Physiological Community Ecology: Variation in Metabolic Activity of Ecologically Important Rocky Intertidal Invertebrates Along Environmental Gradients[1]

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Synopsis.   Rocky intertidal invertebrates live in heterogeneous habitats characterized by steep gradients in wave activity, tidal flux, temperature, food quality and food availability. These environmental factors impact metabolic activity via changes in energy input and stress-induced alteration of energetic demands. For keystone species, small environmentally induced shifts in metabolic activity may lead to disproportionately large impacts on community structure via changes in growth or survival of these key species. Here we use biochemical indicators to assess how natural differences in wave exposure, temperature and food availability may affect metabolic activity of mussels, barnacles, whelks and sea stars living at rocky intertidal sites with different physical and oceanographic characteristics. We show that oxygen consumption rate is correlated with the activity of key metabolic enzymes (e.g., citrate synthase and malate dehydrogenase) for some intertidal species, and concentrations of these enzymes in certain tissues are lower for starved individuals than for those that are well fed. We also show that the ratio of RNA to DNA (an index of protein synthetic capacity) is highly variable in nature and correlates with short-term changes in food availability. We also observed striking patterns in enzyme activity and RNA/DNA in nature, which are related to differences in rocky intertidal community structure. Differences among species and habitats are most pronounced in summer and are linked to high nearshore productivity at sites favored by suspension feeders and to exposure to stressful low-tide air temperatures in areas of low wave splash. These studies illustrate the great promise of using biochemical indicators to test ecological models, which predict changes in community structure along environmental gradients. Our results also suggest that biochemical indices must be carefully validated with laboratory studies, so that the indicator selected is likely to respond to the environmental variables of interest.

INTRODUCTION

One could safely predict that all physiological processes [are] influenced by the tide, could we but read delicately enough the indices.—E. F. Ricketts (Steinbeck, 1951)

The distinct patterns of distribution and abundance that occur along tidal gradients in the wave-swept rocky intertidal region are determined by both the physical environment (Lewis, 1964; Denny *et al.*, 1985; Menge and Sutherland, 1987) and biotic interactions (Paine, 1966, 1969; Dayton, 1971; Connell, 1975; Bertness and Callaway, 1994). Recent ecological studies suggest that the physical environment impacts species interactions (Menge and Olson, 1990), yet we understand little about how impacts on ecological interactions are conveyed via physiological mechanisms. There is a robust literature describing the physiology of many ecologically important members of rocky intertidal communities (Bayne and Widdows, 1978; Hawkins and Bayne, 1984, 1985; Stickle *et al.*, 1985; Stickle and Bayne, 1987). However, until recently few studies have used physiological measurements of metabolic processes to address ecological questions. Here we describe an integrative program for using measures of metabolic activity to explore intertidal eco-physiology. First, we develop the use of biochemical indicators for determination of field condition for several (though not all) species of interest by manipulating food availability under controlled (laboratory or field) conditions. Second, using biochemical indicators that most closely predict changes in metabolic activity and feeding status in the laboratory, we examine ecologically important rocky intertidal invertebrates from communities with distinct physical and oceanographic characteristics. Finally, we test ecological models of physical mechanistic control of community dynamics in the context of physiological impacts on key organisms.

DEVELOPING INDICES OF METABOLIC ACTIVITY IN MODEL SPECIES

Metabolic rate is an excellent predictor of physiological condition (Willmer *et al.*, 2000). It is measured directly using calorimetry, which determines the heat produced as a by-product of the biochemical reactions that constitute metabolism, or indirectly by measuring oxygen consumption. However, both of these methods are intractable for *in situ* studies conducted in the wave-swept rocky intertidal, due to cost, time and safety. Here we use measurements of activity (concentration) of metabolic enzymes and the ratio of RNA to DNA as tools for assessing metabolic activity of rocky intertidal organisms in their natural habitat. Studies of bivalves in aquaculture (Wright and Hetzel, 1985;

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Martinez *et al.*, 1992; Mayrand *et al.*, 1994), as well as extensive studies of marine fishes (Sullivan and Somero, 1983; Lowery and Somero, 1990; Mathers *et al.*, 1992; Yang and Somero, 1993; Pelletier *et al.*, 1995), demonstrate the potential for this approach. Certain biochemical measures (notably the activity of glycolytic and TCA-cycle enzymes), while not a direct measure of metabolic rate, are often correlated with rates of oxygen consumption. Other measures, notably RNA/DNA, are correlated with changes in protein synthesis, which occur during growth and are highest when animals are well fed. We developed biochemical indicators for two rocky intertidal invertebrates, the mussel *Mytilus californianus* and the dogwhelk *Nucella ostrina*, in controlled laboratory or field experiments. We used variation in diet as the treatment variable, though reductions in metabolic rate due to estivation, heat stress or oxygen limitation should also result in a decrease in metabolic enzyme activity or RNA/DNA in nature.

