Gene expression analysis in *Mytilus chilensis* populations reveals local patterns associated with ocean environmental conditions

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**Abstract**

Marine ecosystems involve relationships between genomic interactions of marine populations with shared biogeographic ranges and the environmental conditions. These relationships, studied mainly through neutral DNA markers, are not always consistent with actual biogeographic patterns or the evolutionary history of marine species. In addition, increased information at functional genomic level from non-model species allows the study of adaptive responses in marine populations. This work reports local transcriptomic patterns in populations of the mussel *Mytilus chilensis* and their correspondence with oceanographic variability in southern Chile. Analysis of gene expression patterns was conducted through qPCR of seven candidate genes involved in the response to environmental stress (*Hsp70*, *Hsp90*, iron metabolism (*Ferritin*), pathogens (*Mytilin B, Defensin*) and oxidative stress (*Sod-CuZn, Catalase*) in five study sites located in southern Chile, from Valdivia (39°56′S–73°36′W) to Melinka (43°52′S–73°44′W). Multivariate and correlation analyses were used to assess the relationship between levels of individual gene expression and site features characterized using satellite data on surface temperature, chlorophyll concentration and total suspended sediments. Two main groups of sites with differential patterns of gene expression were identified. Individuals exposed to higher temperatures showed an overexpression of *Hsp70*, *Hsp90* and *Ferritin* genes. The expression of *Sod-CuZn* and Catalase was correlated with local chlorophyll-a (i.e. food availability for mussels), although with opposite correlations. In addition, *Mytilin B* showed higher levels of expression in areas with higher freshwater influence. Patterns of gene expression across the region of interest suggest that spatial variability in environmental conditions induce phenotypic changes in different populations of the same mussel species. In addition, the analysis of expression patterns in candidate genes can reveal local patterns in populations where other molecular markers show no genetic structure.

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1. Introduction

Understanding the effect of environmental variability on the biogeographic patterns of marine species has been one of the main purposes of marine physiology and molecular ecology (Osovitz and Hofmann, 2007). Within this framework, topics such as climate change in oceanic and coastal areas have become very important in studies of marine ecosystem dynamics (Harley et al., 2006; Parmesan, 2006), latitudinal gradients affecting specialization and biodiversity rates (Allen and Gillooly, 2006), larval development under different conditions of temperature and food availability (Hoegh-Guldberg and Pearse, 1995; O’Connor et al., 2007), and relationships between the dispersal ability of species and their biogeographic range (Lester et al., 2007).

Undoubtedly, genetic studies based on molecular DNA markers have made it possible to identify the relationship between biogeographic variables and population genetic structures in marine environments (Ayers and Waters, 2005; Kelly and Eernisse, 2007; Osovitz and Hofmann, 2007). However, few studies have associated phenotypic differences with genotypic changes determined by latitudinal patterns in environmental conditions (e.g. Lee and Petersen, 2002). Therefore, there is currently no certainty about the correlations between phenotypic variations and genomic changes inferred from neutral genetic markers (or markers assumed to be unaffected by natural selection). In some cases, these correlations may occur as a result of genetic processes such as heritability, genetic flow, hybridization among others; however, they are also affected by non-genetic processes, such as disruption of inter-specific interactions and habitat degradation (Ouborg et al., 2010). Furthermore, different types of molecular DNA markers may show contrasting results, or may not detect the population polymorphisms due to low genome coverage. In this context, microsatellite variability is widely used to infer levels of genetic diversity in natural populations.
method to identify candidate genes in different species, allowing the analysis of reactive oxygen species (ROS) in the cell (Mruk et al., 2002), and the innate immune response, such as heat shock proteins (HSP) (Lindquist, 1986). Additionally, other candidate genes are involved in the response to the oxidative stress generated by an excess of free radicals (AMP) (Mitta et al., 2000). Another candidate gene could be Ferritin, which is the main gene involved in iron storage at cellular level, storing up to 4500 iron atoms (Orino and Watanabe, 2008). However, the implication of this gene in different functions at cellular and molecular level is still under discussion. Finally, SOD-CuZn and Catalase are genes involved in the response to the oxidative stress generated by an excess of reactive oxygen species (ROS) in the cell (Mruk et al., 2002), and therefore could be considered as candidate genes in this context.

