

BIOMARKER ASSESSMENT OF ENVIRONMENTAL CONTAMINATION: IN-SITU STUDIES WITH FRESHWATER BIVALVES

Marsha C. Black¹, Stacy M. Westerfield², John I. Belin² and Kelley B. Van Vreede³

AUTHORS: ¹Assistant Professor and ²Graduate students, Environmental Health Science Program; ³Graduate student, Interdisciplinary Toxicology Program, The University of Georgia, Athens, Georgia 30602-2101 (mblack@uga.cc.uga.edu).

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Abstract. Bivalves are effective pollution biomonitors in marine and freshwater environments because of their ability to bioconcentrate many environmental pollutants to levels that greatly exceed those found in water and sediments. However, most research efforts have focused on monitoring chemical accumulation by bivalves, and have not examined the toxic effects of accumulation or exposure to toxic chemicals. In addition, different phases of the mussel life cycle have been sparingly employed for toxicity evaluations.

Our research has focused on developing biomarker assays for adult and juvenile bivalves, primarily DNA strand breakage and the nonspecific biomarkers, growth and condition index. Herein we describe a study using these biomarkers in juvenile and adult *Corbicula fluminea* exposed in a stream contaminated with urban and industrial runoff. Our ultimate goal is to develop and validate appropriate biomarkers for use in assessment of exposure and effects for risk assessments.

INTRODUCTION

Bivalves are particularly vulnerable to exposure and deleterious effects of aquatic pollutants because of their modes of feeding and respiration and close association with sediments. They readily accumulate many environmental pollutants, including heavy metals, hydrocarbons, chlorinated hydrocarbons and radionuclides (Goldberg, 1980; Phillips, 1980; Broman and Ganning, 1985; Muncaster et al., 1990). Accumulation of these substances by bivalves can result in toxicity or mortality, and can ultimately cause population declines and subsequent loss of aquatic ecosystem diversity and stability. Additionally human health can be adversely affected by consumption of contaminated bivalves and the mariculture and freshwater shell industries can suffer economic losses if bivalve populations decline or become chemically contaminated.

Bivalves have often been used as pollution biomonitors in marine and freshwater environments because of their abundance, sedentary habits, long lifespan, and ability to bioconcentrate many environmental pollutants to levels that greatly exceed those present in surrounding water and

sediments (Goldberg, 1980; Leard et al., 1980; Broman and Ganning, 1985). Because of their nearly constant exposure to overlying water, sediment pore water and sediments, bivalves are ideal *in-situ* biomonitors. Most previous work has focused on monitoring chemical accumulation by bivalves collected from contaminated sites (e.g., the International Mussel Watch Program) (Goldberg, 1980). Relatively few studies have examined the toxic effects of exposure to toxic chemicals.

A recent focus of environmental toxicology has been the development of biomarkers -- rapid, toxicological assays that detect sublethal biochemical, physiological, and organismal effects in organisms exposed to environmental contaminants. Biomarkers can be used as a screening tool to detect organism exposure to environmental contaminants, and can also be used to quantify specific toxicological responses of exposed organisms (Huggett et al., 1992). Although many mussel species can survive exposure to numerous environmental contaminants, they often display an array of disturbed physiological and biochemical responses following exposure (Bayne et al., 1985). Biomarker assays can be developed in bivalves upon identification of these responses and their correlation with exposure concentrations. By coupling biomarker measurements with *in-situ* exposures, information on sublethal effects of contamination can be obtained.

Juvenile or larval life stages are often more sensitive to toxicant exposure than adults, as measured by toxicity tests or biomarker responses. Enhanced sensitivities of young organisms may result from their increased metabolic rate, which may affect chemical uptake and distribution rates. Furthermore, young organisms often lack crucial enzymes or pathways for detoxification or repair processes. Thus, additional research needs to address age-related sensitivities of biomarker responses. This will not only elucidate relationships between age or stage of development and biomarker responses, but will also provide important information for determining effects of environmental contaminants on resident mussel populations.

