

BIOCHEMICAL INDICATORS OF STRESS AND METABOLISM: Applications for Marine Ecological Studies

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■ **Abstract** Studies investigating the effects of temperature, food availability, or other physical factors on the physiology of marine animals have led to the development of biochemical indicators of growth rate, metabolic condition, and physiological stress. Measurements of metabolic enzyme activity and RNA/DNA have been especially valuable as indicators of condition in studies of marine invertebrates and fishes, groups for which accurate determination of field metabolic rates is difficult. Properly calibrated and applied, biochemical indicators have been successfully used in studies of rocky intertidal ecology, where two decades of experimentation have generated rigorous, testable models for determining the relative influences of biotic and abiotic factors on species distribution, abundance, and interaction. Biochemical indicators of condition and metabolic activity (metabolic enzymes, RNA/DNA) have been used to test nutrient-productivity models by demonstrating tight linkages between nearshore oceanographic processes (such as upwelling) and benthic rocky intertidal ecosystems. Indices of condition and heat stress (heat shock proteins, or Hsps) have begun to be used to test environmental stress models by comparing condition, activity, and Hsp expression of key rocky intertidal predator and prey species. Using biochemical indicators of condition and stress in natural systems holds great promise for understanding mechanisms by which organisms respond to rapid environmental change.

INTRODUCTION

Understanding the mechanisms by which environmental variation impacts organisms in nature is of great interest to comparative biologists and ecologists and is becoming increasingly important as the effects of climate change cascade through the biosphere. As a result, there has been increasing interest in determining the physiological condition of organisms in a natural context (1–8). Central to the success of many of these integrative studies has been the development of biochemical indicators. Biochemical indicators of metabolic activity are key components of

synthetic or metabolic biochemical pathways that are directly or indirectly linked to processes important for survival or reproduction. The basic premise of their use is that adjustments in rates of physiological processes are necessary to bring metabolic demands into alignment with available energy supply. Biochemical indicators of stress are typically components of the cellular stress response, which are upregulated as a consequence of exposure to environmental conditions that perturb cellular protein structure. The concentration of biochemical components of metabolism or stress shift as a direct result of changing environmental conditions, resulting in alterations in protein synthesis or metabolism that impact performance, growth, or reproductive output. Thus measuring biochemical indicators of stress or metabolism can be used as a “snapshot” of the condition of the organism at the time it was sampled or collected.

Biochemical indicators of condition were first developed by fishery biologists to examine wild populations of economically important fish stocks (9–13). Traditional techniques for determining growth rates or nutritional condition, such as otolith size or liver-somatic index, were time-consuming and could not be used to measure changes in nutritional condition or growth rate on short (e.g., <4 week) timescales (10, 14). Furthermore, these measures were of little utility for assessing survival and growth of early larval stages upon which environmental food availability, temperature, and toxins may have especially large effects (10). These researchers needed a tool with which they could quickly and inexpensively determine the condition of wild fish. It was critical to be able to assay large numbers of individuals and work with preserved specimens or frozen tissue, since sampling typically occurred on vessels with no laboratory facilities. Subsequent studies of wild and hatchery-reared fish (3, 15–19) and marine bivalves and crustaceans in aquaculture (20–22) demonstrate the utility of a suite of biochemical indicators for determining nutritional condition and metabolic activity *in situ*, including metabolic enzyme activities and the ratio of ribonucleic acid to deoxyribonucleic acid (RNA:DNA ratio, or RNA/DNA). A parallel line of investigation resulted in the discovery that organisms exposed to extreme conditions up-regulate the expression of stress-inducible molecular chaperones [heat shock proteins (Hsps) and ubiquitin], which minimize tissue levels of unfolded proteins by repairing them or tagging them for destruction (23–25). As these methods became more widespread in their use, they drew the attention of marine ecologists and eco-physiologists, who have subsequently used biochemical indicators of stress and metabolism to explore mechanistic relationships between organisms and their environment in a natural context. It is these eco-physiological studies that are the focus of this review.

METABOLIC ENZYME ACTIVITIES AS INDICES OF CONDITION

Assessing the physiological condition of ecologically important marine organisms is essential for understanding how environmental change affects survival and predicting how a species or group will interact with competitors or predators.

Metabolic rate is an excellent indicator of physiological condition (26–30). Elegant techniques have been developed to measure *in situ* metabolic rates of mammals, birds, and large fishes such as sharks and tunas (31–36). Unfortunately, determining field metabolic rates is costly and time-consuming, and in some habitats (such as the wave-swept rocky intertidal or the deep sea) logistically challenging. In addition, many ecological studies require much larger sample sizes than are feasible by measuring metabolic rate alone.

It was primarily out of the need to assess metabolic activity in inaccessible habitats or for large numbers of individuals that the use of biochemical indicators was brought into comparative and ecological marine studies. The first indicators developed to determine physiological condition in a natural context were measurements of metabolic enzyme activities in pelagic, Antarctic, and deep-sea fishes (6, 37–39). These studies showed that metabolic rate declines rapidly as a function of minimum depth of occurrence of each species, concomitant with a steep decline in food availability with depth (6, 39). This pattern persists even when the effects of declining temperature and differences in body size are taken into account, suggesting that declining food availability with increased depth explains, in part, declines in metabolic rate. In these studies, the activities of lactate dehydrogenase (LDH), a glycolytic enzyme critical for burst swimming capacity, and citrate synthase (CS), a tricarboxylic acid (TCA) cycle enzyme critical for aerobic metabolism, were also measured and shown to directly correlate with metabolic rate, and hence food availability, for these fish species.

