

Heat-shock protein (HSP70) response in the eastern oyster, *Crassostrea virginica*, exposed to PAHs sorbed to suspended artificial clay particles and to suspended field contaminated sediments

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Abstract

Sediments are a potentially significant source of pollutants, containing not only organic contaminants but heavy metals as well. The heat shock protein response (HSP70 family) in the eastern oyster exposed to suspended clay particles spiked with polynuclear aromatic hydrocarbons (PAHs) and to suspended field contaminated sediments (SFCS) was investigated. In experiment 1, oysters were exposed to 1.0, 1.5 or 2.0 g suspended clay particles with concentrations of 65.6, 159.0 and 242 μg PAHs per g of wet clay particles, respectively, and sampled after 40 days. Controls were exposed to 0, 1.0, 1.5, and 2.0 g suspended unspiked clay particles. In experiment 2, oysters were exposed to 0, 1.0, 1.5, and 2.0 g SFCS and the HSP70 expression was determined after 5, 10, 20 and 40 days exposure. Oysters exposed to suspended clay particles spiked with PAHs showed a significant increase in HSP70 levels, while oysters exposed to 1.0, 1.5 or 2.0 g suspended unspiked clay particles did not show changes ($P = 0.78$) in HSP70 levels compared to the group receiving 0 g clay particles. Exposure to the SFCS resulted in a significant increase in HSP70 as a function of exposure ($P < 0.001$) and treatment ($P = 0.006$). The response, however, was not dose dependent. Compared to the control group (0 g SFCS), groups exposed to 1.0, 1.5 and 2.0 g SFCS reached significantly higher levels in HSP70 at 40 days of exposure, with those exposed to 2.0 g SFCS expressing the highest levels. The HSP70 expression for each treatment showed fluctuations at various time intervals. No mortalities were recorded during the exposure experiments. The major contaminants in the SFCS were PAHs, heavy metals and polychlorinated biphenyls (PCBs). These results reveal that exposure to PAHs sorbed to clay particles and to SFCS induced a HSP70 response in the eastern oyster. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Sediments are a source of toxicity for aquatic organisms, since they contain not only organic

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contaminants but heavy metals as well. As such, sediments are a potentially significant source of pollutants in aquatic ecosystems. Sediments usually contain a higher concentration of hydrophobic contaminants in comparison with the water phase (Eertman et al., 1995). The potential adverse effects of sediment-associated contaminants is generally assessed by determining their concentration directly from sediments, organisms on sites, or determined from bioassays of exposed animals (ASTM, 1993). However, these determinations are not always considered good indicators of effects in all cases (Long et al., 1995) and are time consuming.

Bivalve molluscs such as oysters play an important ecological role in the aquatic system. They filter and ingest suspended particulate matter (e.g. algae and sediment particles) from the water column via filter feeding and in the same process remove contaminants associated with the suspended matter (Björk and Gilek, 1996; Dame and Dankers, 1988). Also, oysters are sessile filter feeders incapable of moving to avoid unfavorable conditions in their immediate environment and are known to bioaccumulate contaminants (Obana et al., 1981; Landrum et al., 1991). Bivalve molluscs have been used as sentinel organisms of chemical contamination and biological effect (Farrington et al., 1983; Page, 1995; Sheehan and Power, 1999). Bioaccumulation of various contaminants (cadmium, phenanthrene, naphthalene, PAHs, etc.) in mussels, *Mytilus edulis* and *M. trossulus* and in the eastern oyster, *Crassostrea virginica* has been documented (Bender et al., 1988; Björk and Gilek, 1996; Pollet and Bendell-Young, 1999). Toxicological studies on aquatic organisms however, typically involve exposure of organisms to water spiked with the contaminant (George and Coombs, 1977; Everaarts, 1990; Monaghan and Bradley, 1993; Satish and Robinson, 2001) or settled sediments under study (e.g. Werner et al., 1998; Werner and Hinton, 1999). These approaches do not consider the potential significance of contaminants associated to suspended particles as an additional route of exposure in filter feeders.

Stress proteins have been proposed as sensi-

tive indicators of sublethal exposure to contaminants in the environment (Sanders et al., 1991; Sanders, 1993; Steinert and Pickwell, 1993). Initially termed “heat-shock proteins” (HSPs), the stress proteins include a number of protein families induced during stress events. Stress proteins are synthesized at higher levels when cells are challenged with certain environmental stimuli such as high temperature and toxic chemicals, making them a potentially useful marker of exposure (Morimoto, 1993; Sanders, 1993). In cells responding to environmental stress, the HSP70 family is preferentially expressed over other HSPs accounting for much of the translational activity. The HSP70 is inducible and highly conserved among all phyla studied including the blue mussel, *M. edulis* exposed to cooper (Sanders, 1993) the Pacific oyster, *Crassostrea gigas*, after heat-shock at 37 °C (Clegg et al., 1998), and echinoderms, *Strongylocentrotus purpuratus* (Stegeman et al., 1992; Sanders and Martin, 1994) after a heat treatment. An increased synthesis of HSP70 was also noted in hemocytes from the eastern oyster, *C. virginica*, for several days after heat treatment at 46 °C (Tirard et al., 1995a). We have also detected the presence of two HSP70 isoforms in the eastern oyster of 69 kDa and 71 kDa 48 h after heat shock for 1 h at 37 °C (Fig. 1).

