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Variation in the transcriptional response of threatened coral larvae to elevated temperatures

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Abstract

Coral populations have declined worldwide largely due to increased sea surface temperatures. Recovery of coral populations depends in part upon larval recruitment. Many corals reproduce during the warmest time of year when further increases in temperature can lead to low fertilization rates of eggs and high larval mortality. Microarray experiments were designed to capture and assess variability in the thermal stress responses of *Acropora palmata* larvae from Puerto Rico. Transcription profiles showed a striking acceleration of normal developmental gene expression patterns with increased temperature. The transcriptional response to heat suggested rapid depletion of larval energy stores via peroxisomal lipid oxidation and included key enzymes that indicated the activation of the glyoxylate cycle. High temperature also resulted in expression differences in key developmental signalling genes including the conserved WNT pathway that is critical for pattern formation and tissue differentiation in developing embryos. Expression of these and other important developmental and thermal stress genes such as ferritin, heat shock proteins, cytoskeletal components, cell adhesion and autophagy proteins also varied among larvae derived from different parent colonies. Disruption of normal developmental and metabolic processes will have negative impacts on larval survival and dispersal as temperatures rise. However, it appears that variation in larval response to high temperature remains despite the dramatic population declines. Further research is needed to determine whether this variation is heritable or attributable to maternal effects.

Keywords: *Acropora palmata*, coral, gene by environment interactions, microarray, thermal stress

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Introduction

Recent declines in coral populations have been driven by anthropogenic impacts, disease and temperature-induced bleaching events (Dudgeon & Aronson 2010). Concurrently, a decline in the number of juvenile recruits has been observed on many Caribbean reefs (Hughes & Tanner 2000; Edmunds 2004; Williams & Miller 2008). The loss of genetic diversity resulting from these population declines, in combination with sexual recruitment failure, may limit corals' ability to adapt to a changing climate (Hoegh-Guldberg & Mumby 2007; van Woesik & Jordán-Garza 2011). Thus, it is important to understand the physiological mechanisms corals use to respond to selective forces such as high temperature, and the extent of intra-population diversity in those traits, especially in the light of severe, recent population, bottlenecks (>80% loss) in some species (Bruckner 2002).

It has been assumed that, like other tropical benthic ectotherms, corals have limited potential to adapt to rising sea surface temperatures due to low thermal stress tolerance, limited genetic variation and long generation times (Hoegh-Guldberg 1999; Somero 2010). Nevertheless, evidence from sites that have experienced recurrent bleaching suggests improved tolerance for thermal stress among surviving colonies during subsequent high temperature events (Glynn & Dcroz 1990; Maynard & Anthony 2008; Thompson & van Woesik

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2009; Guest & Baird 2012). Regardless of whether a colony's higher survival is attributable to its genes or its local environment, genotypes that survive longer can produce more larvae and continue to provide important ecosystem services including shelter (Lirman 1999) and food (coral eggs and larvae are highly preyed upon) for fish and invertebrates. These services are especially critical in the Caribbean with its low functional redundancy among coral species. However, surviving genotypes need to pass on higher stress tolerance to their offspring to contribute to a true adaptive response (Császár & Ralph 2010; Meyer & Aglyamova 2011).

Studies of genotypic variation in corals have been hampered by a lack of observable phenotypes. Further, due to the presence of intra-cellular symbionts, it is difficult to confidently attribute observed phenotypic differences to either the coral host or its endosymbiotic algae (Baums 2008). Such resolution is ultimately necessary to understand the holobiont's stress response and adaptive potential. Gene expression analysis of symbiont-free larvae provides one solution to this problem by enabling observation of many host-specific molecular phenotypes in the form of transcript abundances.

Previous studies have shown that thermal stress has a drastic and immediate effect on coral larval production including reduced fertilization, abnormal embryonic morphology, high larval mortality and a general acceleration of normal embryonic developmental patterns (Randall & Szmant 2009a,b; Voolstra & Schnetzer 2009; Heyward & Negri 2010; Polato & Voolstra 2010). These factors can result in substantial changes to planktonic larval duration and disruption of normal larval dispersal patterns (Edmunds & Gates 2001; O'Connor & Bruno 2007; Randall & Szmant 2009a,b; Shanks 2009; Weersing & Toonen 2009; Heyward & Negri 2010).

The effects of thermal stress appear to be particularly acute as coral larvae approach gastrulation (Randall & Szmant 2009a,b). During this stage, the developing embryo undergoes drastic changes including rapid cell division, differentiation of embryonic tissues and structural rearrangements associated with coordinated embryonic signalling (Hayashibara & Ohike 1997; Momose & Derelle 2008). In *Acropora palmata*, the gastrulation phase of embryonic development is associated with increased mortality even under nonstressful conditions (Randall & Szmant 2009a,b, pers. obs.). Thus, survival through this stage appears to be a bottleneck in the *A. palmata* lifecycle.

To identify transcriptional changes associated with gastrulation that are affected by temperature, and to look for evidence of intra-population variation in transcript abundance of ecologically important genes, we surveyed expression patterns of 65 454 assembled sequences representing an estimated 18 000–20 000 genes in batch cultures of *A. palmata* larvae derived from genotypically distinct parent colonies raised in common-garden aquaria.

Methods

Study species

Acropora palmata is a reef-building coral found throughout the Caribbean and Western Atlantic. This once dominant species has undergone major declines in recent decades (Aronson *et al.* 2008) resulting in its current federal listing as a threatened species (Hogarth 2006). Reproduction of *A. palmata* occurs asexually via fragmentation, or sexually via broadcast spawning. Synchronous mass spawning occurs after the full moon in late summer when gamete bundles containing eggs and sperm are released into the water column and rise to the surface where fertilization occurs (Szmant 1986). Larvae develop rapidly into planulae that exhibit weak swimming behaviour after 4–5 days and are competent to settle and metamorphose into primary polyps after approximately 1 week (Szmant 1986). *A. palmata* larvae are free of photosynthetic endosymbionts; thus, the responses observed here represent that of the coral host alone. Comparisons among species suggest that corals in the genus *Acropora* may be especially sensitive to thermal stress (Negri & Marshall 2007; DeSalvo & Sunagawa 2010).

Larval experimental treatments and RNA extraction

Gametes from multiple parent colonies were collected during the 2009 spawning event on the reef off of Rincon, Puerto Rico. Microsatellite markers were used to determine genotypic identity of each colony targeted for gamete collection (Baums & Hughes 2005). Batch cultures were generated by combining sperm and eggs from selected parent colonies. The two batch cultures used in this study (batches 2 and 5) differed in terms of the four genotypically distinct parent colonies that were used as gamete sources (two other batches derived from a subset of the batch five parents were also generated but not analysed in this study because the genotypic diversity would not have been distributed evenly among all batches). After fertilization (1 h), the zygotes were rinsed with filtered sea water and transferred to temperature-controlled aquaria held at 27 and 29 °C. Twenty-nine degree celsius was chosen as the high temperature because *A. palmata* larvae are particularly sensitive to warm sea surface temperatures (Randall & Szmant 2009a,b) and preliminary experiments at 30 °C with *A. palmata* larvae from the same site in the previous year resulted in 99% mortality within 72 h in our

culture system. Four replicates of each batch culture were kept in separate 1-L plastic containers (with mesh sides to allow for continuous water exchange) at an initial density of five larvae per mL. Replicate pairs of 1-L plastic containers containing the batch larvae were suspended in one of four 45-L polycarbonate bins at both temperatures. Water in each bin was circulated with an aquarium pump and changed daily with filtered sea water preheated to the target temperature. Temperatures were maintained within 0.2 °C (±1 SD) with ¼ hp aquarium chillers (Current USA, CA) and monitored with HOBO data loggers (Onset Co., MA).

