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Stress protein (HSP70 family) expression in intertidal benthic organisms: the example of *Anthopleura elegantissima* (Cnidaria: Anthozoa)*

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ABSTRACT: Both the physiology and distribution of intertidal organisms are strongly influenced by different kinds of physical stress. Temperature, UV radiation and desiccation in low tide conditions are usually considered the most important physical stresses. These factors impact cell metabolism, and the organism's ability to rapidly adapt to altering environmental conditions sets its tidal distribution limits. The role of the HSP70 protein (stress protein) family in thermal stress responses has been widely demonstrated. Although it has been shown that stress protein expression is a useful tool to quantify part of this physical stress, few studies have been made with different *in situ* intertidal organisms. To test differences in HSP70 expression under natural conditions in the intertidal environment, we chose a common cnidarian of the eastern Pacific Ocean: the anemone *Anthopleura elegantissima*. Polyp HSP70 expression depends on the degree of emersion and the extent of physical stress (0.1-3.6 ± 1.5 ng HSP70 μg Protein^{-1} were registered between fully immersed and fully emersed polyps of the same clone). The time of immersion is reflected in the recovery of polyp HSP70 levels from the high tidal exposure (reaching again as shallower clones express more HSP70 (2.5 ng HSP70 μg P^{-1}), than deeper ones (0.1 ng HSP70 μg P^{-1}). Also sunny and foggy environmental situations influence the stress response. Anemone clones exposed to the sunny high intertidal zone express more than three times HSP70 (2.2 ng HSP70 μg P^{-1}) than those on a foggy high intertidal day (0.6 ng HSP70 μg P^{-1}). For *A. elegantissima*, shrinking of polyps, mucus secretion, sand covering, UV absorbing molecules, and a tight patch structure work concurrently with HSP70 expression to alleviate the effects of physical stress in low tidal emersion.

Key words: *Anthopleura*, stress proteins, HSPs, intertidal stress, temperature

RESUMEN: EXPRESIÓN DE LAS PROTEÍNAS DE ESTRÉS (FAMILIA DE LA HSP70) EN ORGANISMOS BENTÓNICOS INTERMAREALES: EL EJEMPLO DE *ANTHOPLEURA ELEGANTISSIMA* (CNIDARIOS: ANTOZOOS). – Tanto la fisiología como la distribución de los organismos intermareales están muy influenciados por distintos tipos de estrés físico. Temperatura, radiación ultravioleta y desecación en condiciones de bajamar se suelen considerar los estreses físicos más importantes. Dichos factores ejercen su impacto sobre el metabolismo celular, y la capacidad del organismo de adaptarse rápidamente a condiciones ambientales alteradas establece sus límites de distribución mareal. Se ha demostrado de forma general el papel de la familia de proteínas HSP70 (proteínas de estrés) en las respuestas al estrés térmico. Aunque se ha demostrado que la expresión de la proteína de estrés es una herramienta útil para cuantificar parte de este estrés físico, se han realizado pocos estudios con diferentes organismos intermareales *in situ*. Para comprobar diferencias en la expresión de la HSP70 bajo condiciones naturales en el ambiente intermareal, elegimos un cnidario común del océano Pacífico oriental: la anémona *Anthopleura elegantissima*. La expresión de la HSP70 en el pólipo depende del grado de emersión y de la importancia del estrés físico (entre pólipos completamente sumergidos y completamente emergidos del mismo clon se registraron 0,1-3,6 ± 1.5 ng HSP70 μg proteína^{-1}). El tiempo de inmersión se refleja en la recuperación de los niveles de HSP70 del pólipo desde la exposición en pleamar: los clones más someros expresan más HSP70 (2,5 ng HSP70 μg P^{-1}) que los más profundos (0,1 ng HSP70 μg P^{-1}). Asimismo, las situaciones ambientales soleadas y neblinosas influyen sobre la respuesta de estrés. Los clones de la anémona expuestos a la zona intermareal elevada y soleada expresan la HSP70 más de tres veces (2,2 ng HSP70 μg P^{-1}) que en el

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caso de un día neblinoso en la misma zona intermareal elevada (0.6 ng HSP70 μg P^{-1}). *A. elegantissima* reduce los efectos del estrés físico en emersión en bajamar mediante varios mecanismos adicionales a la expresión de la HSP70: encogimiento de los pólipos, secreción de mucus, recubrimiento con arena, moléculas que absorben la radiación UV y una distribución a manchas muy densas.

