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DNA STRAND BREAKAGE IN FRESHWATER MUSSELS (*ANODONTA GRANDIS*) EXPOSED TO LEAD IN THE LABORATORY AND FIELD

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Abstract—The sensitivity of a freshwater mussel, *Anodonta grandis*, to DNA damage following lead (Pb) exposure was tested in laboratory and field experiments. Laboratory exposures were conducted for 4 weeks at the following Pb concentrations: 0 (controls), 50, 500, and 5000 μg/L. Mussels were also collected from a strip-mine pond contaminated with trace amounts of lead, cadmium (Cd), and zinc (Zn). Significant DNA strand breakage was observed in foot tissue from mussels exposed in the laboratory to the lowest Pb concentration (50 μg/L). No evidence of strand breakage was observed in any of the analyzed tissues from the mussels exposed to higher Pb concentrations (500 and 5000 μg/L) or from the chronically exposed mussels collected from the strip-mine pond. These data suggest a threshold effect for DNA damage and repair resulting from low-level Pb exposure, whereby repair of DNA strand breaks may occur only if a certain body burden or exposure duration has been achieved.

Keywords—DNA strand breakage Mussels *Anodonta grandis* Lead Heavy metals

INTRODUCTION

Marine and freshwater bivalves readily accumulate many classes of environmental pollutants, including heavy metals, petroleum hydrocarbons, chlorinated hydrocarbons, chlorinated pesticides, and radionuclides because of their intimate association with water and sediments [1–9]. These pollutants are often bioconcentrated to levels that greatly exceed those measured in the surrounding water and sediments, making bivalves well suited as in situ biomonitors for detecting the presence of various toxicants in marine and freshwater ecosystems [1,2,5,10,11].

Although past research with mussels has concentrated on accumulation studies (e.g., International Mussel Watch program [12]), many recent studies with mussels have focused on detecting subtle biochemical and physiological effects of exposure to polluted environments for use as biomarkers of toxicant stress. Because of the ubiquity of DNA-reactive or genotoxic agents in the environment, biomarker assays to detect genotoxicity are being developed in bivalves. Harrison and Jones [13] and Dixon and colleagues [14] have reported the utility of the sister-chromatid exchange assay in detecting genotoxicity in laboratory studies with larvae of the marine mussel, *Mytilus edulis*. The standard metaphase chromosome aberration assay using somatic metaphase cells also has been used to detect chromosomal damage in mussels caged off the coast of Yugoslavia and in controlled laboratory exposures to benzo[a]pyrene [15]. Both of these assays appear to be useful in detecting chromosomal alterations induced by genotoxic agents.

However, there is a need to develop assays that detect more subtle actions of genotoxic agents for use as indicators of potential, and perhaps preventable, damage to chromosomes. Significant increases in repairable DNA strand breakage may occur prior to the permanent chromosomal damage detected by assays, such as the sister-chromatid exchange assay and the standard metaphase chromosome aberration assay. These assays are being developed and used in fish [16–18], but few assays have been adapted for alternate aquatic species. Most of these assays use either alkaline elution techniques [19] or alkaline unwinding followed by fluorometric detection [20]. These techniques have the advantage of being rapid, relatively inexpensive, and requiring little specialized instrumentation other than a spectrofluorometer. Furthermore, because they measure breaks in single-stranded DNA, which occur more frequently than double-stranded breaks, they are very sensitive to the effects of chemical exposure. Neither of these assays, however, directly quantifies DNA strand lengths, in particular the strand lengths of double-stranded DNA. Data from alkaline elution and/or unwinding techniques are expressed as an *F*-value, which represents either the fraction of double-stranded DNA present following alkaline unwinding [16,20] or a strand-scission factor calculated from alkaline elution kinetics [19].

Techniques employing gel electrophoresis allow the direct quantification of DNA fragment lengths and can be used with very a small sample size (5–50 mg tissue, depending on the tissue type). Purified DNA strands applied to neutral or alkaline agarose gels will migrate with an electrical current according to their size. Highly fragmented, low-molecular-weight DNA strands will migrate farther than nondamaged high-molecular-weight DNA strands. DNA migration distances and quantities can be measured by densitometry. Molecular weights of DNA can be calculated by comparison of sample migration distances with those of standards having known molecular weights run on the same gel. Variations of this technique have been used to quantify single-/and double-stranded breaks in DNA isolated from human fibroblast cells exposed to ultraviolet (UV) radiation and in fish blood and liver cells following exposure to genotoxic chemicals [18,21,22; L.K. Martin and M.C. Black, unpublished data].

