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REPORT

Are all eggs created equal? A case study from the Hawaiian reef-building coral *Montipora capitata*

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Abstract Parental effects have been largely unexplored in marine organisms and may play a significant role in dictating the phenotypic range of traits in coral offspring, influencing their ability to survive environmental challenges. This study explored parental effects and life-stage differences in the Hawaiian reef-building coral *Montipora capitata* from different environments by examining the biochemical composition of mature coral colonies and their eggs. Our results indicate that there are large biochemical differences between adults and eggs, with the latter containing higher concentration of lipids (mostly wax esters), ubiquitinated proteins (which may indicate high turnover rate of proteins) and antioxidants (e.g., manganese superoxide dismutase). Adults displayed high phenotypic plasticity, with corals from a high-light environment having more wax esters, lighter tissue δ^{13}C signatures and higher *Symbiodinium* densities than adults from the low-light environment who had higher content of accessory pigments. A green-algal pigment (α-carotene) and powerful antioxidant was present in eggs; it is unclear whether this pigment is acquired from heterotrophic food sources or from endolithic green algae living in the adult coral skel-

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etons. Despite the broad phenotypic plasticity displayed by adults, parental investment in the context of provisioning of energy reserves and antioxidant defense was the same in eggs from the different sites. Such equality in investment maximizes the capacity of all embryos and larvae to cope with challenging conditions associated with floating at the surface and to disperse successfully until an appropriate habitat for settlement is found.

Keywords Biochemical phenotype · Coral eggs · Coral reproduction · Egg provisioning · Gamete variation · Maternal effects · Spawner

Introduction

Currently, coral reefs are experiencing unprecedented pressure and extinction risk due to climate change (e.g., global warming, ocean acidification) and other local impacts associated with anthropogenic disturbances (e.g., pollution, dredging and overexploitation; Hughes et al. 2003; Hoegh-Guldberg et al. 2007; Lough 2008). How these environmental changes will impact the physiological status of adult corals and in turn influence their offspring's phenotype is, however, relatively unexplored.

Adult corals are sessile, but their early life stages are pelagic and therefore experience environmental conditions that can differ from and be more dynamic than those of adults. For example, eggs and larvae float on or swim close to the surface and experience high and variable levels of UV radiation and temperature, and exposure to free radicals (Epel et al. 1999; Marquis et al. 2005; Hamdoun and Epel 2007; Markey et al. 2007; Yakovleva et al. 2009). Thus, parental investment in protective mechanisms and energy reserves for the egg/early embryo is critical to provisioning offspring with the capacity to cope with environmental challenges and develop successfully (Hamdoun and Epel 2007).

Parental effects (e.g., parental investment) occur when the phenotype of offspring is affected by the phenotype or environmental conditions of the parents (Mousseau and Fox 1998; Badyaev and Uller 2009). Parental effects are fundamentally important in biological systems and can impact the life history (Donelson et al. 2009), competitive ability (Wulff 1986), evolutionary trajectories, speciation rates (Wade 1998) and population dynamics (Ginzburg 1998). These effects have been extensively studied in plants, insects and terrestrial vertebrates, but have received much less attention in the marine environment and specifically in natural tropical settings (Mousseau and Fox 1998; Marshall et al. 2008). Indeed, only four studies have focused on the phenotypic relationship between parents and offspring in corals (Michalek-Wagner and Willis 2001; Wellington and Fitt 2003; Alamaru et al. 2009a; Padilla-Gamiño et al. 2012). These studies have revealed pre- and post-zygotic parental effects influencing the physiological characteristics of the egg/larvae (Michalek-Wagner and Willis 2001; Wellington and Fitt 2003; Alamaru et al. 2009a) and the transmission of symbionts to the eggs (Padilla-Gamiño et al. 2012). For example, experimentally bleached parents of the soft coral *Lobophytum compactum* release eggs with lower levels of protein, lipid, mycosporine-like amino acids (MAA) and carotenoid concentrations than those released by healthy corals, which may jeopardize egg and larval viability (Michalek-Wagner and Willis 2001). Furthermore, eggs and larvae of the broadcast-spawning reef corals *Acropora palmata, Montastraea annularis* and *M. franksi* exhibit different levels of photoprotective compounds and survival capabilities depending on the depth of origin of the parent colonies (Wellington and Fitt 2003). Similarly, a strong relationship between the isotopic signatures of parental tissues and those of their planulae has been found in the brooder *Stylophora pistillata* sampled across different depths (Alamaru et al. 2009a), and a recent study has revealed that parental effects may play an important role in the transmission of *Symbiodinium* to the eggs of *Montipora capitata* (Padilla-Gamiño et al. 2012), with corals and eggs from a more challenging environment (high light and temperature) generally associated with *Symbiodinium* clade D, which are known to confer greater thermal tolerance (Rowan 2004).

In this study, we compared biochemical traits (Table 1) in adult colonies of the coral *Montipora capitata* (Family Acroporidae) and their eggs, from two different environments. The goal was to better understand the intraspecific variation in egg composition and the relationship between the biochemical phenotype of parent colonies and the eggs they release. Specifically, we address the following questions: (1) Are the biochemical phenotypes of adults and eggs different between sites? (2) Does the biochemical phenotype of the eggs reflect differences in environmental conditions of the adult?

Life-stage differences and parental investment were explored in the context of energetic (e.g., lipid reservoirs, symbiotic algae, isotopic signatures) and protective (e.g., antioxidants) functions, traits that influence the ability of the embryo/larvae to disperse and cope with variable environmental conditions (e.g., light intensities, oxidative stress, temperature) and settle successfully. These data improve our understanding of what determines the natural biochemical variability of *M. capitata* eggs (host and symbiont), a facet of biology that contributes to the resilience of one of the most important reef-building corals in Hawaii.

Table 1 Traits investigated in adults and eggs of the coral *Montipora capitata* and their biological function

Holobiont trait	Biological function
Lipids	Long-term energy reserves, storage of excess fixed carbon in host tissue
	Triacylglycerols and wax esters are primary energy sources utilized during development and metamorphosis
Isotopic signatures	$\delta^{13}C$ is a marker of carbon sources, $\delta^{13}C$ decreases as photosynthesis decreases and as heterotrophy increases. $\delta^{15}N$ is a marker of trophic level
C:N:P ratios	Carbon, nitrogen and phosphorous are essential bioelements of living organisms
Ubiquitin	Cellular protein that tags proteins for degradation in the proteosomes. Increased levels indicate higher levels of protein degradation
MnSOD	Manganese superoxidase dismutase, a mitochondrial antioxidant enzyme, plays a role in oxidative stress
Fluorescent proteins	Fluorescent proteins serve as antioxidants by scavenging reactive oxygen species (e.g., H_2O_2)
Photosynthetic pigment profiles	Light harvesting compounds: Chl *a* and accessory pigments Chl c_2 and peridinin
	Photoprotective compounds (carotenoids): non-chlorophyll accessory pigments that serve as antioxidants, carotenes (α-carotene, β-carotene) and xanthophylls (dinoxanthin, diadinoxanthin and diatoxanthin)
	a) Pigments/g dw—pigment concentration per holobiont dry mass
	b) Pigment/cell—pigment concentration in each dinoflagellate cell
Dinoflagellate density	*Symbiodinium* dinoflagellate cells provide the host with energy in the form of translocated reduced carbon compounds (glucose, glycerol and aminoacids), which are products of photosynthesis

Materials and methods

Study sites and sample collections

Montipora capitata is a broadcast spawner that has high morphological and physiological plasticity and can inhabit a broad range of environments (Maragos 1972; Grottoli et al. 2004; Palardy et al. 2008; Rodrigues et al. 2008). In contrast to most coral spawners that release asymbiotic eggs, *M. capitata* release eggs with *Symbiodinium* directly transmitted from the parental colony (vertical transmission). Samples of parent colonies and their gametes were collected during the spawning events in summer 2007 from two sites located on the western side of Moku O Lo'e Island in Kane'ohe Bay, O'ahu, Hawai'i, the same sampling event as in Padilla-Gamiño and Gates (2012) and Padilla-Gamiño et al. (2012). Coral colonies were sampled from ~1 to 2 m depth at two sites: Bridge to Nowhere (BTN; 21° 25.893′ N; 157° 47.376′W, $n = 21$) and Gilligan's Lagoon (GL; 21° 25.973′N; 157° 47.392′W, $n = 20$). Colonies located at the GL site were closer to shore than colonies from the BTN site and were shaded by an overstory of trees located on land. *Montipora capitata* colonies at the BTN site were generally branching in morphology (Fig. 1a), whereas colonies at the GL site were predominantly plating in morphology (Fig. 1b).

