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Molluscan Shellfish Safety

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PREFACE

This volume includes a number of presentations at the 4th International Conference on Molluscan Shellfish Safety, which was held in Santiago de Compostela (Galicia, Spain), in June 4 - 8, 2002, and hosted by the Centro de Investigacións Mariñas and the Centro de Control do Medio Mariño, both depending on the "Consellería de Pesca e Asuntos Marítimos da Xunta de Galicia" (Ministry of Fisheries and Maritime Affairs of the Autonomous Government of Galicia). The volume offers a close view of the scientific programme of the Conference, the main objective of which was to establish a forum where useful, enriching debate and exchange of knowledge flowed easily on a broad topic spectrum related to Molluscan Shellfish Safety. The goal was achieved and this volume transmits much of the information handled at the Conference.

Food involves a wide variety of risks for human health. Consistently, involvement of different scientific disciplines is needed to assure food safety. This is also true for molluscan shellfish and very frequently information is too dispersed. The primary objective of this volume, obviously connected with that conference series, is to bring together disperse information available in 2002 and to offer a general scope that serves as a reference. The volume includes information related to phycotoxins, microbiological (viruses, bacteria and protozoa) and chemical contamination, and allergens in molluscan shellfish as matters of public health.

All the manuscripts submitted to be included in this volume have been reviewed by specialists whose opinions have been decisive both for accepting and editing each contribution. We are grateful to them for their generous, independent, and thorough work. This has been an inestimable help. Sponsors listed below furnished financial support and infrastructure for scientific and social events during the Conference as well as for the attendance of scientists and students from countries in delicate economic position. The "Consellería de Pesca e Asuntos Marítimos da Xunta de Galicia" and CAIXANOVA have funded the publication of this volume. Co-publication by the Intergovernmental Oceanographic Commission of UNESCO will ensure that the volume is freely distributed to developing countries and with economies in transition. We deeply thank their sponsorship.

Antonio Villalba, Beatriz Reguera, Jesús L. Romalde, Ricardo Beiras

HSP70 levels in oyster *Crassostrea virginica* exposed to cadmium sorbed to algal food and suspended clay particles

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ABSTRACT

Three experiments were conducted to determine whether or not a HSP70 response is elicited in the oyster gill tissues by the exposure of cadmium (Cd) sorbed to algal food or suspended clay particles. The Cd concentrations in exposed and control oyster gill tissues were determined. In experiment 1, oysters were exposed daily to Cd sorbed to algal paste (Cd-sorbed algal paste, 10.96 or 23.83 ppb Cd per oyster). In experiment 2, oysters were exposed daily to Cd sorbed to suspended clay particles (Cd-sorbed clay particles, 15.69 or 33.58 ppb Cd per oyster). In experiment 3, oysters were exposed daily to Cd-sorbed to algal paste (17.27 or 35.65 ppb Cd per oyster) or suspended clay particles (14.57 or 29.34 ppb Cd per oyster). The exposure time for all three experiments was 40 days. A significant increase in mean HSP70 was found in oysters exposed to Cd-sorbed algal paste in experiment 1, but dose-dependent relationship was not observed. No significance difference in HSP 70 levels was noted between control oysters and oysters exposed to either Cd-sorbed clay particles or Cd-sorbed algal paste in experiments 2 and 3. Compared to the controls, while a significantly higher Cd concentration was found in the gills of oysters exposed to 23.83 ppb, there was no significant increase in gill Cd concentration in oysters exposed to 10.96 ppb in experiment 1. In experiment 2, significantly higher levels of gill Cd were present in oysters exposed to 15.69 ppb and 33.58 ppb than in the controls. In experiment 3, oysters exposed to 35.65 ppb Cd-sorbed algal paste showed a significant increase in Cd concentration compared to controls, but not those exposed to 17.27 ppb. Oysters exposed to Cd-sorbed suspended clay particles also showed a significant increase in gill Cd at 14.57 ppb and 29.34 ppb. Although generally increased Cd concentrations were noted in Cd-exposed oysters, they seem to have a high tolerance for Cd toxicity without eliciting a stress protein response.