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Herein, we report the results of a study utilizing agarose-gel electrophoresis to detect DNA strand breakage (double-stranded breaks) in freshwater mussels exposed to lead (Pb) in acute (laboratory) and chronic (field) exposures. Lead was chosen as the test contaminant because of its stability in aqueous solution, ease in handling and disposal, and the availability of sensitive analytical techniques. Furthermore, although Pb has been classified as a carcinogen by the World Health Organization [23], considerable differences in the genotoxic responses of organisms to Pb have been reported, including no genotoxic responses in some species. Increased sister-chromatid exchanges were detected in Chinese hamster ovary cells exposed to Pb in vivo [24]. Lead-induced chromosomal aberrations were also detected in cultured human leukocytes [25], but not in cultured Chinese hamster cells [26]. No data were found regarding genotoxic responses of aquatic organisms to Pb exposure following laboratory or field exposures. Electrophoretic determinations of DNA strand breakage may offer a more sensitive method to detect genotoxicity due to Pb exposure. Therefore, we used this technique to test the hypothesis that Pb-induced genotoxicity would result in a greater incidence of shorter DNA strand fragments in laboratory- and field-exposed freshwater mussels compared with controls.

MATERIALS AND METHODS

Mussels

Adult *Anodonta grandis* were collected from a noncontaminated pond in Payne County, Oklahoma, and acclimated to laboratory conditions in aerated, filtered (0.4 μm), dechlorinated water in 151-L aquaria for 2 weeks prior to exposure. Mussels were fed Microfeast Plus L-10 larval diet (Fleishmann's Yeast Co., Bartlesville, OK, USA) dissolved in water (0.625 g Microfeast/6 mussels) on alternate days during the acclimation and exposure periods. Mean wet weight (including shell) and length (±SD) of mussels used in the laboratory exposures (n = 24 mussels) were 137.0 (±23.6) g and 110.2 (±5.3) mm, respectively.

Laboratory exposure to Pb

Mussels were exposed for 28 d to Pb, as $Pb(NO_3)_2$ (Fisher Scientific, ACS grade), at 0, 50, 500, and 5000 μg/L Pb. Six mussels of similar size and weight were exposed in each of four 76-L aquaria under a static renewal protocol. Fifty percent of the exposure water (10 L) in each aquaria was replaced on alternate days. Mussels were fed immediately following each water change as described above. Temperature, pH, and ammonia concentrations in each aquarium were measured just prior to each water change. For all treatments, the average pH (±SD) was 6.2 (±0.2), and the average exposure temperature (±SD) was 22.2 (±1.3)°C. Ammonia concentrations varied with the Pb exposure concentration; however, concentrations never reached stressful levels (>2 mg/L). Mean ammonia concentrations (±SD) for the aquaria with 0, 50, 500, and 5000 μg/L Pb were 0.2 (±0.4), 0.2 (±0.5), 0.4 (±0.7), and 0.9 (±0.4) mg/L, respectively. As temperature, aeration, and mussel biomass were approximately the same in all aquaria, differences in ammonia levels may have resulted from increased excretion of metabolic wastes at higher Pb concentrations.

After a 28-d exposure to Pb, mussels were removed and weighed, and the adductor muscle, foot, and mantle were removed and placed in sterile tubes. Tissues were frozen in liquid nitrogen and stored at −80°C until analyzed for DNA strand integrity and metal content.

Chronic field exposure to heavy metals

Six adult mussels, also *Anodonta grandis*, were collected from a strip-mine pond located in Okmulgee County west of Henryetta, Oklahoma, to determine the effects of chronic heavy-metal exposure on DNA strand integrity. Analyses of soils and overburden adjacent to this pond revealed significant quantities of metals, including Pb, Zn, and Cd [27,28]. Upon returning to the laboratory, the mussels were immediately weighed and measured, and the adductor muscle, foot, and mantle were removed, placed in sterile Eppendorf tubes, and frozen in liquid N_2. Tissues were stored at −80°C.

