Temporal analysis of gene expression in a field population of the Scleractinian coral *Montastraea faveolata*

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Abstract

Organisms maintain homeostasis and abate cellular damage by altering gene expression. Coral colonies have been shown to produce unique gene expression patterns in response to different environmental stimuli. In order to understand these induced changes, the natural variation in expression of genetic biomarkers needs to be determined. In this study, an array of genes isolated from Scleractinian coral was used to track changes in gene expression within a population of *Montastraea faveolata* from April to October 2001 in the Florida Keys. The profiles of genes observed in this study can be divided into two groups based on expression over this time period. In spring and early summer, May through July, most of the genes show little deviation from their average level of expression. In August and September, several genes show large deviations from their average level of expression. The physiological and environmental triggers for the observed changes in gene expression have not yet been identified, but the results show that our coral stress gene array can be used to track temporal changes in gene expression in a natural coral population.

1. Introduction

It has been well documented that coral populations around the world are in serious decline (Done, 1992; Wilkinson, 2000; Lesser, 2004). Decreased recovery from bleaching events, increased susceptibility to disease, impacts on reproduction, lowered diversity and death are some of the physiological responses to factors impacting coral populations. Molecular ecology is a rapidly expanding field of biology that is concerned with applying molecular techniques to address traditional ecological questions. The use of genomic technology, such as DNA arrays, can identify coral responses to environmental change before physiological decline is evident (Snell et al., 2003). The unique expression of a specific suite of genes can provide insight into the molecular mechanisms involved in an organism's response to its environment. Edge et al. (2005) review the use of molecular genetic technology as a method to diagnose coral health.

Organisms alter the expression of specific genes in order to maintain homeostasis and abate cellular damage. For example, coral colonies produce unique gene expression patterns in response to different environmental conditions (Edge et al., 2005). In order to understand these induced changes, the natural variation in expression of genetic biomarkers needs to be investigated. While most of the gene expression studies of non-model organisms are conducted in controlled laboratory conditions, field studies are becoming more common (Wiens et al., 2000; Bais et al., 2003; Morgan et al., 2005). However, few of these studies have investigated the natural variation of gene expression within a population (Lejeusne et al., 2006). In order for gene array technology to be a useful tool for detecting population responses in coral, it needs to be determined whether changes in gene expression can be detected

above the natural variation in expression within a population over time (Klaper and Thomas, 2004).

The analysis and interpretation of changes in gene expression by Scleractinian coral may pose challenges not encountered in the study of model organisms due to their colonial morphology, ability to reproduce sexually and asexually, and the blurred distinction between species (Knowlton et al., 1997). In addition, gene expression within a single cell varies in complexity and activation (Levsky and Singer, 2003; Oleksiak et al., 2004; Raser and O'Shea, 2005). Some genes are static, exhibiting little variability in expression over time and under different environmental conditions. Other genes exhibit stochastic expression, fluctuating unpredictably over time in response to a variety of conditions. However, inducible genes fluctuate in a predictive manner in response to specific cues from the extracellular or intracellular environment. The expression patterns produced by a suite of these inducible genes incorporated onto an array can provide information on how a population responds under different conditions.

In this study, an array of genes isolated from coral and tracked changes in gene expression in a population of coral through time. Results show that the targeted DNA array can be used to detect changes in gene expression and that subsets of genes show similar patterns of expression over time. The expression profiles of some of the individual genes revealed significantly different levels of expression which were detectable above natural variation within the population of corals investigated. In addition, other genes whose expression was not detected also provided useful information about the presence or absence of some anthropogenic stressors. For example, genes responsive to organopesticides and other xenobiotics were not detected/induced at the time of sampling.

2. Materials and methods

2.1. Coral collections

Fragments of *Montastraea faveolata*, approximately 2 cm^2, were collected from five colonies at a depth of 4 m from East Turtle Shoal (24°43'15"N, 80°55'50"W) in the middle Florida Keys, USA, in 2001. Samples were collected twice a month from this inshore patch reef during a seven month period (April to October) with the exception of a single collection in late April and early October. Coral fragments were transported to the Florida Keys Marine Laboratory (FKLM) on Long Key in closed containers of natural, recirculating seawater. Samples were then processed for subsequent molecular analysis. Excess skeleton was removed with a hammer and chisel, and the samples were ground in 25–30 ml of a phenol based solution (TRIzol®, Invitrogen™) with a mortar and pestle. Homogenization in TRIzol stops cellular activity for long-term storage and preservation of samples used in molecular analyses.

2.2. Environmental data

Environmental parameters including ocean temperature (degrees Celsius), salinity (ppt), photosynthetically active radiation (PAR, umol/m^2/s), and transmissometry (Formazine Turbidity Units, FTU) for April through October, 2001 were downloaded from NOAA's SeaKeys/C-MAN database recorded by the station at Long Key (LONF1, 24° 50' 24" N, 80° 51' 36" W). If environmental data was not available for a collection date in this study, then a calculated mean consisting of two dates before and two dates after the particular date was used (Fig. 1A and B).

