Received: 5 December 2023 | Accepted: 10 October 2024

DOI: 10.1111/ivb.12447

ORIGINAL ARTICLE

Invertebrate Biology WILEY

Ex situ spawning, larval development, and settlement in massive reef-building corals (*Porites*) in Palau

Matthew-James Bennett[1] | Carsten G. B. Grupstra[2] | Jeric Da-Anoy[2] | Maikani Andres[3,4] | Daniel Holstein[5] | Ashley Rossin[5] | Sarah W. Davies[2] | Kirstin S. Meyer-Kaiser[4]

[1]MARE, Guia Marine Laboratory, Faculty of Sciences, University of Lisbon, Cascais, Portugal

[2]Boston University, Boston, Massachusetts, USA

[3]Palau International Coral Reef Center, Koror, Palau

[4]Woods Hole Oceanographic Institution, Woods Hole, Massachusetts, USA

[5]Lousiana State University, Baton Rouge, Louisiana, USA

Correspondence
Kirstin S. Meyer-Kaiser, Woods Hole Oceanographic Institution, 266 Woods Hole Road, Woods Hole, MA 02543, USA.
Email: kmeyer@whoi.edu

Funding information
This material is based upon work supported by the National Science Foundation under Grant Nos. OCE-2048678 (KMK) and OCE-2048589 (SWD).

Abstract

Reproduction, embryological development, and settlement of corals are critical for survival of coral reefs through larval propagation. Yet, for many species of corals, a basic understanding of the early life-history stages is lacking. In this study, we report our observations for *ex situ* reproduction in the massive reef-building coral *Porites* cf. *P. lobata* across 2 years. Spawning occurred in April and May, on the first day after the full moon with at least 2 h of darkness between sunset and moonrise, on a rising tide. Only a small proportion of corals observed had mature gametes or spawned (14–35%). Eggs were 185–311 μm in diameter, spherical, homogenous, and provisioned with 95–155 algal cells (family Symbiodiniaceae). Males spawned before females, and *ex situ* fertilization rates were high for the first 2 h after egg release. Larvae were elliptical, ~300 μm long, and symbiotic. Just 2 days after fertilization, many larvae swam near the bottom of culture dishes and were competent to settle. Settlers began calcification 2 days after metamorphosis, and tentacles were developed 10 days after attachment. Our observations contrast with previous studies by suggesting an abbreviated pelagic larval period in *Porites* cf. *P. lobata*, which could lead to the isolation of some populations. The high thermal tolerance and broad geographic range of *Porites* cf. *P. lobata* suggest that this species could locally adapt to a wide range of environmental conditions, especially if larvae are locally retained. The results of this study can inform future work on reproduction, larval biology, dispersal, and recruitment in *Porites* cf. *P. lobata*, which could have an ecological advantage over less resilient coral species under future climate change.

KEYWORDS
Pacific, pelagic larval duration, planula, reproduction, stony coral, vertical transmission

1 | INTRODUCTION

Sexual reproduction, larval dispersal, and recruitment are essential processes for the long-term maintenance of coral reefs in the face of myriad threats (Hughes et al., 2017; Hughes & Tanner, 2000; Richmond et al., 2018). Sexually produced larvae can replenish coral populations with genetically variable individuals to withstand present selective pressures. Environmental stressors such as elevated temperatures can result in reduced reproductive capacity in corals, which can lead to reductions in recruitment, abundances, and genetic diversity of corals on a reef (Fisch et al., 2019; Hagedorn et al., 2016; Henley et al., 2022; Humphrey et al., 2008). Improving our understanding of

the fundamental life history stages that influence key population dynamics, such as sexual reproduction, is essential as efforts to manage coral reefs globally expand (Edwards et al., 2024).

Maintaining species and genetic diversity in coral populations is vital to ensuring coral reef longevity and the long-term success of restoration initiatives (Mcleod et al., 2019; Shaver et al., 2022). Recent and past studies on coral reproduction have concentrated on a limited range of species, growth forms, and reproductive modes, with 30% of recent studies involving hermaphroditic branching corals of the genus *Acropora* (Boström-Einarsson et al., 2020). This limited focus presents a challenge for predicting changes in a reef's community structure. Recent work has emphasized the importance of incorporating corals with diverse life histories into reproductive studies and restoration strategies. However, basic information on reproductive biology is missing for many species, making their inclusion in such studies more challenging (Guest et al., 2023). The ability to predict the timing of reproductive events in situ—which can vary across the geographic range of a given species—requires extensive observational data to allow practitioners and managers to effectively plan research operations and limit activities that may disrupt this important natural process (Baird et al., 2009, 2022; Kenyon, 1995; Marhaver et al., 2015).

Active coral reef restoration approaches that use sexual propagation through assisted fertilization and recruitment have the potential to repopulate degraded reefs (dela Cruz & Harrison, 2017). These interventions are increasingly highlighted as an important strategy to promote the survival of coral reefs globally, while efforts to reduce greenhouse gas emissions and implement informed ecosystem management plans continue (Kleypas et al., 2021; Suggett et al., 2024; Vardi et al., 2021). However, the efficacy and productive yield of these initiatives is limited by the lack of available information for many coral species (Boström-Einarsson et al., 2020; Guest et al., 2023).

In corals, sexual reproduction presents a diverse range of strategies and reproductive modes, even within broadcast-spawning species (Guest et al., 2012). Most *ex situ* spawning studies or restoration efforts have concentrated on hermaphroditic species because the gamete bundles produced by these species, which encase both eggs and sperm, facilitate the identification of fecund adult colonies and the collection and concentration of gametes (Boström-Einarsson et al., 2020). In gonochoric species in which sperm is released directly into the water column, the collection of highly concentrated sperm can be more challenging as the sperm dilutes on release. Lower sperm concentrations can result in lower *in vitro* fertilization success (dela Cruz & Harrison, 2020; Nozawa et al., 2015). Sperm limitation has also been proposed as a factor leading to reduced natural fertilization rates as reefs degrade and adult colonies become rarer and more dispersed (Levitan & Petersen, 1995). In response, cryopreservation of sperm has been used in *in vitro* fertilization efforts to boost genetic diversity of corals and promote desirable genotypes (e.g., Grosso-Becerra et al., 2021). External fertilization success in corals can additionally be impacted by the specific timing after release when compatible gametes encounter each other, and the duration that they remain in contact (dela Cruz & Harrison, 2020).

Porites cf. *P. lobata* is a gonochoric broadcast spawning coral (i.e., with separate male and female colonies) that is widely distributed and abundant in the Pacific Ocean (Baums et al., 2012; Glynn et al., 1994; Sale et al., 2019). It is a dominant reef builder, forming massive mounding colonies, and has demonstrated relatively high tolerance to anthropogenic stressors (Barshis et al., 2018; Levas et al., 2013; Loya et al., 2001). A recent study has suggested that in the genus *Porites* massive colonies constitute multiple morphologically similar species that are specialized to different habitats and environmental conditions (Primov et al., 2024). In fact, there are distinct genetic lineages of *Porites* cf. *P. lobata* across habitat gradients in the low-latitude reefs of Palau, with some lineages exhibiting elevated thermal tolerance (Rivera et al., 2022). Little is known about the early life history stages in this population, which may be a species complex. Previous studies show differences in the timing of spawning events across the geographic range of *Porites* cf. *P. lobata*, and this leads to questions about the relevant environmental cues (Table 1). Only one study has reported direct, in situ observations of gamete release in *Porites* cf. *P. lobata* in Palau, and a second study is based on histological observations (Gouezo et al., 2020; Penland et al., 2004).

Given the broad geographic distribution of *Porites* cf. *P. lobata* across diverse habitat types (which suggests a high degree of plasticity and potential for local adaptation), this species' relatively high resilience to thermal stress, and the ecological importance of this species in coral reef systems, *Porites* cf. *P. lobata* represents a promising candidate for restoration initiatives (Humanes et al., 2021). In this study, we expand the body of knowledge on reproduction and embryological development in *Porites* cf. *P. lobata*. We report histological observations of gametogenesis, direct observations of *ex situ* spawning, describe the release of eggs and sperm, assess fertilization with gametes at varying times after release to determine the window for optimal fertilization success, and track larval development as well as settlement behavior, metamorphosis, and development in the first few weeks after settlement. We conducted initial trials with established methods for cryopreservation of coral sperm, which yielded limited success in this species (see Supporting Information Appendix S1). With this study, we aim to provide detailed descriptions and a baseline record of the reproductive biology and early life stages in this species which can enhance the capacity for further research on this species and aid its integration into restoration programs employing sexual coral propagation as a tool for coral reef conservation.

2 | METHODS

2.1 | Environmental data and colony collection

Colonies matching the gross morphology of *Porites* cf. *P. lobata* (minimum diameter 15 cm) were collected from our six study sites ($n = 7$–30 per site) in the Rock Islands Southern Lagoon (Chelbacheb) in the Republic of Palau (Figure 1; Appendix S1). Colonies were collected around the full moon, from 2 days before to 4 days after the full moon. For smaller colonies, (15–25 cm diameter), the whole colony

TABLE 1 Reproductive timing in *Porites* cf. *P. lobata* reported in various geographic locations. Note that many observations are from histological studies in which the night of spawning could not be accurately determined. All other studies are spawning observations denoted as in situ or *ex situ*.

