A unique reproductive strategy in the mushroom coral *Fungia fungites*

Lee Eyal-Shaham¹,² • Gal Eyal¹,²,³,⁴ • Or Ben-Zvi¹,²,⁵ • Kazuhiko Sakai⁵ • Saki Harii⁵ • Frederic Sinniger⁵ • Mamiko Hirose⁶ • Patrick Cabaitan⁷ • Omri Bronstein¹ • Bar Feldman⁴ • Tom Shlesinger¹,⁸ • Oren Levy⁴ • Yossi Loya¹,⁶

Received: 4 March 2020 / Accepted: 11 September 2020 / Published online: 30 September 2020 © Springer-Verlag GmbH Germany, part of Springer Nature 2020

**Abstract** The vast majority of scleractinian corals are either simultaneous hermaphrodites or gonochoric. Exceptions to these are rare. Nevertheless, species belonging to the family Fungiidae are known to exhibit a wide variety of reproductive strategies. We examined the reproductive ecology of the mushroom coral *Fungia fungites* in Okinawa. Our study was conducted as part of a long-term, wide-ranging project (2009–2010 and 2013–2017) which explored the unique reproductive strategies of several species belonging to the family Fungiidae. Here we report the co-occurrence of males, females, and hermaphrodite individuals in a long-term monitored population of the reproductively atypical brooder coral *F. fungites* within the family Fungiidae. *F. fungites* status as a single-polyped solitary coral, was used to perform manipulative experiments to determine the degree of dependence of an individual coral on its conspecific neighbors for reproduction, and examined whether a constant sperm supply is obligatory for the continuous production of planulae. Isolated females of *F. fungites* exhibited a distinctive reproductive strategy, expressed in continuously releasing planulae also in the absence of males. Observations conducted on a daily basis for 2.5 months (throughout the reproductive season of 2015) revealed that some of these individuals released planulae continuously, often between tens and hundreds every day. In an effort to explain this phenomenon, three hypotheses are discussed: (1) Self-fertilization; (2) Asexual production of planulae (i.e., parthenogenetic larvae); and (3) Extended storage of sperm. Finally, we emphasize the importance of continuous and long-term monitoring of studies of coral reproduction; through further genetic studies of coral populations representing a broad range of species and their larval origin.

**Keywords** Scleractinian coral reproduction • Reproductive plasticity • Mixed sexuality • Fungiidae • Sperm storage • Sperm limitation • Self-fertilization

---

Lee Eyal-Shaham and Gal Eyal equal contribution between two first authors.

Topic Editor Morgan S. Pratchett

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s00338-020-02004-7) contains supplementary material, which is available to authorized users.

Yossi Loya
yosiloya@gmail.com

1 School of Zoology, Tel Aviv University, 6997801 Tel Aviv, Israel
2 The Interuniversity Institute for Marine Sciences, 8810569 Eilat, Israel
3 ARC Centre of Excellence for Coral Reef Studies, School of Biological Sciences, The University of Queensland, St. Lucia, QLD 4072, Australia
4 The Mina and Everard Goodman, Faculty of Life Sciences, Bar-Ilan University, 5290002 Ramat Gan, Israel
5 Tropical Biosphere Research Center, University of the Ryukyus, Sesoko, Motobu, Okinawa 905-0227, Japan
6 Department of Environmental and Social Affairs, School of Marine Science and Technology, Tokai University, Odiro 3-20-1, Shimizu, Shizuoka 424-8610, Japan
7 The Marine Science Institute, University of the Philippines, Diliman, 1101 Quezon City, Philippines
8 Institute for Global Ecology, Florida Institute of Technology, Melbourne, FL 32901, USA
**Introduction**

Coral reefs (order: Scleractinia) possess the ability to reproduce both sexually and asexually. There are two basic sexual systems in scleractinians: hermaphroditism (individuals capable of producing both male and female gametes during their lifetime); and gonochorism, known also as dioecy (individuals producing either male or female gametes throughout their lifetime) (Harrison and Wallace 1990; Baird et al. 2009). However, exceptions to these systematic trends are evident in certain coral species that exhibit a more complex sexual pattern (Baird et al. 2009; Harrison 2011), including the occurrence of hermaphroditism in a species otherwise considered to be gonochoric. Such atypical sexual systems have been recorded in various species in which different populations are either gonochoric or hermaphroditic (Glynn et al. 1994), with some populations containing a mixture of females, males, and hermaphrodites (Soong 1991; Santiago-Valentín et al. 2015), or in which a mixture of the three sexual morphs exist within the same colony (Tomascik and Sander 1987; Harrison 2011; Guest et al. 2012; Castrillo-Cifuentes et al. 2015; Glynn et al. 2017). Species belonging to the family Fungiidae are among those exhibiting atypical sexuality (Harrison 2011). Although the majority of species within this family are gonochoric; expressing only one sex throughout their lifetime (Fadlallah 1983; Krupp 1983; Babcock et al. 1986; Harrison and Wallace 1990; Kramarsky-Winter and Loya 1998; Loya et al. 2009; Harrison 2011), some fungiids have been documented to exhibit a unique reproductive strategy, such as the ability to change sex (i.e., sequential hermaphroditism), possibly in response to energetic and/or environmental constraints (Kramarsky-Winter and Loya 1998; Loya and Sakai 2008; Loya et al. 2009); and protandrous sequential hermaphroditism, which has been documented in five solitary fungiid species (Ctenactis echinata, C. crassa, Fungia repanda - now Lithophyllum repanda, F. scruposa - now Danafungia scuposa) (Loya and Sakai 2008) and Herpolitha limax (Loya and Sakai 2008), two of which (C. echinata and C. crassa) changed sex bi-directionally (Loya and Sakai 2008; Loya et al. 2009), and one (H. limax) changed sex multiple times in both directions (i.e., repetitive sex change) (Eyal-Shaham et al. 2019). The reproductive plasticity displayed by this group raises questions regarding the evolution of reproduction in solitary species.

