Spatial and temporal variation in fecundity among populations of *Acropora millepora* on the Great Barrier Reef.

C. H. Tan[1, 2], M. S. Pratchett[1], L. K. Bay[3], E. R. Graham[4], A. H. Baird[1]

[1]ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville QLD 4811 Australia

[2]School of Marine and Environmental Science, Universiti Malaysia Terengganu, Terengganu 20130 Malaysia

[3]Australian Institute of Marine Science, PMB 3, Townsville MC, Townsville QLD 4810 Australia

[4]College of Marine and Environmental Science, James Cook University, Townsville QLD 4811

Corresponding author: andrew.baird@jcu.edu.au

Short Title: coral fecundity variation

ABSTRACT: Sexual reproduction is vital for population persistence, even in organisms that can reproduce asexually, such as corals. Yet information on spatial and temporal variation in reproductive traits is surprisingly rare. Here, we examined spatial and temporal variation in fecundity, defined as the number of oocytes per polyp, in the staghorn coral, *Acropora millepora*, over two years among six populations separated by over 700 km on inshore reefs on the Great Barrier Reef (GBR). Variation in fecundity was greatest at small spatial scales: there were pronounced differences in fecundity within and among colonies at each site but little variation at the site or regional scale. This suggests that fecundity is affected by environmental variables that also vary at small scales, such as light and water flow, rather than variables that vary on a regional scale, such as temperature. Colony fecundity in the first year was a good predictor of colony fecundity in the second year, suggesting that some genotypes are more fecund than others. This research suggest that factors operating at the scale of the individual, such as microhabitat differences in flow or light, or genetic identity, are the main cause of variation in fecundity among coral colonies.

Keywords: coral reefs, demography, life-histories, reproduction

INTRODUCTION

Sexual reproduction is generally considered essential to population persistence, even for organisms, such as corals, that are capable of non-sexual reproduction (e.g. Richmond & Hunter 1990). However, surprisingly few studies have measured reproductive traits in healthy coral populations. Consequently, there is limited information on natural variation in reproductive traits within and among coral species, without which it is

difficult to assess the role of sexual reproduction in regulating coral populations. For example, very little is known about how coral reproductive traits vary in space and time within species because few, if any, studies have quantified reproduction across populations or species at more than one site for more than one time using similar methods. Wallace (1985) followed six *Acropora* spp. over two years at one site on the GBR and found that annual fecundity estimates differed in only two of these species. While there are few estimates of fecundity from more than one location for a small number of species (e.g. Wallace 1999), these data have never been rigorously examined and therefore it is difficult to draw any conclusions about spatial variation in this trait. Only a single study has followed reproductive traits of individual colonies through time, finding that fecundity and sexuality varied between years, possibly in response to available energy reserves in individuals (Loya & Sakai 2008). However, the fungiid species studied by Loya & Sakai (2008) are not typical of scleractinian corals, because they are gonochoric and solitary rather than hermaphroditic and colonial (Baird et al. 2009).

Despite the lack of empirical studies on natural variation in reproductive traits, the response of these traits to stress, including competition (Tanner 1995), injury (Hall 1997), disease (Burns et al. 2011) and bleaching (Michalek-Wagner & Willis 2001a; Mendes & Woodley 2002), suggests that these traits are labile. For example, the proportion of colonies breeding was significant lower following bleaching on the GBR in 1998 in two *Acropora* spp. (Baird & Marshall 2002) when compared to two non-bleaching years. Similarly, gonad size and number were reduced in *Orbicella annularis* following bleaching in the Caribbean (Mendes & Woodley 2002). In addition, the number of oocytes per polyp and the number of gravid polyps were lower in tumorous tissue versus healthy tissue in coral colonies with tumors (Yamashiro et al. 2001; Burns

et al. 2011). These studies provide evidence for plasticity in reproductive traits and therefore suggest these traits should be affected by prevailing environmental conditions, particularly those that might influence resource acquisition, such as light and water flow (Hoogenboom & Connolly 2009).

The aim of this research was to document spatial and temporal variation in fecundity of the coral *Acropora millepora* at two sites in each of three regions separated by over 700 km on the inshore GBR. We also examined the relationship between colony size and fecundity.