Embryos for the microarray experiment were taken from each of the batch containers at 24, 48 and 72 h (due to high mortality in early-stage larvae, sufficient numbers of larvae from multiple replicate bins were only available for samples from batch 2 at 72 h) and preserved in RNAlater (Ambion, TX) followed by storage at −80 °C. Total RNA was extracted from approximately 30–100 larvae from each sample using the RNeasy Mini Kit (Qiagen, CA). Quality and concentration of total RNA were assessed on an Agilent 2100 Bioanalyzer to ensure that high molecular weight RNA was present. RNA extractions of sufficient quality and concentration for further analysis were obtained for two of the four replicates from each batch, treatment and time point (with the exception of the 27 °C sample from batch 2 at 48 h for which only one sample was available). The lower quality of the unused RNA extracts (RNA integrity number <5) was likely due to the presence of dead larvae with degraded nucleic acids in the sample. While mortality was not specifically quantified here, it did occur throughout the experiment in all batches resulting in fewer larvae available at later time points.

Microarray analysis

mRNA was hybridized to custom two-channel microarrays using separate loop designs to compare temperatures and batches for the 24- and 48-h time points. As only batch 2 was useable at 72 h, a dye-swap design was used to compare temperatures at that time. The loop designs were optimized to assess the batch by temperature interaction (Altman & Hua 2006). Separate channel analysis enabled comparisons between time points.

Results for a total of 18 arrays on two 12-plex slides are presented (Table S1, Supporting information). Samples from batches 3 and 4 were included on the slides and included in the overall ANOVA. However, these batches were not used for the main effect and interaction contrasts as their genotypic backgrounds were not independent of the batch 5 samples. Additional details regarding the design of the microarray probes and specifics of the conditions used for sample hybridization can be found in the online supplement. Raw probe intensities and normalized expression data are available on the NCBI Gene Expression Omnibus database (Edgar & Domrachev 2002) through GEO Series accession number GSE36983 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE36983).

Raw probe intensities were read into R for analysis in the Bioconductor package LIMMA (Smyth 2005; R_Development_Core_Team 2008). Probe intensities were background corrected and normalized within arrays using lowess normalization. Normalization between arrays was performed using the Aquantile method, which ensures that the average intensities have the same empirical distribution across arrays (Yang & Thorne 2003). This step is a prerequisite for separate channel analysis of two-colour data (Smyth 2005). Probes that did not show a normalized expression level in any sample that was greater than the mean plus 2 standard deviations of the expression of the random control probes were considered unexpressed and excluded from further analysis. This resulted in 47 705 of 135 185 probes being excluded. The remaining 87 480 probes (representing 65 466 assembled sequences from the *A. palmata* transcriptome) were analysed using separate channel analysis (G. K. Smyth & N. S. Altman, unpublished data) as implemented in LIMMA to compare between temperatures, batches and time points. Mean squared errors were computed using data from all four batches at all time and temperature treatments and were adjusted using the Empirical Bayes method in LIMMA. Contrast t-values were computed using only batches 2 and 5. For each contrast, false discovery rate (FDR) was estimated using the Qvalue package in R (Storey & Taylor 2004). Finally, a log 2-fold change (FC) cut-off of 1.5 (FC = 2.8) and a *Q*-value threshold of 0.05 were used to filter significant results.

Functional analysis

Analysis of the functions associated with differentially expressed probes was performed in two ways to overcome challenges inherent to functional analysis of data from nonmodel species obtained via electronic annotation. Single enrichment analysis of GO terms was run using the program GOEAST (Zheng & Wang 2008) with default parameters and FDR correction. The output was then trimmed of redundant and uninformative terms using a 0.5 semantic similarity score in the program REVIGO (Supek & Bošnjak 2011). The second approach made use of the Ingenuity Pathway Analysis (IPA) software (Ingenuity Systems, CA), which enables the analysis of functional pathways based on a curated database of gene interactions. Additional details regarding

functional enrichment analysis can be found in the online supplement.

Results

After filtering out unexpressed probes, 87 480 probes representing 65 454 unique assembled sequences from the *Acropora palmata* transcriptome (Polato & Vera 2011) remained for analysis. After normalization and model fitting in LIMMA, hierarchical clustering (average clustering of Euclidean distances) showed that samples clustered by time and then by temperature at 24 h, but not at 48 h (Fig. 1). Differentially expressed probes (DEPs) responding to each of the main effects of batch, temperature and time were identified. As expected based on the hierarchical clustering, change with developmental time was responsible for the greatest number of DEPs, followed by temperature, then batch (Fig. 2A). Of particular interest were the regions of intersection between the main factors (i.e. *batch* ∩ *temp*, *temp* ∩ *time*) in which probes responded to more than one factor, and genes showing significant interaction effects for *time* × *temp*, and *batch* × *temp*.

Expression differences between developmental stages

Developmental time drove the majority of expression differences observed in this experiment. A total of 4857 probes (3871 assembled sequences; Fig. 2A-C light yellow circles) showed significant expression differences between 24 h (predominantly late blastulae/early gastrulae) and 48 h larvae (gastrulae). Of these, 1034 probes also differed between temperatures (temp ∩ time; Fig. 2A green plus brown), and 155 differed between batches (batch ∩ time; Fig. 2A orange plus brown). The magnitude of expression differences in terms of fold change (FC) for DEPs between times (mean absolute FC: 28) was greater than that observed for temperature DEPs (mean, 13) or batch DEPs (mean, 30). Separating DEPs based on which time point had higher expression showed that the number of up-regulated probes at each time was roughly equivalent, with 2352 probes showing higher expression at 24 h (24 h > 48 h), and 2505 probes at 48 h (24 h < 48 h).

To distinguish between normal developmental patterns and developmental changes observed only in samples raised at high temperatures, a comparison was made of probes whose expression changed with time under the treatment (29 °C) and those identified at the control (27 °C) temperatures (Fig. 2D). Genes that showed a significant change with time at both temperatures for the batch 2 samples collected over 3 days of development (n = 1689) were also identified. Hierarchical clustering (average linkage clustering using

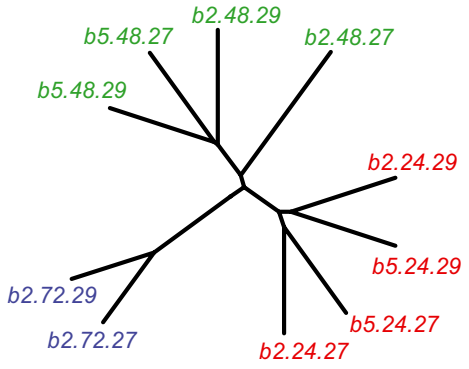

Fig. 1 Hierarchical clustering of gene expression profiles from two batch cultures of *Acropora palmata* larvae (batch 2 and batch 5) in response to temperature (27 and 29 °C) and time (24, 48 and 72 h). Clustering of expression profiles was primarily driven by developmental time. Clustering by temperature was also observed among 24-h samples. Samples are labelled by batch (2/5), time (24/48/72 in hours) and temperature (27/29 in °C).