Country	Site	Year	Month	Days after full moon	Observation type	References
Australia	Heron Island	1978–1979	Dec	N/A	Histology	Kojis and Quinn (1981)
Australia	Orpheus Island	1983	Nov	5	In situ	Babcock et al. (1986)
Chile	Rapa Nui	2013–2014	Feb, Mar	N/A	Histology	Buck-Wiese et al. (2018)
Costa Rica	Caño Island	1985–1991	Apr, May, Sep, Oct	N/A	Histology	Glynn et al. (1994)
Ecuador	Santa Cruz Island	1985–1991	Jan, Apr, May, Sep, Oct	N/A	Histology	Glynn et al. (1994)
Indonesia	Karimunjawa	1995	Oct	3	In situ	Tomascik et al. (1997)
Japan	Sesoko Islands	1986	Jun	2	In situ	Isomura and Fukami (2018)
Palau	Nikko Bay	2002–2003	Apr, May	3–4	In situ	Penland et al. (2004)
Palau	Palau International Coral Reef Center	2017–2018	Apr, May	N/A	Histology	Gouezo et al. (2020)
Palau	Chelbacheb	2022	Apr, May	3–4	*Ex situ*	This study
Palau	Chelbacheb	2023	Apr, May	2–4	*Ex situ*	This study
Panama	Uva Island	1985–1991	Feb, Sep	N/A	Histology	Glynn et al. (1994)
Panama	Taboga Island	1985–1991	Jul, Aug	N/A	Histology	Glynn et al. (1994)
Saudi Arabia	Al Fahal	2012	May	1	In situ	Bouwmeester et al. (2015)
USA (Hawai'i)	Kāne'ohe Bay	1987	Aug, Sep	3–6	Histology	Hodgson (1988)
USA (Hawai'i)	Kāne'ohe Bay	1997	Jun, Jul, Aug	1–4	*Ex situ*	Field (1998); Maté (1998)

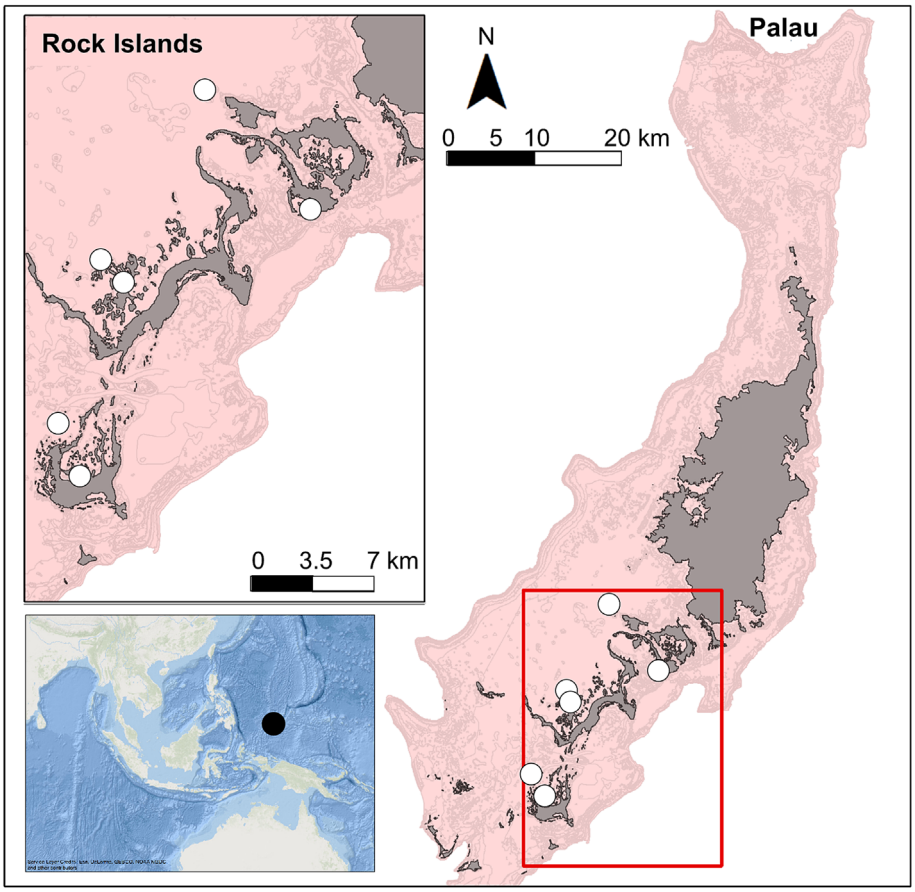

FIGURE 1 Coral collection sites for *Porites* cf. *P. lobata* in the Rock Island Southern Lagoon (Chelbacheb), Palau. Pink indicates the reef platform, and gray represents land.

was collected; for larger colonies (>25 cm diameter), a piece with diameter 15–25 cm was broken off using a hammer and chisel. Corals were housed in individual plastic containers (4.2 L; 17 × 13 × 19 cm) and distributed across flow-through aquariums (3000 L) at the Palau International Coral Reef Center (PICRC) during the observation period. Between observation periods, colonies were returned to nurseries at their site of collection so that the same colonies could be repeatedly observed across years. To increase the likelihood of observing gamete release, additional colonies were collected in each observation period (Table 2, Appendix S1).

Environmental factors that may serve as cues for synchronized gamete release were recorded in situ or sourced from publicly available datasets. Water temperatures were recorded using loggers at ~3 m depth (HOBO TidbiT v2, Onset Corp.) at our six study sites November 2021–May 2023. Temperature measurements were averaged across all sites for each lunar month. The first lunar month of the year begins on the first full moon following the winter solstice, and each lunar month constitutes one lunar cycle (Baird et al., 2022). Tidal amplitude data were obtained from the University of Hawai'i Sea Level Center for the station at Malakal Island, Palau (7°19.80′N, 134°27.28′E; https://uhslc.soest.hawaii.edu/stations/?stn=007#levels). Sunset, moonrise, and twilight times for Koror, Palau, were obtained from the U.S. Naval Observatory (https://aa.usno.navy.mil/data/RS_OneYear).

2.2 | Histological observations

Small tissue samples (~2 cm diameter) were collected with a hammer and chisel either via SCUBA from the same colonies included in the spawning observations (November 2021), or in the laboratory at the Palau International Coral Reef Center (PICRC) after coral collection but prior to spawning (May 2022). Samples were collected from the center of each colony to avoid colony edges where gametes may not be present. Immediately following collection in the lab or the end of the SCUBA dive (i.e., within 20 min of collection), samples were fixed in a solution of 10% neutral buffered formalin in seawater.

Preserved samples were decalcified in 1% EDTA decalcifier solution (5% hydrochloric acid with 5.0 g/L EDTA) for 24–48 h and were stored in 70% ethanol after complete decalcification (Glynn et al., 1991; Szmant-Froehlich et al., 1985). Samples from November 2021 were processed and sectioned at the Seascape Ecology Lab at Louisiana State University, and the remaining samples were processed at the Collaborative Research Laboratory (CoRe) at Boston University. Tissues were dehydrated, cleared, and paraffinized in a Leica ASP6025 tissue processor, embedded in wax blocks using a Leica EG1150H embedding machine, and then cooled in a freezer for 24 h before sectioning. Blocks were sectioned at 5 μm thickness in an oral-to-aboral direction at 300 μm intervals on a Leica RM2125RTS microtome. Three tissue sections for each sample were transferred onto a microscope slide for staining.

Histological tissue sections were stained with hematoxylin and eosin or modified Heidenhain's aniline on a Leica ST5020 multistainer. Sections from 43 individuals were examined for the presence or absence of male and female gametes under a binocular compound microscope (Olympus BH2) with a digital eyepiece camera attachment (Amscope). Gametes (oocytes and spermatocytes) were staged from I to V following the classification of Szmant-Froehlich et al. (1985).

TABLE 2 Observations of *ex situ* spawning in colonies of *Porites* cf. *P. lobata* in Palau. Numbers in parentheses represent colonies that also spawned on the previous night. Number of colonies is the total number of colonies observed in aquariums. Consecutive days with no spawning observed are condensed to a single line.

Days after full moon	Date	Number of colonies	Number of spawning (repeat) males	Start of sperm release (min after sunset)	Number of spawning (repeat) females	Start of egg release (min after sunset)
3	Apr 20, 2022	34	4	90–258	2	190–320
4	Apr 21, 2022	54	1 (1)	58	3 (2)	168–217
5 to 8	Apr 22–24, 2022	54	0	N/A	0	N/A
−2 to +3	May 14–19, 2022	63	0	N/A	0	N/A
4	May 20, 2022	63	1	198	1	172–210
5 to 9	May 21–25, 2022	63	0	N/A	0	N/A
1 to 5	Nov 10–14, 2022	63	0	N/A	0	N/A
−2 to +2	April 4–8, 2023	96	0	N/A	0	N/A
3	April 9, 2023	96	4	149–199	3	179–229
4	April 10, 2023	96	6	70–220	5 (1)	170–335
5 to 8	April 11–14, 2023	96	0	N/A	0	N/A
−2 to +1	May 4–7, 2023	99	0	N/A	0	N/A
2	May 8, 2023	99	1	183	0	N/A
3	May 9, 2023	99	6	139–209	6	159–276
4	May 10, 2023	99	6 (3)	69–149	8 (1)	159–534
5 to 7	May 11–13, 2023	99	0	N/A	0	N/A

2.3 | Spawning timing and gamete release

Ex situ colonies were monitored in April and May 2022, November 2022, and April and May 2023 to observe spawning phenology and behavior. On each observation day, the water level in aquariums was lowered to ~15 cm shortly after sunset. Therefore, each coral was isolated in its own container during the observations, but containers were surrounded by running seawater so that the water remained at ambient ocean temperature. Spawning observations began <1 h after sunset and continued for 4–6 h each day. All colonies were checked for gamete release every 10–20 min, and the frequency and time of gamete release was noted, as well as colony sex. Observations continued for 4–10 days in a given month (Appendix S1).

2.4 | Gamete collection and fertilization

Sperm were collected directly from colonies using a large plastic pipette during release, thereby maintaining high sperm concentrations, and transferred to 2.1-L hydrophobic plastic containers. Eggs were allowed to float to the surface so they could more easily be collected by pipette or by skimming the surface with a plastic tri-pour beaker or petri dish, before being transferred to 400-mL plastic containers with 5 μm filtered seawater (FSW). Gametes were kept separate until a sufficient number of parent colonies had spawned to begin a batch cross (minimum three males and one female). Sperm from selected male colonies were pooled in a single container, and eggs (from $n = 1$ female per batch cross) were gently poured into this pool, allowing fertilization to commence. Gamete transfer was achieved by gently pouring only the eggs from the surface with as little seawater as possible to limit sperm dilution. For all crosses, gametes were separated 45–60 min after fertilization began. The surface layer with fertilized eggs was poured into clean plastic containers, eggs were rinsed with FSW, and then eggs were gently split into multiple containers filled with FSW to dilute any remaining sperm and lower the egg density.

In 2023, a subset of eggs was held apart from sperm until a set time after spawning in order to assess the impact of egg age on fertilization success. A small aliquot (~10 mL) of concentrated sperm from three males and ~1000 eggs from one female were added to triplicate wells of a six-well plate for each time point. Cell strainers (70 μm mesh) were used to separate eggs from sperm ~45 min after fertilization began, and eggs were gently rinsed into a 50 ml beaker filled with FSW. Fertilization success was evaluated visually 2–3 h after fertilization began by observing a subsample of the eggs from each cross under a dissecting microscope (Leica S9i) and counting whole (undivided) eggs and dividing embryos. In addition, we observed motility of a subsample of sperm periodically using a phase-contrast microscope (Amscope).

2.5 | Embryological observations

Developing embryos and larvae were observed at least daily using a dissecting microscope and photographed using white light and blue light with a yellow filter (Nightsea) to detect fluorescence. Embryos and larvae were cultured in hydrophobic plastic containers (2.1 L) in still FSW at ambient temperature (~30°C), and water was changed every 1–2 days.

