist feeders rely on a narrow range of food sources and are thus very vulnerable to changes in their ecosystem. The survival of panda bears, who are extreme specialists, is tightly coupled to the health of bamboo because of their extreme reliance on the plant. Classifying a species as either a specialist or generalist is a common distinction. However, throughout nature, this category is rarely analyzed on an individual basis.

When physiological conditions are optimal and food abundances are high, individuals of generalist species might specialize at the individual level. Studies on foxes, kestrels, sunfish and frogs have all discovered prey choice variability for single individuals (Mather 2012). This behavior is coined in individuals as a 'specialized generalist.' One of the common

reasons for the specialized generalist behavior is due to variation in size, sex and morphology across individuals. These morphological differences are often traced to sub-populations, rather than on an individual level. For example, large adults may have different diets than small adults, despite being a generalist species. Territoriality and social status can also limit the prey size and type an individual can access. For individuals of similar size and social status, prey choices may be dictated by competition with conspecifics. Additionally, what food is accessible or abundant on a local scale can drive individual generalists to specialize. Lastly, if there is excessive food abundances and limited competition, it is possible that an individual's prey choice could be based on personal preference alone.

We examined the specialized generalist phenomenon in the common octopus (*Octopus vulgaris*) in Little Cayman. As nocturnal foragers, the common octopus goes to and from their shelter to retrieve their prey, which they consume and discard outside of the entrance of their dens. Discarding their prey outside of their den forms a pile of shells, called a midden, which makes their home base very conspicuous and easy to find. The

creation of middens allows for an insight into an individual octopus' diet. This accessibility to an individual's prey type makes the common octopus a great species for studying specialization within a generalist population.

In this study, we analyzed the variation in midden composition across octopus' dens found outside of the CCMI research station on Little Cayman Island. Prior research suggests that individual octopus may specialize in the types of organisms on which they select to prey (Anderson 2008). Anderson theorizes that this specialization might be context-dependent, and octopus may switch their preference when the ease of capturing specific prey is altered (Anderson 2008). This would suggest that as a species, the octopus are generalists, but under certain environmental conditions, they may exhibit specialist foraging behavior. Therefore, we hypothesized that octopus will be specialized generalists based on their local environment, and we will observe unique midden compositions depending on what prey availability is around the den. Conversely, we may find that the octopus are specialized generalists regardless of their habitat and den composition will be entirely unique to individuals. Finally, octopuses might be strictly generalists- opportunistically foraging on all the available prey. Thus, we predict that the midden composition will not vary among individuals.

METHODS

To measure individual feeding preference, we utilized the midden making behavior of the common octopus, assessing the variation in the composition of discarded prey outside of the dens. To find octopus dens, we opportunistically sampled a 100 m x 30 m area outside of the CCMI research station on the north side of Little Cayman Island over three consecutive days in March 2020 (Fig. 1). We swam around the designated area searching for accumulations of prey remnants such as conch shells and bivalve, indicating a midden and therefore a den. If a den had a midden, we measured the number of prey and prey composition. To measure midden compositions, we identified the prey down to the lowest taxonomic unit. We distinguished crabs by a segmented carapace, usually the head, and gastropods by shell. Bivalves were usually found separated and were matched where possible but unmatched valves were also counted as an animal

(Mather 2012). To classify the substrate within our sampling area, we laid two 10 m transects radiating out from the den in directions representing the general substrate composition. We swam along 10 m transects and video recorded the ocean floor which we used to categorize the substrate.

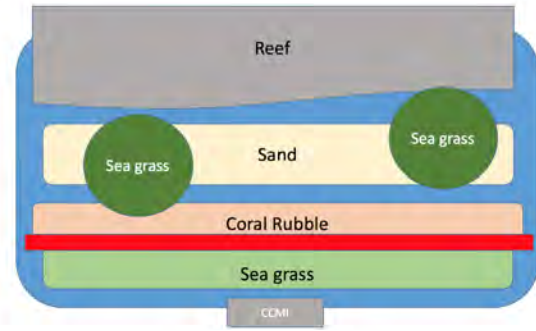


Figure 1: Schematic of sampling plot (100 m x 30 m). A majority of the dens were found along a natural shelf formed between coral rubble and the seagrass substrate types (13/18). This is indicated by the red line.

Statistical methods

To quantify the specialization level of the octopuses, we used a niche-breadth index (Cardona 1991) using the following equation:

$$B' = (\sum(F_i) - \sigma) / (100 * R)$$

F_i is the occurrence frequency for each prey type (proportion of dens containing each prey type), σ is the standard deviation of the occurrence frequencies and R is the number of prey species (11). For this index, B' ranges from 0 to 1, with values closer to zero indicating specialization and values closer to one representing generalization.

To identify the specialists and generalists, we calculated the proportion of prey in each midden into crustaceans, bivalves and gastropods. If one midden contained a majority (i.e. >50%) of one of the three prey classifications, we labeled it as a specialist of that type. If a midden composition did not contain a majority of one prey type, we labeled it as a generalist.

RESULTS

In total, we observed 20 dens, 18 of which had middens. Of the 18 middens, the mean number of prey measured was 9.94 +/- 6.37 SD. We found a total of 11 different prey types including gastropods, bivalves, and crustaceans (Table

1). We classified 13 octopus as specialists and 5 as generalists based on their midden compositions (Fig. 2). Of the 13 specialists, we classified 6 as bivalve specialists, 6 as gastropod specialists, and one as a crustacean specialist. The niche-breadth index value was 0.323 (Table 1).

We found 1.5 dens per 100 m² with a mean distance between dens of 21.4 +/- 7.9 SD, 72% of dens were found along the periphery of coral rubble and sea grass substrates and most were in very shallow water (<1 m). Since the dens were found so close together, we assumed that each octopus had an equal opportunity to hunt a variety of prey. Therefore, we concluded that substrate composition surrounding each den did not influence the prey type.

Table 1: The midden counts represent the number of middens that contained at least one of the prey types. F_i (occurrence frequency) represents the percentage of middens that contained at least one of the prey types.

Species	Midden count	F_i
<u>Mollusca</u>		
Queen Conch	9	45
Faust Tellin	12	12
Tiger Lucine	10	50
Filose Turban	1	5
Reticulated Cowrie-Helmet	4	20
Common Nutmeg	1	5
Ivory Cerith	6	30
Limpet	1	5
Unknown Gastropod	10	50
Unknown Bivalve	11	55
<u>Arthropod</u>		
Crab	6	30

Observational results

Some of the gastropods we counted may have had live hermit crabs in them. One den had eight

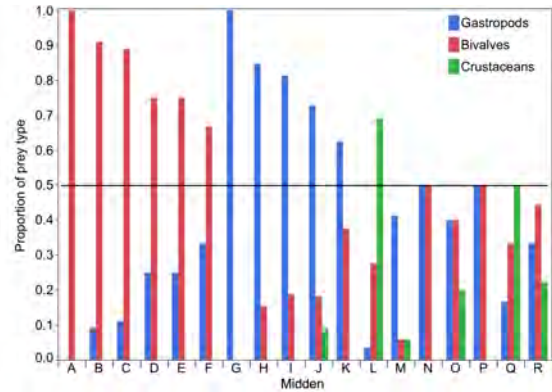


Figure 2: Midden compositions represented by the proportion of prey type; gastropods (blue), bivalves (red), and crustaceans (green). The line across the 0.5 mark defines the octopuses into specialists (above the line) and generalists (below the line). Middens A-F are characterized as bivalve specialists, G-L are gastropod specialists, M is a crustacean specialist and M-R are generalists.

hermit crabs surrounding the midden. Therefore, some of the gastropod shells we counted may have been hermit crabs.

DISCUSSION

Our results show that the common octopuses found outside of CCMI on Little Cayman Island are specialized generalists, and this is behavior may be driven intense competition due to the close proximity of their dens. In the case of an extreme generalist, all prey taxa would be represented in 100% of the middens, resulting in a B' value close to 1.0. In the case of an extreme specialist, there would be one prey taxa that is represented in 100% of the middens, resulting in a B' value close to 0.1. Our calculated B' value of 0.323, leans towards the specialist end of the index, indicating that individuals in this population of the common octopus might be on average specialized generalists. We reject the hypothesis that the specialized generalist behavior is due to variation in substrate type, since some of the dens were only 20 m apart from one another. We assume that all octopuses had equal opportunity to forage on a variety of prey thus, the individual specialization cannot be explained by variation in the abundance of prey in the immediate surrounding of the dens.

A surprising result was the extremely high density of 1.5 common octopus' dens per 100 m². Other studies have found densities which are orders of magnitudes lower than what our data

revealed. Values such as 0.0047 octopus per m³ during daytime sampling and ranges between 0 – 0.688 individuals per 100 m² have been found when sampling night and day (Katsanevakis and de Beer). One potential reason that we observed a shockingly high density of octopus within our sampling area is due to the presence of the coral rubble shelf that runs parallel to the shore. It is well accepted that many octopus' species prefer rock crevices and spaces between rocks and reef (de Beer 2013).

Other factors may define the interplay of octopuses' spatial distributions and specialization. Through specialization, fine scale niche partitioning may determine why so many octopus can occupy a small area; the presence of microhabitats within a sampling area may allow for individuals to exhibit specialist foraging behavior (Bennice 2018). While our data are vastly different than prior work, competition for space and/or resources may also be at play and therefore, individuals which display specialized generalists' behaviors may be more common in high density areas due to the necessity of crafting a unique and sustainable niche. Another explanation is that this sampling area is not resource scarce and individual octopuses are simply picky eaters. The only way to make this distinction is to measure the surrounding prey availability, which is advisable for future research.

There are a few other explanations for the specialized generalist behavior which we were unable to account for in this study. For example, size and age, which are nearly impossible to measure when common octopus are inside their dens may influence diet choice. Although the common octopus are believed to be asocial and solitary for their non-reproductive phase, this is largely understudied and an individual's place within their social group may influence prey choice. A possible experiment may be a measure of individual prey preference in the presence versus absence of other individuals. Middens may not explain the full diet or tell us rates of consumption, as discarded items can be easily moved away from the midden by the current or other animals. This is commonly exemplified by hermit crabs, who opportunistically forage for empty or discarded shells. Therefore, future studies should assess the rates of prey remnant

removal from the middens, which may come both from natural ocean forces and from the reuse of midden content by other marine organisms. Middens are naturally ephemeral, as one study found that 50% of midden contents were lost in 5 days (Mather 1991).

Midden contents serve as a snapshot in time, which we can use to observe current behaviors and predict how individuals might vary in their prey preferences. The observed variation among prey composition outside of dens highlights the unique specialized generalist behavior, which in turn provides insight into whether the species as a whole exhibits specialized generalists behavior and the degree to which individual foraging strategies vary. Nuances in these behaviors may prove to have more complex social implications for the enigmatic species. Further, this highly individualized behavior may begin to emerge as additional foraging studies are conducted on other species in the animal kingdom.

ACKNOWLEDGEMENTS

Thanks to CCMI for unknowingly housing hordes of octopus right in the backyard!

AUTHOR CONTRIBUTIONS

All authors contributed equally to this work.

LITERATURE CITED

- Anderson, R., Wood, J., and Mather, J. 2008. Octopus vulgaris in the Caribbean is a specialized generalist. *Marine Ecology Progress Series* 371: 199–202.
- de Beer, C.L. and Potts, W. M. 2013. Behavioural observations of the common octopus *Octopus vulgaris* in Baía dos Tigres, southern Angola. *African Journal of Marine Science* 35:4. 579–583.
- Bennice, C., Rayburn, A., Brooks, W. R., and Hanlon, R. 2018. Fine-scale habitat partitioning facilitates sympatry between two octopus species in a shallow Florida lagoon. *Marine Ecology Progress Series*. 609.
- Cardona, L. 1991. Measurement of trophic niche breadth using occurrence frequencies. *Journal of Fish Biology*, 39(6), 901–903.
- Katsanevakis, S., and Verriopoulos, G. 2004. Abundance of *Octopus vulgaris* on soft sediment. *Scientia Marina*, 68:4. 553–560.
- Mather, J. A., Leite, T. S., and Batista, A. T. 2012. Individual prey choices of octopuses: Are they generalist or specialist? *Current Zoology*, 58:4. 597–603.

NO SAFETY IN NUMBERS: THE IMPACT OF PREDATION PRESSURE ON PREY AGGREGATION IN A CORAL REEF ECOSYSTEM

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Abstract: Aggregating behavior in prey species is theorized as a response to predation pressure. Communities in coral reefs exhibit high predative pressures and fish prey aggregation behaviors, making them viable study systems for examining predator influence on prey defensive strategies. On Little Cayman Island, we measured densities of top-level fish predators and their aggregating prey fish. Our results showed that predation pressure does not change from the forereef to the backreef, that there is no relationship between predator density and prey density, and that the proportion of fish aggregating significantly decreased with increasing relative predator to prey ratios. This study highlights the complex interspecies interactions which lead to aggregation behaviors and possible limits of dilution effects in prey aggregations under high predation pressure.

Key words: aggregation, coral reef, fish, predation pressure, top-level predators

INTRODUCTION

Reef ecosystems are energy- and nutrient-rich zones in depauperate tropical oceans; since so many resources are concentrated in such a small area, predators and prey are often in close proximity, and high rates of predation in high trophic levels could cause behavioral changes in reef inhabitants (Sale 1991). Top-down controlled reefs, with prey fish abundances limited by predation, can be characterized by abundant and diverse predator assemblages. These typically comprise large proportions of the biomass and species richness in their ecosystems. In an estimate by Goldman and Talbot (1976), 54% of the biomass at One Tree Island on the Great Barrier Reef is comprised of predator species (Sale 1991). For prey species, this pressure necessitates effective choice of defense strategies.

Prey fish exhibit morphological and behavioral adaptations for predator defense in top-down controlled systems. Morphological adaptations indicating high predation pressure include mechanical, aesthetic, and chemical defenses (Sale 1991). Behavioral adaptations like sheltering in safe spaces, crepuscular inactivity while predators are most active, and aggregation in large groups all serve to protect prey against predation, but these adaptations are more flexible. Among such behavioral adaptations, aggregation is one of the most immediately flexible, and is used by a wide range of fish species as a direct predator defense mechanism.

Aggregating may benefit prey fish by several mechanisms, including the following: 1) aggregation dilutes the direct predation pressure on any individual within a group (Bertram 1978); 2) aggregation allows fish to mob predators, which can force predators to relent (Sale 1991); 3) aggregations allow for all individuals to benefit from one another's vigilance while foraging (Bertram 1978). Aggregation may, however, have negative consequences as well; proximity to conspecifics may make aggregating fish more likely to contract disease, or large groups may be more visible targets than individual fish. On the whole, aggregation is often used by prey fish as a predator defense and may serve as a useful proximal indicator of a fish population's perceived predation pressure. Therefore, the degree of aggregation observed may be directly sensitive to predator abundance and density.

The reefs surrounding Little Cayman Island in the Caribbean are a useful system for studying predator influences on prey abundance and behavior. As reef ecosystems are resource islands supported by coral in an oligotrophic sea, fish in reef ecosystems are forced to dwell in the same limited habitat as the predators which will eventually eat them. Top-level predator species have been identified by observation of foraging, which demonstrates a species' prey preference, and by stable isotope tissue analysis, which is widely used to determine the trophic level of consumers (Page et al. 2013). In Little Cayman

reefs, the top trophic guild is comprised of *Epinephelus* (groupers), *Sphyrnaea barracuda* (great barracuda), *Ginglymostoma cirratum* (nurse shark), and *Carcharhinus perezii* (reef shark). Their prey species include *Caranx* (jacks), *Lutjanus* (snappers), and *Haemulon* (grunts).

Our study aims to determine the relationship between top-level predator abundances and the resulting defensive aggregation behavior of their primarily piscivorous prey, hereafter referred to as prey fish. Some prey fish eventually reach sizes beyond which they are too large to safely hide in reef cavities, and forage by roving for long periods of time, exposed to predators as they do so. As a result, the primary behavioral response of these species to perceived predation pressure is likely to be aggregation; if top-down pressure is of great consequence for their survival, this behavior should be sensitive to the predation pressure in a given reef area.

If aggregation is a fundamental predator defense strategy in these prey fish, then in the face of high predator densities and predation pressure, aggregations will become larger and more prevalent. Furthermore, if prey abundances are largely limited by predation, we expect to observe a negative relationship between predator density and prey density. If aggregation is not a fundamental predator defense strategy in these fish, or if this system is not top-down controlled, then we will not observe a relationship between predator and prey densities, nor any change in aggregation size or frequency with predation pressure.

METHODS

Survey Methods

To quantify top-level predators and aggregating prey fish, we snorkeled ten 20-minute transects at three sites (known locally as Cumber's, Mary's Bay, and Central Caribbean Marine Institute (CCMI)) on Little Cayman Island. Cumber's has a relatively deep reef flat, while Mary's Bay and CCMI have a mid-depth and shallow reef flat, respectively. We chose top-level predators and aggregating prey fish based on stable isotope studies and their food web dynamics. Barracuda, grouper, and reef shark all have $\delta^{13}\text{C}$ values between -10.2 and -10.9, placing these species at the same trophic level (Bond et al. 2018). Prey fish were chosen based on their known feeding habits

and natural predators (Page et al. 2013). We took GPS coordinates at the beginning and end of each transect to calculate distance and swam transects in the forereef and backreef at each site. Distance from shore remained approximately consistent between sites and was observed using satellite imagery of our transects (Fig. 1). To calculate densities of top-level predators and aggregating prey fish (Supplemental Fig. 1), we recorded each observation we encountered within 10m of our transect line. If prey fish were aggregating, we also recorded the number of each species present in the aggregation. Prey fish were considered aggregating if they were in a group of two or more, within 5 body lengths of each other, and following the same changes in swimming direction or remaining stationary and closely associated

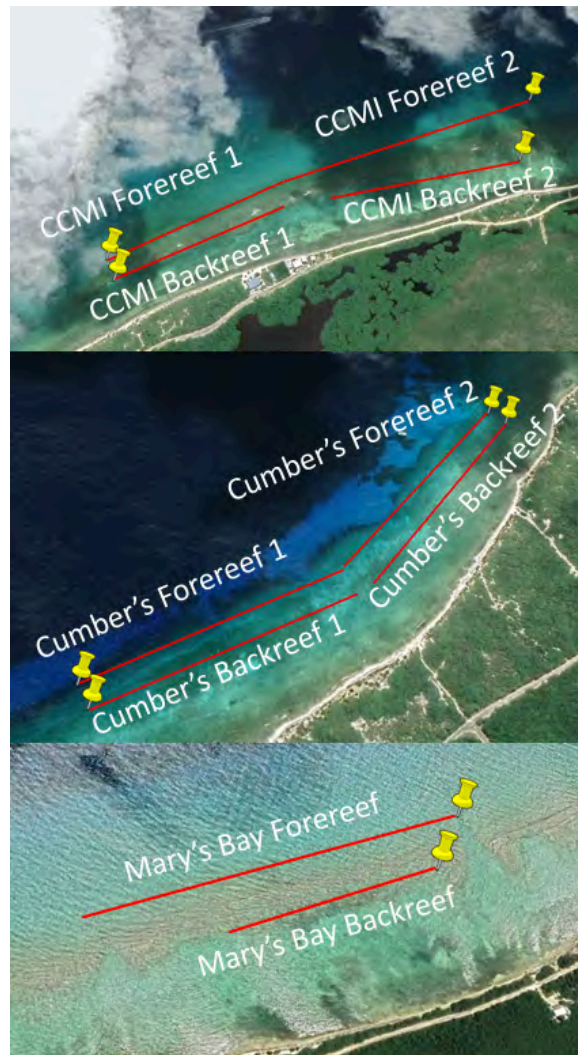


Figure 1: GPS coordinates and distances of each transect at each site.

(Patterson 1998). We also noted if aggregations were observed feeding or displaying unique behaviors. Across the sites, we took a qualitative measurement of depth to test if there was a relationship between predator and prey abundance in deep, mid, or shallow habitats in each site.

Statistical Methods

For each transect, we calculated a predator and aggregating prey density by dividing the number of predator and prey fish we encountered by the distance of the transect. We used distance to calculate densities as the transect width was uniform. We calculated a predator:prey ratio as a metric of predation pressure on prey fish. We also calculated a proportion of prey fish which were aggregating; for example, if 10 total prey fish were on a transect, and 8 of them were in aggregations, the proportion aggregating was 0.8. Mean aggregation size was also calculated by taking the mean number of fish found in each aggregation observed in a transect. To compare predator and prey densities between forereef and backreef transects, we conducted t-tests. We performed an ANOVA to test if predator or prey density varied with reef depth. To test if predator density and predator:prey ratio had an effect on prey density, we conducted linear regressions. To test if predator density and predator:prey ratio affected mean aggregation size and proportion aggregating, we conducted generalized linear mixed models with site as a random effect. All response variables were tested for normality and found to be approximately normal. The residuals were homoscedastic.

RESULTS

Quantitative Results

We found no significant difference across the forereef and backreef in prey densities ($t = -0.59$, $df = 7.46$, $P = 0.57$; Fig. 2a) or in top-level predator densities ($t = 0.44$, $df = 7.08$, $p = 0.67$; Fig. 2b). Additionally, there was no significant difference in densities across different depths of transects for fish prey ($F = 2.29$, $df = 2, 9$, $P = 0.17$). Predator densities were higher at deep sites than at shallow sites ($F = 7.59$, $df = 2, 9$, $P = 0.0177$; Tukey HSD, $P = 0.0187$; Fig. 3); however, there was no difference between the mid and deep or mid and shallow sites. There was no relationship between top-level predator density

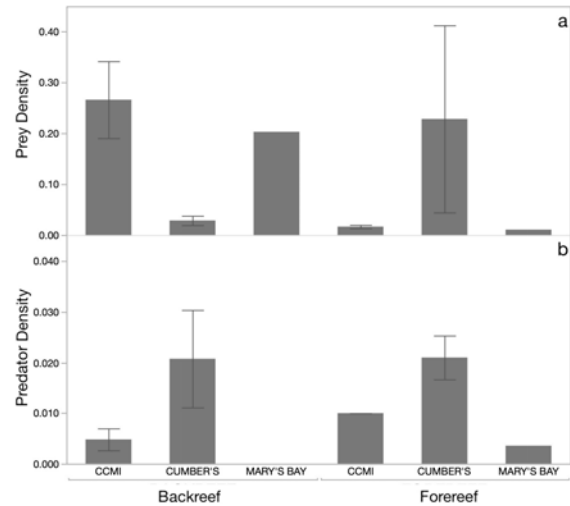


Figure 2: Prey (a) and predator (b) densities by location in the reef, by site.

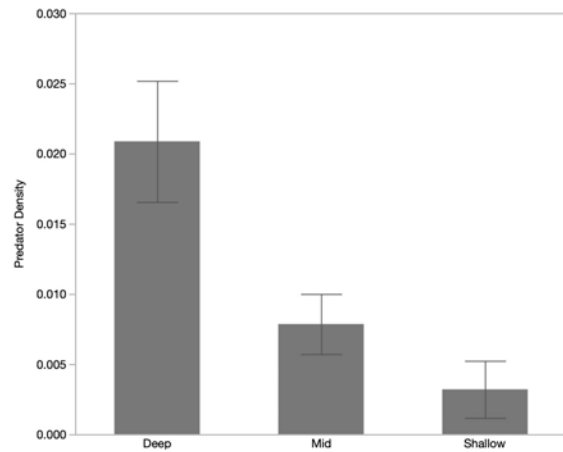


Figure 3: Mean predator density by depth. Mean \pm SE.

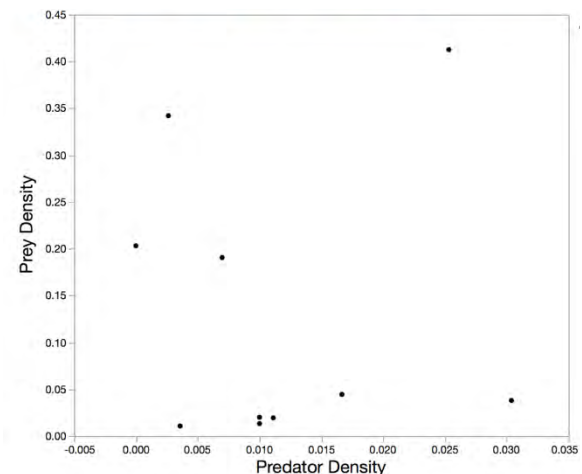


Figure 4: Predator density to prey density.

and prey density (general linear model; prey fish density: $F = 0.0009$, $df = 1$, 6.02 , $p = 0.98$; Fig. 4). Aggregation size did not significantly differ by species (general linear model; species: $F = 1.51$, $df = 6$, 51.06 , $p = 0.19$; Fig. 5). Predation pressure, calculated as the ratio of predators to prey, did not significantly change across the forereef and backreef ($t = 0.597$, $P = 0.57$, $df = 6.97$). We tested site as a random effect and found it to account for 64.75% of the variation.

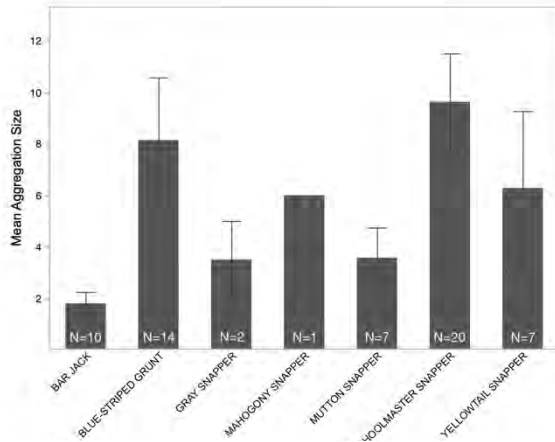


Figure 5: Mean aggregation size by prey species.

There was a negative trend between predator-to-prey ratio and mean aggregation size, though this was not significant (general linear model; predator to prey ratio: $F = 4.21$, $df = 1$, 2.83 , $p = 0.1376$; Fig. 6a). Site, when run as a random effect, accounted for 0.00% of the variation in aggregation size. We found a significant negative relationship between predator-to-prey ratio and proportion of fish that were aggregating (general linear model; predator to prey ratio: $F = 63.4$, $df = 1$, 6.48 , $p = 0.0001$; Fig. 6b.) Site, when run as a random effect, accounted for 49.7% of the variation in proportion of prey fish aggregating.

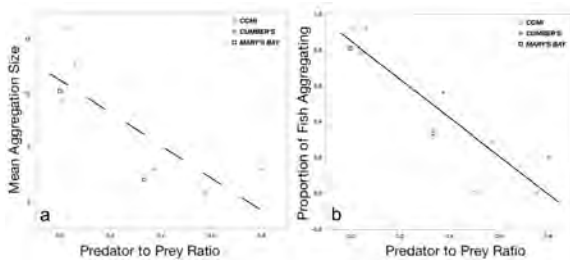


Figure 6: Mean aggregation size (a) and proportion of fish aggregating (b) in relation to predator to prey ratio. Mean aggregation size does not significantly vary with predator to prey ratio.

