PHYSIOLOGY OF THE ROCKY INTERTIDAL PREDATOR *NUCELLA OSTRINA* ALONG AN ENVIRONMENTAL STRESS GRADIENT

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Abstract. To gain insight into suborganismal mechanisms underlying responses of predators to environmental stress, we examined the effects of temperature and food availability on the physiology and feeding activity of the predatory whelk *Nucella ostrina* (formerly *N. emarginata*). Studies were conducted in the mid-low transition zone on rocky reefs at two sites on the Oregon coast: Strawberry Hill (SH) and Boiler Bay (BB). SH is dominated by suspension-feeding invertebrates and their predators, while BB is dominated by macrophytes. In wave-exposed (EX) and wave-protected (PR) habitats at each site, we measured air and water temperatures, whelk body temperatures, and indices of physiological condition (malate dehydrogenase [MDH] activities and RNA : DNA ratios) for wild-caught whelks. The influence of food (±prey) and thermal conditions (±shelter) on prey consumption, physiological performance (oxygen consumption rate), and response to thermal stress (induction of Hsp70-class stress proteins) of whelks was determined in field experiments. Low-tide air temperatures, whelk body temperatures, and biochemical indices of whelk field condition were consistently different between sites (SH > BB) and wave exposures (temperatures: PR > EX, condition: PR < EX). Foraging activity, metabolic rates, and stress protein (Hsp) expression were consistently higher at SH than BB. Respiration rates were lower for all −prey treatments than for +prey treatments; however, whelks in −prey +shade treatments had lower Hsp expression and higher respiration rates (an indication of better physiological condition) than whelks in −prey −shade treatments. Most measures of performance suggested that wave-exposed individuals were more physiologically robust than wave-protected individuals. Temporal physiological responses to stress suggested that high levels of heat stress compromise whelk performance later in the summer. These data are consistent with the hypothesis that physiological stress interacts with prey abundance to modify activity of whelks. Detailed studies of the effects of environmental stress on predator–prey interactions are needed to elucidate the relationship between physiological stress and predator–prey interactions. However, present evidence suggests that whelks alter activity (behavioral modification) and physiology (biochemical modification) in ways that lead to differential impacts by these predators on prey populations at different sites.

Key words: community structure; enzymes; food availability; heat shock proteins; mussels; Mytilus; Nucella; *oxygen consumption rates; RNA : DNA; rocky intertidal; wave exposure; whelks.*

INTRODUCTION

Experimental field studies have taught us much about the ecological processes determining community structure (Paine 1966, 1974, Dayton 1971, Connell 1975, Menge and Sutherland 1976, 1987, Louda and Collinge 1992, Bertness and Callaway 1994). These and other studies suggest that both biotic interactions and environmental stress shape the structure of communities (Menge and Olson 1990). Rocky intertidal communities are ideal model systems for such studies because they harbor organisms that experience large spatial and temporal variation in environmental conditions due to

steep gradients in wave impact, food availability, temperature, and desiccation (Paine 1977, Newell 1979, Denny 1988, Menge and Farrell 1989). Furthermore, key species interactions often vary significantly along these environmental gradients (Connell 1961a, b, Paine 1966, 1974, 1984, Dayton 1971, 1975, Menge 1978a, b, Dayton et al. 1984, Menge and Sutherland 1987, Menge et al. 1994, 1999, Leonard et al. 1998, Bertness et al. 1999). Environmental stress models predict that abiotic stress will differentially affect interacting species, thereby altering the outcome of species interactions along environmental gradients (Menge and Olson 1990). For example, consumer stress models predict that foraging predators are more susceptible than sessile prey to abiotic stress. When foraging predators and/ or sessile prey species have strong direct or indirect effects on community structure, abiotic stress may play a disproportionately large role in shaping community

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structure (Menge 1976, Louda and Collinge 1992, Arnott and Vanni 1993).

Recent studies of the physiology of rocky intertidal invertebrates have begun to yield insight into how environmental stress may impact animals in nature (Bertness et al. 1991, Hofmann and Somero 1995, 1996*a, b,* Dahlhoff and Menge 1996, Roberts et al. 1997, Hofmann 1999, Tomanek and Somero 1999). These and other studies have used either laboratory manipulation of the ''stress'' of interest, or have directly measured physiological indices of stress and performance in nature (Garrity 1984, Stickle et al. 1985, Stickle and Bayne 1987, Bayne and Hawkins 1990, Bayne et al. 1993, Abele et al. 1998). Few studies of marine systems to date have used field manipulations to examine the physiological mechanisms that ultimately underlie patterns of community structure, although this approach has been used quite successfully in other systems (Mooney et al. 1987, Bazzaz 1996).

In the present study, we address the issue of how species interactions vary with environmental stress by testing one prediction of environmental stress models: That a mobile consumer will be physiologically compromised under certain environmental conditions, and these conditions may alter foraging activity. We measured foraging activity, physiological performance, and response to stress in the dogwhelk *Nucella ostrina* (formerly *N. emarginata*; see Palmer et al. 1990, Marko 1998) along naturally occurring and experimentally-manipulated gradients of temperature and food availability at two rocky intertidal sites: Strawberry Hill (SH; 44°N15′ N, 124°07′ W) and Boiler Bay (BB; 44°40′ N, 124°03′ W).

Nucella ostrina is a common midzone predator that preys on mussels (*Mytilus trossulus*) and barnacles (especially *Balanus glandula*) in rocky intertidal communities along the West Coast of North America. Previous studies of the distribution, abundance, and relationships between whelks and other species at SH and BB have shown that *N. ostrina* can have a moderate to strong effect on community structure (Connell 1970, Dayton 1971, Menge 1992, Menge et al. 1994, Navarrete 1996, Navarrete and Menge 1996). *N. ostrina* is thus ideal for examining the effects of stress on the physiology and activity of a foraging predator.

Patterns of species distribution and abundance differ strikingly between SH and BB (Menge et al. 1994, 1997*a, b*). In the mid-low transition zone, sessile invertebrate prey (notably the mussel *Mytilus trossulus* and the barnacle *Balanus glandula*) are larger and more abundant at SH than at BB (Menge et al. 1994, 1997*a, b,* Sanford 1999*a, b,* Sanford and Menge 2000). The whelks *Nucella ostrina* and *N. canaliculata* are also common at this tidal level, due in large part to high prey density (Navarrete et al. 2000). Phytoplankton abundance and productivity are consistently higher at SH than at BB, and thus suspension feeders living at SH have more food available than do suspension-feed-

ers living at BB (Menge et al. 1996, 1997*a, b*). Higher food availability at SH results in higher metabolic rates and higher capacity for protein synthesis in mussels (Dahlhoff and Menge 1996) and barnacles (E. P. Dahlhoff, *unpublished data*).

