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Dartmouth College runs an annual 9-10 week ecological field research program in Costa Rica and the Caribbean. Manuscripts from the research projects in this program have been published in the annual volume “Dartmouth Studies in Tropical Ecology” since 1989. Copies are held in the Dartmouth library and in Costa Rica at the San Jose office of the Organization for Tropical Studies (OTS/OET), at the OTS field stations at Palo Verde, Las Cruces and La Selva, at the Cuerici Biological Station, at the Sirena Station of the Corcovado National Park (through 2010), and at the Monteverde Biological Station. On Little Cayman Island, there are copies at the Little Cayman Research Center.

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

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

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

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

We acknowledge the generous financial support of Dorothy Hobbs Kroenlein.

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 David Peart at david.peart@dartmouth.edu and the administrative assistant is Mary Poulson at mary.poulson@dartmouth.edu. All volumes in this series can be found online at <http://www.dartmouth.edu/~biofsp/Publications.html>

David Peart
Hanover NH, USA
15 March 2011

SCHEDULE FOR DARTMOUTH TROPICAL BIOLOGY PGM - COSTA RICA, W 2011

		<u>Morning</u>	<u>Afternoon</u>	<u>Evening</u>
5 Jan	We	To San Jose	Travel	Arrive in evening
6 Jan		In San Jose	OTS, InBIO	Group dinner in SJ
7 Jan		To Palo Verde	Travel	Lec: Intro CR ecol (RC)
8 Jan		At Palo Verde	Orientation	Lec: Herp Ecol (KD)
9 Jan	Su	At Palo Verde	FP ^b -1 (ant-acacia)	Data analysis/synthesis
10 Jan		At Palo Verde	FP-2	ArthLab(GM/RC)Lec:Behav(RC) FP-1 seminars. Writing.
11 Jan		At Palo Verde	FP-2	VertLab(RC/GM)Lec:DivCoex(RC) Writing. FP1 ms due
12 Jan		At Palo Verde	SIFP ^c plan/proposals	Plant lab (KD) * Data Anal/Writ. FP-2 seminars. Writing
13 Jan		At Palo Verde	SIFP-1	Writing. FP-2 ms due.
14 Jan		At Palo Verde	SIFP-1	SIFP-1/analysis. Revisions.
15 Jan		At Palo Verde	River trip	Writing: SIFP-1 ms due
16 Jan	Su	To Santa Rosa	Travel/walk	Field: Sea turtle nesting
17 Jan		At Santa Rosa	Lec: Mngrv(KD)	* ^d Exploration ^c Field: Sea turtle nesting
18 Jan		To Monteverde	Walk/Trav [Inch MV2pm]	Orientation Writing (revisions)
19 Jan		At Monteverde	Orientation	SIFP-2 planning Lec: Amphibs (A Pounds)
20 Jan		At Monteverde	SIFP-2 pilot /props	SIFP-2 Lec:His/Orig (RC)
21 Jan		At Monteverde	SIFP-2	SIFP-2 Analysis.Writing (rev.)
22 Jan		At Monteverde	SIFP-2	Analysis/synthesis * SIFP-2 seminars
23 Jan	Su	At Monteverde	Writing	Writing SIFP-2 ms due. Bat Jgl
24 Jan		At Monteverde	Writing. Mist netting	Writing; plant quiz Writing
25 Jan		At MV Writing. All fin. mss thru MV due	Exploration (DP arr. SJ)	Free
26 Jan		To Cuerici	Travel	Travel/Orientation Lec: Coevol (RC)
27 Jan		At Cuerici	Orientation	Orient. SIFP-3 plan Lec: Av. Ecol (DP). Metadata
28 Jan		At Cuerici	Trip to paramo	SIFP-3 planning/pilot (RC dep.) SIFP-3 final propos. Corc plans
29 Jan		At Cuerici	SIFP-3	SIFP-3 * Lec: ForConsMgmt (DP)
30 Jan	Su	At Cuerici	SIFP-3	SIFP-3 Analysis.Corc .plans.
31 Jan		At Cuerici	Anaysis, Synthesis	SIFP-3 seminars. Writing Writing SIFP-3 ms due
1 Feb		At Cuerici/travel	Explor. 10am dep.	Travel to La Palma Prep for Corcovado
2 Feb		To Corcovado	Truck. Hike LP trail	Los Patos Trail to Sirena Discussion, night walk
3 Feb		At Corcovado	Nat. hist. of Corc	Nat. hist. of Corc Crit(IE) Walk. Nat his review
4 Feb		At Corcovado	Nat. hist. of Corc	Nat. hist. of Corc Crit(FW) Walk. Nat his review
5 Feb		To Las Cruces	Coast trail to Carate	Travel to Las Cruces Crit LB).Rev Corc nat. his.
6 Feb	Su	At Las Cruces	Self-study pl. fams.	Plant lab/walk Lec:Cons.(Z Zahawi).Revisions
7 Feb		At Las Cruces	Revisions	Plant quiz. Revisions Lec: ForDyn(DP). Writing.
8 Feb		To La Selva	To San Jose	To La Selva Discussion.* Night walk.
9 Feb		At La Selva	Orientation	Biodiversity group walks Crit. (EB). SIFP ideas
10 Feb		At La Selva	SIFP-4 plan/props.	SIFP-4 pilot studies Fmt w/shop. Writing.
11 Feb		At La Selva	SIFP-4 pilot	SIFP4 pilot SIFP-4 proposals/decisions
12 Feb		At La Selva	SIFP-4	SIFP-4 Writing
13 Feb	Su	At La Selva	SIFP-4	SIFP-4 Writing. Final SIFP-3 ms due
14 Feb		At La Selva	SIFP-4	Analysis Writ.Disc. SIFP4 seminars
15 Feb		At La Selva	Writing	Writing. SIFP-4 ms due Free
16 Feb		At La Selva	Copy editing.	Writing. All metadata due Writing
17 Feb		At La Selva	Writ.FinalSIFP4due	* Choc.tour Copy editing.
18 Feb		At La Selva, to SJ	Exploration	Travel to SJ ca. 1pm. Group dinner in SJ

19 Feb Sa Depart for Little Cayman, leave LC 12 Mar

^aOrientation = learning new site/ecosystem/organisms

^bFP = field problems (staff initiated)

^cSIFP = student initiated field problems

^d* = Group meeting times (schedule may change)

^eExploration = Time to explore site

[Initials after lectures = faculty or TA]

CORAL REEF ECOLOGY (BIO 57) - LITTLE CAYMAN – SCHEDULE

* = scheduled lecture

Date		Morning	Afternoon	Evening
19 Feb - Sat	Arrive from CR		<u>13 people arrive 5:40 pm</u>	Dinner, brief orientation, unpack
20 Feb - Sun	Arrive from CR	<u>5 people arrive 8:20 am</u> Main orientation and safety information – Rob, Brad and Perry	Unpack	*Morgan's Lionfish presentation Discussion about program to date and everyone's expectations for LC segment. Schedule overview
21 Feb - Mon		*Coral biology lecture (BT) & *Reef morphology (BT) <i>Assign expert taxonomic groups</i>	Snorkel to see coral followed by <i>Natural history discussion before dinner</i> Unpack equipment Get BCD and regulator from Lowell	Critique: Kelly & Andrew *Invertebrates lecture (KD)
22 Feb - Tue		SCUBA –shore dive at Cumber's Cave (check dive)	*Algae lecture & lab (BT) Snorkel to see invertebrates and collect algae followed by <i>Natural history discussion before dinner</i>	Critique: Zak & Katie *Herbivory & Food webs (BT)
23 Feb - Wed		SCUBA – Sailfin and Meadows	*Zooplankton lecture (GM) & *Fish ecology lecture (BT)	Critique: Suzanne & Fran *Zooplankton lab (GM) & night sampling (BT)
24 Feb - Thu		*Sponge lecture (KD) *Fish ecology (BT) Snorkel to see sponge lab (KD & BT)	Project 1 exploration <i>Natural history discussion before dinner</i>	Critique: Rohan & Marielle Project 1 idea discussion
25 Feb - Fri	Project 1 begins	Beach cleanup Project 1 exploration	3:30pm – Finalize project 1 idea, design, and group members *4:30 – Fish behavior lecture (GM) Weekly Group Meeting	RR: Karaoke for those interested
26 Feb - Sat	Dia libre (OFF)	SCUBA - Coconut Walk & Sara's Set		
27 Feb - Sun		Project 1 - pilot	Project 1	Critique: Ian, Elin & Larry
28 Feb - Mon	Project 1 proposal DUE	Project 1	Project 1	Critique: Jen & Rebecca
1 Mar - Tue		SCUBA – Marilyn's Cut & Soto Trader Project 1	Project 1	Project 1 – data analysis, write methods, stats help
2 Mar - Wed		Project 1	Project 1 - writing and analysis	**Project 1 PRESENTATIONS**

3 Mar - Thu	**Project 1 DUE** Project 2 begins	Project 2 brainstorming & pilot	Project 2 1pm - Project 2 idea discussion	Critique: Sara & Emily Finalize project 2 idea, design, and group members *Conservation & management lecture (BT) Graduate school discussion Grouper Moon video
4 Mar - Fri	Project 2 proposal DUE	Project 2	Project 2 Weekly Group Meeting	RR: Karaoke for those interested
5 Mar - Sat	Dia libre (OFF)			RR: Ball hockey & Karaoke for those interested
6 Mar - Sun		SCUBA – Mixing Bowl & Donna’s Delight	Project 2	**Project 1 revisions DUE**
7 Mar - Mon		Project 2	Project 2	SCUBA - Night dive – Cumber’s Caves
8 Mar - Tue		Project 2	Project 2	**Project 2 – writing and analysis
9 Mar - Wed	**Project 2 DUE @ 6pm**	Project 2	Project 2 – writing and analysis Presentations: Project 2	**Project 2 DUE @ 6pm** Clean up science equipment and field sites
10 Mar - Thu		SCUBA – Baracuda Bight & Great Wall West Clean up	**Project 2 revisions DUE @ 1pm** Revisions and copy editing of all LC projects	Revisions & copy editing of all LC projects Discussion of papers on authorship
11 Mar - Fri		Revisions & copy editing of all LC projects	Clean up & pack	Dinner and campfire (pending wind)
12 Mar - Sat	Depart LC			

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THE EFFECT OF TEMPERATURE ON *PSEUDOMYRMEX SPINICOLA* ACTIVITY

JENNIFER A. FREISE, ROHAN N. CHAUDHARY, ELIN C. BECK, REBECCA M. NIEMIEC,
AND ANDREW N. MERTENS

Project Design: M. C. Duryea. Faculty Editor: Ryan G. Calsbeek

Abstract: Temperature can have strong effects on ectotherm activity. We investigated the effects of temperature on the activity of a tropical ant species which engages in an obligate mutualism with *Acacia collinsii*. The red acacia ant, *Pseudomyrmex spinicola*, is the most abundant of the ant species involved in this mutualism in Costa Rica and occupies both shaded and sunny trees. Little is known, however, about its optimal temperature ranges. We tested the hypothesis that temperature affects *P. spinicola* activity by measuring the rate at which ants return to their home tree. Although we did not find a significant relationship between temperature and activity level, we did find a bimodal distribution in the rates of ant return to home trees.

Key words: *Pseudomyrmex spinicola*, optimal temperatures, pheromones

INTRODUCTION

Variation in temperature can have significant effects on the structure and function of ecosystems. Ectotherms are especially affected by these differences in temperature. Ectotherm activity levels are typically highest within a narrow range of optimal body temperatures (Adolf and Porter, 1993).

The obligate mutualism between ectothermic ants and *Acacia collinsii* in the tropics has been well studied. Several ecologically similar species of ants participate in these mutualisms, but *Pseudomyrmex spinicola* is the most abundant, occupying up to 95% of trees at some sites (Hourdequin et al. 1996).

Previous studies have examined the relationship between sunlight and activity levels of ant species. Hellmuth et al. (2004) found no relationship between defense activity of different ant species and sunlight exposure. Ermentrout et al. (2003) found that *P. nigrocincta* is found primarily in shaded areas, *P. flavicornis* is more abundant in sun-lit areas, and *P. spinicola* was found in both types of habitat. Little is known, however, about how these patterns associated with light availability may be related to the optimal temperature

conditions at which each ant species functions.

We examined the extent to which temperature affects the activity of *P. spinicola*. We chose this species because it is the most abundant acacia ant at our study sites. Since acacia trees are required as host-plants for the ants' survival, we assumed that when displaced from these acacias, ants would return to them quickly. We hypothesized that there would be an optimum temperature at which ants would be most active and return to their home trees fastest.

METHODS

We studied the ant-acacia mutualism in a secondary tropical dry forest near the OTS Biological Station in Palo Verde National Park, Costa Rica on 9 January 2011 from 0800 to 1100. We haphazardly selected thirty *A. collinsii* trees of at least breast height that were inhabited by *P. spinicola*. We measured the temperature of each tree at breast height and on the ground five centimeters away from the base with a Raytek® Raynger® MX™ 635nm laser thermometer gun. We cleared a 10cm radius circle around the base of each tree. At each tree, we removed three ants, one at a

time, and placed them in the cleared space 10cm from the tree's base. Previous work on alarm hormones has suggested that this is a reasonable distance (Holldobler and Wilson 2009). We recorded the time that it took for each ant to return to its home tree. Since ants depend on their home acacias for survival, we assumed an ant's ability to return quickly to its home tree would be a reasonable proxy for its activity level. If an ant took longer than 90 seconds to return, the ant was assigned a return time of 91 seconds. We used JMP 8.0 software to compute regressions between tree temperature and return time and between ground temperature and return time. We also examined the distribution of return times to evaluate our assumption that ants would return to their home tree quickly.

RESULTS

Ants returned 74.4% of the time, which suggests that 10 cm is an appropriate distance.

We found a bimodal distribution of return times, with modes of over 90 seconds (not returning to the tree) and less than 10 seconds (Fig. 1). However, we found no significant

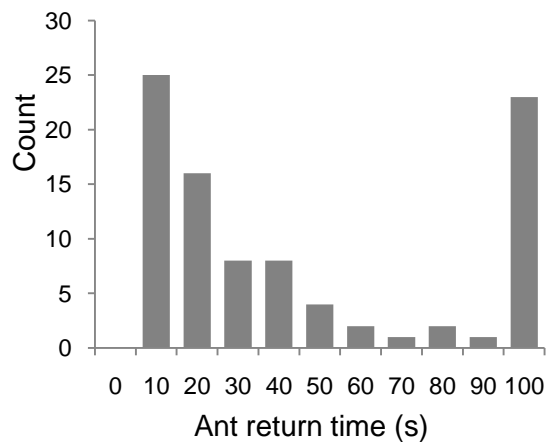


Fig. 1. Bimodal distribution of *P. spinicola* return time (time to return to home tree when displaced) in seconds at Palo Verde Biological Station, Costa Rica. Ants that did not return were assigned a return time of 91 seconds.

relationship between ground or tree temperature and ant return time (ground temperature, $r^2_{89} = 0.01$, $P = 0.32$; tree temperatures, $r^2_{89} = 0.00$, $P = 0.74$; Fig. 2).

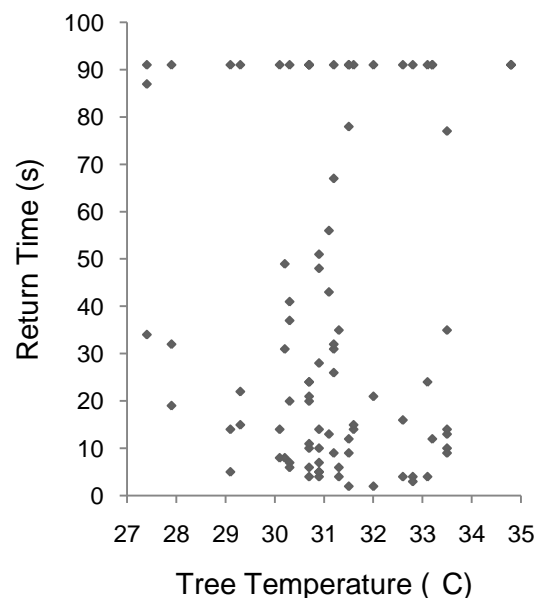
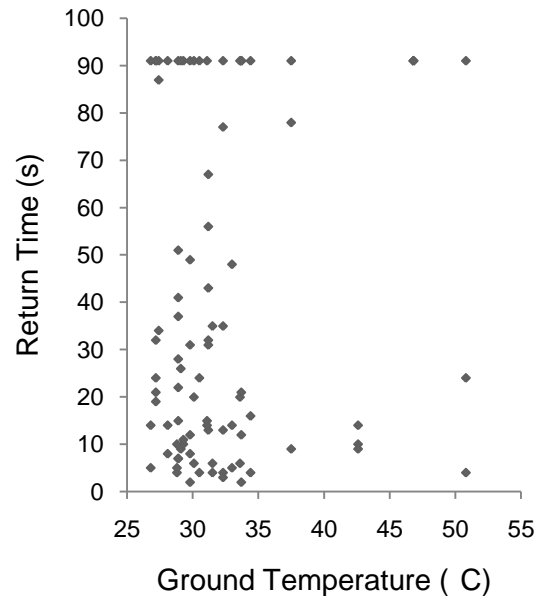


Fig 2. Associations between ground temperature and tree temperature (at breast height) and time for ants to return to their home tree ("Return Time") at Palo Verde Biological Station, Costa Rica ($r^2_{89} = 0.01$, $P = 0.32$; tree temperatures, $r^2_{89} = 0.00$, $P = 0.74$).

DISCUSSION

We found that temperature did not affect the rate at which ants returned to their home acacia trees. Because *P. spinicola* is a habitat generalist it should have a broad optimum temperature range (Ermentrout et al. 2003). Thus, studying temperature variations in *P. spinicola*'s natural environment would not result in significant variations in activity. Further studies should examine both *P. spinicola* and *P. flavicornis* over a larger temperature range to determine if they have distinct optimum temperature ranges. If experiments find that *P. spinicola* has a broader optimum temperature range than *P. flavicornis*, they could show a physiological basis for *P. spinicola* being more of a habitat generalist.

Our study suggests that return time to the home tree may not be an accurate proxy for determining differences in activity level. Other cues in the environment may have had greater effects on ant return rate.

For instance, we noticed that ants that faced the tree when dropped tended to reach it faster than those facing away. Also, while we cleared an equal amount of area around the base of each tree, slope and debris were variable, potentially affecting travel time. Many ants failed to travel in direct paths back to their home trees, often climbing up leaves or twigs in their paths. This could indicate that ants were looking for or attacking intruding plants. Additionally, the removal of ants from their home trees may have affected their navigational abilities.

The bimodal distribution of ant return time may be related to previous ant activity in the area where they were released. Goss et al. (1989) found that ants often take the most efficient path to get back to their home tree based on pheromone trails left by other ants. The probability that an ant will choose a particular path is positively correlated to the densities of

previous pheromone trails (Goss et al. 1989). The high number of ants that returned within 10 seconds may have found such paths leading back to their home trees. Alternatively, the ants that did not return home within 90 seconds may have followed pheromone trails leading away from their home trees, or may have had insufficient pheromones to find their home tree. In addition, ants colonize multiple trees in nearby areas, so pheromone trails could have led to other acacia trees (Janzen 1966).

This study is one of the first to investigate acacia ant behavior on the ground. Further studies should focus on their ground-patrolling behavior, particularly the role played by previous ants' pheromones.

ACKNOWLEDGEMENTS

We would like to deeply thank Young M. C. Duryea for her experimental design that led to this project. Without her, this study would not have been possible.

We would also like to thank Ryan Calsbeek, Gillian Moritz, and Young M. C. Duryea for their editing expertise.

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CONSPECIFIC AND HETEROSPECIFIC AGGRESSION IN ACACIA-ANTS

KELLY S. AHO, KATHERINE L. FITZGERALD, LARRY L. BOWMAN, JR., ZACHARY J. CLARE-SALZLER,
AND IAN D. ENGLER

Project Design: Gillian L. Moritz. Faculty Editor: Ryan G. Calsbeek

Abstract: In order to successfully reproduce and defend themselves, animals must be able to recognize their own species. This is especially important for a species that establishes independent sister colonies. Acacia-ants show aggression in situations that threaten the colony or the *Acacia collinsii* host tree, such as ant predation and *A. collinsii* herbivory. Since different ant species rarely co-colonize, we hypothesize that heterospecific interactions will be more aggressive than conspecific interactions. We observed the response of host ants to introduced conspecifics from the same tree, conspecifics from a different tree, and heterospecifics. Host ants were more aggressive toward heterospecific invaders than conspecific invaders. *Pseudomyrmex spinicola* defended its territory significantly more aggressively than *P. flavicornis*. These results indicate that acacia-ants are able to distinguish between conspecific and heterospecific invasions of their host trees.

Keywords: *P. flavicornis*, *P. spinicola*, *A. collinsii*, Palo Verde, species recognition

INTRODUCTION

Species recognition is important for successful reproduction and defense. A range of organisms from anoles to tits distinguish between conspecifics and heterospecifics in order to gauge the threat posed by other organisms (Macedonia and Stamps 1994, Hansen and Slagsvold 2003). The ability to recognize conspecifics and heterospecifics is especially relevant for colonies defend territories.

A well-documented obligate mutualism exists between *Acacia collinsii* and ants (Janzen 1983). In exchange for nesting structures and food resources, acacia-ants protect *A. collinsii* from herbivores and encroaching vegetation (Janzen 1983). Usually a single-species colony occupies a given tree or clump of trees (Janzen 1983). We studied two acacia-ant species in Costa Rica, *Pseudomyrmex flavicornis* and *Pseudomyrmex spinicola*.

Janzen (1966) found that *P. flavicornis* is less effective than *P. spinicola* at protecting *A. collinsii* against herbivory and encroaching vegetation. *P. spinicola* has been shown to be more aggressive toward members of different

P. spinicola colonies than toward its own colony (DiBiccari et al. 2010). *P. spinicola* has also been shown to be aggressive toward introduced *P. flavicornis* (Schwartz et al. 2006).

Our study examined aggression between ants of the same and different species. We hypothesized that there would be a graded response in aggression by ants to introduce hetero- and conspecifics. Specifically, we predicted that the highest degree of aggression would be directed toward heterospecific ant introductions. There would be an intermediate degree of aggression toward conspecific introductions to foreign trees and the lowest degree of aggression toward conspecific introductions to their host tree. Finally, we predicted that *P. spinicola* would be more aggressive than *P. flavicornis* in response to foreign ants.

METHODS

Our study site was an approximately one-acre plot within Palo Verde National Park, Guanacaste Province, Costa Rica. We examined *P. flavicornis* and *P. spinicola* colonies on *A. collinsii* larger than 10cm diameter at

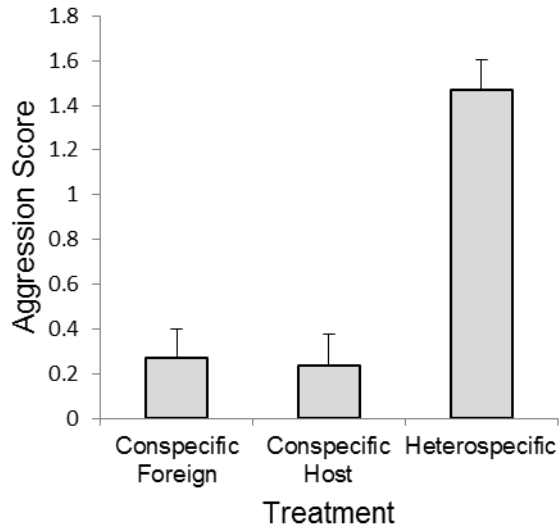


Fig 1. Average aggressive behavior scores for ant introduction treatments on *A. collinsii* in Palo Verde National Park, Costa Rica. Heterospecific introduction aggression was significantly greater than the conspecific introductions.

breast height and distributed at least 10m apart. We employed a full-factorial experimental design with three factors: heterospecific (in which individuals were introduced to a tree of a different species); conspecific-foreign (in which individuals were introduced to a different tree of the same species); and conspecific-host (in which individuals were introduced to a new location on their own tree) and two levels: *P. flavicornis* and *P. spinicola*. Data for all six combinations of level and factor were collected.

To obtain ants for introduction, we shook them off of a branch of their host tree into a plastic bag. We marked the ants by adding fluorescent powder to the bag (pink for *P. flavicornis*, green for *P. spinicola*). After marking, we introduced one ant at a time approximately 23cm from the tip of a branch. Aggressiveness was measured on a 0-3 scale (where 0 = no interaction, 1 = non-aggressive interaction [antennating], 2 = aggressive interaction [grappling], 3 = most aggressive interaction [expulsion or death]) at 0, 30, 60, 90, and 180s.

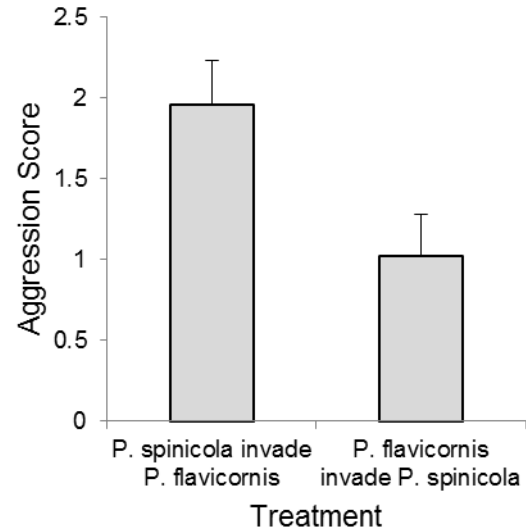


Fig 2. Average aggressive behavior scores for heterospecific ant introduction treatments on *A. collinsii* in Palo Verde National Park, Costa Rica. Aggression was significantly greater with the *P. spinicola* introduction than the *P. flavicornis* introduction.

Time of first interaction was also recorded, along with time of voluntary or involuntary expulsion from the tree, or the time until an ant entered a domatium if applicable.

Statistical Analysis

Ants that jumped off the tree, went into domatia, or were lost during observation were excluded from the data set. A total of 65 trials were included in the final analyses. We used ANOVA to test whether the means of perceived aggression differed across treatments, and we compared means using a Tukey-Kramer test. We performed a Pearson test of the frequency of expulsions by treatments. We used one-sided two-sample t-tests for time before expulsion and to determine whether *P. spinicola* or *P. flavicornis* was the most aggressive host species. Statistical analyses were done using JMP 8.0.

RESULTS

Moving an ant from its home tree to a foreign heterospecific tree resulted in the highest average aggressive behavior score ($F_{2, 62} =$

25.56, $P < 0.0001$, Fig. 1). We detected no significant difference between the behavioral scores for conspecific treatments. There was a significant difference in average aggression scores between the two heterospecific treatments ($t_{19} = -2.51$, $P = 0.01$). Expulsion of foreign ants was significantly more common in heterospecific interactions than conspecific interactions ($\chi^2_1 = 20.53$, $P < 0.0001$). Expulsion was also significantly faster in heterospecific interactions than in conspecific interactions ($t_{15} = -3.35$, $P = 0.002$).

DISCUSSION

The heterospecific introduction of *P. flavicornis* and *P. spinicola* led to significantly greater aggression from the host species than conspecific introductions (Fig. 1). Ants were able to distinguish their own species from other species, likely using chemical cues (such as pheromones) or physical cues (such as visual recognition). This species recognition is necessary to distinguish between colony members and heterospecific intruders. This is especially important in maintaining the cohesion of large colonies. Species recognition should facilitate colony organization and reproductive success, as well as recognition of threats.

In conspecific interactions, some foreign ants entered the domatia, suggesting some level of non-aggressive species and colony recognition. Moreover, heterospecific ants never entered the domatia, indicating aggressive species and habitat recognition.

Expulsion of foreign ants was significantly more common and occurred more quickly in heterospecific interactions than conspecific interactions. This is consistent with our interpretation that expulsion is an aggressive behavior for protection of the colony and tree. More thorough research could determine whether ants aim specifically to protect the

host tree or the colony, potentially by observing behaviors in response to the threat of herbivory and the threat of colony usurpation.

P. spinicola was significantly more aggressive in heterospecific interactions than *P. flavicornis* (Fig. 2). *P. spinicola*'s more aggressive defense combined with its more aggressive removal of basal foliage (Grunseich 2009), indicate that *P. spinicola* is more aggressive overall than *P. flavicornis*. This is consistent with Janzen's conclusion (1966).

These results raise the question of how *P. flavicornis* is able to persist in the presence of a more hostile species. Though our data do not address this, possible explanations include *P. flavicornis* colonizing new *A. collinsii* faster than *P. spinicola*, or *P. flavicornis* thriving when resources are limited. In addition, the cost of attacking *A. collinsii* colonized by heterospecifics may outweigh the benefits. Thus, the more hostile nature of *P. spinicola* might not strongly influence colonization by either species.

Higher levels of aggression in *P. spinicola* may impact the distribution of ants on *A. collinsii* in tropical forest ecosystems. This distribution influences herbivory, survival of competing plants in the vicinity of *A. collinsii*, and other components of the broader forest community.

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We would like to thank Gillian Moritz for designing this study in detail and withstanding countless ant bites in the field. We would also like to thank Ryan Calsbeek, Ph.D. and Katie Duryea for their insightful edits on this manuscript.

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ACACIA ANT ATTACK RESPONSE TO HERBIVORE SALIVARY CUES

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Abstract: Due to the high costs from herbivory, many plants invest in defenses. The bullhorn acacia (*Acacia collinsii*) has developed a unique mutualism with the acacia ant (*Pseudomyrmex spinicola*), which defends the tree from herbivory in exchange for shelter and nutrients. Since defensive attacks against herbivory are energetically costly, ants might benefit from moderating their attacks in proportion to the level of a threat. We investigated ant responses to ctenosaur, human, and horse saliva. Assuming that ctenosaurs are frugivores, humans are omnivores, and horses are herbivores, we hypothesized that the intensity of the ant response would be greater for horse saliva than for ctenosaur saliva, with an intermediate response to human saliva. We applied saliva samples to 20 different trees and measured the intensity of the attack. Our data did not indicate that there were significant differences between treatments, suggesting that ants may not respond to cues in saliva.

Key words: *Pseudomyrmex spinicola*, *Acacia collinsii*, attack response, salivary enzyme cues

INTRODUCTION

Herbivory is a constant threat to the growth and reproduction of plants. Strategies to resist herbivory, such as rapid growth and secondary metabolites, are often energetically expensive for plants (Coley 1985). Some plants have developed mutually beneficial relationships with other organisms that serve as an alternative strategy to herbivore defense.

The relationship between the bullhorn acacia (*Acacia collinsii*) and acacia ant (*Pseudomyrmex spinicola*) is a classic example of a mutualism. In exchange for shelter and nutrients, the ants defend the tree against potential herbivores (Zukowski 2007). Since an aggressive response is energetically costly, ants may benefit from varying their level of attack in proportion to the level of threat. MacIntosh et al. (2000) elicited an ant response to simulated herbivory leaf damage and movement by cutting leaves and tapping branches. While these studies have provided important insights into the cues that provoke ant defenses, other cues that may elicit a defensive response, such as salivary cues in non-human

saliva, remain poorly understood. In this study we simulated herbivory with chemical cues in the form of human, horse, and lizard saliva. We hypothesized that ants would attack with intensity proportional to the threat of the disturbance, with herbivores posing the greatest threat. Since horses are herbivores, humans are omnivores, and ctenosaurs are frugivores, we hypothesized that ants would respond most strongly to horse saliva, less strongly to human saliva, and least strongly to ctenosaur saliva.

METHODS

We collected saliva samples from a human (*Homo sapiens*), a horse (*Equus caballus*), and a ctenosaur (*Ctenosaura similis*) by swabbing the gums of each with filter paper. Samples were stored for 24 hours in sealed vials. Clean, wetted filter paper was used as a control.

Acacia trees of at least breast height, hosting active *P. spinicola* ants, were haphazardly selected from a roadside transect in Palo Verde National Park, Costa Rica. For each tree, we estimated the baseline activity of ants

on a branch. We established a focal location 20 cm from the end of the branch and counted the number of ants that crossed that point in one direction during one minute.

We randomly selected one of the four treatments then rehydrated the filter paper with five drops of water to account for the desiccation of the original saliva. The paper was placed 15 cm from the end of a branch using forceps to avoid introducing human cues. We observed the intensity of the attack for three minutes by measuring the response time—time until the ants first approached the paper—and the number of ants that arrived in the first minute. We rated ant response as aggressive or nonaggressive based on behaviors including approaching, investigating, and biting. Twenty trees in total (five per treatment) were included in the experiment. We used JMP 8.0 to perform ANOVA and regression tests to compare response time and response rate between treatments.

RESULTS

We found no significant difference in ant arrival rate (ANOVA $F_{3,16} = 0.44$, $P = 0.73$; Fig. 1) or response time (ANOVA $F_{3,13} = 1.56$, $P = 0.24$; Fig. 2) among treatments.

Our data showed no significant correlation between baseline activity and the ant arrival rate ($r_{18} = 0.15$, $N = 20$, $P = 0.09$) or between baseline activity and response time ($r_{15} = 0.02$, $N = 20$, $P = 0.55$). Our ranking of attack intensity showed no relationship to either ant arrival rate or response time.

DISCUSSION

We found no significant differences between the responses of ants to lizard, human, or horse saliva. Thus, we could not reject the null hypothesis that ant attack intensity is unrelated to the intensity of the herbivore threat.

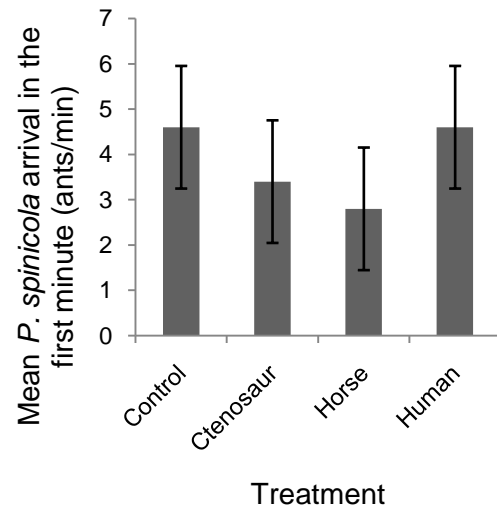


Fig 1. Mean number of ants arriving during the first minute (ants/min) for each treatment on acacia trees in Palo Verde National Park, Costa Rica. Error bars show ± 1 SE from the mean.

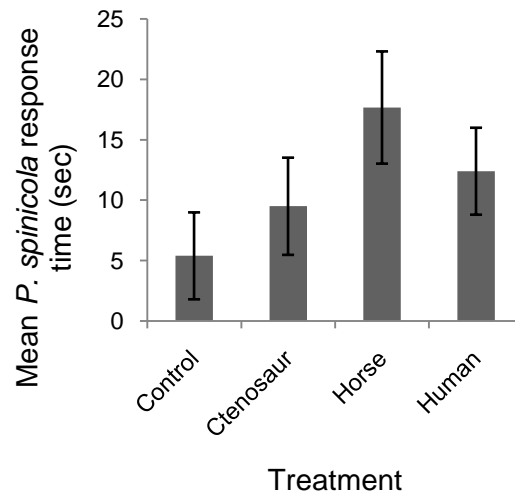


Fig 2. Mean *P. spinicola* response time (s) for each treatment on acacia trees in Palo Verde National Park, Costa Rica. Error bars show ± 1 SE from the mean.

Our study suggests that saliva or chemical cues alone might not be a sufficient representation of an herbivore attack. Because a defensive response is energetically costly, the ants may require more stimuli to illicit an attack, such as leaf damage or movement. Saliva may interact with other cues and physical disturbances to induce a response from the ants.

Alternatively, our results suggest that acacia ants may perceive lizards, humans, and horses as equal threats. While ctenosaurs feed primarily on fruits, they also rely in part on tender, young leaves during the dry season and periods of fruit scarcity (Fitch and Hackforth-Jones 1984). In addition, acacia seeds have been found in ctenosaur droppings (Traveset 1990). Therefore, our original assessment of ctenosaurs as a minimal threat to acacias may be incorrect.

Human and non-human primate saliva have similar chemical compositions (Levine et al. 1977). Because capuchin monkeys are also known to consume parts of the acacia tree (Freese 1983), ants may recognize human saliva as a threat as well.

In addition fossil records indicate that horses and other ungulates have been present in Central America for millions of years (Marshall et al. 1982). It is possible that the ants have evolved to respond similarly to enzyme signals from ctenosaurs, primates, and ungulates.

It is also possible that rehydrated saliva samples were inadequate to provoke an aggressive ant response. The saliva samples were collected twenty-four hours prior to the experiment and were dry by the time of use. Because we found no response to dry saliva in the pilot study, we added water to simulate fresh salivary chemical cues. However, salivary chemicals degrade over time; human saliva samples stored in liquid form at 37°C for twenty-four hours lost 63-92% of important enzyme activity (Kraus and Williams, 1963). With daily temperatures varying between 26-33°C during the course of our study, the loss of enzyme activity could have been occurred. However, personal observations suggest that fresh human saliva does elicit an aggressive attack response.

Future studies could investigate which other characteristics of herbivory, such as animal size, body heat, or movement, provoke the greatest response from acacia ants, and whether salivary cues induce a response in combination with these other characteristics.

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We would like to thank the staff of Palo Verde National Park for their hospitality and Professor Ryan Calsbeek for his experimental design and advice throughout the project. We would also like to express our gratitude for the feedback and support of the students of the FSP and the course TAs, Gillian Moritz, MC Duryea, and Sarah Wengert.

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TERRITORY OPTIMIZATION IN *CTENOSAURA SIMILIS* BY TEMPERATURE AND SUBSTRATE AVAILABILITY

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

Abstract: Accessibility to resources is crucial to territorial animals. Territories with variability in thermal resources are especially important for ectotherms such as lizards, which use environmental temperatures to regulate their body temperature. We hypothesized that these resources are an important determinant to the density and distribution of the territorial lizard *Ctenosaura similis* and that the healthiest lizard will likely hold the territories with the best thermal resources. We surveyed the area around Palo Verde Biological Station for ctenosaurs, mapped their initial location and calculated their sprint speed. We evaluated the substrate along a transect and recorded the temperature and the presence of shade. We found that ctenosaur abundance was positively correlated with thermal habitat heterogeneity, defined as diverse combinations of substrates and shade. However, we did not find any significant relationship between sprint speed, a proxy for lizard health, and mean territory temperature or substrate quality.

Key words: *Ctenosaura similis*, territory optimization, thermal resource, sprint speed

INTRODUCTION

Territorial animals strive to maximize the availability of necessary resources within their habitats. By defending a territory with better resources, an animal is able to improve its fitness and the fitness of its offspring (Verner 1977). Resources often defended in territories include food, shelter, and access to mates.

Lizards, as ectotherms, require external thermal resources to regulate body temperature. Body temperature affects the ability of lizards to be physically active, digest food (Huey and Slatkin 1976), evade predators (Crowley 1985), and reproduce (Licht 2004). Therefore, a lizard able to consistently maintain optimal body temperature has a distinct advantage over lizards with inferior access to thermal resources.

In this study, we investigate how access to different thermal resources influences the density and distribution of the lizard *Ctenosaura similis* and whether lizard health (esti-

mated by sprint speed) is correlated with access to the best territories. We hypothesized that there would be higher ctenosaur densities in areas with greater thermal variance, so lizards could maximize the amount of time they spend within their optimal temperature range (36-37°C) (Fitch and Hackforth-Jones 1983). Furthermore, we predicted that the habitats with the greatest variance in temperature and substrate diversity would be inhabited by ctenosaurs with the fastest sprint speeds.

METHODS

We sampled *Ctenosaura similis* distribution and abundance in Palo Verde National Park in Costa Rica on 11 and 12 January 2011. We spent three hours sampling in both forested and open areas. Every male and female ctenosaur sighting was recorded on a map of the study site (Appendix Fig. 2 and Fig. 3). The map was partitioned into 40 m x 40 m areas because the lizards' territories are on average

22 m in radius (Fitch and Hackforth-Jones 1983).

When possible, ctenosaur sprint speed was recorded by approaching an individual at a constant speed and subsequently forcing the lizard into a sprint. Separate observers recorded the running time and the path of the lizard. Afterwards we measured the distance of the path and calculated an approximate sprint speed.

To evaluate thermal resource variability, we haphazardly selected a transect across our entire sample area. We recorded temperature on both sides of the 150m transect every 5 meters. The temperatures were measured using a Raytek Raynger MX Thermal Gun. We noted the presence of shade or sun and substrate type (leaf litter, bare soil, tile, grass or concrete) for each point. Over the course of two days, we collected the transect temperature data in the early morning (05:30-06:30), mid-morning (09:30-10:30), midday (11:00-12:00), afternoon (14:00-15:00), and evening (17:00-18:00).

Analysis

We calculated the average daily temperature for every 40 m x 40 m territory area using mean substrate temperatures weighted by the percent substrate coverage. We analyzed the linear fit of lizard abundance by average daily temperature.

We also assigned a qualitative score from 1-10 to each of the territories by counting the number of substrate and shade combinations available. We compared mean sprint speed and lizard abundances among each territory score using ANCOVA. The null hypothesis that ctenosaurs were evenly distributed across the study area independently of thermal resource availability was evaluated with a Chi Square test.

We used student t-tests to determine if the mean sprint speed of lizards differed in the sun or shade and also in open or forested area. We did this to determine if initial environmental conditions affected sprint speed. All statistical analyses were performed using JMP 8.0.

RESULTS

We found that different substrates had different mean temperatures throughout the day, indicating that they are distinct thermal resources (Fig. 1).

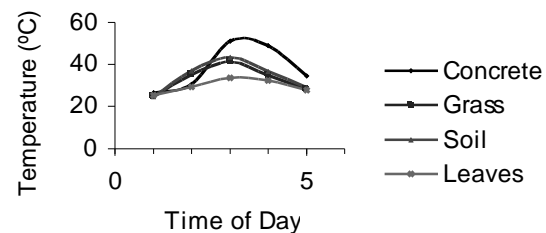


Fig. 1 Daily temperature of substrates within Palo Verde National Park, Costa Rica, where 1=early morning, 2=mid morning, 3=noon, 4=afternoon, and 5=evening.

Ctenosaurs were not evenly distributed and were more likely to be found in open and edge habitats than in forested areas ($\chi^2 = 22.67$, $n = 2$, $P = 0.0001$; Table 1).

Ctenosaur abundance increased with habitat quality score (ANCOVA, $F_{7,18} = 7.42$, $P = 0.0003$). Habitats with a score of 8 or 9 had significantly more ctenosaurs than territories with lower scores (Fig. 2). The same trends were evident for lizards exclusively surrounding the field station, but these results were not significant. This suggests that the presence of the field station was not a large confounding factor. Mean ctenosaur sprint speed did not increase significantly with habitat quality score ($F_{5,4} = 0.13$, $P = 0.98$).

The comparison of weighted mean daily Ctenosaur abundance was not significantly related to weighted mean daily temperature of each territory ($F_{1,24} = 2.093$, $P = 0.17$).

There was no significant difference between the mean sprint speeds of individuals in shaded vs. sunny and open vs. forested areas ($t_{9.76} = -1.36$, $P = 0.21$ and $t_{10.61} = -1.14$, $P = 0.28$, respectively).

Table 1. Results of χ^2 test for distribution of ctenosaurs in Palo Verde National Park, Costa Rica. The null hypothesis that lizards would be evenly distributed was rejected ($n = 2$, $P = 0.0001$).

Environment	Expected Number of Lizard Sightings	Actual Number of Lizard Sightings	χ^2
Forest (>10 m beyond open)	19	2	15.21052632
Transitional (0-10 m beyond open)	19	28	4.263157895
Open (cleared area; grass or bare dirt)	20	28	3.2
Total	58	58	22.67368421

DISCUSSION

Our data suggest that ctenosaurs are not uniformly distributed, but are clumped in open and transitional areas that offer the greatest variety of substrate and shade combinations. Despite the differences between the temperature ranges of the substrates, our data indicate that there is no one substrate type which allows ctenosaurs to be within their preferred temperature range (36-37°C) throughout the entire day. Our data indicate that shaded areas do not reach high enough temperatures to support ctenosaurs' thermoregulatory needs at all times of day. We conclude that territories with the highest levels of substrate diversity provide ctenosaurs with the greatest advantage, allowing them to re-

main close to preferred body temperature for the longest amount of time.

Although Huey et al. (2009) found a relationship between sprint speed and temperature preference, we found that highest sprint speeds were not correlated with the best habitats. This is surprising given the temperature sensitive nature of optimal athletic performance in ectotherms. Additionally, sprint speed data may not accurately indicate lizard health because ctenosaurs that have become habituated to humans may respond differently to an approach by researchers (McLaren et al. 2000).

Our results suggest that if temperatures in tropical forests continue to increase, ctenosaurs, which now prefer open habitats, may be able to live either full- or part-time in the forests, which are currently too cool for them to take up permanent residence. This may have drastic effects on niche organization and interspecies dynamics between forest and open-area tropical lizard species (Huey et al. 2009). Future studies might consider following the protocol used by Huey et al. (2009) for measuring ectotherm temperatures, which involves calculating body temperatures, available operative temperatures, and preferred temperature ranges, to gain a better understanding of how lizard body temperatures relate to their habitat preferences.

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We would like to thank the staff of Palo Verde National Park for their hospitality and permission to conduct research in the park. We also thank the TAs and Dr. Ryan Calsbeek, who aided us in surveying and testing the sprints speeds of lizards.

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APPENDIX

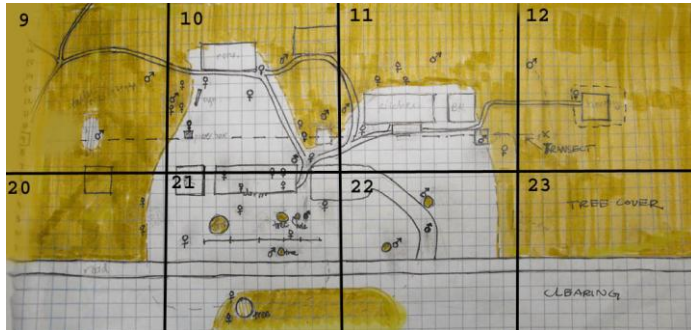


Figure 2. Map of ctenosaur locations and territories.



Figure 3. Map of ctenosaur locations and territories.

EFFECT OF PREDATION AND DENSITY DIFFERENCES ON BEHAVIOR IN *POECILIOPSIS ELONGATA* AND *POECILIA GILLII*

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Abstract: Animals have evolved diverse behavioral mechanisms to reduce the threat of predation. Marsh fish *Poeciliopsis elongata* and *Poecilia gillii* school as a form of predator swamping. We hypothesized that schools of larger numbers of fish would have a reduced response to predation. We observed fish behavior at varying densities with and without simulated predation. Smaller numbers of fish spent more time stationary, probably as a defensive response to predation. Our findings suggest that predation response depends on school size. We also found that larger fish spent more time under shelter. This could indicate a greater need for larger fish to hide because they are more visible in the open.

Key words: fish schooling, predator swamping, shelter, density, activity budget

INTRODUCTION

One social behavior in the animal kingdom that can serve a protective function is predator swamping. Predator swamping occurs when an individual travels with a group of conspecifics, decreasing the probability that the individual will fall victim to a predator (Hamilton 1971). For example, large fiddler crabs cluster together to travel across intertidal flats to feed, thereby reducing their individual predation risk (Ens et al. 1993).

The effectiveness of predator swamping depends on the density of the group. Denser bark beetle aggregations, for example, decrease the proportional effect of predation on the colony (Aukema and Raffa 2004). Also, common terns that nest at high densities lose proportionally fewer chicks to gull predation (Becker 1995).

Poeciliopsis elongata and *Poecilia gillii* are small schooling fish that live in brackish water in the tropics. Many marsh bird species prey upon these fish (Bussing 1998). Their

school sizes vary from only a few fish to more than 30 (Becket al., field observations).

We examined the effect of density on *P. elongata* and *P. gillii* responses to predation. Predation response could be affected by number of fish in a school or the total biomass. We hypothesized that number of fish would have more influence on response to predation. We also hypothesized that larger groups would have a reduced response to predation and would spend less time stationary and under shelter.

METHODS

On 9 and 10 January 2011, we captured *P. elongata* and *P. gillii* from a marsh in Palo Verde National Park, Costa Rica. We estimated the mass of each species by taking the mean of four individuals with an Adam Equipment Highlander Series balance (*P. elongata*: $\bar{x} = 2.22\text{g}$; *P. gillii*: $\bar{x} = 0.62\text{g}$).

On 10 and 11 January, we ran trials in a 95cm x 55cm tank filled 12.5cm deep with tap water. We placed four medium-sized rocks (approx. 5cm x 10cm x 3cm) on the bottom of

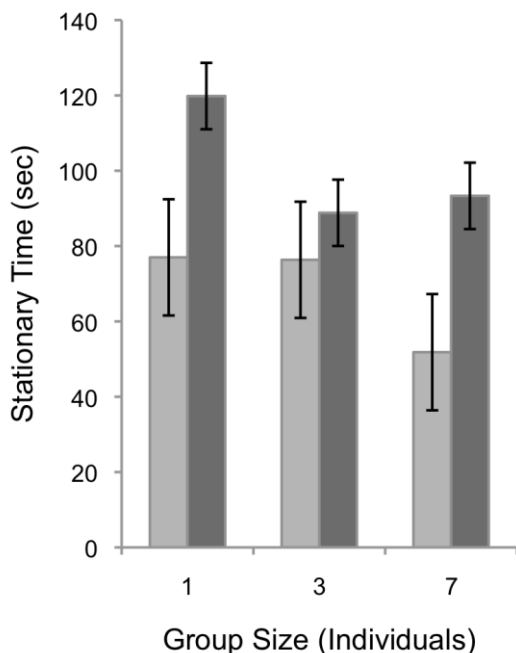


FIG. 1. Mean combined time spent stationary (seconds) by *P. elongata* and *P. gillii* for individual as well as small and large school sizes. Light bars represent no predation and dark bars represent predation. Asterisks indicate treatment pairs with significant differences of mean stationary time between predation and no predation.

one side of the tank to provide a shelter for fish. We monitored the temperature with a YSI model 63 thermometer (approx. 28°C).

We shaded the tank using a dark plastic sheet to keep light constant. We replaced the water after every six trials to control for turbidity. We aerated the water after every trial to ensure substantial dissolved oxygen.

