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Volumen 60 (Supl. 1)
Marzo, 2012
San José, Costa Rica

60 ANIVERSARIO ANNIVERSARY 2012

ISSN-0034-7744

UNIVERSIDAD DE COSTA RICA

REVISTA DE BIOLOGÍA TROPICAL

www.biologiatropical.ucr.ac.cr

Proceedings of the 35th Scientific Meeting of the AMLC
Memorias de la 35ta Reunión Científica de la ALMC

AMLC/ALMC

Association of Marine Laboratories of the Caribbean • Asociación de Laboratorios Marinos del Caribe

San José, Costa Rica • 23-27 de mayo • 2011

Jorge Cortés
Scientific Editor / Editor Científico

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REVISTA DE
BIOLOGÍA TROPICAL

Telefax (506) 2511-5550 • (506) 2511-8982
biologia.tropical@ucr.ac.cr • www.ots.ac.cr/tropiweb • www.biologia.ucr.ac.cr/rbt/

Revista de Biología Tropical • Universidad de Costa Rica • 11501-2060 San José, Costa Rica

Fotografía de portada / Cover photograph:
Fotografías tomadas durante la 35ta Reunión Científica de la Asociación de Laboratorios Marinos del Caribe (ALMC-2011-Costa Rica), Ciudad de la Investigación, Universidad de Costa Rica, San José, Costa Rica, 23-27 de mayo, 2011.
Fotografías por Jaime Nivia Ruiz
Photographs during the 35th Scientific Meeting of the Association of Marine Laboratories of the Caribbean (AMLC-2011-Costa Rica), Campus for Research, University of Costa Rica, San José, Costa Rica, 23-27 May, 2011.
Photographs by Jaime Nivia Ruiz

574.05
R Revista de Biología Tropical / Universidad de Costa Rica. —Vol. 1 (1953)– . — San José, C. R. : Editorial Universidad de Costa Rica, 1953–
v.

ISSN–0034–7744

1. Biología – Publicaciones periódicas, 2. Publicaciones periódicas costarricenses.

BUCR

Edición aprobada por la Comisión Editorial de la Universidad de Costa Rica

Revista de
Biología Tropical

Volumen 60 (Supl. 1) **CONTENIDO** Marzo, 2012

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PROCEEDINGS OF THE 35TH SCIENTIFIC MEETING OF THE
MEMORIAS DE LA 35TA REUNIÓN CIENTÍFICA DE LA
ASSOCIATION OF MARINE LABORATORIES OF THE CARIBBEAN (AMLC)
ASOCIACIÓN DE LABORATORIOS MARINOS DEL CARIBE (ALMC)

San José, Costa Rica, del 23 al 27 de mayo, 2011

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Solciré Martínez Jiménez
Alex Rodríguez Arrieta
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Allan Carillo Baltodano
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Asociación de Laboratorios Marinos del Caribe
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Universidad de Costa Rica

ASOCIACIÓN DE LABORATORIOS MARINOS DEL CARIBE (ALMC)

La ALMC es una confederación de más de 30 instituciones de investigación, educación y gestión marina, dedicada a estimular la producción y el intercambio de información científica y de manejo de recursos, facilitar la cooperación y asistencia mutual entre sus miembros, y fomentar la educación marina y ambiental en la región.

La ALMC fue fundada en 1956 por investigadores interesados en las ciencias marinas del Atlántico tropical y del Caribe. Creada principalmente como una organización científica, la fuerza de la ALMC está en la diversidad de sus miembros institucionales, laboratorios marinos en su mayoría, y de la experiencia de sus miembros individuales, que incluyen profesores, investigadores, estudiantes y otras personas interesadas. Para incorporarse a este diverso grupo, la Asociación ofrece membresías institucionales, individuales, de estudiantes y de miembros patrocinadores.

La ALMC tiene su reunión científica cada dos años (la próxima será en Jamaica en el 2013). Esta es organizada por alguno de los miembros institucionales. La institución organizadora se encarga de la logística de la reunión, incluyendo, invitaciones, información sobre transporte y hoteles, inscripciones, asegurar la sala de sesiones la organización y producción del programa de la reunión y las recepciones. La ALMC no tiene un idioma oficial por lo que los participantes pueden presentar en su lengua materna. En reuniones recientes hemos usado traducción simultanea (Inglés/Español/Inglés) para las presentaciones.

La ALMC publica un boletín en español e inglés, "Ciencias Marinas en el Caribe" dos veces al año para informar a sus miembros y personas interesadas sobre las actividades de la Asociación, conferencias y mesas de trabajo u otros eventos pertinentes, cursos de verano resultados de investigaciones relevantes y nuevos libros de interés.

Los objetivos de la ALMC son:

- Estimular el interés y el avance en las ciencias marinas del Caribe y Atlántico occidental.
- Promover el intercambio de información y resultados de investigaciones.
- Fomentar proyectos cooperativos de investigación.
- Exponer a estudiantes a nueva información y desarrollos tecnológicos en ciencias marinas.
- Facilitar la interacción entre científicos y entre científicos y estudiantes de la región para establecer contactos y relaciones productivas.
- Participar/recomendar en la toma de decisiones de países y organizaciones internacionales concernientes a los ambientes marinos.

Para más información, por favor revise: http://www.amlc-carib.org/index.html

ASSOCIATION OF MARINE LABORATORIES OF THE CARIBBEAN (AMLC)

AMLC is a confederation of more than 30 marine research, education, and resource management institutions endeavoring to encourage the production and exchange of research and resource management information, advance the cause of marine and environmental education in the region, and facilitate cooperation and mutual assistance among its membership.

The AMLC was founded in 1956 by researchers with interests in the marine science of the tropical Atlantic and the Caribbean. Founded primarily as a scientific organization, the strength of AMLC lies in the diversity of its member institutions and the extensive expertise of its membership. Institutional, Individual Scientist, Student, and Sponsoring memberships are available.

Every other year AMLC Scientific meetings (the next will be in Jamaica in 2013) are hosted by member institutions actively conducting marine research in the Caribbean. The host institution provides overall organization of logistics and management of the meeting, securing a venue and accommodations for participants, providing travel, safety and other important information, producing the and organizing the program and logistics for oral and poster presentation, and the coffee breaks and receptions. The AMLC has no designated official language so researchers are free to make their presentations in their native language. In some meetings, simultaneous Spanish/English/Spanish translation has been provided for the presentations.

The AMLC publishes a biannual newsletter in English and Spanish, "Caribbean Marine Science" to inform members of current AMLC activities, meetings, workshops and other pertinent events, relevant research, summer courses and new books.

Goals of AMLC

- To advance common interests in the marine sciences in the Caribbean and western Atlantic.
- To encourage the exchange of information and research results.
- To foster cooperative research projects.
- To expose students to scientists, new research and technological advances in marine sciences in the region.
- To participate/make recomendations in decisions made by national and international organizations concerning the marine environment.

For more information please check: http://www.amlc-carib.org/index.html

San José, Costa Rica, del 23 al 27 de mayo, 2011

P R O G R A M A

Lunes / Monday / 23 Mayo, 2011

8:00-9:00 a.m.		**INSCRIPCIÓN / REGISTRATION**
		CEREMONIA DE APERTURA / OPENING CEREMONY
9:00-9:20	Jorge Cortés	Organizador de la Reunión / Meeting Organizer
		Autoridad Universitaria / University Authority
		PLENARY PRESENTATION
9:20-10:00	Peter Mumby	Measuring and Managing Coral Reef Resilience
10:00-10:20	**Café / Break**	
		CORAL DISEASE I: Laurie Richardson, Moderador/Chair
Hora/Time	**Presentador/Presenter**	**Títulos / Title**
10:20-10:40	Laurie Richardson	Arnfried Antonius, coral diseases, and the AMLC
10:40-11:00	Carolyn Rogers	Are corals growing in mangroves less vulnerable to climate change?
11:00-11:20	Francisco Soto	Prevalence of Caribbean Yellow Band Disease in La Parguera, Puerto Rico
11:20-11:40	Aaron Miller	Mat formation by cyanobacteria in Black Band Disease of corals
11:40-12:00	Chatchanit Arif	Bacterial profiling of White Plague Disease in a comparative coral species
12:00-13:30	**Almuerzo / Lunch**	
		CORAL DISEASE II: Laurie Richardson, Moderador/Chair
13:30-13:50	Ernesto Weil	Species composition and diseases affecting mesophotic coral communities off the southwest shelf edge of Puerto Rico
13:50-14:10	Esteban Soto	*Francisella asiatica* (syn. *F. noatunensis* subsp. *orientalis*) and its imnplications as a marine pathogen
14:10-14:30	Stephen McCauley	Understanding differences in recovering *Diadema antillarum* densities following mass-mortality in the Caribbean: testing hypotheses in St. Thomas, U.S. Virgin Islands
14:30-14:50	Ernesto Weil	Variability and impact of coral disease and bleaching in La Parguera, Puerto Rico 2003-2007
14:50-15:10	Carolina Bastidas	The 2010 bleaching event at Los Roques, Venezuela: An unprecedented loss of live coral
15:10-15:30	**Café / Break**	
		REEF RESILIENCE AND REPAIR: Andrew Bruckner, Moderador/Chair
15:30-15:50	Andrew Bruckner	Measuring ecological resilience of Caribbean coral reefs
15:50-16:10	Mark Ladd	Managing for resilience: refinement and implementation of a resilience index to improve natural resource management
16:10-16:30	Virginia Garrison	Survival of transplanted fragments of *Acropora* and *Porites* over 12 years
16:30-16:50	Aaron Hutchins	Coral nurseries as an approach to reef resilience
		Adjourn Session
17:00-19:00	**Sesión #1 de Afiches y Actividad de Bienvenida / Poster Session #1 and Welcoming Activity**	

Martes / Tuesday / 24 Mayo, 2011

PLENARY PRESENTATION AND DISCUSSION		
Hora/Time	**Presentador/Presenter**	**Títulos / Title**
9:00-9:40	Patricia Miloslavich	Purpose and Progress: Census of Marine Life
9:40-10:00	**Café / Break**	
REEF-ASSOCIATED BENTHOS: Michael Crosby, Moderador/Chair		
10:00-10:20	Dalila Aldana-Aranda	Variabilidad del patrón reproductivo del Ostión Americano, *Crassostrea virginica* (Gmelin, 1791), en el Golfo de México
10:20-10:40	Robert Anderson	Benthic and fish population monitoring in the near shore waters of Grenada, Eastern Caribbean
10:40-11:00	Jose Chavez Villegas	Abundancia y distribución de larvas de *Strombus gigas* (Linnaeus, 1758) durante su period reproductive
11:00-11:20	Alex Mercado-Molina	Demography of the demosponge *Amphimedon compressa*: Evaluation of the importance of sexual *vs.* asexual recruitment to its population
11:20-11:40	Cristina Sanchez-Godinez	Estado actual de los corales duros de algunos arrecifes del Mar Caribe y la estructura bentica asociada
11:40-12:00	Harlan Dean	The polychaetes of the Caribbean
12:00-13:30	**Almuerzo / Lunch**	
APPLICATIONS OF HABITAT MAPPING: David Zawada, Moderador/Chair		
13:30-13:50	David Zawada	Revealing habitat-use patterns of Loggerhead Sea Turtles during inter-nesting periods
13:50-14:10	Sarah Manuel	Environmental assessment and planning for coastal development
14:10-14:30	Maria Liceaga-Correa	Mapping morphometric characteristics of Caribbean seagrasses
14:30-14:50	Natalia Barrantes-Rojas	Environmental interpretation for reef management on Quiribrí Island National Monument, Caribbean, of Costa Rica
14:50-15:10	**Café / Break**	
NOAA SESSION: Brian Walker, Moderador/Chair		
15:10-15:30	Brian Walker	Small-scale mapping of dynamic arborescent coral (*Acropora cervicornis*) thickets
15:30-15:50	Alison Moulding	Coral recruitment to vessel grounding sites off Ft. Lauderdale, Florida USA
15:50-16:10	Paola Espitia	Effectiveness and success of different octocoral reattachment methods
16:10-16:30	María Liceaga-Correa	Coastal morphology, stratigraphy and benthic community characterization north of the Yucatan Peninsula
16:30-16:50	Clark Sherman	Sedimentation patterns at reef sites adjacent to the Guanica Bay watershed, Southwest Puerto Rico
17:00-19:00	**Sesión #2 de Afiches / Poster Session #2**	

Miércoles / Wednesday / 25 Mayo, 2011

OPTIONAL PARTICIPATION: GIRAS DE CAMPO / FIELD TRIPS OR WORKSHOP: MARINE LIFE CENSUS		
Hora/Time	**Presentador/Presenter**	**Títulos / Title**
9:00-4:30	Patricia Miloslavich	Marine Life Census Workshop

Jueves / Thursday / 26 Mayo, 2011

PLENARY PRESENTATION		
Hora/Time	**Presentador/Presenter**	**Títulos / Title**
9:00-9:40	Robin Mahon	The role of the Caribbean Sea Commission (CSC) in regional ocean governance and how the AMLC can contribute
9:40-10:00	Puerto Rico Sea Grant	Opportunities for Inter-laboratory Collaboration
10:00-10:40	Robin Mahon	Free Discussion
10:40-11:00	**Café / Break**	
	INTERNATIONAL COLLABORATION INITIATIVES: Juan José Cruz-Motta, Moderador/Chair	
11:00-11:20	James Mair	Benefits and issues related to marine research collaborative networking at national, regional and international scales
11:20-11:40	Juan José Cruz-Motta	South American Research Group on Coastal Ecosystems: A NAGISA legacy
11:40-12:00	Juan Carlos Silva Tamayo	Introducing the IberoAmerican network on the study of the effects of high atmospheric PCO_2 and ocean acidification on ancient and recent marine ecosystems
12:00-13:30	**Almuerzo / Lunch**	
	FISH & FISHERIES: John Ogden, Moderador/Chair	
13:30-13:50	Stephen Box	Similarities between small scale and commercial coral reef fisheries in Honduras: The need to develop frameworks that can integrate fisheries management across different scales
13:50-14:10	Annemarie Kramer	Habitat complexity and reef fish assemblages in shallow coral reefs in South Caicos, Turks and Caicos Islands
14:10-14:30	Arthur Potts	Evaluating the needs of the fishing and associated livelihoods in the coastal fishing sector of Trinidad & Tobago-plus next steps
14:30-14:50	Helena Molina-Ureña	Marine protected areas and the lionfish invasion of Costa Rican reefs
14:50-15:10	Steven Canty	Defining spatially and temporally explicit fishing mortality rates of fish traps on coral reefs as a crucial step in developing management limits for Caribbean trap fisheries
15:10-15:40	**Café / Break**	
	GENETICS: Nathan Truelove, Moderador/Chair	
15:40-16:00	Nathan Truelove	Genetic connectivity of Spiny Lobster in the MesoAmerican barrier reef
16:00-16:20	Monica Rojas	Genetic population structure of two brittle stars (*Ophiocoma echinata* and *Amphipholis squamata*) with contrasting life histories
16:20-16:40	Daniel Lancheros	Molecular identification of the loggerhead sea turtle *Caretta caretta* (Stejneger, 1901) (Testudines: Cheloniidae) using the gene MTDNA Cytochrome C Oxidase I
16:40-17:00	Nikolaos Schizas	Genetic variation of *Symbiodinium* of the coral host *Agarica lamarcki* between shallow and mesophotic habitats
17:00-18:00	**Encuentro Investigadores-Estudiantes / Researchers-Students Exchange**	
18:30-21:00	**PREMIACIONES Y CENA/CONFERENCE DINNER**	

Viernes / Friday / 27 de Mayo, 2011

PLENARY PRESENTATION		
Hora/Time	**Presentador/Presenter**	**Títulos / Title**
9:00-9:40	Peter Sale	Managing Caribbean coral reefs-the challenge for the next forty years
	OCEANOGRAPHIC INFLUENCES: Clare Fitzsimmons, Moderador/Chair	
9:40-10:00	Eric Alfaro	Atmospheric forcing of cool sea subsurface temperature water events in Bahia Culebra, Costa Rica
10:00-10:20	Tomson Oliver	Significant changes in living polycystine radiolarian and planktonic foraminifera caused by the Orinoco River plume, Southeastern Caribbean Sea
10:20-10:40	**Café / Break**	

	CORAL ECOLOGY: Mark Vermeij, Moderador/Chair	
10:40-11:00	Mark Vermeij	The ecology of coral larvae: Recent findings
11:00-11:20	Brittany Huntington	The influence of spatial reef heterogeneity on coral diversity
11:20-11:40	Benjamin Mueller	The bottom of coral reefs: A net source of DOC
11:40-12:00	Michael Hellberg	Population isolation and introgression in Eastern Pacific Porites
12:00-13:30	**Almuerzo / Lunch**	
	GOVERNANCE & POLICY: Salina Stead, Moderador/Chair	
13:30-13:50	Salina Stead	Linking marine science and socio-economics to governance and policy-spotlight on the Cayos Cochinos, Honduras
13:50-14:10	Eduardo Klein	Planning for the conservation of marine biodiversity in face of offshore gas exploitation in Venezuela
14:10-14:30	Lia Ortiz	From the open sea to a pot of kallaloo: The significance of the USVI fishery in identifying you
14:30-14:50	Ken Lindeman	Erosion of coastal climate adaptation policies in strategically critical regions
14:50-15:10	**Café / Break**	
	GENERAL ECOLOGY: Carlos Carmona, Moderador/Chair	
15:10-15:30	Clark Sherman	Submerged outer-shelf hummock reefs, Southwest Puerto Rico: Record of a regional reef give-up event?
15:30-15:50	Lina Barrios	Responses of Caribbean shallow and deep water marine invertebrates to ocean acidification
15:50-16:10	Ian Sandeman	Evidence that carbon dioxide and exposed carbonic anhydrase in the organic matrix are the basis of calcification on the skeleton surface of living corals
16:10-16:30	Joanne Peel	Growth estimation of the Pink Queen Conch (*S. gigas*) by direct methods in a natural protected area of the Mexican Caribbean
16:30-17:00	**Asamblea General-Premiación Estudiantes y Cierre / General Meeting-Student Awards and Adjourn**	

ADJOURN
Lunes / Monday, 23 de Mayo, 2011

17:00-19:00	**SESIÓN #1 DE AFICHES / POSTER SESSION #1**
Afiche # Poster #	**Presentador(es) & Título / Presenter(s) & Title**
1	**Amaro, María**-Comunidad de esponjas marinas asociadas a substratos rocosos-coralinos de Isla de Tortuga, Dependencia Federal, Venezuela
2	**Alvarez-Barco, Julia A.**-Moluscos asociados al arrecife coralino de Isla Larga, Parque Nacional San Esteban, Carabobo, Venezuela
3	**Arora, S.**-Diversity of bacteria associated with *Montastraea* spp. Across sea water quality gradient in the United States Virgin Islands
4	**Barrios, Jorge**-Evaluación química y actividad biologica de *Caulerpa racemosa* (Chlorophyta Caulerpaceae) proveniente de Isla de Cubagua, Venezuela
5	**Barrios, Jorge**-Evaluación faunística de arrecifes coralinos invidados por *Kappaphycus alvarezii* (Rhodophyta) en Isla Cubagua, Venezuela
6	**Bastidas, Carolina**-Ecological, ecotoxicological and environmental coral reefs monitoring at Los Roques, Venezuela
7	**Broderick, Emily**-What is that pink crust? Making identification of crustose coralline algae more tractable
8	**Carmona-Suárez, Carlos Alberto**-Comparison of methods for indirect estimation of body size in *Cardisoma guanhumi* (Crustacea: Brachyura: Gecarcinidae)
9	**Chávez Villegas, José Francisco-**Efecto climático en la abundancia larval del Caracol Rosa, *Strombus gigas* (Linnaeus, 1758) en el Caribe Mexicano
10	**Cole, Linda**-Diversity and distribution of Tunicata (Urochordata) of Tobago
11	**D'Armas, Haydelba**-Constituyentes químicos bioactivos provenientes de la esponja *Aaptos pernucleata* (Porifera: Demospongiae) recolectada en la Bahía de Mochima, Estado Sucre, Venezuela
12	**Colon-Alvarado, Wilmarie Del C.**-Patterns of dispersion and association of four scleractinian coral species in Culebra Island, Puerto Rico

13	**Fariñas, Milagros**-Evaluación morfológica y ultraestructura de *Cliona varians* (Porifera: Demospongiae) por microscopía electrónica
14	**Fariñas, Milagros**-Es *Cliona varians* (Porifera: Demospongiae) fuente de lectinas?
15	**Gayle, Peter**-Assessing the health status of a coastal habitat: Priorities for impact mitigation and ecosystem restoration
16	**González, Carmen**-Analysis of the spatial-temporal abundance of the sea urchin *Diadema antillarum* in five sites of Puerto Rico
17	**Habegger, Leigh**-The 2010 coral bleaching season and coral recovery in South Caicos, Turks and Caicos Islands
18	**Herrera-Reveles, Ana Teresa**-Crecimiento somático y relación ARN/ADN en estadios juveniles de *Eucinostomus argenteus* (Pisces: Gerridae) dentro de la Bahia de Mochima y en el Golfo de Cariaco, Venezuela
19	**Huete-Perez, Jorge A.**-The Ocean Genome Program: Archiving the genomes of the seas
20	**Klein, Eduardo**-Cartografia y estado de conservación de las comunidades coralinas de la Isla de Blanquilla, Venezuela
21	**Knapp, Charles R.**-Multidisciplinary uses of a public aquarium-owned research vessel
22	**McCoy, Croy**-Quantifying the impact of recreational and artisanal fisheries in the Cayman Islands using of socio-economic questionnaires
23	**Kramer, Annemarie**-Rugosity as a predictor for density of commercially fished grouper (Serranidae) in shallow coral reefs in South Caicos, Turks and Caicos Islands
24	**Liceaga, Correa María de los Angeles**-Distribución potencial del Manatí (*Trichechus manatus manatus*) en Bahía de la Ascensión, Quintana Roo, México
25	**Sandra Schleier-**Abundance and size frequency of corallivores in *Acropora cervicornis* aquaculture farms in Culebra, Puerto Rico

Martes / Tuesday / 24 de Mayo, 2011

17:00-19:30	**SESIÓN #2 DE AFICHES / POSTER SESSION #2**
Afiche # Poster #	**Presentador(es) & Título / Presenter(s) & Title**
1	**Lucas, Matthew**-Acquisition of spectral characteristics of the dominant reef species in Parguera, Puerto Rico to support hyperspectral assessment of reef biodiversity
2	**Lucas, Matthew**-Genetic diversity and connectivity of shallow and mesophotic reefs
3	**Maduro, Dmitri**-Investigation of chemical aggregation cues of Caribbean Spiny Lobsters (*Panulirus argus*)
4	**Maldonado, Antonio**-Detección de norovirus y rotavirus en tejidos de ostras expendidas en Cumaná, Venezuela
5	**Manuel, Sarah**-Distributions of tropical species in Bermuda's benthic habitats in relation to light availability
6	**Manuel, Sarah**-A mangement challenge: Are Green Turtles destroying seagrass beds in Bermuda?
7	**Márquez-Rojas, Brightdoom**-Estructura de la comunidad de Copépodos en el Golfo de Cariaco, Venezuela
8	**Martínez, Kimberly**-Status of *Acropora palmata* populations in Cayo Sombrero, Morrocoy National Park, Venezuela
9	**Martínez, Neidibel** –Does *Diadema antillarum* enhance biodiversity in the coral reefs they inhabit?
10	**Mellein, Jennifer R.**-Juvenile scleractinian coral abundance, composition, and influence on population size frequency distribution (Southeast Florida)
11	**Miloslavich, Patricia**-Comparación de la abundancia, estructura de tallas y fecundidad de *Voluta musica* (Caenogastropoda: Volutidae) an tres sitios de la Costa Norte de la Península de Araya, Venezuela
12	**Montañez Acuña, Alfredo**-Spatial-temporal analysis of coral and algae cover with respect to abundances of the sea urchin *Diadema antillarum* in five sites of Puerto Rico
13	**Nielsen, Vanessa**-2005 coral bleaching event in Cahuita, Caribbean Coast of Costa Rica
14	**Otaño, Abimarie**-Patterns of abundance and diversity of sponge and octocorals along a depth gradient in Mona Island, Puerto Rico
15	**Oxenford, Hazel A.**-Goliath Conch, *Strombus goliath*, in Barbados: Range extension of a Brazilian marine endemic
16	**Oxenford, Hazel A.**-Quantitative assessment of coral diseases in Barbados: A comparison of prevalence between 2002 and 2010
17	**Palmerín Ruiz, Claudia**-Evaluación de plaguicidas organoclorados en Laguna de Alvaredo, Vercruz, México

Afiche # Poster #	Presentador(es) & Título / Presenter(s) & Title
18	**Richardson, Laura**-Estimating marine reserve effects through quantification of macro-algal biomass on a Central Caribbean coral reef
19	**Rodriguez Barreras, Ruber**-Echinoderm communities in the Western Region of the Sabana-Camaguey Archipelago, Cuba
20	**Samper-Villarreal, Jimena**-Seagrass dynamics in Cahuita National Park, Caribbean Coast of Costa Rica
21	**Samper-Villarreal, Jimena**-*Manicina areolata* bleaching in seagrass meadows on the reef flat at Cahuita National Park, Caribbean Coast of Costa Rica
22	**Settar, Christine**-"The reef is closer than you think:" An assessment of a multi-media campaign in the USVI
23	**Tanis, Michael**-Evidence for harvesting sexually immature conch despite marine protection in Little Cayman's Bloody Bay Marine Park
24	**Diaz-Ortega, Geraldine-**Relationship between water quality and densities of the sea urchin *Diadema antillarum*

A sketch of Arnfried Antonius (1934 - 2010)

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Received 28-VI-2011. Corrected 20-IX-2011. Accepted 20-XII-2011.

Arnfried Antonius (Fig. 1) studied Zoology, Paleontology and Ethnology at the University of Vienna, Austria, gradually focusing his attention on the ecology and graphic microanatomical reconstructions of marine invertebrates (Antonius 1965). He completed his Ph.D. in 1967 with a dissertation on convolutid Platyhelminthes from the Red Sea (Antonius 1967). The Marine Biology Institute in Rovinj (then Yugoslavia, now Croatia) and the Museum of Natural History in Venice, Italy, were some of the places where he conducted his work during that time. Arnfried then explored Apulia (Italy), assisting Michele Sarà from the University at Bari, Italy, in search of an adequate location to establish a littoral research field station. Three sites were identified for a final consideration, but Michele Sarà relocated to Genoa and the project was abandoned. Driven to explore and see the world, Arnfried accepted an invitation by the hydrobotanist Fritz Gessner, to work at a new facility in Cumaná, Venezuela, in 1968, and joined the faculty of the Universidad de Oriente. Despite ample funding for the time, the facilities did not have a histology laboratory or microscopes. Arnfried thus started working with "larger" organisms, namely stony corals of the Golfo de Cariaco, where cold-water upwelling made for an interesting environmental setting (Antonius 1968, 1980), and Colombia (Antonius 1972). During the 4th Latin American Zoology Congress, held in Caracas in 1968, Arnfried met Jocelyn López Montes de Oca, who was studying chitons at the Universidad Autónoma de Santo Domingo (incidentally the first graduate of marine biology from that institution), and whom he later married. In 1969, when *Acanthaster planci* outbreaks on coral reefs in the Pacific brought forth panic and funding, Arnfried was invited to join research teams assessing the situation in Ifaluk and Woleei, Micronesia (Antonius 1971).

Fig. 1. Arnfried Antonius at Carry Bow Cay in 2001. Detail from a photo by Mike Carpenter.

In 1972 Arnfried visited Klaus Rützler, a fellow graduate from Vienna, at the Smithsonian Institution's National Museum of Natural History in Washington D.C., to examine the

coral collections. This visit followed previous joint coral reef surveys and developed into a postdoctoral fellowship (Fig. 2). It also marked the beginning of Arnfried's continued involvement in Caribbean marine science, starting with reef surveys off the shore of Belize (then British Honduras) and a historic turn of events involving a briefly disoriented boat crew. Together with Klaus, he was assigned to charter a boat and retrieve an inflatable boat, outboard engine, compressor and other equipment from Glover's Reef. The equipment had previously been deposited there by a group of young, enthusiastic biologists and geologists, convinced that they would receive funding for the establishment of a research facility on one of the Caribbean's few atolls, following extensive surveys and a proposal-development workshop with representatives from approximately 40 institutions (Rützler 2009). Glover's Reef atoll is situated about 20 km east of the Mesoamerican Barrier Reef, Belize; the group's proposition was to compare it with a Pacific atoll, 10 years down the road. That grant never came through. Having retrieved the equipment, Arnfried and Klaus were returning from Glover's Reef when the boat captain lost his orientation and couldn't find Tobacco Cay Entrance. Instead, he found another cut through the reef, next to which there was a small island. Arnfried, Klaus and the rest of the crew went on land. The island was deserted but there was a sign saying, "Welcome to Carrie Bow Caye". And the rest is history, colloquially put, and has been well documented in Rützler's (2009) summary of the studies that have been carried out at the Smithsonian's Carrie Bow Cay Marine Field Station since its inception in 1972.

In 1973 Arnfried joined the Harbor Branch Foundation in Fort Pierce, Florida. At this time he had already started to address reef degradation of all kinds, supplementing field observations with experimentation and methods of pathology. In the same year he co-founded the Florida Reef Foundation and presented his first article on a "coral killing blue-green alga" at the 10th scientific meeting of the Association of Island Marine Laboratories of the Caribbean

Fig. 2. Acklins Island, Bahamas, 1970. Left to right: Walter Adey, Arthur Dahl, Tom Walker, Klaus Rützler and Arnfried Antonius. Photo by Mary Rice.

(now Association of Marine Laboratories of the Caribbean). The paper was published in the meeting's proceedings in 1976 and was the first report on what Arnfried later named Black Band Disease. From this time on, his work on a wide range of coral ailments became well known among coral reef researchers (Antonius 1976, 1977a, 1977b, 1981a, 1981b, 1984a, 1984b, 1985a, 1985b, 1987, 1988a, 1988b, 1989, 1991, 1993, 1995a, 1995b, 1995c, 1995d, 1996, 1998, 1999a, 1999b, 2000a, 2000b, Dahl *et al.* 1974, Antonius *et al.* 1978, 1990, Dodge *et al.* 1983, Rützler *et al.* 1983, Antonius & Riegl 1997, 1998, Antonius & Ballesteros 1998, Antonius & Lipscomb 2000, Verlaque *et al.* 2000, Antonius & Alfonso-Carrillo 2001, Riegl & Antonius 2003). His contributions to our understanding of specific coral diseases and afflictions were summarized by Richardson (2011), and the complete bibliography is included here.

Arnfried accepted a new position in Jeddah, Saudi Arabia, and moved there with Jocelyn in 1980. He joined the faculty at the King Abdulaziz University and began expanding his observations on coral pathology to the Red Sea and the Indo-Pacific (Antonius 1984a). In 1989, Arnfried and his family, now with daughter Anya, moved on to Austria where he continued his studies, gave lectures, was actively involved in the establishment of marine parks in Sinai, Egypt (late 1990s),

regularly visited the Caribbean, and guided graduate students until 2002. He concluded his research endeavors with the examination of "coral-killing" red algae and ciliates. After sudden health complications, which emerged in late December 2009, Arnfried passed away on January 13th, 2010.

The coral pathology session of the 35th AMLC scientific meeting in Costa Rica (2011) was dedicated to Arnfried Antonius, as proposed by Jorge Cortés. Arnfried's contribution in kicking off awareness about coral pathology, amidst the widespread initial indifference about the topic among his peers, is well known and so my account does not pivot on his first descriptions of Black Band Disease. Instead, I intended to outline the path he walked throughout his years of involvement in marine science, many of which he spent in the Caribbean (Fig. 3). Fourteen years ago, at the 28th AMLC meeting, which was also held at the CIMAR in San José, Costa Rica, Arnfried and I spent several hours at a nearby tavern talking about our work experiences, our opinions on the status of coral reefs and research in the Caribbean, among other things. This was not our first or last encounter, but for me it was particularly memorable because it was then that I realized what I appreciated about Arnfried and where I felt a kinship to him. It was his passion for his work and fieldwork in particular, regardless of the logistic and environmental constraints, the popularity of the topic, or the institutional flag, if any, under which work was carried out. He was a kind man, a good listener, and always ready to help. Arnfried was keenly perceptive of regional differences in environmental ethics and was very objective as to the importance of marine research. It is thus not surprising that he had a great sense of humor and never took himself too seriously, or the science circus with which we often surround ourselves.

Fig. 3. Arnfried Antonius in 1980, photographing Black Band Disease progressions at Carry Bow Cay. Photo by Klaus Rützler.

ACKNOWLEDGMENTS

I am deeply grateful to Jocelyn Antonius and Klaus Rützler for the information they have shared with me. Klaus Rützler provided the photos, and Bernhard Riegl shared a few accounts form Sinai. My thanks also go to Jorge Cortés for asking me to write this article, which I happily accepted.

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Arnfried Antonius (1934 – 2010): una semblanza

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Received 28-VI-2011. Corrected 20-IX-2011. Accepted 20-XII-2011.

Arnfried Antonius (Fig. 1) estudio Zoología, Paleontología y Etnología en la Universidad de Viena, en Austria, pero luego centró su atención en Ecología y Reconstrucción gráfica micro-anatómica de invertebrados marinos (Antonius 1965). Completó su doctorado en 1967 con una tesis sobre Platyhelminthes retorcidos del Mar Rojo (Antonius 1967). El Instituto de Biología Marina de Rovinj (antes Yugoslavia, ahora Croacia) y el Museo de Historia Natural de Venecia en Italia eran algunos de los lugares en los que llevaba a cabo su trabajo. Más adelante viaja a Italia donde explora la región de Apulia mientras ayuda a Michele Sarà de la Universidad de Bari a buscar un lugar para establecer una estación para estudiar el litoral. Tres lugares reúnen las condiciones, pero antes de elegir uno M. Sarà se trasladó a Génova y se abandonó el proyecto. En 1968, siguiendo su deseo de ver el mundo y explorarlo, Arnfried acepta la invitación del hidrobotánico Fritz Gessner de la Universidad de Oriente, en Venezuela, para trabajar en un nuevo Centro de Investigación en Cumaná, y pasa a ser profesor de esa institución. Aunque contaba con financiamiento —significativo para la época— el Centro no tenía ni laboratorio de histología ni microscopios, así que Arnfried empieza a trabajar con animales "más grandes", concretamente con corales duros, tanto del Golfo de Cariaco, sitio que posee una zona de afloramiento muy interesante

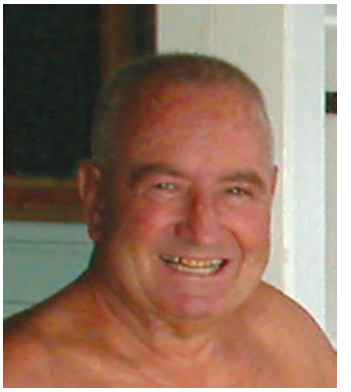

Fig. 1. Arnfried Antonius en Cayo Carry Bow en 2001. Detalle de una foto por Mike Carpenter.

(Antonius 1968, 1980), como de Colombia (Antonius 1972). En 1968, en el IV Congreso Latinoamericano de Zoología celebrado en Caracas, conoce a Jocelyn López Montes de Oca, que en ese entonces estudiaba quitones en la Universidad Autónoma de Santo Domingo (siendo la primera persona que se gradua en biología marina en esa Universidad) y posteriormente se casan. En 1969, la aparición de cantidades masivas de *Acanthaster planci* en el Pacífico genera pánico pero también fondos para evaluar la situación. Arnfried recibe una invitación para viajar a Micronesia y participar de este esfuerzo en las islas de Ifaluk y Woleei (Antonius 1971).

En 1972, Arnfried visita a Klaus Rützler –colega suyo también graduado de la Universidad de Viena–, en el Museo de Historia Natural del Instituto Smithsoniano en Washington D.C., para examinar las colecciones de corales. Anteriormente ya habían colaborado en estudios sobre arrecifes coralinos y la visita se traduce en una posición post-doctoral (Fig. 2). También marca el inicio de la continuada participación de Arnfried en las ciencias marinas del Caribe que comienza con estudios sobre los arrecifes de Belice (British Honduras en ese momento) y con un acontecimiento histórico, involucrando la momentánea pérdida del rumbo por parte de la tripulación. Arnfried y Klaus debían fletar un bote para recuperar un bote de hule, un motor, un compresor y otros aparatos que habían sido dejados en el Arrecife de Glover por un grupo de biólogos y geólogos, jóvenes y entusiastas, que estaban convencidos de que iban a recibir fondos para establecer una estación de investigación en uno de los pocos atolónes del Caribe. Su convencimiento derivaba de los amplios estudios que habían llevado a cabo en la región y del hecho de que se habían reunido con representantes de unas 40 instituciones en un taller cuyo objetivo era formalizar la propuesta para dicha estación (Rützler 2009). El Arrecife de Glover es un atolón situado a unos 20 km al este de la barrera arrecifal de Belice. La propuesta del grupo de investigadores era estudiar el Arrecife de Glover por un período de 10 años, para después compararlo a un un atolón del Pacífico, pero el financiamiento no llegó nunca. Tras retirar el equipo, Arnfried y Klaus emprendieron el regreso. El capitán perdió momentáneamente el rumbo y no encontró la entrada por Cayo Tabaco. Encontró, sin embargo, otro pasaje, al lado del cual había una islita. Arnfried, Klaus y el resto de la tripulación desembarcaron en el lugar. La isla estaba desierta, pero los recibió un rótulo que decía "Bienvenidos a Cayo Carrie Bow". El resto es historia, como se dice en inglés, y está bien documentado en el resumen de los estudios que se han llevado a cabo en la Estación Biológica de Cayo Carrie Bow, desde su establecimiento en 1972 (Rützler 2009).

Fig. 2. Isla Acklins, Bahamas, 1970. De izquierda a derecha: Walter Adey, Arthur Dahl, Tom Walker, Klaus Rützler and Arnfried Antonius. Foto por Mary Rice.

En 1973 Arnfried empieza a trabajar para la Harbor Branch Foundation en Fort Pierce, Florida. Ya había comenzado a ponerle atención a la degradación de los arrecifes en todas sus formas y apariencias, suplementando las observaciones de campo con experimentos y métodos de patología. Ese mismo año cofunda la Florida Reef Foundation y presenta su primer artículo sobre algas que matan corales durante la X Reunión Científica de la Asociación de Laboratorios Marinos Isleños del Caribe (ahora Asociación de Laboratorios Marinos del Caribe). El trabajo se publica en 1976 en las memorias de la reunión. Los trabajos que publica de ahí en adelante constituyen lectura obligatoria entre los investigadores de arrecifes coralinos (Antonius 1976, 1977a, 1977b, 1981a, 1981b, 1984a, 1984b, 1985a, 1985b, 1987, 1988a, 1988b, 1989, 1991, 1993, 1995a, 1995b, 1995c, 1995d, 1996, 1998, 1999a, 1999b, 2000a, 2000b, Dahl *et al.* 1974, Antonius *et al.* 1978, 1990, Dodge *et al.* 1983, Rützler *et al*. 1983, Antonius & Riegl 1997, 1998, Antonius & Ballesteros 1998, Antonius & Lipscomb 2000, Verlaque *et al.* 2000, Antonius & Alfonso-Carrillo 2001, Riegl & Antonius 2003). Sus contribución a nuestro conocimiento de enfermedades especificas de los corales fueron resumidos por Richardson (2011) y aquí se incluye la bibliografía completa.

En 1980, Arnfried y Jocelyn se mudan a Jeddah, Arabia Saudita. Arnfried se une

al cuerpo docente de la Universidad King Abdulaziz y amplía el ámbito de sus estudios sobre patologías coralinas al Mar Rojo y al Indo-Pacífico (Antonius 1984a). En 1989, él y su familia, ahora con una hija, Anya, regresan a Austria, donde el investigador prosigue sus estudios, imparte clases, colabora en el establecimiento de parques marinos en el Sinaí, en Egipto (finales de la década de 1990), continúa visitando el Caribe regularmente y ofrece consejos a estudiantes de posgrado hasta el 2002. Concluye sus esfuerzos de investigación estudiando algas rojas y organismos ciliados que colonizan y matan corales. Su salud se complica súbitamente a finales de diciembre de 2009 y muere el 13 de enero de 2010.

La sesión sobre patología de corales de la XXXV Reunión Científica de la ALMC celebrada en Costa Rica en el 2011 estuvo dedicada a Arnfried Antonius, a petición de Jorge Cortés. La contribución de Arnfried al estudio de las patologías coralinas, pese a la indiferencia con que el tema era mirado en un inicio por sus colegas, es de sobra conocida. De ahí que mis comentarios no hayan girado alrededor de sus primeras descripciones de la "enfermedad de banda negra" que afecta a los corales. Mi intención ha sido, más bien, recorrer la ruta tomada por Arnfried durante los años que dedicó a las ciencias marinas, muchos de los cuales los pasó en el Caribe (Fig. 3). Hace catorce años, durante la XXVIII Reunión Científica de la ALMC, que también se efectuó en el CIMAR en San José de Costa Rica, Arnfried y yo pasamos varias horas en una taberna hablando de nuestras experiencias de trabajo, intercambiando opiniones sobre el estado de los arrecifes y comentando sobre la investigación marina en el Caribe, entre otras cosas. No fue ni nuestro primer encuentro ni el último, pero sí un encuentro memorable, porque ahí me di cuenta de qué era lo que yo más apreciaba de Arnfried y por qué sentía que "éramos de los mismos": era su pasión por el trabajo, sobre todo el trabajo de campo, al que se entregaba sin importar la popularidad del tema, los retos logísticos o ambientales o la bandera institucional, si es que la había. Era un hombre amable, sabía escuchar y siempre estaba dispuesto a ayudar. Su agudeza mental le permitió percibir claramente las diferencias regionales en materia de ética ambiental y ver con objetividad la importancia de las investigaciones marinas. Por eso, no sorprende que tuviera tan buen sentido del humor y que nunca se tomara demasiado en serio, ni a sí mismo, ni al circo científico del que a menudo solemos rodearnos.

Fig. 3. Arnfried Antonius en 1980, tomando fotos de la progresión de la enfermedad de banda negra en Cayo Carry Bow. Foto por Klaus Rützler.

AGRADECIMIENTOS

Quedo profundamente agradecido con Jocelyn Antonius y con Klaus Rützler por la información que compartieron conmigo. Klaus Rützler aportó las fotos y Bernhard Riegl compartió conmigo muchas de las experiencias vividas en el Sinaí. Gracias también a Jorge Cortés, que me pidió que escribiera este artículo, lo que acepté con gran alegría.

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Arnfried Antonius, coral diseases, and the AMLC

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Received 22-VII-2011. Corrected 17-XI-2011. Accepted 20-XII-2011.

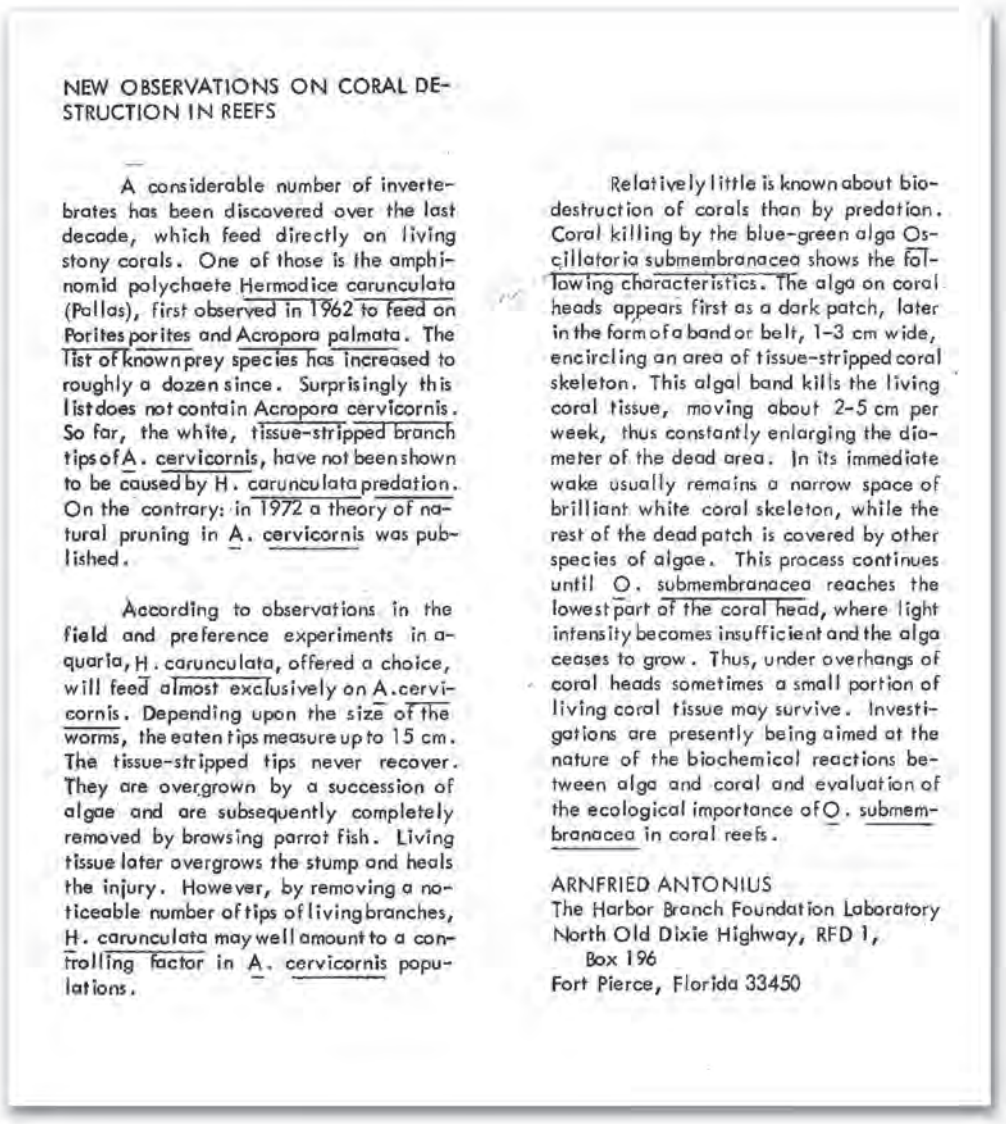
Abstract: The study of coral diseases, coral pathogens, and the effects of diseases on tropical and subtropical coral reefs are all current, high-profile research areas. This interest has grown steadily since the first report of a coral disease in 1973. The author of this report was Arnfried Antonius and the publication was an abstract in the proceedings of a scientific meeting of the Association of Marine Laboratories of the Caribbean, or AMLC (then known as the Association of Island Marine Laboratories of the Caribbean). Since Antonius' pioneering communication he continued working on coral diseases on reefs throughout the world, often documenting the first observation of a novel pathology in a novel location. Each of the coral diseases Antonius first described, in particular black band disease, is the subject of current and ongoing investigations addressing pathogens, etiology, and their effects on coral reefs. Many of the points and observations he made in his early papers are highly relevant to research today. This paper reviews aspects of Antonius' early work, highlighting contributions he made that include the first *in situ* experimental studies aimed at discerning coral epizootiology and the first quantitative assessments of the role of environmental factors in coral disease. Antonius' early findings are discussed in terms of relevant current controversies in this research area. Rev. Biol. Trop. 60 (Suppl. 1): 13-20. Epub 2012 March 01.

Key words: Caribbean coral diseases, black band disease, white band disease, Arnfried Antonius.

In 1973, at the 10th meeting of the Association of Island Marine Laboratories of the Caribbean, Arnfried Antonius gave the first report of a coral disease. Abstracts from this meeting were published in 1976, and Antonius' report, titled "New observations on coral destruction in reefs", is now widely cited as the first published documentation of coral disease. This abstract is reproduced in Fig. 1. Although the publication was produced in 1976, this report has been consistently cited as Antonius (1973) by Antonius himself in his subsequent publications and by subsequent investigators in the field. The actual first publication of a coral disease was by Garrett and Ducklow in 1975.

In the four decades since this report the study of coral diseases has increased steadily and dramatically, with the number of published papers focusing on this topic increasing exponentially since the early 1990s (Sokolow 2009). The increase in both coral disease research and the number of associated papers appearing in the literature has unfortunately been matched by an increase in coral disease incidence and prevalence on tropical and subtropical reefs worldwide, along with an increase in the appearance and spread of novel coral diseases (Sutherland *et al*. 2004, Weil 2004).

Although widely recognized as the "father of coral disease" for his first reports in this research area, Antonius' early work also is highly notable in that he was the first to combine field observations with experimental work in the laboratory using controlled conditions; the first to document the relationships between coral disease incidence and environmental factors (temperature, nutrient enrichment, and pollution); the first to point out that some coral



NEW OBSERVATIONS ON CORAL DESTRUCTION IN REEFS

A considerable number of invertebrates has been discovered over the last decade, which feed directly on living stony corals. One of those is the amphinomid polychaete Hermodice carunculata (Pallas), first observed in 1962 to feed on Porites porites and Acropora palmata. The list of known prey species has increased to roughly a dozen since. Surprisingly this list does not contain Acropora cervicornis. So far, the white, tissue-stripped branch tips of A. cervicornis, have not been shown to be caused by H. carunculata predation. On the contrary: in 1972 a theory of natural pruning in A. cervicornis was published.

According to observations in the field and preference experiments in aquaria, H. carunculata, offered a choice, will feed almost exclusively on A. cervicornis. Depending upon the size of the worms, the eaten tips measure up to 15 cm. The tissue-stripped tips never recover. They are overgrown by a succession of algae and are subsequently completely removed by browsing parrot fish. Living tissue later overgrows the stump and heals the injury. However, by removing a noticeable number of tips of living branches, H. carunculata may well amount to a controlling factor in A. cervicornis populations.

Relatively little is known about biodestruction of corals than by predation. Coral killing by the blue-green alga Oscillatoria submembranacea shows the following characteristics. The alga on coral heads appears first as a dark patch, later in the form of a band or belt, 1-3 cm wide, encircling an area of tissue-stripped coral skeleton. This algal band kills the living coral tissue, moving about 2-5 cm per week, thus constantly enlarging the diameter of the dead area. In its immediate wake usually remains a narrow space of brilliant white coral skeleton, while the rest of the dead patch is covered by other species of algae. This process continues until O. submembranacea reaches the lowest part of the coral head, where light intensity becomes insufficient and the alga ceases to grow. Thus, under overhangs of coral heads sometimes a small portion of living coral tissue may survive. Investigations are presently being aimed at the nature of the biochemical reactions between alga and coral and evaluation of the ecological importance of O. submembranacea in coral reefs.

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Fig. 1. The first report of a coral disease, presented in a talk by Arnfried Antonius in 1973 at the 10th Meeting of the Association of Island Marine Laboratories of the Caribbean (now called the Association of Marine Laboratories of the Caribbean) and published in 1976.

diseases may have similar disease signs but different potential pathogens; and the first to extrapolate coral disease activity on reefs to overall reef ecology. Examples of his contributions in each of these areas are summarized and discussed below.

RECOGNITION AND DESCRIPTION OF THE FIRST CORAL DISEASES

Black Band Disease: The subject of Antonius' first coral disease report (Antonius 1976) was black band disease (BBD). Although not

referred to as a disease in his report (Fig. 1), he conferred this name in the literature in a later publication (Antonius 1981a). In addition to describing the pattern of coral tissue death that results from BBD he identified a blue-green algal (cyanobacterial) pathogen which he identified as *Oscillatoria submembranacea* (Fig. 1) based on a personal communication with Drouet (Antonius 1985a). At that time Drouet was a recognized expert in cyanobacterial taxonomy, using morphological characteristics as the defining criteria. Antonius described the BBD cyanobacterial pathogen as an unbranched filamentous type with "differing end cells, one tapering, the other rounded" (Antonius 1985a). Since this early work this cyanobacterium was redescribed as *Phormidium corallyticum* in three back-to-back papers (Rützler & Santavy 1983, Rützler *et al*. 1983, Taylor 1983) on which Antonius was co-author of one (Rützler *et al*. 1983). In the 2000s the new application of molecular genetics (16S rRNA gene sequencing) to the study of BBD led to much controversy in the literature as to the identification of the dominant BBD cyanobacterium, with various genera proposed that included *Oscillatoria*, *Geitlerinema*, and *Leptolyngbya* (Cooney *et al*. 2002, Frias-Lopez *et al*. 2003, Myers *et al*. 2007) in the Caribbean and *Oscillatoria* and *Pseudoscillatoria* in the Indo-Pacific and Red Sea (Sussman *et al*. 2006, Rasoulouniriana *et al*. 2009, Sato *et al*. 2009). Only very recently has this cyanobacterium been formally described under the International Code of Botanical Nomenclature as the new cyanobacterial genus and species *Roseofilum reptotaenium* (Casamata *et al*. 2012), which translates as "creeping band of red thread". The formal characterization includes description of an identifying morphology, that of one tapering and one rounded end cell, as noted and reported by Antonius (1985a).

As seen in Antonius' first report (Fig. 1), BBD was attributed to the "coral killing" cyanobacterium he described. This contention also became a subject of investigation and controversy, and for many years the BBD pathogen was proposed to be, in addition, to the cyanobacterium, a sulfide-oxidizing bacterium (Ducklow & Mitchell 1979), various heterotrophic bacteria (Cooney *et al*. 2002, Frias-Lopez *et al*. 2004), and a marine fungus (Ramos-Flores 1983). BBD was also proposed as a polymicrobial disease, i.e. caused by a community of bacteria with no primary pathogen (Richardson *et al*. 1997). A recent meta-analysis of clone libraries constructed from 87 BBD samples collected between 2002 and 2010 (Miller & Richardson 2011) revealed that by far the most common 16S rRNA gene sequence was for *R. reptotaenium*, detected in 78% of the clone libraries examined. The three next most abundant sequences, each detected in 13% of clone libraries, were three heterotrophic bacteria, each of which was detected previously in BBD samples (Miller & Richardson 2011). These were an alphaproteobacterium, *Roseovarius crassostreae*, known to be the pathogen of Juvenile Oyster Disease, an uncultured alphaproteobacterium associated with Juvenile Oyster Disease, and a *Cytophaga* sp. (Cooney *et al*. 2002, Frias-Lopez *et al*. 2002, Sekar *et al*. 2008). Recently, a unialgal laboratory culture of *R. reptotaenium* was shown to infect coral in the laboratory and produce BBD (Casamata *et al.* 2012). While the culture has bacterial contaminants, none of these were found in the BBD-derived clone libraries (Miller & Richardson 2011). Since the culture cannot live without associated bacteria (a pure culture could not maintain viability) Koch's Postulates cannot be fulfilled and thus the "proof" of *R. reptotaenium* as the BBD primary pathogen is currently not feasible. For a discussion of this issue in general see Fredricks & Relman (1996). In 1981 (Antonius 1981a) Antonius modified his identification of a single (cyanobacterial) BBD pathogen to a cyanobacterial pathogen "in association with bacteria". It appears that he has been right all along.

White Band Disease: White band disease (WBD), first described by Antonius in 1981 (Antonius 1981a, 1981b), was described as "a band of brilliant white skeleton always visible in the wake of a moving front of tissue

destruction" (Antonius 1981b). As is the case with BBD, WBD has been, since Antonius' first report, the subject of ongoing and controversial research and discussion. In particular, a bacterial pathogen of WBD has been elusive and confusing to the point that it has been suggested that various disease signs given names such white plague and white death, in addition to white band, should be referred to as "white syndrome" (reviewed in Pantos *et al*. 2003). This same point was made by Antonius in his first report of the disease (1981a) in which he states that WBD is "variously called White Death, Plague, etc." Antonius experimentally demonstrated that WBD could not be transmitted between coral hosts, either by direct contact or by injection with fresh disease material (Antonius 1981b), and could not be cured using antibiotics (Antonius 1985b). In contrast, in his 1985 study he successfully demonstrated that BBD could be easily transmitted and was curable with antibiotics. Antonius' overall conclusions about WBD etiology, that it is a "physiological syndrome that needs only a trigger" (Antonius 1981b), remains the most viable working hypothesis today.

Shut Down Reaction: In 1977 Antonius first described the coral disease he termed shut down reaction (SDR). He noted that this disease only occurred in corals maintained in aquaria (Antonius 1977), an observation that holds true today (Sutherland *et al*. 2004). The name is based on the extremely rapid rate of tissue destruction, 10 cm per hour along a moving front, that can be transmitted using infected tissue or contact with an infected colony, but only when the recipient colony is under stress (Antonius 1981b). No definitive work has been carried out to define the etiology of SDR.

Skeleton Eroding Band: The final coral disease that Antonius was the first to describe is skeleton eroding band (SEB) (Antonius & Lipscomb 2000). This disease is caused by a protozoan, *Halofolliculina corallasia*, which appears as a dark band that moves across corals while lysing tissue. The fact that Antonius recognized this as separate from BBD attests to his exceptional observational skills, for it is only by close visual examination that one can tell the difference between the two. Antonius reported SEB on corals in the Indo-Pacific. A similar protozoan infection on Caribbean corals has only relatively recently been noted (Croquer *et al*. 2006)

COMBINING FIELD OBSERVATIONS AND EXPERIMENTAL WORK

While it is well-known that Antonius was the first to report and name several coral diseases, less well know is the fact that he was the first to conduct the combination of field and laboratory experimental work required to understand coral disease etiology. One example will be provided.

In Antonius' ongoing work to determine the pathogens associated with BBD and WBD, summarized above, he made the observations that: i) BBD could be easily transmitted between corals, whereas WBD could not; ii) inoculation of a healthy coral with BBD would produce an infection on a healthy coral whereas inoculation with WBD would not; iii) exposure of infected coral colonies *in situ* to antibiotics would cure a BBD infected coral but not one infected with WBD; and iv) new BBD infections appeared to occur only on corals infected with WBD, specifically at the site of WBD infection.

The last observation (reported in Antonius 1981a, 1981b, and 1985b) was the basis for a remarkable seven year field/laboratory experiment carried out to determine how BBD infections initiate on the reef (Antonius 1985a). The time required to complete the experiment was due to the fact that, since WBD could not be artificially transmitted, experiments relied on finding naturally occurring WBD-infected colonies *in situ* on the reef for experimental use. Furthermore, indicative of Antonius' detailed and exhaustive approach to research, the experiment included corals on reefs of both the Caribbean and the Indo-Pacific. In each region four species of WBD and BBD susceptible corals were studied, a total of eight coral species investigated.

Finally, all experiments were replicated 12 times at three different temperatures (26, 28 and 30˚C, selected based on his observations of temperature thresholds for BBD - see below).

Antonius' experimental design (Antonius 1985a) consisted of field experiments in which a BBD-infected coral was placed near a WBD-infected coral, and laboratory experiments using aquaria in which he manipulated WBD and BBD-infected corals. BBD-infected corals were easy to obtain since he could artificially and easily infect healthy corals, but WBD-infected corals for laboratory experiments had to be collected on the reef. Field experiments consisted of monitoring WBD-infected corals for which BBD-infected corals were placed 0.5-1 m upstream. Control WBD-infected colonies were in areas of the reef that were free of BBD, or had BBD-infected corals placed a minimum of 10 m downstream. Laboratory experiments consisted of placing BBD-infected corals into aquaria with WBD-infected corals, controls being aquaria with WBD-infected corals together with healthy or injured corals (no BBD). All experiments consisted of observing whether or not the WBD-infected corals developed BBD.

Antonius' results were, in his word, "unequivocal" proof of the positive relationship between BBD and WBD. Analysis of the results of the 12 experiments showed that at 30˚C the appearance of a BBD infection on a WBD lesion ranged, for Caribbean corals, from 58-83% on the reef and from 69-92% in aquaria. For Indo-Pacific corals the values were 62-86% on the reef and 71-93% in aquaria. At 28˚C Caribbean corals exhibited 54-78% BBD infection on the reef and 67-85% in aquaria, and Indo-Pacific corals 42-80% and 50-92% respectively. At 26˚C (below the field temperature threshold of BBD) there were zero cases of BBD infection on the reef, and a maximum of 8% infection in aquaria, where stress may have been a factor. Half of the species tested (both Caribbean and Indo-Pacific corals) did not become infected in aquaria with BBD at 26˚C. None of the controls on the reef or in aquaria became infected with BBD.

As remarkable as these experiments is the fact that in the 27 years since this paper was published none of the many investigators in coral disease research has further investigated these fascinating and compelling findings.

CORAL DISEASE INCIDENCE AND ENVIRONMENTAL FACTORS

In the last few decades, as coral disease incidence and prevalence have increased on reefs world-wide, there has been a major focus on the relationship between coral disease and environmental factors, in particular those associated with human activity. Virtually all of the studies that found a positive relationship were preceded by similar work by Antonius.

Antonius was the first to report a temperature threshold for coral disease, specifically BBD and WBD. His first mention of this (Antonius 1981a) was the observation that both BBD and WBD were seasonal on reefs of high latitude (Bermuda and Florida), with diseased colonies present in the summer but not the winter and with the BBD season longer than that for WBD on the same reefs. Based on extensive field surveys on reefs in the Red Sea combined with temperature data, he demonstrated that BBD activity was strongest at 30˚C but did not occur at or below 26˚C (Antonius 1985b). In this same study he noted that WBD was not affected by temperature except for one period when it fell to an unusual low of 22˚C. At this time WBD frequency decreased, but was still present.

Antonius was also the first to report a correlation of a disease (BBD) with nutrients and sewage outflow. His first observation occurred when he was conducting BBD infection experiments to determine coral host species susceptibility and an aquarium water source became "contaminated" (Antonius 1981a). He observed that two normally non-susceptible coral species were infected and killed by BBD and concluded that the newly observed susceptibility was due to a 400x increase in nitrate and 10x increase in phosphate in the water (which

he called "artificial hypernutrition"). He also observed that WBD progression was not affected by exposure to this water. His correlation of the disease with sewage was noted during extensive surveys of disease at 33 sites along the Red Sea (Antonius 1988a). In this study he documented the highest BBD frequency near Jeddah, which he attributed to "sea water pollution, especially eutrophication". Since this work other investigators have shown a positive correlation between BBD and elevated nutrients (Bruckner & Bruckner 1997, Kuta & Richardson 2002, Kaczmarsky *et al*. 2005).