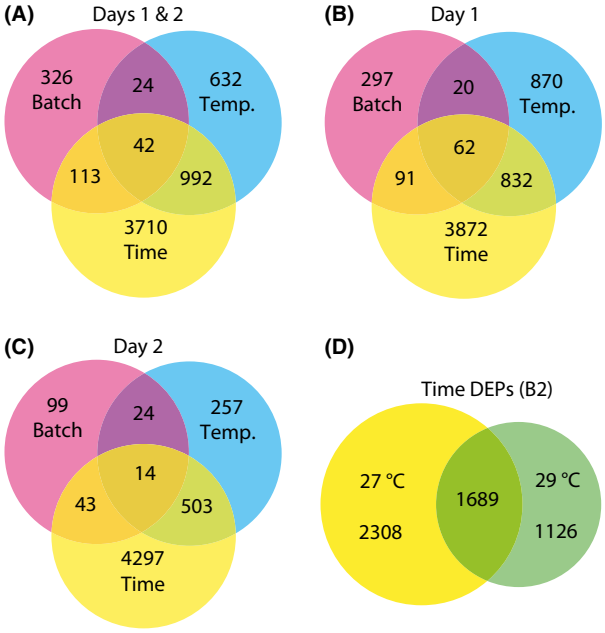

Fig. 2 Venn Diagrams showing (A) the number of differentially expressed probes in *Acropora palmata* larvae responding to each of the main effects of time, temperature and batch when both 24- and 48-h data are considered together. The number of probes responding temperature and batch differed substantially between (B) 24 h and (C) 48 h (time DEPs were calculated by comparing 24- and 48-h samples and are the same 4857 probes in A, B and C). (D) Probes that differed between time points were separated based on which temperature treatment they were observed in, by high temperature (experimental [29 °C] or and control [27 °C] samples) to distinguish between normal developmental patterns and changes observed only at the high temperature.

Spearman correlation coefficients) of treatments based on the expression profiles of these 1689 probes (Fig. 3) showed that high temperature accelerated normal patterns of gene expression in the 48-h sample. This is illustrated by the similarity of expression profiles for this set of genes between the 24- and 48-h samples at 27 °C, whereas at 29 °C, the 48- and 72-h samples were most similar. Differences between temperature treatments observed in some genes may therefore have been driven by faster development at 29 °C rather than involvement in the thermal stress response.

PAM clustering (Partitioning Around Medoids; Kaufman & Rousseeuw 1990) of the probes that changed with time over the first 3 days of development in batch 2 (Fig. 3) identified two strongly supported clusters (orange and others) of which the larger of the two could be broken into three subclusters (light blue, blue and purple). The two main clusters were characterized by a change between 24 and 72 h. The behaviour of the 48-h samples was particularly striking, however. By 48 h at 27 °C, gene expression in the orange, purple and light blue clusters is generally similar to that at 24 h, whereas at 29 °C, the expression profile at 48 h is more similar to the 72-h sample in all clusters.

Probes whose expression changed with developmental time under control conditions (27 °C) were functionally enriched for annotations including embryonic development (*notch homolog SCL, PAX, POU4F2*), *WNT* signalling (*WNT, daple-like, frizzled, δ-catenin*) and peroxisomal fatty acid oxidation (*LTP, ACOX, CAT*; Table 1). Other functions that were up-regulated with time at 27 °C, included cytoskeletal components (*ACT1, DMD, DCTN4*) and redox homeostasis genes (*GRX, TXN2, PRDX5*). Differential regulation of a variety of transcription factors (both up- and down-regulated; Table S4, Supporting information) and down-regulation of histone proteins with time were observed at both temperatures, but a subset of histones were also up-regulated with time at 29 °C only. Among other genes that changed with time at both temperatures were C-type lectins, lectin-binding proteins, red and green fluorescent proteins, and a variety of cell structure and adhesion proteins (*COL27A1, DHC, FLN, MYO, N-COL*).

Only in samples raised at the higher temperature (29 °C) were cytoplasmic ribosomal components and genes involved in RNA processing differentially regulated with time. This included down-regulation of ribosomal proteins and biogenesis factors (*BMS1*), and a variety of RNA splicing factors, tRNA and mRNA processing proteins. Genes involved in cell-cycle regulation (cyclins and cell-cycle checkpoint proteins) also showed greater differences with time at high temperatures. Interestingly, genes with pathogenesis functions were up-regulated by 48 h only in samples raised at 29 °C. These were *venom allergen-like 16 protein*, *hemolytic lectin CEL-III* and a homolog of the potent anemone toxin *PsTX-60B* (a full list of up- and down-regulated probes can be found in Table S3 (Supporting Information) dryad item: doi:10.5061/dryad.t3pr6 at http://datadryad.org/handle/10255/dryad.39350).

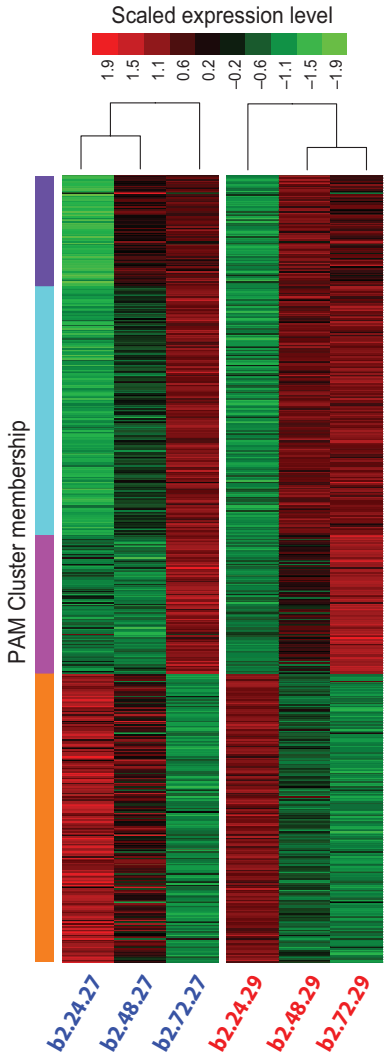

Fig. 3 Profiles of probes (shown as scaled expression coefficients) exhibiting differential expression in *Acropora palmata* larvae between time points in batch 2 samples plotted over 72 h. Samples reared at 27 °C are to the left and 29 °C to the right. The coloured bar on the far left represents the results of clustering by partitioning around medoids (PAM) at $K = 4$. Samples are labelled by batch (b2), time (24/48/72 in hours) and temperature (27/29 in °C).

Expression differences between temperature treatments

Because developmental time was associated with such dramatic change, differential expression between the temperatures was determined separately for each time

Table 1 Enriched Gene Ontology (GO) categories for probes showing differential expression with time