Qualitative Results

While measuring predator and prey densities in our transects, we noticed specific behaviors of top-level predators that could have implications for reef ecosystems. While no top-level predators were observed aggregating with other top-level predators, we noticed territorial behaviors between groupers (we observed a black grouper nipping, ramming, and chasing another yellowtail grouper). Also, novel to the authors, we observed rapid and distinct color-changing morphology in some of the observed great barracuda when barracuda were moving between substrate types. This has been observed in juveniles in other studies in Bimini, Bahamas (De Sylva 1963).

DISCUSSION

The key hypothesis that ratio and sizes of prey fish aggregations would increase with higher predation pressure was not supported. Instead, we actually found the opposite, with less aggregation in areas of higher predation pressure. While only the proportion of fish aggregating decreased significantly with increasing predator to prey ratio, the non-significant trend in mean aggregation size with predator to prey ratio also decreased and hinted at a similar theme. Critically, these findings contradict the prediction that aggregation behavior increases in habitats with more predation. One plausible explanation for this relationship relates back to other primary reasons for aggregations. If the aggregations in this sample set were a function of feeding or mating, it is possible that prey species we observed were preferentially aggregating in areas with lower predator to prey ratios if their food sources or ideal mating habitat happened to be in areas with lower predator to prey ratios. Alternatively, these results could be highlighting the limits of the dilution effect. While aggregating will decrease the probability of predation for an individual in that aggregation, a larger aggregation in a predator-rich environment could increase conspicuousness to a predator. If the increased frequency and damages of predator encounters outweigh the fitness benefits of being in an aggregation, it may no longer be an advantage for prey species to aggregate. Regardless of aggregation type, for feeding, or protection, these data show a selection against aggregation in habitats with high relative predation pressure.

Notably, these results contrast with literature that suggests schoolmaster snapper (*Lutjanus apodus*) are safer in larger aggregations but imply that predation dynamics on reefs could be emblematic of dynamics in other systems (Benjamin 2017). Stream dwelling caddisflies (*Rhyacophila*) in select high-predation environments have been used to prove the concept that aggregations can actually be a fitness detriment when frequencies and damages of predator encounters are significantly higher for aggregations than for individuals (Wrona and Dixon 1991). To confirm if increasingly frequent and damaging predator encounters are driving the aggregation dynamics we found in this system, future studies could specifically investigate the frequency and damage of predation events on prey fish aggregations.

There was no relationship between top-level predator density and prey fish density. We posit two possible reasons for this result: first, predators could hunt down prey fish populations in a non-density dependent fashion. Second, the high primary productivity of these reef ecosystems could bolster the prey population despite heightened top-down pressures in certain localized environments. These findings also support the use of a population-dependent predation metric; if predator density does not affect prey density, it is perhaps more accurate to use the predator density to prey density ratio as a measure of the risk of predation an individual would face. The predator to prey ratio takes into account the relative risk of predation given other prey conspecifics and heterospecifics in the area, whereas predator and prey density primarily look at predation in a spatial context.

The size of aggregations of prey fish varied between species, with the largest mean aggregation sizes in blue-striped grunt, mahogany snapper, and schoolmaster snapper. However, the differences in aggregation size between species were not significant. It is possible that aggregation behavior rather than size varied between species; blue-striped grunt is known to be territorial in most cases, but do aggregate for predation protection, whereas many snappers of the genus *Lutjanus* aggregate for feeding as well as predation protection (Balraj 2016). Differing reasons for aggregating may have affected the sizes and densities of aggregations we observed.

Given our initial observations and the spatial divides a reef crest creates, we expected to see variation in predator density between forereef and backreef environments. Contrary to this prediction, neither top-level predator density nor prey fish density significantly differed between forereef and backreef habitats. However, while there was no significant difference in prey by depth, predators exhibited higher densities in deep water. This is evidence of similar predation pressure and prey availability across forereef and backreef habitats, consistent with the hypothesis that recruitment of juvenile fish to backreef and later forereef habitats leads to high predation in these zones (Shulman 1985).

While ecological theory stipulates that aggregation provides a fitness benefit in areas with high predatory pressure, the results of this study highlight a possible limitation of dilution effects. Resource-rich environments with heavy predation are not limited to the tropical oceans, and the limits of fitness benefits of aggregation could be applied to more systems. The adage “safety in numbers” may be a simplistic explanation of the dilution effect, but in situations when there are high densities of top-level predators, safety in numbers may not be so safe.

ACKNOWLEDGEMENTS

Thanks to the friendly barracuda that followed Max and Ben during initial snorkeling near CCMI that inspired this study. Also thank you to the lovely CCMI staff who support our daily operations even if they do impose strict prohibition. Thank you to Celia and Melissa for putting us through the wringer to make us really consider the purpose of this study, and to Clare for always believing in us.

AUTHOR CONTRIBUTIONS

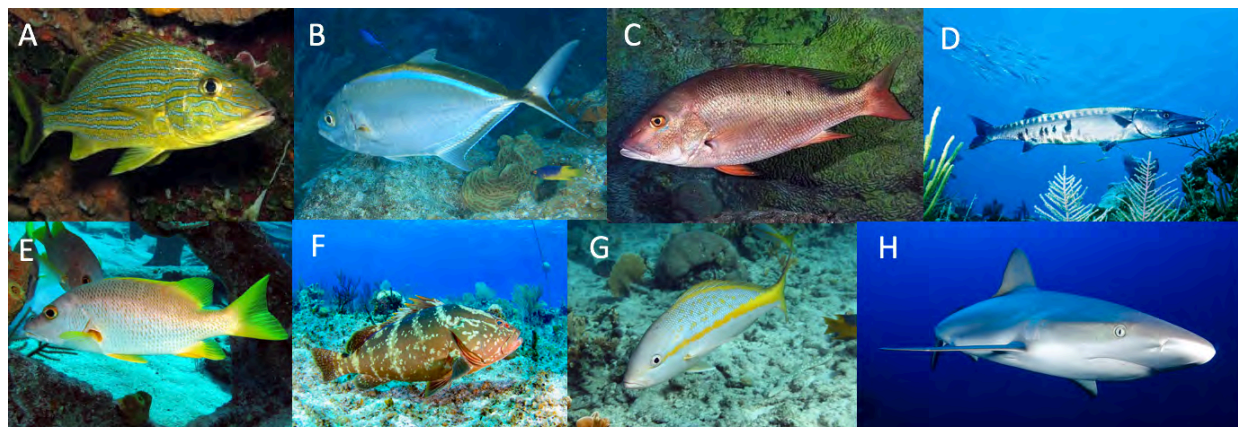
Over the course of this study, Max developed shark-ray vision, Lij explored a burgeoning career in underwater videography, Colton lost the usage of a single ear, and Ben explored the limits of how much data could fit onto a single slate. Truly a team effort, in all metrics.

LITERATURE CITED

Balraj, L. 2016. *Haemulon sciurus* (Blue Striped Grunt). University of the West Indies Life Sciences.

- Benjamin, M. 2017. *Lutjanus apodus* (Schoolmaster Snapper). University of the West Indies Life Sciences.
- Bertram, B. C. 1978. Living in groups: predators and prey. *Behavioural ecology: an evolutionary approach*, 221-248.
- Bond, M.E., Valentin-Albanese, J., Babcock, E.A. et al. 2018. The trophic ecology of Caribbean reef sharks (*Carcharhinus perezii*) relative to other large teleost predators on an isolated coral atoll. *Mar Biol* 165, 67.
- De Sylva, D. P. 1963. Systematics and life history of the great barracuda, *Sphyraena barracuda* (Walbaum).
- Page, H. M., Brooks, A. J., Kulbicki, M., Galzin, R., Miller, R. J., Reed, D. C., ... & Koenigs, C. 2013. Stable isotopes reveal trophic relationships and diet of consumers in temperate kelp forest and coral reef ecosystems. *Oceanography*, 26(3), 180-189.
- Paterson, S. E. 1998. Group occurrence of great barracuda (*Sphyraena barracuda*) in the Turks and Caicos Islands. *Bulletin of Marine Science*, 63(3), 633-638.
- Sale, P. F. (Ed.). 1991. Chapter 17: Predation as a process structuring coral reef fish communities. *The Ecology of Fishes on Coral Reefs*. Academic Press.
- Schulman, M. J. 1985. Recruitment of coral reef fishes: Effects of distribution of predators and shelter. *Ecology*, 66(3), 1056-1066.
- Wrona, F. J., Dixon, R. W. 1991. Group size and predation risk: a field analysis of encounter and dilution effects. *The American Naturalist*, 137(2), 186-201.

APPENDIX



Supplemental Figure 1: Some of the species we encountered during data collection. A: Blue-striped Grunt (*Haemulon sciurus*; prey). B: Bar Jack (*Caranx ruber*; prey). C: Mutton Snapper (*Lutjanus analis*; prey). D: Great Barracuda (*Sphyraena barracuda*; predator). E: Schoolmaster Snapper (*Lutjanus apodus*; prey). F: Nassau Grouper (*Epinephelus striatus*; predator). G: Yellowtail Snapper (*Ocyurus chrysurus*; prey). H: Reef Shark (*Carcharhinus perezii*; predator).

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https://www.inaturalist.org/guide_taxa/942024

https://en.wikipedia.org/wiki/Mutton_snapper

<https://www.dkfindout.com/uk/animals-and-nature/fish/barracuda/>

<https://reefguide.org/keys/pixhtml/schoolmaster2.html>

<https://rollingharbour.com/tag/nassau-grouper-spawning-nassau-grouper-threats/>

https://naturalhistory2.si.edu/smsfp/irlspec/Ocyurus_chrysu.htm

<https://www.robertharding.com/index.php?lang=en&page=search&s=grey%2Breef%2Bsharks&smode=0&zoom=1&display=5&sortby=0&bgcolour=white>

Little Cayman

IMPACT OF COMMUNITY ASSEMBLAGE ON CORAL DISEASE AND MORTALITY

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Abstract: Climate change and other human impacts have dramatically increased environmental stress on coral reefs around the world, which have led to increased rates of disease and death in these communities. While the impact of abiotic factors on coral health has been extensively studied, less is known about the effects of coral community assemblage on coral health. We quantified how alpha and beta coral diversity influence coral health across three different marine protection statuses on Little Cayman Island. Mortality was quantified by the percent cover of dead coral tissue across eighty quadrats. We hypothesized that more diverse coral communities possess a larger range of defense strategies and therefore may have less dead coral. We also hypothesized that areas with the highest level of protection against human interference would have the least dead coral. Surprisingly, we found that percent dead coral increased with higher alpha diversity, suggesting that interspecific competition was prevalent in more diverse quadrats. Of all 17 species of coral observed, seven were found to be diseased at least once, indicating that disease is a prominent cause of death of coral on Little Cayman Island. As climate change and other anthropogenic impacts continue to increase stress on corals, populations will continue to decline, possibly leading to the extinction of coral by the year 2050. By understanding the factors that influence coral health, we can better direct future conservation efforts and work to prevent further loss of such an essential ecosystem.

Keywords: coral, beta diversity, disease, health, Little Cayman Island, Shannon diversity

INTRODUCTION

Coral reefs serve as habitat for a wide variety of marine species. They are the most taxonomically diverse of all marine systems and define the physical structure of coastlines. Additionally, many human populations rely upon coral reef fisheries for sustenance and livelihood. For example, coral reefs fuel tourism, attracting visitors who support local economies. In the Caribbean, the coral reef tourism industry has been valued at \$2.1 billion USD (Burke and Maidens 2004). Though corals have persisted on earth for the last 500 million years, today, accumulating biological, chemical, and physical stressors from anthropogenic climate change and other human influences are causing them to die at alarming rates (Downs et al. 2005; Weis and Allemand 2009; Hughes et al. 2018).

These threats impact corals in many ways. Both corals and their photosynthetic symbionts, zooxanthellae, are highly sensitive to abiotic changes in temperature, acidity, salinity, and light availability (Goreau et al. 1998; Williams 1990). Additionally, chemical pollution has been shown to negatively impact coral health (Goreau et al. 1998). Biotic factors, such as inter- and intraspecific competition and predation also

place significant pressure on corals. Finally, in recent years, the spread of disease throughout coral communities has become increasingly prevalent. Specific diseases such as black band, white band, and white pox have ravaged global coral populations.

While the sensitivity of coral to abiotic stressors has been intensely studied in recent years, less is known about how characteristics of coral community assemblage influence coral susceptibility to disease, predation, or competition. In other systems, an increased diversity of species in a community has been shown to decrease the extent to which stressors such as disease and competition impact that community (Wallace 1971; Grace 2016). Additionally, fishing regulations, including the establishment of marine protected areas and replenishment zones, might influence coral mortality. For example, fishing can not only cause physical damage to coral, but also remove herbivorous fish which keep corals' competitors in check. Lastly, disease and death are affecting coral communities, though they may differentially impact specific coral species.

In this study, we examine how coral health on Little Cayman Island in the Caribbean varies

across community assemblages, sites with different fishing regulations, and for individual species. By studying how coral mortality varies across these levels, we can gain further insight into the trends of coral decline observed today and how these sensitive organisms may tolerate stressors in their changing environments.

Because higher diversity in coral communities leads to a larger range of both defense strategies and quality of different coral species as a host or prey, we hypothesize that areas with high diversity will have less dead coral. However, alternatively, high coral diversity may increase interspecific competition between corals, causing increased coral mortality. Among the different management areas (i.e. marine park, protected area, and unprotected area), the marine park has the highest level of protection against human activity. For this reason, we hypothesize that there will be less dead coral in this area. Alternatively, because non-targeted factors such as ocean acidification and rising sea temperatures might influence coral health more than will different levels of regulation, there may not be a difference in coral health between the areas. Lastly, due to the threats facing corals and the wide range of different characteristics and defenses different coral species use to combat these, we hypothesize that some coral species will be more resistant to death than others, causing differential mortality between species.

METHODS

Observational Design

To assess coral community assemblages, we chose random points along beaches at the Central Caribbean Marine Institute (CCMI) and Mary's Bay on Little Cayman Island. We swam out to the back-reef, and in a line parallel to the beach, placed forty 1 x 1-meter quadrats on the reef, each one meter apart and at a depth of 1.5 meters. At CCMI, all 40 quadrats were in a marine park, the highest level of regulation. At Mary's Bay, 20 of these quadrats were in a replenishment zone, in which some fishing is restricted (hereafter "protected area") and 20 in an unprotected area, where no fishing regulations are in place. We took one top-down photo of each one-fourth of the quadrat.

We assessed the community composition of each photo by estimating percent cover for each of three categories: live coral, dead coral, and non-living coral rubble and summed these for 4 quadrants in each quadrat. For the living coral, we identified the species. Because it was difficult to differentiate between causes of coral death including disease, predation, competition, and repetitive bleaching, we combined all dead coral tissue into a single group, (Loya et al. 2001; Toller et al. 2001; Scheufen et al. 2017). In cases when a disease or bleaching was obvious and distinct, we identified bleaching or the specific disease.

Statistical Analyses

To assess how coral diversity within a plot influences coral health, we used linear regression to compare the Shannon diversity index value of each quadrat to the percent cover of dead coral. To determine how this alpha diversity varied across the protection statuses, we found the Sørensen index of dissimilarity to get beta diversity for each of the three protection statuses using the R package "vegan." We also compared percent of dead coral across the three protection statuses by using an ANOVA to compare between quadrats in each area

RESULTS

The percent dead coral tissue increased with the Shannon diversity index value (Linear Regression: slope = 0.178, ± 0.23 , $P = 0.031$, $r^2 = 0.064$, Fig. 1).

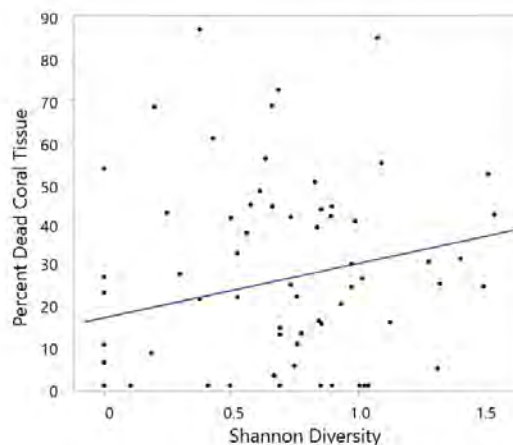


Figure 1: Percent dead coral tissue by Shannon Diversity Index values. Each point represents one m² quadrat, (n=72).

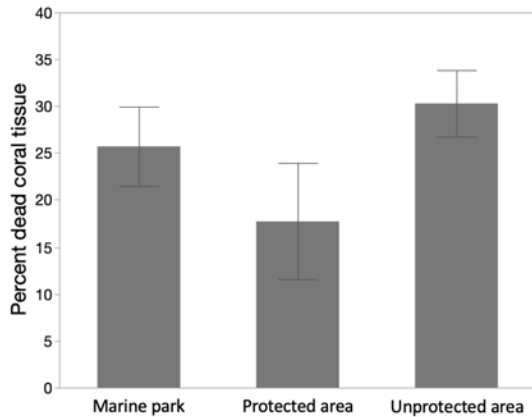


Figure 2: Average percent dead coral tissue in quadrats at each site. The marine park is located at CCMI. The protected area and unprotected area are both at Mary's Bay. Quadrats with no coral are not included ($n = 34$ at marine park area, $n = 18$ at protected area, and $n = 20$ at unprotected area).

The percent of dead coral tissue did not significantly differ by site (ANOVA: $F_{df1, df2} = 2, 71$, $P = 0.25$, Fig. 2). The mean percent dead coral tissue was 26% at the marine park at CCMI, 18% at the protected area at Mary's Bay, and 30% the unprotected area at Mary's Bay.

In the marine park, the average alpha diversity was 0.53, in the protected area it was 0.33, and in the unprotected area it was 0.86. The alpha diversities across the three sites were statistically different (ANOVA: $F_{df1, df2} = 2, 71$, $P = 0.0007$). The alpha diversity of the unprotected area was statistically different from that of the protected area (post-hoc test, $P = 0.0002$) and from that of the marine park (post-hoc test, $P = 0.0057$). The alpha diversities of the marine park and protected area were not different from each other (post-hoc test, $P = 0.09$). In the marine park, the beta diversity was 0.81, in the protected area it was 0.79, and in the unprotected area it was 0.57.

We found differences in the percent dead coral tissue across species (Fig. 3). Fused staghorn and grooved brain coral had no dead tissue, knobby brain coral, elkhorn coral, and blade fire coral had <10% dead tissue, and great star coral had ~80% dead tissue. The remaining 11 species all had between 20 and 50% dead tissue.

Of all 17 species of coral observed, six were affected by disease at least once (Table 1). We found white pox in elkhorn coral at CCMI, black

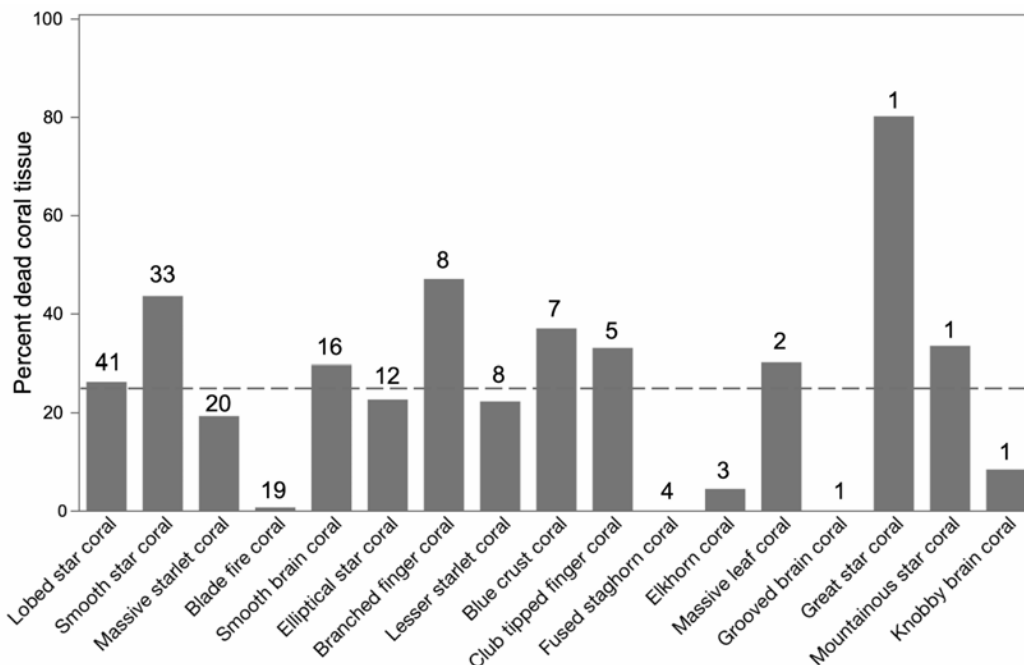


Figure 3: Percent dead tissue for each coral species. Values were found by dividing the total area of dead tissue for a species across all quadrats by the total area of that species found in quadrats. Numbers above each species indicate the number of quadrats in which each species was found, and species are ordered by this value. Dashed line represents average percent dead coral tissue for all species (25.7%).

band disease in lobed star coral at Mary's Bay, an unknown disease in massive starlet coral, and dark spot disease in club tipped finger coral and smooth brain coral at CCMI and in smooth star coral in Mary's Bay. Overall, the most commonly afflicted coral species were elkhorn coral and club tipped finger coral. For each species, 33% of the quadrats they were observed in contained diseased corals of that species.

DISCUSSION

Our results aid in understanding factors influencing coral health at the community and species level on Little Cayman Island. We observed that the proportion of dead coral increased with higher species diversity. However, only 6% of the variation in dead coral was explained by diversity, indicating that factors we did not study also contribute significantly to coral death. This positive relationship may be because, in more diverse quadrats, there is a greater occurrence of interspecific competition. Competition between corals requires energy and reduces the resources an individual can either invest in growth or use to fight off disease, and therefore can lead to greater mortality in these individuals. We observed competition between branched fire coral and lobed star coral as evidenced by fire coral overwhelming the lobed star coral, killing it at points of contact (Supplemental Figure 1).

There was no statistical difference in coral death across the marine park, protected area, and unprotected area. Previous marine studies done at CCMI have shown that there is no difference in temperature, salinity, or dissolved oxygen between CCMI and Mary's Bay (Griffie et al. 2019). Additionally, because our samples were taken at similar depths, there was no difference in light availability across sites. It is therefore unlikely that these abiotic factors differentially affected the proportion of dead coral at each site. In addition, while regulations on fishing have been shown to positively affect fish populations, their effect on coral health is less clear and may not be significant (Bégin 2016).

Although there was no difference in coral death, there was a difference in both alpha diversity and beta diversity between sites. The beta diversity was highest at CCMI, in the marine park, lower in the protected area at

Mary's Bay and even lower than in the unprotected area. This result suggests that the stricter regulations associated with the marine park may promote the diversity of that ecosystem; however higher diversity does not decrease coral death.

In the context of global ecosystem changes that affect coral, such as ocean acidification and rising sea temperature (Weis and Allemand 2009), there are often "winners" and "losers;" species are differentially susceptible to rapid environmental change. Those that can withstand changes better than can others in their community may become more dominant in future years. Elkhorn coral is susceptible to white pox disease and therefore may be an example of a species that will lose in the context of global change as coral disease spreads more rapidly. White pox has been known to decimate populations across the Caribbean and the Florida Keys (Sutherland and Ritchie 2004). In our study, all of the instances of elkhorn coral were at CCMI, and of these quadrats, 33% had white pox disease. The absence of elkhorn coral at Mary's Bay could suggest it has already been completely eliminated there. In contrast, fused staghorn may be an example of a species that can preferentially withstand environmental changes. We found fused staghorn coral in four quadrats and no instances of any dead tissue. Fused staghorn is a hybrid of staghorn and elkhorn coral and is known to be a hardy species, able to survive in large temperature ranges (Fogarty 2012; Aguilar-Perera and Hernández-Landa 2018). Other prevalent coral diseases include black band disease, which rapidly degrades the tissue of primarily massive corals (Frias-Lopez 2004), and dark spot disease, a slowly progressing chronic disease that has affected a wide range of coral species in the Caribbean.

Anthropogenic pressures make coral more susceptible to disease, increasing their mortality rates. Without immediate conservation efforts, coral reefs may be gone by 2050 (Burke 2011). Understanding the susceptibility of an individual species to disease and bleaching will help scientists to develop specialized methods for protecting those species. In addition, a better understanding of which protection statuses are the most effective at preserving coral diversity

will allow governments to tailor regulations to better support coral in a rapidly changing world.

ACKNOWLEDGMENTS

Thank you to all of the CCMI staff for supporting this project with knowledge, patience, and enthusiasm!

AUTHOR CONTRIBUTIONS

All authors contributed equally to this work.

