4-1-2014

Increase of Excavating Sponges on Caribbean Coral Reefs: Reproduction, Dispersal, and Coral Deterioration

Andia Chaves-Fonnegra
Nova Southeastern University, andiachaves@gmail.com

NOVA SOUTHEASTERN UNIVERSITY OCEANOGRAPHIC CENTER

INCREASE OF EXCAVATING SPONGES ON CARIBBEAN CORAL REEFS: REPRODUCTION, DISPERSAL AND CORAL DETERIORATION

By

Andia Chaves Fonnegra

Submitted to the Faculty of
Nova Southeastern University Oceanographic Center
in partial fulfillment of the requirements for
the degree of Doctorate of Philosophy

Nova Southeastern University

April 2014

Dissertation of
Andia Chaves Fonnegra

Submitted in Partial Fulfillment of the Requirements for the Degree of

Doctorate of Philosophy:

Marine Biology/Oceanography

Nova Southeastern University
Oceanographic Center

April 2014

Approved:

Dissertation Committee

Major Professor :_____
Jose V. Lopez, Ph.D.

Committee Member :_____
Mahmood Shivji, Ph.D.

Committee Member :_____
Bernhard Riegl, Ph.D.

Committee Member :_____
Sven Zea, Ph.D.

Dedicated to

My Mom, Gary and Sebastián

My family, friends, and mentors who put all their love, support, dedication, and trust in me.

And

a teacher that trusted I will learn English one day

AKNOWLEDGEMENTS

This dissertation has been like making a big puzzle, and it has been possible thanks to all of you:

The opportunity and mentor. To my supervisor, Joe Lopez, for giving me the opportunity and support to work in his lab. For his open mind and trust.

The financial support. To the UNESCO L´Orèal Fellowship for Young Women in Science, the Billing Fishing Tournament Scholarship - NSU, COLCIENCIAS Colombian Scholarship - LASPAU, Broward Women Club Scholarship - NSU, Presidential Grant - NSU. To Olguita Barrios and Juanito Marquez for being the first loan institution. To Shanoor, Camilo, Leonardo, and aunt Clemencita for supporting my finances. To Mom´s and Gary´s love-science fund.

The microlab team. To the NSU Marine Microbiology and genetics laboratory, especially to Ewelina, Crystal, Karita, Patty, Naoko, Jigu, Dawn, Emily, Alex, Shira, Chuck and Becky for their teaching, support, great lunches, feedback, patience, and birthday cakes. To the pre-med undergraduate students, and to my wonderful volunteer students: Samia, Paola, Jesse, Shira and Renne, for their immeasurable help. Thanks for the great moments, and for testing my teaching skills with all of you.

The perfect collaboration. To Sven Zea for being the light in the darkest moments. To Kevin Feldheim for guiding me down the population genetics path. To Manuel Maldonado for helping me to understand the reproductive biology of sponges. To Pat Blackwelder for teaching me all about electron microscopy. To Bernard Riegl for teaching me how to model populations. To Jesse Secord for your incredible assistance in the lab. To all of you for being always there, any moment, any time.

The technical assistance. To Mamhood Shivji for his suggestions and recommendations. To the Conservation Biology and Genetics Laboratory, especially to Andrea, Becka, Vince, Teagan, Alex, Jen, Christine and Kim, for their feedback on the technical questions about population genetics. To the Population Modeling - GSI - Coral Lab: Dusty, Beranda, Claire, Anesti, Alyson, Gwilym, and Chris, for their feedback. To the Coral Histology lab: Alyson, Abby, Adam, and Jenna, for teaching me new techniques. Special thanks to Pierre Duchesne for guidance with running and interpreting the results

from FLOCK program. To the Co-lab meetings´ members, especially to Nicole Fogarty and Brian Walker for their suggestions.

The field work teams. Dave Gilliam and all members of the OC Coral Reef Restoration and Monitoring Laboratory (CRRAM), especially Liz, Mao, Paola, Stephanie, Alyson, Jenna, Ari, Ian, Cody, and Chuck for including me in your busy monitoring schedule in Florida, and for sharing the seawater temperature and transect data. To Keri for helping me to collect sponge samples during a long year in Broward. To Rob Ruzicka and his team, for taking me to the Middle Keys and helping me to obtain samples in that area.

Joe Pawlik, for the opportunity to join your team in Key Largo, and one of your cruises to the Bahamas. To Tse-Lynn, Jan, Coquito, Lyndsey, John, Inga, Sven and Micha Ilan for sharing your passion about sponges and science, and the memorable moments. To Sven Zea, Universidad Nacional de Colombia and Banda-Dive Shop for their support at San Andrés Island, Colombia.

To Malcom Hill for taking me to the east of Looe Key. To The Porifera Tree of Life Project (The Portol), Bob Thacker, Cristina Diaz, and the Smithsonian Sponge Taxonomy course, for giving me the opportunity to do field work in Panamá. To Ben Müller for sending me samples from Curacao. To Elizabeth Mclean, Klaus Rützler, and all scientists and staff at Carry Bow Cay, for helping me to include Belize in my sampling, and making the experience unforgettable.

The energy. To Swim Fort Lauderdale Masters Team, for their friendship and making me feel part of Florida, and opening a fantastic world to me. To the marvelous water world and its creatures.

The love. To my Mom, Gary, Grandma and Sebastián, and to all my wonderful family and friends for their love and support. To Graeme, for your love and enthusiasm about my studies.

Thank you ☺

TABLE OF CONTENT

GENERAL ABSTRACT

Coral reefs ecosystems are deteriorating and facing dramatic changes. These changes suggest a shift in dominance from corals to other benthic organisms. Particularly in the Caribbean Sea, with corals dying, sponges have become the leading habitat-forming benthic animals. However, little is known about what life-history traits allow organisms to proliferate in a marine system that is undergoing change. Thus, the objective of this dissertation was to try to understand the current increase of encrusting excavating sponges on deteriorating Caribbean coral reefs through the study of reproduction, recruitment and dispersal potential of the widely distributed and currently expanding species, *Cliona delitrix*. Different methodological approaches were used, such as histology, electron microscopy, quantification of sponges in the field, genetics, and mathematical modeling. Results are presented in four different chapters. It was found that *Cliona delitrix* has an extended reproductive cycle in Florida, USA, from April - May to around November - December depending on a >25°C sea-water temperature threshold. *C. delitrix* gametogenesis is asynchronous and it has multiple spawning events. *C. delitrix* is recruiting abundantly on Caribbean coral reefs, preferentially on recent coral mortality than on old coral mortality. The increase in *C. delitrix* and other excavating sponges can be explained by the repeated spawning and by the coincidence in time and space of larval production with the availability of new dead coral, which tend to overlap during the warmest months of the year. Eggs or larvae of *C. delitrix* appear to survive enough to be transported by currents over larger distances. It was found that dispersal ranges for *Cliona delitrix* may reach as far as ~ 315 km in the Florida reef track, and over ~ 971 km in the South Caribbean Sea, between Belize and Panama. Thus, reproduction, dispersal, and recruitment patterns of *C. delitrix* along with oceanographic currents, and eddies that form at different periods of time, are sustaining the spread of this sponge on coral reefs. According to mathematical models carried out, *C. delitrix* increase on reefs fluctuates depending of coral mortality events and available space on old dead coral (colonized by algae and other invertebrates). However, under temperature anomalies, these sponges will

tend to increase and take over the reef system only if heat stress and coral mortality is moderate. Under massive mortality events both corals and sponges will tend to decline, although sponges at a slower rate than corals. In general, coral excavating sponges have been favored by coral mortality, especially during past few decades. However as bioeroders, their success is also limited by the success of calcifying corals. In a reef management context and based on this dissertation's findings, it is suggested that excavating sponges, and especially *Cliona delitrix*, should be more formally included in reef monitoring programs. Their increase can be used to track coral mortality events on reefs (past and future), and also can be used as another major bioindicator of health on coral reefs.

Keywords: life-history traits, coral reefs death, excavating sponges, climate change, bioindicator.

GENERAL INTRODUCTION

In the last three decades coral reef ecosystems have been deteriorating as a result of overfishing, pollution and climate change (Hughes et al. 2003, Knowlton and Jackson 2008, Knowlton 2012). The most critical result of climate change is an increased frequency of bleaching and disease in corals (Glynn 1993, Hoegh-Guldberg 1999) and other marine organisms (Harvell et al. 1999, Lafferty, Porter, and Ford 2004). Increases of as little as 1°C in sea temperature have led to mass coral bleaching and death in most marine tropical areas (Hoegh-Guldberg 1999). Because corals are able to adapt to and survive limited temperature increases, some recovery from bleaching has been observed (West and Salm 2003). In fact, if a coral recovers its associated zooxanthellae within 4 to 6 months after a bleaching event, it will survive (Diaz-Pulido & McCook, 2002). However, most evidence suggests that climate change has exceeded the ability of corals to acclimate and adapt, and that reefs around the world will continue to decline (Gardner et al. 2003). Thus, reef ecosystems are facing dramatic changes.

What are the implications of such changes at an ecosystem level? If corals are dying and leaving free substratum, what kinds of organisms might colonize and perhaps dominate coral reef landscapes? Rapid colonization by algae is a widely-documented phenomenon following bleaching events (Glynn 1993, Hoegh-Guldberg 1999), extreme low tides (Fishelson 1973), crown of thorn starfish predation (Price 1975), and mechanical injuries (Meesters and Bak 1993). Colonization starts with thin algal turfs that later are replaced by a succession of other algae (Diaz-Pulido and McCook 2002). Besides algae, marine sponges are abundant, competitive and able to take advantage of coral death. As an example, the encrusting excavating sponge *Cliona tenuis* (recorded as *C. caribbaea*) has increased in abundance throughout Caribbean wave-swept fore-reef zones after Acroporid corals massively died from disease and bleaching (Cortés et al. 1984). Also, *C. tenuis* spreads more rapidly on Belizean reefs during the warm relative to cool seasons (1.3 - 3.1 versus 0.9 - 1.4 mm · day^{-1}) (Rützler 2002). Weil (2002) predicted that

zooxanthellate encrusting excavating sponges will succeed in competition for space with corals, as reefs continue to be affected by climate change. However, sponges are stalled to completely takeover corals, as their lateral growth is limited to solid and illuminated substratum. Also, some corals can escape by upward growth (López-Victoria and Zea 2005, López-Victoria, Zea, and Weil 2006).

In general, sponges are successful competitors for space through chemical and physical adaptations that help them to dominate reefs (Diaz and Rützler 2001). They are able to modify the structure of coral reefs through bioerosion, calcification or cementation, impacting and changing coral cover (Díaz and Rützler 2001). In addition, most sponge species are able to survive in different environments and can tolerate pollution better than corals, owing to their heterotrophic filtering feeding capacity (Reiswig 1971). Other sponge characteristics that can modify the environment where they live include: secretion of secondary metabolites that can alter water quality, mediation in primary production and nitrification through complex symbioses with other organisms, and conversion of dissolved organic matter into food for reef consumers (Díaz and Rützler 2001, Becerro 2008, de Goeij et al. 2013).

Particularly in the Caribbean Sea, sponges have a high diversity, even surpassing all coral groups combined. Abundance (area coverage) reaches up to 24 % on light-exposed hard substrata reef habitats and 54 % in cryptic habitats (Díaz and Rützler 2001). Sponge biomass in the Caribbean can exceed those values for corals and algae (Rützler 1978), reaching up to five or six times greater than that Great Barrier Reef sponges (Wilkinson and Cheshire 1990, 1989). Consequently, the Caribbean Sea is a good scenario in which to study and predict coral reef traits with respect to sponges as competitors. Why are sponge abundances higher and increasing in some areas? Specifically, why do excavating sponges appear to take advantage of deteriorated or stressed coral reefs?

At least 24 sponge species (18 clionaids, 5 oceanapiids and 1 aplysinellid) constitute the excavating sponge assemblage in the Caribbean Sea. Clionaids: *Cliona amplicavata, C. aprica, C. arenosa, C.barbadensis, C. caribbaea, C. celata, C. cribrosa, C. delitrix, C.*

dioryssa, C. euryphylla, C. flavifodina, C. janitrix, C. lampa, C. laticavicola, C. lobata, C. macgeachii, C. millepunctata, C.paucispina, C. peponacea, C. schmidti, C. spirilla, C. suberea, C. truitti, C. undulatus, C.vastifica, C. vermifera, C. viridis and *C. tenuis;* Oceanapiids: *Siphonodictyon (=Aka) coralliphagum*, *S. brevitubulatum, S. siphonum, S. cachacrouense* and *S. jamaicensis;* Aplysinellid: *Suberea flavolivescens* (Díaz and Rützler 2001; Van Soest and Zea, personal communication, Pang 1973, Hofman and Kielman 1992). Some of these excavating sponges are among the most important coral reef framework bioeroders, and can be responsible for up to 90 % of total boring in live and dead coral heads (MacGeachy and Stearn 1976). Those species which simultaneously encrust and excavate limestone are able to displace and kill live coral tissue (Rützler 2002, López-Victoria, Zea, and Weil 2006).

Among encrusting excavating sponges, *Cliona delitrix,* which lacks associated photosynthetic zooxanthellae, is one of the most destructive species to reef corals. This sponge is able to excavate 10 - 12 cm deep inside coral skeletons and spread laterally at mean rates of ~1.5 cm $\cdot$ y^{-1} (Chaves-Fonnegra and Zea 2011), completely overpowering massive live corals (Chaves-Fonnegra and Zea 2007, Pang 1973). Currently, *C. delitrix* is one of the most conspicuous sponges in the Florida Keys (Ward-Paige et al. 2005), as well as in other areas in the Caribbean Sea (Rose and Risk 1985, Chaves-Fonnegra, Zea, and Gómez 2007). Another encrusting excavating sponge without zooxanthellae, common in Florida and the Bahamas, is *Pione lampa*. This species can penetrate 8 - 10 cm inside the calcareous substrata at a rate equivalent to removing the upper 1 cm-layer of surface per year, and may produce 5 - 6 kg of fine grained carbonate detritus per 1 m^2 in 100 days (Neumann 1966).

Cliona delitrix and Pione lampa have increased their abundance, individual sizes and cover (2 -, 3 -, and 7 - fold, respectively) from 1996 to 2001 in the Florida Keys National Marine Sanctuary. In parallel, stony coral species' number and cover have declined (Ward-Paige et al. 2005). Furthermore, an extensive coral reef monitoring program found that *C. delitrix* cover has also increased 7.7 $cm^2 \cdot m^{-2}$ from 2003 to 2007 in the reef areas from Miami Dade County to Palm Beach County (Commission, Institute, and Center

2008). Excavating sponges increase in the Florida Keys Natural Sanctuary, and in general in the Caribbean Sea, is directly associated with human pollution (Ward-Paige et al. 2005, Rose and Risk 1985, Holmes 1997, Chaves-Fonnegra, Zea, and Gómez 2007).

In the last three decades, cover of encrusting excavating sponges has increased considerably, especially in areas that have experienced massive death of coral tissue from diseases, bleaching and hurricanes (López-Victoria and Zea 2004, Cortés et al. 1984, Rützler 2002). Moreover, *Cliona orientalis*, a zooxanthellate excavating sponge from the Great Barrier Reef, appears more bleaching-resistant than other coral reef organisms (Schönberg et al. 2008, Schönberg and Suwa 2007). It can redistribute its zooxanthellae in a diel rhythm; zooxanthellae are most concentrated and closest to the sponge surface during the day and widely distributed and drawn into the sponge tissue during the night. The ability to move zooxanthellae thus appears to increase bleaching-resistance, at least in this sponge species (Schönberg and Suwa 2007, Schönberg et al. 2008).

Knowledge about excavating sponges in the Caribbean Sea is focused on rates of erosion, abundances, sizes, lateral extension rates, cover, and their changes in relation to sewage pollution. But, no information exists about population level traits such as reproductive timing, recruitment, long term growth, survivorship, and dispersion of these sponges. Thus, considering that corals are being drastically affected by temperature increases and pollution (Hughes et al. 2003), it is important to better understand the mechanisms of sponge dispersal and colonization and how drastic are the implications of these sponge populations at reef community and ecosystem levels.

Thus, the aim of this dissertation was to characterize and better understand the current increase of encrusting excavating sponges on Caribbean coral reefs through the study of the reproduction, recruitment and dispersal potential of one widely distributed and currently expanding species, *Cliona delitrix*. On a wider scope, this study attempts to understand what life-history traits allow an organism to proliferate in a system that is undergoing change. This document has been divided into four chapters, and each of them corresponds to independent manuscripts. Chapter 2 has been submitted for publication

and the other three chapters are now in preparation to be submitted for eventual publication.

-

References

Becerro, M.A. . 2008. "Quantitative trends in sponge ecology research." *Marine Ecology* 29:167-177.

Chaves-Fonnegra, A., and S. Zea. 2007. "Observations on reef coral undermining by the Caribbean excavating sponge *Cliona delitrix* (Demospongiae, Hadromerida)." In *Porifera research: biodiversity, innovation and sustainability*, edited by M.R. Custódio, G. Lôbo-Hajdu, E. Hajdu and G. Muricy, 247-254. Rio de Janeiro: Museu Nacional.

Chaves-Fonnegra, A., and S. Zea. 2011. "Coral colonization by the encrusting excavating Caribbean sponge *Cliona delitrix*." *Marine Ecology* 32:162-173.

Chaves-Fonnegra, A., S. Zea, and M.L. Gómez. 2007. "Abundance of the excavating sponge *Cliona delitrix* in relation to sewage discharge at San Andrés Island, SW Caribbean, Colombia." *Boletín de Investigaciones Marinas y Costeras* 36:63-78.

Commission, Florida Fish and Wildlife Conservation, Fish and Wildlife Research Institute and the National Coral Reef Institute, and Nova Southeastern University Oceanographic Center. 2008. Southeast Florida Coral Reef Evaluation and Monitoring Project 2007 Year 5 Final Report.: 35.

Cortés, J., M. Murillo, H.M. Guzmán, and J. Acuña. 1984. "Pérdida de zooxantelas y muerte de corales y otros organismos arrecifales en el Caribe y Pacífico de Costa Rica." *Revista de Biología Tropical* 32:227-231.

de Goeij, J.M., D. van Oevelen, M.J. Vermeij, R. Osinga, J.J. Middelburg, A.F. de Goeij, and W. Admiraal. 2013. "Surviving in a marine desert: the sponge loop retains resources within coral reefs." *Science* 342 (6154):108-110.

Diaz-Pulido, G., and L.J. McCook. 2002. "The fate of bleached corals: patterns and dynamics of algal recruitment." *Marine Ecology Progress Series* 232:115-128.

Díaz, M., and K. Rützler. 2001. "Sponge: an essential component of Caribbean coral reefs." *Bulletin of Marine Science* 69 (2):535-546.

Diaz, M.C., and K. Rützler. 2001. "Sponges: an essential component of Caribbean coral reefs." *Bulletin of Marine Science* 69 (2):535-546.

Fishelson, L. 1973. "Ecological and biological phenomena influencing coral-species composition on the reef tables at Eilat (Gulf of Aqaba, Red Sea)." *Marine Biology* 19:183-196.

Gardner, T.A., Coté I.M., J.A. Gill, A. Grant, and A.R. Watkinson. 2003. "Long-term region-wide declines in Caribbean corals." *Science* 301 (5635):958-960.

Glynn, P.W. 1993. "Coral reef bleaching: ecological perspectives." *Coral Reefs* 12:1-17.

Harvell, C.D., K. Kim, J.M. Burkholder, R.R. Colwell, P.R. Epstein, D.J. Grimes, E.E. Hofmann, E.K. Lipp, A.D.M.E. Osterhaus, R.M. Overstreet, J.W. Porter, G.W. Smith, and G.R. Vasta. 1999. "Emerging marine diseases climate links and anthropogenic factors." *Science* 285:1505-1510.

Hoegh-Guldberg, O. 1999. "Climate change, coral bleaching and the future of the world´s coral reefs." *Marine and Freshwater Research* 50:839-866.

Hofman, C.C., and M. Kielman. 1992. "The excavating sponges of the Santa Marta area, Colombia, with description of a new species." *Bijdragen tot de Dierkunde* 61 (4):205-217.

Holmes, K.E. . 1997. "Eutrophication and its effect on bioeroding sponge communities." Proc 8th Int Coral Reef Symp, Panamá.

Hughes, T.P., A.H. Baird, D.R. Bellwood, M. Card, S.R. Connolly, C. Folke, Hoegh-Guldberg Grosberg, O., J.B.C. Jackson, J. Kleypas, J.M. Lough, P. Marshall, M. Nyström, S.R. Palumbi, J.M. Pandolfi, B. Rosen, and J. Roughgarden. 2003. "Climate Change, Human Impacts, and the Resilience of Coral Reefs." *Science* 301 (5635):929-933.

Knowlton, N. 2012. "Iconic coral reef degraded despite substantial protection." *Proceedings of the National Academy of Sciences USA* 109 (44):17734-17735.

Knowlton, N. , and J.B.C. Jackson. 2008. "Shifting baselines, local impacts, and global change on coral reefs." *PLoS Biol* 6 (2):e54. doi:10.1371/journal.pbio.0060054.

Lafferty, K.D., J.W. Porter, and S.E. Ford. 2004. "Are diseases increasing in the ocean?" *Annual Reviews of Ecology, Evolution, and Systematics* 35:31-54.

López-Victoria, M., and S. Zea. 2004. "Storm-mediated coral colonization by an excavating Caribbean sponge." *Climate Research* 26:251-256.

López-Victoria, M., and S. Zea. 2005. "Current trends of space occupation by encrusting excavating sponges on Colombian coral reefs." *Marine Ecology* 26:33-41.

López-Victoria, M., S. Zea, and E. Weil. 2006. "Competition for space between encrusting excavating Caribbean sponges and other coral reef organisms." *Marine Ecology Progress Series* 312:113-121.

MacGeachy, J.K., and C. Stearn. 1976. "Boring by macro-organisms in the coral *Montastraea annularis* on Barbados reefs." *Int Revue ges Hydrobiology* 61:715-745.

Meesters, E.H., and R.P.M. Bak. 1993. "Effects of coral bleaching on tissue regeneration potential and colony survival." *Marine Ecology Progress Series* 96:189-198.

Neumann, A.C. 1966. "Observations on coastal erosion in Bermuda and measurements of the boring rate of the sponge, *Cliona lampa*." *Limnology & Oceanography* 11 (1):92-108.

Pang, R.K. 1973. "The systematics of some Jamaican excavating sponges (Porifera)." *Postilla* 161:1-75.

Price, I.R. 1975. "The development of algae on the skeletons of reef-building corals." In *Crown-of-thorns starfish seminar proceedings*, 181-191. Canberra: Australian Government Publishing Service.

Reiswig, H. 1971. "*In situ* pumping activities of tropical Demospongiae." *Marine Biology* 9 (1):38-50.

Rose, C.S., and M.J. Risk. 1985. "Increase in *Cliona delitrix* infestation of *Montastraea cavernosa* heads on an organically polluted portion of the Grand Cayman." *P.S.Z.N.I. Marine Ecology* 6 (1):345-363.

Rützler, K. 1978. "Sponges in coral reefs." In *Coral Reefs: research methods. Monographs in oceanographic methodology*, edited by D.R. Stoddard and R.E. Johannes, 299-313. Paris: UNESCO.

Rützler, K. 2002. "Impact of crustose clionid sponges on Caribbean reef corals." *Acta Geologica Hispánica* 37 (1):61-72.

Schönberg, C.H.L. , and R. Suwa. 2007. "Why bioeroding sponges may be better hosts for symbiotic dinoflagellates than many corals." In *Porifera research: biodiversity, innovation and sustainability.*, edited by M.R. Custódio, G. Lôbo-Hajdu, E. Hajdu and G. Muricy, 569-580. Rio de Janeiro: Museu Nacional.

Schönberg, C.H.L., R. Suwa, M. Hidaka, and W.K. Weng Loh. 2008. "Sponge and coral zooxanthellae in heat and light: preliminary results of photochemical efficiency monitored with pulse amplitude modulated fluorometry." *Marine Ecology* 29:247-258.

Ward-Paige, C.A., M.J. Risk, O.A. Sherwood, and W.C. Jaap. 2005. "Clionid sponge surveys on the Florida Reef Tract suggest land-based nutrient inputs." *Marine Pollution Bulletin* 51:570-579.

Weil, E. 2002. "Sponge-induced coral mortality in the Caribbean. A potential new threat to Caribbean coral reefs." VI Internacional Sponge Conference, Genova.

West, J.M. , and R.V. Salm. 2003. "Resistance and resilience to coral bleaching: implications for coral reef conservation and management. ." *Conservation Biology* 17 (4):956-967.

Wilkinson, C., and A. Cheshire. 1989. "Patterns in the distribution of sponge populations across the central Great Barrier Reef." *Coral Reefs* 8 (1):127-134.

Wilkinson, C., and A. Cheshire. 1990. "Comparasion of sponge populations across the Barrier Reefs of Australia and Belize: evidence for higher productivity in the Caribbean." *Marine Ecology Progress Series* 67 (1):285-294.

CHAPTER 1: REPRODUCTIVE DYNAMICS OF THE CORAL EXCAVATING CARIBBEAN SPONGE *Cliona delitrix*

Abstract

Cliona delitrix is one of the most abundant and destructive coral-excavating sponges on Caribbean reefs. However, basic aspects of its reproductive biology, which largely determine the species propagation potential, remain unknown. We conducted a two year study of the reproductive cycle and gametogenesis of a *C. delitrix* population located in a shallow reef in Florida (USA). Mesohyl (tissue) from tagged and randomly collected sponge individuals was sampled one to several times a month for two years, and analyzed by light microscopy to identify, count, and measure reproductive structures. Transmission electron microscopy (TEM) was used to describe the cytology and ultrastructure of gametogenesis. The relationship between reproductive activity and seawater temperature and moon cycle were examined. *C. delitrix* is oviparous and gonochoric, except for a few simultaneous hermaphroditic individuals. Different stages of gametogenesis were described. Granulose cells were found as nurse cells and phagocytized by oocytes of different sizes, thus contributing to the growth and maturation of female gametes. In late oogenesis, granulose cells formed an envelope around each mature oocyte. Spermatogenesis started by transdifferentiation of choanocytes, with nurse cells also involved in this initial process. The *Cliona delitrix* reproductive cycle in Florida, USA, runs from April - May to around November- December depending on whether water temperature reaches above 25 °C. At a population level, oogenesis and spermatogenesis, occurred in pulses and were asynchronous among individuals. Within individual females, developing oocytes of different cohorts co-occurred. In the males, transdifferentiation of choanocytes into spermatic cysts occurred different times during the reproductive cycle. Mature eggs and spermatic cysts appear to be spawned simultaneously during both full and new moons. Unlike other oviparous sponges, the reproductive cycle of *C. delitrix* does not culminate with a single highly synchronous pulse of gamete spawning each year.

Rather three to five successive spawning events occurred during summer months. This extended spawning suggests a reproductive strategy that decreases the risk of massive offspring mortality during local adverse events, and that may increase the chances for larvae to find corals with recent mortality, its preferred attachment substratum.

Introduction

Over the past three decades, the abundance of excavating sponges has increased, particularly in areas affected by pollution (Rose and Risk, 1985; Holmes, 1997; Ward-Paige et al., 2005; Chaves-Fonnegra et al., 2007), coral mortality and hurricanes (López-Victoria and Zea 2004). These sponges can settle and excavate different calcium carbonate substrata (Wells, Wells, and Gray 1964), and kill coral tissue (Chaves-Fonnegra and Zea 2007). Excavating sponges also can grow quickly in warmer temperatures (Rützler 2002, Cortés et al. 1984, Weil 2002) and increase their boring rates at lower pH (Duckworth and Peterson 2013, Wisshak et al. 2012). These abilities make them very strong space competitors on reefs, especially during expected scenarios of warmer and more acidic environments.

Among excavating sponges, *Cliona delitrix* (Hadromerida, Demospongia), is one of the most destructive to Caribbean reef corals. It is able to excavate 10 - 12 cm deep cavities inside coral skeletons and spread laterally at mean rates of ~1.5 cm $\cdot$ y^{-1} (Chaves-Fonnegra and Zea 2011), eroding the three dimensional structure of massive live corals, and growing on much of the external surface of the coral head (Pang 1973a, Rützler 2002, Chaves-Fonnegra and Zea 2007). Currently, *C. delitrix* is one of the most conspicuous sponges in the Caribbean Sea (Ward-Paige et al. 2005, Commission, Institute, and Center 2008, Chaves-Fonnegra, Zea, and Gómez 2007). Along with *Pione lampa, Cliona delitrix* has increased its abundance, individual sizes, and cover approximately 2 -, 3 -, and 7- fold, respectively, from 1996 to 2001 in the Florida Keys National Marine Sanctuary. At the same time, species number and cover of stony corals

have declined in the area (Ward-Paige et al. 2005). Furthermore, an extensive coral reef monitoring program found that *C. delitrix* cover has also increased 7.7 $cm^2 \cdot m^{-2}$ from 2003 to 2007 in the reef areas from Miami Dade County to Palm Beach County, USA (Commission, Institute, and Center 2008).

To understand the on-going expansion of excavating sponges in the Caribbean Sea, and the potential ecological and environmental implications, it is important to evaluate the strategies these sponges use for successful propagation and the conditions which facilitate this. Therefore, a fundamental step in assessment is to determine the species' reproductive biology, which remains poorly understood to date.

Both seasonal and year round reproduction occur in sponges, and the process has been suggested to be modulated by several environmental variables, with the longest periods of active reproduction tending to occur in environments where warm temperatures and sufficient food availability are relatively constant. In contrast, in areas of strong seasonal temperature changes, reproductive periods are shorter (Maldonado and Riesgo 2008). Temperature shifts may trigger or accelerate gametogenesis, embryogenesis and larval release (Maldonado and Riesgo 2008). In the case of oviparous sponges, lunar phase (Fell 1983) and tides have also been suggested to trigger spawning events (Usher et al. 2004).

Information about the reproductive cycles of sponges in the Greater Caribbean Sea (including the Caribbean Sea, Gulf of Mexico, Florida, Bahamas) is scarce, and is available mostly for viviparous species (e.g. Leong and Pawlik 2011, Maldonado and Young 1996). In Florida, reproduction occurs during the months with warmer water temperatures: March to September, being highest between May and October, and peaking in July and August (Leong and Pawlik 2011, Maldonado and Young 1996).

Excavating sponges of the genus *Cliona* are known to be oviparous (Lévi 1956, Pomponi and Meritt 1990, Nassonow 1883, Piscitelli et al. 2011) (Table 1). However, oocytes can be fertilized internally before expulsion into the water column (Bautista-Guerrero, Carballo, and Maldonado 2013, Maldonado and Riesgo 2008). Viviparity has only been reported in temperate *Cliona lobata* (Topsent 1900). In general, for both oviparous and viviparous sponges, oocytes grow progressively and early oocytes present variable shapes and pseudopodia, whereas late-stage oocytes tend to be spherical, enclosed in a thin envelope of primarily collagen surrounded by a layer of parent cells, also called "nurse cells" (Maldonado and Riesgo 2009, Maldonado 2009). In *C. celata* these are granular cells (Piscitelli et al. 2011, Warburton 1961). Reabsorption of developing oocytes in *Cliona* spp. can occur, particularly in winter (Piscitelli et al. 2011). Egg size in *Cliona* spp. is relatively small, reaching a maximum average diameter of 78.8 µm in *C. viridis,* 40 µm in *C. celata*, 76 µm in *C. tenuis* and 60 µm in *C. vermifera* (Piscitelli et al. 2011, González-Rivero 2011, Bautista-Guerrero, Carballo, and Maldonado 2013). As in other sponges, *Cliona* oocytes typically contain a nucleus with one large nucleolus, and cytoplasmatic inclusions such as yolk granules, lipids, and inclusions with heterogeneous content, and clusters of mitochondria in the cytoplasm (Ereskovsky 2010). Eggs can be isolecithal or oligolecithal, and inclusions may enter the cytoplasm by phagocytosis of whole mesohyl cells or their fragments, phagocytosis of symbiotic bacteria, pinocytosis by condensation and transformation of mitochondrial clusters into yolk granules, and by authosynthesis (Ereskovsky 2010, Riesgo and Maldonado 2009a, Sciscioli et al. 2002). Oocytes in other sponges of the order Hadromerida may originate from archaeocytes of the mesohyl. However an ultrastructural study of the Hadromerid *Suberites massa* found that oocytes originated from choanocytes, with the possibility that large archaeocytes could transdifferentiate from choanocytes (Ereskovsky 2010).

Characterizing spermatogenesis of oviparous sponges can be difficult because of the relatively short time span, from days to weeks (Maldonado and Riesgo 2008). Therefore, investigation of spermatogenesis in the genus *Cliona* remains incomplete. Previous work with *C. celata* and *C. viridis* found that spermatic cysts occur at a high density over a

period of time as short as a week (Piscitelli et al. 2011) and can reach a diameter of 100 µm and 140 µm respectively. Similarly, in *C. vermifera* cysts can be as large as 120 µm. Alternatively, *C. tenuis* spermatic cysts exhibit the fairly small diameter of 21 µm (González-Rivero 2011). The only study in the order Hadromerida devoted to the origin of male gametes showed that spermatocytes development in *Suberites massa* occurs by transdifferentiation of choanocytes, and that not all components in one cyst are developmentally synchronous (Diaz and Connes 1980). In contrast, in *Cliona tenuis* male gametes develop synchronously inside the spermatic cyst (González-Rivero 2011).

Hermaphroditism and gonochorism occur in the family Clionaidae (Maldonado and Riesgo 2008). Specifically the genus *Cliona* exhibits contemporaneous hermaphroditism, in which spermatic cysts coexist with oocytes during the short period of time of spermatogenesis, although both gamete types occur at different locations inside the sponge (Piscitelli et al. 2011). In gonochoric species such as *C. vermifera* females are about 3:1 times more frequent than males (Bautista-Guerrero, Carballo, and Maldonado 2013).

Although fertilization may take place internally either in the sponge tissue or in the exhalant canals (Rosell 1993, Piscitelli et al. 2011), embryogenesis in oviparous sponges is external: eggs are released and embryonic development leads to a free-swimming larval stage (Maldonado and Riesgo, 2008). Thus, collecting fertilized eggs and larvae remains elusive, contributing to the lack of knowledge of embryonic development in oviparous *Cliona*. Cleavage has been observed *in vivo* in *Cliona viridis* (Maldonado and Riesgo 2008) and *C. celata*, in which parent nurse cells (granular cells) move into the clefts between the blastomeres without dividing (Warburton 1961). These granulose cells are present in the mature larvae as well, but without their typical cytoplasmic granules (Warburton 1961). Only larvae from a few *Cliona* spp. have been described to date, and there has been controversy and possible misinterpretation about its description. Initial studies were not clear about which type of larvae, amphiblastulla or parenchymella, *C.*

stationis and *C. celata* had (Brien 1973, Lévi 1956). Later, *Cliona viridis* larvae was described as parenchymella with a short planktonic phase (Mariani, Uriz, and Turon 2000, Mariani, Piscitelli, and Uriz 2001). However, after Maldonado and Berquist (2002) the term amphiblastulla is only valid for Calcarea sponges, and parenchymella refers to a solid larva that develops from a solid embryo (esteroblastula). As *Cliona* larvae develops form a hollow embryo that later fills with maternal cells, it is currently defined as clavablastula (Maldonado 2006, Maldonado and Bergquist 2002).

The reproductive cycle of *Cliona* and *Pione* excavating sponges has been described in five species: *Cliona celata* and *C. viridis* in Porto Cesareo, Italy, Mediterranean Sea (Piscitelli et al. 2011); *Pione truitti* in Chesapeak Bay, USA, Western Atlantic (Pomponi and Meritt 1990); *C. tenuis* in Glover's Reef Atoll, Belize, Caribbean Sea (González-Rivero 2011) and *C. vermifera* in Isabel Island, Mexico, Pacific Ocean (Bautista-Guerrero, Carballo, and Maldonado 2013). In all of these species, annual changes in temperature may represent the main environmental variable influencing oogenesis and spermatogenesis. Also, gamete production in *Cliona* has shown to be synchronized between the individuals of a population, but with oogenesis lasting longer (months) than spermatogenesis (weeks) (Piscitelli et al. 2011, Pomponi and Meritt 1990, González-Rivero 2011). In temperate *C. celata* and *C. viridis*, oogenesis tends to be a nearly continuous process over the year, ceasing only for 1 to 4 months after spawning, while spermatogenesis is a very fast process, taking one week, and usually occurring in one main peak in May and coupled to oocyte maturation (Piscitelli et al. 2011). In the temperate *P. truitti,* oogenesis also occurs when water temperature increases, and extends for a shorter period from March to June. While spermatogenesis, rarely noticed, takes places during May and June (Pomponi and Meritt 1990). In contrast, oogenesis in the tropical *C. tenuis* occurs when water temperature is colder (in Belize: October to May, close to 26 °C), and until temperature start increasing (28 °C); spermatogenesis exhibited an ephemeral peak in June (González-Rivero 2011). Contrary to these patterns, in *C. vermifera* oogenesis and spermatogenesis occur together and have more than one peak

between July and November when maximum temperatures reach 28 - 30 °C (Bautista-Guerrero, Carballo, and Maldonado 2013).

Many sponges including excavating sponges of very different phylogenetic affiliations exhibit asexual reproduction as a common feature (Schönberg 2002). Gemmules have been found in *Siphonodictyon labyrinthicum, Cliona janitrix and Pione vastifica* in the Mediterranean Sea (Topsent 1900, Rosell and Uriz 2002); *Cliona annulifera* and *Thoosa investigatoris* in India (Annandale 1915); *Pione truitti* in North Carolina and Chesapeake Bay, USA (Wells, Wells, and Gray 1964, Pomponi and Meritt 1990), and *Pione lampa* in Bermuda and Florida (Rützler 1974, Schönberg 2002). Gemmules allow sponges to survive and disperse in harsh environments, such as those subject to extreme temperatures, desiccation, and unstable substrates (Schönberg 2002). Asexual reproduction by budding has also been observed in the excavating sponges *Pione vastifica* of New England (Hartman 1958) and *Cliona viridis* in the Mediterranean Sea (Rosell 1993, Rosell and Uriz 2002). In addition, asexual dispersal by fragmentation occurs in *Cliona celata* of Cape Cod (Cobb 1969, 1975), and in *Cliona tenuis* in the Caribbean Sea (López-Victoria and Zea 2004).

Studies of coral excavating sponge reproductive cycles and gametogenesis will help us to understand how these sponges disseminate, how human activities and environmental change may affect sponge abundance on reefs and its contribution to bioerosion. Considering these impacts, this study aims to determine the reproductive cycle and gametogenesis in the coral excavating sponge *Cliona delitrix*. The study is based on these hypotheses: 1) *C. delitrix* is an oviparous, hermaphroditic sponge similar to most other members of the genus *Cliona*; 2) this species has a single spawning pulse over one year, and 3) asexual reproduction by buds and gemmules is also a common strategy.

Table 1. Summary of available information about sexual reproduction in the *Cliona* species. NI: no information. He: Hermaphrodite; Go: gonochoric, ?: not verified. One species of *Pione*, previously considered *Cliona* is included.

Species	**Distribution**	**Gametic status**	**Reproductive time**	**Larval type**	**Substratum**	**Reference**
Cliona celata	North Atlantic Ocean	He	Oogenesis: almost year-round. Eggs are sticky and released in Summer. Spermatogenesis: May.	Parenchymella**?** Amphiblastula**?**	Oyster shells	(Warburton 1958) (Lévi 1956) (Brien 1973) (Piscitelli et al. 2011)
Cliona lobata	Gulf of Mexico	NI	NI	Clavablastula	Oyster shells	(Pang 1973b) (Maldonado 2006)
Cliona microstrongylata	Northwest Pacific. Sea of Cortés	NI	March. Eggs 70- 90µm.	NI	Oyster shells	(Carballo and Cruz-Barraza 2005)
Cliona stationis	Mediterranean Sea	NI	NI	Parenchymella**?** Amphiblastula**?**	NI	(Lévi 1956, Brien 1973)
Cliona tenuis	Caribbean Sea, Belize	Go**?**	Oogenesis: Oct-May Spermatogenesis: June**?**	NI	Branching and massive corals	(González-Rivero 2011)
Piona truitti (formerly of the genus *Cliona*)	Chesapeake Bay (USA)	?	Oogenesis: March-June. Spermatogenesis: May-June.	NI	Oyster shells	(Pomponi and Meritt 1990)
Cliona vermifera	Mexico, Pacific Ocean	Go	Oogenesis and spermatogenesis from July to November	NI. Only zygotes were observed	Branching corals	(Bautista-Guerrero, Carballo, and Maldonado 2013)
Cliona viridis	Mediterranean Sea	He	Oogenesis: almost year-round. Eggs released in spring. Larva develops 2 days after spawning. Spermatogenesis: May.	Parenchymella uniformly flagellated. Yellow≈350µm Phototaxis absent. Release in June	Calcareous and siliceous substrata	(Mariani, Uriz, and Turon 2000, 2006); (Piscitelli et al. 2011)

Methods

Study area and sampling

To determine the reproductive timing of *Cliona delitrix*, tissue samples (3 cm diameter x 1 cm high) were collected for two years from a population located on the inner reef of Broward County (26° 08´ 31.8´´ N; 80° 05´ 47.64´´ W), Florida, USA (Figure 1), at a depth of 18 - 22 m. For a description of the area see Banks et al., (2008). During SCUBA dives, a 3 cm in diameter steel corer and a hammer was used to extract sponge samples from coral skeletons. During the first year (October 2009 to September 2010), samples were collected both from four tagged individuals once a month and from 1 to 10 randomly located individuals once, twice, or three times a month, depending on how intense reproductive activity was inferred to be in the population from the microscopic study of the tissue sample in the preceding sampling. During the second year (October 2010 to September 2011) ten tagged individuals were sampled once a month, except during August, when samples were collected four times. Additionally, we randomly sampled 5 to 10 individuals from April to September, once in April, twice in May, July and September, and four times in June and August (See Appendix1.1, Table 1 for collection times). Tagged and un-tagged individuals were selected haphazardly and the size (i.e., exposed sponge surface) of each individual donor measured. Temperature was recorded every two hours from 2 hobo temperature loggers, placed on the reef at 20 m depth by the South East Florida Coral Reef Monitoring Project (SECREMP). Moon phases were obtained from the U.S. Naval observatory for each year (http://aa.usno.navy.mil/data/docs/MoonFraction.php.).

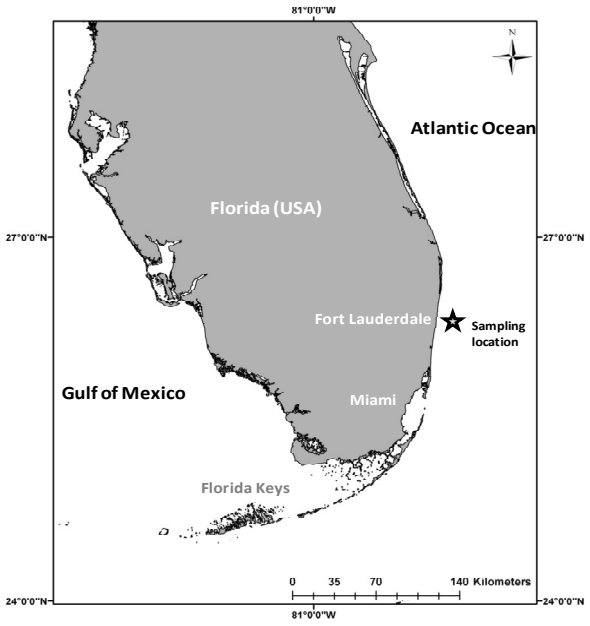


Figure 1. South Florida study area. The star marks the Broward County site where samples were collected for the two years of study.

Histology and light microscopy

All sponge tissue samples were transported immediately upon collection in ambient seawater to the laboratory and fixed for histology in Bouin´s fixative solution for 2 days. Then, samples were rinsed in distilled water (2 times per 15 min), and decalcified using a 10 % solution of HCl/EDTA for 3 days (modified from Renegar et al. 2008, see Appendix 1-B). After decalcification, samples were rinsed with three successive changes of distilled water (20 minutes each) and placed in 50 % ethanol (for at least 1 hour). To further dehydrate tissues, samples were placed in 70 % ethanol for 1 hour. Samples were then desilicified in 4 % hydrofluoric acid overnight at 4 °C, to remove spicules. On the following day, samples were rinsed in 70 % ethanol for 20 minutes, and then rinsed twice in 95 % ethanol for 15 min each, and in two changes of 100 % for 10 min. Fragments

were submerged in xylene for two changes of 15 min each, and placed in two changes of paraffin for 15 min each. Samples were then transferred to fresh paraffin, using a Sakura Tissue-Tek embedding center at the NSU - Oceanographic Center Coral Histology Lab. The paraffin blocks were placed on a cold plate to remove molds, and kept in the refrigerator at 4 °C in fresh water until sectioning. Sections 4 µm - thick were obtained on a Leica RM2125 microtome. Three sections were made, each separated by 1000 µm, and each was placed in a different slide. Thus, a total of three slides per each sample were obtained. All sections were stained with Heidenhain's, and some with Hematoxiline - Eosine for comparison. Although Heidenhain´s is a common stain for corals it is less commonly used for sponges, but it provided a good resolution of sponge structures such as cell nuclei (dark red), collagen and choanocytes (blue) and granulose cells (yellow) (See Appendix1.1 for protocol).

To estimate densities of gametes over time, two photographs (100 x) of each of the three sections per individual were taken. Photographs were at least 210 µm apart from each other to avoid overlapping areas and density overestimation. The area of suitable tissue varied among sections due to the "porosity" of the tissue and the holes left by the HCl-digested coral calcium carbonate. Thus, to obtain equivalent areas of tissue per picture, all pictures were contrasted on a white background using the program Corel Paintshop Pro X4, and a Matlab code was written to calculate the percentage of tissue, as the percentage equivalent to tissue (see Appendix 1.1).

Digital histological images were analyzed to measure and to count gametes and to study cytology. To compare different individuals, the number of reproductive structures of interest (i.e., gametes, embryos, etc) in the tissue were extrapolated to an area of 1 mm^2 in each photograph. Thus, the total tissue area microscopically sampled for each individual was the sum of the area of the 6 pictures taken (6 mm^2). The number of oocytes per mm^3 (O) of tissue was estimated applying the formula proposed by Elvin (1976) and used for sponge gametes estimations by Maldonado and Riesgo (2009):

$$O=(N(t/(d+t))F$$

where **N** is the number of oocytes or spermatocytes counted in the area of the histological sections with tissue per individual (in this case 6 mm^2); **t** is the thickness of section (4 µm), **d** is the average diameter of oocytes for each month, **O** is oocytes per mm^3 of tissue and **F** (**=41.67**) is the factor to convert the volume of the observation to 1 mm^3.

Electron microscopy

Subsamples from the same sponges collected for histology were simultaneously fixed for electron microscopy in 2 % glutaraldehyde in 0.05 M sodium cacodylate buffer prepared in filtered sea water to maintain osmolarity. Then, only some samples that contained reproductive elements in histological sections were rinsed three times for ten minutes each in 0.05 M sodium cacodylate buffer. Then, they were submerged in a post-fixative solution of 1 % osmium tetroxide for 45 minutes, followed by three ten-minute rinses in 0.05 M sodium cacodylate buffer. For dehydration, sponge samples were submerged in a graded series of ethanol at 20 %, 40 %, 60 %, 70 %, 90 % and then in 100 % for three changes of five minutes each. Following dehydration, samples were separated for transmission electron microscopy (TEM), and scanning electron microscopy (SEM) as discussed below.

For TEM, after dehydration samples were embedded in liquid Spurr resin, which was changed three times over a period of three hours. Samples were then placed in flat embedding molds and polymerized overnight at 60 °C in a stable temperature oven. Blocks were trimmed using GEM/STAR knifes to expose the area of tissue of interest. Blocks were then sectioned to 90 nm in a Porter Blum MT - 2 Ultramicrotome, fitted

with a diamond knife. To obtain membrane contrast, sections were placed on copper grids and stained with lead citrate (6 minutes) and uranyl acetate (10 minutes). Sections stained with lead citrate and / or uranyl acetate, and non-stained sections were observed in a Philips 300 TEM at the NSU Oceanographic Center, and micrographs taken with a JEOL JEM 1400 X at the Miller School of Medicine at the University of Miami. For SEM examination samples were dried in HMDS (hexamethyldisilazane) drying solution by rinsing three times for five minutes each. Samples were then placed in trays under the hood for 1 hour. Samples were left to outgas overnight. After approximately 24 hrs each sample was placed on a stub covered with an adhesive tab and sputter-coated with palladium in aTechnics II Sputter Coater. Coated samples were observed and images recorded in a FEI XL-30 ESEM FEG Scanning Electron Microscopy.

Cytological stages and numerical analyses

The development of oocytes and spermatic cysts was described. To avoid confusion in the text, oocyte development is referred as stages I - IV, whereas spermatic cysts development as cysts 1 - 4. To determine that sponge individuals maintained their gonochoric status throughout the year and did not switch sex, as successive hermaphrodites may do, the 10 individuals that were tagged during 2010-2011 were followed and analyzed.

To describe the reproductive cycle and to examine the potential relationship between gametogenetic activity and seawater temperature, we plotted the monthly density and percentages of oocyte and spermatocyte (number of structures mm^{-3}) versus monthly average temperature. Also, the Pearson or Spearman correlations (depending on the normal distribution of the values) were used to test if the density of oocytes and spermatic cells (transdifferentiated choanocytes) correlated with seawater temperature.

To calculate the diameter of reproductive structures, pictures of up to 15 reproductive structures (eggs, sperm or transdifferentiated choanocytes) were taken in each individual, and measured using Image J program (http://rsbweb.nih.gov/ij/index.html).

To evaluate gametogenesis in the population during the peak of reproductive activity prior to gamete release, samples of 20 to 36 sponges (random and tagged individuals) were collected weekly between August 10th and September 8th 2011. Also, individual analyses of tagged individuals were performed to check how many times an individual could engage in spawning, and to visualize in detail the paucity of gamete development over time. For female individuals, percentage of developmental stages were calculated based on the same $n \leq 15$ oocytes used to obtain diameters. For male individuals, we use density or transdifferentiated choanocytes and spermatic cysts to also estimate the time that transdifferentiated choanocytes (sperm cells) were present in each male individual.

Results

Oogenesis

The mechanism by which oocytes originate was not elucidated because it was not possible to capture early oocyte stage images in TEM. However, histological observations suggested oocytes likely originated either from archaeocytes or from choanocytes, as archaeocytes or very early oocytes were observed in the center of choanocyte chambers (Figure 2, A). When clearly formed but small oocytes appeared, choanocyte chambers around them were absent or disorganized (Figure 2, B). These small oocytes were defined as stage I (Table 2), and tended to have a round shape and a dark nucleus. At stage II, oocytes became oval with a nucleolus that stained bright red (Figure 2, C). The size of these oval oocytes was smaller than the initial round younger oocytes. At this stage they started to form pseudopodia becoming amoeboid in shape,

engulfing and likely phagocytizing granular cells, as both inclusions inside the eggs and inclusions inside granular cells look similar in TEM micrographs. Also, yolk and clusters of mitochondria were present in the cytoplasm (Figure 4, D - F). At stage III, the oocytes were larger and the yolk granules and inclusions moved to the peripheral cytoplasm, which appeared darker. Many granulose cells started to surround and enter the oocyte (Figure 3, A - C). At stage IV (Table 2), oocytes became completely circular in shape, and were surrounded by granular cells, all within an ample nesting cavity formed in the mesohyl (Figure 3, D - F). Neither embryos nor larvae were observed in any individual. Female individuals simultaneously contained cohorts of oocytes at different developmental stages. This suggests that oogenesis is asynchronous at the individual level. Observations on when different cohorts co-occurred in the females are quantitatively explained within the reproductive cycle section.

Table 2. Histological features of oogenesis stages in *Cliona delitrix*. Stage I: young oocytes during the first growth phase; Stage II: oocytes undergoing yolk accumulation; Stage III: oocytes about to complete yolk accumulation; stage IV: mature oocytes.

Stage I	Size: 10-45 µm (21.7 ± 7.3; n=170); shape: rounded; no accumulation of yolk granules, or some of them start to be present.
Stage II	Size: 11.2-79.9 µm (34.02 ± 10.6; n=1214); shape: oval to amoeboid with pseudopodia; yolk granules, lipids and inclusions with heterogeneous content are accumulated in the cytoplasm and granulose cells are in contact and being engulfed by the oocyte.
Stage III	Size: 23.8-97.2 µm (56.4 ± 11.0; n=585); shape: rounded, although still forming pseudopodia; yolk granules and inclusions only in peripheral cytoplasm; many granulose cells surrounding or inside the oocyte.
Stage IV	Size: 52.5 – 96.3 µm (73.5± 8.1; n=117); shape: completely round and surrounded with granular cells. Space between the mesohyl and the oocyte-granular cells is formed.