Unlike most of the fungiids studied to date, *Fungia fungites* is unique in its reproductive strategy being a brooding species (Loya et al. 2009). In many brooding coral species, sperm-egg fusion takes place at the mother colony, following sperm release into the surrounding seawater by a conspecific male (Yund 2000; Pemberton et al. 2004). However, the extreme dilution of sperm in seawater often reduces sperm concentration to levels that limit fertilization. For many sperm-dependent marine invertebrates, including corals, reproductive failure due to sperm limitation is a serious problem (Yund 2000; Combosch and Vollmer 2013), which should consequently exert a strong selective pressure on reproductive strategies in order to maximize the success of fertilization and thereby enhance fitness. Numerous adaptive mechanisms by which to enhance reproductive success have evolved in marine organisms, including the ability of females to store sperm. The sperm remains in the females for a finite period of time before being used to fertilize their eggs (Neubaum and Wolfner 1998). Sperm storage results from selection operating on both male (the prolonged survival ability of sperm) and female (the ability to maintain sperm) traits, and is advantageous to females belonging to taxa with uncertain mating opportunities such as sessile organisms (e.g., stony corals; barnacles; sponges) that are lacking motility (Neubaum and Wolfner 1998). Alternatively, reproduction independent of cross-fertilization success or sperm supply can be assured via the mechanism of ‘self-fertilization’ (*Favia fragum* and *Porites astreoides*: (Brazeau et al. 1998)); or by asexual means such as the ‘production of asexual planulae’ (i.e., parthenogenetic larvae), as reported in the coral *Pocillopora damicornis* (Ayre et al. 1997; Combosch and Vollmer 2013), this mechanism is more common when outcrossing is disadvantageous, difficult, or impossible (Pemberton et al. 2004). For predominantly sessile organisms, extrinsic forces impose limits on their mating pool size, which may later be modified through largely intrinsic events that occur within the females themselves (Pemberton et al. 2004). Thus, in order to elucidate the reproductive mechanism involved, particular attention should be directed to these intrinsic ‘cryptic’ events.

A dearth of information on important life-history traits of *F. fungites* prompted a detailed investigation into the reproductive biology of this species including time of spawning and seasonality. Additionally, we have conducted a series of manipulative experiments to determine the degree of dependence of an individual coral on its neighbor conspecífics for reproduction, and whether a frequent sperm supply is obligatory for the production of planulae in this species.

**Methods**

**Study site and sampling**

The study was conducted between June 2009-August 2010 and July 2013–August 2017 and took place at several patch reefs near Sesoko Station (26°39’ N, 127°52’ E), Okinawa (Japan). In this area, the *Fungia fungites* population is...
concentrated at a few sites that are inhabited by tens of thousands of individuals (including approximately more than 12 fungid species) (Loya and Sakai 2008), densely distributed between 1 and 15 m depth. Specimens of F. fungites, ranging from small to very large (2–15 cm in diameter), were randomly collected from the reef and transferred to running filtered seawater containers at the laboratory. Individuals (> 5 cm) were tagged with a numeric plastic tag attached by a nylon fishing cord inserted through a thin hole drilled at the edge of the coral skeleton using a portable drill (Loya and Sakai 2008). No adverse effects on the corals were observed following the tagging procedure (i.e., monitoring of the possible damage to the tagged corals continued throughout the entire reproductive season). To avoid damage to the small-size corals in the studied population (< 5 cm), they were kept individually in marked containers. All corals were measured (diameter and weight; buoyant and dry weight) and photographed (for computerized surface area analysis) immediately after collection. A ruler was placed next to each photographed specimen to enable scale (Fig. 1a). Following each annual set of measurements and observations, which varied between 2–3 months, the tagged corals were returned to their natural habitat (i.e., same reef location, same depth) and retrieved the following year before the expected breeding season.