In this report we present results from studies with juvenile and adult freshwater clams that have employed the biomarker approach. These studies illustrate the utility of using bivalves for biomarker studies, yet also highlight some of their limitations and areas needing further study.

MATERIALS AND METHODS

Experimental organisms

Asiatic clams (*Corbicula fluminea*) were collected from the North Oconee River (Fall 1995 study) or from Lake Chapman, Athens-Clarke County, GA (Fall 1996 study). Clams were collected a month prior to deployment and were held in flowing, aerated dechlorinated water to depurate any potential trace contaminants previously accumulated. Experiments always used clams sampled from a single location to avoid any confounding effects of population-specific sensitivities or tolerances.

Study sites

Trail Creek is an urban first-order stream located in Athens-Clarke County, GA. It was selected as the contaminated site for the *in-situ* studies because it receives municipal and industrial storm water runoff. Furthermore, local natural resource specialists are concerned about its apparent lack of biological diversity (pers. comm., Nancy Smith, Department of Environmental Education, Athens-Clarke County, GA). Table 1 provides a list of toxic chemicals, potentially present in trace quantities in storm water runoff from local industries bordering Trail Creek.

Reference sites for this study included a site on the N. Oconee River at the University of Georgia's Whitehall Forest (1995 study) and a site on Sandy Creek, located behind Sandy Creek Nature Center (1996 study). Both reference sites are located in Athens-Clarke County and are considered to be noncontaminated.

In-situ exposures with adult *C. fluminea*

In two separate field seasons (Fall 1995 and 1996), clams were suspended in sleeves within plastic cages in Trail Creek and a reference site. Prior to caging, clam lengths and weights were measured for comparison with post-exposure lengths and weights.

In Fall 1995, 96 adult *C. fluminea* were caged at each site (16 clams/cage, 3 cages/site). In Fall 1996 the study was expanded to include 80 adult and 160 juvenile clams, with 40 juveniles and 20 adults per cage and 2 cages per site. Clams were removed after 1 and 2 weeks' exposure (Fall 1995) and 2, 4, and 6 weeks' exposure (Fall 1996). Upon sampling clam lengths and weights were measured, foot tissues were excised and snap frozen in liquid N₂, and remaining tissues dried (12 h at 100°C) to enable calculation of dry weights, condition index and tissue water content (Fall 1996 only).

Biomarker Assays

Growth of exposed adult and juvenile *C. fluminea* was measured as a net weight gain or loss, based on pre- and post-exposure mussel weights. Growth rate was calculated from the net weight gain or loss (mg) ÷ exposure time (d). Condition indices of exposed adult and juvenile *C. fluminea* were calculated from clam shell lengths and wet and dry weights

Table 1. Toxic Substances Stored and Used at Industries Bordering Trail Creek

coal tar	sulfuric acid
petroleum emulsion	phosphoric acid
petroleum distillate	sodium tetraborate
propylene glycol	formaldehyde
ammonium hydroxide	dibenzyl toluene
monolauryl ether	phenol
molybdenum	chromium

according to Graney and Giesy (1988): $CI = (\text{tissue dry weight} \div \text{shell length}) \times 100$. Percentage tissue water was calculated from tissue wet and dry weights of exposed adult and juvenile *C. fluminea* by the following equation: $\%TW = [(\text{wet wt} - \text{dry wt}) \div \text{wet wt}] \times 100$.