After giving the fish one minute to acclimate to the tank, we recorded fish activity for two minutes. We measured stationary and shelter time, which we defined as time spent not moving and time spent within the constructed shelter, respectively. We simulated predation by poking the beak of a bird-shaped cardboard cutout into the water at 15s intervals. We visually divided the water surface into six equal regions and rolled a six-

TABLE 1. Mean stationary time of fish for each school size with and without predation.

Mean Stationary Time (SE)	Predation?	Group Size
119.83s (11.68s)	Yes	1
88.83s (13.92s)	Yes	3
93.33s (11.97s)	Yes	7
77.00s (11.68s)	No	1
76.35s (13.92s)	No	3
51.83s (11.97s)	No	7

sided die to randomly assign predator attack locations. We performed each trial using one, three, or seven individuals that were chosen at random from a pool of either 21 *P. elongata* or 17 *P. gillii*. We ran three trials of each density with and without a predator. To test for an effect of school biomass on predator response, we also conducted three trials using 11 individuals of *P. gillii*, which were equivalent to the biomass of three individuals of *P. elongata*.

We used JMP 8.0 to perform statistical analyses.

RESULTS

We found no evidence for a statistical interaction between group size and predator treatment (Fig. 1; for raw data see Table 1). Overall, fish spent more time stationary in the presence of simulated predation than in the absence of simulated predation (with predation: $\bar{x} = 100.67s$, $SE = 7.48s$, $n = 18$; without predation: $\bar{x} = 68.39s$, $SE = 7.48s$, $n = 18$; $t_{34} = 3.05$, $P = 0.002$, Fig. 2). In our test of the effects of group size and predation on stationary time, predation was the only behavior that had a significant effect, and we found no evidence for higher order interactions (predator: $F_{1,30} = 5.81$, $P = 0.02$; group size: $F_{2,30} = 2.15$, $P =$

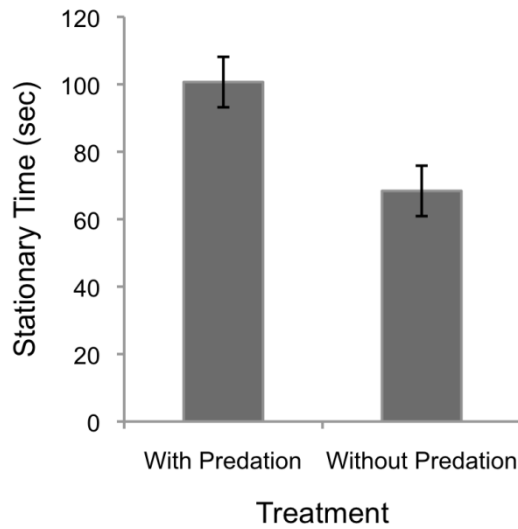


Fig 2. Mean time spent stationary (seconds) by both fish species with and without predation.

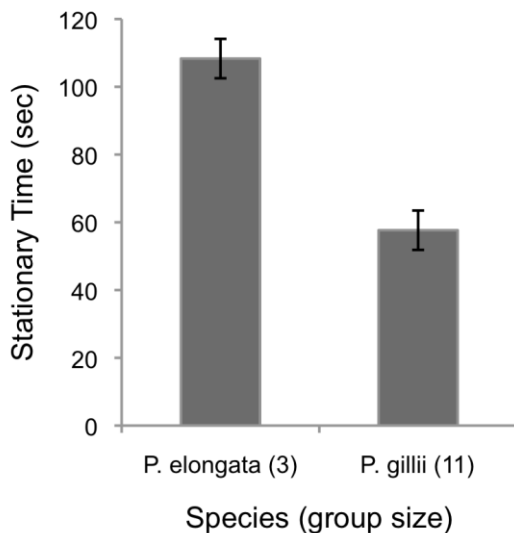


Fig 3. Mean time spent stationary (seconds) by *P. elongata* and *P. gillii* in different size groups with predation and approximately equal total biomass (6.82g).

0.13; predator \times group size: $F_{2,30} = 0.93$, $P = 0.41$).

Comparing treatments of equal biomass (6.82g), a smaller group (3) of *P. elongata* spent significantly more time stationary than a larger group (11) of *P. gillii* in the presence of predation (*P. elongata*: $\bar{x} = 108.33s$, $SE = 5.81s$, $n = 3$; *P. gillii*: $\bar{x} = 57.67s$, $SE = 5.81s$, $n = 3$; $t_4 = -$

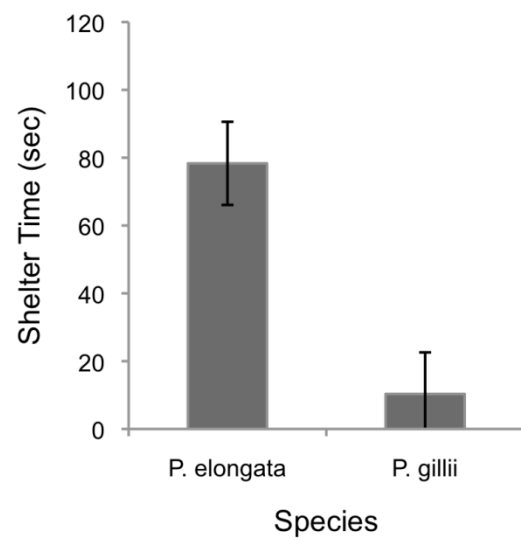


Fig 4. Mean time spent under shelter (seconds) by *P. elongata* and *P. gillii* with predation.

6.16, $P = 0.002$, Fig. 3). However, when we kept number of individuals constant across species, there was no difference in the mean stationary time (one individual: $t_{10} = 0.85$, $P = 0.21$; three individuals: $t_{10} = -1.18$, $P = 0.13$; seven individuals: $t_{10} = -1.66$, $P = 0.07$). Finally, *P. elongata* spent significantly more time in sheltered areas in the presence of predation (*P. elongata*: $\bar{x} = 78.33s$, $SE = 12.26s$, $n = 9$; *P. gillii*: $\bar{x} = 10.33s$, $SE = 12.26s$, $n = 9$; $t_{16} = -3.92$, $P = 0.0006$, Fig. 4) and in the absence of predation ($t_{16} = -1.82$, $P = 0.04$).

DISCUSSION

Both fish species spent more time stationary in the presence of simulated predation (Fig. 1). This suggests that being stationary may be a defensive behavior, as stationary fish should be harder for predators to spot. Similarly, tadpoles suppress foraging in the presence of a predator (Van Buskirk and Yurewicz 1998).

Larger schools of fish were stationary for less time than smaller schools of fish in the presence of a predator (Fig. 2). Because any given individual has a lower probability of

being attacked, larger schools of fish likely see a predator as less of a threat. This is consistent with Domenici's (2010) finding that fish in schools had a delayed reaction to predators. Schools can swim in open waters and continue foraging rather than remaining stationary and hidden from predators. In the absence of predation, there was no significant difference in movement behavior between low and high densities of fish.

Groups of equal fish biomass were stationary for significantly different amounts of time in response to predation (Fig. 3). The number of fish present, not the total biomass of the fish, likely influenced the change in stationary time and the perceived threat of predation. A decreased perception of predation through schooling is consistent with predator swamping theory and has been shown to significantly influence behavior in other species (Aukema and Raffa 2004).

The smaller *P. gillii* spent significantly less time in the shelter than the larger *P. elongata* in the presence of predation (Fig. 4). One explanation may be that *P. gillii* are less visible to predators in open water and do not need to hide in a shelter. Another explanation could be that *P. gillii* protected themselves from predation by schooling instead of hiding in the shelter. This would be consistent with Botham's (2008) finding that a fish of the *Poecilia* genus exhibited tighter schooling in response to predation than without predation.

P. gillii spent significantly less time than *P. elongata* in the shelter without predation, implying that there may be an inherent species difference in shelter usage. *P. gillii* may be preyed upon by larger fish (Bussing 1998). Some fish species are known to avoid shelter because of the likelihood of the presence of larger, possibly predatory fish in shelters (Rahel and Stein 1988). This could explain the

aversion of *P. gillii*, and not *P. elongata*, to the shelter.

P. elongata may have learned where the shelter was; once they found the shelter, they quickly returned to it in subsequent trials. Although we randomized the individual fish used, we used fish for multiple trials due to limited numbers.

P. gillii and *P. elongata* can continue foraging in the presence of a predator when they form schools. Because both of these species show a number-dependent schooling response to predation, our findings may give insight into phenomena that are ubiquitous to schooling fish and other species that exhibit group behaviors.

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AGGRESSION DIFFERENCES BETWEEN SEXES IN NORTHERN JACANAS (*JACANA SPINOSA*)

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Abstract: The reproductive success of some organisms depends on their ability to defend nesting territory. Northern Jacanas have a polyandrous social structure where males defend chicks and females defend harems of adult males. We observed the behaviors of Northern Jacanas and categorized their behaviors by sex. One behavior we observed was wing flashing, a behavior particular to Jacanas. Females exhibited this behavior more often than males, suggesting that these wing flashes may be a signal of territorial aggression. Our data suggest that female Jacanas are more aggressive than males, and support a female-dominated social structure in Jacanas.

Key words: Northern Jacana, polyandry, aggression

INTRODUCTION

One of the guiding tenants of sexual selection studies is Bateman's Principle, which states that eggs are physiologically expensive to produce while sperm is comparatively cheap (Bateman 1948). Therefore, the limiting factor in a female's fitness is how many eggs she can create, whereas for a male the limiting factor is how many mates he can access. A female should thus be choosy, only allowing her eggs to be fertilized by preferred mates, while males should be promiscuous, seeking to mate with as many females as possible. One result of this principle is male territoriality where the strongest male augments his fitness by controlling a harem of females.

As a sex-role reversed case of the Bateman Principle, *Jacana spinosa* (Northern Jacana) are polyandrous; males perform most of the parental care, including nest building and egg incubation (Jenni 1983). Females produce clutches of eggs with one to four males and provide replacement clutches after predator attacks (Betts and Jenni 1991). Females defend large territories composed of smaller plots that males use for breeding and foraging.

Both females and males have carpal spurs and bright feathers, which are revealed during wing flashing, suggesting that this behavior is an aggressive signal (Rand 1954).

We hypothesized that because females must defend their mates from conspecific females, they should exhibit more aggressive behaviors than males.

METHODS

This study focused on *J. spinosa* behavior during the dry season in the marsh habitat at Palo Verde National Park, Costa Rica on 10-11 January 2011. We observed 14 hours of *J. spinosa* behavior from a 10-meter observation tower at the southern edge of the marsh and from a 60-meter boardwalk extending into the marsh. We spent the majority of the time observing from the boardwalk because it offered an extensive view of the marsh and the Jacanas. We haphazardly chose a focal bird and observed each bird for 10 minutes, recording every observed wing-flash. At one-minute intervals, we recorded general behavior (e.g., aggressive vs. non-aggressive), distance to the nearest conspecific (juvenile or adult), and the number of conspecifics within a 10-foot ra-

dius. Behavioral categories were divided into chasing, foraging, preening, scanning, social, and traveling. Social behavior was defined as any non-aggressive interactions between jacanas. Sex was identified by relative size, given that females are 60% larger (Jenni 1983).

We compared aggressive and non-aggressive behavior by sex. Foraging, preening, socializing, and traveling were considered non-aggressive, while chasing and scanning were deemed aggressive. We noted whether aggressive behavior was offensive or defensive, e.g. chasing and being chased. We compared aggressive behavior between sexes using a Chi Square test, excluding birds whose sex was indeterminate. We also recorded wing-flashing as an aggressive behavior but analyzed it separately because these data were recorded continuously. We compared the mean number of wing flashes between the sexes using a t-test. Statistical analyses were performed in JMP 8.0.

RESULTS

We found that Northern Jacana females display aggressive behaviors significantly more often than males ($\chi^2 = 18.687$, $df = 1$, $P < 0.0001$; Table 1). We also found that females had a marginally significant greater mean number of wing flashes per bird per 10 minutes ($t_{257} = 1.97$, $P = 0.07$; Fig. 1).

TABLE 1. Chi Square test illustrating Northern Jacana females exhibit more aggressive behavior than males in Palo Verde National Park, Costa Rica. The top number is the observed behaviors and the bottom number is expected behaviors.

	Aggressive	Non-Aggressive	Sum
F	45 28.4576	116 132.542	161
M	28 44.5424	224 207.458	252
Sum	73	340	413

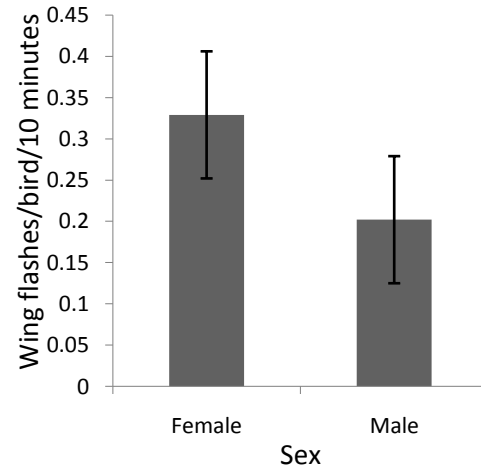


Fig 1. The relationship between sex and mean number of wing flashes per bird per 10 minutes in Northern Jacanas at Palo Verde National Park, Costa Rica. Error bars illustrate one standard error from the mean.

DISCUSSION

Our results suggest that female jacanas spend more time than males behaving aggressively, including scanning, chasing, and wing flashing. These results are expected given that males defend their smaller foraging and breeding territories against other males, while females defend the territories of all their mates (Jenni 1983).

Possible confounding factors in our study include bias or inconsistency in determining the sex of the Jacanas. While we tried to sex solely based on size, we were aware that females tend to be more territorial than males and this may have introduced bias. However, to ensure we did not sex incorrectly, we disregarded birds of ambiguous sex in our data analysis.

Jacanas exemplify a role reversal of the Bateman principle. Jacana eggs are heavily preyed upon; thus, reproduction is limited by egg predation rather than production (Jenni 1983). As a result, female Jacanas mate with and produce eggs from as many males as possible. Males guard their clutch of eggs, so the more clutches and male protectors a female has, the better chance some eggs will

survive predation. This supports our hypothesis that females are more aggressive in an effort to defend their valuable mates.

Since female Jacanas do not have to invest energy into raising young, our study demonstrates they instead invest that energy into aggressive and territorial behaviors. Further studies may specifically investigate how females allocate energy between territoriality, resource acquisition, and egg production to optimize fitness.

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DIFFERENCES IN CAPUCHIN LOCOMOTION BETWEEN SEXES IN RESPONSE TO PREDATION

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Faculty Editor: Ryan G. Calsbeek

Abstract: Group living in primates, as well as other vertebrates, is thought to have evolved to reduce individual predation risk. Primates may have developed differential roles between the sexes to protect the group from predation. The well-studied Old World primates display distinct roles between the sexes. However, sexually distinct behavior is not as well understood in the white-faced capuchin (*Cebus capucinus*), a New World monkey. We hypothesized that in response to predation, males would actively defend the group in return for status within the troop and reproductive success, while females would be more passive. We observed the movement of capuchin monkeys with and without a simulated threat using audio playback experiments. In general, males remained closer to the ground than females. In response to a capuchin alarm call, males jumped more, while females moved to higher levels of the canopy. Despite similar general behaviors between the sexes, males served the protective role in the troop.

Key words: *Cebus capucinus*, predator response, audio cues, kin protection

INTRODUCTION

Many animal species have adapted defense mechanisms to predation. In some species, males and females have evolved different responses to predators, often based on sexual dimorphism in size, behavior, activity level, or role in offspring protection (Shine et al. 2000).

Alarm calls are one response that animals have evolved to alert others to the presence of a predator. White-faced capuchin (*Cebus capucinus*) alarm calls fall into two distinct categories—one to alert others to the presence of aerial predators and other monkeys, and one for terrestrial predators and snakes (Fichtel et al. 2005). Wheeler (2008) found that alarm calls in response to visual models of predators are motivated by parental care and kin protection, with the intention of recruiting individuals to mob the predator. Treves (1999) found that sound playback experiments using predator vocalizations were effective at inducing a response from arboreal primates.

Male capuchins are about 1.5 times larger than females but like other New World mon-

keys they display few other sexually dimorphic traits (Gebo 1992; Fedigan and Baxter 1984). Gebo found only very slight differences in general posture and locomotion between male and female capuchins (1992).

Van Schaik and van Noordwijk (1989) observed that male capuchins display higher rates of vigilance in the presence of predators. They are also more active than females in detecting, approaching, and mobbing real and model predators. We examine sex-specific responses to predation by male and female white-faced capuchins, a species in which males exhibit dominance and display more aggressive behavior than females (Reid 1997).

We hypothesized that male capuchins, as the dominant members of the group, would actively defend the troop in the presence of threats simulated by audio recordings of capuchin alarm calls and of predators. We predicted male defensive behavior would include more active movement, such as jumping and remaining closer to the ground to scan for predators. Females, by contrast, would act more cautiously by moving higher

in the more protected canopy. We also predicted that in response to the sound of a terrestrial predator or an alarm call to a terrestrial threat, both sexes would spend more time higher in the trees to avoid predation.

METHODS

We followed two troops of 15-20 white-faced capuchins on 13 and 14 January 2011 in Palo Verde National Park, Costa Rica. We recorded baseline observational data on 13 January and conducted experimental trials on 14 January.

We haphazardly selected a focal individual and observed it for 300 seconds ($n=132$), noting sex, age (juvenile or adult), horizontal travel (number of trees traveled through per trial), and total number of jumps. Every 30 seconds, we also recorded approximate height above the ground (ground level=0, 1-5m=1, 5-10m=2, 10-15m=3).

We used an iPod Nano (Apple, Inc.) and Tweakers portable speakers (YC Cable, SPKR-R1-BK-D01) to playback three distinct recordings (Emmons et al. 1997). We controlled for a sound response with a recording of a guayaquil squirrel (*Sciurus stramineus*), which is not a predator. We simulated a predator call with a jaguar (*Panther onca*) recording (Digweed et al. 2005). We simulated a threat to the troop with a capuchin alarm call.

We conducted one-sided two-sample t-tests by sex, comparing total number of jumps and rate of horizontal movement per five minutes for the observational group. For the experimental group, we compared height in canopy, total number of jumps, rate of horizontal movement against sex for each treatment: guayaquil squirrel, jaguar, and a capuchin alarm call.

We used JMP 8.0 to perform ANCOVA and t-tests.

RESULTS

Females were significantly higher in the canopy than males ($t_{677} = -4.92$, $P < 0.01$; Fig. 1). Overall, males did not jump significantly more than females ($t_{677} = 0.90$, $P = 0.81$), but males jumped more than females in response to a capuchin alarm call (ANCOVA, Treatment x Sex: $F_{3,56} = 5.58$, $P < 0.01$; Sex: $F_{1,56} = 0.99$, $P = 0.33$; Treatment: $F_{3,56} = 3.52$, $P = 0.04$; Fig. 2). Both males and females moved higher into the canopy in response to capuchin alarm calls and jaguar calls (Sex: $F_{1,1} = 10.26$, $P < 0.01$; Treatment: $F_{2,2} = 20.45$, $P < 0.01$; Fig. 3). Males remained significantly lower than females in response to the capuchin call ($t_{115.17} = -3.97$, $P < 0.01$).

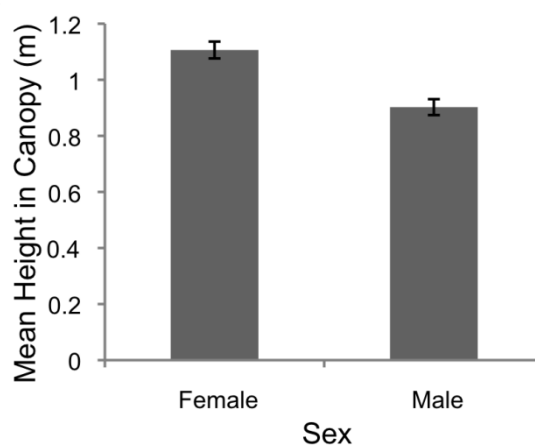


Fig. 1 The height in canopy (m) (means \pm 1 S. E.) of *C. capucinus* by sex estimated in 5-minute observational trials at Palo Verde National Park, Costa Rica. Height in canopy was estimated on an ordinal scale: 0=ground level, 1=1-5m above ground, 2=6-10m, 3=11-15m.

DISCUSSION

Our data support the hypothesis that male capuchins exhibited more locomotive and postural behavior consistent with troop defense than females. Males spent significantly more time lower to the ground than females (Fig. 1), as Fragaszy (1990) observed. Two primary predators of capuchins, jaguars and large snakes, live on the ground. Being close to the ground is dangerous for capuchins, yet

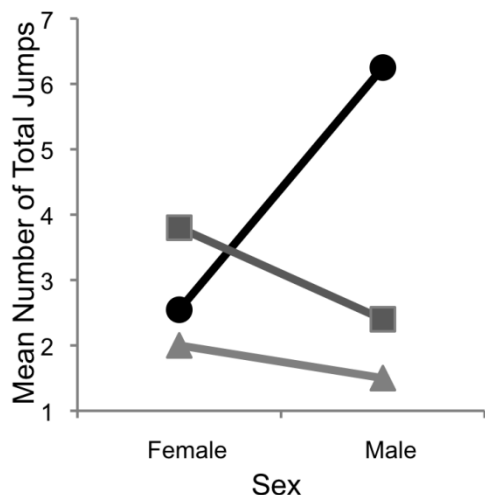


Fig. 2 Mean total jumps per 5-minute observational trial by sex of *C. capucinus* in response to simulated audio cues of a capuchin alarm call (black circles), a jaguar call (dark grey squares), and a squirrel recording (light grey triangles) at Palo Verde National Park, Costa Rica.

they must be close enough to discern predators. Because males spend more time lower to the ground than females, they are more likely to be looking for predators and protecting the troop. Being high in the canopy, by contrast, provides more protection from terrestrial predators (Wright 1998). Thus, females may be able to stay in a safer area higher in the canopy because they are protected by the more vigilant males.

Our data also support the hypothesis that males defend the troop more actively against predation, while females take a more passive approach to defense. In response to a capuchin alarm call, male monkeys jumped more often (Fig. 2), whereas females moved even higher in the canopy (Fig. 3). A capuchin alarm call signifies a predator or other threat, and our data suggest that male monkeys must respond by evaluating this threat. Jumping, while energetically costly, allows males to move around quickly. This enables them to be more aware of the environment, predators, and potential threats. Several males also issued a loud call in response to the capuchin

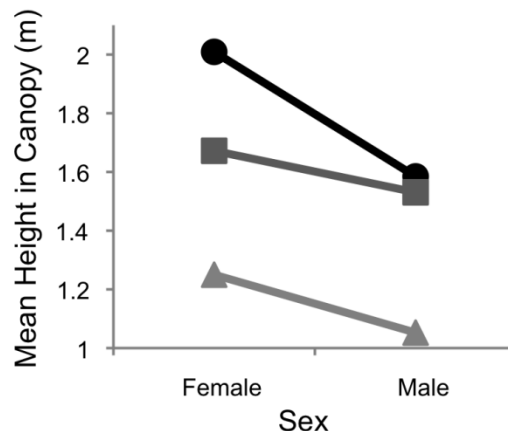


Fig. 3 Mean height in canopy (m) in 5-minute observational trials by sex of *C. capucinus* in response to simulated audio cues of a capuchin alarm call (black circles), a jaguar call (dark grey squares), and a squirrel recording (light grey triangles) at Palo Verde National Park, Costa Rica.

alarm call, which did not occur in response to the squirrel or jaguar calls.

One potential source of error is the recordings used in our experiment. Our portable speakers had limited volume, so only monkeys in the vicinity could hear the sounds. Also we did not know precisely which type of capuchin alarm call we used, e.g. for terrestrial predators, humans, or other monkeys (Fichtel et al 2005).

Defending the troop is dangerous for the males, as they are closer to the ground and more vulnerable to predators. Males may choose to accept that danger if protecting the troop gives them more social status and attracts potential mates. Many of the data we collected were not significantly different between males and females, suggesting that the two sexes exhibit similar behavior overall. Fedigan (1984) concluded that there are minimal sexually distinct behaviors in capuchins, yet our findings show a clear divergence in protective behavior between males and females, revealing a more nuanced view of the social structure of capuchins.

Future studies could compare simulated predation with combinations of audio cues and visual cues, to determine which aspects of predation evoke a response from capuchins. Degree and type of threat from different predators, such as aerial versus terrestrial, could also elicit distinct responses for males and females. Examining other protective behaviors, such as scanning and issuing alarm calls, would give further insight into how protecting the troop is partitioned between the sexes.

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AGGREGATION BEHAVIOR IN COSTA RICAN DRAGONFLIES WITH PROXIMITY TO WATER

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Abstract: Social organisms periodically aggregate in multi-species and mixed sex groups to maximize rates of resource acquisition and social facilitation. We studied the species composition and distribution, as well as the sex ratio of three distinct dragonfly swarms in a tropical marsh environment. We hypothesized that dragonfly swarming behavior is affected by resources, such as prey availability and water proximity. We did not find differences in prey abundance at dragonfly swarm sites. We found that the species composition and sex ratio within a swarm differs with proximity to water, an important resource for breeding. Finally, we found that females are more abundant in areas closest to water. Further studies on dragonflies could expand our understanding of the mechanisms that drive insect species distribution, biodiversity, and social behaviors.

Key words: swarming, sex ratio, resource availability, species evenness, species richness

INTRODUCTION

Many species exhibit grouping behavior in response to uneven resource distribution or to enhance social interactions, such as mate location and predator swamping.

Dragonflies (Suborder: Anisoptera) are common insects that exhibit complex social grouping. Though they spend most of their lives as aquatic larvae, they emerge as flying adults for a few weeks to mate. Following mating, females fly to a nearby body of water and oviposit on the water surface.

Previous studies suggest that dragonfly swarming locations and behavior are related to resource availability, including proximity to water. For example, the dragonfly *Perithemis tenera* exhibits territoriality over resources, occupying waterfront vegetation first (Switzer 2002). This suggests that areas closer to the water are higher quality sites. Switzer also suggests that higher competition is present at the more desirable sites closer to the water, as more intraspecific aggression has been observed in those sites (2002).

Studies have also suggested an inherent male bias present in swarms close to water. Hamilton and Montgomerie (1989) found that

neotropical swarms near streams can be up to 87% male. This may be due to the longer time larval females spend hunting in order to obtain the necessary nutrients to produce eggs. Feeding is a risky behavior for larval females because hunting requires movement in the water, which increases their exposure to predators (Hamilton and Montgomerie 1989). Campanella (1975) found that male dragonflies arrived at water sites before females and outnumber females at all times of day. Because females oviposit in the water, males might increase reproductive success by aggregating near the water.

We hypothesized that proximity to water affects species and sex ratio within a dragonfly swarm. We predicted that swarms closer to the water would have a higher number of males because water is an important resource for reproduction. We also predicted lower species diversity closer to water because competitive species drive out other species to less preferable sites farther from the water.

METHODS

We examined dragonfly aggregations at Palo Verde Biological Station, Costa Rica. We

collected dragonflies along a road transect in the morning and afternoon on 13-14 January 2011. We identified swarms in three sites hereafter referred to as A, B, and C. At least 100 dragonflies were flying in close proximity at each site. Site A was 70 m from the marsh, site B was 2 m from the marsh, and site C was within the marsh, 1 m from shore. We also collected solitary dragonflies at varying distances from the marsh. After the dragonflies were collected, we identified the sex and species (Haber 2010 and Roberson 2007).

On the morning of the first day, we captured, photographed, and marked 20 dragonflies from each site by writing an indelible number on each wing for mark and recapture. We also measured total body length. In the afternoon, we captured 98 additional dragonflies from site C because the swarm was larger and denser than the others. We used the Lincoln Index to estimate the population size of each swarm. We used EcoSim™ to rarify our data (to compensate for smaller sample sizes at sites A and B) and then calculated species abundance.

To test the hypothesis that swarming behavior is driven by preference for a specific site, we transplanted a 99-dragonfly swarm sample from site C 25 m away from its original site. We tracked the dragonflies by marking their abdomens with DayGlo™. We chose a release site that had similar resources to control for environmental variables. We noted the percentage of marked dragonflies at the release site every 30 s for 10 min, and qualitatively observed their behavior.

The following day, we repeated the transplant experiment with 20 dragonflies from site A. The swarm was diminished from the previous day (possibly due to poor weather conditions). The swarm at site B was diminished to a level where collection and release was not possible.

To survey prey availability, we measured the density of small insects at sites A, B, and C, and at three respective control sites with similar vegetation but without dragonfly swarms. We set up three 8.5 x 11 in pieces of paper coated in Tanglefoot™ insect trap at each site. The traps were set out at 10 am and collected at 5 pm.

RESULTS

For the mark-recapture portion of our study, we recaptured one dragonfly from site C. Using the Lincoln Index, we estimate the size of the swarm at site C to contain approximately 2000 individuals. However this approximation may be inaccurate because only one dragonfly was recaptured.

During the transplant experiment at site C, we observed that ~50% of the dragonflies stayed at the release site 10 minutes after release. At site A, all 20 dragonflies dispersed into the surrounding area, though none were identified at the original site.

We rarefied samples to compare relative species richness between sites. Site B was the richest with a calculated 5.56 species per 16 individuals captured, followed by site C with 4.02 species and site A with 2.45 species (Fig. 1). We also calculated Shannon-Wiener indic-

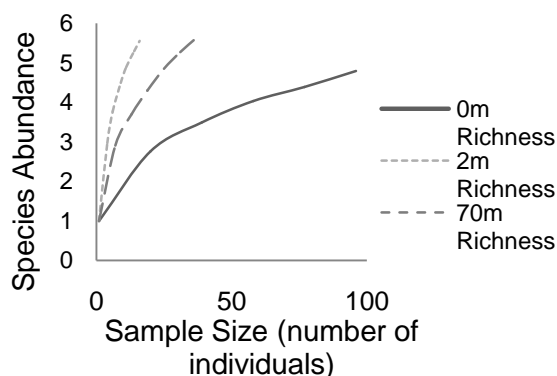


Fig 1. Species abundance data for dragonflies captured at three sites of different distances (0 m, 2 m, and 70 m) from a marsh in Palo Verde, Costa Rica. Data were rarefied through EcoSim™ to compensate for uneven sample sizes at different sites.

es for all three sites. Site A had the lowest diversity with an index value of 0.504, followed by site C with a value of 1.416 and site B with a value of 1.625. Lastly, we created a rank-abundance curve of species across all sites (Fig. 2).

There was no significant difference between mean sex ratios at different distances from water (ANOVA $F_{1, 2} = 4.72$, $P = 0.16$). However, there was a suggestive positive trend indicating a male-biased sex ratio further from the water. Despite a nonsignificant linear regression, there was a positive relationship between proportion of males in a

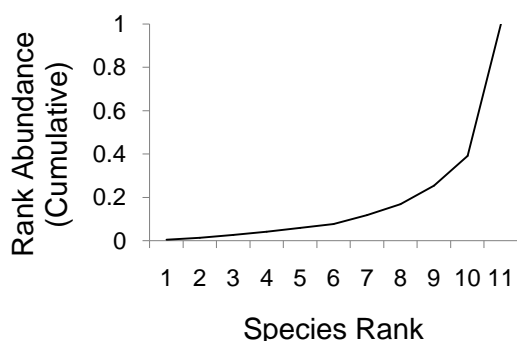


Fig 2. Cumulative species rank abundance curve for dragonflies captured at three sites of different distances from the water in Palo Verde, Costa Rica.

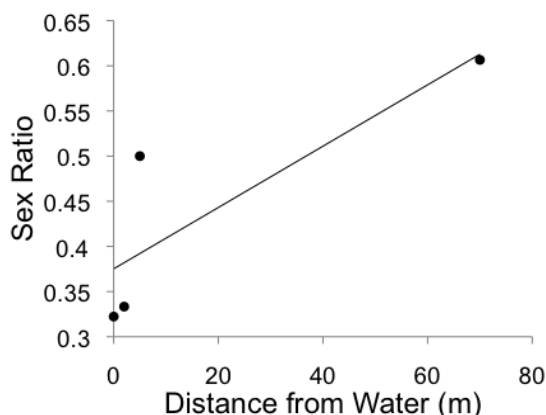


Figure 3. Linear regression of sex ratio (number of males/total number in a swarm) by distance from water of dragonflies captured around a marsh in Palo Verde, Costa Rica.

swarm and distance from water ($r^2_{1, 2} = 0.70$, $P = 0.16$; Fig. 3). Time of day was not significant (2-way ANOVA, $F_{1, 2} = 1.7899$, $P = 0.65$) and was removed from our final analysis. We found no significant differences in the number of prey-sized insects collected between any of the sites or controls captured by the Tanglefoot™ ($F_{12, 15} = 1.22$, $P = 0.36$).

DISCUSSION

Our data support the hypothesis that proximity to water may influence the composition of dragonfly swarms. Although our findings were not significant, they suggest a relationship between distance to water and the sex ratio within a swarm; as distance from water decreases, the proportion of females increases. This highly female-biased sex ratio could be explained by the fact that female dragonflies must deposit their eggs in water; consequently, they may be found in higher concentrations closest to the marsh after mating. Although females migrate to the water to oviposit, males may avoid open water to decrease their susceptibility to potential predators. However, the greater proportion of females caught at the sites closer to the water is surprising, given the highly male-biased sex ratio found in past studies (Hamilton and Montgomerie 1989).

We also found differences in species diversity within swarms at different distances from water. We found that one species, the Red Mantled Dragonlet (*Erthrodiplox fervidia*), was more abundant than other species at the site closest to the water, suggesting that this species was the best competitor. However, we also noticed that it was one of the smallest species ($\bar{x} = 36.13$ mm). As a smaller dragonfly, the Red Mantled Dragonlet may have physiological advantages for evading predators or outcompeting other dragonfly species. Further studies could examine how dragonfly

foraging strategies and defensive mechanisms relate to interspecific competition.

Our study does not support the idea that resource availability alone controls dragonfly swarming behavior. We found no differences between insect abundances across the swarm and control sites, suggesting that dragonflies do not congregate based on prey availability. When dragonflies were released 25 m away from their sites in similar habitat, they did not return immediately, suggesting that dragonfly swarming behavior may be more dependent on water availability or social behavior than specific site preference.

We found very few dragonflies on the open field and road between 10 m and 60 m from water. The marsh shore 0-5 m from the water and the tropical dry forest 70 m from the water, where we found dragonfly aggregations, were heavily vegetated. We noted that the marsh and the open fields between the marsh and forest were subject to continuous high winds during the day (pers. obs.). It is energetically expensive for small insects to fly in high winds, so dragonflies may congregate out of the wind (Ellington 1984). By contrast, the heavy vegetation at dragonfly swarm sites 0 m, 2 m, and 70 m from the water sheltered the sites from the wind. An investigation of the relationship between swarms and other environmental factors, such as wind, may also be important in understanding social grouping behavior.

Our study demonstrates that resource availability alone does not determine the sex and species composition of dragonfly swarms; however, proximity to water may be an influential factor. Further studies could investigate alternate mechanisms that drive swarm formation, including differences in body size with respect to species composition and social hierarchy.

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MORPHOLOGICAL CHARACTERISTICS AND PHENOTYPIC PLASTICITY IN *EICHHORNIA CRASSIPES*

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Abstract: Invasive species reduce biodiversity and harm native communities through rapid growth and adaptation. The common water hyacinth, *Eichhornia crassipes*, is a virulent invasive species throughout North America. Several characteristics, including a high degree of phenotypic plasticity, allow it to colonize a wide variety of environments and outcompete many native species. In this study, we examined the relationship between water depth and phenotypic development and predicted that plants would become shorter and that their leaves would become smaller as water depth increased. We found morphological trends precisely opposite of those we predicted. Surprisingly water depth decreased as distance from shore increased. We reject our original hypothesis and conclude that many factors may play roles in the trends observed.

Key words: Water hyacinths, phenotypic plasticity, invasive plants, light availability

INTRODUCTION

Invasive species are often characterized by high biomass production, reproductive rates, and high adaptability (Penn. DCNR 2010). Because of this, they pose a major threat to ecological communities and human societies worldwide. They often reduce biodiversity and shift ecological relationships by preying on and outcompeting native species. They can also threaten economically important species or cause direct damage to human infrastructure.

Common water hyacinth (*Eichhornia crassipes*) is among the most virulent invasive species in North America, Central America, Africa, Europe, and Australia. Native to South America, water hyacinth was introduced to other continents after humans imported it as an ornamental plant (Barrett 1983). Water hyacinth forms vast free-floating mats at astonishing rates, producing as much as 22.0 g/m² organic matter per day (Pieterse 1978). The plant can reproduce sexually, ei-

ther by outcrossing or autogamous fertilization, or clonally (Barrett 1983).

Perhaps the most striking characteristic of the water hyacinth is its well-documented phenotypic plasticity. *E. crassipes* individuals develop into two distinct forms. Close to shore, individuals tend to be tightly packed with tall, narrow petioles. Farther from shore, individuals are more widely dispersed, squat, have smaller leaves, and have bulbous petioles (Barrett 1983). Light intensity, population density, plant hormone levels, temperature, and rooting have all been correlated with the development of different phenotypes (Pieterse 1978).

In general, depth is an important factor in determining distribution and success of aquatic plants (Xiao et al 2007). The intent of this study was to examine the hypothesis that depth is the dominant driving force behind phenotypic development in *E. crassipes*. Because they cannot root in soil to achieve stability, we predicted that plants in deeper water would have shorter profiles, smaller leaves, and bulbous petioles in order to float.

Moreover, we predicted that plants in shallower water would be more resistant to desiccation because periodic water level changes (e.g., tidal patterns, wet vs. dry year variation) would strand them out of water. Finally, as plants with small leaves have been shown to have higher respiration rates (Davies 1998), we predicted that hyacinths small leaves would use more dissolved oxygen in the dark.

METHODS

We performed our study of *E. crassipes* phenotypic variation in the marsh at Palo Verde National Park, Guanacaste Province, Costa Rica on 14-15 January 2011. We measured a 65 m transect along a boardwalk extending into the marsh. Every five meters, we measured water depth and haphazardly selected ten plants. One leaf and one petiole from each plant were selected haphazardly. We then bisected each petiole into two parts, the bulbous section and the narrow section. The volume of the bulbous part was measured by water displacement. We measured length of the narrow section, leaf area, bulbous part wet mass, leaf wet mass, narrow part wet mass, and leaf dry mass. The leaves were dried in perforated tinfoil packets in the sun.

To test whether desiccation resistance differed between morphological types, we selected 20 representative samples of the two most extreme types we observed: thin, tall petioles with large leaves (Type 1) and bulbous, short petioles with small leaves (Type 2). Samples were selected from areas in which they were dominant; Type 1 samples from the 0 m mark, near the shore, and Type 2 samples from the 65 m mark. These samples were laid out in the sun, and the number of live leaves on each plant was recorded. Leaves became visibly papery and brittle as

desiccation proceeded. Every 30 minutes, we counted the number of non-desiccated leaves, partially desiccated leaves, and completely desiccated leaves on each plant.

To measure respiration, two tanks of marsh water were constructed in the lab and initial dissolved oxygen (DO) concentrations were recorded using a YSI Professional Optical Dissolved Oxygen instrument. A Type 1 plant was placed in one tank, and a Type 2 plant was placed in the other tank simultaneously. Tanks were then covered to prevent oxygen loss, and water volume was kept constant. After 10 minutes, the DO was measured again in both tanks. Fifteen plants of each morphotype were examined.

Analysis

The ratio of the volume (mL) of the petiole's bulbous part to the length (cm) of the petiole's narrow part was used to differentiate petiole morphologies. We performed two different regressions to test whether this ratio was correlated with distance from shore, and to test whether variation in morphotype fluctuated with distance to shore. We also performed regressions of leaf size, specific leaf area (SLA), and leaf dry matter content (LDMC) versus distance from shore. We used a one-sample t-test to determine whether the mean percent change in O₂ (corrected for mass) was different for Type 1 and Type 2 morphotypes. Finally, we used ANOVA to test for differences in Type 1 and Type 2 desiccation over time. All statistical analyses were performed using JMP 8.0.

RESULTS

Water depth was negatively correlated with distance from shore in our transect ($r^2_{1, 12} = 0.72$, $P < 0.0001$; Fig. 1).

There was a significant relationship between the ratio of the bulbous petiole volume to narrow petiole length and the distance

from shore ($F_{1, 12} = 28.9$, $P = 0.0002$; Fig. 2), indicating that the farther from shore a plant was, the larger the bulbous part was relative to the narrow part.

We found a negative relationship between size of leaf and distance from shore ($F_{1, 12} = 18.9$, $P = 0.0009$; Fig. 3). There was no relationship between SLA and distance from shore ($F_{1, 12} = 1.08$, $P = 0.32$) but a positive trend between LDMC and distance from shore ($F_{1, 12} = 2.44$, $P = 0.14$; Fig. 4).

The means of percent of oxygen depleted (corrected for mass) by Type 1 and Type 2 plants were significantly different ($t_{14.3} = -2.42$, $P = 0.03$; Fig. 5). Type 1 plants had the least negative mean, indicating that they depleted less oxygen during respiration trials.

We did not measure any significant difference between desiccation rates of Type 1 or Type 2 plants (repeated measures ANOVA: $F_{7, 32} = 0.07$, $P = 0.95$).

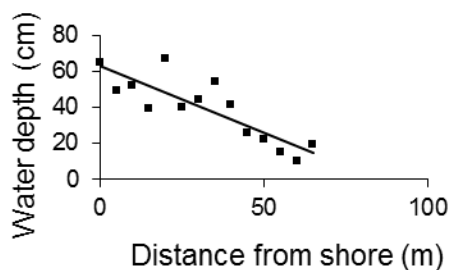


Fig. 1 Relationship between water depth and distance from shore in a marsh in Palo Verde, Costa Rica.

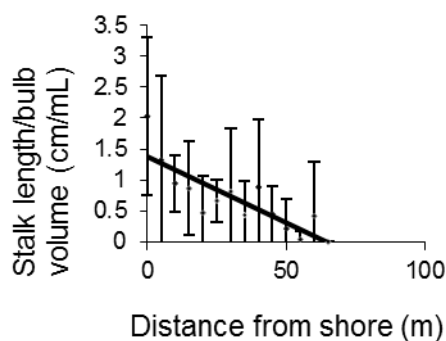


Fig. 2 Relationship between the ratio of stalk length to bulb volume in water hyacinths and distance from shore in Palo Verde, Costa Rica.

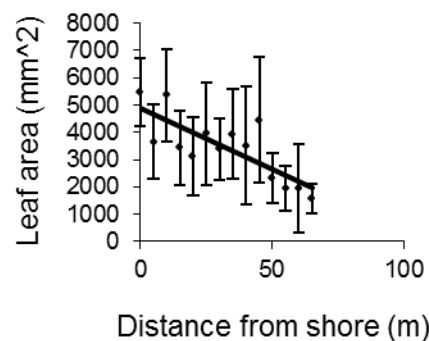


Fig. 3 Relationship between water hyacinth leaf area and distance from shore in a marsh in Palo Verde, Costa Rica.

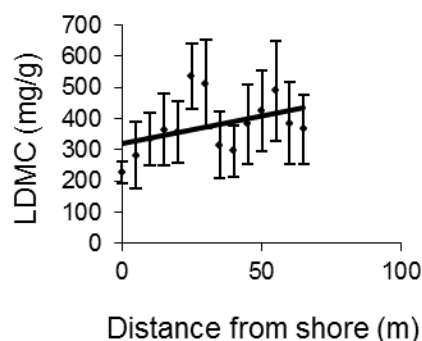


Fig. 4 Trend between water hyacinth leaf dry matter content (LDMC) and distance from shore in Palo Verde, Costa Rica.

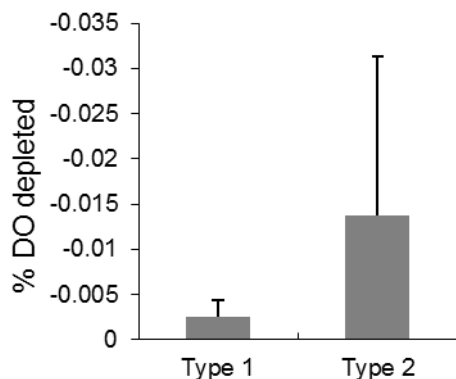


Fig. 5 Mean dissolved oxygen depletion between Type 1 and Type 2 water hyacinth plants in a dark respiration experiment. Type 1 plants had thin, tall petioles with large leaves, while Type 2 plants had bulbous, short petioles with small leaves. Water hyacinths were collected from two sites (near shore for Type 1 and far from shore for Type 2) in a marsh in Palo Verde, Costa Rica.

DISCUSSION

We found a number of significant relationships between water hyacinth morphology and depth. Plants in deeper water had taller profiles, larger leaves, lower respiration rate per gram, and narrower petioles. These results fail to support both of our original hypotheses, that plants in deeper water would have shorter profiles, smaller leaves, and bulbous petioles, and the null hypothesis that there would be no relationship between morphology and depth. However, the depth gradient measured in our study was precisely the opposite of what we expected, with shallower water farther from shore. It is possible that sediment buildup alongside the boardwalk where we gathered our plants created this atypical depth gradient.

The trends in morphology that we saw were typical of other studies that measured morphology in relation to distance from shore (Barrett 1983), but our depth gradient was not. Therefore, we tentatively conclude

that depth is not the driving force behind phenotypic development in *E. crassipes*.

Although our original intention was to examine the correlation between depth and morphology, we found trends that could be related to a variety of other environmental factors. Variation in morphological characteristics in water hyacinth may be related to light availability, as suggested by the trends we found in leaf size and dark respiration. In general, plants in shaded areas grow large, thin leaves to collect light. Because such plants receive less light, they also respire more slowly, conserving their stored energy (Muraoka et al. 2000). However, if light availability is determined by shoreline vegetation, we would expect to see distinctly different morphologies in the sun and shade, where instead we saw a gradient of morphological change. It is possible that light availability and quality are determined not by shore vegetation but by the density of surrounding adult plants. Richards and Lee (1986) found that Type 1 morphologies could be induced in hyacinths by growing clones in the light spectrum found in the midst of a dense Type 1 adult population. Further research could examine the relative contribution of light intensity and spectrum to phenotypic development in water hyacinth.

An alternative explanation for the trends we found is that plants in open water (Type 2) were exposed to stronger wind and water currents and were less well supported by surrounding plants. As a result, they may have developed morphological characteristics to increase stability. Plants found near shore (Type 1) may be more stable and invest more in leaf material than in stabilizing morphologies. In Type 2 plants, we found relatively large floating bladders and short profiles, which could help them to stay upright. From personal observation, however, there

were no perceptible currents in the marsh, and the wind seemed constant over most of our transect. If wind and current were significant factors, we would expect to see a distinct change between morphologies where we saw a smooth gradient. Future studies could examine this idea further.

Finally, it is possible that all of these factors interact in the life history of the water hyacinth. Perhaps dense plants that support each other, root in soil, and create shade produce new plants adapted to low light and high stability. By contrast, high light availability, low stability, disturbance, and the inability to root in soil may induce morphologies adapted for those conditions. To better understand what drives water hyacinth phenotypes, future studies should synthesize the effects of light, depth, and disturbance.

Because the water hyacinth is such an adaptable plant, very few things limit its development. Understanding the driving forces behind hyacinth morphology might illuminate the factors limiting hyacinth growth and aid in finding a way to control it as an invasive species.

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ISLAND BIOGEOGRAPHY AND INTERMEDIATE DISTURBANCE IN BROMELIAD IN- VERTEBRATE COMMUNITIES

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Abstract: Island Biogeography Theory proposes that large islands close to the mainland maximize species richness and diversity. The Intermediate Disturbance Hypothesis suggests that intermediate levels of disturbance can also maximize richness and diversity. We examined the application of these models to invertebrate communities within bromeliad tanks from the cloud forest of Monteverde Biological Reserve, Costa Rica. We predicted that invertebrate feeding group diversity would increase with bromeliad size and number of proximal bromeliads. We also predicted that diversity and richness would be highest in tanks with predation disturbance. We found that feeding group richness and diversity tended to be negatively correlated with bromeliad size, whereas there was no significant trend with number of nearby bromeliads. Diversity and richness were highest in bromeliads at higher elevations with a greater presence of predators. We suggest that the Intermediate Disturbance Hypothesis may be driving diversity and richness within smaller bromeliads. Island Biogeography Theory may not be applicable at the small spatial scale that we measured, but may be relevant to larger scale patterns of bromeliad community diversity.

Key words: feeding groups, diversity, richness, predation, competition

INTRODUCTION

Island Biogeography Theory (IBT) posits that species richness and diversity are positively related to individual island size and number of proximal islands (MacArthur and Wilson 1967). The IBT has been applied to many types of islands. For example, the diversity and richness of aquatic pathogens on organic matter particles is known to increase with particle size and density (Lyons 2010).

Another model, the Intermediate Disturbance Hypothesis (IDH), suggests that diversity is maximized in the presence of intermediate levels of disturbance (Connell 1979). For example, Lepori (2006) found that small-scale sediment disturbances in riverbeds helped maintain diversity, while a large disturbance like flooding had a negative impact on aquatic community diversity. Predation, which can be a form of intermediate disturbance, has

been found to increase diversity at low trophic levels (Menge and Sutherland 1976).

These models can be studied in relation to epiphytic bromeliads (Bromeliaceae) in Costa Rican cloud forests. Bromeliads contain a tank formed by overlapping leaf bases that hold water creating semi-isolated habitats for macro-invertebrates.

Previous studies examining IBT in bromeliads have found no relationship between species richness and size of the plant or tank volume (Bizzarro 1992, Weller 2004). Traditionally, studies on invertebrate interactions examine distinct feeding groups rather than species (LeCraw and Mackereth 2010, Gerino et al. 2003). Because feeding group classifications are directly related to trophic level and resource use, feeding groups should represent diversity more comprehensively than species. Accordingly, we investigated the application of the IBT and the IDH to functional feeding groups within bromeliads.

We predicted that feeding group diversity and richness would increase with size and local (within 2m) bromeliad density, as suggested by the IBT. We also predicted that diversity and richness would be highest in tanks with intermediate levels of predation. On a larger spatial scale (the park landscape), we expected that feeding group diversity and richness would increase with increasing elevation, due to higher density of bromeliads (DiBiccari 2010).