MATERIALS AND METHODS

Study sites, selection of colonies and sampling frequency

This study was conducted on the fringing reefs at two sites in each of three inshore high island groups separated by 5˚ of latitude along the Great Barrier Reef (GBR): Orpheus Island (18.62°S; 146.48°E) and Pelorus Island (18.55°S; 146.48°E) in the Palm Island group; Hook Island (20.17°S; 148.90°E) and Mid-Molle Island (20.23°S; 148.82°E) in the Whitsunday Island group; and Miall Island (23.15°S; 150.90°E) and Halfway Island (23.20°S; 150.97°E) in the Keppel Island group (Fig. 1). All sites were less than 20 km from the mainland and located on the leeward side of islands at depths of between one to three meters (Fig. 1). *Acropora millepora* is a corymbose species that is common in shallow water on most inshore reefs and in protected areas on mid- and outer-shelf reefs along most of the length of the GBR (Veron & Wallace 1984; Wallace 1999). At each site, 30 *A. millepora* colonies were tagged in April or May 2009 and then revisited on another four trips over the next two years with the final trip in April 2011. Only colonies likely to be reproductively mature (maximum diameter > 16 cm; Hall &

Hughes 1996) and with no tissue damage were tagged. The track swam on the first sampling trip was logged using a GPS towed on a body board and the position of each colony was recorded on this track.

Quantifying polyp fecundity

Samples for reproductive analysis were collected in the week before the full moon in October 2009 and 2010 to ensure the samples were collected before spawning, which typically occurs in either November or December at these sites (Willis et al. 1985; unpublished data). One branch, at least 5 cm long, was collected from the centre of each colony to avoid the sterile zone on the periphery of colonies (Wallace 1985). Branches were placed in individual zip-lock bags labelled by colony number while underwater and then transferred to labelled containers containing 10% seawater formalin immediately upon surfacing. Back in the lab, branches were decalcified in 10% formic acid and then placed in 10% seawater formalin until dissection.

Branches were dissected under a stereo-dissecting microscope. First, each branch was cut in half along the sagittal plane to allow visual inspection of the distribution of polyps containing oocytes. Typically, there is an area, commencing at the tip, where no polyps contain oocytes known as the sterile zone (Wallace 1985). Any polyps without oocytes below the sterile zone were visibly smaller than the others, presumably because they had recently been budded, and were therefore deemed immature (Sakai 1998) and excluded from sampling. Ten mature polyps were selected haphazardly from below the sterile zone and dissected out of the branch. Next, each polyp was dissected, and the number of oocytes recorded. Finally, 30 oocytes from each branch were selected haphazardly from those that had been dissected out of the polyps,

and the maximum diameter measured using a stage micrometer under a compound microscope at 40X magnification.

Estimating colony size

On each sampling occasion, all tagged colonies were photographed using a Canon Powershot G11 from approximately 1.5 meters above and perpendicular the surface of the colony to quantify horizontal planar surface area. A pre-calibrated 10 x 10 centimetre white Perspex scale bar was placed on the surface of each colony when photographed. Photographs were corrected for barrel distortion and then horizontal planar surface area was quantified for each coral colony using the software package ImageJ (http://rsbweb.nih.gov/ij/).

Statistical analysis

Contingency tables were used to test for differences in the number of colonies that were and were not breeding at each site and in each year. A three-way ANOVA was used to test for differences in the mean number of oocytes per polyp. Factors were region, site nested within region and colony nested within site and region. All factors were treated as random. Variance components were also calculated using the same model. The analysis was done separately for each year to allow partitioning of variance among the three scales of spatial variation. Only colonies with oocytes were used in the analysis. The fit of the models was explored graphically by comparing the predicted values to the residuals and there was no evidence of bias in the models.

The relationship between colony size and fecundity was tested using linear regression, as was the relationship between fecundity in 2009 versus fecundity in 2010. Colony size was $\log_{10}$ transformed and separate regressions were performed for each

site in each year. All ANOVA were performed with IBM SPSS statistical software version 20 and all regressions in R.

RESULTS

A high percentage of tagged colonies were breeding at all sites in both years, ranging from 82% at Orpheus Island in 2009 to 100% at Pelorus and Hook Islands in 2010 (Table 1). The proportion of colonies breeding did not vary among sites in either year (2009 $\chi^2 = 2.91$, df = 5, p = 0.71; 2010 $\chi^2 = 5.68$, df = 5, p = 0.34), or between years ($\chi^2 = 0.21$, df = 1, p = 0.6403).

