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REPRODUCTION OF MEDITERRANEAN ZOOXANTHELLATE AND AZOOXANTHELLATE SCLERACTINIAN CORALS ALONG ENVIRONMENTAL GRADIENTS

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Esame finale anno 2014

"*Face à la mer le bonheur est une idée simple...*"

Chourmo. Jean Claude Izzo

To my daughter Irene

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Chapter I. General Introduction

Global knowledge provides a wealth of information on the biology and ecology of scleractinian coral reproduction that surpasses most other marine invertebrate groups, and corals could provide an important model for assessing life history and evolutionary theories (Harrison and Booth 2007). This studies focuses mainly on sexual reproductive characteristics in tropical zooxanthellate species due to their key role in coral reefs, which are one of the most biodiverse ecosystems on the planet (Bellwood and Hughes 2001). Additional studies regarding sexual reproduction in temperate and subtropical scleractinian species are crucial, in particular concerning non-zooxanthellate species, to provide new insights on these poorly studied organisms.

Sexual system, reproductive mode and propagation

The range of reproductive processes and modes in corals partially reflects the extraordinary ability of cnidarian cell lines to differentiate and re-differentiate (Holstein et al. 2003), which provides their tissues with remarkable developmental plasticity and adaptability. Corals have two different primary modes of development: broadcast spawning of gametes from their polyps into the water column for external fertilization and development (spawners); or brooding of embryos within their polyps, with subsequently release (brooders; Harrison and Wallace 1990). The vast majority of corals studied to date are broadcast spawning species; hence, external fertilization leading to planktonic larval development is the dominant mode of development (Harrison 2011). The spatial and temporal organization of sex function (male, female or hermaphrodite) within individual organisms, colonies, populations or species is referred to as the sexual system (revised in Guest 2012). Simultaneous hermaphroditic coral species typically produce both male and female gametes at the same time within the same individual (Policansky 1982), and represent the vast majority of known coral species (Harrison 2011). Sequential hermaphrodites have mature gametes at different times, during the same breeding season, or during their lifetime (Policansky 1982). Gonochoric coral species are less commonly reported than hermaphroditic ones and are characterized by colonies or solitary corals that have separate sexes and function only as male or female (Harrison and Wallace 1990).

The extent and importance of asexual versus sexual propagation varies greatly among populations of corals and among coral species (Ayre and Hughes 2000; Baums et al. 2006; Foster et al. 2007). Asexual reproduction generates genetically identical modules that may prolong the survival of the genotype, whereas sexual reproduction allows genetic recombination and production of new coral genotypes that may enhance fitness and survival of the species (Harrison 2011). Corals exhibit a wide range of asexual reproductive processes that produce new clonal solitary polyps or colonies (Harrison and Wallace 1990). These processes include colony fragmentation, colony

fission, polyp expulsion or polyp "bail-out," and budding from an anthocaulus or regenerating tissues (Kramarsky-Winter et al. 1997; Gilmour 2002). Asexual production of brooded planulae is generally uncommon and has been documented in some populations of the reef coral *Pocillopora damicornis* (Ayre and Miller 2004; Sherman et al. 2006), and in *Tubastrea coccinea* and *Tubastrea diaphana* (Ayre and Resing 1986). *Oulastrea crispata* may also brood asexually by producing planulae during periods when sexual reproduction has ceased (Lam 2000). In a recent study, carried out during my PhD course, we have found clues of an agamic production of brooded planulae in the Mediterranean solitary non-zooxanthellate scleractinian *Caryophyllia inornata* (Goffredo et al. 2012; Chapter 2 of present work). This gonochoric species exhibited an unusual pattern of embryogenesis, with embryos produced throughout the whole year also in male and sexually inactive polyps. An asexual origin of embryos was hypothesized, but further molecular analyses are needed to confirm this hypothesis.

Environmental parameters on reproductive timing

Sexual reproductive processes in corals appear to be strongly influenced by various environmental factors regulating and synchronizing reproductive cycles and gamete maturation (Harrison and Wallace 1990). Annual reproductive patterns are typical for the majority of anthozoans, but the timing and the degree of synchronization of spawning events vary geographically, ranging from the highly coordinated mass phenomena described for the Great Barrier Reef (Babcock et al. 1986), to the continuous or random breeding throughout the year reported for a few species in shallow and deep waters (Waller and Tyler 2005). Photoperiod and solar radiation (Babcock et al. 1994), lunar (Levy et al. 2007) and tidal cycles (Babcock et al. 1986), food availability (Coma et al. 1995) and local seasonal environmental phenomena have been suggested as temporal cues or environmental pressures regulating breeding time, as an alternative to or in combination with temperature cycles. In temperate corals, seawater temperature has long been considered as a primary variable providing a reliable cue to reset the biological clock since temperature affects the metabolism, which in turn affects the gametogenesis (Nozawa 2012).

Annual reproductive cycle in the Mediterranean scleractinian corals *Balanophyllia europaea* (Goffredo et al. 2004), *Leptopsammia pruvoti* (Goffredo et al. 2006) and *Cladocora caespitosa* (Kružić et al. 2008; Kersting et al. 2013) show a seasonal gonadal development, induced by the annual variation of seawater temperature and photoperiod. I was involved in a study that described the sexual reproductive cycle (gamete development, planulation timing and its relation to environmental parameters) of *Astroides calycularis* (Goffredo et al. 2011; Chapter 3). This colonial scleractinian, endemic of the southwestern Mediterranean Sea, showed a pattern of sexual

reproduction linked to seasonal variation of photoperiod and temperature confirming the assumption of a marked environmental control on gonadal development in scleractinian corals. This study contributed to increase the paucity of knowledge on sexual reproduction and gonadal output of Mediterranean corals, which is strongly related to their population demography (Goffredo et al. 2007), and is essential for the management and preservation of the Mediterranean marine ecosystem (Fiorillo et al. 2013).

Global climate change and reproduction

Global climate change is having profound and diverse consequences on marine ecosystems. Rising atmospheric carbon dioxide (CO_2) is one of the most critical problems of our times since its effects are globally pervasive and irreversible on ecological timescales (Field et al. 2012). The primary direct consequences are increasing acidity (ocean acidification; Doney et al. 2009) and temperatures (ocean warming; Field et al. 2012). In recent decades, the rates of change have been rapid and may compromise the ability to adapt of many organisms. Furthermore, rates of physical and chemical change in marine ecosystems will almost certainly accelerate over the next several decades in the absence of immediate and dramatic efforts toward climate mitigation (Field et al. 2012). Direct effects of changes in ocean chemistry and temperature may alter the physiological functioning, behavior, and demographic traits (e.g., productivity) of organisms, leading to shifts in size structure, spatial range and abundance of populations (Doney et al. 2012).

In scleractinian corals, the most likely consequences of ocean warming and acidification, are generally regarded as reduced calcification (Andersson et al. 2011) and increased frequency and severity of coral bleaching events (a breakdown of the symbiotic relationship between corals and their endosymbiotic algae; Baker et al. 2008). As the oceans continue to warm and acidify, it becomes essential to understand the repercussions that these changes will have on sexual reproduction.

While many studies on ocean warming and acidification have focused on the sensitivity of adult growth and calcification (Albright and Mason 2013), rising experimental evidence now suggest that numerous early life and reproductive processes may also be negatively impacted. Lower pH seems to negatively influence coral reproduction by reducing sperm motility, fertilization success and larval dispersion (Albright 2011). Harmful effects of ocean warming hit individual fecundity, egg quality, fertilization success and recruitment (McClanahan et al. 2009; Albright and Mason 2013). A growing number of studies investigate the independent effects of temperature and pH on early life history stages in tropical symbiotic corals (Albright 2011). However, few studies

have investigated the interactive effects of these stressors (Albright and Mason 2013), and little concern has been paid to temperate corals.

The Mediterranean is a biodiversity hotspot under intense pressure from anthropogenic impacts (Lejeusne et al. 2010). There are reasons to believe that the Mediterranean is already one of the most impacted seas in the world, since climate change interacts synergistically with many other disturbances (Lejeusne et al. 2010). Climatic models further predict that the Mediterranean basin will be one of the most impacted regions by the ongoing warming trend and by an increase in extreme events (Field et al. 2012). This makes the Mediterranean a potential model of more global patterns to occur in the world's marine biota, and a natural focus of interest for research on climate (Lejeusne et al. 2010).

Most of my PhD research focused on the potential effect of temperature increase on the reproduction of two Mediterranean scleractinians: *B. europaea* (Airi et al. 2014; Chapter 4) and *L. pruvoti* (Airi et al. manuscript in preparation; Chapter 5). Reproductive output in these two species has been quantified in a natural gradient of solar radiation and seawater temperature along the western Italian coast. Coupling these results with previous studies on growth, demography and calcification performed along the same gradient (Goffredo et al. 2004, 2007, 2008, 2010; Caroselli et al. 2011, 2012a 2012b), leads to the conclusion that non-zooxanthellate species may be more tolerant to temperature increase than symbiotic ones.

List of 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, in press. DOI: 10.1371/journal.pone.0091792.

Airi V, Prantoni S, Calegari M, Falini G, Levy O, Dubinsky Z, Goffredo S 2014 Reproductive output of a non-zooxanthellate temperate coral is unaffected by temperature along an 850 km latitudinal gradient. Manuscript in preparation.

Albright R 2011 Reviewing the effects of ocean acidification on sexual reproduction and early life history stages of reef-building corals. Journal of Marine Biology 2011.

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

Andersson AJ, Mackenzie FT, Gattuso JP 2011 Effects of ocean acidification on benthic processes, organisms, and ecosystems. In: Gattuso JP, Hansson L, editors. Ocean Acidification. Oxford University Press. pp. 122-153.

Ayre DJ, Hughes TP 2000 Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. Evolution 54: 1590-1605.

Ayre DJ, Miller KJ 2004 Where do clonal coral larval go? Adult genotypic diversity conflicts with reproductive effort in the brooding coral *Pocillopora damicornis*. Marine Ecology Progress Series 277: 95-105.

Ayre DJ, Resing JM 1986 Sexual and asexual production of planulae in reef corals. Marine Biology 90: 187-190.

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. Marine Biology 90: 379-394.

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

Baker AC, Glynn PW, Riegl B 2008 Climate change and coral reef bleaching: An ecological assessment of long-term impacts, recovery trends and future outlook. Estuarine, Coastal and Shelf Science 80: 435-471.

Baums IB, Miller MW, Hellberg ME 2006 Geographic variation in clonal structure in a reef building Caribbean coral, *Acropora palmata*. Ecological Monographs 76: 503-519.

Bellwood DR, Hughes TP 2001 Regional-scale assembly rules and biodiversity of coral reefs. Science: 292: 1532-1535.

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

Caroselli E, Mattioli G, Levy O, Falini G, Dubinsky Z, Goffredo S 2012b Inferred calcification rate of a Mediterranean azooxanthellate coral is uncoupled with sea surface temperature along an 8° latitudinal gradient Frontiers in Zoology, 9: 32.

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.

Coma R, Ribes M, Zabala M, Gili JM 1995 Reproduction and cycle of gonadal development in the Mediterranean gorgonian *Paramuricea clavata*. Marine Ecology Progress Series 117: 173-183.

Doney SC, Fabry VJ, Feely RA, Kleypas JA. 2009. Ocean acidification: the other CO_2 problem. Annual Review of Marine Science 1: 169-92.

Doney SC, Ruckelshaus M, Emmett Duffy J, Barry JP, Chan F, English C, … Talley L. 2012 Climate Change Impacts on Marine Ecosystems. Annual Review of Marine Science 4: 11-37.

Field CB, Barros V, Stocker TF, Dahe Q 2012 Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation: Special Report of the Intergovernmental Panel on Climate Change. Cambridge and New Yor. Cambridge University Press. 594 p.

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). Marine Biology 160: 719-728.

Foster NL, Baums IL, Mumby PJ 2007 Sexual vs. asexual reproduction in an ecosystem engineer: the massive coral *Montastrea annularis*. Journal of Animal Ecology 76: 384-391

Gilmour JP 2002 Substantial asexual recruitment of mushroom corals contributes little to population genetics of adults in conditions of chronic sedimentation. Marine Ecology Progress Series 235: 81-91.

Goffredo S, Arnone S, Zaccanti F 2002 Sexual reproduction in the Mediterranean solitary coral *Balanophyllia europaea* (Scleractinia, Dendrophylliidae). Marine Ecology Progress Series 229: 83-94.

Goffredo S, Mattioli G, Zaccanti F 2004 Growth and population dynamics model of the Mediterranean solitary coral *Balanophyllia europaea* (Scleractinia, Dendrophylliidae). Coral Reefs 23: 433-443.

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. Marine Biology 148: 923-931.

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. Mainer Biology 152: 351-361.

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, Mattioli G, Zaccanti F 2010 Growth and population dynamic model for the non-zooxanthellate temperate solitary coral *Leptopsammia pruvoti* (Scleractinia, Dendrophylliidae) Marine Biology 157: 2603-2612.

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). Bulletin of Marine Science 87: 589-604.

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? Journal of Morphology 273: 943-956.

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

Harrison PL, Booth DJ 2007 Coral reefs: naturally dynamic and increasingly disturbed ecosystems. In: Connell SD, Gillanders BM (eds) Marine ecology. Oxford University Press, Melbourne, pp 316-377.

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 Coral reefs: an ecosystem in transition. Springer Netherlands pp. 59-85.

Holstein TW, Hobmayer E, Technau U 2003 Cnidarians: an evolutionary conserved model system for regulation? Developmental Dynamics 226: 257-267

Johnston IS 1980 The ultrastructure of skeletogenesis in hermatypic corals. International Review of Cytology 67: 171-214.

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

Kramarsky-Winter E, Fine M, Loya Y 1997 Coral polyp expulsion. Nature 387: 137.

Kružić P, Žuljević A, Nikolić 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. Marine ecology Progress series 205: 101-111.

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 in Ecology & Evolution 25: 250-260.

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.

McClanahan T, Weil E, Corte´s J, Baird AH, Ateweberhan M 2009 Consequences of coral bleaching for sessile reef organisms. In: van Oppen MJH, Lough JM (eds) Ecological studies: coral bleaching: patterns, processes, causes and consequences. Springer-Verlag, Berlin, pp 121-138.

Nozawa Y 2012 Annual variation in the timing of coral spawning in a high-latitude environment: Influence of temperature. The Biological Bulletin 222: 192-202).

Policansky D 1982 Sex change in plants and animals. Annual Review of Ecology, Evolution, and Systematics 13: 471-495.

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.

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

Chapter II. Unusual pattern of embryogenesis of *Caryophyllia inornata* (Scleractinia, Caryophylliidae) in the Mediterranean Sea. Maybe agamic reproduction?

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Unusual Pattern of Embryogenesis of *Caryophyllia inornata* (Scleractinia, Caryophylliidae) in the Mediterranean Sea: Maybe Agamic Reproduction?

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ABSTRACT While knowledge of the reproductive biology of tropical scleractinian corals is extensive, information from temperate zones is limited. The aim of this study is to describe the reproductive biology of *Caryophyllia inornata*, a temperate species, and to increase the understanding of the reproductive strategies of Mediterranean corals. Samples of *C. inornata* were collected during SCUBA surveys at Elba island. Sexually active individuals displayed either male or female germ cells, showing a gonochoric sexuality. *C. inornata* exhibited an unusual pattern of embryogenesis. Embryos appeared throughout the whole year in males and in sexually inactive individuals, and they did not show a seasonal pattern of development, as usually expected for sexual reproduction. This observation suggests the possibility of asexual origin. These embryogenetic sexually inactive individuals were larger in size than the embryogenetic sexually active ones, and they might be senile polyps that preserve the ability to produce embryos only by agamic reproduction. J. Morphol. 273:943–956, 2012.

KEY WORDS: embryo development; gametogenesis; sexual inactive polyps; sexuality; reproductive mode

INTRODUCTION

Knowledge of pattern of sexuality and mode of reproduction are fundamental for the understanding of the macroevolutionary processes of all multicellular organisms (Kerr et al., 2011). Knowledge of the reproductive biology of corals (Scleractinia), gained by studying their sexuality (hermaphroditic or gonochoric), reproductive mode (broadcasting or brooding), embryonic (coeloblastula or stereoblastula), and larval development (benthic or planktonic), is the first step to understanding the population dynamics of marine organisms (e.g., Goffredo et al., 2005).

Despite in-depth studies over the last three decades, which have greatly increased understanding of the reproductive biology of scleractinians, the wide range of reproductive strategies of this group remains enigmatic (Loya and Sakai, 2008). Of the more than 1,500 recognized coral species, characteristics of sexual reproduction have now been recorded in at least 444 species (Harrison, 2011) mainly from tropical and subtropical zones (Fadlallah, 1983; Heltzel and Babcock, 2002; Neves and Pires, 2002; Mangubhai and Harrison, 2008a,b). Information on scleractinians from temperate zones, however, is limited (Szmant-Froelich et al., 1980; Beauchamp, 1993). Data on corals from the Mediterranean Sea come from observations made more than a century ago by Lacaze-Duthiers (1873), a few observations on *Cladocora caespitosa* (Kruzic et al., 2008) and recent studies on *Balanophyllia europaea* (Goffredo et al., 2002), *Leptopsammia pruvoti* (Goffredo et al., 2005, 2006), and *Astroides calycularis* (Goffredo et al., 2010, 2011).

The variety of reproductive processes and modes among coral species partially reflects the extraordinary ability of their cells to differentiate and to provide their tissues with increased plasticity and evolutionary adaptability (Campbell, 1974; Holstein et al., 2003; Harrison, 2011). The abundance

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of information on the biology and reproductive ecology of scleractinians exceeds that of some other groups of marine invertebrates and therefore provides an important model for the understanding of their evolution and life cycles (Harrison, 2011). Complex and sometimes controversial evolutionary forces are the basis for sexual determination in plants and animals. These may present the same sex throughout their lifetime, or change from one functional sex to another, displaying phenomena of sexual inversion (Loya and Sakai, 2008). Reaching sexual maturity depends on a balance between growth and risk of death, which is linked to the age and size of the organisms. Variations in age and size at the first reproductive event and differences in the "sex ratio" influence population growth rates (Fujiwara and Caswell, 2001). These variations are important, as they may represent the beginning of evolutionary divergences (Richmond and Hunter, 1990).

Various studies performed in the 1970s and 1980s showed that in several ovoviviparous anthozoa planulae production can be derived by asexual reproductive processes (Ottaway and Kirby, 1975; Black and Johnson, 1979; Stoddart, 1983; Ayre and Resing, 1986), contradicting the assumption that these are only of sexual origin (Hyman, 1940; Connell, 1973). The selective advantages of sexual versus asexual reproduction change in different conditions, and the energy allocation intended for each reproductive strategy can reflect changes in the environment (Bradshaw, 1965; Jackson and Coates, 1986; Stearns, 1992). Reproductive flexibility and its effect on the structure of the population are often generalized in life-history theory. Theoretical models suggest under favorable conditions and low stress levels, energy investment in asexual propagation predominates (Williams and Mitton, 1973; Warner, 1975; Williams, 1975). Such asexual reproduction would generate a clonal line that might contribute to keeping populations inside the area of the parental habitat, thus propagating well-adapted genotypes at the local level. On the other hand, when local conditions are unfavorable and stress levels are high, more energy will be invested in sexual reproduction and dispersion (Warner, 1975; Williams, 1975; Carvalho, 1994). This produces a genotypically different lineage, which might enable a wide dispersion or recolonization of more heterogeneous habitats (Williams, 1975; Maynard Smith, 1978), thus contributing to an increase in the fitness and survival of the species (Harrison, 2011). Sherman et al. (2006) state that the relationship between stability of the habitat and genetic diversities might be far more complex than has been theorized. According to these authors, asexual reproduction may be an adaptation that allows the exploitation of newly available substrata after a disturbance event. The availability of suitable space after a disturbance event may allow for the rapid recolonization of these areas by the localized recruitment of asexually generated larvae from surviving colonies (Sherman et al., 2006). Gilmour (2002a) observed that the Australian population of the *Fungia fungites* coral, exposed to a high rate of chronic sedimentation, shows up to 30% of asexually derived polyps. The population of *Fungia scutaria*, common in very rough shallow water, shows a more marked asexual budding compared with populations of *Fungia granulosa*, common in calm, deep water, suggesting that the evolution of distinct reproductive strategies in closely correlated species might in part be the consequence of different environmental constraints (Kramarsky-Winter and Loya, 1998; Goffredo and Chadwick-Furman, 2003).

Changes in several biological processes, for example, the consequences of climate changes, are already evident in several ecosystems (Harley et al., 2006). Increases in temperature may cause alterations in gamete release into the environment (Lawrence and Soame, 2004), as well as in the quality of the eggs and survival of the larvae (McClanahan et al., 2009; Randall and Szmant, 2009). The repercussions of climate change are expected to be greater in the temperate and high-latitude zones (Solomon et al., 2007), with marked consequences in organisms that display seasonality in gonadic development (Lawrence and Soame, 2004). Therefore, sexual reproductive processes are sensitive to a wide range of natural and anthropogenic stress factors, which impair or block the critically important phases of reproduction and recruitment required to maintain and replenish coral populations (Harrison, 2011). Without sexual recombination, these populations have little chance of adapting to changes in environmental conditions and, in particular, ocean warming (van Woesik, 2009). Considering that the reproduction of coral seems more sensitive to stress in comparison with other vital functions (Harrison and Wallace, 1990), the presence of ecologically appropriate environmental conditions is essential to ensure reproductive success (Harrison, 2011).

This study, which is part of the European project FP7-IDEAS ERC "Corals and global warming: the Mediterranean versus the Red Sea," aims to investigate the reproduction of *Caryophyllia inornata* (Fig. 1) in the northern Tyrrhenian Sea to increase knowledge on Mediterranean scleractinian corals, key organisms of the brother project. We describe the morphological aspects of gametogenesis and embryonic development, defining the sexual condition, sex-ratio and reproductive mode of *C. inornata*.

The Caryophylliidae family is ubiquitous, formed both by solitary and colonial corals and includes 296 living species divided into 51 genera (Cairns, 1999; Kitahara et al., 2010). Nine species live in the Mediterranean Sea, grouped into five genera

Fig. 1. *Caryophyllia inornata*. Specimens photographed at Elba isle (Leghorn, 42°45′N and 10°24′E). [Color figure can be viewed in the online issue, which is available at wileyonline library.com.]

(Minelli et al., 1995); four of these (*Caryophyllia*, *Ceratotrochus*, *Paracyathus*, and *Trochocyathus*) are solitary (Baird et al., 2009). *Caryophyllia* Lamarck, 1801 is exclusively an azooxanthellate genus and contains 66 species (Kitahara et al., 2010), including *C. inornata* (Cairns, 1999).

The distribution of *C. inornata* is found in the eastern and western part of the Mediterranean sea (Zibrowius, 1980) and extends up to the north-eastern Atlantic coasts (Cairns, 1999), from the Canary Islands to the North Sea (Zibrowius, 1980). It colonizes caves, walls, and wrecks, from the surface down to 100 m deep in dimly lit environments. It is one of the main species that populate the walls and the vaults of caves and in some cases is the dominant species (Zibrowius, 1978).

MATERIALS AND METHODS

Sampling

Samples of *C. inornata* were collected from the aircraft wreck of Elba Isle (Leghorn, Tuscany, 42°45′N and 10°24′E), during 18 monthly collections from May 2009 to October 2010. Using SCUBA, 20 polyps were collected each month, at a depth of 12–15 m. In this study, over 315 polyps were collected in 18 monthly dives. Water temperature was continuously recorded in the field by underwater digital thermometers, and at the time of each specimen sampling with mercury thermometers. The mean population density in the sampling site was 6025 ± 898 individuals m^{-2}, corresponding to 1.669 ± 358 kg m^{-2} of calcium carbonate. Bed coverage was 15.3 ± 2.5%. The color of the polyps varied from pink to brownish. Photoperiod was obtained from the online database EuroMETEO® (http://www.eurometeo.com). 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 Analysis

Histological analysis was performed on 72 polyps (Table 1). The biometric analysis of each polyp was performed by measuring the length (L, major axis of the oral disk), the width (w, minor axis of the oral disk), and height (h, oral–aboral diameter; Fig. 2). The body volume (V) was calculated using the equation: $V = h * (L/2) * (w/2) * \pi$ (Goffredo et al., 2002).

Histological Analysis

Polyps were postfixed in Bouin solution. After decalcification in ethylenediamine tetra acetic acid 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 hematoxylin and eosin.

Cytometric Analysis

Histological observations were made using a light microscope NIKON Eclipse 80i. Cytohistological readings were made with a two image analysis systems: NIKON NIS-Elements D 3.1 and LEICA Q500IW. The maximum and minimum diameters of the spermaries and oocytes in nucleated sections were measured and classified into developmental stages in accordance with earlier studies on gametogenesis in scleractinians (Goffredo et al., 2005, 2010). The presence of embryos in the gastrovascular cavity and mesenterial septa were recorded, and their stage of maturation was identified (Goffredo and Telò, 1998; Goffredo et al., 2005). The size of each reproductive element was determined as the mean of the two diameters (Goffredo et al., 2005, 2010).

The following definitions were used: sexually active polyps, individuals that display gametogenetic activity; spermatogenetic polyps, individuals that display spermaries; oogenetic polyps, individuals that display oocytes; embryogenetic polyps, individuals that display embryos; sexually inactive polyps, individuals that do not display gametogenetic activity.

RESULTS

Sexuality

The sexually active individuals had either oocytes or spermaries; no individual displayed both types of germ cells. Sexual dimorphism was not observed, significant differences were not found in the mean size of spermatogenetic and oogenetic individuals (Student's t-test for L: $t = 1.423$, df = 35, $P = 0.255$; Student's t-test for V: $t = 2.705$, df = 35, $P = 0.073$; Table 2). The sex ratio of sexually active polyps was significantly different from 1 with a 1:3.1 ratio that favored spermatogenetic individuals (chi-square test, $\chi^2 = 9.76$, df = 1, $P < 0.01$). Embryos were found in the coelenteric cavity and/or inside mesenterial septa of 76.4% of the polyps analyzed, suggesting internal development (Table 1). Embryos were identified in all monthly samples and inside oogenetic, spermatogenetic, and inactive individuals. All nine oogenetic polyps had embryos (L = 7.7 ± 0.4 mm; V = 347.1 ± 45.1 mm^3; means ± SE; Table 2). Of the 28 spermatogenetic polyps, 24 had embryos (L = 7.2 ± 0.2 mm; V = 251.5 ± 16.0 mm^3; means ± SE; Table 2) and four were without embryos (L = 6.7 ± 0.5 mm; V = 256.7 ± 19.8 mm^3; means ± SE; Table 2). Of the 35 inactive polyps, 28 were embryogenetic (L = 8.4 ± 0.3 mm; V = 394.64 ± 0.8 mm^3; means ± SE; Table 2) and 7 did not show embryos (L = 6.9 ± 0.6 mm; V = 317.7 ±

TABLE 1. Caryophyllia inornata—Size, sex condition, and reproductive state of analyzed polyps

Date	Polyp code	L (mm)	w (mm)	h (mm)	V (mm^3)	Reproductive state	Oocytes	Spermaries	Embroys			Notes
									early	intermediate	late	
	CI-140509-P1	8.40	7.10	9.05	423.91	I	—	—				R
	CI-140509-P2	7.20	6.45	11.20	408.51	S	—	285	—	6	23	
	CI-140509-P3	5.35	5.00	10.00	210.09	S	—	8	—	—	—	
	CI-140509-P4	7.00	6.20	9.00	306.78	S	—	131	—	—	—	
	CI-140509-P5	6.70	6.35	6.10	203.83	S	—	43	5	16	13	
	CI-140509-P6	7.05	6.40	6.65	235.66	S	—	—				R
	CI-140509-P7	6.25	5.30	6.70	174.31	S	—	27	—	8	9	
	CI-140509-P8	7.00	6.10	8.10	271.65	I	—	—	—	27	8	
14th May 09	CI-140509-P10	6.05	5.30	5.80	146.07	S	—	647	—	4	15	
	CI-140509-P12	9.60	8.10	13.50	824.48	I	—	—	—	—	—	
	CI-140509-P13	8.00	7.45	8.55	400.22	O	2016	—	—	14	36	
	CI-140509-P14	5.00	5.80	7.70	175.38	S	—	124	—	—	12	
	CI-140509-P17	9.45	8.35	10.30	638.33	I	—	—	1	2	18	
	CI-140509-P19	7.65	6.70	7.05	283.80	S	—	909	2	11	7	
	CI-140509-P22	7.35	6.30	7.30	265.49	S	—	—				R
	CI-140509-P23	7.00	6.80	8.10	302.82	S	—	1800	—	1	5	
	CI-140609-P1	7.50	6.20	7.25	264.78	I	—	—				R
	CI-140609-P2	8.05	8.00	9.05	457.75	O	865	—	2	—	61	
	CI-140609-P7	7.35	5.30	10.00	305.95	S	—	442	—	1	30	
14th Jun 09	CI-140609-P10	5.85	5.35	7.25	178.21	O	56	—	2	—	1	
	CI-140609-P12	6.90	5.60	5.40	163.88	S	—	770	—	3	8	
	CI-140609-P13	6.45	5.65	8.40	240.42	O	315	—	1	3	25	
	CI-120709-P1	8.15	7.05	10.65	480.60	I	—	—	—	—	4	
	CI-120709-P2	8.20	7.90	10.70	544.40	O	79	—	—	—	3	
12th Jul 09	CI-120709-P5	8.30	7.80	10.40	528.81	I	—	—	—	—	2	
	CI-120709-P6	7.40	5.35	8.35	259.63	S	—	2	—	—	—	
	CI-120709-P14	5.15	4.60	6.40	119.08	I	—	—	—	—	—	
	CI-140809-P1	8.20	7.80	10.00	502.34	I	—	—	—	—	—	
	CI-140809-P2	6.80	7.15	7.20	274.94	I	—	—	—	—	—	
	CI-140809-P3	6.20	7.30	9.40	334.14	I	—	—	—	1	1	
14th Aug 09	CI-140809-P4	7.15	7.40	8.15	338.68	I	—	—	—	—	—	
	CI-140809-P5	7.60	7.00	8.75	365.60	S	—	39	—	1	—	
	CI-140809-P6	7.70	6.30	4.95	188.59	I	—	—	3	2	18	
	CI-170909-P2	10.25	9.00	11.15	807.85	I	—	—				R
	CI-170909-P3	7.25	6.25	8.00	284.71	I	—	—	4	—	2	
17th Sep 09	CI-170909-P4	8.35	7.20	6.70	316.36	I	—	—	1	—	—	
	CI-170909-P5	11.70	9.30	7.35	628.12	I	—	—	—	2	5	
	CI-170909-P6	6.90	6.30	5.90	201.43	I	—	—	—	1	—	
	CI-191009-P1	8.45	6.30	8.10	338.67	I	—	—	2	6	6	
	CI-191009-P3	7.25	6.25	8.00	871.54	I	—	—	—	1	—	
19th Oct 09	CI-191009-P4	8.35	7.20	6.70	297.55	I	—	—	3	1	1	
	CI-191009-P5	11.70	9.30	7.35	142.35	S	—	1	—	3	1	
	CI-191009-P7	5.40	4.70	3.40	67.77	I	—	—	—	—	—	
	CI-181109-P1	5.35	5.00	4.60	96.64	I	—	—	—	—	—	
	CI-181109-P2	7.05	6.25	6.10	211.10	S	—	7	1	—	16	
18th Nov 09	CI-181109-P3	7.55	6.85	7.00	284.33	I	—	—	—	—	8	
	CI-181109-P4	8.10	7.25	7.05	325.16	I	—	—	—	—	4	
	CI-151209-P1	8.45	7.55	6.95	348.24	S	—	71	2	8	21	
	CI-151209-P2	6.65	6.55	6.30	215.52	S	—	5	2	1	2	
15th Dec 09	CI-151209-P3	7.65	6.70	5.65	227.44	S	—	37	—	—	10	
	CI-151209-P4	7.20	6.35	6.20	222.63	I	—	—	1	—	2	
	CI-130110-P2	9.15	6.90	5.55	275.20	I	—	—	—	—	3	
	CI-130110-P4	7.70	6.60	7.30	291.37	I	—	—				R
13th Jan 10	CI-130110-P5	6.45	5.75	5.30	154.38	I	—	—	—	2	3	
	CI-130110-P7	6.00	5.05	4.35	103.52	I	—	—	1	—	5	
	CI-130110-P10	10.00	8.10	7.45	473.95	I	—	—	—	8	51	
	CI-070210-P1	8.20	6.95	6.00	268.56	I	—	—	4	1	1	
	CI-070210-P2	6.60	6.50	7.15	240.91	S	—	4	2	9	12	
07th Feb 10	CI-070210-P3	6.90	5.95	8.50	274.08	O	3	—	—	—	3	
	CI-070210-P8	7.35	6.40	4.65	171.79	S	—	20	—	—	8	
	CI-120310-P1	7.00	6.70	6.80	250.48	S	—	74	—	—	—	
	CI-120310-P3	11.10	8.75	11.55	881.05	I	—	—	—	22	43	
12th Mar 10	CI-120310-P4	11.60	8.55	8.55	666.01	I	—	—	4	45	69	
	CI-120310-P6	10.00	9.00	7.35	519.54	O	110	—	—	3	14	
	CI-180410-P1	7.75	6.70	7.30	297.71	S	—	1285	—	—	11	
	CI-180410-P2	7.35	6.40	4.65	171.79	S	—	913	—	8	45	

TABLE 1. Caryophyllia inornata—Size, sex condition, and reproductive state of analyzed polyps (continued)

Date	Polyp code	L (mm)	w (mm)	h (mm)	V (mm^3)	Reproductive state	Oocytes	Spermaries	early	intermediate	late	Notes
18th Apr 10	CI-180410-P3	6.75	6.20	6.85	225.15	I	—	—	—	1	9	
	CI-180410-P6	8.60	7.15	7.55	364.62	S	—	1922	—	3	28	
	CI-200510-P1	7.05	6.30	7.00	244.18	O	1573	—	1	4	8	
	CI-200510-P3	7.70	6.65	6.25	251.35	S	—	638	—	—	5	
20th May 10	CI-200510-P5	8.55	7.45	5.30	265.15	O	304	—	—	2	60	
	CI-200510-P6	9.40	7.65	6.40	361.46	S	—	2966	—	—	26	

L: major axis of the oral disk; *w*: minor axis of the oral disk; *h*: oral—aboral diameter; *V*: body volume; O: oogenetic polyp; S: spermatogenetic polyp; I: inactive polyp; R: quantitative analysis not performed, presence of embryos confirmed.