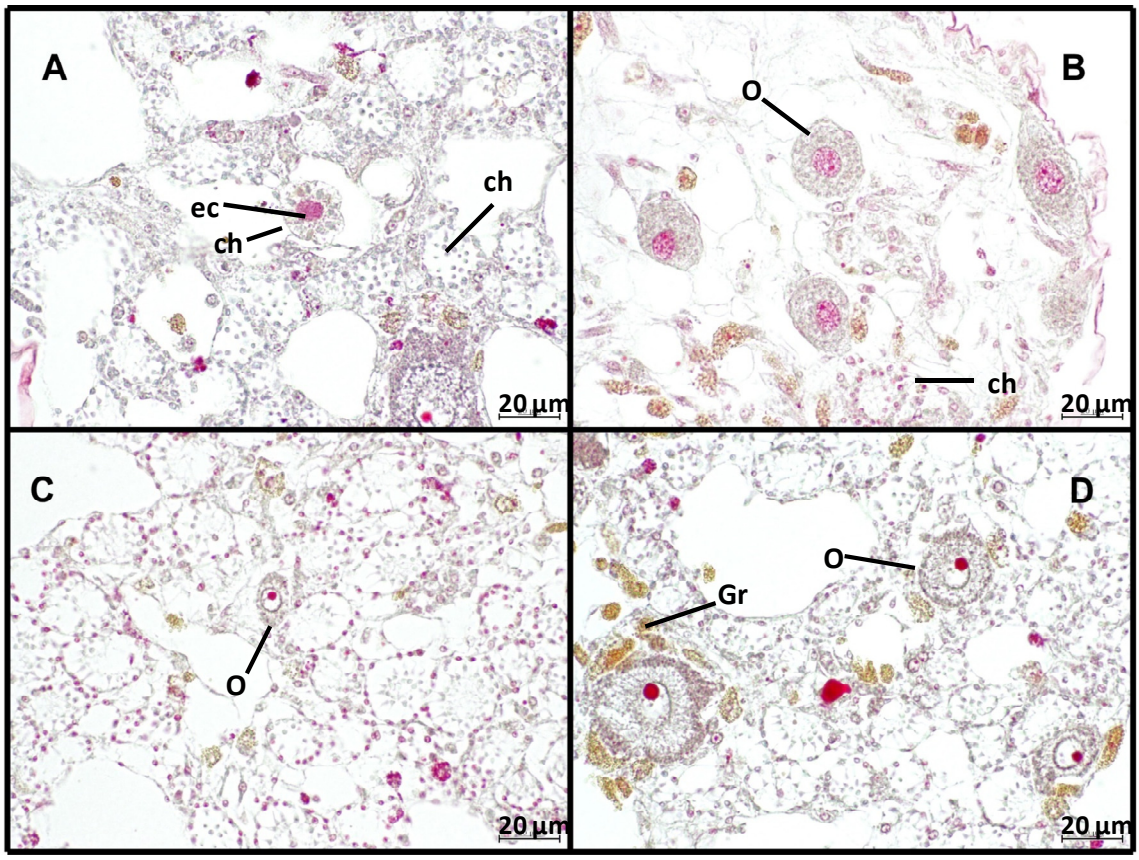


Figure 2. Oogenesis observed by light microscopy. A) Choanocyte chamber enclosing a cell in the center, probably an archaeocyte (ec); B) young oocytes during first growth phase in stage I; C) oval oocyte starting to accumulate inclusions, stage II. D) Oocytes in stages II and III. Abbreviations, ch: choanocytes chambers; Gr: granulose cell; O: oocyte.

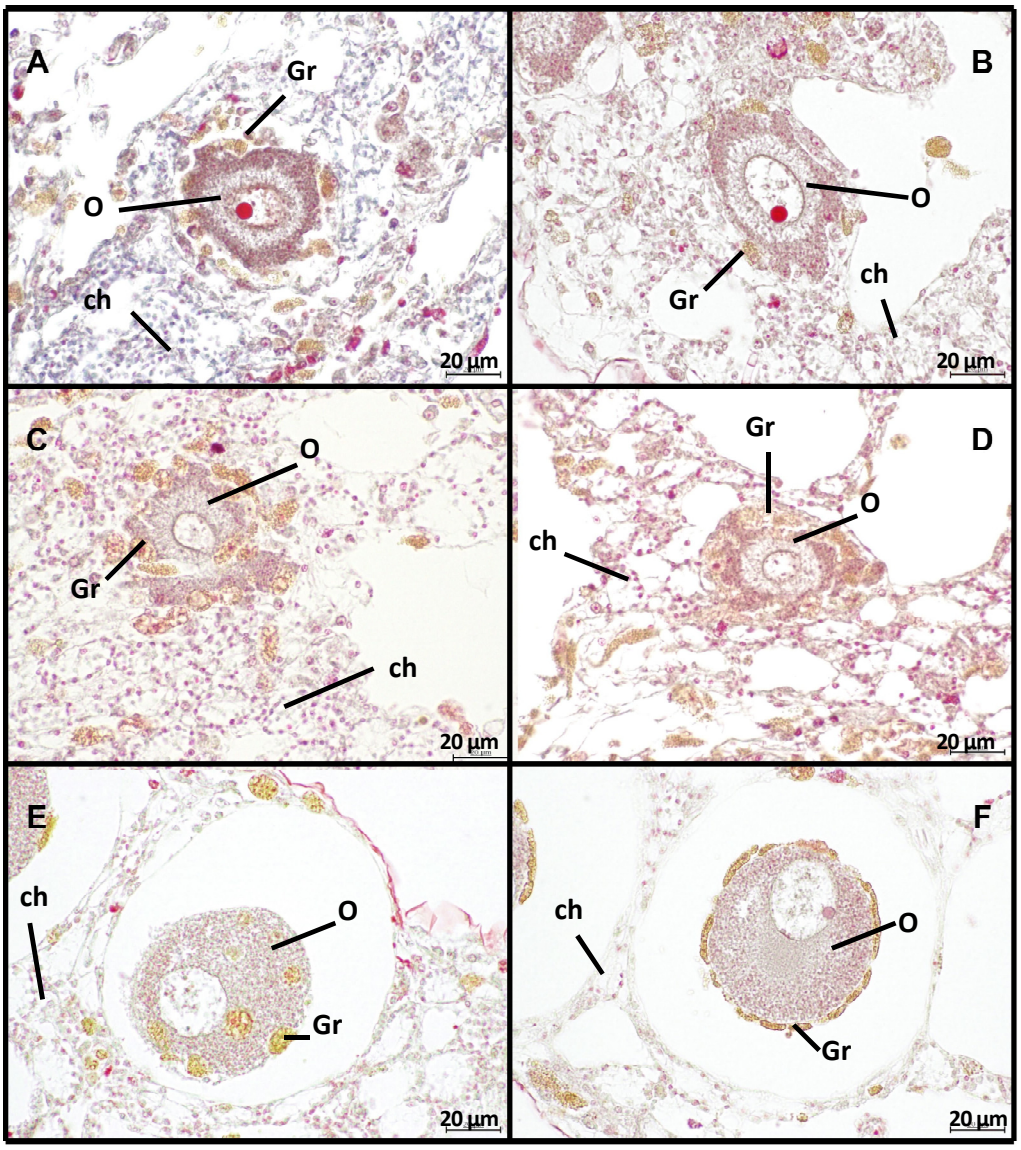


Figure 3. Oogenesis Stages III - IV. A and B) Oocytes in stage III with yolk only in the periphery; pseudopodia and engulfing of granulose cells are clear. C) Granulose cells inside stage III oocytes. D) Stage III oocyte with granulose cells starts to separate from the mesohyl. (E) Separation from the mesohyl is completed and granulose cells start to organize around stage IV oocyte. F) Stage IV oocyte is completely surrounded by granulose cells, forming the egg envelope. Abbreviations as in Figure 2.

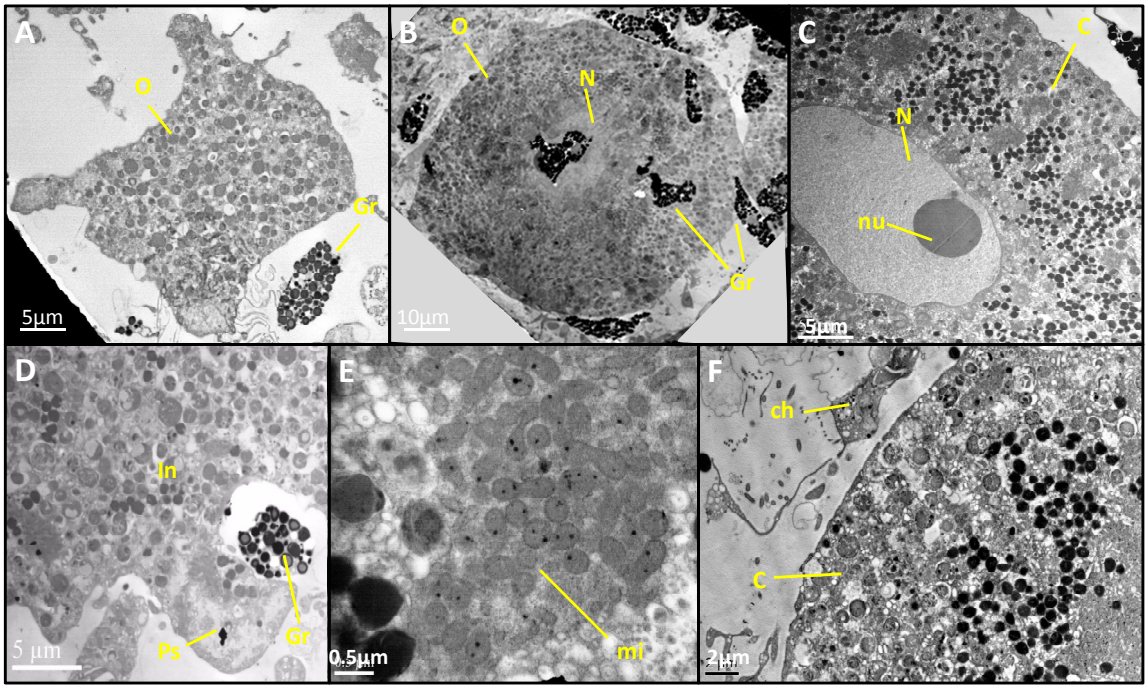


Figure 4. Transmision electron micrographs of oocytes in stage II of development with inclusions in the cytoplam. C: citoplasm; Gr: Granulose cell; In: inclusions;; mi: mitochondria; N: Nucleus; nu: nucleolus; O: oocyte; Ps: pseudopodia.

Spermatogenesis

Histology and TEM results suggested that spermatogenesis started by transdifferentiation of choanocytes, as several individuals that were holding spermatic cysts also exhibited modified choanocyte chambers with cell groups slightly larger than choanocytes (Figure 5, A, B). These cells are considered choanocytes in the process of transdifferentiation into spermatogonia. In TEM they showed a denser and larger nucleus (2 - 2.5 µm) than choanocytes (1 - 1.5 µm), two or three large vacuoles, and some small mitochondria, all having lost the typical collar of choanocytes (Figure 7, A, B). Some other cells with nuclei similar-sized or slightly larger (3 µm) than choanocytes showed synaptonemal complexes, which are typical of Prophase I and correspond to primary spermatocytes (Figure 7, C, D). Flagellum was also observed in some of the primary spermatocytes (Figure 7, D). Transdifferentiation was synchronous within a choanocyte chamber, with most of the choanocytes becoming spermatogonia. These aggregations of

transdifferentiating choanocytes became surrounded by granulose cells, which also occurred in between them (Figure 5, A - D). Four different stage of development were identified in spermatic cysts (Figure 6, 8), being herein referred to as Cysts 1 to 4. Cysts 1 were the smaller cysts, and can contain spermatogonia (Figure 6, A). Cysts 2 were larger than Cysts 1 and possibly contained spermatocytes I (Figure 6, B, C, Figure 8). Cysts 3 contained probably spermatids or spermatozoa; difficult to define as both spermatids and spermatozoa can also present cilium in sponges (Riesgo and Maldonado 2009b, Riesgo, Maldonado, and Durfort 2007), and we were not able to observe them in TEM (Figure 6 C, D, E, Figure 8). Cysts 4 were the largest observed (109 µm ± 34.1), with spermatozoa accumulated at one side and ready to be released (Figure 6, F). Groups of transdifferentiated choanocytes (before forming a cyst) were smaller than any of the spermatic cysts observed (Figure 8). In the tagged male individuals that were followed, the transdifferentiated choanocytes developed into a spermatic cyst 3 in about five days. Male individuals with mature spermatic cysts also simultaneously contained transdifferentiated choanocytes or spermatic cysts in earlier developmental stages. This suggests that spermatogenesis is asynchronous at the individual level, and designed to produce several pulses that appear to match the several oocyte cohorts noticed in the female individuals.

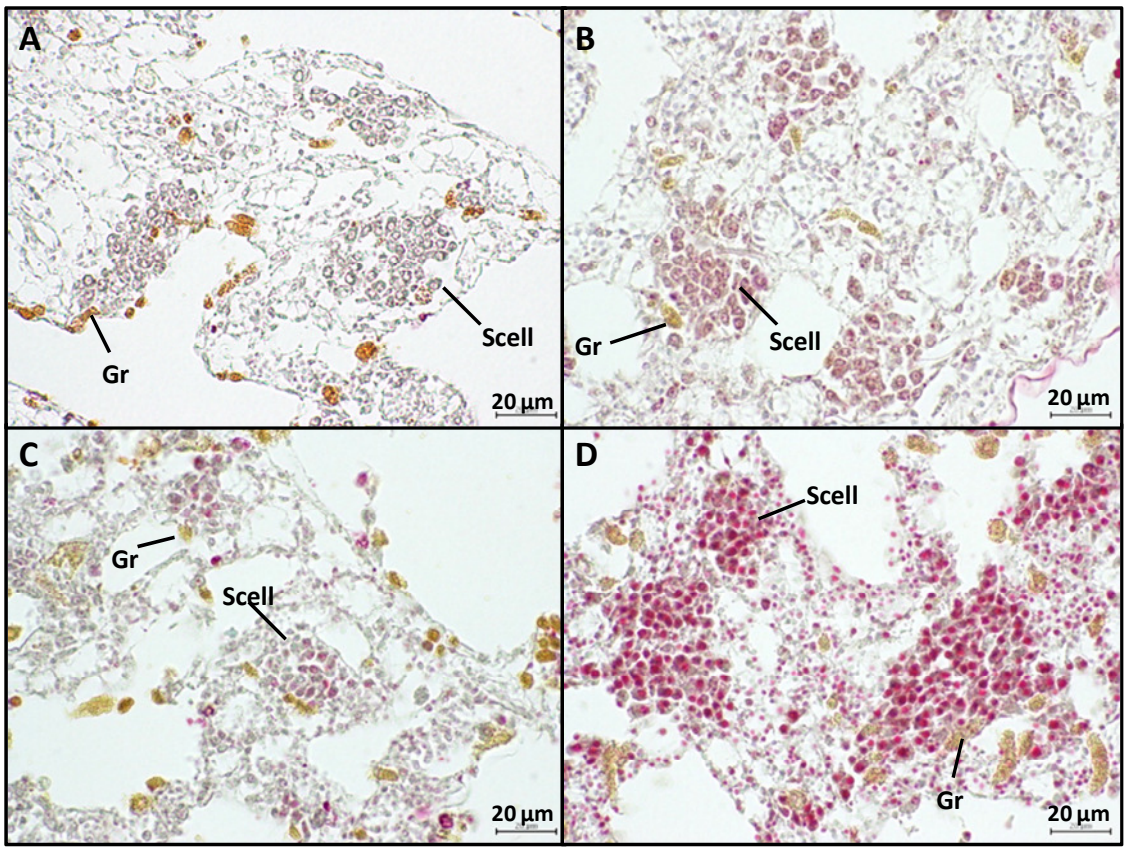


Figure 5. Choanocytes transformation into spermatogonia (Scel). Granulose cells (Gr) surrounding and entering groups of transdifferentiated choanocytes. A and B) Choanocyte chambers disappear and fuse with each other, choanocytes migrate to the center and grow in size. C and D) possible spermatogonia.

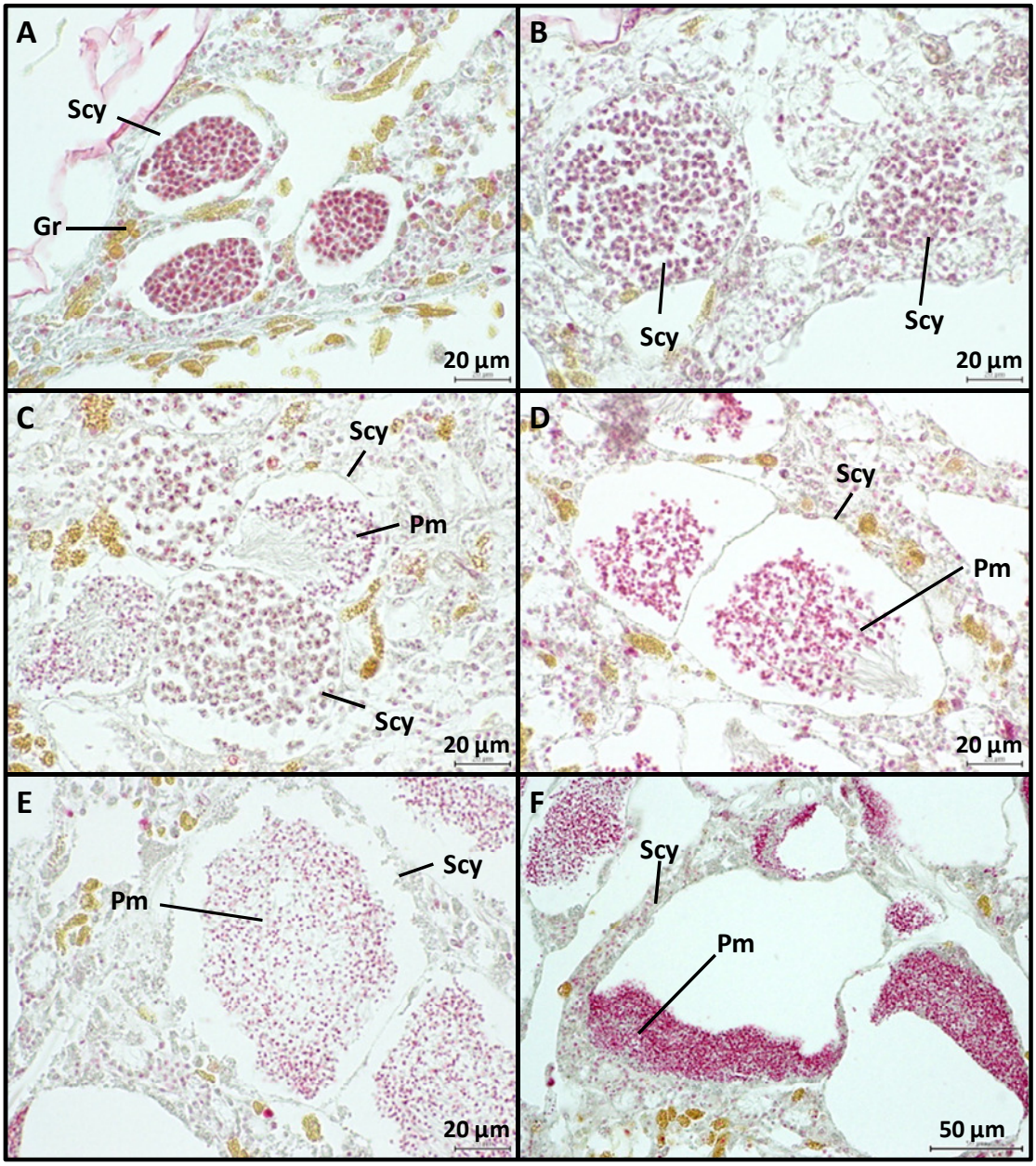


Figure 6. Development of spermatic cysts. A) Cyst 1 (with possible spermatogonia); B) Cyst 2 (spermatocytes I); C) Cyst 2 (spermatocytes I) and Cyst 3 (spermatids or spermatozoa) co-occurr in the same individual; D) Cyst 3 (spermatids or spermatozoa); E) Cyst 3 (spermatids or spermatozoa); F) Cyst 4 (condensed spermatozoa). Gr: granulose cell; Pm: spermatozoa; Scy: spermatic cyst.

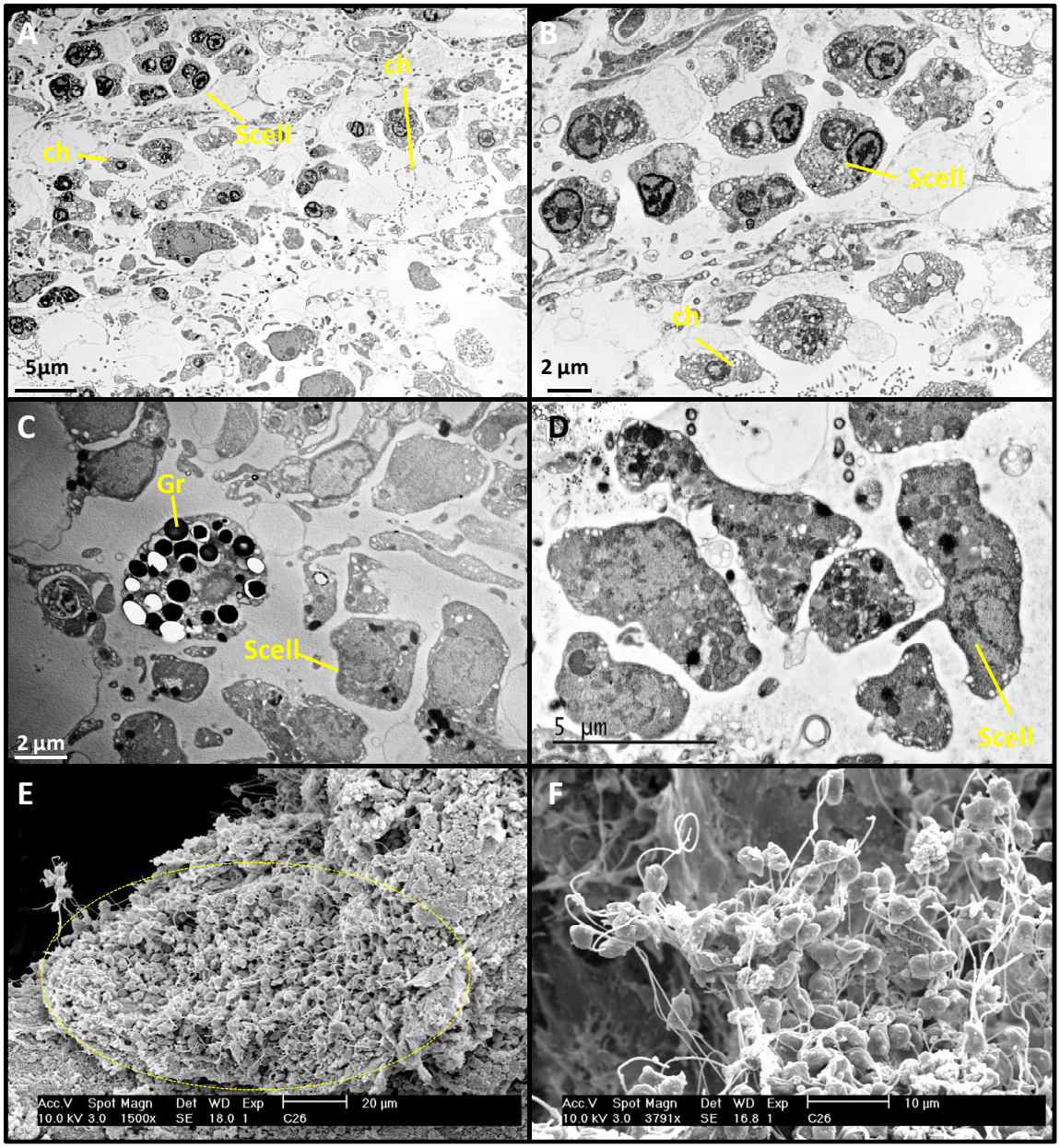


Figure 7. A-D) TEM of spermatogenesis; E) SEM of spermatic cysts; F) SEM of spermatozoa. Ch: choanocyte; Gr: granulose cell; Scell: transdifferentiated choanocyte.

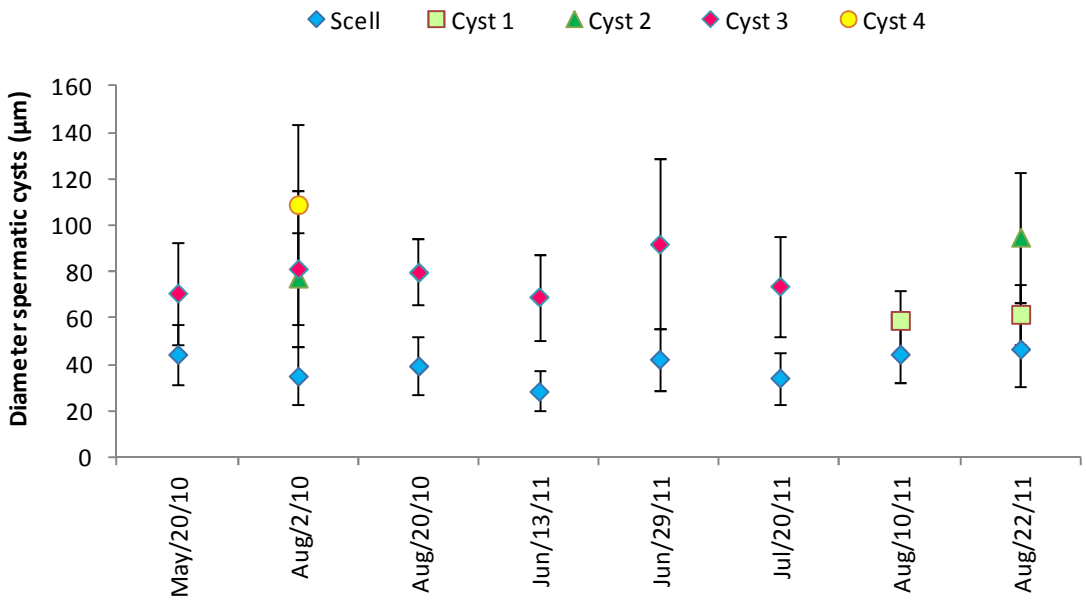


Figure 8. Size distribution of different spermatic cyst stages (Cyst 1-4), and transdifferentiated choanocytes (Scell) throughout the summers of 2010 and of 2011. Measurements were obtained using Image J of light microscopy images. Each dot represents the mean and error bars the standard deviation.

Gametic status

Most *Cliona delitrix* individuals were gonochoric, and sex reversal was never observed in the marked individuals (Figure 9). The estimated sex ratio varied at each reproductive pulse, departing from parity when only spermatic cysts were used to assign males; in this case females were more frequent in most reproductive peaks (Table 3). However, the sex ratio was closer to parity when transdifferentiated choanocytes were also used to assign males (Table 3). Only three individuals (of 650 examined) were simultaneous hermaphrodites, one with sperm as cyst 2 and oocytes in stage II, and the other two both with transdifferentiated choanocytes (early sperm cells) and early oocytes (stage I) (Figures 10, 11). These data support that a small percentage of individuals are simultaneous hermaphrodites in the population. The weekly analysis in August showed 2.9 % of individuals were hermaphrodites with simultaneously oocytes and transdifferentiated choanocytes (Figure 11).

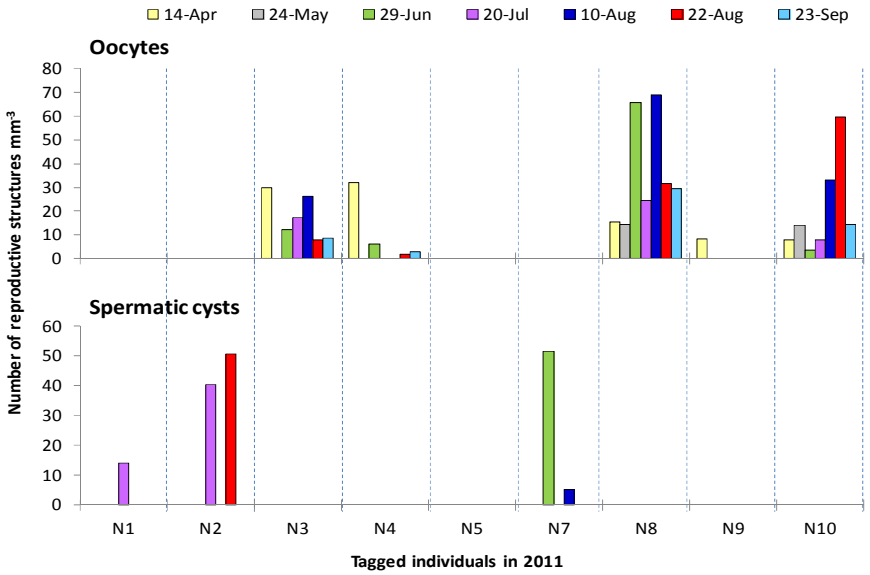


Figure 9. Number of reproductive structures in ten tagged individuals (x axis: N1 – N10) during 2011 (April- September). Females with oocytes (top graph), and males with spermatic cysts (bottom graph). N5 did not exhibit any reproductive structures, and N6 died and it was not included in the analyses.

Table 3. Ratio of female to male individuals at each reproductive peak combining tagged and randomly sampled individuals. n: total number of individuals sampled. r: randomly sampled individuals; t: tagged individuals. Females and males were assigned depending on the presence of only O: oocytes and SC: spermatic cysts, and also for males adding both SC and Scell: transdifferentiated choanocytes. Hermaphrodites were not included in the analysis.

Date (reproductive peak)	**(O: SC)**		**(O: SC + Scell)**	
	Female : Male	n (r+t)	Female : Male	n (r+t)
20-May-2010	1 : 3	7	1 : 3	7
2-Aug-2010	1 : 1	8	1 : 1	8
20-Aug-2010	3 : 1	4	1 : 2	8
13-June-2011	5 : 1	11	3 : 1	12
29-June-2011	4 : 1	10	1 : 1	14
20-July-2011	1 : 2	15	1 : 2	15
10-Aug-2011	4 : 1	10	1 : 1	15
22-Aug-2011	4 : 1	18	1 : 1	26
Total	**3:1**	**83**	**1:1**	**105**

Reproductive cycle

Population level

At a population level, developing oocytes were found in the sponge tissue in all sampled months for both years (although only in April - May and until November - December they pass into mature stages, see next section). Transdifferentiated choanocytes (the precursor spermatic cells) were found in most of the sampled dates during the warmer months, and spermatic cysts only in some of the sampled dates during summer (Figures 10, 12). In both years, about 10 % to 43 % (29 % to 40 % specifically in August, Figure 11) of male individuals had spermatic cysts simultaneously with transdifferentiated choanocytes (Figure 10, A, B).

Presuming that spawning would have taken place shortly after mature spermatic cysts were present in the samples, reproductive pulses occurred several times during summer, in consecutive months and even twice in the same month (Figure 11, 10 and 12, A,B). The first year of study (2009 - 2010) spermatic cysts were observed on three different times: May 20th, August 2nd, and August 20th (Figure 10 and 12, A), whereas during the second year (2010 - 2011) they were observed on five times: June 13th, June 29th, July 20th, August 10th, and August 22nd (Figure 10 and 12, B). Differences between tagged and random individuals were observed; for example, proportions of female and males were different, and the presence of spermatic cysts occurred only simultaneously in tagged and random samples on May 20th 2010, June 29th 2011, and July 20th 2011 (Figure 12, B).

Differences between tagged and random individuals during reproductive peals, and that not all individuals engage in reproduction at each peak, clearly indicates that the population is not reproducing at the same time (Figures 10 and 12, A, B). Thus, reproduction is asynchronous at a population level.

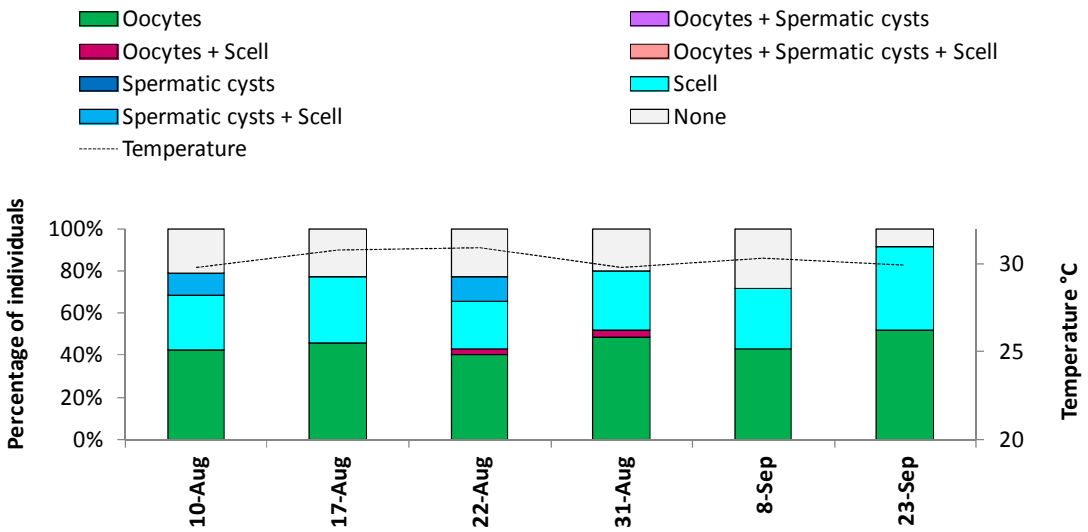


Figure 11. Percentage of individuals containing reproductive structures during August and September 2011, in relation to sea-water temperature. This figure is based on weakly sampling. Data and codes as in Figure 10.

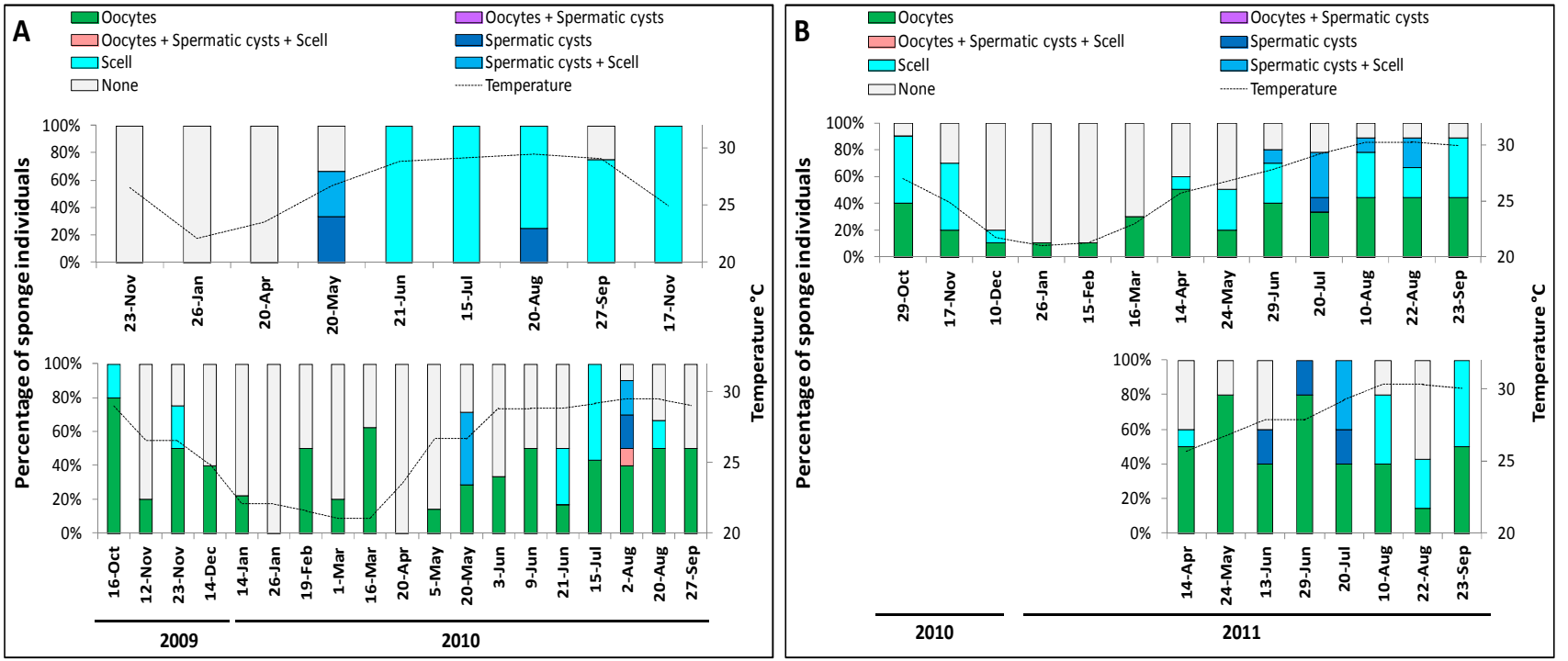


Figure 10. Percentage of individuals containing reproductive structures during the two sampled years, in relation to sea-water temperature. A) 2009-2010; B) 2010-2011. Top graphs in A and B correspond to tagged individuals, and bottom graphs to random ones. Scell: transdifferentiated choanocytes. This percentage depend of the number of samples at each date, thus for random individuals in January 26th and February 19th of year 2009-2010 when samples (n) were 1 or2, the percentage is relatively overestimated.

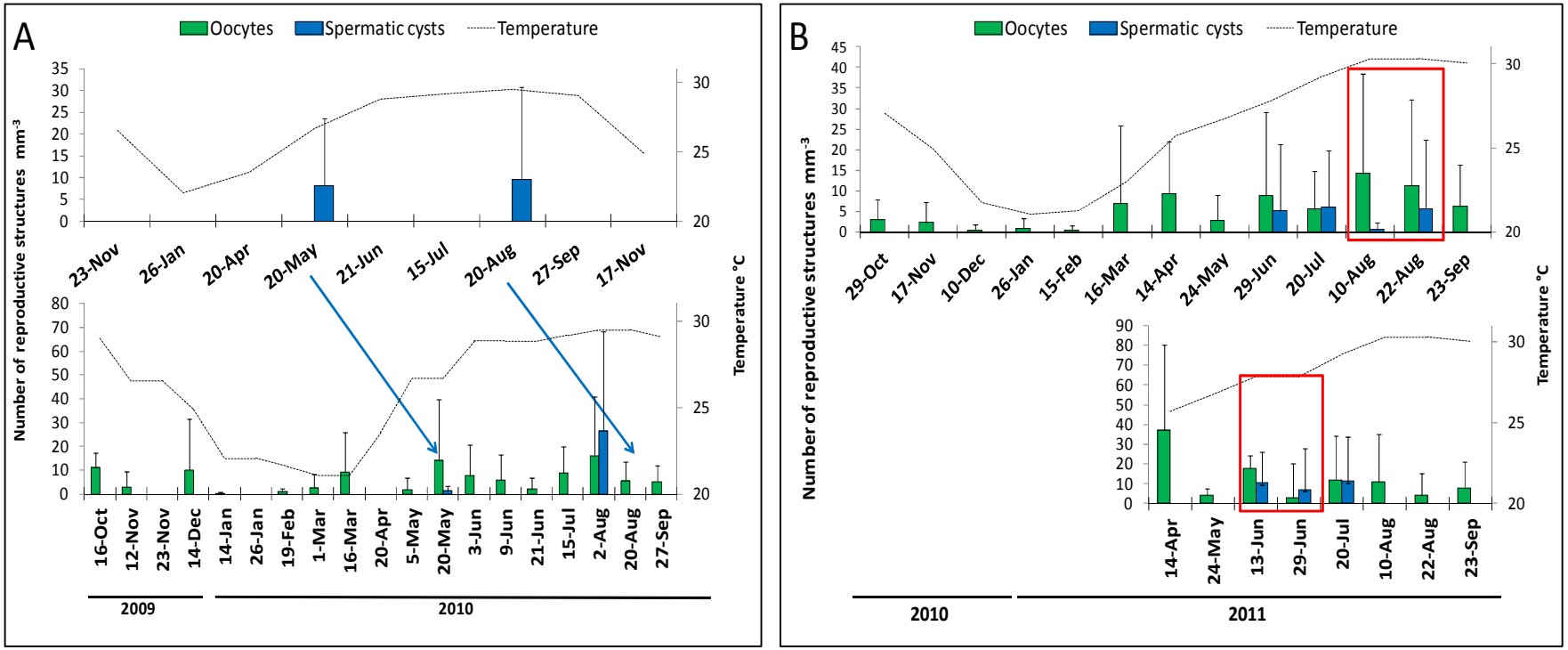


Figure 12. Density of reproductive structures for each sampled year in relation to sea-water temperature. A) 2009 - 2010, and B) 2010 - 2011. Upper graphs are for tagged individuals and lower graphs for randomly sampled individuals. Arrows mark the corresponding collection dates for random individuals in which tagged individuals had spermatic cysts in 2009 - 2010. Red squares show the dates in which spermatic cysts were found twice in the same month.

Individual level

During the warmer months, different cohorts of oocytes were observed to co-occur in the same female individual (Figures 13, 14). For example in October and November 2010, and in April, August and September 2011 stages I and III were present in the same individuals, whereas in June and July 2011 stages I and IV, and II and IV were observed (Figures 13, 14). Also, oocytes in stage III were present in the same individual every week in the same month, and oocytes in stage IV every month and even every two weeks (Figure 14). These results indicate that *Cliona delitrix* has an asynchronous oogenesis within single individuals, which complete a relatively rapid cycle, likely every two weeks to a month, as observed in one of the tagged individuals (Figure 15, female 4). However, a female individual did not necessarily produce oocytes every month (Figure 9). Also, the same female individual could produce gametes during most of the reproductive cycle, showing one or two peaks of higher density (Figure 9), while a few females maintained oocytes all year round (Figure 14).

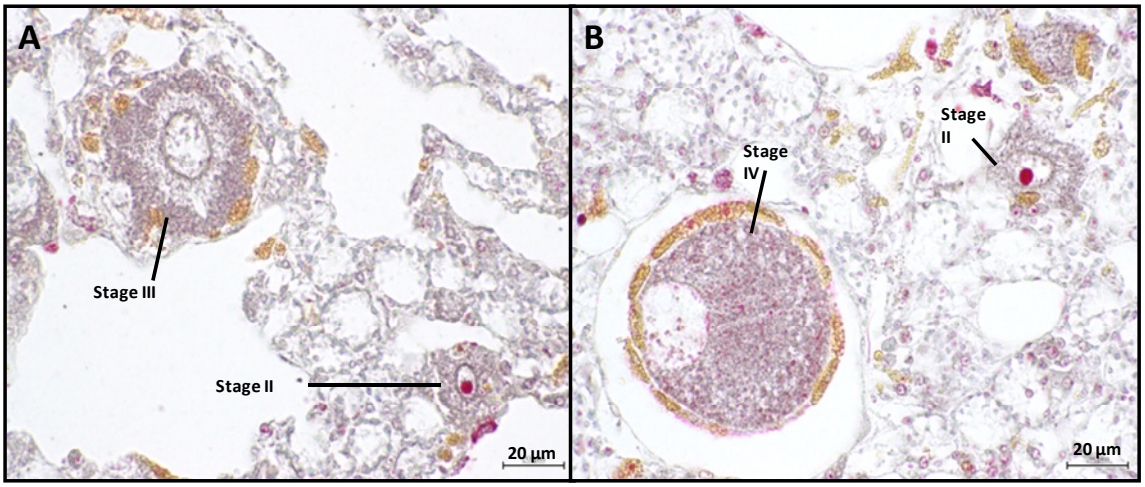


Figure 13. Different oocyte stages in the same *C. delitrix* female individuals. A) Stage II and Stage III together; B) Stage IV and Stage II together.

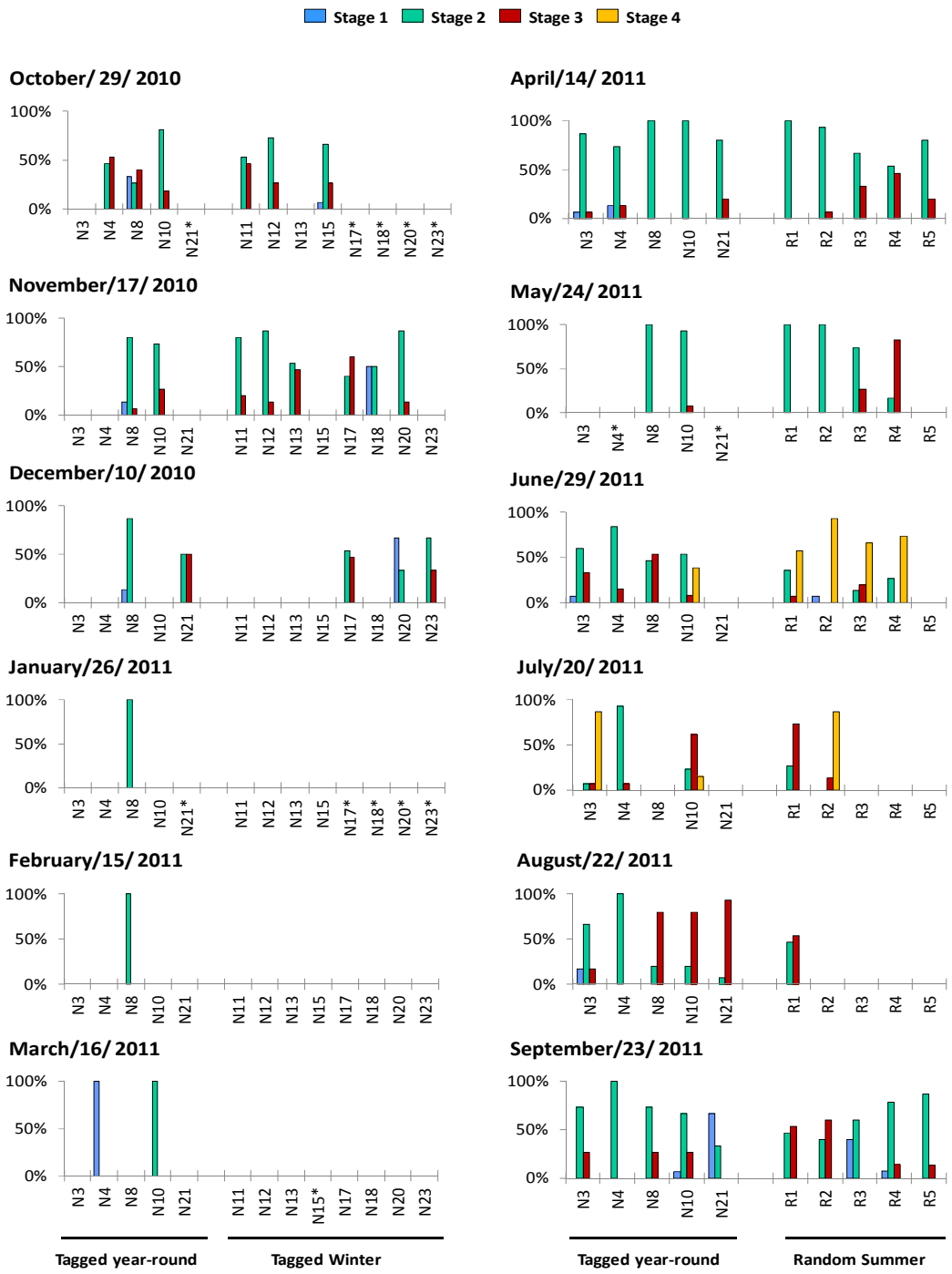


Figure 14. Oogenesis stages in female individuals sampled from October 2010 to September 2011. Samples are marked as either year-round (N1, N4, N8, N10, N21), fall - winter (N11 - N23), or randomly sampled female individuals (R1 - R5). N11 - N23 samples were collected from Oct 2010 to March 2011, while R1 - R5 samples were collected only after April 2011. (*) = individual was not sampled on this date.

At a population level transdifferentiated choanocytes where observed during the entire reproductive cycle (April - December, Figure 10), however at an individual level these cells tended to disappear after the presence of spermatic cysts (Figure 16), showing that these cells are also produced in pulses. Similarly, the development of cysts was not continuous and had a maximum of two peaks per individual during the reproductive cycle (Figures 9, 16).

Although not all the individuals in the population were reproductively active at the same time, the percentage of oocytes at mature developmental stage (stage 4) was higher when all spermatic cysts observed were also more developed (cyst 3 or cyst 4) (Figure 17). This showed that oogenesis and spermatogenesis can be coupled in pulses and males and females individuals tend to have part of their gametes matured in synchrony for spawning. Which as showed by our weekly sampling (July 29th to September 8th 2011), can occur twice a month (Figure 17).

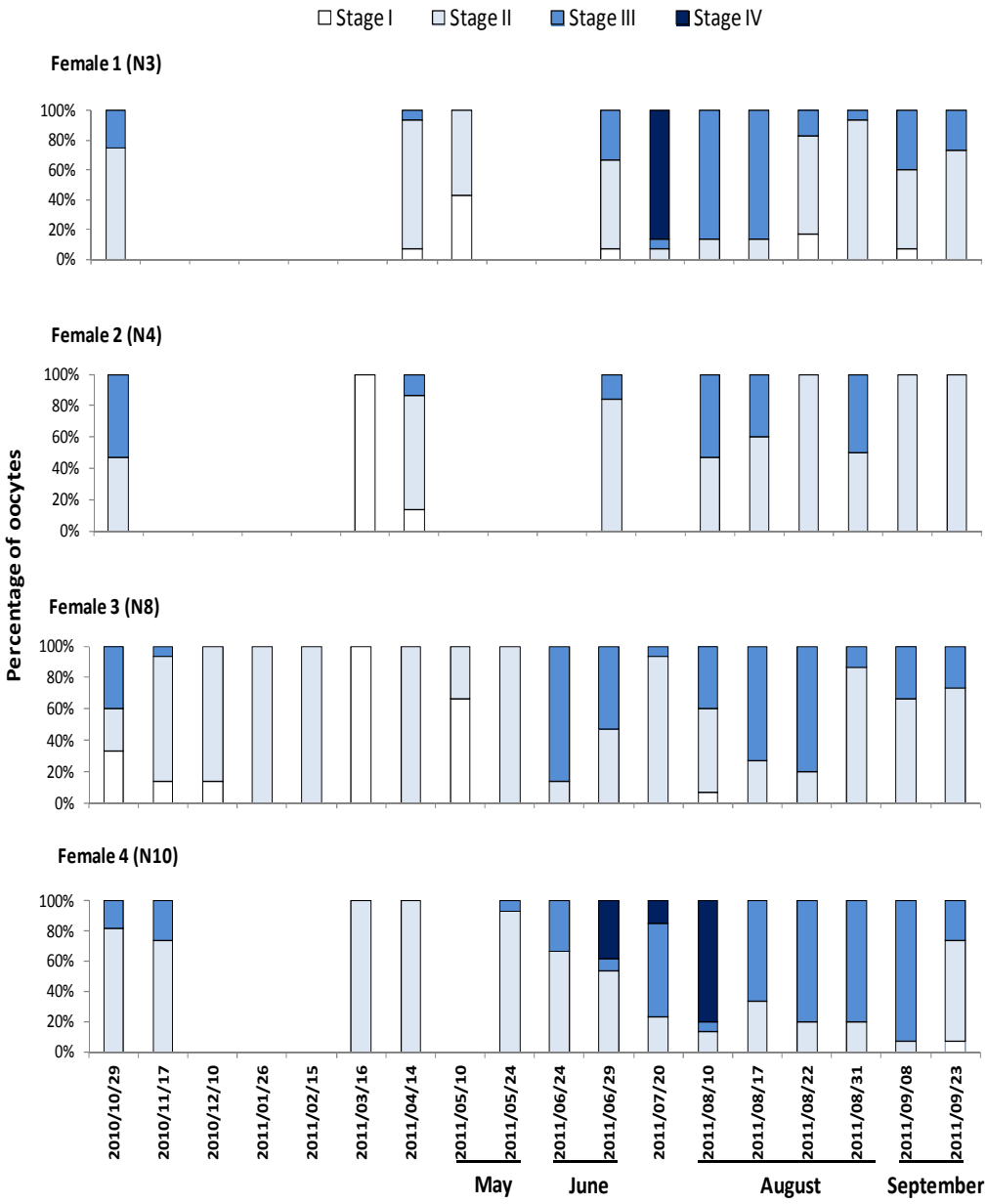


Figure 15. Percentage of oocytes at different developmental stages for the four tagged female in the sampling year 2010-2011.

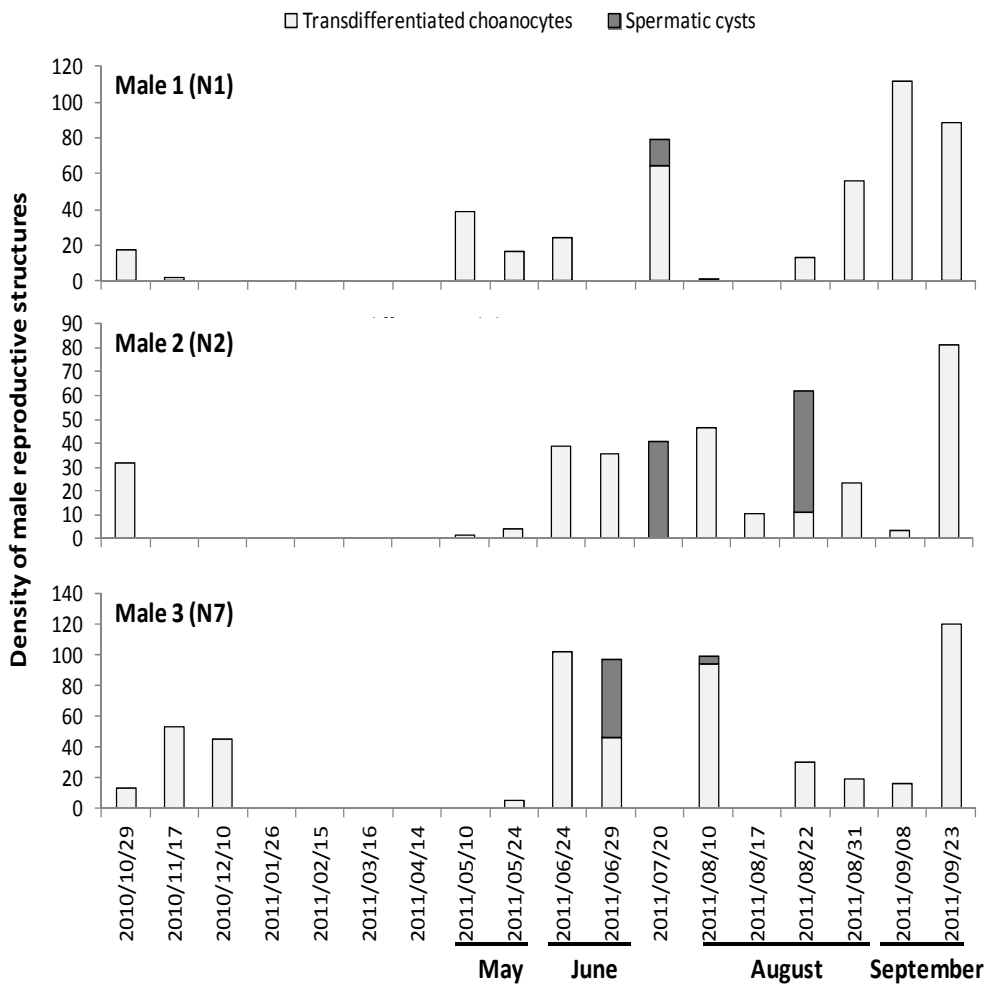


Figure 16. Density of transdifferentiated choanocytes (sperm cells) and spermatic cysts for the three tagged males in year 2010- 2011.