CORAL DISEASES AND CORAL REEF ECOLOGY

Antonius' body of work also illustrates his insight into the effect of coral diseases on coral biology and coral reef ecology. Three examples are summarized. The first, part of his ongoing study on BBD infection and transmission, involved experimentally testing the hypothesis that wounds on corals caused by lysing of tissue by mesenterial filaments of aggressive corals of other species could be infected by BBD (Antonius 1985a). He did document successful infection, which he recorded as lower than BBD infections on WBD lesions (40-50% on the reef, 30-40% in aquaria) – a difference he attributed to the fact that wounds due to aggression were stationary and temporary, whereas WBD induced lesions on infected colonies were present over days to weeks due to the steadily advancing disease front. Of note is his interpretation of his observation in the context of coral biology. He concluded that BBD susceptibility was an "unexpected bonus for the aggressor" in that BBD infection would likely completely remove the encroaching coral from the reef. This outcome confers a much greater benefit when compared to the limited ability of the aggressor to inflict tissue damage on a small area of an immediately adjacent competing coral colony via extrusion of mesenterial filaments (Antonius 1985a).

The second example also arose from BBD infection studies. In this case Antonius noted that BBD often began at the site of a clump of green algae growing near or onto a coral colony. His interpretation was that the pathogenic BBD cyanobacterium resided in the green algal turf, which served as a reservoir, and that as the turf moved against the coral via wave action it abraded the coral surface, resulting in an opening into which the BBD cyanobacterium could invade (Antonius 1985a). This was the first consideration of a reservoir for a coral pathogen on the reef.

Finally, Antonius showed that susceptibility to coral disease could be used as a coral taxonomic tool. In Antonius (1988b) he noted that there was confusion as to whether *Platygyra lamellina* and *P. daedalea* were one or two species. The controversy was resolved when he determined that *P. daedalea* could not be infected with BBD, whereas *P. lamellina* was susceptible. In addition to these three examples there are many more cases of extrapolation of his work to coral reef ecology in his papers.

CORAL DISEASES AND THE AMLC

Since Antonius' first report of a coral disease at the AMLC meeting in 1973 there has been a steady stream of papers presented at AMLC scientific meetings that focused on, or were related to, coral diseases (see proceedings on the AMLC website, http://www.amlc-carib.org/). And, beginning in 1997 and at every AMLC scientific meeting since, there were sessions dedicated to studies on coral diseases. As research in this field continues AMLC marine laboratories and individual AMLC members continue to contribute to the advancement of knowledge in this critical area of research.

ACKNOWLEDGMENTS

Arnfried Antonius passed away in 2010. The coral disease session at the 35th Scientific Meeting of the AMLC, held at the Universidad de Costa Rica, San Pedro, Costa Rica, was dedicated to his memory as is this paper, presented at the meeting.

RESUMEN

El estudio de las enfermedades de los corales, los patógenos de los corales y los efectos de estas enfermedades sobre los arrecifes tropicales y subtropicales son actualmente áreas importantes de investigación. El interés en este tema ha crecido continuamente desde el primer informe sobre una enfermedad de coral que se publicó en 1973. El autor de este informe fue Arnfried Antonius y la publicación fue un resumen en el Libro de Programa y Resúmenes de la Décima Reunión de la Asociación de Laboratorios Marinos Isleños del Caribe (conocida ahora como la Asociación de Laboratorios Marinos del Caribe). Desde esta comunicación pionera, Antonius siguió trabajando sobre las enfermedades de los corales en arrecifes alrededor del mundo, a menudo documentando la primera observación de una nueva enfermedad en un nuevo lugar. Cada enfermedad de coral descrita por primera vez por Antonius es actualmente el objeto de investigaciones actuales en lo que se refiere a patógenos, ecología de las enfermedades y los efectos sobre los arrecifes de coral. Muchas de las observaciones en sus trabajos tempranos siguen siendo relevantes en la investigación actual. Este trabajo examinará ciertos aspectos de los estudios tempranos de Antonius sobre las enfermedades de los corales, poniendo de relieve sus contribuciones novedosas que incluyen los primeros experimentos *in situ* que tenían como objetivo el estudio de la etiología de las enfermedades de los corales y los primeros análisis cuantitativos de la incidencia de las enfermedades de corales y de los patrones de distribución en función de los factores ambientales. Las contribuciones iniciales de Antonius se discuten en términos de las controversias actuales sobre el tema.

Palabras clave: Enfermedades de corales, enfermedad de banda negra, enfermedad de banda blanca, Arnfried Antonius

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Insights into Migration and Development of Coral Black Band Disease Based on Fine Structure Analysis

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Received 15-VII-2011. Corrected 12-XII-2011. Accepted 20-XII-2011.

Abstract: In many diverse ecosystems, ranging from natural surfaces in aquatic ecosystems to the mammalian gut and medical implants, bacterial populations and communities exist as biofilms. While the process of biofilm development has been well-studied for those produced by unicellular bacteria such *Pseudomonas aeruginosa*, little is known about biofilm development associated with filamentous microorganisms. Black band disease (BBD) of corals is characterized as a polymicrobial biofilm (mat) community, visually-dominated by filamentous cyanobacteria. The mat migrates across a living coral host, completely lysing coral tissue and leaving behind exposed coral skeleton. It is the only known cyanobacterial biofilm that migrates across a substratum, thus eliciting questions about the mechanisms and unique characteristics of this system. Fragments of the coral *Montastraea annularis*, five artificially infected with BBD and two collected from a naturally BBD-infected colony, were used to address these questions by detailed examination using scanning and transmission electron microscopy (SEM and TEM). In areas close to the interface of coral tissue and the mature disease band two types of clusters of cyanobacteria were observed, one with random orientation and one with parallel orientation of filaments. The latter exhibited active secretion of extracellular polysaccharide (EPS) while the randomly oriented clusters did not. Within the well developed band cyanobacterial filaments were observed to be embedded in EPS and were present as layers of filaments in parallel orientation. These observations suggest that BBD cyanobacteria orient themselves and produce EPS in a sequential process during migration to form the complex BBD matrix. Rev. Biol. Trop. 60 (Suppl. 1): 21-27. Epub 2012 March 01.

Key words: black band disease, biofilm, microbial mat, corals.

Black band disease (BBD) of corals exists as a thick biofilm, or thin mat, that migrates across coral colonies completely degrading coral tissues (Rützler *et al.* 1983). While the mechanisms that control band migration and development are not known, this horizontal migration of the intact biofilm/mat community, and it's migration across a living coral host, are unique (Richardson 1996). The pathogenicity of a polymicrobial mat dominated by filamentous cyanobacteria is also unique and is an important aspect of the disease.

Biofilms have been studied in depth for decades, and in natural ecoystems they can be found on virtually any surface in aquatic environments. Much interest has been focused on pathogenic biofilms, which are very common for animal (including human) hosts. Evidence suggests that biofilm-forming bacteria exist in a transient, planktonic form, which colonizes a surface to produce a biofilm (Wolcott and Ehrlich 2008). Bacteria growing in biofilms differ markedly from their free-living counterparts. One important difference is the excretion

of extracellular polysaccharides (EPS) by biofilm-associated microorganisms, which facilitates the adhesion of microorganisms within the biofilm (Rickard *et al.* 2003). Such EPS secretion, which is a conspicuous component of BBD, may play an important role in forming the complex polymicrobial structure associated with this disease. It is well-documented that, when associated in biofims, bacteria exhibit an increased resistance to fluctuating environmental conditions, including avoidance of antibiotics and antagonistic cells associated with host immune systems (Govan and Deretic 1996, Costerton *et al.* 1999, O'Toole *et al.* 2000). It has been estimated that 65%-80% of human diseases are caused by biofilms (Wolcott and Ehrlich 2008). The latter fact alone has made the initiation and development of biofilms important areas of research.

Perhaps the most well-studied of pathogenic biofilms are those associated with lung infections in individuals with cystic fibrosis, caused by the unicellular species *Pseudomonas aeruginosa* (O'Toole *et al.* 2000). Polymicrobial biofilm development has also been studied, for example focusing on biofilms associated with dental plaque, a system that may contain as many as 300 different species of microorganisms (Paster *et al.* 2001). Model systems have been used to directly examine the process of biofilm development in the laboratory. Such model systems have made use of both single and multiple species, including *Escherichia coli, P. fluorescens,* and *Vibrio cholerae*, among others (Pratt and Kolter 1998, Watnick *et al.* 1999). However, all of these model systems have focused on unicellular bacteria, and little is known about the development of biofilms associated with filamentous bacteria.

BBD is known to infect at least 64 scleractinian coral species worldwide, and is considered to be an important disease contributing to the loss of coral cover due to its often lethal, preferential infection of massive, reef-building corals (Rützler *et al.* 1983, Edmunds 1991, Kuta and Richardson 1996, Sutherland *et al.* 2004, Voss and Richardson 2006). It is caused by a polymicrobial biofilm, or very thin (<1mm) mat, which can range from a few millimeters to several centimeters in width (Rützler and Santavy 1983, Carlton and Richardson 1995). The biomass of the mat is dominated by gliding, filamentous cyanobacteria of the newly described genus *Roseofilum* (Casamata *et al.*, 2012) and may also contain members of the cyanobacterial genera *Oscillatoria, Geitlerinema, Leptolyngbya* and *Phormidium* (Cooney *et al.* 2002, Frias-Lopez *et al.* 2003, 2004, Sussman *et al.* 2006, Barneah *et al.* 2007, Myers *et al.* 2007). Together, BBD cyanobacteria provide the structural framework for the mat and have recently been shown to be directly involved in BBD pathobiology. BBD-associated coral mortality occurs as the contiguous band migrates across the coral colony, at a rate of three millimeters to one centimeter a day, lysing coral tissue and leaving behind exposed coral skeleton. This coral tissue lysis is aided by a cyanotoxin, microcystin, produced by BBD cyanobacteria (Richardson *et al.*, 2007, 2009; Miller and Richardson 2011). Here we use SEM and TEM to examine the fine structure of the BBD biofilm/mat to elucidate mechanisms that may contribute to the development and progression of the BBD mat across a coral colony.

MATERIALS AND METHODS

Sample collection and preparation for microscopy are described in detail in Miller *et al.* (2011). Briefly, seven BBD-infected fragments of the coral *Montastraea annularis* species complex were used for this study. Five fragments, from aquarium maintained colonies or from apparently healthy colonies on Horseshoe Reef at Lee Stocking Island, Bahamas, were artificially infected with freshly collected BBD. The resultant band was allowed to migrate for a period of 2-3 days after which the fragment was immersed in a fixture composed of 2% glutaraldehyde in sodium cacodylate buffered seawater. The remaining two fragments, collected from a naturally BBD-infected coral colony at Algae Reef in Key Largo, Florida, which appeared to have been infected over several seasons due to the significant tissue

loss observed on the colony, were fixed immediately after collection. Natural and artificial infections have previously been shown to be indistinguishable both macroscopically (Richardson *et al.* 2009) and at the fine structural level (Miller *et al.* 2011). In the laboratory after a buffer wash all fragments were post-fixed in 1% osmium tetroxide, rinsed with buffer, dehydrated in a graded series of ethanols, and processed for SEM (critical point dried) and/or TEM (embedded in Spurr© resin) analysis (see Miller *et al.*, 2011).

RESULTS

Examination of the BBD biofilm/mat on infected fragments using SEM revealed that, despite the homogenous appearance of the disease band macroscopically, the mat exhibited spatial heterogeneity. In both artificially and naturally infected coral fragments cyanobacterial filaments were found millimeters ahead of the mature band (Figure 1). These filaments were present as loose aggregations that formed clusters between and underneath coral tissue layers, and could be seen separating the coral tissue from the coral skeleton. Some of the clusters (Figure 1A) consisted of cyanobacteria that appeared to be randomly oriented relative to each other, with few filaments in alignment, and had no associated EPS. Other clusters were observed to exhibit active EPS secretion that was associated with individual filaments that were oriented primarily in parallel, with groups of filaments generally aligned together (Figure 1B). In some cases, such filaments appeared to be enveloped in EPS, but there was no distinct layer of EPS matrix holding the filaments together.

The differentiation between EPS and non-EPS producing BBD cyanobacteria can be clearly seen in TEM micrographs (Figure 2). Some clusters of cyanobacteria penetrating through coral tissue had no ring of EPS surrounding each cyanobacterial filament (shown in cross-section in Figure 2A), while other cyanobacteria, also present in clusters and penetrating through coral tissue, had no apparent ring of EPS (Figure 2B).

Examination of the BBD in the center of the mat (between the leading edge and the exposed coral skeleton behind the band) revealed a much more organized band structure (Figure 3). Thick layers of cyanobacteria were observed to be oriented in parallel and were in much closer physical association than those in the coral tissue in front of the band, providing a distinct structural framework (Figure 3A). It

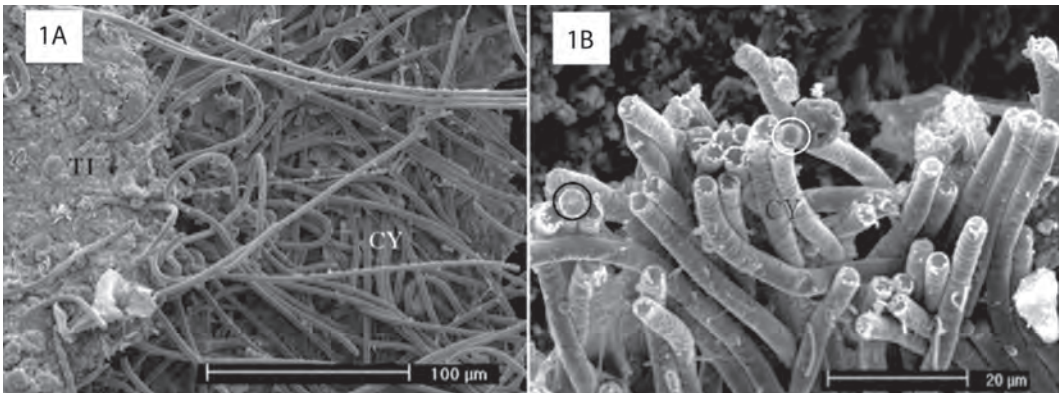

Fig. 1. SEM images of coral tissue in front of the leading edge of the BBD mat. A. Cluster of cyanobacteria exhibiting random orientation, and no apparent EPS, underneath the coral tissue. B. Close up of EPS secretion associated with individual cyanobacterial filaments within a cluster of filaments with parallel orientation. White circle indicates ringed EPS structure around terminal cell; black circle indicates a disk of EPS covering the terminal cell. CY: cyanobacteria, TI: coral tissue.

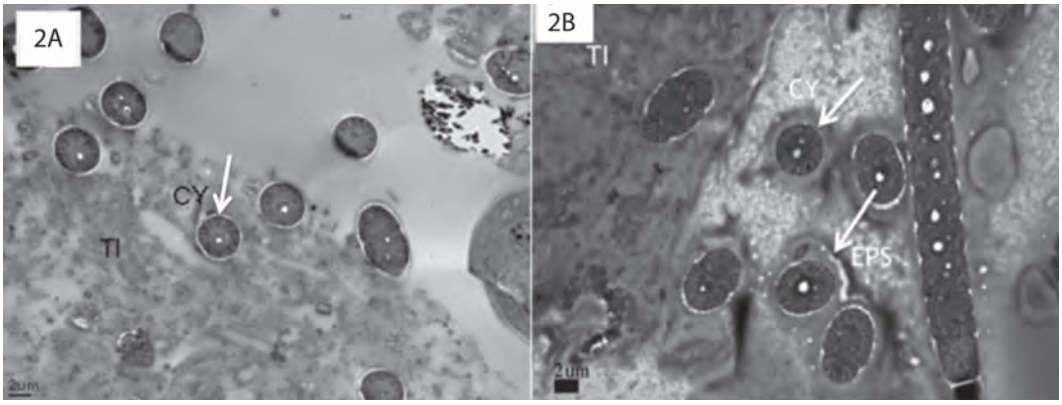

Fig. 2. TEM images of EPS secretion from cyanobacteria at the interface with coral tissue and the disease band. A. Cross-section of cyanobacteria within coral tissue exhibiting no ring of EPS surrounding the filament. B. Cross-section of cyanobacteria within coral tissue surrounded by a ring of EPS. CY: cyanobacteria, TI: coral tissue, EPS: exopolysaccharides.

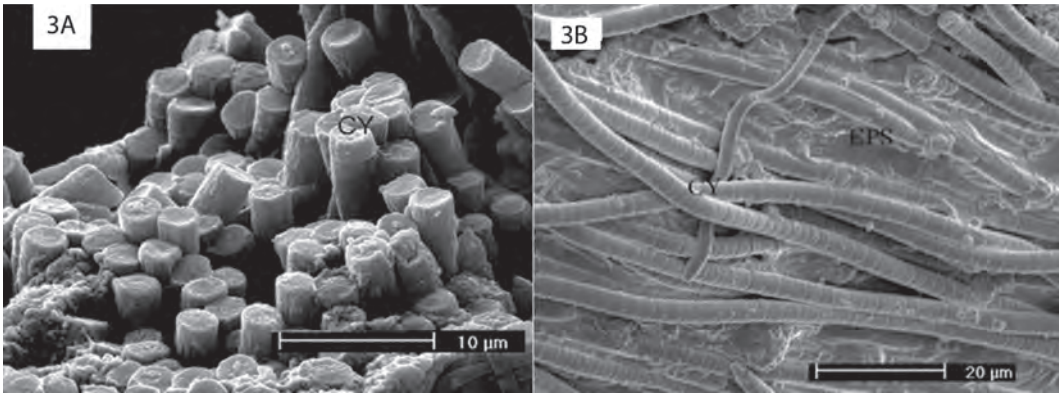

Fig. 3. Development of EPS matrix in the BBD biofilm/mat. A. Layering of cyanobacterial filaments in parallel orientation on top of coral tissue at the interface between coral tissue and the mature BBD mat. B. Parallel BBD filaments embedded in EPS matrix. EPS: exopolysaccharides, CY: cyanobacteria.

was also observed that parallel filaments were embedded in a distinct EPS matrix (Figure 3B).

DISCUSSION

In this study, SEM and TEM examination of BBD-infected coral fragments revealed that clusters of cyanobacteria were present millimeters ahead of the pathogenic disease band. These clusters could be seen penetrating through (Figure 2), and underneath (Figure 1A) coral tissue. Previous studies have shown that BBD cyanobacteria are able to penetrate into coral tissue (Rützler *et al.* 1983, Barneah *et al.* 2007, Sato *et al.* 2009, Miller *et al.* 2011), and into coral skeleton (Miller *et al.* 2011).

The BBD cyanobacteria ahead of the mature band in the current study were aggregated in clusters that exhibited both primarily random (Figure 1A), or primarily parallel (Figure 1B), orientation. In these areas of the infected coral the BBD cyanobacteria either

exhibited no EPS secretion (Figure 1A, 2A), or were associated with EPS on the surface of filaments (Figure 1B, 2B). Some of these clusters were fully embedded in EPS (Figure 3B).

There appeared to be a transition between the clusters of cyanobacteria in the tissue in front of the band, and the fully formed band itself. Specifically, randomly oriented, non-EPS forming clusters appeared to transition into parallel-oriented filaments that produced EPS, which then appeared to transition into more closely packed, layered clusters. In some of the transitional clusters where cyanobacteria exhibited parallel orientation there was significant layering, leading to aggregations that were several filaments thick (Figure 3A), which could provide a distinct structural framework for the growth of associated microorganisms in the BBD mat. These aggregations can be considered to be biofilms, which may further aggregate to form the mature mat.

Previous studies have suggested that BBD cyanobacteria, which are the dominant component of the BBD community, provide the structural framework of the BBD mat (Rützler and Santavy 1983). This hypothesis is supported by the results presented in the current study. Furthermore, the observed layering of filaments, and differentiation in EPS production, are consistent with the results of other studies focused on formation of biofilms. In well-studied, model biofilm systems composed of unicellular bacteria, the development of the biofilm occurs after free-living, planktonic bacteria attach to a surface and begin to secrete EPS. This EPS then embeds and surrounds the biofilm-forming bacteria in an adhesive matrix (Gacesa 1998, Kolenbrander *et al.* 1999, Flemming *et al.* 2000, O'Toole *et al.* 2000, Handley *et al.* 2001, Rickard *et al.* 2003). Mutations in the genes controlling EPS secretion lead to both altered attachment behavior and biofilm formation, further strengthening this relationship between EPS and biofilm formation (Makin and Beveridge 1996, Genevaux *et al.* 1999).

In contrast to the temporal basis of unicellular biofilm formation, it appears that filamentous BBD cyanobacteria form the biofilm/mat in a spatial, longitudinal fashion. As BBD cyanobacteria migrate into tissue ahead of the band, they appear to stop producing EPS. The clusters of filaments behind these leading BBD cyanobacteria produce small amounts of EPS that accumulate and bind the filamentous matrix into new biofilm that thickens to become a mat. This secretion of EPS embeds not only the surrounding cyanobacteria but also the many other BBD-associated bacteria into a distinct layer that constitutes the mature biofilm/mat.

Our results reveal new insights into the development of the BBD biofilm/mat and its association with coral tissue in apparently healthy areas ahead of the band. One of the most intriguing questions about BBD biofilm/mat remains unanswered – what is the cue that causes the mat to migrate across and through coral tissue?

RESUMEN

En muchos ecosistemas diversos, que van desde ecosistemas acuáticos hasta los intestinos de mamíferos e implantes médicos, las poblaciones y comunidades de bacterias existen como biopelículas (biofilms). El proceso de desarrollo de las biopelículas ha sido bien estudiado para aquellos producidos por bacterias unicelulares como *Pseudomonas aeruginosa,* pero se conoce muy poco acerca del desarrollo de biopelículas asociadas con microorganismos filamentosos. La Enfermedad de Banda Negra (EBN) de coral es caracterizada como una comunidad polimicrobiana que forma una biopelícula (lecho), visualmente-dominada por una cianobacteria filamentosa. El lecho migra a través de un huésped de coral vivo, rompiendo completamente el tejido del coral y dejando atrás el esqueleto de coral expuesto. Es la única biopelícula cianobacteriana que migra a través de un sustrato, por lo tanto esto genera preguntas acerca de los mecanismos y las características únicas de este sistema. Fragmentos del coral *Montastraea annularis,* cinco artificialmente infectados con EBN y dos colectados de una colonia EBN-infectada, fueron usados para abordar estas preguntas mediante exámenes detallados con microscopía electrónica de barrido y de transmisión (MEB y MET). En zonas cercanas a la interfaz de tejido del coral y la banda de la enfermedad madura, se han observado dos tipos de grupos de cianobacterias, uno con orientación aleatoria y otro con una orientación paralela de los filamentos. Este último exhibe la secreción activa de polisacáridos extra-celulares (PEC), mientras que los grupos orientados al azar no lo hicieron. Dentro de la banda de filamentos cianobacterianas bien desarrollados se observó que estaban integradas en

PEC y que se presentaban como capas de cianobacteria con orientación paralela. Estas observaciones sugieren que la cianobacteria de EBN se orienta a sí misma y produce PEC en un proceso secuencial durante la migración para formar la matriz complejo de EBN.

Palabras clave: enfermedad banda negra, biopelícula, lecho microbiano, corales

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Massive hard coral loss after a severe bleaching event in 2010 at Los Roques, Venezuela

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Received 15-VII-2011. Corrected 16-XII-2011. Accepted 20-XII-2011.

Abstract: Thermal anomalies have become more severe, frequent and well-documented across the Caribbean for the past 30 years. This increase in temperature has caused coral bleaching resulting in reef decline. At Los Roques National Park, Venezuela, temperature has been monitored at four reef sites. In mid-September 2010, seawater temperature reached 30.85°C at 5 m depth in Los Roques, an archipelago only slightly affected by previous bleaching events. For example, bleaching in Los Roques in 2005 was mild compared to the rest of the Caribbean and to the results in this study. In 2010, seawater temperatures remained above 29.0°C from mid-August until the first week of November, resulting in +16 Degree Heating Weeks by that time. Our annual survey of four reef sites indicated that 72% of 563 scleractinian colonies were partial or totally bleached (white) or pale (discolored) in October 2010. In February 2011, there were still 46% of coral colonies affected; but most of them were pale and only 2% were bleached. By February, coral cover had declined 4 to 30% per transect, with a mean of 14.3%. Thus, mean coral cover dropped significantly from 45 to 31% cover (a 34% reduction). In addition to bleaching, corals showed a high prevalence (up to 16%) of black band disease in October 2010 and of white plague (11%) in February 2011. As a consequence, coral mortality is expected to be larger than reported here. Reef surveys since 2002 and personal observations for more than 20 years indicated that this bleaching event and its consequences in Los Roques have no precedent. Our results suggest that reef sites with no previous record of significant deterioration are more likely to become affected by thermal anomalies. However, this archipelago is relatively unaffected by local anthropogenic disturbance and has a high coral recruitment, which may contribute to its recovery. Rev. Biol. Trop. 60 (Suppl. 1): 29-37. Epub 2012 March 01.

Key words: coral bleaching, mortality, loss of coral cover, Caribbean, coral reefs.

Coral reefs are tropical ecosystems with high productivity and therefore they provide a myriad of ecological goods and services to human societies, particularly in developing countries (Moberg & Folke 1999). Coral reefs world-wide have shown signs of deterioration, mainly reported by the loss of coral cover. Hurricanes, bleaching, coral diseases, thermal anomalies and overfishing are among the factors that have been associated with coral reef deterioration (e.g. Bellwood *et al.* 2004, Rogers 2009). In the Caribbean this phenomenon has been particularly evident, as extensive reductions of coral cover and the concomitant loss of architectural complexity have occurred (Gardner *et al*. 2003, Weil *et al.* 2006, Alvarez-Filip *et al.* 2009).

Mass bleaching events (MBE, events that reduce the density of *Symbiodinium* in its hosts over a large geographic area) have increased

in frequency and intensity (e.g. McWilliams *et al.* 2005); and it appears that they act synergistically with other detrimental factors to produce negative consequences for coral reef organisms, populations and communities (reviewed in Brown 1997). Thus, MBE have been reported across the Caribbean as a factor that reduces coral cover (e.g. Cortés 2003, Gardner *et al.* 2003). However, the effects and consequences of a particular bleaching event may vary between localities depending on oceanographic processes which is partly determined by reef morphology and topography; population size structure and species composition of coral communities (Brandt 2009). In 2005, prolonged thermal anomalies and its associated MBE occurred on a regional scale (e.g. Donner *et al*. 2007, Wilkinson & Souter 2008, Brandt & McManus 2009), but the largest coral cover losses related to this MBE were detected in the eastern Caribbean, where also epizootic events came after a prolonged bleaching period (Miller *et al.* 2006, Muller *et al.* 2008). In contrast, this bleaching event had mild effects in the southern Caribbean (Croquer & Weil 2009); particularly, in Venezuela (Rodríguez *et al.* 2010).

In 2010, another mass bleaching event was recorded worldwide (Normile 2010), although apparently not as severe as in 2005 for the Caribbean. However, coral reefs at Los Roques National Park (LRNP) were severely affected in 2010. Los Roques is a marine protected area characterized by healthy coral reef ecosystems compared to other sites in the Caribbean (Villamizar *et al.* 2003, Croquer *et al.* 2010; but see Croquer *et al.* 2005). The relatively good conditions of coral reefs ecosystems at LRNP (e.g. Schweizer *et al.* 2005) might be explained by low anthropogenic impacts, absence of runoff; and perhaps by the combination of low hurricane activity, low frequency of bleaching events, and thermal anomalies which seldom exceed few weeks over this geographic area. In this paper we provide the first evidence of a mass bleaching event producing significant impacts on coral reefs at Los Roques National Park.

MATERIAL AND METHODS

Study site: LRNP is located about 150 km north of the Venezuelan coast. This marine protected area encompasses 2211.4 km^2 of tropical marine ecosystems including mangroves, seagrass beds, soft-bottom communities, sandy and rocky shores and coral reefs. Reef development in Los Roques is extensive showing high diversity and live cover of scleractinian corals compared with other reef areas in Venezuela (Ramírez-Villarroel 2001) and other areas of the Caribbean (Rodríguez-Ramírez *et al.* 2008). Coral reefs at LRNP also differ within the region because of the presence of recovering populations of the threatened species *Acropora palmata* (Zubillaga *et al*. 2005) and *A. cervicornis*.

Seawater Temperatures: seawater temperatures were monitored with four HOBO data loggers (Pro-V2), at the four reef sites of the archipelago every 12 minutes. The loggers were fixed to the bottom near the coral, at an average depth of 5 m and the data shown here were from the 3rd of March 2010 to the 3rd of February 2011. The Degree Heating Weeks index (DHW) was calculated according to Liu *et al.* (2006). The estimation of monthly mean temperatures was done with a time series from May 2003 till July 2011 obtained from the MODIS-SCAR sensor (data obtained from http://cariaco.ws/).

Surveys of coral bleaching: In order to assess the magnitude of the 2010 MBE and its effects on coral reefs in LRNP, the proportion of bleached or paled colonies as well as the live coral cover was determined along ten, 10-m long, permanent transects located at each reef site, covering a depth interval of 5 to 15 m. This was carried out in October 2010, about one month after the onset of bleaching, and then again five months after the initial survey, in February 2011. During the initial survey, four reef sites (i.e. reefs located in the slope of different cays) were sampled: two located at the south-western edge of LRNP (Dos

Mosquises and Cayo de Agua) and two located in the north-east (Madrizquí and Rabusquí). The maximum distance among reef sites was 36 km, and their location and other characteristics have been described (Zubillaga *et al.* 2008, Croquer *et al.* 2010). Coral cover was estimated from chain transects (CARICOMP 2001), and the condition of coral colonies intercepted were also recorded (coloration, diseased, surface of partial mortality). In February of 2011, two sites were resurveyed (Cayo de Agua and Dos Mosquises Sur) to ascertain the effect of the bleaching event, six months after its onset. Therefore, comparisons between October 2010 and February 2011 were made only for these two sites, whereas results that pertain only to October 2010 were obtained from the observations at the four sites.

Statistical analysis: Null hypothesis of no temporal changes in live coral cover was compared with a repeated measures analysis of variances after checking ANOVA assumptions (i.e., normality and homogeneity of variances). Also a regression analysis was done to test whether the loss of live coral cover in each transect by February 2011 was linearly correlated with the proportion of colonies bleached in October 2010.

RESULTS

Seawater Temperature and Degree Heating Weeks: During 2010, the maximum temperature of 30.85 °C at 5 m depth was reached between the 17 and 19 of September at all four reef sites. At that depth, the seawater temperatures remained above 29.0 °C from mid-August until the first week of November 2010 (Fig. 1A). By that time, Degree Heating Weeks (DWH) reached a maximum value of 16 °C-weeks in the superficial waters of the archipelago (Fig. 1B, from NOAA) and 13 °C-weeks in waters at 5 m (data from in situ loggers).

Percentage of bleached and diseased coral colonies: In October 2010, 72% of the 563 scleractinian colonies were bleached or pale. In February 2011, there were still 46% of coral colonies affected; but the recovery of zooxanthellae density was in place, as most of them were pale, and only 2% were bleached (Fig. 2).

The high prevalence of Black Band Disease (BBD) had no precedent since the beginning of our surveys in 2002. In fact, BBD has been only detected once before, in 2007, when it reached 1% in Dos Mosquises (DMS). In contrast, the prevalence of BBD was 6% in DMS and 16% in Cayo de Agua (AGU) in October 2010. By February 2011, we did not observe any sign of BBD in these localities.

In 2010, the prevalence of White Plague (WP, with no differentiation made between type I and II) was also high compared to previous years. Contrasting with the drop in prevalence of BBD, the prevalence of WP was higher in February 2011 (10 -11%) than in October 2010 (4 - 8%).

Analysis of the cover change: By October 2010, mean coral cover was 42.5% ± 3.97 (± standard error) in AGU and 47.4% ± 3.41 in DMS. By February 2011, it was 32.2% ± 4.60 and 29.1 ± 3.44, respectively. Thus, the coral cover dropped similarly between sites (repeated measures ANOVA Time*Reef $p > 0.05$; Fig. 3) and significantly from a mean of 44.9 to 30.6% (Time $p < 0.05$), with a concomitant loss of 17% in the number of colonies.

Although there could be other causes for the rapid loss of coral cover (including the onset of diseases), a great proportion is certainly attributable to the bleaching event of 2010. This assumption is based on the relatively short time period elapsed between the high seawater temperature anomaly and both surveys. Also, the proportion of bleached or pale colonies explained about 22% of the variability obtained in the net loss of coral cover in each transect ($r^2 = 0.2165$ for the regression shown in Fig. 4).

DISCUSSION

Considering the combined global land and ocean surface temperature, the year 2010 tied with 2005 as the warmest on record (NOAA 2010). Also, both are known as years were mass bleaching events (MBE) have occurred

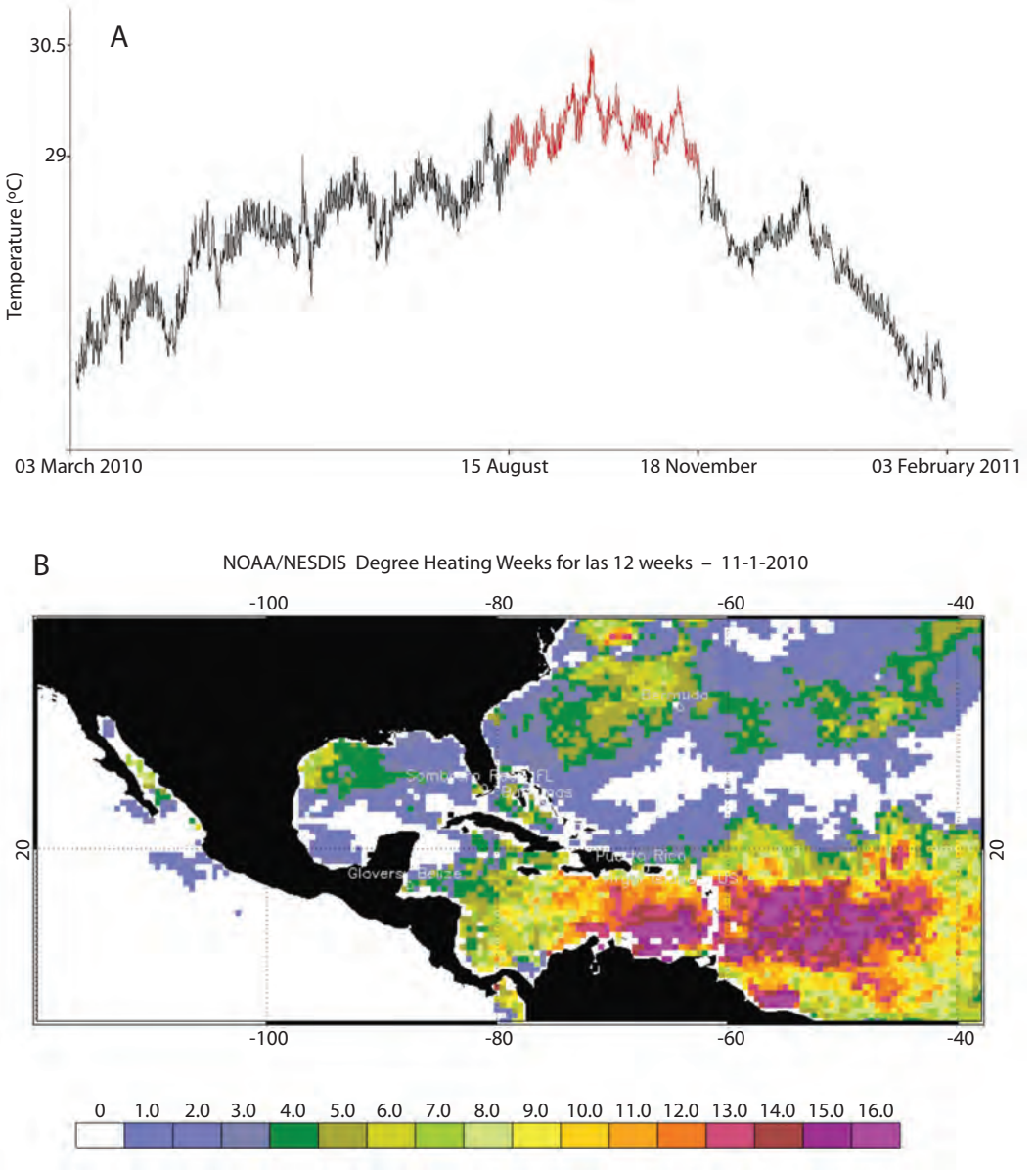

Fig. 1. A) Water temperature in °C at 5 m depth obtained *in situ* by a HOBO logger. Four reef sites were measured but only the data for Rabusquí is shown as it was very similar among sites. B) Degrees Heating Weeks (DHW) for the Caribbean region by November 1, 2010 (obtained from the NOAA, http://www.osdpd.noaa.gov/ml/ocean/cb/dhw_2010.html).

across the Caribbean. However, the intensity of the bleaching event and its consequences in Los Roques, Venezuela, were markedly different between these years. The bleaching event observed in October 2010 in Los Roques, and the consequences recorded up to February 2011, had no precedent since we started our surveys in 2002 and have no match with our observations for more than 20 years in Venezuelan reefs. Thus, these coral reefs fall within

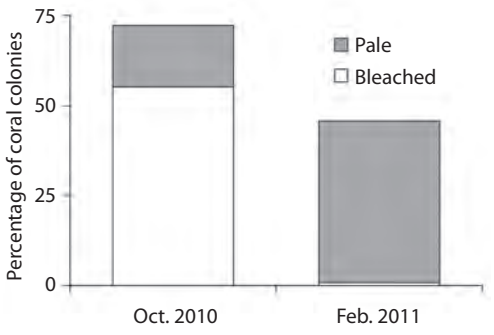

Fig. 2. Percentage of coral colonies that were bleached or pale in the sites surveyed in October 2010 (N= 563; four reef sites) and in February 2011 (N= 208; two reef sites) in Los Roques, Venezuela.

the expectations of a higher incidence and/or frequency of massive event of bleaching as temperatures rise above coral bleaching thresholds (Hoegh-Guldberg 1999).

Previous bleaching events observed in Venezuelan coral reefs have been mild compared with those that occurred during the same periods in other Caribbean localities (e.g. Lang *et al.* 1992, Eakin *et al.* 2010). In Los Roques, in particular, less than 4% of coral colonies were bleached in August and September 2005 (Rodríguez *et al.* 2010), and <10% across the archipelago at the peak of the event in November 2005 (Villamizar *et al.* 2008). In contrast, the 2005 MBE was severe across the Caribbean, particularly in the eastern reefs, where percentage of bleached corals reached up to 80% (Ballantine *et al.* 2008, Oxenford *et al.* 2008, Brandt & McManus 2009, Croquer & Weil 2009, Miller *et al.* 2009, Eakin *et al.* 2010). A series of climatic and oceanic factors contribute to the variability in the thermal stress across the region (e.g. hurricanes, irradiance, water flow), which in turn results in spatial and temporal variations of the bleaching intensity (e.g. Jokiel & Brown 2004).

The distribution of heat in the superficial water masses along the Venezuelan coast was completely different between 2005 and 2010 (Fig. 1A in Eakin *et al.* 2010 versus Fig. 1B in this study) and this can be considered a factor contributing to the differences in bleaching intensity observed between 2005 and

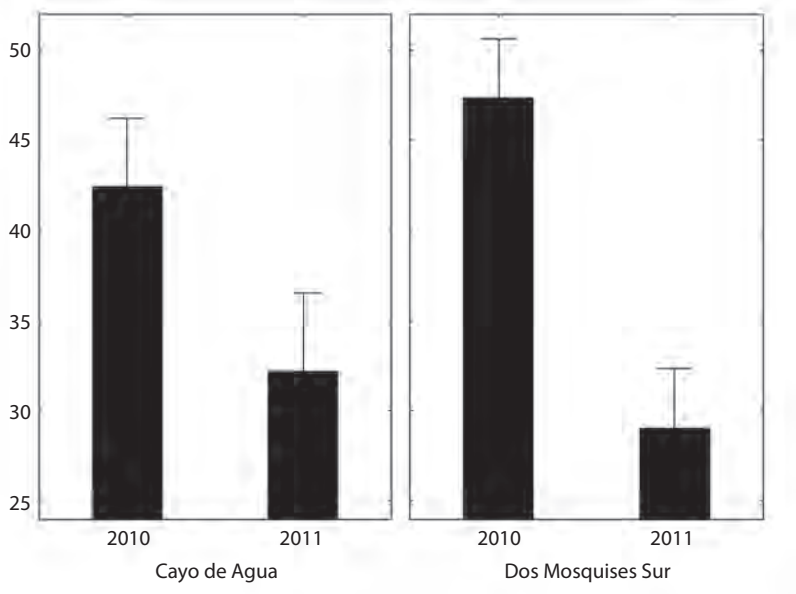

Fig. 3. Hard coral cover (%) measured in October 2010 and in February 2011 at two reef sites (Cayo de Agua and Dos Mosquises); Los Roques, Venezuela.

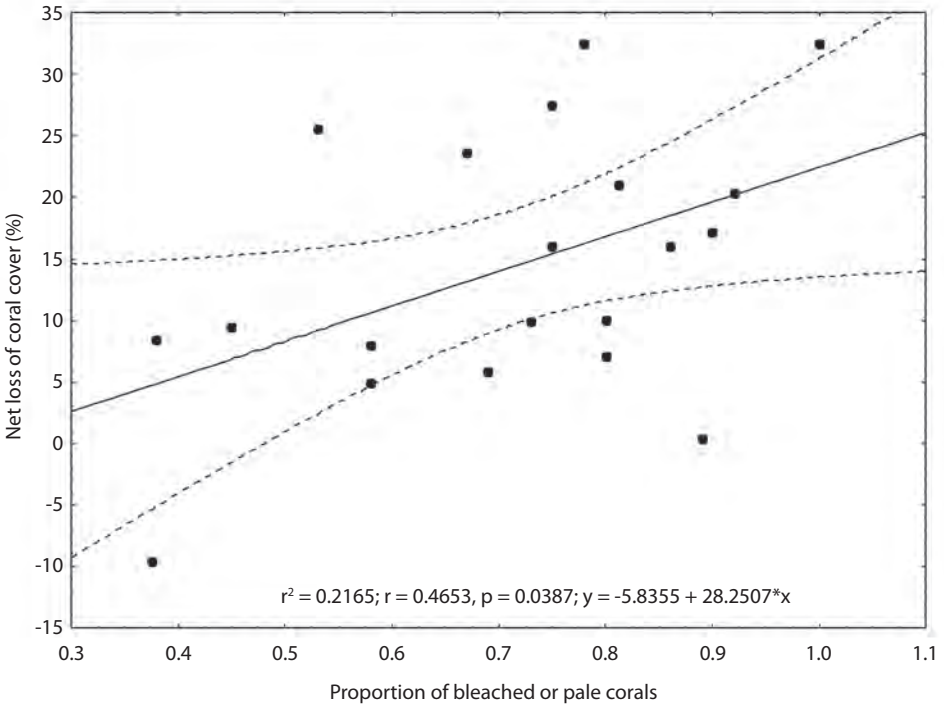

Fig. 4. Relationship between the net coral loss (%) that occurred between October 2010 and February 2011, and the proportion of colonies that were bleached or pale by October 2010 at two reef sites in Los Roques, Venezuela.

2010. It is well known that bleaching severity is determined by a combination of intensity and duration of thermal stress and irradiance conditions (Glynn & D'Croz 1990, Lesser & Farrell 2004). *In situ* measurements indicated that maximum temperatures in the archipelago (excluding the internal lagoon) were about 1°C higher in 2010 than in 2005 for the same period (29.92 °C in 2005 vs. 30.85 °C in 2010). Concomitantly, Degree Heating Weeks (DHW) calculated from *in situ* loggers did not exceed the value of 5 °C-weeks in 2005 (Villamizar *et al.* 2008) for the reefs surveyed in this study, whereas it reached 13 °C-weeks in 2010. It has been generalized that values of over 4°C-weeks cause significant bleaching, and values over 8°C-weeks cause widespread bleaching and some mortality (Liu *et al*. 2006), which was also the case in this study.

Another example of the variability in bleaching intensity is illustrated by the differences found between the 2005 and 2010 MBE in Venezuela. In 2005, bleaching intensity was higher in coastal rather than oceanic reefs (16% vs. <4 of bleached corals, Rodríguez *et al.* 2010). The opposite trend was found in 2010, when intensity seemed higher for the oceanic reefs of Los Roques compared to coastal reefs of Morrocoy (personal observations). These results contrasted with other studies that have consistently found more severe MBE in inshore waters compared to outer reefs (e.g. Berkelmans *et al.* 2004).

Coral cover was severely reduced (by 36%) at our study sites in Los Roques after the 2010 bleaching event, which was similar to the 40% mortality observed in 2005 across the Caribbean (Eakin *et al.* 2010). However, we suspect that the long- term consequences could be worse in Los Roques, because during our last survey (February 2011): a) the bleaching was still ongoing as 46% of colonies remained

pale, despite a drop in seawater temperature; and b) there was a high prevalence of white plague (~10%) by that time. Previous studies indicate that after a MBE, the mortality of corals can span for about two years (e.g. Eakin *et al.* 2010), as well as its detrimental consequences on coral reproduction (e.g. Szmant & Gassman 1990). Also, the onset of diseases associated to the MBE, particularly black band disease, white plague and yellow band disease, can contribute further to the loss of coral cover (e.g. Brandt & McManus 2009, Miller *et al.* 2009, Weil *et al.* 2009).

The loss of coral cover induced by the 2010 MBE in Los Roques has no precedent at least in 20 years. Although we expect that MBE-induced coral mortality to continue for several months after our last survey, a rapid recovery in Los Roques could be possible because a relatively high coral cover (~30%) is still present in many reefs of the archipelago, there is relative low anthropogenic impact (e.g. a permanent population of ~ 2,000 inhabitants), and high coral recruitment rates have been found (Humanes 2009). Venezuelan coastal reefs, that lack these conditions, have shown very slow recovery after massive mortality events (Bastidas *et al.* 2006). Unfortunately, the positive expectations for the recovery of reefs in Los Roques depend on other large scale factors that are difficult to foresee, such as the distribution of thermal anomalies across the Caribbean.

ACKNOWLEDGMENTS

We would like to thank Mitsui Ltd for funding a project that includes the environmental monitoring of Los Roques in 2009 and 2010; the Instituto Nacional de Parques (INPARQUES) for issuing the permits to work in the park; and the useful comments of two anonymous reviewers that greatly helped to improve the manuscript.

RESUMEN

Durante las últimas décadas las anomalías térmicas han sido más frecuentes y severas en el Caribe, quedando pocos arrecifes exentos de eventos masivos de blanqueamiento (EMB). En el Parque Nacional Los Roques, Venezuela, un archipiélago poco afectado previamente por EMB, la temperatura del agua a 5m de profundidad alcanzó 30,85°C en septiembre 2010, y fue >29,0°C entre mediados de agosto y la primera semana de noviembre en cuatro arrecifes. El 72% de 563 colonias de escleractinios estaban blanqueadas o pálidas para octubre de 2010, mientras que para febrero 2011, el 46% de las colonias aún estaban afectadas. Para febrero 2011, la cobertura béntica coralina promedio disminuyó de 45 a 31%. Además, los arrecifes mostraron una alta prevalencia (de hasta 16%) de enfermedad de banda negra en Octubre 2010, y de plaga blanca (11%) en Febrero 2011. Como consecuencia, es probable que la mortalidad coralina resulte mayor a la reportada acá. Sin embargo, Los Roques es poco afectado por perturbaciones antropogénicas y cuenta con un alto reclutamiento de corales, lo cual podría contribuir a su recuperación.

Palabras clave: blanqueamiento coralino, mortalidad, pérdida de cobertura de coral, Caribe, arrecife coralino.

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Static measurements of the resilience of Caribbean coral populations

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Received 18-VII-2011. Corrected 12-XII-2011. Accepted 20-XII-2011.

Abstract: The progressive downward shift in dominance of key reef building corals, coupled with dramatic increases in macroalgae and other nuisance species, fields of unstable coral rubble ,loss of structural relief, and declines of major functional groups of fishes is a common occurrence throughout the Caribbean today. The incorporation of resilience principles into management is a proposed strategy to reverse this trend and ensure proper functioning of coral reefs under predicted scenarios of climate change, yet ecosystem processes and functions that underlie reef resilience are not fully understood. Rapid assessments using the Atlantic and Gulf Rapid Reef Assessment (AGRRA) and the IUCN Resilience Assessment protocol can provide baseline information on reef resilience. A key aspect of these surveys focuses on coral population dynamics, including measures of coral cover, size, partial and whole-colony mortality, condition, and recruitment. One challenge is that these represent static measures involving a single assessment. Without following individual corals over time, it is difficult to determine rates of survival and growth of recruits and adult colonies, and differentiation of juveniles from small remnants of older colonies may not be possible, especially when macroalgal cover is high. To address this limitation, corals assessed in Bonaire in July 2010 were subdivided into two categories: 1) colonies on the reef substrate; and 2) colonies colonizing dead corals and exposed skeletal surfaces of living corals. Coral populations in Bonaire exhibited many features indicative of high resilience, including high coral cover (often 30-50%), high levels of recruitment, and a large number of corals that settled on dead corals and survived to larger size-classes. Overall, the skeletal surfaces of 12 species of corals were colonized by 16 species of corals, with up to 12 settlers on each colony, most (67%) on *M. annularis* (complex) skeletons. Nevertheless, completely dead *M. annularis* colonies were common, survivors were frequently reduced in size and subdivided into smaller tissue remnants, and these species exhibited higher amounts of partial mortality than all other species. A notable absence of sexual recruits and juveniles of *M. annularis* illustrates a progressive shift away from a *Montastraea* dominated system. This shift, characterized by an increasing dominance of smaller, short-lived species such as *Agaricia* and *Porites* and a reduction in size of longer-lived massive corals, is occurring throughout the Caribbean. Monitoring of the survival of recruits is necessary to determine whether Caribbean reefs will retain the same function, structure, identity and feedbacks (key signs of resilience) if the losses of *M. annularis* (complex) continue at present levels. The rapid assessment protocol utilized here allows characterization of colony size structure, partial mortality, recruitment, and whether small corals represent surviving recruits that increased in size or larger (older) colonies that continue to shrink in size. This approach can help determine the history of a site and its resilience. Rev. Biol. Trop. 60 (Suppl. 1): 39-57. Epub 2012 March 01.

Key words: resilience, coral size structure, coral recruitment, survival and growth, coral monitoring and assessment.

Until the late 1970s, benthic substrates on Caribbean reefs were occupied primarily by reef-building corals, with 50-80% benthic cover by living corals. Coral reefs exhibited a generalized zonation pattern with elkhorn coral (*Acropora palmata*) forming large, monospecific stands in the reef crest and shallow fore reef (0-5 m depth); stands of staghorn coral (*A. cervicornis*) at intermediate depths (5-25 m depth) on wave exposed reefs and in shallow, protected environments; massive corals (dominated by *Montastraea annularis* complex)

throughout the fore reef (5-30 m depth) and in back reef and lagoonal areas; and plating agaricids near the base of the reef (20-40 m depth) (Goreau 1959). Coral cover has plummeted on Caribbean reefs primarily due to the near total demise of branching acroporids during the 1990s (Aronson & Precht 2001, Aronson et al. 2002, Bruckner 2003, Gardner et al. 2003), which is now being followed by massive losses of *Montastraea annularis* (complex) (Bruckner & Bruckner 2003, 2006a,b, Edmunds & Elahi 2007, Bruckner & Hill 2009).

Changes to Caribbean reefs, attributed largely to coral diseases, hurricanes, mass mortality of the herbivorous long-spined sea urchin (*Diadema antillarum*), localized human impacts, recent bleaching events and climate change (Lessio 1988, Carpenter 1990, Sutherland 2004, Weil 2004,Grimsdith & Salm 2006) have manifested as dramatic phase shifts characterized by a dominance of macroalgae and other nuisance species, fields of unstable coral rubble, loss of three-dimensional structure, and increases in abundance of shorter-lived brooding corals such as *Agaricia* and *Porites* (Hughes 1994, Edmunds & Carpenter 2001). Many of these stressors have cumulative negative impacts on coral reef ecosystem health and are integrally linked. For example, the virulence and severity of diseases is elevated during and immediately following bleaching events and other periods of elevated environmental stress (Bruno *et al.* 2007, Ballantine *et al.* 2008, Muller *et al.* 2008, Rogers *et al.* 2008, Bruckner & Hill 2009). Other human impacts such as overfishing of groupers, snappers, parrotfish, lobster and other species can have cascading impacts on ecosystem health by removing keystone species that are critical in controlling harmful algae, corallivores and other pests (Jackson *et al.* 2001, Mumby 2006, Green & Bellwood 2009). These "pest" species compromise remaining corals through direct competition and overgrowth, and may prevent recruitment of coral larvae and regrowth of damaged corals.

Although many of the threats affecting coral reefs have been examined in detail since the early 1990s, very few studies have identified a single direct cause of these impacts (a "smoking gun"), or site-specific remedies or treatments for particular problems. Conversely, there has been a proliferation of publications and reports presenting broad scale recommendations on the need to address destructive human activities such as pollution and overfishing, and the benefits associated with the establishment of marine protected areas (Folke *et al.* 1996, Roberts 1997, Roberts *et al.* 2001, Hughes *et al.* 2003, 2005, Almany *et al* 2007, 2009, Jones *et al.* 2009,.. There is good evidence that reduced fishing pressure, and in particular the protection of herbivorous fish populations, can prevent trophic cascades and coral-algal phase shifts (Mumby 2006, Mumby *et al*. 2006). Furthermore, restoration of certain keystone invertebrates, such as *Diadema antillarum*, and increases in density of large herbivorous fishes can trigger a reversal from a macroalgal dominated state and promote coral recruitment (Edmunds & Carpenter 2001, Carpenter & Edmunds 2006, Hughes *et al.* 2009, Norstrom *et al.* 2009). As climate change poses a growing threat to the persistence of these ecosystems, it is imperative that these efforts are scaled up, as degradation is outpacing the potential for recovery in absence of management interventions.

Over the last decade, research efforts have placed a greater emphasis on examining coral reef health and resilience, with a goal to identify strategies to enhance the resilience of these ecosystems (Hughes *et al.* 2003, 2005). *Ecological Resilience* is a term which describes the capacity of a system to absorb, resist or recover from disturbance, or to adapt to change, while continuing to maintain essential functions, structure, identity and feedbacks (Hollings 1973, Nystrom & Folke 2001, Nystrom *et al.* 2008, Obura & Grimsditch 2009). There is considerable evidence that sites with chronic human impacts are the least likely to resist mortality and subsequently recover from acute large-scale events, but by addressing these human impacts, they may be better able to cope with acute events such as climate change and

recover from both natural and anthropogenic impacts (Jennings & Kaiser 1998, Worm *et al.* 2006, Knowlton & Jackson 2008). MPAs, when properly designed and enforced may help promote resilience to disturbances by increasing or maintaining key ecosystem parameters such as fish biomass and coral cover, and help maintain critical ecosystem processes and functions (Cote *et al.* 2001, Halpern 2003). However, in some cases MPAs may not produce tangible conservation benefits, and they are often not accepted by resource users (McClanahan *et al*. 2006), emphasizing the need for alternate management strategies. While great strides have been made in understanding interrelationships among corals, fishes, and algae and the role of certain functional groups in enhancing the health of coral reef ecosystems, large gaps remain in our understanding of the dynamic and complex processes that promote or undermine resilience (Hughes *et al*. 2005).

A detailed assessment of resilience relates to the entire scope of positive and negative factors affecting the ecosystem. This includes ecological, environmental and physical factors as well as social factors such as patterns of resource uses and extraction, type and extent of pollution, presence of invasive species, governance structures, economics, and effectiveness of existing conservation and management efforts (Hughes *et al.* 2005). Quantitative ecological indicators of resilience, that can be measured include: 1) functional group abundance, species diversity and community redundancy, with emphasis on corals, algae, large motile invertebrates and fish communities; 2) the ecological interactions that drive dynamics within and among these groups; 3) habitat and environmental influences that directly affect reef associated organisms and interactions between them; and 4) external drivers of change, including anthropogenic and climate factors, and the level of connectivity with other reefs, (Cowen *et al.* 2006, Lindsey & Bruno 2008, Nystrom *et al.* 2008, Green & Bellwood 2009, Obura & Grimsditch 2009).

The Atlantic and Gulf Rapid Reef Assessment (AGRRA) and the IUCN Resilience Assessment protocols (Obura & Grimsdith 2009, Lang *et al.* 2010) are two different rapid ecological assessment approaches that can provide an indication of the ecological resilience of a coral reef. Both of these share common attributes, and they highlight the importance of the inclusion of ecologically relevant fishes, algal functional group biomass/cover, and coral population structure in rapid assessments. The IUCN protocol includes other measures as well, such as a qualitative assessment of various ecological and biological factors as well as physical factors that promote resilience through shading, screening, cooling and enhanced stress tolerance (Obura & Grimsditch 2009). A detailed resilience assessment also includes characterization of reef processes, such as complex food-web interactions (e.g. herbivory, trophic cascades) reproductive cycles, population connectivity, and coral and fish recruitment, as well as examination of biological characteristics (e.g. genetics of corals and zooxanthellae, symbiont performance, and coral species susceptibility to bleaching, diseases and other stressors).

Because comprehensive measurements of resilience, incorporating the parameters described above and others, are not possible using a single rapid assessment, this study focused solely on one aspect of resilience, coral population dynamics. The approach represents a hybrid between the AGGRA and IUCN protocols, with a few modifications. Detailed measures of colony size (length, width and height) were taken for each coral, along with an estimate of the extent of partial colony mortality. This prevents a potential underestimation of the original colony size that would occur if only the live portion is considered when classifying coral size. The number and diversity of recruits and juveniles (colonies < 4 cm diameter) are also recorded. Recruitment is often considered an indicator of resilience (i.e. signs of recovery following chronic or acute disturbances) (Mumby & Harborne 2010). However, without following recruits over time it is difficult to determine survival rates of these recruits, or whether they are contributing to population

recovery. Survival of recruits, and growth to larger size-classes was assessed by distinguishing between colonies on the reef substrate and those found on exposed skeletal surfaces of other corals; the latter represents both recruits and surviving juveniles. This method was tested in July 2010 in Bonaire, and the results of these assessments are presented here.

MATERIALS AND METHODS

Study site: In July 2011, the Living Ocean Foundation conducted rapid assessments on 24 sites off the leeward side of Bonaire and the adjacent Klein Bonaire, targeting *Montastraea* dominated reefs from 5-15 m depth. All of these sites are fringing reefs that begin close shore and drop to deeper water within a few hundred meters. Many of the areas above 5 m depth were badly damaged by recent hurricanes and are largely devoid of corals, but much of the *Montastraea* structure at mid depths is still intact. The sites were grouped into 3 distinct areas: a) sites north of Kralendijk located off a rocky coastline; b) sites off the small offshore Klein Bonaire; and c) sites south of Kralendijk located off a sand and low relief limestone shoreline, many of which have a double reef system (Fig. 1, Table 1).

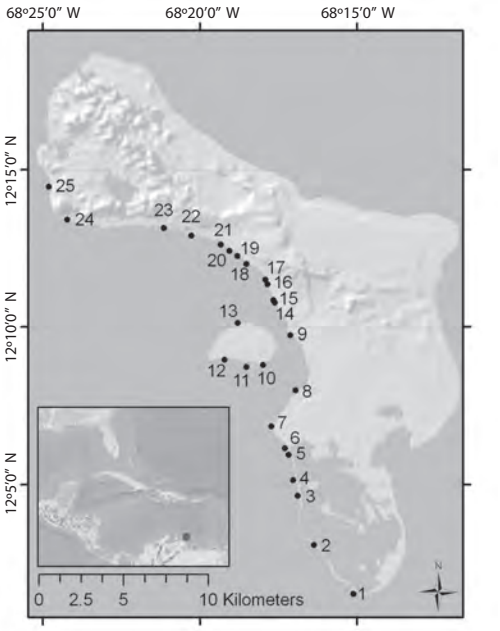

Fig. 1. Locations of reef assessments in Bonaire. Sites are listed from south to north. Numbers correspond to the site names listed in Table 1.

Assessment protocol: The resilience assessments conducted in Bonaire include measures of corals, fish, algae and motile invertebrates through application of attributes of the Atlantic and Gulf Rapid Reef Assessment (AGRRA) protocol, the IUCN bleaching resilience protocol, and several additions. Data were collected using a combination of belt transects, point intercept methods and photographic documentation. While numerous biological, ecological, physical and social measures must be analyzed concurrently to gain a full picture of coral reef resilience, this manuscript focuses specifically on one aspect of resilience (coral population resilience) evaluated through a static measure of coral population dynamics. Seven parameters were recorded for corals: 1) benthic cover; 2) coral diversity and abundance (by species); 3) coral size class distributions (by species) and size of tissue remnants; 4) amount of partial mortality and number of tissue remnants; 5) recruitment; 6) location of settlement of recruits; and 7) coral condition. Belt transects, each 10 m long and 1 m wide (minimum of three per reef), were extended parallel to depth gradients on each reef. Within this belt, each coral 4 cm or larger in diameter was identified to species, measured and assessed for condition. A one meter bar, marked in 1 cm increments was used to measure the maximum diameter, width (perpendicular to the diameter), height, and amount of mortality. Mortality was divided into three categories: recent, transitional and old.

Benthic cover: Cover of substrate and benthic organisms (algae and invertebrates) was estimated using a point intercept method. At each site, a minimum of six 10 meter long transects were deployed. The organism and

TABLE 1
Location of sites examined in Bonaire

Site #	Reef Name	Longitude W	Latitude N
1	Red Slave	-68.251800	12.025550
2	Margate Bay	-68.272867	12.051350
3	The Invisibles	-68.281383	12.077617
4	Jeannie's Glory	-68.283833	12.085733
5	Alice in Wonderland	-68.286217	12.099233
6	Angel City	-68.288267	12.102750
7	Lighthouse Point	-68.295417	12.114350
8	Windsock	-68.282583	12.133317
9	Something Special	-68.285250	12.162250
10	Keep Sake	-68.299760	12.146800
11	Monte's Divi	-68.308710	12.145660
12	South Bay	-68.320133	12.149650
13	Knife	-68.313250	12.168610
14	Small Wall	-68.293483	12.179567
15	Black Durgon Reef	-68.294338	12.180745
16	Andrea I	-68.297483	12.189217
17	Andrea II	-68.298600	12.191600
18	Oil Slick Leap	-68.308633	12.199950
19	Jeff Davis Memorial	-68.313417	12.204133
20	Weber's Joy/Witches Hut	-68.317617	12.206900
21	1,000 Steps	-68.322317	12.210183
22	Tolo/Ol' Blue	-68.337900	12.214883
23	Karpata	-68.352383	12.218967
24	Tailor Made	-68.403630	12.223380
25	Nukove	-68.413333	12.240933

substrate type was recorded every ten cm for a total of 100 points per transect. Substrates were identified as pavement, rubble, sand/silt, dead coral and live coral. All corals were identified to species. Other invertebrates were subdivided into phylum or class and growth form or identified to genus/species when possible. Algae were divided into five functional groups (fleshy macroalgae, erect coralline algae, crustose coralline algae, turf algae, cyanobacteria) with certain nuisance species recorded to genus (e.g. *Microdictyon, Lobophora, Dictyota, Stypopodium, Peyssonnelia*). Key invertebrates that also may become nuisance species recorded to genus or higher taxonomic level included: tunicate (*Tridemnum),* encrusting gorgonian (*Erythropodium, Briareum),* colonial anemone (*Palythoa),* encrusting or bioeroding sponge *(Cliona langae/aprica* complex*, Cliona delitrix, Anthosigmella),* and hydrozoan coral (*Millepora)*.