Intertidal benthic invertebrates from the southern Chile shoreline are an attractive system for biogeographical studies of stress and abundance. Intertidal invertebrates typically have broad geographical ranges that may span thousands of kilometers, whereas their longitudinal and tidal height ranges may be restricted to one degree and a few meters, respectively. Such patterns of geographic distribution can be approximately described as one-dimensional domains with two endpoints (rather than a continuous boundary), and provide simplified systems to draw up hypotheses about the responses of organisms to environmental gradients (Sagarin and Somero, 2006). Moreover, intertidal benthic invertebrates have little ability to find refuge during episodes of extreme environmental conditions, and must cope with sharp variations in temperature and salinity due to alternating periods of emersion and immersion associated to tides (Jones et al., 2010).

The mussel *Mytilus chilensis* is distributed along the Chilean coast and is most abundant in southern shores (Brattström and Johannsen, 1983). Over its wide geographic range of distribution, some individuals inhabit areas with substantial differences in local environmental conditions, such as temperature and freshwater inputs that affect surface salinity, phytoplankton productivity, and marine bacterial loads. Furthermore, anthropogenic factors, such as aquaculture, cause variations in the natural distribution of species due to the displacement of seeds and breeders from one location to another with different environmental conditions. This scenario increases the probability of losing local adaptation due to the reduction of genetic variation at inter-population level (Allendorf et al., 2008). Hence, the aim of this study was to assess the presence of local patterns of gene expression in 5 populations of *M. chilensis* that span a gradient in oceanographic variability and local environmental conditions. Our genetic assessment was based on the expression analysis of the above-described 7 candidate genes involved in environmental stress (HSP70 and HSP90), antimicrobial response (*Mytilin B* and *Defensin*), oxidative stress (*Catalase* and *SOD-CuZn*) and iron metabolism response (*Ferritin*).

2. Materials and methods

2.1. Sample collection

Individuals of *M. chilensis* (50–70 mm shell length) were collected from the following five sites in southern Chile (Fig. 1): Chaihuin, near the mouth of the Valdivia river (39°56′S–73°36′W); Curimán (42°3′S–72°30′W), on the eastern side of the Chiloé Inner Sea (CIS); Chauques Islands, on the eastern side of Chiloé island (42°16′S–73°5′W); Puerto Raul Marín (43°41′S–73°00′W) and Melinka (43°52′S–73°44′W), at the southern end of the CIS. Twenty individuals (n=20) were sampled from each population from September 10 to 12, 2010 (see Supplementary Material 1 to view the time and tide of each sampling site). Approximately 250 mg of gill tissue were extracted from each individual and immediately fixed in 1 ml of RNA Later (Ambion) according to the manufacturer’s instructions. Finally, samples were stored at low temperature (−80 °C) until the RNA was extracted.

Species identification was supported through analysis of ITS-2 gene using primers reported previously for other mollusk species (Aguilera-Munoz et al., 2008) and through analysis of 18S gene using the method and primers reported by Santana et al. (2006) for *Mytilus* spp.

2.2. RNA extraction and cDNA synthesis

Total RNA was extracted from approximately 100 mg of gill tissue using the TRIzol reagent (Invitrogen) according to manufacturer’s protocol. The phase separation was conducted using 100% chloroform and the RNA precipitation was performed with 100% isopropanol previously cooled at −20 °C. Subsequently, the RNA was washed using 75% ethanol prepared with DEPC water and previously cooled at −20 °C, and then resuspended in 50 μl of DEPC water. The concentration and purity of isolated RNA were measured in a ND-1000 spectrophotometer (NanoDrop®Technologies) and its integrity was visualized by electrophoresis in 1.2% MOPS-agarose gel stained with 0.001% ethidium bromide. For cDNA synthesis, 200 ng of total RNA were retrotranscribed for each sample, using the RevertAid H Minus First Strand cDNA Synthesis kit (Fermentas).