Egg size was estimated by measuring the diameter of 160 individual eggs using the straight-line selection tool in ImageJ. Abundances of algal symbiont cells (Symbiodiniaceae) were estimated in eggs and larvae using the cell counter function in ImageJ. Only a projection of the three-dimensional surface area of each egg or larva was visible in the images, so the number of observed algae was used to extrapolate abundance per individual. The plan area of the egg or larva where algae could be clearly discerned in the image was measured using the oval select function. The diameter of the individual was measured using the straight-line function and used to calculate the three-dimensional surface area of the egg or larva as

$$\text{Area} = 4\pi\left(\frac{d}{2}\right)^2$$

For elliptical larvae, two diameters were measured (long and short axes) and used to calculate surface area as

$$\text{Area} = 4\pi\left(\frac{d_1}{2}\right)\left(\frac{d_2}{2}\right)$$

The number of algae observed was divided by the plan area and multiplied by the three-dimensional surface area to yield the approximate number of cells in a larva. This image analysis method was compared to the standard method of quantifying algae from a known number of homogenized larvae using a hemocytometer. Three replicate solutions with 50 larvae homogenized in 100 μL FSW were prepared before adding 10 μL to the counting chamber. Both methods yielded similar ranges of algae per larva, so we report image analysis results for algae per egg and per larva in this study.

2.6 | Settlement observations

Two days post-fertilization, a subset of larvae were exposed to limestone tiles that had been pre-conditioned for 1 month on the PICRC house reef, or to unconditioned microscope slides with or without the addition of crushed crustose coralline algae (CCA, multiple species combined following methods of Pollock et al. (2017)). Approximately 30 larvae were added to a 2.1-L hydrophobic plastic container with settlement substrata (tiles or glass slides). Settlers were observed daily and photographed using a dissecting microscope with white light and fluorescence.

3 | RESULTS

3.1 | Histological observations

Histological analysis of tissues revealed the presence of oocytes and spermatocysts containing spermatocytes in May 2022 but not in

November 2021 (Figures 2A and 3). Out of the 43 colonies sampled in May, after the April and May 2022 spawning events, only 4.65% (2/43) colonies had oocytes and 13.95% (6/43) had spermatocysts (Figure 2A). The majority of the oocytes and spermatocysts were stage IV (Figure 3). The average size of mature (stage IV) oocytes was 186.98 ± 31.35 μm (mean ± *SD*), and stage I–III oocytes were 83.12 ± 23.48 μm (Figure 2B). No hermaphroditic colonies (with both oocytes and spermatocysts) were found (Figure 3).

3.2 | Spawning timing and gamete release

Spawning was observed 3–4 days after the full moon in April and May (Table 2). Gamete release times ranged 1–4 h after sunset with the peak release (~70% of spawning colonies) between 1.5 and 3.5 h after sunset. Males began spawning earlier than females, and gamete release continued in pulses for both sexes for around 30 min. A small proportion of colonies (~8%) released gametes on more than one night in a given month.

In both 2022 and 2023, spawning coincided with increasing water temperatures. Lunar months 3–5 each had higher mean water temperatures than the previous lunar month, and spawning occurred in lunar months 4 and 5 in both years (Figure 4). Spawning occurred exclusively on nights when moonrise was at least 2 h after sunset, providing a significant dark period (Figure 5). Additionally, colonies spawned on a rising tide on nights when low tide occurred after sunset and high tide occurred on or after midnight (Figure 6).

Sperm had a small head with a pointed tip and wider base, plus a long flagellum (Figure 7). Sperm release resembled white smoke and occurred in pulses that were each several minutes apart (Figure 7). Mature oocytes could be observed under a dissecting microscope in tissue chips taken from gravid adult colonies prior to spawning (Figure 7). Female colonies released light pink-brown eggs that were solitary or in clusters of <5 eggs; the eggs were weakly buoyant and floated very slowly to the surface of the water (Figure 7). Eggs were spherical, homogeneous in terms of shape and size, and had a diameter of 233.7 ± 18.1 μm (mean ± *SD*) (Figure 8). The mean algal symbiont concentration was 121 ± 26 cells per egg.

3.3 | Gamete age assays

Fertilization success was high for the first 2–2.5 h after spawning began for a given female (Figure 9). Eggs older than 2.5 h had much lower fertilization success, with nearly zero fertilization 3.5 h after spawning began. We also observed some variation in egg viability,

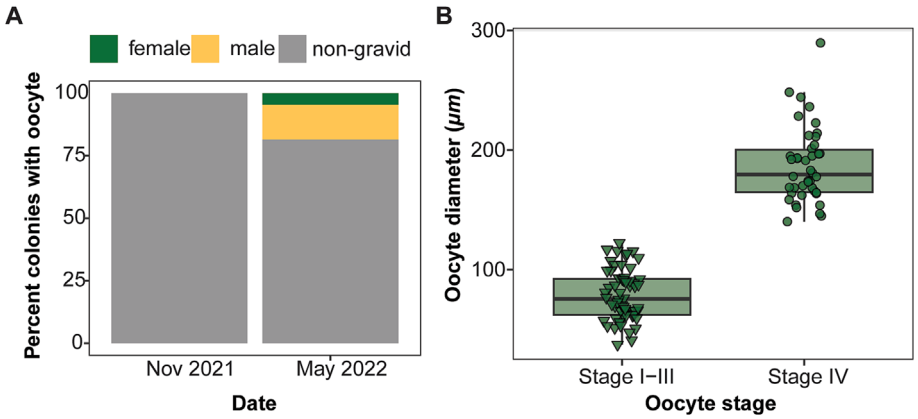

FIGURE 2 Percentage of gravid colonies of *Porites* cf. *P. lobata* and oocyte sizes. (A) Percentage of colonies containing oocytes (female) and spermatocysts (male) on each sampling date, $n = 43$ individuals. (B) Immature (stages I–III) and mature (stage IV) oocyte sizes. Each point represents an oocyte measured in 10 random polyps of two gravid colonies found in May 2022. Horizontal line represents the median; lower and upper bounds of the box represent first and third quartiles.

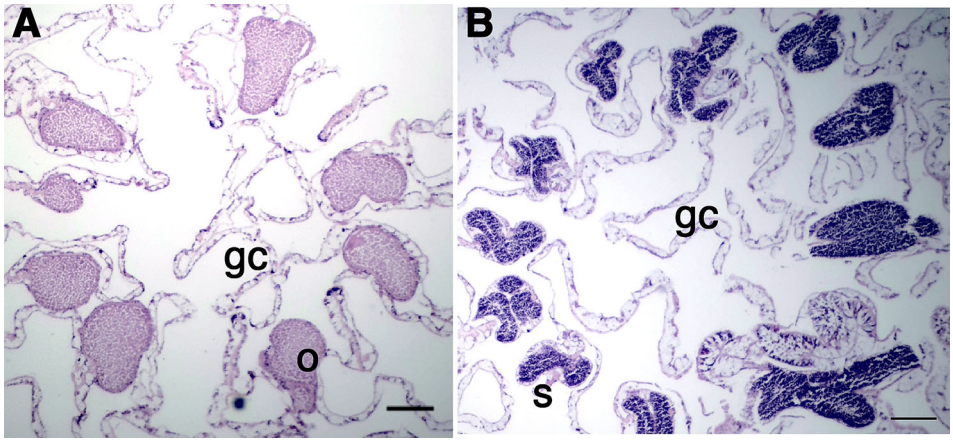

FIGURE 3 Photomicrographs of cross-sectional views showing gametes in tissues of *Porites* cf. *P. lobata*. (A) Histological section of a whole female polyp with oocytes. (B) Stage IV spermatocysts. O, oocyte; s, spermatocyst; gc, gastrovascular cavity. Scale bars = 100 μm.

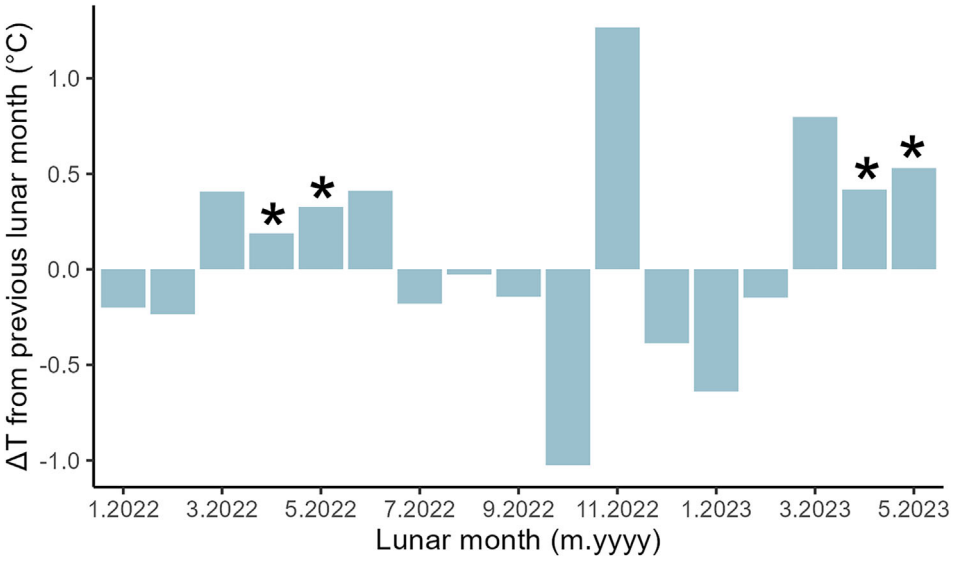

FIGURE 4 Monthly change in water temperature (°C) by lunar month in habitats with *Porites* cf. *P. lobata* in Palau. The first lunar month of the year begins with the first full moon following the winter solstice (Baird et al., 2022). Vertical bars show the difference in mean water temperature for a given lunar month compared to the previous lunar month. Asterisks indicate lunar months in which spawning was recorded. Observations were made in April and May 2022, November 2022 (no spawning observed), and April and May 2023.