Methods

Mussels. Similarly sized mussels (9–15 g) were collected from a wave-exposed area at a site along the Oregon coast (Strawberry Hill; 44°15′15″N, 124°06′40″W) and placed in a flow-through seawater table at 14°C. After 4 days mussels were randomly sub-divided into two groups (fed and starved), which were treated identically except that the fed group was routinely supplied with a ''soup'' containing fish food pellets, mussel tissue and phytoplankton. Water was heavily aerated and changed frequently (every 2–3 days) and food was added (to fed treatment) immediately after each water change. To minimize the effects of elevated metabolic rates that occur immediately after the initiation of a feeding bout (specific-dynamic action), we waited at least 24 hr after the addition of new food to measure mussel metabolic rates.

We measured mussel metabolic rates immediately after field collection, at the initiation of the feeding experiment (day 4), and approximately every 2 wk thereafter for 9 wk. At each time point, mussels were randomly removed from treatment containers, cleaned to remove epibionts from their shell, and placed (individually) in a respiration chamber. Oxygen consumption rates were measured, after 1 hr acclimation, in a closed system using a Clark-type polarographic oxygen electrode. Each individual was measured in three, 20 min recording periods with 10 min breaks between each measurement period to flush the recording chamber with fresh, air-saturated seawater. Immediately following the final measurement period each mussel was dissected and gill, adductor and mantle (devoid of gonad) were flash-frozen on dry ice and stored at −70°C for subsequent biochemical assays. The empty mussel shell was rinsed in seawater and background respiration of the shell, which harbors bacteria, algae and other oxygen-consuming organisms, was measured. Background rates were routinely 1–2% of mussel oxygen consumption rate, and these values

were subtracted from total (mussel in shell) rates. In each tissue sample, we measured the activities of citrate synthase (CS) and malate dehydrogenase, as well as the ratio of RNA to DNA, following published methods (Sullivan and Somero, 1983; Mathers *et al.*, 1992; Pelletier *et al.*, 1995; Dahlhoff and Menge, 1996).

Whelks. Data for dogwhelks (*Nucella ostrina*) reported here were collected as part of another study, some of which we have published elsewhere (Dahlhoff *et al.*, 2001). In that study, similarly sized whelks (15–20 mm length) were collected from an intermediate wave exposure site at Strawberry Hill (SH) in May 1995. Whelks were transplanted into stainless-steel wire mesh cages at wave-exposed and wave protected areas at SH in the presence (fed) or absence (starved) of food (small *Mytilus trossulus*) and monitored for 120 days. Starved whelks were provided with empty mussel shells so that sun-exposure was similar for both treatment groups. Mussels were added to ''fed'' treatments throughout the experiment. Whelks were removed from cages every 30 days throughout the summer and oxygen consumption rates were immediately measured following methods described in Dahlhoff *et al.* (2001). Immediately after determination of metabolic rate, whelks were dissected and foot tissue flash-frozen on dry ice. The activity of malate dehydrogenase was determined for a subset of these individuals following published methods (Dahlhoff and Menge, 1996).

Results

We generated linear regressions between oxygen consumption rate and enzyme activity for each enzyme-tissue combination for laboratory-acclimated mussels (Fig. 1A; Table 1) and MDH activity of foot tissue and field-acclimated whelks (Fig. 1B). We found that citrate synthase (CS) from gill and malate dehydrogenase (MDH) from adductor muscle or foot was significantly correlated with oxygen consumption rate. We also found a high degree of individual variability in these physiological measures in this controlled laboratory experiment, leading to relatively low correlation coefficients (Fig. 1 legend). This variation is not explained by differences in reproductive status or body size among individuals (Stillman *et al.*, 1996; Dahlhoff *et al.*, 2001).

For both mussels and whelks, starvation had a significant impact on oxygen consumption rate (Figs. 1, 2A), RNA/DNA (Fig. 2B), and activities of MDH and CS (Fig. 1). Mussel oxygen consumption rates were initially very high and variable and dropped in both groups in the first week (Fig. 2A). Starvation lowered mussel oxygen consumption rates by 25% within 14 days of the beginning of treatment (One-way analysis of variance, hereafter ANOVA: $P < 0.001$) and differences between oxygen consumption rates of fed and starved individuals remained similar throughout the course of the experiment. Whelk oxygen consumption rates were also highly variable at the beginning of the
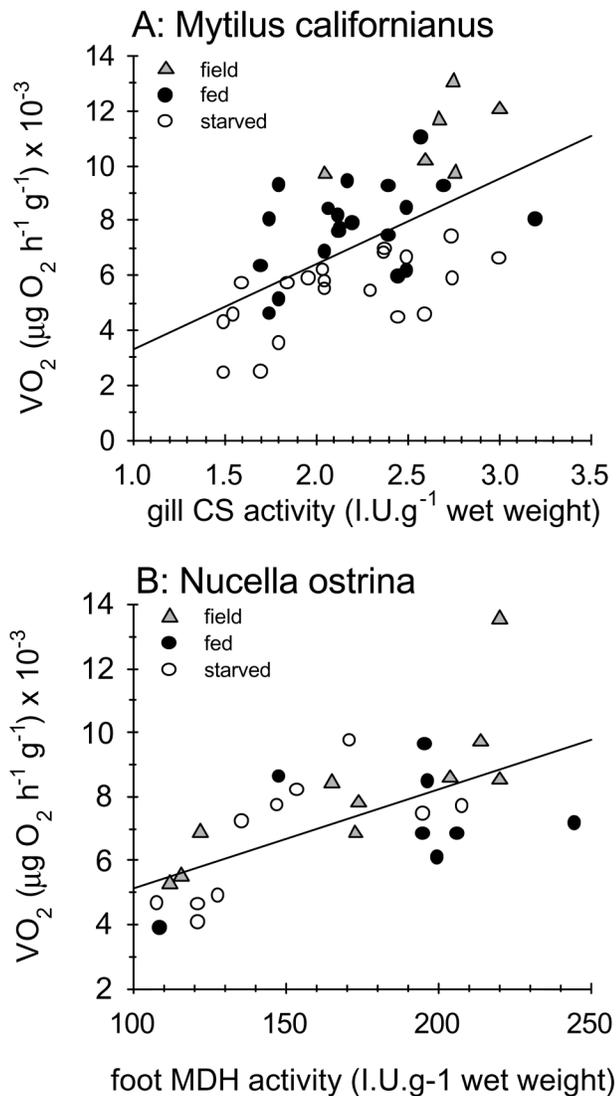
FIG. 1. Relationship between oxygen consumption rate and metabolic enzyme activity for two species of rocky intertidal invertebrates, *Mytilus californianus* (A) and *Nucella ostrina* (B). Data points represent mussels from laboratory acclimation experiments or whelks held under variable feeding conditions in the field. Enzymes presented (gill citrate synthase for mussels and foot malate dehydrogenase for whelks) were those that correlated most closely with oxygen consumption rate. Linear regressions (A: $VO_2 = 3.22$ CS $- 0.26$; $R^2 = 0.30$; $F_{1,46} = 20.4$, $P < 0.001$; B: $VO_2 = 0.032$ MDH $+ 1.25$; $R^2 = 0.56$; $F_{1,26} = 33.2$) are indicated with solid lines. Additional statistical analyses described in text.