Gametogenesis in relation to environmental cues

Temperature

Both density and percentage of individuals with reproductive structures were higher during the warmer months of the year (Figures 10, 12). Increment in mean (per sampling date) oocytes density and transdifferentiated choanocytes (sperm cells) density correlated with an increment in sea water temperature (Oocytes: Pearson $r = 0.4$, $p < 0.05$, $n = 41$ including random and tagged females both years; and Pearson $r = 0.7$, $p < 0.05$, $n = 12$ only

including tagged females from second year. Sperm cells: Spearman r= 0.7, p< 0.05, n= 41 including random and tagged males both years; and Pearson r= 0.6, p< 0.05, n= 12 only including tagged males from second year). During the colder months of the year, December - April for 2009 - 2010, and January - March for 2010-2011, oocytes remained in stage I and II. The winter of the first year of sampling (2009 - 2010) was longer than that of the second year, with a one month delay for the temperature to rise over 25 °C and for oocytes to start stage III of development, i.e., the end of May in 2010, and middle of April in 2011. Similarly, choanocytes started transdifferentiation (to form spermatic cells) exactly at the same time. Thus, we consider that water temperature above 25 °C triggers the final development of oocytes and the transdifferentiation of choanocytes into spermatic cells. Figure 14 shows the change in oocyte stages for year 2010 - 2011.

Relationship to the lunar cycle

In summer 2010, three separate development pulses of spermatic cysts were observed 4 to 8 days before or after the full moon. On May 20th, eight days before the full moon, most spermatic cysts were already at cyst 3, whereas most oocytes were in early development (stage II), thus the spawning event in May probably occurred later, or the sampled females were not ready to spawn (Figure 17). On August 2nd, seven days after the full moon, both mature oocytes and spermatic cysts were present, and it was the only date (both sampled years) in which cysts 4 were observed, suggesting this is the closest sampling date to any of the spawning events. Also, spermatic cysts (cysts 3) and oocytes (stage III) were again observed on August 20th, 4 days before the next full moon, showing a second spawning event in the same month (Figure 17), but in this case before full moon.

During the summer of 2011, five separate development pulses of spermatic cysts were observed, three of them 3 to 5 days before or after the full moon; the fourth was 2 days before the new moon, and the fifth during the first quarter moon (Figure 17). However the fifth one showed spermatic cysts in early development (cysts 1 and 2). Thus, it is

possible that spawning occurred later: before or during the new moon, on August 22nd, similar to August 10th, when spawning probably occurred after, not before the full moon (Figure 17). Therefore, we found that all five pulses of spermatogenesis in summer 2011 appear to be related to full or new moons. The presence of spermatic cysts in males coincided with the presence of oocytes at stage IV in females, except during one event on June 13th, in which is possible that the mature oocytes had already been released (Figure 17).

Although both spermatic cysts and mature oocytes were observed from 3 to 8 days before and after both the full and the new moon, this was not the case in all the sampled dates in which sperm was found. Thus, there is not a clear relationship for all spawning events with the moon cycle. It is possible that the full and new moons trigger the spawning and that only those individuals with mature reproductive gametes will participate at each time. Thus, considering the asynchronous characteristic of the reproduction in *C. delitrix* at the population and the simultaneity of several developmental stages at the individual level, it is possible that in each spawning event only some individuals will be engaged, and that they will start to spawn a couple of days before the full or the new moon, extending it until 8 days after.

During this two year study, asexual reproductive structures typical of *Cliona* sponges such as gemmules, buds, or fragmentation of the sponge were not observed.

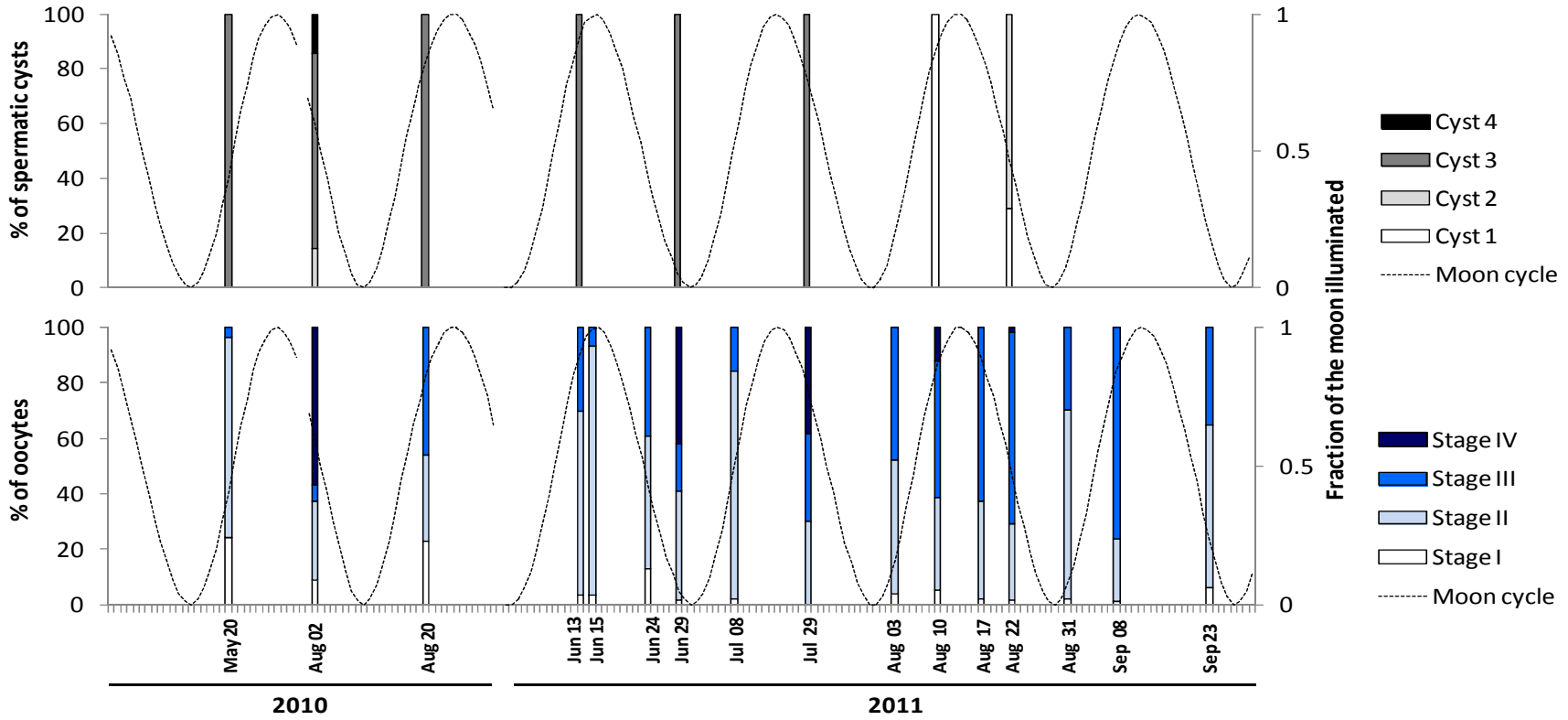


Figure 17. Moon phases and gametogenesis for summer 2010 and 2011. Fraction of the moon illuminated (Y-axis) shows the full moon (peaks) and new moon (valleys). Each bar represents a sampling date in which developmental stages for spermatic cysts and oocytes stages are color coded.

Discussion

This study elucidates a complex reproductive cycle in the excavating sponge *Cliona delitrix*, which includes distinctive gametogenic events, separate sexes (gonochorism), rare hermaphrodite individuals, sexual differences in the duration of gametogenesis, asynchrony of gametogenesis at the individual and at the local population level, and multiple spawning events over the warmest months of the year, possibly associated to the moon cycle.

Oocytes in *C. delitrix* had similar shapes and sizes than those of other *Cliona*. However, we found that granular cells (nurse cells) play an important role since the beginning of oogenesis. Granular cells had been previously reported but only for mature oocytes in *Cliona* adults and larvae (Piscitelli et al. 2011, Warburton 1961). For males, when choanocytes transdifferentiated, granular cells interspersed; both transdifferentiated choanocytes and granular cells appeared to be in constant interaction. However, granular cells did not enter spermatic cysts; therefore, for males granular cells could be involved only during the process of transdifferentiation of choanocytes into spermatic cells.

Cliona delitrix appears to be oviparous as other members of the genus *Cliona* (Piscitelli et al., 2011; Bautista-Guerrero et al., 2013). Neither embryos nor larvae were observed in any individual, suggesting that cleavage occurs once zygotes are expelled from the sponges. Thus, embryogenesis seems to occur outside the sponge mesohyl, while fertilization could occur either externally or internally. In this latter case, the zygotes would be quickly expelled after fertilization for external development, as reported for other clionaids (Maldonado & Riesgo, 2008; Bautista-Guerrero et al., 2013).

Cliona delitrix is similar to *Spongia officinalis*, other gonochoric sponge, in having a few hermaphroditic individuals (Baldacconi et al. 2007). However, *C. delitrix* hermaphrodites

were simultaneous - and not successive - hermaphrodites. Physiological, morphological and behavioral factors together with environmental limits influence the evolution of gonochorism from hermaphroditism (Prevedelli, Massamba n'siala, and Simonini 2006, McCartney 1997, Ghiselin 1987). Previous studies suggest that individuals are often able to assess the reproductive values related with functioning as one sex or the other, and that they can adopt the suitable breeding tactic (Munday, Buston, and Warner 2006, Ghiselin 1969). For example in sequential hermaphrodites, sex change can be favored when fertility increases for one sex compared to the other (Warner 1975). Also, sessile marine organisms that are in low populations, with relaxed sperm competition and localized gamete dispersal may favor the maintenance of hermaphroditism (McCartney 1997). Thus, having a low percentage of simultaneous hermaphrodites may favor *C. delitrix* asynchronous reproductive strategy; helping to equilibrate the ratio of females:males during specific reproductive pulses.

The *Cliona delitrix* female:male ratio was variable and departed from parity in most cases only when spermatic cysts were used to assign males. In this case females were more frequent than males, similar to findings for *C. vermifera* (Bautista-Guerrero, Carballo, and Maldonado 2013). However, the sex ratio was closer to parity when transdifferentiated choanocytes were also used to assign males. This suggests that parity may be the norm, and that a sex ratio departing from parity can be the result of misplacing the sampling time with the moment in which most male individuals have spermatic cysts, as spermatogenesis is usually very fast. Thus, reproduction in *Cliona delitrix* appears to agree with the Düsing - Fisher´s sex ratio principle, wherein the total numbers of each sex tend to be equal, as unbalanced ratios allow the rarer sex to have a greater average reproductive success that will eventually re-establish equilibrium (i.e., frequency-dependent selection) (Queller 2006).

Completion of gametogenesis was dependent on whether water temperature rose above 25 °C, a threshold that triggered the development of early oocytes (stage II) into mature eggs (stages III and IV), and the transdifferentiation of choanocytes into spermatic cells.

Also both oocyte and transdifferentiated choanocyte densities correlated with an increase in sea-water temperature. Considering these aspects, the *Cliona delitrix* reproductive cycle in Florida, USA, goes from April - May to around November - December depending on the 25 °C sea-water temperature threshold. The length of the seasonal reproductive cycle of *C. delitrix* overlaps with that of most sponges in Florida (Leong and Pawlik 2011, Maldonado and Young 1996), but appears to be longer and more continuous.

Although its reproductive cycle is seasonal, *Cliona delitrix* does not follow the typical pattern of a seasonal reproductive species, with a short reproductive phase, high percentage of individuals reproductively active, and synchronized gamete development and spawning within the individual and the population (Witte 1996). Other excavating sponges (*Cliona* or *Pione*), follow the latter pattern, with often only one or two highly synchronic major spawning events or zygote release during the warmest season (Bautista-Guerrero, Carballo, and Maldonado 2013, Pomponi and Meritt 1990, Piscitelli et al. 2011). Rather, *C. delitrix* reproduction has more than one reproductive phase, with possibly several spawning events. Also the percentage of reproductively active *C. delitrix* individuals varies with time (20 to 100 % during summer), and gamete development is asynchronous within the individual; with discrete periods of gamete production that are not synchronized between all the individuals of the population. Therefore, *C. delitrix* reproduction involves only a fraction of the population at different times during the reproductive period, a characteristic that places this species in the category of asynchronous but year - round reproductive organisms (Witte 1996, Gage and Tyler 1991). Indeed, at the population level, oogenesis in *Cliona delitrix* occurs as a continuous process over the year; even during the coldest months early stage (I and II) oocytes were observed in few individuals. Maintaining oocytes in the tissue after the reproductive season is a characteristic that has not been reported in other clionaids, as gametic activity typically ceases after the release of eggs or zygotes (Piscitelli et al. 2011, Bautista-Guerrero, Carballo, and Maldonado 2013). This capability could favor *C. delitrix* to engage in reproduction immediately after the environmental conditions are suitable.

At the individual level, oogenesis is also asynchronous, as females had simultaneous cohorts of oocytes at different developmental stages. Each cohort of eggs would take between two weeks and a month to mature, and eggs could be released in several, perhaps successive spawning pulses. Spermatogenesis was also asynchronous at the individual level; the same male sponge could have spermatic cysts at different developmental stages, which as in females, corroborates the possibility of more than one spawning pulse. However developed inside each cyst was synchronous, as in other clionaids (Bautista-Guerrero, Carballo, and Maldonado 2013). Although at a population level transdifferentiated choanocytes were observed during the reproductive season (April - December), at an individual level these cells emerged and disappeared, in relation to the presence of spermatic cysts. Transdifferentiation of choanocytes appears to take a month. The process of change from transdifferentiated choanocytes into spermatic cyst 3 takes at least five days. This is a faster process, not described before in *Cliona* species.

The pulses of male gametes development are not a continuous process in male individuals, as they had a maximum of two pulses of spermatic cysts during the whole reproductive cycle, which were separated at least by one month. As different male individuals of *Cliona delitrix* produced spermatic cysts close to both full and new moons, it is possible that each moon triggered their development and spawning. This would also permit two spawning events in the same month. A relationship between moon phase and sponge spawning of sponges has been suggested before for some species such as *Chondrilla australiensis,* which spawns 11 days after the full moon (Usher et al. 2004), and *Neofibularia nolitangere* which starts releasing of gametes the third day after the full moon (Hoppe and Reichert 1987).

In comparing reproductive cycles of *Cliona* excavating sponges from the Mediterranean Sea, Caribbean Sea, and Pacific Ocean, both gamete status and reproductive cycle are

different. In the Mediterranean Sea, *C. celata* and *C. viridis* are hermaphrodite with longer oogenesis and a very fast spermatogenesis which end up in one single spawning event in May (Piscitelli et al. 2011, Mariani, Uriz, and Turon 2000). Caribbean Sea species *C. delitrix* and *C. tenuis*, and Pacific Ocean species *C. vermifera* are gonochoric. Both *C. delitrix* and *C. vermifera* can have more than two pulses of spermatogenesis and more than two cohorts of oogenesis. *C. delitrix* between April and December and *C. vermifera* between July and November (Bautista-Guerrero, Carballo, and Maldonado 2013). This scenario has been suggested but not proven for *C. tenuis* (González-Rivero 2011). Why these differences?

Multiple spawning events are a strategy that increases reproductive success by decreasing the risk of massive offspring mortality in the face of local adverse events (Richmond and Hunter 1990). In addition it can increase the chances of successful colonization, and guarantee these species to be able to recruit at some point whenever free space was open on coral colonies. All three *C. delitrix*, *C. tenuis* and *C. vermifera* prefer to colonize corals (Chaves-Fonnegra and Zea 2011, Bautista-Guerrero, Carballo, and Maldonado 2013, López-Victoria and Zea 2005), and specifically *C. delitrix* on recently dead coral where coral skeleton is clean and exposed (Chaves-Fonnegra, Zea, and Lopez Submitted), whereas the Mediterranean species *C. celata and C. viridis* colonize different types of calcareous substrata and limestone (Mariani, Uriz, and Turon 2000, Nassanov 1883). It is possible that having more reproductive pulses can enhances changes of recruitment on a substratum more difficult to find, such as clean coral skeletons (see chapter 2). Overall, *Cliona'*s complex reproductive strategy appears to have contributed to its widening distribution.

With the current increase in coral mortality there is more substratum available for coral excavating sponges to recruit. Therefore, the recent successful spreading of *Cliona delitrix* and other excavating sponges probably stems from the new and more open coral substratum for attachment and recruitment (Chapter 2). Multiple pulses of spawning

events in a year is a strategy to guarantee recruitment. Indeed, as spawning is occurring at the same season of thermal stress and mortality in Caribbean corals (Eakin et al. 2010) the possibility of *C.delitrix* larvae to recruit on deteriorated coral colonies is increasing even more.

Considering that 25 °C is a trigger temperature for the development of reproductive structures in *Cliona delitrix*, we hypothesize that in tropical areas where water temperature is higher than 25 °C, this species could be reproductive year-round. In this case the possibility of colonizing new coral colonies could be higher, as the sponges could have more spawning events in tropical than in subtropical areas of the Greater Caribbean Sea. However, other factors as food availability could be limiting their reproduction (Maldonado and Riesgo 2008). Additional studies of sponges from tropical areas will be necessary to test this hypothesis.

During this two year study, asexual reproductive structures typical of *Cliona* sponges such as gemmules, buds or fragmentation of the sponge (Pomponi and Meritt 1990, Schönberg 2002) were not observed. Also, previous experiments of attachment of *C. delitrix* fragments on corals were not successful (personal data). However, five coral colonies with *C. delitrix* were observed to detach and move after storms (at different locations in Florida - USA), and two of them were able to crash and settle onto other coral colonies allowing the sponge to pass and colonize new coral colonies (A. Chaves-Fonnegra, unpublished observations). This is similar to the reported dispersal of *Cliona tenuis* colonizing *Acropora palmata* branches (López-Victoria and Zea 2004). Therefore, storms and hurricanes can also contribute to the asexual dispersal of *Cliona delitrix*.

Conclusions

Cliona delitrix is a gonochoric and oviparous sponge, with rare hermaphrodite individuals. Contrary to other members of the genus *Cliona* this species presents asynchronous gametogenesis, and reproduction does not occur as a single event in which all individuals in the population participate. Instead, *C. delitrix* present multiple spawning events over the warmest months of the year, possibly associated to the moon cycle. *C. delitrix* did not present asexual reproductive structures such as gemmules or buds. However, full sponge individuals can detach and move during storms. In general the extended reproductive cycle with multiple spawning events suggests that *C. delitrix* has a reproductive strategy that decreases the risk of massive offspring mortality during local adverse events, and that may increase the chances for larvae to find corals with recent mortality. Overall, *Cliona'*s complex reproductive strategy appears to have contributed to its widening distribution.

References

Annandale, N. 1915. "Indian boring sponges of the family Clionidae " *Records of the Indian Museum* 11:1-24.

Baldacconi, R., C. Nonnis-Marzano, E. Gaino, and G. Corriero. 2007. "Sexual reproduction, larval development and release in *Spongia officinalis* L. (Porifera, Demospongiae) from the Apulian coast." *Marine Biology* 152:969-979.

Banks, K.E., B.M. Riegl, V.P. Richards, B.E. Walker, K.P. Helmle, L.K.B. Jordan, J. Phipps, M. Shivji, R.E. Spieler, and R.E. Dodge. 2008. "Chapter 5: The reef tract of continental Southeast Florida (Miami-Dade, Broward, and Palm Beach Counties, USA)." In *Coral Reefs of the USA*, edited by B. Riegl and R.E. Dodge, 175-220. Springer-Verlag.

Bautista-Guerrero, E., J.L. Carballo, and M. Maldonado. 2013. "Abundance and reproductive patterns of the excavating sponge *Cliona vermifera*: a threat to Pacific coral reefs?" *Coral Reefs* "in press" DOI 10.1007/s00338-013-1094-1.

Brien, P. 1973. "Les démosponges morphologie et reproduction." In *Traité de Zoologie, anatomie, systématique, biologie*, edited by P.P. Graseé, 133-461. Paris: Masson and Cie.

Carballo, J.L., and J.A. Cruz-Barraza. 2005. "*Cliona microstrongylata*, a new species of boring sponge from the Sea of Cortés (Pacific Ocean, Mexico) " *Cahiers de Biologie Marine* 46:379- 387.

Chaves-Fonnegra, A., and S. Zea. 2007. "Observations on reef coral undermining by the Caribbean excavating sponge *Cliona delitrix* (Demospongiae, Hadromerida)." In *Porifera research: biodiversity, innovation and sustainability*, edited by M.R. Custódio, G. Lôbo-Hajdu, E. Hajdu and G. Muricy, 247-254. Rio de Janeiro: Museu Nacional.

Chaves-Fonnegra, A., and S. Zea. 2011. "Coral colonization by the encrusting excavating Caribbean sponge *Cliona delitrix*." *Marine Ecology* 32:162-173.

Chaves-Fonnegra, A., S. Zea, and M.L. Gómez. 2007. "Abundance of the excavating sponge *Cliona delitrix* in relation to sewage discharge at San Andrés Island, SW Caribbean, Colombia." *Boletín de Investigaciones Marinas y Costeras* 36:63-78.

Chaves-Fonnegra, A., S. Zea, and J.V. Lopez. Submitted. "Recruitment of the excavating sponge *Cliona delitrix* is favored by recent coral mortality." *Coral Reefs*.

Cobb, W. R. 1969. "Penetration of calcium carbonate substrates by the boring sponge, *Cliona*." *American Zoologist* 9:783-790.

Cobb, W. R. 1975. "Fine structural features of destruction of calcareous substrata by the burrowing sponge *Cliona celata*." *Transactions of the American Microscopical Society* 94:197-202.

Commission, Florida Fish and Wildlife Conservation, Fish and Wildlife Research Institute and the National Coral Reef Institute, and Nova Southeastern University Oceanographic Center. 2008. Southeast Florida Coral Reef Evaluation and Monitoring Project 2007 Year 5 Final Report.: 35.

Cortés, J., M. Murillo, H.M. Guzmán, and J. Acuña. 1984. "Pérdida de zooxantelas y muerte de corales y otros organismos arrecifales en el Caribe y Pacífico de Costa Rica." *Revista de Biología Tropical* 32:227-231.

Diaz, J.P., and R. Connes. 1980. "Etude ultrastructuralè de la spermatogenèse d´une Démosponge." *Biologie Cellulaire* 38:225-230.

Duckworth, A.R., and B.J. Peterson. 2013. "Effects of seawater temperature and pH on the boring rates of the sponge *Cliona celata* in scallop shells." *Marine Biology* 160 (1):27-35.

Eakin, C.M., J.A. Morgan, S.F. Heron, T.B. Smith, G. Liu, L. Alvarez-Filip, B. Baca, Erich Bartels, Carolina Bastidas, Claude Bouchon, Marilyn Brandt, Andrew W.Bruckner, Lucy Bunkley-Williams, Andrew Cameron, Billy D. Causey, Mark Chiappone, Tyler R. L.Christensen, M. James C. Crabbe, Owen Day, Elena de la Guardia, Guillermo Dı´az-Pulido, Daniel DiResta, Diego L. Gil-Agudelo, David S. Gilliam, Robert N. Ginsburg, Shannon Gore, Héctor M. Guzmán, James C. Hendee, Edwin A. Hernández-Delgado, Ellen Husain, Christopher F. G. Jeffrey, Ross J. Jones, Eric Jordán-Dahlgren, Les S. Kaufman, David I. Kline, Philip A. Kramer, Judith C. Lang, Diego Lirman, Jennie Mallela, Carrie Manfrino, Jean-Philippe Mare´ chal, Ken Marks, Jennifer Mihaly, W. Jeff Miller, Erich M. Mueller, Erinn M.Muller, Carlos A. Orozco Toro, Hazel A. Oxenford, Daniel Ponce-Taylor, Norman Quinn, Kim B.Ritchie, Sebastián Rodríguez, Alberto Rodríguez Ramírez, Sandra Romano, Jameal F. Samhouri, Juan A. Sánchez, George P. Schmahl, Burton V. Shank, William J. Skirving, Sascha C. C. Steiner, Estrella Villamizar, Sheila M. Walsh, Cory Walter, Ernesto Weil, Ernest H. Williams, Kimberly Woody Roberson, and Yusri Yusuf. 2010. "Caribbean corals in crisis: record thermal stress, bleaching, and mortality in 2005." *PlosOne* 5 (11):1-9.

Ereskovsky, A. 2010. *The comparative embryology of sponges*. I ed. London New York: Springer.

Fell, P.E. 1983. "Porifera: oogenesis." In *Reproductive biology of invertebrates: oogenesis, oviposition, and oosorption*, edited by K.G. Adiyodi and R.G. Adiyodi. Chichester, UK: John Wiley and Sons.

Gage, J.D., and P.A. Tyler. 1991. *Deep-sea biology. A natural history of organisms at the deep sea floor.* Cambridge: Cambridge University Press.

Ghiselin, M.T. 1969. "The evolution of hermaphroditism among animals." *The Quarterly Review of Biology*
44 (2):189-208.

Ghiselin, M.T. . 1987. "Evolutionary aspects of marine invertebrate reproduction." In *Reproduction of Marine Invertebrates*, edited by C.A. Giese, J.S. Pearse and V.B. Pearse, 609-666. Palo Alto: Blackwell Scientific Publications.

González-Rivero, M.A. 2011. "The ecology of bioeroding sponges on Caribbean coral reefs." University of Exeter.

Hartman, W.D. 1958. "Natural history of the marine sponges of southern New England." *Bulletin of Peabody Museum of Natural History* 19 (3):229-242.

Hoppe, W.F., and M.J.M Reichert. 1987. "Predictable annual mass release of gametes by the coral reef sponge *Neofibularia nolitangere* (Porifera: Demospongiae)." *Marine Biology* 94 (2):277-285.

Leong, W., and J.R. Pawlik. 2011. "Comparison of reproductive patterns among 7 Caribbean sponge species does not reveal a resource trade-off with chemical defenses." *Journal of Experimental Marine Biology and Ecology* 401:80-84.

Lévi, C. 1956. "Etude des *Halisarca* de Roscoff. Embryologie et systématique des demosponges." *Archives de Zoologie Expérimentale et Générale* 93:1-181.

López-Victoria, M., and S. Zea. 2004. "Storm-mediated coral colonization by an excavating Caribbean sponge." *Climate Research* 26:251-256.

López-Victoria, M., and S. Zea. 2005. "Current trends of space occupation by encrusting excavating sponges on Colombian coral reefs." *Marine Ecology* 26:33-41.

Maldonado, M. 2006. "The ecology of the sponge larva." *Canadian Journal of Zoology* 84 (1):175-194.

Maldonado, M. 2009. "Embryonic development of verongid demosponges supports the independent acquisition of spongin skeletons as an alternative to the siliceous skeleton of sponges." *Biological Journal of the Linnean Society* 97:427-447.

Maldonado, M., and P. Bergquist. 2002. "Phylum Porifera." In *Atlas of Marine Invertebrate Larvae*, edited by C. Young, 21-50. Barcelona, España: Academic Press.

Maldonado, M., and A. Riesgo. 2008. "Reproduction in the Phylum Porifera: A synoptic overview." In *Biologia de la reproducció*, edited by M. Durfort and F. Vidal, 29-49. Barcelona, Spain: Societat Catalana de Biología.

Maldonado, M., and A. Riesgo. 2009. "Gametogenesis, embryogenesis, and larval features of the oviparous sponge *Petrosia ficiformis* (Haplosclerida, Demospongiae)." *Marine Biology* 156 (10):2181-2197.

Maldonado, M., and C. Young. 1996. "Effects of physical factors on larval behavior, settlement and recruitment of four tropical demosponges." *Marine Ecology Progress Series* 138 (1-3):169-180.

Mariani, S., M.P. Piscitelli, and M.J. Uriz. 2001. "Temporal and spatial co-occurrence in spawning and larval release of *Cliona viridis* (Porifera: Hadromerida)." *Journal of the Marine Biological Association of the United Kingdom* 81 (4):565-567.

Mariani, S., M.J. Uriz, and X. Turon. 2000. "Larval bloom of the oviparous sponge *Cliona viridis*: coupling of larval abundance and adult distribution." *Marine Biology* 137 (5-6):783-790.

Mariani, S., M.J. Uriz, X. Turon, and T. Alcoverro. 2006. "Dispersal strategies in sponge larvae: integrating the life history of larvae and the hydrologic component." *Oecologia* 146 (1):174-184.

McCartney, M.A. 1997. "Sex allocation and male fitness gain in a colonial, hermaphroditic marine invertebrate." *Evolution* 51 (1):127-140.

Munday, P.L., P.M. Buston, and R.R Warner. 2006. "Diversity and flexibility of sex-change strategies in animals." *Trends in Ecology and Evolution* 21 (1):89-95.

Nassanov, N. 1883. "Zur biologie und anatomie der *Clione*." *Zeitschrift fur Wissenschaftliche Zoologie* 39:295-308.

Nassonow, N. 1883. "Zur Biologie und Anatomie der *Clione*." *Zeitschrift für Wissenschaftliche Zoologie* 39 (pls. 18-19):295-308.

Pang, R.K. 1973a. "The ecology of some Jamaican excavanting sponges." *Bulletin of Marine Science* 23 (2):227-243.

Pang, R.K. 1973b. "The systematics of some Jamaican excavating sponges (Porifera)." *Postilla* 161:1-75.

Piscitelli, M., G. Corriero, E. Gaino, and J. M. Uriz. 2011. "Reproductive cycles of the sympatric excavating sponges *Cliona celata* and *Cliona viridis* in the Mediterranean Sea." *Invertebrate Biology* 130 (1):1-10.

Pomponi, S.A., and D.W Meritt. 1990. "Distribution and life history of the boring sponge *Cliona truitti* in the Upper Chesapeake Bay." In *New perspectives in sponge biology*, edited by K. Rützler, 384-390. Washington: Smithsonian Institution Press.

Prevedelli, D., G. Massamba n'siala, and R. Simonini. 2006. "Gonochorism vs. hermaphroditism: relationship between life history and fitness in three species of *Ophryotrocha* (Polychaeta: Dorvilleidae) with different forms of sexuality." *Journal of Animal Ecology* 75:203-212.

Queller, D.C. 2006. "Sex ratios and social evolution." *Current Biology* 16:R664-R668.

Renegar, D.A., P.L. Blackwelder, J.D. Miller, D.J. Gochfeld, and A.L. Moulding. 2008. "Ultrastructural and histological analysis of Dark Spot Syndrome in *Siderastrea siderea* and *Agaricia agaricites*." Proceedings of the 11th International Coral Reef Symposium, Ft. Lauderdale, Florida.

Richmond, R.H., and C.L. Hunter. 1990. "Reproduction and recruitment of corals: comparisons among the Caribbean, the Tropical Pacific, and the Red Sea." *Marine Ecology Progress Series* 60:185-203.

Riesgo, A., and M. Maldonado. 2009a. "Ultrastructure of oogenesis of two oviparous demosponges: Axinella damicornis and Raspaciona aculeata (Porifera)." *Tissue Cell* 41 (1):51-65. doi: S0040-8166(08)00058-X [pii] 10.1016/j.tice.2008.07.004.

Riesgo, A., and M. Maldonado. 2009b. "An unexpectedly sophisticated, V-shaped spermatozoon in Demospongiae (Porifera): reproductive and evolutionary implications." *Biological Journal of the Linnean Society* 97:413-426.

Riesgo, A., M. Maldonado, and M. Durfort. 2007. "Dynamics of gametogenesis, embryogenesis, and larval release in a Mediterranean homosclerophorid demosponge." *Marine & Freshwater Research* 58 (1):398-417.

Rosell, D. 1993. "Effects of reproduction in *Cliona viridis* (Hadromerida) on zooxanthellae." *Scientia Marina* 57 (4):405-413.

Rosell, D., and M.J. Uriz. 2002. "Excavating and endolithic sponge species (Porifera) from the Mediterranean: species descriptions and identification key." *Organisms Diversity and Evolution* 2:55-86.

Rützler, K. 1974. "The burrowing sponges of Bermuda." *Smithsonian Contributions to Zoology* 165:1-32.

Rützler, K. 2002. "Impact of crustose clionid sponges on Caribbean reef corals." *Acta Geologica Hispánica* 37 (1):61-72.

Schönberg, C.H.L. 2002. "*Clione lampa*, a bioeroding sponge in a worm reef." *Hydrobiologia* 482 (1):49-68.

Sciscioli, M. , E. Lepore, M. Mastrodonato, L. Scalera-Liaci, and E. Gaino. 2002. "Ultrastructural study of the mature oocyte of *Tethya aurantium* (Porifera: Demospongiae)." *Cahiers de Biologie Marine* 43 (1):1-7.

Topsent, E. 1900. "Etude monographique des spongiaires de France: III. Monaxonides Hadromerina." *Archives de Zoologie Expérimentale et Générale* 8:1-331.

Usher, K., D. Sutton, S. Toze, S. Kuo, and J. Fromont. 2004. "Sexual reproduction in *Chondrilla australiensis* (Porifera: Demospongia)." *Marine & Freshwater Research* 55:123-134.

Warburton, F.E. 1958. "The manner in which the sponge *Cliona* bores in calcareous objects." *Canadian Journal of Zoology* 36:558-562.

Warburton, F.E. 1961. "Inclusion of parental somatic cells in sponge larvae." *Nature* 191:1317.

Ward-Paige, C.A., M.J. Risk, O.A. Sherwood, and W.C. Jaap. 2005. "Clionid sponge surveys on the Florida Reef Tract suggest land-based nutrient inputs." *Marine Pollution Bulletin* 51:570-579.

Warner, R.R. . 1975. "The adaptive significance of sequential hermaphroditism in animals." *The American Naturalist* Vol. 109 (965):61-82.

Weil, E. 2002. "Sponge-induced coral mortality in the Caribbean. A potential new threat to Caribbean coral reefs." VI Internacional Sponge Conference, Genova.

Wells, W.H., M.J. Wells, and I.E. Gray. 1964. "Ecology of sponges in Hatteras Harbor, North Carolina." *Ecology* 45 (4):752-767.

Wisshak, M., C.H.L. Schönberg, A. Form, and A. Freiwald. 2012. "Ocean Acidification Accelerates Reef Bioerosion." *PLOSOne* 7 (9):1-8.

Witte, U. 1996. "Seasonal reproduction in deep-sea sponges- triggered by vertical particle flux?" Marine Biology 124 (4):571-581.

Appendix 1.1. Supplementary Information

A- Table 1. Number of individuals used for analyses. Although the total number of samples collected for both years was 650, they all were used only for descriptions of gametogenesis, and some of them for each analysis. Year I: 2009-2010; Year II: 2010-2011. Figures of the analyses correspond to the ones in the document. R: random individuals; T: Tagged individuals.

Observation/Analysis	n (individuals)	n (oocytes/sperm)	Random/Tagged	Year	Temporal scale	Figures
Description Gametogenesis	all 650	NA	R+T	I and II	Monthly/biweekly/weekly	Fig 1-7
Size distribution oocytes	85	(1-15)	R+T	I and II	Monthly/biweekly/weekly	Table 2
Size distribution of spermatic cysts	(7-28)	(1-15)	R+T	I and II	Each reproductive pulse found	Fig 8
Tagged individuals graphs to test gonochorism	10	NA	T	II	Monthly/biweekly	Fig 9
Ratio female:male	(7-26)	NA	R+T	I and II	Monthly/biweekly	Table 3
Percentage of individuals with reproductive structures 2009-2010	R: 1-10; T: 3-4	NA	R+T	I	Monthly/biweekly/weekly	Fig 10
Percentage of individuals with reproductive structures 2010-2011	R: 5-10; T: 10	NA	R+T	II	Monthly/biweekly/weekly	Fig10
Percentage of individuals weekly analysis 2011	R: 5-10; T: 14-26	NA	R+T	II	weekly	Fig 11
Density of reproductive structures year 2009-2010	R: 1-10; T: 3-4	NA	R+T	I	Monthly/biweekly/weekly	Fig 12
Density of reproductive structures year 2010-2011	R: 4-10; T: 10	NA	R+T	II	Monthly/biweekly/weekly	Fig 12
Oogenesis stages in females	R:5; T:13	NA	T	II	Monthly	Fig 14
Percentage oocytes in each tagged female	4	NA	T	II	Monthly/biweekly/weekly	Fig 15
Percentage male reproductive structures each tagged male	3	NA	T	II	Monthly/biweekly/weekly	Fig 16
Size distribution oocytes	85	(1-15)	R+T	I and II	Monthly/biweekly/weekly	Table 2

B- Table 2. Histology protocol to look for reproductive structures in excavating sponges. Andia Chaves Fonnegra Oct 2009. Mix of sponge and coral histological techniques.

Fix tissues overnight, 24h (or for extended periods up to 3 weeks) in Bouin´s fixative . Use gloves and a fume hood, or if in a boat or in the field station, do it outside.
Rinse in distilled water 2x 10-15minutes.
Decalcification : use Hydrochloric acid (HCl) 10% plus EDTA. Leave specimens for 2 to 3 days under the fume hood, use glass beakers of 200ml per each sample (Coral lab do it in a big plastic tray and separate samples with plastic rings) To prepare 3L (3000ml) of 10% HCl use: 300ml of HCl (Concentrated which usually is 37%) 3g of EDTA 2700ml of dH_2O. Mix well in a glass bottle, and put with a stir bar on a mixing plate for 20min to 1h. Depending of the quality of the EDTA it can take longer.
After decalcification rinse specimens 3x30 min to 1hour in Distilled Water prior to ethanol.
Dehydration: Transfer specimens to a glass vial, add and remove each of the following : 50% ethanol – 1 hour (can be stored in 50% ethanol for longer periods). 70% ethanol – 1 hour (then stop here for **Desilicification**: remove most of 70% ethanol and add 4% hydrofluoric acid prepared in 70% ethanol to plastic 50ml falcon tubes (Prepare fresh every time under a fume hood) and add until covering the specimen (usually 3-5ml, but depends on the size of the tissue). Leave the specimen in the 4 °C refrigerator overnight. The following day, remove the HF/Ethanol to a waste container and add fresh 70% Ethanol, enough to ensure the HF has been rinsed out, 20 min). Continue with dehydration series. [HF waste was stored in a 4 °C refrigerator until it could be safely disposed as chemical waste, while carefully following all MSDS protocols]. 95% ethanol – 15 min x 2 100% ethanol – 10 min x 2-3. Depending on the size of the specimen; longer for larger specimens). Note: absolute 100% ethanol causes shrinkage and hardening, so prolonged time in absolute is not good.
Draw off the 100% Ethanol and add Xylene or Toluene – 15 min x 2. Note: Xylene and Toluene melt plastics, use glass beakers or vials. I used Xylene.
Draw off the Xylene and place the tissue in molten paraffin in a beaker on the oven at 60 °C– 15min x 2.
Embedding: Transfer the tissue to a metallic or plastic mold with fresh molten paraffin. Orient the tissue on the bottom of the mold, press the tissue softly to release any trapped bubbles. Place the embedding rings. Pour in more wax, and move the boat off the hot plate to an ice cold plate.

Sectioning: Do sections between 0.4 and 0.5 µm, thicker sections at 10 µm can also be used, but usually are too thick to see the structures clearly. Use good microtome knifes, and be cautious that even desilicifing and decalcifing sometimes does not eliminate other minerals that can break the tissue. Sections should be done slowly and if you find minerals take a dissecting needle and pull them out. This can leave holes in the block and in your sections, so be careful. Use slides with a space to mark and proper markers or pencil that will not get erased during the staining procedure.

C- Table 3. Heidenhain´s Aniline Blue staining protocol used at the NSU Oceanographic Center Coral Histology Lab to stain corals, and used to stain marine sponges. Andia Chaves Fonnegra Oct 2009. Times may change depending on how fresh the stains are. This protocol is longer than the common Harris Hematoxylin/Eosin typically used for sponges.

Heat azocarimine to 56 °C and filter, then maintain in the oven at 60 °C while in use. If not in used keep it in the fridge at 4 °C
De-paraffinized 2 minutes each under hood 3 times xylene 3 times 100% ethanol 1 time 95% ethanol 1 time 80% ethanol 1 time dH_2O
Stain red 1 time Azocarmine- stain in oven at 60 °C – 25 minutes (species dependent) 2 times dH_2O (eliminate excess of stain) – 3 dips/plunges per container 1 time Aniline alcohol (distain) – (2-5 minutes but check under scope how much is distained) 1 time Phosphotungstic acid (fix) – (12-15 minutes) 1 time dH_2O (rinse) – (2 minutes)
Stain blue 1 time Aniline blue – stain in dark- 20-25 minutes 2 times dH_2O (rinse) – 2-3 dips/plunges per container
Dehydrate 1 time in 95% ethanol – 1 minute 3 times in 100% ethanol – 2 minutes 3 times in Xylene – 2 minutes
Cover slides Add two small drops of glue (Cytoseal) Put coverslide and let dry overnight.

D- Matlab code to quantify sponge tissue on pictures

```
tissue=[];

for i=1:1

eval(['pic=imread("C:\Users\Andia C Fonnegra\Desktop\pictures matlab\June 29
2011\pic',num2str(i),'.JPG");']);

% lines=size(trans,1);
% columns=size(trans,2);


level1=pic(:,:,1);
level2=pic(:,:,2);
level3=pic(:,:,3);

hole=[];
for i=1:3
eval(['hhole=find(level',num2str(i),'==255);']);
hole=[hole; hhole];
eval(['level',num2str(i),'(hole)=0;']);
end

picture=cat(3,level1,level2,level3);
figure;imshow(picture);

ttissue=100-(numel(hole)*100/numel(picture));
tissue=[tissue ttissue];

end
%close all;
```

CHAPTER 2: RECRUITMENT OF THE EXCAVATING SPONGE *Cliona delitrix* IS FAVORED BY RECENT CORAL MORTALITY

Abstract

Factors such as thermal stress, contamination, and indiscriminate fisheries kill corals, ultimately changing the community structure and coral reef ecosystem functions. During the past few decades, coral mortality in the Caribbean Sea has concurred with an increase in abundance of other benthic organisms, including strong reef bioeroders such as the sponge *Cliona delitrix*. However, recruitment preferences of this sponge and its relation to coral mortality have not been fully tested. We hypothesized that the preferential attachment of its larvae on recent coral mortality (RM) is one of the main drivers of the increase. To test this hypothesis, belt transects were used to estimate contemporary sponge recruitment and to infer sponge larval substratum preferences in various localities throughout the Greater Caribbean. Also, recruitment was directly quantified in permanent transects in San Andres Island, Colombia, and over dead coral after the 2010 massive coral mortality in Bocas del Toro, Panamá. Results showed that *C. delitrix* current recruitment is higher than other excavating sponge species. We confirmed that *C. delitrix* recruits settle in greater proportion on corals with RM rather than with older mortality (OM). Concomitantly, sponge recruitment was significantly higher at depths where coral mortality was greater. Thus, with this study we establish that settlement and recruitment of *C. delitrix* in the Greater Caribbean is favored by RM. Nevertheless, the spatial-temporal scale in which coral tissue dies and *C. delitrix* reproduction and larvae attachment takes place also play an important role in the settlement and recruitment success of this excavating sponge.

Introduction

An extensive decrease of hard coral cover of around 40 % occurred in Caribbean reefs after the 1980s coral mortality (Hughes 1994, Gardner et al. 2003). Since then, reduction of corals has been followed by an increase of other benthic organisms: macroalgae, sponges, corallimorpharia, soft corals and urchin barrens (Norström et al. 2009). Currently, sponges have higher cover than other benthic organisms (Díaz and Rützler 2001), and are considered the dominant habitat-forming animals of Caribbean reefs (Pawlik 2011). Reports of sponge increases in the past twenty years include the barrel sponge *Xestospongia muta* (McMurray, Henkel, and Pawlik 2010), excavating sponges of the genus *Cliona* (Antonius and Ballesteros 1998, López-Victoria and Zea 2004, Ward-Paige et al. 2005), and the chicken liver sponge *Chondrilla* (Aronson et al. 2002). The last two sponges behave as aggressive and successful space competitors mainly in conditions stressful to corals (Rützler 2003) .

Coral-excavating sponges that have increased in the Caribbean Sea include *Cliona delitrix* and *Pione lampa* (Ward-Paige et al. 2005, Chaves-Fonnegra, Zea, and Gómez 2007, Rose and Risk 1985), and *C. aprica*, *C. caribbea* and *C. tenuis* (López-Victoria, Zea, and Weil 2006, Rützler 2002). The increase of *Cliona* has been correlated with an increase in pollution (Holmes 1997, Ward-Paige et al. 2005, Rose and Risk 1985, Chaves-Fonnegra, Zea, and Gómez 2007), coral mortality, hurricanes (López-Victoria and Zea 2004), and high seawater temperature (Rützler 2002, Cortés et al. 1984).

Excavating sponges are among the most important coral reef framework bioeroders, and can be responsible for up to 90% of total boring activity in live and dead coral heads (MacGeachy and Stearn, 1976). Sponges which simultaneously encrust and excavate limestone are able to displace and kill live coral tissue (Rützler, 2002; López-Victoria et al., 2006). Among encrusting excavating sponges, *Cliona delitrix,* which lacks associated photosynthetic zooxanthellae, is one of the most destructive species to reef corals. This sponge is able to excavate 10 - 12 cm inside coral skeletons and spread laterally at mean rates of ~1.5 cm $\cdot$ y^{-1} (Chaves-Fonnegra, 2006), completely overpowering massive live

corals (Pang, 1973; Rützler, 2002; Chaves-Fonnegra and Zea, 2007). Previous observations suggest that attachment of *Cliona* larvae is on RM (López-Victoria and Zea 2005, Chaves-Fonnegra and Zea 2011). However, no specific information exists about excavating sponge settlement and recruitment preferences (Rützler 2002, López-Victoria, Zea, and Weil 2006, Chaves-Fonnegra and Zea 2011), which are important to understand why excavating sponges are increasingly abundant on Caribbean reefs.

Sponge settlement and recruitment can be highly variable, being present in some years and completely absent in others, and may depend on the maturity of other assemblages on the substratum (Sutherland and Karlson 1977, Zea 1993). Also, sponge recruitment is reduced by filamentous algae, but increased by crustose coralline algae (Zea 1993, Ayling 1980). The ability of marine sponges to settle preferentially on specific substrates has been previously tested with a few marine sponge species. In fact, the larvae of most shallow water sponges have an "exploratory" behavior before settlement (Maldonado 2006). For example, larvae of *Crambe crambe* and *Scopalina lophyropoda* settle preferentially on microrefuges of grooved substrata where survival of later stages is higher (Maldonado and Uriz 1998). Similar behavior to preferentially settle under surfaces (darker areas) was found for *Haliclona loosanoffi* larvae, which settles under *Mytilus* shells probably as a response to surface texture (Fell 1976). Also, successful recruitment of some sponge larvae from *Stylopus* sp. only occurs on natural crustose coralline surfaces (Aylin, 1980).

Cliona species have a preference for different calcium carbonate substrata. Some Clionaidae (Bouchet and Rützler 2003) species preferentially recruit on oyster shells (Pomponi and Meritt 1990). This is the case for *C. truitti*, *C. celata*, *C.viridis* and *C. lobata* whose larvae settlement and metamorphosis occurs on mollusk shells (Wells, Wells, and Gray 1964, Hartman 1958, Pomponi and Meritt 1990). Other excavating sponges such as *Spiroxia* prefer branching corals (*Corallium*) with a magnesium - calcite axis (Calcinai et al. 2008); *C. caribbaea* and *C. delitrix* prefer massive coral colonies to settle (López-Victoria and Zea 2005, Chaves-Fonnegra and Zea 2011).

It is unknown if excavating sponge larvae are able to attach directly onto live coral tissue. However, coral defense mechanisms seem to be sufficient to prevent sponge larvae settlement on live tissue. In fact, previous studies demonstrated that live coral tissue is able to deter excavating sponge colonization, through extracoelenteric digestion and mesenterial filaments (McKenna 1997). Thus, it is possible that any excavating sponge larvae falling onto live coral will be trapped by and digested by its polyps, and if attachment on a dead side is too close to live coral, messenterial filaments may reach them.

In earlier studies carried out between 1960 and 1980, excavating sponges were commonly observed dwelling on or growing from coral colony bases where live coral tissue had died as a result of natural upward coral growth (Goreau and Hartman 1963, Pang 1973, MacGeachy 1977). Conversely, with the extensive coral die-off occurred during the past three decades, as excavating sponges became more abundant they were more frequently observed growing on top of coral colonies, where coral tissue should have been alive (Chaves-Fonnegra et al. 2005, MacGeachy and Stearn 1976, López-Victoria and Zea 2005). Occasional recruits of *Cliona delitrix* have been observed on corals which died by black band disease (Edmunds 2000), and on skeletons of corals that previously had yellow band disease (Bruckner and Bruckner 2006). Thus, it has been hypothesized that coral excavating sponge larvae prefer to attach and settle on surfaces free of other encrusting organisms and on sites with RM (Goreau and Hartman 1963, Chaves-Fonnegra et al. 2005, MacGeachy and Stearn 1976, Hartman 1977, López-Victoria and Zea 2005, Chaves-Fonnegra and Zea 2011). Hence, the increase of excavating sponges could be the result of an augmented settlement and successful recruitment on RM skeletons, which have become increasingly available owing to contemporary coral deterioration. The aim of this study was thus to evaluate current trends of *Cliona delitrix* recruitment, and to determine if larval settlement of this species occurs preferentially on RM. To demonstrate it we tested the following predictions:

1. There should be a larger proportion of small individuals (i.e., recruits) in *Cliona delitrix* populations.

2. *C. delitrix* recruits should be found more frequently on coral substratum of RM in comparison to OM. Thus recruits should occur preferentially on those coral species that are experiencing greater mortality.

3. *C. delitrix* individuals should occur more frequently on the top of coral colonies, where coral tissue should have been naturally alive, than on cracks and colony bases, where live coral tissue is naturally absent.

4. *C. delitrix* recruits should be found preferentially on very recently dead, still clean, coral calyces, near to or surrounded by live coral tissue. These small recruits have not yet been able to grow enough to attack coral polyps around its perimeter.

Methods

Belt and line transects

The proportion of recruits in *Cliona delitrix* populations and the original condition of the coral substratum were their larvae had settled, were estimated from data obtained on 40 m^2 (20 m x 2 m) belt transects. New recruitment was also recorded repeatedly visiting marked transects, and in transects where there had been massive coral death. Transects were haphazardly placed at five locations in the Greater Caribbean Sea: Bahamas (Inagua Islands, n= 4), Belize (Carrie Bow Key, n= 7), Colombia (San Andrés Island, n= 6), Panamá (Bocas del Toro Archipelago, n= 6) and USA (Conch reef, Key Largo, Florida, n= 4) (Figure 1). In each transect, a 20 m tape was stretched along the bottom, and a 1 m PVC rod used as a guide to swim along on both sides of the tape. To avoid possible taxonomical confusion with other orange papillated excavating sponges that typically occur on branching and platy corals (e.g., *Porites porites*, *Agaricia tenuifolia*), such corals were not included in any of the analyses. Also, in each locality 3 to 10 *C. delitrix*

recruits were collected to corroborate taxonomical identification with field identification, and pictures for most recruits were taken in each area to compare and check morphology.

Each *C. delitrix* within belt transects was measured using a caliper for small individuals, or a 1 m measuring tape fixed on the PCV rod for large individuals. *C. delitrix* recruits were arbitrarily defined as those individuals with a maximum diameter ≤ 5cm (the smallest recruit was 0.2 cm). These measurements were used to evaluate size distribution for each locality sampled. The relative proportion of recruits to larger individuals was used to infer the existence and extent of recruitment. Locations were also compared in their density of *C. delitrix* recruits, normalized to area of available coral substratum (live + dead, total and by species,). For this, first the linear distances of each coral species (or category) under the 20 m-long tape (linear transect) were recorded in the field. Then, the percent cover of each coral species (or category) was calculated through the following formula: % linear cover = (sum of distances in cm under the tape * 100 %) / 2000 cm. The area of specific coral species (or category) within the 40 m^2 of each belt transect was calculated as follows: Area in m^2= (% linear cover * 40 m^2) / 100. Density was then calculated with the following formula: Density = number of recruits in specific coral species (or category) / Area of specific coral species (or category). The Statgraphics Centurion program http://www.statgraphics.com/) was used for the statistical analyses.

