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Review

Defining the limits of physiological plasticity: how gene expression can assess and predict the consequences of ocean change

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Anthropogenic stressors, such as climate change, are driving fundamental shifts in the abiotic characteristics of marine ecosystems. As the environmental aspects of our world's oceans deviate from evolved norms, of major concern is whether extant marine species possess the capacity to cope with such rapid change. In what many scientists consider the post-genomic era, tools that exploit the availability of DNA sequence information are being increasingly recognized as relevant to questions surrounding ocean change and marine conservation. In this review, we highlight the application of high-throughput gene-expression profiling, primarily transcriptomics, to the field of marine conservation physiology. Through the use of case studies, we illustrate how gene expression can be used to standardize metrics of sub-lethal stress, track organism condition in natural environments and bypass phylogenetic barriers that hinder the application of other physiological techniques to conservation. When coupled with fine-scale monitoring of environmental variables, gene-expression profiling provides a powerful approach to conservation capable of informing diverse issues related to ocean change, from coral bleaching to the spread of invasive species. Integrating novel approaches capable of improving existing conservation strategies, including gene-expression profiling, will be critical to ensuring the ecological and economic health of the global ocean.

Keywords: climate change; conservation; global warming; marine biology; physiology; transcriptomics

1. OUR CHANGING OCEANS

Our world's oceans are entering an era of profound change [1]. Spurred primarily by anthropogenic activities, contemporary marine species are being challenged with major deviations from the prevailing abiotic and biotic conditions that dominated their evolutionary history [2,3]. These potential threats to marine biodiversity and ecosystem function are multiple and interacting [4,5]. The rapidly increasing concentration of carbon dioxide (CO₂) in the atmosphere, stemming from the human combustion of fossil fuels, not only increases air temperature but also triggers a corresponding increase in sea surface temperature as the added thermal energy is transferred to the world's oceans [6,7]. Concurrently, approximately 50 per cent of atmospheric CO₂ is absorbed by the global ocean [8] and while the dissolution of CO₂ into the marine environment removes this greenhouse gas from the atmosphere and curbs the rate of temperature increase [9], it forms carbonic acid in

seawater and lowers ambient surface pH and calcium carbonate saturation [10]. As these and other environmental characteristics of marine habitats shift, new opportunities for non-native species to colonize and displace native organisms arise [11–13]. In addition, near-shore marine systems, focal points for human settlement and resource use, have become universally degraded by habitat destruction, uncontrolled exploitation and pollution [14,15]. That this confluence of ocean change has and will have significant impacts on ecologically and economically important marine organisms is incontrovertible [16]. Consequently, the dialogue surrounding ocean change is no longer focused on whether or not marine organisms will be affected by climate change or other anthropogenic disturbances, but rather which organisms and ecosystems are most vulnerable [17,18]. Considering the strong connection between human livelihood and the health of marine life, there exists an impetus to develop or modify conservation strategies in order to most accurately model the consequences of ocean change [19].

In this review, we explore the emerging field of conservation physiology by showcasing the potential for gene expression, and in particular transcriptomics (quantifying changes in the cellular mRNA pool), to address issues pertinent to ocean change. To our knowledge,

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gene-expression data has never been directly incorporated into marine conservation decision-making, but warrants exploration given its utility in an expanding number of scientific fields. We begin by describing how physiology can inform marine conservation and how the recent increase in genomic resources for various marine organisms facilitates its integration into a conservation framework. Following sections highlight case studies from the scientific literature to illustrate the many ways in which gene expression can assist in assessing and predicting the impacts of ocean change. Here, we detail two main approaches: (i) how targeting genes involved in the cellular stress response (CSR) can define thresholds for physiological function that underlie multiple responses to ocean change in diverse taxa; and (ii) how coupling gene expression with environmental monitoring and novel statistical analysis can more precisely link physiological responses to patterns of environmental stress. From a broad perspective, disseminating the relevance of gene-expression analyses to problems associated with environmental change may aid in the development of new strategies that can enhance conservation practices.