Studies on the stress protein response to contaminants have focused on the fraction dissolved in the water phase (e.g. Monaghan and Bradley, 1993; Sanders et al., 1994) or to settled sediments (e.g. Werner et al., 1998; Werner and Hinton, 1999). In all of these studies, a stress protein response was observed upon exposure to heavy metals, PAHs, or complex mixtures of contaminants associated with the water phase or deposited sediments.

To our knowledge no study has been conducted to test the effects of pollutants sorbed to suspended particles or suspended field contaminated sediments (SFCS) on the HSP70 response in benthic filter feeders. The present study was conducted to test whether exposure of oysters to various doses of PAHs sorbed to suspended clay particles or SFCS induce a HSP70 response.

2. Methods and materials

2.1. Experiment 1: HSP70 response in oysters exposed to PAHs sorbed to suspended clay particles

The effects of suspended clay particles spiked with PAHs or suspended clay particles alone on the stress protein response were tested. Oysters collected from the Damariscotta River, Maine, an area rarely infected by the protozoan parasite *Perkinsus marinus* were used in this experiment. *P. marinus*, has caused periodic epizootics in the eastern oyster populations in the Chesapeake Bay and resulted in mass mortalities since the 1950s. Induction of HSP70 has been described in *P. marinus* after heat treatment (Tirard et al., 1995b). In mammalian hosts, stress proteins are believed to aid in protecting from microbial stress and are involved in immune functions and host–pathogen interactions (Polla, 1991; Healy et al., 1992; Murray and Young, 1992; Young et

al., 1993). The effects of parasitic infection on the HSP70 response in invertebrate hosts are not known. There is no documentation on the alteration of HSP70 due to *P. marinus* infection. However, to avoid potential confounding effects due to the presence of the parasite, oysters were selected from a region with rare incidence of *P. marinus*.

Experimental oysters were acclimated to local conditions, to a salinity of 18 ppt (York River salinity) and temperatures of 23–24 °C, over 9 days in two 600 L tanks. After acclimation, a subsample of oysters ($n = 10$) was examined for *P. marinus* infection and the results were negative.

Clay particles for the experiments were prepared by pulverizing green shale (Illite 46E0315, Wards/Cenco, Rochester, NY) to an average size of 50 μm . The clay particles were then hydrated in 1 μm filtered York River water (YRW), and stored at 4 °C until use.

A solution containing a mixture of PAHs (fluoranthene, pyrene, benzo(a)pyrene and benzo(e)pyrene; 100 μg PAH/ml of each) was prepared in acetone. The PAH solution was added to hydrated clay particles (HCPs) to obtain a nominal concentration of 400 μg PAHs/g HCP. The mixture was stirred continuously and small amounts of distilled water were added occasionally until the carrier evaporated and the mixture became a slurry. The PAH-spiked clay particle slurry was mixed with unspiked clay particle slurry to obtain three nominal concentrations: 40, 133, and 200 μg PAHs/g of HCPs. These nominal concentrations represented exposure treatments of 40, 200 and 400 μg PAHs/day in 1.0, 1.5 and 2.0 g HCPs, respectively. Subsequent extraction and GC analysis of the spiked clay particles revealed the actual concentrations to be 65.6, 159.0 and 242.0 μg PAHs/g of HCPs. Clay particle suspensions were prepared by stirring the desired amounts of spiked clay particles for treatment groups and unspiked clay particles for control groups into YRW for 2 h to ensure the adequate suspension of the clay particles prior to use.

Acclimated oysters were divided into seven groups. Treatment groups (3) were exposed daily

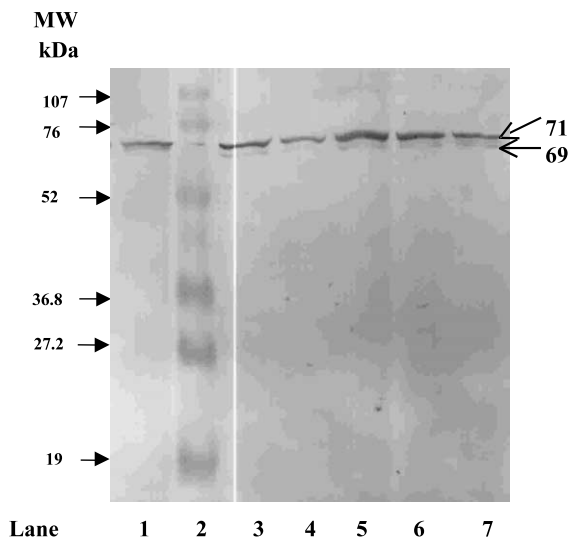


Fig. 1. Representative Western Blot showing two HSP70 isoforms of 71 and 69 kDa in gill tissue of the eastern oyster, *Crassostrea virginica*. Oysters were heat-shocked for 1 h at 37 °C followed by a 48 h accumulation period at ambient temperature. Lanes 1, 3–7 show the two isoforms detected using anti-HSP70 monoclonal antibody (Affinity BioReagents MA3-006). Lane 2 shows low range molecular weight marker (Bio Rad Prestained SDS-PAGE Standards, Low Range).