Reproduction study

An experimental set-up for continuous systematic data collection on the reproduction of F. fungites was initiated in July 2009, based on an histological study performed by Munasik (1999) and preliminary reproductive observations carried out in 2007. The corals, which were kept together in large containers with constant seawater inflow during the day, were transferred to separate individual containers for monitoring their reproductive products. Observations were carried out between 05:00 and 10:00 h, according to observational results reported in Munasik (1999). During 2009–2010 and 2013–2014, the observational procedure was repeated for 3–13 consecutive days after the full moon of June–August (Table 1). In 2015, observations were conducted for 2.5 months (July 1–September 13); on a daily basis for the first six weeks and then, following a reduction in the reproductive output, on a weekly basis until September 13. In 2016–2017, observations were conducted twice a week during July and August. The procedure included documentation of sex and fecundity (i.e., number of planulae released daily by an individual). In the years prior to 2015, observations on timing and quantification of reproductive output release (sperm, eggs, planulae) were performed with the naked eye (planulae) or a microscope (sperm and egg count). From 2015 on, after discovering that the planulae fluoresce, planulae counts were carried out with the aid of a blue-LED torch and a yellow long-pass filter (DFP-1 & FG-RB, NIGHTSEA, USA), which assisted in identifying the fluorescent planulae (Fig. 1c). The determination of a coral’s sex was performed each season separately as follows: Female-individuals that were documented to release only planulae (or in rare cases oocytes) throughout the entire reproductive season (May–September); Male-individuals that were documented to release only sperm throughout the entire reproductive season; Hermaphrodite- individuals that were documented to release both female (i.e., planulae or oocytes) and male (sperm) reproductive outputs throughout the reproductive season, either simultaneously or days to weeks apart.

Isolation of reproductive corals

At the beginning of July 2015, many of the sampled corals were documented as releasing a large number of planulae (hundreds) every day for over two weeks. In order to determine whether the continuous production of planulae depends on a frequent (i.e., daily) sperm supply, we isolated from the studied population seven, previously monitored, highly reproductive females of various sizes in the middle of the reproduction season of 2015 (July 15). Each of the isolated female corals was placed in a separate bucket with a daily change of 0.2 μm filtered seawater (TCR-020-S1FE Filter, Advantec MFS, USA), similar to the filtered water used for the rest of the corals, and under the same light conditions. Each morning we recorded the nature of the reproductive products (sperm, planulae or both), as well as collected and counted the planulae to determine fecundity. The selection of corals for the isolation experiment was performed prior to the beginning of the experiment based on two weeks of daily reproduction observations and female fecundity records of the entire sampled population (n = 203 individuals). The selected corals that were chosen for isolation were highly fecund females (i.e., releasing planulae) as determined during the period that preceded the beginning of the experiment.

Following the isolation of the females, we examined whether a reduction in reproductive longevity and/or reproductive intensity, or complete reproductive cessation, had occurred.

Female fecundity of isolated vs. non-isolated corals

Female fecundity counts were performed on two experimental populations: (1) Highly fecund females (n = 9) from the entire studied population (i.e., “non-isolated”); and (2) Highly fecund females that were isolated from the rest of the sampled population (n = 7) two weeks after
onset of observations (i.e., “isolated”). Measurements were conducted over a period of 2.5 months in 2015 (July 15–September 13). Planulae counts were performed every day from July 1 to August 9 (excluding July 9–10 and August 6–8) and then again on August 14, August 23, September 3, and September 13. Each morning, after planulae release hours (06:30–08:30), we counted the number of planulae released from each of the individual corals. After removing the coral from its container, water was gradually transferred from the container into a long glass-measuring cylinder where fluorescing planulae were counted. The counting was done with the aid of a blue-LED torch and a yellow long-pass filter (DFP-1 & FG-RB, NIGHTSEA, USA). The total number of planulae released from each individual throughout the entire period was recorded. Fecundity was determined as the average planulae number released per individual coral per day, and was normalized to the coral surface area. The surface area was measured from the coral photos taken at the beginning of each reproductive season using ImageJ software (Schneider et al. 2012).

Statistical analyses

Statistical analyses were performed using R software (R Development Core Team 2014) and Sigmaplot 12.2 (Systat Software, USA). Data were checked for normality (Shapiro–Wilk test) and homogeneity of variance (F-test), and
tested accordingly with appropriate parametric or non-parametric tests. Pairwise \( \chi^2 \) test was carried out to compare sex-ratio at different times, and t-test was used to compare fecundity in the isolated vs. non-isolated populations. \( p \) values < 0.05 were considered statistically significant.

**Results**

**Reproduction of *Fungia fungites***

The long-term observational monitoring (2009–2010, 2013–2017) revealed a mixed sexual pattern. The population of *F. fungites* in this area was found to consist of females, males, and hermaphrodites, with a labile sex ratio that varied significantly between years (Pearson’s \( \chi^2 \) test, \( \chi^2 = 287.96, \ p < 0.0001 \)) as well as between months within each reproductive season, (Pearson’s \( \chi^2 \) test, \( \chi^2 = 278.26, \ p < 0.0001 \)) (Tables 1, 2; Fig. 2). In 2009–2010 and 2013 sex ratios were male-biased, varying from 1.0:0.5:0.07 in 2013 to 1.0:0.75:0.0 in 2010; while in 2014–2017 sex ratios were female-biased, varying from 0.0:0.75:0.24 in 2016 to 0.31:1.0:0.13 in 2014 (Table 1). Pairwise \( \chi^2 \) test comparing sex ratios between years and months is provided in Tables 3 and 4. The planulae varied in size (\( \sim 150–400 \) μm) and could be observed as tiny dots swimming in the water column, while the male gametes appeared as a milky cloud, causing the water to appear murky. The planulae contained zooxanthellae when released and settled rapidly (within hours) on preconditioned substrates (Terra-cotta tiles that have been immersed in seawater flow-through tank system for a year; Fig. 1b, c). The data collected in 2015–2017 indicate that most of the reproductive individuals had released planulae (a few to dozens of planulae) and/or ejected sperm for a few days at a time, with pauses in-between during the season; while some individuals released planulae continuously throughout the entire monitoring period (2.5 months), often between tens and hundreds of planulae each day. The percentage of reproduction differed between years (Table 1), but was notably highest in 2015, when observations were carried out continuously for 2.5 months. The duration of the *F. fungites* reproduction season lasts at least four months, with reproductive output release occurring early in the morning (06:30–08:30 h) during June–September and ceasing around mid-September. The data collected from 2015–2017 indicate that the output release had occurred continuously throughout the entire monitoring period (2.5 months). The duration of the *F. fungites* reproduction season peaked in July, with the highest reproductive percentages of corals (Fig. 2, Table 2). In most years, males were more common at the beginning of the season (June) and then (August–September) gradually decreased in number, while female spawning could be observed throughout the entire breeding season (Fig. 2). Daily observations in 2015 revealed that the majority of sperm release ceased toward the end of July (Fig. 3). The hermaphroditic individuals were observed to release both sperm and planulae either simultaneously or at intervals of days/weeks between the two reproductive outputs.