GO term	Time DEPs at 27 °C description	GO term	Time DEPs at 29 °C only description
Biological Process			
0002011	Morphogenesis of epithelial sheet	0006807	Nitrogen compound metabolic process
0006635	Fatty acid beta-oxidation	0031640	Killing of cells of other organism
0046395	Carboxylic acid catabolic process	0042254	Ribosome biogenesis
0006836	Neurotransmitter transport	0045446	Endothelial cell differentiation
0032501	Multicellular organismal process	0007186	G-protein coupled receptor signalling
0032502	Developmental process	0007049	Cell cycle
0044281	Small molecule metabolic process	0043170	Macromolecule metabolic process
0016055	Wnt receptor signalling pathway	0006396	RNA processing
0007166	Cell surface receptor linked signalling	0010467	Gene expression
0006334	Nucleosome assembly	0006139	Nucleic acid metabolic process
0070085	Glycosylation		
0005975	Carbohydrate metabolic process		
0006818	Hydrogen transport		
0006812	Cation transport		
0006525	Arginine metabolic process		
0006486	Protein glycosylation		
Cellular Component			
0000124	SAGA complex	0005730	Nucleolus
0031012	Extracellular matrix	0030677	Ribonuclease P complex
0043204	Perikaryon	0001669	Acrosomal vesicle
0042383	Sarcolemma	0000178	Exosome (RNase complex)
0005777	Peroxisome	0042151	Nematocyst
0000786	Nucleosome	0005634	Nucleus
0032993	Protein–DNA complex	0044422	Organelle part
0005886	Plasma membrane		
Molecular Function			
0003824	Catalytic activity	0004540	Ribonuclease activity
0015075	Ion transmembrane transporter	0008066	Glutamate receptor activity
0004364	Glutathione transferase activity	0043015	Gamma-tubulin binding
0016491	Oxidoreductase activity	0070547	L-tyrosine aminotransferase activity
0016757	Glycosyl group transferase activity	0003842	1-pyrroline-5-carboxylate dehydrogenase
0008194	UDP-glycosyltransferase activity	0005529	Sugar binding
0005326	Neurotransmitter transporter	0005509	Calcium ion binding
0008484	Sulphuric ester hydrolase activity	0003676	Nucleic acid binding
0005267	Potassium channel activity	0016706	Oxidoreductase activity on paired donors
0008889	Glycerophosphodiester - phosphodiesterase activity	0046872	Metal ion binding
		0016796	Exonuclease activity

Columns to the left show terms enriched in DEPs from control samples raised at 27 °C (Fig 2D yellow and yellow-green). Columns to the right show enriched terms from samples raised at the higher experimental temperature only (Fig 2D green). Enrichment determined with GOEAST (false discovery rate corrected $P < 0.1$) and trimmed for >0.5 semantic similarity with REVIGO. Additional redundant GO terms were removed after visual inspection.

point. Parsed this way, 1784 DEPs (1453 assembled sequences; Fig. 2B blue circle) were detected at 24 h, and 798 DEPs (632 assembled sequences; Fig. 2C blue circle) at 48 h. Only 72 probes were consistently differentially regulated between the temperatures at both time points (Table 2). Of these, 21 were down-regulated (27 °C > 29 °C) and 51 were up-regulated (27 °C < 29 °C) at the high temperature. These genes compose a core set of larval thermal stress genes, and as such could play a role in the adult response to thermal stress.

The response to high temperature at 24 h was characterized by up-regulation of heat shock proteins (*HSP70/90/97/b11, smallHSP*), oxidative stress genes (*CAT, PRDX5, GSTO1, TXN2*) and genes involved in cellular signalling. Proteins involved in cell structure and organization were also up-regulated with temperature at both 24 and 48 h. Differential expression of genes involved in protein synthesis, folding and degradation included ribosomal proteins that were up-regulated at the high temperature, and a ribosome production factor

Table 2 Genes that were differentially expressed between temperature treatments at both 24 and 48 h

Expression pattern	24 h FC	48 h FC	Description	Function
27 < 29 °C	−24.9	−33.4	Heat shock protein 70	Protein folding, heat stress response
	−18.0	−74.5	Hemolytic lectin CEL-III	Sugar binding, cytotoxicity
	−14.2	−4.3	Peptidylglycine alpha-hydroxylating monooxygenase (PHM)	Neuropeptide activation
	−9.5	−18.4	Cysteine-rich secretory protein LCCL domain-containing 2 (CRISPLD2)	LPS binding/organ development
	−7.7	−6.1	Thioredoxin 2 (Txn2-prov protein)	Redox homeostasis
	−6.7	−7.1	Choline transporter-like protein 2 (CTL2)	Pos reg of NFkB signalling
	−6.5	−5.5	LIM homeobox protein 1 (LHX1)	Embryonic tissue differentiation
	−5.6	−4.9	Putative malate dehydrogenase 1B (MDH1B)	TCA cycle
	−5.6	−9.9	Isocitrate dehydrogenase (IDH)	TCA cycle
	−4.3	−7.3	LL5 beta protein, putative	Microtubule binding, active in PI3K signalling, inhibits NFkB
	−4.0	−5.1	YALI0E34111p	Calcium binding
	−4.0	−8.9	Ypel2 protein	Cell division
	−3.7	−4.1	LOC494769 protein	Nucleic acid binding
	−3.6	−4.9	Putative 8-lipoxygenase-allene oxide synthase fusion protein	Lipid metabolism
	−3.5	−6.8	Allene oxide synthase-lipoxygenase protein	Lipid metabolism
	−3.4	−5.0	Hepatocellular carcinoma-associated antigen 127-like	
27° > 29 °C	18.3	4.2	Actin-related protein 8 (ARP8)	DNA replication and repair, organization of mitotic chromosomes
	14.2	5.7	Solute carrier family seven member 1 (SLC7A1)	Amino acid transport
	9.6	6.3	ETS activity modulator	Apoptosis, neg reg of cell cycle
	9.2	10.6	Bromodomain adjacent to zinc finger domain protein 2B (BAZ2B)	Transcription regulation
	5.0	3.9	GK21470	
	4.5	4.7	Histone H3	Nucleosome
	4.4	4.9	Fermitin family homolog 2	Cell adhesion
	3.7	6.1	Single-stranded DNA-binding protein (mssp-1)	Interacts with transcription factor MYC
	3.5	3.4	NFkB inhibitor-interacting Ras-like protein 1 (NKIRAS1)	Contributes to inhibition of NFkB
Time × Temp	20.4	−13.1	Ephrin (Efn)	Cell to cell signalling, epithelial development
	15.0	−11.2	Histone H4	Nucleosome
	13.5	<−100	Cholesterol oxidase	Cholesterol metabolism
	6.3	−10.9	Calretinin	Calcium binding
	6.2	<−100	Glucose-methanol-choline oxidoreductase	Metabolism
	6.0	−10.4	Calbindin 2, (Calretinin)	Calcium binding
	4.8	−15.3	GI18443	Calcium binding
	4.1	−4.2	C20orf4 homolog	
	4.1	−4.3	Shab	Potassium transport
	−4.2	6.4	Cys/Met metabolism PLP-dependent enzyme superfamily	Amino acid metabolism

Expression pattern indicates which temperature treatment showed higher expression levels. *Time × Temp* indicates genes that were differentially expressed in alternate directions at the different time points. Only probes with annotation data are shown. FC, Fold Change.

(*RPF1*) that was down-regulated. Histone proteins were down-regulated at high temperatures, as were lipid metabolism genes (*CHOB*, *GMC*, *LPP1*) and the important developmental signalling molecules *formin-like1*, *frizzled*, *hedgling* and *snail2*. At high temperatures, a set of enzymes involved in peroxisomal β-oxidation of fatty acids that did not all meet thresholds for statistical significance individually showed consistent patterns of up-regulation at 24 h and down-regulation at 48 h when viewed from a pathway perspective (Fig. 4; Table 3).