to 1.0, 1.5 or 2.0 g suspended spiked clay particles (corresponding to 65.6, 238.5 and 484.0 μg PAHs/oyster daily, $n = 12$) for 40 days and sampled for determination of HSP70 response. Control groups (4) were exposed daily to 0, 1.0, 1.5 or 2.0 g suspended unspiked clay particles to test the effects of clay particle dosage on HSP70 responses. Oysters were maintained in 2 l containers with aeration and fed 0.2 g algal paste daily. YRW was changed every other day. At the end of the experiment (40 days post-exposure), gills from individual oysters were excised and used for HSP70 analysis. Gills were selected because they are directly exposed to waterborne contaminants and particulates. Aliquots of rectal, mantle and gill, and adductor muscle tissues were also removed to analyze for possible *P. marinus* infection.

2.2. Experiment 2: HSP70 response in oysters exposed to suspended field contaminated sediments (SFCS)

The HSP70 response was part of a larger study examining cellular responses in oysters exposed to SFCS (Chu et al., 2002). These sediments were collected from the Elizabeth River, a highly contaminated estuary in Virginia. Chemical analysis of these sediments revealed the presence of PAHs (70.20 ± 5.95 mg/kg dry sediments), PCBs (0.41 ± 0.09 mg/kg dry sediments) and a variety of heavy metals (Chu et al., 2002). Oysters were received from the Damariscotta River, Maine. After acclimation, 50 oysters were examined for *P. marinus* infection and the rest were divided into four groups, maintained in individual 2 l chambers with aeration, fed 0.2 g algal paste/oyster daily and exposed daily to 0, 1.0, 1.5, or 2.0 g SFCS. *P. marinus* diagnosis on the 50 acclimated oysters was negative. YRW was changed every other day. Oysters were sampled for HSP70 analysis 5, 10, 20, and 40 days post exposure. After collecting hemolymph for measuring cellular responses (Chu et al., 2002), tissues from individual oysters were excised for HSP70 analysis (gill) and examined for possible *P. marinus* infection (rectal, mantle and gill, and adductor muscle).

3. Heat-shock protein analysis

HSP70 in gill tissues was assessed by slot blot. Western blot and immunoassays have been used routinely to analyze stress proteins in target tissues and organisms. While the use of Western blot is effective in evaluating stress protein responses, the assay is time consuming and laborious. In comparison, the slot blot technique for estimating total HSP70 is relatively quick. This technique has been used previously in HSP70 determinations comparing fishes from contaminated and relatively clean sites in southern California (Brown and Bay, 1999), in oysters exposed to algae contaminated with PCBs (Cruz Rodríguez et al., 2000), and in a macroalga exposed to environmental stressors (Lewis et al., 2001). However, because the binding of a secondary antibody to the first antibody is not always 1:1 and the ratio between antigen and antibody is not necessarily constant, the application of the technique used is semi-quantitative. Notwithstanding, the blots serve to obtain an estimate of HSP70 accumulation when comparing treatments.

Oyster gill tissues were homogenized using a hand held blender (Ultraturrax T-25 Homogenizer) at 24 000 rpm for 30 s on ice in 2 ml of buffer (66 mM Tris pH 7.2, 3% Nonidet and 0.1 mM PMSF). The homogenate was centrifuged at $19\,800 \times g$ on a fixed angle rotor for 30 min at 4 °C and the supernatant collected. Total protein concentration was determined using Biorad DC Protein Assay (Lowry et al., 1951).

Prior to using slot blot for evaluation of the HSP70 in oyster gill tissues, the specificity of the primary antibody and the efficacy of the approach were tested. First, the specificity of the primary monoclonal antibody raised against human HSP70 protein (Affinity BioReagents MA3-006) was assessed. After electrophoretic separation in 12.5% SDS-polyacrylamide gel (Laemmli, 1970) and Western blotting, this primary monoclonal antibody was found to recognize two HSP70 isoforms, 69 and 71 kDa, in oyster soluble protein extracts (Fig. 1).