Previous studies have found that species diversity in bromeliads has no relationship with nutrient content or detritus input (Richardson 2000; Licona et al. 2003). Accordingly, we predicted that the IBT and IDH would be more influential in feeding group diversity and richness than temporary abiotic factors such as detrital matter, light availability, pH, and temperature of the tank.

METHODS

We haphazardly sampled 25 small to medium bromeliads from zero to two meters high along trails in Monteverde National Park, Costa Rica on 20 and 21 January 2011.

We measured elevation with a Highgear® wristwatch altimeter. We measured light availability using a digital camera (E8700 Nikon Inc.) with a fisheye lens (FC-E9 0.2x Nikon Inc.) and lens adaptor (UR-E12 Nikon Inc.) and Gap Light Analyzer 2.0 software. We estimated internal diameter—the distance across the top of the inner tank—as a proxy for bromeliad size. We counted number of bromeliads within a 2m radius of the sampled plant. We measured tank water temperature (°C) with a Rayteck® Raynger® MX™ 635nm laser thermometer gun. We measured pH of the tank with Shortrange Alkacid® Fisher Scientific litmus paper.

We removed organic matter with tweezers from each bromeliad. Detritus was air-

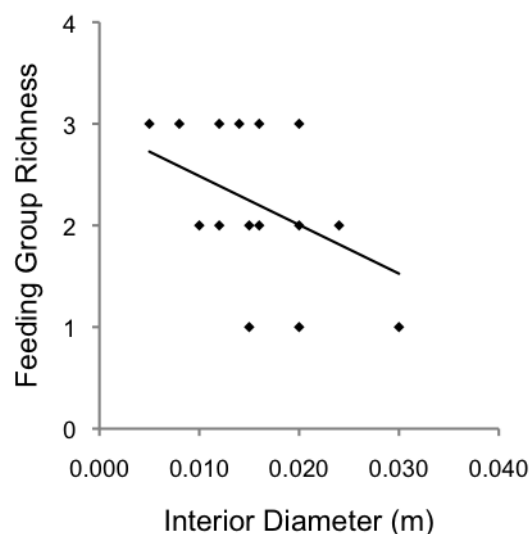


Fig. 1. Negative trend between feeding group richness of bromeliad invertebrate communities and bromeliad interior diameter in the cloud forest of Monteverde Biological Station.

dried and weighed using a digital scale (HCB 123 Adam Equipment). We irrigated each bromeliad tank with 135mL of tap water to remove aquatic invertebrates. We used a coffee filter to strain the sample into a small vial. Using a dissecting microscope (N5-64105 Wild Heerbrugg) with a 120V halogen light (V-Lux 1000 Volpi), we counted invertebrates. We classified invertebrates as shredders, collectors, filter feeders, parasites, or predators (Resh and Cardé). We measured richness as number of feeding groups and calculated diversity using the Shannon-Weiner index.

We used JMP 8.0 to perform regressions.

RESULTS

We found that feeding group richness decreased marginally with bromeliad interior diameter ($r^2_{23} = 0.15$, $P = 0.05$; Fig. 1). Feeding group diversity tended to decrease with increased bromeliad interior diameter but this result was not significant ($r^2_{23} = 0.12$, $P = 0.09$). There was no significant relationship between feeding group diversity or richness and number of bromeliads in a 2m radius ($r^2_{23} = 0.06$, $P = 0.26$; $r^2_{23} = 0.01$, $P = 0.61$).

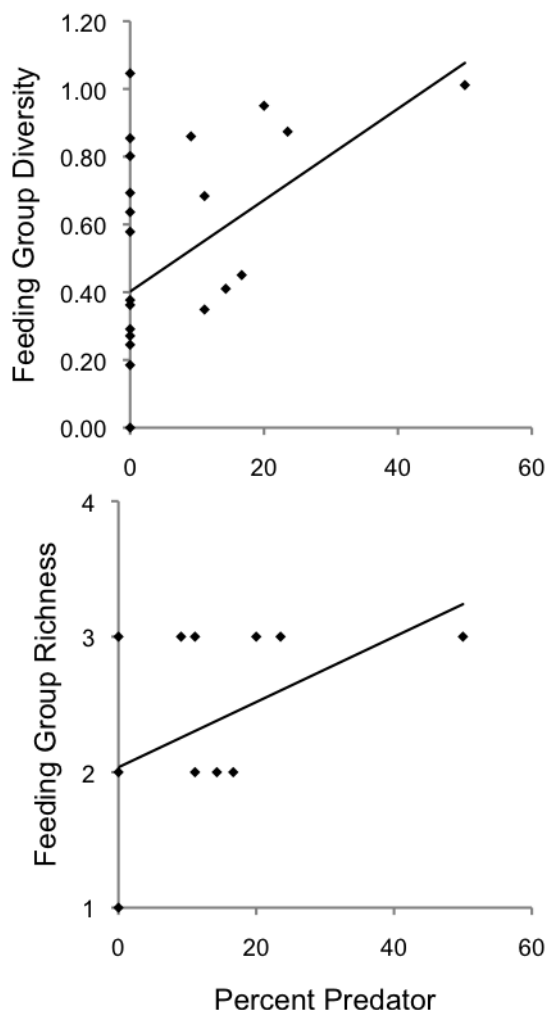


Fig 2. Feeding group diversity and richness increases with percent of invertebrates that were predators in bromeliads in the cloud forest of Monte Verde Biological Station.

We found that feeding group richness and diversity increased significantly with percent of invertebrates that were predators (richness: $r^2_{23} = 0.16$, $P = 0.05$; diversity: $r^2_{23} = 0.22$, $P = 0.02$; Fig. 2).

We also found that feeding group richness and diversity increased with elevation (richness: $r^2_{22} = 0.22$, $P = 0.02$; diversity: $r^2_{22} = 0.17$, $P = 0.04$; Fig. 3).

Finally, we found no significant relationships between feeding group richness or diversity and temperature, pH, light availability, or detrital mass.

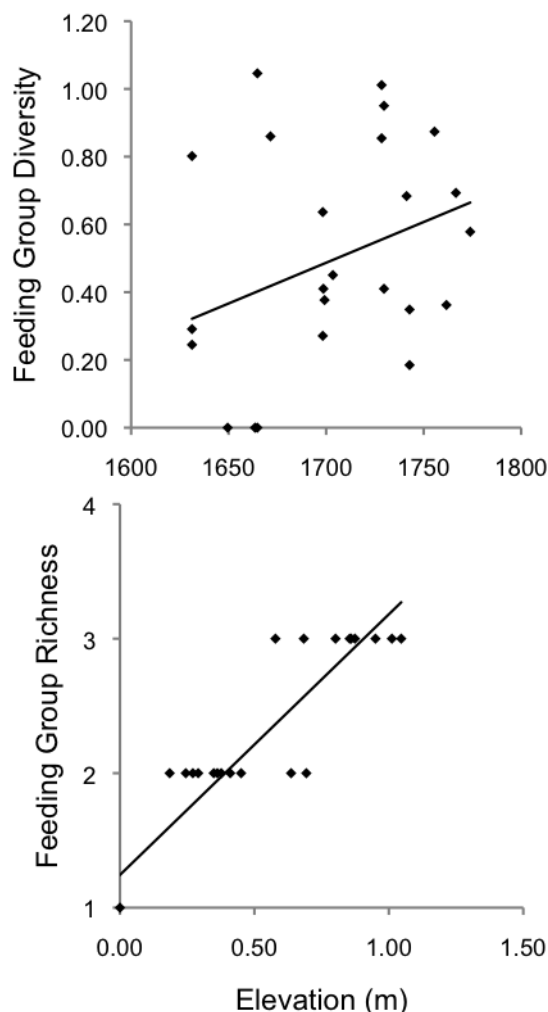


Fig. 3 Feeding group diversity and richness increases with elevation in bromeliads in the cloud forest of Monte Verde Biological Station.

DISCUSSION

We found that bromeliads with smaller inner diameters had higher feeding group richness (Fig. 1). Because this pattern is opposite our prediction, we suggest that the IBT does not explain diversity of functional groups at the spatial scale of small to medium bromeliads measured in this study. Sheffer (2006) also found that small habitat size does not imply less biodiversity. Levels of disturbance may be more influential in determining diversity and richness on a small scale. Larger tanks can maintain a more constant volume of

water, which could reduce the frequency of mild desiccation during the dry season, a possible form of intermediate disturbance. Heino et al. (2003) also demonstrated that frequent disturbances of aquatic communities at small spatial scales prevent the dominance of one species.

Additionally, feeding group richness and diversity increased with increasing percent of invertebrates that were predators (Fig. 2). By consuming prey, predators minimize the potential for competitive exclusion among these lower trophic levels and at the same time open gaps for colonization. Predation on detritivores increases the rate of nutrient cycling by reducing their emergence and nitrogen export (Ngai and Srivastava 2006). Increased nutrient cycling allows rapid turnover of feeding groups, which could maintain greater diversity.

Bromeliads at higher elevation had significantly higher invertebrate feeding group richness and diversity (Fig. 3). Richardson (1999) likewise found that diversity of bromeliad insect communities was reduced at lower elevation sites in Puerto Rican cloud forests. Since humidity increases with elevation in Monteverde (Kyker-Snowman et al. 2011), bromeliads at higher elevations are less likely to experience the large disturbance associated with complete desiccation during the dry season.

We did not find a significant relationship between feeding group diversity and number of other local bromeliads. However, the greater density of bromeliads at higher elevations (DiBiccari 2010) may be contributing to the increased diversity of feeding groups. This relationship suggests that the IBT may apply on a larger geographic scale.

Further studies could also examine how the IBT and the IDH explain changes in aquatic invertebrate communities in bromeliads of

various sizes. The IBT and IDH are important in studying biodiversity at many scales and can be applied to various systems.

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EXTINCTION AND COLONIZATION IN TANK BROMELIADS AS A MICROECOSYSTEM

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Faculty Editor: Ryan G. Calsbeek

Abstract: Island biogeography theory seeks to explain the factors that influence geographic variation in species richness. This study examines bromeliad microhabitats as islands of invertebrate communities to test components of this theory. We removed the top trophic level from bromeliads to simulate extinction and added a top trophic level to others to simulate colonization. We also propose a model to predict trophic level stratification based on island area and species composition. Our model predicted that trophic additions would cause species richness to decline, while trophic removals would cause species richness to increase. All of our empirical trials, including controls, declined in richness, which did not fit the proposed model. Bromeliads remain very useful as a study system for ecosystem ecology, and we suggest that with more research the model proposed would accurately predict species richness changes.

Key words: *Guzmania nicaraguensis*, modeling, species area, island biogeography, invertebrates

INTRODUCTION

Island biogeography is a theory first proposed by MacArthur and Wilson (1967) to explain the incredible species richness and diversity that occurs on archipelagoes. Their proposal of a species-area ratio stimulated many studies to determine if there is a carrying capacity of an ecosystem, where ecosystem ecology is largely dependent on land area.

MacArthur and Wilson's ideas have been applied across a wide variety of geographic scales, defining "islands" as isolated habitats. Deserts surrounded by lush forests, urban parks, or microcosms can all act as biological islands that can foster extinction, colonization, and speciation (Franklin et al. 2002).

We propose a method for applying island biogeography theory to examine the effects of extinction and colonization in a microhabitat, using tank bromeliads as a model system. Previous studies using bromeliads have considered multiple species of bromeliads as the same study subject (Licona et al. 2003). However, by only studying a single species, a control for morphological differences can be

maintained. Here we present data from a study of a common Costa Rican bromeliad *Guzmania nicaraguensis*, which is an epiphytic or terrestrial growing plant that collects detritus and invertebrates for its own nourishment (Morales 1999). The bromeliads also provide a complex habitat for organisms that we propose mimics the composition of larger ecosystems.

We argue that bromeliads act as isolated islands of invertebrate diversity to which island biogeography theory applies. Previous studies have tried to apply bromeliads to this theory but have been constrained by relatively small sample sizes or small bromeliads (Clare-Salzler et al. this volume, Bizarro 1992). By sampling a wide array of individuals with differing vase sizes, our goal was to achieve a more complete picture of bromeliad vase content diversity. With the consideration that each individual's pit vase acts as an island, we test effects of extinction and colonization on a relatively accessible model system (Simberloff 1974).

Because the communities inhabiting bromeliads are largely aquatic, pit vase volume should act similarly to island area. As such,

we hypothesized that plants with tanks of larger volume should sustain more individuals and more trophic levels than tanks of much smaller volume.

As the tanks get larger, the food webs contained within the bromeliad should become more complex. Moreover, these trophic levels should mimic trophic arrangements of larger systems. Modeling extinction and colonization in these microcosms will elucidate many of the underlying theories concerning maintenance of species diversity and species conservation.

We also hypothesize that by removing top predators (extinction) and adding top predators (colonization) to different tanks, our results would mimic extinction and colonization in larger ecosystems, providing further insight into extinction and colonization as mechanisms of changing diversity.

METHODS

We collected 20 study individuals of *G. nicaraguensis* at Monteverde Biological Station, Costa Rica. We collected 10 specimens with vases < 9 cm in depth and 10 specimens with vases > 15 cm in depth. We stored the specimens in hydroponic trays until sampling.

We sampled the detritus and contents of each individual plant by tearing each level of the base and emptying the contents into dissecting trays. We then identified all invertebrates found in the plants to the lowest visually identifiable taxonomic rank with the aid of a dissecting scope (Lehmkühl 1979 and Resh and Cardé 2003). We also recorded vase width and depth, leaf diameter, and whirling pattern of each individual. We used these data to calculate the approximate volume of the bromeliad tanks assuming $V = \pi r^2 h$.

We used vials and ceramic cups to construct vase structures in the laboratory. To mimic the small vases we add 20 mL of local

stream water to vials; for the large vase mimics, we used 200 mL of local stream water in ceramic cups. Both volumes for the laboratory vases were based on the average volume for large and small bromeliads.

Extinction Model Colonization Model

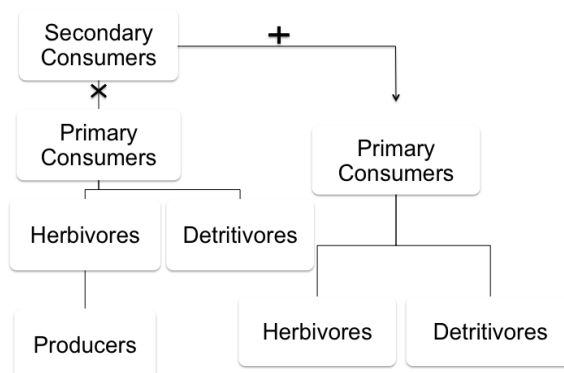


Fig. 1.1. The extinction and colonization models include removing secondary consumers from larger Bromeliad vases and placing them into smaller bromeliad vases.

To simulate extinction, we removed the top trophic level, secondary consumers, from 5 large vases. To model colonization and to test the species-area plasticity, we added top predators from other specimens to 5 of the smaller vases (Fig. 1.1). Five of each (small and large) specimen were also used as controls where all invertebrates removed for sampling were directly added to a vial or container, depending on size of specimen.

All vials and ceramics were left for 48 hours to allow for ecosystem manipulation and stabilization. We then re-sampled all vials and containers and catalogued the changes in species abundance and trophic level composition.

We classified all contents of the vases into trophic groups based on feeding guilds (Fig. 1.2) (Armbruster et al. 2002). Detritivores and herbivores were classified into a single category "herbivores". Omnivores were classified as primary consumers; higher predators,

twice removed from producers, were classified as secondary producers. After 48 hours, we compared the change in individual abundance among functional groups between treatments.

We used Microsoft Excel and JMP 8.0 for our data analyses.

Extinction/Colonization Model

We constructed a model to predict trophic level stratification according to area:

$$PA_P + HA_H + C_1A_x + C_2A_y = \Psi$$

where P corresponds to the ratio of total number of species in the producer trophic level to total number of species found in the system. H corresponds to the ratio of total herbivores in the system to the number of total species found in the system. C_1 and C_2 correspond to the ratios of primary consumers and secondary consumers to the total number of species found in all trophic levels, respectively.

A_P, H, x, y are area constants with values determined by our experimental manipulations.

The constants were found by dividing the total number of individuals of respective trophic levels by the total area, giving the proportional area necessary for a given trophic level to survive. Ψ is the area score for the ecosystem, to which changes in trophic levels can be compared. If $\Psi_f < \Psi_i$, colonization is possible and extinction is not a risk. If $\Psi_f > \Psi_i$, colonization is not possible and extinction will occur.

The area constants were estimated for this system using the collected baseline data of species and numbers of individuals were then averaged to be used in the extinction/colonization model. This model can be modified based on the number of trophic levels and top-down or bottom-up effect.

RESULTS

The species area curve (Fig. 2.1) shows a logarithmic relationship between number of taxonomic groups of arthropods and pit-vase volume ($r^2_{19} = 0.67, P = 0.01$). Log transformation rendered the species-area relationship linear (Fig. 2.2) ($r^2_{19} = 0.57, P = 0.01$).

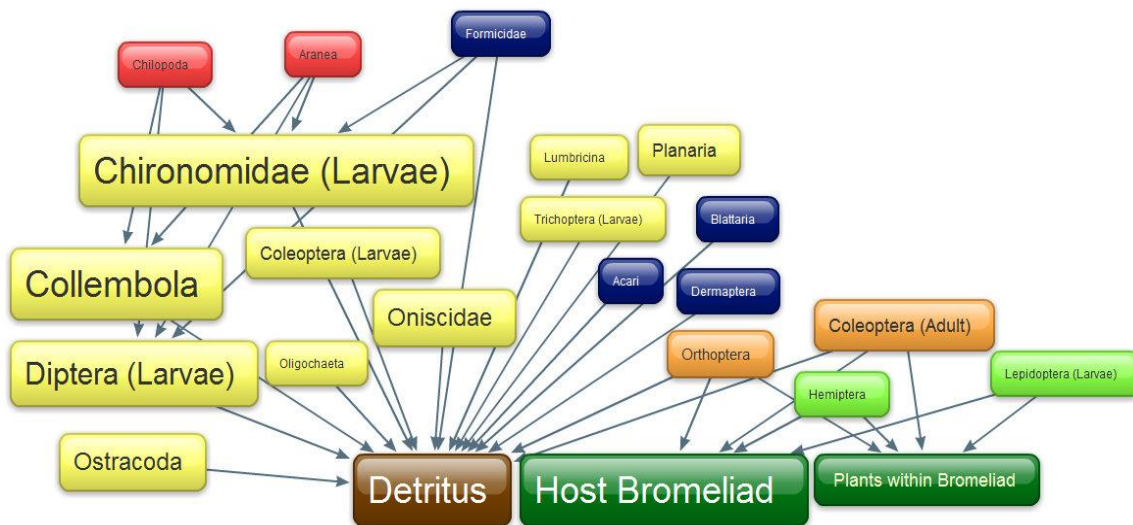


Fig. 1.2. Trophic web of organisms found in bromeliads. Arrows point from a taxonomic group to the most common food items. The size of font is quantitatively correlated with the abundance of the taxonomic group. Box colors identify feeding group as follows: yellow-detritivores, light green-herbivores, orange, detritivores+herbivores, blue-omnivores, red-predators.

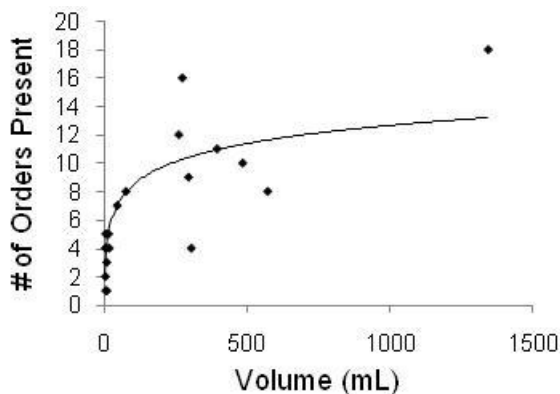


Fig. 2.1. Species-area curve representing the relationship between vase volume and number of taxa present.

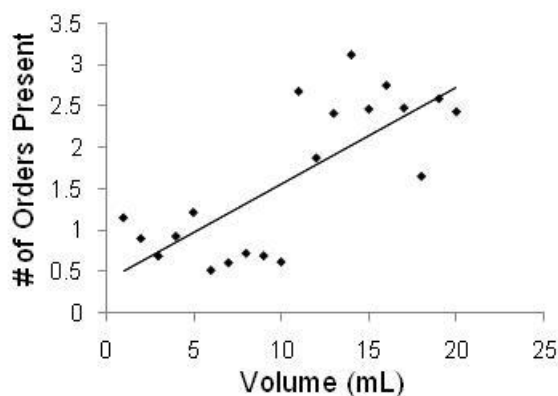


Fig. 2.2. Species-area data log-transformed to represent the linear relationship between vase volume and number of orders present.

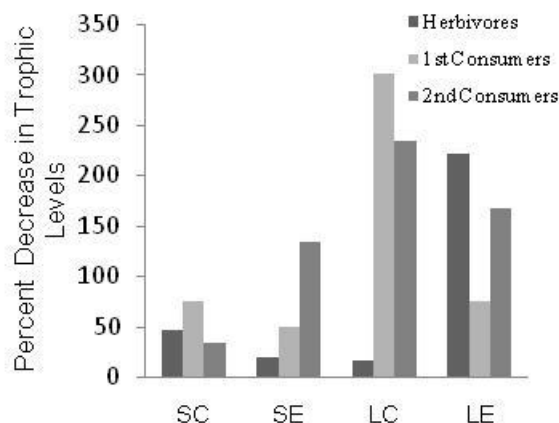


Fig. 2.3. The percent decrease in trophic levels from first sampling to second sampling.

Contrary to our expectations, the changes in control vials of small and large Bromeliad vases were greater than those in our experimental model. There were no significant differences in the experimental additions or re-

movals when compared to the controls (Fig. 2.3).

We compared the change in Shannon-Weiner indices of diversity, between large and small bromeliads, and tested for a treatment by size class interaction. We detected no effect of our treatments as a function of bromeliad size class (control: Trophic level \times size: 2-way ANOVA, $F_{2,29} = 2.06$, $P = 0.15$; experimental: Trophic level \times size: 2-way ANOVA, $F_{2,29} = 1.5$, $P = 0.24$).

MODEL RESULTS

Despite the inconclusive nature of our empirical study, the area constants that our results yielded are as follows: $A_P = 0.0022$, $A_H = 0.058$, $A_x = 0.019$, $A_y = 0.015$. The validity of these area constants should either be verified or refuted by additional studies. If further research supports the validity of these constants, then we propose these constants could be used in the proposed model to accurately estimate species-area relationships in other ecosystems.

DISCUSSION

The composition of bromeliad tank communities follows the equilibrium theory of island biogeography. We found a strong relationship between pit vase volume and the number of taxa the bromeliad contained. This same observation was also true of trophic levels; when bromeliads had larger vases, more trophic levels were present.

Although the manipulations that we performed were not successful, there is strong evidence that further research and refinement of experimental techniques could lead to conclusions about extinction and colonization models. Because the species-area relationship follows MacArthur-Wilson's predictions so closely, it is very probable that a simple system such as tank bromeliads could be used to model ecosystems at the micro level.

Several confounding factors limited the scope of our study including time and equipment. Further research could test *in situ* vases and for longer periods of time, possibly providing a more accurate baseline. Better habitats for top predators would also be a consideration for further research given top predators' habitat is on the plant and not necessarily in the pit vase. Lastly, a greater understanding of invertebrate taxonomy and feeding habits could help create a more accurate model of bromeliad trophic structure.

The area constants estimated by our study represent the relative effects that each trophic level has on its respective ecosystem. The addition or removal of "herbivores" had the greatest effect on the overall area needs of the ecosystem, followed by "primary consumers," "secondary consumers," and "producers," in descending order.

The model proposed in this study has potential for estimating the area needed to support trophic levels at a functional group level. The model should apply to ecosystems where the only change is the top predator removal or addition. By performing our experiment with water filled artificial habitats, we introduced a large disturbance. Any effects of the removal or addition of a trophic level may have been masked by the effects of the disturbance.

More research and consideration is needed to make the model a more accurate estimate of species-area relationships. However, such a model would be immensely useful in conservation and reintroduction efforts, while also allowing us to understand habitat fragmentation and other man-made "islands."

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ENERGY OPTIMIZATION AND FORAGING PREFERENCE IN HUMMINGBIRDS (TROCHILIDAE)

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Faculty Editor: Ryan Calsbeek

Abstract: Optimal Foraging Theory predicts that organisms will maximize their foraging efficiency by balancing time spent feeding and time spent searching for new feeding sites. Foraging efficiency is particularly important for hummingbirds (*Trochilidae*) because of their high metabolism and energy requirements. We hypothesized that hummingbirds would prefer large flowers and clusters with more flowers to optimize their energy expenditure. Hummingbirds should also spend more time at flowers with a higher nectar concentration because of the greater energy reward. We observed hummingbird behavior in response to manipulations of reward size using artificial feeders in Monteverde, Costa Rica. Our results supported the hypothesis that hummingbirds prefer flowers that offer higher energy rewards.

Key words: Hummingbird, foraging strategy, nectar concentration

INTRODUCTION

Foraging animals must constantly make decisions about which patches of food to visit and how long to visit them. Optimal Foraging Theory predicts an energy trade-off: energy is required to locate and travel to a new patch, but as the amount of time spent within a patch increases and resources are consumed, the energy available decreases (Pyke 1978). According to the Marginal Value Theorem, animals forage in a patch until the rate of energy gain is less than or equal to the potential energy gain from other patches within the habitat (Charnov 1976).

Hummingbirds (*Trochilidae*), with a high wingbeat frequency and a heart rate that can achieve up to 1300 beats per minute while hovering, have an extremely high metabolism for their size (Suarez 1992). Hummingbirds feed on nectar because its high sugar concentration provides a quick source of energy (Fogden and Fogden 2005). Hummingbirds are ideal organisms for studying Optimal Foraging Theory because they face a high cost if they do not choose a foraging strategy that

maximizes their energy intake (Fogden and Fogden 2005).

In this study, we investigated the factors that determine hummingbird patch choice and feeding duration. We tested the hypothesis that hummingbirds should not forage randomly but should forage to optimize potential energy intake. We predicted that hummingbird foraging preference would be based primarily on visual cues such as flower size and clustering of flowers. Because larger flowers should contain more nectar (Fenster et al. 2006), we predicted that hummingbirds would prefer large feeders to small feeders and large clusters of feeders to small clusters of feeders. Finally, we predicted that nectar quality would affect hummingbird foraging choice, such that hummingbirds would spend more time at feeders with concentrated nectar over diluted nectar.

METHODS

We studied hummingbird foraging behavior at the Monteverde Biological Station, Costa Rica. We constructed hummingbird feeders using 6mL and 50mL plastic vials. Vials were covered in red tape to attract

hummingbirds (Ödeen and Håstad 2010) and filled with a 30% sucrose solution. The mouths of the vials were also covered in red tape, and a hole punch was used to create a standard opening. The vials were spaced 30 cm apart on tree branches at a height of 1.5-2 m outside the Monteverde Biological Station.

Patch and flower size

To test whether hummingbirds prefer more or larger flowers in a patch, we arranged clusters of different sizes by hanging vials next to each other. We had six different cluster treatments: 1, 3, and 5 vials of both small (6mL) and large (50mL) volume. We randomized two, three, or four clusters of feeders at four sites around the field station.

We conducted twenty-minute observations of all of the sites on 20 and 21 January 2011 between the hours of 06:00 and 18:00, for a total of 24 effort-hours. After three trials, treatments were rotated around our study site to control for site effects. We recorded the number of visits to each cluster and the duration of each visit.

Concentration and volume

We performed two additional experiments on 22 January 2011 for 12 effort-hours each (between the hours of 06:00 and 14:00) to examine why hummingbirds spend more time at larger flowers. To test whether the nectar concentration or the physical size of the feeder was more important to feeding duration, we set up 40% and 10% sucrose solutions in three large and three small individual vials.

To test whether hummingbirds discern the volume of nectar in the feeder, we concealed a small vial inside a large vial to create a treatment with a large physical size and small nectar volume. Four of these compound vials were set up, in addition to four large and four small vials as controls. The sugar

concentration and volume treatments were hung at four sites along the road by the field station's garden. Within each experiment, blind treatments were distributed randomly. We observed the sites for twenty-minute trials, recording data in the same way as in our previous observational study.

Analysis

We performed ANCOVA to evaluate the potential effect of site on the visit duration. For the patch and flower size experiments, we used ANOVA to compare mean number of visits between large and small feeder clusters to establish hummingbird visual foraging preference. We also used ANOVA to compare mean visit duration between large and small feeders to assess whether hummingbirds remain at feeders that offer higher energy rewards. To analyze whether hummingbird visit duration is driven by volume, we performed ANOVA to compare mean visit duration between large, compound, and small feeders. We used ANOVA to examine the effects of sucrose concentration on hummingbird foraging preference by comparing mean visit duration between high and low concentration feeders of both sizes. We then performed post-hoc Tukey HSD tests to determine which comparisons were significant. Statistical tests were conducted with JMP 8.0.

RESULTS

We found no evidence for a statistical interaction between site and hummingbird visitation to each treatment (ANCOVA, $F_{23,196} = 1.33$, $P = 0.19$). Therefore, we pooled the data from all four sites in our analysis.

Hummingbirds made significantly more visits to the largest clusters of feeders than to smaller ones ($F_{2,217} = 24.93$, $P < 0.0001$; Fig. 1). Hummingbirds also spent significantly more time at large feeders than at small feeders ($F_{1,211} = 4.99$, $P = 0.027$; Fig. 2).

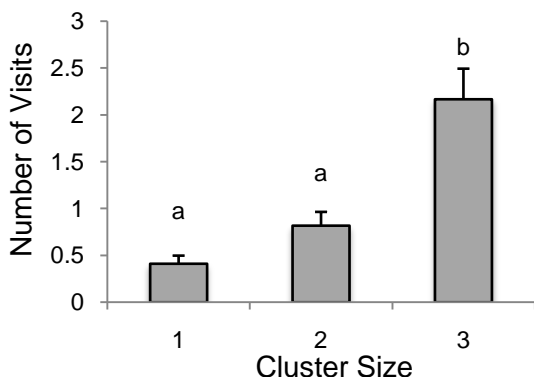


Fig. 1. Mean number of hummingbird visits to clusters of 1, 3, and 5 feeders of 40% sucrose concentration at Monteverde, Costa Rica. Error bars represent one standard error from the mean.

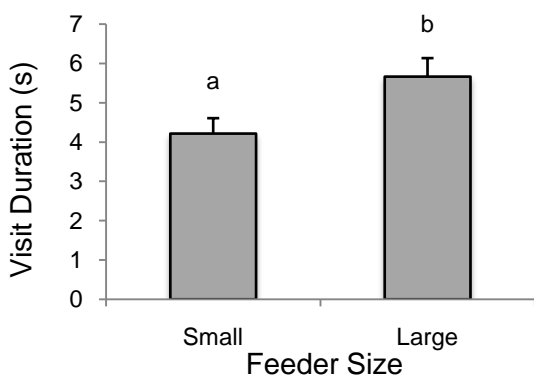


Fig. 2. Mean duration (s) of hummingbird visits to large and small feeders with 40% sucrose concentration weighted by total number of visits to each treatment at Monteverde, Costa Rica. Error bars represent one standard error from the mean.

In the volume experiment, hummingbirds spent significantly more time at large compound feeders than small feeders ($F_{2,9} = 5.18$, $P = 0.032$; Fig. 3). Time spent at large feeders alone was not significantly different from either compound or small feeders.

Hummingbirds stayed significantly longer at high-concentration feeders than low-concentration feeders (10%: $\bar{x} = 2.42$, (SE = 0.56); 40%: $\bar{x} = 4.72$, (SE = 0.56); $F_{2,4} = 8.52$, $P = 0.015$). Hummingbirds spent significantly more time at feeders that were both large and contained high sugar concentrations ($F_{3,8} = 8.47$, $P = 0.0073$, Fig. 4).

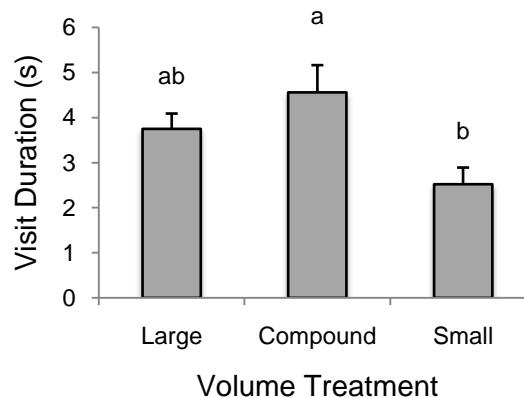


Fig. 3. Mean duration (s) of hummingbird visits to large volume feeders, large volume feeders containing a small volume solution, and small volume feeders weighted by total number of visits to each treatment. Trials were conducted at Monteverde, Costa Rica. Error bars represent the standard error of the mean.

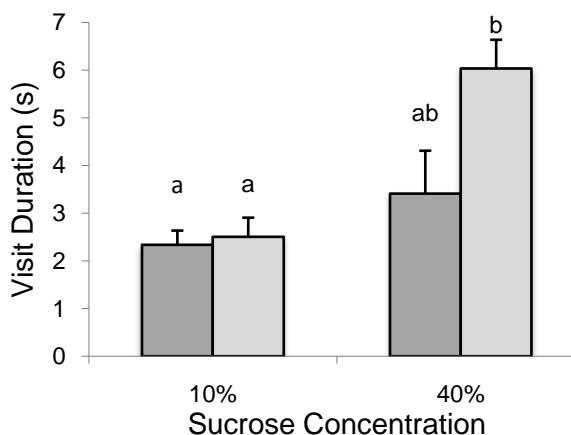


Fig. 4. Mean duration (s) of hummingbird visits to feeders containing 10% sucrose solution and 40% sucrose solution weighted by total number of visits to each treatment. Dark bars represent small feeders and light bars represent large feeders. Trials were conducted at Monteverde, Costa Rica. Error bars represent one standard error from the mean.

DISCUSSION

Because hummingbirds have a high metabolism, they must optimize their energy expenditure. Hummingbirds may increase their foraging efficiency by distinguishing between high- and low-energy flower rewards (Wolf and Hainsworth 1975). In our study, hummingbirds consistently optimized their energy intake by spending the most time at large

feeders, large clusters of feeders, and feeders with high sugar concentrations.

Our data support our hypothesis that hummingbirds visit large clusters of flowers significantly more often than small clusters (Fig. 1). Hummingbirds may be minimizing their travel-associated energy costs by choosing to feed at higher-density patches. Furthermore, when there are more flowers in a cluster, it is more likely that all flowers will not be already depleted. Alternatively, hummingbirds may rely primarily on visual foraging strategies and simply have been able to find larger clusters of feeders more easily (Stiles 1975).

We also found that hummingbirds spend more time per visit at large feeders than small feeders (Fig. 2). Hummingbirds may feed at large feeders longer to maximize their energy intake because larger flowers may contain a greater nectar reward. Fenster et al. (2006) found that ruby-throated hummingbirds prefer larger artificial flowers in a system where larger corolla size was correlated with both more and higher quality nectar.

Consistent with our predictions, we found that hummingbirds discriminated only between the sizes of feeders, not their volumes (Fig. 3). This result may be biased by the fact that no hummingbird in our experiment could deplete all the nectar in a single visit because our vials contained a much greater volume of sugar water than is found naturally in flowers (Feinsinger 1983). However, Salguero-Faria and Ackerman (1999) found that the volume of nectar offered by a Puerto Rican orchid had no effect on hummingbird visitation and pollination success. This is consistent with our results and with the conclusion that hummingbird visit duration is not driven by volume.

We also found that hummingbirds feed longer at large high-concentration feeders

than at small high- and low-concentration feeders and large low-concentration feeders (Fig. 4). By staying longer at high-concentration flowers, hummingbirds can maximize their energy gain while minimizing their effort time and travel costs (Tamm and Gass 1986).

Hummingbirds' clear preference for certain flower characteristics is likely to exert selective pressures on hummingbird-pollinated plants. These plants must balance the energy allocated to attract hummingbirds with energy for metabolic processes, including plant growth. Plants may have developed a balance between quality and quantity of rewards that optimizes their energy investment in exchange for hummingbird pollination. Our results indicate that plants can best attract hummingbirds by investing in large flowers with high-concentration nectar.

Despite hummingbird preference for the highest concentration nectar available in laboratory tests (Bolten and Feinsinger 1978), producing high-concentration nectar may be too energetically costly for plants. Galetto and Bernadello (2004) found that flowers produce only enough nectar to attract a hummingbird long enough to receive pollen. When birds are satiated after one feeding, they will not expend more energy to visit other flowers. If the flowers all have low-concentration nectar, hummingbirds must visit multiple flowers to satisfy their energetic needs, thereby increasing pollination and the plant's reproductive success (Bolten and Feinsinger 1978).

Our results support the hypothesis that hummingbirds follow Optimal Foraging Theory and choose foraging sites to maximize their energy intake. Further studies could investigate how other aspects of flower morphology and nectar production affect patterns of hummingbird foraging preference.

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We would like to thank the staff of Monteverde Biological Station for their hospitality. We would also like to express our gratitude for the feedback and support of the course TAs, the students of the FSP, and Dr. Ryan Calsbeek, who aided us in refining our experimental design and data analysis.

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NUTRIENT AVAILABILITY, SOIL QUALITY AND ELEVATION IN A TROPICAL CLOUD FOREST

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Faculty Editor: Ryan G. Calsbeek

Abstract: Tropical forests are highly productive but often nutrient limited. To offset nutrient limitation, tropical forests cycle nutrients rapidly compared to other ecosystems. Because other abiotic factors, such as wind and temperature, are more limiting at high elevations, we predicted that vegetation in high-elevation elfin forest would be less limited by nutrient availability than lower elevation forest. As a result, we hypothesized that nutrient cycling would be lower at high elevations and that more organic matter and nutrients would accumulate in the soil. We sampled soil cores along an altitudinal transect and recorded humus and litter depth, abundance of fungal groups and invertebrates, vegetation type and percent cover. We found that humus depth increased with elevation and that surface erosion did not vary with elevation. We concluded that elfin forests were less nutrient limited than lower elevation forests, but future studies should look at other aspects of the nutrient cycle such as actual rates of supplemental nutrient uptake.

Key words: Nutrient cycling, elfin forest, humus

INTRODUCTION

Tropical forests are highly productive because of high light and water availability; however, they are often nutrient limited. To offset nutrient limitation, tropical forests rapidly cycle nutrients (Vitousek 1984). Generally in tropical rainforests, nutrients are tied up in the copious aboveground biomass (Osborne 2000). As fine organic litter from vegetation decomposes, nutrients enter the humus layer, and are available for vegetal uptake or are lost to lower soil layers (Osborne 2000). Humus, therefore, functions as a reservoir of nutrients available to vegetation (Natural Resources 2005). Nutrients in the tropics often remain in organic compounds and are not lost to lower soil layers, which allows for rapid nutrient cycling (Vitousek 1984). Rapid cycling occurs in multiple ways: vegetation can retain nutrients via re-absorption, large amounts of carbon can be fixed per unit of nutrient in vegetation, or nutrients released from decomposing vegetation can be rapidly absorbed by roots and decomposers (Vitousek 1984). Organic matter not sequestered in

biomass provides food for decomposers and other soil organisms, including invertebrates (Natural Resources 2005).

Within tropical forests nutrient cycling efficiency varies (Vitousek 1984). Elfin forests, like those found at high elevations within Monteverde Biological Reserve, are characterized by stunted trees (Lawton 1982). Previous studies have suggested that trees at this elevation are stunted because their growth is limited by factors such as wind at the canopy level, water stress, and the steep slopes on which they are found (Lawton 1982). If elfin forests at high elevations are limited by factors other than nutrient availability, we predicted that they would invest less in maximizing their rate of nutrient uptake, with the result that more organic matter and nutrients would accumulate in the soil over time. On the other hand, if nutrients are the most limiting factor to growth at low elevations, we predicted that plants would maximize their rate of nutrient uptake, with the result that less organic matter and nutrients would accumulate over time. Therefore, we predicted that greater amounts of leaf litter, humus,

fungus bodies, and arthropods, as measures of nutrient availability, would be found at higher elevation sites.

METHODS

We gathered data from 1 m radius circular plots every 10 meters of elevation gain between 1547 meters and 1817 meters above sea level in Monteverde National Forest, Costa Rica on January 20-21, 2011. A Garmin GPSmap 76CSx, calibrated to the known elevation of the research station, was used to measure elevation gain. On the first day of the study, we used a Speedtech SM-28 Sky-Master weather station to record air temperature, wind speed, relative humidity, and barometric pressure roughly 2 meters from the ground. We used wind speed as a proxy for erosive forces.

On the second day, at each of the 28 sites we used an Oakfield Apparatus Co. soil corer (model H) to estimate humus depth. We determined humus depth by measuring the distance from the surface to the start of the lower layer of reddish clay. We recorded the presence or absence of clay and/or grey subsoil in each core. Depths were averaged from three cores taken haphazardly within a 1 meter radius at each site.

We used a Physitemp BAT-12 Microprobe Thermometer to evaluate soil temperature. We measured minimum and maximum litter depth and counted fungal blooms in a one-meter circle immediately off the trail every 10 meters in elevation gain. We visually estimated canopy coverage and the relative contributions of different plant types (tree, shrub, or other vegetation) to the ground cover. Finally, we used a soil corer to collect a fourth humus sample to evaluate invertebrate abundance in the lab. In the lab, we weighed each wet soil sample and used a dissecting scope to remove invertebrates and sort them by family.

RESULTS

We found that humus depth increased with elevation ($r^2_{1,26} = 0.20$, $P = 0.018$; Fig. 1). Wind speed was zero at all but 4 sites, and was never greater than 1 m/s. Canopy cover tended to decrease as elevation increased ($r^2_{1,26} = 0.11$, $P = 0.079$; Fig. 2), and maximum leaf litter depth (cm) increased with percent canopy cover ($r^2_{1,26} = 0.17$, $P = 0.031$). However, maximum and minimum leaf litter depth did not vary significantly with elevation ($r^2_{1,26} = 0.06$, $P = 0.21$ and $r^2_{1,26} = 0.04$, $P = 0.33$, respectively).

We found a positive trend between number of fungal blooms and elevation, but this relationship was not significant ($r^2_{1,26} = 0.09$, $P = 0.11$; Fig. 3). We found no relationship between invertebrate abundance and elevation ($r^2_{1,26} = 0.05$, $P = 0.27$) or between vegetative cover type and humus depth ($r^2_{2,25} = 0.11$, $P = 0.24$).

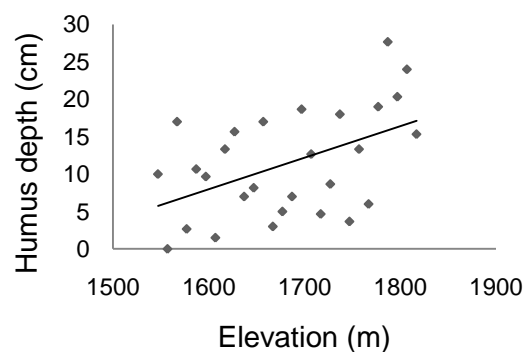


Fig. 1 Average humus depth increases significantly with elevation in Monteverde, Costa Rica.

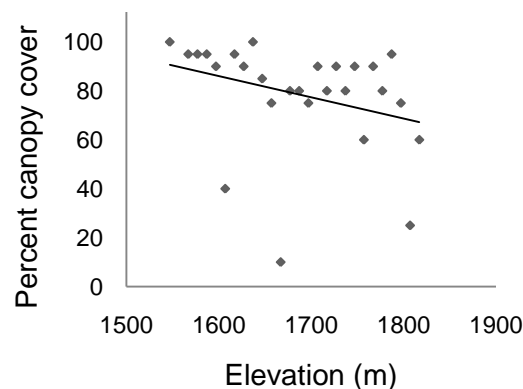


Fig. 2 Negative relationship between percent canopy cover and elevation in Monteverde, Costa Rica.

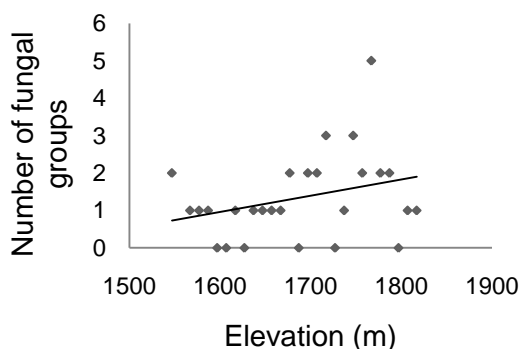


Fig. 3 Positive trend between number of fungal groups (fungal blooms) and elevation in Monteverde, Costa Rica.

DISCUSSION

Elevation and humus depth were positively correlated, indicating more organic matter is present in soil at higher elevations. As humus acts as a nutrient reservoir, its abundance reflects nutrient availability. This supports our original hypothesis that more nutrients are available at high elevations. This trend was especially powerful given that canopy cover decreased with elevation, meaning that trees would deposit less leaf litter on the forest floor at higher elevations.

We found that litter depth did not change significantly with elevation and that wind speed at two meters above the ground was never greater than 1m/s. We conclude that surface erosion in the dry season is relatively constant with elevation, although we could not account for the effects of wet-season rains, soil type, etc. Since wind erosion could affect humus and litter depth measurements, it was important to exclude it as a confounding factor in our study.

We found a positive trend between the number of fruiting fungal clusters and elevation. Since fungi thrive on decomposing organic matter, this trend could indicate that nutrients are available in higher abundances at higher elevations.

The small soil sample collected at each site did not allow us to draw conclusions about

invertebrate populations. Perhaps larger soil samples would have produced enough invertebrates to observe a trend.

Overall, our data suggest that there is more organic matter and available nutrients in the soil at higher elevations. Since plant absorption is a major way that nutrients leave the soil system and other factors that might affect humus accumulation (i.e., erosion) are consistent at all of our sites, we conclude that plants at higher elevations are absorbing nutrients from the humus more slowly. In general, nutrients are a powerful limiting factor in the growth of tropical forests. However, slower nutrient cycling at higher elevations imply that plants at higher elevations are limited by something other than nutrient availability.

The stunted nature of elfin forests is well documented (Lawton 1982). Multiple factors have been shown to contribute to the trees' short stature. Wind at the canopy level, water stress, and the steep slopes on which elfin forests are found have all been suggested as major causes of elfin tree morphology (Lawton 1982). Our study suggests that elfin forests are limited by factors other than nutrient availability. This finding supports the work of Vitousek (1984), in that variation exists even within one type of tropical forest. Future studies should examine other aspects of nutrient cycling within elfin forests, especially nutrient uptake via experimental nutrient supplementation.

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We would like to thank the staff at Monteverde Biological Reserve for their hospitality and permission to conduct research at the site. Also, we express much gratitude to Professor Ryan Calsbeek and the course TAs (Katie Duryea, Gillian Moritz, and Sarah Wengert) and Zachary Clare-Salzler for their feedback and support.

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EFFECTS OF FLOW RATE ON STREAM MACROINVERTEBRATE DIVERSITY IN MONTEVERDE, COSTA RICA.

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Faculty Editor: Ryan Calsbeek

Abstract: Species diversity is an important gauge of ecosystem health. Understanding the driving forces that maintain species diversity is therefore an important means of informing approaches to conservation. In tropical streams, macroinvertebrates often act as such gauges, so it is important to understand which abiotic factors most influence their diversity. In this study we test the hypothesis that invertebrate diversity in streams is primarily limited by flow rate of the stream. We conducted an experimental manipulation of stream flow at 10 sites along a tropical stream in the cloud forest of Monteverde, Costa Rica. We measured flow rates, dissolved oxygen concentrations, and invertebrate diversity before and after constructing dams around the sites. There was a significant positive correlation between flow rate and diversity, suggesting that flow rate strongly influences stream invertebrate diversity.

Key words: benthic macroinvertebrates, stream ecology, disturbance

INTRODUCTION

As anthropogenic impacts on the environment have increased, assessing the health of ecosystems has become increasingly important. One gauge of ecosystem health is species diversity.

In freshwater streams, macroinvertebrate diversity is a widely used indicator of stream health (Connolly and Crossland 2004). Understanding which biotic and abiotic factors drive macroinvertebrate diversity is thus useful in understanding which factors drive stream health.

One factor that can have a large impact on ecosystem health is disturbance, both natural and anthropogenic. In tropical streams, disturbance includes falling logs and other allochthonous inputs, in addition to man-made dams. These disturbances can affect abiotic factors such as stream flow and dissolved oxygen, as well as biotic factors such as the distribution of organic debris.

Previous studies have focused on how allochthonous inputs affect the distribution and abundance of macroinvertebrate communities. Marino et al. (2004) found that the abun-

dance of certain functional feeding groups sampled exclusively from rock riffles, or relatively open, fast-moving stretches of stream, was negatively correlated with distance from debris dams. This suggests that debris dams may be useful to certain macroinvertebrates, potentially as a source of food and shelter. Brown et al. (2010), further investigating this matter, found much greater overall abundances of macroinvertebrates in rock riffles than in debris dams, despite the potential food and shelter provided by dams. Collectively, these studies on tropical streams suggest that a variety of abiotic factors, and not just debris availability, drive macroinvertebrate distribution.

However, it is also possible for detritus levels in streams to strongly affect macroinvertebrate distribution. In contrast to Brown et al. (2010), Schneider and Winemiller (2008), working in the temperate zone, found greater macroinvertebrate abundance and diversity in detritus-rich debris piles than in relatively open stretches of stream. This suggests that detritus may be a limiting resource for invertebrate communities in the temperate zone. In the tropics, however, macroinvertebrate

communities may not be limited by availability of detritus because of the higher level of canopy cover (Mauthuriau 2008).

These studies and others have examined which factors drive abundance and distribution of macroinvertebrates across streams. Few studies, though, have focused on which factors drive and limit macroinvertebrate diversity. Excluding detritus as a limiting factor in the tropics (Mauthuriau 2008), we predicted that neither temperature nor pH would regulate diversity, as they are unlikely to vary greatly in a small stream. However, flow rate usually varies within a stream and could influence the influx of nutrients and dissolved oxygen, as well as benthic composition.

In this study, we tested the hypothesis that macroinvertebrate diversity in tropical streams is mainly regulated by flow rate.

METHODS

We studied stream invertebrate diversity in Monteverde National Park, Costa Rica, between 20 and 22 January 2011.