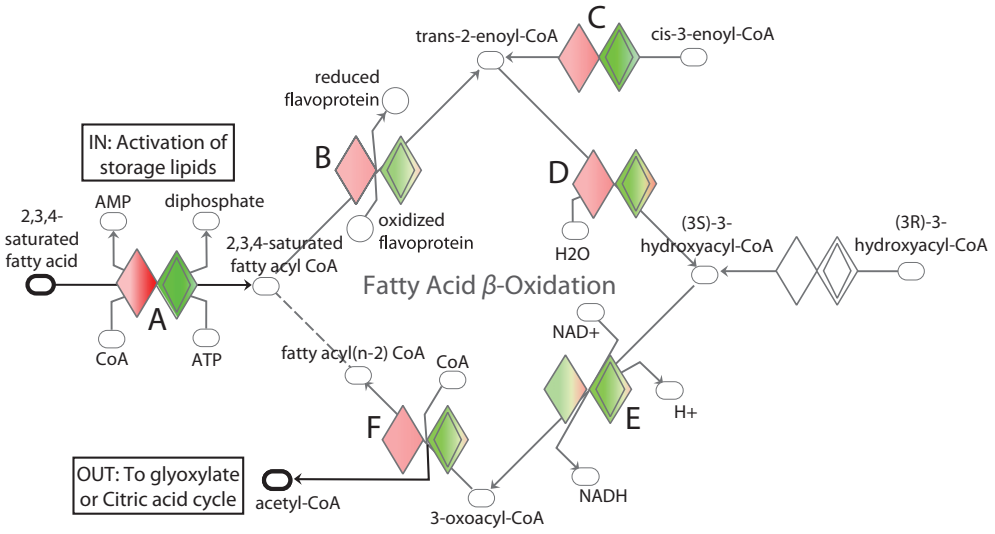

Fig. 4 Enzymes involved in fatty acid β-oxidation were almost uniformly up-regulated (red shading) with temperature at 24 h (single diamonds), and down-regulated (green shading) by 48 h (double diamonds) compared to *Acropora palmata* larvae reared at normal temperatures. This suggests that at 24 h, early mobilization of storage lipids is increased in larvae reared at high temperatures. Subsequently at 48 h, lipid metabolism slowed to a greater degree in larvae reared at high compared to normal temperature. Diamonds represent enzyme classes (see EC numbers below), thus each colour incorporates the expression levels of multiple probes, and a single probe can be associated with multiple enzyme activities. Intensity of red and green shading represents the degree of temperature induced up- or down-regulation, respectively. Sometimes, expression of individual probes varied within an enzyme class at a single time point (indicated by colour gradients). Enzymes that were not found in our data set (unshaded diamonds), other proteins (circles) and endogenous metabolic compounds (ovals) are identified. The dashed line connects the same compound in different stages of polymerization. Enzyme Commission numbers for each enzyme can be found in Table 3. Fatty acid β-oxidation pathway adapted from HumanCyc. 2000–2012 Ingenuity Systems, Inc. All rights reserved.

Table 3 Fold change of enzymes involved in the peroxisomal β-oxidation of fatty acids pathway showed generally consistent temporal patterns of up- and down-regulation with temperature (as illustrated in Fig. 4)

Enzyme class	Symbol	Entrez gene name	FC (24 h)	FC (48 h)
A	ACSBG2	Acyl-CoA synthetase bubblegum family member 2	2.87	−2.30
A	ACSL1	Acyl-CoA synthetase long-chain family member 1	2.63	−4.28
A	ACSL4	Acyl-CoA synthetase long-chain family member 4	1.18	−1.12
A	ACSL6	Acyl-CoA synthetase long-chain family member 6	**5.98**	−3.13
A	SLC27A6	Solute carrier family 27 (fatty acid transporter), member 6	1.27	−2.92
B	ACADM	Acyl-CoA dehydrogenase, C-4 to C-12 straight chain	1.56	1.48
B	IVD	Isovaleryl-CoA dehydrogenase	2.04	−1.66
C	ECI2	Enoyl-CoA delta isomerase 2	2.58	−2.81
C; D; E	EHHADH	Enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase	1.61	−1.54
D	AUH	AU RNA binding protein/enoyl-CoA hydratase	2.51	2.64
D	ECHS1	Enoyl CoA hydratase, short chain, 1, mitochondrial	2.01	3.95
D; E; F	HADHA	Trifunctional protein, alpha subunit	2.21	2.2
D; E; F	HADHB	Trifunctional protein, beta subunit	1.80	−2.18
E	HSD17B4	Hydroxysteroid (17-beta) dehydrogenase 4	−1.44	−1.62
E	HSD17B10	Hydroxysteroid (17-beta) dehydrogenase 10	−1.54	−1.49

The fold change of the probe that met the threshold for statistical significance is denoted in bold. Enzyme Commission numbers for each enzyme are: A: EC 6.2.1.3, B: EC 1.3.8.-, C: EC 5.3.3.8, D: EC 4.2.1.17, E: EC 1.1.1.35, and F: EC 2.3.1.16.

By 48 h, two of the same metabolic genes that were down-regulated at 29 °C at the previous time point were now among the most up-regulated genes at 29 °C (*CHOB*, *GMC*), while another peroxisomal enzyme (*AAO_1*) was strongly down-regulated. Similarly, two histone proteins (*H4 and H2B*) that were strongly down-regulated at 24 h were up-regulated by 48 h. The molecular chaperones *dnaJ* and *SACS* were both down-regu-

Table 4 Genes that were differentially expressed between the batches at both 24 and 48 h

Expression pattern	24 h FC	48 h FC	Description	Function
b2 > b5	>100	>100	Ferritin	Iron ion and redox homeostasis
	7.6	17.8	Chromoprotein	Pigment
	7.2	13.6	Neurotrimin	Neural cell adhesion
	54.1	7.8	Pleckstrin homology domain containing, family G (with RhoGef domain) member 5, transcript variant 1	Signal transduction
	3.6	5.4	DnaJ (Hsp40) homolog, subfamily C, member 28	Heat shock protein binding
	13.0	4.9	Putative cyclase	Enzymatic activity
	5.4	4.6	Nanor b	
b2 < b5	−4.4	−13.8	Cut-like 1 (Drosophila)	Developmental gene expression
	−4.9	−8.2	Testis-specific serine/threonine-protein kinase 6	Signalling
	−13.2	−6.5	Phosphatase, orphan 2	RNA binding
	−3.4	−5.5	Riboflavin-binding protein	Micronutrient binding/transport
	−11.1	−5.5	Pseudouridylate synthase seven homolog	Nucleic acid metabolism
	<−100	−5.3	Green Fluorescent Protein	Fluorescence
	−7.9	−4.6	UPF0399 protein v1g245966	Ribosome biogenesis
	−4.1	−4.1	Highly similar to Autophagy protein 5	Autophagy

Expression pattern indicates which batch (b2 or b5) showed higher expression levels.

lated with temperature at 48 h, as was another ribosome biogenesis protein (*BMS1*). Other molecular chaperones that were up-regulated at 24 h were no longer significantly up-regulated by 48 h, with the exception of *HSP70* which remained highly up-regulated. Consistent up-regulation was also observed for *hemolytic lectin CEL-III*.

Expression differences between genotypic backgrounds

There were 505 probes (from 440 assembled sequences) differentially expressed between the batches. For this analysis, 350 probes with differential expression confounded by development (batch ∩ time; Fig. 2 orange and brown) were included with probes responding to batch alone, so that stage-specific differences could be considered along with constitutive differences. Data from 24 h were again analysed separately from the 48-h data to control for changes of expression with time. Probes that differed between the batches were distributed between the time points with 470 probes (419 assembled sequences) at 24 h, and 180 probes (163 assembled sequences) by 48 h.