2.3. Candidate genes, primers design and PCR standardization

Candidate genes involved in different biological processes for the genus *Mytilus* were selected from GenBank and Mytibase databases (Veneri et al., 2009). Specific primers were designed for these sequences using Primer3 (Rozen and Skeatsky, 2000), included in Geneious Pro software version 5.1.7. Genes, functions and primers are described in Table 1. Subsequently, PCR reactions were conducted in 25 μl of total volume containing 1× PCR buffer (Fermentas), 0.2 μg/μl of BSA, 1.5 mM of MgCl₂, 500 nM of sense and anti-sense primers, 0.06 μl/μl of Taq Polymerase (Fermentas) and 2 μl of mussel cDNA as template. The PCR product was visualized by electrophoresis in 1.2% agarose gel stained with 0.001% ethidium bromide. The corresponding bands were cut using the Gel Extraction Kit (EZNA), cloned by TOPO-TA cloning kit
and sequenced in an ABI3730×l automatic sequencer (Applied Biosystems). After obtaining the sequences, new specific primers were designed to amplify products with a size between 60 bp and 150 bp for qPCR. Sequence results and GenBank accession numbers are shown in Table 1. Sequences of primers designed for qPCR are shown in Table 2.

2.4. Gene expression analysis through quantitative PCR

Five serial dilutions of cDNA stocks ranging from 1 to 1:625 were made to establish the dynamic range for the genes under study. Enzyme efficiency between 90% and 110% in all reactions was obtained. Subsequently, a pool of individuals from each population was used to select the best housekeeping gene according to its stability value inferred by the NormFinder application. Four housekeeping genes were analyzed: 18S, 28S, elongation factor α and α-tubulin, of which the latter was the most stable at the moment of analyzing its stability value and the standard error among two samples per site which were selected randomly in the five sampling sites. NormFinder application select the best housekeeping-gene for a qPCR assay analyzing the Stability value, in which lower values mean most stable between replicates (see Supplementary Material 2 to view the Ct values of the assay and the stability value obtained for each housekeeping gene) (Richard Khan-Malek and Wang, 2011). Subsequently, a gene expression analysis was conducted in the five populations using the comparative ΔΔCt method, with 3 replicates for each individual (n = 20 per sampling site), and normalizing the expression data of each target gene (Table 1) with the α-tubulin endogenous control data, which was the most stable gene. All reactions for dynamic range, endogenous validation and expression analysis were conducted using the Maxima Kit® SYBR Green/ROX qPCR Master Mix (2×) (Fermentas, USA), according to manufacturer’s instructions and using a primer concentration of 500 nM and a 1:5 dilution for each cDNA stock.

Table 1
Amplified and sequenced genes in Mytilus chilensis, function, GenBank accession numbers and length.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primary function</th>
<th>GenBank accession number</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSP70</td>
<td>Molecular chaperone</td>
<td>HQ693569</td>
<td>487 bp</td>
</tr>
<tr>
<td>HSP90</td>
<td>Molecular chaperone</td>
<td>HQ693565</td>
<td>245 bp</td>
</tr>
<tr>
<td>Defensin</td>
<td>Antimicrobial peptide</td>
<td>HQ693570</td>
<td>260 bp</td>
</tr>
<tr>
<td>MytilinB</td>
<td>Antimicrobial peptide</td>
<td>HQ693567</td>
<td>339 bp</td>
</tr>
<tr>
<td>SOD-CuZn</td>
<td>Free-radical catalysis</td>
<td>HQ693564</td>
<td>510 bp</td>
</tr>
<tr>
<td>Catalase</td>
<td>Free-radical catalysis</td>
<td>HQ693566</td>
<td>378 bp</td>
</tr>
<tr>
<td>Ferritin</td>
<td>Iron storage</td>
<td>HQ693568</td>
<td>542 bp</td>
</tr>
</tbody>
</table>

Fig. 1. Study region and geographic location of sampling sites: Chaihuín (CHA), Curamín (CUR), Islas Chauques (CAU), Puerto Raúl Marín (PRM), and Melinka (MEL).
2.5. Statistical analysis