We selected 10 sites along a second-order stream. These sites were characterized by a moderate flow rate (approximately 36 cm/s), a rocky bottom, and an intermediate depth (approximately 15 cm) relative to the overall stream. We chose sites to be narrow enough to be effectively dammed off. Each site was 1.5-2 m long and 0.5-1 m wide.

At each site, we recorded water temperature and dissolved oxygen (mg/L and percent saturation) using a YSI Pro ODO digital instrument and pH using EMD colorpHast indicator strips. We measured the depth in the middle of each site, and surface flow rate was calculated as the time for a float to travel 1m in the main channel. We used surface flow rate as a proxy for overall stream flow rate at each site.

We then collected two rocks of intermediate size (bottom area of approximately 200 cm²) from roughly the center of each site. As we lifted each rock from the stream bottom, we positioned a 30 cm wide kick net immediately downstream to collect the debris freed from the removal of the rock. We scrubbed each pair of rocks for 60 s in a bucket of water to remove macroinvertebrates and detritus and measured the area of the bottom of each rock. We collected all material from the bucket and net and sorted the macroinvertebrates.

We identified the macroinvertebrates down to the family level using aquatic insect keys (Lehmkuhl 1979, Thorpe and Covich, 2001). The few organisms that we could not identify to family were identified to order. We also dry massed the organic material in the debris to quantify detritus present at each site.

Immediately after macroinvertebrate collections, we built dams along the downstream and upstream borders of the site to slow water flow. When the flow was not slowed by 4 cm/s or more, we also built a small dam along the side to further slow flow rate. Dams were constructed out of rocks, sticks, and leaves. Following dam construction, we measured flow rate.

After 22-24 hours, we returned to the sites and measured temperature, dissolved oxygen, and flow rate. We chose this time frame so that flow rate and dissolved oxygen, but not detritus levels, would be affected. We repeated the collection of rocks, debris, and macroinvertebrates, and sorted and identified all macroinvertebrates. Following our experiments, we removed all dams from the stream.

We conducted two-sample t-tests comparing Shannon-Wiener Indices of diversity, dissolved oxygen, flow rate, and g detritus/cm² rock between treatments. We used linear regressions to test for correlations between flow

rate and dissolved oxygen, and flow rate and Shannon-Wiener Indices of diversity.

We used JMP 8.0 to perform t-tests and a linear regression. We sampled a larger area the day after constructing dams than the previous day. Even after controlling for uneven sampling effort between sites using the Shannon-Weiner Index of diversity, we used rarefaction as a more statistically appropriate measure of comparing species diversity by simulating equal sampling sizes before and after our experimental manipulation. We rarefied our data using EcoSim™.

RESULTS

We collected 501 invertebrates before dam construction and 1100 invertebrates after, representing seven orders and sixteen families. Despite the discrepancy in the number of invertebrates collected, the density of macroinvertebrates (number collected/area sampled) was not significantly different before and after damming ($t_{14.6} = 1.75$, $P = 0.10$).

Temperature, pH, and detritus levels did not vary before and after damming (all $P > 0.19$). Stream flow rate ($t_{13.4} = -3.54$, $P = 0.004$) and dissolved oxygen ($t_{13.8} = -2.44$, $P = 0.03$) were significantly lower after damming than before. There was a significant positive correlation between flow rate and dissolved oxygen ($r^2_{19} = 0.22$, $P = 0.04$). There was no significant change in g detritus/cm² of rock after damming ($t_{12.2} = -0.68$, $P = 0.51$).

We found a significantly lower Shannon-Wiener Index of diversity after damming, even though we sampled a larger area after the manipulation ($t_{17.9} = -2.90$, $P = 0.01$; Fig. 1). Moreover, there was a significant positive correlation between flow rate and Shannon-Wiener Index ($r^2_{19} = 0.21$, $P = 0.04$; Fig. 2).

Rarefaction provided a more accurate way of comparing the diversity before and after damming by simulating equal sample sizes.

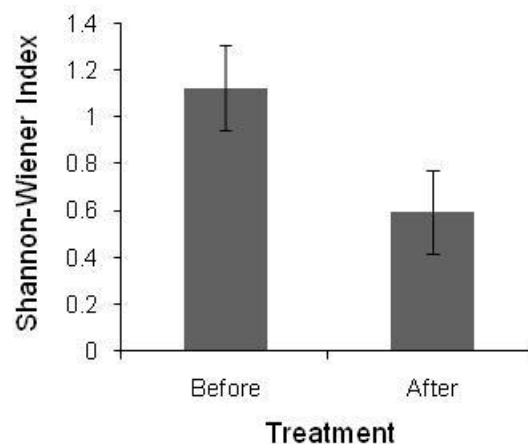


Fig. 1 Shannon-Wiener Index for diversity of stream macroinvertebrates before and after damming in Monteverde, Costa Rica. Error bars ± 1 SE.

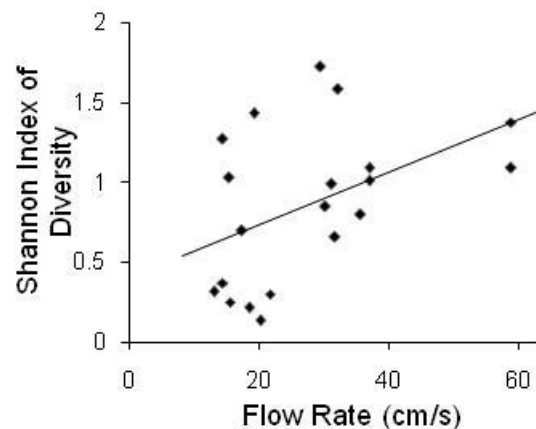


Fig. 2 Shannon-Wiener Index for diversity of stream macroinvertebrates by flow rate in Monteverde, Costa Rica.

Rarefaction further increased the disparity between Shannon-Wiener Indices before (3.45) and after (2.86) damming (Fig. 3 and 4).

DISCUSSION

Our study suggests that decreased stream flow may negatively affect invertebrate diversity in a tropical stream. Diversity was significantly higher before damming than after (Fig. 1). The Shannon-Wiener Index of diversity decreased after damming, both with and without rarefaction. Furthermore, there was a significant positive correlation between flow rate and diversity (Fig. 2). These findings are consistent with previous observational studies suggest that rock riffles with faster flow-

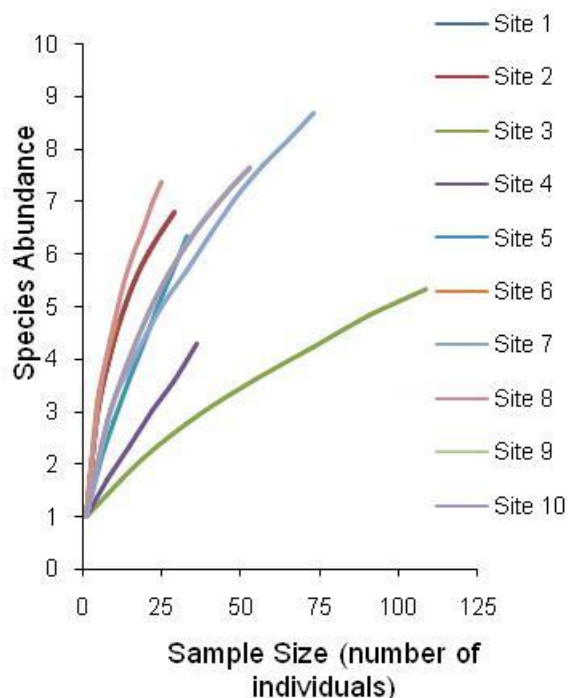


Fig. 3 Rarefied data of macroinvertebrate species abundance by sample size before damming a stream in Monteverde, Costa Rica.

ing water may be more suitable habitats for tropical macroinvertebrates than debris dams (Brown et al. 2010). The consistent temperature, pH, and detritus levels before and after damming suggest that decreases in invertebrate diversity were mostly due to changes in stream flow.

Abundance was not significantly different before and after damming; however species diversity is known to be a better measure of stream health than overall macroinvertebrate abundance (Gaufin and Tarzwell 1952). High abundance of a few species that thrive in unhealthy stream conditions can lead to high overall abundance, whereas diversity will only be high in streams that are healthy enough to support many types of species.

Our finding of decreased diversity in slower flowing streams may be related to the decrease in dissolved oxygen. Dissolved oxygen concentrations decreased significantly from a mean of 75.9% saturation before damming to a mean of 74.1% after damming.

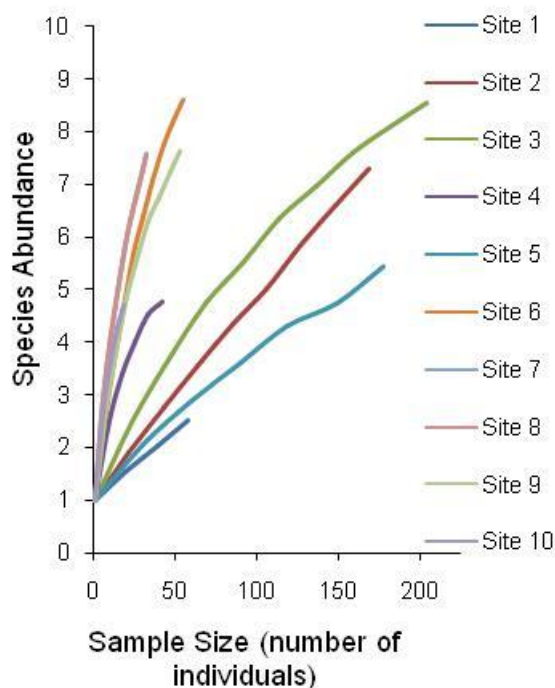


Fig. 4 Rarefied data of macroinvertebrate species abundance by sample size after damming a stream at Monteverde, Costa Rica.

However, this decrease likely had limited effects on invertebrate mortality, as previous studies have found that most taxa in tropical streams can tolerate any dissolved oxygen levels greater than 20% saturation (Connolly et al., 2004). Therefore, it is unlikely that the decrease in diversity in experimental sites was driven by mortality due to decreased dissolved oxygen levels. Dissolved oxygen may have influenced diversity by affecting macroinvertebrate migration and activity. Further experimentation may clarify to what extent small changes in dissolved oxygen affect migration or activity levels of stream invertebrates.

Stream flow may also influence the benthic composition of streams. Decreased stream flow may allow the settlement of sand, silt, and pebbles. Previous studies have linked changes in functional feeding group composition with changes in silt buildup in streams (Pollard and Reed 2004). In addition, increased sand and pebble substrate can significantly reduce the density of stream inverte-

brates (Gurtz and Wallace 1984). Therefore, the decreased diversity in experimental sites may have been due to build up of sand and silt in those areas.

We did not find significantly different levels of detritus before and after our 24-hour study. However, buildup of allochthonous materials due to decreased flow rates may influence macroinvertebrate composition over a longer period of time. This potential detritus buildup should be considered when assessing the long-term impacts of changes in flow rate on stream ecology.

Our study suggests that decreased flow rate in streams, whether due to natural or anthropogenic causes, may decrease macroinvertebrate diversity, and thus greatly affect freshwater stream ecology. Further studies may investigate the extent to which these decreases in invertebrate diversity affect the structure and functioning of stream ecosystems as a whole.

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LACK OF HOST SPECIFICITY IN A HOLOPARASITIC SPECIES OF BALANOPHORACEAE

JENNIFER A. FREISE AND EMILY KYKER-SNOWMAN

Faculty Editor: David R. Peart

Abstract: Many tropical species are highly specialized. For example, holoparasitic plants depend entirely on other plants for nutrients. Because plant parasite-host relationships are chemically and physically complex, we hypothesized that a holoparasitic plant at Cuerici, Costa Rica (*Helosis* sp.) would be adapted to just one or a few hosts. We compared the relative abundance of potential host species within 1 m and 2 m radius circles around tubers to their relative abundance along haphazard transects through their habitat. No tree species occurred disproportionately within the circles. We concluded this is a generalist species, capable of parasitizing multiple hosts.

Key words: *Helosis*, *Secondary tropical forest*, *parasitism*

INTRODUCTION

High competition in the tropics drives many species to specialize. Holoparasites, which depend on parasitism for all essential nutrients, are one example; they have lost ancestral autotrophic structure and function to become heterotrophic. Balanophoraceae is a family of pantropical holoparasitic plants which parasitize tree roots in moist upland forests. Plants in this family have a large central softball-sized root tuber with upward-protruding inflorescences (Mabberley 1993).

It is not clear to what extent Balanophoraceae species are host-specific (Mabberley 1993). However, because the structural and chemical relationships between parasite plants and their hosts are intricate (Gómez 1983), and the protective adaptations of potential host trees are varied, we predicted that Balanophoraceae are adapted to specific host trees.

To test this hypothesis, we examined the degree of host specificity of one species of Balanophoraceae in the habitat where it was locally abundant. We identified the genus as *Helosis* due to its hexagonal scales (Gómez 1983, Mabberley 1993).

METHODS

We surveyed flowering *Helosis* sp. tubers in the upland oak forest near Cuerici Biological Station on 20 and 21 January, 2011. We surveyed three gullies in secondary forest. The gullies, where soils were moist and rich compared to the surrounding primary oak and bamboo forest, appeared to be the optimal habitat of *Helosis* sp. (Carlos Solano, pers. comm.).

Any tubers with live or recently rotted inflorescences or infructescences were included. Tubers without inflorescences were too difficult to locate. Our few shallow excavations revealed that inflorescences within a few meters of each other were connected and possibly ramets of the same genet. We decided to count each ramet as a physiologically independent individual if it was > 30 cm away from its neighbors.

Rather than risk damaging the root systems of tubers through extensive excavation, we chose to examine the proximity of tree species to tubers, under the assumption that tubers would be found more often by their preferred hosts. For each tuber, we noted the abundance and morphological type of each individual tree > 2 m tall within a 1 m radius circle and a 2 m radius circle. Trees which

were above this height were considered large enough to be a significant nutrient and energy resource to the tubers. To compare tree species composition near tubers to that in the gully habitat overall, we walked three haphazard, 2 m wide, 30 m long belt transects for each of our three sites. Along these transects we identified individuals of tree species > 2 m tall to morphological type. *Helosis sp.* individuals along transects that were not found within the tuber-centered circles were counted but not identified. Later, we identified morphological types, initially identified by an assigned letter, to species if possible.

Analysis

One important assumption in our study was that parasitic plants would be denser within the circles than along the transects. If tuber density were the same in circles and transects, any differences in species composition between them could not be attributed to the presence of *Helosis sp.* We used a t-test to compare the density of inflorescences between circles and transects.

To evaluate species composition differences, we ran a contingency analysis comparing the relative abundances of all species in 1 m radius circles, 2 m radius circles, and transects. We considered it unlikely that species that were uncommon overall could be the preferred host of *Helosis sp.*, given that tubers frequently occurred when these species were absent. Therefore, we excluded species with < 15 surveyed individuals overall. To evaluate differences in species composition in more depth, we calculated the relative abundance of each species in each circle and transect. Relative abundance was defined as the number of individuals of a species divided by the total number of tree individuals in a circle or transect. For each species, ANOVAs were performed to test for differences in relative ab-

undances between 1 m radius circles, 2 m radius circles, and transects.

RESULTS

Helosis sp. tubers were denser in both 1 m and 2 m radius circles than in transects. (ANOVA $F_{2,66}=64.17$, $P<0.0001$; Fig. 1).

The contingency analysis indicated differences between the overall composition of 1 m circles, 2 m circles, and transects ($\chi^2_{df=12}=29.57$, $P=0.003$). The ANOVA performed on relative abundance of each species showed that the differences between relative species abundance were non-significant between circles and transects except for one species ("species M"; ANOVA $F_{2,55}=10.28$, $P=0.0002$; Fig. 2). Species M was more abundant in transects than in 1 m circles (Tukey-Kramer HSD, $P=0.0002$) or 2 m circles (Tukey-Kramer HSD, $P=0.0006$).

DISCUSSION

Helosis sp. inflorescences were denser within circles than transects. We inferred that differences in species composition were related to tuber density.

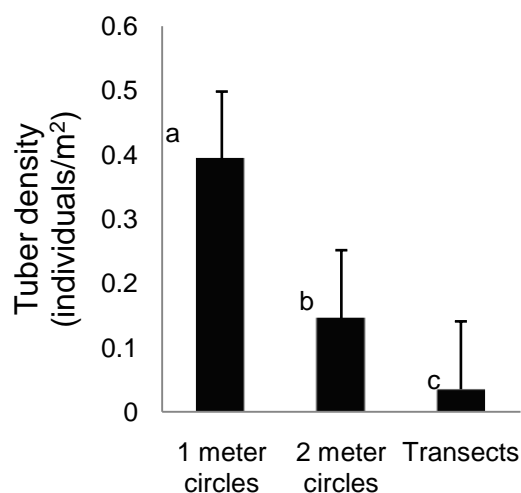


Fig. 4 Mean *Helosis sp.* tuber density (± 1 S.E.) in 1 m radius circles around tubers, 2 m radius circles around tubers, and haphazard transects through secondary forest gullies at Cuerici Biological Station, Costa Rica. Transects contained a significantly lower mean density of tubers than either size circle.

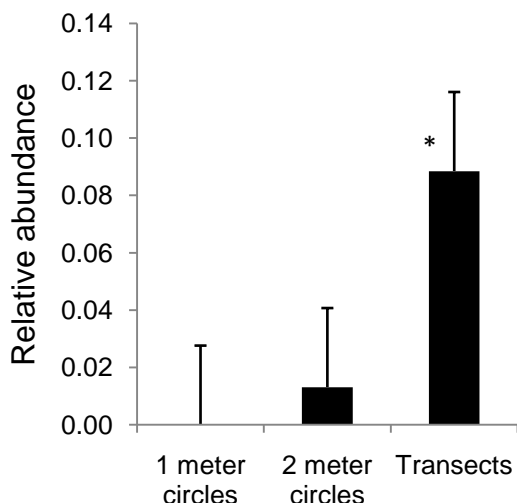


Fig. 2 Relative abundance of species M (\pm 1 S.E.) in 1 m radius circles around *Helosis sp.* tubers, 2 m radius circles around tubers, and haphazard transects through secondary forest gullies at Cuerici Biological Station, Costa Rica. Transects contained a significantly higher relative abundance of species M.

Our results showed that inflorescences are clumped. If tubers were evenly distributed at the average density found in transects, we would expect 15 m between tubers. However, 60% of inflorescences were within 2 m of at least one other. This clumping could be explained by host availability, local dispersal, clonal reproduction, or the availability of other resources.

Our contingency analysis showed that there was a difference between the species composition in circles and transects. However, the only significant relationship between tubers and individual species was a negative association with species M (we were unable to identify species M beyond morphotype). Removing M from the contingency analysis produced species compositions which were not significantly different. We therefore reject our original hypothesis that *Helosis sp.* specializes on a specific host species. Given that species M and *Helosis sp.* are found in the same habitat and that *Helosis sp.* seems to be a generalist, perhaps species M has defenses

against parasitism (e.g., allelopathy, tough roots, or secondary compounds). Future studies could look for evidence of such defenses.

An alternative hypothesis is that *Helosis sp.* is a partial saprophyte. *Monotropa*, or indian pipe, was long considered to be a parasite until it was shown that it sustains itself entirely on decaying organic matter (Gómez 1983). We observed that *Helosis sp.* was frequently found in areas with copious decaying organic matter, such as rotting logs and mats of dead lianas. Even if *Helosis sp.* is partially saprophytic, however, previous research has discovered connections between the roots of *Helosis sp.* and those of trees (Mabberley 1993), indicating that it is at least a partial parasite.

The implications of a generalist parasite are striking. To parasitize multiple hosts, *Helosis sp.* must develop intricate host/parasite relationships with trees whose physical and chemical defenses differ widely. Despite these difficulties, such a plant is less limited by host distribution. If *Helosis sp.* is able to supplement its nutrient uptake through partial saprophytism, the differences in suitability of host trees might not be very important. Future studies could examine the relative importance of parasitism and saprophytism to the success of *Helosis sp.*

ACKNOWLEDGEMENTS

We would like to thank our T.A.s, M.C. Duryea, S. Wengert, and G. Moritz for their help with statistical analysis and Professor D. Peart for his help with plant identification. Special thanks to Carlos Solano for sharing his extensive knowledge of the area surrounding Cuerici Biological Station.

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FACTORS AFFECTING GROWTH AND DISTRIBUTION OF TWO BAMBOO SPECIES (*CHUSQUEA LONGIFOLIA* AND *CHUSQUEA SUBTESSELLATA*) IN A HIGH-ELEVATION TROPICAL FOREST

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Faculty Editor: David Peart

Abstract: At Cuerici Biological Reserve near Cerro de la Muerte, Costa Rica, two species of bamboo (*Chusquea longifolia* and *C. subtessellata*) dominate the forest understory at different elevations. We examined abiotic and biotic factors that could affect the growth and distribution of the two bamboo species. Bamboo biomass was greatest on southeast-facing slopes and with a deep humus layer, and decreased with increasing relative abundance of oak leaves in the surrounding leaf litter. There was also a negative quadratic relationship between bamboo biomass and the basal area of the surrounding forest. *C. subtessellata* had significantly less biomass in the transition zone between the two species than at the high-elevation site where it occurred alone. Our findings suggest that light, nutrients, competition between bamboo species, and competition with oaks may drive growth and distribution of bamboo.

Keywords: light availability, nutrients, interspecific competition, *Quercus*

INTRODUCTION

Oak tree canopy dominance in high-elevation tropical forests is the most pronounced example of single-species dominance in Costa Rica (Hartshorn 1983). This dominance is unusual, as tropical forests generally display high tree species diversity (Hubbell 1979). Janzen (1983) suggested that oaks interact with bamboo, the dominant species in the understory, to influence the distribution, growth, and reproductive success of both species (Hartshorn 1983).

Two species of bamboo grow in the Cuerici Biological Reserve: *Chusquea longifolia* at lower elevations and *C. subtessellata* at higher elevations. The species overlap in a transitional zone between 2800 and 2900 m (Janzen 1983; pers. obs.). The transition between the low-elevation oak *Quercus copeyensis* and high-elevation oak *Q. costaricensis* occurs over a similar elevational range (pers. obs.). This suggests that oak and bamboo distributions may be causally related (C. Solano, pers. comm.).

In the biological reserve, these monocarpic bamboo populations masted and died in 1992, as confirmed by observations at three sites at that time (C. Solano, pers. comm.). Since bamboos at these sites are the same age, we were able to compare their growth rates using estimates of biomass alone. We hypothesized that bamboo would grow best in areas with the greatest light and nutrient availability. We predicted that deeper humus, lower basal area per hectare of surrounding trees, and gentler, eastward-facing slopes would be associated with increased bamboo biomass. We also predicted that the relative abundance of oak leaves in the leaf litter would correlate negatively with bamboo biomass because of antagonistic relationships between the two species (e.g. shading of bamboos by oaks and/or shading of oak seedlings by bamboo). Finally, we predicted that biomass of each species would be lower in the transition zone than in single-species sites because interspecific competition could limit growth where the species overlap.

METHODS

We collected data on 29 and 30 January 2011 near Cerro Cuerici, Cuerici Biological Station, Costa Rica. We sampled at the three sites noted in the Introduction: a *C. longifolia* site at 2700 m, a *C. subtessellata* site at 2900 m, and a transition site at 2800 m where the two species co-occurred. At both of the two single-species sites, we marked six 10 m transects haphazardly selected off the trail. We collected data at three pairs of 1 m² quadrats spaced at 3 m intervals along both sides of each transect. At the transition zone, we marked five transects, each with 2-6 quadrats.

We used a SkyMaster and Garmin76CSX GPS to measure air temperature, humidity, wind speed, and elevation at each quadrat. At each transect, we measured slope with a clinometer and aspect with a compass. At each pair of quadrats, we used a forester's cruising prism to quantify total tree basal area per hectare. In each bamboo clump, we used a representative shoot to estimate the average stem height. We also measured the basal circumference of the clump. To quantify an index of bamboo biomass, we multiplied stem height by basal area. In each quadrat, we summed these biomass indices.

We took cores in each quadrat to measure humus depth. We measured wet mass and dry mass of the humus samples using an Ohaus Valor 3000 Xtreme balance. We dried humus in the sun on aluminum foil for > 12 hr. To quantify soil moisture, we divided the difference between wet mass and dry mass of each sample by its wet mass. We also sampled all of the leaf litter from a 30 x 30 cm area in each quadrat to determine the relative abundance of oak leaves.

Analysis

We ran regressions to determine the relationship between bamboo biomass and the

relative abundance of oak leaves in the leaf litter, and to examine the associations of humus depth and basal area with bamboo biomass. We used a t-test to compare mean biomass at each site by species and ANOVA to determine the effect of slope aspect on bamboo biomass. Finally, we ran regressions to examine the effect of slope on both bamboo biomass and oak leaf relative abundance in the litter. We used JMP 8.0 for all statistical analyses.

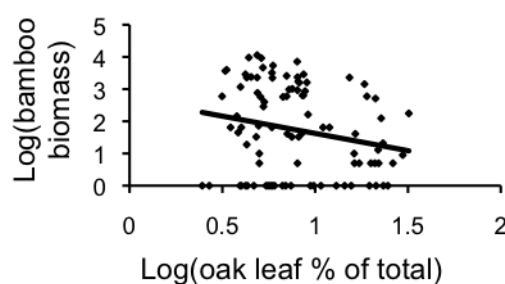


Fig. 1. Bamboo biomass against oak leaf relative abundance in the leaf litter on Cerro Cuerici, Costa Rica.

RESULTS

The proportion of oak leaves in the leaf litter was negatively related to bamboo biomass ($r^2_{94} = 0.05$, $P = 0.04$; Fig. 1). Humus depth varied positively with the biomass of both species of bamboo (*C. longifolia*: $r^2_{32} = 0.26$, $P = 0.002$; *C. subtessellata*: $r^2_{34} = 0.26$, $P = 0.002$; Fig. 2). The biomass of *C. subtessellata* was significantly lower at the transition site than at the higher elevation site ($t_{20,0} = -3.43$, $P = 0.003$; Fig. 3), while the biomass of the *C. longifolia* did not change between the transition and lower elevation site ($t_{5,05} = -1.08$, $P = 0.33$; Fig. 3). There was a weak negative quadratic relationship between bamboo biomass and total tree basal area ($r^2_{93} = 0.09$, $P = 0.01$; Fig. 4). Bamboo biomass was significantly greater on southeast-facing slopes than west- and northwest-facing slopes, with east-facing slopes being intermediate ($F_{3,92} = 4.52$, $P = 0.005$; Fig. 5). The relative abundances of both

oak and bamboo species were related to slope, with oak abundance increasing ($r^2_{94} = 0.47$, $P < 0.0001$) and bamboo abundance decreasing ($r^2_{94} = 0.11$, $P = 0.001$).

DISCUSSION

If bamboo is light-limited, high levels of morning sunlight in the east may explain the greater bamboo biomass on southeast- and east-facing slopes. In the afternoon, Cerro Cu-erici becomes cloudier, so less light reaches west-facing slopes (C. Solano, pers. comm.).

Since bamboos have shallow, fibrous roots (in contrast to the deep, woody roots of oaks), bamboos may not establish and survive as successfully as oaks on steep slopes. This is consistent with our findings that slope has a negative relationship with bamboo biomass and a positive relationship with oak abundance.

The negative quadratic relationship between total bamboo biomass and basal area per hectare implies that intermediate basal area of surrounding trees maximizes bamboo

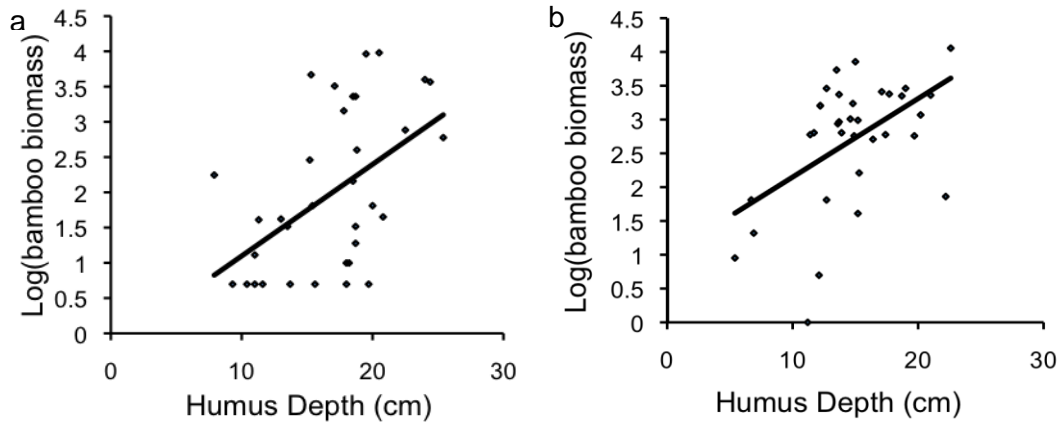


Fig. 2. (a) *C. subtessellata* and (b) *C. longifolia* biomass against humus depth on Cerro Cu-erici, Costa Rica.

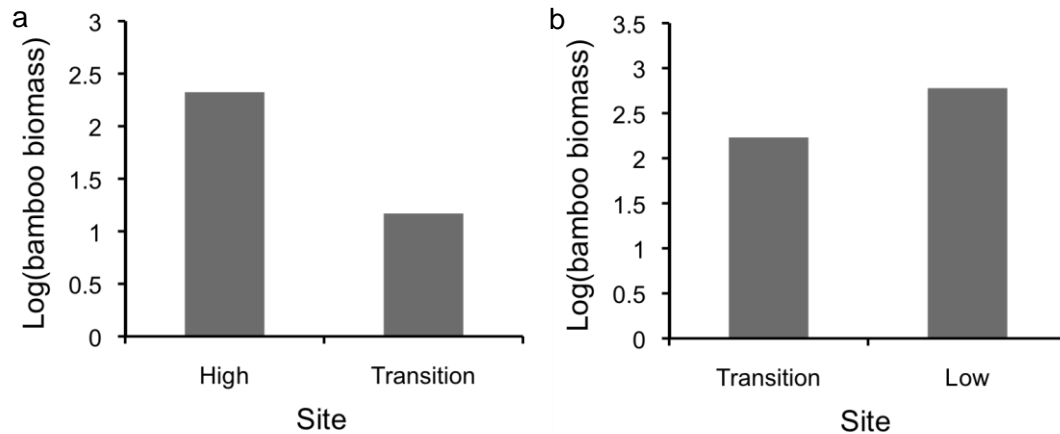


Fig. 3. Biomass of (a) high-altitude bamboo species *C. subtessellata* and (b) low-altitude bamboo species *C. longifolia* at the high elevation *C. subtessellata* site (2900 m), low elevation *C. longifolia* site (2700 m) and transition site where the two species co-occurred (2800 m), on Cerro Cu-erici, Costa Rica. Differences in biomass between sites of (a) *C. subtessellata* were significant but differences in biomass of (b) *C. longifolia* were not significant. Error bars represent ± 1 SE.

growth. Low neighboring tree basal area may indicate a harsh local habitat for all vegetation. When neighboring basal area is high, trees may shade bamboo shoots and compete with them for nutrients. An intermediate density of nearby trees could aid bamboo growth by facilitating nutrient cycling and adding organic matter to the humus layer. Soil humus is a reservoir of nutrients for plants, and deeper humus may promote bamboo growth if nutrients are limiting. Biomass for each species of bamboo was positively correlated with humus depth, consistent with this logic.

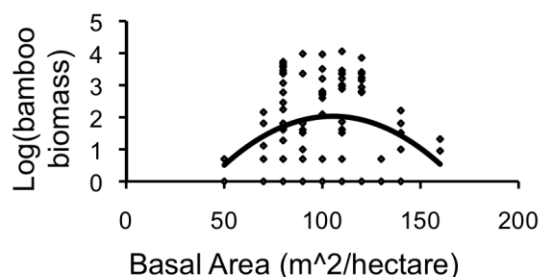


Fig. 4. Relationship between bamboo biomass total tree basal area per hectare on Cerro Cuerici, Costa Rica.

C. subtessellata biomass decreased significantly at the periphery of its range, in the transition zone. *C. longifolia* biomass also decreased in the transition zone, but not significantly. Competition for resources between the two bamboo species may drive decreased biomass in the transition zone and contribute to the abrupt distributional limit in both species.

Braden et al. (2001) found that tannins in oak leaves increase soil acidity, which may negatively affect bamboo growth. This may explain the negative association between bamboo biomass and oak abundance. Also, more oak trees may increase shading, inhibiting growth of bamboo if bamboo is light limited. In turn, bamboo shoots may prevent the regeneration of oaks by blocking light from reaching oak saplings (Hartshorn 1983).

The mass die-off of bamboo periodically clears the understory, providing an approximate ten-year window for oak colonization (Janzen 1983, C. Solano, pers. comm.). In addition, oak seedlings may colonize steep slopes, where, according to our results, bamboos do not dominate the understory.

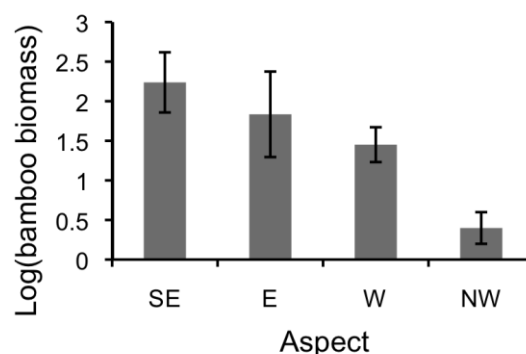


Fig. 5. Bamboo biomass on various slope aspects on Cerro Cuerici, Costa Rica. Error bars represent ± 1 SE.

Other abiotic factors we measured were not associated with bamboo distribution and density, including humidity, wind speed and soil moisture. However, we have low confidence in these relationships because our measurements were taken over a two-day period and therefore are not representative of the local climate.

Future studies could focus on measuring environmental variables over the course of at least one year to build a comprehensive picture of the relationship between bamboo growth and climate. In addition, further research could quantitatively investigate the effects of light on bamboo growth and measure biomass and distribution more accurately. It may be interesting to further examine the tight distributional relationship between these bamboo species and oak trees by studying the factors behind the distribution of *Q. copeyensis* and *Q. costaricensis* in Cuerici. Finally, the next mass flowering event is approaching at Cuerici (C. Solano, pers. comm.), which pro-

vides an opportunity to investigate the effects of mass bamboo mortality on oak regeneration and the dynamics of high elevation tropical forests.

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We would like to thank Carlos Solano at Cuerici Biological Station for sharing his expansive knowledge of local natural history. Also, we express much gratitude to Professor David Peart and the course TAs (Katie Duryea, Gillian Moritz, and Sarah Wengert).

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GRAZING INTENSITY AND OPTIMIZING PRODUCTIVITY IN CATTLE PASTURES

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Faculty Editor: David Peart

Abstract: As agriculture expands to meet growing food demands, sustainable productivity is increasingly important. In a grazing system, this requires balancing presently available vegetation for cattle grazing with nutrients stored in the soil. We hypothesized that in rotationally-managed pastures at low grazing intensity, fields left fallow should accumulate above-ground biomass without attrition of the humic layer. We also hypothesized that a currently-grazed, rotationally managed pasture at low grazing intensity would have more above-ground biomass than a continuously-grazed pasture at higher grazing intensity. We sampled depth and color of humus and collected above-ground biomass in five cattle pastures at Cuerici Biological Station in Costa Rica, four of which were under rotational management at low grazing intensity. The fifth was continuously grazed at higher intensity. As hypothesized, humic depth did not differ among pastures, despite accumulation of biomass in fallow fields. But contrary to our second hypothesis, more intensive grazing was associated with higher above ground biomass, possibly because of accelerated nutrient cycling and increased growth of herbaceous vegetation. We conclude that higher grazing intensity may increase productivity in the rotationally-managed pastures.

Key words: Humus layer, organic matter, cattle grazing, nutrient cycling

INTRODUCTION

Cattle-ranching is resource-intensive, requiring careful management to be sustainable (Tilman et al. 2002). Sustaining pasture productivity requires balancing presently available vegetation for grazing with future resources, such as organic material in the soil.

Maintaining this balance may be achieved by managing the intensity of grazing, manipulating the density of cattle, and rotating cattle between pastures. Pasture rotation allows fallow fields to regenerate leafy vegetation and recover soil nutrients.

In pastures, cattle return nutrients to the soil from above-ground vegetation in the form of manure and urine. Manure is then incorporated into humus, the layer of decomposing organic material that serves as a pool of nutrients for plant uptake (FAO Natural Resources Management and Environment Department 2005). Nutrients in this layer can later be cycled into above-ground plant matter (Wardle et al. 2004). If a pasture is overgrazed, cattle can consume the above-ground

vegetation faster than it can regrow. The lack of regrowth reduces the available nutrients in above-ground biomass, thereby interrupting the nutrient cycle and potentially allowing the soil to erode.

We examined the six-month dynamics of nutrient storage in cattle pastures in a two-day study by sampling pastures at known stages of the fallow cycle. We hypothesized that rotationally-managed fields would accumulate above-ground biomass without loss of humic depth and quality during their fallow periods. We reasoned that because dynamics of the humic layer are slow, the impact of cattle grazing should not be great enough to change the pool of humic material over a six-month period.

We also hypothesized that with more intensive grazing, above-ground biomass should decrease. Specifically, we predicted that a rotationally-managed field at low grazing intensity would have greater above-ground biomass than a continuously-grazed field with a higher grazing intensity.

Finally, we hypothesized that higher qual-

ity humus, represented by darkness of humus, should be positively related to higher above-ground biomass.

METHODS

We sampled five cattle pastures near Cuerici Biological Station, Costa Rica on 29 and 30 January 2011. Four were at various stages in a rotational grazing system with two cattle. Three of these pastures had been left fallow for 1 month, 2-3 months, and 4-5 months, respectively. One of the four was currently being grazed with an estimated intensity of 0.80 cow-hr/m² ("lower grazing intensity"). We quantified grazing intensity using cow-hr/m², where one cow-hr/m² means that over the entire 6-month period of the rotational cycle, each m² of the pasture was grazed on average by one cow for one hour. We also sampled in a pasture that had been grazed for six months by four cattle, in a continuous, non-rotational system, with an estimated intensity of 2.94 cow-hr/m² ("higher grazing intensity").

We used stratified random sampling in each pasture, which we divided into four, eight, or ten approximately equal areas by pacing, depending on the size of the field. Sets of coordinates for quadrat locations in each area, totaling twenty samples per field, were randomly generated using a stopwatch. We removed all above-ground organic material within each 20 cm x 20 cm quadrat using scissors. The collected organic material was sealed in an airtight bag and then wet-massed with a Highland Adam 120 g scale. We took a soil core within each quadrat using an Oakfield (Model H) 2 cm-diameter corer and measured the depth of the humic layer. Darkness of humus was used as an index of humus quality, because more organic matter content creates a darker soil (Romig et al. 1995). We estimated darkness on a scale from 1-4, with 1 representing the lightest soil.

We conducted a two-sample t-test to compare mean above-ground biomass between pastures with lower and higher grazing intensities. We performed ANOVA and Tukey-Kramer tests to compare mean above-ground biomass across pastures and across sites of varying humic darkness. Statistical analyses were performed using JMP 8.0.

RESULTS

In accordance with our hypothesis, the above-ground biomass was significantly higher in the pasture that had been fallow for 4-5 months ($\bar{x} = 75.73$, $SE = 4.72$, $n = 20$) compared to all other, more recently grazed pastures under rotational management (0 months: $\bar{x} = 32.53$, $SE = 4.72$, $n = 20$; 1 month: $\bar{x} = 32.83$, $SE = 4.72$, $n = 20$; 2-3 months: $\bar{x} = 40.65$, $SE = 4.72$, $n = 20$; $F_{3,76} = 18.91$, $P < 0.0001$, Fig. 1). Contrary to our hypothesis, higher grazing intensity led to significantly higher above-ground biomass than lower grazing intensity ($t_{31.50} = 2.54$, $P = 0.02$, Fig. 2). As we hypothesized, there was no significant difference in humic depth ($F_{3,74} = 0.92$; $P = 0.44$) nor humic darkness ($F_{3,74} = 0.26$; $P = 0.85$)

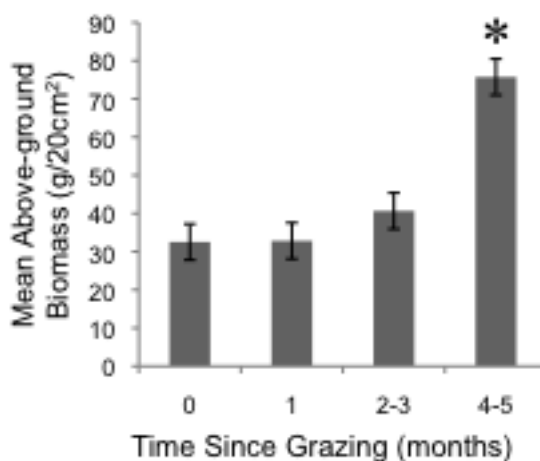


Fig. 1 Above-ground biomass (means \pm 1 S. E.) in pastures at different stages of the rotational cycle, at Cuerici, Costa Rica. The asterisk indicates significant difference from all other bars.

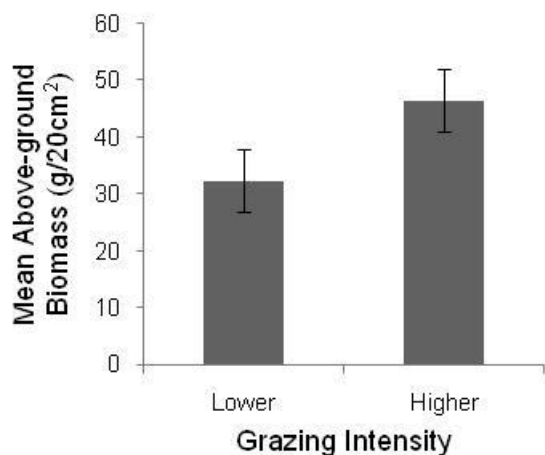


Fig. 2 Above-ground biomass (means \pm 1 S. E.) in two currently grazed pastures with different grazing intensities at Cuerici, Costa Rica. Lower intensity refers to two cattle per pasture and rotation of cattle among pastures. Higher intensity refers to four cattle per pasture and no rotation of cattle among pastures.

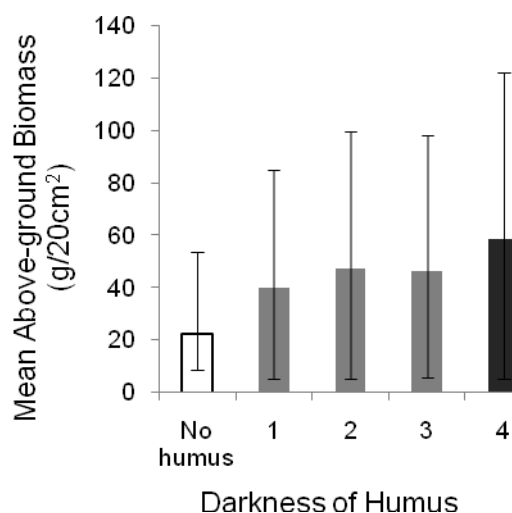


Fig. 3 Above-ground biomass (means \pm 1 S. E.) in patches of varying darkness of humus in cattle pastures at Cuerici, Costa Rica. Humus, when present, was ranked by darkness on an ordinal scale from 1 (lightest) to 4 (darkest). The white and black bars are significantly different from each other; the grey bars are not significantly different from either white or black bars.

across pastures under rotational management. Also consistent with our hypothesis, sites with the darkest humus had significantly higher above-ground biomass than sites with no humic layer ($F_{4, 93} = 3.63$, $P = 0.009$; Fig. 3). Above-ground biomass for sites

with intermediate darkness of humus were not significantly different from values for other sites.

DISCUSSION

Our data support our hypothesis that the fallow fields had more above-ground biomass than currently grazed fields. However, contrary to our hypothesis, there was more above-ground biomass in fields that were grazed more intensively. From this, we conclude that higher grazing intensity may increase the productivity of the rotationally-managed pastures.

In the rotational system, the pasture that had been fallow for 4-5 months had significantly more above-ground biomass than the more recently grazed fields (Fig. 1), in part because of abundant woody blackberries and mature ferns. The nutrients in woody material are less concentrated and less readily available, locked up in more complex, cellulosic carbohydrates that cattle are less likely to consume. Decomposition of woody plants is also slower than for herbaceous plants, reducing the speed of nutrient cycling. As a result, allowing a field to remain fallow for too long and become dominated by woody plants may decrease its productivity. We acknowledge that the fields may have differed in their abundances of blackberries even at the same stage of the rotational cycle. However, we assume that grazing is a larger control of blackberry abundance because cattle are known to graze young blackberry shoots.

Although the fields had different above-ground biomass, all had similar nutrient pools based on similar humic layer depth and quality. In a pasture, above-ground biomass typically represents only a small fraction of total organic matter present; decomposition of current above-ground vegetation would not substantially alter the pool of humic material.

Similarly, leaving a field fallow long enough to accumulate blackberries did not apparently alter the humic layer. Thus, future productivity of these fallow fields has not been degraded, at least on a six-month time scale.

Comparing the currently-grazed pastures, the field with twice as many cattle had more above-ground biomass (Fig. 2), so the vegetation there must have a much higher growth rate than in the field with lower grazing intensity. Higher grazing intensity may increase vegetation growth rate by accelerating nutrient cycling. Cattle release nutrients for new plants in the form of manure, which decomposes more quickly than grass. Much of the biomass in the field with four cows (higher grazing intensity) was grassy, not woody. The nutrients then are held in the more readily available grasses rather than slow-decomposing woody material. Higher grazing intensity may remove young blackberry shoots, reducing competitive pressure on grasses.

Undergrazing, as seen in the rotationally managed fields with two cows, allows woody plants to proliferate in fallow fields and may maintain a slower nutrient cycling rate. A higher grazing intensity may therefore increase productivity by promoting growth of grasses and rapid nutrient cycling.

There is more above-ground biomass associated with the darkest humus (Fig. 3) Because there is a pool of nutrients stored in humus, fields with the darkest humus should support more above-ground biomass in the future. Thus, it is important for managers to monitor the humic quantity and quality as an indicator of the sustainability of grazing practices, rather than focusing entirely on the visible above-ground biomass.

We conclude that the rotationally managed fields, in their currently under-grazed state, appear to be managed sustainably. Be-

cause the more intensely grazed pasture supported greater above-ground biomass without a decline in soil quality, we suggest that increasing grazing intensity in the rotationally-managed fields may increase productivity without compromising sustainability.

ACKNOWLEDGEMENTS

We would like to thank the staff at Cuerici Biological Station for their assistance and support. We would especially like to thank Carlos Solano for sharing his wealth of knowledge.

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WATER QUALITY AND ARTIFICIAL SELECTION IN A TROPICAL HIGH ELEVATION TROUT AQUACULTURE SYSTEM

REBECCA M. NIEMIEC, SARA E. REMSEN, ANDREW N. MERTENS, AND LARRY L. BOWMAN, JR.

Faculty Editor: David R. Peart

Abstract: Aquaculturists use a variety of management strategies to improve productivity. However, small-scale operations may lack data to guide management strategies in response to economic demand and environmental variation. At Cuerici Biological Station, Costa Rica, we sampled water quality and artificial selection in Rainbow trout aquaculture. We determined length and weight cutoffs for selection, measured dissolved oxygen and pH of trout tanks, and created a life-stage diagram of the trout to provide quantitative information for management.

Key words: Rainbow trout, hatchery management, fish farming, artificial selection

INTRODUCTION

Cuerici Biological Station has a small-scale trout hatchery and aquaculture system at 2700 m in the San Jose province of Costa Rica. It is the only privately operated hatchery in Costa Rica. Cuerici trout farmers attempt to mimic natural environmental conditions and artificially select the most robust trout to ensure higher quality fish.

Trout have demanding habitat requirements. Klontz found that a pH outside the 6.5-8.5 range caused slow growth, gill damage, and death (1991). Optimal growth is achieved in a temperature range from 12-25°C. Dissolved oxygen concentrations below 5 mg/L can cause slow growth and a buildup of harmful materials that can lead to infection (Cowx 2005).

We quantified artificial selection from the juvenile stage to the reproductive adult stage. We also measured pH, temperature, and dissolved oxygen levels in the aquaculture system at trout tanks and stream sites to compare conditions over time using previous data (Wengert et al. 2009).

METHODS

We measured temperature, pH, and dissolved oxygen content at sites 0-18 (Fig. 1) throughout the aquaculture system from 9:00-11:00 on 29 January 2011 and from 15:00-17:00 on 30 January 2011. The measurements included both stream sites and the input and output of trout tanks.

We participated in the selection of juveniles that will become reproducing adults. On 29 January 2011, we cleaned the tank at site 9.1 and relocated 200 juveniles from site 4 to site 9.1. We then cleaned tank 4 and refilled it with water. In order to minimize stress, we moved the fish a day prior to selection. On 30 January 2011, we weighed and measured the standard length (snout to caudal peduncle) of each fish.

Fish that were not selected for reproduction were moved to site 10. All other fish were placed in tank 4, where they will remain until reproductive age.

We used the farm's 2010 estimate of trout abundance at each stage to create a life-stage chart (Fig. 5). We used JMP 8.0 for all data analyses.

RESULTS

We found higher dissolved oxygen and pH in 2011 compared to 2009 but no difference in temperature (DO: $t_{64} = 3.80$, $P < 0.0002$; pH: $t_{78} = 31.03$, $P < 0.0001$; temperature: $t_{109} = 0.94$, $P = 0.17$; Fig. 2). We excluded sites 0, 17, and 18 because data were not collected for both years. Using t-tests, we found no difference in the DO, pH, or temperature between the input and output of each respective site in either year.

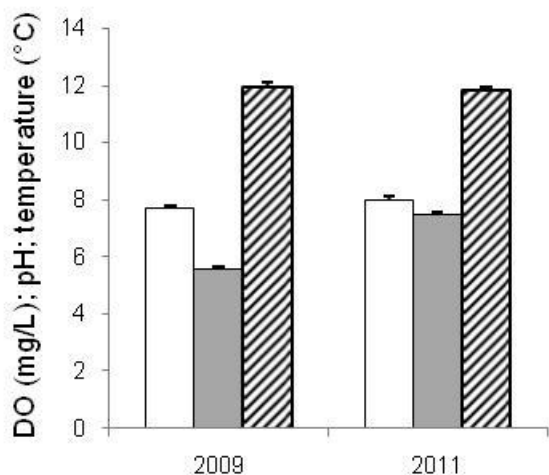


Fig. 2 Comparison of mean dissolved oxygen (white bar), pH (gray bar), and temperature (striped bar) from sites 1-16 between 2009 and 2011 at the Cuerici trout farm, Costa Rica (means \pm 1 S.E.).

