CONTROL STRATEGIES FOR THE ZEBRA MUSSEL, *DREISSENA POLYMORPHA*, AND THE ASIAN CLAM, *CORBICULA FLUMINEA*: COMPARATIVE STRESS RESPONSES AND NONTARGET IMPACT

by

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(ABSTRACT)

The studies described herein focused on the use of intermittent halogenation to control biofouling of water intake systems by the zebra mussel, *Dreissena polymorpha*, the comparative response of zebra mussels and the Asian clam, *Corbicula fluminea*, to a surfactant-based chemical control agent, the nontarget impact associated with the control agent, and the use of the Asian clam as a biomonitor of the control agent.

Effects of intermittent (2-4 hr/day) treatments with chlorine or bromine at levels of 0.5 and 1.0 mg/L (total residual oxidant) upon settling of zebra mussel veligers were examined in studies conducted in a field laboratory on western Lake Erie. Veliger densities in the water column at the field site peaked at 530/L, while mussel densities on settling monitors reached 147,083/m^2 over the course of the study period. Zebra mussel settling in test systems treated with the halogens was reduced by as much as 91% in comparison with controls, although mussel densities of up to 6,044/m^2 still occurred. Treated mussels which remained settled had growth rates similar to controls, and reached 2-4 mm length over 30 days. The intermittent halogen treatments had no significant impact on either adult zebra mussels or Asian clams. The studies indicate that while the treatment regimes may reduce zebra mussel densities within intake systems, the threat of eventual fouling due to cumulative settling remains.

In another series of laboratory and field experiments, zebra mussels and Asian clams were exposed to the cationic surfactant-based molluscicide DGH/QUAT under both static and flow-through conditions. Cumulative mortality of the two bivalves was comparable after 24-hr treatments, but zebra mussels experienced significantly higher mortality in 6-hr exposures conducted at 20-25 °C. The molluscicide induced an increase in tissue

water and a decrease in whole body glycogen levels of both organisms, although these changes occurred at a faster rate in the zebra mussel. While greater sensitivity of zebra mussels to DGH/QUAT may have been due to a longer time spent siphoning during the exposure periods, it may also indicate an enhanced ability of the Asian clam to tolerate the biochemical stress associated with exposure.

DGH/QUAT was toxic to nontarget organisms with acute LC_{50} values ranging from 0.12 - 11.0 mg/L, and chronic lowest observed effects levels ranging between 0.03 and 2.81 mg/L. The adsorptive potential of the surfactant actives in the compound led to differences in effects levels generated with static or flow-through test systems. This adsorptive nature may indicate that field monitoring of an effluent monitoring rather than the derivation of numerical criteria is the more appropriate strategy for regulation of the molluscicide, or other surfactant-based chemicals. Mixing DGH/QUAT with bentonite clay detoxified the molluscicide at ratios of 10:50 mg/L and higher, although the clay itself often had a negative impact on test organisms. Both laboratory and field studies demonstrated that a 50 % effluent dilution eliminated toxic effects of DGH/QUAT, but further indicated that its application may not be compatible with systems in which the effluent comprises the majority of flow.

Tissue water and whole body glycogen levels of Asian clams were not effective response parameters for early warning of the presence of low levels of DGH/QUAT, although the Asian clam has been used successfully in long-term studies that characterized the condition of receiving systems.

DEDICATION

This dissertation is dedicated to the memory of my brother,

John L. Bidwell
1946 - 1992

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1.0 PROLOGUE

The zebra mussel, *Dreissena polymorpha*, is a freshwater bivalve native to the Black and Caspian sea regions of Asia (Stanczykowska 1977). The mussel colonized most of Europe in the 19th century and was discovered in North America in 1988 (Hebert et al. 1989). Initially found in Lake St. Clair, it rapidly spread through the Great Lakes, with its range now extending to most of the larger river systems in the midwest and eastern United States (Mackie et al. 1989; Haag and Garton 1992; New York Sea Grant 1993). The predicted range of the zebra mussel includes southern Canada and most of the U.S., but may exclude southern regions where summer water temperatures approach 28-32 °C (McMahon and Tsou 1990; Strayer 1991).

The Asian clam, *Corbicula fluminea*, is also an introduced bivalve which occurs in North America. A native of Southeast Asia, the clam was first discovered in the Pacific Northwest in 1938, and presently occurs in most of the major drainage basins in the United States (Burch 1944; McMahon 1983). The northern range of Asian clams is limited by a lower temperature tolerance of ~ 2 °C (Mattice and Dye 1976; Cherry et al. 1980).

As with most other exotic plants and animals, the introduction and subsequent spread of zebra mussels and Asian clams has primarily been affected by man, with the ultimate success of these organisms attributed to a lack of ecological restraints in the newly invaded habitat (Morton 1979). For example, the spread of zebra mussels through Europe is attributed to the connection of waterways by an expanding canal network (Kerney and Morton 1970), while its North American introduction probably occurred through the

release of larvae in ballast water from a freighter that originated in a freshwater European port (Mackie et al. 1989; Griffiths et al. 1991; Mackie 1991). The retention of a byssus in the adult zebra mussel has further facilitated its spread, as the organisms can be ferried by attachment to boats or floating debris (McMahon and Tsou 1990; O'Neill and MacNeill 1991). Asian clams are thought to have been introduced into North America by Chinese immigrants who used them as food, with subsequent dispersal due to mechanisms such as the transport and release of water containing larvae, use of clams as bait, and the activities of tourists or aquarium hobbyists (McMahon 1983; Counts 1986).

Since their introductions, both zebra mussels and Asian clams have gained notoriety as pest species with the potential for environmental and economic impact. Ecological impacts are largely due to competitive interactions with other aquatic organisms such as unionid mussels. For further discussion of this aspect, the reader is referred to Kraemer (1974), McMahon (1983), Mackie (1991), Haag et al. (1991), and O'Neill and MacNeill (1991). The economic impact of the bivalves is largely the result of their ability to invade and foul water intake systems (Cherry et al. 1980; O'Neill and MacNeill 1991). This biofouling potential is facilitated by life history characteristics which include high reproductive output, free-living microscopic juvenile stages which are easily entrained by intake water, and rapid growth rates (McMahon 1983; McMahon and Tsou 1990).

Water intake systems provide ideal habitat for the bivalves, affording protection, a continuous supply of food, and often warmer temperatures (Mattice 1983; Klerks et al. 1993) In addition, characteristics of the adults enhance the organisms' ability to live in

this environment. The heteromyarian shell form and byssus of the zebra mussel facilitates existence in flowing water such as that found in intake lines (Morton 1979). Similarly, the infaunal nature of Asian clams allows them to colonize depositional areas within the system (Morton 1979).

Fouling is usually precipitated when live or dead individuals become lodged in pipes or condenser tubes and reduce or completely stop water flow (McMahon 1977). The byssus further exacerbates fouling by the zebra mussel since their attachment makes the organisms difficult to physically remove. In addition, the propensity of the mussels to attach to each other leads to accumulations that can further reduce the diameter of intake pipes, or to aggregations of mussels which slough off and cause clogging (Barton 1993; Kovalak et al 1993). Mackie (1991) stated that the byssate nature of the zebra mussel makes it one of the most tenacious fouling organisms ever introduced into North America. Indeed, the zebra mussel has had a rapid impact as a pest species, with severe fouling incidents reported within 3 years of its introduction into the U.S.. In contrast, Asian clams were present in the U.S. for over 20 years before causing a significant fouling problem (Lamarre 1991). Projected costs incurred by mussel fouling in the Great Lakes alone are estimated at $5 billion within the next decade (Lamarre 1991; Armor and Wiancko 1993). Nationwide costs associated with fouling by Asian clams is around $1 billion/year (Isom 1986).

In light of the potential impact zebra mussels and Asian clams can have on raw water users, the need for control measures is obvious. Similar control strategies have been used against both species and include physical removal or straining, heating system water, use

of coatings which are toxic or inhibit settling, and the application of chemical agents (Mattice 1983; McMahon 1983; Mackie et al. 1989; Armor and Wiancko 1993).

Presently, chemical control agents, particularly chlorine, are most commonly employed to control biofouling by the two organisms (Van Benschoten et al. 1993; Belanger et al. 1991). Continuous chlorination over the course of the spawning season has proven to be an effective method for control of both zebra mussels and Asian clams (Doherty et al. 1986; Belanger et al. 1991; Klerks and Fraleigh 1991; Fraleigh et al. 1993). However, current effluent guidelines for power plants limit chlorine discharge to a 0.2 mg/L total residual oxidant (TRO) concentration for 2 hr/day unless a dechlorination system is in place (USEPA 1980). This treatment has minimal effect on adult bivalves since they can detect chlorine at this level and avoid exposure through valve closure during the application period (Doherty 1986; Whitehouse et al. 1985; Jenner and Janssen-Mommen 1993).

While evidence from previous studies indicates that an intermittent treatment regime could be effective at controlling larval stages of the zebra mussel (Neuhauser et al. 1991, Barton 1993, and Klerks et al. 1993), none have fully addressed aspects such as settling rates and growth potential of the mussels in the face of the halogen treatments. The objective of the study presented in Chapter 2 of this dissertation was therefore to further evaluate the impact of intermittent halogen treatments upon settling, accumulation, and growth of zebra mussels. The study also sought to determine if the exposure regimes had any sublethal effects on adult zebra mussels and Asian clams. This latter aspect was of interest since any degree of impairment to the bivalves could be used to enhance other

control strategies.

While chlorine continues to be the biofouling control agent of choice, largely due to its combination of proven efficacy under certain exposure conditions (i.e. continuous applications) and general familiarity with application (Van Benschoten et al. 1993), concerns about the formation of carcinogenic byproducts or chlorine-induced corrosion of pipe systems continues to stimulate interest in developing alternative control agents (Klerks and Fraleigh 1991; McMahon et al. 1993). Among these alternatives are nonoxidizing chemicals, which have advantages in that they are relatively easy to apply, non-corrosive, and do not form toxic byproducts (McMahon et al. 1993).

In Chapter 3, the efficacy of DGH/QUAT, a nonoxidizing, cationic surfactant-based compound, was compared as a control agent for the zebra mussel and Asian clam. Since this chemical was initially developed for control of the clam, comparative data are important toward optimizing its use against the zebra mussel. Comparison of previous studies (e.g. Anderson et al. (1976), Fisher et al. (1991), Kraak et al. (1992)), demonstrates the potential for zebra mussels and Asian clams to exhibit differential sensitivity to certain chemicals. An examination of selected biochemical variables (tissue water, whole body glycogen) in the two organisms was also conducted following their exposure to DGH/QUAT. These data characterize the sublethal response to the chemical which can help clarify the reasons behind observed differences in sensitivity.

Regardless of the efficacy chemical agents may have against biofouling organisms, they all share the common liability of being nonspecific. Indeed, the current "holy grail" of industries producing biofouling control agents is specificity, and while the compounds

made are commonly registered for use as "molluscicides", they tend to be general biocides with the potential to impact non-target organisms (for the sake of continuity, DGH/QUAT will be referred to as a "molluscicide" in the upcoming chapters although no specificity is implied). In contrast to chlorine which has a large data base pertaining to its impact on nontarget organisms (USEPA 1985), there is limited data in this area for many alternative control agents. As a result, a number of nonoxidizing biocides, including DGH/QUAT, are currently under review by both federal and state regulatory agencies (Howe and Kaplan 1990; Howe et al. 1990; Magni 1990; Mondor and Howe 1993). The study presented in Chapter 4 summarizes research efforts which examined factors affecting availability of DGH/QUAT, impact upon selected non-target organisms, and detoxification potential through combination with bentonite clay. The detoxification aspect takes advantage of the adsorptive potential of surfactants and associated reductions in bioavailability which can occur when this binding takes place (i.e. Pittinger et al. 1989).

Finally, while zebra mussels and Asian clams are primarily known as pest species, they may have useful roles in biological monitoring, which is the process by which living organisms are used to detect pollutants in the environment (Rand and Petrocelli 1985; Cairns and Pratt 1993). Bivalves have been identified as particularly useful biomonitoring organisms because of their sedentary life style, ease in collection, and ability to accumulate pollutants (see reviews by Phillips 1977; Johnston and Hartley 1983; Doherty 1990). Because they are now widespread and abundant in the U.S., Asian clams have been used as biomonitoring organisms in place of native freshwater mussels, which are

becoming endangered or cannot be found in suitable numbers for testing (Foster and Bates 1978; Doherty 1990). Studies with the clams have examined bioaccumulation of contaminants (Rodgers et al 1979; Caldwell and Buhler 1983; Graney et al 1983; Modin 1969; Livingston et al 1978; Elder and Mattraw 1984), and response parameters such as body condition, mortality, impaired growth or reproductive output (Fritz and Lutz 1986; Belanger et al 1986; Farris et al. 1988; Graney and Giesy 1988; Shema et al. 1993).

In Europe, the zebra mussel has been used in bioaccumulation studies, and examination of valve closure and filtration rates also used to monitor water quality (Sloof et al. 1983; Neumann and Jenner 1992). One caveat with respect to conducting these studies with either zebra mussels or Asian clams is that, due to the obvious potential to assist in their spread, they should not be used to monitor systems in which they are not already present.

In Chapter 5, tissue water and whole body glycogen levels of Asian clams were monitored during exposure to low levels of the molluscicide DGH/QUAT. Biochemical parameters such as these have previously been used as "biomarkers" to detect sublethal pollutant stress in the clams (Anderson 1978; Giesy et al 1983; Cantelmo-Cristini et al 1985; Farris 1986; Sappington 1987; Graney and Giesy 1988; Farris et al 1988). Cairns and Niederlehner (1990) also stated that advantages of biomarkers include quick response to pollutants and a potential to provide early warning of contamination. The objective of the study was therefore to determine if the two biochemical variables could be used to detect potentially harmful levels of molluscicide, and thus be used to monitor the receiving system during on-site applications. Because of a relatively short-lived free form

in the environment (Lewis and Wee 1983; Cooper 1988), field monitoring of surfactant based contaminants may also provide a more viable regulatory approach over standardized laboratory tests.

2.0 EFFICACY OF INTERMITTENT HALOGENATION FOR ZEBRA MUSSEL CONTROL

2.1 Introduction

Since its introduction into North America in 1988, the zebra mussel, *Dreissena polymorpha*, has become established as a major pest species due to its potential to foul raw water intake systems. This aspect is facilitated by the mussel's free swimming larval stage (veliger) which is easily entrained by intake water. Once in the system, larvae can settle and, by means of a byssus, securely attach to hard surfaces (Mattice 1984; Barton 1993). Accumulation and growth of the mussels within a water system can lead to a reduction in pipe diameter and impedance of water flow. The industrial implications of a zebra mussel infestation include the loss of electrical generating efficiency, loss of fire protection, and an increase in pipe corrosion rates due to a buildup of bacteria and slime associated with the attached mussels (Dexter 1985; Lamarre 1991; O'Neill and MacNeill 1991). Municipal water plants can also be affected through reductions in water pumping efficiency or impacts on water quality resulting from mussel dieoffs and decay (Clarke 1952; Jenner and Janssen-Mommen 1993).

Currently, the most common technique employed for control of zebra mussels in North America and Europe is the application of chlorine (Mackie et al. 1989; McMahon and Tsou 1990; Van Benschoten et al. 1993). Continuous chlorination over the course of the spawning season has proven to be an effective method for control of both adult zebra mussels and settling postveliger larvae (Jenner 1985; Klerks and Fraleigh 1991; Fraleigh et al. 1993). However, current effluent guidelines for power plants limit

chlorine discharge to a 0.2 mg/L total residual oxidant (TRO) concentration for 2 hr/day unless a dechlorination system is in place (USEPA 1980). Previous studies have demonstrated that adult zebra mussels can detect this level of oxidant and avoid exposure through valve closure during the 2-hr application period (Clarke 1952; Whitehouse et al. 1985; Jenner and Janssen-Mommen 1993), although an on-site evaluation conducted by Barton (1993) has indicated that this treatment regime could be effective at preventing settlement of the postveliger stages.

The objective of the present study was to further evaluate the impact of intermittent (2 hr/day) chlorination upon settling, accumulation, and growth of zebra mussel larvae. Similarly, the efficacy of bromination, a treatment strategy which has been presented as an alternative to chlorine (Mills 1980; Fellers et al. 1988), was also evaluated. Finally, the effect of the halogen treatments on growth and whole body glycogen levels of adult zebra mussels was examined to characterize potential sublethal stress associated with the exposure regimes.

2.2 Methods

2.2.1 Test Site

Experiments were conducted in a field laboratory positioned next to the water intake of a power plant on the western basin of Lake Erie, Monroe County, MI. The forebay area immediately in front of the plant intake was surrounded by land on three sides, with open access to the lake on the south. The laboratory was fitted with 12 flow-through exposure systems (Fig. 2-1) which were joined by a common header and received a

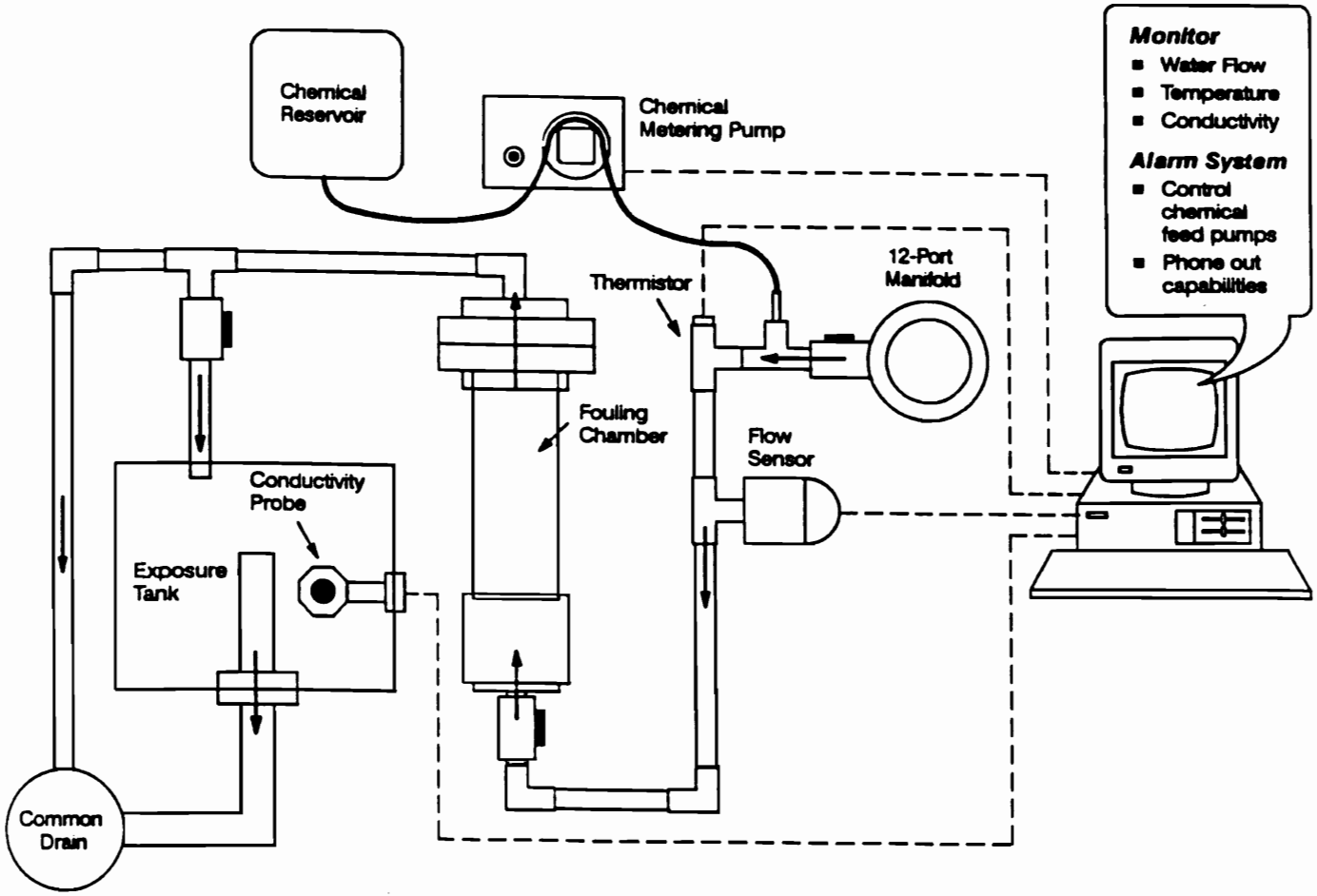


Figure 2-1. Schematic of the flow-through system in which the halogen studies were conducted.

constant supply of Lake Erie water pumped from ~ 3 m in the forebay. Each loop included a 38-L polypropylene treatment tank and a fouling chamber into which a concrete test panel (9 cm width x 30 cm length x 2 cm thick) was placed to track zebra mussel accumulation. Water flow through the treatment tanks was maintained at 38 L/min.

2.2.2 Intake Bay Surveillance

Replicate 1-L water samples were collected weekly from the intake bay at depths of 3 and 5 m for determination of zebra mussel veliger densities. Water sample collection and veliger enumeration followed that described by Mackie (1991a). Microscope slides were suspended in groups of 5 on plastic racks (Wildco Periphytometer) at 3 and 5 m (2 racks/depth) for determining weekly and cumulative larval settling in a method following that described by Marsden (1992). For weekly settling, 2 slides from each rack were collected and the number of accumulated mussels were counted with the aid of a dissecting microscope. New slides were then placed in the racks for the next week's collection. The 3 remaining slides in each rack were left to determine 30 and 60 day accumulation potential. For these longer-term accumulation slides, total counts and length distributions of mussels greater than 1 mm were determined. Length measurements were made to the nearest 0.01 mm with a digital vernier caliper. Slide racks were initially suspended on 6 July 1991.

In addition to the microscope slides, cement panels (10 cm x 20 cm x 2cm, 5/depth) were suspended at 3 and 5 m near the intake to further assess mussel accumulation and

growth. A 24 cm^2 subsample from each panel was collected on days 42, 60, and 100, with densities and length frequencies determined as described for the microscope slides. The cement panels were initially deployed on 11 June 1991.

2.2.3 Halogen Treatments

Daily 2-hr treatments of 0.5 mg/L chlorine, 1.0 mg/L chlorine, and 0.5 mg/L bromine were administered to each of two replicate side-streams, and two streams served as controls. Unless otherwise stated, all halogen levels, including those from other referenced studies, are reported as total residual oxidant (TRO). Halogen applications lasted for 30 days, from 5 July to 5 August 1991. Stock solutions of chlorine and bromine were prepared in 9 L Nalgene carboys and administered to the streams via peristaltic pumps through an injection port located upstream of the fouling chamber. Household bleach (5.25% sodium hypochlorite) was used as the primary chlorine stock, while bromine solutions were prepared by mixing 40% sodium bromide with bleach in a 1:1 molar ratio. Fresh stock solutions were prepared every third day over the course of the study.

Oxidant residuals were measured three times during the 2-hr applications using a DPD colormetric procedure (Hach Chemicals). Target levels were maintained by adjusting peristaltic pump flows as necessary. Temperature, pH, and conductivity of the incoming water was monitored by remote sensor and recorded every six hours for the duration of the study.

A second halogen study was initiated on 14 August and continued through 28 September 1991. This study was essentially a repeat of the first with the addition of a 0.5 mg/L chlorine treatment which was administered for 4 rather than 2 hrs/day, and a 1 mg/L bromine treatment (2 hr/day). Dosing was done as described for the first study.

Settling of Zebra Mussels

The impact of the halogen treatments on settling of zebra mussel juveniles and larvae was monitored on groups of 5 glass microscope slides which were suspended on plastic racks in each treatment tank. Monitoring of larval settling proceeded as described for surveillance of the intake bay i.e., 2 slides taken from each tank (4/treatment) on a weekly basis, and the remaining slides used to monitor accumulation and growth of mussels over the entire study.

Survival, Growth, and Glycogen Levels of Adult Zebra Mussels and Asian Clams

While it was assumed that the intermittent halogen treatments would have little impact on the survival of adult mussels, this objective was designed to characterize possible sublethal effects which could have been indicative of low level stress. Subjecting the mussels to a long-term low level stress could increase susceptibility to other control strategies such as heat treatment or a one time application of other chemical agents. In the first study, growth of zebra mussels alone was monitored, while in the second the response of mussels was compared with that of the Asian clam, *Corbicula fluminea*, another freshwater fouling bivalve which occurs in North America.

In the second study, whole body glycogen was determined for zebra mussels and Asian clams from selected halogen treatments. For zebra mussels, only individuals from the initial 5-7 mm size group were used for determination of these variables.

Prior to glycogen determination, clam or mussel tissues (n=6) were dissected, frozen, and stored in liquid nitrogen. The glycogen assay followed a procedure described by Roehrig and Allred (1974). Tissues were first homogenized in 0.05 M phosphate buffer (pH 4.8; 500 ml/g dry tissue) with a Tekmar Ultra-Turrax homogenizer at 15,000 rpm for 15 seconds. Following a 20 min centrifugation at 5,000 x g, the supernatant was drawn off and the remaining tissue pellet was dried at 60 °C for 24 hrs. Duplicate 100 μl aliquots of supernatant were incubated with 3.5 units of Amyloglucosidase (Sigma Chemical Co.) and phosphate buffer (final volume 0.55 ml) for 2 hrs at 37°C. Glycogen standards (from *Mytilus edulis*) containing 5 to 80 μg glycogen were assayed as described for the samples. Following the enzymatic conversion of glycogen to glucose, 0.5 ml aliquots of sample or standard were incubated with a freshly prepared oxidase-peroxidase dye reagent specific for glucose determination for 30 min at 37°C. Absorbance was measured at 450 nm, and total concentrations were determined from a glycogen standard curve. Free glucose present in the tissues was determined by incubating supernatant aliquots without amyloglucosidase, and then assaying for glucose as previously described. True glycogen content was determined by subtracting free glucose from the total concentrations and expressed as percent dry weight.

Test Organisms

Zebra mussels were collected from concrete blocks which had been suspended in the plant forebay for approximately 2 months prior to the start of the study. Mussels were separated by cutting byssal threads with a scalpel to avoid injury to the organisms. Due to their limited availability in the vicinity of the study site, Asian clams were collected from the New River near Narrows, VA, wrapped in moist paper towels, and shipped in beverage coolers to the Lake Erie field laboratory via overnight mail. This method of shipping has previously been shown to exert minimal stress on the organisms (Cherry et al. 1991). The clams were acclimated to Lake Erie water for at least 2 weeks prior to testing.

Two size classes of zebra mussels with initial shell lengths of either 6-7 or 12-13 mm were used for this aspect of the study. The mussels were separated into subgroups of 10 individuals, and their initial lengths were measured to the nearest 0.01 mm with a digital vernier caliper. A total of 8 subgroups (4/treatment tank) were exposed to each treatment regime, with growth determinations based on the difference between the average initial and final lengths of these groups. In each treatment tank, mussels were held in duplicate polystyrene mesh baskets (30.5 cm x 5 cm x 7.2 cm) which were subdivided into 4 compartments (3.8cm x 5.0cm x 7.2cm) to hold each of the subgroups. Any mussels that did not secrete new byssal thread and securely attach within the baskets within 24 hrs were removed and replaced with a new individual prior to the start of the test.

Initial Asian clam lengths ranged between 10 and 13 mm. Determination of initial lengths and subgroup size followed that described for zebra mussels, although clam

subgroups (10 individuals) were held in nylon mesh baskets which were suspended in the treatment tanks.

2.2.4 Statistical Analyses

All statistical procedures were conducted with a Statistical Analysis Package (SAS 1988). Normality of data was first tested using the Shapiro-Wilk procedure. Data not having a normal distribution (settling data from both studies) were analyzed using a nonparametric Kruskal-Wallis k-sample test followed by multiple comparison with a rank version of Fisher's Protected LSD. Analysis of variance was conducted on normal data (growth data) with a general linear model (GLM), followed also by multiple comparison using Fisher's Protected LSD. Prior to parametric analysis, percentage data from the glycogen assay were transformed by arcsine-squareroot transformation (Zar 1984). All comparisons were conducted at $\alpha=0.05$.

