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Interspecific Hybridization May Provide Novel Opportunities for Coral Reef Restoration

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Climate change and other anthropogenic disturbances have created an era characterized by the inability of most ecosystems to maintain their original, pristine states, the Anthropocene. Investigating new and innovative strategies that may facilitate ecosystem restoration is thus becoming increasingly important, particularly for coral reefs around the globe which are deteriorating at an alarming rate. The Great Barrier Reef (GBR) lost half its coral cover between 1985 and 2012, and experienced back-to-back heat-induced mass bleaching events and high coral mortality in 2016 and 2017. Here we investigate the efficacy of interspecific hybridization as a tool to develop coral stock with enhanced climate resilience. We crossed two *Acropora* species pairs from the GBR and examined several phenotypic traits over 28 weeks of exposure to ambient and elevated temperature and pCO_2. While elevated temperature and pCO_2 conditions negatively affected size and survival of both purebreds and hybrids, higher survival and larger recruit size were observed in some of the hybrid offspring groups under both ambient and elevated conditions. Further, interspecific hybrids had high fertilization rates, normal embryonic development, and similar *Symbiodinium* uptake and photochemical efficiency as purebred offspring. While the fitness of these hybrids in the field and their reproductive and backcrossing potential remain to be investigated, current findings provide proof-of-concept that interspecific hybridization may produce genotypes with enhanced climate resilience, and has the potential to increase the success of coral reef restoration initiatives.

Keywords: hybridization, restoration, coral reefs, climate change, *Acropora*, assisted evolution, genetic rescue, hybrid vigor

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INTRODUCTION

The rapid increase in atmospheric CO_2 to levels not documented for millions of years (Hönisch et al., 2012) and associated ocean warming and acidification have profoundly transformed the marine realm (Pandolfi et al., 2011). Higher-than-usual seawater temperatures can cause coral bleaching, the breakdown of the symbiotic relationship between the coral host and its dinoflagellate endosymbionts (*Symbiodinium* spp.), and associated coral mortality (Hoegh-Guldberg, 1999). Ocean acidification is reducing carbonate ion availability in seawater and can depress calcification rates of calcifying organisms like corals (Langdon et al., 2000; Doney et al., 2009; Chan and Connolly, 2013). These global changes, coupled with local stressors such as pollution, overfishing, and outbreaks of crown-of-thorns starfish, have drastically

altered coral cover and community composition at a global scale. In the last three decades, multiple mass bleaching events have decimated coral reefs worldwide including in 1998, 2010, and 2014–2017 (Eakin et al., 2016; Heron et al., 2016; Hughes et al., 2017, 2018). The Great Barrier Reef (GBR) is no exception with 50–80% coral mortality recorded on many northern reefs following the 2016 mass bleaching event (Great Barrier Reef Marine Park Authority, 2017), followed by another high mortality mass bleaching event in 2017. Climate models predict a <5% chance of reaching the Paris agreement target of limiting the global temperature rise to <2°C compared to pre-industrial times by 2100 (Raftery et al., 2017), and most coral reefs are forecasted to experience annual severe bleaching before the end of the century (van Hooidonk et al., 2016). Several observations of an increase in tolerance of coral bleaching after successive bleaching events suggest that adaptation and/or acclimatization are possible under certain conditions (Maynard et al., 2008; Berkelmans, 2009; Guest et al., 2012; Penin et al., 2013). Nevertheless, over 50% of the world's coral reefs has been lost in the last three decades, with the Caribbean having lost over 80% of its coral cover (50 Reefs, 2017)[1], indicating that the rates of natural adaptation and acclimatization are overall insufficient to keep pace with the rate of environmental changes (van Oppen et al., 2017).

Active reef restoration is one way to assist the recovery of coral reefs that are degraded, damaged or destroyed. Reef restoration is still in its infancy and all of the few successful efforts so far occurred on a small spatial scale (e.g., Nakamura et al., 2011; Omori, 2011; Villanueva et al., 2012; Guest et al., 2014; dela Cruz and Harrison, 2017). Traditionally, locally sourced biological material is used for restoration based on the assumption that these populations are locally adapted and therefore most likely to survive (Breed et al., 2013). However, anthropogenic disturbances are rapidly changing the environment and shifting selection pressures (Becker et al., 2013), and locally sourced stock is therefore potentially mismatched with the altered environment. An effective restoration strategy should thus incorporate an understanding of present day ecological characteristics of species, characteristics of future available habitats, and adaptive potential of species (Becker et al., 2013). The use of non-local and climate resilient materials is controversial, but is gaining traction in wildland restoration (Jones and Monaco, 2009), revegetation (Sgrò et al., 2011; Breed et al., 2013), and coral reef restoration (Rau et al., 2012; van Oppen et al., 2017).

One possible way to improve the adaptive potential of species is via hybridization, which can increase genetic variation, break genetic correlations that constrain evolvability of parental lineages, and assist species to acquire adaptive traits (Hoffmann and Sgrò, 2011; Becker et al., 2013; Carlson et al., 2014; van Oppen et al., 2015; Hamilton and Miller, 2016; Meier et al., 2017). Hybridization can be conducted either via targeted crossing of individuals or species carrying desired phenotypic traits (e.g., high thermal tolerance) or via crossing between species with the goal of increasing genetic diversity and new variation for natural selection to act upon, and potentially generating hybrid vigor. The relative fitness of F1 hybrids (**Figure 1**) depends on whether there are additive (i.e., hybrids are of intermediate fitness between the parental species), dominant (i.e., hybrids are of equal fitness to the dominant parent species), over-dominant (i.e., hybrids are more fit than both parental species), under-dominant (i.e., hybrids are less fit than both parental species) gene effects, and/or maternal effects (i.e., hybrids are of equal fitness to their maternal parent species) (for review, see Lippman and Zamir, 2007; Li et al., 2008; Chen, 2013). Reciprocal hybrids are predicted to have equal fitness, except under maternal inheritance. With maternal effects, the fitness of the hybrids is directly affected by the fitness of the maternal parental species, regardless of the offspring's own genotype (Roach and Wulff, 1987; Bernardo, 1996). In the context of restoration, hybrid vigor which can be driven by dominant or over-dominant mechanisms, is a desirable outcome. The value of hybridization in enhancing fitness has been demonstrated in multiple cases. For instance, hybridization has provided genetic variance in morphology for adapting to changing environments in Darwin's finches (Grant and Grant, 2010), altered chemical defense of hybrid Brassicaceae plants and aided their survival through the Last Glacial Maximum (Becker et al., 2013), and facilitated extensive adaptive radiation in haplochromine cichlid fishes (Meier et al., 2017).

Hybridization is known to occur naturally in some scleractinian corals and has played an important role in the evolution and diversification of the genus *Acropora* (van Oppen et al., 2001; Willis et al., 2006). In the Caribbean, recent environmental degradation and massive population decline in *Acropora cervicornis* and *Acropora palmata* have favored hybridization and expansion of their F1 hybrid, *Acropora prolifera* (Fogarty, 2012). These hybrids either have equivalent or higher fitness relative to the parent species in most life history stages examined (Fogarty, 2012). In recent years, *A. prolifera* has been reported in increasingly high abundance in many reef locations (Fogarty, 2012; Japaud et al., 2014; Aguilar-Perera and Hernández-Landa, 2017) and the hybrid has expanded to marginal environments where parent species are absent (Fogarty, 2012).

Although interspecific hybridization is a potential tool to enhance restoration outcomes, it is often dismissed in restoration initiatives. Concerns raised include the possibility of outbreeding depression in later generations (i.e., F2, F3, backcross), and the loss of diversity through losing part of the parental species' genome (for review, see Hamilton and Miller, 2016). Most examples of outbreeding depression, however, are associated with the admixture of populations or species that are geographically distant, or when life history or phenological differences are large (Hwang et al., 2012; Whiteley et al., 2015). Outbreeding depression can also be transient and can be overcome by natural selection (Jones and Monaco, 2009; Aitken and Whitlock, 2013; Hamilton and Miller, 2016). Instead of reducing genetic diversity, hybridization may conserve diversity by protecting the parental genome from the risk of extinction, and can also increase genetic diversity by combining two divergent genomes within a single organism (Garnett et al., 2011). For example, hybridization has successfully enhanced genetic diversity, improved the population size and rescued the highly inbred, remnant population of Florida

[1]https://50reefs.org/ (Accessed April 3, 2018).