experiment (Fig. 1B, "field" group). Within 30 days, whelks caged at Strawberry Hill that had been provided food had significantly higher oxygen consumption rates than caged whelks deprived of food, independent of wave exposure (One-way ANOVA, feeding; $F_{1,31} = 4.3$; $P < 0.05$; oxygen consumption rates square root-transformed before analysis; see also Table 3 in Dahlhoff *et al.* (2001).

Starvation reduced enzyme activity, though the pattern was not as clear as it was for oxygen consumption rate. For starved mussels, gill CS activities tended to

be lower than for fed mussels, though this trend was not significant (Fig. 1A; One-way ANOVA, feeding; $F_{1,33} = 1.4$; $P = 0.256$). For starved whelks, foot MDH activities were lower for starved individuals than for fed ones (Fig. 1B; One-way ANOVA, feeding; $F_{1,16} = 4.7$; $P = 0.045$). We also found starvation induced significant responses in RNA/DNA in all mussel tissues, an effect that was dependent on acclimation time (Multivariate ANOVA, feeding*acclimation time; Wilks' Lambda $P < 0.0001$). Adductor muscle yielded the strongest and most consistent response to variation in food availability (Fig. 2B), consistent with its role as a location for energy storage. These data are consistent with our previous studies of mussels and whelks and suggest that RNA/DNA is an excellent indicator of dietary status in rocky intertidal marine invertebrates (Dahlhoff and Menge, 1996; Menge *et al.*, 1999; Dahlhoff *et al.*, 2001).

Discussion

Significant correlation between enzyme activity (a measure of enzyme concentration in the tissue of interest) and the rate at which oxygen is consumed may indicate that metabolic rate is higher in individuals with high activities of key metabolic enzymes. If this is the case, then enzymes active in the presence of oxygen should be better predictors of metabolic activity than anaerobic enzymes. In the present study, we found that activities of CS and MDH, which are both active in the presence of oxygen, are significantly correlated with oxygen consumption rate. Furthermore, we found that the activity of a primarily anaerobic enzyme, pyruvate kinase, was not correlated with oxygen consumption rate at all (data not shown). However, MDH is also active in the absence of oxygen, and may actually be a better predictor of total metabolic activity for rocky intertidal animals, which experience routine bouts of environmental hypoxia between periods of oxygenation (De Zwann *et al.*, 1981; Hochachka and Somero, 2002). Significant correlation between oxygen consumption rate, enzyme activities and RNA/DNA may also be due to the fact that high metabolic rate individuals tend to be more robust, with more muscle fibers per gram and thus denser tissue, than individuals with lower metabolic rates (Yang and Somero, 1993). The observation that starved mussels and whelks have lower oxygen consumption rates, lower CS and MDH activities and lower RNA/DNA supports the prediction that well-fed individuals are more robust, and therefore have higher enzyme concentrations in metabolically active tissues. The data presented here do not allow us to distinguish between these different mechanistic explanations, but do suggest that metabolic enzyme activities and RNA/DNA can be used to predict changes in metabolic activity in nature.

VARIATION IN METABOLIC ACTIVITY BETWEEN ROCKY
INTERTIDAL SITES

We examined differences in metabolic activity of ecologically important rocky intertidal invertebrates

TABLE 1. *Using enzyme activity as an index of metabolic rate for the mussel* Mytilus californianus.*

	Enzyme				
	Citrate synthase[1]			Malate dehydrogenase[1]	
Tissue	Regression[2]	R^2		Regression	$R^{2\ 2}$
Gill	$1.6 + 0.13\ VO_2$	0.3****		$50.8 + 1.95$**VO_2	NS
Adductor	$50.8 + 1.95\ VO_2$	NS		$63.2 + 1.40$**VO_2	0.09**
Mantle	$1.2 + 0.08\ VO_2$	0.2***		$34.1 + 0.89$**VO_2	NS

[1] Enzyme activities are in I.U. g^{-1}.