KEY WORDS: *Crassostrea virginica*, oyster, heat shock proteins, Cadmium, bioaccumulation

INTRODUCTION

In the Chesapeake Bay, marked declines in oyster harvests have been experienced since the 1950's (Andrews 1954) with particularly severe reductions in the 1990's (Burreson & Ragone 1996). Factors that may contribute include disease, over harvesting and degradation in water quality (Burreson & Ragone-Calvo 1996, Chu & Hale 1994). Oysters are sedentary filter feeders and play an important role in the ecosystem. They filter water trapping suspended matter and in the process accumulate contaminants present in the suspended matter. Resuspension of particles and sediments back into the water column might expose oysters and other filter feeding bivalves to contaminants long after they are not present in the water. Thus, filter feeders such as oysters are exposed to contaminants not only through the water but also via filtrated and/or ingested matter, affecting bioaccumulation up the trophic levels and posing a health concern if consumed by humans.

Marine organisms, including bivalves, are known for their capacity to accumulate heavy metals (Fowler 1979, Janssen & Scholz 1979, Wikfors et al. 1994) and the accumulated metals can affect negatively the organisms (Shuster & Pringle 1969, Zaroogian & Morrison 1981). Cadmium, a known pollutant of estuaries, is highly toxic and accumulates to high levels in bivalves (Carmichael & Fowler 1981, Roesijadi & Unger 1993). Since many bivalves are sedentary and accumulate metals, they are considered good indicators of metal pollution in the environment (Zaroogian 1980, Farrington et al. 1983, Landrum et al. 1991). Cadmium concentrations greater than 15 ppb have been considered higher than concentrations encountered in chronically stressed natural waters (Zaroogian 1980) and exposure to 40 – 60 ppb Cd is considered to represent areas heavily contaminated (Hung 1982).

Heavy metals associated to suspended matter can enter the food web accumulating in the organisms' tissues and have adverse effects on bivalve filter feeders such as the oyster. Accumulation of Cd was noted in mussels, (*Mytilus edulis*) fed algae (*Dunaliella marina*) contaminated with 100 ppb Cd (Janssen & Scholz 1979). Algae reportedly can accumulate up to 70% of the Cd present in solution (Janssen & Scholz 1979) and oysters (*C.* virginica) can assimilate > 80% of the metal associated to the cytosolic fraction and 36% of the metal associated to membranes of ingested algae (Reinfelder et al. 1997). Larvae of *C. virginica* fed algae (*Isochrysis galbana*) adapted to grow in media containing 15.3 ppm Cd, had higher mortality than larvae exposed to 27 ppb Cd dissolved directly into the water (Wikfors & Ukeles, 1982). Cadmium can also adsorb onto inorganic matter becoming available and possibly toxic to filter feeders. Pollet & Bendell-Young (1999) reported that mussels (*M. trossulus*) exposed to 12.5 ppb Cd sorbed to suspend sediments accumulated more Cd from suspended sediments than from solution in water. Exposing *D. magna* to 90 ppb or 60 ppb Cd sorbed to clay or sand respectively, caused greater than 50% mortality in 48 hours (Weltens et al. 2000).

The stress protein response has been proposed as a general indicator of exposure to stress (Sanders 1993). Stress proteins are synthesized at higher levels when cells are challenged with environmental stimuli such as high temperature, heavy metals or toxic chemicals, making them a potentially useful marker of exposure (Morimoto 1993, Kothary & Candido 1982, Sanders, 1993). HSP70

accounts for much of the translational activity in cells responding to environmental stress and members of this family of proteins are inducible and highly conserved among various phyla (Margulis et al. 1989 Welch 1990, Abukhalaf et al. 1994). The effects of metal exposure on the HSP70 have been tested in aquatic organisms exposed to contaminated water (Veldhuizen-Tsoerkan et al. 1990, 1991, Ryan & Hightower 1994, Sanders & Martin 1994, Werner & Nagel 1997, Tedengren et al. 2000) or settled sediments (Werner et al. 1998, Werner & Hinton 1999). However, these studies have produced mixed results showing increases in the stress protein response (Ryan & Hightower 1994), minimum changes (Tedengren et al. 2000), no change (Veldhuizen-Tsoerkan et al. 1991) or upregulation and downregulation of the stress protein response (Werner & Nagel 1997, Werner & Hinton 1999).

