



FEATURE ARTICLE

Interspecific and interhabitat variation in *hsp70* gene expression in native and invasive kelp populations

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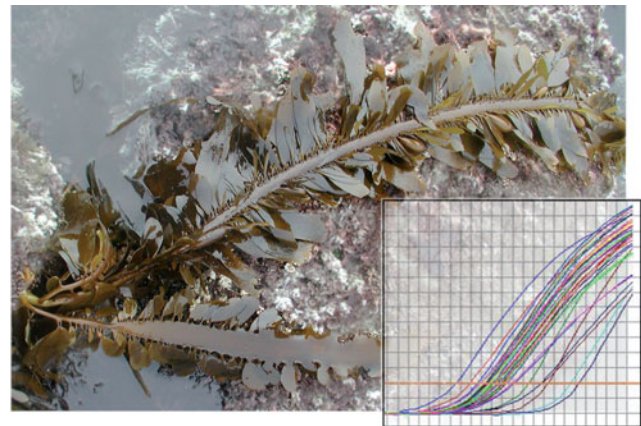
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ABSTRACT: This study characterized the response to thermal stress in 3 kelp species to contribute to the understanding of the role of the heat shock response in species distributions and in native–invasive species interactions. We sampled the invasive kelp *Undaria pinnatifida* in its native range in Japan and its introduced range in California, USA, to investigate small- and large-scale differences in its response to temperature stress. We then conducted similar experiments on native kelp species in different habitats in California to investigate differences in the response among species and habitats. We examined temperature response by measuring the induction of the gene (*hsp70*) that encodes for heat shock protein 70 (Hsp70), which protects cellular proteins from mis-folding and degradation by environmental stress. Individuals of *U. pinnatifida*, and the native California species *Egregia menziesii* and *Pterygophora californica* were heat-shocked at a range of temperatures, and mRNA was extracted and analyzed for expression of *hsp70*. Significant differences in the timing and magnitude of *hsp70* induction were observed between intertidal and harbor populations of *U. pinnatifida* within a few meters of each other in Japan, indicating environmentally driven variability in this response. Similarly, intertidal and subtidal populations of *E. menziesii* showed different responses, with subtidal *E. menziesii* populations responding more like subtidal *P. californica* populations. Native California species showed similar magnitudes of expression across all population, while *U. pinnatifida* collected from California harbors exhibited a more robust *hsp70* response than native California species but was similar in magnitude to Japanese populations sampled.

KEY WORDS: Kelp · *hsp70* · Invasive · Native · Thermotolerance · qPCR · *Undaria*

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Intertidal kelp populations exhibit faster heat shock gene up-regulation than submerged populations. Inset shows PCR amplification vs. cycle.

Photo: S. Henkel

INTRODUCTION

Only a few of the many species introduced to novel habitats become invasive (Williamson 1996), suggesting the existence of a suite of characters that enable invasiveness (Bossdorf et al. 2004). A large body of literature has developed in recent years attempting to identify these key characters (Williamson & Fitter 1996, Dukes & Mooney 1999, Kolar & Lodge 2001, Parker et al. 2003, Neubert & Parker 2004, Stachowicz & Byrnes 2006), and generally, broad tolerance, high reproductive rates, and plasticity (the ability to adjust traits to match different conditions) have been associated with invasive species. Many introduced species also become invasive after persisting for a long time at low abundances in the introduced habitat (Mack et al. 2000,

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Sakai et al. 2001), possibly after evolutionary adjustments to the novel environments (Mooney & Cleland 2001). In order to determine if certain species possess characteristics that enhance invasiveness or if invasive populations have evolved different characteristics than source populations, it is necessary to conduct investigations of an invader in both its introduced and native ranges. To date, the majority of studies on invasive species have been conducted within their introduced ranges, and ecologists often know little about the ecology of such species in their native ranges (Hierro et al. 2005). A few studies have shown that invasive species behave differently in their new range (Perez et al. 1988, Hay & Villouta 1993, Thornber et al. 2004), but whether that is due to a plastic response to a new environment, genetically controlled shifts in traits, founder effects, or various other factors remains uncertain.

Undaria pinnatifida, native to southeast Asia, was first identified in California, USA, in 2000 and has been found primarily in harbors from Monterey Bay, California, to Baja California, Mexico; however, 3 benthic (2 subtidal and 1 intertidal) populations are known to exist outside of harbors (Silva et al. 2002, Aguilar-Rosas et al. 2004). As *U. pinnatifida* is an aquaculture species in Asia, there is a large body of literature on its reproduction (Kimura & Notoya 1996, Liu et al. 2004, Choi et al. 2005), growth (Ishikawa 1993, Oh & Koh 1996), and nutrition (Jurkovic et al. 1995, Goni et al. 2002). However, there is much less literature on the ecology of wild populations in its native range (but see Saito 1956, Saito 1975). Conversely, there has been a large amount of research on *U. pinnatifida* in invaded areas, as it is such a conspicuous introduced species (Boudouresque et al. 1985, Fletcher & Manfredi 1995, Floc'h et al. 1995, Casas & Piriz 1996, Campbell et al. 1999, Forrest et al. 2000, Casas et al. 2004, Schaffelke et al. 2005, Sliwa et al. 2006, Dean & Hurd 2007). Lacking to date are direct comparisons of native and introduced populations. Furthermore, although many studies have investigated growth and reproductive patterns of invasive *U. pinnatifida*, molecular mechanisms for dealing with environmental stressors have been little investigated in this species. Thus, in this study, we aimed to investigate a molecular response to temperature challenges in native and introduced populations of *U. pinnatifida* as well as in native California kelp species.

In the present study we quantified the response to temperature by measuring transcriptional activation, or up-regulation, of the *hsp70* gene which encodes heat shock protein 70, a chaperone protein that protects cellular proteins from mis-folding and degradation by environmental stress (Lindquist 1986). In previous work, we found significant differences in the heat shock response between harbor and benthic, subtidal

populations of introduced *Undaria pinnatifida* in North America (Henkel & Hofmann 2008). For this investigation we characterized 2 main comparisons: the response to thermal stress (1) within species between different habitats, and (2) among species in their native and introduced ranges. Physiological comparisons in response to thermal stress in a variety of habitats and between native species and a competitive invader may help determine if thermal microhabitats are influential in facilitating invasions. This is especially important in benthic species such as kelps where community composition is often influenced by the order in which species colonize a habitat; thus, rising sea temperatures may give invasive species from warmer climates the competitive edge (Stachowicz et al. 2002). We conducted experiments on wild populations of *U. pinnatifida* in its native Japan as well as on introduced populations found in California harbors to investigate whether introduced populations exhibit more or less variation in their pattern of *hsp70* expression in response to temperature and to investigate small and large geographic scale differences in its response to temperature stress.

