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LETTER

Corals in Healthy Populations Produce More Larvae Per Unit Cover

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Abstract

In coral reef conservation and management, the prevailing metric of reef health is percent coral cover, a measurement commonly used with the assumption that each unit of live coral tissue has equivalent ecological value. Here we show that the reproductive output of a coral population is not proportional to the cover of coral present. Instead, when compared to declining populations nearby, high cover coral populations produced up to four times more larvae per square centimeter of tissue, resulting in up to 200 times higher larval production per square meter of reef. Importantly, corals that produced more larvae did not produce smaller larvae, as predicted by resource allocation theory. Instead, higher fecundity corresponded to higher energetic lipid reserves in higher cover coral populations. In the wake of unprecedented global coral bleaching, our findings suggest that the largest reductions in coral reproduction may occur when corals are lost from previously healthy populations.

Introduction

Coral reefs protect trillions of dollars in human assets and generate billions of dollars in tourism revenue annually, making their conservation a priority for modern societies (Cesar *et al*. 2003; De Groot *et al*. 2012; Pendleton *et al*. 2016). The prevailing metric of reef health is coral cover, i.e., the percentage of the seafloor occupied by live coral tissue. This metric is used to assess reef decline across broad geographic scales (Gardner *et al*. 2003; Jackson *et al*. 2014; De'ath *et al*. 2012; Bruno & Valdivia 2016; Hughes *et al*. 2017), to measure whether coral reefs recover after protection (McClanahan 2008; Selig & Bruno 2010), and to compare the health states of different reefs (Hill & Wilkinson 2004; Kaufman *et al*. 2011). This metric's popularity is not surprising given that these standardized data are relatively inexpensive to collect and easy to interpret. Reef scientists also measure benthic decline as lost rugosity, shifts in species composition, and changes in the abundance of calcifiers (Alvarez-Filip *et al*. 2011; Darling *et al*. 2012; Smith *et al*. 2016), while reef health assessments have expanded to incorporate various benthic, fish, and microbial community data (Hill &

Wilkinson 2004; Kaufman *et al*. 2011). Nevertheless, coral cover remains the most common metric of benthic reef health—especially in conservation—and a proxy for reef health overall. In almost all its uses and interpretations, a given amount of coral cover is assumed to have equivalent ecological value whether in a healthy reef ecosystem or in a degraded one (i.e., a reef on which coral cover is declining through time).

In marine conservation, the number and quality of larvae produced by fish populations is considered when siting and sizing marine protected areas (MPAs), with the overall goal of creating sources of larvae that "spill over" to neighboring sites (McClanahan & Mangi 2000). Given this goal, the reproductive behavior of target species is often used when choosing MPA locations; for example, relatively small MPAs have been established around fish spawning aggregations due to the disproportionate importance of these locations to the total reproductive output of a species (Sala *et al*. 2002; Gaylord *et al*. 2005). In contrast, spatial variation in coral fecundity is rarely measured**,** despite the fact that coral fecundity can be one of the most important predictors of larval recruitment (Hughes *et al*. 2000). Individual-level fecundity can be strongly influenced by population size, as has been demonstrated in birds, plants, and insects (Cooch *et al*. 1989; Kery *et al*. 2000, Awmack & Leather 2002). If population degradation similarly reduces the reproductive output of individual coral colonies, the difference in larval production between healthy and unhealthy coral populations could be far greater than differences in their population size.

Accurately quantifying coral reproductive output requires measurements of both larval quantity and quality. In response to acute environmental stresses such as sedimentation and eutrophication, corals have been shown to reduce the number of offspring they produce (Kojis & Quinn 1984; Tomascik & Sander 1987). However, while stressed corals may make fewer larvae, they could potentially maintain overall reproductive success by producing larger larvae (Smith and Fretwell 1974), which would each have an increased likelihood of settlement (Hartmann *et al*. 2013). Therefore, simply producing fewer offspring does not necessarily represent a loss of ecological function. Coral larvae are almost entirely comprised of lipids (Arai *et al*. 1993) and thus parent corals face a resource allocation tradeoff when they reproduce: a higher investment of lipids in larvae reduces the parent's ability to store energy and grow (Ward 1995). Under stress, adult corals are known to rapidly deplete stored energetic lipid reserves (Grottoli *et al*. 2004) suggesting there is less energy available to invest in reproduction. Therefore, to accurately quantify population-level reproductive potential, it is critical to measure parent population size, larval output, and larval quality, as well as parental energetic lipid content.

To test how coral population size affects a population's reproductive health, we measured reproductive output of individual colonies of three common coral species in neighboring healthy populations with stable coral cover and degraded coral populations that have lost coral cover. We then determined whether larval number, larval size, and larval lipid content differed between populations of three species, and we measured the extent to which population-level reproductive output was limited by parental energy reserves. Coral reefs on the Caribbean island of Curaçao created a unique natural experiment in which to conduct these comparisons. Currently, stony coral cover in the region of Oostpunt ("East Point") is more than twice as high as on reefs in the island's nearby capital area of Willemstad (49% vs. 19%; Figure 1). Coral cover at Oostpunt has been stable since measurements began in the 1970s (Jackson *et al*. 2014). Coral cover in Willemstad was similar to that at Oostpunt as recently as the late 1990s, but cover has declined by more than 50% since that time (Jackson *et al*. 2014). The loss of corals on the reefs of Willemstad is attributed to a long history of intensive coastal development, while the persistence and stability of coral cover on Oostpunt reefs, just six kilometers away, is attributed to the virtual absence of any coastal or inland development. The disparate development pressures and reef states, the shared location and oceanography, and the exceptional health of Oostpunt relative to Willemstad and to the wider Caribbean (Jackson *et al*. 2014), make these neighboring regions particularly well-suited for testing whether hidden reductions in coral reproductive performance occur following declines in their natural populations.