Palabras clave: *Anthopleura*, proteínas de estrés, HSPs, estrés intermareal, temperatura.

INTRODUCTION

Intertidal organisms have different mechanisms to confront high tidal fluctuations. There are wide ranges in the extent (in time) of such stresses (temperature, UV radiation or dessication), and the organism's vertical placement depends on its adaptive responses to these physical constraints. Sessile organisms in particular are more susceptible to such exposure, and temperature has long been considered a dominant physical factor regulating intertidal distribution (e.g. Connell, 1961). Individual responses to the above mentioned emersion constraints, as well as differential abilities to set and dominate available free space are responsible for the development and maintenance of structural patterns in the intertidal community (Taylor and Littler, 1982).

The effects of these physical stress cycles may have deleterious consequences on cellular structures and proteins. One mechanism commonly used by cells to counter such deleterious effects is to increase the expression of stress proteins (HSPs) that are found in almost all organisms (Feder and Hofmann, 1999). These proteins are classified into seven or so different families based solely on molecular size ranging from 10-110 kDa. HSP functions are numerous and many remain unknown, specially functions related with ecology (Hofmann, 1999; Feder and Hofmann, 1999). The most well understood roles of HSPs are to chaperone proteins being folded into the correct three-dimensional conformation at the ribosome, to re-fold denatured proteins following severe stress, to aid in targeting damaged proteins for degradation, and to chaperone certain cellular receptors in their unoccupied state (Kiang and Tsokas, 1998).

It is known that HSPs have roles for organisms under natural conditions that encounter large physical stresses such as temperature shifts. Few studies have been performed on organisms in the field to test *in situ* responses to such temperature stress. An example study would be one on an intertidal species facing natural stress fluctuations *in situ* such as large temperature changes resulting from emersion/immersion cycles with tidal regimes. This question has been examined in intertidal mussels, *Mytilus* sp., sampled during single tidal cycles in winter and summer (e.g. Hofmann and Somero, 1995), or over an annual cycle (Helmuth and Hofmann, 2001). Most of the experiments have been made in controlled laboratory situations, where acclimation and HSP regulation is easier to follow (Hofmann and Somero, 1995; Hofmann and Somero, 1996; Roberts *et al.*, 1997).

From these studies, four main points arose: 1) There is a seasonal-induced HSP expression in intertidal organisms; i.e. summer low tides provoke higher HSPs expression than winter low tides (Hofmann and Somero, 1995), and such expression may depend on microhabitat and thermal heterogeneity (Helmuth and Hofmann, 2001); 2) HSPs are maximally expressed at sublethal temperatures, and organisms have a tendency to lower the expression rapidly after re-immersion (Hofmann and Somero, 1996); 3) HSPs are rapidly synthesised and are associated with other mechanisms to avoid lethal protein denaturation (Hofmann and Somero, 1996; Roberts *et al.*, 1997); and 4) The energy costs associated with replacing heat-damaged proteins and with mantaining the concentrations and activities of heat shock proteins may contribute substantially to cellular energy demands (Hoffman and Somero, 1995).

