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REVIEW

The environmentally tuned transcriptomes of *Mytilus* mussels

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ABSTRACT

Transcriptomics is a powerful tool for elucidating the molecular mechanisms that underlie the ability of organisms to survive and thrive in dynamic and changing environments. Here, we review the major contributions in this field, and we focus on studies of mussels in the genus *Mytilus*, which are well-established models for the study of ecological physiology in fluctuating environments. Our review is organized into four main sections. First, we illustrate how the abiotic forces of the intertidal environment drive the rhythmic coupling of gene expression to diel and tidal cycles in *Mytilus californianus*. Second, we discuss the challenges and pitfalls of conducting transcriptomic studies in field-acclimatized animals. Third, we examine the link between transcriptomic responses to environmental stress and biogeographic distributions in blue mussels, *Mytilus trossulus* and *Mytilus galloprovincialis*. Fourth, we present a comparison of transcriptomic datasets and identify 175 genes that share common responses to heat stress across *Mytilus* species. Taken together, these studies demonstrate that transcriptomics can provide an informative snapshot of the physiological state of an organism within an environmental context. In a comparative framework, transcriptomics can reveal how natural selection has shaped patterns of transcriptional regulation that may ultimately influence biogeography.

KEY WORDS: Intertidal, Mussel, *Mytilus*, Stress, Transcriptomics

Introduction

The rocky intertidal zone is a highly variable marine environment, with respect to prevailing abiotic factors. Whilst some abiotic factors, such as the tidal and diel cycle are predictable, others, such as solar heating and wave action, vary greatly on a day-to-day basis and depend on the prevailing climatic and wave conditions (Denny and Wethey, 2001). Other factors vary over longer-term seasonal cycles and in response to global climate change (Helmuth et al., 2002). This unique combination of changing abiotic factors makes the intertidal zone and its inhabitants fascinating subjects for the study of the effects of varying environmental factors on animal physiology (Somero, 2002). Unlike many extreme environments that are difficult to access and study on a continuing basis, rocky intertidal habitats are readily accessible sites for scientists who are interested in the physiological mechanisms that enhance survival in challenging environments.

Among the diverse organisms found in rocky intertidal habitats, sessile species such as mussels, make excellent study systems for examining environmental stress. Mussels of the genus *Mytilus* frequently dominate the low to mid rocky intertidal region in temperate seas of the northern and southern hemispheres and thus are a key organism in coastal ecosystems (Seed, 1992). Whilst some mobile animals are able to reduce the severity of stress by moving to less-exposed positions on the shore, mussels, as sessile organisms, must endure stressful episodes (Halpin et al., 2002) and thus are likely to possess particularly pronounced mechanisms to deal with stress. For example, analysis of the oyster genome revealed an expansion of the genes coding for heat shock protein 70 and apoptosis inhibitors, which was interpreted as an adaptation to intertidal stressors (Zhang et al., 2012). One advantage of studying sessile organisms is that the same mussel assemblage can be sampled repeatedly over periods of days, weeks and even years, thus allowing phenotype to be interpreted within the context of both prevailing and prior abiotic events (Dowd et al., 2013; Gracey et al., 2008; Letendre et al., 2009; Regoli et al., 2004). This has made mussels particularly appropriate animals for investigating adaptive physiological processes that allow life to flourish in a fluctuating environment (Braby and Somero, 2006a; Fields et al., 2006; Hofmann and Somero, 1995; Logan et al., 2012; Williams and Somero, 1996).

There is a rich literature on the physiological adaptations exhibited by *Mytilus* spp. in the intertidal zone, with particular attention placed on the expression of heat shock proteins as an indicator of heat stress between sites and seasons (Buckley et al., 2001; Halpin et al., 2002; Hofmann and Somero, 1995), metabolism and pH regulation during emersion (Bayne et al., 1976a,b; Fan et al., 1991), and ratios of RNA/DNA as indicators of metabolic activity (Dahlhoff, 2004). However, there is still a poor understanding of how different adaptive strategies are integrated within and between tissues to produce the whole organism adaptations to varying environmental conditions. Similarly, we know relatively little about the regulatory mechanisms that underpin acclimatization to local conditions, or the mechanistic basis for differences in the environmental tolerance and biogeography of the different species of *Mytilus*.

The aim of this review is to discuss the insights that gene expression profiling approaches have made on investigations into the intertidal adaptations and environmental tolerance of *Mytilus* spp. These RNA transcript screening approaches have the potential to provide a system-wide perspective of the regulatory processes that are associated with the response to a particular environmental challenge or a particular physiological state (Gracey and Cossins, 2003). While there is no doubt that regulation of transcription is just one of several levels at which biological regulation occurs, and that phenotype results from integration at all levels, the monitoring of global transcript abundance remains one of the best indices of the physiological state of the cell and can unite events at the molecular level with traits that emerge at higher levels of organization (Ghazalpour et al., 2011).

Though we focus our review on *Mytilus* mussels, we would like to emphasize that transcriptomics is a versatile tool that has been used to uncover the molecular bases of physiological responses to the environment in many intertidal species. For example, Ronges et al. (2012) used transcriptomics to characterize the time course of changes in gene expression during cold

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The Journal of Experimental Biology

acclimation in porcelain crabs, *Petrolisthes cinctipes*. Evans et al. (2013) employed transcriptomics to discover that larval sea urchins (*Strongylocentrotus purpuratus*) show robust changes in the expression of genes involved in calcification and pH regulation in response to ocean acidification (elevated P_{CO_2} treatment), thus indicating that there may be some degree of resilience to ocean acidification in populations and/or species that routinely experience upwelling of low pH water. In yet another study, Collén et al. (2007) measured transcriptional responses to multiple stressors (i.e. osmotic stress, heat and high light) in the red seaweed *Chondrus crispus* and their results were among the first to show the prevalence of oxidative stress in intertidal organisms. In addition to describing physiological responses to abiotic factors like temperature and pH, transcriptomics has revealed the molecular mechanisms that underlie responses to predation in intertidal snails (Chu et al., 2014) and larval settlement in barnacles (Chen et al., 2011).

Transcriptomics has also been used in a comparative framework to elucidate evolutionary outcomes of natural selection on transcriptional regulation. For example, Mandic et al. (2014) compared the transcriptional responses to hypoxia of subtidal and intertidal species of sculpin and revealed divergent transcriptional responses that were correlated with inter-specific differences in hypoxia tolerance (Mandic et al., 2014). In a study on intertidal copepods (*Tigriopus californicus*), Schoville et al. (2012) compared transcriptional responses to heat stress between warm- and cold-adapted populations to uncover the molecular basis of thermal tolerance. They found that warm-adapted populations of *T. californicus* have a more robust heat shock response, particularly among genes that encode heat shock proteins (Schoville et al., 2012). In a related study of population divergence, Pespeni et al. (2013) correlated gene expression to growth under common-garden conditions between northern and southern sea urchin populations (*S. purpuratus*) and found that population-level differences in growth were underlined by the differential expression of genes involved in protein synthesis and biomineralization. In Pacific oysters (*Crassostrea gigas*), Lang et al. (2009) showed that transcriptional responses to heat stress varied between selectively bred families in aquaculture, whereas Chaney and Gracey (2011) identified a set of genes whose differential expression was correlated with future mass mortality events. These studies illustrate the power and applicability of transcriptomics to a diverse array of study systems and research questions. We now turn our focus to mussels to gain an in-depth insight into the molecular mechanisms that mediate organism–environment interactions in the genus *Mytilus*.

Mytilus mussels: evolutionary history and modern biogeography

The origins of *Mytilus* can be traced back to the North Pacific Ocean (Seed, 1992; Vermeij, 1991) where the four species, *Mytilus californianus*, *Mytilus trossulus*, *Mytilus edulis* and *Mytilus galloprovincialis*, shared a common ancestor approximately 7.6 mya (Ort and Pogson, 2007). The California mussel *M. californianus* represents a distinct lineage from the other three species, which make up the blue mussel species complex (Ort and Pogson, 2007; Seed, 1992). The blue mussels shared a common ancestor approximately 3.5 mya, when *M. edulis* diverged from *M. trossulus* in the North Atlantic (Heath et al., 1995; Vermeij, 1991). This event was followed by the emergence of *M. galloprovincialis* in the Mediterranean Sea approximately 2 mya (Barsotti and Meluzzi, 1968). There is a fifth *Mytilus* species, *Mytilus coruscus*, that is native to the western Pacific (Seed, 1992).

