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TITOLO TESI

REPRODUCTION AND POPULATION STRUCTURE IN TEMPERATE AND TROPICAL CORALS IN RELATION TO ENVIRONMENTAL PARAMETERS

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"The key is to see ourselves as part of the natural systems that support us."

by the oceanographer Dr Sylvia Earle

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I. General introduction

I.I. Scleractinian corals

The order Scleractinia first appeared in the Triassic period (Veron 1995) and consists of stony corals that produce hard exoskeleton of calcium carbonate crystals. Even though some species are solitary, most are colonial (about 60% of the approximately 1400 extant species; Cairns 1999). These organisms can be ecologically divided into reef-building (hermatypic) corals and non-reef-building (ahermatypic) corals. Both type of corals secrete an exoskeleton, but while hermatypic species construct the primary reef framework and most of them normally contain millions of zooxanthellae (i.e., zooxanthellate), ahermatypic species do not contribute significantly to reef formation and mostly lack zooxanthellae (i.e., non-zooxanthellate; Yonge 1973; Schuhmacher and Zibrowius 1985; Cairns 2007). Specifically, the zooxanthellae are dinoflagellate endosymbionts of the genus *Symbiodinium* sp. that make mutualistic associations with corals, residing within vacuoles in the cells of the host gastrodermis (Trench 1979, 1987). The zooxanthellae serve as primary producers and supply their coral host with up to 95% of their photosynthetic products, such as sugars, amino acids, carbohydrates and small peptides (Trench 1979; Muscatine 1990). These compounds provide the coral with energy for respiration, growth, and the deposition of its $CaCO_3$ skeleton (Muscatine 1990). It is believed that the rapid ecological success of these animals is directly related to the symbiosis with the zooxanthellae that allowed them to survive in oligotrophic and highly irradiated habitats through photosynthesis. Moreover, photoautotrophy is not the only source of nutrition for corals. Heterotrophy (or the direct ingestion of zooplankton and other organic particles in the water column) in corals seems essential for providing nitrogen, phosphorus, and other nutrients necessary for protein synthesis and other essential metabolic requirements (Lesser 2004).

The fossil record suggests that corals as a group are more likely to suffer extinctions than some of the groups associating with them, whose habitat requirements may be less stringent (Bruno and Selig 2007). As with rainforests, the importance of coral reefs lies not so much in the diversity of the corals themselves, but rather in the millions of species that live primarily or exclusively in association with them (Knowlton 2001). Moreover, coral reefs are a source of food and livelihood for at least 100 million people worldwide, support major industries (fishing and tourism) and play a key role in stabilizing coastlines (Connell 1978).

Despite scleractinian corals have been extensively studied in the last 200 years (Harrison and Wallace 1990), knowledge on the extant scleractinian "species" is scarce, probably because the investigation of marine environments is more challenging. For example, the exploration of deeper reefs (e.g. mesophotic) and deep-sea environments is still at its infancy (Cairns 2007). Other complications may derive also from the imperfect taxonomic resolution of highly variable species and potential cryptic species (Veron 1995, 2000). Therefore, further information about both solitary and colonial, hermatipic and ahermatypic, zooxanthellate and non-zooxanthellate scleractinian species is essential, in particular concerning temperate and subtropical regions.

I.II. Coral reproduction

Usually, coral life cycle involves the production of gametes in the mesenteries of the polyp (that is the benthic phase), fertilization, embryo development and a larval phase that actively swim looking for a hard substratum. After the settlement, the planula larvae metamorphoses into a juvenile polyp that starts the formation of the calcium carbonate exoskeleton (Fig. 1). In colonial species, follows the growth of tissues and skeleton by asexual budding (which can be intratentacular and extratentacular) to form new polyps attached to the parent or asexual reproduction for giving rise to new colonies (see Richmond 1997).

Based on the anatomical and physiological simplicity, members of the phylum Cnidaria are evolutionarily plastic (Fautin 2002) and display several patterns in reproductive traits: hermaphroditic broadcast spawners, hermaphroditic brooders, gonochoric broadcast spawners, or gonochoric brooders. However, there are some scleractinian species with a mixed sexuality and /or both reproductive modes (Harrison 2011). Hermaphroditic corals are the most common (74% of the total known species) and develop both male and female gametes within polyps or colonies during their lifetime, whereas gonochoric corals have separate sexes (see Harrison 2011; Baird et al. 2009). Hermaphroditism is usually simultaneous, but there are some forms of hermaphroditism which are more complex to detect, such as the cyclic sequential characterized by oocytes and spermaries that mature at different times in the same breeding season (Waller et al. 2005) and the protandrous or protogynous sequential during the lifetime (in which the first part of life is characterized by the presence of female or male gametes and in the second part there is a sexual inversion; Loya and Sakai 2008). When gametes reach the maturity, they are released in the water column by the broadcast spawning corals for external fertilization and subsequent embryo and larval development, while in brooding corals fertilization takes place within the coelenteric cavity of polyp (Harrison and Wallace 1990; Richmond and Hunter 1990).

Corals have also the ability to reproduce asexually in several ways, developing new solitary corals or colonies genetically identical to the parent (Highsmith 1982; Cairns 1988; Harrison and Wallace 1990). In branching corals, asexual reproduction may take place via colony fragmentation as a result of physical impacts such as wave action and fish predation. Other asexual processes are colony fission, longitudinal and transverse division, polyp expulsion or polyp “bail-out” and budding. In rare cases, the asexual production of brooded embryos has been observed in some tropical and subtropical scleractinians (Stoddart 1983; Ayre and Miller

2004; Sherman et al. 2006; Ayre and Resing 1986; Nakano and Yamazoto 1992; Lam 2000). During my research, a possible agamic production of brooded embryos was found continuously in females, males and sexually inactive individuals of the temperate coral *Caryophyllia inornata*, without a clear seasonal trend (Marchini et al. 2015: Chapter 2). While sexual reproduction allow genetic variability of the populations through recombination and subsequent production of new genotypes that can adapt to changing environment with a wide dispersion or recolonization of more heterogeneous habitats, asexual planulae possess well-adapted genotypes at the local level but with limited dispersal abilities (Williams 1975; Maynard-Smith 1978).

It was observed that sexuality and reproductive mode follow evident biogeographical patterns, perhaps as an adaptation strategy to cope with the different surrounding environmental conditions (Szmant 1986; Baird et al. 2009) since they are unable to move to a more suitable environment (van Oppen et al. 2015). Indeed, in contrast to tropical corals, it seems that most of the Mediterranean species are gonochoric brooders. However, very few studies are available on Mediterranean species, thus, further investigations on their reproduction are essential. The temperate zooxanthellate coral *Balanophyllia europaea* (Family: Dendrophylliidae; Goffredo et al. 1998, 2002; Airi et al. 2014) is hermaphrodite and brooding, while the non-zooxanthellate *Leptopsammia pruvoti* (Family: Dendrophylliidae; Goffredo et al. 2005, 2006), *Astroides calycularis* (Family: Dendrophylliidae; Goffredo et al. 2010, 2011) and *Caryophyllia inornata* (Family: Caryophylliidae; Goffredo et al. 2012; Marchini et al. 2015) are all gonochoric and brooding. Lastly, the zooxanthellate *Cladocora caespitosa* (Family: Oculinidae; Kružić et al. 2008; Kersting et al. 2013) was described as both gonochoric and hermaphrodite and is the only known spawning coral in the Mediterranean Sea.

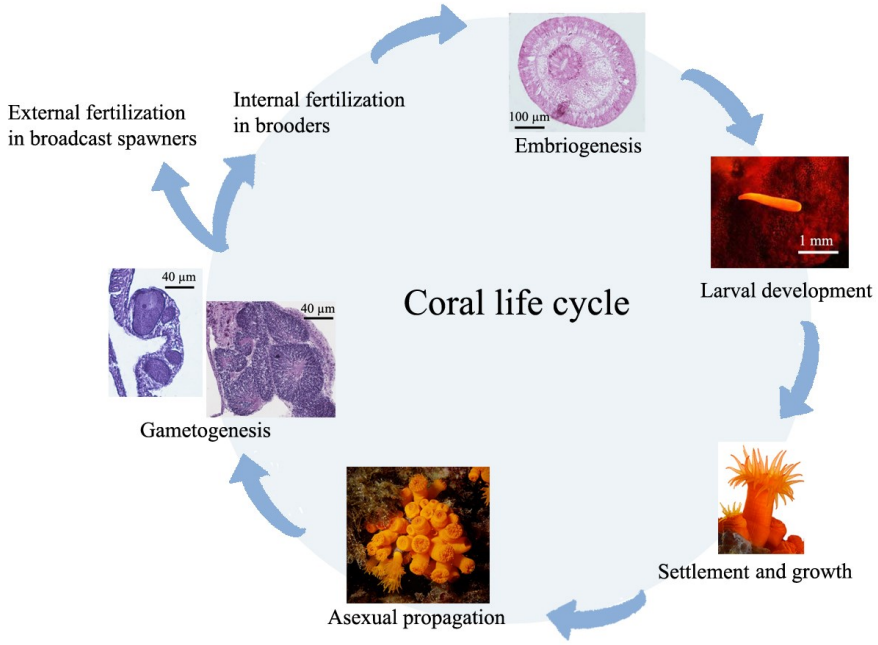

Fig. 1 Main stages of coral life cycle that involves oocytes and spermary development (gametogenesis), which can encounter in the water column for broadcast spawners or inside the body of brooders (fertilization). Subsequently, embryos develop from blastula to gastrula stage (embryogenesis) up to the formation of planula larva that swim looking for a suitable substratum where to settle. The polyp starts to grow and, in many case, forms the colony by budding.

I.III. Coral population structure

Population size structure depend on variations in rates of colony growth, recruitment and mortality, and may indicate individual sensitivities to life-history processes and environmental variation (Goodbody-Gringley et al. 2015). The analysis of coral populations in terms of size-frequency distribution, can provide a snap-shot of current reef condition and if monitored over time may be an indicator for stability or decline (Meesters et al. 1996; Caroselli et al. 2012). However, the population structure of coral species has been little investigated in the field, maybe for the extreme requirements of underwater survey efforts to collect colony-size data (Meesters et al. 2001). Population dynamics of modular organisms are often influenced by the depth at which they live, as depth can affects the feeding abilities of corals and consequently their potential energy reserves (Tsounis et al. 2006; Harland et al. 1992). During my abroad period I was involved in the investigation of population structure of the tropical colonial coral *Montastraea cavernosa* along the South Shore in Bermuda. Colony density, surface area and size-frequency distributions were strongly related to environmental conditions that vary with depth, such as temperature and nutrient levels. In fact, mesophotic reefs are characterized by more numerous but smaller colonies than shallow reefs. However, shallower and deeper populations contribute equally to overall percent cover, suggesting that mesophotic reef in Bermuda is relatively stable (Goodbody-Gringley et al. 2015: Chapter 5). The different size-frequency distributions between depths could have a direct effect on larval recruitment, as a depth-dependent settlement of new recruits has been demonstrated in scleractinian corals (Mundy and Babcock 2000).

I.IV. Environmental influences on reproductive cycle

It is widely recognized that environmental patterns influence many aspects of coral reproduction, such as gamete development, spawning, fertilization and planulation (Michalek-Wagner and Willis 2001; van Woesik 2009; Torrents and Garrabou 2011). Gametogenesis and spawning require coordination with environmental cycles for ensuring synchronization and reproductive success but the degree of reproductive synchrony varies greatly within and among species at different geographic locations (Harrison 2011). Some corals belong to the highly coordinated mass phenomena described for the Great Barrier Reef (Babcock et al. 1986), and some species display biannual or multiple cycles of gametogenesis and breeding throughout the year in shallow and deep waters (Dahan and Benayahu 1997; Ben-David-Zaslow et al. 1999; Cordes et al. 2001, all soft corals; Waller and Tyler 2005; Goffredo et al. 2002, 2006, 2011; Marchini et al. 2015).

Numerous studies state that seawater temperature is the major seasonal cue, partly because it influences many key physiological processes (Hochachka and Somero 2002) and also because broadcast spawning generally takes place when waters are warming or close to the annual maxima (Harrison and Wallace 1990). Other environmental variables can regulate reproductive cycles, in alternative to or in combination with temperature cycles, as food availability (Hartnoll 1975; Coma et al. 1995), seasonal rainfall (Mendes and Woodley 2002), typhoons (Fan et al. 2005), wind or tide patterns (Simpson 1985). Although seawater temperature has been suggested to influence larvae release in corals (Harrison et al. 1984; Babcock et al. 1986), this process appears to be triggered by the level of lunar irradiance (Harrison et al. 1984; Jokiel et al. 1985; Babcock et al. 1994) through sensitive photoreception in the blue region of the spectrum (Gorbunov and Falkowski 2002) and via photosensitive cryptochromes (Levy et al. 2007). Also

photoperiod was described as an environmental determinant of gamete release, capable of phasing the overall reproductive cycle. However, photoperiod might have a greater effect at higher latitudes where the seasonal day-length signal is strongest, but its importance as a natural entraining variable remains largely unknown (Pankhurst and Porter 2003).

The temperate Mediterranean Sea is characterized by annual cycles of photoperiod that in turn influences seasonal patterns of seawater temperature, acting as regulator of the biological clocks of corals (Fiorillo et al. 2013). Gametogenesis in Mediterranean scleractinian corals investigated to date (*Balanophyllia europaea*, Goffredo et al. 2002; *Leptopsammia pruvoti*, Goffredo et al. 2006; *Cladocora caespitosa*, Kružić et al. 2008, Kersting et al. 2013; *Astroides calycularis*; Goffredo et al. 2011) seems related to seasonal variations of photoperiod and seawater temperature. In fact, while the decrease in photoperiod and seawater temperature in autumn and winter could be a cue for gamete development, the increase of the same environmental parameters, during winter and spring, seems to coincide with sperm release and oocyte fertilization (Airi et al. 2016). These results were confirmed by the study on the annual reproductive cycle of the Mediterranean coral *Caryophyllia inornata* at Elba Island, describing a gonadal development strongly influenced by seasonal variations of the environmental parameters. Indeed, gonadal size of both females and males increase significantly from March until May, when both photoperiod and seawater temperature increase after the minimum of the year. Fertilization takes place from April to July, when photoperiod is the longest of the year (Marchini et al. 2015: Chapter 2).

For these reasons, sexual reproduction is considered the most sensitive life process to environmental variations and may indicate how organisms respond to stress (Harrison and Wallace 1990; Ward 1995; van Woesik 2009) such as thermal stress (Negri et al. 2007; Meyer et

al. 2008; Randall and Szmant 2009) or increased sedimentation and turbidity (Gilmour 1999; Fabricius et al. 2003). Moreover, all the phases of coral life cycle suffer the continuous increase of pollutants resulting from human action such as oil (Loya and Rinkevich 1979; Lane and Harrison 2002), metals (Reichelt-Brushett and Harrison 2005; Negri and Heyward 2001) and pesticides (Negri et al. 2005; Markey et al. 2007), which are severely compromising the health of coral reefs together with the impacts of climate changes such as global warming and ocean acidification.

I.V. Corals in a changing ocean

Temperature, pH, pCO_2 and calcium carbonate ($CaCO_3$) saturation are among the most important environmental factors controlling the distribution, physiological performance, morphology and behavior of marine invertebrates (Kinne 1970, Pörtner et al. 2005, Pörtner and Knust 2007, Pörtner 2008, Widdicombe and Spicer 2008, Doney et al. 2009). However, in the last decades there have been some alterations in the balance of these parameters, modifying the marine ecosystem. Currently, nearly 30% of the world's coral reefs are considered severely damaged, and close to 60% are likely to be lost by 2030 for a combination of physical, chemical and biological stresses (Wilkinson 2003). This decline is mostly due to the growing greenhouse gas emissions, which have led large increases in the atmospheric anthropogenic CO_2 emissions to the atmosphere. Since the Industrial Revolution, the atmospheric CO_2 has risen from 280 ppm to today's level of 387 ppm (Pachauri et al. 2014). The current climate models estimated that pCO_2 present levels will likely attain more than 1000 ppm by 2100 (Fig. 2a; Pachauri et al. 2014). About half of the emitted anthropogenic CO_2 still remains in the atmosphere (Houghton 2007), a further 20% is removed from the atmosphere and stored on land and the remaining 30% is absorbed by the oceans, increasing acidity and causing ocean acidification (Sabine et al. 2004).

By dissolving in seawater, CO_2 reacts with H_2O, triggering a series of chemical reactions that alter the seawater carbonate chemistry, resulting in a drop in surface ocean pH. If the oceans continue to absorb CO_2 , a further reductions of 0.3–0.5 pH units are expected by the end of this century (Fig. 2c; Pachauri et al. 2014). As the deposition of $CaCO_3$ by the calcifying scleractinian corals and other reef organisms is partially controlled by the saturation state (Ωarag), a reduction in Ωarag indicates undersaturation and a tendency towards dissolution of the skeletal (Gattuso et al. 1998). Moreover, coral reproduction is described as a sensitive process to ocean acidification and negative effects have already been detected on sperm motility (Morita et al. 2009), fertilization success (Albright et al. 2010) and larval development (Albright et al. 2008, 2010; Cohen et al. 2009; Suwa et al. 2010; Albright and Langdon 2011).

Atmospheric greenhouse gases also trap some of the heat energy that would otherwise re-radiate to space, contributing to warm the planet. In the past century, sea surface temperatures have risen by 0.4–0.8 °C and it was estimated a further increase ranging between 2 and 4.5°C by 2100 (Fig. 2b; Pachauri et al. 2014). Ocean warming is the most serious direct climate change stressor for some regions, including the Mediterranean, southern North Sea, Western Antarctic Peninsula and south-eastern Australia (Ridgway 2007; Barnes and Peck 2008; Coma et al. 2009; Schmalenbach and Franke 2010; Schofield et al. 2010), leading to significant losses of ice sheets and increase in sea level (Pachauri et al. 2014). One of the most dramatic impacts of ocean warming on corals is mass coral bleaching, which is the breakdown of the symbiosis between corals and zooxanthellae (Glynn 1993). The host coral cannot then rely on the energy supplied by photosynthesis of the zooxanthellae, with negative consequences on the costly processes such as reproduction. Impacts of increasing temperature on environmental control of reproduction include reduced individual fecundity, low egg quality, lowered fertilization success and reduced

recruitment through effects on post-fertilization processes (e.g., embryonic and larval development, survival, settlement, metamorphosis, and early post-settlement growth; Albright and Mason 2013; Linares et al. 2008).

It is expected that corals will be among the most threatened organisms on the planet, thus there is an urgent need to understand the susceptibility of the coral life history stages in face of changing ocean to develop management strategies for reproductive success.

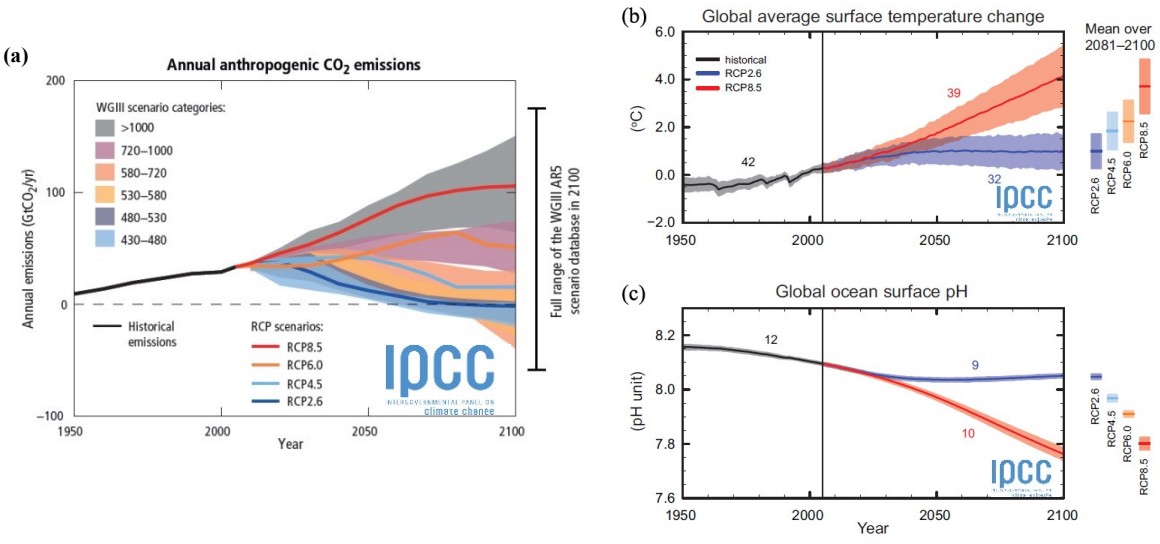

Fig. 2. (a) Emissions of carbon dioxide (CO) alone in the Representative Concentration Pathways (RCPs) (lines) and the associated scenario categories used in WGIII (coloured areas show 5 to 95% range). The WGIII scenario categories summarize the wide range of emission scenarios published in the scientific literature and are defined on the basis of CO2-eq concentration levels (in ppm) in 2100 (b) CMIP5 multi-model simulated time series from 1950 to 2100 for change in global annual mean surface temperature relative to 1986–2005, and (c) global mean ocean surface pH. Time series of projections and a measure of uncertainty (shading) are shown for scenarios RCP2.6 (blue) and RCP8.5 (red). Black (grey shading) is the modelled historical evolution using historical reconstructed forcings (Pachauri et al. 2014).

I.VI. Environmental gradients as "natural laboratories" for climate changes studies

To examine the effects of climate change on the organisms, there are two main empirical methods. In the first case, experiments can be performed in aquaria under controlled conditions (e.g., temperature, light, pCO_2 or pH). This approach enables researchers to avoid the impact of confounding factors and to study organisms' responses to environmental conditions not yet occurring under natural conditions, such as predicted extreme temperatures or pH values. However, they lack the complexity of natural systems, including co-limiting factors (nutrients, currents and irradiance) and are too short to fully address ecosystem-level responses. Another approach implies using environmental gradients (such as latitudinal, acidity and bathymetric) as "natural laboratories", in which however confounding factors such as local adaptation and species interactions (Dunne et al. 2004) cannot be excluded. On the other hand, sites along a natural gradient have evolved with the local climate over years or even centuries, providing a unique opportunity to assess the integrated long-term effects of temperature on marine organisms in a larger ecosystem framework (Rustad 2008). For these reasons, the relative lack of studies using natural gradients merits attention given the different abiotic and biotic processes occurring along all the types of gradients.

In the Mediterranean Sea, the effects of temperature and solar radiation on reproduction have been recently investigate along a latitudinal gradient covering approximately 850 km and a 2°C temperature difference that matches the expected temperature increase for the end of this century (Stocker et al. 2013). The zooxanthellate coral *Balanophyllia europaea* was investigated in six populations located along this gradient. The warmest populations showed a reduced productivity in the gametogenetic process, suggesting that the zooxanthellate *B. europaea* is affected by increasing temperature. It is hypothesized that this sensitivity could depend on the

symbiosis with the zooxanthellae since the photosynthesis of these unicellular algae is inhibited at higher temperatures, reducing the available energy for reproductive processes (Airi et al 2014). In fact, reproductive output of the non-zooxanthellate coral *Leptopsammia pruvoti*, studied along the same latitudinal gradient, is unaffected by higher temperature and solar radiation. My research included a study performed along this temperature gradient on the non-zooxanthellate coral *Caryophyllia inornata*. The reproductive output of this non symbiotic species does not vary along the gradient, indicating that this species might be quite tolerant to increasing temperature and solar radiation (Manuscript in preparation: Chapter 3).

We also assessed, for the first time, the effect of long-term exposure to acidic conditions on the reproduction of a coral naturally occurring at CO_2 vents (Manuscript in preparation: Chapter 4). Continuous and localized CO_2 emissions (99% CO_2; Capaccioni et al. 2007), which naturally acidify the surrounding seawater, are generated by an underwater volcanic crater at 10 m depth, located off Panarea Island (Aeolian Archipelago, southern Tyrrhenian Sea). The CO_2 emissions generate a pH gradient simulating levels matching different IPCC scenarios. The spermatogenesis of *B. europaea* living along the natural pH gradient, was not affected by increasing CO_2, perhaps because in a hypercapnic (high CO_2) environment the photosynthetic efficiency of the zooxanthellae is enhanced, leading to an increase of the available energy for host gonadal development.

Finally, bathymetric gradients are useful to compare the structures between shallow and deep populations to establish criteria that characterize the ‘‘health’’ of coral reefs, and to formulate management plans in response to anthropogenic and natural disturbances (Lesser 2004). As corals of shallow depths (5 to 30 m) are most susceptible to the effects of climate change (increase of sea surface temperatures and storm wave damage) and to the harmful human

activities (sedimentation, nutrient enrichment, physical damage, overfishing), mesophotic populations (> 30 m) may serve as potential sources of larvae for the impacted shallow reef communities. In Bermuda we observed that the colony size of *M. cavernosa* was significantly smaller at mesophotic sites, suggesting that growth rates and maximum colony surface area are limited in conditions of lower light intensity, seawater temperature, and nutrient concentration. Colony density was significantly higher at mesophotic sites, however, the average percent cover was not significantly different (Goodbody-Gringley et al. 2015: Chapter 5). These results suggest that the mesophotic zone, which extends around the perimeter of the Bermuda platform, makes a viable habitat able to support an established population of *M. cavernosa*, leading to the development of the "Deep Reef Refugia Hypothesis". In fact, investigating the health and stability of mesophotic coral populations can give insights on the capability of these reefs to serve as a source of propagules to maintain shallow water reefs and help guide future management and conservation strategies (Lesser et al. 2010).

References

Airi V, Gizzi F, Marchini C, Goffredo S (2016) Sexual reproduction of Mediterranean scleractinian corals. In: Goffredo S, Dubinsky Z (eds) The cnidaria, past, present and future. The world of Medusa and her sisters. Springer, Dordrecht, in press

Airi V, Gizzi F, Falini G, Levy O, Dubinsky Z, Goffredo S (2014) Reproductive efficiency of a Mediterranean endemic zooxanthellate coral decreases with increasing temperature along a wide latitudinal gradient. PLoS ONE 9:e91792

Albright R, Mason B (2013) Projected near-future levels of temperature and pCO_2 reduce coral fertilization success. PLoS ONE 8:e56468

Albright R, Langdon C (2011) Ocean acidification impacts multiple early life history processes of the Caribbean coral *Porites astreoides*. Global Change Biol doi: 10.1111/j.1365-2486.2011.02404.x

Albright R, Mason B, Miller M, Langdon C (2010) Ocean acidification compromises recruitment success of the threatened Caribbean coral *Acropora palmata*. Proc Natl Acad Sci 107:20400-20404

Albright R, Mason B, Langdon C (2008) Effect of aragonite saturation state on settlement and post-settlement growth of *Porites astreoides* larvae. Coral Reefs 27:485-490

Ayre DJ, Resing JM (1986) Sexual and asexual production of planulae in reef corals. Mar Biol 90:187-190

Ayre DJ, Miller KJ (2004) Where do clonal coral larval go? Adult genotypic diversity conflicts with reproductive effort in the brooding coral *Pocillopora damicornis*. Mar Ecol Prog Ser 277:95-105

Babcock RC, Bull GD, Harrison PL, Heyward AJ, Oliver JK, Wallace CC, Willis BL (1986) Synchronous spawnings of 105 scleractinian coral species on the Great Barrier Reef. Mar Biol 90:379-394

Babcock RC, Wills BL, Simpson CJ (1994) Mass spawning of corals on high latitude coral reef. Coral Reefs 13:161-169

Baird AH, Guest JR, Willis BL (2009) Systematic and biogeographical patterns in the reproductive biology of scleractinian corals. Annu Rev Ecol Evol Syst 40:551-571

Barnes DKA, Peck LS (2008) Vulnerability of Antarctic shelf biodiversity to predicted regional warming. Climate Research 37:149-163

Ben-David-Zaslow R, Henning G, Hofmann DK, Benayahu Y (1999) Reproduction in the Red Sea soft coral *Heteroxenia fuscescens*: seasonality and long-term record (1991 to 1997). Mar Biol 133:553-559

Bruno JF, Selig ER (2007) Regional decline of coral cover in the Indo-Pacific: timing, extent, and subregional comparisons. PLoS ONE 2:e711

Cairns SD (1988) Asexual reproduction in solitary scleractinia. In: Proceedings of the 6th international coral reef symposium, vol 2, Townsville, 1988, pp 641-646

Cairns SD (1999) Species richness of recent scleractinia. Atoll Res Bull 459:1-46

Cairns SD (2007) Deep-water corals: an overview with special reference to diversity and distribution of deep-water scleractinian corals. Bull Mar Sci 81:311-322

Capaccioni B, Tassi F, Vaselli O, Tedesco D, Poreda R (2007) Submarine gas burst at Panarea Island, southern Italy, on 3 November 2002: a magmatic versus hydrothermal episode. J Geophys Res 112:B05201

Caroselli E, Zaccanti F, Mattioli G, Falini G, Levy O, Dubinsky Z, Goffredo S (2012) Growth and demography of the solitary scleractinian coral *Leptopsammia pruvoti* along a sea surface temperature gradient in the Mediterranean Sea. PLoS ONE 7:e37848

Cohen AL, Holcomb M (2009) Why corals care about ocean acidification: uncovering the mechanism. Oceanography 22:118-127

Coma R, Ribes M, Serrano E, Jimenez E, Salat J, Pascual, J (2009) Global warming-enhanced stratification and mass mortality events in the Mediterranean. Proc Natl Acad Sci USA 16:6176-6181

Coma R, Ribes M, Zabala M, Gili JM (1995) Reproduction and cycle of gonadal development in the Mediterranean gorgonian *Paramuricea clavata*. Mar Ecol Prog Ser 117:173-183

Connell JH (1978) Diversity in tropical rain forests and coral reefs. Science 199:1302-1310

Cordes EE, Nybakken JW, Van Dykhuizen G (2001) Reproduction and growth of *Anthomastus ritteri* (Octocorallia: Alcyonacea) from Monterey Bay, California, USA. Mar Biol 138:491-501

Dahan M, Benayahu Y (1997) Reproduction of *Dendronephthya hemprichi* (Cnidaria: Octocorallia): year-round spawning in an azooxanthellate soft coral. Mar Biol 129:573-579

Doney SC, Fabry, VJ, Feely RA, Kleypas JA (2009) Ocean acidification: the other CO_2 problem. Ann Rev Mar Sci 1:169-192

Dunne JA, Saleska SR, Fischer ML, Harte J (2004). Integrating experimental and gradient methods in ecological climate change research. Ecology 85:904-916

Fabricius KE, Wild C, Wolanski E, Abele D (2003) Effects of transparent exopolymer particles and muddy terrigenous sediments on the survival of hard coral recruits. Estuar Coast Shelf Sci 57:613-621

Fan TY, Chou YH, Dai CF (2005) Sexual reproduction of the Alcyonacean Coral *Lobophytum pauciflorum* in Southern Taiwan. Bull Mar Sci 76:143-154

Fautin DG (2002) Reproduction of Cnidaria. Can J Zool 80:1735-1754

Fiorillo I, Rossi S, Alva V, Gili JM, López-González PJ (2013) Seasonal cycle of sexual reproduction of the Mediterranean soft coral *Alcyonium acaule* (Anthozoa, Octocorallia). Mar Biol 160:719-728

Gattuso JP, Frankignoulle M, Bourge I, Romaine S, Buddemeier RW (1998) Effect of calcium carbonate saturation of seawater on coral calcification. Global Planet Change 18:37-46

Gilmour J (1999) Experimental investigation into the effects of suspended sediment on fertilisation, larval survival and settlement in a scleractinian coral. Mar Biol 135:451-462

Glynn PW (1993) Coral reef bleaching: ecological perspectives. Coral reefs 12:1-17

Goffredo S, Marchini C, Rocchi M, Airi V, Caroselli E, Falini G, Levy O, Dubinsky Z, Zaccanti F (2012) Unusual pattern of embryogenesis of *Caryophyllia inornata* (Scleractinia, Caryophylliidae) in the Mediterranean Sea. Maybe agamic reproduction? J Morphol 273:943-956

Goffredo S, Gasparini G, Marconi G, Putignano MT, Pazzini C, Airi V, Zaccanti F (2011) Sexual reproduction in the Mediterranean endemic orange coral, *Astroides calycularis* (Scleractinia, Dendrophylliidae). Bull Mar Sci 87:589-604

Goffredo S, Gasparini G, Marconi G, Putignano MT, Pazzini C, Zaccanti F (2010) Gonochorism and planula brooding in the Mediterranean endemic orange coral *Astroides calycularis* (Scleractinia: Dendrophylliidae). Morphological aspects of gametogenesis and ontogenesis. Mar Biol Res 6:421-436

Goffredo S, Airi V, Radetić J, Zaccanti F (2006) Sexual reproduction of the solitary sunset cup coral *Leptopsammia pruvoti* (Scleractinia, Dendrophylliidae) in the Mediterranean. 2. Quantitative aspects of the annual reproductive cycle. Mar Biol 148:923-932

Goffredo S, Radetic J, Airi V, Zaccanti F (2005) Sexual reproduction of the solitary sunset cup coral *Leptopsammia pruvoti* (Scleractinia, Dendrophyllidae) in the Mediterranean. 1. Morphological aspects of gametogenesis and ontogenesis. Mar Biol 147:485-495

Goffredo S, Arnone S, Zaccanti F (2002) Sexual reproduction in the Mediterranean solitary coral *Balanophyllia europaea* (Scleractinia, Dendrophylliidae). Mar Ecol Prog Ser 229:83-94

Goffredo S, Telò T (1998) Hermaphroditism and brooding in the solitary coral *Balanophyllia europaea* (Cnidaria, Anthozoa, Scleractinia). Ital J Zool 65:159-165

Goodbody-Gringley G, Marchini C, Chequer AD, Goffredo S (2015) Population Structure of *Montastraea cavernosa* on shallow versus mesophotic reefs in Bermuda. PLoS ONE 10:e0142427

Gorbunov MY, Falkowski PG (2002) Photoreceptors in the cnidarian hosts allow symbiotic corals to sense blue moonlight. Limnol Oceanogr 47:309-315

Harland AD, Davies PS, Fixter LM (1992) Lipid content of some Caribbean corals in relation to depth and light. Mar Biol 113:357-361

Harrison PL, Babcock RC, Bull GD, Oliver JK, Wallace CC, Willis BL (1984) Mass spawning in tropical reef corals. Science 223:1186-1189

Harrison PL, Wallace CC (1990) Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky Z (ed) Ecosystems of the world: coral reefs. Elsevier, Amsterdam, pp 133-207

Harrison PL (2011) Sexual reproduction of scleractinian corals. In: Dubinsky Z, Stambler N (eds) Coral reefs: An ecosystem in transition. Springer, Netherlands, pp 59-85

Hartnoll RG (1975) The annual cycle of *Alcyonium digitatum*. Est Coast Shelf Sci 3:71-78

Highsmith RC (1982) Reproduction by fragmentation in corals. Mar Ecol Prog Ser 7:207-226

Hochachka PW, Somero GN (2002) Biochemical Adaptation: Mechanism and Process in Physiological Evolution. New York: Oxford University Press

Houghton RA (2007) Balancing the global carbon budget. Annu Rev Earth Planet Sci 35:313-347

Jokiel PL, Ito RY, Liu PM (1985) Night irradiance and synchronization of lunar release of planula larvae in the reef coral *Pocillopora damicornis*. Mar Biol 88:167-174

Kersting DK, Casado C, López-Legentil S, Linares C (2013) Unexpected divergent patterns in the sexual reproduction of the Mediterranean scleractinian coral *Cladocora caespitosa*. Marine Ecology Progress Series 486:165-171

Kinne O (1970) Invertebrates. Temperature effects. In Marine Ecology. Environmental Factors, Part 1 Volume 1, O. Kinne (ed.). Chichester, UK: Wiley-Interscience, 407-514

Knowlton N (2001) The future of coral reefs. Proc Natl Acad Sci USA 98:5419-5425

Kružić P, Zuljevic A, Nikolic V (2008) Spawning of the colonial coral *Cladocora caespitosa* (Anthozoa, Scleractinia) in the Southern Adriatic sea. Coral Reefs 27:337-341

Lam KKY (2000) Sexual reproduction of a low-temperature tolerant coral *Oulastrea crispata* (Scleractinia: Faviidae) in Hong Kong, China. Mar Ecol Prog Ser 205:101-111

Lane A, Harrison PL (2002). Effects of oil contaminants on survivorship of larvae of the scleractinian reef corals *Acropora tenuis*, *Goniastrea aspera* and *Platygyra sinensis* from the Great Barrier Reef. In: Proceedings of the 9th international coral reef symposium, vol 1, Bali, 2000, pp 403-408

Lesser MP (2004) Experimental biology of coral reef ecosystems. J Exp Mar Biol Ecol 300:217-252

Lesser MP, Slattery M, Stat M, Ojimi M, Gates RD, Grottoli A (2010) Photoacclimation by the coral *Montastraea cavernosa* in the mesophotic zone: light, food and genetics. Ecology 91:990-1003

Levy O, Appelbaum L, Leggat W, Gothlif Y, Hayward DC, Miller DJ, Hoegh-Guldberg O (2007) Light-responsive cryptochromes from a simple multicellular animal, the coral *Acropora millepora*. Science 318:467-470

Linares C, Coma R, Zabala M (2008) Effects of a mass mortality event on gorgonian reproduction. Coral reefs 27:27-34

Loya Y (1976) Skeletal regeneration in a Red Sea scleractinian coral population. Nature 261:490-491

Loya Y, Sakai K (2008) Bidirectional sex change in mushroom stony corals. Proc R Soc B 275:2335-2343

Loya Y, Rinkevich B (1979) Abortion effect in corals induced by oil pollution. Mar Ecol Prog Ser l:77-80

Maynard-Smith J (1978) The evolution of sex. Cambridge University Press, Cambridge

Marchini C, Airi V, Fontana R, Tortorelli G, Falini G, Levy O, Dubinsky Z, Goffredo S (2015) Annual reproductive cycle in the temperate coral *Caryophyllia inornata* driven by an unusual embryogenesis. PLoS ONE 10:e0141162