**Fecundity of isolated vs. non-isolated individuals**

Fecundity estimates revealed that there was no significant difference in fecundity between the isolated group prior to its isolation and the non-isolated group (t-test, \( t = -0.708, \ p = 0.491 \)), or between the fecundity of the isolated group

---

**Table 1** *Fungia fungites*: Long-term population reproductive traits in Okinawa

| Years | 2009 | 2010 | 2013 | 2014 | 2015 | 2016 | 2017 |
|-------|------|------|------|------|------|------|------|
| n     | 62   | 111  | 51   | 219  | 203  | 94   | 73   |
| Number/Sex | 30 M | 28 M | 21 M | 35 M | 18 M | 0 M  | 8 M  |
|       | 15 F | 21 F | 5 F  | 112 F| 130 F| 58 F | 27 F |
|       | 2 H  | 0 H  | 0 H  | 14 H | 43 H | 22 H | 12 H |
| NR    | 15   | 62   | 25   | 58   | 12   | 14   | 26   |
| Sex-ratio (M:F:H) | 1:1 | 1:1 | 0.31:1:0.14 | 0:1:0.29 |
|       | 0.5:0.75:0.24 | 1:1 | 1:1 |
|       | 0.07 | 0 | 0 | 0.13 | 0.33 | 0.37 | 0.44 |
| % reproduction | 75.8 | 44.2 | 50.9 | 73.5 | 94.1 | 85.1 | 64.4 |
| % Hermaphrodites (H) | 4.2 | 0 | 0 | 8.7 | 22.5 | 27.5 | 25.5 |

\( n = \) total number of specimens examined; Number/Sex = number and sex of corals observed breeding (e.g., 30 M = 30 Males); NR = number of non-reproductive corals in the examined population; Sex-ratio: Male: Female: Hermaphrodite (M:F:H); % reproduction = percentage of reproductive corals out of the breeding population; % Hermaphrodites (H) = % of corals out of the breeding population releasing both sperm and planulae. For the size-frequency distribution of *F. fungites* individuals for which sex was determined see Supplementary Fig. S1.
Table 2  *Fungia fungites*: Population reproductive traits during 2009–2010, 2013–2017 in Okinawa*

| Year | 2009 | 2010 | 2013 | 2014 | 2015 | 2016 | 2017 |
|------|------|------|------|------|------|------|------|
| Dates | July 12–15 | Aug 8–13 | June 28 - July 9 | July 27-Aug 8 | June 23–29 | July 23–29 | July 14–16 | Aug 12–16 | July 2–6 | Aug 1–5 | Sept. 3 | July 21–24 | Aug 18–23 | July 11–12 | Aug 6,12 |
| Lunar cycle | 7 | 8 | 6 | 7 | 6 | 7 | 7 | 8 | 7 | 8 | 9 | 7 | 8 | 7 | 8 |
| DAFM | 5–8 | 2–7 | 2–12 | 1–13 | 0–7 | 1–8 | 2–4 | 2–6 | 0–5 | 0–5 | 4 | 1–4 | 0–5 | 2–3 | -1,5 |
| n | 62 | 62 | 111 | 111 | 51 | 51 | 102 | 226 | 203 | 188 | 182 | 94 | 94 | 73 | 73 |
| Number/Sex | 30 M | 30 M | 18 M | 0 M | 19 M | 5 M | 37 M | 6 M | 25 M | 1 M | 0 M | 3 M | 0 M | 16 M | 0 M |
| Sex-ratio (M:F:H) | 1:0.5:0 | 1:0.5 | 0.9:1:0 | 0:1:0 | 1:0.26:0 | 1:0.24:0 | 0.9:1 | 0.3:1:1 | 0.18:1 | 0.02:1:0 | 0:1.0 | 0.06:1 | 0.1:0 | 1:0.8 | 0:1:0 |
| % reproduction | 75.8 | 75.8 | 34.2 | 6.3 | 47.1 | 9.8 | 90.2 | 42.0 | 82.7 | 28.7 | 3.8 | 67.0 | 23.4 | 42.5 | 10.9 |
| % Hermaphrodites (H) | 0 | 4.2 | 0 | 0 | 15.2 | 0 | 5.9 | 0 | 20.6 | 0 | 6.4 | 0 |

The data represents reproductive traits recorded only throughout the days after the full moon (DAFM). Sex-ratio: M: F: H—Male: Female: Hermaphrodite. n = total number of specimens examined; Number/Sex = number and sex of corals observed breeding; NR = number of non-reproductive corals; Sex-ratio- Male: Female: Hermaphrodite (M: F: H); % reproduction = % reproductive corals out of the breeding population; % Hermaphrodites (H) = % corals out of the breeding population releasing both sperm and planulae.