The comparison between native and introduced populations can help determine if thermotolerance characteristics observed in introduced populations are species traits of *Undaria pinnatifida* or if they are traits of a potentially invasive ecotype that make it especially successful at surviving transport and colonizing new areas. Further, studying introduced species in multiple habitats in the native range may reveal the potential level of variation in a particular response and provide a window to the future of how introduced populations will respond once they become as genetically diverse as native populations, assuming initial bottlenecks or founder effects. Comparisons to existing native species in the introduced range can help scientists make predictions about the invader's ability to compete with and possibly out-persist native species in the introduced range. If *U. pinnatifida* exhibits a greater or more plastic thermotolerance than California kelp species, then it may have the potential for persisting under scenarios of increasing ocean warming or temperature fluctuations that would negatively impact native species. Thus, we sampled the native kelp *Egregia menziesii* in 2 habitats in California and another native California kelp, *Pterygophora californica*, in a single habitat and made comparisons among all species to further examine the potential role of phylogeny.

Egregia menziesii was chosen as the first native comparison species due to its broad distribution (Alaska, USA, to Baja California, Mexico, intertidal to 15 m; Abbott & Hollenberg 1976), which includes a high degree of variability in temperature, nutrient, and wave exposure, and thus potential for a broad range of

physiological responses. Thus, while a single *E. menziesii* population may not experience the range of temperatures likely experienced by native *Undaria pinnatifida* populations in the Seto Inland Sea of Japan (Yanagi 1984), the species does experience a broad range of temperatures throughout its distribution. *Pterygophora californica* was chosen for the second native comparison since it is in the same kelp group as *U. pinnatifida* (Lane et al. 2006). Comparisons between closely related native and invasive species as well as among those that are most distantly related may be interesting as the amount, array, and kinetics of Hsps produced in response to heat stress can vary widely within phylogenetic lineages (Bosch et al. 1988, Saunders et al. 1991, White et al. 1994, Gehring & Wehner 1995). The use of both *E. menziesii* and *P. californica* as comparisons to *U. pinnatifida* enabled investigations between closely and distantly related kelp species from a variety of habitats.

We hypothesized that the responses of *Undaria pinnatifida* populations in California would be more similar to each other than the responses among *U. pinnatifida* sampled in Japan due to reduced genetic diversity among populations in the introduced range (Voisin et al. 2005, Uwai et al. 2006). Within California native species we hypothesized that we would observe significant differences in intertidal and subtidal population of *Egregia menziesii*, based on differences observed between populations of kelp collected from different habitats in our previous studies (Henkel & Hofmann 2008). For the native versus invasive species comparison, we hypothesized that *U. pinnatifida* would respond more rapidly to temperature stress, as temperatures in the sampled region in Japan fluctuate more widely and have higher maxima than those observed in California coastal waters (Yanagi 1984). We also hypothesized that introduced *U. pinnatifida* would have a higher magnitude of *hsp70* gene expression than California native species, and possibly native Japanese populations of *U. pinnatifida*, indicating a greater capacity to respond to stress events and possibly contributing to invasion success. The results presented here show a trend of heat shock gene regulation being tuned to local conditions and the invasive species, *U. pinnatifida*, indeed, showing higher expression than the native California species.

MATERIALS AND METHODS

Sample collection and treatment. Japan: *Undaria pinnatifida* ($n = 5$ at each site) were collected from 4 sites (2 intertidal and 2 harbor) on Awaji Island in the Seto Inland Sea of Japan. Individuals were collected from one paired submerged (AB-H) and intertidal

(AB-I) site near the Asagiri bus station in Sumoto City, Hyogo Prefecture. An additional intertidal population was sampled at Etoile Oishi Park (EO-I), also in Hyogo Prefecture, and an additional submerged population was sampled at the *Porphyra* culturing facility on Awaji Island (PC-H). 'Submerged' sites were floating concrete dock structures where *U. pinnatifida* was collected near the zero tide line, such that they were never exposed by the tide, hereafter referred to as 'harbor' sites for comparison with *U. pinnatifida* collected from California harbors. Individuals were transported in coolers to the laboratory of Dr. Hiroshi Kawai of Kobe University. For the paired intertidal and harbor sites near the Asagiri bus station (AB-H and AB-I), as well as the second intertidal site at Etoile Oishi Park (EO-I), experiments were begun within 4 h of collection. Transportation issues returning from the *Porphyra* culturing facility on Awaji Island (PC-H) resulted in greater than 5 h of transit time during which samples were inadvertently exposed to heat stress. Thus, the PC-H samples were not suitable for analysis, unfortunately unbalancing our sampling design. Upon return to the laboratory, 150 to 200 mg tissue from each specimen was placed in a 20 ml scintillation vial filled with filtered seawater and placed in aluminum blocks submerged in water baths held at $\sim 12, 17, 22, 26, 30,$ and 33°C for 1 h. After thermal incubation, samples were rinsed in $1\times$ phosphate-buffered saline (PBS), blotted dry, flash frozen in liquid N_2 , stored at -80°C , and then transported on dry ice to the laboratory at UC Santa Barbara.

California: Collections in California were made from 3 regions: Santa Barbara county, Los Angeles county and San Diego county. These sites are all located within the Southern California Bight; thus, they experience similar oceanographic conditions among them. Collections were made from the different regions in order to encompass any population structure that could affect physiological traits. Within each region, 4 individuals of each species were collected from the different sites. *Undaria pinnatifida* were collected by hand from 3 harbors where it has been introduced: Santa Barbara Harbor, Cabrillo Harbor in Los Angeles, and Coronado Island in San Diego Bay. Subtidal *Egregia menziesii* and *Pterygophora californica* were collected on SCUBA and intertidal *E. menziesii* were collected by hand from sites near the harbors. Specific collection locations are listed in Table 1 along with coordinates, date of collection, and temperature data obtained from NOAA buoys ($\pm 1.0^{\circ}\text{C}$), LTER moorings (Tidbit[®]; $\pm 0.4^{\circ}\text{C}$), or i-button[®] ($\pm 0.5^{\circ}\text{C}$) data loggers placed in the intertidal. Individuals were transported in coolers to the laboratory, and experiments were begun within 4 h of collection. Immediately upon return to the laboratory, 150 to 200 mg tissue from each