Mean fecundity differed between sites and among colonies in both years and there were no regional differences in either year (Table 2; Fig. 2). The majority of variation in fecundity occurred among colonies: 51.2% of the total variation occurred at this scale in 2009 and 53.9% in 2010 (Table 2). Only 5.4% and 7.2% of the variation in 2009 and 2010 respectively occurred at the site level (Table 2).

Mean fecundity did not vary with respect to colony size, except at Pelorus Island in 2009, where fecundity increased with colony size (Fig. 3; Table 3).

Mean colony fecundity in 2009 was a good predictor of mean fecundity in 2010 at three of the six sites (Fig. 4; Table 4).

DISCUSSION

Despite the large spatial scale of this study, which compared colonies separated by over 700km on inshore reefs of the GBR, the fecundity of *Acropora millepora* varied mostly at small spatial scales. Fecundity did not vary among regions in either of the two years,

and the difference among sites was small and inconsistent, with the possible exception of Halfway Island, which had the highest mean fecundity in both years. Fecundity was however, often very different among colonies within the same site. Furthermore, the best predictor of colony fecundity was fecundity in the previous year. All of these results suggest that factors operating at the colony scale, such as microhabitat differences in flow or light, or genetic differences among colonies, are the main cause of variation in fecundity among colonies of *A. millepora* on inshore reefs on the GBR.

These results suggest that there are individualistic differences among colonies, caused by intrinsic (e.g. genotype) or extrinsic (microhabitat) factors, which lead to marked differences in fecundity between neighbouring colonies. The importance of microhabitat on colony physiological performance is supported by models suggesting that energy acquisition is strongly influenced by the light and flow regime (Hoogenboom & Connolly 2009; Hoogenboom et al. 2011), and that small differences in colony position, such as distance from the reef crest, can affect colony performance and population abundance (Madin et al. 2012).

The high level of variability among individuals in fecundity suggests that this is not an ideal variable with which to test or monitor the effects of stress, because a large number of individuals or replicates will need to be sampled to detect an effect. Alternatively, differences in the biochemical composition of oocytes (Michalek-Wagner & Willis 2001b), that might affect vital rates such as acquisition of competence and larval mortality might be more informative.

Colony size had no consistent effect on fecundity in *A. millepora* despite theoretical predictions. Kim & Lasker (1997) predicted that average fecundity per polyp of larger colonies should be reduced due to self-shading effects in the centre of colonies. Hoogenboom & Connolly (2009) predicted that larger colonies have a greater net

energy balance over a wider range of light and flow regimes and inferred that this should lead to an increase in colony fecundity with colony size. Neither of these predictions was supported by the relationships between size and fecundity in *A. millepora*. In contrast, the differences among colonies within sites and the fact that fecundity in the first year was a good predictor of fecundity in the second year, suggest that genetic or microhabitat differences are the major driver of variation in fecundity. It is, in fact, rare to find a relationship between colony size and reproductive variables in corals. For example, of six species examined by Hall & Hughes (1996) on the reef crest at Lizard Island, there was a positive relationship between colony size and the size of oocytes in only two species. Colony size had no effect on oocyte or testes number per polyp, or testes volume per polyp, for any species (Hall & Hughes 1996).