*In order to be able to compare reproductive parameters among years throughout the whole study, the data analyzed and presented in this table takes into account only reproductive records noted each month, during the days after full moon (DAFM), although in 2015–2017 data were collected for a longer period of time (see Methods)
post-isolation and that of the non-isolated group at the same time ($t$ test, $t = -0.46$, $p = 0.652$) (Fig. 4). However, a significant difference was found when comparing both groups’ combined fecundity pre-isolation with both groups’ combined fecundity post-isolation (Mann–Whitney test, $T = 354$, $p < 0.001$). The number of planulae released during the reproductive season of 2015 had declined similarly in both groups (i.e., isolated and non-isolated), both prior to isolation and post isolation (Fig. 5).

**Discussion**

**Mixed sexuality in Fungia fungites population**

Despite gonochorism having been reported to date as characterizing the majority of the family Fungiidae (Loya et al. 2009; Harrison 2011), the present study demonstrates that, in addition to males and females, some hermaphroditic individuals also exist among the population of the fungiid coral *Fungia fungites* in Okinawa, Japan. The discovery of this existence came at a relatively late stage of a long-term, wide-ranging project exploring the reproductive strategies of the Fungiidae (Loya and Sakai 2008; Loya et al. 2009; Eyal-Shaham et al. 2019). The unique reproductive strategy of *F. fungites* reported here was confirmed through detailed daily observations of the reproductive output of the monitored *F. fungites* population. These findings contrast those of an histology study carried out in 1998 (Munasik 1999), which demonstrated that this species has separate sexes (i.e., a gonochoric species), as well as contrasting with observational data obtained in 2009–2010 and 2013, in which only two individuals (out of 224 corals observed throughout those years) were documented to release both the reproductive outputs of both sexes (Tables 1, 2). The reported variations in sex ratios (Males: Females: Hermaphrodites) may have derived from different timing and length of observations (Table 1), as well as from the identification method used in previous years (i.e., naked eye observations), which was improved in 2015, enabling identification of planulae by means of fluorescence (see Methods). This, however, does not rule out
a possible natural shift in the sex-ratio of the population as a result of environmental changes. Nevertheless, the existence of hermaphrodites in addition to males and females was evident in five out of the seven years of the present study (Tables 1, 2), while its apparent absence in 2010 and 2013 may have been due to a lack of prolonged observations. The earlier knowledge derived from preliminary work in Okinawa (Loya et al. 2009), suggesting that reproduction in many of the studied fungids peaks after full moon periods during the reproductive season (June to September), led us to monitor the reproduction of F. fungites for several days following the full moon during 2009–2014. However, only during the 2015 reproductive season, we discovered that most of the F. fungites individuals reproduced on a daily basis throughout the entire season. Consequently, some of the individuals recorded as 'females' based on observations made on the days following the full moon, were later redefined as hermaphrodites, after being observed to release sperm with the progression of the reproduction season. The same was true for 'males', which also released planulae at a later stage in the season. Indeed, the sex-ratio data collected in the five days following the full moon in July 2015 significantly differ from those collected for the entire month (Pearson’s χ² test, χ² = 45.234, p < 0.00001). The same holds true for data collected after the full moon in August 2015 versus data collected for the entire month (Pearson’s χ² test, χ² = 18.57, p < 0.00008). These findings emphasize the great importance of continuous and long-term monitoring observations required for the study of coral reproduction.

Most of the ca. 450 coral species whose reproduction is known (Fadlallah 1983; Harrison and Wallace 1990; Baird et al. 2009; Harrison 2011) are either hermaphroditic or have separate sexes (gonochoric). However, even though strong systematic patterns are evident in the sexual patterns of most of the genera studied (Baird et al. 2009; Harrison 2011; Kerr et al. 2011), not all coral species can be strictly classified into these basic patterns, as mixed or contrasting sexual patterns occur in some species (Fadlallah 1983; Harrison and Wallace 1990; Baird et al. 2009; Harrison 2011). Unusual sexual patterns include species that feature both gonochoric and hermaphroditic populations, and
populations containing a mixture of the three sexual morphs—female, male, and hermaphrodite (with varying sex-ratios), such as reported in the families Poritidae and Agariciidae (Delvoye 1988; Soong 1991; Glynn et al. 1994, 2017; Glynn and Ault 2000; Baird et al. 2009; Harrison 2011; Santiago-Valentín et al. 2015). These species can be considered as displaying labile gonochorism (sensu Giese 1974). However, in cases in which hermaphrodites are found to be extremely rare, the species is usually considered as exhibiting stable gonochorism (Giese 1974; Harrison 2011; Glynn et al. 2017). The occurrence of the three sexual morphs can also be found within colonies (Tomascik and Sander 1987; Guest et al. 2012; Castrillón-Cifuentes et al. 2015). For example, Guest et al. (2012) found episodic occurrences of hermaphroditism in Diploastrea heliopora, in which colonies contained male, female, and a low proportion of ‘co-sexual’ polyps (i.e., containing both oocytes and spermaries on the same mesentery). They described a sequential pattern within polyps with overlapping oogenic and spermatogenic cycles, and suggested that polyps of D. heliopora are capable of bi-directional alternation in sexual function. Furthermore, a mixture of male, female, and ‘co-sexual’ polyps was also found in a population of Porites porites in Barbados. In this species, there was a shift from strictly gonochorotic populations at unaffected sites to populations with primarily male-dominated ‘co-sexual’ polyps at polluted sites (Tomascik and Sander 1987). Such an environmentally-induced shift supports the assumption that atypical breeding systems arise as a plastic response in order to enhance reproductive success under poor environmental conditions (Freeman et al. 1976; Zimmerman 1991; Guest et al. 2012). In fungiid corals, sex change, first reported by Loya and Sakai (2008) was assumed to occur in response to energetic and/or environmental constraints. It is thus highly likely that the mixed sexuality found in F. fungites in the present study also results from environmental influences. However, whether this type of sexual system in corals has a genetic/systematic component or, alternatively, is dictated by environmental constraints, is yet to be determined.