102.8 mm^3; means ± SE; Table 2). The mean sizes of the seven inactive polyps without embryos were not significantly different from those of the 37 sexually active polyps analyzed (Student's *t*-test for *L*: $t = 0.793$, df = 42, $P = 0.592$; Student's *t*-test for *V*: $t = 0.746$, df = 42, $P = 0{,}697$; Table 2). The mean sizes of the inactive polyps with embryos were significantly greater than those of the embryogenetic sexually active individuals (Student's *t*-test for *L*: $t = 3.626$, df = 63, $P = 0.001$; Student's *t*-test for *V*: $t = 2.975$, df = 63, $P = 0.010$; Table 2). Quantitative cytohistometric analysis was performed on the nine oogenetic polyps observed, on 26 of the 28 spermatogenetic polyps, and on 31 of the 35 embryogenetic sexually inactive polyps.

Male Gametogenesis

The spermaries were located in the mesenterial septa and were made up of groups of germ cells and delineated by mesogleal envelope (Fig. 3). A total of 13,170 spermaries were identified and measured. Five stages of maturation were identified:

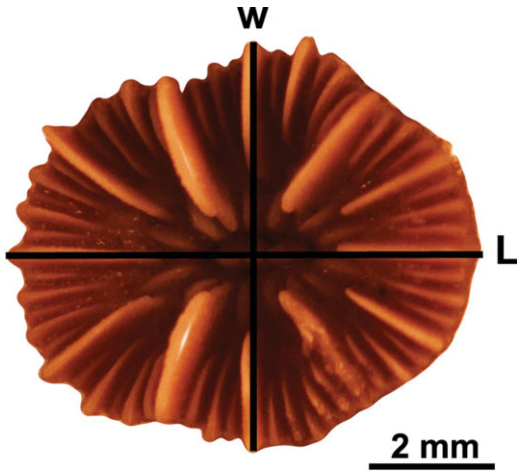

Fig. 2. *Caryophyllia inornata*. Specimens photographed in the laboratory (*L*: major axis, *w*: minor axis of the polyp). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Stage I—undifferentiated germ cells arose in the gastrodermis and then migrated toward the mesoglea of the mesentery where they regrouped forming the spermary. The spermary was made up of an early aggregation of spermatogonia (Fig. 3B). Spermaries had a mean diameter of 28.36 ± 0.77 μm, $N = 152$.

Stage II—the spermary was made up of a group of spermatocytes undergoing meiosis. The mesogleal layer enveloping the spermary had not yet formed a wall. (Fig. 3C). Spermary mean diameter was 51.84 ± 0.88 μm, $N = 530$.

Stage III—the spermary, still made up of a group of spermatocytes undergoing meiosis, was delineated by a clearly differentiated wall formed by the mesoglea (Fig. 3D). Spermary mean diameter was 83.56 ± 0.50 μm, $N = 5{,}791$.

Stage IV—the spermary showed a centripetal maturation gradient: less mature and larger germ cells (spermatocytes) were located at the periphery of the spermary, whereas more mature and smaller ones (spermatids) were located in the center (Fig. 3E,F). Spermary mean diameter was 94.39 ± 0.76 μm, $N = 4{,}310$.

Stage V—the spermary was made up of a mass of spermatozoa with their tails all facing in the same direction (an arrangement known as a "bouquet"; Fadlallah and Pearse, 1982; Fig. 3G). Sper-

TABLE 2. Caryophyllia inornata—mean sizes and standard error of sexually active polyps (S: spermatogenetic, S + E: spermatogenetic with embryos, O: oogenetic, and O + E: oogenetic with embryos) and sexually inactive (I: inactive and I + E: inactive with embryos)

Reproductive state	*L* (mm)	*V* (mm^3)
Sexually active	7.3 ± 0.2 ($N = 37$)	275.3 ± 16.3 ($N = 37$)
S	6.7 ± 0.5 ($N = 4$)	256.7 ± 19.8 ($N = 4$)
S + E	7.2 ± 0.2 ($N = 24$)	251.5 ± 16.0 ($N = 24$)
O	—	—
O + E	7.7 ± 0.4 ($N = 9$)	347.1 ± 45.1 ($N = 9$)
Sexually inactive	8.1 ± 0.3 ($N = 35$)	379.2 ± 38.2 ($N = 35$)
I	6.9 ± 0.6 ($N = 7$)	317.7 ± 102.8 ($N = 7$)
I + E	8.4 ± 0.3 ($N = 28$)	394.6 ± 40.8 ($N = 28$)

L: major axis of the oral disk of the polyp, *V*: body volume of the polyp, and *N*: number of polyps examined.

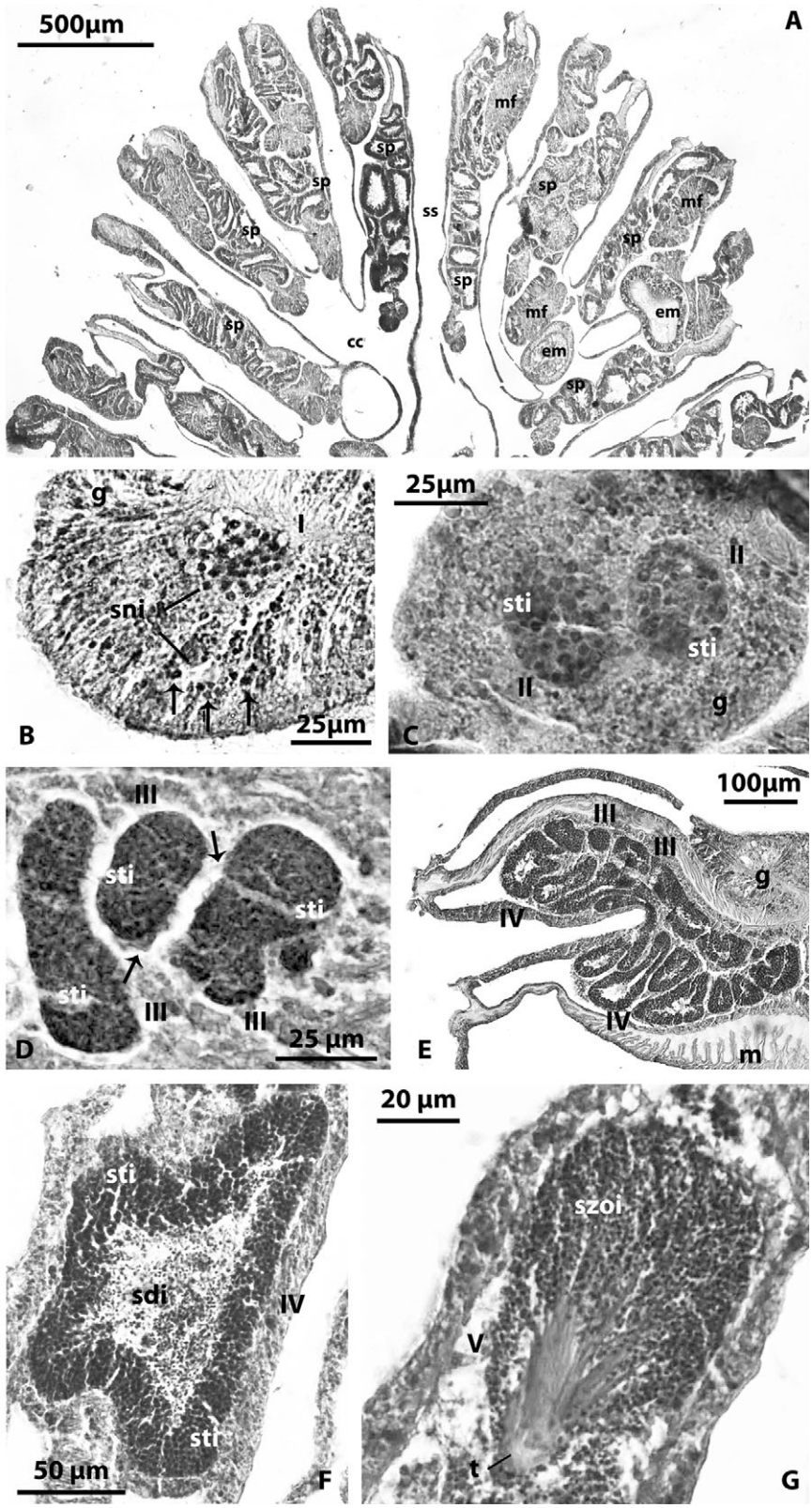

Fig. 3. *Caryophyllia inornata*. Spermatogenesis. **A**: Localization of the spermaries in the mesenterial septa. **B**: Stage I: undifferentiated germ cells disposed in the gastrodermis layers of the mesentery (arrow). The spermaries are made up of a group of spermatogonia. **C**: Stage II: the spermaries are made up by spermatocytes involved in the process of meiosis. **D**: Stage III: the spermary, containing spermatocytes undergoing meiosis, is delineated by a wall that has arisen from the mesoglea (arrows). **E**: Spermaries of stages III and IV located in the mesentery. **F**: Stage IV: the spermary presents an external layer of spermatocytes and an internal mass of spermatids, recognizable by the presence of a tail. **G**: Stage V: the spermary is made up of a mass of spermatozoa. Shortly before leaving the spermary, mature spermatozoa form a bouquet, with their tails all facing in the same direction (arrow). [cc: coelentric cavity; m: mesoglea; ss: skeletal septum; mf: mesenterial filament; sp: spermary; em: embryo; g: gastrodermis; sni: spermatogonia; sti: spermatocytes; sdi: spermatids; szoi: spermatozoa; t: spermatozoa tails; I, II, III, IV, V: spermary developmental stage].

mary mean diameter was 102.19 ± 1.09 μm, N = 2,387.

Female Gametogenesis

The oocytes were oval-shaped and located in the mesenteries (Fig. 4). A total of 5,321 oocytes were identified and measured. The diameter of the oocytes ranged from 11.63 to 141.16 μm, and their mean diameter was 68.75 ± 0.26 μm, N = 5,321. The early stages of oogenesis were visible in the mesentery's gastrodermal layers. Early oocytes had a centrally located spherical nucleus and a high ratio of nucleus to cytoplasm (Fig. 4B). In the intermediate stages, the oocytes still presented a spherical nucleus and the nucleus/cytoplasm ratio decreased due to the accumulation of yolk (Fig. 4C). In the more advanced stages, the nucleus/cytoplasm ratio was further reduced due to the accumulation of yolk (Fig. 4D,E). The nucleus had also migrated to the cell's periphery, and adhering closely to the cell membrane it changed shape, becoming indented and concave (Fig. 4D,E). During oogenesis, the nucleolus was always positioned on the periphery of the nucleus (Fig. 4D,E).

Oral–Aboral Distribution of Gametogenic Processes

Distribution of the germ cells along the oral–aboral axis was significantly different between spermatogenetic and oogenetic polyps (Fig. 5). Although the size of the spermaries was correlated negatively with the distance from the oral pole, that of the oocytes correlated positively (Fig. 6). The mean distance of the spermaries from the oral pole (58.05 ± 0.11%) was significantly greater with respect to that of the oocytes (54.11 ± 0.15%; Student's t-test, t = 20.53; df = 18,489; $P < 0.001$; Fig. 5). Both in spermatogenetic individuals and in oogenetic ones, a third of the polyps corresponding to the oral pole were nongametic.

Embryonic Development

The embryos were located both in the mesenterial septa, inside the mesoglea layer wrapped in the gastrodermis, and in the gastrovascular cavity of oogenetic, spermatogenetic and sexually inactive individuals (Fig. 6A,C,D). A total of 1,056 embryos were identified and measured. Development proceeded through the formation of embryos that did not show a blastocoel cavity (Fig. 6B). Often, early embryos located in the gastrovascular cavity seemed in close morphological continuity with mesenterial septa or detached from it (Fig. 6A detail). Furthermore, early embryos were located also inside the body of embryos in a more advanced stage of development, showing continuity with the host tissues (Fig. 6E). The diameter of the early embryos ranged from 58.49 to 308.08 μm. The mean diameter was 136.27 ± 7.09 μm, N = 44. During the intermediate stage, called stereogastrula, the ectoderm was clearly distinct from the endoderm. The ectodermal layer, formed by multiple layers of cells, seemed clearly differentiated and separate from the endodermal central mass by a well-defined mesoglea layer (Fig. 6F). During the advanced stages of development, the stereogastrula showed an invagination of the ectodermal cells, which led to the formation of the stomodeum (Fig. 6H–J) and the differentiation of the mesenterial septa by the invagination of the mesoglea layer toward the center of the embryo (Fig. 6G). The diameter of the stereogastrula ranged from 82.64 to 853.68 μm. The mean diameter was 307.39 ± 4.21 μm, N = 1,012.

DISCUSSION

Sexuality

All the sexually active polyps examined contained only a single type of germ cell, indicating that *C. inornata* may be gonochoric. Most hermaphrodites are simultaneous (Harrison, 2011), that is, the same organisms develop mature oocytes and spermaries at the same time (Policansky, 1982). Additionally, male and female individuals of *C. inornata* do not display significantly different sizes, which also suggests that the species is gonochoric as, according to Harrison (2011), organisms with gonochoric sexuality do not show any relationship between sex and coral size. Sequential hermaphrodites may exhibit sex change over successive breeding seasons or over their lifetime (Ghiselin, 1974; Policansky, 1982). The direction of sex change (protandrous or protogynous) is determined by the relative reproductive success over the course of a lifetime for the two sexes. The optimal size at sex change is when the potential subsequent lifetime reproductive output as the second sex exceeds that of remaining as the first sex. Charnov's theory of sex allocation (1982) predicts that sex change is favored when reproductive success (fitness) increases more quickly with size or age in one sex than in the other. True protandrous sex change from initial male function in small corals to female function in larger corals has recently been demonstrated for some fungiid mushroom corals (Loya and Sakai, 2008; Loya et al., 2009). In three deep water *Caryophyllia* species, a cyclic hermaphroditic sexuality has been observed. In cyclic hermaphroditism, gonadal development is asynchronous and germ cell maturation does not have seasonality. Male and female germ cells, at different stages of maturation, were identified in the mesentery of the same individual in all the samples of the three species (Waller et al., 2005). In *C. inornata,* mature opposite sex gametes were observed in the same period of the year, always in

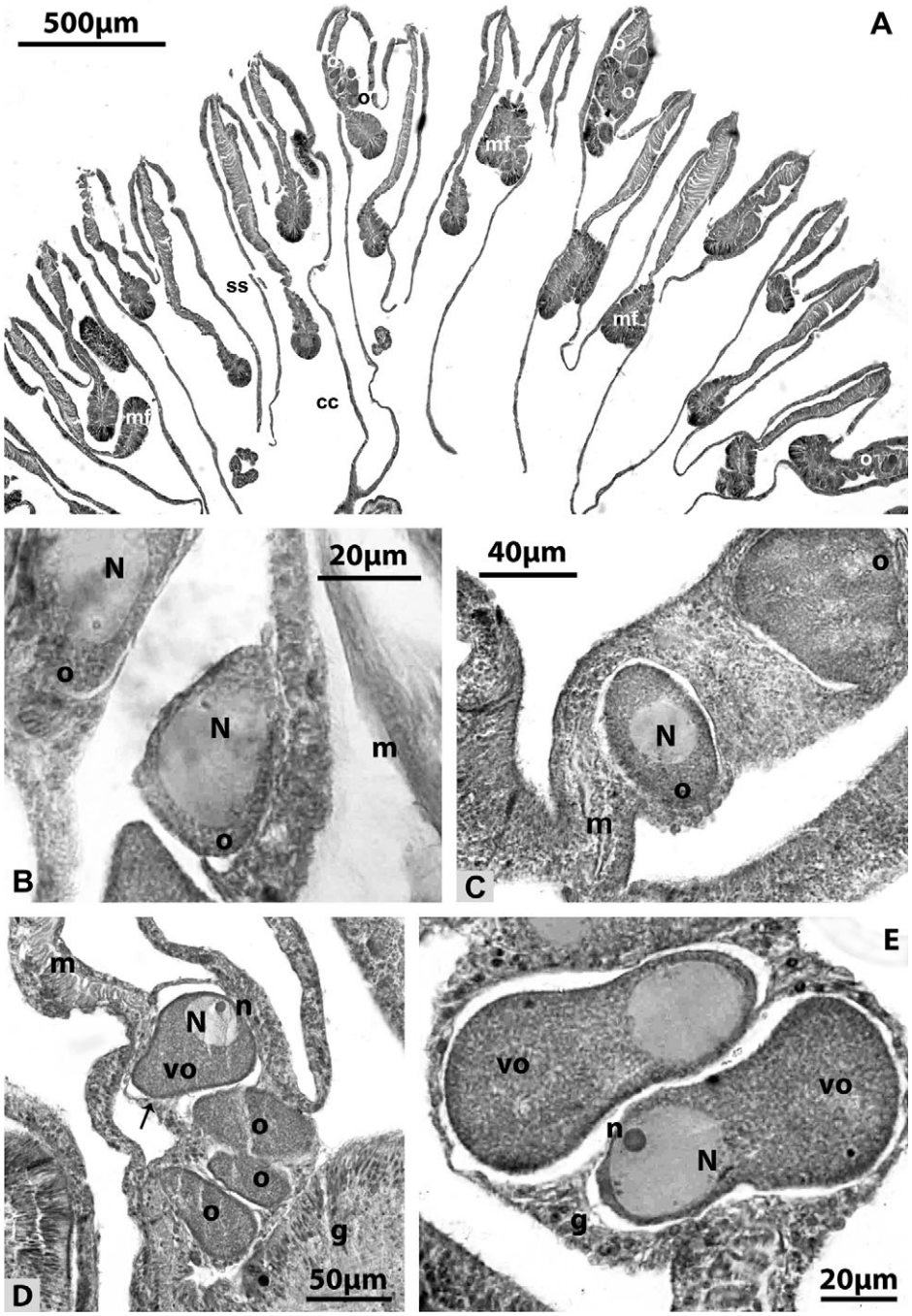

Fig. 4. *Caryophyllia inornata*. Oogenesis. **A**: Localization of the oocytes in the mesenterial septa. **B**: Early stage: two previtellogenic oocytes in the gastrodermis of the mesentery, characterized by a high nucleus/cytoplasm ratio. **C**: Intermediate stage: vitellogenic oocyte located in the mesoglea. The spherical-shaped nucleus is still located in the central portion of the cell. The nucleus/cytoplasm ratio is reduced. Note a larger vitellogenic oocyte in the same section. **D**: Late stage: the nucleus of the oocyte has started to migrate toward the cell's periphery. Note the distinct membrane of the oocyte (arrow). **E**: Late stage: two mature oocytes, located in the mesentery; the nucleus/cytoplasm ratio is greatly reduced. [cc: coelentric cavity; mf: mesenterial filament; ss: skeletal septum; o: oocyte; m: mesoglea; N: nucleus; n: nucleolus; vo: vitellogenic oocyte; g: gastrodermis].

different individuals, showing a marked seasonality and synchronicity, according to the pattern expected for gonochoric conditions.

In the Caryophylliidae family, the gonochoric condition is predominant (7 species out of 11 studied; Baird et al., 2009; Harrison, 2011). Whereas in the genus *Caryophyllia* only one other case of gonochorism is known, *Caryophyllia smithii* (Hiscock and Howlett, 1977; Tranter et al., 1982), three cases of hermaphroditism are known for the deep

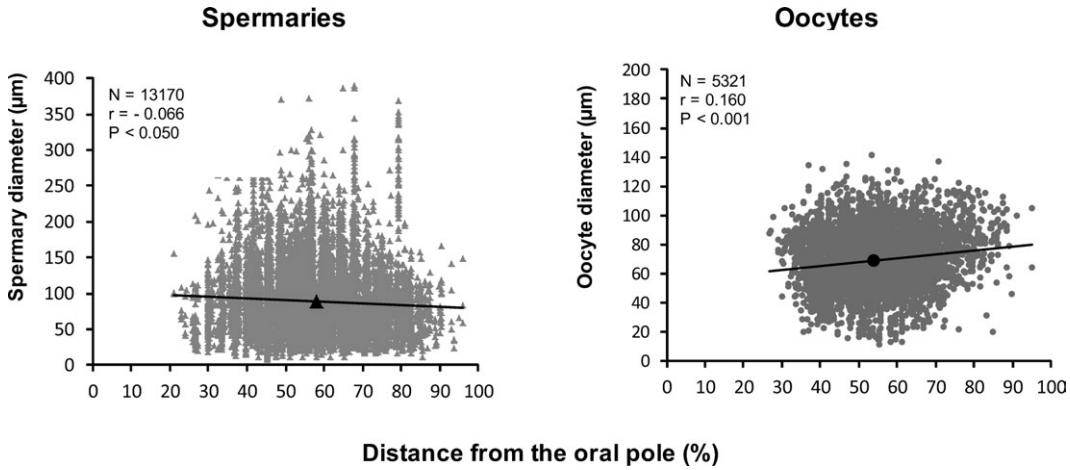

Fig. 5. *Caryophyllia inornata.* Distribution according to size along the oral–aboral axis of spermaries in male polyps and oocytes in female polyps. The distance from the oral pole is expressed as a percentage: 0% = at oral pole level and 100% = at aboral pole level. ▲: the point at which mean spermary distance (58.05 ± 0.11%; mean ± SE) and mean spermary size (88.57 ± 0.40 µm) intersect; •: the point at which mean oocyte distance (54.11 ± 0.15%) and mean oocytes size (68.75 ± 0.26 µm) intersect. Note that the value ranges on the ordinate axes are different.

species, *Caryophyllia ambrosia, Caryophyllia sequenzae*, and *Caryophyllia cornuformis* (Waller et al., 2005). The data available for scleractinians indicate that the sexual condition tends to be constant within genera or families (Harrison, 2011). Of the 105 genera of scleractinians where data of sexual condition are available, 50 contain only hermaphroditic species, 38 only gonochoric species, whereas the remaining 17 genera presented species with both sexual conditions. The genus *Caryophyllia,* displaying mixed sexuality, falls into a particularly interesting group. Szmant (1986) observed that most scleractinians are hermaphroditic, thus suggesting that hermaphroditism is an ancestral condition. Nevertheless, in a more recent morphological and molecular analysis, Daly et al. (2003) found that gonochorism is the ancestral condition of anthozoa hexacorallia, including scleractinians. Supporting these considerations a phylogenetic analysis of the reproductive properties concluded that gonochorism is the ancestral sexual condition of scleractinians. The reproductive mode, instead, evolves faster than sexual condition, and it is too variable between taxa to find the ancestral state among scleractinians (Baird et al., 2009; Kerr et al., 2011).

The sex ratio in a population with random mating is normally 1:1 (Maynard-Smith, 1978). A number of additional forces could play an important role in the deviation from this rule, such as errors during sampling or a clonal propagation (Harrison and Wallace, 1990). While sampling errors may have occurred, they are unlikely given large sample size of 15–20 individuals collected randomly from the population for each monthly sample (over 315 polyps in 18 months). The sex ratio skewed, however, in favor of males, which might therefore be explained by clonal propagation. A sex ratio favoring males has also been shown in other solitary scleractinians of the Fungidae family: *F. scutaria, Diaseris distorta, Fungia concinna, F. fungites* where clonal propagation is well known to occur (Kramarsky-Winter and Loya, 1998; Colley et al., 2000; Gilmour, 2002a,b). Agamic propagation is known in other caryophylliids, where 27 genera are colonial and the remaining 24 solitary (Cairns, 1999). Clonal propagation in the Caryophylliidae family has not still been documented. The morphological profiles described in this study suggest that clonal propagation might occur in the formation of new propagules. Eighty-five point seven per cent of spermatogenetic individuals (males) and 80.0% of sexually inactive individuals presented embryos at different stages of maturation and this production of embryos appeared throughout the entire year and was not characterized by a clear seasonal pattern, as would normally be expected in a reproductive model based on an annual cycle of sexual reproduction. The asexual production of brooded planulae has also been shown in some populations of *Pocillopora damicornis* (Stoddart, 1983), sometimes in combination with gametogenetic activity (Ayre and Miller, 2004; Sherman et al., 2006; Yeoh and Dai, 2010), in *Tubastrea coccinea* (Ayre and Resing, 1986; Glynn et al., 2008), and *Tubastrea diaphana* (Ayre and Resing, 1986). *Oulastrea crispata* is of particular interest, as it can also produce embryos asexually in the period when sexual reproduction ends (Nakano and Yamazoto, 1992; Lam, 2000). Embryogenetic sexually inactive individuals of *C. inornata* were larger in size than the embryogenetic sexually active ones. These polyps might be sexually old individuals that preserve the ability

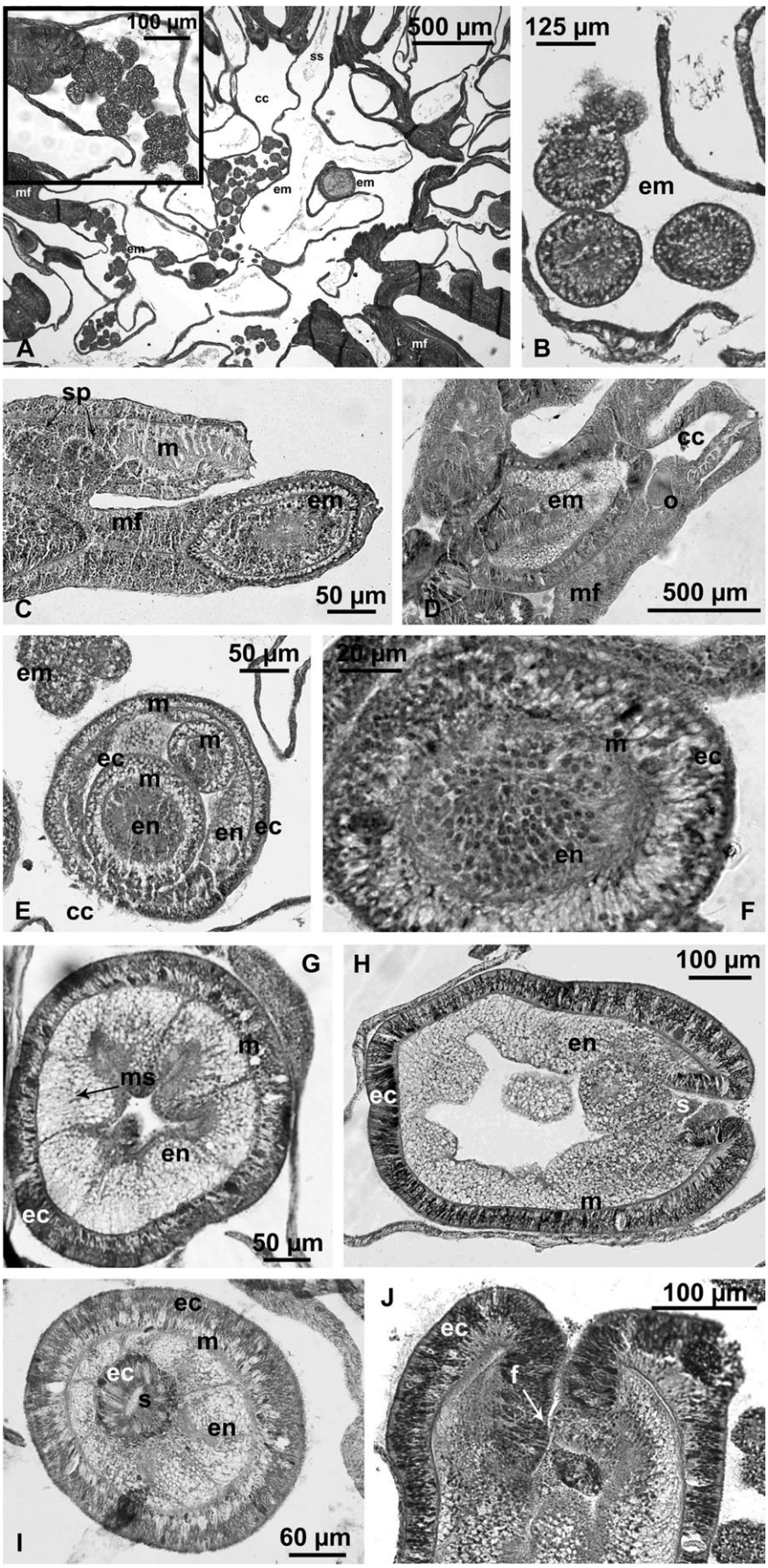
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Figure 6.

to produce embryos agamically. In the scleractinian corals *Stylophora pistillata,* senescence was indicated by the observation of a gradual reduction in the physiological processes of growth and reproduction before the natural death of the colony (Rinkevich and Loya, 1986). Bosch (2009) states that senescence can be observed in metazoa, indicating a progressive decline in physiological functions leading to an increase in the death rate. The same author has observed that degenerative processes in *Hydra* sp., such as decline in reproductive sexual activity, were observed as a clear sign of ageing. To escape population mortality and senescence these organisms reproduce exclusively asexually by budding. The production of asexual brooded planulae by locally adapted genotypes might increase local recruitment and survival in some species (Williams, 1975), whereas sexual reproduction in these species might increase colonization of distant reefs. The high population density of *C. inornata,* in the order of thousands of individuals per m^2, might be the consequence of asexual planulae production whereas the small-sized oocytes and consequent planktotrophic development might promote dispersion and colonization of distant areas. An extensive dispersal minimizes the likelihood of extinction: if local conditions have deteriorated, planktotrophic larvae have an effective means of escape, and this can generally postpone their metamorphosis in the absence of specific environmental cues until not found an appropriate habitat (Pechenik, 1999).