The use of stress proteins is predicated on being more sensitive to sublethal exposure to contaminants than other end points such as scope for growth, feeding and respiration rates (Sanders 1993, Steinert & Pickwell 1993). The present study was conducted to determine whether a HSP70 response is elicited in the eastern oyster, *C. virginica*, exposed to sublethal concentrations (15 – 30 ppb) of Cd sorbed to algal food or suspended clay particles. To our best knowledge, no study has investigated the stress protein response in filter feeders exposed to sublethal levels of metals sorbed to suspended matter.

METHODS AND MATERIALS

Preparation of clay particles

Clay particles for the experiments were prepared by pulverizing green shale (Illite 46E0315, Wards/Cenco, Rochester, New York) to an average size of 50 µm. The clay particles were then hydrated in 1 µm filtered York River water (YRW) and stored at 4^0C until use.

Algal paste

Algal paste, mixed in a 50:50 ratio of *Tretaselmis* sp. and *Isochrysis* sp., was purchased from Reed Mariculture Inc. (San José, CA). This algal mix was used as a diet and as a Cd exposure vehicle for the experimental oysters.

Preparation of Cd-contaminated algal Paste and Cd-contaminated suspended clay particles

The effects of algal paste or suspended clay particles spiked with Cd on the stress protein response were tested. A Cd ($CdCl_2$) stock solution was prepared to a final nominal concentration of 1 ppm (actual concentration 0.94 ppm) in water. Cd sorbed to algae (Cd-sorbed algal paste) was prepared each day by adding stock solution to algal paste to obtain nominal concentrations of 15 and 25 ppb Cd. Cd sorbed to clay particles (Cd-sorbed clay particles) was prepared by adding stock solution to hydrated clay particles to obtain nominal concentrations of 15 ppb Cd (1g clay particles), and 25 or 30 ppb Cd (2g clay particles). The actual Cd concentrations in the Cd-sorbed algal paste and Cd-sorbed clay particles were subsequently determined. All oysters including controls were fed 0.20 g algal paste / oyster daily. All treatments whether algal mix or clay particle suspensions were

prepared by stirring separately the material for treatment groups and control groups into YRW prior to use.

Oysters

Oysters collected from the Damariscotta River, Maine, an area rarely infected by the protozoan parasite, *Perkinsus marinus*, were used in all the following described experiments. The effects of parasitic infection on the HSP70 response on invertebrate hosts are not known. To avoid potential confounding effects due to the presence of the parasite, oysters were selected from a region with rare incidence of *P. marinus*. Oysters for the experiments 1, 2 and 3 were collected in summer (August) 1999, winter (February) 2000 and spring (March) 2001 respectively.

In all experiments, oysters were acclimated to local conditions (York River water salinity = 12-18 ppt; temperatures = 19ºC – 21ºC) over fourteen days in two 600 L tanks. After acclimation, subsamples of oysters (n=10) were examined for *P. marinus* infection. At the end of each experiment all oysters were also examined for *P. marinus* infection. All oysters tested negative for *P. marinus* infection.

Experiments

Experiment 1: Oysters exposed to Cd-sorbed algal paste. Acclimated oysters were separated into 3 groups. Individual oysters were exposed daily to 15 or 25 ppb (actual concentrations were determined to be 10.96 and 23.83 ppb respectively) Cd-sorbed algal paste for 40 days. The control group was fed unspiked algal paste daily. Oysters were maintained in 2L containers with aeration. Water 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.

Experiment 2: Oysters exposed to Cd-sorbed suspended clay particles. The experimental protocol was similar to the one described above except that individual oysters in treatment groups were exposed daily to 15 or 25 ppb (actual concentrations were determined to be 15.69 and 33.58 ppb, respectively) Cd-sorbed clay particles. After 40 days exposure, oysters were analyzed for HSP70. Our previous study (Cruz-Rodríguez & Chu 2002) indicated that exposure up to 2 g suspended clay particles alone did not affect the HSP70 response. Oysters were kept in individual 2L containers with aeration and fed 0.2 g unspiked algal paste daily. The aeration kept the clay particles in suspension. 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.

