ELSEVIER

Journal of Experimental Marine Biology and Ecology
311 (2004) 37–46

Journal of EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

www.elsevier.com/locate/jembe

Genomic approaches to detecting thermal stress in *Calanus finmarchicus* (Copepoda: Calanoida)

Maria Voznesensky[a,b], Petra H. Lenz[c,*], Céline Spanings-Pierrot[d], David W. Towle[b]

[a] *Northwestern University, Evanston, IL 60201, USA*
[b] *Mount Desert Island Biological Laboratory, P.O. Box 35, Salisbury Cove, ME 04672, USA*
[c] *Pacific Biomedical Research Center, University Hawaii Manoa, 1993 East-West Rd., Honolulu, HI 96822, USA*
[d] *Université Montpellier II, UMR 5171, 34095 Montpellier cedex 05, France*

Received 5 February 2004; received in revised form 22 April 2004; accepted 26 April 2004

Abstract

Zooplankton may be subjected to physiological stress as they encounter rapid and large changes in temperature through vertical migration or transfer into different water masses. Induction of one or more heat shock proteins (hsp) is a common protective response to thermal stress in organisms. We looked for evidence for such a response in *Calanus finmarchicus*. We compared hsp70 expression in copepods exposed to temperature stress with that for non-stressed controls. Partial sequences of the amplified cDNA product were obtained and aligned with known hsp70 sequences to establish the identity of the heat shock protein. In one experiment, animals were transferred from their collection temperature (8 °C) to 20 °C for 30 min, and then returned to 8 °C for 4 h before sampling for gene expression levels. In another, the animals were exposed to 18 °C over 48 h before sampling for molecular analysis. A four-fold induction of hsp70 was measured in both groups of heat shocked animals using quantitative real time polymerase chain reaction (PCR). The experimental temperatures, although high for *C. finmarchicus*, are within the range of temperatures experienced by this species in their habitat. In addition to confirming an hsp70-mediated response in *C. finmarchicus*, the findings suggest that a recent history of thermal stress may be assessed in natural populations through a routine molecular assay.

Keywords: Gene expression; Hsp70; Temperature; Stress

* Corresponding author. Tel.: +1-808-956-8003; fax: +1-808-956-6984.
E-mail address: petra@pbrc.hawaii.edu (P.H. Lenz).

0022-0981/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jembe.2004.04.017

1. Introduction

Organisms respond to environmental conditions, such as thermal stress, with rapid changes in gene expression. As molecular tools have become more automated, assessing gene-level regulation of cellular processes has become routinely possible. Thus, functional genomics approaches are now available for assessment of the state of cellular conditions in natural populations. These new tools promise to be able to identify populations and even individuals experiencing stress in their natural habitat (Helmuth and Hofmann, 2001; Halpin et al., 2002).

Organisms have many physiological mechanisms to cope with non-optimal temperatures, and even ultimately lethal temperatures can be tolerated for a short time by most (Pörtner, 2001). Molecular responses to thermal and other stresses typically involve the up-regulation of one or more heat shock protein (hsp) (for review: Somero, 1995; Feder and Hofmann, 1999). These highly conserved groups of proteins are found in nearly all organisms from prokaryotes to mammals and are involved in the repair of proteins essential for cellular function. Hsp70, one of the best studied, functions as a chaperone, assisting in protein folding (Feder and Hofmann, 1999). Induction of hsp70 mRNA and protein synthesis in response to stresses has been documented in a number of marine organisms, including several invertebrates (Koziol et al., 1997; Rossi and Snyder, 2001; Spees et al., 2002; Boutet et al., 2003). An increase in hsp70 protein was detected after a 5-h exposure to an elevated temperature (23 °C) in *Eurytemora affinis*, an estuarine copepod (Bradley et al., 1992). No studies on mRNA expression in copepods have yet been reported.

