Long-term survival, growth, and reproduction of *Acropora palmata* sexual recruits outplanted onto Mexican Caribbean reefs

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Acropora palmata is a foundational yet endangered Caribbean reef-building coral species. The lack of recovery after a disease outbreak and low recruitment has led to widespread use of fragmentation to restore populations. Another option is the production of sexual recruits (settlers) via assisted reproduction to improve the genetic diversity of depleted populations, however the viability of this approach has not been tested over the long term. In 2011 and 2012, *A. palmata* larvae were cultured, settled, and the sexual recruits raised in an *ex-situ* nursery. Survival and growth were monitored over time. In 2014, these two F1 cohorts were moved to an *in-situ* nursery and after one year, a subset (29 colonies) was outplanted onto Cuevones Reef in the Mexican Caribbean. Growth and survival of these colonies were monitored periodically and compared to colonies that remained in the *in-situ* nursery. In 2019, samples were collected and analyzed for fertility and fecundity. 53% of the colonies were gravid and fecundity was 5.61 ± 1.91 oocytes and 3.04 ± 0.26 spermaries per polyp. A further 14 colonies from these two cohorts were outplanted in 2020 onto Picudas Reef and monitored during the subsequent spawning seasons. Two years after outplanting onto Picudas Reef, all colonies were alive and spawning of three of these colonies was recorded in 2022 in synchrony with the wild population. Gametes were collected from two colonies and crossed, with 15% fertilization success. Spermatozoa from wild colonies were then added and fertilization success increased to 95%. The resultant larvae followed normal development and symbiont uptake was visible within two weeks. The F2 generation was settled, maintained in an *ex-situ* nursery, and monitored for survival and growth. Both F1 and F2 generations followed a Type III survival curve with high initial mortality while in the *ex-situ* nursery and low later-stage mortality. The growth rates of these colonies increased three-fold after outplanting when compared to their

growth rates in the *ex-situ* and *in-situ* nurseries. All colonies survived while in the *in-situ* nursery and for an additional nine years after outplanting onto Cuevones Reef. Overall, our results show that colonies produced by assisted breeding, once outplanted, may contribute to the genetic diversity and establishment of self-sustaining sexually-reproducing populations, which is an overarching goal of coral restoration programs.

1 LONG-TERM SURVIVAL, GROWTH, AND REPRODUCTION OF *ACROPORA PALMATA*
2 SEXUAL RECRUITS OUTPLANTED ONTO MEXICAN CARIBBEAN REEFS

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Abstract

21 *Acropora palmata* is a foundational yet endangered Caribbean reef-building coral species. The
22 lack of recovery after a disease outbreak and low recruitment has led to widespread use of
23 fragmentation to restore populations. Another option is the production of sexual recruits (settlers)
24 via assisted reproduction to improve the genetic diversity of depleted populations, however the
25 viability of this approach has not been tested over the long term. In 2011 and 2012, *A.*
26 *palmata* larvae were cultured, settled, and the sexual recruits raised in an *ex-situ* nursery. Survival
27 and growth were monitored over time. In 2014, these two F1 cohorts were moved to an *in-*
28 *situ* nursery and after one year, a subset (29 colonies) was outplanted onto Cuevones Reef in the
29 Mexican Caribbean. Growth and survival of these colonies were monitored periodically and
30 compared to colonies that remained in the *in-situ* nursery. In 2019, samples were collected and
31 analyzed for fertility and fecundity. 53% of the colonies were gravid and fecundity was 5.61 ±
32 1.91 oocytes and 3.04 ± 0.26 spermaries per polyp. A further 14 colonies from these two cohorts
33 were outplanted in 2020 onto Picudas Reef and monitored during the subsequent spawning
34 seasons. Two years after outplanting onto Picudas Reef, all colonies were alive and spawning of
35 three of these colonies was recorded in 2022 in synchrony with the wild population. Gametes were
36 collected from two colonies and crossed, with 15% fertilization success. Spermatozoa from wild
37 colonies were then added and fertilization success increased to 95%. The resultant larvae followed
38 normal development and symbiont uptake was visible within two weeks. The F2 generation was
39 settled, maintained in an *ex-situ* nursery, and monitored for survival and growth. Both F1 and F2
40 generations followed a Type III survival curve with high initial mortality while in the *ex-situ*

41 nursery and low later-stage mortality. The growth rates of these colonies increased three-fold after
42 outplanting when compared to their growth rates in the *ex-situ* and *in-situ* nurseries. All colonies
43 survived while in the *in-situ* nursery and for an additional nine years after outplanting onto
44 Cuevones Reef. Overall, our results show that colonies produced by assisted breeding, once
45 outplanted, may contribute to the genetic diversity and establishment of self-sustaining sexually-
46 reproducing populations, which is an overarching goal of coral restoration programs.
47

Introduction

49 A long-term trend in declining coral cover has been documented in the Caribbean (Gardner *et al.*
50 2003). Since the 1980s, reefs along the Mexican Caribbean have shown signs of degradation due
51 to a combination of disturbances (Gómez *et al.* 2022). *Acropora palmata* is a foundational
52 Caribbean reef-building species and was abundant forming reef crests in the Mexican Caribbean
53 prior to the 1980s (Jordán-Dahlgren and Rodríguez-Martínez 2003). However, currently it is listed
54 as critically endangered on the IUCN Red List (International Union for the Conservation of Nature
55 2023).
56   During the 1980s, white-band disease was the primary cause of the greater than 95%
57 decline in *A. palmata* abundance in the Caribbean (Gladfelter 1982, Aronson and Precht 2001,
58 Precht *et al.* 2002). Unabating anthropogenic disturbances such as the marked increase in coastal
59 development due to the ever-expanding tourism market and associated urbanization (Rioja-Nieto
60 and Álvarez-Filip 2019, Häder *et al.* 2020, Banaszak 2021) combined with hurricanes (Jordán-
61 Dahlgren and Rodríguez-Martínez 1998, Gómez *et al.* 2022) and sea surface thermal anomalies
62 (Bove *et al.* 2022) threaten the viability of this and many other coral species (Miller *et al.* 2000,
63 Rodríguez-Martínez *et al.* 2001, Vermeij *et al.* 2011, Rodríguez-Martínez *et al.* 2014, Richmond
64 *et al.* 2018, Álvarez-Filip *et al.* 2019, González-Barrios *et al.* 2021).
65   One example of such degradation is Picudas Reef located in the Puerto Morelos Reef
66 National Park in the Mexican Caribbean. Monitoring of this reef has shown that it has a 3%
67 prevalence of both White Pox and White Band disease, high cyanobacterial and macroalgal cover
68 as well as small *A. palmata* colonies (Banaszak and Álvarez-Filip 2014). Artisanal fishing is
69 allowed on this reef, which is rarely visited by tourists (Instituto Nacional de Ecología 2000). In
70 recent years the corals on this reef have been severely impacted by the outbreak of Scleractinian
71 Coral Tissue Loss Disease (Guzmán-Urieta and Jordán-Dahlgreen 2021).
72   Reefs can also be damaged by ships. In December 1997, a cruise ship impacted Cuevones
73 Reef located in the Isla Mujeres, Punta Cancún, and Punta Nizuc National Park, primarily affecting
74 the reef-building species *Acropora palmata* growing on the reef crest (Victoria-Salazar *et al.*
75 2023). This grounding affected a total area of 504 m^2 reducing the live coral cover from 14% to
76 1%. By 2016, coral cover had recovered to 4.5% principally composed of *Porites astreoides* and
77 *Agaricia agaricites*, but there was no evidence of natural recruitment of reef-building species such
78 as *A. palmata* (Victoria-Salazar *et al.* 2023).
79   *Acropora palmata* has yet to recover to its historical highs on the reefs in the Mexican
80 Caribbean (Rodríguez-Martínez *et al.* 2014), which has led to widespread interventions to