Rationale for tissue selection

Foot, mantle, and adductor muscle were chosen for the DNA analyses (and subsequent residue analyses) because of their compatibility with the extraction and analysis procedures, their physiological functions, and their physical location within the shell. Foot and mantle tissues are easily sampled and have large surface areas for exposure to toxicants. Furthermore, because mantle tissue provides materials for the shell, it could be a conduit for materials that are ultimately deposited in the shell. Adductor muscle is easily sampled and extracted, has a low surface area, and, perhaps, a lower potential for exposure owing to its location away from the major flow of water siphoned into the shell. Although gill tissue would seem to be an excellent tissue for this experiment because of its constant exposure to toxicants through respiratory and filter-feeding activities, it was not analyzed. Previously, we experienced great difficulty in separating proteins from DNA extracted from the gill tissue, resulting in extremely low DNA extraction efficiencies. Improved gill extraction techniques are currently under investigation.

Metals analyses

Surface sediments from the mussel collection site were collected using a plastic trowel, placed into acid-washed plastic containers, and stored at −20°C for less than 2 weeks. Sediment (1-g samples) and mussel tissues (samples pooled from three mussels to yield approximately 1 g tissue/tissue type), plus the appropriate reference standards, matrix spikes, and blanks, were digested with trace metal-grade nitric acid using U.S. Environmental Protection Agency standard method 3050 [29]. Digests were analyzed for Pb, Cd, and Zn content by inductively coupled plasma atomic emission spectroscopy (ICP). Tissue metal concentrations are expressed as wet weights because extra tissue was not available to subsample for gravimetric moisture determinations. To avoid potential losses of metals by volatilization (J.S. Hausbeck, personal communication), tissue samples were not dried prior to digestion. Sediment metal concentrations are expressed as dry weights by correction for percentage moisture in each sample. The moisture percentage was determined gravimetrically after drying a subsample at 100°C. Metal concentrations in nondigested water samples were also determined by ICP analysis.

DNA extraction, purification, and analysis

DNA from the foot, mantle, and adductor muscle was isolated and purified using a modification of a standard rodent protocol [17,30; L.K. Martin and M.C. Black, unpublished data]. Under ice-cold, sterile conditions, approximately 50 mg of tissue was minced, gently homogenized with a plastic pestle, and extracted with a buffer containing 250 mM NaCl, 100 mM Tris base, and 100 mM ethylenediaminetetraacetic acid (EDTA). Cells in the homogenate were lysed by adding sarcosyl (1% wt/

vol, *N*-lauroyl-sarcosine, sodium salt; Sigma Chemical Co., St. Louis, MO, USA), followed by the addition of 2 to 4 μg ribonuclease A (approx. 100 Kunitz units/mg, Sigma Chemical Co., St. Louis, MO, USA). After incubating on ice for approximately 1 h, the extracts were purified by extracting twice with equal volumes of CIP (50% chloroform : isoamyl alcohol [24:1], 50% Tris-HCl–buffered phenol [Gibco BRL, Grand Island, NY, USA]), followed by 1 or 2 extractions with chloroform to remove all traces of phenol from the aqueous DNA extract.

Absorbances of diluted aliquots of purified DNA were measured on a UV-visible spectrophotometer at 260 and 280 nm, and the 260/280 ratios were calculated. Acceptable ratios were greater than 1.6, but less than 2.0, indicating that DNA was adequately purified (i.e., the extracts were not contaminated with proteins) and that all RNA was enzymatically degraded. Samples whose ratios did not fall within the acceptable range were either reextracted with chloroform or incubated with additional ribonuclease A, and the absorbances were rechecked to assure DNA purity prior to electrophoresis.

Purified DNA extracts were electrophoresed along with a DNA standard (λ-*Hin*dIII fragments, Bio-Rad Laboratories) on a nondenaturing gel containing 0.5% agarose in TBE buffer (final dilution = 65 mM Tris HCl, 22.5 mM boric acid, and 1.25 mM EDTA, prepared from a 10% solution of 10× TBE Extended Range Buffer, Bio-Rad Laboratories, Richmond, CA, USA), with ethidium bromide added to permit visualization of the DNA. Each gel was prepared with 30 sample wells, so that a single gel could contain all extracts of each tissue type at a single Pb concentration (total of 20 or 24 samples) and five or six *Hin*dIII standards. Gels were electrophoresed at 47 V in 0.5× TBE running buffer for 2 to 3 h. DNA was visualized and photographed on a UV transilluminator, and the negative was scanned on a laser densitometer with a computerized image analysis program (PDI Model 25 densitometer with Quantity One software, PDI, Inc., Huntington Station, NY, USA).