Male Gametogenesis

The morphological aspects of male gametogenesis in *C. inornata* correspond to those of other species of the genus *Caryophyllia,* described in hermaphroditic corals with external fertilization (*C. ambrosia*, *C. sequenzae*, and *C. cornuformis;* Waller et al., 2005) and to those of gonochoric *C. smithii*, found with external fertilization for some populations (Tranter et al., 1982) and with internal fertilization for others (Hiscock and Howlett, 1977). Within the Caryophylliidae family, *C. inornata* has the morphological profiles of male gametogenesis like those of the colonial gonochoric species, *Lophelia pertusa* (Waller et al., 2005) as well as those of species belonging to other families: for example, *Fungiacyathus marenzelleri* (gonochoric and brooding, Fungiacyathidae; Waller et al., 2002), the colonial *A. calycularis* (gonochoric and brooding, Dendrophylliidae; Goffredo et al., 2010), *Mussimilia hispida* (hermaphroditic and broadcasting, Mussidae; Neves and Pires, 2002) and in species of the genus *Madracis* sp. (hermaphroditic and brooding Pocilloporidae; Vermeij et al., 2004).

Female Gametogenesis

The morphological profiles of female gametogenesis in *C. inornata* are essentially similar to those described for other species of the same genus. Waller et al. (2005) noticed that in the genus *Caryophyllia* the size of the mature oocyte increases with increases in depth characterizing the habitat of the species. Large oocytes and consequent lecithotrophic development are currently recognized as an adaptation to oligotrophic environments such as abyssal ones (Shilling and Manahan, 1994). Most of the deep water scleractinians studied up to now have lecithotrophic larvae (Burgess and Babcock, 2005). *C. inornata* has relatively small oocytes similar to those of *C. smithii,* that has a depth range matching that of *C. inornata.* Other species of the same genus but from abyssal water, *C. cornuformis*, *C. sequenzae*, and *C. ambrosia* display oocytes with maximum sizes that are 2–5 times greater. The small size of the oocytes suggests planktotrophic development (Pechenik, 1999). The size of the oocytes reflects the energetic balance for dispersion, larval settlement, and metamorphosis (Dahan and Benayahu, 1998; Cordes et al., 2001). Planktotrophic larvae generally have a rather long pelagic larval phase, throughout which they feed in the column of water, and a marked ability to disperse. The production of planktotrophic larvae is often combined with high fecundity, thus increasing the probability of recruitment. The fertility estimated for *C. inornata,* despite involving a small number of samples, appears to be relatively high, in the order of thou-

Fig. 6. *Caryophyllia inornata*. Embryogenesis. **A** (detail in the rectangle): Localization of the embryos within of the gastrovascular cavity and in morphological continuity with mesenterial septa. **B**: Early embryos without blastocoel cavity. **C**: Stereogastrula (intermediate stage) located inside a mesenterial septa. Note the presence of two spermaries in the same section (arrow). **D**: Stereogastrula (intermediate stage) in the gastrovascular cavity surrounded by the mesenterial tissues, note the presence of an oocyte. **E**: Early embryos located inside an embryo in a more advanced stage of development. **F**: Stereogastrula (intermediate stage). At this stage of development, the ectoderm is clearly distinct from the endoderm. The two layers are divided by the mesoglea (arrows). **G**: Late stereogastrula, transversal section. Differentiation of the mesenterial septa due to introflexion of the mesoglea (arrow). **H**: Late stereogastrula, longitudinal section. Detail of the oral pole of the embryo showing the stomodeal invagination. **I:** Transversal section. Note the stomodeal opening, surrounded by the ectodermal layer. **J:** Detail of the late stereogastrula. Ectodermal cells have begun to multiply, forming an invagination at the embryo's oral pole. The arrow indicates the pharynx. [cc: coelentric cavity; ss: skeletal septum; mf: mesenterial filament; em: embryo; sp: spermarium; m: mesoglea; o: oocyte; ec: ectoderm; en: endoderm; ms: mesenterial septa; s: stomodeal invagination; f: pharynx.]

sands of oocytes per polyp, similar to that of *C. smithii*.

Oral–Aboral Distribution of Gametogenic Processes

The different distribution observed between spermaries and oocytes might be caused by the migration of these reproductive elements toward the oral and aboral pole, respectively, during their maturation. The oral–aboral distribution of the gametogenetic processes of *C. inornata* is similar to that found in the Dendrophylliidae *B. europaea,* a simultaneous hermaphrodite (Goffredo et al., 2002). We have hypothesized that this type of arrangement, adopted by hermaphroditic species, may decrease encounters between opposite sex gametes produced by the same individual thus serving as a "statistical barrier" to self-fertilization. (Goffredo et al., 2005). Gonochorism ensures cross fertilization. In this case, the stage of maturation and the sizes of the spermaries progressively increase toward the oral pole of the polyp, to allow dispersion of the spermatozoa into the environment. Conversely, the mature oocytes cluster at the base of the polyp, where the embryos develop.

Reproductive Mode

C. inornata is the first certain record of brooder reproductive mode in the genus. Previously, a possible instance of brooding was shown for *C. smithii* (Hiscock and Howlett, 1977) and *Caryophyllia clavus* (Fadlallah, 1983). The other three species whose reproductive mode is known, the deep species *C. cornuformis, C. sequenzae,* and *C. ambrosia* are all broadcast spawners. The reproductive mode appears to be a relatively flexible and variable characteristic in the genus *Caryophyllia* (Harrison, 2011). Shlesinger et al. (1998) suggest that brooding might be the ancestral reproductive mode in hexacorallia, but consider spawning as a derived reproductive characteristic.

Embryonic Development

In this study, oocytes were found only inside the mesentery, including those at the more mature stages of development. Embryos were also observed in the mesenterial septa, within the mesoglea layer and wrapped in the gastrodermis, and in the gastrovascular cavity. The union of the gametes might occur when the mature oocyte is still inside the mesentery. There is no evidence of a blastocoel formation during embryogenesis; embryonic development proceeds via stereoblastulae, and subsequent gastrulation occurs by delamination, giving rise in the last stages of development to fully formed embryos, with clearly differentiated mouth and pharynx and a gastrovascular cavity divided by the mesenterial septa. To the authors' knowledge, this is the first detailed description of embryogenesis in the genus *Caryophyllia*. The morphological profiles are like those observed in other solitary Mediterranean scleractinian corals (Goffredo and Telò, 1998; Goffredo et al., 2000, 2002, 2005). In general, embryonic development might be correlated to the reproductive mode: in brooding corals, physical restrictions might lead to stereoblastulae formation, whereas in the case of broadcast spawners, where there is no physical restriction, celoblastulae development might be possible (Heltzel and Babcock, 2002).

CONCLUSIONS

The population of *C. inornata* from Elba Island is gonochoric, with a sex ratio in favor of males. Mature oocytes are small-sized, suggesting a possible planktotrophic development of the larvae. Embryos, which do not have a blastocoel cavity, develop inside the mesenterial septa and the gastrovascular cavity of females, males, and sexually inactive individuals, suggesting a possible asexual origin.

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LITERATURE CITED

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

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

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

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 23:171–82.

Black R, Johnson MS. 1979. Asexual viviparity and population genetics of *Actinia tenebrosa*. Mar Biol 53:27–31.

Bosch TCG. 2009. Hydra and the evolution of stem cells. BioEssays 31:478–486.

Bradshaw AD. 1965. Evolutionary significance of phenotypic plasticity in plants. Adv Genet 13:115–156.

Burgess SN, Babcock RC. 2005. Reproductive ecology of three reef-forming deep-sea corals in the New Zealand region. In: Friewald A, Roberts JM, editors. Cold water corals and ecosystems. Berlin, Heidelberg: Springer-Verlag. pp 701–713.

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

Campbell RD. 1974. Development. In: Muscatine L, Lenhoff HM, editors. Coelenterate biology. Reviews and new perspectives. New York: Academic Press. pp 179–210.

Carvalho GR. 1994. Genetics of aquatic clonal organisms. In: Beaumont AR, editor. Genetics and evolution of aquatic organisms. London: Chapman & Hall. pp 291–322.

Charnov EL. 1982. The theory of sex allocation. Princeton: Princeton University Press. 355 p.

Colley SB, Feingold JS, Peña J, Glynn PW. 2000. Reproductive ecology of *Diaseris distorta* (Michelin) (Fungiidae) in the Galápagos Islands, Ecuador. In: Proc 9th Int Coral Reef Symp, Bali, Vol. 1, pp 1–7.

Connell JH. 1973. Population ecology of reef-building corals. In: Jones OA, Endeans R, editors. Biology and Geology of Coral Reefs II. New York: Academic Press. pp 205–246.

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

Dahan M, Benayahu Y. 1998. Embryogenesis, planulae longevity, and competence in the octocoral *Dendronephthya hemprichi*. Invertebr Biol 117:271–280.

Daly M, Fautin DG, Cappola VA. 2003. Systematics of the Hexacorallia (Cnidaria: Anthozoa). Zool J Linn Soc 139:419–437.

Fadlallah YH. 1983. Sexual reproduction, development and larval biology in scleractinian corals: A review. Coral Reefs 2:129–50.

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

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

Ghiselin MT. 1974. The economy of nature and the evolution of sex. Berkeley: University of California Press.346 p.

Gilmour JP. 2002a. Acute sedimentation causes size-specific mortality and asexual budding in the mushroom coral, *Fungia fungites*. Mar Freshw Res 53:805–812.

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

Glynn PW, Colley SB, Mate JL, Cortes J, Guzman HM, Bailey RL, Feingold JS, Enochs IC. 2008. Reproductive ecology of the azoxanthellate coral *Tubastraea coccinea* in the Equatorial Eastern Pacific: Part V. Dendrophylliidae. Mar Biol 153:529–544.

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

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

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, Chadwick-Furman NE. 2003. Comparative demography of mushroom corals (Scleractinia: Fungiidae) at Eilat, northern Red Sea. Mar Biol 142:411–418.

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, 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, 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, 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.

Harley CDG, Randall Hughes A, 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. 2011. Sexual reproduction of scleractinian corals. In: Dubinsky Z, Stambler N, editors. Coral Reefs: An Ecosystem in Transition. Berlin: Springer. pp 59–85.

Harrison PL, Wallace CC. 1990. Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky Z, editor. Coral Reefs, Ecosystems of the World, Vol. 25. Amsterdam: Elsevier. pp 133–207.

Heltzel PS, Babcock RC. 2002. Sexual reproduction, larval development and benthic planulae of the solitary coral *Monomyces rubrum* (Scleractinia: Anthozoa). Mar Biol 140:659–667.

Hiscock K, Howlett RM. 1977. The ecology of *Caryophyllia smithii* Strokes and Broderip on south-western coasts of the British Isles. In: Drew EA, Lythgoe JN, Woods JD, editors. Underwater Research. New York: Academic Press. pp 319–334.

Holstein TW, Hobmayer E, Technau U. 2003. Cnidarians: An evolutionary conserved model system for regulation? Dev Dyn 226:257–267.

Hyman L. 1940. The invertebrates I. Protozoa through Ctenophora. New York: McGraw Hill.726 p.

Jackson JBC, Coates AG. 1986. Life cycles and evolution of clonal (modular) animals. Philos Trans R Soc Lond B 313:7–22.

Kerr AM, Baird AH, Hughes TP. 2011. Correlated evolution of sex and reproductive mode in corals (Anthozoa: Scleractinia). Proc R Soc B: Biol Sci 278:75–81.

Kitahara MV, Cairns SD, Miller DJ. 2010. Monophyletic origin of *Caryophyllia* (Scleractinia, Caryophylliidae), with descriptions of six new species. Syst Biodivers 8:91–118.

Kramarsky-Winter E, Loya Y. 1998. Reproductive strategies of two fungiid corals from the northern Red Sea: Environmental constraints? Mar Ecol Prog Ser 174:175–182.

Kruzic 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.

Lacaze-Duthiers H. 1873. Dèveloppement des coralliaires. Actinaires à Polipiers. Archs Zool Exp Gen 2:269–348.

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.

Lawrence AJ, Soame JM. 2004. The effects of climate change on the reproduction of coastal invertebrates. Ibis 146:29–39.

Loya Y, Sakai K. 2008. Bidirectional sex change in mushroom stony corals. Proc R Soc B: Biol Sci 275:2335–2343.

Loya Y, Sakai K, Heyward A. 2009. Reproductive patterns of fungiid corals in Okinawa, Japan. Galaxea 11:119–129.

Mangubhai S, Harrison PL. 2008a. Asyncronous coral spawning patterns on equatorial reefs in Kenya. Mar Ecol Prog Ser 360:85–96.

Mangubhai S, Harrison PL. 2008b. Gametogenesis, spawning and fecundity of *Platigyra daedalea* (Scleractinia) on equatorial reefs in Kenya. Coral Reefs 27:117–122.

Maynard Smith J. 1978. The evolution of sex. Cambridge: Cambridge University Press. 222 p.

McClanahan T, Weil E, Cortes J, Baird AH, Ateweberhan M. 2009. Consequences of coral bleaching for sessile reef organisms. In: van Oppen MJH, Lough JM, editors. Ecological Studies: Coral Bleaching: Patterns, Processes, Causes and Consequences. Berlin: Springer-Verlag. pp 121–138.

Minelli A, Ruffo S, La Posta S. 1995. Checklist delle specie della fauna italiana. Cnidaria, Ctenophora. III. Bologna: Edizioni Calderini.38 p.

Nakano Y, Yamazoto K. 1992. Ecological study of reproduction of *Oulastrea crispata* in Okinawa. Zool Sci 9:1292.

Neves EG, Pires DO. 2002. Sexual reproduction of Brazilian coral *Mussimilia hispida* (Verril, 1902). Coral Reefs 21: 161–168.

Ottaway JR, Kirby DC. 1975. Genetic relationships between brooding and brooded *Actinia tenebrosa*. Nature 255:221–223.

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

Policansky D. 1982. Sex change in plants and animals. Annu Rev Ecol Syst 13:471–495.

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.

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.

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

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.

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

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

Solomon S, Qin D, Manning M, Marquis M, Averyt K, Tignor MMB, Le Roy Miller H Jr, Chen Z. 2007. 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: Cambridge University Press.40 p.

Stearns SC. 1992. Trade-offs. In: Stearns SC, editor. The Evolution of Life Histories. Oxford: Oxford University Press. pp 72–90.

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

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

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

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

van Woesik R. 2009. Calm before the spawn: Global coral spawning patterns are explained by regional wind fields. Proc R Soc B: Biol Sci 277:715–722.

Vermeij MJA, Sampayo E, Broker K, Bak RPM. 2004. The reproductive biology of closely related coral species: Gametogenesis in *Madracis* from the southern Caribbean. Coral Reefs 23:206–214.

Waller GR, Tyler PA, Gage JD. 2002. The reproductive ecology of the deep-sea scleractinian coral *Fungiacyathus marenzelleri* (Vaughan, 1906) in the Northeast Atlantic Ocean. Coral Reefs 21:325–331.

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.

Warner RR. 1975. Sexual-asexual evolutionary equilibrium? Am Nat 112:960–962.

Williams GC. 1975. Sex and Evolution. Princeton: Princeton University Press.210 p.

Williams GC, Mitton JB. 1973. Why reproduce sexually? J Theor Biol 39:545–554.

Yeoh SR, Dai CF. 2010. The production of sexual and asexual larvae within single broods of the scleractinian coral, *Pocillopora damicornis*. Mar Biol 157:351–359.

Zibrowius H. 1978. Les scléractiniaires des grottes sous-marines en Méditerranée et dans l'Atlantique nord-oriental (Portugal, Madère, Canaries, Açores). Pubblicazioni della Stazione zoologica di Napoli. Mar Ecol 40:516–545.

Zibrowius H. 1980. Les scléractiniaires de la Méditerranée et de l'Atlantique nord-oriental. Mem Inst Oceanogr (Monaco) 11:1–284.

Chapter III. Sexual reproduction in the Mediterranean endemic orange coral, *Astroides calycularis* (Scleractinia, Dendrophylliidae)

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SEXUAL REPRODUCTION IN THE MEDITERRANEAN ENDEMIC ORANGE CORAL *ASTROIDES CALYCULARIS* (SCLERACTINIA: DENDROPHYLLIIDAE)

Stefano Goffredo, Gabriella Gasparini, Giulia Marconi, Maria Teresa Putignano, Claudia Pazzini, Valentina Airi, and Francesco Zaccanti

ABSTRACT

Astroides calycularis (Pallas, 1766) is a common endemic azooxanthellate scleractinian coral living in the southwestern Mediterranean Sea, generally in shaded habitats, below overhangs, or at cave entrances, from the surface to 50 m depth. The annual reproductive cycle of *A. calycularis* (gamete development in relation to environmental parameters, planulation timing, size at sexual maturity, fecundity, and sex ratio) was studied at Palinuro in the southern Tyrrhenian Sea (Italy) from April 2004 to September 2005. Colonies were gonochoric, were mature at 3–4 cm^2 in area, and had a sex ratio of 1:1. Polyps were sexually mature at 3–4 mm length (maximum diameter of the oral disc), and the females brooded their larvae. The maturation of spermaries took 7 mo and that of oocytes took over 12 mo. The rate of gamete development increased significantly from November to March. Fertilization occurred from April to May, with planulation in June. Mature oocytes ranged from 400 to 1590 μm and planulae size was 1850 μm (oral–aboral axis). Seasonal variation in seawater temperature and photoperiod likely play an important role in regulating reproductive events. The amount of energy devoted to male gametogenesis (quantified by gamete index) was significantly higher than female gametogenesis. In relation to other dendrophylliids, *A. calycularis* presents an intermediate reproductive strategy on the r-K continuum.

Understanding the population dynamics and dispersal in marine organisms requires knowledge of their reproductive biology (Stearns 2000), which includes sexuality (hermaphroditic or gonochoric), reproductive mode (brooding or broadcast spawning), embryonic development (coeloblastic or stereoblastic), and larval development (benthic or planktonic).

Sexual maturity, which depends on the size and age of the organism, is determined by a balance between the growth rate and mortality risk. Population growth rates are influenced by the age and size at reproduction, as well as by the sex ratio (Babcock 1991, Fujiwara and Caswell 2001). Complex interactions between intrinsic factors such as size, age, and physiological condition, as well as extrinsic factors such as density, food availability, physical disturbance, and predation regulate the timing of sexual maturity (Harvell and Grosberg 1988). Reproductive capability and mortality of scleractinian coral species generally depend on colony area (Babcock 1991, Hall and Hughes 1996).

In scleractinian corals, the most common form of sexuality is hermaphroditism, while gonochorism occurs only in 25% of the species studied (Hall and Hughes 1996, Kruzic 2008, Baird et al. 2009). In the Mediterranean Sea, sexual reproduction is described for only seven species of scleractinians. Of these, three reports are from the

19th century (Lacaze-Duthiers 1897), with the sexual reproduction of four species, *Astroides calycularis* (Pallas, 1766), *Balanophyllia europaea* (Risso, 1826), *Cladocora caespitosa* (Linnaeus, 1767), and *Leptopsammia pruvoti* (Lacaze-Duthiers, 1897), described more recently (Goffredo and Telò 1998, Goffredo et al. 2002, 2005, 2006, 2010, Kruzic 2008, Baird et al. 2009).

The cycle of gametogenesis usually culminates with a short period in which gametes are released into the environment where external fertilization occurs (Harrison and Wallace 1990, Richmond and Hunter 1990). To maximize fertilization rate and reproductive success, it is important that gamete development and release be synchronous, since the rapid dilution of gametes in the aquatic environment lowers the probability of fertile encounters (Harrison and Wallace 1990, Levitan 1996). Regulation of the reproductive cycle in corals is correlated with several environmental factors, such as seawater temperature and photoperiod (Harrison and Wallace 1990, Richmond and Hunter 1990, Soong 1991, Penland et al. 2004).

The family Dendrophylliidae is cosmopolitan and includes both solitary and colonial corals; 148 living species are described and divided into 19 genera (Cairns 1999). Seven species live in the Mediterranean Sea, and these are grouped into five genera; three of these (*Astroides, Cladopsammia,* and *Dendrophyllia*) are colonial (Minelli et al. 1995). The genus *Astroides* is made up of a single species, *A. calycularis* (Cairns 2001).

Astroides calycularis is gonochoric (male and female colonies) and brooding (planula releasing, Goffredo et al. 2010). The smaller size of peripheral polyps compared to central ones suggests that polyp budding occurs preferentially at the outskirts of the colonies, possibly increasing the competitive advantage for space utilization (Goffredo et al. 2011). Large colonies have polyps that are of a smaller size than small and medium colonies, suggesting that in larger colonies, energy is invested in increasing polyp size only up to the size at sexual maturity, rather than increasing the size of already mature polyps (Goffredo et al. 2011). *Astroides calycularis* is a Mediterranean and Ibero-Moroccan Bay endemic species and is believed to be a warm water species with narrow temperature tolerance (Zibrowius 1995, Grubelic et al. 2004, Goffredo et al. 2010). However, it has also been found outside the Strait of Gibraltar, along the Atlantic coasts of Morocco and Spain (Bianchi 2007), with some recent records in the northeastern part of the Adriatic Sea, along the coasts of Croatia (Grubelic et al. 2004, Bianchi 2007, Kruzic 2008) up to the Gulf of Venice (Casellato et al. 2007). *Astroides calycularis* is found from the surface to 50 m (Rossi 1971), but is typically found in the shallow infralittoral (0–15 m depth), on vertical walls, or inside caves (Kruzic et al. 2002). It is an azooxanthellate species (Cairns 1999, Goffredo et al. 2010), living in both light and dark, and seems to prefer elevated currents (Kruzic et al. 2002, Grubelic et al. 2004). The population density can be high, with colonies covering up to 90% of the rocky walls (S Goffredo, pers obs). Generally, the colonies have an ellipsoid shape with polyps densely crowded or separated, depending on water flow (Kruzic et al. 2002, Goffredo et al. 2010).

One of the fundamental challenges facing ecologists is to understand how natural systems will respond to environmental conditions (Harley et al. 2006). Global warming is likely to alter the phase relationship between environmental cues, such as photoperiod and temperature, that control or synchronize the reproductive cycle of many marine invertebrates, and such changes are likely to be greatest in temperate areas (Solomon et al. 2007). The potential impact of climate change on marine

invertebrate reproduction highlights the need to understand the physiological basis of reproduction in marine organisms (Lawrence and Soame 2004).

Here we report on the quantitative aspects of the annual reproductive cycle of *A. calycularis*, gamete development in relation to environmental parameters, planulation timing, colony and polyp size at sexual maturity, fecundity, and sex ratio. Morphological aspects of spermatogenesis, ovogenesis, embryogenesis, and larval development have been described elsewhere (Goffredo et al. 2010).

Materials and Methods

Sampling.—*Astroides calycularis* samples were collected at Palinuro (Italy, southern Tyrrhenian Sea; 40°01.81′N, 15°16.74′E) during 16 monthly collections from April 2004 to September 2005 at a depth of 7–10 m along a randomly placed transect line, parallel to the coast line; distance between two consecutive colonies was 2 m. The mean time interval between sampling events was 33.2 d (SE = 1.8 d). Water temperature was measured directly in the field at the depth and time of sampling using a mercury thermometer. Photoperiod data were taken from the online database Ciraci P; EuroMETEO®. Rome, Italy: Nautica Editrice Srl; 4 January, 2011, c1995–2011, 14 October, 1995. Available from: http://www.eurometeo.com.

During each sampling period, 10 colonies of *A. calycularis* were collected, fixed in saturated formalin solution (10% formaldehyde and 90% seawater; solution saturated with calcium carbonate), and transferred to the laboratories for histological analysis.

Biometric Analysis.—For each collected colony, colony length (L_C, major axis of the colony) and width (W_C, minor axis of the colony) were measured and used to compute colony area (A_C) using the formula

$$A_C = \pi \frac{L_C \cdot W_C}{4}.$$

Colony surface area was used because this is a more accurate and representative measure of colony size than colony length (Meesters et al. 2001, Nozawa et al. 2008). A biometric analysis of all of the polyps in each collected colony was performed: polyp length (L_P, major axis of the oral disc), width (W_P, minor axis of the oral disc), and height (h, oral-aboral axis) were measured and used to compute body volume (V_P), using the formula

$$V_P = \pi \frac{h \cdot L_P \cdot W_P}{4}$$ (Goffredo et al. 2002).

Histological and Cytometric Analysis.—Polyps were post-fixed in Bouin solution. After decalcification in EDTA and dehydration in a graded ethanol series from 80% to 100%, polyps were embedded in paraffin and serial transverse sections were cut at 7 μm intervals from the oral to the aboral poles. Tissues were then stained with Mayer's hematoxylin and eosin. Histological observations were made under a light microscope and cyto-histological measurements were made with a Leica Q5001 W image analyzer. To calculate the size of the oocytes in nucleated sections and of the spermaries at different stages of maturation, the largest (maximum diameter, *D*) and smallest sizes (minimum diameter, *d*) of each were measured. Gamete size was determined as the average of the two diameters. Spermaries were classified into five morphologically identified developmental stages according to Glynn et al. (2000) and Goffredo et al. (2005, 2010). Similarly, the average of the maximum and minimum diameters of the embryos was used to calculate their size (Goffredo and Telò 1998, Goffredo et al. 2005).

Gamete Index.—Oocytes and spermaries were ellipsoidal in shape, thus the volume (V_0) of oocyte or spermary was estimated using the formula

$$V_0 = \frac{4}{3}\pi\left(\frac{D}{2}\right)\left(\frac{d}{2}\right)^2$$ (Goffredo et al. 2006).

Volume of gametes was calculated as the sum of the volume of each oocyte or spermary and the gamete index was expressed as the percentage of body volume occupied by the gametes (Goffredo et al. 2006).

Size at Sexual Maturity and Fecundity.—The minimum size at sexual maturity of polyps was considered as the size at which 50% of the individuals developed either spermaries or oocytes (Oh and Hartnoll 1999, Roa et al. 1999). Fecundity was expressed both at the polyp and the colony level. At the polyp level, fecundity (F) was expressed as the number of mature oocytes produced per female polyp per reproductive season using the formula

$$F = \frac{A \cdot B}{C},$$

where A is the length of the "ovary" (based on the number of sections in which oocytes were present), B is the observed frequency of mature oocytes, and C is the size of mature oocytes (Goffredo et al. 2006). At the colony level, fecundity was calculated as the sum of the fecundity estimates for each polyp of the female colony.

Results

Sexuality and Reproductive mode.—Histological examination of the 53 colonies revealed no signs of sexual dimorphism at either the polyp or the colony level. There were no significant differences in mean polyp and colony size between males and females (Student's t-test for L_P: t = 0.894, P = 0.373; Student's t-test for V_P: t = 0.031, P = 0.975; Student's t-test for L_C: t = 1.095, P = 0.280; Student's t-test for A_C: t = 0.486, P = 0.630; Table 1). The sex ratio of colonies was 1:1 (Chi-square test: χ^2 = 0.143, df = 1, P = 0.705, was calculated for 15 female colonies and 13 male colonies sampled in the annual period of maximum gamete expression from November to May). Fifty-nine polyps were inactive; 27 of them were from 15 female colonies (L_P = 3.66 mm, SE = 0.23; V_P = 45.40 mm^3, SE = 7.87), four were from four male colonies (L_P = 2.48 mm, SE = 0.36; V_P = 15.57 mm^3, SE = 4.90), and the remaining 28 inactive polyps (L_P = 5.13 mm, SE = 0.13; V_P = 93.17 mm^3, SE = 8.55) were from 14 indeterminate colonies collected in the summer-autumn period, from July to October. The mean size of the 27 inactive polyps from 15 female colonies was significantly smaller than the mean size of the 80 analyzed female polyps (Student's t-test for L_P: t = 7.779,

Table 1. Mean size and standard error of sexually active *Astroides calycularis* (males and females) and inactive/indeterminate polyps or colonies (L_P major axis of the oral disc of the polyp, V_P polyp volume, L_C major axis of the colony, A_C colony area, n number of polyps or colonies examined).

	Sexually active	Males	Females	Inactive polyps / indeterminate colonies
L_P (mm)	5.1 ± 0.1 (n = 123)	5.0 ± 0.1 (n = 43)	5.2 ± 0.1 (n = 80)	4.3 ± 0.2 (n = 59)
V_P (mm^3)	104.5 ± 3.9 (n = 123)	104.3 ± 6.8 (n = 43)	104.5 ± 4.8 (n = 80)	66.1 ± 6.4 (n = 59)
L_C (cm)	5.0 ± 0.3 (n = 39)	5.5 ± 0.4 (n = 13)	4.8 ± 0.4 (n = 26)	5.5 ± 0.6 (n = 14)
A_C (cm^2)	16.8 ± 1.9 (n = 39)	18.1 ± 3.0 (n = 13)	16.2 ± 2.4 (n = 26)	20.3 ± 4.2 (n = 14)

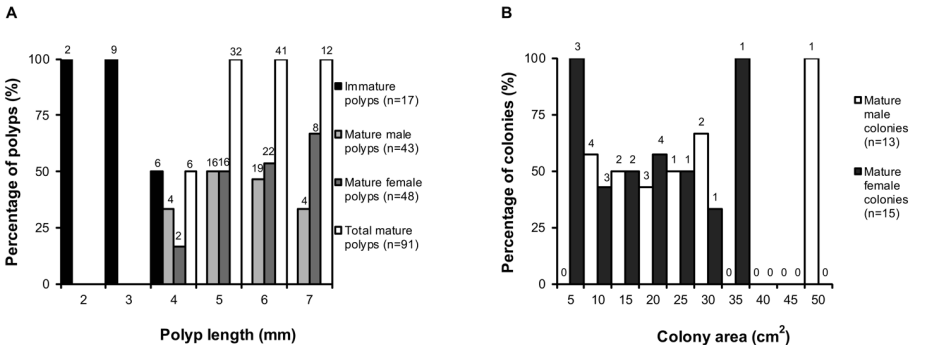


Figure 1. Percentage of sexually mature *Astroides calycularis* per size class from November to May, the period of maximum annual gamete activity. (A) Percentage of mature polyps per size class; total number of polyps measured = 108. (B) Percentage of mature colonies per size class; total number of colonies measured = 28.

P = 5.312 × 10^{-12}; Student's *t*-test for V_P: *t* = 6.305, P = 6.915 × 10^{-9}); the mean size of the four inactive polyps from four male colonies was significantly smaller than the mean size of the 43 analyzed male polyps (Student's *t*-test for L_P: *t* = 6.637, P = 3.525 × 10^{-8}; Student's *t*-test for V_P: *t* = 3.949, P = 2.731 × 10^{-4}); the mean size of the 28 inactive polyps from the 14 indeterminate colonies was not significantly different from the mean size of the 123 sexually active polyps (Student's *t*-test for L_P: *t* = 0.162, P = 0.872; Student's *t*-test for V_P: *t* = 1.241, P = 0.216). The mean size of the 14 indeterminate colonies was not significantly different from the mean size of the 39 sexually active colonies (Student's *t*-test for L_C: *t* = 0.788, P = 0.434; Student's *t*-test for A_C: *t* = 0.868, P = 0.389; Table 1). Embryos were found in the coelenteron of seven out of 10 (70%) female polyps collected in three female colonies of May 2004 and 2005. Polyps were sexually mature at 3–4 mm in length (Fig. 1A). According to biometric analyses (Goffredo et al. 2011), a polyp in this category has W_P = 3–4 mm, *h* = 3–4 mm, V_P = 17–45 mm^3. Colonies were sexually mature at 3–4 cm^2 in area (Fig. 1B). According to biometric analyses (Goffredo et al. 2011), a colony in this category has L_C = 2–3 cm, W_C = 2–3 cm.