Second, the efficacy of the slot blot (Bio-Dot SF Microfiltration Apparatus) was tested. The linearity of the response was confirmed by serial

dilutions of a “reference sample” (soluble protein obtained by heat shocking four oysters for 1 h at 37 °C followed by a 48 h accumulation period at ambient temperature). The linear range of the assay was determined to be between 0.25 and 2.50 µg protein. The gradient showed a strong positive correlation ($r^2 > 0.96$) between the amount of protein loaded and the intensity of the signal. Non-specific reactivity of the antibodies was not visible in the slot blot. In addition, samples were run by both Western blot and slot blot. Correlation between the two methods was good, showing a similar pattern in HSP70 response. Slot blot measures both inducible and constitutive forms of HSP70.

The blotting procedures consisted in directly applying and immobilizing 1.5 µg total protein per tested sample in triplicates onto nitrocellulose (0.45 µm). The “reference sample” gradient (0.25, 0.50, 1.00, 1.50, 2.00 and 2.50 µg protein) was loaded in every blot to adjust for interblot variability. The 1.50 µg dilution in each series was used for data normalization. The blot was blocked with 5% non-fat dry milk in TTBS (0.05% Tween-20, 500 mM NaCl, 15 mM Tris, pH 7.5) for 30 min, followed by two washes in TBS (500 mM NaCl, 15 mM Tris, pH 7.5) for 10 min. Antibody dilutions (1:5000 primary antibody and 1:1000 secondary antibody) used were such that the quantity of antigen, not antibody, was limiting. Primary monoclonal antibody against HSP70 (Affinity Bioreagents, MA3-006) was applied for 90 min, followed by one wash with TTBS and two washes with TBS for 10 min each. The secondary antibody (Goat anti-mouse AP-conjugated) was applied for 90 min. Subsequently, the blot was washed twice with TBS for 10 min, then placed in a developing solution containing NBT (*p*-nitroblue tetrazolium chloride) and BCIP (5-bromo-4-chloro-3-indolyl phosphate). Bands started to develop after 30 min and development was completed after 3 h. The blot was then stored in deionized water until densitometric analysis (Fig. 2).

Densitometric analysis was performed by scanning the blots using SeptraScan software (ISS Enprotech, MA, USA). The areas of the samples were recorded and each sample area normalized

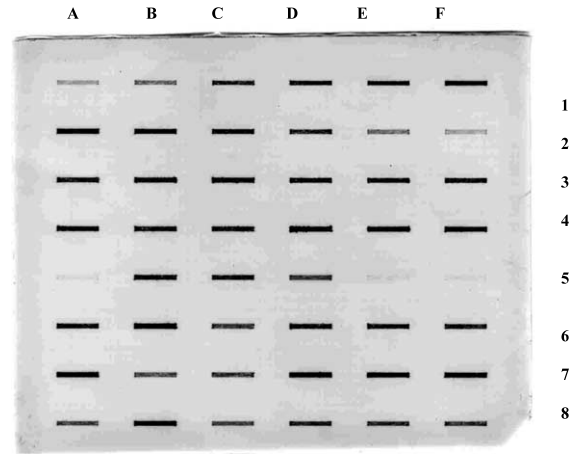


Fig. 2. Representative slot blot of soluble proteins extracted from eastern oyster, *Crassostrea virginica*, gill tissue. Rows 1 and 2 show “reference sample” serial dilution loaded from opposite directions with 0.25, 0.50, 1.00, 1.50, 2.00 and 2.50 µg total protein. The remaining wells were randomly loaded with 1.50 µg protein of samples exposed to 1.00, 1.50 or 2.00 g SFCS. The slot blot technique measures constitutive and inducible levels of total HSP70 in samples.

against the area of the 1.50 µg dilution from the dilution series loaded in each blot. Arbitrary units of HSP70, expressed as units Hsp70, were defined as the normalized values divided by 1.50.

$$\text{Normalized}_{\text{area}} = \frac{\text{Sample}_{\text{area}}}{\text{Reference}_{\text{area}}}$$

$$\text{Units Hsp70} = \frac{\text{Normalized}_{\text{area}}}{1.50}$$

4. Condition index

The condition index (CI) is a measure of physiological condition of the organism. CI was calculated as tissue dry weight divided by shell dry weight multiplied by 100 (Lucas and Beninger, 1985). Results are expressed as the mean and S.D.

5. Bioaccumulation

Organic pollutant burdens in the oysters were determined by freeze drying the samples prior to

analysis. Surrogate standards containing d_8 -naphthalene, d_{10} -fluorene and 1-1' binaphthyl were added. Samples were extracted with dichloromethane at 100 °C and 1500 psi for 10 min in an accelerated solvent extractor (Dionix ASE 200). Extract purification and gas chromatographic analysis procedures have been described previously (Chu and Hale, 1994). Interfering biogenic material was removed by fractionation using solid phase extraction (SPE) column chromatography (2 g silica 100–200 mesh) sequentially eluted with hexane, hexane:dichloromethane (40:60) and dichloromethane:acetone (25:75). The PAHs were recovered in the hexane:dichloromethane fraction. Purified extracts were analyzed by capillary chromatography (DB-5 column, 60m) with flame ionization detection for PAHs. An internal standard (*p*-terphenyl) was added to the extracts immediately prior to GC analysis for quantitation. GC/MS was used for authentication of analyzed peaks. Recoveries of the surrogate standards were typically greater than 77% for high molecular weight PAHs. Results are presented as the mean and SD.