Markey KL, Baird AH, Humphrey C, Negri AP (2007) Insecticides and a fungicide affect multiple coral life stages. Mar Ecol Prog Ser 330:127-137

Mendes JM, Woodley JD (2002) Timing of reproduction in *Montastraea annularis*: relationship to environmental variables. Mar Ecol Prog Ser 227:241-251

Meesters EH, Hilterman M, Kardinaal E, Keetman M, De Vries M, Bak RPM (2001) Colony size-frequency distributions of scleractinian coral populations: spatial and interspecific variation. Mar Ecol Prog Ser 209:43-54

Meesters EH, Wesseling I, Bak RPM (1996) Partial mortality in three species of reef-building corals and the relation with colony morphology. Bull Mar Sci 58:838-52

Meyer E, Davies S, Wang S, Willis BL, Abrego D, Juenger TE, Matz MV (2008) Genetic variation in responses to a settlement cue and elevated temperature in the reef-building coral *Acropora millepora*. Mar Ecol Prog Ser 392:81-92

Michalek-Wagner K and Willis BL (2001) Impacts of bleaching on the soft coral *Lobophytum compactum*. Fecundity, fertilization and offspring viability. Coral Reefs 19:231-239

Morita M, Suwa R, Iguchi A, Nakamura M, Shimada K et al. (2009) Ocean acidification reduces sperm flagellar motility in broadcast spawning reef invertebrates. Zygote 18:103-107

Mundy C, Babcock R (2000) Are vertical distribution patterns of scleractinian corals maintained by pre- or post-settlement processes? A case study of three contrasting species. Mar Ecol Prog Ser 198:109-119

Muscatine L (1990) The role of symbiotic algae in carbon and energy fluxes in reef corals. In: Dubinsky Z (ed) Coral reef ecosystems. Elsevier, Amsterdam, pp 75-87

Nakano Y, Yamazoto K (1992) Ecological study of reproduction of *Oulastrea crispata* in Okinawa. Behav Biol Ecol 1292

Negri AP, Heyward A (2001) Inhibition of coral fertilization and larval metamorphosis by tributyltin and copper. Mar Poll Bull 41:420-427

Negri A, Vollhardt C, Humphrey C, Heyward A, Jones R, Eaglesham G, Fabricius K (2005) Effects of the herbicide diuron on the early life history stages of coral. Mar Poll Bull 51:370-383

Negri AP, Marshall PA, Heyward A (2007) Differing effects of thermal stress on coral fertilization and early embryogenesis in four Indo-Pacific species. Coral Reefs 26:759-763

Oliver JK, Babcock RC, Harrison PL, Willis BL (1988) Geographic extent of mass coral spawning: clues to ultimate causal factors. In: Proceedings of 6th International Coral Reef Symposium, vol 2, Townsville, pp 803-810

Pachauri RK, Allen MR, Barros VR, Broome J, Cramer W, Christ R, Church JA, Clarke L, Dahe Q, Dasgupta P, Dubash NK, Edenhofer O, Elgizouli I, Field CB, Forster P, Friedlingstein P, Fuglestvedt J, Gomez-Echeverri L, Hallegatte S, Hegerl G, Howden M, Jiang K, Jimenez Cisneroz B, Kattsov V, Lee H, Mach KJ, Marotzke J, Mastrandrea MD, Meyer L, Minx J, Mulugetta Y, O'Brien K, Oppenheimer M, Pereira JJ, Pichs-Madruga R, Plattner GK, Pörtner HO, Power SB, Preston B, Ravindranath NH, Reisinger A, Riahi K, Rusticucci M, Scholes R, Seyboth K, Sokona Y, Stavins R, Stocker TF, Tschakert P, van Vuuren D and van Ypserle JP (2014): Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change / R. Pachauri and L. Meyer (editors) , Geneva, Switzerland, IPCC, 151 p, ISBN: 978-92-9169-143-2

Pankhurst NW, Porter MJR (2003) Cold and dark or warm and light: variations on the theme of environmental control of reproduction. Fish Physiol Biochem 28:385-389

Pörtner HO (2008) Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. Mar Ecol Prog Ser 373: 203-217

Pörtner HO, Knust R (2007) Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science 315:95-97

Pörtner HO, Langenbuch M, Michaelidis B (2005) Synergistic effects of temperature extremes, hypoxia, and increases in CO_2 on marine animals: from Earth history to global change. J Geo Res 110:C09S10

Randall CJ, Szmant AM (2009) Elevated temperature reduces survivorship and settlement of the larvae of the Caribbean scleractinian coral, Favia fragum (Esper). Coral Reefs 28:537-545

Reichelt-Brushett AJ, Harrison PL (2005) The effect of selected trace metals on the fertilization success of several scleractinian coral species. Coral Reefs 24:524-534

Richmond RH, Hunter CL (1990) Reproduction and recruitment of corals: comparisons among the Caribbean, the Tropical Pacific, and the Red Sea. Mar Ecol Prog Ser 60:185-203

Richmond RH (1997) Reproduction and recruitment in corals: critical links in the persistence of reefs. In: Birkeland C (ed) Life and death of coral reefs. Chapman and Hall, New York, pp 175-197

Ridgway KR (2007) Long-term trend and decadal variability of the southward penetration of the East Australian Current. Geo Res Let 34:L13613

Rustad LE (2008) The response of terrestrial ecosystems to global climate change: towards an integrated approach. Sci Total Environ 404:222-235

Sabine CL, Feely RA, Gruber N, Key RM, Lee K, Bullister JL (2004) The oceanic sink for anthropogenic CO_2. Science 305:367-371

Sherman CDH, Ayre DJ, Miller KJ (2006) Asexual reproduction does not produce clonal populations of the brooding coral *Pocillopora damicornis* on the Great Barrier Reef, Australia. Coral Reefs 25:7-18

Schmalenbach I, Franke HD (2010) Potential impact of climate warming on the recruitment of an economically and ecologically important species, the European lobster (*Homarus gammarus*) at Helgoland, North Sea. Mar Biol 157:1127-1135

Schofield O, Ducklow HW, Martinson DG, Meridith MP, Moline MA, Fraser WR (2010) How do polar marine ecosystems respond to rapid climate change? *Science* 328:1520-1523

Schuhmacher H, Zibrowius H (1985) What is hermatypic? A redefinition of ecological groups in corals and other organisms. Coral Reefs 4:1-9

Simpson CJ (1985) Mass spawning of scleractinian corals in the Dampier archipelago and the implications for management of coral reefs in Western Australia. West Aust Dept Conserv Environ Bull 244:35

Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds) IPCC 2013: Summary for Policymakers. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA

Stoddart JA (1983) Asexual production of planulae in the coral *Pocillopora damicornis*. Mar Biol 76:279-284

Suwa, R, Nakamura M, Morita M, Shimada K, Iguchi A et al. (2010) Effects of acidified seawater on early life stages of scleractinian corals (Genus *Acropora*). Fish Sci 76: 93-99

Szmant AM (1986) Reproductive ecology of Caribbean reef corals. Coral Reefs 5:43-54

Torrents O, Garrabou J (2011) Fecundity of red coral *Corallium rubrum* (L.) populations inhabiting in contrasting environmental conditions in the NW Mediterranean. Mar Biol 158:1019-1028

Trench RK (1979) The cell biology of plant animal symbioses. Annu Rev Plant Physiol 30:485-531

Trench RK (1987) Dinoflagellates in non-parasitic symbioses. In: Taylor FJR (ed) Biology of dinoflagellates. Blackwell, Oxford, pp 530-570

Tsounis G, Rossi S, Aranguren M, Gili JM, Arntz W (2006) Effects of spatial variability and colony size on the reproductive output and gonadal development cycle of the Mediterranean red coral (*Corallium rubrum* L.) Mar Biol 148:513-527

van Oppen MJH, Oliver JK, Putnam HM, Gates RD (2015) Building coral reef resilience through assisted evolution. Proc Natl Acad Sci 112:2307-2313

van Woesik R (2009) Calm before the spawn: global coral spawning patterns are explained by regional wind fields. Proceedings of the Royal Society B: Biological Sciences 277:715-722

Veron JEN (1995) Corals in space and time. The biogeography and evolution of the Scleractinia. Comstock/Cornell University Press, Ithaca, NY, 321pp

Veron JEN (2000) Corals of the world. Australian Institute of Marine Science, Townsville

Waller RG, Tyler PA (2005) The reproductive biology of two deep-water, reef-building scleractinians from the NE Atlantic Ocean. Coral Reefs 24:514-522

Waller GR, Tyler PA, Gage JD (2005) Sexual reproduction in three hermaphroditic deep-sea *Caryophyllia* species (Anthozoa: Scleractinia) from the NE Atlantic Ocean. Coral Reefs 24:594-602

Ward S (1995) The effect of damage on the growth, reproduction and lipid storage in the scleractinian coral *Pocillopora damicornis*. J Exp Mar Biol Ecol 187:193-20

Wilkinson CR (2003) Coral reefs are facing widespread extinctions: can we prevent these through sustainable management practices? Proc 7th Int Coral Reef Symp 1:11-21

Williams GC (1975) Sex and evolution. Princeton University Press, Princeton

Widdicombe S, Spicer JI (2008) Predicting the impact of ocean acidification on benthic biodiversity: what can animal physiology tell us? J Exp Mar Biol Ecol 366:187-197

Yonge CM (1973) The nature of reef building (hermatypic) corals. Bull Mar Sci 23:1-5

Chapter II

Annual reproductive cycle and unusual embryogenesis of a temperate coral in the Mediterranean Sea

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RESEARCH ARTICLE

Annual Reproductive Cycle and Unusual Embryogenesis of a Temperate Coral in the Mediterranean Sea

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Abstract

The variety of reproductive processes and modes among coral species reflects their extraordinary regeneration ability. Scleractinians are an established example of clonal animals that can exhibit a mixed strategy of sexual and asexual reproduction to maintain their populations. This study provides the first description of the annual reproductive cycle and embryogenesis of the temperate species *Caryophyllia inornata*. Cytometric analyses were used to define the annual development of germ cells and embryogenesis. The species was gonochoric with three times more male polyps than female. Polyps were sexually mature from 6 to 8 mm length. Not only females, but also sexually inactive individuals (without germ cells) and males were found to brood their embryos. Spermaries required 12 months to reach maturity, while oogenesis seemed to occur more rapidly (5–6 months). Female polyps were found only during spring and summer. Furthermore, the rate of gamete development in both females and males increased significantly from March to May and fertilization was estimated to occur from April to July, when mature germ cells disappeared. Gametogenesis showed a strong seasonal influence, while embryos were found throughout the year in males and in sexually inactive individuals without a defined trend. This unusual embryogenesis suggests the possibility of agamic reproduction, which combined with sexual reproduction results in high fertility. This mechanism is uncommon and only four other scleractinians (*Pocillopora damicornis*, *Tubastraea diaphana*, *T. coccinea* and *Oulastrea crispata*) have been shown to generate their broods asexually. The precise nature of this process is still unknown.

Introduction

Reproductive biology is a key feature of an organism's life strategy [1] and is fundamental to understand the population structure and dynamics of sessile animals [2], which are an

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important component of aquatic communities. Corals are modular organisms that can potentially lead to a variety of reproductive processes and modes, reflecting their extraordinary regeneration ability, developmental plasticity, and adaptability [3,4]. However, for reproduction, there are essentially only four combinations of reproductive patterns: propagation mode (sexual or asexual), sexuality (hermaphroditic or gonochoric), reproductive mode (broadcasting or brooding), and embryonic development (coeloblastula or stereoblastula) [4]. These organisms can display a mixed propagation mode of sexual and asexual reproduction in order to preserve their populations [5]. Simultaneous mixed reproduction is rare in animals and is often described as the "best-of-both-worlds" scenario that can help organisms adapt to changing environments [6]. Sexual reproduction requires the production of gametes, fertilization, embryo development, a larval phase and enables genetic recombination and production of new genotypes. This genotypically different lineage might enable a wide dispersion or recolonization of more heterogeneous habitats, increasing the fitness and survival of the species [7,8]. Asexual reproduction may take place via colony fragmentation, colony fission, longitudinal and transverse division, polyp expulsion or polyp "bail-out", budding and, in rare cases, the production of brooded embryos spreading successful genotypes without mating [4]. This clonal line might contribute to keeping populations inside the area of the parental habitat, thus propagating well-adapted genotypes at the local level [8]. It may be also an adaptation that allows the exploitation of newly available substrata after a disturbance event [9].

Concerning sexuality, most of the scleractinians are hermaphrodites and only 26% of the studied species are described as gonochoric [3,4]. The hermaphroditism normally is simultaneous, but there are some forms of hermaphroditism more complex to detect as the cyclic sequential in the same breeding season (as has been described for three deep species of the genus *Caryophyllia*) [10] and the protandrous or protogynous sequential during the life. *Lobactis scutaria* and *Lithophyllon repanda* are predominantly male at small sizes whereas large individuals are all females, suggesting that these fungiids are protandrous hermaphrodites [11,12]. Additionally, *Ctenactis echinata* is a protandrous species but has the capacity for bidirectional sex change between the years as occurs in dioecious plants that display a labile sexuality in response to energetic and/or environmental constraints [12].

Fertilization can be either internal when the embryo is formed and develops within the polyp and is released as a motile planula (brooding), or external when the embryo develops in the water column (broadcast spawning); the first condition is less common within the order Scleractinia and represents the 16% of the total number of known coral species [3,4]. Very few brooders can produce planulae by asexual processes, indeed, it has been shown only in some populations of *Pocillopora damicornis* [13], sometimes in combination with gametogenetic activity [9,14,15], in *Tubastraea diaphana* [16], *T. coccinea* [16,17], and *Oulastrea crispata* [18,19]. These scleractinians were also found to be pioneer species, colonizing unpredictable, short-lived or unexploited habitats as oil and gas platforms [19,20].

The reproductive cycle can be regulated by several environmental factors such as seawater temperature, photoperiod, wind or current patterns, lunar cycles of night irradiance, food availability and seasonal rainfall [1,4]. In particular, photoperiod (therefore solar radiation) and seawater temperature are not mutually exclusive events. In fact, in the Mediterranean Sea there are marked seasonal patterns of seawater temperature driven by photoperiod and irradiance cycles distinctive of temperate latitudes [1]. However, while several studies have shown that seawater temperature strongly influences gametogenesis [17,21–25], the potential role of photoperiod has so far been overlooked.

Although reproduction of scleractinians has been thoroughly studied in the last decades [12,21,22,26–34], the great variety of reproductive strategies within this group is not yet entirely known and even less is known about asexual patterns. Furthermore, knowledge on the

reproductive biology of Mediterranean scleractinian corals is scarce and exclusively linked to aspects of the sexual propagation of *Balanophyllia europaea* [35–39], *Leptopsammia pruvoti* [2,40], *Cladocora caespitosa* [41,42] and *Astroides calycularis* [43–45].

This manuscript describes, for the first time, the quantitative aspects (sex ratio, size of individuals at sexual maturity, fecundity, and seasonal patterns of gonadal development and fertility) of the annual reproductive cycle in the Mediterranean solitary coral *Caryophyllia inornata* (S1 Fig; Duncan, 1878) at Elba Isle (Italy). Some aspects of the reproductive biology of this species have already been described, revealing a gonochoric sexuality and a brooding reproductive mode, driven by an unusual pattern of embryogenesis in which embryos are found in females, males and sexually inactive individuals throughout the year, suggesting a possible asexual origin of the embryos [46].

Materials and Methods

Ethic Statement

According to the European normative (2010/63/EU of 8 August 2010) on the protection of animals used for scientific purposes, there is no active conservation measure for the Mediterranean coral *Caryophyllia inornata*. The species is not protected in Italy, nor it is subject to any regulations. Hence, no permit was needed to collect samples. For this study, sampling was limited strictly to the number necessary and performed where the species is characterized by a high population density to minimize the impact of removing individuals and preserve both the demographic and genetic structure of the natural populations.

Study species, sample collection and environmental parameters

The solitary coral *Caryophyllia inornata* is distributed in the Mediterranean Sea [47] and extends up to the Northeastern Atlantic coasts [48], from the Canary Islands to the Southern coast of the United Kingdom [47]. It colonizes caves, walls and wrecks, from the surface down to 100 m depth in dimly lit or dark environments, representing one of the main species that populate the walls and the vaults of caves and in some cases is the dominant species [49].

Polyps were collected from an aircraft wreck at Elba Isle (42°45'N, 10°24'E), during 18 monthly samplings from May 2009 to October 2010. A minimum of 15 polyps were collected randomly each month at a depth of 12–15 m by SCUBA diving. The population density in the sampling site was 6025 ± 898 (mean ± SE) individuals m^{-2} with a percentage cover of 15.3 ± 2.5% (mean ± SE) [50].

Photoperiod data were obtained from an online database (http://www.eurometeo.com). Water temperature (°C) was continuously recorded every three hours by digital sensors (I-Button DS1921H, Maxim Integrated Products) placed at the depth and site of collection for the entire sampling period. A linear regression was produced between DT (Depth Temperature; °C) and SST (Sea Surface Temperature; °C) data to estimate temperatures during periods in which sensors were lost due to bad weather conditions. In this study we considered the monthly average DT of almost two years of sampling (n = 18 monthly temperatures).

Polyps were fixed in saturated formalin solution (10% formaldehyde and 90% seawater; the solution was saturated with calcium carbonate) and transferred to the laboratories for histological analysis.

Biometric and histological analysis

Biometric analyses were performed on 158 polyps by measuring length (L, maximum axis of the oral disc), width (l, minimum axis of the oral disc) and height (h, oral–aboral axis) of each

sampled polyp. The volume (V) of the individual polyp was calculated using the formula $V = h * (L/2) * (l/2) * \pi$ [37].

Polyps were post-fixed in Bouin solution. After decalcification in EDTA and dehydration in a graded alcohol series from 80% to 100%, polyps were embedded in paraffin and serial transverse sections were cut at 7 μm intervals along the oral-aboral axis, from the oral to the aboral poles. Tissues were then stained with Mayer's haematoxylin and eosin [37].

Cytohistometric analysis

Cytohistometric observations were performed with an optical microscope using the software NIKON NIS-Elements D 3.2. The maximum and minimum diameters of the oocytes in nucleated sections and spermaries were measured and classified into developmental stages according to earlier studies on gametogenesis in scleractinians [11,37,51–54]. The presence of embryos in the gastrovascular cavity and mesenterial septa was recorded, and their stage of maturation identified [2,35]. The size of each reproductive element was determined as the mean of the two diameters [2,43].

Definitions

In accordance with the sexuality described by Goffredo *et al* [46], based on the type of germ cells observed and the presence or absence of embryos, 5 reproductive states have been identified: sexually active individuals that present gametogenetic activity (i.e., females with embryos, males, and males with embryos) and sexually inactive individuals, without germ cells (i.e., inactive individuals and inactive individuals with embryos).

The following reproductive parameters were determined: a) *size at sexual maturity*, defined as the length at which 50% of the analyzed polyps developed spermaries or oocytes; b) *fecundity*, defined as the number of mature oocytes produced per body volume unit (100 mm^3) per reproductive season; c) *gonadal index*, defined as the percentage of body volume occupied by germ cells [37]; d) *fertility*, defined as the number of embryos per body volume unit (100 mm^3).

Results

Sexuality and reproductive mode

The analysis of 158 polyps confirmed that *Caryophyllia inornata* is gonochoric and brooder [46]. The sex ratio of sexually active polyps was significantly different from 1 with a 1:3.5 male biased ratio (chi-square test, $\chi^2 = 20.43$, df = 1, $p < 0.001$).

Embryos were found in all monthly samples and inside females, males, and inactive individuals (Fig 1) [46]. All 15 females had embryos (L = 7.9 ± 0.4 mm; V = 366 ± 47 mm^3; mean ± SE). Of the 52 males, 45 had embryos (L = 8.2 ± 0.3 mm; V = 363 ± 31 mm^3; mean ± SE) and 7 were without embryos (L = 6.5 ± 0.4 mm; V = 219 ± 27 mm^3; mean ± SE). Of the 91 inactive polyps, 60 had embryos (L = 7.9 ± 0.3 mm; V = 341 ± 29 mm^3; mean ± SE) and 31 did not show embryos (L = 5.5 ± 0.4 mm; V = 171 ± 40 mm^3; mean ± SE).

Polyps up to 6 mm in length were immature and size at sexual maturity ranged from 6 to 8 mm in length (Fig 2). According to biometric analyses a polyp in this category has l = 5–7 mm, h = 5–6 mm, V = 146–206 mm^3. The frequency of sexually mature polyps decreased in larger size classes (Fig 2).

Annual reproductive cycle

Female polyps were observed between February and July, while males were found during the entire year (Figs 1 and 3). This suggests that the oogenesis process requires less time to reach

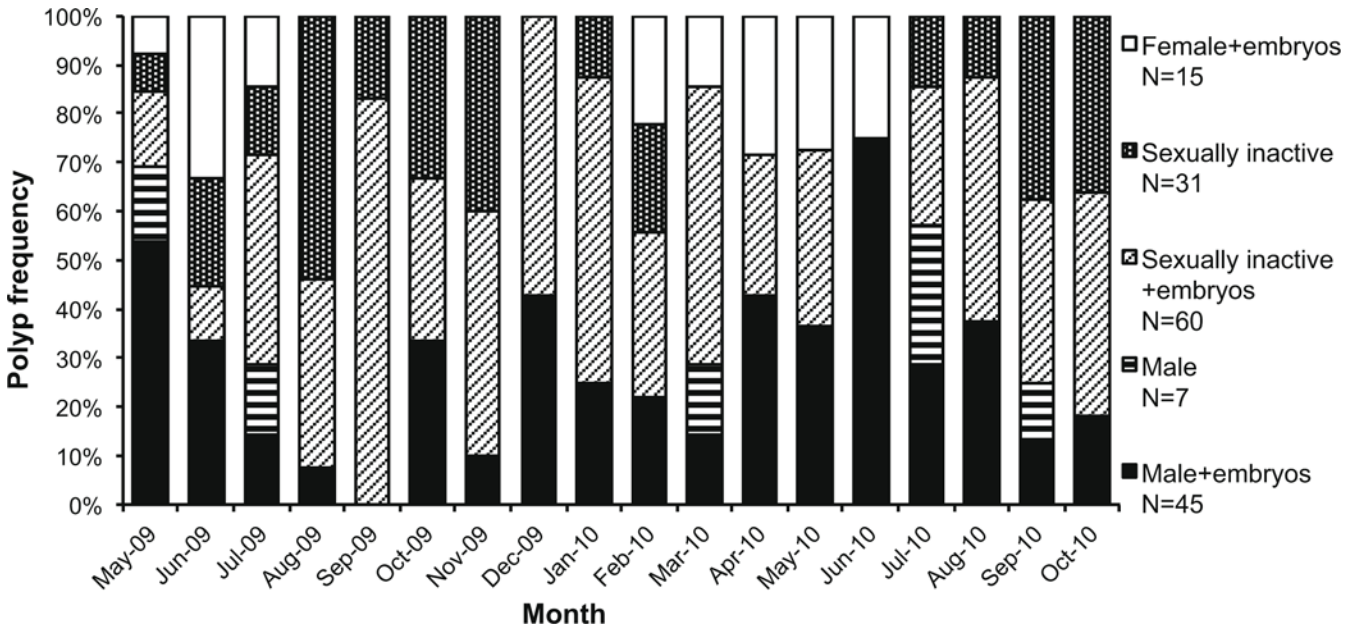

Fig 1. Monthly frequency of the 5 reproductive states. Monthly frequency of the 5 reproductive states (female with embryos, inactive individual, inactive individual with embryos, male and male with embryos) characterizing the population of Elba Isle, between May 2009 and October 2010 (N = 158).

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the final stage of maturation than spermatogenesis, which needed about 12 months (Fig 3). Gonadal size of both females and males increased significantly from March until May, when both photoperiod and water temperature increased after the minimum of the year (Fig 4A and 4B). Fertilization took place from April to July, when photoperiod was the longest of the year (Fig 4A and 4B). Immediately after the fertilization period, we observed the emptying of

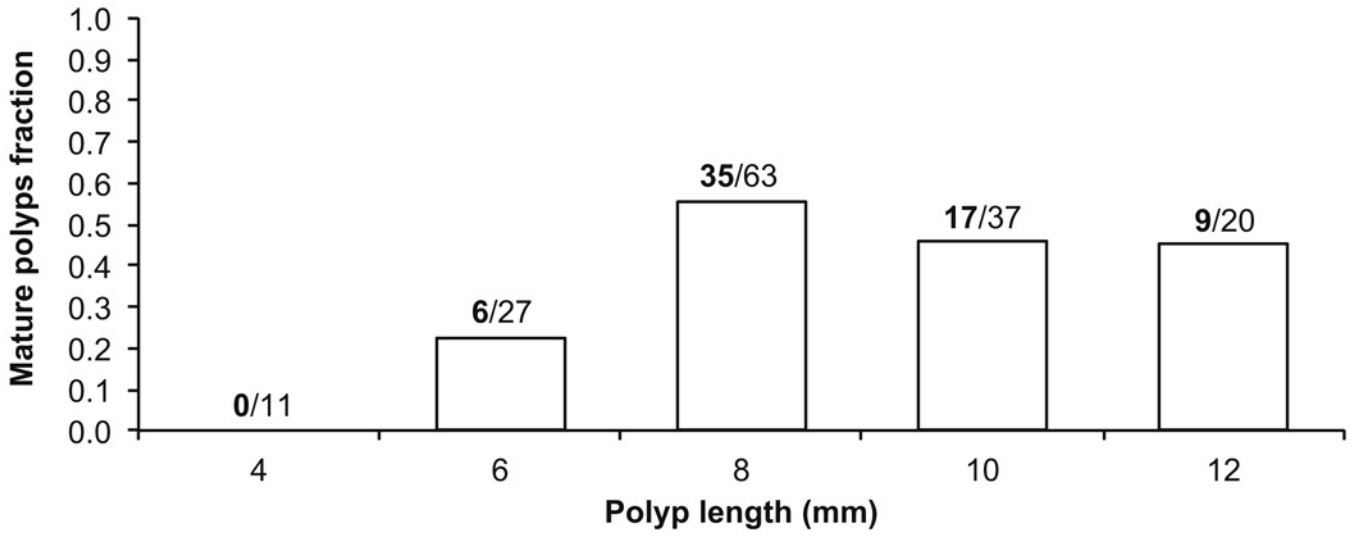

Fig 2. Fraction of sexually mature individuals per size class (mm). Fraction of sexually mature individuals per size class in millimeters, collected at Elba Isle. The values above the bars indicate the number of sexually mature polyps (bold) out of the number of polyps analyzed per size class (N = 158).

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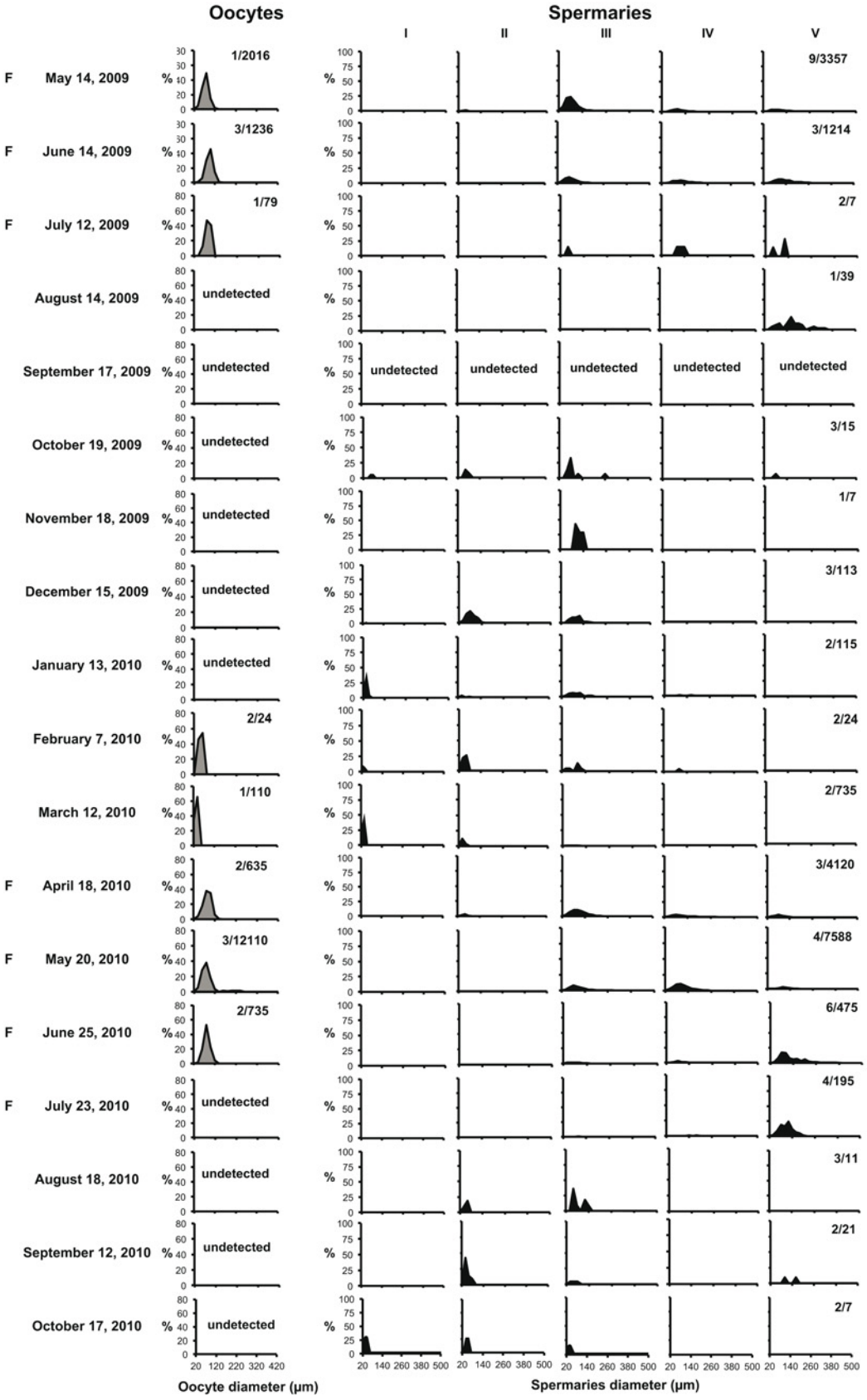
Oocytes
Spermaries
I
II
III
IV
V
F May 14, 2009
1/2016
9/3357
F June 14, 2009
3/1236
3/1214
F July 12, 2009
1/79
2/7
August 14, 2009
undetected
1/39
September 17, 2009
undetected
undetected
undetected
undetected
undetected
undetected
October 19, 2009
undetected
3/15
November 18, 2009
undetected
1/7
December 15, 2009
undetected
3/113
January 13, 2010
undetected
2/115
February 7, 2010
2/24
2/24
March 12, 2010
1/110
2/735
F April 18, 2010
2/635
3/4120
F May 20, 2010
3/12110
4/7588
F June 25, 2010
2/735
6/475
F July 23, 2010
undetected
4/195
August 18, 2010
undetected
3/11
September 12, 2010
undetected
2/21
October 17, 2010
undetected
2/7
%
20 120 220 320 420
20 140 260 380 500
Oocyte diameter (µm)
Spermaries diameter (µm)

Fig 3. Oocytes and spermaries size-frequency distribution. Size-frequency distribution of oocytes and of the five stages of spermary maturation in monthly samples collected at Elba Isle from May 2009 to October 2010. Values reported indicate the number of polyps/the total number of oocytes or spermaries measured per monthly sample. F = fertilization period.

doi:10.1371/journal.pone.0141162.g003

spermaries and we did not register the presence of oocytes (Fig 3). During the autumn months following the fertilization period, we observed the development of early stages of spermaries maturation in males (Fig 3).

Size of mature oocytes and fecundity

All the oocytes of *Caryophyllia inornata* reached maturity during the period from February to July, since we observed their disappearance after fertilization. Mature oocyte size was 69.7 μm (SE = 0.1) and ranged from 12 μm to 382 μm. We found a mean fecundity of 20'106 (SE = 11'715) mature oocytes in averaged-sized females of L = 7.9 mm (SE = 0.4), corresponding to l = 7.0 mm (SE = 0.4), h = 8.0 mm (SE = 0.4), V = 366 mm^3 (SE = 47), N = 15 polyps collected during the period of gonadal development (Fig 4B).

Fertility

Polyps up to 6 mm in length were not fertile and size at embryo production ranged from 6 to 8 mm in length (Fig 5). A continuous production of embryos in different stages of development (early embryos, intermediate and advanced stereogastrulae) [46] was observed during the entire year (Fig 4C). The fertility of females increased significantly from April to June, the same period in which gonadal development increased and fertilization occurred (Figs 4B and 4C and 6A). Embryos inside males and sexually inactive individuals were observed in all sampling months without a clear relation with seasonal variations of water temperature and photoperiod (Fig 6B and 6C).

Discussion

Sexuality and reproductive mode

This study provides the first description of the quantitative aspects of the annual reproductive cycle and embryogenesis of the temperate species *Caryophyllia inornata*.

C. inornata is gonochoric and brooder, as previously described for this species [46]. Histological analyses confirmed that no polyps showed simultaneous male and female gametes in different stages of development, excluding the possibility of a cyclical hermaphroditism, as reported for the three deep species of the genus *Caryophyllia* [10]. Also, protandrous or protogynous sequential hermaphroditism can be excluded, as the size of male and female individuals was not significantly different [46].

The male biased sex ratio observed in *C. inornata* could be explained by a clonal propagation where male clones are more likely to reproduce asexually than females, as has been reported in some solitary scleractinians of the Fungidae family: *Diaseris distorta*, *Lobactis scutaria*, *Lithophyllon concinna* and *Fungia fungites* [11,23,55]. A male biased sex ratio may also increase fertilization success, resulting in an advantage for sessile gonochoric corals with internal or surface fertilization [56–58]. Within the family Caryophylliidae, an agamic propagation by unequal intratentacular budding was observed in the colonial coral *Lophelia pertusa* [59–61]. This cold-water scleractinian also displays sexual reproduction, following an annual cycle of gametogenesis [62]. Evidence of an asexual production of brooded embryos in combination with gametogenetic activity, as it might occur for *C. inornata*, has been demonstrated in some populations of *Pocillopora damicornis*, in Western Australia, Hawaii, and southern Japan

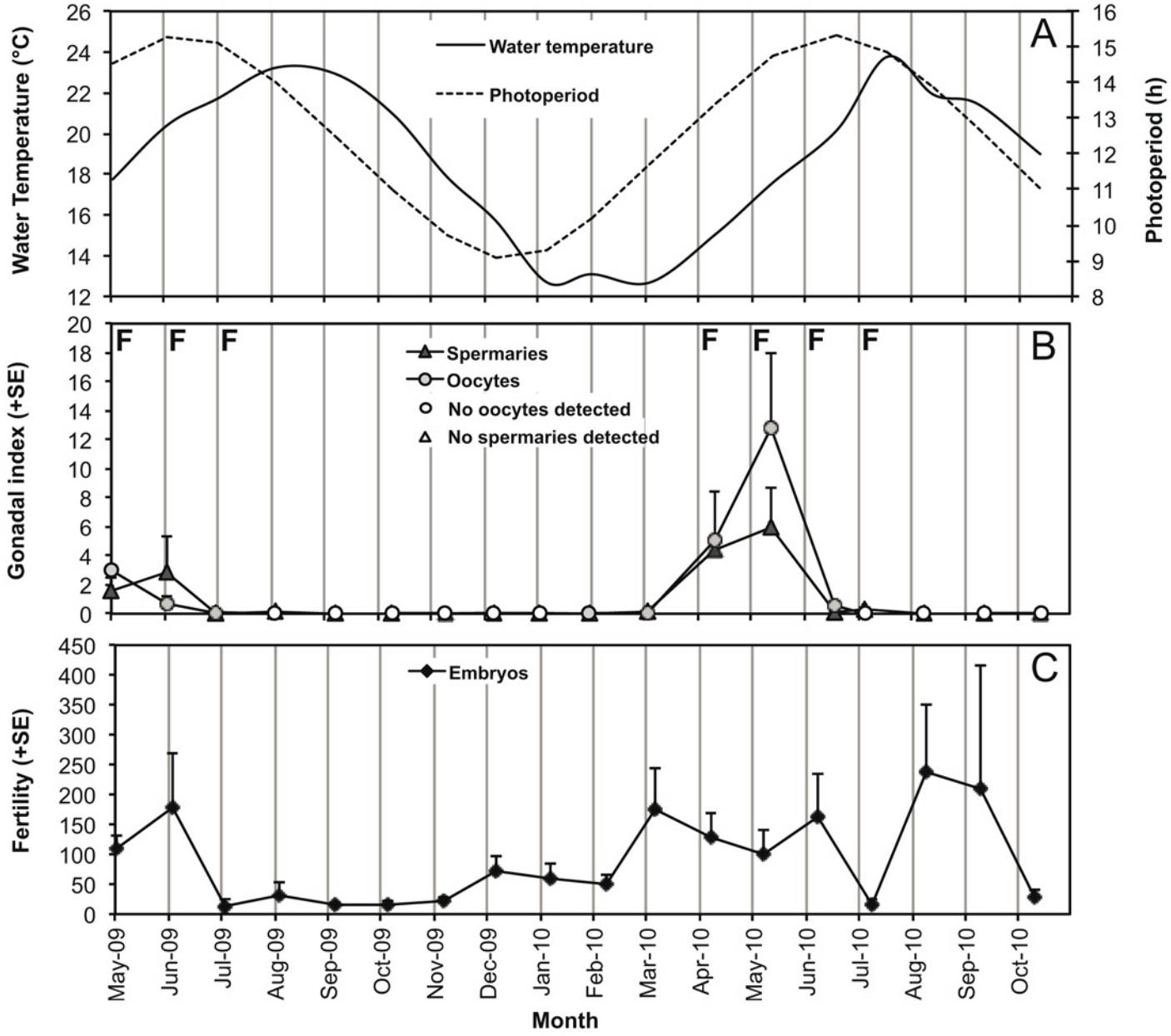

Fig 4. Variation in water temperature, photoperiod, gamete development and fertility. Variation in water temperature and photoperiod (A), gamete development (monthly mean + SE; B), and total fertility (monthly mean + SE; C) from May 2009 to October 2010 at Elba Isle. F = fertilization period.