Table 1. Kelp collection sites and date of collection in California, USA. Temperature ranges are the average daily minimum and maximum and the absolute minimum and maximum water temperatures recorded near the indicated sites during the 2 wk prior to kelp collection. Kelp species: *Undaria pinnatifida*, *Egregia menziesii*, and *Pterygophora californica*. SB: Santa Barbara, LA: Los Angeles, SD: San Diego

Site	Coordinates	Collection date	Temperature range (°C)	
			Average	Absolute
Harbor <i>U. pinnatifida</i>				
Santa Barbara Harbor (SB)	34° 24' 26" N, 119° 41' 33" W	14 June 2006	15.4 – 17.7	14.2 – 20.4
Cabrillo Way Marina (LA)	33° 43' 15" N, 118° 16' 34" W	15 June 2006	19.0 – 20.3	17.9 – 21.3
Coronado Island (SD)	32° 41' 58" N, 117° 10' 07" W	12 June 2006	19.9 – 21.2	19.1 – 21.8
Intertidal <i>E. menziesii</i>				
Ledbetter Beach (SB)	34° 23' 47" N, 119° 42' 19" W	14 June 2006	14.2 – 17.1	12.8 – 18.3
Cabrillo Beach (LA)	33° 42' 30" N, 118° 17' 05" W	15 June 2006	19.0 – 20.3	17.9 – 21.3
La Jolla (SD)	32° 15' 17" N, 117° 15' 13" W	12 June 2006	17.6 – 20.7	15.8 – 21.6
Subtidal <i>E. menziesii</i> and <i>P. californica</i>				
Refugio (SB)	34° 27' 41" N, 120° 04' 21" W	30 June 2007	15.1 – 17.6	13.6 – 19.1
White's Point (LA)	33° 42' 52" N, 118° 19' 06" W	29 June 2007	18.0 – 19.5	17.0 – 20.5
Point Loma (SD)	32° 41' 01" N, 117° 15' 29" W	10 July 2007	20.0 – 21.5	19.3 – 22.8

specimen was placed in 20 ml scintillation vials filled with filtered seawater and placed in an aluminum gradient heat block at 12, 17, 21.5, 26.5, 31, 33, and 36 ± 1°C for 1 h. After thermal incubation, samples were removed from heat, rinsed in 1× PBS, blotted dry, flash frozen in liquid N₂, and stored at –80°C.

Gene expression analysis. For analysis of *hsp70* gene induction, total RNA was extracted, cDNA was transcribed, and quantitative PCR was run as described in Henkel & Hofmann (2008). Primers, volumes, and melt temperatures are listed in Table 2. Reported relative mRNA levels were calculated in the following fashion: (1) *hsp70* and *ribulose* cycle threshold (C_t) values were first normalized to that of the corresponding product of the cDNA standard from each plate; (2) resultant *hsp70* values were then normalized to the resultant *ribulose* values for the each sample. The *ribulose* gene was deemed to be an appropriate internal control because the *ribulose* mRNA levels did not differ significantly across heat incubations.

Statistical analysis. To compare *hsp70* expression within and among species, normalized expression values at each of the incubation temperatures for each

individual were divided by that individual's normalized expression value for T₀ = time zero (non-heat-shocked control). This was necessary to make comparisons among species because of the use of species-specific primers and standards, which could affect the recorded magnitude of expression, and to account for differing levels of transport stress resulting in differing initial conditions of the kelp. Two-way repeated measure ANOVAs (SigmaStat version 3.5) were conducted for each kelp population to assess for differences in *hsp70* expression among sites and across temperatures. Where significant differences were found among sites or temperature incubations, Student-Newman-Keul's (SNK) post-hoc analyses were performed to clarify patterns of the heat shock response. In order to compare the heat shock response among native California kelp as well as native and invasive *Undaria pinnatifida* populations, 2-way repeated measure ANOVAs (JMP version 7.0) were conducted on *hsp70* gene expression data normalized to T₀ to make comparisons among populations. A population was defined as a species–habitat combination, and only those species–habitat combinations that were replicated at 3 sites were used

Table 2. Primers for quantitative PCR (qPCR), including effective volumes in 20 µl reactions and melt temperatures

	Forward	Reverse	Volume (µl)	Melt temp. (°C)
<i>Undaria pinnatifida</i>				
<i>hsp70</i>	CAGGTGTACGAGGGTGAG	GTTAAGGATACCGTTGCG	1.0	50
<i>ribulose</i>	CACAAGGATGTTTTTCGTTTT	GAAGACCCATAATCCCAAT	0.7	58
<i>Egregia menziesii</i>				
<i>hsp70</i>	AAGTTAACCTAGACGGTATCCC	TGGTGATCTTGTTCTCCTTGC	1.0	55
<i>ribulose</i>	ATGTCAAAGGATGGGCTGT	CAGGTTCTGTTGAAGGTCGT	1.0	58
<i>Pterygophora californica</i>				
<i>hsp70</i>	AAGTTAACCTAGACGGTATCCC	TGGTGATCTTGTTCTCCTTGC	1.0	50
<i>ribulose</i>	CTATGGTTATGGCTCGTAATGAAG	GGTGTATCTGTTGAAGTGAATCG	1.0	55

in the analysis (i.e. *Egregia menziesii*–subtidal, *E. menziesii*–intertidal, *Pterygophora californica*–subtidal, *U. pinnatifida*–harbor). SNK post-hoc tests were performed to determine which populations responded to the temperature treatments differently.

T_{max} was identified for each population and defined as the temperature at which *hsp70* was expressed in greatest relative proportion. T_{off} then was defined as the temperature at which *hsp70* expression dropped significantly (according to SNK post hoc analysis) from that at T_{max} . To make comparisons of the rate of up-regulation of *hsp70* among the species and populations, the natural log-transformed fold expression data from 12°C to the T_{max} were plotted against the natural log of the incubation temperatures. Linear regressions were obtained and the slope of the regression was used as a measure of the rate of up-regulation. Where we had replicates of species and habitats (*Egregia menziesii* and *Pterygophora californica*), we conducted 2-way ANOVA with the fixed factors ‘species’ and ‘habitat’ on the regression data.

RESULTS

Overall, heat shock protein gene expression analysis revealed 2 trends in the data: (1) *Undaria pinnatifida* seems to have a higher capacity for tolerating thermal stress than the native California species sampled in this study; and (2) variation in *hsp70* expression seemed to depend more on habitat than phylogeny or geographic region. The sections below describe the analysis for each comparison; the gene expression profile is summarized in Table 3.