Recruitment: Sampling for corals smaller than 4 cm was done using a minimum of five $0.25m^2$ quadrats per transect. Each quadrat located at fixed, predetermined intervals (e.g. 2, 4, 6, 8, 10 m), alternating between right and left side of the transect. Recruits were identified in both point intercept surveys and belt transects. These corals were divided into recruits (0-2 cm diameter) and juveniles (2.1-3.9 cm).

Corals colonizing skeletons of other corals: All corals settling on skeletal surfaces of other colonies (completely dead corals and corals with partial mortality) within each belt

transect were identified and recorded separately from those corals occurring on reef substrates. For each of these colonies, measurements were taken of the size (length, width and height for colonies 4 cm or larger; maximum diameter for corals that were 0-3.9 cm), and an estimate of percent mortality made for those exhibiting partial mortality.

Condition of corals: Visual estimates of tissue loss, using a 1 m bar marked in 1 cm increments, was recorded for each colony over 4 cm in diameter. If the coral exhibits recent tissue loss, the amount of remaining tissue, the percent that recently died and the percent that died long ago were estimated for the entire colony surface. Tissue loss was categorized as recent mortality (occurring within the last 1-5 days), transitional mortality (filamentous green algae and diatom colonization, 6-30 days) and old mortality (>30 days). For each coral with partial or whole colony mortality, the cause of mortality was identified if possible. The diagnosis included an assessment of the type of disease, the extent of bleaching, predation, competition, or overgrowth, or other cause of mortality. Each coral was first carefully examined to identify cryptic predators such as snails (*Coralliophila abbreviata*) and fireworms (*Hermodice carunculata*). Lesions were then diagnosed into four categories: recent tissue loss, skeletal damage, color change, and unusual growth patterns (an individual colony could have multiple characteristics such as color change and recent tissue loss) and when possible a field name was assigned. Diseases were identified according to Bruckner 2010b and Raymundo *et al.* 2008, and included yellow band disease (YBD), white plague (WP), black band disease (BBD), red band disease (RBD), Caribbean ciliate infection (CCI), dark spots disease (DSD) and white band disease (WBD).

RESULTS

Coral cover: On most of the reefs examined in this study, between 5-15 m depth, coral cover was high (40-60%;Fig. 2a). The only exceptions were two sites on northern reefs, Webers Joy/Witches Hut and Jeff Davis Memorial (cover= 30-35%), and in certain locations with outbreaks of white plague. *Montastraea annularis* (complex) were the dominant corals, in terms of living cover, occupying approximately 20-25% of the benthos, and making up over 50% of the total live coral cover. The next most common species, in terms of living cover, where *Agaricia, Madracis* and *Porites* spp (Fig. 2b). Cover of major functional groups of reef building corals showed slight variations between sites and depths. For instance, cover of *M. annularis* complex was lowest on northern reefs (pooled for all depths; 22% vs 24%) while cover of *Agaricia* spp. was lowest on southern reefs (6.6% vs 9%). Klein Bonaire also had a higher cover by *Madracis mirabilis* (>6%); this coral formed large thickets on several reefs that occasionaly extended the length of 10 m transects or more. There were also differences in coral cover between depths. For instance, *Agaricia* spp. and *Eusmilia fastigiata* had the highest cover on all reefs at 15 m. In contrast,cover of *Porites* was higher at 10 m and 15 m depth than at 5 m. *Madracis* spp. was most variable, having the highest cover at 5 m depth on northern and southern reefs, and 10 m depth on Klein Bonaire. *Acropora palmata* and *A. cervicornis* were virtually absent from all point intercept surveys, and only identified infrequently in belt transects. Cover of macroalgae was relatively low on all reefs, but was significantly higher on northern reefs (Fig. 2a). There was no correlation between coral cover and macroalgal cover (r^2=0.01, p=0.56). Cover of turf algae ranged from 7-38%, and was significantly correlated to coral cover (r^2 = 0.44, p=0.0003).

Coral composition: A total of 5957 corals, 4 cm or larger in diameter, were identified within belt transects (10 m X 1 m) on 25 reefs in Bonaire (Fig. 3). *M. annularis* (complex) was the dominant functional group of corals at all sites overall, in terms of numbers of colonies, making up approximately 27% of all corals. *M. annularis* complex was numerically

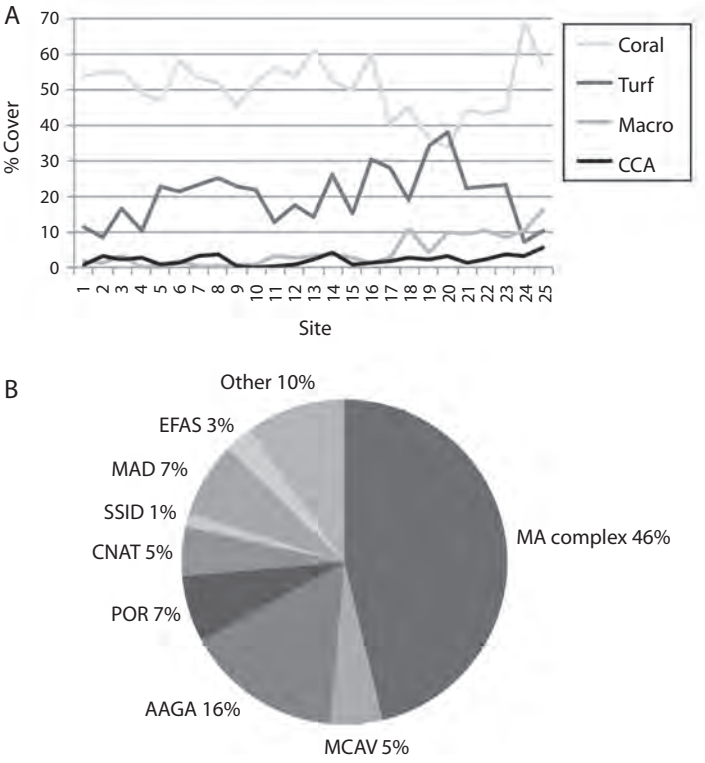

Fig. 2. Benthic cover for 25 reefs examined in Bonaire. A. Mean cover of corals, turf algae, macroalgae, and crustose coralline algae for each reef, from south to north pooled for 5, 10 and15 m depth. B. Cover of each functional group of corals, expressed as a percent of the total living coral cover.

dominant between 5-10 m depth, while *Agaricia* was slightly more abundant at 15 m depth. Reefs in the north and off Klein Bonaire had a higher proportion of *M. annularis* (complex) colonies (30 and 29% respectively), when compared to reefs in the south (21%). When examined by species, *M. annularis* was significantly more abundant than *M. faveolata* and *M. franksi* on northern reefs and Klein Bonaire, but not southern reefs, while abundance of *M. faveolata* and *M. franksi* was very similar in all locations. The second most abundant functional group was the genus *Agaricia* (18-26% of all corals). This genus was the dominant taxon on southern reefs and the second most abundant taxon in other locations. The genus *Porites* was the third most abundant taxon. While the proportion (number of colonies) of brooding species (especially *Agaricia, Porites*) was very high, the contribution to living coral cover was less than *M. annularis* (complex) because these colonies were smaller in size.

Coral size structure: Corals ranged in size from 4 cm (smallest coral assessed in belt transects; 0-3 cm corals assessed separately using quadrats and on exposed skeletal surfaces) to over 450 cm diameter, with a maximum

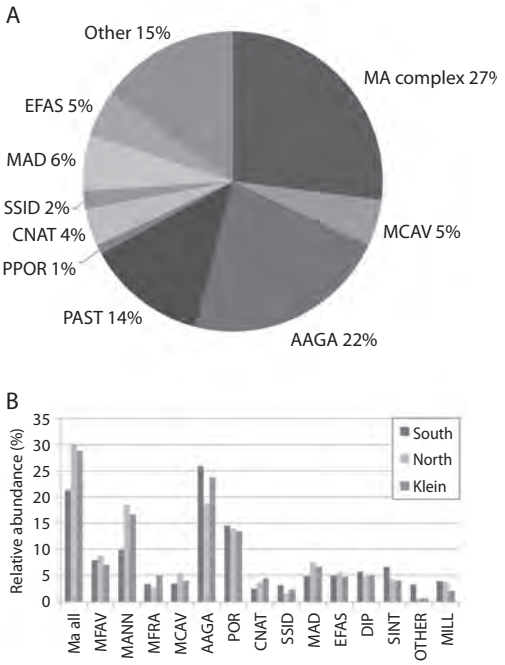

Fig. 3. Coral composition observed in belt transects from 5-15 m depth. A. Composition of corals by functional group pooled for all sites and depths examined in Bonaire. B. The proportion of colonies of each species for northern reefs, southern reefs and Klein Bonaire, pooled for all depths.

height of 460 cm. The size structure of *M. annularis* (complex) shows a bell shaped distribution with few small colonies (<20 cm) and few very large colonies (>200 cm) and a large number of medium-sized corals (30-80 cm diameter); the population structure also exhibited a second peak for colonies that were 150-199 cm diameter (Fig. 4a). Colonies of other species (all species except *M. annularis* complex) were dominated by very small colonies (<20 cm) and populations in all locations (all sites pooled, as well as northern, southern and Klein Bonaire reefs) exhibited a monotonic decline in size with very few colonies over 60 cm in diameter. Colonies of *M. annularis* (complex) were significantly larger than all other species (mean diameter = 58 cm, versus 24 cm for other species pooled; original diameter of the colony; Fig. 4b), although large (> 1 m diameter) colonies of *Siderastrea siderea, Stephanocoenia intersepta, Colpophyllia natans* and extensive (2-5 m wide) thickets of *Porites porites* and *Madracis mirabilis* were seen.

There was a notable absence of smaller colonies (<10 cm) of *M. annularis* (complex). It is important to note that these represent measures of the diameter of the entire corallum (the original skeletal surface area) and not the size of tissue remnants on larger skeletal surfaces. Many colonies in the genus *Montastraea* that had contiguous skeletons,1-3 m in diameter or larger, were reduced in live tissue area, and individual corals often consisted of multiple tissue remnants (mean = 6.6 remnants per colony) that were reduced to a few cm in diameter.

Colony mortality: The amount of partial mortality observed on corals located within belt transects varied from 0-99%, with significant differences between species, colony sizes and locations (Fig. 5). For *M. annularis* complex (n=1602), a total of 73 (4.5%) had completely died, with surviving colonies (n=1529) missing a mean of 28% of their tissue. Tissue loss for these species consisted of 25% old mortality and 3% transitional and recent mortality. When examined by size, colonies of *M. annularis* (complex) with less than 30% partial tissue loss were significantly smaller in size (mean diameter = 48 cm; mean tissue loss=11%; n=889) than colonies with 30-99% partial tissue loss (mean diameter =61 cm; mean tissue loss= 50%; n=639). However, a correlation analysis using the entire range of sizes (no pooling into size classes) showed that tissue loss was not significantly correlated with colony size. This may be due to the fact that colonies exhibited a high range of tissue loss in all size classes: each size class contained colonies with no mortality, moderate levels of mortality and extensive mortality. Individual colonies of *M. annularis* (complex) were also frequently divided into a number of smaller patches of live tissue; on average, each coral was subdivided into 6.6 separate tissue remnants.

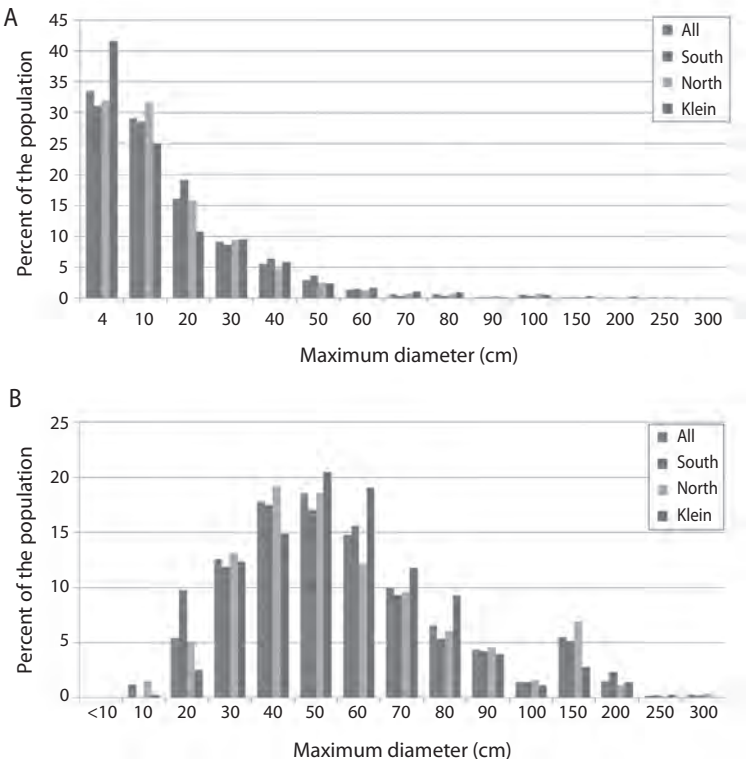

Fig. 4. Population demographics of reef-building corals on 25 reefs examined in Bonaire. A. Size structure of all species except for *M. annularis* complex. B. Size structure of *M. annularis* complex. Blue bars= all reefs; red bars= southern reefs; green bars= northern reefs; purple bars= Klein Bonaire.

Tissue loss for other corals (all species except *M. annularis* complex) was significantly lower (mean partial tissue loss=8%) and fewer dead colonies were identified (n=20, 0.4%). Interestingly, partial mortality for colonies that had colonized reef substrates was higher than partial mortality for colonies that had colonized exposed skeletal surfaces of living corals (9.4% vs 7.6%). These corals were also less frequently subdivided into smaller tissue remnants (mean=1.41 remnants/colony), possibly because they were younger and smaller in size overall.

Based on size structure, abundance, levels of recruitment, and coral condition, coral communities could be divided into two primary groups, the *M. annularis* complex (*M. annularis, M. faveolata* and *M. franksi*) and all other species. Corals lumped into "other species" were small to medium-sized (mean=24 cm), and population structure exhibited a monotonic decline in size; most colonies were < 20 cm in diameter and very few colonies were over 60 cm. Although a small proportion of colonies showed active signs of disease and competition from other biotic stressors, these corals had low levels of partial mortality (8%), few completely dead colonies were observed (0.4%), and they were the predominant species colonizing dead skeletal surfaces of other corals as well as reef substrates.

Recruits on reef substrate: A total of 1688 quadrats, each 0.25 m^2, were examined on 25 reefs in Bonaire. Over 40% of the quadrats contained at least one coral (0-3 cm in

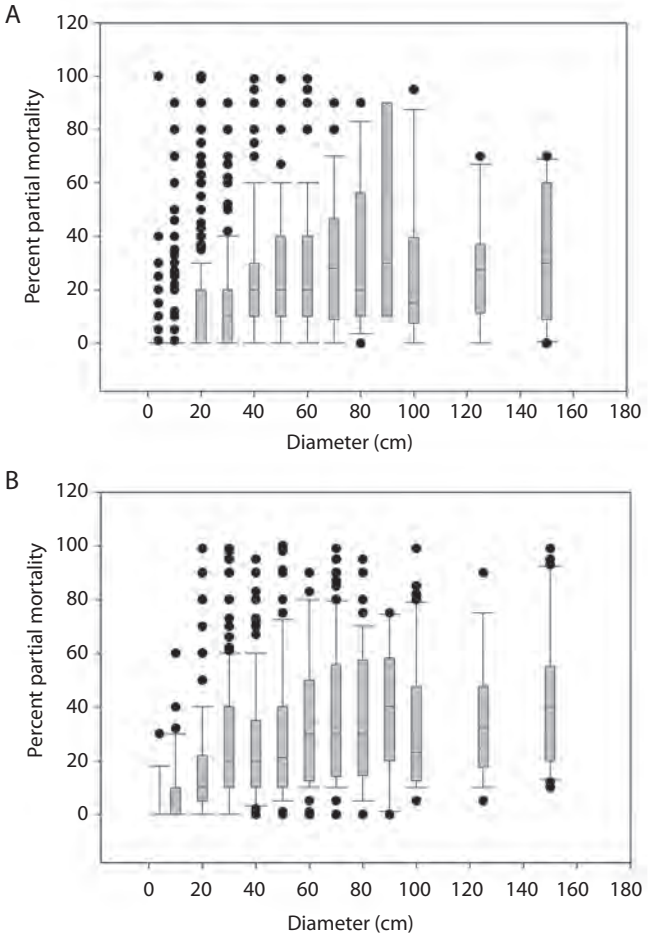

Fig. 5. Relationship between partial mortality and colony size for A. *M. annularis* (complex), and B. All other species (pooled) shown as a box plot. The boundary of the box illustrates the 25th and 75th percentile, the line within the box marks the median, error bars indicate the 10th and 90th percentile, and outlying points are shown as black dots. Colonies are pooled into 13 size classes, 4-9 cm, 10-19 cm, 20-29 cm, 30-39 cm, 40-49 cm, 50-59 cm, 60-69 cm, 70-79 cm, 80-89 cm, 90-99 cm,100-124 cm, 125-150 cm and > 150 cm.

diameter, classified here as a recruit). A higher proportion of quadrats on southern reefs contained recruits (45% vs. 40% and 37% of quadrats on northern reefs and Klein Bonaire, respectively). Individual quadrats contained a maximum of 5 recruits (northern and southern reefs) and 8 recruits (Klein Bonaire) each. Eighteen different species of scleractinian corals and two hydrozoan corals were observed in quadrats. There was a notable absence of sexual recruits of *M. annularis, M. faveolata* and *M. franksi*, although numerous tissue remnants <4 cm in diameter were noted. The dominant corals observed as recruits included *A. agaricites* (mean = 3.9 recruits/m^2), *P. astreoides*(mean = 2.2 recruits/m^2), and *Madracis* spp (mean = 0.95 recruits/m^2) while all other species were at densities of <0.2/m^2 (Fig. 6a). Species that were common as adults within transects, but not observed in quadrats included *Mussa angulosa, Mycetophyllia lamarckiana, M. aliciae, Dendrogyra cylindricus, Isophyllia sinuosa, I.*

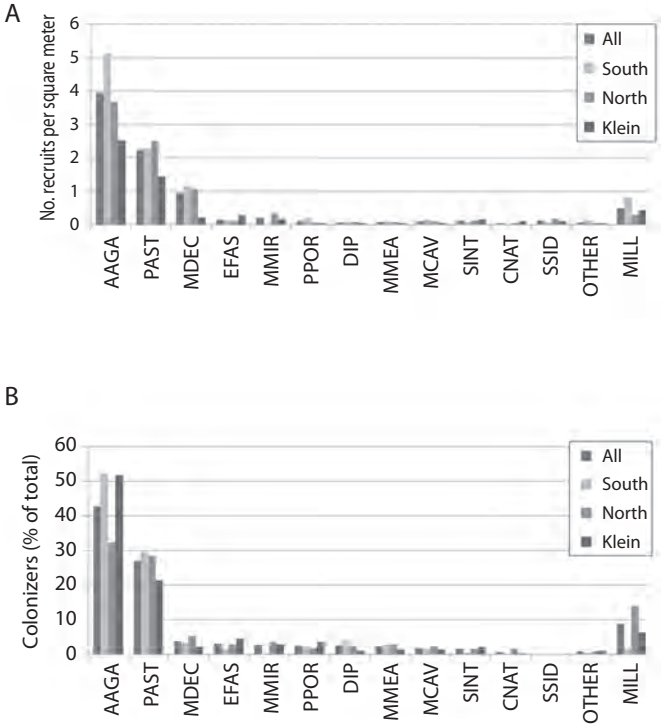

Fig. 6. A comparison between recruits settling on reef substrate (A) and recruits settling on dead coral skeletons (B).

rigida, S. intersepta, Scolymia spp.*, Acropora palmata,* and *A. cervicornis.*

Colonization of coral skeletal surfaces: The exposed skeletal surfaces of 249 corals were colonized by new stony corals. Corals that supported these settlers were missing a mean of 60% of their tissue. Difference in partial mortality occurred between regions, with colonies from southern reefs that were colonized by other corals showing much higher amount of partial mortality overall. *M. annularis* (complex) colonies that supported settlers of other species were similar in size in all three locations, and they were significantly larger than all other species that were colonized by new corals (Table 2).

A total of 12 species of corals supported colonizers (Fig. 7), although most were observed on *M. annularis* (36%) and *M. faveolata* (35%). A much higher proportion of *M. annularis* (complex) skeletons on Klein Bonaire (19%) were colonized by corals as compared to northern (13%) and southern (5.3%) reefs. Exposed skeletal surfaces of other species were less frequently colonized by new corals, with exception of *M. cavernosa* and *S. siderea* on

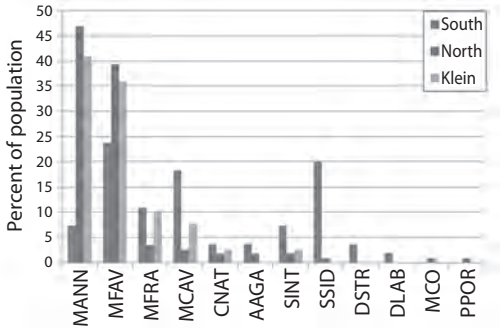

Fig. 7. Species of coral supporting coral settlers on northern, southern and Klein Bonaire reefs.

TABLE 2
Mean size and condition of corals that supported colonization by other species of corals. Corals are pooled into *Montastraea annularis* complex (Ma), other species (other) and all corals (all), subdivided into northern (N), southern (S) and Klein (K)Bonaire reefs.

	N	%	# remnants	Max Diam (cm)	Width (cm)	Max Height (cm)	% old mortality	% recent	% total mortality
All	248	4.7	4.8	66	60	58	58	0.8	59
Ma (S)	23	5.3	3.3	70	61	53	71	1.7	73
Ma (N)	105	12.9	5.9	74	67	66	60	0.9	61
Ma (K)	67	19.3	5.5	65	59	60	51	0.7	52
Other (S)	32	2.2	2.4	49	44	38	63	0.0	63
Other (N)	12	0.8	2.2	49	45	40	49	1.1	50
Other (K)	10	1.6	2.1	51	48	46	52	0.0	52

southern reefs (Fig. 4). No differences in the number of settlers per unit area were noted between reef substratum and exposed *M. annularis* skeletal patches.

Overall, 825 corals of 16 species were identified as colonizers on these corals, with up to 12 observed on a single colony. These settlers were up to 30 cm in diameter, with most (49%) from 4-7 cm in diameter and <10% that were 3 cm or smaller. *Agaricia agaricites* (43%) and *P. astreoides* (27%) were the most common colonizers, although broadcast spawners were also observed (Fig. 6b). Interestingly, some species observed as recruits were not observed to settle on dead skeletons (*S. siderea*). The proportion of colonizers also differed significantly from the proportion of recruits observed on the reef substrate among some species (e.g. fewer *M. decactis* and higher percentage of colonizers of *E. fastigiata, C. natans, Diploria spp., M. cavernosa, P. Porites, M. meandrites*, and *S. interepta*), but not all species (*Agaricia* and *P. astreoides*). There was also a notable absence of *M. annularis* (complex) recruits and colonizers on dead skeletons. While *M. annularis* (complex) was not observed recruiting onto any exposed skeletal surfaces, juvenile corals (4 cm or larger) that had settled on dead coral surfaces represented 17.5% of all corals of other species (all species except *M. annularis*) identified within belt transects.

DISCUSSION

Assessment protocols for stony corals frequently rely on single or repeat assessments of coral cover as the primary metric to characterize reef condition and gauge changes. This approach assumes that reefs with high coral cover are in better shape and exhibit higher resilience (Gardner *et al.* 2003, Bruno & Selig 2007), yet coral cover may actually be one of the last metrics to indicate ecosystem failure (Bellwood *et al.* 2004, McClanahan *et al.* 2011). A second coral metric that has received greater attention in recent years is an assessment of recruitment, whereas high levels of recruitment may suggest a reef is rebounding after a disturbance (Mumby & Harborne 2010). In addition, studies characterizing impacts, such as coral diseases, usually rely on a count the number of colonies in a given area to provide some indication of prevalence or incidence of the particular condition (Raymundo *et al.* 2008). These parameters alone may fail to provide a reliable measure of reef resilience because they fail to take into account factors affecting the fitness and dynamics of coral populations. Namely, corals are modular (clonal) organisms can undergo growth, shrinkage, fission into several ramets, and fusion; subunits can function independently and they exhibit indeterminate growth (Babcock 1991).

Two of the most important parameter to consider when assessing resilience of coral populations is size and amount of partial mortality, as mortality and fecundity rates in corals are strongly size-dependent (Harrison & Wallace 1990). Smaller corals are generally more vulnerable to stressors, while the likelihood of total colony mortality decreases with increasing colony size (Babcock & Mundy 1996, Hall & Hughes 1996). Corals also show a positive relationship between colony size and fecundity, with smaller colonies allocating more energy to non-reproductive life history traits such as growth and maintenance and focusing on reproduction after achieving a critical threshold size (Szmant-Froelich 1985). Reproductively mature colonies may also regress in size below that minimum threshold, becoming non reproductive (Szmant 1991). In addition, competitive abilities and regenerative potential also increases with size (Meesters *et al.* 1994).

Since colony size can influence patterns of mortality and fecundity, population size frequency distributions provide insight into the effects of disturbance and population processes. Coral populations are normally highly positively skewed, with a dominance by the smallest size classes and an exponential decrease with increasing colony size (Bak & Meesters 1998, 1999). Larger colonies typically become rarer in a population, but they have a decreased probably of total colony mortality than smaller colonies (Hughes & Jackson 1985, Soong & Lang 1992). Furthermore, larger colonies show a lower frequency of partial and whole colony mortality, with mortality decreasing in relation to colony size (Meesters *et al.* 1994). One consequence of this is that smaller colonies suffer total mortality more frequently than larger colonies, while larger colonies sustain higher levels of partial mortality over multiple disturbances, but have a larger chance of survival (Soong 1993). Thus, size frequency distributions of coral populations provide a sensitive means of discriminating changes in coral communities in response to acute and chronic disturbances, and may help identify differences between populations exposed to differing degree of environmental stressors.

Scleractinian coral populations normally exhibit a positively-skewed (left) size frequency distribution with populations dominated by smaller colonies. On a Caribbean *Montastraea* reef, a population that has not been exposed to a major disturbance for several decades may progressively exhibit a shift towards the opposite extreme, with a dominance of extremely large colonies (negatively-skewed right). In Bonaire, a northern reef (Taylor Made) with a higher coral cover than all other sites examined in this study was dominated by extremely large colonies of *Montastraea faveolata* and *M. annularis*, many over 200 cm in diameter and 5 m in height. This site had few small corals and colonies exhibited little partial mortality, suggesting an absence of a major acute disturbance for several decades, and minimal chronic impacts from disease or other stressors. This reef appears to be in a stable equilibrium at this time. Nevertheless, this site may be pushed over the tipping point towards a trajectory of coral decline by an acute disturbance (e.g. an outbreak of white plague), as it exhibited low coral diversity, low levels of recruitment ,and few juvenile corals. However, the site may also undergo rapid recovery if other resilience factors are present, such as high rates of herbivory, as recruitment may increase once substratum becomes available.

The demographic structure of a coral population also illustrates the presence of past disturbances, where a population dominated by small size classes may indicate high mortality in intermediate and larger corals (Nystrom *et al.* 2008). Several reefs at the southern end of Bonaire had high coral cover, but they were dominated by a high diversity of small corals, most of which were 4-20 cm diameter. These reefs included a high proportion brooding species such as *Agaricia* and *Porites*, although various brain corals, flower coral, cactus corals and many other species were present. Most *Montastraea* colonies were small (<90 cm diameter), many dead standing colonies of these species were observed, and colonies were

frequently subdivided into small tissue remnants. Although high numbers of recruits and juvenile corals were present, no *Montastraea* colonies below 10 cm diameter were observed. The declining condition of *M. annularis* is indicative of a past disturbance, as well as chronic tissue loss from disease. While coral cover is rebounding, the progressive replacement of *M. annularis* may be indicative of lowered resilience as the community is becoming dominated by smaller, shorter-lived species that are highly susceptible to future perturbations.

By subdividing mortality into three categories, recent, transitional and old mortality, the cause of tissue loss and the timing of a disturbance event may be discernable(Lang 2003). The presence of high levels of recent mortality suggests that a disturbance has occurred and the event is ongoing. Sites containing corals with extensive transitional mortality and little recent mortality were affected by a disturbance in the recent past, but the event has passed and the system may be at the early stages of recovery. At the opposite extreme, if most colonies have extensive patches of old mortality, and little or no recent mortality, it is apparent thatthe site was impacted at some time in the past, but the source of mortality is gone making it difficult to determine when the mortality occurred, or whether it was an acute event or a chronic disturbance. In Bonaire, extent of partial and whole colony mortality was notably different among reefs, with southern reefs showing low levelsof old mortality and the highest amount of recent and transitional mortality. Recent mortality was attributed to an outbreak of white plague; this disease was also present in other locations, but at a lower prevalence.

The challenge faced when assessing colony size and extent of mortality through a single rapid assessment is that it is impossible to determine the growth of a coral population over time. Because corals are clonal animals, they can progressively increase in size with age, theoretically with indeterminate growth, but they can also shrink in size, without dying (Babcock 1991). Through partial mortality colonies are frequently subdivided into isolated tissue remnants, and the original colony boundaries can become obscured by other organisms, especially on reefs with a high cover of macroalgae or other epibionts. A dominanceof small colonies may represent several successful recruitment events, or the small colonies may be surviving remnants of formerly larger corals. A feasible way to differentiate juvenile corals from older surviving remnants, without having to tag individual colonies and return to the site to look at survival over time, is to separate out coral measurements into two categories: those corals found on the reef substrate, and those that have colonized exposed skeletal surfaces of corals with partial or total mortality. All corals that settled onto exposed skeletal surfaces or dead coral skeletons can be considered recruits and not remnants, which is not the case for colonies occurring on the reef substratum. In contrast, small patches of tissue of the same species, that are similar in morphology and coloration to surrounding tissue are likely to be tissue remnants.

In Bonaire, corals frequently settled on most species of massive and plating corals, although the highest settlement and survival rates appear to be on *Montastraea* skeletons. Settlers included 16 important reef-building species, with a notable absence of *Montastraea annularis* (complex). The majority of these corals were completely alive, unlike many of the similar sized corals that occurred on the reef substrate. There were however, no differences noted in the number of recruits recorded on coral skeletons of *M. annularis*, versus the reef platform. While the two substrates are equally attractive to settling larvae, dead skeletal surfaces of *Montastraea* may be optimal, due to high rates of survival and growth into larger size classes (and absence of partial mortality as observed among the same species on reef substratum). Because the settlement surface (coral skeleton) is raised off the bottom, settlers are less affected by sediment transport and siltation, macroalgal competition, and possibly disease. Nevertheless, one species (*Siderastrea siderea*) recruited onto the reef, but it did not colonize coral skeleton. The

proportion of colonizers on skeletons that survived to larger size classes also differed among species, indicating that high recruitment does not necessarily equate to high resilience.

The discrimination of corals settling onto skeletal surfaces of other corals provides a useful metric when using a single rapid assessment to determine levels of recruitment, as well as the potential for longer-term survival and growth. This is not be possible when examining coral population dynamics based solely on colonies on the reef substrate, as large corals that undergo shrinkage and fission may be misinterpreted as juveniles (smaller size classes). One complication with this measure is an absence of information on the age of the coral skeleton necessary to attract settling larvae. Corals did settle on exposed skeletal patches of colonies of *M. annularis* (complex) exhibiting slow, chronic mortality from yellow band disease (e.g. areas that were denuded 30-90 days earlier; Bruckner, unpubl data), suggesting coral skeleton of this species becomes suitable for settlement relatively early. However, for other species only long dead skeletal patches, with an absence of macroalgae and other prominent epibionts, appeared to support coral settlers. Although it is not possible to determine the age of the dead skeleton, it was certainly many months to years, as evidenced by the presence of turf algae, crustose coralline algae, and encrusting invertebrates.

The results presented here provide an indication of the resilience of of coral populations in Bonaire. The presence of high levels of recent recruitment and growth of recruits into larger size classes demonstrates that newly settling corals are surviving and contributing to an increase in the proportion of these taxa. The absence of larger size classes of some coral taxa that colonized exposed skeletal surfaces suggest that certain species are recruiting onto these reefs at levels that exceed survival. In addition, the dominant frame-builder (*M. annularis* complex) on these reefs appears to be highly vulnerable to recent disturbances, as populations of these species are being progressively reduced in abundance and size, and they have not shown substantial levels of recruitment needed to replace colonies that died. As observed in other locations throughout the Caribbean, these reefs are undergoing a transition from a *Montastraea*-dominated system to a community consisting predominantly of other species. Fortunately for Bonaire, these reefs still have unusually high coral cover, high levels of recruitment and good survival of juvenile corals, and several sites contain a high abundance of large, unblemished colonies of *Montastraea annularis* (complex), all which are important indicators of resilience.

While single assessments will never provide the kind of data that would result from repeated visits to a site over time, single rapid assessments can provide valuable data on the resilience of coral populations. For corals, these assessments must expand upon traditional measures of coral cover and abundance, by incorporating data on recruitment, size structure, partial and whole colony mortality, and the extent of survival and growth of recruits.

ACKNOWLEDGEMENTS

The intensive surveys completed In Bonaire between July 19-26, 2010 represent the dedicated efforts of a talented group of scientists and field assistants. I am grateful for the assistance and expertise provided by the team to complete rapid ecological assessments, and extensive time devoted to data entry, with special thanks to Eric Borneman, Robin Bruckner, Kalisi Faanunu, Philip Renaud, Glynnis Roberts, Debbie Santavy and Amanda Williams. Al Catafulmo and the Black Durgon Inn graciously accommodated the research team during this mission. I also thank the Government of Bonaire and the Bonaire Marine Park for assistance with permits and other technical aspects associated with the work. All funding for this work was provided by the Khaled bin Sultan Living Oceans Foundation. Surveys were performed under a research permit #30001380 granted by the Island Council of the Island Territory of Bonaire.

RESUMEN

En la actualidad se está viendo en el Caribe un cambio en la composición de los corales constructores de arrecifes, aumento en la cobertura de macroalgas y otras especies, un aumento en áreas cubiertas por escombros de corales, y una pérdida de relieve. La incorporación de principios de resiliencia en el manejo es una estrategia propuesta para revertir esta tendencia y asegurar la sobrevivencia y el adecuado funcionamiento de los arrecifes de coral bajo escenarios previstos de cambio climático. Sin embargo, todavía quedan grandes vacíos en la comprensión de los factores que promueven la resiliencia. Evaluaciones rápidas realizadas con la metodología AGRRA (Atlantic and Gulf Rapid Reef Assessment) y con el protocolo de Evaluación de Resiliencia para arrecifes coralinos de la IUCN brindan información de línea base sobre la resiliencia de los arrecifes del Caribe. Un aspecto clave de estos estudios se centra en la dinámica de las poblaciones de los corales, incluyendo medidas de cobertura de coral, estructura de tallas, la extensión de la mortalidad parcial y total de toda la comunidad, condición de los corales y reclutamiento. Un reto es que esto representa una medida estática que involucra una única evaluación. Sin seguir las colonias individuales y el reclutamiento en el tiempo, es difícil determinar las tasas de sobrevivencia y crecimiento de los reclutas, y podría no ser posible la diferenciación de los juveniles de los restos pequeños de colonias más viejas, especialmente cuando la cobertura algal es alta. Para abordar esta limitación, los corales monitoreados en Bonaire en julio del 2010 fueron subdivididos en dos categorías: 1) colonias sobre la estructura arrecifal; y 2) colonias creciendo sobre coral muerto o sobre las superficies expuestas del esqueleto de los corales vivos. Los arrecifes en Bonaire exhiben muchas características indicativas de alta resiliencia, incluyendo una alta cobertura de coral (frecuentemente 30-50%), altos niveles de reclutamiento, y un gran número de corales que se asentaron sobre los corales muertos y crecieron. En general, las superficies del esqueleto de 12 especies de corales fueron colonizadas por 16 especies de corales, con un máximo de 12 colonizadores en cada colonia, la mayoría (67%) sobre esqueletos de *Montastraea annularis* (complejo). Colonias completamente muertas de *M. annularis* fueron comunes y los sobrevivientes con frecuencia son más pequeños o subdivididos en pequeños restos de tejido. *Montastraea annularis* es la especie que exhibe una mayor mortalidad parcial en relación con los demás corales. Una notable ausencia de reclutamiento sexual y juveniles de *M. annularis* ilustra el cambio progresivo de cambio de un sistema dominado por *Montastraea.* Este cambio, que se está produciendo en todo el Caribe, se caracteriza por un dominio cada vez mayor de especies más pequeñas y de vida corta como *Agaricia* y *Porites*, y una reducción en el tamaño de los corales masivos longevos. El seguimiento de la sobrevivencia de los reclutas es necesario para determinar si los arrecifes del Caribe mantendrán la misma función, estructura, identidad y retroalimentación (signos clave de la resiliencia), y si las pérdidas de *M. annularis* (complejo) continuarán a los niveles actuales. La evaluación rápida presentada aquí posibilita caracterizar la estructura de tamaño de las colonias, los niveles de reclutamiento y determinar si los corales pequeños representan sobrevivientes de colonias que incrementan su tamaño o colonias grandes (más viejas) que siguen disminuyendo de tamaño. Este enfoque puede ayudar a determinar la historia de un sitio y su capacidad de recuperación.

Palabras clave: resiliencia, estructura en la talla del coral, reclutamiento de coral, sobrevivencia y crecimiento, monitoreo de corales, evaluación de corales

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Transplantation of storm-generated coral fragments to enhance Caribbean coral reefs: A successful method but not a solution

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Received 8-VII-2011. Corrected 16-XII-2011. Accepted 20-XII-2011.

Abstract: In response to dramatic losses of reef-building corals and ongoing lack of recovery, a small-scale coral transplant project was initiated in the Caribbean (U.S. Virgin Islands) in 1999 and was followed for 12 years. The primary objectives were to (1) identify a source of coral colonies for transplantation that would not result in damage to reefs, (2) test the feasibility of transplanting storm-generated coral fragments, and (3) develop a simple, inexpensive method for transplanting fragments that could be conducted by the local community. The ultimate goal was to enhance abundance of threatened reef-building species on local reefs. Storm-produced coral fragments of two threatened reef-building species [*Acropora palmata* and *A. cervicornis* (Acroporidae)] and another fast-growing species [*Porites porites* (Poritidae)] were collected from environments hostile to coral fragment survival and transplanted to degraded reefs. Inert nylon cable ties were used to attach transplanted coral fragments to dead coral substrate. Survival of 75 reference colonies and 60 transplants was assessed over 12 years. Only 9% of colonies were alive after 12 years: no *A. cervicornis*; 3% of *A. palmata* transplants and 18% of reference colonies; and 13% of *P. porites* transplants and 7% of reference colonies. Mortality rates for all species were high and were similar for transplant and reference colonies. Physical dislodgement resulted in the loss of 56% of colonies, whereas 35% died in place. Only *A. palmata* showed a difference between transplant and reference colony survival and that was in the first year only. Location was a factor in survival only for *A. palmata* reference colonies and after year 10. Even though the tested methods and concepts were proven effective in the field over the 12-year study, they do not present a solution. No coral conservation strategy will be effective until underlying intrinsic and/or extrinsic factors driving high mortality rates are understood and mitigated or eliminated. Rev. Biol. Trop. 60 (Suppl. 1): 59-70. Epub 2012 March 01.

Keywords: *Acropora cervicornis, A. palmata*, coral mortality, *Porites porites,* reef restoration, coral transplantation.

Continuing declines and the lack of recovery on coral reefs worldwide have sparked renewed calls for action by the scientific, conservation, and reef management communities (e.g., Bruno & Selig 2007, Mumby & Steneck 2008, Rinkevich 2008, Teplitski & Ritchie 2009). Consensus exists around the need to maintain as much genetic diversity, structural and biological integrity, and ecological services as possible if reefs are to be sustainable over time (e.g., Roberts *et al.* 2006, Shearer *et al.* 2009). Less agreement surrounds how best to achieve those goals and how to proceed in response to declining abundance of corals and other reef organisms. In cases of acute physical damage to reefs, such as in ship groundings, sophisticated engineering methods have been developed to mitigate damage and to maximize recovery and are used in combination with substrate stabilization and colony transplantation (e.g., Jaap *et al.* 2006). But increasingly, loss of live coral has been related to disease and abnormally high water temperatures and not to direct impacts from human activities (e.g., Miller *et al.* 2009). As researchers work to deepen understanding of reef ecology, coral

reproduction, disease processes, and predation and to identify environmental drivers and effects from a changing climate, what is the appropriate response to the continuing loss of coral? What response at the local level will most effectively reduce further losses, minimize species extinctions, guard against loss of reproductive capacity, and maintain reef services such that local reefs and the well-being of human communities are simultaneously maintained (Knowlton 2006)?

Recently, restoration strategies have focused on the broader conservation effort, emphasizing the need to combine local management actions, such as establishment of no-harvest marine reserves and effective management of the coastal zone (both terrestrial and marine), with direct actions, such as transplantation (Epstein *et al.* 2005, Edwards & Gomez 2007, Mumby & Steneck 2008, Young 2000). Transplantation of coral colonies or fragments, whether from aqua-, mariculture or harvesting from a healthy colony, has been the most frequently recommended action for increasing coral abundance on damaged or degraded reefs and for conserving listed or "at-risk" species (e.g., Epstein *et al.* 2005, Edwards & Gomez 2007, Rinkevich 2008, Teplitski & Ritchie 2009). Yet there is a deepening awareness that no habitat, once damaged or degraded, can be restored to its original condition (Young 2000) and that the basic factors causing declines must be addressed if restoration of reefs and conservation of threatened reef species are to succeed over time (Edwards & Clark 1998, Birkeland 2004, Kaufman 2006, Bruno & Selig 2007). It has been suggested that newly developed molecular tools be used to optimize selection of coral propagules for cultivation and transplantation, to deepen our understanding of transplant survival (Baums 2008, Vollmer & Kline 2008), and to identify and maximize the genetic diversity of transplants (Shearer *et al.* 2009), which is considered essential. Debate continues over the effectiveness of transplantation in conserving threatened coral species, increasing coral abundance, and accelerating reef restoration or enhancement at ecologically relevant temporal and spatial scales. This controversy is due in part to the small scale of transplant studies compared to the scale of reef damage (e.g., Edwards & Gomez 2007) and the relatively short duration of most studies. Published research documenting transplant survival for 5 years or more is rare in the scientific literature (Bruckner & Bruckner 2006, Garrison & Ward 2008, Bruckner *et al.* 2009), although reports from proprietary restorations (e.g., ship groundings) exist but are difficult to access. Recently, a few large-scale transplantation studies have been initiated (Normile 2009). Their findings after 5 years, 10 years and beyond will be of great interest.

In 1999, a small-scale coral fragment transplantation project was initiated in a Caribbean marine protected area (Virgin Islands National Park, U.S. Virgin Islands). The primary objectives were to (1) identify a source of coral colonies for transplantation that would not result in damage to reefs, (2) test the feasibility of collecting and transplanting storm-generated coral fragments, and (3) develop a simple, inexpensive method for transplanting fragments that could be conducted by the local community. The ultimate goal was to enhance abundance of threatened reef-building species on local reefs. Although small in scope, this is one of only two long-term studies of coral transplants. The survival and growth of transplant and reference colonies over 12 years are presented, as are lessons learned.

MATERIALS AND METHODS

Study: This study was conducted from May 1999 to April 2011 on four reefs within Virgin Islands National Park (VINP, St. John, U.S. Virgin Islands; Fig. 1). The study sites, experimental design, field methods (coral fragment collection, handling, transport, placement and orientation), criteria for attachment-substrate, data collection and analysis, and statistical models are detailed in Garrison & Ward (2008), as are analysis and interpretation of the first 5 years (1999-2004) of data. Briefly, unattached storm-produced fragments of three

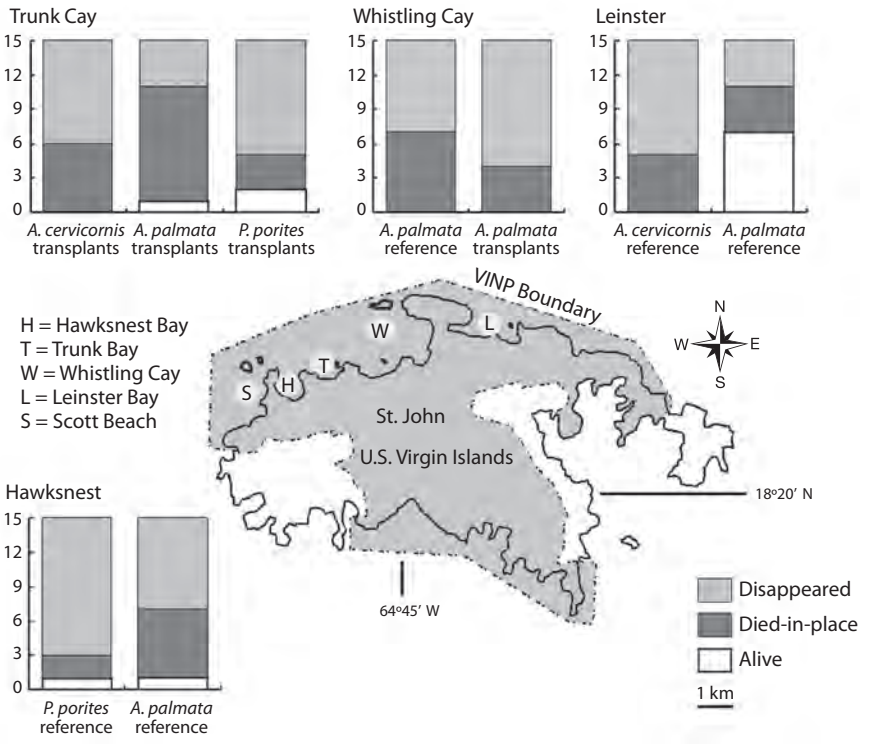

Fig. 1. Map of transplant and donor reefs in Virgin Islands National Park (VINP), St. John, U.S. Virgin Islands. Bar graphs represent the proportion of transplants and reference colonies that survived (white), died in place (dark gray), or were lost to displacement (disappeared; light gray) for each species at each site at 12 years (2011).

fast-growing Caribbean species [*Acropora palmata* (Lamarck, 1816) (elkhorn coral), *A. cervicornis* (Lamarck, 1816)(staghorn coral), and *Porites porites* (Pallas, 1766)(finger coral)] were collected from shallow (1-3m) sandy or bare substrate unfavorable for survival due to abrasion and tumbling (e.g., Bowden-Kerby 2001) and were transplanted to degraded reefs. These three species were chosen based on their life histories and reproductive strategies: all are fast growing; all colonize primarily via fragmentation; and healthy-appearing fragments of all three species were available in sufficient numbers for transplantation. Transplant reefs were selected based on similarity to fragment donor reefs in regard to depth, substrate type, water quality, water-mass turnover, and the presence of dead, intact *A. palmata* skeletons for attachment of transplants. Inert nylon cable ties were selected over uncoated wire, monofilament line, and underwater epoxy to secure the fragments to dead, standing *A. palmata* skeletons or other reef framework (see Garrison & Ward 2008).

To follow the natural mortality rates of each of the three species over the timespan of the experiment and to control for environmental/site effects, reference colonies on donor and transplant reefs were selected to be as similar to transplanted fragments as possible, based on size, depth, and exposure to ocean swells (Table 1). There was no reference colony monitoring at Scott Beach due to hazardous boat traffic, and coral abundance was too low at Trunk Cay and Scott Beach for monitoring. One hundred thirty-five corals (60 transplanted fragments and 75 reference colonies) were tagged, photographed, measured, and qualitatively assessed

TABLE 1
The numbers of monitored reference colonies and transplanted fragments are shown for each species (*Acropora cervicornis*, *A. palmata*, and *Porites porites*) by site. Source reefs of transplanted fragments are indicated (adapted from Garrison & Ward 2008)

St. John, U.S. Virgin Islands	Latitude Longitude	Coral species	Number of reference colonies	Number of fragments transplanted	Source of transplanted fragments
Trunk Cay	18.353 N	*A. cervicornis*		15	Scott Bay
	64.763W	*A. palmata*		15	Leinster Bay
		P. porites		15	Scott Bay
Hawksnest Bay	18.347 N	*A. palmata*	15		
	64.780W	*P. porites*	15		
Whistling Cay	18.372 N	*A. palmata*	15	15	Leinster Bay
	64.747W				
Leinster Bay	18.363 N	*A. cervicornis*	15		
	64.750W	*A. palmata*	15		
Total colonies		*A. cervicornis*	15	15	
		A. palmata	45	30	
		P. porites	15	15	
		All species	75	60	

at 6-month intervals from May 1999 to July 2001, annually from 2001 to 2004, 2009, and 2011. For the year-12 assessment in 2011, all transplanted fragments that were in place on the reef and reference colonies, alive or dead, were photographed, live tissue was measured, and the presence of lesions, disease signs, paling, and predators was recorded.

Data analysis: In survivorship analyses, coral colonies were considered dead and were removed from further inclusion in the dataset if: (1) the entire colony or fragment had disappeared and could not be relocated (physical dislodgement), or (2) live tissue was not observed (100% tissue loss). Differences in survival probability were assessed using the generalized linear model module of Statistica 6.0 with a specified binomial distribution and complimentary log-log (clog-log) link. The clog-log link function is recommended when data are "interval censored" (i.e., mortality occurs in continuous time, but is observed at discrete intervals; Singer & Willett 2003). Logistic regression procedures offer an alternative to ordinary least-squares regression, since bivariate outcomes (e.g., survival or death) seldom meet statistical assumptions required for ordinary regression (Peng *et al.* 2002).

After 12 years, the complexity of the experimental design and size of the data set resulted in categorical independent variables that were often defined by low sample sizes and/or risk sets. In conjunction with coarsening of sampling periods over a relatively long-duration study, a more parsimonious linear time effects model was chosen to avoid problems in model fitting associated with maximum-likelihood algorithms (e.g., model convergence, coefficient stability). Based on previous analyses of the 1999-2004 data set (Garrison & Ward 2008), a linear regression model was chosen to describe risk sets over time and was allowed to interact non-proportionally with time. Parameter effects were included based on significant improvements ($\alpha = 0.05$) in the log-likelihood ratio, against the χ^2-distribution, relative to a constant time-effects model (i.e., intercept only, hazard is constant through time).

RESULTS

Coral survival: After 12 years, only 9% (12) of the initial 135 colonies were alive: 3%

of *A. palmata* transplants (1 of 30) and 18% (8 of 45) of reference colonies; no *A. cervicornis* colonies; and 13% (2 of 15) of *P. porites* transplants and one reference colony (7%; Fig. 2). The mean mortality rates of *A. palmata* and *P. porites* colonies remained constant over the study (31 and 33% yr^{-1} likelihood of colony loss, respectively), whereas for *A. cervicornis* the mortality rate increased on average by a factor of 1.5 annually (Fig. 2; Garrison & Ward 2008). *Acropora palmata* transplants were 2.3 times more likely to die in the first year than reference colonies, but not thereafter (Fig. 2). There was no significant difference between transplant and reference colony survival for *A. cervicornis* ($\chi^2_{(0.05,\ 2}$ =0.359, p=0.836), *P. porites* ($\chi^2_{(0.05,\ 3)}$=1.848, p=0.605), or between *A. palmata* colonies transplanted from Hawksnest Bay to Trunk Cay and reference colonies in Hawksnest Bay ($\chi^2_{(0.05,\ 2)}$ =2.51, p=0.285; Fig. 1). However, the mean probability of *A. palmata* transplant mortality in Whistling Cay was 4.8 times greater than the Leinster Bay reference colonies in the first year of the study ($\chi^2_{(0.05,\ 1)}$ =11.74, p<0.001) but did not significantly differ in the year-to-year rate of decline thereafter.

Over the 12-year study, 56% of the initial 135 corals were lost due to physical dislodgement and 35% died in place. The relative roles of physical dislodgement and mortality in place varied among years (Fig. 2). Physical dislodgement was the major cause of mortality of transplants and reference colonies in the first year (67% and 75%, respectively). Mortality in place played a greater role in *A. palmata* (56%) than in *A. cervicornis* (47%) or *P. porites* losses (27%; Fig. 2) and was most likely the result of disease, predation, high-temperature stress, or some combination. Physical damage was observed on most colonies at all sites and at most assessments, yet damage to colonies did not predict future survival/morality. Many colonies sustained serial damage only to survive and grow while others died despite no visible physical damage.

Survival of *A. palmata* transplants ($\chi^2_{(0.05,\ 3)}$=2.804, p=0.246) and *A. cervicornis* and *P. porites* transplants and reference colonies did not show a site effect, whereas survival of *A. palmata* reference colonies differed significantly among sites (Table 2 and Fig. 1; Leinster 47%, Hawksnest 7%, and Whistling Cay 0%). In the first year, *A. palmata* reference colonies exhibited a 12% yr^{-1} mean probability of mortality at all sites. Reference colonies in Leinster Bay continued on this trajectory for 12 years, whereas the mean mortality rate at Whistling Cay and Hawksnest Bay increased annually by 1.25- and 1.32-fold, respectively ($\chi^2_{(0.05,\ 2)}$=8.688, p<0.013).

The initial log-mean live-tissue size of transplanted coral fragments differed from reference colonies across all species, with

TABLE 2

Reduced linear model, logistic regression results of the survival of 45 *Acropora palmata* reference colonies: 15 colonies each on Leinster Bay, Hawksnest Bay, and Whistling Cay reefs. Survivorship was monitored for 12 years. Probability of mortality of reference colonies at Leinster Bay did not change over time, whereas probability of *A. palmata* reference colony mortality increased annually by a factor of 1.25 on Hawksnest and 1.32 on Whistling Cay reefs

Reduced linear model	β	SE β	Wald's χ^2	d.f.	p	Hazard ratio (e^{β})
Intercept	-2.01	0.23	80.53	1	<0.001	NA
Hawksnest Bay colony survival x time interaction	0.22	0.06	12.79	1	<0.001	1.25
Whistling Cay colony survival x time interaction	0.28	0.07	17.37	1	<0.001	1.32

Logit parameter estimates (ß) and standard errors, Wald's chi-square statistics (Wald χ^2), degrees of freedom (d.f.), significance test results (p), and Hazard ratio [(e^{β}; an estimate of the size of effect of time on the base hazard (in this case, the intercept estimate)].

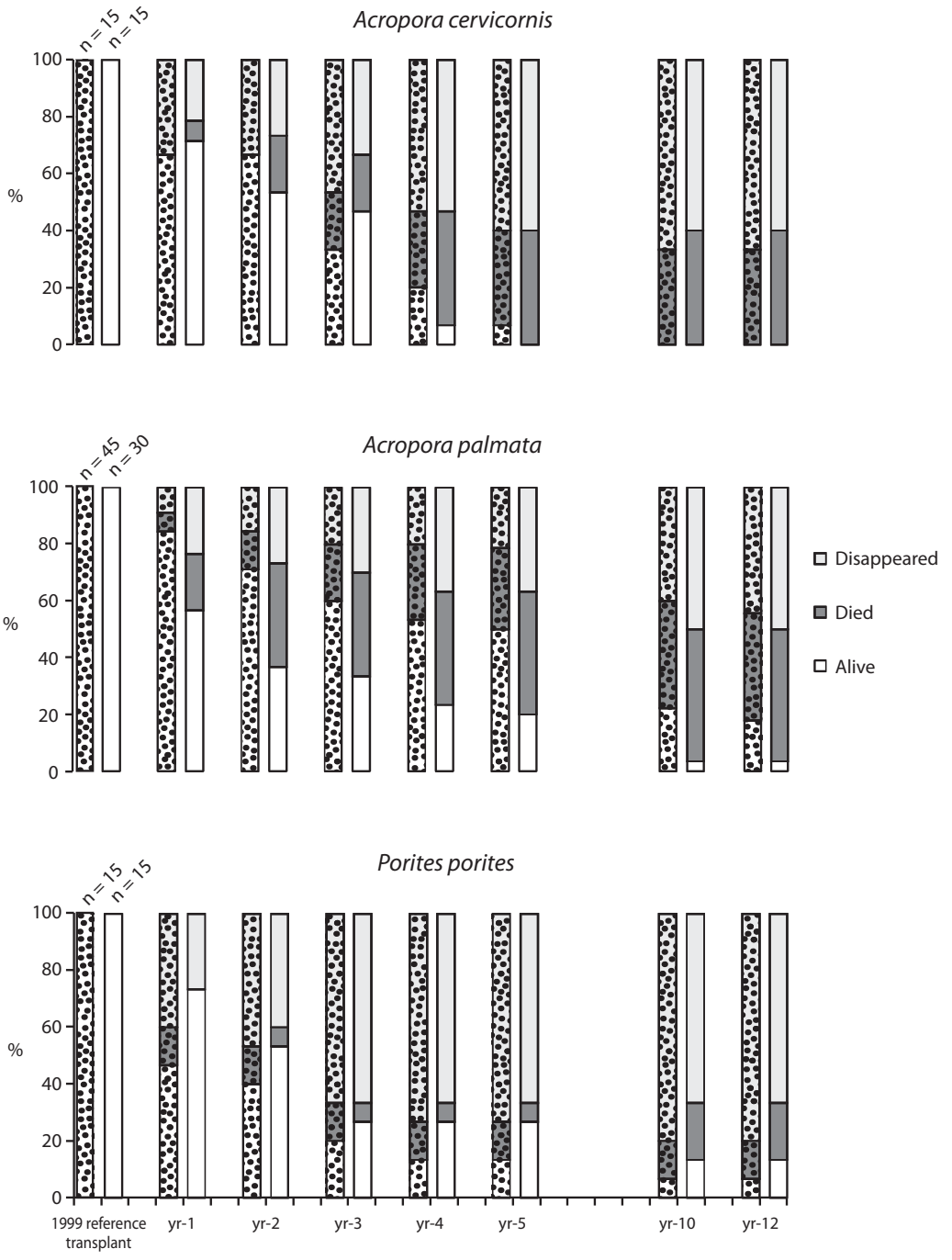

Fig. 2. The percentage of transplants (solid color bars) and reference colonies (pebble-texture bars) that survived (white), died in place (dark gray), or were lost to displacement (light gray) for each species over 12 years.

reference colonies generally larger than transplants (see Table 2, Garrison & Ward 2008). Size was a factor in survival only for *A. palmata* colonies in the first 5 years; the probability of mortality or dislocation of an *A. palmata* colony or transplant in the following year decreased by 15% per year with every 0.1 unit increase in log-maximum colony length over the first 5 years (β=-1.60, 95% C.I.=-2.59, -0.61; Garrison & Ward 2008).

Colony growth: Two of the three transplants and 67% (6/9) of the reference colonies alive at 12 years had increased in size. Maximum diameter of the single surviving *A. palmata* transplant increased more than 6-fold over the 12-year study [from 20 cm in 1999 to 130 cm prior to being physically dislodged in spring 2011 (Fig. 3)].

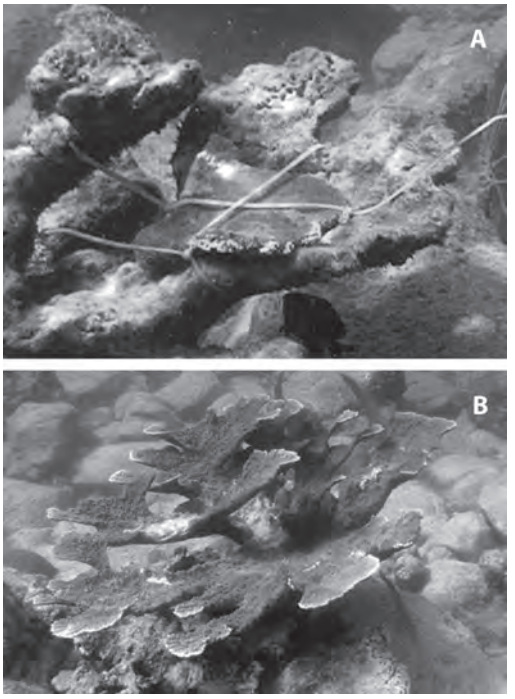

Fig. 3. Images of transplanted *Acropora palmata* fragment that survived 12 years. Top image: fragment when it was transplanted in 1999 (20 cm maximum dimension); bottom image: same transplanted fragment had grown to 130 cm maximum dimension in 2009.

Cost: Transplantation costs were low despite the small scale of the project and use of boats and scuba, all of which would be expected to increase the cost per transplant (Garrison & Ward 2008). Cost of all materials, use of a boat and scuba, and scientist salary totaled US$21 per transplant (Garrison & Ward 2008). Cost would decrease further to a fraction of US$1 per transplant for nylon cable ties if all work was conducted by volunteers on snorkel (Garrison & Ward 2008). Collection, transportation, and attachment of each fragment to a reef 1 - 5 km distant required 0.6 hr per fragment (Garrison & Ward 2008).

DISCUSSION

The transplantation methods and concepts tested in the field over the 12-year study were shown to work. (1) Storm-produced coral fragments of *A. palmata*, *A. cervicornis*, and *P. porites* that were collected, transplanted, and attached to the reef using nylon cable ties had survival rates similar to reference colonies over 12 years. (2) No damage was inflicted on reefs or coral colonies in obtaining coral transplants. (3) Although physical displacement was the primary cause of mortality overall, the loss of a greater proportion of reference colonies than transplants to physical displacement supports the effectiveness of inexpensive and easy-to-use cable ties for transplant attachment and confirms conclusions reached by others (Bruckner & Bruckner 2001, Williams & Miller 2010, Forrester *et al.* 2011). However, concepts and processes shown to work in the field do not necessarily translate into viable solutions for coral reef rehabilitation.