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[6,9,15,16,63–67]. This strategy has been observed in other tropical scleractinians like *Tubastraea diaphana* [16], *T. coccinea* [17] and *Oulastrea crispata*, which can also produce asexual embryos during periods when gametogenesis is not occurring [18,19]. This mixed reproductive strategy might allow colonization of new structures in the sea, in a relatively short period of time [19]. The Australian sea anemone *Actinia tenebrosa* [68,69] and the tropical *A.*

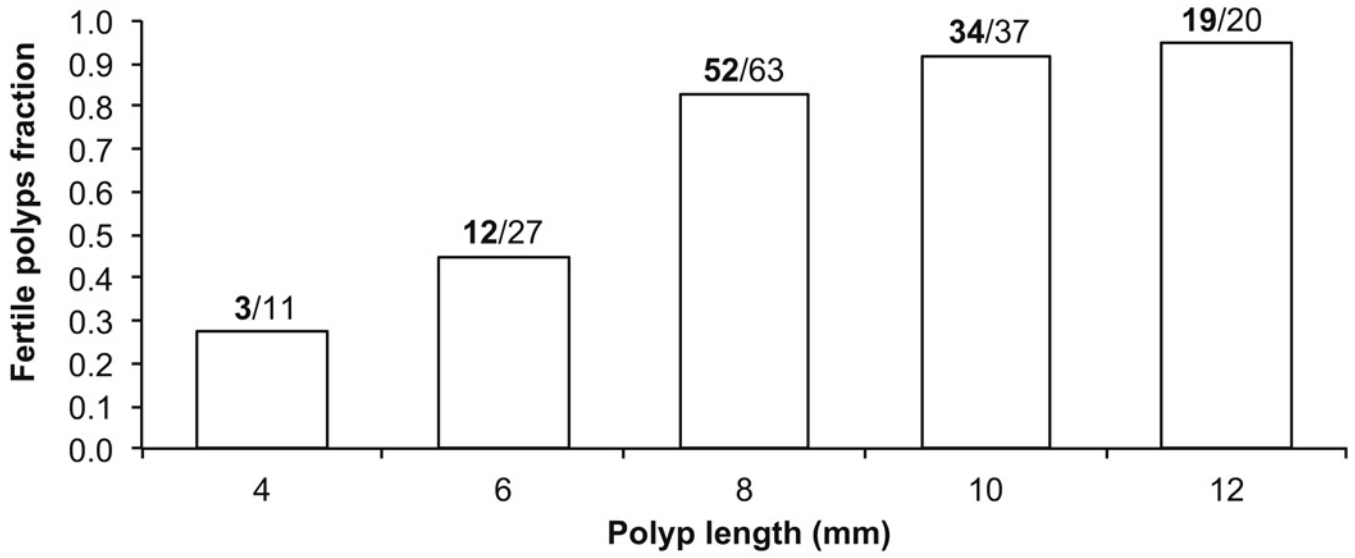

Fig 5. Fraction of fertile individuals per size class (mm). Fraction of fertile individuals per size class in millimeters, collected at Elba Isle. The values above the bars indicate the number of sexually mature polyps (bold) out of the number of polyps analyzed per size class (N = 158).

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bermudensis [70] brood embryos genetically identical to the parent. The same pattern of embryogenesis was observed in the temperate *A*. *equina*, which displays asexual brooded embryos while undergoing a (regular) gametogenetic cycle and reveals genetic variation at isozyme loci, providing clear evidence that sexual reproduction also occurs [71–74]. However, to date, none of these species have been shown to use sexual reproduction to produce brooded larvae. Instead, sexual larvae could be generated by broadcast spawning and external fertilization [72,74–76], probably to produce widely dispersed planktonic progeny [7].

Reaching sexual maturity is a process which depends on size and age of the organism and is one of the main components of reproductive biology [77]. *C. inornata* reached sexual maturity between 6 and 8 mm in length. The fraction distribution of sexually mature individuals has a bell-like shape, where both smaller size and larger size individuals tended to not produce germ cells. Smaller polyps may be immature individuals without the ability to produce gametes, while larger polyps may be sexually old individuals that preserve the ability to produce agamic embryos. In fact, it is possible that this species, after reaching a certain size/age, is affected by senescence [46] leading to a progressive decline in metabolic functions and to an increase in the mortality rate [78]. This phenomenon was demonstrated for the colonial coral *Stylophora pistillata* which shows a significant decrease in the rate of reproduction a few months before the natural death of the colony [79]. However, this hypothesis has to be taken cautiously because sexually inactive individuals with embryos in *C. inornata* were not significantly larger than the embryogenetic sexually active ones. Further studies on reproductive senescence are needed to clarify this peculiar aspect.

Annual reproductive cycle

The size frequency distribution of spermaries observed during monthly samples suggests that spermatogenesis of *Caryophyllia inornata* follows an annual cycle, where male germ cells require about 12 months to mature. A similar spermatogenesis has been documented, within

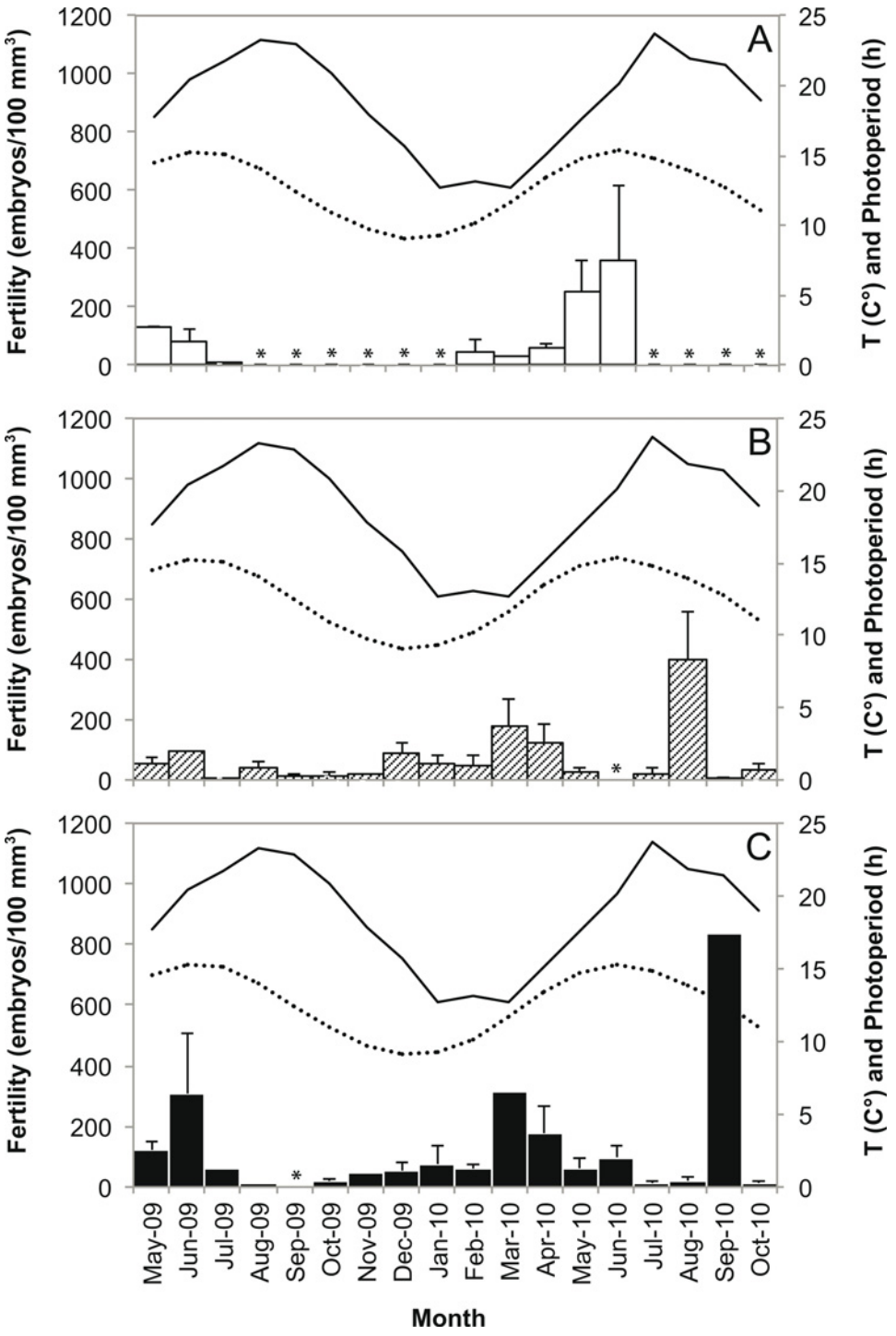

Fig 6. Diagram of the relationship between environmental parameters and fertility. Diagram of the relationship between water temperature (solid line), photoperiod (dotted line) and monthly mean fertility (bars) of females (A), sexually inactive individuals (B) and males (C) from May 2009 to October 2010. Error bars are standard errors (SE). * Reproductive state not detected.

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the Caryophylliidae family, for the deep coral *Lophelia pertusa* in Norway [62]. On the other hand, oocytes were present in only 5–6 months, showing a shorter oogenesis than *L. pertusa*

(13–14 months in duration, with one or two months overlapping between cycles) and shorter than the other temperate scleractinians whose reproductive cycle has been studied in detail: *Balanophyllia europaea* [37], *Leptopsammia pruvoti* [40], *Astroides calycularis* [44]. These three species display an oogenesis of about 24 months with an overlap of the gametogenetic cycle. Is not unusual for scleractinian gametogenetic cycle to differ between males and females but the general trend is a much longer oogenesis [11,21,40,80,81] which needs more time and energetic investment with respect to spermatogenesis [37,82].

Our results showed that the annual reproductive cycle of *C. inornata* is characterized by oogenetic development and fertilization that take place between February and July and appears to be strongly influenced by seasonal variation in photoperiod and water temperature. The increase of photoperiod and water temperature during the spring and early summer coincides with the maximum development of the gonads and might be a potential cue for sperm release and oocytes fertilization. Variations in seawater temperature are often mentioned as an important phenomenon that controls gametogenetic cycles and planula release in many anthozoans [17,21–25,83]. Fewer studies have been shown that even photoperiod could be involved in the reproduction processes [81,84–86]. Histological techniques do not allow to detect with reasonable accuracy the planulation patterns in *C. inornata*. However, the population shows decreased fertility in July, which could indicate the release into the environment of planulae derived from the previous period of fertilization (sexual planulae) and, therefore, a rather short maturation period of planulae. The timing of maturation of sexual planulae is usually of the order of several months, 1–4 months for *L. pruvoti* [2] and 4–5 months for *B. europaea* [37,38]. In *B. elegans*, embryos require 14–15 months of development, presenting an equally long oogenesis [87].

Size of mature oocytes and fecundity

In order to make a comparison within the genus, it has been considered the maximum oocyte size which was greater in *Caryophyllia inornata* (382 μm) than in *C. smithii* (150 μm) [88]. On the other hand, the maximum oocytes size of the deep species *C. sequenzae* (450 μm) and *C. ambrosia* (700 μm) was greater than *C. inornata*, while *C. cornuformis* was approximately the same size (350 μm) [10]. Within the genus *Caryophyllia*, the size of mature oocytes could increase with the increase of depth [10]. Large oocytes and subsequent lecitotrophic development are currently recognized as an adaptation to environments such as the oligotrophic abyss [89]. The larval development mode has not yet been determined for *C. inornata*, but the small size of oocytes (12–382 μm) could suggests a planktotrophic development of the larvae that generally have a rather long pelagic larval phase and a marked ability to disperse [90].

All the oocytes of *C. inornata* were considered potentially fertilizable (therefore mature) as we observed their disappearance after fertilization with oogenesis restricted to a short period of time (February-July). This contrasts with the Mediterranean coral *Leptopsammia pruvoti* whose reproductive cycle has been extensively studied. Fecundity of *L. pruvoti* was estimated considering only mature oocytes (size $>$ 340 μm) since two distinct stocks of oocytes are present, resulting in thousands of times lower (20.2 mature oocytes) fecundity than in *C. inornata* [40]. These results suggest that our species tends to produce many small oocytes concentrated in a few months a year.

Fertility

Caryophyllia inornata was fertile between 6 and 8 mm in length, the same size of sexual maturity. However, it is noteworthy that in the smaller size (between 4 and 6 mm) almost 50% of polyps was able to produce embryos, while less than 30% of the same size class was sexually

mature. It is therefore likely that this species begins to produce embryos before producing germ cells, suggesting again a possible agamic production of brooded embryos. In contrast to the "bell shaped" distribution of sexually mature individuals, the distribution of fertile (embryogenenetic) individuals showed an increasing trend, suggesting that larger/older polyps maintain their ability to produce embryos even without sexual reproduction. Combosch and Vollmer [6] found that bigger colonies of *Pocillopora damicornis* reproduce more asexually than smaller colonies, leading to increased recruitment and survival of the successful genotypes in larval cohorts.

In *C. inornata*, 87% of males and 66% of sexually inactive individuals had embryos at different stages of maturation (66% of total individuals). The production of embryos by these individuals was not related with seasonal variations in water temperature and photoperiod. In fact, these embryogenetic polyps showed all stages of embryo development throughout the year [46]. Embryogenetic sexually inactive individuals, that strongly characterize this population, might be: i) a third reproductive state that reproduces only agamically; ii) sexually old individuals (as observed in *Stylophora pistillata*) [79], with the ability to produce agamic embryos; iii) quiescent males during the months immediately following the fertilization period; iv) cryptic females within the group of sexually inactive individuals. In fact, the high proportion of this group raises the possibility that females could be present in the same abundance as the sexually inactive individuals, but that their gametes develop in a shorter period (5–6 month per year). The sea anemones *Actinia equina*, *A. tenebrosa* and *A. bermudensis* show similarities with *C. inornata* as their populations are characterized by embryogenetic females, embryogenetic males, and embryogenetic sexually inactive individuals that brood embryos throughout the year [69,70,74]. It has been hypothesized that these anemones present a rapid sequential hermaphroditism, producing sexual embryos as females, and continue to brood while they switch rapidly (relative to the duration of brooding) into males, passing through an intermediate sexually inactive phase [91]. However, molecular studies and laboratory experiments demonstrate that embryos inside males and sexually inactive individuals may be produced by some form of agamic internal budding [14,15,18,69,72,73,75,92].

The continuous and high fertility of *C. inornata* in the study area, on the order of about a hundred embryos per polyp, might partially be due to asexual production of planulae, making this species a successful colonizer. As such, the small oocytes and the consequent planktotrophic development may favor the dispersal and colonization of distant areas. However, the effect of habitat stability and varying levels of disturbance on sexual and asexual reproduction might be more complex [15].

Summarizing, *C. inornata* was sexually mature and produced embryos between 6–8 mm in length. Gametogenesis was influenced by temperature and photoperiod and was characterized by a rapid oogenesis. *C. inornata* showed small oocytes and high fecundity. In contrast to gametogenesis, fertility did not show a seasonal trend since embryos were found in females, males and sexually inactive individuals throughout the year, suggesting an agamic origin of the embryos. Further analysis with molecular markers such as hypervariable microsatellites are needed to confirm a possible asexual production of brooded embryos in *C. inornata* at Elba Isle. Although several studies on the production of brooded embryos have been carried out, the precise nature of this reproductive mode is still unknown.

Supporting Information

S1 Fig. Living specimens of *Caryophyllia inornata* photographed at Elba Isle (42°45'N, 10° 24'E).
(EPS)

S1 Dataset. Full overview of the raw data used for this study. Biometric measurements (length, width, height and volume), reproductive state, oocytes fecundity/spermaries abundance, gonadal index and fertility for each polyp analyzed (N = 158).
(XLSX)

S2 Dataset. Environmental data used for this study. Average monthly water temperature (°C) and photoperiod (h) from May 2009 to October 2010 at Elba Isle.
(XLS)

S1 Results. Data used to generate Fig 1. Monthly frequency of the 5 reproductive states characterizing the population of Elba Isle. See figure legend in the manuscript.
(XLSX)

S2 Results. Data used to generate Figs 2 and 5. Fig 2. Fraction of sexually mature individuals per size class in millimeters. See figure legend in the manuscript. Fig 5. Fraction of fertile individuals per size class in millimeters. See figure legend in the manuscript.
(XLS)

S3 Results. Data used to generate Fig 3. Size-frequency distribution of oocytes and of the five stages of spermary maturation in monthly samples. See figure legend in the manuscript.
(XLS)

S4 Results. Data used to generate Fig 4. Variation in water temperature and photoperiod, gamete development (monthly mean + SE) and total fertility (monthly mean + SE). See figure legend in the manuscript.
(XLS)

S5 Results. Data used to generate Figs 4 and 6. Fig 4. See figure legend in the manuscript. Fig 6. Relationship between water temperature, photoperiod and monthly mean fertility (+SE) of each reproductive state. See figure legend in the manuscript.
(XLSX)

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Author Contributions

Conceived and designed the experiments: SG. Performed the experiments: CM SG. Analyzed the data: CM VA RF GT MR. Contributed reagents/materials/analysis tools: SG. Wrote the paper: CM VA GF OL ZD SG. Gave conceptual advice: GF OL ZD.

References

1. Fiorillo I, Rossi S, Alva VJ, Gili M, Lopez-Gonzalez PJ. Seasonal cycle of sexual reproduction of the Mediterranean soft coral *Alcyonium acaule* (Anthozoa, Octocorallia). Mar Biol. 2013; 160:719–728.

2. Goffredo S, Radetic J, Airi V, Zaccanti F. Sexual reproduction of the solitary sunset cup coral *Leptopsammia pruvoti* (Scleractinia, Dendrophyllidae) in the Mediterranean. 1. Morphological aspects of gametogenesis and ontogenesis. Mar Biol. 2005; 147:485–495.
3. Baird AH, Guest JR, Willis BL. Systematic and biogeographical patterns in the reproductive biology of scleractinian corals. Annu Rev Ecol Evol Syst. 2009; 40:551–571.
4. Harrison PL. Sexual reproduction of scleractinian corals. In: Dubinsky Z, Stambler N (eds) Coral reefs: an ecosystem in transition. Springer, Netherlands; 2011. pp. 59–85.
5. Pinzón JH, Reyes-Bonilla H, Baums IB, LaJeunesse TC. Contrasting clonal structure among *Pocillopora* (Scleractinia) communities at two environmentally distinct sites in the Gulf of California. Coral Reefs. 2012; 31:765–777.
6. Combosch DJ, Vollmer SV. Mixed asexual and sexual reproduction in the Indo-Pacific reef coral *Pocillopora damicornis*. Ecol Evol. 2013; 3:3379–3387. doi: 10.1002/ece3.721 PMID: 24223276
7. Williams GC. Sex and evolution. Princeton University Press, Princeton; 1975.
8. Maynard-Smith J. The evolution of sex. Cambridge University Press, Cambridge; 1978.
9. Sherman CDH, Ayre DJ, Miller KJ. Asexual reproduction does not produce clonal populations of the brooding coral *Pocillopora damicornis* on the Great Barrier Reef, Australia. Coral Reefs. 2006; 25:7–18.
10. Waller GR, Tyler PA, Gage JD. Sexual reproduction in three hermaphroditic deep-sea *Caryophyllia* species (Anthozoa: Scleractinia) from the NE Atlantic Ocean. Coral Reefs. 2005; 24:594–602.
11. Kramarsky-Winter E, Loya Y. Reproductive strategies of two fungiid corals from the northern Red Sea: environmental constraints? Mar Ecol Prog Ser. 1998; 174:175–182.
12. Loya Y, Sakai K. Bidirectional sex change in mushroom stony corals. Proc R Soc Biol Sci Ser B. 2008; 275:2335–2343.
13. Stoddart JA. Asexual production of planulae in the coral *Pocillopora damicornis*. Mar Biol. 1983; 76:279–284.
14. Ayre DJ, Miller KJ. Where do clonal larvae go? Adult genotypic diversity conflicts with reproductive effort in the brooding coral *Pocillopora damicornis*. Mar Ecol Prog Ser. 2004; 277:95–105.
15. Yeoh SR, Dai CF. The production of sexual and asexual larvae within single broods of the scleractinian coral, *Pocillopora damicornis*. Mar Biol. 2010; 157:351–359.
16. Ayre DJ, Resing JM. Sexual and asexual production of planulae in reef corals. Mar Biol. 1986; 90:187–190.
17. Glynn PW, Colley SB, Mate JL, Corte´s J, Guzman HM, Bailey RL. Reproductive ecology of the azoxanthellate coral *Tubastraea coccinea* in the Equatorial Eastern Pacific: Part V. Dendrophylliidae. Mar Biol. 2008; 153:529–44.
18. Nakano Y, Yamazoto K. Ecological study of reproduction of *Oulastrea crispata* in Okinawa. Behav Biol Ecol. 1992; 9:1292.
19. Lam KKY. Sexual reproduction of a low-temperature tolerant coral *Oulastrea crispata* (Scleractinia: Faviidae) in Hong Kong, China. Mar Ecol Prog Ser. 2000; 205:101–111.
20. Sammarco PW, Atchison AD, Boland GS. Expansion of coral communities within the Northern Gulf of Mexico via offshore oil and gas platforms. Mar Ecol Prog Ser. 2004; 280:129–143.
21. Harrison PL, Wallace CC. Coral Reefs. Ecosystems of the world. Elsevier, Amsterdam; 1990.
22. Richmond RH, Hunter CL. Reproduction and recruitment of corals: comparisons among the Caribbean, the tropical Pacific, and the Red Sea. Mar Ecol Prog Ser. 1990; 60:185–203.
23. Colley SB, Feingold JS, Pena J, Glynn PW. Reproductive ecology of *Diaseris distorta* (Michelin) (Fungiidae) in the Galapagos Islands, Ecuador. Proc 9th Int Coral Reef Symp. 2000; 1:373–379.
24. Beauchamp KA. Gametogenesis, brooding and planulation in laboratory populations of a temperate scleractinian coral *Balanophyllia elegans* maintained under contrasting photoperiod regimes. Invertebr Reprod Dev. 1993; 23:171–182.
25. Steiner SC. Spawning in scleractinian corals from SW Puerto Rico (West Indies). Bull Mar Sci. 1995; 56:899–902.
26. Fadlallah YH. Sexual reproduction, development and larval biology in scleractinian corals. Coral Reefs. 1983; 2:129–150.
27. Szmant AM. Reproductive ecology of Caribbean reef corals. Coral Reefs. 1986; 5:43–54.
28. Babcock RC, Bull GD, Harrison PL, Heyward AJ, Oliver JK, Wallace CC, Willis BL. Synchronous spawning of 105 scleractinian coral species on the Great Barrier Reef. Mar Biol. 1986; 90:379–394.
29. Kramarsky-Winter E, Fine M, Loya Y. Coral polyp expulsion. Nature. 1997; 387:137–137.

30. Shlesinger Y, Goulet TL, Loya Y. Reproductive patterns of scleractinian corals in the northern Red Sea. Mar Biol. 1998; 132:691–701.

31. Ward S, Harrison P, Hoegh-Gulberg O. Coral bleaching reduced reproduction of scleractinian corals and increases their susceptibility to future stress. Proc 9th Int Coral Reef Symp. 2002; 2:1123–1128.

32. Levy O, Appelbaum L, Leggat W, Gothlif Y, Hayward DC, Miller DJ, Hoegh-Guldberg O. Light-responsive cryptochromes from a simple multicellular animal, the coral *Acropora millepora*. Science. 2007; 318:467–470. PMID: 17947585

33. Okubo N, Mezaki T, Nozawa Y, Nakano Y, Lien Y-T, Fukami H, et al. Comparative Embryology of Eleven Species of Stony Corals (Scleractinia). PLoS ONE. 2013; 8:e84115. doi: 10.1371/journal.pone.0084115 PMID: 24367633

34. Guest JR., Baird AH, Goh BPL, Chou LM. Sexual systems in scleractinian corals: an unusual pattern in the reef-building species *Diploastrea heliopora*. Coral Reefs. 2012; 31:705–713.

35. Goffredo S, Telò T. Hermaphroditism and brooding in the solitary coral *Balanophyllia europaea* (Cnidaria, Anthozoa, Scleractinia). Ital J Zool. 1998; 65:159–165.

36. Goffredo S, Telò T, Scanabissi F. Ultrastructural observations of the spermatogenesis of the hermaphroditic solitary coral *Balanophyllia europaea* (Anthozoa, Scleractinia). Zoomorphology. 2000; 119:231–240.

37. Goffredo S, Arnone S, Zaccanti F. Sexual reproduction in the Mediterranean solitary coral *Balanophyllia europaea* (Scleractinia, Dendrophylliidae). Mar Ecol Prog Ser. 2002; 229:83–94.

38. Goffredo S, Zaccanti F. Laboratory observations on larval behaviour and metamorphosis in the Mediterranean solitary coral *Balanophyllia europaea* (Scleractinia, Dendrophylliidae). Bull Mar Sci. 2004; 74:449–458.

39. Airi V, Gizzi F, Falini G, Levy O, Dubinsky Z, Goffredo S. Reproductive efficiency of a Mediterranean endemic zooxanthellate coral decreases with increasing temperature along a wide latitudinal gradient. PloS ONE. 2014; 9:e91792. doi: 10.1371/journal.pone.0091792 PMID: 24618568

40. Goffredo S, Airi V, Radetic J, Zaccanti F. Sexual reproduction of the solitary sunset cup coral *Leptopsammia pruvoti* (Scleractinia, Dendrophylliidae) in the Mediterranean. 2. Quantitative aspects of the annual reproductive cycle. Mar Biol. 2006; 148:923–932.

41. Kružić P, Zuljevic A, Nikolic V. Spawning of the colonial coral *Cladocora caespitosa* (Anthozoa, Scleractinia) in the Southern Adriatic sea. Coral Reefs. 2008; 27:337–341.

42. Kersting DK, Casado C, López-Legentil S, Linares C. Unexpected patterns in the sexual reproduction of the Mediterranean scleractinian coral *Cladocora caespitosa*. Mar Ecol Prog Ser. 2013; 486:165–171.

43. Goffredo S, Gasparini G, Marconi G, Putignano MT, PazziniC, Zaccanti F. Gonochorism and planula brooding in the Mediterranean endemic orange coral *Astroides calycularis* (Scleractinia: Dendrophylliidae). Morphological aspects of gametogenesis and ontogenesis. Mar Biol Res. 2010; 6:421–436.

44. Goffredo S, Gasparini G, Marconi G, Putignano MT, Pazzini C, Airi V, et al. Sexual reproduction in the Mediterranean endemic orange coral, *Astroides calycularis* (Scleractinia, Dendrophylliidae). Bull Mar Sci. 2011; 87:589–604.

45. Casado-Amezùa P, Gasparini G, Goffredo S. Phenological and morphological variations in the Mediterranean orange coral *Astroides calycularis* between two distant localities. Zoology. 2013; 116:159–167. doi: 10.1016/j.zool.2012.10.005 PMID: 23578936

46. Goffredo S, Marchini C, Rocchi M, Airi V, Caroselli E, Falini G, et al. Unusual pattern of embryogenesis of *Caryophyllia inornata* (scleractinia, caryophylliidae) in the Mediterranean Sea: Maybe agamic reproduction? J Morphol. 2012; 273:943–956. doi: 10.1002/jmor.20039 PMID: 22707320

47. Zibrowius H. Les scléractiniaires de la Méditerranée et del'Atlantique nord-oriental. Mem Inst Oceanogr. 1980; 11:1–284.

48. Cairns SD. Species richness of recent scleractinia. Atoll Res Bull. 1999; 459:1–46.

49. Zibrowius H. Les scléractiniaires des grottes sous-marines en Méditerranée et dans l'Atlantique nord-oriental (Portugal, Madére, Canaries, Açores). Mar Ecol. 1978; 40:516–545.

50. Caroselli E, Nanni V, Levy O, Falini G, Dubinsky Z, Goffredo S. Latitudinal variations in biometry and population density of a Mediterranean solitary coral. Limnol Oceanogr. 2015; 60:1356–1370.

51. Rinkevich B, Loya Y. The reproduction of the Red Sea coral *Stylophora pistillata*. II. Synchronization in breeding and seasonality of planulae shedding. Mar Ecol Prog Ser. 1979; 1:145–152.

52. Szmant-Froelich AM, Yevich P, Pilson MEQ. Gametogenesis and early development of the temperate coral *Astrangia danae* (Anthozoa: Scleractinia). Biol Bull. 1980; 158:257–269.

53. Kruger A, Schleyer MH. Sexual reproduction in the coral *Pocillopora verrucosa* (Cnidaria: Scleractinia) in KwaZulu-Natal, South Africa. Mar Biol. 1998; 132:703–710.

54. Glynn PW, Colley SB, Ting JH, Mate JL, Guzman HM. Reef coral reproduction in the eastern Pacific: Costa Rica, Panama and Galapagos Islands (Ecuador). IV. Agariciidae, recruitment and recovery of *Pavona varians* and *Pavona* sp.a. Mar Biol. 2000; 136:785–805.

55. Gilmour JP. Substantial asexual recruitment of mushroom corals contributes little to population genetics of adults in conditions of chronic sedimentation. Mar Ecol Prog Ser. 2002; 235:81–91.

56. Benayahu Y, Loya Y. Substratum preferences and planulae settlement of two Red Sea Alcyonaceans: *Xenia macrospiculata* Gohar and *Parerythropodium fulvum fulvum* (Forskal). Mar Biol Ecol. 1984; 83:249–261.

57. Farrant PA. Gonad development and the planulae of the temperate Australian soft coral *Capnella gaboensis*. Mar Biol. 1986; 92:381–392.

58. Brazeau DA, Lasker HR. Sexual reproduction and external brooding by the Caribbean gorgonian *Briareum asbestinum*. Mar Biol. 1990; 104:465–474.

59. Cairns SD. The deep-water Scleractinia of the Caribbean Sea and adjacent waters. Stud Fauna Curacao. 1979; 67:1–341.

60. Cairns SD. The marine fauna of New Zealand: Scleractinia (Cnidaria: Anthozoa). New Zealand Oceanographic Institute Memoirs; 1995. pp. 103:210.

61. Roberts JM, Wheeler AJ, Freiwald A, Cairns SD. Coldwater corals: the biology and geology of deep-sea coral habitats. Cambridge University Press, Cambridge; 2009.

62. Brooke S, Järnegren J. Reproductive periodicity of the scleractinian coral *Lophelia pertusa* from the Trondheim Fjord, Norway. Mar Biol. 2013; 160:139–153.

63. Stoddart JA. Genetic differentiation among populations of the coral *Pocillopora damicornis* off southwestern Australia. Coral Reefs. 1984; 3:149–156.

64. Ayre DJ, Hughes TP, Standish RS. Genetic differentiation, reproductive mode, and gene flow in the brooding coral *Pocillopora damicornis* along the Great Barrier Reef, Australia. Mar Ecol Prog Ser. 1997; 159:175–187.

65. Adjeroud M, Tsuchiya M. Genetic variation and clonal structure in the scleractinian coral *Pocillopora damicornis* in the Ryukyu Archipelago, southern Japan. Mar Biol. 1999; 134:753–760.

66. Miller KJ, Ayre DJ. The role of sexual and asexual reproduction in structuring high latitude populations of the reef coral *Pocillopora damicornis*. Heredity. 2004; 92:557–568. PMID: 15100708

67. Whitaker K. Genetic evidence for mixed modes of reproduction in the coral *Pocillopora damicornis* and its effect on population structure. Mar Ecol Prog Ser. 2006; 306:115–124.

68. Ayre DJ. The effects of asexual reproduction and inter-genotypic aggression on the genotypic structure of populations of the sea anemone *Actinia tenebrosa*. Oecologia. 1983; 57:158–165.

69. Sherman CD, Peucker AJ, Ayre DJ. Do reproductive tactics vary with habitat heterogeneity in the intertidal sea anemone *Actinia tenebrosa*? J Exp Mar Biol Ecol. 2007; 340:259–267.

70. Monteiro FA, Russo CA, Solé-Cava AM. Genetic evidence for the asexual origin of small individuals found in the coelenteron of the sea anemone *Actinia bermudensis* McMurrich. Bull Mar Sci. 1998; 63:257–264.

71. Carter MA, Thorpe JP. Reproductive, genetic and ecological evidence that *Actinia equina* var. *mesembryanthemum* and var. *fragacea* are not conspecific. J Mar Biol Assoc UK. 1981; 61:79–93.

72. Orr J, Thorpe JP, Carter MA. Actinia equina. Mar Ecol Prog Ser. 1982; 7:227–229.

73. Carter MA, Miles J. Gametogenic cycles and reproduction in the beadlet sea anemone *Actinia equina* (Cnidaria: Anthozoa). Biol J Linn Soc Lond. 1989; 36:129–155.

74. Perrin MC, Thorpe J, Sole-Cava AM. Population structuring, gene dispersal and reproduction in the *Actinia equina* species group. Oceanogr Mar Biol Annu Rev. 1999; 37:129.

75. Carter MA, Thorp CH. Reproduction of *Actinia equina* var. *mesembryanthemum*. J Mar Biol Assoc UK. 1979; 59:989–1001.

76. Monteiro FA, Solé-Cava AM, Thorpe JP. Extensive genetic divergence between populations of the common intertidal sea anemone *Actinia equina* from Britain, the Mediterranean and the Cape Verde Islands. Mar Biol. 1997; 129:425–433.

77. Fujiwara M, Caswell H. Demography of endangered North Atlantic right whale. Nature. 2001; 414:537–541. PMID: 11734852

78. Bosch TC. Hydra and the evolution of stem cells. BioEssays. 2009; 31:478–486. doi: 10.1002/bies.200800183 PMID: 19274660

79. Rinkevich B, Loya Y. Senescence and dying signals in a reef building coral. Experentia. 1986; 42:320–322.

80. Fan TY, Dai CF. Reproductive ecology of the scleractinian coral *Echinopora lamellosa* in northern and southern Taiwan. Mar Biol. 1995; 123:565–572.

81. Acosta A, Zea S. Sexual reproduction of the reef coral *Montastrea cavernosa* (Scleractinia: Faviidae) in the Santa Marta area, Caribbean coast of Colombia. Mar Biol. 1997; 128:141–148.

82. Baillon S, Hamel JF, Mercier A. Comparative study of reproductive synchrony at various scales in deep-sea echinoderms. Deep-sea Res I. 2011; 58:260–272.

83. Clayton PD, Collins DJ. Reproduction and feeding ethology of a tropical, intertidal sand-dwelling anemone (*Actinoporus selongatus*, Carlgren, 1900). Hydrob. 1992; 237:31–38.

84. Babcock RC, Wills BL, Simpson CJ. Mass spawning of corals on high latitude coral reef. Coral Reefs. 1994; 13:161–169.

85. Soong K. Sexual reproductive patterns of shallow-water reef corals in Panama. Bull Mar Sci. 1991; 49:832–846.

86. Penland L, Kloulechad J, Idip D, Woesik R. Coral spawning in the western Pacific Ocean is related to solar insolation: evidence of multiple spawning events in Palau. Coral Reefs. 2004; 23:133–140.

87. Fadlallah YH, Pearse JS. Sexual reproduction in solitary corals: overlapping oogenic and brooding cycles, and benthic planulas in *Balanophyllia elegans*. Mar Biol. 1982; 71:223–231.

88. Tranter PRG, Nicholson DN, Kinchington D. A description of the spawning and post-gastrula development of the cool temperate coral *Caryophyllia smithii* (Stokes and Broderip). J Mar Biol Assoc UK. 1982; 62:845–854.

89. Shilling FM, Manahan DT. Energy metabolism and amino acid transport during early development of Antarctic and temperate echinoderms. Biol Bull. 1994; 187:398–407.

90. Pechenik JA. On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. Mar Ecol Prog Ser. 1999; 177:269–297.

91. Ottaway JR. Population ecology of the Intertidal Anemone *Actinia tenebrosa* II Geographical distribution, synonymy, reproductive cycle and fecundity. Aust J Zool. 1979; 27:273–90.

92. Carter MA, Funnell ME. Reproduction and brooding in *Actinia*. Development and cellular biology of coelenterates. Elsevier North Holland Inc, New York; 1980.

Chapter III

Reproduction of the solitary coral *Caryophyllia inornata* is unaffected along an 850 km gradient on the western Italian coast

Manuscript in preparation

Reproduction of the solitary coral *Caryophyllia inornata* is unaffected along an 850 km gradient on the western Italian coast

Abstract

The main greenhouse gas produced by human activity is carbon dioxide that traps some of the thermal energy, causing an increase in global surface temperature. The IPCC predicted that ocean surface temperature will rise of 0.6-2.0 °C by 2100. Ocean warming is expected to produce strong impacts on marine ecosystems such as coral reefs, affecting reproductive processes. This study examined variations in reproductive output in relation to seawater temperature and solar radiation along a wide latitudinal gradient on the western Italian coast, in the non-zooxanthellate Mediterranean coral, *Caryophyllia inornata*. Fecundity, spermary abundance, gonadal index and fertility in females, males and sexually inactive individuals were homogeneous along the latitudinal gradient. The non-zooxanthellate species *C. inornata* seems quite tolerant to environmental changes, similarly to the non-zooxanthellate *Leptopsammia pruvoti*, probably because the lack of zooxanthellae would make them less dependent from environmental parameters as solar radiation and temperature.