2. PHYSIOLOGY IN OCEAN CHANGE AND CONSERVATION

Mechanisms for coping with the environment have been traditionally treated as issues of physiology, aiming to understand the bases of lethal and sub-lethal stress and the differences that exist among species in their capacity to respond to the environment [20,21]. Although study systems, methodological approaches and manipulated variables may differ, conclusions drawn from physiological investigation are congruent: organisms have a discrete range of environmental conditions under which performance is optimized and thresholds where physiological function is compromised and eventually lost [22]. In determining how the environment constrains physiological function, reasonably unambiguous and quantitative boundaries can be developed that permit an assessment of the relative vulnerabilities of organisms to environmental change [23–26], information that is certainly useful in the context of conservation [27,28]. To illustrate this concept, we briefly describe three examples from marine species: an intertidal crab, an Antarctic bivalve and coral reef fishes. Cardiac function in the intertidal porcelain crab *Petrolisthes cinctipes* (Randall) collapses at a temperature (31.5°C) very near the maximum habitat temperature currently experienced in nature (31°C), indicating that the survival of *P. cinctipes* could be threatened by even modest increases in temperature stemming from global warming [29]. The Antarctic bivalve *Limopsis marioensis* (Smith) displays an upper critical temperature (beyond which mortality occurs) of approximately 2°C, suggesting this species could perish with warming of only 1–2°C above current Antarctic summer maxima [30,31]. Finally, in two tropical species of coral reef cardinalfishes (*Ostorhinchus cyanosoma* (Bleeker) and *Ostorhinchus doederleini* (Jordan & Snyder)), aerobic scope (the difference between resting and maximal rates of oxygen consumption) was

cut nearly in half following an increase in water temperature from 29 to 31°C, indicating that *O. cyanosoma* and *O. doederleini* will have a reduced capacity to perform aerobic functions (e.g. burst swimming) at summer temperatures expected to occur regularly in their native habitat by the end of this century [32]. Clearly, these types of physiological assessment are of value to conservation, yet each of these experimental approaches possesses inherent limitations that restrict their use across a wider range of organisms. Analyses of cardiac function are restricted to species with a heart, accurate determination of upper critical temperatures requires a reliable gross morphological indicator of death and is largely dependent on experimenter-defined rates of environmental change, and measurements of aerobic scope are confined to species that can be held at least temporarily in the laboratory. More comprehensive approaches to conservation physiology would bypass these obstacles through: (i) incorporating diagnostic tools capable of cross-cutting taxa via evolutionarily conserved metrics of organism performance; (ii) providing reliable indicators of sub-lethal stress; and (iii) being capable of tracking organism performance in natural habitats. Approaches that integrate analyses of gene expression can meet these criteria and advance the field of conservation physiology. While we acknowledge that gene expression is itself associated with a set of limitations, we presume that exploring novel approaches capable of improving existing conservation efforts will be central themes in future conservation strategies [33–37].

3. EXPANDING TOOL-KITS FOR CONSERVATION PHYSIOLOGY

Rapid advances in technology have increased the repertoire of experimental approaches available to investigate responses to the environment. In what many environmental physiologists now consider to be the ‘post-genomic’ era [38–40], tools that exploit the increased availability of DNA sequence information represent valuable assets in characterizing species’ responses to their environment. High-throughput gene-expression profiling, and in particular transcriptomics, has emerged as a powerful tool for physiological investigation, providing an expanded view of the molecular changes that occur when organisms experience environmental stress [41–43]. The advantages of transcriptomic based-approaches are clear: coordinated manipulation of gene expression represents one of the most rapid and versatile reactions available to organisms experiencing environmental stress and monitoring shifts in gene expression across thousands of genes simultaneously provides an integrated picture of the molecular, cellular and physiological mechanisms that collectively constitute an organism’s adaptive response to the environment. Fuelled by data-generating power, increased tractability and applicability across disciplines, the number of studies incorporating transcriptomic data has expanded greatly over the last decade. As a result, statistical approaches to the analysis of large quantities of gene-expression data have been standardized [44], global databases that can house large quantities of gene-expression data have been established [45,46],

the relevance of transcript amount in biological regulation has been empirically addressed [47,48] and the fact that genes identified through these techniques actually impact fitness has been demonstrated repeatedly [49–51]. Such technological and conceptual advances have triggered an important paradigm shift in experimental thinking: researchers no longer need to be convinced of the accuracy or utility of transcriptomics in providing meaningful information as to how organisms respond to the environment and can instead focus more directly on implementing these tools in novel and important ways. This trend is clearly illustrated within the field of ocean change biology. Whereas only a few years ago discussions regarding the use of transcriptomics in climate-change science were largely hypothetical and focused on how to appropriately implement these tools and expand their application to a broader range of target organisms [42,52,53], genomic resources for marine research have increased to the point where many of these goals have been accomplished. In fact, there presently exists a sufficient mass of gene-expression data related to ocean change to infer trends that could be applied more widely in marine research. One trend to emerge is that much like more traditional physiological measurement (e.g. upper critical temperature, cardiac function and aerobic scope), gene expression can be used to define thresholds for physiological function beyond which fitness and long-term species survival are impaired, essential pieces of information for predicting responses to ocean change and developing conservation strategies. Furthermore, recent studies show that gene-expression profiling can bypass some of the obstacles impeding the larger scale application of conservation physiology to marine systems, such as expanding the number of species in which measurements can be taken, tracking physiological performance in the wild under ecologically relevant scenarios and standardizing metrics of sub-lethal stress.