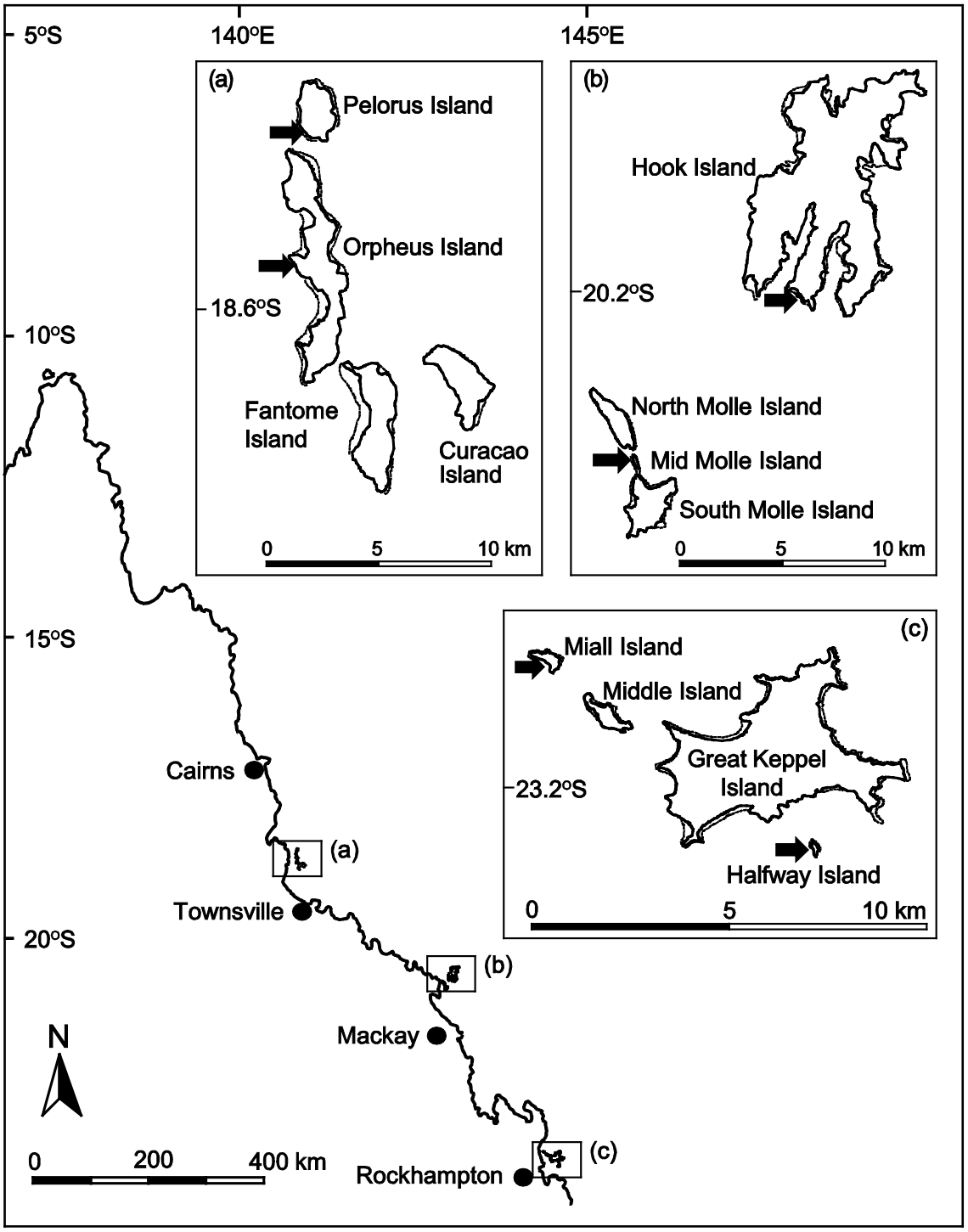
In conclusion, variation in reproductive variables was greatest at small scales and likely to be driven by genetics or microhabitat differences in light and flow based on colony position, in this study. Similarly, theoretical prediction with respect to the relationship between colony size and reproductive variables appear to be overwhelmed by genetic or microhabitat differences among colonies.

Acknowledgments We thank Alison Jones for assistance in the field. This study was supported by Australian Research Council Centre of Excellence Grant CE0561432.