Distribution of Gametogenetic Processes Along the Oral–Aboral Axis.—Gamete distribution along the polyp oral–aboral axis differed significantly between males and females (Fig. 2). While spermary size in males was not correlated with the distance from the oral pole, the size of the oocytes in females had a positive correlation. Furthermore, the mean distance of spermaries from the oral pole was significantly less than that of oocytes (Student's *t*-test: *t* = 16.737, df = 48955, P < 0.001; Mann-Whitney's U test: U = 46406652; Wilcoxon's W test: W = 1124905998, P < 0.001).

Annual Sexual Reproductive Cycle.—Gamete size increased more rapidly in males than in females from November to March, the months with shortest photoperiod and coldest water temperature (Fig. 3). In this period, females had two distinct stocks of oocytes, consisting of small (26–400 μm) or large (400–1590 μm) cells. Meanwhile, in males, there was an acceleration in spermatogenesis with a maturation from I to III/IV stage (Fig. 4, Goffredo et al. 2010). Fertilization took place from February to May, when both photoperiod and water temperature

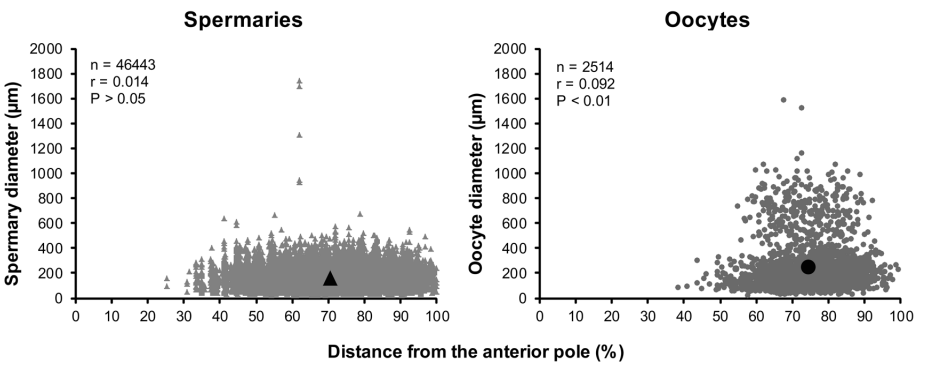


Figure 2. Distribution according to size along the oral–aboral axis of spermaries in *Astroides calycularis* male polyps, and oocytes in female polyps. The distance from the oral pole is expressed as a percentage: 0% = at oral pole level and 100% = at aboral pole level. ▲ the point at which the mean spermary distance (70.58%, SE = 0.06) and mean spermary size (160.36 µm, SE = 0.30) intersect; ● the point at which mean oocyte distance (74.64%, SE = 0.18) and mean oocytes size (242.28 µm, SE = 3.95) intersect.

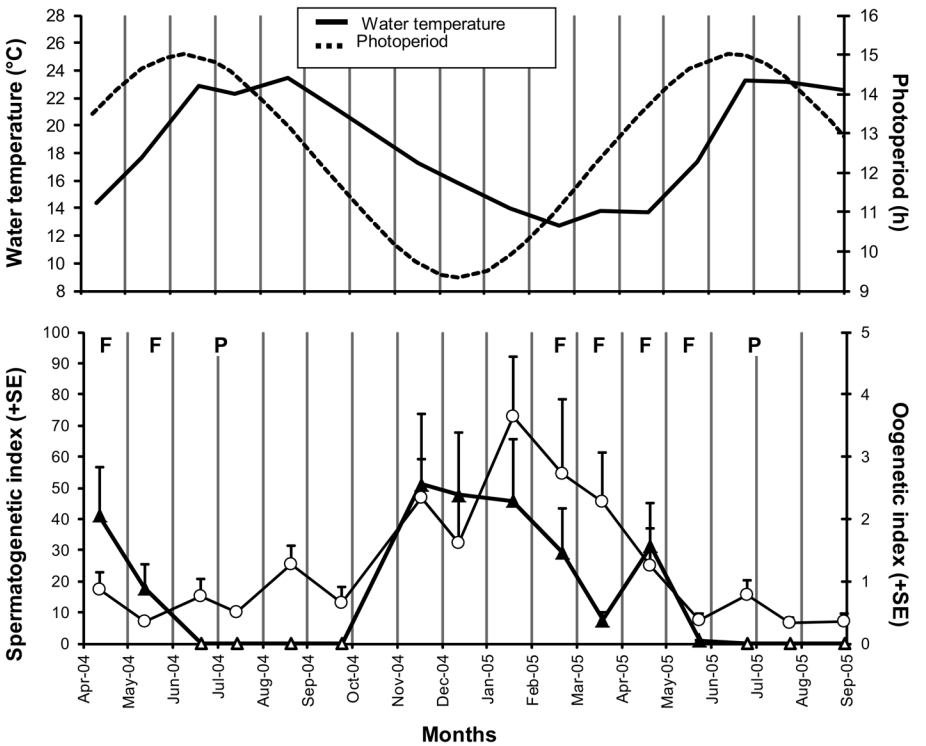


Figure 3. Variation in *Astroides calycularis* gamete development, water temperature, and photoperiod from April 2004 to September 2005 at Palinuro. Note that the value ranges on the ordinate axes are different (○ oocytes; ▲ spermaries; Δ no spermary detected; F = fertilization; P = planulation).

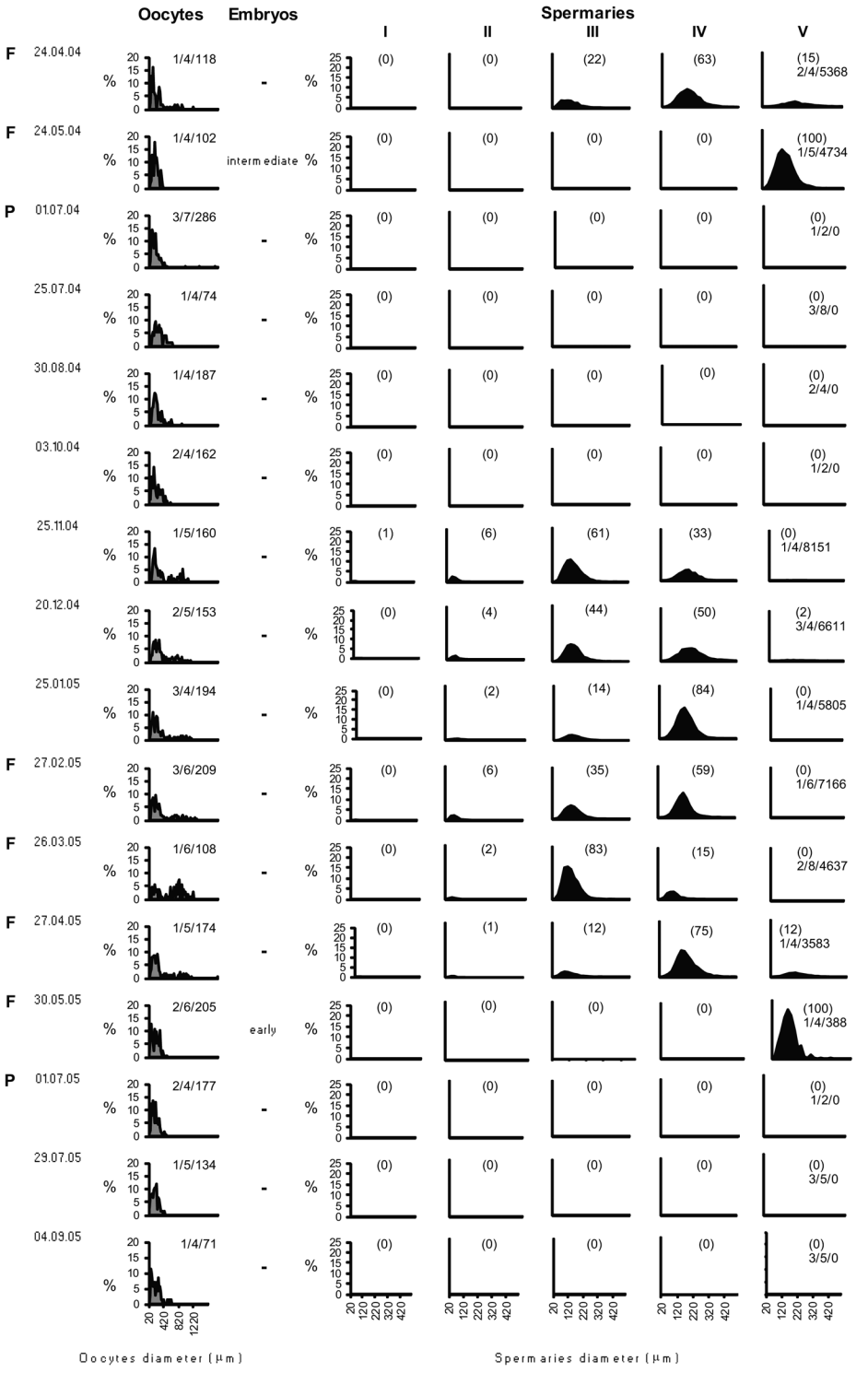
were increasing (Fig. 3). In the samples collected during these months, large-sized oocytes disappeared, while spermaries reached maturity, and early and intermediate embryos were observed in the coelenteric cavity (Figs. 3, 4; Goffredo et al. 2010). During the months immediately following the period of fertilization (June and July), we observed growth of the oocyte stock that remained after the reproductive event and the recruitment of new oocytes in female polyps. Between June and October, the spermaries in male polyps disappeared (Figs. 3, 4). Planulation took place between June and July 2004 and 2005, when photoperiod and water temperatures were at the annual maximum (Fig. 3), and was recognized when mature embryos disappeared from the coelenteric cavity (Fig. 4).

Size of Mature Oocytes and Fecundity.—Mature oocytes ranged from 400 to 1590 μm (Fig. 4). At the polyp level, a mean of 10.1 mature oocytes (SE = 1.9) were found in mean-sized female polyps of V_P = 95.1 mm^3, SE = 7.6 (L_P = 4.7 mm, SE = 0.2; W_P = 4.4 mm, SE = 0.2; h = 5.0 mm, SE = 0.2; n = 58 polyps collected during the period of maximum annual gamete development, from November to May). Polyp fecundity varied with size (Fig. 5A). Specimens of 2–3 mm length contained 0–1 oocytes (n = 13), those of 4–5 mm length contained 10–15 oocytes (n = 36), and those of 6–7 mm length contained 9–23 oocytes (n = 9). At colony level, a mean of 487 mature oocytes (SE = 66) was found in mean-sized female colonies of A_C = 13.5 cm^2, SE = 2.2 (L_C = 4.4 cm, SE = 0.5; W_C = 3.6 cm, SE = 0.3; n = 15 colonies collected during the period of maximum annual gamete development, November–May). Colony fecundity varied with colony area (Fig. 5B). Colonies up to 10 cm^2 contained a mean of 419 oocytes (SE = 101, n = 3), those of 10–20 cm^2 contained a mean of 671 oocytes (SE = 98, n = 6), and those of 20–30 cm^2 contained a mean of 710 oocytes (SE = 54, n = 2).

Discussion

Sexuality and Reproductive Mode.—The sexuality found in *A. calycularis* is typical of Dendrophylliidae, in which gonochorism and brooding are the prevalent reproductive characteristics (Fadlallah 1983, Goffredo et al. 2000, 2005). This systematic pattern in dendrophylliid reproduction has been verified by recent phylogenetic and molecular analyses of the evolution on coral reproductive biology (Baird et al. 2009). Kerr et al. (2010) claim that the organism's reproductive mode (brooding vs spawning) is correlated with the evolution of its sexual system (gonochorism vs hermaphrodism). Harrison (1985) suggests that sexuality is a relatively constant feature within families of scleractinian corals, and defines Dendrophylliidae as a gonochoric family.

Szmant (1986) expected that success in fertilization of a gonochoric brooding species would depend on the population density and its sex ratio. To increase the brooding space in Caribbean coral species, incubation of embryos should yield a sex ratio that favors females. We did not observe this deviation in *A. calycularis*. The size of *A. calycularis* polyps at sexual maturity, compared with that of other solitary dendrophylliids whose reproduction is known, indicates that reproductive activity begins at an intermediate polyp size relative to the range for this family (Table 2). Colony size at sexual maturity observed in *A. calycularis* was higher than in *Tubastraea coccinea* (Lesson, 1829) (Table 2).


Oocytes
Embryos
Spermaries
I
II
III
IV
V
F 24.04.04 1/4/118 - (0) (0) (22) (63) (15) 2/4/5368
F 24.05.04 1/4/102 intermediate (0) (0) (0) (0) (100) 1/5/4734
P 01.07.04 3/7/286 - (0) (0) (0) (0) (0) 1/2/0
25.07.04 1/4/74 - (0) (0) (0) (0) (0) 3/8/0
30.08.04 1/4/187 - (0) (0) (0) (0) (0) 2/4/0
03.10.04 2/4/162 - (0) (0) (0) (0) (0) 1/2/0
25.11.04 1/5/160 - (1) (6) (61) (33) (0) 1/4/8151
20.12.04 2/5/153 - (0) (4) (44) (50) (2) 3/4/6611
25.01.05 3/4/194 - (0) (2) (14) (84) (0) 1/4/5805
F 27.02.05 3/6/209 - (0) (6) (35) (59) (0) 1/6/7166
F 26.03.05 1/6/108 - (0) (2) (83) (15) (0) 2/8/4637
F 27.04.05 1/5/174 - (0) (1) (12) (75) (12) 1/4/3583
F 30.05.05 2/6/205 early (0) (0) (0) (0) (100) 1/4/388
P 01.07.05 2/4/177 - (0) (0) (0) (0) (0) 1/2/0
29.07.05 1/5/134 - (0) (0) (0) (0) (0) 3/5/0
04.09.05 1/4/71 - (0) (0) (0) (0) (0) 3/5/0
%
20 420 820 1220
20 120 220 320 420
Oocytes diameter (µm)
Spermaries diameter (µm)

Oral–Aboral Distribution of Gametogenic Processes.—The observed distribution of reproductive elements along the oral–aboral axis in gonochoric polyps of *A. calycularis* was very similar to that observed in gonochoric polyps of *L. pruvoti* (Goffredo et al. 2006). In these two gonochoric species, the absence of a differential spermary distribution along the oral–aboral axis in males could be related to gonochorism, which ensures the physical separation of male and female gametogenetic processes in separate individuals, and in turn assures cross-fertilization. In contrast, in the simultaneous hermaphroditic polyps of the dendrophylliid *B. europaea,* mature spermaries tend to be distributed toward the oral pole, while mature oocytes are distributed toward the aboral pole (Goffredo et al. 2002). This type of arrangement may reduce the number of encounters between the gametes of the opposite sex in the same individual polyp, producing a "statistical barrier" to self-fertilization (Goffredo et al. 2005).

Annual Reproductive Cycle.—The size frequency distribution of spermaries observed in the different months suggests that spermatogenesis in *A. calycularis* follows an annual cycle, and that male germ cells take 6–7 mo to mature. In the case of females, two oocyte stocks were present, indicating that female germ cells may take longer than 12 mo to mature. Similar gametogenic cycles have been documented for the three other species belonging to the family Dendrophylliidae: the solitary corals *B. europaea* in the Mediterranean Sea (Goffredo and Telò 1998, Goffredo et al. 2002), *Balanophyllia elegans* (Verrill, 1864) along the western coast of North America (Fadlallah and Pearse 1982, Beauchamp 1993), and *L. pruvoti* in the Mediterranean Sea (Goffredo et al. 2005, 2006). Among other azooxanthellate colonial corals, the presence of two oocyte stocks has been observed in *Madrepora oculata* (Linnaeus, 1758) of the family Oculinidae (Waller and Tyler 2005). A longer maturation period for female germ cells compared to male germ cells is typical of gametogenesis in anthozoans (Acosta and Zea 1997, Goffredo et al. 2002, Schleyer et al. 2004, Guest et al. 2005, van Woesik et al. 2006, Ribes and Atkinson 2007, Hellstrom et al. 2010, van Woesik 2010).

The reproductive phase (gamete development, fertilization, planulation) in the annual cycle of *A. calycularis* takes place from October/November to June/July. As the period from June to October was of reproductive quiescence, these summer–autumn months are likely a trophic phase, during which polyps invest in somatic growth.

Reproductive events in this species may occur in relation to seasonal variations in water temperature and photoperiod, which could be the major factor controlling the reproductive activities of corals, as has been suggested for other anthozoans (Glynn et al. 2000, Penland et al. 2004). In winter, photoperiod and water temperature reach their annual minimum and this may act as a signal that could be correlated to gamete development. The subsequent increase in photoperiod and water temperature, during winter and spring, coincides with sperm release and egg fertilization. Recently, blue-light-sensing photoreceptors (cryptochromes) have been detected in the

Figure 4. *(Opposite page)* Size–frequency distribution of oocytes and of the five stages of spermary maturation in monthly samples of *Astroides calycularis* collected at Palinuro from April 2004 to September 2005. Values reported indicate the total number of colonies/total number of polyps/total number of oocytes or spermaries measured per monthly sample. In brackets is the percentage of the stages of spermary maturation. The middle column illustrates the presence and stage of development of embryos found in the coelenteric cavity of female polyps (F = fertilization; P = planulation).

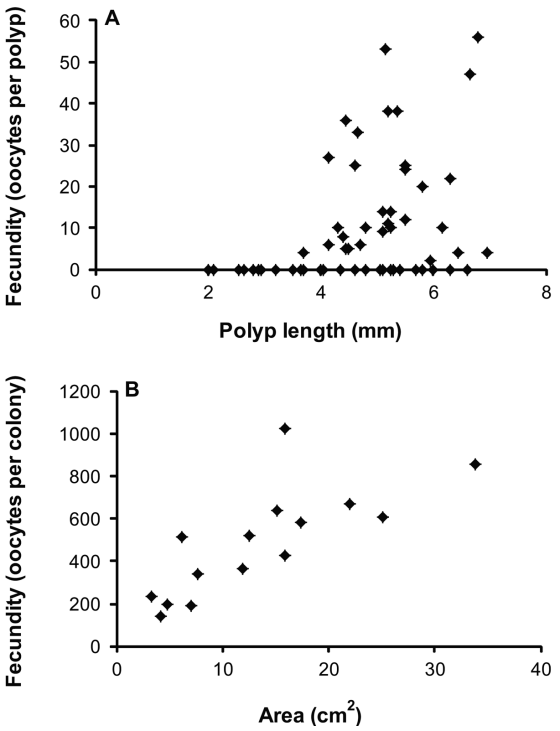


Figure 5. (A) Relationship between fecundity (mature oocytes per polyp) and polyp size (y = 4.447x – 10.952, n = 58, r = 0.369, P < 0.01) in *Astroides calycularis*. (B) Relationship between fecundity (mature oocytes per colony) and colony size (y = 22.341x + 185.360, n = 15, r = 0.759, P < 0.01).

reef building coral *Acropora millepora* (Ehrenberg, 1834) along the Great Barrier Reef. These proteins are ancestral members of the protein family potentially responsible for light perception in animals. In corals, expression patterns of genes coding for photoreceptor proteins vary in response to circadian rhythms, suggesting that mass spawning could be regulated also via photosensitive cryptochromes (Levy et al. 2007).

The period during which germ cells are released varies geographically (Harrison and Wallace 1990, Richmond and Hunter 1990). Comparison of the timing of gamete release within species among localities may reveal population responses to different environmental conditions (Babcock et al. 1994). These environmental factors could also influence reproduction by acting as long-term agents exerting selective pressure on the sexuality of populations (Acosta and Zea 1997).

In May, embryos were found inside the coelentric cavity of female polyps. Planulation occurs during the summer at maximal photoperiod and temperature. Released larvae were observed in the field on the benthos, crawling around the parental polyp, similar to the larvae of *T. coccinea*, another azooxanthellate dendrophylliid coral in the Gulf of California (Paz-García et al. 2007, Goffredo et al. 2010).

There are different interpretations of the role of photoperiod and water temperature in the regulation of the annual reproductive cycle in other dendrophylliids. For example, both of these factors are thought to play a role in regulating major reproductive events in *L. pruvoti* and *B. europaea* (Goffredo et al. 2002, 2005), while

Table 2. Characteristics of the reproductive biology of the species of dendrophylliid corals whose annual reproductive cycle is known.

	Leptopsammia pruvoti	*Tubastraea coccinea*	*Astroides calycularis*	*Balanophyllia europaea*	*Balanophyllia elegans*
Sexuality	Gonochoric	Hermaphroditic	Gonochoric	Hermaphroditic	Gonochoric
Sex ratio	1:1	–	1:1	–	1:1
Polyp size at sexual maturity [fraction of maximum size (observed size, mm)]	32% (3 mm)	– (9 mm)	38% (3 mm)	38% (8 mm)	56% (6 mm)
Maximal polyp size (oral disc maximum diameter, mm)	8	–	8	21	10
Colony size at sexual maturity [fraction of maximum size (observed size, cm^2)]	–	– (1 cm^2)	6% (4 cm^2)	–	–
Maximal colony size (maximum area, cm^2)	–	–	63	–	–
Fecundity (mature oocytes 100 mm^{-3} polyp)	38–114	–	8–13	8–14	2–6
Fecundity (mature oocytes 100 cm^{-2} colony)	–	43,418–68,526	3,222–5,040	–	–
Oocyte volume output (mm^3 mature oocytes 100 cm^{-2} colony)	–	3,420–5,720	299–468	–	–
Embryonic incubation period (mo)	1–4	1–2	1	4–5	14–15
Planulae size (oral–aboral axis, µm)	1,100	1,000	1,850	2,150	4,000
Sources	Goffredo et al. (2005, 2006)	Glynn et al. (2008)	Present study, Goffredo et al. (2010, 2011)	Goffredo et al. (2002, 2004), Goffredo and Zaccanti (2004)	Fadlallah and Pearse (1982), Beauchamp (1993)

the reproductive cycle of *B. elegans* may be regulated by water temperature alone (Fadlallah and Pearse 1982, Beauchamp 1993). Additional studies are necessary to distinguish the role of different environmental factors in regulating reproductive events in these scleractinians.

In our study, information on the annual reproductive cycle was collected over an 18-mo period and therefore inter-annual variation was not examined. However, we observed similar stages in the reproductive cycle during April–September in both years (2004 and 2005). During this period, a significant number of inactive polyps and indeterminate colonies were found (the 32.4% of polyps and 26.4% colonies). The size of the inactive polyps in the indeterminate colonies was not significantly different from those of the active polyps in active colonies. Therefore, it is possible that these elements were in a state of quiescence. In particular, the 14 inactive colonies detected from July to October, when no male colonies were detected, may have been quiescent males after the period of spring fertilization.

The mean annual fecundity of *A. calycularis* (41.3 oocytes cm^{-2} of colony ± 4.6 SE) is lower than in the colonial azooxanthellate brooder dendrophylliid *T. coccinea* in the eastern Pacific (from 227.1 oocytes cm^{-2} of colony ± 1.3 SE in Costa Rica to 897.4 oocytes cm^{-2} of colony ± 0.1 SE in Panama; Glynn et al. 2008). The volume of mature oocytes produced per area unit indicates a lower fecundity for *A. calycularis* with respect to *T. coccinea* (*A. calycularis*: 3.8 mm^3 of oocytes cm^{-2} of colony ± 0.4 SE; *T. coccinea*: from 16.2 mm^3 of oocytes cm^{-2} of colony ± 0.1 SE in Costa Rica to 82.8 mm^3 of oocytes cm^{-2} of colony ± 0.1 SE in Panama; Glynn et al. 2008). *Tubastraea coccinea* has, along with *Porites panamensis* (Verrill, 1866) (Glynn et al. 1994) and *Stylophora pistillata* (Esper, 1797) (Loya 1976, Hall and Hughes 1996), a much higher annual fecundity than other colonial zooxanthellate brooder species (Harrison and Wallace 1990, Glynn et al. 2008). The colonial azooxanthellate but broadcasting species *Lophelia pertusa* (Linnaeus, 1758) has a much higher fecundity than *T. coccinea* (> 3000 oocytes cm^{-2}; Waller and Tyler 2005).

In both the gonochoric *A. calycularis* (the present study) and *L. pruvoti* (Goffredo et al. 2006), the body volume occupied by male gametes was, respectively, 17.7 and 2.6 times greater than that occupied by female gametes. In the simultaneous hermaphroditic *B. europaea,* the body volume used by male gametes is the same as that of female gametes (Goffredo et al. 2000, 2002). Thus, the proportion of energy devoted to male gametogenesis is significantly higher in the gonochoric species than in the hermaphroditic one. This difference could be related to the contrasting sexuality or fertilization biology of these three species. Cross-fertilization likely takes place in the gonochoric *A. calycularis* and *L. pruvoti*, while in the hermaphroditic *B. europaea,* fertilization could be autogamous (Goffredo et al. 2004). To assure successful mating encounters in gonochoric organisms, male sex allocation is greater than in hermaphrodites. Greater male sexual allocation in dioecism or cross-fertilization when compared to hermaphroditism or self-fertilization is common in plants (Charnov 1982, Mione and Anderson 1992, Jurgens et al. 2002).

Reproductive Strategies.—Reproductive strategies of dendrophylliids, in which the reproductive cycle has been described, seem to cover the entire range of the r–K life history strategy continuum (Pianka 1970, Stearns 2000). The gonochoric *L. pruvoti*, having higher levels of fecundity, shorter periods of embryo incubation, and smaller planula size, presents a quantitative strategy (r-reproductive strategy).

In contrast, the gonochoric *B. elegans*, having a longer delay in reaching sexual maturity, lower fecundity, longer embryonic incubation period, and larger planula size, presents a qualitative strategy (K-reproductive strategy). The reproductive strategy of the gonochoric *A. calycularis*, whose reproductive characteristics lie somewhere between the above-mentioned characteristics, is placed intermediate along the r–K continuum.

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Literature Cited

Acosta A, Zea S. 1997. Sexual reproduction of the reef coral *Montastrea cavernosa* (Scleractinia: Faviidae) in the Santa Marta area, Caribbean coast of Colombia. Mar Biol. 128:141–148. http://dx.doi.org/10.1007/s002270050077

Babcock RC. 1991. Comparative demography of three species of scleractinian corals using age- and size-dependent classifications. Ecol Monogr. 61:225–244. http://dx.doi.org/10.2307/2937107

Babcock RC, Willis BL, Simpson CJ. 1994. Mass spawning of corals on high latitude coral reef. Coral Reefs. 13:161–169. http://dx.doi.org/10.1007/BF00301193

Baird AH, Guest JR, Willis BL. 2009. Systematic and biogeographical patterns in the reproductive biology of scleractinian corals. Ann Rev Ecol Syst. 40:531–71.

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. http://dx.doi.org/10.1080/07924259.1993.9672312

Bianchi CN. 2007. Biodiversity issues for the forthcoming tropical Mediterranean Sea. Hydrobiologia. 580:7–21. http://dx.doi.org/10.1007/s10750-006-0469-5

Cairns SD. 1999. Species richness of recent Scleractinia. Atoll Res Bull. 459:1–12.

Cairns SD. 2001. A generic revision and phylogenetic analysis of the Dendrophylliidae (Cnidaria: Scleractinia). Smith Contrib Zool. 615:1–84.

Casellato S, Masiero L, Sichirollo E, Soresi S. 2007. Hidden secrets of the northern Adriatic: "Tegnùe," peculiar reefs. Cen Eur J Biol. 2:122–136. http://dx.doi.org/10.2478/s11535-007-0004-3

Charnov EL. 1982. The theory of sex allocation. Princeton University Press, Princeton.

Fadlallah YH. 1983. Sexual reproduction, development and larval biology in scleractinian corals: a review. Coral Reefs. 2:129–150. http://dx.doi.org/10.1007/BF00336720

Fadlallah YH, Pearse JS. 1982. Sexual reproduction in solitary corals: overlapping oogenic and brooding cycles, and benthic planulas in *Balanophyllia elegans*. Mar Biol. 71:223–231. http://dx.doi.org/10.1007/BF00397039

Fujiwara M, Caswell H. 2001. Demography of endangered North Atlantic right whale. Nature. 414:537–541. PMid:11734852. http://dx.doi.org/10.1038/35107054

Glynn PW, Colley SB, Eakin CM, Smith DB, Cortés J, Gassman NJ, Guzmán HM, Del Rosario JB, Feingold JS. 1994. Reef coral reproduction in the eastern Pacific: Costa Rica, Panamá, and Galápagos Islands (Ecuador). II. Poritidae. Mar Biol. 118:191–208. http://dx.doi.org/10.1007/BF00349785

Glynn PW, Colley SB, Maté JL, Cortés J, Guzman HM, Bailey RL, Feingold JS, Enochs IC. 2008. Reproductive ecology of the azooxanthellate coral *Tubastraea coccinea* in the equatorial eastern Pacific: Part V. Dendrophylliidae. Mar Biol. 153:529–544. http://dx.doi.org/10.1007/s00227-007-0827-5

Glynn PW, Colley SB, Ting JH, Maté JL, Guzman HM. 2000. 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. 136:785–805. http://dx.doi.org/10.1007/s002270000286

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. http://dx.doi.org/10.1007/s00227-005-0137-8

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. http://dx.doi.org/10.3354/meps229083

Goffredo S, Caroselli E, Gasparini G, Marconi G, Putignano MT, Pazzini C, Zaccanti F. 2011. Colony and polyp biometry and size structure in the orange coral *Astroides calycularis* (Scleractinia: Dendrophylliidae). Mar Biol Res. 7:272–280. http://dx.doi.org/10.1080/17451000.2010.492222

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. http://dx.doi.org/10.1080/17451000903428488

Goffredo S, Mattioli G, Zaccanti F. 2004. Growth and population dynamics model of the Mediterranean solitary coral *Balanophyllia europaea* (Scleractinia, Dendrophylliidae). Coral Reefs. 23:433–443. http://dx.doi.org/10.1007/s00338-004-0395-9

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. http://dx.doi.org/10.1007/s00227-005-1567-z

Goffredo S, Telò T. 1998. Hermaphroditism and brooding in the solitary coral *Balanophyllia europaea* (Cnidaria, Anthozoa, Scleractinia). Ital J Zool. 65:159–165. http://dx.doi.org/10.1080/11250009809386740

Goffredo S, Telò T, Scanabissi F. 2000. Ultrastructural observations of the spermatogenesis of the hermaphroditic solitary coral *Balanophyllia europaea* (Anthozoa, Scleractinia). Zoomorphology. 119:231–240. http://dx.doi.org/10.1007/PL00008495

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

Grubelic I, Antolic B, Despalatovic M, Grbec B, Beg Paklar G. 2004. Effect of climatic fluctuations on the distribution of warm-water coral *Astroides calycularis* in the Adriatic Sea new records and review. J Mar Biolog Assoc UK. 84:599–602. http://dx.doi.org/10.1017/S0025315404009609h

Guest JR, Baird AH, Goh BPL, Chou LM, 2005. Reproductive seasonality in an equatorial assemblage of scleractinian corals. Coral Reefs. 24:112–116. http://dx.doi.org/10.1007/s00338-004-0433-7

Hall VR, Hughes TP. 1996. Reproductive strategies of modular organisms: comparative studies of reef-building corals. Ecology. 77:950–963. http://dx.doi.org/10.2307/2265514

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. PMid:16958887. http://dx.doi.org/10.1111/j.1461-0248.2005.00871.x

Harrison PL. 1985. Sexual characteristics of scleractianian corals: systematic and evolutionary implications. Proc Fifth Int Coral Reef Congr, Tahiti.

Harrison PL, Wallace CC. 1990. Reproduction, dispersal and recruitment of scleractinian corals. *In:* Dubinsky Z, editor. Ecosystem of the world. Coral Reefs, Elsevier, Amsterdam. p. 133–207.