Ninety percent of the 190 female trout were selected to become reproductive adults (Fig. 3). All 10 males were selected for reproduction because of the low abundance of males. Cuerici farm has mostly females in its reproductive stock because only a few males are needed to fertilize all the females. We measured standard length (snout to caudal peduncle) in cm and weight in g of every 15-month-old trout that went through selection. Fish were selected if they were >25 cm in length, or weighed >20 g. The mean length and weight of the total population were 26.8 cm and 26.4 g, respectively. The selected fish had mean a length of 27.1 cm and weight of 27.2 g, that

were higher than the mean length and weight of the non-selected fish, at 24.4 cm and 20.8 g, respectively (t-test length: $t_{37} = 6.97$, $P < 0.0001$; weight: $t_{41} = 9.43$, $P < 0.0001$; Fig. 4).

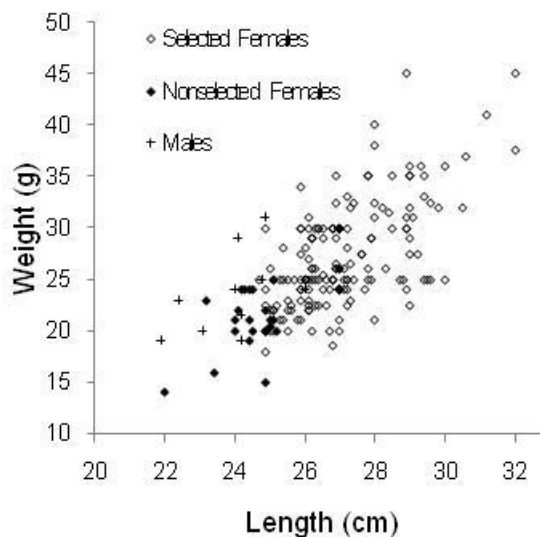


Fig. 3. Selection criteria for juvenile trout (length and weight for selected females, non-selected females, and males) at Cuerici trout farm, Costa Rica.

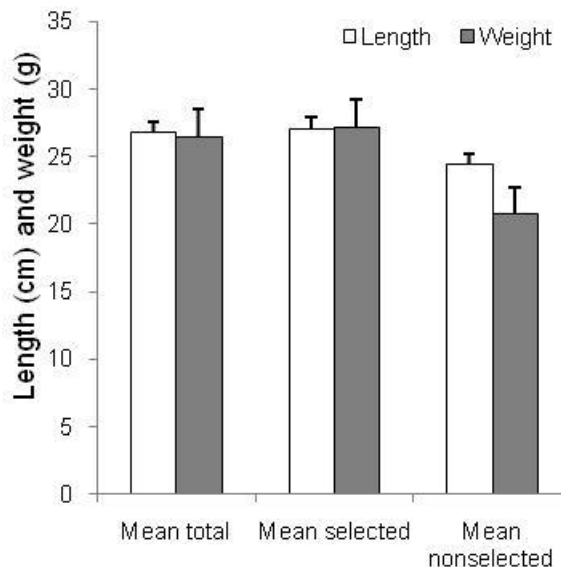


Fig. 4. Mean length and weight for the total population of juvenile trout, trout selected for reproduction, and trout not selected for reproduction at Cuerici trout farm, Costa Rica, in 2011 (means \pm 1 S.E.).

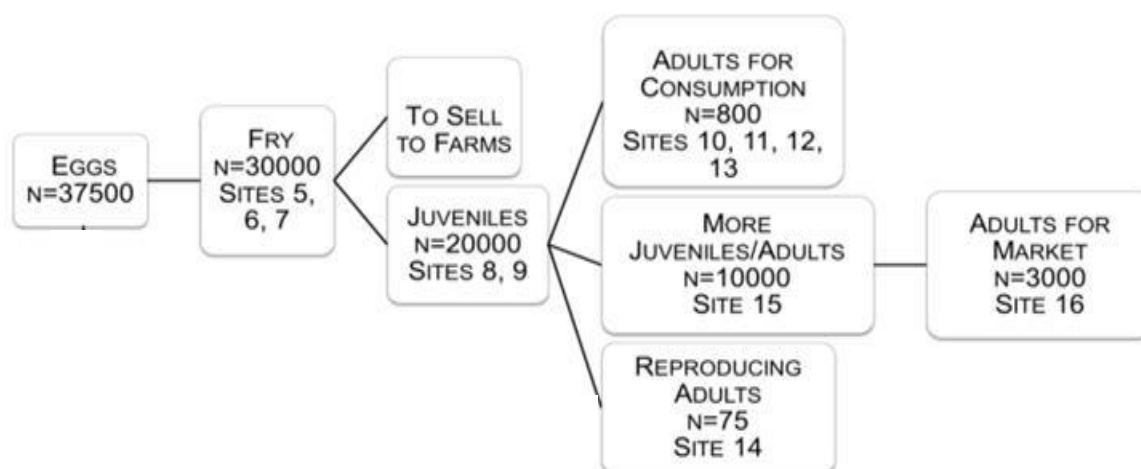


Fig. 5. Trout life stages in the Cuerici trout farm, Costa Rica, and the tanks in which each stage is present. N= number of individuals in each stage and F= number of eggs per reproducing adult.

DISCUSSION

While the measures of oxygen and pH were lower in 2009, we are not sufficiently confident in the accuracy of measurements (especially as the 2009 authors reported difficulties with calibration) to infer a difference in the values between 2009 and 2011. Although the mean dissolved oxygen in 2011 was 0.3 mg/L higher, such small differences in dissolved oxygen do not affect fish health (Klontz, 1991). The mean pH increased from 5.6 to 7.5 from 2009 to 2011, which was surprising given Klontz's (1991) finding that Rainbow trout typically live in a pH range of 6.5-8.5. The 2009 pH values may have had deleterious effects on the trout.

We determined the length and weight selection criteria used by the manager. These data may be used to help consumers make educated decisions about the quality of Cuerici trout and to inform other aquaculture operations about Cuerici's selection strategies.

Finally, our life-stage diagram (available in *Dartmouth Studies in Tropical Ecology* metadata files) documents how trout are moved

through the tanks in the aquaculture system (Fig. 5). There, we provide rough estimates of the numbers selected to proceed to each age class in 2010. Future studies may use this information to create a model of the trout farm that will allow for manipulation of selectivity between stage classes to maximize production.

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Cuerici Biological Station Trout Hatchery

updated January 2011

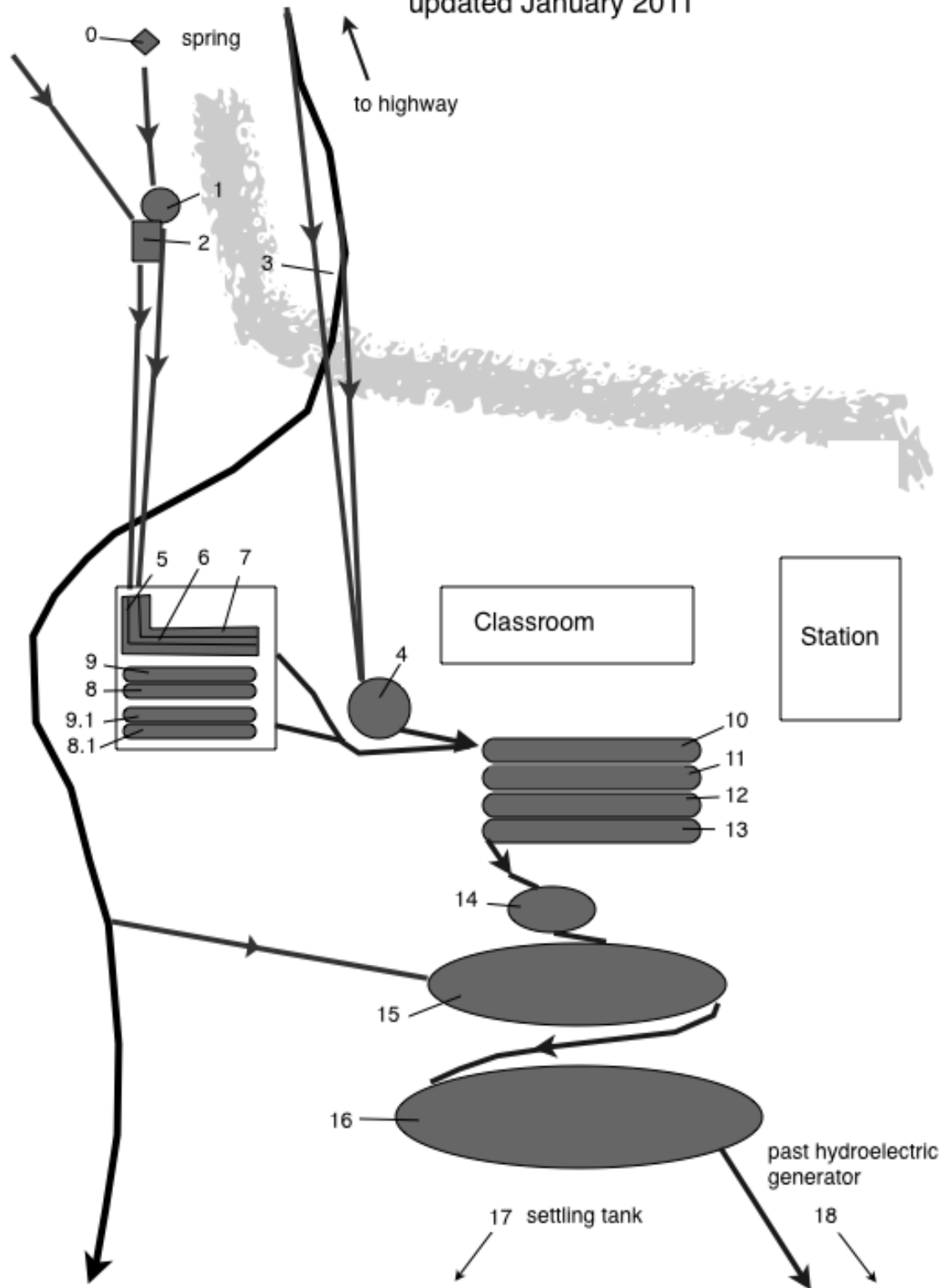


Fig. 1 Map of water tanks sampled at 19 sites at the Cuerici trout farm in Costa Rica, updated with sites 0 and 18 from Wengert et al., 2009. Sites 0-3 were stream sites, sites 4-16 were tanks housing Rainbow Trout, site 17 was the settling tank for trout waste, and site 18 was downstream from the hydroelectric generator.

NAVIGATION STRATEGIES IN FORAGING *P. CLAVATA* ANTS

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Abstract: Bullet ants (*Paraponera clavata*) have a simple social structure and an opportunistic foraging strategy. Individuals usually forage independently using multiple orientation cues. We hypothesized that *P. clavata* use both pheromones and visual cues, flexibly and opportunistically, to navigate while foraging. We manipulated the local environment by relocating and eliminating pheromone trails and rearranging small-scale visual cues between the nest and an artificial nectary. We found that bullet ants can increase their foraging efficiency by utilizing pheromone trails. We also found that they use small-scale visual cues to return to the nest and may use large-scale visuals for orientation.

Key words: Bullet ant, pheromone, orientation, visual cues

INTRODUCTION

Foraging behavior for most species of ants tends to be highly organized among multiple active individuals. For example, eusocial ants, such as leafcutters, maintain organization while foraging by using pheromones to mark and maintain precise trails (Wetterer et al. 1992).

Bullet ants (*Paraponera clavata*) lack a fully developed caste system and individuals forage independently (Breed 2002, Young and Hermann 1988). Bullet ants are flexible, opportunistic foragers because they forage both terrestrially and arboreally for nectar, plant parts, and arthropods (Young 1977, Young and Hermann 1980).

Bullet ant workers may use both pheromone trails and visual cues such as canopy cover to navigate in complex terrain (Baader 1996). However, neither visual cues nor pheromone trails are entirely sufficient for orientation to and from the nest, suggesting that the two cues may complement each other (Ehmer 1999). We hypothesized that bullet ants can utilize a flexible combination of pheromone trails and small-scale visual cues to navigate while foraging.

We predicted that when pheromone trails and small-scale visual cues were removed, the ants would take more time to return to the nest. We also predicted that bullet ants would use pheromone trails to recruit other ants to the nectary and that removal and relocation of these trails would reduce the rate of arrival at the nectary.

As we uncovered more complex navigational strategies in *P. clavata* than we initially expected, we adjusted our treatment manipulations accordingly. We designed experiments to complement previous studies and to examine the relative importance of pheromones and visual cues for navigation between the nest and a food source.

METHODS

We performed four types of manipulations of *P. clavata* foraging behavior on 13 and 14 February 2011. Trials were performed at a colony at 510 m in the arboretum at La Selva Biological Station, Costa Rica. We constructed each nectary by placing six cotton balls saturated with 20% sucrose solution on a small plastic bag 2 m away from the nest. We placed a 55 cm x 75 cm plastic bag covered in homogenous soil on the ground between the

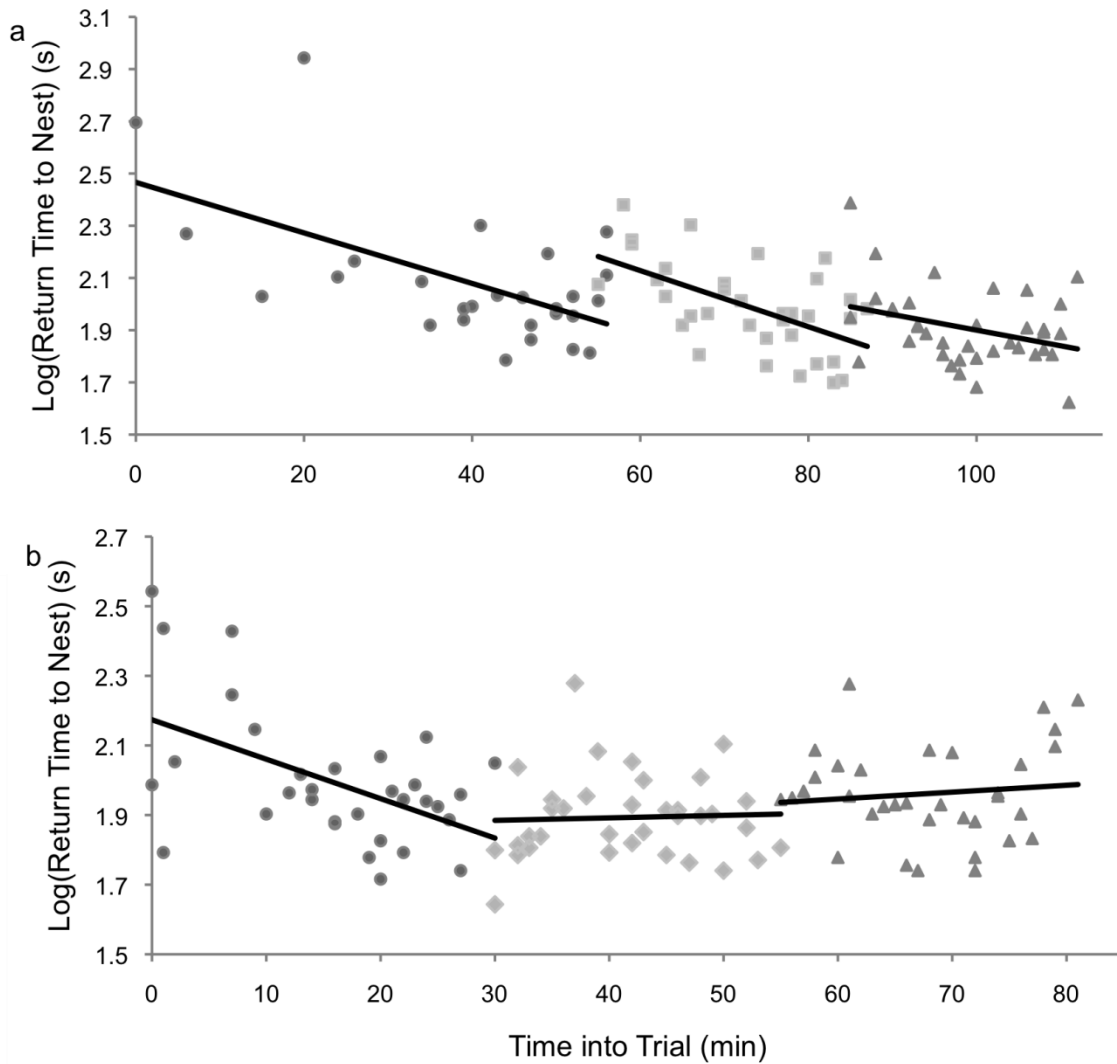


Fig. 1. Return time (s) from an artificial nectary to the nest in (a) the morning and (b) the afternoon of 13 February 2011 in the arboretum of La Selva, Costa Rica. (a) Circles represent ants during the baseline treatment, squares represent ants during the negative control, and triangles represent ants during the visual manipulation. (b) Circles represent ants during the baseline treatment, diamonds represent during the pheromone manipulation, and triangles represent ants during the visual manipulation.

nectary and the nest. We placed two rocks of about 15 cm diameter and two sticks about 50 cm long as small-scale visual cues on the plastic. We assumed that a pheromone trail was established on the soil-covered plastic bag and that baseline activity was reached when the time to return to the nest stabilized, ca. 40–60 min. after the placement of the nectary. For each trial, we measured recruitment rate by counting the number of ants arriving at the nectary every minute after the first ant's ar-

rival. We also recorded the elapsed time of haphazardly selected ants leaving the nectary until they returned to the nest.

Placement Manipulations

In the first treatment (“negative control”), after establishing baseline activity in the morning of 13 February, we disturbed both the newly created pheromone trails and the small visual cues. We carefully removed the plastic with the pheromone trail and replaced it with a new soil-covered plastic sheet. We

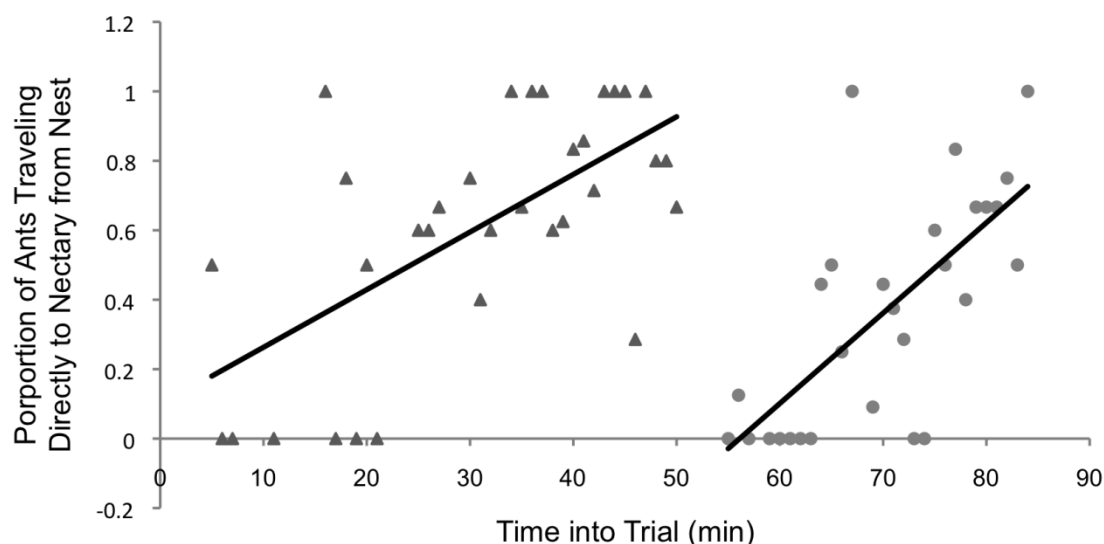


Fig. 2 The proportion of *P. clavata* that travel directly from the nest to an introduced nectary. Triangles represent ants leaving during the baseline treatment, while circles represent ants leaving during the rotation manipulation on the afternoon of 14 February 2011. Experiment was conducted in the arboretum in La Selva, Costa Rica.

rearranged the small-scale visual cues on the new plastic bag in a different configuration. We continued the trial ca. 20-30 min, until return time to the nest was constant.

Next, we rearranged the visual cues, without disturbing the pheromone trail ("visual manipulation" treatment). We again continued the trial until the return time stabilized.

We then removed the nectary for four hours and returned in the afternoon. We replaced the nectary and allowed the ants to establish baseline activity. Next, we removed the soil-covered plastic with the established pheromone trail ("pheromone manipulation" treatment) and replaced it with a new plastic sheet. We replaced the visual cues in the same location.

After the ants had re-established baseline return times, we repeated the visual manipulation.

Rotation Manipulation

In the early afternoon of 14 February 2011, we allowed the ants to establish baseline foraging behavior. Next, we rotated the plastic

bag 90° at the nectary without disturbing the newly created pheromone trails and the small-scale visual cues. We continued to measure ant recruitment rate. We noted whether an ant actively dragged its abdomen along the ground while returning to the nest, presumably laying a pheromone trail. We also measured return time and the proportion of ants that returned directly to the nest instead of following the rotated pheromone trail. We also counted and calculated the proportion of ants leaving the nest that travelled directly to the nectary.

RESULTS

For each of our morning treatments (baseline, negative control, and visual manipulation), return time to the nest declined over time, according to a negative logarithmic curve (baseline $r^2_{25} = 0.34$, $P < 0.01$?; negative control $r^2_{32} = 0.32$, $P < 0.01$); visual manipulation $r^2_{35} = 0.10$, $P = 0.05$; Fig. 1). In the afternoon, there was also a negative logarithmic relationship between return time and time into the trial for the baseline treatment ($r^2_{30} =$

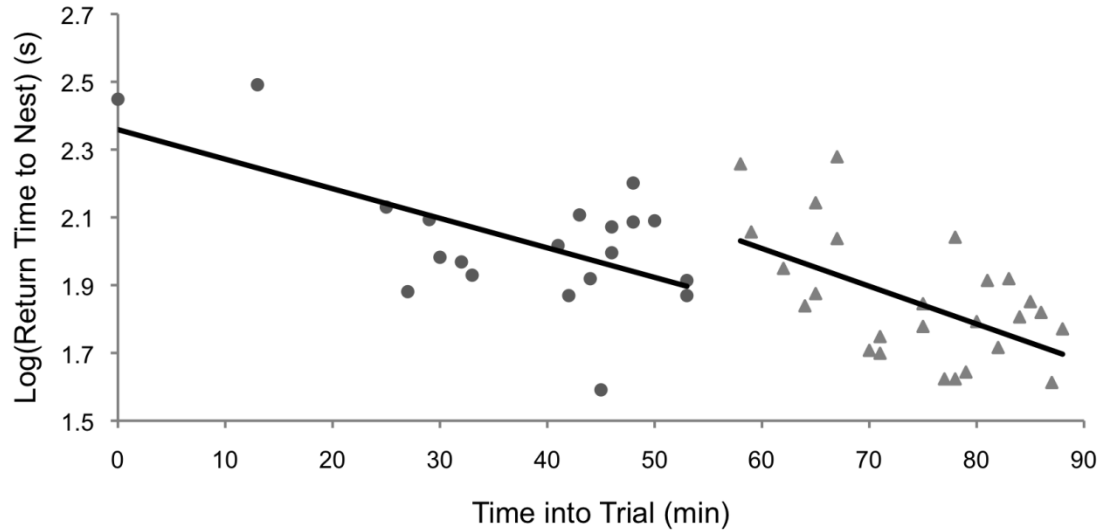


Fig 3. *P. clavata* return time (s) in relation to time into trial (min). Circles represent ants returning to the nest during the baseline treatment. Triangles represent ants returning to the nest during the rotation manipulation. Experiment was conducted on 14 February 2011 in the arboretum in La Selva, Costa Rica.

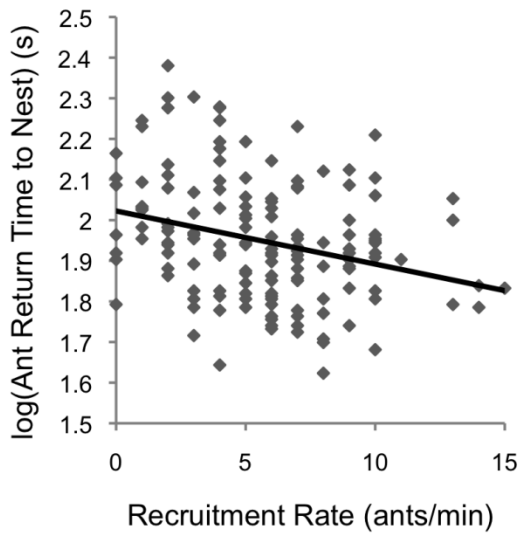


Fig. 4. Relationship between the recruitment rate of ants (ants/min) to a nectary and *P. clavata* return time (s). Ants were observed for 4 hrs along the arboretum in La Selva, Costa Rica on 13 and 14 February 2011.

0.26, $P < 0.01$; Fig. 1). However, for the pheromone and visual manipulations, we saw no significant change in return time over the course of the trial (pheromone manipulation $r^2_{33} = 0.00$, $P = 0.80$; visual manipulation $r^2_{32} = 0.01$, $P = 0.54$; Fig. 1).

The proportion of ants traveling directly to the nectary from the nest increased with time during the establishment of baseline activity ($r^2_{32} = 0.41$, $P < 0.01$; Fig. 2). After the rotation manipulation, there was an initial drop followed by an increase of ants returning to the nectary ($r^2_{27} = 0.49$, $P < 0.01$; Fig. 2).

Return time decreased logarithmically for the baseline treatment ($r^2_{18} = 0.36$, $P < 0.01$; Fig. 3). After an initial increase in return time following the rotation manipulation, we also found a significant decline in the log-transformed value of return time over the course of the trial ($r^2_{26} = 0.27$, $P < 0.01$; Fig. 3). After the rotation manipulation, 96% of the ants returned directly to the nest without following the rotated pheromone trail ($N_{\text{total}} = 27$).

Throughout all treatments, the return time to the nest decreased as ant recruitment rate increased ($r^2_{167} = 0.19$, $P < 0.01$, Fig 4).

The return time to the nest for ants that appeared to lay pheromone trails was significantly slower than those that did not ($t_{49} = 3.09$, $P = 0.01$).

DISCUSSION

We found that bullet ants can flexibly employ different navigation strategies while foraging, depending on available pheromones and visual cues.

P. clavata can use visual cues to navigate back to the nest. After rearranging small-scale visual cues, we found that ants were slower to return to the nest, which indicates a reduced ability to orient themselves (Fig. 1). Furthermore, after the pheromone trails and small-scale visual cues were rotated 90 degrees, 96% of the ants returned directly to the nest instead of following the rotated pheromone trail. This implies that ants may be using large-scale orientation cues, such as surrounding vegetation or canopy light patterns for navigation? (Ehmer 1999, Harrison et al. 1989, Baader 1996). The presence of other ants between the nectary and the nest may have served as an additional visual cue.

Our data also indicate that bullet ants use pheromone trails to return to the nest. After the negative control, visual manipulation, and rotation manipulation, ant return time increased initially followed by a decrease over the course of the trial (Fig. 1 and Fig. 3). A possible reason for this decrease is that ants were reestablishing pheromone trails, which may increase foraging efficiency. Additionally, we found that as ant recruitment rate increased, the return time to the nest decreased (Fig. 4). This trend suggests that ants become more efficient foragers in the presence of more nestmates, possibly due to a reinforced pheromone trail, which is consistent with the findings of Dorigo and Gambardella (1997).

Surprisingly, we found that in the first afternoon trial, ant return time did not increase immediately after the removal of the pheromone trail (pheromone manipulation; Fig. 1). Toward the end of the afternoon trials, ants had been foraging for about seven hours.

Young and Hermann (1980) found that *P. clavata* ant activity decreases after intense foraging bouts of up to four hours. It also rained between morning and afternoon trials, and rain has been found to dramatically decrease the foraging activity of bullet ants (Young and Hermann 1980).

After the rotation manipulation, a lower proportion of the ants travelled directly from the nest to the nectary, which suggests ants could not as easily locate the food source without pheromone trails (Fig. 2). Ants may use pheromones to recruit other members of the colony and to mark the most direct path (Baader 1996, Billen and Morgan 1988, Blum et al. 1964).

It may be beneficial for *P. clavata* to use visual cues in certain situations. Baader (1996) suggests that because pheromone trails degrade over time and are not always available, visual cues are more reliable for independent foraging. Harrison et al. (1989) found that the first ant to discover a food source used local landmarks to return to it, while recruited ants relied on pheromone trails. It may be energetically costly to set a pheromone trail since ants laying a trail took longer to return to the nest. Ants may do so only when needed, such as when recruiting other foragers to a food source.

Based on these experiments, *P. clavata* use both pheromones and visual cues to navigate between the nest and a food source while foraging. Understanding the relationship between the navigation strategies of *P. clavata* and other social ants may shed light on the evolution of social insect behavior.

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SEXUAL STRATEGY AND DORSAL COLORATION IN AN APOSEMATIC FROG

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Abstract: In aposematic species, sexual selection through male competition for females and natural selection due to predation can both affect coloration and may lead to divergence in color. This effect has been well-studied in the dendrobatid frog *Oophaga pumilio*. We examined color divergence in these frogs as it relates to male sexual strategy at La Selva Biological Station, CR on 11-14 February 2011. We investigated three aspects that are likely related to male fitness in *O. pumilio*: coloration, call, and territory type. We found a bimodal distribution of dorsal brightness within a population of the "blue jeans" morph, indicating that *O. pumilio* has diverged within a single morph. We found no relationship between coloration and territory visibility, probably because resident frogs have an advantage in defending territories. We found no relationship between dorsal brightness and call frequency, but there was a negative relationship between dorsal brightness and call pulse rate. This suggests a difference in sexual strategy in differently colored frogs. We infer that disruptive natural selection and sexual selection work together to cause color divergence within a single color morph of *O. pumilio*.

Key words: *O. pumilio*, brightness, disruptive selection, male calls, territory, Dendrobatidae

INTRODUCTION

Aposematic coloration is subject to both natural and sexual selection. Predators recognize and avoid brighter individuals (Noonan and Comeault 2009), while duller individuals are more likely to escape detection. Predation, therefore, imposes disruptive selection, favoring both brightly-colored and more cryptic forms over intermediate coloration (Przeczek et al. 2008; Noonan and Comeault 2009). Coloration also influences mate choice. For example, female dendrobatid frogs preferentially mate with males of similar color (Summers et al. 1999; Noonan and Comeault 2009; Rudh et al. 2011; Wang and Summers 2010; Maan and Cummings 2008; Maan and Cummings 2009). These two forms of selection are reinforcing (Noonan and Comeault 2009; Rudh et al. 2011; Rudh et al. 2007), leading to great diversity of coloration within aposematic lineages (Przeczek et al. 2008).

Probably due to this reinforcement, divergence in color may be the strongest evolutionary trend in the recent history of strawberry poison dart frogs (*Oophaga pumilio*) (Wang

and Summers 2010; Brown et al. 2010; Przeczek et al. 2008; Rudh et al. 2011). Frog behavior may facilitate the strength and direction of these effects (Pröhl and Ostrowski 2011), particularly sexual selection. Several aspects of male frog behavior are essential to their mating success, including territoriality (Bunnell 1973; McVey et al. 1981; Crothers et al. 2011) and calling (Pröhl 2003; Bunnell 1973; McVey et al. 1981). As differently colored frogs have different challenges to overcome in both predator avoidance and mate location, it is likely that they adopt different defensive and sexual strategies.

We examined variation in coloration within the "blue jeans" morph of *O. pumilio* in relation to two other factors involved in sexual selection, male calls and territory visibility. We reasoned that if disruptive selection was acting on coloration, differently colored frogs would have different sexual strategies. We predicted that bright frogs would have more open territories as they invest in coloration to attract mates and that dull frogs would invest in calling.

METHODS

We studied *O. pumilio* on 11-14 February 2011 at La Selva Biological Station, Costa Rica. We located 32 male frogs by their calls and photographed each on a white and black standard with a Pentax W90. We recorded calls of 28 frogs using Olympus digital voice recorders. We assumed that each territory covered a 1.5 m radius from where the frog was first sighted (Bunnell 1973; McVey et al. 1981). To determine visibility in each territory, we measured leaf litter depth and territory openness. We measured leaf litter depth in the center of each territory and 0.5 m away from the center in four directions. Each of us independently scored the openness of each territory on a scale of 1-5, where 1 was too dense for a human to traverse and 5 was as open as a paved trail.

Image Analysis

Photographs of each frog were analyzed in GIMP 2.6 to determine dorsal brightness. An RGB histogram was taken of three small homogeneous red areas on each frog's back. The three red channel peak means were averaged to obtain an overall measurement of redness. The raw redness values were divided by the value of pure red to create a red brightness score of 0-100.

Sound Analysis

Recordings of each frog call were analyzed in Raven Lite 1.0. Number of chirps per minute (call pulse rate) was estimated using six-second clips. The average frequency (pitch) of each call was also measured. We chose to measure these aspects of frog calls because Pröhl (2003) found them to be important to male reproductive success.

Statistical Analysis

We ran regressions in JMP 8.0. We excluded one call pulse rate because it was a clear outlier, significantly lower than all other values.

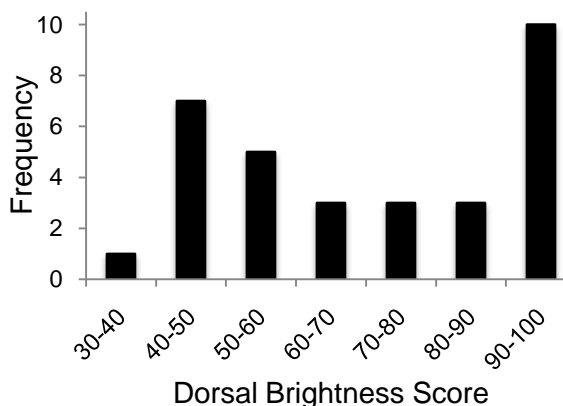


Fig. 1 Bimodal distribution of dorsal brightness (0-100) of *Oophaga pumilio* at La Selva Biological Station, Costa Rica. Dorsal brightness was scored relative to the brightness of true red.

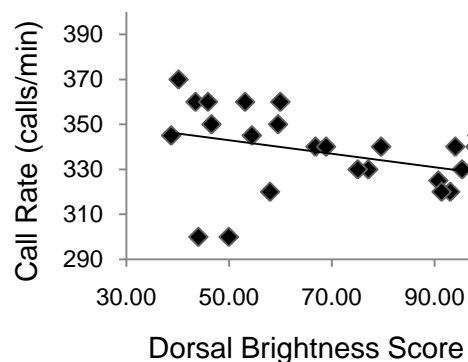


Fig. 2 Relationship between dorsal brightness (0-100) and call pulse rate of *Oophaga pumilio* at La Selva Biological Station, Costa Rica. Dorsal brightness was scored relative to the brightness of true red.

RESULTS

Frog dorsal brightness was bimodal (Fig. 1). Frogs were most commonly either very bright (90-100) or very dull (40-50).

Call pulse rate was negatively related to dorsal brightness ($r^2_{26} = 0.12$, $P = 0.07$; Fig. 2). Call pitch was negatively related to territory openness ($r^2_{27} = 0.11$, $P = 0.08$; Fig. 3).

82 percent of our territory scores were identical among all observers, and those that varied did by only one point. However, there was no relationship between dorsal brightness and either leaf litter depth ($r^2_{31} = 0.01$, $P = 0.54$) or territory openness ($r^2_{31} = 0.01$, $P = 0.64$).

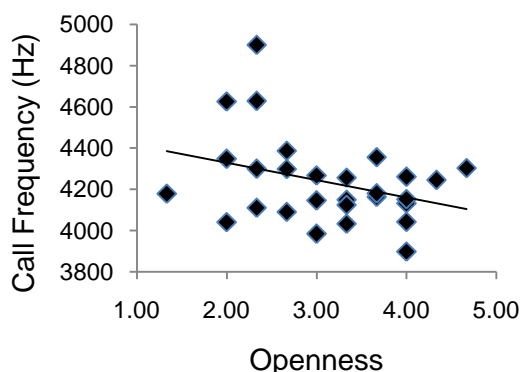


Fig. 3 Relationship between territory openness and call frequency in *Oophaga pumilio* at La Selva Biological Station, Costa Rica. Territory openness was scored on a 1-5 scale where 1 was vegetation too dense for a human to traverse and 5 was as open as a paved trail.

DISCUSSION

Disruptive selection, implied by the bimodal distribution of dorsal brightness in *O. pumilio*, is consistent with the growing literature on the divergence of color in this species.

Pröhl (2003) found that *O. pumilio* call frequency and pulse rate vary only slightly among individuals. Therefore, we believe our trends in call frequency and pulse rate were notable despite their marginal significance.

We interpret the negative relationship between call pulse rate and brightness as evidence that frogs with different coloration differ in their investment in the audio component of mate attraction. Dull frogs are less visible, so they invest more energy in calling to attract mates, whereas bright frogs can call at a slower rate. Calling strategy may also be related to predator avoidance. When visually detected by predators, dull frogs may be more likely to be eaten because they do not advertise toxicity. Thus, they might spend more time hiding and invest more in calling. Pröhl and Ostrowski (2011) found that bright frogs spent more time on conspicuous perches and dull frogs spent more time in the leaf litter. The different calling strategies of bright and dull frogs could attract different

females (Pröhl 2003) and reinforce the divergence in *O. pumilio* color.

Call frequency (pitch) is not related to coloration but is related to the physical properties of the territory. High-frequency sounds have high energy and can penetrate obstacles, so frogs in dense territories tend to use high-frequency calls. Frogs in open territories can use low-frequency calls, which travel farther but cannot penetrate obstacles as well.

Although brighter frogs are more aggressive competitors in the lab (Crothers et al. 2011), it is likely that additional factors influence territory acquisition in nature. Baugh and Forester (1994) found a residence effect in *O. pumilio*; resident males were more aggressive than challengers, regardless of color. The residence effect could explain the absence of a relationship between frog brightness and either leaf litter depth or territory openness.

Our study links *O. pumilio* coloration and behavior. Future studies could further examine effects of frog behavior, such as predator avoidance strategy and female preference for color and call, on color divergence in nature. Understanding the reinforcing effects of behaviors on color divergence in *O. pumilio* is important to understanding the diversity of aposematic species.

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GROUPING BEHAVIOR OF THE FRUGIVOROUS FRESHWATER TROPICAL FISH *BRYCON GUATEMALENSIS*

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Faculty Editor: David R. Peart

Abstract: *Brycon guatemalensis* ("machacas") are frugivorous tropical fish that form groups. We tested the relationship between grouping behavior and foraging of machacas in the Puerto Viejo River at La Selva, Costa Rica. We recorded fish density and behavioral response after dropping edible and inedible inputs into the river in various treatments. We hypothesized and found that grouping would increase with local input of food. We also predicted and found that grouping would be related to habituation, such that a consistent addition of inedible inputs to the river would lead to a decrease in response and grouping over time. We tested the null hypothesis that machaca density would be proportional to the food availability in foraging patches. We found that their density was less than proportional, suggesting that interference competition occurs in groups. We hypothesized and found that grouping benefits individual food detection because the responses of neighboring fish provide sensory cues that allow fish to locate food at distances greater than they can detect alone.

Key words: Social facilitation, habituation, machaca, foraging, interference competition

INTRODUCTION

Conspecific grouping is common in animals, with benefits ranging from foraging efficiency to mate location and predator swamping. While grouping may increase intraspecific competition, the benefits of social facilitation often outweigh the costs.

The benefits of aggregation in fish are well documented. Chum salmon group to gain information about foraging opportunities and predation threats (Ryer and Olla 1991). In salmon schools, as group size increased, probability of predator detection increased, while risk perceived by individual fish decreased (Ryer and Olla 1991). In addition, individual fish in groups consumed more food than solo foragers (Ryer and Olla 1991). Another study by Ryer and Olla (1992) found that fish initiated feeding sooner when grouped than when alone.

Few studies have applied the benefits of grouping to freshwater fish that feed mostly on external inputs to the stream. We examined the grouping behavior of *Brycon guatemalensis*, commonly known as the machaca,

a frugivorous characid fish found in the freshwater rivers and streams of Costa Rican tropical rainforests. Machacas feed mainly on allochthonous material, such as the fruit and leaves of *Ficus glabrata*, and are seed dispersal agents for some trees (Horn 1996). Machacas have been observed gathering beneath ripe fig trees and eating fruits as they fall (Horn 1996).

We do not address the broad question of whether grouping increases individual food intake. Rather, we focus on several specific hypotheses about the relationships between food availability, grouping and foraging behavior.

First, we hypothesized that machacas would group more when food is more locally abundant. Next, we tested whether machacas can increase foraging efficiency by learning not to respond to inedible allochthonous material. Accordingly, we predicted that group density would decline over time with consistent introductions of inedible items.

We further hypothesized that social facilitation occurs because individual fish in

groups can locate food at further distances than when foraging solo (which we define as increased food locating ability). We reasoned that visual cues (neighboring machacas moving towards a food source) would alert nearby machacas to the presence of food further away. An alternative mechanism that could generate the same pattern is the increase in water pressure resulting from the responses of neighboring fish, which can be detected by the sensitive lateral line cells of machacas.

Finally, we tested the null hypothesis that machaca densities would be proportional to the amount of food dropped in adjacent patches of the stream. The null hypothesis would hold if the primary factor limiting food availability for individuals in a patch is exploitative competition (where individuals interact only indirectly through consumption, which reduces overall resource availability). Deviations from this null hypothesis would suggest that other factors drive food availability for individuals, such as social facilitation or interference competition (direct interaction where one fish actively limits foraging success of another). If interference effects are greater than social facilitation, fish densities would increase by a factor less than the proportional increase in food. The reverse would be true if the effects of social facilitation (e.g. due to enhanced food location, as hypothesized above) were greater than those of interference.

METHODS

We conducted our experiments at La Selva Biological Station between 10-13 February 2011, from the bridge spanning the Puerto Viejo River.

We defined fish density as the number of fish in a 3 m radius around a focal point specific to each experiment (see below for details) and used local density as an index of group-

ing behavior. Most fish in aggregations (with or without our experimental manipulations) were within a 3 m radius, so we used the number of fish in this circle to represent group density in all experiments.

Control/Food

We first tested local fish density in response to the presence or absence of food inputs. As a control, we observed two aggregations of machacas that were present without an observable food input. We recorded the density of the group every 15 s. We defined this as one trial, and conducted 15 such trials. Densities were centered at a focal point defined as the middle of the aggregation. For the food treatment, we dropped one piece of fruit (watermelon, approximately 1 cm³) into the river every 15 s and measured the fish density centered at the fruit. We repeated this for 10 trials.

Habituation

We then tested for group learning in response to inedible allochthonous inputs. Every 15 s for 15 min, we dropped a pebble of approximately 1 cm diameter from the bridge to a consistent point near the center of a group and assessed the fish density, centered at this focal point. We also qualitatively assessed the average response intensity of the fish within a 3 m radius of each dropped pebble using an ordinal scale, with 0 (no response), 0.25 (slight movement towards the dropped pebble), 0.5 (minor effort to investigate), 1 (active investigation of the object), 2 (effort to eat the object), and 3 (aggressive effort to eat the object). While there was variation in individual fish response, we focused on assessing how the overall group responded to inedible inputs.

Food Locating

To test if grouping leads to increased individual success in locating food, we dropped fruit into the stream and observed fish densi-

ty and response. We first recorded fish density centered around the focal point of a haphazardly selected focal fish on the periphery of a loosely aggregated group. At approximately 20 s intervals, we dropped in a piece of fruit at varying recorded distances away from the focal fish. The density around the focal fish was reassessed after each dropped fruit, along with the intensity of the focal fish's response using the same ordinal scale as above. We recorded the distance between the fruit and the individual furthest from the fruit to respond.

Response of Group Size to Food Availability

To evaluate if fish density is proportional to the amount of food dropped in a region, we created two “patches” with different amounts of food inputs in each. We defined a patch as the projected area of the river within a 3 m radius of the point at which the food entered the water. The two patches were located on the same side of the bridge to control for possible upstream-downstream effects. On average, they were approximately 7.5 m apart, which we observed to be a distance that allowed for fish exchange between patches. At 15 s intervals, we dropped three pieces of fruit (approximately 1 cm³ each) into one patch and simultaneously dropped one piece into the other. We then recorded the density of the fish centered at the point at which food entered the water immediately after we dropped the food. After 10 trials, we switched which patch we dropped three pieces of fruit into and which patch we dropped one piece into to control for site bias, and we repeated the trials. We repeated the entire experiment at three other sites along the bridge.

RESULTS

Consistent with our hypothesis, fish density was significantly higher when food was added than in the control treatment without

food, after a fixed number of trials ($t_{20.3} = 10.05$, $P < .0001$; Fig. 1). Fish density also increased significantly over time as food was consistently dropped in ($r^2_{19} = 0.27$, $P = 0.02$; Fig. 2).

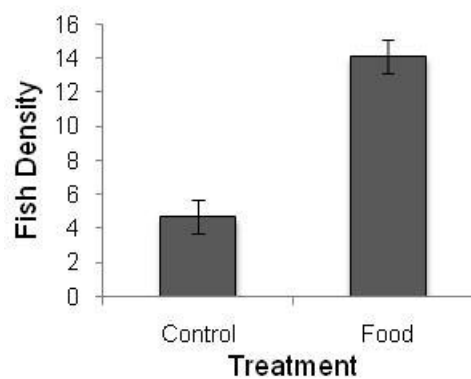


Fig. 1. Mean fish density for areas with no food input (Control) and areas with fruit dropped in every 15 s (Food) in Puerto Viejo River, La Selva, Costa Rica. Density was recorded every 15 s for 15 and 10 trials, respectively. Density was measured in number of fish within a 3 m radius centered at the middle of the group in the control treatment and at the fruit in the food treatment.

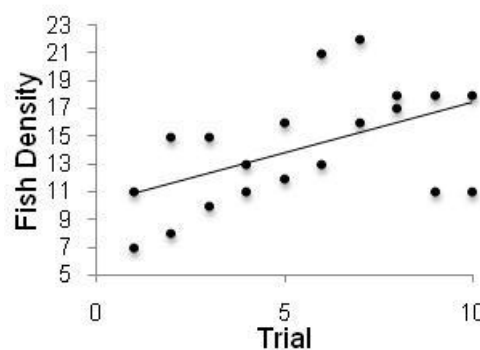


Fig. 2. Fish density as fruit was dropped into a river at 15 s intervals for 10 trials in Puerto Viejo River in La Selva, Costa Rica. Density was measured as number of fish within a 3 m radius of the fruit.

Consistent with our predictions regarding habituation, as fruit-sized pebbles were dropped into the stream over time, fish density ($r^2_{179} = 0.21$, $P < 0.0001$; Fig. 3) and average group response ($r^2_{179} = 0.54$, $P < 0.0001$) decreased significantly.

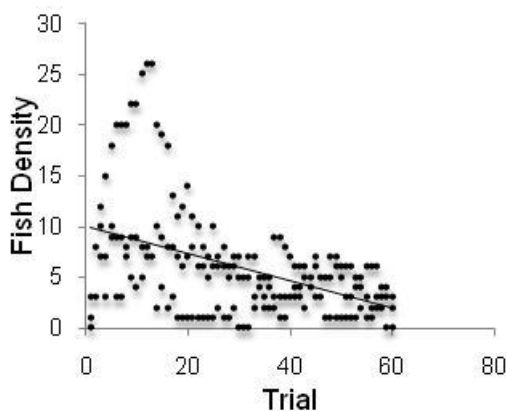


Fig. 3. Fish density after successive habituation trials at 15 s intervals in Puerto Viejo River, La Selva, Costa Rica. One pebble was dropped in each trial, and density was measured as number of fish within a 3 m radius of the pebble.



Fig. 4. Fish density (number of fish within a 3 m radius of fruit dropped) in two patches of a river 7 m apart in Puerto Viejo River, La Selva, Costa Rica. One patch had three pieces of fruit dropped in, and the other had one piece of fruit dropped in.

Fish density did not decrease significantly in patches in which one piece of food was consistently dropped ($r^2_{112} = 0.01$, $P = 0.33$), yet fish density increased significantly in patches in which three pieces were dropped ($r^2_{112} = 0.17$, $P < 0.0001$) over the course of 15 trials. During the last five trials of each run, after fish had potentially adjusted to the change in food availability at each patch, the mean fish density was significantly higher for patches at which three pieces of food were dropped than for patches at which one piece was dropped (three pieces: $\bar{x} = 25.3$; one piece: $\bar{x} = 15.4$; $t_{53.2} = 8.18$, $P < 0.0001$; Fig. 4). However, fish density in the patch with higher food inputs (three

pieces dropped) was much less than three times the density with lower inputs (one piece dropped).

With regard to food locating, as fish density increased, there was no significant increase in the distance from the food to the furthest responding fish ($r^2_{78} = 0.02$, $P = 0.26$). As distance from the food to the focal fish increased, that focal fish's response decreased significantly ($r^2_{79} = 0.63$, $P < 0.0001$; Fig. 5). The best-fit line of these data intersects a focal fish response of 0 at a distance of 7.8 m. In the groups observed, 27/80 trials had furthest fish responding from a distance of 8-12 m from the food dropped.

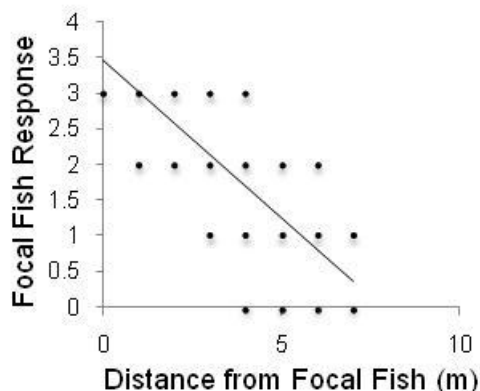


Fig. 5. Response of haphazardly selected focal fish to food dropped. Response was recorded on a qualitative scale from 0 (no interest) to 3 (full attack), as a function of the distance of dropped food from the focal fish, in Puerto Viejo River, La Selva, Costa Rica.