In the present day, *Mytilus* species inhabit a wide range of temperate coastal environments throughout the globe. *M. californianus* has a broad latitudinal range that extends along the eastern Pacific from Baja California to Alaska (Seed, 1992), where it characteristically inhabits wave-exposed sites of the rocky intertidal zone, although it can also be found in subtidal habitats as well. The blue mussels can also be found at wave-exposed sites (Seed, 1992), but in the eastern North Pacific they tend to be more prevalent in protected habitats, such as bays and estuaries (Braby and Somero, 2006b; Lockwood and Somero, 2011a; Schneider and Helmuth, 2007). The biogeographic range of the blue mussels extends across several continents (Hilbish et al., 2000; McDonald and Koehn, 1988; Seed, 1992). *M. trossulus* is found in the eastern and western North Pacific, as well as the western North Atlantic and Baltic Sea. *M. edulis* is found on both sides of the North Atlantic, as well as the southern tip of South America. *M. galloprovincialis*, a native of the Mediterranean Sea, has established invasive populations on the coasts of California, East Asia and South Africa as a result of human transport. In addition, there are native populations of *M. galloprovincialis* (subspecies: *Mytilus galloprovincialis planulatus*) in Australia, Tasmania and New Zealand that are the result of a trans-equatorial migration that took place approximately 1.5 mya (Hilbish et al., 2000).

Biological rhythms in an intertidal habitat

The marine intertidal zone is where terrestrial and marine habitats converge, and the inhabitants are subject to both the 24 h light and dark daily rhythm of the terrestrial earth and also the 12.4 h ebb and flow of the tidal cycle. The ecology of these ecosystems is strongly influenced by the tidal cycle because it determines the duration that inhabitants are emersed, which ultimately influences the vertical distribution of organisms on the shore (Somero, 2002; Suchanek, 1981). To match the rise and fall of the tide, many inhabitants exhibit circatidal biological rhythms, which allow organisms to anticipate and respond accordingly to predictable daily changes in tidal height (Tessmar-Raible et al., 2011; Wilcockson and Zhang, 2008). There is increasing evidence that these rhythms are driven by an endogenous time-keeping circatidal clock because they persist even when tidal cues are removed (Takekata et al., 2012; Zhang et al., 2013). The most thoroughly characterized circatidal rhythms are behavioral and many intertidal inhabitants will exhibit periods of foraging activity that are coordinated with the tidal cycle (reviewed in Palmer, 1973, 1975) and with changes in tidal height (Stillman and Barnwell, 2004).

An effort to investigate the relative contribution of both the light/dark cycle and the tidal cycle on the physiology of *M. californianus* was undertaken by monitoring rhythms of gene expression in both simulated and natural tidal environments (Connor and Gracey, 2011). In the intertidal simulations, mussels were sampled at 2 h intervals under conditions of alternating 6 h episodes of immersion and aerial emersion, and a 12 h:12 h light:dark regime. Statistical analysis identified 2756 rhythmic transcripts, comprised of one group of 236 transcripts that oscillated with a tidal period of 12 h, and a larger group of 2365 transcripts that oscillated at a circadian period of ∼24 h. The tidal transcripts displayed oscillations that peaked at all phases of immersion and emersion, with the majority of transcripts peaking in the middle of each episode of immersion and emersion (Fig. 1). In contrast, circadian transcripts fell into two broad groups, with one expressed around dawn and the other at dusk. A similar gene expression dataset was also collected from mussels located in the field that were acclimated to either subtidal or intertidal conditions and then sampled *in situ* at 2 h intervals over 50 h. These data validated the laboratory simulations and of the 697 rhythmic transcripts identified in the field mussels, only 72 exhibited a tidal periodicity while 501 exhibited a circadian period.

The Journal of Experimental Biology (2015) 218, 1822-1833 doi:10.1242/jeb.118190

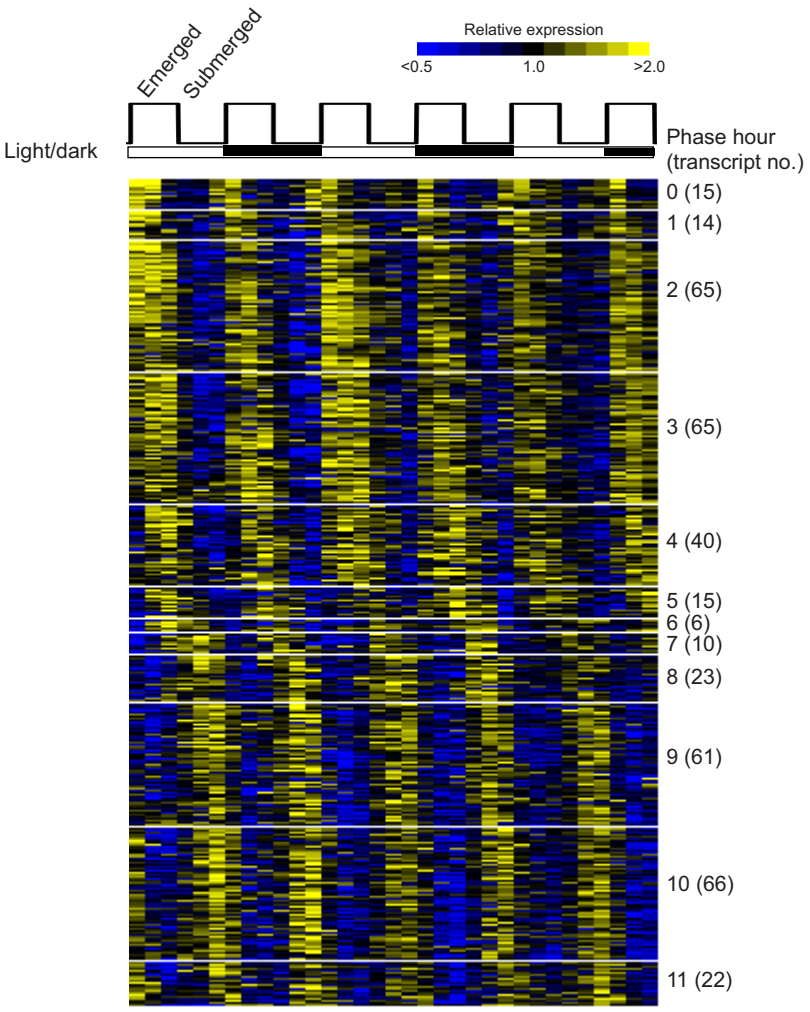

Fig. 1. Gene expression rhythms in *Mytilus californianus* associated with alternating bouts of aerial emersion and immersion. Gene expression profiles of transcripts that exhibited a 12 h period under simulated tidal conditions of 6 h emersion and 6 h immersion over 66 h. Animals were sampled every 2 h at 1, 3 and 5 h intervals within each 6 h bout. Transcripts were grouped and ordered according to the phase of peak expression (0–11 h, with a phase of 0 h indicating transcripts that peak at the transition to emersion, 6 h indicating transcripts that peak at the transition to immersion and 11 h indicating transcripts that peak at the end of the immersion). The number of transcripts assigned to each phase is indicated in brackets on the right of each heatmap. The scale bar represents $\log_2$ ratio, with blue showing down-regulation, yellow up-regulation and black no difference from the control condition. The figure shows a re-analysis of circatidal phase from the data published by Connor and Gracey (2011).

The abundance of circadian transcripts indicates that the 24 h light/dark cycle rather than the 12.4 h tidal cycle is the dominant zeitgeber or exogenous cue that serves to synchronize rhythmic gene expression to the changing environment of *M. californianus*. This discovery was unexpected because emersion represents a dramatic shift in environmental conditions that also causes profound metabolic changes, including physiological hypoxia and a cessation of cardiac activity (Bayne et al., 1976b). The nature of the sensory, transduction and regulatory pathways involved in the entrainment of mussel gene expression rhythms to the environment remains unclear.

As a final component in the study, Connor and Gracey also explored the effect that elevated temperatures, such as those that mussels experience during daytime emersion (Helmuth and Hofmann, 2001), would have on expression rhythms (Connor and Gracey, 2011). This revealed that imposition of a modest heating episode of +7°C during emersion ablated the rhythmicity of 37% of the tidal transcripts and 17% of the circadian transcripts. This finding suggests that thermal perturbations have consequences beyond their direct effect on macromolecular structures (Feder and Hofmann, 1999) by affecting both the timing and amplitude of biological rhythms in intertidal inhabitants.