Introduction

Climate change is today one of the most serious threats to the biodiversity of our planet. Since the Industrial revolution, the human use of fossil fuels, deforestation and the massive advent of the intensive agriculture have dramatically increased the concentration of greenhouse gases in the atmosphere (Sabine et al. 2004). The main greenhouse gas produced by human activity is carbon dioxide (Solomon et al. 2007), which currently has an accumulation rate in the atmosphere that has never been recorded (Kump et al. 2009). Greenhouse gases trap some of the thermal energy, causing an increase in global mean temperature (Harley et al. 2006) and, consequently, in ocean surface temperature that has risen of 0.4-0.8 °C over the last century (Harley et al. 2006). If the anthropogenic emissions keep growing, a further increase of 0.6-2.0 °C is expected by 2100 (Stocker et al. 2013). According to the Intergovernmental Panel on Climate Change (IPCC), ocean warming is producing profound impacts on marine ecosystems, which are among the most ecologically and socio-economically vital on the planet (Harley et al. 2006).

Climatic models predict greater influence of the global warming in the temperate areas than tropical ones (Solomon et al. 2007). In particular, the small enclosed Mediterranean Sea that is considered a 'biodiversity hotspot', is one of the most strongly affected regions by increasing temperature (Field et al. 2012). Indeed, the warming rates of the Mediterranean Sea are three times higher than those of the oceans (Solomon et al. 2007; Vargas-Yáñez et al. 2007). These traits make the Mediterranean Sea one of the most emblematic natural model for studying the interactions between marine life and environmental changes (Feely et al. 2004; Lejeusne et al. 2010).

The increase in seawater temperature will probably have an impact on the biology of coral populations, reducing their reproductive capacity (Baird and Marshall 2002). In fact reproduction, which is steadily exposed to environmental cues, is considered the most sensitive stage of the life cycle and can provide information on how organisms react to stress (Harrison and Wallace 1990; Ward 1995; van Woesik 2009). The reproductive cycle of corals can be affected by ocean warming through reduction of fertility, eggs quality, fertilization and recruitment success, threatening the ability of corals to recover after disturbances (Albright and Mason 2013; Linares et al. 2008). Although fertilization of corals is highly sensitive to water chemistry, some studies indicates that this process in many species can be resistant to near-future ocean warming, suggesting an acclimatization or adaptation to high temperature stress (van Oppen et al. 2015). In general, embryos and larvae were found to be less thermotolerant than gametes and fertilization (Byrne 2011).

While several studies show the effects of ocean warming on sexual reproduction of tropical scleractinians (Harriot 1983; Bassim et al. 2002; Krupp et al. 2006; Nozawa and Harrison 2007, Negri et al. 2007), only a few investigated the response at elevated temperatures in temperate corals and even less in the non-zooxanthellate ones (Airi et al. 2014; Airi *submitted*).

The present study examined the non-zooxanthellate solitary coral *Caryophyllia inornata* (Fig 1), which is widely distributed in the eastern and western Mediterranean Sea (Zibrowius 1980), extending to the northeast of the Atlantic coast (Cairns 1999), and from the Canary Islands to the southern coasts of England (Zibrowius 1980). Its population density can reach thousands of individuals per m^2, varying from 100 to 1500 individuals per m^2 along the western Italian coasts (Caroselli et al. 2015). *Caryophyllia inornata* is gonochoric and brooding (Goffredo et al 2012, Marchini et al. 2015), showing peculiar traits such as a male biased sex

ratio (1:3; Goffredo et al. 2012) and the presence of embryos in coelenteric cavity and mesenteric septa of females, males and sexually inactive individuals (without germ cells), throughout the year (Marchini et al. 2015). Previous studies reveal that temperature and solar radiation not affect population density, growth parameters, net calcification rate, bulk skeletal density and linear extension rate of *C. inornata* (Caroselli et al. 2015; Caroselli et al. 2016a; Caroselli et al. 2016b), while variations have been observed in population dynamics with increasing solar radiation (Caroselli et al. 2016a). The specific aim of this work was to quantify the reproductive output of *Caryophyllia inornata* along a latitudinal gradient of temperature and solar radiation in face of ocean warming.

Materials and Methods

Samples collection and environmental parameters

Specimens of *Caryophyllia inornata* were collected from five sites along a latitudinal gradient, from 44°20'N to 36°46'N (Fig 2). Coral collection occurs from June 2010 to November 2012. During this period, 18 monthly samplings were performed for four populations (Genova: April 2011-September 2012; Calafuria: February 2011-July 2012; Scilla: July 2010-November 2012; Pantelleria: September 2010-November 2012), with a minimum of 15 polyps collected during each excursion. Data from Elba Island population came from a previous study (Marchini et al. 2015) for which samples were collected between May 2009 and October 2010.

Depth Temperature (DT; °C) was measured by digital thermometers (I-Button DS1921H, Maxim Integrated Products), placed at the sampling location for each population. Sensors recorded seawater temperature during the entire experimental period. Sea Surface Temperature data (SST; °C) for each site were recorded hourly from the National Mareographic Network of the Institute for the Environmental Protection and Research (ISPRA, available to

http://www.mareografico.it). These data were measured by mareographic stations, (SM3810 manufactured by the Society for the Environmental and Industrial monitoring; SIAP+MICROS), placed close to the sampling sites. For each location, a linear regression was obtained between DT and SST data to estimate historical at-depth temperatures of the three years preceding the sampling.

Solar radiation (W/m^2) was recorded from the archives of the Satellite Application Facility on Climate Monitoring (CM-SAF/EUMETSAT, available to http://www.cmsaf.eu), using real time data sets based on intersensor calibrated radiances from MFG satellites. Mean annual solar radiation of each site was obtained for the 15 km square associated with each of the five sites. As for temperature, also for solar radiation we considered the average of the three years preceding the sampling.

Biometric and hystological analysis

Biometric analyses were performed by measuring length (L, maximum axis of the oral disc), width (W, minimum axis of the oral disc) and height (h, oral–aboral axis) of each sampled polyp. The volume (V) of the individual polyp was calculated using the formula $V = h * (L/2) * (l/2) * \pi$ (Goffredo et al. 2002).

Polyps were post-fixed in Bouin solution to ensure a better fixation and staining of the tissues. After decalcification in EDTA and dehydration in a graded alcohol series from 80% to 100%, polyps were embedded in paraffin and serial transverse sections were cut at 7 μm intervals along the oral-aboral axis, from the oral to the aboral poles. Tissues were then stained with Mayer's haematoxylin and eosin (Goffredo et al. 2002; Marchini et al. 2015).

Cytohistometric analysis

Cytometric analyses were made with an optical microscope using the image analyzer NIKON NIS-Elements D 3.2. The maximum and minimum diameters of the oocytes in nucleated sections and spermaries were measured and the presence of embryos was recorded. Spermaries were classified into five developmental stages according to earlier studies on gametogenesis in scleractinians (Goffredo et al. 2005; Goffredo et al. 2012).

Definitions

Reproductive output was defined through four reproductive parameters: a) *fecundity rate* and *spermary abundance*, both defined as the number of reproductive elements per body volume unit (100 mm^3); b) *"gonadal" index*, defined as the percentage of body volume occupied by the germ cells; c) *reproductive element size*, defined as the average of the maximum and minimum diameter of spermaries and oocytes in nucleated section; d) *fertility,* defined as the number of embryos per body volume unit (100 mm^3; Marchini et al. 2015).

In accordance with the annual reproductive cycle described by Marchini et al. (2015), gametal development in *C. inornata* was characterized by two gametes activity periods. The *gametes recruitment period* (Korta et al. 2010; Lowerre-Barbieri et al 2011; Airi et al. 2014), occurring between February and April, was characterized by the development of early stages of oocytes and spermaries. The *gametes maturity period* (Korta et al. 2010; Lowerre-Barbieri et al 2011; Airi et al. 2014), between May and July, was characterized by the presence of larger oocytes and advanced stage of maturation of spermaries that reached maturity for fertilization.

Statistical analysis

A 2-sample Kolmogorov-Smirnov test was used to compare the stages/size-frequency distribution of reproductive elements between gametes recruitment and gametes maturity periods

for all populations (Olea and Pawlowsky-Glahn 2009). Data were tested for normality using a single Kolmogorov-Smirnov test and for homogeneity of variance or homoscedasticity using a Levene's test. When assumptions for parametric statistics were not fulfilled, a non-parametric test was used. The Kruskal-Wallis test is a non-parametric alternative to the analysis of variance (ANOVA) and is used to compare groups of means. The non-parametric Kruskal-Wallis test was used to compare reproductive parameters among study sites. Student's *t* test was used to compare the mean oocytes and spermaries size of populations between reproductive periods. The Mann-Whitney *U* test was used as a non-parametric alternative to the Student's *t* test. Spearman's rank correlation coefficient (ρ) was used to calculate the significance of the correlations between reproductive and environmental parameters. Spearman's rank correlation coefficient is an alternative used for data that do not meet the assumptions of Pearson's correlation coefficient (Altman 1991; Potvin and Roff 1993). All analyses were computed using SPSS 22.0 (Apache Computer Software Foundation).

Results

Mean annual depth temperature (DT; °C) and mean annual solar radiation (W/m²) were significantly different among sites (DT, Kruskal-Wallis, $p<0.05$; solar radiation, ANOVA, $p<0.001$; Table 1).

All populations showed gonochoric and sexually inactive polyps in both reproductive periods (Table 2).

Oocytes size/frequency distribution during February-April (gametes recruitment period) was significantly different from that of May-July (gametes maturity period), in all populations (Kolmogorov-Smirnov, $p<0.001$; Fig 3). The mean oocytes size was smaller than 100 μm in both reproductive periods and in all populations (Table 3; Fig 3).

The distribution of spermary maturation stages was significantly different between gametes recruitment and gametes maturity periods in all populations (Kolmogorov-Smirnov, $p<0.001$; Fig 4). From February to April (gametes recruitment period), each population was characterized by small spermaries belonging to the earliest maturation stages (stages I, II and III; Fig 4). During May-July (gametes maturity period) all populations were characterized by more advanced maturation stages (stage III, IV and V; Fig 4). The mean spermaries size during the gametes recruitment period was significantly lower than that of gametes maturity period in all populations (Mann-Whitney *U* test, $p<0.05$; Table 4; Fig 4).

The presence of embryos in the gastrovascular cavity and mesenterial septa were recorded throughout the year without significantly differences between reproductive periods, in females, males and sexually inactive individuals of all populations (Table 5; Fig 5).

Female fecundity and gonadal index were the same during both gametes recruitment and gametes maturity periods in all populations along the latitudinal gradient (Table S1; Figs S5 and S6). The mean oocytes diameter was significantly different during both reproductive periods among populations (Kruskal–Wallis test, $p<0.001$; Table S1; Figs S5 and S6). While in the gametes recruitment period the mean oocytes diameter was negatively correlated with the DT and the solar radiation, during gametes maturity period it was positively correlated with the same environmental parameters (Spearman correlation test, $p<0.01$; Table S1; Figs S5 and S6).

Male abundance and gonadal index did not change among populations in both reproductive periods (Table S2; Figs S7 and S8). Instead, the mean spermary diameter was significantly different during gametes recruitment and gametes maturity periods among populations (Kruskal–Wallis test, $p<0.001$; Table S2; Figs S7 and S8). In southern populations, males showed smaller

spermaries during February-April (Spearman correlation test, $p<0.01$; Table S2; Fig S7), and larger germ cells during May-July (Spearman correlation test, $p<0.05$; Table S2; Fig S8).

In both periods, fertility did not differ in females and inactive individuals in all populations along the gradient (Table S3; Figs S9 and S10). Only males fertility was significantly different among populations during gametes maturity period, without any correlation with solar radiation and DT variations (Kruskal–Wallis test, $p<0.05$; Table S3; Fig S10).

Discussion

All the populations of *C. inornata* analyzed along the latitudinal gradient displayed a rapid oogenesis, mainly represented by oocytes smaller than 100 μm, a spermatogenesis distinct in two reproductive periods (gametes recruitment and gametes maturity periods), the presence of sexually inactive individuals throughout the year and a reproductive cycle influenced by annual variation of seawater temperature and photoperiod, as previously reported for the population of Elba Island (Marchini et al. 2015). Relations between reproductive cycle and environmental conditions have been observed also for the temperate scleractinians *Balanophyllia europaea* (Goffredo et al. 2002), *Leptopsammia pruvoti* (Goffredo et al. 2006), *Astroides calycularis* (Goffredo et al. 2011), *Cladocora caespitosa* (Kružić et al. 2008; Kersting et al. 2013) and *Balanophyllia elegans* in the temperate waters of California (Fadlallah and Pearse 1982; Beauchamp 1993). At present, several studies assert that sexual reproduction of corals, including gametogenesis, gamete release, fertilization and planulation, depends on seasonal environmental cues (Babcock et al. 1986; Harrison and Wallace 1990; Penland et al. 2004; van Woesik et al. 2006). The main environmental factors involved in the control of reproductive processes are seawater temperature and solar radiation, as they may exert a significant influence on physiological processes (Harrison and Wallace 1990; Brown 1996). Despite mean solar radiation

and annual depth temperature were significantly different among sites, the seasonal trend of these parameters seems not produce temporal shifts of the reproductive cycle of *C. inornata*. Indeed, all populations showed a similar periodicity for gamete development during both gametes recruitment and maturity periods, suggesting an overlap in reproductive seasonality of *C. inornata* along the latitudinal gradient. Similarly, *B. europaea* and *L. pruvoti* exhibited an overlap in gonadal development stages among the same populations analyzed for this study (Airi et al. 2014; Airi et al. *submitted*).

It is important to note that *C. inornata* is characterized by a strong male biased sex ratio, thus, the analysis of the oocytes was challenging and performed on a small number of females. Polyps of *C. inornata* showed the same reproductive output in all the analyzed populations, since oocytes fecundity, spermary abundance, female and male gonadal index were not affected by increasing temperature and solar radiation (Supplementary Tables S1, S2 and Figures S1, S2, S3, S4). The absence of an evident relation with environmental parameters displayed by *C. inornata* confirms previous findings on population density, net calcification rate (that is linear extension rate × bulk skeletal density) and growth parameters of this species, which are not influenced by increasing solar radiation and temperature (Caroselli et al. 2015; Caroselli et al. 2016a; Caroselli et al. 2016b). In fact, the reproductive capacity of a species is strongly influenced by the growth rate and the ecological dynamics of populations (Madin et al. 2012). Thus, *C. inornata* seems quite tolerant to environmental variations, as observed for *L. pruvoti*, studied along the same latitudinal gradient (Airi et al. *submitted*). On the contrary, the populations of the zooxanthellate coral *B. europaea* analyzed along the same gradient were less abundant, less stable (with loss of young individuals; Goffredo et al. 2007, Caroselli et al. 2011, Goffredo et al. 2008, Goffredo et al. 2009) and less efficient in using the energy invested for gonadal development with increasing

temperature (Airi et al. 2014). Indeed, the warmest populations showed more and larger oocytes during gametes recruitment period, while at the end of the maturation processes were characterized by the same number of the oocytes compared to the populations with lower solar radiation and temperature. This less efficiency in energy allocation highlighted in the warmer populations, could be due to an inhibition of the photosynthetic activity of the zooxanthellae that, exposed to extreme temperatures, reduces the available energy towards reproduction (Goffredo et al. 2007, 2008; Airi et al. 2014). Contrary to *B. europaea*, *C. inornata* and *L. pruvoti* are both non-zooxanthellate corals and the absence of symbiosis with the dinoflagellates unicellular algae would make them less dependent to solar radiation and temperature variations.

The population of Elba Island of *C. inornata* is characterized by the presence of embryos in all stages of development throughout the year in females, males and sexually inactive individuals. The same pattern was found in all populations studied along the latitudinal gradient. The absence of seasonality in embryo development and the presence of embryos before the fertilization period (gametes recruitment period), suggest the possibility of an agamic production of embryos in this species (Goffredo et al. 2012; Marchini et al. 2015). Fertility (number of embryos per body volume unit) was homogeneous among populations along the latitudinal gradient (Supplementary Tables S3 and Figures S5 and S6) confirming that the reproduction of *C. inornata* does not vary with increasing temperature and solar radiation. A mixed strategy of sexual and asexual reproduction, seems to merge into a single organism the advantages of both propagation modes. Asexual reproduction allows the rapid propagation of a large number of individuals well adapted to local level (Maynard-Smith 1978) and to reproduce in unfavorable habitats, or in absence of the both sexes (Francis 1979). In fact, the scleractinians *Acropora cervicornis* and *A. palmata*, favoring asexual reproduction in disturbed habitats, partly reduce the

genotypic variability of populations (Tunnicliffe 1981; Neigel 1983) and partly they are able to persist even in stress conditions, apparently delaying the response of the populations to environmental variations (Lasker and Croffoth 1999). In contrast, sexual reproduction contributes to the formation of new genotypes (Williams 1975), allowing organisms to adapt to the surrounding environment variations (van Woesik 2009). However, this propagation mode is energy costly (Ward 1995). During sexual reproduction most of the available resources for an organism must be allocated to the formation of gametes (Antonovics 1980), in fact in the oocytes are stored a large quantities of lipids (Leuzinger et al. 2003; Stimson 1987) that are removed from the energy reserves addressed to other metabolic processes (Patton et al. 1977). On the contrary, the cell division and regeneration processes of tissues involved in asexual reproduction require a low energy investment (Francis 1979). A mixed reproduction strategy could therefore offer a reproductive assurance to the organism, which can be independent from the formation and maturation of gametes and fertilization success (Yund 2000), energetically expensive processes and dependent on environmental parameters. These reproductive traits have probably evolved in response to their sessile lifestyle, making *C. inornata* able to regularly adjusts to environmental conditions, since the organism is unable to move in a more suitable environment (van Oppen et al. 2015).

References

Airi V, Prantoni S, Calegari M, Baldi VL, Levy O, Falini G, Dubinsky Z, Goffredo S (2016) Reproductive output of a non-zooxanthellate Mediterranean coral is unaffected by temperature along an 850 km latitudinal gradient. Sci Rep *Submitted*

Airi V, Gizzi F, Falini G, Levy O, Dubinsky Z, Goffredo S (2014) Reproductive efficiency of a Mediterranean endemic zooxanthellate coral decreases with increasing temperature along a wide latitudinal gradient. PLoS ONE 9:e91792

Albright R and Mason B (2013) Projected near-future levels of temperature and pCO_2 reduce coral fertilization success. PLoS ONE 8:e56468

Altman DG (ed) (1991) Practical statistics for medical research. Chapman & Hall, London

Antonovics J (1980) Limits to Action: The Allocation of Individual Behavior. In: JER Staddon (ed). New York: Academic, p 1-25

Babcock RC, Bull GD, Harrison PL, Heyward AJ, Oliver JK, Wallace CC, Willis BL (1986). Synchronous spawning of 105 scleractinian coral species on the Great Barrier Reef. Mar Biol 90:379-394

Baird AH and Marshall PA (2002) Mortality, growth and reproduction in scleractinian corals following bleaching on the Great Barrier Reef. Marine Ecology Progress Series 237:133–141

Bassim K, Sammarco P, Snell TL (2002) Effects of temperature on success of (self and non-self) fertilization and embryogenesis in *Diploria strigosa* (Cnidaria, Scleractinia). Mar Biol 140:479-488

Beauchamp KA (1993) Gametogenesis, brooding and planulation in laboratory populations of a temperate scleractinian coral *Balanophyllia elegans* maintained under contrasting photoperiod regimes. Invertebr Reprod Dev 23:171-182

Brown B (1997) Coral bleaching: causes and consequences. Proc 8th Int Coral Reef Symp 1: 65-74

Byrne M (2011) Impact of ocean warming and ocean acidification on marine invertebrate life history stages: vulnerabilities and potential for persistence in a changing ocean. In: Gibson RN, Atkinson RJA, Gordon JDM (eds) Oceanography and marine biology: an annual review, vol 49, pp 1–42

Cairns SD (1999) Species richness of recent Scleractinia. Atoll Research Bulletin 459:1-46

Caroselli E, Ricci F, Brambilla V, Mattioli G, Levy O, Falini G, Dubinsky Z, Goffredo S (2016a) Relationships between growth, population dynamics, and environmental parameters in the solitary non-zooxanthellate scleractinian coral *Caryophyllia inornata* along a latitudinal gradient in the Mediterranean Sea. Coral Reefs, in press. doi:10.1007/s00338-015-1393-9

Caroselli E, Brambilla V, Ricci F, Mattioli G, Levy O, Falini G, Dubinsky Z, Goffredo S (2016b) Inferred calcification rate of a temperate azooxanthellate caryophylliid coral along a wide latitudinal gradient. Coral Reefs, in press. doi:10.1007/s00338-016-1422-3

Caroselli E, Nanni V, Levy O, Falini G, Dubinsky Z, Goffredo S (2015) Latitudinal variations in biometry and population density of a Mediterranean solitary coral suggest higher tolerance to seawater warming for non-zooxanthellate species. Limnol Oceanogr 60:1356–1370

Caroselli E, Prada F, Pasquini L, Nonnis Marzano F, Zaccanti F, Falini G, Levy O, Dubinsky Z, Goffredo S (2011) Environmental implications of skeletal micro-density and porosity variation in two scleractinian corals. Zoology 114: 255-264

Fadlallah YH and Pearse JS (1982) Sexual reproduction in solitary corals: overlapping oogenic and brooding cycles, and benthic planulas in *Balanophyllia elegans*. Mar Biol 71:223-231

Feely RA, Sabine CL, Lee K, Berelson W, Kleypas J, Fabry VJ, Millero FJ (2004) Impact of anthropogenic CO_2 on the $CaCO_3$ system in the ocean. Science 305:362-366

Field CB, Barros V, Stocker TF, Dahe Q (ed) (2012) Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation: Special Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge and New York

Francis L (1979) Contrast between solitary and clonal lifestyles in the sea anemone *Anthopleura elegantissima*. Am Zool 19:669-681

Goffredo S, Marchini C, Rocchi M, Airi V, Caroselli E, Falini G, Levy O, Dubinsky Z, Zaccanti F (2012) Unusual pattern of embryogenesis of *Caryophyllia inornata* (Scleractinia, Caryophylliidae) in the Mediterranean Sea. Maybe agamic reproduction? J Morphol 273:943-956

Goffredo S, Gasparini G, Marconi G, Putignano MT, Pazzini C, Airi V, Zaccanti F (2011) Sexual reproduction in the Mediterranean endemic orange coral, *Astroides calycularis* (Scleractinia, Dendrophylliidae). Bull Mar Sci 87:589-604

Goffredo S, Caroselli E, Mattioli G, Pignotti E, Dubinsky Z, Zaccanti F (2009) Inferred level of calcification decreases along an increasing temperature gradient in a Mediterranean endemic coral. Limnol Oceanogr 54:930-937

Goffredo S, Caroselli E, Mattioli G, Pignotti E, Zaccanti F (2008) Relationships between growth, population structure and sea surface temperature in the temperate solitary coral *Balanophyllia europaea* (Scleractinia, Dendrophylliidae). Coral Reefs 27:623-632

Goffredo S, Caroselli E, Pignotti E, Mattioli G, Zaccanti F (2007) Variation in biometry and population density of solitary corals with environmental factors in the Mediterranean Sea. Mar Biol 152: 351-361

Goffredo S, Airi V, Radetic J, Zaccanti F (2006) Sexual reproduction of the solitary sunset cup coral *Leptopsammia pruvoti* (Scleractinia, Dendrophylliidae) in the Mediterranean. 2. Quantitative aspects of the annual reproductive cycle. Mar Biol 148:923-932

Goffredo S, Radetic J, Airi V, Zaccanti F (2005) Sexual reproduction of the solitary sunset cup coral *Leptopsammia pruvoti* (Scleractinia, Dendrophyllidae) in the Mediterranean. 1. Morphological aspects of gametogenesis and ontogenesis. Mar Biol 147:485-495

Goffredo S, Arnone S, Zaccanti F (2002) Sexual reproduction in the Mediterranean solitary coral *Balanophyllia europaea* (Scleractinia, Dendrophylliidae). Mar Eco Prog Ser 229:83-94

Harley CDG, Hughes AR, Hultgren KM, Miner BG, Sorte CJB, Thornber CS, Rodriguez LF, Tomanek L, Williams SL (2006) The impacts of climate change in coastal marine systems. Ecol Lett 9:228-241

Harrison PL and Wallace CC (1990) Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky Z (ed) Ecosystem of the world. 25. Coral reefs. Amsterdam, Elsevier, p 133-207

Kersting DK, Casado C, López-Legentil S, Linares C (2013) Unexpected divergent patterns in the sexual reproduction of the Mediterranean scleractinian coral *Cladocora caespitosa*. Mar Ecol Prog Ser 486:165-171

Korta M, Murua H, Kurita Y, Kjesbu OS (2010) How are the oocytes recruited in an indeterminate fish? Applications of stereological techniques along with advanced packing density theory on European hake (*Merluccius merluccius* L.). Fish Res 104:56–63

Kump LR, Bralower TJ, Ridgwell A (2009) Ocean acidification in deep time. Oceanography 22:94-107

Krupp DA, Hollingsworth LL, Peterka J (2006) Elevated temperature sensitivity of fertilization and early development in the mushroom coral *Fungia scutaria* Lamarck 1801. Proceedings of the 10th International Coral Reef Symposium, p 71-77

Kružić P, Zuljevic A, Nikolic V (2008) Spawning of the colonial coral *Cladocora caespitosa* (Anthozoa, Scleractinia) in the Southern Adriatic sea. Coral Reefs 27:337-341

Lasker HR and Coffroth MA (1999) Responses of clonal reef taxa to environmental change. Am Zool 39:92-103

Lejeusne C, Chevaldonné P, Pergent-Martini C, Boudouresque CF, Pérez T (2010) Climate change effects on a miniature ocean: the highly diverse, highly impacted Mediterranean Sea. Trends Ecol Evol 25:250-260

Leuzinger S, Anthony KR, Willis BL (2003) Reproductive energy investment in corals: Scaling with module size. Oecologia 136:524-531

Linares C, Coma R, Zabala M (2008) Effects of a mass mortality event on gorgonian reproduction. Coral reefs 27:27-34

Lowerre-Barbieri SK, Ganias K, Saborido-Rey F, Murua H, Hunter JR (2011) Reproductive timing in marine fishes: variability, temporal scales, and methods. Mar Coast Fish 3: 71–91

Madin JS, Hughes TP, Connolly SR (2012) Calcification, storm damage and population resilience of tabular corals under climate change. PLoS ONE 7:e46637

Marchini C, Airi V, Fontana R, Tortorelli G, Falini G, Levy O, Dubinsky Z, Goffredo S (2015) Annual reproductive cycle in the temperate coral *Caryophyllia inornata* driven by an unusual embryogenesis. PLoS ONE 10: e0141162

Maynard-Smith J (1978) The evolution of sex. Cambridge University Press, Cambridge

Negri AP, Marshall PA, Heyward AJ (2007) Differing effects of thermal stress on coral fertilization and early embryogenesis. Coral Reefs 26:759-763

Neigel JE and Avise JC (1983) Clonal diversity and population structure in a reef-building coral, *Acropora cervicornis*: self-recognition analysis and demographic interpretation. Evolution 37:437-453

Nozawa Y; Harrison P (2007) Effects of elevated temperature on larval settlement and post-settlement survival in scleractinian corals, *Acropora solitaryensis* and *Favites chinensis*. Mar Biol 152:1181-1185

Olea RA and Pawlowsky-Glahn, V (2009) Kolmogorov-Smirnov test for spatially correlated data. Stochastic environmental research and risk assessment 6:749-757

Patton JS, Abraham S, Benson AA (1977) Lipogenesis in the intact coral *Pocillopora damicornis* and its isolated zooxanthellae: evidence for a light-driven carbon cycle between symbiont and host. Mar biol 44:235-247

Penland L, Kloulechad J, Idip D, Woesik R (2004) Coral spawning in the western Pacific Ocean is related to solar insolation: evidence of multiple spawning events in Palau. Coral Reefs 23:133-140

Potvin C, Roff DA (1993) Distribution-free and robust statistical methods: viable alternatives to parametric statistics? Ecology 74:1617-1628

Richardson AJ and Poloczanska ES (2008) Under-resourced, under threat. Science 320:1294-1295

Sabine CL, Feely RA, Gruber N, Key RM, Lee K, Bullister JL, Wanninkhof R, Wong CS, Wallace DWR, Tilbrook B, Millero FJ, Peng T-H, Kozyr A, Ono T, Rios AF (2004) The oceanic sink for anthropogenic CO_2. Science 305:367–371

Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) IPCC 2007: Summary for policymakers. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge and New York: Cambridge University Press

Stimson JS (1987) Location, quantity and rate of change in quantity of lipids in tissue of Hawaiian hermatypic corals. Bull Mar Sci 41:889-904

Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds) IPCC 2013: Summary for Policymakers. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA

Tunnicliffe V (1981) Breakage and propagation of the stony coral *Acropora cervicornis*. Proc Natl Acad Sci USA 78:24272431

van Oppen MJ, Oliver JK, Putnam, HM, Gates RD (2015) Building coral reef resilience through assisted evolution. Proc Natl Acad Sci 112:2307-2313

van Woesik R (2009) Calm before the spawn: global coral spawning patterns are explained by regional wind fields. Proc R Soc Biol Sci Ser B 277:715-722

van Woesik R, Lacharmoise F, Koksal S (2006) Annual cycles of solar insolation predict spawning times of Caribbean corals. Ecol Lett 9:390-398

Vargas-Yáñez M, Garcia-Martinez MC, Moya- Ruiz F, Tel E, Parrilla G, Plaza F, Lavin A (2007) Cambio Climatico en el Mediterraneo espanol. Instituto Espanol de Oceanografia, Ministerio de Educacion y Ciencia

Ward S (1995) The effect of damage on the growth, reproduction and lipid storage in the scleractinian coral *Pocillopora damicornis*. Jour Exp Mar Bio Ecol 187:193-20

Williams GC (1975) Sex and evolution. Princeton, Princeton University Press

Yund PO (2000) How severe is sperm limitation in natural populations of marine free-spawners?. Trends Ecol Evol 15:10-13

Zibrowius H (1980) Les scléractiniaires de la Méditeranée et de l'Atlantique nord-oriental. Mémoires de l'Institut océanographique (Monaco) 11:1-284

Tables

Table 1. Mean annual depth temperature (DT; °C) and solar radiation (W/m²) from the sampled populations. The sites are arranged in order of increasing solar radiation; SE, standard error.

Population	Code	DT (°C) mean ± SE	Solar radiation (W/m²) mean ± SE
Genova	GN	17.65 ±0.62	156.9±3.2
Calafuria	CL	16.56 ±0.55	174.1±1.9
Elba	LB	17.47 ±0.58	184.9±2.3
Scilla	SC	18.61 ±0.60	205.5±1.8
Pantelleria	PN	19.01 ±0.57	218.2±0.5

Table 2. Number of females, males, inactive individuals and proportion of fertile polyps in each population for gametes recruitment and gametes maturity periods. The sites are arranged in order of increasing solar radiation.

Gametes recruitment period				
Population	**Females**	**Males**	**Inactive individuals**	**Proportion of fertile polyps**
Genova	1	7	19	20/27
Calafuria	1	5	25	19/31
Elba	4	7	11	20/22
Scilla	2	4	7	12/13
Pantelleria	1	3	9	12/13
Gametes maturity period				
Population	**Females**	**Males**	**Inactive individuals**	**Proportion of fertile polyps**
Genova	2	10	9	20/21
Calafuria	1	7	9	15/17
Elba	10	28	16	45/54
Scilla	2	9	5	12/16
Pantelleria	2	14	9	23/25

Table 3. Mean fecundity, gonadal index and diameter of oocytes in each population for gametes recruitment and gametes maturity periods. The sites are arranged in order of increasing solar radiation; N, polyps number for fecundity and gonadal index, oocytes number for diameter. SE, standard error.

Gametes recruitment period					
Population	**N**	**Fecundity (#/100 mm^3)**	**Gonadal Index ♀ (%) mean ± SE**	**N**	**Oocytes Diameter (µm) mean ± SE**
Genova	1	41706	0.9	1441	32±0.3
Calafuria	1	7357	0.3	261	43±0.7
Elba	4	5789±4755	2.0±1.6	627	74±0.3
Scilla	2	13114±526	0.2±0.1	1991	28±0.2
Pantelleria	1	861	0.0	29	46±3.2
Gametes maturity period					
Population	**N**	**Fecundity (#/100 mm^3)**	**Gonadal Index ♀ (%) mean ± SE**	**N**	**Oocytes Diameter (µm) mean ± SE**
Genova	2	23879±23739	4.2±4.2	4036	71±0.2
Calafuria	1	180278	19.2	16288	59±0.1
Elba	10	23900±17388	4.5±2.6	16176	68±0.1
Scilla	2	4991±3981	1.9±1.6	3165	96±0.2
Pantelleria	2	67402±27976	15.3	22541	70±0.1

Table 4. Mean abundance, gonadal index and diameter of spermaries in each population for gametes recruitment and gametes maturity periods. The sites are arranged in order of increasing solar radiation. N, polyps number for abundance and gonadal index, spermaries number for diameter. SE, standard error.

Gametes recruitment period					
Population	**N**	**Abundance (#/100 mm^3)**	**Gonadal Index ♂ (%) mean ± SE**	**N**	**Spermary Diameter (µm) mean ± SE**
Genova	7	300±145	0.0±0.0	181	53±1.9
Calafuria	5	46±27	0.0±0.0	37	50±7.3
Elba	7	11362±4125	1.9±0.9	4879	84±0.7
Scilla	4	1780±1594	0.2±0.2	2847	67±0.6
Pantelleria	3	1551±1075	0.0±0.0	501	36±0.3
Gametes maturity period					
Population	**N**	**Abundance (#/100 mm^3)**	**Gonadal Index ♂ (%) mean ± SE**	**N**	**Spermary Diameter (µm) mean ± SE**
Genova	10	2628±1062	1.2±0.5	2863	109±1.1
Calafuria	7	1723±933	0.4±0.2	1267	103±1.0
Elba	28	5564±1994	1.7±0.6	13257	94±0.4
Scilla	9	10602±5081	3.7±1.9	7384	102±0.5
Pantelleria	14	5380 ±1829	2.3±0.9	9667	104±0.4

Table 5. Mean fertility of females (F), males (M) and inactive individuals (I) in each population for gametes recruitment and gametes maturity periods. The sites are arranged in order of increasing solar radiation. SE, standard error.

Gametes recruitment period						
Popolazione	**N**	**F**	**N**	**M**	**N**	**I**
Genova	1	9.4	7	212.3±71.9	13	190.3±67.8
Calafuria	1	225.8	3	69.2±34.8	15	28.6±7.1
Elba	4	48.9±20.0	6	160.1±57.1	9	120.3±45.5
Scilla	2	69.7±34.0	4	67.4±26.7	6	80.2±28.3
Pantelleria	1	0	3	35.9±23.5	8	66.8±25.2
Gametes maturity period						
Popolazione	**N**	**F**	**N**	**M**	**N**	**I**
Genova	2	125.6±81.8	10	73.8±18.9	8	141.8±62.8
Calafuria	1	176.4	6	121.7±48.3	8	84.7±26.3
Elba	10	187.7±62.9	23	116.0±31.6	12	29.3±9.3
Scilla	2	14.9±1.6	6	31.4±7.3	4	12.0±5.5
Pantelleria	2	28.11±16.7	14	105.1±28.0	8	74.0±17.0

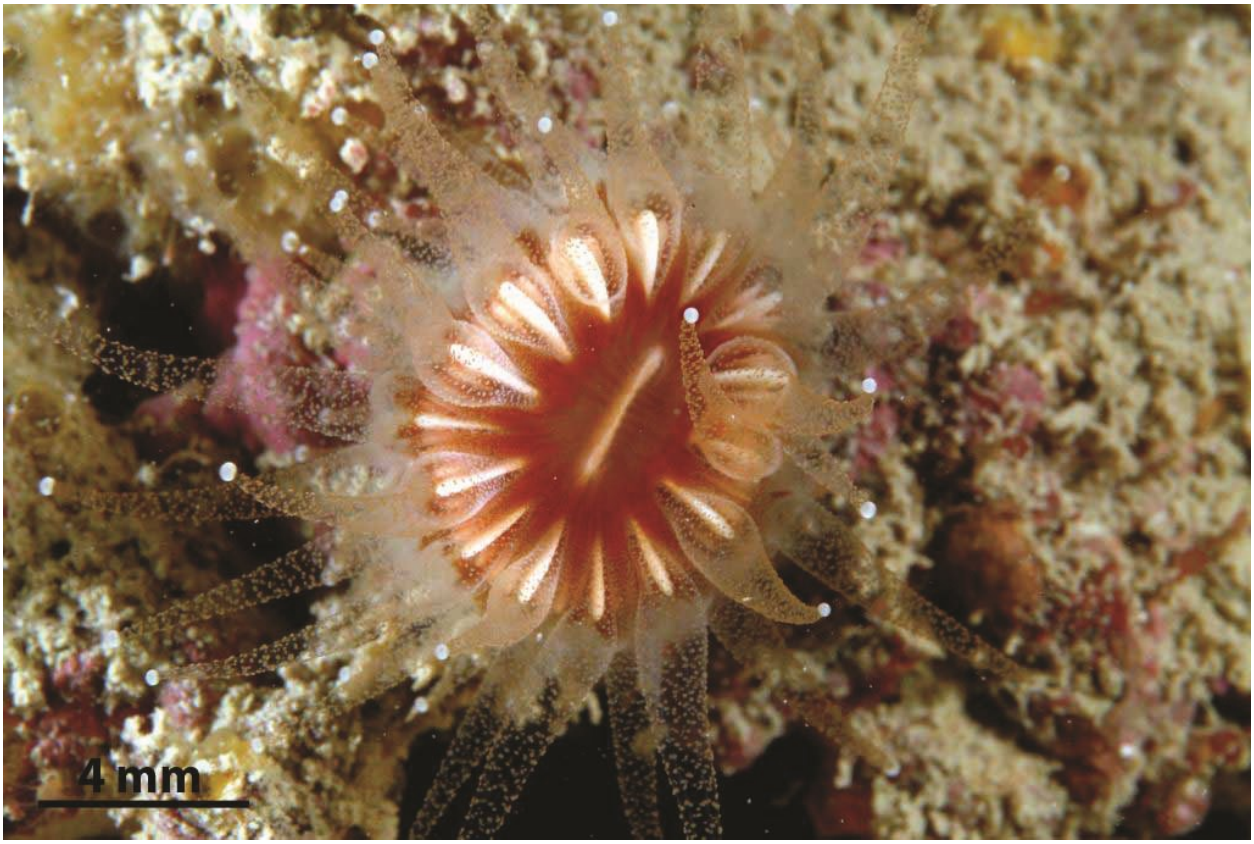

Figure 1. Living specimens of *Caryophyllia inornata* at Elba Isle (42°45'N, 10°24'E) by the courtesy of the nature photographer Gianni Neto.

