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Plasticity in gene expression and fatty acid profiles of *Acropora tenuis* reciprocally transplanted between two water quality regimes in the central Great Barrier Reef, Australia

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ABSTRACT

To investigate plasticity in biochemical and physiological health attributes of corals, identical colony fragments of the coral *Acropora tenuis,* from two inshore populations, were exposed to native and novel environmental regimes. Variation in global gene expression (GE) and lipid and fatty acid (FA) composition of surviving colony fragments were quantified after four months. Major FA classes, with the exception of short-chain (C18) polyunsaturated fatty acids (PUFA), decreased in concentration when coral fragments were exposed to lower water quality regardless of their source population. In contrast, a strong effect of source population was detected in the GE profiles of all coral fragments and was enriched with genes associated with translation, ribosome biogenesis and ribosome cellular components. One cluster of co-expressed genes positively correlated with multiple individual FA and included genes involved in developmental processes and cellular pathways. This study demonstrates the strong influence of a source effect defining gene expression relating to basic biological functions, including biosynthetic processing, translation and ribosome biogenesis. However, there is plasticity in FA composition and specific genes relating to elevated health and immunity, which can respond to changes in environmental conditions. These findings suggest hope for future corals, if we can reduce anthropogenic water quality stressors.

1. Introduction

On Australia's Great Barrier Reef (GBR), nearshore reefs are predicted to be the most affected by local environmental and anthropogenic stressors (Brodie et al., 2011; Wooldridge, 2009), as these corals are subjected to higher turbidity (Anthony, 2006; Fabricius et al., 2013), dissolved nutrients (De'ath and Fabricius, 2010) and temperatures (Berkelmans, 2002) than corals on offshore reefs. To distinguish the effects of chronic versus acute exposure to these stressors, biomarkers are needed to monitor and characterize coral health. Biochemical attributes and gene expression profiles of corals hold promise for measuring how local environmental conditions influence the health of inshore coral populations, but further baseline work is needed to understand the potential for corals from different source populations to modify these attributes in response to variation in environmental conditions.

Organisms can optimize their survival and performance under local environmental regimes through a continuum of mechanisms ranging from physiological acclimatization to genetic adaptation. Physiological acclimatization (also referred to as phenotypic plasticity) describes the capacity of an organism, within its lifetime, to adjust (or re-adjust) its biochemical attributes and physiological performance to a varying environment without genetic change (Brown and Cossins, 2011; Coles and Brown, 2003; Sanford and Kelly, 2011; Weis, 2010). Epigenetic modifications of DNA that affect transcriptional regulation, for example methylation or histone modification, can underpin phenotypic

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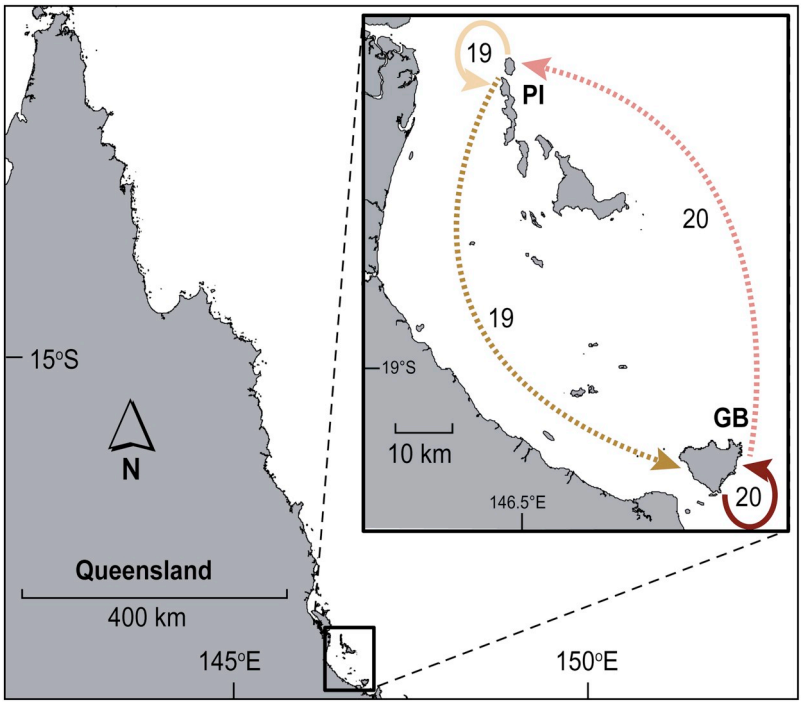

Fig. 1. Map of the coast of Queensland, Australia and the inshore sites on the Great Barrier Reef used for the reciprocal transplant study of *Acropora tenuis*. Colonies for back-transplanting (solid arrows) and cross-transplanting (dashed arrows) were from GB (Geoffrey Bay) and PI (Pelorus Island). Numbers indicate original deployment sample size.

plasticity and can potentially be inherited (Feil and Fraga, 2012), thereby blurring the distinction between acclimatization and adaptation. Genetic adaptation is the result of natural selection, whereby over time, populations optimize fitness traits under local environmental conditions and involves genetic changes between generations (Sanford and Kelly, 2011). Locally adapted genotypes, therefore, are predicted to have greater fitness than foreign genotypes under native conditions and this effect can be measured as a genotype-environment interaction (Brown and Cossins, 2011; Kawecki and Ebert, 2004). Physiological acclimatization, epigenetic modifications and/or genetic adaptation can enable corals to survive in changing local environmental regimes, such as deteriorating water quality, and in response to global environmental perturbations under climate change (Donner et al., 2005).

Reciprocal transplant experiments can reveal the ability of wild populations to respond to novel conditions while controlling for prior environmental history and genetic variability through fragmentation of a single coral genotype (Barshis et al., 2010; Howells et al., 2013; Kenkel et al., 2015). These designs can distinguish among potential responses by revealing either (1) differences in the performance of corals from different source populations regardless of environmental conditions (source effects), indicating no plasticity in phenotypic responses; or (2) plasticity in the performance of corals depending on the environment (environmental effects), leading to similarly expressed phenotypes at a given location through acclimatization of foreign genotypes to local conditions; or (3) a source × environment interaction, indicating local adaptation (Brown and Cossins, 2011; Coles and Brown, 2003; Sanford and Kelly, 2011). It should be acknowledged that a strong source effect or a source × environment interaction could also arise as a consequence of long term acclimatization or developmental canalization, which would not be distinguishable from local adaptation with this design.

Fatty acids (FA) are used in most physiological processes in corals (e.g., respiration, growth, reproduction; Stimson, 1987; Ward, 1995; Yamashiro et al., 1999; Figueiredo et al., 2012; Conlan et al., 2017a, 2017b), and play an important role in the stress resistance and metabolism of corals (Bachok et al., 2006; Hulbert, 2003; Imbs et al., 2015). Corals obtain FA from nutritional sources (e.g., autotrophy and/or heterotrophy; Teece et al., 2011; Baumann et al., 2014), as well as de novo synthesis by both the *Symbiodinium* populations and the coral host (Kabeya et al., 2018). As such, FA profiles can be affected by the surrounding environmental conditions (e.g. Bergé and Barnathan, 2005; Rocker et al., in review) and may provide insight into the biochemical functioning of coral populations across different environments.

Analyses of gene expression (GE) can provide insights into biological processes, molecular functions and cellular components that support healthy functioning of the coral holobiont (Bay et al., 2013; Bay et al., 2009; Kenkel et al., 2014; Moya et al., 2012; Rocker et al., 2015; Wright et al., 2015). Global GE assesses a multivariate, molecular phenotype with no a priori information required and can be used to describe the physiological state of, or physiological processes occurring within, that organism (Dixon et al., 2015). These data can also be used to describe temporal changes in expression occurring within an organism (Barshis et al., 2013) through processes such as up-regulation or front-loading of genes. Global GE is a useful tool for assessing the molecular drivers of biochemical and physiological changes induced by environmental stressors in corals. Detection of changes in gene expression and functional pathways can help to interpret the status and trends in coral health in response to stress.

The capacity for phenotypic plasticity in key biochemical and physiological traits is essential for corals facing future environmental change. Therefore, the aim of this study was to determine the extent to which coral populations could acclimatize to different environmental regimes (specifically water quality) when genotypes were reciprocally exposed to novel and native water quality environments over a four-month period. To partition the effects of source population genetics (and/or canalization) and environmentally-induced phenotypic plasticity, variation in global GE and relative fatty acid (FA) composition (i.e., percentages and concentrations) of surviving fragments of experimental colonies was quantified. Analyses of differential gene expression was conducted using a weighted gene correlation network analysis (WGCNA), which correlated variation in FA composition and global GE profiles, to explore potential molecular mechanisms underpinning variation in biochemical and population attributes and growth.

2. Materials and methods

2.1. Study sites and sampling design

This transplantation experiment has been described previously in Rocker and Brandl (2015). Briefly, this study was conducted at two sites within the Burdekin region of the central sector of Australia's Great Barrier Reef (GBR) from February to June 2013. Twenty partial colonies of the coral *Acropora tenuis* were collected from Geoffrey Bay (GB; Magnetic Island, Australia; S19°09.264′ E146°52.083′; ~ eight km offshore) and nineteen partial colonies were collected from the northwest, leeward corner of Pelorus Island (PI; Palm Island Group, Australia; S18°32.435′ E146°29.326′; ~ sixteen km offshore; Fig. 1). *A. tenuis* is a relatively hardy, shallow water coral that is commonly found at both GB and PI (Rocker et al., 2017). All colonies were therefore collected from two - four m depth at both sites and held on board the research vessel to control for transplantation/handling stress. Coral colonies were halved to produce genetically identical fragments approximately ten × ten cm^2; one genetically identical fragment was left at the native site (source population) and the second was moved to the novel site (transplant location; Fig. 1). Small colonies were used to minimize intra-colony variation due to potential changes associated with spawning and reproduction (Conlan et al., 2018; Figueiredo et al., 2012). Coral fragments were then haphazardly mounted onto a single wire-mesh rack following Berkelmans and van Oppen (2006) at each site at two m depth.

Corals at these two sites typically experience differences in water quality in particulates and dissolved nutrients (Fig. 2; Rocker et al., 2017), with PI defined as 'very good' water quality and GB defined as 'moderate' water quality (Thompson et al., 2014). GB is generally characterized by high turbidity levels (due to total suspended solids and particulates; Fig. 2), as it is closer to the coast and in a shallow bay adjacent to a large suburb. Furthermore, dissolved nutrients are also generally higher at GB (Fig. 2).

