

AN ABSTRACT OF THE THESIS of Valeri A. Lapacek for the Master of Science in Biology presented, July 13th, 2017

Title: Sexual Reproductive Biology of Guam's Staghorn *Acropora*

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Reproductive biology of dominant reef-building Pacific staghorn *Acropora* species (*Acropora aspera*, *A. pulchra*, *A. muricata*, *A. cf. muricata*, and *A. cf. intermedia*) was investigated by dissecting decalcified samples collected from February 2015 through June 2016. One gametogenesis cycle occurred per year per species within 9-10 months. Egg production in Guam was lower for *Acropora aspera*, *A. pulchra*, and *A. cf. intermedia* but similar for *A. muricata*, compared to those of other studies in different locations. Multiple populations were examined for *Acropora pulchra* and *A. muricata*, and mature oocyte diameters varied significantly between study populations for *Acropora pulchra* and spermary sizes varied between populations for *Acropora muricata*. Furthermore, the low reproductive outputs observed may suggest that some species are more sensitive to environmental stressors such as thermal anomalies. Spawning timing among *A. pulchra*, *A. cf. intermedia*, *A. cf. muricata*, and *A. muricata* was relatively predictable, coinciding with the seasonal peak in insolation in April and May of 2016. Spawning in the field was confirmed for *A.*

pulchra and *A. muricata* in April and May of 2017, occurring between 2015-2045

h. Multiple, small-scale spawning events were observed *in situ* between two and seven days after the full moon in *A. pulchra* and *A. muricata*. Furthermore, in 2016, *A. cf. muricata* and *A. muricata* spawned during multiple moon phases within the same month. *Acropora aspera* spawned asynchronously with the other staghorn *Acropora* in this study, occurring at the peak of the rainy season in September and October. Substantial gamete production was observed in this study, but low genotypic diversity and low sexual recruitment has been documented for *A. pulchra*, thus it is likely that Guam's staghorn *Acropora* species rely significantly on asexual fragmentation for population maintenance and expansion, and recovery may be slow after mortality events.

TO THE OFFICE OF GRADUATE STUDIES

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**SEXUAL REPRODUCTIVE BIOLOGY OF GUAM'S
STAGHORN ACROPORA**

BY

Valeri A. Lapacek

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INTRODUCTION/PROBLEM STATEMENT

The coral genus *Acropora* is the most diverse among the scleractinia. The greatest species diversity is found in the Indo-West Pacific (Veron 2000; Wallace 1999). *Acropora* are fast-growing dominant reef builders, making them essential three-dimensional habitats within coral reefs worldwide (reviewed by Harrison and Booth 2007; Veron 1995; Wallace 1999; Wilson and Rosen 1998). Staghorn *Acropora* species exhibit fragile, arborescent, branching morphologies that often form thickets, called ‘stands.’ Staghorn *Acropora* stands are usually found in areas where water is retained at low tide, such as inner reef flat zones, moats, and lagoons (Veron 2000). Guam’s staghorn stands are found within a lagoon, shallow reef flats and back reefs, and harbor shoals. Staghorn stands are an essential refuge for ecologically and commercially important fishes and invertebrates, especially since few mangrove habitats and seagrass beds are found on Guam (Floros and Schleyer 2016).

Many Guam residents depend on coral reefs for food and as a source of income. Additionally, Guam’s tourism industry greatly benefits from coral reef ecosystems, which support activities such as snorkeling, SNUBA, submarine tours, glass-bottom boat tours, and SCUBA diving. Guam’s economy relies heavily on tourism, which accounts for ~20% of the island’s GDP and supports 33% of the island’s jobs (Guam Tourism Satellite Account Economic Report 2015). In Tumon Bay, a marine preserve with a mile-wide stretch of sandy beach dominated by more than 20 large hotels, concerns have been raised about the effects of development on the Bay’s protected coral reef. Similar levels of coastal

development have been shown to have serious detrimental effects on corals and other marine life in the Red Sea (Roberts and Hawkins 1994).

Acropora corals, especially those with branching morphologies (including staghorns), are indicative of healthy, stable reef systems due to their high sensitivity to environmental stress (Glynn 1996; Johnstone and Kahn 1995; Peters 1993; Salvat 1992). Staghorns occupy habitats that are frequently exposed to anthropogenic stressors such as sedimentation, eutrophication, pollution, and physical damage (Guilcher 1988). As a result, staghorn *Acropora* are particularly susceptible to coral bleaching (Guest et al. 2012; McClanahan et al. 2014) and diseases (Sutherland et al. 2004). Guam's acroporids are impacted by brown band disease, white syndrome, and growth anomalies (Myers and Raymundo 2009). Additionally, *Acropora* are preferred prey of corallivorous gastropods (*Drupella*) and the crown-of-thorns starfish (*Acanthaster planci*), which have severely reduced Guam's staghorn populations (Moyer et al. 1982; Burdick et al. 2008; Caballes 2009).

From 2005-2010, Guam's Coral Reef Initiative Long-term Monitoring Program began locating and mapping existing staghorn *Acropora* populations to document the location, species composition, and size of existing thickets. This effort revealed the following common species: *Acropora aspera*, *A. pulchra*, *A. cf. intermedia*, *A. muricata* (formally *A. formosa*), and *A. cf. muricata*; and rarer species *A. vaughani*, *A. teres*, *A. austera*, and *A. virgata* (Burdick et al. 2008) (Figure 1a-i). Currently, the IUCN Red List of Threatened Species lists *A. aspera* as vulnerable (IUCN 2015). Unfortunately, the Mariana's staghorn

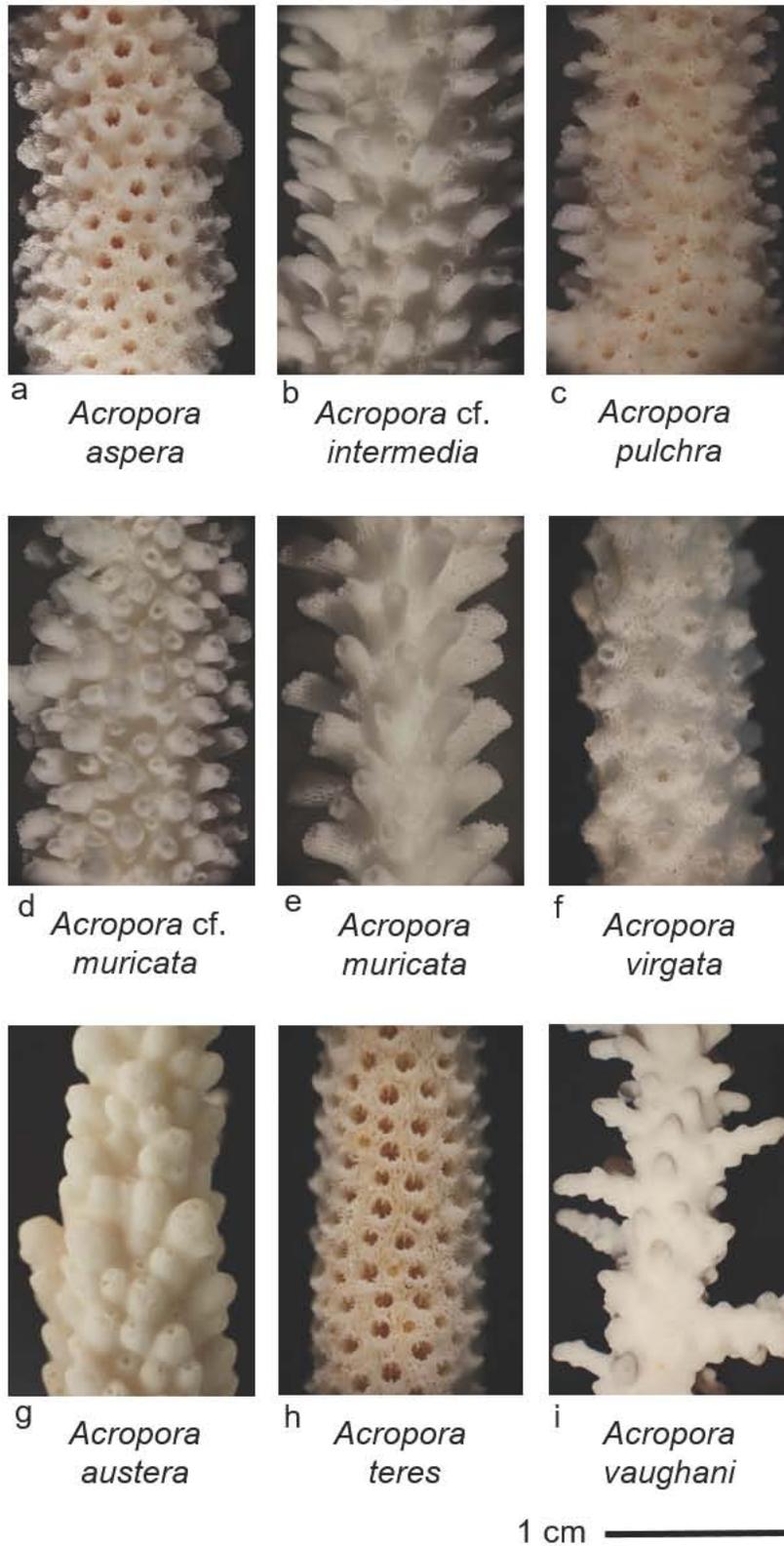


Figure 1: Coral skeleton images of Guam's staghorn *Acropora* species: (a) *Acropora aspera*, (b) *A. cf. intermedia*, (c) *A. pulchra*, (d) *A. cf. muricata*, (e) *A. muricata*, (f) *A. virgata*, (g) *A. austera*, (h) *A. teres*, and (i) *A. vaughani*.

Acropora have been severely impacted by back-to-back bleaching episodes in 2013 and 2014, resulting in extensive mortality (Raymundo et al. 2017). Throughout 2015, the remaining stands were resurveyed to document the extent of bleaching mortality. Approximately 53% (+/- 10%) of Guam's staghorns were lost to bleaching and low-tide exposures within 12 months, and eleven out of the 21 sites showed estimated mortality $\geq 75\%$ (Raymundo et al. 2017). Furthermore, all the sites containing rare staghorns exhibited 80-90% mortality, leaving the survival of these species highly questionable. Due to the extensive loss, Guam's staghorns have become a local management concern and active rehabilitation efforts have been initiated.

Successful coral reproduction is vital for the continued existence of coral reefs, as it provides new recruits and replaces damaged and killed corals. Scleractinian corals can reproduce both sexually and asexually. Currently, there is not sufficient data to describe the reproductive patterns of staghorn *Acropora* in the Mariana Islands. Previous work by Heyward (1988) and Richmond and Hunter (1990) included no information regarding the reproductive timing of staghorn *Acropora* species, and no progress has been made since these publications. *Acropora*, including staghorns, are hermaphroditic broadcast spawners throughout their range (Baird et al. 2009). However, the spawning timing and fecundity have not been documented for Guam staghorns. Furthermore, over the last several decades, larval recruitment throughout the Mariana Islands has declined and remains low, compared to other reefs in the Pacific (Birkeland and Randall 1981; Neudecker 1981; Birkeland 1997; Minton

and Lundgren 2006). In light of the recent, widespread decline of Guam's staghorn *Acropora* populations, information on sexual reproduction is crucial to assess the potential for recovery and persistence of these species. This study will examine Guam's most populous and easily accessible staghorn *Acropora* species: *Acropora aspera*, *A. pulchra*, *A. cf. intermedia*, *A. cf. muricata*, and *A. muricata*.

Coral Reproduction in Acropora

To date, all known *Acropora* are hermaphroditic (Baird et al. 2009), growing both ovaries and testes (spermaries) in polyp mesenteries (Policansky 1982). Sexual reproduction produces new genotypic combinations, allowing populations to maintain genetic traits associated with resilience to environmental variability, thus increasing fitness and survivorship (Reed and Frankham 2003; Blomqvist et al. 2010). Asexual reproduction produces clones, allowing successful genes to persist in a population and, allows coral colonies grow and expand via budding and fragmentation. New clonal polyps are formed when one polyp divides into two polyps, a process called budding, and fragmentation occurs when a coral branch will break off and grow to form a new clonal colony (Harrison and Wallace 1990). In staghorn *Acropora*, asexual fragmentation and budding are the dominant modes of reproduction. However, the importance and extent of asexual vs. sexual reproduction varies between populations and species (Ayre et al. 1997; Ayre and Hughes 2000; Miller and Ayre 2004; Baums et al. 2006; Sherman et al. 2006; Whitaker 2006; Foster et al. 2007).

All corals in the *Acropora* genus are broadcast spawners (Baird et al. 2009), which release gametes into the sea for external fertilization and

development. Acroporids have successive reproductive spawning events throughout their lifetime (iteroparity), consisting of one gametogenesis cycle each year, and most are of reproductive age between 3–5 years (Harrison and Wallace 1990). Oocytes and spermaries mature together, but oogenesis precedes spermatogenesis by a few months (Harrison and Wallace 1990). Early stages of gametogenesis can vary within the colony and the population, but gametogenesis becomes more synchronized as development continues (Kojis and Quinn 1981, 1982; Harriott 1983; Wyers 1985; Szmant 1986; Okubo and Motokawa 2007). Broadcast spawning corals are evolutionarily constrained by only having one mating opportunity each year, thus spawning synchrony is most likely highly adapted to optimize fertilization success.

Spawning in Acropora

One to two weeks before spawning, eggs will develop pigmentation (Babcock 1984; Babcock and Smith 2000; Marshall and Stephenson 1933). Just a few hours before spawning, eggs and sperm become compressed in a vitelline membrane to form one or more egg-sperm bundles within the mouth cavity of the parent colony. These bundles become visible beneath the polyp's oral disk during the 'setting' stage. Egg and sperm bundles are positively buoyant because eggs are largely made up of lipids, thus these bundles float to the sea surface after spawning. These bundles break apart and become concentrated at the sea surface, often creating spawn slicks which increases the likelihood of cross-fertilization (Oliver and Willis 1987). No longer than 4 days after fertilization, competent larvae will form and begin to search for a suitable settlement

substrate. Coral larvae are lecithotrophic, thus rely on the egg yolk for energy. Yolk reserves are largely made up of lipids, which is a long-term energy source that is consumed throughout development to provide energy for larval dispersal, settlement, and metamorphosis (Arai et al. 1993; Harii et al. 2007, 2010). Branching *Acropora* have relatively short pelagic larval durations (PLDs) of ~12 days and, to survive, must settle before then (Babcock and Heyward 1986; Nishikawa 2008; Nozawa and Harrison 2008). Following settlement, corals undergo metamorphosis by forming into a single coral polyp. Then, the polyp will divide into clones and start to form a colony.

Environmental cues that regulate coral spawning range from broad to fine scales, dictating the time of year (i.e. month), the night of spawning, and the time (i.e. hour) of spawning (Babcock et al. 1986). Seasonal fluctuations in temperature, insolation, and rainfall may influence gametogenesis and spawning (Glynn and Ault 2000; Mendes and Woodley 2002; Penland et al. 2004b). Traditionally, sea surface temperatures (SST) were largely considered a broad-scale factor in controlling reproductive timing because there are many locations where broadcast spawning occurs as waters warm to the annual maxima (Harrison and Wallace 1990). However, much evidence points to discrepancies between warming SST and spawning timing (Babcock et al. 1994; Mendes and Woodley 2002; Penland et al. 2004b) and Kojis (1986) suggested that temperatures in shallow water, such as reef flats, were too variable to control gametogenesis. Rainfall decreases salinity, especially at the surface where gametes accumulate after being spawned. Thus, rainfall may explain some of the

geographic variations in spawning timing, although this has not been well examined (Mendes and Woodley 2002). The rate of change of insolation, which coincides with seasonally calm periods, was found to be a good predictor of coral spawning in the Caribbean (van Woesik et al. 2006; van Woesik 2009). Penland et al. (2004b) reported multi-species spawning during the peaks in insolation but, more recently, Keith et al. (2016) showed that SST reliably predicted spawning for *Acropora* in the Indian and Pacific Ocean.

Normally, an entire coral population will spawn at the same time (Harrison and Wallace 1990) and, frequently, the spawning times of many species will synchronize, leading to multi-species spawning events (Babcock et al. 1986). Historically, multi-species spawning events were thought to be restricted to regions with significant temperature and irradiance seasonality (Oliver et al. 1988), but more recent studies from a wider geographical range show that seasonality and synchrony of major coral spawning events is widespread, although the timing and extent of synchrony varies greatly among locations (Baird et al. 2009; Guest et al. 2005; Guest et al. 2002; Hayashibara et al. 1993; Nozawa et al. 2006; Suzuki et al. 2011; Vicentuan et al. 2008; van Woesik et al. 1995). In contrast, some reef regions, such as the Red Sea, Hawaii, central Pacific, and the eastern Pacific, experience little overlap in spawning amongst coral assemblages (Shlesinger and Loya 1985; Richmond and Hunter 1990; Glynn and Ault 2000; Kenyon 2008). For example, Shlesinger and Loya (1985) reported nine species in the Red Sea (Eilat, Israel) that spawn during different seasons (i.e. months) and, in Palau, Penland et al. (2004) documented spawning

activity eight months of the year, observing synchronous spawning of 10 or more broadcast spawners in March, April, May, and September. At a broad level (i.e. month), spawning synchrony is most likely controlled by environmental cues that work best to optimize fertilization success.

Coral spawning is also synchronized on finer scales. Monthly lunar and tidal cycles may control the time of month (i.e. number of days after the full moon) that spawning occurs, and diurnal light cycles control the hour of spawning (Babcock and Heyward 1986). Generally, synchronized spawning occurs within a week after the full moon and between dusk and midnight. Corals contain blue-sensitive photoreceptors, which are known to maintain circadian rhythms (Levy et al. 2007) and are sensitive to the blue region of the light spectrum (i.e. moonlight) (Gorbunov and Falkowski 2002). Furthermore, Oliver et al. (1988) hypothesized that spawning usually occurs at low amplitude neap tides, a period with low water motion and low water volume, to optimize fertilization success, and occurs when the moon rises later in the night, a period when predation is reduced.

Sexual reproduction (spawning) has important implications for population genetic structure, coral evolution, and taxonomy. Synchronized spawning increases the probability of cross-fertilization (Levitan et al. 2004; Oliver and Babcock 1992; Willis et al. 1997) and hybridization (Willis et al. 2006), whereas asynchronized spawning has led to reproductive isolation (Fukami et al. 2003) and genetic and morphological variation (Stobart and Benzie 1994; Dai et al. 2000; Wolstenholme 2004). The time of spawning of some species has been

shown to vary on the scale of hours (Knowlton et al. 1997; Szmant et al. 1997; van Oppen et al. 2001; Hayashibara and Shimoiki 2002; Levitan et al. 2004) or even weeks or months out of phase with mass spawning (Babcock and Heyward 1986; Hayashibara et al. 1993; Wallace 1999; Fukami and Shimoiki 2002). Resultantly, these species may be reproductively isolated because gametes drift away and dilute, rapidly limiting chances for fertilization (Oliver and Babcock 1992). Furthermore, the time it takes for egg-sperm bundles to break apart has been shown to limit opportunities for cross-fertilization and hybridization (Wolstenholme 2004). Thus, both synchronized and asynchronized spawning has contributed to the evolution of coral species.

Coral Fecundity

With limited resources, adult corals must allocate energy between growth and reproduction. Environmental or ecological changes would require more energy for growth and/or maintenance, thus decreasing the amount of energy for reproductive output. Energy allocation trade-offs have been observed between growth and reproduction in corals (Ward 1995; Anthony et al. 2002), making reproductive output a useful indicator of coral health and/or stress. In corals, fecundity is usually measured by the number of eggs per polyp, which provides a useful index (i.e. fecundity index) of reproductive effort. Additionally, smaller egg sizes in *Acropora* have been documented as a result of environmental stressors such as bleaching (Ward et al. 2000; Michalek-Wagner and Willis 2001) and elevated nutrients (Ward and Harrison 2000), thus egg size is also a useful indicator of coral health or stress. Polyp size and density varies widely between

taxa, so fecundity is often compared on a tissue area basis (i.e. one cm⁻²) with respect to the number of gametogenic cycles per year, calculated as mean annual fecundity. Furthermore, oocyte diameter and spermary length is an accurate metric used to document gamete maturation within the gametogenesis cycle. Fecundity and gamete size are easily measured from the dissected polyps from fixed and decalcified coral samples, and has been measured in many *Acropora* species (Wallace 1985; Ward et al. 2000).

Reproductive processes are highly sensitive to natural and anthropogenic stressors (Fabricius 2005; Harrison and Wallace 1990; Richmond 1997), and changes in fecundity and reproductive output are documented responses to stressors. For example, sedimentation caused a 50% decrease in fecundity in *Acropora palifera* (Kojis and Quinn 1984). In *Acropora longicyathus* and *A. aspera*, elevated nutrient levels produced significantly smaller and fewer eggs, and smaller testes than those which were not exposed to nitrogen (Ward and Harrison 2000), and coral bleaching reduced the number of eggs, egg size, and testes size in *Acropora aspera* and *A. nobilis* (Ward et al. 2000). In other genera, crude oil exposure resulted in fewer and smaller gonads in *Siderastrea siderea* (Guzmán and Holst 1993) and *Stylophora pistillata* colonies with broken branches had more sterile branches and fewer female gonads (Rinkevich and Loya 1989). Additionally, many studies have shown reduced fecundity and reproductive success caused by a variety of environmental factors and anthropogenic pollutants. For example, Richmond (1993) found an 86% decrease in fertilization with a 20% decrease in salinity; trace metals (i.e. copper) reduced fertilization success by >50% in *Acropora tenuis* and *A. longicyathus*

(Reichelt-Brushett and Harrison 2005); ultraviolet radiation terminated sexual reproductive processes in *Acropora cervicornis* (Torres et al. 2008); fungicides and insecticides reduced fertilization, metamorphosis, and photosynthesis in *Acropora millepora* (Markey et al. 2007); and coral bleaching reduced fertilization by decreasing sperm concentrations and motility in *Acropora nasuta* (Omori et al. 2001). Thus, coral sexual reproduction is a sensitive life process, which is why it is critical to reduce local stressors and safeguard reproductive success.

GOALS & QUESTIONS

Specifically, this thesis documents the sexual reproductive biology of Guam's dominant staghorn *Acropora* species, *Acropora aspera*, *A. pulchra*, *A. cf. intermedia*, *A. cf. muricata*, and *A. muricata* by 1) describing gametogenesis; 2) determining the level of spawning synchrony; and 3) quantifying fecundity; the following questions were addressed, for each species, to fulfill these goals:

1. What is the length of gametogenesis and the number of gametogenic cycles per year? Furthermore, are seasonal cue(s) (i.e. insolation, SST, and rainfall) correlated with gametogenesis, specifically oogenesis?
2. Do Guam's staghorn *Acropora* species spawn synchronously, releasing gametes during the same month(s), day(s), and time of day (i.e. hour)?
3. What is the reproductive output and how does reproductive output vary between populations or between years, of the same species?

METHODS

Study Area

Guam is a tropical island located between 13.2°N and 13.7°N and between 144.6°E and 145.0°E. Due to Guam's close proximity to the equator, temperature and photoperiod (length of day) remain relatively constant throughout the year, averaging 30 °C and 12 hours, respectively. A dry season occurs from December to June and the wet season occurs from July to November. Guam has an area of 212 square miles (549 km²) and a coral table reef surrounds most of the island. A small, shallow lagoon is located at the southernmost tip of Guam.

The study populations of *Acropora aspera*, *A. pulchra*, *A. cf. intermedia*, *A. cf. muricata*, and *A. muricata* are conspicuous thickets on Guam's shallow reef flats. More than half of Guam's staghorn *Acropora* had recently died from extreme low tides and two back-to-back bleaching episodes in 2013 and 2014 (Raymundo et al. 2017). Four sites (Tumon, Agat, Togcha, and Achang) with easy access and significant remaining staghorn populations were selected for monitoring (Table 1, Figure 2). At these sites, corals are exposed to significant anthropogenic stressors, including seasonally high freshwater influx, wastewater effluent, nutrient loading, sedimentation, and snorkeler/fisherman damage (Guilcher 1988).

Table 1: Site descriptions of sampled populations found at each site.

Site Name	Habitat Description	Staghorn Species	Distance from Shore (m)	Depth (m)	GPS Location	Total Staghorn Population Size (m ²)
14 Tumon	Shallow bay (~2 km across), near significant development (i.e. hotels, roads, etc.)	<i>A. pulchra</i>	260	0.5-1.5	13°31'7.54"N; 144°48'12.66"E	151,423
		<i>A. muricata</i>	30	3-5	13°30'19.55"N; 144°47'21.07"E	
		<i>A. cf. intermedia</i>	260	0.5-1.5	13°31'7.54"N; 144°48'12.66"E	
Agat	Reef flat, with small creek outfall	<i>A. pulchra</i>	290	0.5-1.5	13°22'56.76"N; 144°39'5.87"E	24,488
		<i>A. muricata</i>	290	0.5-1.5	13°22'56.76"N; 144°39'5.87"E	
		<i>A. cf. muricata</i>	290	0.5-1.5	13°22'56.76"N; 144°39'5.87"E	
Togcha	Back reef crevice (~4 m across)	<i>A. pulchra</i>	530	0.5-1.5	13°22'4.74"N; 144°46'30.65"E	5,035
Achang	Reef flat, next to large river channel (~165 m across)	<i>A. pulchra</i>	1,000	0.5-1.5	13°14'43.91"N; 144°41'5.98"E	21,138
		<i>A. aspera</i>	1,000	0.5-1.5	13°14'43.91"N; 144°41'5.98"E	

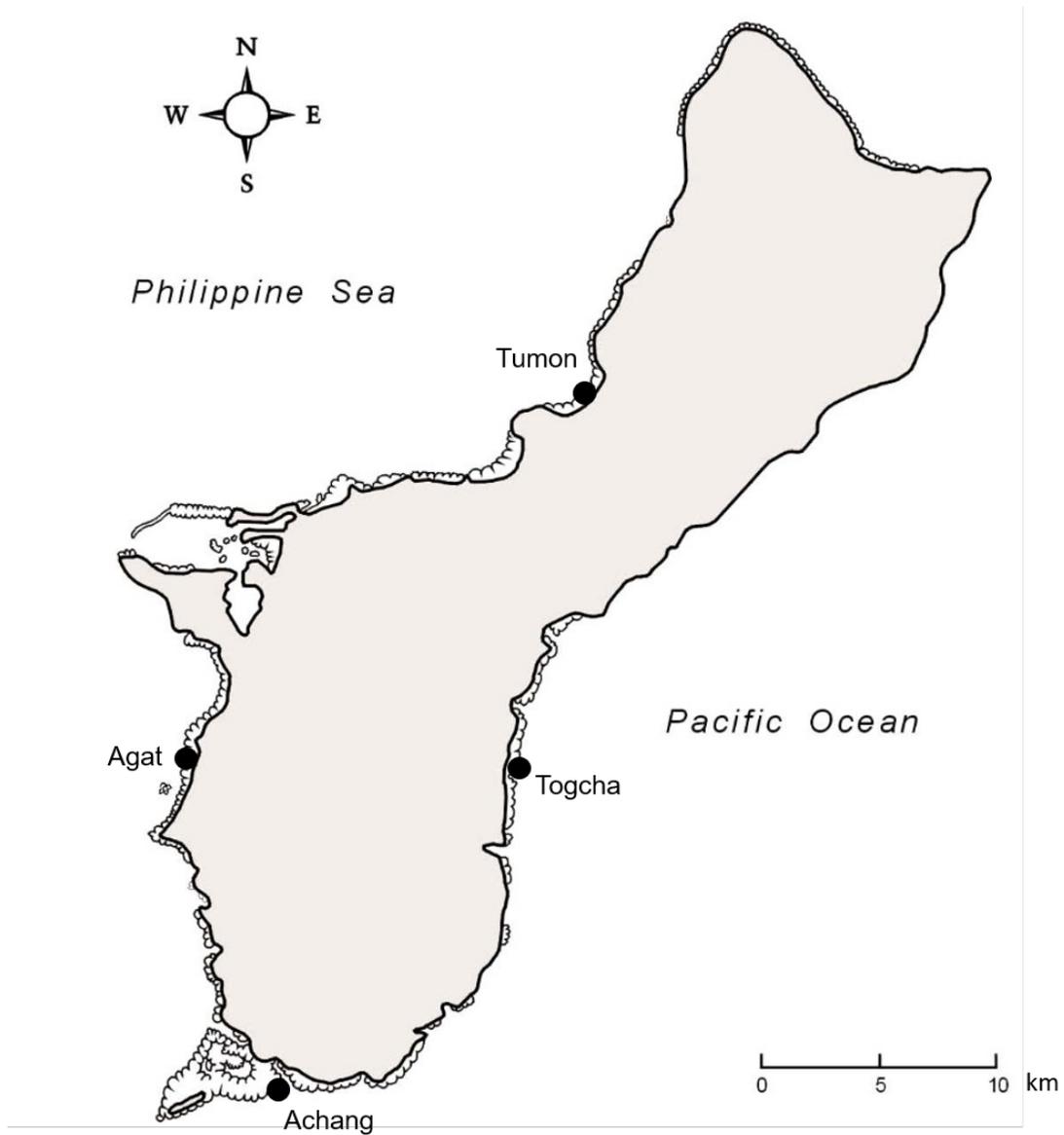


Figure 2: Map of Guam indicating the location of survey sites (black dots).

Monthly examination of Acropora reproductive timing

In February 2015, five colonies per species were tagged, photographed, and mapped at each site to monitor reproduction every month. Beginning February 2015, monthly sampling ('monthly samples') occurred for 14 months, 1-7 days prior to the full moon. Additionally, in 2016, samples were collected weekly ('weekly samples'), for 3-4 weeks, before and after the full moon during the previously observed month(s) of spawning. To sample, wire cutters were used to remove three 3-5 cm long branches/colony, below the growing tips, which are zones of reduced fecundity (Kojis 1986a, b; Oliver 1984; Wallace 1985). These branches (i.e. fragments) were collected in WhirlPak bags with fresh seawater, placed on ice, and transported to the UOG Marine Lab for dissection.