2.3 Results

2.3.1 Intake Bay Surveillance

Water temperatures of the plant intake area ranged between 13.4 and 29.2 °C over the course of the monitoring period (Fig. 2-2A). Temperatures generally stayed above 24 °C until dropping sharply (from 24 to 16°C) between 14 and 21 September 1991. No differences in veliger densities were observed between samples taken from 3 and 5 m, so the data were combined. Maximum veliger densities occurred during the first study, with peaks of 298 and 530 larvae/L observed on 13 and 27 July 1991, respectively.

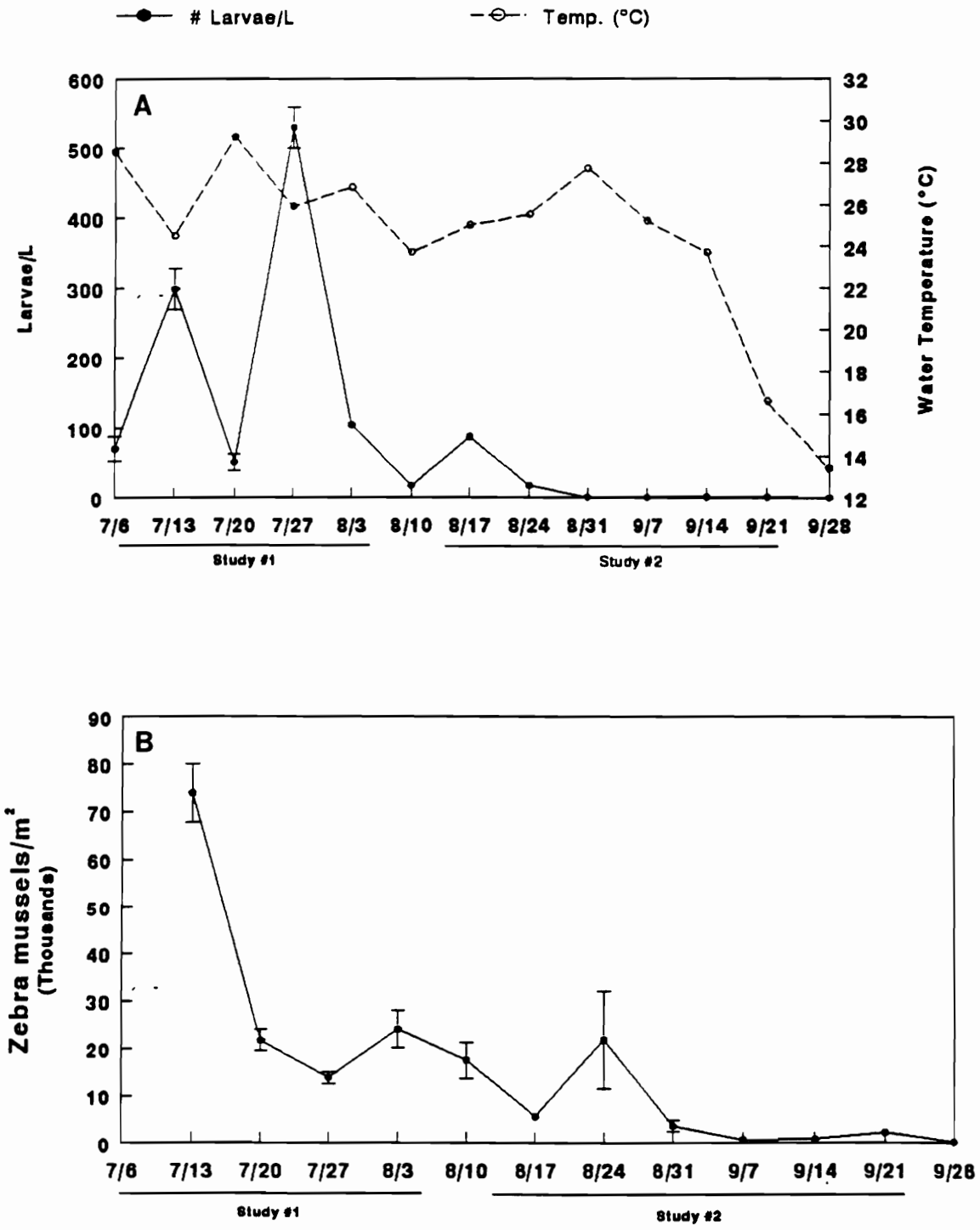


Figure 2-2. Average (± 1 SEM) zebra mussel veliger densities and water temperature (A), and densities of settled mussels on glass microscope slides (B) from the forebay of the power plant.

Larval densities declined between 27 July and 10 August, followed by a third peak of 87/L on 17 August. Densities remained low after this time, averaging less than 1 organism/L through September. No veligers were detected on 28 September 1991, the last day of sampling.

As with the veliger densities in the water column, settling rates of the organisms on glass slides suspended at the intake were highest during the first study (Fig. 2-2B). Settling data from the two sampling depths were similarly combined for presentation and analysis. The maximum density of 73,973 mussels/m^2 was observed on 13 July 1991, the first sampling date of the monitoring period for larval settling. A second peak of 24,080/m^2 was observed on 3 August, followed by a final increase to 21,866/m^2 slide on 24 August. The initial peak in larval settling on 13 July directly coincided with high veliger densities in the water column, while those which occurred on 3 and 24 August followed weeks in which larval densities in the water column had peaked (Fig. 2-2A and B).

Most of the larvae on the slides between 13 July and 24 August appeared to be new postveligers which were settling for the first time. However, from 31 August through the end of the monitoring period, the mussels found on the weekly slides were more often of a larger size class (> 1mm), indicating they may have been individuals exhibiting secondary movement onto the slides from another point of initial attachment.

Average densities ($\pm$ 1 SEM) of zebra mussels on glass slides left in the racks to monitor cumulative settling were 67,700/m^2 (4,612) and 70,533/m^2 (6,533) on days 30 (8/5/91) and 60 (9/4/91), respectively. These values were not significantly different

between the two periods, indicating that settling on the slides may have leveled off after some point. Unfortunately, due to the loss of slides over the course of the monitoring period, samples could not be taken beyond day 60.

Shell lengths of the zebra mussels measured on the 30-day slides ranged from 1 to 12 mm (only mussels $\geq$ 1 mm measured). The majority of these individuals (49 %) fell in the 2 - $<$4 mm grouping followed by the 4 - $<$6 mm size class (Fig. 2-3). Again, the presence of larger mussels on the slides may be indicative of secondary movement by previously settled mussels rather than instances of increased growth in certain individuals. Zebra mussels from the 60-day slides ranged from 1 to 16 mm in length. Compared to those from day 30, the lengths from the 60 day samples had a more even distribution over the various length groupings, an effect which is probably due to the sequential settling and growth of larvae. A majority of individuals sampled fell into the 10 - $<$12 mm group, although this only accounted for 28.6 % of those measured.

Average mussel densities ($\pm$ 1 SEM) on cement panels from the plant intake were 57,000 (5,204), 125,000 (17,625), and 147,083/m^2 (3,041), on days 42 (7-23-91), 60 (8-10-91) and 100 (9-20-91), respectively. The high variability associated with the samples from day 60 were the result of growth of a freshwater bryozoan on certain panels which decreased mussel densities on them. The percentage of mussels falling into higher size classes increased with time (Fig. 2-4). For the 42-day samples, 55% of the individuals measured fell between 4-8 mm. Mussels from the 60-day samples were evenly distributed between 2-10 mm, with a significantly higher percentage of individuals between 10-14 mm as compared to the day 42 samples. The range of shell lengths measured in the day

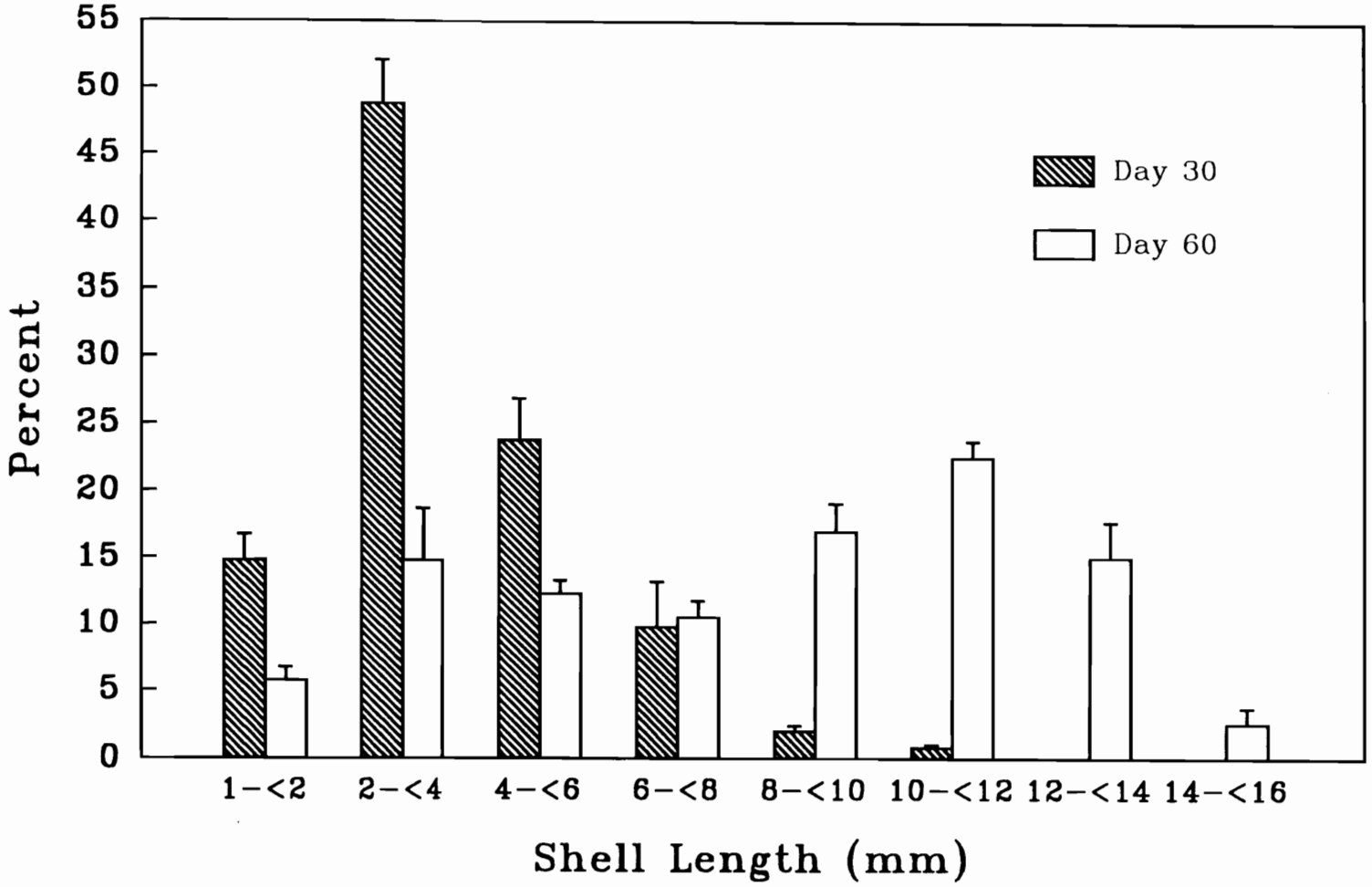


Figure 2-3. Average shell length distribution (± 1 SEM) of zebra mussels (≥ 1mm) taken from glass slides suspended near the power plant intake for 30 or 60 days.

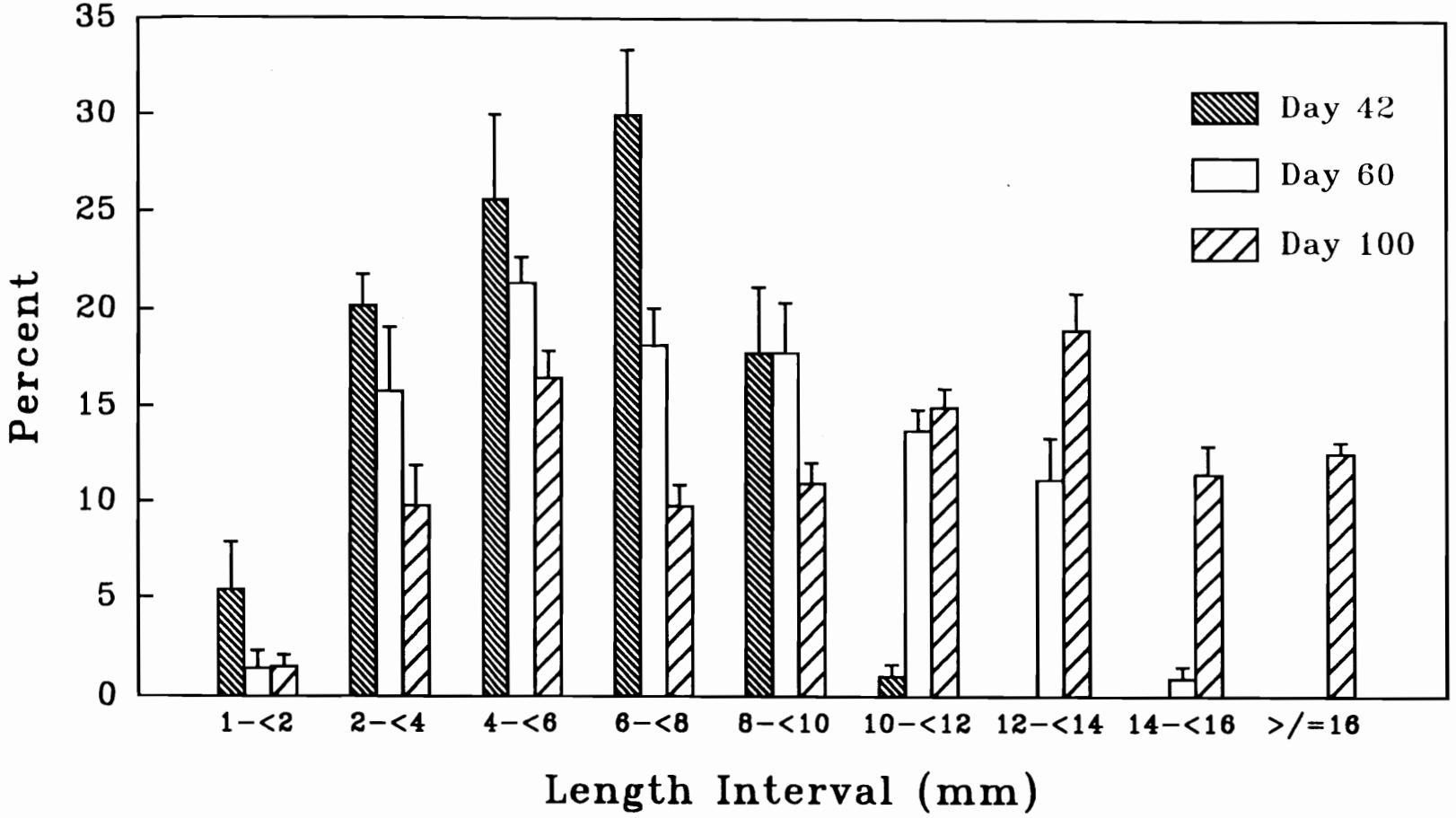


Figure 2-4. Average shell length distribution (± 1 SEM) of zebra mussels (≥ 1mm) subsampled from cement panels suspended near the power plant intake.

100 samples extended to 22 mm. The majority of mussels from these samples fell between 2 and 16 mm with a significant peak at 12 - <14 mm. Once again, the even distribution of zebra mussels over the respective length ranges of both the 60 and 100 day samples was maintained by the presence of individuals in the smaller size classes.

Densities and length distributions of zebra mussels from the day 60 glass slides and cement panels were analyzed together in order to compare the two monitoring methods. Densities of mussels from the cement panels were significantly higher than those obtained on the glass slides (125,000/m^2 $\pm$ 17,625 vs 70,533/m^2 $\pm$ 6,533) even though the surface area sampled on the panels (37 cm^2) was smaller than the total area of the microscope slides (25 cm^2). Length distributions of mussels from the two substrates were similar, extending to 16 mm and with a relatively even distribution over the size classes as previously discussed (Fig. 2-3 and 4).

2.3.2 Halogen Treatments: Study #1

On 2 out of the 4 weeks in Study # 1, as well as for the average weekly settling rate, zebra mussel densities on glass slides suspended at the plant intake were significantly higher than those from the laboratory control treatment tanks (Table 2-1). The halogen treatment which most consistently reduced the weekly settling of postveligers on the slides was the 1.0 mg/L chlorine. Numbers of mussels in this treatment were significantly lower than controls on 3 of 4 weeks in the study, with the exception of week 3 when the control system became fouled by a bryozoan in the water lines that fed the control treatment tanks. This reduced water flow and probably sieved out the veligers.

Table 2-1. Weekly zebra mussel densities on glass microscope slides from Study # 1. Treatments were administered as daily intermittent (2 hr/day) doses over a 4 week period. The asterisk (*) indicates densities significantly different from controls at α=0.05.

	Mean Larval Density/m^2 (± 1 SEM)				
Treatment	Week 1 7/13/91	Week 2 7/20/91	Week 3 7/27/91	Week 4 8/3/91	Settling Rate (Avg. Density/week)
Forebay	73,966* (6,121)	21,766 (2,261)	14,333* (1,102)	24,066 (3,869)	34,885* (5,153)
Control	20,466 (3,241)	18,600 (4,308)	7,066 (1,674)	13,466 (2,542)	14,900 (1,928)
0.5 mg/L Bromine	17,000 (1,933)	9,466 (3,292)	15,466* (2,751)	9,066 (2,054)	12,750 (1,465)
0.5 mg/L Chlorine	8,333* (1,587)	11,666 (2,974)	8,866 (665)	9,666 (1,677)	9,633 (915)
1.0 mg/L Chlorine	1,866* (326)	6,466* (3,687)	4,866 (367)	5,533* (802)	4,683* (960)

To at least some degree, all three halogen treatments reduced fouling of the glass slides by other invertebrates such as the bryozoan and in certain cases this appeared to actually facilitate zebra mussel settling. Mussel densities in the 0.5 mg/L chlorine treatment were significantly lower than controls only during week 1 of the study, while weekly densities in the bromine treatment were never significantly different from controls. In fact, during week 3 of the study, densities of postveligers on slides in the bromine treatments were significantly higher than those from the control.

Perhaps more important than the weekly effects of the halogen treatments were those on glass slides which were left in the tanks for the entire 30-day study (Table 2-2). There was a problem with these data in that a significant difference in the densities of mussels on slides from the two control tanks was observed.

Densities on control "A" were similar to those on slides from the intake (67,933 vs 67,200 mussels/m^2 for control and intake respectively), while control "B" had an average of only 15,022/m^2. One explanation for this effect may be that the input line to control "B" was subject to a greater degree of bryozoan fouling until it was cleaned on week 3. This may have consistently reduced the number of larvae entering the tank and in turn settling on the slides. However, no significant differences between the control tanks were observed in the weekly settling data. The mussels on the slides in control tank "B" may have exhibited a higher rate of movement off the slides as compared to control replicate "A". It was noted that because of its positioning along the instream header, control replicate "B" was at times prone to siltation by the highly eutrophic Lake Erie water.

Table 2-2. Cumulative densities of zebra mussels on glass microscope slides after intermittent (2 hr/day) treatments with chlorine and bromine over a 30-day period. Treatments which do not share at least 1 common letter are significantly different at $\alpha = 0.05$.

Treatment	Mean Mussel Density/m^2 ($\pm$ 1 SEM)
Forebay	67,200 (4,612)**A**
Control A	66,933 (9,986)**AB**
Control B	15,022 (2,003)**CD**
Control A+B	40,977 (12,469)**BC**
0.5 mg/L Bromine	21,822 (4,002)**C**
0.5 mg/L Chlorine	10,133 (2,121)**DE**
1.0 mg/L Chlorine	6,044 (1,066)**E**

In light of the discrepancy between the two control replicates, the data from the treatment tanks were compared against control "A" alone, control "B" alone, and controls "A" and "B" combined (Table 2-2). The densities from control replicate "A" may be more appropriate to compare the treatments against since they more closely match densities on the intake slides. When compared to control A alone, all halogen treatments caused a significant reduction in mussel settling. The order of efficacy in terms of % reduction from control was 1.0 mg/L chlorine (91 % reduction) > 0.5 mg/L chlorine (85 %) reduction > 0.5 mg/L bromine (67 % reduction). When compared to the combined control data, only the densities from the chlorine treatments were significantly reduced, and when compared to control replicate B alone, only the 1.0 mg/L chlorine was significantly reduced. Regardless of comparison or statistical difference from control, all halogen treatments had mussels on the 30-day cumulative slides in densities which ranged from 6,044/m^2 (1.0 mg/L chlorine) to 21,822 /m^2 (0.5 mg/L bromine).

The two chlorine treatments may have had a mild effect on the length distributions of mussels from the 30-day cumulative laboratory slides. A significantly higher percentage of individuals from these treatments fell in the 1- <2 mm size class, while higher percentages of mussels from the intake, control, and 0.5 mg/L bromine treatment fell in the 4- <6 mm size class and higher (Fig. 2-5). However, in terms of the overall length distributions, the highest percentage of individuals, regardless of treatment, were in the 2- <4 mm size range, indicating the majority of the mussels were not affected by the chlorine treatments.

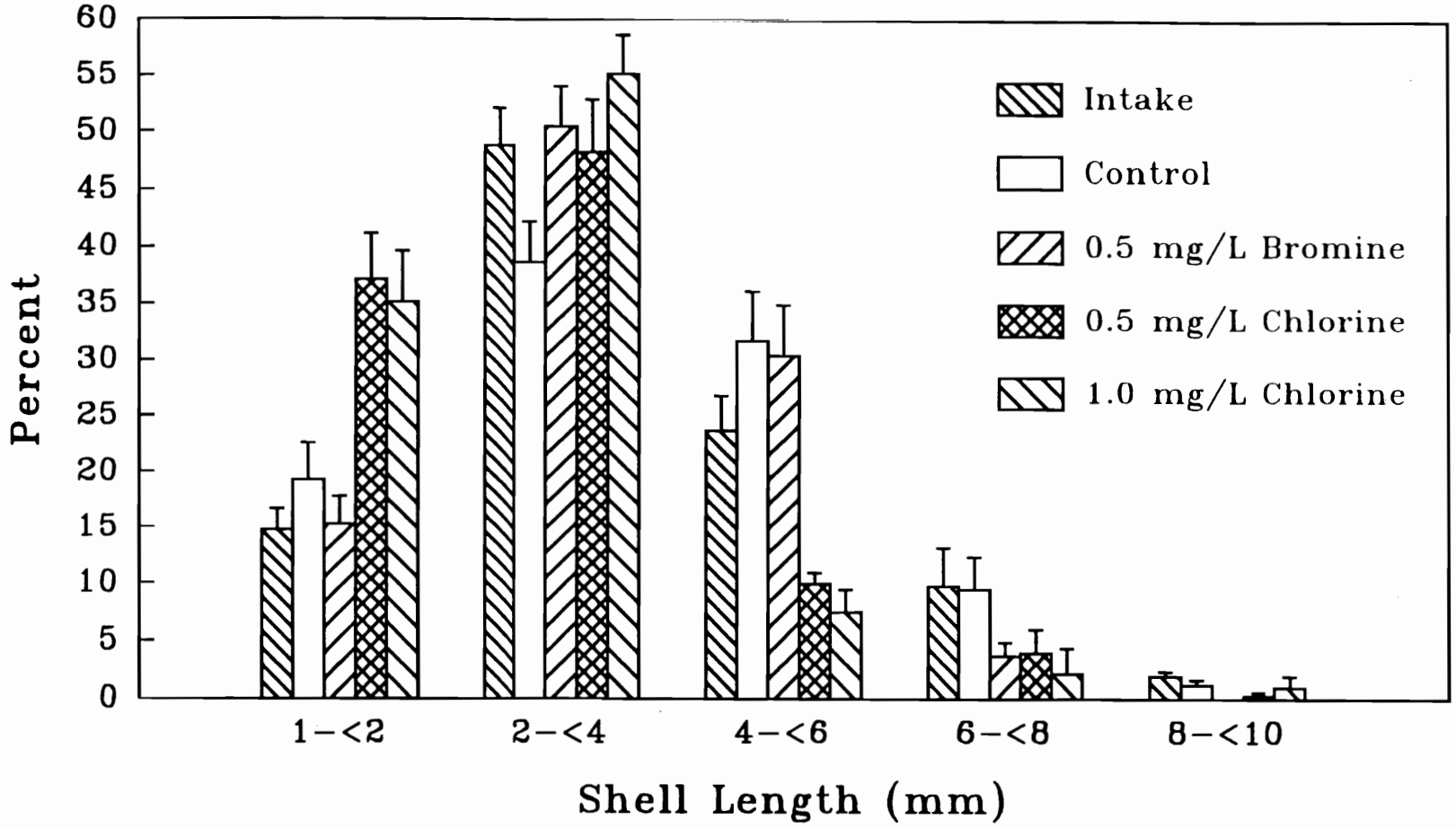


Figure 2-5. Average shell length distribution (± 1 SEM) of zebra mussels (≥ 1mm) taken from glass microscope slides which received intermittent (2 hr/day) treatments with chlorine or bromine for 30 days.

Zebra mussel densities were also determined on 24 cm^2 subsamples taken from the cement panels which had been in the fouling chamber of each sidestream system during the 30 days of Study # 1 (Table 2-3). Unfortunately, breakage of two sample containers led to the loss of one replicate from the bromine and 1.0 mg/L chlorine treatments. As a result, no statistical analysis of the data was possible. Perhaps the most outstanding aspect with respect to these data was that, in contrast to the 30-day cumulative slides, there appears to be no significant difference in mussel densities on the cement panels from each treatment. In fact, the one sample from the 1 mg/L chlorine treatment, which had the lowest density on the microscope slides, had a higher density of mussels than the control (67,500 mussels/m^2 vs an average of 60,625/m^2 in the control). Some differences were also found between the length distributions of mussels from the laboratory slides (Fig. 2-5) and the cement panels taken from the fouling chambers (Fig. 2-6). The majority of mussels from the cement panels fell in the 4 - < 6mm size class while most of the mussels from the slides were 2 - < 4mm. In addition, the range of lengths on the cement panels extended to 16 mm vs 10 mm on the slides. There was also a rather high percentage (37%) of mussels on the cement panels from the 0.5 mg/L bromine treatment which fell in the 6 - < 8mm size range, although these data represent only 1 replicate sample.

The halogen treatments had no significant impact on growth of either size class of adult zebra mussel tested, with all exhibiting a length increase over the course of Study # 1. The increments by which the mussels grew are presented in Figure 2-7. The smaller zebra mussels experienced average increases in shell lengths that ranged from 5.96-7.39

Table 2-3. Average densities of zebra mussels (≥ 1 mm) subsampled from cement panels held in the fouling chambers of the field laboratory. The treated panels received intermittent doses (2 hrs/day) of bromine or chlorine over a 30-day period.