Overall, gonochorotic coral species are less common than hermaphroditic coral species, with the former representing one-third of the scleractinian species for which their sexual pattern is known (Baird et al. 2009; Harrison 2011). The family Fungiidae is one of the few families of corals (along with Agariciidae, Astrocoeniidae, Oculinidae and Poritidae) that are predominantly gonochorotic (Baird et al. 2009). However, some fungids are known to change sex during their lifetime and are therefore labeled sequential hermaphrodites (Loya et al. 2009). In the present study, sexuality was determined by observations of the reproductive release of sexual products. The percentages of all three sexual morphs varied along the period of the study as follows: the percentage of hermaphrodites of the breeding population ranged between 0–8% in 2009–2010 and 2013–2014 (when observations were made only after a full moon and without the aid of fluorescence), to 22–28% in 2015–2017 (when observations were conducted continuously throughout July and August, regardless of the moon phase, and with the aid of fluorescence) (Table 1).

Hypotheses: Self-fertilization/asexual planulae-production/sperm-storage—a unique reproductive tactic

Experimental isolation of F. fungites individuals from their population for an extended period of time (two months) did not affect the reproductive patterns of the isolated individuals. We detected no reduction in planulae production nor a change in the length of the reproductive season or in fecundity values for isolated versus non-isolated individuals (Figs. 4, 5). The comparable fecundity of the two groups suggests that internal cross-fertilization in F. fungites is not dependent upon a constant sperm supply by neighboring conspecifics, and raises questions regarding the possible mechanism involved. To the best of our knowledge, there is currently no documentation of such a phenomenon in scleractinian corals, in which gonochorotic individuals continuously produced planulae for two months during complete isolation from the rest of the population. This finding is particularly surprising given the solitary, single-polyp life-form of F. fungites. Three hypotheses for this phenomenon are suggested: (1) Self-fertilization; (2) Asexual production of planulae; and (3) Sperm storage.

(1) Self-fertilization: The evolutionary advantage of selfing is that it may provide the benefit of ‘reproductive assurance’ in organisms with limited opportunities for sexual encounters, such as in sessile cnidarians (Tomlinson 1966; Schlesinger et al. 2010). This reproductive mechanism provides a way for supplying multiple copies of genotypes that have already proven successful under the prevailing conditions (Schlesinger et al. 2010). However, it also imposes trade-offs with genetic diversity, adaptive ability, effective recombination, and fitness (Charlesworth and Charlesworth 1987; Frank and Mokady 2002; Schlesinger et al. 2010; Caplins and Turbeville 2015). Fertilization trials indicate that self-fertilization is rare in corals (Wallace and Willis 1994; Miller and Babcock 1997; Miller and Mundy 2005; Harrison 2011). Likewise, self-fertilization is even more unlikely to occur in F. fungites due to its solitary nature and the proportionately high percentage of single-sex morphs within the population. Moreover, the individuals examined in the isolation experiment released only planulae with no evidence of...
sperm, neither in the period that preceded the isolation nor in the two months following their isolation. Furthermore, in view of the generally poorer performance of ’selfed’ offspring relative to outcrossed offspring (Barrett et al. 2009), it is not clear how a species could benefit from a high rate of self-fertilization.

(2) Asexual production of planulae: Although fragmentation is the most widely-studied and obvious means of asexual reproduction in reef cnidarians (Coffroth and Lasker 1998; Lirman 2000), genetic comparisons of parents and offspring have revealed that genetically identical coral colonies can also arise through the production of parthenogenetic larvae (i.e., asexual production of planulae) (Ayre and Resing 1986; Ayre et al. 1997; Brazeau et al. 1998; Baums et al. 2005; Harrison 2011; Combsch and Vollmer 2013). In both brooding and broadcast species (Ayre and Resing 1986; Harrison 2011; Combsch and Vollmer 2013), parthenogenetic larvae can be formed simultaneously with sexually-produced larvae from within the same colony, as in the case of Pocillopora damicornis (i.e. mixed reproduction strategy) (Yeoh and Dai 2010; Combsch and Vollmer 2013), thus combining the contrasting fitness disadvantages of cloning and sexual reproduction, while also compromising the respective benefits. Parthenogenesis assures reproduction independent of fertilization success (Combsch and Vollmer 2013) and consequently can overcome reproductive failure due to sperm limitation.