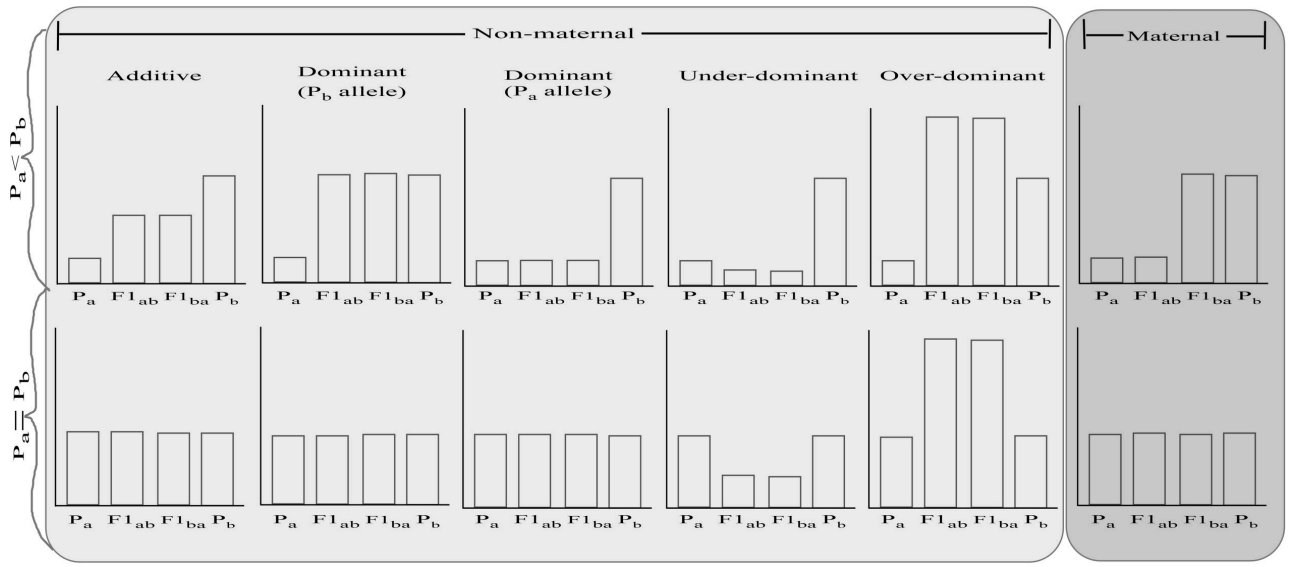

FIGURE 1 | Possible relative fitness of reciprocal F1 hybrids ($F1_{ab}$ and $F1_{ba}$) based on fitness of the parental species (P_a and P_b) and the driving mechanism. In the top graphs, parental species are assumed to differ in phenotype while the bottom graphs indicate a situation where parental species are similar. For further explanation, see text.

panther (Johnson et al., 2010) and the Mt. Buller mountain pygmy-possum (Weeks et al., 2017) from extinction (i.e., genetic rescue).

Here we investigate interspecific hybridization as a novel tool to increase genetic diversity and develop coral stock with increased climate resilience. Parental species were not chosen for their relative climate resilience, but based on our expert knowledge of the probability that they would cross-fertilize as well as their evolutionary relatedness. We examined the performance of hybrids from reciprocal crosses of two *Acropora* species pairs raised under ambient and elevated seawater temperature and pCO_2 conditions, and assessed (1) whether prezygotic barriers exist in interspecific hybrids of *Acropora* corals from the GBR, and (2) whether hybrids show enhanced fitness and resilience compared to the purebreds. Four phenotypic traits (i.e., survival, recruit size, *Symbiodinium* uptake, and photochemical efficiency) were measured in hybrid and purebred offspring as proxies for fitness. Surviving hybrids and purebreds at the end of the experiment were transplanted to long-term grow-out tank for rearing with the aim to allow future assessment of their reproductive and backcrossing potential when they reach sexual maturity at ∼4 years of age. We continued to monitor these survivors for survival and size during the grow-out period.

MATERIALS AND METHODS

Coral Spawning, *in Vitro* Fertilization, and Experimental Design

A detailed timeline of the experiment, sampling and measurement of each trait is shown in Figure S1. Parental colonies were collected from Trunk Reef, central GBR, prior to full moon on 22nd Nov 2015 and maintained in flow-through aquaria of the National Sea Simulator (SeaSim) at the Australian Institute of Marine Science (AIMS). When signs of imminent spawning were observed ("setting," i.e., where the sperm-egg bundles begin to protrude through the mouth of the polyps), colonies were isolated in individual tanks to avoid uncontrolled mixing of gametes prior to *in vitro* crossing. The five most profusely spawning colonies of each parental species were used for crossing to form (1) an *Acropora tenuis* × *Acropora loripes* cross, and (2) an *Acropora sarmentosa* × *Acropora florida* cross (**Figure 2**). These two species pairs were chosen to represent a phylogenetically divergent cross and a phylogenetically closely related cross. The phylogeny of *Acropora* spp. is divided into two distinct groups: the "early spawners" and the "late spawners," where the "late spawners" spawn about 1.5–3 h before the other group (Fukami et al., 2000; van Oppen et al., 2001; Márquez et al., 2002). *A. tenuis* (early spawner) and *A. loripes* (late spawner) are phylogenetically divergent, while *A. sarmentosa* and *A. florida* (both are "late spawners") are closely related and fall within the same phylogenetic clade (Fukami et al., 2000; van Oppen et al., 2001; Márquez et al., 2002). Little information is available from the literature about the relative resilience of these four parental species, but this has limited relevance for this study as our purpose was to increase genetic diversity (and thus adaptive potential) via hybridization, and not to conduct targeted breeding with species of known relative bleaching tolerance. Only two *A. florida* colonies spawned on the same night as *A. sarmentosa,* therefore, only two colonies were used for this species.

Egg-sperm bundles of individual colonies were collected and eggs and sperm were separated using a 100 μm filter. Eggs were washed three times with filtered seawater to remove any residual sperm and placed in a 3 L bowl until crosses were set up (within 3 h). Sperm concentration of every colony was