Coral mortality was striking with loss of all *A. cervicornis* by year-5 and most *A. palmata* and *P. porites* transplant and reference colonies by year-12 (Fig. 2). Although the high rates of mortality observed could be an artifact of small sample size and the dramatic diminishing of sample size through time, the findings are consistent with most Caribbean region research (e.g., Hughes 1994, Aronson & Precht, 1997, 2001, Rogers 1999, Bruckner *et*

al. 2009). Patches of healthy *A. palmata* and *A. cervicornis* exist (e.g., Vargas-Ángel & Thomas 2002, Vargas-Ángel *et al.*, 2003) but the regional picture is one of decline (e.g., Gardner *et al.* 2003, Rogers *et al.* 2009, and references therein). These findings paint an unambiguous picture of dynamic turnover of individual coral colonies on shallow-water Caribbean reefs and present a bleak outlook for the (1) long-term survival of transplants of these species and (2) the viability of two of the study species – *A. cervicornis* and *P. porites*. Both *A. palmata* and *A. cervicornis* have been key reef-building species in the Caribbean for thousands of years (e.g., Aronson & Precht 1997, Pandolfi *et al.* 2005) and even though the life spans of individual colonies may be relatively short, populations may persist over time (Jaap *et al.* 2006). Nonetheless, the accelerating decline of *A. cervicornis* documented in the first 5 years and the high mortality rates of transplants and reference colonies of all three species documented here invoke concern.

Most coral transplantation research spans months, with few studies continuing for more than 2 years. *Acropora palmata* transplant survival documented in this study was similar to but lower than that reported in the only other long-term study of reattached *A. palmata* fragments (Bruckner *et al.* 2009), despite the considerable difference in scale of the studies [n=30 this study (VI); n=1857, Mona Island, Puerto Rico (PR)]. The difference in fragment survival between the two studies narrowed from year-2 (43% VI; 57% PR, Bruckner & Bruckner 2001] to year-10 (3% VI; 6% PR, Bruckner *et al.* 2009), indicating a realistic range of *A. palmata* fragment survival over time that could be expected in restoration efforts in the NE Caribbean. Storm-generated coral fragments were selected as the source of corals to transplant in this study because (1) intact corals were not damaged to create transplants, (2) fragment survival was maximized by attachment to the substrate (Williams & Miller 2010, Forrester *et al.* 2011), and (3) damage to intact colonies from unattached fragment projectiles was reduced. A valid concern is that storm-generated fragments may have lower survival rates as a result of damage sustained during the fragmentation process itself and from subsequent abrasion. However, similar survival rates for transplants and reference colonies were documented for three of the four sets of transplants (*A. cervicornis, P. porites*, and *A. palmata* Trunk transplants/Hawksnest reference colonies), indicating that damage to transplants from the natural fragmentation process, or from collection and transplantation was not a factor in survival.

The high mortality of transplants and reference colonies overall indicates that environmental (extrinsic) and/or organismal (intrinsic genotypic or molecular-level function) factors and not transplant/reference status or experimental methodology were driving mortality. An extrinsic factor, storm-generated swells, appeared to play a key role. Physical dislodgement caused more than one-half of *A. cervicornis* and *P. porites* colony mortality and nearly one-half of *A. palmata* transplant and reference colony mortality (Fig. 2). Surprisingly, the effect of location on survival of *A. palmata* colonies was not related to degree of direct exposure to ocean swells or distance from human activities. The site with highest *A. palmata* transplant and reference colony mortality (Whistling Cay) was the most protected from ocean swells, farthest from the island of St. John and associated pollution, and subjected to the least human activity (Fig. 1). The sites with lowest mortality (Leinster and Hawksnest) were directly exposed to storm swells and human activities (boats and snorkeling) and directly adjacent to the island and a road (Fig. 1). Reef architecture or bathymetric configuration at each site may have differentially enhanced or impeded water movement and have been a factor driving differences in mortality observed among sites. Intrinsic factors such as genotypes that produce faster growth or stronger and less brittle skeletons could account for the apparent robustness of colonies that survived strong storm swells at the lower-mortality sites (Bowden-Kerby 2009). Similarly, although disease-like lesions were observed

at all sites, colonies with disease-resistant genotypes or more robust immune function would be more likely to resist and survive infection (e.g., Ritchie 2006, Rosenberg *et al.* 2007, Vollmer & Kline 2008, Teplitski & Ritchie 2009). Multiple environmental factors that may have adversely impacted corals during the study period include elevated water temperatures, disease, chemical pollutant or nutrient influx, changes in salinity, acidification, sedimentation, predation, and the more subtle effects from loss of top predators or herbivores on reefs. Possible intrinsic factors include impaired immune function due to genotype (disease resistance; Vollmer & Kline 2008); immunosuppressors in the environment; changes in the microbial community of the coral holobiont (Ritchie 2006, Rosenberg *et al.* 2007, Teplitski & Ritchie 2009); impaired calcification; and/or genetic sensitivity to environmental stressors. The factors driving differences in survival among sites remain unknown but multiple factors are likely (e.g., Birkeland 2004, Bruno *et al.* 2007, Muller *et al.* 2008, Nyström *et al.* 2008).

Many restoration scientists have stressed the need for coral transplantation to be sustained over time and at an appropriate scale if it is to be effective (e.g., Rinkevich 1995, 2008, Epstein *et al.* 2005, Edwards & Gomez 2007). Results from this small-scale study bring into question whether such a major effort could be successful if underlying factors driving declines are not also addressed. In cases where coral mortality and reef degradation are primarily due to acute damage from humans, transplantation of coral colonies might help to conserve coral species and help accelerate reef recovery, but only if undertaken in conjunction with other actions such as management of human activities through use of marine protected areas, enforcement of marine and terrestrial regulations, and education (e.g., Epstein *et al.* 2005, Rinkevich 2005, 2008). If chronic global or regional stressors such as abnormal water temperatures, contaminants, or disease are the primary drivers of declines on coral reefs, it is difficult to understand how transplantation, even on the scale of tens of thousands of transplants across tens of hectares, could succeed in conserving species or restoring reefs over time if the drivers of mortality and degradation are not addressed (Birkeland 2004, Kaufman 2006). Is 3% survival of transplants at 12 years an acceptable outcome? Will 3% transplant survival at 12 years be effective in halting declines of threatened coral species, maintaining genetic diversity and ecosystem services and functions, or staving off species extinction if corals on the surrounding reefs are dying at a similar rate?

To retain *A. palmata* genetic diversity when restoring damaged coral reefs, Shearer *et al.* (2009) suggest that fragments from 7-10 donor colonies would retain 50% of allelic diversity and fragments from 30-35 colonies would retain 90% diversity. What fraction of that transplanted genetic diversity can be expected to be retained with 3% survival of transplants at 12 years? Vollmer & Kline (2008) have proposed an interesting integrated strategy for conserving *A. cervicornis*, a threatened species that has been decimated by epizootics, has limited sexual recruitment, and has been shown to have low gene flow (Vollmer & Palumbi, 2007): (1) protect remnant populations that have survived epizootics or other extrinsic insults; and (2) transplant laboratory or maricultured disease-resistant genotypes. This could be extended to *A. palmata*, which in this study at this location in this time period appeared to be more robust than *A. cervicornis*.

CONCLUSIONS

This project was initiated to test the feasibility of using a non-destructive source of coral transplants by collecting storm-produced coral fragments and to develop a simple, inexpensive method for transplanting fragments that could be conducted by the local community. The ultimate goal was to enhance recovery of important reef-building species that were in decline. The major lessons learned from this 12-year study are:

- the larger the transplanted fragment, the greater the probability of survival (Garrison & Ward 2008);
- transplant survival varied among species;
- survival rates of storm-generated coral fragments collected and transplanted to reefs were similar to those of reference colonies;
- inexpensive, inert nylon cable ties effectively attach coral fragments to dead coral skeleton.
- the low survival rates of *A. palmata*, *A. cervicornis*, and *P. porites* transplant and reference colonies at 12 years brings into question the efficacy of transplantation for conservation of coral species or reversal of reef degradation;
- coral transplantation will not be effective in conserving coral species or in assisting reef recovery over time until the underlying factors causing degradation of reefs and mortality of corals are understood, addressed, and eliminated or mitigated;
- community involvement is important in building what Brightsmith *et al.* (2008) call "conservation constituencies," an informed and engaged public that in turn educates the wider community, thereby reducing damage to reefs.

ACKNOWLEDGMENTS

Thanks go to the National Park Foundation, Canon U.S.A., Inc., and the U.S. Geological Survey for funding the project and to Friends of Virgin Islands National Park, the Trust for Public Land, and Virgin Islands National Park for in-kind support. This research was conducted under NPS permits #VIIS-2002-SCI-0012 (VIIS-0217), VIIS-2004-SCI-0023 (VIIS-04020), and VIIS-2009-SCI-0009 (VIIS-09010). We are indebted to: M. Quade, B. Bremser-Nielsen, S. Caseau, D. Downs, J. Garrison, T. Kelley, E. Link, W. Stelzer, R. Waara, 70 community volunteers, and the Pine Peace School 5th- and 6th-grade science classes. Thanks to C. Rogers for insightful comments on project design, and to anonymous reviewers for their thoughtful comments. Any use of trade names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

RESUMEN

En respuesta a la dramática pérdida de corales constructores de arrecifes y la continua falta de recuperación, un proyecto de pequeña escala de transplante de corales, al cual se le dio seguimiento por 12 años, se inició en el Caribe (Islas Vírgenes de EUA) en 1999. Los principales objetivos fueron (1) identificar fuentes de colonias de coral para el trasplante, que no produjeran daños a los arrecifes, (2) evaluar la viabilidad del trasplante de fragmentos de coral generados por tormentas, y (3) desarrollar un método simple y barato para transplantar fragmentos que pudiera ser realizado por la comunidad local. La meta última era aumentar la abundancia de especies constructoras de arrecife amenazadas en los arrecifes locales. Fragmentos de coral producidos por tormenta de dos especies constructoras de arrecife amenazadas [*Acropora palmata* y *A. cervicornis* (Acroporidae)] y otras especies de crecimiento rápido [*Porites porites* (Poritidae)] fueron recolectadas en ambientes no adecuados para la supervivencia de fragmentos de coral y se trasplantaron a los arrecifes degradados. Fajitas de nylon inerte fueron utilizadas para unir los fragmentos de corales transplantados al sustrato de coral muerto. La sobrevivencia de 75 colonias de referencia y de 60 transplantadas fueron monitoreadas por más de 12 años. Sólo el 9% de las colonias estaban vivas tras 12 años, sin presencia de *A. cervicornis*, el 3% de los transplantes de *A. palmata* y el 18% de las colonias de referencia de *Acropora.* El 13% de los transplantes de *P. porites* y el 7% de las colonias de referencia sobrevivieron. El desprendimiento físico resultó en la pérdida del 56% de las colonias, mientras que el 35% murió en el lugar. Solamente *A. palmata* mostró una diferencia en sobrevivencia entre los trasplantes y las colonias de referencia, eso fue solo en el primer año. La ubicación fue un factor en la sobrevivencia sólo para las colonias de referencia de *A. palmata* y después de 10 años. A pesar de que los métodos y los conceptos fueron probados efectivamente en el campo por más de 12 años de estudio, no mostraron ser la solución. Ninguna estrategia de conservación va a ser efectiva hasta que se delimiten y sean entendidos, mitigados o eliminados los factores intrínsecos y/o extrínsecos que conducen a las altas tasas de mortalidad.

Palabras clave: *Acropora cervicornis, A. palmata*, mortalidada de coral, *Porites porites,* restauración de arrecifes, transplantes de corales

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Benthic and fish population monitoring associated with a marine protected area in the nearshore waters of Grenada, Eastern Caribbean

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Received 15-VII-2011. Corrected 1-XII-2011. Accepted 20-XII-2011.

Abstract: Annual benthic and fish population surveys were completed at five locations in the nearshore waters along Grenada´s southwest coast during 2008 - 2010. Two survey sites are located in a newly launched Marine Protected Area (MPA). Photo Quadrat (PQ) and Point Line Intercept (PLI) surveys were used to determine substrate cover. Algae was the primary live cover increasing significantly from 45.9% in 2008 to 52.7% in 2010 (PLI). Algae was also predominant (61.0% - 59.3%) in the PQ surveys although annual variation was not significant. Hard coral cover ranged from 16.5% to 15.4% (PLI) and 11.4% to12.0% (PQ) with no significant differences between years. Branching and encrusting corals occurred more frequently than massive corals. In the three annual surveys neither algal cover nor hard coral varied significantly between MPA and non-protected areas (PLI). Relative abundance of fishes along 30x2m belt transects did not vary significantly among years however density of fishes decreased significantly across years for most major groups. *Chromis* spp. dominated the survey sites at 65.2% in 2008 and 49.8% in 2010, followed by territorial damselfish,11.1% and 15.5%, wrasse increased from 7.3% to 15.5%. Both the substrate cover and fish survey data analyses indicated a stable but degraded community. Annual surveys are planned at these sites for the foreseeable future. Existing and future data from this project will be valuable in determining the efficacy of MPA management, guiding resource management decisions and monitoring the health status of Grenada's valuable reef systems. Rev. Biol. Trop. 60 (Suppl. 1): 71-87. Epub 2012 March 01.

Key words: benthic cover, coral, reef fish, monitoring, Grenada, Eastern Caribbean, marine protected area.

The island nation of Grenada is part of the Eastern Caribbean region recently classified as being at "very high risk" by the Reefs at Risk in the Caribbean report (Bouchon *et al.* 2008). Of the 160 km^2 of reef area in Grenada 41% were listed as having a high-risk threat index and 40% were listed as very high (Burke & Maidens 2004). The primary contributors to this rating were coastal development and fishing pressure.

Coral communities rely on large herbivorous fish species to manage levels of macroalgae (Burkepile & Hay 2010, Ceccarelli *et al.* 2011, Walsh 2011). In an analysis of the Grenadian demersal fish catch and fishing effort from 1986 to 1993, Jeffrey (2000) found that the number of boats employed in the demersal fishery off the west coast of Grenada increased by 200% however the catch declined by nearly 75% during this seven year period. Local overfishing often targets large herbivorous species reducing these fish stocks thus contributing to increased abundance of macroalgae on coral reefs (Hawkins & Roberts 2003). One impact of increased algal abundance is reduced growth and recruitment of coral polyps (Bascompte *et al*. 2005, Arnold 2007, Birrell *et al.* 2008, Mora 2008).

Introduction of excess nutrients to coral reef systems from coastal development further

enhances overgrowth of algae (Lapointe *et al.*1997) and directly inhibits coral recruitment and growth (Littler *et al.* 2009). These local stressors weaken a coral community's resilience (Hughes 1994, Hughes *et al.* 2003, Gardner *et al.* 2003, Wilkinson 2008) making it more vulnerable to global climate change and increased storm activities (Goldenberg *et al*. 2001, Eakin *et al.* 2010, Hughes *et al.* 2010). Grenada has been impacted by two major hurricanes in the past decade: Ivan in September 2004 and Emily in July 2005. Major storms such as these can result in devastating effects on reefs breaking down the basic structure and dislodging corals leaving leveled areas of rubble (Woodley *et al.* 1981).

Many countries have established Marine Protected Areas (MPAs) that restrict some uses of coastal reef systems with the hope that these sections will provide a source of biodiversity to adjacent or "down current" locations. Unfortunately, there is a paucity of data that demonstrates the effectiveness of specific management practices. While the Grenadian government established legislation for the Moliniere-Beausejour MPA on the southwest coast of the island in 2001, no significant management practices were implemented until 2010. Permanent mooring buoys were established in 2009, warden patrols began in 2010 and some fishing practices were restricted from September 2010.

A development plan for Grenada's National Protected Areas System identified the need for external assistance in research and monitoring of Grenada's protected areas (Mac Leod 2007). Initial surveys at nine sites off the southwest coast of Grenada in 2006 and 2007 identified macroalgae was the most abundant substrate cover ranging from 36.5% (± 0.8%) to 53.2 % (± 1.2%). Hard corals covered 23.8% (± 0.9%) to 38.1% (± 1.2%) (Bouchon *et al.* 2008). This 2008-2010 study builds on the initial survey and establishes a foundation upon which the effectiveness of the Moliniere-Beausejour MPA management techniques may be evaluated.

Study Area: Five sites ranging in depth from 5.2m-12.2m, located along Grenada's southwest coast were established in 2008. Similar reefs both inside and outside the MPA that are frequently used by the dive industry were selected. Dragon Bay (12° 5'6.00"N 61°45'45.36"W) and Flamingo Bay (12° 5'30.36"N 61°45'30.60"W) are in MPAs, while Northern Exposure Shallow (12° 1'57.30"N 61°46'14.28"W), Northern Exposure Deep (12° 2'22.14"N 61°46'4.74"W) and Quarter Wreck (12° 1'40.98"N 61°47'0.84"W) are in non-protected areas. Four 30m parallel transects were set up at 5m intervals. Metal stakes mark the beginning and end of each permanent transect.

MATERIALS AND METHODS

The substrate composition of Grenada's southwest coast was surveyed with the Photo Quadrat (PQ) and Point Line Intercept (PLI) methods. The PQ method allows for careful identification of substrate types from a digital photograph. Although identification of substrate types is not always optimal based on digital photos this approach allowed more intense scrutiny of the substrate since time is not a factor in the sampling process. In addition using Coral Point Count with Excel extensions (CPCe) v.3.6 allowed a randomized sampling scheme for each picture (Kohler & Gill 2006). Since there are 60 pictures associated with each transect this increases the total number of observations. The PLI method developed by Crosby & Bruckner in 2002 based on Crosby & Reese (1996) was used to estimate relative abundance of major types of substrate cover and fish species associated with the coral reefs in Grenada's nearshore waters. Four 30m permanent transects were surveyed at each of the five locations. Fish species, as well as *Diadema antillarum*, abundance occurring within a two-meter wide belt (AGRRA Protocol v. 4.0) from the substrate to water surface along each transect were recorded. Benthic substrate was identified and recorded at a point directly below each half-meter mark along each transect.

Divers completed fish data collections along each transect in ten minutes.

Sixty photo quadrats from each transect were processed using Coral Point Count with Excel extensions (CPCe) v.3.6 (Kohler & Gill 2006). A Canon EOS Digital Rebel XTI camera in an Ikelite underwater housing was used to take a picture at every half-meter mark. Attached to the underwater housing was a tube with a calibrated scale used to maintain a consistent distance (60cm) from the substrate and to assist with scaling in the CPCe software program. The images were uploaded into the CPCe software program, and a 20cm by 20cm square was superimposed on the image. Eight points were randomly generated inside the square, and the substrate under each point was identified. Dumas *et al*. (2009) found that whether nine or ninety-nine points were used in a1m^2 area, the difference for large categories was not significant. Thus for the 400 cm^2 area in this study 8 points were deemed sufficient. Also, a Sony HDR-SR8 video camera in an Amphibico underwater housing was used to take video of each location to record a broader perspective of the survey sites.

For both the PLI and PQ data a repeated measure analysis of variance (ANOVAR) using transects as the sampling unit was used to monitor Grenada's southwest benthic community and fish assemblage from 2008 to 2010. To satisfy the assumption of normality, proportional data was arcsine square root transformed and all non-proportional data was log transformed. The Shapiro-Wilk test, as well as skewness and kurtosis values were used to assess normality. Non-normal distributions were examined, and if appropriate outliers were removed (Zar 1999). Mauchly's sphericity test was used to determine sphericity, if violated the Greenhouse-Geisser correction was used to determine significance. Additionally all cases of significance were verified with the multivariate analyses, which do not assume sphericity. A Bonferroni correction (significance value (0.05)/ number of comparisons made) was used when determining significance (Sokal and Rohlf 1995). Also, the Bonferroni correction multiple comparison test was used to determine among which years a significant difference occurred.

To identify interactions between the MPA and non-protected area from 2008 to 2010 a two-way ANOVAR was used. This was only done for the PLI data, because the PQ data had an insufficient sample size. In order to effectively make this comparison the same sample size needed to be used for the MPA and non-protected area. This was accomplished by selecting two of the three non-protected locations, Quarter Wreck and Northern Exposure Shallow. The above criteria for assessing normality, sphericity, and significance were used. When an interaction was found to be significant a follow up one-way analysis of variance (ANOVA) test was used to further examine the interaction. The same criteria for assessing normality were used, and Levene's test of homogeneity was used to evaluate the equality of variances. If the results of Levene's test were found to be significant, then a $p<0.01$ was used to determine significance. The Bonferroni correction was still used when determining significance as well (Sokal and Rohlf 1995).

RESULTS

Substrate (PLI): Algae was the dominant substrate cover found at all locations off Grenada's southwest coast (Fig. 1). Algae increased significantly from 45.9% (SE=1.7; n=35) in 2008 to 52.7% (1.4; 35) in 2010 (ANOVAR, F=7.431, p=0.001). Comparison of major algal groups (macroalgae, turf and coralline) showed that macroalgae consistently dominated the algal community. Turf algae decreased significantly in 2010 and coralline algae increased significantly in 2010 (Tables 1 & 2).

Algal cover in the MPA ranged from 46.3% (3.9; 12) to 51.4% (3.5; 12) over the three years, while in the non-protected area it ranged from 44.0% (3.2; 12) to 50.3% (2.2; 12); no significant interaction between time and location was found (Two-way ANOVAR, F=1.528, p=0.239) (Table 3). Yet the different types of algae experienced significant interaction. Turf algae did have

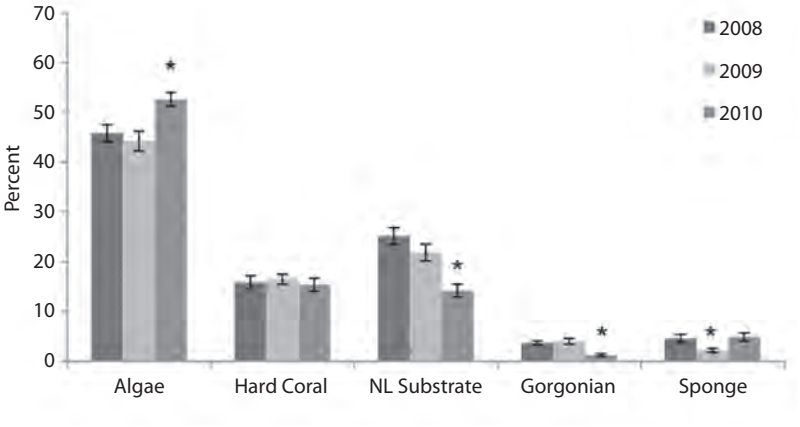

Fig. 1. Mean substrate cover with error bars (SE) based on Point Line Intercept surveys from Grenada's southwest reefs during 2008-2010; * indicates a significant difference (ANOVAR; p<0.017).

TABLE 1

Mean percent cover for algal categories based on Point Line Intercept and Photo Quadrat surveys from Grenada's southwest reefs during 2008-2010. A Bonferroni correction of p<0.017 was used to determine significance

Category	PLI			Bonferroni Comparision	PQ			Bonferroni Comparision
	n	Mean %	SE		n	Mean %	SE	
Macroalgae 2008	32	74.7	2.1		18	70.6	1.9	2010 > 2008 (0.002)
Macroalgae 2009	32	72.4	4.7		18	67.2	1.7	2010 > 2009 (0.001)
Macroalgae 2010	32	71.7	2.2		18	78.0	2.1	
Turf 2008	33	14.8	2.1	2010 < 2008 (p = 0.000)	17	3.8	0.6	2009 < 2008 (0.002)
Turf 2009	33	23.1	4.6	2010 < 2009 (p = 0.000)	17	0.8	0.3	2009 < 2010 (0.001)
Turf 2010	33	2.4	0.6		17	6.0	1.4	
Coralline 2008	24	9.0	1.2	2010 > 2008 (p = 0.000)	16	24.7	2.1	2010 < 2008 (0.001)
Coralline 2009	24	4.2	0.7	2010 > 2009 (p = 0.000)	16	32.6	1.4	2010 < 2009 (0.000)
Coralline 2010	24	23.4	2.1		16	15.9	1.0	

TABLE 2

ANOVAR for major Algal groups based on Point Line Intercept and Photo Quadrat surveys from Grenada's southwest reefs during 2008-2010. Bonferroni correction of p<0.017 was used for determining significance (* indicates significant)

Category	PLI			PQ		
	n	F	p	n	F	p
Macroalgae 2008-2010	32	1.306	0.271	18	13.795	0.000*
Turf 2008-2010	33	14.577	0.000*	17	12.476	0.000*
Coralline 2008-2010	24	31.393	0.000*	16	35.969	0.000*

TABLE 3
Substrate mean based on the Point Line Intercept surveys in non-protected and marine protected areas from Grenada's southwest reefs during 2008-2010. When the Two-way ANOVAR showed a significant interaction between time and location, a one way ANOVA was done for further examination. A Bonferroni correction of p<0.017 was used when determining significance

Substrate	Non-Protected Area			Marine Protected Area			ANOVA	
	n	Mean %	SE	n	Mean %	SE	F	p
Coral 2008	12	15.9	2.6	12	16.4	2.3		
Coral 2009	12	17.6	1.3	12	15.2	2.2		
Coral 2010	12	14.3	1.4	12	19.9	4.3		
Gorgonian 2008	12	3.7	0.9	12	4.6	1.1	0.452	0.508
Gorgonian 2009	12	3.7	1.1	12	6.5	1.3	4.249	0.051
Gorgonian 2010	12	0.7	0.3	12	1.8	0.5	3.137	0.090
Algae 2008	12	47.6	2.6	12	46.9	3.3		
Algae 2009	12	44.0	3.2	12	46.3	3.9		
Algae 2010	12	50.3	2.2	12	51.4	3.5		
NL Substrate 2008	12	21.6	2.5	12	23.2	3.1		
NL Substrate 2009	12	23.1	2.7	12	16.1	2.5		
NL Substrate 2010	12	13.6	1.9	12	15.3	3.1		
Sponge 2008	12	5.6	1.9	12	5.5	0.8	0.857	0.365
Sponge 2009	12	1.4	0.7	12	4.4	1.0	7.511	.012
Sponge 2010	12	4.0	1.2	12	8.0	1.2	7.027	.015

a significant interaction (Two-way ANOVAR, F=6.738, p=0.005), but the follow up tests showed no significant differences between the MPA and non-protected area. Coralline algae also exhibited a significant interaction (Two-way ANOVAR, F=17.752, p=0.000), and for 2010 the 32.4% (3.3; 12) found in the non-protected area was significantly greater than the 18.2% (3.6; 12) in the MPA. Macroalgae did not exhibit any significant interaction (Two-way ANOVAR, F=1.581, p=0.237) (Table 4).

The hard coral cover did not vary significantly from year to year ranging from 16.5% (1.0; 35) to 15.4% (1.3; 35) (Fig. 1) (ANOVAR, F=0.531, p=0.591) (Fig. 1). However the type of hard coral (massive, encrusting and branching) observed did change across the years. Encrusting coral occurred most frequently in 2008 however by 2010 branching coral occurred most frequently (Tables 5 & 6).

Hard coral cover ranged from 15.2% (2.2; 12) to 19.9% (4.3; 12) in the MPA, and 14.3% (1.4; 12) to 17.6% (1.3; 12) in the non-protected area (Table 3). Although hard coral did not differ significantly (Two-way ANOVAR, F=0.072, p=0.931) in overall percent between the MPA and non-protected area encrusting coral did have a significant interaction between time and location (Two-way ANOVAR, F=7.049, p=0.004). Yet in follow up analyses no significant differences between the MPA and non-protected area was found. Massive (Two-way ANOVAR, F=3.555, p=0.046) and branching (Two-way ANOVAR, F=3.170, p=0.091) coral had no significant interaction between time and location (Table 7).

While hard coral cover remained stable, gorgonian cover significantly dropped from 3.7% (0.4; 32) and 4.0% (0.6; 32) in 2008 and 2009 to 1.8% (0.3; 32) in 2010 (ANOVAR, F=19.609, p=0.000). Other significant changes in the substrate were seen in the sponge and non-living categories. Sponge cover saw a sudden decrease from 4.6% (0.8; 35) in 2008 to 2.2% (0.4; 35) in 2009, but recovered to 4.9% (0.8; 35) in 2010 (ANOVAR, F=6.212, p=0.005). Also non-living substrate significantly decreased from 25.2% (1.7; 35) and 21.9% (1.9; 35) in 2008 and 2009 to 14.2% (1.3; 35) in 2010 (ANOVAR, F=14.745, p=0.000) (Fig. 1).

TABLE 4

Algae type mean based on the Point Line Intercept surveys in non-protected and marine protected areas from Grenada's southwest reefs during 2008-2010. When the Two-way ANOVAR showed a significant interaction between time and location, a one way ANOVA was done for further examination. A Bonferroni correction of p<0.017 was used when determining significance

Algae Type	Non-Protected Area			Marine Protected Area			ANOVA	
	n	Mean %	SE	n	Mean %	SE	F	P
Macroalgae 2008	12	70.3	2.9	12	75.6	2.9		
Macroalgae 2009	12	64.0	8.1	12	87.3	2.5		
Macroalgae 2010	12	63.7	3.2	12	78.5	4.1		
Turf 2008	12	17.7	4.1	12	16.1	2.0	0.005	0.945
Turf 2009	12	29.9	7.9	12	8.4	2.0	4.595	0.043
Turf 2010	12	2.1	0.9	12	3.1	1.2	0.237	0.631
Coralline 2008	12	10.6	2.8	12	8.5	1.8	0.143	0.709
Coralline 2009	12	4.9	2.0	12	4.4	1.1	0.161	0.692
Coralline 2010	12	32.4	3.3	12	18.2	3.6	7.329	0.013

TABLE 5

Mean percent cover for hard coral categories based on Point Line Intercept and Photo Quadrat surveys from Grenada's southwest reefs during 2008-2010. A Bonferroni correction of p<0.017 was used to determine significance

Category	Photo Quadrat			Bonferroni Comparison	Point Line Intercept			Bonferroni Comparison
	n	Mean	SE		n	Mean	SE	
Massive 2008	18	36.8	3.6		35	12.9	2.1	2008<2009 (p=0.003)
Massive 2009	18	31.3	3.4		35	27.6	3.1	2008<2010 (p=0.007)
Massive 2010	18	30.5	3.1		35	26.4	3.4	
Branching 2008	19	41.0	6.0		35	32.3	3.9	
Branching 2009	19	43.5	4.1		35	45.8	3.5	2008<2010 (p=0.017)
Branching 2010	19	42.8	4.1		35	44.7	3.9	
Encrusting 2008	19	22.2	4.0		31	47.9	4.5	2008 > 2009 (p=0.000)
Encrusting 2009	19	22.0	2.8		31	21.9	2.9	2008 > 2010 (p=0.000)
Encrusting 2010	19	25.2	2.8		31	21.1	2.9	

TABLE 6

ANOVAR for major coral forms based on Point Line Intercept and Photo Quadrat surveys from Grenada's southwest reefs during 2008-2010. Bonferroni correction of p<0.017 was used for determining significance

Coral Form	Point Line Intercept			Photo Quadrat		
	n	F	p	n	F	p
Massive 2008-2010	35	8.626	0.000*	18	1.630	0.211
Branching 2008-2010	35	4.714	0.012*	19	0.100	0.799
Encrusting 2008-2010	31	13.409	0.000*	19	0.798	0.458

Further comparisons of percent cover in the MPA to non-protected areas revealed that gorgonian cover did have a significant interaction (Two-way ANOVAR, F=13.005, p=0.000). Additional tests of gorgonian cover in the MPA and non-protected areas showed no significant difference among years. Sponge cover also exhibited a significant interaction (Two-way ANOVAR, F=8.654, p=0.002). The sponge cover in the MPA was not significantly different

TABLE 7
Coral Form mean based on the Point Line Intercept surveys in non-protected and marine protected areas from Grenada's southwest reefs during 2008-2010. When the Two-way ANOVAR showed a significant interaction between time and location, a one way ANOVA was done for further examination. A Bonferroni correction of p<0.017 was used when determining significance

Coral Form	Non-Protected Area			Marine Protected Area			ANOVA	
	n	Mean %	SE	n	Mean %	SE	F	P
Massive 2008	12	7.8	2.6	12	19.9	5.0		
Massive 2009	12	23.4	4.9	12	34.1	6.2		
Massive 2010	12	24.6	5.2	12	30.7	6.6		
Branching 2008	12	39.9	9.4	11	29.1	7.0		
Branching 2009	12	58.5	4.7	11	40.6	4.9		
Branching 2010	12	54.1	7.1	11	34.2	9.0		
Encrusting 2008	12	46.9	8.7	12	42.6	8.2	0.195	0.663
Encrusting 2009	12	13.5	3.4	12	17.7	3.3	0.568	0.459
Encrusting 2010	12	18.1	4.0	12	17.7	6.7	0.137	0.714

from the non-protected area in 2008; however sponge cover in 2009 and 2010 showed that the MPA sponge cover was significantly greater than the non-protected area (Table 3).

Substrate (Photo Quadrat): Algae, the dominant substrate cover, ranging from 61.0% (1.5; 19) in 2008, 59.9% (1.9; 19) in 2009 and 59.3% (1.8, 19) in 2010 showed no significant annual differences (ANOVAR, F=0.373, p=0.616) (Fig. 2). Although the percent cover of algae did not change across years, the type of algae observed did. Macroalgae which occurred more frequently than other types of algae increased significantly in 2010. Turf algae dipped significantly in 2009 and coralline algae decreased significantly in 2010 (Table 1 & 2).

Percent hard coral cover remained stable across years ranging from 11.4% (0.7; 18) to 12.0% (1.1; 18) (ANOVAR, F=0.037, p=0.964). Of the three hard coral forms recorded branching coral occurred most frequently with no significant annual variation (Table 3 & 4). Cyanobacteria which was not recorded over the three year sampling period with the PLI method was similar in percent cover to hard coral ranging from 14.7% (1.5; 19) to 11.9% (1.7; 19) (ANOVAR, F=1.314, p=0.277). Percent sponge cover did increase significantly from 1.6% (0.4; 19) in 2008 to 4.2% (0.7; 19) in 2010 (ANOVAR, F=9.478, p=0.002, Bonferroni p=0.004) (Fig. 2).

Fish: A total of 62 fish species were observed at the five sampling locations from 2008 to 2010 (Table 8). The major groups of fish analyzed included *Chromis spp.*, damselfishes, parrotfishes, surgeonfishes, and wrasse. *Chromis spp.* were separated from the damselfishes because of their large number. Diversity indices were quite high and similar across all sites (Table 9).

Relative abundance of all but one of the most frequently occurring groups of fishes did not vary significantly across the three years of this study. *Chromis spp.*, the largest group observed, showed a downward trend going from 65.2% (3.5; 34) to 49.8% (4.2; 34); however the difference was not significant (ANOVAR, F=3.611, p=0.032). Damselfishes ranged from 11.1% (1.7; 32) to 15.5% (1.7; 32) (ANOVAR, F=3.531, p=0.035) and parrotfishes from 10.1% (1.6; 36) to 6.4% (0.7; 36) (ANOVAR, F=1.732, p=0.184) (Fig. 3). Surgeonfishes also remained stable between 0.9% (0.1; 31) and 1.3% (0.2; 31) (ANOVAR, F=0.146, p=0.864). Wrasse however showed a significant increase from 7.3% (1.0; 35) to 15.5% (2.1; 35) (ANOVAR, F=7.341, p=0.001) (Fig. 3).

TABLE 8
Fish species observed during surveys at five sampling locations over Grenada's southwest reefs during 2008-2010

Acanthuridae	**Haemulidae**	**Pomacentridae**
Acanthurus coeruleus	*Haemulon chrysargyreum*	*Holacanthus tricolor*
Acanthurus chirurgus	*Haemulon flavolineatum*	*Chromis cyanea*
Acanthurus bahianus	*Haemulon* spp.	*Chromis multilineata*
Acanthurus spp.	**Holocentridae**	*Stegastes partitus*
Apogonidae	*Myripristis jacobus*	*Abudefduf saxatilis*
Apogon townsendi	*Holocentrus rufus*	*Stegastes leucostictus*
Apogon spp.	*Holocenthus coruscus*	*Stegastes diencaeus*
Aulostomidae	*Holocentrus adscensionis*	*Stegastes planifrons*
Aulostomus maculatus	**Grammatidae**	*Microspathodon chrysurus*
Balistidae	*Gramma loreto*	**Scaridae**
Monacanthus spp.	**Labridae**	*Scarus vetula*
Blenniidae	*Thalassoma bifasciatum*	*Sparisoma aurofrenatum*
Blennidea spp.	*Clepticus parrae*	*Sparisoma viride*
Bothidae	*Halichoeres bivittatus*	*Scarus* spp.
Bothus lunatus	*Bodianus rufus*	**Sciaenidae**
Carangidae	*Halichoeres garnoti*	*Equetus lanceolatus*
Carangoides ruber	*Xyrichtys* spp.	*Equetus punctatus*
Decapterus macarellus	**Lutjanidae**	**Scorpaenidae**
Cirrhitidae	*Lutjanus synagris*	*Scorpaena plumieri*
Amblycirrhitus pinos	*Lutjanus mahogoni*	**Serranidae**
Chaetodontidae	*Ocyurus chrysurus*	*Cephalopholis fulva*
Chaetodon capistratus	*Lutjanus* spp.	*Cephalopholis cruentata*
Chaetodon striatus	**Mullidae**	*Serranus tigrinus*
Chaetodon spp.	*Pseudupeneus maculatus*	*Hypoplectrus* spp.
Diodontidae/Tetraodontidae	*Mulloidichthys martinicus*	*Hypoplectrus guttavarius*
Canthigaster rostrata	**Ophichthidae**	*Hypoplectrus chlorurus*
Gobiidae	*Myrichthys breviceps*	**Synodontidae**
Coryphopterus glaucofraenum	**Ostraciidae**	*Synodus intermedius*
Coryphopterus hyalinus	*Acanthostracion quadricornis*	
Elacatinus genie	*Acanthostracion polygonius*	
Muraenidae	**Priacanthidae**	
Echidna catenata	*Priacanthus arenatus*	

TABLE 9
Shannon-Wiener diversity index based on identified species observed during surveys over Grenada's southwest reefs during 2008-2010. (Chromis were excluded because their large numbers would dominate the index)

Location 2008-10	2008		2009		2010	
	H'	Richness	H'	Richness	H'	Richness
Dragon Bay	2.346	37	2.032	29	2.218	36
Flamingo Bay	2.183	27	2.459	30	2.217	30
N.E Shallow	2.417	29	2.143	32	2.061	25
N.E. Deep	2.392	35	2.608	33	2.411	30
Quarter Wreck	2.527	38	2.309	31	2.333	32
All Locations	2.548	51	2.598	49	2.500	53

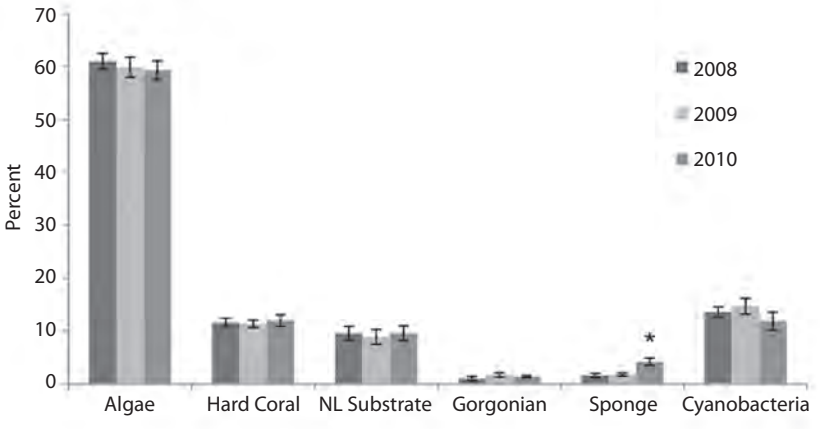

Fig. 2. Mean substrate cover with error bars (SE) based on Photo Quadrat surveys from Grenada's southwest reefs during 2008-2010; * indicates a significant difference (ANOVAR; $p<0.017$).

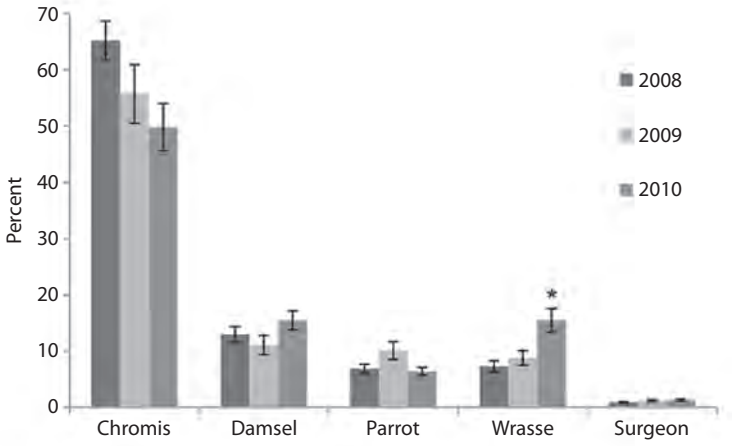

Fig. 3. Mean fish composition with error bars (SE) based on Point Line Intercept surveys from Grenada's southwest reefs during 2008-2010;* indicates a significant difference (ANOVAR; $p<0.017$).

In comparing the MPA to the non-protected area, a significant interaction between time and location was observed for the chromis, which ranged from 47.8% (6.8; 12) to 77.1% (3.7; 12) in the MPA and 42.0% (6.4; 12) to 45.8% (6.7; 12) in the non-protected area (Two-way ANOVAR, $F=6.303$, $p=0.007$). Additional tests revealed that percent chromis observed in 2008 was significantly lower in the non-protected area then the MPA (Table 10). The wrasse group also had a significant interaction between time and location (Table 11). During 2008 the wrasse were significantly higher in the non-protected area at 11.9% (1.9; 12), whereas the MPA only had 3.5% (1.1; 12) wrasse (Table 10). None of the other fish groups showed a significant interaction at time and location (Table 11).

The density of fishes on the other hand did show significant differences for most groups

TABLE 10
Mean Fish percent based on the Point Line Intercept surveys in non-protected and marine protected areas from Grenada's southwest reefs during 2008-2010. When the Two-way ANOVAR showed a significant interaction between time and location, a one way ANOVA was done for further examination. A Bonferroni correction of p<0.017 was used when determining significance

Fish Observed	Non-Protected Area			Marine Protected Area			ANOVA	
	n	Mean %	SE	n	Mean %	SE	F	p
Chromis 2008	12	42.7	6.4	12	77.1	3.7	20.476	0.000
Chromis 2009	12	42.0	7.6	12	63.2	8.2	3.529	0.074
Chromis 20010	12	45.8	6.7	12	47.8	6.8	0.025	0.876
Damselfish 2008	12	21.1	2.9	12	9.7	1.4		
Damselfish 2009	12	15.3	3.3	12	9.3	2.7		
Damselfish 2010	12	18.0	3.0	12	14.6	2.8		
Parrotfish 2008	12	9.6	1.7	12	3.9	0.6		
Parrotfish 2009	12	13.2	2.8	12	6.4	1.8		
Parrotfish 2010	12	7.0	1.1	12	5.3	1.5		
Surgeonfish 2008	11	1.7	0.4	12	0.6	0.1		
Surgeonfish 2009	11	1.2	0.4	12	1.0	0.4		
Surgeonfish 2010	11	0.8	0.3	12	1.7	0.5		
Wrasse 2008	12	11.9	1.9	12	3.5	1.1	18.988	0.000
Wrasse 2009	12	8.0	2.0	12	10.3	2.4	0.522	0.478
Wrasse 2010	12	14.8	2.2	12	23.5	4.5	2.576	0.123

over the years of the study. *Chromis spp.* decreased significantly from 669.3 fish/100m^2 (180.5; 30) to 286.6 fish/100m^2 (78.3; 30) (ANOVAR, F=9.215, p=0.000). Damselfishes density also significantly decreased from 70.3 fish/100m^2 (3.7; 34) in 2008 to 40.6 fish/100m^2 (2.7; 34) in 2009 (Bonferroni, p=0.000) and 55.3 fish/100m^2 (5.2; 34) in 2010 (Bonferroni, p=0.015) (ANOVAR, F=17.994, p=0.000). The density of parrotfishes significantly decreased from 39.5 fish/100m^2 (3.2; 30) and 39.7 fish/100m^2 (4.2; 30) in 2008 and 2009 to 26.3 fish/100m^2 (4.5; 30) in 2010 (ANOVAR, F=10.786, p=0.000). Wrasse density however showed an increase from 37.6 fish/100m^2 (4.6; 34) and 30.2 fish/100m^2 (3.5; 34) in 2008 and 2009 to 68.6 fish/100m^2 (15.4) in 2010 but the change was not significant (ANOVAR, F=3.525, p=0.035). The density of surgeonfishes did not significantly change, however it showed a downward trend going from 6.3 fish/100m^2 (0.8; 25) in 2008 to 5.9 fish/100m^2 (1.4; 25) in 2009, and finally to 4.5 fish/100m^2 (0.5; 25) in 2010 (ANOVAR, F=1.859, p=0.179) (Fig. 4). The only fish group that experienced a significant interaction between time and location for density was damselfish (Two-way ANOVAR, F=7.288, p=0.016) (Table 11). Damselfish in 2010 were significantly higher in the non-protected area at 54.9% (3.5; 11), while only 36.8% (4.8; 12) were observed in the MPA (ANOVA, F=9.600, p=0.005) (Table 12).

The observed fish assemblage was divided into feeding groups based on the classification of Sadin (2008b). Combined data from all sites across years (2008-2010) showed the dominant feeding group of the assemblage to be planktivores at 81.3% (1.8%; 35) to 74.7 (3.5%; 31). Herbivores represented 9.7% (1.0%; 35) to 13.6 (2.0%; 36), while carnivores comprised 10.9 (1.3%; 30) to 14.9% (2.2; 34). Fish feeding groups were not significantly different between years. In addition there was no significant difference between the MPA and non-protected areas in the percent planktivores (Two-way ANOVAR, F=3.891, p=0.036), herbivores (Two-way ANOVAR, F=2.900, p=0.078) or carnivores (Two-way ANOVAR,

TABLE 11
Two-way ANOVAR of Fish percent and density at time and location from non-protected and marine protected areas from Grenada's southwest reefs during 2008-2010. A bonferroni correction factor of p<0.017 was used to determine significance (*indicates significant)

Interaction	Fish Percent			Fish Density		
	n	F	p	n	F	p
Chromis Time*Location	12	6.303	0.007*	10	1.965	0.169
Damsel Time*Location	12	4.424	0.024	11	7.288	0.016*
Parrotfish Time*Location	12	1.784	0.191	12	0.072	0.931
Surgeonfish Time*Location	10	2.279	0.131	10	0.192	0.183
Wrasse Time*Location	12	13.656	0.000*	11	3.595	0.046

TABLE 12
Mean Fish density based on the Point Line Intercept method from non-protected and marine protected areas from Grenada's southwest reefs during 2008-2010. If the Two-way ANOVAR showed a significant interaction between time and location, a follow up one way ANOVA was done for further examination. A Bonferroni correction of p<0.017 was used when determining significance

Fish Group	Non-Protected Area			Marine Protected Area			ANOVA	
	n	Mean (fish/100m^2)	SE	n	Mean (fish/100m^2)	SE	F	p
Chromis 2008	11	439.6	88.6	11	498.6	112.2		
Chromis 2009	11	422.0	192.7	11	972.3	378.6		
Chromis 20010	11	348.3	171.6	11	171.1	26.7		
Damselfish 2008	11	69.4	6.5	12	64.9	4.7	0.294	0.593
Damselfish 2009	11	43.4	4.4	12	27.9	4.7	7.559	0.012
Damselfish 2010	11	54.9	3.5	12	36.8	4.8	9.600	0.005
Parrotfish 2008	12	49.0	6.0	12	22.0	3.5		
Parrotfish 2009	12	41.3	5.4	12	18.9	3.9		
Parrotfish 2010	12	30.5	9.0	12	13.1	2.8		
Surgeonfish 2008	12	4.1	0.6	11	7.3	1.5		
Surgeonfish 2009	12	5.0	1.1	11	6.0	2.9		
Surgeonfish 2010	12	3.1	0.8	11	3.8	0.8		
Wrasse 2008	12	42.8	7.6	12	46.1	9.5		
Wrasse 2009	12	24.0	3.3	12	39.4	6.2		
Wrasse 2010	12	88.7	37.8	12	75.1	21.1		

F=2.490, p=0.111) since none exhibited a significant interaction between time and location.

Combined *Diadema antillarum* density for Grenada's southwest coast exhibited a significant downward trend having 3.1 urchins/100m^2 (0.5; 36) in 2008, to 1.9 urchins/100m^2 (0.5; 36) in 2009 and to only 0.2 urchins/100m^2 (0.1; 36) in 2010 (ANOVAR, F=6.078, p=0.004). It should be noted even after log transformation the data did not fulfill the assumption of normality, however sphericity could be assumed. There was also no significant interaction at time and location for diadema (Two-Way ANOVAR, F=1.853, p=0.197).

DISCUSSION

Data collected during three annual surveys indicates benthic cover in the nearshore waters off the southwest coast of Grenada was similar to many reported findings from across the Caribbean. Algae dominated the substrate (45.9% to 61.0%) and live hard coral coverage (16.5% to 11.4%) was quite low. Algae

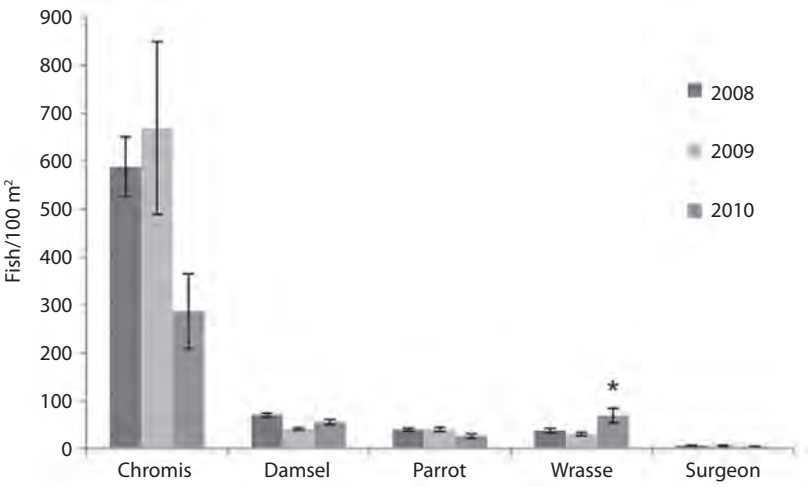

Fig. 4. Mean fish density with error bars (SE) based on Point Line Intercept surveys from Grenada's southwest reefs during 2008-2010; * indicates a significant difference (ANOVAR; $p<0.017$).

dominated systems have been reported for many nearshore communities in the Caribbean (Hughes 1994, Gardner 2003, Burke & Maidens 2004, Bouchon *et al*. 2008, Wilkinson 2008, Mumby 2009, Walsh 2011). Algae has been the dominant substrate cover on Caribbean reefs since a major ecological phase shift occurred in the 1980s. Overfishing, hurricane damage and a disease-induced die-off of *D. antillarum* have been proposed as major factors in this shift (Hughes 1994, Gardner *et al.* 2003).

Low densities of *D. antillarum* in Grenadian nearshore waters may be one of the key factors in the high algal component of this benthic community. The mean *D. antillarum* density found in 2x30m belt transects off the coast of Grenada during this study ranged from 0.002/m^2 to 0.031/m^2 which is much lower than the 4.25/m^2 densities measured in 2003 by Carpenter & Edmunds (2006) for these waters and the 1.7-8.9/m^2 they found associated with reefs of six countries around the Caribbean. Based on general surveys across a spectrum of western Atlantic reefs between 1998-2000 Kramer (2003) reported mean *D. antillarum* densities of 0.029/m^2. Newman *et al*. (2006) found mean densities of 0.019/m^2 at similar depths in the western and northern Caribbean. In both studies fleshy macroalgae generally dominated the reef benthic communities where these low *D. antillarum* densities occurred.

Given the importance of *D. antillarum* in the coral reef community reestablishment of *D. antillarum* may have potential as a management tool to enhance coral growth in algal dominated systems. This potential became apparent when a phase shift reversal was noted on Jamaica's north coast. Coral cover increased from 23% in 1995 to 54% in 2004 with higher growth rates of juvenile corals and higher densities of small juvenile recruits in "dense urchin zones" (Idjadi *et al*. 2006, 2010, Bechtel *et al*. 2006). The potential impact of increased numbers of *D. antillarum* on coral recovery sparked introductions of additional *D. antillarum* into Grenada's MPA from adjacent populations in 2011 (Nimrod personal communication). These relocations will hopefully result in significant increases in local populations of *D. antillarum* that will reduce macroalgae and facilitate an increase in coral recruitment and growth.

Understanding the composition of Grenada's southwest coastal nearshore fish community will also inform existing and future fisheries management practices. Heavy fishing

pressure has been identified as one of the key factors in transformation of coral reefs to algal dominated systems (Hawkins & Roberts 2003). Fishing methods in Grenada include beach seining, trap nets, hand lines and spearing. Target species are mainly carnivores such as Lutjanidae (snapper), Serranidae (groupers) and Carangidae (jacks, pompanos and mackerels and scad) (Finlay 2000). Large herbivores such as Scaridae (parrotfish) and Acanthuridae (surgeonfishes and tang) are also frequently seen in Grenadian fish markets. Observations during 2008-2010 indicated that planktivores (74.7 - 81.3%) dominated the nearshore Grenadian fish community followed by herbivores (9.7 - 13.6%), carnivores (10.9 - 13.9%). The herbivore component of Grenada's nearshore fish assemblage seems low when compared to other studies. Toller *et al*. (2010) found 65% herbivores off Saba Island in habitat types similar to those in Grenada. In a synthesis of Caribbean wide surveys between 1998 and 2000, Kramer (2003) found herbivores made up 64.6% of the fish community sampled. Simply determining that the herbivore component of the Grenadian fish community is low compared to other locations does not allow a full understanding of the impact this has on substrate cover. Burkepile & Hay (2010) pointed out the importance of species level identification in reef fish monitoring. Each herbivorous species can have unique impacts on algal succession and coral growth. A diverse assemblage of herbivorous fishes can reduce development of macroalgae communities and thereby enhance recruitment of coral to open substrates. Ceccarelli *et al*. (2011) divides herbivorous fishes into roving herbivores or "foragers" (parrotfish *Scarus* spp. and surgeonfish *Acanthurus* spp.) and "farmers" (territorial damselfish *Stegastes* spp.) in order to evaluate their potential influence on algal succession and coral reef recovery. "Farmers" tend to suppress algal succession preventing development of the fleshy macroalgae stage. "Foragers" have an intermediate effect allowing development of some macroalgae but not a late-successional assemblage (Ceccarelli *et al*. 2011). In Grenada's nearshore fish community herbivores were dominated by parrotfishes (Scaridae) at 70.2% followed by territorial damselfish fish (*Pomacentrus* spp., *Stegastes* spp., *Microspathodon* spp.) at 17.9% and surgeonfish (*Acanthurus* spp.) at 11.5%. Thus "farmers" comprised only 17.9% in the Grenadian nearshore herbivorous fish community while "foragers" made up 82.1%. It is understandable therefore that turf algae comprised such a small portion of the algal community and fleshy macroalgae made up the majority. Arnold (2007) demonstrated that grazing by scrapers such as parrotfish and urchins facilitate coral recruitment more than territorial damselfishes that maintain low levels of turf algae. Since the species composition of herbivores in Grenada's nearshore waters is primarily comprised of "foragers" rather than "farmers" potential benefits are likely for future coral recruitment if the overall number of herbivores can be increased. It is hoped that newly implemented fishing restrictions in the Moliniere-Beausejour MPA will facilitate increased abundance of herbivorous fishes.

In addition to low numbers of herbivores, algal dominance is also driven by increases in nutrients in nearshore waters. Littler *et al*. (2009) described the importance of taking into consideration the complex interaction of herbivory, nutrient levels and stochastic events in understanding existing conditions and developing management strategies for coral reef communities. Lapointe *et al*. (1997) argued that nutrient input from non-point source pollution related to development and population increases on the island of Jamaica was a major factor in driving the shift from a coral dominated system to an algal dominated community. In a comparative study of reef communities Sandin *et al*. (2008a) saw a shift from dominance by a few large top predator fish species to dominance by small lower trophic level consumers, primarily planktivores, in areas of increasing human populations. The dominance of planktivores (primarily *Chromis* spp.) in Grenadian nearshore waters may be an indicator of excess nutrients into these waters. Two major rivers (St. Johns and Beausejour), flow into the nearshore waters of Grenada's southwest

coast. These rivers drain heavily populated areas as well as agricultural lands and have the potential of delivering excess nutrients into the reef communities. These nutrients have the potential of enhancing macroalgal growth and inhibiting the recruitment and growth of coral (Littler *et al.* 2009).

The three years of monitoring at permanent transects in this study provide a basis for future trend analysis and evaluation of management practices. Hughes *et al*. (2010) advocates long term monitoring of important taxonomic groups as well as identification of mechanisms and feedbacks in order to detect indicators of phase shifts. He also encourages agencies involved in research and management of reefs to take a proactive integrative approach through education of grassroots constituencies, enhancing access to existing information and expertise and strengthening regulations associated with harvesting important species from these communities. This approach is beginning to be implemented in Grenada through the work of the Grenada Government Fisheries Division and the Moliniere-Beausejour Marine Protected Area Stakeholder Group.

This study establishes a baseline of information but long term and more specific monitoring is needed to better understand the trajectory of Grenada's reef communities. Gardner *et al*. (2003) indicated that areas of coral recovery are often dominated by non-framework builders such as *Agaricia* and *Porites* rather than framework builders such as *Acropora* and *Montastrea*. These framework builders that formerly dominated reefs in the Caribbean are essential to surviving the destructive forces of major storms. The coral community in Grenada's nearshore waters is comprised primarily of branching coral much of which is *Agaricia* and *Porites*. Given the importance of framework builders to the resilience of coral reef communities identification of coral species will be added to the monitoring program to better understand the coral community.

The similarity between the MPA and non-protected areas seen in this study may be due to the fact that the Moliniere-Beausejour MPA management plan was not fully implemented until September 2010. After full implementation of the plan wardens began to patrol the area and prevent fishing from boats and enforce the use of permanent mooring buoys by divers and snorkelers in the MPA. Future monitoring efforts will be able to use the results of this study as a basis for comparison in order to assess the impact of the newly implemented management practices in the MPA. Expansion of current studies will allow a better understanding of mechanisms and feedbacks in these reef systems. Video and photographs of transects and surrounding habitats are being incorporated into public presentations for Grenadian resource managers and the general public to encourage a broader understanding of the importance of careful resource management.

In addition to focusing on local environments it is important to connect these studies to broader ecosystem wide analyses. Ogden (2010) encourages moving toward an ecosystem-based management plan for the Caribbean. Ogden cites plans for regional management inspired by the CARICOMP network of marine laboratories and encourages going beyond local problems and addressing issues like the *D. antillarum* die-off, wide spread white band disease and the annual plume of discharge from Venezuela's Orinoco River. Efforts are ongoing to strengthen connections of this ongoing monitoring effort to the network of Caribbean marine laboratories and provide information that will assist regional management.

ACKNOWLEDGEMENTS

Funding for this project was provided by the Fischer Family Foundation and Mr. Gary Stimac and is greatly appreciated. A special thanks to Jacob Krause for playing a major role in developing this program. Thanks are also offered to Jillian Groeschel, Kyle Foster, Svetlana Bornschlegl, Victoria Krueger, Thomas Dietrich, Ben Hermanson, Laurelyn Dexter, Angela Majeskie, Emily Bolda, Allison Page, and Angela Blasezyk for data collection. Kayli Giertych and Stephen Vandenberg are

thanked for assistance with the manuscript. Billie Harrison of the Racine Zoo is also thanked for field assistance.

RESUMEN

Un estudio sobre poblaciones bentónicas y de peces fue realizado en cinco localidades en la zona costera en el suroeste de Grenada entre 2008 y 2010. Dos sitios se ubicaron en una Área Marina Portegida (AMP) recientemente creada. Para determinar la cobertura se utilizaron foto-cuadrantes (FQ) y transectos de intersección de puntos (TIP). Las algas fueron el principal componente del bentos, aumentando significativamente de 45,9% en 2008 a 52,7% en 2010 (TIP). Las algas también fueron predominantes (61,9%-59,3) en los FQ, aunque las diferencias anuales no fueron significativas. La cobertura de corales pétreos tenía un ámbito de 16,5% a 15,4% (TIP) y de 11,4% a 12,0% (FQ), sin diferencias significativas entre años. Los corales ramificados e incrustantes fueron más frecuentes que los corales masivos. En los tres años no hubo diferencias significativas entre las AMPs y las áreas no protegidas. La abundancia relativa de peces a lo largo de un transecto de 30x2m no varió significativamente entre los años, sin embargo, la densidad de peces decreció significativamente a través de los años, para los grupos principales. *Chromis* spp. predominó con 65,2% en 2008 y 49,8% en 2012, seguido por damiselas territoriales, 11,1% y 15,5%, y los lábridos aumentaron de 7,3% a 15,5%. Tanto la coberura del sustrato como los datos de peces indican una comunidad estable pero degradada. Sondeos anuales están planeados para el futuro. Los datos existentes y futuros de este proyecto serán muy útiles para determinar la eficacia de la gestión de las AMPs y el estado de salud de los sistemas arrecifales de Grenada.

Palabras clave: cobertura bentónica, coral, peces de arrecife, monitoreo, Grenada, Caribe Oriental.

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Abundancia y distribución de larvas de *Strombus gigas* (*Mesogastropoda*: *Strombidae*) durante el período reproductivo de la especie en el Caribe Mexicano

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Recibido 14-VII-2011. Corregido 20-X-2011. Aceptado 20-XII-2011.