[2] VO_2 is in units of $\mu l\ O_2\ h^{-1}\ g^{-1}$.

* Data are regression statistics for the relation between citrate synthase or malate dehydrogenase (dependent variables) and oxygen consumption rate (independent variable) for three tissues: gill, adductor muscle, and mantle.

** $P < 0.05$; *** $P < 0.005$ and **** $P < 0.0001$; n = 48 for each regression.

living at sites along the coast of Central Oregon. Most of our studies were conducted at Strawberry Hill (SH) and Boiler Bay (BB: 44°50′00″N; 124°03′42″W). Patterns of species distribution and abundance, particularly in the low and high zones, differ markedly be-
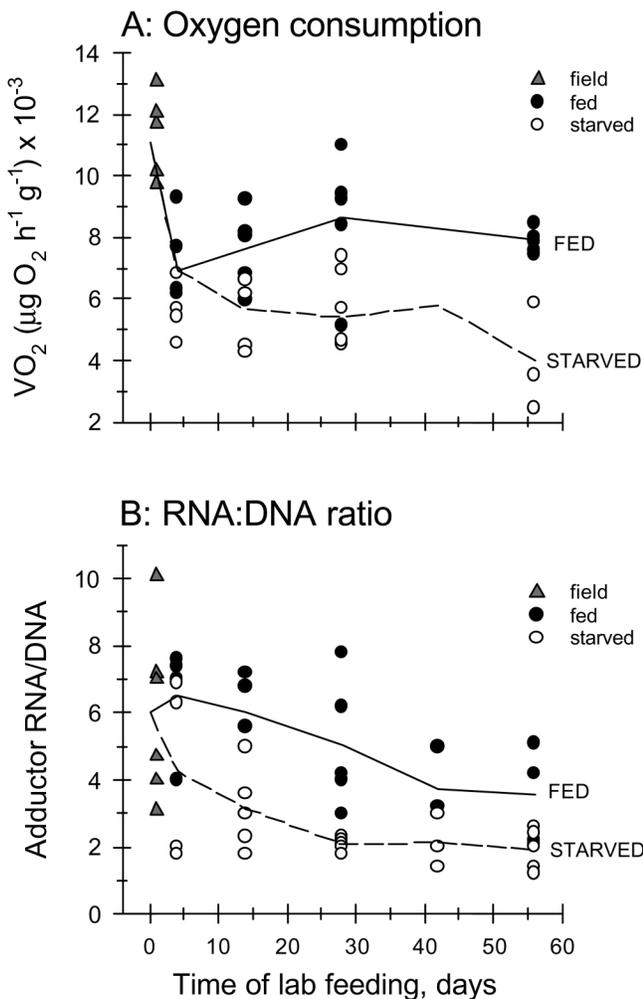

FIG. 2. Effects of dietary state on oxygen consumption rate (A) and RNA:DNA ratio (B) of field-collected *Mytilus californianus* (shaded triangles) and individuals that were either fed (solid line) or starved (dashed line) in the laboratory. For both oxygen consumption rates and RNA:DNA ratios, data points represent individual mussels. Statistical analysis is described in text.

tween SH and BB (Menge *et al.*, 1994, 1997a). Suspension-feeding invertebrates, especially the mussel *Mytilus californianus* and the barnacle *Balanus glandula*, are larger and more abundant at SH than BB (Menge *et al.*, 1994; Menge, 2000). Foraging carnivores, notably the sea star *Pisaster ochraceus* and the whelk *Nucella ostrina*, are also more abundant at SH than BB, especially in the mid-low transition zone, which is usually determined by the upper tidal limit of sea star foraging (Paine, 1966, 1969a). Phytoplankton abundance and productivity are consistently higher at SH than BB (Menge *et al.*, 1997a, b).

The mechanism proposed for the difference in community structure between SH and BB is based on nutrient-productivity models discussed elsewhere in this volume (Menge *et al.*, 2002). The basic premise of these models is that more food is delivered to suspension-feeders at high productivity sites (SH) than low productivity sites (BB). This increased energy input into the suspension-feeder part of the food web cascades up to impact animals at higher tropic levels (''bottom-up effects''), effectively increasing the carrying capacity for animals that consume suspension feeders. At sites with low primary productivity offshore, more nutrients are available to benthic macroalgae and they dominate the low zone. The SH-BB system is therefore ideal for our purposes. Here we have two sites, close together in space, with radically different community structures, and a hypothesis as to what is causing these differences that we can test using measurements of metabolic activity. If ''bottom-up effects'' are important for structuring rocky intertidal communities, then both suspension-feeders and their predators should have higher enzyme activities and RNA/DNA at SH than BB. Conversely, if nearshore productivity is not important in shaping benthic community dynamics, we may still see that suspension feeders are more robust at SH than BB since they get more food at SH. Predators, however, will be able to compensate for lower food quality by foraging longer or making different prey choices.