2.3. Target development

Total RNA was isolated from a 2 ml aliquot of each homogenized coral fragment following the manufacturer's protocol for TRIzol® (based on Chomezynski and Sacchi, 1987). RNA concentrations were estimated by ultraviolet absorbance at 260 nm and integrity of the ribosomal subunits was confirmed by electrophoresis on a 1% formaldehyde agarose gel. Replicate aliquots of up to 2 μg of total RNA from each sample collection were reverse transcribed using SuperScript™ II reverse transcriptase (Invitrogen) and an oligo (dT) primer (Operon Biotechnologies, Inc.). During reverse transcription, DIG labeled dUTPs (digoxigenin-11-2'-deoxy-uridine-5'triphosophate, alkali-labile; Roche Diagnostics) were incorporated into the transcribed cDNA for subsequent detection using chemiluminescence. Specific conditions of the reverse transcription reaction are described in Edge et al. (2005). For each sample collection, an aliquot of cDNA was added to a high sodium-dodecyl-sulfate (SDS) buffer (Roche Diagnostics) resulting in cDNA concentrations ranging from 30–50 ng ml^{-1}. These DIG-labeled cDNA solutions represent the targets used with a coral array to assess differences in gene expression across collection date.

2.4. Expression profiling

An experimentally designed coral gene array was used to evaluate differential gene expression in the field samples. ESTs on the array correspond to 32 different genes isolated from *Acropora cervicornis* and *Montastraea faveolata* (Morgan et al., 2001; Morgan and Snell, 2002; Edge et al., 2005). These gene fragments cover a range of functions including response to xenobiotic exposure and oxidative stress, maintenance of cellular integrity and respiration, post-translational processing and apoptosis. The cDNAs representing each gene were spotted in triplicate onto each nylon array and samples were analyzed using three replicate arrays to estimate technical error. Edge et al. (2005) describe the development of the array and the preparation of probes on the array.

2.5. Hybridization

DIG-labeled targets were hybridized to the array in order to visualize probes expressed in the total RNA from colonies collected at each date. The hybridization protocol is described in Morgan et al. (2001) and Morgan and Snell (2002). Hybridizations of samples from each date were performed three times using labeled cDNA from different colonies. Nylon membranes were used only once to ensure a consistent correlation between spot intensity and transcript concentration. Membranes exposed