4. THE CELLULAR STRESS RESPONSE: UNIVERSAL BIOMARKERS OF REDUCED FITNESS

From an ocean change perspective, organisms generally have three responses to shifting environmental conditions. Firstly, organisms may disperse to more hospitable environments (i.e. migration); secondly, organisms may adapt to new environments over time through genetic change and evolution (i.e. adaptation); and thirdly, organisms may exploit existing capacities to modify their phenotype to better suit new environmental conditions (i.e. phenotypic plasticity) [22]. Perhaps most relevant to conservation is the ability to estimate an organism's phenotypic plasticity, whereby organisms already living in environments that restrict their ability to acclimatize to new conditions are predicted to be most vulnerable to additional environmental change and suitable targets for conservation. Consequently, a means to identify when organisms have exhausted phenotypic plasticity represents a potentially effective tool for marine conservation.

Virtually all cells respond to acute environmental change by inducing a specific set of proteins that function to prevent and repair macromolecular damage

[54–59]. This reaction, termed the CSR, is a key factor in determining the range of environmental conditions an organism can endure [54,55] and therefore holds promise in terms of assessing and predicting responses to ocean change. Three aspects of the CSR facilitate its use within ocean change biology and conservation. Firstly, the mechanistic basis of the CSR has diverged little throughout evolutionary history and even taxonomically distant organisms respond to environmental stress by inducing a highly similar suite of genes [54,55]. This inherent property of the CSR means that a universal set of biomarkers can be used to diagnose fitness across a range of organisms. Secondly, the CSR is induced in a stressor-independent fashion. The CSR is a reaction to macromolecular damage and because many different types of environmental stress cause similar types of damage to macromolecules (e.g. protein unfolding), the same set of expressed genes can be used to diagnose an organism's sensitivity to a variety or combination of abiotic variables [54,55,57–59]. This aspect of the CSR adds a critical degree of ecological relevance, as organisms in nature are likely to encounter a suite of shifting environmental variables simultaneously. Finally, while components of the CSR are highly conserved across taxa, induction thresholds and functional limits vary on a species-by-species basis that reflects environmental conditions most frequently encountered during evolutionary history [26,60,61]. Taken collectively a compelling approach to conservation physiology emerges: monitoring the expression of the highly conserved and stressor-independent CSR provides a means to establish thresholds for phenotypic plasticity that allow predictions to be made as to how species will fare in future environments.

(a) Case study: standardizing sub-lethal stress

Monitoring changes in the expression of genes related to the CSR provides a pathway to assess sensitivity to environmental change, but requires that the relationship between shifts in the expression of specific components of the CSR and the range of environmental conditions an organism can endure be resolved. While it has been assumed that the induction of certain genes involved in the CSR is a function of the magnitude or severity of stress [54,55], empirical evidence for this presumption has been conspicuously absent. Recently, transcriptomic analysis of the intertidal and estuarine goby fish *Gillichthys mirabilis* (Cooper) during laboratory-controlled heat stress confirmed that the CSR is not an all or nothing response, but is instead delineated by diagnostic shifts in gene expression that reflect distinct levels of sub-lethal stress from an onset threshold to a functional limit that encroaches the upper critical temperature [62]. The onset of heat stress is associated with protein denaturation and the increased synthesis of molecular chaperones, including the well-known heat-shock proteins, which work to re-fold proteins to native conformations [63]. Further increases in temperature result in an upregulation of genes involved in proteolysis, whose functions include the organized destruction of irreparably damaged proteins [54,55,64]. Heat stress that approaches the

upper critical temperature triggers changes in the expression of genes that regulate the cell cycle, putatively functioning to arrest division, prevent the replication of damaged DNA and/or stimulate programmed cell death pathways [54,55,62].

Empirically establishing a tiered CSR is advantageous for conservation as it offers a method to both assess the severity of sub-lethal stress and establish an upper-level threshold for physiological function across a range of organisms. The induction of molecular chaperones indicates sub-lethal stress levels that compromise protein function but may be repairable, while the simultaneous expression of genes involved in protein folding, proteolysis and cell cycle regulation would occur closest to organism-tolerance limits and signify the loss of phenotypic plasticity and severely compromised organismal function. In the following sections, we highlight recent studies in marine organisms that have taken steps towards implementing this approach.

(b) Case study: biomarkers for bleaching

Reef-building corals inhabiting shallow tropical waters are keystone species in highly productive and diverse marine ecosystems. Unfortunately, these corals are sensitive to disturbance and are considered highly vulnerable to the environmental shifts associated with ocean change [65–69]. The physiological integrity of reef-building corals is dependent on unique symbioses with photosynthetic dinoflagellate algae known as zooxanthellae [70,71]. However, the coral–algae symbiosis is tenuous and collapses in response to adverse environmental conditions. The dissociation of symbiotic zooxanthellae from the coral host is termed bleaching, as the pale calcareous skeleton shows through the coral's transparent tissues that are deficient in pigment associated with the zooxanthellae [72,73]. Many bleached corals survive only temporarily, and even if zooxanthellae are recovered, these corals often exhibit depressed colony growth, decreased reproductive potential and increased susceptibility to disease [74–76]. Combined with the likelihood that episodes of bleaching will increase in frequency and magnitude in the future [67], these data emphasize the need to characterize resiliency and vulnerability within corals world-wide and develop tools capable of predicting bleaching thresholds and prioritizing targets for conservation.