To assess ecophysiological responses of *Nucella ostrina* to environmental stress, we measured habitat temperatures, whelk body temperatures, and foraging activity. We also quantified indicators of performance known to respond rapidly to variation in food availability and physiological condition: whole animal oxygen consumption ($\dot{V}O_2$), activities of the metabolic enzyme malate dehydrogenase, and RNA : DNA ratio (Hawkins and Bayne 1984, Stickle et al. 1985, Stickle and Bayne 1987, Lowery and Somero 1990, Dahlhoff and Menge 1996, Menge et al. 1997*a,* 1999). To further quantify physiological response to thermal stress, we measured expression of a 70 kD-class stress protein (Hsp70), as Hsp70 expression responds rapidly to temperature variation in rocky intertidal invertebrates (Hofmann and Somero 1996*a, b,* Roberts et al. 1997, Hofmann 1999, Tomanek and Somero 1999). The rationale behind using these physiological measures is summarized in the next section. Using these indices of stress and performance, we addressed the following questions: (1) Do whelks show evidence of being differently affected by stress in distinct thermal and dietary microhabitats? (2) Do whelks compensate for low prey abundance by decreasing their metabolic rates and/or increasing their foraging activity? (3) Are whelks that are ''well-fed,'' either due to high foraging activity or higher prey density, better able to tolerate thermal stress than those that are deprived access to prey?

BACKGROUND: ECOPHYSIOLOGICAL RESPONSES

Physiological response to food availability

Carnivorous invertebrates such as whelks living in rocky intertidal regions may experience variation in food quality, quantity, and feeding opportunities both within and between sites due to variation in wave exposure, substratum topography, and tidal level (Navarro and Winter 1982, Bayne and Hawkins 1990, Hawkins and Bayne 1992). Variation in food availability can have profound effects on the physiology of foraging invertebrates (Wolcott 1973, Underwood and Jernakoff 1984, Stickle et al. 1985, Stickle and Bayne 1987, Branch and Moreno 1994, Trussell 1997). This variation could result from a variety of factors, including wave exposure, availability of shade, competition for food, and food quality. First, whelks living in wave-exposed areas may have longer foraging times than individuals in wave-protected areas because of longer periods of wetness, and thus lower thermal stress at wave-exposed sites (Lewis 1964, Tomanek and Somero 1999). Whelks may also experience limitation in foraging activity in areas of high wave impact due to

the fact that foraging whelks are more susceptible to displacement by heavy waves than nonforaging individuals (Denny 1988). Second, whelks in areas of high prey density may have both more food available and more shelter (among the prey) from both heavy wave activity and heat stress at low tide (Davenport et al. 1996, 1998). Third, there may be greater competition for prey at lower tidal heights, especially when the sea star *Pisaster ochraceus* is present (Navarrete 1996, Navarrete and Menge 1996, Navarrete et al. 2000). Fourth, the "quality" of suspension-feeding prey may be highest in areas where phytoplankton abundance is highest (Bertness et al. 1991, van Erkon Schurink and Griffiths 1992, Kreeger 1993, Kreeger and Langdon 1993, Dahlhoff and Menge 1996).

Variation in food availability may lead to adjustments in metabolic rates to bring metabolic demands of organisms into alignment with available energy supplies (Hawkins and Bayne 1984, 1985, van Erkon Schurink and Griffiths 1992, Bayne et al. 1993, Kreeger 1993). Variation in food availability should thus be reflected in a variation in physiological "condition," indexed by changes in metabolic rate or dietary state. Metabolic rate is most typically determined by measuring oxygen consumption rate (Stillman and Somero 1996), or can be measured indirectly by assaying levels of metabolic enzymes (Yang and Somero 1993, Dahlhoff and Menge 1996, Stillman et al. 1996). Dietary status can be assayed using the RNA : DNA ratio, an index of protein synthetic capacity (Wright and Hetzel 1985, Martinez et al. 1992, Foster et al. 1993, Dahlhoff and Menge 1996). Both direct and indirect indicators of metabolic activity were important to the present study. Malate dehydrogenase activities and RNA : DNA ratios were used as a "snapshot" of field metabolic activity and dietary status, whereas oxygen consumption measurements were used to assess whole animal metabolism for field-manipulated individuals. We expected that physiological condition should be correlated to prey density (SH > BB) and foraging opportunity (EX > PR). However, at low tide in some microhabitats (e.g., Strawberry Hill, wave-protected [SHPR]), thermal stress may ameliorate the benefits of high prey density.

Physiological response to temperature

Temperature has an important and pervasive influence on the distribution and abundance of organisms via its effects on physiological processes (Hochachka and Somero 1984, Prosser 1986, Cossins and Bowler 1987). Rocky intertidal habitats are often characterized by steep thermal gradients. Thus intertidal invertebrates may be particularly susceptible to the physiological effects of environmental temperature variation, especially since, as ectotherms, their body temperatures are determined by environmental temperature (Hochachka and Somero 1984, Cossins and Bowler 1987). Stress proteins (heat shock proteins, or Hsp's) minimize

cellular heat damage by refolding partially unfolded proteins into their functional state (Lindquist 1986, Parsell and Lindquist 1993, Parsell et al. 1993, Hofmann 1999). Temperature extremes often induce Hsp's and enhance thermal tolerance (Parsell et al. 1993, Krebs and Bettencourt 1998, 1999, Krebs and Feder 1998). Not surprisingly, the heat shock response appears to be a critical component of adaptational strategies used by ectothermic animals to acclimatize to variations in body temperature in situ (Hofmann and Somero 1995, 1996*a, b,* Roberts et al. 1997, Chapple et al. 1998, Feder 1999, Hofmann 1999). However, Hsp production can incur a substantial physiological cost (Feder and Hofmann 1999, Hofmann 1999). One may thus expect a trade-off between Hsp production, physiological condition, and foraging activity for animals experiencing natural gradients in environmental stress. Unfortunately, this relationship has not been explored for any intertidal invertebrate, especially one that has potentially strong effects on community structure via its impact on prey distribution and abundance.

METHODS

Quantification of field temperatures

Ambient air and water temperatures were monitored every 30 min throughout the cage transplant studies (see *Field-manipulated populations*) using thermistors encased in dataloggers (Onset Computer Corporation, Pocasset, Massachusetts). We secured loggers to the rock using housings made of stainless-steel mesh, white Vexar mesh, and cable ties. Loggers were placed at the same tidal height as the experiments, and were installed away from algal canopy, rocky ledges, or other sources of shade. Whelk body temperatures were measured using a hand-held digital thermometer (Omega HH-82; Omega Vanzetti, Sharon, Massachusetts) equipped with E-type Teflon-insulated 36 AWG fine-wire thermocouples. A small hole was drilled in the posterior portion of the whelk's shell, and the thermocouple was gently inserted into this hole until it was touching, but not piercing, soft tissue. Modeling clay was used to cover the insertion point, and the whelk was placed back where it was collected and allowed to re-attach to the substratum. Body temperature was recorded every 15 min. Recordings were initiated on consecutive low tides as soon as the water receded at the beginning of low tide, and continued until the water returned.

Natural populations

Animal collections for physiological measurements.—From July 1993 to July 1994, whelks were collected every 90–110 d at wave-exposed areas at SH and BB. Ten individuals were collected at randomly determined locations along a 10-m transect line laid parallel to the shore. Individuals were immediately dissected and frozen on dry ice in the field. Dissected tissues were returned to the laboratory, where they were

stored at −70°C until biochemical analyses were conducted.