Harvell CD, Grosberg RK. 1988. The timing of sexual maturity in clonal animals. Ecology. 69:1855–1864. http://dx.doi.org/10.2307/1941162

Hellstrom M, Kavanagh KD, Benzie JAH. 2010. Multiple spawning events and sexual reproduction in the octocoral *Sarcophyton elegans* (Cnidaria: Alcyonacea) on Lizard Island, Great Barrier Reef. Mar Biol. 157: 383–392. http://dx.doi.org/10.1007/s00227-009-1325-8

Jurgens A, Witt T, Gottsberger G. 2002. Pollen grain numbers, ovule numbers and pollen–ovule ratios in Caryophylloideae: correlation with breeding system, pollination, life form, style number, and sexual system. Sex Plant Reprod. 14:279–289. http://dx.doi.org/10.1007/s00497-001-0124-2

Kerr AM, Baird AH, Hughes TP. 2010. Correlated evolution of sex and reproductive mode in corals (Anthozoa: Scleractinia). Proc R Soc Lond B. 278:75–81. PMid:20659935. http://dx.doi.org/10.1098/rspb.2010.1196

Kruzic P. 2008. First record of *Cladopsammia rolandi* (Cnidaria: Anthozoa) in the Adriatic Sea. Nat Croat. 17:9–14.

Kruzic P, Zibrowius H, Pozar-Domac A. 2002. Actiniaria and Scleractinia (Cnidaria, Anthozoa) from the Adriatic Sea: first records, confirmed occurrences and significant range extensions of certain species. Ital J Zool. 69:345–353. http://dx.doi.org/10.1080/11250000209356480

Lacaze-Duthiers H. 1897. Faune du Golfe du Lion. Coralliaires, Zooanthaires, Sclérodermés. Archives de Zoologie Expérimentale Générale. 5:1–249.

Lawrence AJ, Soame JM. 2004. The effects of climate change on the reproduction of coastal invertebrates. Ibis. 146:29–39. http://dx.doi.org/10.1111/j.1474-919X.2004.00325.x

Levitan DR. 1996. Effects of gamete traits on fertilization in the sea and the evolution of sexual dimorphism. Nature. 382:153–155. http://dx.doi.org/10.1038/382153a0

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. PMid:17947585. http://dx.doi.org/10.1126/science.1145432

Loya Y. 1976. The red sea coral *Stylophora pistillata* is an r strategist. Nature. 259:478–480. http://dx.doi.org/10.1038/259478a0

Meesters EH, Hilterman M, Kardinaal E, Keetman M, deVries M, Bak RPM. 2001. Colony size-frequency distributions of scleractinian coral populations: spatial and interspecific variation. Mar Ecol Prog Ser. 209:43–54. http://dx.doi.org/10.3354/meps209043

Minelli A, Ruffo S, La Posta S. 1995. Checklist delle specie della fauna italiana. Cnidaria, Ctenophora. Edizioni Calderini, Bologna.

Mione T, Anderson GJ. 1992. Pollen–ovule ratios and breeding system evolution in Solanum section Basarthrum (Solanaceae). Am J Bot. 79:279–287. http://dx.doi.org/10.2307/2445016

Nozawa Y, Tokeshi M, Nojima S. 2008. Structure and dynamics of a high-latitude scleractinian coral community in Amakusa, southwestern Japan. Mar Ecol Prog Ser. 358:151–160. http://dx.doi.org/10.3354/meps07342

Oh CW, Hartnoll RG. 1999. Size at sexual maturity, reproductive output, and seasonal reproduction of *Philocheras trispinosus* (Decapoda) in Port Erin Bay, Isle of Man. J Crust Biol. 19:252–259. http://dx.doi.org/10.2307/1549231

Paz-García DA, Reyes-Bonilla H, González-Peralta A, Sánchez-Alcántara I. 2007. Larval release from *Tubastraea coccinea* in the Gulf of California, Mexico. Coral Reefs. 26:433. http://dx.doi.org/10.1007/s00338-007-0219-9

Penland L, Kloulechad J, Idip D, van 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. http://dx.doi.org/10.1007/s00338-003-0362-x

Pianka ER. 1970. On r- and K- selection. Am Nat. 104:592–597. http://dx.doi.org/10.1086/282697

Ribes M, Atkinson MJ. 2007. Effects of water velocity on picoplankton uptake by coral reef communities. Coral Reefs. 26:413–421. http://dx.doi.org/10.1007/s00338-007-0211-4

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. http://dx.doi.org/10.3354/meps060185

Roa R, Ernst B, Tapia F. 1999. Estimation of size at sexual maturity: an evaluation of analytical and resampling procedures. Fish Bull. 97:570–580.

Rossi L. 1971. Cnidari e Ctenofori d'Italia. Quaderni della Civica Stazione Idrobiologica di Milano. 2:77–86.

Schleyer MH, Kruger A, Benayahu Y. 2004. Reproduction and the unusual condition of hermaphroditism in *Sarcophyton glaucum* (Octocorallia, Alcyoniidae) in KwaZulu-Natal, South Africa. Hydrobiologia. 530:399–409. http://dx.doi.org/10.1007/s10750-004-2683-3

Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt K, Tignor MMB, Miller HL. 2007. Climate change 2007: the physical science basis. Cambridge University Press, Cambridge.

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

Stearns SC. 2000. Life history evolution: successes, limitations, and prospects. Naturwissenschaften. 87:476–486. PMid:11151666. http://dx.doi.org/10.1007/s001140050763

Szmant AM. 1986. Reproductive ecology of Caribbean reef corals. Coral Reefs. 5:43–54. http://dx.doi.org/10.1007/BF00302170

van Woesik R. 2010. Calm before the spawn: global coral spawning patterns are explained by regional wind fields. Proc R Soc B Biol Sci. 277:715–722. PMid:19892757. PMCid:2842745. http://dx.doi.org/10.1098/rspb.2009.1524

van Woesik R, Lacharmoise F, Koksal S. 2006. Annual cycles of solar insolation predict spawning times of Caribbean corals. Ecol Lett. 9: 390–98. PMid:16623724. http://dx.doi.org/10.1111/j.1461-0248.2006.00886.x

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. http://dx.doi.org/10.1007/s00338-005-0501-7

Zibrowius H. 1995. The "southern" *Astroides calycularis* in the Pleistocene of the northern Mediterranean—an indicator of climatic change (Cnidaria, Scleractinia). Geobios. 28:9–16. http://dx.doi.org/10.1016/S0016-6995(95)80201-0

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Chapter IV. Reproductive efficiency of a Mediterranean endemic zooxanthellate coral decreases with increasing temperature along a wide latitudinal gradient

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Reproductive efficiency of a Mediterranean endemic zooxanthellate coral decreases with increasing temperature along a wide latitudinal gradient

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Abstract

Investments at the organismal level towards reproduction and growth are often used as indicators of health. Understanding how such energy allocation varies with environmental conditions may, therefore, aid in predicting possible responses to global climatic change in the near future. For example, variations in seawater temperature may alter the physiological functioning, behavior, reproductive output and demographic traits (e.g., productivity) of marine organisms, leading to shifts in the structure, spatial range, and abundance of populations. This study investigated variations in reproductive output associated with local seawater temperature along a wide latitudinal gradient on the western Italian coast, in the zooxanthellate Mediterranean coral, *Balanophyllia europaea*. Reproductive potential varied significantly among sites, where *B. europaea* individuals from the warmest site experienced loss of oocytes during gametogenesis. Most of the early oocytes from warmest sites did not reach maturity, possibly due to inhibition of metabolic processes at high temperatures, causing *B. europaea* to reabsorb the oocytes and utilize them as energy for other vital functions. In a progressively warming Mediterranean, the efficiency of the energy invested in reproduction could be considerably reduced in this species, thereby affecting vital processes. Given the projected increase in seawater temperature as a consequence of global climate change, the present study adds evidence to the threats posed by high temperatures to the survival of *B. europaea* in the next decades.

Introduction

Coral reefs, like many other ecosystems, are currently undergoing changes in biodiversity, ecosystem function, and resilience due to rising seawater temperatures acting in synergy with additional environmental pressures [1]. A rise in global average temperature of 0.7°C since the start of the industrial revolution has caused or contributed to significant losses of global coral cover over the past few decades, and oceans are expected to experience a further warming of 1.1–6.4°C within the 21st century [2]. Climatic models [3] predict that the Mediterranean basin will be one of the most impacted regions by the ongoing warming trend [4]. The Mediterranean is already showing rates of seawater warming that exceed threefold those of the global ocean [2,4], making it a potential model for global scenarios to occur in the world's marine biota, and a natural focus of interest for research [5].

Increasing temperatures are having a strong impact on marine systems [6]. Indeed, temperature is the major environmental factor controlling invertebrate development, marine species distributions and recruitment dynamics [7,8]. Seawater temperature increases will likely affect the population biology of coral species by reducing reproductive capacity [9]. The harmful effects of increasing temperature on coral reproduction include reduced individual fecundity, egg quality, lowered fertilization success and reduced recruitment through effects on post-fertilization processes (e.g., embryonic development, larval development, survival, settlement, metamorphosis, and early post-settlement growth) [10,11]. The combined effects of fertilization failure and reduced embryonic development in some coral species are likely to exacerbate ecological impacts of climate change by reducing biodiversity [12]. Several studies assessed the immediate and delayed impacts of environmental change on Mediterranean gorgonian colonies [11-14 including sublethal impacts on reproductive effort [11,15,16,17], but few studies have examined temperate solitary corals. Research focusing on reproductive processes in regions with peculiar physical conditions is urgently needed as a baseline against which to test the effects of climate change on sexual

reproduction (e.g. fecundity) [10,18] and organismal performance, that are essential to understand population dynamics of marine organisms [19].

Organismal performance under both "normal" and "stressful" conditions is mainly determined by the energetic status of the individual, which can ultimately affect its fitness (i.e. reproductive output). During prolonged periods of stress, the energy balance of a coral is negative and the organism is drawing on all biochemical pools, and thus both storage and structural components for energy could be compromised [20]. Shallow water reef corals strongly rely on energy derived from photosynthesis by its symbiotic zooxanthellae [21]. In particular, key processes like gametogenesis [22], larval longevity and settlement [23] are dependent on the availability of stored energy as lipids that are reabsorbed when resources are limited [24]. If metabolic processes involved in recovery from stress deplete lipid reservoirs in oocytes, then fewer resources are available for new egg production [25], significantly affecting gametogenesis.

This study focused on an endemic zooxanthellate Mediterranean scleractinian, *Balanophyllia europaea* (Fig. S1), a simultaneous hermaphrodite and brooding coral [26]. There is growing concern for the future of this endemic species in light of expected seawater warming, since increasing temperature negatively affects *B. europaea* skeletal density [27] (due to increased porosity [28]), population abundance [29], population structure stability [30], growth and calcification [28]. Our specific aim was to quantify the reproductive output of *B. europaea* along a latitudinal gradient of temperature. We expected to find a similar negative response of reproductive output with increasing temperature.

Materials and Methods

Ethics Statement

This study was carried out following the fundamental ethical principles. According to the European normative, there is no active conservation measure for the Mediterranean scleractinian coral studied here (*B. europaea*). The species is not protected in Italy, nor is it subject to any regulations. Thus,

no permit was needed to sample specimens. For this study, sampling was limited strictly to the number necessary and performed where the species has high population density to minimize the impact of removing individuals and preserve both the demographic and genetic structure of the natural populations.

Specimens of *B. europaea* came from six sites along a latitudinal gradient, from 44°20'N to 36°45'N (Fig. 1). Coral collection began in June 2010 and ended in November 2012. During this period, 18 samples were taken monthly from five populations (Genova: April 2011-September 2012; Elba: December 2010-May 2012; Palinuro: June 2010-November 2011; Scilla: June 2011-November 2012; Pantelleria: June 2011-November 2012), with a minimum of 15 polyps collected during each excursion. Data from Calafuria population came from a previous study [26] in which samples were collected from July 1997 to October 1998.

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 $V = (L/2) * (W/2) * h * \pi$ [26].

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 [26].

Cytometric analyses were made with an optical microscope using the image analyzer NIKON NIS-Elements D 3.2. The maximum and minimum diameters of oocytes in nucleated sections and spermaries were measured and the presence of embryos in the coelenteric cavity was recorded. Spermaries were classified into five developmental stages in accordance with earlier studies on gametogenesis in scleractinians [19,31,32].

Reproductive output was defined through three 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 germ cells [26]); and c) *reproductive element size*, defined as the average of the maximum and minimum diameter of spermaries and oocytes in nucleated section [26].

Based on the reproductive season [26], gametal development in *B. europaea* was divided in two gamete activity periods. The *gametes recruitment period* [33,34] was defined as the post-fertilization period, between June and September, generally characterized by: 1) a stock of smaller oocytes; 2) the recruitment of new oocytes; and 3) the beginning of spermary development [26]. The *gametes maturity period* [33,34] was defined as the pre-fertilization period taking place between December and March and generally characterized by the presence of larger oocytes and advanced stage of maturation of spermaries [26].

Temperature data (Depth Temperature – DT; °C) came from temperature sensors (I-Button DS1921H, Maxim Integrated Products), placed at the sampling location for each population. Sensors recorded temperatures 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 are measured by mareographic stations placed close to the sampling sites using SM3810 manufactured by the Society for the Environmental and Industrial monitoring (SIAP+MICROS). A linear regression was produced between DT and SST data to estimate historical at-depth temperatures. In this study we considered the average DT temperature of the three years preceding the sampling (n = 36 monthly temperatures).

Solar radiation (W/m^2) was collected 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 2.5°-latitude-by-longitude square associated with each of the six sites. As for temperature, also for solar radiation we considered the average of the three years preceding the sampling (n = 36 monthly solar radiation).

Data were checked for normality using a Kolmogorov-Smirnov's test and for variance homoskedasticity using a Levene's test. When assumptions for parametric statistics were not fulfilled, a nonparametric 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; it is useful for data that do not meet ANOVA's assumptions. The non-parametric Kruskal–Wallis test was used to compare reproductive parameters among study sites. The non-parametric Kolmogorov-Smirnov test was used to compare the size-frequency distribution of reproductive elements between populations and between the two periods. Student's *t* test was used to compare the mean oocytes and spermaries size of populations between periods. Spearman's rank correlation coefficient was used to calculate the significance of the correlations between reproductive and environmental parameters. Spearman's rank correlation coefficient is an alternative to Pearson's correlation coefficient [35]. It is useful for data that are non-normally distributed and do not meet the assumptions of Pearson's correlation coefficient [36]. All analyses were computed using PASW Statistics 17.0.

Results

Mean annual solar radiation (W/m²) and mean annual DT (°C) were significantly different among sites (solar radiation, ANOVA, $p < 0.001$; DT, Kruskal-Wallis, $p < 0.05$; Table 1; Fig S2).

All populations contained both oocytes and spermaries during both reproductive periods, while embryos were detected only between June and September (gametes recruitment period). The oocyte size/frequency distribution of June-September (gametes recruitment period) was significantly different from that of December-March (gametes maturity period), in all populations (Kolmogorov-Smirnov, $p < 0.001$; Fig. 2). Within June and September (gametes recruitment period) most oocytes were smaller than 400 µm, in all populations. In the following season (December-March, gametes maturity period), two distinct oocyte stocks appeared in all populations, characterized respectively by small (immature < 400 µm) and large (mature > 400 µm) cells (Fig. 2). The mean oocyte size of June-September (gametes recruitment period) was significantly lower

than that of December-March (gametes maturity period) in all populations (Student's *t*-test, $p < 0.001$; Table 2; Fig. S3).

The distribution of spermary maturation stages in June-September (gametes recruitment period) was significantly different from that in December-March (gametes maturity period), in all populations (Kolmogorov-Smirnov, $p < 0.001$; Fig. 3). Each population was characterized, from June to September (gametes recruitment period), by small spermaries, mainly belonging to the earliest maturation stages (stages I and II). In the period December-March (gametes maturity period), all populations were characterized by more advanced maturation stages (mainly stage III; Fig. 3). The mean spermary size of June-September (gametes recruitment period) was significantly lower than that of December-March (gametes maturity period) in all populations (Student's *t*-test, $p < 0.001$; Table 3; Fig. 3). In all populations, June-September (gametes recruitment period) was characterized by the presence of embryos in the coelenteric cavity.

Fecundity, gonadal index and oocyte size were significantly different among populations, during June-September (gametes recruitment period) (fecundity, Kruskal–Wallis test, $p < 0.01$; gonadal index and oocyte size, Kruskal–Wallis test, $p < 0.001$; Tables 2 and S1). In this period, all oocyte reproductive parameters showed positive correlations with both environmental parameters (DT and solar radiation; Table S1; Fig. S4). During December-March (gametes maturity period), the fecundity and oocyte size were significantly different among populations (fecundity, Kruskal–Wallis test, $p < 0.05$; diameter, Kruskal–Wallis test, $p < 0.001$; Tables 2 and S1). The mean size of oocytes across all populations was negatively correlated with the DT (Table S1; Fig. S5). In the warmest population (Pantelleria island, 19.69 ± 0.05°C; Table 1), the number of mature oocytes at fertilization was three times lower than in the recruitment period, indicating a clear reduction of fecundity during this period (Table 2). In the coldest population (Calafuria, 17.73 ± 0.16°C; Table 1), fecundity was the same during both periods (Table 2).

In both periods, only the spermary size was significantly different among populations (Kruskal–Wallis test, $p < 0.001$; Tables 3 and S2) and in both reproductive periods, spermary size was negatively correlated with both DT and solar radiation (Table S2; Fig. S6 and S7).

Discussion

Traditionally, seawater temperature cycles and solar radiation fluctuations have been related to reproductive timing of gamete development, fertilization and planulation [16,37] providing a reliable cue to reset the biological clock and trigger the physiological changes related to oocyte yolk deposition [38] and spermary development [26,39,40]. The effects of changing photoperiod and seawater temperature on gametogenic cycles of anthozoans have been largely overlooked [15,41,42]. The reproductive biology of *B. europaea*, studied at Calafuria, shows a reproductive seasonality induced by annual variation of seawater temperature and photoperiod [26]. The same pattern seems to appear in other Mediterranean dendrophylliids like *Leptopsammia pruvoti* [39] and *Astroides calycularis* [40] and in the Mediterranean endemic oculinid *Cladocora caespitosa* [43,44]. A similar periodicity for gamete development and embryonic presence during the recruitment period, suggest an overlap of reproductive seasonality in all populations along the latitudinal gradient by *B. europaea*. In broadcasting scleractinian corals, where temperature dependence leads to location-specific synchronous reproductive times [45], temporal variation in spawning events by corals from different latitudes, over two or more consecutive months, is uncommon [18]. In brooding scleractinians, reproductive cycles are protracted over several months coinciding with environmental seasonality change [46,47].

Specimens from the warmer and more irradiated populations of *B. europaea* generated a significantly greater number of oocytes during the initial stages of gametogenesis (gametes recruitment period). Before fertilization (gametes maturity period), however, individual oocyte number was not related to temperature/irradiance along the gradient, while oocyte size was smaller with increasing temperature (Tables 2 and S1). A reduction of photosynthetic efficiency is

documented for several species when temperatures are above optimal [48,49], thereby limiting energetic resources for polyp gametogenesis [9,50]. The onset of gametogenesis (proliferation of germ cells and their differentiation into gametes) may require little energy investment and may, therefore, be less sensitive to selective pressures such as food availability and more reliant on environmental seasonal cycles [51]. In this scenario, warmer populations of *B. europaea* could invest in energetically inexpensive early stages of oogenesis to generate a potential energy resource that would guarantee sufficient metabolic efficiency. On the other hand, the ripening of gametes, especially of oocytes, is an energy consuming process and, therefore, extremely sensitive to selective pressures [51].

Regarding male gametogenesis, during both reproductive periods, the size of spermaries decreased with increasing temperature (Tables 3, S2), while their abundance was not significantly related to environmental parameters. The energetic investment for gametogenesis between males and females is often assumed to differ [52]. For many lower invertebrates, and especially sessile ones, mating effort and parental care are minimal and reproductive output provides a good approximation of the reproductive effort, so most of the energy involved in reproduction is stored in gonads [53]. This "cost of sex" is mainly represented by oogenesis, while the investment of spermary production minimally influences the energetic balance of the organism [52].

For all organisms, energy flow provides an important cost for physiological performance, including maintenance, growth and reproduction, all of which have implications on survival and fitness. Reproductive investment and growth are often used as indicators of health or stress at the organism level (e.g. [54]), and knowledge of how such allocation varies among species or morphological types is crucial for the interpretation of physiological response to environmental factors [53]. Essentially, organisms invest their energy in continuous trade-offs between somatic/skeletal growth and reproduction, which in many species includes the possibility of asexual reproduction [55]. In a changing environment, physiological trade-offs vary through time, reflecting variations in resource availability [56], and the 'energy allocation' explains this partitioning

between the various investment options (e.g. growth, sexual reproduction, defense) [57]. For example, the coral *Montipora digitata* under varying light regimes shows an increase of energy allocated to reproduction versus growth at intermediate light levels. In this species the skeletal growth is less susceptible to environmental variations and during periods of resource shortage, energy is preferentially allocated for skeletal growth [57]. *B. europaea* shows a reduction of skeletal density, due to increasing porosity, and especially of pores with larger size, with increasing temperature [28,29,58]. Also its growth and calcification are negatively related to temperature [27,30]. Warmer populations are less stable, showing a progressive reduction in young individuals and reduced population density [29,30]. It has been hypothesized that the decrease in calcification rate [27] and skeletal density [29] in *B. europaea* with increasing temperature could be due to a reduction of energy input available, maybe due to photosynthetic inhibition of the symbionts [29,30]. Populations of *B. europaea* in warmer sites could potentially resorb earlier oocytes adjusting their energetic budget by reallocating the resources destined to oocyte maturity into other vital functions depleted by the negative effect of temperature. Resorption of oocytes is not fully understood, but it is thought that by breaking down the large amount of lipid vesicles in oocytes, energy can be absorbed back into the coral [59]. In the soft coral *Lobophytum compactum*, fecundity is reduced after an induced bleaching event. In this zooxanthellate coral, early oocytes are resorbed to allow development of remaining ones. Energy allocated to reproduction is apparently shifted towards maintaining fewer eggs than normal to ensure that they reach a mature size [36]. The branching coral *Acropora formosa* shows lower survival rate and a resorption of early vitellogenic oocytes after fragmentation, suggesting that there is a trade-off of energy between reproduction and survival [60].

In conclusion, *B. europaea* shows the highest ecological performance in the coldest part of its distribution, characterized by a higher growth coefficient [30], a greater population density [29,61] and a higher efficiency in partitioning the energy budget (this work; [27-30]). On the contrary, populations in warmer regions appear to invest their energy in the initial stages of

gametogenesis in order to ensure a sufficient gamete number ready for fertilization in the maturity period. Nevertheless, this effort is not enough to guarantee the same reproductive performance at higher temperatures, as adult populations in warmer sites are less abundant, less stable, and contain fewer young individuals [29,30]. This suggests that increasing temperature may negatively influence post-fertilization life stages, such as larval dispersal, survival and settlement. Depressed organismal condition exhibited by the warmer population could be due to their location near the edge of the species distribution range, where species generally show a lower ecological performance with reduced adaptability to variations in climate [62]. Being endemic to the Mediterranean [63], *B. europaea* has limited potential to respond to seawater warming by migrating northward toward lower temperatures, since the latitudinal range considered covers almost the entire northern distribution of this species [27]. This scenario would indicate a possible reduction in the distribution area of this species, with irrecoverable losses in terms of genetic variability, particularly considering the fragmented genetic structure that characterizes the species [64]. The present study, therefore, confirms the concerns for the future of this endemic species [27-30]. In fact, in a progressively warming Mediterranean, the energetic efficiency of this species could be considerably reduced, affecting vital processes (e.g. growth). Thus, an effective allocation strategy will be crucial for ensuring adaptability to a changing environment.

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287 **References**

288 1. Pandolfi JM, Connolly SR, Marshall DJ, Cohen AL (2011) Projecting coral reef futures under
289 global warming and ocean acidification. Science 333: 418-422.

290 2. Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (2007)
291 Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the
292 Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge and
293 New York: Cambridge University Press. 996 p.

294 3. Parry M (2000) Assessment of potential effects and adaptions for climate change in Europe: The
295 europe acacia project (a concerted action towards a comprehensive climate impacts and
296 adaptions assessment for the European Union). Jackson Environment Institute, University of
297 East Anglia. 320 p.

298 4. Field CB, Barros V, Stocker TF, Dahe Q (2012) Managing the Risks of Extreme Events and
299 Disasters to Advance Climate Change Adaptation: Special Report of the Intergovernmental
300 Panel on Climate Change. Cambridge and New York: Cambridge University Press. 594 p.

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

304 6. Brierley A, Kingsford M (2009) Impacts of climate change on marine organisms and ecosystems.
305 Curr Biol 19: 602-614.

306 7. Hoegh-Guldberg OVE, Pearse JS (1995) Temperature, food availability, and the development of
307 marine invertebrate larvae. Am Zool 35: 415-425.

308 8. Gillooly JF, Charnov EL, West GB, Savage VM, Brown JH (2002) Effects of size and
309 temperature on developmental time. Nature 417: 70-73.

310 9. Baird AH, Marshall PA (2002) Mortality, growth and reproduction in scleractinian corals
311 following bleaching on the Great Barrier Reef. Mar Ecol Prog Ser 237: 133-141.

312 10. Albright R, Mason B (2013) Projected near-future levels of temperature and pCO_2 reduce coral
313 fertilization success. PLOS ONE 8: e56468.
314 11. Linares C, Coma R, Zabala M (2008) Effects of a mass mortality event on gorgonian
315 reproduction. Coral reefs 27: 27-34.
316 12. Negri AP, Marshall PA, Heyward AJ (2007) Differing effects of thermal stress on coral
317 fertilization and early embryogenesis in four Indo Pacific species. Coral Reefs 26: 759-763.
318 13. Coma R, Ribes M, Serrano E, Jiménez E, Salat J, Pascual J (2009) Global warming-enhanced
319 stratification and mass mortality events in the Mediterranean. Proc Natl Acad Sci 106: 6176-
320 6181.
321 14. Cupido R, Cocito , Manno V, Ferrando S, Peirano A, Iannelli M, Bramanti L, Santangelo G
322 (2012) Sexual structure of a highly reproductive, recovering gorgonian population: quantifying
323 reproductive output. Mar Ecol Prog Ser 469: 25-36.
324 15. Gori A, Linares C, Rossi S, Coma R, Gili JM (2007). Spatial variability in reproductive cycle of
325 the gorgonians *Paramuricea clavata* and *Eunicella singularis* (Anthozoa, Octocorallia) in the
326 Western Mediterranean Sea. Mar Biol 151: 1571-1584.
327 16. Torrents O, Garrabou J (2011) Fecundity of red coral *Corallium rubrum* (L.) populations
328 inhabiting in contrasting environmental conditions in the NW Mediterranean. Mar Biol 158:
329 1019-1028.
330 17. Kipson S, Linares C, Teixidó N, Bakran-Petricioli T, Garrabou J (2012) Effects of thermal
331 stress on the early development stages of a gorgonian coral. Mar Ecol Prog Ser 470: 69-78.
332 18. Bauman AG, Baird AH, Cavalcante GH (2011) Coral reproduction in the world's warmest
333 reefs: southern Persian Gulf (Dubai, United Arab Emirates). Coral Reefs 30: 405-413.
334 19. Goffredo S, Radetić J, Airi V, Zaccanti F (2005) Sexual reproduction of the solitary sunset cup
335 coral *Leptopsammia pruvoti* (Scleractinia, Dendrophylliidae) in the Mediterranean. 1.
336 Morphological aspects of gametogenesis and ontogenesis. Mar Biol 147: 485-495.

337 20. Lesser MP (2013) Using energetic budgets to assess the effects of environmental stress on
338 corals: are we measuring the right things?. Coral Reefs 32: 25-33.
339 21. Muscatine L (1990) The role of symbiotic algae in carbon and energy flux in reef corals. In:
340 Dubinsky Z, editor. Coral reefs. Ecosystems of the World. New York: Elsiever. vol. 25, pp. 75-
341 87.
342 22. Henry LA, Hart M (2005) Regeneration from injury and resource allocation in sponges and
343 corals - a review. International review of hydrobiology 90: 125-158.
344 23. Graham EM, Baird AH, Connolly SR (2008) Survival dynamics of scleractinian coral larvae
345 and implications for dispersal. Coral Reefs 27: 529-539.
346 24. Weil E, Cróquer A, Urreiztieta I (2009) Yellow band disease compromises the reproductive
347 output of the Caribbean reef-building coral *Montastraea faveolata* (Anthozoa, Scleractinia). Dis
348 Aquat Org 87: 45.
349 25. Tamelander J (2002) Coral recruitment following a mass mortality event. Ambio 31: 551-557.
350 26. Goffredo S, Arnone S, Zaccanti F (2002) Sexual reproduction in the Mediterranean solitary
351 coral *Balanophyllia europaea* (Scleractinia, Dendrophylliidae). Mar Ecol Prog Ser 229: 83-94.
352 27. Goffredo S, Caroselli E, Mattioli G, Pignotti E, Dubinsky Z, Zaccanti F (2009) Inferred level of
353 calcification decreases along an increasing temperature gradient in a Mediterranean endemic
354 coral. Limnol Oceanogr 54: 930-937.
355 28. Caroselli E, Prada F, Pasquini L, Nonnis Marzano F, Zaccanti F, Falini G, Levy O, Dubinsky Z,
356 Goffredo S (2011) Environmental implications of skeletal micro-density and porosity variation
357 in two scleractinian corals. Zoology 114: 255-264.
358 29. Goffredo S, Caroselli E, Pignotti E, Mattioli G, Zaccanti F (2007) Variation in biometry and
359 population density of solitary corals with environmental factors in the Mediterranean Sea. Mar
360 Biol 152: 351-361.

30. 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.

31. 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.

32. 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.

33. 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.

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.

35. Altman DG (1991) Practical statistics for medical research. New York: Chapman & Hall, CRC. 624 p.

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

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

38. McClintock JB, Watts SA (1990) The effects of photoperiod on gametogenesis in the tropical sea urchin *Eucidaris tribuloides* (Lamarck)(Echinodermata, Echinoidea). J Exp Mar Biol Ecol 139: 175-184.

386 39. Goffredo S, Airi V, Radetić J, Zaccanti F (2006) Sexual reproduction of the solitary sunset cup
387 coral *Leptopsammia pruvoti* (Scleractinia, Dendrophylliidae) in the Mediterranean. 2.
388 Quantitative aspects of the annual reproductive cycle. Mar Biol 148: 923-931.
389 40. Goffredo S, Gasparini G, Marconi G, Putignano MT, Pazzini C, Airi V, Zaccanti F (2011)
390 Sexual reproduction in the Mediterranean endemic orange coral *Astroides calycularis*
391 (Scleractinia, Dendrophylliidae). Bull Mar Sci 87: 589-604.
392 41. Ribes M, Coma R, Rossi S, Micheli M (2007). Cycle of gonadal development in Eunicella
393 singularis (Cnidaria: Octocorallia): trends in sexual reproduction in gorgonians. Inv Biol 126:
394 307-317.
395 42. Harrison PL (2011) Sexual reproduction of scleractinian corals. In Coral Reefs: an ecosystem in
396 transition. Springer Netherlands. pp. 59-85.
397 43. Kružić P, Žuljević A, Nikolić V (2008) Spawning of the colonial coral *Cladocora caespitosa*
398 (Anthozoa, Scleractinia) in the Southern Adriatic Sea. Coral Reefs 27: 337-341.
399 44. Kersting DK, Casado C, López-Legentil S, Linares C (2013) Unexpected patterns in the sexual
400 reproduction of the Mediterranean scleractinian coral *Cladocora caespitosa*. Mar Ecol Prog Ser
401 486: 165-171.
402 45. De Putron SJ, Ryland JS (2009) Effect of seawater temperature on reproductive seasonality and
403 fecundity of *Pseudoplexaura porosa* (Cnidaria, Octocorallia): latitudinal variation in Caribbean
404 gorgonian reproduction. Invertebr Biol 128: 213-222.
405 46. Fadlallah YH (1983) Sexual reproduction, development and larval biology in scleractinian
406 corals. Coral reefs 2: 129-150.
407 47. Richmond RH, Hunter CL (1990) Reproduction and recruitment of corals: Comparisons among
408 the Caribbean, the Tropical Pacific, and the Red Sea. Mar Ecol Prog Ser 60: 185-203.
409 48. Nakamura E, Yokohama Y, Tanaka J (2004) Photosynthetic activity of a temperate coral
410 *Acropora pruinosa* (Scleractinia, Anthozoa) with symbiotic algae in Japan. Phycol Res 52: 38-
411 44.