Methods

Study sites and species

Research was conducted at six coral reef sites on the leeward coast of the island of Curaçao (southern Caribbean; 12.1696°N, 68.9900°W; Figure 1). Three study sites were located in the region of Oostpunt (12.036293°N, 68.800692°W; 12.041472°N, 68.7800 61°W; 12.040067° N, 68.755114°W) and three sites were located in the region of Willemstad (12.108794°N, 68.954839°W; 12.102549°N, 68.931171°W; 12.0926 61°N, 68.908719°W). Oostpunt, the undeveloped, easternmost area of the island, has very little human impact on land and sits up-current from the rest of the island. Willemstad, the urban center of the island, has a population of > 153,000, a large industrial harbor, cruise ship terminals, and an oil refinery. The abundance of all major

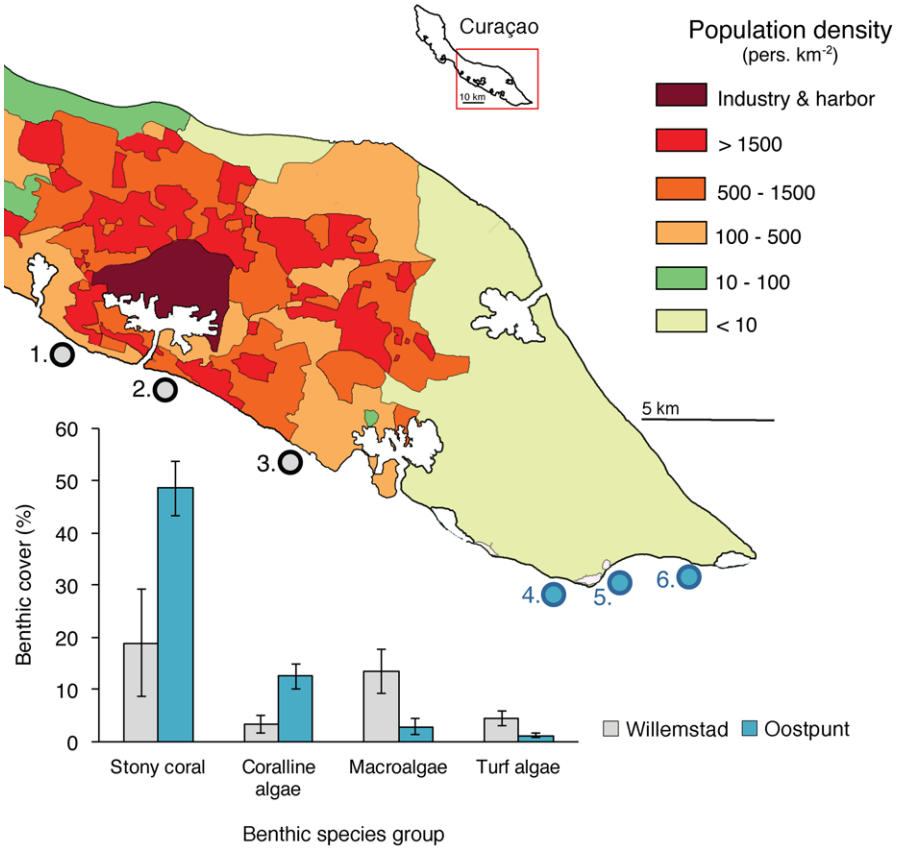

Figure 1 Location and benthic composition of study sites in Curaçao. Reefs in the urban region of Willemstad (Sites 1–3) are marked in grey. Reefs along the undeveloped terrain at Oostpunt (Sites 4–6) are marked in teal. Benthic community composition for each region is shown as the mean percentage of the benthos covered by each functional group (live stony coral, coralline algae, macroalgae, and turf algae). Bars represent 95% confidence intervals. Benthic cover was determined by photoquadrat surveys at all six study sites. Reproductive measures were collected from corals at Sites 2 and 6 (see Figure 2). Energetic lipids were measured from adult corals collected at Sites 1–6 (see Figure 4).

benthic taxa in each region was quantified using SCUBA surveys at each of the three sites within each region. At each site, three 30 m transects with 10 m between each transect were conducted at a depth of 10 m (Figure 1). On each transect, 20 photoquadrat images of 0.90 × 0.55 m (0.5 m^2) were taken at randomly-distributed points. The proportion of each dominant benthic group (live coral, macroalgae, turf algae, coralline algae, sand flat) in each image was assessed using Coral Point Count with Excel Extensions (CPCe; Kohler and Gill 2006). This program displays a specified number of randomly-distributed points over each photoquadrat image. The benthic group under each point is recorded and then averaged across photoquadrat images at the transect and site scales.

The brooding species used in this study were: (1) *Agaricia humilis* (low-relief lettuce coral), a small (< 12 cm in diameter), encrusting to submassive, gonochoric stony coral that releases larvae throughout the year (Van Moorsel 1983); (2) *Favia fragum* (golf ball coral), a small (< 5 cm diameter), simultaneously hermaphroditic, submassive coral that releases larvae over 8–10 days per month coincident with the lunar cycle (Szmant-Froelich *et al*. 1985); and (3) *Siderastrea radians* (lesser starlet coral), a small gonochoric encrusting species that releases larvae on a continual basis (Szmant 1986). The broadcast spawning species used in this study were: (1) *Orbicella annularis* (boulder star coral, previously *Montastraea annularis;* Budd *et al*. 2012), a threatened, massive coral and one of the Caribbean's most significant reef builders (Szmant 1986); and (2) *Acropora palmata* (elkhorn coral), a previously-dominant but now-threatened branching coral (Szmant 1986). Photoquadrat images were taken from the six sites in order to collect species-level data on the spatial coverage of the five coral species studied here (Figures 2A and S1). The abundance and coverage of each species was quantified by outlining each colony of the five studied species in ImageJ and calculating the planar size of each colony.