The analysis of gene expression data was conducted using the Statistica 7 software (Statsoft Inc.). A Shapiro–Wilks test was performed to determine the parametric or nonparametric data distribution. In the case of genes showing a parametric distribution, a one-way ANOVA test was conducted to identify significant differences between populations; while a Kruskal–Wallis test was used in those genes that showed a non-parametric data distribution (Yuan et al., 2006). In both cases, a probability value was established to identify significant differences. To assess the association of changes in gene expression among sites, we performed a Principal Components Analysis (PCA) on the matrix of gene expression data, using the 7 genes as variables and the 5 sites as inputs from rivers and surface runoff. This positive correlation could be explained by the geographical distance. Standardized Euclidian distances were calculated for each pair of sites based on their 7-dimensional and 2-dimensional coordinates for gene-expression and geographic location, respectively. The test statistic r was computed as

\[ r = \sum_{i=2}^{n} \sum_{j=1}^{i-1} a_{ij} b_{ij}, \]

where \(a\) and \(b\) are the elements in the lower-triangular part of both distance matrices. Significance of \(r\) was assessed by comparing its original value \(r_0\) with the empirical probability distribution obtained after \(N=10,000\) iterations of a randomization procedure. On each iteration, the rows and corresponding columns of one of the two matrices were randomly rearranged, and the value of \(r\) subsequently computed. Significance was calculated as \(G/(N+1)\), where \(G\) is the number of iterations in which \(r \geq r_0\).

2.6. Measurements of oceanographic conditions in sampling sites

Data on Sea Surface Temperature (SST) and surface chlorophyll (chl-a) concentrations over the study region were gathered from Level-3 images produced by the MODIS (Moderate Resolution Imaging Spectroradiometer) sensor onboard NASA’s Aqua satellite. Reduced-resolution Level-2 MERIS images with a spatial resolution of ca. 1 km were processed using the SeaWiFS Data Analysis System (SeaDAS) developed at the NASA Goddard Space Flight Center.

3. Results and discussion

3.1. Intra- and interpopulation analysis of gene expression in *M. chilensis*

Relative variation analysis of gene expression at intrapopulation level showed that genes involved in thermal response (among other stressors) had the highest level of transcripts associated mainly with *HSP70* and *HSP90* (Fig. 3a). This pattern was observed for all populations, except for Puerto Raul Marín, where the gene expression of *HSP90* was lower than antimicrobial peptide *Mytilin B* (Fig. 2d). The latter gene was expressed at low levels in the northern sampling sites, whereas it was comparable to *Ferritin* and *HSP90* at southern sites. The gene involved in iron metabolism, *Ferritin*, had low levels of expression relative to the other genes described above, but expressed at higher levels than the genes involved in the oxidative stress response (*SOD-CuZn* and *Catalase*), which showed a relatively low expression pattern in all populations.

Interpopulation analysis showed that genes related to thermal stress response (*HSP70* and *HSP90*) had a higher level of gene expression at lower-latitude areas, such as Chaihuín, followed by Curamin and Islas Chauques. The lowest levels of gene expression for *HSPs* were recorded in Puerto Raul Marin and then in Melinka. Hence, there was a significant (\(p \leq 0.001\)) north–south gradient in *HSP* gene expression (Fig. 3a). Neither of the genes with an antimicrobial function, *Defensin* and *Mytilin B*, showed a significant latitudinal pattern in gene expression (Fig. 3c), although *Mytilin B* increased sharply at Puerto Raul Marin and Islas Chauques (Fig. 3b). Regarding antimicrobial peptide genes, our results showed that *Defensin* had no significant inter-population differences, which was probably due to the high variability in expression observed at the individual level. As for *Mytilin B*, significant interpopulation differences in its expression were independent of spatial patterns in SST, but positively and significantly correlated with total suspended sediments, which may be regarded as a proxy for freshwater inputs from rivers and surface runoff. This positive correlation could be reflecting the genomic-level response of mussels to local changes in riverine influence, which might be driving changes in microbial assemblages. In mussels, differential patterns of expression for these genes have been observed among geographical areas, as well as from experiments in which individuals were injected with different species of bacteria (Li et al., 2010). As for the genes involved in oxidative stress, both *SOD-CuZn* and *Catalase* showed significant among-site differences in gene expression (\(p \leq 0.001\)) but no clear latitudinal pattern (Fig. 3c). Finally, the *Ferritin* gene showed a clear and significant latitudinal gradient (\(p = 0.043\)), with an increase of gene expression towards lower-latitude sites (Fig. 3d). In relation to this gene, Larade and Storey...
(2004) found an overexpression of Ferritin transcripts in Littorina littorea individuals exposed to periods of anoxia; subsequently, Zhou et al. (2008) showed an increase of Ferritin in Litopenaeus vannamei exposed to environmental stress caused by changes in pH. Later, Wang et al. (2009) found significant differences in Ferritin at different larval stages of Meretrix meretrix; and recently, Salinas-Clarot et al. (2011) showed a gene expression pattern of Ferritin that increases under...
thermal stress conditions in *Halotis rufescens*. All of these studies suggest that this gene is associated with several cellular and metabolic processes in different organisms. Our results lend partial support to the hypothesis that *Ferritin* may be involved in the response to changes in ambient temperature, since a clear latitudinal pattern was observed in its gene expression levels, but correlations between gene expression and mean SST were non-significant. We submit that, unlike molecular chaperone genes, this gene was more affected by water temperature at the time of sampling than the local regime of SST variability throughout the years spanned by our EOF analysis. However, further research is needed to explore the association between *Ferritin* and other sources of environmental stress at the sampling localities.