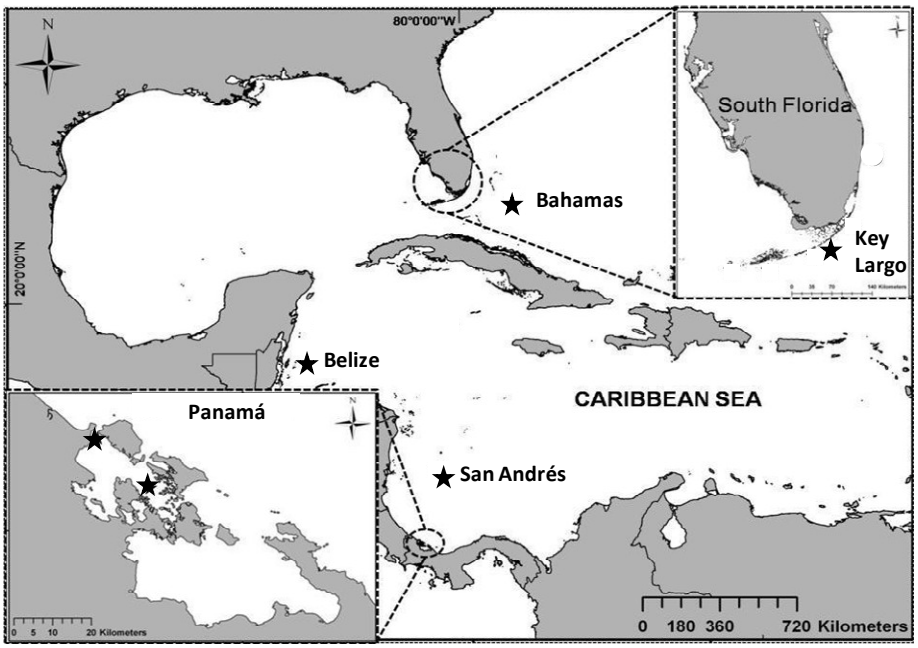


Figure 1. Study Area of Greater Caribbean Sea locations (sampling sites starred): Belize, Carrie Bow Cay (sampled January 2010); Colombia, San Andrés Island (April and November 2011); Panama, Bocas del Toro Archipelago (July 2010 and February 2012); Bahamas, Inagua Islands (July 2011); Florida, Key Largo, Conch reef (November 2010).

Inferring *C. delitrix* larval settlement

Place of larval settlement and the condition of the substratum for each individual sponge was inferred from transect observations as follows: (1) substratum preference, (2) position of the sponge recruits and adults on the coral, and (3) the condition of the coral substratum adjacent to recruits (present and new).

Substratum preference of recruits

Dead coral not covered by erect macroalgae, gorgonians, sponges and other invertebrates was designated as substratum available for *C. delitrix* larval settlement. This dead coral substratum was classified in categories ranging from newly dead to old (see Table 1, below: categories of coral mortality), and the density of *C. delitrix* recruits per unit area of these categories was taken as a measure of larval settlement preferences. Density of

adults was also quantified for comparisons. For this, first the linear distances of each dead coral substratum category under the 20 m-long tape (linear transect) were recorded in the field. Then, the percent cover of each dead coral category was calculated obtaining the % linear cover, then the area of each dead coral category within the 40 m^2 of each belt transect, and finally the density of recruits per coral mortality category, which follows the same formulae as explained in the belt and line transect section.

Categories of coral mortality

Under the linear transect and around sponge recruits dead coral was classified initially in two categories: Recent Mortality (RM) and Old Mortality (OM). Recent Mortality was further divided in two subcategories: New Mortality (NM), and Prior Mortality (PM). Definitions for each category are given in Table 1. When possible, these mortality categories were discriminated by coral species. In some instances, intensive grazing by herbivores (especially sea-urchins) could maintain the OM substratum clean enough to be classified as PM despite its age.

For all localities sampled, differences between recruit densities on Recent (Current + Prior) vs. Old substratum were evaluated by t-tests.

Table 1. Definition of substratum types.

Substratum type	Code	Description
1. Recent mortality	RM	Coral death about 1 to 3* years ago; clean calyces or coral skeleton covered by a thin layer of turf algae.
1a. New mortality	NM	Coral death from days to a few weeks ago; calyces mostly white and clean; dead and live portions are still at the same level (i.e., live polyps have not grown further upwards or dead calyces have not eroded downwards).
1b. Prior mortality	PM	Coral death from a few weeks to about 1 to 3 years ago; calyces eroded, turf algae generally present; neighboring live polyps are slightly higher than dead areas owing to growth of the former and/or grazing and erosion over the latter.
2.Old mortality (Coral rock)	OM	Coral death about > 3 years ago; coral surface has undergone extensive diagenesis and is encrusted by calcareous and turf algae and trapped sediments.

*This is an approximate estimate which probably varies among localities; in Bocas del Toro in 2012 we could still distinguish old mortality from the recent mass mortality occurred in 2010.

Position on coral

On the belt transects carried out in the Bahamas and San Andres, the position of sponge individuals (recruits and adults) on the coral colonies was recorded. To designate position, four categories were established: a) Base (on the coral base where naturally live coral tissue is usually absent); b) Crack (on cracks present in the coral colony); c) Top (on areas where live coral should be present in relation to colony shape). When sponges had grown too large or the entire coral colony was dead (and the coral substratum old), it was often impossible to discern the original position in which settlement occurred, therefore this cases were not included in the analysis.

Condition of coral substratum adjacent to recruits

Coral mortality around recruits was recorded and categorized as previously defined in Table 1. Then, the type of coral mortality around the sponge recruit was used to infer which substratum sponge larval settlement had occurred. We use the term settlement for the moment a larva reaches the substratum, attaches and begins metamorphosis. The term recruitment was defined as the moment in which an observer actually sees the settled individual. There can be mortality or substratum quality changes that go unnoticed by the observer in the time lapse between settlement and recruitment observations. Thus, we carefully observed the microhabitat and position of recruitment to infer the conditions of the substratum when settlement happened as follows: If sponge recruits were found on NM, the two possible options for larval attachment were on live coral tissue or on the same NM. The possibility of attachment on live coral tissue was discarded because it was assumed that live coral would be able to eat the larvae. If sponge recruits were on PM, the two possible options for larval attachment were on NM or the same PM. Whereas, for recruits found on OM the only possible option was that larvae attachment occurred on the same OM; it was consider that the time it takes for RM to become OM is probably longer than the time it takes for a larva to settle and become a recruit.

Estimation of new recruits

Six permanent transects were marked on the San Andrés Island western shallow terrace reef on April 2011 using galvanized stainless steel stakes (4 - 5") and buoys to record *C. delitrix* new recruitment. Initially, diagram maps of each transect including excavating sponges (recruits and adults) and currently unhealthy corals (from bleaching and dark or white spots diseases) were drawn, and pictures of recruits were taken. Recruitment was quantified 7 months later on November 2011. The number of new recruits was recorded on the spatial diagram drawn underwater and based on pictures. Initially unhealthy coral colonies were also checked to evaluate if there was recruitment onto their affected areas.

Study case: sponge recruitment after a stratified massive coral mortality

Bocas del Toro waters in Panama reached a temperature of 31.1 °C at 5 and 10 m deep on August and September 2010 (STRI Bocas station data). Satellite images from NOAA (NOAA, 2000) showed 8 DHW (=degree heating weeks) of thermal stress in this area from August 16 to September 30, 2010. Later we observed and confirmed that most of the coral reefs at 6 and 10 m in depth in one of our stations, Casablanca reef (9° 21´ 27.66´´ N; 82° 16´ 16.38´´ W), suffered bleaching, and later massive mortality. Which was probably produced by a density-driven temperature inversion in the study area (Neal et al. 2013).

As *C. delitrix* had been quantified on 40 m^2 transects one month before the coral bleaching event (July 25th- August 7th 2010), we could build a case study to evaluate the effects of massive coral mortality on *C. delitrix* sponge recruitment by recording recruits and adults one year and a half later (February 2012). By then, we could easily distinguish which coral substratum comprised RM from bleaching, and thus directly test the hypothesis that settlement and recruitment of *C. delitrix* is favored by this substratum. Transects had been placed between 4 and 6 m in depth in Adriana´s reef, and at 6 m, and 9 m in Casa Blanca reef (Figure 2) before the bleaching event, and were repeated thereafter in approximately the same areas.

Results

Recruit vs. adult proportion

C. delitrix of smaller size (0.2 to 5 cm) defined as recruits occurred in a greater number than mid-size (5.1 - 15 cm) and larger sponges (> 15.1 cm), except for Inagua Islands in the Bahamas, where both small and mid-size sponges occurred approximately in the same number (Figure 2). In general, *C. delitrix* had larger sizes in Bahamas, Colombia and Panama than in Belize and Florida. The prevalence of small sponges is thus indicative of on-going recruitment everywhere in the Greater Caribbean.

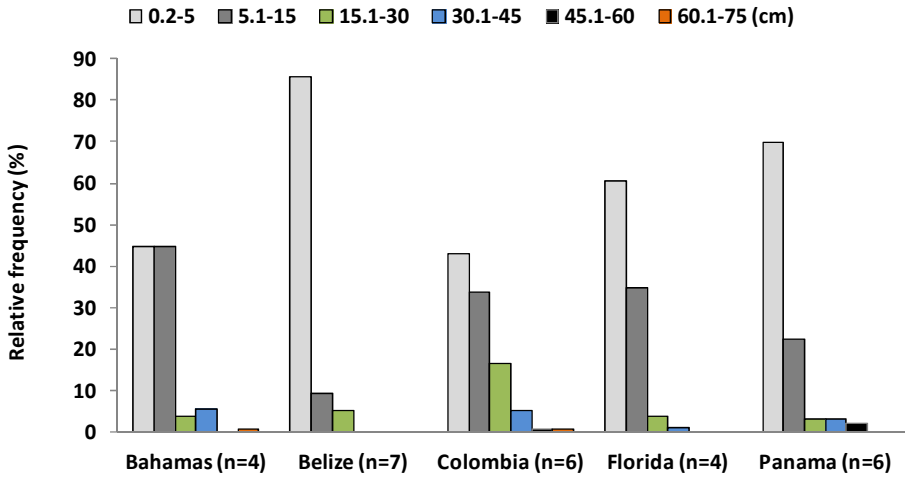


Figure 2. Size-frequency distribution of *Cliona delitrix* individuals in the five locations. The average depth of each location were: Bahamas (Great Inagua: 20 m, Little Inagua: 14 m), Belize (Patch reef: 8.5 m, Barrier reef: 13.5 m), Colombia (Wildlife: 9 m), Florida (Conch reef: 15.5 m, Conch reef Pinnacle: 23 m), Panamá (Adriana's reef: 4 - 6 m, Casa Blanca: 9 m).

Florida and Panama were the areas with higher density of *Cliona delitrix* individuals on coral substratum (live + dead, Table 2). In Belize (Carrie Bow Cay), Florida (Conch reef)

and Panamá (Bocas del Toro) the density of *C. delitrix* recruits was higher than the adults, whereas in Bahamas (Inagua Islands) and Colombia (San Andres Island) it tended to be similar. *C. delitrix* in the Greater Caribbean more often colonized the following coral species: *Diploria strigosa*, *Montastraea annularis, M. cavernosa*, *M. faveolata*, *M. franksi*, *Porites astreiodes* and *Siderastrea siderea*. The latter coral was the most constantly occupied by both recruits and adults (Table 2). The highest density of *C. delitrix* recruits was recorded on *Stephanocoenia intersepta* as a result of its very low cover.

Table 2. Density of *Cliona delitrix* recruits and adults per area (m^2) of massive coral species (live + dead). R: recruits; A: adults; (-): species was not present in the sampled area. The total density of recruits and adults on coral substratum (live + dead) is specified at the end of the table as the mean per location ± 1 standard deviation (of n transects).

	Bahamas (n=4)		**Belize (n=7)**		**Colombia (n=6)**		**Florida (n=4)**		**Panama (n=6)**		**Caribbean (n= 27)**	
Coral species	**R/m²**	**A/m²**	**R/m²**	**A/m²**	**R/m²**	**A/m²**	**R/m²**	**A/m²**	**R/m²**	**A/m²**	**R/m²**	**A/m²**
Colpophyllia natans	-	-	-	-	0	0	-	-	0.5	0.2	0.5	0.2
Diploria labyrinthiformis	-	-	0	0	0.9	0.4	-	-	0	0	0.5	0.2
Dichocoenia stokesi	-	-	-	-	0	0	-	-	-	-	0	0
Diploria strigosa	0.9	1.8	3.3	3.3	0.8	0.8	-	-	0.3	0.6	0.9	0.8
Orbicella annularis	0.3	0.4	-	-	0	0	3.8	2.6	2.2	0.3	0.8	0.4
Montastraea cavernosa	0	10	1.6	0.4	0.04	0.4	0.4	2.1	1.7	0	0.4	0.6
Orbicella faveolata	0	0	0	0	0	0	0	0	1.4	0.2	1.0	0.1
Orbicella franksi	1.7	2.0	-	-	0	0	2.5	1.1	0.4	0.2	1.0	0.9
Meandrina meandrites	-	-	-	-	0	0	0	0	-	-	0	0
Mussa angulosa	-	-	-	-	-	-	-	-	0	0	0	0
Porites astreoides	1.4	0	1.8	0	0.6	0	1.4	2.9	1.2	0	1.1	0.2
Solenastrea bournoni	-	-	-	-	-	-	0	1.0	0	0	0	0.9
Stephanocoenia intersepta	10.0	0	0	0	4.2	0	0	0	-	-	2.0	0
Siderastrea radians	-	-	-	-	0	0	0	0	-	-	0	0
Siderastrea siderea	1.6	0.4	1.7	0.3	1.2	1.6	7.6	7.6	4.6	1.7	1.9	1.6
Dead coral non identified	0.2	0.3	0.2	0.2	0.1	0.2	1.1	0.2	1.1	1.1	0.3	0.3
Total on coral (live+ dead)	**0.53 ± 0.33**	**0.60 ± 0.34**	**0.81 ± 0.94**	**0.24 ± 0.41**	**0.50 ± 0.22**	**0.6 ± 0.25**	**1.96 ± 0.92**	**1.01 ± 0.32**	**0.96 ± 0.84**	**0.43 ± 0.42**	**1.23 ± 1.3**	**0.54 ± 0.43**

Inferring *C. delitrix* larval settlement

Substratum preference of recruits

The mean density of recruits per m^2 was significantly higher on RM coral on all five Greater Caribbean reefs locations, rather than OM coral (Student-t test, Figure 3). Belize (Carrie Bow Cay) showed the highest density of recruits on RM, compared to the other four locations.

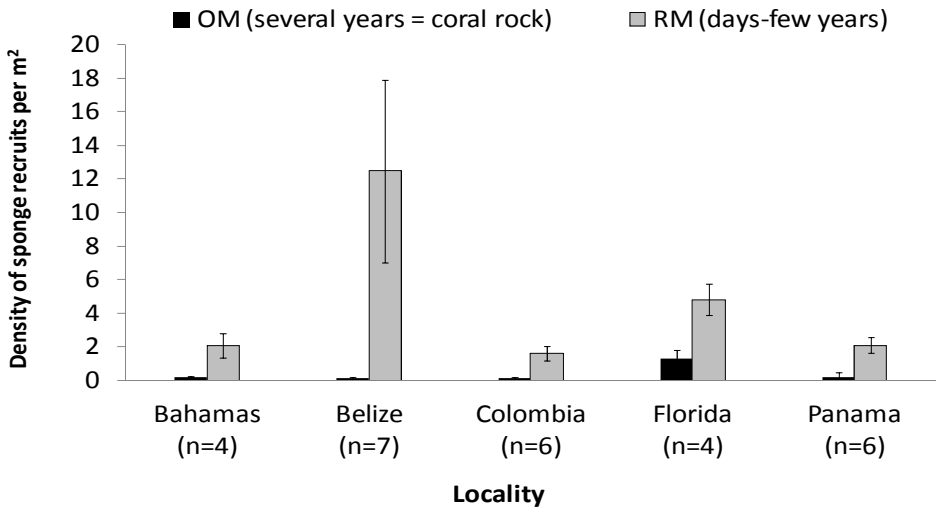


Figure 3. Substrate preferences of *Cliona delitrix* recruits. OM: old coral mortality (coral rock); RM: recent coral mortality (current+ prior coral mortality).

Position on coral and condition of substratum adjacent to recruits

The majority of individuals (recruits and adults) in the Bahamas and San Andrés transects were found on the top of coral colonies (Figure 4). That is, larvae attached mostly on areas where live coral tissue should have been naturally present. In an important number of large sponges we could not discern the possible position of the original place of settlement (ND in Figure 4). More recruits were surrounded by RM than by OM (Figure 5). Thus for presumed larval settlement, we estimate that 27.5 % of larvae attach to OM and 72.5 % on RM. It should be stated that the RM around recruits could not be caused

by the still too small sponge recruits. Also, although most sponge recruits observed on RM were in most cases on PM, some recruits were also on NM, which indicate that sponge settlement can happen at any moment after coral tissue dies.

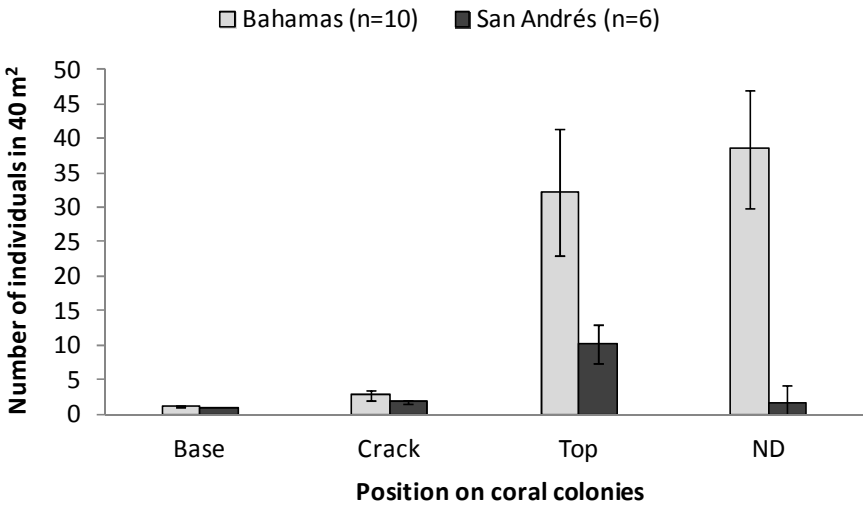


Figure 4. Position of *Cliona delitrix* individuals (recruits + adults) on coral colonies. Data obtained for Bahamas and San Andres Island (n=number of transects; ND=not discernible).

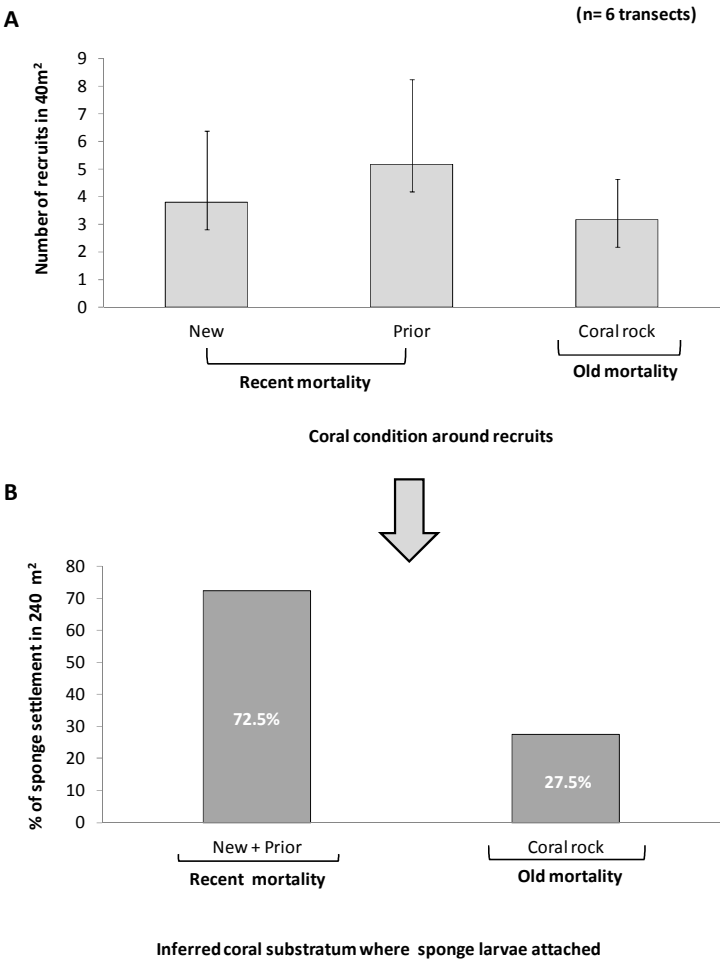


Figure 5. A) Coral tissue condition around *C. delitrix* recruits in San Andrés Island (Colombia) transects. B) Inferred larval settlement based on coral conditions around sponge recruits.

Estimation of new recruits

In April 2011, there were 58 *C. delitrix* recruits on the 240 m^2 of San Andrés Island reef. Seven months later, 15 new recruits were recorded in the same area; 10 on *Siderastrea siderea* coral (6 on RM and 4 OM), 4 on *Porites astreoides* coral (3 on RM and 1 on OM), and 1 on coral rock, species was not identified, (OM) (Figure 6). The recruitment rate was 0.0625 new recruits per m^2 in 7 months, if we extrapolate to a year= $0.1 \cdot m^{-2} \cdot yr^{-1}$. Previously bleached or diseased corals did not have any sponge recruits.

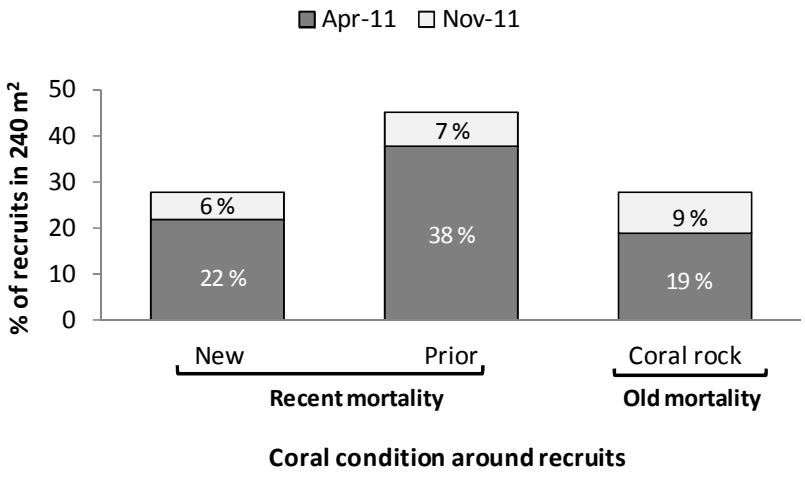


Figure 6. Percentage of the total number of recruits, per type of substratum on April 2011 (Before), plus the new ones observed in November 2011 (seven months later) in San Andrés Island reef.

Study case: sponge recruitment after a stratified massive coral mortality

The increase in water temperature and consequent bleaching and death occurred in a stratified gradient on the reef, with most mortality at mid depths. At Adriana´s reef (4 - 6 m depth), most corals, sponges, and other invertebrates survived. By contrast at Casa Blanca reef at 6 m, some organisms were affected, and below 9 m in depth most of them died. The shallows of Adriana´s reef (4 - 6 m) had almost the same numbers of *C. delitrix* recruits in 2010 and 2012. However at Casa Blanca reef at 6 m and 9 m deep the number of recruits increased in 2012. This increment was higher on RM rather than on OM at 9 m, where the live coral cover (only taking into account massive coral) decreased from 8.8 % to 1.8 % (Figure 7). Also, at 9 m the number of *C. delitrix* adults decreased 59 % from 2010 to 2012; their remains were clearly discernible in 2012, whereas in Adriana´s reef and in Casa Blanca at 6 m all adults of this species were alive in 2012. The rate of recruitment at Adriana´s reef was 0.01 recruits $\cdot$ m^{-2} $\cdot$ yr^{-1}, whereas at Casa Blanca at 6 m was 0.1 $\cdot$ m^{-2} $\cdot$ yr^{-1}, and at 9 m was 0.2 $\cdot$ m^{-2} $\cdot$ yr^{-1}.

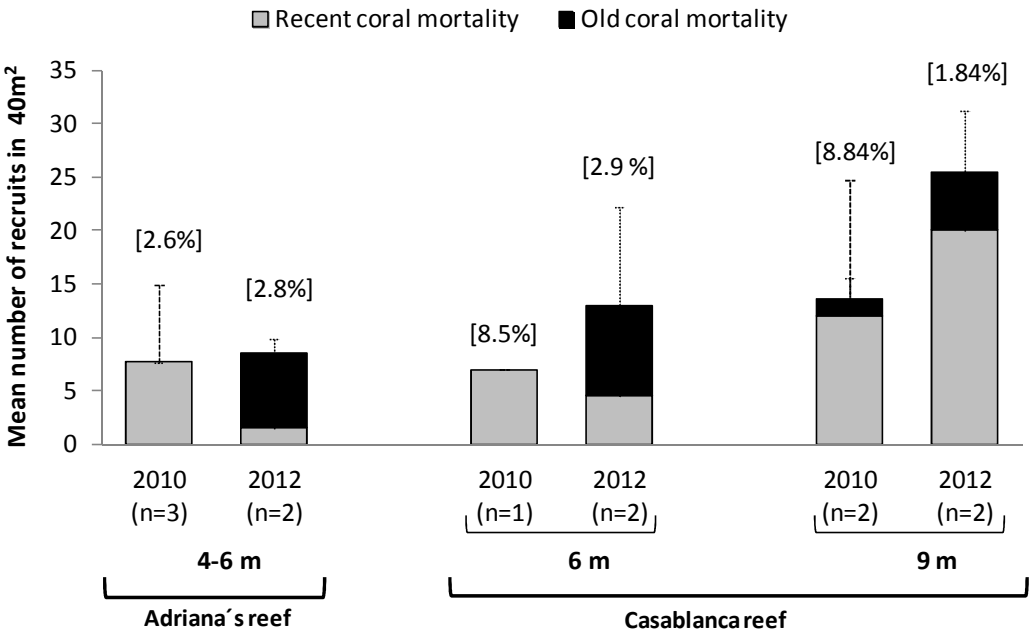


Figure 7. Average number of *Cliona delitrix* recruits on recent coral mortality (RM) and on old coral mortality (OM) in Bocas del Toro, Panamá, prior (August 2010) and after (February 2012) a density-driven temperature inversion in which coral and sponge death occurred below 6 m in depth. In brackets is the average percentage of live coral tissue in each corresponding year and at each depth (4 - 6 m, 6 m, and 9 m). The n value in parenthesis and under each year corresponds to the number of transects.

Discussion

This study contributes to the understanding of why excavating sponges, especially *C. delitrix*, are presently more abundant on Caribbean reefs. First, the size-distribution of *C. delitrix* showed that currently there are more recruits than adults on Caribbean reefs, indicative of a strong on-going recruitment throughout the Greater Caribbean. This pattern has also been found for other excavating sponges such as *C. aprica*, *C. caribbaea* and *C. tenuis*. However, the proportion of small individuals (recruits) to adults varies depending on the studied areas (López-Victoria and Zea 2005, González-Rivero et al. 2013).

In the Caribbean Sea the density of *C. delitrix* recruits and adults was higher on *Siderastrea siderea* coral, a preferred species to colonize by *Cliona* excavating sponges (Chaves-Fonnegra and Zea 2011, López-Victoria, Zea, and Weil 2006). One possible reason for this preference is that Dark Spot Syndrome affects *S. siderea* frequently across most of this coral's distribution range (Gil-Agudelo et al. 2004, Renegar et al. 2008). This disease can kill and open spots of RM. Although we did not find recruitment of *C. delitrix* on mapped corals with diseases or bleaching, other studies found that both corals and *C. delitrix* recruits can settle and recruit on recently dead coral skeletons (Edmunds 2000). Considering the increase and greater survival of excavating sponges over corals under thermal stress and that *C. delitrix* can also preempt space for scleractinian coral larval attachment, it is possible that this sponge is reducing the chances of coral recruits to find suitable substratum to settle.

C. delitrix recruits were preferentially found on RM over OM. During 1960s and 1970s *Cliona* recruitment was recorded on the bases of dead coral where tissue recedes as result of natural coral growth (Goreau and Hartman 1963, Pang 1973, MacGeachy and Stearn 1976). We demonstrated that *C. delitrix* larvae prefer to settle on top of coral colonies where coral tissue should have been live. Thus, contemporary coral mortality opens new space for *C. delitrix* larvae to settle. Why would sponge larvae prefer to settle on RM? We posit that in RM clean coral calyces are exposed and offer larvae sheltered spaces between septa and easy access into coral skeleton. On OM, sponge larvae will have to search for an appropriate space, attaching on or between calcareous algae and other invertebrates, making entrance and excavation harder and longer. Another advantage for *C. delitrix* attaching on RM is that its lateral extension can be faster, as shown by Chaves-Fonnegra and Zea (2011) for adults. This faster establishment on clean calyces allows excavating sponges to undermine and displace surrounding live coral tissue, as they grow laterally overpowering coral heads more rapidly (López-Victoria et al., 2006).

The preference of *C. delitrix* larvae for RM implies that its recruitment is more successful in the time window of coral mortality: from the moment when clean coral calyces are available to before calyces become heavily incrusted by other organisms (from hours to

up to 1 - 3 years after). Indeed, the special study case in Panama showed that after a coral mortality event in 2010, *C. delitrix* recruitment increased where the amount of RM was higher, and in the Bahamas and San Andrés most *C. delitrix* recruits were also found on RM, more on PM than on NM. Which is logical consequence of the fact that NM is transient, becoming PM within weeks. This means that *C. delitrix* larval settlement is probably occurring very soon after coral tissue dies, but it is not always possible to observe it.

It is important to consider that for higher *C. delitrix* recruitment to occur, sponge reproduction, larval development and dispersal have to coincide with coral mortality episodes. Interestingly, *C. delitrix* reproduction in Florida occurs from April to December and can have several reproductive pulses when sea-water temperature reaches its highest during the summer from May to August (see Chapter 1). Multiple spawning events allow for a continuous release of larvae and recruitment if coral mortality is occurring. Summer time of the year is when most thermal stress and consequent coral bleaching and coral mortality events occur in Florida and the Caribbean Sea (Eakin et al. 2010). Thus the spatial-temporal scale in which coral tissue death and attachment of sponge larvae coincide, gives an advantage to this sponge over corals. Although the thermal stress of 8 DHW (NOAA, 2000) can also be lethal for *C. delitrix* sponges, which was evident in Bocas del Toro reefs after the warming event of 2010, sponge mortality did not impede a higher local recruitment. Sponge larvae were probably produced at all depths. Taking into account these conditions, we consider that the current success of *C. delitrix* is due to a higher survivorship during thermal stress events, coupled to a higher success of sponge larvae attachment when the most suitable substratum, NM, becomes more available.

The recruitment rate of *Cliona delitrix* at Bocas del Toro increased in deep areas where coral mortality was higher, and it was similar than at San Andres Island (0.1 to 0.2 recruits $\cdot$ m^{-2} $\cdot$ yr^{-1}). This suggests that chronic coral mortality, smaller and more localized on colonies in San Andrés, creates opportunities for *C. delitrix* larvae to attach in the same way that the 2010 massive coral mortality event did in Bocas del Toro. *C. delitrix* recruitment rate in natural habitat was lower than for excavating sponge *C. tenuis*

($2.5 \cdot m^{-2} \cdot yr^{-1}$) in Glover´s Atoll, Belize (González-Rivero et al. 2013), for non-excavating sponge *Polymastia* sp. ($9.6 \cdot m^{-2} \cdot yr^{-1}$) in Northern New Zealand (Ayling 1980), and for an assemblage of different species of sponges ($3.4 \cdot m^{-2} \cdot yr^{-1}$) in Discovery Bay, Jamaica (Hughes 1996).

As zooxanthellate excavating sponges also appear to be currently settling on the top of coral colonies (López-Victoria et al., 2005), in a constant process (González-Rivero et al. 2013), and may also be more resistant than corals to thermal stress (Márquez, Zea, and López-Victoria 2006, Schönberg and Suwa 2007, Schönberg et al. 2008), our *C. delitrix* findings may apply to them as well.

Adaptations such as migrating zooxanthellae inside the tissue (Schönberg et al. 2008), maintenance of pigmentation at different temperatures (Miller et al. 2010), increase of boring rates at lower pH (Wisshak et al. 2012, Duckworth and Peterson 2012), and increase growth at higher temperatures (Cortés et al. 1984, Rützler 2002, Nicol and Reisman 1976) will give an advantage to these sponges under a more acidic and warmer environment. However, preferential recruitment on calcium carbonate and on specific species of corals or oysters may limit them. Besides thermal stress, other factors which contribute to coral mortality as contamination and indiscriminate fisheries will also open space for sponge larvae to attach. Thus, increasing abundance of excavating sponges on reefs will probably depend on the amount of RM at the time of the sponges' reproductive peaks, but could be limited by possible survivor competitors as algae, other sponges and colonial tunicates with faster growth rates (López-Victoria, Zea, and Weil 2006, Chaves-Fonnegra and Zea 2011).

Conclusions

C. delitrix is recruiting abundantly on Greater Caribbean coral reefs, preferentially on RM than on OM, and on top of coral colonies, where live coral tissue should have been. Clean coral substratum, free of algae and other invertebrates would make it easier for larvae to attach and take root. As contemporary, chronic or massive coral mortality creates new clean space for settlement, increased abundance of *C. delitrix* and other excavating sponges can be explained by the coincidence in time and space of larval production with the availability of new coral death. The apparently lower effect of thermal stress on excavating sponges (adults and larvae) than on corals, may be another factor contributing to the current success of these sponges on Caribbean reefs.

References

Antonius, A., and E. Ballesteros. 1998. "Epizoism: a new threat to coral health in Caribbean reefs." *Revista de Biología Tropical* 46 (5):145-156.

Aronson, R.B. , W.F. Precht, M.A. Toscano, and K.H. Koltes. 2002. "The 1998 bleaching event and its aftermath on a coral reef in Belize." *Marine Biology* 141:435-447.

Ayling, A.L. 1980. "Patterns of sexuality, asexual reproduction and recruitment in some subtidal marine Demospongiae." *Biological Bulletin* 158:271-282.

Bouchet, P., and K. Rützler. 2003. "CLIONIDAE d'Orbigny, 1851 (Porifera, Hadromerida): proposed emendment of spelling to CLIONAIDAE to remove homonymy with CLIONIDAE Rafinesque, 1815 (Mollusca, Pteropoda)." *Bulletin of Zoological Nomenclature* 60 (2):99-102.

Bruckner, A.W. , and R.J. Bruckner. 2006. "The recent decline of *Montastraea annularis* (complex) coral populations in western Curaçao: a cause for concern?" *Revista de Biología Tropical* 54 (3):45-58.

Calcinai, B., C. Cerrano, N. Iwasaki, and G. Bavestrello. 2008. "Sponges boring into precious corals: an overview with description of a new species of *Alectona* (Demospongiae, Alectonidae) and a worldwide identification key for the genus." *Marine Ecology* 29:273–279.

Chaves-Fonnegra, A., M. López-Victoria, F. Parra, and S. Zea. 2005. "Ecología química de las esponjas excavadoras *Cliona aprica*, *C. caribbaea*, *C. delitrix* y *C. tenuis*." *Boletín de Investigaciones Marinas y Costeras* 34 (1):43-67.

Chaves-Fonnegra, A., and S. Zea. 2011. "Coral colonization by the encrusting excavating Caribbean sponge *Cliona delitrix*." *Marine Ecology* 32:162-173.

Chaves-Fonnegra, A., S. Zea, and M.L. Gómez. 2007. "Abundance of the excavating sponge *Cliona delitrix* in relation to sewage discharge at San Andrés Island, SW Caribbean, Colombia." *Boletín de Investigaciones Marinas y Costeras* 36:63-78.

Cortés, J., M. Murillo, H.M. Guzmán, and J. Acuña. 1984. "Pérdida de zooxantelas y muerte de corales y otros organismos arrecifales en el Caribe y Pacífico de Costa Rica." *Revista de Biología Tropical* 32:227-231.

Díaz, M., and K. Rützler. 2001. "Sponge: an essential component of Caribbean coral reefs." *Bulletin of Marine Science* 69 (2):535-546.

Duckworth, A.R., and B.J. Peterson. 2012. "Effects of seawater temperature and pH on the boring rates of the sponge *Cliona celata* in scallop shells." *Marine Biology* xx:xx.

Eakin, C.M., J.A. Morgan, S.F. Heron, T.B. Smith, G. Liu, L. Alvarez-Filip, B. Baca, Erich Bartels, Carolina Bastidas, Claude Bouchon, Marilyn Brandt, Andrew W.Bruckner, Lucy Bunkley-Williams, Andrew Cameron, Billy D. Causey, Mark Chiappone, Tyler R. L.Christensen, M. James C. Crabbe, Owen Day, Elena de la Guardia, Guillermo Dı´az-Pulido, Daniel DiResta, Diego L. Gil-Agudelo, David S. Gilliam, Robert N. Ginsburg, Shannon Gore, Héctor M. Guzmán, James C. Hendee, Edwin A. Hernández-Delgado, Ellen Husain, Christopher F. G. Jeffrey, Ross J. Jones, Eric Jordán-Dahlgren, Les S. Kaufman, David I. Kline, Philip A. Kramer, Judith C. Lang, Diego Lirman, Jennie Mallela, Carrie Manfrino, Jean-Philippe Mare´ chal, Ken Marks, Jennifer Mihaly, W. Jeff Miller, Erich M. Mueller, Erinn M.Muller, Carlos A. Orozco Toro, Hazel A. Oxenford, Daniel

Ponce-Taylor, Norman Quinn, Kim B.Ritchie, Sebastián Rodríguez, Alberto Rodríguez Ramírez, Sandra Romano, Jameal F. Samhouri, Juan A. Sánchez, George P. Schmahl, Burton V. Shank, William J. Skirving, Sascha C. C. Steiner, Estrella Villamizar, Sheila M. Walsh, Cory Walter, Ernesto Weil, Ernest H. Williams, Kimberly Woody Roberson, and Yusri Yusuf. 2010. "Caribbean corals in crisis: record thermal stress, bleaching, and mortality in 2005." *PlosOne* 5 (11):1-9.

Edmunds, P. J. . 2000. "Recruitment of scleractinians onto the skeletons of corals killed by black band disease." *Coral Reefs* 19:69-74.

Fell, P.E. 1976. "The reproduction of *Haliclona loosanoffi* and its apparent relationship to water temperature." *Biological Bulletin* 150 (2):200-210.

Gardner, T.A., Coté I.M., J.A. Gill, A. Grant, and A.R. Watkinson. 2003. "Long-term region-wide declines in Caribbean corals." *Science* 301 (5635):958-960.

Gil-Agudelo, D.L. , G.W. Smith, J. Garzón-Ferreira, E. Weil, and D. Petersen. 2004. "Dark spots disease and yellow band disease, two poorly known coral diseases with high incidence in Caribbean reefs." In *Coral health and disease*, edited by E. Rosenberg and Y. Loya, 337-350. Berlin: Springer.

González-Rivero, M., A.V. Ereskovsky, C.H.L. Schönberg, R. Ferrarie, J. Fromonte, and P.J. Mumby. 2013. "Life-history traits of a common Caribbean coral-excavating sponge, *Cliona tenuis* (Porifera: Hadromerida)." *Journal of Natural History* "in Press" DOI 10.1080/00222933.2013.802042.

Goreau, T.F., and W.D. Hartman. 1963. "Boring sponges as controlling factors in the formation and maintenance of coral reefs." In *Mechanisms of hard tissue destruction*, edited by R. F. Soggneas, 25-54. Washington D.C.: American Association for the Advancement of Science

Hartman, W.D. 1958. "Natural history of the marine sponges of southern New England." *Bulletin of Peabody Museum of Natural History* 19 (3):229-242.

Hartman, W.D. 1977. "Sponge as reef builders and shapers." *American Association of Petroleum Geological Studies in Geology* 4:127-134.

Holmes, K.E. . 1997. "Eutrophication and its effect on bioeroding sponge communities." Proc 8th Int Coral Reef Symp, Panamá.

Hughes, T.P. 1994. "Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef." *Science* 265 (5178):1547-1551.

Hughes, T.P. 1996. "Demographic approaches to community dynamics: a coral reef example." *Ecology* 77 (7):2256-2260.

López-Victoria, M., and S. Zea. 2004. "Storm-mediated coral colonization by an excavating Caribbean sponge." *Climate Research* 26:251-256.

López-Victoria, M., and S. Zea. 2005. "Current trends of space occupation by encrusting excavating sponges on Colombian coral reefs." *Marine Ecology* 26:33-41.

López-Victoria, M., S. Zea, and E. Weil. 2006. "Competition for space between encrusting excavating Caribbean sponges and other coral reef organisms." *Marine Ecology Progress Series* 312:113-121.

MacGeachy, J.K. 1977. "Factors controlling sponge boring in Barbados reef corals." Proc 3rd Int Coral Reef Symp, Miami.

MacGeachy, J.K., and C. Stearn. 1976. "Boring by macro-organisms in the coral *Montastraea annularis* on Barbados reefs." *Int Revue ges Hydrobiology* 61:715-745.

Maldonado, M. 2006. "The ecology of the sponge larva." *Canadian Journal of Zoology* 84 (1):175-194.

Maldonado, M., and M.J. Uriz. 1998. "Microrefuge exploitation by subtidal encrusting sponges: patterns of settlement and post-settlement survival." *Marine Ecology Progress Series* 174 (1):141-150.

Márquez, J.C., S. Zea, and M. López-Victoria. 2006. "Is competition for space between the encrusting excavating sponge Cliona tenuis and corals influenced by higher-than-normal temperatures?" *Boletín de Investigaciones Marinas y Costeras* 35:257-263.

McKenna, S.A. 1997. "Interactions between the boring sponge, *Cliona lampa* and two hermatypic corals from Bermuda." Proc 8th Int Coral Reef Symp, Panama.

McMurray, S. E., T. P. Henkel, and J. Pawlik. 2010. "Demographics of increasing populations of the giant barrel sponge *Xestospongia muta* in the Florida Keys." *Ecology* 91 (2):560-570.

Miller, A.N., K.B. Strychar, T.C. Shirley, and K. Rützler. 2010. "Effects of heat and salinity stress on the sponge *Cliona Celata*." *International Journal of Biology* 2 (2):3-16.

Neal, B.P., C. Condit, G. Liu, S. dos Santos, M. Kahru, B.G. Mitchell, and D. I. Kline. 2013. "When depth is no refuge: cumulative thermal stress increases with depth in Bocas del Toro, Panama." *Coral Reefs* "in Press" DOI 10.1007/s00338-013-1081-6.

Nicol, W.L. , and H.M. Reisman. 1976. "Ecology of the boring sponge (*Cliona celata*) at Gardiner's Island." *Chesapeake Science* 17 (1):1-7.

NOAA. 2000. "NOAA Coral Reef Watch Operational 50-km Satellite Coral Bleaching Degree Heating Weeks Product, Jan. 1, 2001-Dec. 31, 2010." NOAA Coral Reef Watch. Accessed Data set accessed 2011-01-05 at http://coralreefwatch.noaa.gov/satellite/hdf/index.html

Norström, A.V., M. Nyström, J. Lokrantz, and C. Folke. 2009. "Alternative states on coral reefs: beyond coral-macroalgae phase shifts." *Marine Ecology Progress Series* 376:295-306.

Pang, R.K. 1973. "The ecology of some Jamaican excavanting sponges." *Bulletin of Marine Science* 23 (2):227-243.

Pawlik, J.R. 2011. "The chemical ecology of sponges on Caribbean reefs: natural products shape natural systems." *BioScience* 61 (11):888-898.

Pomponi, S.A., and D.W Meritt. 1990. "Distribution and life history of the boring sponge *Cliona truitti* in the Upper Chesapeake Bay." In *New perspectives in sponge biology*, edited by K. Rützler, 384-390. Washington: Smithsonian Institution Press.

Renegar, D.A., P.L. Blackwelder, J.D. Miller, D.J. Gochfeld, and A.L. Moulding. 2008. "Ultrastructural and histological analysis of Dark Spot Syndrome in *Siderastrea siderea* and *Agaricia agaricites*." Proceedings of the 11th International Coral Reef Symposium, Ft. Lauderdale, Florida.

Rose, C.S., and M.J. Risk. 1985. "Increase in *Cliona delitrix* infestation of *Montastraea cavernosa* heads on an organically polluted portion of the Grand Cayman." *P.S.Z.N.I. Marine Ecology* 6 (1):345-363.

Rützler, K. 2002. "Impact of crustose clionid sponges on Caribbean reef corals." *Acta Geologica Hispánica* 37 (1):61-72.

Rützler, K. 2003. "Sponges on coral reefs: a coomunity shaped by competitive cooperation." VI Internacional Sponge Conference, Genova.

Schönberg, C.H.L. , and R. Suwa. 2007. "Why bioeroding sponges may be better hosts for symbiotic dinoflagellates than many corals." In *Porifera research: biodiversity, innovation and sustainability.*, edited by M.R. Custódio, G. Lôbo-Hajdu, E. Hajdu and G. Muricy, 569-580. Rio de Janeiro: Museu Nacional.

Schönberg, C.H.L., R. Suwa, M. Hidaka, and W.K. Weng Loh. 2008. "Sponge and coral zooxanthellae in heat and light: preliminary results of photochemical efficiency monitored with pulse amplitude modulated fluorometry." *Marine Ecology* 29:247-258.

Sutherland, J.P., and R.H. Karlson. 1977. "Development and stability of the fouling community at Beaufort, North Carolina." *Ecological Monographs* 47:425-446.

Ward-Paige, C.A., M.J. Risk, O.A. Sherwood, and W.C. Jaap. 2005. "Clionid sponge surveys on the Florida Reef Tract suggest land-based nutrient inputs." *Marine Pollution Bulletin* 51:570-579.

Wells, W.H., M.J. Wells, and I.E. Gray. 1964. "Ecology of sponges in Hatteras Harbor, North Carolina." *Ecology* 45 (4):752-767.

Wisshak, M., C.H.L. Schönberg, A. Form, and A. Freiwald. 2012. "Ocean Acidification Accelerates Reef Bioerosion." *PLOSOne* 7 (9):1-8.

Zea, S. 1993. "Recruitment of Demosponges (Porifera, Demospongiae) in rocky and coral reef habitats of Santa Marta, Colombian Caribbean " *P.S.Z.N.I: Marine Ecology* 14 (1):1-21.

CHAPTER 3: POPULATION STRUCTURE AND DISPERSAL OF THE CORAL EXCAVATING SPONGE *Cliona delitrix*

Abstract

Excavating sponges of the genus *Cliona* strongly compete with live reef corals, often killing and bioeroding entire colonies. During the past few decades the increase of excavating sponges on Caribbean reefs has been correlated with pollution and coral degradation. However, important aspects affecting their distribution, such as dispersal capability and population structure remain largely unknown. Thus, the aim of this study was to determine levels of genetic connectivity and dispersal of *Cliona delitrix* across the Greater Caribbean (Caribbean Sea, Bahamas and Florida), to understand current patterns and possible future trends in their distribution and effects on reefs. Ten species-specific microsatellite markers were developed *de novo* and used to genotype 504 individuals from 12 different locations. We found high levels of genetic differentiation and six genetic populations: one in the Atlantic (Florida-Bahamas), one specific to Florida, and four in the South Caribbean Sea. In Florida, two independent breeding populations are likely separated by depth. Gene flow and ecological dispersal occurs among other populations in the Florida reef track, and between some Florida locations and the Bahamas. Similarly, gene flow occurs between populations in the south Caribbean Sea but appears restricted between the Caribbean Sea and the Atlantic (Florida-Bahamas). Dispersal of *C. delitrix* was farther than expected for a marine sponge, and results support the influence of ocean current patterns on genetic connectivity in the South Caribbean Sea, within the Florida reef track, and between Florida and the Bahamas. As coral mortality continually creates newly available habitat for recruitment, *C. delitrix* populations will continue to grow. Dispersal should be favored in areas where currents are strong enough to transport sponge eggs or larvae over longer distances.

Introduction

Sponges are the dominant habitat-forming animals in Caribbean coral reefs (Pawlik 2011). Their diversity is higher than all coral groups combined, and their biomass (weight, volume) can exceed corals and algae (Rützler 1978, Díaz and Rützler 2001). The increase of sponges over corals in Caribbean reefs has been attributed to factors such as pollution, overfishing, and coral mortality (Loh and Pawlik In press, Chaves-Fonnegra, Zea, and Lopez Submitted, Ward-Paige et al. 2005). Sponges can tolerate pollution better than corals, owing to their heterotrophic filter capacity (Reiswig 1971). Thus some sponge species have become abundant in areas where sewage pollution occurs (Ward-Paige et al. 2005, Rose and Risk 1985, Holmes 1997). Simultaneously, overfishing has removed sponge predators, which can increase the competition for space between faster-growing palatable sponges and reef-building corals (Loh and Pawlik In press). Higher coral mortality has also opened more habitat for sponge larvae to attach to dead coral skeletons (Chaves-Fonnegra, Zea, and Lopez Submitted, Chapter 2).

In general, many sponges have successful survival strategies and compete for space, modifying coral reefs through bioerosion (Chaves-Fonnegra and Zea 2007, Rützler 1975), and calcification or cementation (Wulff and Buss 1979, Wulff 1984); these impact and change coral cover (Diaz and Rützler, 2001). Coral excavating sponges can bioerode entire coral colonies through chemical and physical mechanisms (Pomponi 1979, Zundelevich, Lazar, and Ilan 2007). These sponges have become more abundant during the past few decades in the Caribbean Sea and Florida (Ward-Paige et al. 2005, Chaves-Fonnegra, Zea, and Gómez 2007, López-Victoria and Zea 2004). Specifically, *Cliona delitrix*, has become one of the most destructive species, with its ability to kill coral tissue (Chaves-Fonnegra and Zea 2007), and excavate 10-12 cm inside coral skeletons, spreading laterally at mean rates of ~1.5 cm y^{-1} (Chaves-Fonnegra and Zea 2011). This sponge currently is one of the most conspicuous in the Florida Keys, as well as in other areas in the Caribbean Sea (Chaves-Fonnegra, Zea, and Gómez 2007, Rose and Risk 1985, Ward-Paige et al. 2005).

The relatively high abundance, species richness, and important ecological functions of sponges establish them as a dominant group that can structure and modify Caribbean reef ecosystems (Pawlik 2011, Díaz and Rützler 2001, de Goeij et al. 2013). Consequently, understanding population dynamics of marine sponges will be vital to potentially predict future changes at a reef level, especially for those sponges that can have a strong impact on reef-building corals, such as excavating sponges. Important aspects affecting excavating sponges' distribution, such as dispersal capability and population genetics structure remain largely unknown. Reproductive modes of *C. delitrix* have recently been characterized (Chaves-Fonnegra et al. In preparation, Chapter 2).The only population genetics study on excavating sponges estimated that the dispersal distance of *C. delitrix* reaches beyond 10-100 m, and appeared to be sexual through larvae, rather than asexually through fragmentation or clonality (Zilberberg et al., 2006).

A prevailing view is that Poriferan species generally exhibit low dispersal since most larvae remain in the water column for a short period of time, minutes to few days, and usually less than 2 weeks (Bergquist and Sinclair 1968, 1973, Maldonado 2006, Mariani, Uriz, and Turon 2000, Ilan and Loya 1990, Meroz and Ilan 1995). The larvae of *Cliona* sponges are currently defined as clavablastula (Maldonado 2006, Maldonado and Bergquist 2002), also with low dispersal (Mariani, Uriz, and Turon 2000, Mariani et al. 2006). Observations of *Cliona viridis* indicate that its larvae are small (300 µm length x 100 µm width) and weak swimmers that can stay in the water column for less than 10 days (Mariani, Uriz, and Turon 2000, Mariani et al. 2006). In laboratory, and inside beakers, larvae from *C. celata* can swim continuously on the surface for only 20 to 30 h with no apparent reaction to daylight or darkness; then they start to creep in the bottom for approximately the same time they spend at surface, until attaching (Warburton 1966).

Early population genetics studies in the phylum Porifera used allozymes to determine phylogenetic and population level differences in allele frequencies, and contributed to the discovery of cryptic species (Solé-Cava and Thorpe 1986, Sara et al. 1988). Mitochondrial DNA (mtDNA), specifically the commonly used COI gene (Folmer et al. 1994), have been useful, but in species-dependent manner due to the relatively low DNA

substitution rates discovered in sponges and many other marine invertebrates (Lavrov et al. 2005, Kayal et al. 2012). In some cases mtDNA was inappropriate for phylogeographic analyses in sponges (Wörheide 2006, Duran, Pascual, and Turon 2004), albeit with exceptions (Lopez-Legentil and Pawlik 2009, Debiasse 2010). Similarly, nuclear markers such as internal transcribed spacer (ITS) and nuclear rDNA sequences have also proven to be useful in some sponge population genetic studies on *Leucetta* (Bentlage and Wörheide 2007). However, in other species such as *Axinella corrugata,* the ITS marker had limited variability (Lopez et al. 2002).

In this context, nuclear DNA microsatellite loci (also known as simple tandem repeats, or STRs) have been considered the more informative and variable nuclear markers for population genetics for many organisms, including marine sponges. Microsatellite loci have been identified in at least five demosponge species to date - *Halichondria panacea, Crambe crambe, Scopalina lophyropoda*, *Xestospongia muta* and *Spongia officinalis* (Dailianis et al. 2011, Knowlton et al. 2003, Richards, Feldheim, and Shivji 2008, Blanquer, Uriz, and Pascual 2005, Duran et al. 2004) - and also one calcareous sponge, *Paraleucilla magna* (Guardiola, Frotscher, and Uriz 2012). Most of these microsatellite markers have been useful for comparisons within the Mediterranean Sea (Dailianis et al. 2011, Guardiola, Frotscher, and Uriz 2012), and between the Mediterranean and Atlantic populations (Duran et al. 2004). Also they have helped find genetic structure at very short spatial scales, between populations separated by only 10 to 50 m (Guardiola, Frotscher, and Uriz 2012), ~25 to 100 m (Blanquer, Uriz, and Caujapé-Castells 2009), and between individuals separated 0 - 7 m of distance from each other (Calderón et al. 2007).