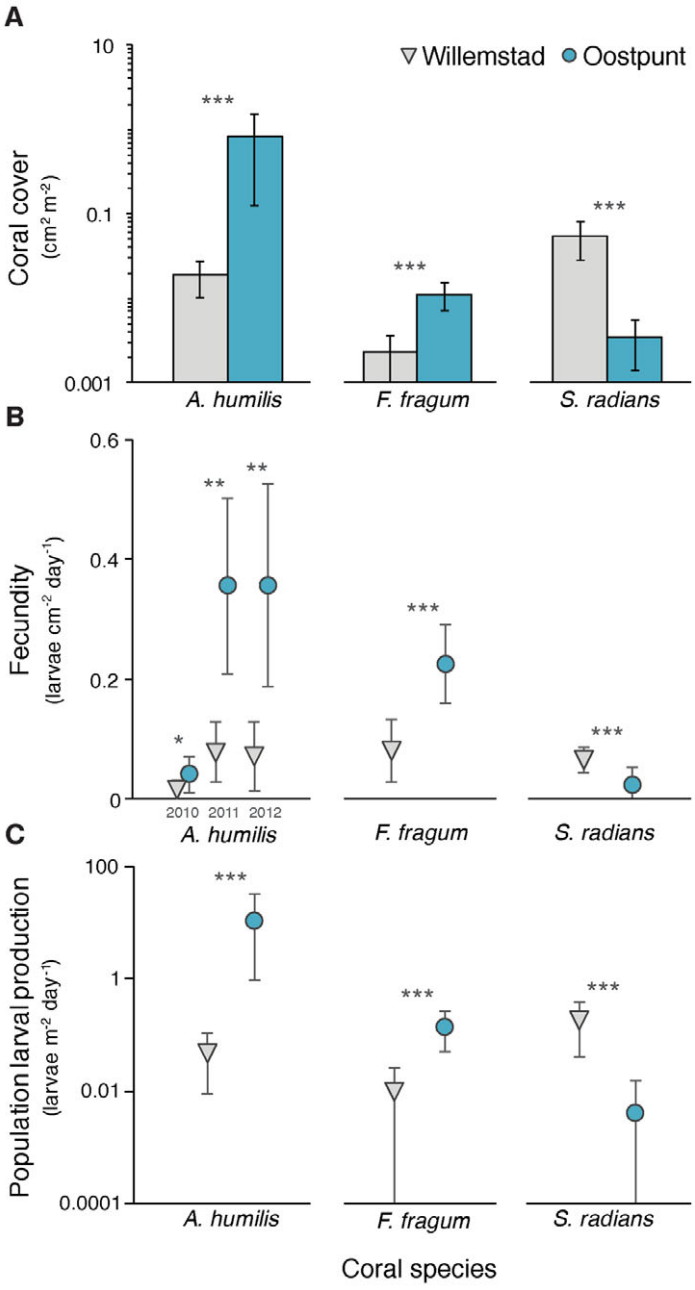

Figure 2 Coral cover and reproductive measures for three brooding coral species collected from Willemstad and Oostpunt coral reefs. (A) Mean coral cover represented as square centimeters of live tissue per square meter of reef. Data from Sites 1–3 in the Willemstad region are shown as grey bars, and data from Sites 4–6 in the Oostpunt region are show as teal bars, see Figure 1). Bars represent the standard error of the population. Regional cover was compared with a t-test. (B) Mean coral fecundity in each region represented as the number of larvae produced per square centimeter of coral tissue per day. Bars represent 95% confidence intervals. Regional fecundity was compared with a Wilcoxon rank sum test. For *A. humilis*, colonies were sampled at the same site in three successive years: 2010, 2011, and 2012. (C) Population-level production of coral larvae per square meter of reef based on measurements of fecundity and coral cover. Bars represent 95% confidence intervals. Regional larval production was compared with bootstrapped products of fecundity and population density. Significant differences between regions are denoted with asterisks according to the level of statistical significance: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Timing of collection for reproductive measures

The timing of larval sampling from *A. humilis*, *S. radians*, and *F. fragum* was chosen to catch each species at its peak reproductive output, if such a time existed. All available evidence suggests it is unlikely that the timing of larval release differs between sites on Curaçao. Year-round production of larvae has been observed in *S. radians* in Jamaica, Panama, and Puerto Rico, demonstrating consistent temporal patterns of reproduction at spatial scales much larger than that of our two study regions (summarized in Soong 1991). The release of larvae by *F. fragum* is strongly tied to lunar periodicity, the timing of which was observed to be consistent across Puerto Rico (Szmant-Froelich *et al*. 1985), Bermuda (Goodbody-Gringley & de Putron 2009), and Curaçao (based on our observations during collections). Finally, *A. humilis* colonies that were sampled monthly less than 0.5 km from Willemstad were found to be most reproductive during the summer and fall (Van Moorsel 1983); hence, we sampled this

Table 1 Corals collected for fecundity measurements. Data included are the month and year of colony collection, the number of days during which adult colonies were tracked in a flow-through aquarium system for larval production, the number of colonies collected per region for measuring larval production, the size of the colonies collected (mean and standard deviation [SD]), and the number and percentage of colonies that produced larvae during the monitoring period. Asterisks represent significant differences in mean colony size between sites by year (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