412 49. Al-Horani FA (2005) Effects of changing seawater temperature on photosynthesis and
413 calcification in the scleractinian coral *Galaxea fascicularis*, measured with O_2, Ca^{2+} and pH
414 microsensors. Sci Mar 69: 347-354.
415 50. Rinkevich B (1989) The contribution of photosynthetic products to coral reproduction. Mar Biol
416 101: 259-263.
417 51. Ramirez-Llodra E (2002) Fecundity and life-history strategies in marine invertebrates. Adv Mar
418 Biol 43: 87-170.
419 52. Hayward A, Gillooly JF (2011) The cost of sex: quantifying energetic investment in gamete
420 production by males and females. PLOS ONE 6: e16557.
421 53. Leuzinger S, Anthony KR, Willis BL (2003) Reproductive energy investment in corals: scaling
422 with module size. Oecologia 136: 524-531.
423 54. Maltby L (1999) Studying stress: the importance of organism-level responses. Ecol Appl 9:
424 431-440.
425 55. Nespolo RF, Halkett F, Figueroa CC, Plantegenest M, Simon JC (2009) Evolution of trade-offs
426 between sexual and asexual phases and the role of reproductive plasticity in the genetic
427 architecture of aphid life histories. Evolution 63: 2402-2412.
428 56. Fischer B, Dieckmann U, Taborsky B (2011) When to store energy in a stochastic environment.
429 Evolution 65: 1221-1232.
430 57. Leuzinger S, Willis BL, Anthony KR (2012) Energy allocation in a reef coral under varying
431 resource availability. Mar Biol 159: 177-186.
432 58. Fantazzini P, Mengoli S, Evangelisti S, Pasquini L, Mariani M, Brizi L, Goffredo S, Caroselli E,
433 Prada F, Falini G, Levy O, Dubinsky Z (2013) Time-Domain NMR study of Mediterranean
434 scleractinian corals reveals skeletal-porosity sensitivity to environmental changes. Environ Sci
435 Technol 47: 12679-12686.

436 59. Lueg JR, Moulding AL, Kosmynin VN, Gilliam DS (2012) Gametogenesis and spawning of
437 *Solenastrea bournoni* and *Stephanocoenia intersepta* in southeast Florida, USA. J Mar Biol
438 2012.
439 60. Okubo N, Motokawa T, Omori M (2007) When fragmented coral spawn? Effect of size and
440 timing on survivorship and fecundity of fragmentation in *Acropora formosa*. Mar Biol 151: 353-
441 363.
442 61. Goffredo S, Mattioli G, Zaccanti F (2004) Growth and population dynamics model of the
443 Mediterranean solitary coral *Balanophyllia europaea* (Scleractinia, Dendrophylliidae). Coral
444 Reefs 23: 433-443.
445 62. Sagarin RD, Gaines SD, Gaylord B (2006) Moving beyond assumptions to understand
446 abundance distributions across the ranges of species. Trends Ecol Evol 21: 524-530.
447 63. Zibrowius H (1980) Les Scléractiniaires de la Méditerranée et de l'Atlantique nord-oriental.
448 Mémoires de l'Institut océanographique, Monaco 11.
449 64. Goffredo S, Mezzomonaco L, Zaccanti F (2004) Genetic differentiation among populations of
450 the Mediterranean hermaphroditic brooding coral *Balanophyllia europaea* (Scleractinia,
451 Dendrophylliidae). Mar Biol 145: 1075-1083.
452

Tables

Table 1.

Mean annual solar radiation (W/m²) and temperature (DT; °C) values of the sampled populations. DT sensors (I-Button DS1921H, Maxim Integrated Products), were placed at the sampling location, at 5–7 m depth in each population. Solar radiation (W/m^2) was collected from MFG satellites. The sites are arranged in order of increasing DT; SE, standard error.

Population	Code	DT (°C) mean ± SE	Solar radiation (W/m²) mean ± SE
Calafuria	CL	17.73 ± 0.16	174.1 ± 1.9
Elba	LB	18.07 ± 0.24	184.9 ± 2.3
Genova	GN	18.13 ± 0.43	156.9 ± 3.2
Scilla	SC	18.73 ± 0.15	205.5 ± 1.8
Palinuro	PL	19.14 ± 0.14	194.6 ± 2.7
Pantelleria	PN	19.69 ± 0.05	218.2 ± 0.5

Table 2. Mean fecundity, gonadal index and diameter of oocytes in each population

Gametes recruitment period (June – September)

Population	N	Fecundity (#/100 mm³) mean ± SE	Gonadal Index (%) mean ± SE	N	Diameter (µm) mean ± SE
Calafuria	18	161 ± 39	0.22 ± 0.07	1135	166.3 ± 3.3
Elba	6	148 ± 37	0.65 ± 0.17	544	193.7 ± 3.8
Genova	8	168 ± 47	0.27 ± 0.12	505	166.0 ± 3.3
Scilla	9	256 ± 58	0.41 ± 0.13	729	166.7 ± 2.8
Palinuro	10	734 ± 194	1.57 ± 0.38	1766	178.4 ± 1.9
Pantelleria	8	663 ± 240	1.43 ± 0.51	1312	188.2 ± 2.6

Gametes maturity period (December – March)

Population	N	Fecundity (#/100 mm³) mean ± SE	Gonadal Index (%) mean ± SE	N	Diameter (µm) mean ± SE
Calafuria	19	117 ± 38	1.04 ± 0.30	1040	350.3 ± 7.5
Elba	8	175 ± 32	0.79 ± 0.16	435	243.4 ± 7.7
Genova	4	411 ± 183	1.37 ± 0.40	532	222.5 ± 6.2
Scilla	4	602 ± 257	2.72 ± 1.50	902	241.1 ± 4.5
Palinuro	7	112 ± 30	0.39 ± 0.15	261	217.7 ± 7.5
Pantelleria	6	236 ± 106	1.25 ± 0.41	445	265.4 ± 7.1

Mean fecundity, gonadal index and diameter of oocytes in each population for both reproductive periods. The sites are arranged in order of increasing DT; SE, standard error. N, polyp number for fecundity and gonadal index, oocyte number for diameter.

467 **Table 3. Mean abundance, gonadal index and diameter of spermaries in each population**

Gametes recruitment period (June – September)

Population	N	Abundance (#/100 mm³) mean ± SE	Gonadal Index (%) mean ± SE	N	Diameter (µm) mean ± SE
Calafuria	17	140 ± 52	0.010 ± 0.003	425	51.4 ± 1.2
Elba	2	169 ± 106	0.010 ± 0.001	44	54.2 ± 2.8
Genova	1	1463	0.080	211	46.3 ± 1.1
Scilla	6	272 ± 80	0.010 ± 0.004	192	40.7 ± 0.8
Palinuro	6	393 ± 133	0.020 ± 0.006	185	40.0 ± 1.0
Pantelleria	5	760 ± 368	0.030 ± 0.020	343	42.0 ± 0.7

Gametes maturity period (December – March)

Population	N	Abundance (#/100 mm³) mean ± SE	Gonadal Index (%) mean ± SE	N	Diameter (µm) mean ± SE
Calafuria	19	1840 ± 609	1.10 ± 0.40	7257	120.5 ± 0.8
Elba	8	595 ± 235	0.47 ± 0.23	830	126.0 ± 1.8
Genova	4	2135 ± 1122	1.95 ± 1.51	1852	124.8 ± 1.3
Scilla	4	981 ± 561	0.16 ± 0.09	499	81.7 ± 1.6
Palinuro	6	1875 ± 1664	0.85 ± 0.80	1755	103.2 ± 1.1
Pantelleria	5	2660 ± 2320	0.93 ± 0.25	1831	92.0 ± 1.0

468 Mean abundance, gonadal index and diameter of spermaries in each population for both
469 reproductive periods. The sites are arranged in order of increasing DT; SE, standard error. N, polyps
470 number for abundance and gonadal index, spermaries number for diameter.

471

Figures

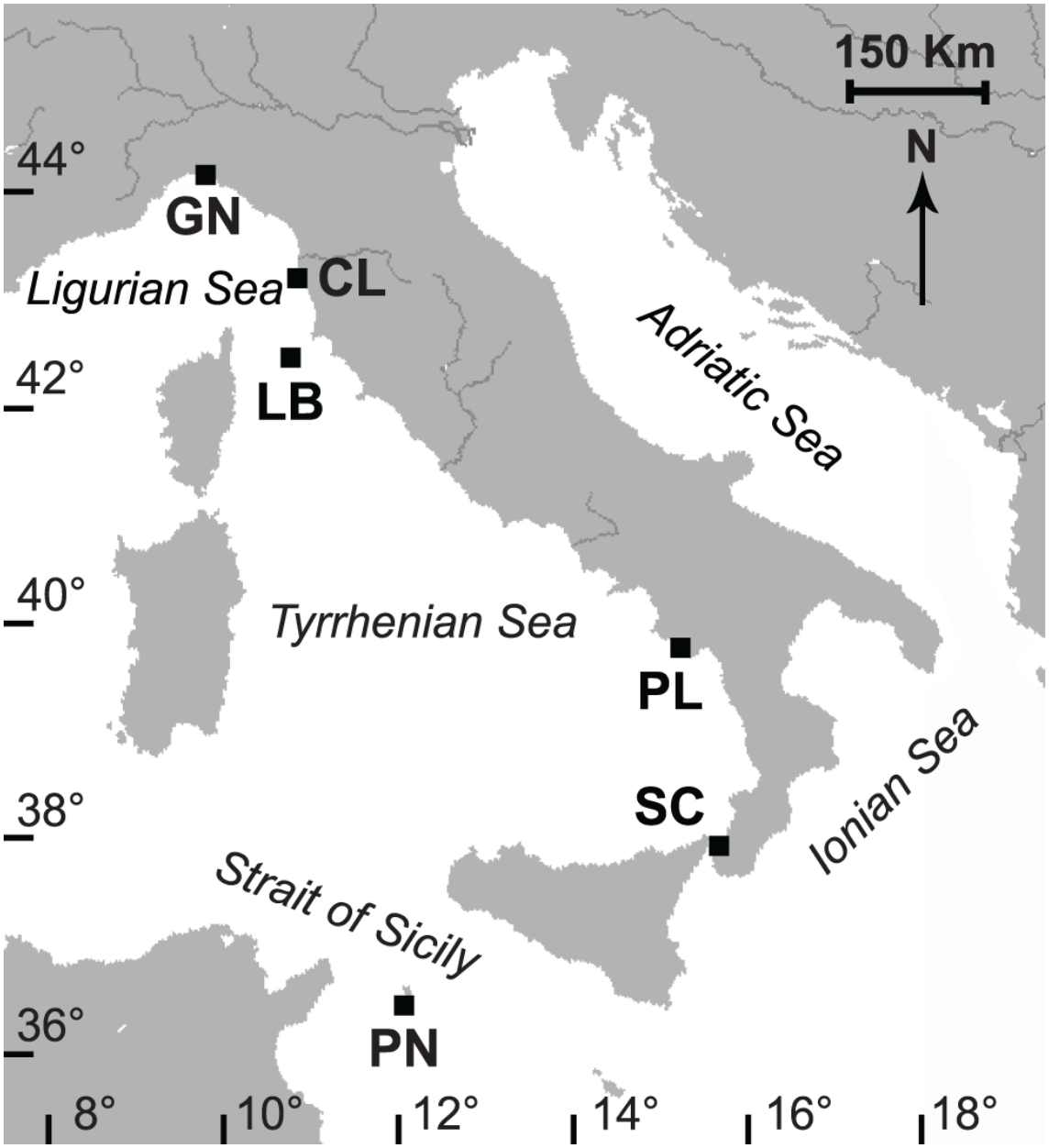


Figure 1. Map of the Italian coastline indicating the sites where corals were collected.

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; PL Palinuro, 40°02'N, 15°16'E; SC Scilla, 38°01'N, 15°38'E; PN Pantelleria Isle, 36°45'N, 11°57'E.

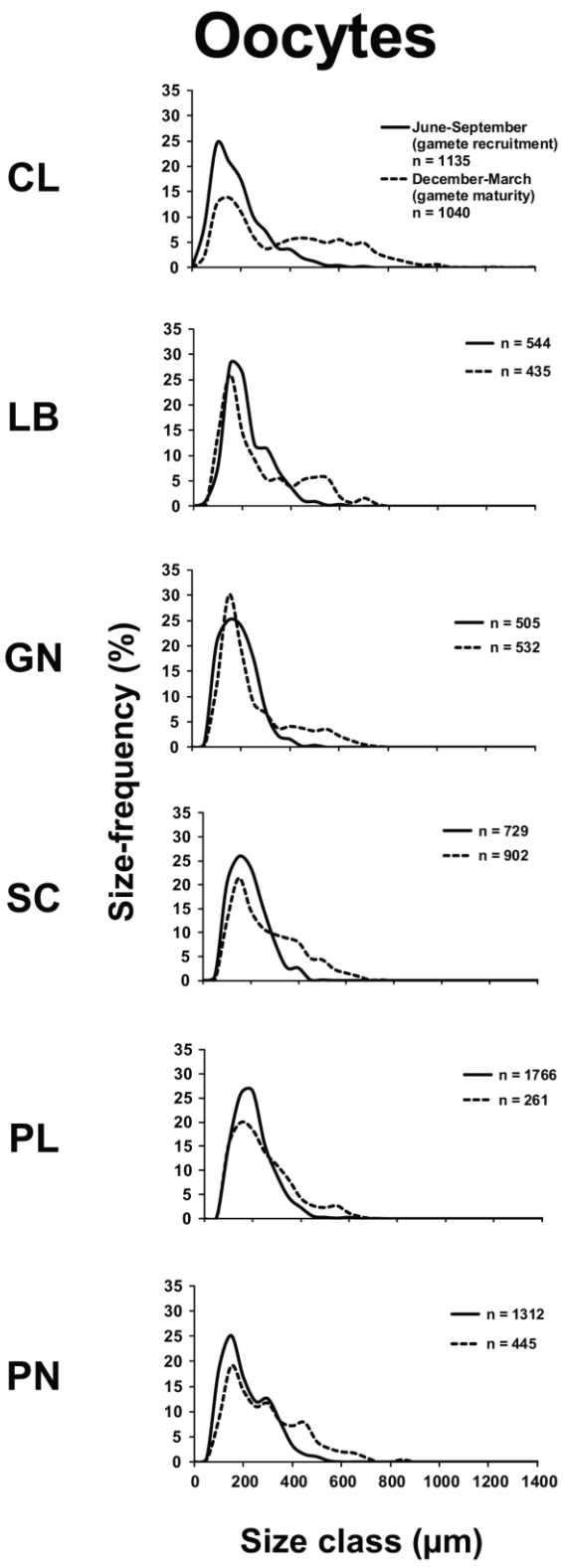


Figure 2. Oocyte size/frequency distribution in the recruitment and maturity periods.

Distribution of the oocytes size during gamete recruitment period (solid line) and gamete maturity period (dashed line).

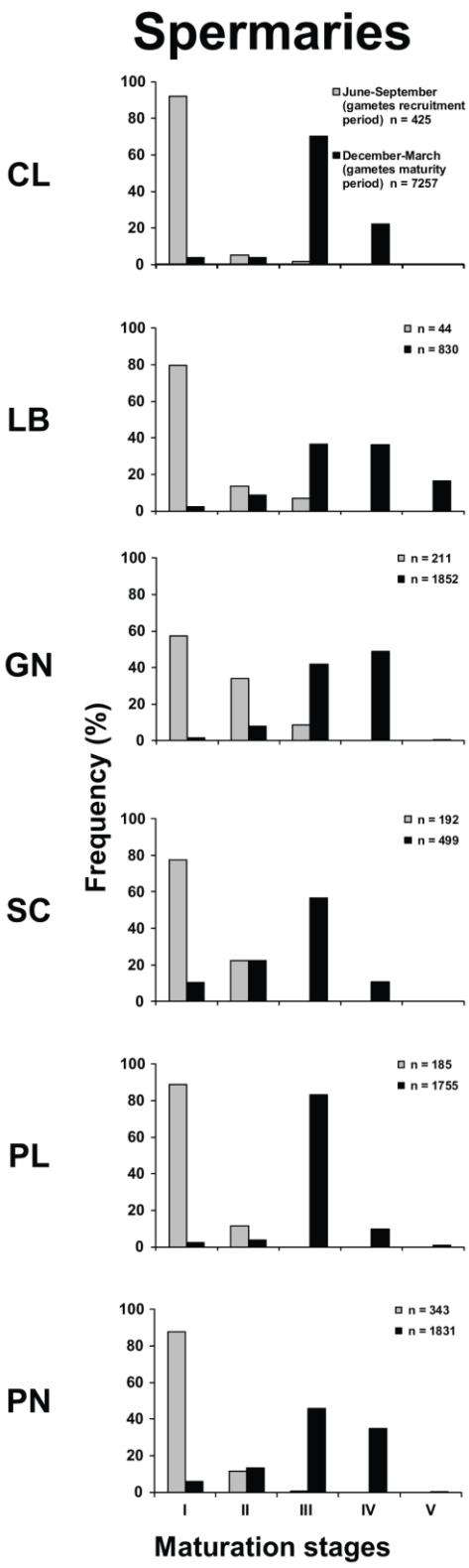


Figure 3. Spermary frequency distribution in the recruitment and maturity periods.

Distribution of the maturation stages during gamete recruitment period (gray histogram bars) and gamete maturity period (black histogram bars).

Supporting Information

Table S1. Oocytes. Kruskal-Wallis test and correlation analyses between reproductive and environmental parameters in the sampled populations, in both periods. K-W, significance of the Kruskal-Wallis test; r_s, Spearman's correlation coefficient; * $p < 0.050$; ** $p < 0.010$; *** $p < 0.001$; ns, not significant.

Gametes recruitment period (June – September)

		DT (°C)	Solar radiation (W/m²)
	K-W	**r_s**	**r_s**
Fecundity (#/100 mm^3)	**	0.500 ***	0.434 ***
Gonadal Index (%)	***	0.575 ***	0.518 ***
Diameter (µm)	***	0.086 ***	0.069 ***

Gametes maturity period (December – March)

		DT (°C)	Solar radiation (W/m²)
	K-W	**rs**	**rs**
Fecundity (#/100 mm3)	*	0.254	0.101
Gonadal Index (%)	ns	-	-
Diameter (µm)	***	- 0.109 ***	- 0.017

496 **Table S2.** Spermaries. Kruskal-Wallis test and correlation analyses between reproductive and
497 environmental parameters in the sampled populations, in both periods. K-W, significance of the
498 Kruskal-Wallis test; r_s, Spearman's correlation coefficient; * $p < 0.050$; ** $p < 0.010$; *** $p <$
499 0.001; ns, not significant.

500

Gametes recruitment period (June - September)

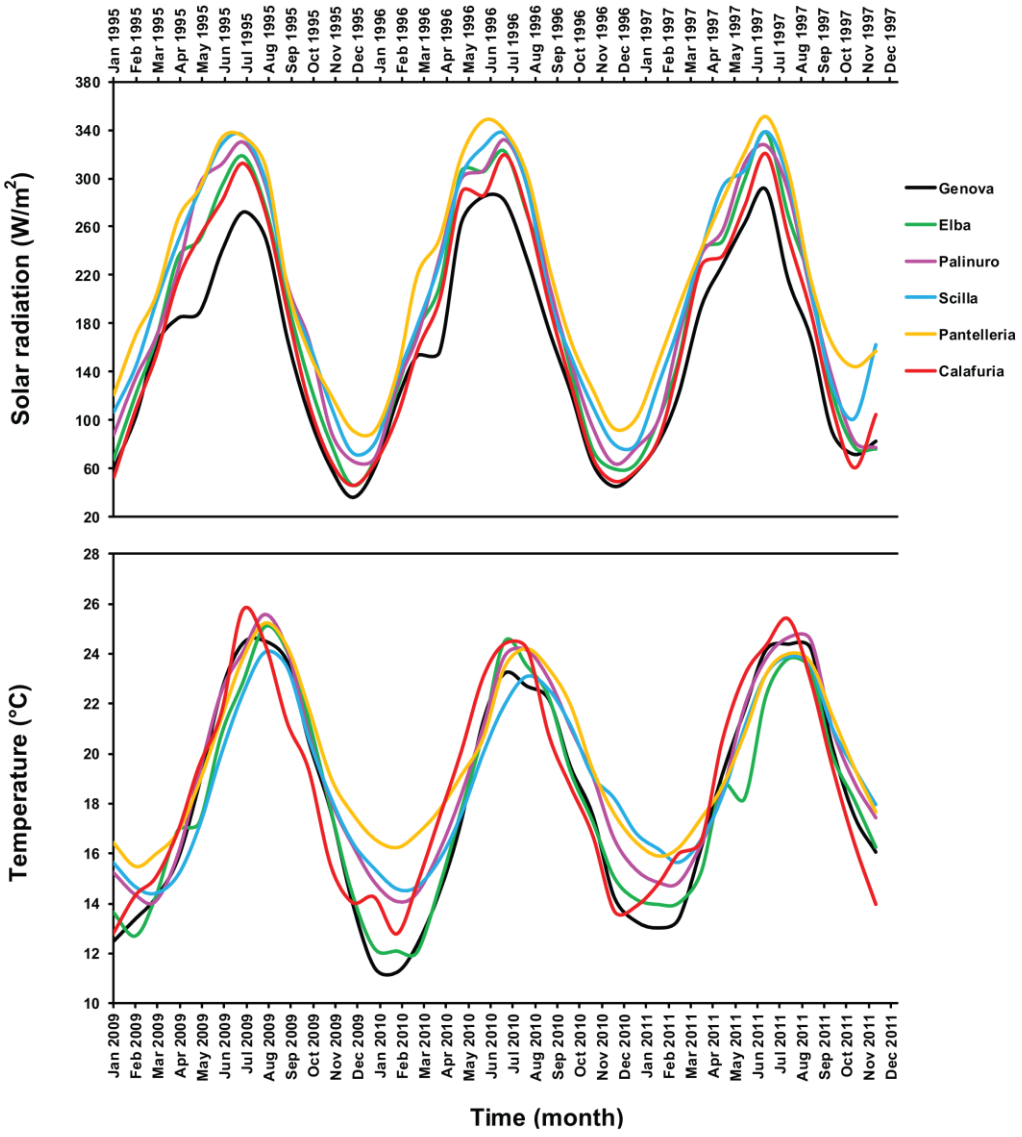
		DT (°C)	Solar radiation (W/m²)
	K-W	**r_s**	**r_s**
Abundance (#/100 mm³)	ns	-	-
Gonadal Index (%)	ns	-	-
Diameter (µm)	***	- 0.191 ***	- 0.154 ***

Gametes maturity period (December – March)

		DT (°C)	Solar radiation (W/m²)
	K-W	**r_s**	**r_s**
Abundance (#/100 mm³)	ns	-	-
Gonadal Index (%)	ns	-	-
Diameter (µm)	***	- 0.131 ***	- 0.176 ***

501

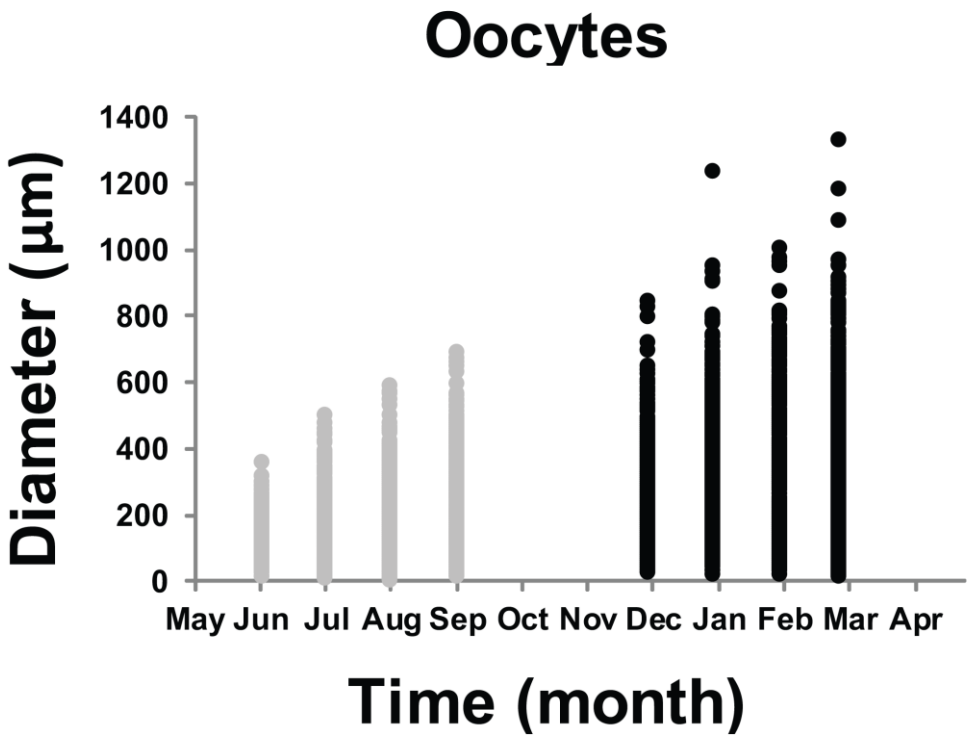
Figure S1. Living specimens of *Balanophyllia europaea* **photographed at Scilla (South Italy, 38°01'N, 15°38'E).**



506

507 **Figure S2. Annual fluctuation of solar radiation and temperature.** Mean monthly solar
508 radiation (W/m²) and temperature (DT; °C) during three years preceding the sampling. Annual
509 fluctuation referred to the triennium between January 1995 and December 1997 in Calafuria
510 population. For the other five populations it referred to the triennium between January 2009 and
511 December 2011.

512



513

514 **Figure S3. Oocyte diameter during recruitment and maturity periods.**

515 Monthly size increase of the oocyte diameter during gamete recruitment period (gray indicators)

516 and gamete maturity period (black indicators).

517

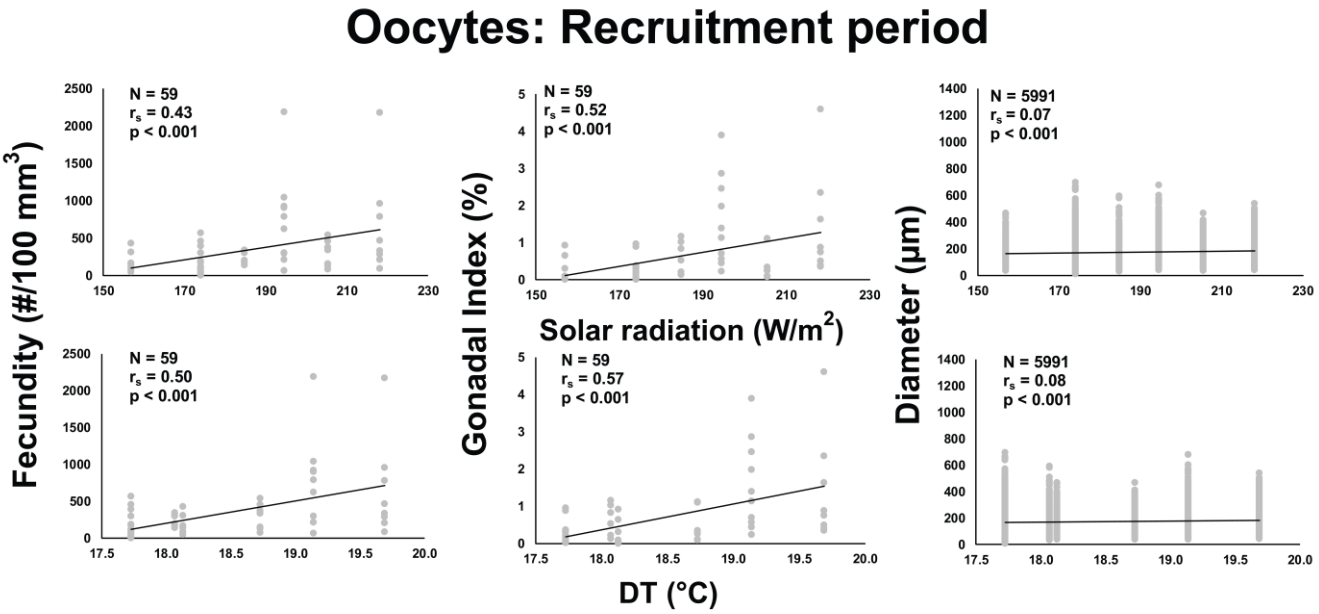


Figure S4. Oocytes. Correlation analyses. Spearman's correlation between reproductive and environmental parameters during gamete recruitment period; N, polyp number for fecundity and gonadal index, oocyte number for diameter; r_s, Spearman's correlation coefficient; p, significance of the correlation test.

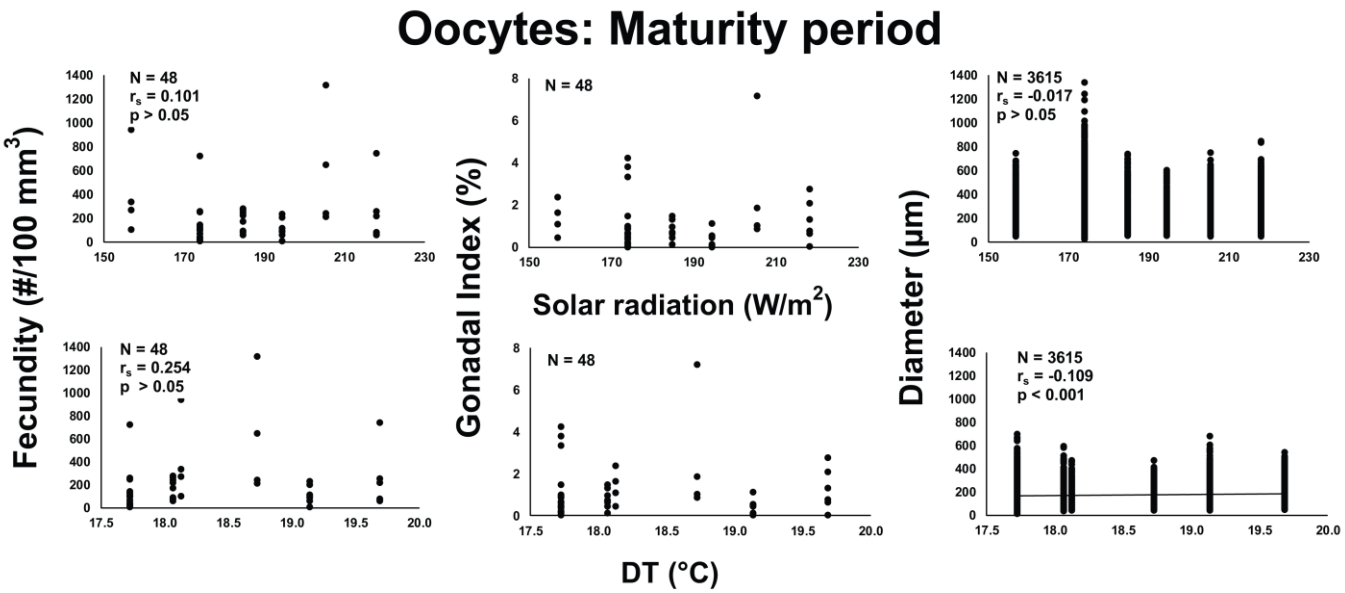


Figure S5. Oocytes. Correlation analyses. Spearman's correlation between reproductive and environmental parameters during gamete maturity period; N, polyp number for fecundity and gonadal index, oocyte number for diameter; r_s, Spearman's correlation coefficient; p, significance of the correlation test.