Treatment	Mean Mussel Density (± 1 SEM)	
	No./sample	No./m^2
Control	145.4 (7.5)	60,625 (3,125)
0.5 mg/L Bromine[1]	150	62,500
0.5 mg/L Chlorine	154.0 (3.0)	64,167 (1,250)
1.0 mg/L Chlorine[1]	162	67,500

[1] = only 1 replicate

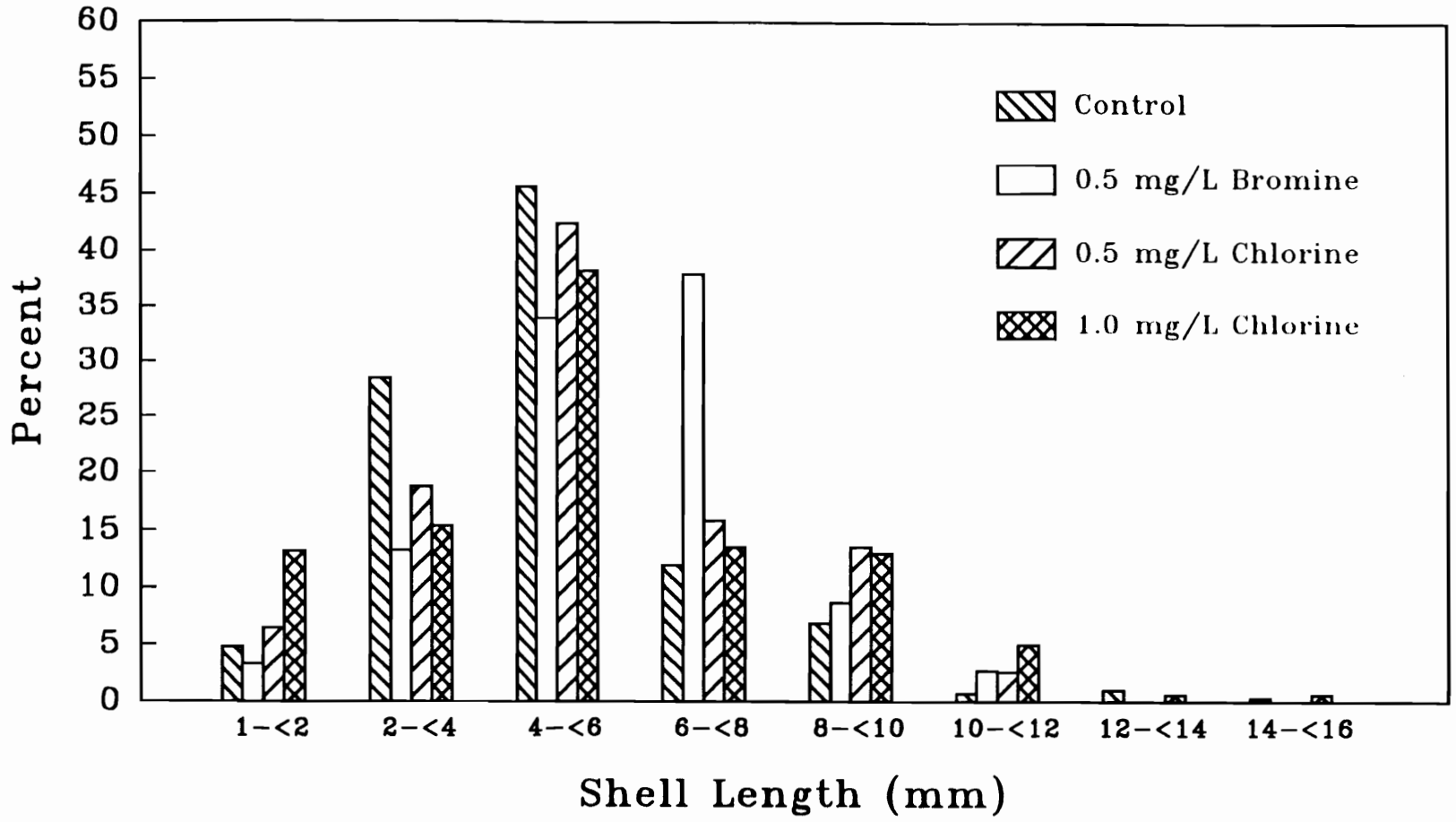


Figure 2-6. Shell length distributions of zebra mussels (≥ 1mm) subsampled from cement panels which received intermittent (2 hr/day) treatments with chlorine or bromine for 30 days.

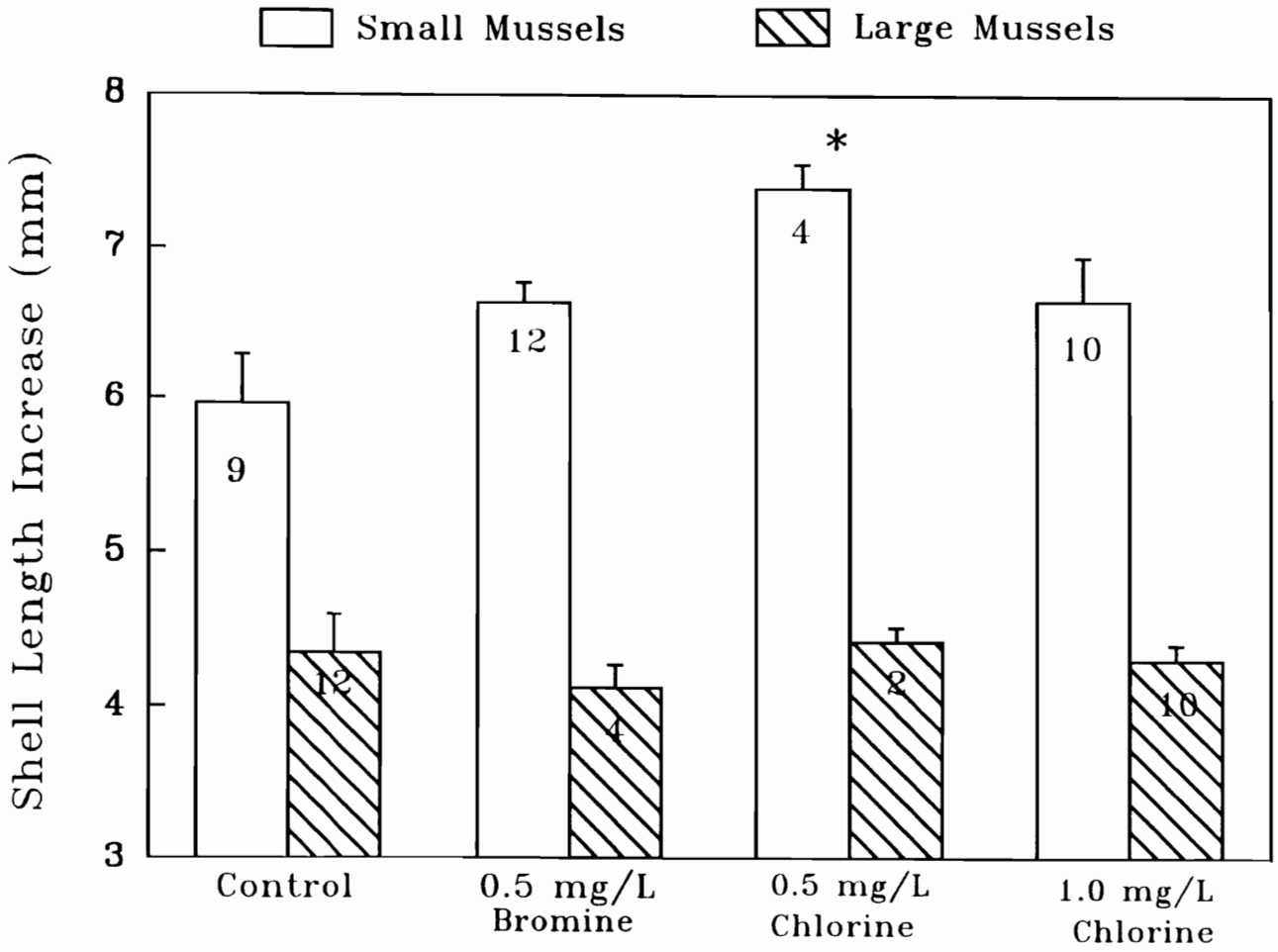


Figure 2-7. Average shell growth (± 1 SEM) for small (6.4-7.4 mm initial length) and large (12.3-12.8 mm initial length) zebra mussels which received intermittent (2 hr/day) halogen treatments in Study # 1. The asterisk (*) indicates significant difference from controls at $\alpha =$ 0.05.

mm, while large mussels grew an average of 4.12-4.35 mm. As determined by comparing the respective increase in shell length, small mussels grew significantly more than large ones. Only one significant treatment difference was observed; the small mussels exposed to 0.5 mg/L chlorine grew more than the controls with an average length increase of 7.39 mm over the 30 day period.

As expected, daily exposure to the halogens had no significant effect on survival of the mussels, with mortality ranging from 2-12 % (Fig. 2-7).

2.3.3 Halogen Treatments: Study #2

Densities and settling of veliger larvae were at detectable levels for the first 2 weeks of Study # 2, but then dropped off sharply. The study was extended to 45 days in an attempt to catch another peak in veliger densities in the early fall. Weekly water samples were taken through the middle of October and no increase in veliger densities was observed. While the larval densities were relatively low during the first two weeks of Study # 2 as compared to Study # 1, there was still sufficient settling on the laboratory slides to compare densities between the treatments. After this time however, settling of larvae within the laboratory system became limited and highly variable. As a result, only data from the first 2 weeks of Study # 2 could be used to compare the impact of the treatments upon veliger settling.

Settling data from weeks 2 and 6 of Study # 2 are presented in Table 4. At the end of week 2, all halogen treatments had significantly reduced zebra mussel densities on the glass slides as compared to controls. The order of efficacy in this study in terms of %

reduction from controls was 0.5 mg/L chlorine, 4 hrs (91% reduction) > 1.0 mg/L chlorine, 2 hrs (80% reduction)= 1.0 mg/L bromine, 2 hrs (80% reduction) > 0.5 mg/L bromine (62 % reduction) > 0.5 mg/L chlorine, 2 hrs (56 % reduction). While most of the treated slides which had been left in the tanks for the entire 6 weeks of the study had appreciably lower mussel densities than the control (Table 2-4), no statistical differences were observed due to high variability between the replicates. Mussel densities on cement panels from the fouling chambers were subject to similar variability.

Growth and survival of adult zebra mussels and Asian clams were determined after 30 days in Study # 2. As in study # 1, smaller zebra mussels grew significantly more than either larger mussels or Asian clams, with the 3 groups exhibiting increases in shell length that ranged from 6.28-6.78 mm, 4.15-5.74 mm, and 1.72-2.34 mm, respectively (Fig. 2-8). Both groups of zebra mussels grew significantly more than did the Asian clams. All chlorine treatments had a significant impact on growth of large mussels with daily 4-hr exposures to 0.5 mg/L chlorine significantly affecting small mussels and Asian clams as well.

There was no significant impact on survival of any of the adult bivalves. The highest level of mortality observed (14%) occurred in the control Asian clams, and may have been due to siltation within the treatment tank.

Whole body glycogen levels were determined for small zebra mussels and Asian clams which had been exposed to the 4 hr daily treatment with 0.5 mg/L chlorine. While these organisms experienced some growth impairment due to the halogen exposure, whole body glycogen levels were not affected. Average ($\pm$ 1 SEM) glycogen levels (as

Table 2-4. Zebra mussel densities on glass microscope slides from Study # 2. Halogens were administered as daily intermittent (2 or 4 hr/day) doses over a 6 week period. The asterisk (*) indicates densities significantly different from controls at α=0.05.

Treatment	Mean Larval Density/m^2 (± 1 SEM)	
	Week 2 8/28/91	Week 6 9/27/91
Forebay	10,285 (2,016)	190 (151)
Control	7,800 (887)	2,400 (1,357)
0.5 mg/L Bromine	3,000 (212)*	567 (264)
1.0 mg/L Bromine	1,566 (320)*	300 (146)
0.5 mg/L Chlorine (2 hr)	3,400 (491)*	2,600 (1,129)
0.5 mg/L Chlorine (4 hr)	667 (202)*	300 (228)
1.0 mg/L Chlorine	1,533 (206)*	167 (100)

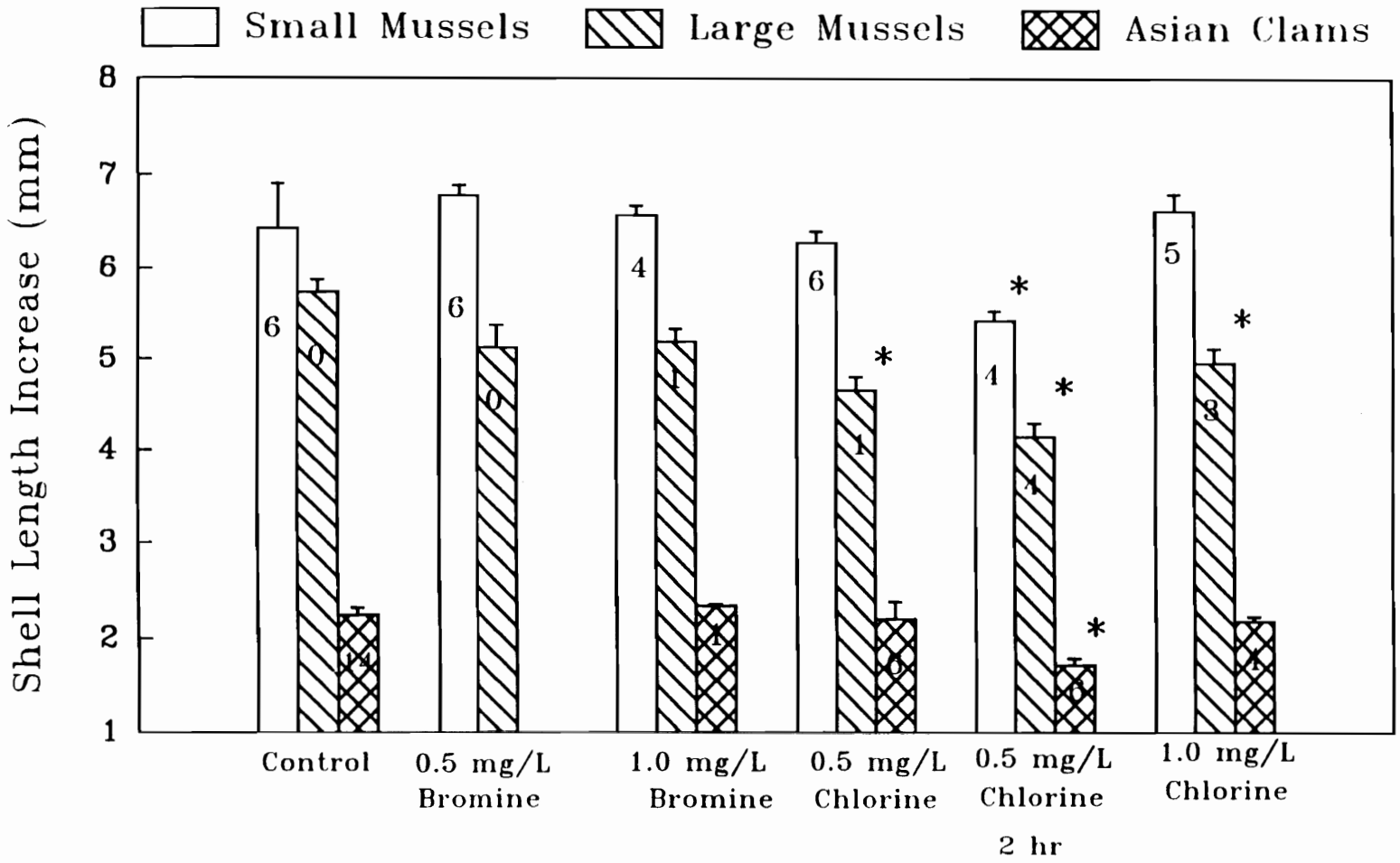


Figure 2-8. Average shell growth (± 1 SEM) for small (8.0-8.2 mm initial length) and large (13.0 mm initial length) zebra mussels and Asian clams (11.8-12.1 mm initial length) which received intermittent (2 or 4 hrs/day) halogen treatments in Study # 2. The asterisk (*) indicates significant difference from controls at $\alpha = 0.05$, and numbers in the bars are percent mortality for the group.

% dry weight) for zebra mussels were 11.9 (4.8) and 14.3 (3.4) % for control and treated groups, respectively. For the Asian clams, glycogen levels of controls averaged 20.4 (2.7) %, while that of the chlorine exposed group was 19.8 (1.6) %.

2.4 Discussion

2.4.1 Forebay Surveillance

Previous studies in Lake Erie reported the first appearance of zebra mussel veliger larvae when water temperatures reached 18 °C (Van Cott et al 1992; Fraleigh et al. 1993a; Garton and Haag 1993). Water sampling for veligers in the present study was not aimed at following mussel population dynamics in the forebay of the power plant, but primarily to provide ancillary information for the halogen studies. Water temperature in the forebay had reached 28 °C, and larvae were present when monitoring began on 6 July 1991.

With a combined total of 4 years (1989-1992) of data collected from the western basin of Lake Erie, Van Cott et al. (1992), Fraleigh et al. (1993), and Garton and Haag (1993) report highest veliger densities in July and August. The peak veliger densities reported in this study fall within the range of those reported in the literature and probably represent the period of maximum densities near the field laboratory for the 1991 spawning season.

In their study on reproductive and settlement patterns of *D. polymorpha* in western Lake Erie, Garton and Haag (1993) observed a strong correlation between densities of veligers in the water and postveliger settlement on glass slides during one sampling year

but little relationship between the two during the next year. In the present study, a high settling rate on 13 July was coincident with equally high veliger densities in the water column on that sampling date. In contrast, the peaks in settling which occurred on 3 and 24 August appeared to follow peaks in veliger densities by one week. Zebra mussels usually spend 8-15 days as planktonic veligers before settling (Marsden 1992; Sprung 1993), and settling rates are probably more dependent on age of veligers in the water rather than on densities alone (Garton and Haag 1993). A higher number of older larvae in the samples from 13 July could explain the correlation with settling on that date, while veligers counted on 27 July and 17 August may have represented a generally younger stock, with associated increases in settling not occurring until roughly 7 days of maturation.

High veliger densities in the water column may also not translate into high settling rates because of factors such as high larval mortality or the action of water currents which serve to carry veligers away before settling occurs (Lewandowski and Ejsmont-Karabin 1983; Sprung 1989). These factors can help to explain why the peak of 530 larvae/L, which was observed at the study site on 27 July, did not correspond with an equivalent degree of settling.

The length distribution data from both the glass slides and cement panels indicated that newly settled postveligers reached a size range of 2-6 mm within 30 days of settling. If it is assumed that larvae settle at approximately 220 μm in length, the average growth rate of a 5 mm mussel over 30 days would be 0.16 mm/day. This growth estimate is supported by the length data from the mussels collected off the cement panels after 100

days. A peak in the length distribution of these samples fell between 10-14 mm. A mussel with a length of 12 mm would have had a growth rate of 0.12 mm/day over the 100 day period. The true growth rate of the mussels may be higher than this estimate since it assumes mussels settled on the substrates the day they (the substrates) were deployed. Formation of a biofilm enhances settling (Lewandowski 1982b), and neither the slides nor panels were preconditioned to develop such a film before they were put out. However, examination of the slides and cement panels indicated that a film usually developed within about 3 days of being submerged due to the highly productive and eutrophic nature of the lake water.

The growth rates and size ranges reported for mussels in the present study are in agreement with literature values. Marsden (1992) states that under optimal conditions, growth rates of settled juveniles can reach 0.21 mm/day, while Mackie (1991b) reported a maximum of 0.5 mm/day. Growth of young-of-the-year mussels is dependent on factors such as food quality and availability, temperature, and physical aspects of the water (Bij de Vaate 1991; Marsden 1992; Smit et al. 1993).

It has been suggested that length frequency distribution can be used to identify year classes in zebra mussels (Morton 1969). However, the successive settling of postveligers over the course of the spawning season and the potential for more than one distinct peak in veliger densities to occur could confound interpretation of such data. For example, two frequency modes were apparent in the length distribution from the day 100 cement panels (Fig. 4), one around 4-6 mm and another at 12-14 mm. These could be interpreted as two distinct year classes rather than as two successive settling events which occurred

during the same spawning season. Based on the date the day 100 samples were taken (9-20-91), and estimated growth rates, these two peaks in the length distribution may correspond with the peaks in mussel settling that were observed 13 July and 24 August.

While veliger densities in the forebay dropped off to near zero after 31 August, low numbers of mussels were still found attached on the weekly glass slides through September. In most cases, the settled individuals were around 1 mm in length or larger. In addition, larger mussels (up to 22 mm) which did not appear to fit into expected length distributions were found in both the cumulative glass slide and cement panel samples. Zebra mussels have the ability to detach from an initial point and exhibit secondary settlement (Griffiths et al. 1991; Mackie et al. 1991; Ackerman and Claudi 1992), and the presence of larger mussels on the forebay monitoring substrates is probably indicative of such a phenomena. This type of secondary movement is another factor which can make it difficult to determine age structure based on length distributions of a population (Griffiths et al. 1991)

Peaks in weekly settling densities ranged between 21,866 and 73,973 mussels/m^2/week. Cumulative densities on slides from the forebay reached 70,533/m^2 by day 60, while those on cement panels reached 147,083/m^2. These values are within the range of settling densities reported by Van Cott et al. (1993) who also conducted a study in 1991.

Selectivity by zebra mussel postveligers settling on artificial substrates has been documented by Walz (1975), Van Diepen and Davids (1986) and Kilgour and Mackie (1993). Marsden (1993) stated that factors such as texture (smooth vs rough) and

orientation were important in determining zebra mussel densities on substrates. The higher densities of zebra mussels found on the cement panels as compared to the glass slides after 60 days may be an indication of selectivity between the pitted surface of the former versus the smooth surface of the latter. However, visual comparison of the two substrates indicated that the initial surfaces of both were completely covered with zebra mussels, and that the added densities on the panels appear to have been due to the successive buildup of mussels upon the initial layer. Such an outward buildup on the glass slides was inhibited due to the design of the slide rack. That is, mussels could only accumulate so far before encountering the adjacent slide. There was also a slight difference in the size distributions between mussels on the glass slides and cement panels, with the majority of the former ranging between 2-4 mm while the latter ranged between 4-6 mm. This could be due to crowding on the glass slides and in turn a limitation of food availability since the cement panels had more surface area exposed to the open water. A similar difference was observed on slides and cement panels from the treatments. While the glass microscope slides may not have been as efficient in tracking the cumulative density of mussels > 1 mm, they were particularly useful when newly settled postveligers were counted since they could easily be scrapped and examined under a dissecting scope.

2.4.2 Halogen Treatments: Settling and Growth of Zebra Mussels

Lower densities of zebra mussels within intake or experimental laboratory systems on Lake Erie as compared to the lake itself have previously been reported (Fraleigh et

al. 1993b; Klerks et al. 1993; Van Cott et al. 1993). The reasons behind such observations include settling of mussels along the intake lines or predation by adult mussels resident within the system (Klerks et al. 1993). A large accumulation of mussels was found in both the intake line and the header which supplied the flow-through systems in the field laboratory. Settling or mortality of postveligers prior to reaching the glass slides in the exposure tanks could then account for the lower numbers of mussels settled on control slides in the laboratory as compared to those suspended in the plant forebay.

Chlorine appeared to be more effective at controlling settling of zebra mussel postveligers than bromine under intermittent treatments. On a weekly basis, 1.0 mg/L chlorine almost always elicited a significant reduction in settling and on 30-day cumulative slides, both 0.5 and 1.0 mg/L chlorine caused significant reductions.

Other studies have examined the impact of chlorine on zebra mussel veligers. In a study conducted at the Perry nuclear plant on Lake Erie, Barton (1993) reported that 2 hr daily chlorination with 0.5 mg/L chlorine for 28 days had no impact on adult zebra mussels, but did appear to control settling of postveligers on monitors located within the plant. Veliger densities coming into the plant during the time the study was conducted appeared low (max 98/L) which may have artificially enhanced the efficacy of the study. In their comparison of 3 oxidizing chemicals for controlling zebra mussel veligers, Klerks et al. (1993) observed greater efficacy of chlorine than permanganate or peroxide. In static experiments, 100 % mortality of veligers was achieved with a 2 hr exposure to chlorine treatments as low as 0.5 mg/L (Klerks et al. 1993). In flow-through experiments conducted at 23 °C during this same study, reductions of veliger densities

as high as 93% were observed in the exposure chambers. This reduction was attributed to both mortality and "inactivation" of the veligers whereby they close their shell to avoid the oxidant and settle from the water column. Similarly, in a flow-through experiment, Neuhauser et al. (1991) reported a 99% reduction in veliger densities in the water column using chlorine levels down to 0.2 mg/L and a contact time of 30 minutes.

With respect to settling, Fraleigh et al. (1993b) examined the impact of continuous application of oxidants in a field study that ran from 11 July to 2 October 1992. They observed greater than 90 % (usually greater than 95 %) reductions from control settling with applications of chlorine, chlorine dioxide, permanganate, and chloramine at minimal concentrations of 0.25, 0.125, 0.25, and 0.25 mg/L, respectively. In the present study using intermittent exposures, the maximum reduction in settling as compared to controls was similar to that observed by Fraleigh et al., at 91% for both 1.0 mg/L chlorine in Study # 1 and the 4 hr treatment with 0.5 mg/L chlorine in Study # 2.

The impact of the halogens on settling of postveligers in Study 2 was similar to that of Study 1, in spite of the fact that veliger densities in the latter were considerably lower than those observed during the former. There was generally good agreement with respect to the % reductions from the control values for the 2 studies. For example, treatment with 0.5 mg/L bromine reduced postveliger settling by 67% in Study 1 and 62% in Study 2. Both a dose and time dependent response were evident, with the 1.0 mg/L levels of each halogen having a greater degree of impact on mussel settling than the 0.5 mg/L treatments, and the 4-hr treatments with 0.5 mg/L chlorine doing the best of all doses. These results agree with those obtained by Matisoff et al. (1990) who found both a dose

and time dependent increase in mortality of veligers in static exposures to chlorine levels between 0.5 and 2.5 mg/L. Efficacy (as measured by % reduction from controls) of 0.5 mg/L chlorine (2-hr treatment) dropped from 85 to 50% between Studies 1 and 2, respectively. This was probably the result of a malfunctioning delivery pump for that chlorine treatment which caused severe fluctuations in the halogen levels during the first 2 weeks of the second study.

In contrast to that observed on the glass slides, settling densities on cement panels from the fouling chambers had no significant between-treatment differences. Settling juvenile zebra mussels prefer textured to smooth surfaces (Marsden 1993), and the densities of mussels settled on the glass slides in the treatment chamber may, in part, reflect this preference. In the face of the daily halogen treatments, mussels on the glass microscope slides may have had a greater propensity to migrate off the less preferable glass substrate than from the more pitted and textured concrete panels. The observed impact of the halogen treatments upon mussel densities may have been magnified by this substrate preference. Unfortunately, the loss of samples from the treated cement panels at the end of Study 1 and the low and variable densities of mussels on them at the end of Study 2 did not permit statistical comparison of the densities, leaving this aspect of the data unresolved.

While the two chlorine treatments in Study 1 may have mildly offset growth of settling mussels, the majority of individuals from treatments and controls alike fell into the 2-4 mm range on slides or the 4-6 mm range on cement panels. It then appears that, once settled, the mussels exposed to the daily 2-hr halogen treatments experienced growth

comparable to controls. Khalanski and Bordet (1980) and Jenner (1985) observed reductions in settling and growth of the marine mussel, *Mytilus edulis*, during 4 hr on/4 hr off (for a total of 12 hr exposure/day) treatments with chlorine levels as low as 0.1 mg/L. The growth reduction was attributed to lack of filtering due to avoidance of the oxidant. Apparently the 2-hr exposure regime used in Study 1 was sufficient to reduce settling but not sufficient to induce an energy demand that resulted in growth reduction.

The actual cause of the reduction of mussel densities observed on the glass slides was not determined in this study. As previously stated, it may in part have been due to migration off the slides. Using the exposure regime described above, Khalanski and Bordet (1980) reported that treatment with 0.5 mg/L chlorine resulted in detachment of approximately 80% of juvenile *M. edulis* from mussel monitors. James (1967) also reported detachment and migration of *M. edulis* exposed to low doses of chlorine (0.05 mg/L). Few, if any, dead postveligers were observed on the slides, although mortality or inactivation and sinking of the free swimming veligers could also have been factors in the density reductions.