Coral species	Month/year of parent colony collection	Number of days of larval collection	Number of colonies collected for larval production		Average colony size in cm^2 (± SD)		Number of colonies that released larvae (%)	
			Oostpunt	Willemstad	Oostpunt	Willemstad	Oostpunt	Willemstad
A. humilis	9/2010	7	30	30	13.0 (11.7)**	17.7 (9.6)	21 (70%)	14 (47%)
A. humilis	9/2011	6	27	27	12.9 (7.0)	15.3 (8.5)	24 (89%)	17 (63%)
A. humilis	9/2012	7	15	15	19.5 (8.9)	22.1 (7.7)	15 (100%)	15 (100%)
S. radians	9/2010	5	30	30	9.9 (6.0)	9.3 (4.1)	4 (13%)	14 (47%)
F. fragum	9/2011	12	27	27	7.9 (2.4)	6.7 (2.5)	27 (100%)	17 (63%)

species at their most reproductive time of the year. In sum, we sampled *F. fragum* and *A. humilis* reproduction at the peak of larval output appropriate for each species. The lack of variation in fecundity in *S. radians* suggests larval output sampled at any point in the year is reflective of the species' overall fecundity. We could not collect offspring of *A. palmata* and *O. annularis* from both regions for comparison because these species reproduce sexually by mass spawning during a very narrow window of time (< 30 minutes, 1–2 times per year) and night diving at both locations simultaneously was not feasible.

Coral collection for reproductive measures at Oostpunt and Willemstad

Colonies of the three brooding species described above were collected from Site 2 in the Willemstad region (Figure 1; 12.108794°N, 68.954839°W) and Site 6 in the Oostpunt region (12.041472°N, 68.780061°W). Only colonies with no signs of diminished condition (e.g., physical damage, disease, or bleaching) were collected, and divers sought to collect colonies of reproductive size and to hold the average size of collected colonies consistent between sites (see Table 1 for colony size details). Divers removed colonies from the reef with a hammer and chisel while taking care to collect the entire colony and to avoid damaging the live tissue. Underwater, colonies were placed in individual plastic bags filled with seawater. The bags were brought to the surface where they were placed in a seawater-filled cooler and transported to CARMABI. Within 2 hours of collection, corals were placed in individual 1 L plastic tri-pour beakers in aquaria with flow-through seawater (100 μm-filtered) supplied to each beaker. Each beaker was fitted with an outflow tube that released water into a partially-submerged cylindrical container with nylon mesh at the bottom (150 μm pore size); this allowed for continuous larval collection while maintaining constant seawater flow.

Corals were collected at Oostpunt and Willemstad in 2010 for *S. radians*, 2011 for *F. fragum,* and in 2010, 2011, and 2012 for *A. humilis* (Table 1). The date of *A. humilis* collection for all 3 years was within the same two-week period each year. In 2011 and 2012, for both *F. fragum* and *A. humilis*, colonies were collected at both sites on the same day. In 2010 only, collection of *S. radians* and *A. humilis* from each site was separated by one week due to space constraints in the aquarium system. Because neither species shows lunar periodicity in larval release (Van Moorsel 1983; Szmant 1986), we presumed that the 1-week difference in collection dates did not affect larval release patterns.

In the laboratory, photographs were taken of adult colonies against a scale bar on the day of collection and at the end of the experiment. The two-dimensional (2-D) surface area of each adult colony was measured from photographs using ImageJ (Rasband 1997–2016). Given that 2-D images can lead to underestimations of 3-D coral surface area, we selected only relatively flat (i.e., encrusting and submassive) coral species that lack structural complexity (i.e., they do not form branches or blades) for this phase of the study. All three species release larvae during the night, therefore larval collections were made between 08:00 and 10:00 every day. Larvae were collected for 5–7 days for *A. humilis* and *S. radians* (which are continuous larvae releasers with no lunar periodicity) and for 12 days for *F. fragum* (in order to ensure larvae were collected for the entire monthly period of larval release; Szmant-Froelich *et al*. 1985). Upon collection, larvae were separated by parent colony into beakers containing 0.45 μm-filtered seawater (FSW). The number of larvae released each day by each colony was recorded. Up to five larvae from each colony were haphazardly selected, examined under a stereomicroscope with a scale bar, and photographed; the 2-D area of each larva was

then measured (Van Moorsel 1983). While we took care to ensure that nonspheroid larvae were measured when the longitudinal axis was in plane with the camera, any slight deviations off-axis would cause an underestimation of size that would be mathematically amplified (with respect to population variance) by calculating larval volume based on a spheroid (Petersen & Van Moorsel 2005). Therefore, larval sizes are reported here in two dimensions (as area) rather than as volume estimates. Afterward, 5–10 individuals released from a single colony on a single day were pipetted onto a precombusted 25 mm diameter glass fiber filter (Whatman, GF/F, Kent, UK), wrapped in aluminum foil, frozen at −20°C for less than two weeks, and then stored at −80°C prior to lipid extraction and analysis.

Coral collection for lipid measurements

To examine energetic resources available to parent corals more generally, adult tissue samples for lipid extraction were taken from two brooding species (*A. humilis* and *S. radians*) and from two spawning species (*O. annularis* and *A. palmata*) from all six sites in Oostpunt and Willemstad (Figure 1) within a 1-month period. For the small brooding species (*A. humilis* and *S. radians*), the entire colony was collected. For the large, mass-spawning species (*O. annularis* and *A. palmata*), a small fragment was collected using hammer and chisel (approximately 3 cm^2). Care was taken to maintain consistency of collection depth across sites and all depths were recorded. Samples were placed in plastic bags underwater and the bags were placed on ice when divers surfaced. Tissue was removed from the skeleton with an airbrush and FSW within two hours of collection. The resulting tissue-seawater slurry was immediately frozen at −20°C and moved to −80°C until lipid extractions were performed.