6. Statistical analysis

When necessary, logarithmic transformation of the data was carried out to comply with normality and equality of variances requirements. One-way ANOVA was used to test for differences as a function of treatment in stress protein expression in experiment # 1. Two-way ANOVA was used to test for differences in HSP70 expression between treatments and over length of exposure in experiment # 2 (SAS Institute, Cary, NC). Tukey-HSD test was used to compare means when ANOVA was significant ($P < 0.05$). Results are expressed as mean and 95% confidence interval.

7. Results

7.1. Experiment 1: HSP70 response in oysters exposed to PAHs sorbed to suspended clay particles

Oysters exposed for 40 days to 1.0, 1.5 or 2.0 g

suspended clay particles containing PAHs (fluoranthene, pyrene, benzo(a)pyrene and benzo(e)pyrene) showed a statistically significant increase in the HSP70 levels compared to controls (Fig. 3). However, no dose dependency was noted. Exposing oysters to 1.0, 1.5 or 2.0 g suspended clay particles alone (controls) did not cause significant increases in HSP70 levels compared to oysters not exposed to suspended clay particles (0 g clay particle, Fig. 3). Oysters exposed to the highest dose of suspended PAH-contaminated particles showed a four-fold increase in the accumulated PAHs compared to the lowest dose (17.2 μg PAH/g tissue DW and 69.0 μg PAH/g tissue DW, respectively) (Fig. 4). PAHs were not detected in control oysters (Fig. 4). The bioaccumulation of PAHs did not appear to directly correlate to the stress response in a dose-dependent nature. There was no mortality in the exposed oysters. No statistically significant difference was observed in the CI between oysters

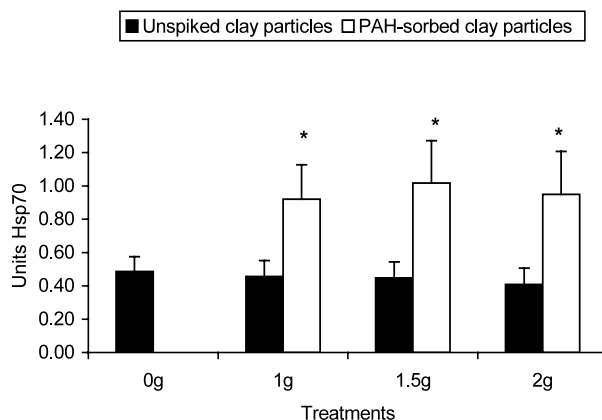


Fig. 3. HSP70 response in oysters *Crassostrea virginica*, after 40 days exposure to suspended unspiked clay particles (0, 1.0, 1.5, or 2.0 g daily), or to suspended PAH-sorbed clay particles (1.0, 1.5 or 2.0 g daily, corresponding to 65.6, 238.5, 484.0 μg PAHs, respectively). No significant differences were observed between treatments for oysters exposed to suspended unspiked clay particles (Mean and CI, $N = 9-11$). Oysters exposed to suspended PAH-sorbed clay particles show a significant increase in HSP70 levels compared to controls. However no dose dependency is observed (mean and CI, $N = 8$). CI = 95% confidence interval. (*) Asterisk denotes significantly different ($P < 0.05$).

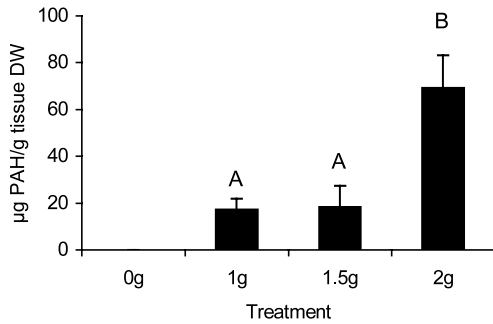


Fig. 4. PAH bioaccumulation ($\mu\text{g PAH/g tissue dry weight}$) in oyster gill tissue after 40 days exposure to suspended PAH-sorbed clay particles. Oysters show a dose related increase in accumulated PAHs (mean and SD, $N = 3$). Bars with different letters denote significant difference ($P < 0.05$).

exposed to different amounts of suspended clay particles compared to the controls. No significant difference in CI was observed between controls and suspended clay particles spiked with PAHs. *P. marinus* infection was not detected in any of the experimental oysters.