The experiment commenced in early February 2013 and was inspected after two weeks (mid-February 2013) to quantify stress (e.g., bleaching) and mortality (e.g., tissue sloughing) from experimental handling and transplantation. Initial assessment after two weeks found no indicators of stress or mortality (Rocker and Brandl, 2015). Final sampling occurred after four months, in June 2013, for both fatty acid (FA) composition (i.e., percentage of total FA and absolute concentrations) and gene expression (GE) analysis. For treatments, one branch (two - six cm long) was collected from the middle of each colony at each site, at each sampling time point, and used for both FA and GE analyses. As partial mortality (missing branches likely from selective predation) occurred on corals transplanted from GB to PI (assessed in Rocker and Brandl, 2015), smaller samples (< two cm long branches) were collected for genetic analyses from ten GB-to-PI transplants in June 2013; samples from only five of these transplants provided sufficient material for biochemical analyses. Samples were immediately snap-frozen in liquid nitrogen (−80 °C) for further analyses.

2.2. Quantification of ash-free dry weight, total lipid content, and fatty acid concentration

Ash-free dry weight and total lipid content were determined following Rocker et al. (2017). Biochemical analyses were conducted on twenty corals from GB at GB, five corals from GB at PI, nineteen corals from PI at GB, and fifteen corals from PI at PI. All corals were sampled after four months of exposure to novel and native environmental conditions. Briefly, samples were crushed in liquid nitrogen, freeze-dried for 48 h, and sub-sampled for total lipid and subsequent fatty acid (~90%) and ash-free dry weight (~10%) analyses. Ash-free dry weight aliquots were incinerated in a muffle furnace for 12 h at 475 °C, placed in a desiccator and subsequently weighed following Fitt et al. (2000). Total lipids were extracted using dichloromethane:methanol (2:1) extractions, washed and partitioned with 3:1 0.44% KCl:methanol, recovered from the lower phase, and dried under nitrogen following Conlan et al. (2018). Total lipid extracts were then weighed and standardized to dry weight (mg lipid g dw^{-1}).

Fatty acid concentration was determined following Rocker et al. (in review). Briefly, following total lipid extraction, fatty acids were esterified using acid catalyzed methylation (Christie, 2003), 100 uL internal standard C23:0 (0.75 mg mL^{-1}) was added to the extracts, and then extracts were analyzed with gas chromatography (Agilent Technologies 7890A, USA; following Conlan et al. (2018). FA content was standardized to weight of total lipid content and expressed as mg FA g $lipid^{-1}$ for quantitative comparisons.

2.3. Global coral gene expression

Tag-based RNA-Seq libraries were prepared following Meyer et al. (2011), with modifications for sequencing on the Illumina HiSeq 2500 platform. This methodology allows for deep sequencing and quantitative analyses of short cDNA reads in organisms without a reference genome (Meyer et al., 2011). The tag-based approach utilizes small quantities of initial RNA, efficiently uses sequencing coverage and only requires an assembled transcriptome as a reference database (Meyer et al., 2011). Furthermore, this tag-based RNA-Seq method outperforms traditional RNA-sequencing for a fraction of the cost (Lohman et al., 2016).

Total RNA was extracted from ten genotypes from GB (i.e., ten fragments at GB and the ten genetically identical fragments at PI) and eleven genotypes from PI (eleven fragments at PI and the eleven genetically identical fragments at GB). All 21 genotypes were sampled after four months of exposure to novel and native environmental conditions ($n = 42$). Total RNA was extracted from the 42 total samples with an Aurum™ Total RNA Mini Kit (Bio-Rad Laboratories, Hercules, California) following the manufacturer's protocol, with the modification that diluted DNase I was incubated on the filter membrane at room temperature for one hour. The concentration and purity of RNA were determined in each sample using Nanodrop 2000 (ThermoScientific) and a 1% agarose gel to confirm two distinct ribosomal RNA bands. Samples were diluted to 100 ng μL^{-1} in Aurum™ Elution Solution (Bio-Rad, Hercules, California) for further analysis.

Sample preparation followed protocols from Meyer et al. (2011) and updates on the Matz website (http://www.bio.utexas.edu/research/matz_lab/matzlab/Methods.html, released for unrestricted use prior to this manuscript). Briefly, one mg of RNA per sample was fragmented at 95 °C for 12 min. Extent of RNA fragmentation was determined by a smear in the ribosomal RNA band region from 1.0 uL (~ 100 ng) of product on a 1% agarose gel (30 min at 100 V). Oligonucleotide 3ILL-30TV (10 μM) primer was incorporated by incubating the fragmented RNA product and primer at 65 °C for three minutes, followed by reverse transcription to single strand cDNA in 10.0 μL reactions containing 1 X SMARTScribe Reverse Transcriptase (Clontech, Mountain View, California) and S-ILL-swMW 10 μM RNA oligonucleotide incubated at 42 °C for one hour and 65 °C for 15 min. Samples of first strand cDNA then underwent amplification to enrich gene concentrations in 50.0 μL reactions containing 1 X Titanium Taq Polymerase (Clontech, Mountain View, California), 10 μM 5ILL oligonucleotide, 10 μM 3ILL-30TV oligonucleotide and 100.0 ng first strand cDNA product. The amplification profile consisted of five minutes at 95 °C and 16 cycles of 40 s at 95 °C, two minutes at 60 °C, and one minute at 72 °C. The cDNA products were treated with PCR Kleen Spin Columns (Bio-Rad Laboratories, Hercules, California) following the manufacturer's protocol to remove unincorporated dNTPs, primers and short primer-dimers.

Clean cDNA products were diluted to 5.0 ng μL^{-1} in 10 mM tris HCl pH 8 prior to barcode labeling for sample identification. Sample barcoding mix was prepared by combining 10 X PCR buffer, 10 μM TrueSeqMpx2n Illumina Universal oligo, 1 μM barcode oligo, 1 X Titanium Taq Polymerase (Clontech, Mountain View, California), and

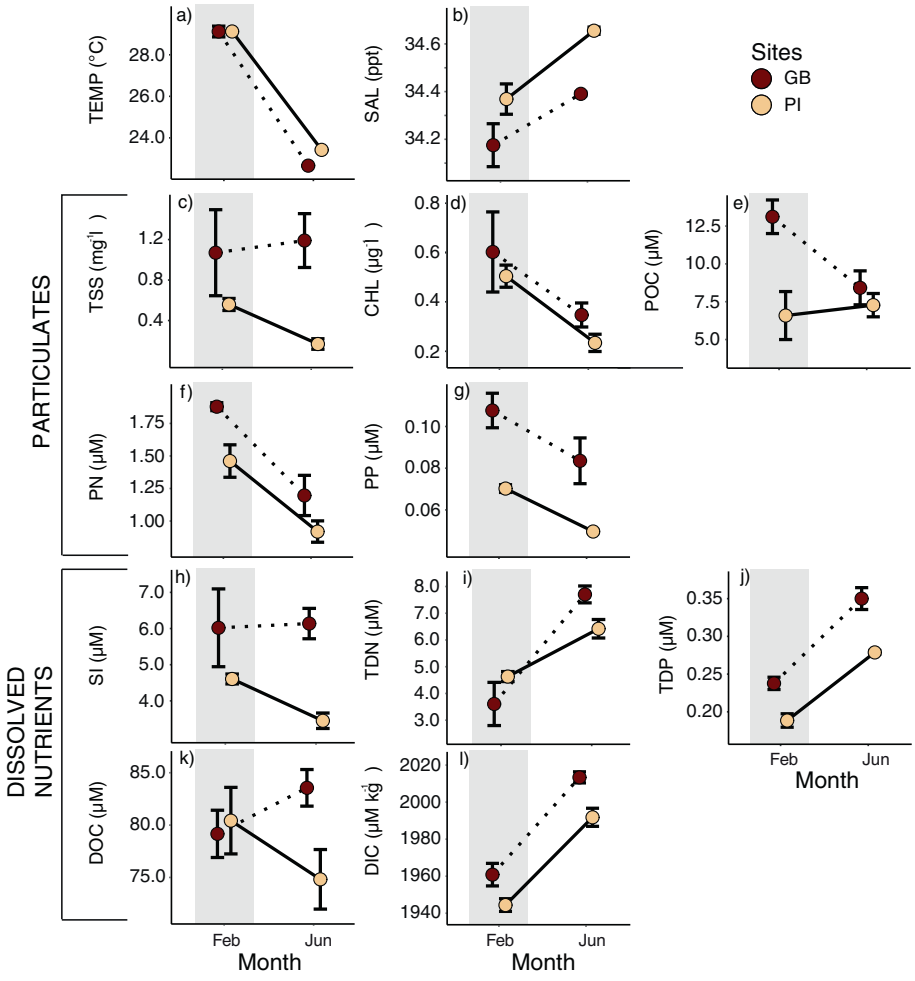

Fig. 2. Variation in environmental water quality parameters at Geoffrey Bay and Pelorus Island. Data points are means ± SE of samples collected in February and June 2013. Environmental water quality parameters include: (a) Temperature (°C); (b) Salinity (ppt); (c) Total Suspended Solids (mg l^{-1}); (d) Chlorophyll a (µg l^{-1}); (e) Particulate Organic Carbon (µM); (f) Particulate Nitrogen (µM); (g) Particulate Phosphorus (µM); (h) Silicate (µM); (i) Total Dissolved Nitrogen (µM); (j) Total Dissolved Phosphorus (µM); (k) Dissolved Organic Carbon (µM); (l) Dissolved Inorganic Carbon (µM kg^{-1}). Wet season is indicated by grey panels.

50 ng cDNA template. The cycling profile was customized to include a five-minute hot start at 95 °C, four cycles of 40 s at 95 °C, two minutes of 63 °C and one minute of 72 °C. Barcoded cDNA products were run on a 2% agarose gel in 1 X TBE buffer, with SYBR Green I nucleic acid gel staining dye adding according to manufacturer's protocol for size selection (400 to 500 base pairs).

2.4. Bioinformatic analyses

A total of 42 libraries were sequenced on three lanes of the Illumina HiSeq 2500 (SE 1 × 50 base pairs) at the Genome Sequencing and Analysis Facility at the University of Texas at Austin. Samples were spread across lanes such that all combinations of source and transplant treatments were represented in each lane. On average, 9.8 million sequences were generated per library for a total of 391 million raw reads. Of these, reads without the 5'-Illumina leader sequence were discarded, and this leader was trimmed from remaining reads. The *fastx_toolkit* (http://hannonlab.cshl.edu/fastx_toolkit) was then used to trim the reads after a homopolymer run of 'A' ≥ 8 bases was encountered, retain reads with minimum sequence length of 20 bases, and quality filter them, which required a PHRED quality of at least 20 over 90% of the sequence. 3.6 million reads per sample on average remained after quality filtering. Filtered reads were mapped to the *A. tenuis* reference transcriptome (http://www.bio.utexas.edu/research/matz_lab/matzlab/Data.html, released for unrestricted use prior to this manuscript) using Bowtie 2 (Langmead and Salzberg, 2012). Overall, 61.7 million reads were mapped for all 42 samples, with 1.5 million mapped reads per sample on average. Read counts were assembled by isogroup (i.e. groups of sequences putatively originating from the same gene, or with sufficiently high sequence similarity to justify the assumption that they serve the same function) using a custom perl script (https://github.com/z0on/tag-based_RNAseq). This script discarded any PCR duplicates, which were defined as reads mapping to the same starting position in the reference and aligning with 100% identity along the length of the shorter read. Reads mapping to multiple isogroups were also disregarded. In total, 1.3 million unique reads per sample, on average, were successfully mapped to 78,000 isogroups (Table S1). Isogroups with counts < 10 in > 90% of the samples (defined as low coverage reads; Langfelder and Horvath, 2008) were removed, resulting in 25,000 isogroups remaining for statistical analysis (i.e., removal of 53,000 low coverage isogroups).