No samples were collected in January of 2015 and 2016 due to high surf advisories, which restricts field activities. In addition, for *Acropora pulchra*, Togcha was only sampled in April and August of 2015 because of dangerous field conditions and high bleaching-related mortality (>90%). Also, Achang was only sampled in 2016 because the population was only recently discovered. Repeated sampling of the same colonies (>10% of colony volume) can have impacts on colony growth and reproduction (Rinkevich 2000). Considering this, the original tagged colonies were only sampled for six months and new colonies from the same population were tagged, photographed, mapped, and sampled thereafter.

Coral Tissue Dissection

Branches for dissection were fixed in 100 ml of 10% formalin in seawater for 24-48 hours, rinsed with tap water, and decalcified using 250 ml of 10% hydrochloric acid until skeleton was dissolved. Depending on the density of the skeleton, ~1-2 acid washes (~24 hours/wash) were required. After decalcifying, branches were soaked in tap water for ~24 hours and stored in 70% ethanol until dissection. For 'monthly samples,' five polyps per branch were haphazardly selected and dissected using a dissecting microscope (Wild Makroskop M420 1,25x Type 400076 & Olympus SZX2-ILLT t5 SN) with a camera attachment (Canon EOS 60D with microscope adapter). The number and diameter of oocytes were recorded in each polyp (Figure 3_{a,b}). All measurements were taken using a calibrated micrometer eyepiece. Spermaries are only large enough (i.e. easily visible) to dissect 1-2 months before spawning. Thus, both short and long pairs of spermaries were dissected from 'weekly samples' (before predicted spawning in 2016), recording the maximal spermary diameter and medial spermary diameter to calculate the maximum geometric mean diameter (GMD) (i.e. square root of the maximal diameter multiplied by the medial diameter) (Figure 3_{a,b}). Additionally, from 'weekly samples,' the presence or absence of oocytes were recorded.

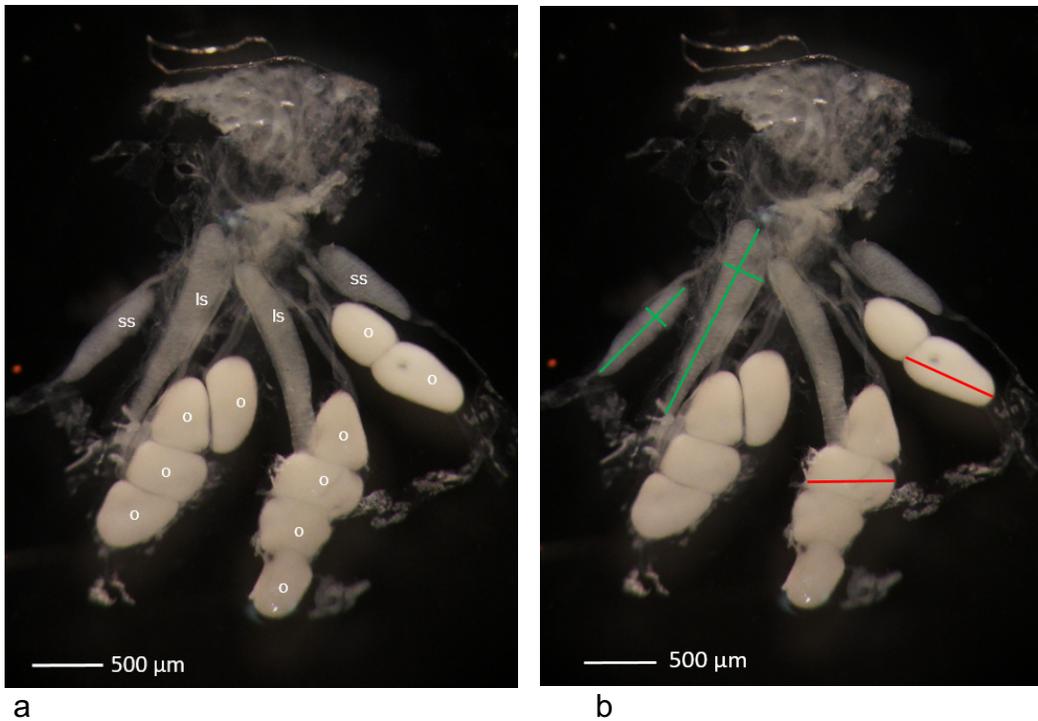


Figure 3: (a) Labeled dissected coral polyp indicating long and short spermaries as “ls” and “ss,” respectively, and oocytes as “o.” (b) Green lines represent the lengths and widths of the long and short spermaries to calculate GMD and red lines represent the oocyte measurements.

Data Analysis

Gametogenesis

The length of gametogenesis and the number of gametogenic cycles per year were determined using ‘monthly samples’ from 2015 and 2016, aggregated by species. The length of gametogenesis was calculated as the difference, in number of months, between the first appearance of oocytes and the disappearance (i.e. due to spawning) of mature eggs from dissected branches. Additionally, gametogenesis was similarly characterized by the five developmental stages described by Fan and Dai (1998): I) few oogonia in mesoglea with prominent nucleolus and little cytoplasm, II) developing oocytes undergoing vitellogenesis, III) developing oocytes and early spermaries that were

distinct sacs with few spermatogonia, IV) developing oocytes and elongated, oval spermaries that increased in diameter and in the number of spermatocytes, and V) mature oocytes and a spermaries with bouquets of spermatozoa.

To determine if seasonal cue(s) are correlated with gametogenesis, Pearson product-moment correlations and linear regressions were calculated (separately for each species) to measure the strength and direction (i.e. positive or negative) of association between mean monthly oocyte diameters and, separately, mean monthly insolation, sea surface temperatures (SST), and rainfall. Oocyte diameters were determined using ‘monthly samples’ from 2015 and 2016, aggregated by species. However, oocyte diameters from *Acropora pulchra* from Tumon were consistently smaller than those from all other populations, but the reasons for this were unclear, therefore, I omitted these from all regressions. Additionally, this study did not take into account the the unexplained variation in oocyte size due to differences among population, colonies, or fragments. Insolation (kWh/m²), on a clear day, was calculated from a 22-year period obtained online from the Surface Meteorology and Solar Energy (SSE) project, sponsored by the National Aeronautics and Space Administration (NASA). SSTs (°C) were calculated from daily averages from NOAA’s satellite-derived SST dataset (2014-16); missing data were filled in the zonal direction using the National Center for Atmospheric Research (NCAR) Command Language (NCL) Poisson grid fill. Rainfall (mm) was calculated from the NOAA National Weather Service 50-year Rainfall Database (Lander and Guard 2006) from 1958-2000 and the NOAA Online Weather Data (NOWdata) precipitation records from 2000-2017.

Spawning Synchrony

Spawning synchrony was determined on broad to fine scales: the time of year (i.e. month), the night of spawning, and the time (i.e. hour) of spawning. Spawning synchrony occurred when the majority of gametes were released at the same time (e.g. month, night, and time), and any deviation was defined as asynchronous. The month of spawning was determined using ‘monthly samples’ from April and May of 2015 and ‘weekly samples’ from April and May of 2016. The disappearance, from dissected samples, of mature eggs (‘weekly’ and ‘monthly’ samples) and spermaries (‘weekly’ samples only) indicated spawning. The night of spawning was determined using the sampling windows (i.e. number of days in between sampling) from ‘weekly samples’ from April and May of 2016 and *in situ* observations for *Acropora pulchra* and *A. muricata* populations in Agat and Tumon populations in 2017. The night of the full moon was defined as “day 0” and subsequent days after were defined in sequence (e.g. day 7=one week after the full moon). The time of spawning was determined using *in situ* observations for *Acropora pulchra* and *A. muricata* in Agat and Tumon in 2017, recording the time range when bundles were observed floating upwards in the water column.

Reproductive Output

Reproductive output was accessed using the following measures: gamete size, number of eggs per polyp (i.e. fecundity index), proportion of reproductive branches, and mean annual fecundity. More specifically, differences within these measures of reproductive effort were examined between populations of the same species (i.e. *Acropora pulchra* and *A. muricata*) and between 2015 and 2016. All

measures of reproductive effort were determined using dissected samples within two months of spawning (i.e. late gametogenesis) because both gametes are easily detected (i.e. measurable), it is unlikely that oocytes become adsorbed, and to discern that gametogenesis has synchronized. Additionally, this study did not take into account the the unexplained variation due to colonies or fragments.

First, analyses of covariance (ANCOVA) were performed using linear regression models to measure the strength and direction (i.e. positive or negative) of association between gamete sizes (i.e. stage IV and V oocyte diameters, GMD of long spermaries, GMD of short spermaries, and GMD of both spermaries) and between gamete sizes and the number of eggs per polyp. Only mature eggs (i.e. stage IV and V) and spermaries sizes were used since spermaries grow significantly one month before spawning (Harrison and Wallace 1990; Fan and Dai 1998). For *Acropora aspera*, ‘monthly samples’ and ‘weekly samples’ from October of 2016 were used. For all species except for *Acropora aspera*, ‘monthly samples’ and ‘weekly samples’ from April of 2016 were used, aggregated by population. However, for *Acropora pulchra* and *A. cf. intermedia* in Tumon, spermaries were only found in March. All data were randomized to generate the largest equal sample size for each test, separately (Minitab 2010).

Comparisons of the central tendencies (i.e. means and/or median values) were used to determine differences between populations of the same species (i.e. *Acropora pulchra* and *A. muricata*) for gamete sizes (i.e. stage IV and V oocyte diameters, GMD of long spermaries, and GMD of short spermaries), the number of eggs/polyp, and the proportion of reproductive branches. In addition, comparisons of the central tendencies (i.e. means and/or median values) were

used to determine differences between years, 2015 and 2016, for egg sizes (i.e. stage IV and V oocyte diameters), the number of eggs/polyp, and the proportion of reproductive branches. For *Acropora aspera*, ‘monthly samples’ from August and September of 2015 and ‘weekly samples’ from September and October of 2016, aggregated by year, were used. For all species except *Acropora aspera*, ‘monthly samples’ from April and May of 2015 and ‘weekly samples’ from March and April of 2016 were used, aggregated by population and year. Comparisons between populations of spermary sizes were made between sampling dates that were no more than 5 days apart to account for rapid growth immediately prior to spawning. All data distributions were checked for normality using Shapiro-Wilks tests. Data sets for oocyte size and the number of eggs/polyp were often large (i.e. $n > 100$) and, therefore, failed normality tests (i.e. Shapiro-Wilks test) because even the smallest deviations from perfect normality can lead to a significant result (even though every dataset has some degree of randomness). For all skewed and non-normal data, Box Cox transformations (Box and Cox 1964) were applied to transform skewed data into a normal shape (Minitab 2010). For data that passed a Shapiro-Wilks normality test, comparisons were made using one-way ANOVAs (SigmaPlot 11). If data could not be adequately transformed to a normal shape and/or pass a Shapiro-Wilks normality test, nonparametric tests were used: Mann–Whitney Rank Sum tests, Kruskal-Wallis, and Kruskal-Wallis test on Ranks (SigmaPlot 11). For data with comparisons between more than two groups, post hoc pairwise comparisons (i.e. Dunn’s comparisons methods) were used to compare median values between each group. Then, complementary analyses using effect size tests were used to quantify (i.e.

assess) the size of the difference between groups (i.e. two) and test whether there is a true significance difference of the means and/or median values. Effect size was determined using Cohen's d effect size formula for data meeting parametric statistical requirements and Cliff's Delta effect size for nonparametric data using R (2013) package `effsize` (0.7.1). For Cohen's d , the magnitude of the effect size was assessed using the following thresholds: $|d| < 0.2$ =negligible, $|d| < 0.5$ =small, $|d| < 0.8$ =medium, and $|d| > 0.8$ =large. For Cliff's Delta estimate, the magnitude of the effect size was assessed using the following thresholds: $|d| < 0.147$ =negligible, $|d| < 0.33$ =small, $|d| < 0.474$ =medium, and $|d| > 0.474$ =large. Furthermore, for Cliff's Delta and Cohen's d , the sign (i.e. \pm) was determined by which value in the formula was first. Therefore, when for effect size, the sign does not indicate magnitude or direction.

Occasionally, only a few polyps per branch contained eggs. Thus, the proportion of reproductive branches was determined from dissected branches with at least five gravid polyps. For nonreproductive branches, '0' was recorded and for reproductive branches '1' was recorded. For *Acropora aspera*, 'monthly samples' from August and September of 2015 and 'monthly' and 'weekly samples' from September and October of 2016 were used. For all species except *Acropora aspera*, 'monthly samples' from April and May of 2015 and 'monthly' and 'weekly samples' from March and April of 2016 were used, aggregated by population. Chi-square tests and, for small counts (i.e. < 5), Fisher's exact tests were used to compare the equality of proportions of reproductive branches between populations of the same species and between 2015 and 2016 (SigmaPlot 11).

Mean annual fecundity is an estimation of reproductive effort used to make comparisons between species. Since polyp size and density varies among taxa, mean annual fecundity is calculated by dividing the average number of eggs/polyp by the average number of polyps per cm², multiplied by the number of gametogenic cycles/year (Harrison and Wallace 1990). The average number of polyps per cm² was determined from ten branches for each species. In addition, the average number of eggs/polyp was determined for each population. For *Acropora aspera*, ‘monthly samples’ from August and September of 2015 and ‘monthly samples’ from September and October of 2016 were used. For all species except *Acropora aspera*, ‘monthly samples’ from April and May of 2015 and ‘monthly samples’ from March and April of 2016 were used.

RESULTS

Acropora aspera

Changes in mean oocyte diameters over each month indicated a clear annual gametogenic cycle that was ~9-10 months for *Acropora aspera* (Figure 4a). No gametes were found in November and December; oogonia first appeared in February. The onset of gametogenesis was staggered among colonies and branches, exhibiting ~1-month delay. Oocyte diameters gradually increased linearly throughout oogenesis (Figure 4a). Occasionally, immature oocytes were observed with mature oocytes within the same reproductive mesentery; thus, large ranges were observed for mature oocyte diameters (Table S4), suggesting that oogenesis is highly variable. Spermatogenesis generally started approximately 3 months later than oogenesis, but this was difficult to observe

without using histological methods. In 2016, spermaries were observed in September and October (Figure 4a).

Oocyte diameter was significantly and positively correlated with rainfall (Pearson product-moment correlation; $R^2=0.91$, $p<0.001$), which explained 91% of the variation, and with SST (Pearson product-moment correlation; $R^2=0.822$, $p=0.007$), which explained 82.2% of the variation, whereas insolation showed no correlations (Pearson product-moment correlation; $R^2=-0.165$, $p=0.672$; Figure 4a,b). Furthermore, there was a very strong linear relationship between mean monthly oocyte diameter and rainfall ($R^2=0.824$, $p<0.001$) (Figure 5a) and a strong linear relationship between mean monthly oocyte diameter and SST ($R^2=0.675$, $p=0.007$; Figure 5b), whereas insolation showed no linear relationship with mean monthly oocyte diameter ($R^2=0.027$, $p=0.672$; null results not shown).

Acropora aspera spawns asynchronously with the other species in this study, spawning in September and October (Figure 4a), whereas the other species spawned in April and May. Furthermore, in 2016, approximately half of the gametes spawned after the September full moon and the other half of gametes spawned after the November full moon (i.e. split spawning) (Figure 6). There were no in field observations of spermaries, pigmented eggs, or spawning for *Acropora aspera*.

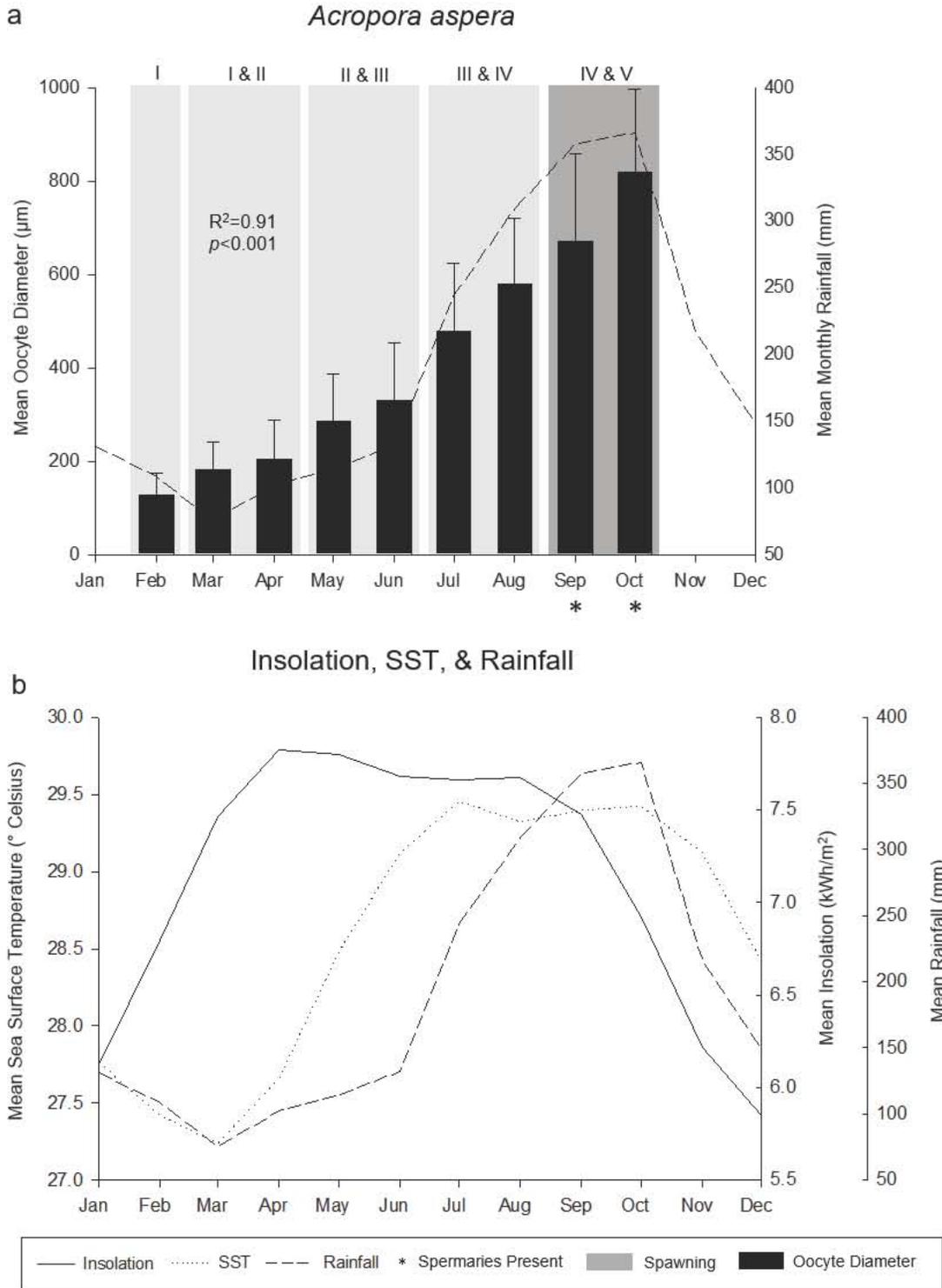


Figure 4: **(a)** Mean monthly oocyte diameter for *Acropora aspera*. Error bars indicate the standard deviation. R^2 =Pearson moment correlation, with R^2 and p -values, between mean monthly oocyte diameter and mean monthly rainfall (see Methods -> Data Analysis for details). **(b)** Temporal changes in insolation, SST, and rainfall (monthly means) in Guam.

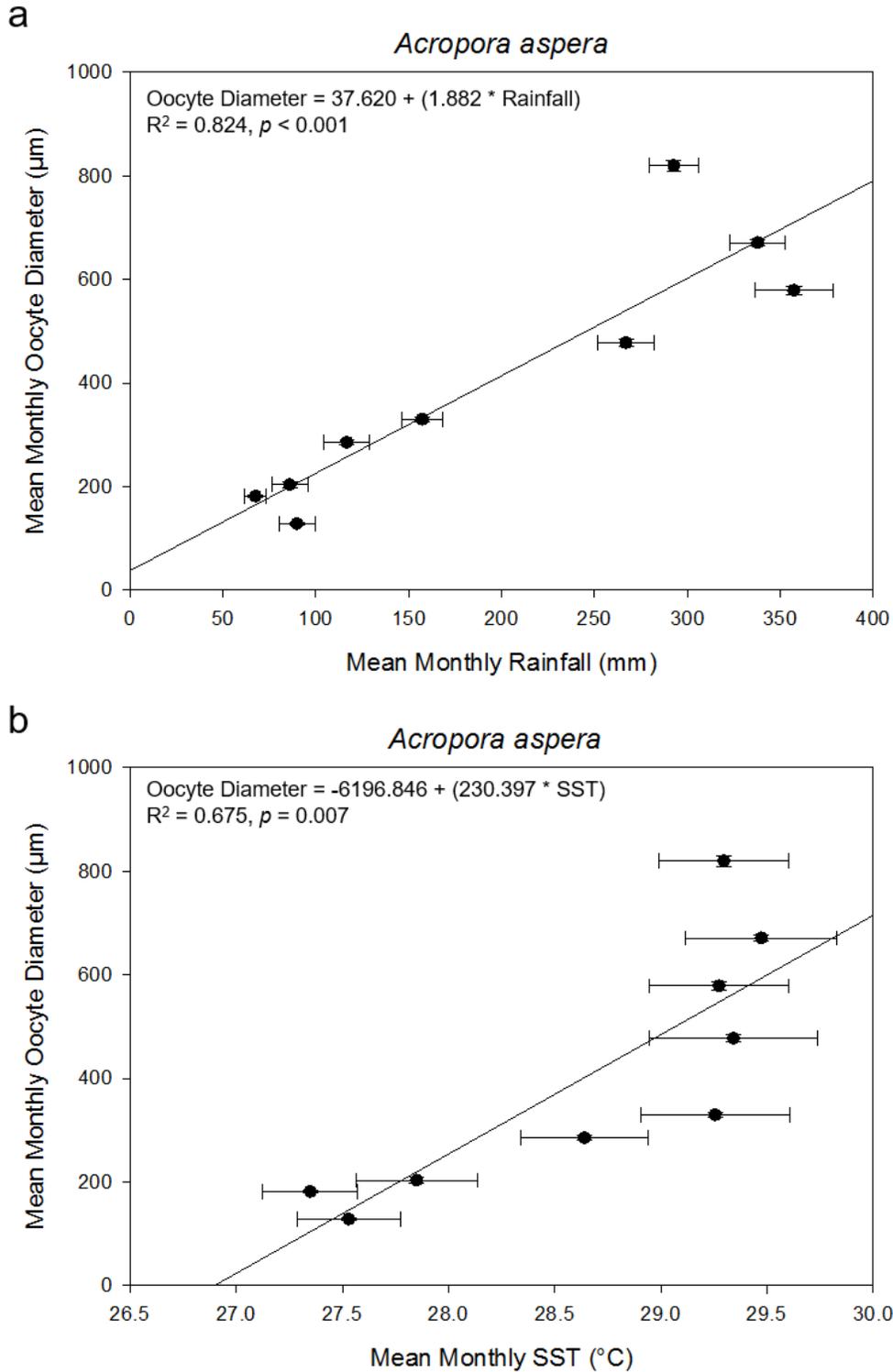


Figure 5: Linear regressions, with R^2 and p -values and regression line equations, between (a) mean monthly oocyte diameter and mean monthly rainfall and (b) mean monthly oocyte diameter and mean monthly SST, for *Acropora aspera* (see Methods -> Data Analysis for details). Bi-directional error bars indicate the standard error.

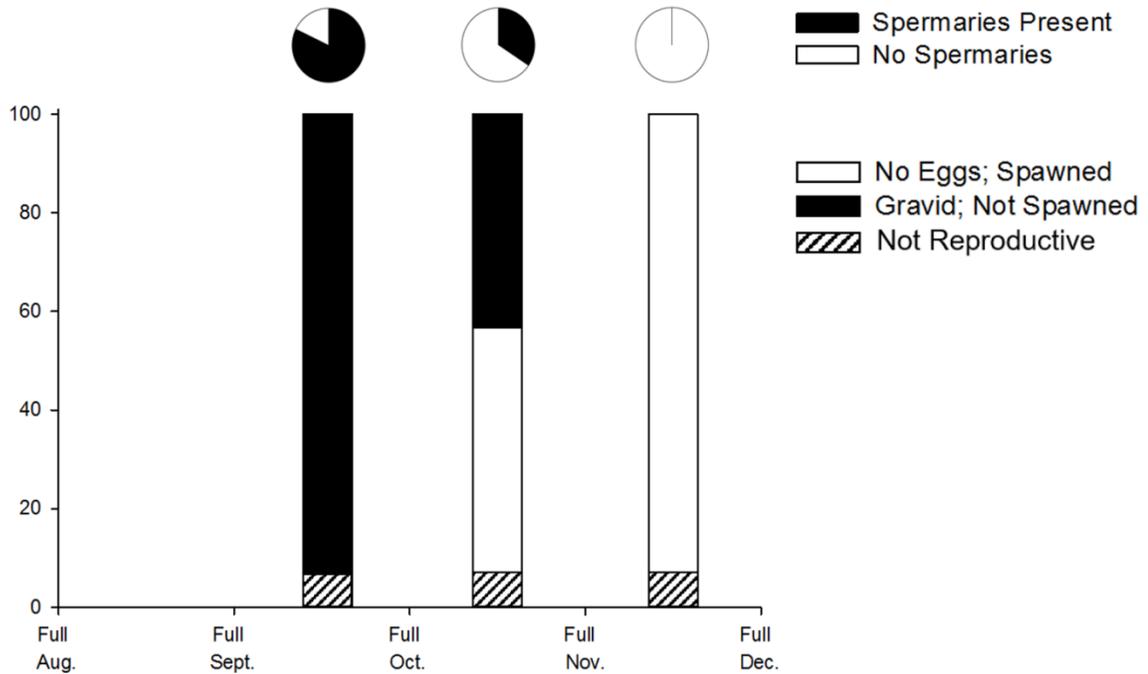


Figure 6: Proportions of gamete presence in *Acropora aspera* during spawning months in 2016. Bars and pie graphs indicate the presence or absence of eggs and spermaries, respectively. “Full” represents the full moon for corresponding month. Proportion of reproductive branches was calculated in late gametogenesis (≤ 2 months before spawning) and when at least five polyps contained eggs.

There was no linear relationship between the sizes of spermaries (i.e. long, short, and total spermaries) and eggs, and no linear relationship between the fecundity index and size of gametes (eggs and long, short, and total spermaries) (ANCOVA; Figure S2_{a-g}). Mean egg diameters for 2015 and 2016 were $627.8 \mu\text{m} \pm 146.8 \mu\text{m}$ and $728.8 \mu\text{m} \pm 207.8 \mu\text{m}$, respectively (Table S3). Comparisons of median values revealed that there were significant differences between egg diameters in 2015 and 2016 ($p < 0.005$, Mann-Whitney Rank Sum), but a negligible effect size was observed (Figure 7; Table S1_c). Thus, the significant difference was attributed to a large sample size with inherent variability, and not

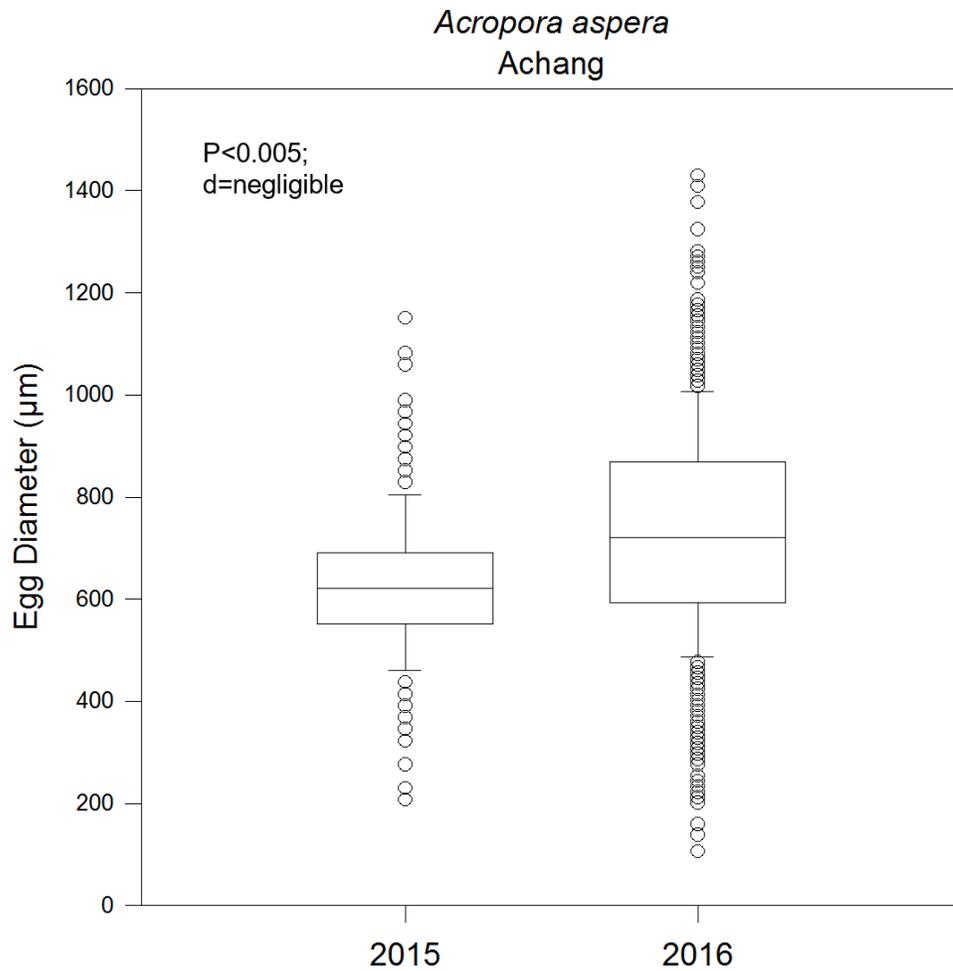


Figure 7: Maximum egg diameters for years 2015 and 2016 for *Acropora aspera*. Boxes represent the 25th and 75th quartile range, horizontal cross lines represent the median values, whiskers represent the range, and points represent 5th and 95th percentile outlier values. p -value and the effect size magnitude (d) indicate comparisons of median values between 2015 and 2016. See Methods -> Data Analysis for details.

a true significant difference in the median values. The maximum mean lengths of long and short spermaries were $1390.0 \mu\text{m} \pm 335.6 \mu\text{m}$ and $747.7 \mu\text{m} \pm 180.1 \mu\text{m}$, respectively (Table S4). Furthermore, the GMD of long and short spermaries were tracked within one month of spawning and, in that month, no geometric growth was detected. Therefore, for *Acropora aspera*, the rate of spermary growth appears to slow in the last month of spermatogenesis (Figure 8). The number of eggs/polyp differed significantly between 2015 and 2016 ($p < 0.001$, Mann-Whitney Rank Sum), resulting in a large effect size (i.e. a large difference between the median values) (Table S1_f). In 2016, *Acropora aspera* had ~4 more eggs per polyp than in 2015 (Figure 9). The proportion of reproductive branches did not differ between 2015 (80%) and 2016 (93%) ($p = 0.258$, Fisher's) (Table S2_d, Figure S2). Lastly, the mean annual fecundity doubled from 2015 (105 eggs/cm^2) to 2016 (211 eggs/cm^2) (Figure S2).