In a companion study, changes in metabolism were investigated by measuring changes in heart rate and by analysis of metabolite levels in the gills of the same mussels using mass-spectrometry-enabled metabolomics (Connor and Gracey, 2012). The analysis of heart rates showed that animals enter a period of dramatically reduced cardiac activity, or bradycardia, during bouts of emersion, with the drop in heart rate occurring within minutes of being exposed to air. The concurrent screen of metabolite abundance patterns identified 24 metabolites (of 172 identified metabolites) that exhibited a rhythmic change in abundance, with all 24 metabolites oscillating with a period of 12 h. Thus, both cardiac activity and changes in abundance of gill metabolites were driven by the tidal cycle of alternating episodes of immersion and emersion. The most striking theme detected within the metabolome data was evidence of a shift between anaerobic and aerobic metabolism during the simulated periods of low and high tide, respectively. For example, the relative abundance of both malate and succinate increased dramatically during low tide, a pattern that is consistent with the previously described induction of anaerobiosis in emersion or hypoxic mussels (Bayne et al., 1976b). Upon reimmersion, malate and succinate levels declined whereas citrate levels climbed, indicating that these anaerobic end products were rapidly metabolized by the TCA cycle upon the resumption of aerobic metabolism. The switch between anaerobic and aerobic metabolism appears to be under the physical control of valve movement, with the valves being closed during emersion to prevent desiccation, but this also restricts access to environmental oxygen, leading to a rapid decline in tissue oxygenation (Fan et al., 1991). The metabolomic screen also revealed a hitherto unanticipated role that carnitine metabolism may play in the orchestration of this metabolic shift,

by revealing that many intermediate metabolites accumulated as carnitine conjugates during low tide emersion. These intermediate metabolites are normally metabolized by the mitochondria. However, during emersion-induced anaerobiosis, the levels of intermediate metabolites increase and they are rapidly conjugated to carnitine rather than accumulating as their CoA derivatives. Elevated acyl-CoA levels can be damaging to cells (Song et al., 2009) and so the conjugation of carnitine to these intermediate metabolites during emersion may serve to protect the cell from the accumulation of acyl-CoA (Bremer, 1990).

Together, these 'omics' studies provide the most comprehensive overview to date into how transcript and metabolite levels are orchestrated in mussels under a tidal regime, and show that ~40% of the transcriptome exhibits rhythmic changes in abundance, with 80–90% of the rhythmic transcripts exhibiting a circadian 24 h oscillation (Connor and Gracey, 2011), while rhythmic changes in metabolite abundance are associated solely with a tidal 12 h oscillation (Connor and Gracey, 2012). Given the preponderance of circadian transcriptional rhythms in the intertidal mussels, it is surprising that no metabolites were detected that oscillate with a circadian rhythm. In terrestrial organisms, most metabolites oscillate with a circadian period that appears to be linked to daily feeding regimes with the ingestion of food serving as a potent exogenous cue (Eckel-Mahan et al., 2012). Given that, for mussels, feeding is restricted to periods of immersion, rhythms of metabolite and transcript abundance may turn out to be driven by tidal-controlled access to both food and oxygen during immersion. These studies further emphasize the importance that the light/dark cycle has played in the evolution of daily patterns of transcriptional regulation (Edgar et al., 2012) and highlight how daily rhythms resonate, even in organisms that today experience strong sub-daily external environmental cues, such as those arising from the tidal cycle.

Orchestration of life at intertidal extremes

Most mussel beds on the western coast of North America are located in the mid intertidal range where they experience a mixed semidiurnal tidal cycle of two high and two low tides of different size every lunar day. Although rare along this coast, mussel assemblages that reside at higher positions in the intertidal zone are present, which, as a result, are only inundated once a day during the larger of the high tides (Gracey et al., 2008). To explore how life is orchestrated in this extreme intertidal environment Gracey et al. (2008) sampled mussels located in beds in either this upper exposed intertidal zone or a lower mid intertidal site at 20 time points across 3 days and then analyzed RNA expression patterns in gill, adductor muscle and hepatopancreas tissues. This revealed a striking pattern of gene expression periodicity in the higher intertidal mussels, with the identification of two groups of genes that displayed anti-correlated patterns of gene expression, such that, as the expression of one set of genes increased, the other fell and vice versa. This pattern was observed in all three tissues, suggesting that transcriptional programs were synchronized across tissues. Functional interpretation of the types of genes that comprised these two groups revealed that one group was dominated by genes involved in a variety of metabolic processes, such as the TCA cycle, electron transport, metabolism and proteolysis, and whose products were often located in the mitochondria and cytoplasm, while the other group comprised genes involved in cell division, transcription, mRNA processing and the ubiquitin cycle, and included many gene products that are located in the nucleus. Thus, these high intertidal mussels were temporally switching between expressing genes that could be broadly classified as involved in metabolism and mitochondrial function versus another group that were involved in cell division and gene regulation.

One hypothesis arising from the observed patterns is that mussels located at high sites alternate between periods of growth and cell proliferation, interspersed with intervals of recovery during which the cellular energy charge is restored. This temporal pattern may be analogous to the discovery in yeast that metabolic and cell division events are compartmentalized in time (Tu et al., 2005). Rhythmic patterns of cell proliferation have been reported in *M. galloprovincialis*, and revealed that epithelial cell turnover in the digestive gland (a tissue that is constantly undergoing renewal) were synchronized with the tidal cycle, increasing during low tide and decreasing during high tide (Zaldibar et al., 2004). It has been suggested that the functional rationale behind the temporal compartmentalization of physiological processes is to ensure that processes that might be deemed sensitive to oxidative stress are temporally separated from periods when reactive oxygen species may be present. For example, in yeast, DNA replication is temporally separated from bursts in respiratory activity to prevent the occurrence of DNA damage caused by free radicals released by active mitochondria (Chen et al., 2007).

Investigating habitat and biogeographical patterns of abiotic stress using transcriptome data collected from field assemblages

Abiotic factors vary both within and between rocky intertidal locations, with dramatic differences in environmental variables being recorded at spatial scales varying from centimeters (Denny et al., 2011) to hundreds of kilometers (Helmuth et al., 2002). One of the promises of transcript screening approaches is that environmental differences within sites and between geographical locations can be investigated through the prism of the differential gene expression of the inhabitants. According to this perspective, similarities and differences between expression signatures of mussels sampled at different places – either within a site or between locations – are a direct consequence of the historical and the prevailing environmental conditions at the sample sites.

Within-site microhabitat differences are predicted to have profound effects on the abiotic factors that an intertidal mussel experiences, particularly with respect to the extent that individuals experience solar heating during emersion (Helmuth et al., 2010). Comparisons of body temperatures and gene expression profiles of mussels sampled simultaneously from higher exposed and lower sheltered sites on the same intertidal habitat indicated that animals at lower sites were exposed routinely to lower temperatures over a 3 day sampling period (Gracey et al., 2008). This difference in thermal regime was supported by the finding that genes related to protein folding and the proteotoxic stress response were consistently more highly expressed in animals at higher sites, regardless of the time point at which the samples were collected. These data suggest that differences in day-to-day thermal regimes are manifested by changes in the transcriptome and support the long-held hypothesis that the elevated expression of protein chaperones serves a preparative or protective function in the inhabitants of the high intertidal zone (Hofmann and Somero, 1995; Roberts et al., 1997).

Other studies have attempted to use expression data to investigate biogeographical differences across broad spatial scales. Comparisons of *M. californianus* sampled at locations ranging from Baja Mexico up to British Columbia suggested that thermal variation between locations was the most plausible explanation for the observed differences in gene expression (Place et al., 2008). When studies also took into account within-site variation (higher versus lower on the

The Journal of Experimental Biology (2015) 218, 1822-1833 doi:10.1242/jeb.118190

shore), they revealed that mussels sampled at the same location tended to show expression signatures that were more similar to one another, than to mussels sampled at the same tidal height but at different locations (Place et al., 2012). This finding indicates that there exists a complex interaction between microhabitat differences and broader scale oceanographic process, for example, food availability, and that many environmental variables need to be accounted for when comparing expression data collected from different geographical locations.