An increased understanding of the physiological mechanisms that underlie bleaching is imperative to predicting its occurrence in future environments. One potential strategy involves the use of transcriptomics to determine changes in gene expression that precede bleaching and subsequently using these genes as biomarkers to better predict future bleaching events. Forays into this approach suggest that the bleaching occurring in response to heat stress is associated with the induction of a CSR by the coral holobiont (i.e. the coral, symbionts and associated micro-organisms). Transcriptomic profiling across 2059 genes in the elkhorn coral *Acropora palmata* (Lamarck) [77], a major reef-building coral in the Caribbean where coral reef declines have been especially pronounced [78], revealed that the heat-induced shifts

in gene expression that precede bleaching are comprised primarily of genes associated with a severe CSR, including those involved in protein folding, proteolysis and growth arrest. These changes in gene expression mirror those occurring in *G. mirabilis* as it nears its upper critical temperature [62] and imply that bleaching is triggered when environmental conditions exceed the capacity of the holobiont to buffer or repair heat-induced macromolecular damage. Importantly, comparison of the transcriptomic response to heat stress and bleaching in *A. palmata* to that of distantly related coral, *Montastraea faveolata* (Ellis & Solander) [79], is suggestive of a conserved response in scleractinian corals despite the fact that bleaching occurs at different temperatures in these species. If the transcriptional response to bleaching is indeed conserved between distantly related species and between species that bleach at different temperatures, monitoring the CSR could serve as a bellwether for predicting temperature tolerance, bleaching events or resilience across coral lineages. Such an approach may also improve existing management strategies. For example, the use of cumulative degree days to predict bleaching does not incorporate heating rate [80], a critical variable that governs the ability of an organism to tolerate heat stress [81], leading to potentially inaccurate predictions that could be offset by integrating metrics of stress that incorporate heating rate such as gene expression. Non-lethal sampling procedures would also minimize additional impacts on these already threatened coral species. With respect to *A. palmata*, non-lethal sampling in the wild could be facilitated by the frequent fragmentation that occurs naturally in branching corals [82].

(c) Case study: predicting mass mortality

A major aim of aquaculture practice is to decouple food production from the environment by controlling growing conditions, feed input and disease. Despite these efforts, most aquaculture operations are not completely quarantined from the natural environment, meaning that even farmed species may be susceptible to ocean change and require conservation-like interventions to ensure a reliable supply of seafood in the future [83]. The cultivation of the Pacific oyster, *Crassostrea gigas* (Thunberg), responsible for 95 per cent of the world's billion dollar (USD) oyster industry, is dependent on their suspension in natural inter-tidal or sub-tidal marine environments, meaning that farmed oysters are exposed to ambient environmental conditions and rely on natural ecosystems to provide the nutritional requirements for growth [84]. However, ocean change may have caused a shift in thermal regimes that negatively impact survival during grow-out. For the last 50 years, cultivated and wild *C. gigas* have experienced frequent and extreme mortality events, where 30–70% of oyster assemblages perish [85]. A correlation between the onset of mortality events and the presence of elevated water temperatures has led to the hypothesis that mass mortality is related to the adverse effects of heat stress on oyster physiology [86].

Identifying changes in gene expression that precede mass-mortality events has the potential to provide mechanistic insight into oyster mortality and generate

biomarkers predictive of future mortality events. In a test of this hypothesis, a *C. gigas* microarray was used to establish a consistent pattern of gene expression that distinguished oysters that survived mass-mortality events from individuals that perished [87]. This discovery was facilitated by an innovative sampling regime, whereby a continuous time series of haemolymph was gathered non-lethally from individual oysters at regular intervals. Concurrent tracking of mortality isolated a set of individuals from which haemolymph was collected in the period that preceded their death. Gene-expression patterns between these samples and survivors could then be compared to resolve a genetic signature predictive of mortality. The mass-mortality-related signature isolated in *C. gigas* bears the hallmarks of a severe CSR and was statistically enriched for genes involved in cell death and protein degradation. These data imply, especially in the absence of a pathogenic infection [88], that mortality was at least in part related to the inability of the CSR to restore homeostasis during environmental stress and reiterate that genes involved in destructive processes are expressed nearest organism tolerance limits. Relevant to the conservation of wild oysters and the development of aquaculture practices that more effectively manage mortality events, the predictive pattern of gene expression occurred in samples taken four weeks prior to mortality. The fact that oyster health may have deteriorated progressively and death was not sudden raises the possibility that early detection of mortality biomarkers could afford time for interventions that promote survival, such as transplanting animals to an alternative site. Even if the animal's fate is fixed and mortality cannot be ameliorated, diagnosis could inform harvesting practices that would maximize survival across the largest possible proportion of oysters. The innovative sampling method developed for these oysters paves the way for similar non-lethal procedures to be used in other mollusc species of ecological or economic importance.