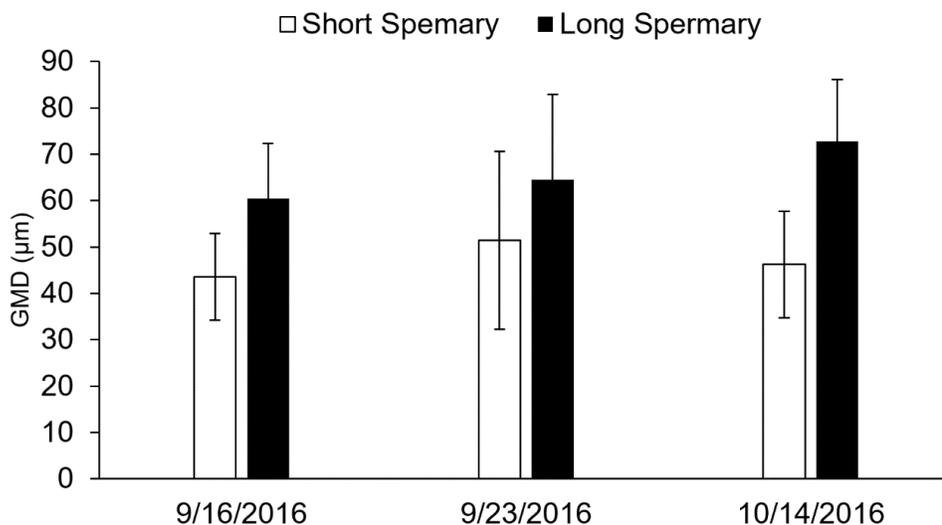


Figure 8: GMD (μm) of long and short spermaries for *Acropora aspera*. X-axis represents the sampling date and error bars indicate the standard deviation. The full moon occurred on 9/17/2016 and 10/16/2016. See Methods -> Data Analysis for details.

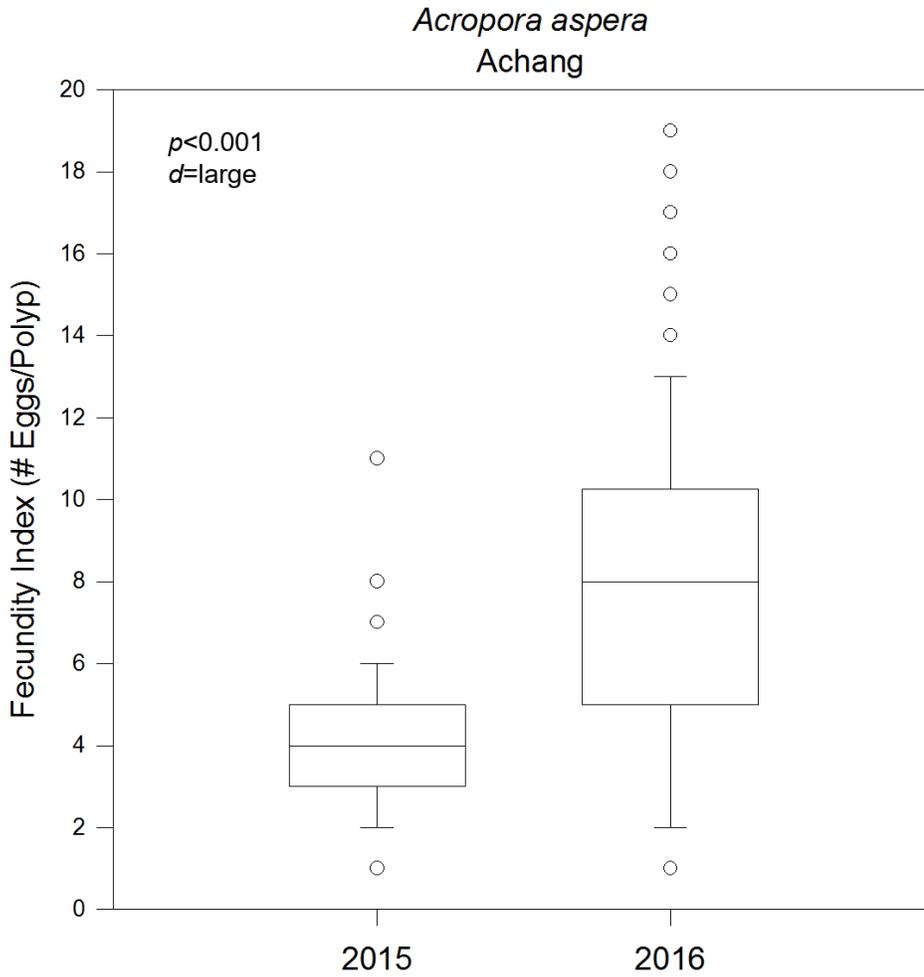


Figure 9: Comparison of the fecundity index (i.e. number of eggs per polyp) between years for *Acropora aspera*. Boxes represent the 25th and 75th quartile range, horizontal cross lines represent the median values, whiskers represent the range, and points represent 5th and 95th percentile outlier values. p -value and the effect size magnitude (d) indicate comparisons of fecundity index median values between 2015 and 2016. See Methods -> Data Analysis for details.

Acropora pulchra

Changes of mean oocyte diameters over each month indicated a clear annual gametogenic cycle that was ~9-10 months for *Acropora pulchra* (Figure 10a). No gametes were found in June and oogonia first appeared in July and August. The onset of gametogenesis was staggered among colonies and branches, exhibiting ~1-month delay. Oocyte diameters gradually increased in early gametogenesis (stage I and II), then increased exponentially in stage IV and V (Figure 10a). Occasionally, immature oocytes were observed with mature oocytes within the same reproductive mesentery; thus, large ranges were observed for mature oocyte diameters (Table S4), suggesting that oogenesis is highly variable. Spermatogenesis generally started approximately 3 months later than oogenesis, but this was difficult to observe without using histological methods. In 2016, spermaries were observed in March, April, and May (Figure 10a).

Oocyte diameter was significantly and negatively correlated with rainfall (Pearson product-moment correlation; $R^2=-0.673$, $p=0.033$), but there was no significant relationship with insolation (Pearson product-moment correlation; $R^2=0.410$, $p=0.239$) or SST (Pearson product-moment correlation; $R^2=-0.507$, $p=0.134$) (Figure 10_{a,b}; null results not shown). Furthermore, there was a weak negative linear relationship between oocyte diameter and rainfall ($R^2=0.453$, $p<0.001$), whereas insolation and SST showed no linear relationship with oocyte diameter ($R^2=0.168$, $p=0.435$ for insolation; $R^2=0.257$, $p=0.109$ for SST) (Figure 11; null results not shown).

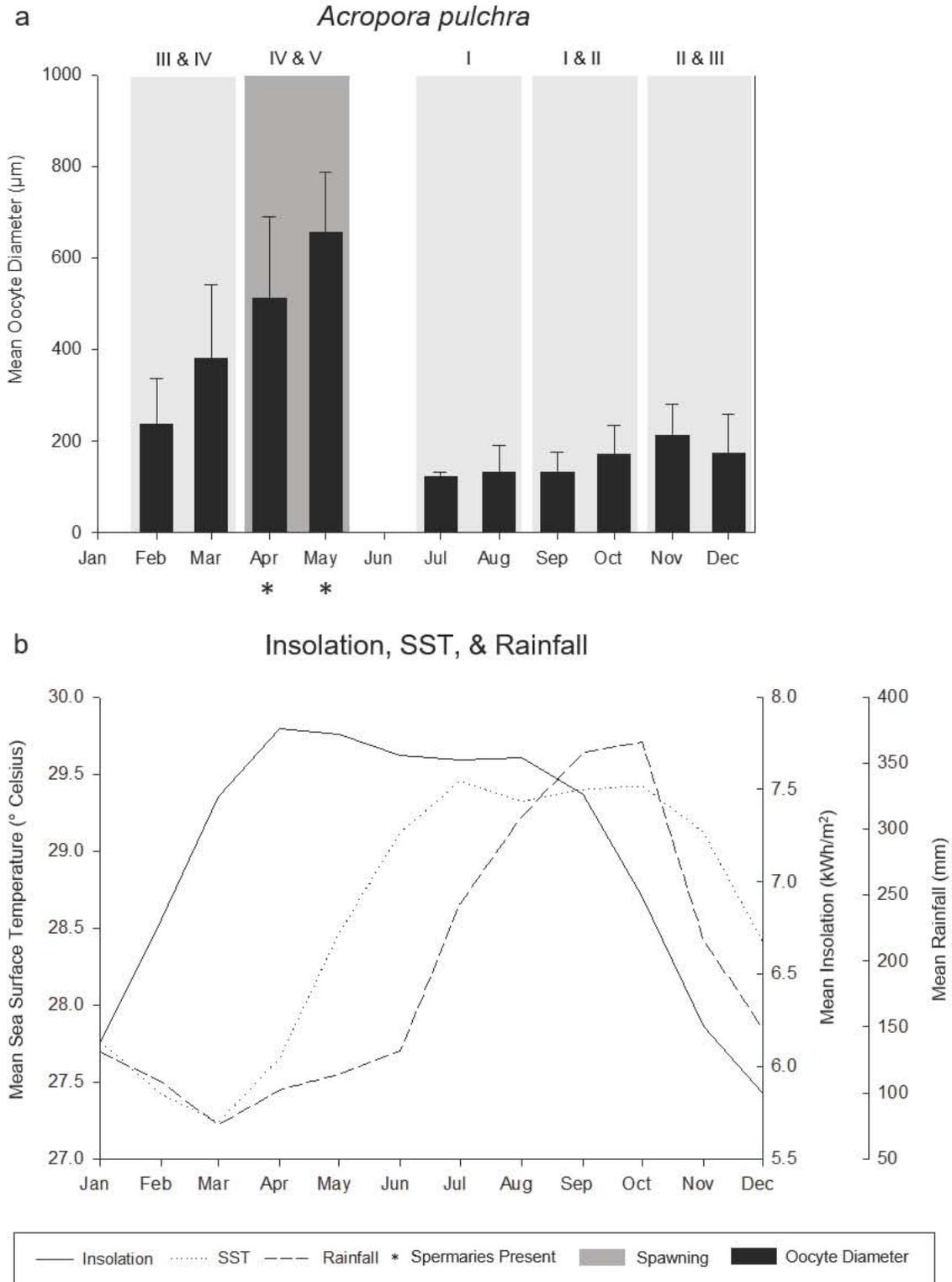


Figure 10: **(a)** Mean monthly oocyte diameter for *Acropora pulchra*. Error bars indicate the standard deviation. See Methods -> Data Analysis for details. **(b)** Temporal changes in insolation, SST, and rainfall (monthly means) in Guam.

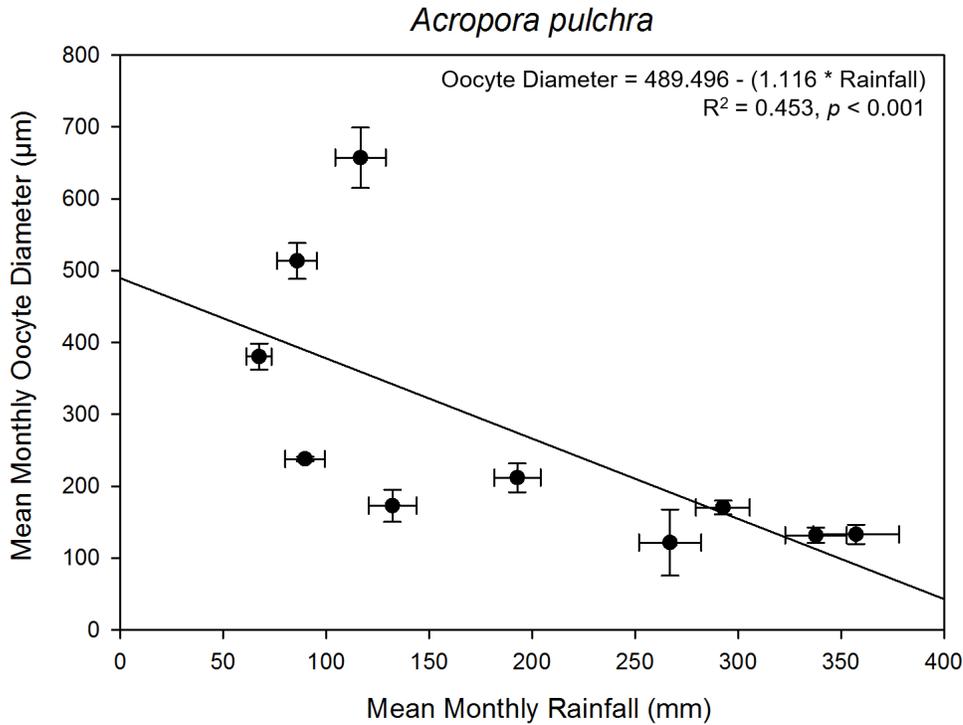


Figure 11: Linear regression, with R^2 and p -values and regression line equation, between oocyte diameter and rainfall for *Acropora pulchra* (see Methods -> Data Analysis for details). Bi-directional error bars indicate the standard error.

In 2015, all *Acropora pulchra* populations spawned in May and, in 2016, *Acropora pulchra* spawned after the full moon in April and May, differing between populations. For example, in Tumon, *Acropora pulchra* spawned approximately half of its gametes in April and the other half in May (split spawning). In Agat, it spawned in April, whereas in Achang *A. pulchra* spawned in May (Figure 12). Except for *Acropora aspera*, all other species spawned in April or May; thus, spawning of *Acropora pulchra* was synchronous (with respect to the month) with the other species in this study. Sampling data indicated that, in 2016, spawning occurred within a 9-day window, 5 days before and 4 days after the full moon. A consensus among the literature suggests that *Acropora* spawn synchronously after the full moon, so it could be assumed that spawning

on Guam occurred within the first 4 days after the full moon. Thus, spawning in 2016 was relatively synchronized (with respect to the number of days after the full moon) and concise (i.e. ≤ 4 days).

Oocyte coloration changed from ivory to a light pink or peach color ≤ 14 days prior to spawning (Figure S3; Table S3). In 2017, *in situ* spawning was observed at Agat and Tumon between 2015-2100 h, but no egg-sperm bundle setting was observed in the polyp gastrovascular cavity. In Agat, the largest release of bundles occurred in April on day 2 during high tide, but multiple small-scale spawnings were observed on days 3, 4, and 7 (Table S3). In Tumon, small-scale spawning was observed on day 4 in April and day 0 (i.e. full moon) in May. Branches were haphazardly cracked in May to check for pigmented eggs in Tumon on day 11 (n=100), day 20 (n=50), and day 26 (n=25), and approximately 75%, 40%, and 12% of branches contained pigmented eggs, respectively. Thus, in 2017, gametes were intermittently and inconsistently released throughout the month of May, before the full moon. Therefore, in 2017, spawning was asynchronous (with respect to the number of days after the full moon), but the time (i.e. hour) of spawning was synchronized. An anecdotal report stated that *Acropora pulchra* spawned in Saipan (a northern, neighboring island in the Marianas) on day 6 (i.e. 6 days after the full moon) in May of 2017 and setting was observed (L. Johnson, pers. comm.)

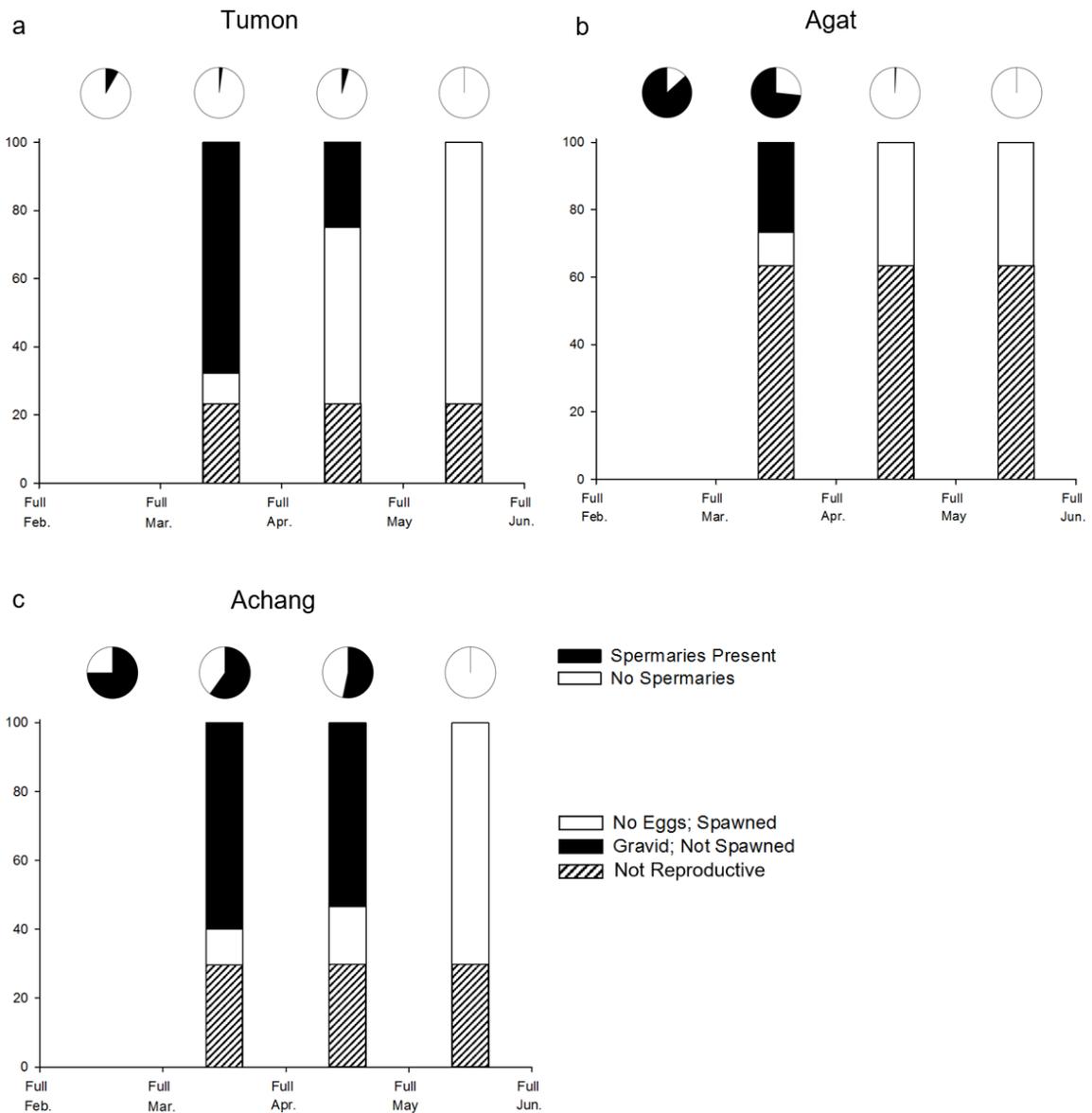


Figure 12: Proportions of gamete presence in *Acropora pulchra* during spawning months in 2016. Bars and pie graphs indicate the presence or absence of eggs and spermaries, respectively. “Full” represents the full moon for corresponding month. Proportion of reproductive branches was calculated in late gametogenesis (≤ 2 months before spawning) and when at least five polyps contained eggs.

No linear relationship existed between the sizes of spermaries (i.e. long, short, and total spermaries) and eggs, and no linear relationship between the fecundity index and size of gametes (eggs and long, short, and total spermaries) (ANCOVA; Figure S2_{a-g}). A comparison of the median values revealed that egg diameters significantly differed between years at Tumon ($p < 0.001$, Mann-Whitney Rank Sum) and Agat ($p < 0.001$, Mann-Whitney Rank Sum). In 2016, egg diameters for Tumon and Agat were ~ 125 - $225 \mu\text{m}$ smaller than in 2015, resulting in large effect sizes (Table S1_a, Figure 13_{a,b}). Further, in 2015, pairwise comparisons among populations revealed significant differences in egg diameters between Agat ($695.1 \mu\text{m} \pm 132.9 \mu\text{m}$), Tumon ($297.8 \mu\text{m} \pm 146.5 \mu\text{m}$), and Togcha ($575.9 \mu\text{m} \pm 78.9 \mu\text{m}$) for *Acropora pulchra* ($p < 0.05$, Dunn's method; Table S4). A medium effect size was observed between Agat and Togcha, and large effect sizes were observed between Agat and Tumon and Tumon and Togcha, indicating that egg diameters differed substantially between populations (Table S1_a, Figure 14_a). In 2016, pairwise comparisons among populations revealed significant differences in egg diameters between Agat, Tumon, and Achang ($p < 0.05$, Dunn's method). However, the differences observed between Agat and Achang were negligible (Table S1_a, Figure 14_a), meaning that the significant difference between Agat and Achang was attributed to a large sample size and not a true significant difference of the median values. However, Tumon's egg diameters were ~ 150 - $250 \mu\text{m}$ smaller than Agat and Achang, resulting in large effect sizes (Table S1_b, Figure 14_b).

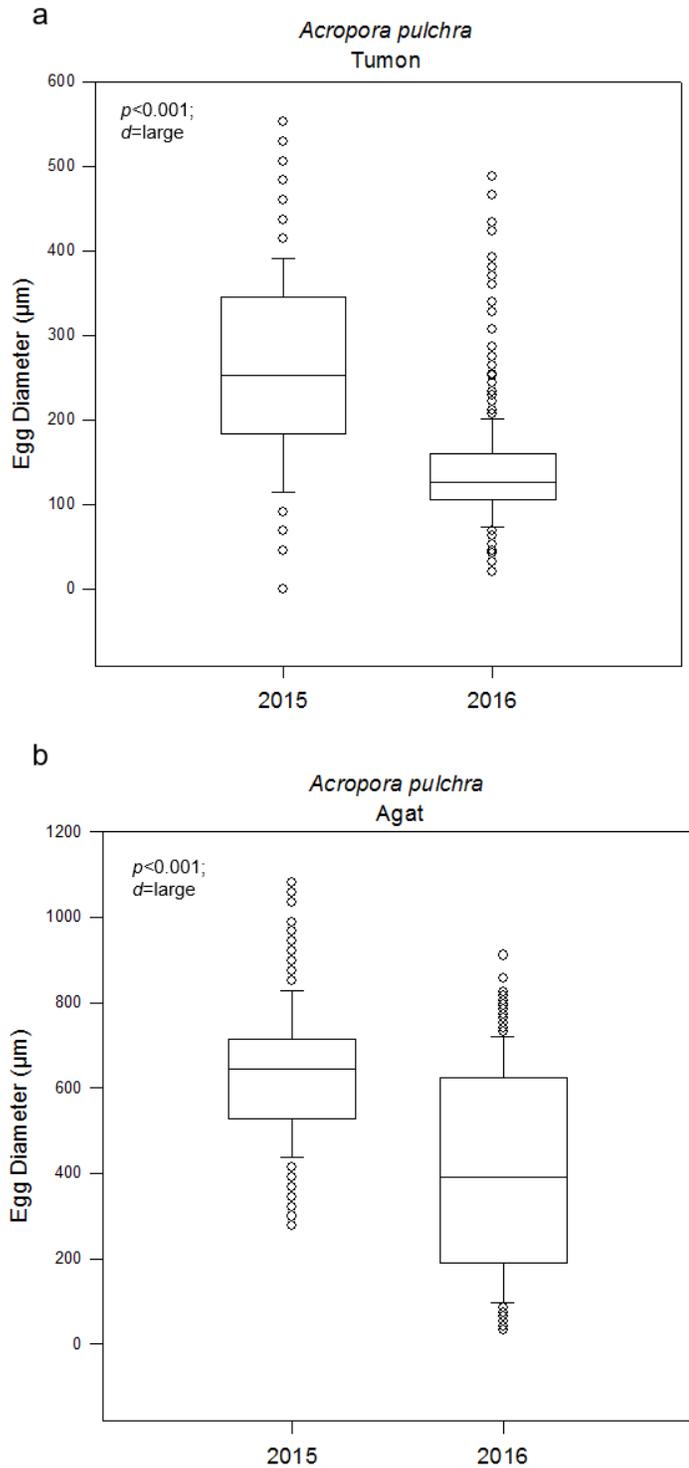


Figure 13: Comparison of maximum egg diameters between years for *Acropora pulchra* populations in (a) Tumon (b) Agat. Boxes represent the 25th and 75th quartile range, horizontal cross lines represent the median values, whiskers represent the range, and points represent 5th and 95th percentile outlier values. p -values and the effect size magnitude (d) indicate comparisons of median values between 2015 and 2016. See Methods -> Data Analysis for details.

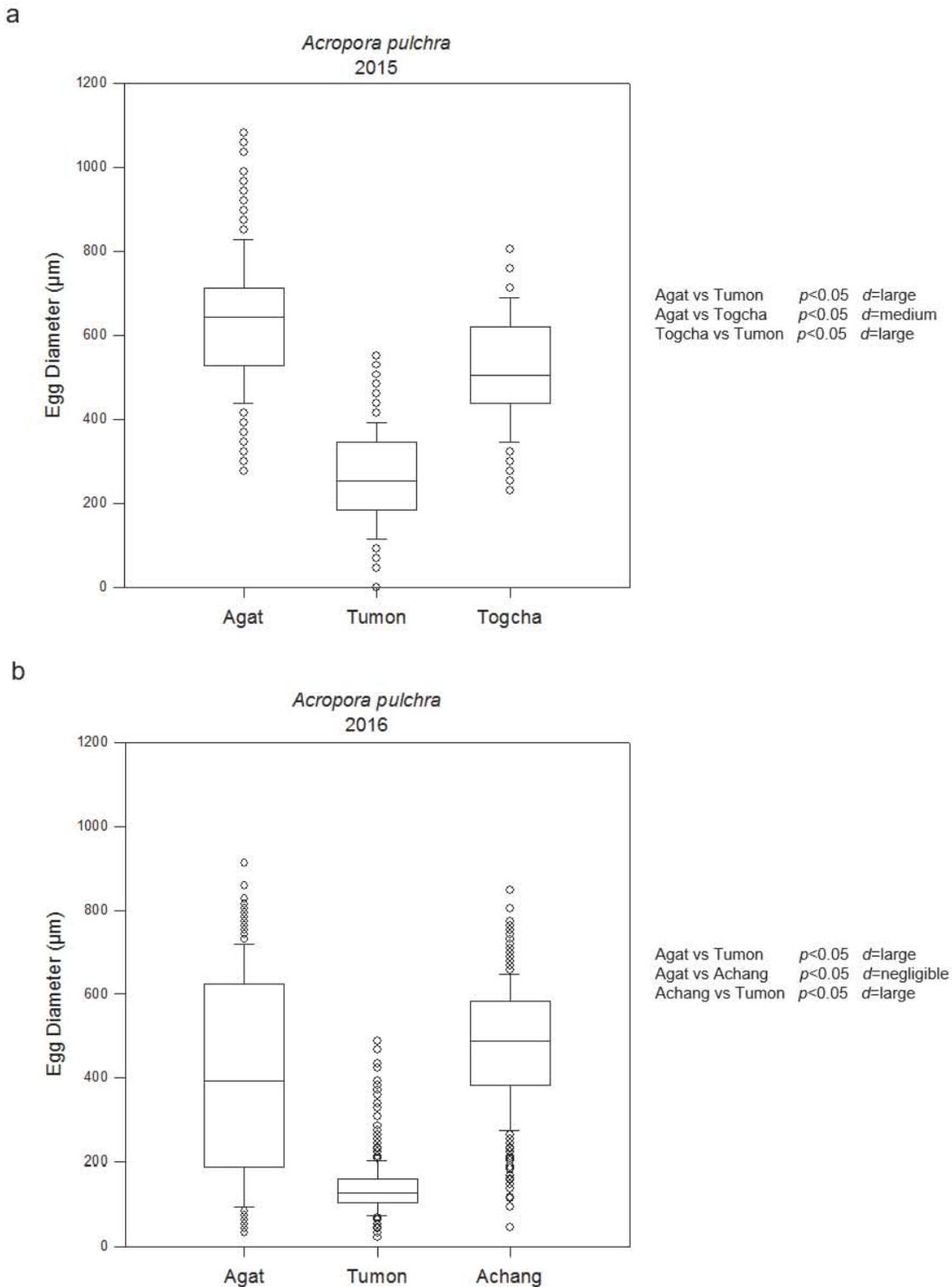


Figure 14: Comparison of the maximum egg diameters between *Acropora pulchra* populations in (a) 2015 and (b) 2016. Boxes represent the 25th and 75th quartile range, horizontal cross lines represent the median values, whiskers represent the range, and points represent 5th and 95th percentile outlier values. p -values and the effect size magnitude (d) indicate comparisons of median values between populations. See Methods -> Data Analysis for details.