81   replenish depleted populations, particularly using coral fragments (Bayraktarov *et al.* 2020). The
82   assisted production of sexual recruits is also important for population replenishment to maintain
83   or potentially increase genetic diversity and enable adaptation to changing environmental stressors
84   (Baums 2008, Baums *et al.* 2013) as well as to improve population connectivity (Baums *et al.*
85   2013, Chamberland *et al.* 2015, Baums *et al.* 2019).
86   *Acropora palmata* is a hermaphrodite, housing oocytes and spermaries in the same polyp
87   (Szmant 1986). Spawning of oocytes and sperm, packaged together in gamete bundles, occurs in
88   the summer months after the full moon, typically in August (Szmant 1986, Jordan 2018, Fogarty
89   and Marhaver 2019). *Acropora palmata* colonies have been reared from larvae, outplanted and
90   shown to be reproductively active at four years of age (Chamberland *et al.* 2015, 2016). A
91   knowledge gap that remains is whether it is viable to produce *A. palmata* colonies using assisted
92   fertilization and coral breeding to contribute to the re-establishment of sexually reproducing
93   populations by producing viable offspring.
94   Here, we describe the results of long-term tracking of *A. palmata* colonies, produced
95   through assisted reproduction of wild-caught gametes, reared in land-based facilities, then
96   transferred to *in-situ* nurseries, and finally outplanted onto two reefs in the Mexican Caribbean.
97   This F1 generation of colonies has reached sexual maturity on both reefs and spawned
98   synchronously with wild colonies. An F2 generation of *A. palmata* juveniles from the outplanted
99   colonies was also produced providing evidence that colonies raised in the laboratory and
100   outplanted onto reefs have the potential to naturally contribute to the replenishment of genetically-
101   diverse populations, which is critical for restoration programs to be successful over the long term.
102

Materials & Methods

Gamete collection and larval culture

105   *Acropora palmata* gamete bundles (containing eggs and sperm) were collected from wild parent
106   colonies at La Bocana Reef (20.99° N, 86.8° W) in the Puerto Morelos Reef National Park,
107   Mexican Caribbean. Spawning occurred 5 nights after full moon in August 2011 and September
108   2012. Collecting nets with containers at the top were deployed on presumed genetically distinct
109   colonies by divers. In 2011, spawn was collected from 10 parent colonies and from 4 parent
110   colonies in 2012. The collecting containers were lidded and taken to the dive boat, where the
111   gamete bundles from all parents were combined for fertilization in 1 μm filtered and UV-C
112   sterilized sea water (FSW). The gametes were gently mixed for 30 seconds at five-minute intervals
113   to assist the fertilization process and transported to the laboratory at the Unidad Académica de
114   Sistemas Arrecifales of the Universidad Nacional Autónoma de México campus in Puerto
115   Morelos, Quintana Roo, México. After two hours, the mixture was rinsed to remove excess sperm
116   and predators, using fat-separating pitchers, and placed into two 100 L coolers containing FSW
117   maintained at 28° C until settlement. After four hours, two samples of the mixture were examined
118   under a dissecting microscope at 60X magnification (Motic SMZ-161, USA), and the number of
119   developing embryos relative to the total number of eggs was determined to estimate fertilization
120   success. Daily 60% water changes were performed for each culture to eliminate unfertilized eggs,

121 metabolized lipids, organic matter, and mucus to reduce bacterial proliferation (Banaszak *et al.*
122 2018).

123

124 **Settlement and post-settlement care in *ex-situ* nurseries**
125 Substrates in the shape of plugs were made using a 1.5 cm high section of 1" PCV pipe as a mould,
126 using a mixture of Portland cement and beach sand (1:1) and then conditioned in the sea, on
127 pavement in the back reef, for approximately two months prior to larval settlement to promote the
128 growth of coralline algae and biofilm that induce larval settlement and metamorphosis (Negri *et*
129 *al.* 2001). The substrates were scrubbed to remove sediment and overgrowing algae, and placed in
130 the culture bins once the larvae began to swim.
131   Initial settlement was determined at five to eight days post-settlement. At 10 days post-
132 settlement, the F1 settlers were inoculated with symbiotic dinoflagellates freshly isolated from *A.*
133 *palmata* fragments using FSW and an air brush (Tytler and Spencer 1983, Baird *et al.* 2006).
134 During the inoculation period, the water level in the larval culture bins was reduced and a
135 concentration of 1×10^6 symbiont cells/mL was added. After 16 h, a partial water change was made,
136 and three days later the settlers were inspected for signs of symbiont uptake using a dissecting
137 microscope and fluorescent light.
138   The F1 settlers were maintained in outdoor aquaria either at UNAM or at Xcaret Aquarium
139 (Fig. 1, 2). Both *ex-situ* nurseries were outfitted with flowing sea water at an average temperature
140 of 29 ± 0.6 °C and peak noon irradiance ranging from 300 to 600 µmol m^{-2} s^{-1}. Temperatures were
141 measured using HOBO water temperature data loggers (HOBO UA-001-64 Pendant Temp) and
142 irradiance was measured using a LI-COR LI-192 underwater quantum sensor connected to a LI-
143 COR 1400 data logger. The settlers were fed daily, initially using commercially available feed
144 (Kent Marine, USA) according to the recommendations of the manufacturer, and later with rotifers
145 and fresh *Artemia* nauplii. The aquarium walls and floors were cleaned twice a week to remove
146 filamentous algae, whereas the substrates were carefully cleaned with the naked eye to reduce algal
147 overgrowth on a weekly basis. Detailed monitoring of the settlers was begun at the third month
148 post-settlement (Table 1). For the 2012 cohort, colony area and number of polyps were obtained
149 from photographs analysed with ImageJ software.

150

151 **Transfer and monitoring of F1 colonies in *in-situ* nurseries**
152 In April 2014 a subset of colonies from the two cohorts (2011 and 2012) was transferred from the
153 two *ex-situ* nurseries to two *in-situ* nurseries (Fig. 1, 2): one was located in the Puerto Morelos
154 Reef National Park (20.88 N, 87.85 W) at 5 m depth and the other at Las Ruinas (20.42° N, 87.11°
155 W) in Playa del Carmen at 3.8 m depth. The *in-situ* nurseries consisted of 45 cm high vertical
156 supports made from 2.5 cm diameter PVC tubes incorporated into cement plates that measured 50
157 cm x 50 cm x 20 cm (L x W x H). Each support had a PVC connector at its free end which was
158 used to mount the coral colony via a compatible connector (Johnson *et al.* 2011). Each nursery
159 received the same number of juveniles from each cohort: 12 each from the 2011 cohort and 18
160 each from the 2012 cohort.

161   The average daily temperature in the Puerto Morelos *in-situ* nursery, measured by a HOBO
162 water temperature data logger (HOBO UA-001-64 Pendant Temp), was 29 ± 0.5 °C and 28 ± 0.3
163 °C in the Las Ruinas nursery. Irradiance at solar noon, measured by an Odyssey Waterproof
164 Photosynthetic Active Radiation Logger, ranged between 450 and 600 µmol m^{-2} s^{-1} in the Puerto
165 Morelos *in-situ* nursery and between 230 and 710 µmol m^{-2} s^{-1} in the Las Ruinas nursery.
166   Survival and growth were determined at the same intervals in the Puerto Morelos and Las
167 Ruinas nurseries; for colonies from the 2011 cohort at 2.6, 3, and 3.5 years old, and for colonies
168 from the 2012 cohort at 1.6, 2, and 2.4 years old (Table 1). For growth measurements, the
169 maximum diameter was recorded using a vernier caliper. We compared growth rates between *ex-*
170 *situ* and *in-situ* nurseries via a parametric t-test (RStudio Team 2020).
171

Outplanting of F1 colonies onto Cuevones Reef

173 The lack of natural *A. palmata* recruitment at Cuevones Reef (21.15° N, 86.74° W) 20 years after
174 a ship grounding (Victoria-Salazar *et al.* 2023) prompted the selection of Cuevones Reef as an
175 outplant site for *A. palmata* juveniles. In February 2015, 29 colonies from both the 2011 and 2012
176 cohorts were harvested from the two *in-situ* nurseries and outplanted onto Cuevones Reef (Fig. 1,
177 2) at 10 m depth. The overall temperature averaged 29 ± 0.5 °C and average peak noon irradiance
178 ranged between 300 and 400 µmol m^{-2} s^{-1}. Each colony was attached to the reef using a mixture of
179 cement and sand. The colonies were monitored for survival and growth as described in Table 1.
180