DNA appears on the gel as either distinct bands of uniformly sized, nonfragmented strands or smears of fragments with a range of sizes. Average mobilities, densities, and relative quantities (%) of DNA present in each band or smear were determined using the image analysis software. A standard curve was prepared for each gel by plotting the average mobilities (R_f) of each λ-*Hin*dIII fragment versus the logarithm of each strand length (23.1, 9.9, 6.6, 4.4, or 2.3 kilobases), yielding a linear relationship between DNA mobility and the logarithm of strand length. Strand lengths for fragments detected in samples were calculated from their mobilities using a linear regression of the standard curve (modified from [21]). Sample data are reported as the average strand length. This is a weighted average calculated for each sample by summing the products of the relative quantities (%) of each fragment multiplied by its strand length.

Statistical analyses

DNA strand length data were first subjected to the Bartlett's test to determine homogeneity of sample variances. Because sample variances were determined to be homogeneous, analysis of variance (ANOVA) was used to determine if differences in DNA strand lengths could be attributed to Pb exposure concentrations [31]. Significant differences in DNA strand lengths between Pb-exposed mussels and controls were determined by the method of least significant difference at the significance level of 0.05 [32]. Tissues exhibiting a significant decrease in average DNA strand length compared with controls were assumed to have significant DNA strand breakage.

Table 1. Tissue lead concentrations (μg/g wet weight) in mussels exposed to $Pb(NO_3)_2$ in laboratory exposures. Tissues from three mussels were pooled for digestion and analysis (n = 2)

Lead exposure	Mantle	Foot	Adductor muscle
0 (control)	<5	5 ± 7	<5
50 μg/L	<5	<5	<5
500 μg/L	6 ± 1	11 ± 1	<5
5000 μg/L	48 ± 27	48 ± 5	18 ± 8

Data are presented as mean ± SD.

RESULTS AND DISCUSSION

Laboratory study

Lead accumulation by mussels varied with exposure concentration and tissue type (Table 1). Mussels accumulated Pb in all tissues (mantle, foot, and adductor muscle) when exposed to 5000 μg/L. Highest Pb concentrations were measured in mantle and foot. Lead was detected only in foot and mantle tissues of animals exposed to 500 μg/L Pb, and no Pb accumulation was observed in any tissue following exposure to 50 μg/L Pb. Consistently, Pb residues in adductor muscle were less than those measured in foot and mantle, tissues with large surface areas and presumably higher potential for exposure.

Our results compare favorably with copper (Cu) accumulation by *Anodonta cygnea* exposed for 35 days to 10.9 μg/L Cu [8]. Copper concentrations measured in mantle and foot tissue were more than twice those measured in adductor muscle. In a separate 28-d exposure with Pb (57 μg/L $PbCl_2$), the same authors found no distinct accumulation patterns for Pb in mussel tissues. However, depuration of Pb was more rapid from the adductor muscle than other tissues, perhaps indicating a lower capacity for storing Pb in the adductor muscle over chronic exposure durations [8]. Similar mechanisms could have limited Pb accumulation in our laboratory-exposed mussels and the chronically exposed mussels collected from the strip-mine pond. Possible explanations for lower Pb residues measured in adductor muscle include lower net accumulation by the tissue due to its small surface area, limited exposure to siphoned water, or greater elimination rates by adductor muscle. Further experiments designed specifically to measure accumulation and depuration rates over semichronic to chronic exposure durations are needed to adequately test these hypotheses. Future experiments should also measure tissue water contents to ensure that apparent differences in Pb residues are not due to differences in tissue-water retention.