7.2. Experiment 2: HSP70 response in oysters exposed to suspended field contaminated sediments (SFCS)

Exposure to SFCS resulted in a general increase in total HSP70 levels ($P < 0.001$) in oysters in all treatment groups. Oysters exposed to 0 g SFCS did not show statistically significant changes in HSP70 levels (Fig. 5a). Treatment groups receiving SFCS showed increases in HSP70 levels over time, but these were not strictly linear in each case. Oysters exposed to 1 g SFCS showed a minor increase after 20 days, with a subsequent significant increase after 40 days (Fig. 5b). Oysters exposed to 1.5 g SFCS showed a sustained increase after 20 days (Fig. 5c). Oysters exposed to 2 g SFCS showed fluctuations in the levels with a series of increases and decreases, and an eventual significant increase after 40 days (Fig. 5d). The oysters in this group reached the highest levels of HSP70 expression of any treatment group (Fig. 5b,c,d).

HSP70 levels remained high in all the exposed oysters after 40 days. Compared to the 0 g SFCS,

the HSP70 levels in oysters exposed to 1.0, 1.5 and 2.0 g SFCS were statistically significantly higher. Even when a treatment effect was observed ($P = 0.006$), no dose dependency was present. There were no mortalities in the exposed oysters. Oysters exposed to SFCS showed no statistical significant difference in CI between treatment and control groups. *P. marinus* infections were not detected in any of the oysters. Analysis of PAHs revealed concentrations of 0, 1.45, 1.49, 2.46 mg PAHs/kg wet tissues in 0, 1.0, 1.5, and 2.0 g SFCS after 40 days in exposed oysters (Chu et al., 2002).

8. Discussion

Bioavailability of sediment or particle-associated contaminants plays a decisive role in toxicity. Contaminants bound to suspended particles have been shown to be bioavailable (Björk and Gilek, 1996; Pollet and Bendell-Young, 1999) and to pose potential adverse effects to filter feeders (Hermsen et al., 1994; Weltens et al., 2000) and other aquatic organisms (Van Brummelen and Stuijzand, 1993; Farag et al., 1998). Pollet and Bendell-Young (1999) showed Cd^{2+} accumulation in the mussel, *M. trossulus*, exposed for 4 h to spiked suspended particulate matter. Björk and Gilek (1996) showed phenanthrene uptake in the blue mussel, *M. edulis*, from ingested suspended particulate organic matter concurrent with decreases in body CI.

Few studies have tested the effects of contaminants associated with suspended particles on organisms (Van Brummelen and Stuijzand, 1993; Hermsen et al., 1994; Van den Belt et al., 1999; Peeters et al., 2000; Weltens et al., 2000). The effects of contaminants sorbed to suspended particles on organisms have been reported using indicators at the organismal level such as CI, feeding rate, survival and growth. Van Brummelen and Stuijzand (1993) showed reductions in available energy for growth in terrestrial isopods, *Onniscus asellus* and *Porcellio scaber*, exposed to benzo(a)pyrene. Peeters et al. (2000) showed decreased growth rate in water louse, *Asellus aquaticus* exposed to suspended sediments spiked

with B(a)P. Hermesen et al. (1994) reported that sediments spiked with lindane reduced feeding rates in the mussel *M. edulis*. However, indicators at the molecular level such as the stress proteins, have been proposed as more sensitive indicators of sublethal exposure to contaminants in the environment than physiological responses such as scope for growth and filtration rates (Sanders et al., 1991; Sanders, 1993; Steinert and Pickwell, 1993).

The effects of contaminants bound to suspended particulate matter may be particularly acute to filter feeders such as oysters. Filter feeding has been a successful strategy, opening vast

food resources to bivalves and many other aquatic organisms. However, this also has exposed these organisms to contaminants via suspended particles in addition to direct uptake from water through body surfaces. Bivalve molluscs are capable of bioaccumulating contaminants to many thousands times background levels making them useful sentinels of chemical contamination (Sheehan and Power, 1999). The present study demonstrated that exposing oysters to either suspended clay particles spiked in the laboratory with PAHs or to SFCS elicited a stress protein response (HSP70). The bioavailability of PAHs associated with suspended clay particles and