Fertility and Fecundity of F1 colonies at Cuevones Reef

182 In August 2019, tissue samples were collected from the edge of 10 colonies (2011 cohort) and 5
183 colonies (2012 cohort), placed in ziploc bags with seawater, and transported to the laboratory. The
184 samples were fixed in 10% formaldehyde in FSW for 24 hours at 4 °C, then rinsed with FSW.
185 Then, all samples were decalcified by progressively increasing the concentration of hydrochloric
186 acid (HCl, buffered with EDTA, Sodium Tartrate and Potassium) from 1 to 8%. The carbonate-
187 free tissue was preserved in 70% ethanol. Between 10 and 14 polyps were dissected from each
188 colony to document fertility (% polyps with oocytes, Van Veghel and Kahmann 1994) and
189 fecundity (defined as the number of oocytes per polyp, Kojis and Quinn (1981) and spermaries per
190 polyp). Polyp density, gonadal index (number of oocytes per cm^2), and oocyte diameter were
191 estimated in randomly-selected polyps.
192

Outplanting of F1 colonies onto Picudas Reef

194 In September 2020, two colonies from the 2011 cohort and 14 colonies from the 2012 cohort were
195 outplanted onto Picudas Reef (20.88 N, 87.85 W) in the Puerto Morelos Reef National Park (Fig.1,
196 2). Picudas Reef is 3 to 5 meters deep, with an overall average temperature of 29 ± 0.2 °C and a
197 peak noon irradiance ranging between 400 and 550 µmol m^{-2} s^{-1}. The colonies were attached to
198 the reef using a mixture of cement and sand. The survival, growth, and spawning pattern of all 16
199 colonies were monitored in the summer during the subsequent two years (Fig. 2). The benthic
200 cover of the reef was obtained following the AGRRA method (Lang *et al*. 2012) at one and 18
201 months after outplanting.

202
203 **Spawning of F1 colonies at Picudas Reef**
204 In August 2022, gametes from two of these F1 colonies were collected (Fig. 2) and cross-fertilized
205 at 85 min after spawning using 2 mL of gamete bundles from each colony. Separately, an extra
206 cross was made 110 min after spawning with 1 mL of gamete bundles from an unrelated wild
207 colony and 30 mL of the sperm batch from the initial cross of F1 colonies. Embryos were cultured
208 and settled as described above. The F2 settlers were maintained in the UNAM *ex-situ* nursery
209 under natural solar conditions.
210

211 ## Results
212 **Fertilization and settlement success in *ex-situ* conditions**
213 Gamete bundles were collected from two of the 10 colonies that spawned in 2011 and all four
214 colonies that spawned in 2012. Fertilization success was 90% in 2011 and 99% in 2012.
215 Approximately 2,000 settlers from the 2011 cohort and 1,000 settlers from the 2012 cohort were
216 inoculated with symbionts and raised in aquaria maintained under natural sunlight. During the first
217 three months post-settlement, survival of the settlers was 95% after which there was a decrease to
218 31% at 4 months and 0.3% at 18 months for the 2011 cohort (Table 1). Survival of the 2012 cohort
219 was 38% at 9 months and 12% at 18 months. Mortality was due to overgrowth by filamentous
220 algae, failure to take up symbionts despite multiple inoculation attempts, or competition between
221 the coral settlers. In some cases, two or more coral settlers would fuse to form one larger colony.
222 The number of polyps ($R^2 = 0.96$) and colony area ($R^2 = 0.94$) increased exponentially over time
223 in *ex-situ* culture (Fig. 3). Growth rates in terms of the maximum diameter of the colonies in the
224 *ex-situ* nursery were 2.5 mm ($\pm$ 0.7) per month for the 2012 cohort (Fig. 4).
225

226 **Survival and growth of F1 colonies in the *in-situ* nursery and after outplanting**
227 The survival of the 60 juvenile colonies placed in the *in-situ* nursery was 100% for both cohorts
228 during the 10-month period (Table 1). Twenty-nine colonies were outplanted onto Cuevones Reef
229 in the west Coast of Isla Mujeres, Punta Cancún and Punta Nizuc National Park, 16 colonies were
230 outplanted to Picudas Reef in the Puerto Morelos Reef National Park and 15 colonies remained in
231 the *in-situ* nursery in the Puerto Morelos Reef National Park. Growth rates as determined by
232 following the maximum diameter over time were 1.9 mm $\pm$ 0.5 per month just prior to outplanting
233 onto the reef. This was slightly lower but not significantly different (t= 2.16, p=0.0791) to the
234 growth rates recorded in colonies maintained in the *ex-situ* nursery during the same period (Fig.
235 4).
236 Three and a half years after the 29 colonies were outplanted onto Cuevones Reef, survival
237 of both cohorts was 100%. Growth rates as determined by the maximum diameter increased to 5.6
238 mm $\pm$ 1.8 per month, whereas the growth rates of the 15 colonies from the same cohort that
239 remained in the *in-situ* nursery were 1.9 mm $\pm$ 0.5 per month (Fig. 4).
240 Prior to outplanting onto Picudas Reef, macroalgal cover was approximately 34% with
241 23% turf algae, 23% crustose coralline algal and 3% live coral cover. One and a half years after
242 the outplanting the macroalgal cover was 21%, with 27% turf algae, 27% crustose coralline algal

243 and 5% live coral cover. Of the 16 colonies that were outplanted onto Picudas Reef, all colonies
244 remained alive 2.5 years after outplanting. In the same period, the range of growth rates determined
245 by the maximum diameter was wide, from -260 mm to 350 mm per month. The negative growth
246 is presumably due to partial damage to the colonies due to branch breakage from the impact of
247 hurricanes Delta and Zeta that affected the Puerto Morelos Reef National Park in 2020 (Fig. 5).
248

249 **Fertility and fecundity of outplanted colonies at Cuevones Reef**
250 Based on the dissections of the polyps from the 15 F1 colonies (Fig. 6A, 6B) that were sampled at
251 Cuevones Reef, eight (53%) contained gonads. Of the 96 polyps that were dissected from the eight
252 colonies with gonads, 76% contained oocytes and/or spermaries (Fig. 6C, 6D). Fecundity was 4.03
253 ± 2.7 (n = 387) oocytes per polyp and there were 2.30 ± 1.5 (n = 221) spermaries per polyp (Fig.
254 7A). The gonadal index was 63.63 ± 43.35 (n = 8) oocytes cm^{-2} (Fig. 7B) and 35.03 ± 19.39 (n =
255 8) spermaries cm^{-2}(Fig. 7B). The mean maximum diameter of the oocytes (Fig. 7C) was 769 μm
256 ± 141 (n = 42) and the mean diameter of the spermaries (Fig. 7C) was 1.52 mm ± 0.423 (n = 14).
257

258 **Spawning, fertilization, and settlement success from colonies at Picudas Reef**
259 In August 2022, spawning was recorded in 1 colony from the 2011 cohort and 3 colonies from the
260 2012 cohort (Fig. 8A, 8B, Table 2) and 18 other nearby colonies, 8 wild and 10 colonies of asexual
261 origin, three nights after full moon at Picudas Reef. Two mL of gamete bundles were collected
262 from each of two colonies of the 2012 cohort that spawned. Cross fertilization of these two colonies
263 yielded low fertilization success at 15%. However, when their sperm were crossed separately with
264 bundle gametes from a wild colony, fertilization success increased to 95%. The embryos and larvae
265 produced in the second cross were maintained in *ex-situ* aquaria with a settlement yield of 2.4% at
266 13 days after settlement (Fig. 8C). The settled larvae successfully took up symbionts (Fig. 8D).
267 Survival at three months was 1.76%. The average maximum diameter of the settlers was 1.2 ± 0.3
268 mm (n = 19) and the average area was 1.0 ± 0.4 mm^2 (n = 19).
269

270 # Discussion

271 In this study, we not only show that F1 colonies of *Acropora palmata* produced by crossing wild-
272 caught gametes can be reared in the laboratory and outplanted onto coral reefs, but that they are
273 reproductively viable and spawn synchronously with wild colonies. Moreover, this is the first
274 report of the production of viable F2 offspring of this species. Thus, the goal to produce potentially
275 self-sustaining breeding populations of this critically endangered species is achievable
276 approximately within a 10-year span.
277   Both F1 cohorts reared in the laboratory, even after maintenance for extended periods in
278 *ex-situ* nurseries prior to outplanting onto a degraded site such as Picudas Reef or a damaged area
279 such as Cuevones Reef, released gametes within the known spawning window for this species and
280 on the same night as their wild counterparts. Coinciding in spawning date and time is significant
281 because it means that it is highly probable that fertilization between the laboratory-produced and
282 natural colonies can occur; in fact, we showed that such a combination results in fertilization
283 success of 95%, at least in our laboratory setting.