To compare temperature and light conditions at the two collection sites, temperature was measured at 10-min intervals for ~1 year (July 2007–August 2008) using StowAway Tidbit data loggers (Onset Computer) accurate to ±0.2 °C. Light was measured during two 2-week periods in 2008 (22 September–1 October and 25 November–5 December 2008). Light measurements were taken at 10-min intervals using Odyssey Photosynthetic Irradiance Recording Systems (Odyssey).

Montipora capitata released (spawned) egg–sperm bundles (Fig. 1c) between 20:45 and 21:15 h during the first quarter of the new moon in June 2007. Gametes were collected on the reef using a novel net system specifically designed to collect spawn from shallow colonies with minimum damage to both adult coral colonies and released gametes. The cylindrical nets surrounded the coral colony, allowing the collection of egg–sperm bundles at the water surface after release (for details see Padilla-Gamiño and Gates 2012). The nets were placed over the corals 1–2 h before spawning and removed each night after spawning. The positively buoyant egg–sperm bundles were collected using scoop nets, transferred to plastic beakers and broken apart by rinsing with 0.2-µm-filtered seawater (Fig. 1d). A subset of the freshly collected and unfertilized eggs were observed and photographed immediately upon collection using dissecting and compound microscopes (Olympus SZX7 and BX51, respectively) (Fig. 1c–f). Most eggs were stored at −80 °C until further analysis. Samples from adult coral colonies were collected 5 days prior to spawning by breaking small fragments (~25 cm^2) at least 4 cm away from the tips and edges of the colonies where polyps were anticipated to be reproductively active (Wallace 1985; pers obs). Samples were immediately placed in dry ice and stored at −80 °C for further analysis. Of the 41 colonies followed throughout the reproductive season, 25 colonies spawned in June 2007 (Padilla-Gamiño and Gates 2012). Reproductive output was highly variable between colonies providing us with different amounts of egg material for biochemical measurements. For specific details on the spawning dynamics of *M. capitata* in 2007, see Padilla-Gamiño and

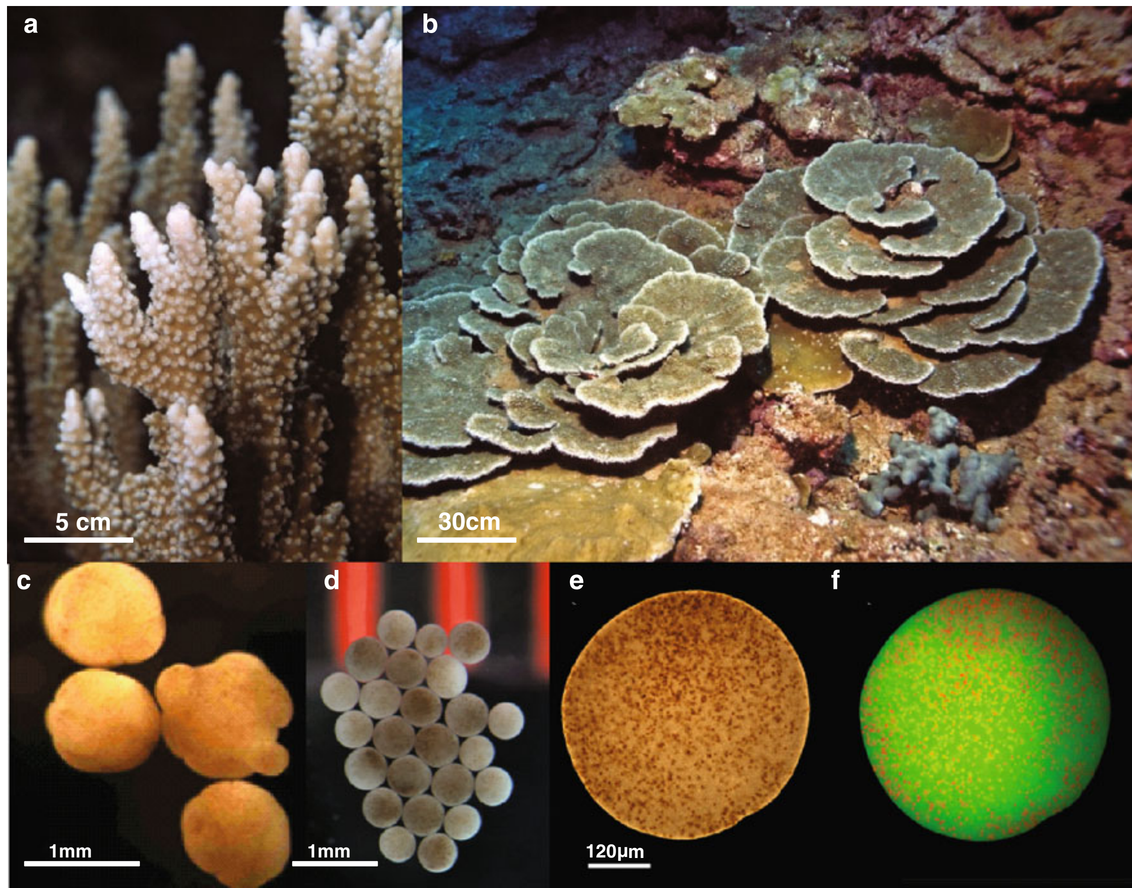


Fig. 1 *Montipora capitata* is a simultaneous hermaphrodite that releases egg–sperm bundles. **a** Branching and **b** plating morphologies of *Montipora capitata*. **c** Bundles contain approximately 14 eggs and a sperm mass. **d** Eggs contain *Symbiodinium* cells transferred from the parent. A *Montipora capitata* egg under **e** *white light* and **d** *blue light*. In **f** the *green* fluorescence is from fluorescent proteins and the *red* fluorescence is from the chlorophyll in the symbiotic dinoflagellates

Gates (2012). Sample size differed among the biochemical analyses (n = 5–11 per site for both adults and eggs) and was constrained by the availability of eggs and instrumentation.

Laboratory analysis

Total lipids and lipid classes

Total lipids (n = 8 per site for both adults and eggs) were extracted according to Rodrigues and Grottoli (2007). In brief, ground samples were extracted in a 2:1 chloroform/methanol solution, the organic phase washed using 0.88 % KCl, and the extract dried to a constant weight. Lipids were normalized to total ash-free dry tissue biomass of the organic fraction (host and symbionts). Triacylglycerol (TAG) and wax ester (WE) concentrations in total lipid extracts were determined by high-performance liquid chromatography/evaporative light-scattering detection (HPLC/ELSD) using triolein (Sigma-Aldrich, #1787-1AMP) and oleyl oleate (Sigma-Aldrich, # O3380) as reference standard (Silversand and Haux 1997). The percentage of "other lipids" (OLs; polar lipids, free fatty acids, sterols, diacylglycerols and monoacylglycerols) was estimated as the difference between total lipids and the sum of TAG and WE.

Stable isotopes and element ratios

Coral samples (n = 6 per site for both adults and eggs) were ground in 0.2-μm-filtered sea water (FSW). If skeleton was present, tissue was separated from the ground skeleton in several washes with FSW. Isolated tissue was then homogenized, and aliquots from the same homogenate were taken for different analyses (stable isotopes, algal pigments, *Symbiodinium* densities and ash-free dry weight determination). Coral δ^{13}C and δ^{15}N analyses were performed using standard methods (i.e., Rodrigues and Grottoli 2006; Hughes et al. 2010). Briefly, pre-burned Glass Fiber Filters (0.7 μm pore size, GF/F; Whatman) containing the homogenized coral tissue were dried at

60 °C prior to isotopic and phosphorus analyses. Samples were analyzed on a Costech Elemental Analyzer coupled to a Finnigan Delta IV Plus stable isotope ratio mass spectrometer under continuous flow using a CONFLO III interface in the Stable Isotope Biogeochemistry Laboratory at Ohio State University. Approximately 10 % of all samples were run in duplicate. Stable carbon ($\delta^{13}C =$ ‰ deviation of the ratio of ^{13}C: ^{12}C relative to the Vienna Peedee Belmenite Limestone standard) and stable nitrogen ($\delta^{15}N =$ ‰ deviation of ^{15}N:^{14}N relative to air) measurements were made where the average standard deviations of repeated measurements of the USGS24 and IAEA-N1 standards were 0.06 ‰ for $\delta^{13}C$ and 0.12 ‰ for $\delta^{15}N$. Total phosphorus was obtained using the modified high-temperature ashing/hydrolysis method (Monaghan and Ruttenberg 1999). For each coral tissue homogenate (1–0.5 ml), 40 µL of $MgNO_3$ was added; then, the solution was dried and burned at 550 °C for 2 h. After combustion, the residual material was hydrolyzed in 1 mL of 1 M HCl and analyzed via the molybdenum blue method (Grasshoff et al. 1983). Stable isotopes, algal pigments and *Symbiodinium* densities were normalized to total ash-free dry tissue biomass of the organic fraction (host and symbionts).