Another significant challenge to interpreting observations collected across wide geographical ranges is how to control for the temporal changes in gene expression that are driven by local tidal and diel factors. Calibrating and normalizing between-site data so that rhythmic changes in transcript abundance do not degrade the statistical power of the study is a formidable task and has yet to be properly addressed. For example, a biogeographical study reported that both citrate synthase and G2/M mitotic-specific cyclin B were differentially expressed between locations (Place et al., 2012), whereas another study reported that these same genes exhibited extensive hourly variation within a site (Gracey et al., 2008). Caution needs to be exercised when interpreting these patterns because temporal expression changes are likely to confound efforts to identify genes that are intended to serve as molecular markers for site-to-site environmental differences. Similarly, thermal regimes will vary seasonally within a site and the thermal experiences of intertidal inhabitants will be driven by the complex interplay between the prevailing climatic conditions and the tidal cycle (Helmuth et al., 2002). For example, analysis of gene expression in mussels sampled opportunistically following an unusually hot low tide revealed a transcript signature that was enriched for cell damage response genes whose expression was not normally elevated during routine low tide heating events (Gracey et al., 2008). Given the plasticity of the transcriptional patterns and their sensitivity to the prevailing and immediately preceding conditions, there will be challenges in extrapolating between gene expression patterns collected across a limited range of time points and the perceived ‘average’ level of environmental stress associated with a particular geographical location.

The blue mussels: physiology influences biogeography

Because environmental factors influence physiology, physiological traits are targets of natural selection that can ultimately shape patterns of biogeography. *Mytilus* blue mussels have been a powerful system for demonstrating this phenomenon. A good example is the clinal pattern of leucine aminopeptidase *Lap* alleles among populations of *M. edulis* in Long Island Sound in the Northwest Atlantic. Koehn and Hilbish did classic work, reviewed by Koehn and Hilbish (1987), that connected clines in *Lap* allele frequencies in Long Island Sound to functional biochemical and whole-organism survival data to show that particular alleles of this gene are favored in salty ocean habitats but disadvantageous in brackish habitats. To summarize, these authors found one *Lap* protein variant in *M. edulis* (i.e. *Lap-94*) that produces more free amino acid osmolytes inside cells and this allele confers greater survival in ocean habitats because it allows mussels to more efficiently osmoconform. However, *Lap-94* variants have decreased survival in brackish habitats because a higher osmolyte concentration leads to a metabolic cost, as cells need to actively transport these osmolytes outside of the cell to achieve the correct osmotic balance in habitats with lower salinity.

More recently, the blue mussels have emerged as a comparative system for studying the physiological factors that influence biogeographic patterns of native versus invasive species in the Northeast Pacific. Although the blue mussels are closely related species, they evolved under disparate abiotic conditions (Mann and Hamilton, 1995; Thunell, 1979; Vermeij, 1991), which likely influences their modern biogeographies. The evolutionary history of *M. galloprovincialis* has predisposed it to being a successful invasive species in environments similar to that of its home range in the Mediterranean Sea (Lockwood and Somero, 2011a). In the Northeast Pacific, *M. galloprovincialis* has displaced the native *M. trossulus* along the southern end of its former range in central and southern California (Geller, 1999). Currently, the southern range limit of *M. trossulus* is Monterey Bay. South of Monterey, populations of blue mussels are entirely *M. galloprovincialis* (McDonald and Koehn, 1988; Rawson et al., 1999). The two species co-occur and form un-fit hybrids (Brannock et al., 2009; Rawson et al., 1999) from Monterey north to Humboldt Bay, and north of Humboldt, *M. trossulus* is the dominant species (Heath et al., 1995; McDonald and Koehn, 1988). The northern range limit of *M. galloprovincialis* fluctuates according to the Pacific Decadal Oscillation (Hilbish et al., 2010), further lending support to the idea that environmental factors such as temperature establish biogeographic patterns in these species.

Thermal responses in *Mytilus trossulus* versus *Mytilus galloprovincialis*

Mytilus trossulus and *M. galloprovincialis* differ markedly in thermal tolerance. The intertidal zone is a dynamic environment that undergoes daily and seasonal shifts in temperature, salinity, pH, and oxygen concentration. These environmental shifts disrupt molecular structures and perturb cellular processes (Kültz, 2005) and *Mytilus* spp. have adapted to cope with these changes via mechanisms that preserve cellular homeostasis (Banni et al., 2011; Gilby et al., 1997; Hofmann and Somero, 1995). However, each species is adapted to cope with the range of conditions that they most often encounter in their native environments. Therefore, abiotic factors, such as temperature and salinity, are likely to play a role in setting biogeographic range limits (Tomanek, 2008). Indeed, where *M. trossulus* and *M. galloprovincialis* co-occur in central California, *M. galloprovincialis* out-competes the native species at sites that are warmer and saltier (Braby and Somero, 2006b; Schneider, 2008; Schneider and Helmuth, 2007). In addition, *M. trossulus*, *M. edulis* and *M. galloprovincialis* maintain a greater scope for growth at cold, cool and warm temperatures, respectively (Fly and Hilbish, 2013).

Hofmann and Somero (1996) were the first to demonstrate that *M. trossulus* and *M. galloprovincialis* possess different heat tolerances, as evidenced by differences in the expression of heat shock protein 70 (Hsp70). They found that *M. trossulus* had higher constitutive levels of Hsp70 but that *M. galloprovincialis* was able to induce Hsp70 expression to a greater degree than *M. trossulus* upon exposure to acute heat stress. Following up on this study, Braby and Somero (2006a) compared the cardiac physiology of these two species. They found that *M. trossulus* maintains a higher resting heart rate – consistent with the thermal compensation necessary for living in cooler environments. However, they found that the critical heart rate temperature (H_{crit}), i.e. the temperature at which heart rate collapses during a heat ramp, was on average three degrees lower for *M. trossulus* than for *M. galloprovincialis*. This indicates that *M. galloprovincialis* has a higher thermal tolerance than *M. trossulus* because there is a tight correlation between H_{crit} and the lethal temperature of a mussel (Logan et al., 2012). Conversely, *M. trossulus* appears to have a substantially better cold tolerance

than *M. galloprovincialis*, because *M. trossulus* can maintain cardiac performance at extremely low temperatures, even as low as 0°C, whereas *M. galloprovincialis* cannot (Braby and Somero, 2006a). These striking differences in thermal physiology reflect the disparate evolutionary histories of these species and are likely to influence the range of environments in which they exist in the present day.

One of the major questions that came out of the work by Braby and Somero (2006a) was the extent to which the differences in cardiac physiology were underlined by molecular differences, such as gene and protein expression. The study by Hofmann and Somero (1996) begins to address this question, but this study only provides quantitative measurements of one protein, i.e. Hsp70. As such, the studies by Hofmann and Somero (1996) and Braby and Somero (2006a) laid the groundwork for two sets of companion studies by Lockwood et al. (2010), Lockwood and Somero (2011b) and Tomanek, Zuzow and Fields (Fields et al., 2012; Tomanek, 2010) that compared the transcriptomic and proteomic responses of *M. trossulus* and *M. galloprovincialis* after exposure to acute heat and low salinity (hypo-osmotic) stressors.

Lockwood et al. (2010) compared the transcriptomic responses of common-garden acclimated *M. trossulus* and *M. galloprovincialis* to acute heat stress. They exposed animals to a heat ramp similar to that of Braby and Somero (2006a) and measured global gene expression with microarrays. Perhaps the most remarkable finding from this comparison was that *M. trossulus* and *M. galloprovincialis* did not exhibit major differences in their transcriptional responses to acute heat stress (Lockwood et al., 2010). In fact, the vast majority of genes that showed significant responses were largely similar in the two species. Only 6% of the genes that showed significant responses to heat stress were different between the species. Moreover, most of the common stress-response genes, including nearly all of the molecular chaperones, showed shared responses between the species. Despite the overall similarities, there were important differences between the species. Most notably, *M. trossulus* induced genes involved in proteolysis to a greater degree than *M. galloprovincialis*, such as genes encoding subunits of the proteasome. Meanwhile, *M. galloprovincialis* strongly induced a gene encoding the molecular chaperone small heat shock protein 24 (Hsp24), whereas *M. trossulus* showed only a muted response of this gene. These results suggest that wholesale evolution of transcriptional regulation across the genome is not necessary to achieve the degree of thermal tolerance that distinguishes *M. galloprovincialis* from *M. trossulus*.

This is an important finding that has implications for the future adaptation of populations and species to climate change. The degree of differentiation that separates these two species in terms of their thermal tolerances to acute heat stress is approximately 3°C (Braby and Somero, 2006a). Therefore, species may be able to adapt to rising global temperatures, at least in the short term, because all that may be required to climb the thermal tolerance curve is the differential regulation of a handful of genes (Lockwood et al., 2010). In fact, organisms like *Mytilus* that have large effective population sizes are likely to be able to adapt quickly in response to future climate change because larger populations have access to a greater degree of standing genetic diversity for natural selection to act on. In addition, *Mytilus* populations on the whole should have access to a wide range of mutational events across all genomes in the population, so beneficial mutations may be encountered often and spread quickly through the population (Karasov et al., 2010).