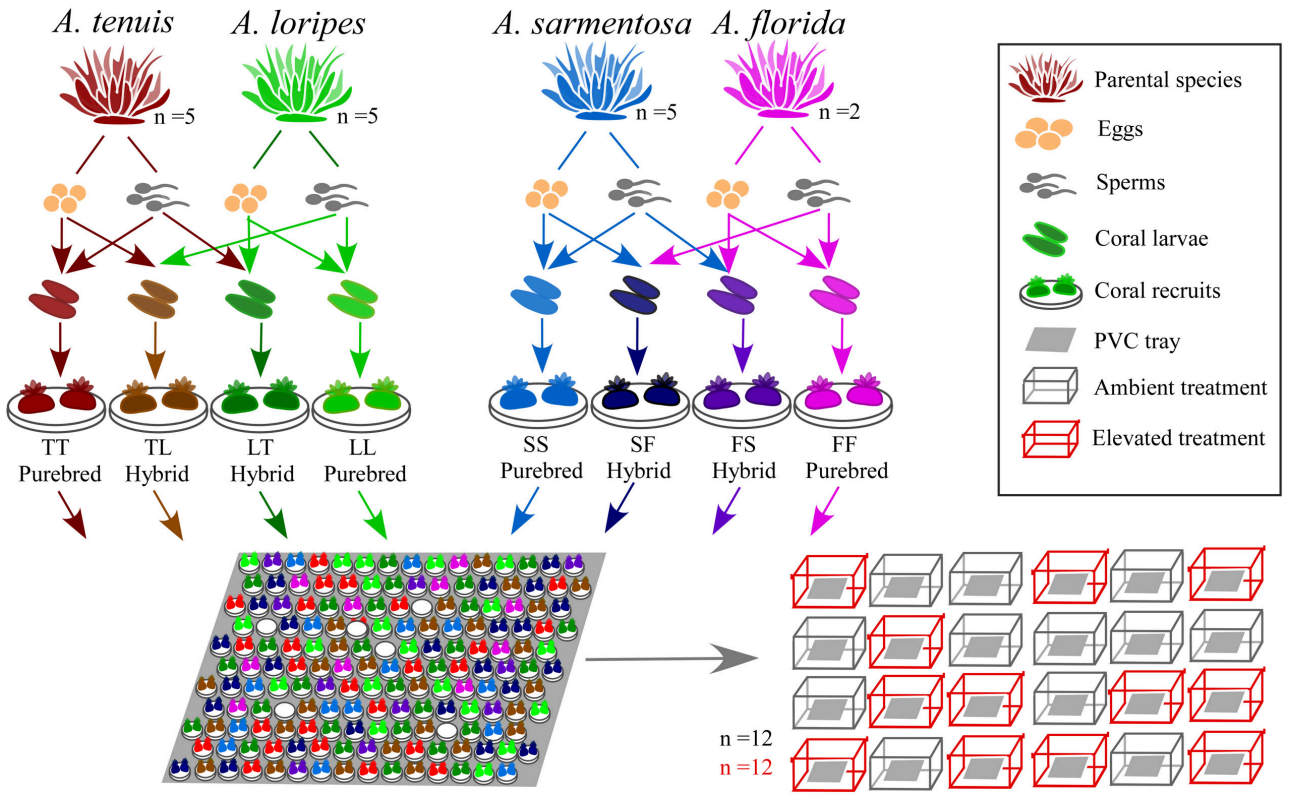

FIGURE 2 | Experimental set up showing the two interspecific crosses [i.e., *A. tenuis* (T) × *A. loripes* (L) and *A. sarmentosa* (S) × *A. florida* (F)], the four resultant offspring groups from each cross (TT, TL, LT, LL; and SS, SF, FS, FF, respectively), larval settlement, and comparison of hybrid and purebred fitness under ambient and elevated conditions. The abbreviation of the offspring groups throughout this paper is that the first letter represents the origin of the eggs and the second letter the origin of sperm [e.g., SF is a hybrid of *A. sarmentosa* (S) eggs with *A. florida* (F) sperm]. The different colors used for the offspring groups in the figure reflect differently colored settlement plugs used for each offspring group.

measured with a hemocytometer on a compound microscope with 40x magnification. Similar quantities of sperm from each conspecific colony were pooled to create a mixed sperm solution. For the hybrid crosses, the mixed sperm solution of the other parental species was added to the eggs of each interspecific colony. This method prevented intraspecific fertilization by possible remaining sperm that was not washed off the eggs (note that no self-fertilization was observed in any of the crosses performed). Fertilization was conducted under ambient conditions at a sperm concentration of 10^6 sperm mL^{-1}. Three samples of 100 eggs were collected for each species as a self-fertilization test and a "no sperm" control. Each species pair cross produced four offspring groups, two purebreds and two hybrids (**Figure 2**). Embryos of each offspring group were then placed in rearing tanks for development under ambient conditions.

Fertilization Rates and Embryonic Development

Fertilization rates were assessed at 3.5 h, and embryonic development at 9, 15, 21, 33, 45, 57, and 93 h after sperm was added to the eggs. All embryos had reached planula stage and were ready to settle by 93 h. Triplicate samples of 100 embryos of each offspring group were collected and fixed in 4% formaldehyde. Developmental stages were assessed and counted under a dissecting microscope based on the stages described in Randall and Szmant (2009).

Larval Settlement and *Symbiodinium* Uptake

Prior to coral spawning, ceramic plugs of eight different colors were preconditioned in the outdoor SeaSim flow-through aquaria under ambient conditions for 6 weeks to develop crustose coralline algae (CCA) and a microbial biofilm to provide a larval settlement cue. Five days after fertilization, planula larvae of the eight offspring groups were each settled onto one assigned color of the pre-conditioned plugs under ambient conditions. Plugs of eight different colors were used so that each offspring groups could easily be identified and randomized in the experimental PVC trays holding the plugs (**Figure 2**). During settlement, *Symbiodinium* (i.e., algal symbionts) isolated from the parent colonies were added to achieve a final density of 2×10^6 cells mL^{-1} in each settlement tank. Larvae of each offspring group only received *Symbiodinium* from their parental

species. To isolate the *Symbiodinium*, an ∼6 cm fragment with three branches was removed from each parental colony with a bone cutter. Soft tissues of the fragment were then removed using an airbrush. The mixed soft tissues/seawater solution was collected and centrifuged at 200 g for 5 min to pellet the *Symbiodinium*. The *Symbiodinium* cells were resuspended and washed three times with filtered seawater before being added to the larvae. *Symbiodinium* uptake was assessed under a dissecting microscope prior to exposure to elevated conditions. Recruits ($n = 20$ per offspring group) were categorized as either with or without *Symbiodinium*.

Settled recruits were randomized and evenly distributed on 24 tailor-made PVC trays to rear under (1) ambient conditions of 27°C, 415 ppm pCO_2, or (2) elevated conditions of ambient +1°C, 685 ppm pCO_2 (**Figure 2**). Recruits for the elevated conditions were ramped to the target temperature and pCO_2 from ambient at a rate of +0.2°C and + ∼50 ppm per day. There were 12 replicate tanks for each of the two treatment conditions and tank positions in the experiment room were randomized (**Figure 2**). Every tank held one PVC tray with 20 plugs of each offspring group, with the exception of *A. florida* purebred (FF) and hybrid (FS) which had only 10 plugs due to fewer larvae being available. To avoid sediments from accumulating on top of the recruits, the trays were placed at an approximately 45° angle. Experimental conditions followed Davies Reef (18.83°S, 147.63°E) diurnal and annual temperature variations, a reef in proximity to Trunk Reef were the adult corals used for spawning were collected. A mixed marine microalgae diet of *Isochrysis*, *Pavlova*, *Tetraselmis*, *Chaetocerous calcitrans*, *Thalassiosira weissflogii,* and *Thalassiosira pseudonana* was fed to the recruits twice a day at a final concentration of ∼5,000 cells mL^{-1} in the tank.

Survival and Recruit Size

Recruits from each tank were imaged using a high-resolution camera (Nikon D810) mounted on a quadpod with a waterproof case. Imaging was conducted fortnightly in the first 8 weeks of the experiment, thereafter every 4 weeks until 28 weeks. The numbers of surviving recruits were visually counted and recorded. Detailed images were taken at 28 weeks for size measurement. Recruit size was estimated as surface area of a circle from the measured recruit diameter since recruits were circular in shape and were not yet forming upright branches. Measurements were made using the software ImageJ and calibrated on the scale chart presented on every image. Recruits were maintained under the treatment conditions for 28 weeks. Surviving juveniles were thereafter relocated to long-term grow-out tanks to accommodate their larger size and maintained under ambient raw water (i.e., unfiltered seawater) to cater for higher feeding demand. Due to the small size of some recruits at 28 weeks and therefore difficulty to make comparisons, size was again measured at 1 year of age (i.e., about 5 months after all surviving recruits were moved to ambient raw water conditions) using the same measurement method. Furthermore, a set of photos of the median sized juvenile were taken at 2 years of age.

Photochemical Efficiency

Photochemical efficiency (i.e., dark adapted maximum photosystem II quantum yield, Fv/Fm) was measured at week 28 as a proxy for coral health. Measurements were made using Imaging- Pulse Amplitude Modulation (I-PAM) derived by the software ImagingWin (v2.40b). Recruits ($n = 15$ per offspring group per treatment) were dark adapted overnight, and remained submerged in the treatment seawater during imaging. A recruit would only be measured if: (1) it was not obscured by filamentous algae, and (2) its size was no smaller than the software's area of interest requirement.

Seawater Chemistry

Automated controls of seawater chemistry were provided by SeaSim via the SCADA (Supervisory Control and Data Acquisition) system. Experimental conditions are summarized in **Table 1**. Seawater temperature and pH were recorded every hour using resistance temperature detector (RTD) and a pH probe (Tophit CPS471D). pCO_2 was measured bi-weekly using a CO_2 equilibrator calibrated to a standard gas of 500 ppm. Total alkalinity (A_T) was measured using VINDTA calibrated to Dickson's Certified Reference Material. Salinity was measured weekly with an HACH IntelliCAL™ CDC401 Standard Conductivity Probe calibrated with IAPSO Standard Seawater. Seawater carbonate chemistry parameters, including Ω_{arag}, DIC (dissolved inorganic carbon), CO_3^{2-}, and HCO_3^- were calculated using the measured values of seawater A_T, pCO_2, temperature and salinity, with the program CO2SYS (Lewis and Wallace, 1998 as implemented in Microsoft Excel by Pierrot et al., 2006).

Statistical Analysis

Survival

Generalized linear mixed models (GLMM) (McCulloch and Neuhaus, 2013) for binomial data with logistic link functions were used to estimate the effects of treatment and offspring group on recruit survival at week 28. Analyses were conducted separately for the offspring groups of the *A. tenuis* × *A. loripes*

TABLE 1 | Experimental conditions of the ambient and elevated treatment.