284   At seven and eight years old, the outplanted F1 colonies were shown to be fertile, producing
285   both oocytes and spermaries. The size of the oocytes is large at 769 μm ± 141 in diameter, which
286   falls within the values for nine species of *Acropora* from the Great Barrier Reef that ranged from
287   601 to 728 μm diameter (Wallace 1985). The fecundity of the outplanted F1 colonies was 5.61 ±
288   1.91 oocytes per polyp and falls within the values reported for four species of *Acropora* from the
289   Great Barrier Reef that ranged from 4.67 ± 0.60 to 6.52 ± 0.84 oocytes per polyp (Pratchett *et al*.
290   2019).
291   The performance of F1 and F2 larvae and settlers was similar to that of other generations
292   raised in our *ex-situ* larval culture facility (Banaszak *et al*. 2018, Miller *et al*. 2021). As shown in
293   this study, reproductively active adult colonies can be produced from wild caught gametes by
294   applying a series of protocols that optimize culture conditions. Intensive care under controlled
295   conditions during the early developmental stages and after settlement can result in colonies that
296   thrive in the reef environment. However, the growth rates of the colonies while in *ex-situ* nurseries
297   was relatively low compared to the growth rates on the reef, possibly due to limitations associated
298   with cleaning and maintaining corals in *ex-situ* cultures. The growth rates were higher than those
299   in *in-situ* nurseries, which may also be influenced by environmental factors such as competition
300   with algae and predation by fish (Mendoza-Quiroz, Pers. Obs.). The growth rates of the colonies
301   once outplanted onto the reef increased approximately three-fold, when compared to their growth
302   rates in *ex-situ* or *in-situ* nurseries.
303   The outplanted colonies reached sexual maturity at least within eight years in both
304   Cuevones Reef and Picudas Reef. Low fertilization success (15%) in the crossing of two of the F1
305   colonies outplanted at Picudas reef indicates that these may be siblings or half-siblings. Both
306   colonies are from the 2012 cohort, when gametes from four parents were crossed. However,
307   fertilization success increased to 95% once the sperm from these two colonies was crossed with
308   eggs from a wild colony.
309
310   Recommendations
311   Based on our experience and the results of this study, we present ten recommendations for
312   restoration practitioners who wish to establish a coral larval propagation program for population
313   replenishment. 1. Collect from as many unrelated colonies as possible to increase fertilization
314   success; 2. Collect from distant colonies due to the high clonality characteristic of this species to
315   ensure maximal genetic diversity; 3. Use equal volumes of gametes from each colony during
316   assisted fertilization; 4. Maintain cultures as clean as possible during fertilization and embryonic
317   and larval development; 5. Use static conditions for early phase culturing; 6. Precondition
318   settlement substrates for a minimum of one month to improve settlement success; 7. Provide
319   intensive care post-settlement by feeding recruits and cleaning substrates of algal overgrowth; 8.
320   Include an intermediate nursery phase; 9. Outplant sexual recruits in a well-spaced pattern and
321   interspersed with unrelated wild colonies to improve fertilization potential and importantly, reduce
322   the chances of inbreeding. If wild colonies are not available, then we suggest that cohorts from

323 different generations and from as many collection sites as possible be intermixed.; and 10.
324 Undertake long-term monitoring of growth, survival, reproductive capacity and spawning.
325

Conclusion

327 Overall, our results show that coral colonies produced using assisted fertilization and *ex-situ* coral
328 breeding are able to reproduce synchronously with wild populations and thus have the potential to
329 contribute to the re-establishment of self-sustaining, sexually-reproducing populations of
330 *Acropora palmata* in the Mexican Caribbean. Innovative techniques to scale up these efforts are
331 needed to achieve the long-term goal of re-establishing the structure and function of coral reefs.
332

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339

Conflict of interest statement

341 The authors declare that the research was conducted in the absence of any commercial or financial
342 relationships that could be construed as a potential conflict of interest.
343

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491

Manuscript to be reviewed# Figure 1

Location of sites where the phases of this study were performed.

A) Distribution of sites along the Quintana Roo coast. B) Cuevones Reef where F1 juveniles of *A. palmata* were outplanted in February 2015 and gravid colonies were recorded in 2019. C) La Bocana Reef where gametes were collected in 2011 and 2012; UNAM where gametes were fertilized and recruits were bred in an *ex-situ* nursery; *In-situ* nursery where juvenile colonies were maintained; and Picudas Reef where outplanted F1 colonies spawned in 2022. D) Xcaret where an *ex-situ* nursery was installed and an *in-situ* nursery was installed at Las Ruinas. Map data © 2023 Google, Landsat/Copernicus, Maxar Technologies.

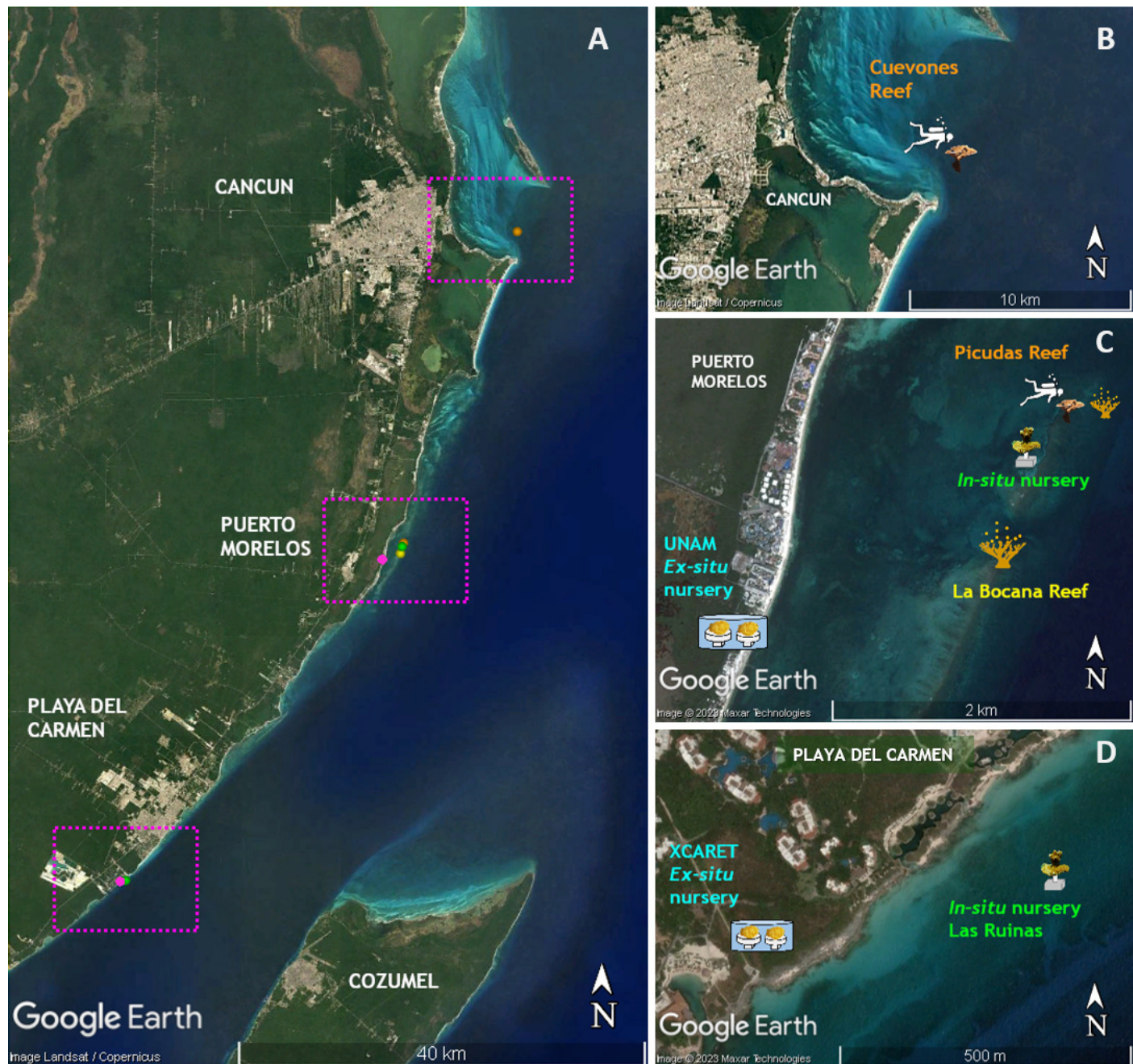



Figure 2

Timeline of the phases described in this study for the two cohorts of *Acropora palmata* produced via assisted sexual reproduction.