Transcriptomic studies provide a comprehensive view of the genomic response of an organism to environmental stress, and in a comparative framework, transcriptomics elucidates the degree of evolution that has occurred in regulatory regions of the genome. However, transcriptomic studies are limited in that they may or may not reflect the full range of molecular physiological responses. Therefore, taking the comparative approach in *Mytilus* one step further, Tomanek and Zuzow (2010) measured the proteomic responses of mussels that were exposed to the same heat stress, and were in fact from the same experiment, as the Lockwood et al. (2010) study. Overall, similar to the transcriptomic study, Tomanek and Zuzow (2010) found that the two species shared common proteomic responses to heat stress. However, some of the details of the proteomic responses differed from the transcriptional responses. In particular, while the overall responses between the two mussels were shared at the level of the protein, the onset temperatures for the expression of heat shock proteins were lower in *M. trossulus*. Consistent with the transcriptomic data, the expression of proteasome subunits was higher in *M. trossulus*. Indeed, it is overwhelmingly apparent that *M. trossulus* is more heat sensitive than *M. galloprovincialis*.

The overall shared responses of heat shock protein expression in *M. trossulus* and *M. galloprovincialis*, as discovered by Lockwood et al. (2010) and Tomanek and Zuzow (2010), is in contrast to the findings of Hofmann and Somero (1996). In the previous study, Hofmann and Somero (1996) showed substantial differences between *M. trossulus* and *M. galloprovincialis* in the heat shock response, particularly in the expression of Hsp70. This discrepancy can be reconciled by considering the details of the experimental designs. In the study by Hofmann and Somero (1996), gill tissue was sampled immediately after an acute heat stress exposure of 3 h, whereas Tomanek and Zuzow (2010) allowed their samples to recover at a non-stressful temperature for 24 h before sacrifice. As such, Hofmann and Somero (1996) quantified the immediate proteomic response to acute heat stress, which, not surprisingly, identified primary differences in the expression of Hsp70. In contrast, the second study examined the cumulative effect of heat stress on protein expression across a full day of recovery and therefore represents a more holistic view of different classes of proteins involved in a broad range of cellular processes. In addition, Tomanek and Zuzow (2010) had greater resolution to identify proteins via 2D gel electrophoresis and mass spectrometry; therefore, this more recent study gives a more comprehensive snapshot of the heat-stress-induced proteomes of *M. trossulus* and *M. galloprovincialis*. Taken together, these two studies complement one another and describe the proteomic underpinnings of the differences in acute thermal tolerance between *M. trossulus* and *M. galloprovincialis*.

Another factor that is likely to play a role in determining the relative success of *M. trossulus* versus *M. galloprovincialis* is the ability to acclimate to different thermal environments. This is a particularly relevant question with regard to how these two species will respond to climate change. Fields et al. (2012) compared the proteomic responses of *M. trossulus* and *M. galloprovincialis* to acclimation for 4 weeks to 7°C, 13°C and 19°C, and they found that the cold-adapted *M. trossulus* induced molecular chaperones when acclimating to 19°C while *M. galloprovincialis* did not. Conversely, *M. galloprovincialis* showed a more significant proteomic response to cold acclimation than *M. trossulus* and changed the expression of molecular chaperones as well as a number of proteins involved in cytoskeletal remodeling and oxidative stress (Fields et al., 2012).

Responses to salinity stress in *Mytilus trossulus* versus *Mytilus galloprovincialis*

There is evidence that suggests that tolerance to changes in salinity may also differ between these two species and may thus contribute

The Journal of Experimental Biology (2015) 218, 1822-1833 doi:10.1242/jeb.118190

to their distinct biogeographic ranges. In fact, at some sites where the two species co-occur in central California, the frequency of *M. galloprovincialis* is more correlated with salinity than with temperature (Braby and Somero, 2006b). The physiological reason for this may, in part, be explained by the fact that *M. galloprovincialis* hearts respond in a similar way to low salinity stress (hypo-osmotic shock) as *M. trossulus* hearts respond to heat stress (Braby and Somero, 2006a). That is, *M. galloprovincialis* hearts collapse more readily than *M. trossulus* hearts during acute hypo-osmotic stress exposure. This indicates that salinity tolerance may be an additional factor that limits the spread of *M. galloprovincialis* in northern latitudes because seasonal rainfall is a significant source of freshwater input into coastal habitats in the Northeast Pacific (Braby and Somero, 2006b).

To shed light on the possible effects of a sudden change in salinity on cellular responses that underlie whole-organism physiological responses, Lockwood and Somero (2011b) compared the transcriptomic responses of *M. trossulus* and *M. galloprovincialis* to an acute salinity stress, i.e. a hypo-osmotic shock or sudden drop in salinity. Probably the most striking finding of this study was the relatively minor transcriptional responses to salinity treatment compared with the robust responses to heat stress that these authors found in their previous study (Lockwood et al., 2010). In fact, less than 2% of the genes represented on the microarray showed significant responses to salinity stress (Lockwood and Somero, 2011b). This is in contrast to the 36% of the transcriptome that changed in response to heat stress (Lockwood et al., 2010). Among the genes that changed expression in response to acute salinity stress, most showed a shared response between the species (Lockwood and Somero, 2011b). These shared response genes were characterized as being involved in osmoconformation and cell cycle control. Conversely, very few genes were different between the species in their transcriptional responses and were categorized as being involved in mRNA splicing, polyamine synthesis, exocytosis, translation, cell adhesion and cell signaling.

Perhaps this lack of transcriptional response was because the mussels were not that stressed, i.e. that the exposure conditions were not harsh enough to elicit a robust response. In contrast to this idea, the companion proteomics study by Tomanek et al. (2012), which was conducted on the same set of samples as those used for the transcriptomics study, found robust changes at the level of the proteome in response to this salinity treatment. A large body of work on osmoregulation confirms that the major loci of action for the cellular response to osmotic shock constitute post-translational modifications that modulate protein activity (Evans and Somero, 2010; Kültz and Avila, 2001; Kültz and Burg, 1998). These types of responses are likely to be much faster than transcriptional responses (Evans and Somero, 2009), which can take hours to produce protein, with the exception of the heat shock response that induces changes in protein expression in a matter of minutes (de Nadal et al., 2011; Weake and Workman, 2010). Indeed, phosphorylation events are a major part of the cellular stress response of *Mytilus* to osmotic stress (Evans and Somero, 2010). Moreover, there are substantial differences between the phosphorylation responses of *M. trossulus* and *M. galloprovincialis*, with *M. trossulus* consistently able to elicit a more robust phosphorylation response than *M. galloprovincialis* in response to salinity stress (Evans and Somero, 2010).

Transcriptomics has been an important discovery-based method for elucidating molecular patterns that underlie whole-organism tolerances to environmental stress. To follow up on this work, there are perhaps two lines of study that are particularly relevant. First, how do multiple environmental variables interact to affect physiological responses? Second, what are the effects of environmental stress across life stages? It is important to consider the effects of multiple stressors because in nature abiotic factors,