195 REFERENCES

196 Baird AH, Guest JR, Willis BL (2009) Systematic and biogeographical patterns in the
197 reproductive biology of scleractinian corals. Annual Review of Ecology,
198 Evolution, and Systematics 40:551-571
199 Baird AH, Marshall PA (2002) Mortality, growth and reproduction in scleractinian
200 corals following bleaching on the Great Barrier Reef. Mar Ecol Prog Ser
201 237:133-141
202 Burns JHR, Takabayashi M (2011) Histopathology of growth anomaly affecting the
203 coral, Montipora capitata: Implications on biological functions and population
204 viability. Plos One 6:e28854
205 Hall VR (1997) Interspecific differences in the regeneration of artificial injuries on
206 scleractinian corals. J Exp Mar Biol Ecol 212:9-23
207 Hall VR, Hughes TP (1996) Reproductive strategies of modular organisms:
208 Comparative studies of reef-building corals. Ecology 77:950-963
209 Hoogenboom MO, Connolly SR (2009) Defining fundamental niche dimensions of
210 corals: synergistic effects of colony size, light, and flow. Ecology 90:767-780
211 Hoogenboom MO, Connolly SR, Anthony KRN (2011) Biotic and abiotic correlates of
212 tissue quality for common scleractinian corals. Mar Ecol Prog Ser 438:119-
213 U143
214 Kim K, Lasker HR (1997) Flow-mediated resource competition in the suspension
215 feeding gorgonian *Plexaura homomalla* (Esper). J Exp Mar Biol Ecol 215:49-64
216 Loya Y, Sakai K (2008) Bidirectional sex change in mushroom stony corals.
217 Proceedings of the Royal Society B: Biological Sciences 275:2335-2343
218 Madin JS, Hoogenboom MO, Connolly SR (2012) Integrating physiological and
219 biomechanical drivers of population growth over environmental gradients on
220 coral reefs. Journal of Experimental Biology 215:968-976
221 Mendes JM, Woodley JD (2002) Effect of the 1995-1996 bleaching event on polyp
222 tissue depth, growth, reproduction and skeletal band formation in *Montastraea*
223 *annularis*. Mar Ecol Prog Ser 235:93-102
224 Michalek-Wagner K, Willis BL (2001a) Impacts of bleaching on the soft coral
225 *Lobophytum compactum*. I. Fecundity, fertilization and offspring viability. Coral
226 Reefs 19:231-239
227 Michalek-Wagner K, Willis BL (2001b) Impacts of bleaching on the soft coral
228 *Lobophytum compactum*. II. Biochemical changes in adults and their eggs. Coral
229 Reefs 19:240-246
230 Richmond RH, Hunter CL (1990) Reproduction and recruitment of corals: Comparisons
231 among the Caribbean, the Tropical Pacific, and the Red Sea. Mar Ecol Prog Ser
232 60:185-203
233 Sakai K (1998) Effect of colony size, polyp size, and budding mode on egg production
234 in a colonial coral. Biol Bull 195:319-325
235 Tanner JE (1995) Competition between scleractinian corals and macroalgae - an
236 experimental investigation of coral growth, survival and reproduction. J Exp
237 Mar Biol Ecol 190:151-168
238 Veron JEN, Wallace CC (1984) Scleractinia of Eastern Australia. Part V. Family
239 Acroporidae, Vol 6. ANU Press, Canberra
240 Wallace CC (1985) Reproduction, recruitment and fragmentation in nine sympatric
241 species of the coral genus *Acropora*. Mar Biol 88:217-233
242 Wallace CC (1999) Staghorn corals of the world. CSIRO, Collingwood

243 Willis BL, Babcock RC, Harrison PL, Oliver JK (1985) Patterns in the mass spawning
244 of corals on the Great Barrier Reef from 1981 to 1984. Proc 5th Int Coral Reef
245 Symp 4:343-348
246 Yamashiro H, Oku H, Onaga K, Iwasaki H, Takara K (2001) Coral tumors store
247 reduced level of lipids. J Exp Mar Biol Ecol 265:171-179
248



249

250 **Figure 1** Map of the three sampling regions on the Great Barrier Reef. Inserts: a) Palm

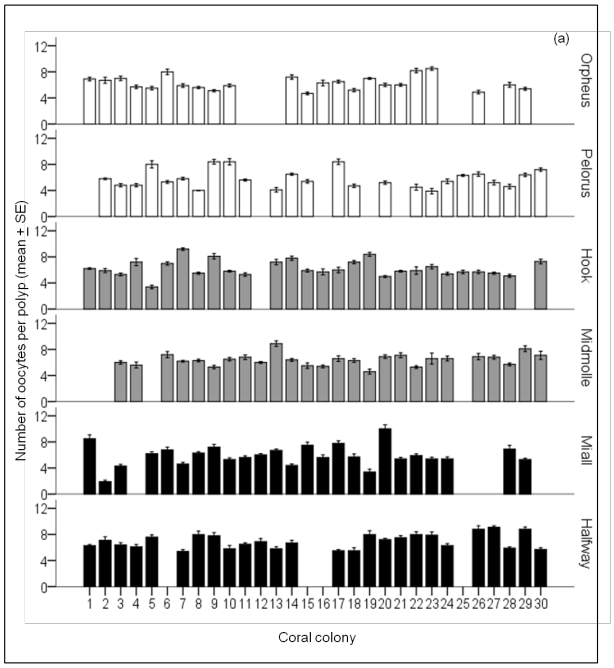
251 Islands (18° S), b) Whitsunday Islands (20° S) and c) Keppel Islands (23° S). Arrows

252 indicate the position of sampling sites within regions.