(d) Case study: pathways to invasion

Invasive species pose numerous threats to marine ecosystems [89]. As ocean change continues to alter the environmental characteristics of marine habitats, an expected outcome is that species more tolerant of new conditions will expand biogeographic ranges and displace native species [11–13,18]. Native and invasive species of blue mussel (genus *Mytilus*) along the west coast of North America exemplify this potential consequence of ocean change [90]. Historical records show that native *Mytilus trossulus* (Gould) were once abundant within protected sites along much of the coastline [91], but current distributions are mostly limited to central California and northward [92,93]. This biogeographical shift has been attributed to the introduction of the European blue mussel *Mytilus galloprovincialis* (Lamarck), a vigorous invader native to the Mediterranean Sea, where habitats are characterized by warmer water temperatures, lower magnitude tidal fluxes and less seasonal variation than is found in the north-eastern Pacific. As a reflection of this evolutionary history, *M. galloprovincialis* has evolved a warm-adapted physiology that allows it to competitively displace

Mytilus trossulus along its warmer southern range, an effect that may become exacerbated as oceans continue to warm. Little is known about the role of physiology in governing the invasive potential of *M. galloprovincialis* or other marine invaders, but knowledge of the mechanisms that underlie invasive success will be salient as ocean change continues to present opportunities for invasive species to expand biogeographic ranges [10].

Comparative transcriptomics [94] and proteomics [95] of *M. galloprovincialis* and *M. trossulus* during heat stress (13–32°C at +6°C h⁻¹) demonstrates that modifying the expression of components of the CSR is a facet of increased thermotolerance in *M. galloprovincialis* and that relatively small changes in the expression of a few key genes underlie pronounced physiological differences that facilitate invasive success. One such change occurs in the small heat-shock protein Hsp24, which was expressed at dramatically higher levels in *M. galloprovincialis* than in *M. trossulus* during heating [94]. This additional chaperoning activity aids in promoting protein function at elevated temperatures. Conversely, relative sensitivity to heat stress in *M. trossulus* is at least partially a function of proteins suffering irreparable damage at relatively cooler temperatures. Expression levels of genes involved in proteolysis, an integral part of the CSR that degrades proteins terminally damaged by environmental stress, were elevated in *M. trossulus* relative to *M. galloprovincialis* at 32°C [94]. The ability of proteins to maintain function during severe heat stress in *M. galloprovincialis* but not *M. trossulus* establishes an important threshold that can be used to predict interspecies dynamics as a consequence of ocean warming and help manage the further spread of *M. galloprovincialis*. For example, using the 32°C threshold as part of ecological niche models (based on the concept that species will be able to establish populations only in areas that match the set of ecological conditions to which they are limited in native habitats) may assist in identifying routes and arrival sites for invasion, key information in developing an effective strategy against a future invasion [96–99]. In more general terms, modification of the CSR may be a conserved feature of invasiveness and characterizing patterns of gene expression under different environmental conditions in other invasive species may assist in developing models or predictions of invasive success that could aid in marine conservation.

(e) Case study: hotspots of vulnerability

Determining in which biogeographic areas organisms are most vulnerable to environmental change is integral to predicting responses to future change and implementing effective conservation strategies [100]. Anadromous species of fish undertake migrations from marine to fresh water environments for reproductive purposes and in doing so use large geographical areas and occupy habitats that vary considerably in environmental characteristics. The capacity of anadromous fish to acclimatize to new environments during migration is dependent on adaptations to environmental conditions most frequently encountered during evolutionary history [101–103]. As a consequence, recent shifts in the

environmental characteristics of either fresh- or salt water habitats, such as those stemming from global climate change, are anticipated to exert substantial stress on anadromous fish populations [104,105].

In the Fraser River, British Columbia, Canada there are a number of ecologically and economically important sockeye salmon populations (*Oncorhynchus nerka* (Walbaum)) that exhibit an anadromous lifestyle. However, thermal regimes within the Fraser River have been falling increasingly outside ranges to which these salmon populations are adapted and are hypothesized to be driving unprecedented rates of premature mortality during spawning migration [106,107]. These trends suggest that contemporary environments already threaten salmon physiology [102,108] and that additional warming will exacerbate mortality. Should the current warming trend continue (the Fraser River has seen a 2°C elevation in average peak summer temperatures over the past 40 years [109,110]), there is little doubt that the long-term survival of sockeye within the Fraser, the billion dollar (CAD) sockeye salmon industry and the ecological vitality of the Fraser River watershed will all be at risk.