Short-term reductions in growth have been measured in juvenile and adult mussels exposed to many heavy metals, including tributyltin [33,34], Cu [35], cadmium (Cd) [36], and zinc (Zn) [35]. However, in our laboratory exposure to Pb, mean mussel weights (±SD) before and after the exposure were 137.0 g (±23.6) and 136.8 g (±24.4), respectively. Mean mussel lengths were also unchanged during the exposure. These results may imply that Pb had no measurable effect on adult mussel growth at the experimental concentrations and exposure durations. However, the use of whole-animal wet weights to measure growth may not yield true measures of mussel weights, because of the inclusion of siphoned water and the influence of tissue water content in these measurements. Therefore, this method would detect only gross changes in weight due to toxicant exposure. Measures of tissue protein and glycogen contents will indicate the status of tissue energy reserves. For short-term,

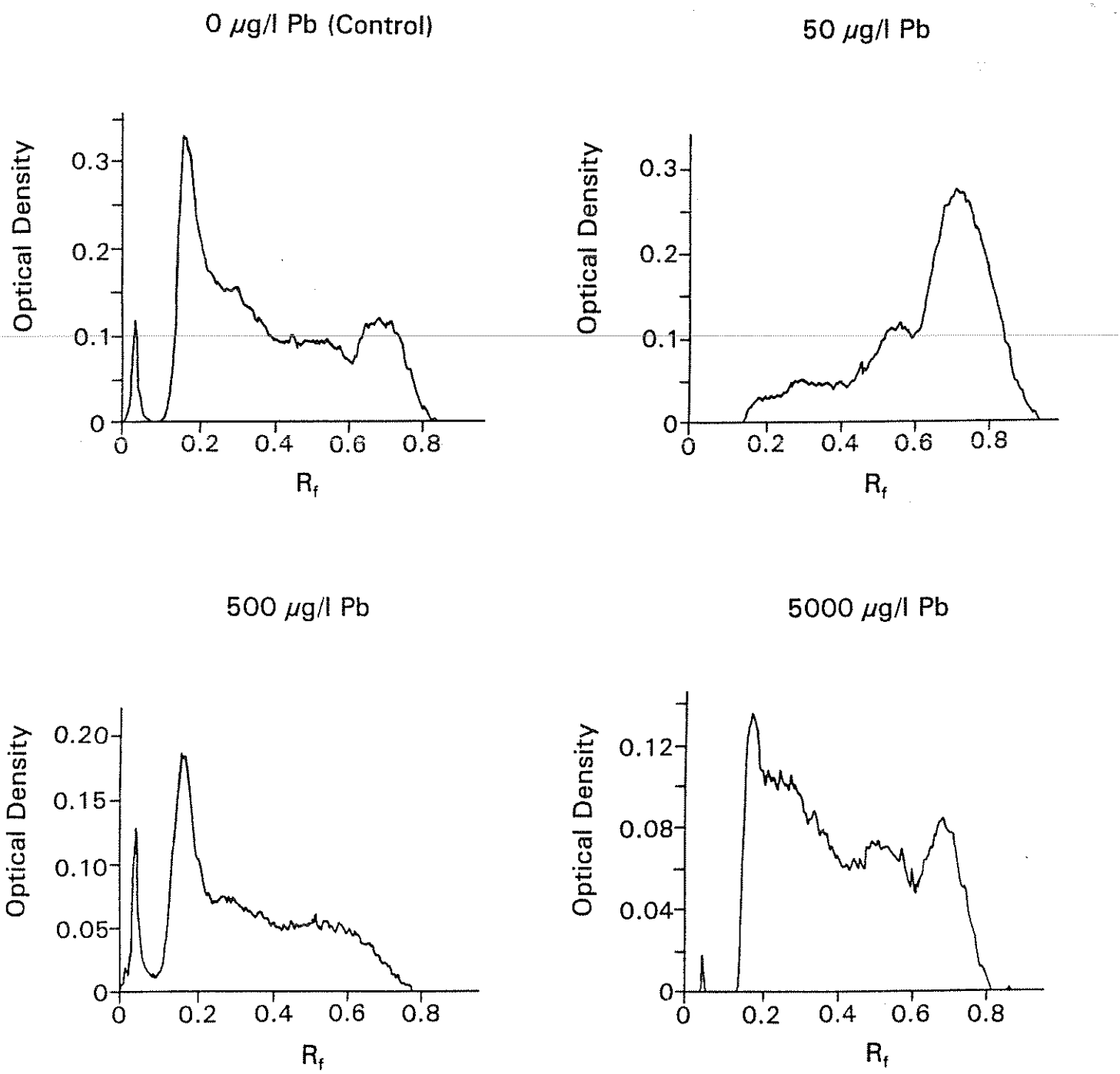

Fig. 1. Absorbance spectra from densitometry scans showing DNA size distributions in foot tissue extracted from four mussels exposed to 0, 50, 500, and 5000 μg/L lead. Each spectra represents results from one individual. Average DNA strand lengths calculated from these scans are 30.2 kb (0 μg/L Pb), 1.2 kb (50 μg/L Pb), 34.2 kb (500 μg/L Pb), and 24.7 kb (5000 μg/L Pb). Spectra show the downfield absorbance shift (measured as higher R_f values) associated with lower average strand length in mussels exposed to 50 μg/L lead.

sublethal toxicant studies with adult organisms (such as our laboratory study), protein and glycogen determinations may provide a more sensitive way to measure a toxicant's effect on growth, by evaluating an organism's capacity for growth rather than net changes in weight or length.

Concentration-dependent differences in electrophoretic mobility for double-stranded DNA were apparent in foot tissue exposed to Pb. Mussels exposed to 50 μg/L Pb showed large zones of smaller DNA fragments, which migrated farther from sample wells than larger fragments. Most of the DNA extracted from the same tissue of mussels exposed to 0, 500, and 5000 μg/L Pb consisted of longer strands. Absorbance