2.5. Statistical analyses

All analyses were performed using R 2.15.3 (R Core Team 2015). To test for differences in means of FA concentrations of source and transplant corals from the two populations, generalized linear mixed effect models (GLMMs) were used in the R package 'MASS' (Venables and Ripley, 2002). The model was run with explanatory fixed effects of source and transplant locations and a random effect of genotype. Corals back-transplanted to their source site (PI) were set as 'intercept' in

GLMM analyses due to low sample size (and large error margins) of GB colonies transplanted to PI. GLMMs were performed on total lipid, FA classes and individual FA concentrations using Gamma error distributions to control for normality and homogeneity of variance. FA health indicator ratios were tested with Gaussian error distributions, as assumptions of normality and homogeneity of variance were met. All model fits were assessed through examination of the distribution of Pearson residuals and fitted residuals in diagnostic plots.

Variation in FA percentage composition of corals among source populations and transplant locations were characterized with multivariate principal component analyses (PCA) using the package 'vegan' (Oksanen et al., 2015). Analyses of variance (ANOVAs) were performed on the four principal components considered important (i.e., when standard deviations were > 1.0), with two, fully crossed fixed factors – source population and transplant location.

Gene expression patterns of *A. tenuis* were *regularized logarithm* (rlog) transformed, in order to shrink together values of different samples for genes with low counts, while $\log_2$ transforming genes with high counts, using the package 'DESeq2' (Anders and Huber, 2010). GE expression patterns were analyzed in R using the 'WGCNA' package following methods in (Langfelder and Horvath, 2008) and 'DESeq2' package following methods in (Love et al., 2014). 'WGCNA' allows for analyses of large, high dimensional data sets, such as global gene expression, and correlates gene expression data with multiple traits of the samples (i.e. coral phenotypic and health attributes; Langfelder and Horvath, 2008; Wright et al., 2015). Furthermore, 'categorical' traits or traits that are defined as experimental conditions (i.e., source populations, transplant location, etc.) can also be included and assessed through 'WGNCA', providing an alternative to traditional 'DESeq' analyses. One outlying sample (P15.2; coral sourced from PI and transplanted to GB) was detected from a sample network with a standardized connectivity score of < -2.5 and removed from further analyses (Fig. S1). Remaining samples were not considered outliers and hence retained in further analyses. A signed co-expression network was constructed using Pearson correlations of all genes across all treatments. Similarities in expression were transformed into connection strengths using a soft threshold power of 13, based on scale-free topology fit index. Linkage hierarchical clustering and a topological overlap matrix were used to identify groups of genes (or network modules) with highly positive correlations of relative expression levels. Modules with > 85% similar expression profiles and < 30 genes were merged. Coral source and transplant locations, coral colony area change (from Rocker and Brandl (2015)), individual FA concentrations and FA health indicator ratios were correlated to the uniquely identified co-expression modules. Gene ontology (GO) enrichment was performed on modules with significant correlations to coral attributes. If no significant GO terms were detected, differential expression of individual genes within the modules was examined directly.

3. Results

3.1. Fatty acid composition of source and transplant colonies of Acropora tenuis

A PCA of the FA percentage compositions of reciprocally transplanted colonies of *Acropora tenuis* identified four important principal components that best represented variation in 17 individual FA and explained 51.5, 13.6, 11.1 and 6.0% of the variance, respectively (Fig. 3; Table 1; Fig. S2; Table S2). Source populations separated along PC1, with GB-sourced corals generally having negative PC1 scores and PI-sourced corals generally having positive PC1 scores (Fig. 3). Overall, GB-sourced corals were defined by higher percentages of FA 14:0, 16:0, 16:1n-7, 18:1n-9, 18:2n-6, 18:3n-6, 20:3n-6, 18:4n-3 and 22:6n-3 (negative PC1 values; Fig. 3). In contrast, PI-sourced corals were defined by higher percentages of FA 17:0, 18:0, 21:0, 20:1n-9, 20:5n-3, 22:5n-3, 20:4n-6 and 22:4n-6 (positive PC1 values; Fig. 3). Although significant

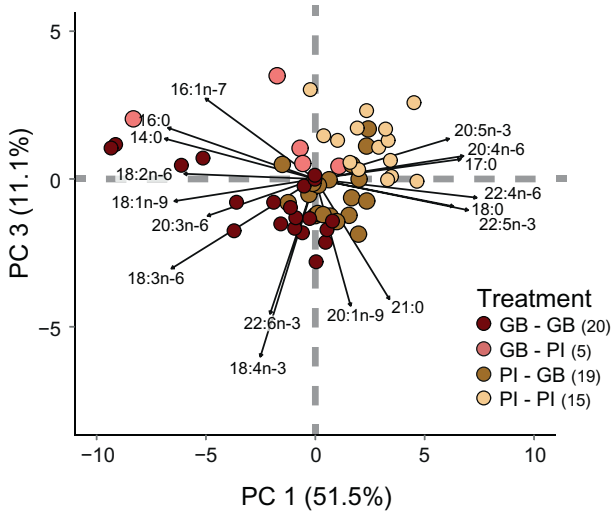

Fig. 3. Biplot of principal component analysis of 17 FA measured in coral colonies reciprocally transplanted between Geoffrey Bay (GB) and Pelorus Island (PI), located in the Burdekin region, in June 2013. PC1 and PC3 are plotted as indicators of source and transplant effects, respectively. FA are expressed as percentage composition of total FA. Lettering for treatments denote 'source population – transplant location'. Sample sizes for FA analyses are in parentheses.

Table 1
Standard deviation and proportion of variance explained in a principal component analysis (PCA) of the percentage composition of 17 FA quantified in *Acropora tenuis* reciprocally transplanted between GB and PI within the Burdekin region. * denotes important principal components defined by > 1.0 standard deviation.

Importance of components				
	PC1	*PC2*	*PC3*	*PC4*
Standard deviation	2.96 *	1.52*	1.38*	1.01*
Proportion of variance	0.515	0.136	0.111	0.060
Cumulative proportion	0.515	0.651	0.762	0.823

variance was found to attribute to PC2 (13.6%; Table 5.1), no significant effects, of source, transplant, nor their interaction, on FA percentage composition were found along PC2 (Fig. S2; Table S2). PC3 separated variation attributable to significant source and transplant effects (Fig. 3; Table S2). Generally, coral colonies transplanted to the GB site had negative PC 3 scores, whereas coral colonies transplanted to the PI site had positive PC3 scores. FA that characterized corals transplanted to the more turbid GB environment were 18:0, 21:0, 18:1n-9, 20:1n-9, 18:3n-6, 20:3n-6, 22:4n-6, 18:4n-3, 22:5n-3 and 22:6n-3 (negative PC3 values; Fig. 3). Coral colonies transplanted to the clear water PI environment were defined by 14:0, 16:0, 17:0, 16:1n-7, 18:2n-6, 20:4n-6 and 20:5n-3 (positive PC3 values; Fig. 3).

Significant effects of transplantation to a novel environment were found, despite the large standard errors (SE) associated with mean FA concentrations of the GB to PI transplants. SE's in this treatment are large because of the small number of samples retrieved as consequence of selective predation on this group of corals (see (Rocker and Brandl, 2015)); Fig. 4; Table 2; Fig. S3; Fig. S4; Fig. S5; Table S3). In general, coral transplants adjusted their FA concentrations to local concentrations, with most FA classes significantly higher at PI than GB (Fig. 3a-f). In contrast, short-chain (C18) polyunsaturated fatty acids (SC PUFA), which can be broken down into short-chain omega-3 polyunsaturated fatty acids (SC n-3 PUFA) and short-chain omega-6 polyunsaturated fatty acids (SC n-6 PUFA), displayed source by transplant effects that were significant in two comparisons (Table 2). In these latter two cases,

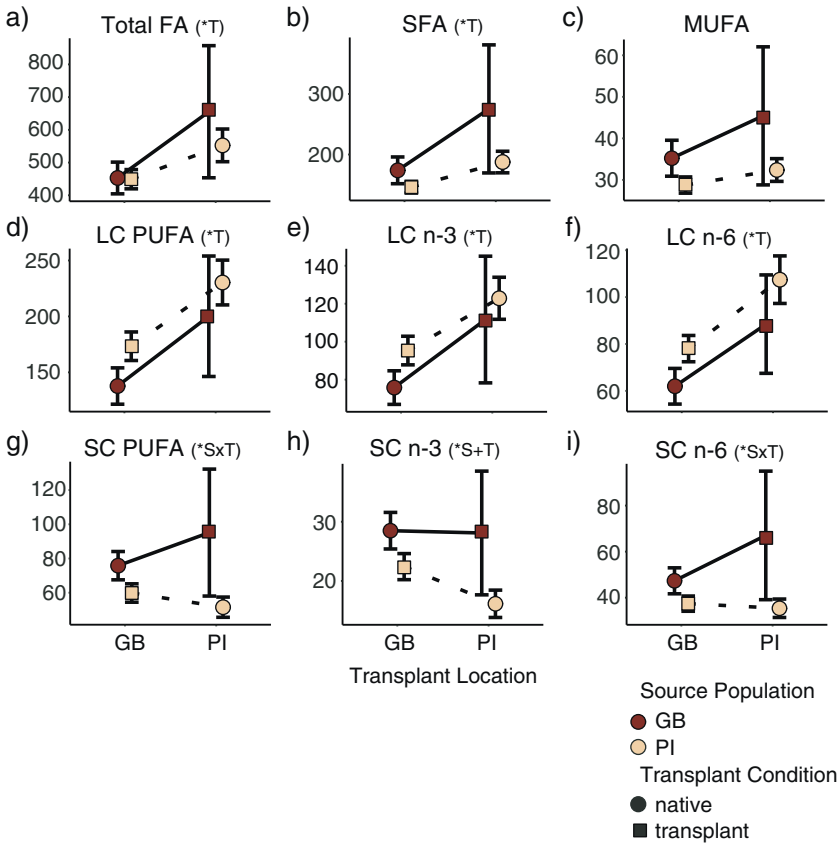

Fig. 4. Mean concentrations (± SE) of total FA and eight FA classes for corals reciprocally transplanted between GB (Geoffrey Bay) and PI (Pelorus Island) in June 2013. Source population is indicated by the color of the symbols: GB (dark red) and PI (light yellow). Transplant treatment is indicated by the shape of symbols: native (O) and transplant (□). Concentrations of FA classes are standardized to mg FA g lipid^{-1}. Sample sizes for FA analyses: native GB (n = 20), transplanted GB (n = 5), native PI (n = 15) and transplanted PI (n = 19). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

mean concentrations of SC PUFA and SC n-6 PUFA were greater at PI for corals sourced from moderate water quality (GB-sourced corals), but greater at GB for more clear-water corals (PI-sourced corals; Fig. 4g, i but not 4 h).