2.4.3 Halogen Treatments: Growth, Survival, and Glycogen Levels of Adult Bivalves

Neither chlorine nor bromine had a significant effect on survival of either size class of zebra mussel or Asian clams in the two halogen studies conducted. These results are consistent with the findings of previous work which investigated intermittent chlorination as a control strategy for adult zebra mussels (Klerks and Fraleigh 1991; Barton 1993) and Asian clams (Mattice et al. 1982). Jenner and Janssen-Mommen (1993) demonstrated that

zebra mussels can detect chlorine levels well below 0.5 mg/L and avoid the oxidant through valve closure. Similar results were obtained for the Asian clam (Doherty et al. 1986; Sappington 1987). Review of the literature indicates that continuous chlorination is necessary for control of adult zebra mussels or Asian clams. Jenner (1985) reported that a field application of 0.4 mg/L TRO chlorine achieved complete mortality of zebra mussels in 2 weeks at water temperatures around 15 °C. In contrast, Asian clams exposed to chlorine at 0.34 or 0.42 mg/L TRO in two separate pipe circuits of an industrial facility on the New River, Virginia, experienced mortality of only 13 and 21%, respectively after a 28-day continuous application at 15°C (Doherty et al. 1986). The differential sensitivity to chlorine observed between these two studies may be due to lower activity levels of *Corbicula* at 15°C as compared to *Dreissena*.

Smaller zebra mussels had a significantly higher growth rate than larger mussels in Studies 1 and 2. The average growth rate for smaller mussel controls and treatments which were not significantly different from controls was 0.21 mm/day. The average rate for the large mussels was 0.14 mm/day in Study 1 and 0.18 mm/day in Study 2. A negative relationship between length and rate of growth has previously been reported for zebra mussels in both Europe and North America (Nichols et al. 1990; Bij de Vaate 1991; Neumann et al. 1993). Smit et al. (1992) suggested that this difference could be due to a greater energy allocation toward reproduction in the larger mussels. Reported lengths at which the mussels become sexually mature range from 6 to 10 mm (Mackie 1991; Marsden 1992), so the smaller mussels used in the present study with an average initial length of 7.5 mm were probably sexually mature. Since the 2 halogen studies were

conducted during the spawning season of the mussels, it is possible that the observed growth differential between the small and large mussels reflects a difference in energy allocation.

Growth rates observed for the adult mussels in the present study fall within the maximal range of 0.21 - 0.5 mm/day reported for the organisms under optimal conditions by Marsden (1992) and Mackie (1991), respectively.

With an average initial length of 11.9 mm, the Asian clams used in Study 1 can be considered mature individuals (reproductive activity begins at around 8 mm, McMahon 1983). As seen with zebra mussels, growth rates of *C. fluminea* are negatively correlated with size (McMahon and Williams 1986). Growth rates of control and non-significant treated individuals in the present study averaged 0.075 mm/day. This rate of growth was greater than that observed in similar sized clams by Britton et al. (1979), but is comparable to the results of Schema et al. (1993) for a study conducted in the Ohio River.

As determined by increase in shell length, both size classes of zebra mussels grew more than Asian clams. Since the shell of the Asian clam is heavier than that of the zebra mussel, this difference in growth could be in part due to a greater energy demand associated with shell elaboration in the former as compared to the latter. There are probably also differences in energy allocation between the two, with the byssus-attached mussel moving less and thus having more energy for growth than the infaunal clam which actively moves in the sediments with its foot.

With respect to the halogen treatments, no impact on growth was observed in the zebra mussels during Study 1, but in Study 2 the 4-hr treatment with 0.5 mg/L chlorine caused a significant reduction in growth of both the mussels and Asian clams. It is unfortunate that length distributions for mussels which settled on the glass slides is not available since impacts on their growth may also have been apparent in this treatment. As Khalanski and Bordet (1980) proposed for the growth reductions observed in *M. edulis*, the effect observed in the mussels and clams is probably due to a reduction in food intake related to the time the organisms spent with the valves shut rather than siphoning.

Whole body glycogen levels for small mussels and Asian clams in this treatment were not significantly different from controls, indicating no impact on the energy reserves and making it difficult to determine if the observed growth reductions had any biological significance with respect to the long term survival or reproductive capacity of the organisms. It is also not clear why all three chlorine treatment regimes caused significant growth impairment of the larger mussels in Study 2. While it could relate to the energy demands associated with reproduction, an effect from both chlorine levels used in Study 1 should have elicited a similar effect.

2.5 Summary

Intermittent treatments with chlorine and bromine at levels of 0.5 and 1.0 mg/L TRO caused significant reductions in settling of zebra mussel postveligers, with an effect that was both dose and time dependent. Density reductions as high as 91% from control

values were observed. The efficacy of the treatments was similar between the two studies conducted, even though larval densities in the second study were reduced. None of the treatments were completely effective at inhibiting larval settling, and the final density of mussels that accumulated in the treatment tanks depended on larval densities in the water (higher densities in the water led to higher numbers settled). Growth comparisons indicated that mussels which remained settled within the system were able to grow comparably to control individuals. The intermittent treatments had no effect on survival of adult zebra mussels or Asian clams, and all showed positive growth over the course of the studies. Growth impairment of both mussels and clams was evident in the 4-hr treatment with 0.5 mg/L chlorine, an effect probably due to avoidance of the oxidants and in turn a reduction in feeding. No impact on glycogen levels of selected groups of the bivalves was observed, indicating that under the exposure conditions used, the halogen treatments had no effect on energy reserves. This may have been due to replenishment of glycogen through feeding when halogens were not being dosed.

The intermittent exposure regimes used in this study can reduce, but will not prevent, zebra mussel settling. Mussels that do settle are largely unaffected by the treatments, so additional application of a more effective biocide would be necessary to prevent eventual fouling problems.

3.0 COMPARATIVE RESPONSE OF ZEBRA MUSSELS AND ASIAN CLAMS TO A NONOXIDIZING MOLLUSCICIDE

3.1 Introduction

The zebra mussel, *Dreissena polymorpha*, (= mussel) and the Asian clam, *Corbicula fluminea*, (= clam) are freshwater bivalves which, after accidental introductions into North America, have become established as major pest species due to their potential to foul raw water intake systems. Zebra mussels, native to the Black and Caspian sea regions of western Asia, were initially discovered in Lake St. Clair in 1988 and have since spread throughout the Great Lakes, with populations now extending to larger river systems in the midwest and eastern United States (Mackie et al. 1989; O'Neill and MacNeill 1991; NY Sea Grant 1992). The Asian clam, a native of Southeast Asia, was first reported on the west coast of North America in 1938 and now occurs throughout most of the U.S. (Morton 1979; Counts 1986).

Both species share common life history characteristics which lead to their ability to invade and cause fouling problems in water intake systems. These include high reproductive output, free-living juvenile stages which are easily entrained in intake water, and high growth rates (McMahon 1983; McMahon and Tsou 1990). Fouling by the zebra mussel is exacerbated by the presence of a functional byssus in the adult stage which allows attachment to various hard surfaces. This attachment not only makes the mussels difficult to physically remove, but their propensity to attach to each other leads to accumulations that can seriously reduce the diameter of intake pipes and impede water flow (Barton 1993; Kovalak et al. 1993). As compared to Asian clams, the zebra mussel

has had a much more rapid impact as a pest species, causing severe fouling incidents within 3 years of its introduction into the U.S.. In contrast, Asian clams were reportedly present in this country for over 20 years before causing a significant fouling problem (Lamarre 1991).

Similar strategies have been employed to control biofouling by the two organisms, and the application of chlorine is the most widespread chemical treatment currently employed for either species (Belanger et al. 1991; Van Benschoten et al. 1993). Unfortunately, problems associated with chlorination are the same regardless of the organism targeted for control. Specifically, unless dechlorination is possible, current effluent guidelines limit chlorine applications to 2 hrs/day with a maximum total residual oxidant concentration of 0.2 mg/L at the point of discharge (USEPA 1980). Previous studies have demonstrated that such an application is inadequate to control either zebra mussels or Asian clams (Mattice et al. 1982; Lyons et al. 1992). When permitted, continuous chlorination provides effective control, but concerns about the formation of carcinogenic byproducts or chlorine-induced corrosion of pipe systems continue to stimulate interest in developing more environmentally acceptable control agents (Klerks and Fraleigh 1991; McMahon et al. 1993).

As an alternative to chlorine, a number of nonoxidizing molluscicides have been developed. These compounds are relatively easy to apply, non-corrosive, and do not form toxic byproducts (McMahon et al. 1993). In addition, the bivalves generally do not detect these molluscicides as they do an oxidizing agent such as chlorine, and so do not exhibit

avoidance through valve closure. The result is a potential for an acceptable level of efficacy with a much shorter application time.

In the present study, the efficacy of DGH/QUAT, a nonoxidizing surfactant-based molluscicide, was compared for control of the zebra mussel and Asian clam. This compound was initially developed as a control agent for Asian clams. In addition to recording mortality following exposure, whole body glycogen and tissue water levels were monitored in the two species to characterize and compare the sublethal stress response to the molluscicide.

3.2 Methods

3.2.1 Test Sites

Bioassays with zebra mussels and Asian clams were conducted at a university laboratory, Virginia Tech, Blacksburg, VA, and at a field laboratory located at a coal-fired power plant on the western basin of Lake Erie near Erie, MI. The field laboratory received a continuous supply of Lake Erie water which was pumped from the forebay of the power plant. The university laboratory bioassay was a 24-hr static exposure, while a series of short-term (6-12 hr) exposures were conducted under flow-through conditions at the Lake Erie field laboratory.

3.2.2 Test Organisms

Zebra mussels were collected from concrete blocks which had been suspended in the forebay of the power plant. For the university study, the mussels were wrapped in moist

paper towels and shipped overnight in a double cooler to Blacksburg, VA. Ice packs were used to keep the organisms cool during shipping. Asian clams were collected from the New River near Narrows, Virginia. Due to their limited availability in the vicinity of the Lake Erie study site, clams were shipped to the field laboratory from Virginia in the fashion described for zebra mussels. The study at Virginia Tech was conducted during the spring of 1991, while the work on Lake Erie was done during the summer of that same year.

At Virginia Tech, the two species were maintained in separate 96-L holding tanks and were allowed to acclimate to laboratory water (dechlorinated Blacksburg tap water) for at least three weeks prior to the start of the bioassay. Water in the holding tanks was continuously aerated, and approximately half the volume was renewed each week. The holding tanks were equipped with a small chilling unit and side-mounted power filter. Holding temperatures were 7-8 and 15 °C, for zebra mussels and Asian clams, respectively. The week prior to the start of the bioassay, the temperature in the zebra mussel tank was increased at approximately 1 °C per day until the target test temperature of 15 °C was reached. Both species were fed a dense mixture of live *Chlamydomonas*, live *Ankistrodesmus*, and dried *Chlorella* (Nichols 1991; 1993).

In the field laboratory, the organisms were held in two large flow-through troughs which received a constant supply of Lake Erie water at a rate of 38 L/min. Holding temperature matched that of the incoming water. Asian clams were acclimated to the Lake Erie water for two weeks prior to the start of the first bioassay, after which time clams used for testing were continually maintained in the troughs. A large group of zebra

mussels was collected on the day the clams arrived from Virginia, so the organisms were held in the laboratory system for the same amount of time before the start of a bioassay.

Adult individuals were used in both the Virginia Tech and Lake Erie studies. Zebra mussel and Asian clam shell lengths ranged between 10.0 to 19.3 and 10.9 to 17.0 mm, respectively.

3.2.3 Bioassay Procedures

DGH/QUAT

DGH/QUAT is a nonoxidizing, surfactant-based molluscicide which is 13% active by weight. The active components of the compound are dodecylguanidine hydrochloride (DGH), and n-alkyl dimethylbenzyl ammonium chloride (Quat). All molluscicide concentrations are reported as nominal levels of concentrated product. Target levels were validated with an analytical procedure which included mixing samples of test solution with a methyl-orange buffer followed by extraction with 1,2 dichloroethane. The absorbance of the extract was then read on a spectrophotometer at 415 nm, and concentrations of toxicant were calculated from a standard curve.

Virginia Tech Study

Static exposures with clams and mussels were conducted in three replicate, 15-L polycarbonate containers with aeration and a test volume of 8 L. Both species were placed in the test containers 24 hrs prior to the start of the bioassay to allow the zebra mussels time to attach to the floor or walls of the containers. Only those mussels which

had securely attached were used in the test. The organisms were exposed to target levels of 0 (control), 3.75, 7.5, 15, and 20 mg DGH/QUAT/L, with laboratory water as the diluent at 15 °C. Exposures lasted 24 hrs, after which the organisms were transferred to another set of polycarbonate containers which held 12 L of fresh laboratory water. Mortality was recorded over a 20-day period during which time the bivalves were offered the previously described algal mixture on a daily basis. Recovery water was changed every third day. The organisms were considered dead if they did not respond to gentle probing of the mantle tissue (zebra mussels) or exhibited no resistance to a blunt probe inserted between the valves (Asian clams) (McMahon et al. 1993). A total of 30 individuals (10/replicate) from each species was used to monitor mortality.

Dissolved oxygen, pH, and conductivity were measured in each test container at the start and end of the exposures, and every other day in each recovery container.

All testing and handling of zebra mussels conducted at Virginia Tech was done in accordance with a permit that had been obtained from the Virginia Department of Game and Inland Fisheries. Containment protocols followed those developed by Bidwell et al. (1991), and are included in Appendix 1.

Lake Erie Field Laboratory

All bioassays were conducted under flow-through conditions in a series of PVC troughs located in the field laboratory. Each trough received a continuous supply of Lake Erie water at a rate of 38 L/min, and two replicate troughs were used for each level of DGH/QUAT tested. Stock solutions were prepared by mixing molluscicide with

dechlorinated municipal tap water obtained from the power plant. The stocks were administered via peristaltic pumps through an injection port located on the inflow pipe to each trough. Control troughs were similarly dosed with a supply of dechlorinated water only. Turbulence within the trough inflow pipes sufficiently mixed the molluscicide and lake water as validated by measuring molluscicide levels along the length of the troughs.

A series of three bioassays were conducted in the field laboratory. The first bioassay was conducted when water temperature was 25°C, with a DGH/QUAT level of 15 mg/L. The second test was run at 20 °C and included DGH/QUAT concentrations of 5, 10, and 15 mg/L. The third test was conducted at 15 °C and also included DGH/QUAT concentrations of 5, 10, and 15 mg/L. For tests 1 and 2, exposures lasted 6 hrs, while exposures for test 3 included 6 and 12 hr treatments. In all bioassays a pair of troughs which received only Lake Erie water were designated as controls. Dosing was terminated by turning off peristaltic pumps and allowing the troughs to flush with lake water. At the flow rate described, the entire volume of the troughs was renewed within minutes.

A total of 30 individuals (15/trough) from each species were used to monitor mortality. Prior to the start of the bioassays, zebra mussels were allowed to attach to semi-circular sheets of PVC which were then placed into each exposure trough. Asian clams were dispersed throughout the troughs. In test 3, a second group of 20 Asian clams was exposed to DGH/QUAT under the 15 mg/L, 12 hr regime. A small piece of toothpick was glued between the valves of these clams so they could not avoid exposure to the molluscicide through valve closure. A group of 20 clams was similarly altered and

placed in the control troughs. This procedure was done to determine if differential avoidance lead to observed differences in sensitivity to the molluscicide.

Dissolved oxygen and pH were monitored at the beginning and end of the dosings, and then at 3-day intervals following dosing. Mortality of the organisms was monitored through day 20 post-dose, and was determined as described for the Virginia Tech study.

3.2.4 Biochemical Variables

Whole body glycogen and tissue water levels were determined for both zebra mussels and Asian clams following the DGH/QUAT exposures conducted at Virginia Tech, and test 1 (25 °C, 15 mg/L) of the field lab tests. These assays were performed to compare the sublethal effects of the molluscicide. Since glycogen is a primary energy store of bivalves (Cantelmo-Cristini et al. 1985), it was chosen as an indicator of general stress. Whole body tissue water levels were monitored due to the potential osmotic effects which surfactants can induce in aquatic organisms (Abel 1974).

Whole Body Glycogen

Prior to glycogen determination, clam or mussel tissues (n=6) were dissected and quick frozen with liquid nitrogen. Samples were than stored in liquid nitrogen until analysis. The glycogen assay followed a procedure described by Roehrig and Allred (1974). Tissues were first homogenized in 0.05 M phosphate buffer (pH 4.8; 500 ml/g dry tissue) with a Tekmar Ultra-Turrax homogenizer at 15,000 rpm for 15 seconds. Following a 20 min centrifugation at 5,000 x g, the supernatant was drawn off and the

remaining tissue pellet was dried at 60 °C for 24 hrs. Duplicate 100 μl aliquots of supernatant were then incubated with 3.5 units of Amyloglucosidase (Sigma Chemical Co.) and phosphate buffer (final volume 0.55 ml) for 2 hrs at 37°C. Glycogen standards (from *Mytilus edulis*) containing 5 to 80 μg glycogen were assayed as described for the samples. Following the enzymatic conversion of glycogen to glucose, 0.5 ml aliquots of sample or standard were incubated with a freshly prepared oxidase-peroxidase dye reagent specific for glucose determination for 30 min at 37°C. Absorbance was measured at 450 nm and total concentrations determined from a glycogen standard curve. Free glucose present in the tissues was determined by incubating supernatant aliquots without amyloglucosidase, and then assaying for glucose as previously described. True glycogen content was determined by subtracting free glucose from the total concentrations, and was reported as percent dry weight.

For the bioassay at Virginia Tech, specimens were sampled for glycogen levels on days 1, 5, 10, and 20 post-dose. In the field laboratory, the organisms were sampled at 12 hrs (zebra mussels only), and then on days 1, 5, and 10 post-dose.

Tissue Water

Whole body tissue water levels were determined in the organisms by excising the visceral mass (n=10), determining the wet weight to the nearest 0.0001g on a Mettler electronic balance, drying the tissue at 60°C for 24 hrs, and then reweighing. Tissue water was calculated as wet weight-dry weight/wet weight, and then expressed as a percentage.

Organisms were sampled for determining tissue water levels on the same days as glycogen in the Virginia Tech bioassay, and at hour 1, 3, 6, during dosing, and then 6 hrs and days 1, 5, and 10 postdose for the field study.

3.2.5 Statistical Analyses

Whole body glycogen and tissue water levels were analyzed by analysis of variance (ANOVA) following arcsine-squareroot transformation of the data (Zar 1984). Differences among the treatment groups were determined by Fisher's Protected LSD at $\alpha = 0.05$. The time necessary for 50% mortality (LT_{50}) was calculated using probit analysis, with significant differences determined by non-overlapping confidence intervals. All analyses were performed using a Statistical Analysis Systems package (SAS 1985).

3.3 Results

3.3.1 Mortality

In the bioassay conducted at Virginia Tech (24-hr static exposure, 15 °C), DGH/QUAT levels of 7.5 mg/L and higher were toxic to zebra mussels and Asian clams, and post-dose mortality was similar by day 20 (Table 3-1). By comparing LT_{50} values, however, the rate of mortality was significantly greater for zebra mussels. For example, at 7.5 mg/L the LT_{50} for the mussel was 1.4 days while that for Asian clams was 10.6 days (Table 3-2). At concentrations of 15 and 20 mg/L, all zebra mussels died within the 24-hr exposure period. The LT_{50} of Asian clams at these levels was 8.7 and 8.9 days, respectively.

TABLE 3-1. Mortality of the zebra mussel, *Dreissena polymorpha*, and the Asian clam, *Corbicula fluminea*, following a 24-hr static exposure to the molluscicide DGH/QUAT at 15 °C. Z= zebra mussel, A= Asian clam.

DGH/QUAT (mg/L)	% Mortality (Post-Dose)							
	Day 1		Day 5		Day 10		Day 20	
	Z	A	Z	A	Z	A	Z	A
0	0	0	0	0	0	0	0	0
3.75	0	0	3	0	3	0	3	0
7.5	30	0	90	3	90	63	90	87
15.0	97	0	97	3	100	100	100	100
20.0	100	0	100	0	100	100	100	100

TABLE 3-2. Lethal time to 50 % mortality (LT_{50} values ± 95 % CI) for zebra mussels and Asian clams following a 24-hr static exposure to DGH/QUAT at 15 °C.

DGH/QUAT (mg/L)	LT_{50} (Days)	
	zebra mussel	Asian clam
0	---	---
3.75	*	*
7.5	1.4 (1.1 - 1.7)	10.6 (9.9 - 11.4)
15.0	< 1 Day	8.7 (8.4 - 9.1)
20.0	< 1 Day	8.9 (8.6 - 9.2)

* = LT_{50} not computed due to insufficient mortality

At 15 °C, mortality of both species was relatively low in the 6-hr exposures, although 15 mg DGH/QUAT/L did cause 23 % mortality in zebra mussels as compared to no mortality for Asian clams (Table 3-3). Increased efficacy at this temperature was observed when the exposure time was increased to 12 hr. Zebra mussels were more sensitive, experiencing 73 % mortality at 15 mg/L by day 20 as compared to 55 % mortality of the Asian clams. LT_{50} values for the two organisms were also significantly different under this treatment regime. It took 3.8 days for 50% of the Asian clams to die versus 1.8 days for zebra mussels (Table 3-4). Pegging open the valves of the Asian clams did not significantly increase mortality of the organisms to the molluscicide. In fact, at the 12 hr, 15 mg/L exposure, mortality of the pegged clams (35 %) was less than that observed for the clams which had not been pegged open (55%).

Differences in sensitivity of zebra mussels and Asian clams were particularly evident in the 6-hr exposures between 20 and 25 °C. At these temperatures, molluscicide levels of 10 or 15 mg/L resulted in nearly complete mortality of zebra mussels within 24 hrs of exposure (Table 3-3). Conversely, the highest level of mortality observed for the Asian clams was only 43%, so no LT_{50} values were generated (Table 3-4).

3.3.2 Biochemical Variables

Due to 100% mortality of zebra mussels which occurred within 24 hrs at 15 and 20 mg DGH/QUAT/L in the bioassay conducted at Virginia Tech, no biochemical data were available at these levels. Molluscicide levels of 7.5 mg/L and higher induced a

Table 3-3. Mortality of the zebra mussel and the Asian clam following exposure to DGH/QUAT under flow-through conditions.
Z= zebra mussel, A= Asian clam, AP= Asian clam with valves pegged open

DGH/QUAT (mg/L)	% Mortality (Post-Dose)								
	Day 1			Day 5			Day 10[1]		
	Z	A	AP	Z	A	AP	Z	A	AP
	15 °C, 6-hr exposure								
0	0	0		0	0		0	0	
5	0	0		0	0		0	0	
10	0	0		3	0		3	0	
15	0	0		23	0		23	0	
	15 °C, 12-hr exposure								
0	0	0	0	0	0	0	0	0	0
5	0	0	---	0	0	---	0	0	---
10	3	0	---	27	25	---	30	25	---
15	30	0	0	50	55	35	73	55	35
	20 °C, 6-hr exposure								
0	0	0		0	0		0	0	
5	0	0		0	0		3	25	
10	90	5		97	5		100	25	
15	100	5		100	5		100	35	
	25 °C, 12-hr exposure								
0	0	0		0	0		0	0	
15	100	10		100	43		100	43	

[1]Day 20 mortality identical to day 10

TABLE 3-4. Lethal time to 50 % mortality ($LT_{50\ values}$ ± 95 % CI) for zebra mussels and Asian clams exposed to DGH/QUAT under flow-through conditions. Z = zebra mussel, A = Asian clam

Temperature (°C)	Exposure (Hrs)	DGH/QUAT (mg/L)	LT_{50} (Days)	
			Z	A
15	6		*	*
	12	0	---	---
		5	*	*
		10	*	*
		15	1.8 (1.4 - 2.4)	3.8 (3.3 - 4.5)
20	6	0	---	---
		5	*	*
		10	0.72 (0.62 - 0,84)	*
		15	0.21 (0.20 - 0.22)	*
25	6	0	---	---
		15	0.24 (0.21 - 0.27)	*

* = LT_{50} not computed due to insufficient mortality

significant increase in the whole body tissue water content of both organisms (Fig. 3-1 and 3-2).

The 7.5 mg/L treatment also caused a reduction in whole body glycogen levels of the mussels, although not a statistically significant response (Fig. 3-3). Exposure to 15 and 20 mg DGH/QUAT/L resulted in significant reductions in whole body glycogen levels of the Asian clam by the end of the 24-hr exposure period, with all treatments significantly reduced by day 5 (Fig. 3-4). Glycogen levels of those clams exposed to 3.75 and 7.5 mg/L recovered to control levels by day 10, although mortality in the latter was not complete until day 20 (Table 3-1).

As in the university laboratory, the 6-hr exposure caused significant increases in the tissue water content of the bivalves, even before dosing had stopped (Fig. 3-5). Asian clams appeared able to regulate water content after dosing, with levels returning to control values within 1 day. Overcompensation in the clams was evident on days 5 and 10, when tissue water levels of dosed clams dropped below that of controls. No regulation of tissue water prior to death was evident for zebra mussels.

Glycogen levels of both organisms were significantly reduced relative to controls in the 6-hr exposure to 15 mg/L at 25 °C (Fig. 3-6). An effect was apparent in zebra mussels within 12 hrs after dosing. Whole body glycogen levels in Asian clams returned to levels comparable to controls by day 5 post-dose (Fig. 3-5).

Under both exposure regimes, the impact of the molluscicide upon whole body tissue water and glycogen was similar for both zebra mussels and Asian clams; namely, tissue water levels increased and glycogen levels decreased. The rate at which these changes

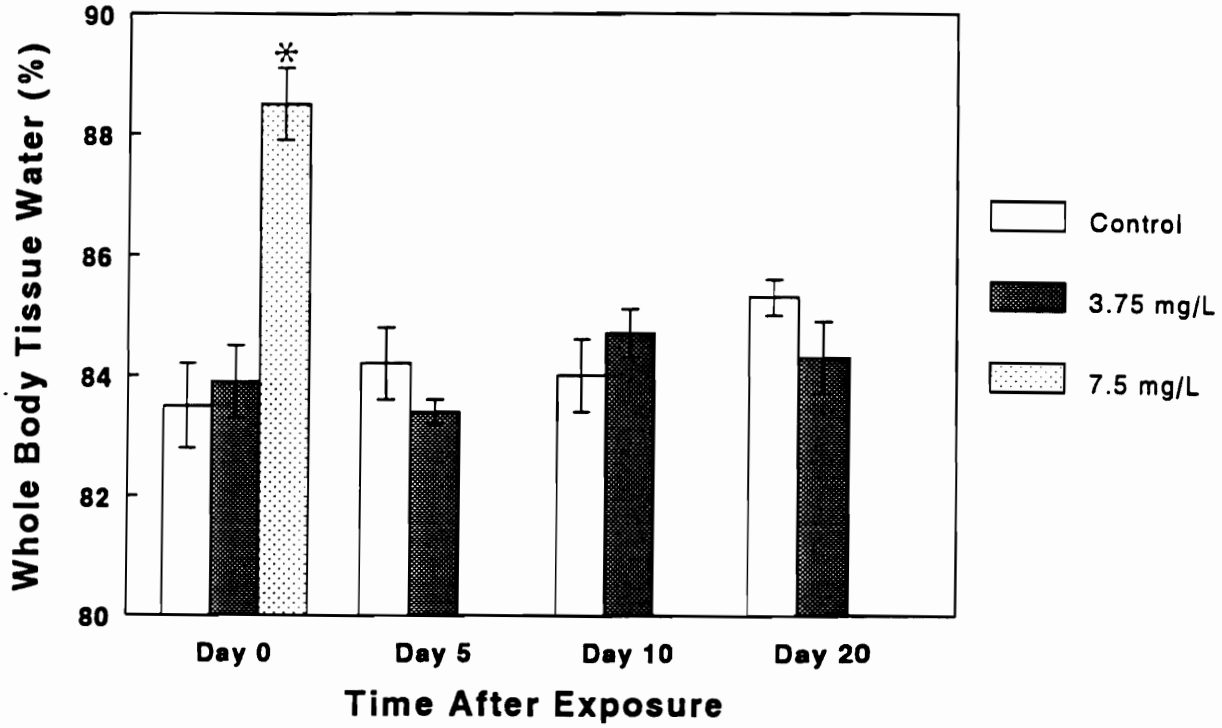


Figure 3-1. Zebra mussel tissue water content following a 24-hr exposure to DGH/QUAT at 15 °C. Samples for Day 0 were taken at the end of dosing. The asterisk (*) represents those values significantly different from controls at $\alpha=0.05$, and error bars represent $\pm$ 1 SEM.