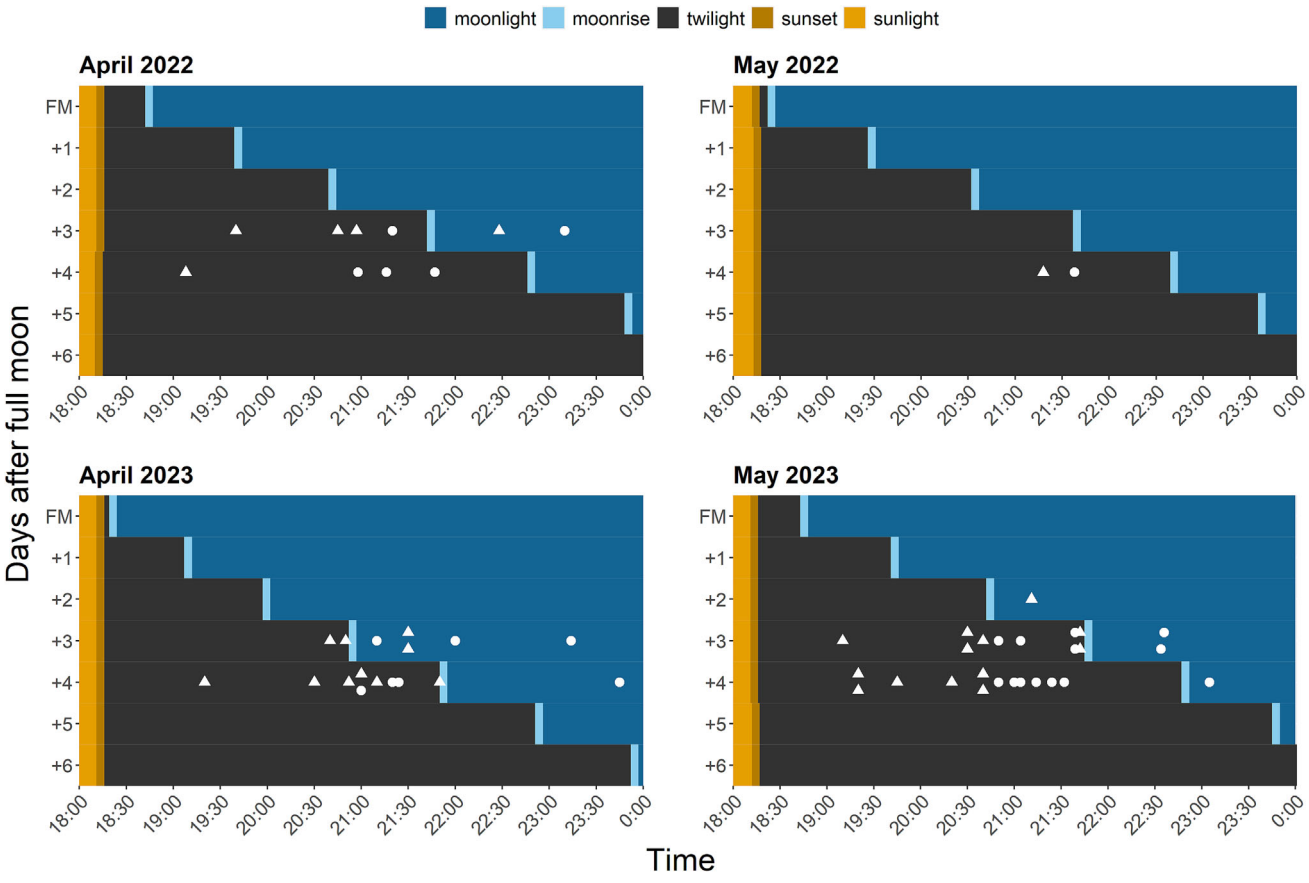

FIGURE 5 Spawning times for *Porites* cf. *P. lobata* in relation to moonlight conditions in Palau. White shapes indicate observed gamete release times in each year of observation, with triangles representing male colonies and circles representing female colonies.

with eggs from one female showing a precipitous decline in fertilization success earlier than other females tested (Figure 8). Sperm were observed actively swimming up to 4 h after spawning began for a given male, indicating that sperm motility was likely not the limiting factor in fertilization success for eggs older than 2.5 h.

3.4 | Embryological observations

The first cellular division (two-cell stage) was observed ~1.5 h after sperm, and eggs were mixed. Cellular divisions rates varied among fertilized eggs, and batch crosses had individuals in multiple stages of

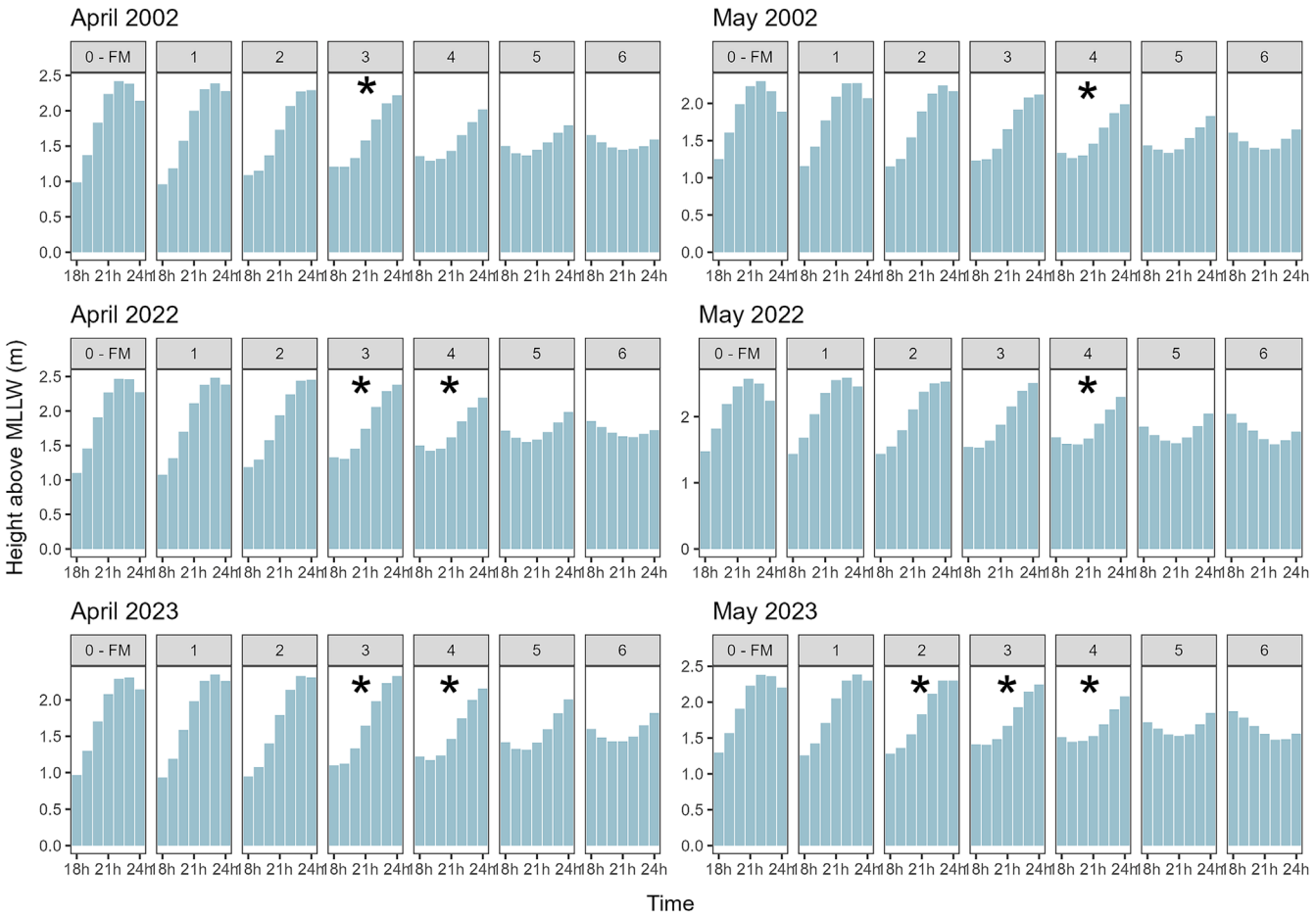

FIGURE 6 Observations of spawning in *Porites* cf. *P. lobata* in relation to tidal state. Hourly measurements of water level above mean low low water (MLLW) at Malakal Island, Palau (UHSLC Station #7), are shown from 6:00 p.m. to midnight on the night of the full moon and up to six nights afterward in each month. Asterisks indicate nights when spawning was observed in 2002 (Penland et al., 2004), 2022, and 2023 (present study).

embryonic development at the same time (Figure 8). Subsamples of eggs maintained under a microscope in an air-conditioned room (27°C) took longer to divide than eggs maintained at ambient temperature (~31°C), and many did not complete embryogenesis. Fertilization success and embryological development may be sensitive to temperature in this species, although further dedicated study would be required to confirm our observation.

There were 207 ± 76 algal cells in 5-day-old larvae. Larvae were elliptical, 312 ± 39 μm long, and 254 ± 41 μm in the shorter diameter (Figure 8). Larvae were neutrally or negatively buoyant and were evenly distributed throughout the water column in culture containers 36 h after fertilization. Most larvae had slow, directional or meandering swimming, although some barely swam at all in the still water cultures.

3.5 | Settlement observations

Larvae older than 2 days began to display characteristic settlement behaviors, which comprised repeated exploration of the bottom of culture dishes or swimming in helices. The mean diameter of settled individuals was 580 ± 13 μm ($n = 19$, range 486–675 μm; Figure 10). Larvae readily settled on limestone tiles and glass microscope slides placed in plastic culture containers. Higher proportions of larvae settled on substrata where crushed CCA had been added (~60%), relative to substrata without CCA (<20%). Some larvae in each culture settled in a narrow groove on the bottom of the culture containers, even without the addition of CCA as a settlement cue. Most larvae in settlement assays attached to the substratum within 24 h, and calcification began 2 days after attachment (Figure 10). Tentacles were first observed 8 days after attachment to the substratum (Figure 10).

4 | DISCUSSION

Our results confirmed that *Porites* cf. *P. lobata* in Palau are gonochoric broadcast spawners with a spawning season spanning multiple months (Gouezo et al., 2020; Penland et al., 2004). Spawning occurred in the fourth and fifth lunar months in Palau, on nights with at least 2 h of darkness between sunset and moonrise, on a rising tide. By relating spawning observations to environmental conditions, this