3.2. Correlations between gene expression modules and coral attributes

Seventeen unique groups of genes or network modules were identified in linkage hierarchical clustering ('WGCNA' analysis; modules correspond to the 17 colors listed vertically in Fig. 5). Of these, 12 modules were correlated with a range of coral attributes and experimental parameters, specifically source population or transplant location, principal components of FA analyses, colony growth (total change in area), and concentrations of individual FA (Fig. 5; Fig. S6). According to DESeq analyses, 5873 genes had a significant origin effect, no genes had a significant transplant effect, and 75 genes had an interactive origin × transplant effect (Fig. S7). This analysis found comparable qualitative patterns to 'WGCNA' analysis.

Of the total 24,848 genes assigned to the 17 unique modules (Fig. 5), 9113 genes were annotated (36.7%). Modules ranged from being 21.1% up to 55.3% annotated. Eigengenes (defined as the first principal component of a given module or a representative of the gene expression profile within a module; Langfelder and Horvath, 2008) of the black module (the seventh largest module containing 1365 genes, 29.3% of which were annotated) were strongly correlated with source population. GO (gene ontology) enrichment analysis of biological processes and cellular components indicated that the gene enrichment of this module was associated with ribosomal cellular components, macromolecule biosynthetic processing, translation, and ribosome biogenesis (Fig. 6). Genes in the black module were expressed 11.7% (0.12-fold) higher, on average, in corals sourced from GB compared to PI (Fig. 7). The pink module (63 genes) was also correlated with source population and FA 14:0, 16:0, 18:1n-9 and 18:3n-6 (Fig. 8; Fig. S8). Average GE associated with the pink module was 46.0% (0.46-fold) higher in corals sourced from GB, regardless of location (native versus novel; Fig. 8; Fig. S9).

Eigengenes of the purple module (49 genes; 46.9% annotated) were strongly correlated with transplant location and had higher expression in colonies at PI compared to colonies at GB (Fig. 9a). Additionally, the purple module was the only module to be significantly correlated with total change in area of coral colonies (Fig. 5). Average GE decreased by 12.3% (0.12-fold) in corals when exposed to GB conditions compared to PI conditions (Fig. 9a; Fig. S10). This module included genes related to mitochondrial functioning (i.e. NADH ubiquinone oxidoreductase, UDP-glucose 4-epimerase, and succinate dehydrogenase flavoprotein subunit), as well as chitinase and trypsin.

The red module (35 genes) was strongly correlated with both source population and transplant location (Fig. 5) and was 31.4% annotated. Expression was lower in GB natives and higher in PI natives, whereas both GB and PI transplants had individuals with both higher and lower expression in a novel environment compared to corals in their native environments (Fig. 9b). Corals sourced from GB had 35.2% (0.35-fold) lower average expression in their source environment when compared to PI corals in their source environment (Fig. 9b; Fig. S11). GE was 11.9% (0.12-fold) higher than the local optimum when corals were transplanted from PI to GB. Comparatively, GE was 10.0% (0.10-fold) lower than the local optimum when corals were transplanted from GB to PI. Genes within this module were related to tissue and muscle structure (collagen, immunoglobulin C-2 type and myosin heavy chain and transcription factor of HNF3 family), or associated with immunity (C-type lectin/mannose receptor, transcription factor of HNF3 family and immunoglobulin C-2 type).

Table 2
Generalized linear mixed model (GLMMs) analyses of FA class concentrations (mg FA g lipid^{-1}; June 2013 samples) to determine the effects of reciprocally transplanting colonies of *Acropora tenuis* between Burdekin sites GB and PI, with genotype considered a random effect. FA classes use a Gamma error distribution and the "intercept" parameter corresponds to the predicted response variable for native coral colonies at PI. *** denotes significance at $\alpha = 0.001$. ** denotes significance at $\alpha = 0.01$. * denotes significance at $\alpha = 0.05$.

	Estimate	Std Error	*t*-statistic	P	
Total fatty acids					
(Intercept)	0.0020	0.0002	8.185	< 0.001	***
Source (GB)	−0.0005	0.0004	−1.180	0.238	
Transplant (GB)	0.0004	0.0002	2.307	0.021	*
Source*Transplant (GB)	0.0005	0.0004	1.592	0.112	
Saturated Fatty Acids					
(Intercept)	0.0059	0.0008	7.477	< 0.001	***
Source (GB)	−0.0022	0.0013	−1.676	0.094	
Transplant (GB)	0.0016	0.0005	2.783	0.005	**
Source*Transplant (GB)	0.0020	0.0011	1.854	0.064	
Monounsaturated Fatty Acids					
(Intercept)	0.0336	0.0042	8.074	< 0.001	***
Source (GB)	−0.0109	0.0067	−1.630	0.103	
Transplant (GB)	0.0047	0.0029	1.570	0.117	
Source*Transplant (GB)	0.0095	0.0055	1.736	0.825	
Long Chain Polyunsaturated Fatty Acids					
(Intercept)	0.0048	0.0007	7.149	< 0.001	***
Source (GB)	0.0002	0.0012	0.157	0.875	
Transplant (GB)	0.0015	0.0005	2.922	0.003	**
Source*Transplant (GB)	0.0016	0.0012	1.355	0.175	
Long Chain Omega-3 Fatty Acids					
(Intercept)	0.0090	0.0013	7.039	< 0.001	***
Source (GB)	−0.0001	0.0023	−0.048	0.962	
Transplant (GB)	0.0025	0.0010	2.629	0.009	**
Source*Transplant (GB)	0.0033	0.0022	1.501	0.133	
Long Chain Omega-6 Fatty Acids					
(Intercept)	0.0103	0.0015	6.916	< 0.001	***
Source (GB)	0.0010	0.0028	0.354	0.723	
Transplant (GB)	0.0036	0.0012	3.051	0.002	**
Source*Transplant (GB)	0.0030	0.0027	1.105	0.269	
Short Chain Polyunsaturated Fatty Acids					
(Intercept)	0.0207	0.0024	8.626	< 0.001	***
Source (GB)	−0.0094	0.0036	−2.605	0.009	**
Transplant (GB)	−0.0023	0.0022	−1.060	0.289	
Source*Transplant (GB)	0.0070	0.0034	2.064	0.039	*
Short Chain Omega-3 Fatty Acids					
(Intercept)	0.0647	0.0074	8.694	< 0.001	***
Source (GB)	−0.0276	0.0111	−2.499	0.013	*
Transplant (GB)	−0.0156	0.0073	−2.150	0.032	*
Source*Transplant (GB)	0.0203	0.0108	1.882	0.060	
Short Chain Omega-6 Fatty Acids					
(Intercept)	0.0311	0.0038	8.282	< 0.001	***
Source (GB)	−0.0142	0.0058	−2.474	0.013	*
Transplant (GB)	−0.0012	0.0032	−0.393	0.695	
Source*Transplant (GB)	0.0111	0.0051	2.163	0.031	*

4. Discussion

Substantial variation in gene transcripts and fatty acid (FA) concentrations were detected in colonies of the coral *Acropora tenuis* reciprocally transplanted between two water quality regimes in the Burdekin region, i.e., between a turbid, moderate water quality environment (Geoffrey Bay; GB) and a comparatively good water quality environment (Pelorus Island; PI; see Rocker et al., 2017 for further characterization of the two water quality regimes). Analyses indicate that variation in these biochemical and molecular attributes are a function of both genetic differences associated with source populations and phenotypic responses when exposed to a new environment at novel sites, although the relative importance of these influences varied among attributes. Source population effects were stronger for transcriptomic profiles: nine gene expression (GE) modules were correlated with source population, whereas only two gene modules were correlated with transplant location. In contrast, changes in FA concentrations and overall FA composition in novel environments indicate that variation in these biochemical attributes is strongly driven by environmental conditions. The strong influence of source population on gene expression may limit the capacity of these two coral populations to acclimatize and/or adapt to future conditions. However, restrictions imposed by population-level genotypic differences may be at least partially mitigated by the capacity of corals to vary their FA metabolism in response to the surrounding environment.

4.1. Source effects on gene expression of Acropora tenuis

Effects of source populations occur when colonies transplanted to a novel environment express phenotypic responses similar to those at their native site and/or different to those of native corals in the novel environment, indicating a limited capacity of the transplants to respond to environmental change (Weis, 2010). For example, genes associated with ribosomal structures, translation and biogenesis were uniformly expressed at lower levels in corals sourced from PI (good water quality site), regardless of whether they were located in their native or the novel environment. These gene ontology (GO) categories describe basic biological functions that may be ubiquitously expressed and lack dynamic regulation (Dixon et al., 2014). Having the differential expression of these underlying biological functions defined by the source population, with limited capacity for phenotypic plasticity, could potentially underpin the slower growth rates of corals from the good water quality site (see Rocker et al. (2017)). However, down-regulation of ribosomal- and translation-related genes has been found in heat-stressed larvae of *Acropora millepora* (Dixon et al., 2015; Meyer et al., 2011), indicating that regulation of genes defining basic biological functioning can be responsive to environmental variation at an early life stage when conditions are extreme. These findings suggest that the expression of ribosomal-related genes may become fixed under local environmental conditions during the early life stages of corals examined here, or that expression is stable unless corals are exposed to more extreme environmental conditions.

The strong source effects on gene expression detected here are consistent with local adaptation leading to population genetic divergence. Gene flow may be limited or restricted among populations of broadcast-spawning corals across geographic distances like those between the two reefs studied here (~ 60 km). Limited gene flow has been found at regional scales (e.g. < 100 km; Baums et al., 2005) and at shorter distances (e.g. < 35 km) for reefs separated in a cross-shelf direction (Mackenzie et al., 2004). Interestingly, even coral populations in close proximity (separated by < 5 km) can have divergent population structures related to nuclear ribosomal genes of the coral host (Barshis et al., 2010), providing further support for findings from this study that suggest source effects associated with population-level genetic differences are limiting the dynamic responses of corals transplanted to novel water quality regimes. However, the magnitude of differential regulation between populations in this study was relatively small (~10% or 0.1-fold) compared to multiple-fold changes reported elsewhere (e.g. Barshis et al., 2013; Kenkel et al., 2014).