spectra from the densitometer scans of four samples from this gel (Fig. 1) indicated that high-molecular-weight, nonbroken mussel DNA have low mobilities (R_f values ≤ 0.2, as observed in the spectra from the 0, 500, and 5000 μg/L Pb exposures). Tissue from the 50 μg/L Pb exposure had very little high-quality DNA, but had a large absorbance peak at $R_f \approx 0.7$, signifying a large quantity of highly fragmented DNA in that sample. Calculations of the average DNA strand lengths confirmed the presence of highly fragmented DNA in all mussels exposed to 50 μg/L Pb.

Average DNA strand lengths were reduced by 58% in foot tissue from mussels exposed to the lowest Pb exposure concentration (50 μg/L), compared with average values from con-

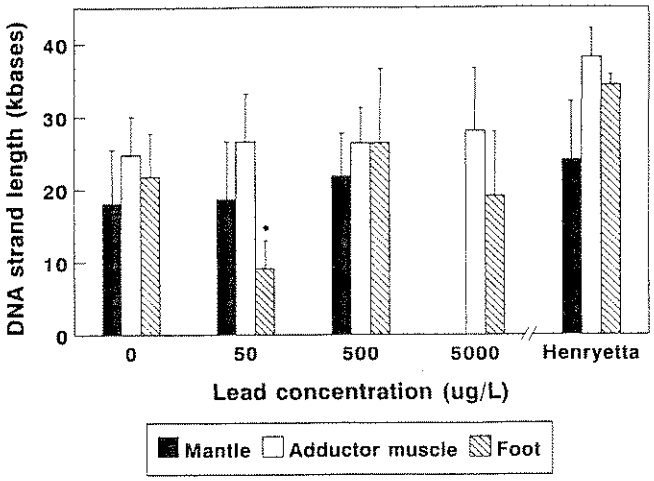

Fig. 2. Mean DNA strand lengths (±SD) in mantle, adductor muscle, and foot tissues of mussels exposed to 0 (controls), 50, 500, and 5000 μg/L lead in laboratory exposures (n = 5 observations for 0 μg/L exposures; n = 6 for the 50, 500, and 5000 μg/L exposures). The * indicates a significant difference in DNA strand length versus controls ($p < 0.02$).

trols. No significant differences in DNA strand lengths were observed between any tissues from mussels exposed to the highest concentrations of Pb (500 and 5000 μg/L) and any tissues from controls (Fig. 2). Bihari and colleagues [19] found a similar pattern of damage to single-stranded DNA in the marine mussel *Mytilus galloprovincialis* following exposures to benzo[a]pyrene and 4-nitroquinoline-*N*-oxide. For both compounds, higher doses resulted in lower incidences of single-strand breakage than low doses after a 48-h exposure, although a dose–response relationship was observed for DNA damage after a 1.5-h exposure. Faster and more efficient DNA repair rates at higher exposure concentrations were suggested as reasons for the ultimate reduction in strand breakage at high concentrations compared with lower concentrations. In a similar study with Sprague-Dawley rats exposed to endrin, Bagchi and colleagues [37] reported evidence of significant repair of single-stranded DNA by 24 h postexposure. Maximum repair occurred at the highest exposure concentrations by 72 h postexposure.