Acropora palmata colonies were transferred from two *ex-situ* nurseries (UNAM and Xcaret) to two *in-situ* nurseries (Puerto Morelos and Las Ruinas) and later to Cuevones Reef and Picudas Reef. The ages of the two cohorts are shown in blue (2011 cohort) and red (2012 cohort) at the top and the corresponding coloured arrows (with N = number of recruits) depict the transfer to the next phase. Samples for fecundity analysis were collected from Cuevones Reef and spawning observations and gamete sampling were undertaken at Picudas Reef. Note: The Las Ruinas *in-situ* nursery was decommissioned in 2015 when colonies were outplanted to Cuevones Reef and the remaining coloines were transferred to the Puerto Morelos nursery (dashed black arrow).

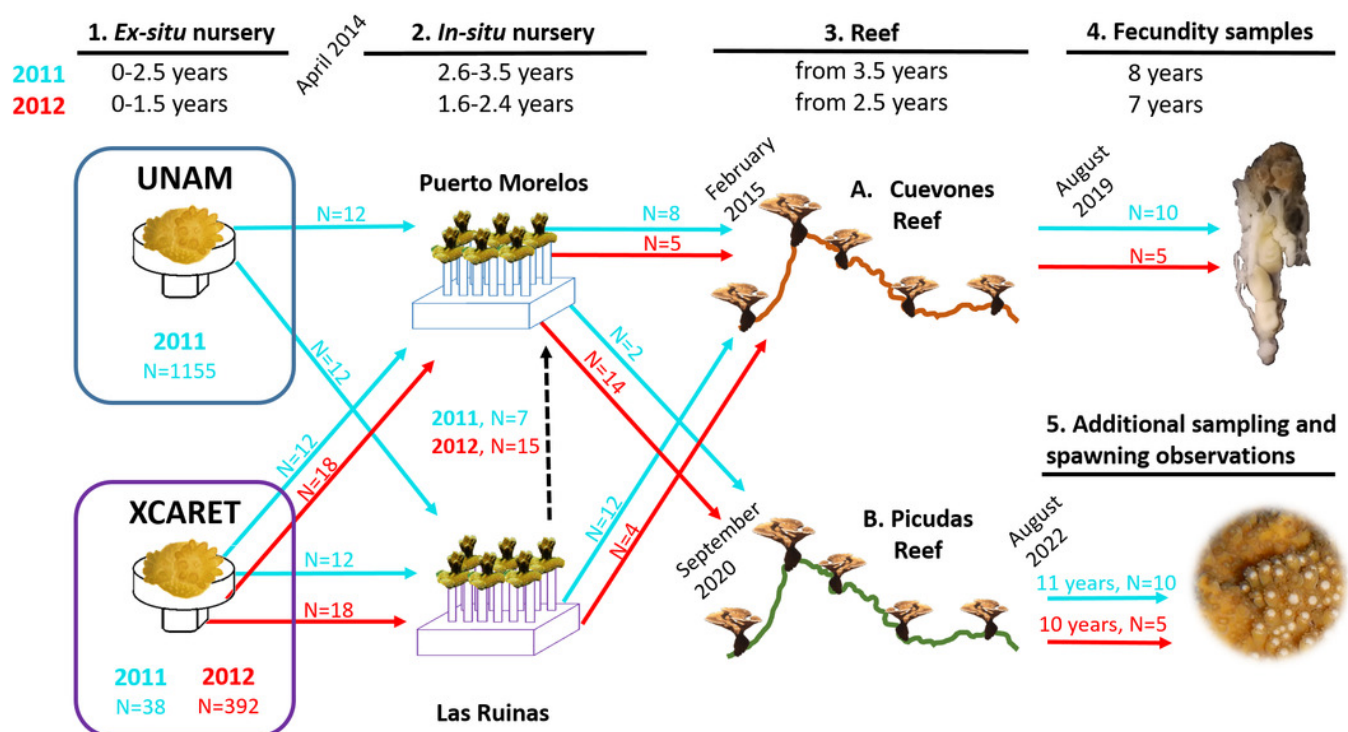



Figure 3

Growth of *Acropora palmata* recruits in *ex-situ* culture during the first 12 months after settlement.

The number of polyps (triangles) and colony area (circles) show an exponential function of recruit age (R^2 = 0.9345, y = $0.0975x^{2.2486}$). The broken trend line refers to polyp number and the solid line to colony area. Error bars are standard errors of the mean.

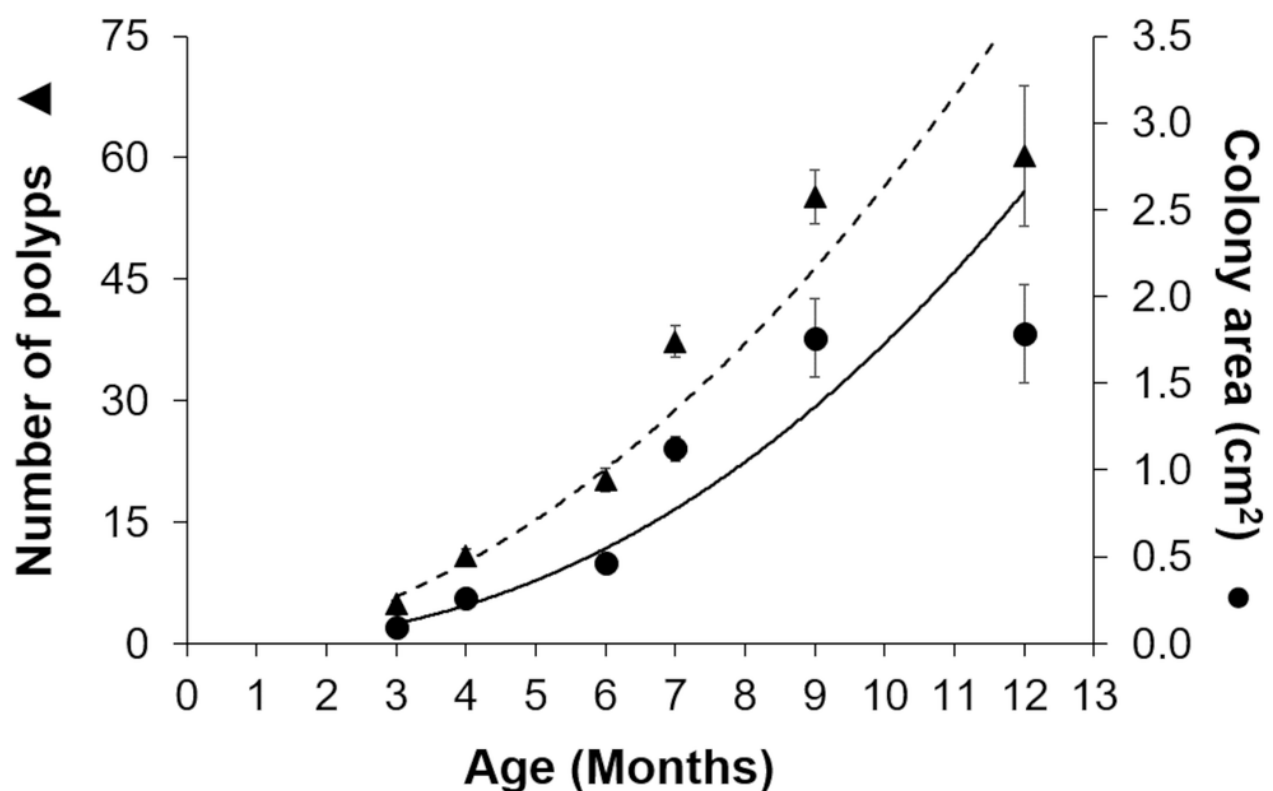



Figure 4

Comparison of growth rates of the two cohorts (2011 and 2012) of *Acropora palmata* F1 colonies.