The multivariate analysis (PCA) of gene expression by site produced 2 main components that together explained 90% of total variability (PC1 = 71%, PC2 = 19%). While the PC1 axis separated northern and southern localities, the second principal component (PC2) separated the Chalcaes Islands (CAU) from the other two northern sites (Fig. 4). Along this PC2 axis, the two CIS sites (CAU and CUR) were more distant to each other than any other pair of sites (Fig. 4). An inspection of the vectors of coefficients obtained for PC1 and PC2 (Fig. 4, blue lines and symbols) indicated that site ordination along the two main axes of variability responded mostly to discrepancies in the expression of *Mytilin B* and *SOD-CuZn* versus other genes. More specifically, the separation of sites responded primarily to differences in the expression of the *Ferritin* and thermal stress response genes *HSP70* and *HSP90*, and secondarily to the expression of *Catalase*, *Mytilin B* and *SOD-CuZn* (Fig. 4).

Finally, the Mantel randomization test showed a non-significant correlation (p = 0.07) between distance matrices, indicating that among-site differences in gene expression cannot be simply ascribed to geographic distance among sites.

### 3.2. Patterns in oceanographic conditions and correlation with gene expression

Satellite-derived mean fields for surface conditions (SST, chl-a and total suspended sediments) showed clear and very distinct spatial structures, which in no case corresponded to monotonic changes as a function of latitude (Fig. 5). For instance, the warmest surface waters were not observed at the northernmost site, but instead at site CUR on the northeastern section of the CIS (Fig. 5a). Mean chlorophyll-a concentrations were high (>5 mg m\(^{-3}\)) at all sites, though significantly higher (p < 0.0001) at the two sites located in the CIS (>9.5 mg m\(^{-3}\), Fig. 5b). The pattern of total suspended sediments, on the other hand, was more diffuse but showed increased concentrations near the mouth of rivers and fjords, on the southwestern part of Chiloe Island, and throughout the northern section of the CIS (Fig. 5c). Although not apparent from Fig. 5c, mean sediment loads at PRM (1.37 g m\(^{-3}\)) were significantly higher (p = 0.0026) than at all other sites (<1 g m\(^{-3}\)).