Protein analysis

The levels of Manganese Superoxide Dismutase (MnSOD) and ubiquitin-conjugated proteins were studied ($n = 11$ per site for both adults and eggs) via western blot according to Barshis et al. (2010). All western blots were standardized using a standard curve dilution of a single, reference coral extract; hence, concentrations of specific experimental samples are relative to the concentration of the ubiquitin-conjugated proteins or MnSOD contained in the reference extract. 10 µg of total protein for each experimental extract was loaded on the gel in triplicate. Antibodies against ubiquitin (Cat, #SPA-200), manganese superoxide dismutase (MnSOD; Cat.#SOD-110) and anti-rabbit- and anti-mouse-conjugated (Cat. #SAB-300, SAB-100) horseradish peroxidase were obtained from Stressgen Biotechnologies (Victoria, British Columbia, Canada). Primary antibody dilutions were 1:5,000 for MnSOD and 1:2,000 for ubiquitin; secondary antibody dilutions were all 1:10,000. All images were recorded using a ChemiDoc XRS molecular imager (Bio-Rad) and analyzed and quantified with ImageJ software (Abramoff et al. 2004).

Fluorescent properties of eggs and sperm were explored using epifluorescence microscopy. Emission characteristics of coral fluorescent proteins (FP) in eggs and adults were examined using a fluorescence spectrophotometer (SpectraMax M2, Molecular Devices, Sunnyvale, California).

Algal pigments

Glass Fiber Filters (0.7 µm pore size, GF/F; Whatman) containing the coral and algal tissue homogenate ($n = 6$ per site for both adults and eggs) were extracted in 3 mL of HPLC-grade acetone in culture tubes along with 50 µL of an internal standard (canthaxanthin) at 4 °C in the dark for 24 h. The extracts were processed according to Bidigare et al. 2005; for details, see methods in the electronic supplemental material, ESM. Pigment concentrations and ratios at the colony and algal cellular level were computed by normalizing to sample dry tissue biomass (µg pigment/g dw and symbiont density (pg pigment/cell) (see Table 1 to review the differences between pigment normalizations).

Symbiodinium densities

Symbiodinium cells ($n = 5$ at BTN and $n = 6$ at GL for both adults and eggs, an adult–egg set of BTN samples was lost) were separated from the tissue homogenate by centrifuging and performing multiple washing steps using FSW. After separation, the *Symbiodinium* pellets were resuspended in FSW ($n = 5$ at BTN and $n = 6$ at GL for both adults and eggs) and homogenized, and three subsamples counted manually with a hemocytometer and a light microscope (Olympus BX-51). A linear regression was used to estimate the number of bundles (or eggs) per ash-free dry tissue mass of the egg sample to provide an estimate of the amount of symbiont cells per egg (ESM Appendix I).

Statistical analyses

Prior to analysis, data were normalized as necessary using logarithmic or inverse transformations to achieve homogeneity of variances and normality. The xanthophylls DDX and DTX had similar patterns throughout and were combined for the statistical analysis. All variables were analyzed using a general linear model, with developmental stage and site as fixed factors and colony modeled as a random factor nested within site to account for the repeated measurements between adults and eggs. When significant effects were identified, Tukey's post hoc tests were performed to determine differences between groups (i.e., differences between sites within life stage). Parental effects were recognized if the interaction between site and stage factors was significant and/or if significant differences between the eggs reflected differences between the adults. Means, standard deviations and ranges (minimum–maximum) of temperature and light were calculated for each site during the periods sampled. Temperature and light measurements were compared between sites using a Mann–Whitney test. All statistical analyses were performed using

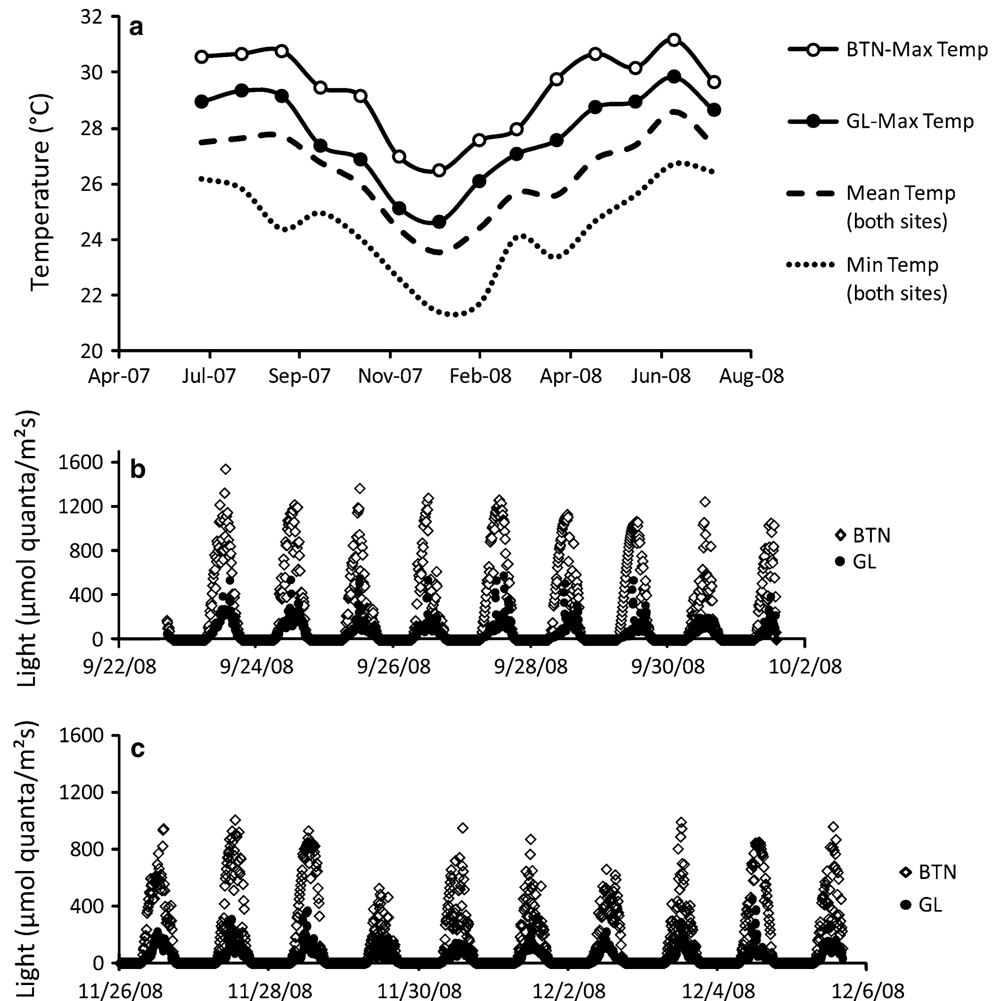


Fig. 2 **a** Temperature (°C) and **b**–**c** light (μmol quanta/m^{2}s) data from the two study sites in Moku O Lo'e Island, Kane'ohe Bay Hawaii, **b** 22 September–1 October and **c** 25 November–December 5, 2008

Minitab statistical software (version 15). p-values were considered significant below an alpha of 0.05.