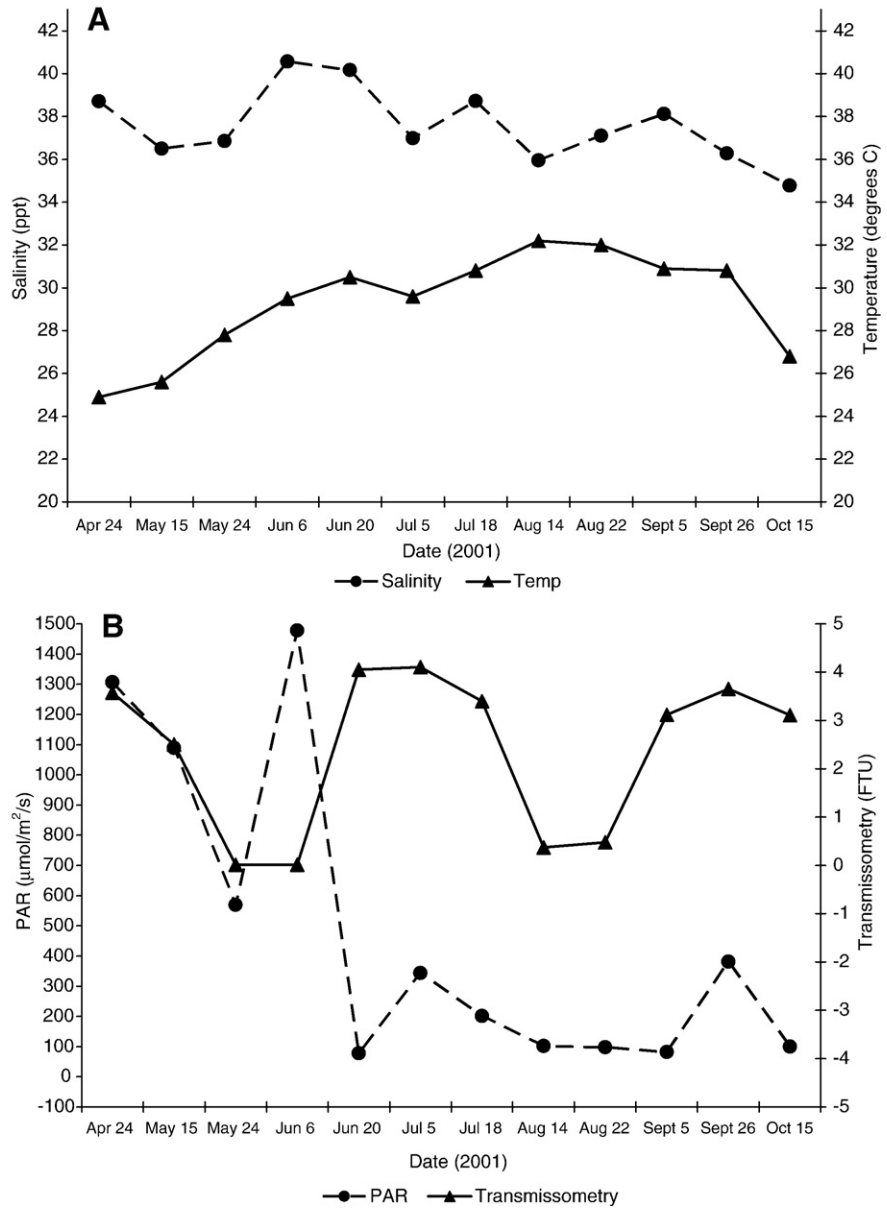

Fig. 1. Environmental data collected from NOAA SeaKeys/C-Man station at Long Key for 2001. A) Salinity (ppt) and ocean temperature (°C). B) Photosynthetically active radiation (PAR, umol/m^2/s) and transmissometry (Formazine Turbidity Units, FTU).

overnight to X-ray film produced dark spots corresponding to expressed transcripts in target cDNA. The level of expression between samples collected at each time point was compared. Array analysis to determine levels of probe expression is described in Edge et al., 2005.

2.6. Data analysis

Global mean normalization was performed across all arrays. After background signals had been subtracted, all detectable signals were log 2 transformed. Statistical analyses (Univariate ANOVA, SPSS v.15) were performed on log 2 transformed data. Levene's Test of Equality of Error Variances was used to determine if all data groups had similar variances. If Levene's Test revealed significant differences in sample variances, the Tahmane's T2 posthoc test was perform to determine which sample groups were significantly different from each other. If the variances were similar, then Student–Neuman–Keuls test was used to determine if there were any significant subsets of sampling groups.

2.7. Clustering

Hierarchical clustering was used to develop a preliminary characterization of the genes and their corresponding expression profiles during the time course in this study. Mean values of log transformed expression data were calculated for each gene on every date. Hierarchical clustering was performed on these

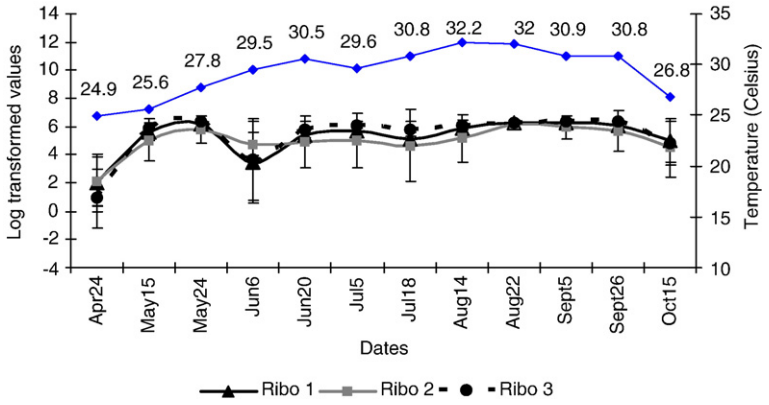

Fig. 2. Cluster 1. Gene expression levels of three ribosomal proteins with a Pearson's correlation coefficient=0.99. Sample collection dates in 2001 are indicated on the *X*-axis and log-transformed expression levels in arbitrary units on the *Y*-axis. Error bars represent standard deviation. Small diamonds represent water temperature (°C) on dates of sampling.

mean values in order to generate clusters of genes with similar expression patterns (Eisen et al., 1998).

3. Results

During this investigation, 12 of the 32 genes on the array were detected. Three independently prepared replicate membranes did not vary significantly in their expression signals ($P>0.05$, Univariate ANOVA, $F_{2,26}=0.843$). Detectable expression signals ranged from a −3.84 to 6.48 log base 2 arbitrary units (au) above the background. Detectable signals with values less than 1 transformed into negative expression signals. Descriptive statistics of log transformed data indicated unequal variances for the 12 genes analyzed in this study (Levene's Test of Equality of Error Variances, $F_{415,629}=4.892$; $P<0.5$). Since Univariate ANOVA is generally insensitive to heteroscedasticity, Tamhane's T2 posthoc test was applied (Tamhane, 1979).

3.1. Mean expression of individual genes

Of the 12 genes analyzed, the three ribosomal genes used in this study exhibited the highest average expression levels ranging from 4.94 to 6.31 au. Results of Tamhane's test revealed that the mean expression levels of these genes are not significantly different from each other ($P>0.05$). UC1 (uncharacterized 1) had the second highest expression signal (=2.55 au), especially on 22nd Aug and 5th Sept which were significantly different ($P<0.05$, Tamhane's T2 Test) from the levels expressed on 20th June and 18th July. Thioredoxin, uPAR and UC2 (uncharacterized 2) had the third highest mean expression signals ranging from 0.77 to 2.22 au. All of the remaining genes (TRAP, Mt, Ft, UC3, and PUcope17A) represented the fourth group which had the smallest mean expression signals ranging from 0.35 to 0.41 au.