Many studies have now demonstrated a close link between the activity of enzymes critical to energy metabolism, metabolic rate, and food availability for fishes (19, 38, 40–42) and marine invertebrates (2, 43–47). One key to the success of these studies is selection of the appropriate enzyme-tissue combinations for each species of interest. An excellent example of this point is the use of the glycolytic enzyme LDH as an index of condition (40, 48–52). Changes in food availability strongly affect LDH activity in white muscle. However, LDH activity (and that of other metabolic enzymes) tends to remain constant in brain, independent of changes in environmental food quality or quantity (42, 53). LDH is central to burst swimming performance because its activity allows for the continuance of energy production critical for muscle contraction during functional hypoxia (54). A decrease in LDH activity because of low food availability directly impacts swimming performance, causing a decline in the ability of an individual to escape from predators or capture prey. Conversely, brain LDH activity, while low, is conserved during starvation, presumably to allow the individual to survive until conditions are more ideal for active movement and growth. Similar tissue-specific responses are observed for other metabolic enzymes (such as CS or pyruvate kinase). In addition, linkages between metabolic enzymes and condition also depend on the natural history of the organism of interest (19, 50, 52, 55, 56). Thus calibration of biochemical indicators with laboratory studies, as well as using multiple measures whenever possible (biochemical indicators, metabolic rate measurements, growth rates, etc.), is critical.

Another important point regarding the use of metabolic enzymes as biochemical indicators of metabolism is that while variation in enzyme activity may correlate with metabolic rate and environmental food availability, the underlying cause for metabolic variation may not be related to food availability per se, but to some other environmental variable. A powerful example of this point has been demonstrated by recent work conducted on deep-sea pelagic cephalopods (57, 58). For deep-sea squids and octopi, metabolic rate is highly correlated with CS activity from mantle tissue, and activities of mantle CS and octopine dehydrogenase (ODH, an invertebrate analog of LDH) decline precipitously with minimum depth of occurrence, as is observed for teleost fishes (6, 39). However, unlike many of the fishes used in those landmark studies, cephalopods are active visual predators. The fact that CS and ODH activities decline with depth implies that burst swimming ability is being lost with depth, either due to changes in food availability or to decreased availability of light for visual predation (57). Enzymatic activities of other swimming tissues (fin and arm) in these deep-sea cephalopods are actually quite high, implying no temperature-independent decline in metabolic rate with depth, but instead a shift in locomotor strategy from burst swimming (where mantle is primarily used) to sustained, aerobic swimming, where arm and fin movement is most prevalent. Thus in this case, a decline in CS and ODH activity in mantle tissue with depth is not strictly a function of changes in food availability but is also a relaxation in the need for burst locomotion (to catch prey or escape predators) in the light-limited deep sea (58). This example underscores the importance of understanding the natural history and physiology of one's study organisms when using biochemical indicators for ecological studies.

RNA/DNA AS INDICATOR OF CONDITION

Another technique used to index condition (often in combination with aforementioned enzyme activities) is the RNA:DNA ratio, or RNA/DNA. The basic principle of using RNA/DNA as a measure of condition is that total RNA content is primarily a function of ribosome number and is correlated with new protein synthesis, whereas DNA content remains constant in an individual because it is a function of chromosome number (9, 10, 59). The ratio of RNA to DNA (rather than total RNA content) is used because changes in cell size or water content alter RNA concentration independently of any change in protein synthesis. A number of studies have shown that RNA/DNA is directly related to both tissue growth and nutritional status (reviewed in 16). Altering feeding ration in the laboratory leads to significant shifts in ribosomal RNA content and protein synthesis for a number of groups, including cod, trout, sandbass, shrimp, octopi, and sea urchins (41, 60–65). Changes in protein synthesis directly correlate with RNA/DNA, although this pattern depends on tissue type and the extent of laboratory treatment. For example, studies of the barred sand bass *Paralabrax nebulifer* and the Atlantic cod *Gadus morhua* suggested that protein synthesis, measured both directly and as indicated

by RNA/DNA, was more sensitive to starvation in white than in red muscle (10, 61). Published studies show that starved individuals tend to have lower growth rates, higher mortality rates, and lower RNA/DNA than fed controls. Changes in protein synthetic capacity, either measured directly or indexed by RNA/DNA, respond to changes in food availability on the order of days, illustrating the power of this technique for assaying organisms in a rapidly changing natural environment (9, 59).

Analysis of data published for three distinct groups of marine animals, teleost fishes (10, 66–68), crustaceans (7, 62), and molluscs (22, 46, 69), held in the laboratory and either food-deprived or fed ad lib on a high-quality diet demonstrate that RNA/DNA significantly correlates with nutritional condition (Figure 1A: results of nested ANCOVA given in figure caption). Determination of food availability in situ in marine systems is difficult for animals at higher trophic levels, but for suspension-feeding invertebrates and fishes, regional primary productivity is an excellent indicator of food availability (70–74). Results from published studies in