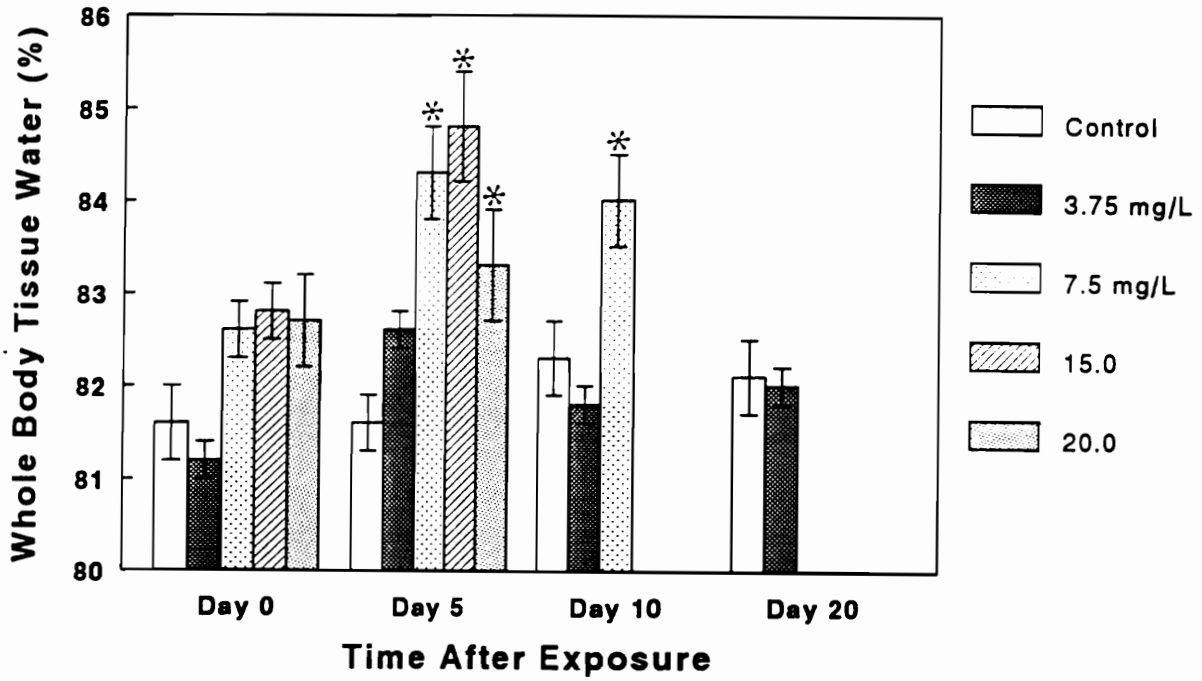


Figure 3-2. Asian clam tissue water content following a 24-hr exposure to DGH/QUAT at 15 °C. Samples for Day 0 were taken at the end of dosing. The asterisk (*) represents those values significantly different from controls at $\alpha=0.05$, and error bars represent $\pm$ 1 SEM.

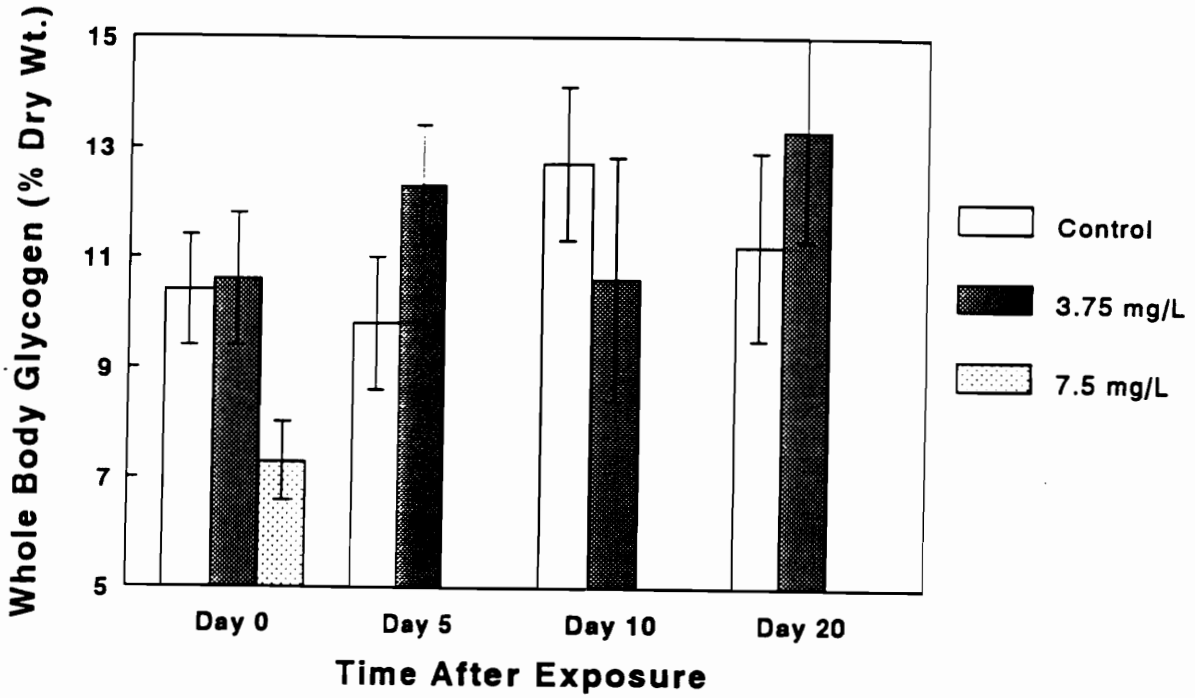


Figure 3-3. Zebra mussel glycogen levels following a 24-hr exposure to DGH/QUAT at 15 °C. Samples for Day 0 were taken at the end of dosing. The asterisk (*) represents those values significantly different from controls at $\alpha=0.05$, and error bars represent $\pm$ 1 SEM.

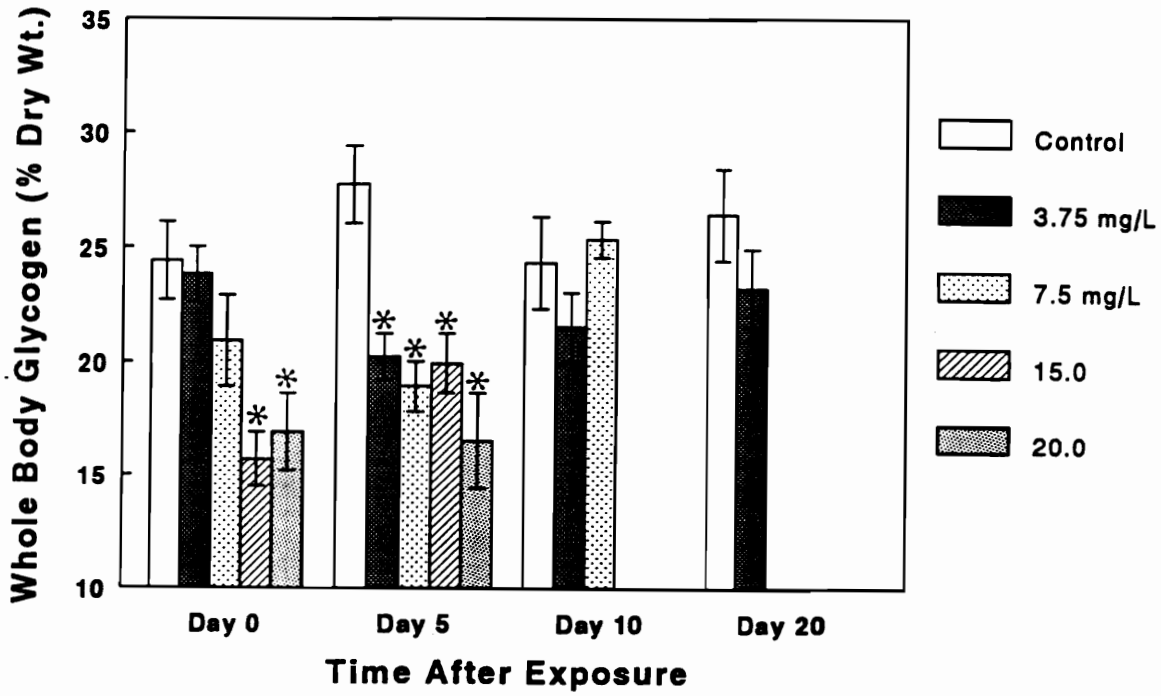


Figure 3-4. Asian clam glycogen levels following a 24-hr exposure to DGH/QUAT at 15 °C. Samples for Day 0 were taken at the end of dosing. The asterisk (*) represents those values significantly different from controls at $\alpha=0.05$, and error bars represent $\pm$ 1 SEM.

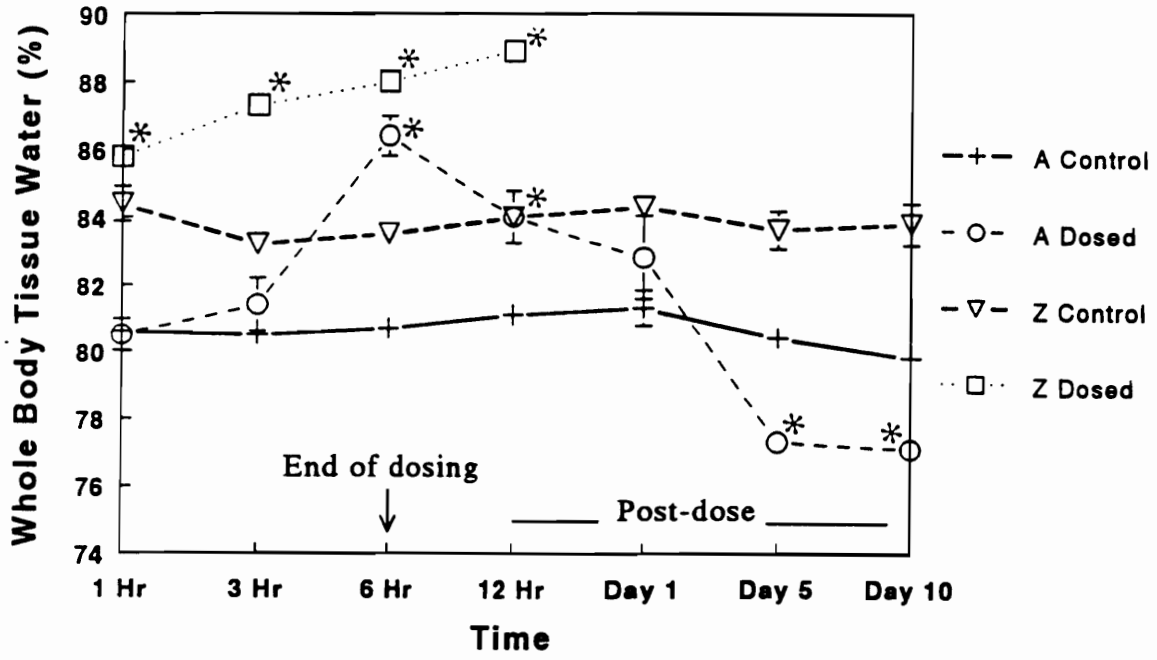


Figure 3-5. Tissue water content of the zebra mussel (Z) and the Asian clam (A) during and after a 6-hr exposure to 15 mg/L DGH/QUAT at 25 °C. Samples for Day 0 were taken at the end of dosing. The asterisk (*) represents those values significantly different from controls at $\alpha=0.05$, and error bars represent $\pm$ 1 SEM.

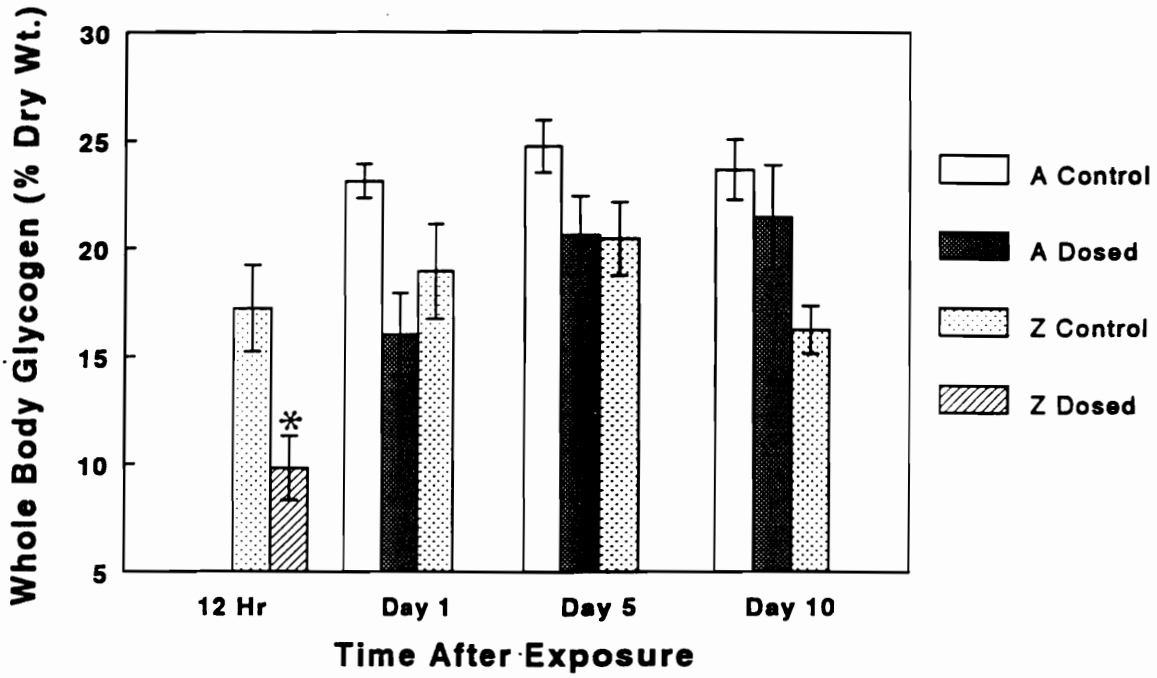


Figure 3-6. Glycogen levels of the zebra mussel (Z) and the Asian clam (A) following a 6-hr exposure to 15 mg/L DGH/QUAT at 25 °C. Samples for Day 0 were taken at the end of dosing. The asterisk (*) represents those values significantly different from controls at $\alpha=0.05$, and error bars represent $\pm$ 1 SEM.

took place appeared to differ between the two, further indicating a difference in sensitivity to the molluscicide. For example, in the 24-hr exposure, maximum increase in tissue water levels of the zebra mussel was observed in the 7.5 mg/L treatment at the end of the dosing period, representing an approximate increase of 5 % over control levels (Fig. 3-1). Maximum levels in Asian clam tissue water were not reached until day 4 post-dose, and represented an increase of 4% over controls (Fig. 3-2). In the 6-hr exposure, tissue water levels of dosed zebra mussels were significantly higher than controls within 1 hr of the start of dosing and continued increasing up to 6 hrs post-dose at which time mortality was too high to continue sampling (Fig. 3-5). Tissue water levels of Asian clams increased by 3 hrs into the exposure, but did not significantly differ from controls until the end of the 6-hr dose.

The control range of tissue water levels for either species did not significantly differ between the two studies. Zebra mussel whole body tissue water levels ranged between 83-84%, while control Asian clams levels ranged between 81 and 82% (Fig. 3-1 and 2, Fig. 3-5).

Control whole body glycogen levels did not significantly differ between clams used in the two studies and ranged between 24-27 % dry weight (Fig. 3-4 and 3-6). Control glycogen levels of zebra mussels were significantly higher in those individuals tested in Lake Erie water as compared to those held at Virginia Tech (ranges: 18-20%, Lake Erie; 10-13%, VA Tech, Fig. 3-4 and 3-6). This difference may be indicative of stress or dietary deficiency in the laboratory-held animals. Control Asian clams had significantly

higher levels of whole body glycogen than did control zebra mussels, probably due to the larger mass of foot muscle and associated muscle glycogen in the clam.

3.4 Discussion

3.4.1 Mortality

Previous studies (Lyons et al. 1988; 1990) have demonstrated the efficacy of DGH/QUAT as a control agent for zebra mussels and Asian clams. Molluscicide levels of 7.5 mg/L induced similar levels of mortality in the two species after a 24-hr static exposure at 15 °C. Total mortality of both organisms occurred at 15 mg/L under the same exposure regime. These bivalves are comparably sensitive to molluscicide exposures which extend beyond 12-24 hrs. For example, the 72-hr LC_{50} at 20 °C was determined to be 2.3 and 2.7 mg/DGH/QUAT/L for zebra mussels and Asian clams, respectively (J.C. Petrille, Betz Laboratories, Trevose, PA, pers. comm.).

While the end result of the 24-hr exposure was similar for both species (e.g. comparable levels of mortality at the same levels of chemical), the rate at which this mortality occurred was different. Zebra mussel LT_{50} values were always significantly lower than those for Asian clams, and these results provided an initial indication that sensitivity differences existed.

These differences were readily apparent in the 6-hr exposures conducted at the Lake Erie field laboratory. Here, zebra mussels experienced higher mortality than did Asian clams in exposures to 10 or 15 mg/L.

The results of the present study differ from those of McMahon et al. (1993), who reported greater sensitivity of Asian clams in both LT_{50} and time to 100 % mortality as compared to zebra mussels under static exposures to another surfactant-based molluscicide. They state that reduced tolerance of the clams may have been due to a higher filtration rate and increased uptake of the molluscicide, and increased susceptibility to oxygen depletion caused by molluscicide-induced gill damage. However, in that study zebra mussels were tested at 20°C while Asian clams were exposed at 25°C, and this temperature differential may have played a role in the sensitivity difference. Mortality of Asian clams exposed to copper increases substantially with temperature. Belanger et al. (1991) reported that the LT_{50} of clams exposed to 100 ug Cu/L decreased more than 50% from 25.1 to 11.8 days as temperature increased from 20.1 to 26.5°C.

Differences in sensitivity to other toxicants have previously been observed between the zebra mussel and the Asian clam. Fisher et al. (1991) reported a 24-hr LC_{50} of 92 mg/L for zebra mussels exposed to KH_2PO_4, while no LC_{50} was generated for Asian clams exposed to KH_2PO_4 at levels up to 2,000 mg/L under the same test conditions. Similarly, comparison of LC_{50} data generated for Asian clams exposed to K_2SO_4 (Cherry et al. 1980) and KCL (Anderson et al. 1976) with that generated for zebra mussels exposed to these salts (Fisher et al. 1991), indicate significantly greater sensitivity of the zebra mussel. It should be noted, however, that sublethal responses (gaping, foot immobilization) observed in clams exposed to various potassium salts (Anderson et al.

1976; Daum et al. 1979) occur at thresholds below reported LC_{50} values for zebra mussels.

Under at least some exposure conditions, differences in sensitivity to chlorine may also exist between the two bivalves. Jenner (1984) reported that a field application of 0.4 mg/L TRO chlorine achieved complete mortality of zebra mussels in 2 weeks at ~15 °C. In contrast, Asian clams exposed to chlorine at 0.34 or 0.42 mg/L TRO in two separate piping circuits of an industrial facility on the New River, Virginia, experienced mortality of only 13 and 21 %, respectively after a 28-day continuous application at 15°C (Doherty et al. 1986; Belanger et al. 1991). The differential sensitivity to chlorine observed between these two studies may be due to lower activity levels of Asian clams at 15°C as compared to zebra mussels or to differences in water chemistry which favored the more toxic free residual oxidant in the zebra mussel study.

One unanticipated result with respect to mortality was that pegging open the shells of Asian clams during the 12-hr exposure to 15 mg/L DGH/QUAT did not significantly increase mortality compared to unpegged clams. In another study which examined the effects of a nonoxidizing biocide on Asian clams, McMahon and Lutey (1988) altered the ability of the clams to avoid the molluscicide by filing the valves such that a small gap remained between them when closed. Mortality of normal clams was similar to that of filed clams continually exposed to the molluscicide. The results were attributed to an equivalent degree of exposure between the two groups, indicating the normal clams did not avoid the molluscicide through valve closure. The present study may similarly indicate that Asian clams do not avoid DGH/QUAT. However, the fact that mortality of

pegged clams was 20% lower than unaltered clams could also indicate that this method of keeping the valves parted interfered with siphoning and lead to less molluscicide exposure.

3.4.2 Biochemical Variables

When aquatic organisms are exposed to surfactants, the primary target of effect is the gill (Abel 1974; Knezovich et al. 1989). Previous studies which have examined the effects of both anionic and cationic surfactants on fish gills have reported thickening and vacuolization of the epithelial tissue (Abel 1976; Biesinger and Stokes 1986). This impact leads to death of the organism through suffocation (Knezovich et al. 1989). Some of this damage may be facilitated by the influx of water into the gill tissue. In a study on perfused gill of the rainbow trout (*Oncorhynchus mykiss*), Jackson and Fromm (1977) found that exposure to the anionic surfactant linear alkyl sulphate increased water permeability and, in turn, water uptake of the tissue. An increase in the water permeability of the gills can explain the increase in levels of whole body water content observed in both zebra mussels and Asian clams in the present study. Graney and Giesy (1988) also observed an increase in the whole body water levels of Asian clams exposed to the anionic sodium dodecyl sulphate (SDS).

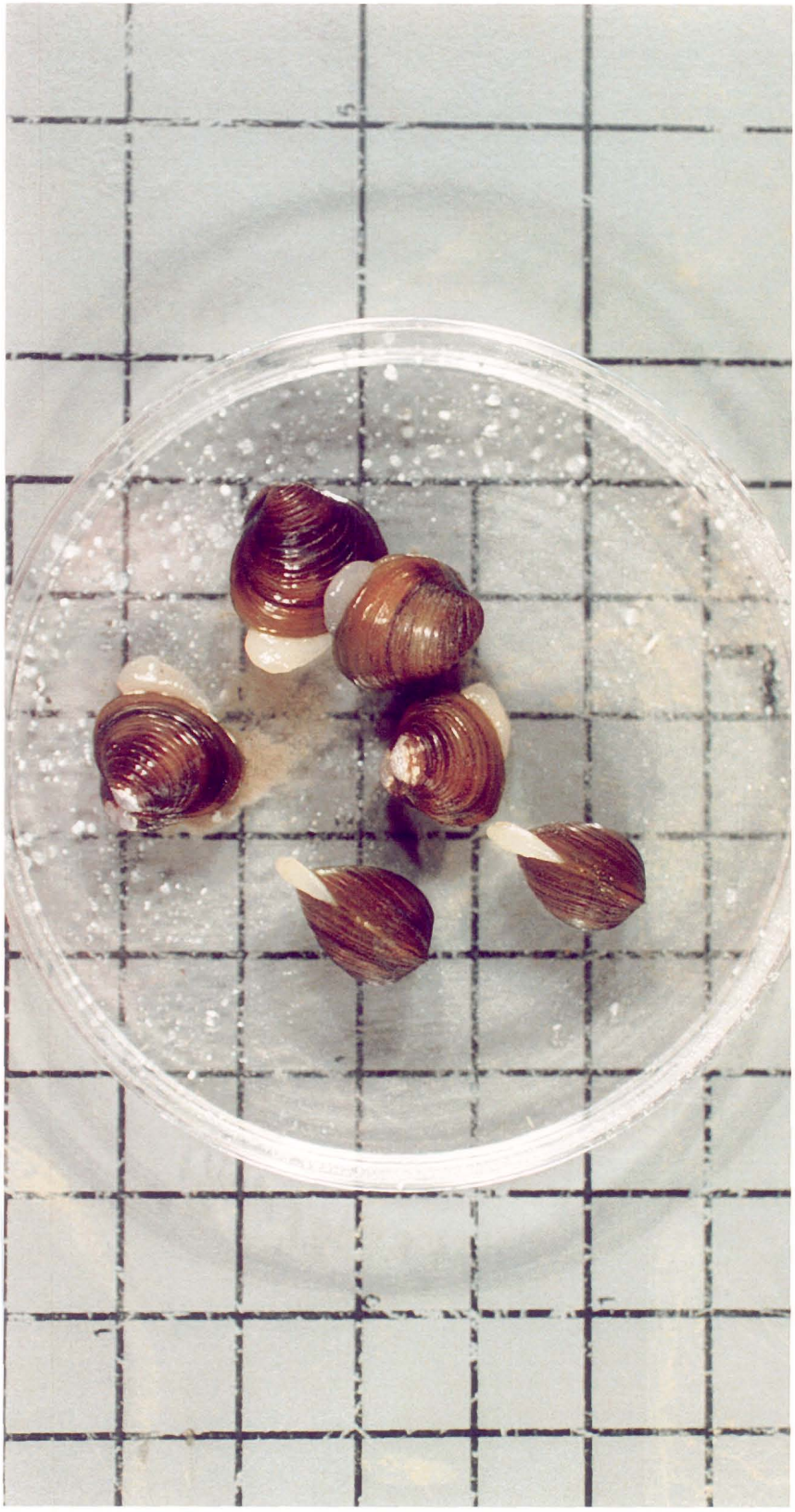
Interestingly, the toxic mode of action of surfactants may be similar to that of potassium. Through microscopic examination of gill tissue of zebra mussels exposed to potassium, Fisher et al. (1991) reported vacuolization indicative of intracellular fluid accumulation. They attributed these effects to structural changes in the plasma membrane

of the epithelial cells. Both Daum et al. (1979) and Anderson et al. (1978) have described the "foot response" in Asian clams exposed to potassium which is characterized by a distension and swelling of the foot muscle such that it protrudes from the shell. Daum et al. (1979) further determined the cause of the response to be at least partly due to fluid accumulation within the muscle. Similar distension of the foot was observed in Asian clams exposed to DGH/QUAT (Fig. 3-7).

Graney and Giesy (1988) observed a decline in the condition index of Asian clams which had received a chronic exposure to SDS. The authors stated that increased energy demand associated with toxicant-induced stress may have lead to this effect. In the present study, the reduction in whole body glycogen could similarly have occurred as the bivalves mobilized this energy reserve to maintain homeostasis in the face of molluscicide induced stress. Glycogen may have specifically been used in the production of the amino acid alanine, which has previously been shown to play a role in osmotic regulation of bivalves (Gainey 1978; Zurburg and De Zwaan 1981).

The glycogen results obtained for Asian clams here illustrate the importance of dose in controlling the rate at which a biochemical response is elicited. For example, in the study done at Virginia Tech, clams exposed to 3.75 and 7.5 mg/L treatments showed a more delayed response (e.g. did not exhibit a significant drop until day 4 post-dose vs a significant drop by the end of the 24-hr exposure period as found in 15 and 20 mg/L dosed individuals). Obviously the higher levels of molluscicide induced a more rapid energy demand than observed at lower levels. The speed at which clam glycogen levels dropped probably was influenced by temperature and metabolic rate of the organisms.

Figure 3-7. Distension of the foot of Asian clams following exposure to DGH/QUAT.