Oceanic copepods, such as *Calanus finmarchicus*, inhabit thermally stable environments. *C. finmarchicus* dominates the zooplankton community in spring and summer in the North Atlantic (Meise and O'Reilly, 1996). During the summer and early fall, depending on latitude, population numbers in the surface waters decline and large numbers of subadult individuals (copepodid stage C5 = CV) descend to deep waters (below 200 m) to diapause. Between early spring and summer, *C. finmarchicus* inhabits cool waters between 0 and 12 °C. Low temperature stress may occur at and below 0 °C, although population and individual variability in tolerance for cold temperatures have been observed (Pedersen and Tande, 1992; Hirche et al., 1997). Individuals may experience warmer temperatures through advection into coastal waters or into warm water masses. Cold-water adapted *C. finmarchicus* can occur in waters near 20 °C during the summer (e.g., Turner et al., 1993), which is near their thermal tolerance limit (LT_{50}: 22 °C; Hirche, 1987). Our objective was thus to determine the change in hsp70 mRNA in *C. finmarchicus* following thermal stress exposure. We here report the first gene sequence for hsp70 in a copepod and investigate how it may be used to monitor stress levels of marine plankton.

2. Materials and methods

2.1. Experimental design

C. finmarchicus CV were sorted into 3.6-l jars from mixed plankton tows in the Gulf of Maine on June 25, 2003. Care was taken to maintain animals at collection temper-

atures (6 to 8 °C), during transfer to a temperature-controlled incubator at 8 °C. Two groups of ca. 25 animals were immediately preserved in RNA*later*® (Ambion) and frozen at − 20 °C. After return to the laboratory, the heat shock experiments were set up as shown in Table 1. The control and short-term heat shock treatments were set up with two replicates each with ca. 25 individuals in 3.6-l jars. All animals survived the short heat shock treatments well, although they showed evidence of behavioral changes, being easier to catch than the control animals. After treatment, animals were returned to 8 °C for 4 h, then were preserved in RNA*later*® and frozen at − 20 °C until further analysis. Due to a malfunctioning of the incubator, a second group of animals experienced an extended period (48 h) of up to 18 °C water (measured when the malfunction was discovered). Two sets of 25 animals from this group were collected and preserved in RNA*later*®. The remaining individuals were returned to 8 °C water and maintained in the laboratory.

2.2. RNA purification and first-strand synthesis of cDNA

Total RNA was purified from all groups of samples using RNAgents® Total RNA Isolation System from Promega. The purified RNA was sized and quantified using the Agilent Technologies 2100 Bioanalyzer. Poly-A mRNA in 2 μg of total RNA per sample was reverse transcribed using an oligo-dT primer and SuperScript II reverse transcriptase (Invitrogen). The resulting cDNAs were used as templates for conventional PCR and real-time quantitative PCR.

2.3. Primer design, amplification of cDNA templates and sequencing

Primers were designed based on homology with known hsp70 sequences and commercially synthesized (Integrated DNA Technologies). A GenBank search for hsp70 in crustaceans produced sequence data for *Artemia franciscana* (AAL27404). This amino acid sequence was used in a BLAST search, which showed the closest matches with the nematode *Caenorhabditis elegans* (Q05036), and the insects *Manduca sexta* (Q9U639),

Table 1
Experimental design

Group	Treatment	Sampling
Field animals RL1, RL2	No treatment	10 min after collection with plankton net
Control C1, C2	Temperature: 8 °C, water level changes similar to short term heat shock	4 h after 2nd water level manipulation
Short-term heat shock E1, E2	Temperature: 20 °C for 30 min, return to 8 °C	4 h after return into 8 °C water
Long-term heat shock 48-1, 48-2	Temperature: initial 8 °C, with an increase in temperature to 18 °C over 48-h period	At 18 °C after incubation period, sample of animals that survived

Four duplicate groups of animals experienced different treatments as indicated in the treatment column. Sampling indicates the time that individuals were transferred into RNAlater® after the "treatment".