RNA : DNA ratios.—Concentrations of RNA and DNA in foot tissue were determined by ethidium bromide fluorescence following the method of Bentle et al. (1981) as modified by Dahlhoff and Menge (1996). Tissues were thawed on ice, weighed, and homogenized in 30 volumes 2 mol/L NaCl with a hand-driven glass homogenizer (Kontes Duall, Vineland, New Jersey, USA). Fifty μL of each sample were incubated in 1.5 mL of 0.005 mg/mL ethidium bromide and 0.10 mg/mL Proteinase K at 37°C for 90 min. After incubation, 0.5 mL buffer (80 mmol/L Tris-Cl, pH 7.5 at 20°C) was added, and fluorescence was recorded at 365 nm excitation and 590 nm emission using a Perkin-Elmer (Oakbrook, Illinois) LS-5B luminescence spectrofluorometer. RNA and DNA concentrations of tissues were estimated from a standard curve calculated using known quantities of RNA and DNA (Sigma calf thymus DNA, 1–4 μg; Sigma calf liver RNA, type IV, 2–8 μg; Sigma Genosys, The Woodlands, Texas).

Malate dehydrogenase activities.—To assay malate dehydrogenase activities, foot tissue was thawed on ice, weighed, and immediately homogenized in 10 vol ice-cold 50 mmol/L potassium phosphate buffer (pH 6.8 at 20°C). The homogenate was centrifuged (4°C, 5 min at 14 000g [137 293.1 m/s^2]) and supernatant collected. Enzymes were assayed spectrophotometrically following the methods of Dahlhoff and Menge (1996). Enzymatic activities are reported in International Units per gram wet mass (IU/g).

Field-manipulated populations

Cage transplants, foraging rates and animal collections.—All experimental whelks (15–20 mm length) used at both sites were collected in May from a location of intermediate wave exposure at SH away from the experimental reef area. Time and logistical constraints did not allow a reciprocal set of experiments using BB whelks. Although this constrains generalizations from our experiments (i.e., we cannot infer that BB whelks would have necessarily shown similar responses), it does reduce the likelihood that any changes in experiments were due to genetic differences between whelk populations. Moreover, sampling studies (RNA : DNA ratios, malate dehydrogenase [MDH]) from natural populations native to each site and exposure provided results consistent with those observed in experimental (SH-derived) whelks. Nonetheless, we remain sensitive in our interpretations to possible effects of whelk source on our results.

Whelks were taken to the laboratory, and oxygen consumption rates were measured for 30 randomly selected individuals. All remaining individuals were marked with nail polish, and transplanted into 10 × 10 × 5 cm stainless-steel wire-mesh cages with 6-mm openings at BB and SH. Cages were secured to the rock with stainless-steel screws. Ten marked whelks were

placed in each cage, which were assigned to one of three treatments: +prey +shelter, −prey +shelter, and −prey −shelter. Whelks in +prey +shelter treatments were placed in cages into which small *Mytilus trossulus* had been translocated two weeks beforehand. Mussels were added throughout the experiment to these cages to maintain continuous availability of food. In +prey cages, mussels were held against the rock with plastic mesh. Whelks had access to mussels at all times because they could feed by extending their proboscises through the mesh. Whelks in −prey +shelter treatments were placed in cages over bare, scraped rock to which empty mussel shells had been attached with marine epoxy (Z-spar, Kop-Coat, Pittsburgh, Pennsylvania). These shells provided shelter from the sun to whelks, controlling for the fact that *M. trossulus* provide both food and shelter to the whelks in the +prey +shelter treatments. Whelks in −prey −shelter treatments were placed in bare-rock cages without either food or shelter. Each block of three treatments was replicated five times at each site × exposure combination.

Two whelks were removed from each cage every 30 d. Collected individuals were placed in a cooler on ice, and brought immediately to the laboratory at Newport, Oregon. Whelks were placed in seawater for two hr at ambient seawater temperature (11°–12°C) to allow them to recover from collection and handling. Collected whelks were replaced with unmarked, wild-caught individuals to maintain a constant number of whelks per cage throughout the experiment. During the first collection (June), the total number of empty *M. trossulus* shells was counted in each +food +shelter treatment to determine foraging activity. Mussel shells could not fall out of the cages, and most mussel shells showed evidence of whelk-induced damage (small drill holes). Foraging rates were calculated using the total number of empty shells in the +food +shelter cages. Predation rates reported here for June represent a slight overestimate of the actual rate because mussels protected from whelks suffer a low level (≤5%) of nonpredator-caused mortality (E. P. Dahlhoff and B. A. Menge, *unpublished data*). Background mortality rates did not differ between sites, so adjusting rates to account for background mortality was deemed unnecessary. Post-June foraging rates were not analyzed because nonpredator-related mortality (as determined by the absence of drill marks) of transplanted mussels was high and variable.

Oxygen consumption rates.—Oxygen consumption rates ($\dot{V}O_2$) were determined using a Gilson differential respirometer (Gilson Medical Electronics, Middleton, Wisconsin) to measure metabolic rate of whelks held in cages under different field treatments. Snails were placed in glass chambers containing 5 mL sterile-filtered seawater, and the chambers were connected to the respirometer. Whelks were allowed to equilibrate to the assay temperature (10°C) for 45 min. This assay tem-

perature was selected to approximate the seawater temperature the whelks would experience upon the return of the water at the end of low tide, when the animals become active in situ. Each measurement was conducted in triplicate 60-min time periods, with 10 min between each period, during which time the respirometer was opened to equilibrate the system with fresh air. At the end of each triplicate, snails were removed from chambers, dissected, weighed, and frozen at −70°C. The consumption of oxygen was linear with time, suggesting specimens did not experience oxygen limitation during the experiment. The slope of the resulting line was used to calculate mass-specific oxygen consumption rate (Stillman and Somero 1996).

Tissue levels of Hsp70.—Tissue levels of Hsp70 were measured throughout the summer for a subset of whelks that were held in the experimental cages under all treatments. Whelks were randomly sampled every 30 d from cages in each site × exposure combination, and foot tissue was dissected from whelks in the field and immediately frozen on an aluminum block chilled on dry ice. Samples were transported back to the lab on dry ice and stored at −70°C until processing. We determined tissue levels of Hsp70 for field-manipulated whelks by Western Blot analysis following published methods (Roberts et al. 1997, Dahlhoff and Rank 2000). We quantified Hsp70 by chemiluminescence using a commercially available anti-Hsp70 antibody that recognizes several isoforms of Hsp70 (MA3-001, Affinity Bioreagents, Golden, Colorado) and a ''secondary'' antibody, conjugated with peroxidase, used to locate anti-Hsp70 antibody using x-ray film. We measured band area and density using Sigma Scan Pro (release 4.0 for PC; SPSS Science, Chicago, Illinois), and determined Hsp70 levels by linear regression to a serial dilution curve of four concentrations of a known quantity of pure Hsp72 (Human recombinant, SPP-855, Stressgen Biotechnologies, Victoria, British Columbia, Canada) on each blot. We routinely observed a single band at 72 kD on experimental blots. However, both noninducible (70 kD) and stress-inducible (72 kD) isoforms of Hsp70 may have been present. We remain sensitive to this issue in our interpretation of Hsp data.