Parameter*	Ambient Mean	Ambient *SD*	Elevated Mean	Elevated *SD*
Temperature (°C)	26.5	2.1	27.5	2.1
pCO_2^- (μatm)	399	5	666	36
pH_T	8.04	0.00	7.86	0.02
A_T (μmol kg^{-1})	2327	21	2327	21
Ω_{arag}	3.6	0.3	2.7	0.3
HCO_3^- (μmol kg^{-1})	1766	25	1915	21
CO_3^{2-} (μmol kg^{-1})	226	15	167	15
DIC (μmol kg^{-1})	2004	15	2100	12
Salinity (ppt)	35.5	0.8	35.5	0.8

Means and standard deviations (SDs) are given.

**pCO_2, partial pressure of CO_2 of air in equilibrium with seawater; pH_T, pH in total scale; A_T, total alkalinity; Ω_{arag}, aragonite saturation state; HCO_3^-, bicarbonate ion concentration; CO_3^{2-}, carbonate ion concentration; DIC, dissolved inorganic carbon.*

cross and the offspring groups of the *A. sarmentosa* × *A. florida* cross using R Core Team (2016) with packages *lme4* (Bates et al., 2014) and *multcomp* (Hothorn et al., 2008). In order to account for tank differences in the experimental design, a random tank effect was included in the models. Models were checked for overdispersion using a Chi-square test (Bolker et al., 2009) and goodness of fit using Akaike Information Criteria (Akaike, 1974). AIC of the GLMM of the *A. tenuis* × *A. loripes* cross was 397, the *A. sarmentosa* × *A. florida* cross was 331. Tukey's pairwise comparisons were then conducted and *p*-values were corrected using the Benjamini–Hochberg method (Benjamini and Hochberg, 1995). To obtain a visual overview of survival over time, longitudinal generalized linear models (GLM) for binomial data were used to estimate the survival for the offspring groups across all time points, and the combined hybrid offspring vs. purebred offspring. A summary table of the mean survival is provided in **Table 7**. Survival data were also analyzed with Cox proportional hazards regression as a comparison to GLMM. Results of the Cox regression were very similar to those of the GLMM and are shown in the Supplementary Methods and Results section (Table S1).

Size

Statistical analyses of size were conducted separately for the offspring groups of the *A. tenuis* × *A. loripes* cross and the offspring groups of the *A. sarmentosa* × *A. florida* cross at 28 weeks and at 1 year of age. For the 28-week time point, the absence of growth in a large number of samples under elevated conditions resulted in non-normality of the data, and non-parametric Kruskal–Wallis tests were undertaken followed by Dunn's pairwise comparisons (Dunn, 1964). *P*-values for the multiple pairwise comparisons were adjusted with the Benjamini–Hochberg method. For the 1 year time point, due to the absence of survivors in some offspring groups, not all size comparisons could be undertaken. Offspring groups with no or less than three survivors were excluded from the analyses. The remaining size data were normally distributed (tested using Shapiro–Wilk tests; Shapiro and Wilk, 1965) and variances were homogeneous (tested by Levene's tests; Levene, 1960) and five pairwise comparisons were undertaken using combined variance *t*-tests. For the *A. tenuis* × *A. loripes* cross, three *t*-tests were possible for offspring groups that were previously exposed to ambient conditions (note they have been relocated to long-term grow-out tank under raw ambient seawater after 28 weeks). The *p*-values of these comparisons were adjusted using the Benjamini–Hochberg method. The above analyses were run in R (version 3.3.1). A summary of the mean sizes of the recruits is shown is **Table 7**.

Symbiodinium Uptake and Photochemical Efficiency

Generalized linear models (GLM) (McCulloch and Neuhaus, 2013) were used to test the effect of offspring group on rates of *Symbiodinium* uptake, which was treated as a binomial distributed variable (i.e., *Symbiodinium* taken up/not taken up). Treatment was not included in this model as *Symbiodinium* uptake was assessed prior to the start of treatment. For photochemical efficiency (i.e., dark adapted yield, Fv/Fm), generalized linear models were also used to test the effects of offspring group and treatment on the response. Tukey pairwise comparisons were then used and the *p*-values were adjusted with the Benjamini–Hochberg method. These analyses were run with R packages *lme4* (Bates et al., 2014) and *multcomp* (Hothorn et al., 2008).

RESULTS

Spawning Time, Fertilization Rates, and Embryonic Development

The date and time of spawning of the *Acropora* spp. used in this study are summarized in **Table 2**. The *A. tenuis* × *A. loripes* cross was conducted on the 6th day after the full moon, where *A. tenuis* spawned at ∼19:00–19:30 and *A.* loripes at ∼21:45. The *A. sarmentosa* × *A. florida* cross was conducted on the 7th day after the full moon, where *A. sarmentosa* spawned at ∼20:30–20:45 and *A. florida* at ∼21:15–21:30. Fertilization rates of all but one hybrid offspring group were high (averaged 93%) (**Figure 3**). Hybrid LT had lower fertilization rates (averaged 79%) compared to all other offspring groups. No fertilization was observed in the "no-sperm" control and self-fertilization tests. Purebred and hybrid embryos developed normally and reached the planula stage 93 h after fertilization (Figure S2). The hybrid LT also had a slower initial embryonic development rate with the majority of the LT embryos being at the 2–4 cell stage at 3.5 h after fertilization, while embryos of all other offspring groups were at the 8–16 cell stage (Figure S2). From 9 h onwards, however, all offspring groups developed at similar rates.

Survival

Offspring Groups

Overall, maternal effects were observed in the hybrid offspring groups of the *A. tenuis* × *A. loripes* cross and over-dominance in the *A. sarmentosa* × *A. florida* cross, with some variations between treatment conditions (**Figure 4**). Offspring groups differed significantly for survival both in the *A. tenuis* × *A. loripes* cross (GLMM, $\chi^2 = 252.2$, df = 3, $p < 0.001$), and the *A. sarmentosa* × *A. florida* cross (GLMM, $\chi^2 = 32.2$, df = 3, $p < 0.001$). The values present below are mean survival and

TABLE 2 | Spawning date and time of the *Acropora* spp. from Trunk Reef, central GBR.

Date	Species	Days after full moon	Setting time	Spawning time
30/11/2015	*A. tenuis*	4	1,815	1900–1930
1/12/2015	*A. tenuis*	6	1,830	1,900
1/12/2015	*A. loripes*	6	2,000–2,045	2,145
2/12/2015	*A. loripes*	7	1,930	2,145
2/12/2015	*A. sarmentosa*	7	1,930	2,030–2,045
2/12/2015	*A. florida*	7	2,030	2,130
3/12/2015	*A. florida*	8	2,000	2,115

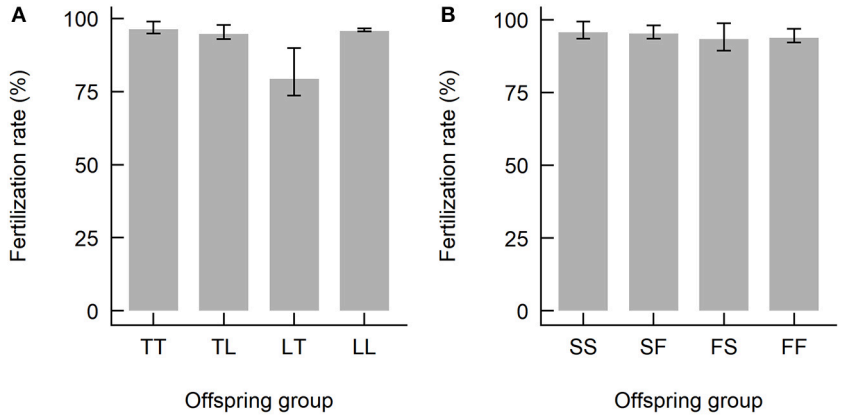

FIGURE 3 | Fertilization rates of the offspring groups from **(A)** the *Acropora tenuis* (T) × *Acropora loripes* (L) cross, and **(B)** the *Acropora sarmentosa* (S) × *Acropora florida* (F) cross. The abbreviation of the offspring groups is that the first letter represents the origin of the eggs and the second letter the origin of sperm, where TL, LT, SF, FS are hybrids and TT, LL, SS, FF are purebreds.Values are mean and error bars represent 95% CI calculated using the angular transformed data back-transformed into percentages.

