

Heat shock protein responses in thermally stressed bay scallops, *Argopecten irradians*, and sea scallops, *Placopecten magellanicus*

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Abstract

The effects of thermal stress on the induction of heat shock proteins (HSPs) were examined in northern bay scallops, *Argopecten irradians irradians*, a relatively heat tolerant estuarine species, and sea scallops, *Placopecten magellanicus*, a species residing in cooler, deeper water. Polyclonal antibodies used in this work for analysis of inducible HSP70 and HSP40 only recognized proteins of 72 and 40 kDa respectively from the mantles of both scallop species. Additionally, HSP quantification using the antibody to HSP70 was equally effective by either immunoprobings of western blots or ELISA, demonstrating that either approach could be successfully employed for analysis of thermal response in scallops. Sea scallop HSP70 and HSP40 did not change when animals were heat-shocked for 3 h by raising the temperature from 10 °C to 20 °C; however, a 24 h treatment of the same magnitude elicited a significant response. Conversely, bay scallops displayed rapid and prolonged HSP70 and HSP40 responses during the recovery period following a 3 h heat shock from 20 °C to 30 °C. Temperature reduction from 20 °C to 3 °C for 3 h also caused significant HSP70 and HSP40 increases in bay scallops; this represents the first time cold shock was shown to induce HSP synthesis in bivalve mollusks. The onset of the HSP40 response was more rapid than for HSP70, occurring at the end of the cold shock itself prior to transfer to a recovery temperature. Both proteins responded maximally during recovery at control temperature. HSP responses of sea and bay scallops to thermal stress may be related to their habitat in the natural environment and they suggest a differential capacity for adaptation to temperature change. This is an important consideration in assessing the response of these scallops to different culture conditions. Crown Copyright © 2008 Published by Elsevier B.V. All rights reserved.

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1. Introduction

Sea scallops, *Placopecten magellanicus*, (Gmelin, 1791) and bay scallops, *Argopecten irradians*, (Lamarck, 1819) are commercially important species on the Atlantic coast of North America. Sea scallops inhabit colder and deeper waters on the continental shelf and are distributed from Labrador to Cape Hatteras between depths of 10 and 100 m (Shumway et al., 1987; Pilditch and Grant, 1999). *P. magellanicus* is particularly sensitive to elevated temperatures (Gould and Fowler, 1991; Pilditch and Grant, 1999) but is well adapted to temperatures

ranging from 5 to 15 °C, with optimum growth occurring at ~10 °C (Young-Lai and Aiken, 1986). Bay scallops occur along the eastern coast of the USA, from Massachusetts to Texas (Clark, 1965). The northern subspecies, *A. irradians irradians*, inhabits shallow subtidal areas of less than 10 m between Maine and New Jersey, USA, with an unconfirmed report of a relic, isolated population off the Nova Scotia (NS) coast in eastern Canada (Rhodes, 1991), and more recent establishment of a self-sustaining population in Melmerby Bay, northern NS. *A. irradians concentricus*, a more southerly subspecies, occurs from North Carolina to Florida (Brand, 1991). Bay scallops are normally subjected to considerable fluctuations in temperature in their estuarine habitat (Nelson et al., 1977). Temperatures above 15 °C ensure good bay scallop growth, but the species temporarily withstands temperatures in the range of –2 °C

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to 30 °C (Tettelbach, 1991). Cold tolerance may limit the distribution of bay scallops and the areas in which they may be successfully cultivated. For example, significant mortalities of cultured bay scallops on the Atlantic coasts of Canada and the USA occurred in conjunction with low water temperatures during winter months (Tettelbach and Wenczel, 1993; Couturier et al., 1995; Barber and Davis, 1997).

Sea and bay scallops are also cultured inshore, where they may be exposed to considerable changes in water temperature. Temperature fluctuations of approximately 11 °C over an 8 day cycle have been recorded in coastal bays of eastern Nova Scotia (Pilditch and Grant, 1999). Inshore waters of the Baie des Chaleurs, the Gulf of St. Lawrence, and the Atlantic coast of Nova Scotia are subject to relatively strong thermal stratification as warming occurs during the summer months, and oscillations in the thermocline lead to temperature fluctuations of up to 15 °C in the water column (Dadswell and Crawford-Kellock, 1989; Côté et al., 1993; Pearce et al., 2004). In this context, mass mortalities of *P. magellanicus* in Atlantic Canada have been attributed to sudden increases in water temperature resulting from wind-induced shifts in the depth of the thermocline (Dickie, 1958; Dickie and Medcof, 1963; Côté et al., 1993). In the coastal waters of Georgia, mortalities of juvenile bay scallops held in pearl nets at a depth of 30 cm were observed during summer upon a rapid increase in temperature from 26 to 29.7 °C (Heffernan et al., 1988). Cultured sea and bay scallops may also be exposed to sudden temperature variations during husbandry practices, including transfer from cooler rearing temperatures on the bottom to warmer temperatures at the surface, and vice versa (Lafrance et al., 2002).