The strongest difference between the batches at 24 h was driven by higher expression (>100 FC) of a group of *ferritin* homologs in batch 2. This was accompanied by higher expression of *HSP40*, intra- and extra-cellular structural proteins, protein synthesis and degradation enzymes, and multiple genes involved in DNA replication and repair. Differences were also observed in developmental regulators (*WNT2/5a, SPRY*) and signalling molecules (*TRAF5*, *PLECKHG*, protein kinases). Up-regulated in batch 5 at 24 h were some alternative genes with similar functions including iron ion homeostasis (*ferric-chelate reductase*), signalling (*TRAF2/6*, protein kinases), and protein synthesis and degradation (*KLHL4*, *RPS3A*). Also, up-regulated in batch 5 were the autophagy gene *ATG5* and *histones H1/3/4/5*. By 48 h, several of the same genes observed at 24 h remained up-regulated, representing genes that were constitutively differentially regulated between batches through the first 2 days of development (Table 4). Homologs of *HSP70* were also expressed at much higher levels in batch 2 by this time.

Genes with significant interaction effects

Tests were conducted to detect three classes of interactions; time by temperature, batch by temperature and batch by time. Probes with significant interactions for all but batch × time were detected.

Batch × Temp. There were 85 probes (78 assembled sequences) that showed a significant interaction between batch and temperature in the 48-h samples (Table 5; no *Batch × Temp* interactions were observed at 24 h). Homologs of proteins involved in the *WNT* signalling pathway (*Daple-like*, *PFTAIRE1*), and cytoskeletal components required for cellular integrity and organization were among the genes with significant *Batch × Temp* interactions. Others included the peroxisome-associated antioxidant *catalase*, and cholesterol transport and metabolism genes (*NPC1*, *LCAT*).

Time × Temp. We found 735 probes (615 assembled sequences) that displayed a significant interaction

Table 5 Genes that showed a significant interaction between batch and temperature

Expression Pattern	Fold change	Description	Function
27 °C: b2 > b5	21.4	Catalase (CAT)	Peroxisomal oxidoreductase
29 °C: b2 < b5	19.6	Cell wall protein, putative (putative adhesin/flocculin)	Cell adhesion
	17.3	Zinc finger RNA-binding protein (ZFR)	Embryonic development/gastrulation
	17.1	PERQ amino acid-rich GYF domain-containing 1 (GIGYF1)	Regulates tyrosine kinase receptor signalling
	16.6	Striatin-3 (STRN3)	Calmodulin binding/scaffolding/signalling
	13.6	Eukaryotic translation initiation factor 3, 10 theta(EIF3)	Protein synthesis
	12.8	Trafficking protein particle complex subunit 3 (TRS3)	Vesicular transport from ER to Golgi
	10.0	Serine/threonine-protein kinase Sgk1 (EC 2.7.11.1)	Protein modification/signalling
	9.4	Schwannomin-interacting protein, putative (SCIP1)	Signalling
	9.4	Calcium/calmodulin-dependent protein kinase IV (CAMK4)	Protein modification/signalling
	9.3	Niemann-Pick C1 protein (NPC1)	Cholesterol transport
	8.8	Sodium channel and clathrin linker 1 (SCLT1)	Signalling
	8.7	Daple-like protein	Regulates WNT signalling
	8.3	Poly (ADP-ribose) polymerase family, member 14 (PARP14)	Cell proliferation
	8.0	Epsilon-tubulin (TUBE1)	Cytoskeleton
	7.9	Sorting nexin-30 (SNX30)	Intracellular trafficking
	7.8	Protein GDAP2 homolog	Regulates neuronal differentiation
	7.8	Beta-G spectrin (BGS1)	Cell membrane organization and stability
	7.5	Vinculin (VCL)	Cytoskeleton
	7.4	F-box and WD repeat domain containing 7 (FBXW7)	Protein degradation
	7.1	Lecithin:cholesterol acyltransferase family protein (LCAT)	Cholesterol metabolism
	6.9	Dystroglycan, putative (DAG)	Cytoskeleton ECM linker
	6.8	AN1-type zinc finger protein 6 (ZFAND6)	Stress response
	6.4	Transmembrane emp24 domain-containing 3 (TMED3)	Protein trafficking
	6.1	Polycomb group ring finger 3 (PCGF3)	Transcription repression
	6.0	Si:dkey-91i17.1	rRNA processing
	6.0	Cyclin-D1-binding protein 1 homolog (CCNDBP1)	Negative regulation of cell-cycle progression
	5.5	Serine/threonine-protein kinase PFTAIRE-1	Cell-cycle regulator of WNT signalling
	5.2	NADPH oxidase regulator NoxR	Regulates production of ROS by Nox
	4.9	Actin-binding protein (α-Catenin; CTNNA)	Protein binding
27 °C: b2 < b5	−18.6	LOC100125106 protein (bub1b)	Mitotic checkpoint
29 °C: b2 > b5	−8.9	Lztr-1, putative	Putative transcriptional regulator
	−6.8	MAP kinase activating protein C22orf5, putative	Response to stimulus/ signalling
	−6.1	Ribosomal protein S27 (MRPS27)	Protein synthesis
	−4.0	Reverse transcriptase-like protein (RTL)	RNA dependent DNA replication

Expression pattern indicates which batch (b2/5) showed higher expression levels at each temperature (27°/29 °C).

between developmental time and temperature. Enrichment of functional terms included cellular response to stimulus, signalling, protein metabolism and positive regulation of transcription. Signalling genes included many with calcium-binding properties. Genes involved in cholesterol and lipid metabolism were among those showing the strongest time × temp interactions, as were *C-type lectins* and *WNT* homologs 2 and 5a.

Discussion

Temperature stress presents a problem for marine ectotherms (including coral larvae) when metabolic and developmental rates rise with temperature resulting in a trade-off between physical growth and stress management (Fitt & Costley 1998; Edmunds & Gates 2001; Gillooly & Brown 2001; Gillooly & Charnov 2002; Clarke & Fraser 2004). This increase in respiration rate represents a cost for the organism as the extra energy used (derived from limited stores of maternally provisioned lipids in the case of lecithotrophic coral larvae) does not fully contribute to growth or development. Further, once temperatures reach levels that trigger a stress response, transcriptional priorities shift from expression of growth-targeted genes (i.e. ribosome biogenesis and energy metabolism) to stress response pathways (HSPs, antioxidants and damage repair) (Kültz 2005; López-Maury & Marguerat 2008). Our data showed that coral larvae growing under thermally stressful conditions experienced modulation of normal developmental rates,

and a shift from growth-targeted to stress-responsive expression profiles to balance the demands of organismal growth with protection from environmental stress.

Expression differences between developmental stages

Initially, *Acropora* embryos are maternally provisioned with many of the proteins and mRNAs required for early embryonic development (Grasso & Maindonald 2008; Schwarz & Brokstein 2008). Chromatin condensation state and histone modifications play a key role in regulation of these maternal factors during early embryonic stages (Shechter & Nicklay 2009; Lindeman & Winata 2010), thus explaining the observed changes in expression of nucleosome proteins and histone modifiers with time. At the 24-h time point, we also observed up-regulation of genes involved in cellular development, DNA replication, gene expression and embryonic tissue organization in agreement with studies of early gene expression in this and other coral species (Grasso & Maindonald 2008; Portune & Voolstra 2010). Up-regulation of genes with these functions suggests priming of the embryonic transcriptional machinery as control of transcription shifts away from maternal factors.