Results

Study site environment Temperature was higher and more variable at the BTN site than the GL site throughout the year ($W = 4.5 \times 10^{10}$, $p < 0.0001$, Mann–Whitney, Fig. 2a), with up to 3 °C fluctuations observed over a single 24-h period. The BTN site was also characterized by the highest and broadest light intensities in late summer and late autumn sampling times compared with the GL site ($W = 1773932$, $p < 0.0001$, $W = 1413959$, $p < 0.0001$, September and November, respectively, Fig. 2b–c). The GL site had 23 % of the light levels of the BTN site.

Effects of stage

Total lipids and lipid composition Total lipid concentrations were higher in eggs than in adults (Fig. 3a, Table 2) representing 80 and 25 % of the total dry tissue weight, in eggs and adults, respectively. Lipid composition differed between life stages, with WE representing approximately 56 % of the total lipid weight in the eggs and only 9–17 % of the total lipid weight in adults (Fig. 3b, d, Table 2). In contrast, TAG was found in higher concentrations in adults than in eggs (Fig. 3c–d, Table 2). The "other lipids" category ranged between 36 and 92 % (mean 74.9 %) in adults and 25–57 % (mean 45.5 %) in eggs (Fig. 3d).

$\delta^{13}C$, $\delta^{15}N$, %C, %N, %P and elemental ratios (C:N, C:P, N:P) Adult colonies were enriched in ^{13}C compared with eggs ($p = 0.009$, Fig. 4a, Table 2). Average $\delta^{15}N$ values of adults and eggs from both sites ranged between 3 and 5.7 ‰ and were not different between life stages or sites (ESM Appendix IIa, Table 2). Carbon content was higher in eggs than in adult corals ($p < 0.0001$, Fig. 4b, Table 2). In contrast, nitrogen and total phosphorus contents were higher in the adult coral tissue than in the eggs (Fig. 4c–d, Table 2). C:N and C:P ratios in eggs were higher than in the adult colonies (Fig. 4e–f, Table 2). N:P ratios did not differ

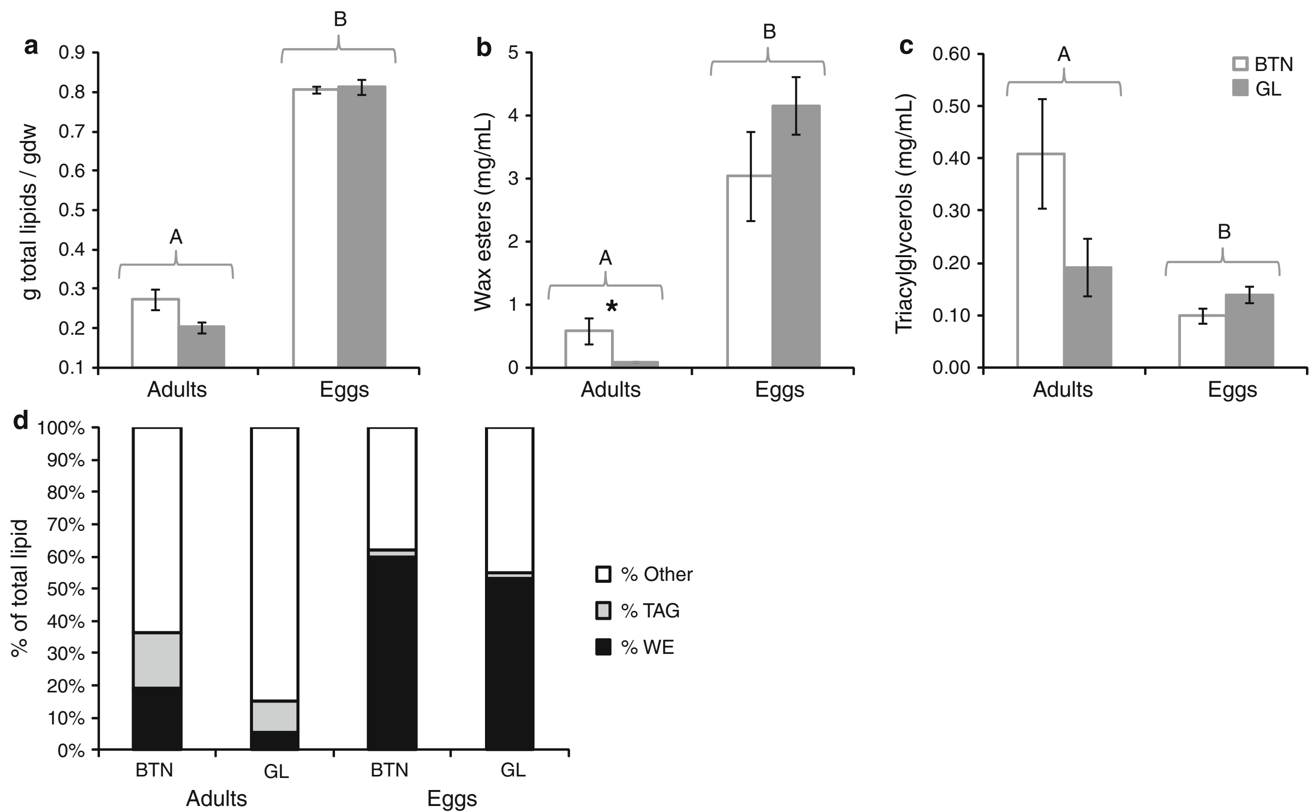


Fig. 3 **a** Total lipids, **b** wax esters (WE), **c** triacylglycerols (TAG) and **d** percentage of lipid classes in adults and eggs of *Montipora capitata*. Means ± SE. *Capital letters* indicate differences between stages, and *asterisks* indicate differences between sites within stage

Table 2 Results of the general linear model testing the effects of life stage and site on the traits investigated

	Effect of stage		Effect of site		Effect of colony (site)		Effect of site × stage	
Physiological parameter	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Lipid (*df* = 31)	1006.77	**<0.0001**	2.93	0.109	1.00	0.497	4.59	0.050
Wax esters (*df* = 25)	189.65	**<0.0001**	3.33	0.093	2	0.122	24.37	**<0.0001**
Triacylglycerols (*df* = 25)	5.4	**0.04**	1.59	0.234	0.5	0.868	4.57	0.056
δ^{13} C (*df* = 23)	10.61	**0.009**	1.74	0.216	7.80	**0.002**	3.93	0.076
δ^{15} N (*df* = 23)	0.52	0.489	3.98	0.074	0.81	0.627	2.43	0.150
C (%) (*df* = 23)	35.19	**<0.0001**	0.09	0.773	1.42	0.294	5.44	**0.042**
N (%) (*df* = 23)	22.88	**0.001**	1.37	0.269	1.18	0.398	0.02	0.903
Total P (*df* = 23)	22.45	**0.001**	0.84	0.382	1.3	0.353	0.01	0.935
C:N (*df* = 23)	233.55	**<0.0001**	6.63	**0.028**	0.73	0.685	4.83	0.053
C:P (*df* = 21)	64.24	**<0.0001**	0.01	0.935	1.29	0.355	1.49	0.253
N:P (*df* = 21)	0.18	0.680	1.15	0.312	1.51	0.273	0.00	0.986
MnSOD (*df* = 35)	63.57	**<0.0001**	1.11	0.308	1.44	0.236	4.13	0.059
Ubiquitin-conjugates (*df* = 34)	34.84	**<0.0001**	6.26	**0.024**	0.93	0.559	0.22	0.648
Symbiont no. of cells (*df* = 21)	42.31	**<0.0001**	7.68	**0.022**	1.34	0.336	4.03	0.076

Significant values at 95 % confidence ($p < 0.05$) are in bold, *df* degrees of freedom

between life stages (ESM Appendix IIb, Table 2).Thus, the major differences in tissue composition between life stages are higher levels of carbon–lipid in the eggs.