Adult and larval coral lipid measurements

Lipids were extracted from adult and larval tissues using the protocol employed in Bligh & Dyer (1959). GF/F filters with larvae were submerged in a sequential 2:1:0, 2:2:0, 2:2:1.8 chloroform:methanol:water (v:v:v) solvent system. After separation of the polar and nonpolar phases, the phase containing nonpolar lipids was isolated and dried under a stream of N_2 gas. For adult samples, the tissue-seawater slurry was homogenized with a handheld electric homogenizer, after which a 1 mL aliquot was placed in a combusted aluminum weigh boat for tissue mass measurements and a 3 mL aliquot was placed into a combusted glass vial for lipid extraction. The extraction solvent system described above was adjusted to account for water already contained in the sample when lipids were extracted from adult tissue. Total tissue of each adult sample was measured by drying samples at 70°C for at least 48 hours, and measuring mass before and after ashing samples at 450°C for 4 hours (ash-free dry weight).

To measure lipid class concentrations, bulk lipid extracts were resuspended in a known volume of chloroform from which 1 μL was spotted onto each of three quartz Chromarods (S-III, Iatron Laboratories, Inc., Tokyo, Japan). Lipid classes were then separated using thin layer chromatography in a two-solvent system. First the chromarods were placed in a mixture of hexane:diethyl ether:acetic acid (99:1:0.05, v:v:v) for 25 minutes, after which they were dried, then placed in hexane:diethyl ether:acetic acid (80:20:0.1, v:v:v) for 25 minutes; we have previously used this protocol to quantify coral lipid classes (Carilli *et al.* 2012). Separated lipid classes were immediately quantified using an Iatroscan TLC-FID MK-5 (Iatron Laboratories, Inc., Tokyo, Japan) that pyrolized lipids along the entire length of each rod. The retention time and area of each peak were recorded with LabView software (National Instruments, Texas, USA). Lipid class identities and concentrations (Figure 4) were determined based on retention times and calibration curves generated using the following standards: 5-α-cholestane for hydrocarbons, palmitic acid palmityl ester for wax ester, tripalmitin for triacylglycerols, stearic acid for free fatty acids, stigmastanol for sterols, and L-α-phophatidylcholine for phospholipids.

Statistical analyses

Coral cover by species was normally distributed ($P > 0.05$; Shapiro-Wilk test) and thus was compared between regions using a two-tailed t-test (Figures 2A and S1). Fecundity data were normalized across species by the duration of the month. The distribution of values for fecundity (larvae cm^{-2} day^{-1} of colony area) and larval size (mm^2) were not normally distributed, so regional differences in fecundity, larval size, and larval lipid content were tested using a nonparametric Wilcoxon rank sum test (Figures 2B and 3).

The combined demographic measures of reproductive output per region were calculated with a bootstrapping approach to capture the sampling distributions of the two variables of interest: the number of larvae produced (larvae cm^{-2} day^{-1} of colony area) replicated across individual coral colonies and the area of the reef covered by that species replicated across photoquadrats (Figure 2C). For each region a random draw from each distribution (fecundity and benthic cover) was multiplied and then subtracted from an equivalent draw from the other

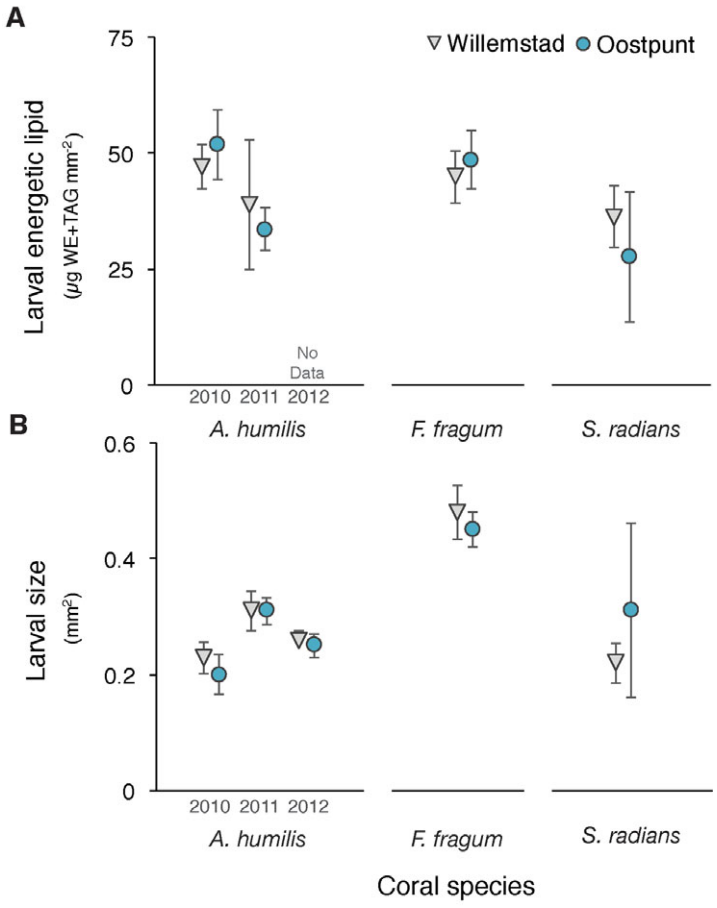

Figure 3 Larval energetic lipid content and larval size in three brooding coral species sampled from Willemstad and Oostpunt coral reefs. Larvae were analyzed from one site in Willemstad (grey triangles) and one site in Oostpunt (teal circles). For each site, 27–30 colonies were sampled, except for *A. humilis* in 2012, when 15 colonies were sampled per site. All data are shown as the mean and 95% confidence intervals around the mean. (A) Larval energetic lipid content. Data are reported as the sum of wax ester (WE) and triacylglycerols (TAG) normalized to the two-dimensional larval surface area (mm^2). (B) Larval size. Data are reported as the two-dimensional surface area per larva (mm^2). *A. humilis* colonies were sampled at the same sites in each of 3 years: 2010, 2011, and 2012, but larval lipid content was not measured in 2012. There were no significant differences in larval size or energetic lipid content between regions in any species.