The colonies were produced by crossing wild-caught gametes and culturing the embryos and larvae through settlement and grow out in an *ex-situ* nursery. Growth (maximum diameter of the colonies) was measured while in the *ex-situ* nursery (solid grey bar, n = 24), after transfer to an *in-situ* nursery (solid white bar, n = 17), and after outplanting onto Cuevones Reef (solid black bars, n_{2011} = 9, n_{2012} = 10). Error bars indicate standard deviation of the mean. Means with the same letters are not significant (p > 0.05).

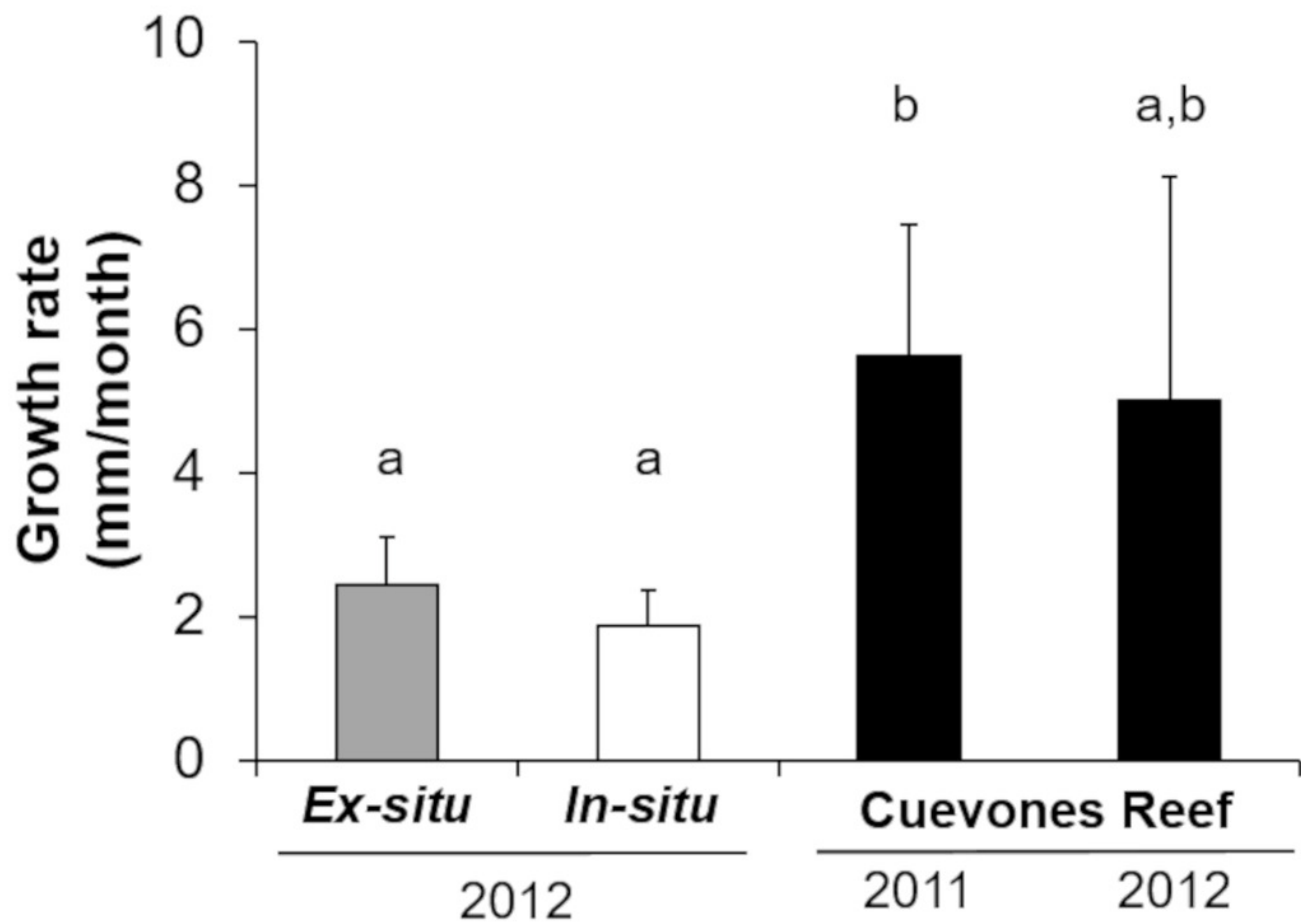



Figure 5

Comparison of growth rates of F1 *Acropora palmata* colonies outplanted on Picudas Reef.

Growth (maximum diameter of the colonies) was measured *in-situ* for two years after outplanting at Picudas Reef. Error bars indicate 10th and 90th percentiles of the median. Dashed line denotes zero growth rate.

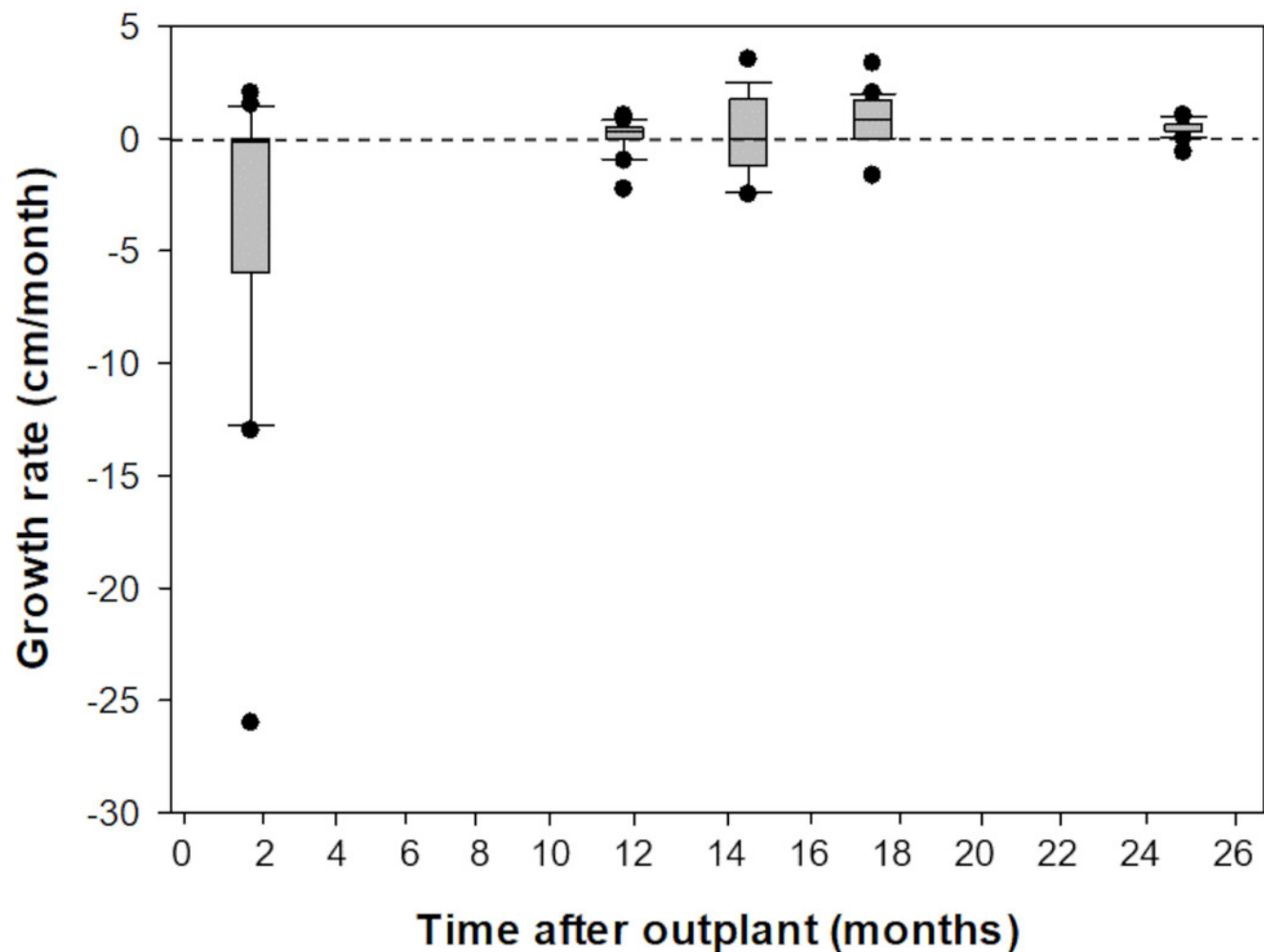



Figure 6

Reproductive capacity of *Acropora palmata* colonies produced in the laboratory and outplanted onto Cuevones Reef.