Anthopleura elegantissima is widespread along the Eastern Pacific coast, and there have been many studies on its distribution (e.g. Sebens 1982, 1983), physiology (e.g. Shick, 1981; Dykens and Shick, 1984), ecology (e.g. Francis, 1973a,b; Sebens, 1982, 1983) and ethology (e.g. Francis, 1973a,b; Ayre and Grossberg, 1995). Dessication and thermal extremes clearly limit *A. elegantissima* distribution in the higher intertidal as individuals are smaller and have the lowest rates of asexual reproduction in this zone (Sebens, 1983). Many experiments on *A. elegantissima* physiology were performed to study the effects of high intertidal colonization in this cnidarian, and all of them show low metabolic rates under lengthy emersion conditions (Shick, 1981; Zamer, 1986; Zamer and Shick, 1987). Under such stressful conditions, the low metabolic rates have to be invested in mechanisms to counter adverse cellular

damage induced by extreme conditions (i.e. high temperatures, UV radiation, dessication, etc.). In the subtidal and lower intertidal, *A. elegantissima* metabolic rates are higher (Zamer and Shick, 1987) and its distribution is limited by fish, nudibranch, and sea star predators (Sebens, 1983), as well as by interactions with a number of sessile space competitors (Francis, 1973b; Sebens, 1983; Rossi and Snyder, 2001). The major advantage of this organism as a model for stress biology study is the large clonal aggregations of genetically identical individuals (polyps). This species often exists in clonal aggregations that extend ≥1 m above the high intertidal level (Sebens, 1982). *A. elegantissima* is also an excellent organism to study single genome responses across environmental stress gradients that occur naturally with tidal cycles. Such genetic variation differences in inter- and intraclonal sea anemones have been examined in physiological studies (e.g. Shick and Dowse, 1985).

The aim of this work was to quantify relationships between perceived physical stress and HSP70 protein levels in the cnidarian *Anthopleura elegantissima* under different intertidal conditions.

MATERIALS AND METHODS

Measurements of *A. elegantissima* body temperatures

In order to compare the range of stress protein responses (see below), we were interested in examining intertidal *A. elegantissima* internal body temperatures during emersion on sunny or foggy days. This information provides a framework of body temperature changes under differential physical stress that can be compared with HSP70 levels. A six-channel portable temperature/voltage datalogger equipped with Kapton Type-T 30-gauge thermocouple probes with an accuracy of 0.1°C and response time of 0.5 sec (Cole-Parmer, Veron Hills, IL USA) was used. All thermocouples were calibrated against a NIST-calibrated thermometer before use in the field. A single *A. elegantissima* clone from the high intertidal (1 m above the mean low water level) was identified and three outside and three interior polyps were measured over the tidal emersion cycle on sunny versus foggy summer days. Thermocouple probes were placed 0.5 cm inside the oral cavity of individual anemones and internal body temperatures recorded every 5 min until re-immersion.

Sampling of *A. elegantissima* in different intertidal conditions

Anemones were sampled in September-October 1998 and 1999, to avoid potential seasonal differences in HSP expression (e.g. Hofmann and Somero, 1995 for *Mytilus trossulus*). Field collection sites were located either next to the Bodega Marine Laboratory (BML), or at Bodega Harbour Jetty (3 km from BML).

To assay specific sites of HSP70 production in individual *A. elegantissima* tissues, four individuals were sampled following 3 h emersion on a sunny day. Pieces of tentacle crown, oral disk, column, and pedal disk were dissected, immediately frozen in liquid nitrogen, and stored at -70°C.

As a method to assess whether outer clone individuals (warriors) or inside polyps of extended *A. elegantissima* clones expressed higher HSP70 under emersion stress, we sampled three *A. elegantissima* clones at BML (N = 4 outside or inside polyps/clone), with different emersion periods. It is important to state that all outside polyps chosen were always non-interacting (intra or interspecific competition). We recently demonstrated that competition for space may stimulate HSP70 protein level increases in this anemone (Rossi and Snyder, 2001). The first patch was completely emersed (2-3h), the second was in the surf zone (<30 min immersed), and the third one was always immersed.

To assess the importance of immersion time, three clones (BML, N = 2 outside and N = 2 inner polyps/clone) were sampled when fully emersed and 2 hours after full immersion.