Table 2
Primers used to amplify hsp70 in *C. finmarchicus*

Sense primers	Antisense primers
F2: 5′GCNAARAAYCARGTNGCNATGAA3′	R2: 5′YTTYTCNGCRTCRTTNACCAT3′
F4: 5′CAAGATGAAGGAGACTGCTGA3′	R3: 5′ATGCCTCATCAAGAGGAACACC3′
R5: 5′ACAAAGGAGGGTTGTCTAAGGAGGA3′	F5: 5′GGTACAGCTTGGTAATAACGGGACT3′
R6: 5′GGTATCCTCAATGTTCAGGCTAATG3′	

F2 and R2 are the degenerate primers designed from amino acid sequences from other organisms. Degenerate primers have alternate nucleotides in several positions to increase the probability of annealing to a cognate cDNA. The other primers are the species-specific primers designed from initial nucleotide sequence information of the product amplified by the degenerate primers. Nucleotide code—G: guanine; C: cytosine; A: adenine; T: thymine; Y (pyrimidine) = T or C; R (purine) = A or G; N (any nucleotide) = A, G, C or T.

Trichoplusia ni (AAB06239), *Bombyx mori* (BAB92074) and *Cotesia rubecula* (AAN73310). Amino acid sequences were aligned with MultiAlin (http://www.toulouse.inra.fr/multalin.html; Corpet, 1988), and conserved regions were used as the basis for designing degenerate primers (F2 and R2; Table 2).

Putative hsp70 cDNA was then amplified by the polymerase chain reaction (PCR) using first degenerate primers (F2, R2). PCR products were tested for purity and molecular size by agarose gel electrophoresis (0.8%). The gel bands were excised, purified, and extracted using the MinElute gel extraction protocol (Qiagen). Purified products were prepared for direct sequencing on an ABI Prism 3100 automated sequencer (Marine DNA Sequencing Center, Mount Desert Island Biological Laboratory). Partial sequences were edited using Chromas software, analyzed for open reading frames (DNASIS) and identified by BLAST analysis (Altschul et al., 1997). These sequences were then used to design specific primers (F4, R5 and R6, and R3 and F5) for PCR amplification under more stringent conditions and for additional sequence information and quantitative PCR.

2.4. Analysis of gene expression levels

In real-time quantitative PCR (RT-QPCR), hsp70 cDNAs, analyzed in 1-μl triplicate aliquots, were amplified in the presence of Stratagene Brilliant SYBR Green Master Mix using the Stratagene MX4000 Multiplex Quantitative PCR System. Two sets of primers (R5/F5 and R6/F5) were used in two separate RT-QPCR runs to quantify relative mRNA expression levels between each group of animals. The relative abundances of hsp70 mRNA in test samples were compared to a standard copepod mRNA obtained from control animals. For the statistical analysis, the triplicate samples were averaged for univariate analysis of variance with a post hoc Tukey test to identify homogenous groupings (General Linear Models, SPSS 11.0 for Windows).

3. Results

The quantification of total RNA with the Bioanalyzer demonstrated one single peak at 18S for ribosomal RNA in the copepod, as opposed to the three customary ones for

crustaceans (Skinner, 1968). Gel electrophoresis of the PCR products using primers F2 and R2 showed a single band estimated at 1500 bp, which corresponded to an expected length of ca. 500 amino acids (Fig. 1). Sequence comparison confirmed homology with known hsp70. The amplified product using specific primers F4 and R3 produced a sequence that contained an open reading frame of 558 nucleotides (Table 3). This sequence was homologous to other hsp70 sequences, and the open reading frame was used to predict the most likely amino acids encoded by the amplified cDNA. Alignment of the *C. finmarchicus* amino acid sequence with corresponding sequences from *A. franciscan*a and other arthropods is shown in Fig. 2. The partial sequence obtained for *C. finmarchicus* codes for the sequence at the carboxy-terminus. In the conserved region (amino acids 1 to 157), the amino acid sequence for *C. finmarchicus* had an agreement of 81% with at least three of the four sequences (Fig. 2; 127 out of 157 amino acids). In the non-conserved carboxy-terminus region (amino acids 158 to 186), *C. finmarchicus* showed similarity with the insects, *Locusta migratoria, M. sexta* and *Chironomus tentans* (Fig. 2).