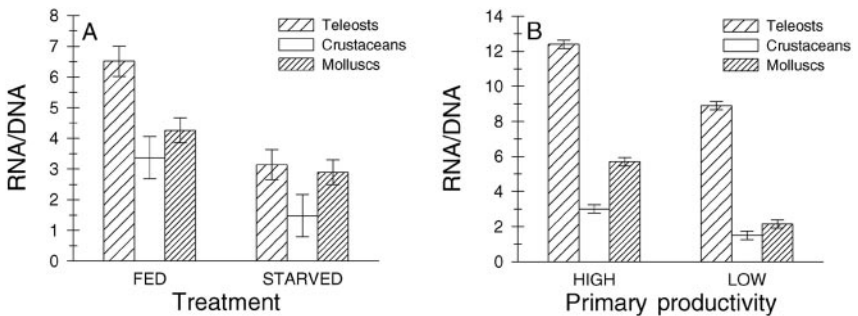


Figure 1 Correlation between food availability and RNA/DNA of muscle tissue from lab-acclimated (A) and field-acclimatized (B) fishes, crustaceans, and molluscs. Sources of data given in text. LAB: Least squares means (\pm SEM) were determined using nested analysis of covariance (ANCOVA), with phylogenetic group ($N \geq 2$ species, nested within group), and treatment (fed, fasted) as main effects. RNA/DNA is correlated with lab nutritional condition (ANCOVA, treatment: $F_{1,11} = 24.75$, $p < 0.05$) independent of phylogenetic group (food \times group interaction NS). FIELD: Least squares means (\pm SEM) were determined using nested ANCOVA, with phylogenetic group ($N \geq 2$ species, nested within group) and food availability (high PP, low PP) as main effects. In this analysis, primary productivity variation was grouped as high or low on the basis of measurements provided by the authors in each publication. Studies were excluded from literature survey if primary productivity data were not provided or easily accessed from another publication. RNA/DNA is correlated with food availability in nature (ANCOVA, condition: $F_{1,13} = 79.83$, $p < 0.0001$) independent of phylogenetic group (food availability \times group interaction NS), although there are significant differences in RNA/DNA between phylogenetic groups (ANCOVA, group: $F_{2,13} = 175.08$, $p < 0.0001$).

which primary productivity drives food-web dynamics (upwelling coastal ecosystems and the Antarctic) were compared (Figure 1B) and show that RNA/DNA is linked to food availability for planktivorous or larval fishes (43, 75), crustaceans (7, 45), and molluscs (2, 47, 76) living in a variety of natural systems. This analysis suggests that RNA/DNA is correlated with food availability in nature and is thus a good indicator of condition, whether variation in food availability is related to differences between seasons (2, 7, 45, 76) or study localities (43, 47, 75). However, there are significant differences in RNA/DNA between phylogenetic groups, emphasizing the importance of laboratory calibration using species of interest whenever possible.

There is still considerable debate about the acceptability of using metabolic enzymes or RNA/DNA as indicators of condition for animals in a natural context. First, it is difficult to distinguish between effects of food availability on protein synthesis from other factors that may influence the rates of metabolic processes. In particular, environmental temperature variation profoundly impacts metabolic rate, as most marine animals are ectotherms, leading to under or over estimation of metabolic activity when temperature is variable (53, 77–79). The use of multiple indicators, as well as close monitoring of temperature, can minimize (but not eliminate) this consideration. Second, individuals held under identical conditions in the laboratory, or exposed to similar conditions in nature, may have different metabolic enzyme activities or RNA/DNA owing to genetic variation within populations, differing availability of stored carbon, or other factors (80–83). Third, DNA content and protein expression will vary among different phylogenetic groups and between different tissue types, making comparisons between species potentially difficult. Fourth, for RNA/DNA determinations, results often depend on the technique used to determine nucleic acid content, in part because RNA is much more sensitive to enzymatic degradation than DNA. Recent advances in nucleic acid quantification techniques have minimized this final problem (84, 85), but the other points remain considerations for applying these techniques to ecological studies.

HEAT SHOCK PROTEINS AS INDICATORS OF THERMAL STRESS

The increased importance of determining effects of environmental extremes on the physiology of animals in nature, as well as recent advances in biochemical and molecular techniques, have led to an explosion of studies investigating stress-inducible molecular chaperones (Hsps and ubiquitin) in an ecological context (47, 86–90). Temperature extremes, pH extremes, anoxia, or high heavy metal concentrations can induce molecular chaperones, and repeated exposure to sublethal temperatures may induce expression of stress proteins that enhance thermal tolerance (25, 91–93). Hsp induction benefits organisms by enhancing their ability to recover from environmental stress and to cope with subsequent stress (25, 86, 94), although there may be a fitness cost associated with over-expressing Hsps (95–97). When stress leads to accumulation of irreversibly damaged proteins, the ubiquitin

response is activated. Ubiquitin is a small protein that attaches to proteins beyond repair and tags them for destruction by proteases (94). Western blots treated with antiubiquitin antibody reveal a heterogeneous population of tagged proteins or ubiquitin conjugates. Although increases in diversity of proteins conjugated to ubiquitin have been used as an index of thermal stress *in situ* (5, 98), differential expression of Hsps (especially the 70 kDa class) are the most commonly used biochemical indicator of heat stress in eco-physiological studies (47, 87, 99–101).

Variation in expression of Hsps in nature is correlated with differences in average environmental temperature and degree of environmental heterogeneity across large and small-scale geographic thermal gradients and through time (5, 47, 88, 89, 99, 102). The threshold at which stress-inducible forms of Hsps are upregulated in nature depends on genetic differences among populations or between species and upon recent thermal history (87, 103–106). However, as with indices of metabolic activity and condition, results must be put into informed physiological context. First, whereas some forms of Hsps are expressed only in response to conditions that unfold cellular proteins (so called stress-inducible isoforms), other forms of Hsps are present at all times as an integral part of the regular protein synthetic machinery. These constitutively expressed Hsps (cognates) are critical for folding nascent polypeptides as they are being synthesized by the ribosome (23, 24). Many techniques routinely used for Hsp quantification in natural systems (in particular, immunocytochemical analysis of Western blots) do not easily distinguish between inducible forms and cognate. Second, tissue levels of cognate Hsps may increase in response to temperature-induced elevations in metabolic rate, independently of the presence of damaged proteins. Third, genetic differences among populations may lead to differences in Hsp expression among different genotypes experiencing identical environmental conditions (89, 103, 105). Finally, although most studies are interested in one particular stressor on their study organism of interest, Hsp expression is upregulated in response to any condition that unfolds protein (23, 24). In nature, different physiological stressors often co-vary. For example, in the wave-swept rocky intertidal, where organisms living in the high zone experience both elevated body temperatures and desiccation at low tide, it is difficult to distinguish the relative importance of these two factors in upregulating Hsps. As with biochemical indicators of metabolism, these concerns can be minimized with a good understanding of natural history and ecology of the study system, careful laboratory calibration, and good experimental design.