The species we selected for this study were those that were known to have key roles in shaping rocky intertidal community structure. The suspension-feeding mussel *Mytilus californianus* and its smaller con-

TABLE 2. *Metabolic variation of key invertebrates living at two Oregon rocky intertidal sites, Strawberry Hill and Boiler Bay, with distinct nearshore food availability and low-tide air temperature.*

Species	RNA/DNA[1]			CS activity[2]			MDH activity[2]		
	SH	BB	$F_{1,38}$	SH	BB	$F_{1,38}$	SH	BB	$F_{1,38}$
Mytilus californianus	6.14	4.26	5.0*	2.31	1.56	4.9*	205	188	4.2*
Balanus glandula	2.40	1.43	57.0***	—	—	—	—	—	—
Nucella ostrina	3.33	1.28	36.7***	4.42	3.92	NS	188	160	4.3*
Pisaster ochraceus	1.64	1.62	NS	—	—	—	89.0	89.1	NS

* $P < 0.05$, *** $P < 0.0001$ for one-way analysis of variance for effects of site on RNA/DNA or enzyme activity. n = 20 individuals for each species, collected in July 1994. RNA/DNA values were square root transformed and CS, MDH activities log transformed before analysis.
[1] RNA/DNA is a unit-less ratio.
[2] CS and MDH activities are in I.U. g^{-1}.

gener *M. trossulus* are competitors for space at many wave-swept sites along the west coast of North America, including SH and BB (Paine, 1984; Menge, 1992; Menge *et al.*, 1994). The barnacle *Balanus glandula* is also an important player in community dynamics, especially in the high intertidal (Connell, 1961*a*, 1972). *Nucella ostrina* is a common mid-zone predator that preys on barnacles and small mussels and has a moderate to strong effect on community structure (Connell, 1970; Menge *et al.*, 1994; Navarrete, 1996). The sea star *Pisaster ochraceus* is a low-zone predator that defines a keystone species: its presence controls the lower limit of mussels and thus it has a very strong impact on community structure (Paine, 1966, 1969*a*). We have done controlled feeding studies of two of these species (*Mytilus californianus* and *Nucella ostrina*), described earlier, as models to relate differences in metabolic activity to distinct oceanographic conditions exemplified by SH and BB.

Methods

For comparisons between SH and BB, two areas near the mid-low transition zone and moderately exposed to waves were selected. These localities were sampled for all species of interest (where present) every 30 days from May–August. Data shown are for July 1994, which is our most complete (in terms of species) data set. For each sampling time point, specimens were collected from SH and BB on the same day. Tissues from n = 5 individuals per locality were collected (n = 10 per site). Individuals were haphazardly selected and tissues field-dissected. The following tissues were collected: mussels—gill and posterior adductor; whelks—gill and foot; barnacles—cirri closer-muscle (as gonad free as possible); sea stars—tube feet (removed from the sea star; animal was not destructively sampled). After dissection, tissues were wrapped tightly in foil, flash-frozen and stored on dry ice until returned to Santa Clara University, where they were stored at $-70°C$ until biochemical assays were conducted. For each specimen, the ratio of RNA to DNA was measured following methods described elsewhere (Dahlhoff and Menge, 1996). MDH activity from muscle tissue (mussels, whelk and sea stars) and CS activity from gill (mussels, whelks) were measured following published methods (Sullivan and Somero,

1983; Pelletier *et al.*, 1995). This multivariate approach has been used successfully in other systems and offers both statistical and physiological rigor (Mathers *et al.*, 1992; Pelletier *et al.*, 1995).

Differences in metabolic activity among *Mytilus californianus* at other rocky intertidal sites along the Oregon coast were also measured. In July of 1999 and 2000, n = 10 mussels were collected from moderately wave-exposed regions of the mid-zone of seven sites characterized by differing amounts of phytoplankton delivered to the nearshore. These were Fogarty Creek, Boiler Bay and Cape Arago (low productivity sites), Gull Haven and Cape Blanco (moderate productivity sites) and Yachats Beach and Strawberry Hill (high productivity sites). Mussels were field dissected, adductor muscle sampled and stored at $-70°C$ and RNA/DNA measured as described above.

Results

We found significant differences in metabolic activity between sites that varied among species. Overall, mussels, barnacles and whelks all had higher metabolic activities at SH than BB, whereas there was no difference in metabolic activity for sea stars (Table 2). These differences were most consistent for mussels, which showed significant variation in CS and MDH activity, as well as RNA/DNA. RNA/DNA was consistently different between sites, as indicated by both the multiple species comparison between SH and BB (Table 2), and by the single species (mussel) comparison between multiple sites and years (Fig. 3). RNA/DNA is an indicator of protein synthetic capacity and is highest in animals that are well fed. Our results support the prediction that suspension feeders at high productivity sites like SH are routinely receiving greater amounts of food, which results in higher metabolic activities for suspension-feeders at such sites.