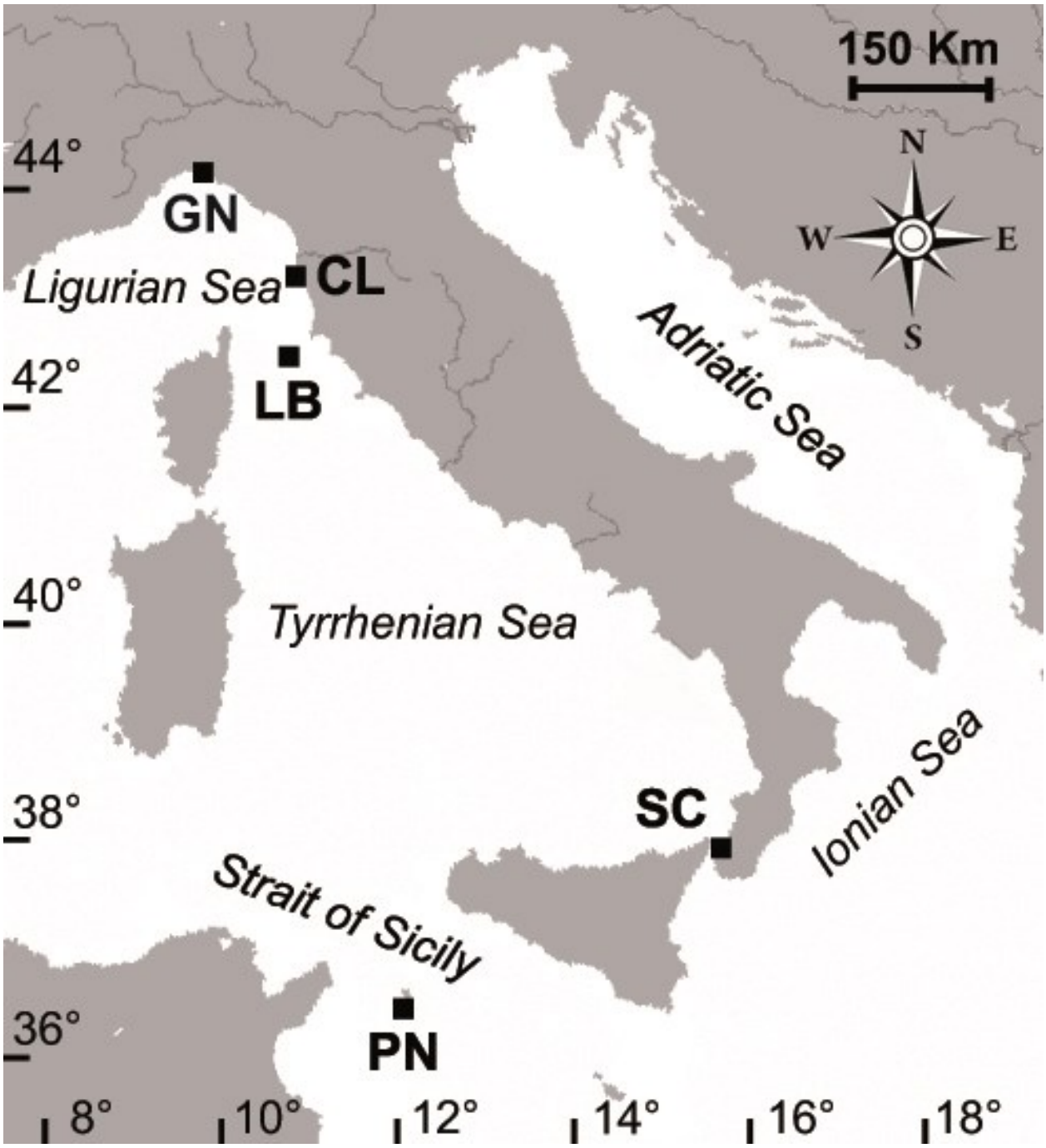

Figure 2. Map of the Italian coastline indicating the sites where corals were collected. Abbreviations and coordinates of the sites in decreasing order of latitude: GN Genova, 44°20'N, 9°08'E; CL Calafuria, 43°27'N, 10°21'E; LB Elba Isle, 42°45'N, 10°24'E; SC Scilla, 38°01'N, 15°38'E; PN Pantelleria Isle, 36°45'N, 11°57'E. Map was created by the authors using the software Adobe® Illustrator® CS3.

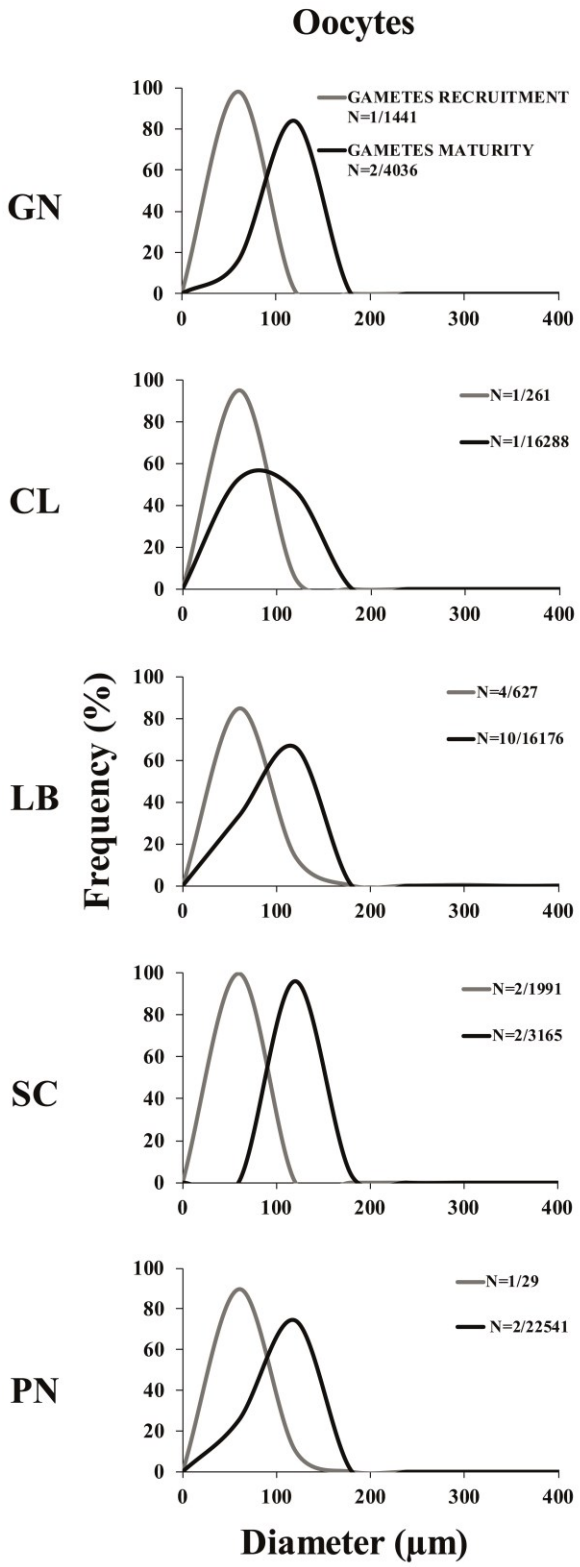

Figure 3. Oocyte size/frequency distribution in the recruitment and maturity periods. Distribution of the oocytes size during gametes recruitment period (gray line) and gametes maturity period (black line).

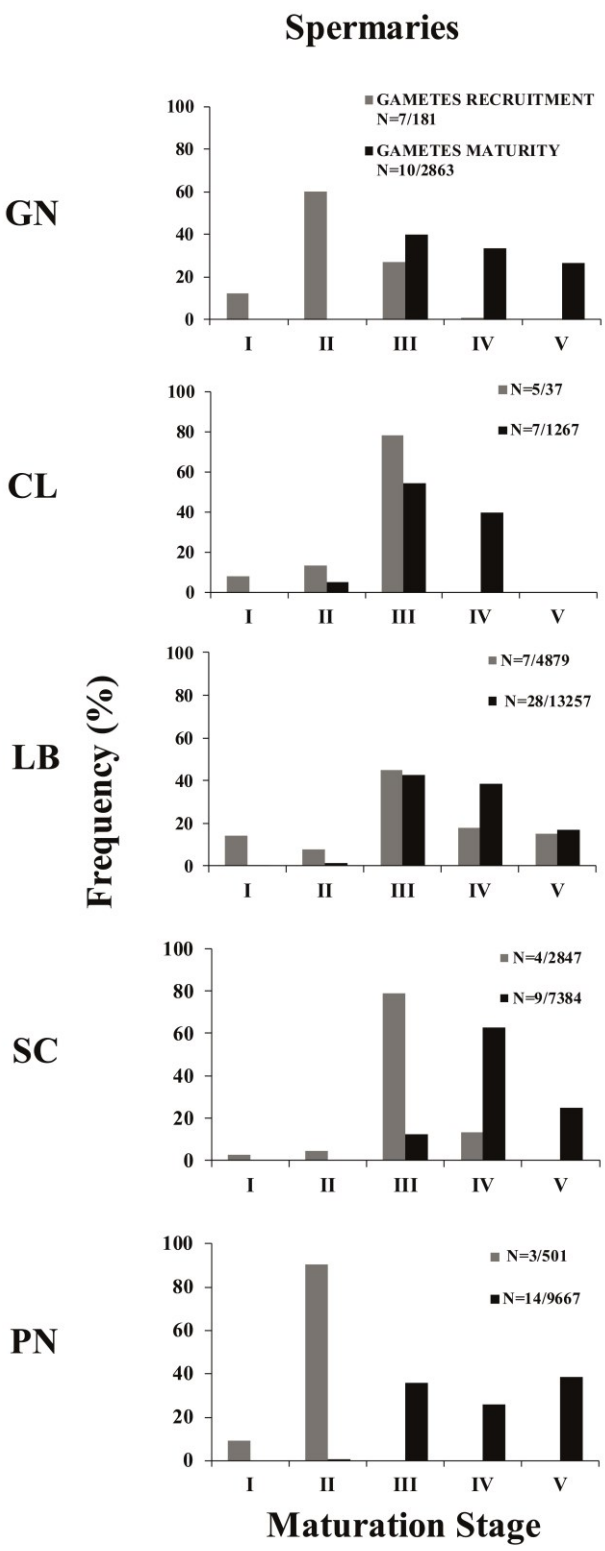

Figure 4. Spermary frequency distribution in the two reproductive periods. Distribution of the maturation stages during gametes recruitment period (gray bars) and gametes maturity period (black bars).

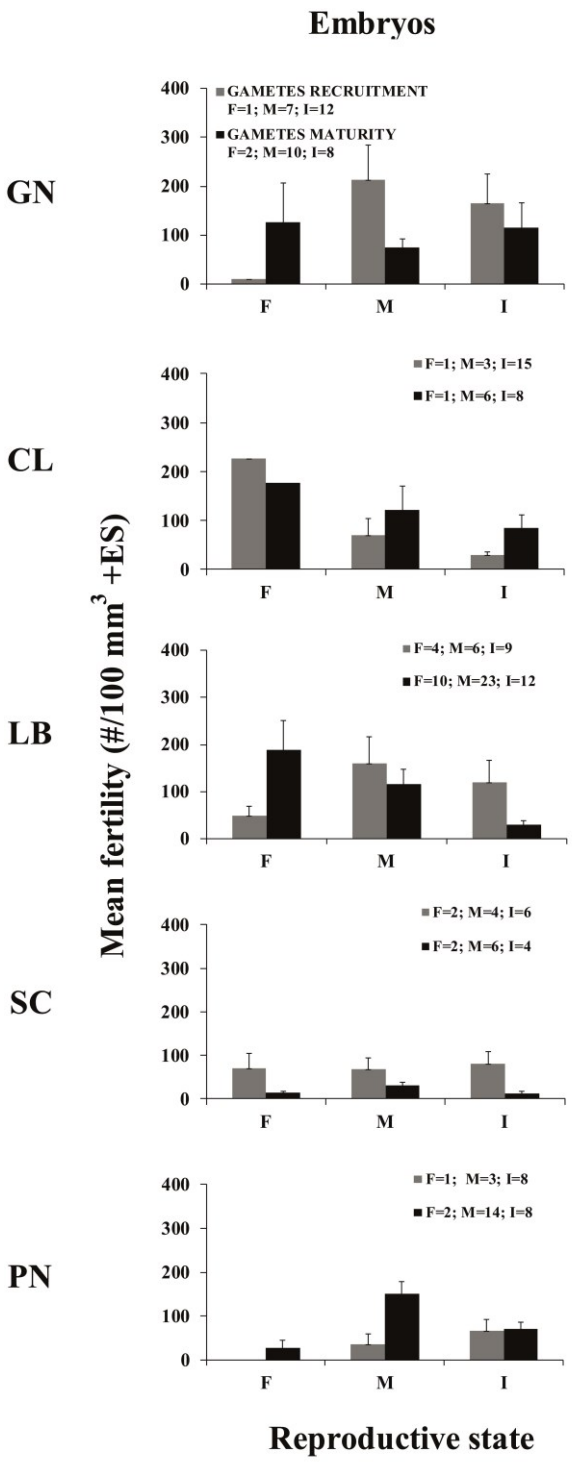

Figure 5. Mean fertility in feamles (F), males (M) and sexually inactive individuals (I) ± standard error (ES) for the two reproductive periods. Mean fertility during gametes recruitment period (gray bars) and gametes maturity period (black bars). N = number of analyzed polyps.

Supporting Information

Table S1. Oocytes. ANOVA/Kruskal-Wallis test and correlation analyses between reproductive (fecundity, gonadal index, oocytes diameter) and environmental parameters (DT, solar radiation) for gametes recruitment and gametes maturity periods.

Gametes recruitment period			
		DT(C°)	**Solar radiation (W/m^2)**
	ANOVA/K-W Test	**ρ**	**ρ**
Fecundity (#/mm^3)	ns	-	-
Gonadal index ♀(%)	ns	-	-
Oocytes diameter (µm)	***	-0.360**	-0.148**
Gametes maturity period			
		DT(C°)	**Solar radiation (W/m^2)**
	ANOVA/K-W Test	**ρ**	**ρ**
Fecundity (#/mm^3)	ns	-	-
Gonadal index ♀(%)	ns	-	-
Oocytes diameter (µm)	***	0.286**	0.247**

ANOVA/K-W Test, significance of the ANOVA/Kruskal-Wallis test; ρ, Spearman's correlation coefficient; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns $p > 0.05$.

Table S2. Spermaries. ANOVA/Kruskal-Wallis test and correlation analyses between reproductive (abundance, gonadal index, spermary diameter) and environmental parameters (DT, solar radiation) for gametes recruitment and gametes maturity periods.

Gametes recruitment period			
		DT(C°)	Solar radiation (W/m^2)
	ANOVA/K-W Test	ρ	ρ
Abundance (#/mm^3)	*	ns	0.414*
Gonadal index ♂ (%)	ns	-	-
Spermary diameter (µm)	***	-0.230**	-0.198**
Gametes maturity period			
		DT(C°)	Solar radiation (W/m^2)
	ANOVA/K-W Test	ρ	ρ
Abundance (#/mm^3)	ns	-	-
Gonadal index ♂ (%)	ns	-	-
Spermary diameter (µm)	***	0.106**	0.073**

ANOVA/K-W Test, significance of the Anova/Kruskal-Wallis test; ρ, Spearman's correlation coefficient; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns $p > 0.05$.

Table S3. Embryos. ANOVA/Kruskal-Wallis test and correlation analyses between fertility in females (F), males (M), inactive individuals (I) and environmental parameters (DT, solar radiation) for gametes recruitment and gametes maturity periods.

Gametes recruitment period			
		DT(C°)	Solar radiation (W/m^2)
	ANOVA/K-W Test	ρ	ρ
Fertility (#/mm^3) F	ns	-	-
Fertility (#/mm^3) M	ns	-	-
Fertility (#/mm^3) I	ns	-	-
Gametes maturity period			
		DT(C°)	Solar radiation (W/m^2)
	ANOVA/K-W Test	ρ	ρ
Fertility (#/mm^3) F	ns	-	-
Fertility (#/mm^3) M	ns	-	-
Fertility (#/mm^3) I	ns	-	-

ANOVA/K-W Test, significance of the ANOVA/Kruskal-Wallis test; ρ, Spearman's correlation coefficient; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns $p > 0.05$.

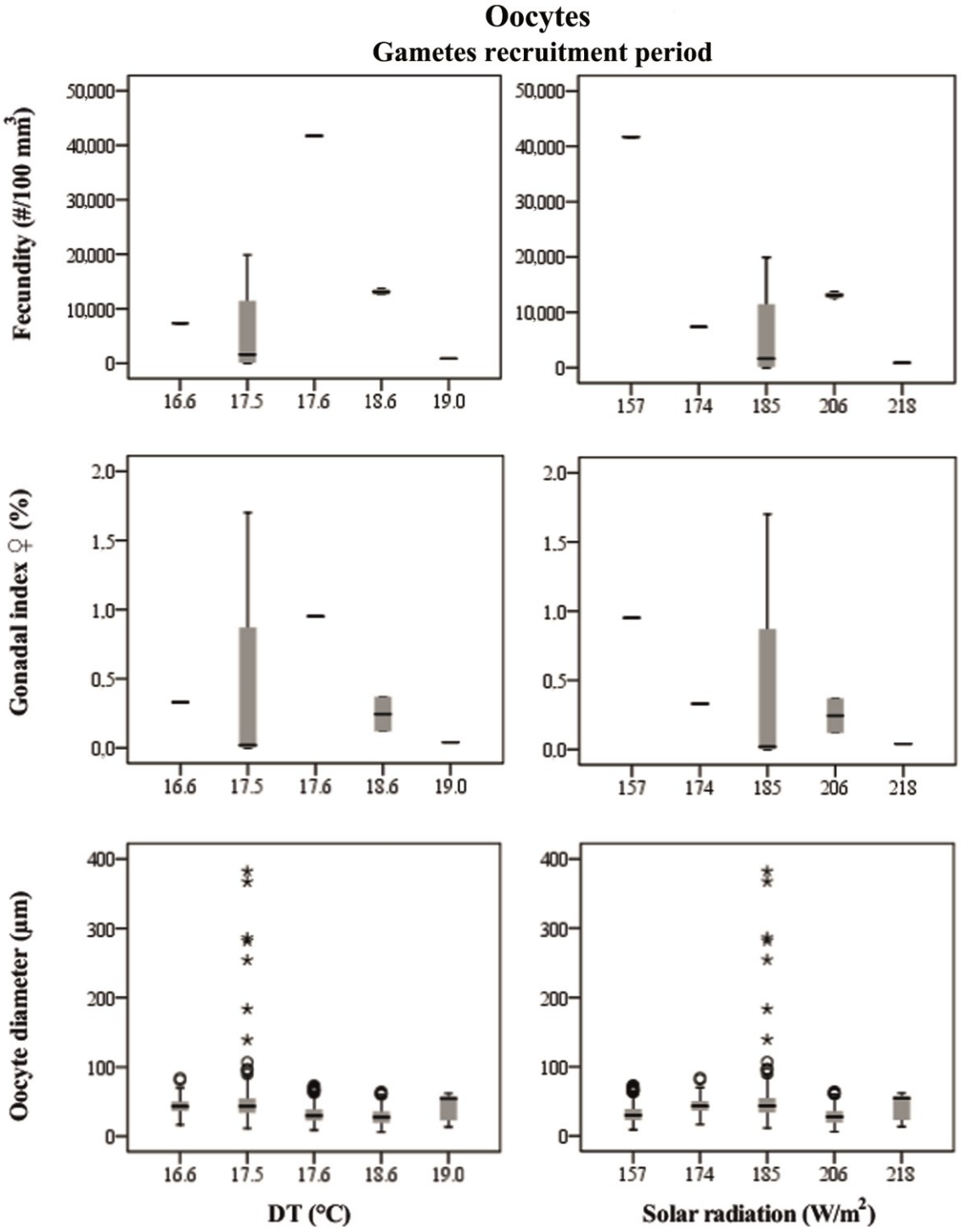

Figure S1. Oocytes. Boxplot of Spearman's correlation between reproductive and environmental parameters during gametes recruitment period. Median (solid horizontal line), first and third quartiles (box outline), minimum and maximum values (whiskers) and outliers (circles). See Table 3 for the number of polyps and oocytes analyzed and Table S1 for Spearman's correlation test.

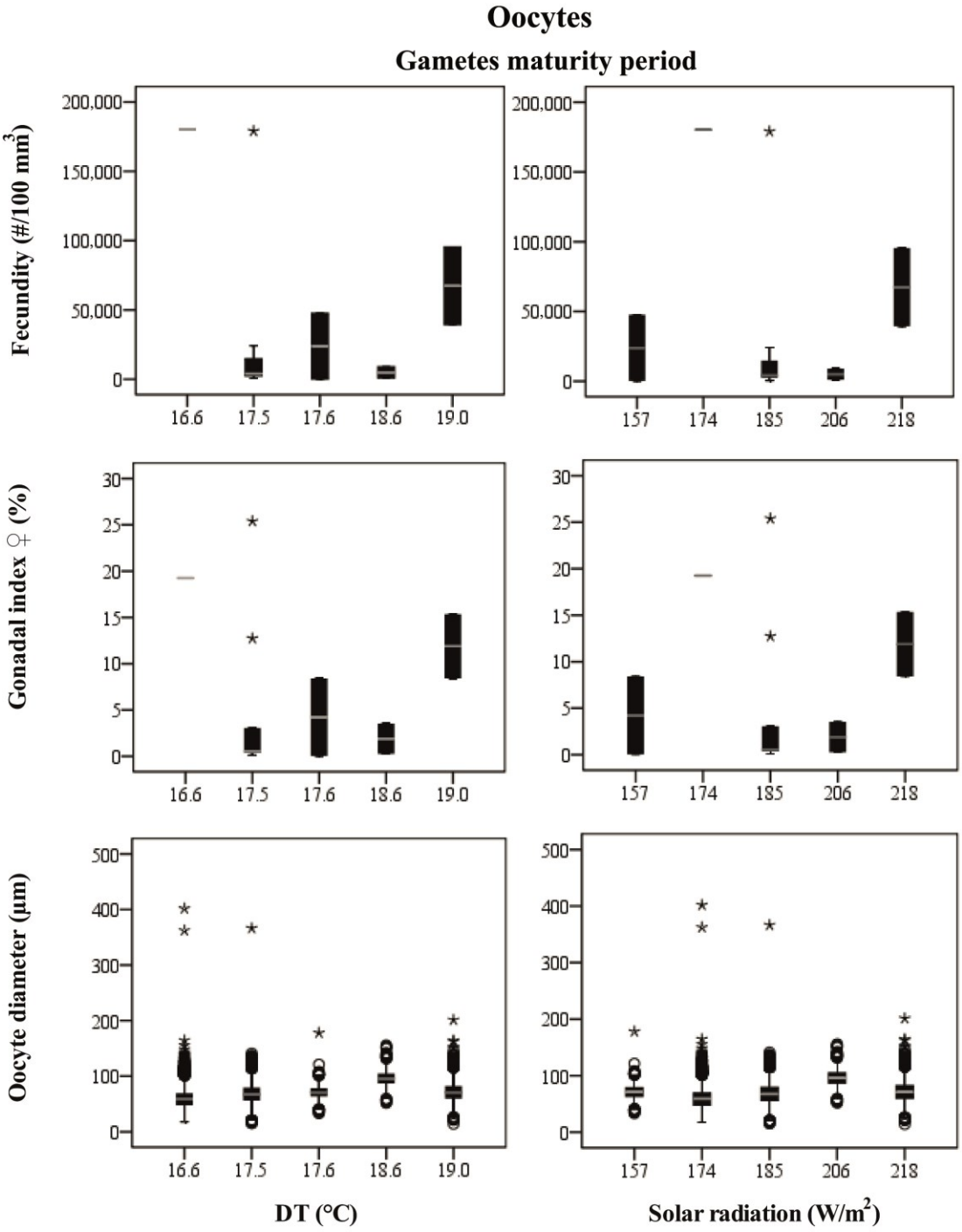

Figure S2. Oocytes. Boxplot of Spearman's correlation between reproductive and environmental parameters during gametes maturity period. Median (solid horizontal line), first and third quartiles (box outline), minimum and maximum values (whiskers) and outliers (circles). See Table 3 for the number of polyps and oocytes analyzed and Table S1 for Spearman's correlation test.

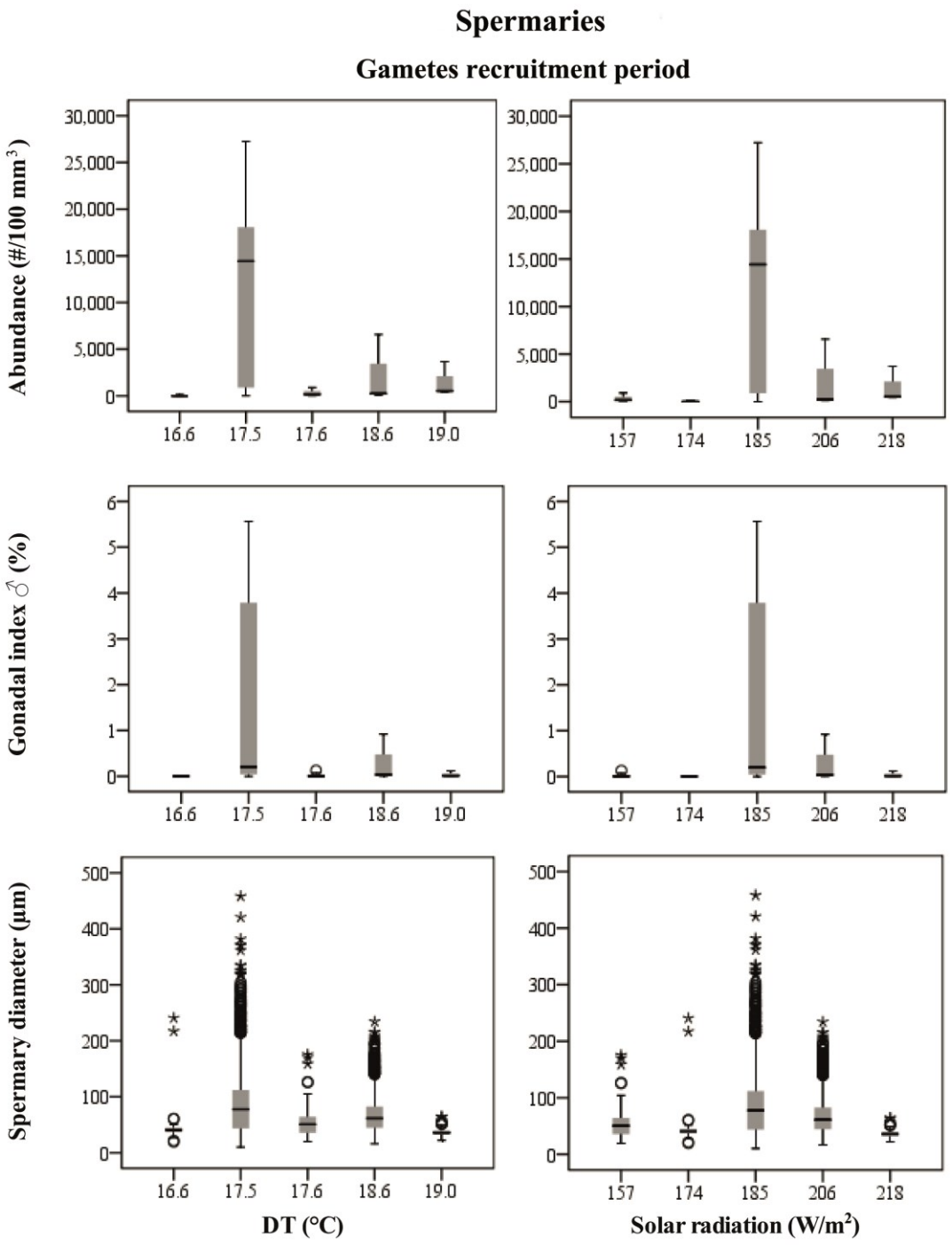

Figure S3. Spermaries. Boxplot of Spearman's correlation between reproductive and environmental parameters during gametes recruitment period. Median (solid horizontal line), first and third quartiles (box outline), minimum and maximum values (whiskers) and outliers (circles). See Table 4 for the number of polyps and spermaries analyzed and Table S 2 for Spearman's correlation test.

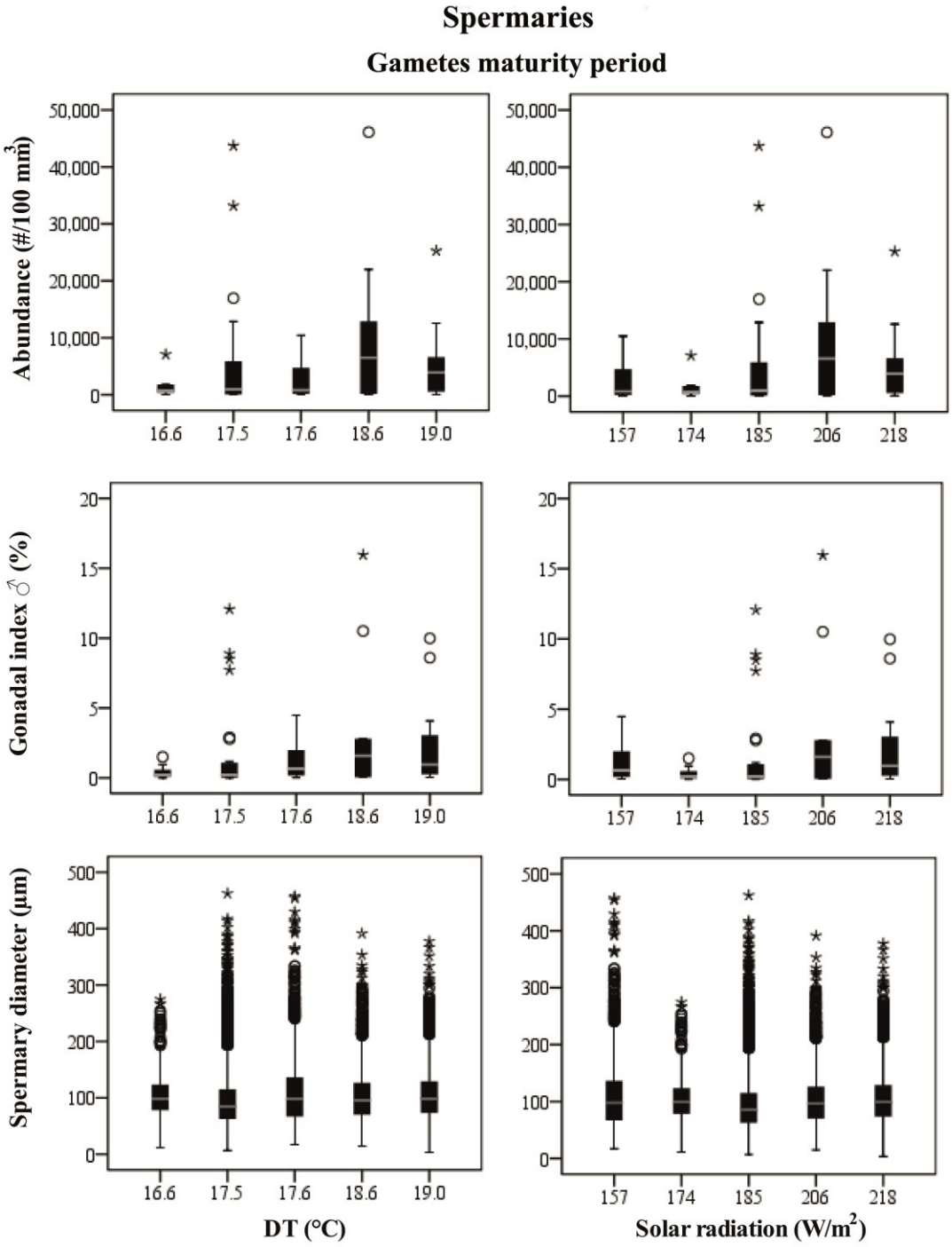

Figure S4. Spermaries. Boxplot of Spearman's correlation between reproductive and environmental parameters during gametes maturity period. Median (solid horizontal line), first and third quartiles (box outline), minimum and maximum values (whiskers) and outliers (circles). See Table 4 for the number of polyps and spermaries analyzed and Table S2 for Spearman's correlation test.

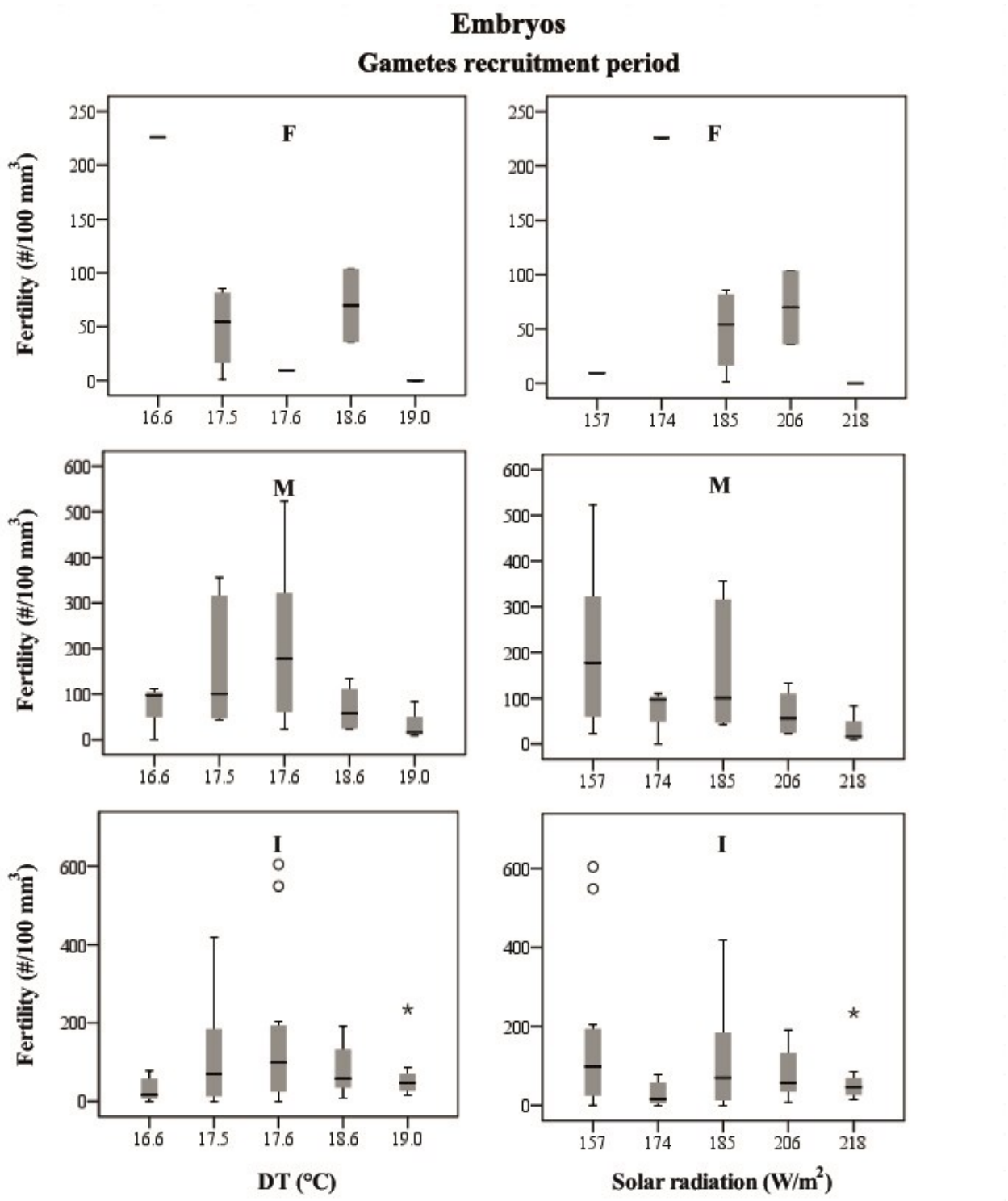

Figure S5. Embryos. Boxplot of Spearman's correlation between reproductive and environmental parameters during gametes recruitment period. Median (solid horizontal line), first and third quartiles (box outline), minimum and maximum values (whiskers) and outliers (circles). See Table 5 for the number of polyps and embryos analyzed and Table S3 for Spearman's correlation test.