A) A 9-year old *A. palmata* colony on Cuevones Reef. Scale bar = 10 cm. B) Section of an *A. palmata* colony showing pink-coloured oocytes. Scale bar = 1 cm. C) Cross section and (D) longitudinal section of developing oocytes (O) and spermaries (S) in a decalcified coral polyp. Scale bar = 1 mm. Photo credits: A and B by Sandra Mendoza Quiroz, C and D by Daniela Muñoz Villareal.

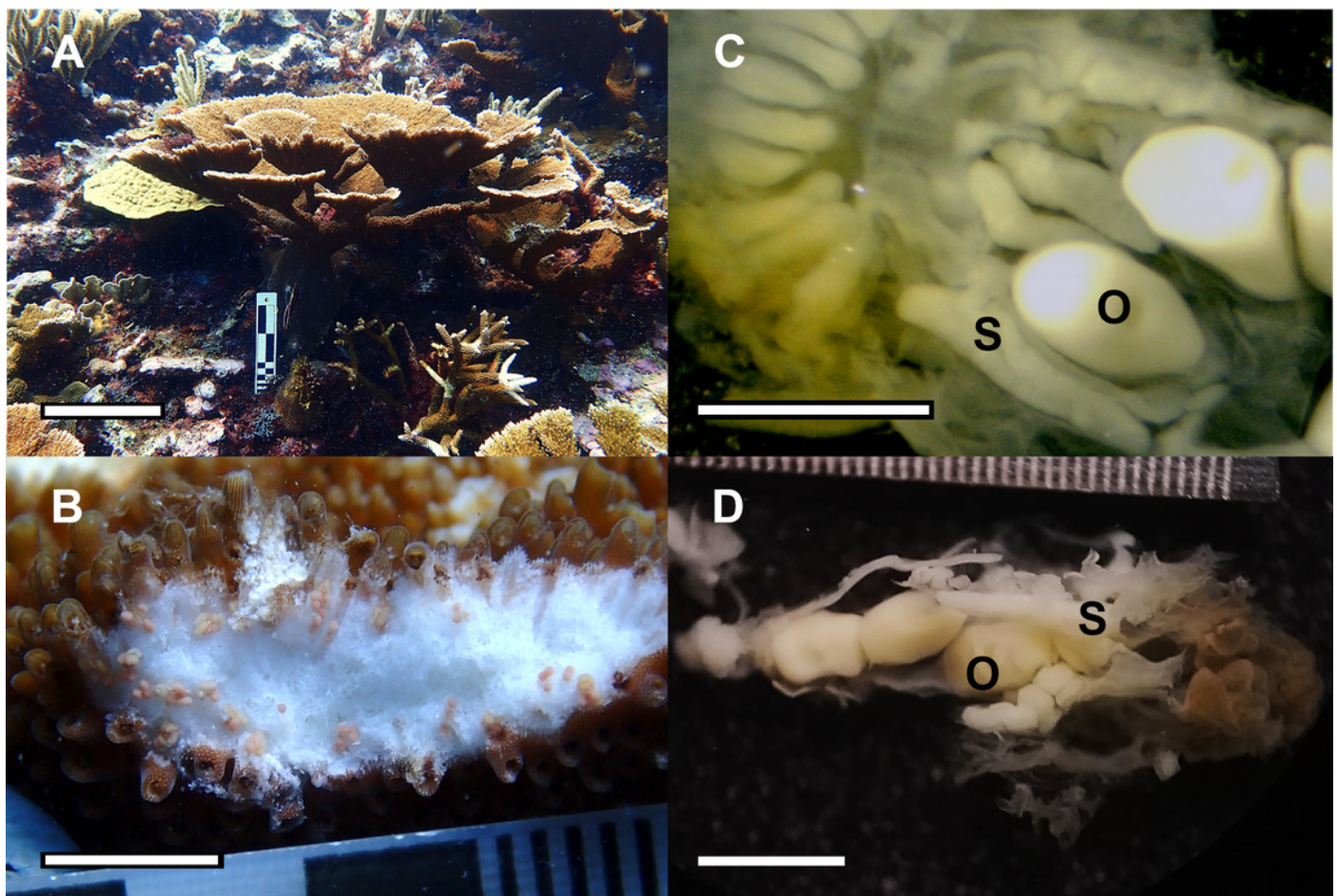



Figure 7

Fecundity and gonadal index from eight F1 colonies.

(A) Boxplot of oocytes (black bars) and spermaries (white bars) per polyp. Boxes represent 25th and 75th percentiles and error bars indicate 10th and 90th percentiles. The grey line represents the mean and diamonds indicate outliers. (B) Gonadal index per cm^2. (C) Maximum diameter of oocytes (black bar) and spermaries (white bar). Error bars indicate standard deviation of the mean.