Discussion

We show here and elsewhere that SH whelks, mussels and barnacles have consistently higher enzyme activities and ratios of RNA to DNA than BB specimens, suggesting metabolic activities are higher at SH than BB for these species (Dahlhoff and Menge, 1996; Menge *et al.*, 1997*a*). We have also shown that for mussels and whelks, reciprocal transplantation causes
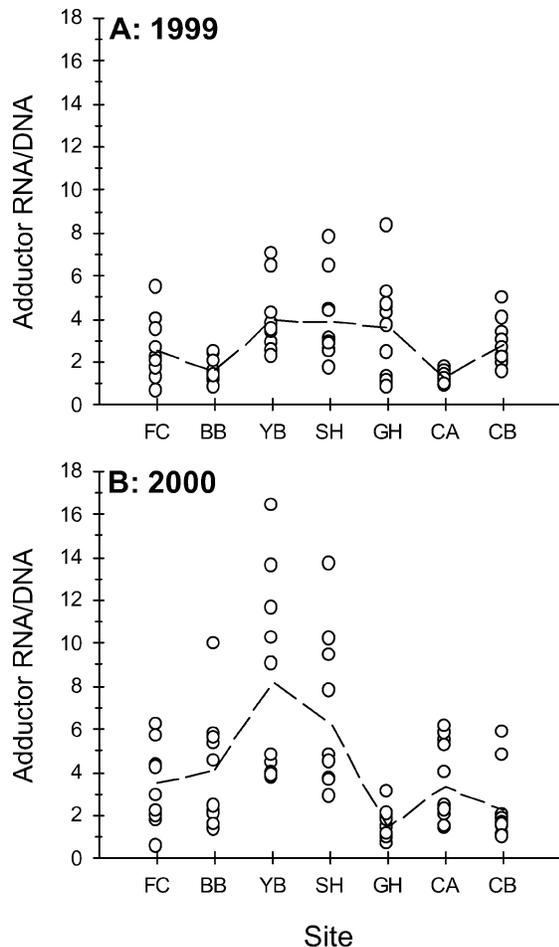
FIG. 3. Variation in adductor muscle RNA/DNA for *Mytilus californianus* from rocky intertidal sites along the Oregon coast in July of 1999 (A) and 2000 (B). Data points represent individual mussels. Site means are indicated by dashed line. Site abbreviations are as follows. FC: Fogarty Creek; BB: Boiler Bay; YB: Yachats Beach; SH: Strawberry Hill; GH: Gull Haven; CA: Cape Arago; CB: Cape Blanco.

among sites. First, the physiology of the small predator (whelk) may be more tightly linked to the physiological state of its prey than a large predator. This postulated tighter physiological linkage between whelks and their prey could be due to a more limited whelk foraging range. If a whelk hits a food-poor prey patch, it may take much longer to locate a better patch of prey than it would a sea star because whelks are smaller and have more limited foraging ranges. A sea star, on the other hand, can move many meters at high tide and may therefore be less limited if it encounters a food-poor habitat. Second, this pattern may be due to differences in prey responses to food limitation. For example, barnacles (whelk prey) may be more susceptible to short-term food deprivation than mussels (sea star prey) because of their small size. Third, physical conditions (such as low tide air temperature) are different between SH and BB. Low tide air temperature is probably less of a factor for sea stars than for whelks, as sea stars can avoid locations where body temperatures are elevated, whereas whelk body temperatures are closely related to air temperatures (Dahlhoff *et al.*, 2001). While we cannot, at this point, distinguish among these alternatives, our data do suggest that bottom-up effects are important in determining differences between SH and BB, at least for three of these four ecologically important community components.

What is the generality of links between community structure and metabolic activity of key ecological players in that community? We have found that in a similar paired comparison between sites along the East and West coasts of the south island of New Zealand, several species of mussels living at high productivity sites have higher metabolic activities, as reflected in RNA/DNA, than conspecific mussels at low productivity sites (Menge *et al.*, 1999). We have not yet measured metabolic activities for foragers at these sites, and it remains to be seen if differences in forager metabolism relating to size or strategy are observed in these homologous studies. We have also greatly expanded our study of rocky intertidal communities along the west coast of North America, beginning once again with measurements of metabolic activity of the mussel *Mytilus californianus* (Fig. 3). We find that mussels at high productivity sites (*e.g.*, SH, YB) tend to have both higher and more variable metabolic activities than those at low productivity sites (*e.g.*, BB, FC). These data also indicate that differences in metabolic activity within sites (or even within a single mussel bed) may be as great (or greater) than differences between sites.

METABOLIC VARIATION ALONG WAVE EXPOSURE GRADIENTS

Environmental differences within a single locale are another important cause of shifts in rocky intertidal community dynamics, the most dramatic and pervasive of which are differences along wave exposure gradients. Key species interactions vary along wave-exposure gradients (Connell, 1961*a*, *b*; Paine, 1966, 1974;

specimens to rapidly acclimatize to the site of transplant, suggesting differences in metabolic measures between sites are linked to conditions at that site (Dahlhoff and Menge, 1996; Dahlhoff *et al.*, 2001). We are just now beginning to understand the implications of these patterns for interactions among species. For example, we have observed distinctions in patters of metabolic activity between a small foraging predator (whelk) and a large forager (sea star; Table 2). If bottom-up effects (food supply) were not important, there should be no differences in the metabolic activity of either predator between sites, as predators are mobile and should be able to compensate for small differences in prey quality by increased foraging or by the selection of other prey. Conversely, if bottom-up effects were very important, then both whelks and sea stars should have higher metabolic activities at SH than BB.