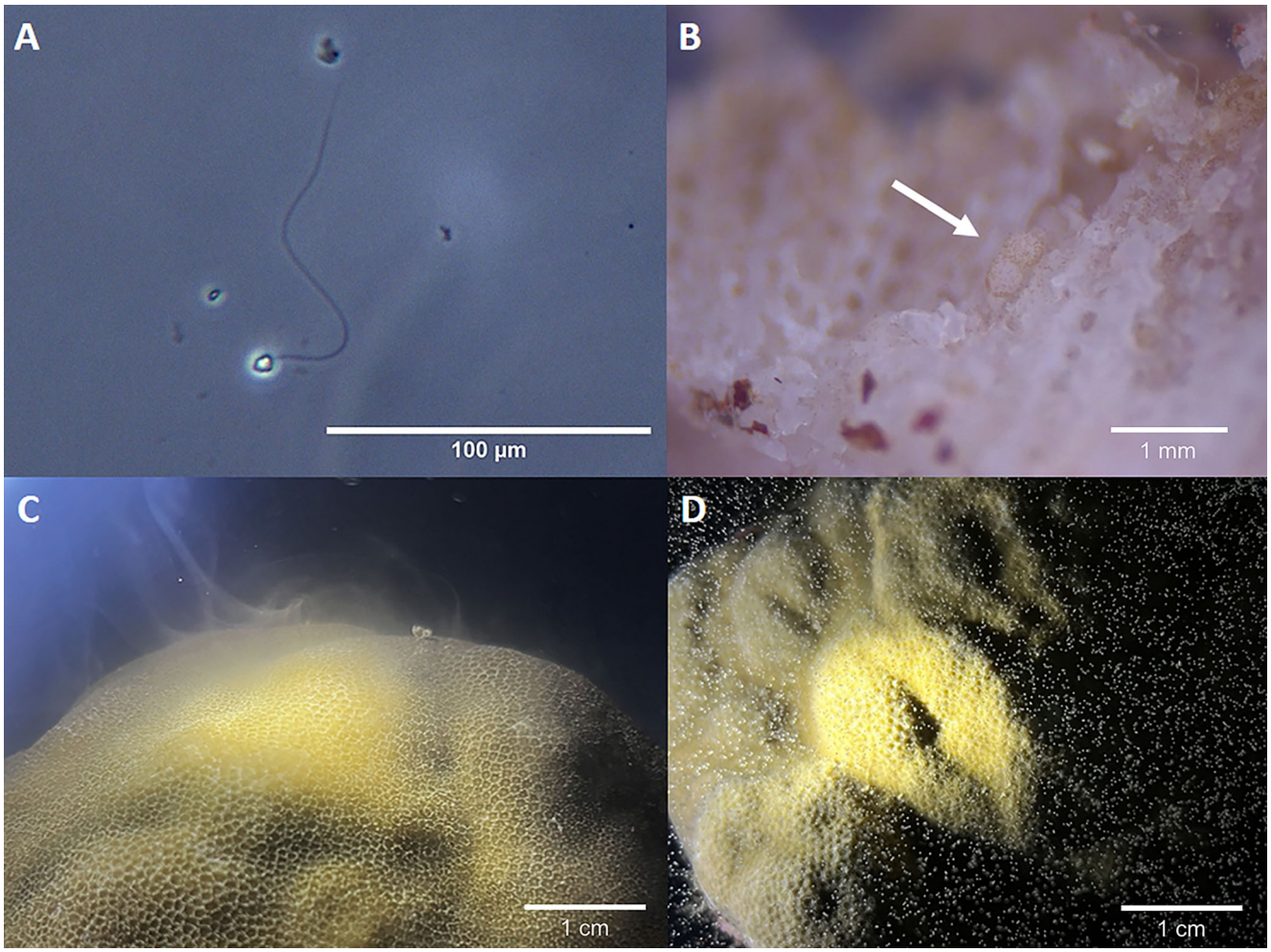

FIGURE 7 *Ex situ* spawning in *Porites* cf. *P. lobata*. (A) Phase contrast light micrograph of sperm. (B) Mature oocytes (white arrow) observed in tissue sample from an adult colony. (C) Male beginning to release sperm with the appearance of white smoke. (D) Female releasing pink-brown eggs, some of which occur in small clusters.

study can aid in predicting spawning events in populations of *Porites* cf. *P. lobata* elsewhere across its broad geographic range.

We observed spawning in lunar months with a higher mean temperature than the previous lunar month. Rising water temperature is an important cue for coral gametogenesis and spawning across multiple spatial and temporal scales (Guest et al., 2005; Keith et al., 2016; Lin & Nozawa, 2023). Increasing sea surface temperatures as a result of climate change could alter the timing of coral spawning (Paxton et al., 2016). However, we found spawning patterns in relation to environmental factors were remarkably consistent between our study (2022, 2023) and observations made 20 years prior (Penland et al., 2004). Water temperatures in shallow lagoons and coral reefs in Palau have risen ~0.5°C in the last two decades (Colin, 2018). This increase may not be sufficient to cause a significant shift in timing of spawning in *Porites* cf. *P. lobata*, or the night of spawning within a given month might be determined by other factors, such as moonlight and tide. Corals rely on environmental cues on multiple temporal scales to fine-tune spawning timing and maximize fertilization success (Guest et al., 2008).

A period of darkness between sunset and moonrise appeared to be important for spawning in the colonies of *Porites* cf. *P. lobata* in this study. Exposure to light after sunset can disrupt spawning synchrony in corals, and in fact, darkness between sunset and moonrise is an important factor determining the night of spawning in multiple species (Kaniewska et al., 2015; Lin et al., 2021). Spawning midway between low and high tide is common among corals in Palau (Kenyon, 1995). In bathymetrically complex areas such as our study sites in the Rock Island Southern Lagoon (Chelbacheb), spawning on an incoming tide could facilitate gamete mixing between reefs (Kenyon, 1995).

Only a small proportion of coral colonies observed in this study spawned (2022, 14%; 2023, 35%) or had gametes in histological sections (2022, 18%). It is possible that we missed some spawning in April 2022, particularly for corals collected 4 days after the full moon. Nonetheless, colonies in this study were selected haphazardly (i.e., without regard to possession of gametes), so our observations in other months could be indicative of the proportion of individuals participating in spawning events in situ. A previous study in the eastern Pacific found that only 30%–67% of colonies of *P. lobata* sampled had

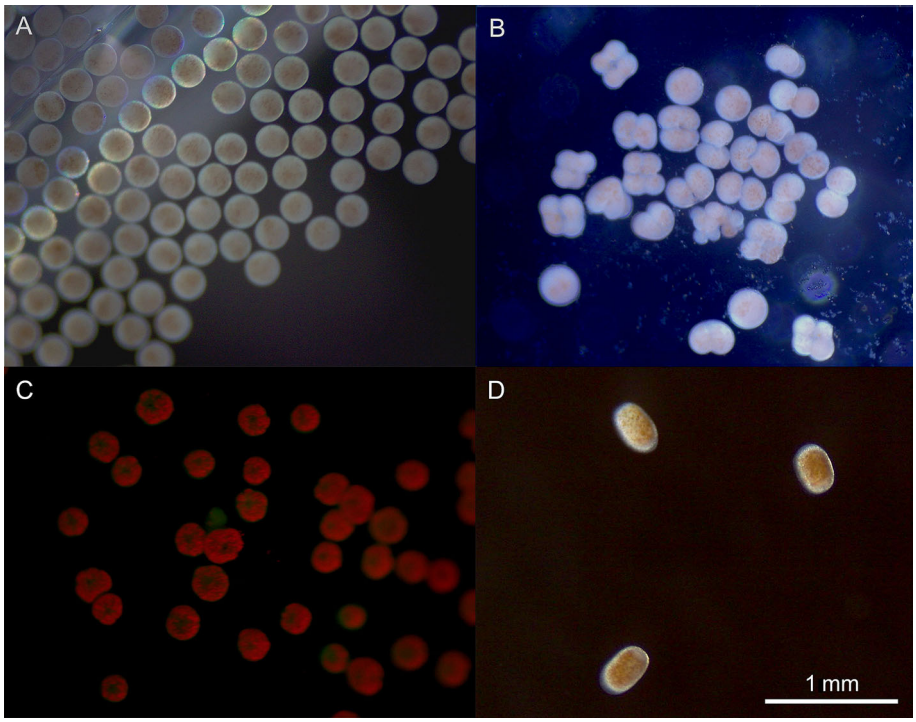

FIGURE 8 Development of larvae of *Porites* cf. *P. lobata*. (A) Representative picture of eggs directly after collection. (B) Fertilized embryos with dividing two- and four-cell stages. The first embryos observed to complete the first division did so ~90 min after gametes were mixed, and the same embryos completed their second division ~40 min later when kept at ambient temperature (~30°C). (C) Fluorescence in 3-day-old larvae. (D) Four-day-old larvae. Scale bar applies to all figure panels.

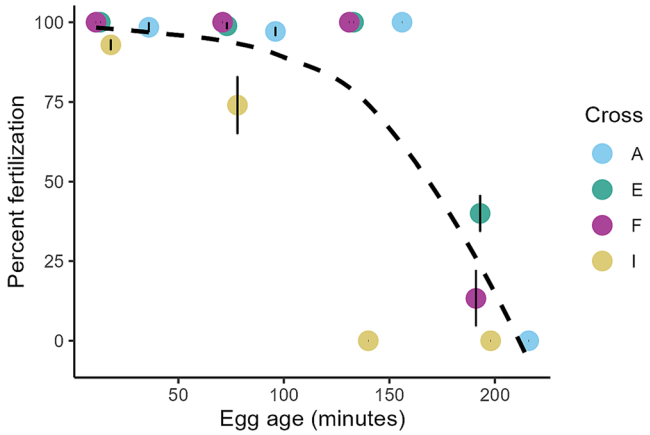

FIGURE 9 Increasing egg age reduces fertilization in *Porites* cf. *P. lobata*. Each cross included eggs from one female and pooled sperm from three males. Error bars represent standard error. Dashed line represents best-fit curve. Colors represent the four different crosses of this assay. Each cross had three technical replicates per time point, at which eggs from the same egg donor were added to three separate containers holding sperm from the three pooled sperm donors. Fertilization in each container was assessed separately and averaged to obtain the fertilization rate for a given cross and time point.

gonads (Glynn et al., 1994), although a study from Rapa Nui (Easter Island) reported very high (50%–100%) proportions of gravid colonies (Buck-Wiese et al., 2018). It is unclear why such a low proportion of colonies would be reproductive in a given year. No colonies were observed to switch sex between spawning years, so sex switching does not explain the low proportion of colonies spawning. Environmental stress, including bleaching, has carryover effects on reproduction, leading to reduced fecundity, smaller oocytes, and a lower proportion of gravid colonies (Johnston et al., 2020; Leinbach et al., 2021). It is therefore possible that the low proportion of reproductive colonies in our study was a latent effect of environmental stress. Some coral bleaching occurred in Palau in November 2022 and could have affected the 2023 spawn, but no bleaching was observed in 2021 (authors, unpubl. data). The proportion of colonies spawning *ex situ* varied greatly among study sites, with no colonies from Merchechar spawning in either year. In contrast, 52% of colonies collected from Taoch released gametes in April or May 2023 (Appendix S1). This could be related to differences in localized environmental conditions and recent history, or differences among the populations found at each site.

One consistent observation in our data was that males commenced gamete release before females. Males spawning before females have also been reported for other gonochoric species (Hagman et al., 1998; Marhaver et al., 2015). Because we isolated colonies in individual containers to separate gametes, it is possible that females missed some chemical or environmental cues—such as the presence of sperm in the water column—that could have induced spawning sooner. A delay in gamete release *ex situ* has been reported when compared with concurrent field observations of the same species (Neely et al., 2021; O'Neil et al., 2021). Therefore, our observations should be confirmed by in situ spawning studies.