Protein biomarkers Relative concentrations of MnSOD were higher in eggs than in adults (Fig. 5a, Table 2). Relative concentrations of ubiquitin conjugates were also

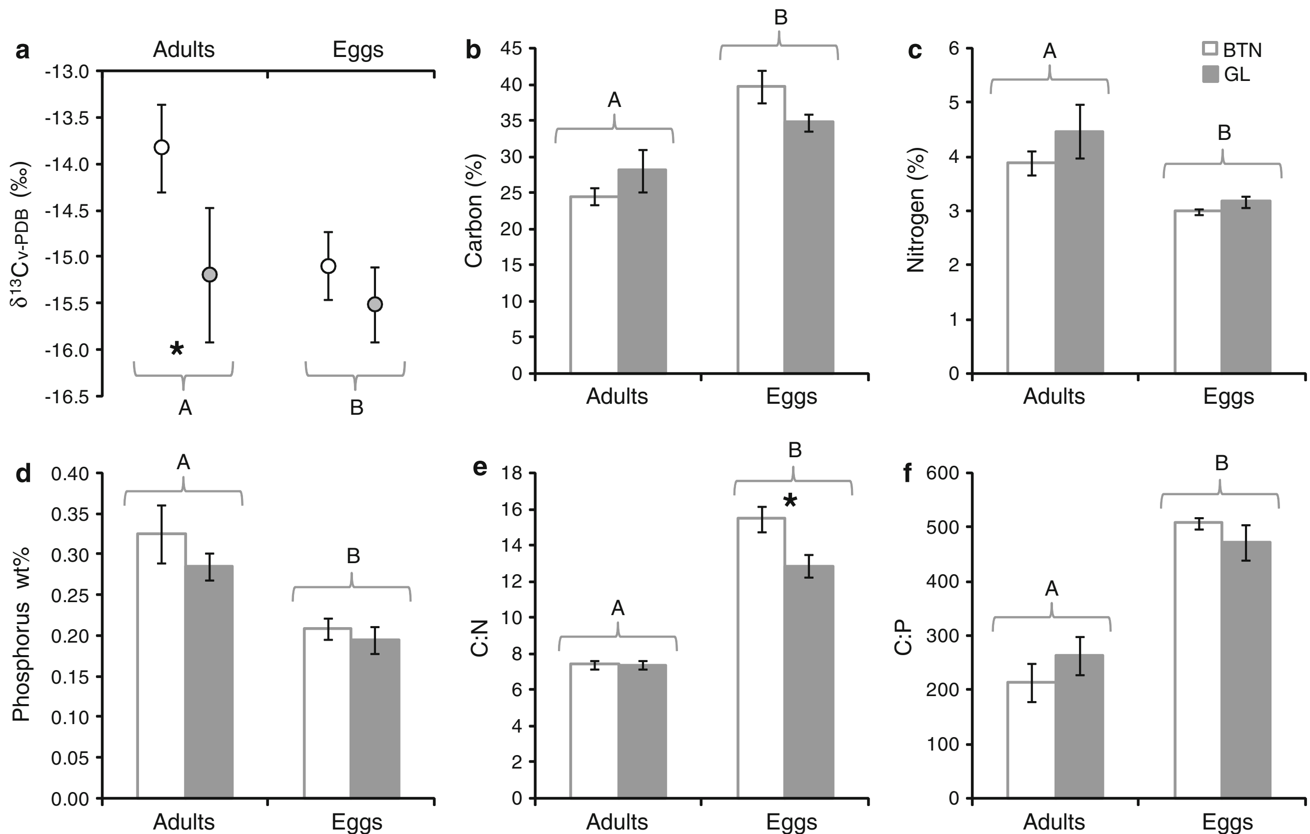


Fig. 4 **a** δ^{13}C, **b** % carbon, **c** % nitrogen, **d** % phosphorus, **e** C:N and **f** C:P ratios in adults and eggs of *Montipora capitata*. Means ± SE. *Capital letters* indicate differences between stages, and *asterisks* indicate differences between sites within stage

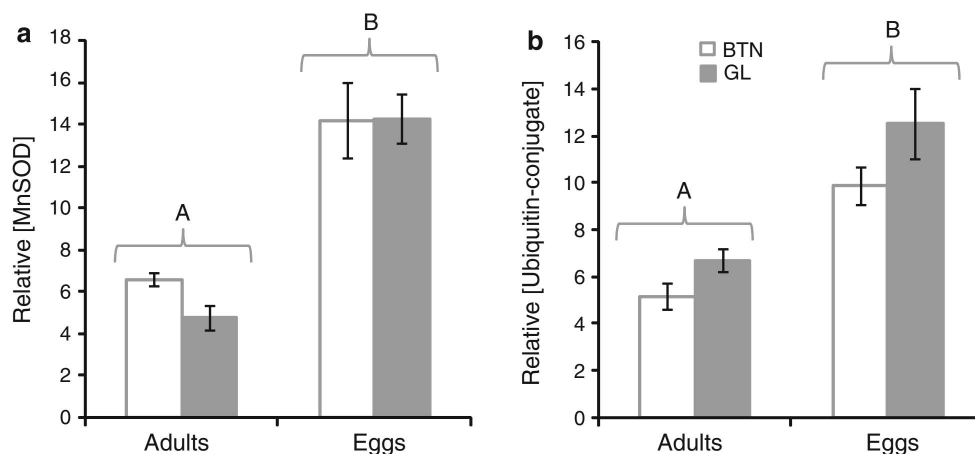


Fig. 5 **a** Manganese superoxide dismutase (MnSOD) and **b** ubiquitin conjugate levels in adults and eggs of *Montipora capitata*. Means ± SE. *Capital letters* indicate differences between stages

higher in eggs than in adult colonies (Fig. 5b, Table 2). Fluorescent proteins were detected in both eggs and adult tissues. Both emitted cyan-green light, characteristic of fluorescent proteins ($\lambda = 491$, 3 nm resolution), and far-red light from chlorophyll ($\lambda = 682$, 3 nm resolution) when excited by blue light (450 nm) (Fig. 1 c, d).

Algal pigments and Symbiodinium *densities* The dinoflagellate pigments Chl *a*, Chl c_2, peridinin, β-carotene, dinoxanthin, diadinoxanthin (DDX) and diatoxanthin (DTX) were detected in samples from all colonies for both adults and eggs. Dinoflagellate pigment concentrations per g ash-free dry tissue were always higher in the adults