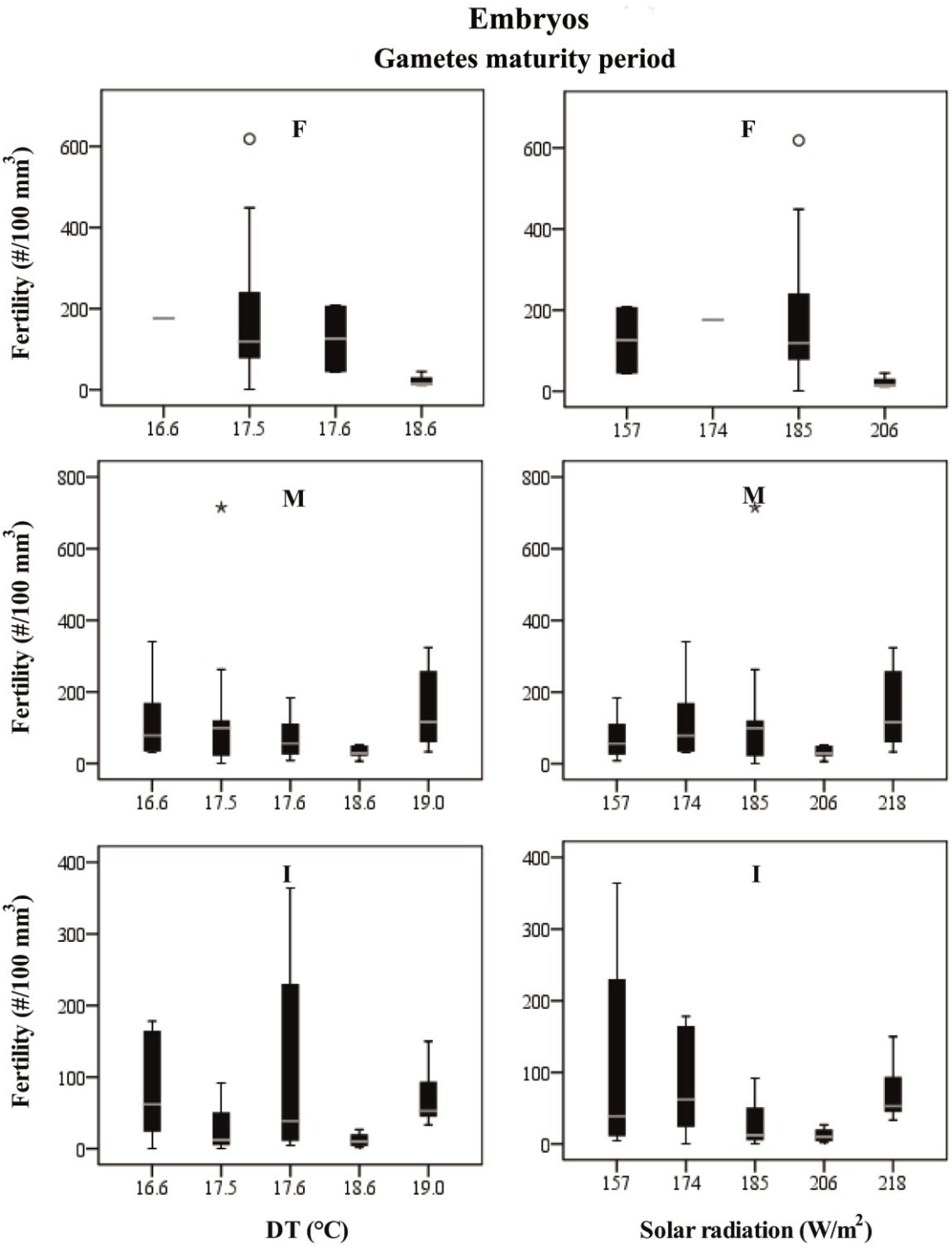

Figure S6. Embryos. Boxplot of Spearman's correlation between reproductive and environmental parameters during gametes maturity period. Median (solid horizontal line), first and third quartiles (box outline), minimum and maximum values (whiskers) and outliers (circles). See Table 5 for the number of polyps and embryos analyzed and Table S3 for Spearman's correlation test.

Chapter IV

Spermatogenesis of a temperate zooxanthellate coral is not influenced by long-term CO_2 exposure at a volcanic vent

Manuscript in preparation

Spermatogenesis of a temperate zooxanthellate coral is not influenced by long-term CO_2 exposure at a volcanic vent

Abstract

In recent years, there is a growing awareness concerning the effects of climate change on marine ecosystems. The progressive increase in ocean acidification is a major threat on calcifying organisms such as scleractinian corals. This study examines the effects of pCO_2 on spermatogenesis of the temperate and zooxanthellate coral *Balanophyllia europaea*, which lives along a natural pCO_2 gradient in close proximity to an underwater crater near Panarea island (Tyrrhenian Sea, Italy). Specimens were collected from three sites along the pCO_2 gradient generated by continuous CO_2 bubbles from the crater that ranged from pH 8.07 to pH 7.74. Increasing pCO_2 along this gradient did not affect spermatogenesis of *B. europaea*, probably due to the symbiosis with the zooxanthellae, which increase the photosynthetic efficiency, providing additional energy to be allocated towards reproduction. Therefore, this symbiosis could be an advantage for the temperate *B. europaea*, allowing the coral to persist in face of ocean acidification.

Introduction

The planet is undergoing a drastic increase in atmospheric carbon dioxide (pCO_2) concentrations (from 280 ppm to 390 ppm since the Industrial Revolution) due to anthropogenic activity such as the burning of fossil fuels, cement production, and land use changes (Stocker et al. 2013). Around one-third of all CO_2 emissions from the past 200 years have been absorbed by the oceans (Sabine et al. 2004), driving changes in seawater carbonate chemistry and leading to a reduction of about 0.1 pH units in ocean surface waters (Caldeira and Wickett 2003). Ocean acidification (OA) is in progress and a further pH decline by 0.3–0.4 units is expected by 2100 (Pachauri et al. 2014).

OA is causing alterations to marine ecosystems and, in particular, represents a major threat for numerous calcifying marine organisms such as mollusks and corals (Jokiel et al. 2008), arousing serious concerns among scientists. Calcification may be especially sensitive because altered carbonate chemistry directly affects the deposition and dissolution rates of the $CaCO_3$ used for shells and skeletons (Gattuso and Buddemeier 2000).

However, other physiological and biological processes including reproduction of these marine organisms can be vulnerable to an increase in pCO_2 (Kurihara and Shirayama 2004; Havenhand et al. 2008; Parker et al. 2009; Havenhand and Schlegel 2009; Morita et al. 2009; Byrne et al. 2010; Albright et al. 2010; Reuter et al. 2011). Due to their sensitivity to water chemistry, marine gametes and embryos have long been used as a bioassay system for monitoring of environmental pollutants (Dinnel et al. 1987, Ringwood 1992, Carr et al. 2006, Byrne et al. 2008). A compromised gamete production could have negative consequences on larval recruitment and growth, reflecting on marine population dynamics (Doherty and Fowler 1994).

Even if susceptibility of corals to increased pCO_2 varies with species (Fabricius et al. 2011), it seems that many structurally complex corals will be lost, leading to a decline in habitat available to a variety of other species and changes in structure and function of the marine ecosystem (Fabricius et al. 2014). Despite the growing awareness about the threats of coral reefs, the little information available regarding the effects of OA on coral sexual reproduction includes sperm motility (Morita et al. 2009), fertilization success (Albright et al. 2010), and larval development and/or growth (Albright et al. 2010; Albright et al. 2008; de Putron et al. 2011).

Assessing effects of acidification on gamete development is hard as gametogenesis can extend even up to 24 months for some temperate species (Goffredo et al. 2002, 2006, 2011), and maintaining corals under experimental conditions for this period of time can prove challenging (Albright 2011). This research, therefore, investigated the effect of OA on spermatogenesis in the Mediterranean scleractinian coral *Balanophyllia europaea* (Fig. 1) naturally living along a pCO_2 gradient. The species under study is solitary and zooxanthellate with a simultaneous hermaphroditic sexuality and a brooding reproductive mode (Goffredo et al. 2002). Previous works reveals that biomineralization control (Goffredo et al. 2014), skeletal nano and microstructural features, linear extension rate, interseptal volume fraction and corallite biometry (Fantazzini et al. 2015) of *B. europaea* did not change significantly with decreasing pH, despite a clear reduction in net calcification rates. This reduction in net calcification rate was complemented by an increase in skeletal porosity and a consequent decrease in skeletal bulk density and stiffness (Fantazzini et a l. 2015), which could result in increased mortality and the observed population density decline (Goffredo et al. 2014). *B. europaea* was also studied in relation to increasing temperature, which negatively affects skeletal density (Goffredo et al. 2009) [(due to increased porosity (Caroselli et al. 2011)], growth and calcification (Caroselli et

al. 2011), population abundance (Goffredo et al. 2007), population structure stability (Goffredo et al. 2008) and reproductive output (Airi et al. 2014).

Materials and Methods

Study site

Fieldwork was conducted close to an underwater volcanic crater located in the southeast of the islet of Bottaro, near the island of Panarea (Sicily, Italy, 38°38′16″N 15°06′37″E; Fig. 2). The crater (20 m x 14 m) situated at roughly 10 m depth (Capaccioni et al. 2007), generates hydrothermally stable CO_2 emissions (99%; Capaccioni et al. 2007; Goffredo et al. 2014) creating a natural pH gradient that extends about 34 m from the center of the crater (Goffredo et al. 2014), where there is a condition of greater acidity (pH_{TS} 7.40), to the periphery characterized by a condition normal pH (pH_{TS} 8.07; Prada 2014). For this study, three Sites along the pH gradient have been selected: the control site (Site 1: mean Total Scale pH_{TS} 8.07) that is 34 m away from the crater, the intermediate site (Site 2: mean Total Scale pH_{TS} 7.87) located 13 m away from the crater, and the highest pCO_2 site (Site 3: mean Total Scale pH_{TS} 7.74) that is 9 m from the crater.

Seawater carbonate chemistry

pH (NBS scale), temperature (T), salinity (Sal) and total alkalinity (TA) were measured at each Site during several surveys between July 2010 and May 2013 with a multi-parametric probe (600R, YSI Incorporated, USA; Tab. 1) and operated by SCUBA divers. Measured pH_{NBS} was converted in total scale (TS) using CO2SYS software (Mehrbach et al. 1973; Dickson 1990; Dickson and Millero 1987). Temperature sensors (Thermochron iButton, DS1921G, Maxim Integrated Products, USA) were placed near each Site and recorded depth temperature (T; °C)

every three hours from June 2011 to May 2013 and replaced to each field campaign. The pH, total alkalinity, salinity and temperature were used to calculate other carbonate system parameters using the software CO2SYS with referenced dissociation constant (Tab. 1). The study site has stable hydrothermal–chemical properties and only pCO_2 concentration differed significantly across sites (Capaccioni et al. 2007; Goffredo et al. 2014).

Sampling

Specimens of *Balanophyllia europaea* were randomly collected by SCUBA diving at the three study sites along the pH gradient (10 from Site 1, 10 from Site 2 and 10 from Site 3) on April 28th 2013. Based on a previous work on the sexual reproduction of *B. europaea* (Goffredo et al 2002), the reproductive cycle displays the maximum gonadal development and is characterized by advanced maturation stages of spermaries (just before the fertilization process) during this sampling period. The sample size was chosen to limit damage on the natural population, which significantly diminishes in the most acidic sites (Goffredo et al. 2014) and was considered suitable for properly describing the trend and properties of spermatogenesis.

Polyps were fixed in saturated formalin solution (10% formaldehyde and 90% seawater; the solution was saturated with calcium carbonate) and transferred to the laboratories for histological analysis.

Biometric and cytometric analysis

Biometric analyses were performed on 20 polyps by measuring length (L, maximum axis of the oral disc), width (l, minimum axis of the oral disc) and height (h, oral–aboral axis) of each sampled polyp. The volume (V) of the individual polyp was calculated using the formula $V = h * (L/2) * (l/2) * \pi$ (Goffredo et al. 2002).

Polyps were post-fixed in Bouin solution. After decalcification in EDTA and dehydration in a graded alcohol series from 80 to 100%, polyps were embedded in paraffin, and serial transverse sections were cut at 7µm intervals along the oral–aboral axis, from the oral to the aboral poles. Tissues were stained with Mayer's hematoxylin and eosin (Goffredo et al. 2002).

Cytometric analyses were made with a light microscope NIKON Eclipse 80i using an image analysis systems: NIKON NIS-Elements D 3.1. All spermaries, classified into developmental stages in accordance with earlier studies on gametogenesis in scleractinians (Goffredo et al. 2002, 2005, 2010, 2012), were measured. The size of each spermary was determined as the mean of the two diameters (Goffredo et al. 2002).

Reproductive parameters

Spermatogenesis was studied through the following reproductive parameters: 1) *spermary abundance*, defined as the number of spermaries per body volume unit (100 mm^3); 2) *gonadal index*, defined as the percentage of body volume occupied by spermaries; 3) *spermary size*, defined as the average of the maximum and minimum diameter of spermaries.

Statistical analyses

A one-way permutation analysis of variance (PERMANOVA, Anderson 2001) based on Euclidean similarity was performed to test differences of spermary maturation stage distributions among Sites. This analysis was performed using PRIMER version 6. Levene's test was used for testing homogeneity of variance, and one-sample Kolmogorov-Smirnov's test was used to check for normality. The comparison with the normal distribution was test also using the powerful Shapiro-Wilk *W* test when the sample size was lower than 2000 (Shapiro and Francia 1972, Royston 1991). To compare reproductive parameters among Sites the non-parametric Kruskal-

Wallis equality-of-populations rank test (Kruskal and Wallis 1952) was used, as the assumptions for parametric statistics were not fulfilled. Spearman's rank correlation coefficient was used to calculate the significance of the correlations between spermary diameters and pH. Spearman's rank correlation coefficient is an alternative to Pearson's correlation coefficient (Altman 1991). It is useful for data that are non-normally distributed and do not meet the assumptions of Pearson's correlation coefficient (Potvin and Roff 1993). The analyses were computed using PASW Statistics 17.0.

Results

The analysis of 20 polyps revealed that sexuality of *Balanophyllia europaea* is hermaphroditic in all Sites along the pCO_2 gradient of the underwater crater. Spermary maturation stage distribution was the same among the three Sites (Fig 3), showing both early and advanced stages of maturation (stage: I, II, III, IV, V; Fig. 3; Fig. 4), with a prevalence of III, IV, V stages. Spermaries abundance and gonadal index did not show variations among the three Sites (Tables 2 and 3; Fig 5). Conversely, the diameter of spermaries was significantly different among Sites (Kruskall-Wallis test, $p < 0.001$) and increased with increasing of pCO_2 (Tables 2 and 3; Fig 5).

Discussion

This is the first long-term study on the influence of OA on spermatogenesis in a temperate zooxanthellate coral, naturally exposed to a pCO_2 gradient. Although there are available studies on coral reproduction, they describe the effect of the pH decrease exclusively by manipulative experiments with constant treatment conditions that eliminate natural variability (Albright 2011). Moreover, most of the researches regard mollusks (Kurihara et al. 2007; Parker et al. 2009; but

see Havenhand and Schlegel 2009; Ellis et al. 2009), crustaceans (Arnold et al. 2009; McDonald et al. 2009; Findlay et al. 2009, 2010) and echinoderms (Kurihara and Shirayama 2004; Havenhand et al. 2008; Dupont et al. 2008; Morita et al. 2009; Clark et al. 2009; Reuter et al. 2011; but see Byrne et al. 2010; Brennand et al. 2010; O'Donnell et al. 2010) with very little information available for corals, which are exclusively tropical (Albright et al. 2008; Morita et al. 2009; Cohen et al. 2009; Albright et al. 2010; Suwa et al. 2010; Albright and Langdon 2011; Albright and Mason 2013).

Specimens were collected in the maximum gonadal development period (just before fertilization) and thus advanced maturation stages of spermaries were expected. Increasing pCO_2 did not affect male germ cells, since the presence of all five spermary maturation stages have been observed in all the three Sites. Increasing pCO_2 did not show an influence on either spermary development or production (abundance and gonadal index) among Sites. Only spermary diameter was significantly different among Sites, displaying a positive correlation with increasing pCO_2. However, the medians of the three box plots almost overlap, providing no biological interpretation. Available studies indicate that gamete production may show resistance to acidification, although this question deserves further attention. Similarly, the corals *Montipora capitata*, *Oculina patagonica* and *Madracis pharensis* did not show a decrease in gamete production under acidified conditions, even though the former was exposed to lower pH only for 6 months and the other two corals for 12 months (Jokiel et al. 2008; Fine and Tchernov 2007). While gametogenesis may proceed normally, the spawning process (in particular of females) in *Astrangia poculata* was found to be susceptible to ocean acidification (Holcomb et al. 2011), suggesting that the energetically costly process of egg production leaves little energy available to the coral to sustain "normal" calcification rates in more acidic conditions (Cohen and Holcomb

2009). Also fertilization success can be negatively affected by elevated pCO_2, as observed for other two species of coral, *Acropora palmata* (Albright et al. 2010) and *Montastraea faveolata* (Albright 2011), but in these studies the effect of pCO_2 was dependent on the sperm concentration.

The results of this work are in agreement with a similar analysis for the same population of *B. europaea* performed on the oogenesis, confirming that gonadal development of this zooxanthellate coral is unaffected by an increase in pCO_2 (Gizzi 2016). However, its population density decreased in more acidic Site, with also a reduction in the number of immature polyps (Caroselli et al. in prep.). A constant gametogenesis but with a decline in juveniles could be explained by a possible vulnerability of the fertilization process or the larval phase or settlement to increase acidity. Moreover, a previous analysis performed along the same pH gradient on skeletal properties of *B. europaea* describes a decrease in net calcification rate (that is, gross calcification minus dissolution) due to an increase in skeletal porosity under low pH conditions, probably to maintain a constant linear extension rate, which is essential for reaching size at sexual maturity, thus for reproductive process (Fantazzini et al. 2015). The increase in skeletal porosity could be explained by a short-term study conducted in a different experimental site on *B. europaea*, which show an increase in gross calcification and also in dissolution in the most acidic condition, leading to a decrease in net calcification (Metalpa et al. 2011). Moreover, another short-term experiment along the pH gradient close to the underwater crater at Panarea, showed an increase in zooxanthellae photosynthetic efficiency of *B. europaea* under low pH conditions (Dubinsky personal observation), providing extra available energy that could be allocated towards reproduction.

The non-zooxanthellate coral *L. pruvoti*, transplanted at the same study site (short-term experiment), displayed a spermatogenesis negatively affected by increasing pCO_2, resulting in a delay of fertilization and planulation process (Gizzi 2016). Thus, the symbiosis with the zooxanthellae could be a benefit for *B. europaea* to cope with the increased pCO_2 expected by the end of this century.

References

Airi V, Gizzi F, Falini G, Levy O, Dubinsky Z, Goffredo S (2014) Reproductive efficiency of a Mediterranean endemic zooxanthellate coral decreases with increasing temperature along a wide latitudinal gradient. PLoS ONE, 9:e91792

Albright R (2011) Reviewing the effects of ocean acidification on sexual reproduction and early life history stages of reef-building corals. J Mar Biol Article ID 473615, pp 14

Albright R (2011) Effects of ocean acidification on early life history stages of Caribbean scleractinian corals, Ph.D. thesis, University of Miami/Rosenstiel School of Marine and Atmospheric Science

Albright R, Langdon C (2011) Ocean acidification impacts multiple early life history processes of the Caribbean coral *Porites astreoides*. Global Change Biol 17:2478-2487

Albright R, Mason B, Miller M, Langdon C (2010) Ocean acidification compromises recruitment success of the threatened Caribbean coral *Acropora palmata*. Proc Natl Acad Sci USA 107:20400-20404

Albright R, Mason B, Langdon C (2008) Effect of aragonite saturation on settlement and post-settlement growth of *Porites astreoides* larvae. Coral Reefs 27:485-490

Altman DG (1991) Practical Statistics for Medical Research. Chapman & Hall, London

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32-46

Anderson MJ, Gorley RN, Clark K (2008) PERMANOVA + for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth

Arnold KE, Findlay HS, Spicer JI, Daniels CL, Boothroyd D (2009) Effect of CO_2-related acidification on aspects of the larval development of the European lobster, *Homarus gammarus* (L.). Biogeosciences 6:1747-1754

Brennand HS, Soars N, Dworjanyn SA, Davis AR, Byrne M (2010) Impact of ocean warming and ocean acidification on larval development and calcification in the sea urchin *Tripneustes gratilla*. PLoS One 5:e11372

Byrne M, Soars N, Selvakumaraswamy P, Dworjanyn SA, Davis AR (2010) Sea urchin fertilization in a warm, acidified ocean and high pCO_2 ocean across a range of sperm densities. Mar Env Res 69:234-239

Byrne M, Oakes DJ, Pollak JK, Laginestra E (2008) Toxicity of landfill leachate to sea urchin development with a focus on ammonia. Cell Biol Toxicol 24:503-51

Caldeira K, Wickett ME (2003) Anthropogenic carbon and ocean pH. Nature 425:365

Caldeira K, Archer D, Barry JP, Bellerby RGJ, Brewer PG, Cao L, Dickson AG, Doney SC, Elderfield H, Fabry VJ, Feely RA, Gattuso JP, Haugan PM, Hoegh-Guldberg O, Jain AK, Kleypas JA, Langdon C, Orr JC, Ridgwell A, Sabine CL, Seibel BA, Shirayama Y, Turley C, Watson AJ, Zeebe RE (2007)

Comment on "Modern-age buildup of CO_2 and its effects on seawater acidity and salinity" by Hugo A. Loáiciga. Geophys Res Lett 34:L18608

Capaccioni B, Tassi F, Vaselli O, Tedesco D, Poreda R (2007) Submarine gas burst at Panarea Island, southern Italy, on 3 November 2002: a magmatic versus hydrothermal episode. J Geophys Res 112:B05201

Caroselli E, Prada F, Pasquini L, Nonnis Marzano F, Zaccanti F, Falini G, Levye O, Dubinskye Z, Goffredo S (2011) Environmental implications of skeletal micro-density and porosity variation in two scleractinian corals. Zoology 114:255-264

Carr RS, Biedenbach JM, Nipper M (2006) Influence of potentially confounding factors on sea urchin porewater toxicity tests. Arch Environ Contam Toxicol 51:573-579

Clark D, Lamare M, Barker M (2009) Response of sea urchin pluteus larvae (Enchinodermata: Echinoidea) to reduce seawater pH: a comparison among a tropical, temperate, and a polar species. Mar Biol 156:1125-1137

Cohen AL, Holcomb M (2009) Why corals care about ocean acidification: uncovering the mechanism. Oceanography 22:118-127

Cohen AL, McCorkle DC, De Putron S, Gaetani GA, Rose KA (2009) Morphological and compositional changes in the skeletons of new coral recruits reared in acidified seawater: insights into the biomineralization response to ocean acidification. Geochem Geophys Geosyst 10:1-12

de Putron SJ, McCorkle DC, Cohen AL, Dillon AB (2011) The impact of seawater saturation state and bicarbonate ion concentration on calcification by new recruits of two Atlantic corals. Coral Reefs 30:321-328

Dickson AG (1990) Thermodynamics of the dissociation of boric acid in synthetic sea water from 273.15 to 298.15 K. Deep-Sea Res A 37:755-766

Dickson AG, Millero FJ (1987) A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep-Sea Res A 34:1733-1743

Dinnel PA, Link JM, Stober QJ (1987) Improved methodology for a sea urchin sperm cell bioassay for marine waters. Arch Envic Contamin Toxicol 16:23-32

Doherty P, Fowler T (1994) An empirical test of recruitment limitation in a coral reef fish. Science 263:935-939

Dupont S, Havenhand J, Thorndyke W, Peck L, Thorndyke M (2008) Near-future level of CO_2-driven ocean acidification radically affects larval survival and development in the brittlestar *Ophiothrix fragilis*. Mar Ecol Prog Ser 373:285-294

Ellis RP, Bersey J, Rundle SD, Hall-Spencer JM, Spicer JI (2009) Subtle but significant effects of CO_2 acidified seawater on embryos of the intertidal snail, *Littorina obtusata*. Aquatic Biology 5:41-48

Fabricius KE, De'ath G, Noonan S, Uthicke S (2014) Ecological effects of ocean acidification and habitat complexity on reef-associated macroinvertebrate communities. Proc R Soc B 281:20132479

Fabricius KE, Langdon C, Uthicke S, Humphrey C, Noonan S, De'ath G, Okazaki R, Muehllehner N, Glas MS, Lough JM (2011) Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. Nature Clim Chang 1:165-169

Feely RA, Orr J, Fabry VJ, Kleypas JA, Sabine CL, Langdon C (2009) Present and future changes in seawater chemistry due to ocean acidification. Carbon Sequestration and Its Role in the Global Carbon Cycle 175-188

Findlay H, Kendall M, Spicer J (2009) Future high CO_2 in the intertidal may compromise adult barnacle *Semibalanus balanoides* survival and embryonic development rate. Mar Ecol Prog Ser 389:193-202

Findlay HS, Kendall MA, Spicer JI, Widdicombe S (2010) Post-larval development of two intertidal barnacles at elevated CO_2 and temperature. Mar Biol 157:725-735

Fine M, Tchernov D (2007) Scleractinian coral species survive and recover from decalcification. Science 315:1811

Gattuso JP, Buddemeier RW (2000) Ocean biogeochemistry: calcification and CO_2. Nature 407:311-313

Gizzi F (2016) Reproduction, growth and biomineralization of calcifying marine organisms and their relationships with environmental parameters. Dissertation Ph.D. thesis, University of Bologna

Goffredo S, Prada F, Caroselli E, Capaccioni B, Zaccanti F, Pasquini L, Fantazzini P, Fermani S, Reggi M, Levy O, Fabricius KE, Dubinsky Z, Falini G (2014) Biomineralization control related to population density under ocean acidification. Nat Clim Chang 4:593-597

Goffredo S, Marchini C, Rocchi M, Airi V, Caroselli E, Falini G, Levy O, Dubinsky Z, Zaccanti F (2012) Unusual pattern of embryogenesis of *Caryophyllia inornata* (Scleractinia, Caryophylliidae) in the Mediterranean Sea. Maybe agamic reproduction? J Morph 273: 943-956

Goffredo S, Gasparini G, Marconi G, Putignano MT, Pazzini C, Airi V, Zaccanti F (2011) Sexual reproduction in the Mediterranean endemic orange coral, *Astroides calycularis* (Scleractinia, Dendrophylliidae). Bull Mar Sci 87:589-604

Goffredo S, Gasparini G, Marconi G, Putignano MT, Pazzini C, Zaccanti F (2010) Gonochorism and planula brooding in the Mediterranean endemic orange coral *Astroides calycularis* (Scleractinia: Dendrophylliidae). Morphological aspects of gametogenesis and ontogenesis. Mar Biol Res 6:421-436

Goffredo S, Caroselli E, Mattioli G, Pignotti E, Dubinsky Z, Zaccanti F (2009) Inferred level of calcification decreases along an increasing temperature gradient in a Mediterranean endemic coral. Limnol Oceanogr 54:930-937

Goffredo S, Caroselli E, Mattioli G, Pignotti E, Zaccanti F (2008) Relationships between growth, population structure and sea surface temperature in the temperate solitary coral *Balanophyllia europaea* (Scleractinia, Dendrophylliidae). Coral Reefs 27:623-632

Goffredo S, Caroselli E, Pignotti E, Mattioli G, Zaccanti F (2007) Variation in biometry and population density of solitary corals with environmental factors in the Mediterranean Sea. Mar Biol 152:351-361

Goffredo S, Airi V, Radetić J, Zaccanti F (2006) Sexual reproduction of the solitary sunset cup coral *Leptopsammia pruvoti* (Scleractinia, Dendrophylliidae) in the Mediterranean. 2. Quantitative aspects of the annual reproductive cycle. Mar Biol 148:923-932

Goffredo S, Radetić J, Airi V, Zaccanti F (2005) Sexual reproduction of the solitary sunset cup coral *Leptopsammia pruvoti* (Scleractinia, Dendrophylliidae) in the Mediterranean. 1. Morphological aspects of gametogenesis and ontogenesis. Mar Biol 147: 485-495

Goffredo S, Arnone S, Zaccanti F (2002) Sexual reproduction in the Mediterranean solitary coral *Balanophyllia europaea* (Scleractinia, Dendrophylliidae). Mar Ecol Prog Ser 229:83-94

Havenhand JN, Fenina-Raphaela B, Thorndyke MC, Williamson JE (2008) Near-future levels of ocean acidification reduces fertilization success in a sea urchin. Curr Biol 18:R651-R652

Havenhand JN, Schlegel P (2009) Near-future levels of ocean acidification do not affect sperm motility and fertilization kinetics in the oyster *Crassostrea gigas*. Biogeosci Discuss 6:4573-4586

Holcomb M, Cohen AL, McCorkle DC (2011) A gender bias in the calcification response to ocean acidification. Biogeosci Discuss 8: 8485-8513

Jokiel PL, Rodgers KS, Kuffner IB, Andersson AJ, Cox EF, Mackenzie FT (2008) Ocean acidification and calcifying reef organisms: a mesocosm investigation. Coral Reefs 27:473-483

Kruskal WH, Wallis WA (1952) Use of ranks in one-criterion variance analysis. J Am Stats Assoc 47:583-621

Kurihara H, Kato S, Ishimatsu A (2007) Effects of increased seawater pCO_2 on early development of the oyster *Crassostrea gigas*. Aquatic Biology 1:91-98

Kurihara H, Shirayama Y (2004) Effects of increased atmospheric CO_2 on sea urchin early development. Mar Ecol Prog Ser 274:161-169

Lueker TJ, Dickson AG, Keeling CD (2000) Ocean pCO_2 calculated from dissolved inorganic carbon, alkalinity, and equations for K1 and K2: validation based on laboratory measurements of CO_2 in gas and seawater at equilibrium. Mar Chem 70:105-119

McDonald MR, McClintock JB, Amsler CD, Rittschof R, Angus RA, Orihuela B, Lutostanski K (2009) Effects of ocean acidification over the life history of the barnacle *Amphibalanus amphitrite*. Mar Ecol Prog Ser 385:179-187

Mehrbach C, Culberson CH, Hawley JE, Pytkowicz RM (1973) Measurements of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol Oceanogr 18: 897-907

Morita M, Suwa R, Iguchi A, Nakamura M, Shimada K, Sakai K, Suzuki A (2009) Ocean acidification reduces sperm flagellar motility in broadcast spawning reef invertebrates. Zygote 18:103-107

O'Donnell MJ, Todgham AE, Sewell MA, Hammond LM, Ruggiero K, Fangue NA, Zippay ML, Hofmann GE (2010) Ocean acidification alters skeletogensis and gene expression in larval sea urchins. Mar Ecol Prog Ser 398:157-171

Pachauri RK, Meyer LA (eds.) IPCC 2014: Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland

Parker LM, Ross PM, O'Connor WA (2009) The effect of elevated pCO_2 and temperature on the fertilization and embryonic development of the Sydney rock oyster *Saccostrea glomerata* (Gould 1850). Global Chang Biol 15:2123-2136

Potvin C, Roff DA (1993) Distribution-free and robust statistical methods: viable alternatives to parametric statistics. Ecology 74:1617-1628

Reuter KE, Lotterhos KE, Crim RN, Thompson CA, Harley CDG (2011) Elevated pCO_2 increases sperm limitation and risk of polyspermy in the red sea urchin *Strongylocentrotus franciscanus*. Glob Chang Biol 17:163-171

Ringwood AH (1992) Comparative sensitivity of gametes and early developmental stages of a sea urchin species (*Echinometra mathaei*) and a bivalve species (*Isognomon californicum*) during metal expostures. Arch Environ Contam Toxicol 22:288-295

Royston P (1992) Approximating the Shapiro-Wilk W-test for nonnormality. Statist Comput 2:117-119

Sabine CL, et al. (2004) The oceanic sink for anthropogenic CO_2. Science 305:367–371

Shapiro SS, Francia RS (1972) An approximate analysis of variance test for normality. J Am Stats Assoc 67:215-216

Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) IPCC 2007: Summary for policymakers. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge and New York: Cambridge University Press

Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds) IPCC 2013: Summary for Policymakers. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA

Suwa R, Nakamura M, Morita M, Shimada K, Iguchi A, Sakai K, Suzuki A (2010) Effects of acidified seawater on early life stages of scleractinian corals (Genus Acropora). Fish Sci 76:93-99

Tables

Table 1. **Seawater carbonate chemistry measurements for each Site.** The pH, temperature (T), total alkalinity (TA) and salinity (S) were used to calculate all the other parameters using CO2SYS software with dissociation constants. Mean pH values were calculated after conversion of data to hydrogen ion concentrations. Mean values are reported with the min and max values in brackets.

Measured Parameters

Treatment	pH (total scale)	T (°C)	TA (µmol kg^{-1})	S (‰)
Site 1	8.07 (7.82-8.45)	20.5 (14.3-26.0)	2438 (2368-2600)	37 (33-38)
Site 2	7.87 (7.54-8.25)	20.7 (14.4-26.0)	2429 (2334-2618)	37 (33-38)
Site 3	7.74 (7.05-8.21)	20.6 (14.4-26.0)	2426 (2343-2610)	37 (34-38)

Calculated Parameters

Treatment	*pCO_2 (µatm)	*HCO_3^- (µmol kg^{-1})	*CO_3^{2-} (µmol kg^{-1})	*DIC (µmol kg^{-1})	*Ω_{arag}
Site 1	391 (127-780)	1869 (1466-2144)	232 (120-398)	2114 (1867-2291)	3.6 (1.8-6.3)
Site 2	672 (234-1561)	2030 (1664-2264)	163 (68-314)	2214 (1984-2383)	2.5 (1.1-5.0)
Site 3	907 (262-5100)	2073 (1835-2365)	144 (25-243)	2246 (2089-2552)	2.2 (0.4-3.9)

pH (n = 103-110 per site), T (n = 112-115 per site) and S (n = 107-110 per site) were measured in July 2010, September 2010, November 2010, March 2011, June 2011, July-August 2011, November-December 2011, April-May 2012, June 2012 and May 2013. TA (n = 14 per site) was measured in September 2010, November 2010, March 2011, June 2011, July-August 2011, November-December 2011, April-May 2012, June 2012 and May 2013. pCO_2 = carbon dioxide partial pressure; HCO_3^- = bicarbonate; CO_3^{2-} = carbonate; DIC = dissolved inorganic carbon; Ωarag = aragonite saturation.

Table 2. **Mean abundance, gonadal index and diameter value of spermaries in each Site along the pH_{TS} gradient.**

Site	pH_{TS}	N_p	Abundance (#/100 mm^3) mean ± SE	Gonadal Index (%) mean ± SE	N_s	Diameter (µm) mean ± SE
1	8.07	7	1483 ± 537	0.9 ± 0.3	1935	116 ± 0.9
2	7.87	7	2855 ± 1209	2.1 ± 1.1	3874	122 ± 0.8
3	7.74	6	1077 ± 343	0.9 ± 0.3	1550	133 ± 1.4

N_p , number of polyps; N_s number of spermaries.

Table 3. **Kruskal-Wallis test and correlation analyses between reproductive parameters and pH_{TS} in the sampled Sites.**

Reproductive parameters	K-W	rho_S
Abundance (#/100 mm^3)	NS	-
Gonadal Index (%)	NS	-
Diameter (µm)	***	***

K-W, significance of the Kruskal-Wallis test; r_s, Spearman's correlation coefficient; *** $p < 0.001$; NS, not significant.

Figures

Figure 1. Living specimens of *Balanophyllia europaea* photographed at Scilla (South Italy, 38°01'N, 15°38'E). Photo by courtesy of Francesco Sesso.

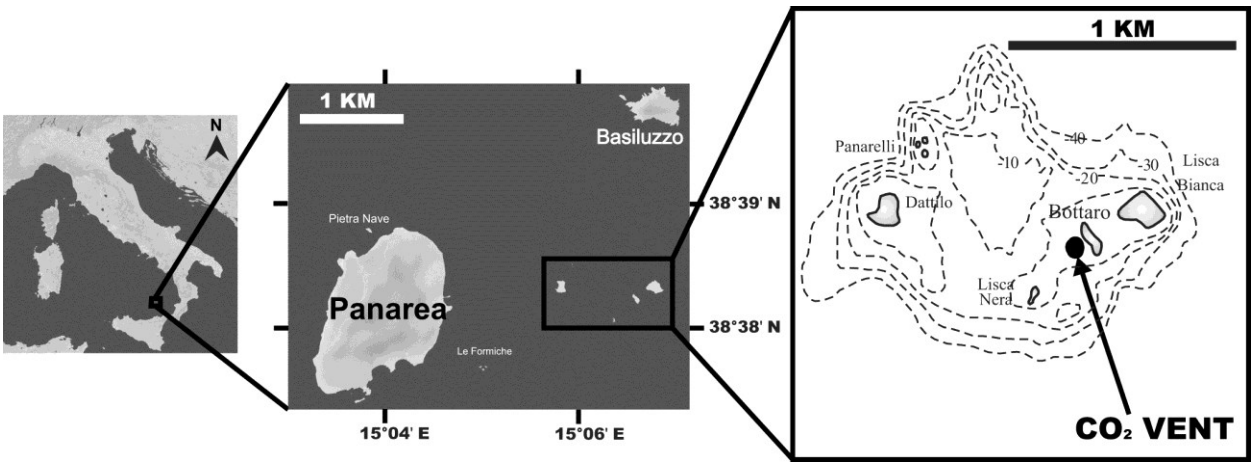

Figure 2. Map of the study site off Panarea Island (Aeolian Archipelago) with a close-up on the location of the vent area, Southeast of Bottaro, where corals were collected.