Native and invasive *Undaria pinnatifida* responses

For native *Undaria pinnatifida* in Japan, significant differences were observed between individuals from the floating dock at Asagiri (harbor) and the 2 intertidal sites (Fig. 1). Two-way repeated measures ANOVA results revealed that both temperature ($F_5 = 4.629$, $MS = 9.616$, $p = 0.001$) and site ($F_2 = 4.578$, $MS = 18.080$, $p = 0.033$), but not the interaction ($F_{10} = 0.830$, $MS = 1.724$, $p = 0.602$), were significant effects for *hsp70* expression (residual = 59). SNK post-hoc analysis revealed that the intertidal sites, AB-I and EO-I were statistically indistinguishable while the harbor site (AB-H) was significantly different from both. For the harbor site (AB-H) the T_{max} was 22°C, while the 2 intertidal sites had a higher T_{max} of 26°C (Table 3). The rate of *hsp70* up-regulation (determined using linear regression) was similar between the 2 populations at Asagiri (AB-H and AB-I) and much higher in the inter-

Table 3. *Undaria pinnatifida*, *Egregia menziesii*, and *Pterygophora californica*. *hsp70* expression parameters for native and invasive kelp species in different habitats. Up-regulation rate: slope of the linear regression of *hsp70* expression versus incubation temperature; T_{max} : temperature with the highest magnitude of *hsp70* expression; T_{off} : temperature at which *hsp70* expression was significantly less than T_{max} ; ns: not significant. AB-H and AB-I: harbor and intertidal site near the Asagiri bus station in Sumoto City, Hyogo Prefecture; EO-I: intertidal site at Etoile Oishi Park. Other abbreviations as in Table 1

Collection	Up-regulation rate	T_{max}	T_{off}	Collection month
Harbor				
<i>U. pinnatifida</i> SD	2.89	26.5	31	June
<i>U. pinnatifida</i> LA	2.80	26.5	31	June
<i>U. pinnatifida</i> SB	3.45	26.5	31	June
<i>U. pinnatifida</i> Japan (AB-H)	3.02	22	30	March
Intertidal				
<i>U. pinnatifida</i> Japan I (AB-I)	2.99	26	30	March
<i>U. pinnatifida</i> Japan II (EO-I)	5.13	26	30	March
<i>E. menziesii</i> SD	1.59	26.5	31	June
<i>E. menziesii</i> LA	2.14	21.5	ns	June
<i>E. menziesii</i> SB	2.00	26.5	31	June
Subtidal				
<i>E. menziesii</i> SD	0.84	33	None	July
<i>E. menziesii</i> LA	1.44	31	None	June
<i>E. menziesii</i> SB	1.26	31	None	June
<i>P. californica</i> SD	1.48	26.5	None	July
<i>P. californica</i> LA	1.25	26.5	None	June
<i>P. californica</i> SB	1.93	31	None	June

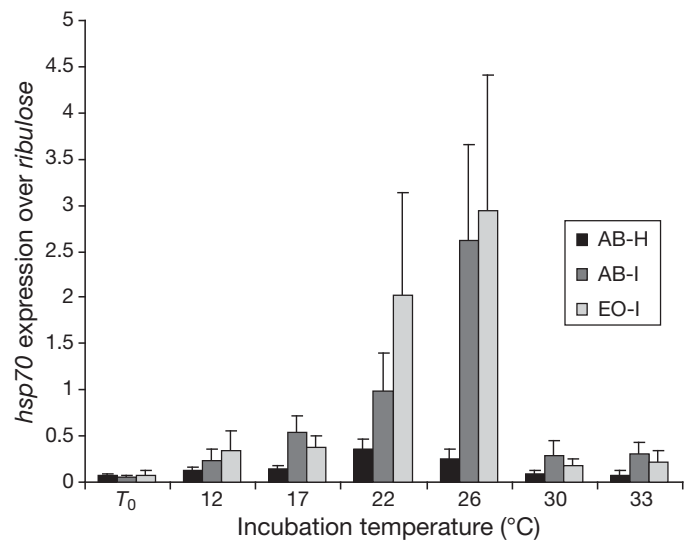


Fig. 1. *Undaria pinnatifida*. *hsp70* expression (+SE) in native populations in Japan; $n = 5$ individuals for each temperature at each site. AB-H and AB-I: harbor and intertidal site near the Asagiri bus station in Sumoto City, Hyogo Prefecture; EO-I: intertidal site at Etoile Oishi Park. T_0 : non-heat-shocked control

tidal population collected from Etoile Oishi Park (EO-I) (Table 3), although the 2 intertidal populations had a higher magnitude of expression overall than the harbor population (Fig. 1).