In order to contrast clones immersed at different times on the same day, three boulders (Bodega harbour Jetty, different depths) with three different *A. elegantissima* clones were sampled as follows: 4 outside (base of the boulder) and 4 inner (top of the boulder) polyps/clone were sampled, put in plastic bags in ambient seawater (13°C) and frozen in liquid nitrogen after a further 30 min SCUBA collection period. We previously demonstrated that the 30 min extra tissue incubation in ambient seawater before freezing has no significant effect on tentacle HSP70 levels (Rossi and Snyder, 2001). All three boulders were immersed at the moment of the polyp collection, but the depth of the base and the top was different. The depths for the first boulder were 2 m (bottom, outside polyps) and 1.5 m (top, inner polyps); for the second, 1.5 and 1 m, and for the third, 1 and 0.5 m depth.

The solar irradiance conditions (indirectly as temperature changes) were also tested between sunny and foggy days. The same *A. elegantissima* patches were sampled (N = 2 outside and inside polyps/clone) during days of complete sunlight and under thick coastal fog (>2h emersed polyps). All samples were dissected, immediately frozen in liquid nitrogen, and stored at -70°C, except for those from the boulder experiment (see above).

HSP70 measurements

The following protocol was used in western immunoblotting for HSP70 expression. Frozen tentacle samples (stored at – 70°C) were individually homogenized in 0.2 ml of buffer K containing 5 mM $NaHP0_4$, 40 mM HEPES, pH 7.4, 5 mM $MgCl_2$, 70 mM potassium gluconate, 150 mM sorbitol, and 1% SDS. Homogenates were centrifuged 10 min at 10,000g, and the supernatants were combined with equal volumes of SDS sample buffer (Laemmli, 1970) and boiled for 5 min. Supernatant protein levels were determined by BioRad DC assay, and 20 µg of tentacle protein were loaded in each gel lane. Discontinuous SDS gels (1 mm) were 6.2% for the stacking gel and 12% for the resolving gel. After running for 2 h at 150 volts, SDS gels were electroblotted onto PDVF membranes (for 1 h at 100 volts). The protein band quality of each western blot was checked by visualization of proteins by Ponceau S staining. HSP70 protein was detected by western blotting using mouse monoclonal anti-HSP70 (SPA-822, StressGen, Victoria, B.C.). The use of SPA-822 HSP70 antiserum can possibly underestimate the number of HSP70 isoforms, and consequently may explain the finding of single HSP70 proteins by our methods. However, we have successfully used the same antiserum and measured 2 and 3-4 different HSP70 isoforms in larval lobsters, *Homarus americanus*, juvenile abalone, *Haliotis rufescens*, and adult mussels, *M. galloprovincialis*, respectively (Snyder and Mulder, 2001; Snyder *et al.*, 2001). The secondary antibody was goat-anti-mouse IgG, conjugated to peroxidase (Sigma). Visualization was performed using ECL reagents (Amersham) and exposure of blots to X-ray film.

Blot band intensities were compared by scanning the X-ray films and analysed with the NIH Image software package. For each blot, 50 ng of standard HSP70 protein (human, StressGen) was included. Scanned intensities of all HSP bands were compared against the intensities of the HSP70 protein standard from each blot. Each scanned NIH Image datum point is divided by the intensity of the HSP70 standard from that particular western blot. One-way ANOVA with post-hoc Scheffé Test was used to show significant differences between field experiments.

RESULTS

Figure 1 shows internal *A. elegantissima* body temperatures monitored in the same intertidal clone on hot sunny and foggy days. Under foggy conditions, anemone temperatures reached 19-21°C from 12°C ambient seawater within 4 h of emersion just prior to re-immersion by the incoming tide. On a sunny day, anemones attained temperatures near 30°C within a similar 4 h emersion period.