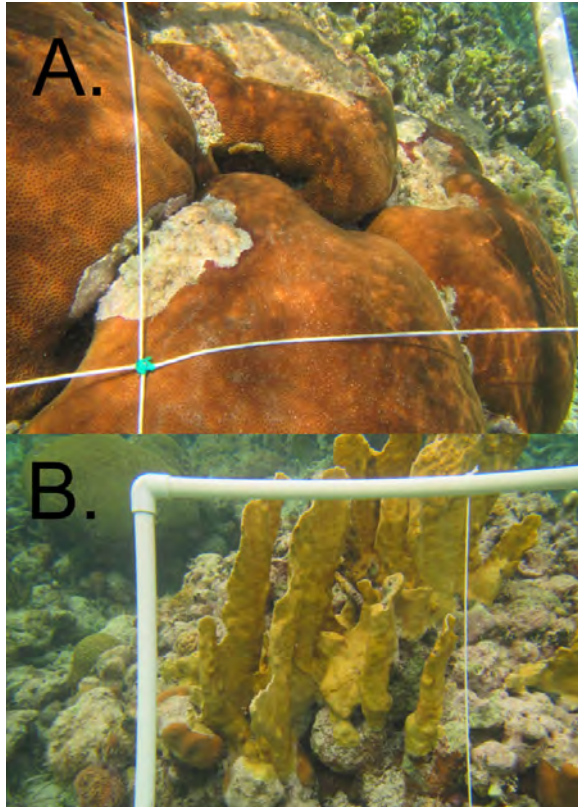
LITERATURE CITED

- Aguilar-Perera, A., Hernández-Landa, R.C. 2018. Occurrence of large thickets of *Acropora prolifera* (Scleractinia: Acroporidae) in the southern Gulf of Mexico. *Mar Biodiv* 48, 2203–2205.
- Bégin, Chantale, et al. 2016. Effects of Protection and Sediment Stress on Coral Reefs in Saint Lucia. 2016. *PLoS ONE*. 11 (2).
- Burke, L. and J. Maidens. 2004. Reefs at Risk in the Caribbean. World Resources Institute, Washington, DC.
- Burke, L., Reyntar, K., Spalding, M., Perry, A. 2011. Reefs at Risk Revisited. World Resource Institute.
- Downs, C., Fauth, J., Robinson, C., Curry, R., Lanzendorf, B., Halas, J., Woodley, C. 2005. Cellular diagnostics and coral health: Declining coral health in the Florida Keys. *Marine Pollution Bulletin*. 51(5–7): 558–569.
- Fogarty, N.D. 2012. Caribbean acroporid coral hybrids are viable across life history stages. *Mar Ecological Program Ser* 446:145–159.
- Frias-Lopez, Jorge, et al. 2004. Bacterial Community Associated with Black Band Disease in Corals. *Applied and Environmental Microbiology*, 70(10):5955–62.
- Goreau, T., et. al. 1998. Rapid spread of diseases in Caribbean coral reefs. *Revista de Biología Tropical*. 46(5):157–171.
- Grace, J. 2016. Biodiversity Critical to Maintaining Healthy Ecosystems. USGS.
- Hughes, T.P., Kerry, J.T., Baird, A.H. et al. 2018. Global warming transforms coral reef assemblages. *Nature* 556, 492–496.
- Kevin Griffiee, Hannah Marr, Callie Page, and Jenna Salvay 2019. Effects of reef zone and protection status on reef community composition and health. *Dartmouth Studies in Tropical Ecology* 2019. Dartmouth College.
- Loya, Y. et al. 2001. Coral bleaching: the winners and the losers. *Ecology Letters*. 4, 122–131.
- Patterson, K., Ritchie, K., Polson, J., Mueller, E., Peters, E., Santavy, D, and Smith, G. 2002. The etiology of white pox, a lethal disease of the Caribbean elkhorn coral, *Acropora palmata*. *Proceedings of the National Academy of Sciences*. 99 (13) 8725–8730.
- Scheufen, T., Krämer, W., Iglesias-Prieto, R., and Enríquez, S. 2017. Seasonal variation modulates coral sensibility to heat-stress and explains annual changes in coral productivity. *Nature*.
- Sutherland K., Ritchie K. 2004. White Pox Disease of the Caribbean Elkhorn Coral, *Acropora palmata*. In: Rosenberg E., Loya Y. (eds) *Coral Health and Disease*.
- Springer, Berlin, Heidelberg Teaching Geography 2018. Threats and challenges to the Cayman Islands reefs. Geographical Association.
- Toller, W., Rowan, R. and Knowlton, N. 2001. Zooxanthellae of the *Montastraea annularis* species complex: Patterns of distribution of four taxa of *Symbiodinium* on different reefs and across depths. *Biology Bulletin*. 201, 348–359.
- Wallace, H.R. 1971. The influence of the density of nematode populations on plants. *Nematologica*. 17(1): 154–166.
- Weis, Virginia and Allemand, Denis 2009. What Determines Coral Health? *Science*. 324 (5931): 1153–1155.
- Work, Thierry and Aeby, Greta 2006. Systematically describing gross lesions in corals. *Diseases of Aquatic Organisms*. 70: 155–160.
- Work, Thierry M., and Ernesto Weil 2015. Dark-Spots Disease. *Diseases of Coral*, John Wiley & Sons, Ltd, pp. 354–60.

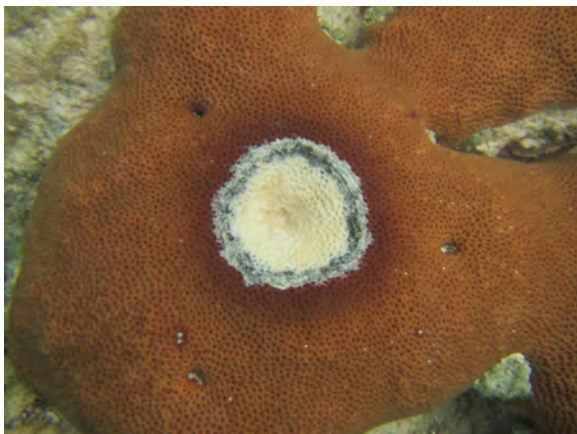
APPENDIX

Table 1: Out of all quadrats in which a species of coral was found, the proportion of those quadrats where the species was diseased. NAs indicate zero observations of a species at that site.

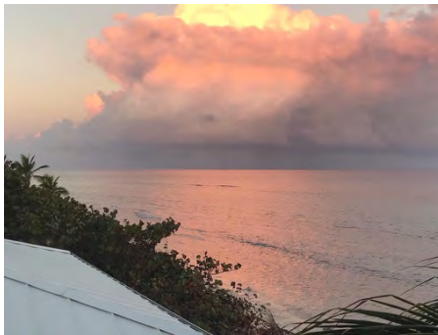
Species	Species ID	Number of Quadrats where diseased/present (CCMI)	Number of quadrats where diseased/presents (Mary's Bay)	Disease
Elkhorn coral	APAL	0.33	NA	White Pox
Fused staghorn Coral	APRO	0.00	NA	
Grooved brain coral	DLAB	0.00	NA	
Elliptical star coral	DSTO	0.00	0.00	
Great star coral	MCAV	NA	0.00	Blackband disease, bleaching
Blade fire coral	MCOM	0.00	0.00	
Lobed star coral	OANN	0.00	0.08	
Mountainous star coral	OFAV	0.00	NA	
Blue crust coral	PBRA	0.00	0.00	Dark spot disease
Knobby brain coral	PCLI	0.00	NA	
Massive leaf coral	PDEC	NA	0.00	
Branched finger coral	PFUR	0.00	0.00	
Club tipped finger coral	PPOR	0.33	0.00	Dark spot disease
Smooth brain coral	PSTR	0.08	0.00	
Smooth star coral	SBOU	0.00	0.23	Dark spot disease
Lesser starlet coral	SRAD	0.00	0.00	
Massive starlet coral	SSID	0.08	0.00	Unknown



Supplemental Figure 1: Four massive starlet coral (*Siderastrea siderea*) colonies at Mary's Bay. Regions of dead tissue observed where individuals are in direct contact (Panel A). Blade Fire (*Millepora complanata*) competing with Lobed Star (*Orbicella annularis*) for substrate at CCMI (Panel B).



Supplemental Figure 2: Massive Starlet Coral (*Siderastrea siderea*) at Mary's Bay with Black Band Disease.



Little Cayman





THE SCENT OF DEATH: CONSPECIFIC AND HETEROSPECIFIC RESPONSE TO CHEMICAL CUES FROM DEAD LIONFISH

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Abstract: Chemical sensing is a dominant sensory modality in the marine world that can greatly affect the fitness of individuals. These cues impact a number of the behaviors exhibited by individuals, from foraging behaviors to predator avoidance. This study examines the effect of chemical cues emitted by a dead lionfish on the behavior of conspecifics and silverside fish, a heterospecific prey species. Lionfish were found to be attracted to the dead conspecific, perhaps recognizing it as a potential food source. The chemical cue was found to have no impact on silverside behavior, perhaps due to the invasive nature of the lionfish or the lack of relevance to the silverside's fitness. Because lionfish are attracted to chemical cues emitted by a dead conspecific, spearing lionfish during culling outings remains an effective method of controlling invasive lionfish populations.

Key words: chemical cues, fission-fusion, lionfish, silverside fish, Little Cayman

INTRODUCTION

Chemical sensing is a dominant modality in the marine world. Chemical cues can influence various aspects of an organism's behavior underwater, including identification of food, mates, and predators (Hay 2009). Chemicals emitted by an individual can be received by another member of the same species (conspecific), or an individual of a different species (heterospecific). How these cues are interpreted and the behavioral responses they illicit vary based on the individual receiving them and their relationship to the individual emitting them. Effective behavioral responses to varying chemical cues can be essential to the fitness and success of an organism.

One commonly utilized cue known to incite behavioral change is that of "the smell of death." Reactions to an olfactory cue of an injured or dead individual vary widely across species, but two commonly observed behaviors are fleeing from the cue, if it is identified as the presence of a predator, or attraction to the cue, if it is identified as an easy meal becoming available. For example, when blue crabs pick up on chemical cues released from the body of an injured conspecific, they avoid the area where their conspecific was attacked (Hay 2009). In contrast, other species are known to be attracted to the scent of conspecific death, as it can signal available food, for cannibalistic species, or a newly available habitat (Valdez 2019).

However, little is known about how lionfish react to cues emitted by a dead conspecific.

We investigated the use of chemical cues by conspecifics, and by one species of prey, to examine the impact of interspecific versus intraspecific chemical information on fish behavior. Specifically, we studied the response of lionfish (*Pterois volitans*) and silversides (*Menidia beryllina*) from Little Cayman Island to the chemicals released by a recently killed lionfish. As a pervasively invasive species throughout the Caribbean, lionfish pose a significant threat to the survival of native fish and coral reef ecosystems. With a lack of natural predators and exceptionally high rates of fecundity, lionfish can kill up to 80% of fish present at a reef in just five weeks (Albins and Hixon 2008). Understanding how chemical cues affect lionfish and other heterospecifics can inform culling efforts and population management strategies. If lionfish are repelled by alarm cues from dead or injured conspecifics, culling practices involving netting fish may be more effective than the current tactic of spearing. Contrastingly, if lionfish are attracted to the scent of a dead conspecific, spearing lionfish could come with the added benefit of luring other lionfish to the culling area. Additionally, understanding the sensitivity of a prey species to chemical cues from dead lionfish can inform our understanding of how prey

species are reacting to cues emitted by an invasive predator.

Because many fish utilize intraspecific chemical cues to infer predation danger (Atema 1995), chemicals released from a recently killed conspecific may act as an alarm cue to other lionfish, deterring them from the area with the chemical cue. If lionfish perceive olfactory cues from the death of a conspecific as a sign of danger, they would move away from the chemical cue. Alternatively, because lionfish are carnivorous fish known to eat other juvenile lionfish (Dahl et al. 2018), chemical cues released by a killed conspecific may indicate a tasty meal and attract other lionfish. If lionfish perceive this olfactory cue as an indicator of food, they would move towards the chemical cue. If lionfish are not sensitive to chemical cues released by a dead conspecific, there would be no behavioral response to the presence of a cue.

Because lionfish and silversides share a number of predators, such as the grouper, chemicals released by a killed lionfish may indicate predation danger to silversides. If silversides use olfactory cues from dead lionfish as an indicator of predation threat, they would move away from the chemical cue. Alternatively, recognition of this interspecific chemical cue may not occur between lionfish and silversides. Lionfish have only recently been introduced to the Caribbean, therefore recognition of the association between the cue and the potential danger may not have had time to evolve. If this is the case, there would be no difference in behavioral response of silversides to presence or absence of the cue.

METHODS

Setting up the flume

We tested the response of fish to the chemical cues from dead lionfish by using a two-channel choice flume, with dimensions of 0.95 x 0.40 x 0.42 m, as used in previous studies (Welch 2014; Figure 1). Water from two sources was pumped into each side of the flume. Water flowed out of the flume from one source at a flow rate of 35 mL per second.

A Drip Test™ was used before running trials to ensure that water was flowing into both sides of the flume at an even rate. For this test, we altered the flow rate of each input source to

the flume until each had a flow rate of 17.5mL/s. We then used a dye test, as used in previous studies, to ensure that water flowing into the “cue present” zone of the flume was not mixing with water in the “cue absent” zone (Welch 2014). We added one drop of food coloring to one side of the “input zone”. Over the course of five minutes, the food coloring was pushed through that side of the flume. Water from one side of the flume did not mix with water from the other side. We repeated the dye test after every time the input or output of water to the tank was turned off.

Conspecific response

To test whether or not lionfish were attracted to the chemical cues emitted by a dead conspecific, we first had to kill a lionfish by blunt force trauma to the head. We then lacerated the fish several times across the body with a knife. The dead fish was placed behind a permeable physical barrier into one side of the input zone (Figure 1). The chemical cues emitted from the dead lionfish were given three minutes to disperse through the side of the flume where it was placed.

Due to the sedentary nature of the lionfish, it was necessary to incentivize them to move in trials. Because lionfish are nocturnal and prefer to spend their time in shady areas, we placed two lights over the intermediate zone and covered the cue present, cue absent, and input zone with wooden planks to create artificial shade (Figure 1).

For each trial, we placed one lionfish into the intermediate zone of the flume (n=14). To minimize the effect of a lionfish being placed into the flume oriented towards or away from one side, we prevented the lionfish from moving towards the shade for the first ten seconds after it had been placed into the flume. We then allowed the lionfish to acclimate to the tank for two minutes before starting the trial. Over the course of ten minutes, the time that the lionfish spent in each of three zones of the flume was recorded. These zones were the cue present zone, the cue absent zone, and the intermediate zone.

At the end of each trial, we removed the living lionfish from the flume. Because the chemical cues emitted from a dead lionfish

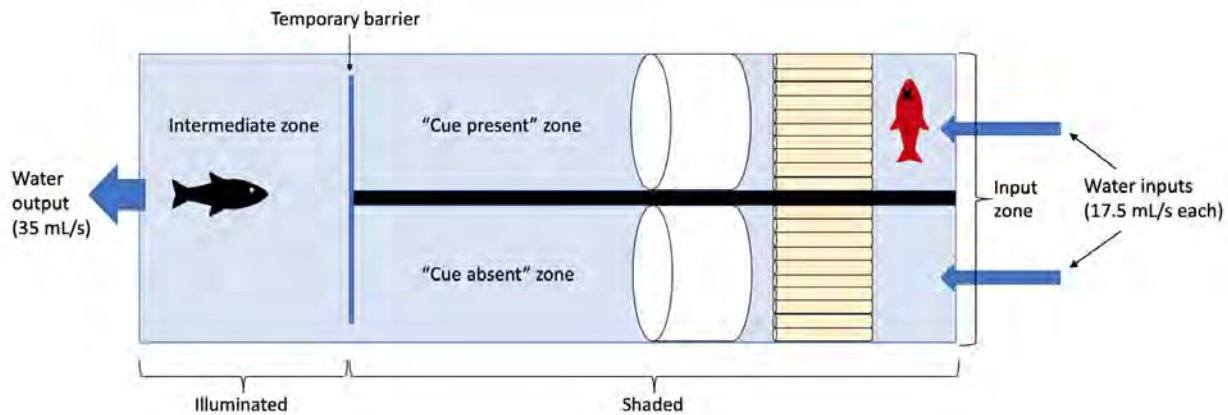


Figure 1: Schematic of the flume apparatus used in this study. Half of the trials were performed with the dead lionfish on each side of the input zone. The temporary barrier was removed 10 seconds after a living lionfish was placed in the tank, and 5 seconds after a silverside was placed in the tank.

degrade over time, we also removed the dead lionfish from the flume. The living lionfish used in the preceding trial was then killed to be used as the cue in the next trial. To control for behavioral lateralization, seven trials were conducted with the dead lionfish on the left side of the flume, and seven trials were conducted with the dead lionfish on the right side of the flume.

Heterospecific response

To test whether or not silverside fish were attracted to the chemical cues emitted by a dead lionfish, we killed and lacerated a lionfish and then placed it behind a permeable physical barrier into one side of the input zone (Fig. 1). The chemical cues emitted from the dead lionfish were given thirty seconds to disperse through the side of the flume where it was placed.

For each trial, we placed one silverside into the intermediate zone of the flume ($n=19$). To minimize the effect of a silverside being placed into the flume oriented towards or away from the side with the cue, we prevented the silverside from moving towards either side of the flume for the first five seconds after it had been placed into the flume. Over two minutes, the time that the silverside spent in each of three zones of the flume was recorded. These zones were the cue present zone, the cue absent zone, and the intermediate zone. We recorded silversides for a shorter period of time than the lionfish because

silversides were much more active. We did not create an artificially shaded area of the flume for the silversides because the silversides were very active and did not require incentive to move.

At the end of each trial, we removed the living silverside from the flume. To control for behavioral lateralization, ten trials were conducted with the dead lionfish on the left side of the flume, and nine trials were conducted with the dead lionfish on the right side of the flume. Because the chemical cues emitted from a dead lionfish degrade over time, a new lionfish was killed after ten silverside trials were conducted.

Statistical Methods

We utilized two non-parametric Wilcoxon Signed Rank Paired t-tests to analyze the effect of presence or lack of chemical cue on both live lionfish and silverside fish flume side choice. We utilized two non-parametric Wilcoxon Ranked Sums tests to test for the effect of lateralization in both fish species.

RESULTS

Lionfish spent significantly more time in the side of the flume with the cue present than they did in the side without the cue (Wilcoxon Signed Rank Paired t-test: $S = 38.00$, $df = 13$, $p = 0.015$; Fig. 2). On average, lionfish spent 70% of their time in the side of the flume with the cue present, and 11% in the side without the cue. Proportion of time spent in the side with or without the cue was not affected by which side

of the tank the dead lionfish was placed in (Wilcoxon Ranked Sums: $Z = 0.14$, $df = 1$, $p = 0.888$).

Silverside fish displayed no preference for the side of the flume with cue present or the cue absent (Wilcoxon Signed Rank Paired t-Test: $S = 39.50$, $df = 18$, $p = 0.108$; Fig. 3). On average, silversides spent 43% of their time in the side of the flume with the cue present, and 21% in the side without the cue. Proportion of time spent in the side with or without the cue was not affected by which side of the tank the dead lionfish was placed in (Wilcoxon Ranked Sums: $Z = 0.00$, $df = 1$, $p = 1.00$).

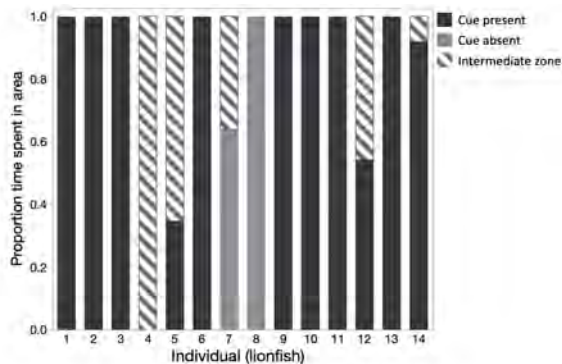


Figure 2: The proportion of time spent by each lionfish in the area of the flume with the dead lionfish present, the dead lionfish absent, and the intermediate zone where the flume was not divided ($n = 14$).

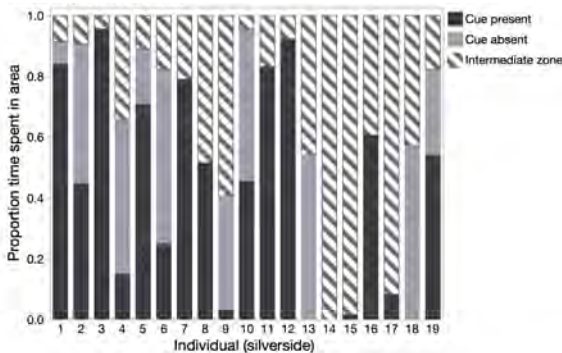


Figure 3: The proportion of time spent by each silverside in the area of the flume with the dead lionfish present, the dead lionfish absent, and the intermediate zone where the flume was not divided ($n = 19$).

DISCUSSION

Lionfish displayed a preference for the side of the flume containing the dead conspecific. As the live lionfish could not see the dead lionfish, we conclude that live lionfish picked up on, and were attracted to, a chemical released by dead

lionfish. Though not observed in this study, lionfish are known to eat conspecifics up to half their size (Mihalitsis and Bellwood 2017). As a result, it is likely that the lionfish are attracted to the chemical cue as it may indicate a meal and perceiving and acting upon the cue likely provides the lionfish with a fitness advantage.

Though our results suggest the lionfish are attracted to the chemical cues of a dead conspecific, the biological relevance of the cues must be considered within the context in which they were received. Because we had to incentivize the lionfish to choose between the two sides of the tank by illuminating the intermediate zone, this created a stimulus the lionfish would seek to avoid and should be taken into account when thinking about the importance of chemical cues from a dead conspecific in informing the lionfish behavior. Of note, silversides did not require the same incentive to move.

Unlike the live lionfish, silverside fish did not prefer the side of the flume with a dead lionfish. Rather, they displayed no preference for either side of the flume. This indicates that the chemical cues of dead lionfish perceived by live lionfish are not utilized by silverside. It is possible that Native Caribbean species have not evolved the mechanisms which would allow them to detect the cues produced by a dead lionfish as the red lionfish is an invasive species, native to the Indo-Pacific, that was introduced to the Caribbean around 12 years ago.

The perception of chemical cues has a significant impact on predator-prey interactions and the fitness of an organism (Hay 2012). Several animals depend on chemical cues to avoid predation. Various species of larval amphibians have been shown to use chemical cues to detect predatory fish (Petranka et al., 1987). Damselflies recognize predators by chemical cues from a predator's diet that indicate the individual has recently eaten other damselflies (Chivers et al., 1996). Many fish involuntarily secrete specialized alarm pheromones from their skin when attacked, signaling to conspecifics a predation threat in the area (Verheggen et al., 2010). Appropriate behavioral responses to these chemical alarm cues, such as avoiding an area, reducing movement, or increased shoaling, can be critical

to an individual's survival. An inability to detect such vital chemical cues can be detrimental to the fitness of an animal.

The ability of predators and prey to detect relevant chemical cues may be hindered when the cues originate from an invasive species. As native species have not evolved alongside the invasive species, there is a greater chance that they have not yet developed the sensory mechanisms or recognition templates to identify the invasive species. In this case, prey may be negatively impacted by the absence of warning cues. Future studies may compare the behavioral responses of Caribbean and Indo-Pacific prey species towards chemical cues emitted by lionfish, dead and alive. It is much more likely that Indo-Pacific prey species would be repelled by chemical cues emitted by lionfish, as these species have had time to develop the capability to do so.

The results of this study can help guide culling practices for lionfish. Having demonstrated that lionfish are attracted to dead conspecifics, spearing may be a useful culling technique as it could attract more lionfish to the culling area. However, in this study, we also observed the sedentary nature of the lionfish. Though attracted to the scent, lionfish may not move towards a dead conspecific in the wild at a rate that would result in an added culling benefit. At the very least, the lionfish are not repelled by the scent of a dead conspecific and extra measures do not need to be taken to avoid the dispersal of these chemical cues.

ACKNOWLEDGEMENTS

Thanks to Maisy and all of the CCMI staff for the accommodations, hospitality, and support of our lionfish project.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this work.

LITERATURE CITED

- Albins, M. A., and M. A. Hixon. 2008. Invasive Indo-Pacific lionfish *Pterois volitans* reduce recruitment of Atlantic coral-reef fishes. *Marine Ecology Progress Series* 367: 233-238.
- Atema, J. 1995. Chemical signals in the marine environment: Dispersal, detection, and temporal signal analysis. *Proceedings of the National Academy of Sciences of the United States of America* 92: 62-66.
- Chivers, D. P., Wisenden, B. D., Smith, R. F. 1996. Damselfly larvae learn to recognize predators from chemical cues in the predator's diet. *Animal Behaviour* 52: 315-320.
- Dahl, K. A., D. S. Portnov, J. D. Hogan, J. E. Johnson, J. R. Gold, and W. F. Patterson III. 2018. Genotyping confirms significant cannibalism in northern Gulf of Mexico invasive red lionfish, *Pterois volitans*. *Biological Invasions* 2018: 3513-3526.
- Hay, M. 2009. Marine Chemical Ecology: Chemical Signals and Cues Structure Marine Populations, Communities, and Ecosystems. *Annual Review of Marine Science* 1: 193-212.
- Mihalitsis, M. and D. Bellwood. 2017. A morphological and functional basis for maximum prey size in piscivorous fishes. *PloS one* 12.
- Petranka, J. W., Kats, L. B., Sih, A. 1987. Predator-prey interactions among fish and larval amphibians: use of chemical cues to detect predatory fish. *Animal Behaviour* 35: 420-425.
- Valdez, L. and M. Laidre. 2019. Scent of death: Evolution from sea to land of an extreme collective attraction to conspecific death. *Ecology and Evolution* 9: 2171-2179.
- Verheggen, F. J., Haubruge, E., Mescher, M. C. 2010. Alarm Pheromones - Chemical Signaling in Response to Danger. *Vitamins and Hormones* 83: 215-239.
- Welch, M. 2014. Effects of elevated CO₂ on fish behaviour undiminished by transgenerational acclimation. *Nature Climate Change* 4: 1086-1089.

PRIME REAL ESTATE: SOME CLEANING STATIONS ARE MORE LUCRATIVE THAN OTHERS FOR THE CAYMAN CLEANER GOBY

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Abstract: A unique mutualism defined by the necessary removal of parasites from one fish, for consumption by a different fish, exists between the Cayman cleaning goby (*Elacatinus cayman*) and a variety of Caribbean reef fish. The cleaner-client symbiosis is exhibited throughout multiple species around the world, but the system is consistently driven by market principles in which external factors influence both the viability and competitive edge of any given cleaner or cleaning station. This study assessed the effect of real estate on market success, as well as competition and duration of cleaning on propensity to cheat. We hypothesized that certain station locations may be more favorable to clients, so some stations may receive more traffic than others. We found that client visitation rates, as well as species richness, was significantly higher at stations which were situated either far from or an intermediate distance from a reef crest sampling site, than those near a reef crest. The number of neighbors an individual station has and the time a client spends at a station do not significantly affect the cheating rate of the cleaners. We do observe a trend indicating that a higher number of neighbors and the more time spent at a station increase the likelihood of cheating occurring during the cleaning interaction. These results suggest that Cayman cleaner gobies do not compete with one another by changing their service quality but may compete for valuable real estate.

Keywords: *asymmetry, Cayman cleaner goby, cheating, choice power, ectoparasite, market, mutualism, territory*

INTRODUCTION

Sequestering territories is crucial for resource retention in numerous taxa. Reasons for this spatial fidelity are numerous. Some species bear vulnerable young and must keep them protected in a safe space to which they return, as do many egg-laying birds. Others use possession of a space as an attractant for mates or use certain areas as arenas to prove their worth as mates (Pratt et al., 1992; Vanderbilt et al. 2015). Perhaps most notably, however, food resources are often concentrated in certain areas, and consumers benefit from sole access to such resources, as many predator species like felines and canines do. Some territories are safer, more attractive, or denser in food than others, and so the acquisition of “prime real estate” confers direct fitness benefits on its owners.

Cleaner gobies in the genus *Elacatinus* serve “clients” such as damselfish, grunts, parrotfish, and planktivores by eating ectoparasites off of their skin (Soares et al. 2013). Cleaners maintain consistent “cleaning stations,” which persist from day-to-day and offer ectoparasite food as a steady resource for the cleaners. In this case, the food source available to a territorial animal is

retained by a mutualist which gets to *choose* which stations receive the food. These gobies sometimes “cheat” by biting clients’ surface mucus instead of parasites, but they generally prefer to eat parasites rather than mucus i.e. cheating (Soares et al. 2013). Other cleaner fish in Egypt and the Indo-Pacific, the bluestreak wrasse (*Labroides dimidiatus*), prefer to cheat rather than eat client mucus, and, as a result, their clients evolved to employ “choice power,” wherein they choose to visit clients who cheat less often, forcing bluestreak wrasse to cooperate to earn repeat customers (Bshary & Grutter, 2003; Bshary & Shcafffer, 2002). There was no such pressure in Caribbean reefs selecting for gobys’ clients to employ sanction power, and as a result, no repeat patronage or choice power are observed in this system (Soares et al. 2013). However, cleaner gobies still do cheat at times, and some stations appear to receive more traffic than others (Authors’ observations 2020), despite no variation in service quality (Soares et al. 2013). If, as has been established, gobies do not prefer cheating over cooperation, then why do they do it? Furthermore, if clients in this system always

select stations on a one-off basis without considering service quality, what motivates them to choose one station over another? Are some station spaces more valuable to cleaners than others?