Statistical analyses.—Regression analysis was used to analyze diurnal measurements of whelk body temperature. For all other data we used analysis of variance (ANOVA), with ''site'' (SH and BB), ''exposure'' (EX and PR), time, food (prey), and shelter as treatments. All were regarded as fixed effects; our null hypotheses were that whelk responses did not differ between these sites of known ecological conditions, or between contrasting conditions of exposure, time, food, or shelter. This experimental design constrains statistically based inferences about whelk responses beyond these sites, wave exposures, or experimental conditions, but does not prevent consideration of more general implications of our results based on these studies, and other ecological and physiological knowledge in this system.

TABLE 1. Ambient air temperatures at Strawberry Hill (SH) and Boiler Bay (BB) in wave-protected (PR) and wave-exposed (EX) regions, June–August 1995.

Site and exposure	Air temperature (°C)		No. days above 25°C
	Mean	1 SE	
SHPR	25.0	0.7	28
BBPR	21.9	0.7	22
SHEX	20.3	0.6	19
BBEX	15.9	0.6	3

Two-way ANOVA was used to compare seasonal and site-related variation of RNA : DNA ratios and enzymatic activities of whelks. Enzyme concentrations were square-root transformed for analysis, whereas RNA : DNA ratios were used without transformation because assumptions of normality and independence of error terms were met by the ratio data. Three-way and four-way ANOVA were used to analyze $\dot{V}O_2$ (site, wave-exposure, prey, and time) and Hsp70 (site, exposure, and time) data. Oxygen consumption rates were square-root transformed for analysis, whereas Hsp70 values were not transformed because assumptions of ANOVA were met by the nontransformed data. Homogeneity of variances was confirmed using Cochran's test (Winer et al. 1991). Statistical analyses were conducted using SYSTAT (Release 7.0 for PC [Wilkinson 1996]) and Jmp (Release 3.1 for PC, SAS Institute).

RESULTS

Habitat thermal regime and whelk body temperature

Data obtained from electronic loggers indicate that the thermal environment of whelks varies with site and exposure. Table 1 shows mean habitat temperatures (with SE) at each locale, as well as the number of days that maximal air temperature exceeded 25°C for at least 30 min. Strawberry Hill is warmer than Boiler Bay, and wave-protected areas are warmer than wave-exposed areas (Two-way ANOVA, Site: $F = 34.71$, $P < 0.0001$; Exposure: $F = 70.74$, $P < 0.0001$; site × exposure: NS). Air temperatures were higher in July and August than in June (data not shown for brevity). The number of days that maximal air temperature exceeded 25°C, a temperature known to induce Hsp70 expression for whelks in the lab, was greatest at wave-protected areas. Interestingly, the number of days that air temperature exceeded 25°C was similar at Strawberry Hill, wave-exposed [SHEX] and Boiler Bay, wave-protected [BBPR] (Table 1). Minimum temperature at these sites was equivalent to sea-surface temperature, which varied from 9.5 to 12.2°C throughout the study (as judged from high tide temperatures taken from loggers). Higher air temperatures at wave-protected areas are likely due to lower water flow and lower wave splash. At wave-exposed sites, wave splash can cool the surface of the rock (and thus animals) by 10°–15°C in a few minutes (E. P. Dahlhoff, *unpublished data*). Other conditions that influence air and rock temperature, includ-

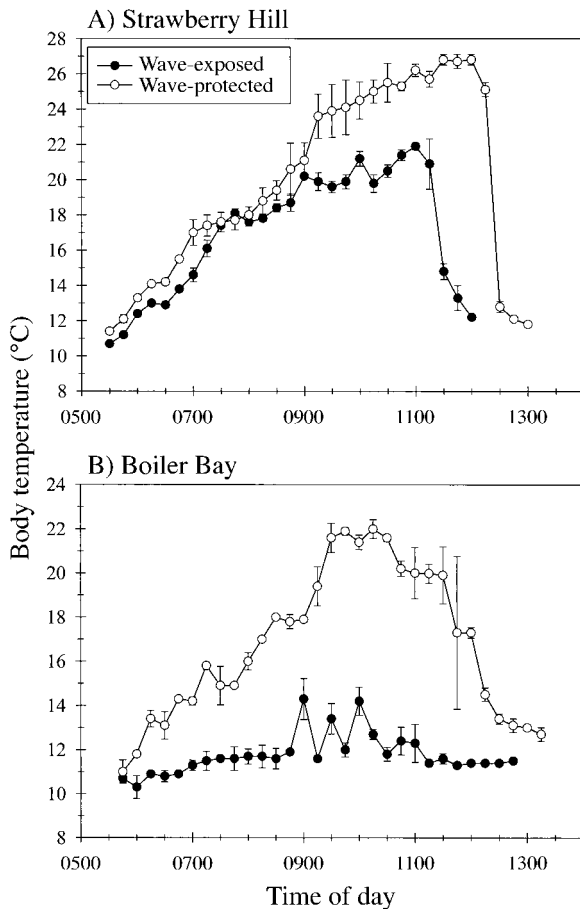## A) Strawberry Hill

B) Boiler Bay

FIG. 1. Measurements of whelk body temperatures during low tide at (A) Strawberry Hill and (B) Boiler Bay, on two consecutive days in July (2 July SH, 3 July BB). Body temperatures were recorded in randomly selected whelks approximately every 15 min using hand-held digital thermometers. Data points are means ($\pm$ 1 SE) of four individuals at each wave exposure.
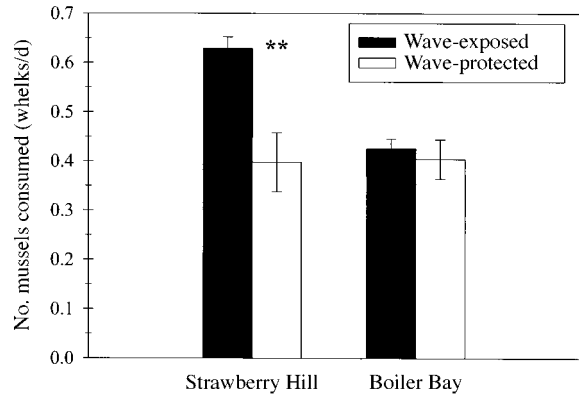