Relative expression levels were measured in heat-shock experiments using the sequence-specific primers obtained from the initial sequence: R5/F5 and R6/F5 (Fig. 3). Hsp70 mRNA expression was evident in all measured samples. It was markedly enhanced in both heat-shock treatments. Analysis of variance indicated significant effects by treatment ($p < 0.001$). Lowest expression of hsp70 was found in the individuals preserved immediately after collection (RL1 and RL2). The control animals in the experiments also had very low expression levels of hsp70 mRNA, not significantly different from RL1 and RL2 (Table 4). Mean expression levels in the heat-shocked samples (30 min [E] and 48 h [48]) were approximately four-fold higher than the controls (RL and C). The heat-shocked groups were significantly different from the control and freshly caught groups (Table 4; primers R6F5 at $p < 0.05$). Results using primers R5F5 indicate 2 overlapping homogeneous groupings statistically: field (RL), control (C) and 30-min heat shocked (E) groups, and the 2 heat-shocked groups (30 min [E] and 48 h [48]) at $p = 0.05$ (Table 4). Hsp70 mRNA expression levels were slightly higher in animals that experienced the 48-h than the

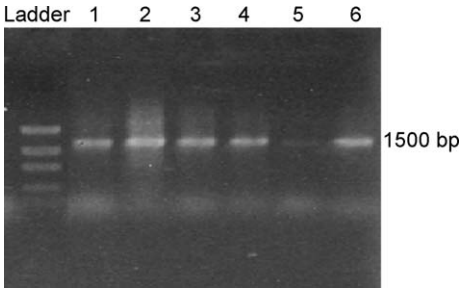

Fig. 1. PCR products amplified in *C. finmarchicus* using primers F2 and R2. Note the prominent band at molecular size of approximately 1500 bp. Sample identification 1: E1, 2: E2; 3: 48-1; 4: 48-2; 5: RL1; 6: RL2. Molecular weights in base pairs of the ladder start at top with bands at 2000, 1200, 800, 400, 200 bp. The 200-bp band is present although it is very faint.

Table 3
Nucleotide sequence of PCR product amplified with F4 and R3 primers identified as hsp70 using BLASTN (*e* value: $8e^{-17}$)

CFINCOMP.SEQ																
1	GAC	CTC	ACT	GGC	ATT	CCA	CCA	GCC	CCA	AGA	GGT	GTT	CCT	CAA	ATT	GAG	48
1	D	L	T	G	I	P	P	A	P	R	G	V	P	Q	I	E	16
49	GTC	ACC	TTT	GAT	ATT	GAT	GCA	AAC	GGT	ATC	CTC	AAT	GTT	CAG	GCT	AAT	96
17	V	T	F	D	I	D	A	N	G	I	L	N	V	Q	A	N	32
97	GAC	AAG	TCA	ACT	GGA	AAG	CAG	AAT	AAG	ATC	ACC	ATC	ACC	AAT	GAC	AAA	144
33	D	K	S	T	G	K	Q	N	K	I	T	I	T	N	D	K	48
145	GGA	AGG	TTG	TCT	AAG	GAG	GAT	ATT	GAA	AGA	ATG	GTC	AAT	GAT	GCA	GAA	192
49	G	R	L	S	K	E	D	I	E	R	M	V	N	D	A	E	64
193	AAG	TTC	AAG	GCT	GAT	GAT	GAA	AAG	CAG	AAG	GAC	AGA	ATT	TCT	GCA	AAG	240
65	K	F	K	A	D	D	E	K	Q	K	D	R	I	S	A	K	80
241	AAT	GGC	CTT	GAG	TCA	TAT	TGC	TTT	AAC	ATG	AAA	ACT	ACC	ATT	GAA	GAT	288
81	N	G	L	E	S	Y	C	F	N	M	K	T	T	I	E	D	96
289	GAA	AAG	GTC	AAG	GAT	AAG	ATA	TCA	GAT	GAT	GAC	AAG	AAA	AAG	ATA	TCG	336
97	E	K	V	K	D	K	I	S	D	D	D	K	K	K	I	S	112
337	GAA	AAA	TGT	GAA	GAA	GCC	ATA	AAA	TGG	CTC	GAT	GCT	AAC	CAA	CTT	GCT	384
113	E	K	C	E	E	A	I	K	W	L	D	A	N	Q	L	A	128
385	GAA	GTT	GAT	GAG	TTC	AAT	GAG	AAA	CAG	AAA	GAG	GTC	GAG	GCG	GTC	TGC	432
129	E	V	D	E	F	N	E	K	Q	K	E	V	E	A	V	C	144
433	AGT	CCC	GTT	ATT	ACC	AAG	CTG	TAC	CAG	TCT	GCT	GGA	GGT	ATG	CCC	GGT	480
145	S	P	V	I	T	K	L	Y	Q	S	A	G	G	M	P	G	160
481	GGT	ATG	CCA	GGT	GGT	ATG	CCA	GGT	GGA	ATG	CCG	GGC	GGA	GCT	CCC	GGA	528
161	G	M	P	G	G	M	P	G	G	M	P	G	G	A	P	G	176
529	GCT	GGA	GGA	GNG	GGA	CCA	ACT	ATT	GAA	GAA							558
177	A	G	G	X	G	P	T	I	E	E							186