The wide time spans over which we observed spawning could serve as temporal pre-zygotic barriers in *Porites* cf. *P. lobata*. We observed spawning over 2 months per year, on multiple nights in each month, and over multiple hours on each night. While sperm swam actively for several hours, the time delay between sperm and egg

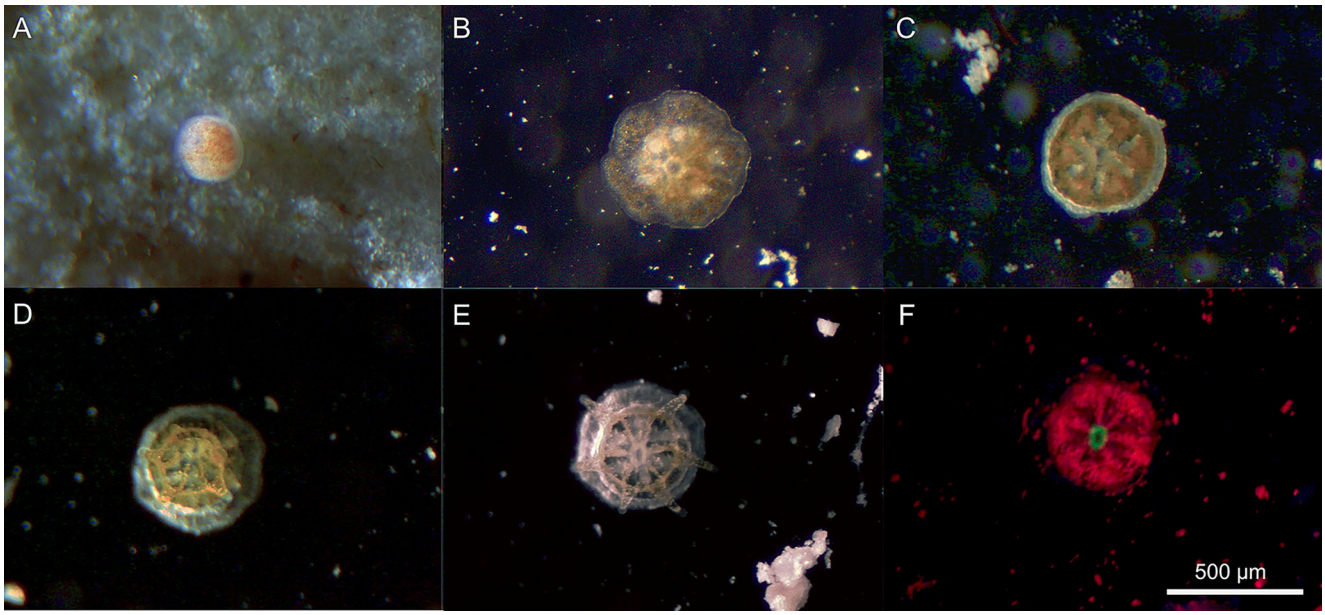

FIGURE 10 Settlement in *Porites* cf. *P. lobata*. (A) Larva attached to limestone tile. (B) Settler 24 h after attachment. (C) Settler 5 days after attachment. (D) Settler 7 days after attachment with rudimentary tentacles. (E) Settler 10 days after attachment. (F) Green fluorescence limited to polyp mouth in a settler 10 days after attachment. Scale bar applies to all figure panels.

release could result in sperm dilution and restricted fertilization rates in situ (Oliver & Babcock, 1992). We were unable to assess the impacts of sperm concentration in this study, but this is a potential avenue for future research. Furthermore, eggs had high fertilization for only the first 2–2.5 h after release. Other gonochoric coral species have similarly narrow windows for egg fertilization, which may serve as a reproductive barrier for sympatric species (Levitan et al., 2004). It is well documented that samples identified as *Porites* cf. *P. lobata* include high genotypic diversity and multiple cryptic lineages (Boulay et al., 2013; Forsman et al., 2015; Rivera et al., 2022; Schweinsberg et al., 2016). Future studies should examine the potential for spatial and temporal reproductive isolation among lineages.

Previous studies have assumed a long pelagic larval duration in *P. lobata* because of vertical symbiont transmission in this species (Baums et al., 2012; Field, 1998). However, we observed competency to settle in larvae just 2 days post-fertilization. While individual larvae have been kept in culture for up to 53 days in a prior study (Field, 1998), many individuals in our study settled on culture dishes in the absence of a settlement cue, suggesting "desperate" settlement (Toonen & Pawlik, 1994). Factors leading to "desperate" behavior in symbiotic larvae could be related to algal physiology and should be further explored. Our observations indicate that the vast majority of individuals settle shortly after achieving competency. In addition to the abbreviated larval period, neutral or negative buoyancy was observed in most larvae within 24 h of fertilization, with many individuals distributed throughout the water column or swimming at the bottom of the culture dishes. Variations in dispersal depth could lead to differences in dispersal distance for larvae of *Porites* cf. *P. lobata*, because currents are slower, less directional, and more turbulent in the benthic boundary layer (Vogel, 1996).

Palau's Rock Island Southern Lagoon (Chelbacheb) is characterized by restricted circulation and long water residence times (Golbuu et al., 2016). Most reefs in this area are likely to be self-seeding (Golbuu et al., 2012, 2016). With a pelagic larval duration of just 2 days, highly localized recruitment would be expected in *Porites* cf. *P. lobata* (Golbuu et al., 2012). Nonetheless, model results indicate that coral dispersal and recruitment are heavily influenced by variations in current direction and wind speed (Gouezo et al., 2021). Molecular studies of population genetic structure have suggested limited dispersal in *Porites* cf. *P. lobata* on multiple scales: between semi-isolated lagoons and outer patch and barrier reefs in Palau (Rivera et al., 2022), between the Hawai'ian Islands and Johnston Atoll (Polato et al., 2010), and at the largest scale, across the East Pacific Barrier (Baums et al., 2012). Future studies should address how variations in pelagic larval duration and oceanographic conditions influence dispersal in *Porites* cf. *P. lobata*.

Overall, the results of this study contribute to the body of knowledge on the spawning, embryonic development, and larval settlement for a key reef-building coral species. We demonstrated that high fertilization success and high settlement rates can be achieved with methods commonly practiced in *ex situ* reproductive studies. Furthermore, the short pelagic larval duration and high settlement achieved in our study, together with the high thermal tolerance and genetic diversity in *Porites* cf. *P. lobata* shown elsewhere, make it a promising candidate for *ex situ* assisted reproduction programs (Guest et al., 2023). By reporting specific timing for spawning events, and determining the optimal range for introducing gametes in *in vitro* fertilization procedures, our study should further enable restoration practitioners to include this species in future initiatives and optimize their fertilization success. Finally, the development of a coral

spawning calendar for Palau would rapidly accelerate research and enhance the capacity for larval propagation in this archipelago with additional coral species (e.g., Baird et al., 2022).

AUTHOR CONTRIBUTIONS

Study design—Matthew-James Bennett, Kirstin S. Meyer-Kaiser, Carsten G. B. Grupstra, Jeric Da-Anoy, Sarah W. Davies; data collection and spawning observations—Matthew-James Bennett, Maikani Andres, Kirstin S. Meyer-Kaiser, Carsten G. B. Grupstra; histology—Jeric Da-Anoy, Ashley Rossin, Daniel Holstein; data analysis—Matthew-James Bennett, Carsten G. B. Grupstra, Jeric Da-Anoy, Ashley Rossin, Daniel Holstein; manuscript drafting—Matthew-James Bennett, Kirstin S. Meyer-Kaiser, Carsten G. B. Grupstra, Jeric Da-Anoy; manuscript editing—all.

ACKNOWLEDGMENTS

We are grateful to Joy Shmull-Sam and the Palau International Coral Reef Center staff for facilitating field and lab work for this study in Palau. Comments from two anonymous reviewers improved a previous version of this manuscript. This material is based upon work supported by the National Science Foundation under Grant Nos. OCE-2048678 (KMK) and OCE-2048589 (SWD). Sample collection was authorized by scientific research permits issued by the Palau Ministry of Natural Resources, Environment, and Tourism (RE-22-17, RE-22-24, RE-23-09) and Koror State Government (permit nos. 72, 78, and 83) to KMK.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data in this manuscript are largely observational and not well-suited to inclusion in a repository. Data from the gamete age assays are available through BCO-DMO (https://www.bco-dmo.org/dataset/926315), and spawning observations are listed in the Supplementary Material.

ORCID

Matthew-James Bennett https://orcid.org/0000-0002-2299-4582
Kirstin S. Meyer-Kaiser https://orcid.org/0000-0003-2219-6086

REFERENCES

Babcock, R. C., Bull, G. D., Harrison, P. L., Heyward, A. J., Oliver, J. K., Wallace, C. C., & Willis, B. L. (1986). Synchronous spawning of 105 scleractinian coral species on the Great Barrier Reef. *Marine Biology*, *90*, 379–394. https://doi.org/10.1007/BF00428562

Baird, A. H., Bhagooli, R., Ralph, R. J., & Takahasi, S. (2009). Coral bleaching: The role of the host. *Trends in Ecology and Evolution*, *24*, 16–20. https://doi.org/10.1016/j.tree.2008.09.005

Baird, A. H., Edwards, A. J., Guest, J. R., Harii, S., Hatta, M., Lachs, L., Mera, H., Sinniger, F., Abrego, D., Ben-Zvi, O., Bronstein, O., Cabaitan, P. C., Cumbo, V. R., Eyal, G., Eyal-Shaham, L., Feldman, B., Figueiredo, J., Flot, J. F., Grinblat, M., … Yamazato, K. (2022). A coral spawning calendar for Sesoko Station, Okinawa, Japan. *Galaxea Journal of Coral Reef Studies*, *24*, 41–49. https://doi.org/10.3755/galaxea.G2021_S10O

Barshis, D. J., Birkeland, C., Toonen, R. J., Gates, R. D., & Stillman, J. H. (2018). High-frequency temperature variability mirrors fixed differences in thermal limits of the massive coral *Porites lobata*. *Journal of Experimental Biology*, *221*, jeb188581. https://doi.org/10.1242/jeb.188581

Baums, I. B., Boulay, J. N., Polato, N. R., & Hellberg, M. E. (2012). No gene flow across the Eastern Pacific Barrier in the reef-building coral *Porites lobata*. *Molecular Ecology*, *21*, 5418–5433. https://doi.org/10.1111/j.1365-294X.2012.05733.x

Boström-Einarsson, L., Babcock, R., Bayraktarov, E., Ceccarelli, D., Cook, N., Ferse, S., Hancock, B., Harrison, P., Hein, M., Shaver, E., Smith, A., Suggett, D., Stewart-Sinclair, P., Vardi, T., & McLeod, I. (2020). Coral restoration – A systematic review of current methods, successes, failures and future directions. *PLoS ONE*, *15*, e0226631. https://doi.org/10.1371/journal.pone.0226631

Boulay, J. N., Hellberg, M. E., Cortés, J., & Baums, I. B. (2013). Unrecognized coral species diversity masks differences in functional ecology. *Proceedings of the Royal Society B*, *281*, 20131580.