Expression of genes within the pink module was significantly affected by the source population. Higher expression of these genes, which are involved in developmental processes, cellular pathways and immunity (e.g. protein kinase, histone methyltransferase, and aryl hydrocarbon receptor), in corals sourced from the moderate water quality site (GB) than in corals from the good water quality site (PI) may be a response to more stressful environmental conditions at GB. Positive correlations between the expression of these genes and FA concentrations (14:0, 16:0, 18:1n-9 and 18:3n-6) suggest that gene and FA functions may be linked. FA and other lipids have been implicated in the activation and regulation of protein kinases and growth factors

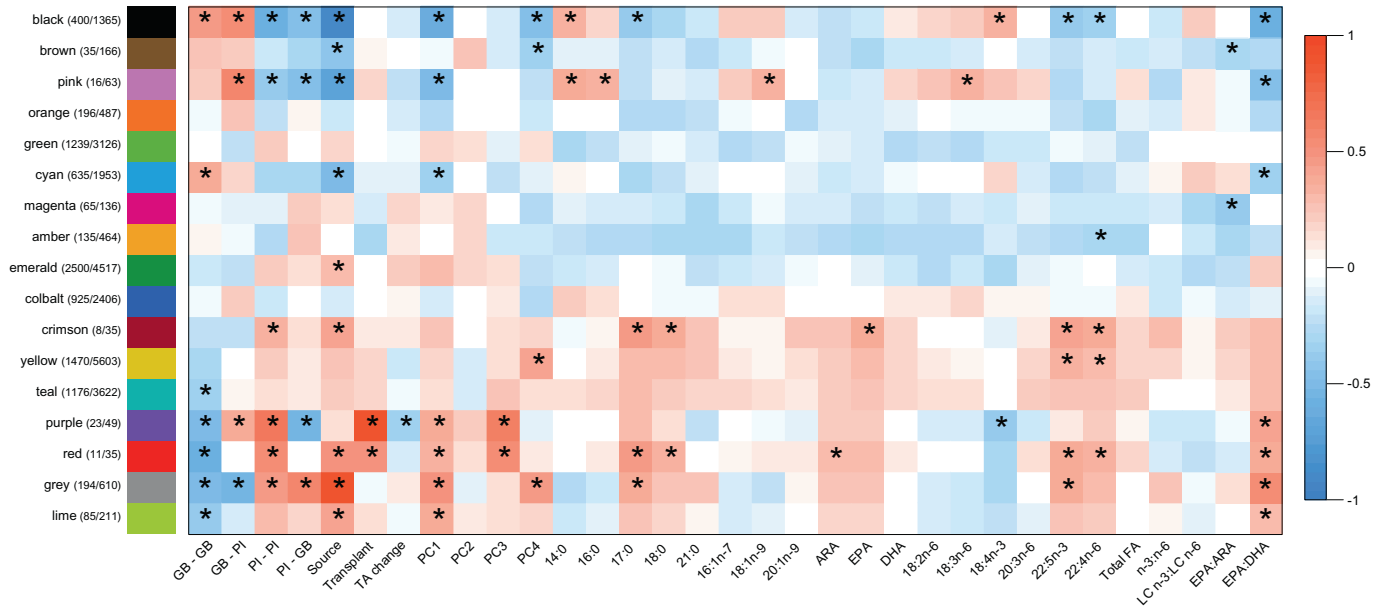

Fig. 5. Gene module – coral attribute matrix showing correlations between 17 unique gene modules (colors listed vertically) and 33 coral health attributes or experimental parameters (listed horizontally). Unique modules were determined by WGCNA analyses. Number of annotated genes and total number of genes in each module are indicated by numbers following module color, respectively. * denotes a significant correlation at $p < .05$; heatmap colors indicate the magnitude and direction of the relationship. Coral and experimental attributes include four treatments (lettering denotes 'source population – transplant location'), source and transplant effects, total area change, principal components from PCA of FA percentage composition, individual and total FA concentrations, and coral health indicator ratios.

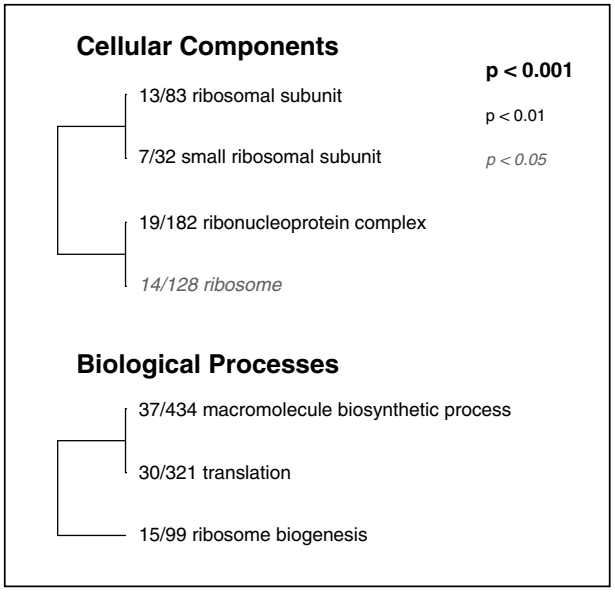

Fig. 6. Gene ontology (GO) for categories within the black module, which was significantly correlated with source effects. For reciprocally transplanted coral samples, gene enrichment was associated with cellular components and biological processes. Font type and boldness indicate the significance of the term. The fraction preceding the GO term indicates the number of genes annotated with the category that passed an unadjusted *p*-value threshold of 0.05. The trees indicate sharing of genes among GO categories. Terms on the same branch represent subsets.

(Merrill Jr and Schroeder, 1993). Furthermore, 18:3n-6 and its metabolites can affect the expression of genes associated with immune functions and apoptosis (Kapoor and Huang, 2006), such as the aryl hydrocarbon receptor (AhR). Activation of AhR is suggested to be important for immunological responses, as well as for the inhibition of inflammation (Li et al., 2011). 18:3n-6 is an omega-6 polyunsaturated fatty acid (n-6 PUFA), which decreases membrane fluidity and increase cellular inflammation (Nettleton, 1995; Sargent et al., 1990). At the moderate water quality site, genes associated with immunological responses, including AhR, may be activated to counteract the negative effects of n-6 PUFA.

Source population effects (associated with the black and pink modules) suggest macromolecule synthesis is a biological process regulated by the coral host and specific to the coral source population. The correlations found between source populations and FA 16:0 and 18:1n-9 suggest that certain populations may be more efficient in the synthesis of macromolecules. This hypothesis is further supported as FA 16:0 and 18:1n-9 are essential precursors to PUFA (Monroig et al., 2013), and invertebrates (including corals) are now known to be capable of de novo synthesis of PUFA (Kabeya et al., 2018). Future studies should aim to further elucidate the pathways and genes associated with de novo FA synthesis in the coral animal.

4.2. Effect of transplant location on coral biochemical attributes and gene expression

The reciprocal transplant design identified a number of traits that are phenotypically plastic. After four months, FA concentrations of corals transplanted to a novel environment were similar to those of natives at each site, for both transplanted populations. Total FA

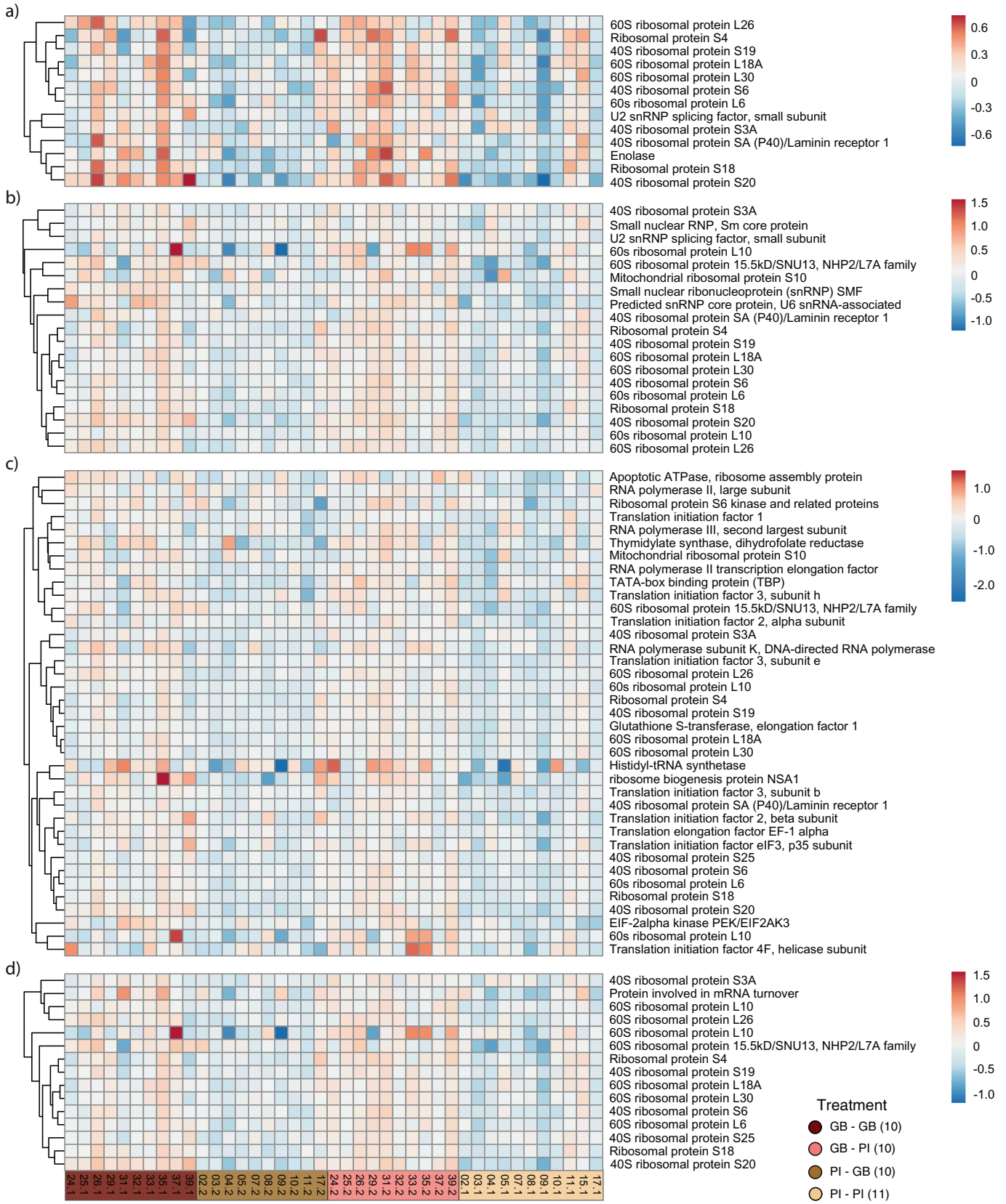

Fig. 7. Heatmaps depicting the relative expression of co-expressed genes within the black module (significantly correlated with source effects) that were enriched for cellular components and biological processes: (a) ribosomal and small ribosomal subunits; (b) ribonucleoprotein complex and ribosome; (c) macromolecule biosynthetic process and translation; and (d) ribosome biogenesis. The trees are hierarchical clustering of genes based on Pearson's correlation of their expressions across samples. Rows are genes; columns are samples ordered by treatment. Treatment is indicated by 'source population – transplant location'; sample sizes for GE analyses are in parentheses.