Most of HSP70 protein is expressed in tentacle tissue (Fig. 2). The mean percentage of the total HSP70 found in the tentacle is 90.9% on a per tissue protein basis. None of the other tissues contained more than 6.5% (oral disk, column, pedal disk) of the total HSP70 per tissue protein.

Depending on the degree of emersion, *A. elegantissima* shows different levels of HSP70 protein expression (Fig. 3). The more exposed clones (fully emersed) expressed higher HSP70 concentrations (3.7 ± 1.5 ng HSP70 µg P^{-1}) than those fully immersed (0.1 ± 0.2 ng HSP70 µg P^{-1}; $F_{2,9}$=15.41, p<0.001). The recently re-immersed (<30 min) patches also show significant differences with the non-exposed ones (2.0 ± 0.2 ng HSP70 µg P^{-1}), but not with the fully emersed ones.

Similar HSP70 concentrations were found in outside (2.2 ± 0.4 ng HSP70 µg P^{-1}) and inner (2.8 ± 0.5

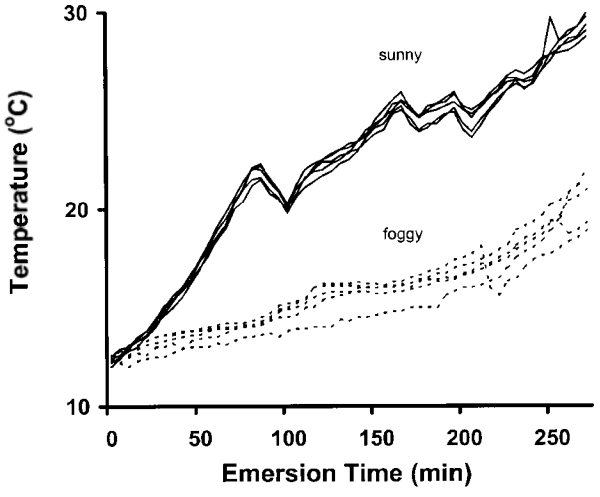

FIG. 1. – Temperature profiles from *A. elegantissima* oral cavities (n = 6 from each individual for each time point) 0.5 cm below the oral disk during tidal emersion on a sunny or foggy day in summer.

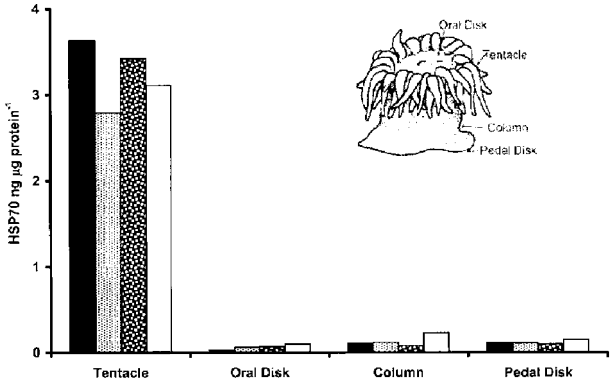

FIG. 2. – HSP70 protein levels in *A. elegantissima* tentacle crown, oral disk, column, and pedal disk. Each color pattern represents one of four individuals sampled on a summer afternoon.

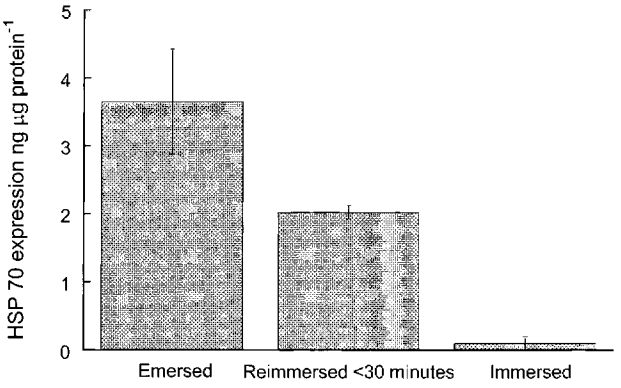

FIG. 3. – HSP70 protein levels in *A. elegantissima* warrior tentacles sampled from different clones either fully emersed and exposed to direct sunlight for several hours, partially emersed and then re-immersed for < 30 min prior to sampling, or immersed for the entire day. Mean ± SD.