There are several explanations as to why whelks and sea stars exhibit different patterns in metabolic activity

Table 3. *Metabolic variation of key invertebrates living along a steep wave exposure gradient at Strawberry Hill.*

Species	RNA/DNA[1]			CS activity[2]			MDH activity[2]		
	EX[3]	PR[4]	$F_{1,18}$	EX	PR	$F_{1,18}$	EX	PR	$F_{1,18}$
Mytilus californianus	7.58	4.55	6.4*	2.2	1.9	7.6*	217	194	5.6*
Mytilus trossulus	3.22	1.41	4.8**	5.1	2.8	46.6***	493	306	173.4***
Nucella ostrina	4.54	2.12	24.4***	3.7	2.8	20.4**	224	156	15.0**
Pisaster ochraceus	3.86	3.60	0.5	1.1	1.3	7.8*	92	86	0.7

* $P < 0.05$, ** $P < 0.005$, *** $P < 0.0001$ for one-way analysis of variance for effects of exposure on RNA/DNA or enzyme activity. n = 10 individuals for each species, collected in July 1994. RNA/DNA values were square root transformed and MDH, CS activities log transformed before analysis.

[1] RNA/DNA is a unit-less ratio.
[2] CS and MDH activities are in I.U. g^{-1}.
[3] EX, wave-exposed.
[4] PR, wave-protected.

Dayton, 1975; Menge and Sutherland, 1987), in part due to differences in how organisms respond to physical conditions that reduce performance or compromise survival. Environmental stress models predict that stress will differentially affect interacting species and thus alter the outcome of species interactions along environmental gradients (Menge and Olson, 1990; Menge *et al.*, 2002). Using indices of metabolic activity, as well as additional measures of physiological stress such as the heat shock response, the ecological implications of which are discussed elsewhere in this volume (Halpin, 2002; Tomanek, 2002), we can gain mechanistic insight into consumer-prey interactions, which underlie variations in species distribution and abundance along stress gradients.

Methods and results

We compared variation in metabolic activity of mussels, whelks and sea stars along a wave-exposure gradient at SH. Methods and dates for collecting specimens and assaying metabolic enzyme activities and RNA/DNA were identical to those described for comparisons among sites, except that *Mytilus trossulus* was also measured in the wave-exposure study, as it is important prey for both whelks and sea stars. With the exception of sea stars, metabolic activity (indexed by CS and MDH activities and RNA/DNA) of mussels and their predators was consistently higher at wave-exposed than wave-protected localities (Table 3). As with site-related effects, differences between wave exposures were most pronounced in the summer, were similar from year to year, and were similar to those seen at other rocky intertidal sites (Dahlhoff and Menge, 1996; Menge *et al.*, 1997*a, b*; Dahlhoff *et al.*, 2001).

Discussion

These data suggest that individuals living in wave-exposed areas are physiologically more robust than those in wave-protected areas. There are several potential explanations for this pattern. Animals living higher on the shore or protected from wave splash routinely experience higher body temperatures at low tide than wave-exposed individuals (Roberts *et al.*, 1997; Dahlhoff *et al.*, 2001). These elevated temperatures in-

duce the expression of heat shock proteins (Hsps), which repair damaged metabolic proteins or prepare them for recycling (Feder and Hofmann, 1999). In general, there is a correlation between exposure gradient and Hsp expression, though patterns can be complex (Roberts *et al.*, 1997; Dahlhoff *et al.*, 2001; Halpin, 2002). Because the heat shock response is energetically taxing (Krebs and Feder, 1998), animals making large quantities of Hsps must reduce energetic expenditures elsewhere, such as in growth or activity. In addition, animals living low on the shore will receive more wave splash and will generally have more foraging opportunities than wave-protected individuals, even at the same relative tidal height, so high temperatures in areas of low wave splash may be compounded by reduced feeding opportunities.

High temperature and low food/foraging opportunities work in concert to reduce metabolic activity for animals in wave-protected areas. However, we cannot discern the relative importance of two concomitant stressors from data shown here (Table 3). To differentiate the relative importance of temperature and diet for clines in metabolic activity that impact community structure, we conducted whelk field caging experiments where we manipulated the level of food (and thermal stress) experimentally in nature (Dahlhoff *et al.*, 2001). We collected whelks from a single source population at SH, enclosed them in cages in the field with or without prey (*Mytilus trossulus*) at wave-exposed and wave-protected areas, and monitored foraging activity, oxygen consumption rate and Hsp70 levels throughout the summer. In this study, we measured the activities of CS and MDH and RNA/DNA (Dahlhoff *et al.*, 2001), some of which we described earlier. Whelks foraged more actively, had higher metabolic rates and lower levels of Hsp70 at wave-exposed sites (Table 4), consistent with our predictions that wave-protected areas are physiologically stressful. Of note, starvation appeared to lead to a reduced ability to mount a heat shock response at wave-protected areas where heat stress is highest.