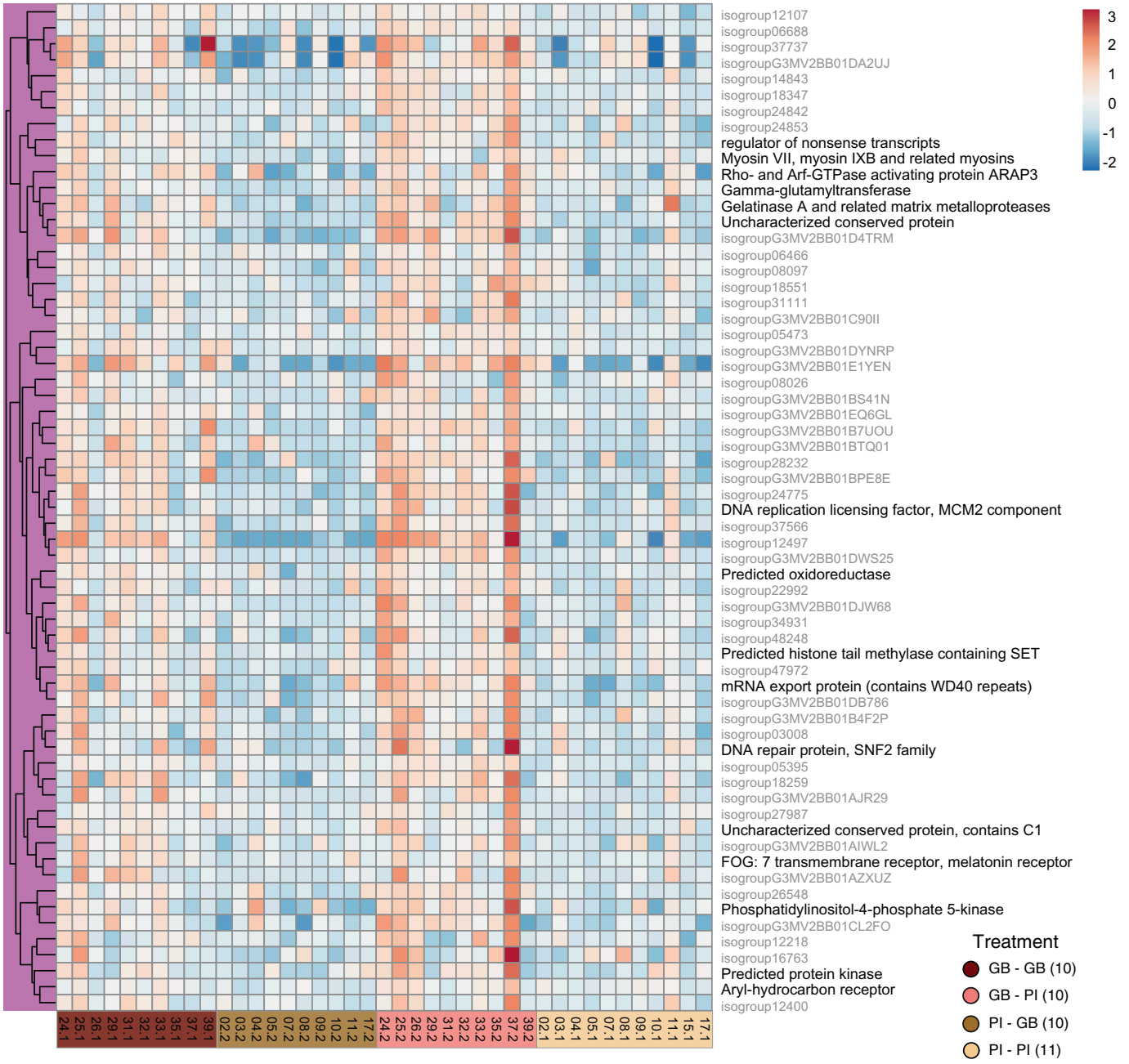

Fig. 8. Gene expression heatmap of differentially expressed genes within the pink module (significantly correlated with source effects and individual FA 14:0, 16:0, 18:1n-9 and 18:3n-6). Trees are hierarchical clustering of genes based on Pearson's correlation of their expression across samples. Columns are samples ordered by treatment. Treatment is indicated by 'source population – transplant location' and sample sizes for GE analyses are in parentheses. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

concentrations increased by ~ 20% and long chain polyunsaturated fatty acids (LC PUFA), which include essential FA, increased by ~ 30% when GB corals were transplanted to PI. Plasticity in FA concentrations enables corals to optimize their energy stores, cellular membrane characteristics and biological functioning (Francis et al., 2014; Tchernov et al., 2004). The higher concentrations of LC PUFA at PI may be a hallmark of a healthier coral holobiont population (as previously found in healthy, unbleached corals; Bachok et al., 2006) or a greater heterotrophic food supply (Conlan et al., 2017a), as these FA provide a higher level of protection against environmental stressors by maintaining cellular membrane fluidity and by protecting the photosynthetic machinery of the *Symbiodinium* (Los et al., 2013). Furthermore, 20:5n-3 (EPA) and 20:4n-6 (ARA), which are FA proposed to be involved in immune responses (Kaur et al., 2011), decreased in all corals when transplanted to a moderate water quality environment. Reefs closer to shore are considered to be more stressful environments (Fabricius et al., 2005; Kenkel et al., 2015), with reduced water quality decreasing coral fitness. Therefore, it is possible that corals exposed to deteriorated water quality are catabolizing n-3 PUFA (EPA & DHA). In terms of FA maintenance and production, corals at PI (the site further from shore with good water quality) are healthier compared to corals at the more inshore GB site with reduced water quality.

Although *Symbiodinium* communities were unable to be assessed in this study, *Symbiodinium* populations have been found to differ between these two sites (both in *Symbiodinium* abundance and type; Rocker et al., 2017). Colonies of *Acropora tenuis* at GB have significantly higher

Fig. 9. Gene expression heatmaps of differentially co-expressed genes within the (a) purple module (significantly correlated with transplant environment), and (b) red module (significantly correlated with source and transplant effects). The trees are hierarchical clustering of genes based on Pearson's correlation of their expression across samples. Columns are samples ordered by treatment. Treatment is indicated by 'source population – transplant location' and sample size for GE analyses is in parentheses. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

symbiont densities dominated by type C1, while colonies at PI have lower symbiont densities with either type C2 or mixed C1 and C2 (Rocker et al., 2017). Differences in coral holobiont FA concentrations may be attributable to this variation in symbiont communities; however, > 50% of coral holobiont FA can be found within the skeleton (Conlan et al., 2017) suggesting the coral animal tissues (within and surrounding the skeleton) contribute the majority of FA to the coral holobiont. Furthermore, FA concentrations generally decreased in corals at GB (the site where highest density symbiont populations were found) compared to corals at PI (Rocker et al., 2017), further supporting population-specific differences in symbiont communities, as well as in coral animal tissues.

Interestingly, colony morphology differed between the two source populations, likely as a consequence of differences in colony extension rates and skeletal density. Corals from GB were significantly less dense and extended faster than corals from PI (Rocker et al., 2017). However, comparisons of changes in these biochemical attributes between transplants from the two sites were unable to be conducted, as corals from GB were affected by selective predation when transplanted to PI (Rocker and Brandl, 2015); instead mean change in coral colony area was compared between the transplant groups. The purple module was the only gene module that was correlated with change in colony area, and one of two modules correlated with transplant site, suggesting that this gene module was the only one to be affected by the predation event. Genes in the purple module had higher relative expression, by an average of 12.5%, within transplants outgrown at PI compared to transplants outgrown at GB. Corals native to PI had 15.4% (0.15-fold) higher expression compared to corals native to GB. Two genes, chitinase and trypsin, had higher expression within corals at PI. Both these genes are inherent to digestive processes (Dahiya et al., 2006; Rawlings and Barrett, 1994) and have implications for defense and symbiotic interactions within corals (Harvell et al., 2007; Wood-Charlson et al., 2006). Higher expression of these genes could indicate higher levels of control over *Symbiodinium* populations within coral hosts at the good water quality site (PI), as well as greater functional capacity to break down nutritional sources. Genes related to mitochondrial functioning (i.e. NADH ubiquinone oxidoreductase, UDP-glucose 4-epimerase, and succinate dehydrogenase flavoprotein subunit), which are suggested to have a role in stress tolerance (Dixon et al., 2015), also had higher expression in corals outgrown at PI. Overall, transplant effects detected in this study suggest higher molecular and biochemical performance, leading to increased fitness, in corals exposed to cleaner water quality, regardless of origin.

4.3. Interactive and additive effects on gene expression and fatty acid composition

SC PUFA, including SC n-6 PUFA, and a single module (red) were significantly correlated with both source population and transplant location. Of the 35 genes in the red module, only 11 were annotated. GE was 12% higher than the local optimum in corals transplanted from PI to GB and 10% lower than the local optimum in corals transplanted from GB to PI. Annotations were related to tissue and muscle structure (i.e. collagens, immunoglobulin C-2 type, myosin heavy chain and transcription factor of HNF3 family) and immunity (i.e. C-type lectin/mannose receptor, transcription factor of HNF3 family, immunoglobulin C-2 type). Collagens are the main structural protein in connective tissues within animal bodies (Exposito et al., 2008). Corals both originating from, and transplanted to, a good water quality environment exhibited higher expression of collagens and other genes annotated as having a role in tissue and muscle structure. Higher expression of these genes at PI could be interpreted as higher investment in the structural integrity of coral host tissues when water quality regimes are comparatively good. Contrary to these results, other studies have reported that collagen was expressed at higher levels in inshore-sourced corals compared to offshore corals (Kenkel et al., 2013) and in corals from variable environments (tide pools with highly variable temperatures; Barshis et al., 2013). However, another study found that when environmental conditions are stressful enough to cause bleaching, collagens are compromised (Moya et al., 2012), suggesting that investment in genes associated with tissue and muscular structures is strongly influenced by environmental conditions.

C-type lectin/mannose receptor and immunoglobulin C-2 type genes, which have putative roles in immunity and symbiosis, were more highly expressed in corals sourced from, and transplanted to, a cleaner water environment (PI). Lectins are commonly down-regulated in corals exposed to acute heat stress (Barshis et al., 2013; Kenkel et al., 2014) and immunoglobins are down-regulated in bleached corals (Desalvo et al., 2008), suggesting that these genes are also be involved in pathogen and symbiont control (Jimbo et al., 2010; Kvennefors et al., 2010; Wood-Charlson et al., 2006). Immunoglobins, which were front-loaded in heat-tolerant corals in tide pools (Barshis et al., 2013), had 15.8% lower expression in PI corals transplanted to GB compared to the local optimum and 20.4% lower expression in GB corals transplanted to PI than the local optimum. This relatively higher expression suggests corals exposed to and sourced from a cleaner water quality environment (PI) may be front-loading immunity-related genes allowing for a higher stress tolerance if environmental conditions deteriorate.