region. This calculation was repeated 10,000 times with replacement to generate a distribution of values for the difference in reproductive output between regions. Statistical significance was determined based on the proportion of these values that crossed 0 (i.e., no difference between regions). The null hypothesis was supported if 0 fell within the middle 95% of values and was rejected if 0 fell in the upper or lower 2.5% of values (i.e., two-tailed test, alpha = 0.05). For each region, the mean of the 10,000 estimates was used to estimate fecundity. Adult energetic lipid content was normally distributed based upon a Shapiro-Wilk test of normality ($P > 0.05$). Differences within and between regions were determined using a nested analysis of variance (ANOVA) with independent variables: sites within region and region (Figures 4 and S2).

Results

In population assessments, the cover of adult colonies of *A. humilis* and *F. fragum* was 47 and 5 times higher at Oostpunt, respectively ($P < 0.001$; Figure 2A), while *S. radians* had higher cover on the reefs of Willemstad (14 times greater; $P < 0.001$). Higher cover of *S. radians* at Willemstad is consistent with its known preference for stressful and marginal habitats such as eutrophic bays on Curaçao (Vermeij *et al*. 2007). Thus, we assessed reproductive metrics across three species with similar life histories but different optimal habitats.

Across all adults collected, *F. fragum* colonies from Oostpunt produced nearly three times more larvae per square centimeter of living tissue than colonies from Willemstad (0.22 vs. 0.08 larvae cm^{-2} day^{-1}, $P < 0.001$; Figure 2B; Table 1). This difference was driven by two factors: first, 96% of *F. fragum* colonies from Oostpunt released larvae, compared to only 63% of colonies from Willemstad ($P < 0.01$). Second, among colonies that released larvae, individuals from Oostpunt produced nearly twice as many larvae per square centimeter of surface area compared to conspecifics from Willemstad, i.e., the region with declining coral cover (0.23 vs. 0.13 larvae cm^{-2} day^{-1}, respectively; $P < 0.05$). Similarly, *A. humilis* colonies produced four times more larvae at Oostpunt

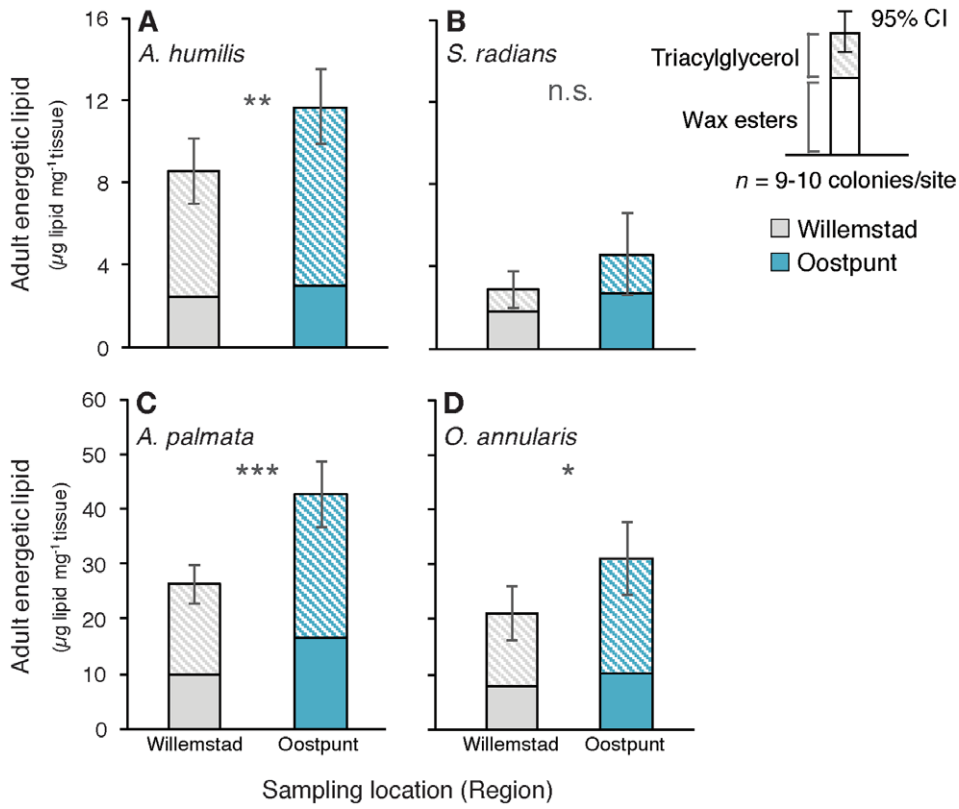

Figure 4 Energetic lipid content of adult corals collected from Willemstad and Oostpunt coral reefs. Four species of adult corals were collected at six sites, three sites in the degraded region of Willemstad (grey bars) and three sites in the healthy region of Oostpunt (teal bars). There was no statistical difference in lipid content among sites within a region in any species, therefore only regional comparisons are shown (alpha = 0.05). Data are shown as the mean lipid concentrations per mass of coral tissue (μg lipid mg^{-1} tissue) for two lipid classes that provide energy to corals: wax ester (lower bars, solid colors) and triacylglycerols (upper bars, crosshatched colors). Lipids were measured from the following species: (A) *A. humilis*, (B) *S. radians*, (C) *A. palmata*, and (D) *O. annularis*. For each site, 10 colonies were sampled per species (i.e., 30 colonies were sampled per region for each species), except for *S. radians*, which was absent at one Oostpunt site ($n = 20$ total colonies from Oostpunt). Bars represent 95% confidence intervals around the mean of the sum of both lipid classes. A nested analysis of variance was used to compare lipid content of corals at sites within a region and between region for each species. A lack of statistical significance is denoted by "n.s." and asterisks denote the degree of statistical significance: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