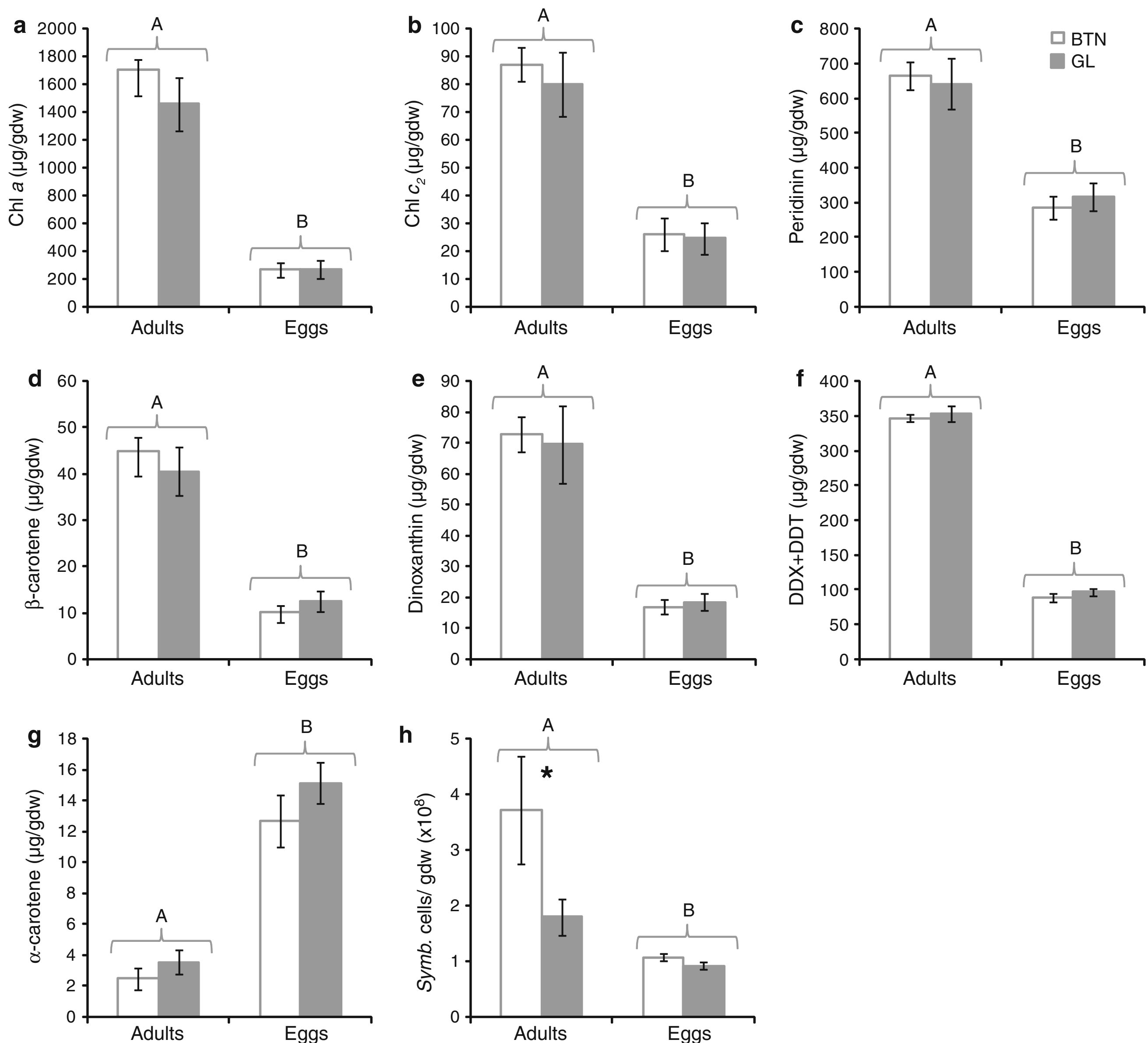


Fig. 6 Photosynthetic pigment concentrations and *Symbiodinium* densities of adults and eggs of *Montipora capitata* normalized by tissue ash-free dry weight. **a** Chl *a*—chlorophyll *a*; **b** Chl c_2—chlorophyll c_2; **c** peridinin; **d** *β*-carotene; **e** dinoxanthin; **f** DDX + DDT-diadinoxanthin + diatoxanthin, **g** α-carotene and **h** *Symbiodinium* density. Means ± SE. *Capital letters* indicate differences between stages, and *asterisks* indicate differences between sites within stage

compared with the eggs (Fig. 6a–f, Table 3). The pigment alpha-carotene (α-carotene), characteristic of green algae, was also detected in all colonies and both life stages. In contrast to dinoflagellate (brown algae) pigments examined here in which pigment concentrations range between 2.5 and 8 fold higher in parent tissue (Fig. 6a–f), α-carotene concentration was approximately sixfold higher in the eggs compared with the adults (Fig. 6g, Table 3). In addition, *Symbiodinium* densities (cell number normalized by tissue dry mass) were higher in adult corals than in eggs (Fig. 6h, Table 2). *Symbiodinium* densities in the eggs were estimated to range between 2.3 and 4.2 × 10^3 symbiont cells per egg. The concentrations of Chl *a*/cell, *β*-carotene/cell, dinoxanthin/cell, DDX + DTX/cell were greater in adults than in eggs (Fig. 7a, d, e, f, Table 3).

Effects of site

Total lipids and lipid composition Total lipid content did not change between sites for both adults and eggs (Fig. 3a, Table 2). Eggs from both sites had similar levels of WE, even though parents from the BTN site had higher levels of

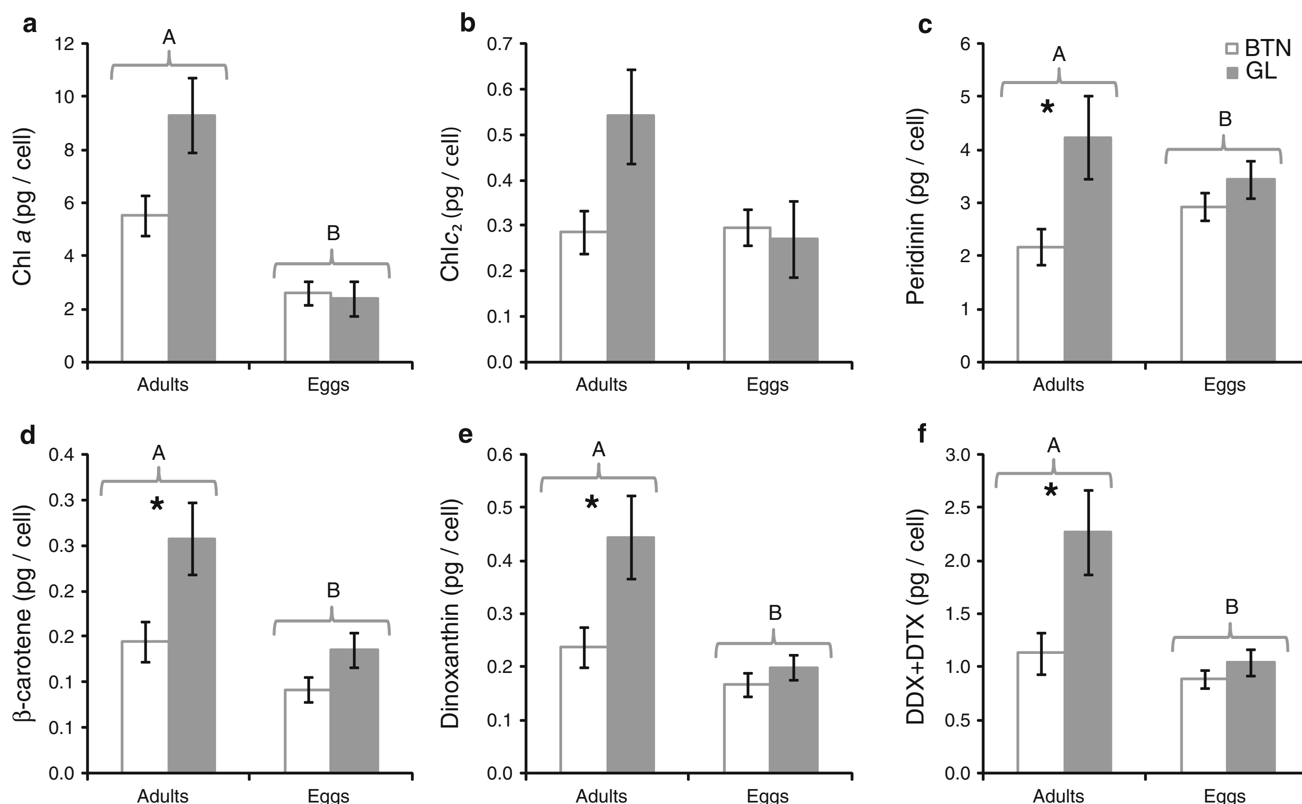


Fig. 7 Photosynthetic pigment concentrations of adults and eggs of *Montipora capitata* normalized by density of *Symbiodinium* cells. **a** Chl *a*—chlorophyll *a*; **b** Chl c_2—chlorophyll c_2; **c** peridinin; **d** *β*-carotene' **e** dinoxanthin' **f** DDX + DDT-diadinoxanthin + diatoxanthin. Means ± SE. *Capital letters* indicate differences between stages, and *asterisks* indicate differences between sites within stage

WE (post hoc test $p < 0.001$, Fig. 3b, d, Table 2). No differences in TAG between sites were found in either stage (Fig. 3c, Table 2).

δ^{13}C, δ^{15}N, %C, %N, %P and elemental ratios (C:N, C:P, N:P) δ^{13}C was significantly higher in adults from the BTN site (post hoc test $p = 0.011$, Fig. 4a, Table 2), but no difference was found in δ^{13}C between the eggs from different sites. No site effects on adults or eggs were observed in δ^{15}N, % carbon, % nitrogen, % phosphorous, C:P and N:P (Fig. 4b–d, f, Table 2). Only C:N had a significant site effect, which was primarily driven by the differences in the eggs between sites (post hoc test $p = 0.017$, Fig. 4e, Table 2).

Protein biomarkers Adults and eggs from both sites had similar concentrations of MnSOD (Fig. 5a, Table 2). A significant effect of site was found in the relative concentrations of ubiquitin–conjugates. Overall, ubiquitin conjugates were higher at the GL site in both stages; however, post hoc tests did not detect differences between sites within stages (post hoc test $p = 0.534$, $p = 0.220$, adults and eggs respectively, Fig. 5b, Table 2).