Our study aimed to determine what pressures structure the goby cleaning market in the Caribbean, and how the goby's preference for cooperation and clients' lack of choice power differentiates this system from the bluestreak wrasse market. We used the reefs off the North shore of Little Cayman and the Cayman cleaner goby (*Elacatinus cayman*) as a study system. We then determined if certain stations receive higher visitation than others, and if this difference in visitation can be attributed to any qualities of the station locations themselves. We posited that shoreward distance from the reef crest is advantageous, sheltering clients from the gaze of predators and making the station easily visible to fish feeding on coral and coral-associated algae. If so, then stations far from the reef crest would exhibit higher visitation rates. If there is no station preference, and all space is equally valuable, then no visitation difference will be observed between stations.

Regarding goby cheating, previous research has indicated that client parrotfish in the Caribbean carry fewer parasites than Indo-Pacific parrotfish. As a result, gobies may cheat because they *must*, despite not preferring to do so, simply due to scarcity of their parasite food source (Soares et al. 2013). If this is true, then cleaner gobies will likely eat any parasites available on the skin of a client, only cheating at the very *end* of a cleaning interaction. Alternatively, due to scarcity of parasites, cleaner gobies may actually benefit from aggravating a client after removing all parasites from it, signaling the end of an interaction in order to move on to the next client as quickly as possible. If either of these is true, then the longer a cleaning interaction continues, the more likely the client is to be cheated.

METHODS

We opportunistically sampled a 200 m section of the reef crest at Cumbers cove on the north shore of Little Cayman Island. Six sites were identified, each containing multiple cleaning stations. The stations were approximately 30 m

apart. To assess the relative competition each station faces, the “neighbors” of each station were counted within a 30 m radius from the study station (Fig. 1). Additionally, to categorize the position of the stations with relation to the reef crest, we mapped the stations within each site. Individual station was classified as being either, near, an intermediate distance from, or far from the reef crest.

To assess the relative popularity of certain cleaning stations, as well as the relative qualities of service provided, we conducted focal studies on multiple cleaning stations within each site for 15 minutes each. Within a focal study, we recorded each client that visited, noting its species and if a jolting behavior was observed. Further, we noted how long each client stayed at the station. Assessment of cleaners' competitors and scoring of service quality follows the paradigm laid out by Hayse (2018).

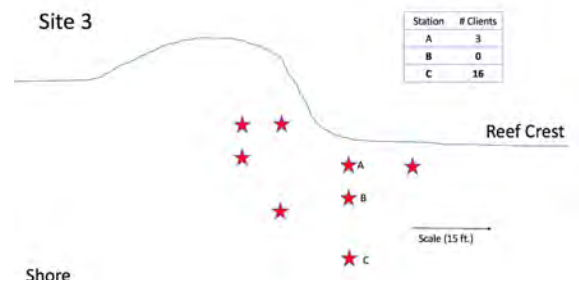


Figure 1: Schematic of site three with a subset of data on the number of client visits. Stars indicate observed cleaning stations within the site and the stations labelled A, B, and C were the stations on which focal studies were conducted. Station neighbors were counted if they fell within a 30 m radius of the focal study stations. B and C are bolded to indicate that these focal studies were conducted simultaneously.

Statistical methods

To quantify the popularity of a given station, we utilized a generalized linear model to compare the number of clients visiting a station in 15 minutes to the distance of the station from the reef crest. Distance from the reef crest was nested within site for this analysis. A generalized linear model was also used to compare species richness to distance from the reef crest, again nesting this distance within site. Additionally, a generalized linear model was used to analyze the relationship between the

number of neighbors around a given station and that station's cheating rate (jolts/client/15 min.). The number of neighbors was nested within site. Lastly, we analyzed the change in likelihood of cheating by duration of a cleaning visit, using an ordinal logistic regression with binomial responses of cheating occurring (1) or no cheating occurring (0). The duration of a visit was nested within the station. All data was analyzed using JMP Pro 14.

RESULTS

A majority of the client fish observed were stoplight parrotfish (60%). Other observed client species included the princess parrotfish, mahogany snapper, initial phase queen parrotfish, red hind, black durgon, initial phase redbtail parrotfish, striped parrotfish, and the blue angelfish (Table 1).

Table 1: Breakdown of client species observed during all focal studies.

Species	Observed	Proportion
Stoplight Parrotfish	60	61.22
Striped Parrotfish	7	7.14
Queen Parrotfish	10	10.2
Initial Phase		
Blue Angelfish	1	1.02
Redtail Parrotfish	2	2.04
Initial Phase		
Red Hind	4	4.08
Black Durgon	3	3.06
Mahogany Snapper	1	1.02
Princess Parrotfish	10	10.2

The cleaning stations situated in positions far from and an intermediate distance from the reef crest received significantly more clients than stations situated near the reef crest (GLM: $X^2 = 37.04$, $df = 11$, $p = 0.0001$, Fig. 2). Variation in visitation among sites was also significant, but more of the variation was explained by distance than by site (GLM: $X^2 = 17.10$, $df = 5$, $p = 0.0043$). Species richness was highest at the stations situated far from and an intermediate distance from the reef crest (GLM: $X^2 = 54.73$, $df = 11$, $p < 0.0001$, Fig. 3). Variation in species richness among sites was also significant but did not explain more of the

variation than distance (GLM: $X^2 = 36.40$, $df = 5$, $p < 0.0001$). There was no relationship between the number of neighbors that a station had and the number of clients the station received (GLM: $X^2 = 9.41$, $df = 8$, $p = 0.31$, Fig. 4). Difference among sites was not significant (GLM: $X^2 = 9.07$, $df = 5$, $p = 0.106$). Further, the likelihood that a cleaner fish cheated increased as the time spent by the client fish at

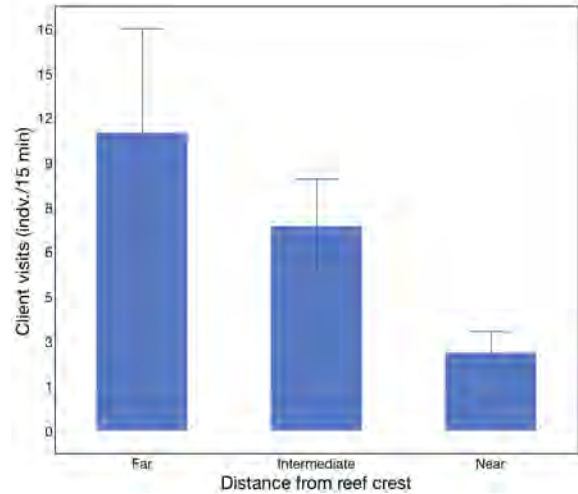


Figure 2: The average number of individual clients per 15-minute focal study, which visited stations categorized as being far from ($n = 33$), an intermediate distance from ($n = 50$), or near the reef crest ($n = 15$). $SE \pm$ Mean.

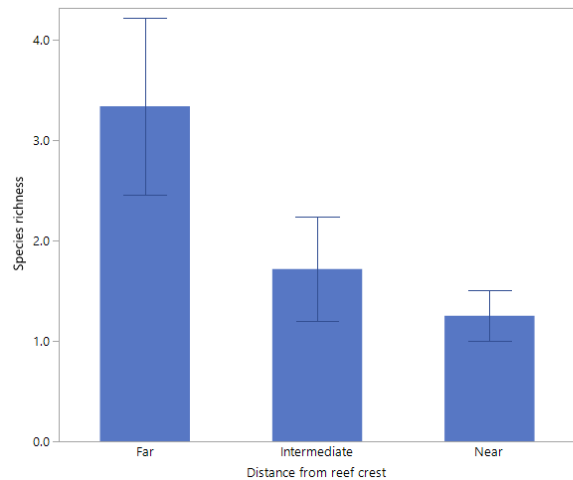


Figure 3: The average number of species which visited stations categorized as being far, an intermediate distance from, or near the reef crest, $n = 99$ clients, $n = 9$ species. $SE \pm$ mean.

the station increased (OLF: $X^2 = 4.66$, $df = 3$, $p = 0.199$, Fig. 5).

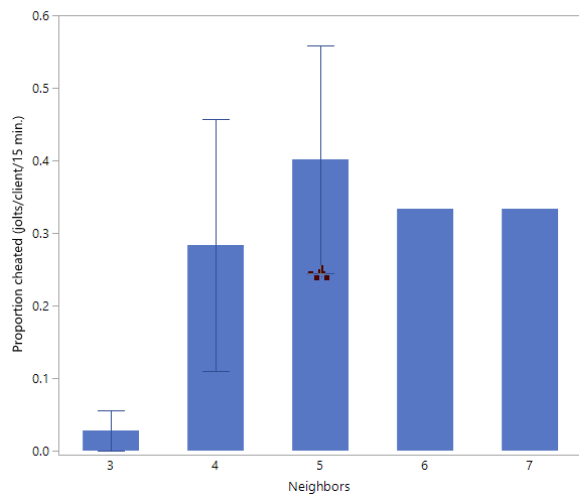


Figure 4: The average proportion of the clients which were cheated, indicated by the number of jolts per client per 15-minute focal study, by the number of neighbors observed around a given station, $n = 14$ stations ($n = 2$ stations with 3 neighbors; $n = 4$ stations with 4 neighbors; $n = 5$ stations with 5 neighbors; $n = 2$ stations with 6 neighbors; $n = 1$ station with 7 neighbors). Error bars constructed using one standard error from the mean.

DISCUSSION

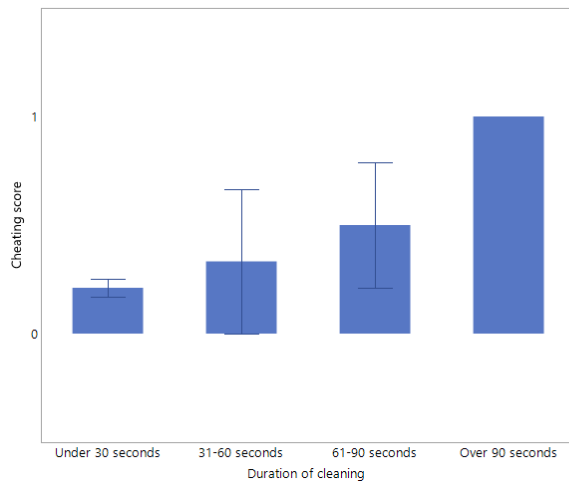


Figure 5: The average cheating score, indicated with a binary yes (1) or no (0) with regards to the time a client spent at a station, $n = 98$ ($n = 90$ clients served under 30 seconds; $n = 3$ clients served 31-60 seconds; $n = 4$ clients served 61-90 seconds; $n = 1$ client served over 90 seconds). Error bars constructed using one standard error from the mean.

Our results indicate that client fish do have site preferences: sites farther from the reef crest received higher visitation and exhibited greater client species richness. These results provide support for our first hypothesis, which posited clients prefer to visit certain stations over others. As cleaning interactions are perceived as one-offs by cleaners and clients in this system, and the low parasite load of Caribbean fish makes cleaning interactions low stakes for clients, any significant variation in clients' station selection is likely based on characteristics of the station space. This suggests that particular station spaces in these reefs are more valuable than others.

Cheating rate did not decrease with competitor presence, and thus clients do not appear to exert choice power in this system. However, our results suggest that Cayman cleaner gobies likely compete for valuable real estate. In our study, station locations far from the reef crest seem to be strategically valuable. It may be that the visibility to clients or the safety from predators provided by these far stations entice clients to visit them. Given the immense benefit of highly visited stations to their occupant cleaner gobies, and the potential scarcity of such a resource, it becomes curious how cleaners acquire exclusive access to these stations. It may be that cleaners compete directly for these station areas and must defend them territorially. Alternatively, it may be that older, larger, or otherwise dominant pairs of gobies occupy the best stations, and new occupants overtake them after current occupants die or leave. If the groups occupying a station are not static, but rather experience fission and fusion, it may be that gobies age into a station space, or that mated goby pairs alternate between high- and low-traffic sites. Future research may illuminate the means by which cleaner gobies acquire their station spaces, and if or why some gobies retain ownership of highly valuable station space; for example, removal of some or all gobies from certain stations may reveal if or how these station spaces are then repopulated.

Though cleaning interactions are low-stakes for clients in this system, the scarcity of parasites makes interactions very high-stakes for cleaners. Though the trend was not statistically significant, longer cleaning interactions were

more likely to result in cheating. Given the high stakes of cleaning from the cleaners' perspective, and gobies' preference for cooperative feeding, our results suggest that cleaner gobies indeed only cheat by necessity, eating all available parasites on a client and only cheating afterward. This may serve as an added nutrient bonus for cleaners once the consequence of losing a client's parasites is gone or may help a cleaner to cycle through more parasite-bearing clients by promptly sending away cleaned clients. These results match our predictions for parasite acquisition being the resource limitation for these gobies. Though this trend was not statistically significant in our study, likely due to low statistical power, it may be biologically relevant and warrants further study.

The mechanics of mutualistic relationships are highly determined by the resources limiting one or both of the mutualists involved. In the highly parallel cleaning markets in the Mediterranean and Caribbean, the parasite loads of client fish reverse the cleaner-client power dynamic in a way that shifts the emergent structure of the entire system. Client fish in the Mediterranean deal with parasites that are an utter detriment to fitness, while their cleaners face an abundant and replenishing resource, if clients are properly managed. Meanwhile, client fish in the Caribbean face parasites as a mere nuisance, while their cleaners must deal with a scarce food resource placing them in constant jeopardy, searching for the next meal. Asymmetries like this usually destabilize mutualistic relationships, and yet the cleaner-biased mutualism in the Mediterranean and the client-biased system in the Caribbean persisted by selecting for compensatory behavioral adaptations, client choice power in the Mediterranean case, and, potentially, cleaner competition for prime real estate in the Caribbean (Bshary & Grutter 2002).

The idea that asymmetry inherently destabilizes mutualisms may not capture the full scope of mutualists' potential for adaptation. Mutualistic relationships are often asymmetrical at first; in the case of Attine ants' agricultural mutualism with fungi, for example, the fungi initially benefited from a relationship with ants, but could survive without them, while their ants

were *obligated* to engage in this mutualism to survive (Schultz & Brady, 2008). This inherent asymmetry did not lead to cessation of the mutualism, but instead selected for ants' active removal and sterilization of its fungus' parasite *Escovopsis*, a behavior which in turn selected, eventually, for the fungus' dependence upon the ants (Little et al., 2006; Schultz & Brady, 2008). Meanwhile, yeast-farming ants do not face the same pressures their *Leucocoprineae*-farming counterparts must address, as yeast has no predators as aggressive as *Escovopsis*; as a result, neither these ants nor their yeast species developed such complex or interwoven adaptations (Schultz & Brady, 2008). Asymmetry in a mutualism may, in fact, simply provide selective pressure for the evolution of complex social behaviors, with the *direction* of the asymmetry causing different behaviors to emerge in different ecosystems.

ACKNOWLEDGEMENTS

Thank you to Clare and Celia for supporting our data collection at the beautiful Cumbers. RIP Colton's feet.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this work.

LITERATURE CITED

- Bshary, R. & Grutter, A.S. 2002. Asymmetric cheating opportunities and partner control in a cleaner fish mutualism. *Animal Behavior*, 63, 547-555.
- Bshary, R. & Grutter, A.S. 2003. Cleaner fish prefer client mucus: Support for partner control mechanisms in cleaning interactions. *Proc. Biol. Sci.*, 270, S242-S244.
- Bshary, R. & Schaffer, D. 2002. Choosy reef fish select cleaner fish that provide high service quality. *Animal Behavior*, 63, 557-564.
- Hayse, C. G. W. (2018). Cleaner Wrasse & Clients: Market Structure and Manipulation of Clients' Choice Power. *Biology 23 at Dart College*.
- Little, A. E. F., Murakami, T., Mueller, U. G., & Currie, C. R. 2006. Defending against parasites: fungus-growing ants combine specialized behaviours and microbial symbionts to protect their fungus gardens. *Biol. Lett.*, 2, 12-16.
- Pratt N. C., et. al. 1992. Behavioral, physiological, and morphological components of dominance and mate attraction in male green iguanas. *Zoo Biology* 11: 153-163

- Schultz, T. R., & Brady, S. G. 2008. Major evolutionary transitions in ant agriculture. *PNAS*, 105, 14, 5435-5440.
- Soares, M. C., Cardoso, S. C., Nicolet, K. J., Cote, I. M., & Bshary, R. 2013. Indo-Pacific parrotfish exert partner choice in interactions with cleaner fish but Caribbean parrotfish do not. *Animal Behaviour*, 86, 611-615.
- Vanderbilt, C. C., Kelley, J. P., & Duval, E. H. 2015. Variation in the performance of cross-contextual displays suggests selection on dual-male phenotypes in a lekking bird. *Animal Behaviour*, 107, 213-219.

THE ROLE OF OCEAN CURRENTS AND LOCAL WIND PATTERNS IN DETERMINING ONSHORE TRASH ACCUMULATION ON LITTLE CAYMAN ISLAND

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Abstract: Each year, humans deposit billions of pounds of plastic and other trash into the ocean. These ocean plastics are distributed worldwide and pose a significant threat to marine ecosystems. The Caribbean Islands are the largest plastic polluter per capita, and the interconnected nature of the Caribbean Sea promotes trash transport among all of the islands. We quantified the onshore accumulation of plastics and other trash over four days at four sandy beaches on Little Cayman Island (two on the north side of the island and two on the south) to determine whether the deposition of trash on the island is driven by ocean currents or local wind patterns. Though previous research suggests that major ocean currents play a considerable role in the accumulation of trash on beaches, other studies have found that powerful local winds can overcome the influence of ocean currents. On Little Cayman Island, where ocean currents come from the southeast, we might expect to see higher trash accumulation rates at sites on the southern side of the island. Alternatively, if local wind patterns have a greater influence than do ocean currents on where trash is deposited, we would expect to see trash accumulation variation on each side of the island depending on local wind patterns. We found that more southerly wind increases trash accumulation rate on the south side of the island and more northerly wind increases trash accumulation rate on the north side of the island. Based on our findings, we recommend focusing beach cleanup efforts on Little Cayman on recent wind patterns.

Key Words: ocean, currents, wind, plastic, trash, Little Cayman Island, onshore accumulation

INTRODUCTION

Plastic has become pervasive in every aspect of our lives. Each year, humans deposit over five million tonnes of plastic and other trash into the ocean, which later washes up on beaches or converges in oceanic gyres (Jambeck et al. 2015). Plastic waste has even been found in the guts of amphipods in the deepest parts of the sea (Jamieson et al. 2019). However, the widespread distribution of plastic and other trash is not without consequences. Oceanic plastic pollution poses a huge threat to marine ecosystems. Seabirds, turtles, seals, and other wildlife are dying at alarming rates from ingesting plastic or getting tangled in plastic products (Laist 1997).

A large portion of these plastics and other trash has accumulated in the five major oceanic gyres. The largest of these is the Great Pacific Garbage Patch, which is located between the coast of California and Hawaii and contains an estimated 79 thousand tonnes of plastic brought there by ocean currents (Lebreton et al. 2018). However, aside from accumulating in oceanic gyres, plastic also washes up on shorelines around the world. In the Caribbean islands, which produce the most plastic pollution per capita (Ritchie &

Roser 2020), plastic waste is easily transported among islands due to the connected nature of the Caribbean Sea. The once pristine beaches in the Caribbean islands now have substantial plastic and other trash, causing alarm to residents and visitors, harming wildlife, and potentially negatively affecting the tourism industry.

One of the areas receiving large amounts of plastic and trash from the other Caribbean islands is Little Cayman Island. Though environmental activists on Little Cayman are vying for a single-use plastics ban on the island (Young 2020), the issue of onshore trash accumulation still persists. Researchers from the Central Caribbean Marine Institute on Little Cayman believe that most of the plastic and other trash that washes onto the island is brought primarily to the southeastern faces of the island from Haiti, Jamaica, and the Dominican Republic by major ocean currents (personal communication, L. Forbes). In response to this accumulation, there have been consistent grassroots efforts to remove the tonnes of plastic and other trash that washes up onto the beaches of Little Cayman. We aimed to develop baselines for trash accumulation to determine where and when to focus local cleaning efforts.

We examined the role of ocean currents and local wind patterns in determining where and how much trash accumulates by quantifying the trash accumulation rate on various parts of the island. The Caribbean current, similar to that which created the Great Pacific Garbage Patch, is relatively constant throughout the year, and likely plays a major role in the accumulation and density of trash in the ocean. However, the local, prevailing winds on the island change seasonally, and in the winter, notably, vary in magnitude and direction on short time scales (Burton 1994). Studies have shown that strong, persistent local winds can overcome the influence of prevailing climate patterns on trash accumulation, affecting or even reversing them (Swanson & Zimmer 1990). If ocean currents are the primary determinant of trash deposition, we would expect more onshore trash accumulation on the southern side of the island regardless of wind direction due to the southeastern current hitting Little Cayman. However, if daily wind changes drive trash deposition, we would not expect a difference in trash accumulation among sites overall, but instead in trash accumulation among sites by day depending on wind direction.

METHODS

Observational Design

We studied trash abundance at four beaches on Little Cayman Island, two on the south side of the island: The Department of Environment and Nighthawk; and two on the north side of the island: Bloody Bay, and Cumber's (Fig. 1). Substrate can have a large influence on the quantity and composition of trash accumulation (Pace 1996). To normalize for these variations, we conducted all studies on sandy beaches.

On the first day of our study (considered "day 0"), we cleared all trash present along three ten-meter transects at each of the four sites. Transects ran parallel to the waterline, two meters above the waterline, and were four meters wide. Specifically, we removed trash visible to the naked eye. Then, for four subsequent days, we counted and collected new trash pieces accumulated at each transect (N=48).

We categorized the trash we picked up each day as either plastic, glass, Styrofoam, or "other," in the lab. We also counted the number of bottles, bottle caps, and plastic bags. Because pieces we

picked up may have broken down in the process of transporting them back to the lab, the number of trash pieces at each site were sometimes greater than our counts in the field. Therefore, we opted to use our field values for analyses.

To estimate the number of microplastics present at each site, we took 10 cm cores (N=32) and used two- and five-mm sieves to separate and count microplastics between these sizes. Microplastics smaller than two mm were the same size or smaller than sand and therefore could not be sieved or identified easily. We took three cores, each in the middle of a transect, two meters up from the waterline. We also took five cores per site, four meters up from the waterline, to avoid the area where microplastics would be washed away by waves.

We used wind data taken every five minutes from Tropical Runaway Station ICAYMANB3, located on the west end of Cayman Brac (Tropical Runaway 2020). Ocean current direction was determined from historical hydrographic surveys (Roemmich 1981).



Figure 1: Sites chosen for trash surveys on Little Cayman Island

Statistical Analyses

To analyze the differences in daily trash accumulation between the sides of the island and among days, we performed an ANOVA on $\log(1+x)$ transformed data. To test the significance of the different sites on the trash accumulation, we nested site within the side of the island.

For each period between trash collections, we found both the average north-south component and the east-west component of wind. For each site, we related the number of new pieces of trash averaged across transects to the average wind values since the previous collection. We made two general linear models, one for each wind component, to test how this daily trash

accumulation rate over this period was affected by 1) the side of the island and north-south wind, and 2) the side of the island and east-west wind. We nested site within side of the island in both of these analyses.

RESULTS

We found 1,773 pieces of trash in total. Of these, 1424 (80%) were plastic, 275 (16%) were Styrofoam, 61 (3%) were glass, and 15 (1%) were “other” (Fig. 2). Of the plastic, 523 pieces (36%) were below 1 centimeter in size. In addition, we found 78 bottle caps, 21 straws, and 144 pieces of plastic packaging, including items such as plastic bags. The average accumulation rate per meter per day was 2.5 pieces for the Department of Environment, 1.6 pieces for Bloody Bay, 3.4 pieces for Nighthawk, and 3.6 pieces for Cumber’s. On average at our sites, 2.7 pieces of trash are deposited per meter of shoreline per day. In total, we collected 2.01 kg of trash. On average at our sites, 1.95 g accumulated per meter of shoreline per day.

The total pieces of new trash per day did not differ between days (ANOVA: $F_{3,11}=2.68$, $p=0.06$; Fig. 3) nor the side of the island (ANOVA: $F_{1,11}=3.77$, $p=0.06$). The interaction between day and side of the island was significant (ANOVA: $F_{3,11}=21.80$, $p<0.0001$). Site had no influence on the trash accumulation within the sides of the island (ANOVA: $F_{2,11}=0.11$, $p=0.89$).

The east-west component of wind had no effect on onshore trash accumulation on either side

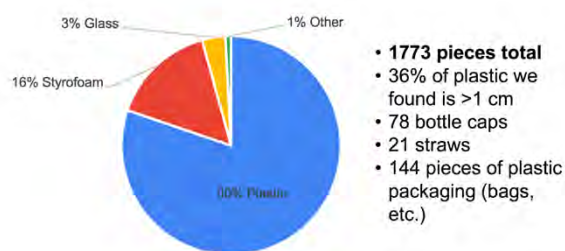


Figure 2. Composition of total trash items collected separated into plastic, Styrofoam, glass, and other.

of the island (general linear model: $F_{1,11}=0.92$, $p=0.36$). Trash accumulation on the north side of the island increased with northerly winds, and trash accumulation on the south increased with southerly winds (general linear model: $F_{1,11}=17.40$, $p=0.0019$; Fig. 4). Site nested within the

side of the island had no significant effect (general linear model: $F_{2,11}=0.88$, $p=0.44$).

The average wind speed during our study was 6.1 mph, and average wind direction was ESE. Wind speeds ranged from 0-16 mph, from northwest to south wind directions. 6.1 mph is slightly weaker wind than the average wind speed for Little Cayman which is 13 mph out of the east.

No micro plastics were found in any of the core samples.

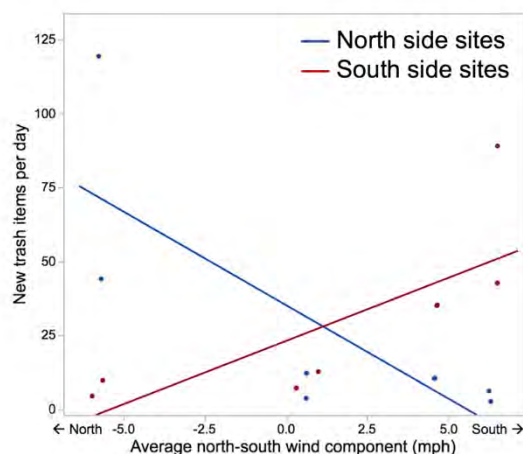


Figure 4: Linear relationships between north-south wind component and trash accumulation rate, by side of the island. Points represent average trash accumulation at one site for one day, located either on the north or south side of the island. Negative wind component values correspond to northerly wind, and positive values correspond to southerly wind.