Open reading frame for nucleotide sequence obtained from amplified PCR product with corresponding amino acid sequence. Partial amino acid sequence was predicted by DNASIS and is given in single-letter notation.

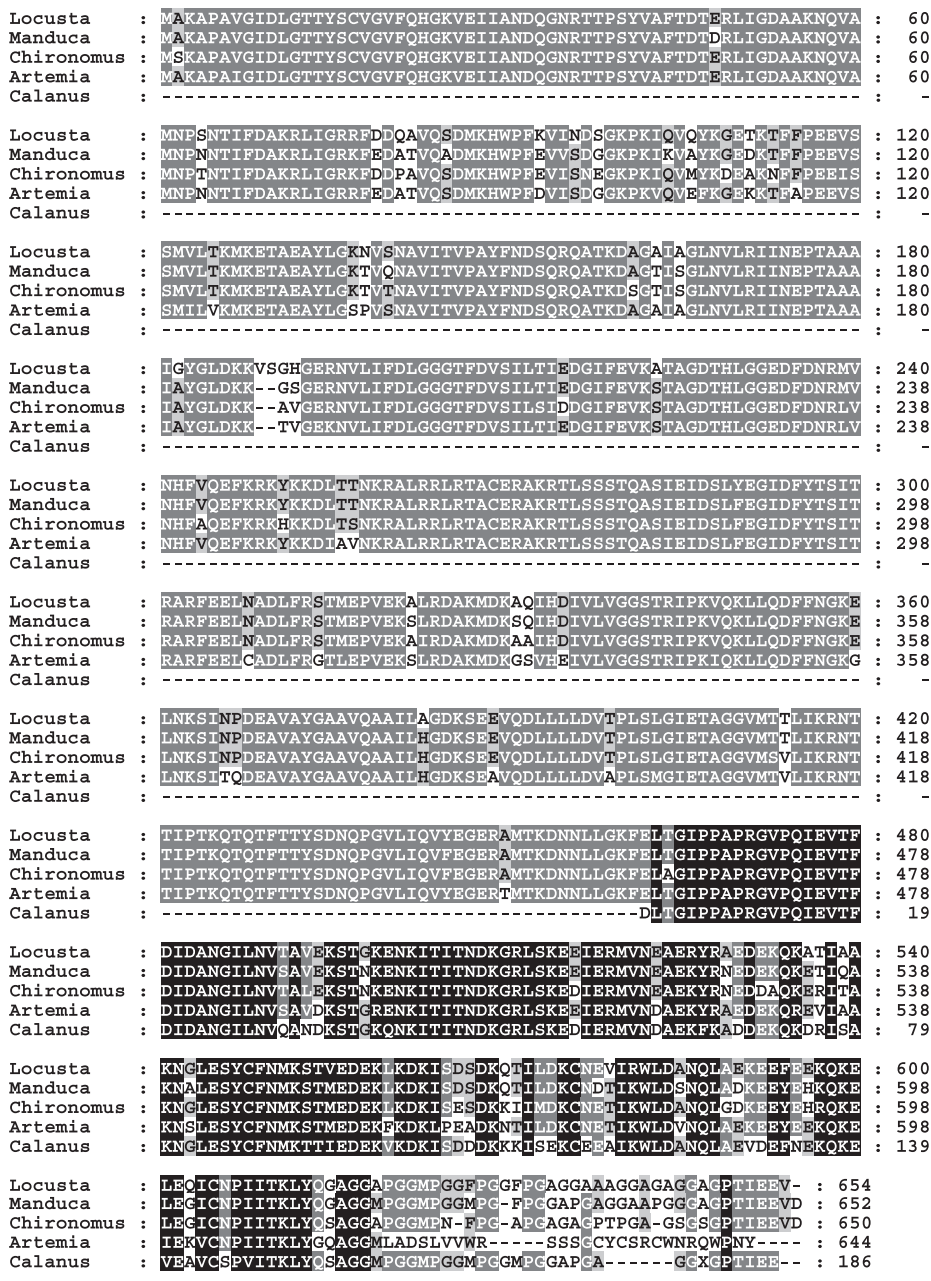

Fig. 2. Alignment of hsp70 amino acid sequences of several arthropods compared to partial sequence from *C. finmarchicus*. Hsp70 amino acid sequences were obtained from GenBank: *L. migratoria* (AAF09496), *M. sexta* (Q9U639), *C. tentans* (AAN14525), *A. franciscana* (AAL27404). Similarity groups were enabled. White letters on black background indicate conserved regions. White letters on grey background show conserved areas in four out of five sequences, and black letters on grey background indicate agreement of three out of five sequences.

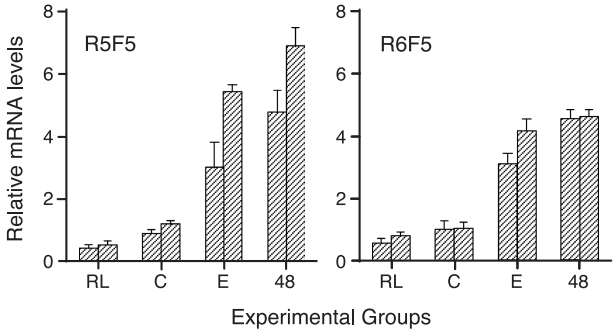

Fig. 3. Real-time quantitative PCR results showing relative hsp70 mRNA levels. RL are the field animals preserved immediately after collection, C are the two control groups, E animals were subjected to short term thermal stress of 30-min incubation at 20 °C, and 48 are the two groups with an extended heat treatment at 18 °C. Hsp70 mRNA levels are represented in relation to a dilution series of standard RNA prepared