In both the university laboratory and Lake Erie exposures, recovery of Asian clam glycogen to control levels was observed in at least certain dosed groups. For the 6-hr exposures, this recovery may have been indicative of the cessation of stress associated with the molluscicide and replenishing glycogen levels by feeding. The assumption that stress ceased when dosing ended is further supported by the fact that clam tissue water levels began to decline when the exposure stopped.

The data from the 24-hr exposure are a bit more difficult to interpret, specifically with respect to the clams from the 7.5 mg/L treatment. Glycogen levels of these clams returned to control levels by day 10 even though a continuing stress was indicated by both elevated tissue water levels and nearly complete mortality by day 20 post-dose. A possible explanation is that daily feeding of the organisms which resumed after dosing was adequate for the clams to both replenish carbohydrate stores while also dealing with the increased energy demand associated with general stress. The adequacy of the trialgal diet to adequately maintain (e.g. illicit growth) Asian clams in the laboratory has previously been documented by Foe and Knight (1986) and is further supported by the glycogen levels of control clams which remained stable in over the 20 days these levels were monitored in the laboratory.

The biochemical changes observed in zebra mussels and Asian clams after exposure to DGH/QUAT are primarily due to structural effects of the molluscicide upon the gill of the organisms and the stress-induced energy demands. The aspects needing clarification are differences in the rate of mortality between the two, and the greatly enhanced efficacy of DGH/QUAT upon zebra mussels during the 6-hr exposures.

Similarly, the rates of biochemical changes often appeared more rapid in the mussel than in clams.

Zebra mussels may have simply siphoned more during the respective exposure periods than Asian clams and so succumbed to the effects of the molluscicide at a faster rate because of increased exposure. While not quantified, zebra mussels in the present study appeared to spend more time siphoning than did Asian clams in either the long or short-term exposures. In their study, McMahon et al. (1993) quantified siphoning time of the two bivalves and found that zebra mussels siphoned longer than Asian clams.

Differential time spent siphoning could also explain why mortality of the two bivalves was similar after the 24-hr exposure and different with only 6 hr of exposure. In long-term exposures, Asian clams may experience a critical level of gill damage which eventually "wears the animals down" and results in death. Under the 6-hr exposure, this critical level may not be reached, with some individuals able to recover from the effects of the molluscicide. The reason why the two bivalves may spend a different amount of time siphoning is not clear. Fisher et al. (1991) stated that the differential sensitivity to potassium observed between zebra mussels and Asian clams may have been a function of the latter's ability to avoid exposure through detection and valve closure. Asian clams do not seem to avoid surfactant-based molluscicides during at least the initial stages of exposure (McMahon and Lutey 1988), but perhaps they begin to respond as the exposure progresses.

Differences in sensitivity to the molluscicide could also be due to the physiological tolerance of the two bivalves. During the 6-hr exposure, Asian clams appeared able to

regulate tissue water levels while no regulation was observed in zebra mussels. Short-term exposures to the molluscicide may result primarily in a disruption of water balance through alteration of gill membrane permeability, and Asian clams may be better able to deal with this osmotic stress. Critical impacts on gas exchange may occur secondarily as exposure continues and a sufficient area of gill surface is damaged.

Previous studies have demonstrated that Asian clams have a greater capacity for osmoregulation (particularly under hyperosmotic stress) than other freshwater bivalves, an ability attributed to the clam's brackish ancestry (Gainey 1978a; 1978b). The physiological mechanism behind this attribute appears to involve the shuttling of free amino acids in and out of the cells to maintain cell volume (Gainey 1977a; McMahon 1983). This same mechanism could similarly enhance the ability of the Asian clam to tolerate the hypo-osmotic stress associated with exposure to DGH/QUAT. The zebra mussel has an evolutionary history similar to that of Asian clams. That is, both organisms are relatively recent immigrants to the freshwater environment (Moore 1969). If differences in sensitivity observed in the present study are indeed due to a difference in the ability of these organisms to physiologically handle hypo-osmotic stress, an additional challenge would be to determine why these physiological capabilities were retained in the clam but lost in the mussel. Unfortunately, comparison of the osmoregulatory ability of zebra mussels and Asian clams based on salinity tolerance is difficult because of variation in reported literature values (i.e. Evans et al. 1979; MacNeill 1991; Strayer and Smith 1993). Additional laboratory testing will be necessary

in order to properly characterize and compare the ability of the two bivalves to handle osmoregulatory dysfunction.

3.5 Summary

Cumulative mortality of zebra mussels and Asian clams was comparable after a 24-hr exposure to a surfactant-based molluscicide, DGH/QUAT, although the rate at which this mortality occurred was significantly greater for the zebra mussel. At temperatures of 20 - 25 °C, 6-hr exposures to 10 or 15 mg/L resulted in 100 % mortality of the mussel but only induced 43% mortality in Asian clams. Biochemical responses to the molluscicide were similar in both organisms, with whole body tissue water increasing and whole body glycogen decreasing. These effects were attributed to gill tissue damage and general stress associated with exposure to the molluscicide. Greater sensitivity to DGH/QUAT by the zebra mussel is probably due to the longer time spent siphoning during dosing. Differences in tolerance to the biochemical effects induced by the molluscicide may also be a factor, although additional comparative work is needed to fully evaluate the importance of this aspect.

A large data base pertaining to control methods exists for the Asian clam, and comparative sensitivity data will be useful in adapting these strategies for use against the zebra mussel in North America. In addition, examination of biochemical responses associated with toxicant exposure will provide a way to evaluate and optimize control strategies for both of these macrofouling organisms.

4.0 IMPACT OF A NONOXIDIZING MOLLUSCICIDE ON NONTARGET ORGANISMS

4.1 Introduction

The zebra mussel, *Dreissena polymorpha*, is now well established as a pest species in the Great Lakes system, with populations extending to most larger river systems in the midwest and eastern United States. The impact of the mussel upon raw water users stems from its ability to invade water intake systems and impede or obstruct water flow (Kovalak et al. 1993; Barton 1993). Within one year of its discovery in the Great Lakes, water plants and other industries were experiencing fouling problems due to mussel infestations (Nalepa and Schloesser 1993). Projected costs incurred by mussel fouling in the Great Lakes alone are estimated at $5 billion within the next decade, with this figure inflating as the mussel spreads throughout North America (Armor and Wiancko 1993).

In light of the potential impact of the zebra mussel, the need for control measures is obvious. Mechanical, thermal, coating, and chemical techniques are all possible (Mackie et al. 1989; Armor and Wiancko 1993), although chemical methods will probably provide the current control option until non-chemical methods are better developed and implemented (McMahon et al. 1993).

Currently, the most commonly applied chemical control agent in either North America or Europe is chlorine (Mackie et al 1989; McMahon and Tsou 1990; Van Benschoten et al 1993), largely due to its combination of proven efficacy under certain exposure conditions (i.e. continuous applications) and general familiarity with application (Van Benschoten et al. 1993). However, due to restrictions on chlorine use in once

through cooling systems and increasing concern over human health effects of chlorinated by-products, a number of alternative chemical control methods have been examined (Cameron et al. 1989; Klerks et al. 1993). Among these alternatives are nonoxidizing chemicals which have advantages in that they are relatively easy to apply, non-corrosive, and do not form toxic byproducts (McMahon et al 1993).

While nonoxidizing biocides have proven efficacy as biofouling control agents (Cherry et al. 1990; Lyons et al. 1990; McMahon et al 1993), impacts on nontarget organisms have also been documented (D.L. Waller, USFWS, pers. comm.; Waller et al. 1991). In light of nontarget impact, these chemicals are currently under review by both federal and state regulatory agencies (Howe and Kaplan 1990; Howe et al. 1990; Magni 1990; Mondor and Howe 1993).

The biocide DGH/QUAT is a non-oxidizing, FIFRA (Federal Insecticide, Fungicide and Rodenticide) registered biocide which has been used in field applications to control the zebra mussel (Lyons et al. 1990) and the Asian clam, *Corbicula fluminea*, another macrofouling bivalve (Cherry et al. 1990). General application procedures for DGH/QUAT include a dosage of 10 - 15 mg/L for 6-24 hrs (Lyons et al. 1990). The control strategy is aimed at newly settled mussels so the number of applications (usually 2 to 4 times/year) depend on duration of the spawning season, mussel settling, and growth. Prior to release into a receiving system, bentonite clay is added to the treated effluent as a detoxifying agent for the cationic surfactants which are the actives in the compound. The present study summarizes research efforts which examined factors

affecting availability of DGH/QUAT, impact upon selected non-target organisms, and detoxification potential through combination with bentonite clay.

4.2 Methods

4.2.1 Test Organisms

Cladocerans (*Ceriodaphnia dubia, Daphnia magna*), fish (Fathead minnow, *Pimephales promelas*), midges (*Chironomus riparius*), amphipods (*Hylallea azteca*), algae (*Selenastrum capricornutum*), and the marine shrimp (*Mysidopsis bahia*) used in the bioassays came from in-house cultures kept at Virginia Tech. Starter cultures for cladocerans, fish, amphipods, and algae were obtained from the U.S. Environmental Protection Agency (EPA) Environmental Research Laboratory at Duluth, Minn. Starter stock for mysid shrimp cultures were obtained from the EPA research laboratory at Gulf Breeze, FL. Midge cultures were initially started from egg masses collected in the field and identified to species by personnel from the Entomology Department at Virginia Tech. Culture methods for the species followed the respective protocols found in Credland (1973), Lee et al. (1980), Lawrence (1981), ASTM (1989), and USEPA (1991). Snails (*Goniobasis* sp.) were collected from Crab Creek, Montgomery County, VA and maintained in artificial stream systems receiving laboratory water (dechlorinated Blacksburg tap) in the Ecosystem Simulation Laboratory (ESL) on the Virginia Tech campus (Fig. 4-1). Snails were acclimated to laboratory conditions for 1 week prior to testing and were continuously supplied with periphyton-covered rocks on which to feed.

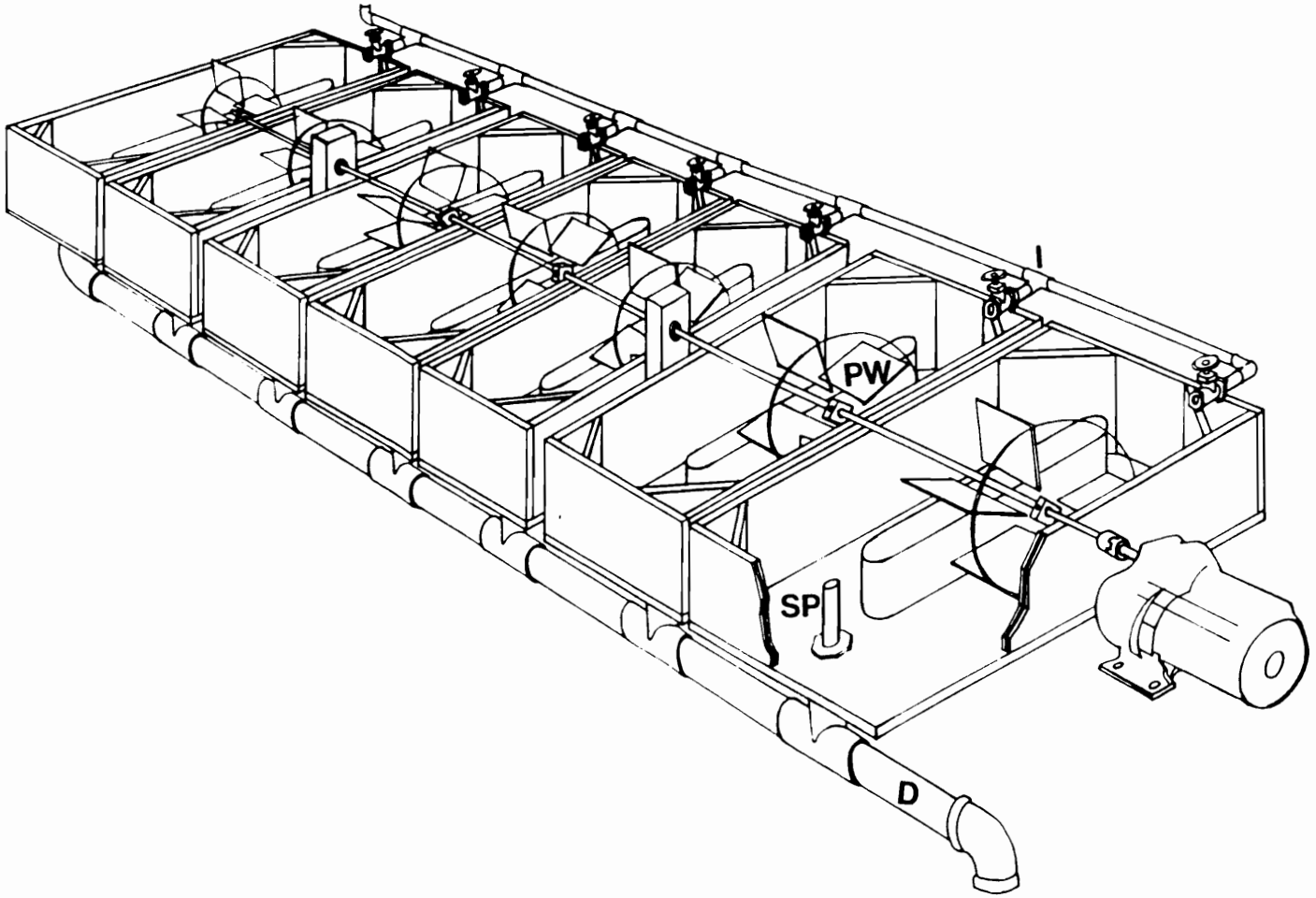


Figure 4-1. Schematic of the artificial stream system used for some nontarget bioassays. I=input for raw river water, PW=paddlewheel, SP=stand pipe, D=drain.

4.2.2 DGH/QUAT and Analytical Methods

DGH/QUAT is a nonoxidizing, surfactant-based molluscicide which is 13% active by weight. The active components of the compound are dodecylguanidine hydrochloride (DGH) and N-alkyl dimethylbenzyl ammonium chloride (Quat). Free levels of molluscicide were determined colormetrically by complexing samples of test solution with a methyl-orange buffer, extracting with 1,2 dichloroethane, and determining the absorbance of the extract on a spectrophotometer at 415 nm. Concentrations of toxicant were then calculated from a standard curve. Levels of molluscicide were determined at the beginning of bioassays, and when enough volume of test solution was available, at the end of the tests or renewals. For flow-through studies, test solution was collected from the overflow and analyzed on a daily basis. The level of detection for this analytical method is 0.2 mg/L. When nominal exposure levels were below this limit, serial dilutions of the lowest measurable concentration were used to prepare the test solutions and an estimate of their measured value was reported. Unless otherwise stated, all reported molluscicide levels are based on measured values.

4.2.3 Bioassays with DGH/QUAT Alone

A summary of test conditions for bioassays conducted with DGH/QUAT is presented in Tables 4-1A and B. Toxicant stocks were derived from a concentrated sample of DGH/QUAT obtained from BETZ Laboratories, Trevose, PA. Acute and chronic (including renewal and feeding regimes) static tests with common test species closely followed those protocols described in USEPA (1989) and USEPA (1991). Tests with

Table 4-1A. Summary of general test conditions for exposures to DGH/QUAT. The following abbreviations describe test types and endpoints:

S: Static, FT: Flow-through, A: Acute, C: Chronic, M: Mortality, R: Reproduction, G: Growth

Species	Age	Test Type	Test Chamber		Test Temperature	# Organisms Per Chamber/ # Replicate	Endpoint
			Size	Volume			
Ceriodaphnia dubia	< 24 hrs	S, A	50 ml	25 ml	25 ± 1 °C	10/2	M
		FT, A	45 ml	35 ml	25 ± 1 °C	5/5	M
		S, C	50 ml	25 ml	25 ± 1 °C	1/10	M, R
		FT, C	45 ml	35 ml	25 ± 1 °C	1/10	M, R
Daphnia magna	< 24 hrs	S, A	100 ml	50 ml	20 ± 1 °C	10/2	M
Fathead minnow	< 24 hrs	S, C	400 ml	250 ml	25 ± 1 °C	10/4	M, G
		FT, C	450 ml	300 ml	25 ± 1 °C	10/4	M, G
Chironomus riparius (midge)	2nd Instar	S, A	100 ml	50 ml [1]	20 ± 1 °C	5/3	M
Hyallela azteca (amphipod)	< 1 week	S, A	400 ml	250 ml [2]	20 ± 1 °C	10/2	M
Goniobasis sp. (snail)	Adult	S, A	1 L	500 ml	20 ± 1 °C	10/2	M
Mysidopsis bahia (marine shrimp)	< 24 hrs	FT, A	45 ml	35 ml	25 ± 1 °C	5/5	M
Selenastrum capricornutum (algae)	Log Phase	S, C	125 ml	50 ml	25 ± 1 °C	11 x 10^4 cells/3	G

[1] 10 ml of glass beads (212-300 μm, Sigma Chem.) added for substrate.

[2] 10-cm piece of PVC pipe (2.5 cm diam.) in each chamber for cover, daily renewal of 80 % of test solution.

Table 4-1B. Summary of specific test conditions for exposures to DGH/QUAT.

S: Static, FT: Flow-through, A: Acute, C: Chronic

Species	Test		Diluent	Toxicant Range (mg DGH/QUAT/L)
	Type	Duration		
C. dubia	S, A	48 hrs	New River	0.10 - 1.0
	FT, A	48 hrs	Synthetic [1]	0.05 - 0.80
	S, C	7 days	Dechlorinated Tap	0.03 - 1.0
	S, C	12 Hr exposure, 6.5 days recovery	Dechlorinated Tap	0.05 - 1.6
	FT, C	12 Hr exposure, 6.5 days recovery	Synthetic [1]	0.1 - 1.6
D. magna	S, A	48 hrs	New River	0.1 - 1.0
Fathead minnow	S, C	7 days	Dechlorinated Tap	0.4 - 12.8
	FT, C	7 days	Dechlorinated Tap	0.05 - 0.8
	FT, C	12-Hr exposure, 6.5 days recovery	Dechlorinated Tap	0.4 - 6.4
C. riparius	S, A	48 hrs	Dechlorinated Tap	1.0 - 16.0
H. azteca	S, A	96 hrs	Dechlorinated Tap	0.1 - 1.6
Goniobasis **sp.**	S, A	96 hrs	Dechlorinated Tap	2.5 - 15.0
M. bahia	FT, A	96 hrs	Natural Seawater [2]	0.1 - 1.6
S. capricornutum	S, C	96 hrs	Algal Media [3]	0.16 - 2.5

[1] US EPA Moderately Hard Water (USEPA 1991)
[2] Collected at Coast Guard Pier, Wrightsville Beach Inlet, Wrightsville Beach, NC
[3] USEPA (1989)

other species followed general methods outlined in Powlesland and George (1986) and APHA (1985). The diluent listed in Table 1B also served as the control treatment for the bioassays. A summary of selected chemistry parameters for the test waters is presented in Table 4-2.

Flow-through methods followed standard operating procedures described by Lauth and Cherry (1993), using 2 exposure systems. In bioassays with *C. dubia* or *M. bahia*, fresh toxicant was combined with diluent by drawing the two solutions through a static mixer with a peristaltic cartridge pump. The resultant test solution was then delivered to glass exposure troughs at a flow rate of 600 ml/hr. Each trough contained 5 replicate test chambers constructed from 45-ml polystyrene cups which held 35 ml at maximum volume. Nitex screen (120 μm mesh) was fixed over each of two 2.5 cm squares cut into opposite sides of the cups, a design which allowed test solution to flow freely through the test chambers. For acute tests, 5 organisms were placed in each cup for a total of 25 organisms/concentration. Test solutions for bioassays with the fathead minnow were delivered as described above to individual test chambers constructed from 450-ml polystyrene jars. Each chamber had an incurrent port located approximately 1 cm from the bottom, and a Nitex-covered rectangular (2.5 cm x 5 cm) overflow hole approximately 2 cm from the top. Flow rates through this system were maintained at 10ml/min. Toxicant:diluent ratios were mediated by using different tubing sizes for delivery of toxicant which allowed test solutions to be derived from a common, concentrated stock.

Table 4-2. Selected water chemistry parameters of dilution waters used in the bioassays.

Dilution Water	pH	Conductivity (μmhos)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
Synthetic	8.09	296	89	62
New River	7.94	111	50	40
Dechlorinated Tap	7.70	145	60	49

For the chronic tests with *C. dubia*, a single daphnid was placed in each test chamber, with two exposure troughs used to allow ten replicates/treatment. Stock solutions of algae (*S. capricornutum*) and Yeast-Cerophyll-Trout Chow (YCT) were added to the diluent reservoir in aliquots calculated to achieve the appropriate concentrations (as recommended in USEPA 1989) in the test chambers at all times. The daphnids were transferred to fresh exposure chambers on a daily basis. Fathead minnows were fed brine shrimp (*Artemia salinia*) by adding the organisms directly to the test containers. Each container was cleaned by removing accumulated debris from the bottom and draining off approximately 75 % of the test solution. Other general test parameters followed those described by USEPA (1991).

As seen in Table 4-1B, certain chronic bioassays with *C. dubia* and the fathead minnow had a 12-hr exposure to DGH/QUAT followed by 6.5 days of recovery in the diluent water. The molluscicide is commonly applied for 12 to 24 hrs under actual conditions, and the modified exposure used in the bioassays was chosen to determine the impact of this "real world" dosing regime upon the nontarget organisms.

4.2.4 Bioassays with DGH/QUAT and Clay

These tests were conducted to assess the detoxification potential of the molluscicide through mixing with bentonite clay, and also to begin preliminary work addressing the ultimate fate and effects of the molluscicide and clay complex once released into a receiving system. In the exposures, a standardized level of DGH/QUAT (usually 10 mg/L) previously shown to be effective for macrofouling control (Cherry et al. 1990;

Lyons et al. 1990) was mixed with aliquots of a 5% clay slurry to achieve various ratios of the two components. The clay slurry was prepared by first mixing bentonite clay with deionized water in a Waring blender for 5 min, followed by an additional 30-min mixing on a stirplate. The DGH/QUAT solution was prepared in test diluent and mixed with the appropriate clay aliquots for an additional 30 min before addition of the organisms.

Bioassays followed the test conditions outlined in Tables 4-3A and B. The protocol for the 21-day *D. magna* survival and reproduction bioassay closely followed that described by ASTM (1989). Test solutions were renewed every other day and the individual daphnids were fed 0.2 mg (dry weight) of a tri-algal mixture which included *Chlamydomonas rheinhardti, Ankistrodesmus falcatus*, and *Chlorella vulgaris*. Methods for nonstandardized bioassays are as follows:

Fathead Minnow 7-Day Flow-through Chronic

Test protocol followed that described for the flow-through test of fathead minnows with DGH/QUAT alone. Peristaltic pumps and static mixers were used to combine premixed stock solutions of DGH/QUAT and clay to obtain the ratios listed in Table 4-3B. Retention time of the two solutions within the static mixer averaged 18 sec.

Daphnia magna 48-hr Flow-through Acute Tests at Varying Water Hardness

This test was conducted to determine if water hardness influenced the binding of molluscicide and bentonite clay. Test chambers and dosing strategy were as described for the fathead minnow flow-through bioassays, with 20 organisms (10/replicate) per

Table 4-3A. Summary of general test conditions for exposures to DGH/QUAT and bentonite clay.

S: Static, FT: Flow-through, A: Acute, C: Chronic, M: Mortality, R: Reproduction, G: Growth

Species	Age	Test Type	Test Chamber Size	Test Chamber Volume	Test Temperature	# Organisms Per Chamber/ # Replicates	Endpoint
C. dubia	< 24 hrs	S, A	50 ml	25 ml	25 ± 1 °C	10/2	M
D. magna	< 24 hrs	S, A	100 ml	50 ml	20 ± 1 °C	10/2	M
		S, A	100 ml	50 ml	20 ± 1 °C	1/10	M, R
		FT, A	450 ml	300 ml	20 ± 1 °C	10/4	M, G
Fathead minnow	< 24 hrs	S, C	400 ml	250 ml	25 ± 1 °C	10/4	M, G
		FT, C	450 ml	300 ml	25 ± 1 °C	10/4	M, G
C. riparius	2nd Instar	S, C	450 ml	250 ml	20 ± 1 °C	10/3	M, G
Leaf Pack Study	-------	S	Artificial Stream		Amibient	10 Leaves per pack/3 packs	Microbial Conditioning

Table 4-3B. Summary of specific test conditions for exposures to DGH/QUAT and clay.

S: Static, FT: Flow-through, A: Acute, C: Chronic

Species	Test		Diluent	Toxicant Ratio DGH/QUAT : Clay (mg/L)
	Type	Duration		
C. dubia	S, A	48 hrs	New River	0:100, 10:0, 10:10, 10:20, 10:30, 10:50, 10:100
	S, A	48 hrs	New River	20, 30, 50, 100, 200[1]
D. magna	S, A	48 hrs	New River	100:0, 10:0, 10:100
	S, C	21 days	Dechlorinated Tap	0:100, 10:0, 10:10, 10:20, 10:30, 10:50, 10:100
	FT, A	48 hrs	Synthetic [2]	10:0, 10:20, 10:30, 10:50, 10:100
Fathead Minnow	S, C	7 days	Dechlorinated Tap	0:100, 10:0, 10:10, 10:30, 10:50, 10:100
	FT, C	7 days	Synthetic [2]	10:0, 10:10, 10:20, 10:30, 10:50
C. riparius	S, C	2 day exposure, 8 day recovery[3]	Dechlorinated Tap	0:100, 10:0, 10:10, 10:30, 10:50, 10:100
	S, C	10 days [4]	Dechlorinated Tap	0:100, 10:0, 10:10, 10:20, 10:30, 10:50, 10:100
Leaf Pack Study	S	Single 12-Hr exposure, or four 12-hr exposures over 9 days	New River	10:0, 10:30, 10:50

[1] Bioassay conducted with exposure to clay alone
[2] US EPA Moderately Hard Water (USEPA 1991)
[3] 20 ml of glass beads (212-300 μm, Sigma Chem.) added to test chambers for substrate.
[4] 2 bioassays, 20 ml of glass beads or natural sediment used for respective substrate.

treatment. Synthetic water at hardness levels of 50, 100, 200, and 400 mg/L as $CaCO_3$ was used as diluent in separate bioassays, with water formulations following that described by USEPA (1991).

Chironomus riparius 10-day Survival and Growth Tests

Midge larvae were exposed to DGH/QUAT and in 400-ml Pyrex storage containers holding 250 ml of test solution. An artificial substrate of glass beads (20 ml, 212-300 μm dia., Sigma Chem.) was placed in each exposure chamber for the larvae to construct tubes and burrow. To start a test, 10 larvae were pipetted into each of three replicate test chambers for a total of 30 organisms per concentration. Each test chamber received a 0.5 ml aliquot of a 0.06 g/ml suspension of flake fish food on a daily basis. Daily renewal of 200 ml of test solution was continued until day 10, at which time all surviving organisms were removed from the test chambers, dried for 24 hours at 60 °C, and weighed to 0.0001 g on a Sartorius micro-electronic balance. In the first bioassay conducted, larvae were exposed to the DGH/QUAT and clay ratios for only the first 2 days of the test followed by 8 days of recovery. As previously mentioned, this altered exposure regime was chosen to match realistic dosing conditions. An additional pair of bioassays were conducted in which the organisms received exposure to the molluscicide and clay for a full 10 days. These 2 tests were run side-by-side and differed only in that natural sediment was used in one instead of artificial beads. The natural sediment (obtained from Sinking Creek in Newport, VA) was sieved to obtain a particle size range similar to that of the glass beads.

Leaf Pack Study

This study was conducted at ambient river temperature between October and November 1992, and indirectly examined the impact of DGH/QUAT and clay upon a natural microbial fauna by monitoring leaf conditioning in an artificial stream system (Fig. 4-1). Artificial streams were constructed such that paddlewheels driven by an electric motor provided constant current. A full description of the system can be found in Farris (1986).