Abstract: Abundance and distribution of *Strombus gigas* (*Mesogastropoda*: *Strombidae*) larvae during their reproductive period in the Mexican Caribbean. The Queen Conch (*Strombus gigas Linnaeus*, 1758) is a species of economic importance in the Caribbean Sea, which, in the 1980's represented the second fishery after de spiny lobster, reason that is currently in a state of overfishing. In order to determine the larval abundance variation during the reproductive season, four locations of the Mexican Caribbean "MC" (Mexico: Puerto Morelos, Sian Ka'an, Mahahual; Belize: San Pedro) were sampled. Monthly, from May to October 2008, planktonic net drags (300µm) were carried out at each location. Temperature (°C), salinity (ppm) and dissolved oxygen (mg L^{-1}) were recorded for each site. A mean larval density of 0.34±0.87 (larvae 10 m^{-3}) was registered between locations, with a peak in August and September (0.82±1.00 and 0.76±1.68 larvae $10m^{-3}$, respectively). The larval density was 60% correlated with salinity ($r=0.6063$, $p<0.05$). A one-way ANOVA showed significant statistical larval density in time ($p<0.05$) and space ($p<0.05$), where Puerto Morelos displayed the higher records during the study (0.54±1.49 larvae $10m^{-3}$). An average larval size of 332.44±59.66µm was recorded. Larval sizes differed significantly between locations ($p<0.05$), but not considering months ($p>0.05$). A 100% of the captured larvae correspond to stage I, showing local reproductive activity, that might indicate the sampled sites in the MC are a source of larvae to *S. gigas*. Rev. Biol. Trop. 60 (Suppl. 1): 89-97. Epub 2012 March 01.

Key words: Reproductive season, larval densities, *Strombus gigas.*

El Caracol rosa *Strombus gigas* (L.) se distribuye en el Mar Caribe del sureste de Florida al norte de Sudamérica, incluyendo las Antillas menores y Bermudas (Randall 1964, Stoner 1997). En el Caribe, la duración de la temporada reproductiva presenta un periodo de 5 meses observado en Bermuda (Berg & Olsen 1989) a 12 meses reportado en México (Corral & Ogawa 1987), con mayor incidencia de mayo a octubre (de Jesús Navarrete 1999, Aldana Aranda & Pérez Pérez 2007; de Jesús Navarrete & Pérez Flores 2007, Bravo Castro 2009).

La larva veliger de *S. gigas* tiene un lapso de desarrollo de 21-30 días (Davis *et al.* 1993, Aldana Aranda & Patiño Suárez 1998). Los estudios de abundancia larval de *S. gigas* inician en los 90, citándose los países de Bahamas (Chaplin & Sandt 1992, Stoner & Davis 1997a, Stoner & Davis 1997b), Florida (Stoner *et al*. 1997, Delgado *et al*. 2008), México (de Jesús Navarrete 1999, de Jesús Navarrete & Aldana Aranda 2000, Oliva Rivera & de Jesús Navarrete 2000, de Jesús Navarrete 2001, Pérez Pérez *et al*. 2003, de Jesús Navarrete & Pérez

Flores 2007, Aldana Aranda & Pérez Pérez 2007, Pacheco Archundia 2007, Bravo Castro 2009), Venezuela (Posada 2003) y Puerto Rico (Appeldoorn 1993).

La mayoría de estos estudios se han realizado de forma puntual, así como en un ciclo anual, y unos pocos han sido realizados durante la temporada reproductiva de esta especie (Stoner & Davis 1997a, Stoner & Davis 1997b, Stoner *et al*. 1997) mientras que regionalmente se cita sólo un trabajo para el norte de la Península de Yucatán (Pérez Pérez *et al*. 2003),en razón de ello, se propusó está investigación, cuyo objetivo fundamental es el conocimiento de la distribución y abundancia espacio-temporal de larvas de *S. gigas*, en cuatro localidades del Caribe Mexicano (CM) durante la temporada reproductiva de la especie.

MATERIALES Y MÉTODOS

Área de estudio: De mayo a octubre de 2008 se realizaron muestreos mensuales en cuatro sitios del Caribe Mexicano: México (Puerto Morelos: 20°49'21"-20°51'21"N, y 86°51'50"-86°52'45"W; Sian Ka'an: 19°44'28"-20°00'58"N, y 87°27'10"-87°28'10"W; Mahahual: 18°42'15"-18°42'57"N, y 87°42'10"-87°42'30"W) y Belice (San Pedro: 17°50'44"-18°06'41"N, y 87°50'09"-88°01'14") (Fig. 1).

Análisis de muestras: Se efectuaron arrastres de plancton (n=3) en cada sitio de muestreo desde una embarcación con motor fuera de borda a una velocidad constante de 5m min^{-1}, con una duración de 5 minutos y a una profundidad máxima de 1m de la línea costera hacia la barrera arrecifal. Se utilizó una red cónica de 30cm de diámetro de boca, 1.5m de largo y una abertura de malla de 300µm. Se empleó un flujómetro *General Oceanics* para conocer el volumen colectado en cada muestra. En cada sitio de muestreo se registró temperatura (°C), salinidad ($^{0}/_{00}$) y oxígeno disuelto (mg L^{-1}) con un medidor YSI 85. Las muestras obtenidas fueron fijadas en formol salino al 4% y llevadas al laboratorio para su posterior análisis e identificación. Las larvas de gasterópodos fueron separadas del plancton y preservadas en alcohol al 70%. Las larvas de *S. gigas* se identificaron y clasificaron en clases de tallas de acuerdo a Davis *et al*. (1993). Se midió la longitud sifonal (µm), empleándose un micrómetro ocular instalado en el objetivo 10x de un microscopio Carl Zeiss AxioStar Plus. El

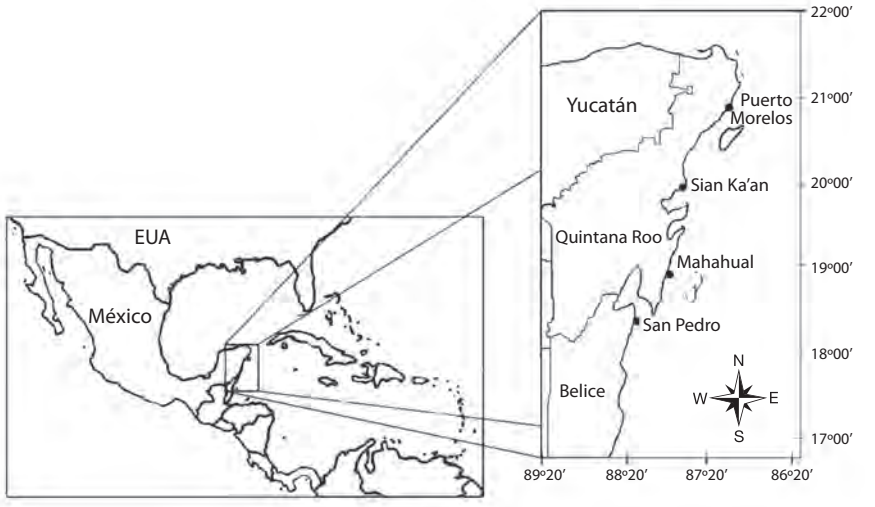

Fig. 1. Área de estudio. / **Fig. 1.** Study area.

número de larvas registradas fue estandarizado para obtener la densidad larval (larvas $10m^{-3}$).

Análisis estadístico: Se calculó media y desviación estándar para los parámetros fisicoquímicos, densidad larval y tallas. Análisis de varianza de una vía ($p_{\alpha 0.05}$) (Sokal y Rohlf 1995) fue empleado para conocer la variación espacio-temporal de densidad larval, tallas y parámetros fisicoquímicos. Se empleó la prueba de Tukey ($p \leq 0.05$) para conocer la variación de la abundancia larval entre sitios y la prueba de Duncan ($p \leq 0.05$) para evaluar la variación de las tallas entre localidades. Se realizó un análisis de correlación (*Spearman*) entre parámetros fisicoquímicos y densidad larval. Los análisis se realizaron en InfoStat/ Profesional Versión 1.1.

RESULTADOS

Parámetros fisicoquímicos: La salinidad (n=12) no presentó variación significativa en el período de estudio (Media: 34.82±0.82 ppm, p=0.2870). La densidad larval presentó 60% de asociación con la salinidad (r=0.6063; p=0.0036), mientras que temperatura y oxígeno disuelto se asociaron en 16% y 9%, respectivamente (Cuadro 1).

Abundancia espacial: En la Figura 2 se muestra la densidad larval de *S. gigas* para las diferentes localidades. La mayor abundancia media se presentó en Puerto Morelos, seguido de San Pedro y Sian Ka'an (0.54±1.38, 0.47±0.88 y 0.30±0.57 larvas $10m^{-3}$, respectivamente). El análisis de varianza de las medias de la abundancia presentó diferencias significativas (p=0.0439). El Cuadro 2 muestra los resultados de la prueba de Tukey, observándose que Puerto Morelos fue estadísticamente diferente del resto de las localidades.

Abundancia temporal: La Figura 3 muestra la densidad larval de *S. gigas* para los meses analizados. En general, se observó la presencia de larvas de mayo a octubre, con mayor abundancia de agosto a septiembre (0.82±1.00 y 0.76±1.68 larvas $10m^{-3}$, respectivamente). El ANOVA mostró diferencias significativas (p=0.0105). La Figura 4 muestra la densidad media larval para las diferentes localidades en el tiempo. En Puerto Morelos se observó larvas en tres meses, mientras que las otras localidades sólo en dos meses. Se observaron dos períodos de abundancia larval sólo para Puerto Morelos. Septiembre presentó la mayor incidencia de larvas (n=3 larvas), seguido de agosto (n=2 larvas).

Tallas de larvas de *S. gigas*: La talla media de larvas de *S. gigas* por localidad se presenta en la Figura 5a, con tallas entre 285.00±58.55µm (Sian Ka'an) y 396.67±46.19µm (Mahahual).

CUADRO 1

Medias y valor de probabilidad para salinidad (ppm), temperatura (°C), oxígeno disuelto ($mg \cdot l^{-1}$) y densidad larval ($larvas \cdot 10m^{-3}$); n, número de datos analizados; $p_{(\alpha 0.05)}$, valor de probabilidad del anova entre parámetro y localidades; r, coeficiente de correlación de Spearman. P: Valor de significancia entre parámetro fisicoquímico y densidad larval

TABLE 1

Means and probability value for salinity (ppm), temperature (°C), dissolved oxygen ($mg \cdot l^{-1}$) and larval density ($larvae \cdot 10m^{-3}$); n, number of analyzed data; $p_{(\alpha 0.05)}$: ANOVA probability value between parameters and localities; r: Spearman correlation coefficient. P: value of significance between physicochemical parameters and larval density

Parámetro	Localidades				Media (n: 12)	$p_{(\alpha 0.05)}$	R	P
	Puerto Morelos	Sian Ka'an	Mahahual	San Pedro				
ppm	*35.03±0.085*	*35.00±0.00*	*35.00±0.00*	*34.25±1.6*	*34.82±0.82*	*0.2870*	*0.6063*	*0.0036*
°C	*27.5±1.12*	27.77±1.29	28.18±1.57	*27.89±0.80*	*27.83±1.17*	*0.8138*	*0.1604*	*0.4416*
$mg \cdot L^{-1}$	*5.20±1.48*	*4.98±1.20*	*5.60±1.20*	*5.17±1.49*	*5.24±1.41*	*0.9074*	*0.0920*	*0.6592*
$larvas \cdot 10m^{-3}$	*0.54±1.38*	0.30±0.57	0.09±0.28	0.47±0.88	0.34±0.87	0.0105	1.0000	1.0000

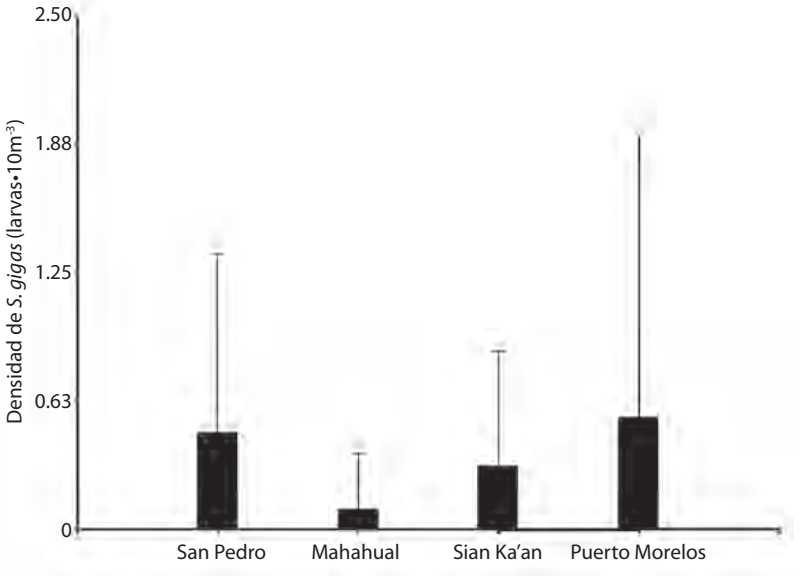

Fig. 2. Densidad larval (larvas·10m^{-3}) entre localidades.
Fig. 2. Larval density (larva·10m^{-3}) between locations.

CUADRO 2
Prueba de Tukey entre abundancia media larval de *S. gigas* y localidades; n_1, número total de muestras.
Prueba de Duncan entre talla media larval y localidades; n_2, número de larvas; A y B, diferencias significativas ($p \leq 0.05$)

TABLE 2
Tukey test between mean abundance of larval *S. gigas* and localities, n_1, number of samples.
Duncan test between average larval size and locations, n_2, number of larvae, A and B, significant differences ($p \leq 0.05$)

Localidad	Prueba de *Tukey* Abundancia media larval			Prueba de *Duncan* Talla media		
	Media larvas·10m^{-3}	n_1	Grupos	Media µm	n_2	Grupos
Puerto Morelos	0.54 ± 1.38	18	A	333.89 ± 32.38	30	A
San Pedro	0.47 ± 0.88	18	B	353.75 ± 71.45	12	A
Sian Ka'an	0.30 ± 0.57	18	B	285.00 ± 58.55	10	B
Mahahual	0.09 ± 0.28	18	B	396.67 ± 46.19	3	A

El análisis de varianza entre tallas y localidades presentó diferencias significativas (p=0.0060). La prueba de Duncan mostró que Sian Ka'an difiere de los otros sitios al presentar larvas de menor talla (Cuadro 2). En la Figura 5b se presenta la talla media en el tiempo. No se registró variación significativa entre meses (p=0.6989). El 100.00% de las larvas observadas corresponden al estadio I (150.00-450.00µm de LS).

DISCUSIÓN

Randall (1964), Weil y Laughlin (1984) y Stoner *et al*. (1996), señalan que el inicio de la actividad reproductiva de *S. gigas* está controlado por la temperatura, asimismo de Jesús Navarrete (1999) señala que este parámetro determina la puesta de masas de huevo, el tiempo de maduración de los embriones,

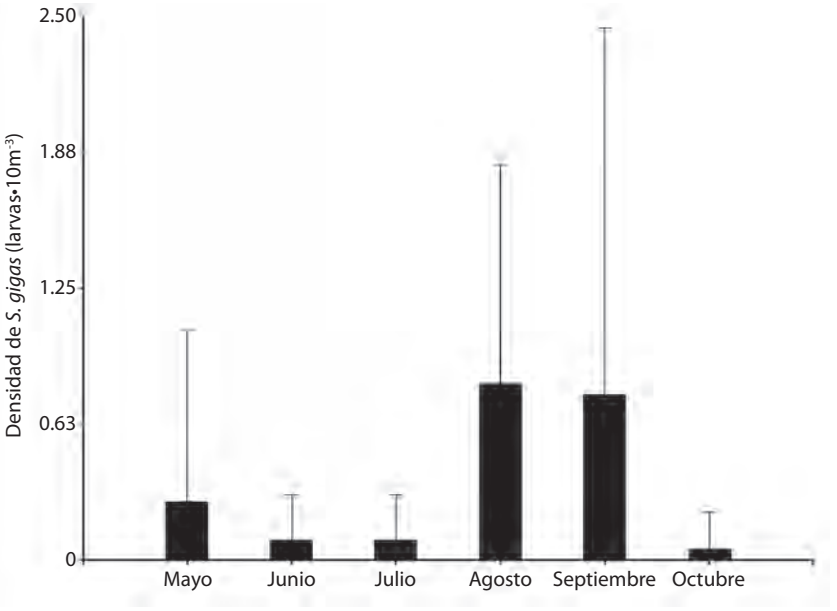

Fig. 3. Densidad larval (larvas·10m^{-3}) entre meses.
Fig. 3. Larval density (larvae·10m^{-3}) between months.

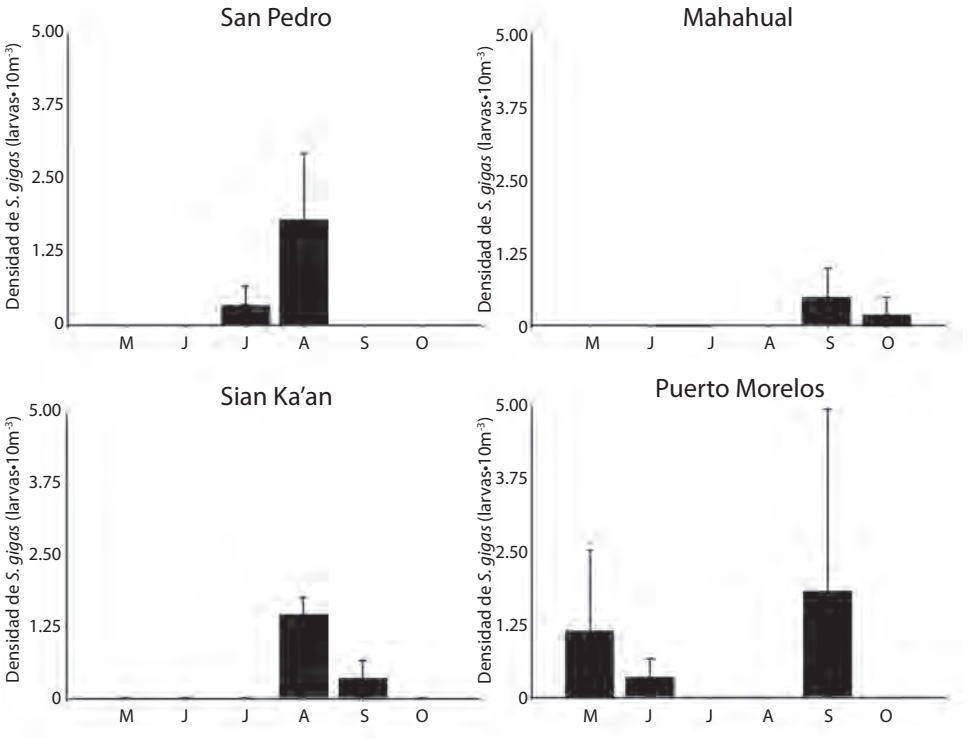

Fig. 4. Densidad larval (larvas·10m^{-3}) entre meses por localidad de estudio.
Fig. 4. Larval density (larvae·10m^{-3}) between months by study site.

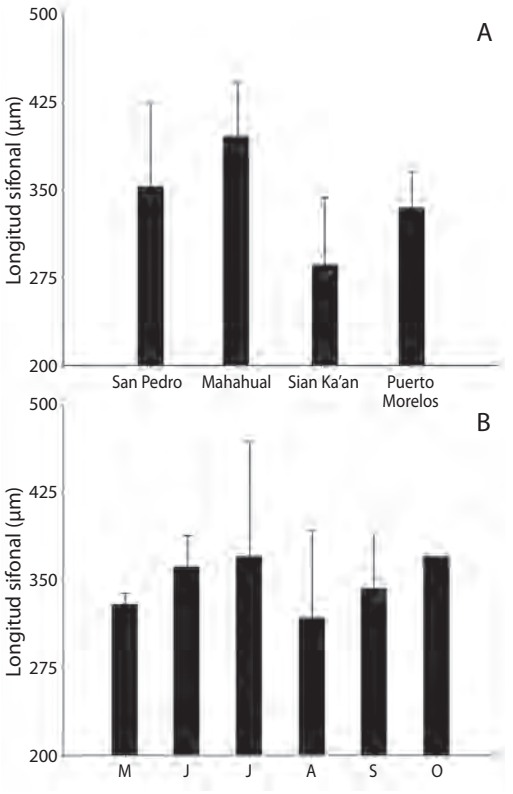

Fig. 5. Tallas medias de longitud sifonal (µm) registradas: **(A)** por localidades de estudio; **(B)** entre meses (M, J, J, A, S y O).

Fig. 5. Mean siphonal length (µm) recorded: **(A)** by sampling localities; **(B)** between months (M, J, J, A, S and O).

las tasas de crecimiento de las velígeras y su tiempo de residencia en la columna de agua. Stoner *et al*. (1992) y Barile *et al*. (1994) señalan que la abundancia larval de *S. gigas* está asociada a la temperatura y al fotoperiodo. Contrariamente, de Jesús Navarrete (1999) no registró relación entre la abundancia y la temperatura, salinidad y el oxígeno disuelto. En el presente estudio la prueba de *Spearman* entre abundancia larval y temperatura mostró 16% de asociación, observándose mayor correlación con la salinidad (60%).

En cuanto a la abundancia larval, los mayores registros han sido para el Arrecife Alacranes con 3.40±6.73 larvas $10m^{-3}$ (Aldana Aranda & Pérez Pérez 2007), seguido por Bahamas con 1.44±1.80 larvas $10m^{-3}$ (Stoner & Davis, 1997b). Los registros más bajos están reportados para la Bahía de la Ascensión con 0.02±0.07 larvas $10m^{-3}$ (de Jesús Navarrete & Pérez Flores 2007). En este estudio para el SAM, la abundancia larval media fue de 0.34±0.87 larvas $10m^{-3}$ (n=64), valor similar a lo reportado por Pérez Pérez *et al*. (2003) para la Península de Yucatán (0.34±0.87 larvas $10m^{-3}$). Puerto Morelos fue la localidad con mayor abundancia, presentando valores menores a los reportados por Bravo Castro (2009), pero mayores a los observados por Pérez Pérez *et a*l. (2003) para el norte de Cozumel (1.03±1.16 y 0.02±0.03 larvas $10m^{-3}$, respectivamente).

Respecto a la abundancia larval temporal reportada en la literatura, los autores registran larvas durante todo el año, con mayor incidencia de junio a agosto (4.70±7.03-17.80±24.77 larvas $10m^{-3}$) (Appeldoorn 1993, Stoner *et al*. 1997, Stoner & Davis 1997a, b, Aldana Aranda & Pérez Pérez 2007, de Jesús Navarrete & Pérez Flores 2007). Mientras febrero y diciembre presentan las más bajas densidades (0.70±0.38 y 0.81±0.31 larvas $10m^{-3}$) (Aldana Aranda & Pérez Pérez 2007). En este estudio se observaron larvas de mayo a octubre, donde la mayor densidad (0.76±1.68 larvas $10m^{-3}$) correspondió a septiembre, coincidiendo con los estudios realizados por Pérez Pérez (2004) y Bravo Castro (2009) para esta región. De acuerdo a los estudios realizados en 2005 por Bravo Castro (2009) y los resultados del presente trabajo, en Puerto Morelos se observa la presencia de dos ciclos con presencia de larvas (mayo y agosto-septiembre), sin embargo, de 2005 a 2008 se ha observado que la abundancia larval para esta localidad ha disminuido de 1.03±1.16 a 0.54±1.38 larvas 10 m^{-3}.

Las tallas de larvas registradas en el presente estudio oscilaron entre 285.00±58.55 y 396.67±46.19µm, donde el 100.00% de las larvas correspondió al estadio I, de acuerdo a la clasificación de Davis *et al*. (1993). Resultado que es similar a los registrados para Banco Chinchorro (89.10%) por de Jesús Navarrete & Aldana Aranda (2000), para el

Arrecife Alacranes (86.42%) por Aldana Aranda & Pérez Pérez (2007) y para el norte de Quintana Roo (96.16%) por Bravo Castro (2009). De acuerdo a la clasificación de Stoner (1997), las localidades del presente trabajo son sitios fuente, con larvas provenientes de reproductores locales.

La distribución de *S. gigas* en su fase larval está regida por las corrientes superficiales, de Jesús Navarrete (1999) señala que existe dependencia del flujo larval en el Caribe, pudiendo la larva de *S. gigas* ser transportada desde sitios ubicados en Belice y México hasta Florida al presentarse corrientes de 0.8 $m.s^{-1}$. En el Caribe se ha registrado un patrón de circulación de sur a norte con deriva hacia el oeste (Kinder 1983, Carrillo González *et al.* 2007),presentándose velocidades de 0.70ms^{-1} a 2.72ms^{-1} para el CM, así como un incremento de hasta el doble en el Canal de Yucatán (Merino Ibarra 1986), sin embargo para la zona norte del CM se ha determinado mayor efecto de los vientos en las masas de agua (Merino Ibarra 1986) provocándose un fenómeno de contracorriente, así, para Puerto Morelos se ha reportado la presencia de giros los cuales producen circulación interna en la laguna arrecifal, así como transporte hacia la costa y mayor tiempo de residencia del agua (Coronado 2007).

Con base en los resultados obtenidos en este trabajo resalta la necesidad de realizar los estudios de monitoreo larval con la medición de corrientes que ayudaran a determinar el grado de dispersión y/o retención entre larvas y sitios. Se recomienda proteger el parque Nacional Arrecifes de Puerto Morelos dado que en el presente estudio, así como en los realizados por Pérez Pérez (2004) y Bravo Castro (2009) se ha observado que las larvas se concentran en esta región, siendo un potencial sitio de reclutamiento para *S. gigas*.

CONCLUSIÓN

Se detectó la presencia de larvas de *S. gigas* en todas las estaciones de estudio, con mayor incidencia en la zona norte, observando mayor abundancia entre julio y septiembre, así como dos picos de abundancia para Puerto Morelos. La salinidad influyó en la distribución de *S. gigas* más que la temperatura. Se observó que el 100% de las larvas corresponde a la clase I establecida por los autores Davis *et al.* (1993) y Stoner y Davis (1997a), razón por la cual se clasifican los sitios como fuente de larvas de *S. gigas*, en base a este resultado, se considera que el Caribe Mexicano puede suministrar larvas a las poblaciones de *S. gigas* de la zona norte del Caribe, sin embargo, es necesario realizar nuevas investigaciones incluyendo más puntos del CM para reafirmar este resultado, así como estudiar patrones de corrientes en el área, a fin de conocer el grado de dispersión larval a nivel espacio-temporal.

AGRADECIMIENTOS

Al Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, por el apoyo para participar en el congreso. Al Consejo Nacional de Ciencia y Tecnología (Conacyt) por la beca N° 340911/240157 y al proyecto "Variación espacio temporal del patrón reproductivo del caracol rosado *Strombus gigas* en diferentes hábitats y su modelo biofísico de conectividad para el Caribe" (Clave: 50094). A Manuel Sánchez Crespo por su colaboración en la colecta de muestras. A Uriel Ordoñez López y a Montserrat Bravo Castro por la asesoría en el análisis e identificación de larvas. A Ximena Renán por sus valiosos comentarios hacia este trabajo.

RESUMEN

El caracol rosa (*Strombus* gigas, *Linnaeus*, 1758) es una especie de importancia económica en el Mar Caribe, por lo cual, en la década de 1980 representó la segunda pesquería después de la langosta espinosa, razón por la que actualmente se encuentra en estado de sobrepesca. Con el objetivo de determinar la variación en la abundancia de larvas durante la época reproductiva, cuatro localidades del Caribe Mexicano "CM" (México: Puerto Morelos, Sian Ka'an, Mahahual; Belice: San Pedro) fueron muestreadas. Mensualmente, de mayo a octubre del 2008, se realizaron arrastres de plancton en cada localidad empleando una red cónica (300µm). Temperatura (°C), salinidad (ppm) y oxígeno disuelto (mg L^{-1}) fueron registrados para cada

sitio. Una densidad media larval de 0.34±0.87 larvas·10m^{-3} fue registrada entre localidades, con un pico de abundancia entre agosto y septiembre (0.82±1.00 y 0.76±1.68 larvas 10m^{-3}, respectivamente). La densidad larval tuvo una correlación del 60% con la salinidad ($r=0.6063$, $p<0.05$). El ANOVA de una vía mostró significancia estadística en tiempo ($p<0.05$) y espacio ($p<0.05$), donde Puerto Morelos tuvo los mayores registros durante el estudio (0.54±1.49 larvas 10m^{-3}). Fue registrada una talla media de 332.44±59.66µm. Las tallas variaron significativamente entre localidades ($p<0.05$), pero no entre meses ($p>0.05$). El 100% de las larvas capturadas corresponden al estadio I definido por Davis *et al* (1993), mostrando actividad reproductiva local, de esta manera, se considera que los sitios muestreados en el CM son fuente de larvas para la especie *S. gigas.*

Palabras clave: Temporada reproductiva, densidad larval, *Strombus gigas.*

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Coral recruitment to two vessel grounding sites off southeast Florida, USA

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Received 12-VII-2011. Corrected 22-XI-2011. Accepted 20-XII-2011.

Abstract: Over the last two decades, more than 10 major vessel groundings have occurred on coral reefs offshore southeast Florida. Lack of any published information on coral settlement, post-settlement survival, and juvenile coral growth in the southeast Florida region inhibits efforts to determine if coral populations will be able to effectively re-establish themselves. The goal of this study was to examine these processes to obtain background data needed to determine the potential for natural recovery. Over a three year period annual coral recruitment, juvenile growth, and mortality rates were measured in 20 permanent quadrats at each of two ship grounding and two control sites. The density of new recruits was generally low, ranging from 0.2±0.1 (SE) to 7.1±1.0 recruits m^{-2}. Although the density of coral recruits was generally higher at the grounding sites, mortality rates were high at all sites during the study period. Growth rates of individual colonies were highly variable, and many of the colonies shrank in size due to partial mortality. Results indicate that corals are able to recruit to the damaged reefs but that slow growth rates and high mortality rates may keep these areas in a perpetual cycle of settlement and mortality with little or extremely slow growth to larger size classes, thus inhibiting recovery. Rev. Biol. Trop. 60 (Suppl. 1): 99-108. Epub 2012 March 01.

Key words: recruitment, reef recovery, vessel groundings, growth rates, post settlement survival.

The southeast Florida reef tract consists of four shore-parallel ridges with a diverse coral reef community similar to that found in the Western Atlantic and Caribbean (Moyer *et al.* 2003, Banks *et al.* 2008). Though similar in fauna, community structure of this high latitude (26° N) region differs from that found in other areas of the Caribbean and Florida Keys (Moyer *et al.* 2003). *Montastraea cavernosa* Linnaeus, 1767 replaces the *Montastraea annularis* (Ellis & Solander, 1786) species complex as the dominant species. Additionally, average coral colony size is typically small, and cover is generally low (Moyer *et al.* 2003).

Coral reefs in southeast Florida are located near a highly developed and heavily populated coastline. As a result, they are subjected to a number of anthropogenic stressors, one of which is physical impact from vessel groundings and anchor damage. Over the past 17 years, at least ten large ship groundings and six anchor damage events associated with the Port Everglades anchorage area have occurred near Ft. Lauderdale, Florida. Impacts include flattening of reef topography, removal of living benthic resources, and creation of large rubble fields. Restoration of these sites has been generally limited to reattachment of scleractinian corals. Removal of rubble was initiated at grounding sites occurring in 2004 and later in an effort to reduce further damage due to high mobility of debris that could hinder recovery of benthic communities. In general, there has been little attempt to restore topography at

the damaged sites with the exception of some localized efforts initiated in 2004 that have accompanied rubble stabilization (e.g., cementing larger sized rubble created by the grounding of the *M/V Eastwind*) (Marine Resources Inc. 2005). However, efforts have been relatively small scale as compared to the larger scale efforts to stabilize reef framework and restore reef topography further south in the Florida Keys that have included use of artificial structures such as prefabricated cement modules and placement of limestone boulders (Jaap *et al.* 2006).

Recovery of damaged sites is generally defined as the return of both community structure and function to a state similar to that which existed pre-impact (Edwards & Gomez 2007). Although there are thousands of organisms which contribute to this structure and function, scleractinian corals are often used as indicators of recovery since they are the main builders of habitat. One of the first steps of recovery after damage has occurred is the recruitment of new colonies to the area. Re-establishment of coral populations is highly dependent on settlement of coral planulae, post-settlement survival, and juvenile coral growth. However, coral recruitment in the Caribbean and western Atlantic is typically low (Rogers *et al.* 1984, Hughes & Jackson 1985, Smith 1997, Miller *et al.* 2000). Furthermore, slow growth rates of generally less than 1 cm yr^{-1} for many Caribbean species (Bak & Engel 1979, Rogers *et al.* 1984, van Moorsel 1988, Edmunds 2000) can influence recovery times. Currently no information on coral recruitment rates or juvenile survival and growth rates has been published for southeast Florida. Therefore, the goal of this study was to examine these processes on a small number of sites off Ft. Lauderdale, Florida to obtain background data needed to determine the potential for natural recovery from damage due to vessel groundings that have occurred in this area.

METHODS

Two ship grounding sites were chosen for study: the Eastwind (26°7'2"N, 80°5'32"W) and Federal Pescadores (26°6'44"N, 80°5'30"W). The *M/V Eastwind* grounded in March 2004 and resulted in approximately 11 000 m^2 of damage on the inner reef just north of Port Everglades (Hudson Marine Management Services 2004a). The *M/V Federal Pescadores* created approximately 23 400 m^2 of damage 0.4 km south of the Eastwind grounding site when it ran aground in October 2004 (Hudson Marine Management Services 2004b). Both groundings destroyed the existing relief, creating large rubble fields from which large rubble was subsequently removed or stabilized during restoration activities (Marine Resources Inc. 2005), but smaller sized debris remained on site.

Two control sites were chosen for comparison to the grounding sites. Because most of the large vessel groundings and anchor damage events off Ft. Lauderdale have occurred just west of the Port Everglades anchorage, this area was avoided when control sites were selected due to the potential for previous impact. Using GIS, a grid consisting of 15x15 m plots was overlaid on the inner reef just north of the anchorage area, and two plots were randomly picked for the control sites (control sites one: 26°9'37"N, 80°5'18"W and two: 26°10'4"N, 80°5'15"W).

Twenty permanently marked 1 m^2 quadrats at each of the four sites were surveyed annually between May 2006 and June 2009. Quadrats were marked at the corners using a stainless steel pin and galvanized nails. A 1 m^2 pvc frame subdivided into 100 squares 10x10 cm in size was placed over the pins to accurately locate corals within the quadrat. All juvenile corals (≤5 cm in diameter) in each quadrat were identified, measured, and mapped. Juvenile corals appearing in the quadrats that were not present in the previous years and were not a product of colony fission were considered coral recruits. Any corals that appeared in the quadrats but were absent in subsequent years were assumed to be dead. The change in maximum diameter of all corals that appeared in multiple years was calculated and used for measures of coral growth.

Differences in density of recruits among sites and among years were tested using a two-way ANOVA on ranks after data transformations failed to achieve normality due to the presence of multiple zero values. Differences in average annual mortality among sites were tested using ANOVA. Post hoc pair-wise comparisons were performed with Tukey tests when significant differences were found.

To further examine similarity in species composition of recruits among sites, a Bray-Curtis similarity index was determined. Species abundance data were pooled for all quadrats within a site during a survey year, and the data were square-root transformed (PRIMER v6). The results were also used to construct a non-metric, multi-dimensional scaling (MDS) plot.

RESULTS

Mean juvenile coral density ranged from a low of 0.5±0.1 (SE) juveniles m^{-2} at control site 1 to a high of 11.6±1.4 juveniles m^{-2} at the Eastwind grounding site (Fig. 1). The density of recruits ranged from 0.2±0.1 recruits m^{-2} at control site 2 to 7.1±1.0 recruits m^{-2} at the Eastwind grounding site (Fig. 2). The results of a two-way ANOVA on ranks indicated that there was a significant difference in density of new recruits both among sites ($p<0.001$) and years ($p<0.001$). A Tukey pair-wise comparison test indicated that the two grounding sites each had a significantly higher density of recruits than either control site ($p<0.001$) and that density of recruits was significantly higher at the Eastwind grounding site than at the Federal Pescadores grounding site ($p<0.001$). There was no significant difference in recruit density between the two control sites. Recruit density was significantly higher in 2008 than in 2007 (Tukey, $p<0.001$) or 2009 ($p<0.01$).

Annual mortality rates of juvenile corals ranged from 11% to 58%. Mean annual mortality rates were higher at the grounding sites than the control sites during the study period, but the difference was not significant (Fig. 3). Survival of juvenile corals for longer than one year ranged from 0 to 30% of the total corals observed at each site in 2008 and 2009 (Fig. 4).

A total of 18 species recruited to the study sites, but the maximum number of species that recruited to any one site was 15 (Table 1). Twice as many species recruited to the grounding sites compared to the control sites (Table 1). Four common species, *Siderastrea siderea* (Ellis & Solander 1786), *Siderastrea radians* (Pallas 1766), *Porites astreoides* Lamarck, 1816, and *Montastraea cavernosa*, recruited to all the sites. Over half of the recruits at the grounding sites were *S. siderea*. *Porites astreoides* and

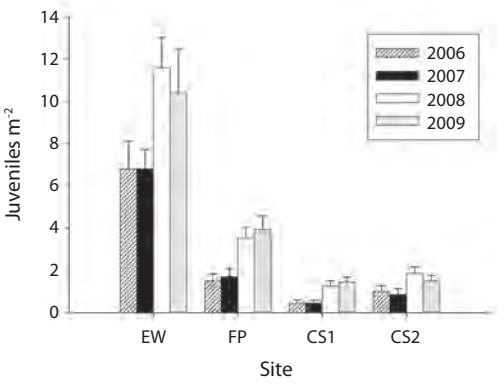

Fig. 1. Mean (+SE) juvenile coral density in quadrats from 2006 to 2009. EW=Eastwind grounding site, FP=Federal Pescadores grounding site, CS1=control site 1, CS2=control site 2.

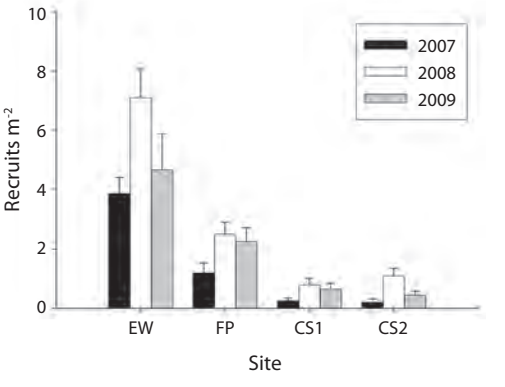

Fig. 2. Mean density (+SE) of coral recruits in quadrats from 2007 to 2009. EW=Eastwind grounding site, FP=Federal Pescadores grounding site, CS1=control site 1, CS2=control site 2.

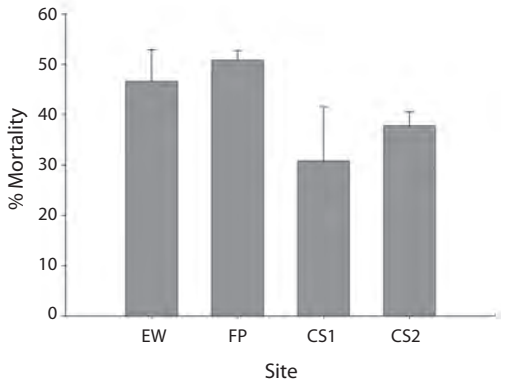

Fig. 3. Mean (+SE) annual mortality of juvenile corals in quadrats 2006-2008. EW=Eastwind grounding site, FP=Federal Pescadores grounding site, CS1=control site 1, CS2=control site 2.

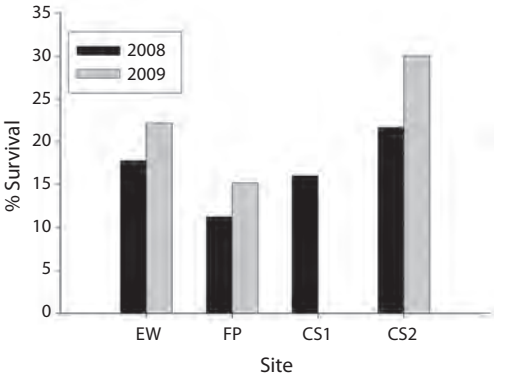

Fig. 4. Percentage of corals observed in 2008 and 2009 that had survived for more than one year. EW=Eastwind grounding site, FP=Federal Pescadores grounding site, CS1=control site 1, CS2=control site 2.

TABLE 1
Percentage of recruits settling in the quadrats from 2007 to 2009

Species	EW[1]	FP[2]	CS1[3]	CS2[4]
Acropora cervicornis	-[5]	-	-	2.9
Agaricia agaricites	1.0	0.8	-	-
Diploria strigosa	3.8	-	-	-
Diploria spp.	1.0	0.8	-	-
Dichocoenia stokesi	2.9	4.2	5.9	-
Eusmilia fastigiata	1.6	1.7	2.9	-
Favia fragum	1.0	-	-	-
Montastraea cavernosa	5.1	7.6	20.6	14.3
Meandrina meandrites	2.2	1.7	5.9	-
Mycetophyllia sp.	0.3	-	-	-
Oculina diffusa	1.3	-	-	-
Porites astreoides	5.4	4.2	35.3	28.6
Porites porites	1.0	0.8	-	2.9
Phyllangia americana	-	0.8	-	-
Solenastrea bournoni	14.1	5.0	-	-
Scolymia spp.	-	0.8	-	-
Stephanocoenia intersepta	2.6	1.7	-	2.9
Siderastrea radians	3.2	1.7	2.9	8.6
Siderastrea siderea	52.6	68.1	26.5	40.0
unidentified	1.0	-	-	-
Number of recruits	312	119	34	35
Number of species	15	14	7	7

1. EW=Eastwind.
2. FP=Federal Pescadores.
3. CS1=control site 1.
4. CS2=control site 2.
5. Dashed line indicates no recruits of that species.

S. siderea comprised a majority of the total recruits (64% to 67% combined) at the control sites though *M. cavernosa* also contributed to a relatively large portion (14% to 21%) compared to the grounding sites (5% to 8%).

A non-metric multi-dimensional scaling (MDS) plot was used to visualize the Bray-Curtis similarity of sites based on species abundance of recruits (Fig. 5). Two main clusters with 60% similarity were formed, one consisting of all the grounding sites (minus the 2007 Federal Pescadores) and the other consisting of 2008 and 2009 control site1 and 2008 control site 2. The 2007 Federal Pescadores data and 2009 control site 2 clustered with the above mentioned data at 40% similarity, and the 2007 data for both control sites clustered with the rest of the sites at 20% similarity.

Growth rates were highly variable both within and between species and often between sites (Table 2). Most species grew less than 1 cm yr^{-1} except for *Agaricia agaricites* (Linnaeus 1758) which was only observed at the grounding sites. Many colonies (23%) suffered partial mortality, resulting in smaller colony size in subsequent years, and 6% of the colonies did not grow between survey periods. For some species, the number of corals measured was low, so in these cases, rates should be interpreted cautiously.

DISCUSSION

Coral reef recovery from disturbances such as ship groundings is dependent on coral settlement, survival, and growth to larger size classes. There are many factors that can affect these processes and, thus, affect recovery ability. In the present study, annual coral recruitment rates were low (0.2 to 7.1 recruits m^{-2}) at all sites as generally seen in other studies of recruitment to natural substrate in the Caribbean (Rogers *et al.* 1984, Hughes & Jackson 1985). Recruitment rates have been observed to decline at higher latitudes (Harriott & Banks 1995, Harriott 1999, Hughes *et al.* 2002), which is consistent with the findings of the current study. Rates observed were similar but slightly lower than those reported for nearby areas to the south such as Biscayne National Park (2-13 recruits m^{-2}: Miller *et al.* 2000) and the Florida Keys (<10 m^{-2}: Smith 1997). A possible reason for reduced recruitment rates to our study sites in comparison to sites further south is reduced larval supply. Although larval supply was not specifically studied here, reduced fecundity

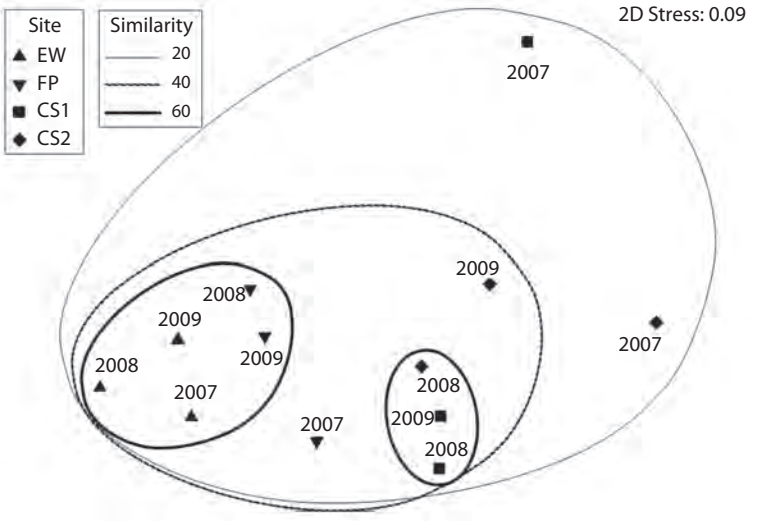

Fig. 5. Non-metric multi-dimensional scaling (MDS) plot of Bray-Curtis similarity index of recruit species composition.

TABLE 2
Mean (± SE) annual growth rates (mm yr^{-1}) for juvenile corals

Species	EW[1]	FP[2]	CS1[3]	CS2[4]	All sites pooled
Agaricia agaricites	14.9±9.6 (3)[5]	19.6±2.3 (2)	-[6]	-	16.8±5.4 (5)
Diploria spp.	4.7±1.6 (6)	8.2±0 (1)	-	-	5.2±1.4 (7)
Dichocoenia stokesii	1.6±1.2 (3)	3.9±0 (1)	2.8±4.0 (4)	-2.9±2.5 (3)	1.0±1.7 (11)
Diploria strigosa	5.8±1.6 (8)	1.9±0 (1)	-	-	5.4±1.5 (9)
Eusmilia fastigiata	3.7±0 (1)	7.7±1.4 (3)	-	-	6.7±1.4 (4)
Montastraea cavernosa	6.7±1.6 (9)	-	0.9±1.0 (7)	2.2±2.1 (12)	3.3±1.1 (28)
Meandrina meandrites	1.0±3.9 (2)	6.1±2.7 (5)	5.1±2.5 (5)	-	4.0±1.5 (12)
Oculina diffusa	-1.0±0 (1)	-	-	-	-1.0±0 (1)
Porites astreoides	9.1±1.8 (12)	11.1±6.3 (2)	2.7±4.7 (5)	4.7±2.3 (5)	7.0±1.5 (24)
Porites porites	5.7±3.8 (4)	-	-	9.1±2.3 (3)	7.2±2.3 (7)
Solenastrea bournoni	1.9±0.6 (54)	0±0.9 (2)	-	-	1.8±0.6 (56)
Stephanocoenia intersepta	9.0±3.3 (5)	8.7±2.1 (3)	-	6.7±0 (1)	8.7±1.8 (9)
Siderastrea radians	2.8±2.7 (4)	-	5.6±0 (1)	8.5±7.5 (2)	4.8±2.4 (7)
Siderastrea siderea	2.9±0.4 (147)	2.1±0.7 (46)	3.3±4.4 (3)	-2.1±6.6 (14)	2.4±0.4 (210)

1. EW=Eastwind
2. FP=Federal Pescadores
3. CS1=control site 1
4. CS2=control site 2
5. Number in parentheses represents number of colonies measured.
6. Dashed line indicates no growth rates were measured.

has been observed at higher latitudes (Hughes *et al.* 2000, St. Gelais 2010), which may contribute to lower recruitment rates. Differential recruitment among sites in the current study is probably not impacted by larval supply since recruitment was significantly higher at the grounding sites compared to the controls. Higher recruitment to the grounding sites may be the result of the greater availability of less-colonized substrate. The presence of well-established communities at the control sites may have resulted in lower settlement through the preemption of space and lower survival through competitive interactions (Birkeland 1977, Bak & Engel 1979, Maida *et al.* 2001, Kuffner *et al.* 2006, Vermeij 2006, Arnold *et al.* 2010).

As is the case in other studies (Rylaarsdam 1983, Babcock 1985, van Moorsel 1988, Vermeij 2006), growth rates of individual colonies in this study were highly variable. Nevertheless, mean growth rates were within the range reported in the literature though on the lower end for most species (Bak & Engel 1979, van Moorsel 1988, Edmunds 2000). The vast majority of colonies grew less than 1 cm yr^{-1}. However, many of the colonies (23%) experienced negative growth and shrank in size due to partial mortality. Average colony size in the region tends to be small (< 50 cm, Moyer *et al.* 2003) in comparison to other areas, and partial mortality and slow growth rates observed in the current study likely contribute to these patterns.

Although mean annual mortality rates were not significantly different among sites, total mortality of juvenile corals was about 50% at the grounding sites and 40% at the control sites during the study period, indicating a slightly higher mortality of juvenile corals at the grounding sites over the three year study period. Additionally, the percentage of corals that survived more than one year was generally low (< 30%), indicating low longer-term (> 1 year) survival across both grounding and control sites.

Low survival of corals was likely mediated by different processes at the grounding and control sites due to the differences in habitat. Although no physical measurements were taken, qualitatively it appeared that sand and rubble movement occurred at the grounding sites at an increased rate in comparison to undisturbed sites. Some of the quadrats that were initially established on hard substrate at the grounding sites were noted to be covered with sand and rubble during subsequent surveys. The movement and accumulation of sand and rubble was likely facilitated by the flattening and destruction of the relief by the grounded ships. The presence and movement of sand and rubble likely inhibited recruit survival at the grounding sites through both burial and scour (Bak & Engel 1979).

Low survival at the control sites may have been affected by competitive interactions with more established benthic organisms and algae (Birkeland 1977, Bak & Engel 1979, Maida *et al.* 2001, Kuffner *et al.* 2006, Vermeij 2006). Though no quantitative data of percent cover were taken within the quadrats, visually the grounding sites appeared more barren in comparison to the control sites (Fig. 6). In contrast, Thanner *et al.* (2006) found that community structure on artificial reefs in southeast Florida reached between 45% to 58% similarity to natural reefs in benthic species composition 4 to 5 years after deployment and that scleractinian corals on the artificial reefs had similar cover, though higher abundance of juvenile corals, compared to the natural reef after the same period. The impoverished benthic community at the grounding sites 5 years post-grounding provides a sharp contrast to the moderately high similarity in benthic community structure between the natural reef and artificial reef structures 5 years post deployment that Thanner *et al.* (2006) reported and indicates that topographic complexity may be important for the recovery of the grounding sites.

Community structure of newly settled recruits was distinct at the grounding sites. More species recruited to the grounding sites (14-15 species) compared to the reference sites (7 species), but settlement was heavily dominated (53-68%) by *S. siderea*. *Siderastrea* spp. are able to live in conditions generally considered inimical to coral survival such as fluctuating salinity, low temperatures, and high sedimentation (Macintyre & Pilkey 1969, Muthiga & Szmant 1987, Lirman & Manzello 2007). Their dominance at the grounding sites might be a result of their tolerance to the high sedimentation observed due to the destruction of topography from the ship groundings. *Montastraea cavernosa*, the dominant species in the region (Moyer *et al.* 2003), comprised a much smaller proportion of the recruits at

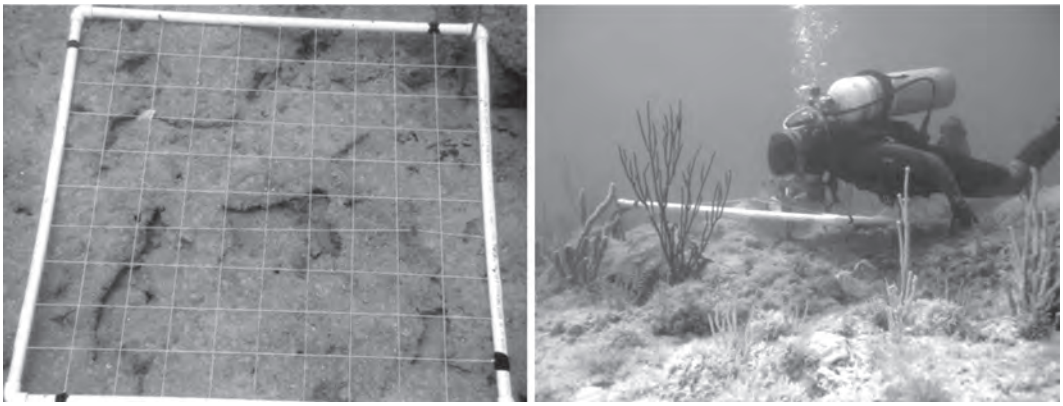

Fig. 6. Photographs of the Federal Pescadores grounding site (left) and control site 1 (right) showing the visual difference in cover of the substrate.

the grounding sites (<8%) compared to the reference sites (14-20%).

The concern is that even though corals are recruiting to the grounding sites, they are not surviving and growing to larger size classes. This phenomenon has been observed in other studies where corals have recruited to damaged sites but have failed to achieve larger size classes even on a decadal time scale (Gittings *et al.* 1990, Rogers 1984, Cook *et al.* 1994, Rogers & Garrison 2001). Corals that have low recruitment and growth rates, as many of the broadcast spawning, reef building corals do, may take even longer to recover as models have determined that convergence to pre-disturbance levels are highly dependent on recruitment rates (Hughes & Tanner 2000, Lirman & Miller 2003). Given the pattern of low recruitment and slow growth of the broadcast spawning corals observed in the present study, natural recovery is likely to be slow. Additionally, the low survival of all corals beyond a year observed at the study sites will contribute to slow recovery.

Recovery is generally defined as the return of community structure and function of the injured site to conditions similar to those that existed pre-disturbance (Edwards & Gomez 2007). However, injured sites have been known to recover to alternate community states distinct from those present pre-disturbance (Hatcher 1984). Aronson and Swanson (1997) found that a decade after the injury occurred, the Wellwood grounding site in the Florida Keys was more similar to a hard bottom community than to its original spur and groove structure. The grounding sites in the current study were visually distinct from control sites. They were noticeably flat and devoid of larger coral colonies and other benthic invertebrates such as sponges and gorgonians common at the control sites. Though return of benthic community structure, if possible, is expected to take on the order of decades, return of topography is expected to take much longer and may never occur. Thus, it is possible that the grounding sites may stabilize to some alternate state that is dissimilar from what was previously present before impact.

In conclusion, ship groundings on coral reefs cause not only serious damage to the reef builders, but also result in a loss of habitat for other organisms, consequently leading to barren areas. They often flatten the topography, leaving a source of sand and rubble that may move over the area and inhibit benthic organism settlement, growth, and survival. This study has shown that corals are able to recruit to damaged areas but that slow growth rates and high mortality rates may keep these areas in a perpetual cycle of settlement and mortality with little or extremely slow growth to larger size classes, thus inhibiting recovery. While the exact processes underlying differences in recruitment, growth, and mortality, both among sites in this study and in comparison to other areas of Florida, are unknown, we believe that the loss of topography at the grounding sites will negatively impact coral recovery. Therefore, methods of restoration compensating for increased sediment mobility and flattened relief are recommended to enhance natural recovery potential of grounding sites.

ACKNOWLEDGMENTS

We thank Nova Southeastern University graduate students for their assistance with field work and Brian Walker for GIS assistance. The Hillsboro Inlet District and Florida Department of Environmental Protection provided funding for this project.

RESUMEN

En las dos últimas décadas, más de 10 grandes encallamientos de embarcaciones se han producido en los arrecifes de coral mar afuera en el sureste de Florida. La falta de información publicada sobre el asentamiento de corales y sobrevivencia post-asentamiento y de los corales juveniles que crecen en la región, limita los esfuerzos para determinar si las poblaciones de coral serán capaces de restablecerse por ellas mismas. El objetivo de este estudio fue examinar estos procesos para obtener la información necesaria para determinar el potencial de recuperación natural. Se midió el reclutamiento anual de coral joven, el crecimiento y las tasas de mortalidad por un período de tres años, mediante 20 cuadrantes permanentes en cada uno de los dos encallamientos de barcos y dos sitios de control.

La densidad de nuevos reclutamientos fue generalmente baja, de 0.2±0.1 a 7.1±1.0 reclutamientos m^{-2}. Aunque la densidad del reclutamiento fue generalmente más alta en los sitios de encallamiento también hubo mayor mortalidad de corales juveniles en esos sitios durante el período de estudio. Las tasas de crecimiento de las colonias individuales fueron altamente variables, y muchas de las colonias se redujeron en tallas debido a mortalidad parcial. Los resultados indican que los corales presentan una disposición a reclutarse en arrecifes dañados, pero las lentas tasas de crecimiento y la alta mortalidad pueden mantener esas áreas en un perpetuo ciclo de asentamiento y mortalidad con poco o extremadamente lento crecimiento hacia las clases de tallas largas, por lo tanto inhibiendo la recuperación.

Palabras clave: reclutamiento, recuperación de coral, encallamiento de barcos, tasas de crecimiento, sobrevivencia post-asentamiento

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Preliminary results with a torsion microbalance indicate that carbon dioxide and exposed carbonic anhydrase in the organic matrix are the basis of calcification on the skeleton surface of living corals

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Received 23-VI-2011. Corrected 14-XII-2011. Accepted 20-XII-2011.

Abstract: Ocean acidification is altering the calcification of corals, but the mechanism is still unclear. To explore what controls calcification, small pieces from the edges of thin plates of *Agaricia agaricites* were suspended from a torsion microbalance into gently stirred, temperature controlled, seawater. Net calcification rates were monitored while light, temperature and pH were manipulated singly. The living coral pieces were sensitive to changes in conditions, especially light, and calcification was often suspended for one or two hours or overnight. The mean calcification rate increased from 0.06 in the dark to 0.10 $mg.h^{-1}.cm^{-2}$ (T test, n=8, $p<0.01$) in low light (15 $\mu mol.s^{-1}.m^{-2}$) and showed a positive linear relationship with temperature. With a reduction of mean pH from 8.2 to 7.6 the mean calcification rate in the light (65 $\mu mol.s^{-1}.m^{-2}$) increased from 0.19 to 0.28 $mg.h^{-1}.cm^{-2}$ (T test, n=8, $p<0.05$) indicating a dependency on carbon dioxide. After waterpiking and exposure of the skeletal surface/organic matrix to seawater, calcification showed an astonishing initial increase of more than an order of magnitude then decreased following a non-linear generalised Michaelis-Menten growth curve and reached a steady rate. Calcification rate of the freshly waterpiked coral was not influenced by light and was positively correlated with temperature. For a mean pH reduction from 8.1 to 7.6 the mean calcification rate increased from 0.18 to 0.32 $mg.h^{-1}.cm^{-2}$ (T test, n=11, $p<0.02$) again indicating a dependency on carbon dioxide. Calcification ceased in the presence of the carbonic anhydrase inhibitor azolamide. Staining confirmed the presence of carbonic anhydrase, particularly on the ridges of septae. After immersion of waterpiked corals in seawater for 48 hours weight gain and loss became linear and positively correlated to temperature. When the mean pH was reduced from 8.2 to 7.5 the mean rate of weight gain decreased from 0.25 to 0.13 $mg.h^{-1}.cm^{-2}$ (T test, n=6, $p<0.05$) indicating a dependence on carbonate. At a pH of 6.5 the skeleton lost weight at a rate of 1.8 $mg.h^{-1}.cm^{-2}$. The relationship between net calcification and pH (n=2) indicates that wt gain turns to loss at pH 7.4. These experiments confirm that calcification is a two-step process, involving secretion of a layer of organic matrix incorporating carbonic anhydrase to produce an active calcifying surface which uses carbon dioxide rather than carbonate. It is also unlikely that the calcifying surface is in direct contact with seawater. Inorganic deposition or dissolution of the skeleton in exposed dead areas of coral is a different phenomenon and is carbonate related. The wide range in results from this and other studies of calcification rate and carbon dioxide may be explainable in terms of the ratio of "live" to "dead" areas of coral. Rev. Biol. Trop. 60 (Suppl. 1): 109-126. Epub 2012 March 01.

Key words: coral calcification, CO_2, pH, temperature, organic matrix, carbonic anhydrase.

The processes involved in the production of an aragonite ($CaCO_3$) skeleton in corals are poorly known compared to calcification in other animals (Allemand *et al*. 2011). Seawater contains about 10.3 $mmol.kg^{-1}$ of calcium ions (Ca^{2+}), dissolved inorganic carbon is present in seawater as carbon dioxide (CO_2), carbonic acid (H_2CO_3), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}), with the equilibria between them described by their equilibrium constants and their relationships with pH , alkalinity and temperature. What is still not clear is in what form dissolved inorganic carbon (DIC) reaches the site of calcification, how DIC and Ca^{2+} get

there and to what extent, if at all, the calcifying surface is in direct contact with seawater. It seems to have been taken for granted that the formation of aragonite crystals would result from the combination of Ca^{2+} and CO_3^{2-} dissolved in seawater. However, Lee *et al*. (2010) have reported crystalline aragonite deposition from a solution of $CaCl_2$ containing carbonic anhydrase using CO_2 directly from the atmosphere. The sources of calcium and carbon and how they reach the calcification site have been reviewed by Cohen & McConnaughey (2003), Furla *et al*. (2000) and Allemand *et al*. (2004) and Allemand *et al*. (2011). Carbon-dioxide which can move freely through cell membranes was proposed by McConnaughey (1989) as a substrate for calcification to account for ^{18}O and ^{13}C deficiencies in coral skeletons (McConnaughey, 2000). The response of corals to changes in CO_2 partial pressure and temperature was reviewed by Reynaud *et al*. (2003). While calcification and photosynthesis may compete for the same DIC pool they are sometimes regarded as complementary (Gattuso *et al*. 1999) rather than in competition (Langdon & Atkinson, 2005). With a focus on ocean acidification as the result of increased anthropogenic carbon dioxide in the atmosphere, calcification has been increasingly linked to the calcium-carbonate saturation state (Ω) which is the ratio of the ion concentration product ($[Ca^{2+}] \times [CO_3^{2-}]$) to the solubility product of the mineral deposited, in this case aragonite (Allemande *et al*. 2004). As acidity increases the relative concentration of carbonate in seawater is reduced and has been used to predict decreased calcification at the organism and community levels (Kleypas *et al*., 1999; Gattuso *et al*., 1999, Marubini & Thake, 1999, Langdon, 2000, Langdon *et al*., 2000 Langdon *et al*. 2003 and Erez *et al*. 2011). However problems have emerged with the link between calcification and carbonate concentration or saturation state (Jury et al 2010, De Putron *et al*. or with the predictions that have followed. From the banding of Florida corals from 1937 to 1996 Helmle *et al*., 2011 found that calcification was stable and the average rate during the most recent decade was not significantly different from those of the preceding 5 decades. Fabricius *et al.* (2011) found that as pH declined from 8.1 to 7.8 (the expected change by the end of the century), found reductions in coral diversity but not all corals were affected. Krief *et al*. 2010 kept coral fragments in controlled aquarium conditions with normal and raised $C0_2$ levels equivalent to pH values of 8.09, 7.49 and 7.19 and the fragments all survived and added new skeleton. The Mediterranean coral *Cladocera* in low pH level did not show predicted reduced calcification (Rodolfo-Metalpa *et al.* 2010, 2011). There is a lot of information on the growth and dissolution kinetics of finely divided aragonite (Gutjahr *et al.*, 1996, Cubillas, 2005) but little information on how dead coral behaves. Rodolfo-Metalpa *et al*.(2011) found that dead corals did not dissolve at a pH of 7.8 and measured dissolution rates at pH 7.4 (0.3-0.6 $mg.g^{-1}.d^{-1}$) and 6.8 (3.7-4.2 $mg.g^{-1}.d^{-1}$). Rodolfo-Metalpa *et al*., (2011) suggested that coral tissues protect the skeletons from corrosive ($\Omega < 1$) seawater. Villegas-Jiminez *et al*. (2009) reported large consistent proton/Ca^{2+} exchanges which may have far reaching implications for the interpretation of kinetic and equilibrium exchanges. As the growth and dissolution kinetics are similar for calcite and aragonite (Gutjahr *et al*., 1996) the same problems of interpretation are likely to exist for aragonite.