Transcriptomic analyses of wild Pacific sockeye salmon moving between ocean and river habitats during spawning migration show that fish migrating through the river during the summer months induce a characteristic CSR soon after entering fresh water [111]. Genes functioning within chaperone, proteolytic, DNA repair and cell death pathways were significantly differentially expressed in fish sampled in the lower Fraser River relative to fish sampled in the marine environment. These data indicate that fish having only recently entered fresh water exhibit compromises in cellular function that impair performance with almost all of their extremely challenging upstream migration remaining. While the induction of a CSR demonstrates that salmon possess adaptive mechanisms to cope with environmental stress, the onset of a CSR also indicates that salmon having entered fresh water possess little reserve phenotypic plasticity to respond to additional environmental change [26,62]. River phases of migration may then represent an area of enhanced vulnerability to mortality and implementing conservation efforts that maximize survival in fresh water may be critical to the long-term survival of sockeye in the face of warming. Tracking the CSR in salmon over space and time will aid in implementing this type of conservation strategy. Despite investing approximately \$40 million (CAD) annually in salmon conservation, there is still considerable uncertainty in predicting population dynamics on a yearly basis [106,112] and salmon managers may be more willing than ever to explore novel tools capable of improving current management models [113,114]. Identifying when (i.e. life-cycle stages) and where (i.e. biogeographic areas) marine organisms are most vulnerable to environmental stress will become critical conservation issues as aquatic ecosystems continue to change.

(f) *Case study: adaptations to acidification?*

While often referred to as the other CO₂ problem [115], ocean acidification has recently been recognized as a pervasive and damaging anthropogenic influence

on marine life [15]. With the progressive uptake of fossil fuel CO₂ by seawater, pH and the relative abundances of carbonate ions decrease. These changes in ocean chemistry have direct implications for the physiological function of marine organisms, most prominently among calcifying organisms that depend on the presence of carbonate ions to manufacture their shells and spines [10,116].

While acidification is a global-scale phenomenon [8], its impact on marine organisms is not expected to be homogeneous [117,118]. Coastal zones along the west coast of the United States are highly variable environments that experience natural fluctuations in pCO₂/pH as a result of up-welling events that mix deeper CO₂-rich waters with shallow surface layers. While in 2007, the Intergovernmental Panel on Climate Change (IPCC) projected levels of pCO₂ in the world's oceans to reach approximately 1020 µatm by 2100 ('business as usual' scenario), actual measurements of pCO₂ dynamics along the coasts of California and Oregon demonstrate that fluctuations in pCO₂ associated with up-welling events already encroach this level [119,120] and may have driven evolutionary adaptations that enhance tolerance towards pCO₂/pH in organisms inhabiting up-welling zones. That there may be species resistant to shifts in pCO₂/pH is critical information when attempting to forecast the consequences of ocean acidification and conserve potentially vulnerable species.

The purple sea urchin (*Strongylocentrotus purpuratus* (Stimpson)) is a key benthic invertebrate in temperate marine ecosystems along the west coast of North America whose larvae are exposed to up-welling events during their lengthy period in the plankton [121]. Gene-expression profiling demonstrates that *S. purpuratus* prism stage larvae cultured at 540 and 1020 µatm do not induce a CSR to defend cellular homeostasis against elevated pCO₂ [122]. Although 251 genes were significantly differentially expressed between larvae cultured at 380 µatm and those grown at 540 and 1020 µatm, indicating at least an impact on physiology, genes involved in protein folding, proteolysis and apoptosis were downregulated or unchanged. The apparent lack of a CSR in larval urchins raised under elevated pCO₂ implies that current pCO₂ regimes do not encroach on tolerance limits and that *S. purpuratus* larvae possess additional phenotypic plasticity to cope with pCO₂ beyond 1020 µatm. While making conclusions based on the absence of an effect is obviously problematic, the facts that *S. purpuratus* occupies habitats that experience regular shifts in pCO₂ and that other marine calcifiers exhibit major effects on gross morphology at similar magnitudes of hypercapnia [123] present circumstantial evidence for resilience. These data reiterate that predictions regarding responses to ocean change need to be made in the context of what organisms experience in the environment and how their evolutionary history might determine their response to potential stressors.