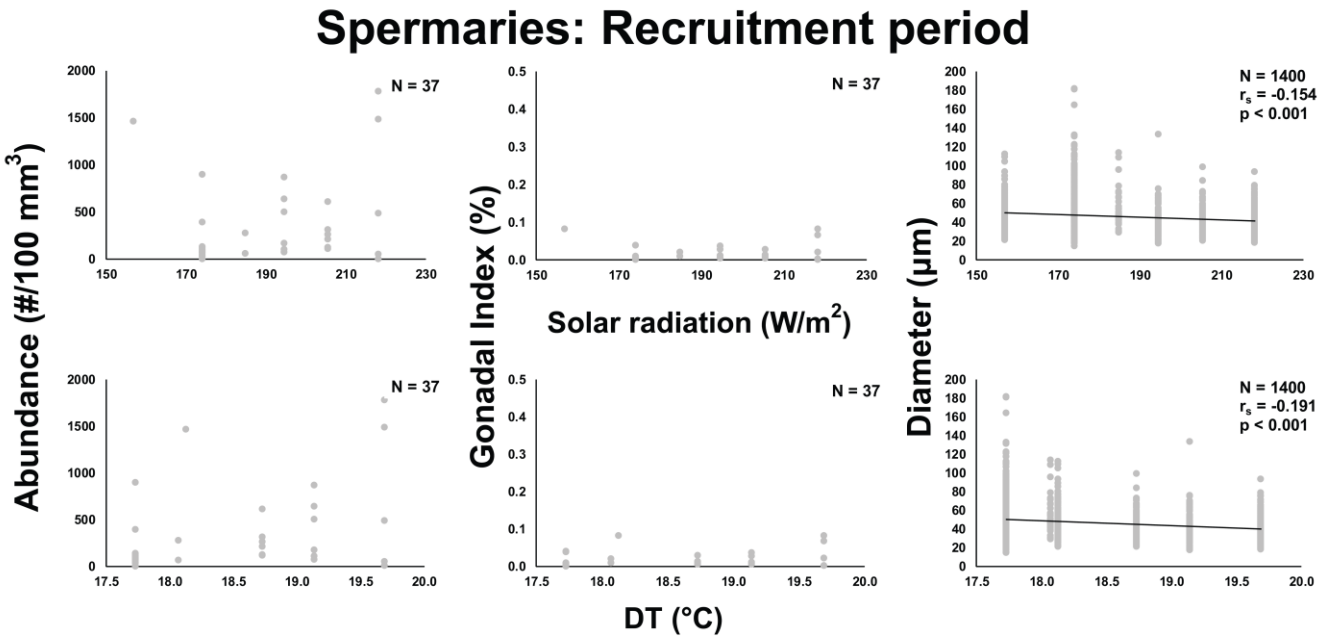


Figure S6. Spermaries. Correlation analyses. Spearman's correlation between reproductive and environmental parameters during gamete recruitment period; N, polyps number for abundance and gonadal index, spermaries number for diameter; r_s, Spearman's correlation coefficient; p, significance of the correlation test.

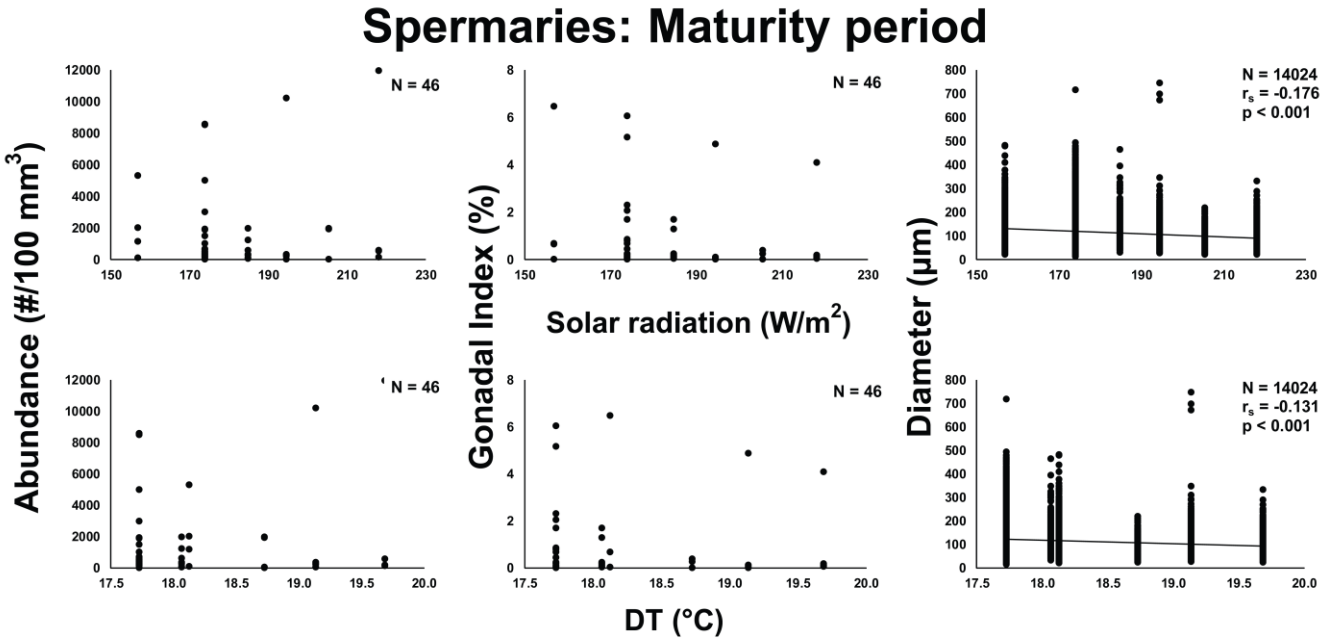


Figure S7. Spermaries. Correlation analyses. Spearman's correlation between reproductive and environmental parameters during gamete maturity period; N, polyps number for abundance and gonadal index, spermaries number for diameter; r_s, Spearman's correlation coefficient; p, significance of the correlation test.

Chapter V. Reproductive output of a non-zooxanthellate temperate coral is unaffected by temperature along an 850 km latitudinal gradient

Manuscript in preparation

Reproductive output of a non-zooxanthellate temperate coral is unaffected by temperature along an 850 km latitudinal gradient

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Abstract

In marine ecosystems, global environmental change is associated with concurrent shifts in temperature, circulation, stratification, and nutrient input, with potentially wide ranging biological effects. Variations in seawater temperature may alter the physiological functioning, reproductive efficiency and demographic traits of marine organisms, leading to shifts in size and abundance of populations. Differences in temperature tolerances between organisms can identify individual and ecological characteristics of "winners" and "losers" in a climate change context. Here we define the reproductive output in the non-zooxanthellate Mediterranean scleractinian *Leptopsammia pruvoti*, along an 8° latitudinal temperature gradient on the western Italian coasts. Coupling our results with previous studies on growth, demography and calcification of *L. pruvoti* in the same temperature gradient, and comparing with another sympatric zooxanthellate coral, *Balanophyllia europaea*, leads to the conclusion that this non-zooxanthellate species may be quite tolerant to temperature increase. To our knowledge, this is the first field investigation of the relationship between reproductive output and temperature of a non-zooxanthellate coral. This study provides new insights on the responses to temperature increase on biological traits of the poorly studied non-zooxanthellate scleractinians.

Key-words: scleractinian, *Leptopsammia pruvoti*, seawater temperature, reproduction, global warming.

Introduction

Humans influence climate primarily through fossil-fuel, industrial, agricultural, and other land use changes that alter atmospheric composition [1]. Anthropogenic derived global change is the main source of environmental perturbation on a global scale, with an accelerated rate of temperature rise that exceeds many pessimistic projections [2]. If global greenhouse gas (GHG) emissions are not restricted, further increases in global temperatures are expected, beyond levels tolerable for corals and calcifying algae, the main reef builders (e.g. [3]). Combined with rising sea levels and shifting weather patterns, warming will have significant impacts on global biodiversity and ecological functioning [2,4]. Given the substantial impact of climate change on ecological communities [5] accounting for how climate change affects population persistence, community structure, and the sustainable delivery of ecosystem services presents a major challenge for conservation biology and ecosystem management [6].

The Mediterranean is a peculiar sea that can serve as a giant mesocosm of the world's oceans, with various sources of disturbances interacting synergistically [7]. The Mediterranean is one of the fastest warming regions affected by climate change [1,7], since interacting synergistically with many other disturbances, extreme climatic events are becoming more frequent, faunas are shifting, and invasive species are spreading [1]. Given these facts, the Mediterranean Sea represents an excellent natural laboratory for exploring the responses of temperate marine biota impacted by climate change [1,7].

Temperature is a key environmental factor that affects organisms at all organization levels by controlling their physiological and ecological processes [8]. The effects of rising sea surface temperature on the organism physiology and behavior in marine ecosystems has been confirmed by several studies (see a review in [9]). Seawater temperature increase will likely affect the population biology of coral species by reducing reproductive efficiency [10]. One of the best tools for marine conservation is a better knowledge of the reproductive cycles and the potential gonadal output in marine species [11]. Sexual reproduction is the foundation of coral population persistence, without

successful reproduction, coral populations are unable to replenish lost individuals and are destined for regional extinction [12]. Because coral reproduction is governed by environmental rhythms that are vulnerable to stochastic events (including heat waves, droughts, and intense tropical and mid-latitude storms), reproduction is generally regarded as the most sensitive life process [13]. Knowledge of the reproductive biology of temperate scleractinian species is relatively scarce, especially for the Mediterranean Sea [14]. The numerous studies describing reproductive biology of anthozoans on tropical reefs (e.g. [15,16]) are mainly on symbiotic corals, that seem particularly sensitive to elevated temperatures [17,18]. On the other hand, the effects of temperature increase on non-zooxanthellate corals have been poorly investigated.

This study focused on the non-zooxanthellate Mediterranean dendrophylliid, *Leptopsammia pruvoti* (Fig. S1), a gonochoric and brooding coral [19,20]. Temperature has been reported not to significantly influence neither its population abundance, nor skeletal architecture features such as corallite length, width, height, and bulk density [21, 23], its population dynamics [22], or its calcification [27]. The density of the calcium carbonate crystals of its skeleton even seems to increase with increasing temperatures [23]. Our specific aim was to quantify the reproductive output of *L. pruvoti* along a latitudinal gradient of temperature, considering the harmful conditions reported for the endemic Mediterranean solitary dendrophylliid coral *Balanophyllia europaea*, studied in the same sites, during the same time interval, and using the same methods as the present study. *Balanophyllia europaea* experienced lower ecological performance in warmer populations, showing a lower growth coefficient [24], lower population density [21,25] and a loss of oocytes during gametogenesis, maybe reabsorbing them and using the energy for other vital functions [26]. In the light of these previous findings, we expect to find a controversial response of *L pruvoti*: doesn't temperature affect reproductive output, according to its growth and population dynamics, or does it negatively affect its gametogenesis, as shown by its sympatric zooxanthellate coral *B. europaea*?

Materials and Methods

Ethics Statement

This study was carried out following the fundamental ethical principles. According to the European normative, there is no active conservation measure for the Mediterranean scleractinian coral studied here (*L. pruvoti*). The species is not protected in Italy, nor it is subject to any regulations. Thus, no permit was needed to sample the specimens. For this study, sampling was limited strictly to the number necessary and performed where the species has high population density to minimize the impact of removing individuals and preserve both the demographic and genetic structure of the natural populations.

Sample collection

Specimens of *L. pruvoti* were collected from six sites along a latitudinal gradient, from 44°20'N to 36°45'N (Fig. 1). Latitude is the main factor influencing the variation in temperature [41], which is the environmental parameter considered in this study and that has already shown correlations with biologic parameters of *L. pruvoti* [23] and other Mediterranean dendrophilliids [21,23-26] in previous studies. Coral collection began in June 2010 and ended in November 2012. During this period, 18 samples were made monthly in five populations (Genova: April 2011-September 2012; Elba: December 2010-May 2012; Palinuro: June 2010-November 2011; Scilla: June 2011-November 2012; Pantelleria: June 2011-November 2012), in each of these almost 15 polyps were collected. Data from Calafuria population came from a previous study [20] and samples were collected from July 2001 to September 2002. Sampling was performed at depths known to have high population densities and where the reproductive biology, biometry, population density, growth (calcification rate, linear extension rate, and skeletal density), population dynamics, and genetics of the species had previously been studied [19-22,27-29].

Sample analysis

Biometry and histological analysis of *L. pruvoti* were done using methods described in detail in [19,20].

Briefly, 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 $V = (L/2) * (W/2) * h * \pi$.

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 [20].

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 in the coelenteric cavity was recorded. Spermaries were classified into five developmental stages in accordance with earlier studies on gametogenesis in scleractinians [19,30,31].

Reproductive parameters

Reproductive output was defined through three 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 the body volume occupied by the germ cells [20]); and c) *reproductive element size*, defined as the average of the maximum and minimum diameter of spermaries and oocytes in nucleated section [20].

The reproductive year was characterized by two gamete activity periods [20]. The *gametes recruitment period* [26] was defined as the post-fertilization period, between June and September, generally characterized by: 1) a stock of smaller oocytes; 2) the recruitment of new oocytes; 3) the beginning of spermary development [20]. The *gametes maturity period* [26] was defined as the pre-

fertilization period taking place between December and March and generally characterized by the presence of larger oocytes and advanced stage of maturation of spermaries [20].

Environmental parameters

Depth Temperature (DT; °C) was measured by digital thermometers (I-Button DS1921H, Maxim Integrated Products, Dallas Semiconductors), placed close to the experimental site, in each population. Sensors recorded seawater temperature every 3 hours during the entire experimental period. Thermometers were replaced every 3 months to avoid problems of encrustation and overgrowth by marine organisms.

Sea Surface Temperature data (SST; °C) for each site were obtained from the National Mareographic Network of the Institute for the Environmental Protection and Research (ISPRA, available to http://www.mareografico.it). These data are measured by mareographic stations, (SM3810 manufactured by the Society for the Environmental and Industrial monitoring; SIAP+MICROS), placed close to the sampling sites at a depth of 1 m below minimum low tide level. Mean annual SST values were computed from hourly measurements.

A linear regression was obtained between DT and SST data to estimate historical at-depth temperatures. In this study we considered the average DT temperature of the three years preceding the sampling (n = 36 monthly temperatures).

Solar radiation (W/m^2) was taken from 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 2.5°-latitude-by-longitude square associated with each of the six sites. As for temperature, also for solar radiation we considered the average of the three years preceding the sampling (n = 36 monthly solar radiation).

Statistical analyses

One-way analysis of variance (ANOVA) was used to compare environmental and reproductive parameters among sites. Levene's test was used for testing homogeneity, and Kolmogorov-Smirnov's test was used for testing normality of variance. When assumptions for parametric statistics were not fulfilled, the non-parametric Kruskal-Wallis equality-of-populations rank test was used. The non-parametric Kolmogorov-Smirnov test was used to compare the size-frequency distribution of reproductive elements between populations and between the two periods. Student's *t* test was used to compare the mean oocytes and spermaries size of populations between periods. Spearman's rank correlation coefficient was used to calculate the significance of the correlations between reproductive and environmental parameters. Spearman's rank correlation coefficient is an alternative to Pearson's correlation coefficient [32]. It is useful for data that are non-normally distributed and do not meet the assumptions of Pearson's correlation coefficient [33]. All analyses were computed using PASW Statistics 17.0.

Results

Mean annual DT (°C) and mean annual solar radiation (W/m²) were significantly different among sites (solar radiation, ANOVA, $p < 0.001$; DT, Kruskal-Wallis, $p < 0.05$; Table 1; Fig. S2).

All populations showed gonochoric polyps in both reproductive periods. Oocytes displayed different means size between periods. Size/frequency distribution during June-September (gametes recruitment period) was significantly different from that of December-March (gametes maturity period), in all populations (Kolmogorov-Smirnov, $p < 0.001$; Fig. 2). Within June and September (gametes recruitment period) most oocytes were smaller than 400 µm, in all populations. In the following season (December-March, gametes maturity period), two distinct oocyte stocks appeared in all populations, characterized respectively by small (immature < 400 µm) and large (mature > 400 µm) cells (percentage increase in Fig. 2). The mean oocyte size of June-September (gametes recruitment period) was significantly lower than that of December-March (gametes maturity period) in all populations (Student's *t*-test, $p < 0.001$; Table 2).

Spermaries maturation occurred progressively during two periods. Each population was characterized, from June to September (gametes recruitment period), by small spermaries, mainly belonging to the earliest maturation stages (stages I and II). During December-March (gametes maturity period), all populations were characterized by more advanced maturation stages (mainly stage III; Fig. 3). The distribution of spermary maturation stages in June-September (gametes recruitment period) was significantly different from that in December-March (gametes maturity period), in all populations (Kolmogorov-Smirnov, $p < 0.001$; Fig. 3). The mean spermary size of June-September (gametes recruitment period) was significantly lower than that of December-March (gametes maturity period) in all populations (Student's *t*-test, $p < 0.001$; Table 3). Elba population was excluded from this analysis since male polyps were not found during the reproductive periods considered in this study.

Gonadal index and oocyte diameter were significantly different along the latitudinal gradient, during both periods (gonadal index, Kruskal–Wallis test, $p < 0.05$; diameter, Kruskal–Wallis test, $p < 0.001$; Tables 2 and S1). Gonadal index showed a positive relation to DT only in June-September (gametes recruitment period; Table S1, Figure S3). Oocyte size was negatively related to DT in June-September (gametes recruitment period) and positively related to both environmental parameters (DT and solar radiation) in December-March (gametes maturity period; Table S1; Figure S3).

Spermary abundance was significantly different along the latitudinal gradient, during both periods (June-September, Kruskal–Wallis test, $p < 0.05$; December-March, ANOVA test, $p< 0.01$; Table S2). Gonadal index was significant different among population in June-September (gametes recruitment period, Kruskal–Wallis test, $p < 0.05$; Table S2). Spermary size changed along the latitudinal gradient in period (Kruskal–Wallis test, $p < 0.001$; Table S2), showing an increase of the diameter with increasing DT and solar radiation in June-September (gametes recruitment period;

Table S2, Figure S4) and a decrease in December-March (gametes maturity period; Table S2; Figure S4).

Discussion

Reproductive timing in corals, as gamete development, fertilization and planulation, may reflect environmental conditions [34,35]. In these organisms, seawater temperature has long been considered as a primary variable providing a reliable cue to reset the biological clock since temperature affects the metabolism, which in turn affects the gametogenesis [36]. In the Mediterranean Sea, marked seasonal patterns of seawater temperature, the primary variable influencing the timing on coral gametogenesis, are ultimately driven by the photoperiod and irradiance cycles characteristic of intermediate latitudes [11]. Considerable attention has been paid to the effects of changing photoperiod and sea temperature on gametogenic cycles of anthozoans in the last two decades (e.g. [18]). Annual reproductive cycle on *L. pruvoti* shows a seasonality for gonadal development, induced by the annual variation of sea temperature and photoperiod [20]. The same pattern appears in other Mediterranean dendrophylliids like *B. europaea* [37], *Astroides calycularis* [38] and in the Mediterranean endemic oculinid *Cladocora caespitosa* [14,39]. Gametogenesis and reproductive timing on *L. pruvoti* showed a similar seasonality along the latitudinal gradient monitored. This finding was expected, since in the geographical areal considered (intermediate latitudes) the reproductive time was regulated by the same environmental seasonality change, and a similar trend was observed in *B. europaea*, in the same sites, with a similar temperature (Kruskal-Wallis, $p > 0.05$) and time interval [26].

Polyps of *L. pruvoti* seems to show the same reproductive output in all the studied populations, while oocytes fecundity, spermaries abundance and gonadal index in male organisms were not influenced, with increasing temperature (Tables S1 and S2; Figures S3 and S4). Therefore, female gonadal index seems increase with temperature (Tables S1; Figures S3). The absence of a clear correlation with environmental parameters exhibited by the reproductive output in the present study

confirms previous findings on the population density, growth and population structure stability of this species, where these parameters resulted unrelated to solar radiation and SST [21-23,27]. *L. pruvoti* seems to be quite tolerant to temperature compared to the zooxanthellate *B. europaea*, studied along the same gradient, whose populations were less abundant, less stable (with loss of young individuals) [21,23,24,40] and were characterized by reduced efficiency in partitioning energy during gametogenesis with increasing temperature [26].

A schematic model (Fig. 4) resumes the main findings on reproductive output and population density of these two species belonging to the same family and sharing a wide range of their distribution area. Reproductive output can be defined as the average product of fecundity (e.g. number of gametes), representing the reproductive effort of an individual [41], which is strongly related to intrinsic population growth rate and demography [42]. The non-zooxanthellate *L. pruvoti* was characterized by the same fecundity along the temperature gradient in both periods (Fig. S3 and Fig 4). Fecundity in the zooxanthellate *B. europaea* increased with temperature along the gradient during the recruitment period but was constant during the maturity period (Fig. 4; [26]). Both species showed lower fecundity immediately before fertilization (maturity period), maybe due to an oocytes degeneration, common during earliest stages of gametogenesis [43]. The oocyte decrease was constant along the gradient in *L. pruvoti* whereas *B. europaea* showed a loss in warm populations greater than 37% (Fig. 4). A possible explanation for this greater loss experienced by the zooxanthellate species, could be the inhibition of some biological processes (such as growth [24], skeletal density [21], and calcification rate [40]) in the southern populations due to a least efficient symbiotic system [22], leading *B. europaea* to reabsorb a greater amount of oocytes in order to reallocate the energy resources into other vital functions [26]. Both species showed the same reproductive output before fertilization, in all populations (Fig. 4). Therefore, we expect to have the same population density along the gradient, which is the case for *L. pruvoti* [21] but not for *B. euroapaea*, characterized by lower population densities in the warmer populations, perhaps due to larval mortality [21]. *Leptopsammia pruvoti* seems to be less sensitive to increasing temperature

compared to *B. europaea*, which may be explained by the absence of symbionts in the former, and thus the lack of an inhibition of host physiological processes [21].

Environmental change may benefit some species, that may experience higher survival, growth, and reproduction, and may thus be "winners" in a changing world [9]. In many cases, however, a shift toward environmental conditions outside the normal range of variability is stressful, causing suboptimal physiological performance and thus creating the "losers" of environmental change [44,45]. For such individuals, more stressful conditions may lead to higher mortality, reduced growth, smaller size, and reduced reproduction [9]. For example, in zooxanthellate species thermal tolerance is primarily governed by the obligate relationship between the coral animal and its photosymbiotic partner [46] showing different efficiency at different environmental condition, such as light and turbidity [47]. In turbid habitats, heterotrophic metabolism is more advantaged [47] and in this conditions non-zooxanthellate organisms could show grater adaptability. There is some evidence that enhanced stratification of coastal waters due to global warming is occurring in the Mediterranean Sea [48], causing turbidity of the water column and consequently limited light dispersal, negatively influencing primary productivity of photosynthetic organisms [49]. This fact could lead non-zooxanthellate and zooxanthellate species to a different performance and adaptability to environmental change, suggesting that the heterotrophic *L. pruvoti* and the zooxanthellate *B. europaea* seem to have different ecological responses to the same temperature regime.

Concluding, *L. pruvoti* seems to be quite tolerant to the natural temperature range experienced in the field, since its organismal performance, such as population abundance, skeletal architecture and density, calcification rate [21,23,27], population dynamics [22], and reproductive output (present study) did not vary with temperature along the latitudinal gradient examined. However, the temperature threshold that will still be tolerable by this species is still unknown. Testing other environmental parameters not considered in this study, such as nutrients, zooplankton

availability and turbidity, is crucial to verify the hypothesis that different responses between non-zooxanthellate (*L. pruvoti*) and zooxanthellate (*B. europaea*) species might depend on their different trophic system. Further investigations on the effects of other environmental parameters, such as nutrients and turbity, are needed to better understand the environmental controls on the ecology of these species, that may shed light on its potential resistance in a progressive warming Mediterranean.

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References

1. Field CB, Barros V, Stocker TF, Dahe Q (2012) Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation: Special Report of the Intergovernmental Panel on Climate Change. Cambridge and New York: Cambridge University Press. 594 p.
2. Ateweberhan M, Feary DA, Keshavmurthy S, Chen A, Schleyer MH, Sheppard CR (2013) Climate change impacts on coral reefs: Synergies with local effects, possibilities for acclimation, and management implications. Marine pollution bulletin, 74: 526-539.
3. Veron JEN, Hoegh-Guldberg O, Lenton TM, Lough JM, Obura DO, Pearce-Kelly P, Sheppard CRC, Spalding M, Stafford-Smith MG, Rogers AD (2009) The coral reef crisis: The critical importance of 350ppm CO_2. Marine Pollution Bulletin, 58: 1428-1436.
4. Hansen J, Sato M, Ruedy R (2012) Perception of climate change. Proceedings of the National Academy of Sciences 109: E2415-E2423.
5. Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, Folke C, … Roughgarden J (2003) Climate change, human impacts, and the resilience of coral reefs. Science 301: 929-33.
6. Baskett ML, Nisbet RM Kappel CV, Mumby PJ, Gaines SD (2010) Conservation management approaches to protecting the capacity for corals to respond to climate change: a theoretical comparison. Global Change Biology, 16: 1229-1246.
7. 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 in Ecology & Evolution 25: 250-260.
8. Linares C, Cebrian E, Kipson S, Garrabou J (2013) Does thermal history influence the tolerance of temperate gorgonians to future warming? Marine Environmental Research, 89:45-52.
9. Doney, SC, Ruckelshaus M, Emmett Duffy J, Barry JP, Chan F, English C, … Talley L. (2012) Climate Change Impacts on Marine Ecosystems. Annual Review of Marine Science, 4: 11-37.
10. Baird AH, Marshall PA (2002) Mortality, growth and reproduction in scleractinian corals following bleaching on the Great Barrier Reef. Marine Ecology Progress Series 237: 133-141.

11. 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). Marine Biology, 160: 719-728.

12. Roth L., Koksal S, van Woesik R (2010) Effects of thermal stress on key processes driving coral-population dynamics. Marine Ecology Progress Series, 411: 73-87.

13. Graham JE, van Woesik R (2013) The effects of partial mortality on the fecundity of three common Caribbean corals. Marine Biology, 1-5.

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

15. McClanahan TR, Ateweberhan M, Muhando CA, Maina J, Mohammed MS (2007) Effects of climate and seawater temperature variation on coral bleaching and mortality. Ecological Monographs 77: 503-525.

16. Baird AH, Guest JR, Willis BL (2009) Systematic and biogeographical patterns in the reproductive biology of scleractinian corals. Annual Review of Ecology, Evolution, and Systematics 40: 551-571.

17. Kahng SE, Benayahu Y, Lasker HR (2011) Sexual reproduction in octocorals. Marine Ecology Progress Series 443: 265-283.

18. Harrison PL (2011) Sexual reproduction of scleractinian corals. In *Coral reefs: an ecosystem in transition* Springer Netherlands pp. 59-85.

19. 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. Marine Biology 147: 485-495.

20. 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. Marine Biology 148: 923-931.

21. 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. Marine Biology 152: 351-361.

22. 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.

23. 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.

24. 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.

25. Goffredo S, Mattioli G, Zaccanti F (2004) Growth and population dynamics model of the Mediterranean solitary coral *Balanophyllia europaea* (Scleractinia, Dendrophylliidae). Coral Reefs 23: 433-443.

26. 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, in press. DOI: 10.1371/journal.pone.0091792.

27. Caroselli E, Mattioli G, Levy O, Falini G, Dubinsky Z, Goffredo S 2012 Inferred calcification rate of a Mediterranean azooxanthellate coral is uncoupled with sea surface temperature along an 8° latitudinal gradient Frontiers in Zoology, 9: 32.

28. Goffredo S, Di Ceglie S, Zaccanti F 2009 Genetic differentiation of the temperate-subtropical stony coral *Leptopsammia pruvoti* in the Mediterranean Sea Israel Journal of Ecology and Evolution, 55: 99-115.

29. Goffredo S, Caroselli E, Mattioli G, Zaccanti F 2010 Growth and population dynamic model for the non-zooxanthellate temperate solitary coral *Leptopsammia pruvoti* (Scleractinia, Dendrophylliidae) Marine Biology 157: 2603-2612.

30. 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. Marine Biology Research 6: 421-436.

31. 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? Journal of Morphology 273: 943-956.

32. Altman DG (1991) Practical statistics for medical research. New York: Chapman & Hall, CRC. 624 p.

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

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

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

36. Nozawa Y (2012) Annual variation in the timing of coral spawning in a high-latitude environment: Influence of temperature. The Biological Bulletin 222: 192-202.

37. Goffredo S, Arnone S, Zaccanti F (2002) Sexual reproduction in the Mediterranean solitary coral *Balanophyllia europaea* (Scleractinia, Dendrophylliidae). Marine Ecology Progress Series 229: 83-94.

38. 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). Bulletin of Marine Science 87: 589-604.

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

40. 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. Limnology and Oceanography 54: 930-937.

41. Metz JAJ, Nisbet RM, Geritz SAH (1992) How should we define fitness for general ecological scenarios? Trends in Ecology & Evolution 7: 198-202

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

43. Kruger A, Schleyer MH (1998) Sexual reproduction in the coral Pocillopora verrucosa (Cnidaria: Scleractinia) in KwaZulu-Natal, South Africa. Marine Biology, 132: 703-710.

44. Somero GN (2010) The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine "winners" and "losers". The Journal of Experimental Biology, 213: 912-20.

45. Van Woesik R, Sakai K, Ganase A, Loya Y (2011) Revisiting the winners and the losers a decade after coral bleaching. Marine Ecology Progress Series 434: 67-76.

46. Howells EJ, Berkelmans R, van Oppen MJH, Willis BL, Bay LK 2013. Historical thermal regimes define limits to coral acclimatization. Ecology 94: 1078-1088.

47. Anthony KRN, Fabricius KE (2000) Shifting roles of heterotrophy and autotrophy in coral energetics under varying turbidity Journal of Experimental Marine Biology and Ecology 252: 221-253

48. Coma R, Ribes M, Serrano E, Jiménez E, Salat J, Pascual J. (2009) Global warming-enhanced stratification and mass mortality events in the Mediterranean. Proceedings of the National Academy of Sciences 106: 6176-6181.

49. Behrenfeld MJ, O'Malley RT, Siegel DA, McClain CR, Sarmiento JL, Feldman GC, ... Boss ES (2006) Climate-driven trends in contemporary ocean productivity. Nature, 444: 752-755.

Tables

Table 1. Mean annual depth temperature (DT) and solar radiation (W/m²) values of the sampled populations.

Population	Code	DT (°C) mean ± SE	Solar radiation (W/m²) mean ± SE
Calafuria	CL	17.33 ± 0.08	174.1 ± 1.9
Elba	LB	17.57 ± 0.23	184.9 ± 2.3
Genova	GN	17.69 ± 0.33	156.9 ± 3.2
Scilla	SC	18.14 ± 0.27	205.5 ± 1.8
Palinuro	PL	18.74 ± 0.12	194.6 ± 2.7
Pantelleria	PN	18.99 ± 0.06	218.2 ± 0.5

The sites are arranged in order of increasing DT; SE, standard error.