3.2. Mean expression of all genes by date

One of the fundamental modes of characterizing gene expression profiles over a time course is to compare the collective signal intensities of all genes for each time period. There was a significant difference in the combined expression levels of all genes on different dates (Univariate ANOVA, $F_{120,237}$; $P<0.05$). Gene expression on 24th April was significantly different from all other dates except 18th July ($P<0.05$, Tamhane's T2 Test). Gene expression on 18th July

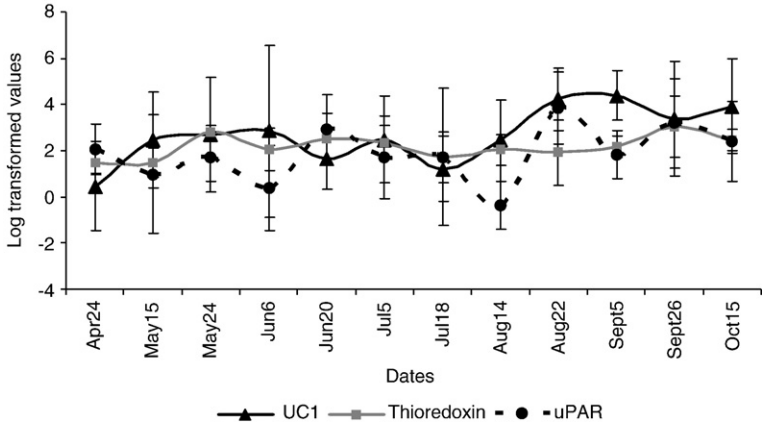

Fig. 3. Cluster 2. Pearson's correlation coefficient=0.87. A negative value represents a log base 2 transformation of a signal that was <1.0 but still above the background. Error bars represent standard deviation.

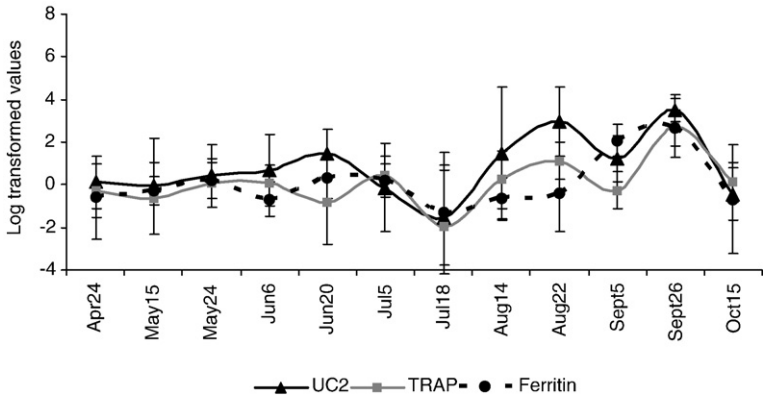

Fig. 4. Cluster 3. Pearson's correlation coefficient = 0.62. A negative value represents a log base 2 transformation of a signal that was <1.0 but still above the background. Error bars represent standard deviation.

(water temperature: 30.8 °C) was not significantly different from any of the dates examined in this study.

3.3. Clustering analysis

Hierarchial clustering (parameters: uncentered correlation with average linkage) was used to provide a basis for a preliminary characterization of the expression profiles for genes in this study. The analysis revealed that some of the genes could be grouped into clusters with varying degrees of similarity based upon their expression patterns. The genes in an individual cluster were either directly linked, or closely joined by nodes, indicating a high degree of similarity based on the cluster analysis. This form of cluster analysis provides a platform for organizing the collective gene expression profiles of noisy population level data into discrete subgroups that can highlight the profile of each subgroup. The expression profile for the ribosomal genes produced cluster 1 which had significant similarities (Pearson's correlation coefficient = 0.99) and further illustrates how these three genes responded in a similar manner over the time course in this study (Fig. 2). Hierarchal clustering grouped UC1, Thioredoxin, and uPAR into cluster 2 (Pearson's correlation coefficient = 0.88) (Fig. 3). Cluster 3 was composed of UC2, TRAP-D, and Ferritin (Pearson's correlation coefficient = 0.62) (Fig. 4). The final, and fourth, cluster included the UC3 (uncharacterized 3) and PUcope17A (polyubiquitin) genes (Pearson's correlation coefficient = 0.22) (Fig. 5). These two genes had the smallest expression signals and lowest correlation coefficients to any nearest neighbors. The MT gene did not cluster strongly with any other gene but was linked by two nodes to its nearest neighbor, which was cluster 3 (Pearson's correlation coefficient = 0.53) (Fig. 6). Hierarchical clustering produces a final cluster that encompasses the entire group of genes. The calculated Pearson's correlation coefficient of this cluster (all twelve genes) was −0.45. However, when the genes for cluster 4 were excluded from the collective cluster, the Pearson's correlation coefficient increased to 0.41.

3.4. Characterization of gene expression profiles by cluster groups

3.4.1. Cluster 1

A comparison of the expression across dates of the ribosomal genes in cluster 1 using Tamhane's T2 Test indicates significantly lower levels of expression on 24th April, 6th June, and 15th Oct compared to the three dates with the highest levels of expression (24th May, 22nd Aug, and 5th Sept) ($P < 0.05$) (Fig. 2). Temperatures on 24th April (24.9 °C) and 15th Oct (26.8 °C) represent two of the lowest temperatures recorded during this study (see Fig. 1).