The study was conducted at a field laboratory located at Glen Lyn, Virginia. Leaf packs were constructed by placing 20 leaves (sugar maple, *Acer saccharum*) in nylon mesh bags which were then randomly placed in the streams which received a constant supply of New River water. The leaves were collected at fall abscision and allowed to dry at room temperature before use. Each artificial stream was filled with approximately 3 cm of a natural river sediment, and three replicate streams were used for each treatment. Treatment regimes included a single 12-hr exposure to 10 mg/L DGH/QUAT (10:0 ratio) and DGH/QUAT:clay at a 10:30 ratio. Multiple exposures were conducted with molluscicide and clay ratios of 10:30 and 10:50. These treatments included the initial dose followed by 3 additional 12-hr exposures at 3 day intervals (for a total of 4 dosings). This strategy was used to simulate multiple inputs to a receiving system such as that along heavily industrialized zones with a number of facilities dosing with the molluscicide.

Static dosing of the system was initiated by making stocks of DGH/QUAT and clay in separate 208 L polystyrene drums using raw New River water as the diluent. Clay

stocks were derived from 5% slurries which were prepared as previously described. Flow of river water to the artificial streams was stopped, the streams were drained, and the volume replaced with appropriate amounts of molluscicide and clay stocks. Mixing in the streams was maintained with the paddle wheels, and test solutions were renewed as described above every 4 hrs during the 12-hr exposure period. At the end of dosing, flow of river water to the streams was restarted. The rate of water entering the 30-L artificial streams was maintained at 2 L/min to completely renew the stream volume in 15 min. After this flushing period, flow was set to 100ml/min. Control streams were subjected to the same changeover schedule as treatment streams but were dosed with river water only.

Initially, and on days 15, 30, and 60 following the end of the dosing periods, 5 leaves were collected from each replicate stream, and four measurements of toughness or penetrance were taken from each leaf as described by Feeney (1970). In this method, a leaf is placed between two plexiglass blocks through which a cylindrical hole had been drilled and a metal rod is placed through this hole to contact the leaf body. A plastic beaker is placed on a base on top of the punching rod and steel shot is added to the beaker until the rod pierces the leaf body. The weight of the shot is then recorded as the penetrance. The principal behind this method is that as the leaf material becomes softer due to the digestive processes of microbes, it takes less pressure to penetrate. Differences in microbial activity between the treatments would be detected as differences in penetrance of leaves.

4.2.5 Effect of River Turbidity on Free Levels of DGH/QUAT

The extent to which naturally occurring suspended matter in river water binds with the molluscicide was evaluated. Measured concentrations of free DGH/QUAT were determined after mixing stock solutions of 10 and 15 mg molluscicide/L in water with levels of total suspended solids (TSS) ranging between 5 and 340 mg/L. Water samples originated from an initial highly turbid grab taken from Stroubles Creek on the Virginia Tech campus following a heavy rain. TSS levels were determined as described by APHA (1985), and adjusted by dilution with deionized water. Water samples were dosed with DGH/QUAT and mixed for 30 min before analysis. The samples were read against blanks with corresponding TSS but no molluscicide.

4.2.6 Water Chemistry

Dissolved oxygen, pH, temperature, conductivity, alkalinity, and hardness were measured in acute and chronic bioassays as recommended by US EPA (1989; 1991).

4.2.7 Statistical Analyses

A trimmed Spearman-Karber (Hamilton et al. 1978) method was used to generate acute LC_{50} (toxicant concentration lethal to 50% of the test organisms) values and associated confidence intervals. Chronic toxicity data were analyzed as described by US EPA (1989), with endpoint estimates of the NOEC (no observed effects concentration) and LOEC (lowest observed effects concentration) made with either a Dunnett's or

Steel's Rank test. Leaf penetrance data were analyzed with a general linear model (GLM) followed by multiple comparison using Fisher's Protected LSD (SAS 1985).

4.3 Results

4.3.1 Bioassays with DGH/QUAT Alone

LC_{50} values from the acute bioassay data ranged from 0.12 and 0.14 mg/L for *H. azteca* and *C. dubia*, respectively, to 6.49 and 11.0 for *C. riparius* and the snail *Goniobasis* sp. (Table 4-4).

In chronic bioassays, *C. dubia* was more sensitive than the fathead minnow for mortality and sublethal impairment (reproduction in the cladoceran, growth in the fish, Tables 4-5A and B). As expected, mortality of both test species was higher in exposures which lasted for 7 days as compared to those which lasted 12 hr. Differences in sensitivity were apparent between bioassays conducted under static versus flow-through conditions. For example, the LOEC in the fathead 7-day test was 5.4 mg/L under static conditions, and 0.23 mg/L under flow-through. This trend did not hold for the 12-hr exposure bioassays with *C. dubia*, since similar measured lowest effect concentrations (for both survival and reproduction) of 0.41 and 0.39 mg DGH/QUAT/L, were obtained for flow-through and static tests, respectively. The agreement between the two test designs was probably the result of the shorter exposure time. Like *C. dubia*, the algae, *S. capricornutum,* was very sensitive to DGH\QUAT, with an LOEC of 0.10 mg/L (Table 4-5B). As with other concentrations of molluscicide which were below the analytical detection limit of 0.2 mg/L, this concentration was based on dilution of the

Table 4-4. Summary of acute bioassays with DGH/QUAT alone. Unless otherwise indicated, all reported values are based on measured concentrations.

S: Static, FT: Flow-through

Species	Test		LC_{50} (mg/L)	95 % Confidence Interval
	Type	Duration		
C. dubia	S	48 hrs	0.45 [A]	0.42 - 0.49
	FT	48 hrs	0.14	0.12 - 0.16
D. magna	S	48 hrs	0.51 [A]	0.48 - 0.54
C. riparius	S	48 hrs	6.49	5.33 - 8.11
H. azteca	S	48 hrs	0.20	0.13 - 0.26
		96 hrs	0.12	0.10 - 0.17
Goniobasis sp.	S	96 hrs	11.0	10.3 - 11.9
M. bahia	FT	96 hrs	0.34	0.28 - 0.41

[A] Based on nominal values

Table 4-5A. Summary of survival in the control, no observed effects concentration (NOEC), and lowest observed effects concentration (LOEC) in chronic bioassays with DGH/QUAT. Divided test durations represent exposure time to molluscicide \ recovery time in diluent only.

S: Static, FT: Flow-through

	Test		Control	NOEC (mg/L)		LOEC (mg/L)	
Species	Type	Duration	% Mortality	Nominal \ Measured	% Mortality	Nominal \ Measured	% Mortality
C. dubia	S	7 days	0	0.125 \ 0.057[A]	0	0.250 \ 0.11[A]	90
	S	12 hr \ 6.5 days	0	0.40 \ 0.22	10	0.80 \ 0.41	100
	FT	12 hr \ 6.5 days	0	0.20 \ 0.27	10	0.40 \ 0.39	30
Fathead minnow	S	7 days	5	3.2 \ 2.8	12	6.40 \ 5.43	100
	FT	7 days	5	0.10[B]	25	0.20 \ 0.23	38
	FT	12 hr \ 6.5 days	5	0.82 \ 0.63	18	1.6 \ 1.4	68

[A] Values based on dilution of lowest measurable concentration
[B] Nominal concentration only

Table 4-5B. Summary of reproductive output or growth of test organisms in the control, no observed effects concentration (NOEC), and lowest observed effects concentration (LOEC) in chronic bioassays with DGH/QUAT. Divided test durations represent exposure time to molluscicide / recovery time in diluent only.

S: Static, FT: Flow-through

Test		NOEC (mg/L)	LOEC (mg/L)	Parameter Measured		
Type	Duration	Nominal \ Measured	Nominal \ Measured	Control	NOEC	LOEC
C. dubia				**Average Neonate Production (± 1 SEM)**		
S	7 days	0.06 \ 0.02 [A]	0.12 \ 0.03 [A]	36.7 (1.1)	32.5 (1.2)	27.9 (2.0)
S	12 hr/6.5 days	0.40 \ 0.22	0.80 \ 0.41	25.5 (1.2)	22.3 (2.2)	0
FT	12 hr/6.5 days	0.20 \ 0.27	0.40 \ 0.39	26.0 (1.3)	22.0 (1.5)	17.2 (1.3)
Fathead minnow				**Average Dry Weight (± 1 SEM)**		
S	7 days	1.60 \ 0.95	3.20 \ 2.81	0.294 (0.021)	0.273 (0.010)	0.211 (0.010)
FT	7 days	0.10 [B]	0.20 \ 0.23	0.421 (0.031)	0.310 (0.044)	0.273 (0.020)
FT	12-hr/6.5 days	0.80 \ 0.61	1.60 \ 1.44	0.334 (0.011)	0.323 (0.020)	0.187 (0.051)
S. capricornutum				**Average Relative Absorbance [C] (± 1 SEM)**		
S	96 hrs	0.16 \ 0.05A	0.31 \ 0.10	23.5 (1.1)	20.4 (3.0)	1.45 (0.3)

[A] Values based on dilution of lowest measurable concentration
[B] Nominal concentration only
[C] Fluorometric Absorbance

lowest measurable concentration in the bioassay.

4.3.2 Bioassays with DGH/QUAT and Clay

Mixture of DGH/QUAT with bentonite clay effectively removed free levels of the molluscicide from the water column, and a ratio of 1:3 dropped the concentration below detection (Table 4-6). However, binding with clay did not always translate into a reduction in toxicity to test organisms. In acute bioassays, nearly 100 % mortality of *C. dubia* was observed at all molluscicide and clay ratios tested (Table 4-7A). In order to gain further insight into these results, a 48-hr acute bioassay in which *C. dubia* were exposed to solutions of bentonite clay was conducted (Table 4-8). This test (48-hr LC_{50} =33.2 mg/L clay using concentrations from 30 - 200 mg/L) clearly demonstrates the sensitivity of the cladoceran to the clay itself.

In contrast to *C. dubia*, clay reduced toxicity of the molluscicide to the larger *D. magna* in static tests (Table 4-7A). Under flow-through conditions, the impact of the molluscicide and clay ratios appeared to correlate with water hardness. At a hardness of 100 mg/L (as $CaCO_3$) a ratio of 10:100 was necessary to reduce mortality to control levels, while at 200 to 400 mg/L a 1:5 ratio was sufficient. No bioassay was conducted at a hardness of 50 mg CaCO3/L due to poor survival and reproduction of brood stock. Clay accumulated in all test chambers at ratios of 10:30 and above, and in some cases the organisms were trapped in a clay layer on the chamber bottom. Those daphnids surviving at 10:100 level were completely trapped below the clay layer but were still alive. Finally, due to clogging of the static mixer with clay, measurable levels of

Table 4-6. Free levels of DGH/QUAT remaining in solution after mixing with various levels of bentonite clay.

Ratio DGH/QUAT : Clay (mg/L)	Measured Concentration of DGH/QUAT (mg/L)
0 : 0	0
10 : 0	10.9
10 : 10	1.59
10 : 20	0.22
10 : 30	Below Detection (< 0.2 mg/L)
10 : 50	Below Detection (< 0.2 mg/L)
10 : 100	Below Detection (< 0.2 mg/L)

Table 4-7A. Mortality of test organisms in both acute and chronic bioassays with DGH/QUAT mixed with bentonite clay. Test conditions are listed under species, S = Static, FT = Flow-through.

	% Mortality										
			D. magna FT, 48 Hr Hardness							*C. riparius* S, 10 Day	
DGH/QUAT : Clay (mg/L)	*C. dubia* S, 48 Hr	*D. magna* S, 48 Hr	100	200	400	*D. magna* S, 21 Day	Fathead Minnow S, 7 Day	Fathead Minnow FT, 7 Day	*C. riparius* S, 2 Day/8 Day	A[1]	N[1]
0 : 0	0	0	0	0	0	0	5	8	0	3	10
10 : 0	100	100	100	100	100	100	100	100	80	70	10
0 : 100	75	0	*	*	*	10	0	*	0	7	3
10 : 10	100	*	*	*	*	100	13	78	10	13	0
10 : 20	100	*	100	100	100	100	*	40	*	7	0
10 : 30	100	*	100	100	60	90	7	50	0	0	10
10 : 50	100	*	60	15	5	40	18	65	3	0	0
10 : 100	95	0	10	10	0	10	10	*	0	7	3

* Ratio not tested

[1] A = tested on artificial substrate of glass beads
N = tested on natural substrate

Table 4-7B. Average reproductive output or growth (± 1 SEM) of test organisms in chronic bioassays with DGH/QUAT mixed with bentonite clay. Divided tests durations represent exposure time to molluscicide and clay \ recovery time in diluent only. Test conditions are listed under species, S = Static, FT = Flow-through, AS = Artificial Substrate (Glass Beads), NS = Natural Substrate. The dagger (†) represents treatments which are significantly different from the control at $\alpha = 0.05$.

Ratio DGH/QUAT : Clay (mg/L)	*D. magna* S, 21 Day Neonate Production	Fathead Minnow S, 7 day Dry Weight (mg)	C. riparius S, 2 Day/8 Day Dry Weight (mg)	*C. riparius* S, 10 Day Dry Weight (mg) AS	*C. riparius* S, 10 Day Dry Weight (mg) N S
0 : 0	183.0 (6.0)	0.310 (0.024)	0.905 (0.012)	0.575 (0.034)	0.835 (0.058)
0 : 100	108.7 (7.4)†	0.286 (0.002)	0.979 (0.014)†	0.678 (0.048)	0.893 (0.051)
10 : 0	CM	CM	0.812 (0.014)†	0.064 (0.010)†	0.678 (0.029)†
10 : 10	CM	0.188 (0.024)†	0.875 (0.012)†	0.227 (0.011)†	0.707 (0.053)†
10 : 20	CM	*	*	0.390 (0.053)†	0.759 (0.070)
10 : 30	0†	0.303 (0.005)	0.916 (0.011)	0.548 (0.044)	0.837 (0.025)
10 : 50	26.7 (9.4)†	0.336 (0.005)	0.928 (0.012)	0.556 (0.011)	0.792 (0.026)
10 : 100	80.4 (7.4)†	0.281 (0.011)	0.950 (0.018)	0.476 (0.037)	0.751 (0.010)

* = Ratio not tested
CM = complete mortality at this level

Table 4-8. Mortality of *Ceriodaphnia dubia* in a 48-hr acute bioassay with bentonite clay.

Clay (mg/L)	% Mortality
0	0
10	30
20	80 (85)[1]
30	45 (55)[1]
50	70
100	80
200	90

[1] number in parentheses is the mortality in a repeat bioassay

DGH/QUAT were occasionally observed in the 1:3 ratio, although the concentration never exceeded 0.24 mg/L.

In the static 21-day test with *D. magna* a 1:10 ratio of molluscicide and clay was necessary to significantly reduce mortality, although reproductive output was impaired at all levels (Table 4-7A and B). Interactions between the organisms and clay were apparent as daphnids in the higher clay levels were often observed with conglomerations of clay and algae sticking to their carapace.

Mortality was reduced by the clay at all ratios tested in the static fathead minnow test, although a significant reduction in growth was observed at a ratio of 10:10 (Table 4-7A and B). As seen in the *D. magna* acute tests, mortality of the fatheads was much higher in flow-through as compared to static bioassays. In the former, significant levels of mortality were observed at all molluscicide and clay ratios tested, so no growth analysis was done. Again, at least part of the observed mortality in this test may have been due to the accumulation of clay and associated stress upon the organisms in the test chambers.

In both 2 and 10-day exposures, molluscicide-induced mortality of the midge, *C. riparius*, was reduced or eliminated at all ratios of clay tested (Table 4-7A and B). Growth impairment was eliminated at ratios of 1:3 and higher and 1:2 and higher on artificial and natural substrates, respectively. In the latter, toxicity of the molluscicide was probably modified by its adsorption to the sediment itself (Fig. 4-2). Such an effect was not observed on the inert glass beads. Chironomids had significantly higher dry weights on natural as compared to artificial sediments, and in both tests conducted with

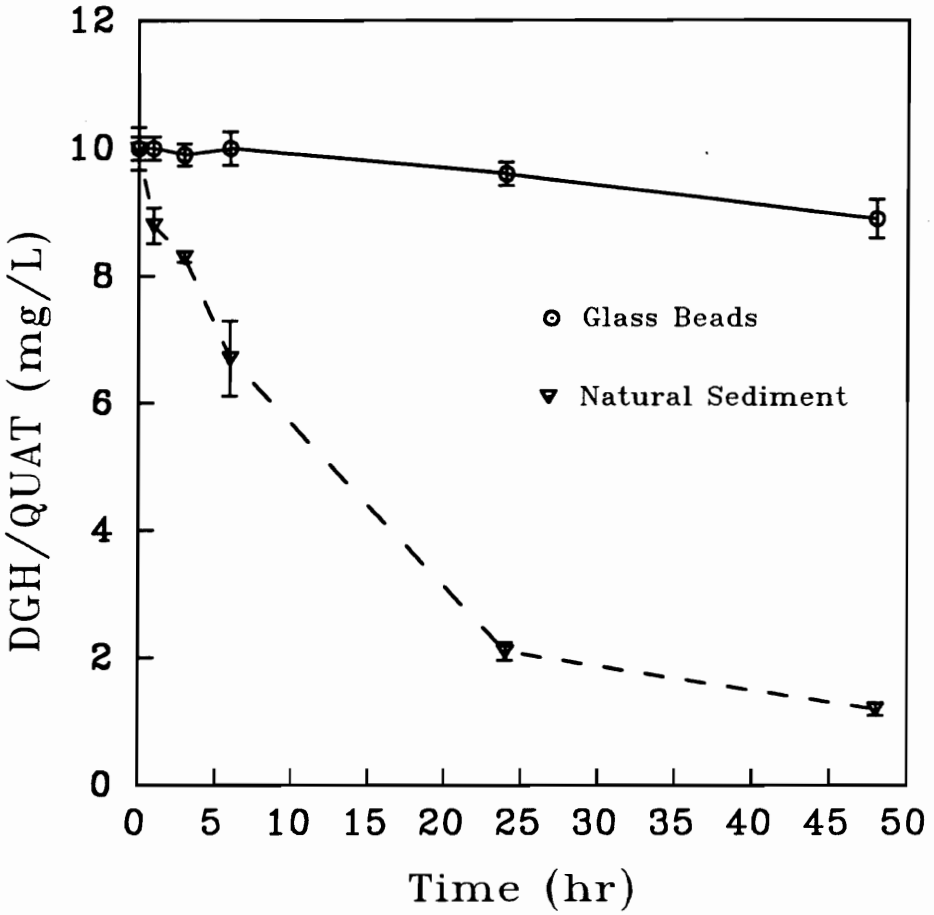


Figure 4-2. Uptake of free levels of DGH/QUAT from water overlying either a natural gravel substrate or an artificial substrate of glass beads. Both substrate types had a particle size range of 212-300 μm. The reported molluscicide level is the average of three replicate samples and error bars represent $\pm$ 1 SEM.

the glass beads, had higher weights as compared to controls in the treatment with 100 mg/L clay alone. These results further illustrate the sterile nature of the glass beads.

Leaf Pack Study

Concentrations of DGH/QUAT in the artificial streams were measured at the beginning and end of renewals which were done at 4-hr intervals during the 12-hr dosing period. Suspended matter in the river water and the natural sediment in the artificial stream systems served to substantially reduce DGH/QUAT levels between these renewals (Table 4-9). For example, average molluscicide levels in the initial 12-hr treatment with DGH/QUAT alone were 7.0 and 3.2 mg/L at the beginning and end of dosing, respectively. Measurable levels of DGH/QUAT were detected in some beginning samples at the 10:30 ratio (0.29 mg/L maximum), although samples taken at the end of the 4-hr intervals were always below detection. No detectable levels of molluscicide were found in the 10:50 streams.

Temperature of New River water running through the artificial streams ranged between 17.0 and 9.6 °C over the 60-day study period. The initial penetrance of leaf packs at the start of the test was 386.9 g, which subsequently declined significantly in all treatments by day 60 (Fig. 4-3). An apparent lag in the rate of leaf processing was observed in treated streams. Penetrance of control leaves was significantly lower than that of all treated leaves on day 15, but by day 30, impairment (as determined by a higher penetrance) was only apparent in the multiple dosed streams. By day 60 none of the systems treated with DGH/QUAT and clay differed from controls, although a

Table 4-9. Measured levels of DGH/QUAT (initial\final) at renewal on each of the treatment dates of the leafpack study.

Date	DGH/QUAT:Clay (mg/L)	DGH/QUAT (mg/L)/ Time Interval (Hr) 0-4	4-8	8-12	12-hr Average
10/21/92	0:0	---	---	---	---
	10:0	8.0\1.5	7.8\4.9	5.1\3.1	7.0\3.2
	10:30	0.23\<0.20	0.29\<0.20	0.27\<0.20	0.26\<0.20
	10:50	<0.20\<0.20	<0.20\<0.20	<0.20\<0.20	<0.20\<0.20
10/24/92	0:0	---	---	---	---
	10:0 (stock)	9.0 = used to prepare 10:30 and 10:50 ratios			
	10:30	0.23\<0.20	<0.20\<0.20	<0.20\<0.20	<0.20\<0.20
	10:50	<0.20\<0.20	<0.20\<0.20	<0.20\<0.20	<0.20\<0.20
10/27/92	0:0	---	---	---	---
	10:0 (stock)	10.9			
	10:30	<0.20\<0.20	0.24\<0.20	<0.20\<0.20	<0.20\<0.20
	10:50	<0.20\<0.20	<0.20\<0.20	<0.20\<0.20	<0.20\<0.20
10/30/92	0:0	---	---	---	---
	10:0 (stock)	7.7			
	10:30	<0.20\<0.20	<0.20\<0.20	<0.20\<0.20	<0.20\<0.20
	10:50	<0.20\<0.20	<0.20\<0.20	<0.20\<0.20	<0.20\<0.20

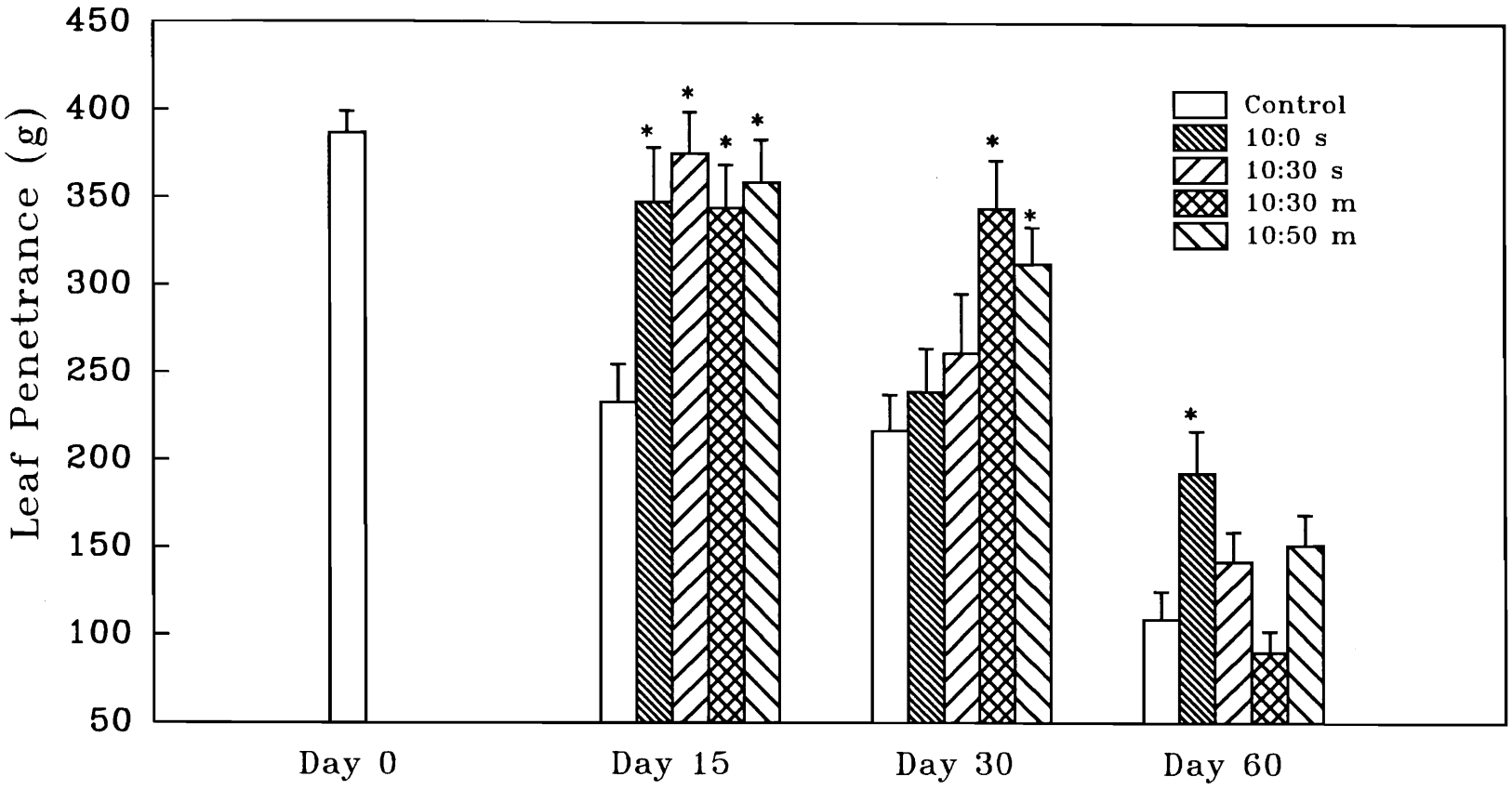


Figure 4-3. Average penetrance of leaf packs following single (s) or multiple (m) treatments with DGH/QUAT and bentonite clay. Levels of molluscicide and clay are expressed in mg/L with the molluscicide concentration first in the ratio. Error bars represent $\pm$ 1 SEM, and an asterisk (*) above the error bar indicates significant difference at $\alpha = 0.05$.

difference between the leaves that had received a single dose of 10 mg/L molluscicide alone and controls was observed. No difference between the control and molluscicide only treatment had been observed on day 30.

4.3.3 Effect of Total Suspended Solids on DGH\QUAT Levels

The capacity of naturally occurring suspended matter to bind with DGH/QUAT was apparent as free levels of molluscicide were inversely related to water turbidity, with some leveling noted above 150 mg TSS/L (Fig. 4-4).

4.4 Discussion

4.4.1 Bioassays with DGH/QUAT Alone

As previously stated, the cationic surfactants dodecylguanidine hydrochloride (DGH) and n-alkyl dimethylbenzyl ammonium chloride (QUAT) form the active portion of the molluscicide (DGH/QUAT) in the present study. Alkyldimethylybenzylammonium compounds are primarily used as biocides, sanitizers, and disinfectants (Boethling 1984; Schaeufele 1984). Vallejo-Freire et al. (1954) described the molluscicidal properties of these chemicals which are a subgroup of the cationic quaternary ammonium compounds or QACs (Boethling 1984).