Empirical Orthogonal Functions calculated for the satellite-derived SST time series produced a first and second EOF that explained 82.1% and 7.4% of spatial-temperature SST variability. The temporal mode of EOF1 corresponded to the annual SST cycle (not shown), and exhibited its greatest amplitude at CUR, where it explained 91.5% of temporal SST variability. The weakest annual signal was recorded at the northernmost site CHA (55.2% of temporal SST variability), whereas intermediate and very similar amplitudes were observed at CAU, PRM, and MEL (81–87% of temporal SST variability). Thus, SST variability over most of the study region appeared as mostly driven by the annual cycle in solar radiation and atmospheric conditions, with the exception of the northernmost site (CHA), where higher-frequency sources of physical forcing may be as important as the seasonal change in surface heating as drivers of thermal variability. As for spatial-temporal patterns in surface chlorophyll-a concentrations, the first and second EOFs explained 46.3% and 19.5% of total chl-a variability, respectively. The temporal mode of EOF1 corresponded to an annual signal, and explained >65% of temporal chl-a variability at both CIS sites (CUR and CAU), whereas it only explained 1.4% of temporal chl-a variability at CHA, and 10% and 27% at PRM and MEL. Therefore, the annual cycles of both SST and surface chlorophyll-a concentration were most predictable at the sites located in the Chiloe Inner Sea, where the highest long-term means were also observed.

Finally, rank correlations calculated for site-specific gene expression and oceanographic conditions (Table 3) indicated a significant positive association between both thermal stress related genes (*HSP90, HSP70*) and the long-term mean SST at each site (Fig. 6a,b), although the association was stronger for *HSP90* (Fig. 6a). This finding supports the hypothesis that molecular chaperone genes are highly inducible by local environmental conditions, since *HSP70* showed expression levels significantly higher than the rest of the genes, followed by *HSP90* in all populations, except PRM. Previous studies have reported that these genes are overexpressed under conditions of environmental stress, regardless of whether these are caused by changes in temperature (Cotton et al., 2010; Dong et al., 2008), salinity (Choi, 2010), or presence of pollutants (Hamers et al., 2004). From the study of four populations of *Mytilus galloprovincialis*, Dutton and Hofmann (2009) reported differential patterns, and more specifically a latitudinal gradient, in the expression of genes involved in the *HSP70* thermal shock response. Regarding both oxidative stress related genes, they showed opposite-sign but significant associations with median chlorophyll-a concentrations, with higher expression levels for the *SOD-CuZn* gene at lower-chlorophyll sites such as PRM (Fig. 6c) and higher expression of the *Catalase* gene at higher-chlorophyll sites such as CAU (Fig. 6d).

In this study, both genes involved in oxidative stress were less expressed than the other five, and no latitudinal gradient was observed, which may indicate that there is no significant oxidative stress in the populations studied. Changes in the expression of *SOD-CuZn* have been reported for gastropods exposed to TBT (Zhang et al., 2009), Aroclor 1254 (De Zoya et al., 2009), thermal stress and heavy metals (Kim et al., 2007). However, no gene expression analyses have been conducted in mussels, and studies conducted thus far had been limited to measurements of the enzymatic activity of this gene in response to several conditions such as temperature and food availability (Lesser et al., 2010). As for the *Catalase* gene, differential levels of expression have been reported in *M. galloprovincialis* exposed to *Vibrio anguillarum* injections (Canesi et al., 2010), consistent with other results that link different genes involved in oxidative stress to innate immunity mechanisms of marine invertebrates (Burge et al., 2007). Our findings suggest that the *SOD* gene is overexpressed at sites with low food availability for mussels...
higher-frequency fluctuations in SST. As for the negative correlation found for \textit{HSP70} expression and median suspended sediments, this result reflects the fact that mean SST and suspended sediments are negatively correlated in this region, as the input of turbid freshwater is driven by rainfall and glacier melting.

3.3. Approaches to identify biogeographic variation effects in marine populations

The implications of biogeographic variations for marine populations evaluated through various molecular markers have been subject of study for several years. In the beginning, allozyme markers and neutral DNA markers were used. Later, molecular markers associated with transcriptome, such as Expressed Sequence Tags–Simple Sequence Repeats (EST–SSR) and Single Nucleotide Polymorphism (SNP) were used. These markers have allowed increased detection of changes at