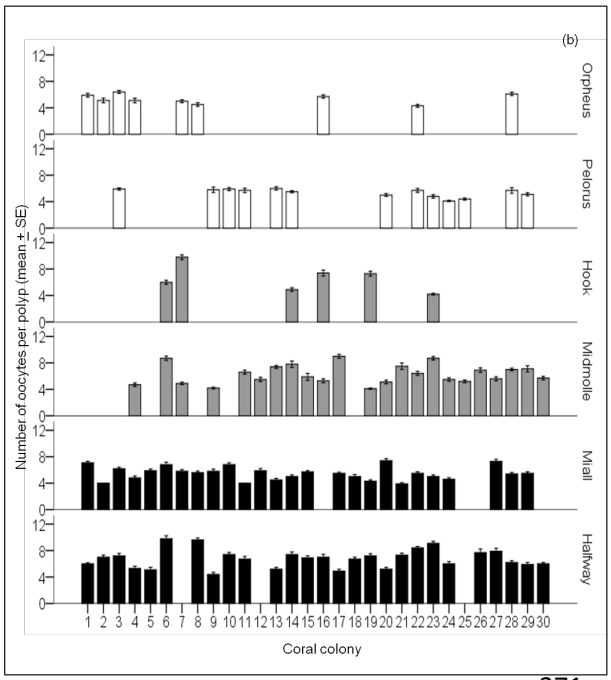
253

254

255



263



271

272 Figure 2. Fecundity of *Acropora millepora* colonies in (a) 2009 and (b) 2010. White
273 bars represent Palm Islands, grey bars represent Whitsunday Islands and black bars
274 represent Keppel Islands. Empty spaces represent dead or missing colonies.