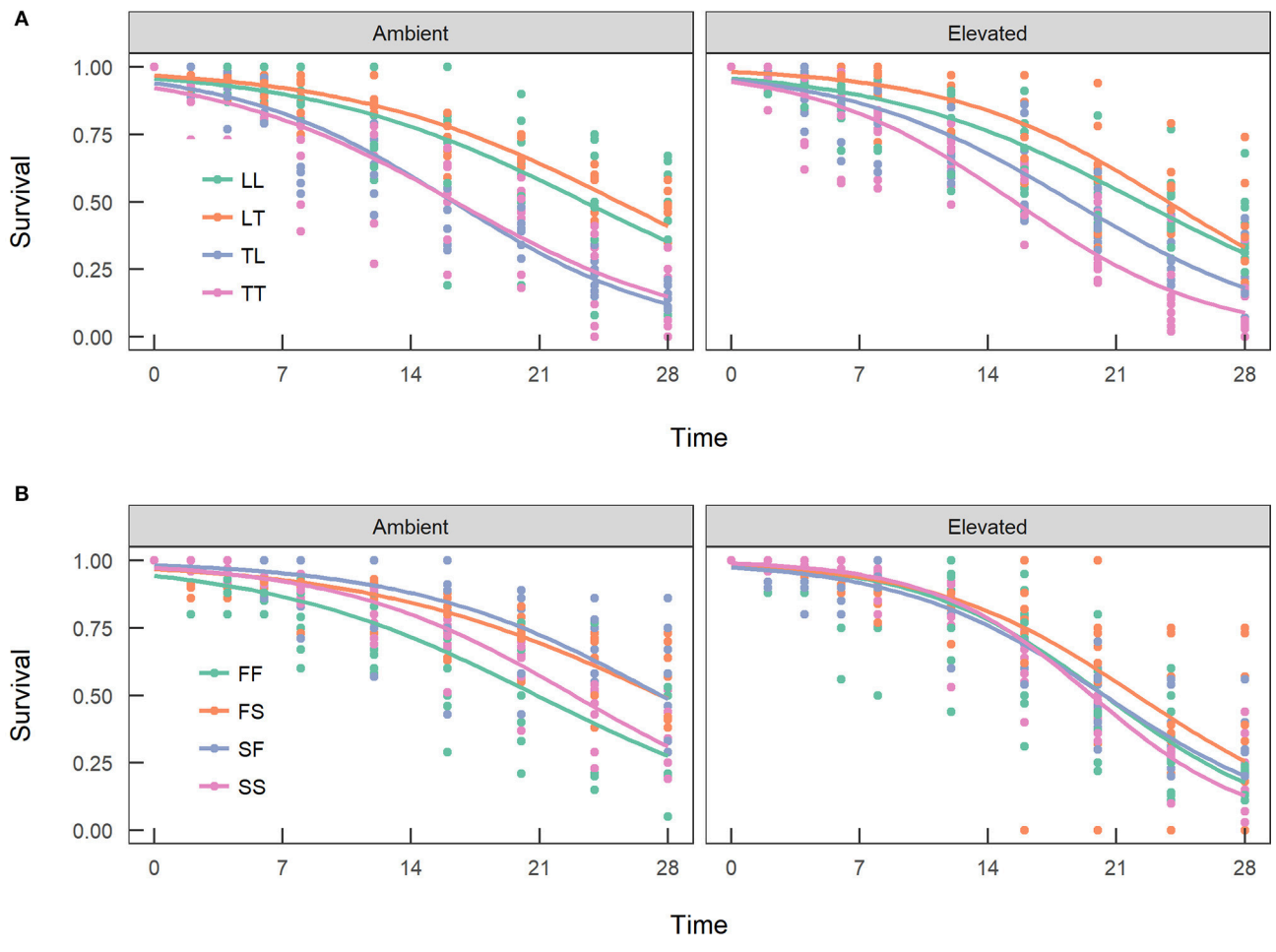

FIGURE 4 | Survival of the offspring groups of **(A)** the *Acropora tenuis* (T) × *Acropora loripes* (L) cross, and **(B)** the *Acropora sarmentosa* (S) × *Acropora florida* (F) cross under ambient and elevated conditions across 28 weeks. The abbreviation of the offspring groups is that the first letter represents the origin of the eggs and the second letter the origin of sperm. Lines represent the estimates of the longitudinal generalized linear models.

the associated 95% confidence intervals are shown in **Table 3**. For the *A. tenuis* × *A. loripes* cross, survival of hybrid LT (49%) and purebred LL (46%) was higher than that of TT (13%) and TL (16%) under ambient conditions ($p < 0.001$ for all) (**Tables 3**, **4**, **7**). Under elevated conditions, survival of hybrid LT (41%) and purebred LL (36%) was also higher than that of TT (7%) and TL (23%) (**Tables 3**, **4**, **7**). Survival of hybrids was similar to that of their maternal parental purebred offspring. Under elevated conditions, survival of hybrid TL (23%) was also higher than that of purebred TT (7%) ($p < 0.001$; **Tables 3**, **4**, **7**).

For the *A. sarmentosa* × *A. florida* cross, survival of both hybrid SF (51%) and FS (53%) was higher than that of the purebred FF (31%) ($p = 0.014$, $p = 0.006$, respectively) and SS (35%) ($p = 0.022$, $p = 0.007$, respectively) under ambient conditions (**Tables 3**, **4**, **7**). Under elevated conditions, only hybrid FS (32%) had higher survival than purebred SS (18%) ($p = 0.007$; **Tables 3**, **4**, **7**). When combining the data for all hybrid and purebred offspring groups for an overall comparison, hybrid offspring had a consistently higher survival than purebred offspring (Figure S3).

Treatments

Treatment had a significant effect on survival of both the *A. tenuis* × *A. loripes* cross (GLMM, $\chi^2 = 26.9$, df = 1, $p < 0.001$), and the *A. sarmentosa* × *A. florida* cross (GLMM, $\chi^2 = 13.6$, df = 1, $p < 0.001$). Tukey pairwise comparisons suggest that TT, SS, SF, and FS had lower survival under elevated conditions compared to ambient conditions at week 28 ($p = 0.025$, 0.002, 0.007, 0.015, respectively; **Table 5**).

TABLE 3 | Mean survival, SE, as well as lower and upper 95% CI of offspring groups from the *Acropora tenuis* (T) × *Acropora loripes* (L) cross and the *Acropora sarmentosa* (S) × *Acropora florida* (F) cross under ambient and elevated conditions.

Treatment	Offspring group	Effect	SE	Lower CI	Upper CI
Ambient	TT	0.13	0.23	0.09	0.19
	TL	0.16	0.21	0.11	0.22
	LT	0.49	0.20	0.39	0.58
	LL	0.46	0.23	0.35	0.57
Elevated	TT	0.07	0.23	0.04	0.10
	TL	0.23	0.18	0.17	0.29
	LT	0.41	0.17	0.33	0.49
	LL	0.36	0.19	0.28	0.45
Ambient	SS	0.35	0.17	0.28	0.43
	SF	0.51	0.26	0.39	0.64
	FS	0.53	0.23	0.41	0.64
	FF	0.31	0.24	0.22	0.41
Elevated	SS	0.18	0.19	0.13	0.24
	SF	0.26	0.24	0.18	0.37
	FS	0.32	0.22	0.23	0.42
	FF	0.20	0.25	0.13	0.29

The first letter of the abbreviation of the offspring group indicates the origin of the eggs and the second letter the origin of sperm.

Recruit Size

Twenty-eight Weeks

For the *A. tenuis* × *A. loripes* cross, treatment had a significant effect on recruit size (Kruskal–Wallis, $\chi^2 = 33.6$, df = 1, $p < 0.001$) but offspring group did not (Kruskal–Wallis, $\chi^2 = 6.9$, df = 3, $p = 0.096$; **Figure 5**). For the *A. sarmentosa* × *A. florida* cross, treatment also had a significant effect on recruit size (Kruskal–Wallis, $\chi^2 = 38.2$, df = 1, $p < 0.001$). Offspring group had a significant effect on size under ambient conditions (Kruskal–Wallis, $\chi^2 = 18.2$, df = 3, $p < 0.001$), but not under elevated conditions (Kruskal–Wallis, $\chi^2 = 1.0$, df = 3, $p = 0.793$). Under ambient conditions, the mean size of hybrids FS (41 mm^2) and SF (43 mm^2) was larger than that of the purebred SS (16 mm^2) ($z = 3.19$, $p = 0.003$; $z = 3.56$, $p = 0.001$, respectively), but not different in size from FF (56 mm^2; **Table 7**).