DNA was extracted using a standard rodent protocol (Maniatis et al., 1982), modified by Black et al. (1996). Foot tissue samples (35 to 50 g from adult clams) were extracted in a high salt buffer, treated with RNase and proteinase K (60°C for 30 min) and purified by sequential extractions with a 1:1 chloroform:isoamyl alcohol (24:1)/phenol mixture, chloroform:isoamyl alcohol, and chloroform. The DNA was precipitated overnight in ice-cold ethanol, reconstituted in TE (tris-EDTA) buffer, and its purity and quantity was determined spectrophotometrically at 260 and 280 nm. Samples were electrophoresed by field inversion gel electrophoresis (FIGE) under nondenaturing conditions, using a 0.75% pulsed field agarose gel. Electrophoresis was conducted at 180 and 120 V forward and reverse voltages, with a ramped switching time of 0.1 to 0.8 seconds for a run time of 11 h. Gels were stained with ethidium bromide and photographed under UV light. Negatives were scanned into a laser densitometer with an image analysis program (Quantity One®, PDI, Huntington Station, NY). Weighted average strand lengths were calculated from densities and mobilities of sample bands for each lane as described by Black et al. (1996).

RESULTS

a. Growth

Although intended to be a 6 week exposure, the Fall 1995 *in-situ* study was terminated after 3 weeks due to the damaging effects of Hurricane Opal on both study sites. Therefore, data from only one and two weeks' exposure were collected. During the two-week exposure no significant differences were detected in clam lengths or weights between the reference and Trail Creek exposure sites. However adult clam weights were significantly reduced at all sites after one and two weeks' exposure, compared to preexposure weights (Table 2). No significant changes in clam length were observed at either location over the 2 week exposure.

Preliminary data from the initial 6 weeks' sampling of the

Table 2. Pre- and Post-exposure Weights and Growth Rates for Caged Adult Clams (Fall 1995 Study).

Site/Time	Pre (g)	Post (g)	Rate (mg/d)
Trail Creek			
Week 1	4.00	3.92	-11.4
Week 2	3.38	3.34	-2.9
Reference			
Week 1	3.49	3.43	-8.6
Week 2	3.53	3.41	-8.6

Fall 1996 study also indicated no significant changes in growth patterns measured as either weight or length increases in Trail Creek-exposed adult or juvenile clams compared with reference clams. However, as observed in the 1995 study, regardless of caging location, 78% of the caged adult clams lost an average of 5% of their total individual weight by 2 weeks. After 2 and 4 week's exposure significant weight losses were observed at all locations, but diminished with time (Fig.1). All juvenile clams gained weight over the duration of the 6 week exposure; although, as observed in adults, no differences were observed in juvenile clam growth (weight change/d) between study sites (Fig.1).

b. CI and % TW

No significant differences were observed in condition index (CI) or tissue water (%TW) between sites or with increasing exposure duration during the first 6 weeks' exposure. Furthermore, no effects were observed in either age class, although in juvenile clams %TW was nearly half that measured in adults.

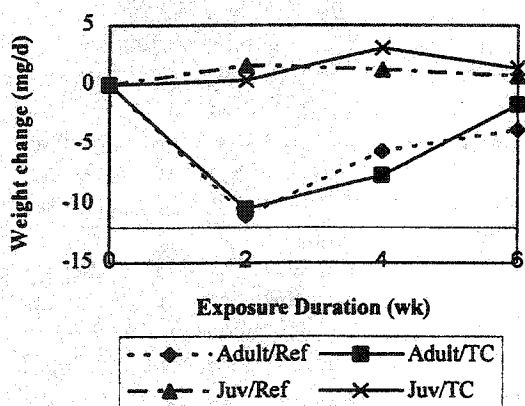


Figure 1. Growth of juvenile (juv) and adult clams caged in Trail Creek (TC) and the reference site (Ref) during Fall 1996. No site-specific differences were detected.