The size of long spermaries did not significantly vary between Achang (n=126), Agat (n=25), and Tumon (n=13) ($p=0.415$, Kruskal-Wallis). In contrast, short spermaries in Achang were significantly larger than those in Tumon ($p<0.001$, one-way ANOVA) and Agat ($p<0.001$, one-way ANOVA), differing by $\sim 12 \mu\text{m}$ (i.e. GMD) which represents large effect sizes (Table S1_d, Figure 15).

For *Acropora pulchra*, the number of eggs/polyp differed significantly between 2015 and 2016 in Tumon ($p=0.006$, Mann-Whitney Rank Sum) and Agat ($p=0.003$, Mann-Whitney Rank Sum). In 2016, *Acropora pulchra* populations had ~ 1 more egg per polyp than in 2015, resulting in small effect sizes (Table S1_f, Figure 16). Multiple comparisons of the number of eggs/polyp between populations in 2015 revealed significant differences between Tumon-Agat and Togcha-Agat ($p<0.05$, Dunn's method) (Figure 17_a). Agat had ~ 1 less egg per polyp than Togcha and Tumon, which represents medium effect sizes between Agat and Tumon (Table S1_f, Figure 17_a). In 2016, multiple comparisons of the fecundity index between populations in 2016 revealed significant differences between Tumon and Achang and Tumon and Agat ($p<0.05$, Dunn's method) (Figure 17_a). Tumon had ~ 1 more egg per polyp than Achang and Agat, which represents small effect sizes (Table S1_f, Figure 17_b).

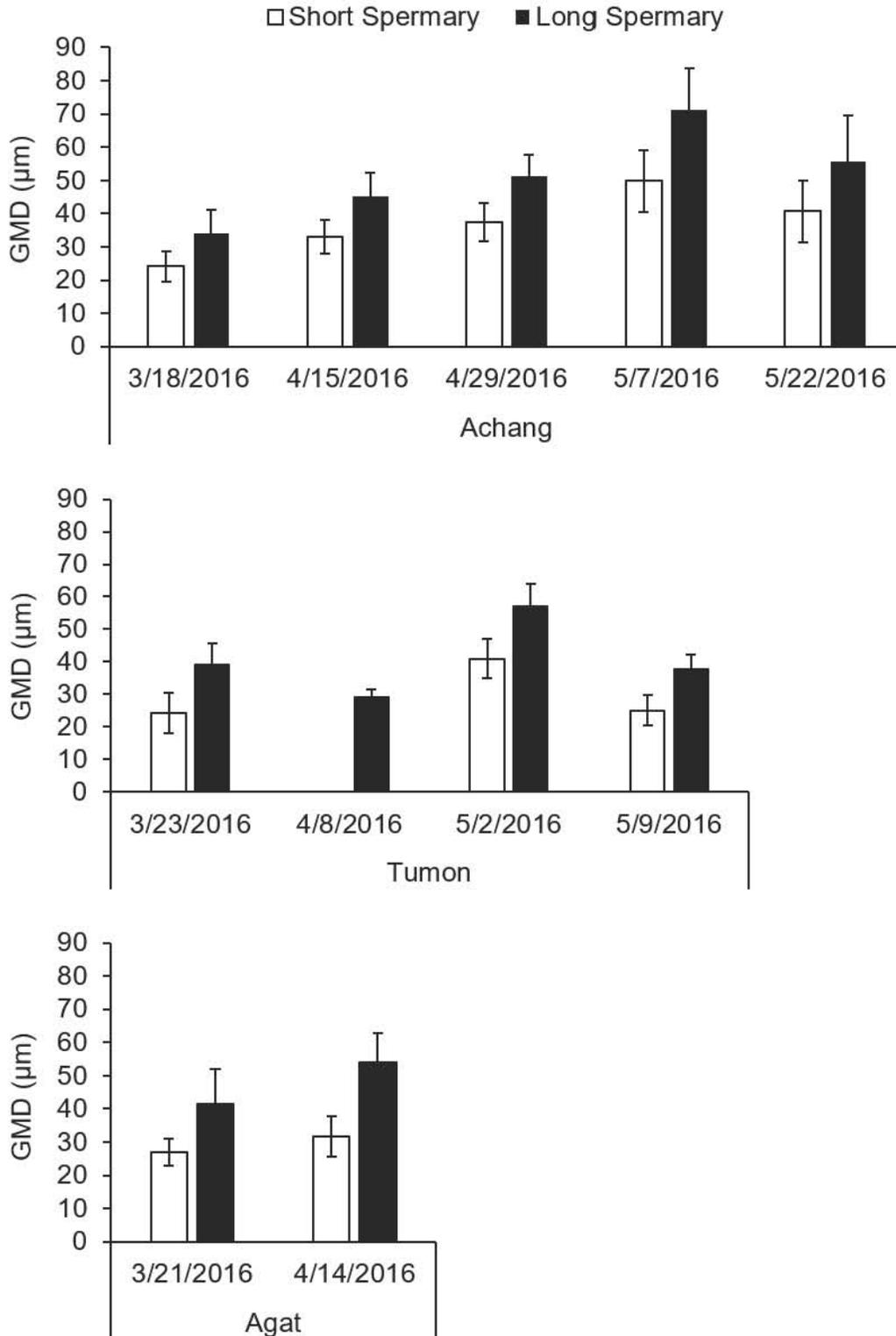


Figure 15: GMD (μm) of long and short spermaries for *Acropora pulchra*. X-axis represents the sampling date and error bars indicate the standard deviation. The full moon occurred on 4/22/2016 and 5/22/2016. See Methods -> Data Analysis for details.

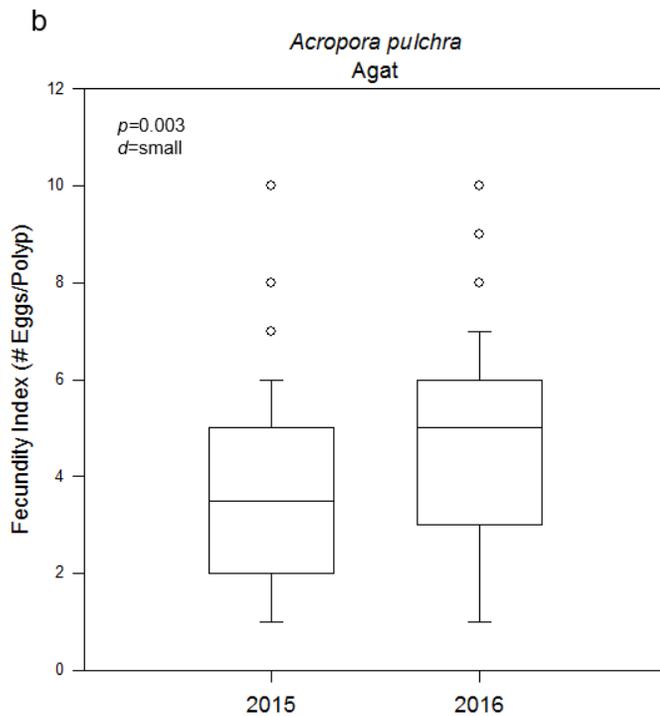
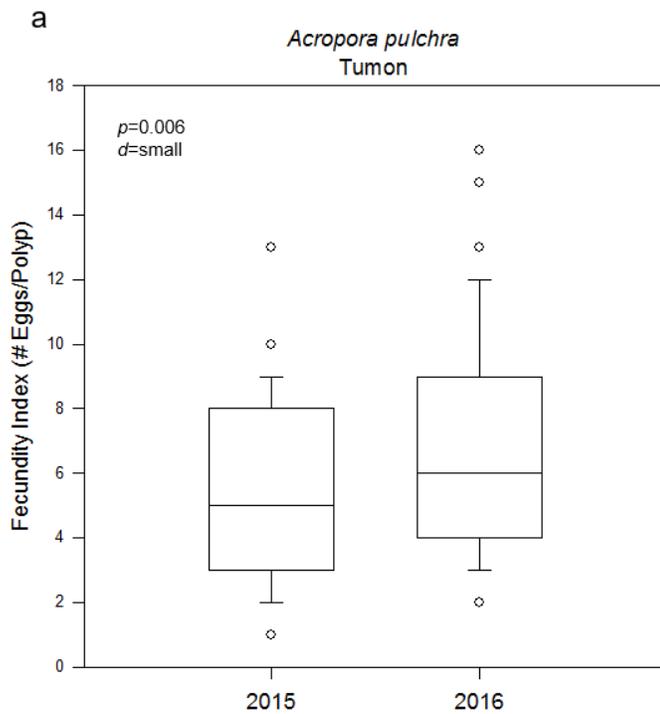


Figure 16: Comparison of the fecundity index (i.e. number of eggs per polyp) between years for *Acropora pulchra* populations in **(a)** Tumon **(b)** Agat. Boxes represent the 25th and 75th quartile range, horizontal cross lines represent the median values, whiskers represent the range, and points represent 5th and 95th percentile outlier values. p -values and the effect size magnitude (d) indicate comparisons of fecundity index median values between 2015 and 2016. See Methods -> Data Analysis for details.

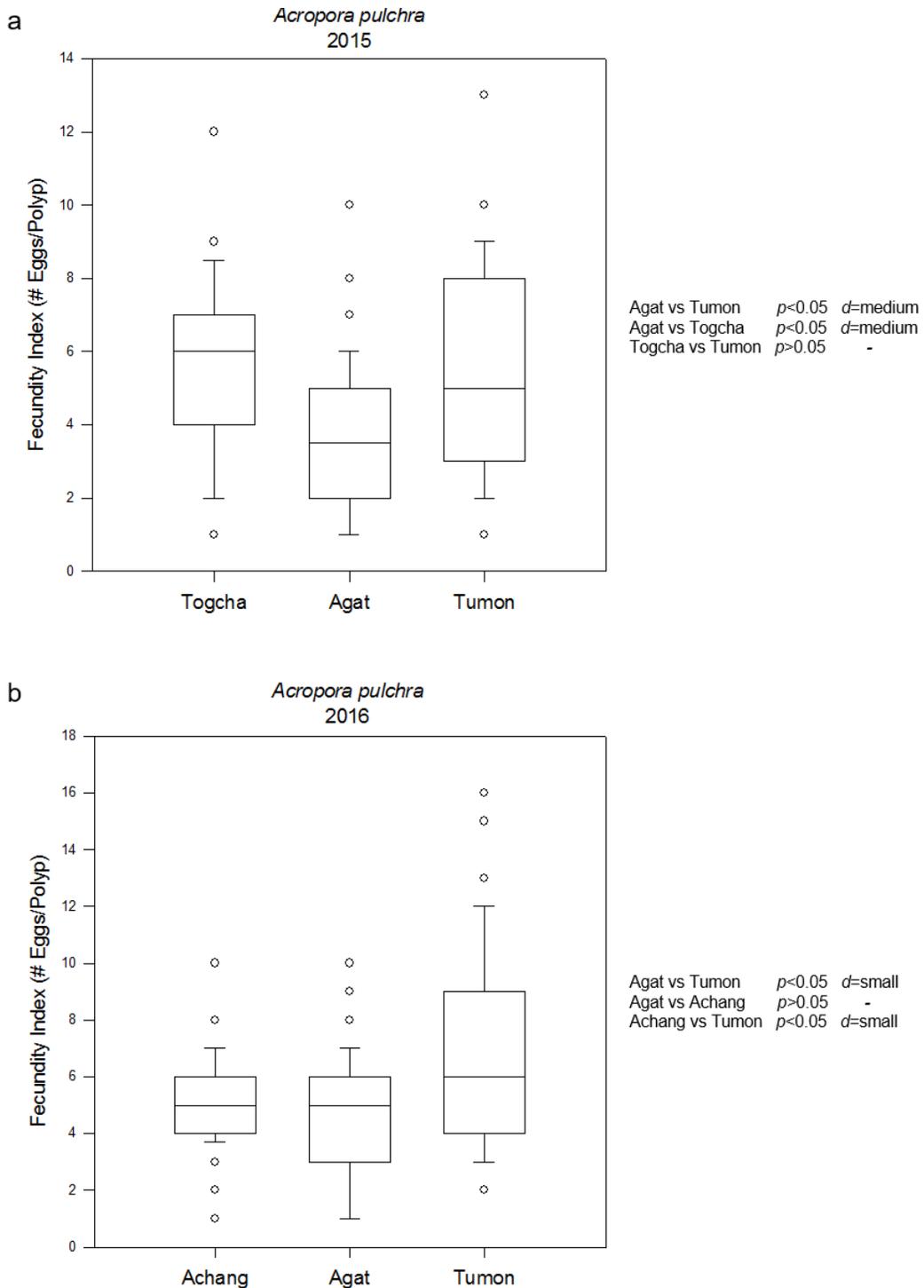


Figure 17: Comparison of the fecundity index (number of eggs per polyp) between *Acropora pulchra* populations in **(a)** 2015 and **(b)** 2016. Boxes represent the 25th and 75th quartile range, horizontal cross lines represent the median values, whiskers represent the range, and points represent 5th and 95th percentile outlier values. p -values and the effect size magnitude (d) indicate comparisons of fecundity index median values between 2015 and 2016. See Methods -> Data Analysis for details.

The proportion of reproductive branches did not differ between 2015 and 2016 for *Acropora pulchra* populations in Tumon and Agat ($p=0.96$, Chi-square) (Table S2_a, Figure S2). However, in 2015, the proportion of reproductive branches differed significantly between Tumon and Agat ($p=0.007$, Chi-square) (Table S2_a). Only 42% of branches were reproductive in Agat, whereas, 83% of branches were reproductive in Tumon (Figure S2). Also, in 2016, the proportion of reproductive branches in Agat (37%) differed from Achang (70%) ($p=0.029$, Chi-square) and Tumon (77%) ($p=0.006$, Chi-square) (Table S2_a; Figure S2). Lastly, the mean annual fecundity for all sites was higher in 2016 (221 eggs/cm²) than in 2015 (180 eggs/cm²) and in, both, 2015 and 2016 it was highest in Tumon and lowest in Agat (Table S6).

Acropora muricata

Changes of mean oocyte diameters over each month indicated a clear annual gametogenic cycle that was ~9-10 months for *Acropora muricata* (Figure 18_a). No gametes were found in June and oogonia first appeared in July and August. The onset of gametogenesis was staggered among colonies and branches, exhibiting ~1 month delay. Oocyte diameters gradually increased in early gametogenesis (stage I and II) and then increased exponentially in stage IV and V (Figure 18_a). Occasionally, immature oocytes were observed with mature oocytes within the same reproductive mesentery; thus, large ranges were observed for mature oocyte diameters (Table S4), suggesting that oogenesis is highly variable. Spermatogenesis generally started approximately 3 months later than oogenesis,

but this was difficult to observe without using histological methods. In 2016, spermaries were observed in March, April, and May (Figure 18a).

Oocyte diameter was significantly and negatively correlated with rainfall (Pearson product-moment correlation; $R^2=-0.719$, $p=0.029$), but there was no significant relationship with insolation (Pearson product-moment correlation; $R^2=0.416$, $p=0.266$) or SST (Pearson product-moment correlation; $R^2=-0.581$, $p=0.101$) (Figure 18a,b). Furthermore, there was a weak negative linear relationship between oocyte diameter and rainfall ($R^2=0.517$, $p<0.001$), whereas insolation and SST showed no linear relationship with oocyte diameter ($R^2=0.173$, $p=0.427$ for insolation; $R^2=0.338$, $p=0.080$ for SST) (Figure 19; null results not shown).

In 2015, all monitored *Acropora muricata* populations spawned in May and, in 2016, they spawned in April. In this study, spawning of *Acropora muricata* occurred at two lunar phases within the same month in 2016 (Figure 20). In Tumon, ~70-80% of gametes spawned by day 4 and the remaining (~20-30%) gametes spawned between day 15 and day 29. In Agat, ~85-90% of gametes spawned before day 7 and the remaining 10-15% of gametes spawned between day 7 and day 16. Except for *Acropora aspera*, all other species spawned in April or May; thus, spawning of *Acropora muricata* was synchronous with the other species in this study (with respect to the month). With respect to the number of days after the full moon, spawning in 2016 was asynchronous because spawning was detected twice in one month for both populations.

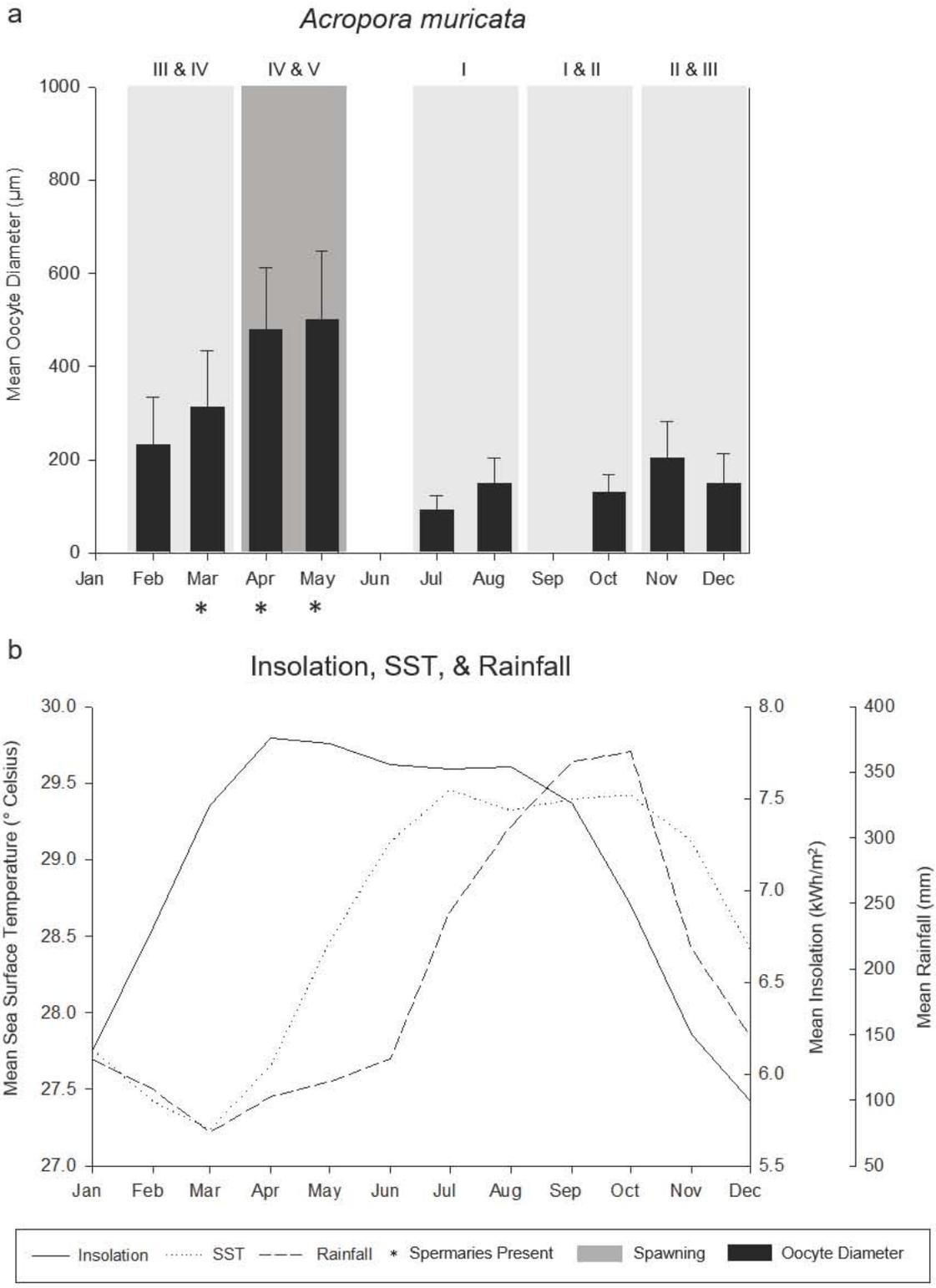


Figure 18: **(a)** Mean monthly oocyte diameter for *Acropora muricata*. Error bars indicate the standard deviation. See Methods -> Data Analysis for details. **(b)** Temporal changes in insolation, SST, and rainfall (monthly means) in Guam.

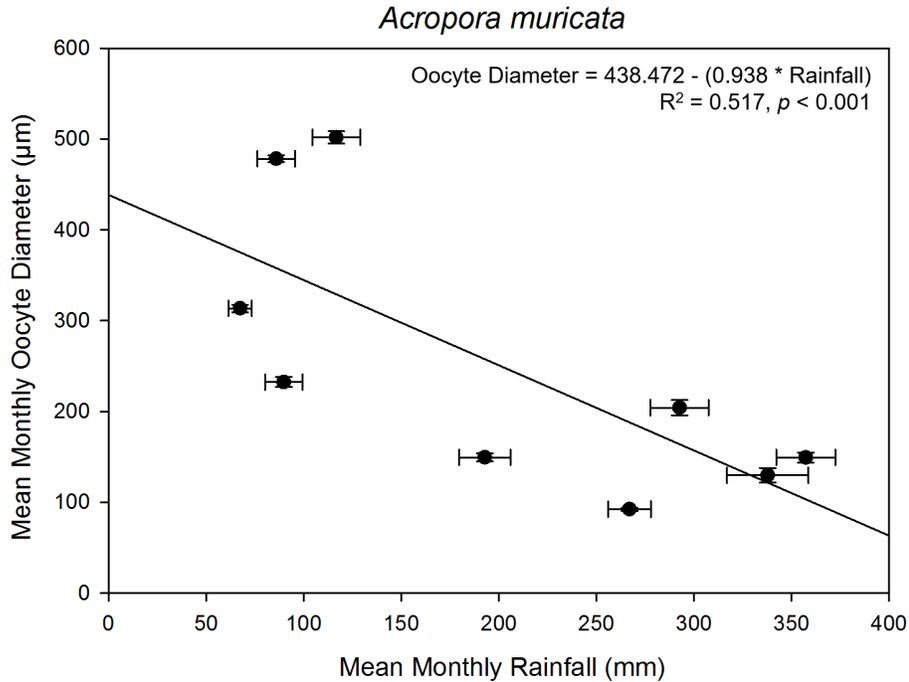


Figure 19: Linear regression, with R^2 and p -values and regression line equation, between oocyte diameter and insolation for *Acropora muricata* (see Methods -> Data Analysis for details). Bi-directional error bars indicate the standard error.

Oocyte coloration changed from ivory to a light pink or peach color ≤ 14 days prior to spawning (Figure S3; Table S3). In 2017, *in situ* spawning was observed in Agat on day 4, between 2030-2100 h and, in Tumon, on day 0, between 2015-2045 h (Table S3). No egg-sperm bundle setting was observed in the polyp gastrovascular cavity. *Acropora muricata* populations spawned for 30 minutes, about fifteen minutes apart, thus, in 2017, spawning was synchronized with respect to the time (i.e. hour).

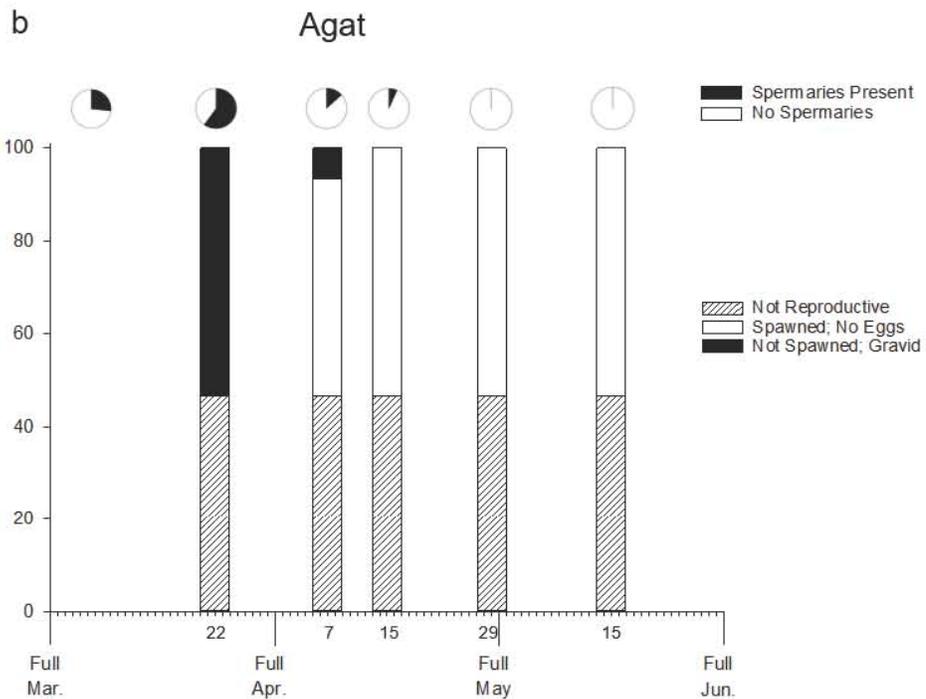
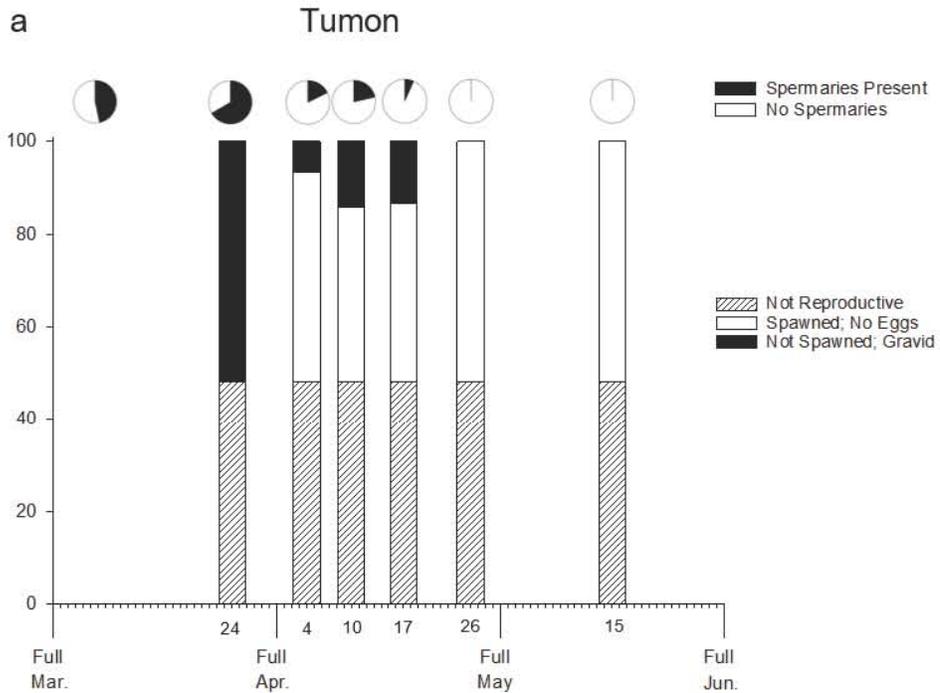


Figure 20: Proportions of gamete presence in *Acropora muricata* during spawning months in 2016. Bars and pie graphs indicate the presence or absence of eggs and spermaries, respectively. “Full” represents the full moon for corresponding month. Proportion of reproductive branches was calculated in late gametogenesis (≤ 2 months before spawning) and when at least five polyps contained eggs.

No linear relationship existed between the sizes of spermaries (i.e. long, short, and total spermaries) and eggs, and no linear relationship between the fecundity index and size of gametes (eggs and long, short, and total spermaries) (ANCOVA; Figure S2_{a-g}). A comparison of the median values revealed that egg diameters were significantly different between years 2015 and 2016, at Tumon ($p=0.003$, Mann-Whitney Rank Sum) and Agat ($p<0.001$, Mann-Whitney Rank Sum). In 2016, egg diameters for Tumon and Agat were ~ 25 - $50 \mu\text{m}$ smaller than in 2015, resulting in medium effect sizes (Table S1_a, Figure 21_{a,b}). In 2015 and 2016, a significant difference was detected between egg diameters in Tumon ($p=0.002$, Mann Whitney Rank Sum) and Agat ($p=0.009$, Mann Whitney Rank Sum), which resulted in negligible effect sizes (i.e. not a true significance difference of the median values) indicating no biological significance (Table S1_b, Figure 4_{c,d}). Mean egg diameters were as follows: Tumon, 2015: $539.4 \mu\text{m} \pm 188.5 \mu\text{m}$; 2016: $561.3 \mu\text{m} \pm 121.4 \mu\text{m}$ and Agat, 2015: $441.8 \mu\text{m} \pm 88.6 \mu\text{m}$; 2016: $488.1 \mu\text{m} \pm 71.7 \mu\text{m}$ (Table S4).