APPLICATION OF BIOCHEMICAL INDICATORS TO FIELD STUDIES

There are a number of other biochemical indicators that are used in marine studies, including total protein content, stable isotope ratios, and nitrogen/carbon ratios (3, 107–111). However, the majority of studies using biochemical indicators to

address ecological problems have used some combination of metabolic enzyme activities, RNA/DNA, and Hsp expression, and those studies are the focus of the balance of this review. One of the most compelling applications of biochemical indices in ecological studies has been in testing models that predict changes in the structure of ecological communities—species distribution, species abundance, and the strength of interactions between competitors or between predators and prey—along environmental gradients in the rocky intertidal region. Two classes of models have been developed and tested using biochemical indicators of metabolism and stress: nutrient productivity models (N/P models) and environmental stress models or ESMs (73, 112–115). These models assume that species interactions ultimately drive community structure but that communities are organized along environmental gradients, and severe habitats will have fewest number of species and lowest productivity, whereas the mildest habitats will have higher productivity, biomass, and species diversity. The two models are complementary: N/P models emphasize impacts of forces at the base of the food web on higher trophic levels (bottom-up effects), whereas ESMs assume that community structure is driven by variation in strength of interactions between basal species (algae, suspension-feeding invertebrates) and foragers along environmental stress gradients (top-down effects). Both models have been important to a qualitative understanding of rocky intertidal community dynamics, and the application of biochemical indicators to these classical ecological problems has allowed for rigorous quantification of the relationships between environmental variation and community structure.

Using Biochemical Indicators of Metabolism to Demonstrate the Importance of Bottom-Up Effects in Coastal Upwelling Ecosystems

The testing of N/P models has been in the form of comparisons between well-described rocky intertidal regions with highly distinct community structures where a clear understanding of nearshore oceanographic processes, which may be driving differences in community structure via bottom-up effects, has been developed (114–120). Studies at two sites along the Oregon coast, Strawberry Hill (SH) and Boiler Bay (BB), have been particularly illustrative (2, 46, 47, 121–123). Patterns of species distribution and abundance differ tremendously between these two sites (Figure 2). Strawberry Hill is a site with high invertebrate biomass, high primary productivity in nearshore waters, and a potentially high rate of delivery of that productivity to nearshore suspension-feeders owing to current patterns and topography. Boiler Bay has relatively lower invertebrate biomass, lower primary productivity in nearshore waters, and oceanographic features that inhibit phytoplankton delivery to the nearshore. In the mid-low zone at SH, space is dominated by suspension-feeding invertebrates and foraging carnivores; at BB, the mid-low zone is dominated by red and brown macroalgae, as well as by foraging herbivores, principally limpets and urchins (117, 118). Suspension-feeding invertebrates, such as the mussels *Mytilus californianus* and *M. trossulus* and the

barnacles *Balanus glandula* and *Chthamalus dalli*, are larger, more abundant, and have higher growth rates at SH than at BB. Foraging carnivores (the sea star *Pisaster ochraceus* and the dogwhelks *Nucella ostrina* and *N. canaliculata*) are larger and more abundant at SH than BB (121, 123, 124). Whelk foraging rates are higher at SH than BB (47). However, foraging rates of whelks and sea stars are strongly influenced by water temperature, which is not different between sites but varies tremendously during upwelling events (125–127).

If nearshore primary productivity variation is a factor driving differences in community structure between BB and SH, then this should be reflected in the condition of suspension-feeding benthic invertebrates. One example is the mussel *M. californianus*. This suspension-feeding bivalve inhabits the mid-low zone at SH and BB, is a major competitor for space, and an important prey species for foraging whelks and sea stars. It is thus central to community dynamics at these sites. Metabolic enzyme activities (CS and malate dehydrogenase, an enzyme of critical importance for anaerobic metabolism in bivalves experiencing routine environmental hypoxia) and RNA/DNA are consistently higher at SH than BB in adductor (shell) muscle of these mussels (2). This finding is generally consistent with patterns of phytoplankton availability between sites (discussed below). Reciprocal transplants of mussels between sites result in convergence of mussel condition to those native to transplant destination, suggesting a high degree of physiological plasticity in response to environmental variation in food availability between sites.