DISCUSSION

Our finding of a significantly higher density of machacas around food dropped into the river (Fig. 1) supports our hypothesis that the fish aggregate around local food sources. Additionally, density increased over time with consistent food input (Fig. 2), indicating that social facilitation mechanisms, such as sensory cues from group activity, encourage the movement of individual fish to that foraging site.

We also found evidence that machaca grouping behavior is influenced by habituation. As rocks were dropped into the water, the machacas initially grouped densely around the input site, reacting to the rocks as if they were edible. However, after several trials, density and group response to the dropped rocks decreased over time and returned to pre-experimental densities, suggesting that habituation occurred (Fig. 3). Further studies may investigate whether increased group density increases the rate of habituation and social learning.

Using data of focal fish responses to food inputs, we extrapolated the average expected limit of an individual fish's response range (furthest distance at which a fish responds to a falling input) to be 7.8 m (Fig. 5). Using the data on the furthest distance of responding fish, we found that 34% of our trials involved a fish response from outside of their expected individual response range. This suggests that grouping facilitates food locating and allows grouped machacas to detect food at a further distance than they can detect alone. This expanded range may be attributed to individuals responding to movement of fish inside their response range towards food outside their response range. These sensory cues may increase foraging efficiency of individuals within a group by extending the range over which they can respond to food. However, since the correlation between density and furthest distance of responding fish was not significant, other factors, such as competition, may reduce this benefit of grouping.

The density of machacas was less than proportional to the amount of food in patches; there were fewer fish in the patch of high food availability than predicted by the null hypothesis (Fig. 4). Fish on the periphery of groups are obstructed by other fish and may not have access to food, suggesting the

presence of interference competition. Further studies could examine the extent to which interference competition limits individual foraging success of fish in a group.

Our methods of determining distance and area measurements may have been a source of error in our study. Because our study was conducted on a bridge approximately 15 m above the water, we were unable to take exact distance measurements. We attempted to control for error by having the same person make distance estimates for all trials across all experiments.

Future studies may examine the trade-offs between the benefits of social facilitation, such as increased response range and food locating, and the costs of competition that arise from foraging in a group. While we evaluated one benefit of grouping, an examination of whether group foragers are more successful than solo foragers overall may provide additional insight into machaca grouping behavior.

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AGGREGATION, DOMINANCE HIERARCHY, AND MATE CHOICE IN GOLDEN ORB-WEAVING SPIDERS (*NEPHILA CLAVIPES*)

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Faculty Editor: David R. Peart

Abstract: Female golden orb-weaving spiders occasionally aggregate, increasing availability of mates and food. We experimentally and observationally investigated the mass, web placement, and mating behavior in female spiders to assess the benefits and costs of aggregating with different-sized females. We hypothesized that larger females would hold a central position in the aggregation with more males nearby. We found that females do aggregate centrally in a hierarchical system based on size, but we detected no pattern in the pairing of males with females. Larger females lost less body mass than smaller females. Although grouping behavior may pose inherent risks, especially to smaller females, the benefits in naturally high-resource environments must outweigh the costs.

Key words: female gigantism, male dwarfism, *Nephila clavipes*, *Araneidae*

INTRODUCTION

Although most orb-weaving spiders are solitary, golden orb-weavers (*Nephila clavipes*) occasionally aggregate (Lloyd and Elgar 1997). In an aggregation, females build and reside in individual webs, using neighboring webs for structural support (Rypstra 1985). Female orb weavers who participate in aggregation behavior catch more prey and attract more mates (Rypstra 1985; Elgar and Fahy 1996). However, female spiders may choose not to aggregate because doing so potentially increases egg sac parasitism and heterospecific kleptoparasitism (Lloyd and Elgar 1997; Smith 1982).

Orb weaving spiders display extreme sexual dimorphism where the female is often an order of magnitude larger than the male (Hormiga et al. 2000). Because male orb weavers are polygamous and select their mates (Vollrath 1997), they increase their fitness in female aggregations due to increased mate access (Christenson and Goist, Jr. 1979). Proximity to the center of the aggregation increases reproductive success but does not in-

crease the risk of predation or sexual cannibalism (Christenson and Goist, Jr. 1979).

We hypothesized that aggregating females would construct a hierarchy based on size as suggested by Farr (1977). We predicted that larger females would build webs in more central locations to maximize web size and prey capture. We also hypothesized that males would position themselves closer to larger, more central females to increase fitness.

METHODS

We collected 12 female and 10 male *N. clavipes* spiders near the La Selva Biological Station, Costa Rica on 11-14 February 2011. Females were individually marked, massed (Ohaus Valor 3000 Xtreme balance) and measured (thorax and total body length). We placed 3 females in each of 4 butterfly enclosures (d = 35 cm, h = 85 cm), and allowed them to acclimate and construct webs for an hour. Each female's position in the enclosure was measured as distance from the closest edge (laterally) and from the top of the cage (vertically).

We then introduced males, in treatments of 1, 3, or 5 individuals, to each of the four enclosures. We ran 5 replicate trials of each treatment for 3 hours each. We assumed proximity to female was an indication of male mate choice. At 1-hour intervals during each trial, we measured each male's distance to the closest female, and each female's distance from the edge and top of the enclosure. Between trials, webs were destroyed and females were haphazardly reassigned to a new enclosure. Spiders were replaced in the case of cannibalism or other cause of death.

To determine female choice of position in the absence of aggregation, we placed 12 females individually in the nets and recorded web placement after one hour. We re-massed the females at the end of the experiment.

To assess natural aggregations of orb-weavers, we located 14 females in the field, and for each recorded mass, thorax and body length, web radius, and number of associated males.

Analysis

We calculated proportional female body mass (individual mass divided by mass of all individuals in same enclosure) in each trial and ran a regression between proportional body mass and lateral and vertical female position. We also ran a regression between absolute female body mass and number and distance of proximal males. We determined each female's change in mass throughout the experiment and ran a regression between initial mass and this change. We performed a regression between *in situ* female mass and mean web radius. We ran two other regressions on field data: one between number of males on each web and female mass and one between number of males and web radius. We used Microsoft Excel and JMP 8.0 for all data analysis.

RESULTS

We found positive relationships between the proportional mass of females and their distance from the lateral and vertical edges of the enclosures, respectively ($r^2_7 = 0.16$, $P = 0.0001$; Fig. 1, $r^2_7 = 0.11$, $P = 0.0002$; Fig. 2).

Larger females were more often the nearest neighbor to males than were smaller females ($r^2_7 = 0.43$, $P = 0.01$; Fig. 3)

There was a positive relationship between average web radius and both female mass ($r^2_7 = 0.68$, $P = 0.01$; Fig. 4) and thorax length ($r^2_7 = 0.52$, $P = 0.03$) for spiders in the field. Number of males in each female's web was unrelated not significantly related to female mass ($r^2_{12} = 0.02$, $P = 0.66$) or mean web radius ($r^2_7 = 0.00$, $P = 0.94$).

Females with greater initial mass lost a smaller proportion of body mass than smaller females ($r^2_7 = 0.46$, $P = 0.05$; Fig. 5).

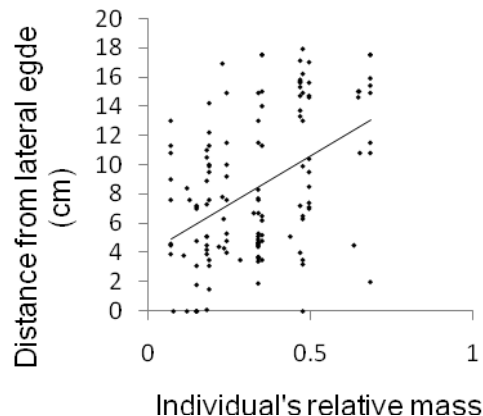


Fig. 1. Female golden orb weaver spiders with higher relative masses were located farther from the lateral edges of the enclosure, closer to the lateral center of the net. Positional data were collected over a series of 5 experimental trials, with three female spiders in each of 4 butterfly cages at La Selva Biological Station, Costa Rica. Relative mass was calculated as mass of individual spider divided by mass of all females within a trial. Distance from the lateral edge was recorded as distance from each spider to the nearest lateral wall of the net.

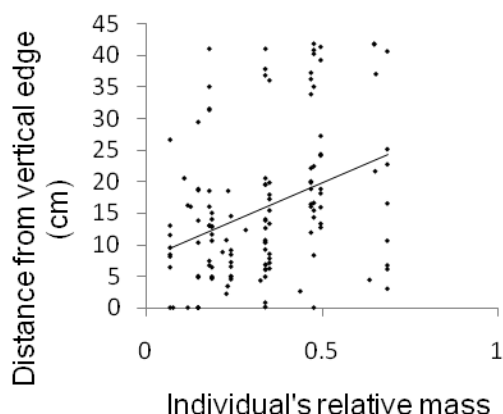


Fig. 2. Female golden orb weaver spiders with higher relative masses were located farther from the vertical edges of the enclosure closer to the vertical center of the net. Positional data were collected in each of 5 experimental trials, with three female spiders in each of 4 butterfly cages at La Selva Biological Station, Costa Rica. Relative mass was calculated as mass of individual spider over mass of all females within a trial. Distance from the vertical edge was recorded as distance from each spider to the nearest vertical boundary (top or bottom) of the net.

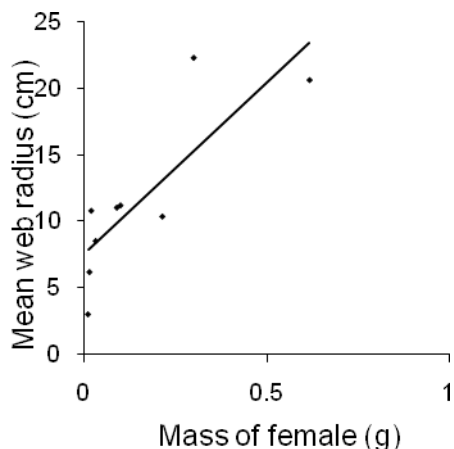


Fig. 4. Mean web radius increased with masses of female. *A. nephila* spiders observed around La Selva Biological Station, Costa Rica.

DISCUSSION

We considered that female size in relation to proximal females is a better determinant of position in a social hierarchy than absolute size. Using this metric, larger females tended

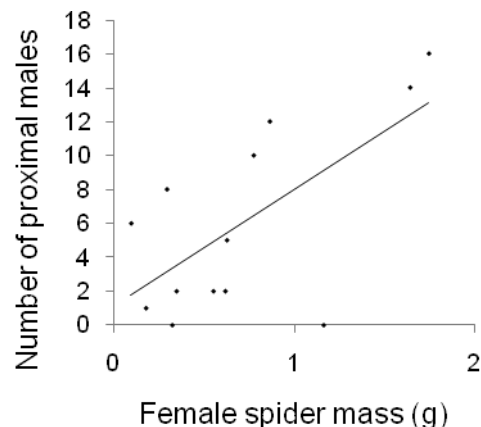


Fig. 3. Females with greater absolute mass had an increased number of proximal males associated with them. Positional data were collected over a series of 5 experimental trials, with three female spiders in each of 4 butterfly cages at La Selva Biological Station, Costa Rica. 10 male spiders were introduced in treatments of 1, 3, or 5 individuals and male distance from nearest neighbor female measured every hour during each trial.

to occupy the center of the enclosure both laterally and vertically.

Males prefer aggregations of females because of increased mate access (Christenson and Goist, Jr. 1979). Small females in aggregations also have more access to mates than solitary females (Rypstra 1985). Although larger females were more often the nearest neighbor to males than smaller females (Fig. 3), we found no close associations in distance between males and individual female spiders. Because larger females were more centrally located in the enclosure, we cannot determine whether male mate choice is due to female size or position.

We observed that larger females made larger webs in the field, but we did not find more males in larger females' webs. Perhaps location of the female may be more important to mate choice than female size alone.

Larger females lost a significantly smaller proportion of their mass over the experimental period, suggesting that they are competitively dominant over smaller females for food. This supports Rypstra (1985), who

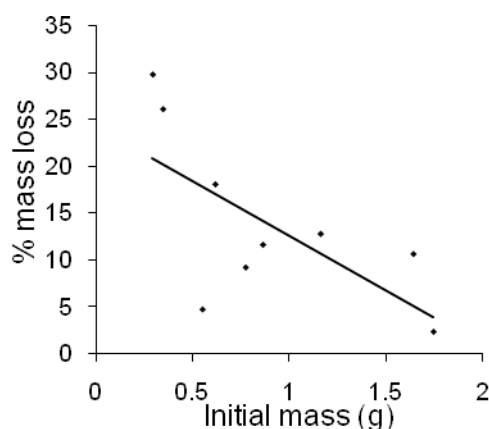


Fig. 5. Percent mass loss of *N. clavipes* spiders over a 3-day experiment at La Selva Biological Station, Costa Rica.

found that golden orb-weaver aggregations are most advantageous in environments where food is not the limiting resource.

Aggregations may be detrimental to smaller females because larger females cannibalize smaller females, an event we witnessed twice. We speculate that the foraging and sexual benefits of aggregating in the field, which were not present in our experiment, may outweigh the potential detriments of cannibalism.

The dangers associated with aggregations that we observed in our study may explain why most orb-weaving spiders continue to display solitary behavior. *N. clavipes*, however, has been known to form natural aggregations. This suggests that although grouping behavior poses inherent risks, especially to smaller females, the benefits in naturally high-resource environments must be substantial enough for aggregations to remain a viable social strategy. Thus, it would be interesting to investigate what patterns of female dominance exist within natural aggregations.

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FACTORS INFLUENCING WING MORPHOLOGY IN COSTA RICAN PASSERINE UNDERSTORY BIRDS

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Abstract: Wing morphology greatly affects a bird's ability to utilize a niche space and is often correlated with factors such as migratory behavior and foraging guild. The ability to fly through tight spaces in vegetation is important for understory birds. I hypothesized that habitat openness would be a strong predictor of aspect ratio and wing loading, two measures of wing morphology, and that habitat openness would be a better predictor than either migratory behavior or foraging guild. I used unpublished data on the wing morphology of 81 species of understory passerine birds mist netted at biological stations throughout Costa Rica by Sarah Wengert and students of the Dartmouth Biology FSP. Overall, migratory status was the best predictor of wing morphology, followed by foraging guild and then habitat openness.

Key words: Aspect ratio, wing loading, habitat openness

INTRODUCTION

For flying birds, wing morphology is an important determinant of niche space (Fig. 1, Saville 1956). Factors associated with wing morphology include migratory behavior and foraging guild. Migratory birds must have wings adapted to flying long distances efficiently because flight is energetically costly. (Winkler and Leisler 1992, Senar et al. 1994, Mila et al. 2008) Different foraging guilds require wings that are efficient for different flight styles. For example, hummingbirds that forage on flying insects tend to be adapted for quicker, more maneuverable flight than birds that forage on terrestrial insects (Stiles 1995).

Aspect ratio and wing loading are two facets of wing morphology. Aspect ratio is defined as the square of wingspan over "total wing area" (including wing and root box area); low aspect ratio is associated with maneuverability. Wing loading is mass per unit wing area. Low wing loading results in more efficient flight because each unit of a bird's mass is supported by a large wing area, but represents a trade-off between wing area and mass that performs other important functions,

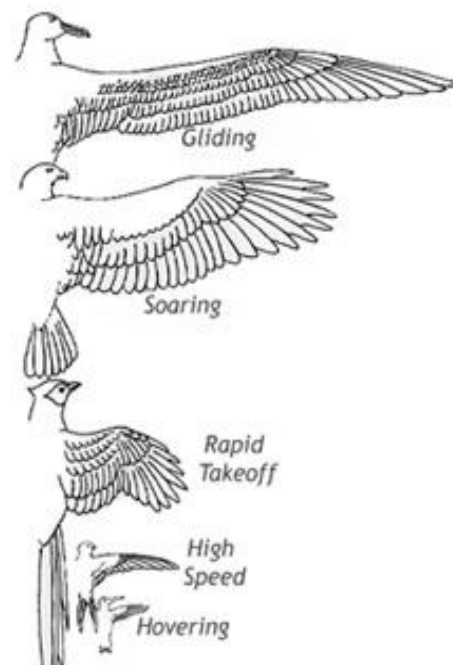


Fig. 5 Examples of morphological wing types. Note the high aspect ratio of the gliding wing and low aspect ratio of the rapid takeoff wing (Ritchison 2007).

such as energy storage. Birds whose niche space requires sustained flight, such as soaring birds, tend to have very low wing loading (Saville 1956).

Understory birds face the difficult challenge of flying safely through forested areas.

Avoiding closely spaced vegetation requires very slow flight speeds and high maneuverability, which in turn requires small wings with low aspect ratios. Low wing loading and efficient flight are very difficult to achieve with small wing area, so understory birds tend to be very small in order to maximize energy efficiency during short flights. (Saville 1956).

Given the importance of vegetation avoidance to the flight style of understory birds, it seems reasonable that vegetation openness in a bird's habitat would be a strong predictor of wing morphology. I hypothesized that birds in habitats with more open vegetation would have higher aspect ratios and lower wing loading as adaptations to more sustained flight. I also hypothesized that migratory behavior and foraging guild would be predictors of wing morphology, but that habitat openness would be a better predictor because of the relative importance of vegetation avoidance to understory birds.

METHODS

The data in this study were gathered by Sarah Wengert and the students of the FSP in 2009 and 2011 as part of a long-term study with Ernesto Ruelas (unpub. data). In both years, mist nets were used to sample populations of birds along the edges of forested areas in Corcovado National Park, Cuerici Biological Station, Monteverde Biological Station, Palo Verde Biological Station, and La Selva Biological Station in Costa Rica. Each bird caught was identified to species and massed by suspension in a cone of known weight from a spring scale.

In 2009, bird wings were traced on graph paper and later analyzed to determine single wing area and single wing length. Specimens at Harvard University were used to measure root box (torso) width and length and to cal-

culate root box area for each species. Measurements taken in the field and from museum specimens were used to calculate wingspan for each species.

In 2011, half-wingspan was measured in the field. Photographs of each wing were taken against a background of graph paper with a ruler visible as a scale and later analyzed for wing area, wing length, and root box width using ImageJ software.

For each individual bird, I calculated aspect ratio and wing loading. Measurements for birds of the same species were averaged. 81 species were compared.

I assigned each species to a foraging guild based on Gomez de Silva and Medellin (2002), defined it as migratory or non-migratory, and assigned it to one of four ordinal habitat openness classes using entries in *A Guide to the Birds of Costa Rica* by Gary Stiles and Alexander Skutch.

Analysis

I performed a MANOVA including both dependent variables (wing loading and aspect ratio) and all three independent variables (habitat openness, foraging guild and migratory behavior) to examine the relative importance of each independent variable as predictors of aspect ratio and wing loading. All statistics were performed using JMP 8.0.

RESULTS

Migratory status ($P < 0.0001$) was a better predictor of wing morphology aspects than foraging guild ($P = 0.04$) and a much better predictor than habitat openness (MANOVA: $F_{10, 70} = 4.62$, $P = 0.76$).

DISCUSSION

Migratory status was the best predictor of wing morphology, followed by foraging guild. Habitat was a very poor predictor of wing morphology. This contradicts my origi-

Table 1. Tabulated P values from MANOVA comparing the relative importance of foraging guild, migratory status, and habitat openness in predicting wing morphology characteristics (aspect ratio and wing loading). Migratory status was a very significant predictor of aspect ratio, foraging guild was a significant predictor of wing morphology, and habitat openness was not a significant predictor.

	Wing morphology
Foraging guild	P = 0.04
Migratory status	P < 0.0001
Habitat openness	P = 0.76

nal hypothesis that habitat openness is a strong predictor of wing morphology.

Given the difference in flight requirements of migratory and non-migratory birds, it follows that migratory status would be a strong predictor of wing morphology. Additionally, given the amount of time understory birds spend foraging and how energetically expensive short flights are (Nudds and Bryant 2000), it follows that foraging flight style would be a strong predictor. Since all understory birds face similar problems with avoiding vegetation, perhaps the differences in openness between areas of forest are not enough to drive wing morphology.

Future studies could examine the relative importance of foraging guild, migratory status, and habitat openness to birds in temperate zones or habitats with greater variance in vegetation openness. Future investigators could also experimentally manipulate wing loading and aspect ratio (by trimming feathers) to observe the impact of both factors on the ability of birds to avoid vegetation, sustain flight, or forage.

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FISH COLONIZATION OF ARTIFICIALLY CREATED *ACROPORA* STRUCTURES IN A TROPICAL PATCH REEF

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Abstract: *Acropora* corals disappeared throughout the Caribbean in the 1980s and consequently the region lost rigid, three-dimensional reef structures that had been habitat for a species-rich fish fauna. To test the potential effects of *Acropora* loss on fish abundance and diversity, we created a gradient of increasing structural complexity mimicking *Acropora* in an area of fringing reef off of Little Cayman Island, BWI. We hypothesized that fish abundance and species richness would increase linearly with structural complexity, and that there would be a greater number of juvenile fish within our structures compared with reference sites on the adjacent fringing reef. We found that fish abundance, evenness, and richness increased along the habitat structural complexity gradient. However, fish diversity metrics and abundance were higher on reference sites than at artificial structures. Consistent with our hypotheses, we found a higher percentage of juvenile fish within artificial structures compared to sites along the intact fringing reef. This suggests that *Acropora*-like structures may be preferred by juvenile fish, and their reintroduction may provide an important nursery habitat.

Key words: species declines, habitat complexity, juvenile fish, Elkhorn coral, Staghorn coral

INTRODUCTION

Staghorn and elkhorn corals (genus *Acropora*) dominated reefs throughout the Caribbean for the past 220,000 years (Pandolfi and Jackson 2006). Since the 1980s, however, *Acropora* has virtually disappeared from the area due to climate change, mass bleaching events, hurricane damage, and disease (Lesser 2007; Aronson and Precht 2001; Sheppard et al. 2009). Both elkhorn (*A. cervicornis*) and staghorn (*A. palmata*) corals are currently listed as threatened under the Endangered Species Act of 1973 (National Marine Fisheries Service 2008), with recent reduction in live coral cover of these species sometimes exceeding 90% (Sheppard et al. 2009).

Consequently, many reefs in the Atlantic have lost rigid, three-dimensional structures that were previously host to a species-rich fish fauna (Sheppard et al. 2009). Lirman (1999) found that abundance of multiple fish species such as damselfish and snappers was higher in reef areas containing *Acropora*, and

that schools of certain species such as grunts were almost exclusively found within the unique branching structure of *Acropora*. Abundance of certain fish species was also correlated with higher densities of *Acropora* corals, as the coral was a source of food or protection, and once *Acropora* reefs died off, coral-feeding species disappeared (Ohman and Rajasuriya 1998; Sano et al. 1984). In addition, the shallower areas these corals formerly occupied are now reduced to rubble, as other coral species cannot tolerate the high disturbance and extensive macroalgae growth characteristic of this area (Aronson and Precht 2001; Sheppard et al. 2009).

To investigate the effects of *Acropora* loss on diversity of coral reef fishes, we compared fish abundance and species diversity metrics along a gradient of increasing structural complexity that was designed to mimic the hard, branching structure provided by *Acropora*. We observed fish recruitment at *Acropora*-like artificial reef structures, at control structures containing a cement block that was used to

anchor all structures, and at reference sites on a fringing reef lacking *Acropora* corals. We hypothesized that our metrics of fish diversity (species evenness and species richness) and abundance would increase linearly with increasing structural complexity. We alternately hypothesized that fish diversity and abundance would increase exponentially with complexity because a minimum threshold of structural complexity may be required for fish to benefit from the refuge structures provided by *Acropora* mimics. We predicted that species diversity and abundance would be lower on our artificial reefs than on the coral fringing reefs, in part due to the presence of edible algae and coral on living reefs. We also predicted that species composition would differ between our structures and reference sites on the fringing reef, with a higher number of juveniles on our structures compared with the reference sites, as these lost structures were once important fish nursery habitats (Nagelkerken et al. 2000).

METHODS

We placed 14 artificial reef structures on the near-shore side of the fringing reef in front of Little Cayman Research Center (LCRC), Little Cayman Island, BWI from 27 February to 2 March 2011. We constructed the structures from PVC pipe, tree branches, and wire with cinderblocks as a weighted base

(Fig. 1.). PVC pipes of 1 m length and 1 inch diameter were used to form the main trunk and a varying number of cross beams, made from PVC pipe of 60 cm and 15 cm lengths and 0.5 inch diameter, were attached according to the diagram. A 0.5 m long tree branch with 5-6 branching twigs was attached to each cross beam. We placed these structures approximately 1 m from existing patch reefs, and spaced them approximately 5 m apart.

After the structures had been in the water for at least 6 hours, we observed number of species, number of individual fish, and number of individual fish of each species at each structure and 10 reference sites. Reference sites were located on the fringing reef, between two artificial structures. We conducted instantaneous scans every 2 minutes for 8 minutes. We observed each structure and reference site 4 separate times (3:30-6pm on 28 Feb., 1 March, and 2 March and 9-11am of 2 March) for a total of 16 observations.

Analysis

We averaged all observations to obtain a mean species richness and fish abundance for each artificial reef and reference site, which were our units of replication. We used simple linear regression to test the effect of number of branches on species richness and fish abundance. We calculated species richness as mean number of species observed at each site, and fish abundance as mean number of fish at

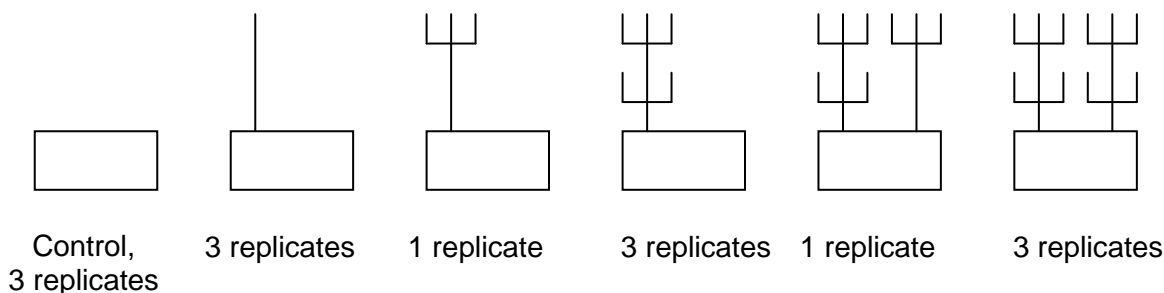


Fig. 1. Experimental design of artificial *Acropora* structure with rectangles representing cinderblocks and lines representing PVC pipes. Half-meter long tree branches were attached to all cross-beams.

each site regardless of species. We used total number of fish observed to calculate species richness and percent juveniles. We ran student's t-tests to determine the difference in species richness, fish abundance, species evenness, and percent juveniles between artificial reef and reference sites. We also used simple linear regression to test the relationship between species evenness and percentage of juveniles and structural complexity. All statistics were performed using JMP 8.0.

RESULTS

The percentage of juvenile fish present was 41.3% higher at the artificial structures than at reference sites ($t_{18,4} = 5.20$, $P < 0.0001$; Fig. 2). Our regressions showed a statistically non-significant but weak positive trend between both species richness and fish abundance and the number of branches of artificial structures (richness: $r^2_{1,12} = 0.09$, $P = 0.29$; ab-

undance: $r^2_{1,12} = 0.12$, $P = 0.24$; Fig. 3). A student's t-tests showed that species richness and fish abundance were significantly higher on reference sites than on artificial structures (richness: $t_{15,5} = -4.04$, $P = 0.01$; abundance: $t_{12,5} = -2.48$, $P = 0.03$; Fig. 4).

Species evenness was slightly lower on artificial structures compared to reference sites

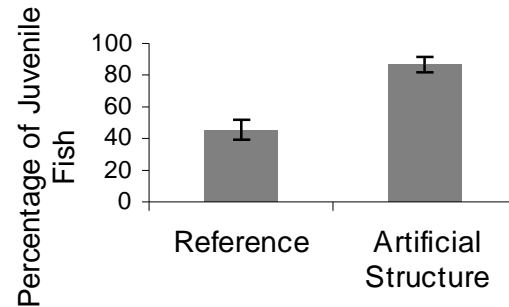


Fig. 2. The percentage of juvenile fish increased on artificial *Acropora*-like structures compared with reference sites on the fringing reef near Little Cayman Research Center.

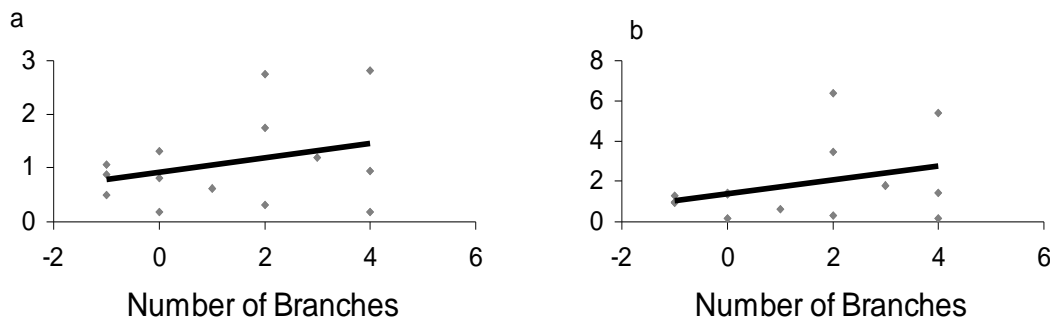


Fig. 3. Positive trend in species richness (a) and fish abundance (b) with increasing number of branches on artificial structures on artificial reefs near Little Cayman Research Center.

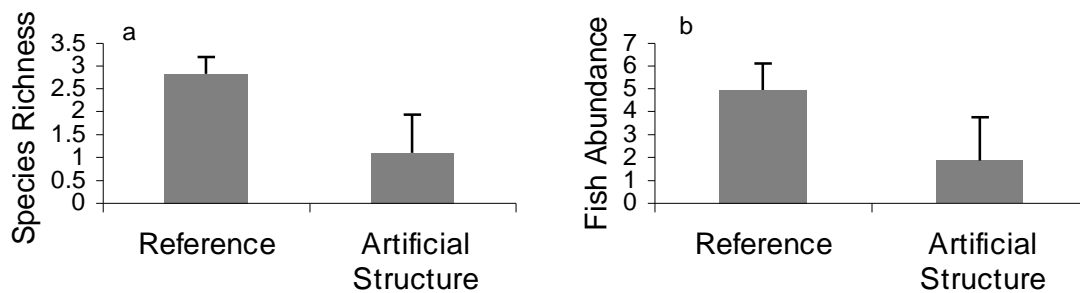


Fig. 4. Species richness (a) and fish abundance (b) were higher at reference reef sites on the fringing reef than on artificial structures near Little Cayman Research Center.

but this difference was not significant at $P < .05$ ($t_{18,1} = -1.61$, $P = 0.13$). Evenness and percent juveniles increased with increasing structural complexity, but these relationships were non-significant (evenness: $r^2_{1,12} = 0.06$, $P = 0.39$; percent juveniles: $r^2_{1,12} = 0.06$, $P = 0.42$).

DISCUSSION

Habitat structural complexity has been positively correlated with fish abundance (Grigg 1994) and species diversity regardless of the material composition of the substrate (Risk 1972). We found a linear trend, though not statistically significant, between number of branches on our artificial reefs, a measure of structural complexity, and fish species richness, evenness, and abundance. Our results suggest that along our gradient of structural complexity, any additional structure was of increased benefit to the fish, and there was no minimum threshold required for recruitment.

The higher rate of colonization of our artificial reefs by juvenile fish compared with reference sites indicates that *Acropora*-like structures may be superior nursery habitat. *Acropora* acts as a barrier to wave energy in shallow waters (Sheppard et al., 2009). Juvenile fish use this sheltered environment as a nursery, as it also provides protection from predators (Nagelkerken et al. 2000). As *Acropora* are dominant species in shallower water where most other corals cannot grow, they provide a unique habitat for juvenile fish, which are uncommon in deeper water (Nagelkerken et al. 2000).

In some cases aggressive or predatory species of fish colonized our artificial reefs, which may have had an effect on the overall species richness and abundance at those sites. For instance, barracuda, a large predatory fish, and juvenile cocoa damselfish, an aggressive territorial herbivore, were the only

fish spotted near some artificial reefs during several observations. We also found several fish such as bottom-dwelling gobies colonizing the cinderblock structures at control and artificial reef sites, suggesting that the branching structure of *Acropora* may not be the only structure important to reef fish.

In addition, the structures may not have been in the ocean long enough to attract the richness and abundance that they could have supported given a longer time for colonization. A natural *Acropora* coral would remain standing and accumulate algae after death, providing a source of food for its inhabitants (Sheppard et al. 2009). Although our structures began to accumulate algae after 2-3 days, this source of food was probably low compared to the intact reef, and perhaps too low to attract many of the former inhabitants of *Acropora*. Randall (1963) found little colonization had occurred on artificial concrete reef structures after a week of exposure to fish, and yet after two years and four months, 2754 fish were collected from the reefs; he also found that the increase in fish biomass with time was not linear. We found a lower richness and abundance of fish on artificial structures than at reference sites, indicating that there were many species of fish that would have the opportunity to colonize these reefs in the future. However, we did not expect that richness and abundance would be identical to the natural fringing reef, as *Acropora*-like structures may be used by a subset of reef fish. In addition, many artificial reef studies cover much larger areas, and larger reef structures may be necessary to find a significant increase in species diversity and composition (Randall 1963).

Many of the former inhabitants of *Acropora* may have relied so heavily on these corals as a habitat that they have become locally extinct, which may explain the low variation we

found in species richness and evenness along our structural gradient. Sano et al. (1987) found that corallivorous species completely disappeared from *Acropora* reefs that died or were reduced to rubble, and numbers of fish species and individuals found on dead and rubble reefs were lower than on live reefs. Species that remain in an area after *Acropora* die-off may now use other coral species or marine objects for structure, and thus may not have colonized our *Acropora* mimics.

In future studies, it would be interesting to explore the long-term impact of artificial *Acropora* structures on juvenile recruitment and survival, especially between foraging guilds or taxonomic groups. Some fish may rely more heavily on shallow reefs as nurseries, whereas others utilize other nurseries such as mangroves and seagrass beds. The introduction of a food source to artificial reefs may also have effects on fish species abundance and diversity.

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AN EXPERIMENTAL TEST OF THE EFFECTS OF ALGAL COVER ON AGGRESSIVE INTERACTIONS OF DUSKY DAMSELFISH

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Abstract: Damselfish are aggressive territorial fish that protect and cultivate algae gardens. Because their gardening has a major effect on reef algae composition, their territorial behavior has bottom-up effects on coral reefs. We experimentally tested how aggression of dusky damselfish toward other fishes changed as a function of the amount of algae in their territories. In patch reefs near Little Cayman Research Center, damselfish were significantly more aggressive after we reduced algal cover. Interestingly, greater damselfish aggression at lower resource levels may indicate that aggression is a response to resource limitation or energy investment in algae cultivation.

Key words: *Stegastes fuscus*, *algae cultivation*, *resource availability*

INTRODUCTION

Damselfish are territorial coral reef fish that cultivate algae (Brawley and Addy 1977). Because they cultivate algae, damselfish are functionally important herbivores on coral reefs where they promote preferred algae growth and, as a result, indirectly deplete coral (Jones et al. 2006). Damselfish cultivation results in sections of the reef being in constant states of early succession, making the damselfish a major biotic cause of reef disturbance (Ferreira et al. 1998).

Damselfish can also remove and transplant algae from corals as part of their cultivation behavior (Jones et al. 2006). Damselfish can increase species richness and biomass of algae in their territories (Hata et al. 2002); their removal has consequently shown reduced biodiversity and biomass in algal species (Mahoney 1990). Axline-Minotti (2003) proposed that damselfish serve as keystone species on reefs, where their effects on algae composition affect diversity in three out of four functional feeding groups in the reef system.

Damselfish territories (usually 2 m²) are crucial to reef systems and are heavily defended (Osório et al. 2006). We examined the aggressive behavior of dusky damselfish in response to experimental removal of algae in their territories. We hypothesized that dusky damselfish would increase their defensive behaviors following a reduction in algae in their territories. Alternatively, aggression could decrease after a reduction in algae if damselfish shift their defensive efforts to nearby patches with undisturbed algae.

METHODS

We surveyed two patch reefs on Little Cayman, Cayman Islands, BWI. Point o' Sand (POS) is located on the southeastern end of Little Cayman and the Little Cayman Research Center (LCRC) is located on the northern side of Little Cayman between Grape Tree Bay and Jackson Point.

On 27 February 2011, we located 30 dusky damselfish territories at LCRC and 22 territories at POS. We marked each damselfish's territory with a numbered dead coral cobble.

On 28 February, from 0800-1100 at LCRC and from 1300-1600 at POS, we observed

baseline damselfish aggression of all 52 territories. For each fish, we conducted two trials; trials were 1 hour apart and lasted for 2 minutes. For each trial, we recorded the number of fish passing within 15 cm of the damselfish and whether they were attacked. A damselfish attack was defined as a sudden direct dart at an invader. We randomly selected 15 of 30 damselfish territories at LCRC and 10 of 22 at POS for experimental algal removal. To reduce algal cover, we exhaustively removed algae by hand, with pliers, and with scissors from 0.5 m² quadrats in the center of territories on 28 February. As a control for the removal method, we disturbed 15 LCRC territories and 12 POS territories by tapping the area with scissors, which did not remove ambient algae. The following day, we re-surveyed aggressive interaction in all territories.

We performed paired t-tests to compare before and after mean attack rates in ambient and removal territories at the two sites. Data did not significantly differ from normality and were not improved by a log transformation. Thus, we used untransformed data in our analyses. We performed two-tailed t-tests

to compare number of invaders between the two days at each site. Statistical analyses were performed in JMP 8.0.

RESULTS

At the reef near LCRC, there was a significantly higher (35%) attack rate in algae removal territories compared to baseline (baseline vs. removal: paired- $t_{14} = 5.80$, $P < 0.0001$; Fig. 1), but no difference in attack rate between baseline and ambient territories, where algae was left intact (baseline vs. ambient: paired- $t_{14} = 0.44$, $P = 0.33$; Fig. 1). Damselfish were significantly more aggressive in the removal treatment than in the ambient treatment at LCRC (removal vs. ambient: paired- $t_{29} = -3.49$, $P = 0.002$; Fig. 1). However, at the Point o' Sand site there were no differences in attack rate between baseline and where algae was left intact (ambient territories) or removed (baseline vs. ambient: paired- $t_{13} = 0.72$, $P = 0.25$; baseline vs. removal: paired- $t_8 = 0.17$, $P = 0.44$; Fig. 1). Damselfish were just as aggressive in the removal treatment as in the ambient treatment (removal vs. ambient: paired- $t_{23} = -0.28$, $P = 0.78$; Fig. 1).

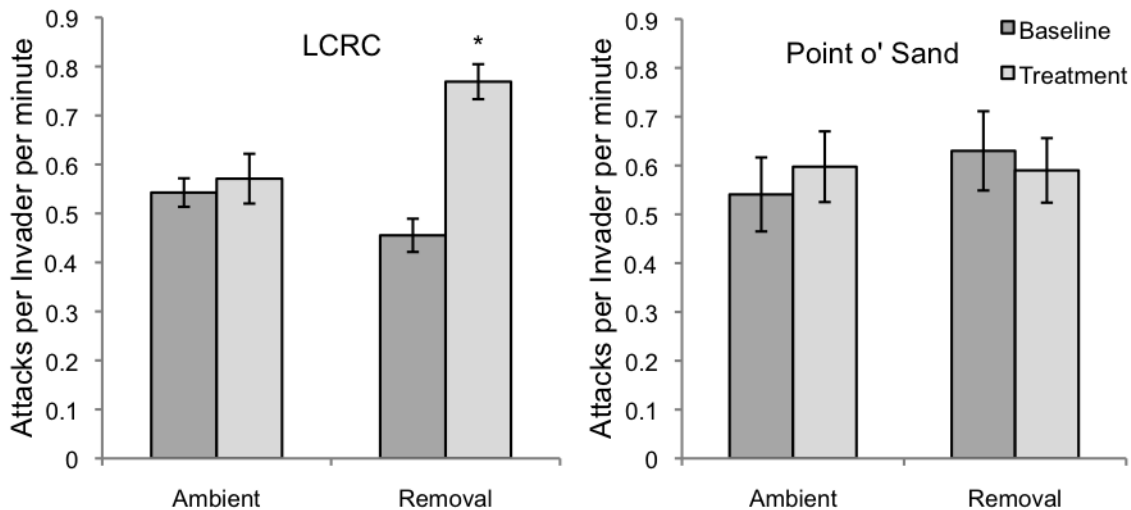


Fig. 1. Attack rates per min before (baseline) and after removal of algae (treatment) at each patch reef site in Little Cayman. Dark bars represent baseline damselfish attack rate per invader ± 1 SE. Light bars represent attack rate after algae removal treatment ± 1 SE. Asterisk (*) indicates that attack rate significantly increased with algae removal at LCRC site.

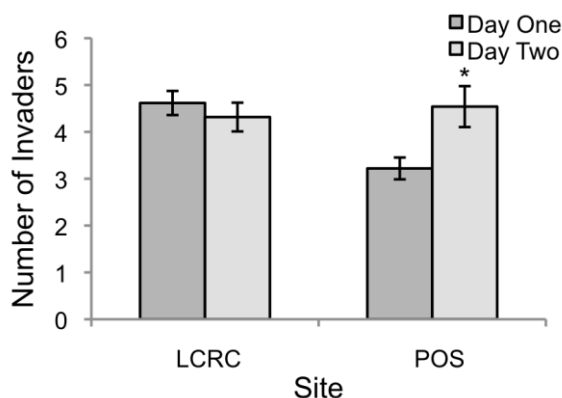


Fig. 2. Mean number of fish that invaded damselfish territories in two minutes at each site ± 1 SE. Dark bars represent the first day of observations and light bars represent the second day of observations. There were significantly more invaders at Point o' Sand on the second day than the first.

There was no significant difference in number of invaders before and after algal removal at LCRC ($t_{58} = 0.75$, $P = 0.46$; Fig. 2). However, there were significantly more (41%) invaders at Point o' Sand on the day after algal removal ($t_{43} = 2.17$, $P = 0.04$; Fig. 2).

DISCUSSION

Our data suggest that damselfish aggressive interactions are negatively related to algal cover. After algae were removed at LCRC, damselfish were more aggressive. Resources were more limited in the removal territories, so damselfish may have increased aggression to better compete for them and protect their algal gardens more strongly to promote rapid regrowth. The increase in aggression may also be related to the farming activity of the damselfish. Damselfish actively cultivate algae (Jones et al. 2006; Brawley and Adey 1977; Hata et al. 2002; Ferreira et al. 1998; Hoey and Bellwood 2010) and attack invading herbivores (Losey 1982; Itzkowitz 1990; Mahoney 1981; Harrington and Losey 1990). This aggression has been interpreted as active protection of the algae garden (Mahoney 1981; Jones et al. 2006). Therefore, damselfish may in-

crease their aggression after algae removal to allow depleted resources to recover.

Interestingly, there was no effect of algal removal on attack rates at POS. At POS, damselfish density appeared to be lower, and perhaps fish compensated for algae removal by foraging in a larger area (EB, RC, ZCS, LB, JF, pers. obs.). In contrast, damselfish density at LCRC appeared higher; hence, fish may have been unable to shift to a nearby unoccupied area after algae was removed from their territory (EB, RC, ZCS, LB, JF, pers. obs.). Therefore, at the LCRC site damselfish defended their territories more aggressively after algal removal than before removal.

We also observed varying water currents at LCRC and POS. The water current at POS was strong on the first day of observations (baseline) and weaker on the second day (post-treatment). While the number of invading fish was temporally consistent at LCRC, there were significantly more invaders at POS on the second day of observations than the first (Fig. 2), perhaps due to differences in water current. According to Osório et al. (2006), damselfish aggression is positively related to the number of invading fish in the area, so levels of aggression on day two should have been higher. However, damselfish at POS attacked the same proportion of fish on the second day regardless of increased invaders or treatment, so an abiotic effect seems likely.

At both sites, the composition of invaders was dominated by algivorous fish (EB, RC, ZCS, LB, JF, pers. obs.). LCRC appeared to have higher damselfish densities suggesting more competition for space and resources. Therefore, the removal of a 0.5 m² area of algae may have a greater influence on damselfish behavior at LCRC. Damselfish at POS appeared to be less common, possibly resulting in less competition for space and resources. Even though there were more invad-

ers at POS, removal of a 0.5 m² area of algae may not have been enough to increase damselfish aggression. Due to natural variation in damselfish territory size, removal of a set amount of algae may not warrant the same response from all damselfish.

As coral reefs are degraded and algae replace corals, we predict that damselfish will become less aggressive. This may have cascading effects on the structure of reef communities. If damselfish density decreases, other algivorous fish may become more locally abundant. Future work could examine interactions among damselfish and their impact on other fish species of different functional feeding groups to determine how increasing algal cover will affect their aggressive interactions.

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DIEL BEHAVIOR AND IMPACT OF THE INVASIVE RED LIONFISH (*PTEROIS VOLITANS*) ON NATIVE CORAL REEF FISH IN THE CARIBBEAN

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Abstract: The Red Lionfish (*Pterois volitans*) is a rapidly spreading invasive fish species in the Caribbean. Little research exists on the behavior and ecology of lionfish because the invasion is recent and lionfish are uncommon in their native Indo-Pacific range. We conducted diel behavior observations of lionfish in reefs near Little Cayman Research Center. We measured the species composition of native fish communities at sites with lionfish present and reference sites without lionfish. We hypothesized that lionfish would be most active at dawn and dusk, and that, as a generalist predator, they would reduce the local abundance and diversity of native fish. Lionfish were most active at dawn (06:00-08:00), afternoon (15:00-18:00), and dusk (18:00-20:00). We found 39% fewer small, potential prey fish at sites with lionfish compared to sites without lionfish. However, taxa richness, dominance, and composition of native reef fish did not differ between sites with and without lionfish. Our results add basic information to the limited knowledge of the activity of the invasive lionfish and its potential impacts on native reef fish communities.

Key words: Richness, dominance, abundance, taxa composition, species invasion

INTRODUCTION

Invasive species have destructive impacts on communities worldwide. They have the potential to alter the evolutionary trajectories of native species through competitive exclusion, niche displacement, and predation (Mooney and Cleland 2001). Molnar et al. (2008) found that 84% of marine environments have reported invasions, making marine ecosystems important for studying the effects of invasions on native species.

The Red Lionfish (*Pterois volitans*), native to the Indo-Pacific, is an invasive species to the western Atlantic that is threatening coral reefs. It is believed that the lionfish in the Caribbean originated from at least six individuals that escaped from an aquarium in Florida in 1992 (Whitfield et al. 2002). Lionfish are generalist predators that can consume a large number and a wide diversity of reef fish and crustaceans, and can reduce native fish recruitment by up to 79% (Albins and Hixon

2008). Lionfish can reproduce every four days under ideal conditions (Morris 2009) and are now found in high abundances throughout the Caribbean (Green and Cote 2009).

Despite the increasing presence of lionfish in the Caribbean, their behavior and impact on reef communities are relatively unknown (Morris et al 2009). There have been no published studies on lionfish in the Cayman Islands since their arrival in 2008.

One important aspect of lionfish behavior is the temporal variation in their activity. Fishelson (1975) found that lionfish are crepuscular in their native range. Morris et al. (2009) suggested that invasive lionfish are most active at dawn, while many divers have reported that lionfish in Little Cayman are active later in the day (M. Edwards and S. Barry, *pers. comm.*). We hypothesized that lionfish would be most active at dawn and dusk.

The composition of native fish communities in the presence and absence of lionfish may be a metric of lionfish impacts on coral

reefs. Since lionfish have been known to reduce native fish recruitment, lionfish presence may have strong effects on the abundance and diversity of native fish. Lionfish may have high site fidelity, and thus would have impacts in their localized area. We hypothesized that lionfish would reduce the abundance and diversity of native reef fish in localized areas.

METHODS

We conducted our study from 27 February – 2 March 2011 in patch and fringe reefs between Jackson Point and Grape Tree Bay near the Little Cayman Research Center, Little Cayman Island, BWI.

Survey

To quantify the distribution of lionfish, we searched for lionfish along three transects: patch reefs 0-40 m from shore, patch reefs 40-80 m from shore, and fringe reef 80-120 m from shore. All transects were 500 m long, 40 m wide, and parallel to shore from the Marine Park marker east of the Little Cayman Research Center to Rock Bottom House west of Little Cayman Research Center. We searched from 9:30-11:00 AM on 27 February 2011, totaling 12.8 observation hours among 7 people. We marked the fish locations with numbered flagging so we could return to the sites.

Behavior

At five different time intervals throughout the day (dawn – 6:00-8:00, morning – 8:00-12:00, afternoon – 12:00-17:00, dusk – 17:00-19:00, dark – 19:00-21:00), we observed lionfish behavior. We allowed the focal fish to acclimate for five minutes to an observer located 1-2 m away. During each time interval, we observed fish four times for five minutes each, with three-minute non-observation intervals between trials. During each trial we recorded changes in behavior and the time at which the behavior changed. Behaviors were

described as resting (no effort to move or drifting passively with the current), tracking (visually or physically following fish), moving (actively swimming), striking (making a sudden movement with mouth open), gaping (opening mouth with no other visible movements), and benthic foraging (nibbling the surface of the reef). We also recorded the location of each fish, noting whether it was in a crevice, on the bottom, or in open water.

Species Composition and Abundance

To assess the species richness and abundance of native fish, we conducted four instantaneous scans within a 1 m radius of each lionfish site. During the 15 s scans, we identified all the fish present to taxonomic family and age class (juvenile or adult). We also conducted four identical scans at reference sites without lionfish and with similar coral structure, water depth, and substrate approximately 15 m away from each lionfish site.

Analyses

To examine the activity of lionfish throughout the day, we calculated the percent time out of the total 20 minutes spent on all 4 trials that a fish exhibited each behavior. Because the data were not normally distributed and could not be normalized with a transformation, these percentages were compared among the different times of day using Wilcoxon non-parametric tests in JMP 8.0.