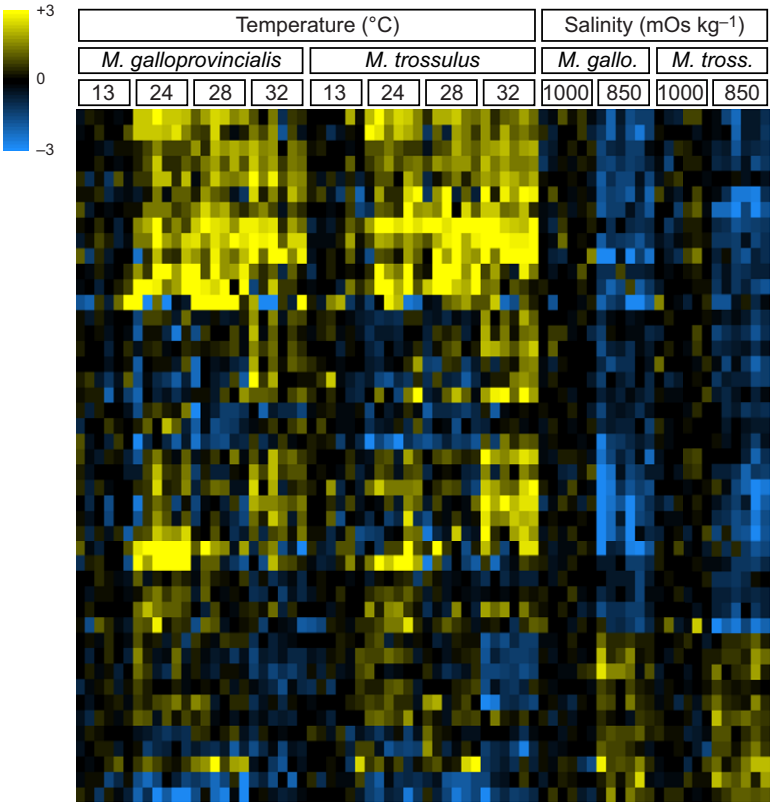

Fig. 2. Heat and low-salinity stressors lead to opposite directions of response in gene expression. Heat map of 45 genes that showed significant responses to acute heat and low salinity stressors in *Mytilus galloprovincialis* and *Mytilus trossulus* (Lockwood et al., 2010; Lockwood and Somero, 2011a,b). Many of these genes are involved in ion transport. In the heat stress study, mussels were common-garden acclimated to 13°C and then exposed to a heat ramp (+6°C h^{-1}) where samples taken at 24°C, 28°C, and 32°C for transcriptomic analysis. In the salinity stress study, mussels were common-garden acclimated to seawater at an osmolality of 1000 mOs kg^{-1} (35‰) and exposed to a salinity of either 1000 mOs kg^{-1} (control) or 850 mOs kg^{-1} (29.75‰) for 4 hours prior to sacrifice and subsequent transcriptomic analysis. The scale bar represents $\log_2$ ratio, with blue showing down-regulation, yellow up-regulation and black no difference from the control condition. *N*=6 for each species and experimental treatment. Modified from Lockwood and Somero (2011b).

The Journal of Experimental Biology (2015) 218, 1822-1833 doi:10.1242/jeb.118190

such as temperature and salinity, are never experienced in isolation. A comparison of Lockwood et al. (2010) and Lockwood and Somero (2011b) reveals a small set of genes that showed significant changes in expression in response to both temperature and salinity in *M. trossulus* and *M. galloprovincialis*. Forty-five genes make up this set of genes, many of which are involved in ion transport, and overwhelmingly, these genes showed opposite directions of response to the two stressors (Fig. 2) (Lockwood and Somero, 2011b). This suggests that, for some genes, simultaneous transcriptional responses to temperature and salinity stressors may actually cancel each other out, a phenomenon that may be explained by the effect of rising temperature on membrane permeability (Swan and Watson, 1999). Indeed, most of the ion-transport-related genes were induced in response to heat stress but repressed in response to hypo-osmotic salinity stress. Other transcriptomic studies of heat stress have reported similar patterns of induction among genes that encode ion transporters (Buckley et al., 2006; Gao et al., 2004). In contrast to the reciprocal responses that were observed among most of the genes in this set, one gene involved in redox balance and oxidative stress (i.e. the gene that encodes thioredoxin reductase 3) was induced in response to both temperature and salinity stressors. This result reflects the importance of redox regulation during episodes of cellular stress (Kültz, 2005) and is consistent with proteomic studies of *Mytilus* that found common responses to temperature and salinity stressors among proteins involved in oxidative stress (Fields et al., 2012; Tomanek and Zuzow, 2010; Tomanek et al., 2012). These trends in transcriptional responses to temperature or salinity stressors in *M. trossulus* and *M. galloprovincialis* pose a question that warrants further study: how will these species respond to concurrent changes in temperature and salinity?

It is important that we also consider the effects of environmental stress across life stages, which has largely been neglected in the field of environmental physiology. In the *Mytilus* system, the adult stage has received the most attention, probably because of experimental convenience. However, it is pertinent to also study earlier larval and juvenile stages. In particular, as a result of the long pelagic larval durations (Strathmann, 1985) and great distances that larvae and juveniles are transported (Addison et al., 2008), pre- and post-

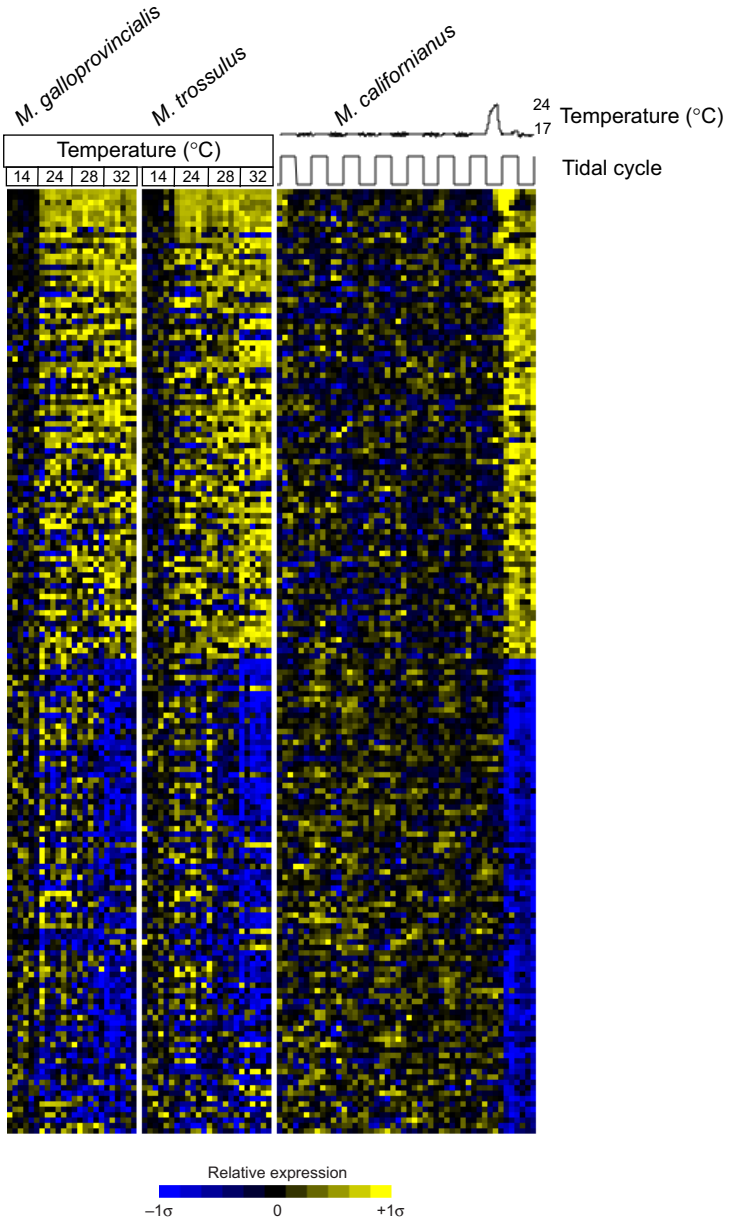

Fig. 3. *Mytilus galloprovincialis*, *Mytilus trossulus* and *Mytilus californianus* share a common transcriptional response to heat stress. Genes that exhibited statistically significant changes in genes expression in response to heat in the datasets of Lockwood et al. (2010) and Connor and Gracey (2011) were ranked according to the sum of their ranks of statistical significance in both datasets, with the most significantly induced genes being positioned at the top of the heatmap. Up-regulated common response genes are shown first followed by the common response down-regulated genes. In order to present the data on the same scale the gene expression data for each species were normalized to obtain a mean of zero and an s.d. of one. Yellow indicates higher levels of expression compared with the mean and blue indicates lower level of expression compared with the mean, with the s.d. indicated (σ).

The Journal of Experimental Biology (2015) 218, 1822-1833 doi:10.1242/jeb.118190

settlement mortality among juvenile *Mytilus* is likely to be a major force that shapes biogeography. Indeed, larval *M. trossulus*, *M. edulis*, and *M. galloprovincialis* are likely to have different tolerances to salinity stress (Hrs-Brenko et al., 1977; Qiu et al., 2002). It remains to be seen what molecular and cellular processes underlie differences in the physiology of whole larvae.

Common responses to heat stress in *Mytilus*

Despite the complexity of *Mytilus* transcriptomic responses to environmental stress, there are common themes that are consistent among species. In particular, there is a core set of 175 genes that exhibit robust changes in expression in response to acute heat stress and these patterns are consistent between labs (Connor and Gracey, 2011; Lockwood et al., 2010). In this section, we compare two datasets (Connor and Gracey, 2011; Lockwood et al., 2010) to reveal the common transcriptomic responses to acute heat stress of *M. californianus*, *M. trossulus* and *M. galloprovincialis*.