One Year

At the 1-year time point, several offspring groups no longer had survivors (**Figure 6**, **Table 7**). Note that the treatment condition

TABLE 4 | Tukey's pairwise comparisons of survival between the offspring groups from the *Acropora tenuis* (T) × *Acropora loripes* (L) cross and the *Acropora sarmentosa* (S) × *Acropora florida* (F) cross following generalized linear mixed models.

Treatment	Offspring group	Log odds ratio	SE	*z*-value	*p*-value	Odds ratio
Ambient	LT–LL	0.13	0.23	0.577	0.564	1.14
	TT–TL	−0.19	0.22	−0.831	0.437	0.83
	TT–LT*	−1.83	0.22	−8.426	<0.001	0.16
	TL–LT*	−1.64	0.20	−8.376	<0.001	0.19
	TT–LL*	−1.70	0.25	−6.786	<0.001	0.18
	TL–LL*	−1.51	0.23	−6.451	<0.001	0.22
Elevated	LT–LL	0.19	0.18	1.063	0.323	1.21
	TT–TL*	−1.41	0.22	−6.328	<0.001	0.24
	TT–LT*	−2.28	0.22	−10.523	<0.001	0.10
	TL–LT*	−0.87	0.17	−5.212	<0.001	0.42
	TT–LL*	−2.09	0.23	−8.887	<0.001	0.12
	TL–LL*	−0.68	0.19	−3.600	<0.001	0.51
Ambient	SF–FF*	0.88	0.32	2.772	0.014	2.42
	FS–FF*	0.93	0.29	3.160	0.006	2.54
	SS–FS*	−0.74	0.24	−3.064	0.007	0.48
	SS–SF*	−0.69	0.27	−2.546	0.022	0.50
	SF–FS	−0.05	0.31	−0.154	0.878	0.95
	SS–FF	0.19	0.25	0.776	0.533	1.21
Elevated	SF–FF	0.38	0.32	1.209	0.302	1.47
	FS–FF	0.65	0.30	2.148	0.052	1.91
	SS–FS*	−0.77	0.26	−3.033	0.007	0.46
	SS–SF	−0.51	0.27	−1.878	0.094	0.60
	SF–FS	−0.26	0.29	−0.903	0.466	0.77
	SS–FF	−0.13	0.28	−0.445	0.707	0.88

*The abbreviation of the offspring groups is that the first letter represents the origin of the eggs and the second letter the origin of sperm. An odds ratio of >1 indicates higher survival, and <1 indicates lower survival of the first cross in the comparison. *Indicates significant difference between this offspring group pair.*

in this section refers to the treatment conditions that the recruits were exposed to during the 28 week period following settlement, but that they were transferred to long-term grow-out tanks with ambient raw (i.e., unfiltered) seawater afterward. For recruits that were previously under ambient conditions, there were no survivors of purebreds TT and FF, while all hybrid groups had survivors. The mean size of LT (362 mm^2) and LL (366 mm^2) offspring was larger than that of TL offspring (47 mm^2) (*t*-test, $p = 0.008$, 0.015, respectively, **Tables 6**, **7**). The size of the LT hybrids was the same as that of the maternal parent species LL (i.e., maternal effect). The mean size of FS hybrids (304 mm^2) was larger than that of the pure breds SS (30 mm^2) (*t*-test, $p = 0.004$, **Tables 6**, **7**). The mean size of hybrid SF (245 mm^2, average of 2 recruits) was also relatively larger than SS (30 mm^2), however, statistical comparison was not possible due to the low sample size for SF ($n = 2$; **Table 7**). For recruits that were previously under elevated conditions, there were no survivors of purebreds TT and SS as well as hybrids TL and SF. The mean size of the hybrid LT recruits (326 mm^2) was the same as that of the maternal parent species LL (290 mm^2) (**Tables 6**, **7**). Median sized survivors of hybrid and purebred juveniles at 2 years of age and are shown in Figure S4.

TABLE 5 | Tukey's pairwise comparisons of treatment effect within an offspring group from the *Acropora tenuis* (T) × *Acropora loripes* (L) cross and the *Acropora sarmentosa* (S) × *Acropora florida* (F) cross following generalized linear mixed models.

Treatment	Offspring group	Log odds ratio	SE	*z*-value	*p*-value	Odds ratio
Elevated vs. Ambient	TT*	−0.77	0.32	−2.394	0.025	0.46
	TL	0.46	0.27	1.678	0.119	1.58
	LT	−0.32	0.26	−1.227	0.257	0.73
	LL	−0.38	0.30	−1.253	0.256	0.68
Elevated vs. Ambient	SS*	−0.90	0.26	−3.531	0.002	0.41
	SF*	−1.08	0.36	−3.033	0.007	0.34
	FS*	−0.87	0.32	−2.720	0.015	0.42
	FF	−0.59	0.35	−1.686	0.135	0.56

*The abbreviation of the offspring groups is that the first letter represents the origin of the eggs and the second letter the origin of sperm. An odds ratio of >1 indicates higher survival, and <1 indicates lower survival under elevated treatment. *Indicates significant differences in survival under different treatments in this offspring group.*

Symbiodinium Uptake and Photochemical Efficiency

There was no significant difference in *Symbiodinium* uptake between the offspring groups of the *A. tenuis* × *A. loripes* cross (GLM, $\chi^2 = 3.25$, df = 3, $p = 0.354$) or the offspring groups of the *A. sarmentosa* × *A. florida* cross (GLM, $\chi^2 = 5.35$, df = 3, $p = 0.148$; Figure S5, **Table 7**). For the *A. sarmentosa* × *A. florida* cross, neither treatment nor offspring groups had a significant effect on photochemical efficiency (Treatment: GLM, $\chi^2 = 0.51$, df = 1, $p = 0.477$; offspring group: GLM, $\chi^2 = 4.28$, df = 3, $p = 0.233$). For the *A. tenuis* × *A. loripes* cross, treatment had a significant effect on photochemical efficiency (GLM, $\chi^2 = 6.87$, df = 1, $p = 0.009$) but offspring groups did not (GLM, $\chi^2 = 2.43$, df = 3, $p = 0.488$; Figure S6). Tukey pairwise comparisons show that purebreds TT and LL under elevated conditions had lower photochemical efficiency than their counterparts under ambient conditions ($p = 0.035$, 0.002, respectively).

Summary Table

The results of the various traits measured are summarized in **Table 7**.

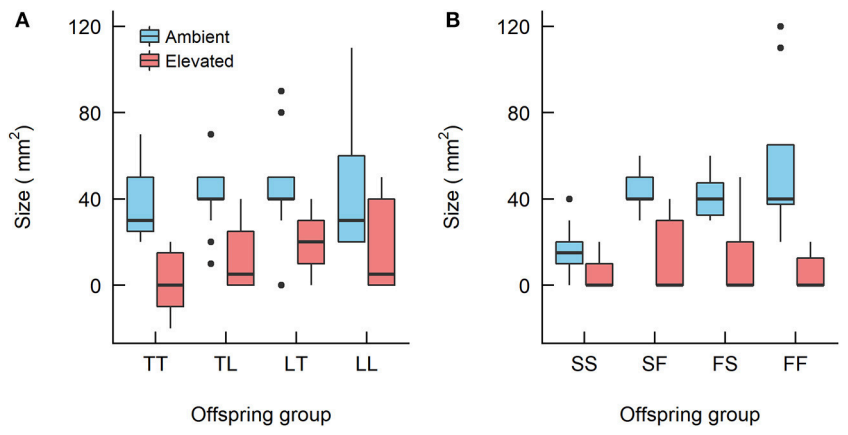

FIGURE 5 | Boxplots showing the size of the *Acropora* offspring groups at 28 weeks since treatment began from **(A)** the *Acropora tenuis* (T) × *Acropora loripes* (L) cross and **(B)** the *Acropora sarmentosa* (S) × *Acropora florida* (F) cross. The first letter of the offspring groups' abbreviation represents the origin of the eggs and the second letter the origin of sperm. The horizontal bars represent median values, box length represents the interquartile range, and the small circles denote unusual points.