from control animals (Controls = 1). R5F5 and R6F5 refer to the primer pairs used in the amplification and the sequences are given in Table 2. Quantitative PCR was run in triplicate and bars indicate standard deviation for each sample.

30-min temperature shock, although this difference was not significant with either sets of primers.

4. Discussion

Measurable levels of hsp70 are not unusual in individuals collected from field conditions (Helmuth and Hofmann, 2001; Halpin et al., 2002). The induction of hsp70 in *C. finmarchicus* was rapid (within 4 h) and in response to a short heat shock (30 min), comparable to the response seen in *A. franciscana* (30 min at 37 °C, Frankenberg et al., 2000). Although the temperature in the long-term experiment, 18 °C, is not reported to be lethal in experiments run for 24 h (Hirche, 1987), significant mortality was found after longer exposure (48 h). Surviving individuals showed up-regulation of hsp70, and

Table 4
Statistical analysis of real time quantitative PCR results with primers R5F5 and R6F6

	Field (RL)	Control (C)	Heat Shock	
			30-min (E)	48-h (48)
Field (RL)	–	0.919	0.089	0.029*
Control (C)	0.694	–	0.156	0.046*
30-min heat shock (E)	0.004*	0.007*	–	0.553
48-h heat shock (48)	0.001*	0.002*	0.186	–