than at Willemstad (0.22 vs. 0.05 larvae cm^{-2} day^{-1}; $P < 0.001$). Again, more *A. humilis* colonies were reproductive at Oostpunt than in Willemstad (87% vs. 66%, respectively) and, among fecund colonies, *A. humilis* from Oostpunt produced over three times more larvae per tissue area as those from Willemstad (0.26 vs. 0.08 larvae cm^{-2} day^{-1}, respectively; $P < 0.01$). To determine whether these observed reproductive differences were reproducible, *A. humilis* colonies at the same location were sampled in September in three successive years. In all 3 years, fecundity was significantly higher at Oostpunt than at Willemstad (Figure 2B). The number of larvae produced per square centimeter of live coral tissue at Oostpunt was 2.5, 4.6, and 5.1 times greater in 2010, 2011, and 2012, respectively, demonstrating consistently elevated reproductive output at Oostpunt.

As with *F. fragum* and *A. humilis*, *S. radians* produced more larvae per unit tissue area in the region where its cover was higher. However, *S. radians* had higher cover at Willemstad, where the population produced 0.06 larvae cm^{-2} day^{-1} (Figure 2B), as compared to 0.02 larvae cm^{-2} day^{-1} at Oostpunt ($P < 0.001$). As with the other two coral species, higher fecundity at the population level was the result of both a larger number of colonies releasing larvae (87% at Willemstad and 30% at Oostpunt, $P < 0.001$) and higher fecundity per square centimeter of surface area in the colonies that were reproductive (0.074 vs. 0.071 larvae cm^{-2} day^{-1}, respectively, $P < 0.05$). Thus, we found that all three coral species were significantly more fecund *per square centimeter of coral tissue* in the regions where their cover was higher, i.e., where each of their populations were healthier.

Differences in per-area fecundity could be driven by differences in parent coral size, but this was not the case in our study. For both *F. fragum* and *S. radians*, colony size (cm^2) was not significantly different between sites ($P = 0.10$ and $P = 0.89$, respectively; Table 1). For *A. humilis*, colony size was not significantly different between sites in 2011 or 2012 ($P = 0.22$ and $P = 0.65$, respectively). In 2010, sampled *A. humilis* colonies were 36% larger at Willemstad ($P < 0.01$). However, corals at this site had *lower* fecundity per unit area. Thus, parent colony size was not a confounding factor in the fecundity differences we observed between Oostpunt and Willemstad.

By combining reproductive metrics and coral cover data, we estimated each species' total reproductive output per square meter of coral reef for each region (Figure 2C). Because each species produced more larvae per unit of live tissue (Figure 2B) in the region where it also had higher cover at the species level (Figure 2A), the differences in total fecundity between regions were considerable. Compared to Willemstad, within 1 m^2 of reef at Oostpunt, *F. fragum* produced 15 times more larvae (0.140 vs. 0.009 larvae m^{-2} day^{-1}, $P < 0.001$) and *A. humilis* produced 203 times more larvae (10.18 vs. 0.05 larvae m^{-2} day^{-1}, $P < 0.001$). Meanwhile *S. radians* produced 45 times more larvae per square meter in Willemstad, where its cover was higher (0.180 vs. 0.004 larvae m^{-2} day^{-1}, $P < 0.001$). Therefore, relative to what would be expected from regional differences in coral cover alone, *S. radians* produced over three times as many larvae, *F. fragum* produced three times as many larvae, and *A. humilis* produced more than four times as many larvae.

Importantly, the corals we studied could have increased the number of larvae they produced through an energetic resource tradeoff, i.e., by making more small offspring rather than fewer large offspring. To test whether more fecund coral populations offset increases in larval production by reducing larval quality, we measured the size and energetic lipid content of the larvae that were collected from all three species. There were no significant differences between offspring from Willemstad and Oostpunt in either metric of larval quality ($P > 0.05$ for all species; Figure 3; Table S1). Therefore, the reproductive differences between coral populations at Oostpunt and Willemstad represent changes in offspring production at the parent level without any apparent tradeoff in offspring quality.

To determine how adult corals could have produced more larvae without making larvae smaller, we measured energetic lipids (wax ester and triacylglycerols) in colonies of four coral species at three sites each in each region. We collected two brooding species: *A. humilis*, which had higher cover in the Oostpunt region (Figures 2A and 4A) and *S. radians*, which had higher cover in Willemstad (Figures 2A and 4B). We also collected two broadcast-spawning species, *A. palmata* and *O. annularis* (Figures 4C–D, respectively), which both had higher cover on the healthy reefs at Oostpunt (9 and 4 times greater coral cover, respectively; Figure S1). These spawning species are both listed under the U.S. Endangered Species Act and contribute more to reef building relative to the brooding species due to their large size and long lifespans. Energetic lipid content was significantly different between, but not within, each region for three of the four species ($P > 0.05$ for all species, $n = 9$–10 colonies $site^{-1}$; Figure 4; Figure S2). Consistent with our measurements of increased fecundity, the energetic lipid content of *A. humilis* colonies was 27% higher on the reefs of Oostpunt relative to the reefs of Willemstad ($P < 0.01$; Figure 4A). Further, for the spawning species *A. palmata* and *O. annularis*, adult energetic lipid content was 38% and 32% higher, respectively, at Oostpunt ($P < 0.001$ and $P < 0.05$, respectively; Figure 4C–D). Therefore, the lipid energy content of adult corals at Oostpunt was higher in all three species that also had higher cover on the reefs at Oostpunt.