Among the 175 genes that show common responses to heat stress, 87 were induced and 88 were repressed in *M. californianus*, *M. trossulus*, and *M. galloprovincialis* (Fig. 3) (Connor and Gracey, 2011; Lockwood et al., 2010). These genes show similar patterns of expression across species and in response to different heating regimes (Fig. 3). Many of these genes have been shown to respond to environmental stress in a diverse array of organisms (Buckley et al., 2006; Dilly et al., 2012; Gasch et al., 2000; Place et al., 2012; Podrabsky and Somero, 2004). Among the genes that were induced, genes encoding heat shock protein 70 (Hsp70) and cAMP-responsive element-binding protein-like 2 (CREB-like 2) top the list as showing the most consistent responses among species (Fig. 4 and supplementary material Table S1). Other commonly induced genes include CCAAT/enhancer-binding protein, cathepsin C, c-jun protein and peroxiredoxin 6 (supplementary material Table S1). These genes encode proteins that are important players in the unfolded protein response (Lindquist and Craig, 1988) and the general cellular stress response (Kültz, 2005). Showing opposite directions of response, genes encoding spectrin β-chain and dynein heavy chain 10 were consistently repressed (Fig. 4 and supplementary material Table S1). Spectrin β-chain participates in the capping of actin filaments during actin polymerization (Machnicka et al., 2012; Pinder et al., 1975) and dynein heavy chain 10 is a subunit of dynein – the motor protein that binds microtubules to coordinate cellular movement (Schroer, 2004). Also of note among the list of commonly repressed genes are 14-3-3-like protein 1, DNA polymerase ζ catalytic subunit and homeobox protein Meis1 (supplementary material Table S1). The repression of these genes reflects the importance of regulating, and in some cases halting, certain cellular processes during heat stress, including protein

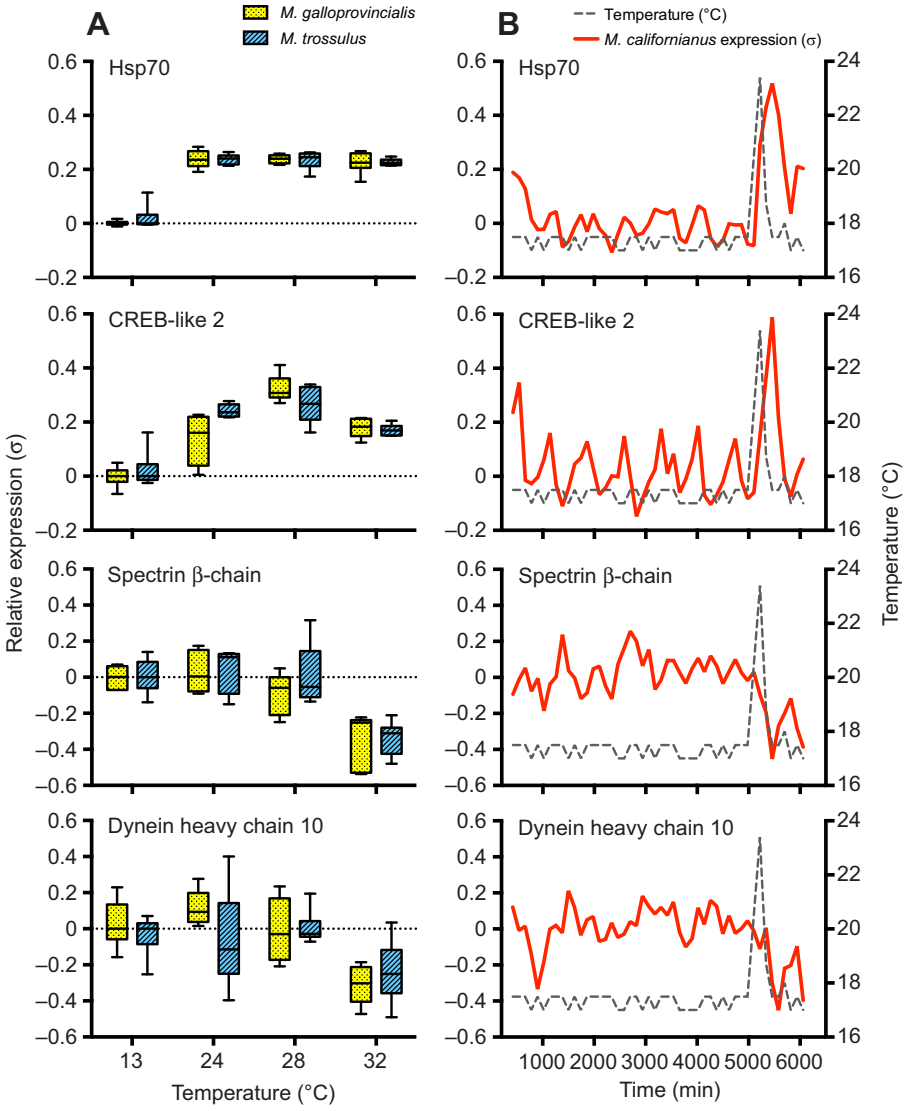

Fig. 4. Common *Mytilus* transcriptional responses to heat stress. Two induced genes Hsp70 and CREB-like 2 (top two panels in A and B) and two repressed genes, spectrin β-chain and dynein heavy chain 10 (bottom two panels in A and B). These data are a subset of those presented in Fig. 3; expression was normalized to obtain a mean of zero and a standard deviation of one. Relative expression is presented as the standard deviation around the mean (as in Fig. 3). (A) Box plots of gene expression in *M. galloprovincialis* and *M. trossulus* at 13°C and during a heat ramp (+6°C h^{-1}) at 24°C, 28°C, and 32°C. Data are from Lockwood et al. (2010). (B) Line plots of gene expression in *M. californianus* along a time course of temperature treatment. Data are from Connor and Gracey (2011).

trafficking, the cell cycle, signal transduction, metabolism and transcription.

It is interesting to note that the heating rate and maximum temperature of exposure were more modest in the study by Connor and Gracey (2011) compared with the study by Lockwood et al. (2010). Yet, the degree of change in gene expression was more pronounced in *M. californianus* than in *M. trossulus* and *M. galloprovincialis* at 24°C (Fig. 3). This difference in transcriptional response may be attributed to differences in the experimental designs of these two studies. Indeed, Connor and Gracey (2011) acclimated *M. californianus* mussels to a 12 h tidal cycle at 17°C for 4 weeks. Subsequently, they exposed these mussels to an aerial heat stress exposure in which the temperature was steadily increased to 24°C over a period of 6 h. In contrast, Lockwood et al. (2010) common-garden acclimated *M. trossulus* and *M. galloprovincialis* in submerged tanks at 13°C for 4 weeks and subsequently exposed submerged animals to a heat ramp at a rate of +6°C h^{-1}. Therefore, the greater response in *M. californianus* at 24°C might have been due to the slower heating rate inherent in aerial exposures (Bjelde and Todgham, 2013; Tomanek and Somero, 2000), thus allowing the mussels in this experiment to mount a robust heat shock response prior to incurring thermal damage to the transcriptional apparatus (DiDomenico et al., 1982). However, both *M. trossulus* and *M. galloprovincialis* showed robust induction of thousands of genes in response to heat stress; thus, there is no evidence that the heating regime employed by Lockwood et al. (2010) led to decreased function of the transcriptional apparatus. Alternatively, acclimation to a tidal rhythm may have primed *M. californianus* to elicit a more robust response. In support of this idea, lower intertidal *M. californianus* show smaller and less-consistent rhythmic changes in gene expression than higher intertidal mussels that experience regular bouts of aerial emersion (Gracey et al., 2008). Moreover, Hsp70 induction levels have been shown to be higher in field-acclimatized than in lab-acclimated *M. trossulus* (Buckley et al., 2001). These observations suggest that the conditions of acclimation and heat exposure should be taken into consideration when designing relevant experiments to test the effects of heat stress in the intertidal zone, where mussels routinely encounter heat stress during aerial exposures at low tide.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
B.L.L., K.W.C. and A.Y.G. conceived the organization and presentation of the literature review. B.L.L. and A.Y.G. analyzed and interpreted the results presented herein. B.L.L. and A.Y.G. drafted and revised the manuscript.

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Table S1. **Annotations and directions of response of the 175 genes that show common responses to heat stress in *M. californianus*, *M. trossulus* and *M. galloprovincialis*.** The order of genes matches that of Fig. 3. A plus sign indicates induction and a minus sign indicates repression. An asterisk indicates genes with expression patterns plotted in Fig. 4. Data are from Lockwood, Sanders and Somero (Lockwood et al., 2010) and Connor and Gracey (Connor and Gracey, 2011).