Experiment 3: Concurrent exposures of oysters to Cd-sorbed algal paste and Cd-sorbed suspended clay particles. This experiment was carried out to expose parallel groups of oysters to Cd- sorbed algal paste or Cd-sorbed suspended clay particles. Acclimated oysters were separated into 5 groups, four treatment and one control groups. Two of the four treatments groups were exposed daily to 15 or 30ppb (actual concentrations were determined to be17.27 and 35.65 ppb, respectively) Cd-sorbed algal paste/oyster and the other two were exposed daily to 15 or 30 ppb (actual concentrations determined to be 14.57 and 29.34 ppb, respectively) Cd-sorbed suspended clay particles/oyster. The control group was fed

unspiked algal paste. All treatments were continued for 40 days after which gills were sampled for determination of HSP70 levels.

Heat-shock protein analysis

HSP70 in gill tissues was assessed by slot blot. This technique has been used previously in HSP70 determinations comparing fishes from contaminated and relatively clean sites in southern California (Brown & Bay 1999), oysters exposed to algae contaminated with PCBs (Cruz Rodríguez et al. 2000) and a macroalga exposed to environmental stressors (Lewis et al. 2001).

Oyster gill tissues were homogenized using a hand held blender (Ultraturrax T-25 Homogenizer) at 24,000 rpm for 30 seconds 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 x *g* on a fixed angle rotor for 30 minutes at 4ºC and the supernatant collected. Total protein concentration was determined using Biorad DC Protein Assay (Lowry et al. 1951).

The blotting procedures consisted of 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) (Cruz Rodríguez & Chu, 2002) 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 minutes, followed by two washes in TBS (500 mM NaCl, 15 mM Tris, pH 7.5) for 10 minutes. 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 (MA3-006, Affinity Bioreagents Inc., Golden, CO, USA) was applied for 90 minutes, followed by one wash with TTBS and two washes with TBS for 10 minutes each. The secondary antibody (Goat anti-mouse AP-conjugated) was applied for 90 minutes. Subsequently, the blot was washed twice with TBS for 10 minutes, 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 minutes and development was completed after three hours. The blot was then stored in deionized water until densitometric analysis.

Densitometric analysis was performed by scanning the blots using SepraScan software (ISS Enprotech, MA, USA). The areas of the samples were recorded and each sample area normalized against the area of the 1.5 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 1.5.

$$\text{Normalized}_{area} = \frac{\text{Sample}_{area}}{\text{Reference}_{area}} \qquad \text{Units Hsp70} = \frac{\text{Normalized}_{area}}{1.5}$$

Cadmium analysis

Samples were freeze-dried and weighted. Cadmium extractions were performed in a microwave accelerated reaction system (MARS 5, CEM corporation, NC, USA). Samples were placed into reaction vessels with 10mL ultra pure nitric acid. Digestion took place at 200ºC and 100 psi for 1 hour in a closed vessel digestion system followed by a cool down period over two hours.

After extraction samples were brought to 25mL final volume in water and analyzed using a PerkinElmer atomic absorption spectrophotometer model AA800 with Zeeman correction in a graphite furnace (GFAA). The wavelength was 228.8 nm, 0.7 slit width. The injector temperature was 20ºC, initial furnace temperature of 110ºC with maximum of 2450ºC and internal gas flow of 250mL/min. Data were collected and quantified using PerkinElmer AAWinLab software.

Statistical analysis

When necessary, logarithmic transformation of the data was carried out to comply with normality and homogeneity of variance requirements. One-way ANOVA was used to test for differences as a function of treatment in stress protein expression (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.

RESULTS

HSP70 levels

In experiment 1 oysters exposed to Cd-sorbed algal past showed a significant increase in mean HSP70 (0.48 ± 0.12 and 0.50 ± 0.10 Units in 10.96 ppb and 23.83 ppb respectively) compared to the controls (0.31 ± 0.06 Units) (Fig. 1A, ANOVA $F=11.54$, $p<0.001$), but a dose-dependent relationship was not observed.