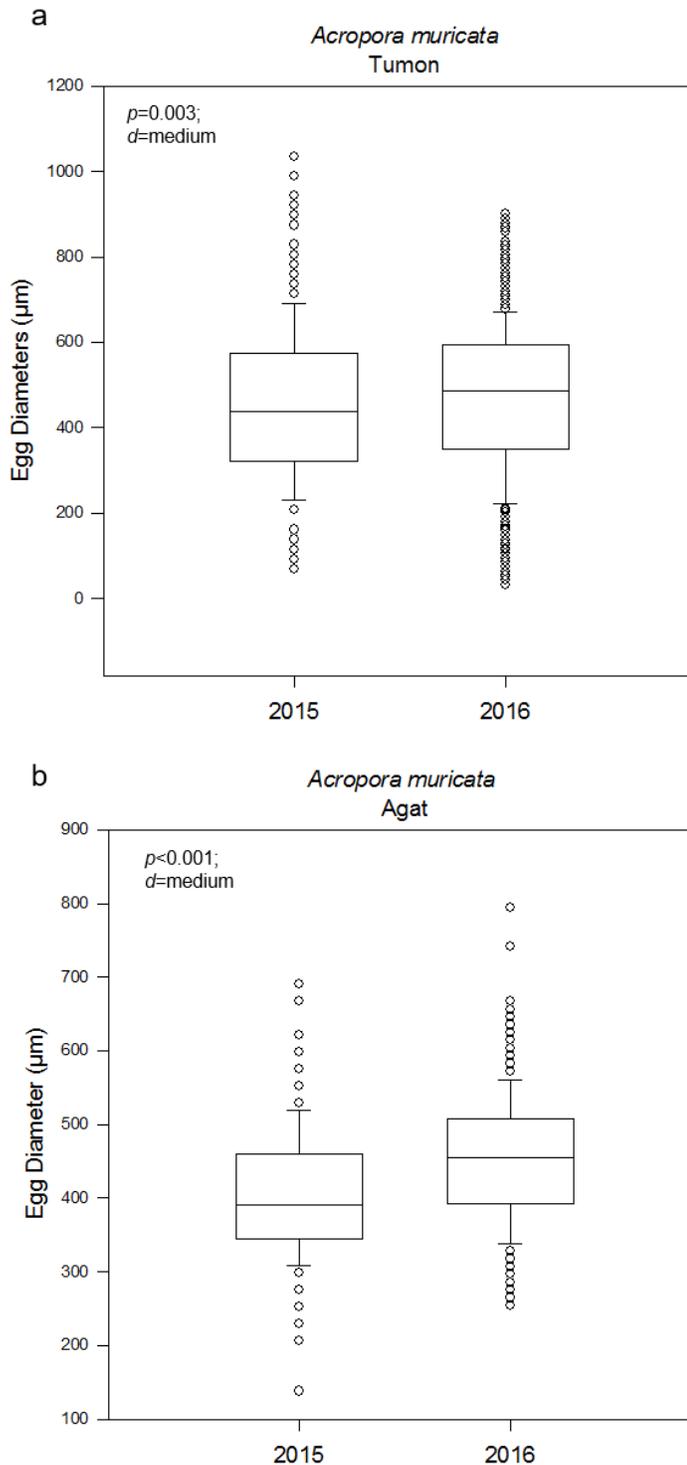


Figure 21: Comparison of maximum egg diameter between years for *Acropora muricata* populations in **(a)** Tumon **(b)** Agat. Boxes represent the 25th and 75th quartile range, horizontal cross lines represent the median values, whiskers represent the range, and points represent 5th and 95th percentile outlier values. p -values and the effect size magnitude (d) indicate comparisons of median values between 2015 and 2016. See Methods -> Data Analysis for details.

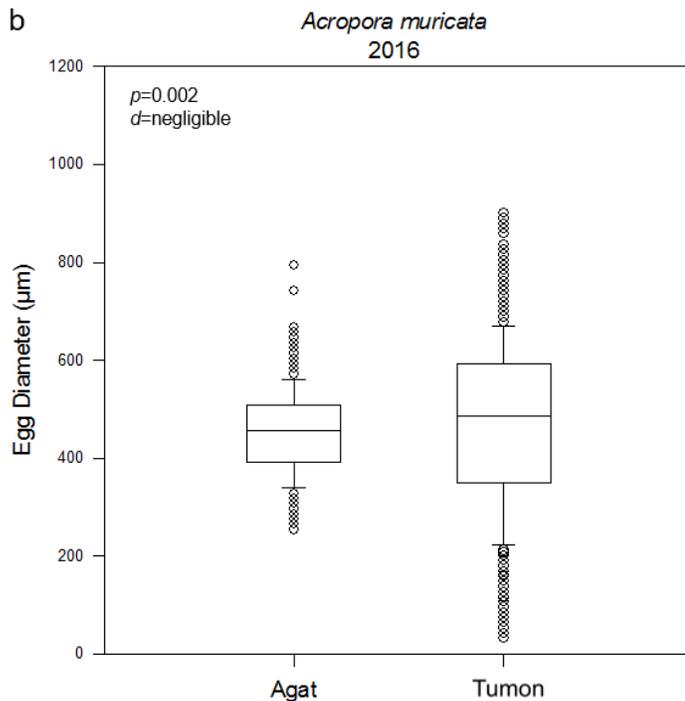
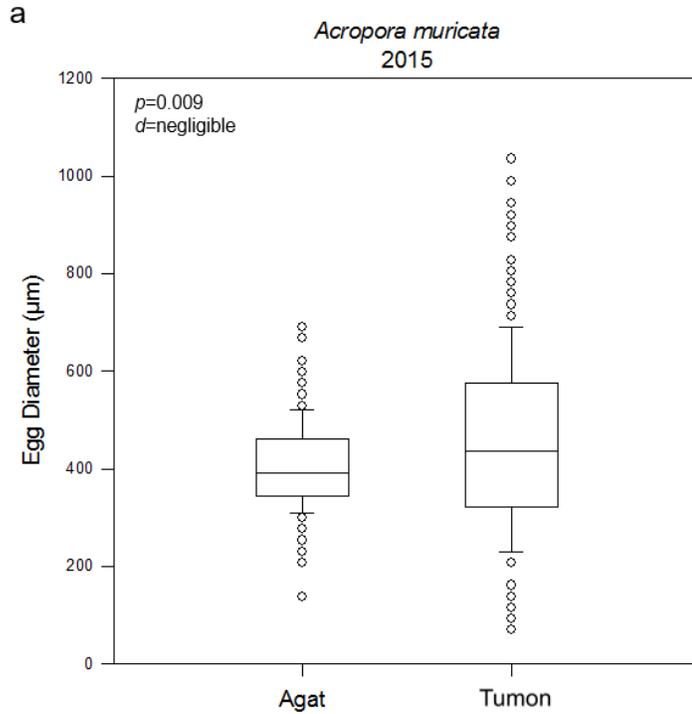


Figure 22: Comparison of the maximum egg diameter between *Acropora muricata* populations in (a) 2015 and (b) 2016. Boxes represent the 25th and 75th quartile range, horizontal cross lines represent the median values, whiskers represent the range, and points represent 5th and 95th percentile outlier values. p -values and the effect size magnitude (d) indicate comparisons of median values between populations. See Methods -> Data Analysis for details.

The size (i.e. GMD) of long and short spermaries differed significantly between Tumon and Agat ($p < 0.001$, Mann-Whitney Rank Sum for long spermaries; and $p < 0.001$, Mann-Whitney Rank Sum for short spermaries), which resulted in large effect sizes (Table S1_e). Tumon's short and long spermaries were $\sim 25 \mu\text{m}$ and $\sim 20 \mu\text{m}$ larger, respectively (Figure 23). Furthermore, the GMD of long and short spermaries were tracked within one month of spawning and, in that month, geometric growth was detected (Figure 23).

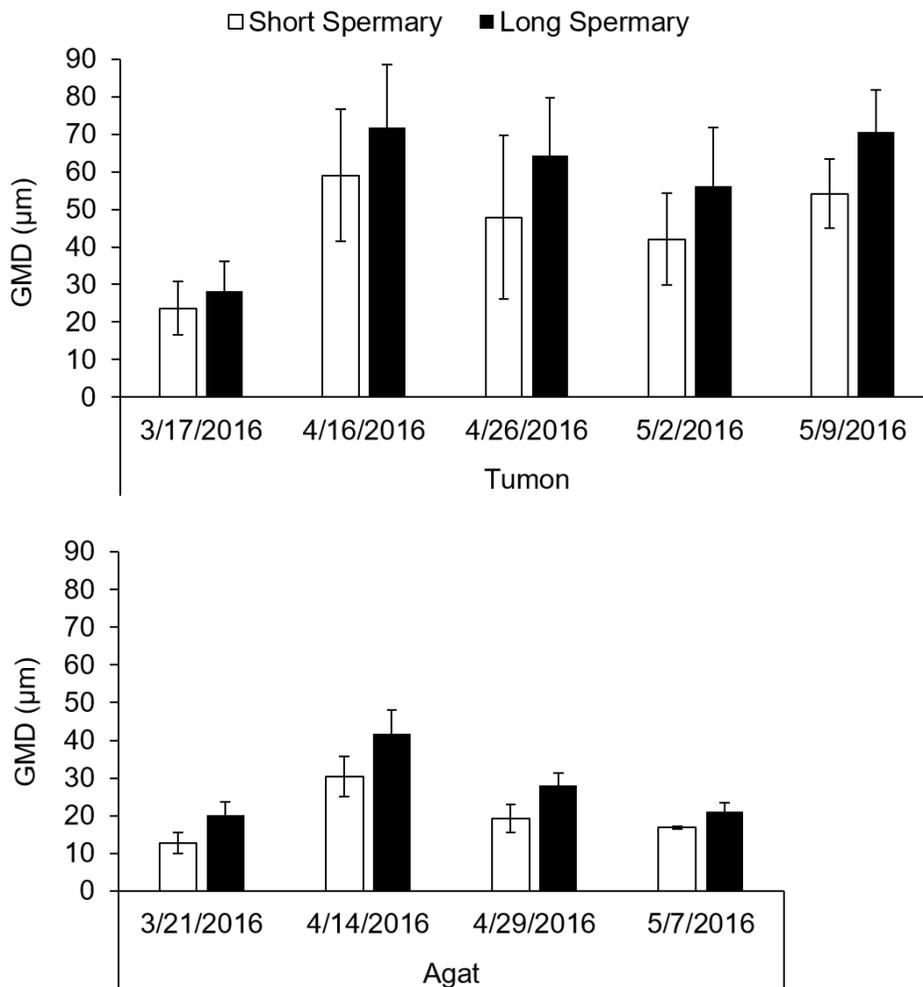


Figure 23: GMD (μm) of long and short spermaries for *Acropora muricata*. X-axis represents the sampling date and error bars indicate the standard deviation. The full moon occurred on 4/22/2016 and 5/22/2016. See Methods -> Data Analysis for details.

The number of eggs/polyp did not differ between years (2015 and 2016) in Tumon ($p=0.479$, Mann-Whitney Rank Sum) and Agat ($p=0.091$, Mann-Whitney Rank Sum) (Figure 24_{a,b}). However, Tumon had ~2 more eggs per polyp than Agat in 2015 ($p<0.001$, Mann-Whitney Rank Sum) and in 2016 ($p<0.001$, Mann-Whitney Rank Sum), which represents a small effect size for 2015 and a large effect size for 2016 (Table S1_g, Figure 25_{a,b}).

Finally, the proportion of reproductive branches did not differ between years for populations in either Tumon ($p=0.426$, Chi-square) or Agat ($p=0.864$, Chi-square) (Table S2_d, Figure S2). Furthermore, the proportion of reproductive branches did not vary between Tumon and Agat populations in 2015 ($p=0.371$, Chi-square) and in 2016 ($p=0.887$, Chi-square) (Table S2_d, Figure S2). On average, ~49% of branches were fecund for both sites and years (Table S2_b, Figure S2). The mean annual fecundity was higher in Tumon (2015: 143 eggs/cm²; 2016: 152 eggs/cm²) than Agat (2015: 115 eggs/cm²; 2016: 101 eggs/cm²).

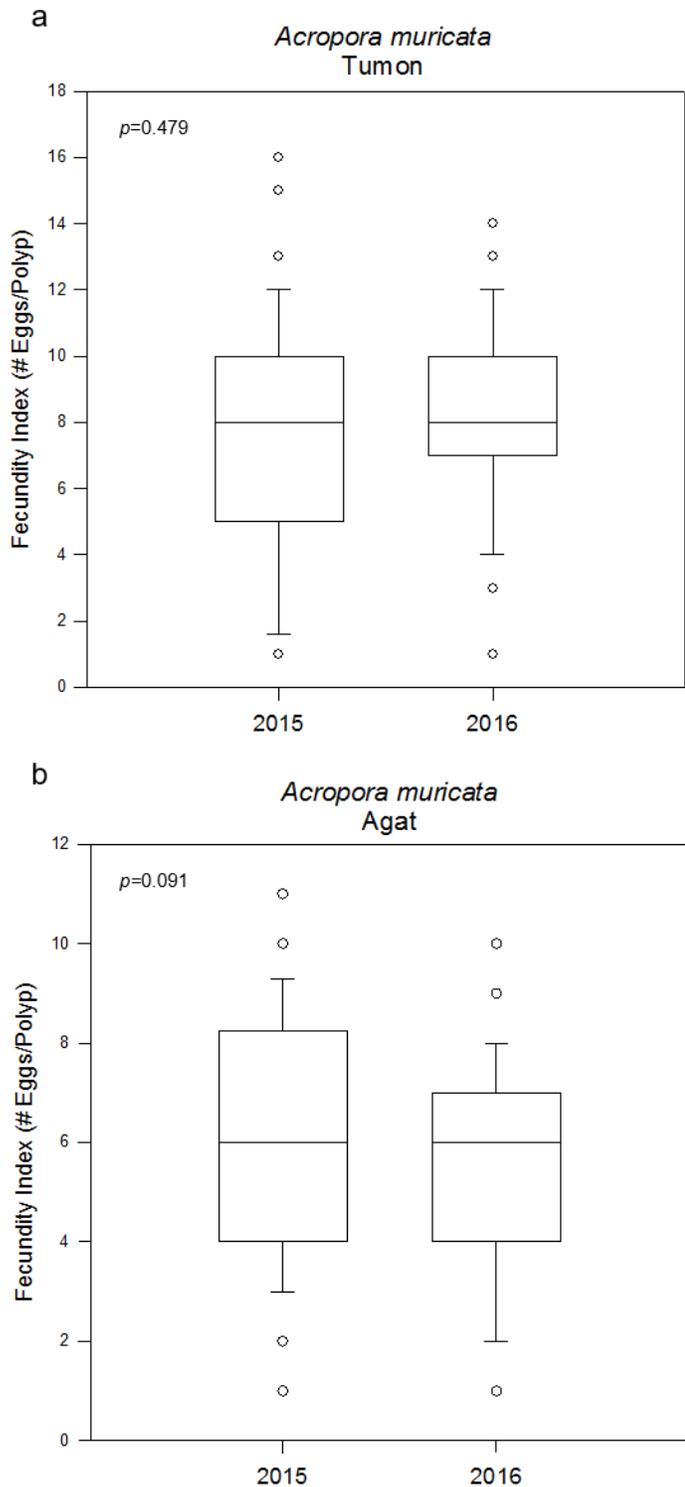


Figure 24: Comparison of the fecundity index (i.e. number of eggs per polyp) between years for *Acropora muricata* populations in (a) Tumon (b) Agat. Boxes represent the 25th and 75th quartile range, horizontal cross lines represent the median values, whiskers represent the range, and points represent 5th and 95th percentile outlier values. p -values indicate comparisons of fecundity index median values between 2015 and 2016. See Methods -> Data Analysis for details.

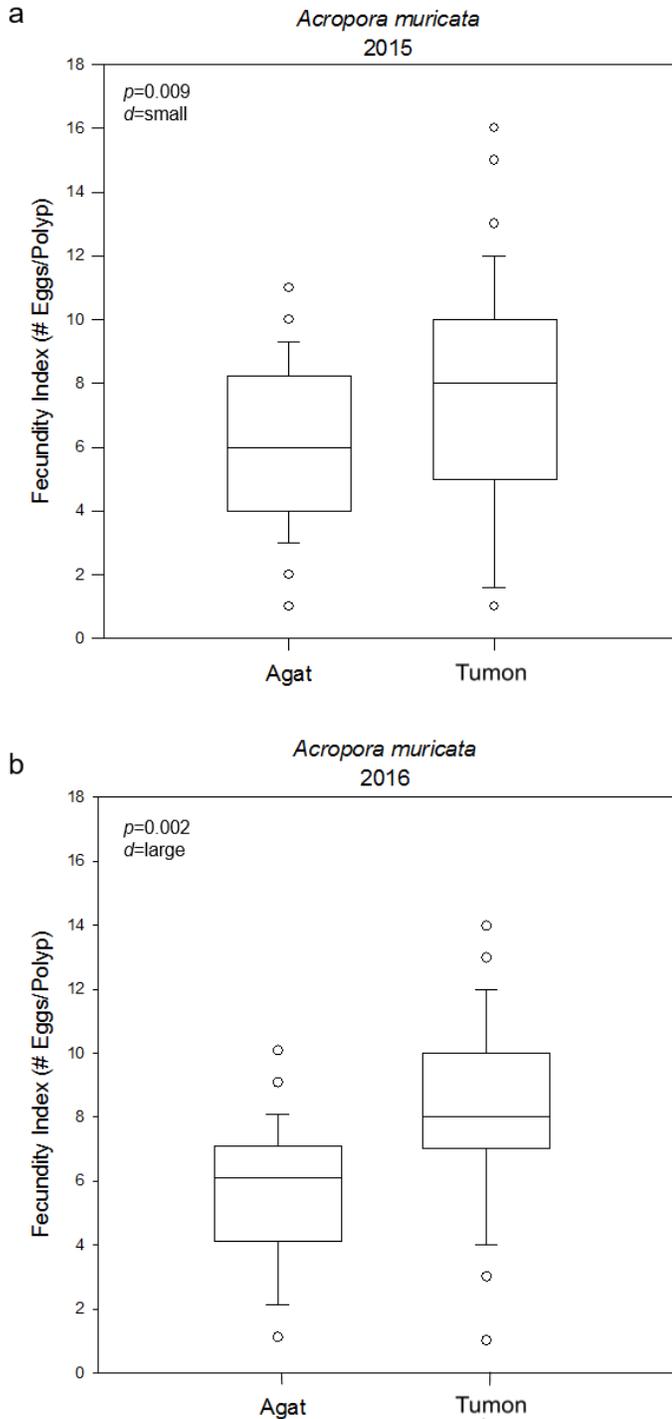


Figure 25: Comparison of the fecundity index (number of eggs per polyp) between *Acropora muricata* populations in (a) 2015 and (b) 2016. Boxes represent the 25th and 75th quartile range, horizontal cross lines represent the median values, whiskers represent the range, and points represent 5th and 95th percentile outlier values. p -values and the effect size magnitude (d) indicate comparisons of fecundity index median values between 2015 and 2016. See Methods -> Data Analysis for details.

Acropora cf. muricata

Changes of mean oocyte diameters over each month indicated a clear annual gametogenic cycle that was ~9-10 months for *Acropora cf. muricata* (Figure 26a). No gametes were found in June and oogonia first appeared in July and August. The onset of gametogenesis was staggered among colonies and branches, exhibiting ~1 month delay. Oocyte diameters gradually increased in early gametogenesis (stage I and II) and then increased exponentially in stage IV and V (Figure 26a). Occasionally, immature oocytes were observed with mature oocytes within the same reproductive mesentery; thus, large ranges were observed for mature oocyte diameters (Table S4), suggesting that oogenesis is highly variable. Spermatogenesis generally started approximately 3 months later than oogenesis, but this was difficult to observe without using histological methods. In 2016, spermaries were observed in March, April, and May (Figure 26a).

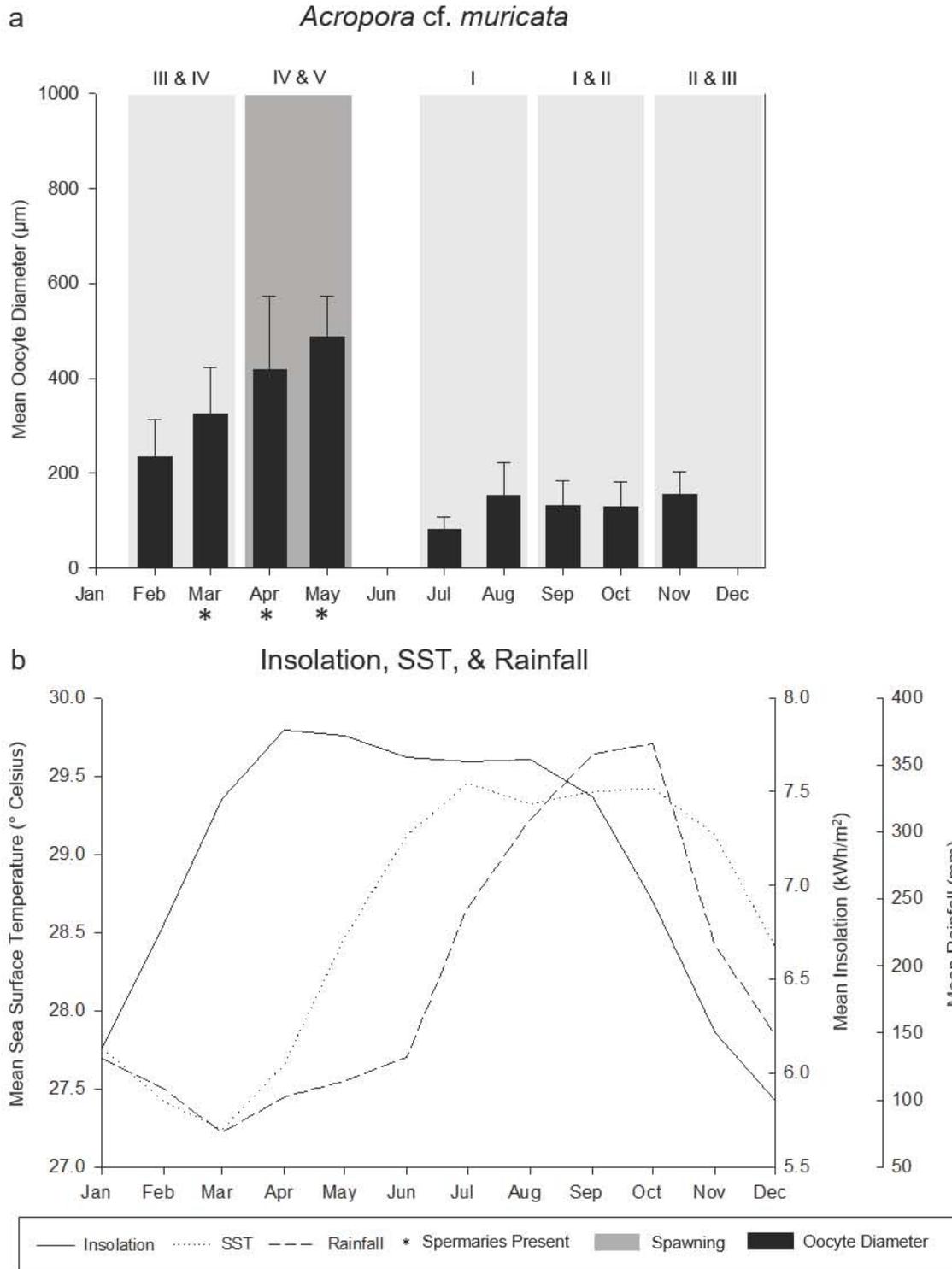


Figure 26: **(a)** Mean monthly oocyte diameter for *Acropora cf. muricata*. Error bars indicate the standard deviation. See Methods -> Data Analysis for details. **(b)** Temporal changes in insolation, SST, and rainfall (monthly means) in Guam.

Oocyte diameter was significantly and negatively correlated with rainfall (Pearson product-moment correlation; $R^2=-0.760$, $p=0.018$) and there was no significant relationship with insolation (Pearson product-moment correlation; $R^2=0.414$, $p=0.268$) or SST (Pearson product-moment correlation; $R^2=-0.630$, $p=0.069$) (Figure 26_{a,b}). Furthermore, there was a weak negative linear relationship between oocyte diameter and rainfall ($R^2=0.577$, $p<0.001$), whereas insolation and SST showed no linear relationship with oocyte diameter ($R^2=0.171$, $p=0.428$ for insolation; $R^2=0.396$, $p=0.054$ for SST) (Figure 27; null results not shown).

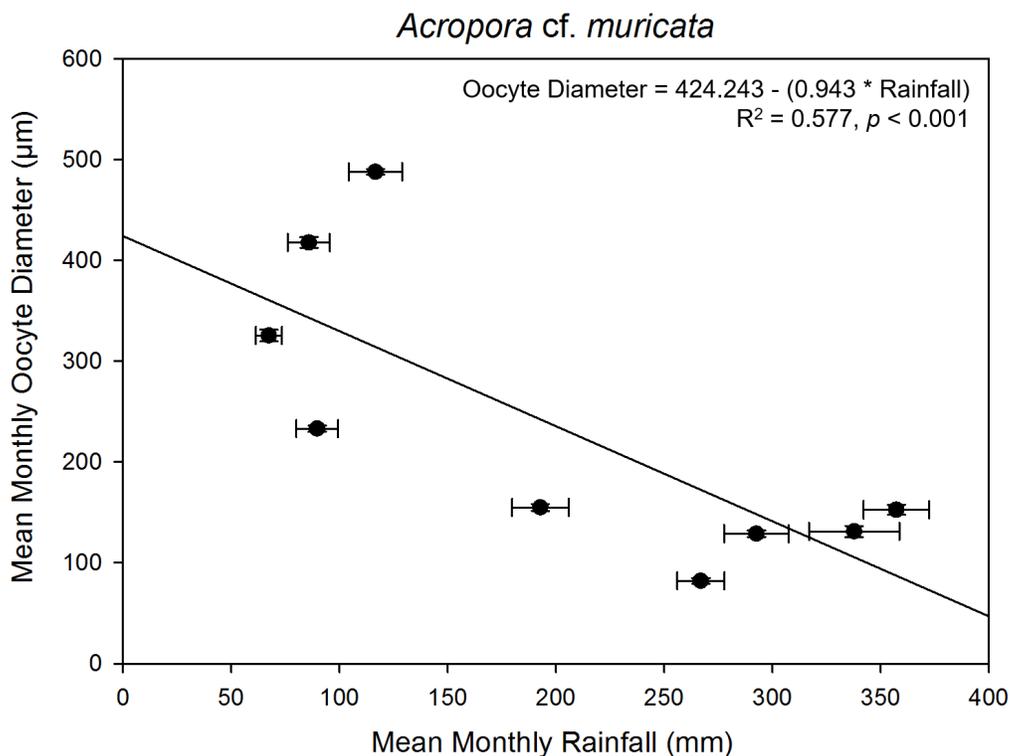


Figure 27: Linear regression, with R^2 and p -values and regression line equation, between oocyte diameter and insolation for *Acropora cf. muricata* (see Methods -> Data Analysis for details). Bi-directional error bars indicate the standard error.

In 2015, *Acropora cf. muricata* spawned in May and, in 2016, it spawned in April. In this study, spawning of *Acropora cf. muricata* occurred multiple times throughout April in 2016 (Figure 28). Approximately 43% of gametes spawned by day 7, an additional 28.3% spawned by day 15 and the last 28.5% spawned by day 29. Except for *Acropora aspera*, all other species spawned in April or May; thus, spawning of *Acropora muricata* was synchronous with the other species in this study (with respect to the month). With respect to the number of days after the full moon, spawning in 2016 was asynchronous because spawning occurred over several weeks in 2016. There were no in field observations of spermaries, pigmented eggs, or spawning for *Acropora cf. muricata*.

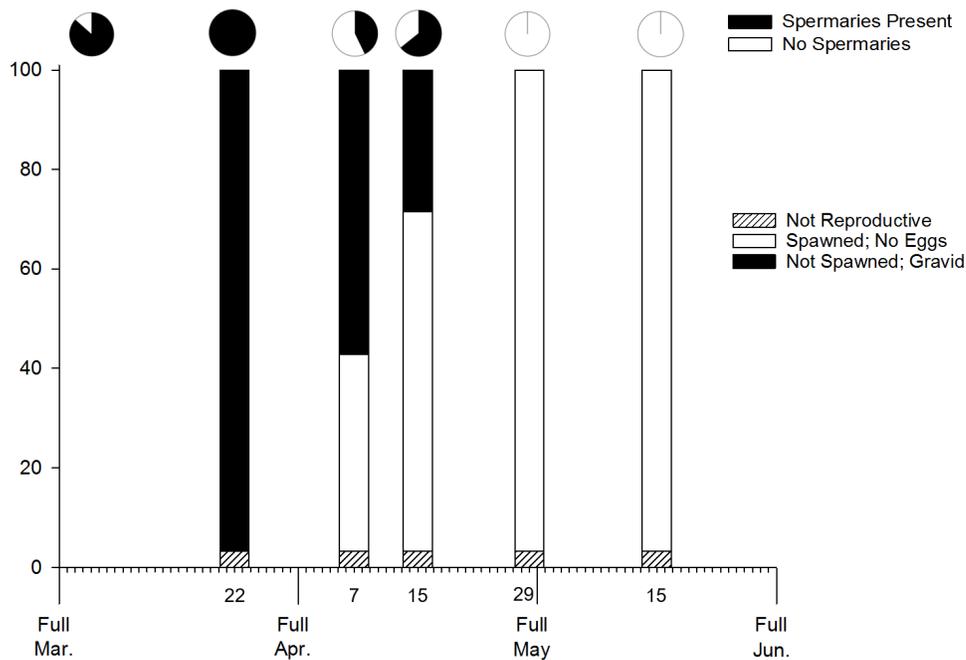


Figure 28: Proportions of gamete presence in *Acropora cf. muricata* during spawning months in 2016. Bars and pie graphs indicate the presence or absence of eggs and spermaries, respectively. “Full” represents the full moon for corresponding month. Proportion of reproductive branches was calculated in late gametogenesis (≤ 2 months before spawning) and when at least five polyps contained eggs.