DISCUSSION

On the time scale of our study, trash accumulation on Little Cayman Island was primarily driven by wind strength and direction. Because trash accumulation did not differ between sides of the island, we can conclude that ocean currents had little influence on trash amounts relative to wind during the course of our study. Additionally, because the amount of trash accumulated did not differ across days, over which there was varying wind, we can conclude that wind did not influence overall trash amounts. However, the amount of trash accumulated on one side of the island is dependent on the day—and therefore the direction and strength of the wind. Though we did not find any micro plastics in the sand, we cannot conclude that there are no micro plastics on Little Cayman Island—this was likely due to a sampling error.

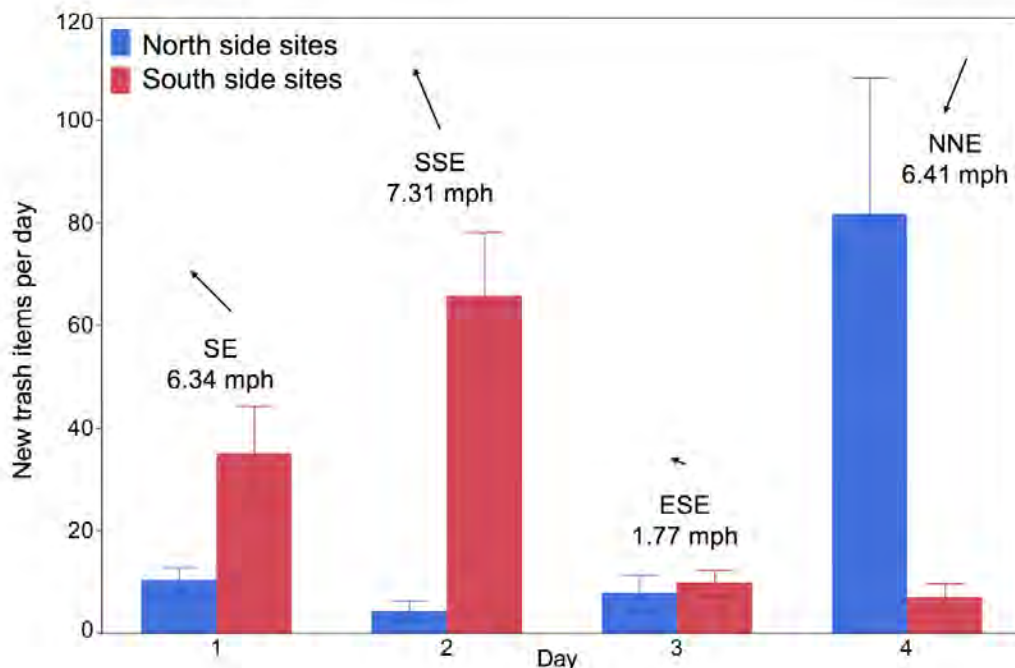


Figure 3: New trash items per day for north and south side sites. Values are per 10 meters. The six transects from the north side sites (three from each Bloody Bay and Cumber's) are shown in blue and the six transects from the south side sites (three from each Department of Environment and Nighthawk) are shown in red. Wind vectors represent the average wind, found by averaging the wind components, over the day leading up to the time of the survey. They are shown in miles per hour, with the length and angle of the arrow corresponding with the speed and direction of the wind, respectively. Mean \pm SE.

The influence of wind direction and magnitude on trash accumulation was also evident in looking at trash accumulation over the course of this study (Supplemental Figure 1). On March 6th (Day 3), the leveling off in cumulative trash amounts was concurrent with the small wind vector on that day. If our calculated rates of 2.7 pieces of trash and 1.95 g per meter per day are representative of all of Little Cayman Island, we could expect almost 100,000 pieces and 74.4 kg of trash to be deposited each day over the roughly 37 kilometers of shoreline of Little Cayman. This accumulation rate is much higher than the results of a similar study, showing an average of 0.0034 pieces per meter per day of anthropogenic debris stranding on Atlantic Ocean shores (Barnes and Milner 2005).

It is possible that these rates are not representative of all shoreline on Little Cayman. Because we normalized for substrate by specifically choosing sandy beaches, we did not capture the diverse shorelines of the island, which include rocky substrates, mangrove forests, and other vegetated areas. Additionally, our samples

were not evenly spaced around the island; Bloody Bay, Cumber's and the Department of the Environment are all located on the west side of the island, while Nighthawk is on the east side. A future study could choose more sites located around the perimeter of the island to learn more about how wind influences this entire area.

Our sample period also may not represent how trash is accumulated all year on Little Cayman. In winter, the winds are more variable than they are in the summer. Given our results, these seasonal wind patterns could cause seasonal trash accumulation patterns. Future studies should survey during different seasons and for longer periods of time. This will help reveal the effects of ocean currents on trash accumulation over longer timescales.

Nevertheless, given the magnitude of trash accumulation on Little Cayman, a strong contingent of conservationists gathers weekly to clean up local beaches. Though these efforts are already very successful at cleaning affected areas, studies such as ours can help inform these practices to be as efficient as possible because they

can predict where trash will accumulate on a short time scale. In the future, trash clean-ups on Little Cayman should focus on areas that have been receiving more wind.

On-shore accumulation studies are also important for understanding the concentration and movement of trash around the world's oceans. Mid-ocean trash concentrations steadily increased from the 1960-1990, however, from 1990-2010 there was no trend despite increases in human trash (Law et al. 2010). This suggests that the sinks and sources of marine plastic are poorly understood. By studying onshore trash accumulations, we can contribute to our understanding of how marine trash concentrations, and our world's oceans, are changing.

ACKNOWLEDGEMENTS

Biggest of all shout-outs to the Central Caribbean Marine Institute staff, namely Lowell, Niki, and Miriam, who were absolutely essential in all parts of this study. Also, great thanks to our Professor and TA's, especially Clare, who spent several hours waiting in a boiling-hot van for us to finish picking up plastic and drove us to the store several times to get snacks when we were tired, hot, and hungry.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this study. Benjamin Schelling was especially useful in finding tiny, tiny, tiny pieces of plastic as well as accurately counting everything, and Shannon Sartain was our sand-core and sand-sieving guru.

LITERATURE CITED

Barnes, D. K. A. and Milner, P. 2005. Drifting plastic and its consequences for sessile organism dispersal

in the Atlantic Ocean. *Marine Biology* 146: 815–825.

Burton, F. J. 1994. Climate and tides of the Cayman Islands. In *the Cayman Islands*. Springer, Dordrecht. 51-60.

Eriksson, C., Burton, H., Fitch, S., Schulz, M., & van den Hoff, J. 2013. Daily accumulation rates of marine debris on sub-Antarctic island beaches. *Marine pollution bulletin*, 66: 1-2, 199-208.

Jambeck, J.R., Geyer, R., Wilcox, C. Siegler, T.R., Perryman, M. Andrady, A., Narayan, R., Law, K.L. 2015. Plastic Waste Inputs from Land into the Ocean. *Science*. 347 6223: 768-71.

Jamieson, A. J., Brooks, L. S. R., Reid, W. D. K., Piernney, S. B., Narayanaswamy, B. E., & Linley, T. D. 2019. Microplastics and synthetic particles ingested by deep-sea amphipods in six of the deepest marine ecosystems on Earth. *Royal Society open science*, 6(2), 180667.

Lebreton, L., Slat, B., Ferrari, F. et al. 2018. Evidence that the Great Pacific Garbage Patch is rapidly accumulating plastic. *Sci Rep* 8, 4666.

Pace, L. 1996. Factors that influence changes in temporal and spatial accumulation of debris on an estuarine shoreline, Cliftwood beach, New Jersey, USA. *Theses*. 1076.

Ritchie, H. and Roser, M. 2020. "Plastic Pollution" Our World in Data.

Roemmich, D. (1981). Circulation of the Caribbean Sea: A well-resolved inverse problem. *Journal of Geophysical Research: Oceans*, 86(C9), 7993-8005.

Swanson, R. L., & Zimmer, R. L. 1990. Meteorological conditions leading to the 1987 and 1988 washups of floatable wastes on New York and New Jersey beaches and comparison of these conditions with the historical record. *Estuarine, Coastal and Shelf Science*, 30(1), 59-78.

Tropical Runaway - ICAYMANB3 (2020). *Weather Underground*.

Young, K. 2020. The plastics problem: Cayman contends with a regional menace. *Cayman Compass*.

SUBSTRATE INFLUENCES OCTOPUS *SPP.* DEN DISTRIBUTION AND ANTI-PREDATOR BEHAVIOR

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TA Editors: Melissa DeSiervo and Clare Doherty
Faculty Editor: Celia Y. Chen

Abstract: Anti-predator evasion or defense are used by all organisms in the animal kingdom. The common octopus (*Octopus vulgaris*) and the Caribbean two spot octopus (*Octopus filiosus*) are two species of coleoid cephalopods that have developed two unique anti-predator evasive behaviors: hiding and camouflage. While *Octopus spp.* have been studied extensively in the Caribbean, there have been few studies investigating how substrate type influences their distribution and anti-predator behaviors. We looked at the distribution of *Octopus spp.* across different substrates and depths in the ocean near Central Caribbean Marine Institute (CCMI) on Little Cayman Island, using den as a proxy for presence. Additionally, we conducted a lab experiment measuring behavioral response of individuals to natural vs unnatural substrates. Our survey at CCMI suggested that *Octopus spp.* prefer coral rubble substrate and are potentially most densely distributed in water with a depth less than 140 cm. Our behavioral study suggested that natural substrate is more conducive to camouflage and that camouflage ability varies based on size. As anthropogenic effects continue to alter the world in irreversible ways, natural substrates and water depths will change and man-made products and colors will become more prevalent in marine ecosystems. These results offer insight into the factors that influence *Octopus spp.* distribution and anti-predator behavior as well as how human impact will influence these highly adaptive creatures in the future.

Key words: Anti-predator behavior, den, distribution, substrate, *Octopus filiosus*, *Octopus vulgaris*

INTRODUCTION

The selecting pressure of predation is ubiquitous throughout marine ecosystems. Over evolutionary time, marine organisms have developed different strategies to increase their fitness. Schooling, increased vigilance, and mimicry are just a few examples of anti-predator behaviors (Reebs, 2008). Behavioral plasticity, or the change in an organism's behavior resulting from exposure to some stimulus or experience, is also important in marine species. Recent studies have shown that individuals from the same population may differ in their level of behavioral plasticity (Dingemanse & Wolf, 2013; Kim, 2016). While some studies have shown plasticity among individuals for anti-predator evasion strategies such as schooling and boldness, there are other strategies that are less often studied.

Coleoid cephalopods, a group that includes squid, octopus and cuttlefish, have developed two anti-predator behaviors: hiding and camouflage. Cephalopods spend most of the day in dens to avoid predation and use camouflage to blend into their surroundings when they leave (Katsanevakis & Verriopoulos 2004). The common octopus (*Octopus vulgaris*) and the Caribbean two spot

octopus (*Octopus filiosus*) are two species of cephalopod that are native to the Caribbean and use both of these anti-predator strategies (Aronson 1991; Aronson 1998; Gilmore, *et al.*, 2016). *O. vulgaris* and *O. filiosus* inhabit seagrass, coral reefs and rubble. However, they differ greatly in size- *O. vulgaris* can be up to 3 feet long while *O. filiosus*' maximum length is 10 inches. One study from southern Angola has shown that octopuses inhabiting shallow, small-boulder substratum make extensive modifications to their habitat, excavating dens of up to 1 m deep and are capable of retracting small boulders over their den entrances during the day (Beer & Potts, 2013). They have been shown to use man-made structures such as PVC pipes as shelters. As benthic foragers, octopus are likely to navigate through anthropogenic refuse. Research has also been done on the specialized organs and tissues *Octopus spp.* use to camouflage. For example, chromatophores are organs in the skin that contain red, yellow and brown pigment sacs that expand and contract to match the patterns around them (Hanlon *et al.*, 2011). Octopus contain other specialized tissue that allow their skin to raise and create three dimensional textures (Gilmore *et al.*, 2016).

The use of dens and camouflage are clearly important and essential in the biological fitness of *Octopus spp.* but there have been few studies on how substrate type influences anti-predator behaviors. To determine substrate and depth preference of *Octopus spp.* we quantified the octopus' dens across common substrates at CCMI on Little Cayman Island. We also quantified the proportion of time that *Octopus spp.* spend hiding and camouflaging in a lab setting on natural and unnatural substrates.

We hypothesized that some substrates, such as turtle grass, allow for an octopus den to be more hidden and are located further away from predators in the reef. This is supported by previous research which suggests that substrate is one of the main factors influencing den density and distribution (Guerra *et al.*, 2013; Axel *et al.*, 2020). Alternatively, there may not be a preferred substrate because other abiotic factors such as water depth may have a greater influence on *Octopus spp.* distribution. As for the anti-predator behavior of camouflage, man-made materials and some colors are not naturally occurring in reef communities. Therefore, we hypothesized that substrate color and morphometry will have an effect on the proportion of time *Octopus spp.* spend camouflaging and hiding. Alternatively, when we consider that some may already be acclimated to materials that are not naturally occurring, we hypothesize that the octopus will not change their behavior based on substrate morphology or color.

METHODS

Field Study

To quantify the number of octopus dens on different substrates, we surveyed seven 50 x 2-m transects in the ocean near CCMI, on Little Cayman Island (Fig. 1). Transects ran parallel to shore and were located at distances progressively closer to the reef, so depth within a transect varied minimally. We measured the depth and categorized the substrate every two meters along each transect and recorded presence or absence of octopus dens. We then calculated the proportion of turtle grass, sand, and coral rubble in the surveyed area.



Figure 1: Satellite view of CCMI overlaid with transect lines.

Experimental Study

To better understand the ability and propensity of *Octopus spp.* to perform anti-predator strategies on different substrates, we conducted experimental manipulations of substrate in the lab. *Octopus* (N=4) were collected from Mary's Bay on Little Cayman Island, from submerged polyvinyl chloride (PVC) tubes they had colonized. After collection, each individual was housed separately in a holding tank on a water table. We set up three experimental tanks containing different substrates: A) pieces of white PVC pipe wrapped in clear scotch tape, B) coral rubble, and C) PVC pipe wrapped in colorful flagging tape (supplemental Fig. 2). Each individual was tested in each of the 3 experimental tanks in a random order. They were given a 30 second acclimation period, and then observed for two minutes and thirty seconds. At each thirty second interval, the anti-predator behavior of the individual octopus was recorded as either hiding, camouflaging, hiding and camouflaging, or neither.

Statistical Analysis

We used JMP Pro 14 for all statistical analyses. To determine if dens were distributed uniformly or influenced by substrate type, we ran a Fisher's exact test. To determine if depth was a significant factor in the presence or absence of dens, we ran a logistic regression with depth nested within transect number. Due to low sample size, we did not perform statistical analyses on our experimental data, but we report means and standard errors of time spend camouflaging and hiding and we discuss potential differences among treatments and individuals.

RESULTS

Field study

Substrate type had a significant effect on den distribution (Fisher's exact test: two-sided; $p = 0.0155$; Fig. 2). 60% of the dens were found in coral rubble despite it composing only 22% of the substrate. 30% of the dens were found in grass (44% of the substrate) while 10% of the dens were found in sand (34% of the substrate). There was no effect of depth on the presence or absence of dens (nominal logistic fit; depth [transect number]: chi-square = 3.313, $df = 7$, $p = 0.855$); however, all dens in our survey were in water <120 cm deep (Fig. 3). We did observe some dens in the deeper zone (>120 cm) that were outside of our transects.

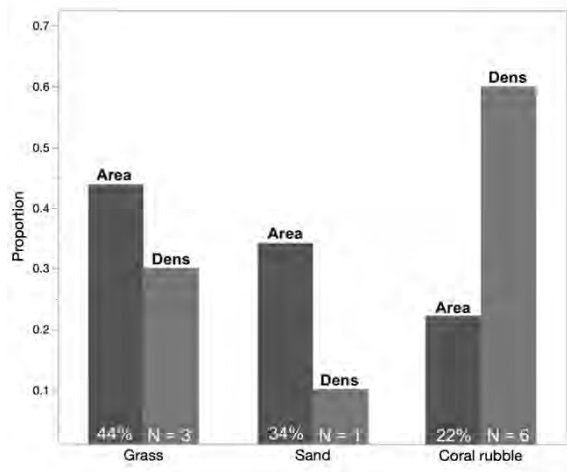


Figure 2: Proportion of area and dens found by substrate.

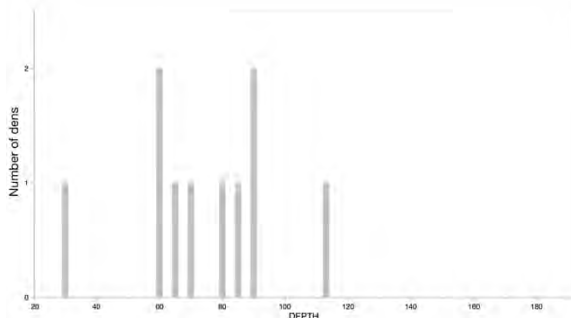


Figure 3. Number of dens by depth (cm).

Experimental study

For our experimental trials, we used four octopus individuals: two common octopuses and two Caribbean two spot octopuses. The common octopuses were 19.43g and 1.91g while the

Caribbean two spot octopuses were 20.63g and 13.00g. Size may have had an impact on behavior. The smallest individual always hid, while the larger individuals camouflaged more often. Across the treatments, octopus camouflaged most often with the natural coral rubble substrate (62.5% of the time), and the least often with the colored PVC (25% of the time), the most unnatural substrate (Fig. 4). Furthermore, the octopus never camouflaged with the colored PVC, but instead would manipulate their environment and camouflage with the white bottom of the tank. Across all treatments, octopus were hiding over half the time. The octopus exhibited neither behavior 4% of the time in the natural substrate, 25% of the time in the white PVC substrate, and 33% of the time in the colored PVC (Fig. 4). One *O. filiosus* spent a majority of the trials swimming around the tank trying to escape, rarely attempting to hide or camouflage. It was also the only individual who used the evasion option of inking—a behavior associated with high stress. The smallest individual we tested hid for the entire trial, regardless of substrate type. A limited sample size ($N=4$) restricts the robustness of any conclusions we can draw about trends in behavior.

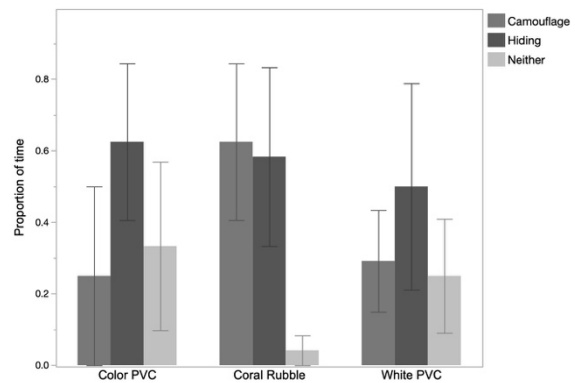


Figure 4: Proportion of time spent camouflaging, hiding, or neither across three different substrate types.

DISCUSSION

Octopus appear to be affected by the substrates on which they build their middens and display hiding or camouflage behavior. One potential reason is that larger predators, such as sharks or barracudas may have trouble navigating shallow waters, providing the octopuses with increased safety. Alternatively, shallower depths in this system are

also at a greater distance from predators in the reef. It is also possible that the majority of octopus' prey live in the shallower, seagrass habitats, and they tend to form dens where their resources are most abundant. Additionally, we observed that all of the dens in the second transect were under a coral overhang, created by a remnant reef flat. This particular structure may serve as the ideal dwelling for an octopus, and spatial limitation of den availability may have a greater influence than the depth of the water on octopus' distribution.

In our experiment, we did not observe the octopus attempting to camouflage on the colored PVC, but they did camouflage on the white PVC. We suggest this difference in behavior is due to the novel coloration of the colored PVC and may indicate that octopuses are limited in their color changing ability. High amounts of human trash building up near the shore and increasing implementation of coral nurseries using man-made materials could greatly impact the survival of octopus if these materials are made of colors not naturally found in marine habitats.

Despite only having a few octopuses for our experiments, our trials illustrate potential behavioral plasticity among individuals in choosing to camouflage or hide, as some of the octopuses had clear tendencies across the three trials. For example, the *O. filiosus* individual who inked might have prior experience that led it to prefer fleeing over hiding, such as its camouflaging failing to work on a predator. In addition, because this *O. filiosus* was in the upper size range of the species, it could have more experience with predator avoidance than a same sized juvenile *O. vulgaris*. Physical features such as size and age might also impact whether an individual chooses to hide or camouflage. These initial trials and observations can serve as a pilot study and a model for future experiments to build off of. For example, a future study could use our methodology with different naturally occurring substrates, such as seagrass, coral rubble, and sand.

Variation in behavior can mean life or death for individuals. Anthropogenic effects alter marine habitats, which will inevitably impact these behaviors. Species that utilize habitats where plastics and other waste accumulate may be especially affected. Natural substrates and water

depths will continue to change over time, and man-made products will become more prevalent. These shifts in habitat could result in widespread changes in anti-predator evasion strategies. Research examining organisms' evasion tactics will be crucial in understanding predation as humans continue to manipulate and impact marine ecosystems.

ACKNOWLEDGEMENTS

Dagny hands down saved this project when it was on the verge of completely fizzling out—so a huge thank you there. This work could not have been done without John's plumbing and operational help with water tables. Thank you, Ms. "M" and Maxine for nutritional support during this project. We would like to thank our four test cephalopods, of which, none were harmed in this study. Thank you, Clare, for the immense help in statistical analysis; additionally, thank you Robert Alter for admitting a wrong position was originally held. Thank you, Melissa and Celia, for everything, always. Finally, we would like to thank the entire CCMI cast (despite octopus permit politics) for everything these past three weeks.

AUTHOR CONTRIBUTIONS

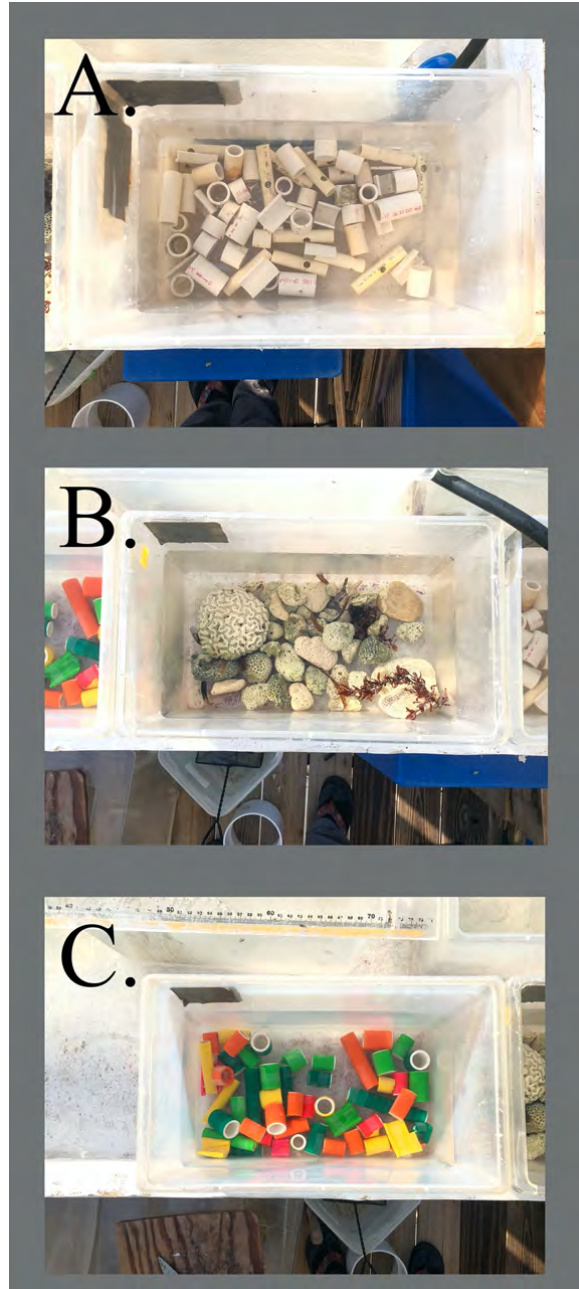
All authors contributed equally, in variable ways.

LITERATURE CITED

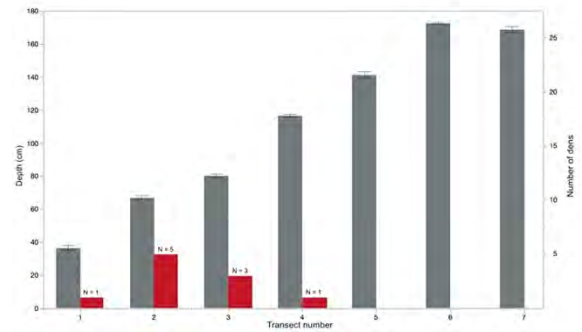
- Aronson, R. 1991. Ecology, paleobiology and evolutionary constraint in the octopus. *Bulletin of Marine Science*, 49: 245-255.
- Aronson, R. 1998. Life history and den ecology of *Octopus briareus* Robson in a marine lake. *Journal of Experimental Marine Biology and Ecology*. 95(1): 37-56.
- Bergmann, S., Lieb, B., Ruth, P., and Markl, J. 2006. The hemocyanin from a living fossil, the cephalopod *Nautilus pompilius*: Protein structure, gene organization and evolution. *Journal of Molecular Evolution*, 62: 362-374.
- Cooper, K., Hanlon, R. 1986. Correlation of iridescence with changes in iridophore platelet ultrastructure in the squid *Lliguncula brevis*. *Journal of Experimental Biology*, 121: 451-5.
- Cloney, R. & Brocco, S. 1983. Chromatophore organs, reflector cells, iridocytes, and leucophores. *American Zoologist*, 23: 581-592
- Beer, C and Potts, W. 2013. Behavioral observations of the common octopus *Octopus vulgaris* in Baía dos Tigres, southern Angola, *African Journal of Marine Science*, 35(4), 579-583.

- Gilmore, R., Crook, R., and Krans, J. 2016. Cephalopod Camouflage: Cells and Organs of the Skin. *Nature Education*, 9(2):1.
- Guerra, Ángel et al. 2014. Dwellers in dens on sandy bottoms: Ecological and behavioral traits of *Octopus vulgaris*. *Scientia Marina*. 78(3): 405-414.
- Hanlon, R. 2007. Cephalopod dynamic camouflage. *Current Biology* 17: 400-404.
- Mathger, L., Denton, E., Marshall, N., Hanlon, R. 2009. Mechanisms and behavioral functions of structural coloration in cephalopods. *Journal of the Royal Society Interface* 6: S149-S163.
- Mather, J. A., Leite, T. S., and Batista, A. T. 2012. Individual prey choices of octopuses: Are they generalist or specialist? *Current Zoology*, 58:4. 597–603.
- Reebs, S. 2008. How fishes try to avoid predators. Université de Moncton.
- Katsanevakis, S., and Verriopoulos, G., 2004. Den ecology of *Octopus vulgaris* Cuvier, 1797, on soft sediment: availability and types of shelter. *Scientia Marina*, 68(1).