Active calcification in scleratinian corals is a two stage process which involves secretion of a layer of organic matrix (reviewed by Allemand *et al*.1998 and Allemand *et al*. 2011) and calcium carbonate, crystallized in the form of aragonite, is deposited in the organic matrix with the involvement of structural proteins with a catalytic role similar to that of carbonic anhydrase (Tambutté *et al*. 2007), calcium binding compounds (Isa & Okazaki, 1987, Constantz & Wiener 1988 and Puverel *et al*. 2005) and proteins regulating the biomineralization process along with Mg^{2+} which inhibits calcite formation (Rahman & Oomori, 2009). Aragonite deposition takes place from the extracellular calcifying fluid (ECF) or hydrogel-like

medium (ECM) (Bryan & Hill, 1941 and Cuif *et al*. 2004a) onto the organic matrix framework on the surface of the skeleton. Charged ions such as Ca^{2+}, HCO_3^- and CO_3^{2-} cannot move passively through cell membranes so either active transport via a transcellular route, passive diffusion of ions or seawater using a paracellular route at the boundaries of cells, or some combination of these routes may be involved (Allemand *et al*. 2011, Cohen & McConnaughey, 2003). Ca^{2+}, for example, may arrive from the calicoblastic layer as the result of the Ca^{2+}ATPase pump such that protons are moved in the opposite direction enhancing the diffusion of CO_2 to the ECM (Adkins *et al*. 2003, Sandeman, 2008a). Allemand *et al*. 2011 reviewed the possible light enhancement mechanisms for calcification. The range of ratios observed for calcification rates in the light versus in the dark is large and the median value of enhanced calcification in light (LEC) is around 3 (Gattuso *et al*. 1999).

Calcification rates of corals are commonly derived by buoyant weighing techniques (Davies, 1989), uptake of the radioactive isotope ^{45}Ca (eg Moya *et al*. 2006, Al-Horani, 2007), the alkalinity anomaly technique (Smith & Key, 1975) or using a sclero-chronological technique (eg by Gischler & Oschmann 2005). Each of these methods has its advantages and disadvantages, with some requiring destruction of the coral. The buoyant weighing technique used by Franzisket (1964) and developed by Davies (1989) for nubbins of *Porites porites* has been used to measure calcification rates over time periods of less than a day. In physiological studies one disadvantage of this technique is that the coral or a coral 'nubbins' has to be transferred to and from a balance for weighing. For physiological experiments over shorter time periods it is desirable to minimise any physical disturbance and provide stable conditions while still being able to change experimental parameters such as light, temperature or pH. Analytical balances are expensive, sensitive to sea air and because they tend to drift they may require re-zeroing regularly which requires disturbing the organism. For this study, a stable torsion microbalance was developed, following Kesling & Crafts (1962), from which small pieces of coral could be continuously suspended in seawater for periods of several hours or days while the seawater medium was manipulated, a parameter at a time, with minimum disturbance to the coral. Kesling & Crafts (1962) outlined the physics of the torsion balance. In our version of the balance the beam and lengths of wire are used are shorter and piano wire was replaced with thinner wire of stainless steel, tungsten or silicon carbide with a central core of tungsten, all of which were found to be less affected by seawater . Mirrors and laser beams were used to achieve magnification of the angular rotation of the torsion wire. Early evaluation indicated that wire of 0.05-0.15mm gave a satisfactory sensitivity, that tension did not effect the sensitivity but the sensitivity decreased with heavier pieces of coral. Davies (1989) established with his buoyant weighing technique that changes in coral tissue weight over the time span of an experiment were small and could be corrected for, and could be ignored altogether for imperforate corals such as *Agaricia.* A method was developed for calibration of the balance without the need to apply the complex equations (Davies, 1989 and Jokiel et al, 1978) used for determining the air weight of coral skeleton from its weight in seawater.

This study was started initially to test and improve the balance and to evaluate its potential for the measurement of weight change in coral calcification. The method clearly has major limitations in that the system is open and the seawater medium can exchange CO_2 with the air. Also, removal of water for analysis changes the water level and interferes with balance function, the only means available of monitoring chemistry of the seawater in situ was with a pH probe. Thus with the system open to the air it can only provide an approximate indication of the carbonate status of the seawater. The aims of the study were to compare net calcification of live coral, of the skeleton after removal of the tissue and of dead coral, and to investigate how net calcification

varies with temperature, irradiance and pH, the latter with a potential to indicate whether CO_2 or CO_3^{2-} are used as the substrate for calcification. The early results were so unexpected, and had the potential to contribute to mechanisms of calcification that in spite of the limitations of the methods and the fact that time constraints meant that some experiments were not repeated, it seemed important to communicate the results more widely.

MATERIALS AND METHODS

Small pieces of *Agaricia agaricites* were snipped from the edge of thin plates of young colonies growing near the reef crest opposite the Discovery Bay Marine Laboratory. The pieces were immediately transported to the seawater tables where they were trimmed to a suitable size (1-2 cm^2) then suspended by loops of thin (0.025 mm diam.) polyester monofilament in gently flowing seawater that had been passed through frequently changed filters of cheesecloth and activated charcoal. The coral pieces were held for two or three days until used, in a regime of dark (6.30pm-6.30am) and light (6.30am-6.30pm) at about 200µmol photons under a mercury halide floodlight (Philips 25W, 25°). Seawater used in the experimental chambers was collected from outside the bay on the fore reef, passed through activated charcoal and millepore filtered (0.45µ). Salinity was measured with a Pinpoint Salinity Monitor (American Marine Inc.). Total Alkalinity of the seawater was not measured directly but was estimated from salinity using a formula for northern Caribbean waters (TA=57.3 x Sal. + 296.4 ± 19.3) from Cai *et al.* (2010). [CO_2], [CO_3^{2-}] and Ω were estimated (Table 1) using the CO@SYS program (Lewis and Wallis 1998) and the NBS Buffer scale.

TABLE 1
Estimated carbon parameters for the seawater used in experiments

Parameter	Control pH (means)		Treatment pH (Means)	
pH (NBS)	8.2	8.1	7.6	7.5
CO_2 (µmol kg^{-1})	10.1	13.4	49.7	63.6
CO_3^{2-} (µmol kg^{-1})	241.5	202.5	75	60.5
Ω_{arag}	3.9	3.25	1.2	0.97

For all experiments, single pieces of coral were suspended by the monofilament loop and a 5-6 cm length of 0.05 mm diam. stainless steel (s/s) wire from the beam of the torsion balance (Fig. 1) into a vessel with 1 or 2l of seawater that had been passed through activated charcoal and millepore filtered (0.45µ). The temperature of the water in the chamber except where otherwise stated was maintained at 28ºC and was regulated to within 0.1ºC by a temperature sensing thermistor, regulator circuit

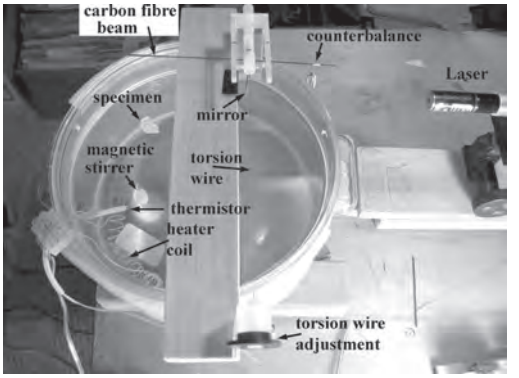

Fig. 1. The torsion balance. The ends of a 15cm length of torsion wire (tungsten, diam. 0.05-0.15mm) are embedded in short pieces of stainless steel tubing (0.5mm OD) with cyanoacrylate. One end is held by friction fit in a polyethylene cylinder which can be rotated in its mount (torsion wire adjustment). The other end of the torsion wire is inserted into a 1cm cylinder (diam. 0.62cm) of low density polyethylene with a cylindrical rare earth magnet (0.31 x 0.31cm diam.) embedded in its other end. The wire is held under tension by a second similar magnet embedded in the end of a threaded 6.35mm diam. polyethylene rod. This rod can be turned to change the separation between the two magnets. A carbon fibre beam is inserted through a hole drilled through the polyethelyene cylinder at right angles to the torsion wire. A second carbon fibre rod inserted into the polyethylene cylinder supports a small piece (2x4mm) of thin cover glass which acts as a mirror. On one end of the beam is a small vertical wire hook from which coral samples can be suspended. The other end of the beam supports a small weight that can be slid along the beam and acts as a counterbalance. The beam from a laser pointer is reflected by the mirror on to a scale at a distance of about 3 m and enables rotation of the torsion wire to be measured.

and insulated heater coil. A small magnetic stirrer (1.0x0.7cm) gently and continuously circulated the water in the vessel. The pH was changed by adding hydrochloric acid (adjusted to the density of seawater), or by exchanging some of the seawater in the vessel with seawater that had a high dissolved CO_2 or $NaCO_3$ content. For higher pH levels water in the vessel was exchanged with seawater that had been bubbled with CO_2 free air. A pH meter (IQ200, Scientific Instruments), accurate to 2 decimal places, and calibrated daily, was used to monitor changes of pH. Room lighting was 15 $\mu mol.s^{-1}.m^{-2}$ and additional light was provided by a metal halide spotlight (Philips 25W, 10°) above the beaker. Irradiance levels were measured with a LI-COR Quantum/Radiometer (LI-250). The room was otherwise darkened and draughts were excluded as far as possible. The balance was protected from air movement by cardboard baffles and readings were only taken when the room air-conditioning unit was not active. The magnetic stirrer was turned off about three minutes before readings were taken. After suspending a piece of coral in the system the position of the balance beam was adjusted by the counter balance weight and/or rotating the fixed end of the torsion wire so that the laser beam was near the appropriate end the scale. Changes in weight as aragonite skeleton was deposited result in movement of the laser beam/spot on the scale and readings were taken at intervals of about ten minutes. The course of an experiment was followed by plotting the readings on graph paper. Rates of weight gain or loss were calculated by regression analysis of series of 4-6 readings. After a set of control reading a single experimental parameter (irradiance, temperature or pH) was then changed and after an hour for acclimation a new set of reading could be taken at 10 minute intervals to give a new rate. When rates of change were very low, longer periods between reading and more readings were taken.

A series of small solid aragonite cubes, cut from *Agaricia* skeletons (1-3mg) of which the dry weights were accurately known were prepared beforehand. Each cube was attached to a 20 cm length of extremely fine monofilament consisting of a single strand from dental floss. Attachment was by dipping the end of the filament into cyanoacrylate and touching it to the cube. A cube was used to calibrate the system for each experiment by dropping it, suspended by its monofilament, carefully onto the coral's surface. The position of the laser spot on the scale with and without the aragonite cube in position was recorded. This was repeated several times and the mean displacement for the cube was calculated. From this the equivalent dry wt. of aragonite per scale unit could be calculated. The aragonite cubes were also used to verify the linearity of the scale (corresponding to a total rotation of < 4º of the wire). This procedure also permitted malfunction of the balance to be detected. Generally the system was very stable and the laser spot returned to the same place even overnight, but if the laser spot did not return to its original position it was usually because of contamination on the s/s suspension wire at its point of entry into the seawater. Replacement of the wire usually corrected the problem. Following any change of temperature, pH or irradiance at least an hour for acclimation was allowed. When an experimental temperature was changed and controlled to a new level the density of the seawater also changed and a recalibration of the balance was required. Corals were carefully inspected before each experiment and were rejected if there were any sign of epiphytes, encrusting organisms or unhealthy areas on the upper or lower surfaces, or if mucus was present. Once placed in the chamber the live coral was permitted to acclimate for a period of two hours, at which point adjustments were made to the balance and placement of the laser so that reading could be taken on an appropriate part of the scale. If detectable calcification was taking place an experiment could commence. If calcification was not detected (no movement in the laser spot for an hour) the coral fragment was left in the equipment until calcification started. Occasionally an overnight period of acclimation was required before calcification started. During experiments corals were

inspected for oxygen bubbles, which tended to appear in high light conditions and if mucus was present. The balance proved to be less sensitive with larger pieces of coral so pieces of coral used in the study were kept small (mean area 1.74 cm^2, mean wt 0.42 g, n=56). The vessels used in the study were large (1 or 2 l beakers), so that changes in the composition of the sea water during an experiment were minimised. The greatest change affecting experiments was probably due to evaporation increasing the density of the seawater and its level relative to the torsion balance, however the changes were slow and the rate of change constant. During experiments the pH changed as skeleton is deposited but because the vessels were open and exchange with the surrounding air could take place and because of slow drift of the pH meter during longer experiments, it was felt that the pH could not be used other than as an approximation of conditions during an experiment. Unless stated otherwise pH of the seawater was generally (8.1-8.2. The pH was continuously monitored and the pH recorded for an experiment was the average of the pH at the beginning and end of each period of measurement. The approximate [CO_2] and [CO_3] at the control and treatment pH used can be seen in Table 1.

To examine the role of the organic matrix in the calcification process, coral skeletons that had been stripped of their live tissue were exposed directly to seawater. A commercial dental waterpik that had been modified with a narrower jet and to work at higher pressure was used to blast away the living tissue with a jet of seawater (waterpiking). This exposes organic matrix and most recently deposited aragonite on the surface of the skeleton. Inspection with a dissecting microscope established that tissue was completely removed even from the deepest polyp cavities

For the higher calcification rates of the waterpiked coral thicker wire was used in the balance to reduce its sensitivity so that the laser spot stayed on scale. The calcification rates of the freshly waterpiked coral were not linear and a different procedure had to be used to compare control and post treatment rates. Readings of weight were taken for 4-6 hours to establish the shape of the curve. The experimental parameter was changed (temperature, pH or acetazolamide added) and after acclimation readings were taken to obtain the new calcification rate. Using the NCSS software, a predicted rate was obtained from the shape of the initial shape of the curve to cover the same time period as the post treatment rate. The two rates were then compared.

In order to see if there were any longer term changes in calcification rate pieces of coral skeleton that had been freshly waterpiked were suspended in seawater for at least 48 hours. Weight gain and loss were measured at normal seawater pH and at reduced pH by replacing some medium with seawater with dissolved CO_2. Dried skeletons taken to Trent University, Canada, were used to determine the relationship between weight gain and loss across the pH range 8.2 - 6.0. For these experiments "Coralife" artificial seawater was used (for chemical analysis and chemistry see Atkinson and Bingham, 1999). A modified balance which was closed to the outside air was used. For the lower rates of loss/gain time periods of 6-12 hours were used. At least two readings of pH and weight gain/loss were taken at the beginning and end of each time period. Rates were calculated using regression analysis. The pH was measured with an Omega PHB-212 Bench pH Meter accurate to three decimal places and calibrated daily with Omega buffers. Again the average of the readings at the beginning and end of each time period was recorded as the pH. Coral surface areas were estimated with aluminium foil (Marsh, 1970).

NCSS statistical software (Number Crunching Statistical Systems, Dr Jerry Heintze, Kaysville, Utah) was used for obtaining a best fit for the growth curves.

RESULTS

Live coral: Pieces of live *Agaricia* did not start calcifying until about 24 hours after collection but if newly collected pieces were

suspended on the torsion balance overnight in the dark calcification usually started before morning. Corals appeared to be very sensitive to any changes in conditions. Changes in light level or chemistry of the water were often followed by the cessation of calcification for an hour or two or longer and many experiments had to be disbanded because calcification ceased completely, although if left overnight calcification often restarted. High light levels (> 300 μmol.s^{-1}.m^{-2}) were not used in the study because of the formation of oxygen bubbles which were sometimes formed and interfered with the functioning of the balance. With irradiance of 65 μmol.s^{-1}.m^{-2} calcification was positively correlated with temperature (Fig. 2). Over the range 27-29.5ºC the calcification rate increased by about 15% per 1ºC change (n=1). The mean calcification rate for *Agaricia agaricites* increased by 60% from 0.063 mg.hr^{-1}.cm^{-2} in the dark to 0.101 mg.hr^{-1}.cm^{-2}.in ambient laboratory lighting of 15 μmol.s^{-1}.m^{-2} (T test, n=8, $p < 0.01$). When the irradiance level was increased to 65 μmol.s^{-1}.m^{-2} further calcification often ceased for an hour or two then sometimes increased to a higher level but often to a lower level (mean 0.80 mg.hr^{-1}.cm^{-2}, T test, n=10, $p > 0.05$) than under the laboratory lighting. When the mean pH of the seawater was lowered from 8.2 to 7.6 the mean calcification rate increased from 0.19 mg.hr^{-1}.cm^{-2} to 0.28 mg.hr^{-1}.cm^{-2} (T test, n=7, $p< 0.05$).

Waterpiked coral: Freshly waterpiked pieces of coral suspended in seawater on the torsion balance were found to have an astonishing initial calcification rate (n=30) more than an order of magnitude higher than the same piece of coral when alive. For example *Agaricia* #20 had a calcification rate of 0.029-0.063 mg.hr^{-1}.cm^{-2} when alive but after waterpiking the initial calcification rate was over 1.0 mg.hr^{-1}.cm^{-2}. The rate however decreases exponentially with time (Fig. 3). The weight/time data give a good fit (R^2 = 0.9952) to the monomolecular growth equation, $W_t = W_{max} (1- e^{k.t})$ and a better fit (R^2 = 0.9987) to the generalized Michaelis-Menten equation from enzyme kinetics (Lopez *et al.* 2000), with time replacing substrate level: $Wt = (W_{max} .t)/(K + t)$

(Note: W_t is the increase of weight at time t, W_{max} is the asymptotic or maximum potential value of W and K is the time for half maximum growth).

With longer (t > 6 hours) experiments (Fig. 4A, B, C) It became apparant that Wt does not actually reach an asymptote or maximum but continues to rise at a constant rate. The Michaelis-Menten general formula was

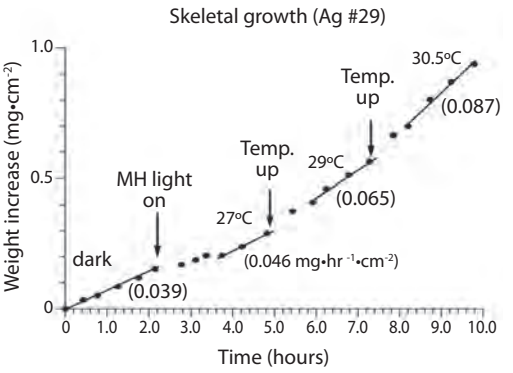

Fig. 2. Weight increase plotted against time. Calcification rates expressed in mg.$^{-1}$.cm^{-2} calculated from the regression lines are given in parentheses. Live *Agaricia* #29 in the dark, and in the light (65 μmol s^{-1} m^{-2}) at 27ºC, 29ºC and 30.5ºC.

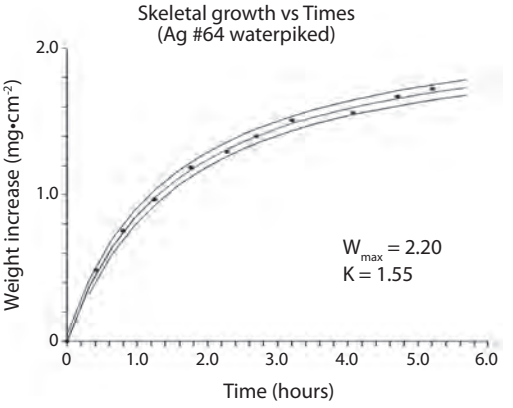

Fig. 3. *Agaricia* #64, Weight increase plotted against time for a freshly waterpiked skeleton. Best fit curve for $W_t = (W_{max}.t)/(K + t)$ with p = .05 lines.

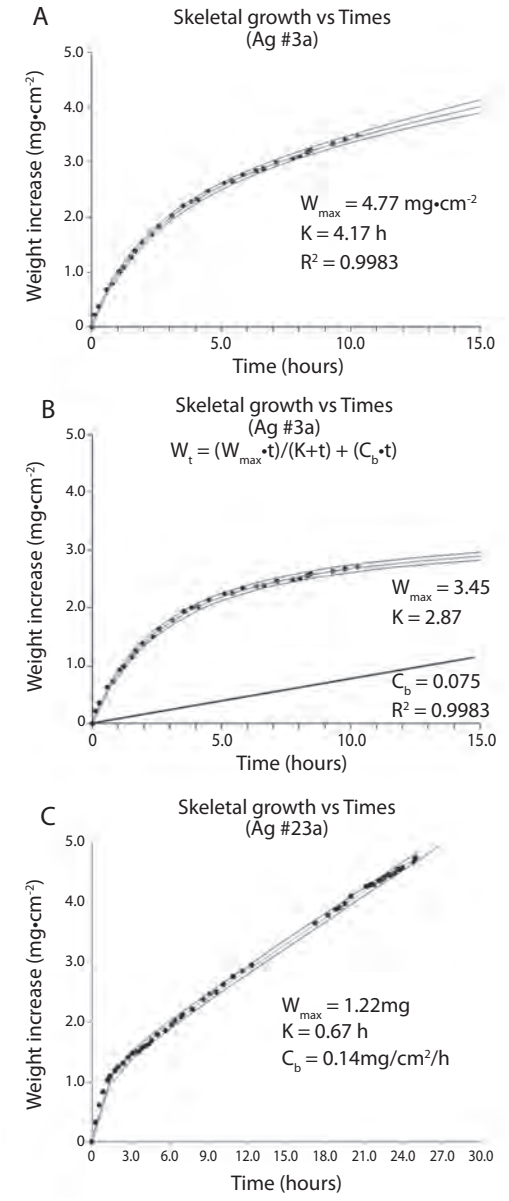

Fig. 4. Waterpiked *Agaricia agaricites,* Weight increases plotted against time for longer running experiments. (**A**) #3a best fit curve plotted with p = 0.05 lines for $W_t = (W_{max}.t)/(K + t)$. (**B**) #3a best fit curve plotted for $W_t = (W_{max}.t)/(K + t) – C_b.t$ with the contribution of $C_b.t$ plotted separately. (**C**) #23a best fit curve for $W_t = (W_{max}.t)/(K + t) + C_b.t$ showing the steady weight gain ($C_b.t$) continuing after the varying component $(W_{max}.t)/(K + t)$ has reached its asymptote.

modified to: $Wt = ((W_{max}.t)/(K + t)) + (C_b . t)$ where Cb is the constant rate of increase or slope of the line (Fig. 4B) and an even better fit to the data is obtained. For coral # 3a (Figs. 4A, B.) the fit to the modified formula (R_2 = 0.9987) is better than that of the unmodified formula (R^2 = 0.9983).

At 27ºC (Fig. 5A) the predicted calcification rate was 0.48 $mg.hr^{-1}.cm^{-2}$, compared to the actual calcification rate at 29 ºC of 0.65 $mg.hr^{-1}.cm^{-2}$. This represents a 17.7 % increase per ºC which is fairly close to the 15% change per ºC for calcification obtained for live corals. The effect of changes of pH can be seen in Fig. 5B for a typical experiment. For a mean change of pH from 8.1 to 7.6 *Agaricia* responded with mean predicted calcification rate of 0.18 $mg.hr^{-1}.cm^{-2}$ compared to an actual mean calcification rate 0.32 $mg.hr^{-1}.cm^{-2}$. This represents a 78% increase in calcification rate (T test, n=11, $p < .02$). Light did not affect the calcification rate. The initial (first hour) calcification rates of freshly waterpicked coral obtained from the NCSS curve-fitting software (Fig. 7) are shown plotted against the time of day at which each coral piece was waterpiked (n=20). These results show a steady decrease in activity through the daylight light hours but a massive increase in the late afternoon.

Carbonic anhydrase: Experiments were undertaken to verify the presence of carbonic anhydrase in the exposed skeletal surface. When the carbonic anhydrase inhibitor, acetazolamide, was added to give a 100µMol solution calcification ceased completely (Fig. 5C). Pieces of *Agaricia* skeleton that had been waterpiked and dried were also tested for carbonic anhydrase with the technique of Ridgeway & Moffatt (1986). The tips of ridges and septae showed the quite distinctive blackening that indicates the presence of carbonic anhydrase (Fig. 6).

Dead coral (Waterpiked coral after soaking in seawater)**:** A third series of experiments was undertaken to investigate what happens

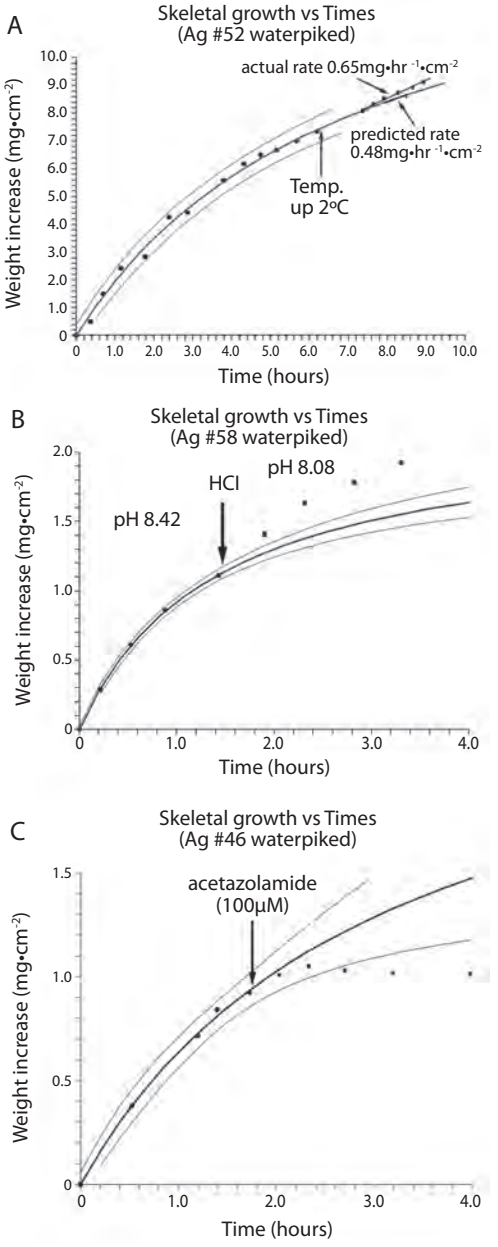

Fig. 5. Water-piked *Agaricia agaricites*. (**A**) #28, temperature raised 2ºC after 2h. (**B**) #58f, HCl added after 1.5h. (**C**) acetazolamide added after 1.75h.

Fig. 6. Photograph of waterpiked *Agaricia* skeleton stained (Ridgeway and Moffett, 1986) for carbonic anhydrase. (incubation for 10 minutes in 1.75 mM $CoSO_4$, rinsing and development in 0.5% ammonium sulphide for 3 minutes. The tips of ridges and septae show blackening that indicates the presence of carbonic anhydrase. Scale bar 0.05cm.

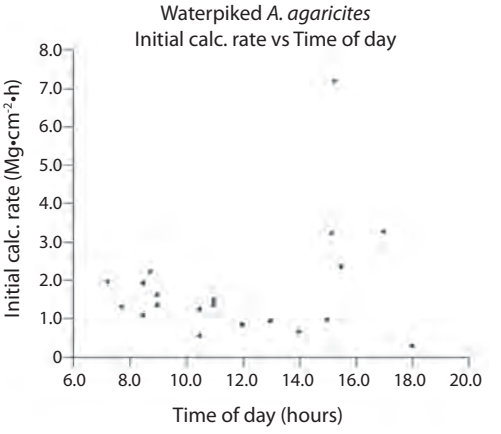

Fig. 7. Waterpiked *Agaricia agaricites*. Initial calcification rates plotted against time of day when waterpiked.

after a waterpiked coral has reached its asymptotic maximum W_{max}. After waterpiking and suspension in seawater for 48-72 hours it was found, surprisingly, that rates of deposition were linear and the same order of magnitude as those of the live coral. Unlike the situation with freshly waterpiked corals the rate of deposition this time was positively correlated with pH. (Figs. 8A, B). When the mean pH of the seawater was reduced from 8.2 to 7.5 the mean calcification rate decreased from 0.25 to 0.13 $mg..h^{-1}.cm^{-2}$. This 50% reduction is statistically significant (T test, n=6, $p < 0.05$). With a larger change in pH from 8.25 to 6.5 the deposition

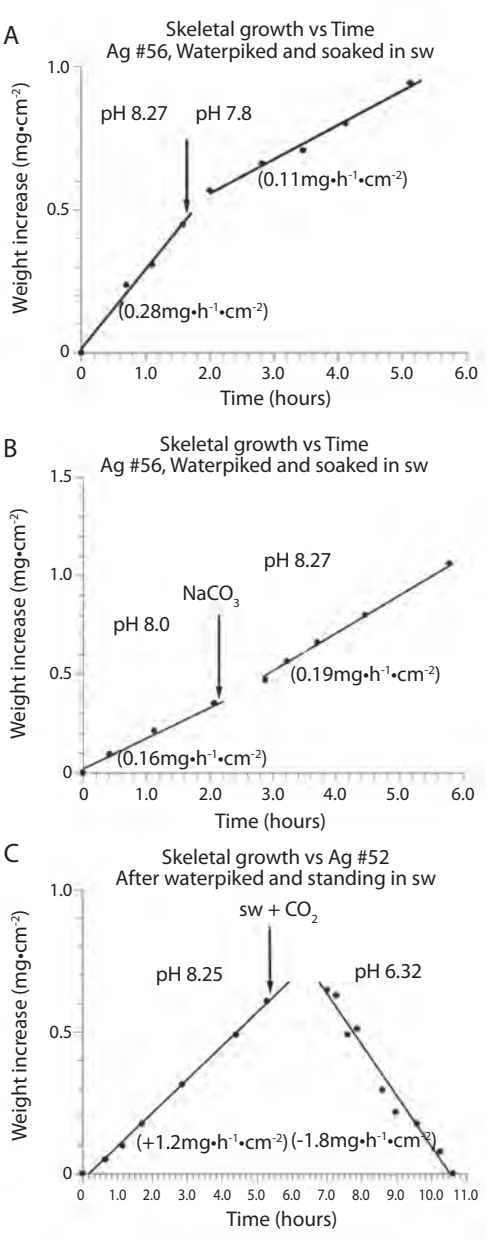

Fig. 8. *Agaricia agaricites*, skeleton after waterpiking and soaking in seawater, Weight increases plotted against time. Calcification rates, expressed in mg^{-1} cm^{-2}, calculated from the regression lines, are given in parentheses. **(A)** #56, before and after addition of HCl. **(B)** #64 before and after addition of $CaCO_3$. **(C)** #52 before and after a large reduction in pH.

rate (n=1) (Fig. 8C) changed from a gain of 1.2 $mg.hr^{-1}.cm^{-2}$ to a loss of 1.8 $mg.hr^{-1}.cm^{-2}$. Interpolation between the two rates indicates that the point at which the change from deposition to dissolution is at around pH 7.5. Again the deposition rate of $CaCO_3$ was very sensitive to temperature (n=1), showing a 57% increase per ºC but there was no change in response to changes of light. (n=6). In the first of two more detailed explorations of the relationship of calcification/dissolution rate and pH the plot of calcification rate against pH (Fig. 9A) had a sigmoidal form similar to a hyperbolic sine function $\sinh = \frac{1}{2}(e^{x} - e^{-x})$. The data (20 points)

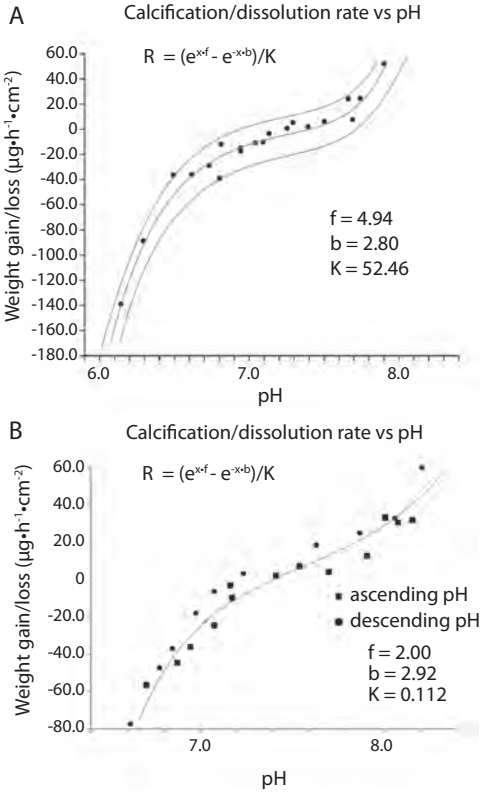

Fig. 9. *Agaricia agaricites*, skeleton after waterpiking and soaking in seawater. **(A)**. Best fit curve with p = 0.05 lines. (B) Best fit curve and data points for ascending and descending pH change and hysteresis effect.

give a good fit (R^2 = 0.965) to the formula R = $(e^{x.f}-e^{-x.b})/K$ where R is the net wt gain/loss, x is pH_r - pH_0 (pH_0 the pH at which R = 0) and f and b are constants (comparable to reaction order or dissolution rate constants) for the forward (weight gain) and backward (weight loss) parameters and K is a constant. Growth/dissolution results are commonly presented as a function of Ω, for example $r = K(1-\Omega)^n$ (Cubillas *et al*. 2005) but the treatment used here is similar to the approach of Lopez *et al*. (2009) and DePaulo (2011) who regard the net calcification rate R as the sum of the forwards rate (gain) R_f and backwards rate (loss) R_b. The relationship between growth/dissolution and pH of a second *Agaricia* skeleton (Fig. 9B) showed the same hyperbolic sine shape but the data (23 data points) gave a less good fit to the formula (R^2 = 0.730). However the difference in position of the data points from the descending pH changes and the ascending pH series are suggestive of the hysteresis effect, depending on which way their experiments were run, described by Gutjahr *et al*. (1996) for finely divided aragonite and calcite. In their comparison of the growth and dissolution rates of finely divided calcite and aragonite plotted against pH the curves, especially for aragonite, are similar to those found in this study. The pH at which R was zero for both skeletons in this study (Fig. 9A, B) was very close to a pH of 7.4. The same crossover point found by Gutjahr *et al*. (1996) was close to a pH of 7.8. One effect noticed in this study but not followed up was that when a piece of coral was moved into seawater of lower pH (eg from 8.1 to 7.5) the pH increased by about $0.01.h^{-1}$.

DISCUSSION

In the experiments in which the pH of the experimental medium was reduced from an average of 8.2 to 7.6 (estimated $[CO_2]$ increases from 10.1 μmol kg^{-1} to 49.7 while estimated $[CO_3^{2-}]$ decreases from 241.5 μmol kg^{-1} to 75) calcification in the living corals increased significantly This provides good evidence that CO_2 is the substrate for calcification rather than CO_3^{2-}. The report by Lee *et al*. (2010) that crystalline aragonite is deposited from a solution of $CaCl_2$ containing carbonic anhydrase using CO_2 directly from the atmosphere gives further support for the possibility that CO_2 can be the substrate for calcification.

Light enhanced calcification has been shown to be 3x the dark level (Allemand *et al*. 2011). This study confirms that for living *Agaricia agaricites* the dark calcification rate is almost doubled in very low light, but for higher light levels, results were inconsistent. In this study when light levels were increased calcification usually ceased immediately for a period of hours then settled at a new level which could be higher or lower that the original. At higher light levels oxygen bubbles interfered with the experimental technique and the hyperbolic tangent relationship between irradiance and calcification rate found by Marubini *et al*. (2004) or Moya *et al*. (2006) could not be confirmed. Sandeman (2008a) showed that the Ca^{2+}ATPase/proton pump may be light sensitive as suggested by Cohen & McConnaughey (2003) and proposed (Sandeman, 2008b) that H_2O_2 produced by zooxanthellae in high light conditions makes the plasma membrane leaky to Ca^{2+} with the result that more Ca^{2+} could reach the ECM. The inconsistent response of calcification to light found in this and other studies is difficult to explain. However, if CO_2 is indeed the substrate for calcification then the responses of corals to light (i.e. initially turning off calcification then adjusting to a new level) found in this study are not inconsistent with what one might expect if there is competition for CO_2 between calcification and photosynthesis.

The (x25) higher initial calcification rate of the freshly waterpiked coral skeletons compared to that of the same surface while alive was astonishing. The possibility that it is the result of air bubble entrapment following waterpiking and the gradual solution of the bubbles seems unlikely given the imperforate nature of the skeleton of the thin pieces of *Agaricia* and the response to acetazolamide (Fig. 5C). Inspection showed the accumulation of a thin

layer of white solid, especially on the ridges of the septa, which when scraped off contained needle shaped rather than rounded crystals, indicating aragonite rather than calcite. Another possibility is that that an enzyme or catalyst is incorporated in the exposed surface which is responsible for the high initial rate. This would be in line with the two-step mechanism proposed by Cuif & Dauphin (2005a,b) in which the biomineralization process starts with secretion of a proteoglycan matrix in which mineralization takes place. The matrix has been shown to contain structural proteins which play a catalytic role similar to that of carbonic anhydrase (Tambutté *et al*. 2007), has calcium binding properties (Isa & Okazaki 1987; Constantz & Wiener, 1988 and Puverel *et al*. 2005) and acidic proteins regulating the biomineralization process and the mineralization process(Rahman & Oomori, 2009) may involve all of these. Confirmation for the presence of carbonic anhydrase on the surface of waterpiked corals comes from the experiment involving inhibition of calcification by acetazolamide (Fig. 5C) and the demonstration by staining of carbonic anhydrase in the most rapidly growing areas of the skeleton (Fig. 6). The basic shape of the deposition versus time curves for waterpiked corals (Figs. 3, 4A, B) is probably the result of the exposed carbonic anhydrase or other active ingredients of the organic matrix being covered as new aragonite is formed. The asymptote W_{max} is reached when the deposition rate is reduced to zero when all the carbonic anhydrase is obliterated by deposited aragonite. Some confirmation for this comes from the lowering of the initial calcification rate (Fig. 7) that takes place during the day. The increases seen in corals waterpiked in the late afternoon may indicate that new organic matrix may be layed down at that time. The linear component of the weight gain C_b is of the same order of magnitude as the rate of weight gain of coral skeleton after soaking in seawater and this abiotic mineralization based on carbonate source appears to start directly after the removal of live tissue by waterpiking and takes place simultaneously. When pH was reduced from an average of 8.1 to 7.6 (estimated $[CO_2]$ increases from 13.4 μmol kg^{-1} to 49.7, while estimated $[CO_3^{2-}]$ decreases from 202.5 μmol kg^{-1} to 75) the actual calcification rates showed an increase over the predicted rate which was significant at $p< 0.02$ level. This indicates that, as for living coral, CO_2 rather than CO_3^{2-} is the form in which DIC reaches the site of calcification as suggested by McConnaughey (1989). As reported by Dauphin *et al*. (2006) and Tambutte *et al*. (2007) the enzyme activity of the organic matrix is stable, it was hardly affected by immersion in boiling water or ethyl alcohol and skeletons of corals collected the previous year showed the same ability to deposit skeleton in this non-linear manner. A small piece of *Montastrea annularis* skeleton from the museum collection showed some activity.

If the (x25) higher initial calcification rate of the freshly waterpiked coral skeletons compared to that of the same surface while alive is indeed the result of an enzyme or catalyst incorporation in the exposed surface it indicates that the calicoblastic layers restrict calcification and contact between the living calcifying surface and seawater delivery via a paracellular route must be small. A model that does not require a paracellular pathway is that of McConnaughey (1989) and Adkins *et al*. (2003). It is based on dissolved CO_2 as the substrate for calcification reaching the ECM by diffusion and Ca^{2+} ions are transported into the ECM and protons transported out by the Ca^{2+}ATPase/proton pump. CO_2 which can pass freely through lipid membranes reaches the ECM directly, with its passage enhanced by the pH difference between the ECM and calicoblastic layer created by movement of protons as the result of by the Ca^{2+}ATPase/proton pump. In the presence of calcium binding proteins and carbonic anhydrase in the organic matrix calcium carbonate is deposited as aragonite: $Ca^{2+} + H_2CO_3 \rightarrow CaCO_3 + 2H^+$

After waterpiking and soaking in seawater the pieces of dead coral skeleton showed a quite different response to lower pH. When the average pH was reduced from 8.2 to 7.5

(estimated [CO_2] increases from 10.1 µmol kg^{-1} to 49.7, while estimated [CO_3^{2-}] decreases from 241.5 µmol kg^{-1} to 75) calcification rate decreased, indicating involvement of carbonate rather than carbon dioxide. At a pH of seawater greater than 7.4 pieces of coral skeleton (Figs. 9A,B) gain weight and below 7.4 lose weight. The weight gains found in this study seem to be problematical. Emerson & Hedges (2009) state what appears to be a commonly held belief that when $\Omega > 1$ precipitation should occur "but this is rare because high concentrations of Mg. block nucleation sites on the mineral surface", the results of this study suggest otherwise. The linear component of the weight gain C_b of freshly waterpiked coral is of the same order of magnitude as the rate of weight gain of coral skeleton after soaking in seawater and this abiotic mineralization based on carbonate source appears to start directly after the removal of live tissue by waterpiking and appears to be a property of non-living exposed coral. The slopes of the curves (Fig. 9A, B) above and below 0, (described by b and f in the equations) are different. This was also found for finely divided aragonite by Gutjhar *et al*. (1996). The hysteresis-like effect seen in the relationship between growth/deposition rates and pH (Fig. 9B), also reported by Gutjhar *et al*. (1996) may be the result of the Ca^{2+}/proton exchange reported by Villegas-Jiménez *et al*. (2009). In this study when coral was moved from seawater of pH 8.1 to seawater of pH 7.5 there was an initial increase in pH ($0.01.h^{-1}$) indicating uptake of protons from the medium as described by Villegas-Jiménez *et al*. (2009). This effect as pointed out by Villegas-Jiménez *et al*. (2009) should be investigated because it could have important consequences for the interpretation of data in calcification studies using calcium isotopes, buoyant weighing or the alkaline anomaly techniques. Further investigation is also needed to better understand the growth/dissolution dynamics of dead coral over a range of temperatures and pH. This should probably be done in a closed flow-through system rather than in the open system without flow that was used in this study.

This study confirms that there are two distinct processes involved in calcification and skeletal growth. First, a "biologically controlled mineralization" process (Mann, 1983) or "organic matrix-mediated mineralization" (Allemand *et al*. 2011). This was seen in living and and freshly waterpiked coral skeleton and is based on the enzyme carbonic anhydrase in the organic matrix, dissolved CO_2 and Ca^{2+}. Second, the deposition and dissolution seen in the freshly waterpiked pieces soaked in seawater (dead coral skeleton) with a non-living mineralization process which is carbonate-based and probably linked to the calcium carbonate saturation state ω.

In their survey of the response of corals to elevated CO_2 Reynaud *et al*. (2003) found -3% to -79% changes in calcification rate. Their own experiments indicated that, at normal temperatures, there was no response to elevated pCO_2 but when temperature and pCO_2 were both elevated, calcification dropped by 50%. Yii *et al*. (2009) found an increase in calcification rate for *Galaxea fascicularis* and a decrease for *Porites cylindrica* with increased CO_2 levels. Muehllehner & Edmunds (2008) reported a small (+5%) increase in calcification rate at 29ºC for *Porites rus* (n=11) and a larger (+100%) increase for *Pocillopora meandrina* (n=9) with increased pCO_2; however at 27ºC there were reductions in calcification rate for both species. All other recent studies have all found reduced calcification with lower pH and have related calcification to [CO_3^{2-}] and the carbonate-saturation state ω. In this study live corals and freshly waterpiked skeletons responded to lower pH or (elevated CO_2) with increased calcification indicating that the substrate for calcification is CO_2. It is perhaps important to consider why this result is at odds with the majority of studies that have reported coral calcification to decrease with increased pCO_2. The explanation for the different results may lie in the fact that in the present study small pieces from the growing edges of thin plates of the *Agaricia agaricites*, were used. These, except for the thin broken edges, were completely covered with living tissue. In other

studies the coral colonies used in calcification studies may have had dead exposed skeleton or a more porous skeleton with internal structural spaces, or channels and cavities from boring animals and which may present a significant area of skeleton in direct contact with seawater. The rate of non-biotic carbonate based deposition is higher and it may outweigh calcification by living coral tissue and thus account for the results obtained. Using the deposition rates per unit area obtained in this study for a coral with a low live calcification rate (comparable to the mean dark rate) and for the abiotic deposition on "dead" areas at two pH levels it is possible to estimate the rates for different combinations of live and dead areas of skeleton (Table 2). The difference between calcification rates in seawater of pH 8.2 and 7.8 range from +30% for coral with no dead areas to -21.5% for coral with 30% dead exposed surface. Increased temperature would increase this range and the difference in temperature coefficients of the two processes may explain the differing for results for different temperatures obtained for example by Muehllehner & Edmunds (2008).

Cohen & McConnaughey (2003) posed the question "Why do coral reefs calcify so fast?". One might also ask why corals calcify so slowly? In this study calcification rates of living pieces of *Agaricia* varied, at normal pH, from a mean of 0.063 $mg.hr^{-1}.cm^{-2}$ in the dark to 0.19 $mg.hr^{-1}.cm^{-2}$ in light while potential rates for newly exposed surface is over 1.0 $mg.hr^{-1}.cm^{-2}$. The reason is fairly clear, living corals build complex three dimensional structures. Structures such as the septae need high rates of deposition during formation with deposition restricted in other areas. Where and how much skeleton is deposited is controlled by the organic matrix and pattern with which the carbonic anhydrase is laid down. Sandeman (2008b) pointed out that vesicles present in the layers in contact with the skeleton are probably associated with secretion of the organic matrix, and the calicoblastic layers had the highest concentration in regions of the coral with the highest calcification rates. Similarly stained carbonic anhydrase seen in Fig. 6 is distributed with the highest concentration on the fast growing septal ridges. This adds weight to the views of Wainwright (1963) and Allemand *et al*. (1989, 2011) that it is the organic matrix that determines the patterns of calcification and provides a mechanism for realizing the complex architecture of corals. The results obtained in this study are also consistent with the hypothetical mechanism presented by Allemand *et al*. (2011) for the assembly of nanograins within the dynamic interface (Colfen & Mann, 2003) provided by the organic matrix.

There are implications from this study for understanding what is likely to happen to corals as the result of acidification and/or warming of the world's oceans. The findings suggest that lower pH, because $[CO_2]$ is higher, and increased temperature, will both enhance active biotic calcification, at least to a point at which other processes such as bleaching take over. For skeleton directly exposed to seawater (dead areas of the coral), as pCO_2 increases and

TABLE 2

Calcification rates (mg h^{-1} cm^{-2}) at pH 8.2 and 7.8 calculated for 5-30% dead area. Rates for 0% and 100% dead area for a coral with a low calcification rate were measured, other values by interpolation. Calcification Rates, mg h^{-1} cm^{-2}

% dead area	pH 8.2	pH 7.8	Difference	% Difference
0	0.092	0.120	0.028	+ 30.4
5	0.100	0.119	0.019	+18.6
10	0.108	0.117	0.009	+0.08
15	0.116	0.116	0.000	0.0
20	0.124	0.114	0.100	-0.08
25	0.132	0.113	0.019	-14.4
30	0.141	0.111	0.030	-21.5
100	0.250	0.090	0.160	-64

ω becomes lower, dissolution will eventually take place. Once past this point temperature increase will only increase the rate of dissolution. Because of the rates and temperature coefficients involved, it is unlikely that an increase in live calcification due to temperature can outpace losses due to abiotic weight loss. For many corals, even if active live calcification is taking place dissolution of skeleton at exposed dead areas, as suggested by Rodolfo-Metalpa *et al*. (2011) and Ries (2011) will affect survival by seriously weakening the supporting structure. Those species with smaller areas of exposed dead surface and a stronger Calcium/ proton pump (Ries 2011) may have a better chance of survival as pH levels drop.

RESUMEN

La acidificaión de los océanos está alterando la calcificón de los corales. Sin embargo, el mecanismo no es todavía claro. Para explorar que controla la calcificación piezas pequeñas del borde de láminas delgadas de *Agaricia agaricites* fueron suspendidas de una microbalanza de torsión en agua de mar ligeramente agitada y con temperatura controlada. La tasa neta de calcificación fue monitoreada mientras se manipulaba la luz, temperatura y pH. Las piezas de coral vivo fueron sensibles a cambios en las condiciones, especialmente de luz, y la calcificación se suspendía por una o dos horas o de un día para otro. La tasa media de calcificación aumentó de 0.06 en la oscuridad a 0.10 mg h^{-1} cm^{-2} (prueba T, n=8, p<0.01) en luminosidad baja (15 µmol s^{-1} m^{-2}) y mostró una relación lineal positiva con la temperatura. Con una reducción en el pH promedio de 8.2 a 7.6 la tasa de calcificación media en la luz (65 µmol.s^{-1}.m^{-2}) aumentó de 0.19 a 0.28 mg h^{-1} cm^{-2} (prueba T, n=8, p<0.05) indicando una dependencia de dióxido de carbono. Después de remover el tejido y exponer la superficie de los esqueletos/matriz orgánica a agua de mar, la calcificación tiene un marcada aumento inicial de más de un orden de magnitud y después decrese siguiendo una curva generalizada Michaelis-Menten de crecimiento no-lineal hasta alcanzar una tasa estable. La tasa de calcificación de esqueletos recién limpiados no estaba influenciada por la luz y estaba positivamente correlacionado con la temperatura. Pra una reducción media de pH de 8.1 a 7.6 la tasa media de calcificaión aumentó de 0.18 a 0.32 mg h^{-1} cm^{-2} (prueba T, n=11, p<0.02) de nuevo indicando la dependencia en el dióxido de carbono. La calcificación cesó en la presencia de azolamida un inhibidor de la anhidrasa carbónica. Tinciones confirmaron la presencia de anhidrasa carbónica, particularmente en las crestas de los septos. Después de sumergir esqueletos sin tejido en agua de mar por 48 horas la ganancia y pérdida de peso se volvió lineal y relacionada positivamente con la temperatura. Cuando el pH promedio se reducía de 8.2 a 7.5 la tasa media de ganacia de peso decrecía de 0.25 a 0.13 mg h^{-1} cm^{-2} (prueba T, n=6, p<0.05) indicando una dependencia en carbonato. A un pH de 6.5 la tasa de pérdida de peso esquelético fue de 1.8 mg h^{-1} cm^{-2}. La relación entre calcificaión neta y pH (n=2) indican que la gancia de peso se vuele pérdida a pH 7.4. Estos experimentos confirman que la calcificación es un proceso de dos pasos, involucrando la secreción de la capa de matriz orgánica que incorpora anhidrasa carbónica para producir una superficie de calcificación activa que usa dióxido de carbono en vez de carbonato. Es también poco probable que la superficie de calcificación esté en contacto directo con el agua de mar. La depositación o disolución inorgánica del esqueleto en áreas expuestas de corales muertos en un fenómeno diferente y está relacionado a los carbonatos. El gran ámbito de resultados de este y otros estudios sobre tasas de calcificación y dióxido de carbono pueden ser explicados en términos de la razón entre las zonas vivas y muertas de los corales.

Palabras clave: calcificación de corales, CO_2, pH, temperatura, matriz orgánica, anhidrasa carbónica

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Growth and population assessment of the queen conch *Strombus gigas* (Mesogastropoda: Strombidae) by capture mark-recapture sampling in a natural protected area of the Mexican Caribbean

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Received 14-VI-2011. Corrected 02-XII-2011. Accepted 20-XII-2011.

Abstract: The Inlet of Xel-Ha is used as a park for ecotourism, representing a sanctuary for the conservation of Pink Queen Conch. Increasing fishing pressure has led to the inclusion of the species in CITES. Most knowledge about the growth of the queen conch was generated through aquaculture, ocean enclosures or obtained using estimates derived from population dynamics. In this study we estimated the growth rate of juvenile *S. gigas* in a natural protected area by direct methods, during the period of April 2009 to January 2011. Data was obtained by capture-mark-recapture sampling. 1418 individuals were tagged and growth of 714 conchs was analyzed. Population size and density was estimated using Schnabel's method. The average density was estimated at 0.1694 ± 0.0996ind. m^{-2}, while the highest density was estimated for September 2010 (0.3074ind. m^{-2}). The highest growth rate (0.27 ± 0.10mm day^{-1}) was detected in juveniles with an initial size between 100-149mm, followed by conch <100mm, with an increase of 0.24 ± 0.05mm day^{-1}. The growth rate decreased for individuals with an initial size between 150-199mm (0.18 ± 0.09mm day^{-1}) and for organisms > 200mm (0.08 ± 0.07mm day^{-1}). Variability in growth rate was high in conch 100-149mm and showed seasonal differences, with the highest growth rate in May 2010. Recruitment of juveniles was highest in October 2009 and February 2010. The population of Xel-Ha has grown in size and more large and juvenile conch could be found than in previous studies, indicating that Xel-ha park is working as a sanctuary for the conservation of the queen conch in Mexico's Riviera Maya. The growth rate of juvenile conch in Xel-Ha is high and exhibits large variations in individuals, reflecting the natural conditions of foraging and aggregation. Seasonal differences in growth rate may be associated with water quality and availability of nutrients for primary production. We conclude that the direct method is useful for the assessment of growth in juvenile *S. gigas* and that growth in natural conditions may be higher than in aquaculture systems. This information may be applied to fishery management as well as rehabilitation programs and aquaculture. Rev. Biol. Trop. 60 (Suppl. 1): 127-137. Epub 2012 March 01.

Keywords: Population size**,** Population density, Growth, Mark-Recapture, Recruitment, Nursery, Xel-Ha.

The queen conch (*Strombus gigas* Linnaeus, 1758) is a large gastropod which represents an important food and economic resource in the Caribbean, being the second largest fishery after the spiny lobster (Appeldorn 1994) with landings of 6 000 metric tons, worth $60 million Dollars (Chakalall & Cochrane 1997). The increasing fishing pressure caused populations to decline in the 1980's and led to the inclusion of this mollusk in the Convention on International Trade of Endangered Species (CITES) and the list of commercially threatened species. In Mexico, on the Peninsula of Yucatan, the conch fisheries reached a peak in 1983, with landings of 1 250 tons. In the late 1980's the majority of the stocks were reported to be overexploited, especially in the state of Yucatan where a permanent fishing ban was implemented in 1988 (Baquiero-Cárdenas 1997). In Quintana Roo captures reached their maximum in the early eighties and by the end of the decade catch volumes started to show signs

of overexploitation (Carta Pesquera Nacional 2006). This has led on one hand to the implementation of different management programs to protect the conch (INP-SAGARPA 2008) and on the other hand, to the development of its aquaculture (Berg 1976, Brownell 1977, Brownell & Stevely 1981, Rathier 1987, Glazer *et al*. 1997, Davis 2000, Moreno de la Torre & Aldana-Aranda 2007). The inlet of Xel-Ha is a natural marine protected area, which has been used since 1995 as a park for ecotourism. The main attraction is the observation of marine fauna in its natural environment; hence the removal of any flora or fauna is prohibited. Xel-Ha is considered a sanctuary for the conservation of the queen conch in the Mexican Riviera Maya, hosting an important number of juvenile conch (Peel *et al*. 2010).

Sound management of a resource such as *S. gigas*, as well as its rehabilitation, protection and the development of aquaculture require biological and ecological knowledge of the species, including growth rate, density and population structure. In this study we determined the rate of growth of juvenile *S. gigas* by direct methods in a natural protected area. Data was obtained through capture-mark-recapture methods, allowing the natural foraging behavior, resource selection and dispersal of the animals.

MATERIAL AND METHODS

Study Site: Xel-Ha is located on the east coast of the Yucatan Peninsula (20°19'15''-20°18'50''N and 87°21'41''-87°21'15''W). The main oceanic current is the Caribbean Current (Organismo de Cuenca Península de Yucatán Dirección Técnica 2008). The area is characterized by medium wave energy and input of fresh water by underground rivers due to karstic conditions in the Peninsula (Organismo de Cuenca Península de Yucatán Dirección Técnica 2008). Xel-Ha is a creek that consists of a mix of fresh groundwater with seawater. The Inlet is connected to the Caribbean Sea by a 100 m wide channel and has a total surface of 14 Ha with a center area and three appendices: Bocana, North Arm and South Arm. Depth ranges from 1.75-4.0m (Organismo de Cuenca Península de Yucatán Dirección Técnica 2008). The climate in the region is warm and subhumid, with rains during summer and winter. The average annual temperature is 26°C. Average annual rainfall is 1 079mm (Organismo de Cuenca Península de Yucatán Dirección Técnica 2008). The sampling site "*Cueva*" is located in the south-arm of the Inlet and includes a small bay surrounded by mangroves (*Rhizophora mangle).* Persistent upwelling of cold freshwater from underground caves, maintains a permanent thermo-and halocline (1.25m). The site has a depth 1.5-3.5m. The bottom is composed of fine mud and sand formed of fragments of calcareous algae, mixed with rocks and dense isolated patches of macroalgae (*Padina* sp*., Halimeda* sp*. Penicillus* sp*. Amphiroa* sp*. Acanthophora* sp*., Caulerpa* sp*., Dictyota* sp.), decaying mangrove leaves and inverted jellyfish (*Cassiopea* sp.) may be found.

Population Parameters: Between April 2009 and January 2011, nine surveys were conducted at the site of *Cueva* in the Inlet of Xel-Ha sampling a total area 6 000m². Three samplings were conducted during 2009, five in 2010 and one in January 2011. All organisms were collected in free-dive, by three divers during 3 hours. We used mark-recapture method, marking all individuals with a plastic Dymo® tag, bearing a consecutive number, which was fixed to the spire of the conch with a plastic cable binder. In order to evaluate the size distribution and growth rate, shell length (SL) was determined for each individual, as well as lip thickness, using a precision vernier caliper (1mm). With the abundance data of recaptured and unmarked individuals we estimated population size using Schnabel's method (Schnabel 1938):

$$N_t = \Sigma (C_t M_t) / \Sigma R_t$$

Relative density of conch in *Cueva* was derived from population size. To determine the growth rate of conch per day, we only used

the measurements of individuals which were recaptured for the first time after being marked in the previous sample (n=706).

Environmental parameters: Parameters for dissolved oxygen (O_2), water temperature (°C), salinity, total nitrogen (N total), total phosphates (P total), nitrate (NO_3^-) and nitrite (NO_2^-), measured at the study site between February 2009 and September 2010 were provided as a courtesy of Xel-Ha Park and were used to aid interpretation of growth rates over time. Data was obtained through the laboratory LAB-ACAMA (Laboratorio de Análisis de Calidad de Agua y Medio Ambiente S.A. de C.V) applying the procedures specified by the Mexican Normative for Water quality NMX-AA-26-SCFI-2001 for sampling and analysis.

Statistical Analysis: Using the program Infostat/S, we calculated the mean daily growth and standard deviation per size class (<100mm, 100-149mm, 150-199mm and ≥200mm) and the average growth per day in each class over time. Growth data was subjected to analysis of variance (ANOVA) to detect significant differences in growth rate over time and between classes, with a confidence level of 95%, followed by Tuckey's honestly significant difference test (Saville 1990). Population structure was determined using histograms, with 10mm intervals for size classes from 45-245mm. We calculated the percentage of individuals with flaring lip per size class. A correlation analysis using Pearson coefficient (Pearson 1896) between average growth rates from each sample and corresponding density was executed.

RESULTS

An average of 52.83%, of a total of 1418 individuals tagged, was recaptured in each sample. The recapture success was lowest in October 2009 with 19.93% and highest in November 2010 with 74.74% (Table 1). Using the method of Schnabel, population size was estimated for each month as well as the relative density of conch in the *Cueva*. The relative average density was estimated at 0.1694 ± 0.0996ind. m^{-2}, while the highest density was estimated for September 2010 with 0.3074 conches per square meter (Table 1).

Conches with SL less than 100mm were the scarcest in *Cueva* representing less than 5%, except in October 2009 and February 2010, when 11.32% and 42.81% were captured, respectively. Relative abundance was highest for the class of conch between 150

TABLE 1
Abundance, estimated abundance using Schnabel Method and density of *S. gigas* in Xel-há (Cueva)

Sample	Ct	Rt	%Rt	Ut	Mt	Nt	Density (ind. m^{-2})
Apr. 09	127	0		127	0		
Jun. 09	106	68	64.15	38	127	198	0.0330
Oct. 09	306	61	19.93	245	165	496	0.0826
Feb. 10	406	193	47.54	213	410	716	0.1193
May. 10	382	195	51.05	187	623	906	0.1510
Jul. 10	566	305	53.89	261	810	1128	0.1879
Sep. 10	545	319	58.89	226	1071	1844	0.3074
Nov. 10	479	358	74.74	121	1297	1422	0.2370
Jan. 11	265	265		0	1418	1422	0.2369

1. Ct= Number of *S. gigas* caught in each sampling.
2. Rt= Number of recaptures in each sample.
3. % Rt= Percentage of recapture per sample.
4. Ut= Number of untagged conch in each sample.
5. Mt= Total of marked animals at time.
6. Nt= Estimated population size using the Schnabel method.