Specifically for the Greater Caribbean (defined here as the Caribbean Sea, Bahamas and Florida), population genetic studies of marine sponges are scarce. Three studies exist to date: one with the common branching vase sponge *Callyspongia vaginalis* (Debiasse 2010), and two with the giant barrel sponge *Xestospongia muta* (Lopez-Legentil and Pawlik 2009, Richards 2010). Viviparous *C. vaginalis* populations appeared highly structured in the Florida reef system, on a scale as small as tens of kilometers in some locations. However, these populations were not isolated by distance, with some larval

dispersal and gene flow appearing to have occurred across this geographic area (Debiasse 2010). For the oviparous *Xestospongia muta*, both mitochondrial and microsatellites markers showed low population differentiation between Honduras/Belize and the Florida reef tracks, separated by at least 1000 km (Richards 2010, Lopez-Legentil and Pawlik 2009). This finding suggests that the Caribbean and loop currents allow for larval transportation between these areas. In contrast, higher levels of differentiation across roughly 300 km were found between Florida locations and the Bahamas, which was attributed to the strong flow of the Florida Current that impedes larval exchange between these geographic locations (Lopez-Legentil and Pawlik 2009, Richards 2010).

With evidence that excavating sponges continue to increase in abundance on Caribbean coral reefs (Ward-Paige et al. 2005, Rützler 2002, Cortés et al. 1984, Chaves-Fonnegra, Zea, and Lopez Submitted, Chapter 2), we have evaluate levels of genetic connectivity and dispersal of *Cliona delitrix* across the Greater Caribbean using microsatellite DNA markers. This study represents the first in-depth investigation of population connectivity on any excavating sponge using this type of nuclear gene marker, which is a valuable tool to monitor population genetics dynamics of this important coral competitor on currently deteriorating coral reefs.

The hypotheses were: 1) *Cliona delitrix* populations will be highly structured; each of the geographic areas selected in the study will constitute isolated populations. 2) *Cliona delitrix* dispersal will be larger than 100 m distances based on Zilberberg et al. (2006) estimates with allozymes markers in Bahamas reefs, contrasting with the general consensus about sponge dispersal.

Methods

Sample collection and DNA extraction

A total of 540 samples were collected from coral reefs in 12 locations within the Great Caribbean (see Table 1). All samples were placed in 15 ml falcon tubes and preserved in 95 % ethanol and kept at -20 °C.; three changes in 95 % ethanol after 1 h, 24 h and 3 days were made to eliminate some secondary metabolites before DNA extractions. Genomic DNA was extracted from 50 mg of *Cliona delitrix* tissue (mostly from oscula to avoid calcium carbonate) using the DNeasy Tissue Kit (Qiagen Inc.). During the incubation step with proteinase K, samples were agitated for 12 h at 60 °C at 150 rpm in a Sheldon VWR 1575 Incubator. One modification to the kit was made: after incubation in proteinase K, instead of vortexing the sample (tissue + calcium carbonate + spicules), we only took the supernatant from each sample, avoiding mixing with any residue at the bottom of tubes, to eliminate spicules or calcium carbonate that can clog the filter-columns in subsequent steps.

Table 1. Number (n) of *Cliona delitrix* samples collected, amplified, and analyzed from the Greater Caribbean (including Caribbean Sea, Bahamas and Florida). Amp - total number of samples amplified at least with 7 of the 10 microsatellites; Analyz - total number of samples analyzed after eliminating clones; Collect - total number of samples collected in each specific geographic area.

Geographic Region	Location	Specific area	Label	Depth (m)	Collect	Amp	Analyz
Atlantic Ocean	Bahamas	Various areas (Bimini – Cat Cays – New Providence – Little San Salvador - San Salvador –Inaguas)	BH	4-18	46	43	42
Atlantic Ocean	Florida	Dania Beach (shallow reef)	DB	9	31	30	25
Atlantic Ocean	Florida	Dry Tortugas (Texas Rock - 8 Fathom - The Maze)	DT	14-22	34	33	27
Atlantic Ocean	Florida	Fort Lauderdale (shallow reef)	FTL	8	54	48	46
Atlantic Ocean	Florida	Key Largo (North Dry Rocks – Conch Wall)	KL	5-18	50	47	40
Atlantic Ocean	Florida	Looe Key (East, outside sanctuary)	LK	11	53	41	39
Atlantic Ocean	Florida	Miami (shallow reef)	MIA	6	19	19	19
Atlantic Ocean	Florida	Middle Keys (Marathon Thor patch –Long Key foot mound reef)	MK	8	31	30	25
Southwest Caribbean	Belize	Carrie Bow Cay (Patch and fore reefs)	BEZ	8-25	77	72	60
Southwest Caribbean	Panama	Bocas del Toro (Punta Caracol – Adrianas)	BT	6-10	65	54	41
Southwest Caribbean	Colombia	San Andres Island (Wildlife)	COL	1-9	70	70	63
Southern Caribbean	Curacao	Reef in front Carmabi Research Station	CUZ	5-15	10	8	8
				Total	**540**	**495**	**435**

De novo development of *Cliona delitrix*-specific microsatellite markers

Genomic DNA (gDNA) from one individual (originally from Carry Bow Cay, Belize) was used to isolate microsatellites markers using the protocol by Glenn and Schable (2005). The gDNA was digested using RsaI and XmnI restriction enzymes, and following digestion the SuperSNX24 linkers were ligated onto the ends of gDNA fragments. These linkers are sites for primers in subsequent polymerase chain reactions (PCR). To obtain fragments of gDNA enriched with microsatellite sequences, twelve biotinylated probes in three separated reactions: one ($ACAT_8$, $AAGT_8$, $AAAT_8$, $AGAT_8$, $AACT_8$), two (TG_{12}, AG_{12}), and three ($ACTC_6$, $ACAG_6$, $AAAC_6$, $AAAG_8$, and $ACCT_6$) were hybridized to the gDNA. The gDNA - biotinylated probe complex was added to magnetic beads coated with steptavidin (Invitrogen). This complex was washed with two hybridization buffers, twice with 12 X saline-sodium citrate (SSC) + 0.2 % Sodium dodecyl sulfate (SDS), and four times with 6 X SSC + 0.1 % SDS incubating at 53 °C per 1 minute each time. To separate the biotinylated probes from the enriched fragments, the latter were denatured at 95°C and precipitated with 3 M sodium acetate and 95 % ethanol.

To increase the amount of enriched gDNA fragments, a PCR "recovery" was performed in a 25 µl reaction containing 1X PCR buffer (10 mM Tris–HCl, 50 mM KCl, pH 8.3), 1.5 mM $MgCl_2$, 2.5 µl of 10X bovine serum albumin (BSA), 0.16 mM of each dNTP, 10X BSA, 0.52 mM of the SuperSNX24 forward primer, 1 U *Taq* DNA polymerase, and approximately 25 ng enriched gDNA fragments. Thermal cycling was performed in a Bio-Rad DYAD, as follows: 95 °C for 2 min, followed by 25 cycles of 95 °C for 20 s, 60 °C for 20 s, and 72 °C for 90 s, and a final elongation step of 72 °C for 30 min. PCR gDNA fragments then were inserted into vectors using the TOPO-TA Cloning®kit (Invitrogen), and vectors were incorporated into *Escherichia coli* competent cells, and incubated overnight at 37 °C on ampicillin LB Agar plates. Bacterial colonies containing a recombinant vector (i.e. white colonies) were isolated and their DNA with gDNA inserts was used as a template for subsequent PCR in 25 µl reaction containing 1 X PCR buffer

(10 mM Tris-HCl, 50 mM KCl, pH 8.3), 1.5 mM $MgCl_2$, 0.12 mM of each dNTP, 10 X BSA, 0.25 mM of the M13 primers, and 1 U *Taq* DNA polymerase. Thermal cycling conditions were 95 °C for 7 min, followed by 35 cycles of 95 °C for 20 s, 50°C for 20 s, and 72 °C for 90 s. Resulting PCR products were cleaned with EXO-SAP enzymes, and sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing reactions were precipitated with ethanol and 125 mM EDTA, and run on an ABI 3730 DNA Analyzer (Applied Biosystems).

Primers pairs were developed for 32 core microsatellites repeats using Primer 3 v.4.0. on the web (Koressaar and Remm 2007, Untergrasser et al. 2012). Ten of these loci amplified reliably and showed evidence of polymorphism. These loci were used to genotype 495 individuals of the coral excavating sponge *Cliona delitrix* and their cross-amplification utility was examined with 8 individuals of *Cliona laticavicola*. Amplification of microsatellites was performed in total PCR reaction volumes of 25 µl. Two of the ten loci amplified better with Promega hot start enzyme, and the other eight with Qiagen hot start enzyme (Table 2). The PCR reaction contained 5 µl 5X PCR buffer, 3 µl 25 mM $MgCl_2$, 1.2 µl 10 mM dNTPs mix, 1 µl of 10 µM fluorescently labeled universal M13 primer (5´-GTAAAACGACGGCCAGT-3´) (Schuelke 2000), 0.5 µl of 10 µM species-specific forward primer attached to M13 primer, 1 µl of the 10 µM reverse species-specific primer, 8.05 µl of dH_2O, 5 µl of DNA template (0.1-2 ng · μl^{-1} which was diluted from stock gDNA), and 0.125 µl of 5U Hot-Start TaqDNA Polymerase (Promega). The Qiagen PCR reaction contained 2.5 µl 10X PCR buffer, 1.5 µl 25mM $MgCl_2$, 1.2 µl 10mM dNTPs mix, 1 µl of 10 µM fluorescently labeled universal M13 primer (5´-TGTAAAACGACGGCCAGT-3´)(Schuelke 2000), 0.5 µl of 10 µM species-specific forward primer attached to M13 primer, 1 µl of the 10 µM reverse species-specific primer, 12.175 µl of dH_2O, 5 µl of DNA template (0.1-2 ng/µl which was diluted from stock gDNA), and 0.125 µl 2.5U of Hot-Start TaqDNA Polymerase (Qiagen). PCR was performed in a gradient thermal cycler (Bio-RadC1000) as follows, for Promega: 94 °C for 4 min, followed by 30 cycles of 94 °C for 15 sec, 15 sec at the primer annealing temperature (Table 1), and 72 °C for 45 sec, followed by 8 cycles of 94 °C for 15 sec, 53 °C for 15 sec, and 72 °C for 45 sec, and a final elongation step of 72 °C

for 10 min. For Qiagen: 95 °C for 5 min, followed by 34 cycles of 94 °C for 1 min, 30 sec at the primer annealing temperature (Table 1), and 72 °C for 1 min, and a final elongation step of 72 °C for 10 min. Products were cleaned using EXO-SAP enzymes and visualized on a 3730 DNA Analyzer (Applied Biosystems). Alleles were sized using the internal standard GeneScan 500 LIZ (Applied Biosystems), and electropherograms were analyzed using GENEMAPPER version 3.7 (Applied Biosystems), see Appendix 1.3, Figure 1.

Summary statistics

Number of alleles , F_{IS}, and Levels of observed (H_O) and expected (H_E) heterozygosity across microsatellite loci were obtained with the Microsatellite toolkit (Park 2001) and FSTAT 2.9.3.2 (Goudet 1995). Test for linkage equilibrium (LE) and deviations from Hardy-Weinberg (HWE) for each locus and location were estimated using unbiased exact tests (Markov chain method) with 10000 dememorizations, 1000 batches and 10000 iterations per batch (Guo and Thompson 1992), as implemented in GENEPOP v.4.2 on the web (Raymond and Rousset 1995a, Rousset 2008). Significance level (≤ 0.05) was adjusted using the sequential Bonferroni correction for multiple pairwise testing (Rice 1989). The number of private alleles per population was estimated using GenAlEx 6.5 (Peakall and Smouse 2012). The frequency of null alleles was estimated using the expectation maximization (EM) algorithm (Dempster, Laird, and Rubin 1977) as implemented in FREENA (Chapuis and Estoup 2007).

Population differentiation

Four different statistical tests were performed to test for population differentiation. Two of them at a population level (Pairwise exact test and multivariate analysis) and three at an individual level (multivariate, Bayesian, and iterative reallocation analyses). The final

consensus about the number of population was decided based on the higher number of test that support the same result, and at the same level of analysis.

Pairwise exact test F_{ST} –populations

F_{IS}, and F_{ST} were calculated using Weir & Cockerham's (1984) statistic implemented in FSTAT 2.9.3.2 (Goudet 1995). Fisher exact tests (Raymond and Rousset 1995b), implemented in FSTAT version 2.9.3.2 (Goudet 1995), were used to estimate significant population differentiation. This test uses the sampling location as *a priori*, and was performed not assuming HWE within samples, set for 1000 permutations, and with α=0.05 as a baseline to make the Bonferroni correction. Following the *ad hoc* procedure proposed by Waples and Gaggiotti (2006), sampling sites connected through a chain of non-significant results were considered to come from the same genetic population. To test the possible effect that linked loci could have on population differentiation, the linked loci were removed and the analyses were repeated. Similarly, to test the possible effect of null alleles on population differentiation, the locus with highest frequency of null alleles was removed systematically (10 to 1 loci), and tests were repeated. Schematic diagrams were drawn to visualize results. Considering that null alleles have the potential to increase F_{ST} values (Chapuis and Estoup 2007), the locus with highest null allele frequency was systematically removed from the data set, and then the standard F_{ST}, corrected F_{ST} for null alleles, F_{IS}, and mean of null allele frequencies per locus (10 to 1) were recalculated and compared.

Multivariate statistical analysis – populations and individuals

As a second approach to evaluate population differentiation we conducted a Principal Coordinates Analysis (PCoA) both at population and individual levels using the GenAlex 6. Software (Peakall and Smouse 2006). PCoA is a multivariate statistical analysis that uses summarized genetic distances between individual multi-locus genotypes to cluster individuals relative to each other in a multidimensional space, without the assumptions of HWE and LE. For the PCoA two types of genetic distance matrices were calculated: a

pairwise, individual-by-individual (*N x N*), and a population-level pairwise F_{ST} matrix. Also a geographic distance matrix was calculated based on the GPS coordinates after conversion to decimal degrees. Both genetic and geographic matrices were used to perform the PCoA. Depending of the initial results at the Greater Caribbean level, subsequent analyses at different sublevels were carried out.

Bayesian algorithm – Individuals

To determine the most likely number of genetically discrete populations [Ln Pr (*X*|*K*)] we used the Bayesian algorithm as implemented in STRUCTURE v2.31 (Pritchard, Stephens, and Donnellya 2000). Similar to the PCoA, we did and initial analysis at the Greater Caribbean (including all sampled sites) and, based on the results, we did subsequent analyses at different levels. Two different admixture models were used, one without *a priori* sampling location information (admixture), and one including the sampling location for each individual (admixture + *locprior*) (Hubisz et al. 2009). These two models were run with 10 and 6 microsatellites (after eliminating linked loci), and the parameters included 200000 burn-in iterations, in ten replicate sets, followed by 200000 Markov chain Monte Carlo (MCMC) repetitions. The number of genetically homologous groups (K) was determined using the *ad hoc* statistics ΔK (Evanno, Regnaut, and Goudet 2005) implemented in STRUCTURE Harvester (Earl and vonHoldt 2012), and results were plotted in DISTRUCT (Rosenberg 2004). STRUCTURE was first run with the total data set (435 individuals) and K= 1 - 20 to determine strong trends for population subdivision at the Greater Caribbean level and then, based on initial results, only with the genotypes corresponding to Florida-Bahamas (263 individuals, K= 1 - 15), and South Caribbean (172 individuals, K= 1 - 10).

Iterative reallocation method – individuals

We also used a new approach, the iterative reallocation method *a la* Paetkau, which uses multilocus maximum likelihood and the "leave-one-out" procedure implemented in FLOCK 3.1. (Duchesne and Turgeon 2012). This method is performed without any a

priori information and does not make use of the sampling design (Duchesne and Turgeon 2012). The partition of the sampled specimens was done in a random mode, with 20 iterations and 50 runs for each *k* assessed (*k*= 2 - 10) for allocations at the Greater Caribbean and, based on initial results, we repeated the analysis including only individuals for Bahamas - Florida, and only for South Caribbean Sea. The stopping and estimation rules where used to determine the number of clusters (K) following Duchesne and Turgeon (2012).

Dispersal patterns

Genetic isolation by geographic distance- populations

Isolation by geographic distance was evaluated between the pairwise F_{ST} / (1-F_{ST}) values and the logarithm of the geographic distances (Latitude/Longitude in decimal degrees) between locations (Rousset 1997). To test for significant correlation, a Mantel test with 9999 permutations was used as implemented in GenAlex 6.5. (Peakall and Smouse 2012). The test was performed at the Greater Caribbean including all locations. Then, considering results, at this level we repeated the test only for Florida and Bahamas, and only for the south Caribbean locations.

Detection of first generation migrants- individual

To evaluate the extent of contemporary (ecological timescale) dispersal of *Cliona delitrix* among geographic locations, the number of first generation immigrants into each location was inferred using the Bayesian assignment method by Rannala & Mountain (1997) implemented in GENECLASS2 (Piry et al. 2004). This method computes the probability that the multilocus genotype of each individual will be encountered in a given population. It was selected as it works better when population differentiation is low and data deviates from Hardy Weinberg equilibrium (Rannala and Mountain 1997). For the analysis, the database included only individuals that amplified with all 10 loci (total: 348 individuals). Considering that this analysis assumes linkage equilibrium we used all 10 loci, and also

repeated the analysis using only 6 loci, after eliminating the linked ones. The statistical criterion computed for likelihood estimation was L_{home}, as it was considered that some source populations for immigrants were probably not sampled (Piry et al. 2004). For the probability of computation we combined the Monte Carlo re-sampling procedure of Paetkau et al. (2004) with the likelihood criteria of Rannala and Mountain (1997) with 10000 simulated individuals and $\alpha = 0.01$, and $\alpha = 0.05$. Specifically for *Cliona delitrix*, migration of whole individuals rarely occurs. Therefore, we consider that these first migrants correspond to the dispersal of eggs or larvae.

Results

De novo development of *Cliona delitrix* microsatellite markers

Development of cloned microsatellites libraries took two weeks, and from the 210 fragments sequenced, 59 did not present any microsatellite. A total of 124 fragments with microsatellites were of excellent quality, from which we tested 32. A total of 22 did not amplify at any annealing temperature or showed an excess of peaks in electropherograms, making them impossible to genotype (see Appendix 1.3, Table 1). The 10 final primers (Table 2) were the ones that amplified at least at one annealing temperature, and showed peak patterns that we were able to genotype in over 495 individuals. These 10 loci from *Cliona delitrix* (Table 2) were also useful for cross-amplification on 8 individuals of *Cliona laticavicola* (Table 3).

Table 2. Characteristics of the 10 new microsatellites loci isolated from *Cliona delitrix*. T: optimized annealing temperature; He: expected heterozygosity under Hardy-Weinberg equilibrium; Ho: observed heterozygosity; Fis inbreeding coefficient. Pro: Promega hot start enzyme; Qia: Qiagen hot start enzyme.

Locus	Forward and reverse primer sequences (5´-3´)	Repeat Motif	T (°C)	Enzyme	N° Alleles	Size range (bp)	He/Ho	Fis
Cd14	F:GGACACAGCCTTTGAGAGGA R:CATGGGAGGTCATGTGAGTG	$(GA)_{19}(CAGA)_6\ldots(GA)_6(GAGT)_6$	50	Qia	48	374-466	0.95/0.82	0.047
Cd23	F:AACACTGAGCCAGCCAAGAG R:ATGACATGAAGCCACCAACA	$(GA)_7(GT)_7$	50	Qia	25	171-227	0.85/0.61	0.165
Cd39	F:ATGAATGCACATGGAGGTGT R:CCACACCCACCTCTTCTAGG	$(TC)_{12}$	56	Qia	78	235-405	0.95/0.75	0.116
Cd41	F: TGAGGCCACGTATGAAAGAG R: ACGGGAGTAGGTCCTGGAAT	$(CA)_{12}$	56	Qia	39	226-287	0.89/0.71	0.088
Cd63	F: TGTGCAAACATGAACTATGTCAA R: TCAGCCACACTGACCTCTCTT	$(GTGA)_{11}(GA)_4GT(GA)_{18}$	58	Pro	57	173-288	0.94/0.61	0.288
Cd81	F: ACCCTTGTGGATTGAGACCA R: TCATCCACACAGTCTAACACCA	$(TACA)_6$	58	Qia	27	224-283	0.90/0.57	0.195
Cd106	F: CCAATGACCCCACCTACAAA R: CAAGCCAGCTGCAACTTAGA	$(TC)_{16}ACT(CA)_{27}(CT)_{10}$	54.6	Pro	22	212-242	0.90/0.70	0.109
Cd114	F: GAGTAACTGGTCCCGTGGAA R: TTCAAATGGCTTGTGTTCGT	$(GAA)_6\ldots(GTT)_2\ldots(GCA)_3(GAA)_3(GGA)_5$	56	Qia	5	231-241	0.51/0.17	0.299
Cd137	F:TTCAGAAATTGGTAGGACTGACG R: GCATTGCATGGTTTTCACAC	$(CT)_{31}$	56	Qia	31	107-172	0.76/0.56	0.196
Cd141	F: TACAGCTGGTGCAGCACTTC R: CTTTTCAATGCCCTCTCCTG	$(TATG)_5(TG)_5$	58	Qia	18	164-257	0.55/0.33	0.311

Table 3. Cross amplification success of the 10 loci from *Cliona delitrix* on eight individuals (n= 8) of *C. laticavicola*. T: optimized annealing temperature; *Na*: number of alleles; *size*: size range of amplicons in base pairs.

Locus	**Cd14**	**Cd23**	**Cd39**	**Cd41**	**Cd63**	**Cd81**	**Cd106**	**Cd114**	**Cd137**	**Cd141**
T (°C)	50	50	56	56	58	58	54.6	56	56	58
Na	7	9	12	7	6	4	5	2	9	7
size	403-441	180-215	254-326	240-281	174-214	256-271	220-234	232-235	121-170	168-206

Summary statistics

From a total of 495 individuals of *Cliona delitrix* genotyped, 60 were found to have identical genotypes (potential clones), and were eliminated from population genetics estimators. The total number of alleles per locus ranged from five to 78 (Table 4), and the average alleles per population varied from 5.4 (Curacao) to 16.3 (Bahamas). Private alleles were present in all studied locations. South Caribbean locations and Bahamas had greater number (11 - 36) and higher average frequencies (3.0 %-14.2 %) of private alleles, than any of the Florida locations (1-8 private alleles with average allele frequencies between 1.4 % and 2.6 %), except for Dania Beach which had only two private alleles with an average frequency of 5.2%.

Four of the 10 loci (Cd14, Cd81, Cd114, and Cd137) showed linkage disequilibrium (LD) in a global analysis. However, linkage between pairs of loci was not consistent across all populations. Considering that LD can cause an allele to behave in an unexpected manner and produce , for example, false non- HWE loci (Freeland 2005), all the analyses that assume LD were done with both 10 and 6 loci, after eliminating the four linked loci. For analyses that did not require the assumption of LD, all 10 loci were used.

The total inbreeding coefficient (F_{IS}) values per population were positive and significant ($p< 0.00042$), except for Curacao. F_{IS} in Belize was positive but not significant after Bonferroni correction. This indicates a heterozygote deficit in most locations (Table 4), suggesting non-random mating between individuals. The exact test for HWE confirmed these results by showing significant deviations in each location, except Curacao (Table 4). Curacao instead showed negative F_{IS} values suggesting outbreeding. The average frequency of null alleles for each locus ranged from 0.033 to 0.122, and for each population ranged from 0.022 to 0.089, overall 0.068, Table 4. The F_{ST} corrected values were slightly lower than the standard F_{ST} values and the difference between both parameters fluctuated between 0.004 and 0.008 (Figure 1). F_{IS} values were higher when null allele frequency was higher (using all 10 loci), and decreased when loci with higher

null allele frequency where removed (Figure 1). However, the standard F_{ST} and corrected F_{ST} values remained relatively stable until a fifth locus (Cd23) was removed. Then a decrease of 0.054 units occurred, and both values continued decreasing until all loci, except one, were removed.

Table 4. Summary of genetic variation and null allele frequencies at each locus. All possible clones were eliminated for these calculations, and only individuals that amplified with 7 or more loci were included. (n) number of genotyped individuals; (Na) number of alleles; (H_E) expected heterozygosity; (H_O) observed heterozygosity; (F_{IS}) inbreeding coefficient; (HWE) probability of conformation to Hardy-Weinberg expectations (p-value); (*pA*) number of private alleles; (Null) frequency of null alleles; (^{m}) mean/locus. BEZ: Belize (Carrie Bow Cay); BT: Panama (Bocas del Toro); COL: Colombia (San Andrés Island); CUZ: Curacao (Carmabi); BH: Bahamas (Various reefs); DB: Florida (Dania Beach); DT: Florida (Dry Tortugas); FTL: Florida (Fort Lauderdale); KL: Florida (Key Largo); LK: Florida (Looe Key); MIA: Florida (Miami); MKY: Florida (Middle Keys). (--) not sufficient variability at locus to determine HWE; (**bold values**) indicate nominal significance at p <0.05; (*) significance after sequential Bonferroni correction (α/118).

Locus	**BEZ**	**BH**	**BT**	**COL**	**CUZ**	**DB**	**DT**	**FTL**	**KL**	**LK**	**MIA**	**MKY**	**Total/locus Mean/locus**
	n=60	**n=42**	**n=41**	**n=63**	**n=8**	**n=25**	**n=27**	**n=46**	**n=40**	**n=39**	**n=19**	**n=25**	**n=435**
Cd14													
Na	17	22	11	14	7	17	14	13	15	19	10	9	48
H_E	0.841	0.947	0.798	0.889	0.858	0.918	0.906	0.829	0.883	0.878	0.771	0.787	0.950
H_O	0.831	0.975	0.683	0.839	1.000	0.833	0.926	0.651	0.925	0.889	0.790	0.609	0.822
F_{IS}	0.013	-0.030	0.146	0.057	-0.179	0.094	-0.022	0.217	-0.048	-0.013	-0.025	0.231	0.047
HWE	**0.000***	0.782	0.055	0.133	0.969	**0.000***	0.742	**0.000***	**0.000***	0.055	**0.010**	**0.000**	**0.000***
pA	2	2	2	5	5	0	2	1	0	3	0	1	23
Null	0.000	0.000	0.065	0.039	0.000	0.028	0.000	0.077	0.000	0.000	0.021	0.087	0.026^m
Cd23													
Na	7	11	6	10	8	10	10	11	7	10	10	8	25
H_E	0.662	0.713	0.687	0.695	0.883	0.862	0.793	0.777	0.557	0.764	0.839	0.803	0.851
H_O	0.550	0.571	0.487	0.672	1.000	0.720	0.630	0.691	0.375	0.579	0.842	0.652	0.607
F_{IS}	0.170	0.201	0.295	0.034	-0.143	0.168	0.209	0.112	0.330	0.244	-0.003	0.191	0.165
HWE	0.153	0.058	**0.005**	0.278	0.475	**0.000***	0.010	**0.003**	**0.000***	**0.000***	0.213	**0.026**	**0.000***
pA	0	0	0	2	4	0	0	1	0	0	0	0	7
Null	0.054	0.080	0.121	0.024	0.000	0.074	0.071	0.043	0.129	0.109	0.000	0.095	0.066^m
Cd39													
Na	29	35	16	28	8	15	23	18	22	23	9	11	78
H_E	0.958	0.959	0.882	0.946	0.875	0.888	0.947	0.514	0.922	0.953	0.460	0.560	0.951
H_O	0.966	0.795	0.854	0.953	1.000	0.520	0.815	0.370	0.757	0.763	0.368	0.520	0.749
F_{IS}	-0.008	0.173	0.032	-0.007	-0.155	0.420	0.142	0.283	0.181	0.202	0.203	0.073	0.116
HWE	**0.000***	**0.000***	**0.008**	**0.033**	0.911	**0.000***	**0.000***	**0.023**	**0.000***	**0.000***	**0.031**	0.295	**0.000***
pA	5	10	1	5	0	1	0	1	0	1	1	2	
Null	0.005	0.076	0.008	0.005	0.000	0.173	0.062	0.091	0.077	0.092	0.000	0.000	0.049^m
Cd41													
Na	13	20	8	8	5	12	12	13	13	16	7	6	39
H_E	0.847	0.910	0.718	0.713	0.733	0.773	0.844	0.731	0.780	0.814	0.729	0.703	0.895

H_O	0.895	0.892	0.346	0.475	1.000	0.875	0.778	0.622	0.825	0.769	0.647	0.609	0.714
F_{IS}	-0.057	0.020	0.523	0.336	-0.400	-0.135	0.079	0.150	-0.059	0.056	0.116	0.137	0.088
HWE	**0.004**	0.307	**0.000***	**0.000***	0.639	0.755	0.379	0.183	0.647	0.290	**0.021**	**0.023**	**0.000***
pA	4	6	0	0	1	0	1	2	0	1	0	0	15
Null	0.000	0.000	0.215	0.138	0.000	0.000	0.021	0.005	0.000	0.000	0.000	0.021	0.033[m]
Cd63													
Na	18	29	12	16	7	13	15	19	21	23	11	11	57
H_E	0.831	0.892	0.764	0.789	0.879	0.883	0.874	0.882	0.906	0.887	0.879	0.898	0.937
H_O	0.649	0.475	0.683	0.620	0.833	0.440	0.667	0.522	0.703	0.590	0.722	0.652	0.611
F_{IS}	0.221	0.471	0.107	0.215	0.057	0.507	0.240	0.411	0.226	0.338	0.183	0.278	0.288
HWE	**0.000***	**0.000***	**0.006**	**0.000***	0.733	**0.000***	**0.000**	**0.000***	**0.000***	**0.000***	**0.004**	**0.001**	**0.000***
pA	1	7	1	3	0	0	3	0	2	1	0	0	18
Null	0.103	0.212	0.033	0.097	0.000	0.228	0.117	0.186	0.118	0.149	0.091	0.123	0.122[m]
Cd81													
Na	10	11	5	8	3	14	11	11	11	14	7	5	27
H_E	0.845	0.846	0.096	0.613	0.425	0.888	0.816	0.743	0.836	0.883	0.742	0.675	0.904
H_O	0.864	0.833	0.098	0.571	0.250	0.440	0.815	0.544	0.525	0.667	0.353	0.360	0.574
F_{IS}	-0.023	0.015	-0.019	0.069	0.429	0.510	0.001	0.271	0.375	0.248	0.532	0.472	0.195
HWE	**0.000***	0.497	1.000	**0.015**	0.385	**0.000***	0.158	**0.002**	**0.000***	**0.000***	**0.001**	**0.000***	**0.000***
pA	4	2	1	2	0	0	0	0	0	0	0	0	9
Null	0.000	0.000	0.000	0.005	0.124	0.226	0.008	0.093	0.159	0.112	0.218	0.170	0.093[m]
Cd106													
Na	9	17	7	14	7	9	8	9	11	9	7	8	22
H_E	0.784	0.876	0.742	0.715	0.883	0.798	0.863	0.772	0.868	0.834	0.499	0.828	0.903
H_O	0.833	0.786	0.600	0.836	0.875	0.500	0.609	0.679	0.641	0.595	0.412	0.846	0.703
F_{IS}	-0.063	0.104	0.194	-0.172	0.010	0.379	0.299	0.123	0.264	0.290	0.179	-0.023	0.109
HWE	**0.002**	**0.011**	**0.008**	0.787	0.499	**0.000***	**0.025**	**0.000***	**0.002**	**0.000***	0.056	**0.000***	**0.000***
pA	0	6	0	2	1	0	0	0	0	0	0	0	9
Null	0.000	0.024	0.059	0.000	0.000	0.145	0.125	0.072	0.120	0.130	0.000	0.000	0.056[m]
Cd114													
Na	3	3	1	2	1	3	4	3	4	4	4	2	5
H_E	0.156	0.219	0.000	0.047	0.000	0.566	0.373	0.419	0.379	0.375	0.514	0.115	0.507
H_O	0.100	0.238	0.000	0.048	0.000	0.160	0.185	0.289	0.325	0.290	0.368	0.120	0.173
F_{IS}	0.362	-0.088	NA	-0.016	NA	0.721	0.508	0.313	0.145	0.231	0.288	-0.043	0.299
HWE	**0.004**	1.000	--	1.000	--	**0.000***	**0.005**	**0.038**	0.299	0.194	0.243	1.000	**0.000***
pA	1	0	0	0	0	0	0	0	0	0	0	0	1
Null	0.093	0.000	0.001	0.000	0.001	0.247	0.144	0.094	0.051	0.075	0.077	0.000	0.065[m]
Cd137													
Na	9	12	10	5	5	8	13	15	7	10	9	10	31
H_E	0.712	0.747	0.635	0.436	0.733	0.747	0.818	0.823	0.718	0.778	0.788	0.760	0.755
H_O	0.638	0.425	0.575	0.397	0.750	0.739	0.519	0.675	0.514	0.641	0.750	0.478	0.563

F_{IS}	0.105	0.434	0.095	0.090	-0.024	0.011	0.371	0.182	0.287	0.178	0.050	0.376	0.196
HWE	**0.000***	**0.000***	0.281	0.063	0.122	0.518	**0.000***	0.086	**0.000***	**0.007**	0.244	**0.000***	**0.000***
pA	1	2	2	0	0	1	2	2	0	0	0	2	12
Null	0.070	0.186	0.071	0.026	0.033	0.017	0.150	0.075	0.103	0.056	0.000	0.135	0.077[m]
Cd141													
Na	7	3	7	5	3	8	6	9	4	8	7	9	18
H_E	0.237	0.070	0.596	0.389	0.492	0.729	0.430	0.791	0.272	0.579	0.788	0.842	0.551
H_O	0.181	0.071	0.100	0.323	0.375	0.280	0.269	0.674	0.175	0.368	0.737	0.800	0.329
F_{IS}	0.233	-0.017	0.834	0.173	0.250	0.621	0.378	0.150	0.360	0.367	0.067	0.051	0.311
HWE	**0.000***	1.000	**0.000***	**0.028**	0.590	**0.000***	**0.011**	0.076	**0.017**	**0.000***	0.063	0.768	**0.000***
pA	0	1	3	1	0	0	0	0	0	0	0	0	5
Null	0.000	0.000	0.319	0.064	0.063	0.256	0.113	0.047	0.099	0.136	0.047	0.000	0.095[m]
Multilocus													
He^m	0.687	0.718	0.592	0.623	0.676	0.805	0.766	0.728	0.712	0.774	0.701	0.697	0.707
Ho^m	0.651	0.606	0.443	0.573	0.708	0.551	0.621	0.572	0.577	0.615	0.599	0.565	0.591
F_{IS}	**0.054**	**0.157***	**0.255***	**0.081***	-0.051	**0.321***	**0.192***	**0.217***	**0.193***	**0.208***	**0.149***	**0.194***	0.164[m]
HWE	**0.000***	**0.000***	**0.000***	**0.000***	0.856	**0.000***	**0.000***	**0.000***	**0.000***	**0.000***	**0.000***	**0.000***	**0.000***
Na^m	12.2	16.3	8.3	11	5.4	10.9	11.6	12.1	11.5	13.6	8.1	7.9	10.7
pA	18	36	10	20	11	2	8	7	2	6	1	5	126
$Null^m$	0.033	0.058	0.089	0.040	0.022	0.139	0.081	0.078	0.086	0.086	0.045	0.063	0.068

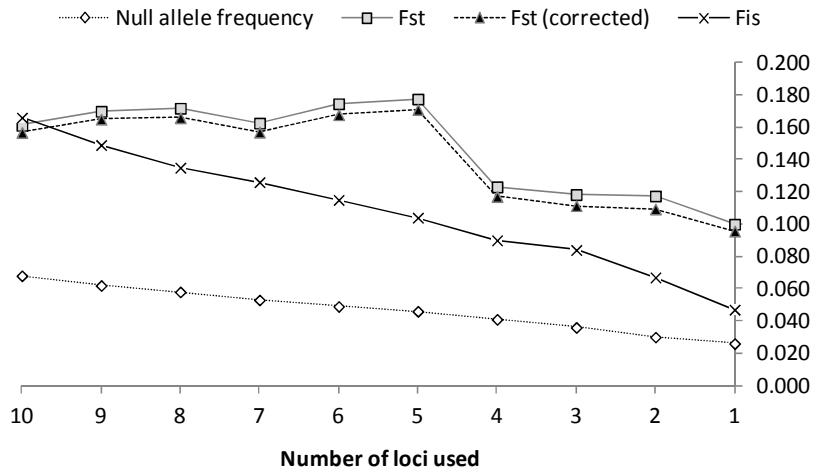


Figure 1. Total Null allele frequencies and F-statistics standard (Weir 1996) and corrected for null alleles (Chapuis and Estoup 2007) for different number of loci used. The frequency of null alleles, standard $F_{ST,}$ and F_{ST} corrected for null alleles were calculated using the software FreeNA (Chapuis and Estoup 2007) and F_{IS} in FSTAT.

Population genetic structure of *Cliona delitrix*

Pairwise exact test and F_{ST} values–populations

Using 10 loci, five *Cliona delitrix* populations were defined: Belize, Bahamas, Colombia, Panamá, and all Florida locations as one population together with Curacao (Table 5, Figure 2 A, B). However, when the four linked loci were eliminated, six populations were found: Belize, Bahamas, Colombia, and Panamá, and the Florida-Curacao divided in two groups, 1: Dania Beach, Dry Tortugas, Key Largo and Looe Key, 2: Fort Lauderdale, Miami, Middle Keys and Curacao (Figure 2 C). When the locus with highest frequency of null alleles was sequentially eliminated (using 9 to 1 loci), six populations were again defined: Belize, Bahamas, Colombia, Panamá, Curacao and Florida; in this case all Florida locations were grouped together and Curacao was delineated as a population significantly different from Florida and other populations (Figure 2 D and E).

Pairwise F_{ST} values (for both ten and six un-linked microsatellites) showed high differentiation (0.14 - 0.27) among Atlantic (Florida, Bahamas) and South Caribbean (Belize, Colombia, Panamá, Curacao) locations, and moderate to high differentiation (0.10 - 0.26) within South Caribbean locations. F_{ST} values showed low differentiation within two groups of locations in the Florida reef track: 1) Dania Beach, Dry Tortugas, Key Largo and Looe Key (-0.0026 – 0.06) and 2) Fort Lauderdale, Miami, Middle Keys (0.03-0.05). Between these two groups differentiation ranged from moderate to high (0.06 – 0.2046). Also, low to moderate differentiation (0.03-0.08) was found between Bahamas and Florida group 1 (Dania Beach, Dry Tortugas, Key Largo, Looe Key), but there was higher differentiation (0.19 – 0.22) between Bahamas and Florida group 2 (Fort Lauderdale, Miami, and Middle Keys).

Table 5. F_{ST} Values between populations using all 10 loci (top number), or only 6 non LD loci (bottom number in parenthesis). Genetic differentiation based on the F_{ST}: little= 0-0.05; moderate= 0.05-0.15; great = 0.15-0.25; pronounced >0.25 (Freeland 2007). For exact test after sequential Bonferroni correction (α 0.000758): (*) significant differences between geographic locations, BEZ: Belize (Carrie Bow Cay); BT: Panama (Bocas del Toro); COL: Colombia (San Andrés Island); CUZ: Curacao (Carmabi); BH: Bahamas (Various reefs); DB: Florida (Dania Beach); DT: Florida (Dry Tortugas); FTL: Florida (Fort Lauderdale); KL: Florida (Key Largo); LK: Florida (Looe Key); MIA: Florida (Miami); MK: Florida (Middle Keys).

	BEZ	**BH**	**BT**	**COL**	**CUZ**	**DB**	**DT**	**FTL**	**KL**	**LK**	**MIA**
BH	0.1667*										
	(0.0999)*										
BT	0.179*	0.2503*									
	(0.1453)*	(0.1204)*									
COL	0.1164*	0.222*	0.0987*								
	(0.0999)*	(0.1284)*	(0.1052)*								
CUZ	0.1842*	0.2604*	0.2585*	0.2416*							
	(0.2157)*	(0.2076)*	(0.1838)*	(0.2214)*							
DB	0.1337*	0.0805*	0.2041*	0.1828*	0.1712*						
	(0.1416)*	(0.0824)*	(0.1354)*	(0.1552)*	(0.1587)*						
DT	0.143*	0.0399*	0.2274*	0.1985*	0.2177*	0.0349*					
	(0.0891)*	(0.0382)*	(0.1093)*	(0.1093)*	(0.1637)*	(0.0327)*					
FTL	0.1669*	0.1861*	0.2413*	0.2222*	0.2051*	0.0549	0.1398*				
	(0.1961)*	(0.1752)*	(0.2039)*	(0.2152)*	(0.2158)*	(0.0616)*	(0.121)*				
KL	0.1725*	0.0384*	0.2351*	0.2176*	0.2567*	0.0526*	0.0083	0.1689*			
	(0.1285)*	(0.0359)*	(0.1281)*	(0.1534)*	(0.2163)*	(0.055)*	(0.0127)	(0.159)*			
LK	0.1517*	0.0469*	0.2241*	0.2006*	0.2096*	0.0202	0.0002	0.1243*	0.0117		
	(0.1059)*	(0.0511)*	(0.1114)*	(0.1219)*	(0.1559)*	(0.0147)	(-0.0026)	(0.1093)*	(0.0174)		
MIA	0.1981*	0.192*	0.2696*	0.244*	0.236*	0.079*	0.1554*	0.0543	0.1797*	0.139*	
	(0.2311)*	(0.2004)*	(0.2301)*	(0.2445)*	(0.2454)*	(0.1054)*	(0.1604)*	(0.0779)	(0.1951)*	(0.1449)*	
MKY	0.1815*	0.2211*	0.2428*	0.2129*	0.2056	0.0743*	0.1705*	0.0318*	0.2046*	0.155*	0.0335
	(0.2146)*	(0.1791)*	(0.2011)*	(0.2095)*	(0.1986)	(0.0545)	(0.1206)*	(0.0273)	(0.1673)*	(0.1031)*	(0.0391)

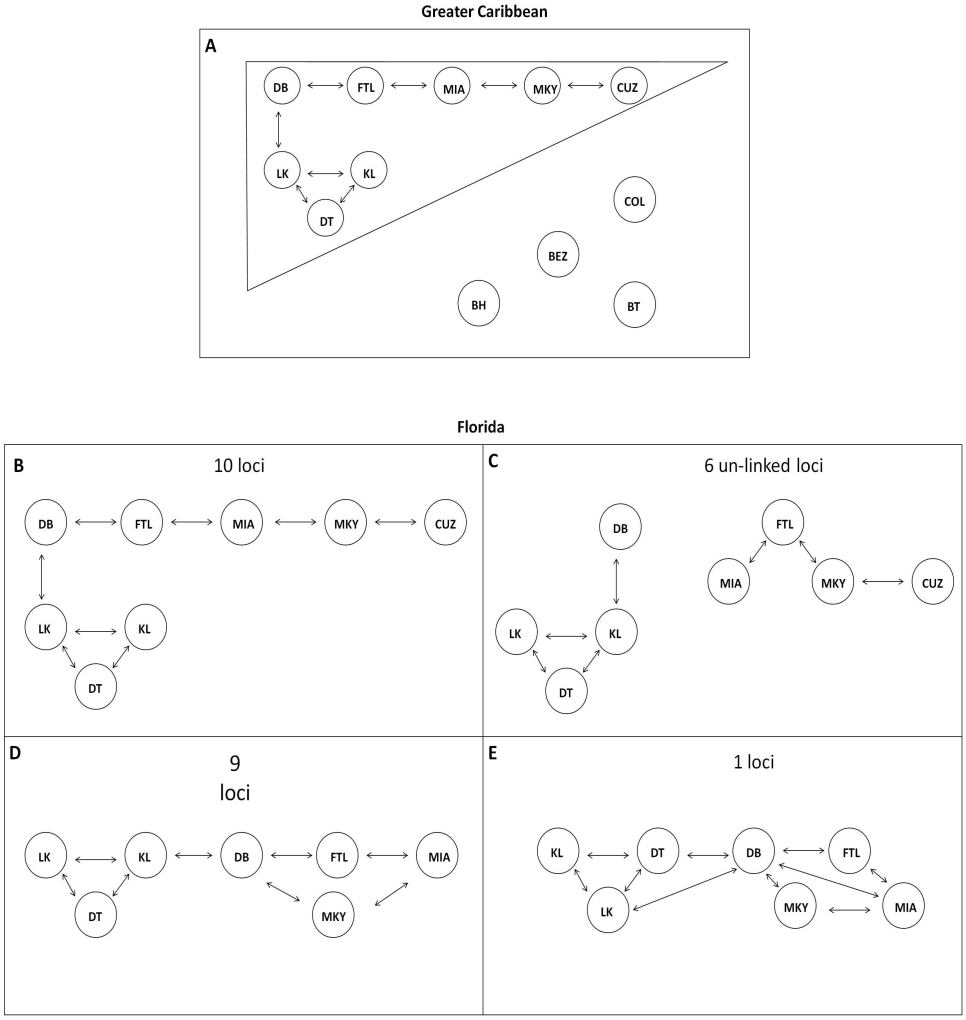


Figure 2. Schematic diagrams of population differentiation based on the Fisher exact test (Raymond and Rousset 1995b). Each circle represents a collection of samples from the same geographic location. Arrow lines represent non-significant differentiation between pairs of locations. Notice that only with six un-linked loci (C) the subdivision in Florida was clear, whereas all other combination of loci joined all Florida location as one genetic population. A) all ten loci at Greater Caribbean level, B) ten loci Florida C) six non linked loci changes only in Florida, D) 9 loci after eliminating the locus with higher null allele frequency, E) one locus after eliminating 9 alleles with higher null alleles frequencies. BEZ: Belize (Carrie Bow Cay); BT: Panama (Bocas del Toro); COL: Colombia (San Andrés Island); CUZ: Curacao (Carmabi); BH: Bahamas (Various reefs); DB: Florida (Dania Beach); DT: Florida (Dry Tortugas); FTL: Florida (Fort Lauderdale); KL: Florida (Key Largo); LK: Florida (Looe Key); MIA: Florida (Miami); MKY: Florida (Middle Keys).

Multivariate statistical analysis – population and individual levels

The PCoA analysis suggested four genetic groups in the total microsatellite dataset: 1) Atlantic, 2) Florida, 3) South Caribbean, and 4) Curacao (Figure 3). Axis 1 explained 30.9 % of the variation found in the distance matrix, whereas Axis 2 and Axis 3

explained 58.1 % and 74.8 % respectively. Axis 1 separated the South Caribbean populations (Belize, Bocas del Toro, Colombia, and Curacao) from Atlantic (Bahamas, Key Largo, Looe Key, Dry Tortugas). Axis 2 separated some of the Florida populations (Dania Beach, Fort Lauderdale, Miami, and Middle Keys) together with Curacao. Axis 3 separated Curacao from the rest of populations analyzed (Figure 3).

Individual-based PCoA analyses at Greater Caribbean level also separated the three main groups observed at a population level: 1) Atlantic, 2) Florida, and 3) South Caribbean. This division was mainly explained by Axis 1 in 11.8 % and Axis 2 in 21.0 %. However, some Belize individuals were found in between the South Caribbean and Florida-Bahamas clusters, and all Curacao individuals in between the South Caribbean and Florida clusters. Some Individuals from the Florida cluster (Dania Beach, Fort Lauderdale, Miami, and Middle Keys) where mixed with the Atlantic cluster (Bahamas, Key Largo, Looe Key, Dry Tortugas). However, only some individuals from the Atlantic cluster (from Looe Key) were also found in the Florida cluster (Figure 4 A, B, C). This was also supported when analyses where done only including individuals from Florida and Bahamas (Figure 4 D, E, F) in which axis 1 explained 18.2 % of the variation found in the distance matrix, and axis 2 and 3, 22.5 % and 25.9 % respectively. At the South Caribbean level, axis 1 separated Colombia and Panama populations from Belize and Curacao at 11.3 %. Axis 2 and 3 which explained 17.8 % and 23.5 % of the variation, did not reveal any substructure between the fourth populations (Belize, Colombia, Curacao and Panamá) (see figure 4 G, H, I).

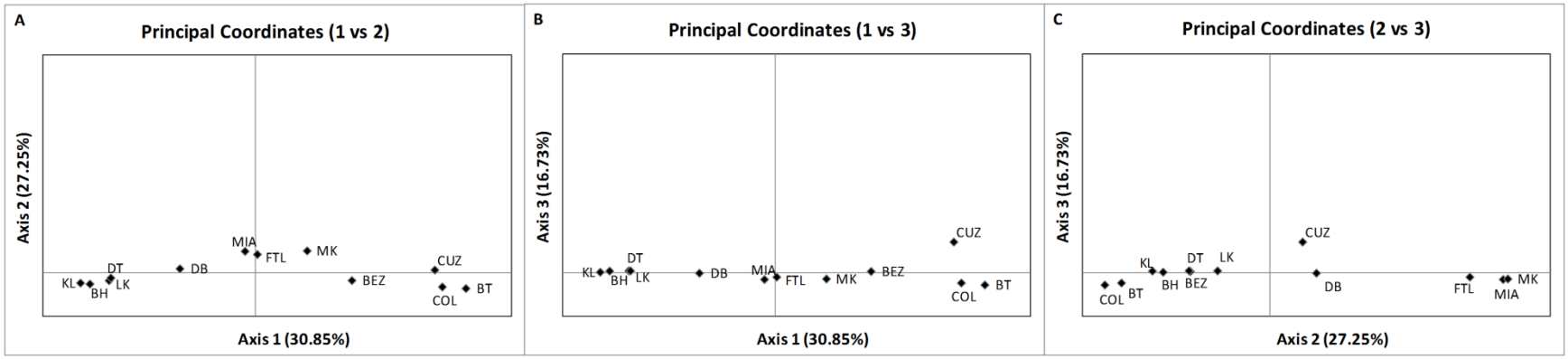


Figure 3. Principal component analyses (PcoA) using genetic distances from a population-level pairwise F_{ST} matrix. A (biplot axis 1vs axis 2); B (biplot axis 1vs axis 3); C (biplot axis 2 vs axis 3). BEZ: Belize (Carrie Bow Cay); BT: Panama (Bocas del Toro); COL: Colombia (San Andrés Island); CUZ: Curacao (Carmabi); BH: Bahamas (Various reefs); DB: Florida (Dania Beach); DT: Florida (Dry Tortugas); FTL: Florida (Fort Lauderdale); KL: Florida (Key Largo); LK: Florida (Looe Key); MIA: Florida (Miami); MK: Florida (Middle Keys).

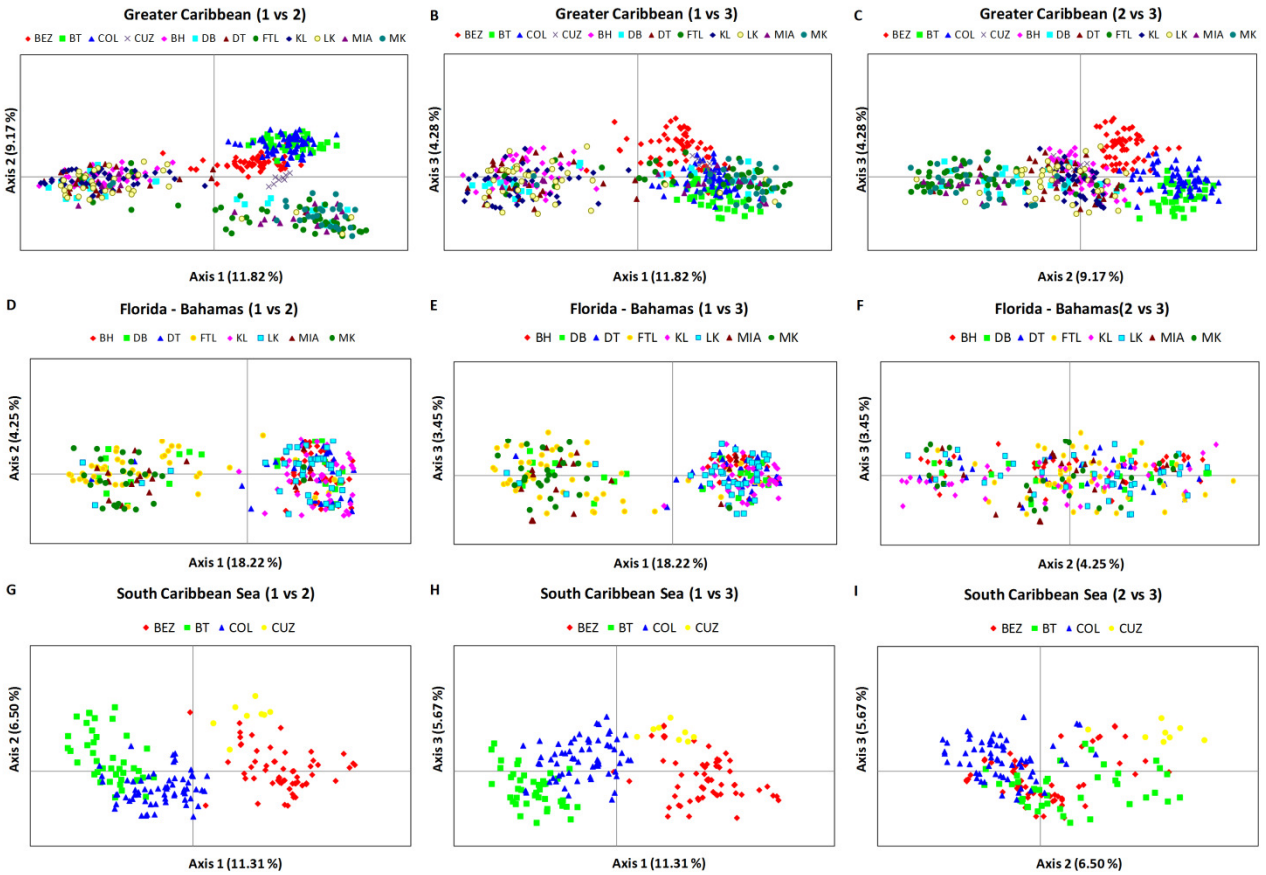


Figure 4. Results of the principal component analyses (PcoA) using genetic distances between individuals and a geographic distance matrix in GenAlex 6.501. The three axis comparisons are showed per analysis level, and the percentage of variation explained by each axis is in parenthesis. Greater Caribbean Sea Level (A,B,C); Florida and Bahamas (D,E,F); South Caribbean Sea (G,H,I). BEZ: Belize (Carrie Bow Cay); BT: Panama (Bocas del Toro); COL: Colombia (San Andrés Island); CUZ: Curacao (Carmabi); BH: Bahamas (Various reefs); DB: Florida (Dania Beach); DT: Florida (Dry Tortugas); FTL: Florida (Fort Lauderdale); KL: Florida (Key Largo); LK: Florida (Looe Key); MIA: Florida (Miami); MK: Florida (Middle Keys).