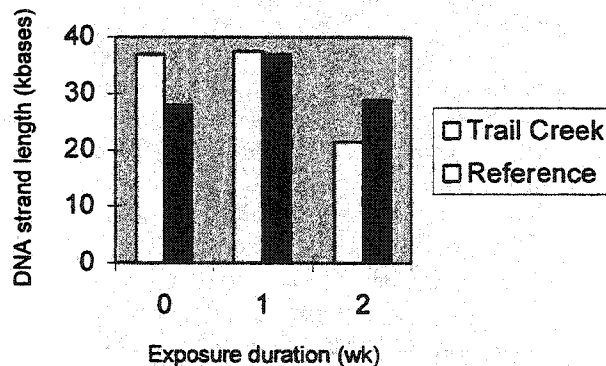


Figure 2. DNA strand lengths in foot tissue of clams caged at Trail Creek (contaminated site) and the N. Oconee River (reference site) during the 1995 study. Average DNA strand lengths were significantly shorter after two week's exposure in Trail Creek versus those caged at the reference site (p = 0.081, ANOVA).

c. DNA Strand breakage

In the Fall 1995 study average DNA stand lengths were 30% shorter in clams sampled from Trail Creek after two weeks' exposure compared with controls (Fig. 2). This effect may have been more pronounced if not for the high variation observed among individuals. DNA strand lengths ranged from 4.1 to 38 kbases, with an average of 21 kbases in Trail Creek-exposed individuals. This represents a two-fold increase in standard deviation in Trail Creek samples after two weeks' exposure, compared with reference and previous Trail Creek samples.

DISCUSSION

During the first 6 weeks of exposure (1996 study) juvenile clams at both caging locations gained weight, with no differences observed due to caging location. Mean growth rates were variable and ranged from 0.4 to 3.1 mg/d, and reflect additions of between 0.03 to 0.2% of total mussel weight per day. Salazar and colleagues (1995) found growth to be a sensitive biomarker in juvenile *Mytilus trossulus* exposed *in-situ* at a Superfund site in Puget Sound, WA. Mean growth rates were reduced 2-3x the rate observed mussels caged at a reference site. This Superfund site was heavily contaminated with heavy metals, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls, whereas the Trail Creek site in our study should contain only trace amounts of contaminants. Variable results, with no definable pattern as observed in our study implies that contaminant concentrations were possibly not sufficient to consistently affect growth rates of caged juvenile mussels.

All adult clams lost weight in our experiments, regardless

of the caging location. Significant periods of reduced siphoning could result in weight losses, as no food intake occurs without siphoning. Reduced siphoning could have resulted from caging stress. Decreased siphoning activity has been correlated with a number of physical stressors, including changes in temperature, salinity and oxygen tension (reviewed in Higgins, 1980). Heavy metal exposure has also caused reduced siphoning rates *C. fluminea* and *Anodonta cygnea* during acute and subchronic exposures (Doherty et al., 1987; Sálanki and V.-Balogh, 1989). Interestingly in our 1996 study, weight losses diminished as the exposure progressed, which may reflect acclimation of clams to exposure conditions.

Belanger and colleagues (1990) reported *in-situ* growth rates of 0.004 to 0.04 mm per day in *C. fluminea* collected from noncontaminated sites. However, in our experiments, clams were suspended above the sediments, which may have reduced the amount of food available to the clams, thus reducing growth rates. Furthermore, mesh sleeves containing clams sampled from all sites were usually covered with fine silt, which may have reduced the flow of food through the enclosures. A wide-pore mesh was used in the 1996 study, which should have prevented this from happening.

In our experience an overall trend of reduced DNA strand lengths with a wide range of responses usually occurs just prior to the observance of significantly shorter strand lengths in organisms exposed to genotoxic agents (unpublished data). This is precisely what occurred in the 1995 study after 2 week's exposure. We hope that with the increased exposure durations and number of clams exposed in the 1996 study (2,4,6 and 10 weeks), more significant DNA strand breakage may be observed.

CONCLUSIONS

Adult and juvenile Asiatic clams have promise as biomonitor organisms for *in-situ* studies; however, exposure times longer than 6 weeks may be necessary to observe significant effects on growth, CI and %TW. More work needs to be done to characterize and validate dose-response relationships between biomarker responses and exposures to chemicals, effluents, and natural waters. Finally, any potential deleterious effects of caging on exposed organisms must also be identified.

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