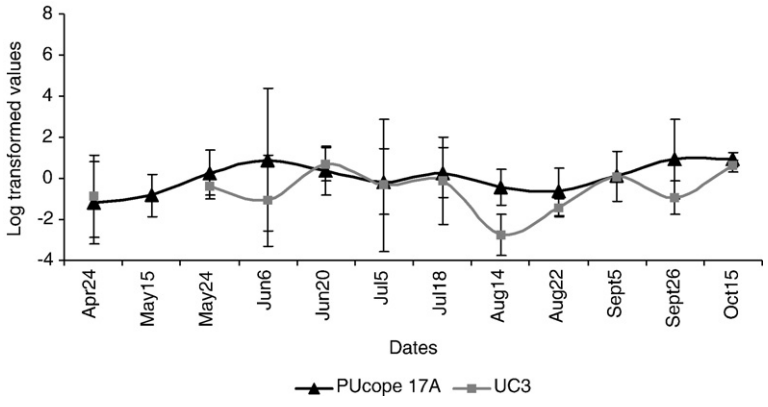

Fig. 5. Cluster 4. A negative value represents a log base 2 transformation of a signal that was <1.0 but still above the background. No signal was detected for UC3 on 15th May since. Error bars represent standard deviation.

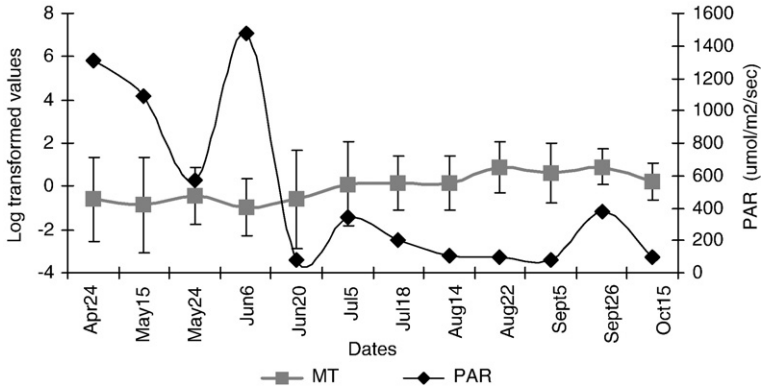

Fig. 6. Expression profile of the metallothionein gene compared to PAR data recorded by NOAA's Seakeys monitoring station. Pearson's correlation coefficient was −0.71.

3.4.2. Cluster 2

The genes in cluster 2 differed in their expression levels over time. The uncharacterized gene UC1 showed significantly higher levels of expression on 22nd Aug and 5th Sept as compared to 20th June and 18th July ($P<0.05$, Tamhane's T2 Test)(Fig. 3). Expression on 24th April appears lower in its expression level than either 20th June or 18th July, but is not statistically significant ($P=0.06$) because of greater variance (Fig. 3). Thioredoxin had elevated expression on 24th May compared to 24th April and 15th May ($P<0.05$, Tamhane's T2 Test) (Fig. 3). The uPAR gene was significantly lower in its level of expression on 14th Aug compared to 24th April, 20th June, 22nd Aug, 5th Sept, and 26th Sept. ($P<0.05$, Tamhane's T2 Test) (Fig. 3).

3.4.3. Cluster 3

Signals on 18th July, 22nd Aug, and 26th Sept represent the greatest deviation from the mean level of expression for genes in cluster 3 (Fig. 4). Although the expression levels for the genes in this cluster are not identical, they share the common characteristic of elevated expression at some point in time spanning the dates of 22nd Aug through 26th Sept. PUsal showed significantly higher levels of expression on 22nd Aug and 26th Sept compared to 18th July ($P<0.05$, Tamhane's T2 Test) (Fig. 4). The TRAP gene was significantly elevated on 26th Sept compared to 6th June, 5th Sept, and 15th Oct ($P<0.05$, Tamhane's T2 Test) (Fig. 4). The Ferritin gene had an elevated expression level on 5th Sept compared to 15th May and 6th June, while expression on 26th Sept was significantly elevated compared to 15th May, 6th June, and 14th Aug ($P<0.05$, Tamhane's T2 Test) (Fig. 4).

3.4.4. Cluster 4

Both genes (PUcope17A and UC3) exhibited no significant differences in their expression levels at any date during this study (Fig. 5). These two genes also exhibited the overall lowest levels of expression of any of the genes detected in this study (Fig. 5). The UC3 gene had no signal detected on 15th May (Fig. 5).

3.4.5. Non-clustered gene

The expression profile for MT (metallothionein) revealed that there were no significant differences in its expression levels during all the sampling dates (Fig. 6). Levene's Test revealed similar variances between all samples, and Student–Neuman–Keuls test confirmed that all samples were not significantly different ($P>0.05$).