5. Conclusions

Biochemical and genetic responses of the coral *A. tenuis* transplanted between two sites with different water quality regimes were a function of both their source population and the transplant environment. GE profiles demonstrated strong source population effects in genes relating to basic biological functions, consistent with either population-level genetic divergence or canalization in early development. Although local adaptation is beneficial when environments are stable, differential regulation of GE in local source populations may reduce the capacity of corals to respond to a changing environment. Plasticity in FA concentrations and specific genes relating to improved health and immunity highlight alternative pathways for corals to respond to future changes in environmental conditions. The genetic and biochemical responses of inshore corals to variation in water quality environments can aid in understanding the ability of corals to acclimatize to future deteriorated or ameliorated conditions.

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Author contributions

Conceptualization, M.R., B.W., and L.B.; Methodology, M.R., C.K., and D.F.; Formal Analysis, M.R. and C.K.; Writing-Original Draft Preparation, M.R.; Writing-Review & Editing, M.R., C.K., D.F., B.W., and L.B.; Funding Acquisition, M.R., B.W., and L.B.

Conflicts of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jembe.2018.11.004.

References

Anders, S., Huber, W., 2010. Differential expression analysis for sequence count data. Genome Biol. 11. https://doi.org/10.1186/gb-2010-11-10-r106.

Anthony, K.R.N., 2006. Enhanced energy status of corals on coastal, high-turbidity reefs. Mar. Ecol. Prog. Ser. 319, 111–116. https://doi.org/10.3354/meps319111.

Bachok, Z., Mfilinge, P., Tsuchiya, M., 2006. Characterization of fatty acid composition in healthy and bleached corals from Okinawa, Japan. Coral Reefs 25, 545–554. https://doi.org/10.1007/s00338-006-0130-9.

Barshis, D.J., Stillman, J.H., Gates, R.D., Toonen, R.J., Smith, L.W., Birkeland, C., 2010. Protein expression and genetic structure of the coral *Porites lobata* in an environmentally extreme Samoan back reef: does host genotype limit phenotypic plasticity? Mol. Ecol. 19, 1705–1720. https://doi.org/10.1111/j.1365-294X.2010.04574.x.

Barshis, D.J., Ladner, J.T., Oliver, T.A., Seneca, F.O., Traylor-Knowles, N., Palumbi, S.R., 2013. Genomic basis for coral resilience to climate change. Proc. Natl. Acad. Sci. 110, 1387–1392. https://doi.org/10.1073/pnas.1210224110.

Baumann, J., Grottoli, A.G., Hughes, A.D., Matsui, Y., 2014. Photoautotrophic and heterotrophic carbon in bleached and non-bleached coral lipid acquisition and storage. J. Exp. Mar. Bio. Ecol. 461, 469–478. https://doi.org/10.1016/j.jembe.2014.09.017.

Baums, I.B., Miller, M.W., Hellberg, M.E., 2005. Regionally isolated populations of an imperiled Caribbean coral, *Acropora palmata*. Mol. Ecol. 14, 1377–1390. https://doi.org/10.1111/j.1365-294X.2005.02489.x.

Bay, L.K., Nielsen, H.B., Jarmer, H., Seneca, F.O., van Oppen, M.J.H., 2009. Transcriptomic variation in a coral reveals pathways of clonal organisation. Mar. Genomics 2, 119–125. https://doi.org/10.1016/j.margen.2009.07.004.

Bay, L.K., Guérécheau, A., Andreakis, N., Ulstrup, K.E., Matz, M.V., 2013. Gene expression signatures of energetic acclimatisation in the reef building coral *Acropora millepora*. PLoS One 8, e61736. https://doi.org/10.1371/journal.pone.0061736.

Bergé, J.-P., Barnathan, G., 2005. Fatty acids from lipids of marine organisms: molecular biodiversity, roles as biomarkers, biologically active compounds, and economical aspects. Adv. Biochem. Eng. Biotechnol. 96, 49–125.

Berkelmans, R., 2002. Time-integrated thermal bleaching thresholds of reefs and their variation on the Great Barrier Reef. Mar. Ecol. Prog. Ser. 229, 73–82. https://doi.org/10.3354/meps229073.

Berkelmans, R., van Oppen, M.J.H., 2006. The role of zooxanthellae in the thermal tolerance of corals: a "nugget of hope" for coral reefs in an era of climate change. Proc. R. Soc. B Biol. Sci. 273, 2305–2312. https://doi.org/10.1098/rspb.2006.3567.

Brodie, J.E., Devlin, M.J., Haynes, D., Waterhouse, J., 2011. Assessment of the eutrophication status of the Great Barrier Reef lagoon (Australia). Biogeochemistry 106, 281–302. https://doi.org/10.1007/s10533-010-9542-2.

Brown, B.E., Cossins, A.R., 2011. The potential for temperature acclimatisation of reef corals in the face of climate change. In: Dubinsky, Z., Stambler, N. (Eds.), Coral Reefs: An Ecosystem in Transition. Springer Netherlands, Dordrecht, pp. 421–433. https://doi.org/10.1007/978-94-007-0114-4.

Christie, W., 2003. Isolation, separation, identification and structural analysis of lipids, in: Lipid Analysis. The Oily Press, Bridgewater.

Coles, S.L., Brown, B.E., 2003. Coral bleaching-capacity for acclimatization and adaptation. Adv. Mar. Biol. 46, 183–223. https://doi.org/10.1016/S0065-2881(03)46004-5.

Conlan, J.A., Humphrey, C.A., Severati, A., Francis, D.S., 2017a. Influence of different feeding regimes on the survival, growth, and biochemical composition of *Acropora* coral recruits. PLoS One 12, e0188568. https://doi.org/10.1371/journal.pone.0188568.

Conlan, J.A., Rocker, M.M., Francis, D.S., 2017b. A comparison of two common sample preparation techniques for lipid and fatty acid analysis in three different coral morphotypes reveals quantitative and qualitative differences. PeerJ 5, e3645. https://doi.org/10.7717/peerj.3645.

Conlan, J.A., Humphrey, C.A., Severati, A., Francis, D.S., 2018. Intra-colonial diversity in the scleractinian coral, *Acropora millepora*: identifying the nutritional gradients underlying physiological integration and compartmentalised functioning. PeerJ 6, e4239. https://doi.org/10.7717/peerj.4239.

Dahiya, N., Tewari, R., Hoondal, G.S., 2006. Biotechnological aspects of chitinolytic enzymes: a review. Appl. Microbiol. Biotechnol. 71, 773–782.

De'ath, G., Fabricius, K.E., 2010. Water quality as a regional driver of coral biodiversity and macroalgae on the Great Barrier Reef. Ecol. Appl. 20, 840–850. https://doi.org/10.1890/08-2023.1.

Desalvo, M.K., Voolstra, C.R., Sunagawa, S., Schwarz, J.A., Stillman, J.H., Coffroth, M.A., Szmant, A.M., Medina, M., 2008. Differential gene expression during thermal stress and bleaching in the Caribbean coral *Montastraea faveolata*. Mol. Ecol. 17, 3952–3971. https://doi.org/10.1111/j.1365-294X.2008.03879.x.

Dixon, G.B., Bay, L.K., Matz, M.V., 2014. Bimodal signatures of germline methylation are linked with gene expression plasticity in the coral *Acropora millepora*. BMC Genomics 15, 1–11. https://doi.org/10.1186/1471-2164-15-1109.

Dixon, G.B., Davies, S.W., Aglyamova, G.V., Meyer, E., Bay, L.K., Matz, M.V., 2015. Genomic determinants of coral heat tolerance across latitudes. Science 348, 1460–1462. https://doi.org/10.1126/science.1261224.

Donner, S.D., Skirving, W.J., Little, C.M., Oppenheimer, M., Hoegh-Guldberg, O., 2005. Global assessment of coral bleaching and required rates of adaptation under climate change. Glob. Chang. Biol. 11, 2251–2265. https://doi.org/10.1111/j.1365-2486.2005.01073.x.

Exposito, J.Y., Larroux, C., Cluzel, C., Valcourt, U., Lethias, C., Degnan, B.M., 2008. Demosponge and sea anemone fibrillar collagen diversity reveals the early emergence of A/C clades and the maintenance of the modular structure of type V/XI collagens from sponge to human. J. Biol. Chem. 283, 28226–28235. https://doi.org/10.1074/jbc.M804573200.

Fabricius, K.E., De'ath, G., McCook, L.J., Turak, E., Williams, D.M., 2005. Changes in algal, coral and fish assemblages along water quality gradients on the inshore Great Barrier Reef. Mar. Pollut. Bull. 51, 384–398. https://doi.org/10.1016/j.marpolbul.2004.10.041.

Fabricius, K.E., De'ath, G., Humphrey, C., Zagorskis, I., Schaffelke, B., 2013. Intra-annual variation in turbidity in response to terrestrial runoff on near-shore coral reefs of the Great Barrier Reef. Estuar. Coast. Shelf Sci. 116, 57–65. https://doi.org/10.1016/j.ecss.2012.03.010.

Feil, R., Fraga, M.F., 2012. Epigenetics and the environment: emerging patterns and implications. Nat. Rev. Genet. 13, 97–109. https://doi.org/10.1038/nrg3142.

Figueiredo, J., Baird, A.H., Cohen, M.F., Flot, J.F., Kamiki, T., Meziane, T., Tsuchiya, M., Yamasaki, H., 2012. Ontogenetic change in the lipid and fatty acid composition of scleractinian coral larvae. Coral Reefs 31, 613–619. https://doi.org/10.1007/s00338-012-0874-3.

Fitt, W.K., Mcfarland, F.K., Warner, M.E., Chilcoat, G.C., 2000. Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. Limnol. Oceanogr. 45, 677–685.

Francis, D.S., Salmon, M.L., Kenway, M.J., Hall, M.R., 2014. Palinurid lobster aquaculture: Nutritional progress and considerations for successful larval rearing. Rev. Aquac. 6, 180–203. https://doi.org/10.1111/raq.12040.

Harvell, D., Jordán-Dahlgren, E., Merkel, S., Rosenberg, E., Raymundo, L., Smith, G., Weil, E., Willis, B.L., 2007. Coral disease, environmental drivers, and the balance between coral and microbial associates. Oceanography 20, 172–195. https://doi.org/10.5670/oceanog.2007.91.

Howells, E.J., Berkelmans, R., van Oppen, M.J.H., Willis, B.L., Bay, L.K., 2013. Historical thermal regimes define limits to coral acclimatization. Ecology 94, 1078–1088. https://doi.org/10.1890/12-1257.1.