5. THE CELLULAR STRESS RESPONSE: CAVEATS FOR CONSERVATION

Monitoring the CSR under different environmental conditions provides a tractable means to evaluate

issues concerning ocean change and conservation in a wide range of marine species. However, as with any experimental approach, there are caveats that need to be considered in order to ensure that results are analysed appropriately and accurate conclusions are generated. Firstly, phenotypically plastic organisms may possess the capacity to overcome aspects of ocean change through physiological acclimatization (or acclimation). In these species, exposure to chronic but moderate environmental stress triggers acclimatory changes that facilitate the remodelling of cells, tissues and organs such that physiological optima are shifted to better perform under new environmental regimes [124]. In this situation, the CSR would be transient (especially at the transcript level), expressed at the onset of stress but disappearing as acclimatization processes restore cellular homeostasis [54,55]. Acquiring samples across spatial and temporal scales is an essential feature of applying the CSR to conservation-related issues [100,125]. Secondly, while the presence of a CSR is a reliable indicator that the current environment is approaching organismal tolerance limits, CSR induction thresholds may not be fixed within an entire population and could also shift across generations with natural selection. In fact, the plasticity of the CSR itself may be an important predictor of the capacity to cope with ocean change. Acquiring samples from a sufficient number of individuals both within and between generations would aid in resolving population-level trends through time. Finally, it is important to realize that not all consequences of ocean change will manifest with the induction of a CSR or with changes in gene expression. For example, ocean acidification has been shown to compromise anti-predator responses in reef damselfish [126] and negatively affect shell selection and assessment in hermit crabs [127].

6. A MULTI-STRESSOR WORLD

While the previously described examples affirm the merit of approaches that narrow focus towards a defined set of genes, this method becomes less informative in natural settings where multiple abiotic and biotic factors interact in complex ways to influence physiology [128]. For example, while migrating salmon induce a classic CSR upon entry to fresh water, how specific environmental variables interact to trigger this response is largely speculative. The efficacy of high-throughput gene-expression profiling is maximized when screens of environmentally regulated gene expression are unbiased. Novel approaches that can more accurately disentangle or partition genetic responses to environmental variables represent meaningful advances in applying gene-expression data towards questions of ocean change and marine conservation.

(a) Case study: separating the wheat from the environmental chaff in oysters

Knowledge as to which factors pose the greatest threat to physiological performance, organism survival or ecosystem health is critical to implementing appropriate conservation measures. However, this task is

complicated by the heterogeneous nature of many of the marine habitats potentially impacted by ocean change. Estuarine environments are marked by considerable variation in abiotic factors across spatial and temporal scales that interact in complex ways to influence physiology. Estuaries in the southeastern United States exemplify this trend, as creeks separated by only a few kilometres can differ in daily averages of temperature by 3–5°C, pH by as much as one unit, salinities by as much as 20 parts per thousand and dissolved oxygen by 20–50% [129] (<http://cdmo.baruch.sc.edu/>). Furthermore, these natural stressors are overlain by anthropogenic stressors, such as the presence of toxic chemical compounds and heavy metals. Such dynamic environments challenge organisms to acclimatize and/or adapt to physiologically demanding conditions. Consequently, estuary ecosystems are considered sensitive to future environmental disturbance [130,131].

The eastern oyster *Crassostrea virginica* (Gmelin) inhabits estuaries of the southeastern United States, and innovative analyses of *C. virginica* in its natural habitat are providing exceptional insight into the relationship between gene expression and the environment [129]. Using machine learning tools (computer programs that analyse user-input data to develop patterns, regularities or rules that are predictive of future responses), transcriptomic data from wild *C. virginica* were mapped to a set of 18 environmental variables (including water-quality measures: temperature, salinity, pH, turbidity, dissolved oxygen, chlorophyll a, ammonium and tissue levels of: As, Cd, Cu, Cr, Fe, Hg, Pb, chloropyrifos, naphthalene, total dichlorodiphenyltrichloroethane (DDT) and total polycyclic aromatic hydrocarbons) collected across each of 11 tidal creeks. The resulting gene–environment matrix was then used to assess the impact of environmental factors on endogenous patterns of gene expression in *C. virginica*. Transcriptional responses to 17 of the 18 environmental variables were resolved with high accuracy, demonstrating that physiological function in *C. virginica* is extremely sensitive to environmental change. Elevated temperature and reduced pH had by far the greatest influence on gene expression and *C. virginica* may be vulnerable to the expected increases in temperature and decreases in pH occurring as a result of ocean warming and ocean acidification, respectively. Responses to the pesticides DDT and chloropyrifos also resulted in robust transcriptomic responses, but, importantly, occurred in response to tissue concentrations well below Environmental Protection Agency actionable levels. These findings are relevant to the conservation of *C. virginica* and its estuarine habitats, serving to isolate disproportionately important environmental variables to monitor more closely in the future. The diagnostic patterns of gene expression isolated in *C. virginica* may also be conserved and allow insight into how specific environmental variables influence the physiology of other organisms residing in different habitats. However, the most significant contribution of this study to the field of conservation physiology probably lies in the dissemination of a powerful approach to more precisely define organism–environment interactions in other genomics-enabled marine species.