3.5. Correlations with environmental metrics

The expression profiles were compared to the available environmental data. For each gene, the mean values of expression per date were compared to the mean values recorded for water temperature, salinity, transmissometry and PAR on the corresponding date. Pearson's coefficient correlations were calculated and summarized in Table 1. Genes in clusters 1 and 2 (ribosomal, UC1, Thioredoxin, and uPAR) demonstrated positive correlations with changes in temperature. These same genes also showed similar correlations with changes in the salinities over the time course of this investigation. In addition to their strong correlation to water temperature, two genes (Thio and uPAR) also exhibited positive correlations >0.80 with transmissometry. The genes in cluster 3 (TRAP, PUsal, and Ferritin) and cluster 4 (UC3, and PU cope 17A) did not exhibit strong correlation with any of the environmental metrics.

3.5.1. Cluster 1

Ribosomal gene expression appears to correlate with changes in water temperature (Peason's correlation coefficient=0.98). There is a temporary drop in ribosomal expression

Table 1
Pearson's correlation coefficients for up-regulated genes and corresponding environmental metric

Gene ID	Temperature	Salinity	Transmissometry	PAR
Ribos	0.98	0.97	0.79	0.59
UC1	0.92	0.90	0.71	0.47
Thio	0.97	0.97	0.83	0.61
uPAR	0.85	0.83	0.81	0.45
UC2	0.54	0.51	0.32	0.21
Ferritin	0.29	0.26	0.32	−0.05
TRAP	0.14	0.11	0.05	0.03
UC3	0.02	0.02	0.11	−0.33
PUcope17A	0.21	0.17	0.12	−0.12
Mt	0.06	0.01	0.08	−0.70

during 6th June before stabilizing at an elevated rate from 20th June to 26th September (see Fig. 2). The greatest temperature change (3.9 °C) for any three consecutive dates occurs between 15th May and 6th June (see Fig. 1). It should also be noted that the water temperature recorded on 6th June is the first of the sampling dates where the temperature is above 29 °C. In addition, while 6th June water temperature (29.5 °C) is not the highest water temperature on the sampling dates of this study, the 6th June was the date of the highest salinity recorded during the time course of this study. Ribosomal expression and water temperature both drop on 15th Oct (see Fig. 2).

3.5.2. Cluster 2

The expression profile of cluster 2 is different from cluster 1 (see Figs. 2 and 3). The highest expression levels for the uncharacterized gene were evident on 22nd Aug and 5th Sept and lowest on 24th April, 20th June and 18th July. However, expression on 24th April was not significant from the highest levels detected on 22nd Aug and 5th Sept even though it was the lowest for any of the dates ($P=0.06$). This may be a function of the small sample size and the high variability associated with a population level study. The highest water temperatures were recorded on 14th Aug and 22nd Aug, while the highest expression levels of the uncharacterized gene occurred on 22nd Aug and 5th Sept. The expression level of this gene remains elevated on 15th Oct even though there is a 4 °C drop in temperature between 26th Sept and 15th Oct (see Figs. 1 and 2).

3.5.3. Cluster 3

Cluster 3 has an expression profile that does not fluctuate from 24th April through 5th July (Fig. 3). On 18th July, there is a temporary drop in expression of all three genes (UC2, TRAP-D, and Ferritin) followed by elevated expression levels over the span of 22nd Aug to 26th Sept. The pattern of expression for these genes does not strongly correspond to changes in water temperature (Pearson's correlation coefficients, UC2=0.54; TRAP-D=0.14; Ferritin=.29) (Table 1).

3.5.4. Remaining genes

The uncharacterized gene (UC3) and the PUcope17A gene in cluster 4 (see Fig. 5) show no significant correlation to any of the environmental metrics. However, the metallothionein gene (MT) shows a negative correlation to PAR measurements over the time course of this study (Pearson's correlations coefficient=−0.71) (Table 1) (see Fig. 6).

4. Discussion

The profiles of genes observed in this study can be divided into two groups based on time period of expression. During spring and early summer, May into July, most of the genes show little deviation from their average level of expression with the exception of the ribosomal genes which show the greatest deviations in April and early June. However, in late summer, August and September several of the genes show larger oscillations around their average expression (Fig. 7). These changes in expression across time reveal the natural variation of genes on this array within a coral population. In addition, detection of significant changes on certain dates above the average level of expression reveals the capability of this cDNA array to detect fluctuations in gene expression within a natural population of coral that may not be associated with natural variation.

4.1. Differential expression

During August and September a stimulus, or multiple stimuli, may be inducing gene expression. Several genes on the array exhibit different expression patterns during these sampling dates. The exact cause of these changes in gene expression is not currently evident; however hypotheses include environmental stimuli, such as elevated temperature, or physiological events, such as spawning. Three of the genes that fluctuate in August and September are uncharacterized, so there is no data on their molecular functions. Of these, UC1 correlates strongly with temperature and salinity data indicating response to environmental stimuli (Table 1). These results suggest hypotheses regarding which environmental stressors are having the greatest impact on field populations of coral at our collection sites. To confirm these hypotheses, tests in controlled conditions need to be quantitatively compared to the expression profiles observed in this field study.