275

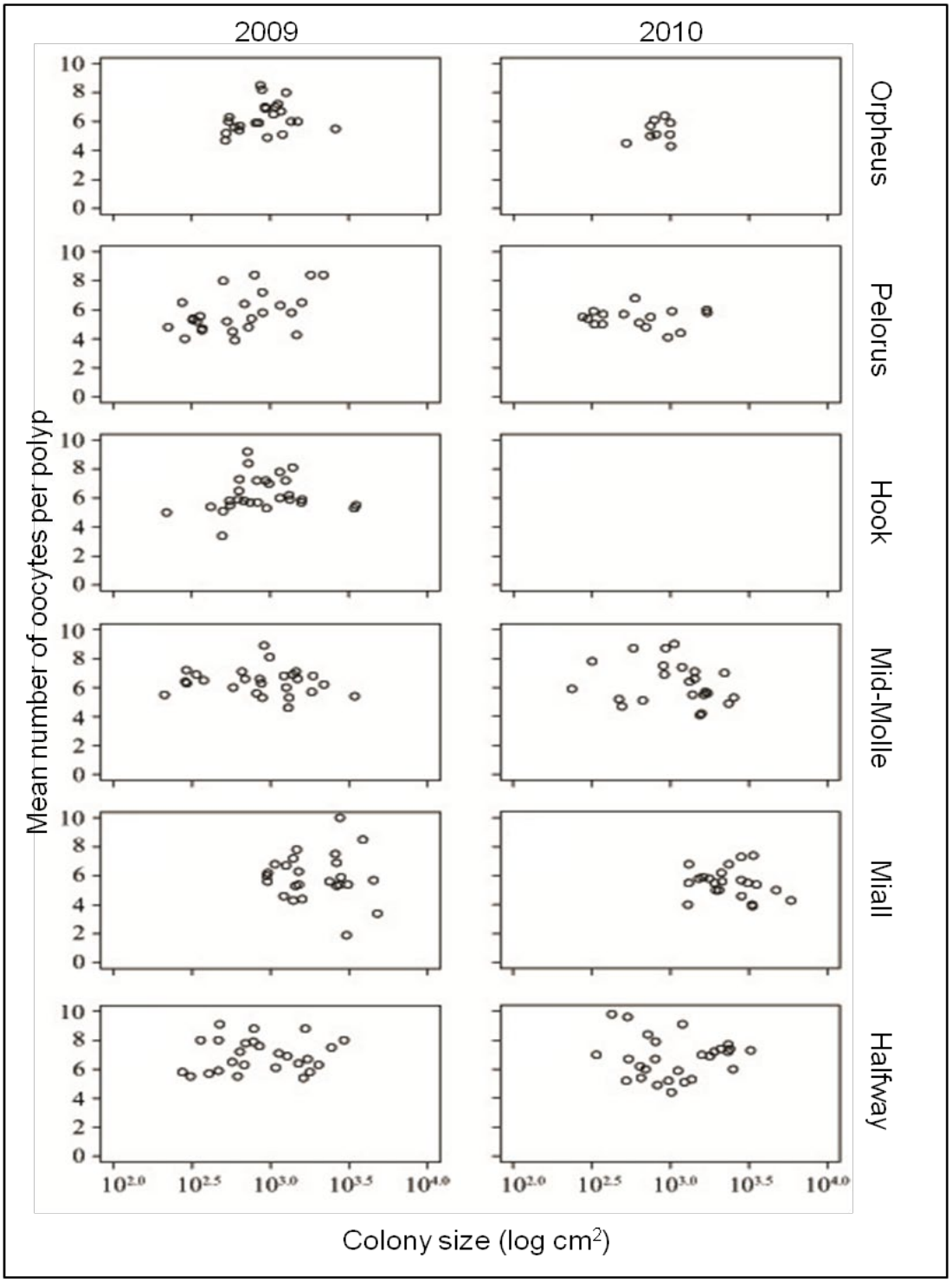
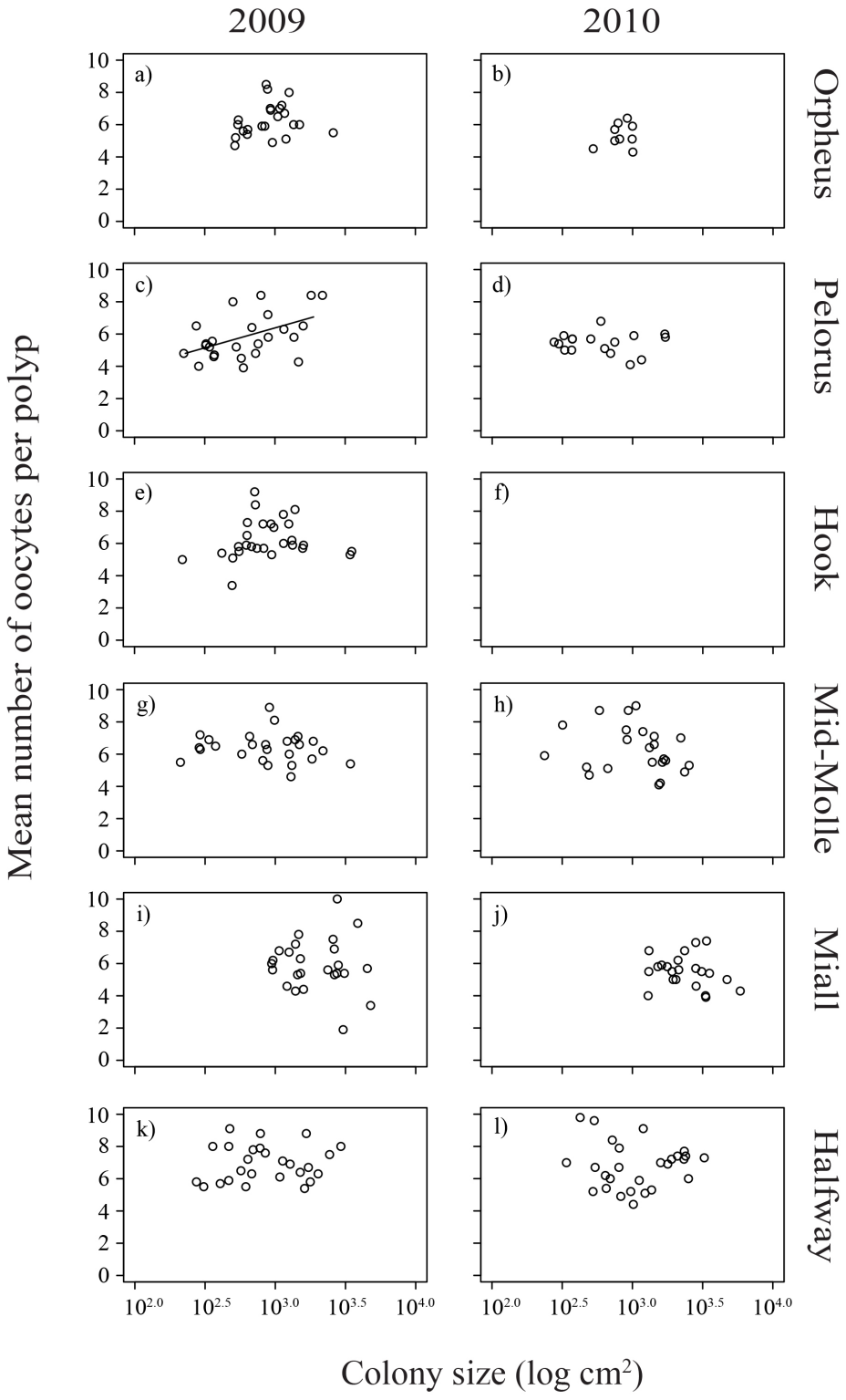