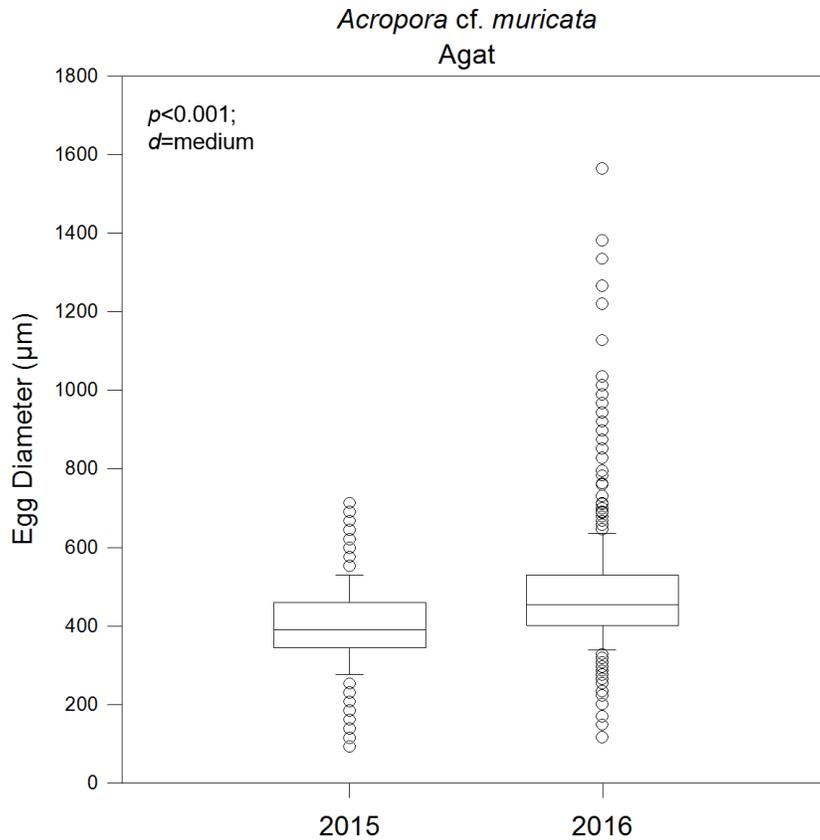


Figure 29: Maximum egg diameter for years 2015 and 2016 for *Acropora cf. muricata*. Boxes represent the 25th and 75th quartile range, horizontal cross lines represent the median values, whiskers represent the range, and points represent 5th and 95th percentile outlier values. p -value and the effect size magnitude (d) indicate comparisons of median values between 2015 and 2016. See Methods -> Data Analysis for details.

The maximum mean length of long and short spermaries were $824.2\mu\text{m} \pm 236.0\mu\text{m}$ and $451.3\mu\text{m} \pm 149.9\mu\text{m}$, respectively (Table S4). The GMD of long and short spermaries were tracked within one month of spawning and, in that month, geometric growth was variable (Figure 30). The number of eggs/polyp differed significantly between 2015 and 2016 ($p=0.020$, Mann-Whitney Rank Sum), resulting in a small effect size (i.e. small difference between the median values) (Table S1_f). Polyps had ~1 more egg per polyp in 2015 than in 2016 (Figure 31). The proportion of reproductive branches did not differ between 2015 (90%) and 2016 (97%) ($p=0.556$, Fisher's) (Table S2_d, Figure S2). Lastly, the mean annual fecundity was 201 eggs/cm² in 2015 and 177 eggs/cm² in 2016.

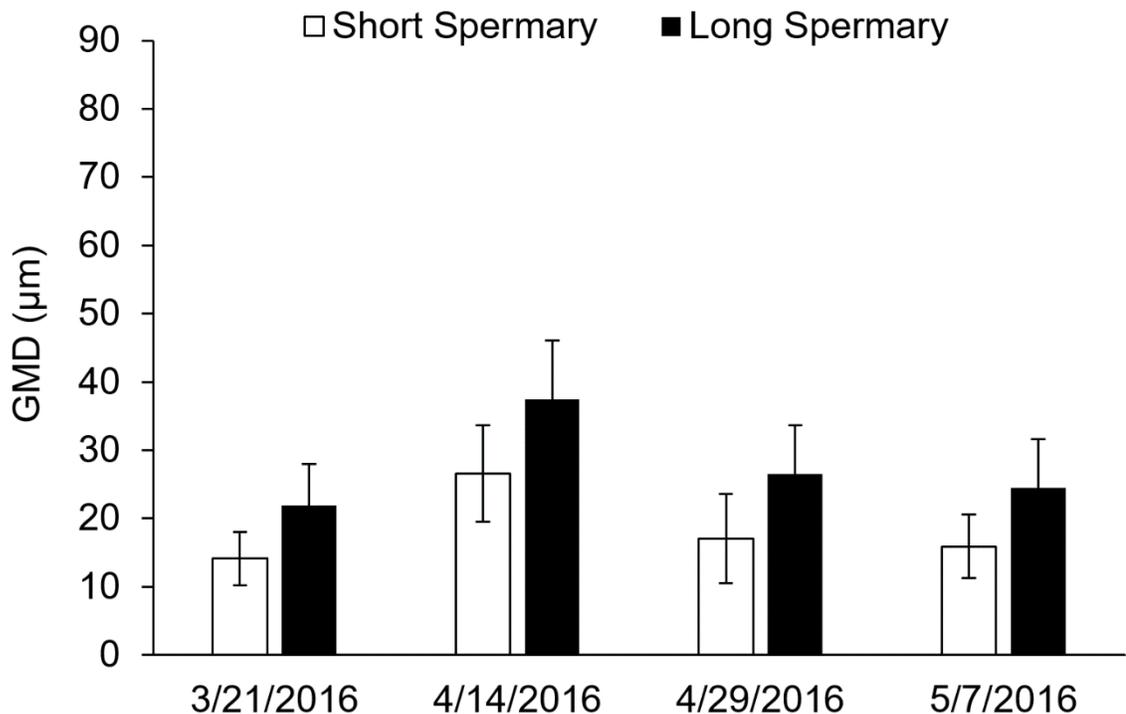


Figure 30: GMD (μm) of long and short spermaries for *Acropora* cf. *muricata*. X-axis represents the sampling date and error bars indicate the standard deviation. The full moon occurred on 4/22/2016 and 5/22/2016. See Methods -> Data Analysis for details.

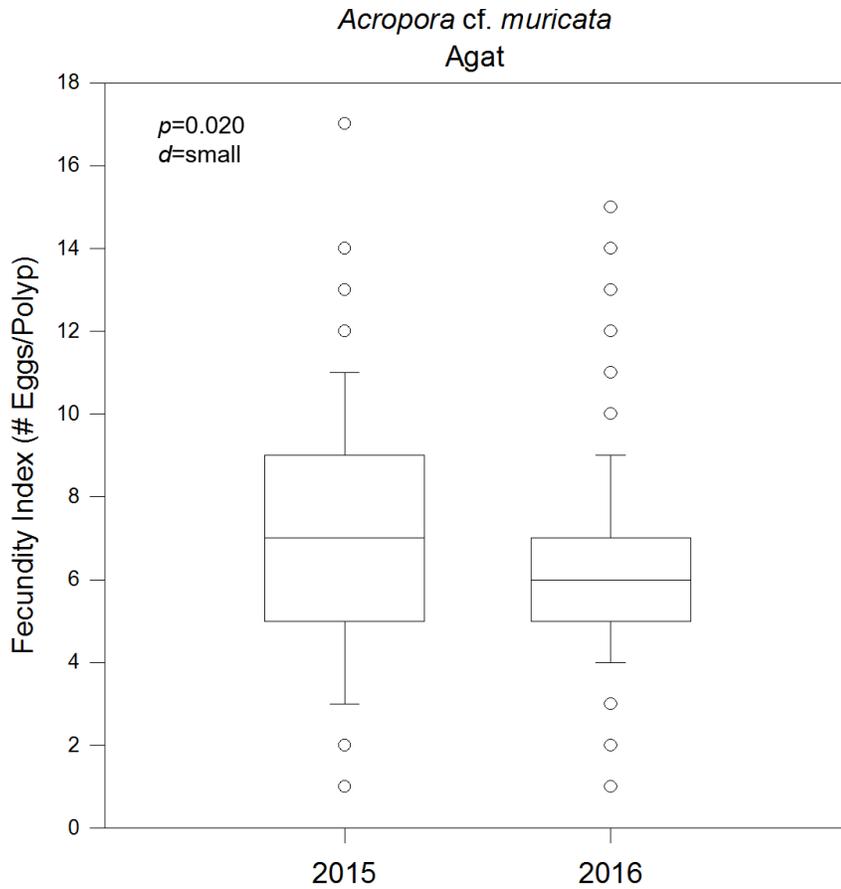


Figure 31: Comparison of the fecundity index (i.e. number of eggs per polyp) between years for *Acropora cf. muricata*. Boxes represent the 25th and 75th quartile range, horizontal cross lines represent the median values, whiskers represent the range, and points represent 5th and 95th percentile outlier values. p -value and the effect size magnitude (d) indicate comparisons of fecundity index median values between 2015 and 2016. See Methods -> Data Analysis for details.

Acropora cf. intermedia

Changes of mean oocyte diameters over each month indicated a clear annual gametogenic cycle that was ~9-10 months for *Acropora cf. intermedia* (Figure 32a). No gametes were found in June and oogonia first appeared in July and August. The onset of gametogenesis was staggered among colonies and branches, exhibiting ~1-month delay. Oocyte diameters gradually increased in early gametogenesis (stage I and II) and then increased exponentially in stage IV and V (Figure 32a). Occasionally, immature oocytes were observed with mature oocytes within the same reproductive mesentery; thus, large ranges were observed for mature oocyte diameters (Table S4), suggesting that oogenesis is highly variable. Spermatogenesis generally started approximately 3 months later than oogenesis, but this was difficult to observe without using histological methods. In 2016, spermaries were observed in March (Figure 32a).

No significant relationship existed between oocyte diameter and rainfall (Pearson product-moment correlation; $R^2=-0.425$, $p=0.221$), insolation (Pearson product-moment correlation; $R^2=0.400$, $p=0.253$) or SST (Pearson product-moment correlation; $R^2=-0.183$, $p=0.613$) (Figure 32a,b; null results not shown). Furthermore, there was a significant linear relationship between oocyte diameter and rainfall but with a lot of unexplained variability ($R^2=0.180$, $p=0.020$), whereas insolation and SST showed no linear relationship with oocyte diameter ($R^2=0.160$, $p=0.394$ for insolation; $R^2=0.033$, $p=0.556$ for SST) (Figure 33; null results not shown).

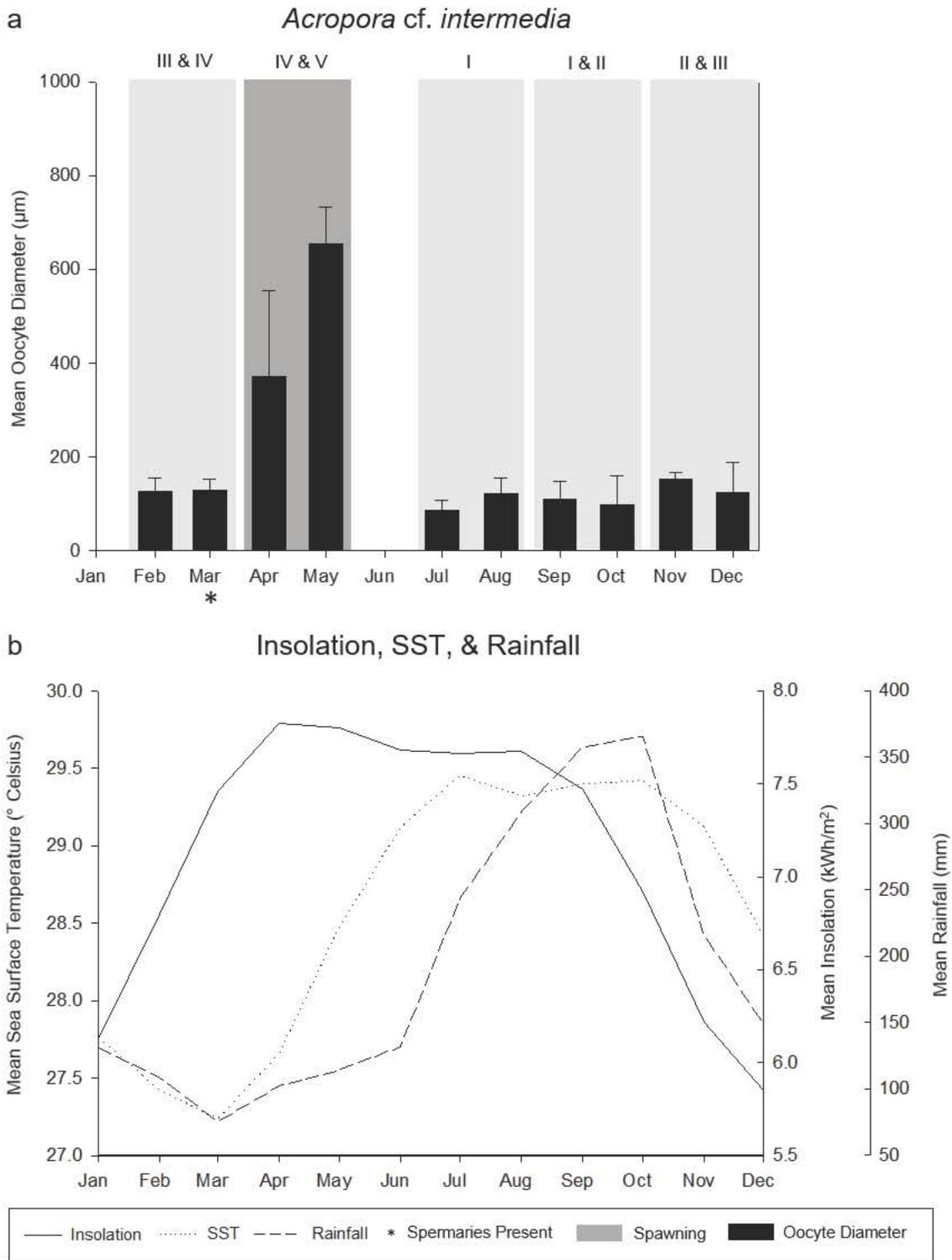


Figure 32: **(a)** Mean monthly oocyte diameter for *Acropora cf. intermedia*. Error bars indicate the standard deviation. See Methods -> Data Analysis for details. **(b)** Temporal changes in insolation, SST, and rainfall (monthly means) in Guam.

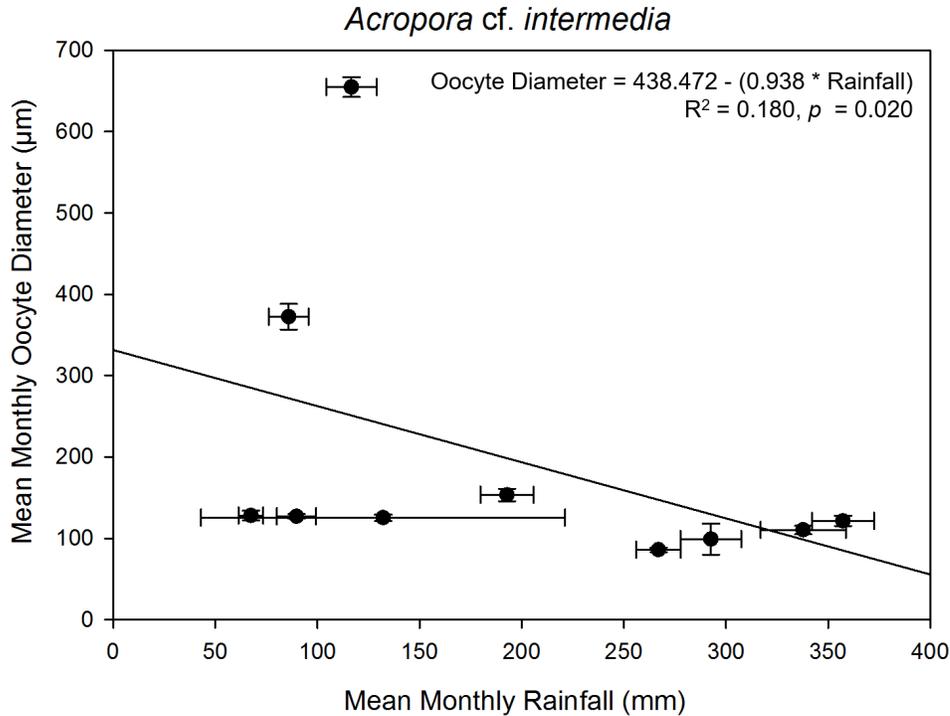


Figure 33: Linear regression, with R^2 and p -values and regression line equation, between oocyte diameter and insolation for *Acropora cf. intermedia* (see Methods -> Data Analysis for details). Bi-directional error bars indicate the standard error.

In 2015 and 2016, *Acropora cf. intermedia* spawned in April. Except for *Acropora aspera*, all other species spawned in April or May; thus, spawning of *Acropora cf. intermedia* was synchronous with the other species in this study (with respect to the month). In 2016, spawning occurred within a 9-day window, 5 days before to 4 days after the full moon. Normally *Acropora* spawn synchronously after the full moon, so it could be assumed that spawning on Guam occurred within the first 4 days after the full moon. Thus, spawning in 2016 was relatively synchronized (with respect to the number of days after the full moon) and concise (i.e. ≤ 4 days). There were no in field observations of spermaries, pigmented eggs, or spawning for *Acropora cf. intermedia*.

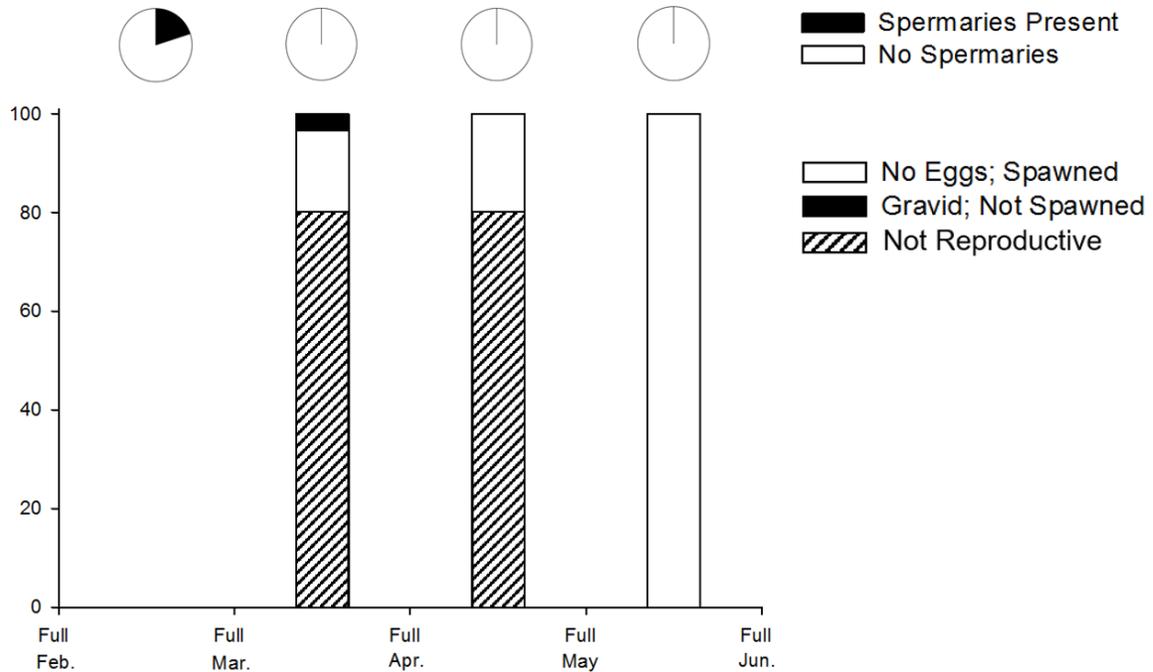


Figure 34: Proportions of gamete presence in *Acropora cf. intermedia* during spawning months in 2016. Bars and pie graphs indicate the presence or absence of oocytes and spermaries, respectively. “Full” represents the full moon for corresponding month. Proportion of reproductive branches was calculated in late gametogenesis (≤ 2 months before spawning) and when at least five polyps contained eggs.

No linear relationship existed between the sizes of spermaries (i.e. long, short, and total spermaries) and eggs, and no linear relationship between the fecundity index and size of gametes (eggs and long, short, and total spermaries) (ANCOVA; Figure S2_{a-g}). Mean egg diameters for 2015 and 2016 were $563.0 \mu\text{m} \pm 93.9 \mu\text{m}$ and $656.6 \mu\text{m} \pm 78.4 \mu\text{m}$, respectively (Table S3). A comparison of the median values revealed that there were significant differences between egg diameters in 2015 and 2016 ($p=0.003$, Mann-Whitney Rank Sum). Egg diameters in 2015 were $\sim 125 \mu\text{m}$ larger than in 2016, which resulted in a medium effect size (Table S1_c, Figure 29).

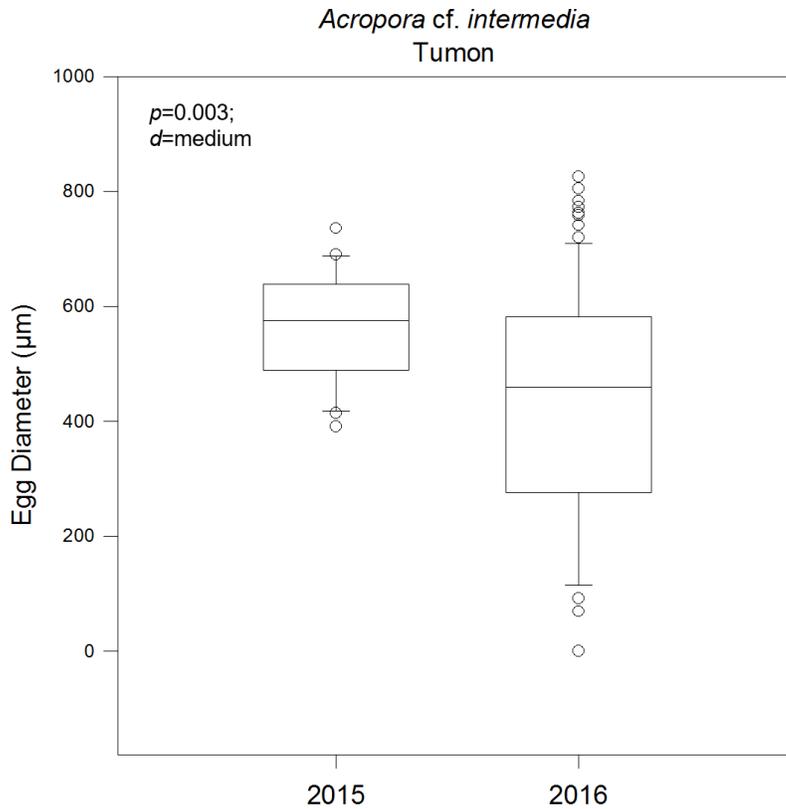


Figure 35: Maximum mean egg diameter for years 2015 and 2016 for *Acropora cf. intermedia*. Boxes represent the 25th and 75th quartile range, horizontal cross lines represent the median values, whiskers represent the range, and points represent 5th and 95th percentile outlier values. p -value and the effect size magnitude (d) indicate comparisons of median values between 2015 and 2016. See Methods -> Data Analysis for details.

Spermaries were detected in March, but not in April. The maximum mean length of long and short spermaries were $1168.2\mu\text{m} \pm 189.8 \mu\text{m}$ and $681.2 \mu\text{m} \pm 101.0 \mu\text{m}$, respectively (Table S4). The GMD of long and short spermaries were $57.3 \mu\text{m} \pm 6.43 \mu\text{m}$ and $37.4 \mu\text{m} \pm 5.63 \mu\text{m}$, respectively (Figure 36). The number of eggs/polyp did not differ between 2015 and 2016 ($p=0.174$, Mann-Whitney Rank Sum), averaging ~ 3 -4 eggs/polyp (Figure 37). The proportion of reproductive branches did not differ between 2015 (4%) and 2016 (20%) ($p=0.117$, Fisher's) (Table S2_d, Figure S2). Lastly, the mean annual fecundity was 83 eggs/cm², in 2015, and 116 eggs/cm², in 2016.

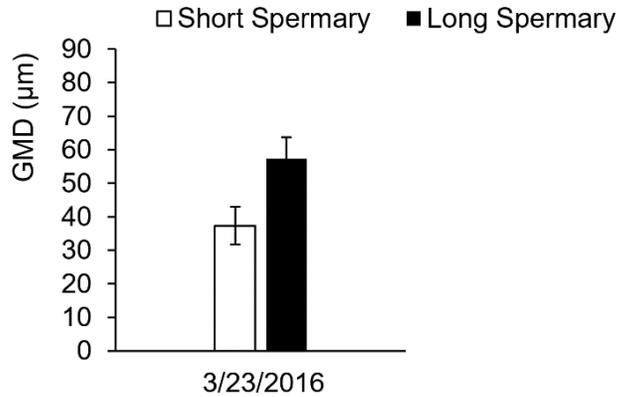


Figure 36: GMD (μm) of long and short spermaries for *Acropora cf. intermedia*. X-axis represents the sampling date and error bars indicate the standard deviation. The full moon occurred on 4/22/2016 and 5/22/2016. See Methods -> Data Analysis for details.

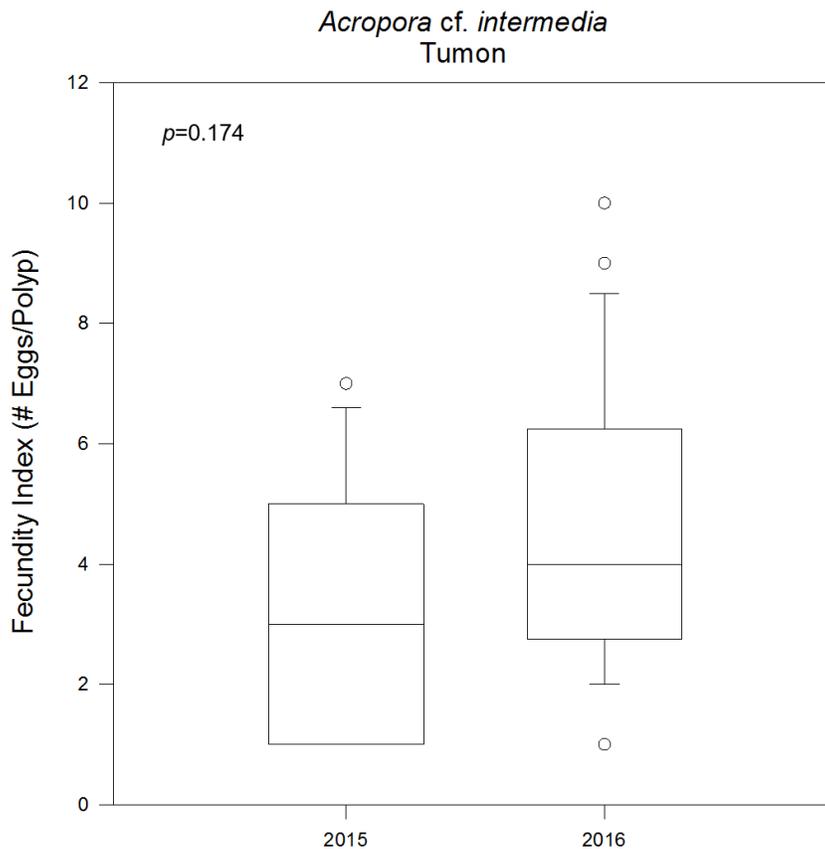


Figure 37: Comparison of the fecundity index (i.e. number of eggs per polyp) between years for *Acropora cf. intermedia*. Boxes represent the 25th and 75th quartile range, horizontal cross lines represent the median values, whiskers represent the range, and points represent 5th and 95th percentile outlier values. p -value indicates comparisons of fecundity index median values between 2015 and 2016. See Methods -> Data Analysis for details.

DISCUSSION

Gametogenesis

The Guam staghorns studied have a similar reproductive cycle to other Caribbean and Indo-Pacific *Acropora*; they are hermaphroditic, broadcast spawners that form egg-sperm bundles with a single spawning season that lasts ~9-10 months (Wallace 1985; Dai et al. 1992; Ward and Harrison 2000; Vargas-Ángel and Thomas 2002). Generally, all eight mesenteries (four male and four female) were reproductive, and two of the four female mesenteries contained between 3–5 ova and the other two usually contained 1–3 ova, which was consistent with findings of Vargas-Ángel and Thomas (2002), Heyward (1989), and Policansky (1982). Furthermore, the top 2-4 cm of each branch (apical zone) was sterile and no gonadal development was observed (Figure 38), which was consistent with Szmant (1986).