Bayesian algorithm – Individual level

The Bayesian algorithm in STRUCTURE also recovered three genetic clusters across the Greater Caribbean level when using ten loci: 1) Atlantic (Bahamas, Dry Tortugas, Key Largo and Looe Key), 2) Florida (Fort Lauderdale, Miami and Middle Keys), and 3) South Caribbean (Belize, Panama, Colombia and Curacao). Dania Beach shared both individuals belonging to the Atlantic and Florida clusters, Figure 5 A. When both models were run only with six loci, Curacao individuals were included within the Atlantic cluster, instead to the South Caribbean (Figure 5 A). When the analyses where preformed only for Florida and Bahamas locations, both models and both ten and six loci showed the same two genetic clusters already found at the Greater Caribbean level: 1) Atlantic (Bahamas, Dry Tortugas, Key Largo and Looe Key), 2) Florida (Fort Lauderdale, Miami and Middle Keys) and Dania Beach having almost the same amount of individuals belonging to the Atlantic and to the Florida clusters (Figure 5 B). At the South Caribbean level, the admixture model with both 10 and 6 loci differentiate four genetic clusters each of them corresponding to the sampling locations (Belize, Panama, Colombia and Curacao), Figure 5 C. However, the admixture model with both 10 and 6 loci, found only two genetic clusters, one including individuals from Belize and Curacao and the second one including individuals from Colombia and Panama (Figure 5 C).

Although the Bayesian method required loci to be in HWE and linkage equilibrium as a priori information, this analysis was not affected by the presence of linked loci, as it showed similar results to the multivariate and iterative methods that do not require HEW or LD as a priori.

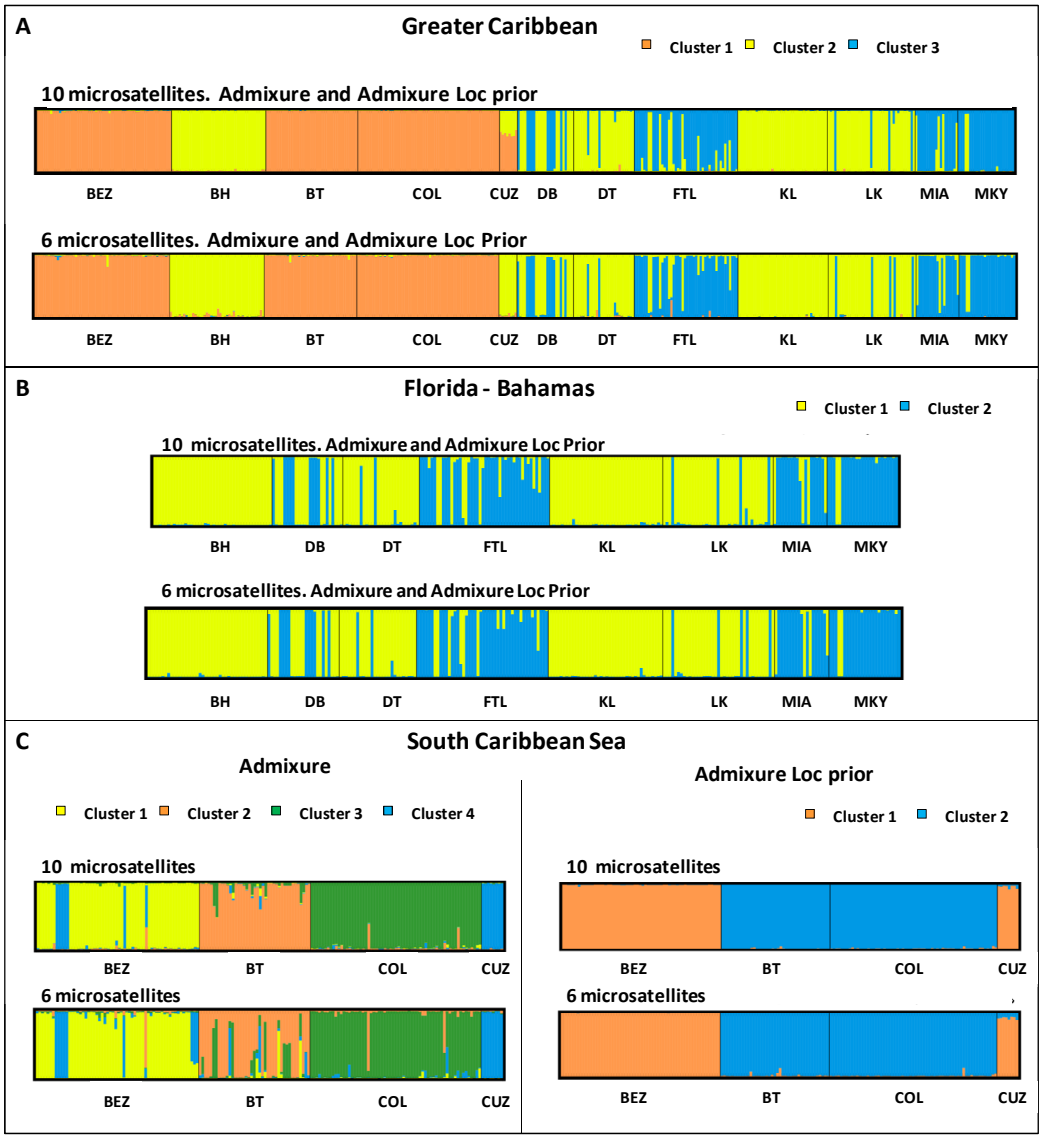


Figure 5. Assignment of *Cliona delitrix* individuals genotypes to genetically homologous groups (K) as inferred by Bayesian analyses using admixture and admixture + *locprior* models in STRUCTURE, both run with all 10 loci and 6 un-linked loci for 200000 burn-in iterations, in ten replicate sets, followed by 200000 Markov chain Monte Carlo (MCMC) repetitions. Each individual is represented by a vertical bar partitioned into K-colored segments that represents its estimated membership fraction in each of the inferred groups. A) Greater Caribbean (all locations); B) Florida and Bahamas locations; C) South Caribbean Sea locations. BEZ: Belize (Carrie Bow Cay); BT: Panama (Bocas del Toro); COL: Colombia (San Andrés Island); CUZ: Curacao (Carmabi); BH: Bahamas (Various reefs); DB: Florida (Dania Beach); DT: Florida (Dry Tortugas); FTL: Florida (Fort Lauderdale); KL: Florida (Key Largo); LK: Florida (Looe Key); MIA: Florida (Miami); MKY: Florida (Middle Keys).

Iterative reallocation method – individual level

The iterative reallocation analyses by FLOCK were also in agreement with both Bayesian and multivariate analyses in finding three main genetic clusters at the Greater Caribbean level: 1) Atlantic (Bahamas, Dry Tortugas, Key Largo and Looe Key), 2) Florida (Fort Lauderdale, Miami and Middle Keys), and 3) South Caribbean (Belize, Panama, Colombia and Curacao) (Figure 6 A, 7). Dania Beach was a location that contained almost the same amount of individuals assigned to either the Atlantic and Florida clusters (Figure 6 A, 7). When analyses were performed including only individuals from Florida and Bahamas, the same two clusters for this area were found (Atlantic and Florida) (Figure 6 B, 7). However, when only the South Caribbean locations were analyzed, four different clusters were found, with each representing a specific geographical location: Belize, Colombia, Curacao and Panama (Figure 6 C). See Appendix 1.3 - Table 2 for the plateau records and specific decision flow used to assign the number of clusters.

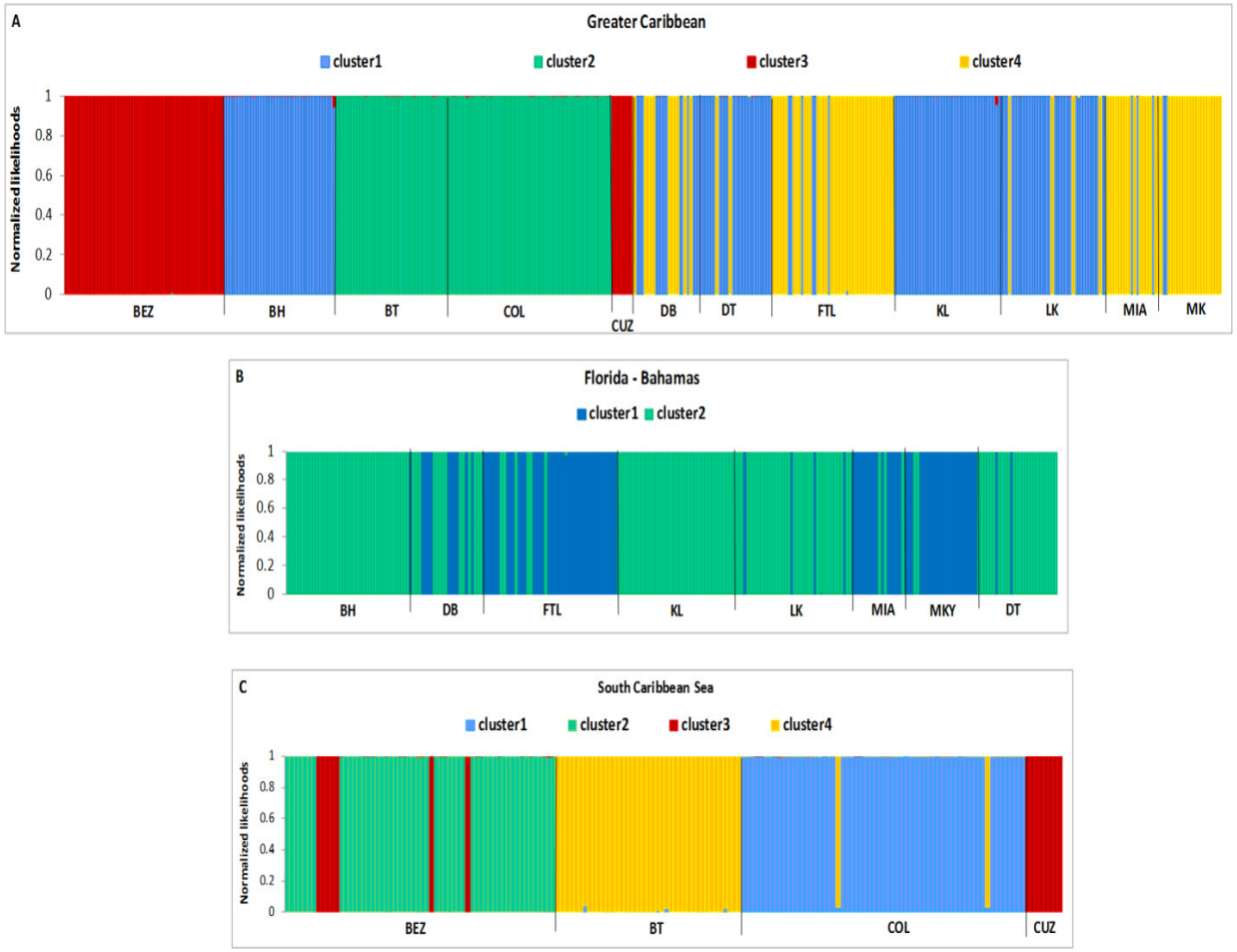


Figure 6. Assignment of individuals based on the normalized likelihood using the Iterative reallocation method of FLOCK. Partition of the sampled specimens was done in a random mode, with 20 iterations and 50 runs for each *k* assessed. A) Greater Caribbean, B) Florida-Bahamas, C) South Caribbean Sea. Each line represents each individual. Geographic locations are marked in the x axis. BEZ: Belize (Carrie Bow Cay); BT: Panama (Bocas del Toro); COL: Colombia (San Andrés Island); CUZ: Curacao (Carmabi); BH: Bahamas (Various reefs); DB: Florida (Dania Beach); DT: Florida (Dry Tortugas); FTL: Florida (Fort Lauderdale); KL: Florida (Key Largo); LK: Florida (Looe Key); MIA: Florida (Miami); MKY: Florida (Middle Keys).

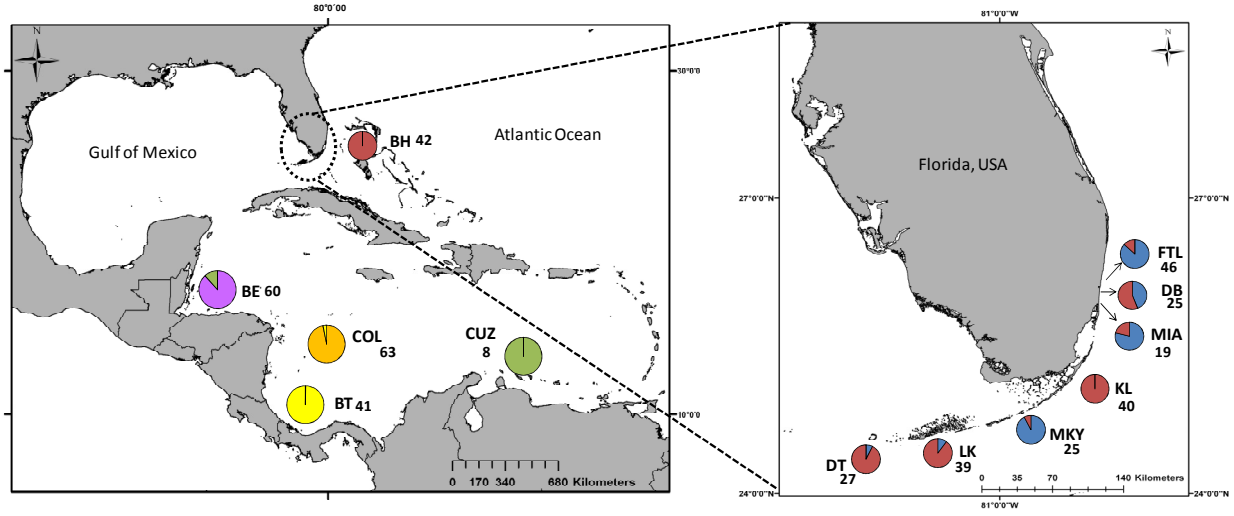


Figure 7. Average normalized likelihood per location obtained using the Iterative reallocation method in FLOCK. Partition of the sampled specimens was done in a random mode, with 20 iterations and 50 runs for each *k* assessed. Each color in the graph represents a different cluster, red: Atlantic cluster, blue: Florida cluster, purple: Belize cluster, orange: Colombia cluster, yellow: Panama cluster and green: Curazao cluster. Numbers of individuals analyzed on the side of each location. BEZ: Belize (Carrie Bow Cay); BT: Panama (Bocas del Toro); COL: Colombia (San Andrés Island); CUZ: Curacao (Carmabi); BH: Bahamas (Various reefs); DB: Florida (Dania Beach); DT: Florida (Dry Tortugas); FTL: Florida (Fort Lauderdale); KL: Florida (Key Largo); LK: Florida (Looe Key); MIA: Florida (Miami); MKY: Florida (Middle Keys).

Dispersal patterns

Genetic isolation by geographic distance- populations

At the Greater Caribbean level, the correlation was positive (R^2= 0.4121) and significant for genetic isolation due to geographic distances ($p \leq 0.002$). The greatest isolation was found between the Atlantic (Florida - Bahamas) and South Caribbean locations (Figure 8A). This was supported by subsequent tests within the Atlantic (R^2= 0.0091; $p \leq 0.392$), and within South Caribbean locations (R^2= 0.5785; $p \leq 0.124$), which did not show significant evidence of isolation by distance (Figure 8 B, C). Therefore, the amount of gene flow appears low between the Caribbean Sea and the Atlantic (over ~1100 km from Belize to Dry Tortugas), whereas higher gene flow occurred between Florida and Bahamas locations (min distance ~88 km from Dania Beach to Bimini). Although not a significant correlation in the South Caribbean region, more isolation by distance and

restriction of gene flow appeared in this area (max distance ~2100 km between Belize and Curacao) than within the Florida reef track (max distance ~318 km from Dry Tortugas to Fort Lauderdale), or between Florida and Bahamas locations.

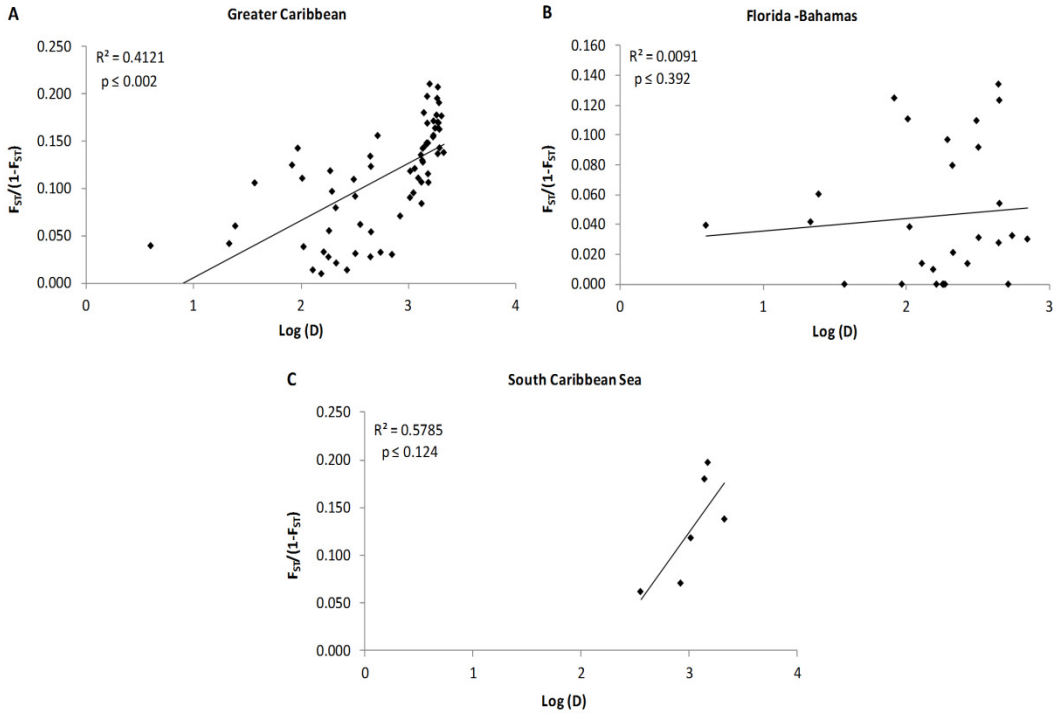


Figure 8. Genetic isolation by distance for *Cliona delitrix* samples inferred from multilocus estimates of genetic differentiation $F_{ST}/(1-F_{ST})$ and the logarithm of the geographic distance (D) using a Mantel test. A) Greater Caribbean; B) Florida and Bahamas, and C) the South Caribbean Sea.

Detection of first generation migrants- individuals

From the 348 individuals included in the assignment analysis, 15.2 % and 16.4 % of the individuals (for 10 and 6 loci respectively, $\alpha = 0.01$) were first generation migrants to a new location. When α was set at 0.05 a slight increment was recorded, 17 % and 18.1 % (10 loci and 6 loci respectively).

Considering 10 loci and α= 0.01, first migrants were only detected in all Florida locations and in the Bahamas (Table 6). Dispersal from Florida to the Bahamas occurred specifically from two locations, Dania Beach (1 individual) and Key Largo (3 individuals), whereas dispersal from the Bahamas to Florida only was recorded towards Dania Beach (1 individual) (Table 6). Among the Florida reef track, most of the dispersal occurred from four locations, Dania Beach, Key Largo, Looe Key and Middle Keys, and most of the "migrants" from Key Largo and Looe Key were found in Dry Tortugas, whereas dispersal from Dania Beach occurred almost in the same amount towards Fort Lauderdale, Dry Tortugas, Key Largo, Looe Key and Miami. Most of migrants from Middle Keys were found in Fort Lauderdale and Miami (Table 6). Along the Florida reef track dispersal was in the same proportion either direction (47 % to the south and 53 % to the north, 10 loci, α= 0.01).

Using the 6 loci (less linked), first migrants were also recorded among Panamá (α= 0.01, 0.05) Belize, and Colombia (α= 0.05) (see Table 6). Also, dispersal between the South Caribbean Sea locations and the Atlantic (Florida-Bahamas) occurred (3 migrants) mostly towards the north, from the Caribbean Sea (Curacao, Panama and Belize) to the Bahamas, whereas only one individual originated in Florida (Looe Key) was found as a migrant into the South Caribbean (Colombia). Within Florida and Bahamas, both Key Largo and Looe Key where the origin of most migrants.

Table 6. Number of first generation migrants with 10 loci (top table) and 6 loci (bottom table). First column α= 0.01 and second column α= 0.05. BEZ: Belize (Carrie Bow Cay); BT: Panama (Bocas del Toro); COL: Colombia (San Andrés Island); CUZ: Curacao (Carmabi); BH: Bahamas (Various reefs); DB: Florida (Dania Beach); DT: Florida (Dry Tortugas); FTL: Florida (Fort Lauderdale); KL: Florida (Key Largo); LK: Florida (Looe Key); MIA: Florida (Miami); MK: Florida (Middle Keys).

10 loci	Populations of origen																	
	BH		DB		DT		FTL		KL		LK		MIA		MKY		Total	
Found in	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05
BH			1	1					3	3							4	4
DB							2	2	5	5	1	1			2	2	10	10
DT			1	1					2	**3**	3	3					6	**7**
FTL			1	1					1	1	2	2		**1**	4	4	8	**9**
KL	1	**2**	1	**2**	3	3					1	1					6	**8**
LK			2	**3**				**1**	6	**7**					2	2	10	**13**
MIA			2	2											3	3	5	5
MKY							1	1					2	2			3	3
Total	1	**2**	8	**10**	3	3	3	**4**	17	**19**	7	7	2	**3**	11	11	52	**59**

6 loci	Populations of origen																							
	BH		BEZ		BT		COL		CUZ		DB		DT		FTL		KL		LK		MIA		MKY		Total	
Found in	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05
BH			1	1	1	1			1	1				**1**			3	**4**							6	**8**
BEZ					1	1																			1	1
BT							1	1																	1	1
COL																				**1**						**1**
DB													1	1	3	3	1	1	3	3					8	8
DT											1	1					3	3	3	3					7	7
FTL											1	1					1	1	3	3			1	**2**	6	**7**
KL	1	**2**									1	1	3	3					3	**4**					8	**10**
LK											3	3		**1**	1	1	4	4					2	2	10	**11**
MIA											2	2											3	3	5	5
MKY															2	2					2	2			4	4
Total	1	**2**	1	1	2	2	1	1	1	1	8	8	4	**6**	6	6	12	13	12	14	2	2	6	7	56	**63**

Discussion

Summary statistics

We developed 10 new microsatellite markers for the excavating sponge *Cliona delitrix* which appear effective for population genetic studies. These markers showed high levels of polymorphism, allowing the determination of distinct population subdivisions across the greater Caribbean sampling area. Although four of the microsatellite loci were in linkage disequilibrium (LD), this was not evident across all populations. LD can cause loci to behave in an unexpected manner, such as affecting HWE (Freeland 2005). However, LD has been previously found in microsatellite markers of marine sponges (Guardiola, Frotscher, and Uriz 2012, Richards 2010, Blanquer and Uriz 2010), and even with this condition they have been useful to differentiate populations at temporal and spatial scales (Guardiola, Frotscher, and Uriz 2012, Blanquer and Uriz 2010). Also, loci under LD may not necessarily maintain in the LD condition over time (Guardiola, Frotscher, and Uriz 2012). In addition, linkage at a global level does not necessarily reflect LD across all populations. As we found for *C. delitrix*, and Blanquer and Uriz (2010) for the sponge *Scopalina lophyropoda*.

Mean expected heterozygosities for all loci in *Cliona delitrix* (H_E = 0.592 - 0.805) were within the range of other marine sponges: *Crambe crambe* (H_E = 0.422 - 0.748) (Duran et al. 2004), *Paraleucilla magna* (0.609 - 0.698) (Guardiola, Frotscher, and Uriz 2012), *Spongia officinalis* (H_E = 0.73 - 0.90) (Dailianis et al. 2011), and *Xestospongia muta* (0.640 - 0.719) (Richards 2010). Also, as commonly observed in sponges, *C. delitrix* showed departure from HWE, and significant heterozygote deficiency for all 10 loci and within all locations, except for Curacao (Table 4). This deficiency was revealed in high positive F_{IS} values, which could have resulted for technical reasons such as the presence of non-amplifying alleles (i.e. null alleles). Null alleles can commonly occur in invertebrates due to mutations in the flanking regions of microsatellites (Callen et al. 1993, McGoldrick et al. 2000, Reece et al. 2004, Brownlow et al. 2008), or to biological

reasons such as inbreeding, selfing, and Wahlund effects (Allendorf and Gordon 2007, Freeland 2005). When exploring the effects of null alleles, we found that the presence of average null alleles for each locus was lower (6.8 % overall) than for the sponge *Xestospongia muta* (13.1 %) (Richards 2010), or other invertebrates as the Barnacle *Pollicipes elegans* (12 %) (Plough and Marko 2014). Nevertheless, the higher F_{IS} values, and heterozygosities deficiencies in *C. delitrix* could be due to biological reasons. For example, inbreeding cannot be completely ruled out. If we consider that *C. delitrix* larvae behave similarly to other *Cliona* larvae (crawling with low dispersal capabilities), then philopatry could be generated (Mariani, Uriz, and Turon 2000, Warburton 1966). This is a typical condition of sponge larvae that structure populations (Guardiola, Frotscher, and Uriz 2012, Dailianis et al. 2011, Debiasse 2010). In addition, *C. delitrix* populations have a small percentage of hermaphrodite individuals (Chapter 1) and inbreeding could be the result of self-fertilization. Although larvae in *Cliona* sponges can fuse before starting to swim (Warburton 1958), and may form chimeras, heterozygosity deficiencies reflect that this may not be the case for *C. delitrix*. Sponge chimeric individuals, despite their philopatric larvae and small patchy populations, exhibit heterozygote excess and high genetic diversity (Blanquer and Uriz 2011).

In addition, heterozygote deficiency in *Cliona delitrix* can be explained by a temporary Wahlund effect, as a consequence of different breeding subunits within each sampled location. This may be possible since *C. delitrix* has an asynchronous reproduction with multiple peaks of gametes released (three to five pulses a year), in which not all individuals in each location engage in reproduction (Chapter 1). Therefore each location could have several breeding subunits of individuals reproducing at different times over years. This characteristic has been suggested previously for *Crambe crambe*, a sponge that may present a reproductive lag between subpopulations, allowing the formation of different breeding units (Duran et al. 2004, Uriz et al. 1998).

The South Caribbean locations and Bahamas had a greater number and higher average frequency of private alleles than any of the Florida locations. This can indicate a recent expansion of the species to Florida reefs, which are marginal to the distribution range of *C. delitrix* (see map in van Soest 2013). This has been suggested for *Crambe crambe* in the Mediterranean (see Duran et al. 2004). Or, as proposed by Slatkin (1985), private alleles can be an estimator of gene flow. Thus, it is possible that the low number and frequency of private alleles in Florida locations are the result of high gene flow between these locations, whereas little migration occurs in the Bahamas and South Caribbean Sea which, as a result, have numerous private alleles.

Greater Caribbean

The Fisher exact test showed all Florida locations together with Curacao as one population, whereas Bahamas, Belize, Colombia, and Panama as independent populations. However, eliminating the locus with higher null alleles showed that the pairwise test joining Curacao with Florida became non-significant. Thus, the link between Curacao and Florida locations could be an artifact due to low sampling (Curacao n= 8) and presence of null alleles. Fst values, Bayesian, multivariate, and iterative reallocation methods supported differentiation between Atlantic locations (Florida, Bahamas) and the South Caribbean locations (Belize, Colombia, Curacao and Panama), but not between Florida and Bahamas. There was also isolation by distance between the Atlantic and South Caribbean locations. However, first migrant analyses (6 loci) suggest limited dispersal may be possible between these geographic areas (over 1100 km). Previous genetic studies through mtDNA sequences and microsatellite allele frequencies showed that dispersal of *Xestospongia muta* between Honduras / Bahamas locations and Florida had occurred for thousands of generations (Richards 2010, Lopez-Legentil and Pawlik 2009). However, as for *C. delitrix*, contemporary dispersal for *X. muta* between these geographic locations (over 1000 km apart) is unlikely (Richards 2010). The Caribbean and Loop currents are the main surface circulation able to disperse pelagic larvae of invertebrates and connect populations between the Caribbean Sea and Florida

(Mitton, Berg Jr, and Orr 1989, Silberman, Sarver, and Walsh 1994). However, for sponges with low dispersal larvae (Maldonado 2006, Uriz et al. 1998, Mariani, Uriz, and Turon 2000), the speed of the Caribbean current vs. the time of the larvae in the water column are not enough to maintain a constant genetic flow between the Caribbean Sea and Florida-Bahamas area. Indeed the Caribbean current decreases as it flows over the Nicaragua Rise, as most of the northwestward flow moves through the southwest of Jamaica (Fratantoni 2001). Therefore crossing this passage can take longer for larvae until the Caribbean current intensifies in the Yucatan Peninsula and reaches the Loop Current towards the Straits of Florida (Lee et al. 1994, Fratantoni 2001). The Caribbean-Loop current speed is in average 31 cm s^{-1}, between Aves Ridge (65° W) and the Florida Straits, depending upon the path and mean current speed (Fratantoni 2001). Thus, if *C. delitrix* larvae behave similarly to *C. celata* and *C. viridis* (retention in the water column between 24h - 10 days) distances of dispersal for *C. delitrix* from Curacao to Florida could be between 27 km and 269 km. This suggests that direct interchange between Curacao and Florida locations through the Caribbean Current (distance over ~2023 km) is unlikely, as is gene flow between Belize and Florida (over ~1000 km).

Atlantic (Florida-Bahamas)

For the Fisher exact test, eliminating the linked loci yielded a significant differentiation within Florida locations, separate two groups: 1) Dry Tortugas, Key Largo, Looe Key and Dania Beach, and 2) Fort Lauderdale, Miami and Middle Keys. Fst values, Bayesian, multivariate, and iterative reallocation methods also supported two genetic clusters in Florida, but one of them also including the Bahamas: 1) Atlantic (Bahamas, Dry Tortugas, Key Largo, Looe Key); 2) Florida (Fort Lauderdale, Miami and Middle Keys). Dania Beach was a location sharing the same amount of individuals from both clusters. In the Atlantic cluster, the Florida locations (Dry Tortugas, Key Largo, and Looe Key) were all deeper sites (11 - 22 m depth) and farther from the coast line (7.18 - 121 km). In contrast, locations in the Florida cluster (Fort Lauderdale, Miami and Middle Keys) were shallow sites (6-8 m depth) closer to the coast line (0.54 - 3.38 km). Thus, depth appears

as one of the factors differentiating the two genetic populations of *C. delitrix* in Florida. These results agree with a recent study on corals in Florida, in which *Montastraea cavernosa* is differentiated in two genetic clusters, one shallow (≤ 10 m) and one deeper (≥ 15 - 25 m), whereas in *Porites astreoides* the depths at which the two genetic clusters differentiate varied regionally: in the Upper and Lower Keys at ≥ 15 m, while in the Dry Tortugas at ≥ 25 m (Serrano 2013). Thus, our findings with *C. delitrix* support depth as an important factor structuring invertebrate populations in the Florida reef track. This is also the first time in which depth is found as a potential factor affecting the structure of sponge populations. For example, individuals occurring in deeper locations in Florida cluster with individuals from Bahamas, suggesting connectivity between Florida and Bahamas may occurs through exchanges of deep water currents. In Dania Beach location (at 9 m depth; Table 1), our analyses showed a clear overlap of the two genetic clusters. Thus, we posit that besides depth, a Wahlund effect may be occurring for *C. delitrix* populations in the Florida reef track. In this geographic area it is possible that each genetic cluster (shallow / deep) represent different breeding subunits that reproduce at different times. This could be a result of the asynchronous multi-spawning reproduction in *C. delitrix* (see Chapter 1). The overlap between these breeding subunits tends to be greater in the northern locations (Dania Beach and Fort Lauderdale), where the continental shelf is narrow (< 5km) (Lee 1975) and both deep and shallow reefs are closer to each other and to the coast line than in the Florida Keys (~7 - 10km) (Lee and Williams 1999).

Isolation by distance was not significant within the Florida reef track; dispersal and connectivity occurs along the ~315 km of the Florida reefs track sampled. *Cliona delitrix* showed higher dispersal than *Xestospongia muta* (Richards 2010) and *Callyspongia vaginalis* (Debiasse 2010) along Florida reefs. Dispersal occurred between locations spanning a minimum distance of ~3 km (Dania Beach to Fort Lauderdale), and up to 315 km (Dania Beach to Dry Tortugas). These results support previous studies suggesting higher connectivity of sponges and corals along the Florida coast (Andras, Rypien, and Harvell 2013, Richards 2010, Baums et al. 2010). In this coast the maximum average speeds of the Florida Current (200 cm $\cdot$ s^{-1}) and the large Tortugas Gyre (40 cm $\cdot$ s^{-1}) (Lee et al. 1994, Lee and Williams 1999), could be responsible for transporting in a fast

rate the eggs or fertilized larvae of *C. delitrix* along the Florida reef track. Previous studies showed that the Florida Current and the Dry Tortugas Gyre are important for larvae dispersal of invertebrates and fish (Richards 2010, Lee et al. 1994, Lee and Williams 1999, Criales and Lee 1995, Serrano 2013). If *C. delitrix* larvae is alive and retained in the water column between 24 h and 10 days (Mariani, Uriz, and Turon 2000, Warburton 1966), dispersal through the Florida Current could be between 173 km and 1728 km, whereas in the Tortugas Gyre between 35 km and 346 km. The strong connectivity and dispersal between Dry Tortugas and Looe Key may be due to the Tortugas Gyre that moves from Dry Tortugas into the vicinity of Looe reef (Lee et al. 1994). Then the gyre continues decreasing in size forming the Pourtales Gyre on the Middle Keys and later decaying before reaching the Upper Keys (Lee et al. 1994, Lee et al. 1995). From the middle Keys to the Upper, Keys the Florida Current takes a northward direction (Lee and Williams 1999). Thus, connectivity between the south reefs from Looe Key and Dry Tortugas to Key Largo could possibly be facilitated by the Tortugas Gyre and the Florida current. Key Largo, Looe Key and Middle Keys were indeed the locations among the Florida reef track, besides Dania Beach (North side of the sampled reef track), to have greater dispersal. Interestingly, the Tortugas Gyre influences on water motions in the Keys extend from May to November (see map and descriptions in Lee et al. 1994, Lee et al. 1992, Lee and Williams 1999), the same period in which the reproductive cycle of *C. delitrix* occurs in Florida (Chapter 1).

Our study also supports connectivity between the Florida reef track and the Bahamas across the Florida Current (over ~88 km distance) as is seen in sea fan *Gorgonia ventalina* (Andras, Rypien, and Harvell 2013). However, these results contrast with previous studies of the area, which found the Florida Current as a barrier that impedes larvae exchange in sponges and corals between these locations (Vollmer and Palumbi 2007, Richards 2010, Lopez-Legentil and Pawlik 2009, Baums et al. 2010). Dispersal between Bahamas and Florida was found in one direction from Dania Beach, and in both directions from and to Key Largo (Upper keys). At the Upper Keys and continuing from Miami to Palm Beach, the flow of the Current often results in a northeasterly direction

(Lee and Williams 1999), which could facilitate the interchange of larvae outside Florida and specifically from Key Largo to the Bahamas. A previous study with the sponge *Xestospongia muta* found that the Key Largo population of this species was genetically distinct from other populations in Florida (Richards 2010). This difference was attributed to the northerly transport of the Florida current in this area and potential larvae interchange from locations outside the Florida reef track (Richards 2010), although not directly from the Bahamas (Lopez-Legentil and Pawlik 2009, Richards 2010). Due to the change and intensification of the Florida Current in the Upper Keys (Lee and Williams 1999), we consider this area is exposed to more larval exchange from populations outside the Florida reef track area.

C. delitrix dispersal was in equal proportions in both directions (south / north) of the Florida reef track. Connectivity in both directions and between the Upper Keys and Northern reef track locations (Miami, Dania Beach and Fort Lauderdale) could be possible through the Florida Current, whereas dispersal from the North locations to the South could be attributed to the Coastal Countercurrent, eddies and gyres that form closer to the coast (Lee and Williams 1999, Lee 1975, Lee and Mayer 1977).

South Caribbean Sea

We found two to four genetic clusters in the South Caribbean Sea. The PCoA method separated two genetic populations: 1) Colombia, Panamá and Belize as one population and 2) Curacao. In contrast the Bayesian and iterative analyses together supported the Fisher exact test findings of four South Caribbean geographic locations as genetic populations: 1) Belize, 2) Colombia, 3) Curacao, and 4) Panama. Therefore, we consider that South Caribbean locations form independent populations, but Colombia, Panamá and Belize (Southwest Caribbean) showed more connectivity and some admixture between them. It is possible that the Belize - Curacao cluster suggested by the *loc prior* Bayesian analysis is an artifact due to insufficient sampling from Curacao (n= 8), as both PCoA

and iterative methods designated this location an independent genetic population. Our results are consistent with previous studies of the Sea fan coral *Gorgonia ventalina*, which also showed apparent admixture among Panamá and the Mesoamerican Barrier Reef (localities among Belize and Mexico coast) (Andras, Rypien, and Harvell 2013).

Although the four sampled locations in the South Caribbean Sea constitute different genetic populations, isolation by distance was not significant between them (along ~2100 km). Gene flow in this area is present and first generation migrants between Colombia and Panama, and Panama and Belize is possible. Connectivity in this area appears to be higher, since the Caribbean Current velocities along the coasts of Venezuela and the Netherland Antilles can reach 70 cm $\cdot$ s^{-1} (Fratantoni 2001). Also an intense recirculation gyre in the southwest corner of the Colombian basin (12° N, 80° W), which is more than 200 km wide and drives strong currents (60 cm $\cdot$ s^{-1}) occur along the Panamanian and Colombian coasts (Fratantoni 2001). Besides, in this area Cyclonic eddies of ~700 km can form and dissipate on the Southwestern Caribbean (Andrade and Barton 2000). If we consider the speed of the gyre and estimate the time of larvae retention between 24 h and 10 days (Warburton 1966, Mariani, Uriz, and Turon 2000), dispersal distances could reach 51 km to 518 km; therefore it is likely that larvae of *C.delitrix* is being interchanged between Bocas del Toro, Panama, and San Andres Island, Colombia (~345 km linear distance, and ~542 km semi-circle perimeter). These locations are under the recirculation gyre. Although less feasible, larvae may be transported by Eddies between Panama and Colombia and the Mesoamerican Barrier reef.

Dispersal of *C. delitrix* on coral reefs

Although Zilberberg et al. (2006) suggested dispersal ranges for *Cliona delitrix* to be only 10 - 100 m, we found that *C. delitrix* dispersal may reach as far as ~315 km in the Florida reef track. Moreover, dispersal may be possible over distances as high as 971 km in the South Caribbean Sea, between Belize and Panama. Excavating sponge eggs and larvae of

C. delitrix appear to survive enough to be transported by currents over larger distances. Thus, both dispersal and reproductive patterns of *C. delitrix* (Chapter 1) permit gametes, eggs and larvae to be exposed to different current and eddies that form at different periods of times (Andrade and Barton 2000). *C. delitrix* is not an invasive species, but rather a resident species to coral reefs. However, this sponge constitutes a strong competitor of corals that is taking advantage of the recent coral mortality (Chaves-Fonnegra, Zea, and Lopez Submitted, Chapter 2). Consequently, as new individuals continue to engage in reproduction, populations will grow. Also, dispersal could be favored especially in areas were currents are strong enough to transport sponge eggs or larvae longer distances (i.e., along the Florida reef track and Bahamas, and between Colombia and Panama). *C. delitrix* increase has been mainly associated to land-based pollution (sewages) and in reefs close to coast lines. However, this sponge could also become an important reef community component in habitats farther from human influence as corals continue to decline.

Conclusions

This study reveals significant genetic structure for *Cliona delitrix* populations between and within locations in the Greater Caribbean. Six genetic populations were defined in the Greater Caribbean combining Fisher exact test, Bayesian, iterative and multivariate approaches: one in the Atlantic (Florida-Bahamas), one specific to Florida, and four in the South Caribbean Sea. The two genetic populations in Florida were differentiated by depth and possibly correspond to two independent breeding populations that can overlap in some locations. Dispersal of *C. delitrix* was farther than expected for a marine sponge, and results support the influence of water currents patterns on genetic connectivity in the South Caribbean Sea, and within the Florida reef track.

References

Allendorf, F.W., and L. Gordon. 2007. *Conservation and the genetics of populations*: Blackwell Publishing.

Andrade, C.A. , and E.D. Barton. 2000. "Eddy development and motion in the Caribbean Sea." *Journal of Geophysical Research: Oceans* 105 (C11):26191-26201.

Andras, J.P., K.I. Rypien, and C.D. Harvell. 2013. "Range-wide population genetic structure of the Caribbean sea fan coral, *Gorgonia ventalina*." *Molecular Ecology* 22:56-73.

Baums, I.B., M.E. Johnson, M.K. Devlin-Durante, and M.W. Miller. 2010. "Host population genetic structure and zooxanthellae diversity of two reef-building coral species along the Florida Reef Tract and wider Caribbean." *Coral Reefs* 29:835-842.

Bentlage, B., and G. Wörheide. 2007. "Low genetic structuring among *Pericharax heteroraphis* (Porifera: Calcarea) populations from the Great Barrier Reef (Australia), revealed by analysis of nrDNA and nuclear intron sequences." *Coral Reefs* 26 (4):807-816.

Bergquist, P., and M.E. Sinclair. 1968. "The morphology and behaviour of larvae of some intertidal sponges." *New Zealand Journal of Marine and Freshwater Research* 2:426-437.

Bergquist, P.R., and M.E. Sinclair. 1973. "Seasonal variation in settlement and spiculation of sponge larvae." *Marine Biology* 20 (1):35–44.

Blanquer, A., and J. M. Uriz. 2011. ""Living together apart": The hidden genetic diversity of sponge populations." *Molecular Biology and Evolution* 28 (9):2435-2438.

Blanquer, A., J. M. Uriz, and J. Caujapé-Castells. 2009. "Small-scale spatial genetic structure in *Scopalina lophyropoda*, an encrusting sponge with philopatric larval dispersal and frequent fission and fusion events." *Marine Ecology Progress Series* 380:95-102.

Blanquer, A., and M. J. Uriz. 2010. "Population genetics at three spatial scales of a rare sponge living in fragmented habitats." *BMC Evolutionary Biology* 10:13. doi: 1471-2148-10-13 [pii]

10.1186/1471-2148-10-13.

Blanquer, A., M.J. Uriz, and M. Pascual. 2005. "Polymorphic microsatellite loci isolated from the marine sponge *Scopalina lophyropoda* (Demospongiae: Halichondrida)." *Molecular Ecology* 5:466-468.

Brownlow, R.J., D.A. Dawson, G.J. Horsburgh, J.J. Bell, and J.D. Fish. 2008. "A method for genotype validation and primer assessment in heterozygote-deficient species, as demonstrated in the prosobranch mollusc *Hydrobia ulvae*." *BMC Genetics* 19:9:55.

Calderón, I., N. Ortega, S. Duran, M. Becerro, M. Pascual, and X. Turon. 2007. "Finding the relevant scale: clonality and genetic structure in a marine invertebrate (*Crambe crambe*, Porifera)." *Molecular Ecology* 16:1799-1810.

Callen, D.F., A.D. Thompson, Y. Shen, H.A. Phillips, R.I. Richards, J.C. Mulley, and G.R. Sutherland. 1993. " Incidence and origin of 'null' alleles in the (AC)n microsatellite markers." *American Journal of Human Genetics* 52:922-927.

Chapuis, M.P., and A. Estoup. 2007. "Microsatellite null alleles and estimation of population differentiation." *Molecular Biology and Evolution* 24:621-631.

Chaves-Fonnegra, A., M. Maldonado, P.L. Blackwelder, and J.V. Lopez. In preparation. "Reproductive dynamics of the coral excavating Caribbean sponge *Cliona delitrix*."

Chaves-Fonnegra, A., and S. Zea. 2007. "Observations on reef coral undermining by the Caribbean excavating sponge *Cliona delitrix* (Demospongiae, Hadromerida)." In *Porifera research: biodiversity, innovation and sustainability*, edited by M.R. Custódio, G. Lôbo-Hajdu, E. Hajdu and G. Muricy, 247-254. Rio de Janeiro: Museu Nacional.

Chaves-Fonnegra, A., and S. Zea. 2011. "Coral colonization by the encrusting excavating Caribbean sponge *Cliona delitrix*." *Marine Ecology* 32:162-173.

Chaves-Fonnegra, A., S. Zea, and M.L. Gómez. 2007. "Abundance of the excavating sponge *Cliona delitrix* in relation to sewage discharge at San Andrés Island, SW Caribbean, Colombia." *Boletín de Investigaciones Marinas y Costeras* 36:63-78.

Chaves-Fonnegra, A., S. Zea, and J.V. Lopez. Submitted. "Recruitment of the excavating sponge *Cliona delitrix* is favored by recent coral mortality." *Coral Reefs*.

Cortés, J., M. Murillo, H.M. Guzmán, and J. Acuña. 1984. "Pérdida de zooxantelas y muerte de corales y otros organismos arrecifales en el Caribe y Pacífico de Costa Rica." *Revista de Biología Tropical* 32:227-231.

Criales, M.M. , and T.N. Lee. 1995. "Larval distribution and transport of penaeoid shrimps during the presence of the Tortugas Gyre in May-June 1991." *Fishery Bulletin* 93:471-482.

Dailianis, T., C.S. Tsigenopoulos, C. Dounas, and E. Voultsiadou. 2011. "Genetic diversity of the imperilled bath sponge *Spongia officinalis* Linnaeus, 1759 across the Mediterranean Sea: patterns of population differentiation and implications for taxonomy and conservation." *Molecular Ecology* 18:3757 - 3772.

de Goeij, J.M., D. van Oevelen, M.J. Vermeij, R. Osinga, J.J. Middelburg, A.F. de Goeij, and W. Admiraal. 2013. "Surviving in a marine desert: the sponge loop retains resources within coral reefs." *Science* 342 (6154):108-110.

Debiasse, M. 2010. "Genetic assessment of connectivity in the common reef sponge, *Callyspongia vaginalis* (Demospongiae: Haplosclerida) reveals high population structure along the Florida reef tract." *Coral Reefs* 29 (1):47-55.

Dempster, A.P., N.M. Laird, and D.B. Rubin. 1977. "Maximum likelihood from incomplete data via the EM algorithm." *Journal of the Royal Statistical Society Series B (Methodological)* 39:1-38.

Díaz, M., and K. Rützler. 2001. "Sponge: an essential component of Caribbean coral reefs." *Bulletin of Marine Science* 69 (2):535-546.

Duchesne, P., and J. Turgeon. 2012. "FLOCK provides reliable solutions to the ‘‘number of populations’’ problem." *Journal of Heredity* 103 (5):734-43.

Duran, S., M. Pascual, A. Estoup, and X. Turon. 2004. "Strong population structure in the marine sponge *Crambe crambe* (Poecilosclerida) as revealed by microsatellite markers." *Molecular Ecology* 13 (3):511-522.

Duran, S., M. Pascual, and X. Turon. 2004. "Low levels of genetic variation in mtDNA sequences over the western Mediterranean and Atlantic range of the sponge *Crambe crambe* (Poecilosclerida)." *Marine Biology* 144 (2):31-35.

Earl, D.A., and B.M vonHoldt. 2012. "STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method." *Conservation Genetics Resources* 4 (2):359-361.

Evanno, G., S. Regnaut, and J. Goudet. 2005. "Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study." *Molecular Ecology* 14:2611-2620.

Folmer, 0., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. "DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates." *Molecular Marine Biology and Biotechnology* 3 (5):294-299.

Fratantoni, D.M. 2001. "North Atlantic surface circulation during the 1990's observed with satellite-tracked drifters." *Journal of Geophysical Research* 106:22067-22093.

Freeland, J.R. 2005. *Molecular Ecology*. England: John Wiley and Sons Ltd.

Freeland, J.R. 2007. *Molecular Ecology*. Chichester, Inglaterra: John Wiley y Sons

Glenn, T.C., and N.A. Schable. 2005. "Isolating microsatellite DNA loci." *Methods in Enzymology* 395:202-222.

Goudet, J. 1995. "FSTAT (Version 1.2): A computer program to calculate F-statistics." *Journal of Heredity* 86 (6):485-486.

Guardiola, M., J. Frotscher, and M.J. Uriz. 2012. "Genetic structure and differentiation at a short-time scale of the introduced calcarean sponge *Paraleucilla magna* to the western Mediterranean." *Hydrobiologia* 687 (1):71-84.

Guo, S.W., and E.A. Thompson. 1992. "Performing the exact test of Hardy-Weinberg proportion for multiple alleles." *Biometrics* 48:361-372.

Holmes, K.E. . 1997. "Eutrophication and its effect on bioeroding sponge communities." Proc 8th Int Coral Reef Symp, Panamá.

Hubisz, M.J., D. Falush, M. Stephens, and J.K. Pritchard. 2009. "Inferring weak population structure with the assistance of sample group information." *Molecular Ecology Resources* 9:1322-1332.

Ilan, M., and Y. Loya. 1990. "Sexual reproduction and settlement of the coral reef sponge *Chalinula* sp. form the Red Sea." *Marine Biology* 105 (1):25-31.

Kayal, E., B. Bentlage, A.G. Collins, M. Kayal, S. Pirro, and D.V. Lavrov. 2012. "Evolution of linear mitochondrial genomes in medusozoan cnidarians." *Genome Biology and Evolution* 4 (1):1-12.

Knowlton, A.L., B.J. Pierson, S.L. Talbot, and R.C. Highsmith. 2003. "Isolation and characterization of microsatellite loci in the intertidal sponge *Halichondria panicea*." *Molecular Ecology Notes* 3:560-562.

Koressaar, T., and M. Remm. 2007. " Enhancements and modifications of primer design program Primer3." *Bioinformatics* 23 (10):1289-1291.

Lavrov, D.V., L. Forget, M. Kelly, and B. Franz. 2005. "Mitochondrial genomes of two demosponges provide insights into an early stage of animal evolution." *Molecular Biology & Evolution* 22 (5):1231-1239.

Lee, T. N. 1975. "Florida Current spin-off eddies." *Deep-Sea Research* 22:753-763.

Lee, T.N, M.E. Clarke, E. Williams, A.F. Szmant, and T. Berger. 1994. "Evolution of the Tortugas Gyre and its influence on recruitment in the Florida Keys." *Bulletin of Marine Science* 54 (3):621-646.

Lee, T.N, C. Rooth, E. Williams, M. McGowan, A.F. Szmant, and M.E. Clarke. 1992. "Influence of Florida Current, gyres and wind-driven circulation on transport of larvae and recruitment in the Florida Keys coral reefs." *Continental Shelf Research* 12:971-1002.

Lee, T.N., K. Leaman, E. Williams, T. Berger, and L. Atkinson. 1995. "Florida Current meanders and gyre formation in the southern Straits of Florida." *Journal of Geophysical Research: Oceans* 100 (C5):8607-8620.

Lee, T.N., and D.A. Mayer. 1977. "Low-frequency current variability and spin-off Southeast Florida." *Journal of Marine Research* 35:193-220.

Lee, T.N., and E. Williams. 1999. "Mean distribution and seasonal variability of coastal currents and temperature in the Florida Keys with implications for larval recruitment." *Bulletin of Marine Science* 64 (1):35-56.

Loh, T.S., and J. Pawlik. In press. "Chemical defenses and resource trade-offs structure sponge communities on Caribbean coral reefs." *Proceedings of the National Academy of Sciences USA* doi: 10.1073/pnas.1321626111.

Lopez-Legentil, S., and J. R. Pawlik. 2009. "Genetic structure of the Caribbean giant barrel sponge Xestospongia muta using the I3-M11 partition of COI." *Coral Reefs* 28 (1):157-165. doi: DOI 10.1007/s00338-008-0430-3.

López-Victoria, M., and S. Zea. 2004. "Storm-mediated coral colonization by an excavating Caribbean sponge." *Climate Research* 26:251-256.

Lopez, J.V., C.L. Peterson, R. Willoughby, A.E. Wright, E. Enright, S. Zoladz, J.K. Reed, and S.A. Pomponi. 2002. "Characterization of genetic markers for *in vitro* cell line identification of the marine sponge *Axinella corrugata*." *The Journal of Heredity* 93 (1):27-36.

Maldonado, M. 2006. "The ecology of the sponge larva." *Canadian Journal of Zoology* 84 (1):175-194.

Maldonado, M., and P. Bergquist. 2002. "Phylum Porifera." In *Atlas of Marine Invertebrate Larvae*, edited by C. Young, 21-50. Barcelona, España: Academic Press.

Mariani, S., M.J. Uriz, and X. Turon. 2000. "Larval bloom of the oviparous sponge *Cliona viridis*: coupling of larval abundance and adult distribution." *Marine Biology* 137 (5-6):783-790.

Mariani, S., M.J. Uriz, X. Turon, and T. Alcoverro. 2006. "Dispersal strategies in sponge larvae: integrating the life history of larvae and the hydrologic component." *Oecologia* 146 (1):174-184.