FIG. 2. Mussel-consumption rates of caged whelks at SH and BB. Foraging rates were determined by counting the number of mussels present per cage at the beginning of the experiment and those still alive after 14 d, and dividing the number of consumed mussels by the number of whelks present in the cage. Data are means ($\pm$ 1 SE) of data from five cages per site $\times$ exposure. Asterisks indicate significance of comparisons between site by exposure (one-way ANOVA; **$P < 0.005$).
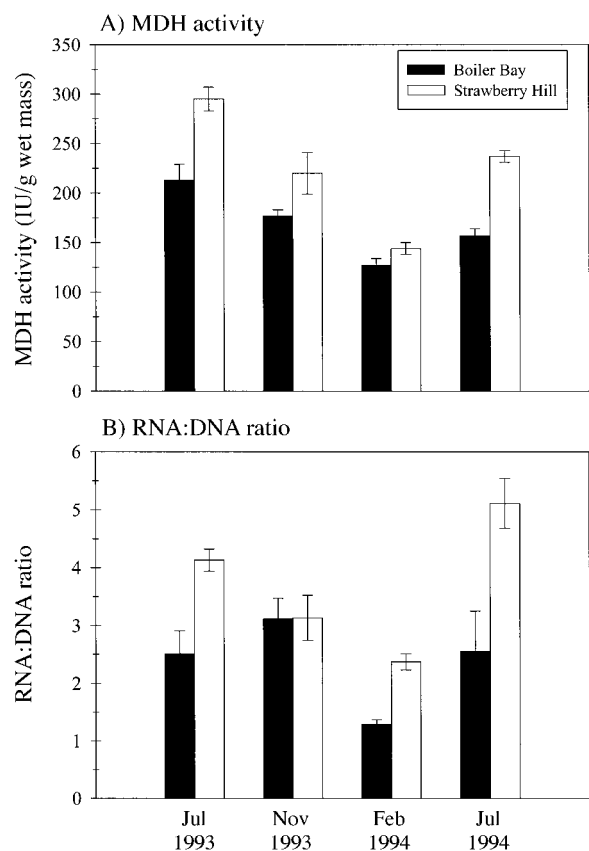

FIG. 3. Differences in (A) malate dehydrogenase activity and (B) RNA : DNA ratio of natural populations of whelks at SH and BB. Data are means ($\pm$ 1 SE) of 10 individuals collected 90–110 d apart. Statistical analyses are reported in Table 2.

ing fog, rain, and degree of sun exposure, do not differ between wave exposures, although they vary between SH and BB (Menge et al. 1997b).

Whelk body temperatures at low tide mirrored patterns observed in air temperature (Fig. 1). SHEX whelks had higher body temperatures than Boiler Bay, wave-exposed [BBEX] whelks (compare filled symbols on Fig. 1A and B). Regression analysis suggests that rates of body temperature increase varied with site and exposure (site $\times$ exposure interaction was significant, $F = 3.116$, $P < 0.05$). EX whelks at BB warmed up more slowly than did PR whelks or whelks at SH.

Mussel consumption

Mussel consumption by whelks varied with site and exposure (Fig. 2). Consumption rates were higher at EX areas at SH (Fig. 2, site $\times$ exposure: $F = 5.704$, $P < 0.05$). Recent studies suggest that whelks have similar impacts on barnacle abundance at SH and BB

TABLE 2. Two-way ANOVA on the effect of site and time on enzymatic activities and RNA : DNA ratios of *Nucella ostrina*.

Source of variation	df	ss	F	P
MDH activity				
Site	1	72.55	43.82	<0.0001
Time	3	178.35	35.91	<0.0001
Site × time	3	15.885	3.198	<0.05
Error	72	119.16		
RNA : DNA ratio				
Site	1	3.074	38.74	<0.0001
Time	3	3.675	15.44	<0.0001
Site × time	3	1.233	5.149	<0.005
Error	72	5.7096		

Notes: Sites were Strawberry Hill and Boiler Bay; time corresponds to measurements made approximately every 90 d from July 1993 to July 1994. Site and time were treated as fixed variables.

(B. A. Menge, *unpublished data*). Whelks at SHEX may thus feed faster but for less time than at other sites or exposures.

Biochemical variation in natural populations.—Metabolic activity of *N. ostrina,* as indexed by malate dehydrogenase activities of foot tissue, was higher at SH than at BB, particularly in summer (Fig. 3, Table 2; site × time interaction was significant). RNA : DNA ratios of foot for SH whelks were also greater than those of BB whelks most times of the year (Fig. 3B, Table 2; site × time interaction was significant). These data suggest that whelks at SH had higher somatic tissue growth rates than whelks at BB, as RNA : DNA ratio is an excellent indicator of protein synthetic capacity in animals with indeterminate growth. The observation that whelk MDH activities and RNA : DNA ratios are higher at SH than at BB suggests that whelks at SH have higher metabolic rates than BB whelks.

Physiological changes in transplanted whelks

Metabolic activity.—Initial oxygen consumption rates of whelks collected in May from SH were 8.8 mmol O_2 per hour per gram wet mass ($n = 30$). Thereafter, oxygen consumption rates varied with site, exposure, prey availability, and time (Table 3). Prey availability affected oxygen consumption as a main effect, while the effects of site, exposure, and time were manifested through two-way interactions (Table 3). To simplify the presentation of the complex results of this experiment, we focus on responses of +prey +shelter whelks by site, exposure, and time (Fig. 4), and responses to prey availability (+prey vs. −prey) in the presence of shelter (Fig. 5). Responses of −prey whelks to shade (+shelter vs. −shelter) are shown in Fig. 7.

Within 30 d, transplanted, +prey +shelter whelks tended to have lower metabolic rates at BB than at SH, especially at wave-exposed locations (Fig. 4, Table 3; site × exposure interaction). Oxygen consumption rates were higher in July than June, and were low in August at both sites, although greater changes occurred at SH (Fig. 4, Table 3; site × time interaction). The effects of wave-exposure on oxygen consumption rates were both site and time dependent (Table 3; exposure × site, $P < 0.005$; exposure × time, $P < 0.05$). Except for August, oxygen consumption rates were similar at wave-protected areas throughout the summer. The fact that whelk metabolic rates were lower in August suggests that elevated temperatures over the course of the summer may lead to accumulated stress damage, resulting in compromised whelk physiological performance.

Experimental reduction in prey availability resulted in lower metabolic rates for all treatments (Fig. 5). Although oxygen consumption rates were still higher at SH, the effects of feeding regime were independent

TABLE 3. Four-way ANOVA on the effect of site, exposure, prey availability, and time on oxygen consumption rate ($\dot{V}O_2$) of *Nucella ostrina*.

Source of variation	df	ss	F	P
Site	1	14.96	95.05	<0.000
Exposure	1	0.01	0.05	NS
Prey availability	1	8.30	26.36	<0.000
Time	2	13.59	43.19	<0.000
Site × exposure	1	1.74	11.07	<0.00
Site × prey availability	1	0.72	2.30	NS
Site × time	2	5.91	18.78	<0.00
Exposure × prey availability	1	0.39	1.23	NS
Exposure × time	2	2.50	7.94	<0.05
Feeding × time	2	1.89	3.01	NS
Site × exposure × prey availability	1	0.46	1.47	NS
Site × exposure × time	2	0.79	2.50	NS
Site × prey availability × time	2	1.08	1.72	NS
Exposure × prey availability × time	2	0.40	0.63	NS
Site × exposure × prey availability × time	2	0.92	1.46	NS
Error	237	37.29		