Differences in mussel condition between SH and BB persist through much of the year but are most pronounced in summer, during coastal upwelling events. Upwelling is a process that occurs along ocean shores in summer, where prevailing winds are in the same direction as prevailing currents (128, 129). With these winds (and the Coriolis effect), warm water, which has been depleted of nutrients by phytoplankton, is pushed offshore, causing cold, nutrient-rich water to upwell from the depths. During times when the winds slow (or change direction), upwelling forces dwindle, leaving the nutrient-rich water to pool at the surface. Phytoplankton in surface waters responds to upwelling relaxation by rapidly increasing growth rate until nutrients are again depleted. This phytoplankton tends to be more nitrogen-rich than phytoplankton present at other times, which means that upwelling-supported phytoplankton is of higher quality for suspension feeders than typical phytoplankton (130–132). At SH and BB, differences in surface current features are also important. During upwelling, a large eddy forms in the nearshore water off SH. This feature retains nutrient and phytoplankton-rich water in the nearshore at SH, whereas at BB, surface water is moved rapidly southward and offshore (133). Thus, more upwelling phytoplankton is delivered to suspension-feeders at SH than BB. Changes in nearshore phytoplankton concentration and quality can be rapid in intermittent upwelling ecosystems such as the SH-BB system. Mussels seem to respond rapidly to upwelling-induced influxes of phytoplankton; in some cases, mussel RNA/DNA increases within 48 h of upwelling relaxation (2, 117). Because upwelling relaxation is usually accompanied by

elevated sea surface temperature, rapid increases in protein synthesis (indexed by RNA/DNA) are probably enhanced by temperature-dependent elevation of mussel metabolic rate.

Could variation in suspension feeder condition, driven by differences in near-shore primary productivity and by the delivery of high-quality phytoplankton during upwelling events, result in the radically different community structures of SH and BB? If N/P variation is driving community structure, then increased energy input into suspension-feeder growth and reproduction increases their size and abundance, effectively increasing the carrying capacity of foraging predators by increasing their food supply, assuming space is not limiting the abundance of foragers. Thus the condition of both suspension-feeders and their predators (sea stars, whelks) should be robust at high primary productivity sites. Conversely, if variation in nearshore productivity is not shaping community dynamics, then suspension-feeders may still be more robust at high productivity sites, but the condition of foraging predators, which are able to compensate for lower food quality by foraging longer or making different prey choices, would be independent of nearshore food availability.

Expanding the SH-BB California mussel comparison to other key invertebrate species at these sites, it was observed that mussels (*M. californianus*, *M. trossulus*), barnacles (*B. glandula*), and whelks (*N. ostrina*) are more physiologically robust at SH than BB: They have higher metabolic rates, metabolic enzyme activities, and/or tissue RNA/DNA. In contrast, metabolic activity of the sea star *Pisaster ochraceus* does not vary between sites (46, 47). If bottom-up effects were not important, there should be no differences in the metabolic activity of sea stars or whelks between sites. Conversely, if bottom-up effects were always important, then both whelks and sea stars would be more robust at SH than BB. These data suggest that bottom-up effects are important for some (whelk-barnacle, whelk-mussel), but not all (mussel-sea star) predator-prey interactions. Along with ecological studies conducted at these sites, physiological data suggest that performance of prey (and some predators) is greater in areas of high N/P input. This may ultimately negatively impact prey abundance, as a more robust predator is a more effective forager. In contrast, it may enhance prey abundance, as predator foraging may be more difficult, since robust condition of suspension feeders leads to higher growth rates (allowing prey to escape predation by getting big faster) or to more resistance to predation (the ability of a mussel to keep its shell closed during a sea star attack, for instance).

Studies of other rocky intertidal systems have supported the idea that regions with periods of high upwelling have more robust suspension-feeders and predators than regions of low upwelling. Rocky intertidal communities on the east and west coasts of the south island of New Zealand experience distinct oceanographic conditions (intermittent upwelling on the west coast, but consistently low N/P input on the east coast). West coast sites are characterized by high suspension-feeder (mussel and barnacle) recruitment and growth rates, consumer abundance, and predation rates, relative to east coast sites. As was observed in the Oregon system,

upwelling input is tightly linked to suspension-feeder condition, as indexed by RNA/DNA (119, 134).

The importance of suspension-feeder condition for bringing nearshore primary productivity into the rocky intertidal food web is clearly present in upwelling-driven ecosystems. In these systems, performance and fitness of dominant suspension-feeders is linked with environmental variation via physiological condition, which varies predictably between sites or over time. But many other physical and biological factors are in play in rocky intertidal ecosystems, and the interaction between somewhat predictable environmental factors, such as temperature and food availability, can impact community dynamics in unpredictable ways. A large-scale investigation of marine ecosystems along the west coast of North America, conducted by PISCO (Partnership for Interdisciplinary Studies of Coastal Oceans) demonstrates this point (Figure 3). Fourteen rocky intertidal sites in Oregon and California were selected for study by PISCO on the basis of nearshore oceanographic conditions (high versus low upwelling, proximity to key features of the California current), community structure (dominated by kelps versus dominated by invertebrates in the mid-low zone), and a good understanding of community dynamics at each site. Primary productivity is highly variable between regions and sites. Generally, phytoplankton concentration in nearshore water is much higher in Oregon than in central or southern California, a pattern consistent with more intermittent upwelling events in Oregon than California, where upwelling (and offshore transport) is more persistent. Growth rates and RNA/DNA of the ecologically important suspension-feeding mussel *M. californianus* generally correlate with differences in primary phytoplankton productivity. Mussels at low phytoplankton productivity sites in central California had the lowest growth rates and RNA/DNA; mussels at high phytoplankton productivity sites in Oregon had the highest RNA/DNA and moderate growth rates.