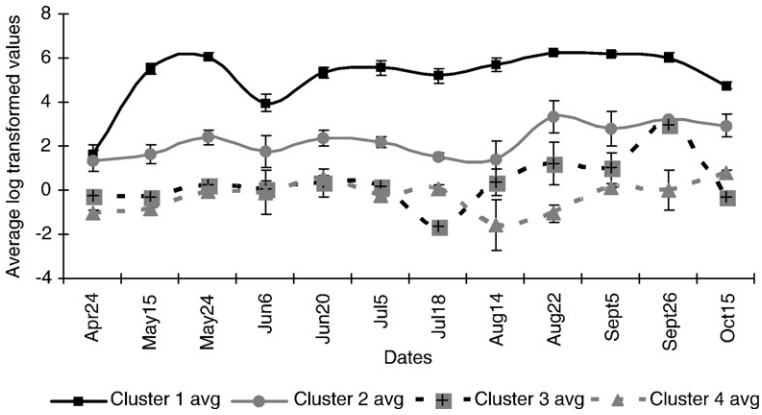

Fig. 7. Averaged cluster profiles. The average log transformed values for each cluster on every sampling date +/− standard error.

Ferritin is elevated during both collection dates in September but does not correlate strongly with any of the recorded environmental data. Ferritin is involved in iron homeostasis, regulation of cell proliferation, and antioxidant defense (Aust, 1995; Orino et al., 2001; Kuo et al., 2004). Oxidative stress and exposure to ultraviolet radiation have been shown to increase ferritin expression (Cairo et al., 1995; Applegate et al., 1998; Pourzand et al., 1999; Tsuji et al., 2000). In marine organisms, oxidative stress is an important part of the stress response and is associated with multiple environmental insults including thermal stress and exposure to ultraviolet radiation (Lesser, 2006).

Although uPAR fluctuates across all collection dates, its highest level of expression occurs in late August. The uPAR gene also exhibits relatively strong correlations with three of the four environmental parameters in this study (Table 1). Previous experiments have demonstrated elevated expression of uPAR in response to acute increases in temperature, salinity and UV exposure (Edge et al., 2005). This gene has multiple functions including signal transduction (Behrendt, 2004), regulation of proteolysis, cytokine activity and cellular adhesion (www.geneontology.org). It is found in several cell types, but is mostly expressed in tissue undergoing remodeling, since it is especially important in wound healing and matrix degradation (Behrendt, 2004). For example, human epithelial cells induce the expression of uPAR mRNA in response to UV light (Marschall et al., 1999). In addition, increased expression of uPAR has been detected in coral colonies from areas known to be experiencing elevated levels of sedimentation (Morgan et al., 2005).

Thioredoxin exhibits significantly different levels of expression during the first three sampling dates (Fig. 2C). Thioredoxin is up-regulated in response to oxidative stress, UV exposure, hypoxia and acute exposure to elevated salinity; it is also involved in cell proliferation, growth and development, and signal transduction (Arner and Holmgren, 2000; Das and Das, 2000; Das and White, 2002; Edge et al., 2005). The significant differences in levels of expression of this gene coupled with its correlation to water temperature and/or salinity early in the sampling dates suggest it may be a sensitive indicator of corals beginning to respond to seasonal changes in their environment.

4.2. Environmental factors

The rapid rise in water temperature and its sustained elevation for several months is one possible stressor impacting corals during the sampling period. Undoubtedly, there could be a multitude of other environmental factors, such as sedimentation or UV, which may have influenced the observed expression profiles. It is noteworthy that 5 of the genes (UC1, Thio, uPAR, Ferritin, Ribo) that show elevated expression on a few, if not all, collection dates are known to be induced by UV exposure (Marschall et al., 1999; Pourzand et al., 1999; Didier et al., 2001; Wang and VandeBerg, 2004; Edge et al., 2005) and are involved in the oxidative stress response (Cairo et al., 1995; Lee et al., 1999; Gasch et al., 2000; Didier et al., 2001; Walker et al., 2002). Ultraviolet radiation and the oxidative stress response are intimately connected. Exposure to ultraviolet (UV) radiation leads to protein damage, tissue inflammation, DNA damage and cell death either directly, or by generating reactive oxygen species (Miralles et al., 1998; Lesser et al., 2001). Organisms respond by up-regulating suites of genes that code for transcription factors, growth factors and proteases, which have been characterized in mammals as the UV response (Devary et al., 1992; Miralles et al., 1998).

It is important to point out that changes in mean expression of other genes on the array were not detected. For example, genes that have previously demonstrated sensitivity to pesticide and polycyclic aromatic hydrocarbon exposure were not expressed in corals collected during this study. The lack of induction for these groups of genes suggests that, if present, these pollutants were below concentrations necessary to trigger stress gene expression. It is also possible that these genes may have responded to a brief pollutant exposure during the intervals between the sampling dates in this study. Alternatively, the coral population may have physiologically responded to a pollutant exposure without noticeable changes in gene expression.