Algal pigments and Symbiodinium densities Algal pigment concentrations per g ash-free dry tissue did not vary between sites (Fig. 6a–g, Table 3). However, there was an effect of site on *Symbiodinium* densities, mostly driven by the differences in the adults between sites. Higher *Symbiodinium* densities were found in adults from the BTN site as compared to counterparts from GL (post hoc test, $p = 0.021$, Fig. 6h, Table 3), and eggs had similar *Symbiodinium* densities between sites (post hoc test, $p = 0.831$). Concentrations of some pigments normalized per *Symbiodinium* cell showed differences between sites (Fig. 7c–f, Table 3), which were primarily driven by the differences between adults and not the eggs. Peridinin/cell, *β*-carotene/cell, dinoxanthin/cell and DDX + DTX/cell were higher at the GL site as compared to BTN (post hoc test, $p = 0.033$, $p = 0.050$, $p = 0.025$, $p = 0.018$, $p = 0.$ respectively, Fig. 7c–f).

Discussion

This study explored the natural variability of biochemical traits in the adult coral colonies and eggs of *M. capitata.* Although considerable differences in the parental phenotype were observed, the biochemical composition and photochemical characteristics of *Symbiodinium* in their

Table 3 Results of the general linear model testing the effects of life stage and site on the photosynthetic pigments of *Montipora capitata*

	Effect of stage			Effect of site		Effect of colony (site)		Effect of site × stage	
Pigment	*df*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Pigment/tissue									
Chl *a*/mg dm	22	220.99	**<0.0001**	0.53	0.481	2.00	0.155	0.35	0.566
Chl c_2/mg dm	22	112.65	**<0.0001**	0.01	0.917	3.74	**0.030**	1.25	0.292
Peridinin/mg dm	23	129.66	**<0.0001**	0.00	0.949	4.04	**0.019**	0.79	0.394
β-carotene/mg dm	23	113.23	**<0.0001**	0.07	0.801	1.27	0.358	1.76	0.214
Dinoxanthin/mg dm	23	283.93	**<0.0001**	0.00	0.945	4.66	**0.011**	1.39	0.266
DDX + DTX/mg dm	23	346.24	**<0.0001**	0.01	0.914	5.97	**0.005**	0.77	0.402
α-carotene/mg dm[a]	23	85.13	**<0.0001**	2.21	0.168	1.03	0.483	0.35	0.568
Pigment/cell									
Chl *a*/cell	22	25.72	**0.001**	3.75	0.079	0.82	0.620	3.74	0.085
Chl c_2/cell	22	2.31	0.163	2.07	0.177	0.84	0.606	2.44	0.153
Peridinin/cell	22	0.01	0.934	5.75	**0.036**	1.54	0.263	2.63	0.139
β-carotene/cell	22	11.4	**0.008**	9.17	**0.012**	0.98	0.516	1.44	0.261
Dinoxanthin/cell	22	14.83	**0.004**	5.26	**0.043**	1.63	0.238	3.72	0.086
DDX + DTX/cell	22	12.07	**0.007**	6.35	**0.029**	1.5	0.278	4.23	0.070

Pigment concentrations were normalized to dry mass (dm), chlorophyll *a* (Chl *a*) and *Symbiodinium* cell density

Significant values at 95 % confidence ($p < 0.05$) are in bold

Chl *a* chlorophyll *a*, Chl c_2 chlorophyll c_2, *DDX* diadinoxanthin, *DTX* diatoxanthin, *df* degrees of freedom

[a] alpha-carotene is a green-algal pigment

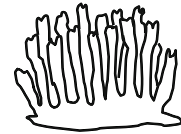
eggs were similar. Thus, regardless of the parental condition, eggs are provisioned with similar energy reserves and antioxidant levels. Such equality of provisioning would allow offspring to settle in a variety of habitats, rather than being restricted to the same habitat as the adults, and may explain, in part, why this species has such a broad distribution in the West Pacific (Veron 2000).

Energy reserves and acquisition

Lipids, particularly wax esters (WE), were a major component in *M. capitata* eggs as compared to adults. WE are largely responsible for the positive buoyancy of the eggs, which is critical for successful fertilization, and represent a long-term energy store (major source of fatty acids) that is consumed throughout development to provide energy for larval dispersal, settlement and metamorphosis (Arai et al. 1993; Lee et al. 2006; Harii et al. 2010). Eggs of *M. capitata* had similar WE content (53–59 % of total lipid weight) to symbiotic propagules of other species (56–69 %, *Montipora digitata,* 52–60 %, *Pocillopora damicornis*) (Arai et al. 1993; Harii et al. 2007, 2010). This is generally lower than the WE content of asymbiotic propagules, which can account for up to 58–85 % of total lipid weight (Figueiredo et al. 2012). Low WE content in symbiotic propagules may reflect the fact that *Symbiodinium* have the capacity to provide additional carbon and energy for dispersal (Harii et al. 2010; Figueiredo et al. 2012) and may also serve to decrease buoyancy and reduce oxidative stress in algal symbionts caused by exposure to high temperature and UV (Yakovleva et al. 2009; Nesa et al. 2012).

Although energy reserves (total lipids) were similar in adults from different sites, WE were higher at the BTN (high light) site, which is consistent with the idea that storage lipids in *Montipora capitata* are composed of photosynthetically derived carbon (Rodrigues et al. 2008). Eggs did not differ in their δ^{13}C signatures between sites, suggesting that similar carbon sources are used for egg production at the two sites. Overall, eggs had lower δ^{13}C values than adults, most likely due to the high concentrations of lipids (Bodin et al. 2007; Alamaru et al. 2009a), which typically have lower δ^{13}C values (DeNiro and Epstein 1977). Lighter δ^{13}C signatures in the adults at the GL (low light) site suggest that these corals have lower rates of photosynthesis or higher heterotrophy, or a combination of both, as compared to corals from the BTN site (Grottoli and Wellington 1999; Rodrigues and Grottoli 2006; Alamaru et al. 2009b). It is likely that the differences in δ^{13}C in adults were mostly driven by lower rates of photosynthesis due to lower light availability at the GL site, which receives only 23 % of the light levels of the BTN site. Increases in heterotrophy (due to lower light availability) at the GL could also contribute to the differences in δ^{13}C. However, this is less likely since previous work has shown that healthy *M. capitata* colonies located

Table 4 (a) Differences in traits between life stages (adult vs. eggs) and (b) between adults from different sites of *Montipora capitata*

a. Differences between stages		b. Parental differences
Higher in adults	Higher in eggs	Higher in high light environment
Triacylglycerol	Total lipids	Wax esters
$\delta^{13}C$	Wax esters	$\delta^{13}C$
Total phosphorous	Carbon	Symbiont cells/mg dw
Symbiont cells/mg dw	C:N	
Chl *a*/mg dw	C:P	
Chl *c*/mg dw	Ubiquitin	
β-carotene/mg dw	SOD	
Peridinin/mg dw	α-carotene/mg dw	Higher in low light environment
Dinoxanthin/mg dw	Chl *c*/Chl *a*	Peridinin/symb cell
DDX + DTX/mg dw	β-carotene/Chl *a*	β-carotene/symb cell
Chl *a*/symb cell	Peridinin/Chl *a*	Dinoxanthin/symb cell
Chl *c*/symb cells	Dinoxanthin/Chl *a*	DDX + DTX/symb cell
β-carotene/symb cell	DDX + DTX/Chl *a*	
Peridinin/symb cell		
Dinoxanthin/symb cell		
DDX + DTX/symb cell		

at 1 m depth have similar feeding rates as colonies at 6 m depth (Palardy et al. 2008), which were receiving less than 42 % of the photosynthetically active radiation (PAR) received by the colonies at 1 m depth (Jokiel et al. 1997). Isotopic nitrogen signatures ($\delta^{15}N$) of *M. capitata* ranged between 4 and 5 ‰ and were similar across sites and between life stages, suggesting that adults from all these sites were feeding at similar trophic levels and that similar nitrogen sources are passed from the parents to the eggs.