(b) Case study: linking humans and oysters through physiology

Coastal zones are focal points of human settlement and marine resource use [14], providing an estimated annual \$14 trillion (USD) worth of ecosystems goods and services in the form of food, raw materials, disturbance regulation and nutrient cycling worldwide [132]. Despite the overt value of coastal ecosystems, these areas continue to be degraded as anthropogenic demands for space and resources rise [15]. Predicting how coastal ecosystems will react to the suite of environmental disturbances that accompanies human occupancy is of critical importance to ensure the long-term economic and ecological health of these ecosystems. However, empirical methods that translate anthropogenic activities into species- or ecosystem-level impacts are imprecise: severe or negligible impacts are much more easily resolved than conditions intermediate to these extremes and predictions as to the direction and speed of change are similarly unrefined [133,134]. The tight connection between gene expression and the environment provides a means to more precisely define the ecological impact of anthropogenic stressors by offering access to a vastly increased number of potential environmental sensors (i.e. genes).

The biogeographic range of the eastern oyster *C. virginica* extends across a spectrum of anthropogenically influenced habitats along the southeastern coast of the United States from areas extensively exploited for human use to areas with a relatively minor anthropogenic footprint. Transcriptomic signatures of wild *C. virginica* demonstrate that gene expression can serve as a sensitive indicator of human presence [134]. In this study, a geographical information system (GIS) was first used to calculate percentage impervious cover in regions surrounding each of 11 estuaries. Impervious cover includes areas that are devoid of vegetation and soil that can no longer effectively absorb rainfall and is tightly correlated with human infrastructure such as roads and parking lots. Estuaries were then classified into four groups based on the percentage impervious cover (less than 10%, 10–30%, 30–50% and greater than 50%) and gene-expression patterns of *C. virginica* inhabiting estuaries bordering these areas compared using a machine-learning-based approach. Transcriptomic profiles from *C. virginica* provided a high level of discriminatory power in classifying estuaries based on anthropogenic influence in the surrounding watershed. Gene-expression signatures were resolved that were diagnostic of even very low anthropogenic land-use (less than 10% impervious cover). A robust response to mild anthropogenic stress suggests that estuarine habitats cannot be altered without a discernible impact on the physiology of resident biota. This conclusion corroborates others in suggesting that these estuarine ecosystems are susceptible to the consequences of ocean change and may require enhanced protection to maintain their ecological health [135]. Repeating these experiments over time will establish a much needed direction of change in each estuary, helping to evaluate current conservation interventions or prioritize future endeavours. As was noted in the previous case study, the lasting impression

made by this investigation in the context of conservation physiology probably resides in providing a methodological blueprint for future investigation targeting other marine organisms and habitats affected by anthropogenic activity.

7. CONCLUDING REMARKS

As the environmental characteristics of our world's oceans continue to change, determining in which species ocean change will push conditions beyond the limits of physiological function has become a major objective of marine conservation. In an era when genomic resources are rapidly increasing, high-throughput approaches to monitoring gene expression are proving effective tools in addressing challenges that bridge marine conservation and ocean change. As highlighted in this review, innovative approaches to sampling, experimental design and statistical analyses have greatly expanded the number and types of problems in which gene-expression-based approaches are now relevant. The functional limits of the highly conserved CSR appears to underlie responses to ocean change across taxa and monitoring the CSR may provide a metric of sub-lethal stress that will aid in the identification of species most vulnerable to future perturbation. However, more unbiased approaches that exploit the extreme sensitivity of gene expression to changes in the environment offer perhaps the greatest potential for marine conservation physiology.

While the studies described here emphasize the relevance of gene expression to ocean change and conservation, incorporating these types of data into marine conservation initiatives on a wider-scale will require both an increase in genomic resources for marine organisms and a much more comprehensive understanding of the abiotic environment. Somewhat surprisingly, of these two requirements it may be the lack of spatially and temporally explicit environmental data that is most limiting the ability to accurately predict patterns of physiological stress and the consequences of ocean change. While genomic information is being acquired with greater frequency in key marine organisms, for instance through the development of next-generation sequencing technology, the complexity of the marine environment is hindering the ability to mimic natural conditions in the laboratory or frame conclusions based on what animals actually experience in nature. For example, investigations regarding the biological consequences of ocean acidification have been driven almost exclusively by projections developed by the IPCC based on measurements of pH and pCO₂ in a single habitat, the open ocean [136]. Recent data have shown that these estimations do not accurately model future pH/pCO₂ regimes in many regions [117–119]. Furthermore, relying on broad-scale environmental measurement can severely under-estimate the influence of localized environmental conditions, which in many cases creates counterintuitive patterns of physiological stress [137]. Coupling physiological measurement with fine-scale environmental monitoring will be a guiding principle for predicting responses to ocean change.

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