Table 2. Mean fecundity, gonadal index and diameter of oocytes in each population

Gametes recruitment period (June – September)

Population	N	Fecundity (#/100mm³) mean ± SE	Gonadal index (%) mean ± SE	N	Diameter (µm) mean ± SE
Calafuria	19	581 ± 166	0.94 ± 0.18	617	177.0 ± 3.7
Elba	3	228 ± 81	0.70 ± 0.11	222	189.7 ± 5.9
Genova	6	1263 ± 297	2.98 ± 0.76	1261	185.0 ± 2.5
Scilla	4	1501 ± 604	4.25 ± 1.73	885	203.1 ± 3.4
Palinuro	6	706 ± 173	1.30 ± 0.41	414	170.1 ± 4.7
Pantelleria	5	1119 ± 310	2.18 ± 0.82	980	173.8 ± 2.7

Gametes maturity period (December – March)

Population	N	Fecundity (#/100mm³) mean ± SE	Gonadal index (%) mean ± SE	N	Diameter (µm) mean ± SE
Calafuria	11	631 ± 177	2.20 ± 0.48	322	224.7 ± 7.2
Elba	5	642 ± 129	3.62 ± 1.02	556	251.7 ± 6.0
Genova	2	422 ± 143	1.32 ± 0.11	213	206.2 ± 6.8
Scilla	3	757 ± 306	5.94 ± 1.93	495	286.5 ± 6.2
Palinuro	6	606 ± 141	3.11 ± 0.85	414	246.2 ± 7.2
Pantelleria	4	157 ± 17	1.31 ± 0.43	95	303.5 ± 18.8

Mean fecundity, gonadal index and diameter of oocytes in each population for both reproductive periods. The sites are arranged in order of increasing DT; SE, standard error. N, polyp number for fecundity and gonadal index, oocyte number for diameter.

Table 3. Mean abundance, gonadal index and diameter of spermaries in each population

Gametes recruitment period (June – September)

Population	N	Abundance (#/100mm³) mean ± SE	Gonadal index (%) mean ± SE	N	Diameter (µm) mean ± SE
Calafuria	27	3954 ± 777	0.588 ± 0.129	3019	68.0 ± 0.5
Elba	-	-	-	-	-
Genova	2	11628 ± 6543	0.610 ± 0.370	914	44.9 ± 0.6
Scilla	4	14787 ± 5138	2.415 ± 0.783	6079	69.1 ± 0.3
Palinuro	6	2042 ± 1064	0.132 ± 0.072	527	53.9 ± 0.9
Pantelleria	1	363	0.002	15	19.2 ± 1.2

Gametes maturity period (December – March)

Population	N	Abundance (#/100mm³) mean ± SE	Gonadal index (%) mean ± SE	N	Diameter (µm) mean ± SE
Calafuria	13	12645 ± 2720	7.57 ± 1.45	4473	121.9 ± 0.9
Elba	-	-	-	-	-
Genova	2	3336 ± 2650	6.65 ± 1.55	3101	138.2 ± 1.1
Scilla	2	41461 ± 499	16.86 ± 3.80	9642	109.8 ± 0.5
Palinuro	1	6121	3.04	581	107.4 ± 1.7
Pantelleria	3	13091 ± 2252	3.96 ± 1.08	2421	88.9 ± 0.8

Mean abundance, gonadal index and diameter of spermaries in each population for both reproductive periods. The sites are arranged in order of increasing DT; SE, standard error. N, polyps number for abundance and gonadal index, spermaries number for diameter.

Figures

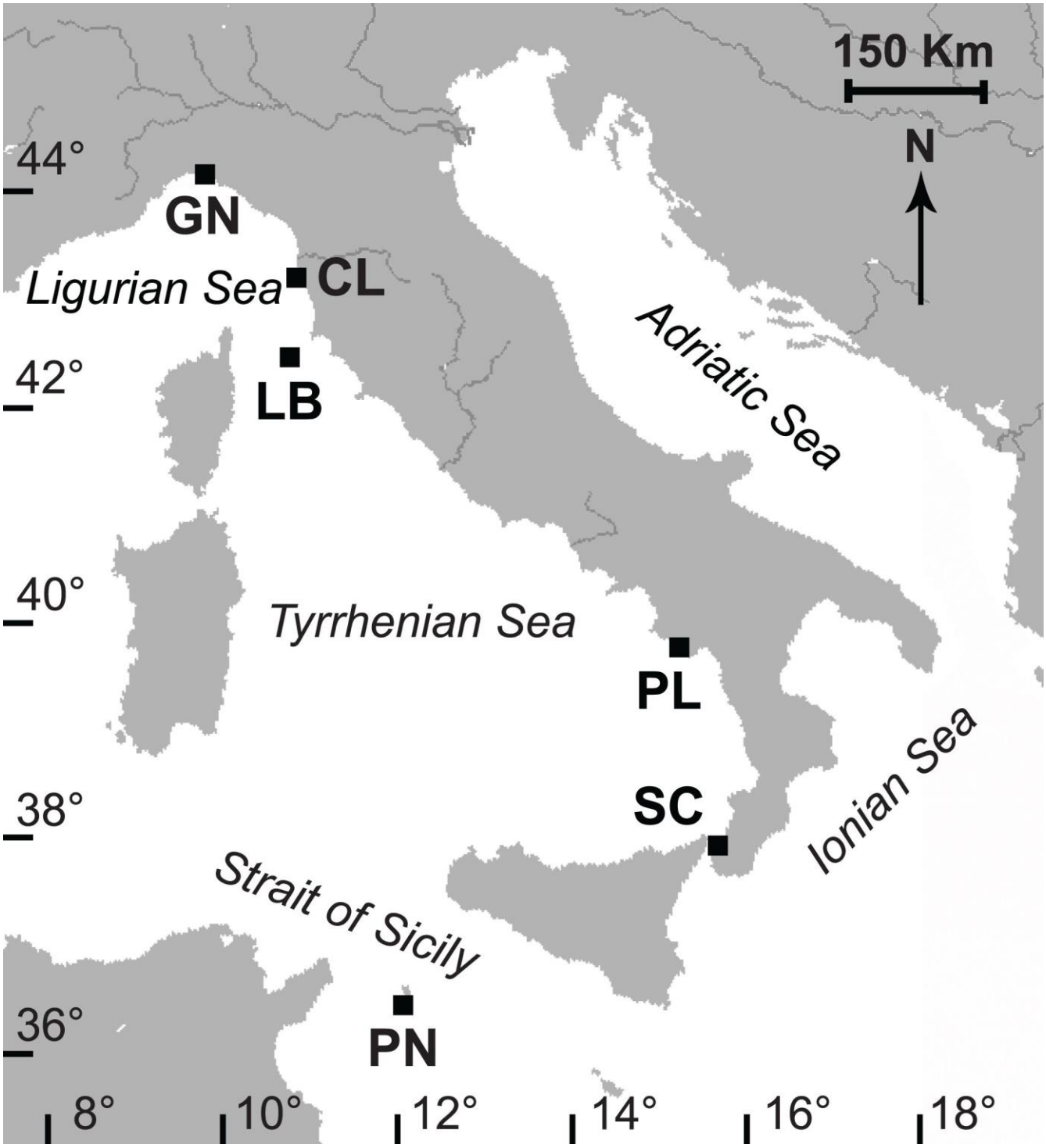

Figure 1. Map of the Italian coastline indicating the sites where corals were collected.

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; PL Palinuro, 40°02'N, 15°16'E; SC Scilla, 38°01'N, 15°38'E; PN Pantelleria Isle, 36°45'N, 11°57'E.

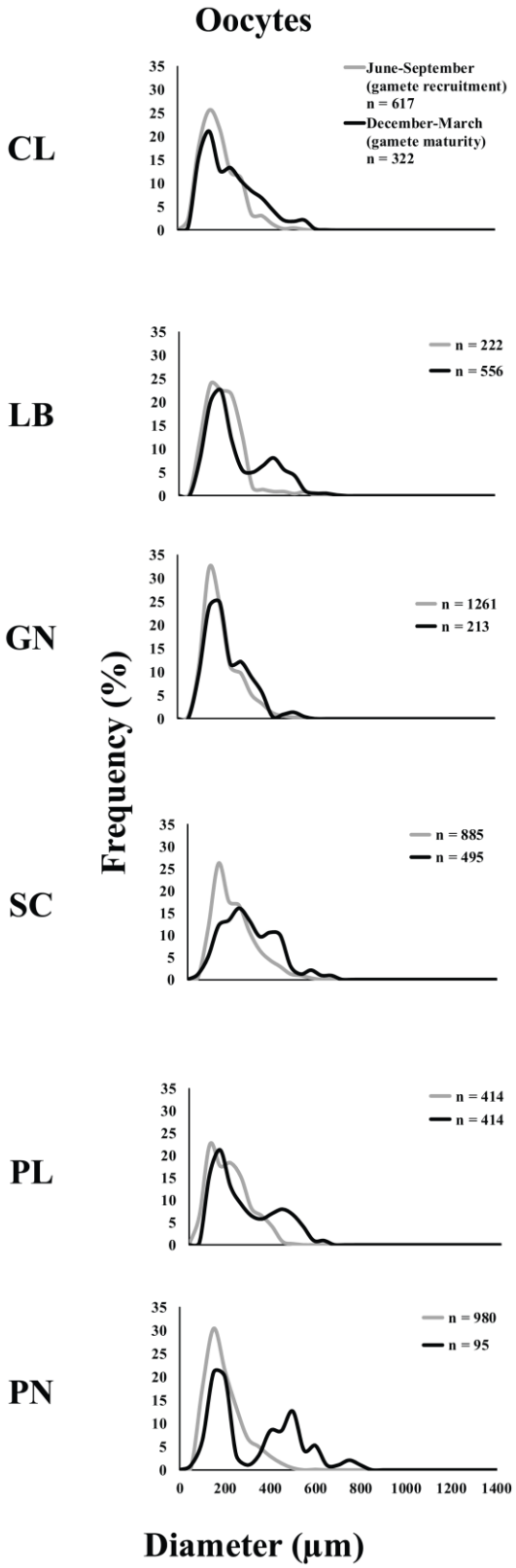

Figure 2. Oocyte size/frequency distribution in the recruitment and maturity periods.

Distribution of the oocytes size during gamete recruitment period (grey line) and gamete maturity period (black line).

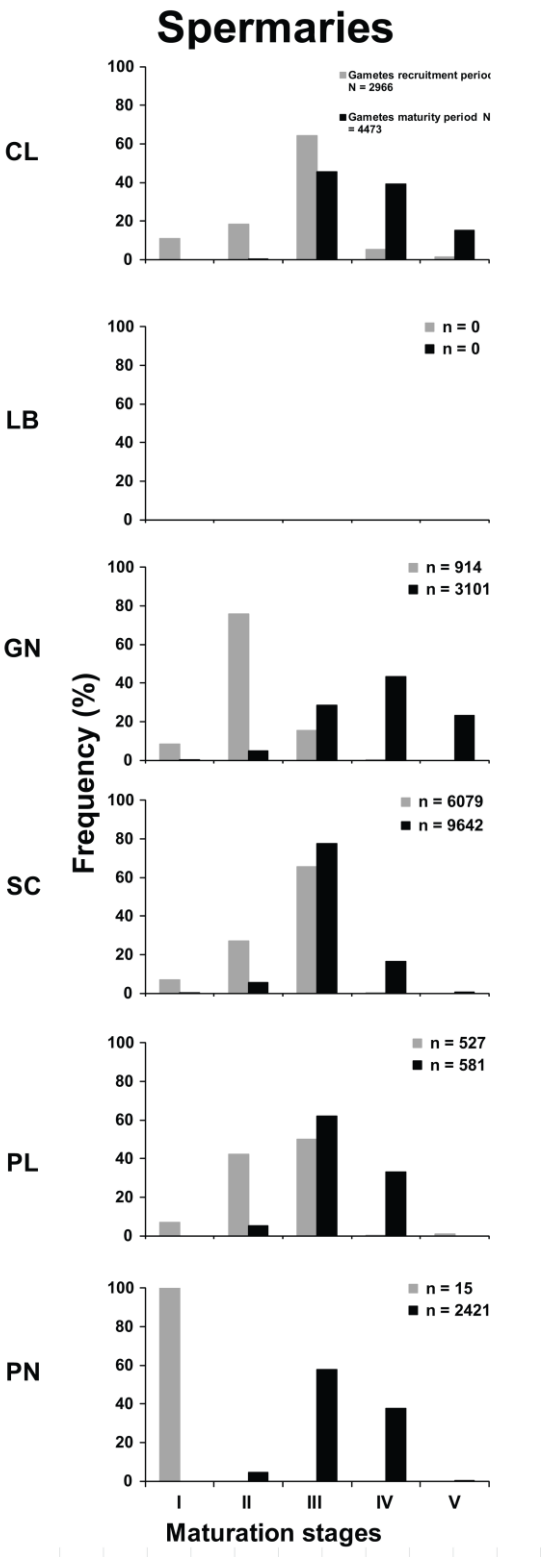

Figure 3. Spermary frequency distribution in the recruitment and maturity periods.

Distribution of the maturation stages during gamete recruitment period (gray histogram bars) and gamete maturity period (black histogram bars). Elba population was excluded from this analysis since male polyps have not been found, during the reproductive periods considered in this study.

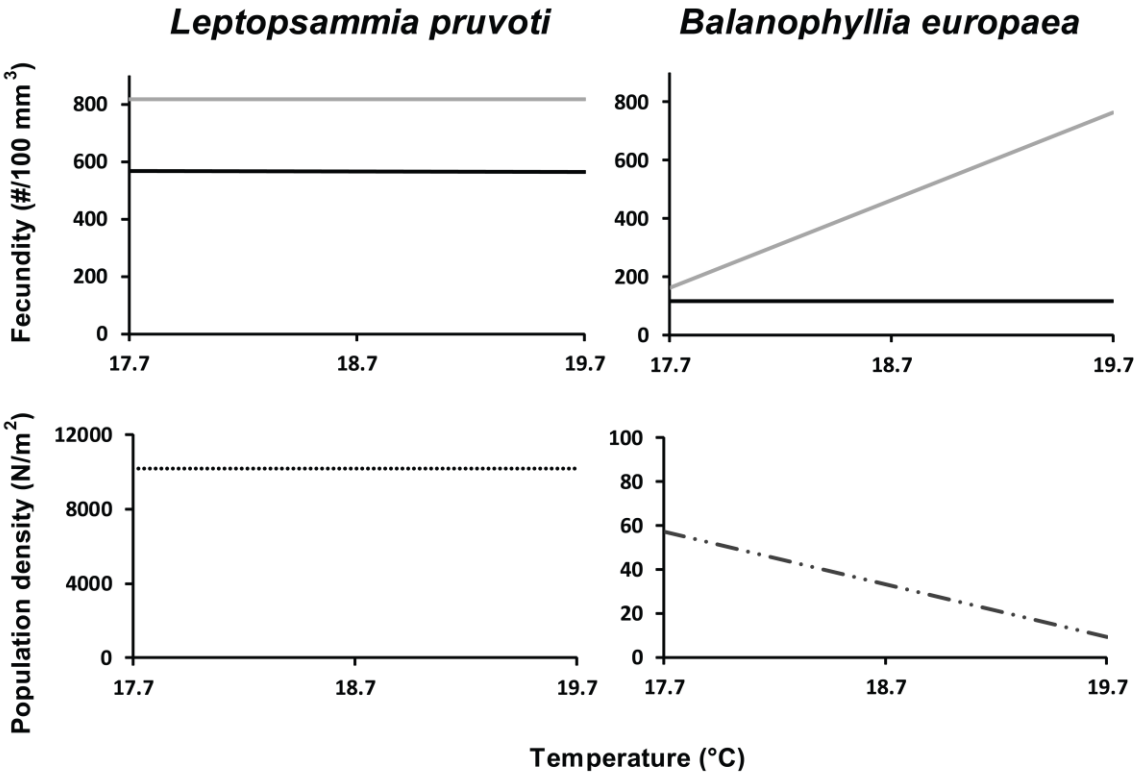

Figure 4. Fecundity and population density in *L. pruvoti* **(non-zooxanthellate) and** *B. europaea* **(zooxanthellate).** Fecundity during recruitment period (gray line) and maturity period (black histogram bars) in *L. pruvoti* (present study) and in *B. europaea* [26] and population density (dashed lines) in the same species [21] along the temperature gradient.

Supporting Information

Table S1. Oocytes. ANOVA/Kruskal-Wallis test and correlation analyses between reproductive and environmental parameters in the sampled populations, in both periods.

Gametes recruitment period (June – September)			
		DT (°C)	**Solar radiation (W/m²)**
	ANOVA		
	K-W	**r_s**	**r_s**
Fecundity (#/100 mm³)	ns	-	-
Gonadal index (%)	*	0.025 *	ns
Diameter (µm)	***	-0.030 *	-0.017
Gametes maturity period (December – March)			
		DT (°C)	**Solar radiation (W/m²)**
	ANOVA		
	K-W	**r_s**	**r_s**
Fecundity (#/100 mm³)	ns	-	-
Gonadal index (%)	*	ns	ns
Diameter (µm)	***	0.080***	0.173***

r_s, Spearman's correlation coefficient; * $p < 0.050$; ** $p < 0.010$; *** $p < 0.001$; ns, not significant.

Table S2. Spermaries. Kruskal-Wallis test and correlation analyses between reproductive and environmental parameters in the sampled populations, in both periods.

Gametes recruitment period (June - September)

		DT (°C)	Solar radiation (W/m²)
	ANOVA K-W	r_s	r_s
Abundance (#/100 mm³)	*	ns	ns
Gonadal index (%)	*	ns	ns
Diameter (µm)	***	0.031 **	0.188 ***

Gametes maturity period (December – March)

		DT (°C)	Solar radiation (W/m²)
	ANOVA K-W	r_s	r_s
Abundance (#/100 mm³)	**	ns	ns
Gonadal index (%)	ns	-	-
Diameter (µm)	***	- 0.196 ***	- 0.251 ***

r_s, Spearman's correlation coefficient; * $p < 0.050$; ** $p < 0.010$; *** $p < 0.001$; ns, not significant.

Figure S1. Living specimens of *Leptopsammia pruvoti* **photographed at Scilla (South Italy, 38°01'N, 15°38'E).**

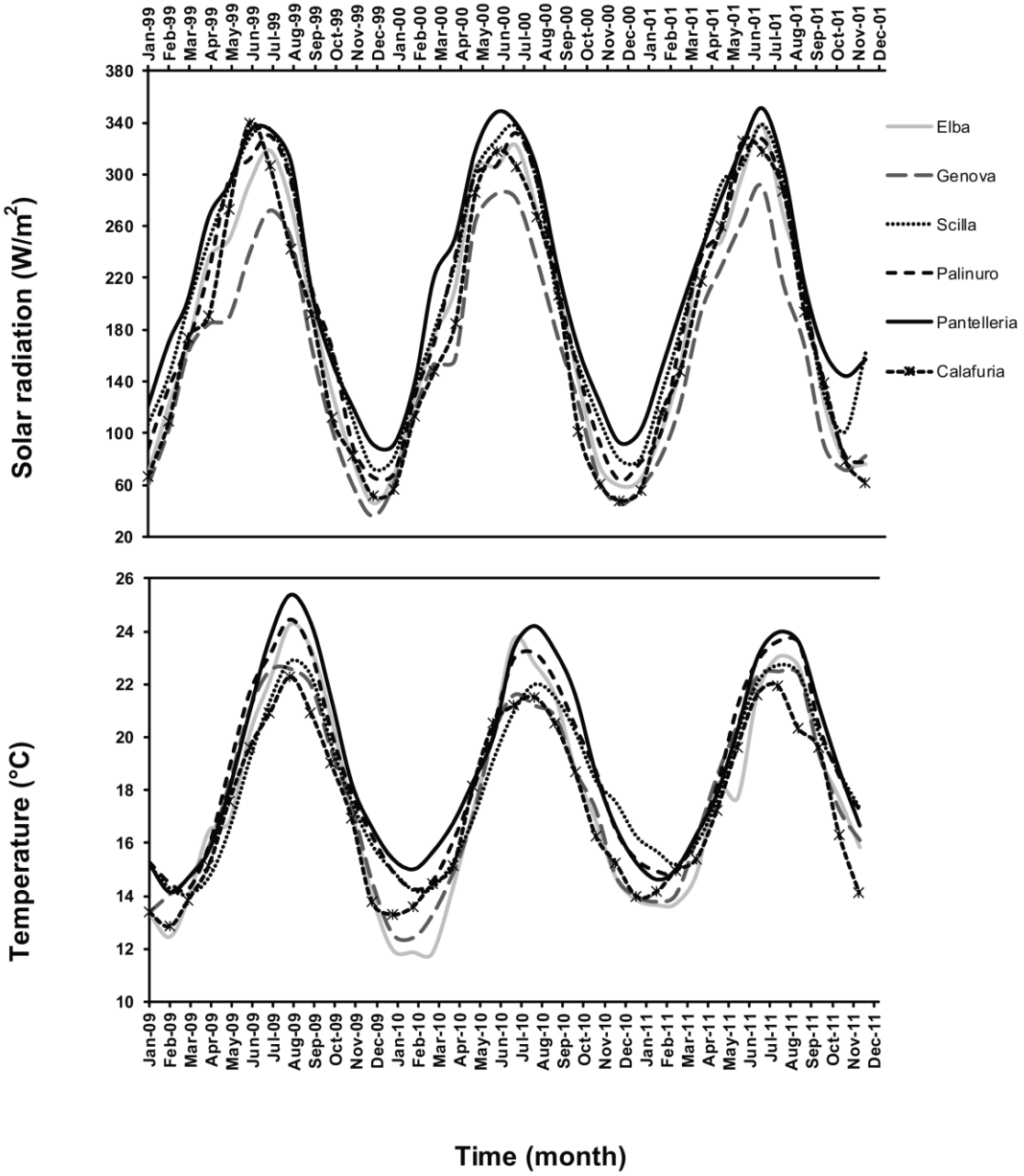

Figure S2. Annual fluctuation of solar radiation and temperature. Mean monthly solar radiation (W/m²) and temperature (DT; °C) during three years preceding the sampling. Annual fluctuation referred to the triennium between January 1999 and December 2001 in Calafuria population. For the other five populations it refers to the triennium between January 2009 and December 2011.

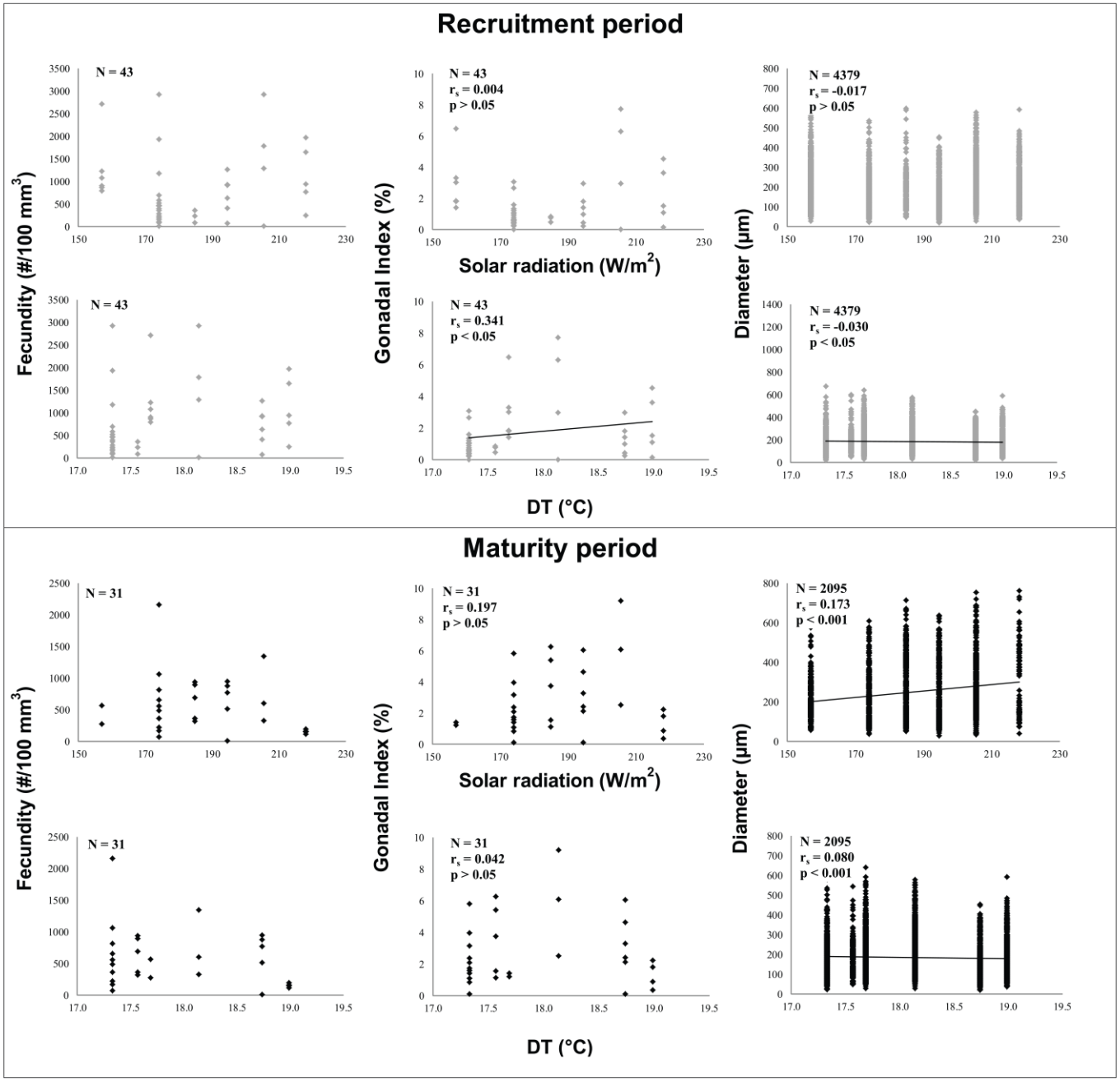

Figure S3. Oocytes. Correlation analyses. Spearman's correlation between reproductive and environmental parameters during recruitment (grey indicators) and maturity (black indicators) periods; N, polyp number for fecundity and gonadal index, oocyte number for diameter; r_s, Spearman's correlation coefficient; p, significance of the correlation test.

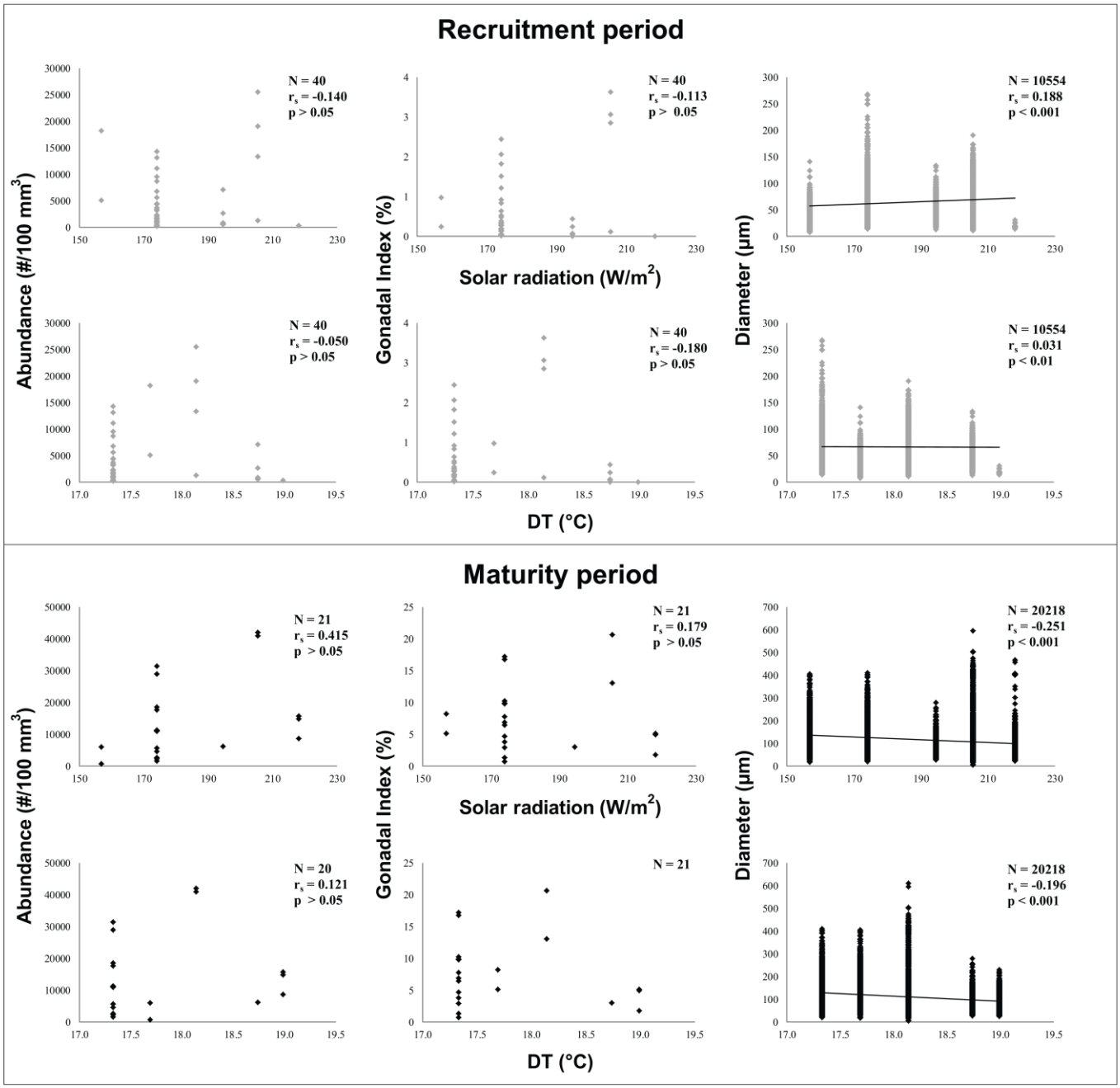

Figure S4. Spermaries. Correlation analyses. Spearman's correlation between reproductive and environmental parameters during recruitment (grey indicators) and maturity (black indicators) periods under study; N, polyps number for abundance and gonadal index, spermaries number for diameter; r_s, Spearman's correlation coefficient; p, significance of the correlation test. Elba population was excluded from this analysis because male polyps were not found in either reproductive periods.

Chapter VI. Conclusions

The present research contributed to increase knowledge on reproductive modes and sexuality of temperate scleractinian corals, highlighting the extraordinary plasticity that characterizes these organisms, showing different forms of propagation and different responses to environmental change.

For the first time, sexuality and reproductive mode in *Caryophyllia inornata* were determined. An unusual embryogenesis without a clear seasonal pattern was observed, suggesting the possibility of an asexual origin. Sexual reproduction of *Astroides calycularis* was governed by annual changes in seawater temperature, as observed for other Mediterranean dendrophylliids. Defining the reproductive biology of these species is the starting point for studying their potential response to variations of environmental parameters, on a global climate change context.

The results on the influence of temperature on reproductive output of *Leptopsammia pruvoti* and *Balanophyllia europaea* suggest that the non-zooxanthellate species may be quite tolerant to temperature increase, since the zooxanthellate species resulted less efficient at warm temperatures. A possible explanation could be related to their different trophic system. In *B. europaea* thermal tolerance is primarily governed by the obligate relationship between the coral and its photosymbiotic partner (zooxanthellae), making it more sensitive to temperature changes. On the contrary, the absence of symbionts in *L. pruvoti* might make this coral more resistant to temperature.

This hypothesis was tested in the experiment performed during the abroad period at the Bar-Ilan University (Israel) under the supervision of Prof. Zvy Dubinsky and of Dr. Oren Levy. The experimental design aimed to investigate the effect of a long-term exposure in controlled conditions of elevated temperatures (T and T+3°C, where T was the *in situ* temperature, at the depth where corals were collected) and pCO_2 (400 ppm and 700 ppm) on the photosynthesis, calcification, metabolic reactions and reproductive output in *B. europaea, L. pruvoti* and *A. calycularis*. The main goal of this research was to understand how different species, showing different metabolisms and reproductive strategies respond to synergistic or antagonistic effects of pH and temperature. By integrating these results, currently under analysis, with previous findings on reproductive output along the temperature latitudinal gradient, we could have a clearer picture on the potential effects of environmental change on these corals.

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