High Pb concentrations (500 and 5000 μg/L) may have induced repair processes for double-stranded DNA, so that DNA damage was not detectable in the tissues examined by the end of our exposure period (28 d). At the lowest exposure concentration, DNA strand breakage may have occurred so gradually over the 28-d exposure that either the "threshold" for repair induction was not reached and little or no repair occurred, or repair processes were not maximally induced by 28 d, resulting in incomplete repair by that time at the lowest Pb exposure. Another possible reason for the high incidence of DNA strand breakage may have been localized cellular necrosis of the foot, which would not be detected by our dissections or assays.

Why damage was observed only in foot tissue, and not in other tissues, is less apparent. The foot was often observed to be extended outside the shells of all mussels (regardless of exposure concentration), especially at night when mussels would move to different positions in the aquaria. Foot extension does increase the surface area of this tissue and may have resulted in increased exposure for the foot tissue, compared with other tissues that are exposed only through siphoning actions. However, this is not entirely supported by the residue analyses, inasmuch as Pb concentrations in the mantle were nearly identical to those in the foot at the highest exposure concentrations. Furthermore, at the exposure concentration where damage was evident in foot tissue (50 μg/L), Pb concentrations in all tissues were below our detection limits (Table 1). However, tissue Pb concentrations reflect the net effects of accumulation, distribution, and elimination, and may be low or not detectable in tissues with efficient elimination by 28 d of exposure to high Pb concentrations. Conversely, for low-level exposure to Pb, DNA damage may have occurred prior to elimination of Pb residues and may have been nonrepairable if repair threshold concentrations were not attained.

Field exposures to heavy metals

Three heavy metals were detected in water, sediment, and mussel tissues collected from the strip-mine pond (Table 2). Zinc was detected in all samples and was present in mantle and foot tissues at nearly twice the concentration found in adductor muscle. This is the same accumulation pattern found for Pb in our laboratory exposures with *A. grandis*. Lead was only detected in sediment samples, whereas trace quantities of Cd were detected in sediment and adductor muscle samples.

Although mussels were exposed to all three metals through contaminated sediments, it is not surprising that little accumulation of Pb and Cd in the soft tissues was measured. In mammals, Pb is deposited and stored ultimately in bone [38]. A comparable storage location in the mussel is the shell. Sturesson [36,39] demonstrated significant accumulation of waterborne Pb and Cd in the shell of the marine bivalve *Mytilus edulis* following a 25-d exposure. Thus, shell may be an effective sink for Pb and Cd, removing them from soft tissues where they may more easily exert toxic effects. Furthermore, once Pb or Cd is trapped in the shell matrix, little metal release is possible unless breakdown of the crystalline matrix occurs [36]. Other potential chronic storage locations for Cd include kidney or liver tissue [38]. Neither hepatopancreas nor kidney was analyzed for heavy metals or DNA damage in this experiment.

Table 2. Heavy-metal concentrations measured in water, sediments, and pooled mussel tissues collected from the strip-mine pond

Sample	Pb	Cd	Zn
Water (mg/L)	<0.05	<0.01	0.02
Sediment (μg/g dry weight)	22.5 ± 2.1	0.6 ± 0.1	0.16 ± 0.1
Mussel tissues[a]			
Mantle (μg/g wet weight)	<5	<1	19.0 ± 5.6
Foot (μg/g wet weight)	<5	<1	22.0
Adductor muscle (μg/g wet weight)	<5	1.6 ± 1.1	12.5 ± 0.7

[a]Tissues from three mussels were pooled for digestion and analysis (n = 2), except for foot, where tissues from six mussels were pooled into one sample.