Bouwmeester, J., Baird, A. H., Chen, C. J., Guest, J. R., Vicentaun, K. C., & Berumen, M. L. (2015). Multi-species spawning synchrony within scleractinian coral assemblages in the Red Sea. *Coral Reefs*, *34*, 65–77. https://doi.org/10.1007/s00338-014-1214-6

Buck-Wiese, H., Burgués, I., Medrano, A., Navarrete-Fernandez, T., Garcia, M., & Wieters, E. A. (2018). Patterns in sexual reproduction of the dominant scleractinian corals at Rapa Nui (Easter Island): *Pocillopora verrucosa* and *Porites lobata*. *Aquatic Biology*, *27*, 1–11. https://doi.org/10.3354/ab00691

Colin, P. (2018). Ocean warming and the reefs of Palau. *Oceanography*, *31*, 126–135. https://doi.org/10.5670/oceanog.2018.214

dela Cruz, D. W., & Harrison, P. L. (2017). Enhanced larval supply and recruitment can replenish reef corals on degraded reefs. *Scientific Reports*, *7*, 13985. https://doi.org/10.1038/s41598-017-14546-y

dela Cruz, D. W., & Harrison, P. L. (2020). Optimising conditions for in vitro fertilization success of *Acropora tenuis*, *A. millepora* and *Favites colemani* corals in northwestern Philippines. *Journal of Experimental Marine Biology and Ecology*, *524*, 151286. https://doi.org/10.1016/j.jembe.2019.151286

Edwards, A., Guest, J., & Humanes, A. (2024). Rehabilitating coral reefs in the Anthropocene. *Current Biology*, *34*, R399–R406. https://doi.org/10.1016/j.cub.2023.12.054

Field, S. (1998). Settlement biology of larvae of *Montipora verrucosa* and *Porites lobata* in Hawai'i. In E. Cox, D. Krupp, & P. Jokiel (Eds.), *Reproduction in reef corals: Results of the 1997 Edwin W. Pauley Summer Program in Marine Biology* (pp. 111–119). Technical Report No. 42.). Hawai'i Institute of Marine Biology, University of Hawai'i.

Fisch, J., Drury, C., Towle, E. K., Winter, R. N., & Miller, M. W. (2019). Physiological and reproductive repercussions of consecutive summer bleaching events of the threatened Caribbean coral *Orbicella faveolata*. *Coral Reefs*, *38*, 863–876. https://doi.org/10.1007/s00338-019-01817-5

Forsman, Z., Wellington, G. M., Fox, G. E., & Toonen, R. J. (2015). Clues to unraveling the coral species problem: Distinguishing species from geographic variation in *Porites* across the Pacific with molecular markers and microskeletal traits. *PeerJ*, *3*, e751. https://doi.org/10.7717/peerj.751

Glynn, P. W., Colley, S. B., Eakin, C. M., Smith, D. B., Cortés, J., Gassman, N. J., Guzmán, H. M., Del Rosario, J. B., & Feingold, J. S. (1994). Reef coral reproduction in the eastern Pacific: Costa Rica, Panamá, and Galápagos Islands (Ecuador). II. Poritidae. *Marine Biology*, *118*, 191–208. https://doi.org/10.1007/BF00349785

Glynn, P. W., Gassman, N. J., Eakin, C. M., Cortes, J., Smith, D. B., & Guzman, H. M. (1991). Reef coral reproduction in the eastern Pacific:

Costa Rica, Panama, and Galapagos Islands (Ecuador) – I. Pocilloporidae. *Marine Biology*, *109*, 355–368. https://doi.org/10.1007/BF01313501

Golbuu, Y., Gouezo, M., Kurihara, H., Rehm, L., & Wolanski, E. (2016). Long-term isolation and local adaptation in Palau's Nikko Bay help corals thrive in acidic waters. *Coral Reefs*, *35*, 909–918. https://doi.org/10.1007/s00338-016-1457-5

Golbuu, Y., Wolanski, E., Idechong, J. W., Victor, S., Isechal, A. L., Oldiais, N. W., Idip, D., Richmond, R. H., & van Woesik, R. (2012). Predicting coral recruitment in Palau's complex reef archipelago. *PLoS ONE*, *7*, e50998. https://doi.org/10.1371/journal.pone.0050998

Gouezo, M., Doropoulos, C., Fabricius, K., Olsudong, D., Nestor, V., Kurihara, H., Golbuu, Y., & Harrison, P. (2020). Multispecific coral spawning events and extended breeding periods on an equatorial reef. *Coral Reefs*, *39*, 1107–1123. https://doi.org/10.1007/s00338-020-01941-7

Gouezo, M., Wolanski, E., Critchell, K., Fabricius, K., Harrison, P., Golbuu, Y., & Doropoulos, C. (2021). Modelled larval supply predicts coral population recovery potential following disturbance. *Marine Ecology Progress Series*, *661*, 127–145. https://doi.org/10.3354/meps13608

Grosso-Becerra, M. V., Mendoza-Quiroz, S., Maldonado, E., & Banaszak, A. T. (2021). Cryopreservation of sperm from the brain coral *Diploria labyrinthiformis* as a strategy to face the loss of corals in the Caribbean. *Coral Reefs*, *40*, 937–950. https://doi.org/10.1007/s00338-021-02098-7

Guest, J., Baria-Rodriguez, M. V., Toh, T. C., de la Cruz, D., Vicentaun, K., Gomez, E., Villanueva, R., Steinberg, P., & Edwards, A. (2023). Live slow, die old: Larval propagation of slow-growing, stress-tolerant corals for reef restoration. *Coral Reefs*, *42*, 1365–1377. https://doi.org/10.1007/s00338-023-02440-1

Guest, J. R., Baird, A. H., Clifton, K. E., & Heyward, A. J. (2008). From molecules to moonbeams: Spawning synchrony in coral reef organisms. *Invertebrate Reproduction and Development*, *51*, 145–149. https://doi.org/10.1080/07924259.2008.9652264

Guest, J. R., Baird, A. H., Goh, B. P., & Chou, L. M. (2005). Seasonal reproduction in equatorial reef corals. *Invertebrate Reproduction and Development*, *48*, 207–218. https://doi.org/10.1080/07924259.2005.9652186

Guest, J. R., Baird, A. H., Goh, B. P. L., & Chou, L. M. (2012). Sexual systems in scleractinian corals: An unusual pattern in the reef-building species *Diploastrea heliopora*. *Coral Reefs*, *31*, 705–713. https://doi.org/10.1007/s00338-012-0881-4

Hagedorn, M., Carter, V. L., Lager, C., Camperio Ciani, J. F., Dygert, A. N., Schleiger, R. D., & Henley, E. M. (2016). Potential bleaching effects on coral reproduction. *Reproduction Fertility and Development*, *28*, 1061–1071. https://doi.org/10.1071/RD15526

Hagman, D. K., Gittings, S. R., & Deslarzes, K. J. P. (1998). Timing, species participation, and environmental factors influencing annual mass spawning at the Flower Garden Banks (northwest Gulf of Mexico). *Gulf of Mexico Science*, *16*, 170–179. https://doi.org/10.18785/goms.1602.06

Henley, E. M., Bouwmeester, J., Jury, C. P., Toonen, R. J., Quinn, M., Lager, C. V. A., & Hagedorn, M. (2022). Growth and survival among Hawaiian corals outplanted from tanks to an ocean nursery are driven by individual genotype and species differences rather than preconditioning to thermal stress. *PeerJ*, *10*, e13112. https://doi.org/10.7717/peerj.13112

Hodgson, G. (1988). Potential gamete wastage in synchronously spawning corals due to hybrid inviability. In J. H. Choat (Ed.), *Proceedings of the 6th international coral reef symposium* (Vol. 2, pp. 707–714). 6th International Coral Reef Symposium Executive Committee.

Hughes, T. P., Jackson, J. B. C., Cumming, G. S., Cinner, J. E., Palumbi, S. R., Bellwood, D. R., van de Leemput, I. A., Morrison, T. H., Kleypas, J., van Nes, E. H., Barnes, M. L., Lough, J. M., & Scheffer, M. (2017). Coral reefs in the Anthropocene. *Nature*, *546*, 82–90. https://doi.org/10.1038/nature22901

Hughes, T. P., & Tanner, J. E. (2000). Recruitment failure, life histories, and long-term decline of Caribbean corals. *Ecology*, *81*, 2250–2263. https://doi.org/10.1890/0012-9658(2000)081[2250:RFLHAL]2.0.CO;2

Humanes, A., Beauchamp, E. A., Bythell, J. C., Carl, M. K., Craggs, J. R., Edwards, A. J., Golbuu, Y., Lachs, L., Martinez, H. M., Palmowski, P., Paysinger, F., Randle, J. L., van der Steeg, E., Sweet, M., Treumann, A., & Guest, J. R. (2021). An experimental framework for selectively breeding corals for assisted evolution. *Frontiers in Marine Science*, *8*, 669995. https://doi.org/10.3389/fmars.2021.669995

Humphrey, C., Weber, M., Lott, C., Cooper, T., & Fabricius, K. (2008). Effects of suspended sediments, dissolved inorganic nutrients and salinity on fertilisation and embryo development in the coral *Acropora millepora* (Ehrenberg, 1834). *Coral Reefs*, *27*, 837–850. https://doi.org/10.1007/s00338-008-0408-1

Isomura, N., & Fukami, H. (2018). Coral reproduction in Japan. In A. Iguchi & C. Hongo (Eds.), *Coral reef studies of Japan*, *Coral Reefs of the World* (Vol. 13, pp. 95–110). Springer Nature. https://doi.org/10.1007/978-981-10-6473-9_7

Johnston, E. C., Counsell, C. W. W., Sale, T. L., Burgess, S. C., & Toonen, R. J. (2020). The legacy of stress: Coral bleaching impacts reproduction years later. *Functional Ecology*, *34*, 2315–2325. https://doi.org/10.1111/1365-2435.13653

Kaniewska, P., Alon, S., Karako-Lampert, S., Hoegh-Guldberg, O., & Levy, O. (2015). Signaling cascades and the importance of moonlight in coral broadcast mass spawning. *eLife*, *4*, e09991. https://doi.org/10.7554/eLife.09991

Keith, S. A., Maynard, J. A., Edwards, A. J., Guest, J. R., Bauman, A. G., van Hooidonk, R., Heron, S. F., Berumen, M. L., Bouwmeester, J., Piromvaragorn, S., Rahbek, C., & Baird, A. H. (2016). Coral mass spawning predicted by rapid seasonal rise in ocean temperature. *Proceedings of the Royal Society B*, *283*, 20160011. https://doi.org/10.1098/rspb.2016.0011

Kenyon, J. C. (1995). Latitudinal differences between Palau and Yap in coral reproductive synchrony. *Pacific Science*, *49*, 156–164.