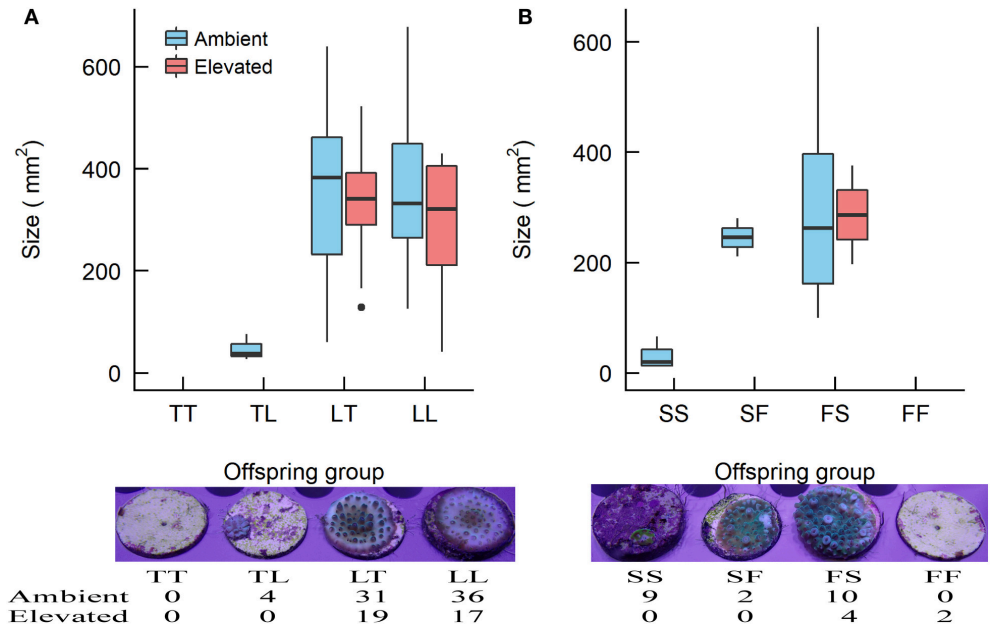

FIGURE 6 | Boxplots showing the size of the *Acropora* offspring groups at 1 year of age (i.e., ∼5 month since relocation to long-term grow-out tank under raw ambient seawater. **(A)** The *Acropora tenuis* (T) × *Acropora loripes* (L) cross and **(B)** the *Acropora sarmentosa* (S) × *Acropora florida* (F) cross. The first letter of the offspring groups' abbreviation represents the origin of the eggs and the second letter the origin of sperm. Where no data are presented there were no survivors in that offspring group. The horizontal bars indicate the medians, box length indicates the interquartile range, and the small circles indicate unusual points. Images below the graphs show examples of median size recruits of the offspring groups reared under ambient conditions in the experiment, and the number of survivors of each offspring group.

TABLE 6 | Results of *t*-tests comparing size at the 1-year time point for remaining offspring groups of the *Acropora tenuis* (T) × *Acropora loripes* (L) cross, and the *Acropora sarmentosa* (S) × *Acropora florida* (F) cross.

Treatment	Offspring group	*t*	df	*p*
Ambient	TL–LT*	3.204	17	0.008
	TL–LL*	2.911	8	0.015
	LT–LL	0.054	21	0.478
	SS–FS*	3.372	10	0.004
Elevated	LT–LL	−0.597	18	0.721

*The first letter of the offspring groups' abbreviation represents the origin of the eggs and the second letter the origin of sperm. *Indicates significant difference between this offspring group pair.*

DISCUSSION

Limited Prezygotic Barriers to Interspecific Hybridization in *Acropora* Corals

To understand the value of hybridization for coral reef restoration, it is important to establish whether prezygotic barriers exist. Interspecific hybridization among *Acropora* spp. has been shown to occur in experimental crosses, with varying degrees of prezygotic barriers (Willis et al., 1997; Van Oppen et al., 2002; Fogarty et al., 2012; Isomura et al., 2013). Among multiple pairs of *Acropora* spp. from the central GBR, crossing resulted in eight pairs with high fertilization (50–80%), seven pairs with moderate fertilization (10–50%) and three pairs with low fertilization (3–10%) (Willis et al., 1997; Van Oppen et al., 2002). The high fertilization rates and normal embryonic development of the interspecific hybrids produced in this study indicate prezygotic barriers are limited in these species pairs. This was unexpected in the case of the *A. tenuis* × *A. loripes* cross which involved an "early spawner" and a "late spawner." These "early spawners" and "late spawners" are believed to have diverged 6.6 Mya (Fukami et al., 2000). We hypothesize that our observations can be explained by the fact that the gametes of these species do not normally encounter one another in the field due to a 2 h difference in spawning times, and selection on prezygotic barriers has therefore been absent. Conversely, *A. sarmentosa* and *A. florida* are phylogenetically closely related, occur sympatrically and spawned ∼30 min to 1 h apart. Our results indicate a prezygotic barrier has not evolved to maintain reproductive isolation of these two species either, and that hybridization may occasionally occur in nature.

Lower fertilization in one direction in *Acropora* hybrid crosses, as was observed for hybrid LT, is not uncommon (Fogarty et al., 2012; Isomura et al., 2013). The likelihood of *A. palmata* eggs being fertilized by *A. cervicornis* sperm, for instance, is smaller than the likelihood of *A. cervicornis* eggs being fertilized by *A. palmata* sperm (Fogarty et al., 2012). The lower fertilization rate of the hybrid LT, however, did not affect recruit size or survival of this offspring group. The slight delay in embryonic development as observed in the hybrid LT was similar to observations for another *Acropora*

TABLE 7 | Summary of the traits measured in the two offspring groups.

Treatment	Cross	Survival-28 weeks (%)	Size-28 weeks (mm^2)	Size-1 year (mm^2)	Photochemical efficiency	Symbiodinium uptake^
Ambient	*A. tenuis* × *A. loripes*	TT: 13 TL: 16 LT: 49 LL: 46	No difference	TT: no survivor TL: 47 LT: 362 LL: 366	No difference[#]	No difference
Elevated	*A. tenuis* × *A. loripes*	TT: 7 TL: 23 LT: 41 LL: 36	No difference	TT: no survivor TL: no survivor LT: 326 LL: 290	No difference[#]	
Ambient	*A. sarmentosa* × *A. florida*	SS: 35 SF: 51 FS: 53 FF: 31	SS: 16 SF: 43 FS: 41 FF: 56	SS: 30 SF: 245[+] FS: 304 FF: no survivor	No difference	No difference
Elevated	*A. sarmentosa* × *A. florida*	SS: 18 SF: 26* FS: 32 FF: 20*	No difference	SS: no survivor SF: no survivor FS: 287[+] FF: 582[+]	No difference	

Values are provided when significant differences between offspring groups were detected.

**Values are not significantly different from other offspring groups of this set. Values are provided for information only.*

[+]*Statistical comparison was not possible due to low sample sizes (ambient SF: n = 2; elevated FS: n = 2, elevated FF n = 2). Values are provided for information only.*

[#]*There was no offspring group effect (i.e., no difference between offspring under the same treatment). However, there was a treatment effect, where purebreds TT and LL under elevated conditions had lower photochemical efficiency than their counterparts under ambient conditions.*

^*Symbiodinium uptake was assessed before treatment commenced, hence recruits were not under treatment conditions.*

cross in Japan (Isomura et al., 2013). This delay, however, was only limited to one early time point and no aberrant development was observed in the hybrids at any time point. The results of this and previous studies suggest that the degree of prezygotic barriers varies between *Acropora* species, and a range of species with limited prezygotic barriers can be used for hybridization with the aim to enhance climate resilience. Interspecific hybridization may also be applied to several other coral genera. Experimental crosses have successfully hybridized species within the genera *Montipora* and *Platygyra* (Willis et al., 1997), but were unsuccessful for species in the genus *Ctenactis* (Baird et al., 2013). Future studies to test the success of interspecific hybridization in additional coral genera will be valuable to determine the extent to which this approach for coral reef restoration can be applied.