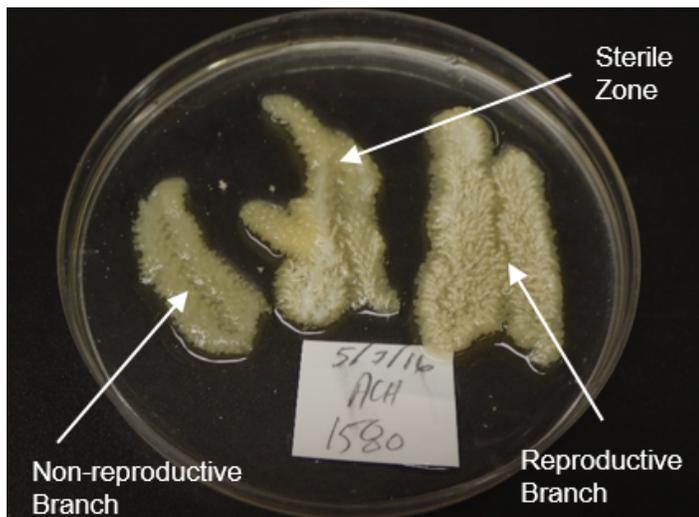


Figure 38: Reproductive and non-reproductive dissected branches with sterile zone at branch tip

Reproductive Seasonality

Seasonal fluctuations in insolation and SST (i.e. monthly means) did not correlate with the increase in oocyte diameters (i.e. monthly means) for *A. pulchra*, *A. cf. intermedia*, *A. cf. muricata*, and *A. muricata*, but an increase in rainfall was negatively and significantly correlated with the increase of oocyte diameters. In this study, *Acropora cf. intermedia* consistently showed low reproductive output, which resulted in lower sample sizes; thus, the negative correlation with rainfall was not as strong for this species. For *Acropora aspera*, the primary factor driving the linear growth of oocyte diameters was the increase in rainfall. The reproductive seasonality (leading to reproductive synchrony) previously recorded for *Acropora aspera* are inconsistent with the results in this study. At Heron Reef (Eastern Australian), Bothwell (1981) reported reproductive asynchrony of *Acropora aspera* compared to nine other *Acropora* species, where *A. aspera* spawned during the dry season and during the maximum for insolation. However, other studies reported reproductive synchrony for *Acropora aspera* with many other species at Orpheus Island (Eastern Australia) and in Western Australia, where spawning occurred during the wet season and the maximum for insolation (Babcock et al. 1994; van Oppen et al. 2002). Also, *Acropora aspera* spawns synchronously in April with over 35 other species at the maximum for insolation during the dry season in Palau (M. Gouezo, unpublished data/personal observation). I hypothesize that *Acropora aspera* on Guam has responded differently to environmental drivers underlying reproductive success. For example, the study site with *Acropora aspera* is

located along a large channel and, during Guam's monsoon season, adult corals may rely on heterotrophic feeding of particulate matter from subsequent run-off, which would increase energy reserves allocated for reproduction. However, *Acropora pulchra* was sampled at the same location and oogenesis was negatively correlated with rainfall. Also, southern Guam is volcanic; thus, southern staghorn populations may have increased heterotrophic feeding. Further, Richmond (1993b) showed that fertilization success decreases by 86% when salinity levels are reduced by 20%. Thus, rainfall is important environmental variable for corals to exert selection pressure. Further work, such as a time series multiple regression model with multiple predictor variables (e.g. neap tide cycles, SST, rate of change for insolation, time, etc.), is needed to better determine the drivers of gametogenesis.

Vargas-Ángel et al. (2006) showed correlations between gamete size and both SST and insolation in Broward County, Florida for *Acropora cervicornis* and Mendes and Woodley (2002) showed correlations between gamete size and light intensity (i.e. photoperiod) at 19 locations for *Montastrea annularis*. Also, Rinkevich and Loya (1989) suggested that lower light availability limits the energy from zooxanthellae for gametogenesis. Mendes and Woodley (2002) suggested that in places with little year-round variation in temperature (e.g. Maldives, Guam, Solomon Islands, Yap, and Palau), spawning would always occur at peak temperatures, prior to the peak in annual rainfall. Penland et al. (2004b) showed that coral spawning in Palau correlated with insolation trends, which is consistent with this study. [Richmond and Hunter (1990) and Heyward

(1988) have reported many coral species (including *Acropora* spp.) in Guam that spawn in July and August during the rainy season and peak SST, which is consistent with Keith et al. (2016), which showed that the rate of change of SST was the most important predictor of coral spawning for *Acropora* in the Indo-Pacific. To summarize, this study shows some degree of reproductive seasonality and some degree of multi-species spawning (i.e. reproductive synchrony), coinciding with near-maximum solar insolation in April and May. I hypothesize that an additional multi-species spawning season occurs in July and August, coinciding with the increase in SST. However, this study shows that exceptions exist and reproductive cycles may change due to local environmental variables.

Spawning

Spawning synchrony is advantageous because it increases fertilization success by increasing the chances that gametes meet in the water column. In contrast, asynchronous spawning decreases fertilization success because there are fewer gametes available for fertilization. Additionally, synchrony allows for interbreeding, which allows for natural hybridization. For example, at Orpheus Island (Northeastern Australia), semi-permeable species boundaries have been observed between *Acropora pulchra* and *A. aspera*, indicating that natural hybridization can occur (van Oppen et al. 2002). In this study, the majority of staghorns spawned in May in 2016 and in April in 2016, with the exception of *Acropora aspera* that spawns in September and October. Thus, temporal barriers in spawning timing restrict interbreeding between *Acropora aspera* and the other species in this study. Some of Guam's staghorn species identifications

are unresolved (e.g. *Acropora muricata*, *A. cf. muricata*, and *A. virgata*) due to slight morphological differences. Potential hybridization events may have occurred, thus spawning timing is an important factor to consider for species identification on Guam.

There may be evolutionary advantages in some degrees of asynchrony because environmental conditions are not always favorable for fertilization and subsequent settlement. For example, at Magnetic Island (Queensland, Australia), all coral gametes and larvae (i.e. mass-spawning event) on the water surface died or became destroyed after heavy rainfall (Harrison et al. 1984). Thus, in some cases, one spawning event can be risky for optimized fertilization success. In this study, *Acropora aspera* and *A. pulchra* (from the Tumon population), spawned half of their gametes one month and the other half the next month, allowing for multiple mating opportunities. Willis et al. (1985) suggested that split spawning might occur when there are deviations from the lunar calendar, such as years with 13 full moons (i.e. the year 2015). Thus, future monitoring of *Acropora aspera* and *A. pulchra* spawning trends is needed to determine if this is a recurrent pattern. Additionally, asynchrony can impede gene flow and result in genetic divergence, making synchrony an important mechanism influencing speciation and genetic diversity (at a population level) (Coyne 1992). For example, the difference in spawning time, on the scale of hours, acted as a temporal barrier, resulting in rapid speciation for some *Acropora* and *Montastrea* species (Knowlton et al. 1997; Fukami et al. 2003). Additionally, two genetically distinct yet cryptic groups of *Acropora tenuis* colonies spawned

during different seasons (i.e. due to genetic differences) in north-western Australia (Gilmour et al. 2016). Thus, the reproductive timing observations in this study should be incorporated into studies of genetic structure Guam's staghorns.

This study documented varying scales of asynchrony. In 2016, *Acropora muricata* and *A. cf. muricata* spawned during multiple, but different, periods of the lunar cycle (Figure 20, 28). Spawning of *Acropora* has occurred before the full moon in Palau and the Great Barrier Reef, although this is an unusual occurrence (PICRIC, M. Gouezo, unpublished data/personal observations). Thus, due to the limited sampling in this study, the possibility of an alternative spawning lunar cue (i.e. last quarter moon, day 22) for *Acropora pulchra* cannot be eliminated. Szmant (1991) suggested that spawning timing depends on where the lunar cycle falls with regards to the onset of gametogenic development, making spawning less synchronized with the full moon. I hypothesize that asynchrony, with respect to the lunar day, is a common pattern as a means of diversifying and/or speciation; however, the power to detect these patterns is often limited by sampling. Further, spawning of *Acropora pulchra* at Agat was asynchronous in 2017, extending for over a week after the full moon on April 11. In addition, after checking branches for pigmented eggs in Tumon, it became evident that corals were spawning over several weeks and the majority of pigmented eggs disappeared before the full moon in May of 2017. Vargas-Ángel et al. (2006b) documented spawning for several weeks in *Acropora cervicornis* and suggested that the pattern may have resulted from the full moon occurring

early in the month. However, no fertilization barriers occurred at the time (i.e. hour) of spawning for *Acropora pulchra* and *A. muricata*. Some suggest that fragmentation outweighs the low success in sexual reproduction in staghorn corals (Tunncliffe 1981; Highsmith 1982). High rates of fragmentation would result in constant size class changes that could lead to cessation of sexual reproduction and subsequent asynchronous spawning until reproductive size is reached again. Thus, with the increased capacity for asexual reproduction in staghorn, there may be less evolutionary pressure to ensure spawning synchrony.

Reproductive Output

This study is the first record of reproductive output for *Acropora aspera*, *A. pulchra*, *A. cf. intermedia*, *A. cf. muricata*, and *A. muricata* in Guam. *Acropora* typically have large eggs between 400-800 μm in diameter when mature (Wallace 1985). In this study, mean mature egg sizes (i.e. stage IV and V oocyte diameters) were comparable to other studies; previously documented oocyte diameters were 575 μm for *Acropora pulchra*, 420 μm , 510 μm , 630 μm , and 571 μm for *A. intermedia* (sometimes referred to as *A. nobilis*), and 389 μm and 414 μm for *A. muricata* (sometimes referred to as *A. formosa*) (Wallace 1985; Dai et al. 1992; Babcock et al. 2003; Kenyon 2008). The oocyte diameters for *Acropora pulchra* in Tumon were \sim 150-250 μm smaller and more translucent (i.e. containing fewer lipids) than that of other populations in both years (Figure 39), whereas egg sizes for *Acropora muricata* did not differ between populations. While literature regarding spermary sizes for the species in this study is generally lacking, Ward and Harrison (2000) documented the maximum GMD of

Acropora aspera spermaries as 37.4 μm and Kenyon (2008) documented maximum lengths for *Acropora intermedia* (sometimes referred to *A. nobilis*) as 2000-2500 μm . However, these studies did not specify whether the ‘maximum’ spermary measurements referred to large and/or short spermaries. In this study, the GMD for *Acropora aspera* for long and short spermaries was 64.92 $\mu\text{m} \pm 5.1 \mu\text{m}$ and 44.54 $\mu\text{m} \pm 10.2 \mu\text{m}$, respectively. For *Acropora cf. intermedia*, the maximum long and short spermary lengths were 1168 $\mu\text{m} \pm 190 \mu\text{m}$ and 681 $\mu\text{m} \pm 101 \mu\text{m}$, respectively. Additionally, spermary sizes in Tumon were much larger than in Agat for *Acropora muricata*, but spermary sizes were generally more consistent for *A. pulchra*, which is the opposite pattern observed for egg sizes.

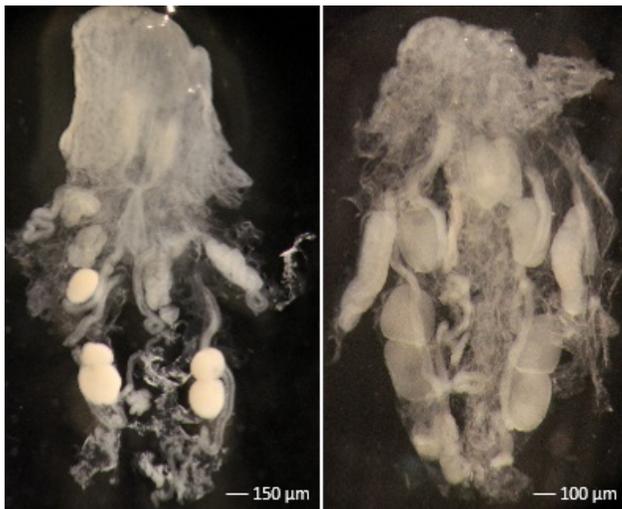


Figure 39: Small, translucent *A. pulchra* eggs from Tumon in April of 2016.

Environmental or ecological changes, such as bleaching or anthropogenic stressors, would require more energy for growth and/or maintenance of an adult coral, thus decreasing the amount of energy for reproductive output. For example, elevated nutrient levels produced significantly smaller eggs and

contained smaller testes in *Acropora longicyathus* and *A. aspera* than those which were not exposed to nitrogen (Ward and Harrison 2000), and coral bleaching reduced egg and testes size in *Acropora aspera* and *A. nobilis* (Ward et al. 2000). Additionally, in other genera, crude oil exposure resulted in smaller gonads in *Sidastrea siderea* (Guzmán and Holst 1993). Thus, populations with smaller gamete sizes (e.g. *Acropora pulchra* in Tumon) likely have poorer overall health than other populations (of the same species) in this study. Further, coral larvae rely on lipids from egg yolk reserves, which is a long-term energy source that is consumed throughout development to provide energy for larval dispersal, settlement, and metamorphosis (Arai et al. 1993; Harii et al. 2007, 2010). Thus, a small egg yolk (i.e. egg size) may decrease larval survivorship and shorten settlement-competency periods (i.e. pelagic larval duration), which plays a role in a species distribution. Also, in late spermatogenesis, ~1-2 months before spawning, the cell size is reduced and the number of cells greatly increases (Vargas-Ángel and Thomas 2002). Therefore, it may be reasonable to assume that smaller spermaries (e.g. *Acropora muricata* spermaries in Agat) contain less sperm than larger spermaries, making fertilization less likely for populations with smaller spermaries.

In general, the fecundity indices (i.e. number of eggs/polyp) in this study were lower compared to those of other studies (Table 2). Kenyon (2008) recorded sizeable variations in the number eggs per polyp sampled two years apart for several *Acropora* species. Thus, differences among the fecundity indices between years may characteristic of *Acropora* species. Furthermore, the

annual egg production for *Acropora intermedia* (sometimes referred to *A. nobilis*) in Guam ranged from 83-116 eggs/cm² (Figure S6), whereas Wallace (1985) estimated mean annual fecundity to be 261 eggs/cm² for *A. intermedia*. Additionally, Wallace (1985) estimated 209 eggs/cm² for *A. florida* and Szmant (1986) estimated 500 eggs/cm² for *A. cervicornis*. Thus, the mean annual fecundities in Guam are lower compared to other staghorn *Acropora*, but these differences may be due to the species differences in the number of polyps/cm². Lastly, for species and/or populations with high fecundity indices and large eggs (e.g. *Acropora* cf. *muricata*), occasionally 1–2 eggs remained inside the polyp after spawning (with spermaries no longer present) (Figure 40). I hypothesize that these remaining eggs did not fit into the bundle due to the space constraints of the coral skeleton. No relationship was observed between egg size and the fecundity index, which suggests that there is little evolutionary pressure to limit egg size in order to increase the fecundity index and vice versa, but there are physical constraints on egg size.

Table 2: Comparisons of the number of eggs per polyp with other studies of the same species.

	Guam	Other Studies		
	Number of Eggs/Polyp	Number of Eggs/Polyp	Location	Reference
<i>Acropora pulchra</i>	4-5	8	Eastern Australia	Ward et al. 2000
<i>Acropora muricata</i>	6-8	6.2, 6.6	Phillipines, Japan	Vicentuan (unpublished data), Okubo et al. 2007
<i>Acropora</i> cf. <i>intermedia</i> (also referred to <i>A. nobilis</i>)	3-4	11.3, 5.3-6.6	Eastern Australia, Central Pacific (Kingdom Atoll)	Ward et al. 2000, Kenyon 2008
<i>Acropora aspera</i>	4-8	11.8, 11-14	Eastern Australia, Eastern Australia	Ward et al. 2000, Ward and Harrison 2000

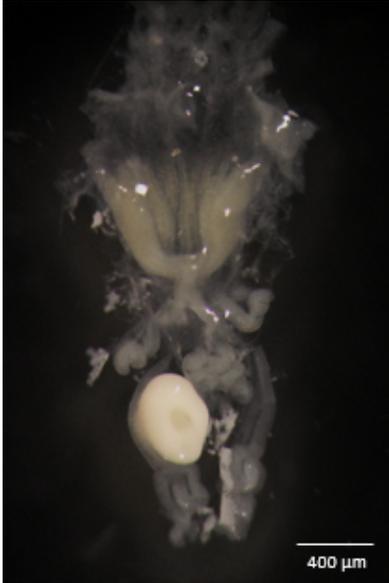


Figure 40: Large egg remaining in the polyp after spawning with no spermaries present.

Here, I report that most of the staghorn *Acropora* species in Guam showed lower reproductive outputs compared to other studies, but this is possibly due to bleaching events in 2013 and 2014 that subsided 5 months prior to this study. Ward et al. (2000) reported fewer and smaller eggs and less testes material 6 weeks and 9 months after a bleaching event for *Acropora aspera*, *A. pulchra*, and *A. intermedia* (sometimes referred to as *A. nobilis*). Also, *Lobophytum compactum* (soft coral) contained approximately half the number of eggs/polyp, and egg volumes were 59% smaller, 20 months post-bleaching (Michalek-Wagner and Willis 2001). In this study, *Acropora pulchra* and *A. cf. intermedia* exhibited low fecundity indices compared to the same species in other studies; this suggests that these species are not allocating as much energy into reproduction as other locations, possibly due to the recent bleaching stress. Furthermore, for *Acropora aspera*, egg diameters were larger in 2016 than in

2015 and the fecundity index nearly doubled in 2016 compared to 2015, suggesting that this species is recovering from previous bleaching in 2013 and 2014. However, without a reproductive baseline for each species, the impacts of bleaching can only be speculated on. For example, Szmant and Gassman (1990) documented that bleached *Montastrea annularis* colonies that did not undergo gametogenesis after a bleaching event. Thus, a low number of reproductive branches in this study may be a result of Guam's 2013-14 bleaching events. For example, only 12% of branches were reproductive for *Acropora cf. intermedia*, which was the lowest compared to the other species in this study (Figure S2). In contrast to the other species in this study, *Acropora muricata* has likely recovered from bleaching stress because the numbers of eggs per polyp were comparable to multiple studies and egg diameters were larger in 2016 for both populations. However, ~49% of the branches for *Acropora muricata* were fecund for Tumon and Agat in 2015 and 2016. Given that this study observed such high proportions of nonreproductive branches, I speculate staghorn branches may not spawn every year (for some species), but our ability to verify this is challenging. I hypothesize that localized environmental factors (e.g. water quality) and genetic differences, such as those involved with stress responses, are driving the differences in reproductive output between populations and/or species, although this was not examined in this study. Furthermore, population-related differences of gamete size were not consistent among eggs and spermaries; therefore, I speculate that gametes are affected by environmental factors differently.

Guam's reefs have some of the lowest coral recruitment rates in the world (Minton and Lundgren 2006) and have shown a considerable decline over the past decades for many coral species (Birkeland and Randall 1981; Neudecker 1981; Birkeland 1997). Boulay (2016) documented *Acropora pulchra* populations to be highly clonal at the 'local patch scale' (i.e. low connectivity between populations) in Guam, which suggests low recruitment rates for *A. pulchra* at each patch (e.g. Tumon, Agat, etc.). The low reproductive outputs observed in this study support all of these findings and show, specifically, that *Acropora pulchra* on Guam has low reproductive success (i.e. sexual reproduction) (Boulay 2016). For example, the low number of reproductive branches and low fecundity indices result in fewer eggs being spawned, thus decreasing the chances that gametes will fertilize, settle, and grow. Additionally, populations with smaller egg sizes (i.e. *Acropora pulchra* in Tumon) likely have shorter larval durations and, therefore, cannot disperse as far. However, staghorn *Acropora* have high growth rates and a colony structure that increases their ability to fragment. Thus, this study and Boulay's findings suggest that Guam's staghorn *Acropora* mainly propagate via asexual reproduction.

Management Implications & Future Research

The results of this study will support more effective management of shallow staghorn *Acropora* species as coastal development continues. Processes such as dredging affect the reproductive success of corals by increasing sediment and turbidity, thus compromising coral recruitment and the recovery of degraded reefs (Babcock et al. 1991; Birrell et al. 2005; Erftemeijer et al. 2012). Sediment

can smother corals and cover substrates, making it difficult for coral larval to settle, and turbidity (from suspended sediment) greatly reduces the amount of light needed for coral-algal symbionts. For example, chronic exposure to elevated sedimentation caused a 50% decrease in fecundity in *Acropora palifera* in Papua New Guinea (Kojis and Quinn 1984). Currently, dredging projects in Western Australia are required to halt operations 5 days before spawning to 7 days afterward when corals with short pelagic larval durations (PLDs) are spawning (Western Australia EPA, 2011). However, this window does not protect the entire PLD or years when spawning is asynchronous (i.e. corals spawning for several weeks). Currently, Guam has spawning moratoriums to impede dredging for 21 days during coral spawning in July, but this is not a permanent regulation and it is not legally binding, except for the Navy. Furthermore, the timing of this moratorium does not protect Guam's staghorns. Fortunately, dredging is site-specific and we have documented the locations of Guam's staghorn *Acropora*, thus dredging moratoriums should be updated to include Guam's staghorn spawning season. Lastly, April and May are most likely Guam's most prominent spawning season because reproductive seasonality has been strongly correlated with the rate of change of insolation worldwide (van Woesik et al. 2006). Thus, a spawning moratorium in April and May would likely protect many other species of corals, such as massive *Porites*.

As previously stated, the reproductive output for *A. cf. intermedia* and *A. pulchra* in Tumon (in front of the Outrigger hotel) was significantly lower than at other sites (i.e. populations). Flooding continues to be an issue in Tumon and,

resultantly, storm water is regularly pumped from parking lots into Tumon Bay. This storm water run-off may contain sewage and/or other pollutants such as oil, gas residue, and other hydrocarbons, that has been shown to reduce fecundity (Tomascik and Sander 1987; Guzmán and Holst 1993; Ward and Harrison 1997). Also, salinity changes have killed corals in Hawai'i, especially on shallow water reef flats which are most likely to be impacted by freshwater runoff (Jokiel et al. 1993). Richmond (1993b) showed that rainfall, at the time of spawning, reduced fertilization success by over 50%. However, the effects of decreased salinity on coral gametogenesis are not well understood. Tumon Bay has reduced salinity due to natural springs and adult corals may be adapted to withstand these conditions. Future research should be aimed to determine how coral health and water quality impacts fecundity and reproductive output at the colony and branch level. Lastly, proper management action should be taken to protect adult corals by preventing flooding and subsequent stormwater run-off onto coral reefs, especially bay areas, such as Tumon, that have longer flushing times.

Additionally, these results will allow for more effective rehabilitation efforts for staghorn *Acropora* on Guam. The low fecundity observed for *A. cf. intermedia* may explain the narrow distribution of this species on Guam, thus active management may be needed for this species. Further, populations with low reproductive output, such as *Acropora pulchra* and *A. cf. intermedia* in Tumon, are likely best managed through restoration methodologies that use asexual fragmentation (i.e. coral nurseries) to maintain and expand these populations. Furthermore, the extensive examinations of fecundity in this study

can help identify which species and populations are best for collecting gametes for sexual rehabilitation efforts. The highest reproductive outputs were observed for *Acropora pulchra* and *A. aspera* at Achang and for *Acropora cf. muricata* at Agat. However, the accessibility of sites and predictability of spawning challenges to consider when collecting gametes. Future research should further document the spawning synchrony (with respect to the lunar day and hour of spawning) and fertilization success of these species.

CONCLUSION

Due to the widespread decline of Guam's staghorn *Acropora* populations, sexual reproduction was studied to assess the potential for recovery and persistence for these species. Guam's staghorn *Acropora* species (*Acropora aspera*, *A. pulchra*, *A. muricata*, *A. cf. muricata*, and *A. cf. intermedia*) are hermaphroditic, broadcast spawners with an annual gametogenic cycle completed in ~9-10 months. This study shows some degree of reproductive seasonality and some degree of multi-species spawning (i.e. reproductive synchrony), coinciding with near-maximum solar insolation in April and May. However, *Acropora aspera* spawning coincided with the peak of annual rainfall in September and October, although a correlation with a single environmental driver does not imply cause. *Acropora muricata* and *A. cf. muricata* released gametes throughout multiple phases of the lunar cycle, whereas the other species released gametes within four nights after the full moon. However, for *Acropora pulchra*, many small-scale spawning events were observed. Except for *Acropora muricata*, the reproductive output for Guam's staghorns were low compared to

those reported in other studies, which may be a result of severe bleaching events prior to this study. Recent bleaching-related mortality, combined with the low reproductive output of surviving staghorn *Acropora* suggests that recovery to former levels of abundance will be slow. Thus, active restoration methods using asexual fragmentation are likely needed to expand these populations. Furthermore, it is imperative that we safeguard annual spawning events by regulating dredging activities during spawning and protect existing adult populations from anthropogenic stressors that reduce fecundity and reproductive success.

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SUPPLEMENTAL TABLES & FIGURES

Table S1: Effect size estimates and magnitudes for gamete sizes and fecundity indices for *Acropora aspera*, *A. pulchra*, *A. cf. intermedia*, *A. cf. muricata*, and *A. muricata* and corresponding year and/or site. See Methods -> Data Analysis for details.

a	Oocyte size for <i>A. pulchra</i> between populations	Sample Size	Effect Size Estimate	Effect Size Magnitude	Test
	Agat vs. Achang (2016)	n _{Agat} =297, n _{Achang} =558	-0.077	negligible	Cliff's delta
	Agat vs. Tumon (2016)	n _{Agat} =297, n _{Tumon} =860	0.669	large	Cliff's delta
	Tumon vs. Achang (2016)	n _{Tumon} =860, n _{Achang} =558	-0.936	large	Cliff's delta
	Agat vs. Togcha (2015)	n _{Agat} =276, n _{Togcha} =209	0.433	medium	Cliff's delta
	Agat vs. Tumon (2015)	n _{Agat} =276, n _{Tumon} =511	0.952	large	Cliff's delta
	Tumon vs. Togcha (2015)	n _{Tumon} =511, n _{Togcha} =209	-0.872	large	Cliff's delta
b	Oocyte size for <i>A. muricata</i> between populations	Sample Size	Effect Size Estimate	Effect Size Magnitude	Test
	Agat vs. Tumon (2015)	n _{Agat} =523, n _{Tumon} =308	-0.108	negligible	Cliff's delta
	Agat vs. Tumon (2016)	n _{Agat} =457, n _{Tumon} =866	-0.102	negligible	Cliff's delta
c	Oocyte size between years for all populations	Sample Size	Effect Size Estimate	Effect Size Magnitude	Test
	<i>Acropora pulchra</i> - Agat	n ₂₀₁₅ =276, n ₂₀₁₆ =297	0.531	large	Cliff's delta
	<i>Acropora pulchra</i> - Tumon	n ₂₀₁₅ =511, n ₂₀₁₆ =860	0.679	large	Cliff's delta
	<i>Acropora muricata</i> - Tumon	n ₂₀₁₅ =523, n ₂₀₁₆ =547	-0.336	medium	Cliff's delta
	<i>Acropora muricata</i> - Agat	n ₂₀₁₅ =308, n ₂₀₁₆ =866	-0.456	medium	Cliff's delta
	<i>Acropora cf. muricata</i> - Agat	n ₂₀₁₅ =1167, n ₂₀₁₆ =937	-0.411	medium	Cliff's delta
	<i>Acropora cf. intermedia</i> - Tumon	n ₂₀₁₅ =20, n ₂₀₁₆ =154	0.409	medium	Cliff's delta
	<i>Acropora aspera</i> - Achang	n ₂₀₁₅ =410, n ₂₀₁₆ =1002	0.003	negligible	Cliff's delta
d	Short spermary size for <i>A. pulchra</i> between populations	Sample Size	Effect Size Estimate	Effect Size Magnitude	Test
	Agat vs. Achang	n _{Agat} =25, n _{Achang} =42	1.11	large	Cohen's d
	Tumon vs. Achang	n _{Tumon} =12, n _{Achang} =42	1.66	large	Cohen's d
e	Spermary size for <i>A. muricata</i> between populations	Sample Size	Effect Size Estimate	Effect Size Magnitude	Test
	Agat vs. Tumon (Long)	n _{Agat} =96, n _{Tumon} =65	-0.602	large	Cliff's delta
	Agat vs. Tumon (Short)	n _{Agat} =47, n _{Tumon} =63	-0.626	large	Cliff's delta
f	Fecundity index for <i>A. pulchra</i> between populations	Sample Size	Effect Size Estimate	Effect Size Magnitude	Test
	Agat vs. Tumon (2016)	n _{Agat} =63, n _{Tumon} =136	-0.323	small	Cliff's delta
	Tumon vs. Achang (2016)	n _{Tumon} =136, n _{Achang} =106	-0.215	small	Cliff's delta
	Agat vs. Tumon (2015)	n _{Agat} =74, n _{Tumon} =94	-0.348	medium	Cliff's delta
	Agat vs. Togcha (2015)	n _{Tumon} =74, n _{Togcha} =24	0.431	medium	Cliff's delta
g	Fecundity index for <i>A. muricata</i> between populations	Sample Size	Effect Size Estimate	Effect Size Magnitude	Test
	Agat vs. Tumon (2015)	n _{Agat} =86, n _{Tumon} =55	-0.261	small	Cliff's delta
	Agat vs. Tumon (2016)	n _{Agat} =85, n _{Tumon} =88	-1.088	large	Cohen's d
h	Fecundity index between years	Sample Size	Effect Size Estimate	Effect Size Magnitude	Test
	<i>Acropora pulchra</i> - Agat	n ₂₀₁₅ =74, n ₂₀₁₆ =63	-0.294	small	Cliff's delta
	<i>Acropora pulchra</i> - Tumon	n ₂₀₁₅ =94, n ₂₀₁₆ =136	-0.211	small	Cliff's delta
	<i>Acropora cf. muricata</i> - Agat	n ₂₀₁₅ =124, n ₂₀₁₆ =148	0.162	small	Cliff's delta
	<i>Acropora aspera</i> - Achang	n ₂₀₁₅ =180, n ₂₀₁₆ =130	-0.612	large	Cliff's delta

Table S2: Post-hoc test results for the proportion of reproductive branches for *Acropora aspera*, *A. pulchra*, *A. cf. intermedia*, *A. cf. muricata*, and *A. muricata* and corresponding year and/or site. See Methods -> Data Analysis for details.

a	Reproductive branches for <i>A. pulchra</i> between populations	Sample Size	<i>p</i> -value	df	Test
	Agat vs. Achang (2016)	n _{Agat} =27, n _{Achang} =27	0.029	1	Chi-square (4.77)
	Agat vs. Tumon (2016)	n _{Agat} =27, n _{Tumon} =30	0.006	1	Chi-square (7.60)
	Tumon vs. Achang (2016)	n _{Tumon} =30, n _{Achang} =27	0.812	1	Chi-square (0.057)
	Agat vs. Togcha (2015)	n _{Agat} =24, n _{Togcha} =6	1	-	Fisher Exact
	Agat vs. Tumon (2015)	n _{Agat} =24, n _{Tumon} =24	0.007	1	Chi-square (7.20)
	Tumon vs. Togcha (2015)	n _{Tumon} =24, n _{Togcha} =6	0.12	-	Fisher Exact
b	Reproductive branches for <i>A. muricata</i> between populations	Sample Size	<i>p</i> -value	df	Test
	<i>Acropora muricata</i> (2015)	n _{Agat} =18, n _{Tumon} =22	0.371	1	Chi-square (0.80)
	<i>Acropora muricata</i> (2016)	n _{Agat} =28, n _{Tumon} =27	0.887	1	Chi-square (0.02)
c	Reproductive branches between years for all populations	Sample Size	<i>p</i> -value	df	Test
	<i>Acropora pulchra</i> - Agat	n ₂₀₁₅ =24, n ₂₀₁₆ =27	0.96	1	Chi-square (0.003)
	<i>Acropora pulchra</i> - Tumon	n ₂₀₁₅ =24, n ₂₀₁₆ =30	0.736	-	Fisher Exact
	<i>Acropora muricata</i> - Tumon	n ₂₀₁₅ =22, n ₂₀₁₆ =27	0.426	1	Chi-square (0.63)
	<i>Acropora muricata</i> - Agat	n ₂₀₁₅ =18, n ₂₀₁₆ =28	0.864	1	Chi-square (0.029)
	<i>Acropora cf. muricata</i> - Agat	n ₂₀₁₅ =20, n ₂₀₁₆ =30	0.556	-	Fisher Exact
	<i>Acropora cf. intermedia</i> - Tumon	n ₂₀₁₅ =24, n ₂₀₁₆ =30	0.117	-	Fisher Exact
	<i>Acropora aspera</i> - Achang	n ₂₀₁₅ =30, n ₂₀₁₆ =27	0.258	-	Fisher Exact

Table S3: Summary of dates and times of *Acropora aspera*, *A. pulchra*, *A. cf. intermedia*, *A. cf. muricata*, and *A. muricata* spawning activity and egg pigmentation around Guam.