We tested effects of lionfish on fish taxa diversity by comparing richness, dominance, and taxa similarity indices between sites with and without lionfish. Mean expected taxa richness and dominance and 95 % confidence intervals were estimated by rarifying to an abundance of 457 individuals using EcoSim software version 7.00. A t-test was used to compare the abundance of fish between sites with and without lionfish. Abundances were normally distributed.

We used Morisita's similarity index to calculate similarity of fish taxa between sites with and without lionfish. To calculate the mean similarity of fish taxa within lionfish sites and the mean percent similarity within reference sites, a random number generator was used to pair lionfish sites with each other and reference sites with each other. We calculated the similarity index for random pairs and performed an ANOVA to test for differences between lionfish and non-lionfish sites, lionfish to lionfish sites, and non-lionfish to non-lionfish sites.

To test for the effects of lionfish on potential prey taxa, we divided the native reef fish into potential prey and non-prey categories. Because lionfish are known to consume prey up to 48% of their own body length (Morris and Akins 2009), and the largest lionfish in our study was estimated to be 22 cm, we established maximum possible prey size at 10 cm. Average fish length within a taxa was

taken from the literature (Humann and DeLoach 2003). We considered fish longer than 10 cm and fish taller than 3 cm too large to be consumed. Abundance was compared among sites using a t-test, and taxonomic composition was compared using ANOVA.

RESULTS

Percent of time spent hiding was significantly related to time of day (Wilcoxon test, $c^2_{4,26} = 7.01$, $P = 0.026$; Fig. 1). We did not find a significant relationship between time of day and percent of time spent active (Wilcoxon test, $c^2_{4,26} = 7.67$, $P = 0.10$; Fig. 1) or percent of time spent resting in the open (Wilcoxon test, $c^2_{4,26} = 11.00$, $P = 0.13$; Fig. 1).

After rarefaction, we found that the mean expected taxa richness of sites with lionfish was 19 taxa (95% confidence interval: 18-19), and the mean expected taxa richness of reference sites was also 19 taxa (95% confidence interval: 19-20, Fig. 2). Thus, there were no

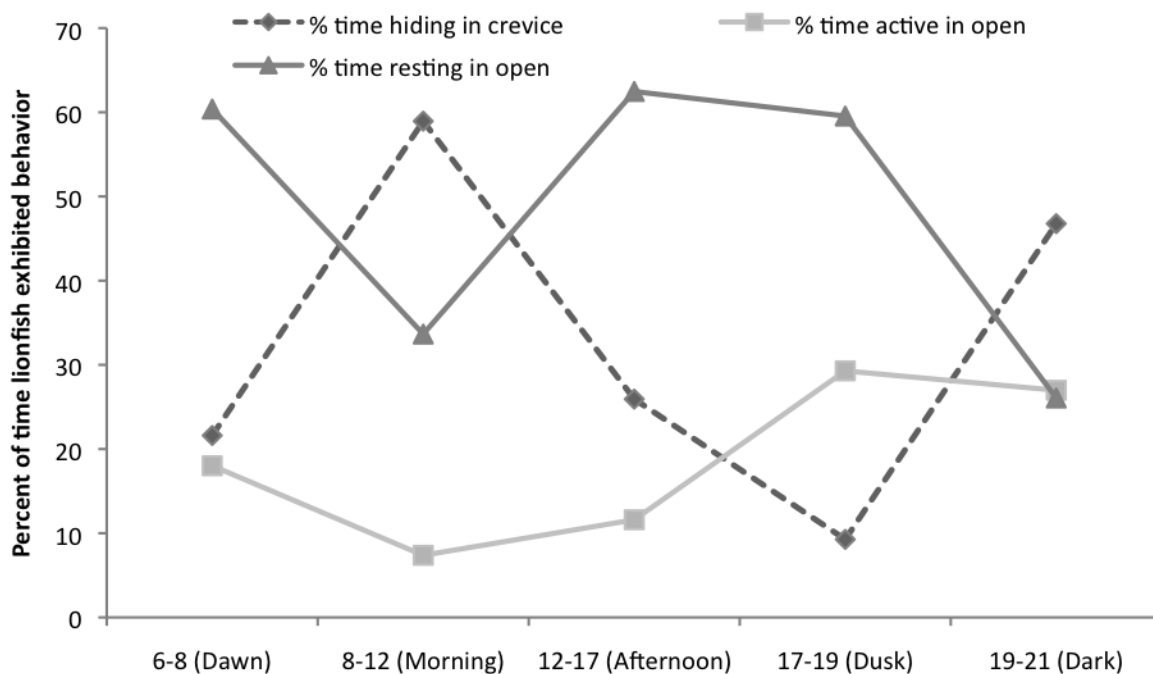


Fig. 1. Diel behavioral activity of lionfish. Average percent of time lionfish spent hiding in a crevice, resting in the open, and active in the open per time interval in offshore patch and fringe reefs near the Little Cayman Research Center, Little Cayman Island. Behaviors were recorded during the time intervals of dawn (6:00-8:00), morning (8:00-12:00), afternoon (15:00-17:00), dusk (17:00-19:00), and dark (19:00-21:00) on varying days from 27 February – 2 March 2011.

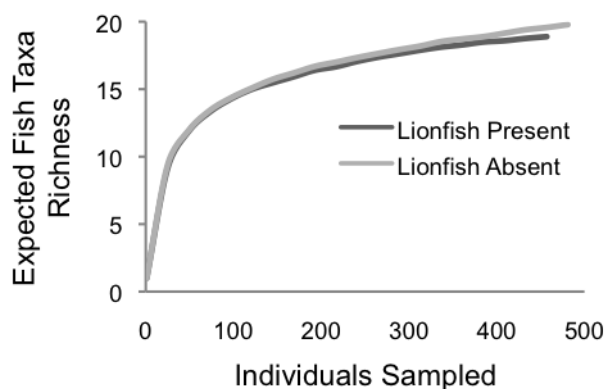


Fig. 2. Rarefied taxa richness of native reef fish at lionfish sites and reference sites without lionfish was similar on patch and fringe reefs near the Little Cayman Research Center, Little Cayman Island. Data were collected from 27 February – 2 March 2011.

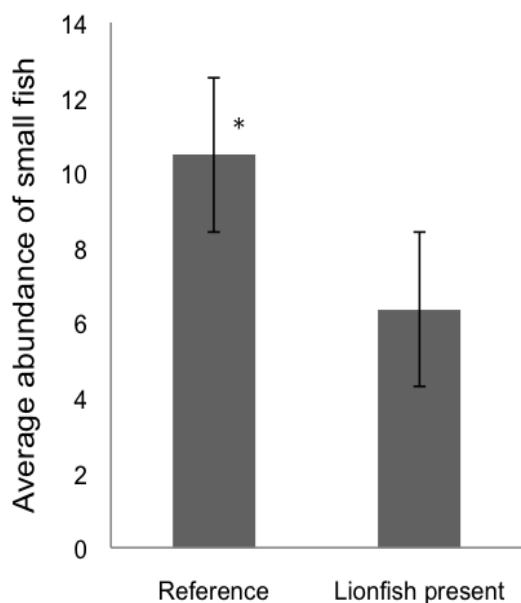


Fig. 3. Average abundance of small prey fish (less than 10.0 cm) at sites with lionfish and reference sites without lionfish at patch reefs near Little Cayman Research Center, Little Cayman Island. Reference sites had an average of 10.5 prey fish while lionfish sites had an average of 6.4.

significant differences in richness between sites with and without lionfish. Using data with all of the fish taxa, the dominance index of lionfish sites was 0.35 (95% confidence interval: 0.33-0.35) and the dominance index of reference sites was 0.27 (95% confidence interval: 0.26-0.28), indicating that the sites with lionfish have significantly higher species dominance. However, there was no significant

difference in richness of prey species between sites with and without lionfish.

Using Morisita's similarity index, we found that similarity of the taxonomic composition at sites with and without lionfish (39.5%) did not differ from percent similarity of randomly paired lionfish sites (32.1%), or from randomly paired reference sites (27.3%) (ANOVA: $F_{2,39} = 1.92$, $P = 0.16$). Similarity indices of small, prey taxa were also not significantly different between pairs of sites with lionfish and pairs without lionfish (ANOVA: $F_{2,38} = 1.94$, $P = 0.15$).

Out of the 16 sites, we returned to five of them three or four times each and found lionfish in their original site 78% of the time.

DISCUSSION

We found that lionfish activity exhibited a distinct diel behavioral pattern. The percentage of time lionfish spent hiding was significantly related to time of day, with least time spent hiding at dawn, afternoon, and dusk. This indicates that lionfish may be foraging on fish species that are active during these times. These results support Fishelson's (1975) conclusions that Indo-Pacific lionfish are crepuscular. Their temporal activity patterns may be a relic from their native habitat, where their prey species, but not their predators, may be active at dawn and dusk (Eggers 1978). We also observed lionfish foraging behavior throughout the day, which may suggest that they are, in part, opportunistic feeders.

Contrary to our hypothesis, we did not find differences in the diversity of native reef fish in sites inhabited by lionfish compared to nearby sites without lionfish. Because fish are highly mobile, any impact of lionfish on native fish species may be distributed across the reef community. Our reference sites may not be independent of the effects of lionfish; for

example, we later discovered lionfish in close proximity to two of the reference sites. We did not exclude these sites from our analyses because lionfish may have influenced our other reference sites as well. Future studies could ensure independence of reference sites by comparing reefs with lionfish to reefs with no documented lionfish invasion, such as those in the Gulf of Mexico (Schofield 2009). In addition, the statistical power of this analysis was low, so a larger sample size may detect additional differences in fish diversity indices.

Similarity in taxonomic composition between lionfish sites and reference sites may indicate that native fish do not avoid lionfish. Native fish may not perceive lionfish as a threat, possibly because it is a recent invader and prey have not had time to evolve in response (Freeman and Byers 2006). In 11.3 hours of observation, we witnessed only five discrete displays of fish aggression, all of which were initiated by the lionfish. More often the native reef fish swam past the lionfish without exhibiting a visible reaction. Similarly, Freeman and Byers (2006) demonstrated that mussels exposed to a recent invasive predator do not exhibit defensive responses.

We found that species dominance differed between lionfish and reference sites. In lionfish sites the large French Grunt was the dominant species, while in reference sites the small Bluehead Wrasse was the dominant species. Furthermore, the abundance of prey fish less than 10 cm long was significantly lower in the presence of lionfish, and small fish have been found in the stomachs of lionfish (Morris and Akins 2009). Lionfish consumption may be reducing the abundance of small fish nearby, but we witnessed no predation events on native fish during our study. While lionfish may be preying on fish outside

of our observations, we did observe lionfish, especially juveniles, foraging near coral and algae, possibly consuming small invertebrates. Since we found that lionfish have high site fidelity, they may consume many invertebrates in one area, thereby indirectly deterring small fish from foraging for invertebrates near them.

Understanding the behavior and the effects of lionfish on reef ecosystems is essential to managing their invasion in the Caribbean. Little Cayman Island is attempting to manage the lionfish invasion through regular culling efforts (M. Edwards and S. Barry, *pers. comm.*). Our findings may improve the efficiency of the culling process by identifying times when lionfish are active or resting in the open, making them easier to find and capture. Additionally, our study is a preliminary step towards understanding the impacts of lionfish on Little Cayman reef fish communities. Future long-term, large-scale studies comparing whole reef ecosystems with and without lionfish may provide insight into the effects of this invasive species.

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CORAL BLEACHING BY ALGAL ALLELOPATHY

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Faculty Editor: Brad W. Taylor

Abstract: Allelopathy is one common type of plant defense against herbivory; however, algae use allelopathy to regulate competing coral recruitment and growth via bleaching. We hypothesized that algae that do not recruit on coral would also possess these allelopathic compounds as an exaptation—preventing recruitment of other algae and corals but also inducing coral bleaching. We exposed corals to species of algae that do (*Dictyota divaricata*) or do not (*Halimeda incrassata*) recruit on coral to test bleaching events on the lobed star coral *Montastraea annularis*. Our hypothesis was not supported by our findings. Algae that naturally recruit on corals (*D. divaricata*) bleached corals, while non-coral recruiting species (*H. incrassata*) did not, suggesting that *D. divaricata*, and possibly similar coral recruiting species, have allelochemicals that bleach corals to outcompete corals for space. Though allelochemicals may not be the only factors leading to coral bleaching, our study suggests that algae that recruit on coral heads use allelopathy to increase algal recruitment through bleaching. Algae may become an important mechanism in driving the architecture of coral reefs as corals are threatened by multiple stressors.

Key words: *Dictyota divaricata*, *Halimeda incrassata*, *lobed star coral*, *Montastraea annularis*, *recruitment*

INTRODUCTION

Secondary compounds are often present in plant species to prevent herbivory but can also have allelopathic effects on competitors. In marine ecosystems, Rasher and Hay (2010) showed that some seaweed species poison corals through direct contact and the release of secondary compounds, causing corals to expel their zooxanthellae resulting in bleaching. Little is known about allelopathy among marine organisms (Graneli and Pavia 2006); however, Kakisawa et al. (1988) showed that some brown algae produce a fatty acid that has allelopathic properties. Corals also produce allelopathic substances contemporaneously with seaweed spore releases (Kim et al. 2004), indicating that there is a competition for recruitment between corals and algae.

With a strong arms race existing between algal and coral recruitment, Rasher and Hay (2010) found that calcifying algae (*Dictyota divaricata*) that grow on deceased coral heads not only produce secondary compounds that protect against recruiting coral polyps but

also that those same compounds kill corals via bleaching. We hypothesized that all algae possess allelochemicals to increase algae recruitment due to the intense competition among conspecific algae. Given Rasher and Hay's (2010) findings, we further hypothesized that species that do not naturally recruit on corals (*Halimeda incrassata*) would produce allelochemicals that would have the same bleaching effects as those that do naturally recruit on corals (*D. divaricata*). We predict that coral recruiting algae coral recruiting algae, such as *D. divaricata*, use these allelochemicals as an exaptation for the added benefit of coral bleaching, which inherently increases recruitment by killing coral heads.

We used these two macroalgae to test coral bleaching events due to algal allelochemicals. We measured bleaching on coral heads that were put in direct contact with algae.

METHODS

We identified five coral patch reef heads and ran experiments at Point of Sand (POS), Little Cayman Island, Cayman Islands from 7-

9 March 2011. On each coral head, we identified patches of lobed star coral (*Montastraea annularis*), and placed two replicates of three different treatments on these lobes. The three treatments were: *D. divaricata*, *H. incrassata*, and control. The algae were collected on 7 March 2011 from the same POS site. To standardize the amount of algae used, we measured volume of the algae with a graduated cylinder. Algal clumps of 20 mL were attached to fishing sinkers and labeled with flagging tape. Control treatments were sinkers with flagging tape without algae.

We placed two replicates of each treatment (*D. divaricata*, *H. incrassata*, and control) on each of the five coral heads. Each replicate was placed on haphazardly chosen locations on the lobes and at least 20 cm apart from other replicates. The sinkers were placed on top of the algae to ensure direct contact between algae and coral. We also measured the depth of each replicate at mid-tide to deter-

mine if depth was a confounding factor for the different treatments. After 48 hours, algae were collected, and their circular bleaching areas (defined as white areas under treatments) were measured to the nearest mm with calipers at their largest diameter.

We natural-log transformed the area bleached and used ANOVA to test for differences in bleaching among treatments. We also used ANOVA to test for differences in depth among treatments, which could have affected the degree of bleaching. All statistical analyses were completed using JMP 8.0.

RESULTS

There was a 3.7 fold increase in bleaching between *D. divaricata* treatments and controls and a 6.0 fold increase in bleaching between *D. divaricata* treatments and *H. incrassata* treatments (ANOVA, $F_{2, 25} = 11.72$, $P = 0.0003$; Fig. 1). Water depth did not differ among treatments (ANOVA, $F_{2, 26} = 0.91$, $P = 0.42$; Fig. 2).

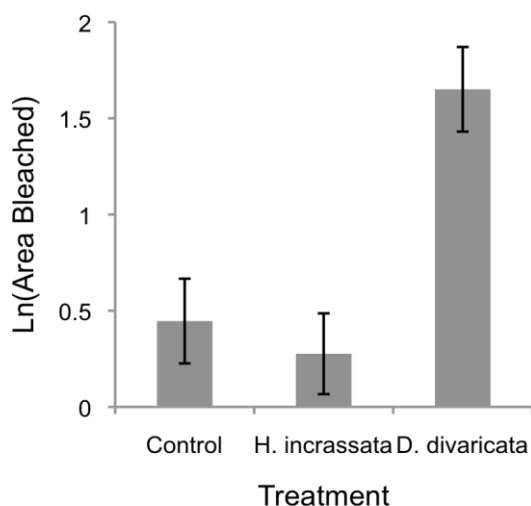


Fig. 1. Average area of lobed star coral (*M. annularis*) bleached in response to direct contact with algae (*H. incrassata* and *D. divaricata*) at Point of Sand, Little Cayman, Cayman Islands. *H. incrassata* is an algal species that does not recruit on coral, while *D. divaricata* recruits on coral. Controls were sinkers without algae in direct contact with coral. Values are mean \pm 1 SE.

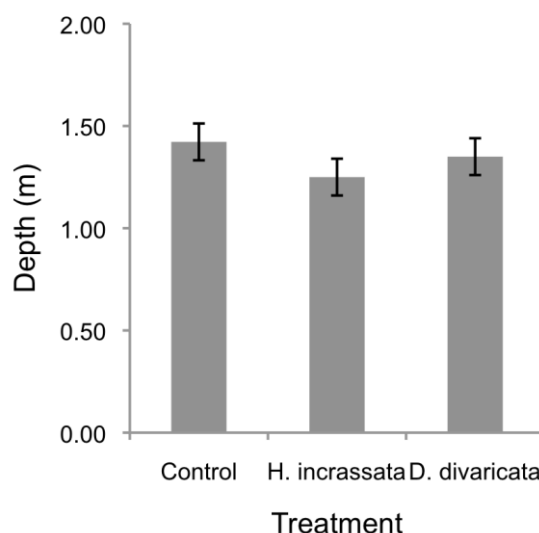


Fig. 2. Average depth of lobed star coral (*M. annularis*) at sample sites (Point of Sand, Little Cayman, Cayman Islands) of treatments: direct contact with algae (*H. incrassata* and *D. divaricata*). Controls were sinkers without algae in direct contact with coral. Values are mean \pm 1 SE.

DISCUSSION

We found that *D. divaricata* bleached significantly more coral than *H. incrassata* or our control treatment. This suggests that *D. divaricata*'s allelopathic bleaching compounds may be an adaptation. We cannot conclude whether the bleaching result is an adaption specifically for bleaching; however, our results suggest that coral recruiting algae have some mechanism that bleaches corals, directly or indirectly.

H. incrassata's lack of bleaching effects on corals suggests that this species lacks bleaching allelochemicals, despite having other similar secondary compounds to coral recruiting algae (Rasher and Hay 2010). Competition among algae may be why *H. incrassata* still produces allelochemicals to prevent recruiting algae. However, *H. incrassata* does not recruit on coral, thereby not needing coral bleaching compounds.

Rasher and Hay (2010) suggested that algae have allelopathic compounds that may bleach coral to make algal recruitment more successful. Our results suggest that coral bleaching compounds are not present in all algae species but only in those that specifically settle on coral heads.

We measured the depth of replicates to determine that depth was not a confounding factor on bleaching among treatments. To control factors such as current and light availability as much as possible, we chose coral heads at similar depths, assuming that bleaching could differ in intensity based on local abiotic factors. We found no effect of depth on bleaching.

Further studies could examine whether bleaching compounds are inducible when algae are in direct contact with coral or if they are produced constantly as a preventive agent against settling polyps. Many stressors affect corals, leading to their current decline; though

bleaching via allelochemicals may not be a leading cause of coral decline, we believe that algal allelopathy may also affect coral recovery. Preventing new polyps from recruiting is likely to slow coral recovery rates from stressors like bleaching. By preventing recruitment of new polyps and bleaching existing coral, coral recruiting algae may have significant effects on coral's ability to recover from declines.

Our study suggests that as coral recruiting algae increase in abundance, coral health will likely decline as corals are bleached by algal allelopathy. *D. divaricata* and similar species may eventually dominate the architecture of coral reef ecosystems because of their allelochemical effects on corals. *D. divaricata* and similar species have an increased effect because these species can not only prevent coral from recruiting but also kill adult corals, preventing polyps from even being released.

However, *H. incrassata* and similar species may become the dominant species as they can prevent coral recruitment but do not rely on coral as a substrate for recruitment. Thus, with increasing coral declines and decreased availability of substrate for new algae, negative feedback may be exerted on allelochemical production in coral recruiting algae. If an equilibrium point is not reached and coral recruiting algae deplete their available coral substrates, the future of declining corals may bring rise to a reef dominated by *H. incrassata* and similar species.

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LIONFISH (*PTEROIS VOLITANS*) HABITAT USE AND EFFECTS ON FISH SPECIES COMPOSITION IN THE CARIBBEAN

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Abstract: Lionfish (*Pterois Volitans*) are an invasive species in the Caribbean that arrived to the Cayman Islands in 2008. While previous studies have demonstrated that lionfish can reduce native fish recruitment, little is known about their habitat preferences and their effect on local fish communities in Little Cayman. We conducted an observational study of lionfish habitat characteristics and an experimental study of the effects of lionfish removal on local fish communities. We examined lionfish habitat characteristics, such as reef patch size and percent coral cover. We hypothesized that the removal of lionfish would increase diversity of native reef fish. We found that lionfish size and site fidelity were positively associated with coral patch circumference, but this trend was not statistically significant. We also found that the index of reef fish dominance was lowest where lionfish were absent, intermediate where lionfish were removed, and highest where lionfish were present. We did not find any differences in species richness or similarity among sites at which lionfish were removed, present, or absent. Further studies with access to more lionfish and more habitats may better test the impacts of lionfish on reef communities.

Key words: invasive species, reef, richness, dominance

INTRODUCTION

Invasive species introductions have severe ecological and economic impacts on their host environments. The Red Lionfish (*Pterois volitans*) invasion in the Western Atlantic began with the accidental introduction of at least six lionfish from a Florida aquarium in 1992 (Whitfield et al. 2002). Since then, lionfish have spread along the east coast of the United States and in the Caribbean, and they have been spotted as far north as New York (Hare and Whitfield 2003).

Morris and Whitfield (2009) predicted that without natural or anthropogenic limitations, lionfish would eventually spread west into the Gulf of Mexico, north to Virginia, and south along the eastern coast of South America. Furthermore, they predicted that the local lionfish densities would continue to increase, exacerbating problems in coral reef systems (Morris and Whitfield 2009).

Because lionfish are voracious generalist feeders, their presence as a dominant reef predator can dramatically decrease the biomass of small fish and shrimp (Fishelson 1997). This may lift grazing pressures on algae, leading to increased algal growth (Morris and Akins 2009). Lionfish may also outcompete native species, especially juvenile fish, affecting the community structure of these populations (Eggleston et al. 1998). Albins and Hixon (2008) found that lionfish in the Bahamas can reduce recruitment of native reef fish by 79%.

To decrease the density of lionfish, several management strategies have been developed, largely focused on culling, which involves removing lionfish with nets or spears (Morris and Whitfield 2009). Effective culling efforts depend on the ability to locate lionfish efficiently.

Few studies have examined lionfish behavior, habitat preferences, and impact on local fish communities in the Caribbean. Remsen et

al. (2011) found that the native reef fish species composition near lionfish is not significantly different than at reference sites (sites without lionfish that have similar characteristics to sites with lionfish). However, due to possible confounding factors, including the presence of unaccounted for lionfish in reference sites, these results may not reflect the full impact of lionfish presence on local reef fish community structure. This study expands on Remsen et al. (2011) by experimentally examining how the presence or removal of lionfish affects the species composition of local reef fish. We measured species composition at sites with lionfish and then removed the lionfish to directly measure the effects of lionfish removal on local native species richness and dominance index. We predicted that sites with lionfish present would have a higher dominance and lower richness of native reef fish before removal than after. Lionfish may deplete prey species locally, thus increasing dominance of non-prey species and decreasing richness.

Another purpose of this study is to investigate the trends in lionfish habitat characteristics. Knowing these trends would facilitate lionfish location, increasing the efficiency of culling efforts. We observed certain habitat characteristics, including patch reef size, percent of coral composition, and specific location of the lionfish in a reef (crevice, ledge, or reef surface). We also noted lionfish size because lionfish of different sizes may occupy different habitats.

METHODS

We conducted our study from 4 - 8 March 2011 in patch and fringe reefs west of Grape Tree Bay near the Little Cayman Research Center, Little Cayman Island, BWI.

Within a 100 m long, 80 m wide stretch of patch and fringe reef, we found 11 lionfish

and marked their sites with flagging tape on 4 March 2011.

On 6 March 2011, in the morning and afternoon, we recorded characteristics of these sites and the lionfish within them, if the lionfish was present. Characteristics included lionfish presence; estimated lionfish length (tip of nose to base of tail; Fig. 1); whether the lionfish was in a crevice, under a ledge, or on the surface of the reef; water depth; lionfish height from the substrate; circumference of the patch reef containing the site, if on a patch reef; estimation of degrees out of 360 that the lionfish was enclosed by the reef; and estimated percent coral cover in a 3 m radius around the lionfish.



Fig. 1. Lionfish adult at a patch reef offshore from the Little Cayman Research Center, Little Cayman Island, BWI on 4 March 2011. Photo by Ian D. Engler.

We then recorded species composition of the reef fish within a 2 m radius of each lionfish site. After allowing 2 min for reef fish to habituate to our presence, we performed four 15 s scans of reef fish species and abundance per site, with 2 min between each scan. We identified reef fish to species, as well as to age class (adult or juvenile).

Four lionfish were removed using SCUBA, hand nets, and a spear from the previously marked sites. Fish to be removed were selected opportunistically based on our ability to relocate them.

Table 1. The range and mean values for lionfish size and habitat characteristics for 11 lionfish sites in fringe and patch reefs offshore from the Little Cayman Research Center, Little Cayman Island, BWI from 4 – 8 March 2011.

Characteristic	Range	Mean
Lionfish size (cm)	6 - 25	12
Water depth (m)	0.6 - 1.2	1
Lionfish height from substrate (m)	0 - 0.6	0.2
Patch circumference (m)	6.6 - 26.0	16
Degree enclosure (°)	45 - 270	178
Percent coral cover (%)	40 - 80	44
Percent site fidelity (%)	20 - 100	63

The following day, in the morning and afternoon, we recorded species composition of reef fish at all sites and categorized sites as lionfish absent, lionfish present, and lionfish removed.

Analyses

We calculated the range and mean of lionfish size, water depth, lionfish height from substrate, patch circumference, percent of coral in a 3-meter radius of the lionfish, and site fidelity of the eleven lionfish observed. Site fidelity was recorded as the percent of times lionfish were present out of the five times that we visited each site. At the sites where lionfish were removed, site fidelity was measured as the percent of times lionfish were present out of the three times we visited each site before removal. We used linear regressions to test the relationships among lionfish size, site fidelity, and habitat characteristics in JMP 8.0. We log transformed lionfish size and arcsine square root transformed site fidelity to normalize these data.

We rarefied the species composition data to standardize for differences in abundance and calculated dominance and richness using EcoSim version 7.00.

We used Morisita's Similarity Index to calculate percent similarity of fish taxa of sites

with lionfish present, lionfish absent, and following lionfish removal. A random number generator was used to pair present sites with each other, absent sites with each other, and removed sites with each other. We calculated the similarity index for these random pairs as well as random pairs of present to removed sites, present to absent sites, and absent to removed sites. We compared them with ANOVA, testing if differences in the percent similarity were caused by random chance rather than by the presence of the lionfish.

RESULTS

The average lionfish size was 12 cm, the average patch circumference was 15.5 m, and the average percent of coral cover was 44% for the eleven sites observed (Table 1). While not significant, we found a weak positive trend between patch circumference and log transformed lionfish size ($r^2_{11} = 0.07$, $P = 0.38$; Fig. 2). We also found a non-significant positive trend between patch circumference and arcsine square root transformed lionfish site fidelity ($r^2_{11} = 0.11$, $P = 0.27$; Fig. 3). We found that 46% of the lionfish observed were found in a crevice, 36% under a ledge, and 18% on the surface of the reef.

After rarefying the data to a sample size of 257 individuals, the mean expected species richness of sites where lionfish were absent was 26.06 species (95% Confidence Interval: 23-28), the mean expected species richness of sites where lionfish were present was 26.00 species, and the mean expected species richness of sites where the lionfish were removed was 22.69 (95% Confidence Interval: 21-23). The overlapping confidence intervals given by EcoSim indicate there were no significant differences in mean expected richness of sites with lionfish absent, lionfish present, and lionfish removed.

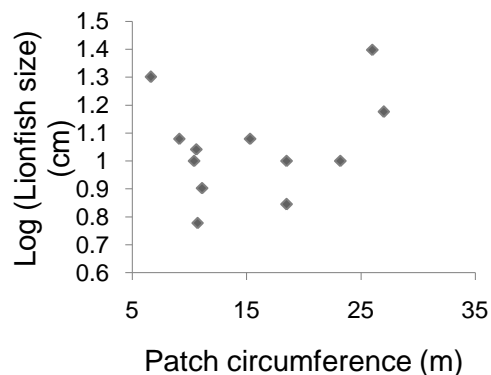


Fig. 2. The suggested, but not significant, positive relationship between patch circumference (m) and lionfish size (cm). Data were collected from 11 lionfish at sites in fringe and patch reefs offshore from the Little Cayman Research Center, Little Cayman Island, BWI from 4 - 8 March 2011. Lionfish size was approximated underwater using a ruler and then log transformed.

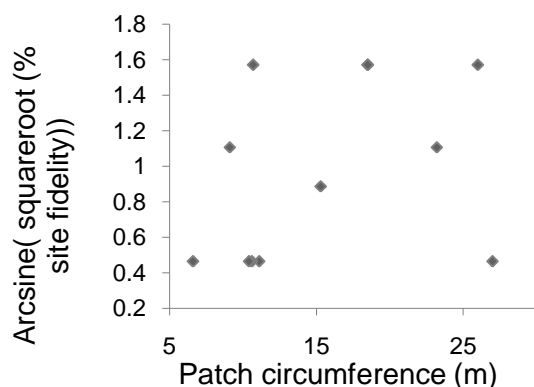


Fig. 3. The suggested, but not significant, positive relationship between patch circumference (m) and arcsine square root-transformed lionfish site fidelity. Data were collected from 11 lionfish at sites in fringe and patch reefs offshore from the Little Cayman Research Center, Little Cayman Island, BWI from 4 - 8 March 2011. Site fidelity was calculated from the number of times lionfish were observed at their original site divided by the number of visits to the site.

The dominance index of sites where lionfish were absent (0.14; 95% Confidence Interval: 0.125-0.160) was significantly lower than the dominance index of sites where lionfish were removed (0.20; 95% Confidence Interval: 0.187-0.218; Fig. 4). The dominance index of sites with lionfish present was 0.31, which was significantly higher than both the dominance indices of sites with lionfish absent and

the sites where lionfish were removed. Significance was determined by examining the overlap in 95% Confidence Intervals given by Ecosim.

The ANOVA found all comparisons of similarity across conditions (present-present, present-absent, present-removed, absent-absent, absent-removed, and removed-removed) were not significantly different ($F_{5,27} = 1.48$, $P = 0.23$).

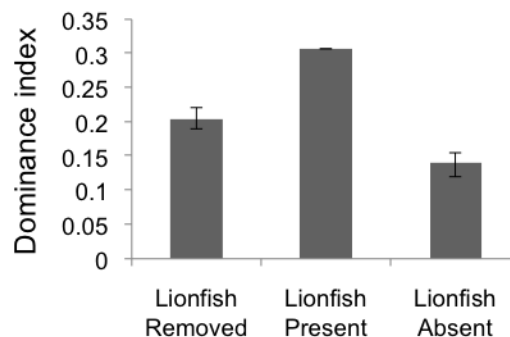


Fig. 4. Dominance indices of all native reef fish observed for sites with lionfish removed, lionfish present, and lionfish absent, calculated using rarified data collected from 11 lionfish at sites in fringe and patch reefs offshore from the Little Cayman Research Center, Little Cayman Island, BWI from 4 - 8 March 2011.

DISCUSSION

We examined site characteristics and found patterns between patch size, lionfish size, and site fidelity. Contrary to our hypothesis, we found differences in native fish dominance, but not richness, among present, absent, and removed lionfish sites.

In this patch/fringe reef environment, lionfish can occupy a wide range of patch sizes with circumferences ranging from 6.6 to 26.0 m (Table 1). Larger patches may offer increased structural complexity, which previous studies have linked to increased abundances of native reef fish (Garpe et al. 2006), which may be prey species for lionfish. Therefore, the weak positive trend between patch circumference and lionfish site fidelity may be explained by an increased abundance of prey

in larger patches. In addition, we found a weak positive trend between patch circumference and lionfish size. Larger lionfish require greater amounts and different types of prey (Morris and Akins 2009) that may be more available at larger patches. Since larger lionfish may occupy larger patches, our study provides information that may be used if culling efforts target specific age classes. Greater sampling effort would provide more insight into these trends.

There were no significant differences in native fish species richness in the presence, absence, or removal of lionfish, possibly because their impact is not localized around their habitat. These findings were consistent with Remsen et al. (2011), which found no differences in native reef fish richness between sites where lionfish were consistently observed and sites on the same reef where no lionfish had been observed during the study. Due to their highly variable site fidelity (Table 1), the impact of lionfish might be spread out over a greater area, thereby diluting their local effect. Additionally, as mentioned in Remsen et al. (2011), lionfish are relatively recent invaders, and prey fish may not avoid them because they have not had enough time to develop an awareness of the danger of lionfish (Hare and Whitfield 2003).

Our data demonstrate that sites where lionfish were absent and sites where lionfish were removed had significantly lower dominance indices than sites where lionfish were present, possibly because the lionfish may be decreasing the abundances of prey species. Thus, richness might not be impacted because the prey species are still present, but evenness is decreased because certain prey species are less abundant (Remsen et al. 2011). Our data also show that the dominance index of sites where lionfish were absent was significantly lower than that of sites with lionfish

removed. This is likely due to the fact that our study only permitted one day of readjustment after lionfish were removed, so native reef fish may not have had sufficient time to reoccupy the area.

The presence of lionfish does not appear to be related to specific species of reef fish, as there were no significant differences among all comparisons of species similarity across all site conditions (lionfish present, absent, or removed). These findings are consistent with Remsen et al. (2011). A possible explanation for this is that lionfish may have a minimal impact upon local reef fish communities, contrary to Albins and Hixon (2008). Alternatively, the impact of lionfish on reef communities may be extensive enough that lionfish removals from small patch reefs do not cause significant changes in reef fish composition because these removals would only detect localized effects on reef communities. In addition, if lionfish do indeed affect community composition, reef fish may not have had sufficient time to respond to their removal in the single day that passed between removal and our data collection on species composition. This could also apply to our findings of daily variation in lionfish presence at a given site; as long as a lionfish visits a site somewhat regularly, it may have a consistent impact upon reef fish communities because lionfish are efficient and voracious predators (Morris and Akins 2009). Future studies could examine the effects of lionfish removal over a longer period of time.

While our findings regarding lionfish impact on local reef fish composition were consistent with the observational study by Remsen et al. (2011), our experimental study was more rigorous because it examined the effects of the removal of lionfish from select sites. In addition, our study provides data on patterns among habitat characteristics, lionfish size,

and site fidelity, which may be used to increase the efficiency of locating lionfish in future culling efforts.

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We would like to thank Greg Locher, who volunteered to cull the lionfish for the experimental manipulation of our study. Without his help, our study could not have happened. We would also like to acknowledge the staff of Little Cayman Research Center for support of experiment logistics.

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EFFECTS OF OCEAN ACIDIFICATION ON A TURTLE GRASS MEADOW

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Faculty Editor: Brad Taylor

Abstract: With increasing atmospheric CO₂ concentrations, the oceans are expected to increase in acidity during the next century. Increasing ocean acidity has been shown to negatively affect many marine ecosystems, particularly calcifying organisms. We investigated acidification effects on the turtle grass communities of Little Cayman Island, BWI. We hypothesized that acidified seawater would decrease turtle grass growth, the presence of calcifying epiphytic algae, and the metabolism of snail grazers. We placed turtle grass and snails in tanks with acidified or natural seawater for 4 days. We found that turtle grass growth decreased, leaf senescence increased, and epiphytic algal cover was strongly reduced in acidified seawater. We also found that snail activity was negatively affected by acidic seawater. Our results suggest that continuing ocean acidification could be detrimental to the productivity and health of turtle grass meadows.

Key words: *Thalassia*, *Littorina*, calcifying algae, CO₂ concentrations, pH

INTRODUCTION

Oceans act as important carbon sinks for anthropogenic emissions, reducing atmospheric concentrations of carbon dioxide by up to one-third (Doney et al. 2009). As oceans become saturated with carbon dioxide, the pH of seawater is predicted to drop from 8.1 to 7.7-7.8 by the end of this century (Le Quéré et al. 2007; Orr et al. 2005). This increased acidity may have dramatic effects on marine organisms, in part because it reduces the concentration of carbonate ions and the solubility of aragonite, a form of calcium carbonate used to create biological structures (Orr et al. 2005; Doney et al. 2009).

Turtle grass (*Thalassia testudinum*), the most common marine plant in the Caribbean (Littler et al. 1989), supports an ecosystem with a variety of organisms, including epiphytic algae and snails, that will likely be impacted by ocean acidification. Hendriks et al. (2010) found that in acidic environments gastropod survival and growth rates decreased by 93% and 63%, respectively. Some invertebrates depress their metabolic rates to maintain an internal acid-base balance in response

to increased acidity (Portner et al. 2004). While metabolic rates of other marine heterotrophs decrease with acidification, the metabolic response of gastropods has not yet been studied (Hendriks et al. 2010).

Additionally, growth and recruitment of several species of unattached crustose coralline algae decrease at lowered pH levels (Jokiel et al. 2008; Kuffner et al. 2008). However, few studies have investigated the effects of acidification on epiphytic calcifying red algae, although they are important to the function of coastal seagrass meadows. The epiphytic algae that grow on turtle grass leaves provide protection from desiccation and herbivory (van Montfrans et al. 1984).

Here we investigate the effects of ocean acidification on growth and metabolism of turtle grass and presence of epiphytic crustose coralline algae (*Hydrolithon farinosum*). We also examined how the metabolic rates and grazing behavior of snails (*Littorina* sp.) were impacted by lowered pH. We hypothesized that lowering the pH of seawater would stress turtle grass, thereby reducing its growth and metabolic rate. We predicted that

the percent cover of calcifying algae on turtle grass would decrease in a lower pH environment because calcium carbonate structures necessary for algal growth would degrade. We predicted that acidified treatments with snails added would have the least amount of algal cover, which would further reduce turtle grass growth rates. Finally, we hypothesized that snail metabolic rates and activity would decrease with increased acidity because snails would be under greater stress.

METHODS

We conducted an experiment simulating the effects of ocean acidification on turtle grass communities at Little Cayman Research Center, BWI, from 6 to 9 March 2011.

We created an acidic environment by filling 12 clear, square tanks of 7.5 L capacity with 5 L of seawater and 100 mL of 5% white vinegar. Acid was added daily to maintain the tanks at pH 7, measured with pH strips. We created 12 control tanks filled with 5 L of seawater (pH 8). Tanks maintained a constant temperature of 26°C, which is similar to the average afternoon temperature of 29.5°C that we measured at the turtle grass collection site.

Turtle grass mats including belowground biomass were collected using a shovel from the seagrass meadow in front of the Little Cayman Research Center, Little Cayman, BWI. Because Corlett and Jones (2007) found that the coralline red algae *Hydrolithon farinosum* was the most abundant epiphyte on turtle grass growing near Grand Cayman, BWI, we assumed that this was the same epiphytic calcifying algae we found in our experiment. Snails were collected from the seagrass meadow at South Hole Sound, Little Cayman Island, BWI.

We massed turtle grass samples and placed mats of similar biomass in 12 tanks of acidified seawater and 12 tanks of natural

seawater. We added 20 snails to six of the acidified tanks and six of the control tanks.

We analyzed the cover of calcifying algae on 10 haphazardly sampled turtle grass leaves from each tank daily. We measured total length of the leaf and the length of the leaf that was white due to coverage by epiphytic algae (cm). Because algal cover was not uniform on the white area of the leaf, we approximated percent cover on this portion to the nearest 25%. We also noted leaf senescence daily as evidenced by browning of leaves.

We estimated growth on turtle grass blades by poking holes with a toothpick at the base of individual leaves at the beginning of the experiment. We measured growth of 10 haphazardly chosen blades in each tank at the end of 4 days by recording the distance (mm) from the base of the leaves to the growth scar.

Snail metabolic rate was estimated once daily by measuring changes in dissolved oxygen after snails were added. We removed all snails from the tanks and placed them together in 125 mL Nalgene® bottles filled completely with seawater. Dissolved oxygen (% and mg/L) was measured before and 30 min after adding snails using a YSI Inc. Pro Optical Dissolved Oxygen™ meter.

We also estimated respiration rate of the turtle grass each afternoon by measuring dissolved oxygen change. Dissolved oxygen was measured before and 30 min after the tanks were covered with airtight black plastic bags. Snail activity was estimated daily by recording the location of each snail in the tanks as on the tank wall, on the leaves, or on the sandy substrate. We classified snails on the leaves and wall, where they must exert effort to maintain suction with the surface, as displaying active behavior.

Analysis

We multiplied the proportion of leaf length covered in algae by the percent cover of those algae to estimate overall leaf algal cover. Algal cover data were arcsine square root transformed and the ten leaves observed per replicate were averaged across treatments. Plant respiration was weighted by initial plant mass.

We used ANOVA to examine differences in growth between treatments. We used a repeated-measures ANOVA to quantify the effect of treatment and time on algal cover. We used student's t-tests to determine the difference in senescence and plant oxygen consumption in acidified and control treatments. We also used student's t-tests to examine differences in snail activity and respiration between treatments.

RESULTS

We found that turtle grass growth differed significantly among treatments, with control treatments experiencing twice the growth of acidified treatments ($F_{3,20} = 22.9$, $P < 0.0001$; Fig. 1). A Tukey-Kramer HSD test showed that both control treatments (those with and without snails) grew more than both acidified treatments. Respiration rates showed no difference between acidified and control treatments ($t_{28.0} = -1.38$, $P = 0.18$).

We found a significant treatment by time interaction on algal cover among treatments, with algal cover decreasing over time in acidified but not in control treatments (Wilks' lambda value = 0.174, $F_{9, 41.5} = 4.85$, $P = 0.0002$; Fig. 2). Visual observations confirmed this finding, as algal cover was reduced in acidified treatments more quickly and to a greater degree than in control treatments (Fig. 3). We also found that leaf senescence was 28% high-

er in acidified treatments ($t_{21.9} = -7.96$, $P < 0.0001$, Fig. 4).

Snails were significantly more active in control treatments ($t_{21.5} = 7.66$, $P < 0.0001$; Fig. 5). There was no significant difference in snail respiration rates between acidified and control treatments (snail: $t_{33.9} = 0.64$, $P = 0.52$).

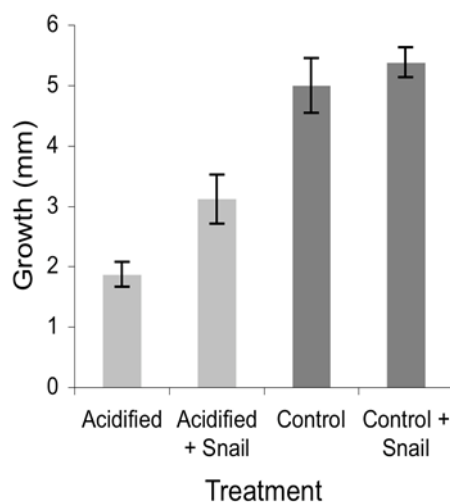


Fig. 1. Turtle grass (*T. testinudum*) growth in control tanks of seawater (with and without snails) is significantly higher than in acidified tanks in an ocean acidification simulation experiment at Little Cayman Research Center, BWI (means \pm 1 SE).



Fig. 3. Cover of epiphytic calcifying red algae (primarily *H. farinosum*) on turtle grass (*T. testinudum*) in tanks of acidified seawater (left) is less than ambient seawater (right) after two days of the ocean acidification experiment conducted at Little Cayman Research Center, BWI.

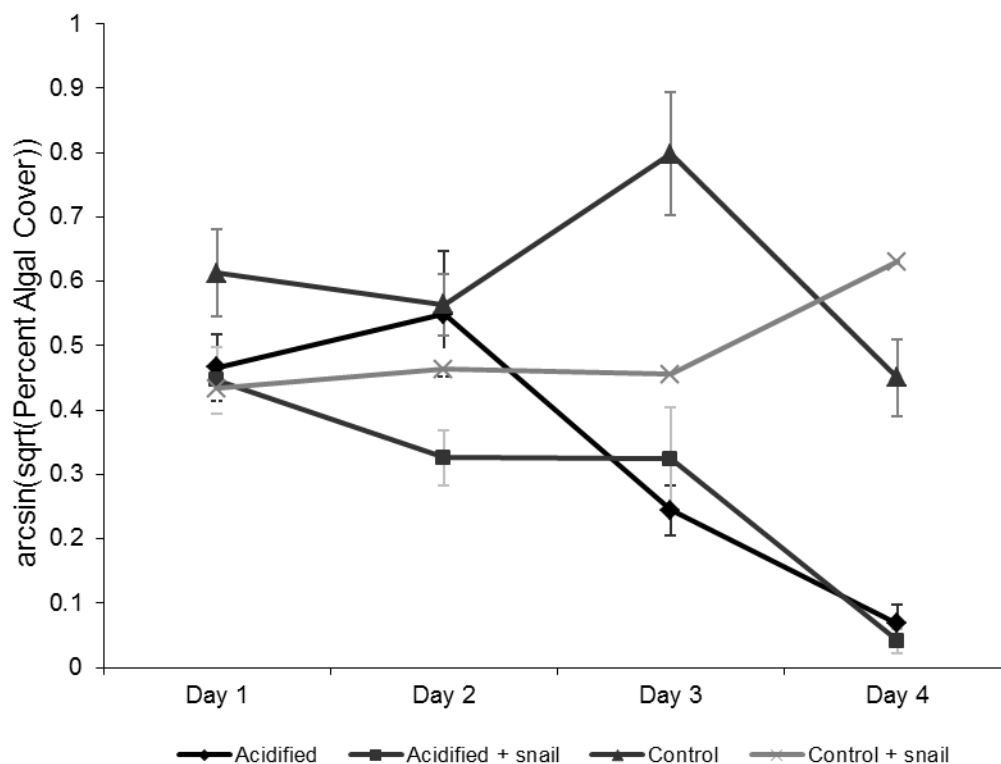


Fig. 2. The effect of acidified and ambient seawater on epiphytic calcifying algal cover on turtle grass (*T. testudinum*) in tanks with and without snails over time (means \pm 1 SE). Algal growth decreased in acid treatments. Algal cover was analyzed visually in an ocean acidification simulation experiment conducted at Little Cayman Research Center, BWI.

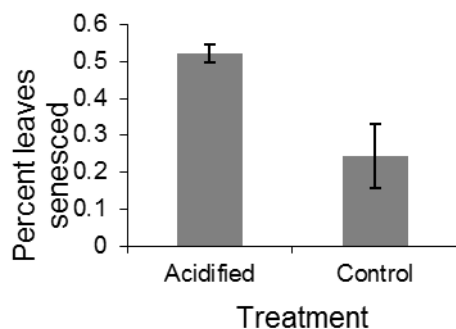


Fig. 4. Leaf senescence of turtle grass (*T. testudinum*) increased in acidified seawater in an ocean acidification experiment conducted at Little Cayman Research Center, BWI (means \pm 1 SE).

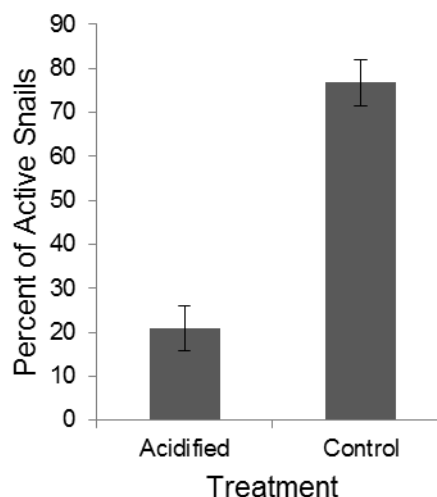


Fig. 5. Percent of active snails in tanks of acidified seawater and ambient seawater (means \pm 1 S.E) in an ocean acidification simulation experiment conducted at Little Cayman Island, BWI. Snails were placed in tanks of turtle grass for four days. Snails were determined to be active if they were attached to turtle grass leaves or the walls of the tank.

DISCUSSION

We conclude that ocean acidification will negatively affect the health of turtle grass because we found a decrease in growth and an increase in senesced leaves of plants in acidified treatments.

The acidic environment also appears to be detrimental to epiphytic calcifying algae, as algal cover on turtle grass leaves was reduced in the acidified tanks. The acid may have dissolved the algal calcium carbonate structures, as acid washes have been known to be effective in reducing epiphyte loads on seagrasses (Dauby and Poulicek 1995). Additionally, the change in chemical composition of the seawater may have prevented the algae from producing new structures and growth.

The reduction in epiphyte cover on the turtle grass may have negatively affected the turtle grass through several mechanisms. The reduced cover of algae may have contributed to the increased leaf senescence in the acidified tanks because epiphytes can protect seagrass from desiccation and harmful UV radiation (Trocine et al. 1981; van Montfrans et al. 1984). Herbivores may also eat older leaves with greater cover of nutrient-rich epiphytic algae, which reduces consumption of younger, photosynthetic basal seagrass leaves (van Montfrans et al. 1984).

We found that both acid and control tanks with snails had less algal cover than those without snails (Fig. 3); Frankovich and Ziemann (2005) also found that snails reduce epiphyte cover. However, snail grazing, even by snails in natural seawater, may not reduce epiphyte cover enough to affect the turtle grass, as we found the growth of turtle grass was not lower in control and acid tanks with snails versus without them (Fig. 1).

We conclude that increasing ocean acidity will be detrimental to snails because we found fewer active snails in the acidified

tanks, and many died (Fig. 5). The decreased activity of the snails in acidic tanks may be due to the reduced ability of the snails to maintain acid-base regulation in their body tissue (Portner et al. 2004). Snail health may be further threatened by acidification as their shells degrade due to the difficulty of creating calcified structures (Doney et al. 2009; Orr et al. 2005).