More importantly, these data demonstrate that primary phytoplankton productivity, mussel growth, and mussel condition are not always coupled. For example, mussel growth rates at the southern California sites (Alegria and Jalama) were as high (or higher) than for mussels living at sites in Oregon rich in phytoplankton delivery (e.g., SH and Gull Haven); RNA/DNA values were high at Jalama and moderate at Alegria both years. In this case, further sampling indicated that southern California mussels were relying on an alternate food source, i.e., decaying algal matter (PISCO consortium, unpublished results). Mussels at sites in Oregon with high algal densities (e.g., BB) do not routinely have high growth rates, metabolic enzyme activities, or RNA/DNA (2, 123), so algal input alone may not drive these very high growth rates in southern California mussels. However, unlike the other sites in this study, Alegria and Jalama are south of Point Conception, and sea surface temperature is significantly higher than that for central California or Oregon. Elevated mussel body temperature will likely lead to an increase in metabolic rate. This effect, in combination with an alternate food source, means that suspension-feeders may be more robust in areas of low primary productivity input if algal cover and temperatures are both high. The fact that RNA/DNA values are higher

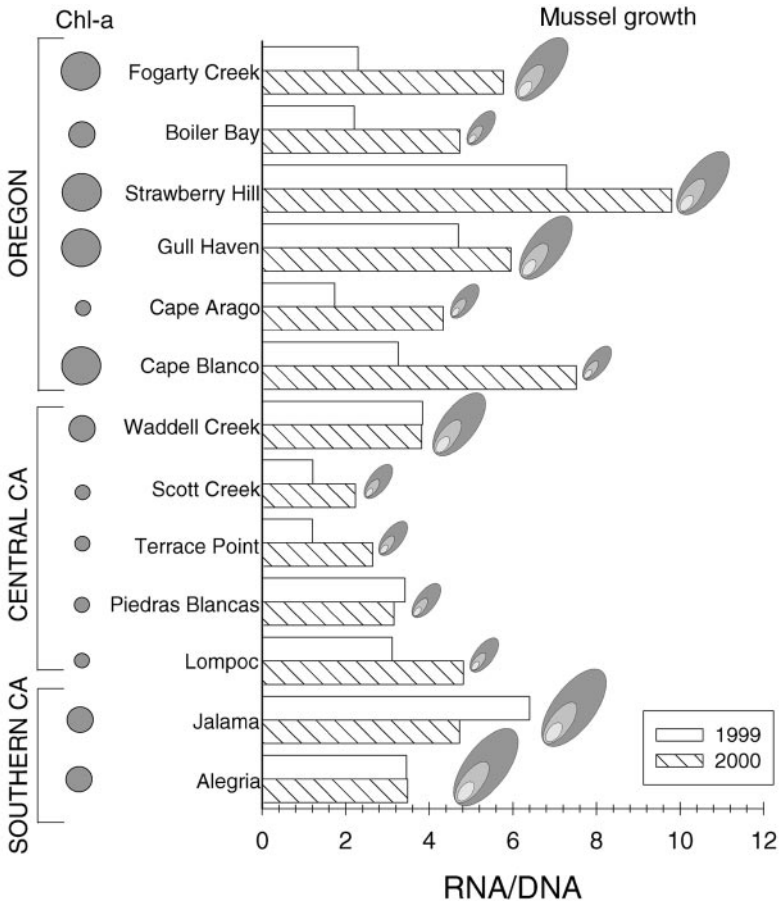


Figure 3 Nearshore primary productivity, growth rate, and RNA/DNA of adductor muscle from the dominant mussel *Mytilus californianus* at 14 rocky intertidal sites in Oregon and California. Average summer chlorophyll-a concentrations (1999, 2000) in nearshore water were determined as described in (118) and are illustrated as relative circle size graphic: low, 0.8–1.5 $\mu\text{g/L}$; moderate, 1.8–3.0 $\mu\text{g/L}$; high, 3–12 $\mu\text{g/L}$. RNA/DNA shown are for mussels collected in July of 1999 and 2000 and were determined as described in (2). Annual mussel growth rates (1999–2000) are illustrated as relative size of mussel graphic: low, 0.1–0.49 cm; moderate, 0.5–1.0 cm; high, 1.0–1.6 cm. Nearshore primary productivity predicted mussel condition, as indexed by RNA/DNA ($y = 2.68,161 + 0.54788x$; $F_{1,11} = 5.0$, $p < 0.05$), but not as indexed by mussel growth because of very high mussel growth rates at two sites in southern California (see text). Data shown here were collected by scientists associated with the Partnership for Interdisciplinary Studies of Coastal Oceans, PISCO (B. Menge, C. Blanchette, E. Dahlhoff, P. Raimondi, S. Gaines, J. Lubchenco, D. Lohse, G. Hudson, M. Foley, J. Pamplin, C. Sorte, A. Keil, J. Freeto, J. Mello, K. Knierim & D. Ricci).

than expected for a low primary phytoplankton productivity site supports this assertion.

Using Biochemical Indicators of Stress and Metabolism to Test Environmental Stress Models

The rocky intertidal region is characterized by steep stress gradients created by the ebb and flow of the tide and by differences in exposure to wave splash. When low tide coincides with elevated summertime temperatures (or nighttime subzero temperatures in winter at high latitude), the amount of stress experienced by intertidal organisms can be extreme (5, 46, 47, 98, 102, 104, 135–138). Key species interactions vary along tidal or wave-exposure gradients in part because of differences in how organisms respond to physical conditions that reduce performance or compromise survival (112, 139–143). It is a classic axiom of rocky intertidal ecology that the upper tidal distribution limit of most species is determined by intolerance of physical stress, whereas the lower distribution is determined by biological interactions, such as predation or competition (112, 144–146). However, many species interactions in the rocky intertidal occur between these two extremes or between the edges of tolerance for two interacting species.