McGoldrick, D.J., D. Hedgecock, L.J. English, P. Baoprasertkul, and R.D. Ward. 2000. " The transmission of microsatellite alleles in Australian and North American stocks of the Pacific oyster (*Crassostrea gigas*): selection and null alleles." *Journal of Shellfish Research* 19:779-788.

Meroz, E., and M. Ilan. 1995. "Life history characteristics of a coral reef sponge." *Marine Biology* 124:443-451.

Mitton, J.B., C.J Berg Jr, and K.S. Orr. 1989. "Population structure, larval dispersal, and gene flow in the queen conch, *Strombus gigas*, of the Caribbean. ." *Biological Bulletin* 177:356-362.

Paetkau, D., R. Slade, M. Burden, and A. Estoup. 2004. "Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power." *Molecular Ecology* 13:55 - 65.

Park, S.D.E. 2001. "Trypanotolerance in West African cattle and the population genetic effects of selection. PhD Dissertation." Doctorate, University of Dublin.

Pawlik, J.R. 2011. "The chemical ecology of sponges on Caribbean reefs: natural products shape natural systems." *BioScience* 61 (11):888-898.

Peakall, R., and P.E. Smouse. 2006. "GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research." *Molecular Ecology Notes* 6:288-295.

Peakall, R., and P.E. Smouse. 2012. "GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update." *Bioinformatics* 28:2537-2539.

Piry, S., A. Alapetite, J.M. Cornuet, D. Paetkau, L. Baudouin, and A. Estoup. 2004. "GeneClass2: A Software for Genetic Assignment and First-Generation Migrant Detection." *Journal of Heredity* 95:536-539.

Plough, L.V., and P.B. Marko. 2014. "Characterization of microsatellite loci and repeat density in the gooseneck barnacle, *Pollicipes elegans*, using next generation sequencing." *Journal of Heredity* 105 (1):136-142.

Pomponi, S.A. 1979. "Cytochemical studies of acid phosphatase in etching cells of boring sponges." *Journal of the Marine Biological Association of the United Kingdom* 59:785-789.

Pritchard, J.K., M. Stephens, and P. Donnellya. 2000. "Inference of population structure using multilocus genotype data." *Genetics* 155:945-959.

Rannala, B., and J.L. Mountain. 1997. "Detecting immigration by using multilocus genotypes." *Proceedings of the National Academy of Sciences USA* 94:9197-9201.

Raymond, M. , and F. Rousset. 1995a. "GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism." *Journal of Heredity* 86:248-249.

Raymond, M., and F. Rousset. 1995b. "An exact test for population differentiation." *Evolution* 49 (6):1280-1283.

Reece, K. S., W. L. Ribeiro, P. M. Gaffney, R. B. Carnegie, and S. K. Allen. 2004. "Microsatellite marker development and analysis in the eastern oyster (*Crassostrea virginica*): confirmation of null alleles and non-mendelian segregation ratios " *Journal of Heredity* 95 (4):346-352.

Reiswig, H. 1971. "*In situ* pumping activities of tropical Demospongiae." *Marine Biology* 9 (1):38-50.

Richards, V. 2010. "Genetic Connectivity and Microevolution of Reef Associated Taxa." PhD, Oceanographic Center, Nova Southeastern University.

Richards, V., K. Feldheim, and M. Shivji. 2008. "Contrasting Patterns of Population Structure and Dispersal for the Giant Barrel Sponge (*Xestospongia muta*) within the Florida Reef Tract and Caribbean. Abstracts." 11th Int. Coral Reef Symp. Fort Lauderdale, Florida.

Rose, C.S., and M.J. Risk. 1985. "Increase in *Cliona delitrix* infestation of *Montastraea cavernosa* heads on an organically polluted portion of the Grand Cayman." *P.S.Z.N.I. Marine Ecology* 6 (1):345-363.

Rosenberg, N.A. 2004. "DISTRUCT: a program for the graphical display of population structure." *Molecular Ecology Notes* 4:137-138.

Rousset, F. 1997. "Genetic differentiation and estimation of gene flow from Fstatistics under isolation by distance." *Genetics* 145:1219-1228.

Rousset, F. 2008. "Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux." *Molecular Ecology Resources* 8:103-106.

Rützler, K. 1975. "The role of burrowing sponges in bioerosion." *Oecologia* 19 (1):203-216.

Rützler, K. 1978. "Sponges in coral reefs." In *Coral Reefs: research methods. Monographs in oceanographic methodology*, edited by D.R. Stoddard and R.E. Johannes, 299-313. Paris: UNESCO.

Rützler, K. 2002. "Impact of crustose clionid sponges on Caribbean reef corals." *Acta Geologica Hispánica* 37 (1):61-72.

Sara, M., P. Mensi, R. Manconi, G. Bavestrello, and E. Balleto. 1988. "Genetic variability in the Mediterranean populations of *Tethya* (Porifera: Demospongiae)."

In *Reproduction, genetics and distribution of marine organisms*, edited by J.S. Ryland and P.A. Tyler, 293-298. Fredesnborg, Denmark: Olsen and Olsen.

Schuelke, M. 2000. "An economic method for the fluorescent labeling of PCR fragments." *Nature Biotechnology ,* 18:233-234.

Serrano, X.M. 2013. "Horizontal vs. vertical connectivity in Caribbean reef corals: identifying potential sources of recruitment following disturbance." Doctorate, University of Miami.

Silberman, J.D., S.K. Sarver, and P.J. Walsh. 1994. "Mitochondrial DNA variation and population structure in the spiny lobster *Panulirus argus*. ." *Marine Biology* 120:601-608.

Slatkin, M. 1985. "Rare alleles as indicators of gene flow." *Evolution* 39:53-65.

Solé-Cava, A.M., and J.P. Thorpe. 1986. "Genetic differentiation between morphotypes of the marine sponge *Suberites ficus* (Demospongiae: Hadromerida)." *Marine Biology* 93 (2):247-253.

Untergrasser, A., I. Cutcutache, T. Koressaar, J. Ye, B.C. Faircloth, M. Remm, and S.G. Rozen. 2012. "Primer3 - new capabilities and interfaces." *Nucleic Acids Research* 40 (15):e115.

Uriz, M.J., M. Maldonado, X. Turon, and R. Martí. 1998. "How do reproductive output, larval behaviour, and recruitment contribute to adult spatial patterns in Mediterranean encrusting sponges?" *Marine Ecology Progress Series* 167 (1):137-148.

van Soest, R. 2013. "Cliona delitrix Pang, 1973. World Porifera database at http://www.marinespecies.org/porifera/porifera.php?p=taxdetails&id=170437 on 2014-03-25." In *(2013) World Porifera database.*, edited by R.W.M van Soest, N. Boury-Esnault, J.N.A. Hooper, K. Rützler, N.J. de Voogd, B. Alvarez de Glasby, E. Hajdu, A.B. Pisera, R. Manconi, C. Schoenberg, D. Janussen, K.R. Tabachnick, M. Klautau, B. Picton, M. Kelly, J. Vacelet, M. Dohrmann and M. Cristina Díaz.

Vollmer, S.V., and S.R. Palumbi. 2007. "Restricted gene flow in the Caribbean staghorn coral *Acropora cervicornis*: Implications for the recovery of endangered reefs." *Journal of Heredity* 98 (1):40-50.

Waples, R.S., and O. Gaggiotti. 2006. "What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity." *Molecular Ecology* 15 (6):1419-1439.

Warburton, F.E. 1958. "Reproduction of fused larvae in the boring sponge *Cliona celata* Grant." *Nature* 181:493-494.

Warburton, F.E. 1966. "The Behavior of Sponge Larvae." *Ecology,* 47 (4):672-674.

Ward-Paige, C.A., M.J. Risk, O.A. Sherwood, and W.C. Jaap. 2005. "Clionid sponge surveys on the Florida Reef Tract suggest land-based nutrient inputs." *Marine Pollution Bulletin* 51:570-579.

Weir, B.S. 1996. *Genetic Data Analysis II.* . Sunderland, Massachuset: Sinauer Associates Inc.

Weir, B.S., and C.C. Cockerham. 1984. "Estimating F-Statistics for the Analysis of Population Structure." *Evolution* 38:1358-1370.

Wörheide, G. 2006. "Low variation in partial cytochrome oxidase subunit I (COI) mitochondrial sequences in the coralline demosponge *Astrsoclera willeyana* across the Indo-Pacific." *Marine Biology* 148:907-912.

Wulff, J. 1984. "Sponge-mediated coral reef growth and rejuvenation." *Coral Reefs* 3:157-163.

Wulff, J., and L. Buss. 1979. "Do sponges help hold coral reefs together?" *Nature* 281 (1):474-475.

Zilberberg, C., M. Maldonado, and A.M. Solé-Cava. 2006. "Assessment of the relative contribution of asexual propagation in a population of the coral-excavating sponge *Cliona delitrix* from the Bahamas." *Coral Reefs* 25:297-301.

Zundelevich, A., B. Lazar, and M. Ilan. 2007. "Chemical versus mechanical bioerosion of coral reefs by boring sponges – lessons from *Pione* cf. *vastifica*." *Journal of Experimental Biology* 210:91-96.

Appendix 1.3. Supplementary Information

Table 1. Primer sequences not successful for the amplification and genotyping of 22 microsatellites loci isolated from *Cliona delitrix*.

Locus	Forward and reverse primer sequences (5´-3´)	Repeat Motif	Product Size (bp)
Cd1	F: TCTATGCTGATGAACCCAAGAA R: CAAATGAATGAGCAGCCTAACA	$(GA)_{19}$	391
Cd11	F: ACGTTCATGCTAACTAATGGGTA R: AAACTGCCTCAAGGCATAACA	$(TGTA)_4TGCA(TGTA)_6$	139
Cd12	F: GGTGCCATAAATGGGTCAAG R: TGACCAACCAGGGTAAAAGTG	$(TATG)_7$	187
Cd16	F: GGATAATGTGCGTGTGTTGG R: ACTTGATCTCCGACCTCTGG	$(GA)_{26}$	151
Cd25	F: AGCAGCAATCGATCTTTACCA R: CGAGGCATTGAATTACATACAG	$(TACA)_6$	150
Cd30	F: CCGACCTTCTCTCTCTGTGG R: CAATGGCAAAACAGATGAGG	$(CT)_{25}$	247
Cd52	F: TCCATTGTTCCACCACTCTTT R: GTCGTGTACCATCGGGTCTT	$(CACT)_5(CA)_{28}$	209
Cd55	F: GCACGAGGGTTACGGTAGTT R:TTCAGAGATCAGCGTAAAATCG	$(GTCT)_{14}$	154
Cd74	F: GCTGTAAACACGTGGACGAC R: CGCATATGAACTCAGTCACACA	$(CATA)_8$	205
Cd80	F: TGCTGCAAACAGACACACAG R: GGAAGTGGGATTGGGAATTT	$(GA)_{30}$	180
Cd91	F: CCCGTGAAGCTGGATGTAAT R: GCTTCACAGAGCTCCCAAAC	$(TA)3…(TAAA)_4$	196
Cd95	F: GTCCATTCCAGCTTCTGCAT R: GCCTGCATTATATGGGCTGT	$(TCTG)_5$	352
Cd119	F: GACAAAGTATCATTCGTCTTCTTCC R: GGACACAGCCTTTGAGAGGA	$(CACT)_6(CT)_5…(CTGT)_8(CT)_{38}$	228
Cd131	F: ATCCACCCATGAATGAGAGC R: TTTCCAATCAGCAGTCGTTG	$(TG)_{14}$	177
Cd132	F: CGACAGCCTTAGTGGTGATG R: TTTGAGCCAAAGCTGCACTA	$(CA)_{11}$	325
Cd134	F: CCTGCATATCCGTGTTCTCA R: CACGAGGGGGAATAAACAAA	$(TG)_{41}$	194
Cd135	F: GCACACGAGGGTTACGGTAG R: TAAAAATGGCCGACCTAGCA	$(GTCT)_7$	219
Cd140	F: GCCCTTGTTTAAGGCCTAGC R: TATCCAGTGGGAACCTCTGC	$(TG)_8CA(TG)_4TA(TG)_4$	191
Cd147	F: GCGCAGCAGGGTTACAGTAG R: AGCCTAGCCACGAAATTCAA	$(GTCT)_8GTTT(GT)_9$	205
Cd150	F: TTCATTACCCATTGGATTTGG R: TTCCAACTCTTGACCAACCA	$(TGTA)_6$	152
Cd152	F: GGGAAACCAAAATGGATGTG R: TCTTGCTTTTCGAGTGGGTAA	$(CA)_{10}$	246
Cd159	F: TGAAAGCTTTTGAGTGCTGCT R: GCTGCTGCTGAGTCAGTTCTT	$(CACAGA)_6(CA)_{18}$	156

Table 2. Plateau records to define the number of populations based on iterative reallocation by Paetkau multilocus maximum likelihood implemented in FLOCK 3.1. (Duchesne and Turgeon 2012). *k*= number of reference groups. K=number of estimated clusters. 1A: value of k with a single plateau of length ≥6; 2A: take the value of K = *k* when stopping rule 1A applies; 1B: four consecutive k=0; 2B: Values of k with a plateau of length ≥6; i: largest plateau value will be the lower bound estimate for K (K≥ *k*). Bold numbers correspond to the values determining point (K= *k*) or lower bound (K≥ *k*).

Greater Caribbean			**Florida - Bahamas**			**South Caribbean Sea**		
k	runs	Plateau sequence	*k*	runs	Plateau sequence	*k*	runs	Plateau sequence
2	50	5, 17, 2, 2, 3, 2, 3	2	50	**49 Stop** 1A, 2A	2	50	34, 5, 4
3	50	6, 19, 2, 5, 7, 2, 4, 2	3	50	2, 2, 2, 4, 2, 4	3	50	4, 5, 4, 3, 3, 2
4	50	**11**, **11**, 3, 2 2B,i	4	50	0	4	50	**21**, 2, 2, 3, 3, 2, 3, 3 2B,i
5	50	2, 5, 3, 3	5	50	0	5	49	0
6	50	0	6	50	0	6	45	0
7	50	0	7	50	0	7	23	0
8	42	0	8	42	0	8	4	0 **Stop** 1B
9	50	0 **Stop** 1B	9	50	0	9	1	0
10	50	0	10	50	0	10	0	0
Decision flow: 1B-2B; K=4			Decision flow: 1A-2A; K=2			Decision flow: 1B-2B; K=4		

Converters used for the GPS coordinates:

The Federal Communication commission http://transition.fcc.gov/mb/audio/bickel/DDDMMSS-decimal.html and Earth Point http://www.earthpoint.us/convert.aspx.

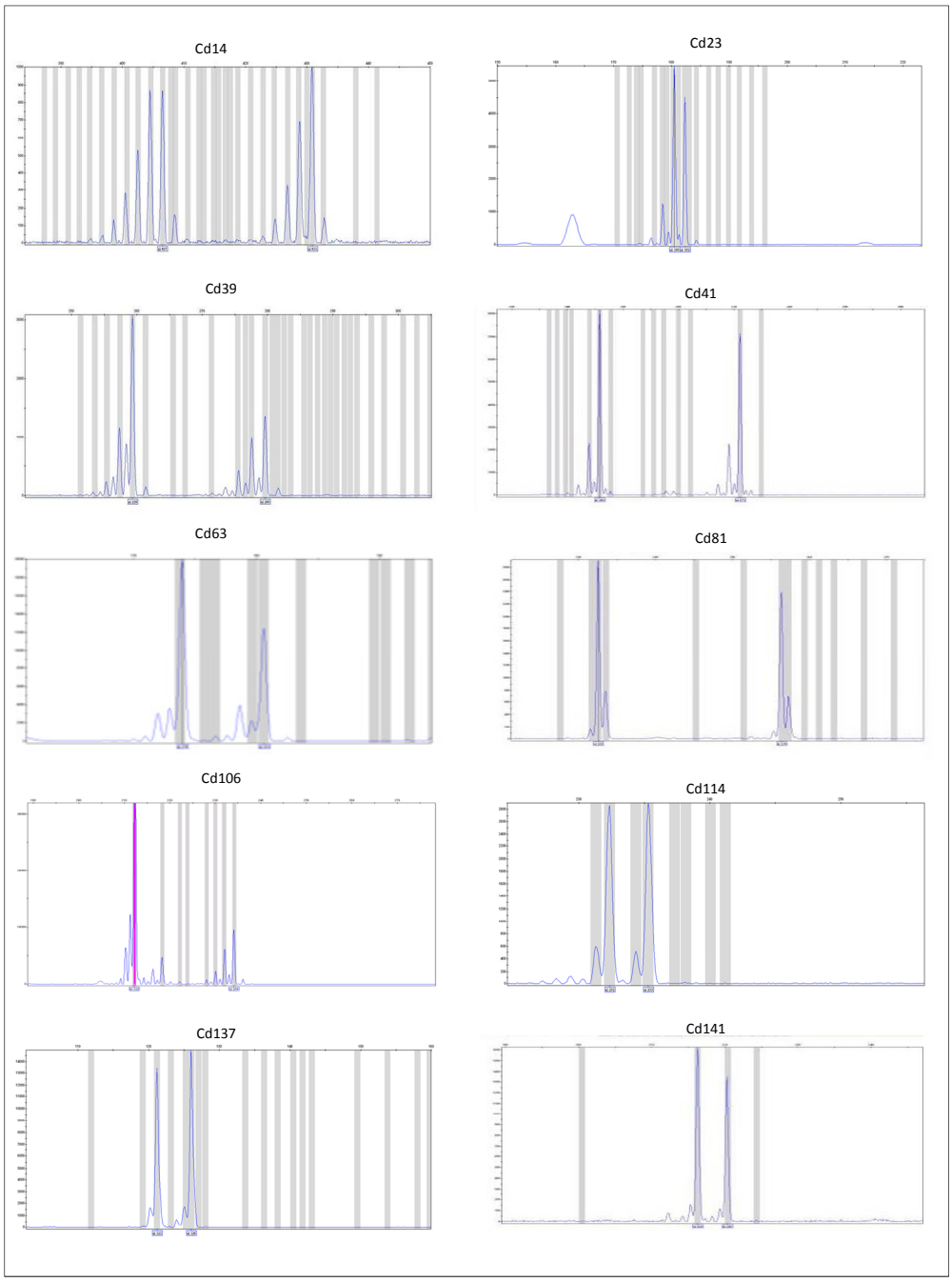


Figure 1. Electropherograms examples for each microsatellite marker. They were analyzed using GENEMAPPER version 3.7 (Applied Biosystems).

CHAPTER 4: FROM CORALS TO EXCAVATING SPONGES? A MARKOV CHAIN MODEL APPROACH

Abstract

Coral reef communities in the Caribbean Sea have changed during the past three decades, shifting to other ecological phases or states. Although sponges do provide habitats on Caribbean reefs, destructive excavating sponges, such as *Cliona delitrix*, also compete with and take over space from deteriorating corals. Thus, we developed a Markov chain model based on field data previously obtained from 100 coral colonies and over a period of 10 years in a Broward County reef, Florida. The model takes a multi-taxa approach in which corals, sponges, and algae are included into the analyses. The state transition model was described, and projections of the model were built in different scenarios of coral recruitment. Also, we used the model and mean transitions matrix together with two disturbance matrices (moderate and massive coral mortality events from heat stress) to project their possible effect on the reef system. The real data showed that in Broward County reef, *Cliona delitrix* sponge started to increase in 2002 and its increase was accentuated after the 2005 heat stress-related massive coral mortality event. Our projections of the mean model and sensitive analysis suggest that for the continuous survival of this coral reef, coral recruitment has to be maintained or increased in the system. When disturbance was added we found that in most projections coral death (ie. overgrowth by algae + other invertebrates) was the final dominant state on reefs. However, in cases in which consecutive moderate coral mortality events occur, sponges could reach percentage cover values as higher as dead coral. Thus, the final reef system will be dominated by dead coral (algae + invertebrates) and the excavating sponges. Under heat stress-induced massive mortality events, both corals and sponges tended to decline, although sponges at a slower rate. During past decades, coral excavating sponges have been favored by coral mortality, opportunistically taking the space newly left by

corals. However, even though excavating sponges appear to be more tolerant to heat stress, they can also face decline, which will depend of the intensity of heat stress and coral mortality events.

Introduction

Caribbean coral reefs have declined, changing their structure in response to persistent disturbances such as disease, thermal stress, overfishing, hurricanes, and pollution (Hughes, 1994; Maliao et al., 2008; Norström et al., 2009; Dudgeon et al., 2010). Reef decline is often associated with a shift from hard coral-dominated to macroalgae-dominate communities (Mcmanus & Polsenberg, 2004; Norström et al., 2009). However, this is not the only change in structure that reflects coral deterioration. Shifts also can occur from hard coral-dominated to other communities such as corallimorpharians, soft corals, urchin barrens, sea anemones, ascidians, and sponges (Maliao et al., 2008; Norström et al., 2009). After corals die, it is difficult to reverse to the initial hard coral-dominated reef, thus some shifts are considered to become stable states (Norström et al., 2009). However, some authors consider that shifts do not necessarily become alternative stable states in coral reefs, but only phases that can be reversed (Dudgeon et al., 2010).

Recently, a variety of models have been used to determine how temperature anomalies affect coral reefs around the world (Pandolfi et al., 2003; Riegl & Purkis, 2009). Some climate change models take into account competition between coral species (Stone et al., 1996; Riegl & Purkis, 2009), and between coral and algae (Renken & Mumby, 2009; Lowe et al., 2011). However, there are not specific multi-species models about sponge-coral interactions under climate change scenarios. The only multispecies approach evaluates the effect of sponge competition on coral stable states (González-Rivero et al., 2011). Other models have been applied to predict the effects of the environment on commercial species and their potential harvest (Cropper and DiResta, 1999), or to

describe changes in demography over time (Mcmurray et al., 2010). Wulff (2006) developed a model to understand pathogen progression within individual sponges.

In Caribbean reefs, sponges are the dominant habitat-forming animals (Pawlik, 2011). Their diversity is higher than all coral groups combined, and their biomass (weight, volume) can exceed that of corals and algae (Rützler, 1978; Díaz & Rützler, 2001). *Cliona* excavating sponge abundances have increased in coral reefs during the past decades (López-Victoria & Zea, 2004; Ward-Paige et al., 2005). Among these sponges, *C. delitrix* is one of the most destructive species on Caribbean reefs, able to excavate deeply (10-12 cm) inside coral skeletons, and to spread laterally at mean rates of ~1.5 cm y^{-1} (Chaves-Fonnegra, 2006), completely overpowering massive live corals (Pang, 1973; Rützler, 2002; Chaves-Fonnegra and Zea, 2007). *Cliona* excavating sponges are in constant competition for space with live corals and algae (turf, calcareous, macroalgae) (López-Victoria et al., 2006; Chaves-Fonnegra & Zea, 2011). Also, these sponges can grow at faster rates when other organisms such as the sea-urchin *Diadema antillarum* rasp surrounding algae (Chaves-Fonnegra & Zea, 2011), or when parrotfish *Sparisoma viride* bite surrounding corals (Márquez & Zea, 2012).

The only population model describing interactions between corals, macroalgae and excavating sponges, suggests that their stable coexistence is not possible, and that excavating sponges tend to favor a low coral cover stable state (González-Rivero et al., 2011). Excavating sponge-coral interactions have been previously considered an epizootic phenomenon (Antonius & Ballesteros, 1998) or an infestation (Glynn, 1997). But, their interaction is best described as asymmetrical competition for space, because sponges usually win, with occasional escape by corals due to their tridimensional growth (Acker & Risk, 1985; Rützler, 2002; López-Victoria et al., 2006).

The increase of these sponges and possible transitions from coral-dominated to excavating sponge- dominated reefs appear to be linked to eutrophication, either through coral mortality or sponge nutrients enrichment (Rose & Risk, 1985; Holmes et al., 2000; Ward-Paige et al., 2005; Chaves-Fonnegra et al., 2007). Thus, phase shifts from hard coral to excavating sponges communities are driven by bottom-up forces linked to a decline in water quality (Norström et al., 2009), but see Pawlik et al., (2013) and Lesser & Slattery (2013) . However, other factors besides eutrophication are favoring the occurrence of excavating sponges, such as better tolerance to high water temperature and coral mortality (Cortés et al., 1984; Rützler, 2002; Miller et al., 2010; Chaves-Fonnegra et al., Submitted), and hurricanes (López-Victoria & Zea, 2004). Thus, anthropogenic and environmental factors are increasing the number of sponge bioeroders (Rose & Risk, 1985; Holmes, 1997; López-Victoria & Zea, 2004; Ward-Paige et al., 2005; Chaves-Fonnegra et al., 2007), possibly accelerating reef bioerosion (Glynn, 1997).

Excavating sponges are the most important coral reef framework bioeroders, and can be responsible for up to 90% of total boring activity in live and dead coral heads (Neumann, 1966; Macgeachy & Stearn, 1976; Glynn, 1997). Being able to bioerode scallop shells at rates of 0.586 kg $\cdot$ $m^{-2}\cdot$ $year^{-1}$ (pH: 7.8) (Duckworth & Peterson, 2013), and corals at rates from 2.23 to 23 kg $\cdot$ $m^{-2}\cdot$ $year^{-1}$ (Neumann, 1966; Rützler, 1975; Glynn, 1997; Wisshak et al., 2012). Sponges bore into calcareous skeletons using amoebocytes (etching cells) that etch and chip minute calcareous fragments (Rützler & Rieger, 1973; Pomponi, 1979). Cutting of the chips is accomplished by enzymes able to dissolve calcium carbonate and the organic matter inside skeletons. Both chips and etching cells are expelled from the sponge, contributing to sediments (Rützler & Rieger, 1973; Pomponi, 1979; Glynn, 1997). Bioerosion and reef growth have always been inseparable, and moderate levels of bioerosion may benefit coral reefs, by contributing towards topographical complexity that serve to increase biodiversity. However, on the other hand, extreme bioerosion can alter reef structure (Glynn, 1997).

Interaction between corals and excavating sponges imply the presence of healthy coral colonies that can transition to a colonies completely overpowered by an excavating sponge (usually one, although in some cases can be more than one, pers. observations). A state transition model of the Markov type could be used to understand the trends and changes in reef structure after an increase of coral excavating sponges. The multi-species Markov chain (MC) models are helpful to characterize ecological dynamics using transitions of ecological states over time (Owen-Smith, 2007; Castillo-Nelis & Wootton, 2010). These models can accurately predict compositions in both, the system that is parametricized, and in novel situations (Wootton, 2001; Wootton, 2004). Application of the Markov chain model began in the 1970´s and helped described successional dynamics of forest communities (Waggoner & Stephens, 1970; Price, 1975). Since then, MC models been applied to other systems, including the marine environment (Hill et al., 2005). For example, Lowe et al., (2011) focused on understanding hard coral and macroalgae dynamic using the probability of transitions between different coral reef states.

Since excavating sponges have increased in abundance in recent years, it is important to predict how many hard corals will be colonized and eventually killed by excavating sponges. Also, it is important to test if coral reefs can become "excavating sponge reefs" over a long period of time (100 years) as environmental changes such as temperature increases and consequent coral mortality occur. Thus, the aim of this study was to evaluate how much excavating sponges can take over currently deteriorated coral reefs and under climate change scenarios. For this we used a state-transition (Markov chain) modeling approach base on field data to build transition probabilities of change from massive scleractinian corals to excavating sponges. This type of biological model could be used as a tool to understand, describe, and predict changes in the reef. Also, it could serve as a tool for environmental management decisions.

The hypotheses to test with the model were:

1) *Cliona delitrix* populations have increased during the past ten years, while live corals decreased, and this pattern will continue over next 100 years.

2) *Cliona delitrix* populations will grow and replace live corals under temperature anomalies.

3) *Cliona delitrix* population will be limited by the increase of dead coral encrusted by algae (turf, macroalgae, calcareous).

Methodology

Definition of ecological states in the coral-sponge interaction

The possible states of change in a healthy coral colony to become an excavating sponge over time were defined based on observations. For this, a total of 43 interactions of massive coral colonies with the excavating sponge *Cliona delitrix* were followed over a period of seven years on San Andres Island, Colombia. These colonies corresponded to the same interactions previously followed for one year by Chaves-Fonnegra and Zea (2011), with the same methodology used. Changes after seven years from coral to only sponge, and information about sponge recruitment and substrata preferences for attachment (chapter 2) were used to establish ecological sates and direction of transitions in the model.

State transition model

a) Data collection and model parameterization

A 30 m^2 photo-transect taken on Broward County middle reef (26° 8' 37.68" N, 80° 04' 01.57´´ W, 11 m deep, DB2 station) was used to monitor 100 coral colonies for a period of ten years (2000 - 2010) (Figure 1 A). Photographs were taken by the Coral Reef Restoration and Monitoring Laboratory (CRRAM, NSU Oceanographic Center) with an Olympus C5060WZ camera, on 40 PVC quadrants of 100 x 75cm (area of quadrant= 0.75m^2) (Figure 1 B). Each photograph was used to count all massive scleractinian corals and all excavating sponges (*Cliona delitrix*), and then they were assigned to an ecological state.

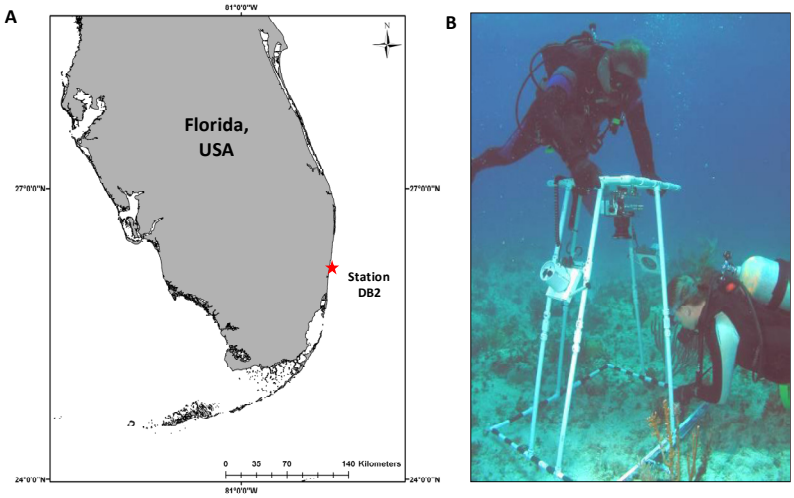


Figure 1. A) Location of the studied area; B) methodological approach to take pictures in a quadrant by the CRRAM Laboratory-NSU Oceanographic Center.

b) Model analysis

For the Markov chain model, the analysis was based on the frequency of all coral colonies (100) within the 30 m^2 transect. This transect was sampled each year (2000 to 2010, excluding year 2009 that was not monitored). In each photograph, all healthy corals, deteriorated corals, dead corals, corals with sponge, and sponges were counted. Once each coral colony was assigned to mutually exclusive ecological states in each year, the transition frequencies were calculated between each ecological state for each pair of consecutive years. A total of nine transition matrices were obtained. These matrices were used to build the transition Markov Chain Model in excel following Owen-Smith (2007). Matrices were organized as square arrays, with columns representing the initial states and the rows the following states. Cell entries constituted the transition probabilities from each starting stage towards each possible next state. Probabilities in all cases summed to unity. In the Markov chain model if *pi(t)* is the proportion of corals in state *i* at time *t*, and *pij* the probability that a reef in state *j* at time *t* will be in state *I* at time *t*+1. Then, proportion of coral colonies in each state can be represented as the vector **c:**

$$\mathbf{c}\,(t) = \begin{bmatrix} ps1(t) \\ ps2(t) \\ . \\ . \\ . \\ . \\ psx(t) \end{bmatrix}$$

and the transition probabilities as a matrix **C**:

$$\mathbf{C} = \begin{bmatrix} ps1s1 & ps2s1 \ldots\ldots & psxs1 \\ Ps1s2 & ps2s2 \ldots\ldots & psxs2 \\ . & . & . \\ . & . & . \\ . & . & . \\ . & . & . \\ Ps1sx & ps2sx & psxsx \end{bmatrix}$$

Thus, the proportions in each state at time t+1 will be given by the linear equation:

$$p(t+1) = c \cdot C(t)$$

c) Present dynamics

To estimate present dynamics of a Broward Florida reef, we started the model with the proportions of colonies found in 2000, and ran each transition matrix connecting consecutive years. Then the model was run based on proportions. In addition, to estimate the corresponding percentage in cover of each state, we assigned two sizes to both sponges and corals in the system: adult diameter = 30 cm^2 and recruit diameter = 5 cm^2. Area of adults and recruits was estimated from a circle ($A= \pi \cdot r^2$). Proportions of sponge and coral recruitment on dead coral were multiplied by the area of recruits (=0.0019 m^2). Similarly, proportions of coral with sponge were considered all new sponge recruits and multiplied by a recruit´s area. The rest of the transitions were considered as adults, and multiplied by adults' area (= 0.071 m^2). Then, the new proportions based on area were used to build new matrices to run the model again. The final outcome of the 10 year data was expressed as proportions of corals and percent cover.

d) System projections and sensitivity analysis

A mean matrix of all ten years of data (2000-2010) was built (Appendix 1, Table 3). Proportions (based on frequency of coral colonies) obtained by the mean matrix were also standardized to percent cover as explained before. To project general trends of the model over 100 years, we used as starting point existing average percentages values of Caribbean reefs (see Appendix 1, Table 1). This information comes from 20 m^2 linear transects, and data was collected as explained in Chapter 2.

Projections of the mean model were performed with four different rates of coral recruitment: i) no coral recruitment (0 recruits $\cdot$ $m^{-2} \cdot y^{-1}$), ii) low coral recruitment (0.018 recruits $\cdot$ $m^{-2} \cdot y^{-1}$), iii) average natural coral recruitment (0.12 recruits $\cdot$ $m^{-2} \cdot y^{-1}$, as quantified on transects over the 10 years), and iv) doubling of the natural recruitment

(0.24 recruits · m^{-2} · y^{-1}). Rate (iv) is a hypothetical case in which coral recruitment may be increased assuming higher dispersal from other reefs. These projections assumed all new coral and sponge recruits will reach at least 5 cm in diameter, and all adults 30cm.

A sensitivity analysis was applied to the mean matrix of the 10 years studied. For this, all proportions at each state and its transitions to other states were changed by the same amount using PopTools 3.2.5 (Hood, 2010). Zero values were kept only for the transitions that we considered not possible. For example, after a coral dies and already has a sponge, it is not feasible that it will revive to a living, healthy or even a deteriorated state. After running, results of the model were presented as proportion of coral colonies, and also they were adjusted to percent cover as explained in the Present dynamics section.

e) Climate change effect

A coral mortality event occurred on August and September 2010 in Bocas del Toro reefs, Panama, where seawater temperature reached 31.1°C at 5 and 10 m deep. Satellite images of the area showed 8 DHW (=degree heating weeks) of thermal stress in this area (NOAA, 2000). To evaluate the effect that climate change can have on the reef dynamics, we used four 40 m^2 (20 x 2 m) transects placed in July 2010, and later on February 2012 (see chapter 2). In each 20 m transect the frequency of coral colonies at different ecological stages was estimated, whereas corals with sponge and solo sponges were quantified on 40 m^2 (as explained in Chapter 2). Base on differences before and after, we estimated transitions probabilities of two disturbance matrices: 1) Moderate mortality event, based on data at 6 m depth, and 2) Massive mortality event, based on data at 10 m depth (see Appendix 1, Tables 4, 5). At each depth, 2 transects were used. The definition of the impact produced by high temperature at each depth was defined based on results from Chapter 2.

To model the effect of moderate and massive mortality events, we used three different matrices: 1) Present reef (Before mortality; based on the mean transitions matrix of a present reef previously obtained from 10 years data at Broward), 2) Disturbance matrix (Moderate or Massive mortality transition matrices as explained before), and 3) Recovery matrix (a matrix in which we use same initial transitions for healthy corals as in the mortality event, but we allowed all other transitions to be as in a present reef), see matrices in Appendix 1,Tables 4 and 5. These three matrices were then run for 100 years. The first 10 years applied the current reef matrix, and then we included mortality events every 10 years (using the moderate mortality matrix). Between mortality events the recovery matrix was allowed to run (Appendix 1, Table 4). Also, we considered that after a first mortality event and its recovery, the next mortality event will probably take longer to get into recovery phase. This considers that with less healthy corals, longer time periods will be needed to reach a recovery phase. Thus, at each new mortality event we lagged the disturbance matrix by one year more than in the previous event.

Also a combination of a moderate and massive mortality models, referred to here as 'mix' model (See Appendix 1, Table 6 for matrices) was applied to obtain a more realistic scenario in which both disturbances occur. In this case each disturbance matrix (moderate or massive) was alternated every five years for 35 years and then randomly assigned until 100 years.

The Moderate mortality model, and mixed (moderate + massive) model, explained before, were run allowing low coral recruitment (0.02 $r \cdot m^{-2} \cdot y^{-1}$), and almost half of the recruitment found in the Broward reef (we use 0.13 $r \cdot m^{-2} \cdot y^{-1}$, Broward reef is 0.15 $r \cdot m^{-2} \cdot y^{-1}$). For the model of massive coral mortality we assumed that coral recruitment will be maintained low as most corals will be affected; two values of recruitment were tested in the model (0.02 and 0.07 $r \cdot m^{-2} \cdot y^{-1}$).

f) Bioerosion estimate

As an estimate of bioerosion produced by the excavating sponge *Cliona delitrix* on corals, we used the volume of sponge tissue inside coral skeleton fragments. These fragments were selected depending of the degree of the excavation by the sponge (36 different types were used). The initial volume of each fragment with sponge was calculated using a cylinder with sea water (50 ml ± 0.05). Then, different types of fragments were grouped in 8 replicates, and submerged in a solution of commercial bleach (NaClO). After approximately 30 minutes, the coral skeleton was free of sponge tissue. The volume of coral fragments without sponge tissue was measured in the cylinder, and the volumetric difference (= initial volume – final volume) was used to established the volume of the sponge. This sponge volume corresponds to the amount of coral skeleton removed by the sponge, thus it can be used as an estimative of bioerosion.

To estimate the amount of bioerosion produced at the reef level, we calculate the volume of sponge recruits and adults at Broward County reef using their area, as explained before in the *Present Dynamics* section. Then, to obtain their volume ($V = \pi \cdot r^2 \cdot h$) we multiplied the area by a conservative mean excavation depth of 2 cm for recruits and 5 cm for adults (Chaves-Fonnegra & Zea, 2007). To obtain the loss of coral skeleton produced by the sponge, we used the percentage of erosion produced by the sponge in the coral skeleton matrix from the fragments submerged in NaClO (Appendix 1, Table 2). Then the loss of coral skeleton produced by each sponge recruit or adult was multiplied by the cover percent of CS (those still competing with corals) and adults S (sponges that already took all coral colony). The final value of coral skeleton loss was given in cm^3 per m^2 of reef, and is equivalent to the accumulated bioerosion that the whole volume of sponges produced. Thus, is not a rate of bioerosion *per se*. Instead is a bioerosion estimate that each sponge generated in relation to its size. The rate per year is in relation to bioerosion generated by new recruits and recruits that became adults, minus the sponges that died and do not bioerode the reefs any longer.

Results

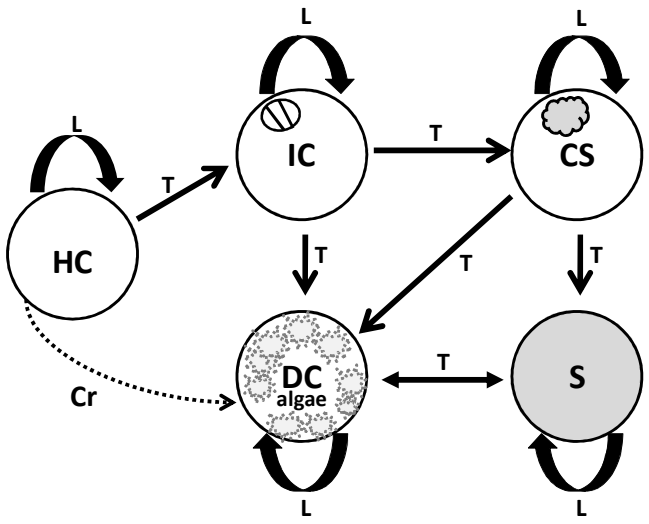
Description of the model

Five different transition states were defined: healthy coral (HC), deteriorated coral (IC), dead coral (DC), coral with sponge (CS) and sponge (S). Table 1 shows specific characteristics of each state. Based on recruitment data, we found that *Cliona* recruitment does not occur on live coral tissue (Chapter 2), but, rather only on recently dead coral and old coral mortality substratum (Chapter 2). Also, from our field observations we found that coral recruitment does not occur on top of *Cliona* sponges. Thus, we assumed that coral recruitment occurs on dead corals that had been covered by calcareous algae (Morse et al., 1988; Heyward & Negri, 1999).

The model posits that a healthy coral (HC) needs to die or deteriorate (partly dead, IC) before any sponge recruit can attach to it. Thus, the first transition occurs from a healthy coral to a deteriorated (IC) or dead coral (DC) (Figure 2). Although deterioration always has to occur before death, it can happen quickly, and may not be observed or quantified in the field. Once a coral deteriorated (IC), sponge recruitment can occur on these colonies (CS). Also, sponge recruits can attach on dead coral (S). The coral colonies with sponge (CS) can compete for several years (> 7 years), before all coral tissue dies, and the sponge is not competing any more with coral (S) (Figure 2). At this stage, the most plausible result is that the sponge takes over the whole coral colony, even if it has to compete with algae or other invertebrates. However, sponges can die due to overgrowth of macroalgae or high sedimentation. In this case the skeleton of the coral colony goes back into a dead coral stage (DC), at this time more bioeroded, but as ecological state, similar to what happen after coral died. It will be coral skeleton covered by algae or other invertebrates (DC). Also this same DC can be again colonized by the sponge. Thus transition between DC and S occur in both ways. Our observations suggest that after corals reach CS state it is not possible that the coral will return to a deteriorated state (IC). Coral tissue died in most cases, or both coral and sponge died; in this latter case the CS will change into a DC state.

Table 1. Ecological states defined in the coral-excavating sponge interaction and used for Markov chain analysis

Healthy coral (HC)	Most of the coral colony is alive, and color and texture of tissue are homogeneous and typical for each species when they are in good physiological condition.
Deteriorated coral (IC)	The colony is not completely covered by tissue (has some dead patches or spots, some usually recent), is bleached, or with other signs of diseases.
Dead Coral (DC)	All coral tissue has disappeared, and coral skeleton is covered by turf algae, calcareous algae, macroalgae, or other invertebrates. This is termed old coral mortality.
Coral with Sponge (CS)	The coral colony still has live tissue and the excavating sponge (small recruits to medium size sponges) is attached and competing with coral tissue.
Sponge (S)	No live coral tissue remains and the sponge lives by itself on the coral head. At this state the sponge can be of any size, and even with algae around. The most plausible result is that the sponge will take over the whole coral colony skeleton.



	Starting state				
Next state	HC	IC	DC	CS	S
HC	$P_{h,h}$	$P_{i,h}$	$P_{d,h}$	$P_{c,h}$	~~$P_{s,h}$~~
IC	$P_{h,i}$	$P_{i,i}$	$P_{d,i}$	$P_{c,i}$	~~$P_{s,i}$~~
DC	$P_{h,d}$	$P_{i,d}$	$P_{d,d}$	$P_{c,d}$	$P_{s,d}$
CS	$P_{h,c}$	$P_{i,c}$	$P_{d,c}$	$P_{c,c}$	~~$P_{s,c}$~~
S	$P_{h,s}$	$P_{i,s}$	$P_{d,s}$	$P_{c,s}$	$P_{s,s}$

Cell entries represent the state transition probabilities.
Ph,i for example represents the probability that a colony initially healthy (*h*) will exist in state deteriorated one time step later (*i*)

Figure 2. Structure of the model between massive corals and excavating sponges. Arrows show the direction of an interaction. HC: Healthy coral; IC: deteriorated coral; CS: Coral with sponge; DC: dead coral; S: sponge; Cr: coral recruitment; L: loops represent the proportion of corals/sponges that remain within a state; T: represents the proportion of corals/sponges that will change state. Coral recruitment was included in the model as new recruits attached on dead corals and are included in $P_{d,h}$. Sponge recruitment occurs when a coral colony has a sponge for the first time; this can be a healthy coral ($P_{h,c}$), deteriorated coral ($P_{i,c}$), or dead coral ($P_{d,c}$). After a coral becomes a sponge (S), there is no reversal from Sponge (S) to HC, IC or CS (crossed out in the table).

Present dynamics

Over the ten year period that photoquadrats were followed (2000 to 2010) at Broward County reef, the proportion and cover of healthy corals increased during the first two years, and then started to decrease. This decrease was accentuated after 2005, and the number of deteriorated and dead corals doubled their amount between 2005 and 2010. In 2005, a bleaching and mortality event occurred across the tropical western Atlantic and

the Caribbean Sea (Eakin et al., 2010), and its consequences were reflected in our data (Figure 3 A-C). The proportion and cover of *Cliona delitrix* and its accumulated bioerosion started increasing in 2002, and reached their maximum between 2005 and 2007. After 2007 the proportion and cover of this sponge, as well as its accumulated bioerosion decreased (Figure 3 A-C). Between 2002 and 2005 the increment in *C. delitrix* was related to a decrease in dead coral, more than to a decrease in healthy corals or increase in deteriorated corals. This is possible, since the sponge can also recruit on dead coral (DC), when algae or other invertebrates die and leave open space (see Chapter 2). After 2005 the increase in this sponge appeared correlated to an increase in deteriorated corals and dead coral, and to a decrease in healthy coral.

System projections

If conditions remained as they were during the observed 10-year period, the reef system projections would not approach equilibrium after 100 years. It can be observed in Figure 4 that although all scenarios of coral recruitment appear to reach equilibrium, actually they all continue slight decreasing or increasing. Under the scenarios of no coral recruitment (0 recruits $\cdot$ $m^{-2} \cdot y^{-1}$) or low coral recruitment (0.018 recruits $\cdot$ $m^{-2} \cdot y^{-1}$) the system will be dominated by a dead coral state (DC). Corals with sponges (CS) will be rare, but sponges (S) will be able to increase their percent cover in a period of 15 to 20 years, cover remaining above that of healthy (HC) and deteriorated coral (IC). HC and IC will continue decreasing, reaching levels below 5% of cover in 100 years (Figure 4 A, B).

However, if coral recruitment remained at the natural rates we observed on the reef (0.12 recruits $\cdot$ $m^{-2} \cdot y^{-1}$), the HC cover can be maintained close to 20%, slightly above *C. delitrix* cover (S) (Figure 4 C). DC will maintain its dominance in the reef, but the percent cover will be lower than in scenarios with no or low coral recruitment. If the natural coral recruitment is hypothetically doubled (0.24 recruits $\cdot$ $m^{-2} \cdot y^{-1}$), the amount of HC will dominate, and DC cover will be reduced to almost a half than in scenarios with no recruitment (Figure 4 D). The percentage of CS will become lower, but S will

tend to occupy almost 20% of the reefs. In the latter scenario, although more dead coral may be available, *C. delitrix* did not necessary take over this space. Rather, in most cases algae had already colonized the available space on DC, pointing to the competition between algae and sponges in the model. Sponge colonization appears to be limited by the amount of HC and IC that is dying.

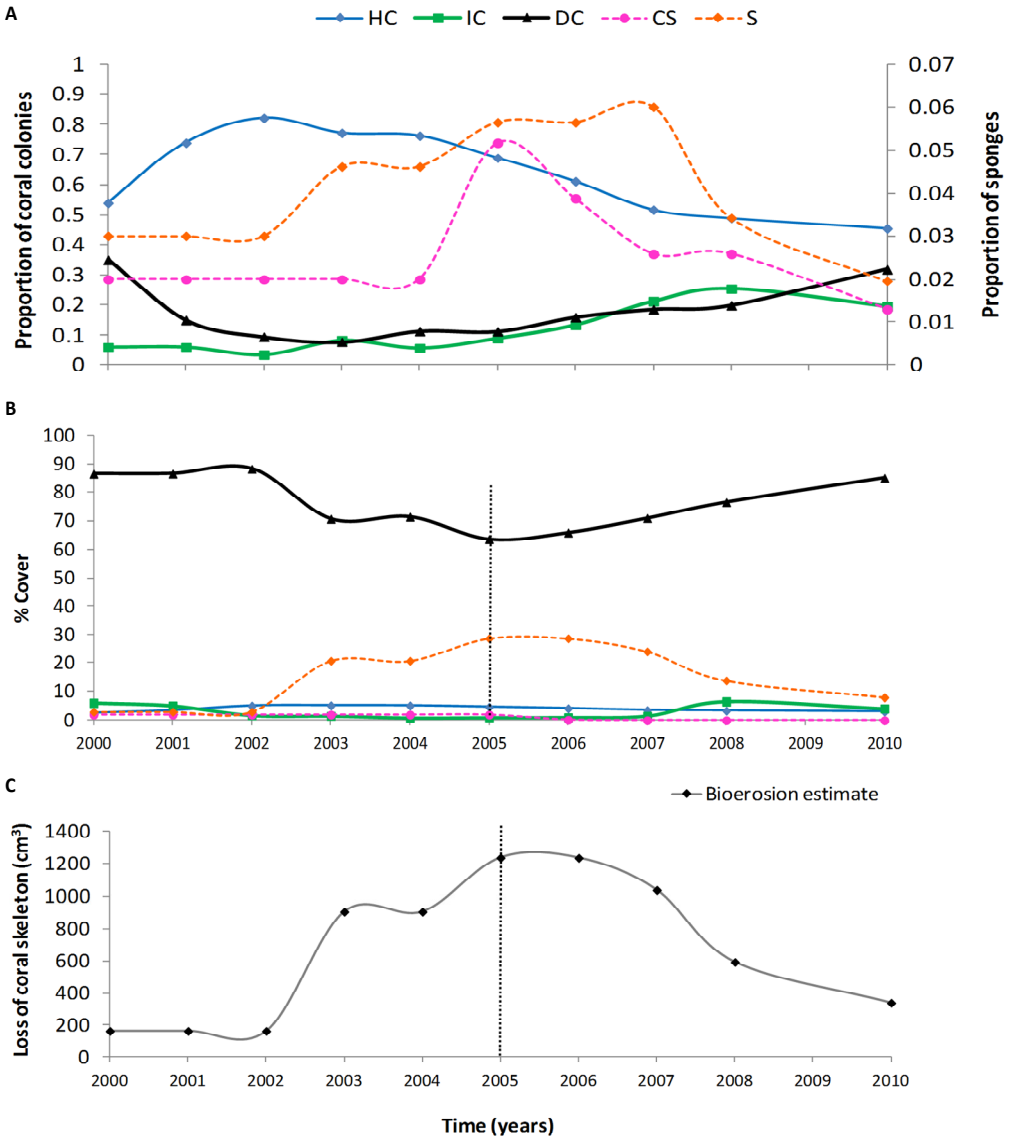


Figure 3. Present trends of ecological states on a Broward County reef. Abbreviations are the same as in Fig 2. A) Proportion of colonies (based on frequencies) including adults and new recruits (corals and sponges) observed each year. B) Percent cover of each state based on proportions standardized by adults and recruits areas. C) Accumulated bioerosion produced by sponges, based on the total volume of adults and recruits for each year. Vertical dotted line on 2005 symbolizes the mortality event.

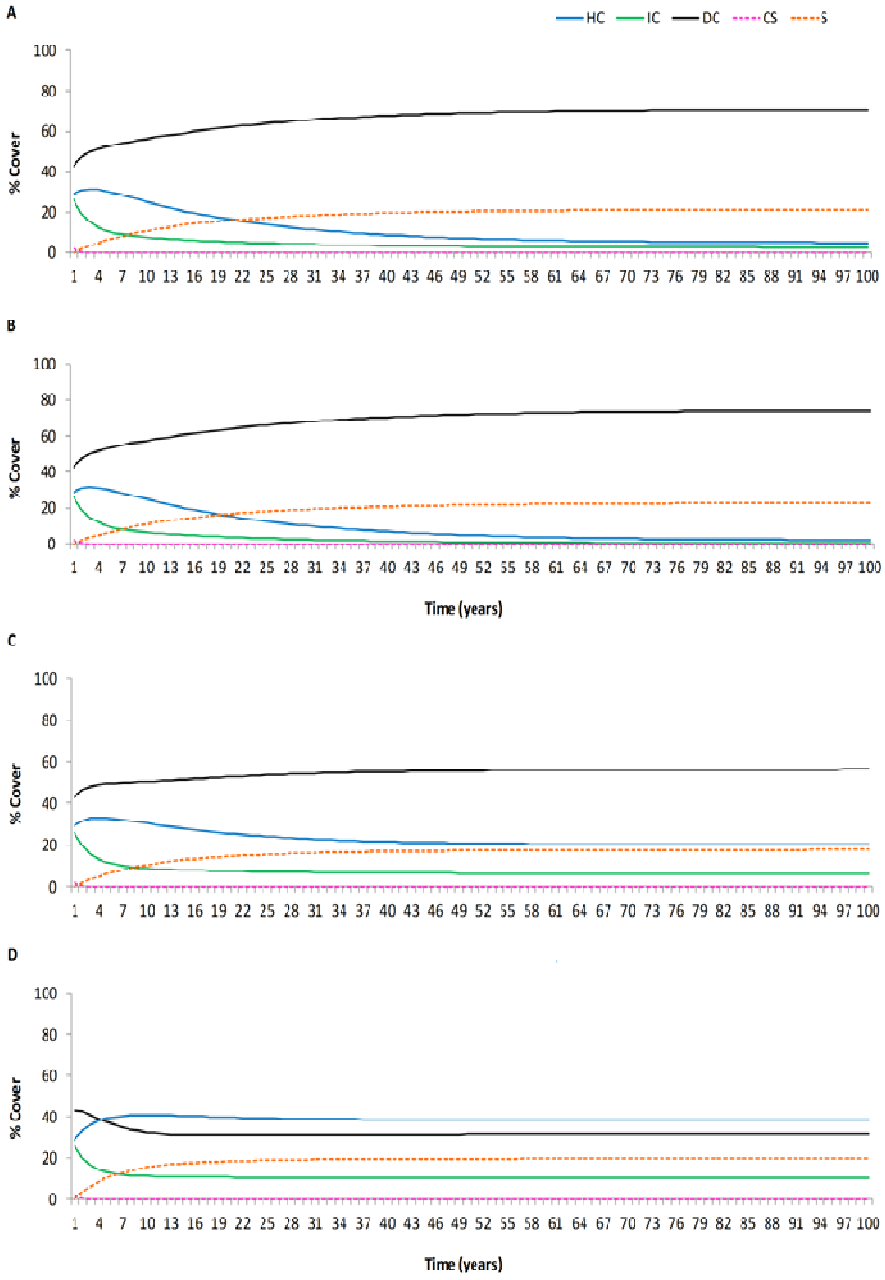


Figure 4. Projected percentage cover using the mean transition probabilities matrix. HC: Healthy coral; IC: deteriorated coral; CS: Coral with sponge; DC: dead coral; S: sponge. A) Without coral recruitment (0 recruits · m^{-2} · y^{-1}); B) with low recruitment (0.018 recruits · m^{-2} · y^{-1}) ,C) with natural mean recruitment found in Broward per year (0.12 recruits · m^{-2} · y^{-1}), and D) doubling the natural recruitment (0.24 recruits · m^{-2} · y^{-1}).