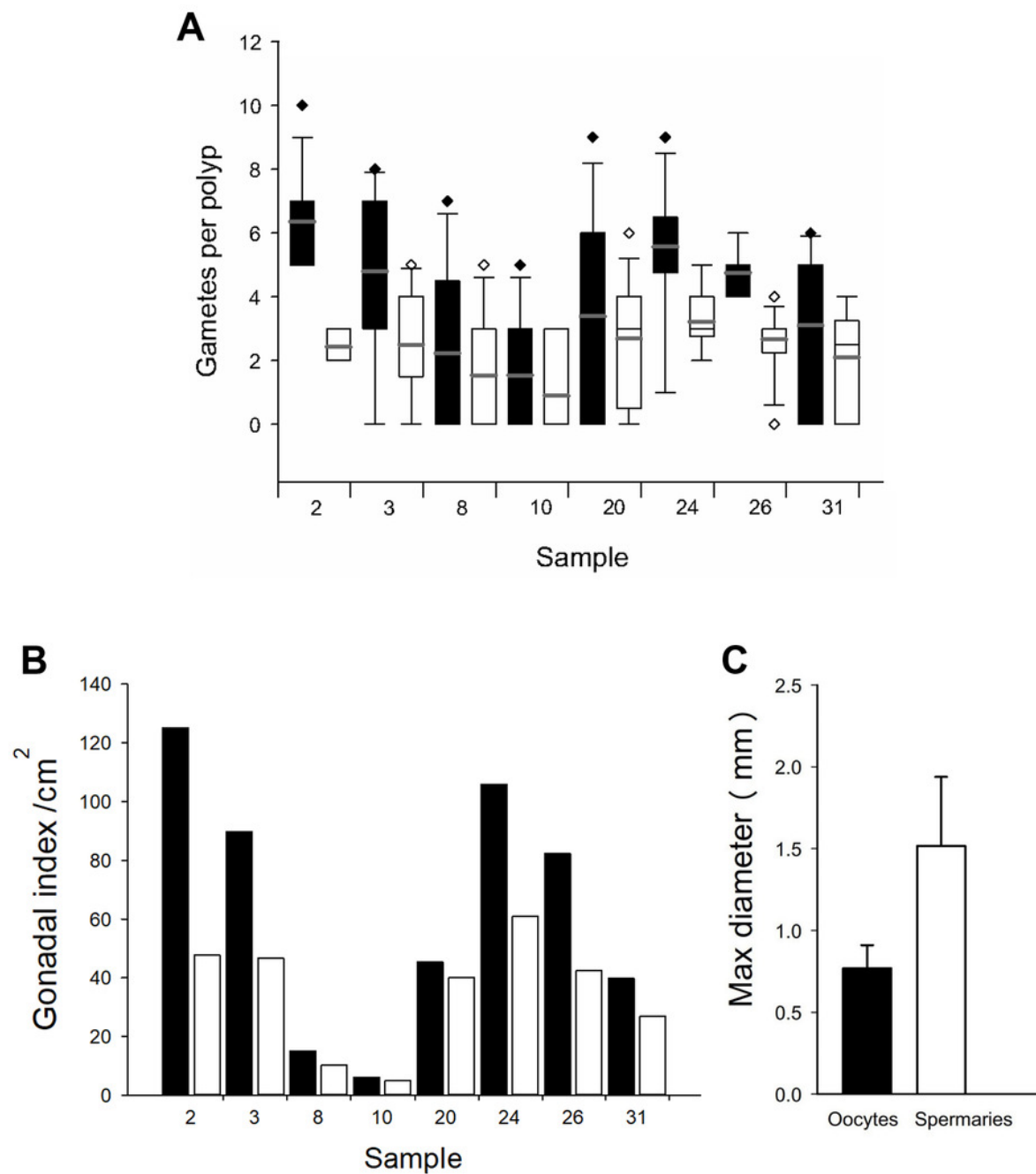
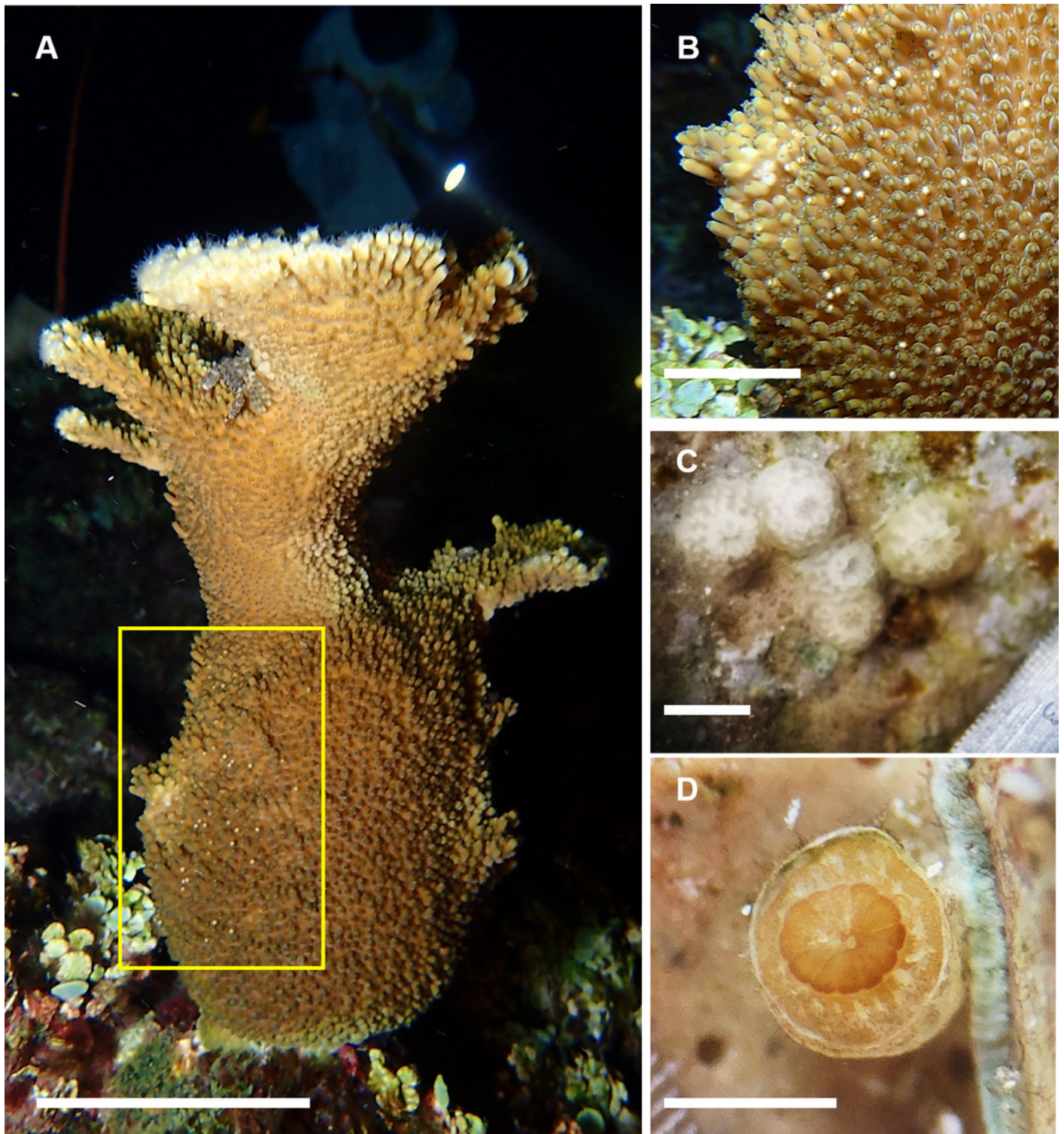



Figure 8

Spawning by the F1 generation of *Acropora palmata* colonies and their offspring.

A) Spawning by an F1 generation *A. palmata* colony that was produced by collecting wild gametes, assisting fertilization, culturing embryos and larvae through to settlement, raising to juveniles and outplanting onto Picudas Reef. Scale bar = 10 cm. B) Closeup of photo A to show setting of gamete bundles in the polyps´ mouths just prior to spawning. Scale bar = 5 cm. C) F2 generation of 10-day-old recruits of *A. palmata* without symbionts and with skeleton clearly visible. Scale bar = 1 mm. D) Five-month-old F2 generation *A. palmata* juvenile in *ex-situ* culture. Scale bar = 1 mm. Photo credits: A and B by Esmeralda Pérez Cervantes, C by Sandra Mendoza Quiroz, and D by Victoria Grosso.

Survival of *Acropora palmata* cohorts

The number of colonies of *A. palmata* produced in 2011 and 2012 that survived, at each time point, in *ex-situ* facilities, after transfer to an *in-situ* nursery, and after outplanting onto Cuevones Reef.

	2011 Cohort				2012 Cohort			
	Date	Age	UNAM	XCARET	Date	Age	UNAM	XCARET
Ex-situ	Sep 2011	1 mo	1155	-	Dec 2012	3 mo	-	393
	Feb 2012	6 mo	119	35	Jun 2013	9 mo	-	151
	May 2012	9 mo	12	12	Sep 2013	12 mo	-	112
	Aug 2012	12 mo	12	12	Nov 2013	14 mo	-	107
	Sep 2013	25 mo	12	12	Mar 2014	18 mo	-	46
In-situ	Apr 2014	2.6 y	12	12	Apr 2014	1.6 y	18	18
	Aug 2014	3 y	12	12	Sep 2014	2 y	18	18
	Feb 2015	3.5 y	12	12	Feb 2015	2.4 y	18	18
Reef	Feb 2015	3.5 y	8	12	Feb 2015	2.5 y	5	4
	Aug 2017	6 y	8	12	Aug 2017	5 y	5	4
	Aug 2020	9 y	8	12	Aug 2020	8 y	5	4

Characteristics of the F1 *Acropora palmata* colonies that spawned in August 2022.

The size, age, and condition of *A. palmata* colonies as well as the volume of gametes collected from each colony (mL) are shown.

Cohort	ID Colony	Spawn Collection	Gamete volume (mL)	Length (cm)	Width (cm)	Height (cm)	Number of branches	Colony condition
2011	120	no	~50 bundles	25	20	30	3	Healthy
2012	324	yes	2	22	17	15	4	Healthy
2012	441	yes	2	40	35	45	7	Healthy
2012	125	yes	0.1	16	18	23	5	Healthy