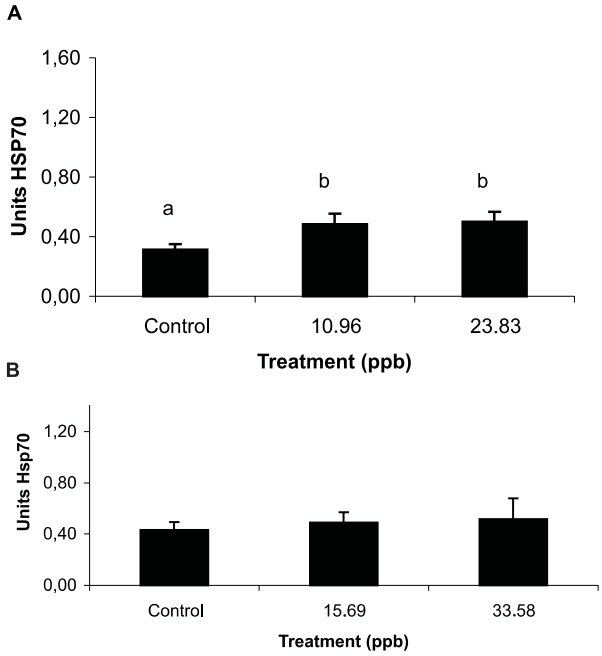

Fig. 1. HSP70 levels (Mean ± 95% confidence interval, n=8-10) in gill tissues of oysters exposed to Cd-sorbed algal paste (**A**, Experiment 1) or Cd-sorbed suspended clay particles (**B**, Experiment 2). In experiment 1, individual oysters were exposed daily to 10.96 ppb and 23.83 ppb Cd-sorbed algal paste. In experiment 2, individual oysters were exposed daily to 15.69 ppb and 33.58 ppb Cd-sorbed clay particles. Different letters represent significant difference ($p<0.05$).

In experiment 2 there was an increase in mean HSP70 levels in oysters exposed to Cd-sorbed suspended clay particles (0.49 ± 0.14 and 0.55 ± 0.26 Units in 15.69 ppb and 33.58 ppb, respectively), but was not statistically significant, compared to controls (0.43 ± 0.09 Units) (Fig. 1B). In both experiment 1 and 2, Cd-sorbed algal paste or Cd-sorbed suspended clay particles, the mean HSP70 of exposed groups reached similar levels (Fig. 1A and B). The HSP70 of the control oysters in experiment 2 was higher than the control oysters for experiment 1 (0.43 ± 0.09 and 0.31 ± 0.06 Units respectively).

In experiment 3, the mean HSP70 levels in the Cd-sorbed algae exposed groups (Fig. 2A) were slightly higher (0.96 ± 0.46 and 1.04 ± 0.54 Units in 17.27 ppb and 35.65 ppb, respectively) than those exposed to suspended clay particles (Fig. 2B) (0.84 ± 0.26 and 0.93 ± 0.24 Units in 14.57 ppb and 29.34 ppb respectively). Although there was an increase in the mean HSP70 levels with treatments, the increase was not statistically significant, compared to controls (0.78 ± 0.12 Units) (Figs. 2A and B).

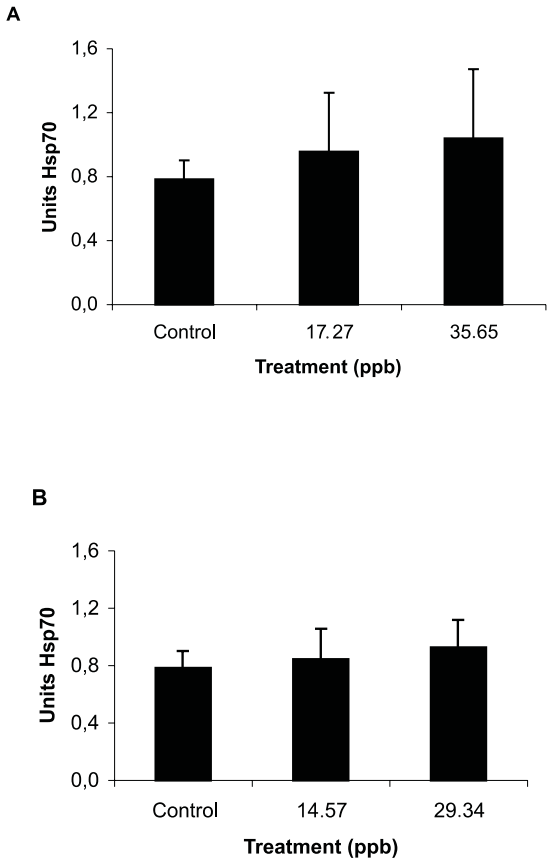

Fig. 2. HSP70 levels (Mean ± 95% confidence interval, n=8-10) in gill tissues of oysters in Experiment. 3. Individual oysters were exposed daily to Cd-sorbed algal paste (**A,** 17.27 ppb and 35.65 ppb per oyster, respectively) or to Cd-sorbed suspended clay particles (**B,** 14.57 ppb and 29.34 ppb per oyster, respectively).