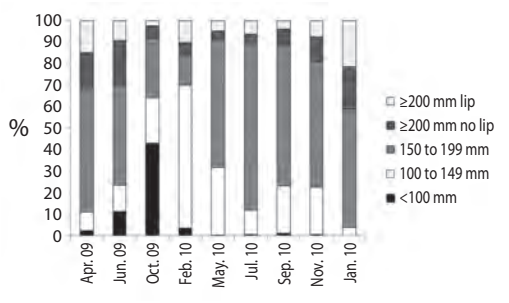

Fig. 1. Distribution of relative size class frequencies of queen conch *S. gigas,* sampled at Xel-ha, Mexico, showing the relative abundance (%) of animals which were <100mm, between 100mm and 149mm, between 150 and 199mm, ≥200mm without lip and ≥200mm with lip.

and 199mm, which made up an average of 51.18% ± 19.57% of the population. Conch between 100 and 149mm represented on average 22.13% ± 18.69% of the population. Their abundance was highest in February 2010 with 66.50%. Organisms with length ≥ 200mm contributed on average 19.73% ± 11.80% to the population. This class had the least variation in abundance compared with other classes. 48.01% ± 12.90% of the organisms ≥200mm showed a formed lip (Fig. 1).

In April 2009, 94% of the conchs were ≥145mm (Fig. 2). The size classes with highest frequency of abundance in that month were the animals with SL between 215mm and 225mm, and the animals between 185mm and 195mm, making up 26% of the population. In June 2009 a significant increase in organisms <145mm was observed, representing 24% of the organisms sampled. Throughout October 2009 and February 2010 abundance of individuals <145mm increased dramatically, recording 63.42% and 67.73%. Modal class in October was 85-95mm (16.34%) and 125-135mm (17.24%) in February. In May the number of conch <145mm captured decreased again, with 22.5% and the modal class were juveniles between 145mm and 155mm, which represented 25.65% of the population. Modal class shifted in July to the class of 155-165mm (22.79%) and the abundance of conch <145mm continued to decline, reaching 9.35%. In September of the same year, we could detect the emergence of new recruits, with 21.65% of conch <145mm and modal class was 185-195mm (16.88%). Recruits kept emerging

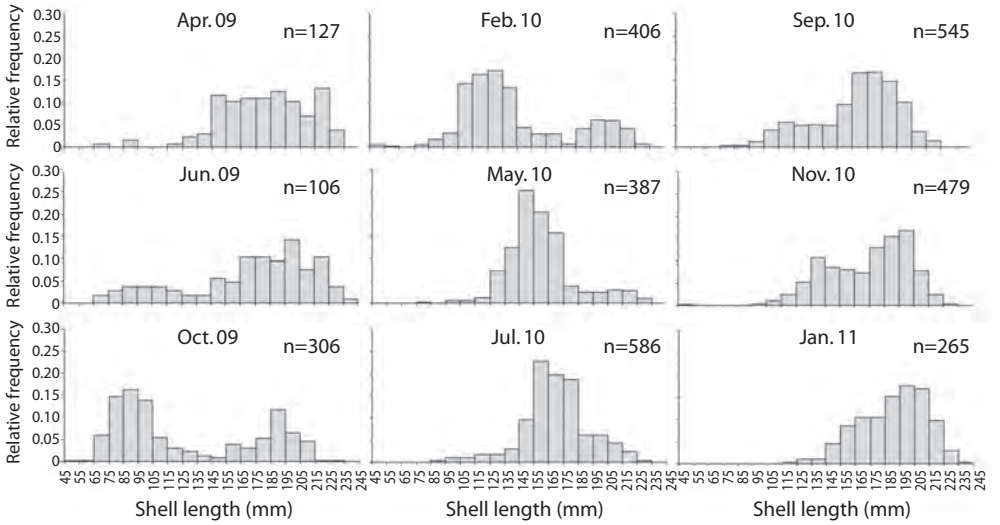

Fig. 2. Histograms showing relative frequency of abundance per size class, modal progression and recruitment patterns of queen conch *S.gigas* sampled at Xel-Ha, Mexico, between April 2004 and January 2011.

throughout November, with 20.05% of the animals <145mm and modal class shifted to 195-205mm (16.7%). In January 2011 no conchs smaller than 115mm were found and 97.37% of the sampled population had a size ≥145mm and the class with highest frequency was 195-205mm (17.74%).

For the growth rate analysis the population was divided into 4 size classes (Fig. 3). Growth was highest in animals of 100-149mm (n=306) with an average growth of 0.27 ± 0.1mm day^{-1}. The animals of this size class showed at the same time the greatest variation in growth rates, with values between 0.01 and 0.63mm day^{-1}. The growth rate had normal distribution (Shapiro-Wilks p=0.0856) and the median coincided with the mean. In juvenile conch smaller than 100mm (n=96) we calculated a growth rate of 0.24 ± 0.05mm day^{-1}. Growth rate decreased to 0.18 ± 0.08mm day^{-1} in the class of 150 - 199mm (n=268) and was lowest with 0.08 ± 0.07mm day^{-1} in conch with a size ≥ 200mm (n=36) (Fig. 3). The growth rate showed significant differences between size classes (ANOVA $F_{3, 705} = 80.08$, $p < 0.0001$), but was similar between the classes <100mm and 100-149mm (Tuckey, $p < 0.05$).

The growth showed significant differences over time in classes of <100mm ($F_{5, 95} = 8.01$, $p < 0.0001$), 100mm to 149mm ($F_{7, 305} = 36.98$, $p < 0.0001$) and 150mm to 199mm ($F_{7, 267} = 9.14$; $p < 0.0001$). There were no significant differences in the class of animals ≥ 200mm ($F_{6, 35} = 1.46$; $p = 0.2262$). The highest growth rate was observed in May 2010, while the lowest growth was observed in October 2009 and November 2010 (Fig. 4 and Table 2).

No significant association was detected between growth rate and the relative density of conch ($R=0.17$; $p = 0.69$).

Xel-Ha Park occasionally carries out surveys to monitor water quality. The results corresponding to the area of *Cueva* are shown in Figure 5.

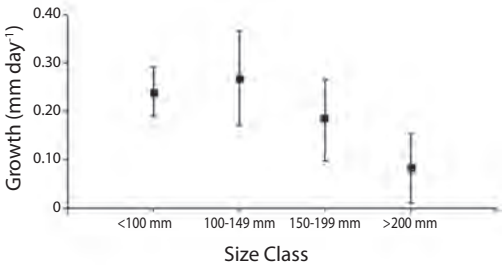

Fig. 3. Mean growth rate (mm day^{-1}) and its standard deviation (Bars) of queen conch *S.gigas* with Shell length <100mm, between 100mm and 149mm, between 150 and 199mm, and ≥200mm, sampled at Xel-Ha, Mexico.

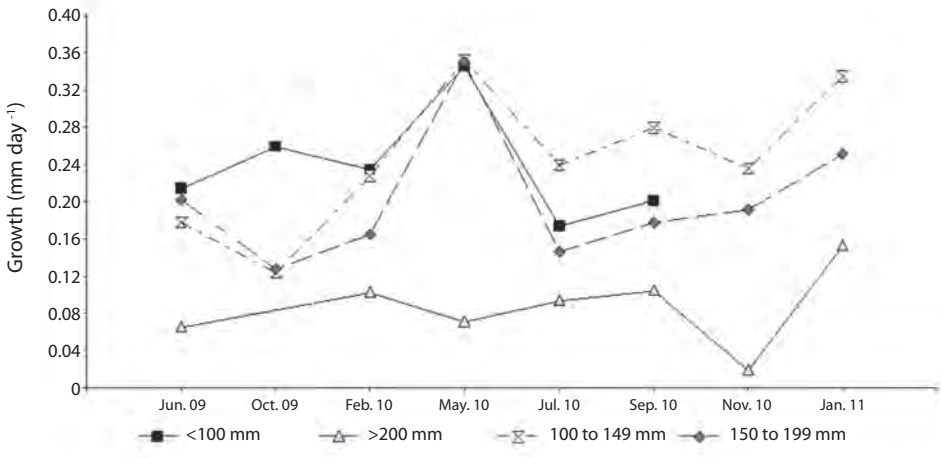

Fig. 4. Average growth rate (mm day^{-1}) over time in pink queen conch *S.gigas* with a shell length of <100mm, 100mm to 149mm, 150mm to 199mm and ≥ 200mm, at *Cueva* (Xel-Ha, Mexico).

TABLE 2
Average growth (mm day^{-1}) of queen conch *S.gigas* with a shell length of <100mm, 100mm to 149mm, 150mm to 199mm and ≥ 200mm, over time, at Xel-Ha, Mexico

Size Class / Growth	Jun. 09	Oct. 09	Feb. 10	May 10	Jul. 10	Sep. 10	Nov. 10	Jan. 11
<100(mm)	0.21	0.26	0.23	0.35	–	0.17	0.2	–
100 to 149(mm)	0.18	0.12	0.23	0.35	0.24	0.24	0.28	0.33
150 to 199(mm)	0.2	0.13	0.16	0.35	0.19	0.15	0.18	0.25
≥200mm	0.06	–	0.1	0.07	0.02	0.09	0.1	0.15

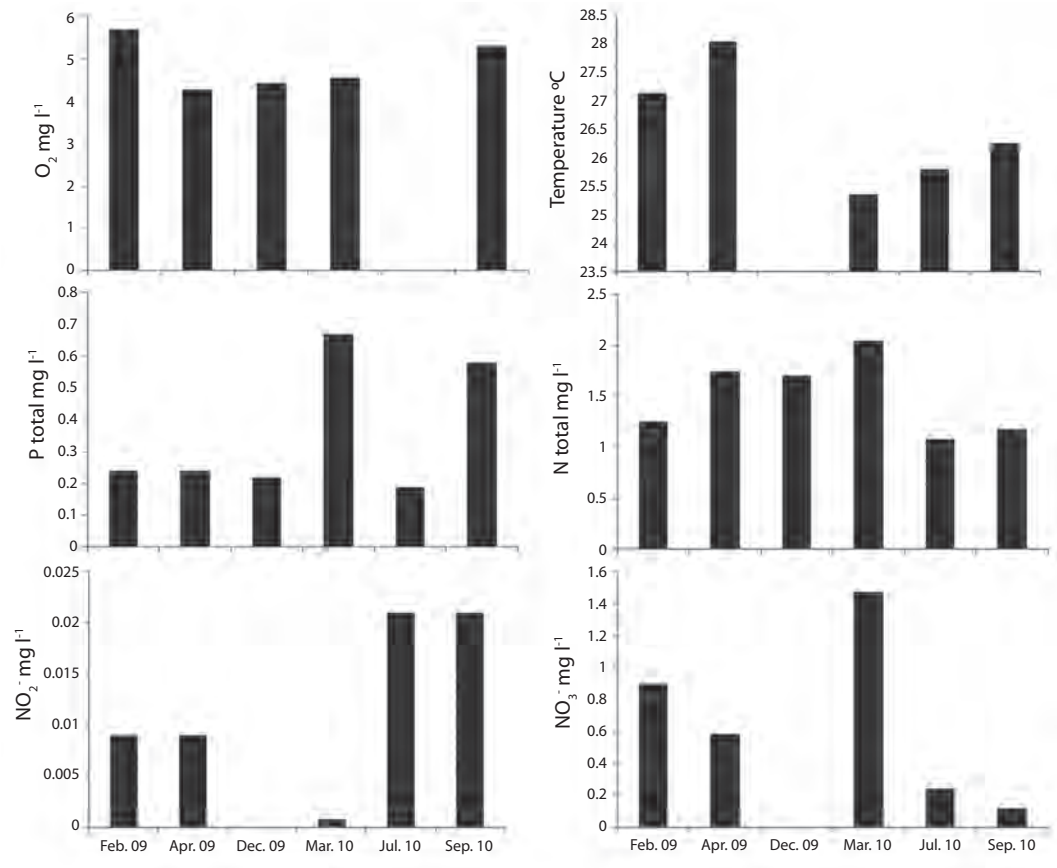

Fig. 5. Water quality parameters, showing surface temperature, dissolved oxygen at the surface, total nitrogen (N total), Nitrate (NO), Nitrite (NO) and total phosphate (total P) at Xel-Ha's *Cueva*. Data was provided by courtesy of the administration of Xel-Ha Park.

DISCUSSION

In the present work conch larger than 210mm were present in all samples and a total of 268 observations could be made, representing 8.42% of the population. Aldana-Aranda *et al.* (2005) reported the absence of animals larger than 210mm at *Cueva* in the majority of the samples taken in a study conducted between 2001 and 2002. Xel-Ha initiated the Monitoring Program, as well as the actions for conservation and rehabilitation of the queen conch in October 2001. The conch population of Xel-Ha has grown in size and more large and

juvenile conch could be found than in previous studies, suggesting that the park of Xel-Ha is providing effective protection for the species.

Apparent recruitment was detected throughout most of the year, but was highest from June 2009 to May 2010 and September 2010 to November 2010. Aldana-Aranda *et al.* (2003) documented recruitment throughout most of the year and high recruitment during October 2001. In a subsequent study Aldana-Aranda *et al.* (2005) documented higher recruitment in October 2002, February 2002 and February 2003, consistent with recruitment peaks observed in this study, in February and October.

In the present study population structure was: <100mm = 7.0%; 100-149mm = 22.1%; 150-199mm = 51.2%; ≥200mm = 19.7%. Aldana-Aranda *et al.* (2005) reported 4.2% of the population <100mm, 23.8% in the class of 100-150mm, 51.2% conch 150-200mm and 23.4% of the population >200mm in the period from November 2001 to August 2003, applying the same methodology. It can be noted that relative proportion of adults and juveniles has remained similar in comparison with previous studies, despite the increase in numbers of individuals, indicating the population is in an equilibrium state.

In the present study, the population was small initially; however, we observed substantial recruitment of juveniles from October 2009 onwards, reaching a maximum density of 0.3074 conches per square meter in September 2010. Aldana-Aranda *et al.* (2005) estimated a population size of 632.15 ± 49.40 individuals in the period from 2001 to 2003, using Schnabel's method. Peel *et al.* (2010) reported average catches of 82.09 conches per sample in the period from January 2004 to January 2008 at the sampling site *Cueva*. The observed population growth was attributed to increased recruitment of juveniles.

The Density at Xel-Ha's Cueva is high, compared to other areas in the Caribbean (Table 3). The densities documented at *Cueva* were higher than densities reported for Alacranes Reef, where conch fishery was banned in 1988 (Pérez-Pérez & Aldana-Aranda 1998, Ríos-Lara *et al.* 1998, Pérez-Pérez & Aldana-Aranda 2000, Pérez-Pérez & Aldana-Aranda 2003), ranging from 0.0047 to 0.018 conch m^{-2}. They were also higher than the densities reported for the two most important commercial

TABLE 3
Average densities of *S. gigas* in the Caribbean

Author, Year	Location	Density (ind. m^{-2})
Ríos-Lara *et al*. 1998	Alacranes Reef, Yucatán, México	0.0047
Pérez-Pérez & Aldana-Aranda 1998	Alacranes Reef, Yucatán, México	0.0072
Pérez-Pérez & Aldana-Aranda 2000	Alacranes Reef, Yucatán, México	0.0084
Pérez-Pérez & Aldana-Aranda 2003	Alacranes Reef, Yucatán, México	0.018
De Jesús-Navarrete *et al*. 1992	Punta Gavilán, Quintana Roo, México	0.003
De Jesús-Navarrete & Oliva-Riviera 1997	Punta Gavilán, Quintana Roo, México	0.0052 ± 0.0023
INP-SAGARPA 2008	Banco Chinchorro, Quintana Roo, México	0.0211 ± 0.035
INP-SAGARPA 2008	Banco Cozumel, Quintana Roo, México	0.0079 ± 0.01653
Berg & Glazer 1991	Florida Keys, Florida, USA	0.000109-0.000298
Friedlander *et al*. 1994	Virgin Islands, USA	0.00171
Stoner & Ray 1993	Exuma Cays, Bahamas	0.2
Stoner & Schwarte 1994	Lee Stocking Island, Bahamas	0.0018-0.0088
Stoner 1996	Exuma Cays (unfished zones), Bahamas	0.0034-0.0147
Stoner 1996	Exuma Cays (fished zones), Bahamas	0.00022-0.0088
Stoner & Ray 1996	Exuma Park,Exuma Cays, Bahamas	0.027
Posada *et al*. 1999	Jaragua National Park, Dominian Republic	0.0004-0.01142

queen conch fishery grounds in Quintana Roo, Banco Chinchorro (0.0211 ± 0.035ind m^{-2}) and Banco Cozumel (0.0079 ± 0.01653ind m^{-2}). In Punta Gavilán, a coastal area without commercial fishing, densities range from 0.003 to 0.0052ind. m^{-2} (De Jesús-Navarrete *et al.* 1992, De Jesús-Navarrete & Oliva-Riviera 1997). Berg & Glazer (1994) reported in the Florida Keys, USA, densities between 0.000109ind. m^{-2} and 0.000298ind. m^{-2}, where a permanent fishing ban has been implemented since 1985 and sanctuaries with surveillance have been created due to the rapid depletion of stocks of Queen Conch. The density at Xel-Ha's *Cueva* is similar to the relatively natural populations in the Exuma Cays (Table 4) (Stoner & Ray 1993, Stoner 1996) and can be compared to the high-density aggregation nursery grounds (Stoner & Ray 1993, Stoner & Lally 1994) in terms of population structure and density.

The growth rate of juvenile conch in *Cueva* was high in comparison with those mentioned by other studies (Table 4). De Jesús-Navarrete (2001) obtained an average increase of 3.21mm $month^{-1}$ (~0.1052mm day^{-1}) in Punta Gavilán and 2.30mm $month^{-1}$ (~0.075mm day^{-1}) in Banco Chinchorro, maintaining conch in enclosures at density of 0.4ind. m^{-2}. In other areas of the Caribbean similar increases were observed (Randall 1964, Alcolado 1976, Brownell 1977, Ray & Stoner 1994). Growth rates measured during this study were comparable to other studies conducted under natural conditions using mark-recapture methods. Gibson *et al.* (1983) determined a rate of 7.2mm $month^{-1}$ (~0.236mm day^{-1}) in Belize, while in Venezuela an increase of 15mm $month^{-1}$ (~0.492mm day^{-1}) was measured (Weil & Laughlin 1984) and in Punta Gavilán, juveniles grew an average of 10mm $month^{-1}$ (~0,327mm day^{-1}) (De Jesús-Navarrete & Oliva-Rivera 1997). The growth rate of juvenile queen conch at Xel-Ha's *Cueva* was comparable to the growth measured by Moreno de la Torre & Aldana-Aranda (2007) under experimental conditions, using artificial diets, who obtained an increase of 0.16-0.23mm day^{-1}. However, the conch used in their study had an initial size inferior to 40mm and were smaller than the organisms in the present study.

Growth rates declined in the 150-199mm size class and tended towards null in conch ≥200mm (Fig. 3). Conches grow in shell length only until maturation. At this time the flared shell-lip is formed. Subsequent shell growth occurs as a progressive thickening of the shell-lip (Appeldoorn 1988). Most conch reach sexual maturation when the shell lip is thicker than 5mm (Appeldorn 1988, Aldana-Aranda & Frenkiel 2007). Shell morphology and maximum size can vary considerably (Alcolado 1976, Appeldoorn 1994) and may not represent a good indicator for maturity (Aldana-Aranda & Frenkiel 2007). The maximum SL observed at Xel-Ha in our study was 239mm and less than the half of the organisms in the ≥200mm class had developed a flaring lip. It may be deduced that more than half of the conch

TABLE 4
Comparative Table of mean growth rates of *S. gigas*

Author, Year	Location	Method	Growth rate (mm $month^{-1}$)	Growth rate (mm day^{-1})
Randall 1964	Virgin Islands, USA	Enclosure	4.16	~0.136
Alcolado1976	Cuba	Enclosure, different environments	3.3	~0.108
Brownell 1977	Florida Keys, USA	Enclosure	4.5	~0,147
Gibson *et al.* 1983	Belize	Mark-Recapture	7.2	~0.236
Weil & Laughlin 1984	Venezuela	Mark-Recapture	15	~0.492
Ray & Stoner 1994	Exuma Cays, Bahamas	Enclosure	–	0.058-0.139
De Jesús-Navarrete & Oliva-Rivera 1997	Punta Gavilán, México	Mark-Recapture	10	~0,327
De Jesús-Navarrete 2001	Banco Chinchorro, México	Enclosure, different environments	3.21	~0.1052
De Jesús-Navarrete 2002	Punta Gavilán, México	Enclosure, different environments	2.30	~0.075
Moreno de la Torre & Aldana-Aranda 2005	México	Laboratory conditions, artificial diet	–	0.16-0.23

≥200mm are still immature and may represent some considerable growth.

Growth rate of the queen conch showed large individual variations, especially in animals of the class of 100-149mm. Alcolado (1976) showed that growth may vary according to environmental variability between sites; however, the study area of the *Cueva* is a relatively small area, making it more likely that all organisms have been exposed to the same conditions. Ray & Stoner (1994) suggested that juvenile conch are vulnerable to predation and may choose lower quality habitat in terms of resources, compromising maximum ingestion and growth, by aggregating or sheltering in dense vegetation, to reduce the risk of predation and increase survival probabilities. The high growth rate of juvenile conch in Xel-Ha and the large variations in individuals likely reflects the natural conditions of foraging and aggregation.

We could detect significant variation in the rate of growth over time (Fig. 4). There was an increase in nutrients important for production of biomass (P, N and NO) in the sample of March 2010, for which it is likely that the increase in growth rate during May 2010 might be the result of higher primary productivity.

We conclude that the direct method is useful for the assessment of growth in juvenile *S. gigas* and that growth in natural conditions is higher than in enclosures and aquaculture systems. This information may be applied to fishery management as well as to rehabilitation programs and aquaculture.

ACKNOWLEDGMENTS

We gratefully acknowledge financial support by CONACYT through Grant Conacyt SEP 24210 Connectivity of the Conch in the Caribbean, CONACYT student scholarship (374674/243375) and CINVESTAV-IPN. Also we thank Xel-Ha Park's administration, Elizabeth Lugo Monjarras and Ricardo Saenz Morales for providing unlimited access and logistic support during our field work. We thank Xel-Ha staff Enrique May, Teresa Rivas, Angel Chavarria and Mario Hoil for their field support.

RESUMEN

La Ensenada de Xel-Ha es usada como parque para ecoturismo y representa un santuario para la conservación del caracol rosado. El incremento en la presión de la pesca ha llevado a la inclusión de esta especie en CITES. Mucho del conocimiento acerca del crecimiento del caracol rosado ha sido generado a través de la acuicultura, encierros oceánicos o usando estimaciones derivadas de las dinámicas poblacionales. En este estudio estimamos la tasa de crecimiento de *Strombus gigas* juvenil en un área natural protegida, por métodos directos durante el período de abril 2009 a enero 2011. Los datos fueron obtenidos por muestras de captura-marca-recaptura. Un total de 1418 individuos fueron marcados y el crecimiento de 714 caracoles fue analizado. La talla de la población y la densidad relativa fue estimada usando el método de Schnabel. La densidad promedio relativa fue estimada en 0.1694±0.0996ind. m^{-2}, mientras que la densidad más alta fue estimada para septiembre 2010 con 0.3074ind. m^{-2} . La tasa de crecimiento más alta (0.27±0.10mm $día^{-1}$) fue detectada en juveniles con una talla inicial entre 100-149mm, seguida por juveniles <100mm, con un incremento de 0.24±0.05mm $día^{-1}$. La tasa de crecimiento disminuyó para individuos con una talla inicial entre 150-199mm (0.18±0.09mm $día^{-1}$) y para organismos >200mm (0.08 ± 0.07mm $día^{-1}$). La variabilidad en la tasas de crecimiento fue alta en individuos entre 100-149mm y mostró diferencias estacionales; con la tasa de crecimiento más alta en mayo 2010. El reclutamiento de juveniles más alto se dio en octubre 2009 y en febrero 2010. La población de Xel-Ha ha crecido en tamaño y se pudo encontrar más adultos y juveniles que en estudios anteriores, lo que demuestra que el Parque de Xel-Há está funcionando como un santuario para la conservación del caracol rosado del Caribe en la Riviera Maya de México. La tasa de crecimiento de juveniles en Xel-Ha es alta y presenta grandes variaciones en los individuos, lo cual refleja las condiciones naturales de la alimentación y la agregación. Las diferencias estacionales en las tasas de crecimiento pueden estar asociadas con la calidad del agua y la disponibilidad de nutrimentos para la producción primaria. Concluimos que el método directo es útil parar monitorear el crecimiento en juveniles de *S. gigas* y que el crecimiento en condiciones naturales es mayor que en sistemas de acuicultura. Esta información puede ser aplicada al manejo de pesquerías así como también en programas de rehabilitación y acuicultura.

Palabras clave: *Strombus gigas*, Área Marina Protegida, densidad poblacional, crecimiento, marcaje-recaptura, reclutamiento

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Comparison of three quick methods to estimate crab size in the land crabs *Cardisoma guanhumi* Latreille, 1825 and *Ucides cordatus* (Crustacea: Brachyura: Gecarcinidae and Ucididae)

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Received 15-VII-2011. Corrected 30-XI-2011. Accepted 20-XII-2011.

Abstract: Quick, reliable and non destructive methods are necessary to estimate size structure on commercial land crabs, in order to acquire relevant information concerning the health of their populations. *Cardisoma guanhumi* and *Ucides cordatus* are two land crabs that are exploited at a high scale and also in an artisan way in the Caribbean area and in the coasts of Brazil, which populations are endangered due to uncontrolled exploitation. The purpose of this work is to provide various methods to estimate indirectly crab body size. Sampling was carried out in Carenero (*C. guanhumi*) and Cumaná (*U. cordatus*) (Venezuela). For each species, three methods were used to measure burrow diameter (Vernier, internal spring caliper and photograph), and these were correlated with real body size of the crabs. Model II linear regression analyzes, i.e. Ordinary Least Squares and Mayor Axis, were used to build and test the performance of forecasting models. *Cardisoma guanhumi* showed a high bivariate data dispersion using Vernier and photo measuring methods, increasing these towards larger animals. Less dispersion was achieved with the spring caliper method; this resulted in the most accurate measurements of indirectly estimated body size in *C. guanhumi* (r^2= 0.61), whereas Vernier measurements were the least precise. On the other hand, all three methods gave reliable estimates for *U. cordatus*, being the Vernier method the most accurate (r^2= 0.71). However, in both species, all forecasting equations overestimated the size of smaller crabs (those below the mean) but underestimated the size of larger crabs. Nevertheless, all three methods were statistically significant for each of the species, and looking at the above mentioned under- and overestimations, they can serve as reliable and fast non-destructive tools to be used by resource managers and field biologists to acquire size structure information concerning these two species. Vernier and internal spring caliper methods are recommended for relative small sampling areas, while photo method is suggested to be used in very extensive sampling regions. Rev. Biol. Trop. 60 (Suppl. 1): 139-149. Epub 2012 March 01.

Key words: Indirect body size measuring methods, *Cardisoma guanhumi*, *Ucides cordatus*, Vernier measurement, internal spring caliper measurement, photographic measurement.

Population structure and density estimates of commercial land crabs are a need of considerable importance, in order to establish their population status and define management politics. The land crab *Cardisoma guanhumi* Latreille 1825 has been the target of commercial exploitation and in an artisan way in several Caribbean countries and in Brazil. In Puerto Rico, there has been a decline of *C. guanhumi* since 1960 (Matos 1997). Moreover, in Cuba, there was a 75% drop and more in the fisheries of this land crab between the decades of 1980 and 1990 (Baisre 2000). Furthermore, in Brazil, *C. guanhumi* has also been under strong fishery pressure, also being endangered and overexploited (Amaral & Jablonski 2005). Specifically in Venezuela, *C. guanhumi* has been commercially captured and exported since the seventies (Taissoun 1974) without official harvesting statistics. The second largest land

crab in the Caribbean and in Brazil, *Ucides cordatus*, has also been extensively exploited in this country (which exhibits the highest populations of this species in the American continent) (Nóbrega Alves & Nishida 2003, Jankowsky et al. 2006, Pimentel Rocha et al. 2008) and in the Orinoco Delta-Venezuela (Novoa 2000). In the latter, *C. guanhumi* and *U. cordatus* are illegally extracted and exported to Trinidad without any regulations (Novoa 2000).

Although there are several studies dealing with the population biology of these land crabs, population estimations have been mainly done through the extraction of the animals and destruction of their habitats (Warner 1969, Oliviera Botelho et al. 2001). Only in few cases population structure was determined using indirect methods to estimate the body size of *C. guanhumi* (Govender & Rodríguez-Fourquet 2000) and as well as of *U. cordatus* (Schmidt et al. 2008). Indirect crab size estimation has been recently applied with a higher frequency to several burrowing species, such as in the fiddler crabs *Uca spinicarpa* Rathbun, 1900 and *U. longisignalis* Salmon and Atsaides, 1968 (Mouton & Felder 1996), *U. tangeri* Leach, 1814 (Lourenço et al. 2000), and *U. annulipes* H. Milne Edwards, 1937 (Skov & Hartnoll 2001), in the ocypodid crabs *Dotilla myctiroides* Stimpson, 1858 (Lee & Lim 2004) and the soldier crab *Heloecius cordiformis* H. Milne-Edwards, 1837 (Macfarlane 2002). In all the works mentioned above, Vernier caliper was used to measure the diameter of the burrow entrance.

Direct capturing of animals and visual census methods for assessing population structure are difficult to perform, because it involves personnel and equipment costs, destruction of burrows and long observation times. On the other hand, methods for indirect estimation of body size are more efficient to achieve this goal and avoid the destruction of crab habitats. For these reasons, the purpose of this research is to test and compare three types of burrow measurements that can estimate carapace length (in mm) of the land crabs *C. guanhumi* and *U. cordatus*, and determine which is more suitable to estimate body size. Moreover, it is intended to prove that these methods can serve as reliable measurements to assess crab body size structure in a fast, cheap and non-destructive manner.

MATERIALS AND METHODS

Field Measurements: Sampling and measurement of crabs and its associate burrows were performed on two Venezuelan localities: Carenero (Miranda state) (10° 32' 5.97"-N, 66° 7' 45.78"-W) for *Cardisoma guanhumi*, and Cumaná (Sucre state) (10°27' 43.11"-N, 64° 6' 07.99" -W) for *Ucides cordatus*. Both sites are characterized by a predominant vegetation of *Avicennia germinans*. With the help of local fishermen, two different capture methods were applied. For *C. guanhumi*, wooden baited traps were used between May and October 2010; for *U. cordatus*, fishing net pieces were placed over the burrow entrances, between February and April 2011. Normally, burrows from *C. guanhumi* are mainly occupied by only one crab (Taissoun, 1974, Moreno, 1980). Similarly, burrows from *U. cordatus* are also occupied by solely one specimen (personal observations). After confirming that only one crab was captured from each burrow, these were sexed and their carapace length measured with a 0.05-mm-precision Vernier caliper. Nevertheless, due to the fact that the purpose of this work was to compare crab size with estimated burrow size through three different measuring methods, we considered unnecessary to separate male and female carapace measurements. Corresponding burrows of captured animals were measured using three different methods: 1.- Superficial crab burrow diameter (in mm) was mensurated with a 0.05-mm-precision Vernier caliper (from now on defined as "Vernier"), taking into account that crabs (*C. guanhumi*, as well as *U. cordatus*) entered and got out their burrows sideways and that their carapace length was well lined up with the diameter of the burrow; 2.- The internal diameter of the burrow was estimated with an inside spring caliper (from now on defined as "caliper") (Fig. 1), taking the same considerations when

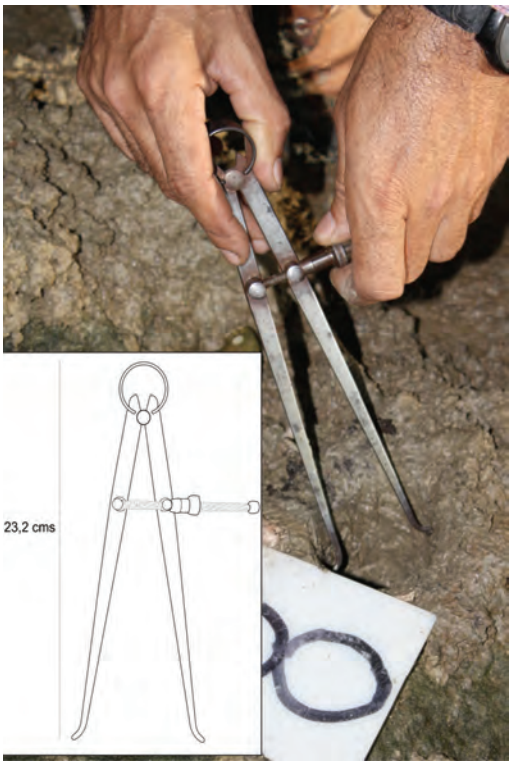

Fig. 1. Photograph and drawing detailing the internal spring caliper used to measure the diameter of the burrows of the crabs *Cardisoma guanhumi* and *Ucides cordatus*.

burrows were measured with the Vernier and being very cautious that the tips of the spring caliper touched the walls of the burrow without penetrating these. The length between the tips was measured with the Vernier; 3.- Each burrow was photographed with a measuring scale placed on its side, and photographs were analyzed with tpsDig v.2 software (Rohlf 2004) (a computer program for statistical analysis of morphometric data) to calculate burrow diameter. This software has been used successfully to quantify size and shape of spermathecae in the beetle *Onthophagus taurus* Schreber 1759 (Simons & Kotiaho 2007), as well as studying the geometric morphometry of two species of the beetle *Erodiontes* spp. (Taravati et al. 2009) and to determine body size preferences in the seahorse *Hippocampus abdominalis* Lesson 1827 (Mattle & Wilson 2009). A total of 118 *C. guanhumi* (73 males and 45 females) were trapped and their carapace length measured (in mm), showing a mean body size of 58.79±18.03 (max= 140.7; min= 32.8); furthermore, 118 vernier measurements, 89 spring caliper measurements and 112 photographs were taken, but only paired data (81) were considered for the analyzes. For *Ucides cordatus*, 105 crabs were captured (mean body size= 35.99±7.34; max= 54.2; min= 18.6) (52 males and 53 females), with the same numbers of Vernier measurements, spring caliper measurements and photographs taken.

Statistical Analyzes: Model II Linear regressions analyzes were calculated using a subset of random measurements and Ordinary Least Squares (OLS) in order to generate predictive models for size estimation for each crab species, in accordance to the suggestions given by Laws & Archie (1981) and Quinn & Keough (2002) to analyze bivariate data subjected both to sampling error. To validate the best forecasting model, Model II via Mayor Axis (MA) regression analyzes was applied using the remaining data. Before theses analyzes, data was first checked for normality with the Shapiro-Wilks test, and the homogeneity in the bivariate dispersion was approached with scatterplots (Quinn & Keough 2002). In both species, individuals and burrows with standardized residuals larger than 3.0 were considered outliers and excluded from the analyzes in order to generate more robust models (Sokal & Rohlf 1995, Quinn & Keough 2002). In the case of *C. guanhumi*, from 81 samples (individuals and their respective burrow sizes), 50 were randomly chosen to build the linear models. To evaluate the predictive capacity of the resulting models, the estimated crab body size of the remaining 31 samples was plotted and analyzed against the real size using MA analyzes. For *U. cordatus*, 29 samples were randomly chosen from a set of 58 to construct the linear model. Crab body size from the remaining 29 samples was estimated and compared with the real ones, using the MA linear regression. For both species, if the predictive models are good, then

the regression lines with MA analyzes should show slopes= 1, intercepts= 0 and angle of 45º (Legendre 2001).

RESULTS

Statistical results for *C. guanhumi*: Graphs with all data included for each of the burrow measuring methods and carapace length of crabs are shown in Fig. 2. Both bivariate data between carapace length and measured burrows with the Vernier (Fig. 2A), and carapace length and measured burrows with the photo-method (Fig. 2C) showed a noticeable increase in the dispersion towards larger sizes. Bivariate data of carapace length and burrow measure with the spring caliper showed less dispersion (Fig. 2B).

For the construction of the predictive model, none of the sampled data (body size and burrow measurements with the different methods) adjusted to normality. Thus, tests based on permutations were applied (Legendre 2001). Estimation variability increased as body size increased, particularly data coming from the Vernier and photo measurements (Fig. 2A and 2C, respectively). After excluding the outliers and using the 50 random selected sample pairs for each of the measuring methods, carapace length adjusted to normality (Shapiro-Wilks test, $p>0.05$), but burrow size with different methods did not (Shapiro-Wilks test, $p<0.05$). Nevertheless, homogeneity was achieved in the bivariate distribution in the three data sets from each of the measuring methods (i.e. points around the line are equally distributed). Vernier method as well as photo method showed a reduced dispersion of the data (Fig. 3A and 3C, respectively), but the spring caliper method still evidences the smallest dispersion of all (Fig. 3B). The results of the linear regression analyzes are shown in Table 1A. The three models are statistically significant. The spring caliper measuring method is the best adjusted model ($r^2= 0.61$) and with the less unexplained variability.

Results of the evaluation of the predictive capacity of the regression models (MA) are shown in Table 2A. All regressions present intercepts that are significantly higher than 0 and slopes significantly lower than 1 (Table 2A). This indicates that all methods overestimate smaller crab body size compared to the real body size mean and underestimate larger size. The imprecision can be observed in Fig. 4, in which the MA linear regression lines show angles above the referential line of 45%. Nevertheless it apparently seems to be that the caliper method revealed a better performance,

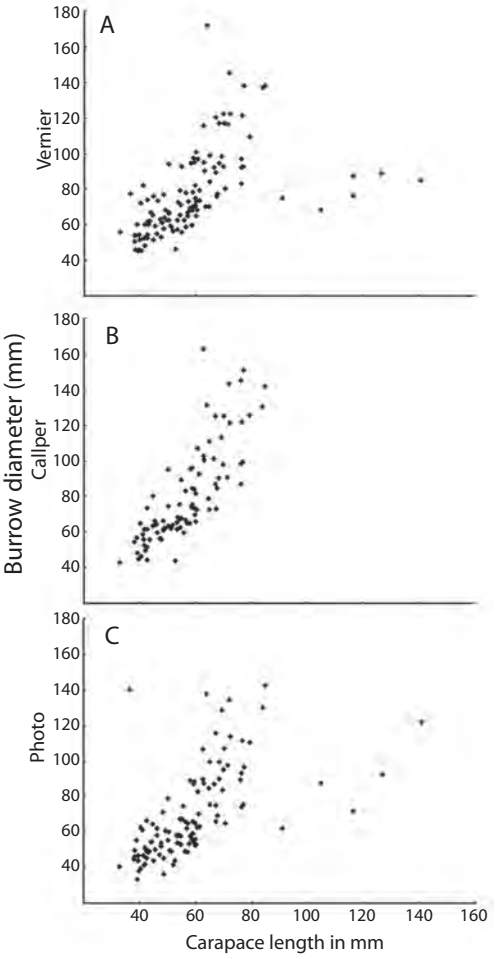

Fig. 2. Bivariate scatterplots of carapace length (mm) and burrow diameter (mm) of *Cardisoma guanhumi*. A: Burrow diameter measured with Vernier; B: Burrow diameter measured with spring caliper; C: Burrow diameter measured from digital photograph.

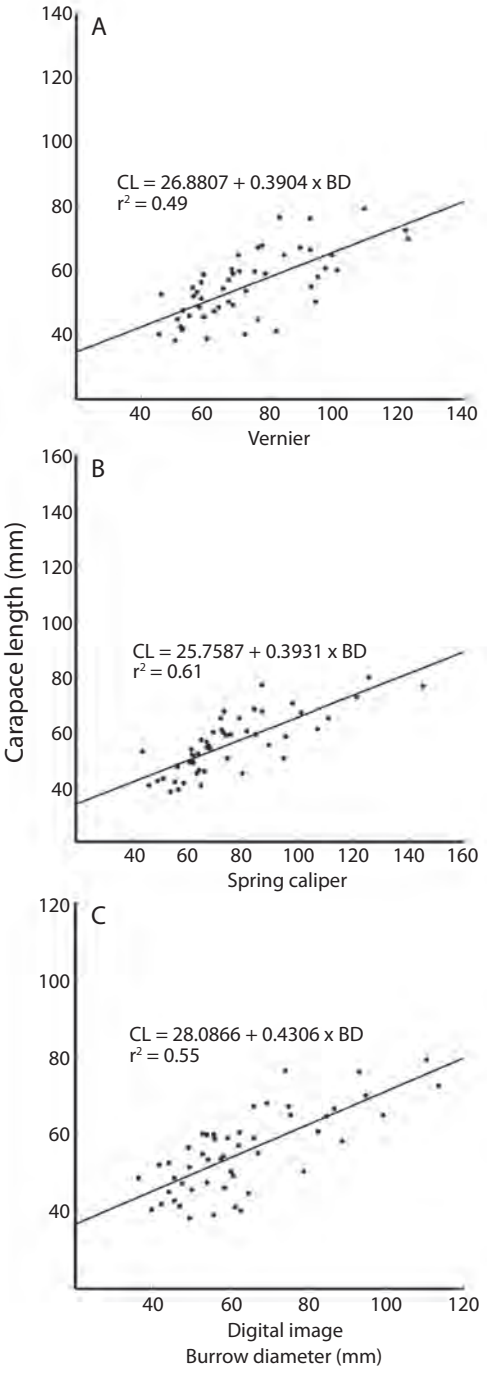

Fig. 3. Model II OLS linear regression method for carapace length (mm) and burrow diameter (mm) in *Cardisoma guanhumi*. A: Burrow diameter measured with Vernier; B: Burrow diameter measured with spring caliper; C: Burrow diameter measured from digital photograph.

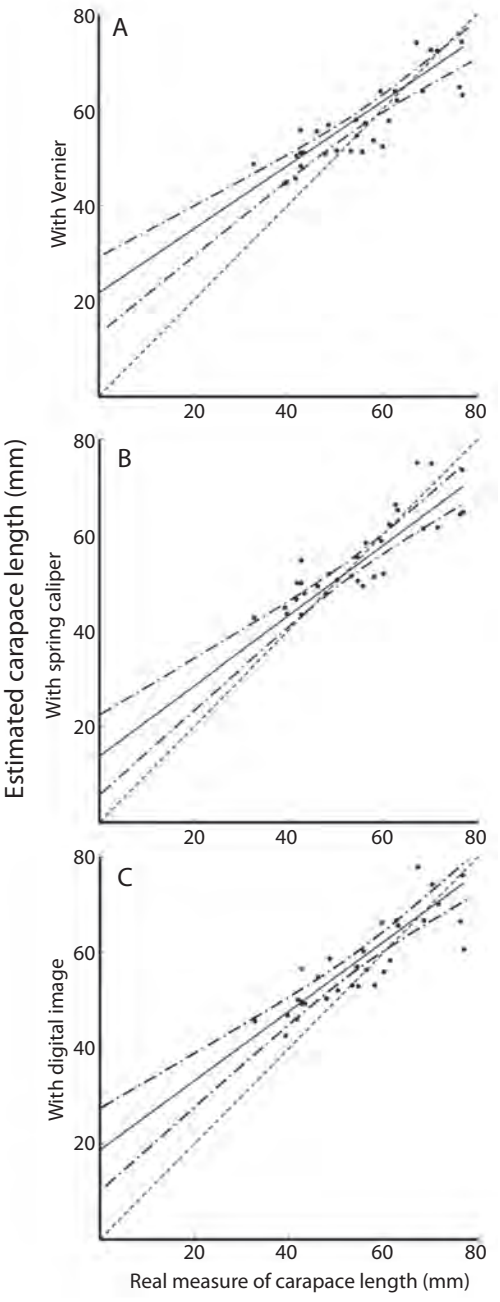

Fig. 4. Model II MA linear regression for the validation of the three measuring methods in *Cardisoma guanhumi*. Estimated carapace length (mm) vs. real carapace length (mm). A: Vernier method; B: Spring caliper method; C: Digital photograph method.

TABLE 1
Linear regression analysis between carapace length of *Cardisoma guanhumi* (A; n= 50) and *Ucides cordatus* (B; n= 29), and the three burrow measuring methods, using Model II (OLS). Significance of slope was tested using t-test and 4999 permutations

A

Method	Intercept	Slope	*t*-test	r^2
Vernier	26.88	0.39	p<0.05	0.49
Spring caliper	25.76	0.39	p<0.05	0.61
Photo	28.08	0.43	p<0.05	0.55

B

Method	Intercept	Slope	*t*-test	r^2
Vernier	2.38	0.71	p<0.05	0.71
Spring caliper	8.14	0.57	p<0.05	0.61
Photo	7.40	0.68	p<0.05	0.53

since the confidence intervals include the referential line along almost all the measures.

Statistical results for *U. cordatus*: Graphs with all data included for each of the burrow measuring methods and carapace length of crabs are shown in Fig. 5. In each of the graphs, no strong dispersion was observed. Crab body size, as well as burrow measurements with the three methods adjusted to normality (all Shapiro-Wilks tests, p>0.05). This satisfies the assumption of distribution for the conventional tests, but tests were applied based on permutations. Similarly, homogeneity was maintained in the bivariate distribution in all the Vernier and caliper measuring methods, but variation increases with increasing body size in the photo method (Fig. 5). After excluding outliers and randomly choosing 29 measurements, body size as well as burrow measures from all estimating methods were still adjusted to normality (Shapiro-Wilks tests, p>0.05). Similarly, homogeneity was maintained in the bivariate distribution in the three methods (Fig. 6). All linear regressions built with Model II OLS method are statistically significant (Table 1B). The Vernier measuring method showed the best adjustment and the less unexplained variation (r^2= 0.71, Table 1B). MA linear regressions analyzes are shown in Table 2B. The three methods have an intercept significantly above zero and slopes that include 1 or approach it significantly. All three methods slightly overestimate smaller crab body size and underestimate the average sizes greater than the population average. Such imprecision can be seen in Figure 7, where the regression lines presented MA angles below the baseline of 45°. Estimates presented by the photo method show the greatest portion of the referential line outside the IC 95%, followed by the caliper and finally by the Vernier methods, the latter having apparently the lowest degree of imprecision in estimating sizes.

TABLE 2
Validation model between real and estimated carapace length for *Cardisoma guanhumi* (n= 31) and *Ucides cordatus* (n= 31), using Model II and MA method

A

Method	Intercept	I.C. 95 % Intercept		Slope	I.C. 95 % slope		Line angle
Vernier	20.89	12.06	28.60	0.65	0.51	0.81	33.20
Spring caliper	15.47	5.95	23.64	0.73	0.58	0.90	35.96
Photo	17.97	8.20	26.30	0.72	0.57	0.90	35.84

B

Method	Intercept	I.C. 95 % Intercept		Slope	I.C. 95 % Slope		Line angle
Vernier	6.26	-1.62	12.70	0.82	0.63	1.04	39.22
Spring caliper	9.35	0.37	16.54	0.73	0.51	0.99	35.98
Photo	11.84	2.42	19.37	0.65	0.43	0.93	33.14

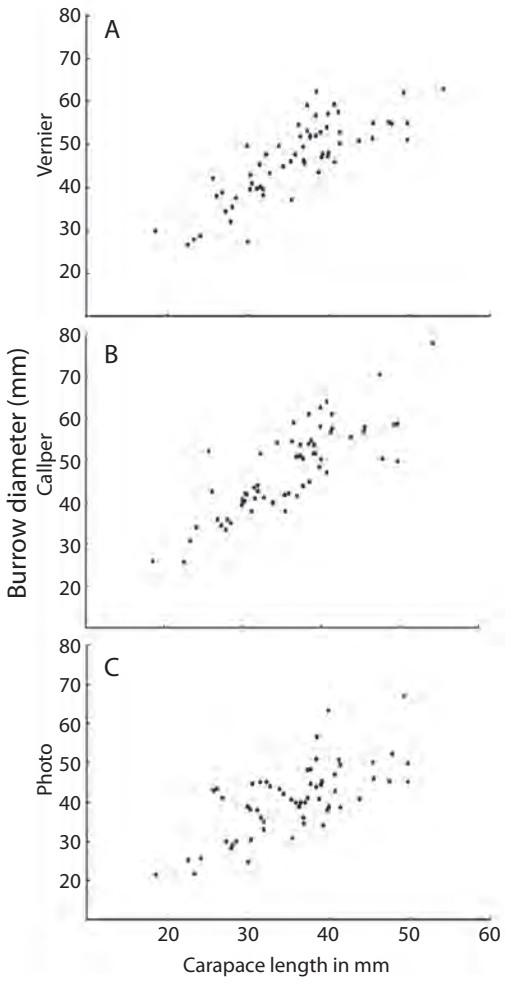

Fig. 5. Bivariate scatterplots of carapace length (mm) and burrow diameter (mm) of *Ucides cordatus*. A: Burrow diameter measured with Vernier; B: Burrow diameter measured with spring caliper; C: Burrow diameter measured from digital photograph.

DISCUSSION

Although OLS linear regressions for each of the applied measuring methods in each species resulted statistically significant (see Tables 1A and 1B), the MA analyzes reflect the same bias in the estimations: these methods overestimate sizes of smaller crabs and underestimate larger ones. The spring caliper method proved

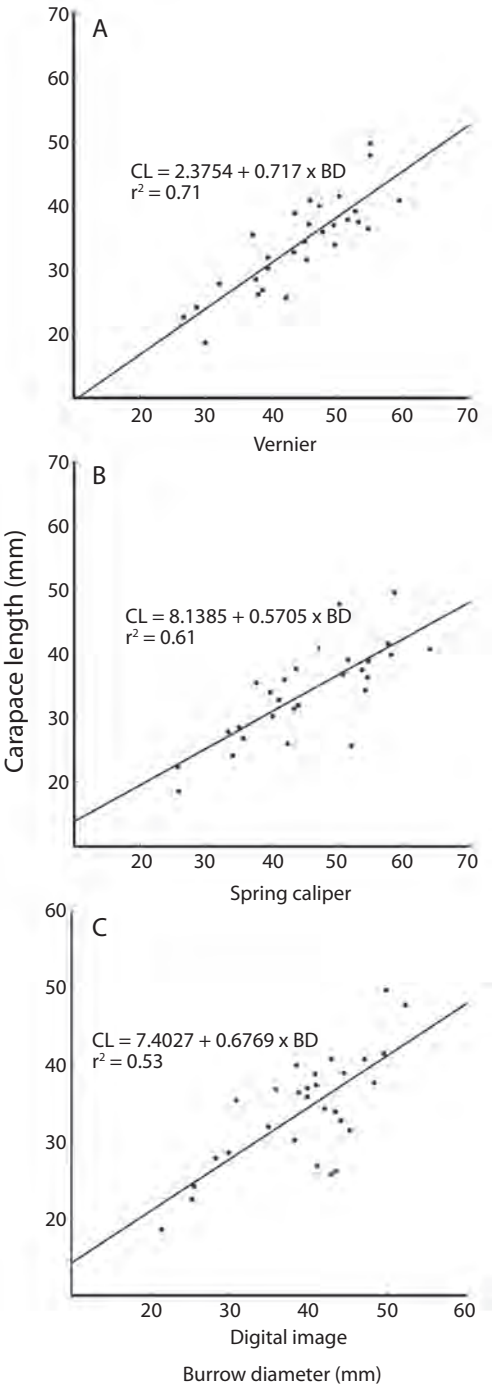

Fig. 6. Model II OLS linear regression method for carapace length (mm) and burrow diameter (mm) in *Ucides cordatus*. A: Burrow diameter measured with Vernier; B: Burrow diameter measured with spring caliper; C: Burrow diameter measured from digital photograph.

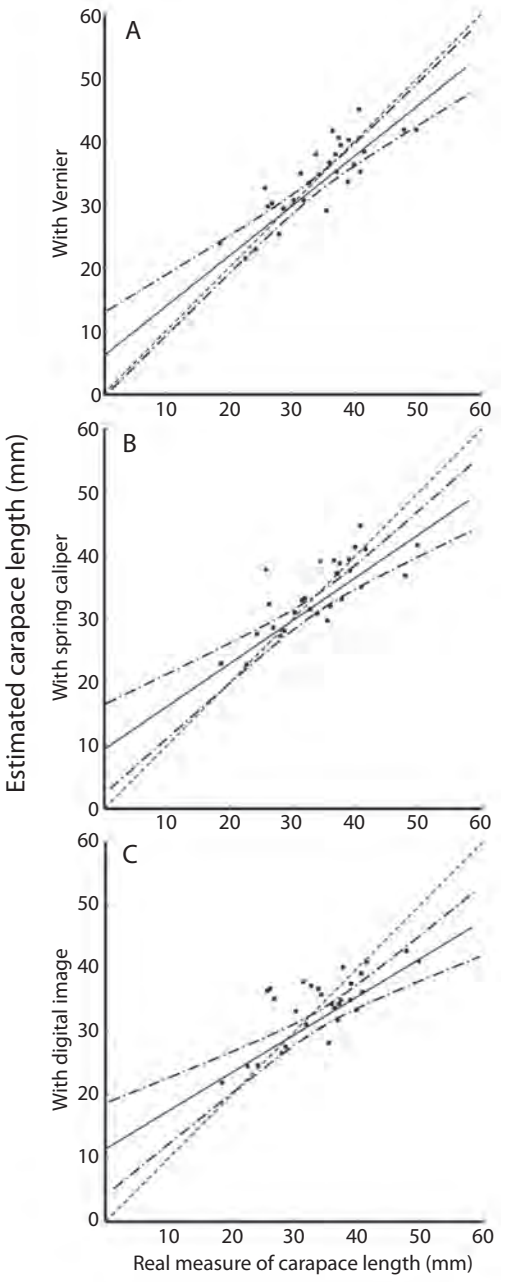

Fig. 7. Model II MA linear regression for the validation of the three measuring methods in *Ucides cordatus*. Estimated carapace length (mm) vs. real carapace length (mm). A: Vernier method; B: Spring caliper method; C: Digital photograph method.

to be the most accurate and with the lowest degree of bias to be applied in *Cardisoma guanhumi*, and the Vernier method in *Ucides cordatus*. In *C. guanhumi*, Vernier method showed a correlation r^2= 0.49, apparently being lower than Govender & Rodríguez-Fourquet (2008) results using the same measuring methodology, where they obtained a high correlation between carapace width and burrow diameter (r^2= 0.89). Moreover, body size of several burrowing crab species have also been estimated using values obtained with a Vernier, achieving high correlations, such as in *Heloecius cordiformis* (ln carapace length r^2= 0.83, MacFarlane 2002) and *Dotilla myctiroides* (carapaces length r^2= 0.89). Although we used carapace length as the reference size, and not carapace width, as used by Govender & Rodríguez-Fourquet (2008), we consider that this is not relevant to the discrepancy between the two correlations, but rather has to do with two aspects: the nature of the substrate and the statistical methodology employed. First, smaller crabs probably using previously abandoned burrows from larger animals, induce a size overestimation and dispersion increase; furthermore, the burrow entrances of *C. guanhumi* individuals that were sampled in Carenero-Venezuela can vary greatly in their shape and form (round, elongated, amorphous), as well as in their consistency (very hard to very soft mud at the entrance of the burrows), causing measuring errors (e.g. tips of the Vernier and the spring caliper penetrating in the very soft mud) that could have produced the lower correlation in the samples taken with the Vernier in the present work. But these measuring problems were not encountered with *U. cordatus*, where burrows were easier to measure. There was less dispersion in data from *U. cordatus* than in *C. guanhumi* and the regression analyzes were much more accurate. On the other hand, from the statistically perspective, the difference could be produced by the statistical methodology employed in the analyzes. Most studies in

marine biology typically use Model I regression, but this is inappropriate (Laws & Archie 1981). It is strongly recommended that Model II regression analyzes should be the methodology to relate two set of variables when both are subjected to sampling error (Sokal & Rohlf 1995, Legendre 2001, Quinn & Keough 2002). In this study, two methods of Model II regressions analyzes were applied following the recommendations of Legendre (2001). The above cited references do not make neither mention about the diversity of burrows nor on the Model of regression analyzes used.

Considering the significance of the measuring technique that was analyzed in the present work, at the moment of deciding which one should be utilized, it will depend on how large is the area to be surveyed and the amount of persons that will accomplish the task. If the area is not very large and more than one person is taking the measurements, we strongly suggest using the spring caliper technique in *C. guanhumi* and the Vernier method in *U. cordatus*, for fine estimation when an accurate body size population structure is required; but if the area is substantially large and few persons are measuring, then the photo technique should be regarded as the most efficient method (although less accurate r^2= 0.55 for *C. guanhumi* and 0.53 for *U. cordatus*). The latter is less time consuming in the field, and saves sampling time. This should be taken in mind, especially in areas where they occupy large geographical extensions. In Venezuela, for instance, there are mainly three regions were *C. guanhumi* is reported to inhabit wide land extensions: from San Juan de Los Cayos to Boca de Yaracuy (8 340 ha, Taissoun 1974), from Higuerote to Boca de Unare (more than 8 340 ha, Taissoun 1974), and the region of the Orinoco Delta (98 802 to 455 298 ha, Conde & Alarcón 1993). Also in the latter geographical area, *U. cordatus* is also reported occupying vast mangrove regions (Novoa 2000 and personal observations). But likewise in Brazil, where mangrove extensions are considerably large (1 012 376 ha; Lacerda et al. 1993), both land crabs are being commercial exploited due to their substantial presence in these forests. Finally, due to the ecological and economical importance to estimate body size structure in both land crabs, it is justified the application of the measuring methods that were analyzed in the present work, even when the bias in estimations is a subject to be taken into account in the interpretation of data.

ACKNOWLEDGMENTS

We are very thankful to the fishermen, especially to Nicolás Suárez, and as well as to Arianny Camejo and Patricia Medina, who helped us trapping and measuring the crabs. We also express our sincere thanks to Hermes Piñango, who showed us the internal spring caliper methodology and gave us critical reviews on the manuscript, as well as direct help in the field.

RESUMEN

Para la estimación de la estructura de tamaños en cangrejos terrestres comerciales y la obtención de información relevante para su manejo, es necesario utilizar métodos rápidos, confiables y no destructivos. *Cardisoma guanhumi* y *Ucides cordatus* son dos cangrejos terrestres que son explotados comercialmente en el Caribe y en Brasil. El propósito de este trabajo es suministrar métodos indirectos para la estimación del tamaño del caparazón de los cangrejos y por consiguiente, de la estructura de tallas. Los muestreos se llevaron a cabo en Carenero (*C. guanhumi*) y en Cumaná (*U. cordatus*) (Venezuela). Se utilizaron tres métodos para estimar el diámetro de sus madrigueras: Vernier, compás y fotografía. Estos se correlacionaron con el tamaño real del cangrejo. Se aplicó el análisis de regresión Ordinary Least Squares Model II y la capacidad de predicción se probó utilizando el modelo II Mayor Axis para las regresiones. *Cardisoma guanhumi* mostró una fuerte dispersión de sus datos en los métodos de Vernier y fotografía. Menos dispersión se obtuvo con el método del compás y fue el más preciso (r^2= 0.61). Para *U. cordatus* las medidas con Vernier fueron la más adecuadas (r^2= 0.71). Sin embargo los tres métodos fueron confiables. Los diferentes métodos mostraron ventajas y desventajas y dependerá del que aplique los métodos, decidir cuál será el más adecuado para sus propósitos.

Palabras clave: Métodos indirectos para medir tamaño corporal, *Cardisoma guanhumi*, *Ucides cordatus*, medidas con Vernier, medidas con compás, medidas con fotografía.

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Crecimiento somático y relación ARN/ADN en estadios juveniles de *Eucinostomus argenteus* (Pisces: Gerreidae) en dos localidades del Caribe de Venezuela

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Recibido 15-VII-2011. Corregido 14-XI-2011. Aceptado 20-XII-2011.

Abstract: Somatic growth and RNA/DNA rate of *Eucinostomus argenteus* (Pisces: Gerreidae) juveniles stages at two localities of the Venezuelan Caribbean. In order to evaluate the association among growth indices of marine fishes at early life stages, the somatic growth rate and physiological conditions of *Eucinostomus argenteus* were estimated at two Venezuelan North-East zones: Mochima Bay and Cariaco Gulf. The age and somatic growth rate were estimated based on daily growth increments in sagitta otoliths. The physiological conditions were evaluated with proteins concentrations and RNA/DNA rate, which were estimated by spectrofluorometric and fluorometric techniques, respectively, on muscle tissue. Juvenile standard length ranged from 9.80 to 39.20mm from 21 to 73 days of age. At all the study localities there were significant and positive correlations between age, otolith diameter and body size, and fitted to a linear regression model. The values of recent growth rate ranged from 0.178 to 0.418mm day^{-1}, backcalculated growth rate oscillated between 0.295 - 0.393mm day^{-1}, and RNA/DNA rate ranged from 1.65 to 6.97. Differences were not found between study zones, but there were differences between localities. Despite the fact that there was no correlation between juvenile´s somatic growth and RNA/DNA rates, the reported values suggesting a *E. argenteus* juvenile's positive growth in their natural habitat at localities studied. Nevertheless, in some localities values that indicate poor nutritional conditions were registered, which could affect other future demographic rates as survivor and fecundity. Rev. Biol. Trop. 60 (Suppl. 1): 151-163. Epub 2012 March 01.

Key words: Gerreidae, growth rate, nutritional condition, otoliths, RNA/DNA.

Estudios sobre la edad y tasa de crecimiento de las especies de peces en sus estadios tempranos permiten entender procesos vitales en la persistencia de las poblaciones, entre los que se pueden mencionar la mortalidad e identificación de cohortes. Al mismo tiempo, las tasas de crecimiento de los individuos se encuentran directamente relacionadas a la dinámica poblacional a través de su influencia a las tasas de supervivencia, madurez y fecundidad (Jones 2002).

El empleo de diversas metodologías para la estimación del crecimiento de los peces puede generar el historial completo de crecimiento de los individuos e incluso relacionarlo con las condiciones ambientales (Stevenson & Campana 1992, Clemmesen & Doan 1996, Gilliers *et al.* 2006). Actualmente dos de las herramientas que han sido ampliamente empleadas para la evaluación la condición y crecimiento de los peces es el estudio de la micro-estructura de los otolitos y los índices bioquímicos.

La interpretación de la relación entre la longitud del cuerpo de los peces y del tamaño de su otolito, así como también el análisis de los incrementos diarios y/o anuales de crecimiento en los otolitos, se ha empleado desde los años 70 para estimar tallas y edades, así como para revelar diversos procesos ecológicos y oceanográficos (Panella 1971, Stevenson & Campana 1992, Sponaugle 2010). Específicamente, a través de análisis de retrocálculo se puede reconstruir la historia de crecimientos de un pez (Secor & Dean 1992, Stevenson & Campana 1992)

Por su parte, la relación ARN/ADN genera un índice del metabolismo celular y ha sido empleado como una medida de la tasa de crecimiento y condición nutricional de los individuos (Buckley 1984). Esto se debe a que las concentraciones de ARN varían ampliamente dependiendo de la tasa metabólica del tejido y de la condición fisiológica del organismo, mientras que las concentraciones de ADN tienden a ser constantes (Leslie 1955).