(3) Sperm storage: Female sperm storage is a biological process, and often a type of sexual selection in which spermatozoa can be stored by the recipient prior to fertilization (Neubaum and Wolflner 1998; Bishop and Pemberston 2006). In the female, sperm become sequestered in specialized storage organs or reservoirs, where they may remain for several days, weeks, months, or years before being used to fertilize the eggs. The variety of animals that store sperm is large and spans several taxa (Neubaum and Wolflner 1998), including sessile marine invertebrates (Bishop 1998; Hughes et al. 2002; Bishop and Pemberston 2006). For example, in the colonial ascidian Diplosoma listerianum sperm can be stored in the female reproductive tract for over seven weeks prior to fertilization (Bishop 1998). In the bryozoan Celleporella hyaline the passage of inseminated eggs into the ovicells in order to commence development may occur up to several weeks after the uptake of sperm by adult colonies (Manriquez 1999). Additionally, in hermaphroditic gastropods, sperm storage can last for months (Reviewed in (Nakadera and Koene 2013). Sperm storage can be advantageous for females of taxa with uncertain mating opportunities, a feature attributed to cross-fertilizing sessile animals such as stony corals. These females are likely to have a high reproductive output because they can continually produce fertilized eggs without re-mating (Neubaum and Wolflner 1998). Other benefits are expressed in prolonged and sustained female fertility (den Boer et al. 2009) and the ability of sperm to remain viable for longer periods (i.e., paternal longevity) (Nakadera et al. 2014). Histological and observational data obtained by Munasik (1999) indicated multi-gametogenic cycles in both male and female F. fungites. However, unlike females, which were reproductive throughout the entire season, male reproductive performance was observed to be sparse from August until mid-September. Those results are compatible with our current findings, which demonstrate a decrease in the number of males as the reproductive season progressed, along with no evidence of sperm release in September (Fig. 2, Table 2). The early decrease in the number of males, while planulae continue to be released until mid-September, may support the ‘sperm storage’ hypothesis. Interestingly, the suggested alternatives do not contradict each other because sperm-storing hermaphrodites potentially have two different sperm types available to them for the fertilization of their eggs: their own sperm (autosperm), and their mating partner’s sperm (allosperm). Thus, after using up the stored allosperm they can continue to reproduce by using autosperm; or instead selectively use autosperm despite the presence of allosperm (Nakadera et al. 2014). Furthermore, sperm storage and parthenogenesis have been documented to occur simultaneously in the same individual (Orconectes limosus, Order: Decapoda) (Bučić et al. 2013). Clearly, the conclusions regarding these possible alternatives rest on assumptions that merit further investigation of larval origin, in order to elucidate the mechanisms that govern multiple planulae release by isolated F. fungites individuals.

Finally, we emphasize the importance of continuous and long-term monitoring of coral reproduction studies, since we may have overlooked much of the plasticity and complexity in coral reproduction by focusing on narrow reproductive periods. Additionally, to better understand the reproductive strategies and sex allocation of scleractinian corals, we need more genetic studies of coral populations representing a broad range of species and their larval origin.

Acknowledgments We thank Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, for the logistical support. We are indebted to I. Hosgin, Y. Nakano, R. Prasetia, Y. Rosenberg, J. Bergman and T. Eyal for help with the fieldwork in Okinawa. This study was supported by the Israel Science Foundation (ISF) No. 1191/16 to YL. LES was partially supported by the WDHOF graduate scholarships in marine conservation, the MERCI travel grant, the PADI Foundation and by the Ministry of Science, Technology & Space fellowship. GE was partly supported by the European Union’s Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No. 796025. All
samples were collected and treated according to regulations of Okinawa Prefecture, Japan.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