For the second brooding species, *S. radians*, adult energetic lipid content was not significantly different within or between regions ($P > 0.05$; Figure 4B). This was not entirely surprising because fecund colonies had similar larval output between Oostpunt and Willemstad (0.071 and 0.074 larvae cm^{-2} day^{-1}, respectively). In this species, the higher fecundity of the population in Willemstad was driven primarily by its higher cover and the larger proportion of colonies in the population that were reproductive at Willemstad. The reproductive success of *S. radians* at Willemstad could explain why weedy coral species can become proportionally dominant in degraded ecosystems, especially when degradation depresses the reproduction of other less weedy coral species, as we found here. Despite these differences, all coral species considered here had a higher rate of larval production *per unit* of coral cover in the habitat where their cover was also higher overall.

Discussion

Using a rare natural experiment on Curaçao, where very healthy, stable corals reefs and reefs with declining coral cover are found in close proximity to one another, we found that all three coral species examined were more reproductive at the colony level in the regions where their coral cover was higher at the species level. One species, *A. humilis*, was measured in three consecutive years and showed interannual variation in fecundity. In

2010, a major thermal stress and coral bleaching event affected Curaçao in the months following this study. None of the collected colonies showed signs of bleaching at the time, but accumulating thermal stress below the bleaching threshold may explain the depressed fecundity at both regions in 2010 relative to 2011 and 2012. Nevertheless, the difference between regions was observed in all three years. Thus, in both a bleaching year and a non-bleaching year, fecundity was higher at the high cover site of Oostpunt.

Increased coral fecundity was not offset by decreased larval quality. Rather, in higher cover populations, individual corals produced three to four times more larvae per unit of live coral surface area while maintaining larval quality. In the four coral species sampled across all six sites, lower energetic lipid content in adult corals was a pervasive characteristic of the sites in Willemstad, where coral cover has declined, representing a physiological explanation for why coral individuals in the degraded populations produced fewer offspring at the polyp level. This mechanism may also explain why acute environmental stressors have been associated with reduced coral fecundity in other locations (Kojis & Quinn 1984; Tomascik & Sander 1987).

At the population level, total reproductive output was *not* proportional to coral cover. This disproportionate difference in fecundity shows that measures of coral cover, taken at face value, are unlikely to reflect the magnitude of potential differences in larval export from coral source populations. When quantifying ecosystem decline and planning conservation measures based on coral cover, it is prudent to acknowledge that reefs with twice as much live coral cover may have the potential for many times greater reproductive output. Similarly, when corals are lost from a healthy, growing population, this may represent a far greater loss in larval production than when the same amount of coral is lost from a stressed population that has already suffered significant declines. In other words, the loss of ecosystem functioning will often be larger than what is reflected by metrics of population decline alone.

Oostpunt is not an enforced protected area, but its lack of coastal development and its up-current location help to maintain low levels of local environmental stressors (especially sewage and nutrient pollution, terrestrial runoff, physical damage, and overfishing) relative to the urbanized areas of Curaçao; this de facto marine protection at Oopstunt fosters the health of its coral populations and demonstrates the potential for reefs to survive the modern era when local stress is minimized. Importantly, while reef conservation projects generally target large areas for protection, our results show that protecting relatively small areas with higher-cover coral populations, especially when community health is attributable to low levels of local environmental stressors, may also help to protect equal or higher amounts of larval production and expected larval export, a primary goal of MPAs (McClanahan & Mangi 2000).

Another goal of MPAs is to stop and to reverse the loss of coral cover within the MPA boundaries. However, in studies using coral cover data, MPAs have been shown to prevent further declines in existing coral communities, but already-degraded coral communities generally exhibit only small or undetectable increases in cover after protection (McClanahan 2008; Selig & Bruno 2010; Mumby and Harborne 2010; Graham *et al*. 2011). The data presented here show that reef recovery in MPAs may be hampered by undetected, depressed levels of coral reproduction, especially when reef protection is more symbolic than practical and thus fails to effectively reduce the severity of local stressors. Given the limited evidence for coral regrowth in MPAs, and the evidence we present here of the reproductive advantages of high-cover coral populations, our results provide an additional incentive to protect and preserve the healthiest remaining coral populations. By stabilizing coral populations on high-cover reefs, and securing their larval output for nearby regions, these protections will help resource managers buy time while approaches for restoration and mitigation in declining systems continue to be developed, optimized, and expanded.

Based on our findings, we believe that the loss of coral reproductive output is an early, yet underappreciated, "ecological ratchet" in the so-called "ratcheting down" of coral reefs (Birkeland 2004). Once lost, high fecundity may be extremely difficult to recover on typical conservation timescales. However, our results hint at an intriguing alternative possibility: that coral fecundity might be positively density-dependent at the species level, even if all other factors such as local stress are held equal. A follow-up study could investigate this by testing whether the removal of stressors at regional scales would lead to increased fecundity, even without increases in coral cover. Given the short reproductive cycles of the species studied here, such a study would be feasible if stressors could be removed at a sufficiently large scale. Such data on the nature and the trajectory of functional recovery will help to aim conservation effort at the actions most likely to improve ecosystem services such as larval output. In the meantime, the unseen reproductive benefits of corals living in high cover populations—the greater larval output per unit of live tissue multiplied by larger population size—represents an underappreciated yet critical reason to direct protection and effective management towards the world's remaining healthy coral populations.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Figure S1 Coral cover of two broadcast spawning coral species collected from Willemstad and Oostpunt coral reefs.

Figure S2 Energetic lipid content of adult corals collected from Willemstad and Oostpunt coral reefs.

Table S1 Sample collections for lipid measurements.

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