Con la finalidad de evaluar la asociación de índices de crecimiento en estadios tempranos de peces marinos a las condiciones ambientales en las que se encuentran, se estimó la tasa de crecimiento somático y las condiciones fisiológicas de una especie común en las costas del oriente venezolano (*Eucinostomus argenteus*) en dos zonas con diferentes condiciones ambientales en cuanto a extensión, exposición de corrientes y productividad.

MATERIALES Y MÉTODOS

Recolección de peces: la mojarra *E. argenteus* fue empleada como organismo modelo de estudio, ya que son individuos comunes en los litorales arenosos y frecuentemente es una especie dominante (Cervigón 1993). Aunque no es una especie de importancia comercial, juega un papel fundamental en la cadena trófica en las costas venezolanas, siendo un enlace importante entre el bentos y grandes depredadores pelágicos (Randall 1967, Rivas *et al.* 1999).

Considerando la importancia ecológica de los ecosistemas marino-costeros de la región oriental de Venezuela (Martín 1995, Miloslavich *et al.* 2003), se seleccionaron dos zonas de estudio: la Bahía de Mochima, Parque Nacional Mochima (10º 24' 30" N, 64º 19' 30" W) y el Golfo de Cariaco (10°31'00" N, 64° 02'96" W). La Bahía de Mochima se considera una zona de alta productividad relativamente cerrada a corrientes externas, mientras que el Golfo de Cariaco es una zona más extensa, con mayor circulación y entrada de corriente externas, aunque con menores valores de productividad (Okuda *et al.* 1968, 1978, Expósito 1997, Marín *et al.* 2004, Quintero *et al.* 2004).

Dentro de la Bahía de Mochima, los ejemplares de estudio fueron recolectados en dos ensenadas internas: Ensenada de Reyes (10°20'22,473" N, y 64°21'42,99" W) y Ensenada Mochimita (10°20'33,42" N, y 64° 21'04,99" W). En el caso del Golfo de Cariaco, la recolecta de ejemplares se realizó en la Ensenada de Turpialito (10°26'56" N, y 64°02'00" W). Estas localidades se caracterizan por litorales de arena fina (250μm), con bajas concentraciones de materia orgánica (datos aún no publicados suministrados por el Laboratorio de Ecofisiología, IOV-UDO), con presencia de raíces sumergidas de mangle y praderas de fanerógamas.

Las capturas fueron realizadas en noviembre 2009 y para ello se empleó un chinchorro de arrastre con abertura de malla de 500μm. Los ejemplares fueron colocados en hielo y transportados al laboratorio donde se mantuvieron congelados hasta el momento de la disección.

Edad y tasa de crecimiento somático: estas estimaciones fueron realizadas por medio del análisis de los otolitos de los ejemplares capturados y su relación con su morfometría. Para ello, a cada ejemplar se le calculó su peso (P) y se les realizaron mediciones de: longitud total (L.T.) y longitud estándar (L.E.). Posteriormente, se realizó una incisión sobre la cápsula ótica de cada ejemplar, con la finalidad de extraer los otolitos sagitta del lado derecho del individuo, siguiendo los pasos de ruptura, arrastre y aislamiento sobre una placa portaobjeto (Secor *et al.* 1992).

Los otolitos fueron colocados sobre una placa de resina sintética termoplástica transparente (Crystalbond™) previamente calentada (>100 ºC) en un portaobjeto, y posteriormente se siguió la metodología de raspado propuesta por Secor *et al.* (1992) para realizar conteo, medición y visualización de los anillos desde el núcleo hasta el borde del otolito. Seguidamente, a través de un programa de procesamiento de imágenes (Sigma Scan Pro 5.0.0), se midió el diámetro del otolito (Ot), se realizó el conteo del número de anillos del otolito y la medición del grosor de cada uno estos anillos, así como también del núcleo del otolito.

A pesar de que en este estudio no se realizó la validación de la formación de los incrementos diarios en los otolitos, se trabajó bajo la hipótesis de que cada banda o anillo representa un día de vida del individuo, considerando que la mayoría de las especies de peces marinos presentan una frecuencia de formación diaria (Campana 1992). De esta forma, la edad de los individuos se estableció según el número de bandas contadas en los otolitos. Por otra parte, el análisis de las variaciones de los anchos de cada anillo permitió discernir las bandas pertenecientes a los períodos de larva e inicio del estadio juvenil, y por ende hacer una estimación aproximada de los períodos de tiempo larvarios de la especie en estudio (Brothers *et al.* 1983)

La tasa de crecimiento reciente de los individuos, C. Rec (mm día^{-1}), fue estimada al relacionar la L.E. de los individuos con la edad (E) en días. De esta forma el valor de la tasa de crecimiento de los individuos corresponde a la pendiente del modelo de crecimiento que mejor ajuste (Thorrold & Williams 1989, Neuman *et al.* 2001). La tasa de crecimiento retrocalculada (C. Retro) se obtuvo utilizando las tallas pretéritas (Thorrold & Williams 1989), considerando que el tamaño del otolito es proporcional al tamaño del individuo. Para el cálculo de dichas tallas pretéritas se empleó el ajuste al relacionar el diámetro del otolito con el tamaño del pez. Así, se empleó la ecuación resultante para determinar las longitudes pretéritas a cada edad, cuya pendiente representó la tasa pretérita media de crecimiento para cada día de la vida del ejemplar.

Condición nutricional: a cada ejemplar se le retiró la cabeza, abdomen y aletas, para utilizar el tejido restante en los análisis de concentraciones de proteínas, ADN y ARN. Cada tejido fue colocado en 100µl de solución de sarcosina (N-lauroy-sarcosina) 1% preparada con buffer TRIS-EDTA (5.0mmol TRIS-HCl; 0.5mmol EDTA; pH 7.5) y se homogeneizó, al momento de colocarle la sarcosina, a los 30 minutos y a los 60 minutos posteriores. Seguidamente, se agregaron 900µl de buffer y se centrifugó a 2 500rpm por 15 minutos, obteniéndose así para cada individuo una solución de 1ml. Todo lo mencionado anteriormente se preparó manteniendo el tejido frío. Del sobrenadante se tomaron muestras por duplicado para la cuantificación de ARN y ADN utilizando la metodología de Clemmesen (1993). Las proteínas totales fueron determinadas mediante la metodología descrita por Bradford (1976).

Análisis Estadísticos: se realizaron análisis de correlación y regresión (Sokal & Rohlf 1995), para establecer relaciones entre las variables medidas en cada juvenil para cada localidad: L.T., L.E., P, Ot, E, proteínas, ADN, ARN y ARN/ADN. Igualmente, se realizaron correlaciones y regresiones entre C. Retro y el índice ARN/ADN por localidad. Todo esto revisando previamente la normalidad y homogeneidad de varianza.

Las distintas tasas de crecimiento se compararon a través de un análisis de covarianza (Sokal & Rohlf 1995). Comparaciones entre localidades fueron realizadas a través de un ANOVA de una vía, para detectar diferencias entre las concentraciones de proteínas, índice ARN/ADN y la tasa de crecimiento retrocalculada, revisando previamente la normalidad y homogeneidad de varianza. Posteriormente, se realizó un test de Tukey para discernir las localidades que mostraban diferencias con un nivel de significancia de 0.05. En el caso de la evaluación del cambio de ancho de las bandas diarias, debido a la naturaleza de los datos,

se realizó la comparación del promedio del ancho de estos incrementos diarios por localidad a través de una prueba Kruskal-Wallis. Estos análisis fueron realizados a través del programa SPSS 16.0.

Para una mejor visualización de las posibles diferencias entre localidades se realizó una ordenación no métrico de escalas multidimensionales (MDS, PRIMER 5), basado en el índice de distancia euclideana (Clarke 1993). Seguidamente, para establecer la significancia del MDS se realizó un análisis de similitud (ANOSIM, PRIMER 5), el cual es un método de permutación aleatoria en la matriz de distancias. El intervalo de similitud global *R* es una medida comparativa del grado de separación entre grupo ($0 \leq R \leq 1$), de forma tal que cuando *R* se aproxima a cero no existe separación entre grupos (Clarke 1993).

RESULTADOS

Los caracteres morfométricos para los juveniles de *E. argenteus* presentan intervalos similares en las tres localidades de estudio, así como también la edad y el peso de los individuos (Cuadro 1). En cada una de las localidades de estudio, los análisis de regresión y correlación de las variables morfométricas con el tamaño del otolito, el peso y la edad de los juveniles se ajustaron a un modelo lineal y fueron significativos ($p< 0.001$) (Cuadro 2).

Tasa de crecimiento reciente

La edad fue un parámetro relacionado con la longitud estándar en las tres localidades (Cuadro 2). De esta forma, con esta relación se determinó la tasa de crecimiento reciente de las tallas en los estadios tempranos de *E. argenteus* (mm día^{-1}), la cual es diferente entre localidades (ANCOVA, $F= 20.16$, $p< 0.0001$) encontrándose los valores mayores dentro de la Bahía de Mochima (Cuadro 3).

Igualmente, en las tres localidades se observó correlación entre la edad y el peso de los individuos (Cuadro 2), y por ende se estimó la tasa de crecimiento en peso para los estadios tempranos de la especie. Los valores de crecimiento se registraron entre 0.003 y 0,013g día^{-1}, sin encontrarse diferencias entre localidades (ANCOVA, $F= 1.53$, $p= 0.2214$).

Tasa de crecimiento retrocalculado

Debido a la proporcionalidad encontrada entre la longitud estándar de los ejemplares y el diámetro del otolito, así como la correlación existente entre la edad y la longitud estándar, se pudo modelar el crecimiento retrocalculado en cada una de las localidades evaluadas (Cuadro 3). De esta forma, se estimó el valor menor dentro de la Ensenada de Reyes, una de las localidades dentro de la Bahía de Mochima (ANOVA, $F= 8.415$, $p= 0.001$).

CUADRO 1
Variables morfométricas, peso y edad de *E. argenteus*

TABLE 1
Morophometrics variables, weight and age of *E. argenteus*

Variable	E. Reyes (Bahía Mochima)	E. Mochimita (Bahía Mochima)	Turpialito (Golfo Cariaco)
L.T.	20.520 ± 7.910 (n= 33)	22.05 ± 7.122 (n= 47)	21.573 ± 8.638 (n= 46)
L.E.	16.045 ± 6.073 (n= 33)	18.757 ± 5.947 (n= 47)	17.708 ± 6.909 (n= 46)
Ot	0.883 ± 0.498 (n= 26)	0.875 ± 0.270 (n= 31)	0.999 ± 0.341 (n= 41)
P	0.174 ± 0.296 (n= 32)	0.172 ± 0.155 (n= 47)	0.165 ± 0.167 (n= 46)
E	30.250 ± 9.261 (n=25)	32.650 ± 6.235 (n=20)	43.520 ± 13.135 (n=23)

L.T.: Longitud total (mm), L.E.: Longitud estándar (mm), Ot: diámetro del otolito (mm), P: Peso (g), E: Edad (Días), D.E.: Desviación estándar; n: número de mediciones.

CUADRO 2
Relaciones entre la longitud estándar, el diámetro del otolito, la edad y el peso en juveniles de *E. argenteus* en las localidades de estudio

TABLE 2
Relationships among standard lenght, otoliths diameter, age and weight of *E. argenteus* juveniles at study localities

Localidad	Relación	Ecuación	r^2	r	n
E. Reyes (Bahía Mochima)	L.E. *vs* Ot	L.E.=11.856Ot + 6.472	0.817**	0.684**	26
	L.E. *vs* E	L.E.=0.4186E + 2.9363	0.568**	0.754**	24
	P *vs* Ot	P=1.6773Ot + 0.6333	0.862**	0.705**	26
	P *vs* E	P=0.0133E – 0.319	0.589**	0.428**	24
E. Mochimita (Bahía Mochima)	L.E. *vs* Ot	L.E.=14.145Ot + 5.8778	0.473**	0.643**	32
	L.E. *vs* E	L.E.=0.3446E + 4.8002	0.496**	0.647**	18
	P *vs* Ot	P=1.277Ot + 0.666	0.456**	0.639**	32
	P *vs* E	P=0.0063E – 0.0996	0.302**	0.584**	20
Turpialito (Golfo Cariaco)	L.E. *vs* Ot	L.E.=18.207Ot - 0.0011	0.812**	0.798**	41
	L.E. *vs* E	L.E.=0.178E + 6.734	0.677**	0.620**	23
	P *vs* Ot	P=1.277Ot + 0.666	0.823**	0.811**	41
	P *vs E*	P=0.0038E - 0.0817	0.660**	0.684**	23

L.E.: Longitud estándar, Ot: diámetro del otolito, E: Edad, P: Peso. r^2: coeficiente de determinación, r: índice de correlación, n: número de individuos ** p< 0.001; ns: no significativo.

CUADRO 3
Valores de crecimiento reciente (C. Rec) obtenidos del modelo lineal ajustado a los datos de longitud estándar –edad y peso– edad, y valores promedio y desviación estándar de la tasa de crecimiento retrocalculada (C. Retro), relación ARN/ADN, concentraciones de proteínas de *E. argenteus* en las tres localidades de estudio

TABLE 3
Values of recent growth (C. Rec) from the lineal model fitted to the data of standard length –age and weight–age, and mean values with their standard deviation of backcalculated growth rate (C. Retro), RNA/DNA rate, proteins concentrations of *E. argenteus* at three study locations

Localidad	C. Rec (g día^{-1})	C. Rec (mm día^{-1})	C. Retro (mm día^{-1})	ARN/ADN	Proteínas (mg g^{-1} tejido húmedo)
E. Reyes (Bahía Mochima)	0.013 (n= 24)	0.418 (n= 24)	0.295 ± 0.050 (n= 24)** ^{A}	2.46 ± 1.36 (n=17) ^{X}	25.75 ± 17.33 (n=28) ^{1}
E. Mochimita (Bahía Mochima)	0.006 (n= 20)	0.334 (n= 18)	0.352 ± 0.080 (n= 18) ^{B}	6.97 ± 5.68 (n=11) **^{Y}	12.56 ± 5.68 (n=29) **^{2}
Turpialito (Golfo Cariaco)	0.003 (n= 23)	0.178 (n= 23)	0.393 ± 0.083 (n= 16) ^{B}	1.65 ± 2.59 (n=16) ^{X}	21.01 ± 2.59 (n=29) ^{1}

n: número de individuos. Se utilizaron pruebas estadísticas para observar diferencias entre localidades para la tasa de crecimiento retrocalculada (C. Retro), ARN/ADN y concentraciones de proteínas y éstas son marcadas por ** y representadas por diferencias en letras o números (usando A, B; X, Y; 1,2)

Cambios en la microestructura del otolito

Los otolitos sagitta de los ejemplares evaluados presentaron un núcleo redondeado de 10 micras de diámetro, aproximadamente. Se observó un patrón de bandas similar en las tres localidades, en donde el ancho de las primeras 8-12 bandas presentaron los menores valores (<5μm), posteriormente se registró un aumento gradual en las siguientes 5-10 bandas y finalmente se puede observar pequeñas oscilaciones en los valores del resto de las bandas de crecimiento

(entre 4 y 8µm, aproximadamente) (Fig. 1 y 2). El ancho de las bandas en los otolitos de los estadios iniciales de *E. argenteus* mostraron diferencias entre las localidades de estudio, específicamente a partir de la banda 15, en donde los mayores valores fueron reportados para Turpialito (Kruskal-Wallis, H= 651.69, p< 0.0001)

Condición Nutricional

El índice de relación ARN y ADN, y las concentraciones de proteínas, presentaron diferencias entre localidades (ANOVA; F= 48.73 p = 0.0000; F= 8.99 P= 0.0007; respectivamente). Los mayores valores del índice ARN/ADN se encontraron en una de las localidades de la Bahía de Mochima (Ensenada Mochimita) (Tukey Test; *P*= 0.0000), mientras que los mayores valores de concentración de proteínas estuvieron presenten en la localidad del Golfo de Cariaco (Turpialito) y Ensenada de Reyes (Bahía de Mochima) (Tukey Test; *P*= 0.0000) (Cuadro 3).

Fig. 1. Otolito sagitta de un ejemplar *E. argenteus* de 11mm de longitud estándar, mostrando el núcleo y 53 líneas de crecimiento.
Fig. 1. Sagitta otolith of a 11mm standard length *E. argenteus* showing the core and 53 growth increments.

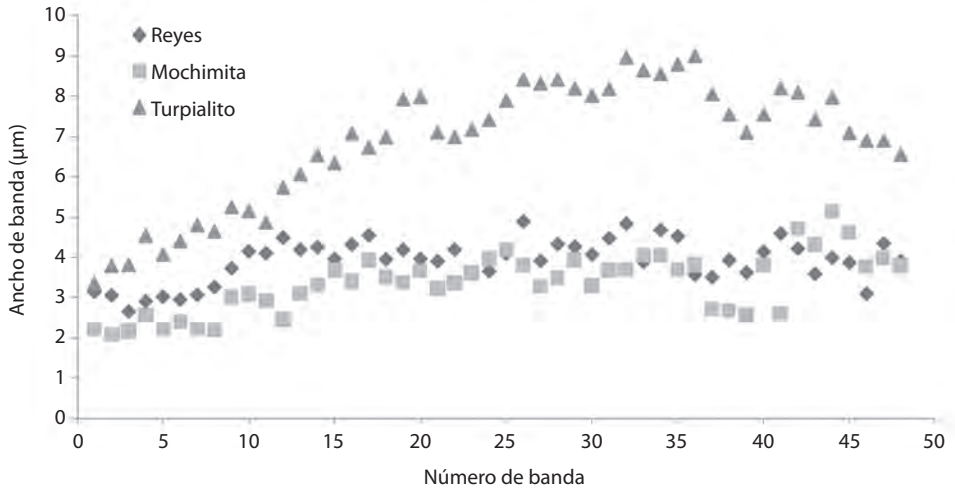

Fig. 2. Variación del ancho promedio de las bandas registradas en los otolitos sagitta de estadios iniciales de *E. argenteus*.
Fig. 2. Variation of the mean increments widths in sagitta otoliths of *E. argenteus* at early stages.

Al relacionar las proteínas, ácidos nucléicos y el índice ARN/ADN con ciertas variables morfométricas de los individuos, se observó que en Ensenada de Reyes y Turpialito existen correlaciones significativas entre la concentración de proteínas y peso corporal, así como también entre la concentración de proteínas y longitud estándar, lo cual no se evidencia para Ensenada Mochimita (Cuadro 4). Igualmente, se buscó una relación entre las tasas de crecimiento somático retrocalculado y el índice ARN/ADN; sin embargo, a pesar de parecer adaptarse a un modelo lineal esto no fue significativo para ninguna de las tres localidades de estudio (Cuadro 4).

Un análisis de ordenación, considerando los valores obtenidos de las concentraciones de proteínas, el índice ARN/ADN y la tasa de crecimiento somático retrocalculado, presenta separación de los individuos entre localidades, con un valor de stress de 0.04 (Fig. 3). Esta separación es significativa, ya que la prueba de análisis de similaridades arrojó un R global igual a 0.467, con un nivel de significancia igual a 0.01. Específicamente, los resultados indican que la Ensenada Mochimita es altamente diferente a las otras localidades, mientras que Ensenada de Reyes y Turpialito son

Fig 3. Ordenación multivariada (MDS) de los ejemplares evaluados en las localidades de E. Reyes (1), E. Mochimita (2) y Turpialito (3), empleando los valores de tasa de crecimiento retrocalculado (C. Retro), concentración de proteínas y relación ARN/ADN.

Fig. 3. Metric dimensional scaling (MDS) ordinations of juveniles evaluated from E. Reyes (1), E. Mochimita (2) and Turpialito (3), using the values of backcalculated growth rate (C. Retro), proteins concentrations and RNA/DNA rates.

CUADRO 4

Relaciones entre las concentraciones de proteínas, ácidos nucléicos y el índice ARN/ADN con variables morfométricas y la tasa de crecimiento somático retrocalculada en juveniles de *E. argenteus* en las localidades de estudio

TABLE 4

Relationships among proteins concetrations, nucleics acids, RNA/DNA rates and morphometrics variables, somatic backcalculated growth rate of *E. argenteus* at study localities

Localidad	Relación	Ecuación	r^2	r	n
E. Reyes (Bahía Mochima)	P *vs* Pr ◊	$P= 1.9379Pr^{-1.217}$	0.773**	-0.879**	29
	L.E *vs* Pr ◊	$L.E.= 34.203Pr^{-0.246}$	0.636**	-0.590**	29
	E *vs* Pr ◊	$E= 51.037Pr^{-0.16}$	0.342 ns	-0.392 ns	20
	Gr *vs* ARN:ADN ◊◊	Gr=-0.0731(ARN:ADN)+ 1.079	0.457 ns	0.473 ns	11
E. Mochimita (Bahía Mochima)	P *vs* Pr ◊◊◊	$P=0.1148e^{-0.029Pr}$	0.218 ns	-0.426**	29
	L.SE. *vs* Pr ◊◊◊	$L.SE.=17.773e^{-0.01Pr}$	0.151 ns	-0.449**	29
	E *vs* Pr ◊◊◊	$E=4.525e^{-0.004Pr}$	0.036 ns	-0.363 ns	20
	Gr *vs* ARN:ADN◊◊	$Gr= 1.10^{-5}(ARN/ADN) + 0.038$	0.422 ns	0.700 ns	5
Turpialito (Golfo Cariaco)	P *vs* Pr ◊◊◊	$P=0.1005e^{-0.032Pr}$	0.511**	0.747**	29
	L.SE. *vs* Pr ◊◊	L.SE.= -0.1502Pr + 18.167	0.334**	-0.665**	29
	E *vs* Pr ◊◊	E= -0.0027Pr + 33.36	0.000 ns	-0.328**	22
	Gr *vs* ARN:ADN ◊◊	Gr= 0.01(ARN:ADN) + 0.122	0.431 ns	0.467 ns	10

L.E.: Longitud Estándar, E: Edad, P: Peso, Pr: Proteínas, Gr: tasa de crecimiento retrocalculado, r^2: coeficiente de determinación, r: índice de correlación, n: número de individuos ** p< 0.001; ns: no significativo. ◊ Relación ajustada a un modelo potencial. ◊◊ Relación ajustada a un modelo lineal ◊◊◊ Relación ajustada a un modelo polinómico.

bastante similares entre si, con respecto a las variables evaluadas (Cuadro 5).

CUADRO 5
Análisis de similitud (ANOSIM). Prueba entre localidades de estudio. El R global de similitud fue de 0.467 con nivel de significancia estadística p= 0.01

TABLE 5
Analysis of similarities (ANOSIM). Test among study localities. The similitude global R was 0.467 with statistic significance level p= 0.01

Localidades	*R*	P
E. Reyes - E. Mochimita	0.889	0.001
E. Reyes – Turpialito	0.127	0.410
E. Mochimita – Turpialito	0.700	0.001

DISCUSIÓN

En esta evaluación, se estableció para *E. argenteus* una relación lineal entre la longitud estándar de los individuos capturados y el tamaño de su otolito, lo cual coincide con varias evaluaciones realizadas a otras especies de peces dentro y fuera del Caribe, en donde esto se observa claramente en los primeros estadios de vida (Jones 2002). La existencia de una relación entre la longitud corporal y el tamaño del otolito, aunado a reportes de la validación sobre la relación entre el crecimiento somático y los incrementos diarios en los otolitos, soportan el uso del ancho o amplitud de los incrementos como una medida de la tasa de crecimiento somático (Secor & Dean 1989, Campana & Thorrold 2001). De esta forma, se pudo estimar la tasa de crecimiento promedio de *E. argenteus* en las localidades de estudio, para la cual hasta la fecha no se había evaluado.

Las tasas de crecimiento en peso de *E. argenteus* son relativamente bajos a los valores estimados para otras especies de mojarras. Estudios en laboratorio han estimado que la tasa de crecimiento de la masa corporal de los gerreidos es lento durante sus primeros estadios, tales como *Eugerres plumieri* para el Caribe (0.06 g día^{-1}), *E. currani* (0.26 – 0.36 g día^{-1}) y *Diapterus peruvians* (0.31- 0.45 g día^{-1}) para el Pacífico (Tucker & Jory 1991, Rubio *et al.* 2004). Por otra parte, en el caso del crecimiento de tallas, diversos estudios en adultos de la dinámica espacio-temporal de ciertas especies de gerreidos, los han identificado como peces de rápido crecimiento. En estos estudios se han evaluado los parámetros de la ecuación de crecimiento de von Bertalanffy, en donde los valores reportados del coeficiente de crecimiento (k) de dicha ecuación se encuentra alrededor de 1, tal es el caso de *E. plumieri* (k= 1.27 año^{-1}) (Venezuela), *Gerres longirostris* (k= 1.1 año^{-1}) (Golfo de Arabia), *Pentaprion longimanus* (k= 1.8 año^{-1}) (Mar Java) (Sadhotomo 1983, Grandcourt *et al.* 2006, Montaño 2009).

Al querer realizar comparaciones con otras especies de la zona de estudio, se tiene que para el área sólo se han hecho estudios similares para el clupeido *Sardinella aurita*, reportándose valores mayores a *E. argenteus* dentro del Parque Nacional Mochima (0.66 y 1.10mm día^{-1}), mientras que en Turpialito las tasas de crecimiento son similares (0.32mm día^{-1}) (Balza & Marín 2000, Balza *et al.* 2006, Ramírez & Marín 2006).

En cuanto al crecimiento de los otolitos, las variaciones diarias encontradas en el ancho de las bandas refleja los cambios en el crecimiento somático de los individuos de *E. argenteus.* En general, las variaciones en el ancho de las bandas presentes tienden generalmente a aumentar con la edad (Thorrold & Williams, 1989), y por lo tanto se puede estimar los períodos de las diferentes fases o estadios de los individuos. De esta forma, del patrón de ancho de las bandas de *E. argenteus* en las localidades de estudio, y siguiendo el patrón propuesto por Brothers & McFarland (1981), se puede distinguir que por el pequeño grosor de las primeras bandas el período larvario de la especie se encuentra entre 12 y 15 días. Por su parte, a partir de la banda número 16 no se evidencia un patrón que permita estimar cambios de post-larva a pre-juvenil y de pre-juvenil a juvenil, lo cual puede estar asociado a cambios de factores internos o externos del individuo (Wilson & McCormick 1999).

Los períodos de las fases mencionadas anteriormente, coinciden con diversas especies marinas, y han sido considerados como períodos relativamente cortos (Swearer *et al.* 1999, Claro & Lindeman 2003, Balza & Marín 2000; Raventós & Macpherson 2001, Marín 2009). Estos se ajustan a las hipótesis de que crecimientos rápidos y mayores presentan ventajas en la sobrevivencia de los individuos (Houde 2009).

En el caso de los mayores tamaños de las bandas de crecimiento de los otolitos de los individuos de Turpialito con respecto a los de la Bahía de Mochima, pueden deberse a factores exógenos o endógenos particulares que se encuentran afectando el crecimiento de las bandas sin que aparentemente se afecte drásticamente el crecimiento somático, tal cual ha sido observado para otras especies de la región (Ramírez & Marín 2006).

Igualmente, al considerar los valores de la relación ARN/ADN obtenidos, se puede inferir que los individuos de *E. argenteus*, de las localidades evaluadas, se encuentran en crecimiento, ya que se ha estimado que el valor límite inferior de ARN/ADN necesario para sobrevivir es 1.0, debido a que individuos que se encuentren en períodos de inanición prolongados presentan decrecimiento en la síntesis de ribosomas e incluso procesos de degradación de los mismos, lo cual indica pérdida de ARN (Alfaro *et al.* 2002). No obstante, se encuentran diferencias entre localidades en cuanto a su condición nutricional, en donde los individuos de Turpialito y Ensenada de Reyes presentan valores que han sido considerados bajos en otras especies, e incluso en evaluaciones experimentales se ha llegado a concluir que para ciertas especies valores por debajo de 3 indican condiciones nutricionales pobres (Bulow 1987, Richard *et al.* 1991, Cunha *et al.* 2003, Caldarone 2005).

Varios estudios de individuos silvestres han encontrados correlación positiva entre el crecimiento de los últimos incrementos de los otolitos y la proporción de ARN/ADN (Clemmesen & Doan 1996, Balza *et al.* 2006, Balza *et al.* 2007). Sin embargo, al igual que en este estudio, hay otros reportes en donde no se ha encontrado correlaciones entre los ácidos nucléicos y el crecimiento de los individuos, sugiriendo que el índice ARN/ADN se encuentra más relacionado a la condición nutricional de los individuos que a su crecimiento (Bergeron 1997, Gilliers *et al.* 2006, Frommel & Clemmesen 2009). Esto pudiera ser explicado por medio de evidencias de transiciones de la composición bioquímica de los individuos durante sus cambios ontogénicos y su reorganización celular.

El contenido proteico de los peces tiende a cambiar durante su crecimiento, en donde las larvas requieren grandes cantidades de proteínas para un rápido crecimiento, y estos tienden a decrecer a medida que llegan a la madurez, pudiendo llegar a ser la concentración de lípidos y no las proteínas los responsables del incremento en masa y de tallas de los individuos (Caldarone *et al.* 2006, Frommel & Clemmesen 2009). De esta forma, las relaciones entre el crecimiento somático y el índice ARN/ADN puede cambiar dependiendo del estadio que se esté evaluando (Peck *et al.* 2003, Caldarone 2005). Al mismo tiempo, la explicación mencionada anteriormente, se ve fortalecida con las relaciones negativas encontradas entre las concentraciones de proteínas de los individuos y los pesos, longitudes estándares y edades de los mismos.

Al considerar que las zonas estudiadas presentan masas de aguas, patrones hidrodinámicos y productividad primaria diferente (Okuda *et al.* 1968, 1978, Expósito 1997, Marín *et al.* 2004, Quintero *et al.* 2004, Márquez *et al.* 2007), los parámetros biológicos evaluados sobre *E. argenteus* sugieren poco efecto de estas diferencias sobre la tasa de crecimiento y la condición nutricional en dicha especie.

Por otra parte, las diferencias expuestas entre las localidades estudiadas dentro de la Bahía de Mochima, reflejan la posible interferencia de factores locales sobre la tasa de crecimiento y la condición nutricional de los estadios iniciales de *E. argenteus.* Entre los factores particulares del hábitat que pudieran explicar las diferencias encontradas entre las

localidades de la Bahía de Mochima, pudieran destacarse la presión de depredadores, temperatura, concentración y composición de presas, turbidez, efecto antropogénico, tal como han sido propuestos para otras regiones (Gibson 1994, Meekan *et al.* 2003, Gilliers *et al.* 2006, Sponaugle *et al.* 2009, Sponaugle *et al.* 2010). Específicamente al sur de la Bahía de Mochima, y muy cercana a la Ensenada Mochimita, se encuentra un sistema de tratamiento de aguas residuales que descarga directamente al mar, de forma tal que a esta área junto a sus adyacencias se le ha caracterizado como un ambiente mesotrófico - eutrófico por las altas concentraciones de nutrimentos y un elevado crecimiento del plancton (Expósito 1997, Narváez 2011).

Al mismo tiempo, en zonas muy cercanas a la laguna de oxidación se han encontrado diversas concentraciones de ciertos metales (datos aún no publicados, suministrados por el Laboratorio de Ecofisiología, IOV-UDO), lo cual pudiera explicar los valores elevados de la relación ARN/ADN en los individuos de la Ensenada Mochimita, puesto que en caso de exposición de contaminantes, un incremento en las concentraciones de ARN pudiera relacionarse a la inducción del sistema de desintoxicación proteico, tal y como lo reportaron Fonseca *et al.* (2009) en evaluaciones experimentales sobre juveniles de *Solea senegalensis* a diferentes concentraciones de cobre. De esta forma los juveniles de *E. argenteus* pudieran estar presentando una movilización de proteínas musculares destinada a compensar el efecto de factores estresantes que posiblemente estén causando las descargas de la laguna de oxidación cercana.

Evaluaciones futuras deberían incrementar el número de localidades dentro de las zonas de estudio, así como también realizar experimentos de crecimiento y condición nutricional en condiciones controladas que permitan dilucidar los patrones de crecimientos somáticos y del otolito, así como también las condiciones nutricionales de los individuos, bajo diferentes condiciones de temperatura, alimento, contaminantes e incluso por presión de depredadores.

AGRADECIMIENTOS

Al Instituto Oceanográfico de Venezuela de la Universidad de Oriente por el apoyo prestado para la realización de esta investigación. A María Elena Amaro, Luis Ariza, Adriana López, Marlin Medina, Lisette Molins y José L. Prin por la colaboración prestada durante el trabajo de campo y laboratorio. Igualmente, los autores desean agradecer a evaluadores anónimos que realizaron aportes interesantes en la elaboración del artículo.

RESUMEN

Con la finalidad de evaluar la asociación de índices de crecimiento en estadios tempranos de peces marinos, se estimó la tasa de crecimiento somático y las condiciones fisiológicas de *Eucinostomus argenteus* en dos zonas del nor-oriente venezolano: Bahía de Mochima y Golfo de Cariaco. La edad y el crecimiento fueron estimados basados en análisis de otolitos sagitta. Las condiciones fisiológicas fueron evaluadas por medio de las concentraciones de proteínas y la relación ARN/ADN, empleando técnicas espectofotométricas y fluorométricas sobre tejido muscular. Las relaciones entre tallas con la edad y el diámetro de los otolitos resultaron positivas, significativas y ajustadas a un modelo de regresión lineal. Los valores de la tasa de crecimiento reciente oscilaron entre 0.178 y 0.418mm día^{-1}, la tasa de crecimiento retrocalculado varió entre 0.295 y 0.393mm día^{-1}, y la tasa ARN/ADN osciló entre 1.65 y 6.97. No se registraron diferencias entre las zonas de estudio, sin embargo se reportaron diferencias entre localidades. A pesar de no encontrarse correlación entre la tasa de crecimiento y la relación ARN/ADN, los valores reportados sugieren crecimiento positivo de los individuos silvestres en las localidades evaluadas. No obstante, ciertas localidades mostraron valores que indican pobres condiciones nutricionales, pudiendo afectarse a futuro otras tasas vitales.

Palabras claves: Gerreidae, tasa de crecimiento, condición nutricional, otolitos, ARN/ADN.

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Comparación de la abundancia, estructura de tallas y fecundidad de *Voluta musica* (Caenogastropoda: Volutidae) en tres sitios de la costa norte de la Península de Araya, Venezuela

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Recibido 20-VII-2011. Corregido 13-XII-2011. Aceptado 20-XII-2011.

Abstract: Abundance, size structure and fecundity of *Voluta musica* (Caenogastropoda: Volutidae) in three sites of the north coast of Araya Peninsula, Venezuela. Considering the intensive artisanal fishing activity and the consequent carrion discard found at Isla Caribe, in relation to other two sites with no intensive artisanal fishing activity, we expect different effects on some features of *V. musica* life history (larger egg capsules, larger organisms, higher abundance of adult organisms). In this paper we compare some population parameters of *Voluta musica* at three localities in the north coast of the Araya Peninsula in Venezuela under different fishing exploitation regimes. The samples were taken monthly during 2008 and 2009 at Isla Caribe, Isla Lobos and Bajo Cuspe. At each site, samples were taken within three areas of 40m^2. The abundance of *V. musica* ranged between 5 ind/120m^2 to 30 ind/120m^2 with significant differences between sites ($F=7.77$; $p<0,01$). Organisms from Isla Caribe were larger in size ($p=0,045$), than those in the other two sites. There is a significant differences in the number of egg capsules between sites and between months, and there is clear evidence that Isla Caribe has the largest abundance of egg capsules ($p<0,01$) suggesting that the extra feeding source (carrion) found at Isla Caribe could have a positive effect on the reproductive potential of the *V. musica* population at this site. Rev. Biol. Trop. 60 (Suppl. 1): 165-172. Epub 2012 March 01.

Key word: Volutidae, artisanal fisheries, egg capsules, fecundity, South Caribbean.

Voluta musica (Linné, 1758) es un gasterópodo marino endémico del Caribe sur que se encuentra en el Libro Rojo de la Fauna Venezolana en la categoría "Insuficientemente conocido" (Rodríguez & Rojas-Suárez 2008). Es una especie gonocórica, de desarrollo directo, que adhiere sus ovicápsulas al substrato, usualmente en la parte interna de conchas vacías de bivalvos (e.g., *Anadara* sp., *Atrina* sp., *Pinna* sp.). Las cápsulas son hemi-esféricas y miden aproximadamente 18mm de diámetro basal. Hay de 3 a 5 embriones dentro de cada cápsula, los cuales se alimentan del fluido intracapsular (Gibson-Smith 1973, Penchaszadeh & Miloslavich 2001).

Isla Caribe, Isla Lobos y Bajo Cuspe son tres sitios ubicados al noreste de la Península de Araya donde se hallan poblaciones de *V. musica*. Isla Caribe (10°41'19"N; 63°51'05"W), es un pequeño islote donde existe un asentamiento permanente de pescadores artesanales con un tráfico marino relativamente alto llevado a cabo por seis botes de 6m de eslora cada uno, adicionalmente es una zona de paso de otras embarcaciones pesqueras y turísticas. Los mismos diariamente realizan faenas de pesca, descartando especies no comerciales de peces, bivalvos y cangrejos en zonas someras de la playa. Cabe destacar que los botes utilizados están pintados con pinturas antiincrustantes,

que sumado a los desechos de los motores (hidrocarburos) son un factor de contaminación que afecta a los caracoles produciendo imposex en hembras de *V. musica* (A. C. Peralta, en prep.). Isla Lobos (10º41'26"N; 63º52'28"W), es otro pequeño islote localizado a 2km del primero. El ecosistema marino es semejante al de Isla Caribe, pero el tráfico marino es de bajo a nulo ya que al sitio llega un bote de 6m de eslora por semana y actualmente no hay asentamientos, botes permanentes ni descartes diarios de pesca, donde A. C. Peralta (en prep.) encontraron una baja incidencia de imposex de 4%. Bajo Cuspe (10º43´53´´N; 63º51´10´´W), es un sitio que se encuentra a 3km de Isla Caribe e Isla Lobos y no presenta botes permanentes ni descartes diarios de la pesca artesanal y en el cual no se han registrado casos de imposex (A.C. Peralta, en prep.) (Observ. pers.).

En Isla Caribe, la presencia de la pesca artesanal con frecuencia diaria genera una fuente alóctona de alimento por descarte pesquero, el cual es aprovechado por las especies carroñeras (gasterópodos, crustáceos, peces, etc.). *V. musica* tiene tanto hábitos carroñeros como depredatorios (von Cosel 1976), bajo este escenario se pretende estudiar el efecto que tendría una fuente adicional de alimento sobre algunos rasgos de la historia de vida en las poblaciones de esta especie. Esta mayor disposición de alimento podría estar relacionada con una abundancia mayor, hembras de tallas mas grandes y un mayor numero de cápsulas por hembra en relación a Isla Lobos y Bajo del Cuspe donde no existe tal fuente de alimento y en donde *V. musica* se alimenta principalmente por depredación, con un mayor costo energético (A.C. Peralta, obs. pers.).

El objetivo del presente trabajo es conocer la abundancia, estructura de talla y fecundidad de *V. musica* en tres sitios de la costa norte de la Península de Araya en las que existen actividades de pesca diferenciales.

MATERIALES Y MÉTODOS

Se realizaron muestreos mensuales entre marzo de 2008 y diciembre de 2009 en Isla Caribe, Isla Lobos y Bajo Cuspe. En cada sitio se delimitaron tres transectas de 20m x 2m ($40m^2$) paralelas a la línea de costa sobre la zona habitada por *V. musica*, a una profundidad de 1,5 m para Isla Caribe e Isla Lobos y a nueve metros en Bajo Cuspe. Se contaron todas las ovicápsulas y los individuos encontrados a lo largo de cada transecta y se determinó la talla y el sexo de cada uno. Sabiendo que las hembras comienzan a madurar a los 57mm de longitud de la concha (A.C. Peralta, en prep) se registró la proporción de hembras mayores a 60mm en cada sitio y en cada mes, con el fin de realizar una estimación indirecta de la fecundidad como una relación entre el número de ovicápsulas encontradas/hembra en cada mes. Se evaluaron las diferencias en la abundancia de hembras maduras a lo largo de los meses del año a través de un análisis de la varianza.

Se registró mensualmente la temperatura en los tres sitios de muestreo mediante sonda digital *YSI 85* y como complemento se utilizó la serie de tiempo de la base de datos del Observatorio Oceanográfico Digital de Venezuela (Klein, E. & J.C. Castillo, 2009) para establecer las épocas de surgencia (temperatura del agua de 23ºC a 26ºC) y relajación (temperatura del agua de 27ºC a 30ºC).

Una vez obtenidos los datos del número de ovicápsulas, número de especímenes, tallas y sexo se aplicó un análisis de la varianza (ANOVA) para cada una de las variables (abundancia total, abundancia de hembras mayores a 60mm, fecundidad y estructura de tallas) con la finalidad de encontrar diferencias significativas entre los tres sitios muestreados. En algunos casos se realizó una prueba *a posteriori* de Tukey para verificar cuál de los sitios estaría generando las diferencias.

RESULTADOS

Las poblaciones de *Voluta musica* demostraron tener una densidad de 0,09ind/m^2 (±7,99) en Isla Caribe; 0,04 ind/m^2 (±3,68) en Isla Lobos y de 0,10 ind/m^2 (±10,64) en Bajo Cuspe. La abundancia varió de 5 a 30ind/$120m^2$ dependiendo del sitio y de la fecha en que se

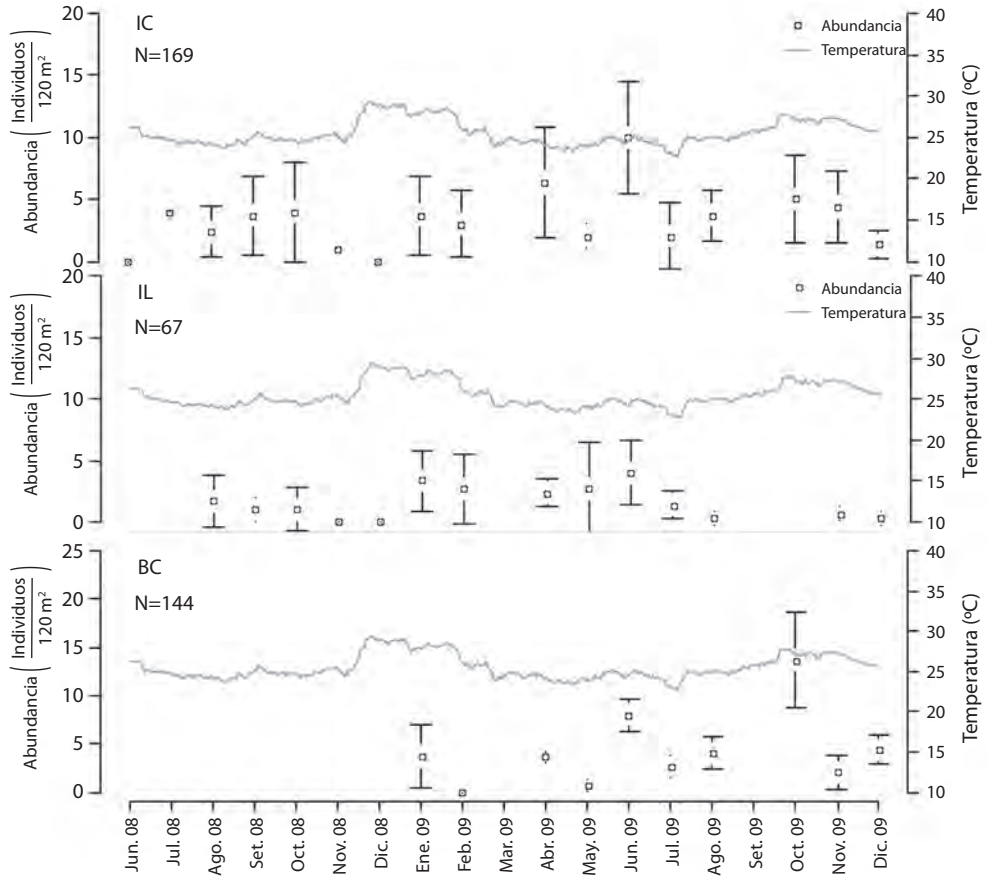

Fig. 1. Comparación de la abundancia de *V. musica* en los tres sitios de muestreo a lo largo de dos años (2008-2009). IC: Isla Caribe; IL: Isla Lobos; BC: Bajo Cuspe.
Fig 1. Comparison of *V. musica* abundance in three sites during 2008-2009. IC: Isla Caribe; IL: Isla Lobos; BC: Bajo Cuspe.

tomaron las muestras (Fig. 1). De acuerdo al análisis de la varianza existen diferencias significativas en la abundancia entre los sitios ($F=7.77$; $p<0.05$) y entre los meses del año a excepción de Isla Lobos ($p<0.05$).

La prueba *a posteriori* mostró que la abundancia es igual para Bajo Cuspe e Isla Caribe (Tukey, $p=0.39$) pero distinta y menor en Isla Lobos ($p=0.008$; 0.0149).

El análisis de la abundancia de hembras maduras (mayores a 60mm de largo) demostró que existen diferencias significativas entre los tres sitios y entre los meses del año ($p<0,05$) (Fig. 2). Igualmente, existen diferencias significativas entre los sitios y entre los meses en cuanto al número de ovicápsulas ($p<0,001$), siendo Isla Caribe el sitio que genera la diferencia (Tukey, $p<0,001$) con un mayor numero de ovicápsulas en relación a los otros dos sitios (Fig. 3).

La fecundidad de *V. musica* estimada no evidenció diferencias significativas entre sitios ni entre los meses ($p>0,5$). Los datos indican que la fecundidad de *V. musica* varió de 1 a 32 en Isla Caribe mientras que en Isla Lobos fue de 1 a 12 y en Bajo Cuspe de 1 a 8 ovicápsulas/hembra/mes (Fig. 3).

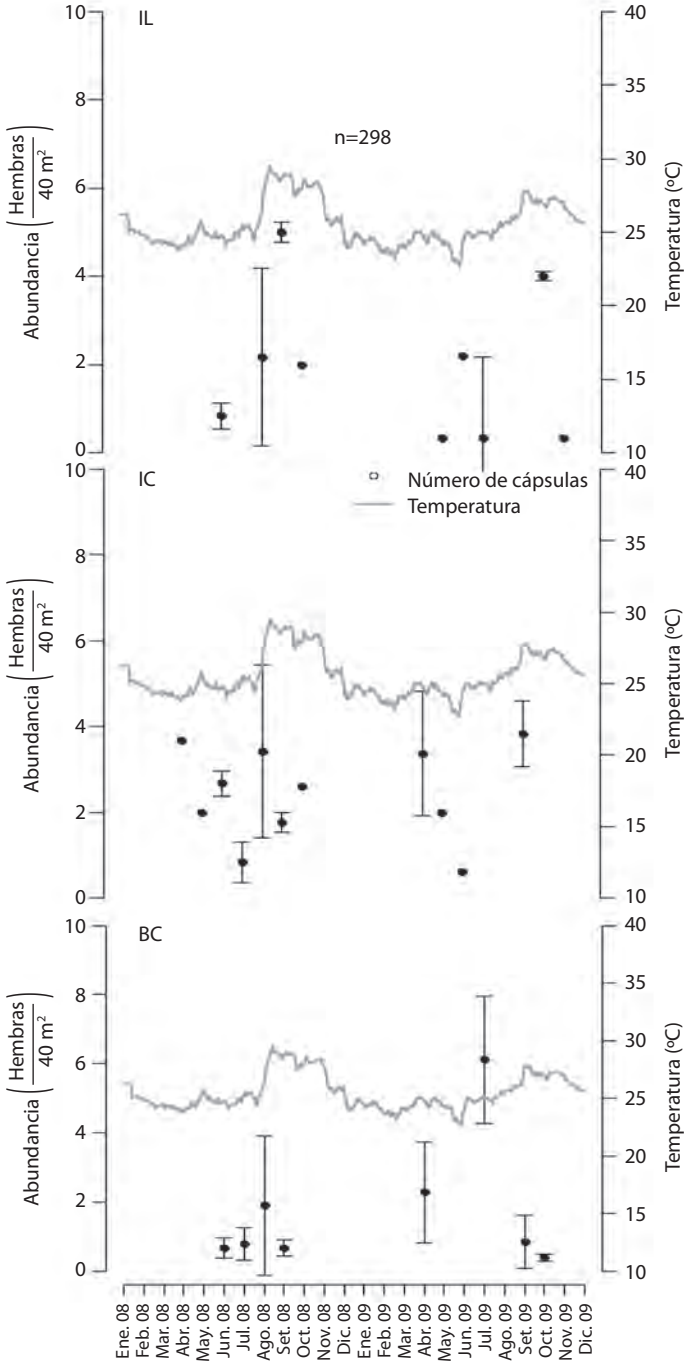

Fig. 2. Abundancia de hembras maduras en tres sitios a lo largo de dos años. IC: Isla Caribe; IL: Isla Lobos; BC: Bajo Cuspe.
Fig. 2. Abundance of mature female specimens in three sites during two years. IC: Isla Caribe; IL: Isla Lobos; BC: Bajo Cuspe.

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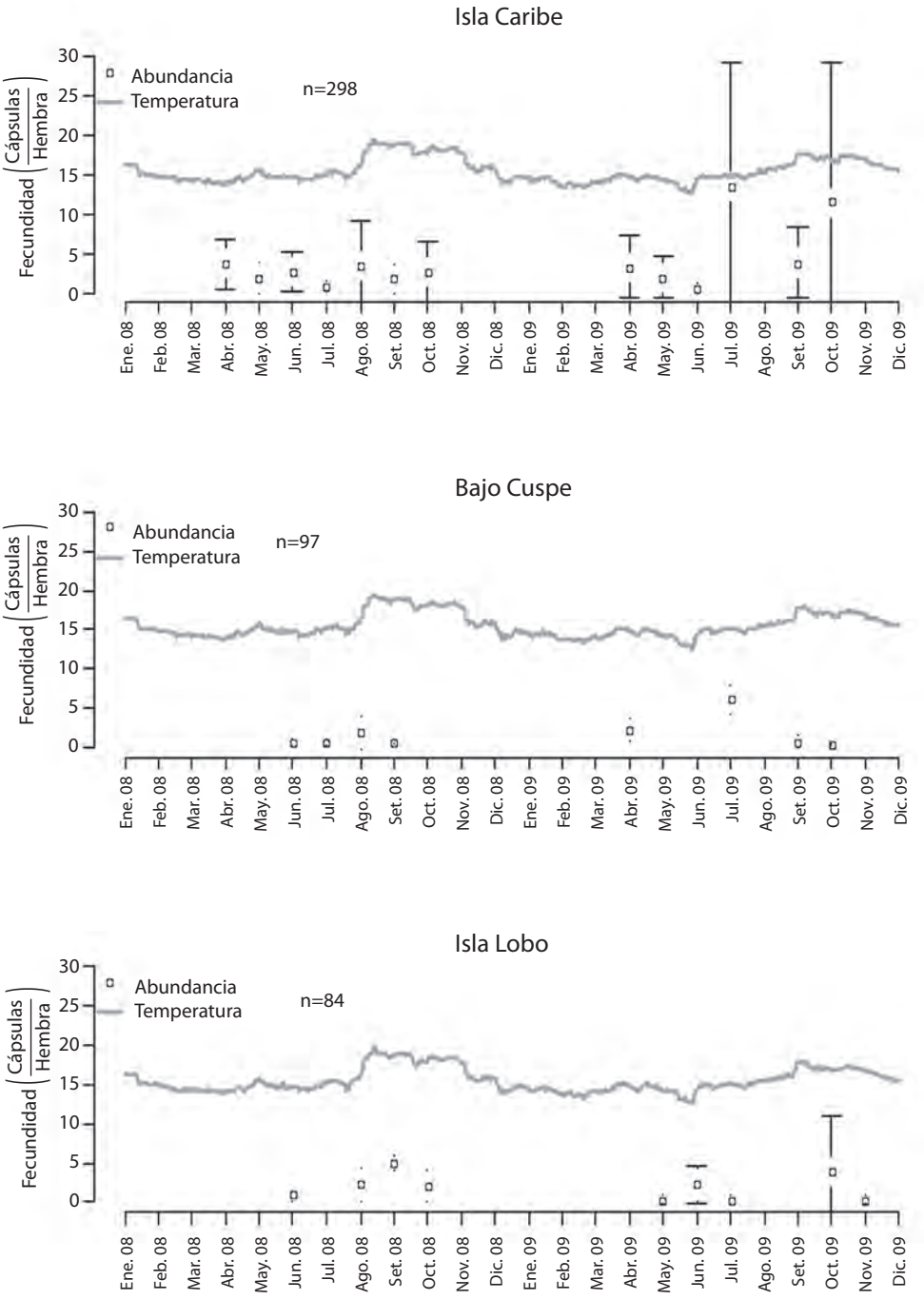

Fig. 3. Número de ovicápsulas por hembra/mes en tres sitios.
Fig. 3. Ovicapsules per female/month in three sites.

La talla de los individuos medida como largo total de la concha varió de 38,7 a 95mm en Isla Caribe, de 34 a 88mm en Isla Lobos, y de 34,4 a 89mm en Bajo del Cuspe. La talla promedio del largo total de la concha de la población, sin distinción de sexos, fue de: 70,6mm en Isla Caribe; 67,25mm en Isla Lobos y de 68,4mm en Bajo del Cuspe. La comparación de tamaño entre los sexos en cada uno de los sitios indica que los machos son significativamente mas pequeños que las hembras ($p<0,05$). No se hallaron individuos menores a 30mm de largo total, salvo uno cuya talla fue de 9mm, encontrado en Isla Caribe (Fig. 4). Se encontraron diferencias significativas en relación al tamaño de los individuos entre los tres sitios ($p<0.05$). En una prueba *a posteriori* se obtuvo que los individuos de Isla Caribe son

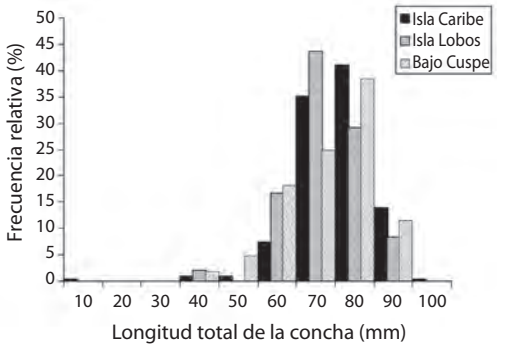

Fig. 4. Estructura de tallas de *V. musica* en tres sitios.
Fig. 4. *V. musica* size structure in three sites.

más grandes en cuanto a la longitud la concha (Tukey, IC >BC >IL, p=0.34; 0.39 y 0.045).

DISCUSION

Se conoce poco sobre la densidad y abundancia de caracoles de la familia Volutidae, en el caso de *Odontocymbiola magellanica,* de las costas norpatagónicas, se ha demostrado que a pesar de pasar gran parte del tiempo enterrada tiene cierta capacidad de desplazamiento. No existen grandes bancos en las zonas donde se halla esta especie, sin embargo se encuentran agregaciones de individuos en las épocas reproductivas donde la densidad se calculó en 0,09ind/m^2 (18ind/200m^2) siendo la densidad en épocas no reproductivas de 0,015ind/m^2 (3indiv/200m^2) (Bigatti 2005). Por otro lado Carranza *et al.* (2008) analizaron la distribución y ecología de gasterópodos de la plataforma continental uruguaya y la zona exterior del Río de la Plata, en profundidades desde 4 a 62m donde el volútido *Adelomelon beckii* fue representado por un solo espécimen en todos los casos, sugiriendo bajas densidades poblacionales para la zona de estudio. En el presente trabajo las poblaciones de *V. musica* también demostraron tener bajas densidades de individuos lo que sugiere que las especies de la familia Volutidae tienen distribuciones amplias con pocos individuos, pero que tienen tendencia a agruparse en épocas del año donde ocurren los eventos de cópula y oviposición. Tal y como se demuestra en el presente trabajo, *V. musica* presentó diferencias en cuanto a su densidad en función de los meses del año en dos de los sitios. Cuando analizamos la densidad de las hembras maduras se observa una tendencia a incrementar el número de individuos por área en relación a los meses de oviposición: abril, mayo, junio, agosto, septiembre y octubre (ver Fig. 3).

Hay una clara estacionalidad en la oviposición en los tres sitios muestreados siendo los meses de abril a octubre los meses donde las hembras oviponen, lo que a su vez coincidiría con los meses de aguas más cálidas. El número de ovicápsulas totales fue mayor en Isla Caribe. Sin embargo, el número de ovicápsulas por hembra por mes dentro de cada sitio mostró una alta variabilidad, por lo que el resultado del análisis de la varianza no evidencia diferencias significativas entres los sitios ni entre los meses. Las diferencias en el número de cápsulas entre las áreas muestreadas dentro de cada sitio/mes nos estaría indicando que hay otro factor que determina la ubicación de las ovicápsulas y que éstas no se encuentran presentes en toda el área muestreada. Varios autores han descrito las cápsulas de *V. musica* las cuales son

colocadas dentro de conchas vacías de bivalvos (Clench & Turner 1970, Gibson-Smith 1973, von Cosel 1976, Penchaszadeh & Miloslavich 2001). En algunos casos se encontraron cochas vacías de *Atrina seminuda* con hasta 30 ovicápsulas adheridas mientras que otras especies de bivalvos como *Arca zebra*, *Codakia sp,* y *Perna perna*, contenían de dos a cuatro ovicápsulas adheridas. Para un próximo estudio, se sugiere considerar el tipo de sustrato disponible para la oviposición en cada uno de los sitios con el fin de minimizar las diferencias que esto podría generar en las determinaciones de abundancia de ovicápsulas dentro de cada mes/sitio.

La estructura de tallas que se obtuvo incluye organismos mayores a 34mm lo cual se podría explicar por el hecho de que son organismos crípticos, viven enterrados en el sustrato y solamente exhiben el sifón inhalante apenas unos milímetros fuera del sedimento. Es sabido que la mayoría de los volútidos viven enterrados emergiendo en busca de alimento o en el momento de reproducirse (Clench & Turner 1964, Clench & Turner 1970, Poppe & Goto 1992), esto hace que sea muy difícil encontrar individuos pequeños con la metodología aplicada.

Morton (2006) describe que en ciertas localidades de Hong Kong en donde existe una pesca artesanal intensiva, el murícido *Ergalatax contractus* es mas abundante que en otros lugares en donde no existe tal actividad, siendo éste un depredador generalista que ocasionalmente consume carroña. Los gasterópodos carroñeros parecen verse favorecidos por los ambientes perturbados (Britton & Morton 1994, Taylor 1994, Morton 1995, Ramsay *et al.* 1997, 1998, Morton 2006) debido a una mayor disponibilidad de alimento en forma de carroña. En el presente trabajo se observan individuos de *V. musica* de tallas más grandes en Isla Caribe y con mayor número de oviposturas, probablemente debido a que los individuos de esa población aprovechan la energía que provee el material de desecho de la pesca artesanal compuesto principalmente por pescado, moluscos y crustáceos. Otros trabajos que han reportado a *V. musica* como especie carrroñera son von Cosel (1976) y A.C. Peralta (en prep.), este último determinó que esta especie sea un depredador generalista que presenta hábitos carroñeros, ante la presencia de los descartes de la actividad pesquera. Estos resultados se asemejan a lo observado en los volútidos *Adelomelon ancilla* (Bigatti *et al.* 2009) y *O. magellanica* de la Patagonia argentina (Bigatti *et al.* 2010), donde se observó que consumen principalmente presas vivas y ocasionalmente carroña (Bigatti *et al.* 2010).

AGRADECIMIENTOS

Centro de Investigaciones Ecológicas de Guayacán (Universidad de Oriente) por el apoyo en las salidas de campo. A Eduardo Klein por su valiosa orientación en el análisis de los datos y Emanuel Valero por su colaboración en el uso de paquetes estadísticos. Al Decanato de Investigación y Desarrollo-USB, y al Decanato de Postgrado-USB por el financiamiento otorgado.

RESUMEN

Teniendo en cuenta la intensa actividad pesquera artesanal y subsecuente fuente adicional de alimento como carroña en Isla Caribe, se esperaría un efecto sobre algunos parámetros poblacionales de *V. musica* como: mayor número de ovicápsulas, individuos de tallas mayores y densidades de caracoles adultos mayores. Con el presente trabajo se desea conocer la abundancia, estructura de talla y fecundidad de *Voluta musica* en tres sitios de la costa norte de la Península de Araya en las que existen actividades de pesca diferenciales. Se realizaron muestreos mensuales entre 2008 y 2009 en Isla Caribe, Isla Lobos y Bajo Cuspe, en cada uno con 3 áreas de 40m^2. La abundancia varió de 5ind/120m^2 a 30ind/120m^2, con diferencias significativas entre los sitios ($F= .77$; $p<0,01$), siendo igual para Bajo Cuspe e Isla Caribe ($p=0,39$) pero distinta y menor en Isla Lobos, ($p=0,008$; 0,0149). Los individuos de Isla Caribe demostraron ser más grandes ($p=0,045$). Existen diferencias significativas entre sitios y entre meses en el número de ovicápsulas ($p<0,01$), siendo Isla Caribe el sitio con mayor abundancia de ovicápsulas ($p<0,01$). Esto sugiere que el alimento suplementario en forma de carroña podría incrementar el potencial reproductivo de la población en Isla Caribe.

Palabras claves: Volutidae, pesca artesanal, ovicápsulas, fecundidad, Caribe Sur.

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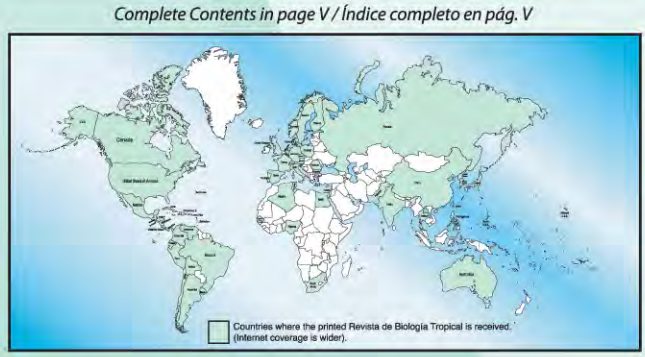

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