Kleypas, J., Allemand, D., Anthony, K., Baker, A. C., Beck, M. W., Hale, L. Z., Hilmi, N., Hoegh-Guldberg, O., Hughes, T., Kaufman, L., Kayanne, H., Magnan, A. K., Mcleod, E., Mumby, P., Palumbi, S., Richmond, R. H., Rinkevich, B., Steneck, R. S., Voolstra, C. R., … Gattuso, J. P. (2021). Designing a blueprint for coral reef survival. *Biological Conservation*, *257*, 109107. https://doi.org/10.1016/j.biocon.2021.109107

Kojis, B. L., & Quinn, N. J. (1981). Reproductive strategies in four species of *Porites* (Scleractinia). In E. D. Gomez, C. E. Birkeland, R. W. Buddemeier, R. E. Johannes, J. A. Marsh, Jr., & R. T. Tsuda (Eds.), *The reef and man: Proceedings of the fourth international coral reef symposium, Manila 2* (pp. 145–151). Marine Sciences Center, University of the Philippines.

Leinbach, S. E., Speare, K. E., Rossin, A. M., Holstein, D. M., & Strader, M. E. (2021). Energetic and reproductive costs of coral recovery in divergent bleaching responses. *Scientific Reports*, *11*, 23546. https://doi.org/10.1038/s41598-021-02807-w

Levas, S. J., Grottoli, A. G., Hughes, A., Osburn, C. L., & Matsui, Y. (2013). Physiological and biogeochemical traits of bleaching and recovery in the mounding species of coral *Porites lobata*: Implications for resilience in mounding corals. *PLoS ONE*, *8*, e63267. https://doi.org/10.1371/journal.pone.0063267

Levitan, D., & Petersen, C. (1995). Sperm limitation in the sea. *Trends in Ecology and Evolution*, *10*, 228–231. https://doi.org/10.1016/S0169-5347(00)89071-0

Levitan, D. R., Fukami, H., Jara, J., Kline, D., McGovern, T. M., McGhee, K. E., Swanson, C. A., & Knowlton, N. (2004). Mechanisms of reproductive isolation among sympatric broadcast-spawning corals of the *Montastraea annularis* species complex. *Evolution*, *58*, 308–323.

Lin, C. H., & Nozawa, Y. (2023). The influence of seawater temperature on the timing of coral spawning. *Coral Reefs*, *42*, 417–426. https://doi.org/10.1007/s00338-023-02349-9

Lin, C. H., Takahashi, S., Mulla, A. J., & Nozawa, Y. (2021). Moonrise timing is key for synchronized spawning in coral *Dipsastraea speciosa*. *Proceedings of the National Academy of Sciences*, *118*, e2101985118. https://doi.org/10.1073/pnas.2101985118

Loya, Y., Sakai, K., Yamazato, K., Nakano, Y., Sambali, H., & van Woesik, R. (2001). Coral bleaching: The winners and the losers. *Ecology Letters*, *4*, 122–131. https://doi.org/10.1046/j.1461-0248.2001.00203.x

Marhaver, K. L., Vermeij, M. J. A., & Medina, M. M. (2015). Reproductive natural history and successful juvenile propagation of the threatened Caribbean pillar coral *Dendrogyra cylindrus*. *BMC Ecology*, *15*, 9. https://doi.org/10.1186/s12898-015-0039-7

Maté, J. L. T. (1998). New reports on the timing and mode of reproduction of Hawaiian corals. In E. Cox, D. Krupp, & P. Jokiel (Eds.), *Reproduction in reef corals: Results of the 1997 Edwin W. Pauley Summer Program in Marine Biology* (p. 7). Technical Report No. 42.). Hawai'i Institute of Marine Biology, University of Hawai'i.

Mcleod, E., Anthony, K. R. N., Mumby, P. J., Maynard, J., Beeden, R., Graham, N. A. J., Heron, S. F., Hoegh-Guldberg, O., Jupiter, S., MacGowan, P., Mangubhai, S., Marshall, N., Marshall, P. A., McClanahan, T. R., Mcleod, K., Nyström, M., Obura, D., Parker, B., Possingham, H. P., … Tamelander, J. (2019). The future of resilience-based management in coral reef ecosystems. *Journal of Environmental Management*, *233*, 291–301. https://doi.org/10.1016/j.jenvman.2018.11.034

Neely, K. L., Lewis, C. L., Lunz, K. S., & Kabay, L. (2021). Rapid population decline of the pillar coral *Dendrogyra cylindrus* along the Florida reef tract. *Frontiers in Marine Science*, *8*, 656515. https://doi.org/10.3389/fmars.2021.656515

Nozawa, Y., Isomura, N., & Fukami, H. (2015). Influence of sperm dilution and gamete contact time on the fertilization rate of scleractinian corals. *Coral Reefs*, *34*, 1199–1206. https://doi.org/10.1007/s00338-015-1338-3

Oliver, J., & Babcock, R. (1992). Aspects of the fertilization ecology of broadcast spawning corals: Sperm dilution effects and in situ measurements of fertilization. *Biological Bulletin*, *183*, 409–417. https://doi.org/10.2307/1542017

O'Neil, K. L., Serafin, R. M., Patterson, J. T., & Craggs, J. R. K. (2021). Repeated ex situ spawning in two highly disease susceptible corals in the family Meandrinidae. *Frontiers in Marine Science*, *8*, 669976. https://doi.org/10.3389/fmars.2021.669976

Paxton, C. W., Baria, M. V. B., Weis, V. M., & Harii, S. (2016). Effect of elevated temperature on fecundity and reproductive timing in the coral *Acropora digitifera*. *Zygote*, *24*, 511–516. https://doi.org/10.1017/S0967199415000477

Penland, L., Kloulechad, J., Idip, D., & van Woesik, R. (2004). Coral spawning in the western Pacific Ocean is related to solar insolation: Evidence of multiple spawning events in Palau. *Coral Reefs*, *23*, 133–140. https://doi.org/10.1007/s00338-003-0362-x

Polato, N. R., Concepcion, G. T., Toonen, R. J., & Baums, I. B. (2010). Isolation by distance across the Hawai'ian archipelago in the reef-building coral *Porites lobata*. *Molecular Ecology*, *19*, 4661–4677. https://doi.org/10.1111/j.1365-294X.2010.04836.x

Pollock, F. J., Katz, S. M., van de Water, J. A. J. M., Davies, S. W., Hein, M., Torda, G., Matz, M. V., Beltran, V. H., Buerger, P., Puill-Stephan, E., Abrego, D., Bourne, D. G., & Willis, B. L. (2017). Coral larvae for restoration and research: A large-scale method for rearing *Acropora millepora* larvae, inducing settlement, and establishing symbiosis. *PeerJ*, *5*, e3732. https://doi.org/10.7717/peerj.3732

Primov, K. D., Burdick, D. R., Lemer, S., Forsman, Z. H., & Combosch, D. J. (2024). Genomic data reveals habitat partitioning in massive *Porites* on Guam, Micronesia. *Scientific Reports*, *14*, 17107. https://doi.org/10.1038/s41598-024-67992-w

Richmond, R. H., Tisthammer, K. H., & Spies, N. P. (2018). The effects of anthropogenic stressors on reproduction and recruitment of corals and reef organisms. *Frontiers in Marine Science*, *5*, 226. https://doi.org/10.3389/fmars.2018.00226

Rivera, H. E., Cohen, A. L., Thompson, J. R., Baums, I. B., Fox, M. D., & Meyer-Kaiser, K. S. (2022). Palau's warmest reefs harbor thermally tolerant corals that thrive across different habitats. *Communications Biology*, *5*, 1394. https://doi.org/10.1038/s42003-022-04315-7

Sale, T. L., Hunter, C. L., Hong, C., & Moran, A. L. (2019). Morphology, lipid composition, and reproduction in growth anomalies of the reef-building coral *Porites evermanni* and *Porites lobata*. *Coral Reefs*, *38*, 881–893. https://doi.org/10.1007/s00338-019-01811-x

Schweinsberg, M., Tollrian, R., & Lampert, K. P. (2016). Genetic variation in the massive coral *Porites lobata*. *Marine Biology*, *163*, 242. https://doi.org/10.1007/s00227-016-3022-8

Shaver, E. C., McLeod, E., Hein, M. Y., Palumbi, S. R., Quigley, K., Vardi, T., Mumby, P. J., Smith, D., Montoya-Maya, P., Muller, E. M., Banaszak, A. T., McLeod, I. M., & Wachenfeld, D. (2022). A roadmap to integrating resilience into the practice of coral reef restoration. *Global Change Biology*, *28*, 4751–4764. https://doi.org/10.1111/gcb.16212

Suggett, D. J., Guest, J., Camp, E. F., Edwards, A., Goergen, L., Hein, M., Humanes, A., Levy, J. S., Montoya-Maya, P. H., Smith, D. J., Vardi, T., Winters, R. S., & Moore, T. (2024). Restoration as a meaningful aid to ecological recovery of coral reefs. *Npj Ocean Sustainability*, *3*, 20. https://doi.org/10.1038/s44183-024-00056-8

Szmant-Froehlich, A., Reutter, M., & Riggs, L. (1985). Sexual reproduction of *Favia fragum* (Esper): Lunar patterns of gametogenesis, embryogenesis, and planulation in Puerto Rico. *Bulletin of Marine Science*, *37*, 880–892.

Tomascik, T., Mah, A. J., Nontij, A., & Moosa, M. K. (1997). *The ecology of the Indonesian seas, part I*. Periplus Editions.

Toonen, R. J., & Pawlik, J. R. (1994). Foundations of gregariousness. *Nature*, *370*, 511–512. https://doi.org/10.1038/370511a0

Vardi, T., Hoot, W. C., Levy, J., Shaver, E., Winters, R. S., Banaszak, A. T., Baums, I. B., Chamberland, V. F., Cook, N., Gulko, D., Hein, M. Y., Kaufman, L., Loewe, M., Lundgren, P., Lustic, C., MacGowan, P., Matz, M. V., McGonigle, M., McLeod, I., … Montoya-Maya, P. H. (2021). Six priorities to advance the science and practice of coral reef restoration worldwide. *Restoration Ecology*, *29*, e13498. https://doi.org/10.1111/rec.13498

Vogel, S. (1996). *Life in moving fluids: The physical biology of flow*. Princeton University Press.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Bennett, M.-J., Grupstra, C. G. B., Da-Anoy, J., Andres, M., Holstein, D., Rossin, A., Davies, S. W., & Meyer-Kaiser, K. S. (2024). *Ex situ* spawning, larval development, and settlement in massive reef-building corals (*Porites*) in Palau. *Invertebrate Biology*, e12447. https://doi.org/10.1111/ivb.12447