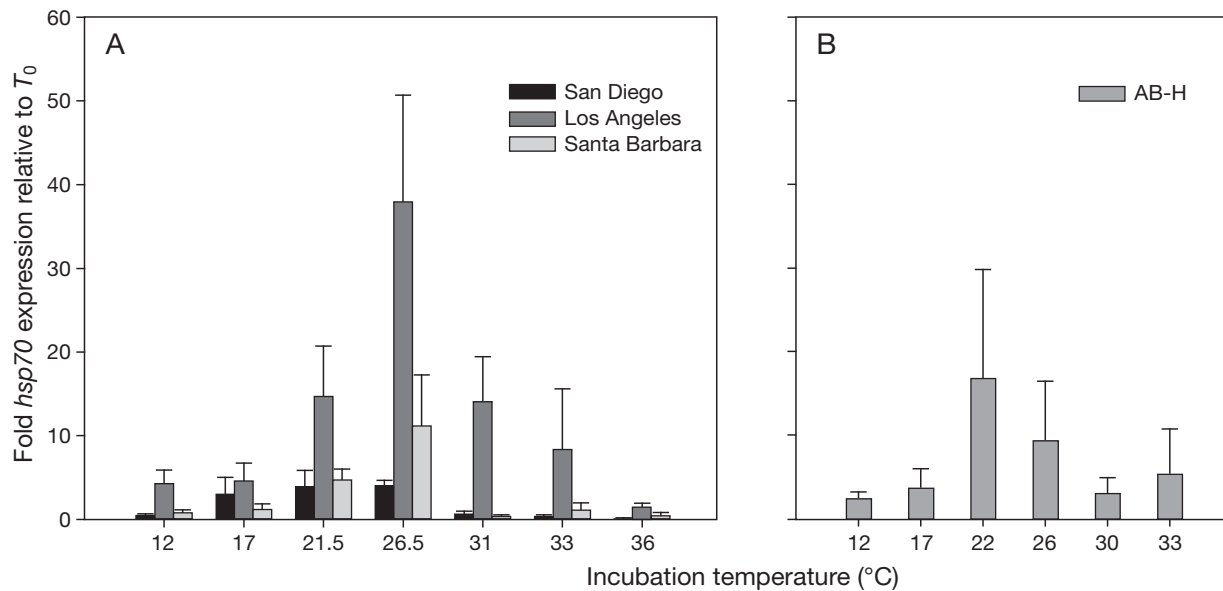


Fig. 2. *Undaria pinnatifida*. *hsp70* expression (+SE) relative to the non-heat-shocked control (T_0) in (A) populations invasive to California harbors and (B) the native Japanese harbor population. AB-H: harbor site near the Asagiri bus station in Sumoto City, Hyogo Prefecture

Undaria pinnatifida collected from California harbors exhibited an *hsp70* expression pattern that matched neither the harbor nor intertidal populations from Japan (Fig. 2A). The California harbor populations had a T_{max} of 26.5°C (like the Japanese intertidal populations); however, the shape of the induction profile was much more similar to that observed for the Japanese harbor population. The rate of *hsp70* up-regulation in the California harbors (2.80 to 3.45) was very similar to that observed in the harbor population in Japan (3.02) as well as the intertidal population from that site (2.99) (Table 3).

Two-way repeated measure ANOVA indicated that both incubation temperature and collection site were significant factors in California *Undaria pinnatifida* *hsp70* expression (\log_{10} -transformed to meet variance requirements) but there was no significant interaction effect between the 2 factors (Table 4). The significant site differences among the California harbor populations of *U. pinnatifida* were the result of the Los Angeles harbor samples having a much greater magnitude of expression than the San Diego or Santa Barbara Harbor samples. However, the site differences were likely not related to water temperatures recorded in the harbors since Los Angeles water temperatures (17.9 to 21.3°C) were intermediate to both Santa Barbara (14.2 to 20.4°C) and San Diego (19.1 to 21.8°C).

In order to make direct comparisons of the magnitude of response between California and Japan, quantitative PCR (qPCR) was repeated on the Japanese *Undaria pinnatifida* harbor population (AB-H) samples using the same standard as used for the California har-

bor *U. pinnatifida*. We then calculated the fold *hsp70* expression for each of the Japanese harbor individuals relative to T_0 expression. Japanese harbor *U. pinnatifida* responded with a very similar fold induction as California harbor *U. pinnatifida* (Fig. 2). When 2-way repeated measure ANOVA and SNK post-hoc analyses were conducted for the 4 (3 California and 1 Japan) harbor populations together, again only the Los Ange-

Table 4. *Undaria pinnatifida*, *Egrecia menziesii*, and *Pterygophora californica*. Two-way repeated measures ANOVA results for individual species populations sampled in California. *Significant at the 0.05 level

	df	F	p
Harbor <i>U. pinnatifida</i>			
Temperature	6,3	15.895	<0.001*
Site	2,8	9.603	0.006*
Site × Temperature	12,6	1.378	0.206
Intertidal <i>E. menziesii</i>			
Temperature	6,4	2.865	0.017*
Site	2,9	1.456	0.283
Site × Temperature	12,8	1.288	0.253
Subtidal <i>E. menziesii</i>			
Temperature	6,3	2.258	0.052
Site	2,8	3.247	0.087
Site × Temperature	12,6	1.229	0.289
Subtidal <i>P. californica</i>			
Temperature	6,3	2.388	0.042*
Site	2,8	1.429	0.289
Site × Temperature	12,6	1.242	0.282

les population was different from the other California populations and no significant differences were observed among SB, SD, and AB-H or between AB-H and LA (Table 5). The magnitude of *hsp70* expression when this gene was ‘turned on’ in both California and Japan *U. pinnatifida* was considerably higher than that observed for the native California species discussed below.

California species responses

Intertidal *Egregia menziesii* exhibited a classic bell-shaped curve of *hsp70* induction, peak, and decline (Fig. 3); however, the magnitude of expression was much lower overall than that observed for the various *Undaria pinnatifida* populations (Fig. 2A). Two-way repeated measures ANOVA indicated that temperature was a significant factor in the *hsp70* gene expression response among individuals, while differences observed among sites were not significant nor was there an interaction of site and temperature (Table 4). The observed T_{max} for the intertidal *E. menziesii* Los Angeles population was 21.5°C and was statistically indistinguishable (SNK post-hoc) from the response at 26.5°C, which was the observed T_{max} for the San Diego and Santa Barbara populations; the T_{off} for intertidal *E. menziesii* was observed to be 31°C, which was approximately that which was observed for *U. pinnatifida* (Table 3). The rate of up-regulation (determined using linear regression) for intertidal *E. menziesii* populations ranged from 1.59 to 2.14 (Table 3).

Table 5. *Undaria pinnatifida*. (A) Results of 2-way repeated measures ANOVA to compare harbor-collected populations of *U. pinnatifida* in California and Japan. (B) Pairwise multiple comparison procedure (Student-Newman-Keuls method) comparisons for factor Site. LA: Los Angeles; SD: San Diego; SB: Santa Barbara; AB-H: harbor site near the Asagiri bus station. *Significant at the 0.05 level

(A) Source of variation	df	MS	F	p
Site	3	5.227	4.761	0.019*
Individual(Site)	13	1.116		
Temperature	6	5.403	22.854	<0.001*
Site × Temperature	18	0.395	1.670	0.065
Residual	74	0.236		
Total	114	0.764		
(B) Comparison	Diff. of means	p	q	p
LA vs. SD	0.995	4	4.986	0.017*
LA vs. SB	0.802	3	3.965	0.037*
LA vs. AB-H	0.514	2	2.573	0.092
AB-H vs. SD	0.482	3	2.413	0.240
AB-H vs. SB	0.289	2	1.427	0.332
SB vs. SD	0.193	2	0.953	0.512

Subtidal *Egregia menziesii* populations had a similar magnitude of gene expression as intertidal populations; however, the expression profile was different than for intertidal *E. menziesii* (Fig. 4B) with the T_{max} found at higher temperatures in the subtidal populations than the intertidal (Table 3). There appeared to be a trend in the subtidal *E. menziesii* populations with