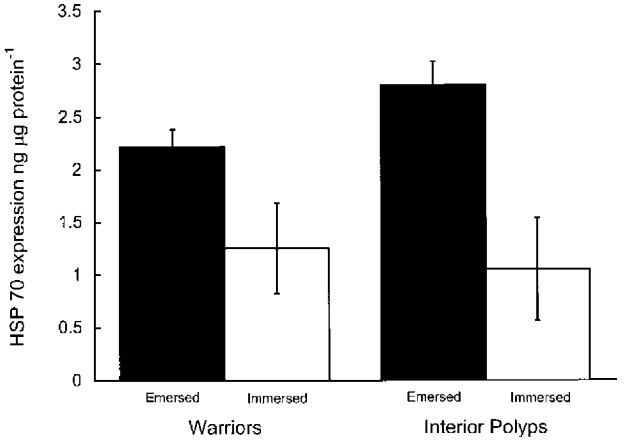

FIG. 4. – HSP70 protein levels in *A. elegantissima* warrior versus interior clonemate tentacles emersed in direct sunlight (> 2 h, black bars), or the same clones resampled 2 h following re-immersion (white bars). Mean ± SD.

ng HSP70 µg P^{-1}) polyps of *A. elegantissima* clones when they are emersed (Fig. 4), or when the same patches were analysed after 2 h of re-immersion (outside 1.3 ± 1.1 ng HSP70 µg P^{-1}, inner 1.1 ± 1.2

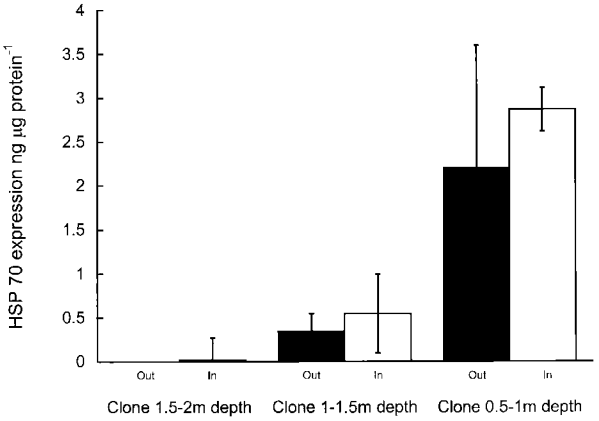

FIG. 5. – HSP70 protein levels in *A. elegantissima* warrior (black bars) versus interior (white bars) clonemate tentacles from boulders existing either at 0.5-1 m, 1-1.5 m, or 1.5-2 m below the mean low water height. Mean ± SD.

ng HSP70 µg P^{-1}, Fig. 4). However, significant differences were found when emersion-immersion was tested in this experiment ($F_{2,22}$=14.85, p<0.001).

No significant differences were found between outside and inside polyps within each clone on the boulders (Fig. 5; $F_{2,18}$=0.15, p=0.865), but significant differences were found between the shallower boulder (at 0.5-1 m depth, 2.5 ± 1.9 ng HSP70 µg P^{-1}) and the deeper ones (at 1-1.5m depth, 0.5 ± 0.7 ng HSP70 µg P^{-1}, and 1.5-2m depth, 0.0 ± 0.0 ng HSP70 µg P^{-1}; $F_{2,21}$=10.60, p<0.001).

Different solar irradiance conditions also resulted in significant HSP70 concentrations differences. Outside polyps had more HSP70 concentration during emersion in a sunny day (after the same time of emersion, 2.2 ±0.3 ng HSP70 µg P^{-1}) than on a foggy day (0.6 ±0.9 ng HSP70 µg P^{-1}; $F_{1,10}$=16.89, p<0.002).