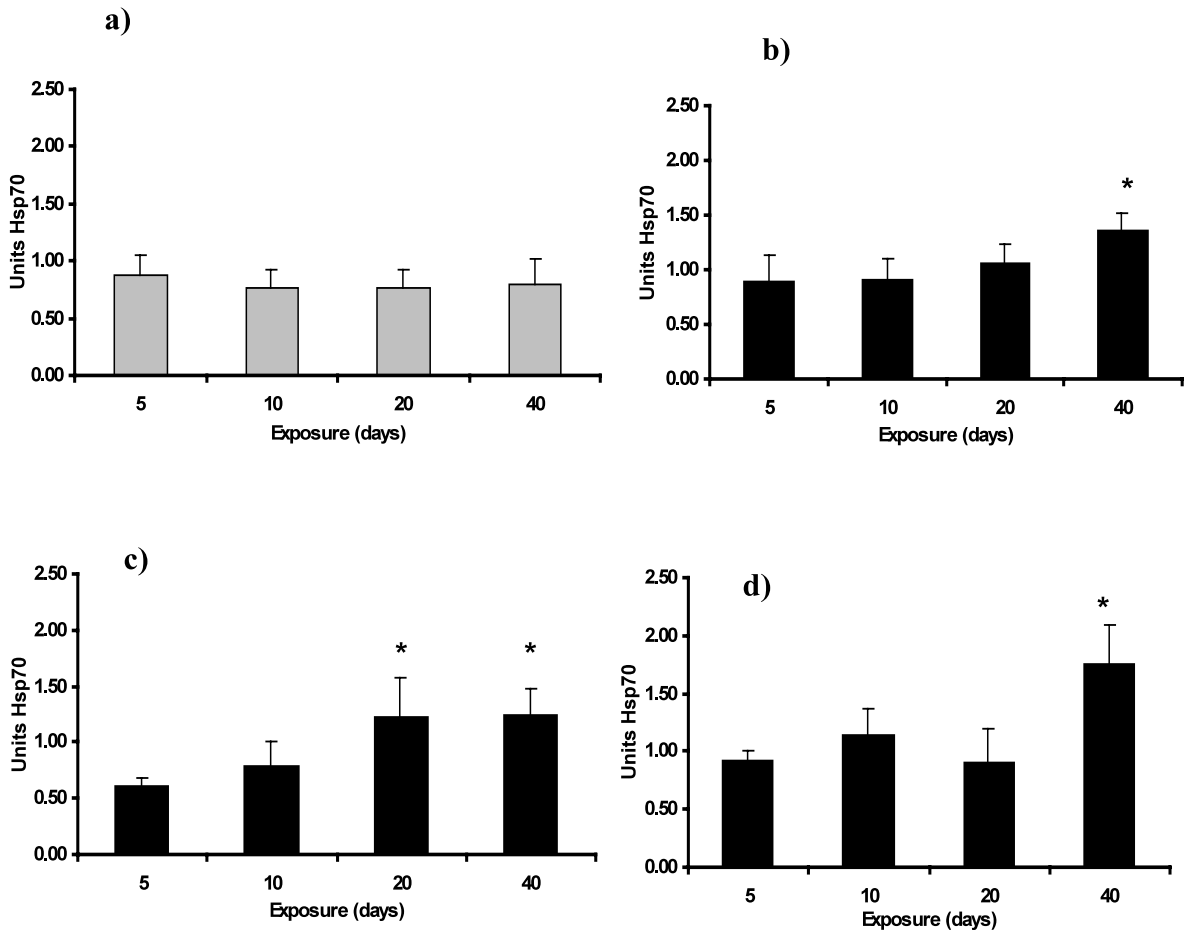


Fig. 5. HSP70 response in the eastern oysters, *Crassostrea virginica* exposed to (a) 0 (b) 1.0 (c) 1.5 and (d) 2.0 g SFCS for 5, 10, 20, and 40 days (mean and CI, $N = 7-9$). The asterisk (*) denotes significantly different from day 5 within each treatment ($P < 0.05$, CI = 95% confidence interval).

SFCS is supported by their accumulation in the oyster's tissues. However, the potential adverse effects of sediment-associated contaminants could not be assessed from alterations in the CI as no difference in this indicator was observed between exposed and control oysters.

Although the bioaccumulation of PAHs shows a dose-related increase in the oyster's tissues, no dose dependency in the stress protein (HSP70) response was noted. Stress proteins have been observed to exhibit a fluctuating response with time, dose or both (Theodorakis et al., 1992; Köhler et al., 1999; Lewis et al., 2001). In a study using the terrestrial isopod, *O. asellus*, exposure to soil samples containing hexachlorocyclohexane (δ -HCH), pentachlorophenol (PCP), benzo(a)pyrene and 2,2',5,5'-tetrachlorobiphenyl (PCB52) in soil samples did not produce a dose dependency in the heat-shock protein response (Köhler et al., 1999). These authors showed HSP70 levels fluctuating between induction and suppression in exposed isopods. They speculated that rapid metabolism/excretion of the contaminants via cytochrome P₄₅₀-like enzymes, or similar bioavailable doses in all treatment groups were responsible for the lack of a significant change in stress protein response. Lewis et al. (2001) reported a significant increase in HSP70 levels in the macroalga, *Enteromorpha intestinalis*, exposed up to 200 $\mu\text{g/l}$ Cu^{+2} , but no dose dependency was observed. In studies with the bluegill sunfish, *Lepomis macrochirus*, Theodorakis et al. (1992) reported increases in HSP70 levels for the first 2 weeks followed by decreases below control levels after 16 weeks. Possible explanations proposed included: acclimation (genetic), compensatory mechanisms or adaptation (physiological) to the stress. The apparent upregulation and downregulation of the HSP70 response observed in the SFCS is difficult to explain. The SFCS is a complex mixture of contaminants, whose synergism or antagonism may influence the nature of the stress protein response. Werner et al. (1998) exposed *Ampelisca abdita* to sediments collected from San Francisco Bay. They reported that HSP64 levels were positively correlated to total PAHs, but HSP71 levels were negatively correlated to benzo(b,k)fluoranthene and benzo(g,h,i)perylene. Either of these responses,

upregulation or downregulation, could be considered as a potential indicator of adverse effects in field situations (Werner and Hinton, 1999). Continuing studies investigating complex mixtures of relevant contaminants are necessary to fully understand the stress protein response and its utility as a general biomarker of contamination.