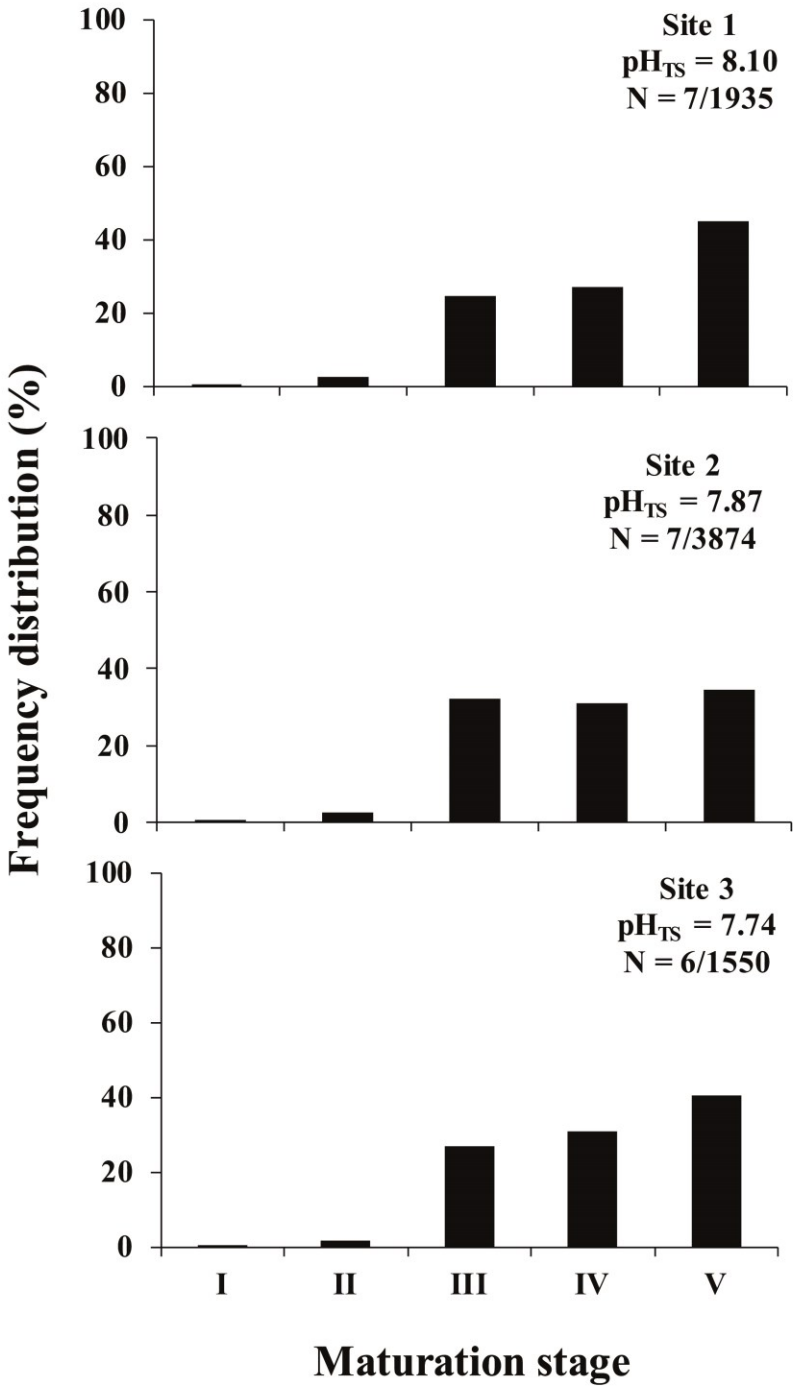

Figure 3. Spermary maturation stages distribution. Distribution of the five stages of spermary maturation in the three Sites along the pCO_2 gradient collected at Panarea Island in April 2013. N indicate the number of polyps/the total number of spermaries measured per Site.

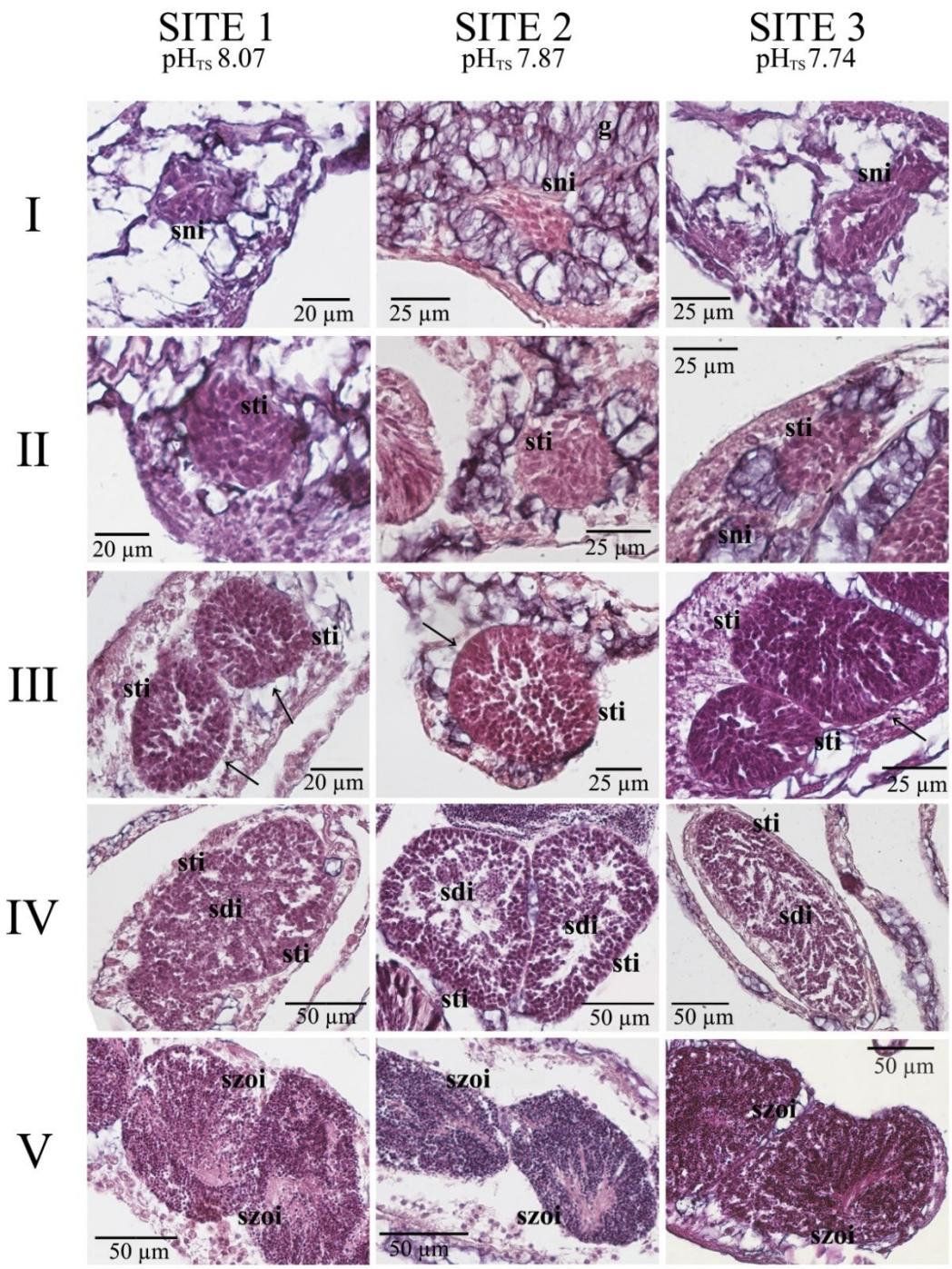

Figure 4. Spermatogenesis of *Balanophyllia europaea*. Five spermary maturation stages (I, II, III, IV, V) in the three study Sites along the pCO_2 gradient. Stage I: undifferentiated germ cells (spermatogonia) disposed in the gastrodermis layers of the mesentery. Stage II: the spermary is made up of a group of spermatocytes involved in the meiosis process. Stage III: the spermaries are delineated by a wall that has arisen from the mesoglea (arrows). Stage IV: the spermary presents an external layer of spermatocytes and an internal mass of spermatids. Stage V: the spermary is made up of a mass of spermatozoa. [g: gastrodermis; sni: spermatogonia; sti: spermatocytes; sdi: spermatids; szoi: spermatozoa].

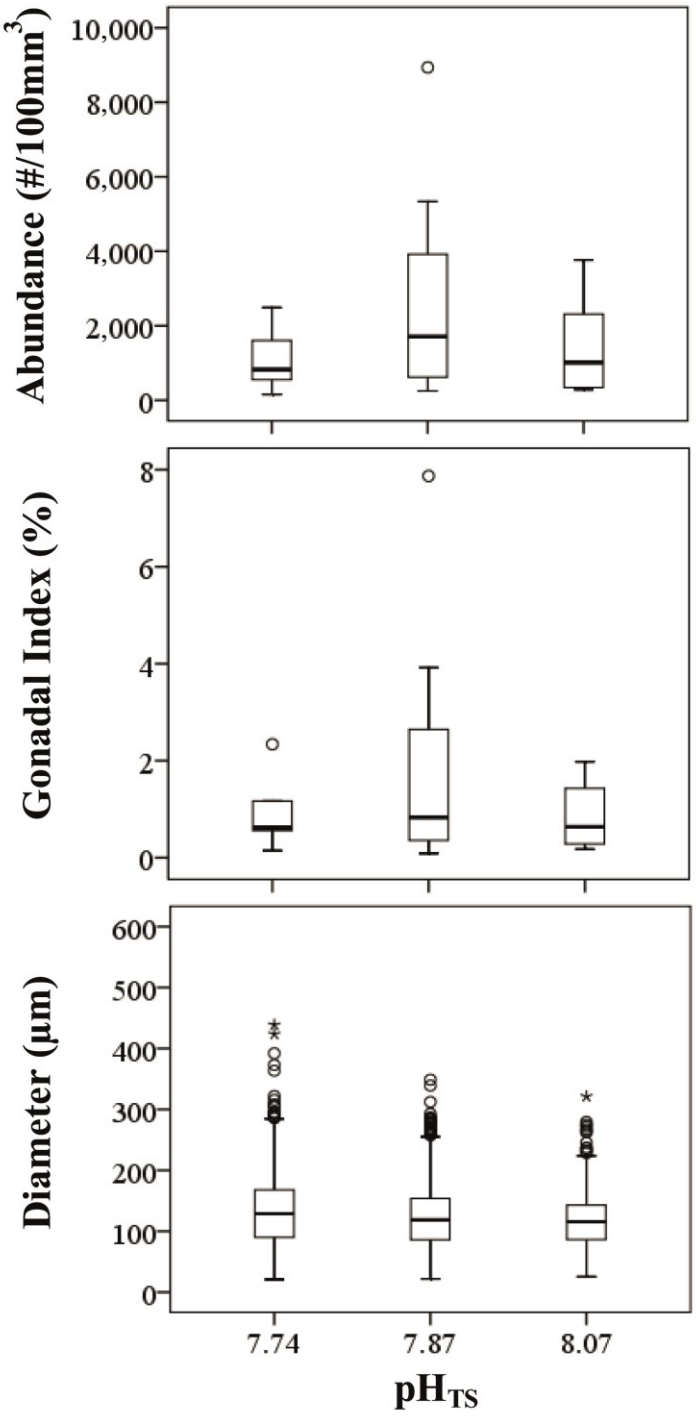

Figure 5. Spermary abundance, gonadal index and diameter of *Balanophyllia europaea*. Box plot of spermary abundance, gonadal index and diameter in the three study Sites showing median (solid horizontal line), first and third quartiles (box outline), and minimum and maximum values (whiskers) and outliers (circles and stars), recorded at Panarea Island in April 2013. See Table 2 for the number of polyps and spermaries analyzed and mean abundance, gonadal index and spermary diameter. See Table 3 for Spearman's correlation test.

Chapter V

Population structure of *Montastraea cavernosa* on shallow versus mesophotic reefs in Bermuda

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RESEARCH ARTICLE

Population Structure of *Montastraea cavernosa* on Shallow versus Mesophotic Reefs in Bermuda

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Abstract

Mesophotic coral reef ecosystems remain largely unexplored with only limited information available on taxonomic composition, abundance and distribution. Yet, mesophotic reefs may serve as potential refugia for shallow-water species and thus understanding biodiversity, ecology and connectivity of deep reef communities is integral for resource management and conservation. The Caribbean coral, *Montastraea cavernosa*, is considered a depth generalist and is commonly found at mesophotic depths. We surveyed abundance and size-frequency of *M*. *cavernosa* populations at six shallow (10m) and six upper mesophotic (45m) sites in Bermuda and found population structure was depth dependent. The mean surface area of colonies at mesophotic sites was significantly smaller than at shallow sites, suggesting that growth rates and maximum colony surface area are limited on mesophotic reefs. Colony density was significantly higher at mesophotic sites, however, resulting in equal contributions to overall percent cover. Size-frequency distributions between shallow and mesophotic sites were also significantly different with populations at mesophotic reefs skewed towards smaller individuals. Overall, the results of this study provide valuable baseline data on population structure, which indicate that the mesophotic reefs of Bermuda support an established population of *M*. *cavernosa*.

Introduction

In recent years, coral reefs have undergone drastic decline due to numerous anthropogenic impacts to environmental conditions including eutrophication, disease, the loss of herbivory, and bleaching associated with ocean warming [1–4]. Currently, nearly 30% of the world's coral reefs are considered severely damaged, and close to 60% are in danger of being lost by 2030 [5]. These losses are particularly pronounced on shallow water reefs of the Caribbean, where the comprehensive study by Jackson et al. [1] reports an overall decline in coral cover of 59%, from an average of 33% before 1984 to 14.3% since 2005. Deep reef systems in the mesophotic zone (>30m), however, have not experienced the same trend, displaying relatively stable coral

populations over time [6]. Yet, in comparison to shallow-water coral reefs, mesophotic reefs have received little attention [7].

Mesophotic coral ecosystems (MCE's) are comprised of a variety of taxa, including sponges, macroalgae, and azooxanthellate corals, as well as light-dependent zooxanthellate corals that exist in zones between approximately 30m and 150m in tropical and subtropical zones [8–10]. These regions tend to exist in low energy deep fore-reef zones that are characterized by steep gradients in light and temperature [8]. Typically the depth at which light is reduced to 1% of the available surface light defines the lower limits of the mesophotic zone [11]. Previous technological limitations have presented major challenges to conducting research on MCE's, resulting in limited understanding of the bathymetric and geographic extent of MCE's and the biodiversity and community structure they support across regions. Even basic taxonomic and systematic characterization of these communities is unknown, underscoring the importance of establishing baseline information on species assemblages and the roles they play in ecosystem function [10, 12].

Analyses of size-frequency distributions can reveal characteristics of species populations as they represent stages of population growth and decline [13]. Population size structure results from variations in rates of colony growth, recruitment and mortality, and may indicate individual sensitivities to life-history processes and environmental variation. The life cycle of modular organisms such as scleractinian corals, however, is complicated by processes such as fragmentation, fission, fusion, and partial mortality, making the relationship between surface area and age difficult to interpret [14]. Yet coral colony surface area can be correlated to age if partial mortality is low, and thus characterizations of population size-frequency distributions may provide critical demographic information, particularly for massive, non-branching colonies [13, 15–19]. Describing coral populations in terms of population size-frequency, therefore, can provide a snap-shot of current reef condition and if monitored over time may serve as an indicator for stability or decline [17, 20].

The aim of this study is to provide baseline characterization of population structure for the dominant zooxanthellate coral at adjacent shallow and mesophotic reefs in Bermuda. As such, this study provides an initial assessment of mesophotic reef condition in relation to environmental conditions that vary with depth, such as temperature and nutrient levels. Fricke and Meischner [21] conducted the only comprehensive study of mesophotic reef composition in Bermuda using submersible video transect surveys. Their study found species diversity decreased drastically below 40m. Among the species found in these mesophotic zones include *Agaricia fragilis*, *Stephanocoenia michelini*, *Madracis decactis*, *Scolymia cubensis*, *Montastraea cavernosa* and *Orbicella franksii*, with *M. cavernosa* and *O. franksii* being the dominant representatives below 30m. Using *in situ* diver-led surveys we examine variations in colony density, surface area, percent cover and size-frequency distributions and provide baseline data on the population structure of *M. cavernosa* on mesophotic reefs in Bermuda.

Materials and Methods

Ethics Statement

Surveys for this study were conducted in public areas outside of any marine reserves and did not require approval or permitting. No specimens were manipulated or collected from reef sites in completing this study and care was taken to avoid contact with benthic substrata.

Site and Species Selection

Located 32°N, 64°W, Bermuda's sub-tropical coral reefs represent the northernmost reef system in the Atlantic. The shallow rim reefs of this pseudo-atoll encircle the platform, dropping

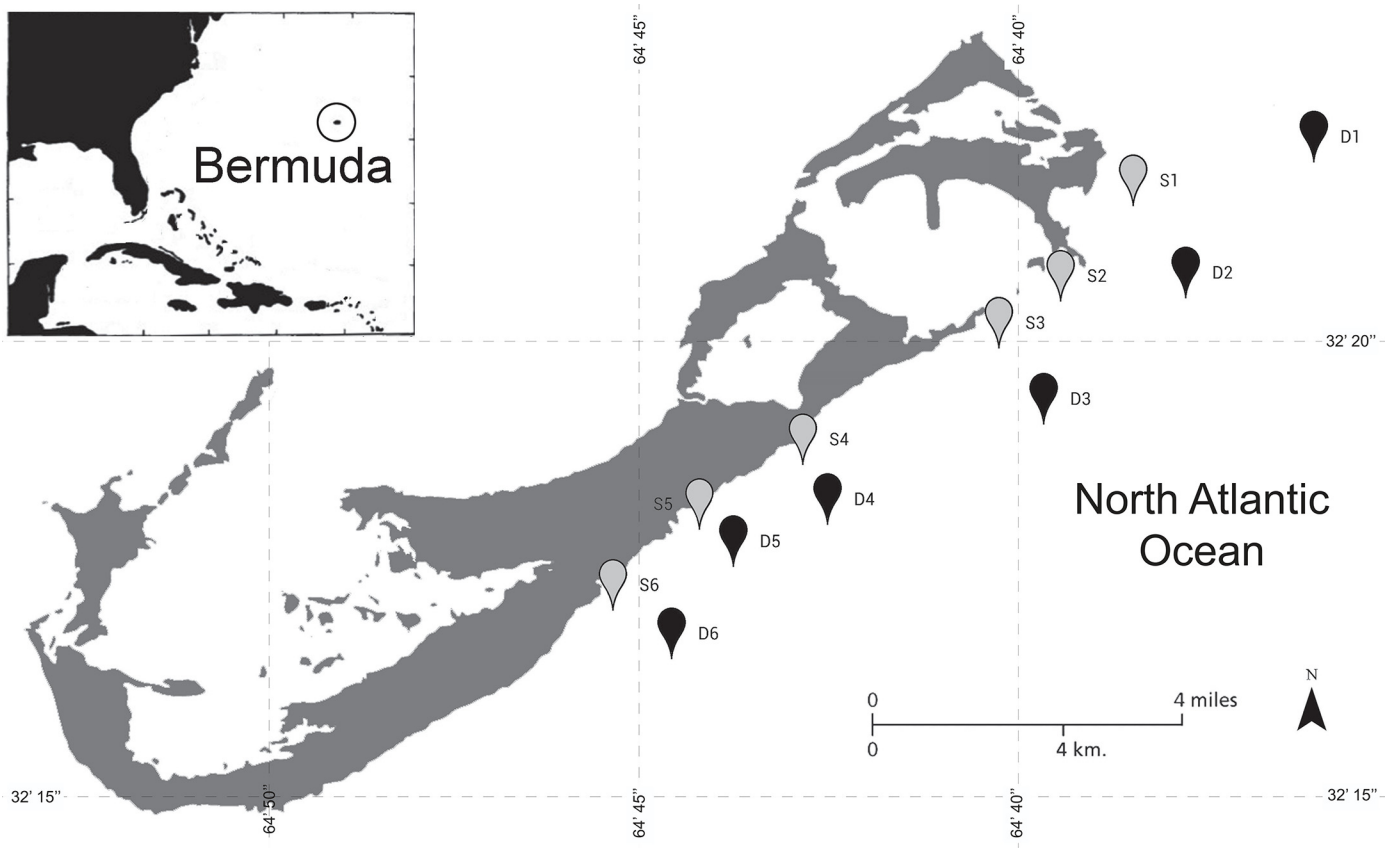

Fig 1. Survey Map. Survey locations on the south shore of Bermuda at shallow (10m; gray markers) and mesophotic (45m; black markers) sites. S1: Rita, 10m; D1: XL, 45m; S2: Coopers, 10m; D2: Coopers, 45m; S3: Tuckers, 10m; D3: Tuckers, 45m; S4: Spittal, 10m; D4: Spittal, 45m; S5: Devonshire, 10m; D5: Devonshire, 45m; S6: Hungry Bay, 10m; D6: Hungry Bay, 45m.

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quickly to deep mesophotic reefs. Thus, deep reefs are easily accessible in Bermuda and corals surviving in these zones are both at their latitudinal and bathymetric limits. Furthermore, shallow water coral cover in Bermuda ranks among the highest in the Caribbean with an estimated cover of 38.6% [1]. Bermuda, therefore, is an ideal and important location in which to study coral community composition and connectivity across a depth gradient.

M. cavernosa (Linnaeus, 1767) is a common reef building coral on fore reef slopes throughout the Caribbean and western Atlantic, extending from Bermuda to Brazil and the West African coast [22, 23]. *M. cavernosa* is considered an 'extreme' depth-generalist [9], as it inhabits depths from 3–100m across its geographical range [21, 24, 25]. Along its bathymetric distribution, *M. cavernosa* exhibits significant phenotypic plasticity in morphology, rates of respiration, and primary productivity [25–27], and is the only hermatypic species documented to survive below 70m in Bermuda [21].

Surveys

Twelve coral surveys were performed between August 17th and December 28th 2014 to estimate abundance and surface area of *M. cavernosa* colonies between shallow and mesophotic reef sites in Bermuda (Fig 1). Six surveys were conducted at shallow sites (10m depth), and six were conducted at nearby mesophotic sites (45m depth). Site names, map labels, GPS coordinates, and survey dates are included in Table 1. Site locations were selected based on accessibility and

Table 1. Survey Locations. Details of site locations surveyed including site map label (Fig 1), corresponding site name, depth (m), date surveyed, and GPS location (latitude and longitude).

Map Label	Site Name	Depth (m)	Date	Latitude	Longitude
S1	Rita	10	17-Aug-14	N32° 21' 29.3"	W64° 38' 29.3"
S2	Coopers	10	17-Aug-14	N32° 20' 28.4"	W64° 39' 28.1"
S3	Tuckers	10	5-Sep-14	N32° 19' 57.7"	W64° 40' 16.5"
S4	Spittal	10	5-Sep-14	N32° 18' 42.3"	W64° 42' 53.4"
S5	Devonshire	10	28-Dec-14	N32° 18' 0.7"	W64° 44' 16.1"
S6	Hungry Bay	10	28-Dec-14	N32° 17' 8.5"	W64° 45' 26.1"
D1	XL	45	17-Aug-14	N32° 21' 58.0"	W64° 36' 5.3"
D2	Coopers	45	17-Aug-14	N32° 20' 29.6"	W64° 37' 47.2"
D3	Tuckers	45	5-Sep-14	N32° 19' 8.8"	W64° 39' 41.0"
D4	Spittal	45	5-Sep-14	N32° 18' 3.7"	W64° 42' 34.7"
D5	Devonshire	45	21-Dec-14	N32° 17' 36.5"	W64° 43' 48.9"
D6	Hungry Bay	45	28-Dec-14	N32° 16' 37.5"	W64° 44' 39.4"

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visual identification of reef structure at mesophotic sites. Paired shallow sites were selected as the nearest site encountered at 10m depth traveling up the reef slope perpendicular to the shoreline. At each site, all *M*. *cavernosa* colonies with greater than 50% of the colony located within 1m of either side of a 30m transect tape (60m^2 total area per survey) were counted and largest surface diameter measured (to nearest cm). Diameter was chosen as a metric for ease of completing surveys at depth with minimal bottom time (maximum bottom time of 25 min.). Transects at each site were laid along the reef slope to ensure a constant depth, beginning at the closest non-living reef structure encountered upon reaching the benthos to which the tape could be secured. A colony was defined as any autonomous coral skeleton with living tissue as described by Meesters et al. [19].

Population Structure Analyses

Density of *M*. *cavernosa* colonies (# of colonies 60m^{-2}) met the assumptions of normality and equal variance and was analyzed by depth using a Student's *t*-test. Mean colony diameter was used to calculate surface area of each colony using the following equation: surface area = 2π (diameter/2)^2. Colony surface area was logarithmically transformed to reduce non-normality and heteroschedasticity and for each site, geometric mean (μ), standard deviation (SD), skewness (g_1), and kurtosis (g_2) were calculated. Mean colony surface area, standard deviation and skewness were compared by depth using the Student's *t*-test (n = 6), and kurtosis was compared by depth using a Mann-Whitney *U*-test [28–30]. These statistics describe the shape of a distribution and allow comparisons between populations at different depths independent of colony surface area [13, 19]. The total surface area per 60m^2 transect was also used to calculate percent cover of *M*. *cavernosa* (% cover 60m^{-2}). Data was transformed to arcsine values and compared by depth using a Student's *t*-test.

Mean size-frequency distributions were generated for each depth zone (shallow and mesophotic) and compared with each other by a Kolmogorov-Smirnov test and to a normal distribution using a Shapiro-Wilk W test [31–33]. Additionally, size-frequency distributions within each shallow and mesophotic site were compared using a Kolmogorov-Smirnov test. Similarity of size-frequency distributions between shallow and mesophotic sites was calculated with the Spearman rank-correlation coefficient by dividing colony numbers into 10 surface area size classes based on a logarithmic scale (class borders were < 0.5, 1.0, 1.5, 2.0. 2.5, 3.0, 3.5, 4.0, 4.5,

and >4.5 cm^2). Correlation coefficients were not normally distributed, and group means were tested with the Mann-Whitney *U*-test. All analyses were computed using PASW Statistics 17.0.

Size-frequency distributions within sites were also examined with a principal coordinate ordination (PCO) analysis based on Euclidean similarity, which generates a two-dimensional plot. PCO analysis is an equivalent to principal component analysis (PCA), but with more flexibility of resemblance measures [34] and allows spatial visualization of dissimilarities among sites and between depths. This analysis was performed using PRIMER version 6.

Nutrient and Temperature Analyses

Seawater samples were collected at two shallow sites and two mesophotic sites during survey dives (Tuckers and Spittal). Four replicate samples were collected at each site. Analysis of nitrate (NO_3), nitrite (NO_2), and silicate ($SiO4^{-2}$) were conducted at BIOS with a Seal Analytical AA3 continuous flow analyzer. Concentrations of nitrogen (NO_3 + NO_2) and silicate at each site met the assumptions of normality and equal variance and were analyzed by depth using Student's *t*-tests (n = 4 per site). Seawater temperature readings were recorded at each of the surveyed shallow and mesophotic sites between July 2014 and January 2015 using a Shearwater Petrel dive computer. Each site was visited twice during this time period for a total of 12 paired temperature readings. Mean temperatures were compared by depth using a Student's *t*-test (n = 6).

Results

Distribution Parameters

Table 2 gives the geometric mean surface area, skewness, kurtosis, maximum colony surface area, standard deviation, the probability that the sample is from a normal distribution, and the sample size at each site. Each parameter is also given for all shallow sites and all mesophotic sites combined.

Colony abundance, surface area and percent cover

The mean density of colonies varied significantly by depth (p = 0.002, Students *t*-test, $F = 0.106$, n = 6), with higher colony density at mesophotic sites compared with shallow sites

Table 2. Distribution Parameters. *M. cavernosa* population distribution parameters including site name, depth (m), geometric mean surface area (μ; cm^2), skewness (g_1), kurtosis (g_2), standard deviation (SD), maximum colony surface area (95%; cm^2), the probability that the populations is from a normal distribution (Pnorm), and the sample size (n) for each site surveyed and for all shallow sites and all mesophotic sites combined.

Site	Depth	μ	g_1	g_2	SD	95%	P_{norm}	n
Rita/XL	10	2508	-0.638	0.497	0.834	16343	0.042	26
Coopers	10	1731	0.389	-0.369	0.518	8836	0.012	12
Spittal	10	1503	-0.535	-0.281	0.457	5655	0.006	21
Tuckers	10	1991	-1.904	4.283	0.737	5284	0.000	6
Devonshire	10	1808	-0.154	-1.161	0.555	7697	0.005	29
Hungry Bay	10	2145	0.046	-1.052	0.617	13586	0.010	31
Rita/XL	45	522	-0.755	0.992	0.687	7697	0.009	62
Coopers	45	330	-0.763	0.630	0.555	2389	0.010	66
Spittal	45	639	-0.304	-0.178	0.709	3927	0.043	36
Tuckers	45	349	-1.088	0.945	0.767	3041	0.031	58
Devonshire	45	322	-0.260	0.004	0.749	2513	0.152	96
Hungry Bay	45	441	-0.614	0.273	0.719	2513	0.078	108
Shallow	**10**	**1933**	**-0.499**	**0.452**	**0.620**	**16343**	**0.000**	**125**
Deep	**45**	**434**	**-0.627**	**0.369**	**0.713**	**7697**	**0.000**	**426**

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(Fig 2A). Colony surface area also varied significantly between depths ($p<0.0001$, Students *t*-test, $F = 0.082$, n = 6), where mean colony surface area was smaller at mesophotic sites compared with shallow sites (Fig 2B; Table 2). Mean colony surface area at shallow sites was typically 4.5 times greater than at deeper sites, and maximum surface area was 2.1 times greater at shallow sites (16343cm^2) compared with mesophotic sites (7697cm^2). This large discrepancy in individual colony surface area resulted in relatively equal contributions to mean percent cover at each depth (p = 0.322, Students *t*-test, $F = 0.091$, n = 6), despite the higher density of colonies at mesophotic sites (Fig 2C).

Standard deviation, skewness and kurtosis

Standard deviations of colony surface area data did not differ significantly between shallow and mesophotic sites (Fig 3; p = 0.262, Student's *t*-test). This suggests that variation in colony surface area is similar at shallow and mesophotic sites.

The asymmetry around the mean of a size-frequency distribution is described as the skewness (g_1; Table 2); where a negative g_1 describes a distribution skewed to the left and a positive g_1 distribution is skewed to the right. In a perfectly symmetrical distribution, g_1 is zero [19]. Skewness did not vary significantly by depth (Fig 3; p = 0.649, Student's *t*-test). Distributions at mesophotic and shallow sites were negatively skewed, indicating a lower frequency of colonies in the smaller size classes.

The degree of peakedness of a distribution around its central mean is described as kurtosis (g_2), where a population can be either over centralized (leptokurtic, $g_2 > 0$) or flatter than normal (platykurtic, $g_2 < 0$). Kurtosis did not vary significantly by depth (Fig 3; p = 0.150, Mann-Whitney *U*-test), where the average kurtosis was 0.45 and 0.37 for shallow and mesophotic sites, respectively.

Size-Frequency Distributions

Mean size-frequency distributions for shallow versus mesophotic sites are given in Fig 4. Logarithmically transforming colony surface area data greatly improved normality. Mean distribution patterns from shallow and mesophotic sites were bell-shaped, yet differed significantly from a normal distribution (Table 2; $p<0.05$, Shapiro-Wilk W test). Furthermore, mean distribution differed significantly between shallow versus mesophotic sites, being skewed towards larger colonies at shallow sites compared with mesophotic sites (Fig 4; $p<0.001$, Kolmogorov-Smirnov test).

Distributions within each of the mesophotic sites were bell-shaped, and 2 out of six sites did not differ from normal distribution (Table 2, Fig 5; $p>0.05$). Distributions within the shallow sites were more variable due to the lower density of individuals, with distributions at all sites differing from a normal distribution (Fig 5; $p<0.05$). Similarity of size-frequency distributions from each site were compared using the Spearman rank-correlation coefficient. These comparisons showed that distributions from the same depths (from distant sites) were more similar than those from adjacent sites at different depths (Table 3). The mean correlation coefficient of distributions from sites at the same depths was 0.29 (SD = 0.08, n = 30), while the mean correlation coefficient of comparisons from adjacent sites at different depths was 0.24 (SD = 0.28, n = 6). These means are significantly different (p = 0.006, Mann-Whitney *U*-test). The high degree of similarity between distributions from the same depth suggests that the population structure of *M. cavernosa* has depth specific characteristics. The PCO results are provided in Fig 6, confirming a clear separation of the size-frequency distributions between depths and more similarity among sites of the same depth than between paired sites at different depths.

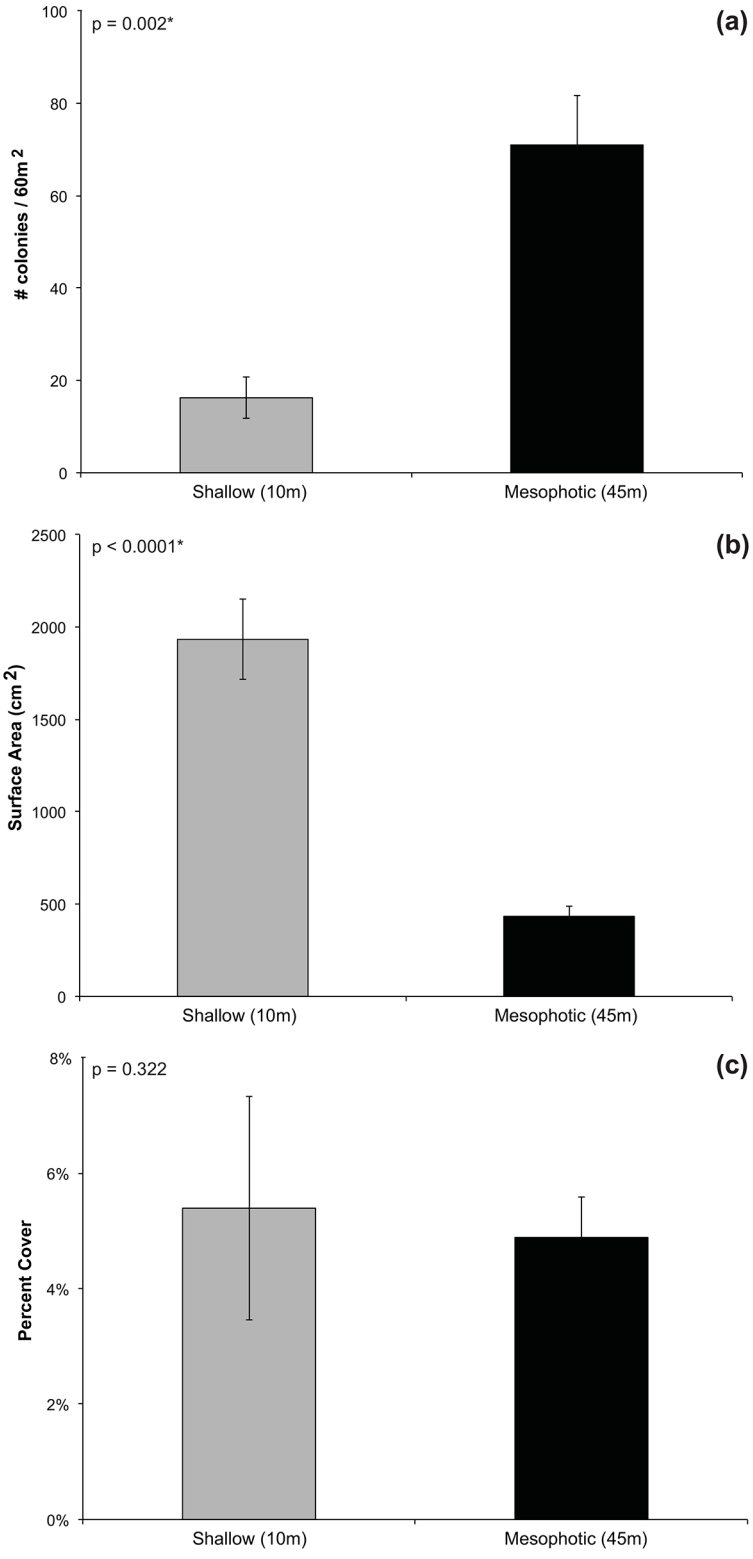

Fig 2. Colony Abundance, Surface Area, and Percent Cover by Depth. (a) mean number of *M*. *cavernosa* colonies per $60m^2$ ± SE at shallow (10m; gray bars) versus mesophotic (45m; black bars) sites (Rita/XL, Coopers, Tuckers, Spittal, Devonshire, Hungry Bay); (b) mean *M*. *cavernosa* colony surface area (cm^2) ± SE at shallow (10m; gray bars) versus mesophotic (45m; black bars) sites; (c) mean percent cover ± SE of *M*. *cavernosa* at shallow (10m; gray bars) versus mesophotic (45m; black bars) sites (n = 6 per depth).

doi:10.1371/journal.pone.0142427.g002

Fig 3. Distribution Parameters by Depth. Mean standard deviation, skewness, and kurtosis (± SE) of *M. cavernosa* population size-frequency distributions from measured colonies at shallow (10m; gray squares) and mesophotic (45m; black triangles) sites (Rita/XL, Coopers, Tuckers, Spittal, Devonshire, Hungry Bay).

doi:10.1371/journal.pone.0142427.g003

Nutrients and Seawater Temperature

Nutrient concentrations were higher on shallow sites compared with mesophotic sites (Fig 7A), with significant differences found in concentrations of nitrate and nitrite between depths ($p<0.0001$, Tuckers; $p = 0.019$, Spittal; Student's *t*-tests, $n = 4$) and silicate between depths at Tuckers ($p = 0.001$, Student's *t*-test, $n = 4$), but not at Spittal ($p = 0.058$, Student's *t*-test, $n = 4$). Mean seawater temperatures also differed significantly by depth, being higher on shallow sites compared with mesophotic sites ($p<0.0001$, Student's *t*-test, $n = 6$). Likewise, variation in temperature was more pronounced on shallow sites, ranging from 22.8 to 29.5°C, compared with mesophotic sites, ranging from 22.2 to 27.8°C (Fig 7B).