DISCUSSION

In our field experiments, even in foggy emersion conditions, *Anthopleura elegantissima* body temperatures reached 8°C above ambient seawater. *A. elegantissima* body temperatures on sunny days reach values 17°C above that of ambient seawater, similar to that reported for emersed mussels (*Mytilus* sp.) and limpets (*Patella vulgata*) in temperate intertidal zones (Davies, 1970; Elvin and Gonor, 1979; Hofmann and Somero, 1995, 1996; Roberts *et al.*, 1997; Helmuth, 1998). Thermal profiles of gelatin-filled shells of high intertidal snails, *Tegula funebralis*, also indicate that this shelled mollusc can undergo equivalent emersion temperature ranges (Tomanek

and Somero, 1999). It can no longer simply measure the air temperature and directly relate those readings to the organism's response. Without such direct measurements of organisms body temperatures, it is difficult to adscribe physiological alterations due to environmental changes. Furthermore, Helmuth (1998, 1999) stressed the importance of considering not only weather cycles but also the microscale heterogeneity of intertidal environments to provide physical force scales that set limits to the distribution of particular organisms in such habitats.

Evidence for specific expression of HSP70 in *A. elegantissima* tentacle tissue is shown in the sunny versus foggy day samples. It is clear that maximum level of expression is reached when hot sunny day conditions are encountered, and in such situation the HSP70 expressed is equal throughout the clone polyps. On the foggy day, expression is more random (see the Standard Deviation compared to the sunny day), suggesting a heterogeneous response to different microenvironmental situations. In the same high tide, there are differences in the HSP70 expression between recently emersed clones, depending on depth. But it is somewhat surprising that the entire anemone clone (with 0.5m differences between bottom and top of the boulder) has very similar HSP70 levels when compared with the other clones (Fig. 5). It would be interesting to examine a wide range of *A. elegantissima* clones to determine if there are genetic differences in the stress protein response that may explain the preliminary observations of variations in clonal susceptibility to high temperatures (Swanson and Edmunds, 1993).

Stress protein responses have been examined in other cnidarians (Bosch and Praetzel, 1991; Miller *et al.*, 1992; Sharp *et al.*, 1994, 1997; Black *et al.*, 1995; Fang *et al.*, 1997; Brennecke *et al.*, 1998; Wiens *et al.*, 2000), but, to our knowledge, direct measurements of HSP protein responses have not been performed on intact cnidarians *in situ* during natural stress cycles such as tidal fluctuations. All studies have involved laboratory acclimated organisms or organisms brought to the laboratory and sampled under artificial conditions. There are few studies of variations in HSP expression over the course of a stress cycle in any organism, especially those in intertidal environments. In intertidal *A. elegantissima*, HSP70 increases during tidal emersion, and recovery to lower levels appears to occur within several hours of re-immersion (Fig. 3). This situation is a similar to that found in *Mytilus trossulus* where HSP68 increases many fold within 5 h of emersion during which body temperatures were increased by 20°C (Hofmann and Somero, 1995). However, recovery from thermal stress during such emersion in mussels is associated with increasing HSP70 (to 4-fold) and HSP90 (to 2-fold) synthesis following tidal re-immersion (Hofmann and Somero, 1996). We were not able to detect HSP90 in *A. elegantissima* with several different commercial antisera (data not shown), and cannot judge the potential role of this stress protein in the thermal stress response in this organism.