As this example suggests, using biochemical indicators in ecological studies may afford the opportunity to tease apart the effects of food, temperature, or wave

TABLE 4. *Variation in activity and physiology of the foraging predator* Nucella ostrina *exposed to different wave exposures and dietary regimes at Strawberry Hill.*

Treatment	Foraging activity[1]			Metabolic rate[2]			Expression of Hsp70[3]		
	EX[4]	PR[5]	$F_{1,8}$	EX	PR	$F_{1,14}$	EX	PR	$F_{1,22}$
Fed	0.63	0.43	12.3**	9.81	6.82	5.2*	108	165	26.2***
Starved	—	—	—	8.20	6.60	5.3*	114	144	NS

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$ for one-way analysis of variance for effects of wave exposure on activity, metabolic rate or Hsp70 expression. n = 5 blocks for foraging data; n = 8 and 12 individuals for metabolic rate and Hsp70 measurements respectively. Metabolic rates and Hsp70 levels were log transformed before statistical analysis.

[1] Foraging activity is in number of mussels consumed whelk^{-1} day^{-1}.
[2] Metabolic rate is indexed by oxygen consumption, reported as mmol O_2 h^{-1} g^{-1}.
[3] Hsp70 levels are reported in ng Hsp70 per g total protein.

stress on species interactions and allow a more effective test of environmental stress models than ecological methods alone (Menge and Olson, 1990; Menge *et al.*, 2002). Data presented here and elsewhere in this volume suggest that enzyme activities and RNA/DNA of some intertidal invertebrates respond to changes in food availability, and Hsps respond to changes in thermal experience. However, many other factors may cause variation in Hsps, enzyme activities or RNA/DNA observed in nature, and we will discuss these briefly, as they are important to consider when designing experiments that use physiological methods to address ecological questions.

First, in many animals, metabolic rate is linked to body size, a phenomenon known as scaling (Willmer *et al.*, 2000). In our laboratory studies, we found that there was no significant effect of body size on the relationship between oxygen consumption, dietary status and enzyme activities. This was in part because we selected individuals of similar size for those studies. In our field studies, we selected specimens of similar size, and recommend this approach for future ecological comparisons, to minimize scaling effects. Second, reproductive status, sex, and size of the gonad may cause variability in enzyme activities or RNA/DNA of somatic tissue, which are unrelated to environmental food availability or other ecological factors of interest. In our controlled feeding studies, we did not find an effect of reproductive status on metabolic rate for mussels or whelks, but those populations were collected from the field at the same time and held under identical conditions in the laboratory. In the field, reproductive status was difficult to control. Although we avoided tissue that may have been contaminated with gonad, some of the variation in our results (especially for small species like barnacles) may be due to the impacts of reproduction on the energy available for protein synthesis in somatic tissue. Future studies of this type should include measures of reproductive state.

Third, there is the problem of heat stress. Low food availability due to low wave splash often correlates with high low-tide air temperature. Thus, an individual may have low metabolic enzyme activities because it is starved, or because it is synthesizing metabolically expensive Hsps and cannot spare the energy to make other metabolic proteins. The ratio of RNA to DNA should not decline with an up-regulation of Hsp, since HSPs utilize the same protein synthetic machinery as other cellular proteins. Thus, measuring RNA/DNA along with metabolic enzyme activities may help moderate the confounding effects of elevated temperature when using biochemical measures to index metabolic activity of animals in nature. Finally, there is the fact that animals with higher body temperatures tend to have higher metabolic rates (Willmer *et al.*, 2000). To compensate for this effect, animals routinely experiencing high temperatures may actually have lower activities of metabolic enzymes, which could contribute to lower enzyme activities or RNA/DNA values for animals living in wave-protected regions.

Our approach to addressing these and other potentially confounding issues has been the following. First, conduct good, controlled laboratory studies of as many of the species of interest as possible. Second, attempt to anticipate as many environmental variables as possible and explore a variety of biochemical indicators to match variables of interest to the situation in nature. Third, measure a number of indicators for each question and species, to give good statistical rigor to the design. We recommend this approach to other investigators interested in using biochemical indicators in broad ecological studies.

CONCLUDING REMARKS

There is a high degree of variation in metabolic activity among individuals in nature. Others have noted high variability in biochemical measures, especially RNA/DNA (Ota and Landry, 1984; Frantzis *et al.*, 1992) and have suggested that these indicators are not useful because the assays are unreliable. Our data suggest a different interpretation: metabolic activity is highly variable for animals living in habitats characterized by extreme physical heterogeneity. The fact that variation in enzyme activity or RNA/DNA observed in nature is reduced (but not eliminated) by laboratory acclimation suggests that both genetic and environmental components are in play. Genetic differences in key enzyme loci may be the source of some metabolic variability, even if no population structure is apparent (Johannesson *et al.*, 1990, 1995; Bayne *et al.*, 1999; Schmidt and Rand, 1999, 2001). Additionally, microhabitat conditions may vary for individuals

living in close proximity, leading to very different environmental experiences for individuals that we think are having similar experiences (Helmuth, 1998; Helmuth and Hofmann, 2000). Therefore, even though biochemical indicators can be used as an index of metabolic activity, to use these indicators to address ecological questions, we must design and analyze our experiments so that they satisfy the assumptions of both physiologists and ecologists. It is our hope that with properly designed and executed studies, understanding how environmental variation is conveyed through organismal physiology to ecological processes will allow us to predict the consequences of environmental change on natural systems, knowledge that will become increasingly valuable as global climate change continues.

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