By 48 h post-fertilization, organized structures, such as the epithelial sheet that encloses the interior of the developing embryo, were forming (Raich & Agbunag 1999; Kraus 2006); thus cell–cell interactions became increasingly important (Table 1). Development of organized tissues is driven by the expression of genes for structural proteins and signalling molecules involved in embryonic tissue organization (Williamson & Henry 1997; Etienne-Manneville & Hall 2002). Up-regulation of several *WNT* homologs and associated pathway components (*CAD2, Frizzled 6*) indicated activation of the highly conserved *WNT* signalling pathway controlling cell proliferation, pattern formation and determination of cell fate. *WNT* is essential for initiation of gastrulation and differentiation of germ layers (Wikramanayake & Hong 2003; Nelson & Nusse 2004; Lee *et al*. 2006; Momose & Derelle 2008).

Lipid metabolism genes (*LTP, ALOX, ACSL, PECI*) were up-regulated with time in larvae raised at both control and treatment temperatures. These genes are needed to process maternally provisioned storage lipids that are the only energy source for developing *Acropora palmata* larvae. In addition to these lipid metabolism genes, several enzymes associated with the conversion of storage lipids via peroxisomal β-oxidation of fatty acids showed consistent patterns of early up-regulation followed by downregulation at high temperatures (Fig. 4; Table 3). Other enzymes related to peroxisomal β-oxidation and energy production via the glyoxylate cycle (*citrate synthase*, *isocitrate lyase*, *and malate dehydrogenase*) were also differentially expressed, or constitutively expressed at high levels (*malate synthase*) (Popov & Volvenkin 2001; Yoshihara & Hamamoto 2001). This is notable because the glyoxylate cycle is rare among higher metazoans and has been detected in only a few metazoan taxa including nematodes and anemones (Patel & McFadden 1978; Kondrashov & Koonin 2006). It is likely that the generation of reactive oxygen species (ROS) during peroxisomal β-oxidation of fatty acids also contributed to up-regulation of redox homeostasis proteins (*CAT, GRX, PRDX5, TRYX*), needed to prevent damage to cellular membranes, proteins and nucleic acids (Schrader & Fahimi 2006).

The larval transcriptional response to temperature

In contrast to the temporally stable stress response of adult corals (DeSalvo & Sunagawa 2010), the larval thermal stress response varied dramatically with time (Fig. 3) in agreement with other studies of larval corals (Rodriguez-Lanetty & Harii 2009; Portune & Voolstra 2010; Meyer & Aglyamova 2011). Such temporal variation in gene expression is a result of interactions between developmental changes in the larvae and the thermal stress response.

Comparison of developmental changes at 27 and 29 °C: Many of the DEPs that varied with time were also significantly affected by temperature. Accelerated patterns of development (Randall & Szmant 2009a,b; Baums, *et al*. 2012) and differential regulation of many important developmental genes in response to temperature (Voolstra & Schnetzer 2009; Portune & Voolstra 2010) have been previously observed in coral larvae.

This temperature-induced acceleration of normal gene expression patterns was clearly observed in the heat map of batch 2 expression profiles (Fig. 3), and the differences in the DEPs between the control (27 °C) and treatment (29 °C) samples identified between time points (Fig. 2D, Table 1). Genes with cytotoxicity functions including hemolytic *lectin CEL-III*, *Venom Allergen-like16* and homologs of the toxin *PsTX-60B*, as well as *a nematocyst outer wall antigen* changed with time only at the high temperature. *PsTX-60B* is a toxin characterized from specialized cnidarian stinging cells (nematocysts) (Satoh & Oshiro 2007). Planula larvae of some cnidarians (including other Acroporids) do possess mature nematocysts used for defence and settlement (Vandermeulen 1974; Chia & Koss 1983; Hayashibara & Ohike 1997). Our data suggest that production of nematocysts had begun in 48-h samples raised at high temperatures, but not at the control temperature. Other functional changes with time that depended on temperature included down-regulation of transcription-related genes (RNA processing and ribosomal proteins), changes in cell-cycle checkpoint proteins, cyclins and histones, all of which are mechanisms for dealing with DNA damage caused by high tempera-

ture or accumulation of ROS (Su & Gao 2004; Harper & Elledge 2007; Nesa & Hidaka 2008). Such temperature-dependent changes in gene expression patterns through time have also been shown in larvae of the congeneric species *Acropora millepora* where genes related to translation (including many ribosomal proteins) were down-regulated after 5 days of exposure to high temperature (Meyer & Aglyamova 2011).

Transcriptional response to temperature across life stages

A major goal of this study was to identify genes that were differentially regulated in response to temperature in corals. In adults, changes in gene expression with temperature are confounded by interactions with their algal symbionts. By working with symbiont-free larvae, we ensured that expression differences were directly related to temperature. However, the thermal stress response of larvae might differ from that of adult colonies because of the very different selective pressures faced by each stage of the coral's biphasic life cycle. The selective environment of a larva whose primary function is dispersal and settlement (*A. palmata* larvae possess no symbionts and do not feed) is profoundly different from that of an adult whose primary function is growth and reproduction. Thus, only a subset of the larval stress response may be relevant to the adult form. To identify genes that are most likely to represent important thermal stress responders in adult corals, we distinguished between probes that showed constitutive differences in expression levels between temperatures consistently across time points (Table 2), and probes that differed significantly between temperatures at one of the two time points (Fig. 2B and C, blue and purple).

As mentioned earlier, *A. palmata* larvae derive their energy from peroxisomal β-oxidation of storage lipids. Temperature induced expression changes of enzymes involved in peroxisomal β-oxidation (Fig. 4; Table 3), and other genes responsible for fatty acid transport and metabolism (*APOL7A*, *ACSL1*) suggested that larvae catabolized their lipid stores more rapidly at elevated temperatures. This in turn drove the need for additional redox homeostasis proteins (*PRDX5*, *TNX5*) to deal with ROS produced during oxidation of fatty acids. While we did not directly measure lipid content in the larvae used in this study, such measurements will be needed to test this hypothesis.

Mobilization of storage lipids could also play an important role in the thermal stress response of adult corals, where thermal stress can lead to the expulsion of photosynthetic endosymbionts known as bleaching (Brown 1997). A breakdown of the symbiotic partnership would require a shift from metabolism of photosynthetically derived carbohydrates to catabolism of storage lipids. Evidence for oxidative degradation of lipids in the form of lipid peroxides has been previously observed in thermally stressed adult colonies of *Montastrea faveolata* in response to high temperature (Downs & Mueller 2000), but it was unclear whether lipid oxidation in adults was a consequence of photosynthetically produced ROS acting on cellular membranes, or a metabolic shift to lipid oxidation. We propose that under starvation conditions associated with bleaching, adult corals switch to energy production derived from catabolism of storage lipids via the glyoxylate cycle. Such a phenomenon is consistent with observations of differential expression of the glyoxylate pathway gateway enzyme isocitrate lyase (DeSalvo & Sunagawa 2010), genes involved in peroxisomal fatty acid metabolism (Ganot & Moya 2011; Meyer & Aglyamova 2011) and reduced tissue biomass following the stressful conditions of peak summer heat and bleaching in this species and others (Fitt & McFarland 2000).

Studies of adult corals have also shown increases in protein chaperones, calcium signalling, DNA and protein damage response genes and a decrease in ribosomal proteins (Fang & Huang 1997; Downs & Mueller 2000; DeSalvo & Voolstra 2008), all of which were observed here. In larval and adult acroporids, expression of heat shock proteins is typically characterized by short-term up-regulation followed by a decline in expression after prolonged exposure to high temperatures (Rodriguez-Lanetty & Harii 2009; DeSalvo & Sunagawa 2010). Here, as in previous studies of coral larvae (Polato & Voolstra 2010; Portune & Voolstra 2010; Meyer & Aglyamova 2011), up-regulation of many HSPs was observed only at early time points, reflecting the complex roles of HSPs in organismal development as well as stress response (Sorensen & Kristensen 2003; Rutherford & Hirate 2007). Of note, however, was the continued up-regulation of *HSP70* by 48 h. This protein plays an important role in the development and protection from stress in mammalian embryos (Luft & Dix 1999), and it appears that such functions are conserved in developing coral larvae.