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|c|}
\hline
\textbf{Source of stress} & \textbf{Ambient HSP70} & \textbf{HSP90} & \textbf{Oxidative Catalase} & \textbf{SOD-Cu/Zn} & \textbf{Fe metabolism Ferritin} & \textbf{Pathogens Mytilin B} & \textbf{Defensin} \\
\hline
\textbf{Sea surface temperature} Mean & 0.90 & 1.00 & 0.70 & -0.70 & 0.80 & -0.50 & 0.73 \\
Median & 0.90 & 1.00 & 0.70 & -0.70 & 0.80 & -0.50 & 0.73 \\
SD & -0.40 & 0.00 & -0.20 & 0.20 & -0.20 & 0.50 & -0.18 \\
Amplitude EOF1 & -0.30 & 0.10 & -0.10 & 0.10 & -0.40 & 0.20 & -0.33 \\
\hline
\textbf{Surface chlorophyll-a} Mean & 0.30 & 0.50 & 0.90 & -0.90 & 0.10 & 0.00 & -0.08 \\
Median & 0.60 & 0.70 & 1.00 & -1.00 & 0.50 & -0.10 & 0.33 \\
SD & 0.40 & 0.70 & 0.80 & -0.80 & 0.20 & -0.10 & 0.08 \\
Amplitude EOF1 & 0.00 & 0.40 & 0.60 & -0.60 & -0.10 & 0.30 & -0.18 \\
\hline
\textbf{Total susp. sediment} Mean & -0.90 & -0.80 & -0.30 & 0.30 & -0.70 & 0.90 & -0.63 \\
Median & -0.90 & -0.80 & -0.30 & 0.30 & -0.70 & 0.90 & -0.63 \\
SD & 0.00 & -0.30 & 0.30 & 0.30 & 0.13 & -0.60 & -0.50 \\
\hline
\end{tabular}
\caption{Rank-correlation coefficients calculated for site-specific gene expression and descriptive statistics of satellite-derived data on sea surface temperature, chlorophyll-a concentration, and total suspended sediments. Significant correlations (\(\alpha = 0.05\)) are shown in boldface.}
\end{table}
In mussels, molecular markers have been developed through the analysis of EST databases. Vidal et al. (2009) found 1274 SSR markers in EST sequences for the genus Mytilus, which were tested through cross-amplification in 5 species of the genus. Later, Vera et al. (2010) found 25 potential SNPs markers in the EST database for the species M. galloprovincialis, which were genotyped, with 12 of them successfully amplified. These approaches have allowed the characterization of molecular markers that can be potentially related to gene expression changes, since they are found in coding regions of the genome. Furthermore, several gene expression studies have been conducted in M. californianus over biogeographic scales. In this context, Place et al. (2008) showed differential patterns of gene expression in four North Pacific populations of this species through the implementation of cDNA microarrays, and found that the spatial differences in gene expression are not directly related to a latitudinal gradient, since there are different environmental variables that may be related to these changes. Furthermore, Dutton and Hofmann (2009) characterized the thermal shock response in four populations of the same species through gene expression analysis of HSP70 and HSC71, finding acclimatization through the expression levels of both genes involved in mediating thermal stress.

In the case of our model species, M. chilensis, previous studies have reported that although this species has a wide distribution range, it is not possible to find significant differences in genetic structure using allozymes in populations from southern Chile (Toro et al., 2006). This study is the first in characterizing and revealing spatial patterns of gene expression in populations of M. chilensis, as well as their relationship with local patterns of variability in environmental conditions. An intriguing possibility raised by this study is that expression of these candidate genes in M. chilensis may show acclimatization to environmental variability over a certain temporal scale, and serve as an indicator for non-lethal effects of climate change on coastal benthic communities. Unfortunately, limitations of the data currently available preclude a full test of such hypothesis. A future study involving reciprocal transplant experiments, as well as the analysis of gene expression in mussels collected under contrasting conditions, should shed light on these pending questions.

Gene expression data are a powerful tool to evaluate transcriptomic changes associated with environmental conditions, allowing to conduct biogeographic studies that focus on the behavior and/or response of natural populations to environmental variability. Additionally, the quantification of variability in the expression of candidate genes can be used to search for molecular markers, such as EST-SSR and SNP, due to its association with significant variations in gene expression, making it possible to observe local adaptation processes in populations with wide biogeographic ranges.

**Author contributions**

C.G. conceived the ideas; G.N. and F.T. collected field data; G.N., P.H., F.T. and C.G. analyzed data and C.G. led the writing process.

**Supplementary data related to this article can be found online at doi:10.1016/j.jembe.2012.03.024.**

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