Antioxidant defense and photoprotection

Oxidative stress is caused by the production and accumulation of reactive oxygen species—ROS—($^1O_2$, O_2^-, H_2O_2, $HO^\bullet$) which can damage cellular components such as lipids, proteins and DNA (Lesser 2006). In corals, oxidative stress can cause bleaching due to elevated temperature and high UV levels and in coral symbiotic larvae, the presence of *Symbiodinium* has been associated with higher vulnerability to oxidative stress than asymbiotic larvae (Yakovleva et al. 2009; Nesa et al. 2012). In our study, eggs from both sites (which contain *Symbiodinium*) seem to be similarly preconditioned to cope with oxidative stress and possessed elevated antioxidant levels and increased turnover rates of proteins when compared to the adults (Table 4a). Fluorescent proteins in the eggs may serve as antioxidants by scavenging ROS such as hydrogen peroxide (H_2O_2) (Palmer et al. 2009), which is able to move easily through biological membranes and cause lipid peroxidation (Halliwell and Gutteridge 1999). In addition, the higher concentrations of MnSOD in the eggs (two fold higher than adults, Table 4a) also suggest that MnSOD may prevent the accumulation of the ROS superoxide in the early life stages of the coral holobiont and reduce oxidative damage. Higher levels of ubiquitin-conjugated proteins in eggs as compared to adults suggest greater rates of protein damage and/or enhanced capacity for turnover (Table 4a). In sea urchin embryos, synthesis rates of ubiquitin have been associated with selective protein degradation during embryogenesis where total ubiquitin content of the embryo increased almost tenfold between fertilization and the pluteus larva stage (Pickart et al. 1991).

The photochemical characteristics of coral adults reflect acclimatization responses to the different light conditions between sites/morphologies. By increasing accessory pigments relative to chlorophyll, corals at the GL site increase the amount of photoprotection and reduce their susceptibility to photodamage and oxidative stress (Lesser et al. 1990). Although corals at the BTN site are experiencing significantly higher light levels, corals in the low-light environment have a plating morphology which may make them more vulnerable to light stress than corals at the BTN site, which have a branching morphology and thus self-shade more. It is important to note that corals at these two sites can harbor different assemblages of *Symbiodinium* (Padilla-Gamiño et al. 2012), and this may also play an important role in the acclimatization strategies and photobiological characteristics of the coral holobionts from the different environments (Little et al. 2004; Rowan 2004; Abrego et al. 2008; Cantin et al. 2009). In contrast to the adults, eggs did not differ in their *Symbiodinium* densities, which suggest that adults provision the egg similarly in

terms of the number of *Symbiodinium* cells transferred and/or there is a limit to the number of *Symbiodinium* cells that an egg can contain. To date, only one study has explored the process of entry of dinoflagellate endosymbionts into the eggs of reef-building corals (Hirose et al. 2001). These authors found that *Symbiodinium* appear to enter mature eggs through follicle cells that surround the eggs in temporary gaps formed in the mesoglea. This process may limit the number of dinoflagellate symbionts that can be transferred and may account for the similar quantities found in eggs from different sites.

Lower pigments (per *Symbiodinium* cell) in the eggs suggest that *Symbiodinium* cells with lower pigmentation are preferentially transferred to the eggs or that *Symbiodinium* cells photoacclimatize differently when in eggs. *Symbiodinium* in eggs are embedded in lipid droplets (the main component of egg cytoplasm), which might add an extra barrier to solar energy acquisition and influence light microenvironment of the symbiont. Lower *Symbiodinium* densities (per ash-free dry weight) and pigment content in the *Symbiodinium* cells may help the early embryo/larvae to reduce the risk of photodamage and subsequent oxidative stress (Lesser et al. 1990). This would be advantageous since *M. capitata* larvae swim near the surface (Hodgson 1985), and in culture, this species has been reported to maintain a high ability to settle for $\sim$6 weeks and in a few cases to delay the onset of settlement competency for 7 months or longer (Kolinski 2004). Chl c_2 and peridinin (per cell) were the only two pigments that had similar concentrations among life stages. In dinoflagellates, these two pigments are efficient light-harvesting accessory pigments that transfer excitation energy to chlorophyll *a* (Chl *a*) (Govindjee et al. 1979) and are part of the Chl-protein complexes (peridinin-Chl *a*-protein and Chl *a*-Chl c_2-peridinin-protein complexes, PCP and acpPC, respectively) (Jeffrey 1976).

Interestingly, a pigment characteristic of green algae (α-carotene) was also detected in both coral adults and eggs. This carotenoid is a fat-soluble pigment (unsaturated hydrocarbon) and a powerful antioxidant that can stop free radicals from causing cells to break down in algae (Niyogi et al. 1997) and may therefore serve to prevent oxidative stress inside the coral eggs. It is important to note that α-carotene is also a major carotene pigment in endolithic green algae (*Ostreobium*) (Jeffrey 1968) which are known to reside in the skeletons of the coral genus *Montipora* (Fine et al. 2005; Magnusson et al. 2007). The presence of high α-carotene levels in the eggs with respect to adults may be due to the fact that pigment extractions were performed using mostly coral homogenate tissue with very small remnants of skeleton (where endolithic algae reside). Furthermore, it remains unclear whether the presence of α-carotene in the eggs is due to (1) translocation from endolithic algae (2) parent's heterotrophy or (3) egg contamination. The first scenario is more likely since there is evidence that endolithic algae can translocate photosynthetic materials to the eggs (Schlichter et al. 1995; Fine and Loya 2002). This pigment could be actively translocated by the host from the gastrodermis or tissue within the skeleton or acquired by the eggs due to similarities in solubility, since lipid is a major component of the egg cell (Arai et al. 1993). If α-carotene is transferred to coral eggs by endolithic algae, this could represent the first evidence of vertical transmission of endolithic algal pigments/metabolites to the coral eggs and highlight an important role for endolithic algae in coral reproduction. A second scenario is that α-carotene is obtained by coral's feeding on the plankton and then transferred to the egg. Arachidonic acid, for example, is a fatty acid that can be present in significant amounts in coral eggs (Arai et al. 1993; Figueiredo et al. 2012) and is most likely acquired by heterotrophy since this fatty acid cannot be synthesized de novo or provided by *Symbiodinium* (Papina et al. 2003; Zhukova and Titlyanov 2003). Finally, an alternative explanation is that endolithic algae "contaminate" eggs by attaching on the surface rather than being incorporated into the eggs. Future research is necessary to identify the localization and sources of this pigment in the eggs.

Despite broad physiological and morphological differences displayed by adult coral colonies, our results indicate that eggs released by corals that are acclimatized/adapted to different environments are provisioned similarly in terms of stored lipids, capacity for light utilization and protection from photodamage. In contrast, Wellington and Fitt (2003) found that larvae released by shallow-water corals had higher concentrations of UVR-protective compounds (e.g., mycosporine-like amino acids) and survived better than larvae from deeper environments when exposed to ambient surface levels of ultraviolet radiation (UVR). In our study, the only difference in the eggs between sites was found in the C:N ratios, and there was no difference in the parental C:N ratios. Eggs from the BTN (high light) site had higher C:N ratios as compared to eggs from the GL site. Because lipid content did not differ between eggs from different sites, we propose that the extra carbon in the eggs from the BTN site reflects higher amounts of carbohydrates, a conclusion consistent with the idea that photosynthetically derived carbon can be incorporated into the propagule (Rinkevich 1989; Gaither and Rowan 2010). This may be due to higher light levels penetrating the tissues and reaching the eggs and/or differences in carbon fixation that link to taxonomic variability in the symbiont assemblages in the eggs (Stat et al. 2008; Padilla-Gamiño et al. 2012). Further research is needed to understand how the physiological capabilities of different *Symbiodinium* clades influence the dispersal of larvae and successful recruitment of the offspring.

Conclusion

Although parental effects were observed previously in the *Symbiodinium* diversity patterns of *Montipora capitata* (Padilla-Gamiño et al. 2012), parental investment in the context of energy reserves and antioxidant protection was the same in adults of *M. capitata* from sites characterized by different light and temperature regimes. This strategy is particularly advantageous because it ensures that coral eggs and early embryos, regardless of the environment of origin, have similar mechanisms available to reduce the damaging effects of ultraviolet radiation and successfully disperse until a habitat suitable for settlement is found. Less variability in the biochemical phenotype of eggs would also have an evolutionary advantage if there was a high cost of gamete plasticity or if offspring fitness was severely affected by an excessive variation in the "optimal biochemical phenotype" of the eggs (Moran and McAlister 2009; Jacobs and Podolsky 2010). In other sedentary organisms, like plants, homeostasis for seed provisioning has been observed between parents growing under optimal and resource-deprived conditions, suggesting that parents can regulate the resource allocation to their offspring in response to environmental conditions (Sultan 1996). Further research is necessary to understand how parental provisioning and egg biochemical composition may change in reef-building corals with a compromised state of health (e.g., bleaching), which may have important consequences for the parental investment in future generations and resilience and persistence of coral reef ecosystems.

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