As mentioned above, ESMs are used to understand differences in community dynamics that are based on variation in strength of species interactions along environmental stress gradients (113, 115, 145). These models are typically used to describe the effects of grazers and foraging predators on the abundance of basal species (e.g., algae and suspension-feeding invertebrates), which are in competition with each other for space and other resources; thus ESMs describe top-down effects in rocky intertidal ecosystems. The predictions of ESMs depend on whether the consumer (consumer stress model) or prey (prey stress model) is most susceptible to stress. For the simple example of a two-species interaction (foraging predator, suspension-feeding prey), if the consumer is more susceptible to stress than prey (the consumer is less mobile, unable to shelter when stressful conditions arise, and/or has a less than robust physiological stress response), then in areas of high stress (e.g., higher in the intertidal, areas of low wave splash), the consumer will not control prey abundance because the majority of its energy will be used to survive the stress event. In contrast, if the prey is more susceptible to stress than the consumer (consumer is smaller than prey, able to shelter or hide from stressful conditions more easily than prey, especially if prey is sessile), in areas of high stress the prey will be weakened, leading to higher consumption rates in high stress than low stress areas. Using indices of metabolic activity (RNA/DNA, metabolic enzymes activities, metabolic rate), along with measures of the cellular stress response (Hsp expression), to study species interactions along tidal and wave-exposure gradients offers tremendous power for testing ESMs because these indicators are useful for determining physiological condition, as well as quantifying the qualitative variable stress.

Recent studies of consumers and prey at rocky intertidal sites in central Oregon (the SH-BB system discussed earlier) are a starting point for these applications. At SH, invertebrate suspension-feeders (*M. californianus*, *M. trossulus*, and *B. glandula*) are more physiologically robust (indexed by activities of metabolic enzymes and RNA/DNA) at wave-exposed (low stress) than wave-protected (high stress) areas (2, 46, 47). In addition, mussels appear to experience less heat stress (indexed by tissue levels of inducible and constitutively expressed isoforms of Hsp70) at wave-exposed than at wave-protected localities (88). Differences between wave exposures are most pronounced in summer, when mid-day low tides often coincide with relatively high air temperatures and sunny conditions, especially at SH (46, 123). One important predator-prey interaction is between the dogwhelk *Nucella ostrina* and two prey species (*M. trossulus* and *B. glandula*). Based on the premises of the prey stress model described above, the fact that dogwhelks are small, mobile, and able to avoid potentially stressful conditions would lead to the prediction that they are more resistant to stress than their prey in high-stress zones. Field experiments where both food availability and thermal stress were manipulated experimentally showed that whelks forage more actively, have higher metabolic rates, and lower levels of total Hsp70 (cognate and inducible) at wave-exposed sites, consistent with the qualitative prediction that wave-protected areas are physiologically stressful. Complete food-deprivation reduces the ability of dogwhelks to mount an effective heat shock response at wave-protected areas where heat stress is highest, suggesting that at low prey concentrations, whelks cannot tolerate elevated stress levels (47). These data suggest that although whelks are small and may be able to behaviorally avoid stressful conditions, they still experience enough stress to become physiologically compromised at the upper edge of their tidal distribution. The relative experience of stress by whelks and their prey has yet to be quantified, but preliminary studies suggest whelks may actually be more susceptible to stress than their prey [barnacles and mussels (D. McMillan & E.P. Dahlhoff, unpublished observations)], which would imply consumer stress is driving the interaction in this case.

Another important predator-prey interaction at these sites is between the foraging predator *Pisaster ochraceus* and the mussels *M. californianus* and *M. trossulus* (123, 125). As mentioned above, indices of stress and condition in mussels suggest that wave-exposed individuals are more robust than wave protected individuals. However, sea stars show no variation in metabolic activity (46) or Hsp70 expression (E.P. Dahlhoff, unpublished data) along a wave-exposure gradient. One possibility is that sea stars are so effective at behaviorally avoiding potentially stressful conditions that the time they do spend out of the water does not result in energetically significant physiological stress. In addition, recent studies of sea star (and whelk) foraging behavior suggest that sea surface temperature is a much more important determinant of ecological activity in these foragers than low-tide air temperature. During coastal upwelling, declines in sea surface temperature result in a rapid decline in sea star foraging activity. During upwelling relaxation, sea surface temperature is elevated, and foraging activity increases (125). Subsequent laboratory

studies show that this decline in foraging activity is a direct consequence of temperature-induced reductions in metabolic rate (126, 127). These data suggest that foragers experiencing episodic thermal variation may have higher foraging rates at high temperature, and when temperature is low, the resulting reductions in metabolic rate lead to lower energetic demand. Thus location on the shore at low tide does not necessarily imply how much stress mobile individuals are experiencing. Again, integrating ecology with measures of cellular biochemistry and whole-organism physiology has proven highly illustrative.