Tukey test used for post hoc analysis (General Linear Models, SPSS 11.0 statistical package for Windows). Multiple comparisons between experimental groups for R5F5 primers (above diagonal) and for R6F5 primers (below diagonal). Numbers indicate probability (p value) for each comparison. *Indicates significance at $p = 0.005$. Using the R6F5 primers, the field and control groups were not significantly different from each other, but were significantly (5% level) different from the heat-shocked groups (30 min and 48 h). The heat-shocked groups were not significantly different from each other. Using the R5F5 primers, there were two homogeneous groupings: field, control and 30-min heat shocked groups; and the heat shocked groups (30 min and 48 h).

individuals that were returned to cool temperatures showed good survival over the next month at 6–9 °C. *C. finmarchicus* thus exhibits the heat-shock response that protects against exposures to high temperatures. Elevated levels of hsp70 and other heat-shock proteins may thus be predicted in *C. finmarchicus* collected from warmer waters during the summer (Turner et al., 1993). The heat-shock response may increase survival in this species by allowing time for individuals experiencing high temperatures to vertically migrate to deeper waters with more optimal thermal conditions.

Thermal tolerances vary widely among species, and temperature has been implicated in contributing to geographical distributions (for review: Somero, 1995). Temperature tolerance ranges can be extended through the induction of heat shock protein (Somero, 1995). Elevated heat-shock protein concentrations can persist for days after a short period of thermal stress (Tomanek and Somero, 2000; Piano et al., 2002). Although mostly studied at high temperature, heat shock proteins offer protection at low as well as high temperatures. Within the genus *Calanus*, *C. finmarchicus* has a broad temperature tolerance in the range from 0 to 20 °C (Hirche, 1987). Thus, induction of heat shock proteins may be predicted to occur with low temperature stress in this species. It may offer protection to individuals emerging from diapause in late winter.

5. Conclusion

Molecular approaches may be used as effective tools to investigate ecological questions in marine zooplankton. Copepod RNA is well preserved in RNA*later*®, which facilitates collection of individuals. RNA/DNA ratios have been used successfully as indicators for growth rates in *C. finmarchicus* in the laboratory (Wagner et al., 1998) and in the field (Campbell et al., 2001). The present study shows that gene expression levels can be measured in wild-caught as well as experimental animals. Such approaches may provide additional and more detailed information about physiological state in natural populations. In addition to confirming the heat shock response in *C. finmarchicus*, the present findings suggest that a recent history of thermal stress may be assessed in natural populations through a routine molecular assay.

Acknowledgements

We would like to thank R. Patrick Hassett, Gabriel Rodrigues and Daniel Burdick for the assistance with collections and experiments; R.P. Hassett for loan of equipment; Daniel K. Hartline for the discussions and review of the manuscript; Pat Couvillon for the assistance with the statistics, and Chris Smith for the DNA sequencing data. College of the Atlantic kindly allowed use of their research vessel *R/V Indigo* and we would like to thank Andrew Peterson and crew for their assistance. This work was supported by NSF grant OCE99-06223 to PHL; NSF DBI01-00394 to DWT; NSF REU01-39190 to Mount Desert Island Biological Laboratory; and by a New Investigator Award from the Salisbury Cove Research Fund of MDIBL to CSP. **[SS]**

References

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3401.

Boutet, I., Tanguy, A., Rousseau, S., Auffret, M., Moraga, D., 2003. Molecular identification and expression of heat shock cognate 70 (*hsc70*) and heat shock protein 70 (*hsp70*) genes in the Pacific oyster *Crassostrea gigas*. Cell Stress Chaperones 8 (1), 76–85.

Bradley, B.P., Lane, M.A., Gonzalez, C.M., 1992. A molecular mechanism of adaptation in an estuarine copepod. Neth. J. Sea Res. 30 (1), 5–10.

Campbell, R.G., Runge, J.A., Durbin, E.G., 2001. Evidence for food limitation of *Calanus finmarchicus* production rates on the southern flank of Georges Bank during April 1997. Deep-Sea Res. II 48 (1–3), 531–549.