Suspended solids in the water column may exert direct mechanical effects on filter feeders by increasing abrasion, clogging the respiratory surfaces of gills, and/or interfering with feeding mechanisms (Hughes, 1976; Hellowell, 1986). This in turn might reduce the intake of suspended particles. Negative responses (closing of valves) has been noted in oysters exposed to silt concentrations above 1 g/l for periods longer than 48 h, with opening of valves at various time intervals apparently to test the water conditions (Loosanoff and Tommers, 1948; Loosanoff, 1962). However, habitat and species feeding ecology may play a role in the responses of organisms to high inputs of suspended sediment (Peeters et al., 2000). Weltens et al. (2000) reported that exposure to uncontaminated sediments up to 500 mg/l did not cause mortalities in the filter feeder, *Daphnia magna*. Van den Belt et al. (1999) showed no mortalities in the zebra fish, *Danio rerio*, exposed to clay particles up to 2000 mg/l. In the eastern oyster, no harmful effects caused by suspended silt have been reported. Engle (1952), McKinney and Case (1973) did not observe apparent detrimental effects in oysters suspended in baskets next to dredging operations. Makin and Hopkins (1961) showed that turbidities up to 0.7 g/l did not cause apparent harmful effects on oysters. Similarly, the amounts of suspended clay particles used in the present study did not appear to produce any negative impact on the exposed oysters since no changes were noted in either HSP70 levels or condition indices in oysters exposed to 0–2 g suspended unspiked clay particles. In addition, no mortalities in the oysters exposed to suspended unspiked clay particles were recorded. However, enhanced HSP70 expression was noted in oysters exposed to suspended clay particles sorbed with PAHs and SFCS. Exposure to SFCS was also found to elevate *P. marinus* disease expression and modulation of cellular responses in

oysters (Chu, et al., 2002). Thus, it is believed that the increased levels in HSP70 response is due to the uptake of PAHs sorbed to the suspended clay particles and contaminants present in the SFCS. However, while the enhanced HSP70 in oysters exposed to PAH-sorbed particles was apparently a response to the presence of PAHs, the increased HSP70 response in SFCS exposed oysters probably reflects a response to the combined effect of various contaminants including PAHs, metals and trace amounts of PCBs, rather than PAHs alone.

In the Chesapeake Bay region, declines in populations of the eastern oyster have been observed since the 1950s (Andrews, 1954) with drastic reductions since 1992 (Ragone and Burreson, 1993). These reductions have been attributed to factors such as disease, over harvesting and a decrease in water quality due to pollution (Chu and LaPeyre, 1993; Ragone and Burreson, 1993; Chu and Hale, 1994; Burreson and Ragone-Calvo, 1996; Anderson et al., 1996; Fisher et al., 1999). The results from the present study indicate that contaminants associated to suspended sediments are stressful to filter feeders such as the eastern oyster at sublethal concentrations. Increases in HSP70 levels in response to the presence of contaminants associated to suspended sediments were clearly demonstrated. The resuspension of sediments could continually expose filter feeders to contaminants long after these compounds are clear from the water column. Thus, water quality must be a concern particularly to filter feeders as shown by the stress response exhibited in the oyster (i.e. increases in HSP70 levels).

The HSP70 response seems to be a sensitive indicator of toxicity to contaminants sorbed to suspended sediments to benthic filter feeders such as oysters. In the present studies, while oysters did not show changes in CI or mortalities, a stress response was detected by the stress protein analysis. The stress protein response appears to be a potentially useful marker, in combination with a suite of other biomarkers, in aquatic toxicological studies.

In summary, results from the present studies exposing oysters to artificial suspended clay particles, contaminants sorbed to suspended clay particles or SFCS give a clear indication of a possible

sublethal toxic effect caused by the presence of suspended contaminated sediments in the water column. To our knowledge, this is the first report on HSP70 response as a measure of a stressful condition in filter feeders exposed to contaminants associated to suspended sediments and clay particles. Because of the bioaccumulation potential of bivalve filter feeders, contaminants could be magnified up trophic levels posing serious consequences for top consumers and human health. The contribution of suspended contaminated particles must be taken into consideration when evaluating polluted waters. Baseline studies are also needed to assess natural variations in the HSP70 response, as well as the mechanisms involved in the stress protein response to various contaminants (i.e. complex mixture of contaminants).

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