While we did not see a change in plant or snail respiration rates between treatments, our methods for measuring respiration were limited, as we could not completely seal the tanks and prevent water from gas exchange. However, Bibby et al. (2007) found that rates of oxygen uptake decrease in snails under stress of both lowered pH and predation.

However, the addition of acetic acid alone may not accurately simulate the effects of ocean acidification due to increased CO₂ concentrations. For instance, while a higher concentration of CO₂ may decrease the pH of seawater, it may also increase photosynthetic rates of seagrass (Zimmerman et al. 1997), causing higher reproductive outputs and biomass production (Palacios and Zimmerman 2007). We suggest that further studies examine the long-term combined effects of increased CO₂ concentrations and acidity in seawater.

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EFFECTS OF EPIPHYTE COVER ON SEAGRASS GROWTH RATES IN TWO TIDAL ZONES

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Faculty Editor: Brad W. Taylor

Abstract: Epiphytic algae are the most important primary producers in seagrass ecosystems; yet, the impact of epiphytic algae on seagrass is not well understood. We experimentally tested the effects of epiphyte cover on the growth rate of turtle grass, *Thalassia testudinum* at Little Cayman Island, BWI. We removed epiphytes from seagrass blades to reduce epiphyte cover and excluded grazers to increase epiphyte cover. We compared the effects of these treatments in a shallow water seagrass bed that is exposed to air at low tide and a seagrass bed in deeper water where seagrass is always submerged. Epiphyte cover had a positive effect on seagrass growth in shallow water but had no effect on growth in deeper water. The effect of epiphyte cover on seagrass growth varies with small-scale environmental differences, which has repercussions for understanding the response of seagrass beds to larger-scale disturbances.

Key words: *Thalassia testudinum*, *mutualism*, *grazer exclusion*, *epiphyte removal*

INTRODUCTION

Epiphytes are extremely important to the ecology of seagrass beds, contributing more production than the grasses on which they grow (Mazzella and Alberte 1986; Moncreiff and Sullivan 2001). The epiphytes benefit from this relationship with seagrasses by gaining a structure on which to grow and by consuming nutrients that seagrasses leach outk (McRoy and Goering 1974; Harlin 1975; Moncreiff and Sullivan 2001). However, the

net effect of epiphytes on seagrass has not been completely determined.

The negative effect of epiphytes on seagrass growth and production is well known. For instance, epiphytes can be so dense that they impact the photosynthesis of their host seagrass, decreasing growth or increasing mortality (van Montrants et al. 1984; Hughes et al. 2004; Cebrián et al. 1999; Fong et al. 2000). In undisturbed seagrass beds and coral reefs, herbivory prevents epiphytic algae from outcompeting seagrasses and corals

Table 1. Growth rate predictions for each treatment and habitat type.

Habitat type	Treatment		
	Ambient epiphytes	Epiphytes Removed	Grazers Excluded
Above Low Tide Line (dry at low tide)	Control	Seagrass will experience slower growth than control because decreased epiphyte cover decreases protection from desiccation or UV radiation.	Seagrass will experience faster growth than control because increased epiphytes will provide protection from desiccation or UV.
Below Low Tide Line (wet at low tide)	Control	Seagrass will experience faster growth than control because decreased epiphyte cover will decrease shading of the leaves.	Seagrass will experience slower growth than control because increased epiphytes will shade the leaves.
Comparison		Growth will be slower above the low tide line after epiphyte removal because the threat of desiccation is higher for these individuals.	Growth will be slower below the low tide line after grazer exclusion because the effect of epiphyte shading will be greater in deeper waters, where light is more limiting.

(Tomas et al. 2005; Fong et al. 2006; Rasher and Hay 2010; Hughes et al. 2004; van Montfrans et al. 1984; Howard and Short 1986; Hootsmans and Vermaat 1985; Fong et al. 2000). Thus, in systems with natural levels of grazing, epiphytic algae cover does not significantly affect seagrass photosynthesis (Bultuis and Woelkerling 1983; Mazzella and Alberte 1986).

Epiphyte cover may benefit seagrass by reducing desiccation during low tide (van Montfrans et al. 1984; Penhale and Smith 1977; Richardson 1980) or by protecting it from UV radiation (Trocine et al. 1981). Therefore, epiphyte cover may be more beneficial to seagrasses in shallow water, where there is a higher probability of desiccation and greater UV exposure.

We tested the positive and negative effects of epiphytes on growth rates of turtle grass, *Thalassia testudinum*. We hypothesize that the positive effects of epiphyte cover would be greater than the negative effects in shallow water; whereas, the negative effects would be greater than the positive effects in deep water. To test these hypotheses, we compared treatments with ambient epiphyte cover, reduced epiphyte cover (achieved by removing epiphytes), and increased epiphyte cover (achieved by excluding large grazers). The specific predictions are provided in Table 1.

METHODS

We studied seagrass at Little Cayman Research Center (LCRC), Little Cayman Island, BWI, on 4-8 March 2011. There are two seagrass beds in front of the LCRC center: one in the shallows close to shore, which is occasionally exposed to air at low tide, and one farther out which is always submerged. The deeper seagrass bed is on average 35 cm deeper than the shallower bed, and has a more

constant, cooler temperature. We used these two habitat types to test the effects of epiphytes on *T. testudinum* growth.

We manipulated *T. testudinum* on 4 March and 6 March 2011. Ten plants were chosen for each treatment. The six treatments were: (1) ambient epiphyte cover above the low tide line (4 and 6 March), (2) ambient epiphyte cover below the low tide line (4 and 6 March), (3) epiphyte removal above the low tide line (4 March), (4) epiphyte removal below the low tide line (4 March), (5) grazer exclusion above the low tide line (6 March), and (6) grazer exclusion below the low tide line (6 March).

All of the blades of each focal plant were punctured with a pin at their bases to measure growth over the experimental period. We removed epiphytes from every blade of epiphyte removal plants by gently scraping them with a razorblade. We visually estimated that at least 90% of epiphytes were removed by this method. Grazer exclosures were constructed using Gutter Guard™ plastic mesh with 1 cm openings. Each exclosure was a 10 cm diameter cylinder by 15 cm in height with a mesh top sewn together with fishing line. Wooden dowels and clothespins were used to hold each exclosure against the sediment. On 8 March 2011, we retrieved all experimental plants and measured the growth from the pin scar to the base of the plant. Growth rates were measured for the youngest blade of each plant, which is the most central blade (Cox and Tomlinson 1988), as this blade exhibited the most rapid growth and the most detectable differences in growth rate.

We analyzed these data with a two-way ANOVA in JMP 8.0 followed by a Tukey's post hoc test to determine significant differences among the three means.

RESULTS

Above the low tide line, *T. testudinum* with more epiphytes grew 25% faster than *T. testudinum* with ambient cover of epiphytes and 67% faster than *T. testudinum* with epiphytes experimentally removed (epiphyte treatment effect: $F_{2,35} = 3.86$, $P = 0.030$, Tukey's post hoc test $P < 0.05$; Fig. 1). In contrast, below the low tide line there was no effect of epiphyte cover on *T. testudinum* growth rate (tide level effects: $F_{2,33} = 0.30$, $P = 0.75$; Fig. 1). The effect of epiphyte cover on *T. testudinum* growth rate depended on water depth (epiphyte treatment \times tide level interaction: $F_{4,69} = 3.53$, $P = 0.035$; Fig. 1). This interaction was driven by the difference in the effect of grazer exclusion between the two tidal zones.

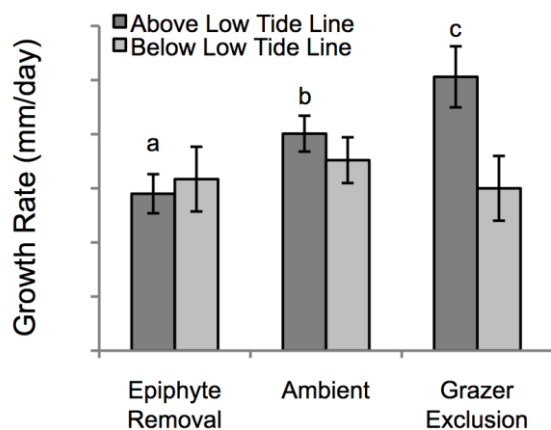


Fig. 1. *T. testudinum* growth rates \pm 1 SE in two tidal zones and three epiphyte treatments at Little Cayman Island. Different letters indicate significant differences based on Tukey's post hoc test $P < 0.05$.

DISCUSSION

Above the low tide line, *T. testudinum* grew faster with greater epiphyte cover and slower when epiphytes were experimentally removed. This is consistent with our hypothesis that epiphytes increase growth rate in shallow zones because they protect seagrass from desiccation or UV radiation above low tide level. The removal of epiphytes may diminish the protection they provide *T. testudi-*

num, stressing the seagrass and reducing growth rates. In contrast, the grazer exclusion allowed epiphytes to accumulate above ambient levels, and the fastest growth rates in this treatment are consistent with beneficial effects of epiphytes.

The effect of epiphytes on *T. testudinum* growth varied with tidal zone. Below the low tide line, in deeper water, there was no relationship between epiphyte load and *T. testudinum* growth rate. Our results show that the effect of epiphyte cover is less important to *T. testudinum* growth below the low tide line, in deeper water, where other factors such as *T. testudinum* density may be more important. The effect of grazer exclusion is greater in deep water than in shallow water. Perhaps grazing pressure is greater in shallow water, so excluding herbivores there led to more epiphyte accumulation than in deep water, leading to a greater increase in protection from desiccation or UV radiation. Alternatively, the shading effect of increased epiphyte cover may have more impact in deep water, where light is more limited. If this is true, the greater negative effect of increased epiphyte cover could have balanced any positive effects, such as UV radiation protection.

The effect of epiphytes on *T. testudinum* growth rates differs in magnitude and perhaps even direction under different environmental conditions. The threats of desiccation and UV radiation are not as pronounced in deeper water and therefore epiphyte cover may not benefit seagrasses, such as *T. testudinum*, below the low tide line as strongly as it benefits seagrasses in shallower water. The shading of seagrass leaves by epiphytes may be more important in more light-limited deeper waters than in the shallows; however, we did not detect this effect on growth rate.

The relative effects of epiphytes (i.e., positive or negative) on seagrasses have also been

shown to vary on a larger spatial scale. In systems that have undergone eutrophication, shading by a dense mat of epiphytes may increase seagrass mortality and lead to the collapse of the seagrass beds (Hughes et al. 2004; Fong et al. 2000). However, shading does not seem to threaten seagrass beds in undisturbed conditions (Bulthuis and Woelkerling 1983; Mazzella and Alberte 1986). Integrating such large-scale research with examinations of the effects of epiphyte cover on smaller, within-site scales could further our understanding of how a seagrass bed responds to changes in nutrient availability.

Furthermore, the effect of epiphytic cover varies even within a single seagrass plant. Constant recruitment of epiphytes to seagrass blades means that older blades have a higher epiphyte load than younger ones (Sand-Jensen 1976). The accumulation of epiphytes on older blades decreases the amount of light and changes the wavelength of that light (Cebrian et al. 1999; Sand-Jensen 1977), and thus may contribute to rapid senescence of older leaves (Bulthuis and Woelkerling 1983; Fong et al. 2000). Moreover, if epiphyte covered leaves are preferred by grazers, then accumulation on older leaves may increase survivorship of seagrass because it diverts grazing pressure away from new growth (van Montfrans et al. 1984). Future studies could compare growth rates of blades of different ages with epiphyte manipulations to better understand the effects of epiphytes on the life history of seagrass.

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DIEL VARIATION IN PHOTOTAXIS IN MARINE INVERTEBRATES

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Abstract: Light cues are important to the diel behavior of many organisms. Phototaxis is an important adaptation that allows marine organisms to navigate to or away from the surface at different times of day or night. We examined temporal variation in phototaxis in seven marine organisms of the families Brachyura, Phyllodocidae, Cumacea, Macrura, Syllidae, and Anomura, and we predicted when organisms would be phototactic based on their published behaviors. We tested the phototactic response of each organism during day and night by exposing individuals to a light source in a dark tank and measuring their distance from the light source after one minute. We tested for phototaxis by comparing observed distances and expected distances, assuming a uniform distribution from the light source. We found that many organisms displayed positive phototaxis during the day, night, or both. We suggest that positively phototactic adult organisms use light cues for finding mates, while positively phototactic larvae were so for diel vertical migration.

Key words: polychaete, epitoke, crustacean larvae, moonlight

INTRODUCTION

Light cues are important in determining the diel behavior of many organisms. For example, many marine zooplankton use light cues to regulate the diel vertical migration between the water column at night, where they feed, and the substrate during the day, where they hide from predators (Lampert 1989). Some polychaete annelids use moonlight as a cue to synchronize nocturnal reproductive aggregations on the ocean surface (Naylor 2001). The surface-aggregating life stage of these annelids, called an epitoke, grow larger eyes, indicating that they may use positive phototaxis towards moonlight for navigating to the surface (Bentley 2001). If all of these organisms are navigating by light cues, the directionality (positive or negative) of phototaxis may change during the day because they do not migrate towards both moonlight and sunlight. Laboratory studies of Crustacean larvae have found that the directionality of phototaxis is influenced by changes in light intensity (Forward 1977), developmental stage (Thorson 1964), level of

feeding (Singarahah 1968), water pressure (Sulkin 1971), and circadian rhythm (Enright and Hamner 1967).

We studied several species of marine organisms that congregate around dive lights at night. We did not observe these organisms in the water column during the day, indicating that they likely undergo diel vertical migration. Because they appeared to be positively phototactic towards our dive lights at night yet remain in the benthos during the day, we posited that they possess an endogenous rhythm set by photoperiod. If they do possess this rhythm, we hypothesized that they would display positive phototaxis at night and negative phototaxis during the day.

Anomura, Brachyura, and Macrura are all pelagic zooplankton that undergo diel vertical migration (Queiroga and Blanton 2005). Therefore, we hypothesized that these organisms would exhibit positive phototaxis (move towards a light stimulus) at night but would exhibit negative phototaxis during daylight hours. Polychaetes of the Syllidae and Phyllodocidae families aggregate on the surface in reproductive swarms (Beesley et al.

2000, Bentley 2001, Naylor 2001). Reproductive swarms often aggregate after a full moon, as individuals may use light cues from the moon (Bentley 2001, Naylor 2001); hence, we predicted that these worms would display positive phototaxis at night and negative phototaxis during the day.

METHODS

We captured several species of crustaceans (families Anomura, Brachyura, Cumacea, and Macrura) and polychaete worms (families Phyllodocidae and Syllidae [with sub-taxa A and B]) congregated around a dive light at night in the patch reefs between Jackson Bay and Grape Tree Bay, Little Cayman on the nights of 5-8 March 2011. During the course of the experiment, organisms were maintained in the laboratory in 85x50x35cm tanks containing seawater and a bubbler. The holding tanks were exposed to natural light during the day and covered in black plastic at night to avoid complications with artificial lab light. The invertebrates were separated by morphology and identified to the lowest possible taxonomic levels using available literature (Beesley et al. 2000, Todd and Laverick 1991). Sex of individuals of Syllidae B was determined by the presence or absence of an egg sack (Todd and Laverick 1991).

For each taxonomic group of organisms, the response to a consistent set of light stimuli was measured once during daylight hours (between 8 am and 6 pm) and once during night hours (between 7 pm and 2 am). For all trials, each organism was placed in a tank covered in black plastic that blocked out ambient light. An observer watched the tank from under the plastic. Larger organisms (Phyllodocid polychaetes) were placed in a 90x45x40cm tank, while smaller organisms (syllid polychaetes and crustacean larvae) were placed in a 25x22.5x22.5cm tank. A

Princeton Tec Torrent LED dive light (Max-bright LED lamp, 95 lumens) was placed against one side of the tank, directed horizontally. The light was turned on, and after 1 minute, the distance of the individual organism from the light source was measured to the nearest 10 cm in the larger tank or the nearest 2.5 cm in the small tank. Based on the observed mobility of all the organisms used, one minute was determined to be sufficient for the organisms to move between any two points in the tank. The light was turned off, the tank was swirled, and the organism was left in the dark for 1 minute to allow it to re-distribute within the tank. This process was repeated 3 times for each individual. Between 4 and 10 individuals were evaluated for each morphological group.

Analysis

All 3 trials for each individual were averaged. For each morphological group with 10 individuals, a Kolmogorov-Smirnov (KS) test was used to test for a difference between the measured distribution of individual distances from the light source after 1 minute and a uniform distribution of distances with 10 points. KS tests were performed for both day and night trials. We used a KS test calculator available online at <http://www.physics.csbsju.edu/cgi-bin/stats/KS-test.n.plot>.

For all morphological groups at different times of day, we tested whether the distributions of distances to the light were normal or non-normal. For normally distributed data, a t-test was used to test for differences between the means of the data and a uniform distribution. An unpooled t-test was used because our data and the uniform distribution had unequal variances. A Wilcoxon non-parametric test was used to compare the means of non-normally-distributed groups to a uniform distribution. The distribution ana-

lyses, t-tests, and Wilcoxon non-parametric tests were calculated in JMP 8.0.

Overall, organisms were considered positively phototactic if one or both tests produced a significant P value and if the mean position of the organism was within 5 cm of the light in the small tank or within 22.5 cm of the light in the large tank (in both cases, the first quarter of the tank). Organisms were considered negatively phototactic if one or both tests produced a significant P value and if the mean position of the organism was within 5 cm of the non-lit end of the small tank or within 22.5 cm of the non-lit end of the large tank. In all cases, results for Kolmogorov-Smirnov tests and either t-tests or Wilcoxon tests agreed on significance, and the mean positions of all groups found to be phototactic were within 5 cm of the light in the small tank or within 22.5 cm of the light in the large tank.

RESULTS

The following groups of data were normally distributed and were therefore examined with an unpooled t-test: Phyllodocidae at day, Phyllodocidae at night, Cumacea at day, Cumacea at night, Macrura at day, Syllidae B at day, Syllidae B with eggs, Syllidae B without eggs, and Anomura at night. The following groups of data were not normally distributed and were tested using a Wilcoxon non-parametric test: Brachyura at night, Macrura at night, Syllidae B at night, Syllidae A at day, Syllidae A at night, and Anomura at day.

Trends in phototaxis for each organism are summarized in Table 1. Brachyura were positively phototactic during day (Kolmogorov-Smirnov: $D = 0.9$, $P < 0.0001$; Wilcoxon: $\chi^2_1 = 13.86$, $P = 0.0002$; mean position = 0.00 cm) and night (Kolmogorov-Smirnov: $D = 0.9$, $P < 0.001$; Wilcoxon: $\chi^2_1 = 12.92$, $P = 0.0003$; mean position = 0.17 cm). Phyllodocidae were not

phototactic during the day (Kolmogorov-Smirnov: $D = 0.3$, $P = 0.63$; $t_{10.01} = -4.23$, $P = 0.80$; mean position = 42.22 cm), but were positively phototactic at night ($t_{12.66} = 0.26$, $P = 0.002$; mean position = 3.33 cm). Cumacea were not phototactic during the day (Kolmogorov-Smirnov: $D = 0.4$, $P = 0.31$; $t_{16.18} = 1.86$, $P = 0.08$; mean position = 5.8 cm), but were positively phototactic at night (Kolmogorov-Smirnov: $D = 0.6$, $P = 0.03$; $t_{11.06} = -2.80$, $P = 0.02$; mean position = 4.2 cm). Macrura were not phototactic during the day ($t_{12.13} = 0.94$, $P = 0.36$; mean position = 8.8 cm) or night (Wilcoxon: $\chi^2_1 = 1.49$, $P = 0.22$; mean position = 6.5 cm). There were not enough individuals of Syllidae B to divide data both by time of day and by sex, so sexes were grouped to test differences among day and night and times of day were grouped to test for differences among sexes. Syllidae B were not phototactic during the day (Kolmogorov-Smirnov: $D = 0.4$, $P = 0.24$; $t_{14.27} = 1.94$, $P = 0.07$; mean position = 5.6 cm) or night (Kolmogorov-Smirnov: $D = 0.5$, $P = 0.11$; Wilcoxon: $\chi^2_1 = 3.17$, $P = 0.08$; mean position = 5.5 cm) when males and females were grouped together. Females of Syllidae B were not phototactic ($t_{10.82} = -0.07$, $P = 0.50$; mean position = 13.67 cm), but males of Syllidae B were positively phototactic ($t_{10.56} = -3.03$, $P = 0.01$; mean position = 3.68 cm). Syllidae A were positively phototactic during both day (Kolmogorov-Smirnov: $D = 0.8$, $P = 0.001$; Wilcoxon: $\chi^2_1 = 9.83$, $P = 0.002$; mean position = 0.92 cm) and night (Wilcoxon: $\chi^2_1 = 5.25$, $P = 0.02$; mean position = 1.25 cm). Anomura were positively phototactic during the day (Kolmogorov-Smirnov: $D = 0.7$, $P = 0.007$; Wilcoxon: $\chi^2_1 = 7.97$, $P = 0.005$; mean position = 1.20 cm) but not at night (Kolmogorov-Smirnov: $D = 0.3$, $P = 0.68$; $t_{17.33} = -1.13$, $P = 0.27$; mean position = 7.8 cm).

TABLE 1. Summary of phototaxis of marine invertebrates at day or night (or with and without eggs, in the case of Syllidae B). $P < 0.05$ indicates significant differences from an even distribution in organisms' position to a light source. Organisms were considered positively phototactic if $P < 0.05$ for both tests and if the mean position of the organism was within 5 cm of the light in the small tank or within 22.5 cm of the light in the large tank (only Phyllodocidae). Shaded rows indicate individuals who are not phototactic.

Organism	Day or night	Kolmogorov-Smirnov test(P values)	t test or Wilcoxon test(P values)	Mean position (cm)	Conclusion (Positive, negative, or no phototaxis)
Brachyura	Day	< 0.0001	0.0002	0	P+
	Night	< 0.0001	0.0003	0.167	P+
Phyllodocidae	Day	0.626	0.8	42.222	P=
	Night		0.0018	3.333	P+
Cumacea	Day	0.313	0.081	5.8	P=
	Night	0.031	0.017	4.2	P+
Macrura	Day	---	0.36	8.8	P=
	Night	---	0.22	6.5	P=
Syllidae B	Day	0.237	0.072	5.6	P=
	Night	0.111	0.08	5.5	P=
	Eggs	---	0.50	13.67	P=
	Eggless	---	0.01	3.68	P+
Syllidae A	Day	0.001	0.002	0.92	P+
	Night	---	0.022	1.25	P+
Anomura	Day	0.007	0.005	1.2	P+
	Night	0.675	0.27	7.8	P=

DISCUSSION

We found a high degree of variation in phototactic responses among marine invertebrates. This is likely due to the diverse roles of phototaxis among marine organisms with very different life histories.

Phyllodocidae were positively phototactic at night and not phototactic during the day. This may be because they are only reproductively active at night. Phyllodocidae were captured in the water column, and Phyllodocidae are benthic except during reproductive periods, so the individuals were likely reproductive (Beesley et al. 2000). During daytime trials, Phyllodocidae individuals were not observed swimming, possibly because they had resumed sedentary benthic feeding behavior after a night in the reproductive phase. Thus, phototaxis is likely important for reproduc-

tion in Phyllodocidae.

The syllid epitokes showed strong positive phototaxis. Syllidae A was significantly positively phototactic during day and night. When all syllid B individuals of both sexes were grouped, no significant phototaxis was seen. However, when the sexes were looked at individually, females (identified by the egg sacks present) were not phototactic, while males were positively phototactic. The epitokes probably use phototaxis to follow moonlight to the surface to join reproductive swarms. The females may have been phototactic prior to mating, but if they had already mated they may not have responded to light cues because they no longer needed to navigate to the surface. Even though epitokes are known only to aggregate at night (Beesley et al. 2000), the epitokes studied were always positively phototactic, indicating that they

lack an endogenous rhythm in phototaxis. It is possible that environmental cues other than photoperiod, such as temperature or tidal variation, regulate the periodicity in epitoke phototaxis which causes them to congregate at night but not at day.

The larval crustaceans showed a wide variation in phototactic responses. Brachyura was always phototactic, Macrura was never phototactic, and Anomura was phototactic during the day and not the night. This is not consistent with our hypothesis that crustacean larvae will only respond to light at night because they have an endogenous rhythm causing diel vertical migration towards moonlight. Existing literature suggests that all three of these families undergo similar diel vertical migration in the field (Alldredge and King 2003, Dos Santos et al. 2008, Forward 1985,). The inconsistency in the ways different families responded to light in the lab likely means that the ways in which these organisms regulate diel vertical migration are complex, dissimilar, and dependent on many environmental factors.

Cumacean individuals, the only adult crustaceans tested, were positively phototactic at night and not phototactic during the day. Cumaceans are strong swimmers and are often found in pelagic tows, even though they are benthic feeders (Todd and Laverick 1991). Also, unlike the other crustacean larvae studied, only males exhibit a diel vertical migration (Akiyama and Yamamoto 2004). Sexually mature males develop an enlarged antenna, and they possibly use moonlight to navigate upwards into the water column to better sense female pheromones and locate females for mating (Sulkin 1984).

Overall, we found incredible variety in the phototactic response of marine organisms collected in Jackson Bay, Little Cayman. Organisms differed in the strength of their pho-

totactic response, in the times of day in which they were phototactic, and in the way their response changed over the course of the day. Even within similar groups of organisms (such as pelagic zooplankton), there was variation, and it was very difficult to apply general trends in phototaxis to any subset of organisms. This variation is indicative of the diversity of ways in which organisms use light to regulate behavior and movement within the water column. Future studies could focus on specific taxa within each family and examine a greater number of species in order to discern general trends.

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GUIDE TO SPONGES (PHYLUM PORIFERA, CLASS= DEMOSPONGIAE) OFF THE COAST OF LITTLE CAYMAN ISLAND

Marielle F. Battistoni

1. Giant Barrel Sponge

Xestospongia muta



These sponges range in size from 2-6 ft. tall, and typically grow at depths of 50-130 ft, but can be seen on several dives around Little Cayman at shallower depths (especially abundant on Barracuda Bite). They are fairly common in the Caribbean, and can be identified by its large barrel shape. *X. muta* also has cyanobacterial symbionts that give the sponge its reddish brown color. These sponges undergo normal cyclic bleaching events, possibly after reproducing, but in recent years there have been fatal bleaching events due to sponge orange band (SOB), likely caused by a pathogen (Coward et. al 2006). Fun fact: the largest giant barrel sponges in the Caribbean may be over 2,300 years old (McMurray et. al 2008). Another fun fact: Isolates from giant barrel sponges may inhibit HIV proteases (Patil et. al 1992). Facing a view inside a giant barrel sponge.



2. Touch-Me-Not Sponge

Neofibularia nolitangere



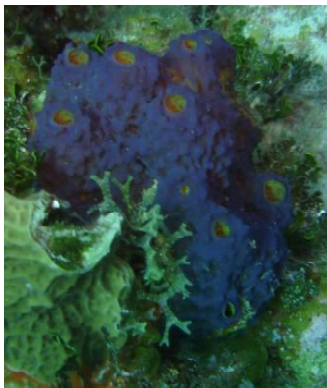
These sponges are quite common in the Caribbean and in reefs off the shore of Little Cayman, often at shallower depths, although their range is from 10-130 ft. They are reddish-black, maroon, or dark brown in color (look for them to change color on the night dive!), and around Little Cayman their morphology is often that of a large tube or vase, although the sponge may take on several growth forms. These sponges get their name from a toxin they express that can cause severe dermatitis (localized pain, redness, inflammation) if a diver comes into contact with their silica spicules. If touched, divers should treat the rash with vinegar and then hydrocortisone cream, although if symptoms are more severe go to the island's clinic! Touch-Me-Nots are one of several sponge species that have been found to release gametes simultaneously in mass spawning events (over 90% of local population at once), often driven by cues from phases of the moon (Hoppe and Reichart 1987).

3. Branching Tube Sponge

Pseudoceratina crassa



This sponge comes in many color variations, including olive green purple, yellow-orange and purple/yellow mixture. All forms are extremely common at Little Cayman dive sites. This variation in color among branching tube sponges may be caused by differences in water chemistry, depth, light, or algal presence, but this species can be identified by the raised bumps on their outer surface and their structure, as a clump of tubes all extending from a single base.



4. Brown Encrusting Octopus Sponge

Ectyoplasia ferox



This sponge can be identified by its orange-brown color, with its excurrent openings ringed by a lighter yellow color. It can be found at depths of 40-75 feet, and usually is found in small clumps or long sprawling formations, encrusted on reefs around Little Cayman, although it gets its name from the fact that it can form long “arms” that look like octopus tentacles.



5. Netted Barrel Sponge

Verongula gigantea



This sponge may be from 2-6 ft in size (smaller than giant barrel sponge), and is occasionally found in the Caribbean, although quite common at dive sites around Little Cayman. Located at depths of around 35-130 ft., this sponge can be recognized by its large barrel shape, very wide diameter with a mesh-like exterior, and is often yellowish, olive, or yellow-brown in color. Below: a view inside a netted barrel sponge (look inside: often there are polychaete worms and other invertebrates living inside).



6. Yellow Tube Sponge

Aplysina fistularis



These sponges are identified by their vibrant yellow color (due to fluorescent pigments), soft texture, and giveaway tube shape, although they have slightly different forms depending on depth (the tubes with antler-like extensions shown on the left in photo below are usually found in shallower water). They are extremely common on dives around Little Cayman, and may be located at depths of only 15 feet. Because sponges are filter feeders, yellow tube sponges have a powerful filter that can handle up to five times their own volume of water per minute, and can discriminate between tiny microscopic organisms (BBC, 2005).



7. Pink Vase Sponge

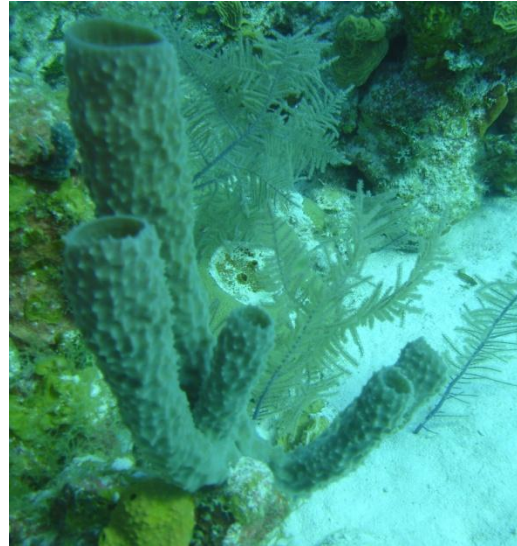
Niphates digitalis



These sponges are distinguished by their lovely pink or lavender color and distinct vase shape, with their excurrent opening and exterior covered in transparent fingerlike projections. It is occasionally found in the Caribbean, but quite common around Little Cayman, from depths of 25-75 ft. This sponge showed one of the fastest rates of wound healing among sponges, regenerating 6% of the wounded area per day, possibly due to its lack of chemical defenses against predators, which might shift its focus towards healing attacks rather than preventing them (Walters and Pawlik 2005).

8. Branching Vase Sponge

Callyspongia vaginalis



These sponges look similar to pink vase sponges (left), although they are generally taller and usually exhibit a branching structure, and may be lavender to gray-brown in color. They may also grow in a fan shape if under stress from currents, as they may be found as shallow as 6 ft below the surface, and their exterior is usually covered with *Parazoanthus parasiticus*, a zoanthid. Also look inside—several species of brittle stars preferentially inhabit these sponges, including *Ophiothrix lineata* and *O. suensonii* (Henkel and Pawlik 2005).



9. Orange Sieve Encrusting Sponge

Diplastrella sp.



This species of sponge is somewhat more rare, and difficult to spot due to its presence in hidden, shaded areas, but it may be noticed encrusting huge reef walls on Little Cayman dives or encrusting other coral surfaces from depths of 20-75 ft. Its bright orange/red-orange color makes identification easier, as well as the dense clusters of pores (lighter dots in photo) found dotting the sponge surface, especially around excurrent openings.

10. Stinker Sponge

Ircina felix



This sponge gets its name from the pungent garlic odor it gives off, especially noticeable when removed from the water. Interestingly, the volatile compounds (including dimethyl sulfide, methyl isocyanide, methyl isothiocyanate) that create this odor have no effect on predatory fishes, while instead a combination of other compounds the sponge produces (furanosesterterpene tetrionic acids) have been shown to provide defense against predators (Pawlik et. al. 2002). It is often seen as an encrusting, white-gray mass, with a network of points and lines crisscrossing its hard surface. It is quite common around Little Cayman and may be seen at shallower depths (ranging from 5-65 ft).

**11. Row Pore/Scattered Pore Rope
Sponge**

Aplysina cauliformis/fulva



It is difficult to distinguish between these two species within the genus *Aplysina*, as both form narrow rope-like, branching structures, with pores and excurrent openings all along each arm. These sponges may be a multitude of different colors, including lavender, red, yellow, brown, and purple, all of which are common around Little Cayman at depths of approximately 40-130 ft. Rope sponges reveal another example of the many as yet unknown benefits to humans that may exist among sponges: extracts from *Aplysina* were found to greatly inhibit bacterial attachment, needed for bacterial colonization of host cells, and these chemicals may contribute to further developments in human medicine, especially in the prevention of infection and spoiling of alimentary substances (Kelly et. al 2003).

12. Green Finger Sponge

Iotrochota birotulata



The name of this sponge gives away its identifiable characteristics— it is green and forms knobby fingerlike projections, often hanging sideways or upside down from walls or growing upright in larger branching structures. This sponge is seen frequently on dives around Little Cayman at depths from 15-60 ft., and often in the presence of other rope and encrusting sponges, and may be mistaken for coral except for its excurrent openings and distinct shape; it is also often covered with golden zoanthids that give it a golden-green color. Don't touch, obviously for ecological reasons, but also because a blue-black dye is released that may stain fingers for several days.



13. Lumpy Overgrowing Sponge

Holopsamma helwigi



This sponge is light pink or lavender in color, and forms a small lumpy, overgrowing structure, as its name suggests. Although more difficult to spot on dives because of its pale color and smaller size (4-16 in), this sponge is relatively common in the Caribbean, including Little Cayman, and usually found at depths of 15-75 feet.

****All sponge photos taken on 2/26/11 at Little Cayman dive sites Coconut Walk (max depth 42 ft.) and Sarah's Set (max depth 64 ft)****

All photos taken by Perry Oftedahl

Sponge guide compiled by Marielle Battistoni

Much thanks to the divemasters Perry Oftedahl, Rob Hedges, Greg Locher and Lowell Forbes for leading all of the dives for the Bio FSP group on Little Cayman.

14. Pitted Sponge

Verongula rigida



Identification of these sponges is made obvious, as their name suggests, from their pitted texture, with many pores and openings on their surface. They are brown-tan in color and found at depths of 20-80 feet. Around Little Cayman they form branching clumps (above) or singular volcano-like structures (below).



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FISH OF THE REEF AT LITTLE CAYMAN RESEARCH CENTER

Compiled by Suzanne Kelson, Ian Engler, Emily Kyker-Snowman

Small Common Fish

Skinny Fish

Spotted goatfish (*Pseudupeneus maculatus*) The spotted goatfish participates in mass spawning aggregations, in local spawning areas that are only a few hours from their resident areas. It is often found on sandy substrate next to the reef burrowing its nose into the ground. Note the goat-like beard. The active color phase is pictured the left, the inactive on the right.



Slippery Dick (*Halichoeres bivittatus*) The slippery dick feeds carnivorously on almost anything that moves, including hermit crabs. You can often see it on sandy substrate off the reef, foraging with the spotted goatfish. The dark center stripe trails off into spots as it nears the tail.



Bluehead Wrasse (*Thalassoma bifasciatum*) The bluehead wrasse forms herds to opportunistically raids the nests of other fish to eat their eggs. The males are territorial, while the females roam freely. They are able to change from females to males when the primary male disappears. Males can spawn 25 times a day, while females can spawn once every other day for two years. The bluehead is very common, as is the juvenile.

Round Fish



Sergeant Major (*Abudefduf saxatilis*) Members of the Damselfish family, sergeant majors do not defend algal farms like some of their relatives. Their diet consists largely of benthic invertebrates, small fish, and zooplankton, though they feed on plants as well. Like other damselfish, they are benthic egg-brooders, with exclusively the male clearing the nest and protecting and caring for the eggs.

Beaugregory (*Stegastes leucostictus*) The juveniles (pictured) of this species are bright yellow with a blue wash from nose to dorsal fin and are very common around rubble in the sand flats. Juveniles look very similar to those of the cocoa damselfish and longfin damselfish, but cocoa juveniles have a dark spot on the upper base of the tail and longfin juveniles have blue stripes rather than a wash of blue. The color of the juveniles indicates that they are 'cleaner fish' who eat the ectoparasites off other fish. Adults are olive-brown, sometimes with yellow on the belly or tail. Adults are mainly carnivorous, and do not guard algae patches like other damselfish. Male adults will carefully maintain and defend nest sites with small refuges during breeding periods and enthusiastically herd one or more females to deposit their eggs there.



Dusky Damselfish (*Stegastes adustus*) (not pictured). These fish are fairly common in front of the station and at Point of Sands within complex coral structures. Adults are dark olive-brown to black and intensely territorial, defending a carefully cultivated patch of algae from much larger fish and even divers. Juveniles are silvery-gray to blue with an orange wash from nose to dorsal fin.



Banded Butterflyfish (*Chaetodon striatus*) Common in Little Cayman, banded butterfly fish often swim around the reef tops, usually in pairs. They have protruding mouths to pluck food from the substrate, including polychaete worms. Their dark bands conceal their eyes, which, along with their narrow bodies, deter predators. They generally spawn in pairs between February and May. When two females are involved in mating, the male spawns with the second less than a minute after spawning with the first.

Larger Common Fish



Blue Tang (*Acanthurus coeruleus*) Members of the Surgeonfish family, blue tangs have a white or yellow spine (as sharp as a scalpel, hence the family name) on the base of their tail. They are very common in Little Cayman at all times of day and are one of the few visible fish species at night. Adults can range from bright blue to dark blue, while juveniles can be blue, yellow, or white. Found alone and in schools, they are herbivores and algae specialists.

Squirrelfish (*Holocentrus adscensionis*)

The huge eyes of the squirrelfish are used to hunt crabs and shrimp at night on sand flats and seagrass beds. During the day, squirrelfish will hide in holes in coral or rock, vigorously defending these refuges from other fish. Squirrelfish use their air bladders to produce very low-frequency (beyond human hearing) calls to communicate with other fish.



Fish that Look Like Fish



Schoolmaster (*Lutjanus apodus*) The schoolmaster often travels in schools, around large heads of patch coral. Note yellow on fins, and not elsewhere on the body.

Lane Snapper (*Lutjanus synagris*) The lane snapper is common in the reef, and not necessarily found in schools. The black spot is larger than the spot on the mahogany snapper, and the fish is smaller.



French Grunt (*Haemulon flavolineatum*) Common in Little Cayman, french grunts often congregate in schools. They feed on invertebrates. The most common grunt is yellow and silver with stripes. Other grunt species have blue stripes and black tails.



Yellowtail Snapper (*Ocyurus chrysurus*) The yellowtail snapper can feed on gamete clouds in the open ocean by following mating pairs. Note the yellow tail and large yellow stripe across the body. Often seen in open water.

Parrotfish



Striped Parrotfish (*Scarus iserti*) The black-and-white-striped initial stage of this fish (right) is very common on the sand around coral heads. The initial phase often has a yellow smudge on the nose. The terminal phase (left) has a yellow stripe or spot above the pectoral fin. Initial-phase fish are predominantly female but terminal phase fish are entirely male, and females are capable of changing sex and becoming terminal-phase males. Initial-phase fish forage in groups on algae on rocky or sandy substrate, sometimes within the territory of a dominant initial-phase female, who can be distinguished from subservient females by its yellow ventral fins.

Redfin Parrotfish (*Sparisoma rubripinne*) The initial phase (pictured) is occasional in Little Cayman, the terminal phase, with green-blue shades, is uncommon. Also commonly known as yellowtail parrotfish, they are shy and lunge away quickly, leaving behind a cloud of sediment. They can change



sexes from females to males (protogynous hermaphroditism) such that all males are sex-changed females. There are then many young males, leading to consistent aggression. Parrotfish have strong beaks to consume algae and dead coral from the reef. Because they ingest 75% inorganic matter, they often excrete waste as sand while they swim.



Stoplight Parrotfish (*Sparisoma viride*)

The initial phase (pictured) is occasional in Little Cayman, while the terminal phase, which is emerald green with yellow, purple, and pink accents, is less common. They adapt their feeding, mating, and social behavior to take advantage of their local environments. Stoplight parrotfish are protogynous hermaphrodites, though initial phase females, as opposed to the largest females, become male when the dominant male disappears. They use

their strong jaws to gouge out algae growing beneath the surface of dead coral on reefs, leading to increased consumption of inorganic matter and increased bioerosion.

Fish with Weird Bodies



Porcupine fish (*Diodon hystrix*)

The porcupine fish is more often seen in the open water near patch reefs, and may be found hiding under ledges. May inflate.

Spotted Trunkfish (*Lactophrys bicaudalis*) The spotted trunkfish can be found swimming in the open, checking out snorkelers, or looming underneath reef overhangs. The remains of sea cucumber intestines have been found in the stomach of a trunkfish. It can also eat gastropods. The juveniles are really cute.





Mushroom Scorpionfish (*Scorpaena inermis*) The scorpionfish is a lie-and-wait predator. It is very difficult to spot, but not uncommon. It hides on the bottom and looks like a rock. As its name suggests, the scorpionfish is poisonous.

Fish with Long and Skinny Bodies



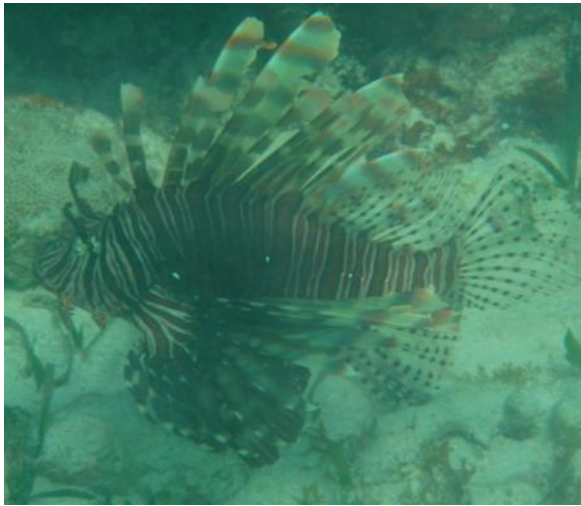
Great Barracuda (*Sphyraena barracuda*) The piscivore has a primitive mouth with sharp teeth, that can kill fish in a single strike. It can strike at other shiny objects, such as jewelry and watches. It is often seen in the reef stalking snorkelers. Watch your back.

Keeltail Needlefish (*Platybelone argalus*) These are often found just under the surface of the calm water between the station and the fringe reef. Easily distinguishable by their slender body and needle-sharp jaw, keeltail needlefish eat mainly smaller fish and attach their eggs to floating objects.



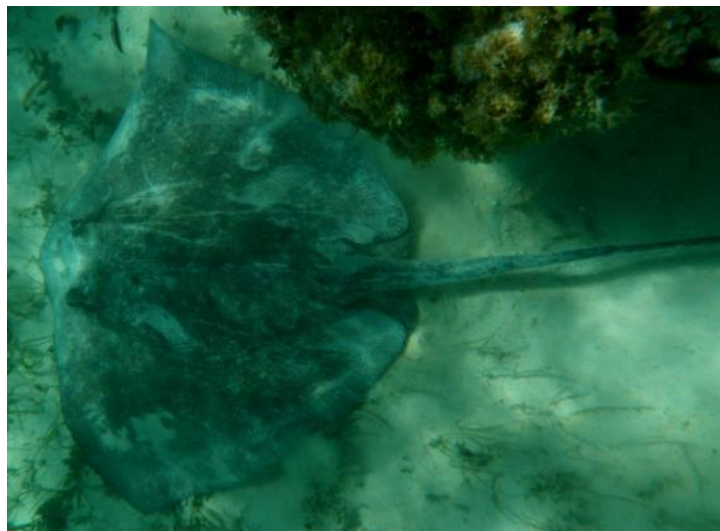
Other Fish

French Angelfish (*Pomacanthus paru*) Occasional in Little Cayman, french angelfish are closely related to grey angelfish. Juveniles (left; intermediate on right) are cleaners of ectoparasites from reef fish, which is advertised by their coloration and protects them from predation. Juveniles primarily consume algae. Sponges make up 70% of the adult diet; only 11 Caribbean fishes are known to regularly feed on sponges due to the fibrous supports and repellent chemicals. French angelfish have strong jaws and many teeth, and they coat lumps of food in mucus to protect their stomachs during digestion. They are territorial to defend mates and are believed to be monogamous.



Red Lionfish (*Pterois volitans*) The lionfish is an invasive species from the Indo-Pacific; they invaded Little Cayman in 2008. It is uncommon in its native range and a recent invader, so there is not much research on the species. Their spines are poisonous and increase their size, both of which aim to deter predation. Lionfish have no predators in the Caribbean and are a threat to reef fish communities because they consume large amounts of prey. Culling efforts on Little Cayman are in place to decrease their numbers.

Southern Stingray (*Dasyatis americana*) The southern stingray is the most common ray in Little Cayman. Often seen with a silver or black bar jack or other fish swimming on top or alongside it as these fish shadow-feed. You can find it foraging in the sand flats or near patch reefs.



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Acknowledgements

Thanks to Frances Wang for a few photographs.

Student Review of the Corcovado Experience 2011

What do you do with only four days in one of the most wild and diverse areas in Costa Rica? How can you most fully experience its endless tropical beaches, dense forests housing big cats and poisonous snakes, and estuaries full of crocodiles and bull sharks? The 2011 FSP spent Feb 2nd- 5th in Corcovado National Park on the Osa Peninsula of Costa Rica. As soon as we started our journey with a bumpy ride in a cattle truck, swerving in and out of rivers, past scarlet macaws and blue *Morpho* butterflies, we knew that Corcovado would be an experience like none other.

Because we spent two of the days hiking in and out of the park, we did not have enough time to conduct full research projects and produce papers. Instead, we chose to take advantage of Corcovado's incredibly diverse system to study the broader ecology of the rainforest.

To maximize the breadth of our learning experience, we divided into five specialist groups focused on mammals, herps, arthropods, plants, or birds, based on our personal interests. During the few days prior to our arrival in Corcovado, each group researched their study groups' basic life history, major taxonomic groups, and the families and species common in Corcovado. On the hike to the Sirena Biological Station, we opportunistically shared knowledge with our fellow students about the organisms we specialized in as we spotted them. This led to enthusiastic discussions about helicopter damselflies, leaf-litter frogs, *Melastomataceae*, howler monkeys and many other organisms. By the time we stumbled into Sirena at the end of the day, we had learned a lot from each other and expanded our knowledge of the organisms in Corcovado.

Over the next two days, we explored the trails around Sirena in small groups, each of which included a representative from each of the specialist teams. We practiced sharing our knowledge in an interactive group setting. Our curious exploration was academically enriching because we were able to share what we were passionate about in a low-pressure but engaging environment. In addition, we were able to spend all of our time outdoors enjoying and learning from the natural system around us. This was a refreshing break from more narrowly focused research, where completing projects was prioritized over appreciating the rare systems surrounding us. As we spent more time in the field, we saw even more organisms, from caimans to spider monkeys to tapirs (seen only by the TAs!). We found that the more we knew and understood, the more we were able to see and appreciate.

Our time in Corcovado was a wonderful addition to our learning experience on the FSP. We also have some suggestions for how it could be developed and improved for future FSP groups. First, more time for preparation within specialist groups would allow students to research and consolidate more information. Since we had no internet and limited materials in Cuerici, our preparation was mostly focused on taxonomy. Given more time, our knowledge could have expanded to include interesting facts or recent research on the focus organisms. We propose that in the future, students should choose their specialist groups earlier, perhaps by the second week of the program. The concept and practice of the biodiversity "expert

groups” worked so well at Corcovado that it could be incorporated into the entire Costa Rica program (and perhaps Little Cayman as well).

On the first full day at the station, students could take a half-day with their specialist group to solidify their knowledge and plan a short lesson about their specialist organism group (roughly a half hour long). These lessons would utilize the incredible natural system around the station to convey knowledge in an engaging, interactive way. The specialist presentations could include a hike towards an area where a particular organism or group is present or a habitat where it is typically found. Alternatively, student specialists could plan a scavenger hunt through the trails or a skit about rare and difficult-to-find organisms.

In addition to sharing knowledge with their cohort, the students might consider creating a comprehensive FSP field guide and informational book to be shared with future FSP groups. The '09 FSP group produced a short booklet on Corcovado that was donated to the National Parks field station, so we know students can do an excellent job along these lines. Every year, specialist groups could add more to the book through an Excel spreadsheet or a shared Google document. Beyond species identification, information would include interesting facts and recent articles on the organisms. Each year, students would have access to the book, which would give a conveniently organized, comprehensive look at the entire breadth of Costa Rican organisms. Specialist groups, projects, and papers would create a greater depth of knowledge. The book may also include project ideas that previous years have come up with but not pursued. The evolution of this book (which could be developed Wiki-style) would help FSP groups build on the knowledge and research of previous years and develop ideas for future groups.

Looking back on our Corcovado experience, we realize that all of the risks that Professor David Peart warned us about at the beginning of our 20 km trek to Sirena, such as the half-dozen species of poisonous snakes, were worth taking. We will never forget the gorgeous swimming hole in the Rio Claro; the ocean cave full of leaf-nosed bats, adjacent to a rocky intertidal zone teeming with chitons and fish; walking through a group of foraging coatis squabbling over roots; coming face to face with inquisitive squirrel monkeys; or watching basilisk lizards run across water. We hope all future FSP groups will have the opportunity to enjoy rich, rugged, and unforgettable Corcovado.

With warmth in our hearts and chiggers in our socks,
We want you all to know that Corcovado rocks.
Don't put your foot in a hole that you cannot see,
Or else you'll end up as an amputee.
Life there's tough with the bugs and the heat
But with your FSP friends, it can't be beat.

With love,

The 2011 FSP