Because Zn is an essential trace element, it is more easily accumulated and stored in soft tissues by bivalves than nonessential heavy metals (e.g., Pb and Cd) on chronic exposure to contaminated water and sediments [2,40–42]. Our data are consistent with the finding that accumulation of Zn occurs primarily in bivalve soft tissues, where it is usually associated with proteins or membrane-bound vesicles in the hemolymph [43,44]. Zinc deposition in the shell is considered to be insignificant [45].

No DNA damage was detected in tissues analyzed from chronically exposed mussels collected from the strip-mine ponds (Henryetta mussels, Fig. 2). DNA isolated from foot and adductor muscle of Henryetta mussels had significantly longer strand lengths than controls. Although mussels were chronically exposed to low concentrations of Pb and Cd in the sediments, several explanations are possible for the absence of strand breakage in these mussels. Exposure concentrations for Pb and Cd were very low. Over a lifetime of exposure, mussels with adequate repair capabilities may have been able to repair any DNA damage that occurred. Enhanced tolerance, which is often heritable, has been observed in organisms chronically exposed to Pb and other heavy metals [46]. Tolerance can afford protection against heavy-metal toxicity by altering toxicant uptake, distribution, and storage or by increasing the rates of toxicant biotransformation and elimination. DNA repair is under genetic control [47]; thus, enhanced DNA repair capabilities could also be acquired through similar mechanisms of tolerance acquisition.

SUMMARY AND CONCLUSIONS

Although no Pb accumulation was measured in foot, adductor muscle, or mantle tissue isolated from mussels exposed to low Pb concentrations (50 μg/L), significant DNA strand breakage was observed in these organisms after a 28-d laboratory exposure. Higher Pb concentrations yielded measurable tissue Pb concentrations in laboratory-exposed mussels (Table 2), yet no DNA strand breakage was observed. Furthermore, mussels experiencing a lifetime exposure to very low concentrations of Pb in the sediments also exhibited no DNA strand breakage in any of the excised tissues. These results emphasize the importance of exposure length and concentration to the progression of damage to double-stranded DNA and also to possible repair following sublethal exposure to Pb.

Lead toxicity is caused by its ability to interact with nucleophilic sites on proteins, DNA, and RNA (reviewed in [48]). These covalent interactions, or adducts, may weaken DNA strands and cause strand breakage at the point of adduction. If strand breakage is repaired via enzymatic processes, no net damage can be observed. Our findings imply that the incidence of DNA damage following low-level exposure to Pb may not follow a dose–response relationship, in which DNA strand breakage would increase with higher exposure concentrations. However, this observation may be explained in terms of DNA damage and repair processes.

If DNA is damaged via adduction or other mechanisms, and repair enzymes are not present, damage will persist until toxicant levels are sufficient to induce enzymatic repair. The residue data from our laboratory exposures support a dose–response relationship between Pb exposure concentrations and Pb accumulation by freshwater mussels. Our DNA damage data, however, do not support a dose–response relationship between Pb accumulation and measurements of DNA strand breakage data following a 28-d exposure to Pb. However, measurements of DNA damage (regardless of the method used) measure the net result of two opposing processes: damage and repair. Although it cannot be verified by our data, a dose–response relationship may exist between Pb accumulation by mussels and the actual interaction of Pb with DNA strands in the absence of repair processes. Perhaps the process without a dose–response relationship is DNA repair. Because repair occurs by an enzymatic process [49,50], it may be subject to a threshold effect, requiring sufficient toxicant to be present for induction or activation. Also, repair may be saturated or inactivated at very high toxicant concentrations. Thus, we propose that perhaps strand breakage determinations should be coupled with indicators of DNA repair to yield a more complete picture of how an organism is coping with genotoxic stressors.

Clearly, more research is needed to identify the progression and etiology of DNA damage and repair in mussels exposed to Pb and other toxicants. Future studies should include analyses of activities of DNA repair enzymes to determine how enzymatic repair processes and rates of repair are affected by toxicant exposure. Actual documentation of the DNA-damaging event (presumably Pb adduction) would aid in the understanding of the nature and timing of DNA damage by Pb. Microscopic analyses of affected tissues might reveal whether high occurrences of strand breakage coincide with necrotic zones in tissue. Finally, conducting experiments with a longer study duration, more frequent sampling intervals, and a wider range of toxicant concentrations would reveal more information on the basic mechanisms of DNA damage and repair.

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