Positive Effects of Hybridization Were Observed in Some F1 Hybrids

Given only limited prezygotic barriers exist, we explored whether hybrid offspring had increased resilience and may be used to enhance coral reef restoration efforts. If hybrids are comparatively resilient, interspecific hybridization may be combined with methods being developed for deploying coral larvae or recruits onto reefs requiring restoration (e.g., Nakamura et al., 2011; Omori, 2011; Villanueva et al., 2012; Guest et al., 2014; dela Cruz and Harrison, 2017). Overall, maternal effects were observed in hybrids of the *A. tenuis* × *A. loripes* cross and over-dominance in hybrids of the *A. sarmentosa* × *A. florida* cross, with some variations between traits and treatment conditions. Possible benefits of hybridization in enhancing reef restoration can be observed in both crosses (**Table 7**). In the *A. tenuis* × *A. loripes* cross, hybrids of both directions exhibited ∼16–34% higher survival than purebred *A. tenuis* under conditions with elevated temperature and pCO_2 (**Table 7**), suggesting hybrids have higher climate resilience than the purebred species. Both purebred species also showed reduced photochemical efficiency under elevated compared to ambient conditions while both hybrid species did not. Furthermore, purebred *A. tenuis* had no survivors at the 1-year time point, yet hybrids from both directions survived. For the *A. sarmentosa* × *A. florida* cross, hybrids of one or both directions showed ∼14–22% higher survival and were larger in size than both or one parental purebred species (**Table 7**). One hybrid offspring group (FS) had survivors in both ambient and elevated conditions at the age of 1 year, while both purebred species had no survivors in one of these conditions (**Table 7**). The FS hybrid was also 10 times larger in size than the only surviving purebred species (*A. sarmentosa*) at 1 year of age under ambient conditions (**Table 7**).

Across all traits measured, hybrids were either equivalent to or more fit than at least one parent, and none of the hybrids performed worse than both parents. These patterns are similar to those seen in some other comparisons. The natural hybrid *A. prolifera* in the Caribbean (Fogarty, 2012) and experimentally produced hybrids of *A. millepora* × *A. pulchra* (Willis et al., 2006) had equivalent or higher fitness compared to their parental species. Experimentally produced hybrids of *A. millepora* × *A. pulchra* grow larger in size than purebreds in the reef-flat environment (Willis et al., 2006). In this study, photochemical efficiency was the same between offspring groups in the same treatment, suggesting that (1) the observed differences in recruit size of the offspring groups were unlikely caused by carbon translocation from *Symbiodinium*, and (2) there was no coral host effect on photochemical efficiency of the *Symbiodinium*.

Hybrid Fitness and Its Relevance to Coral Reef Restoration

A comprehensive assessment of the value of interspecific hybridization to coral reef restoration requires multi-generation fitness examinations of hybrids and backcrosses. Such an assessment will require years given the long generations time of corals (3–7 years to reach reproductive maturity). The present study is one of the few studies that examines the long-term fitness of F1 hybrids and provides detailed assessments from fertilization to embryonic development, *Symbiodinium* uptake, photosynthesis efficiency, survival and size. The results provide evidence that hybridization may have value to reef restoration. From the restoration point of view, hybridization increases genetic variation which can potentially enhance adaptive capacity and release a population from adaptive limits (Hoffmann and Sgrò, 2011; Becker et al., 2013; Carlson et al., 2014; van Oppen et al., 2015; Hamilton and Miller, 2016; Meier et al., 2017). In this study, genetic diversity would have increased in the F1 hybrids and positive effects on survival and recruits size were observed in some cases. Furthermore, none of the hybrid offspring groups performed worse than the purebreds across all traits, suggesting that there was no negative effect of hybridization in the F1. Higher survival and larger recruit size as observed in some hybrids can enhance reef restoration initiatives by reducing post-settlement mortality. Moreover, reproductive maturity of a coral is related to its size (Soong and Lang, 1992; Smith et al., 2005). Corals that achieve a large size earlier can begin to reproduce sooner, which may further assist the recovery of degraded reef systems.

In the *A. tenuis* × *A. loripes* cross, maternal effects were observed in fitness. Hybrid survival and size were similar to that of the maternal purebred species, although it exceeded purebred values in some occasions/conditions. Maternal effects have previously been shown for survival of interspecific hybrid larvae from an *A. florida* × *A. intermedia* cross (Isomura et al., 2013), and for thermal tolerance and gene expression levels of intraspecific *A. millepora* hybrid larvae from a higher and lower latitude cross (Dixon et al., 2015). It is unclear whether the observed fitness for the F1 hybrids from our study is due to nuclear or cytoplasmic maternal effects. If survival and size are governed by nuclear maternal effects, F2 hybrids of both directions will have similar fitness to each other, which will be different from their maternal parent. If survival and size are controlled by cytoplasmic maternal effects, fitness of the F2 hybrids will follow maternal fitness (Roach and Wulff, 1987; Bernardo, 1996). In this case, species that are known to carry desirable traits under climate change may therefore be ideal candidates as a source of eggs for creating coral stock for restoration via interspecific hybridization.

When selecting species pairs for hybridization to facilitate reef restoration, both targeted crossing with species or individuals that carry phenotypic traits of value under climate change (e.g., high thermal tolerance) and non-targeted crossing between species could be considered. Species with high climate resilience (e.g., *A. loripes* in the present study) may be useful for targeted hybridization efforts. Alternatively, non-targeted crossing among related species could be used to generate hybrid vigor and increase genetic diversity for future adaptation.

Knowledge Gaps and Future Studies

This study provides the first steps toward the assessment of interspecific hybridization as an approach to create coral stock with augmented climate resilience. While our findings are supportive of this novel strategy, additional research is required. Three important outstanding questions are: (1) The fitness of hybrids vs. purebreds in the field. While laboratory studies are ideal to investigate the responses of corals to one or two specific stressor(s), corals in the wild are subjected to other selection pressures difficult to simulate in the laboratory. An important next stage of this research would involve outplanting the hybrids and purebreds to the field and monitoring their relative fitness. (2) The reproductive and backcrossing potential of F1 hybrids. Isomura et al. (2016) have shown that experimentally produced *A. intermedia* × *A. florida* F1 hybrids were fertile and able to produce F2 offspring with high fertilization rates. Transgressive segregation can happen in F2 hybrids, where segregating variation of parental species recombines in hybrids at multiple loci to produce extreme phenotypes and may result in some F2 hybrids with extremely high fitness (for review, see Hamilton and Miller, 2016). Conversely, outbreeding depression may become apparent in the F2 generations and result in hybrids with low fitness. F1 hybrids of the *A. intermedia* × *A. florida* cross were able to backcross with either both parent species or the maternal parent species only, depending on the direction of the hybrid cross (Isomura et al., 2016). In the Caribbean, molecular evidence has shown unidirectional gene flow from *A. palmata* into *A. cervicornis*, suggesting that their hybrid *A. prolifera* is fertile and able to backcross with at least one parental species (Vollmer and Palumbi, 2002, 2007). Current knowledge on fertility of F1 coral hybrids remains limited and future studies in the area will be invaluable. (3) The fitness of advanced generation hybrids and backcrosses. If F1 hybrids are fertile and sexual reproduction is successful, high fitness will have to be maintained in the F2, backcrosses and advanced generation hybrids for interspecific hybridization to be beneficial to reef recovery and resilience in the long-term.

F1 hybrids can theoretically propagate via asexual reproduction, and via sexual reproduction with other hybrids or the parental species. The likelihood of the latter depends on the spawning time of the F1 hybrids and the parental species. The *A. tenuis* × *A. loripes* cross in the present study had 2 h difference in spawning time and the *A. sarmentosa* × *A. florida* cross had 30 min to 1 h difference. While the spawning time of the hybrids remains unknown until they reach reproductive maturity, Isomura et al. (2016) showed that F1 *Acropora* hybrid spawned at the same time as their maternal parental species. This suggests that the F1 in the present study will likely be able to at least backcross with the maternal parent species for the *A. tenuis* × *A. loripes* cross, and potentially with both parental species for the *A. sarmentosa* × *A. florida* cross due to closer spawning

time. Asexual reproduction (i.e., fragmentation, polyp bail-out) is a common reproductive strategy of broadcast spawning scleractinian corals (Highsmith, 1982; Sammarco, 1982) and F1 hybrids may also persist in the wild via this method. *A. prolifera*, the natural F1 hybrid in the Caribbean for example, is known to persist and colonize large reef areas through asexual reproduction (Irwin et al., 2017).

In sum, it is likely that interspecific *Acropora* hybrids are able to propagate over extended periods of time, either sexually, asexually, or via both reproductive methods. Before hybrids can safely be used as stock for restoration, however, it must be demonstrated that the risk of this strategy is low by showing that the fitness of later generations remains equal or superior to that of the parental species in the wild.

AUTHOR CONTRIBUTIONS

WC, MvO, LP, and AH: designed the experiment; MvO: developed the concept for this study; WC and LP: conducted the experiment and collected the data; PM, WC, and AH: undertook statistical analyses; WC and MvO: wrote the manuscript and all authors contributed to the final edited version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2018.00160/full#supplementary-material

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