Figure 3. Relationship between colony size in April and the mean number of oocytes per polyp in October for each year at each site. Note no size data exits for Hook in 2010 because the colonies could not be located in the April survey. The regression line was drawn when the relationship was significant



281

282 Figure 4. Relationship between the mean number of ooctyes per polyp in 2009 versus
283 2010 at each site. The regression line was drawn when the relationship was significant.

Table 1. Number (n) and percentage of colonies with oocytes in six populations of *Acropora millepora* on the Great Barrier Reef in October 2009 and 2010.

Region	Site	2009		2010	
		n	Percent with oocytes	n	Percent with oocytes
Palm Islands	Orpheus	26	88	11	82
	Pelorus	26	96	13	100
Whitsunday	Hook	29	97	6	100
Islands	Mid-Molle	28	93	24	96
Keppel Islands	Miall	28	89	27	96
	Halfway	27	96	28	96
	Total	164	93	109	95

Table 2. Summary of ANOVA testing for spatial differences in the mean number of oocytes per polyp in populations of *Acopora millepora* in 2009 and 2010.

Source of variation	2009				
	df	MS	*F*-value	*p*	Var comp (%)
Region	2	20.811	0.385	0.729	0.0
Site (Region)	3	5066	3.512	0.018	5.4
Colony(Site*Region)	147	15.395	13.141	<0.001	51.2
Error	1377	1.172			43.4
Source of variation	2010				
	df	MS	*F*-value	*p*	Var comp (%)
Region	2	70.019	1.026	0.454	5.9
Site (Region)	3	7190	841	0.003	7.2
Colony(Site*Region)	98	15.325	17.379	<0.001	53.9
Error	936	0.882			33.0

Table 3. Summary of linear regression model results for colony size versus fecundity for each site.

		2009				2010			
Region	Site	Slope	r^2	Intercept	*P*	Slope	r^2	Intercept	*P*
Palm Islands	Orpheus	0.91	0.02	3.45	0.50	00	0.18	-6.33	0.26
	Pelorus	2.24	0.24	-0.88	0.01	-0.37	0.01	6.55	0.68
Whitsunday	Hook	0.63	0.02	32	0.49	--	-	-	-
Islands	Mid-Molle	-0.34	0.01	7.46	0.65	-1.15	0.05	9.88	0.32
Keppel Islands	Miall	-0.05	0.00	6.12	0.98	-1.27	0.03	9.85	0.42
	Halfway	0.82	0.02	30	0.44	-1.25	0.04	10.65	0.32

Table 4. Summary of linear regression model for the relationship between fecundity in 2009 vs 2010.

Region	Site	Slope	r^2	Intercept	*P*
Palm Islands	Orpheus	-0.07	0.01	5.77	0.846
	Pelorus	0.05	0.01	5.08	0.710
Whitsunday Islands	Hook	0.85	0.29	0.28	0.267
	Mid-Molle	0.77	0.28	1.40	0.012
Keppel Islands	Miall	0.35	0.37	3.38	0.002
	Halfway	0.49	0.17	3.19	0.045