Species	Population	Spawning Month(s)		No. of Days After Full Moon when Spawning Observed <i>in situ</i> ^c	Time of Spawning (h) ^d	<i>In situ</i> Observation Date of Pigmented Eggs ^e
		2015 ^a	2016 ^b			
<i>Acropora pulchra</i>	Achang	-	May	-	-	5/22/2016
	Agat	May	April	2, 3, 4, 7	2030 - 2100	4/13/2017
	Togcha	May	-	-	-	-
	Tumon	May	April May	0,3	2015 -2045	5/16/2016 4/13/2017 4/22/2017 5/7/2017 5/11/2017
<i>Acropora muricata</i>	Agat	May	April	2	2030 - 2100	4/13/2017
	Tumon	May	April	0	2030 - 2045	4/16/2016 4/15/2017 4/22/2017
<i>Acropora cf. muricata</i>	Agat	May	April	-	-	-
<i>Acropora cf. intermedia</i>	Tumon	April	April	-	-	4/17/2016
			April	-	-	5/18/2016
<i>Acropora aspera</i>	Achang	September	September October	-	-	-

a: Spawning inferred from dissected oocytes from samples collected in 2015

b: Spawning inferred from dissected oocytes and spermaries from samples collected in 2016

c: Determined from *in situ* observations at Agat and Tumon in April of 2017 and at Tumon in May of 2017

d: Determined from *in situ* observations at Agat and Tumon in April of 2017 and at Tumon in May of 2017

e: Determined by haphazardly cracking and observing branches for pigmentation 2015-2017

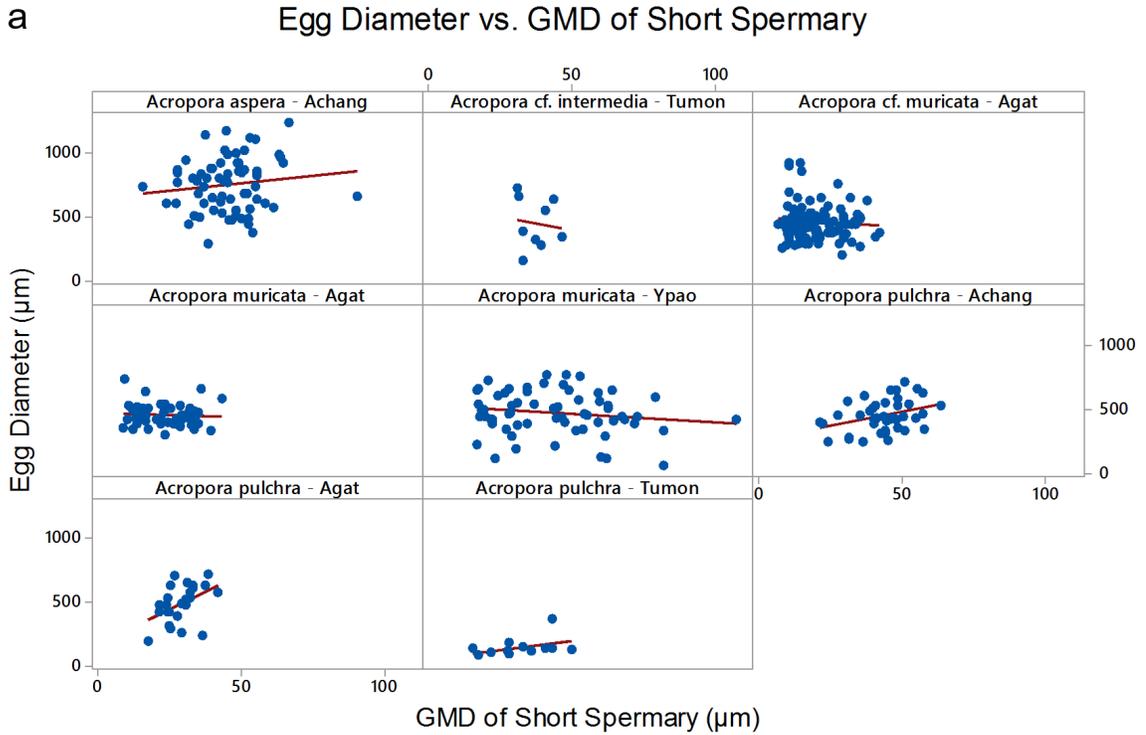


Figure S1: Linear regressions between **(a)** egg diameter and the GMD of short spermaries, **(b)** egg diameter and the GMD of long spermaries, **(c)** egg diameter and the fecundity index, **(d)** the GMD of short spermaries and the fecundity index, **(e)** the GMD of long spermaries and the fecundity index, **(f)** the GMD of both spermaries (total) and egg diameter, **(g)** the GMD of both spermaries (total) and the fecundity index, for *Acropora aspera*, *A. pulchra*, *A. cf. intermedia*, *A. cf. muricata*, and *A. muricata* at each site. See Methods -> Data Analysis for details.

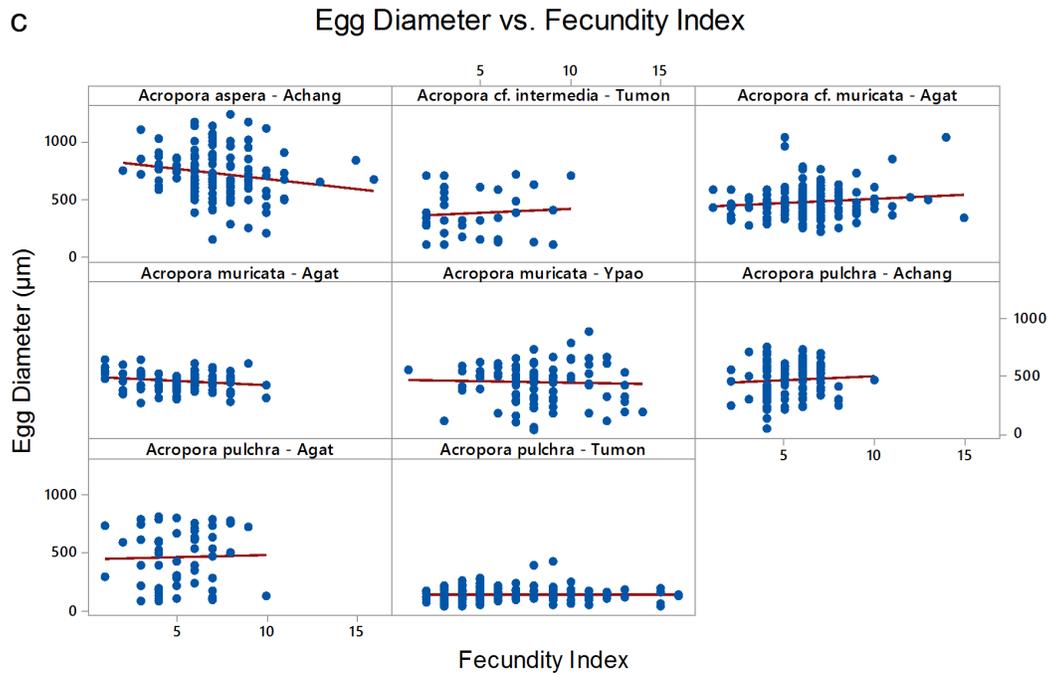
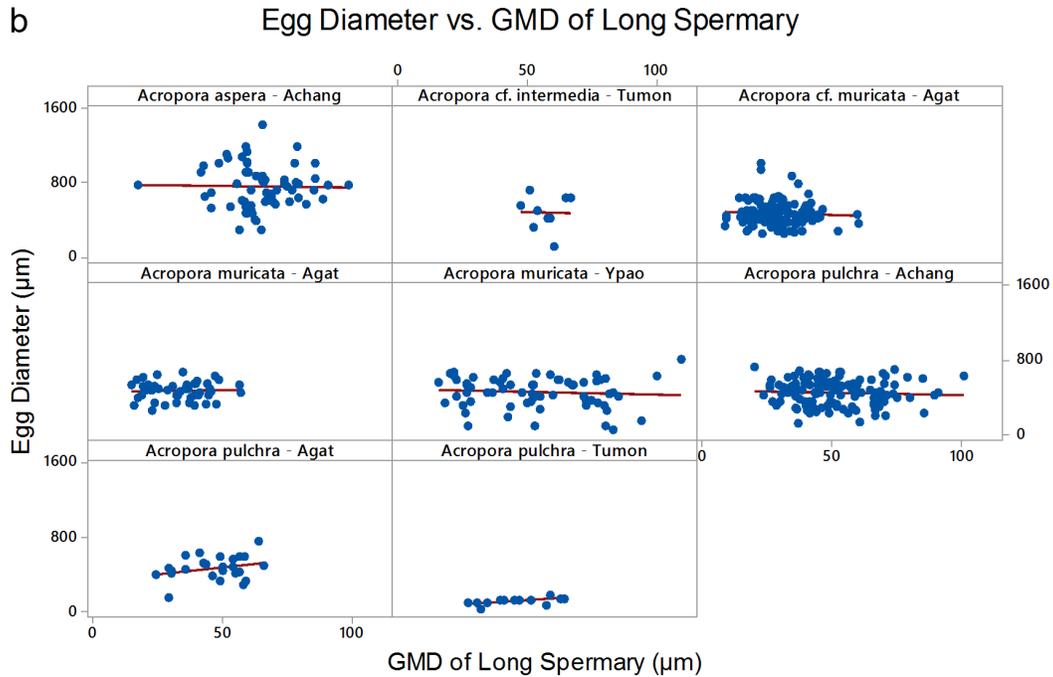


Figure S1: Linear regressions between (a) egg diameter and the GMD of short spermaries, (b) egg diameter and the GMD of long spermaries, (c) egg diameter and the fecundity index, (d) the GMD of short spermaries and the fecundity index, (e) the GMD of long spermaries and the fecundity index, (f) the GMD of both spermaries (total) and egg diameter, (g) the GMD of both spermaries (total) and the fecundity index, for *Acropora aspera*, *A. pulchra*, *A. cf. intermedia*, *A. cf. muricata*, and *A. muricata* at each site. See Methods -> Data Analysis for details.

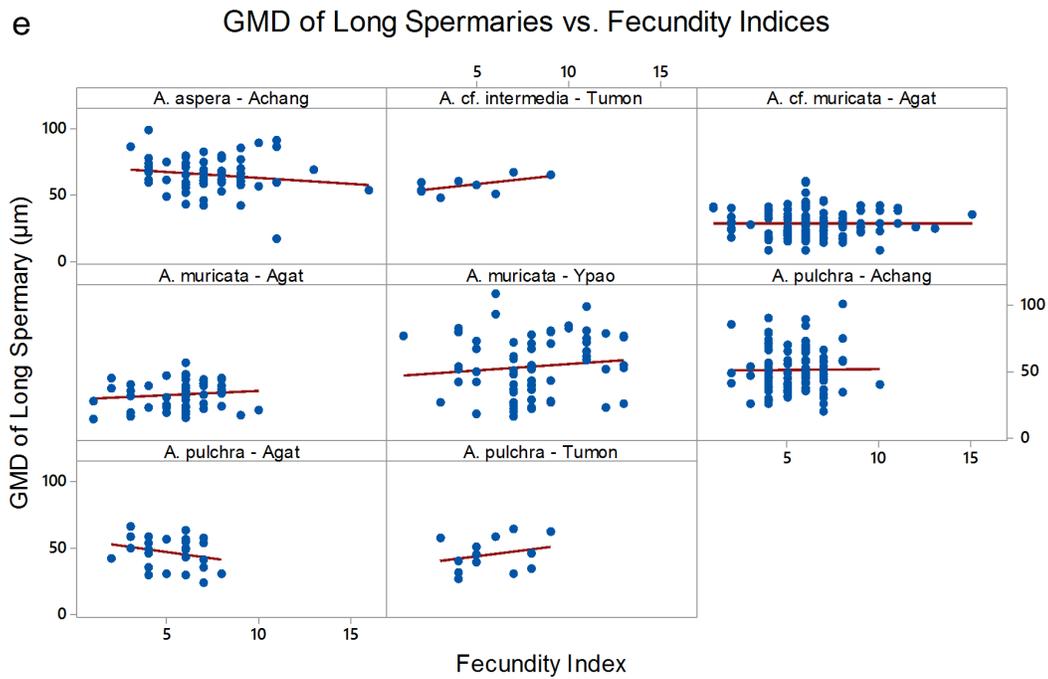
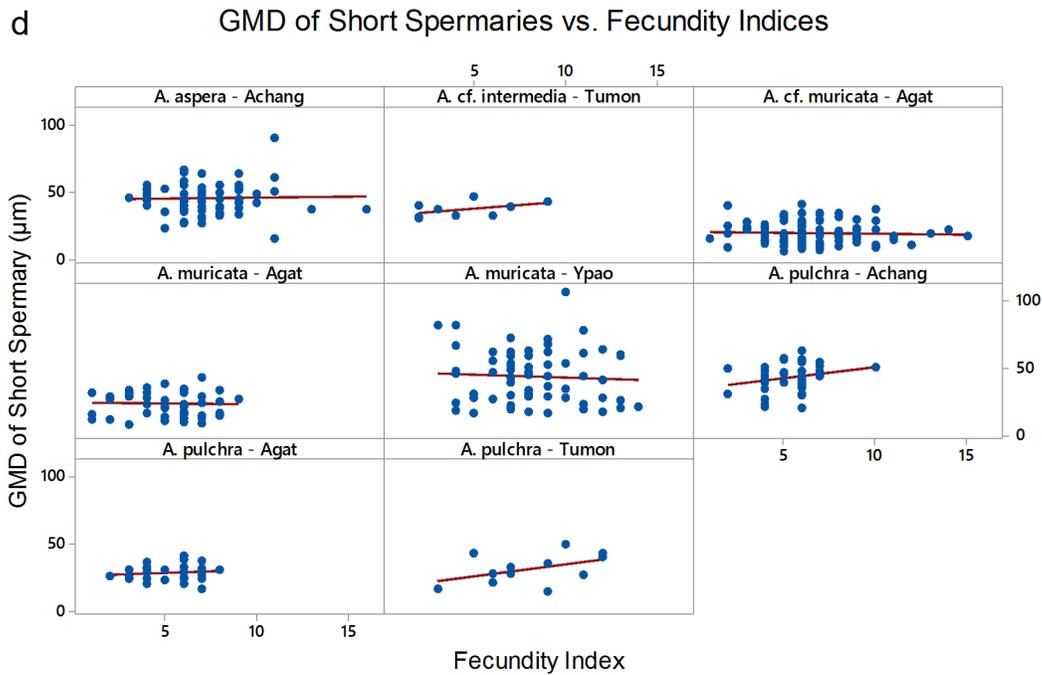


Figure S1: Linear regressions between (a) egg diameter and the GMD of short spermaries, (b) egg diameter and the GMD of long spermaries, (c) egg diameter and the fecundity index, (d) the GMD of short spermaries and the fecundity index, (e) the GMD of long spermaries and the fecundity index, (f) the GMD of both spermaries (total) and egg diameter, (g) the GMD of both spermaries (total) and the fecundity index, for *Acropora aspera*, *A. pulchra*, *A. cf. intermedia*, *A. cf. muricata*, and *A. muricata* at each site. See Methods -> Data Analysis for details.

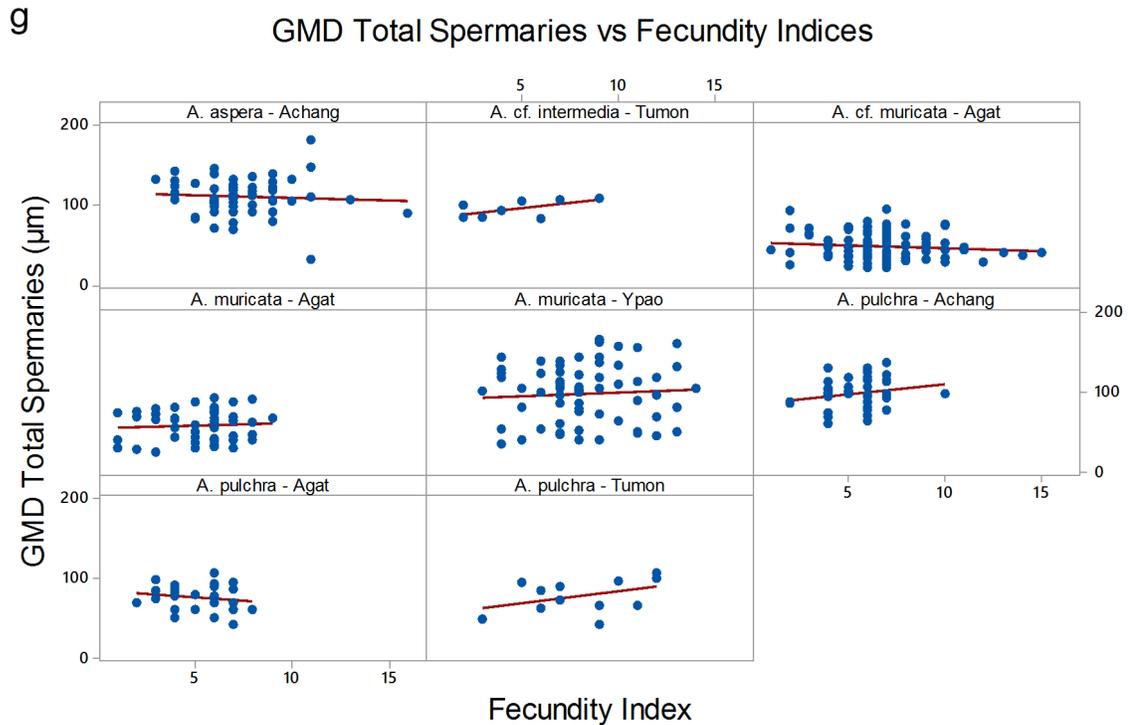
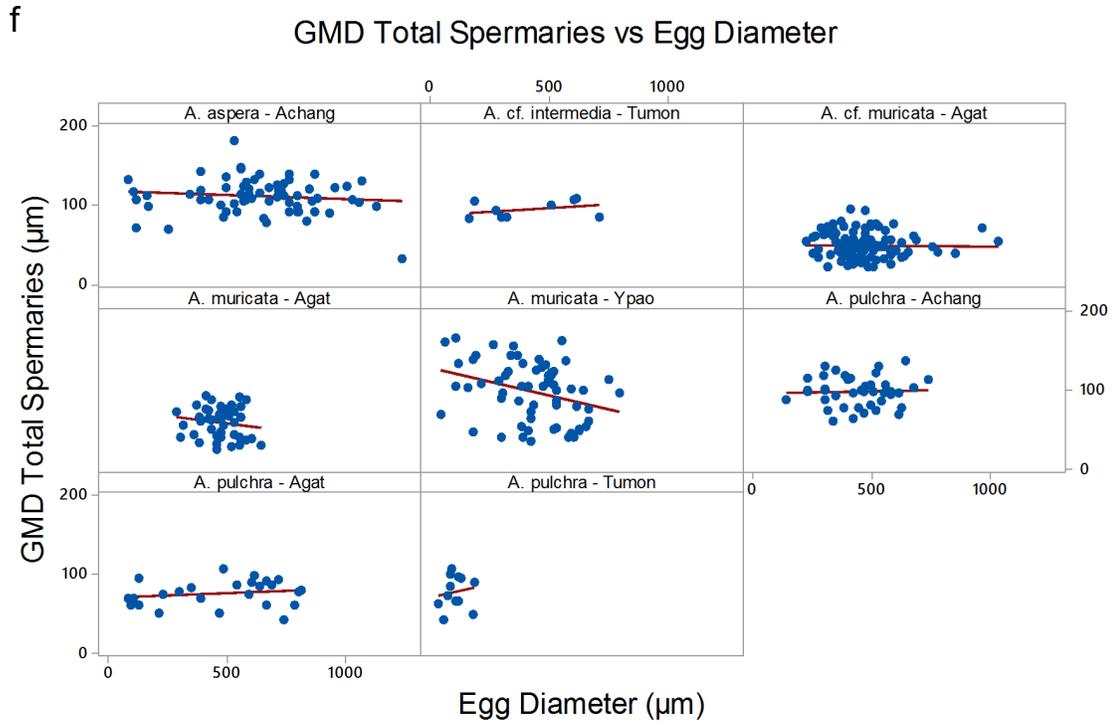


Figure S1: Linear regressions between (a) egg diameter and the GMD of short spermaries, (b) egg diameter and the GMD of long spermaries, (c) egg diameter and the fecundity index, (d) the GMD of short spermaries and the fecundity index, (e) the GMD of long spermaries and the fecundity index, (f) the GMD of both spermaries (total) and egg diameter, (g) the GMD of both spermaries (total) and the fecundity index, for *Acropora aspera*, *A. pulchra*, *A. cf. intermedia*, *A. cf. muricata*, and *A. muricata* at each site. See Methods -> Data Analysis for details.

Table S4: Maximum (i.e. stage IV and V) mean egg diameters and egg diameter range, in 2015 and 2016, for *Acropora aspera*, *A. pulchra*, *A. cf. intermedia*, *A. cf. muricata*, and *A. muricata* populations.

		2015		2016	
		Maximum Mean Egg Diameter (µm)	Egg Diameter Range (µm)	Maximum Mean Egg Diameter (µm)	Egg Diameter Range (µm)
<i>Acropora pulchra</i>	Togcha	575.9 +/- 78.9 (n=78)	414 - 759 (n=78)	-	-
	Achang	-	-	522.0 +/- 138.3 (n=268)	116.5 - 847.0 (n=268)
	Agat	695.1 +/- 132.9 (n=166)	437 - 1081 (n=166)	506.2 +/- 261.8 (n=159)	31.8 - 910.6 (n=159)
	Tumon	297.8 +/- 146.5 (n=56)	46 - 552 (n=56)	139.2.3 +/- 67.5 (n=606)	21.2 - 487.0 (n=606)
<i>Acropora muricata</i>	Agat	441.8 +/- 88.6 (n=261)	230 - 690 (n=261)	488.1 +/- 71.7 (n=298)	317.6 - 794.1 (n=298)
	Tumon	539.4 +/- 188.5 (n=130)	69 - 1035 (n=130)	561.3 +/- 121.4 (n=524)	127.1 - 890.0 (n=524)
<i>Acropora cf. muricata</i>	Agat	468.1 +/- 88.2 (n=453)	253 - 713 (n=453)	504.4 +/- 78.4 (n=535)	338.8 - 794.1 (n=535)
<i>Acropora cf. intermedia</i>	Tumon	563.0 +/- 93.9 (n=20)	391 - 736 (n=20)	656.7 +/- 77.5 (n=40)	487.0 - 825.9 (n=40)
<i>Acropora aspera</i>	Achang	627.8 +/- 146.8 (n=410)	207 - 1150 (n=410)	728.8 +/- 207.8 (n=1002)	105.9 - 1429.4 (n=1002)

		Maximum Mean Long Spermary Length (µm)	Long Spermary Length Range (µm)	Maximum Mean Short Spermary Length (µm)	Short Spermary Length Range (µm)
<i>Acropora pulchra</i>	Achang	1003.9 +/- 158.8 (n=27)	391.8 - 1715.3 (n=27)	624.9 +/- 139.4 (n=54)	285.9 - 889.4 (n=54)
	Agat	973.2 +/- 219.5 (n=12)	714.2 - 1461.1 (n=12)	501.2 +/- 84.7 (n=12)	391.8 - 688.2 (n=12)
	Tumon	741.2 +/- 90.5 (n=3)	645.9 - 825.9 (n=3)	402.3 +/- 107.5 (n=3)	285.9 - 497.3 (n=8)
<i>Acropora muricata</i>	Agat	876.5 +/- 147.8 (n=27)	645.9 - 1154.1 (n=27)	516.5 +/- 106.7 (n=27)	338.8 - 741.2 (n=27)
	Tumon	1605.0 +/- 539.0 (n=24)	815.3 - 2647.0 (n=24)	1080.0 +/- 358.9 (n=24)	540.0 - 1736.4 (n=24)
<i>Acropora cf. muricata</i>	Agat	824.2 +/- 236.0 (n=45)	338.8 - 1471.7 (n=45)	451.3 +/- 149.9 (n=42)	158.8 - 751.7 (n=42)
<i>Acropora cf. intermedia</i>	Tumon	1168.2 +/- 189.8 (n=9)	931.7 - 1503.5 (n=9)	681.2 +/- 101.0 (n=9)	540.0 - 825.9 (n=9)
<i>Acropora aspera</i>	Achang	1390.0 +/- 335.6 (n=58)	402.3 - 2329.4 (n=58)	747.7 +/- 180.1 (n=58)	233.0 - 1185.9 (n=58)

Table S5: Maximum mean long and short spermary lengths and spermary ranges, in 2016, for *Acropora aspera*, *A. pulchra*, *A. cf. intermedia*, *A. cf. muricata*, and *A. muricata* populations.

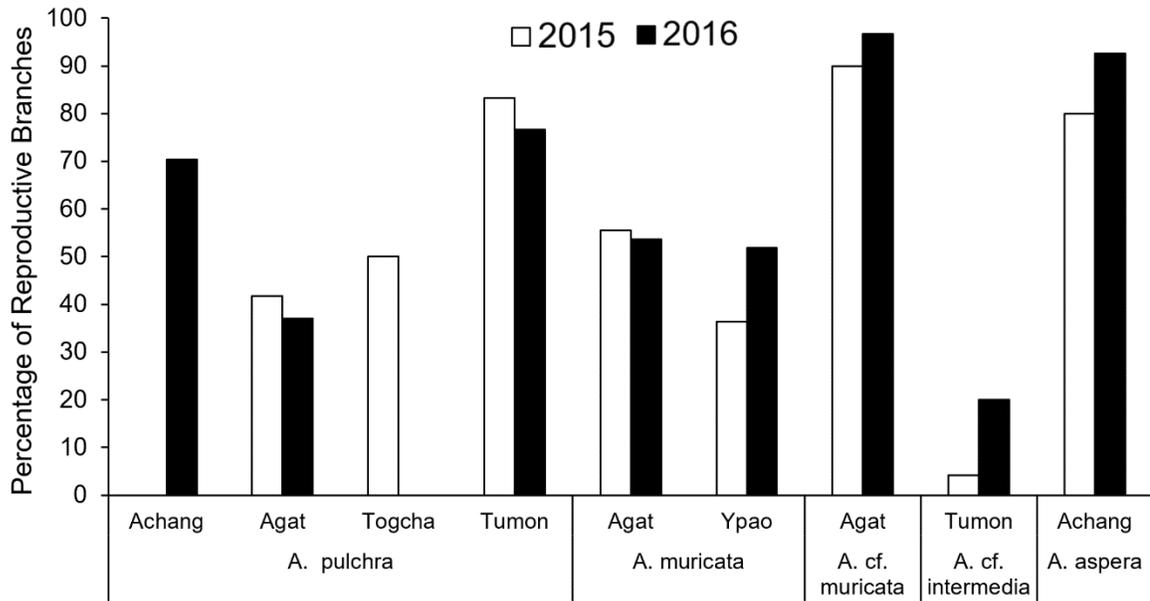


Figure S2: Proportion of reproductive branches for *Acropora aspera*, *A. pulchra*, *A. cf. intermedia*, *A. cf. muricata*, and *A. muricata* at each site in 2015 and 2016. Proportion of reproductive branches was calculated in late gametogenesis (≤ 2 months before spawning) and when ≤ 5 polyps contained eggs.

Table S6: Mean annual fecundity (estimated number of eggs/polyp per cm^2) for *Acropora aspera*, *A. pulchra*, *A. cf. intermedia*, *A. cf. muricata*, and *A. muricata* at each site in 2015 and 2016. Calculated by multiplying the average number of eggs per polyp by the average number of polyps in one cm^2 .

	Species by Year		Species by Population	
	2015	2016	2015	2016
<i>Acropora pulchra</i>	180	221	Agat 140	Agat 179
			Tumon 205	Tumon 258
			Togcha 206	Achang 198
<i>Acropora muricata</i>	126	127	Agat 115	Agat 101
			Tumon 143	Tumon 152
<i>Acropora cf. muricata</i>	201	177		
<i>Acropora cf. intermedia</i>	83	116		
<i>Acorpora aspera</i>	105	211		



Figure S3: Egg pigmentation (i.e. light pink and peach coloration) prior to spawning for Guam's staghorn *Acropora*