Studies on stress physiology and thermotolerance of aquatic animals suggest that the synthesis of heat shock proteins (HSPs) reflects environmental conditions and that these proteins are effective stress biomarkers (Sanders, 1990, 1993; Feder and Hofmann, 1999; Sørensen et al., 2003). Species occupying widely varying distribution patterns and/or thermal habitats differ in HSP responses to stress and HSPs are believed to play an important ecological role in the adaptation of ectotherms to fluctuating environments (Sanders et al., 1991a; Hofmann and Somero, 1996; Feder and Hofmann, 1999; Tomanek and Somero, 1999; Tomanek, 2002, 2005).

HSP70 is ubiquitous, highly conserved and abundant (Parsell and Lindquist, 1994). This HSP is studied extensively because of its importance in protein folding (Gething and Sambrook, 1992; Frydman et al., 1994) and for the protection it affords cells during environmental perturbation (Parsell and Lindquist, 1993; Feder and Hofmann, 1999). HSP40, another molecular chaperone, binds directly to nonnative polypeptides and protects cells from stress by preventing protein aggregation (Cheatham and Caplan, 1998). Most HSPs have the ability to function independently as molecular chaperones, but interactions occur frequently between chaperones (Fink, 1999; Fan et al., 2003). For example, HSP70 and HSP40 are often found in the same cell compartments, and HSP40 assumes co-chaperone status with HSP70. Although the purpose of these interactions remains to be fully elucidated, one of the proposed roles of HSP40 is to direct HSP70 to denatured proteins (Hartl, 1996;

Fink, 1999; Fan et al., 2003). HSP70 is reported in many species, including algae, sea urchins, crustaceans, fish and bivalves (Chapple et al., 1997; Clegg et al., 2000; Matranga et al., 2000; Piano et al., 2002; Lund et al., 2003; Ireland et al., 2004), whereas HSP40 has been documented in mussels, snails and fish (Lyons et al., 2003; Tomanek, 2005; Dong et al., 2006).

Bivalve mollusks play important roles in aquatic environments, and research on the HSP response in these organisms is increasing because of their broad biogeographical distribution and capacity to bioaccumulate heavy metals and organic contaminants. Exposure of mussels to tributyltin compounds and/or heavy metals correlates with tissue-specific HSP increases and the organism's physiological condition, as measured by either filtration rate or scope for growth (Sanders et al., 1991b; Steinert and Pickwell, 1993; Sanders et al., 1994; Bradley et al., 1998). In oysters, proteins of the HSP70 family are the best studied, with HSP70 gene expression characterized in several oyster species (Gourdon et al., 2000; Rathinam et al., 2000; Boutet et al., 2003a; Hamdoun et al., 2003; Piano et al., 2005). Furthermore in oysters, sublethal heat shock enhances survival to subsequent lethal heat stress, a physiological condition known as induced thermotolerance (Shamseldin et al., 1997; Clegg et al., 1998; Brown et al., 2004). Results of these studies suggest that increased HSP synthesis generated by the initial heat treatment confers cell protection which enables oysters to survive a normally lethal insult.

Very limited information on the HSP response is available for scallops. In the bay scallop, *A. irradians*, full-length HSP70 cDNA was cloned and used to measure hemolymph HSP70 mRNA in response to bacterial challenge and organic contaminants (Song et al., 2006). Bay scallop HSP70 mRNA expression was up-regulated 2 h following injection with 2×10^8 cells ml⁻¹ of *Vibrio anguillarum*, and reached a maximum at 8 h before declining gradually after 16 h. Bay scallop HSP70 mRNA gene expression was enhanced with increasing naphthalene exposure from 100 to 700 µg l⁻¹. In the only published work on HSPs in thermally shocked scallops, HSP70 expression was examined in Japanese scallops, *Patinopecten yessoensis*, stressed for 12 h at varying temperatures (Oguma et al., 1998). HSP70 in scallops that were heat-shocked by raising the temperature from 12 °C to 22 °C for 30 min increased approximately 1.5-fold as compared to controls held at 22 °C. HSP70 decreased to control values after 1 h of recovery. In contrast, scallops subjected to a 12 h, sublethal temperature increase from 12 °C to 27 °C displayed significant amplification of HSP70 by 30 min, which then decreased after 2 h only to rise significantly again by 12 h. Additionally, the expression of HSPs in response to cold shock has been examined in several organisms, including human cells, insects, nematodes and bacteria (Joplin et al., 1990; Liu et al., 1994; Salotra et al., 1995; Nunamaker et al., 1996; Martinez et al., 2002), but has not been investigated in bivalve molluscs.

The objective of the current study was to inspect HSP responses of two commercially important scallop species that vary in their habitat and temperature tolerance, and thus assess whether these are attributed to differential induction of HSPs. Sea and bay scallop HSP responses were examined upon

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