FUTURE DIRECTIONS—INVESTIGATING PHYSIOLOGICAL CONSEQUENCES OF ENVIRONMENTAL CHANGE

There is an urgent need to increase our understanding of mechanisms by which animals respond to their environment, especially for organisms that occur in stressful habitats or at the edge of their range. Recent studies indicate that in both terrestrial and marine habitats, species ranges are changing substantially as a consequence of anthropogenic climate change (147–151). Whereas in many cases shifts in distribution are toward higher latitude or altitude, implying a response to overall warming of global climate, this is not uniformly the case; the impact of climate change on the distribution or abundance of given species across an environmental cline is not predictable. For example, the fact that daytime low tides tend to occur at high latitudes in summer suggests that in some ecosystems (e.g., the rocky intertidal), high-latitude sites may actually be more stressful than south temperate or subtropical localities, causing local extinction of sensitive species at high-latitude sites as a result of global warming (152). Second, although it has long been postulated that a given species reaches its highest abundance in the center of its range and that decline in abundance at the edge of a range is a result of biotic or abiotic intolerance of conditions outside that range, recent studies in the rocky intertidal region of the west coast of North America suggest that species abundance may actually be highest at the edge of a range (153, 154). Thus small changes in condition caused by shifts in climate may have profoundly negative implications for many ecologically and commercially important species that are abundant at the edge of their geographic distribution. Third, the highly chaotic nature of atmospheric and oceanic conditions during times of rapid climate flux may lead to counter-intuitive shifts in local climate. Persistent warming may elevate sea level through melting of polar sea ice and thermal expansion of water, thereby changing wave-stress patterns and increasing the frequency of extreme events (storms, droughts, spring tides), thus causing shifts in tidal distribution and abundance of important ecological species (155–158). However, in terrestrial habitats, drought conditions may lead to a decline in winter precipitation, which in turn can cause increased exposure to lethal cold stress in high-altitude or latitude regions where snow pack typically protects over-wintering animals and plants from extreme cold (159, 160).

How can biochemical indicators of stress or condition be used to gain mechanistic insight into the effects of environmental change? Recent studies of intertidal snails (genus *Tegula*) provide an excellent example. In central California, two species of *Tegula* occupy distinct vertical zones on the shore: *T. brunnea* is common in the subtidal to low intertidal, whereas *T. funebris* is very abundant in the mid-intertidal (161). Heat stress appears to limit *T. brunnea* from occupying the mid-intertidal zone, in part due to distinct differences between species in the expression and induction of Hsps (87, 101, 104). Field-caging transplant experiments (in the presence and absence of shade) were conducted in part to mimic increased thermal stress, which would result from elevated summertime temperatures co-occurring with extreme low tides, a prediction of several climate change models (155, 156). These data show that moving the subtidal species (*T. brunnea*) into the zone occupied by the intertidal species (*T. funebris*) rapidly increased expression of at least one isoform of Hsp70, and negatively impacted survival; the intertidal species generally did not show an increase in cellular stress, as indexed by Hsp70 expression (162). Providing shade resulted in a less extreme heat shock response for *T. brunnea* than inunshaded controls, suggesting that sun exposure (rather than desiccation or reduction in feeding time) was the most critical environmental factor causing physiological stress. Up- and down-regulation of Hsp70 expression occurred on the order of days, demonstrating the extreme physiological plasticity of the heat shock response. This study illustrates the power of using biochemical indicators of stress and condition for predicting which species will be susceptible to local extinction because of environmental change, as biochemical indicators (such as Hsp70 expression) are much more sensitive to changes in the environment than growth rate or survival rates alone. Also, it demonstrates that we can learn something about the process by which animals respond to climate change. Using these indicators concomitantly with organismal and ecological measures of responses to climate change (distribution, abundance, activity, metabolic rate, survival, fecundity, etc.) will allow for the development of more rigorous and realistic predictions of the effects of anthropogenic climate change on natural systems.

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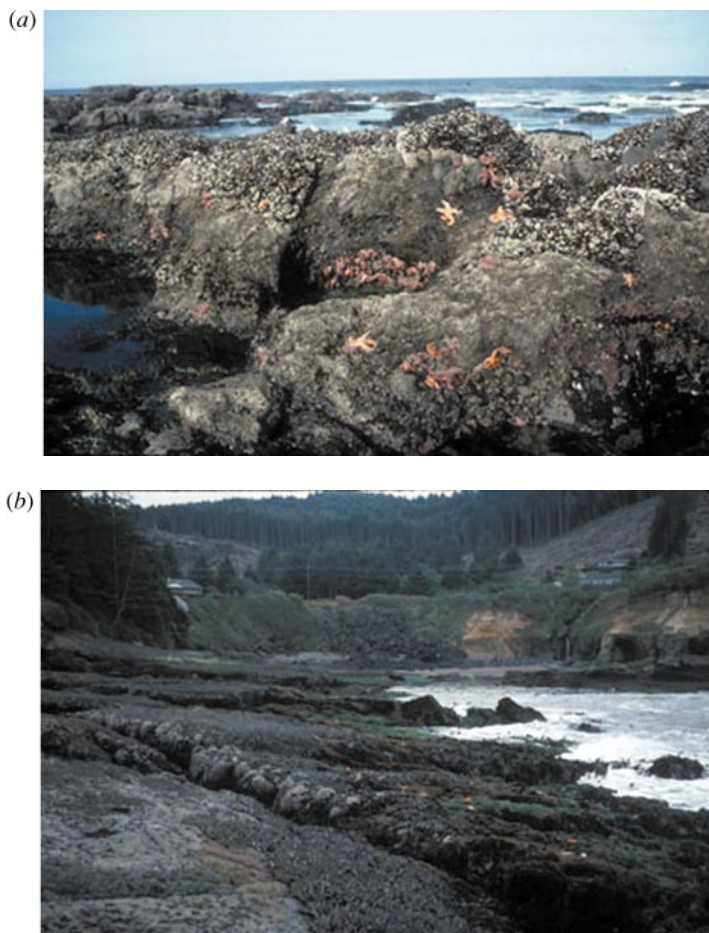


Figure 2 Contrasting patterns of community structure at rocky intertidal sites along the Pacific coast of North America have been the focus of a number of recent eco-physiological studies, including research conducted at the central Oregon sites Strawberry Hill (*upper panel*) and Boiler Bay (*lower panel*).