Sensitivity Analysis

The sensitivity analysis showed that if the probabilities of transition from one state to other are changed by the same amount (see Table 1), both dead coral (DC) and sponges (S) will dominate the reef (Figure 5 A, B). While both proportion and cover of healthy (HC) and deteriorated corals (IC) will decrease. Over the last ten years, Broward County reefs have a greater proportion of HC over those of corals with sponge (CS) or sponges (S) (Figure 3). We found that the main driver in changes is coral recruitment. As previously observed, altering the amount of coral recruitment will shift balance of the system dramatically (Figure 4). From the sensitivity analysis we also found that to avoid the system shift into S and DC, it was necessary for the probability of recruitment of sponges on corals (CS) and transitions from CS to S to be much lower than for other transitions.

Table 1. Sensitivity transitions matrix. HC: Healthy coral; IC: deteriorated coral; CS: Coral with sponge; DC: dead coral; S: sponge.

	Starting state				
Next state	**HC**	**IC**	**DC**	**CS**	**S**
HC	0.2	0.2	0.2	0	0
IC	0.2	0.2	0.2	0	0
DC	0.2	0.2	0.2	0.33333333	0.5
CS	0.2	0.2	0.2	0.33333333	0
S	0.2	0.2	0.2	0.33333333	0.5
Total	1	1	1	1	1

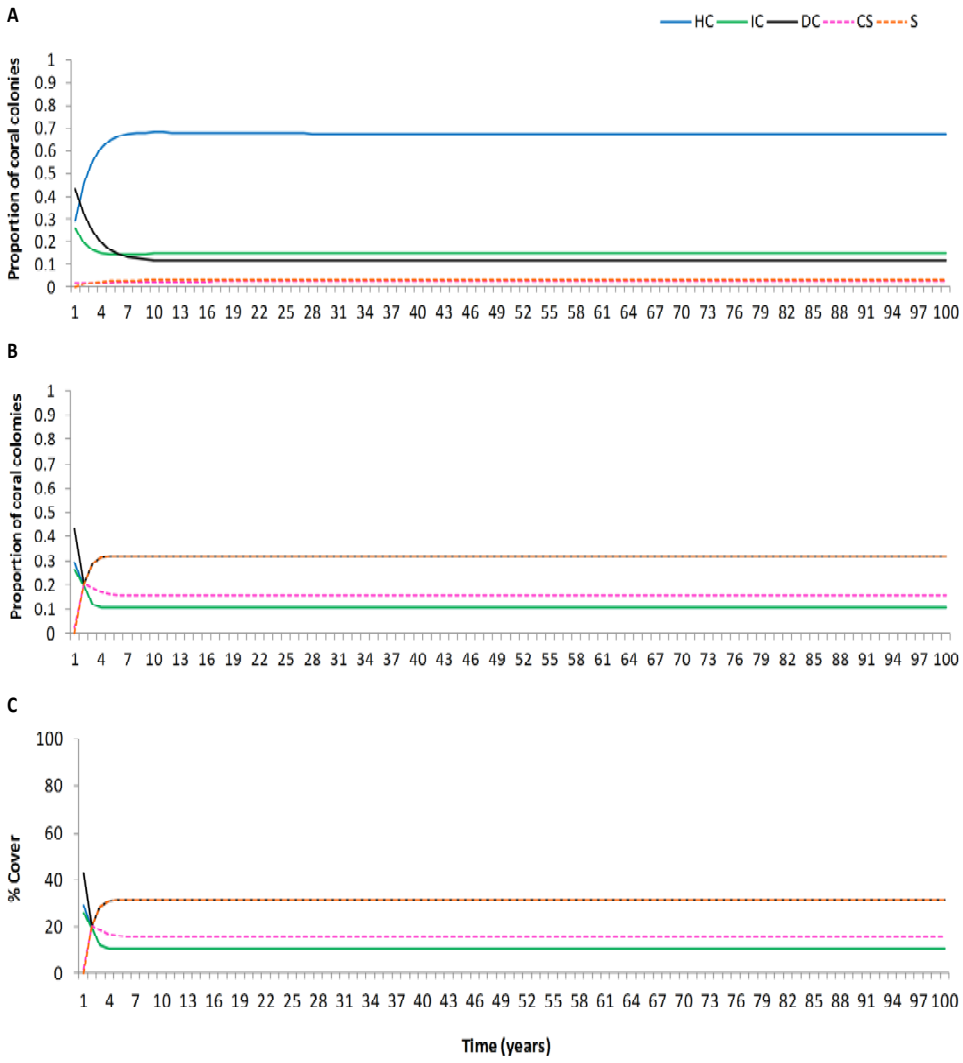


Figure 5. Sensitivity analysis on Broward mean matrix. A) Original proportion of coral colonies. B) Proportions of colonies after the sensitivity analysis. C) Sensitivity analysis expressed as percent of cover. HC: Healthy coral; IC: deteriorated coral; CS: Coral with sponge; DC: dead coral; S: sponge.

Climate change effect

a) Moderate coral mortality

In a reef system with moderate coral mortality every 10 years, and with low and moderate coral recruitment, the projections showed that both DC and S tend to dominate and alternate (Figure 6 A, B). After the first moderate mortality event, the proportion of Healthy coral (HC) decreased, whereas deteriorated coral (IC), dead coral (DC), coral

with sponge (CS) and sponge (S) increased. The IC state rapidly decreased, whereas DC, CS and S increased. In later mortality events, while HC decreased, DC increased each time. After DC peaked to its highest, it slightly dropped, while IC, CS and S increased. Although the general trend of the sponges (CS and S) in this model increased over time, by pulses after each mortality event. In terms of percent cover, DC always dominated, and after the second mortality event S increased above HC and IC. Sponge cover was maintained for a long time, with small decreases. The moderate mortality produced coral deterioration (IC) that was reversed to healthy corals (HC) during periods in which disturbance did not occur. Sponge cover did not reach over 20% and did not surpass the amount of coral mortality produced (Figure 6 C). However its constant increase allowed for a constant increase of bioerosion in the reef (Figure 6 D). Bioerosion and S tend to decrease in years in which dead coral increase.

As in this moderate mortality simulation, in Broward reef, S cover increased after the mortality event (Figure 3), but it was not able to pass over DC.

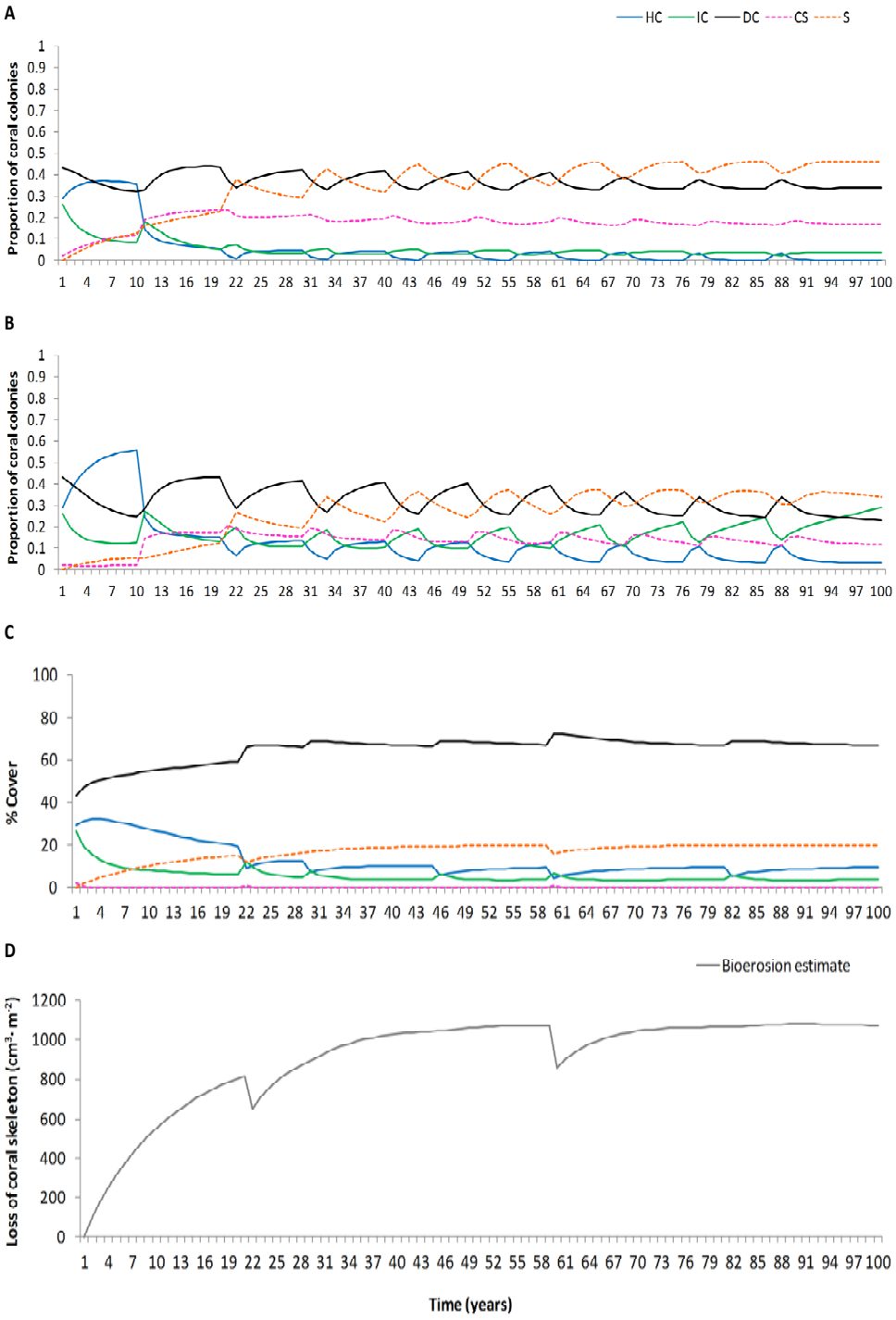


Figure 6. Effects of a moderate coral mortality event, A) with low coral recruitment (0.04 $r \cdot m^{-2} \cdot y^{-1}$), B) with almost half of the mean coral recruitment in the Broward reef (0.13 $r \cdot m^{-2} \cdot y^{-1}$). C) Percent cover of each state based on proportions standardized by adults and recruits areas. D) Accumulated bioerosion produced by sponges, based on the total volume of adults and recruits for each year. HC: Healthy coral; IC: deteriorated coral; CS: Coral with sponge; DC: dead coral; S: sponge.

b) Massive coral mortality

In the massive mortality model, the reef system became dominated by dead coral (DC) both in terms of proportions (frequency of colonies) and percent cover (Figure 7 A-C). Healthy coral (HC) and deteriorated corals decreased and reached values close to zero, in both scenarios: with moderate or low coral recruitment (Figure 7 A, B). Sponges (CS and S) increased in proportion. However, S percent cover increased, which included the adults and larger-size sponges. Thus although sponge recruitment was occurring, and was relatively high in terms of proportion of individuals, owing to their size and transitions into adults sponges, their increase was not reflected in percent cover. During mortality events, 50% of the sponges died (see Chaves-Fonnegra et al., In preparation, Chapter 2). This contributed to a reduction of the sponge cover over time, while dead coral increased. Thus, both healthy corals and sponges were affected; first the HC and then over long period of time the sponges. Bioerosion followed the same pattern as adult sponges, which were primarily responsible of this process. Bioerosion also started increasing after a massive mortality event, while sponges grew over time. Then after each new massive mortality event the sponge population decreased, and the bioerosion rates in the reef decreased accordingly (Figure 7 D).

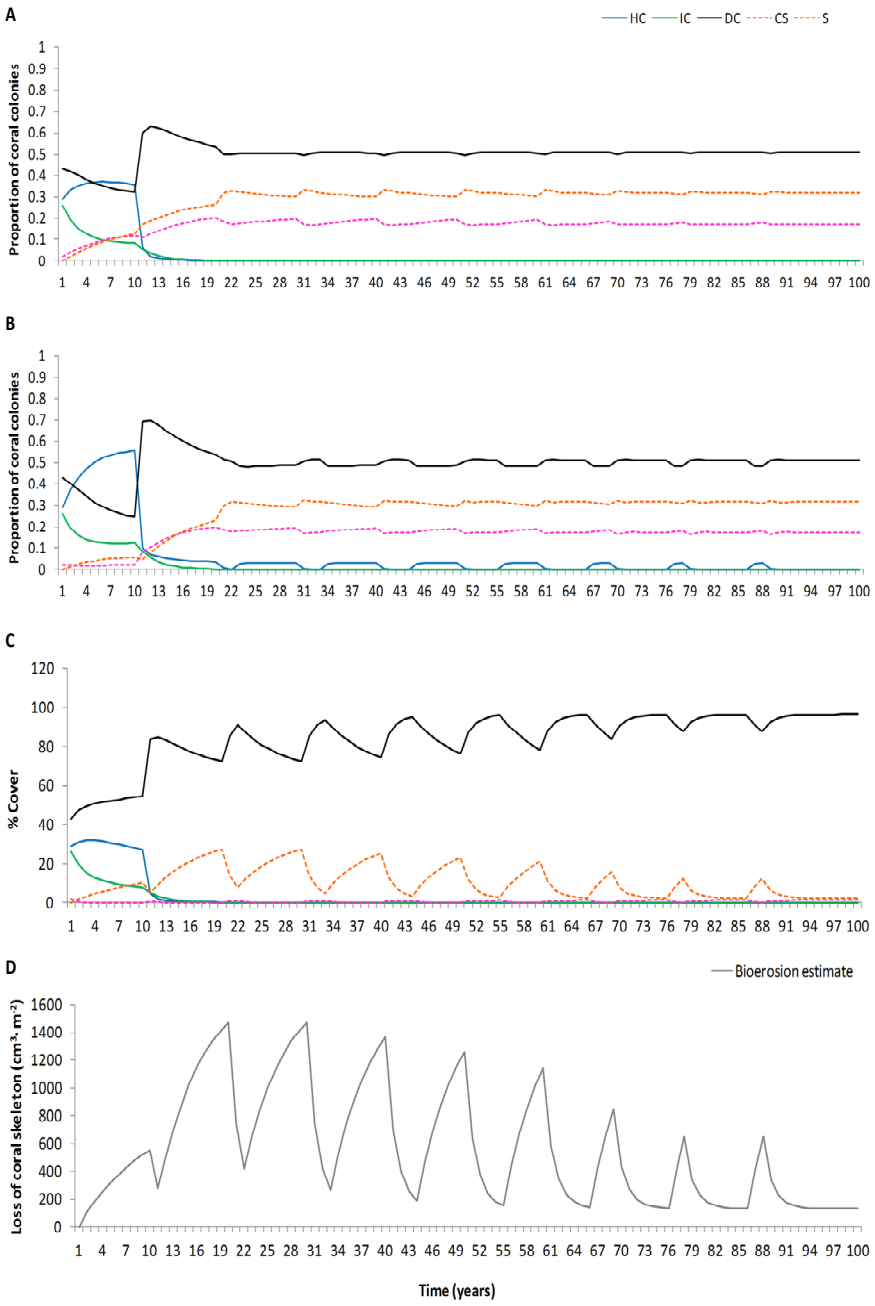


Figure 7. Effect of a massive coral mortality event, A) with low coral recruitment (0.02 r · m^{-2}· y^{-1}), B) with slightly more recruitment (0.07 r · m^{-2} · y^{-1}), C) Percent cover of each state based on proportions standardized by adults and recruits areas. D) Accumulated bioerosion produced by sponges, based on the total volume of adults and recruits for each year. HC: Healthy coral; IC: deteriorated coral; CS: Coral with sponge; DC: dead coral; S: sponge.

c) Successive moderate and massive coral mortality events

In the mixed coral mortality model, dead coral (DC) and sponges (S) alternated their dominance in the reef system (Figure 8). The first mortality event drastically reduced healthy corals (HC) and their recovery depended on coral recruitment. If coral recruitment was low, the proportion of HC was close to zero (Figure 8 A), but if coral recruitment increased, HC colonies also increased (Figure 8 B). However percent cover was kept low (Figure 8 C). It was more difficult than under continuous mortality events to HC cover recovering to previous values. The disturbed reef depended on recruitment from other reefs, and on survival of these recruits before the next mortality event. Sponge proportion, cover and bioerosion fluctuated, and sponges appeared to take advantage during the longer recovery times that occurred from years 70 to 100 after moderate coral mortality events, than after massive events (Figure 8).

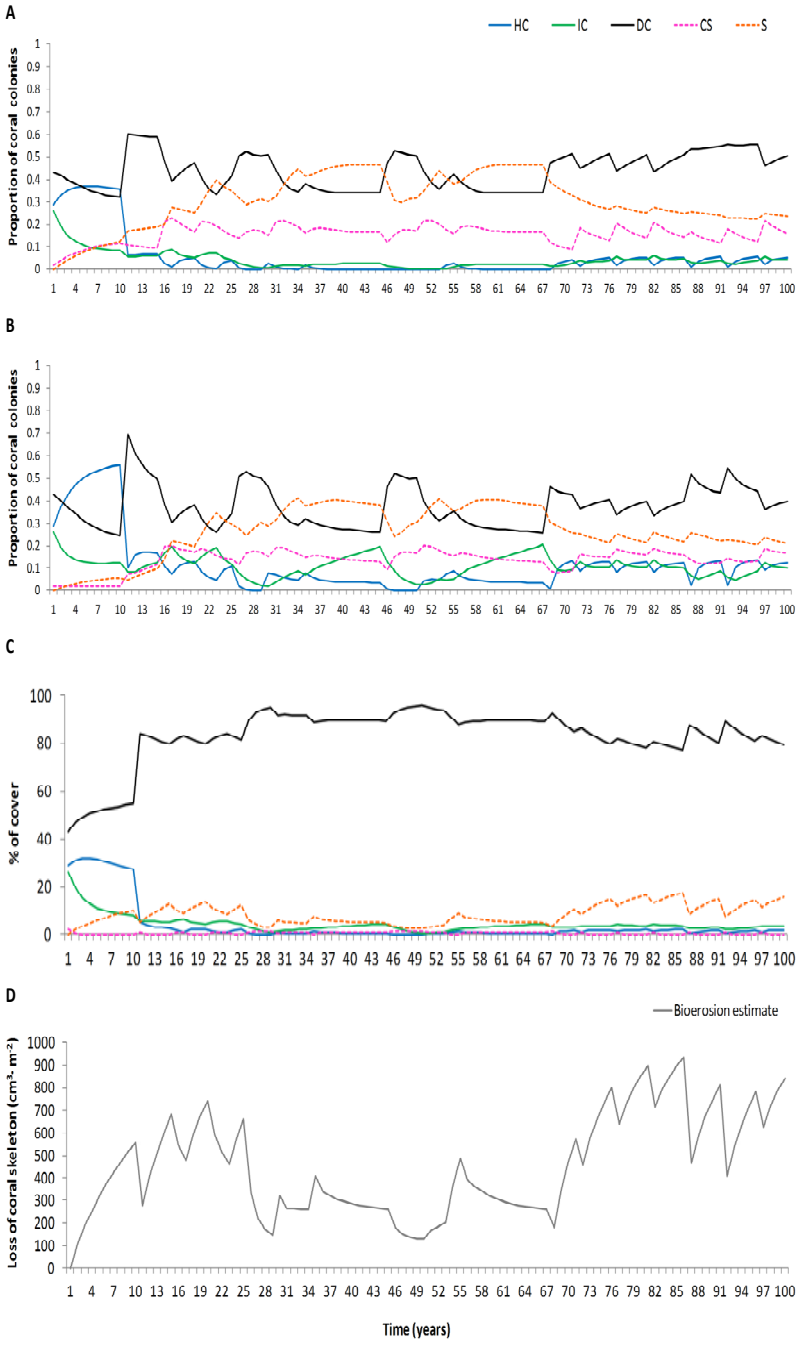


Figure 8. Effect of moderate and massive coral mortality events, A) with low coral recruitment (0.04 r · m^{-2}· y^{-1}), B) with almost half of the recruitment than in Broward reef (0.13 r · m^{-2} · y^{-1}), C) Percent cover of each state based on proportions standardized by adults and recruits areas. D) Accumulated bioerosion produced by sponges, based on the total volume of adults and recruits for each year. HC: Healthy coral; IC: deteriorated coral; CS: Coral with sponge; DC: dead coral; S: sponge.

Discussion

The Markov chain model was useful to describe and predict the general patterns of transitions from healthy massive corals to excavating sponges (represented in this case by *Cliona delitrix*). We found that in most cases dead coral (covered by algae + other invertebrates) will be the dominant state on reefs, except, when moderate coral mortality events occur. Here excavating sponges reached percent cover values as high as dead coral, and the reef system was dominated by dead coral (algae) and excavating sponges. In this scenario competition for dead coral between algae + other invertebrates and excavating sponges increased (see López-Victoria et al., 2006; Chaves-Fonnegra & Zea, 2011).

Moderate disturbance allowed enough mortality for the sponge to recruit (CS), which increases the proportion of sponges (S) on the reef. Thus if moderate disturbance allowed at least 80% of the sponges to survive, Caribbean coral reefs where *C. delitrix* could dwell could become temporal "excavating sponge reefs". This, depending on how extreme is the competition with algae. Massive mortality events tended to affect at least 50% of adult sponges of this species (Chaves-Fonnegra et al., Submitted). Thus, if heat stress is as extreme as occurred in Bocas del Toro in 2005 at 9 m depth (Chaves-Fonnegra et al., Submitted), and previously in 2005 at higher depth (Neal et al., 2013), *C. delitrix* will decline with coral, although, at a slower rate. Dead coral covered by algae will be the most dominant state.

Resistance of excavating sponges to temperature stress appear to be higher than in corals (Chaves-Fonnegra et al., Submitted), and can give them advantage. For example in the sponge *C. celata* no significant pigments loss was detected at temperatures of 18°C, 25°C, 31°C, and 33°C, suggesting a higher threshold to thermal stress (Miller et al., 2010). Also, other Caribbean species such as *Cliona tenuis* (recorded as *C. caribbaea*) have increased in abundance on Costa Rican coral reefs affected by temperature-triggered

bleaching (Cortés et al., 1984). *C. tenuis* spread more rapidly on Belizean reefs during the warm relative to cool seasons (1.3-3.1 versus 0.9-1.4 mm day^{-1}) (Rützler, 2002). In contrast, *C. tenuis* rates of lateral advance in the Colombian Caribbean were similar when corals were partly bleached, regardless of temperature; rates also varied widely among individual sponges and coral species (Márquez et al., 2006). Moreover, *Cliona orientalis*, a zooxanthellate excavating sponge from the Great Barrier Reef, is more bleaching-resistant than other coral reef organisms (Schönberg & Suwa, 2007; Schönberg et al., 2008). It can redistribute vertically its zooxanthellae in a diel rhythm; zooxanthellae are most concentrated and closest to the sponge surface during the day and widely distributed and drawn into the sponge tissue during the night. The ability to move zooxanthellae thus appears to increase bleaching-resistance, at least in this sponge species (Schönberg & Suwa, 2007; Schönberg et al., 2008).

Specifically in Broward County reefs (Florida), *Cliona delitrix* has increased during the past decade. However, its increase between 2002 and 2005 appears to be related to a decrease in dead coral more than to a decrease in healthy corals. This can be possible if algae or other invertebrates died, and open space for the sponge to recruit. The increase of sponges was accentuated after the 2005 bleaching and mortality event, which occurred across the tropical western Atlantic and the Caribbean Sea (Eakin et al., 2010). The event was the result of thermal stress that exceeded any observed in the Caribbean in the prior 20 years, and was the warmest in over 150 years (Eakin et al., 2010). After the mortality event, our model noticed the increase in deteriorated and dead corals, as well as in sponges in Broward reef, while healthy coral cover and frequency decreased.

After 2006, sponges started to decrease on Broward reefs, in apparent relation to an increase in dead coral, which probably can be attributed to an increase of algae, that can impact the growth and recruitment of excavating sponges (López-Victoria et al., 2006; Chaves-Fonnegra & Zea, 2011; Chaves-Fonnegra et al., Submitted). Although the data showed that sponges decreased from 2007 to 2010, it is possible that this trend changed

again, and towards an increase in 2011, after the 2010 massive coral mortality event in the Florida reef track (Lirman et al., 2011). The mortality during this winter event was due to a cold-water anomaly in January 2010, and while some species of sponges also died (Lirman et al., 2011), we did not notice major mortality of *C. delitrix* in the Broward population (monitored for a reproductive study, see Chapter 1).

In all models (Broward reef and its projections) *Cliona delitrix* excavating sponges increase was greater when new deteriorated corals appeared and healthy corals decreased. Thus, any mortality event opens new space for the sponge to attach and grow. Also, during mortality events macroalgae can die if temperature reaches 33°C (Pakker et al., 1995), which also could open new space for sponges to attach. However macroalgae appear to take over free space faster than excavating sponges. Taking into consideration that in the long term algae can compete with the excavating sponges (López-Victoria et al., 2006; Chaves-Fonnegra & Zea, 2011), sponges' growth in the reef will be limited by dead coral covered by alga and other invertebrates. Therefore, sponge increments in the reef will fluctuate, increasing after coral mortality events or new space on dead coral opens, and decreasing when dead coral gets covered by algae (turf, macroalgae, calcareous) and possible by other encrusting invertebrates.

In contrast to González-Rivero et al. (2011), we assert that macroalgae dominance is not favored by *C. delitrix*. In most cases macroalgae is the results of an increment in dead coral, and their prevalence appear independent of the presence of *Cliona delitrix* excavating sponges. Sponge contribution to dead coral (macroalgae) will only happen after sponges die and become again dead coral and new substratum for macroalgae to grow. Also, dead coral (algae + other invertebrates) is usually a dominant state on present deteriorated reefs that will only become completely occupied by *C. delitrix* under consecutive moderate coral mortality events. Only the case of all sponges dying, will contribute to the increase of dead coral (macroalgae). However, we agree with González-Rivero et al. (2011) that competition between algae and corals is more advantageous for

the persistence of corals, than competition between the excavating sponges and corals. This is because live corals competing with excavating sponges will likely end up being overpowered by sponges. Also, because excavating sponges are not available substrata for corals to recruit, whereas dead coral (algae) may be.

From projections and sensitive analyses we observed that coral recruitment is the main driver in the reef system. Also at Broward reef, sponge recruitment is lower enough to allow the system to maintain more frequency and cover of HC than CS or S. However, if coral recruitment decreases, the reef system will be out of balance. Sponges will grow and continue bioeroding the reef, only limited by dead coral (algae). Indeed, in the present system and in projections of this model, sponges never surpassed 20% of cover or 10 individuals (per $30m^2$). Only in the sensitivity analysis when sponge recruitment was intentionally increased to test the response of the system, sponges colonized over 30 coral colonies and a cover close to 40%. Thus, under the present conditions there appear to be a balance between the new coral recruitment and new sponge recruitment in the reef.

Bioerosion will mostly fluctuate depending on the amount of recruits that can become adults, which can produce higher accumulated bioerosion rates over time. Also it will depend on how long it will take for an adult will to excavate the whole coral colony. In addition to the amount of sponges that will die over time. Thus, as previously considered by Glynn (1997), the dynamic balance between reef growth and bioerosion, in this case by excavating sponges, will depend on the vitality of calcifying species, in this specific case, massive corals.

Considering that *Cliona delitrix* becomes abundant especially when recent coral mortality is available, the most obvious management measure is to reduce stressors that contribute to coral mortality. Also, any measure focused on improving coral recruitment will be useful. Some of these new recruits will get the sponge, but not all of them. This will

provide a balance. Bioerosion and reef growth have always been inseparable, and if kept under moderate levels it can be helpful to reef biodiversity (Glynn, 1997). Coral excavating sponges have been favored by coral mortality, especially during past decades. However as bioeroders, their success is also limited by the success of calcifying corals. This suggests a frequency dependent selection factor. If corals are declining, excavating sponges can also face decline, and this will depend of the intensity of heat stress and coral mortality events.

Conclusions

Cliona delitrix has increased during past years in Broward reefs, but this increase fluctuates depending on coral mortality and available space on dead coral.

Under temperature anomalies sponges tend to increase and take over the reef system only if heat stress and coral mortality is moderate. Under massive mortality events both corals and sponges tend to decline, although sponges at a slower rate than corals. If both moderate and massive mortality events occur, sponges will fluctuate depending of the level and how successive mortality events will be.

Cliona delitrix populations appeared to be limited by dead coral, which tends to be the dominant ecological state on Caribbean reefs. Dead coral, as we observed in the field, is usually covered by algae (turf, macroalgae, calcareous and other invertebrates), which in turn can slow the growth rates of sponges, and hamper their recruitment.

References

Acker, K.L, and M. Risk. 1985. "Substrate destruction and sediment production by the boring sponge *Cliona caribbaea* on Grand Caiman Island." *Journal of Sedimentary Petrology* 55 (5):705-711.

Antonius, A., and E. Ballesteros. 1998. "Epizoism: a new threat to coral health in Caribbean reefs." *Revista de Biología Tropical* 46 (5):145-156.

Castillo-Nelis, L., and J.T. Wootton. 2010. "Treatment-based Markov chain models clarify mechanisms of invasion in an invaded grassland community." *Proceedings of the Royal Society of London Series B* 277:539-547.

Chaves-Fonnegra, A., M. Maldonado, P.L. Blackwelder, and J.V. Lopez. In preparation. "Reproductive dynamics of the coral excavating Caribbean sponge *Cliona delitrix*."

Chaves-Fonnegra, A., and S. Zea. 2007. "Observations on reef coral undermining by the Caribbean excavating sponge *Cliona delitrix* (Demospongiae, Hadromerida)." In *Porifera research: biodiversity, innovation and sustainability*, edited by M.R. Custódio, G. Lôbo-Hajdu, E. Hajdu and G. Muricy, 247-254. Rio de Janeiro: Museu Nacional.

Chaves-Fonnegra, A., and S. Zea. 2011. "Coral colonization by the encrusting excavating Caribbean sponge *Cliona delitrix*." *Marine Ecology* 32:162-173.

Chaves-Fonnegra, A., S. Zea, and M.L. Gómez. 2007. "Abundance of the excavating sponge *Cliona delitrix* in relation to sewage discharge at San Andrés Island, SW Caribbean, Colombia." *Boletín de Investigaciones Marinas y Costeras* 36:63-78.

Chaves-Fonnegra, A., S. Zea, and J.V. Lopez. Submitted. "Recruitment of the excavating sponge *Cliona delitrix* is favored by recent coral mortality." *Coral Reefs*.

Cortés, J., M. Murillo, H.M. Guzmán, and J. Acuña. 1984. "Pérdida de zooxantelas y muerte de corales y otros organismos arrecifales en el Caribe y Pacífico de Costa Rica." *Revista de Biología Tropical* 32:227-231.

Díaz, M., and K. Rützler. 2001. "Sponge: an essential component of Caribbean coral reefs." *Bulletin of Marine Science* 69 (2):535-546.

Duckworth, A.R., and B.J. Peterson. 2013. "Effects of seawater temperature and pH on the boring rates of the sponge *Cliona celata* in scallop shells." *Marine Biology* 160 (1):27-35.

Dudgeon, S.R., R.B. Aronson, J.F. Bruno, and W.F. Precht. 2010. "Phase shifts and stable states on coral reefs." *Marine Ecology Progress Series* 413:201-216.

Eakin, C.M., J.A. Morgan, S.F. Heron, T.B. Smith, G. Liu, L. Alvarez-Filip, B. Baca, Erich Bartels, Carolina Bastidas, Claude Bouchon, Marilyn Brandt, Andrew W.Bruckner, Lucy Bunkley-Williams, Andrew Cameron, Billy D. Causey, Mark Chiappone, Tyler R. L.Christensen, M. James C. Crabbe, Owen Day, Elena de la Guardia, Guillermo Dı´az-Pulido, Daniel DiResta, Diego L. Gil-Agudelo, David S. Gilliam, Robert N. Ginsburg, Shannon Gore, Héctor M. Guzmán, James C. Hendee, Edwin A. Hernández-Delgado, Ellen Husain, Christopher F. G. Jeffrey, Ross J. Jones, Eric Jordán-Dahlgren, Les S. Kaufman, David I. Kline, Philip A. Kramer, Judith C. Lang, Diego Lirman, Jennie Mallela, Carrie Manfrino, Jean-Philippe Mare´ chal, Ken Marks, Jennifer Mihaly, W. Jeff Miller, Erich M. Mueller, Erinn M.Muller, Carlos A. Orozco Toro, Hazel A. Oxenford, Daniel Ponce-Taylor, Norman Quinn, Kim B.Ritchie, Sebastián Rodríguez, Alberto Rodríguez Ramírez, Sandra Romano, Jameal F. Samhouri, Juan A. Sánchez, George P. Schmahl, Burton V. Shank, William J. Skirving, Sascha C. C. Steiner, Estrella Villamizar, Sheila M. Walsh, Cory Walter, Ernesto Weil, Ernest H. Williams, Kimberly Woody Roberson, and Yusri Yusuf. 2010. "Caribbean corals in crisis: record thermal stress, bleaching, and mortality in 2005." *PlosOne* 5 (11):1-9.

Glynn, P. 1997. "Bioerosion and coral reef growth: a dynamic balance." In *Life and Death of Coral Reefs*, edited by C. Birkeland, 68-95. New York: Chapman and Hall.

González-Rivero, M., L. Yakob, and P.J. Mumby. 2011. "The role of sponge competition on coral reef alternative steady states." *Ecological Modelling* 222 1847-1853.

Heyward, A.J. , and A.P. Negri. 1999. "Natural inducers for coral larval metamorphosis." *Coral Reefs* 18 (3):273-279.

Hill, M.S., N.A. Lopez, and K.A. Young. 2005. "Anti-predator defenses in western North Atlantic sponges with evidence of enhanced defense through interactions between spicules and chemicals." *Marine Ecology Progress Series* 291 (1):93-102.

Holmes, K.E. . 1997. "Eutrophication and its effect on bioeroding sponge communities." Proc 8th Int Coral Reef Symp, Panamá.

Holmes, K.E. , E.N. Edinger, H. Hariyadi, G.V. Limmon, and M.J. Risk. 2000. "Bioerosion of live massive corals and branching coral rubble on indonesian coral reefs." *Marine Pollution Bulletin* 40 (7):606-617.

Hood, G.M. . 2010. "PopTools version 3.2.5. Available on the internet. URL http://www.poptools.org."

Hughes, T.P. 1994. "Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef." *Science* 265 (5178):1547-1551.

Lesser, M.P., and M. Slattery. 2013. "Ecology of Caribbean Sponges: Are Top-Down or Bottom-Up Processes More Important?" *PLoS One* 8 (11):e79799.

Lirman, D., S. Schopmeyer, D. Manzello, L.J. Gramer, W.F. Precht, F. Muller-Karger, K. Banks, B. Barnes, E. Bartels, A. Bourque, J. Byrne, S. Donahue, J. Duquesnel, L. Fisher, D. Gilliam, J. Hendee, M. Johnson, K. Maxwell, E. McDevitt, J. Monty, D. Rueda, R. Ruzicka, and S. Thanner. 2011. "Severe 2010 cold-water event caused unprecedented mortality to corals of the Florida reef tract and reversed previous survivorship patterns." *PLoS One* 6 (8):e23047.

López-Victoria, M., and S. Zea. 2004. "Storm-mediated coral colonization by an excavating Caribbean sponge." *Climate Research* 26:251-256.

López-Victoria, M., S. Zea, and E. Weil. 2006. "Competition for space between encrusting excavating Caribbean sponges and other coral reef organisms." *Marine Ecology Progress Series* 312:113-121.

Lowe, P.K., J.F. Bruno, E.R. Selig, and M. Spencer. 2011. "Empirical models of transitions between coral reef states: effects of region, protection, and environmental change." *PLoS One* 6 (11):1-15.

MacGeachy, J.K., and C. Stearn. 1976. "Boring by macro-organisms in the coral *Montastraea annularis* on Barbados reefs." *Int Revue ges Hydrobiology* 61:715-745.

Maliao, R.J., R.G Turingan, and J. Lin. 2008. "Phase-shift in coral reef communities in the Florida Keys National Marine Sanctuary (FKNMS), USA." *Marine Biology* 154:841–853.

Márquez, J.C., and S. Zea. 2012. "Parrotfish mediation in coral mortality and bioerosion by the encrusting excavating sponge *Cliona tenuis*." *Marine Ecology* 33 417-426.

Márquez, J.C., S. Zea, and M. López-Victoria. 2006. "Is competition for space between the encrusting excavating sponge Cliona tenuis and corals influenced by higher-than-normal temperatures?" *Boletín de Investigaciones Marinas y Costeras* 35:257-263.

McManus, J.W. , and J.F. Polsenberg. 2004. "Coral–algal phase shifts on coral reefs: ecological and environmental aspects." *Progress in Oceanography* 60:263-279.

McMurray, S. E., T. P. Henkel, and J. Pawlik. 2010. "Demographics of increasing populations of the giant barrel sponge *Xestospongia muta* in the Florida Keys." *Ecology* 91 (2):560-570.

Miller, A.N., K.B. Strychar, T.C. Shirley, and K. Rützler. 2010. "Effects of heat and salinity stress on the sponge *Cliona Celata*." *International Journal of Biology* 2 (2):3-16.

Morse, D.E. , N. Hooker, A.N.C. Morse, and R.A. Jensen. 1988. "Control of larval metamorphosis and recruitment in sympatric agariciid corals." *Journal of Experimental Marine Biology and Ecology* 116:193-217.

Neal, B.P., C. Condit, G. Liu, S. dos Santos, M. Kahru, B.G. Mitchell, and D. I. Kline. 2013. "When depth is no refuge: cumulative thermal stress increases with depth in Bocas del Toro, Panama." *Coral Reefs* "in Press" DOI 10.1007/s00338-013-1081-6.

Neumann, A.C. 1966. "Observations on coastal erosion in Bermuda and measurements of the boring rate of the sponge, *Cliona lampa*." *Limnology & Oceanography* 11 (1):92-108.

NOAA. 2000. "NOAA Coral Reef Watch Operational 50-km Satellite Coral Bleaching Degree Heating Weeks Product, Jan. 1, 2001-Dec. 31, 2010." NOAA Coral Reef Watch. Accessed Data set accessed 2011-01-05 at http://coralreefwatch.noaa.gov/satellite/hdf/index.html

Norström, A.V., M. Nyström, J. Lokrantz, and C. Folke. 2009. "Alternative states on coral reefs: beyond coral-macroalgae phase shifts." *Marine Ecology Progress Series* 376:295-306.

Owen-Smith, N. 2007. *Introduction to modeling in wildlife and resource conservation*. MA, USA: Blackwell Publishing.

Pakker, H. , A.M. Breeman, W.F. Prud'homme van Reine, and C. Van den Hoek. 1995. " A comparative study of temperature responses of Caribbean seaweeds from different biogeographic groups." *Journal of Phycology* 31:499-527.

Pandolfi, J.M., R.H. Bradbury, E. Sala, T.P. Hughes, K.A. Bjorndal, R.G. Cooke, D. McArdle, L. McClenachan, M.J.H. Newman, G. Paredes, R.R. Warner, and J.B.C. Jackson. 2003. "Global trajectories of the long-term decline of coral reef ecosystems." *Science* 301:955-958.

Pawlik, J.R. 2011. "The chemical ecology of sponges on Caribbean reefs: natural products shape natural systems." *BioScience* 61 (11):888-898.

Pawlik, J.R., T.S. Loh, S.E. McMurray, and C.M. Finelli. 2013. "Sponge communities on coral reefs are controlled by factors that are top-down, not bottom-up." *PLoS ONE* 8:e62573.

Pomponi, S.A. 1979. "Cytochemical studies of acid phosphatase in etching cells of boring sponges." *Journal of the Marine Biological Association of the United Kingdom* 59:785-789.

Price, I.R. 1975. "The development of algae on the skeletons of reef-building corals." In *Crown-of-thorns starfish seminar proceedings*, 181-191. Canberra: Australian Government Publishing Service.

Renken, H., and J. Mumby. 2009. "Modeling the dynamics of coral reef macroalgae using a Bayesian belief network approach." *Ecological Modelling* 220:1305-1314.

Riegl, B., and S.J. Purkis. 2009. "Model of coral population response to accelerated bleaching and mass mortality in a changing climate." *Ecological Modelling* 220:192-208.

Rose, C.S., and M.J. Risk. 1985. "Increase in *Cliona delitrix* infestation of *Montastraea cavernosa* heads on an organically polluted portion of the Grand Cayman." *P.S.Z.N.I. Marine Ecology* 6 (1):345-363.

Rützler, K. 1975. "The role of burrowing sponges in bioerosion." *Oecologia* 19 (1):203-216.

Rützler, K. 1978. "Sponges in coral reefs." In *Coral Reefs: research methods. Monographs in oceanographic methodology*, edited by D.R. Stoddard and R.E. Johannes, 299-313. Paris: UNESCO.

Rützler, K. 2002. "Impact of crustose clionid sponges on Caribbean reef corals." *Acta Geologica Hispánica* 37 (1):61-72.

Rützler, K., and G. Rieger. 1973. "Sponge burrowing: fine structure of *Cliona lampa* penetrating calcareus substrata." *Marine Biology* 21:144-162.

Schönberg, C.H.L. , and R. Suwa. 2007. "Why bioeroding sponges may be better hosts for symbiotic dinoflagellates than many corals." In *Porifera research: biodiversity, innovation and sustainability.*, edited by M.R. Custódio, G. Lôbo-Hajdu, E. Hajdu and G. Muricy, 569-580. Rio de Janeiro: Museu Nacional.

Schönberg, C.H.L., R. Suwa, M. Hidaka, and W.K. Weng Loh. 2008. "Sponge and coral zooxanthellae in heat and light: preliminary results of photochemical efficiency monitored with pulse amplitude modulated fluorometry." *Marine Ecology* 29:247-258.

Stone, L., E. Eilam, A. Abelson, and M. Ilan. 1996. "Modelling coral reef biodiversity and habitat destruction." *Marine Ecology Progress Series* 134:299-302.

Waggoner, P.E., and G.R. Stephens. 1970. "Transition probabilities for a forest." *Nature* 225:1160-1161.

Ward-Paige, C.A., M.J. Risk, O.A. Sherwood, and W.C. Jaap. 2005. "Clionid sponge surveys on the Florida Reef Tract suggest land-based nutrient inputs." *Marine Pollution Bulletin* 51:570-579.

Wisshak, M., C.H.L. Schönberg, A. Form, and A. Freiwald. 2012. "Ocean Acidification Accelerates Reef Bioerosion." *PLOSOne* 7 (9):1-8.

Wootton, J.T. 2001. "Prediction in complex communities: analysis of empirically derived Markov models." *Ecology* 82:580-598.

Wootton, J.T. 2004. "Markov chain models predict the consequences of experimental extinctions." *Ecology Letters* 7:653-660.

Appendix 1.4. Supplementary Information

Table 1. Percentages of the existing established ecological states for five different areas in the Caribbean Sea. Data was collected using linear transects of $20m^2$ and scuba diving. For specific methodology see Chapter 2. Specific areas of study include: Belize (Carrie Bow Cay); Bahamas (Iguana Islands); Colombia (San Andrés Island); Florida (Conch Reef- Key Largo); Panamá (Bocas del Toro Archipelago).

Ecological State	Belize n=4	Bahamas n=4	Colombia n=6	Florida n=4	Panama n=6	Average
Healthy coral	31.36%	12.78%	29.84%	20.72%	53.55%	29.65%
Deteriorated coral	8.07%	20.02%	33.57%	16.24%	39.38%	23.46%
Dead coral	59.91%	66.32%	35.23%	61.32%	4.10%	45.38%
Sponge	0.66%	0.88%	1.35%	1.72%	2.97%	1.52%

Table 2. Percentages of sponge *Cliona delitrix* inside coral skeleton (different species of corals). x: average; s.e: standard error; c.v: coefficient of variation.

Replicate	1	2	3	4	5	6	7	8	x	s.e.	c.v
%	44,73	52,63	52,63	56,41	47,36	40	41,66	38,33	**45,87**	**2,37**	**14,6**

Table 3. Mean transition matrix obtained from 65 to 100 coral colonies in a 30m^2 transect. HC: Healthy coral; IC: deteriorated coral; DC: dead coral; CS: Coral with sponge; S: sponge.

	Starting state				
Next state	**HC**	**IC**	**DC**	**CS**	**S**
HC	0.90027422	0.18152958	0.34181969	0	0
IC	0.07609383	0.64810005	0.00740741	0	0
DC	0.01900232	0.17037037	0.62696338	0.08333333	0.11375661
CS	0.00462963	0	0	0.87962963	0
S	0	0	0.02380952	0.03703704	0.88624339

Table 4. Transition matrices used to build for the moderate coral mortality model. HC: Healthy coral; IC: deteriorated coral; DC: dead coral; CS: Coral with sponge; S: sponge.

	Starting state				
Next state	**HC**	**IC**	**DC**	**CS**	**S**
a. PRESENT REEF					
HC	0.90027422	0.18152958	0.15006059	0	0
IC	0.07609383	0.64810005	0.00740741	0	0
DC	0.01900232	0.17037037	0.81391248	0.08333333	0.11375661
CS	0.00462963	0	0	0.87962963	0
S	0	0	0.02861953	0.03703704	0.88624339
b. RECOVERY MATRIX					
HC	0.4	0.18152958	0.15006059	0	0
IC	0.26666667	0.64810005	0.00740741	0	0
DC	0.2	0.17037037	0.81391248	0.08333333	0.11375661
CS	0.13333333	0	0	0.87962963	0
S	0	0	0.02861953	0.03703704	0.88624339
c. MODERATE MORTALITY					
HC	0.4	0	0.08333333	0	0
IC	0.26666667	1	0	0	0
DC	0.2	0	0.66666667	0	0.2
CS	0.13333333	0	0.25	0.45	0
S	0	0	0	0.55	0.8

Table 5. Transition matrices used to build for the massive coral mortality model. HC: Healthy coral; IC: deteriorated coral; DC: dead coral; CS: Coral with sponge; S: sponge.

	Starting state				
Next state	**HC**	**IC**	**DC**	**CS**	**S**
a. PRESENT REEF					
HC	0.90027422	0.18152958	0.15006059	0	0
IC	0.07609383	0.64810005	0.00740741	0	0
DC	0.01900232	0.17037037	0.81391248	0.08333333	0.11375661
CS	0.00462963	0	0	0.87962963	0
S	0	0	0.02861953	0.03703704	0.88624339
b. RECOVERY MATRIX					
HC	0.17948718	0.18152958	0.05	0	0
IC	0	0.64810005	0	0	0
DC	0.82051282	0.17037037	0.85	0.08333333	0.11375661
CS	0	0	0.05	0.87962963	0
S	0	0	0.05	0.03703704	0.88624339
c. MASSIVE MORTALITY					
HC	0.17948718	0	0	0	0
IC	0	0.66666667	0	0	0
DC	0.82051282	0.33333333	0.6875	0	0.5
CS	0	0	0.3125	0.07142857	0
S	0	0	0	0.92857143	0.5

Table 6. Transition matrices used to build a mix model including moderate and massive coral mortality. HC: Healthy coral; IC: deteriorated coral; DC: dead coral; CS: Coral with sponge; S: sponge.

	Starting state				
Next state	**HC**	**IC**	**DC**	**CS**	**S**
a. PRESENT REEF					
HC	0.90027422	0.18152958	0.15006059	0	0
IC	0.07609383	0.64810005	0.00740741	0	0
DC	0.01900232	0.17037037	0.81391248	0.08333333	0.11375661
CS	0.00462963	0	0	0.87962963	0
S	0	0	0.02861953	0.03703704	0.88624339
b. RECOVERY MATRIX					
HC	0.4	0.18152958	0.15006059	0	0
IC	0.26666667	0.64810005	0.00740741	0	0
DC	0.2	0.17037037	0.81391248	0.08333333	0.11375661
CS	0.13333333	0	0	0.87962963	0
S	0	0	0.02861953	0.03703704	0.88624339
c. MODERATE MORTALITY					
HC	0.4	0	0.08333333	0	0
IC	0.26666667	1	0	0	0
DC	0.2	0	0.66666667	0	0.2
CS	0.13333333	0	0.25	0.45	0
S	0	0	0	0.55	0.8
d. MASSIVE MORTALITY					
HC	0.17948718	0	0	0	0
IC	0	0.66666667	0	0	0
DC	0.82051282	0.33333333	0.6875	0	0.5
CS	0	0	0.3125	0.07142857	0
S	0	0	0	0.92857143	0.5

GENERAL CONCLUSIONS

Why and how life-history traits of excavating sponges allow them to take advantage of deteriorated coral reefs?

Through the study of the reproduction, recruitment and dispersal potential of one widely distributed and currently expanding species, *Cliona delitrix*, it was found:

Cliona delitrix has an extended reproductive cycle in Florida, USA, that goes from April - May to around November - December depending on a >25°C sea-water temperature threshold. Its reproductive cycle is divided into multiple spawning events, suggesting that this species has a reproductive strategy that decreases the risk of massive offspring mortality during local adverse events. This strategy may increase the chances for larvae to find corals with recent mortality where the larvae can settle. This complex reproductive strategy could be contributing to its widening distribution.

C. delitrix is recruiting abundantly on Caribbean coral reefs, preferentially on recent coral mortality than on old coral mortality, and on top of coral colonies, where live coral tissue should have been. Contemporary, chronic or massive coral mortality is creating new clean space for sponge settlement. Thus, the increase in *C. delitrix* and other excavating sponges can be explained by the coincidence in time and space of larval production with the availability of new dead coral, which tend to overlap during the warmest months of the year. The apparently lower effect of thermal stress on excavating sponges (adults and larvae) than on corals, may be another factor contributing to the current success of these sponges on Caribbean reefs.

Six genetic populations were defined in the Greater Caribbean: one in the Atlantic (Florida-Bahamas), one specific to Florida, and four in the South Caribbean Sea. The two genetic populations in Florida were differentiated by depth and possibly correspond to two independent breeding populations that can overlap in some locations. These populations appeared to be structured mostly by local and regional oceanic currents.

Dispersal ranges for *Cliona delitrix* may reach as far as ~315 km in the Florida reef track. Moreover, dispersal may be possible over distances of ~ 971 km in the South Caribbean Sea, between Belize and Panama. Excavating sponge eggs or larvae of *C. delitrix* appear to survive enough to be transported by currents over larger distances. Thus, reproduction, dispersal, and recruitment patterns of *C. delitrix* along with different current and eddies that form at different periods of times are sustaining the spread of this sponge on coral reefs. Consequently, as new individuals continue to engage in reproduction, populations will grow.

Although *Cliona delitrix* is not an invasive species, but rather a resident species to coral reefs, it constitutes a strong competitor of corals that is taking advantage of the recent coral mortality.

According to models applied, its increase on reefs fluctuates depending of coral mortality events and available space on old dead coral (colonized by algae and other invertebrates). However, under temperature anomalies sponges will tend to increase and take over the reef system only if heat stress and coral mortality is moderate. Under massive mortality events both corals and sponges will tend to decline, although sponges at a slower rate than corals.

Although *C. delitrix's* current spreading has been mainly associated with land-base pollution (sewage) and in reefs close to coast lines, this sponge could also become an important reef community component in habitats farther from human influence as corals continue to decline. Its spread will mainly depend on oceanic currents and on the presence of recent mortality on corals.

In general, coral excavating sponges have been favored by coral mortality, especially during past few decades. However as bioeroders, their success is also limited by the success of calcifying corals. If corals are declining, excavating sponges can also face decline, and this also depends of the intensity of heat stress and coral mortality events.

To decrease the amount of excavating sponges on reefs, we need conservation strategies that will enhance the recruitment and maintenance of massive corals. Excavating sponges have always been part of the reef, and they depend on corals. In a reef management context and based on this dissertation's findings, we suggest that excavating sponges, and especially *Cliona delitrix*, be more formally included in reef monitoring programs. Their increase can be used to track coral mortality events on reefs (past and future), and also can be use as another major bioindicator of health on coral reefs.

"A discovery is like falling in love and reaching the top of the mountain after a hard climb all in one, an ecstasy induced not by drugs but by the revelation of a face of nature that no one has seen before."

Max Perutz. Nobel Prize in Chemistry 1962

"Sponges are more perfect than man for two reasons. Firstly, they are enormously older, millions of years. Secondly they have survived all kinds of environmental conditions without changing their body plan."

G.P. Bidder 1937. The Perfection of Sponges

No mires cuan profundo es el océano y sigue nadando…