Gene annotation (BLASTx)	GenBank Accession	Direction of response
heat shock protein 70*	ES391114	+
heat shock protein 70	ES735872	+
cAMP-responsive element-binding protein-like 2*	ES404678	+
sporozoite threonine-asparagine-rich protein	ES391808	+
Sequestosome 1	ES403532	+
CCAAT/enhancer-binding protein gamma	ES738577	+
heat shock protein 70	ES407087	+
glyoxylate reductase hydroxypyruvate reductase	ES400468	+
Sestrin-3	ES391506	+
No Significant Hit	ES398477	+
cathepsin c	ES399013	+
Protein FAM195A	ES738750	+
Toll-like receptor 13	ES388151	+
nf-kappa-b inhibitor	ES401131	+
latrophilin 3	ES391336	+
small heat shock protein 24	ES398112	+
No Significant Hit	ES391539	+
c-jun protein	ES405647	+
No Significant Hit	ES392021	+
transcription factor hes-1	ES406050	+
No Significant Hit	ES407069	+
glycyl-trna synthetase	ES738115	+
neuroendocrine protein 7b2	ES391363	+
BTB/POZ domain-containing protein KCTD3	ES404701	+
rna binding motif x-linked-like 1	ES397376	+
iq motif containing k	ES397389	+
No Significant Hit	ES389407	+
Protein bicaudal D homolog 1	ES401580	+
rtdr1-prov protein	ES393888	+
pdz domain protein	ES405641	+

Gene annotation (BLASTx)	GenBank Accession	Direction of response
luteinizing hormone isoform cra_a	ES397159	+
No Significant Hit	ES403660	+
OTU domain-containing protein 4	ES400572	+
Endoplasmic reticulum resident protein 29	ES396968	+
Uncharacterized protein C2orf65	ES396702	+
wd repeat domain 12	ES401411	+
proteasome (macropain) beta 6	ES393950	+
PREDICTED: similar to AGAP010221-PA [*Tribolium castaneum*]	ES404749	+
dre1 protein	ES398398	+
ef-hand domain (c-terminal) containing 2	ES404829	+
ccaat enhancer binding protein	ES399510	+
cdc42 protein	ES389122	+
No Significant Hit	ES394544	+
No Significant Hit	ES396035	+
calmodulin	ES404659	+
heat shock protein 70	ES393037	+
norepinephrine transporter	ES407243	+
dead (asp-glu-ala-asp) box polypeptide 41	ES391493	+
nadh-ubiquinone oxidoreductase ashi subunit	ES399520	+
peptidase (mitochondrial processing) beta	ES389068	+
e2f transcription factor 8	ES402411	+
histocompatibility 13	ES736950	+
gtp-binding nuclear protein ran	ES388802	+
transmembrane bax inhibitor motif containing 6	ES738513	+
lin-24 (twenty-four) like family member (lntl-1)	ES394680	+
No Significant Hit	ES396503	+
cholecystokinin a receptor	ES738358	+
hypothetical protein BRAFLDRAFT_74499 [*Branchiostoma floridae*]	ES395010	+
peroxiredoxin 6	ES397059	+
actin-related protein 2/3 complex subunit 5	ES406440	+
mapmodulin cg5784-pc	ES389000	+
glutaredoxin-1	ES389121	+
regulatory protein	ES401162	+

Gene annotation (BLASTx)	GenBank Accession	Direction of response
yip1 domain member 5	ES394757	+
tctex1d1 protein	ES738040	+
octopine dehydrogenase	ES398049	+
electron-transfer-beta polypeptide	ES736201	+
angiopoietin 4	ES738575	+
No Significant Hit	ES389771	+
No Significant Hit	ES393695	+
ring finger protein	ES402785	+
similar to Col protein	ES389368	+
Headcase protein	ES407873	+
No Significant Hit	ES403599	+
briggsae cbr-nekl-4 protein	ES405475	+
loc495409 protein	ES397215	+
No Significant Hit	ES393811	+
heat shock protein 25	ES737726	+
Carbohydrate sulfotransferase 15	ES394067	+
mgc80611 protein	ES407248	+
Hillarin	ES394886	+
proteasome subunit beta type 3	ES405577	+
sec13-like 1 (*cerevisiae*)	ES396393	+
heat shock cognate 71	ES390558	+
glutathione transferase omega-1	ES737628	+
No Significant Hit	ES401889	+
No Significant Hit	ES392870	+
No Significant Hit	ES393542	-
No Significant Hit	ES404473	-
Serine/arginine-rich splicing factor 10	ES388931	-
No Significant Hit	ES405837	-
No Significant Hit	ES400561	-
fibronectin type iii domain protein	ES388402	-
No Significant Hit	ES738178	-
Disks large homolog 5	ES403331	-
No Significant Hit	ES388508	-
No Significant Hit	ES406332	-

Gene annotation (BLASTx)	GenBank Accession	Direction of response
Myophilin	ES390846	-
A-kinase anchor protein 9	ES394182	-
No Significant Hit	ES396191	-
No Significant Hit	ES400419	-
No Significant Hit	ES400506	-
CD109 antigen	ES402037	-
Spectrin beta chain*	ES392490	-
appr-1-p processing domain protein	ES736599	-
No Significant Hit	ES407863	-
E3 ubiquitin-protein ligase RNF13	ES737088	-
No Significant Hit	ES391695	-
adp-ribosylation factor-like 15	ES399086	-
PAB-dependent poly(A)-specific ribonuclease subunit 3	ES399428	-
CCR4-NOT transcription complex subunit 4	ES390255	-
nup205 protein	ES394577	-
Dynein heavy chain 10*	ES400211	-
RuvB-like helicase 1	ES395942	-
No Significant Hit	ES736674	-
No Significant Hit	ES389174	-
No Significant Hit	ES388486	-
sterile alpha motif domain containing 1	ES399580	-
chromosome 2 open reading frame 65	ES396695	-
Transient receptor potential cation channel subfamily M member 2	ES393487	-
Autophagy-related protein 16-1	ES401483	-
DNA fragmentation factor subunit alpha	ES738725	-
von willebrand factor a domain containing 3a	ES388760	-
Chromobox protein homolog 1	ES399636	-
No Significant Hit	ES390971	-
membrane alanine aminopeptidase	ES400562	-
No Significant Hit	ES402090	-
Uncharacterized serine-rich protein C215.13	ES737098	-
No Significant Hit	ES403820	-
Protein SMG8	ES390045	-

Gene annotation (BLASTx)	GenBank Accession	Direction of response
Cytochrome P450 CYP12A2	ES400127	-
No Significant Hit	ES406619	-
No Significant Hit	ES397322	-
nol1 nop2 sun domain family 2 protein	ES397429	-
ciliary dynein heavy chain 7	ES394142	-
No Significant Hit	ES397211	-
T-complex protein 1 subunit delta	ES391328	-
No Significant Hit	ES402863	-
pol polyprotein	ES389530	-
Slit homolog 3 protein	ES388036	-
No Significant Hit	ES395870	-
No Significant Hit	ES397829	-
structural maintenance of chromosomes 1a	ES397399	-
No Significant Hit	ES396662	-
No Significant Hit	ES398134	-
No Significant Hit	ES396550	-
No Significant Hit	ES391886	-
centrosome protein 4	ES389450	-
No Significant Hit	ES404735	-
ninein-like protein	ES389180	-
No Significant Hit	ES398919	-
kiaa1410 protein	ES401378	-
dre1 protein	ES402685	-
pol polyprotein	ES394531	-
cg6729 cg6729-pa	ES392320	-
structural maintenance of chromosomes 3	ES393614	-
No Significant Hit	ES394055	-
No Significant Hit	ES397621	-
PREDICTED: hypothetical protein [*Strongylocentrotus purpuratus*]	ES738872	-
No Significant Hit	ES388431	-
PREDICTED: similar to predicted protein [*Ciona intestinalis*]	ES391180	-
Tripartite motif-containing protein 3	ES391097	-
Homeobox protein Meis1	ES390651	-

Gene annotation (BLASTx)	GenBank Accession	Direction of response
No Significant Hit	ES392818	-
Protein FAM50A-A	ES390376	-
Polyadenylate-binding protein 2-B	ES393444	-
kiaa1598 protein	ES395677	-
cq098_human ame: full=uncharacterized protein c17orf98	ES396423	-
dna polymerase zeta catalytic subunit	ES394872	-
No Significant Hit	ES391047	-
14-3-3-like protein 1	ES405440	-
leucine-rich transmembrane protein	ES390285	-
cdk5 regulatory subunit associated protein 1	ES403039	-
Transcription intermediary factor 1-alpha	ES390925	-
Serine/threonine-protein phosphatase 1 regulatory subunit 10	ES407772	-