Cd accumulation

There was a significant increase ($P<0.05$) in Cd concentration in gills (6.18 ppm ± 1.07) of oysters exposed to 23.83 ppb, compared to controls (3.18 ppm ± 0.93) in experiment 1. Cadmium concentration (3.33 ppm ± 0.99) in oysters exposed to 10.96 ppb was not different from controls (Fig. 3A).

In experiment 2, oysters exposed to Cd-sorbed suspended clay particles had a significant higher Cd concentration in gill tissue (26.43 ppm ± 10.10 and 17.67 ppm ± 6.15) than control oysters (6.25ppm ± 1.47) (Fig. 3B). The concentration of Cd in the oysters exposed to 33.58 ppb Cd-sorbed clay particles showed lower values compared to oysters exposed to 15.69 ppb, although this is not statistically significant (Fig. 3B).

In experiment 3, oysters exposed to 35.65 ppb Cd-sorbed algal paste (Fig. 4A) showed a significant increase in Cd concentration (10.37 ppm ± 1.03, $P<0.05$), compared to controls (5.68 ppm ± 1.15). But, there was no difference between oysters exposed to 17.27 ppb Cd (7.37 ppm ± 0.99) and controls (Fig. 4A). Oysters exposed to 14.57 ppb Cd-sorbed suspended clay particles showed a significantly higher Cd concentration (14.30 ppm ± 3.95, $P<0.05$) in the gills than controls (Fig. 4B). However, oysters exposed to 29.34 ppb (7.79 ppm ± 2.20) were not significantly different from controls (Fig. 4B).

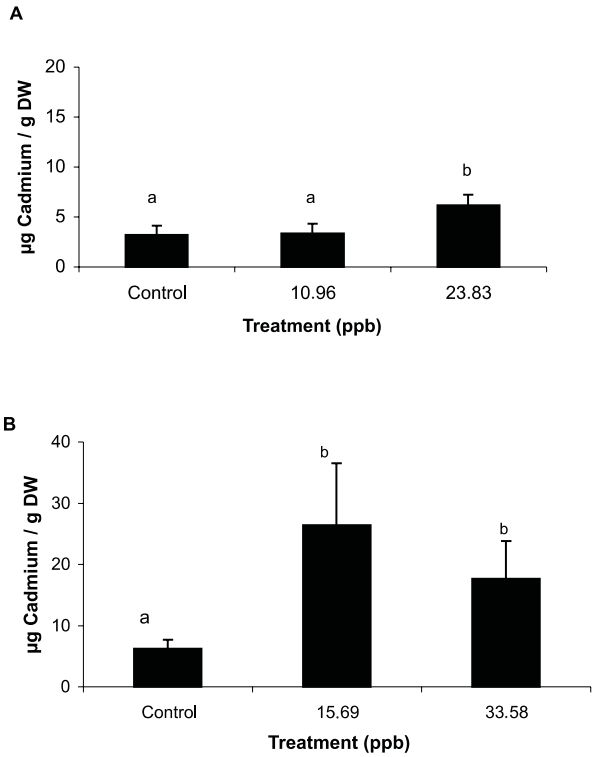

Fig. 3. Cadmium concentration (Mean ± SD, n=6) in gill tissues of oysters exposed daily to Cd-sorbed algal paste (**A**, Experiment 1; 10.96 ppb and 23.83 ppb per oyster, respectively) or Cd-sorbed suspended clay particles (**B,** Experiment 2; 15.69 ppb and 33.58 ppb per oyster, respectively). Different letters represent significant difference ($p<0.05$).

The comparatively lower Cd concentrations in oysters exposed to the highest dose of suspended contaminated clay particle was observed in both experiments 2 and 3. In all of the three experiments, control oysters showed cadmium present in their tissues with values between 3.18 ppm ± 0.93 and 6.25 ppm ± 1.47 (Figs. 3 and 4).