APPENDIX



Supplemental figure 1: Tank with white PVC pipe (panel A), tank with coral rubble (panel B), and tank with colored PVC pipe (panel C).



Supplemental figure 2. Average depth by transect (grey) and number of dens per transect (red). Transect number one was closest to shore.

MANAGEMENT-ORIENTED POPULATION MODELING OF INVASIVE LIONFISH OFF LITTLE CAYMAN ISLAND

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Abstract: Humans have caused rapid global species exchange, bringing invasive species dynamics to a high level of relevance in science and management. Due to their high fecundity, rapid consumption, and numerous strong defenses, lionfish are a highly effective invasive species. Since their discovery offshore of Little Cayman in 2008, lionfish have posed a direct threat to the dominant economy of diving and ecotourism on the island. To better understand lionfish population dynamics and to aid in control of the species, we developed a stage-based matrix model describing the population of lionfish off of Little Cayman. This model incorporates three age classes (larvae, juveniles, and adults) and uses a step size of one year. Transition matrix values were calculated using information from the literature about lionfish life history, long term data collected from the Central Caribbean Marine Institute (CCMI) about culling practices on Little Cayman, and data obtained from lionfish dissections. Five possible transition matrices describing the growth of the population were created given different estimates for batch fecundity, egg viability, and culling pressure. Given each of these possible transition matrices, *STELLA*® was used to model how the population of lionfish on Little Cayman would change over time from estimated abundances in 2012. The transition matrix incorporating the observed culling pressure of 0.37 %/ mo and a batch fecundity viability of 0.21 was used to project a population abundance in 2019 that matched our estimates given CCMI's long term dataset. Assuming this batch fecundity viability of 0.21, increasing culling efforts from 0.37 %/mo to 2 %/mo projects a near extinction of lionfish around Little Cayman by 2050. Sensitivity analysis of these transition matrices suggests that decreasing adult survivorship should be the primary focus of culling efforts.

Key Words: lionfish, invasive species, population viability analysis, culling, Little Cayman

INTRODUCTION

As rapid expansion of global trade and travel lead to the increased movement of species into novel and unprepared systems, scientific inquiry into the impacts and management of invasive species is increasingly relevant. While these species do not upset ecosystem balance when in their native environment, when they are introduced to a new system with abundant food resources and no natural predators (Enemy Release Hypothesis, Keane 2002) they have the potential to be extremely successful. Invasive species can dominate available resources, decimate entire populations, and ultimately be a driving factor of steep declines in biodiversity within an ecosystem.

Lionfish (here referring to *Pterois miles* and *Pterois volitans*) are an example of a highly effective invasive species. Lionfish can disperse quickly across long distances in their pelagic larvae form, and are thought to have spread from one population of just six aquarium-released fish in Florida in the 1980s to concentrations of up to

1,000 individuals per acre in locations across the Eastern coast of the Americas and in the Caribbean (Ruiz et al. 2006). This invasion poses many serious ecosystem-level implications. Lionfish decimate populations of algivorous fish that reduce interspecific algae-coral competition, possibly causing additional harm to already-fragile coral reef ecosystems. Lionfish also target the same prey species as some commercially relevant fish species, possibly resulting in negative impacts to fisheries and resounding ecological and economic impacts.

In the Indo-Pacific, where lionfish are an endemic species, populations are limited by predation and have limited recruitment due to low temperatures (Côté et al. 2013, Gardener 2015). When lionfish were introduced to tropical zones in the Atlantic, they experienced rapid population growth because the abiotic and biotic controls on the population were lessened. High and relatively consistent tropical temperatures allow them to reproduce all year long, with

females producing approximately 30,000 eggs every three days, and even more rapidly in months with ideal temperatures (22-28 ° C, Fogg et al. 2014). Lionfish can also store large amounts of energy in fat deposits, potentially enhancing their survival (Costello et al. 2012). In addition, their stomachs can expand up to 30 times in size, allowing for rapid energy intake of small fish and crustaceans, which in the Atlantic are unadapted to lionfish predation (Weis and Sol 2016). In invasive ranges, they have few predators and possess impressive defenses including a wide halo of sharp needle-like spines containing the neuromuscular toxin acetylcholine, which is released upon contact (Vetrano et al. 2002).

On Little Cayman Island in the Caribbean, which lionfish reached just 12 years ago, locals are struggling to keep the population under control with volunteer-driven and community-funded culling (organized, systematic elimination of unwanted wildlife species) efforts. Here, lionfish pose a direct threat to the livelihood of the local dive and ecotourism businesses that sustain most residents.

This paper aims to better understand the ecosystem-level effects of lionfish and to inform management tactics for mitigating the effects of this invasive species off Little Cayman Island. In conservation and invasive species management, maximum efficiency is reached by determining and targeting the life stage at which changes will most drastically affect the population growth rate. To address this, we developed a matrix population model for lionfish and modeled population responses to changes in culling pressure and batch fecundity viability. We also determined the temporal and spatial distribution of lionfish juveniles and adults around the island to inform temporal and spatial culling targets. We also analyzed the gut contents of culled individuals to understand lionfish diet and speculate about impacts of lionfish population growth or decline on lower trophic levels, specifically in the waters of Little Cayman.

METHODS

Dissections

Lionfish used in dissections were sourced from community cull events organized by Central

Caribbean Marine Institute (CCMI), individual culls, and juvenile lionfish used in a separate lionfish behavior study (Alter et al. 2020). A total of 30 lionfish (male: N = 15, female: N = 13, unidentifiable: N = 2) were dissected over the course of 3 days. In addition to batch fecundity, we measured gape height, gape to length ratio, gamete development, and stomach contents. If a lionfish had distinguishable organisms in its stomach, prey type (fish, shrimp, or crab) was recorded and the length of each prey was measured.

Matrix Model

A stage-based matrix model of the population of female lionfish was constructed using three age classes: larvae (L), juveniles (J), and adults (A). The sex ratio for lionfish on Little Cayman is 1:1 (Edwards 2012). This stage-based matrix model accounts for the fecundity, survival, and growth of each age class over the time step of one year.

$$\begin{pmatrix} 0 & 0 & R_A \\ G_L & P_J & 0 \\ 0 & G_J & P_A \end{pmatrix} \times \begin{pmatrix} L_T \\ J_T \\ A_T \end{pmatrix} = \begin{pmatrix} L_{T+1} \\ J_{T+1} \\ A_{T+1} \end{pmatrix}$$

The parameters defining the transition matrix are annual adult fecundity (R_A), larval recruitment to the juvenile age class (G_L), the probability of juveniles surviving the year and remaining in the same age class (P_J), the recruitment of juveniles to the adult age class (G_J) and the probability of adults surviving the year and remaining in the same age class (P_A).

Parameter Values

To determine transition matrix parameters for the life table model, we used a combination of field data from the long-term culling program conducted by CCMI and values from lionfish fecundity and life table literature to derive the most accurate parameters for Little Cayman Island, as detailed below.

Annual Fecundity: R_A

To calculate the annual fecundity of lionfish, we dissected fish to determine batch fecundity (BF) and estimated spawning frequency (SF). We then created equations for several scenarios of

annual fecundity and annual survival, integrating estimates of the average number of eggs per female per year while accounting for mortality due to predation.

BF estimates were calculated from dissections. However, of the 30 dissected lionfish, only 3 were mature, reproductive females that could be used for fecundity estimations. For each reproductive female, the left and right ovaries were weighed. Then, an approximately 0.15 g subsample of ovarian tissue was viewed under 4 x magnification and the number of eggs was counted. The number of eggs and subsample weight were used to calculate total eggs per female. Egg counts ranged from 29,551 to 38,221 with an average egg count of 31,777, which falls within the expected BF for lionfish (Fogg et al. 2017).

To estimate SF on Little Cayman, we assumed that Little Cayman lionfish spawn year-round (Pers. comm. several Little Cayman residents) (Edwards 2012). We then followed the methods of Fogg (2017) and used surface-sea temperature (SST) to find months of higher and lower relative spawning frequency. We used SST collected by a weather station on Little Cayman to count the number of months with ideal SST for lionfish, between 22 and 28 ° C (Fogg et al. 2017) (seatemperature.info). For months with ideal SST, we assumed an average spawning frequency of once every 2 days, and for months with non-ideal SST, we assumed an average spawning frequency of once every 4 days (Fogg et al. 2017). We then calculated an average yearly spawning frequency of 2.67 from the monthly average spawning rates.

To determine the total number of eggs produced per female per year, we multiplied the average BF by annual SF to calculate the number of eggs per female per year. We then assumed that half of these eggs would be female, so we multiplied the total egg production by 0.5.

The number of eggs produced by any given female per year does not accurately reflect the R_A , or annual fecundity of a female lionfish, since over the course of a year, almost half of the female lionfish in a population will perish, even without culling efforts (Morris 2009). To get a more accurate estimate of the annual fecundity, we integrated two functions, $F(T)$ fecundity at a given time, and $S(T)$ survival at a

given time, to determine the annual fecundity of a female while taking into account the annual mortality of female lionfish. We represented $F(T)$ in three ways, (1) with a maximum egg viability estimate, assuming 75% of eggs will be viable and fertilized, (2) with a more conservative egg viability of 25%, and (3) with an even more conservative egg viability estimate of 21%. These conservative estimates are biologically relevant as not all eggs may be viable at a given spawning event, some females may not spawn at every given opportunity, and not all eggs may be fertilized during a spawning event (Fogg 2017). $S(T)$ was also represented in two ways, based on absence and presence of culling pressure (see Recruitment of Juveniles to Adult Age Class). From these four equations, we integrated $\int_0^1 F(T)S(T)dT$ for four different scenarios (Cases 1 through 5) to get a range of likely R_A values (in millions). Case 5 was a transition matrix with an altered R_A to match the population growth (λ) of the population estimates of females from the long-term dataset (see population estimates section). Cases 6, 7, and 8 used the batch fecundity viability in case 5 with increased monthly culling pressure of 1%, 2%, and 3% respectively to model the population under increased culling.

Table 1: $F(T)$ and $S(T)$ equations based on categories of fecundity estimates and culled vs. uncultured

Categories	Equation
F, Max estimate	$F(T) = 1.619 * T$ (in millions)
F, Conservative estimate	$F(T) = 0.5389 * T$ (in millions)
S, Uncultured	$S(T) = e^{-0.6408 * T}$
S, Culled (0.38 % culling)	$S(T) = e^{-0.6877 * T}$

Table 2: Integrals for R_A under cases of fecundity estimates and culled vs. uncultured

Case	Equation values in millions	R_A values in hundred thousand
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Case 1: Max estimate, unculled	$\int_0^1 (1.619T * e^{-0.6408 * T} dt)$	533,6
Case 2: Max estimate, culled	$\int_0^1 (1.619T * e^{-0.6877 * T} dt)$	518,1
Case 3: Conservative estimate, unculled	$\int_0^1 (0.539T * e^{-0.6408 * T} dt)$	177,9
Case 4: Conservative estimate, culled	$\int_0^1 (0.539T * e^{-0.6877 * T} dt)$	172,7
Case 5: R _A modeled to fit field data, culled	Modeled to fit field data	230,0

Larval Recruitment: G_L

For our estimate of larval recruitment to juvenile age class we used a recruitment rate of 0.00003, an estimate derived from other tropical fish that also have pelagic larva and are broadcast spawners (Arenholz and Morris 2010, Morris 2009). While this value is based off of a one-month iterative population model, the G_L can be scaled to a year since all larval lionfish will mature to the juvenile life stage in approximately 30 days (Morris 2009). Thus, G_L only applies in the first month of development.

Probability of Juveniles Remaining Juveniles: P_J

Since this model uses a one-year step and the juvenile age class of lionfish only lasts 1 year, the probability of a juvenile staying a juvenile over a one-year period is 0 (Edwards 2012).

Recruitment of Juveniles to Adult Age Class: G_J

Since all juveniles will mature to adults over a one-year period, juvenile recruitment to the adult age class is equivalent to the juvenile survival over a one-year period. From Morris (2009), we

estimate the monthly mortality rate of juvenile lionfish to be 0.165 with no culling efforts. We determined the increase in annual mortality from the addition of a monthly culling pressure of 0.0037 by finding the percent of lionfish culled out of the total population estimate per year, and then back-solving using compounded survival rates to find the value of 0.0037. We assumed juvenile and adult lionfish were culled proportionally to their respective population sizes, as all lionfish (juveniles and adults) are targeted during culling events. We calculated compounded yearly survival rates for both culled and uncultured populations with adjusted finite survival rate equations Krebs (2014).

$$(1 - 0.615)^{12} = 0.115 \text{ //unculled}$$

$$(1 - (0.615 + 0.0037))^{12} = 0.109 \text{ //culled}$$

Probability of Adults Remaining as Adults: P_A

We used the same adjusted finite survival rate equation to calculate the proportion of adults surviving the year under culled and uncultured conditions. A monthly finite survival rate of 0.052 was taken from Morris (2009).

$$(1 - 0.052)^{12} = 0.527 \text{ //unculled}$$

$$(1 - (0.052 + 0.0037))^{12} = 0.503 \text{ //culled}$$

Temporal and Spatial Distribution (Depth, Time, Location)

We took values for maximum depth of cull, size class distribution, cull site, month in which the cull was conducted, and total lionfish encountered per cull from the 2012-20 culling dataset collected by CCMI at 36 different cull sites around Little Cayman.

Data for total lionfish encountered were log transformed to meet assumptions of normality. We sorted the size class distribution into age classes of adults and juveniles using a length to age relationship defined for Little Cayman by Edwards (2012). We then used JMP 15 to conduct regressions to analyze the relationship between maximum depth and number of lionfish of each age class encountered. We also conducted a regression on cull site with the number of lionfish encountered from 2012-2019, a regression on month with the number of lionfish encountered, and an ANOVA to analyze culling catch differences between dive sites.

Estimating Population Size

We used the 2012-2020 culling dataset collected by CCMI at 36 different sites around Little Cayman. We first estimated the area covered by each cull dive using the equation:

$$A = N_p \cdot S \cdot V \cdot (T - 7)$$

A = total area sampled in culling event in m²

N_p = number of participants

S = average speed of diver = 15 m/min

V = view width of diver = 4 m

T = Duration of dive (min)

t = time for descent, ascent, and safety stop = 7 min

We then divided the total number of lionfish encountered per culling dive by this calculated area covered to estimate the population density. Total available lionfish habitat was calculated using ImageJ to find area from shore to an isoline at 300m depth around Little Cayman Island. Density was then multiplied by total available lionfish habitat area and averaged for all sites on a per year basis to give a total population:

We used the 1:1 sex ratio of Little Cayman lionfish (Edwards 2012) to find the total population of female lionfish.

$$P = \frac{H}{A} L_E$$

P = total population size

A = total area sampled in culling event (in km²)

H = total available area of lionfish habitat around LC = 27.25 km²

L_E = Lionfish encounters on culling event

*STELLA® Modelling*Building MangoTango

Our population model for female lionfish, MangoTango, was developed using STELLA® from High Performance System® (Tixier et al. 2004). STELLA® is a software package that can be used to model populations by creating a pictorial diagram of a given system and assigning the appropriate formulas and parameters to the system (Yao-Nan & Mei-Yu 2009). MangoTango runs with a yearly step and

is structured with three age classes: larvae, juveniles, and adults (Fig. 1).

For each step, the model calculates births into the larvae age class using the following equation:

$$Births = INT(R_A * Adult)$$

For this and all subsequent equations, the STELLA® INT function was used, which selects the closest integer equal to or less than the value produced by the product of two values representing the input or output to the age class in the population for that time step.

For each step, the model calculates larval deaths using the following equation:

$$e_{ij} = \frac{a_{ij} \partial \lambda}{\lambda \partial a_{ij}}$$

where M_L is multiplied by the number of larvae present in the population.

For each step, the model calculates the number of larvae that advance to the juvenile age class using the following formula:

$$Growth\ to\ Juvenile = INT(G_L * Larvae)$$

where G_L is multiplied by the number of larvae present in the population.

For each step, the model calculates juvenile deaths using the following equation:

$$Juvenile\ Deaths = INT(M_J * Juveniles)$$

where M_J is multiplied by the number of juveniles present in the population.

For each step, the model calculates the number of juveniles that advance to the adult age class using the following formula:

$$Growth\ to\ Adult = INT(G_J * Juveniles)$$

where G_J is multiplied by the number of juveniles present in the population.

For each step, the model calculates adult deaths using the following equation:

$$Adult\ Deaths = INT(M_A * Adults)$$

where M_A is multiplied by the number of adults present in the population.

Population modelling over time given different transition matrices

For the transition matrices associated with cases 1-5, MangoTango was used to determine how the population would change over the course of 38 years given the estimated population on Little

Cayman in 2012. While this Year 0 (2012) population value, which includes both juveniles and adults, was taken from the estimated population size in 2012, the number of larvae (10,000,000) and the proportion of juveniles (30,304) to adults (2,153) were assumed.

For the parameters defined by the transition matrices associated with cases 6-8, MangoTango was used to determine how the population would change over the course of 31 years given the estimated population on Little Cayman in 2019. The numbers of larvae (338 000 000), juveniles (11 400), and adults (2660) in Year 0 (2019), was determined by running MangoTango from 2012 to 2019 using the case 5 transition matrix, which produced population estimates most closely matching those observed.

Sensitivity and Elasticity

To find the sensitivity matrix for the transition matrix that most closely matches the trends observed in the actual data (case 5), we first calculated the population growth rate (λ) by finding the dominant eigenvalue of the transition matrix. To find the sensitivities of λ to each of the transition matrix elements (a_{ij}), we used the following equation:

$$\frac{\partial \lambda}{\partial a_{ij}} = \frac{v_i w_j}{\langle \mathbf{w}, \mathbf{v} \rangle}$$

In this equation, \mathbf{v} is the left eigenvector associated with the transition matrix, \mathbf{w} is the right eigenvector associated with the transition matrix, and $\langle \mathbf{w}, \mathbf{v} \rangle$ is the scalar product of the left and right eigenvectors.

Table 3: Lambda values and 2050 population estimates for each case. Cases 1 through 5 began with a population size of 32,500 females, while cases 6 through 8 began with a population size of 14,100 females. (Please note that our model does not take into account density dependence and its effect on the ability of a population to grow exponentially over long time periods. Cases 1 through 5 were run on a 38-year model (2012 to 2050) and cases 6 through 8 were on a 31-year model (2019 to 2050).

Case	Batch Fecundity Viability	Monthly Culling Pressure	λ	Females in 2050
1	0.75	0%	1.429	15,400,000,000
2	0.75	0.37%	1.067	168,000
3	0.25	0%	1.386	4,310,000,000
4	0.25	0.37%	1.033	51,700
5	0.21	0.37%	0.989	9,6300
6	0.21	1%	0.935	1,780
7	0.21	2%	0.856	124
8	0.21	3%	0.784	12

Elasticity matrices represent the differential contributions of each value in the transition matrix to λ . They allow for a direct comparison of the importance of each of the elements within the transition matrix, particularly when elements differ by orders of magnitude. To determine the elasticity (e_{if}) of each value in the transition matrix, we used the following equation:

RESULTS

When lionfish populations were modeled over time given transition matrices calculated for cases 1-5, the projected population after 38 years varied (Table 3). To create a model that most closely matched the trends in population between 2012 and 2019, a batch fecundity viability of 0.21 was assumed and the culling pressure of 0.38% in Little Cayman was added. The annual growth rate, λ , for case 5 was 0.989.

According to the elasticity matrix calculated from the transition matrix used in case 5, the parameter that has the greatest effect on the population growth rate of lionfish is the proportion of adults that are surviving on an annual basis.

If a batch fecundity viability of 0.21 was assumed and the culling pressure was increased to 1.0%/mo, λ was 0.935 and the projected female population in 2050 was 1,780. When this culling pressure was increased to 2.0%/mo, λ was 0.856 and the projected female population in 2050 was 124. When this culling pressure was increased to 3.0 %/mo, λ was 0.784 and the projected female population in 2050 was 12.

Supplemental results

While there is variation in total lionfish population estimates between years (Figure 4), this does not result in an overall trend of population increase or decrease since culling began in 2012 (slope = - 344.5, $p=0.8$, $R^2=0.00008$).

There tended to be more adult lionfish at deeper depths than at shallower depths (slope=0.0045, $p=0.059$, $R^2=0.12$), and there tended to be more juveniles in shallow water than in deeper water (slope=- 0.0018, $p=0.49$, $R^2=0.11$). However, neither of these relationships were statistically significant.

January tended to have higher numbers of lionfish encountered per cull, with significantly higher monthly cull values than May, June, July, August, October, and November (DF = 11, $F=4.6$, $p < 0.0001$); ($p < 0.04$ for all post-hoc comparisons).

The four cull sites with the lowest number of lionfish encountered were all on the Bloody Bay Wall, the most frequented and accessible dive area where culling occurs most frequently. The four cull sites with the highest number of lionfish encountered were all at less frequented, less accessible dive areas (info on site accessibility and popularity from Giacomo, CCMI, personal communication.).

In the dissections, 10 out of 30 lionfish had identifiable stomach contents. We found crab, shrimp, and fish ranging from 0.2 cm - 4 cm in length. No prey was identifiable to the species level. The longest fish we found was in the stomach of the lionfish with the largest gape size (5.5 cm). The gape:length ratio was 0.2, which is typical for fish (Montaña et al. 2010).

DISCUSSION

Interannual variation in estimated historical population sizes on Little Cayman could be due to annual variation in culling pressure, migration of individuals from other populations, biotic factors including increased predation or depleted food sources, or abiotic environmental factors.

Our models demonstrate that using the fairly standardized methodology of estimating R_A based on egg numbers alone gives an overestimate of the real R_A value when a yearly time step is used. Even in our model that most

closely fits the trends in field data, the conservative R_A value with culling factored in is still an overestimate given other transition matrix values. When we alter the R_A to fit the population trends from our field data in case 5, incorporating current culling pressure of 0.37 %/mo., we find an R_A of 230,000 which is far less than the total possible egg production in a year for a female lionfish, even when accounting for mortality. In case 5, 21% of total eggs released by one female are assumed to be viable and fertilized every year.

There are numerous logical reasons that the R_A value would be under a quarter of the total number of eggs, especially considering that the R_A for lionfish is a very high value when compared with other fish species. It is possible that: (1) only some of the viable eggs are released at each spawning event, (2) all eggs are released, but only some are ripe, and (3) all eggs are released but only some are fertilized. Another possibility for this overestimate in fecundity could be missed spawning events. This model, like most other lionfish studies, assumed that all eggs are released in each spawning event. It has been proven that lionfish exhibit differential vitellogenesis; not all their eggs mature at the same time, and not all eggs are released from the ovarian interstitial tissue at the same time (Fogg et al. 2017). This implies that lionfish females may not release all their eggs at each spawning event, as is the case for other tropical fish species like Pigmy Angelfishes (Bauer 1981). If female lionfish do not release all their eggs in every spawning event, then using batch fecundity multiplied by the annual spawning frequency will cause an overestimate of annual fecundity.

We tried to address a range of possibilities in our study with a span of conservative estimates for annual egg production. In contrast to the projected gradual lionfish population decline in case 5 (where batch fecundity is 0.21), case 4 (where batch fecundity is 0.25) projects a possible increase in population size given no change in the current culling rate. If the true batch fecundity of lionfish is closer to 0.25 than 0.21, then this suggests that culling practices would need to increase on Little Cayman to keep the population of lionfish from increasing significantly over time. If future research could

accurately determine the number of eggs released at each spawning event, the proportion of viable eggs to total egg count, the approximate rate of fertilization during spawning, and proportion of spawning events missed, R_A could be calculated with higher accuracy.

Based on our experimental model projections assuming a batch fecundity of 0.21, we also found that a six-fold increase in culling pressure on both adults and juveniles on Little Cayman, from 0.37 %/ mo to 2 %/ mo, would lead to a projected population of only 124 around the island by 2050. It is important to note that maintaining a culling rate of 2 %/ mo, which equates to 11.9 %/ yr, would be increasingly difficult as the Little Cayman lionfish population decreases.

While Morris (year) defines a necessary culling rate of 27% of adults annually in order to achieve a negative population growth rate, our model (case 6) projects a negative growth rate to the lionfish population when only 1% of both juveniles and adults are culled monthly (6.3% annually). While an annual culling rate of 6.3 % is a threefold increase from current culling efforts, it is much more attainable than projections from other models. Especially given the resource constraints of the Little Cayman culling community, this finding was particularly promising.

It is also possible that some of the other parameters in the transition matrix could be altered to be more accurate for Little Cayman. Rates of larval recruitment to juvenile age classes, juvenile survival, and adult survival were all taken from values calculated for fish populations found in other areas. Further, some of these values, including larval recruitment to the juvenile age class, were values true for other pelagic marine fish unspecific to lionfish. Additionally, neither our model nor any other existing models quantify or account for immigration of lionfish from other populations or rapid selection for decreased sexual maturation size due to culling. If more of these parameters could be derived specifically for Little Cayman, perhaps by tagging individuals and tracking their survival over time, this would increase the accuracy of the model for Little Cayman specifically.

Our spatiotemporal results can be used to inform lionfish culling tactics on Little Cayman. We found more lionfish at less popular, harder-to-access dive sites, which could indicate that though it requires more resources, it is worthwhile to reach more remote sites to cull. We also see a trend of more adult lionfish in deeper water and more juveniles in shallower water, which is in line with local cullers' theory that the older lionfish hide in deeper water, potentially to avoid human hunting. Finally, if lionfish populations were to rapidly increase as projected when culling effort is removed from the model, the populations of small shrimp, crab, and krill on Little Cayman could be decimated due to lionfish predation. These species act as a primary food source for many other reef species, so any significant impact to their abundances would have rippling effects throughout the ecosystem.

Our model of lionfish population dynamics on Little Cayman underscores the fact that the population of the invasive species will rapidly increase in the absence of any culling pressure. It appears that maintenance of the current culling efforts is necessary to constrain the current lionfish population around the island to their current numbers. However, to eliminate the population, it will be necessary to increase culling efforts on the island in future years. Addressing all locally manageable negative pressures on coral reef ecosystems is only becoming more important as locally unmanageable ocean-wide pressures, most notably ocean acidification, increased water temperatures, and microplastic pollution further stress and endanger the world's coral reefs.

ACKNOWLEDGEMENTS

We would like to thank all of the staff at CCMI who worked with us to cull and dissect the lionfish, and for the ability to use some of their stored fish for science.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this project.