4.3. Spawning

A recent article indicates that coral spawning in the Caribbean correlates with the average temperature during the month of spawning and that all corals release gametes at 28–30 ° C, except *M. annularis*, which releases at 27–30 °C (van Woesik et al., 2006). According to our data, these temperatures occurred during the months of June and July in 2001. However, historical data show that coral spawning of *Montastraea* species in the Florida Keys tends to occur in the months of August and September, which were the warmest of our collection (30.8–32.2 °C) (Szmant et al., 1997; Mendes and Woodley, 2002). Several genes on the array show elevated activity from mid-August through September and, for some, their expression patterns correlate with temperature (thioredoxin, ribosomal, UC1, UC2, uPAR). Expression of these genes could be an indication of thermal stress; however it could also be a result of spawning activity, since it is unknown how coral spawning affects the expression of the genes used in this study. Although environmental stimuli of coral spawning have been described, the biochemical mechanisms that elicit physiological responses have not been characterized (Tarrant 2005). Further investigations are required to determine how spawning events affect the overall expression of these genes within the coral transcriptome.

4.4. Natural variation in gene expression

4.4.1. Ribosomal genes

The regulated expression of ribosomal genes is essential in maintaining homeostasis. Ribosomal genes are involved in protein biosynthesis, RNA binding, and transcription regulation among other crucial cellular functions. It is expected that they would be consistently expressed in organisms across time. However, ribosomal genes have also been shown to fluctuate in response to stress (Causton et al., 2001; Edge et al., 2005). In response to acute exposures to stress, ribosomal gene expression increases with elevated salinity and exposure to UV, but

decreases with elevated temperature (Edge et al., 2005). This study is consistent with previous results and reveals that ribosomal expression increases as temperature increases, with a significant drop in expression in early June which represents the greatest change in temperature (+4.6 °C) observed during any four sampling dates in this study. This drop in expression may be due to the rapid rate of increase in temperature, similar to the shock of acute exposure in Edge et al. (2005). As temperature peaks and begins to level off, ribosomal expression also levels off before dropping again in October as temperature decreases. Expression profiles are not available from November to March during this study. This information, along with temperature data for the same period, could clarify how ribosomal expression correlates with changes in temperature for coral populations at this site.

4.4.2. Other genes

Polyubiquitin is induced by DNA damage but also regulates protein degradation, location, activity and interaction with other proteins (Fornace et al., 1989; Nenoi, 1992; Schnell and Hicke, 2003; Varshavsky, 2006). Metallothionein is most well known for its role in heavy metal detoxification (Sato and Kondoh, 2002), but it also functions in a number of biochemical processes including gene expression, apoptosis, proliferation and differentiation (Kagi and Schaffer, 1988; Vallee, 1995; Palmiter, 1998; Davis and Cousins, 2000). The expression levels of metallothionein and polyubiquitin do not fluctuate significantly across collection dates. The observed profiles may be the natural variation in expression for these genes since they are consistently 'on'. However, the possibility that during this study corals were undergoing continuous exposure to a stressor or suite of stressors cannot be ruled out.

4.5. Enhancing the diagnostic capabilities of the coral cDNA array

The coral cDNA array used in this study has previously demonstrated a sensitivity for detecting changes in gene expression profiles of coral exposed to laboratory controlled stressor experiments (Edge et al., 2005) as well as investigations in the field (Morgan et al., 2005). One means of further improving the diagnostic sensitivities of the array is to characterize how individual genes respond on different temporal scales. Once variances in patterns of expression are established for genes on the coral array, deviations beyond these levels can be used as indicators of physiological conditions requiring closer investigation. Characterizing the average expression levels for these genes for over six months helps to identify the constitutive variance of expression and concurrently establishes a predictive model for normal seasonal physiology (basal metabolism). A predictive model would hypothesize that similar reefs with comparable environmental conditions and exposures to anthropogenic stressors should respond with similar patterns of expression. As a result, detecting deviations in these patterns offers researchers a new and sensitive diagnostic assay for identifying altered physiologies/metabolisms. Once seasonal variance is established by repeated measurements through additional studies, resource managers can begin to monitor the expression of these genes and gain additional insight into the physiological status of the targeted populations.

4.6. Biological relevance of expression profiles

The data generated in this study demonstrate how a small group of genes vary in their expression patterns over time in one coral population. At this preliminary stage, it is unknown whether any of these genes can be directly linked in their expression to any of the observed environmental parameters. Important clues about the biological relevance of the expression patterns observed in this study can be obtained when correlation coefficients are calculated for individual genes and various environmental metrics. This study revealed that the genes in Cluster 1 (see Fig. 2) showed a high positive correlation to water temperatures and that the Mt gene showed a relatively high negative correlation to the PAR data (see Fig. 6). Correlating relevant environmental data with observed gene expression profiles narrows the list of stimuli potentially responsible for altered gene expression and generates testable hypotheses for future studies.

The genes used in this study were initially isolated from corals exposed to laboratory induced stress conditions (Edge et al., 2005). Although all of these genes are known to function during the stress response, they also have roles in general cellular metabolism. Subsequently, acute exposures of coral to elevated temperature, salinity and UV provided a first glimpse at how these genes behave under controlled conditions in coral. In addition, some of the genes used in this study have demonstrated differential expression in corals from sites at varying distances to a point source of pollution and with different sedimentation profiles (Morgan et al., 2005). This is the first study to investigate the expression of these genes in a natural coral population across time and demonstrates the sensitivity of a cDNA array to detect changes in gene expression within the targeted coral populations.

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