Intertidal *A. elegantissima* clones have a number of behavioral and physiological mechanisms to respond to fluctuating tidal (exposure) conditions in addition to stress protein production, such as excess of mucus (Shumway, 1978), specialised structures (verrucae adapted to attach pebbles, Hart and Crowe, 1977) and contraction of the column (Shick and Dykens, 1984; Dykens and Shick, 1984). A number of biochemical changes have also been measured in anemones following either field collection or experimental stresses in the laboratory. For example, changes in total adenylate levels (ATP, ADP, AMP) have been measured in whole *A. elegantissima* following temperature, dessication, mechanical disturbance, and starvation in the laboratory (Smith and Watt, 1994) or alterations in lysosomal activity have been noted in the anemone *Anemonia viridis* following heat shock to laboratory-acclimated animals (Suharsono *et al.*, 1993). The potential tissue damage resulting from UV exposure in *A. elegantissima* is ameliorated by the production of UV-absorbing micosporine-like amino acids (Stochaj *et al.*, 1994; Banaszak and Trench, 1995), and further protection from UV damage in this species occurs via elevated catalase and superoxide dismutase activites in animal tissue (Dykens and Shick, 1984). It will be of great interest to test HSP expression under different field UV exposures, as UV can potentially damage anemones (*Metridium senile*: Westholt *et al.*, 2001). Although it has been surmised that outside *A. elegantissima* polyps encounter more stress due to more exposed surfaces during emersion, this is not reflected in our results (Fig. 4). The warrior polyps on the outside borders of clones have equivalent amounts of HSP70 in their tentacles versus those in the interior of the clones presumably under less dessication stress due to the influence of crowding by clone mates in the interior. Our data suggest that in the high tide the energy costs to counter the effects of thermal stress may be similar irrespective to the polyp position within the clone.

The investment of energy to avoid degradation in sublethal conditions (here the high tide situation) has to be well regulated. Zamer (1986) and Zamer and Shick (1987) reported that higher intertidal *A. elegantissima* individuals had both higher prey ingestion rates and assimilation efficiencies, leading to faster growth rates versus lower intertidal conspecifics. Presumably, the extra energy incorporation of the higher intertidal individuals can be used to counter the higher stress regimes encountered when emersed for up to 18 h per day. Metabolic rate (as O_2 consumption or calculated energy budget) is lower in higher intertidal *A. elegantissima* clones during emersion as a form of 'conservationist strategy' (Shick *et al.*, 1979; Shick, 1981; Zamer and Shick, 1987). One of the energetic costs has to be the increased HSP expression to avoid lethal cellular damage. Energetic consequences of high HSP expression, thermotolerance, growth, and survival have been explored in laboratory acclimated organisms. Fitness of bacteria (*E. coli*) is reduced with higher temperature acclimation while survival, associated with increased HSP production, is greatly enhanced (Leroi *et al.*, 1994). Wild *Drosophila* can be significantly (> 10% adults) developmentally defective when temperatures reach extremes (Roberts and Feder, 1999) and the fly extra copy strain shows reduced abnormalities under these conditions. The equilibrium between high and low HSP levels could be a key point to understand why some organisms can live in particular levels within the intertidal. Higher intertidal clones would invest less energy in space competition, as few organisms can afford such environmental stress (Raffaelli and Hawkins, 1996). Tomanek and Somero (1999, 2000) have examined the HSP responses of congeneric snails (*Tegula* sp.) from both different intertidal heights and latitudinal distributions. Snails from the higher intertidal and more tropical latitude had more robust HSP synthesis at higher temperatures. The authors suggested that thermotolerance and both distribution within the intertidal and in warmer habitats may be in part mediated by genetically-fixed stress protein responsiveness.

Monitoring of stress effects in natural populations has been described as an important approach in conservation biology (Parsons, 1996). UV radiation has substantially increased in the Northern Hemisphere due to seasonal widening of the ozone layer and apparent cycling of solar radiation (Shindell *et al.*, 1999). We wish to know if components of the stress response in specific nearshore species can be predictive of their potential disappearance as tied to demonstrated alterations in intertidal community species composition with global warming phenomema (Sagarin *et al.*, 1999). Stress response pathways, including HSP expression, could be essential clues to future interpretations of intertidal organism distributions (e.g. with respect to increasing UV radiation). It may be possible to extend this suggestion to many different intertidal species, as HSP quantification is relatively simple and levels of expression of these proteins may be a comparable index among organisms.

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