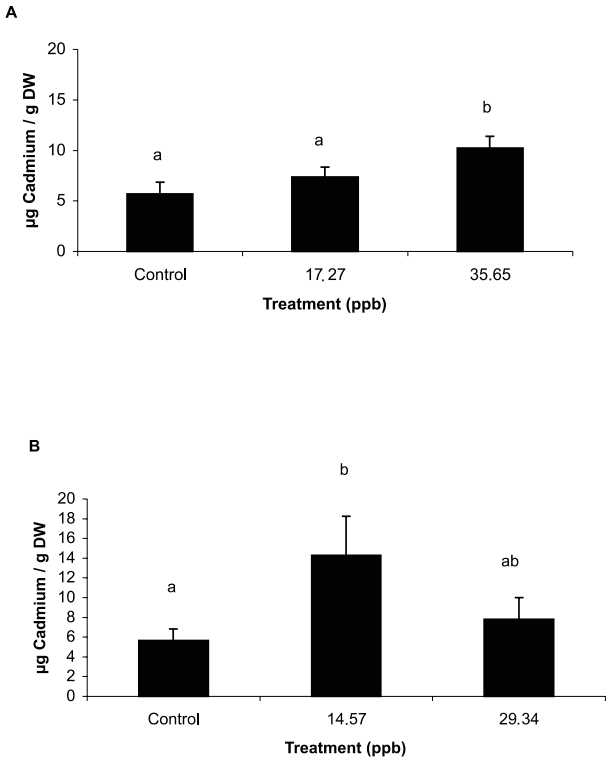

Fig. 4. Cadmium concentration (Mean ± SD, n=6) in gill tissues of oysters in Experiment 3. Individual oysters were exposed daily to Cd-sorbed algal paste (**A,** 17.27 ppb and 35.65 ppb per oyster, respectively) or Cd-sorbed suspended clay particles (**B,** 14.57 ppb and 29.34 ppb per oyster, respectively). Different letters represent significant difference ($p<0.05$).

DISCUSSION

The use of stress proteins as biomarkers has been proposed on the premise that they are more sensitive than other biomarkers (Sanders 1993, Steinert & Pickwell 1993, Ryan & Hightower 1994). The stress protein response in bivalves has been described following exposure to contaminants in solution (e.g., Randall et al. 1989, Veldhuizen-Tsoerkan et al. 1990, 1991, Sanders & Martin 1994) or associated to settled sediments (Werner & Hinton 1999). Sanders and Martin (1994) demonstrated increases in chaperonin 60 (Cpn60) and HSP70 in mussels exposed to Cu^{2+}. Exposing eastern oysters to suspended clay particles spiked in the laboratory with PAHs or to suspended field contaminated sediments (SFCS) elicited a HSP70 response (Cruz-Rodríguez & Chu, 2002). In the present study, however, clear changes in HSP70 levels in oysters exposed to Cd-sorbed suspended matter (i.e. algae or clay particles) were not observed in experiments 2 and 3. It is possible that the concentrations used might not have been high enough to induce a stress protein response. In the Asian clam, *Potamocorbula amurensis*, exposure to Cd concentrations up to 40 ppb in the water

did not elicit changes in HSP70 levels (Dr. Inge Werner, University of California, Davis, personal communication). However, in the present study a pattern of increase in mean HSP70 with exposure and a significant increase in HSP70 in oysters exposed to Cd-sorbed algal paste in experiment 1 suggest possible and potential effects.

Despite the effects demonstrated by heavy metals reported in the literature, bivalves seem capable of coping with exposure and accumulation of trace metals. Shuster and Pringle (1969) and Engel (1999) presented data indicating death occurring only at elevated concentrations (>100 ppm wet weight) in the tissues of oysters exposed to Cd in the water. Exposure to Cd concentrations of 10, 20, and 30 ppm in solution for 6 weeks resulted in accumulation of 165, 220 and 445 ppm respectively with only reductions in body weight as implication of physiological stress in the freshwater bivalve, *Lamellidens marginalis* (Jana & Das, 1997). Long-term exposure (11 months) to 16.5 ppb Cd dissolved in water did not cause changes in HSP70 levels, condition index and adenylate energy charge in mussels (*M. edulis*) (Veldhuizen-Tsoerkan et al. 1990, 1991). Similarly, in the present study, with the exception of experiment 1, no change in mean HSP70 levels of oysters exposed to Cd-sorbed suspended matter was observed.