Discussion

This study documents the population structure of *M. cavernosa* at mesophotic versus shallow reefs in Bermuda and reveals depth specific characteristics of these populations. Our analyses show that size-frequency distributions of populations at shallow reefs vary significantly from those at mesophotic reefs (Fig 4), with colonies from neighboring reefs at the same depths being more similar to one another than to those from adjacent populations at different depths

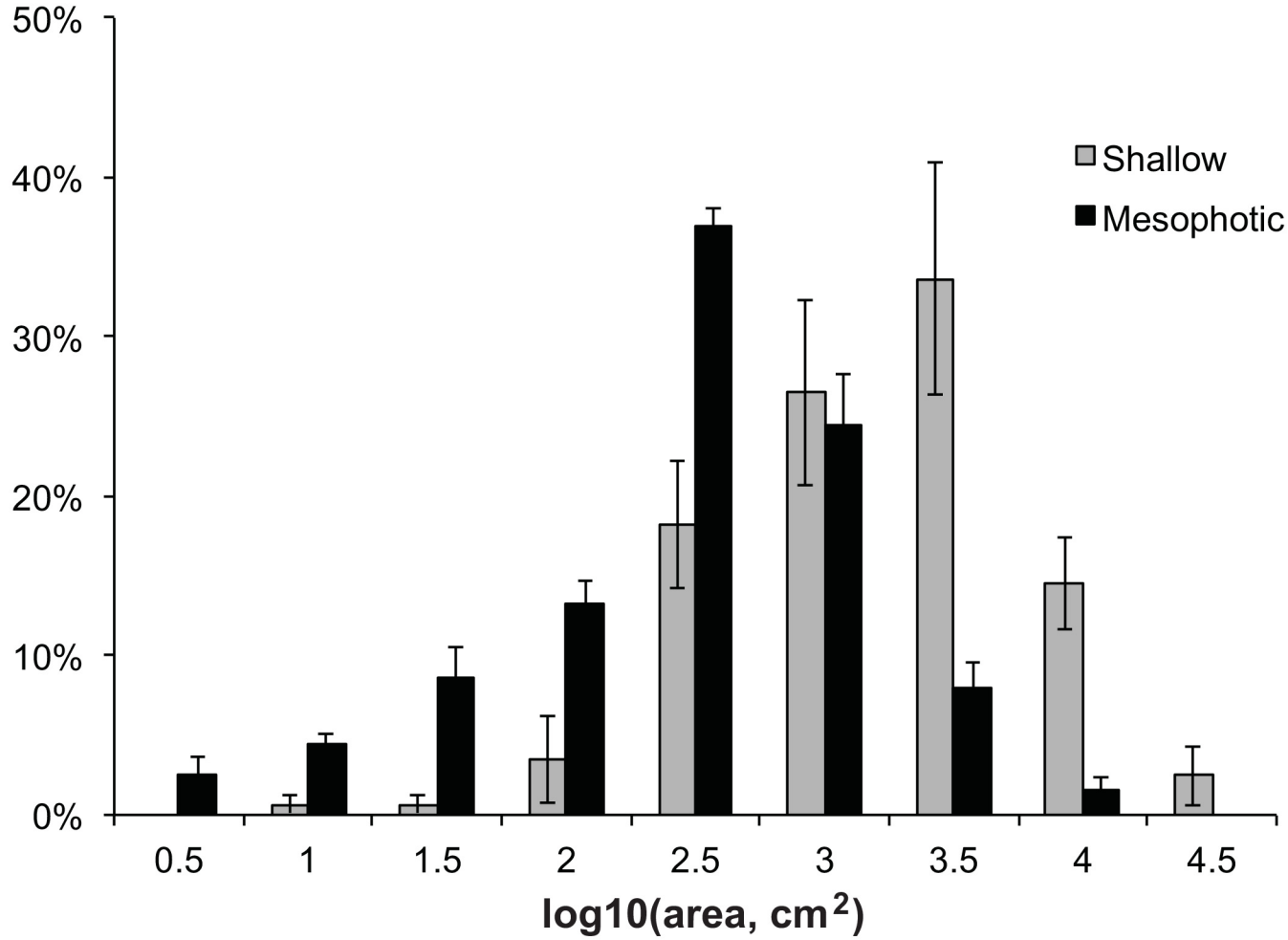

Fig 4. Mean Size-Frequency by Depth. Size-frequency distributions of *M. cavernosa* on a logarithmic scale represented as the mean proportion of individuals (± SE) within each log transformed size class for measured colonies from all shallow (10m; gray bars) and all mesophotic (45m; black bars) survey locations (Rita/XL, Coopers, Tuckers, Spittal, Devonshire, Hungry Bay).

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(Figs 4 and 5, Table 3). These results suggest that conditions that vary with depth, such as light, seawater temperature, and nutrient concentration, likely influence *M. cavernosa* population structure. Overall, this study found the distribution of populations at the mesophotic reef sites examined on Bermuda's south shore is shifted towards smaller individuals relative to shallow reefs (Fig 4). Likewise, the average colony surface area at these mesophotic reefs was significantly smaller than at shallow reefs (Fig 2A). However, it is important to note that a large degree of variation in colony morphology was observed at the different depths, with colonies at mesophotic sites being predominately flat and disc shaped, compared with colonies at shallow sites that varied from flat, to encrusting, to massive boulders. Thus, using diameter to estimate surface area likely underestimated total surface area of shallow-water colonies, resulting in a conservative estimate of colony size differential by depth in this study. These data indicate, therefore, that growth and maximum colony surface area may be limited on mesophotic reefs and suggests that maximum *M. cavernosa* colony surface area is likely controlled by environmental conditions that may limit energetic resources, such as light and nutrient availability.

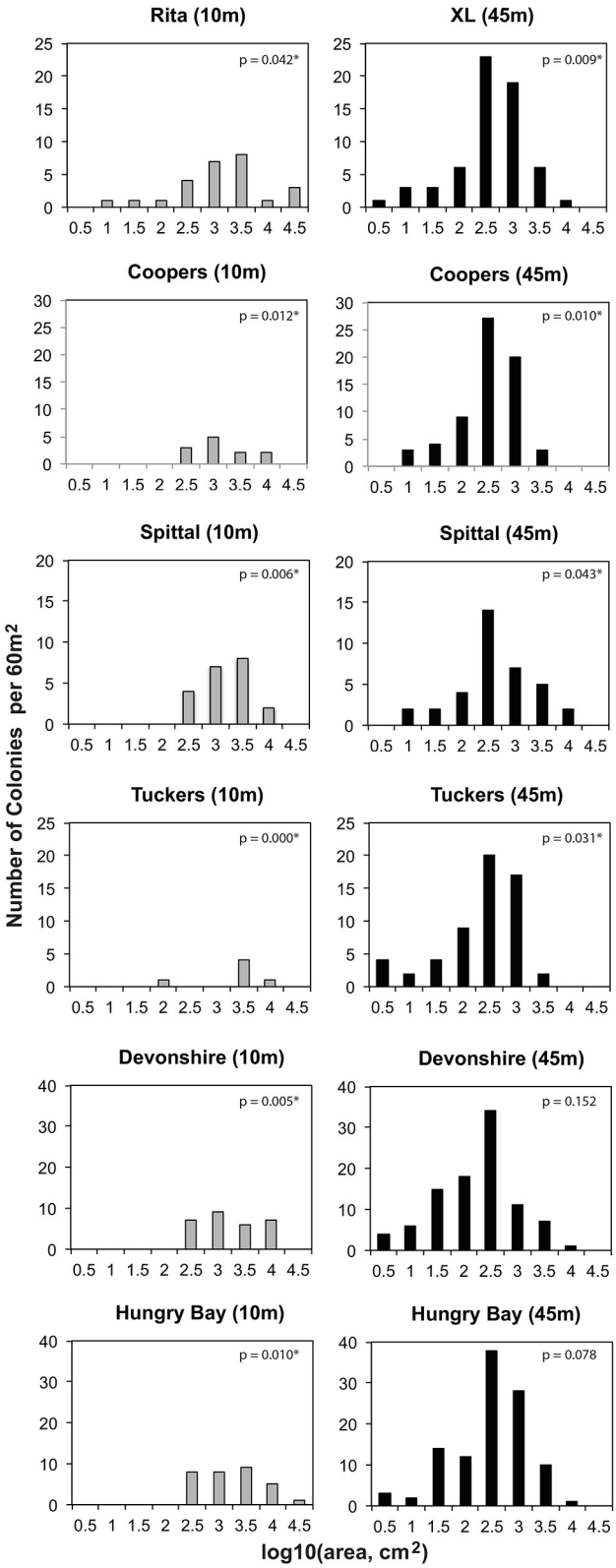

Fig 5. Size-Frequency by Site. Size-frequency distributions of *M. cavernosa* on a logarithmic scale represented as the number of individuals within each log transformed size class for colonies from each

survey location (Rita/XL, Coopers, Tuckers, Spittal, Devonshire, Hungry Bay) at 10m (gray bars) and 45m (black bars) depths. Sites that differed significantly from a normal distribution are indicated with an asterisk (*; $\alpha<0.05$).

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The smaller surface areas of *M*. *cavernosa* colonies found at mesophotic reefs in this study conforms to previous studies that document a decrease in coral colony surface area with depth [35] and the predominance of small colonies at deeper depth distributions [36, 37]. Smaller colony surface area may be a result of nutrient limitation at mesophotic reefs as nutrient analyses of adjacent mesophotic and shallow reefs in this study indicate that nutrients are significantly reduced at these mesophotic sites compared with shallow sites (Fig 7A). Likewise, Lesser et al. [25] document a reduction in phytoplankton availability and marked decreases in light-dependent productivity with depth, indicating that energy required for calcification and growth may indeed be limited for mesophotic corals [38, 39]. Under low-light and nutrient-limited conditions such as those present at mesophotic reefs, corals decrease metabolic demand through reduced respiration [40], slower growth, and morphological adaptations. For example, Grigg [41] found skeletal extension rates of *Porites lobata* declined exponentially with PAR from 3 to 50m in Hawaii. Likewise, Fricke et al. [35] report skeletal extension rates in *Leptoseris fragilis* at 90 to 120m of 0.5–0.8mm $year^{-1}$, which is significantly lower than typical rates reported for other non-branching shallow water corals ranging from 1.0–8.5mm $year^{-1}$ [42]. Alternatively, Bongaerts et al. [43] report an average growth rate of 22.0mm $year^{-1}$ for *Agaricia grahamae* fragments transplanted to 60m in Curacao, which is similar to growth rates in the congeneric species *A*. *humilis* and *A*. *agaricites* from shallow reefs (<30m) in the same region [44, 45]. Additionally, metabolic demands may be met through increased reliance on heterotrophy in conditions where primary production is limited. In the Bahamas, Lesser et al. [25] document a transition from autotrophy to heterotrophy with depth in populations of *M*. *cavernosa* between 45 and 61m associated with significant declines in primary productivity. Whether the energy consumed through heterotrophy is substantial enough to compensate for the reduction in primary production and maintain metabolic rates similar to shallow corals, however, is unclear. Thus, future studies of *M*. *cavernosa* on mesophotic reefs should include examinations of skeletal extension rates to determine rates of growth.

Despite the smaller surface area per colony of *M*. *cavernosa* at mesophotic sites, the relatively high density resulted in equal contributions to percent cover at mesophotic and shallow reefs (Fig 2). The high density of *M*. *cavernosa* colonies at mesophotic reefs may be related to lack of competition with other coral species that are unable to adapt to conditions at this depth.

Table 3. Correlation Coefficients. Spearman rank correlation coefficient values for comparisons of size-frequency distributions of *M*. *cavernosa* between sites. Values above the staggered line are comparisons among shallow sites; values below the staggered line are comparisons among mesophotic sites; values between the staggered lines are between adjacent shallow and mesophotic sites. Significant correlations (statistically similar; $\alpha = 0.05$) are indicated in bold.

	Spearman rank-correlation coefficient					
	Rita/XL	**Coopers**	**Spittal**	**Tuckers**	**Devonshire**	**HungryBay**
Rita/XL	**0.641**	**0.717**	0.279	**0.795**	**0.653**	**0.873**
Coopers	**0.934**	0.504	0.203	**0.934**	**0.988**	**0.880**
Spittal	**0.962**	**0.881**	**0.756**	0.418	0.229	0.306
Tuckers	**0.856**	**0.879**	**0.715**	-0.160	**0.903**	**0.943**
Devonshire	**0.905**	**0.931**	**0.816**	**0.892**	0.296	**0.847**
HungryBay	**0.917**	**0.919**	**0.829**	**0.941**	**0.939**	0.332

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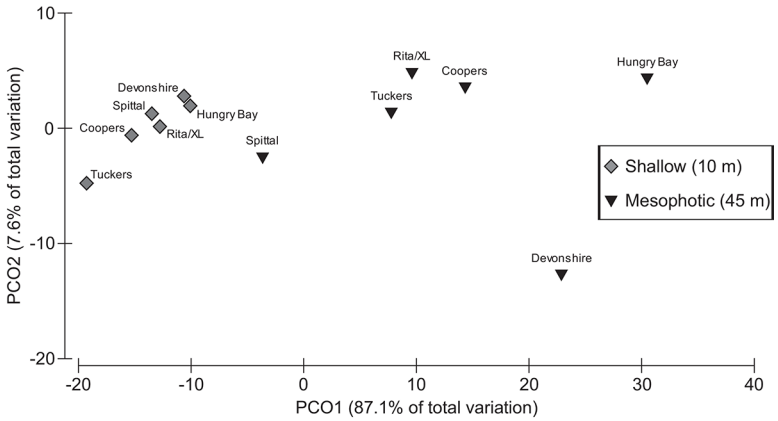

Fig 6. PCO of Population Structure by Site. Principal coordinates analysis (PCO) of *M. cavernosa* size-frequency distributions for each survey location (Rita/XL, Coopers, Tuckers, Spittal, Devonshire, Hungry Bay) at 10m and 45m depths. PCO1 and PCO2 axes together capture 94.7% of the total variation in size-frequency distribution.

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Diversity decreases dramatically at mesophotic reefs with only a handful of scleractinian species known to reside at these depths in Bermuda, including *A. fragilis*, *M. carambi*, *M. decactis*, *M. cavernosa*, *O. franksii*, *P. porites*, *S. michelini*, and *S. cubensis*. Among them, *M. cavernosa* is

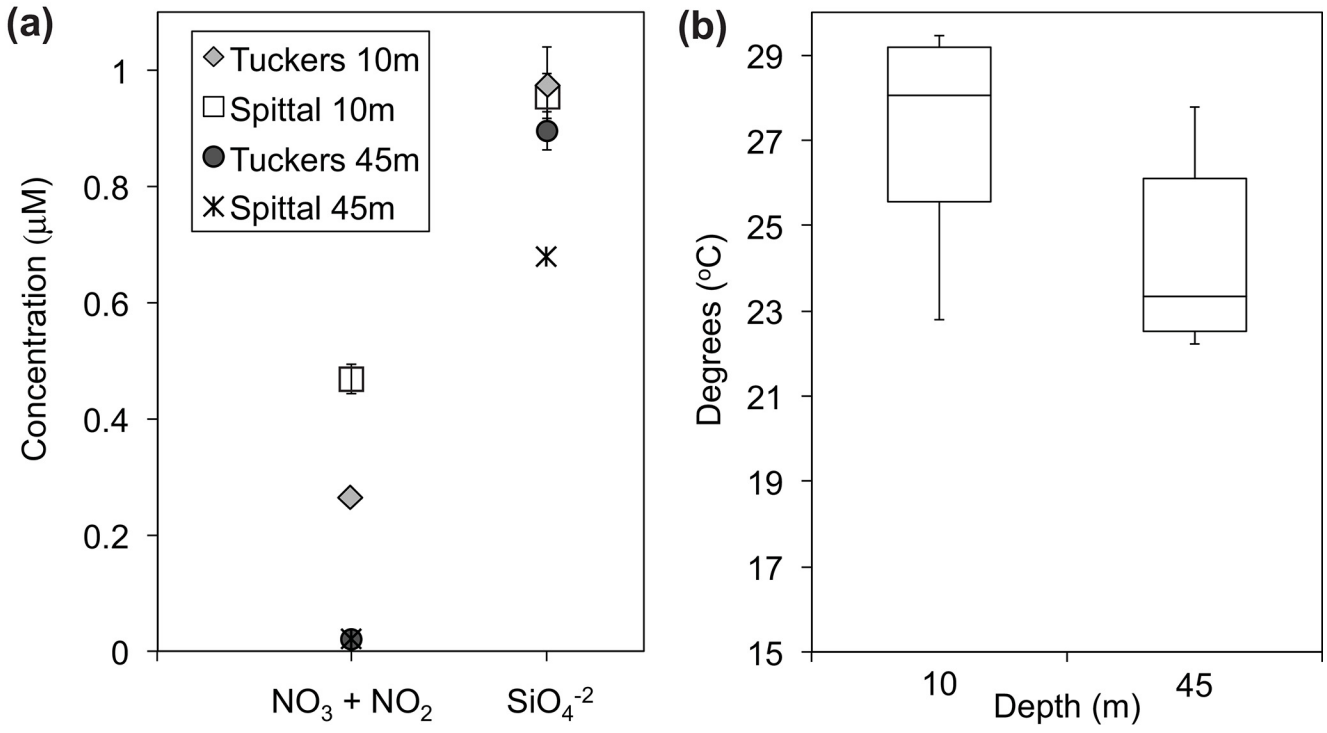

Fig 7. Nutrient Concentration and Temperature by Depth. (a) mean (±SD) concentration (μM) of nitrate (NO_3) + nitrite (NO_2) and silicate (SiO_4^{-2}) on shallow (10m; n = 4 per site) versus mesophotic sites (45m; n = 4 per site) from water samples collected September 5, 2015 ($NO_3 + NO_2$, $p<0.0001$, Tuckers, $p = 0.019$, Spittal; SiO_4^{-2}, $p = 0.001$, Tuckers, $p = 0.058$, Spittal; Student's *t*-tests); (b) box blot of seawater temperature at shallow (10m) and mesophotic (45m) sites showing median values (solid horizontal line), 25th and 75th percentile values (box outline), and minimum and maximum values (whiskers) recorded between July 2014 and January 2015 from 6 paired shallow (10m) and mesophotic (45m) survey sites (2 dives per site); Rita/XL, Coopers, Tuckers, Spittal, Devonshire, and Hungry Bay ($p<0.0001$, Students *t*-test, n = 6).

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the most predominant and is the only coral documented to survive in Bermuda below 70m [21, 46]. On surveys conducted for this study, *M*. *cavernosa* was the most abundant scleractinian species at these mesophotic sites, while *A*. *fragilis* was the second most abundant species with low rates of occurrence (Goodbody-Gringley, pers. obs.). Furthermore, recruitment for most taxa is documented to decline dramatically below 50m, indicating that competitive exclusion has less influence on community structure at depth [47]. Thus, competition for space is likely not a limiting factor for population density on mesophotic reefs, as restricted light and nutrient availability reduces the abundance and diversity of competitive species allowing *M*. *cavernosa* to become well established [47, 48].

Species distribution and population structure are highly influenced by characteristics of the physical environment such as temperature and wave energy, which vary with depth and thus affect coral population dynamics on mesophotic reefs. Thermally induced coral bleaching is known to cause significant mortality on shallow-water reefs [49], however mesophotic corals appear to be well insulated from the effects of increased sea surface temperature (SST), which may be in part due to the lower degree of variability in SST experienced on mesophotic reefs compared with shallow reefs (Fig 7B) [8, 50, 51]. Although increased SST is a reliable indicator of increasing temperature at mesophotic depths >30m [52], Lesser and Slattery [51] report bleaching to be virtually absent on corals inhabiting mesophotic reefs. Reduced occurrences of bleaching events on mesophotic reefs is likely due to a lower maximum SST (Fig 7B) and solar isolation [53] at depth, despite the potential for cold-water stress to induce bleaching as reported elsewhere on Caribbean reefs [54–56]. Likewise, hydrodynamic disturbance and exposure to wave energy are major factors influencing community structure in shallow reef systems. Such disturbances are minimal on mesophotic reefs, however, as surface wave energy attenuates with depth [57, 58]. Corals inhabiting mesophotic reefs are, therefore, buffered from direct physical damage from rough hydrodynamic conditions, which may contribute to the long-term stability of these ecosystems, although episodic storm events may cause fragmentation of branching, foliose and columnar coral colonies at depth [9, 59]. Additionally, human-mediated stresses appear to be reduced on mesophotic reefs due primarily to increased distance from human populations and greater depths than nearby shallow reef systems [60]. Therefore, corals inhabiting mesophotic zones may be protected from biotic and abiotic impacts that typically occur on shallow-water coral reefs.

In fact, the results of this study indicate that colonies of *M*. *cavernosa* appear to form relatively stable populations on mesophotic reefs in Bermuda. Mean population size structure was bell-curved (Fig 4), and standard deviation, skewness, and kurtosis did not vary greatly by site (Table 2). These results suggest that the mesophotic zone, which extends around the perimeter of the Bermuda platform, creates a viable habitat able to support an established population of *M*. *cavernosa*.

Likewise, the mean size-frequency distribution of shallow reef populations was also bell curved, however, the overall size structure of shallow sites was shifted towards larger individuals with the smallest size classes underrepresented (Fig 4). Previous studies on coral population structures suggest that environmental deterioration may skew populations towards a greater proportion of larger individuals [13, 19]. While the results of the present study may indicate that the shallow reef environment in Bermuda is less stable than mesophotic regions, there was no statistical difference in skewness of the populations preventing any conclusive remarks as to the stability of shallow sites versus mesophotic sites.

These findings support previous survey work conducted with submersibles and ROV's in other regions that show stable populations of scleractinian corals on mesophotic reefs, which have not undergone declines similar to those seen on their shallow water counterparts [6]. This apparent stability has led to the development of the "Deep Reef Refugia Hypothesis", which

posits that coral populations at depths greater than 30m could serve as a source/sink for genetic diversity and future repopulation of shallow regions [9]. Several recent studies have undertaken comparisons of conspecifics at neighboring deep and shallow reefs, and show that while a slight degree of genetic discontinuity appears to be present at certain locations, other shallow/deep populations display evidence of genetic connectivity, supporting the possibility of repopulation of deteriorating shallow reefs by deep reef populations [61–63]. Understanding the degree of genetic connectivity among shallow and mesophotic corals will, therefore, ultimately indicate the ability of deep reefs to contribute to shallow reef resilience. Likewise, determining the health and stability of mesophotic coral populations through demographic analyses will suggest the viability of these reefs to serve as a source of propagules to maintain shallow water reefs and help guide future management and conservation strategies [25].

The results presented here represent a baseline assessment of coral population structure and reef condition on MCE's in Bermuda. As the technology of mixed-gas closed circuit diving advances, it is anticipated that research on MCE's will rapidly increase. Access to baseline data on community structure and reef condition will be imperative for future examinations of population demography, assessments of connectivity, projections of ecosystem change, and the overall resilience of global coral reef systems.

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Author Contributions

Conceived and designed the experiments: GGG CM ADC SG. Performed the experiments: GGG CM ADC. Analyzed the data: GGG CM. Contributed reagents/materials/analysis tools: GGG ADC. Wrote the paper: GGG CM ADC SG.

References

1. Jackson JBC, Donovan M, Cramer K, VV L. Status and trends of Caribbean coral reefs: 1970–2012. Gland, Switzerland: Global Coral Reef Monitoring Network, IUCN; 2014.
2. Pandolfi JM, Bradbury RH, Sala E, Hughes TP, Bjorndal KA, Cooke RG, et al. Global trajectories of the long-term decline of coral reef ecosystems. Science. 2003; 301:955–8. PMID: 12920296
3. Hughes TP, Rodrigues LJ, Bellwood DR, Ceccarelli D, Hoegh-Guldberg O, McCook L, et al. Phase shifts, herbivory, and the resilience of coral reefs to climate change. Curr Biol. 2007; 17:360–5. PMID: 17291763
4. Carpenter KE, Abrar M, Aeby G, Aronson RB, Banks S, Bruckner A, et al. One-third of reef-building corals face elevated extinction risk from climate change and local impacts. Science. 2008:560–3. doi: 10.1126/science.1159196 PMID: 18653892
5. Wilkinson CR. Coral reefs are facing widespread extinctions: can we prevent these through sustainable managment practices? Proc 7th Int Coral Reef Symp. 2003; 1:11–21.
6. Bak RPM, Nieuwland G, Meesters EH. Coral reef crisis in deep and shallow reefs: 30 years of constancy and change in reefs of Curacao and Bonaire. Coral Reefs. 2005; 24:475–9.
7. Kahng SE, Copus JM, Wagner D. Recent advances in the ecology of mesophotic coral ecosystems (MCEs). J Exp Mar Biol Ecol. 2014; 7:71–81.
8. Lesser MP, Slattery M, Leichter JJ. Ecology of mesophotic coral reefs. J Exp Mar Biol Ecol. 2009; 375:1–8.

9. Bongaerts P, Ridgway T, Sampayo EM, Hoegh-Guldberg O. Assessing the deep reef refugia hypothesis: focus on Caribbean reefs. Coral Reefs. 2010; 29:309–27.
10. Hinderstein LM, Marr JCA, Martinez FA, Dowgiallo MJ, Puglise KA, Pyle RL, et al. Theme section on "Mesophotic Coral Ecosystems: Characterization, Ecology and Management". Coral Reefs. 2010; 29:247–51.
11. Clarke GL. Light penetration in the Western North Atlantic and its application to biological problems. Rapp. p.-v. réun.—Cons. int. explor. mer. 1936; 101:3–14.
12. Menza C, Kendall M, Hile S. The deeper we go the less we know. Rev Biol Trop. 2008; 56:11–24.
13. Bak RPM, Meesters EH. Coral population structure: the hidden information of colony size-frequency distributions. Mar Ecol Prog Ser. 1998; 162:301–6.
14. Hughes TP, Jackson JBC. Population dynamics and life histories of foliaceous corals. Ecol Monogr. 1985; 55:141–66.
15. Hughes TP, Jackson JBC. Do corals lie about their age? Some demographic consequences of partial mortality, fission and fusion. Science. 1980; 209:713–4. PMID: 17821194
16. Chadwick-Furman NE, Goffredo S, Loya Y. Growth and population dynamic model of the reef coral *Fungia granulosa* Klunzinger, 1879 at Eilat, northern Red Sea. J Exp Mar Biol Ecol. 2000; 249:199–218. PMID: 10841935
17. Meesters EH, Wesseling I, Bak RPM. Partial mortality in three species of reef-building corals and the relation with colony morphology. Bull Mar Sci. 1996; 58:838–52.
18. Meesters EH, Wesseling I, Bak RPM. Coral colony tissue damage in size species of reef-building corals: partial mortality in relation with depth and surface area. J Sea Res. 1997; 37:131–44.
19. Meesters EH, Hilterman M, Kardinaal E, Keetman M, deVries M, Bak RPM. Colony size-frequency distributions of scleractinian coral populations: spatial and interspecific variation. Mar Ecol Prog Ser. 2001; 209:43–54.
20. Caroselli E, Zaccanti F, Mattioli G, Falini G, Levy O, Dubinsky Z, et al. Growth and demography of the solitary scleractinian coral *Leptopsammia pruvoti* along a sea surface temperature gradient in the Mediterranean Sea. PLoS ONE. 2012; 7(6):e37848. doi: 10.1371/journal.pone.0037848 PMID: 22675495
21. Fricke HW, Meischner D. Depth limits of Bermudan scleractinian corals: a submersible survey. Mar Biol. 1985; 88:175–87.
22. Szmant AM. Reproductive ecology of Caribbean coral reefs. Coral Reefs. 1986; 5:43–53.
23. Veron J. Corals of the World. Stafford-Smith M, editor. Townsville, Australia: Australian Institute of Marine Science; 2000. 490 p.
24. Reed JK. Deepest distribution of Atlantic hermatypic corals discovered in the Bahamas. Proc 5th Int Coral Reef Symp. 1985; 6:249–54.
25. Lesser MP, Slattery M, Stat M, Ojimi M, Gates RD, Grottoli A. Photoacclimation by the coral *Montastraea cavernosa* in the mesophotic zone: light, food and genetics. Ecology. 2010; 91:990–1003. PMID: 20462114
26. Davies PS. Respiration in some Atlantic reef corals in relation to vertical distribution and growth form. Biol Bull. 1980; 158:187–94.
27. Lasker HR. Phenotypic variation in the coral *Montastraea cavernosa* and its effects of colony energetics. Biol Bull. 1981; 160:292.
28. Bendel RB, Higgins SS, Teberg JE, Pyke DA. Comparison of skewness coefficient, coefficient of variation, and Gini coefficient as inequality measures within populations. Oecologia. 1989; 78:394–400.
29. Sokal RR, Rohlf FJ. Biometry. The principles and practice of statistics in biological research, 3rd edn. New York: WH Freeman & Company; 1995.
30. Sokal RR, Rohlf FJ. Biometry, 2nd edn. New York: WH Freeman and Company; 1981.
31. Shapiro S, Francia R. An appropriate analysis of variance test for normality. J Am Statist Assoc. 1972; 67:215–6.
32. van Woesik R, Done T. Coral communities and reef growth in the southern Great Barrier Reef. Coral Reefs. 1997; 16:103–15.
33. McClanahan T, Ateweberhan M, Omukoto J. Lont-term changes in coral colony size distributions on Kenyan reefs under different management regimes and across the 1998 bleaching event. Mar Biol. 2008; 153:755–68.
34. Anderson M, Gorley R, Clarke K. PERMANOVA+ for PRIMER: Guide to software and statistical methods. Plymouth, United Kingdom: PRIMER-E Ltd; 2008.
35. Fricke HW, Vareschi E, Schlichter D. Photoecology of the coral *Leptoseris fragilis* in the Red Sea twilight zone (an experimental study by submersible). Oecologia. 1987; 73:371–81.

36. Maragos JE, Jokiel PL. Reef corals of the Johnston Atoll: one of the world's most isolated reefs. Coral Reefs. 1986; 4:141–50.
37. Kahng SE, Maragos JE. The deepest, zooxanthellate scleractinian corals in the world? Coral Reefs. 2006; 25:254.
38. Barnes D, Chalker B. Calcification and photosynthesis in reef-building corals and algae. In: Dubinsky Z, editor. Ecosystems of the world 25: coral reefs. Amsterdam: Elsevier; 1990. p. 109–31.
39. Falkowski PG, Jokiel PL, Kinsey RR. Irrandiance and corals. In: Dubinsky Z, editor. Ecosystems of the world 25: coral reefs. Amsterdam: Elsevier; 1990. p. 89–107.
40. Anthony KRN, Hoegh-Guldberg O. Variation in coral photosynthesis, respiration, and growth characteristics in contrasting light microhabitats: an analogue to plants in forest gaps and understoreys. Funct Ecol. 2003; 17:246–59.
41. Grigg RW. Depth limit for reef building corals in the Au'au Channel, S.E. Hawaii. Coral Reefs. 2006; 25:77–84.
42. Crabbe MJC. Scleractinian coral population size structures and growth rates indicate coral resilience on the fringing reefs of North Jamaica. Mar Environ Res. 2009; 67:189–98. doi: 10.1016/j.marenvres.2009.01.003 PMID: 19269026
43. Bongaerts P, Frade PR, Hay KB, Englebert N, Latijnhouwers KRW, Bak RPM, et al. Deep down on a Caribbean reef: lower mesophotic depths harbor a specialized coral-endosymbiont community. Scientific Reports. 2015; 5(7652).
44. Bak RPM. The growth of coral colonies and the importance of crustose coralline algae and burrowing sponges in relation to carbonate accumulation. Neth J Sea Res. 1976; 10:285–337.
45. Van Moorsel GWM. Disturbance and growth of juvenile corals (*Agaricia humilis* and *Agaricia agaricites*, Scleractinia) in natural habitats on the reef of Curacao. Mar Ecol Prog Ser. 1985; 24:99–112.
46. Venn AA, Weber FK, Loram JE, Jones RJ. Deep zooxanthellate corals at the high latitude Bermuda Seamount. Coral Reefs. 2009; 28:135.
47. Avery WE, Liddell WD. Sessile community recruitment patterns on shallow- and deep-reef hard substrata. Proc 8th Int Coral Reef Sym. 1997; 2:1179–84.
48. Liddell WD, Avery WE. Temporal change in hard substrate communities 10–250 m, the Bahamas. Proc 10th Int Coral Reef Symp. 2000; 1:437–42.
49. Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck R, Greenfield P, Gomez E, et al. Coral reefs under rapid climate change and ocean acidification. Science. 2007; 318:1737–42. PMID: 18079392
50. Bongaerts P, Bridge TCL, Kline D, Muir P, Wallace C, Beaman R, et al. Mesophotic coral ecosystems on the walls of Coral Sea atolls. Coral Reefs. 2011; 30:335.
51. Lesser MP, Slattery M. Phase shift to algal dominated communities at mesophotic depths associated with lionfish (*Pterois volitans*) invasion on a Bahamian coral reef. Biol Invasions. 2011; 13:1855–68.
52. Donner SD, Skirving WJ, Little CM, Oppenheimer M, Hoegh-Guldberg O. Global assessment of coral bleaching and required rates of adaptation under climate change. Global Change Biol. 2005; 11:2251–65.
53. Loya Y, Sakai K, Yamazato K, Nakano Y, Sambali H, Woesik Rv. Coral bleaching: the winners and the losers. Ecol Lett. 2001; 4:122–31.
54. Lirman D, Schopmeyer S, Manzella D, Gramer LJ, Precht WF, Muller-Karger F, et al. Severe 2010 cold-water event caused unprecedented mortality to corals of the Florida reef tract and reversed previous survivorship patterns. PLoS ONE. 2011; 6(8):e23047. doi: 10.1371/journal.pone.0023047 PMID: 21853066
55. Menza C, Kendall M, Rogers C, Miller J. A deep reef in deep trouble. Cont Shelf Res. 2007; 27:2224–30.
56. Kobluk DR, Lysenko MA. "Ring" bleaching in southern Caribbean *Agaricia agaricites* during a rapid water cooling. Bull Mar Sci. 1994; 54:142–50.
57. Huston MA. Patterns of species-diversity on Coral Reefs. Ann Rev Ecol Syst. 1985; 16:149–77.
58. Kahng SE, Garcia-Sais JR, Spalding HL, Brokovich E, Wagner D, Weil E, et al. Community ecology of mesophotic coral reef ecosystems. Coral Reefs. 2010; 29:255–75.
59. White KN, Ohara T, Fujii T, Kawamura I, Mizuyama M, Montenegro J, et al. Typhoon damage on a shallow mesophotic reef in Okinawa, Japan. PeerJ. 2013; 1:e151. doi: 10.7717/peerj.151 PMID: 24032094
60. Riegl B, Piller WE. Possible refugia for reefs in times of environmental stress. Int J Earth Sci. 2003; 92:520–31.

61. Serrano X, Baums IB, O'Reilly K, Smith TB, Jones RJ, Shearer TL, et al. Geographic differences in vertical connectivity in the Caribbean coral *Monstastraea cavernosa* despite high levels of horizontal connectivity at shallow depths. Mol Ecol. 2014; 23:4226–40. doi: 10.1111/mec.12861 PMID: 25039722

62. Bongaerts P, Riginos C, Ridgway T, Sampayo EM, Van Oppen MJH, Englebert N, et al. Genetic divergence across habitats in the widespread coral *Seriatopora histrix* and its associated *Symbiodinium*. PLoS ONE. 2010; 5(5):e10871. doi: 10.1371/journal.pone.0010871 PMID: 20523735

63. Brazeau DA, Lesser MP, Slattery M. Genetic structure in the coral *Montastraea cavernosa*: assessing genetic differentiation among and within mesophotic reefs. PLoS ONE. 2013; 8(5):e65845. doi: 10.1371/journal.pone.0065845 PMID: 23734263

Chapter VI

Conclusions

VI. Conclusions

This Ph.D. thesis investigated reproduction and population structure of scleractinian corals naturally occurring along environmental gradients, which may be used as natural laboratories for climate change studies. In particular, the research mainly focused on Mediterranean corals, which are still poorly studied compared to tropical species.

Reproduction of the Mediterranean coral *Caryophyllia inornata* is strongly influenced by seasonal variations of temperature and photoperiod, presenting a rapid oogenesis and an annual spermatogenesis. However, a peculiar embryogenesis without a clear seasonal trend was observed in females, males and sexually inactive individuals, suggesting a possible agamic development of the embryos. This research has been used as a pilot study in order to have a useful point of comparison and extend the analysis to other populations of the same species along a latitudinal gradient of temperature and solar radiation.

Reproductive traits of the non-zooxanthellate coral *Caryophyllia inornata* were homogeneous among the investigated populations, with no effect of temperature or solar radiation on their reproductive potential along the latitudinal gradient. Therefore, this species seems to be quite tolerant to environmental changes, as observed for another Mediterranean non-zooxanthellate species, *Leptopsammia pruvoti*, which was studied along the same gradient. This tolerance could depend on the lack of symbiosis with the zooxanthellae, making these species less susceptible to increasing temperature. Conversely, the zooxanthellate *Balanophyllia europaea* showed reduced reproductive efficiency in warmer populations along the same latitudinal gradient. An inhibition at high temperatures of zooxanthellae photosynthesis is

hypothesized, which could lead to reduced available energy for regeneration processes such as reproduction.

These different trophic strategies (mixotrophic/zooxanthellate versus heterotrophic/non-zooxanthellate) may also explain the different biological responses of corals to ocean acidification. The spermatogenesis of the zooxanthellate *B. europaea* collected along a natural pCO_2 gradient was not affected by low pH. This result, combined with the oogenesis results (obtained by another Ph.D. student of the research group) showed the same trend, suggesting that the reproductive activity of *B. europaea* seems quite tolerant to increasing pCO_2. In this case the symbiosis with the zooxanthellae may be an advantage, leading to an increase in photosynthetic efficiency when pH decreases due to rising pCO_2.

The ability to survive and reproduce under particular conditions is strongly related to population structure, which provides information on coral responses to the environment. In light of the rapid decline of coral reef health over the past several decades, the population structure of the tropical zooxanthellate coral *Montastraea cavernosa* was investigated along a depth gradient. This study was performed during my abroad period at the Bermuda Institute of Ocean Science (Bermuda) under the supervision of Dr Gretchen Goodbody Gringley. Mesophotic reefs are hypothesized to serve as refugia for shallow coral species exposed to environmental perturbations, where propagules from mesophotic reefs may repopulate shallow zones following disturbance events. Size frequency distribution was found to be depth dependent since the mesophotic zones were represented by smaller colonies but more numerous than the shallow zones. However, the percent cover was consistent between the two depths, indicating that the mesophotic population of *M. cavernosa* in Bermuda is quite stable. This analysis provided an initial assessment of mesophotic reef state in relation to environmental conditions that vary with

depth, such as temperature and nutrient levels. This is a preliminary study which envisions future comparisons among additional depths, including an analysis of the reproductive traits of this species. Understanding coral reproduction and population structure has major implications for the resilience and conservation of coral reef ecosystems.

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