Notes: Independent variables were site (Strawberry Hill and Boiler Bay), exposure (wave-exposed and wave-protected), prey availability (+prey and −prey), and time (every 30 d from May to August 1995). Site, exposure, feeding, and time were treated as fixed variables. NS, not significant ($P > 0.05$).
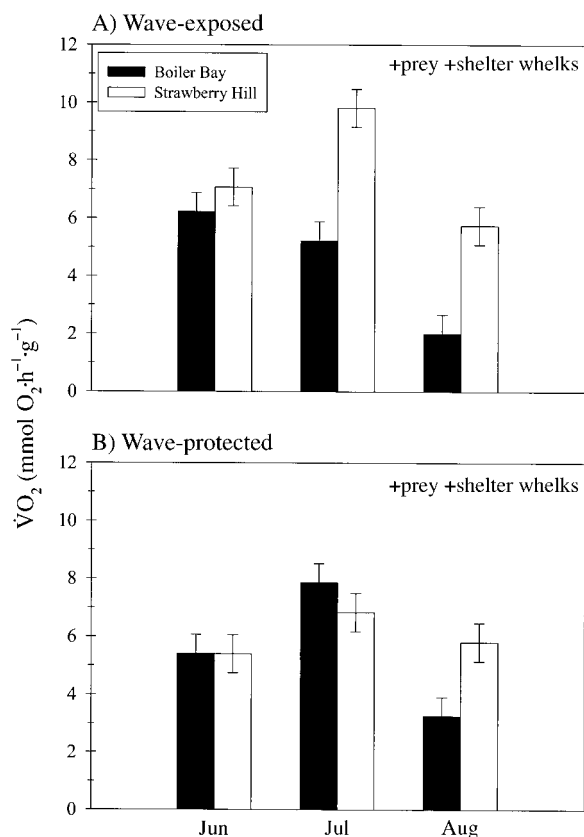
FIG. 4. Oxygen consumption rates ($\dot{V}O_2$) of +food +shelter whelks at (A) wave-exposed and (B) wave-protected areas at BB and SH. Snails in this treatment were given food (small mussels) and shelter (shades built from empty mussel shells). Data are least-squares means ($\pm$ 1 SE) of 10 individuals. Statistical analyses are reported in Table 3.

FIG. 5. Effects of prey availability on the metabolic rates of SH-derived whelks at SH and BB. Data shown are combined least-squares means ($\pm$ 1 SE) of 20 individuals per treatment and exposure. All individuals were given shelter. Statistical analyses are reported in Table 3.

of site or time (Table 3; no interactions involving prey availability were significant). The site-independence of feeding effects was probably due to the fact that fed animals at both SH and BB were supplied an abundance of prey items in their experimental treatments. However, natural abundance of prey is less in the mid-low zone at BB than at SH, and may be responsible in part for lower metabolic activities for natural populations of whelks (Fig. 3). Thus low metabolic rates for experimentally manipulated whelks at BB (Fig. 4) cannot be explained solely by lower prey availability, but rather may be the result of lower foraging activity (Fig. 2). Note that the reduced metabolic rates at BB were from whelks that were native to SH; thus the differences represent short-term shifts by whelks from SH-like physiology to BB-like physiology.

Heat stress.—Tissue levels of a 70 kD heat shock protein (hereafter termed Hsp70) were quantified as a direct index of the effect of thermal stress on +prey +shelter whelks (Fig. 6). Whelks at SH had significantly higher tissue levels of Hsp70 than whelks at BB throughout the summer at both wave-exposed and wave-protected areas (Fig. 6, Table 4). These data,
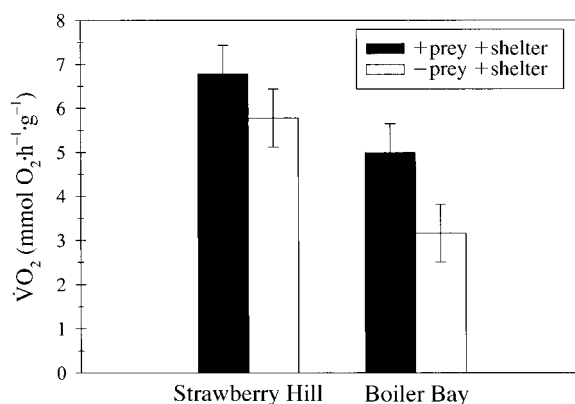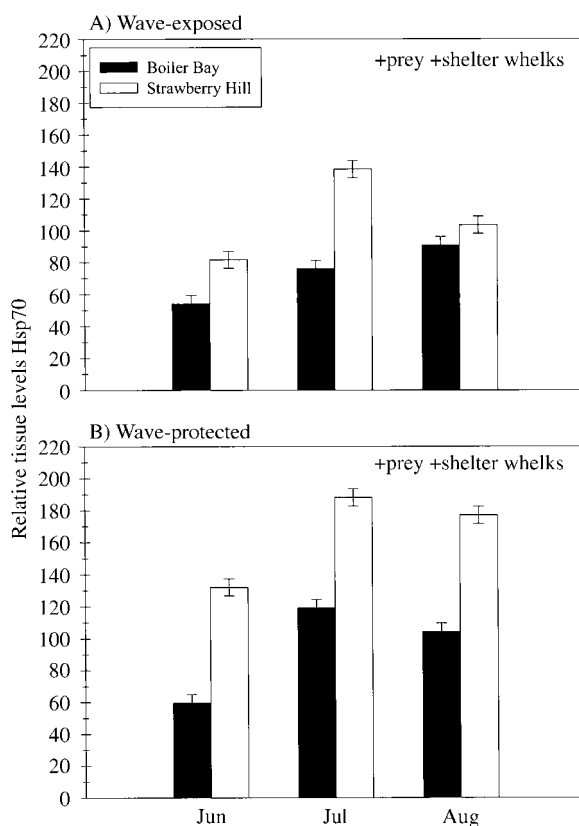

FIG. 6. Changes in Hsp70 expression for SH snails transplanted to (A) wave-exposed and (B) wave-protected areas at BB and SH. Snails in these treatments were given access to prey and shelter. Data are means ($\pm$ 1 SE) of four individuals haphazardly sampled from five cages for each time point. Statistical analyses are reported in Table 4.