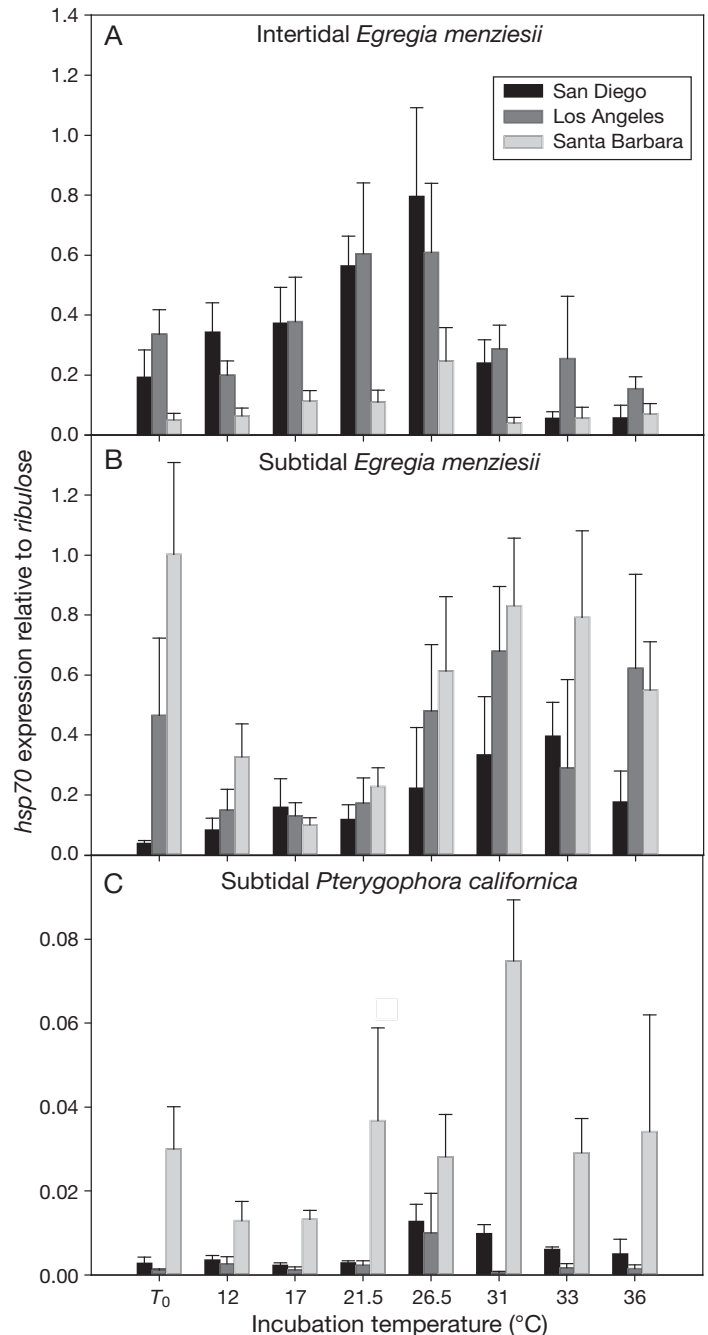


Fig. 3. *Egregia menziesii* and *Pterygophora californica*. *hsp70* expression (+SE) relative to the non-heat-shocked control (T_0) for (A) intertidal *E. menziesii*, (B) subtidal *E. menziesii*, and (C) subtidal *P. californica*

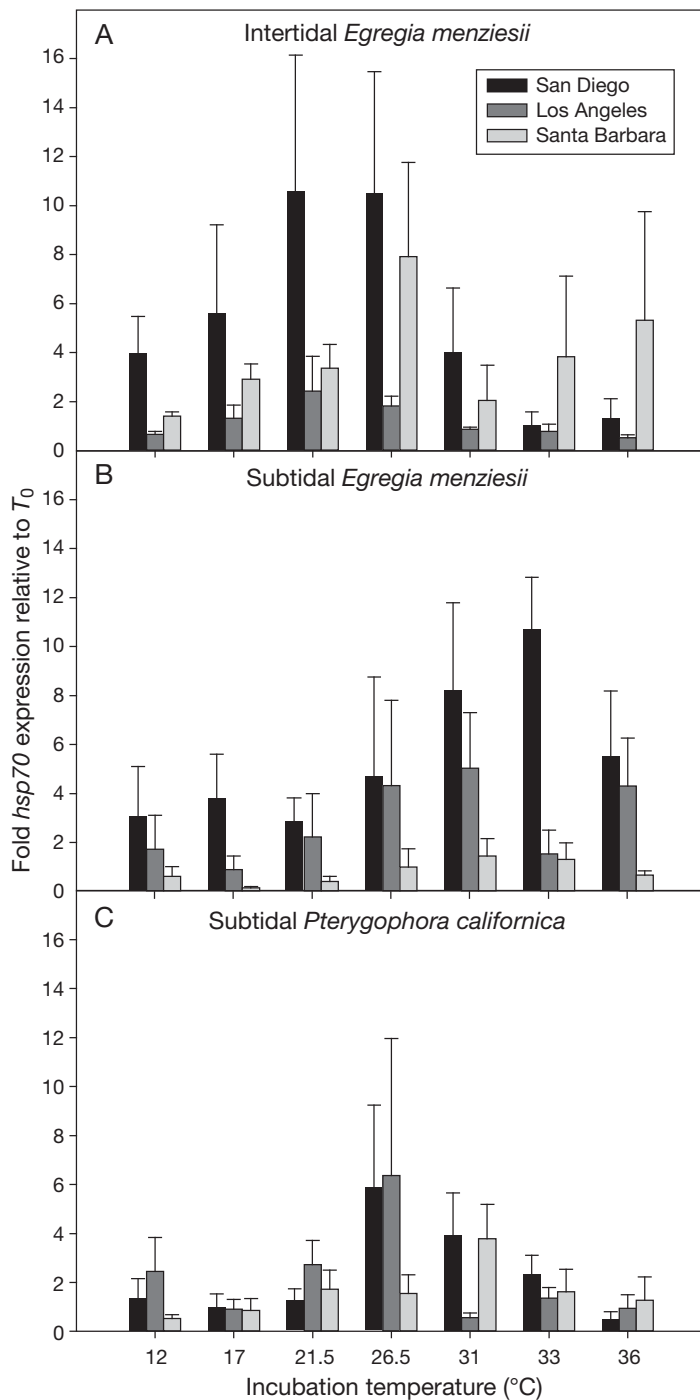


Fig. 4. *Egregia menziesii* and *Pterygophora californica*. *hsp70* expression (+SE) relative to *ribulose* for (A) intertidal *E. menziesii*, (B) subtidal *E. menziesii*, and (C) subtidal *P. californica*

the magnitude of *hsp70* induction increasing with decreasing latitude (Fig. 4B). Temperature was a marginally significant factor and significant differences were not observed among sites (Table 4). Interestingly, no T_{off} was observed in subtidal *E. menziesii*.