LITERATURE CITED

Ahrenholz, D. W., Morris, J.A. 2010. Larval duration of the Lionfish *Pterois volitans* along the

- Bahmanian Archipelago. *Environment Biology of Fish*, 88:305-309.
- Bauer, Jr. J. A., Bauer, S. E. 1981. Reproductive biology of pigmy angelfishes of Genus *Centropyge* (Pomacanthidae). *Bulletin of Marine Sciences*, 31(3), 495-513.
- Copyright Global Sea Temperatures - A-Connect Ltd. (n.d.). *World Water Temperature: Sea Temperatures*.
- Côté, I. M., Green, S. J., & Hixon, M. A. 2013. Predatory fish invaders: Insights from Indo-Pacific lionfish in the western Atlantic and Caribbean. *Biological Conservation*, 164, 50–61.
- Costello R, Frankel N, Gamble M 2012. Allometric scaling of morphological feeding adaptations and extreme sexual dimorphism in energy allocation in invasive lionfish. *Dartmouth Undergrad J Sci* 12:39–41
- Edwards, M. A. 2012. Age, Growth, and Diet of Lionfish (*Pterois* Spp.) from Little Cayman Island, B.W.I. University of Florida.
- Fogg, Alexander & Peterson, Mark & Brown-Peterson, Nancy. 2014. Northern Gulf of Mexico Lionfish: Distribution and Reproductive Life History Trajectories. *Proceedings of the Gulf and Caribbean Fisheries Institute*. 66. 206-207.
- Fogg, A. Q., Brown-Peterson, N. J., Peterson, M. S. 2017. Reproductive characteristics of invasive red lionfish (*Pterois volantis*) in the northern Gulf of Mexico. *Bulletin of Marine Science*, 93(3), 791-813.
- Gardner, Patrick & Frazer, Thomas & Jacoby, Charles & Yanong, Roy. 2015. Reproductive biology of invasive lionfish (*Pterois* spp.). *Frontiers in Marine Science*. 2.
- Keane, R. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution*, 17(4), 164–170.
- Krebs. 2014. *Estimation of Survival Rates* (2nd ed.). UBC Zoology.
- Montaña, Carmen & Layman, Craig & Winemiller, Kirk. 2010. Gape size influences seasonal patterns of piscivore diets in three Neotropical rivers. *Neotropical Ichthyology*. 9. 647-655.
- Morris, J. A., Shertzer K. W., Rice, J. A. 2009. A stage-based population model of invasive lionfish with implications for control. *Biological Invasions*, 13: 7-12.
- Ruiz, R., Matheson, R. E., Roberts, D. E., Whitfield, P. E. 2006. The western Pacific red lionfish, *Pterois volitans* (Scorpaenidae), in Florida: Evidence for reproduction and parasitism in the first exotic marine fish established in state waters. *Biological Conservation*. 3. 384-390.
- Tixier, P., E. Malezieux, and M. Dorel. 2004. SIMBA-POP: a cohort population model for long-term simulation of banana crop harvest. *Ecological Modelling*, 180: 407-417.
- Zhang, Y. and M. Hao. 2009. Simulation of Population Dynamics of Bar-Headed Geese (*Anser Indicus*) around Qinghai Lake Region with STELLA. *First International Conference on Information Science and Engineering*: 5104-5108.
- Vetrano, S. J., Lebowitz, J. B., & Marcus, S. 2002. Lionfish envenomation. *The Journal of Emergency Medicine*, 23(4), 379–382.
- Weis, J. S., & Sol, D. 2016. *Biological invasions and animal behaviour*. Cambridge: Cambridge University Press.

APPENDIX

Supplemental Figure 1: Matlab® coded model for fecundity and other life table parameters

% Ben Zdasiuk, Ashlyn Morris, Reyn Hutten

% RA (reproductive potential) model for female lionfish

% Little Cayman Island

BF = 31777; %Egg count in a given sample

SF = 2.69; %Spawn frequency in days

PropViable = 0.25; %Proportion of viable eggs to generate case estimates, 0.75 high, 0.25 low

SpawnEvents = 365/SF;

AnnualEggs = SpawnEvents * BF * PropViable;

FemaleEggs = AnnualEggs/2;

CullingEffect = (0.0037); %percent of the adult and juvenile population reduced by culling

AdultMortality = 0.052; %Native adult mortality (NO CULLING!)

JuvenileMortality = 0.165;

TotalAdultMortality = AdultMortality + CullingEffect; %monthly adult mortality

AdultAnnualSurvival = (1 - (TotalAdultMortality))^12; %monthly survival compounded to a yearly rate

TotalJuvenileMortality = JuvenileMortality + CullingEffect;

JuvenileAnnualSurvival = (1 - TotalJuvenileMortality)^12;

ExpSurvivalParam = 12 * log(1 - TotalAdultMortality); %Calculates the parameter for exponential survival equation

fun = @(T) FemaleEggs .* T .* exp(ExpSurvivalParam .* T); %Creates F(T)S(T) function

RA = integral(fun,0,1); %Integrates F(T)*S(T) function from 0 to 1

Supplemental Figure 2: Matlab® coded model for fecundity and as a function of egg viability

% Ben Zdasiuk

% RA as a function of Egg Potential (Linear)

% Little Cayman Island

BF = 31777; %Egg count in a given sample

SF = 2.69; %Spawn frequency in days

SpawnEvents = 365/SF;

RA = ones(1,100);

ViabilityMatrix = ones(1,100);

n = 1; while n < 101

PropViable = 0.01 * n;

ViabilityMatrix(n) = PropViable;

AnnualEggs = SpawnEvents * BF * PropViable;

```

FemaleEggs = AnnualEggs/2;

CullingEffect = (0.0037); %percent of the adult and juvenile population reduced by culling

AdultMortality = 0.052; %Native adult mortality (NO CULLING!)

TotalMortality = AdultMortality + CullingEffect;

%AnnualSurvival = (1 - (TotalMortality))^12;

ExpSurvivalParam = 12 * log(1 - TotalMortality); %Calculates the parameter for exponential survival equation

fun = @(T) FemaleEggs .* T .* exp(ExpSurvivalParam .* T); %Creates F(T)S(T) function

RA(n) = integral(fun,0,1); %Integrates F(T)*S(T) function from 0 to 1

n = n + 1;
end
plot (ViabilityMatrix, RA,'k', 'linewidth', 2)
title ('Reproductive potential (RA) as a function of proportion of viable eggs')
xlabel('Proportion of eggs that are viable')
ylabel('Reproductive potential (RA)'); grid on;

```

Supplemental Figure 3: Matlab® coded model for fecundity and as a function of proportion of population culled (on a monthly basis)

```

% Ben Zdasiuk
% RA (reproductive potential) model for female lionfish
% Little Cayman Island

BF = 31777; %Egg count in a given sample

SF = 2.69; %Spawn frequency in days

SpawnEvents = 365/SF;

RA = ones(1,100);

CullingMatrix = ones(1,100);

n = 1;

while n< 101

    PropViable = 0.25;

    CullingMatrix(n) = 0.01 * n;

    AnnualEggs = SpawnEvents * BF * PropViable;

    FemaleEggs = AnnualEggs/2;

    CullingEffect = CullingMatrix(n); %percent of the adult and juvenile population reduced by culling

    AdultMortality = 0.052; %Native adult mortality (NO CULLING!)

    TotalMortality = AdultMortality + CullingEffect;

    %AnnualSurvival = (1 - (TotalMortality))^12;

    ExpSurvivalParam = 12 * log(1 - TotalMortality); %Calculates the parameter for exponential survival equation

```

Little Cayman

```

fun = @(T) FemaleEggs .* T .* exp(ExpSurvivalParam .* T); %Creates F(T)S(T) function

RA(n) = integral(fun,0,1); %Integrates F(T)*S(T) function from 0 to 1

n = n + 1;

end

plot (CullingMatrix, RA,'k','linewidth', 2)
title ('Reproductive potential (RA) as a function of percent of the population culled')
xlabel('Percent of the population culled (per month)')
ylabel('Reproductive potential (RA)'); grid on;

```

Case 6 Transition Matrix	Case 7 Transition Matrix	Case 8 Transition Matrix
$\begin{pmatrix} 0 & 0 & 138,000 \\ 0.00003 & 0 & 0 \\ 0 & 0.099 & 0.464 \end{pmatrix}$	$\begin{pmatrix} 0 & 0 & 127,400 \\ 0.00003 & 0 & 0 \\ 0 & 0.086 & 0.408 \end{pmatrix}$	$\begin{pmatrix} 0 & 0 & 117,700 \\ 0.00003 & 0 & 0 \\ 0 & 0.074 & 0.358 \end{pmatrix}$

Supplemental Figure 4: Transition matrices for cases 6 through 8. Case 6 is 1% monthly culling pressure, 7 is 2% monthly culling pressure, and 8 is 3% monthly culling pressure.

QUEEN CONCH MONITORING PROJECT

2020 BIO FSP COHORT

TA Editors: Melissa Desiervo and Clare Doherty

Faculty Editor: Celia Chen

INTRODUCTION

Queen Conch (*Lobatus gigas*) is a biologically and culturally important organism on Little Cayman Island. Their population is in rapid decline as a result of overfishing, ill-informed fishing regulations, and poaching. Additionally, like other marine shell-building organisms, they are threatened by ocean acidification, which affects their ability to construct shells. Their populations could also be impacted by increasing ocean temperatures as they have a narrow range of temperatures during which they can reproduce.

Some efforts have been made to better protect Queen Conch populations in Little Cayman, including the establishment of Marine Protected Areas (MPAs) and replenishment areas (areas of more stringent fishing regulation). Current fishing regulations do not take into account their two-stage growth; Queen Conchs first grow in length, then in shell lip width. As a result, regulations focusing only on one metric are not adequate and negatively impact their population.

Multiple studies have been conducted on Little Cayman Queen Conchs, including annual studies by Dartmouth. While independently these studies provide important insight on Queen Conchs, their disjointed results cannot be linked to create the long-term picture of population change necessary for effectively evaluating impacts on conchs around Little Cayman. It is challenging to procure the resources to conduct a long-term study, but as Dartmouth Bio FSP returns to Little Cayman annually, we are in a unique position to offer this continuity. In this paper, we outline the framework for an easily repeatable and potentially expandable long-term Queen Conch monitoring study to be conducted annually by Dartmouth Bio FSP students, with the first dataset gathered in February 2020.

METHODS

To estimate the number of Queen Conch per hectare in Little Cayman, we surveyed conch at South Hole and Mary's Bay. At each site, we surveyed 10 transects within the harvested zone and 10 transects within the replenishment zone. Each of the transects was 30 x 2 m. We began laying transects 75 m from the boundary line between the harvested and replenished zones. Within each zone, transects were broken up into two columns of five each, with 15 m between the two rows. Within each column, transects were spaced 15 m apart from each other. GPS coordinates were taken at the corners of each zone (Fig. 1). To lay each transect, we used weighted transect tape. Two swimmers then swam the length of the tape, stopping whenever a conch was found within 1 m of either side of the tape. In Mary's Bay on the north side of Little Cayman, the two columns of transects on the protected zone were 25 m, and not 15 m, apart (Fig. 2). In South Hole, the we started from Owen's Island, not the main island; the protected/unprotected line starts from the western tip of the shore on Owen's Island.

In future years, at each site, researchers should first find the protected/unprotected zone line. They should take a compass bearing along this line to orient themselves and use the right angle to inform what directions to swim transects in on either side of the protected/unprotected line. Then, researchers can use our GPS coordinates to inform where to start laying transects. We recommend that researchers locate one of the corners in each zone as a starting point, and then measure out the distances outlined here to place the other transects. See Figure 2. We recommend designating two researchers as "transect organizers," whose sole job is to locate each transect starting point and explain to other researchers what compass bearing to follow.

Two teams of transect organizers could be employed if there are two GPS devices, one team in each (protected/unprotected) zone.

Using these methods, theoretically a group could survey as many sites as they'd like, anywhere there is a protected/unprotected boundary line. More sites could be beneficial as we aim to continue monitoring the health and abundance of conch in Little Cayman.

To determine the health of conch in each protection status, we assessed whether each conch was living or dead. If dead, we also noted whether the conch had been harvested. Harvested conch has a square slit near the apex of the shell. To assess the demographics of the conch in each protection status, we used rulers to measure the length of the conch, and we used calipers to measure the thickness of the lip of each shell (Fig. 3). All conch with a lip thickness greater than or equal to 10 mm were considered

adults, while those with a lip thickness less than 10 mm were considered juveniles. Surveyors also noted the substrate the conch was found in sand, turtle grass, or coral rubble.

RESULTS

Table 1: Average lip thickness and length by site

Site	Average lip thickness	Average length	Average adult lip thickness	Average adult length	Average juvenile lip thickness	Average juvenile length
Mary's Bay	3.44	16.07	9.33	20.73	15.57	2.81
South Hole	3.77	17.72	14.9	19.33	17.52	2.37

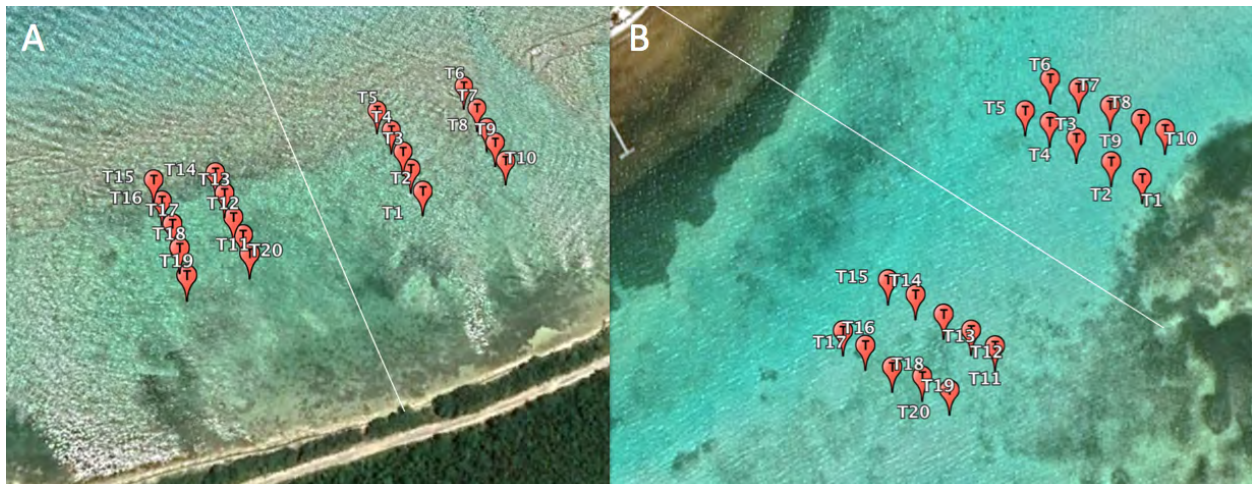


Figure 1: A: Mary's Bay transect points. Transects 1-10 are in the protected zone, transects 11-20 are in the unprotected zone; B: South Hole transect points. Transects 1-10 are in the protected zone, transects 11-20 are in the unprotected zone.

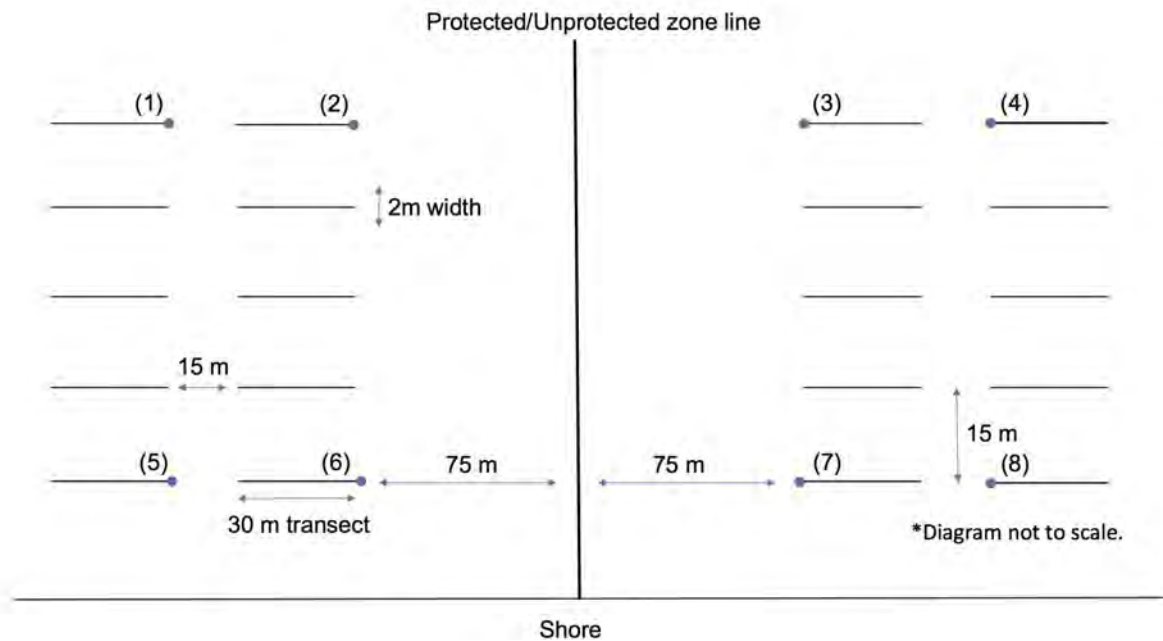


Figure 2: Distances we used for laying transects. GPS coordinates were taken at the start of the transects on the corners (represented with a dot and a number). Latitude, Longitude GPS coordinates for each site are as follows:

- *Mary's Bay*: (1): 19.707618, -79.998436; (2): 19.707232, -79.997082; (3): 19.708108, -79.997351; (4): 19.708276, -79.996435; (5): 19.707021, -79.998175; (6): 19.707108, -79.998045; (7): 19.707535, -79.996659; (8): 19.707000, -79.996106

- *South Hole*: (1): Not observed; (2): 19.665035, -80.066329; (3): Not observed; (4): Not observed; (5): 19.664478, -80.066237; (6): 19.664606, -80.065945; (7): 19.665458, -80.065198; (8): 19.665664, -80.065094

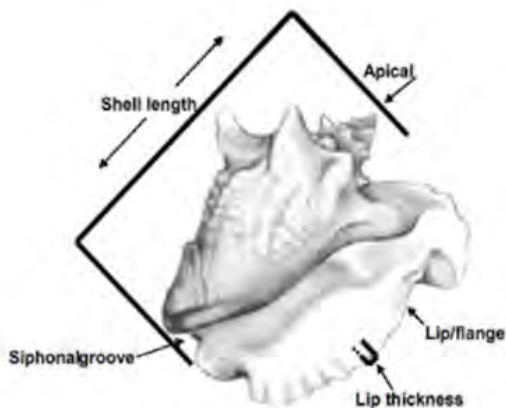


Figure 3: The shell of an adult *L. gigas* with measured dimensions and key features indicated (Collins and Harrison 2007, from 2017 FSP book pg. 101)

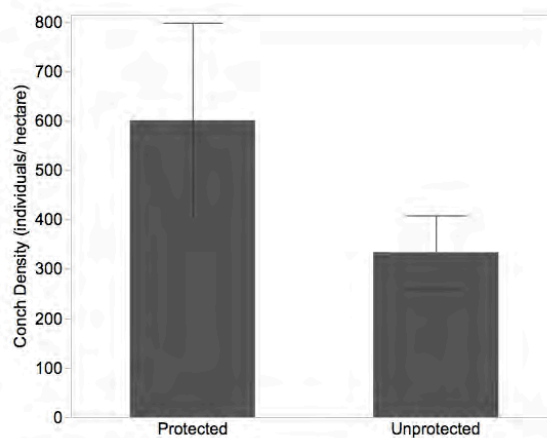


Figure 6: Conch density vs protection status in South Hole

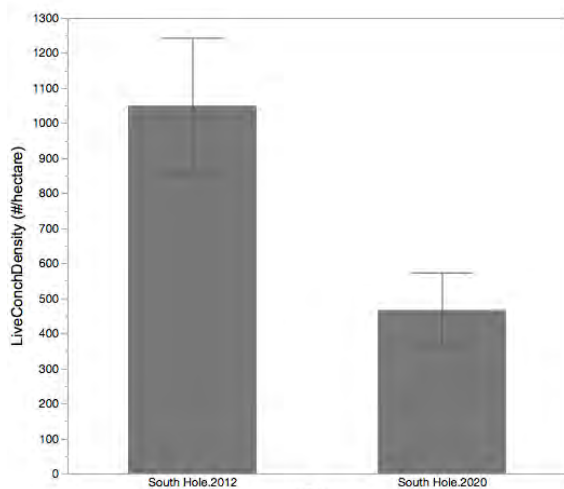


Figure 4: Conch density in south hole over time.

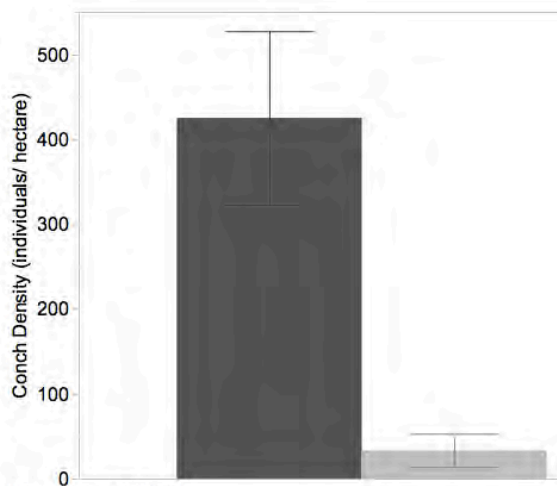


Figure 7: The demographics of Conch found in South Hole. The dark grey bar shows the number of juveniles observed and the light grey bar represents the number of adult Conch observed.

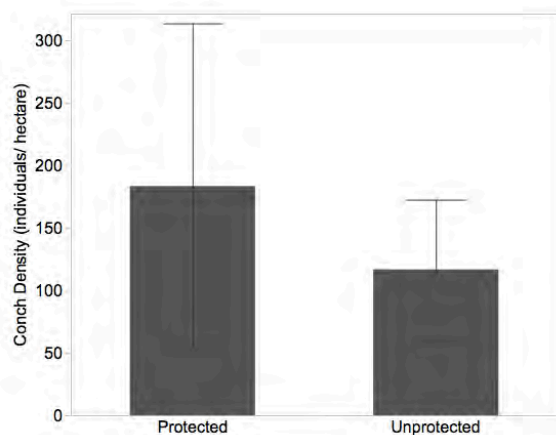


Figure 5: Conch density vs protection status in Mary's bay

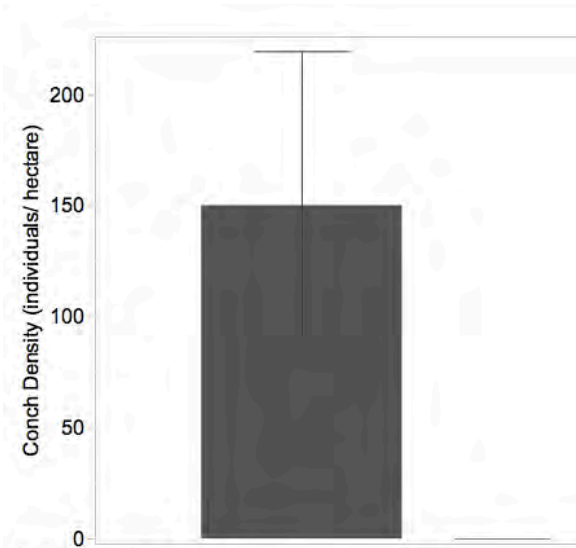


Figure 8: The demographics of the Conch found in Mary's Bay. The dark grey bar represents the number of juveniles observed and the light grey bar shows the number of adults found.