Ayre DJ, Resing JM (1986) Sexual and asexual production of planulae in reef corals. Mar Biol 90:187–190
Ayre DJ, Hughes TP, Standish RJ (1997) Genetic differentiation, reproductive mode, and gene flow in the brooding coral Pocilloporadamicornis along the Great Barrier Reef, Australia. Mar Ecol Prog Ser 159:175–187
Babcock RC, Bull GD, Harrison PL, Heyward AJ, Oliver JK, Wallace CC, Willis BL (1986) Synchronous spawnings of 105 scleractinian coral species on the Great Barrier Reef. Mar Biol 90:379–394
Baird AH, Guest JR, Willis BL (2009) Systematic and biogeographical patterns in the reproductive biology of scleractinian corals. Annu Rev Ecol Evol Syst 40:551–571
Barrett SCH, Ness RW, Vallejo-Marín M (2009) Evolutionary pathways to self-fertilization in a tristylos plant species. New Phytol 183:546–556
Baums IB, Hughes CR, Hellberg ME (2005) Mendelian microsatellite loci for the Caribbean coral Acropora palmata. Mar Ecol Prog Ser 288:115–127
Bishop JDD (1998) Fertilization in the sea: are the hazards of broadcast spawning avoided when free–spawned sperm fertilize retained eggs? Proc R Soc B Biol Sci 265:725–731
Bishop JDD, Pemberton AJ (2006) The third way: spermcast mating in sessile marine invertebrates. Integr Comp Biol 46:398–406
Brazeau DA, Gleason DF, Morgan ME (1998) Self-fertilization in brooding hermaphroditic Caribbean corals: Evidence from molecular markers. J Exp Mar Biol Ecol 231:225–238
Bučič M, Kouba A, Kozák P (2013) Reproductive plasticity in freshwater invader: from long-term sperm storage to parthenogenesis. PLoS ONE 8:e77597
Caplins SA, Turbeville JM (2015) High rates of self-fertilization in a marine ribbon worm (Nemertea). Biol Bull 229:255–264
Castrillo´n-Cifuentes AL, Muñoz CG, Zapata FA (2015) Reproductive patterns of the coral Pocillopora damicornis at Gorgonia Island, Colombian Pacific Ocean. Mar Biol Res 11:1065–1075
Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. Annu Rev Ecol Syst 18:237–268
Coffroth MA, Lasker HR (1998) Population structure of a clonal gorgonian coral: the interplay between clonal reproduction and sexual reproduction. Evolution 52:379–393
Comброс DJ, Vollmer SV (2013) Mixed asexual and sexual reproduction in the Indo-Pacific reef coral Pocillopora damicornis. Ecol Evol 3:3379–3387
Delvoye L (1988) Gametogenesis and gametogenic cycles in Agaricia agaricites (L.) and Agaricia humilis Verriére and notes on gametogenesis in Madracis mirabilis (Duchassaing & MicheU•liot) (Scleractinia). Foundation for Scientific Research in Surinam and the Netherlands Antilles 123:101–134
den Boer SPA, Baer B, Dreier S, Aron S, Nash DR, Boomsma JJ (2009) Prudent sperm use by leaf-cutter ant queens. Proc R Soc B Biol Sci 276:3945–3953
Eyal-Shaham L, Eyal G, Sakai K, Nozawa Y, Harri S, Sinniger F, Bronstein O, Ben-Zvi O, Shlesinger T, Loya Y (2019) Repetitive sex change in the stony coral Herpolitha limax across a wide geographic range. Sci Rep 9:2936
Fadlallah YH (1983) Sexual reproduction, development and larval biology in scleractinian corals. Coral Reefs 2:129–150
Frank U, Mokady O (2002) Coral biodiversity and evolution: recent molecular contributions. Can J Zool 80:1723–1734
Freeman DC, Klikoff LG, Harper KT (1976) Differential resource utilization by the sexes of dioecious plants. Science 193:597–599
Giese A (1974) Introduction: general principles. In: Giese AC, Pearse JS (eds) Reproduction of marine invertebrates, vol. 1. Academic press, New york
Glynn PW, Ault J (2000) A biogeographic analysis and review of the far eastern Pacific coral reef region. Coral Reefs 19:1–23
Glynn PW, Colley SB, Carpiozito-Uartea E, Richmond RH (2017) Coral reproduction in the Eastern Pacific. In: Glynn PW, Manzello DP, Enochs IC (eds) Coral reefs of the Eastern tropical Pacific: persistence and loss in a dynamic environment. Springer, Dordrecht, pp 435–476
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
Guest JR, Baird AH, Goh BPL, Chou LM (2012) Sexual systems in scleractinian corals: an unusual pattern in the reef-building species Diplastrea heliopora. Coral Reefs 31:705–713
Harrison PL, Wallace CC (1990) Reproduction, dispersal and recruitment of scleractinian corals. Ecosystems of the world 25:133–207
Harrison PL (2011) Sexual reproduction of scleractinian corals Coral reefs: an ecosystem in transition. Springer, Berlin, pp 59–85
Hughes RN, Manriquez PH, Bishop JDD (2002) Female investment is retarded pending reception of allosperm in a hermaphroditic colonial invertebrate. Proc Natl Acad Sci 99:14884–14886
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
Kramarsky-Winter E, Loya Y (1998) Reproductive strategies of two fungiid corals from the northern Red Sea: environmental constraints? Mar Ecol Prog Ser 174:157–182
Krupp DA (1983) Sexual reproduction and early development of the solitary coral Fungia scutaria (Anthozoa: Scleractinia). Coral Reefs 2:159–164
Lirman D (2000) Fragmentation in the branching coral Acropora palmata (Lamarck): growth, survivorship, and reproduction of colonies and fragments. J Exp Mar Biol Ecol 251:41–57
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. J Coral Reef Stud 11:119–129
Manriquez PH (1999) Mate choice and reproductive investment in the cheilotesto bryozoan Celleporella Hyalina (L.). University of Wales, Bangor
Miller KJ, Babcock RC (1997) Conflicting morphological and reproductive species boundaries in the coral genus Platygyra. Biol Bull 192:98–110
Miller KJ, Mundy CN (2005) In situ fertilisation success in the colonial invertebrate. Proc R Soc B 274:2335–2343
Munasaki, (1999) Sexual reproduction of the solitary coral Fungia fungites in Okinawa. University of Ryukyus, Japan, p 33
Nakadera Y, Koene JM (2013) Reproductive strategies in hermaphroditic gastropods: conceptual and empirical approaches. Can J Zool 91:367–381

Coral Reefs (2020) 39:1793–1804 1803