Table 6. *Egregia menziesii*. Three-way repeated measures ANOVA for *E. menziesii*, comparing populations from different habitats. *Significant at the 0.05 level

	df	F	p
Temperature	6, 12	3.2609	0.0386*
Temperature × Site	12, 24	1.6799	0.1350
Temperature × Habitat	6, 12	4.2201	0.0163*
Temperature × Site × Habitat	12, 24	2.2754	0.0418*
Site	2, 17	1.6408	0.2231
Habitat	1, 17	0.2318	0.6363
Site × Habitat	2, 17	1.0316	0.3777

In comparing the *hsp70* response among all *Egregia menziesii* populations, there appeared to be a strong influence of the habitat. Overall, the rate of up-regulation in subtidal populations of *E. menziesii* was lower (ranging from 0.84 to 1.44; Table 3) than the intertidal populations. Three-way repeated measure ANOVA with temperature, site, and habitat as factors resulted in a significant interaction effect between habitat and temperature as well as habitat, site, and temperature (Table 6), indicating that populations from different habitats responded differently to the temperature treatments.

Subtidal *Pterygophora californica* exhibited a similar magnitude (Fig. 4C) and rate of up-regulation (Table 3) of *hsp70* expression to subtidal populations of *Egregia menziesii*. In San Diego and Los Angeles, *P. californica* had lower observed T_{max} than subtidal *E. menziesii*, while the T_{max} were the same in Santa Barbara (Table 3). Also similar to the subtidal *E. menziesii*, no T_{off} was observed for subtidal *P. californica* (Table 3). Temperature was identified as a significant factor in *hsp70* gene expression for subtidal *P. californica*; however, differences among populations were not statistically significant (Table 4).

Overall, both native and invasive populations of *Undaria pinnatifida* were significantly different than native California populations. Two-way repeated measures ANOVA followed by SNK post-hoc analysis indicated that at 22°C ($p = 0.002$) and 26°C ($p = 0.030$) all populations of *U. pinnatifida* were significantly different from all populations of California native species; however, no significant differences among native California kelp populations were observed. Based on the comparisons of the rate of up-regulation of *hsp70* among species and populations, it appeared that habitat drove similarity in *hsp70* gene expression (Table 3): subtidal California kelp populations had the slowest up-regulation rate, while native California intertidal populations of the same species had faster rates. Harbor populations of *U. pinnatifida* had still faster up-regulation, while the intertidal *U. pinnatifida* from Etoile Oishi had the fastest up-regulation rate of all.

Where we had replicates of species and habitats (*Egregia menziesii* and *Pterygophora californica*) 2-way ANOVA on the regression data indicated that habitat ($p = 0.029$) but not species ($p = 0.196$) was a significant factor.

DISCUSSION

Invasive versus native responses

We hypothesized that California populations of *Undaria pinnatifida* would be less variable than native Japanese populations due to founder effects which might limit the genetic variability within and across populations in the new range. This hypothesis is supported by studies which indicate invasive populations of *U. pinnatifida* in California are a single haplotype (Voisin et al. 2005, Uwai et al. 2006). Within California, the 3 populations did respond to temperature with the same pattern of *hsp70* expression, the only difference being that Los Angeles *U. pinnatifida* populations had a greater magnitude of expression. While the Japanese populations demonstrated greater differences in the response among populations, this was due to the fact that more variable habitats were sampled. Within a habitat (intertidal) in Japan, the responses were indistinguishable. Thus, we were not able to conclude from this study whether invasive populations of *U. pinnatifida* are more (leading to their invasiveness) or less (due to founder effects) variable than native populations. Since the magnitude of expression in the Japanese harbor population was extremely similar to the magnitude of expression in the San Diego and Santa Barbara, California, harbor populations, it does not seem that the invasive populations of *U. pinnatifida* are any more 'robust' in their ability to up-regulate the heat shock pathway in response to thermal stress than are native *U. pinnatifida*. This lack of difference between native and introduced populations is similar to trends observed for native and invasive tropical shrub populations in terms of growth rates, photosynthetic rates, or biomass allocation (DeWalt et al. 2004).

The invasive species did exhibit a greater magnitude of response overall than the 2 native California species sampled. This trait, resulting in a greater capacity for dealing with thermal stress, may be what contributes to the widespread success of *Undaria pinnatifida* on nearly every continent to date, as the amount of heat shock protein expression is positively correlated with thermotolerance (Krebs & Feder 1998). The greater expression of *hsp70* in *U. pinnatifida* seems to be a trait of the species as a whole since the magnitude of expression was similarly high from both California and Japan populations collected from harbor sites and the

intertidal Japanese populations exhibited still greater expression. Furthermore, the rates of up-regulation for all populations of *U. pinnatifida* were higher than those observed for native California kelp species, even when collected from similar habitats.

Greater *hsp70* mRNA expression may be achieved via 2 main mechanisms. First, *Undaria pinnatifida* may contain more copies of the *hsp70* gene than the other species sampled in this study. Species of *Drosophila* with cosmopolitan distributions have greater *hsp70* copy number, express more Hsp70 protein, and are more thermotolerant than are other members (Krebs & Bettencourt 1999). Furthermore, laboratory strains of *D. melanogaster* that contained additional *hsp70* gene copies were found to have increased Hsp70 concentrations and stress tolerance (Welte et al. 1993, Feder et al. 1996, Roberts & Feder 2000). A second mechanism for greater expression of *hsp70* mRNA in *U. pinnatifida* may be variation in the heat shock factor rather than more copies of the gene. This mechanism is discussed in detail in the following section.