Corpet, F., 1988. Multiple sequence alignment with hierarchical clustering. Nucleic Acids Res. 16, 10881–10890.

Feder, M.E., Hofmann, G.E., 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu. Rev. Physiol. 61, 243–282.

Frankenberg, M.M., Jackson, S.A., Clegg, J.S., 2000. The heat shock response of adult *Artemia franciscana*. J. Therm. Biol. 25 (6), 481–490.

Halpin, P.M., Sorte, C.J., Hofmann, G.E., Menge, B.A., 2002. Patterns of variation in levels of hsp70 in natural rocky shore populations from microscales to mesoscales. Integr. Comp. Biol. 42 (4), 815–824.

Helmuth, B.S.T., Hofmann, G.E., 2001. Microhabitats, thermal heterogeneity, and patterns of physiological stress in the rocky intertidal zone. Biol. Bull. 201 (3), 374–384.

Hirche, H.-J., 1987. Temperature and plankton: II. Effect on respiration and swimming activity in copepods from the Greenland Sea. Mar. Biol. 94 (3), 347–356.

Hirche, H.-J., Meyer, U., Niehoff, B., 1997. Egg production of *Calanus finmarchicus*: effect of temperature, food and season. Mar. Biol. 127 (4), 609–620.

Koziol, C., Batel, R., Arinc, E., Schröder, H.C., Müller, W.E.G., 1997. Expression of the potential biomarker heat shock protein 70 and its regulator, the metazoan DnaJ homolog, by temperature stress in the sponge *Geodia cydonium*. Mar. Ecol. Prog. Ser. 154, 261–268.

Meise, C.J., O'Reilly, J.E., 1996. Spatial and seasonal patterns in abundance and age-composition of *Calanus finmarchicus* in the Gulf of Maine and on Georges Bank: 1977–1987. Deep-Sea Res. II 43 (7–8), 1473–1501.

Pedersen, G., Tande, K.S., 1992. Physiological plasticity to temperature in *Calanus finmarchicus*. Reality or artefact? J. Exp. Mar. Biol. Ecol. 155 (2), 183–197.

Piano, A., Asirelli, C., Caselli, F., Fabbri, E., 2002. Hsp70 expression in thermally stressed *Ostrea edulis*, a commercially important oyster in Europe. Cell Stress Chaperones 7 (3), 250–257.

Pörtner, H.O., 2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwissenschaften 88 (4), 137–146.

Rossi, S., Snyder, M.J., 2001. Competition for space among sessile marine invertebrates: changes in HSP70 expression in two Pacific cnidarians. Biol. Bull. 201 (3), 385–393.

Skinner, D.M., 1968. Isolation and characterization of ribosomal ribonucleic acid from the crustacean, *Gecarcinus lateralis*. J. Exp. Zool. 169, 347–356.

Somero, G.N., 1995. Proteins and temperature. Annu. Rev. Physiol. 57, 43–68.

Spees, J.L., Chang, S.A., Snyder, M.J., Chang, E.S., 2002. Thermal acclimation and stress in the American lobster, *Homarus americanus*: equivalent temperature shifts elicit unique gene expression patterns for molecular chaperones and polyubiquitin. Cell Stress Chaperones 7 (1), 97–106.

Tomanek, L., Somero, G.N., 2000. Time course and magnitude of synthesis of heat-shock proteins in congeneric marine snails (genus *Tegula*) from different tidal heights. Physiol. Biochem. Zool. 73 (2), 249–256.

Turner, J.T., Tester, P.A., Strickler, J.R., 1993. Zooplankton feeding ecology: a cinematographic study of animal-to-animal variability in the feeding behavior of *Calanus finmarchicus*. Limnol. Oceanogr. 38 (2), 255–264.

Wagner, M.M., Durbin, E.G., Buckley, L.J., 1998. RNA:DNA ratios as indicators of nutritional condition in the copepod *Calanus finmarchicus*. Mar. Ecol. Prog. Ser. 162, 173–181.