Hulbert, A.J., 2003. Life, death and membrane bilayers. J. Exp. Biol. 206, 2303–2311. https://doi.org/10.1242/jeb.00399.

Imbs, A.B., Dang, L.P.T., Rybin, V.G., Svetashev, V.I., 2015. Fatty acid, lipid class, and phospholipid molecular species composition of the soft coral *Xenia sp.* (Nha Trang Bay, the South China Sea, Vietnam). Lipids 50, 575–589. https://doi.org/10.1007/s11745-015-4021-0.

Jimbo, M., Yamashita, H., Koike, K., Sakai, R., Kamiya, H., 2010. Effects of lectin in the scleractinian coral *Ctenactis echinata* on symbiotic zooxanthellae. Fish. Sci. 76, 355–363. https://doi.org/10.1007/s12562-009-0204-z.

Kabeya, N., Fonseca, M.M., Ferrier, D.E.K., Navarro, J.C., Bay, L.K., Francis, D.S., Tocher, D.R., Castro, L.F.C., Monroig, Ó., 2018. Genes for de novo biosynthesis of omega-3 polyunsaturated fatty acids are widespread in animals. Sci. Adv. 4, eaar6849.

Kapoor, R., Huang, Y.S., 2006. Gamma linolenic acid: an antiinflammatory omega-6 fatty acid. Curr. Pharm. Biotechnol. 7, 531–534.

Kaur, G., Cameron-Smith, D., Garg, M., Sinclair, A.J., 2011. Docosapentaenoic acid (22:5n-3): a review of its biological effects. Prog. Lipid Res. 50, 28–34. https://doi.org/10.1016/j.plipres.2010.07.004.

Kawecki, T.J., Ebert, D., 2004. Conceptual issues in local adaptation. Ecol. Lett. 7, 1225–1241. https://doi.org/10.1111/j.1461-0248.2004.00684.x.

Kenkel, C.D., Meyer, E., Matz, M.V., 2013. Gene expression under chronic heat stress in populations of the mustard hill coral (*Porites astreoides*) from different thermal environments. Mol. Ecol. 22, 4322–4334. https://doi.org/10.1111/mec.12390.

Kenkel, C.D., Sheridan, C., Leal, M.C., Bhagooli, R., Castillo, K.D., Kurata, N., McGinty, E.S., Goulet, T.L., Matz, M.V., 2014. Diagnostic gene expression biomarkers of coral thermal stress. Mol. Ecol. Resour. 14, 667–678. https://doi.org/10.1111/1755-0998.12218.

Kenkel, C.D., Almanza, A.T., Matz, M.V., 2015. Fine-scale environmental specialization of reef-building corals might be limiting reef recovery in the Florida Keys. Ecology 96, 3197–3212. https://doi.org/10.1890/14-2297.1.

Kvennefors, E.C.E., Leggat, W., Kerr, C.C., Ainsworth, T.D., Hoegh-Guldberg, O., Barnes, A.C., 2010. Analysis of evolutionarily conserved innate immune components in coral links immunity and symbiosis. Dev. Comp. Immunol. 34, 1219–1229. https://doi.org/10.1016/j.dci.2010.06.016.

Langfelder, P., Horvath, S., 2008. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics 9. https://doi.org/10.1186/1471-2105-9-559.

Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9, 357–359. https://doi.org/10.1038/nmeth.1923.

Li, Y., Innocentin, S., Withers, D.R., Roberts, N.A., Gallagher, A.R., Grigorieva, E.F., Wilhelm, C., Veldhoen, M., 2011. Exogenous stimuli maintain intraepithelial lymphocytes via aryl hydrocarbon receptor activation. Cell 147, 629–640. https://doi.org/10.1016/j.cell.2011.09.025.

Lohman, B.K., Weber, J.N., Bolnick, D.I., 2016. Evaluation of TagSeq, a reliable low-cost alternative for RNAseq. Mol. Ecol. Resour. 16, 1315–1321. https://doi.org/10.1101/036426.

Los, D.A., Mironov, K.S., Allakhverdiev, S.I., 2013. Regulatory role of membrane fluidity in gene expression and physiological functions. Photosynth. Res. 116, 489–509. https://doi.org/10.1007/s11120-013-9823-4.

Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 1–21. https://doi.org/10.1186/s13059-014-0550-8.

Mackenzie, J.B., Munday, P.L., Willis, B.L., Miller, D.J., van Oppen, M.J.H., 2004. Unexpected patterns of genetic structuring among locations but not colour morphs in

Acropora nasuta (Cnidaria; Scleractinia). Mol. Ecol. 13, 9–20. https://doi.org/10.1046/j.1365-294X.2003.02019.x.
Merrill Jr., A.H., Schroeder, J.J., 1993. Lipid modulation of cell function. Annu. Rev. Nutr. 13, 539–559. https://doi.org/10.1146/annurev.nu.13.070193.002543.
Meyer, E., Aglyamova, G.V., Matz, M.V., 2011. Profiling gene expression responses of coral larvae (*Acropora millepora*) to elevated temperature and settlement inducers using a novel RNA-Seq procedure. Mol. Ecol. 20, 3599–3616. https://doi.org/10.1111/j.1365-294X.2011.05205.x.
Monroig, Ó., Tocher, D.R., Navarro, J.C., 2013. Biosynthesis of polyunsaturated fatty acids in marine invertebrates: recent advances in molecular mechanisms. Mar. Drugs 11, 3998–4018. https://doi.org/10.3390/md11103998.
Moya, A., Huisman, L., Ball, E.E., Hayward, D.C., Grasso, L.C., Chua, C.M., Woo, H.N., Gattuso, J.-P., Forêt, S., Miller, D.J., 2012. Whole transcriptome analysis of the coral *Acropora millepora* reveals complex responses to CO2-driven acidification during the initiation of calcification. Mol. Ecol. 21, 2440–2454. https://doi.org/10.1111/j.1365-294X.2012.05554.x.
Nettleton, J.A., 1995. Introduction to Fatty Acids, in: Omega-3 Fatty Acids and Health. Chapman & Hall, pp. 1–63.
Oksanen, J., Guillaume Blanchet, F., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2015. Vegan: Community Ecology Package.
Rawlings, N.D., Barrett, A.J., 1994. Families of serine peptidases. Methods Enzymol. 19–61.
Rocker, M.M., Brandl, S.J., 2015. Transplantation of corals into a new environment results in substantial skeletal loss in *Acropora tenuis*. Mar. Biodivers. 45, 321–326. https://doi.org/10.1007/s12526-014-0239-y.
Rocker, M.M., Noonan, S.H.C., Humphrey, C., Moya, A., Willis, B.L., Bay, L.K., 2015. Expression of calcification and metabolism-related genes in response to elevated pCO2 and temperature in the reef-building coral *Acropora millepora*. Mar. Genomics 24, 313–318. https://doi.org/10.1016/j.margen.2015.08.001.
Rocker, M.M., Francis, D.S., Fabricius, K.E., Willis, B.L., Bay, L.K., 2017. Variation in the health and biochemical condition of the coral *Acropora tenuis* along two water quality gradients on the Great Barrier Reef, Australia. Mar. Pollut. Bull. 119, 106–119. https://doi.org/10.1016/j.marpolbul.2017.03.066.
Rocker, M.M., Francis, D.S., Fabricius, K.E., Willis, B.L., Bay, L.K., in review. Temporal and spatial variation in fatty acid composition in *Acropora tenuis* corals along water quality gradients on the Great Barrier Reef, Australia.
Sanford, E., Kelly, M.W., 2011. Local adaptation in marine invertebrates. Annu. Rev. Mar. Sci. 3, 509–535. https://doi.org/10.1146/annurev-marine-120709-142756.
Sargent, J.R., Bell, M.V., Hendersen, R.J., Tocher, D.R., 1990. Polyunsaturated fatty acids in marine and terrestrial food webs. In: Nutrition, Animal, Processes, Tranport (Eds.), Mellinger, J. Karger, Basel, pp. 11–23.
Stimson, J.S., 1987. Location, quantity and rate of change in quantity of lipids in tissue of Hawaiian hermatypic corals. Bull. Mar. Sci. 41, 889–904.
Tchernov, D., Gorbunov, M.Y., de Vargas, C., Narayan Yadav, S., Milligan, A.J., Haggblom, M., Falkowski, P.G., 2004. Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. Proc. Natl. Acad. Sci. 101, 13531–13535. https://doi.org/10.1073/pnas.0402907101.
Teece, M.A., Estes, B., Gelsleichter, E., Lirman, D., 2011. Heterotrophic and autotrophic assimilation of fatty acids by two scleractinian corals, *Montastraea faveolata* and *Porites astreoides*. Limnol. Oceanogr. 56, 1285–1296. https://doi.org/10.4319/lo.2011.56.4.1285.
Thompson, A.A., Lonborg, C., Costello, P., Davidson, J., Logan, M., Furnas, M.J., Gunn, K., Liddy, M., Skuza, M., Uthicke, S., Wright, M., Zagorskis, I., Schaffelke, B., 2014. Marine Monitoring Program. Annual Report of AIMS Activities 2013–2014 - Inshore Water Quality and Coral Reef Monitoring.
Venables, W.N., Ripley, B.D., 2002. Modern Applied Statisticas with S, Fourth. Springer, New York.
Ward, S., 1995. Two patterns of energy allocation for growth, reproduction and lipid storage in the scleractinian coral *Pocillopora damicornis*. Coral Reefs 14, 87–90.
Weis, V.M., 2010. The suceptibility and resilience of corals to thermal stress: Adaptation acclimatization or both? Mol. Ecol. 19, 1515–1517. https://doi.org/10.1111/j.1365-294X.2010.04575.x.
Wood-Charlson, E.M., Hollingsworth, L.L., Krupp, D.A., Weis, V.M., 2006. Lectin/glycan interactions play a role in recognition in a coral/dinoflagellate symbiosis. Cell. Microbiol. 8, 1985–1993. https://doi.org/10.1111/j.1462-5822.2006.00765.x.
Wooldridge, S.A., 2009. Water quality and coral bleaching thresholds: Formalising the linkage for the inshore reefs of the Great Barrier Reef, Australia. Mar. Pollut. Bull. 58, 745–751. https://doi.org/10.1016/j.marpolbul.2008.12.013.
Wright, R.M., Aglyamova, G.V., Meyer, E., Matz, M.V., 2015. Gene expression associated with white syndromes in a reef building coral, *Acropora hyacinthus*. BMC Genomics 16, 1–12. https://doi.org/10.1186/s12864-015-1540-2.
Yamashiro, H., Oku, H., Higa, H., Chinen, I., Sakai, K., 1999. Composition of lipids, fatty acids and sterols in Okinawan corals. Comp. Biochem. Physiol. 122, 397–407. https://doi.org/10.1016/S0305-0491(99)00014-0.