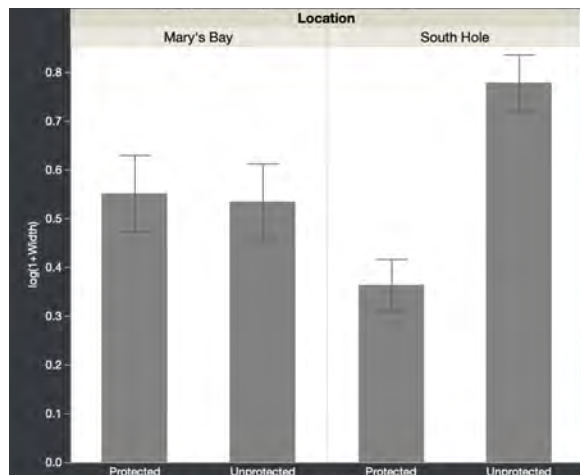
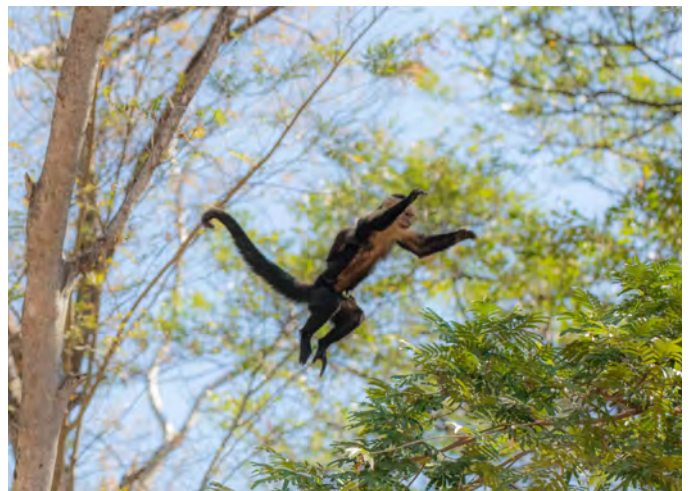
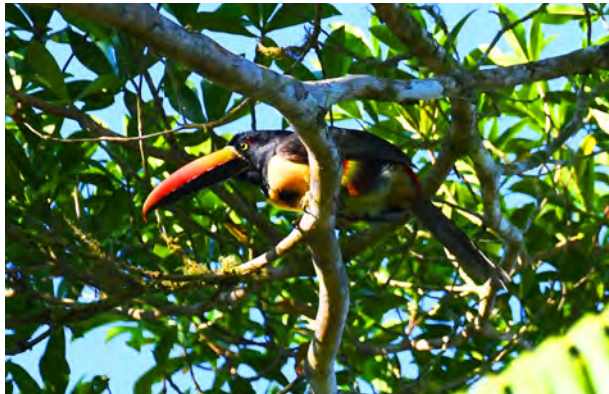
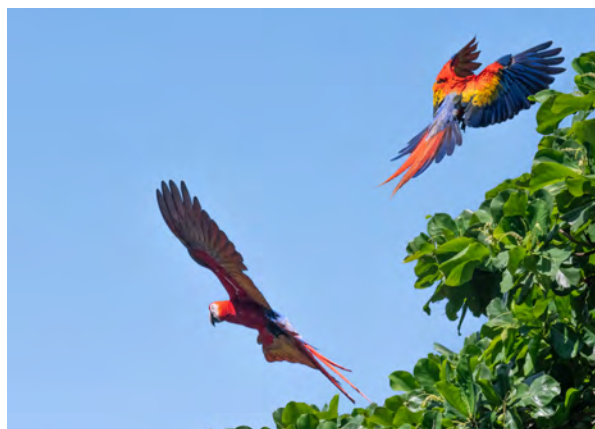
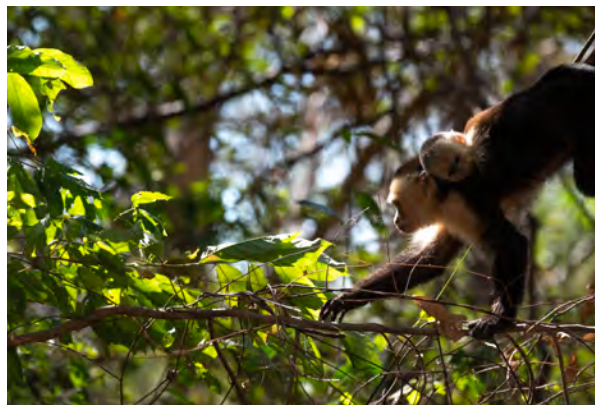
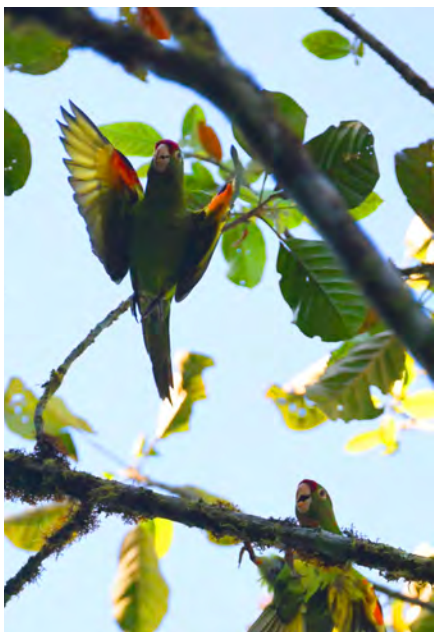
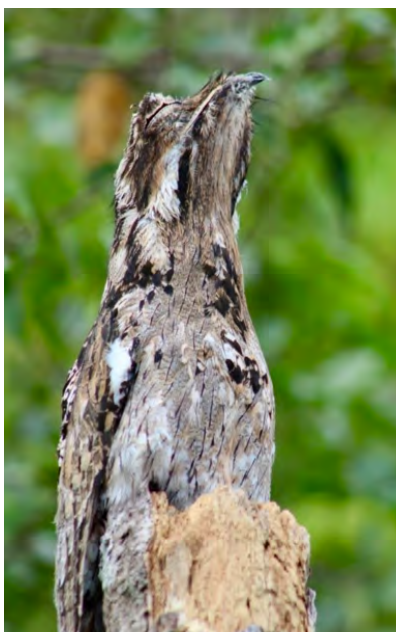
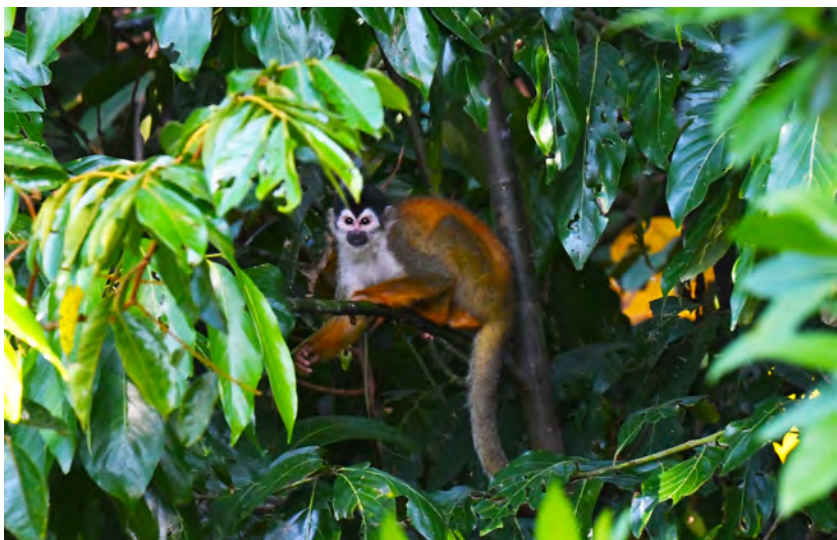


Figure 9: Conch age vs protection status.





WHO SHIT ON THE BUS?

NATHAN G. E. E. EASTMAN-GIFFARD

Faculty Editor: Dan Janzen

“Grown-ups poop, children poop too,” -Gomi, 2001

Abstract: In a world ridden with the effects of climate change, we search for champions to fight for the Earth. But how does knowing that all the coral will die by 2050 affect the human mind and body? This ground-breaking research follows a thirty-year study on the influences of climate change on human psychology, using elements of the original study to broaden our understanding of the human digestive tract using a paired t-test. “Who shit on the bus?” aims to push the boundaries of our understanding to create a new definition of what it means to be human.

Keywords: global warming, internal cooling, a dwindling magnetic field, asteroids, solar flares, poop

INTRODUCTION

There are infinite particles in the known universe. Some of those particles created Earth. Climate change occurs on Earth.

Human activities are exerting increasing impacts on the environment on all scales and these impacts outcompete natural processes. As carbon dioxide concentrations have increased past historic levels over the past sixty years, unnatural events like coastal flooding and The Bachelor in Paradise have become more prevalent in our society (Supplemental Figure 1). “We are living in the Anthropocene,” (personal communication with a sea turtle). While the effects of climate change have been documented in all ecosystems, increased carbon emissions also put psychological stress on young humans aged 20-22 years old. One ongoing, long-term study has offered a “study abroad” once a year to further explore this phenomenon. In this study, fourteen young humans are subjected to sixteen-hour workdays, seven days a week for ten weeks. They are only fed rice and beans and every day they are told that humans are destroying everything nice about the world. Although this study has run for 30 consecutive years, scientists aren’t paid enough, so no one has ever looked at any of the data from this study. Therefore, we assume the effects of climate change are negative on the psyche with 0% random variation accounting for sadness. While this study focuses on the psychological damage that climate change is causing in young humans, there is no research on how climate change influences the physiology of students.

Many decades of research have proven that the human digestive tract exists. Humans eat when they’re hungry and excrete their waste into toilets or convenient holes (Twitter, 2010). But little is known about the deep complexities of the intestines or how digestion is impacted by the stress of knowing that sea levels are rising, frogs are disappearing, and coral is dying. My pilot study aims to answer this question even though no one asked me.

My multimillion-dollar lab full of researchers and research assistants hypothesize that climate change is a stressor that will have a quantifiable, undeniable, and irrevocable effect on the human digestive tract, resulting in defecation. Alternatively, there will be no effect. The null in this instance is that humans don’t poop. To test these hypotheses, we did something complicated and unnecessary.

METHODS

The study design

In order to quantify the physiological stress of climate change in humans living in the Anthropocene, we trapped fifteen Americans, one Canadian, one Irish woman, and 30 boxes full of scientific equipment on a charter bus. The charter bus was ~~45 feet~~ 13 meters in length and 25,000 kg in weight. It was 85 degrees Fahrenheit outside. It was a Tuesday. This experiment was performed in Costa Rica to control for the fact that it’s an election year in the United States and elections are stressful.

Once the doors were locked on the charter bus, we turned off the sewage system, and told

all individuals that they couldn't use the bathroom. We told the group that palm oil production is ruining Costa Rican ecosystems, and then drove the group through the countryside for 15 consecutive hours. The bus traveled south. This was only replicated once due to time constraints.

Statistical analyses

I don't "do stats", so here's a picture of a turtle (Supplemental Figure 2).

RESULTS

We saw a 100% increase in toilet usage among individuals when treated with the charter bus (Pearson's t-test: $t = 30$, $df = 2$, $p = 0.0010$, Fig. 1). Qualitatively, no one admitted to pooping on the bus and no one ever will.

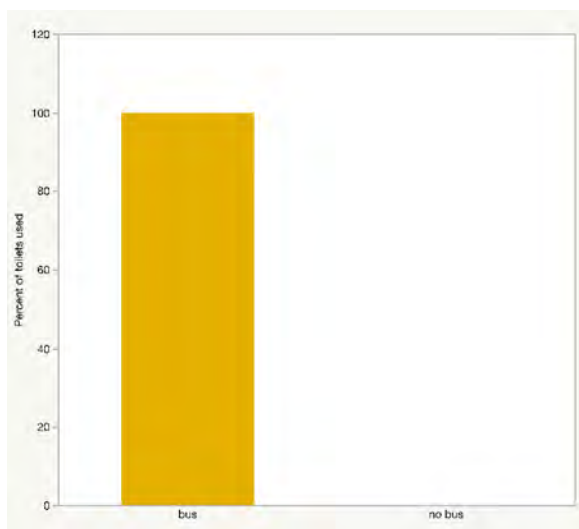


Figure 1. Percent of toilets used by bus treatment.

DISCUSSION

My pet hypothesis was correct. These findings are biologically significant because we were told not to use the toilet on the charter bus, but we did. The only confounding variable of my study is that the individual who used the toilet remains unidentified. This can be rectified by placing a camera in the bus bathroom in future studies. Future studies may also work to answer similar questions such as who threw up on the bus, why am I hungry, and who's on the WIFI?

BROADER: Bathrooms may be forever, but the Earth is temporary. Even if we do manage to flee to distant stars, we're just delaying the inevitable march toward our own annihilation. Time and events work to destroy all we have ever comprehended, and humans are working to accelerate that process. The decay of all matter as we know it. The decay of all life as we know it. One really has to wonder, what is the point of anything? Why continue? Why progress? Life even at its most advanced stage is doomed to nonexistence because our self-proclaimed superiority over the natural world blinds us to reality. Why? Why go on?

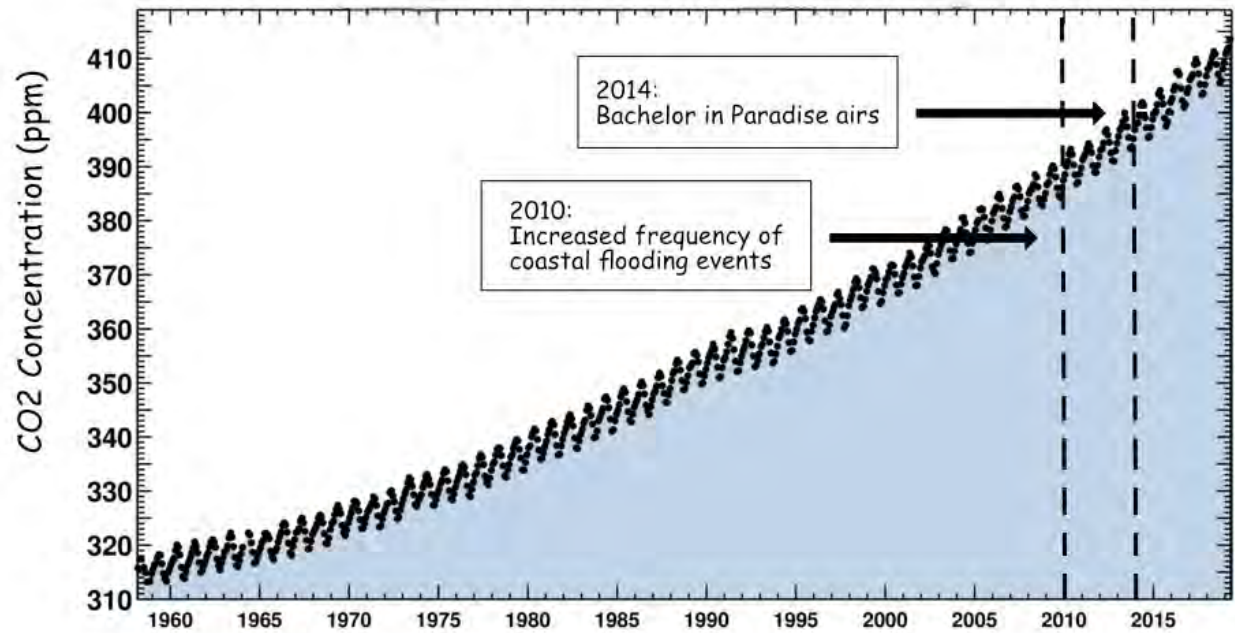
ACKNOWLEDGEMENTS

Thank you to our TA, Clare, for cleaning the bus bathroom. We apologize to our bus driver, Armando. We know you hated us, and for good reason.

LITERATURE CITED

Gomi, T. and Stinchecum, A. 2001. Everyone poops. Kane/Miller Book Publishers.

APPENDIX



Supplemental figure 1. Atmospheric CO₂ concentration (ppm) over time. Dotted lines represent years when biologically significant anomalies occurred in the United States.



Supplemental figure 2. "Stop putting plastic in the ocean"

Not Dartmouth

THE FISSION-FUSION NATURE OF GROUP-FORMING ECOLOGY STUDENTS

[THE FIRST AUTHOR]

Keywords: coronavirus, cost-benefit analysis, fission-fusion, generalized specialized generalist, niche partitioning, ORPs, Poisson distribution, plant practicum, threshold, wifi moratorium

INTRODUCTION

Humans, like many [other parallel biological organisms], are social creatures.

METHODS

Study Design

We placed fourteen 20 to 22-year-olds on a nine-week “Dartmouth” “tropical” “foreign” “studies” “program” in Panama, “Costa Rica,” and Little Cayman Island to observe the nature of their group-forming. The students created purposeless “original research projects” (ORPs): projects seemingly about ecological systems but really just an excuse for the students to convince themselves they are capable of writing scientific papers and performing statistical analyses. They were guided by two graduate student teaching assistants (TAs), who the students thought were there to guide them in science but really were there to point out their flaws at random intervals, and one of three professors at a time.

We specifically instructed the first professor to simultaneously never discuss the difficulty of group work and use the term “fission-fusion” at least three times per day, to at least plant the idea in the students’ brains. We specifically instructed the second professor to refrain from discussing group dynamics even more. However, we allowed this professor to hold one “module” in which the words “diversity” and “gender” were included exactly once. Finally, we specifically instructed the third professor to remind the students of all the nurturing, social and, frankly, human qualities that were missing in the first two.

Notably, the students were responsible for forming their own groups, which for some reason seemed like a good idea at the time. We have already done more than 30 replications of this study so we cannot change our model now. In the middle of our study, we removed the individuals’ access to wifi in an attempt to exacerbate negative group dynamics. We also

collected anecdotal evidence about the quality of the group.

We kept track of pairs of students that never worked with each other as well as those who worked with each other an exceptionally great amount (four or five times out of seven projects). The latter of these students we considered specialists. We also measured which group members preferred extremely small or large groups.

Statistical Analyses

Because we felt certain individuals had not yet fully developed skills for inter-gender communication, we compared the male-to-female ratio for groups in which this individual was present versus when they were not present using a t-test for each.

RESULTS

Of 77 possible pair combinations, 14 were never executed (Fig. 1). KA did not work with five individuals, likely because she only worked with people she wanted to work with. All other individuals did not work with three or fewer individuals. BS and NGEEEG were the only group members to work with all other individuals, probably because they were the most pleasant to be around. Four pairs were specialists: SJ & NGEEEG, SS & BS, MB & BZ, and RA & AM. While three of these four worked together four times, RA and AM worked together five times—the most of any pairing. KA and HT preferred the smallest groups as the only individuals with an average group size of less than 3.5. EL and RH preferred the largest groups as the only individuals with an average group size above 4.

In our analysis of male to female ratio with a given group member, there was not a significant difference for 10 out of the 14 members. The male-to-female ratio was significantly lower when the group contained either APR (t-test: $t =$

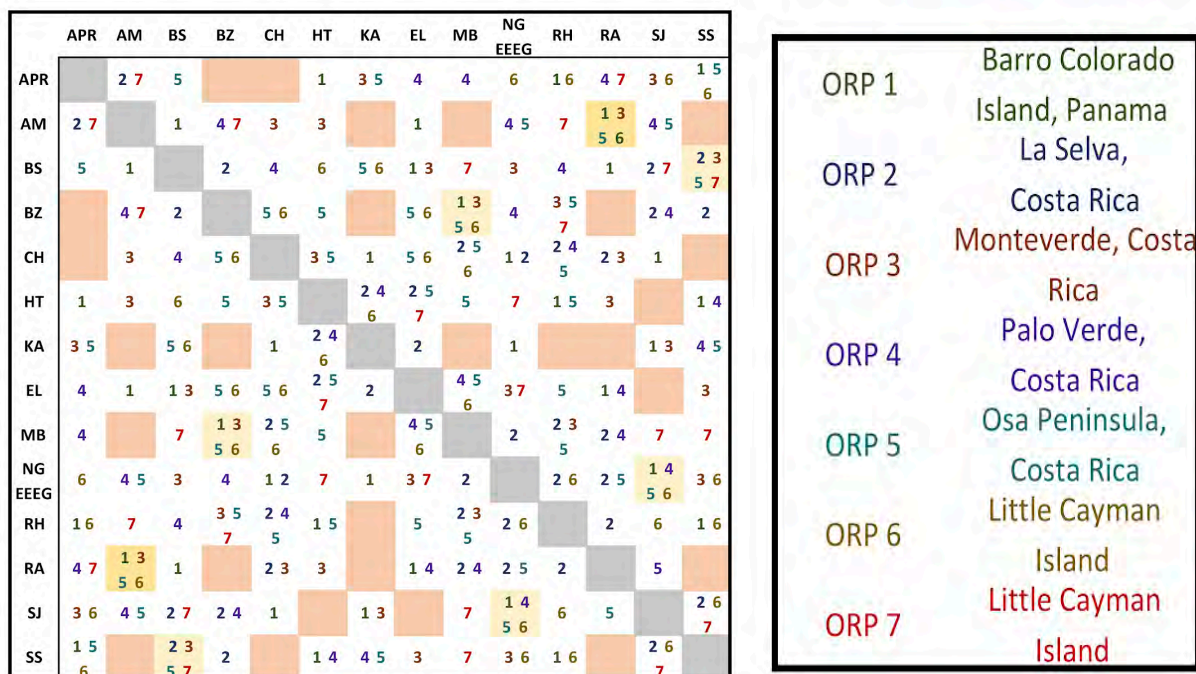


Figure 1. Pairings of individuals. Each study site is represented in a different color. Orange boxes indicate pairs that never worked together. Light yellow boxes indicate pairs that worked together four times, and the dark yellow box indicates the pair that worked together five times.

-4.31, $P = 0.001$) or SJ (t-test: $t = -2.82$, $P = 0.01$) because they are women who prefer working with women. In contrast, the male-to-female ratio was significantly higher when the group contained either EL (t-test: $t = 3.38$, $P = 0.004$) or MB (t-test: $t = 2.67$, $P = 0.018$) because they are men who need validation from other men. These are real results that I actually used JMP for, not actors.

Interviews with an individual closely observing the group concluded they were “the most disrespectful Bio FSP group ever” ($n = 1$; Fig. 2).

DISCUSSION

KA’s decision not to work with the greatest number of individuals is respectable, because who even wants to work with everyone, anyway. SJ and NGE EEGG likely worked together most often because they were the two most beautiful individuals in our study. SS and BS likely worked together most often because they enjoyed each other’s presence and engaging in scientific curiosity together. RA and AM likely

worked together most often so RA could do the stats and AM could present the methods. It should be noted that even given the appearance of the trend between this pair early on in the study, they still worked together on the final three projects. Finally, MB and BZ likely worked together the most as they were simply inseparable buds.

KA and HT preferred the smallest groups, likely because they always worked with each other plus one more individual. BS, SS, and EL were all part of this lucky group of individuals. EL and RH preferred the largest groups likely because they needed the most people to hear their unwavering opinions.

Despite the comment by the individual about the superlative quality of the group, all other individuals surveyed described them as “delightful,” “funny,” and quite surprisingly, “the best group we’ve ever had at this fine establishment.”

Our most interesting finding came when we eliminated the students’ access to wifi on the Osa Peninsula. Rather than forming four groups,

like the students usually did, they formed three, each unique in its own way. The first group performed an entire experiment on snails, which have been entirely studied before, all without any statistics or graphs. Despite the simple nature of their project, one of their group members almost died in the process. The second group had their TA design their entire experiment before they even got to the peninsula and instead of doing research, killed hermit crabs and watched movies. The third group collected data at 4 am even though their question was not related to the behavior of their study system at 4 am. They also created their own language for communication, coming up with new names for each other and only speaking in loud, indistinguishable noises over walkie talkies.

ACKNOWLEDGMENTS

We thank the students for participating in this study. Though we physically and mentally exploited them just to be published in a

mediocre journal, we believe they are better off for it and will use the “skills” they learned for a lifetime. We also thank the two individuals we hired as TAs for the study. Though we definitely did not pay them enough for their job, they exceeded our expectations and taught the students more about “science” between the aforementioned random intervals than we would have ever expected. We thank all of the other people, field stations, and organizations that housed the students. They made the group comfortable and served them delicious food (likely only after they read the human subject research waivers, we made them sign). Finally, we thank the “professors,” without whom this study would not have happened. Though no one is perfect, we think that if the strengths of the three professors were combined into one person, they actually might be.



Figure 2. A photo clearly displaying the disrespectful nature of the study subjects.

Not Dartmouth

AVIAN-FELINE SKINWALKER: YELLOW WARBLER AND HOUSE CAT MORPH

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Abstract: Skinwalkers, also known as shape shifters, have been living among us for millions of years. At the Central Caribbean Marine Institute (CCMI) on Little Cayman Island, we observed a new morph; that between a Yellow Warbler (*Setophaga petechia*) and a house cat (*Felis catus*). Our main line of evidence is that we have not seen the Yellow Warbler or *F. catus* at the same time. Similar to how the United States convicts' criminals - innocent until proven guilty - we identify skin walkers as skin walker until proven not.

Key words: Skinwalker, shape shifter, yellow warbler, setophaga petechia, house cat, felis catus

INTRODUCTION

A skinwalker is a harmful witch that has the ability to take the form of many animals (Internet 2020). So far, most identified morphs take a wolf or cayote (Fig. 1), animals associated with death. In the case of the avian-feline morph, the feline portion is closer to death than the avian portion since they often have nine lives. The fact that this morph has ten lives (nine from the feline half and one from the avian half) makes this subject extremely dangerous. We measured the proportion of time each side of the morph was present to conclude that we in fact dealing with a new Skinwalker morph. Since this morph has never been observed, we took the proper safety measures when conducting this experiment. We always had on full SCUBA gear including wetsuit, BCD, regulator, mask, snorkel, and fins.



Figure 1: The common wolf morph.

METHODS

Experimental design

To test the amount of time each side of the Skinwalker was present, we observed its foraging grounds for 24 hours a day for 3 weeks. When we observed the Yellow Warbler, we recorded the duration of its visit. When we observed *F. catus*, we recorded the duration of its visit.

Statistical Analysis

To analyze the proportion of time when both halves of the morph were observed and any potential overlap, we used a chi-squared test.

RESULTS

When it took the *F. catus* form, it preferred to eat Fancy Feast™ when it was left out on the steps by the dining area. When it took the form of the Yellow Warbler, it foraged on insects inside the classroom. We observed the *F. catus* 50% of the time and the Yellow Warbler 50% of the time with no overlap (Fig. 2). Another scientist at CCMI, Ashlyn Morris, observed the Yellow Warbler exhibiting “evil behavior” and documented it (Fig. 3).

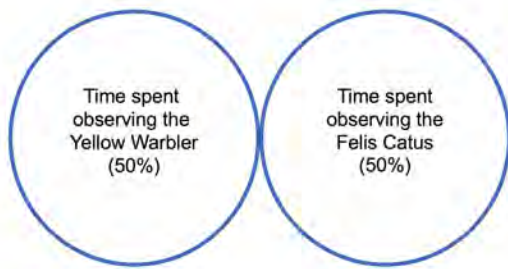


Figure 2: A Venn diagram showing the distribution of time sent observing the Yellow Warbler and F. catus. Note the lack of overlapping time.



Figure 3: A detailed sketch of the Yellow Warbler exhibiting "evil behavior."

DISCUSSION

We can say with absolute confidence that we have observed a new morph of Skinwalker; the avian-feline morph. Since we did not observe the Yellow Warbler and the F. catus at the same time, it must be a Skinwalker. The key observation of the Yellow Warbler displaying "evil behavior" further supports our conclusion since shapeshifters are inhuman and feed on fear. Despite this disturbing finding, the brave Dr. Shannon Sartain removed her safety gear and attempted to interact with the feline, the more dangerous form. Interestingly, this is when we observed the F. catus leave and the Yellow Warbler appeared seconds later (Fig. 4).

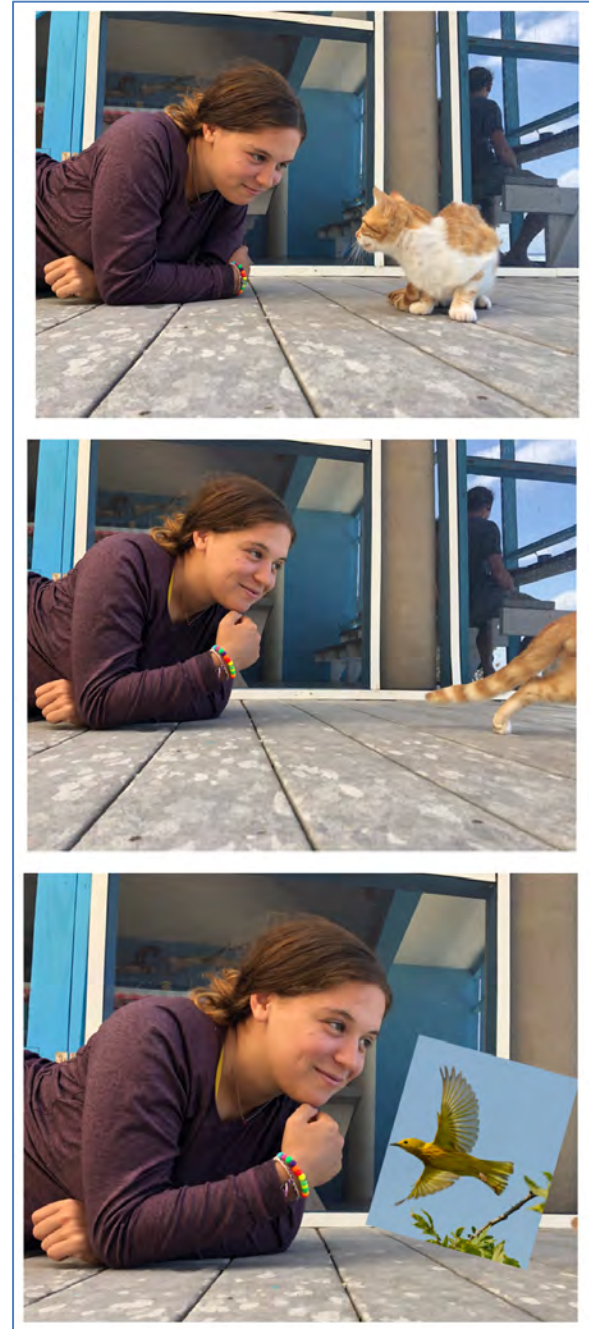


Figure 4: The sequence of events leading us to believe we observed a shape shift.

ACKNOWLEDGEMENTS

Thank you to Ashlyn Morris for providing us with an important sketch.