The presence of the metal binding proteins, metallothionein (Mt) or metallothionein-like proteins and specialized cells (brown cells, amebocytes) might help explain the tolerance of oysters and other bivalve filter feeders to metal toxicity. A series of studies by Ruddell and Rains (1975), Carmichael and Fowler (1981), Zaroogian and Yevich (1994), and Engel (1999) have provided evidence of a multiple component system to cope with metal toxicity in bivalves.

Cadmium, a known pollutant in the marine environment, is accumulated by bivalve filter feeders (e.g., Janssen & Scholz 1979, Zaroogian 1980, Barak et al. 1999, Tedengren et al. 2000). Zaroogian (1980) reported accumulation up to 292 ppm Cd in adult oysters (*C. virginica*) exposed to 5, 10 and 15 ppb Cd for 40 weeks in flowing seawater. Barak et al (1999) described accumulation of trace metals including Cd in three bivalve species (*Mactra corallina*, *Donax* sp and *M. edulis*), and two gastropod species (*Patella* sp and *Cellana rota*), along the Mediterranean, Red and North Seas. Tedengren et al. (2000) have shown uptake up to approximately 24 ppm Cd in mussels (*M. edulis*) exposed to 20 ppb Cd. Janssen and Scholz (1979) showed that algae (*D. marina*) sorbed about 70% of the Cd spiked into the media and metal accumulated in mussels (*M. edulis*) fed the algae. Similarly, in the present study the presence of Cd in oyster gills exposed to the contaminated algal paste and suspended clay particles was observed. However, control oysters showed Cd concentrations between 3.184 – 6.247 ppm in all of the three experiments. These values are similar to those reported by Tedengren, et al. (2000) of 4 – 5 ppm in mussels kept in seawater tanks. In the eastern oyster values ranging from 0.39 – 13 µg / g tissue dry weight of Cd have been reported from relatively uncontaminated sites to contaminated sites (NOAA, 1987).

Differential Cd accumulation was observed among experiments in the present study. These experiments were performed in different times of the year and in different years. It is not certain whether the differences in Cd concentration in gill tissues were because the studied oysters were collected in different times of the year

and different years. Seasonal differences in tissue Cd concentrations have been reported in the eastern oyster (Frazier 1975, Zaroogian 1980, Páez-Osuna et al. 1995).

In the present study, lower gill Cd concentration at the highest exposure concentrations were observed in oysters exposed to Cd-sorbed suspended clay particles in experiments 2 and 3, but not in oysters exposed to contaminated algal food in experiments 1 and 3. This observation was unexpected and difficult to explain. However depuration is not believed to be involved due to the slow loss and long half-life of Cd in bivalves' tissues (Engel 1999, Greig & Wenzlo 1978, George & Coombs 1977). Data from previous studies (Engel 1999, Greig & Wenzlo 1978, George & Coombs 1977) suggest that bivalves do not have an effective depuration capacity for cadmium, copper or zinc. Oysters with high concentrations of accumulated metals lost virtually none of the accumulated metal after 22 weeks of depuration in uncontaminated water (Greig & Wenzlo 1978). Engel (1999) showed that the eastern oyster did not lose significant amounts of Cd after 28 days of depuration following 28 days exposure to 100 ppb Cd. George and Coombs (1977) reported that in exposed mussels the loss rate for Cd was 18 times slower than the rate of accumulation.

In summary, exposure to Cd-sorbed algal paste or suspended clay particles generally did not cause a significant increase in the HSP70 levels in oysters at the exposure concentrations presently used, although a pattern of increase in mean HSP70 with exposure and a significant increase in oysters exposed to Cd-sorbed algal paste in experiment 1 suggest possible and potential effects. In bivalves, mechanisms other than the stress proteins may have more relevance in metal toxicity. The effective sequestration and removal of metals from the cell's cytosol might prevent further interactions with protein components or other structures, thus preventing stimulation of a stress protein response in chronically stressed environments.

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