Habitat differences

Habitat differences observed in *Undaria pinnatifida* in Japan are likely due to population level variability in the heat shock response. The 2 intertidal populations located further apart exhibited statistically indistinguishable induction profiles and magnitude of expression, while the paired intertidal and harbor populations (located approximately 5 m apart) exhibited different expression profiles and magnitude. This suggests that local acclimatization to habitat-specific environmental conditions may have a more significant effect on the induction response than shared genetics, as the paired populations likely have a shared gene pool due to their close proximity. Such local acclimatization is to be expected since species inhabiting intertidal habitats generally encounter greater temperature variability than those that are continually submerged; thus, they must be able to tolerate or respond to temperature fluctuations. This may result in different physiological phenotypes between intertidal and subtidal species or populations, as the pattern of the heat shock response can be set by acclimation to different environmental conditions (Barua & Heckathorn 2004). The fast up-regulation rate observed in the intertidal *U. pinnatifida* population at Etoile Oishi Park (EO-I) may be a response to the more fluctuating conditions found in the intertidal, while the greater magnitude of expression observed in intertidal *U. pinnatifida* populations relative to the harbor population may indicate a greater capacity for dealing with overall temperature increases.

Within the California native species, *Egrecia menziesii*, *hsp70* expression exhibited a significant response to the interaction of habitat and temperature, indicating that populations from different habitats responded to temperature differently. This can be seen in the specific induction parameters where subtidal *E. menziesii* populations had lower up-regulation rates, higher T_{max} , and did not exhibit a T_{off} as compared to intertidal populations. (The lack of significance for habitat as a single factor is likely due to the fact that overall magnitude of *hsp70* expression was similar between the 2 habitats.) *E. menziesii* exhibits morphological variability among sites that seems to be a response to environmental conditions (Henkel et al. 2007) and exhibited population specific responses to heat stress that varied with latitude in previous *hsp70* investigations (Henkel & Hofmann 2008). Thus, the significant differences observed in this physiological measure between habitats in our study were not unexpected.

The other native species, *Pterygophora californica*, had not been previously investigated in terms of the heat shock response, so the findings in this work were novel. *P. californica* is in the same kelp group as *Undaria pinnatifida* (Lane et al. 2006); however, it shares the same range and subtidal habitat as *Egrecia menziesii*, which is regularly used as an out-group to all other kelp species (Lane et al. 2006). Thus, we were interested to learn whether the expression profile of *P. californica* was more similar to the closely related *U. pinnatifida* or if it better matched the pattern for populations of a distantly related species in the same habitat. The expression pattern for *P. californica* was not significantly different from that of *E. menziesii* collected from the same habitat; however, the *hsp70* expression parameters for *P. californica* were quite different from the closely related *U. pinnatifida*. This large difference was likely due both to the fact that they were collected from different habitats and a result of the apparent greater thermotolerance of the introduced species.

The habitat-specific heat shock response observed in this study in both *Undaria pinnatifida* and *Egrecia menziesii* is consistent with a number of studies in which differences were seen within the same species collected from different habitats (Helmuth & Hofmann 2001, Halpin et al. 2002, Manitssev et al. 2007), latitudes (Osovitz & Hofmann 2005, Fangué et al. 2006), or seasons (Roberts et al. 1997, Buckley et al. 2001, Hamdoun et al. 2003, Manitssev et al. 2007). This pattern was previously observed between harbor and subtidal populations of *U. pinnatifida* subjected to a period of constant temperatures in the laboratory followed by experiments similar to those in this work (Henkel & Hofmann 2008). Thus, in kelps, while aspects of the heat shock response might be tuned to the local envi-

ronment, it does not appear to be easily altered by short-term exposure to different conditions such as attempted laboratory acclimations.

Inducible heat shock protein expression in all organisms is regulated by heat shock transcription factors (HSFs); this is referred to as the cellular thermometer model of gene regulation since heat shock proteins play an autoregulatory role in governing their own synthesis by determining the levels of free HSFs that exist in the cell (Tomanek & Somero 2002). The cellular thermometer model predicts that elevated concentrations of HSFs would, other things such as Hsp70 protein levels being equal, favor lower T_{on} values for *hsp* gene transcription because of larger amounts of free HSFs for available to bind to the heat shock element and promote transcription (Tomanek & Somero 2002). Cellular pools of HSFs are known to vary among species (Tomanek & Somero 2002), and greater amounts of HSFs in *Undaria pinnatifida* could be what drives its greater expression of *hsp70* than the sampled native species. There has been conflicting evidence whether HSFs are subject to thermal acclimation/acclimatization within a species (see Buckley et al. 2001, Buckley & Hofmann 2002, Tomanek & Somero 2002), but evidence from Buckley & Hofmann (2004) suggests that endogenous levels of HSF can be changed by acclimation to different environmental conditions. In our system of different kelp populations with differing degrees of stressful thermal history (intertidal > harbor > subtidal), increased concentrations of HSF leading to increased rates of HSF activation and *hsp70* synthesis would be a mechanism by which populations more frequently exposed to thermal stress could have a faster rate of induction and a lower T_{max} as the need for more chaperone proteins is met more quickly.

Alternatively, differences in *hsp70* expression may lie in the activation temperature of HSFs as observed in *Gillichthys mirabilis* (Buckley & Hofmann 2004) and in *Mytilus californianus* (Buckley et al. 2001). It may be that HSFs bind to the heat shock element (HSE) at a lower temperature under certain conditions and are kept inactive on the promoter until a stressful temperature is reached. Thus, differences in induction temperature among populations would be a result of differences in the activation of the HSE where intertidal populations may have developed mechanisms for activating pre-bound HSF more quickly. Future work in this system should include measuring both HSF and Hsp70 protein concentrations to have a more complete picture of the mechanisms driving the *hsp70* expression patterns seen in this study. If such studies revealed that intertidal populations have high endogenous levels of the HSF, it would provide an explanation for the lower temperature of *hsp70* induction. How-

ever, if differences in HSF are not seen among populations with different thermal histories, the controlling steps that underlie the adjustment in gene transcription induction probably occur after the HSF has bound to the promoter.

In the present study, we had the unique opportunity to make comparisons among species and habitats to see if phylogeny or habitat seemed to be the stronger determinant of the pattern of heat shock response. We found that the heat shock response seems to be shaped by environment as the response was more similar within habitat than within species or closely related species. While this study is one of the first to make cross-species and cross-habitat comparisons, our findings have been long predicted. Krebs & Bettencourt (1999) stated, 'Expression in each species may differ for many reasons, or be similar for different reasons; in the end, I predict that variation in Hsp70 expression will follow more microclimatic differences rather than either large-scale phylogenetic or climatic patterns.' Experimental tests of the degree of plasticity of the response between native and invasive species and populations will contribute to the discussion of whether invasive species or populations indeed possess special characters or greater plasticity and lead to greater understanding of how invasive species persist and proliferate and of the dynamics between native and invasive species.

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