A) Oxygen consumption at SH

C) Oxygen consumption at BB

B) Expression of Hsp70 at SH

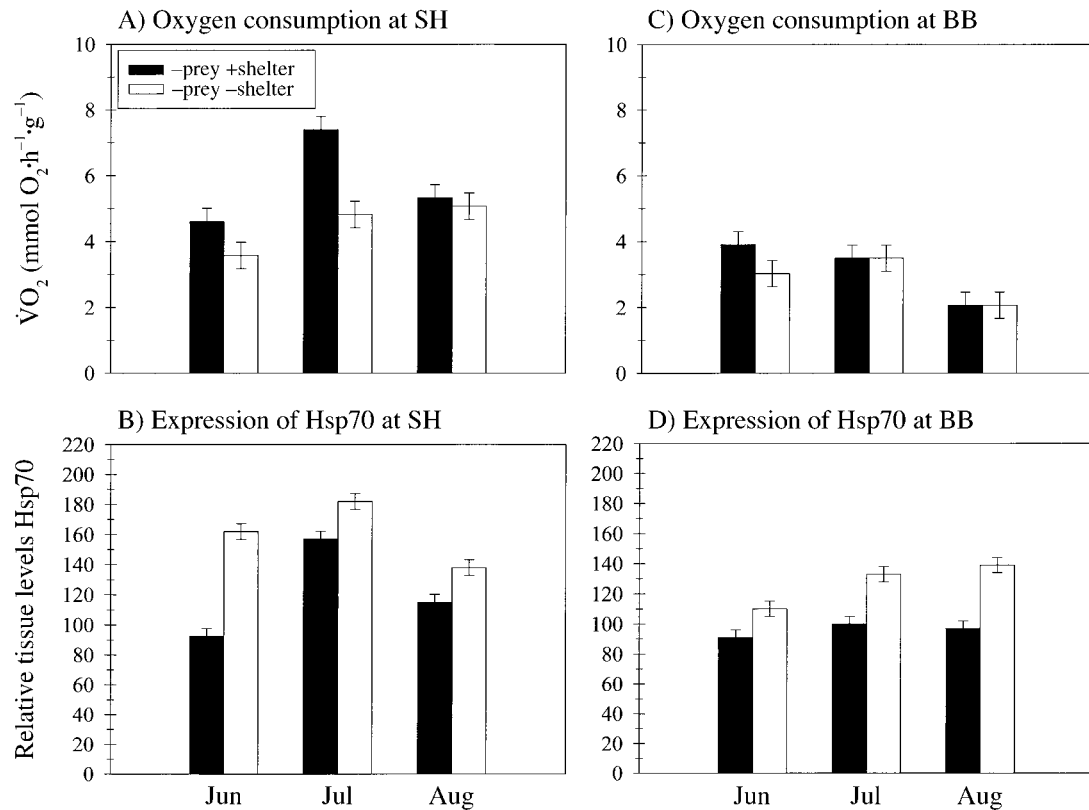## D) Expression of Hsp70 at BB

FIG. 7. Changes in oxygen-consumption rate and Hsp70 expression for snails transplanted to SH (A, B) and BB (C, D), deprived of prey, and held in the absence or presence of sun-shelters. $\dot{V}O_2$ data (A, C) are least-squares means ($\pm$ 1 SE) of data combined from wave-exposed and wave-protected areas ($n = 5$ per block); Hsp70 data (B, D) are least-squares means ($\pm$ 1 SE) of data combined from wave-exposed and wave-protected areas ($n = 4$ per block). Statistical analyses are reported in Table 5.

combined with the fact that body and habitat temperatures are higher at SH (Table 1, Fig. 1) suggest that SH whelks may experience greater heat stress on a routine basis than BB whelks. Furthermore, lower air temperatures, body temperatures, and tissue levels of Hsp70 in wave-exposed whelks, especially at SH, suggests that wave-protected whelks experience greater heat stress than wave-exposed whelks later in the summer (Fig. 6, Table 4; site $\times$ exposure, time $\times$ exposure significant).

Availability of shelter from sun had a strong effect on whelk physiology (Fig. 7, Table 5). *Nucella* caged without prey or shelter ($-$prey $-$shelter treatment) had lower oxygen consumption rates and higher levels of Hsp70 than whelks caged without prey but with shelter ($-$prey $+$shelter treatment). As before, there was a strong effect of site on metabolic rate and Hsp70 expression (Table 5; $P < 0.0001$ for site effects). These data suggest that sun exposure may reduce metabolic activity when prey availability is low, especially later

TABLE 4. Three-way ANOVA on the effect of site, wave-exposure, and time on Hsp70 levels of $+$prey $+$shelter *Nucella ostrina*.

Source of variation	df	ss	F	P
Site	1	23 845.51	34.73	<0.0001
Exposure	1	44.01	0.06	NS
Time	2	17 621.02	12.83	<0.0001
Site $\times$ exposure	1	21 330.84	31.07	<0.0001
Site $\times$ time	2	10 528.77	7.67	<0.001
Exposure $\times$ time	2	8 982.90	6.54	<0.005
Site $\times$ exposure $\times$ time	2	4 287.56	3.12	<0.05
Error	84	57 666.88		

Notes: Independent variables were site (Strawberry Hill and Boiler Bay), exposure (wave-exposed and wave-protected), and time (every 30 d from May to August 1995). Site, exposure, and time were treated as fixed variables. NS, not significant ($P > 0.05$).

TABLE 5. Three-way ANOVA for effects of site and shelter of Hsp70 levels and $\dot{V}O_2$ of −prey whelks at Strawberry Hill and Boiler Bay.

Source of variation	df	ss	F	P
Oxygen consumption				
Site	1	12.82	87.61	<0.0001
Treatment	1	0.26	1.80	NS
Time	2	4.88	33.32	<0.0001
Site × treatment	1	0.85	5.83	<0.05
Site × time	2	1.42	9.72	<0.005
Treatment × time	2	0.00	0.02	NS
Site × treatment × time	2	1.42	9.67	<0.005
Error	162	25.74		
Levels of Hsp70				
Site	1	23 845.51	34.73	<0.0001
Treatment	1	44.01	0.06	NS
Time	2	17 621.02	12.83	<0.0001
Site × treatment	1	21 330.84	31.07	<0.0001
Site × time	2	10 528.77	7.67	<0.001
Treatment × time	2	8 982.90	6.54	<0.005
Site × treatment × time	2	4 287.56	3.12	<0.05
Error	84	57 666.88		

Notes: Independent variables were site (Strawberry Hill and Boiler Bay), treatment (+shelter and −shelter), and time (measured every 30 d from May to August 1995). Site, treatment, and time were treated as fixed variables. NS, not significant ($P > 0.05$).

in the summer after many consecutive days of stressfully high temperatures. This reduction in metabolic activity may be caused by a shift of cellular energy from foraging activity to tissue repair, as evidenced by high levels of Hsp70 concomitant with lower metabolic rates in −prey −shelter whelks in July and August (Table 5; three-way ANOVA, $P < 0.001$ for three-way interaction of site, time, and treatment).

DISCUSSION

Our results suggest that physiological stress is tightly linked to whelk metabolism over a range of environmental conditions at two rocky intertidal sites with distinct community profiles. Modification of whelk activity due to physiological stress may lead to differential impacts of these predators on prey abundance, which may ultimately impact community dynamics. We feel that when combined with recent results demonstrating the effects of physiological stress on major prey species (Hofmann and Somero 1995, Dahlhoff and Menge 1996, Roberts et al. 1997), our results set the stage for studies detailing the physiological mechanisms that underlie the community impacts of predator–prey interactions.

Environmental conditions and whelk ecophysiology

Habitat thermal regime and whelk body temperatures.—The thermal regime of Strawberry Hill (SH) and Boiler Bay (BB) was characterized by determining the substratum surface air temperature. Our data revealed that temperatures are warmest at SHPR, coolest at BBEX, and intermediate at SHEX and BBPR. We used near-substratum air temperature rather than water temperature to characterize thermal habitat because air temperatures reach greater extremes, and are thus more

likely to be more stressful. For example, overall variation in water temperature during our study was small in magnitude (9.5°–12.2°C) relative to variation in air temperature (5.2°–36.4°C). Our direct measurements of whelk body temperatures, while limited in scope, were consistent with results using electronic loggers, although maximal whelk body temperatures were lower than maximal substratum temperatures. Whelks are thus likely to experience physiological stress during exposure to elevated air temperatures that may compromise their ability to forage when the tide returns.