Variation in expression of multiple cytoskeletal components and cell adhesion proteins suggests that thermal stress has a drastic effect on the tissue morphology of both adults and larvae (DeSalvo & Sunagawa 2010). In adults, such a phenomenon may be explained by loosening of the mesoglea to allow for repair of damaged gastrodermal tissues after bleaching (Moya & Ganot 2012). In larvae, misshapen or disorganized individuals are more frequent at high temperatures (Negri & Marshall 2007; Randall & Szmant 2009a,b,a,b; Polato & Voolstra 2010). These morphological problems

could result from changes in embryonic tissue organization at high temperatures and may represent an example of conflicting selective pressures across life history stages where a response that is normal in the adult is pathological in larvae (Schluter & Price 1991; Moran 1994; Pechenik 1999; Crean & Monro 2011).

Expression differences between genotypic backgrounds

Variation in expression of genes important for larval development and survival in the face of thermal stress was identified between the batches observed here. However, characterizing the true population and species-wide extent of this variation, as well as the identity of genes involved will require analysis of larger number of larval cultures representing diverse genetic backgrounds. In this study, each of the eight parent colonies used was heterozygous for a majority of the five microsatellite markers analysed (mean number of alleles over all loci: 4.6; mean observed heterozygosity: 0.80), indicating little inbreeding in the source population. With such high levels of diversity, the larval batches generated here would have consisted of thousands of different genotypes. Thus, despite the small number of parent colonies used, the observation of significant differences between the batches suggests the presence of substantial intra-population variation in gene expression responses to high temperature. This is particularly remarkable considering the extent of recent population declines (Bruckner 2002).

Intra-population diversity in gene expression has been observed in other coral species in response to thermal stress (Császár & Seneca 2009; Meyer & Davies 2009; Meyer & Aglyamova 2011; Souter & Bay 2011), high light (Kenkel & Aglyamova 2011), bleaching (Seneca & Forêt 2010) and sedimentation (Bay & Ulstrup 2009). These studies identified genotypic variation in expression levels of genes involved in energy metabolism and ion transport, as well as heat shock proteins, fluorescent proteins and several transcription factors (Bay & Ulstrup 2009; Meyer & Aglyamova 2011). Targeted expression profiling also revealed genotypic variation of genes with oxidoreductase activity in response to high temperature (Császár & Seneca 2009; Souter & Bay 2011).

Our results indicated that parental effects contributed to differences among batches for expression of fluorescent proteins, energy metabolism genes, developmental regulators and transcription factors, largely consistent with the findings above. Our data showed especially strong expression differences between parental backgrounds in *ferritin* and *HSPs*. The *ferritin* protein, which controls localization of intracellular iron and iron-associated ROS production (Orino & Lehman 2001), is strongly up-regulated in response to abiotic stressors including heat (Atkinson & Blaker 1990), and shows evidence of positive selection in *A. palmata* (Schwarz & Brokstein 2008). Heritability of expression for both of these genes was found to be low in some populations of *A. millepora* (Császár & Ralph 2010). However, such context-dependent measurements can vary greatly among populations, habitats and species, and may be affected by the presence of gene-by-environment interactions, thus calling for further study of the heritabilities of gene expression in *A. palmata*.

Genotype by environment interactions. Genotype-by-environment (GxE) interactions were identified by testing for significant interactions between the factors of batch and temperature on gene expression levels. Genes showing an interaction between batch and temperature are important because they specifically represent genes where the parental backgrounds of the larvae result in different reaction norms to environmental stress (Fig. S2I, Supporting information).

The set of genes that showed a significant interaction between batch and temperature included cytoskeletal proteins, cell–cell adhesion proteins and regulators of the *WNT* signalling pathway, among others (Table 5). These important structural and developmental elements are functionally connected by the *α-catenin* (*CTNNA1*) protein, which also showed a significant GxE interaction. *α-Catenin* anchors transmembrane proteins to the underlying actin cytoskeleton, a key component of cell–cell adhesion. *α-Catenin* can also bind β-*catenin*, an essential signalling protein in the *WNT* pathway, thus acting as a suppressor of *WNT*-activated transcription (Nelson & Nusse 2004; Scott 2006). Therefore, disruption of normal *α-catenin* expression has severe consequences for developing embryos including dissociation of ectodermal cells during gastrulation, and duplication of the dorso-anterior axis (Sehgal & Gumbiner 1997). It is striking to note that both of these phenotypes, in the form of disassociating embryos and planulae with bifurcated oral pores, have been observed among *A. palmata* larvae raised at high temperatures (Randall & Szmant 2009a,b; pers. obs.). This suggests a critical role for plasticity in developmental signalling related to cell proliferation and embryonic growth in the ability of coral larvae to develop properly under thermal stress.

Conclusion

The transcriptional response of coral larvae to thermal stress represents a dynamic balance between mitigation of the harmful effects of thermal stress and maintenance of proper growth and development patterns. The dramatic acceleration of stage-specific expression profiles at

high temperature is consistent with observations of accelerated morphological development and suggests that as global sea surface temperatures rise, nonfeeding larvae will use their energy reserves faster and be competent to settle sooner. Premature depletion of energy reserves would have consequences for larval fitness and metamorphosis, and accelerated development might ultimately result in alteration of dispersal patterns with associated changes in gene flow among populations.

Expression differences in key developmental pathways, cell signalling and adhesion processes, and numerous transcription factors showed that thermal stress results in disruption of developmental processes in *Acropora palmata* larvae. Such changes during gastrulation have severe consequences for survival through this sensitive developmental phase, and high larval mortality at elevated temperatures provides a powerful selective force. This could result in rapid adaptation to high temperature if heritable genetic variation in relevant functional traits exists. Our results provide evidence that larvae from different parental backgrounds show expression differences in genes expected to directly impact performance under thermal stress conditions. Thus, despite dramatic population declines in *A. palmata*, intra-population variation in larval thermal stress tolerance remains.

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N.R.P's research uses genetic techniques to understand how environmental stress, connectivity and biogeography impact the evolution and ecology of populations. N.S.A. studies high dimensional data, multiple testing and statistical methods for bioinformatics. I.B.B. studies the ecology and evolution of deep and shallow marine invertebrates. She is interested in adaptation, dispersal, recruitment and asexual reproduction of clonal cnidarians.

Data accessibility

Microarray Data: Raw Probe intensities and normalized expression data are available on the NCBI Gene Expression Omnibus database through GEO Series accession number GSE36983 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE36983).

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Hybridization design used for the microarray experiment.

Table S2 Table of contrasts performed in Limma to determine differential expression.

Table S3 Differentially expressed genes responding to each of the main factors and their interactions (available as doi:10.5061/dryad.t3pr6 at http://datadryad.org/handle/10255/dryad.39350).

Table S4 Differentially expressed transcription factors (TFs) associated with each of the main contrasts of time, temperature and batch.

Fig. S1 Expression profiles of selected genes showing profiles characteristic of the main groupings discussed in the results.

Appendix S1 Additional details regarding the methods used for microarray development and functional enrichment analysis.