The lower body temperatures of whelks compared to logger temperatures may be due in part to the fact that whelks behaviorally regulate their body temperatures. Avoidance of potentially lethal temperatures is a key thermal regulatory strategy for many ectothermic animals (Prosser 1986, Cossins and Bowler 1987). At low tide we observed whelks in microsites that were both exposed to and sheltered from thermal stress, including wedged in cracks, under large mussels, in the midst of barnacles or small mussels, and on open rock surfaces. In the experimental treatments, whelks were observed inside the mussel shelters that were provided, but were also observed either out on open substratum or on top of their mussel prey. Thus although shelter was available, whelks did not always avoid high sun exposure under either natural conditions or in experimental treatments. Our data suggest that whelks can experience large excursions in body temperature (11°–27°C) during low tide, between sites, and among wave-exposures, and that they do not always avoid exposure to high air temperatures.

Physiological variation in natural populations.—Malate dehydrogenase activities and RNA : DNA ratios were higher for whelks at SH than at BB. Differences

in food availability and quality may underlie differences in whelk physiology between sites. Suspension feeders at SH are more abundant and have higher growth rates, RNA : DNA ratios, metabolic activities, and tissue-protein contents than those at BB (Menge 1992, Menge et al. 1994, 1997*a, b,* Dahlhoff and Menge 1996, Stillman et al. 1996). These earlier data suggest that SH prey have higher nutritional content than BB prey, as previous studies of mussel dietary physiology have shown that protein content of prey correlates with its dietary value (Bayne and Hawkins 1990, Bayne et al. 1993, Kreeger 1993, Kreeger and Langdon 1993, Kreeger et al. 1995).

Since suspension-feeders and their predators are important players in rocky intertidal food webs, variation in both the density and caloric content of prey could ultimately have important impacts on community structure. Previous research revealed that the overall impact of predation in the low intertidal zone was weaker at BB than SH (Menge 1992, Menge et al. 1994, 1996, Navarrete 1996, Navarrete and Menge 1996). Not surprisingly, most of the difference in predation impact was due to *Pisaster ochraceus,* not to *Nucella.* Field experiments suggested that whelk predation, both on a per population and per capita basis, was similar in magnitude at wave-exposed sites at BB and SH (Navarrete and Menge 1996). Since foraging time is limited at both sites by tidal flux, and may be limited by high temperatures in wave-protected regions, SH whelks may obtain higher energetic return on their foraging activity by foraging faster and on prey with higher caloric value. Therefore, our results suggest that physiological acclimatization of predators to variation in food quality or prey density may be an important factor underlying variation in the impact of predation on community dynamics.

Differences in metabolic activity between natural populations of whelks at SH and BB were most pronounced during the summer. There was significant seasonal variation in both RNA : DNA ratio and MDH activity at both sites, with metabolic activity being lowest in February and highest in July. Several factors are likely to contribute to this result. First, like many ectothermic animals, whelks become less active during the winter, and their metabolic activity is suppressed, independent of spatial variability in physical factors or biological interactions (Newell 1979). Second, prey abundance and prey quality are highest in the summer (Dahlhoff and Menge 1996, Menge et al. 1997*a, b*). Third, temperatures are highest in the summer, which may lead to elevated activity. We discuss these possibilities below with respect to our measurements from experimentally manipulated individuals.

Metabolic activity of experimentally manipulated whelks.—Whelks at SH had higher metabolic rates (as indexed by oxygen consumption) than whelks at BB. At wave-exposed areas, this pattern developed within 30 d of transplantation, and was especially pronounced

in July. At wave-protected areas, metabolic rates were similar for whelks in both BB and SH experiments until August. Whelks are ectotherms, and increased body temperatures in ectotherms usually increases activity, at least on a short-term basis (Prosser 1986). The higher body and habitat temperatures observed for SH whelks may result in greater foraging activity. Conversely, the lower temperatures and foraging rates at BB may have produced the lower metabolic rates there. However, elevated body temperatures cannot directly explain higher experimental oxygen consumption rates. Although whelks were acclimatized to different thermal conditions in the field, temperatures at which oxygen consumption rates were measured were identical for all specimens. If whelks differentially altered metabolic rates in response to temperature, we would actually expect lower metabolic rates (at any given temperature) for warm-acclimated animals (the Q_{10} effect; Prosser 1986). A more likely scenario is that high body temperatures led to higher activity in SH whelks, which then led to high ingestion of prey, which resulted in high metabolic rates. However, this dietary model does not explain the fact that whelk metabolic rates were highest in July and dropped dramatically in August. This effect may be due to decreases in prey quality, but is more likely due to accumulated physiological stress due to several months of exposure to elevated air temperatures.

Tissue levels of Hsp70 in experimentally manipulated whelks.—Tissue levels of Hsp70 were higher for whelks at SH than at BB, and were higher at wave-protected areas than wave-exposed areas. Since Hsp70 is a direct indicator of physiological stress, Hsp70 production is likely to have a strong impact on whelk activity, especially late in the summer after several months of exposure high air temperatures during low tide. Heat shock proteins, expressed to repair proteins damaged by high temperature, are energetically costly to produce (Lindquist 1986, Parsell and Lindquist 1993). High tissue levels of Hsp70 in August whelks may mean that energy that was being used for activity (and possibly growth) early in the summer has been shifted to housekeeping and repair functions later in the summer.

The data presented here are consistent with other studies of Hsp70 in natural populations of rocky intertidal invertebrates (Hofmann and Somero 1995, 1996*a, b,* Roberts et al. 1997, Tomanek and Somero 1999). Those studies showed that Hsp70 expression was higher for mussels and snails at wave-protected sites than wave-exposed sites, and higher in the summer than in the winter. It was somewhat surprising that we observed similar patterns of variation for mobile predators. Although some mobile ectotherms can escape high temperatures behaviorally (Prosser 1986), our data are more consistent with the hypothesis that whelks are exposed to significant site- and exposure-related gradients in temperature. Recent studies of

snails in the genus *Tegula* also suggest that mobility does not guarantee a rocky intertidal invertebrate escape from physiological heat stress (Tomanek and Somero 1999). This observation may be due to the fact that mobile foragers are time-limited by tidal flux and wave activity in many rocky intertidal systems. Thus an animal may "sacrifice" its ability to avoid heat stress to remain in an area where prey density is high or abundance of competitors is relatively low. In these cases, the ability of an individual to recover from heat stress (by launching the heat shock response) may be a key component of its success in any given region on the rocky intertidal.

Conclusions

The data presented in this study show strong, consistent patterns in physiological variation for the rocky intertidal predator *Nucella ostrina* over a range of temperatures, wave-exposures, and prey densities. Whelks living in areas with high prey abundance are more physiologically "robust" (i.e., have higher energy stores) even when conditions are physiologically stressful. These results are consistent with the hypothesis that bottom-up ecological effects can be a major determinant of the relative success of foraging predators, as they are for suspension-feeding prey. Although the present study does not provide definitive evidence that community differences between, or within, SH and BB are caused by variation in whelk physiology, it suggests that whelks are able to alter the intensity of their activities to respond to heterogeneous environmental conditions. This study lays the groundwork for future investigations exploring the differential impacts of predators along environmental stress gradients and how that may ultimately affect community dynamics.

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