The toxicity of surfactants as a group can vary according to charge (cationic, anionic, nonionic) and structure (alkyl chain length, straight versus branched chain, accessory groups) (Maki and Bishop 1979; Lewis and Perry 1981; Karpinska-Smulikowska 1984; Lewis 1991). These factors, in addition to the inherent variability which can exist

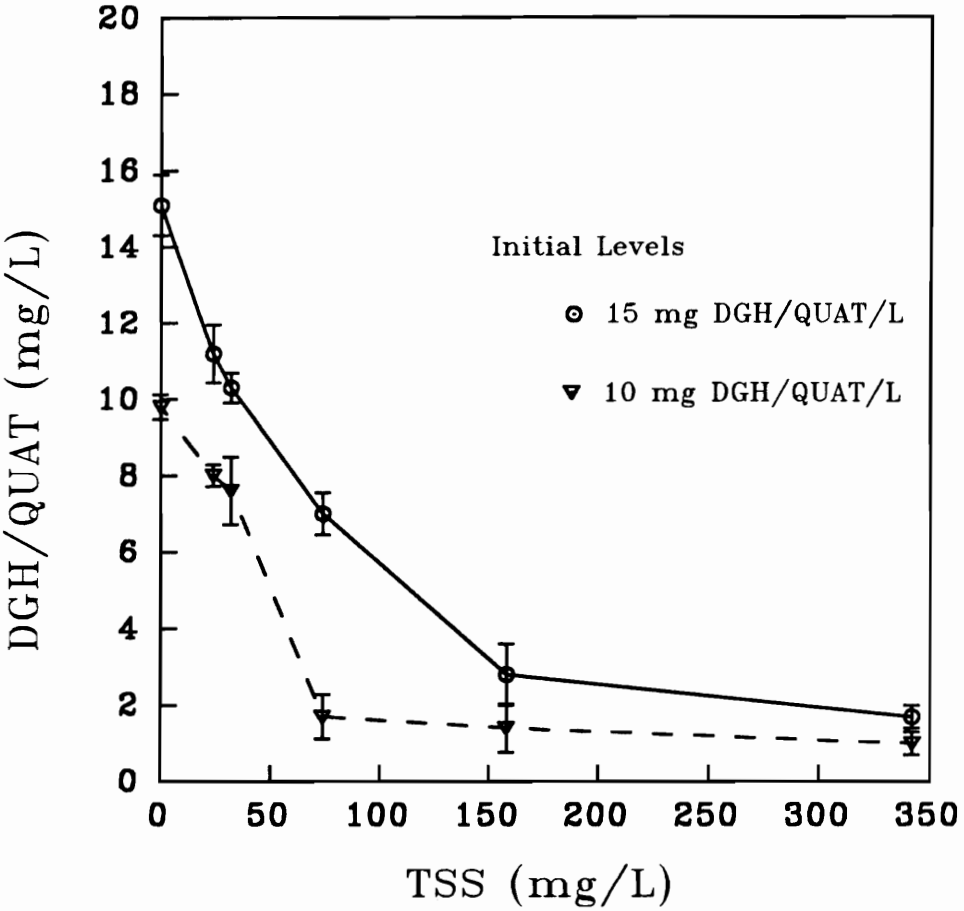


Figure 4-4. Effect of varying total suspended solids (TSS) on free levels of DGH/QUAT in solutions with initial concentrations of 10 or 15 mg molluscicide/L. The reported molluscicide level is the average of three replicate samples and error bars represent ± 1 SEM.

between bioassays, make it difficult to directly compare toxicity data between studies, although comparison of general trends is possible.

Of the species tested in acute bioassays with DGH/QUAT, the cladoceran, *C. dubia*, and the amphipod, *H. azteca*, were most sensitive (LC_{50} values of 0.14 and 0.12 mg/L, respectively), while the midge, *C. riparius*, and the snail, *Goniobasis* sp., were most resistant (LC_{50} values of 6.49 and 11.0 mg/L, respectively). *D. magna* was among the most sensitive organisms tested with DGH/QUAT in previous studies (summarized in Table 10). Beisinger and Stokes (1986) found amphipods, *H. azteca*, and daphnids to be most adversely affected in a microcosm study with a cationic polymer. In this same study, a species of midge, *Paratanytarsus parthogeneticus*, was one of the more resistant organisms in comparative acute testing. Literature values pertaining to the acute toxicity of cationic surfactants to the snail, *Goniobasis* sp., were lacking, although Hendricks et al. (1974) reported a 24-hr LC_{50} of 19.4 mg/L for this species. During the bioassay with DGH/QUAT, snails would sometimes close their operculum and thus avoid exposure to the molluscicide. While this behavior probably afforded the organisms some resistance, it is not clear if the molluscicide actually caused the response.

With only mortality data available, it is not possible to make definitive conclusions concerning differences in sensitivity to DGH/QUAT. In both the present work, and in previous studies of surfactant toxicity (reviewed by Abel 1974; Margaritis and Creese 1979; Lewis and Suprenant 1983), cladocerans were among the most sensitive species examined. Exposure to both anionic and cationic surfactants caused gill damage in fish which can result in osmoregulatory imbalance and eventual asphyxiation (Abel 1974;

Beisinger and Stokes 1986). Osmoregulatory imbalance as indicated by changes in tissue water levels was observed in Asian clams and zebra mussels following exposure to DGH/QUAT (Bidwell et al. 1993). Organisms with greater sensitivity to the molluscicide may succumb more quickly to the physiological effects of exposure just as more resistant forms may better handle these effects. Different modes of toxic action could also play a role. Lewis and Perry (1981) suggested that, based on comparative toxicity data, the anionic and cationic surfactants they tested may have had a different target site or mode of action for *D. magna* as compared to the bluegill, *Lepomis machrochirus*.

C. dubia and the algae *S. capricornutum* exhibited greater sensitivity than the fathead minnow under chronic exposures to DGH/QUAT. Again, differences in species sensitivity can relate to differences in physiological tolerances or modes of action. *C. dubia* was found to be more sensitive than the fathead minnow in previous work with other toxicants (Lewis et al. 1989; Walsh et al. 1980). Cationic surfactants have often been found to be more, or at least equally, toxic to algal species than either anionic or nonionic forms (Lewis 1990), with toxicity probably due to disruption of the cell membrane and alteration of permeability to nutrients and other chemicals (Parishkova and Negrutsky 1988; Lewis 1990).

The effects levels reported for DGH/QUAT in both acute and chronic studies illustrate the differences in molluscicide concentration which can be observed between static and flow-through test strategies, or when using nominal versus measured levels for calculations. In some static tests, free levels of DGH/QUAT dropped to near zero within 24 hr, reducing the actual exposure time the organisms experienced. Cationic surfactants

have the propensity to bind with negatively charged particles or surfaces (Law and Kunze 1966; Lewis and Wee 1983; Huber 1984), so the loss of molluscicide in static testing was probably due to binding of the actives to glassware or particulate matter in the test water. A solution to this problem is the use of flow-through systems which allow demand to be overcome or compensated for, resulting in lower variability of test concentrations.

4.4.2 Comparison with Other Biocides

Table 4-10 summarizes acute, nontarget toxicity data generated in previous studies with DGH/QUAT, two other nonoxidizing biofouling control agents; Bulab 6002 and H130, and chlorine and bromine (additional comparative data should be obtained from Table 4). All biocides were acutely toxic to the majority of species tested, although comparisons between chlorine and DGH/QUAT indicated greater toxicity of the former.

4.4.3 Bioassays with DGH/QUAT and Clay

In addition to loss observed in static test solutions, the adsorptive potential of DGH/QUAT was illustrated by drops in free levels of molluscicide in the presence of bentonite clay, naturally occurring suspended solids, or natural sediment. Since the charged end of a surfactant is the "business end" with respect to toxicity (the charge causes the attraction to gill membrane, etc.), binding of these compounds with alternate surfaces can reduce bioavailability and toxicity (Abel 1974; Lewis and Wee 1983). Reductions in surfactant toxicity have been attributed to suspended matter in natural river water or to binding with food placed in test containers during bioassays (Biesinger et al.

Table 4-10. Summary of acute data from studies previously conducted with DGH/QUAT, other nonoxidizing commercial biocides, chlorine, or bromine.

Species	Biocide	Duration	LC_{50} (mg/L), (95 % C.I.)	Reference
Rainbow Trout (***Oncorhynchus mykiss***)	DGH/QUAT	48 hr	2.12, (1.61 - 2.80)	D.L. Waller, USFWS, La Crosse, WI, pers. comm.
		96 hr	8.1, (*)	Betz Laboratories (1993)
	Bulab 6002	48 hr	0.044, (0.041 - 0.048)	D.L. Waller, pers. comm.
	H130	48 hr	0.75, (0.71 - 0.79)	D.L. Waller, pers. comm.
		96 hr	1.1, (*)	Calgon (1991)
	Chlorine	96 hr	0.059, (0.050 - 0.071)	Bongers et al. 1991
		A	0.062[B], (*)	USEPA (1985)
	Bromine	96 hr	0.068, (0.054 - 0.081)	Bongers et al. 1991
Fathead Minnow	DGH/QUAT	96 hr	4.4, (3.2 -5.4) 100 day old fish	Betz (1993)
	H130	96 hr	0.29, (*)	Calgon (1991)
	Chlorine	A	0.105[B], (*)	USEPA (1985)
Ictalurus punctatus (Channel Catfish)	DGH/QUAT	48 hr	0.83, (0.75 - 0.92)	D.L. Waller, pers. comm.
	Bulab 6002	48 hr	3.35, (2.82 - 3.96)	D.L. Waller, pers. comm.
	H130	48 hr	0.71, (0.68 - 0.73)	D.L. Waller, pers. comm.
		96 hr	2.6, (*)	Calgon (1991)
	Chlorine	A	0.090[a]	USEPA (1985)
C. dubia	Chlorine	100 % mortality after 1 hr exposure to 0.2 mg/L		Cherry (1993)

Table 4-10. (continued)

Species	Biocide	Duration	LC_{50} (mg/L), (95 % C.I.)	Reference
D. magna	DGH/QUAT	48 hr	0.20, (0.16 - 0.23)	Betz Laboratories (1993)
	H130	48 hr	0.20, (*)	Calgon (1991)
	Chlorine	48 hr	0.032, (0.001 - 0.036)	Bongers et al. 1991
		A	0.028[B], (*)	USEPA (1985)
	Bromine	48 hr	< 0.038, (*)	Bongers et al. 1991
H. azteca	Chlorine	96 hr	0.078, (0.062 - 0.096)	Bongers et al. 1991
	Bromine	96 hr	< 0.032, (*)	Bongers et al. 1991
M. bahia	Chlorine	96 hr	0.062, (0.052 - 0.074)	Bongers et al. 1991
	Bromine	96 hr	0.092, (0.074 - 0.113)	Bongers et al. 1991
Goniobasis sp.	Chlorine	A	0.069, (*)	USEPA (1985)
Obliquaria reflexa (Threehorn wartyback mussel)	DGH/QUAT	48 hr	7.70, (6.92 - 8.94)	D.L. Waller, pers. comm.
	Bulab 6002	48 hr	> 100	D.L. Waller, pers. comm.
	H130	48 hr	3.71, (2.73 - 5.06)	Calgon (1991)

A= Combined studies
B= Genus mean acute value
*= No confidence intervals available

1976; Lewis and Wee 1983). In tests with fathead minnows and *D. magna*, Beisinger and Stokes (1986) and Cary et al. (1987) found the toxicity of cationic polymers was reduced in the presence of various clay types. Cary et al. (1987) also observed that bentonite clay was one of the more effective substances for detoxification in their study.

Detoxification of DGH/QUAT was most apparent in static acute tests with *D. magna*, and static subchronic tests with fathead minnows and the midge, *C. riparius*. In the latter two bioassays, molluscicide and clay ratios of 1:3 sufficiently reduced or eliminated molluscicide toxicity. In addition to the clay, detoxification in one of the midge bioassays was enhanced by the presence of natural sediment which helped bind the cationic surfactants. In an earlier study, Pittinger et al. (1989) found that adsorption onto sediment reduced the bioavailability of surfactants and mitigated their toxicity to *C. riparius*. Reductions in toxicity in all static tests was probably aided by loss of molluscicide to glassware surfaces as previously discussed. Clay did not appear to have an impact on the organisms in these tests.

The results of the other laboratory detoxification tests were influenced by the impact of bentonite clay on the test organisms. This was particularly true in acute tests with *C. dubia*, as high mortality of the organisms was observed in not only the treatments with DGH/QUAT and clay, but in an acute bioassay with the clay. The impact was probably due to clay adhering to the daphnids and causing stress by weighing them down or clogging their feeding apparatus. The bioassay with clay alone indicated a bimodal effect which was supported by repeating exposure to selected clay concentrations. At 20 mg/L, it appeared that a maximum level of clay was in suspension, with the 30-mg/L test

solution actually clearer than the 20 mg/L. This may have been due to settling of clay at higher concentrations which caused precipitation of additional clay that would normally have remained in solution. As clay levels increased (50 and 100 mg/L), a noticeable layer collected on the bottom of the test chamber and the organisms came in contact with this clay layer when moving near the bottom.

The results of the *D. magna* flow-through acute test could also have been influenced by bentonite clay. Nearly 100 % mortality was observed in the 1:3 DGH/QUAT to clay ratio at all hardness levels. While DGH/QUAT was detected in the test chambers of this treatment on two occasions (probably due to partial clogging of a clay feed tube), levels did not exceed 0.2 mg/L. This concentration (corresponding with the calculated 48 hr LC_{50} for *D. magna* under constant flow-through conditions) should not have resulted in complete mortality of the organisms if the effect was due to molluscicide alone. The daphnids may have succumbed to lower than normal levels of DGH/QUAT due to additional stress imposed by the clay. *D. magna* survived at ratios of 1:5 and 1:10, although most of the 20 organisms used at each level were completely buried under a layer of settled clay by the end of the 48-hr bioassay. Additionally, at a hardness level of 100 mg/L, the 60% mortality noted for daphnids in the 1:5 ratio was the result of their having been trapped at the water surface in clumps of floating clay. In their detoxification studies, Cary et al. (1986) also observed *D. magna* becoming entrapped within a floc created by cationic polymers bound to materials such as clay.

Detoxification potential of DGH/QUAT in the *D. magna* flow-through acute bioassay appeared related to water hardness. That is, the amount of clay needed to

eliminate mortality decreased from 100 mg/L to 50 mg/L as hardness went from 100 to 200 mg/L CaCO3. Water hardness can affect toxicity of surfactants (Hokansen and Smith 1971; Abel 1974; Maki and Bishop 1979), although in the present study it may have been more important in determining the condition of the *D. magna* rather than mediating toxicity of molluscicide. In the culture broods, healthier organisms were indicated as reproductive output increased with hardness. In fact, a 50 mg/L CaCO3 hardness level was to have been tested but was not because of poor survival of the test organisms. Healthier daphnids may have been more resistant to stress from the clay, and so survival was greater as hardness increased.

Over a 21-day exposure period, a molluscicide and clay ratio of 1:10 was necessary to reduce mortality of *D. magna* to 10 %, versus 60 % at 1:5. Again, the effects here could have been the result of greater settling and less stress from the clay at the 1:10 ratio, or to toxic effects of very low levels of DGH/QUAT still present at 1:5. Effects of stress on the organisms were manifested in poor reproductive output at all treatment levels. Strands of clay and algae used to feed the daphnids (possibly held together by the molluscicide) were observed adhering to the carapace of the organisms, and mortality at the higher clay levels usually coincided with molting, perhaps due to exhaustion brought on as the organisms attempted to shed the carapace. Clay probably also added to mortality observed at all treatment levels in the 7-day flow-through fathead minnow test. Clay accumulated in the tests chambers, and was taken up by the fish. Growth of surviving fathead minnows was suboptimal and microscopic examination indicated the presence of clay in the gut which probably reduced absorption of food.

Leaf Pack Study

Breakdown of allochthonous matter (leaves, woody debris, etc.) includes initial colonization by microbes which, through the release of external digestive enzymes, soften or "condition" leaf material (Suberkropp and Klug 1976). Conditioning of leaves in stream systems is also facilitated by the activity of shredding macroinvertebrates in addition to physical abrasion due to contact with rock and other debris (Bird and Kaushik 1992; Hill et al 1992). Conducting this study in artificial streams allowed exclusion of macroinvertebrates as determined by the lack of organisms in the system. Abrasion was also limited because of the way the leaf packs were held in the streams. Thus it was assumed that softening of the leaf material was largely a function of the microbial fauna that became associated with the leaf packs.

Since classes of quaternary ammonium compounds (including the surfactants in the DGH/QUAT) have commercial uses stemming from their antimicrobial activity (Schaeufele 1984; Parishkova and Negrutskiy 1989), it is possible that the observed reductions in leaf decomposition indicates a toxic effect. The lag time observed in conditioning of treated leaf packs could have been the result of recolonization with organisms from the incoming river water after the initial fauna was removed during the dosing. Unfortunately, those studies which have demonstrated antimicrobial activity of surfactants used exposure levels well above the concentrations of DGH/QUAT measured in the artificial streams, so extrapolation of potential effect levels cannot be made.

Another possibility is that, during the first 15 to 30 days after dosing, the microorganisms used the bound surfactant as a carbon source to a greater extent than the

leaves, resulting in the lag in leaf processing. Microbial degradation of cationic surfactants is well documented (Baleux and Caumette 1977; Larson and Vashon 1983; Huber 1984), although variability in degree of degradation has been observed (Baleux and Caumette 1977; Boethling 1984). Alkyl dimethylbenzyl ammonium compounds (QUAT) are intermediate in biodegradability (Boethling 1984). Microbial degradation of the molluscicide would also depend on the ability of existing organisms to metabolize the compound, or for community adaption to occur. The rate of biodegradation of long chain QACs did not reach a maximum until approximately day 15 in exposures conducted with an unadapted microbial community (Ventullo and Larson 1986).

In retrospect, some analysis of bacterial density or respiration rates would have enhanced this study, since based on leaf softening data, it is possible only to say that the functional aspect of leaf degradation was impaired. Additional studies are needed to clarify actual community effects, as is work to fully characterize breakdown of the active ingredients in DGH/QUAT. However, given the importance of allochthonous energy input in stream systems (Cummins 1974; Anderson and Sedell 1979), impairment of this functional aspect could have significant implications. While larger river systems are not as dependent on this external energy source, the microbial fauna still has an important functional role in carbon cycling, similarly making the observed impact an important aspect to consider.

Results of the detoxification studies must be interpreted with respect to "real-world" conditions. Generally, test organisms received at least a 48-hr exposure to molluscicide and clay which exceeds the 24-hr dosing usually employed in applications of

DGH/QUAT (Lyons et al. 1990). Exposures with DGH/QUAT and clay were designed to mimic situations in which an effluent containing the components would make up 100 % of the receiving system flow, with no account for dispersion or dilution made. The detoxification of DGH/QUAT would also be influenced by naturally occurring suspended solids in a receiving system. Lewis and Wee (1983) observed the need for considering the characteristics of surface waters which, particularly in the case of surfactants, can affect the toxicity of a chemical to a greater extent than the high quality laboratory waters commonly used in bioassays. These factors clearly support the views of Chapman (1983), Woltering and Bishop (1989), and Cairns (1990) who all commented on the utility of field validation to support results of laboratory bioassays.

4.4.4 Results of an On-Site Application of DGH/QUAT

Cherry et al. (1990) conducted an extensive study in conjunction with a DGH/QUAT application to control fouling by the Asian clam at a nuclear generating facility on the Ohio River near Pittsburgh, PA. The 24-hr summer dosing was monitored by flow-through field laboratory studies, in-river benthic monitoring, and static laboratory toxicity testing of plant effluent which contained molluscicide and bentonite clay at a ratio of 1:3. Testing strategy utilized standard laboratory test organisms in addition to resident species to establish both lethal and sublethal endpoints.

Exposure to 100 % effluent containing molluscicide and clay caused no mortality of *D. magna*, the snail, *Physa* sp., or the mayfly, *Isonychia bicolor*. Successful emergence of the mayfly was noted within 40 days of exposure. Similarly, the effluent had no

impact on 7-day growth and survival of the fathead minnow in laboratory bioassays. Sediments collected in specialized traps located above, within, and below the plant outfall during and up to three weeks following dosing had no impact on growth or survival of the midge, *C. riparius*. There were also no significant changes in composition of the benthic macroinvertebrate community at stations located downstream from the plant outfall.

Growth of Asian clams was not impaired by exposure to the effluent, although a sublethal indicator of stress, cellulolytic enzyme activity, did indicate an effect when measured 30 days after dosing in individuals which had been exposed to 50 % effluent. No significant differences in enzyme activity were apparent in clams sampled on day 40 post-dose. Survival and reproduction of *C. dubia* were impaired in 50 % effluent, and acute studies with various dilutions of dosed effluent or Ohio River water dosed with bentonite clay indicated sensitivity to clay alone. In bioassays which followed the molluscicide application, variable toxicity of the Ohio River water itself to *C. dubia* was apparent. The instream waste concentration (IWC) for this particular facility is 5 % effluent. Based on the definition of the IWC as described by the USEPA (1991), during the lowest seven day average conditions for a ten year period (7Q10), the effluent of the power plant would make up 5% of the Ohio River flow. Since the lowest effluent dilution toxic to the organisms tested was 50%, Cherry et al. (1990) concluded that integrity of the river system was not compromised by the molluscicide application.

4.4.5 Regulatory Considerations

Both state and federal regulatory agencies have indicated a desire to establish numerical water quality based effluent limits for specific water treatment chemicals such as DGH/QUAT (Howe and Kaplan 1990; Howe et al. 1991; Magni 1991). Surfactants in general pose a difficult class of chemicals for which to establish numeric criteria because of the difficulty in determining what available concentrations could exist in natural systems (Abel 1974; Cooper 1988). Regulatory aspects of treatment chemicals such as DGH/QUAT are further confounded by relatively short, sporadic dosing periods (commonly 6-24 hrs for 3-4 dosings/year), and by combining these chemicals with substances such as bentonite clay prior to release.

One approach toward these regulatory concerns may be a more precise description of appropriate application (considering flow characteristics or dilution qualities of the receiving system, etc.) within the Federal Insecticide, Fungicide, and Rodenticide (FIFRA) registration of these chemicals. This approach will be facilitated by a better understanding of effluent effects levels. In the present study full strength mixtures of molluscicide and clay (representing 100 % effluent) had an impact upon a number of the test organisms, supporting the view of Howe et al. (1991), who stated that these types of biocides may not be compatible with systems where effluent makes up a major component of stream flow. Additional dilution studies must be conducted to determine what percentage of effluent should be considered a "major component".

4.5 Summary

Laboratory and field bioassays were conducted to examine the toxicity and detoxification potential of the surfactant-based molluscicide DGH/QUAT. Like the majority of other available biocides, DGH/QUAT exhibited acute toxicity with LC_{50} values ranging from 0.12 - 11.0 mg/L. Comparison of these data with available literature values for oxidizing biocides (chlorine and bromine) indicates greater toxicity of oxidizing biocides. Chronic lowest observed effects levels for DGH/QUAT ranged between 0.03 and 2.81 mg/L, with differences between static and flow-through test strategies apparent. As observed with other cationic polymers and surfactants, free levels of the molluscicide dropped in the presence of bentonite clay, naturally suspended solids, or sediment. The presence of natural sediments mitigated molluscicide toxicity to the midge *C. riparius*, while results of detoxification studies using bentonite clay were more variable. This was primarily because of clay effects on the test organisms. Microbial conditioning of leaf material was also impacted by dosing with full strength mixtures of DGH/QUAT and clay, although it is not clear if this effect was due to toxicity or use of the surfactants as an alternate carbon source.

The propensity for adsorption onto clay or naturally occurring solids in receiving systems indicate that free DGH/QUAT will be minimal, and that regulation of the chemical through numerical criteria may not be the most appropriate strategy. Laboratory studies indicate that, due to apparent stress associated with bentonite clay itself, application of DGH/QUAT may not be compatible with systems having low levels of effluent dilution. An effluent dilution of 50% or higher eliminated nontarget effects of

DGH/QUAT during an on-site application. Data from additional field validations will further clarify environmentally safe dilution levels.

5.0 SHORT-TERM BIOMONITORING OF A SURFACTANT-BASED MOLLUSCICIDE WITH THE ASIAN CLAM

5.1 Introduction

Biological data are essential components of pollution assessment because chemical analyses alone cannot predict toxicity or environmental impact (Cairns and Niederlehner 1990). To that end, biological monitoring is a technique by which living organisms are used to detect pollutants in the environment (Rand and Petrocelli 1985; Cairns and Pratt 1993). Bivalve molluscs have been identified as useful biomonitoring organisms because of characteristics which include a sedentary life style, ease in collection, and ability to accumulate pollutants (see reviews by Phillips 1977; Johnston and Hartley 1983; Doherty 1990). In freshwater systems, the Asian clam, *Corbicula fluminea*, has been utilized as a biomonitoring organism in part because of its now widespread range and abundance (Foster and Bates 1978; Doherty 1990). This species, a native to Southeast Asia, was introduced into North America some 50 years ago, and is now found throughout most of the lower 48 United States (McMahon 1983; Counts 1986). It has been suggested that Asian clams be used as pollutant monitors in place of native freshwater mussels which are becoming endangered or cannot be found in suitable numbers for testing (Foster and Bates 1978; Doherty 1990).

Monitoring studies with Asian clams have examined bioaccumulation of metals and organics from both water and sediment (Rodgers et al 1979; Caldwell and Buhler 1983; Graney et al 1983; Modin 1969; Livingston et al 1978; Elder and Mattraw 1984), and response parameters such as body condition, mortality, impaired growth or reproductive

output following field exposures to industrial effluents and laboratory exposures to metals or organics (Fritz and Lutz 1986; Belanger et al 1986; Farris et al. 1988; Graney and Giesy 1988; Shema et al. 1993). Biochemical or physiological "biomarkers" have also been used to detect sublethal pollutant stress in the clams (Anderson 1978; Giesy et al 1983; Cantelmo-Cristini et al 1985; Farris 1986; Sappington 1987; Graney and Giesy 1988; Farris et al 1988). Cairns and Niederlehner (1990) state that advantages of biomarkers include quick response to pollutants and a potential to provide early warning of contamination.

In Chapter 3 it was established that whole body glycogen and tissue water of Asian clams were affected by exposure to the surfactant-based molluscicide DGH/QUAT at levels between 3.75 and 15 mg/L. The objective of the present study was to determine if these variables respond similarly to the low levels of surfactant found to have acute and chronic effects on non-target invertebrates tested in the studies described in Chapter 4. This information will then be used to evaluate the potential use of these biomarkers in monitoring receiving systems during applications of the molluscicide. In addition, since the adsorptive potential of surfactants in general (Abel 1974; Cooper 1988) could confound laboratory testing of effluents containing these chemicals (through a reduction in bioavailability during transport, etc.), validation of these markers in the Asian clam could lead to their application in field monitoring programs aimed at detecting other surfactant-based contaminants.

5.2 Methods

5.2.1 DGH/QUAT and Analytical Methods

DGH/QUAT is a nonoxidizing, surfactant-based molluscicide which is 13% active by weight. The active components of the compound are dodecylguanidine hydrochloride (DGH), and n-alkyl dimethylbenzyl ammonium chloride (QUAT). Free levels of molluscicide were determined colormetrically by complexing samples of test solution with a methyl-orange buffer, extracting with 1,2 dichloroethane, and determining the absorbance of the extract on a spectrophotometer at 415 nm. Concentrations of toxicant were then calculated from a standard curve. The level of detection for this analytical method is 0.2 mg/L. Unless otherwise stated, all reported molluscicide levels are based on measured values.

5.2.2 Test Organisms

Asian clams were collected from the New River near Narrows, Virginia, and transported to the Ecosystem Simulation Laboratory (ESL) on the Virginia Tech campus. In the laboratory, they were maintained in a flow-through artificial stream system (Farris 1986) receiving laboratory water (dechlorinated Blacksburg tap water) at 20 °C. During holding and testing the clams were fed a concentrated (800 cells/ml) tri-algal mixture of *Chlamydomonas rheinhardti*, *Ankistrodesmus falcatus*, and *Chlorella vulgaris*. The organisms were acclimated to laboratory conditions for 1 wk prior to testing. Adult clams with shell lengths between 12 to 16 mm were used in the study.

5.2.3 Bioassay Procedures

Clams were exposed to DGH/QUAT in replicate troughs constructed from polyvinyl chloride (PVC) rain gutter and endcaps. Troughs measured 46 cm in length, 5.9 cm in depth, and had an average width of 9.7 cm (due to tapered sides). Inflow and overflow ports were located approximately 3.5 cm from the base on opposite ends of the troughs. The bioassay was conducted at 20 °C under flow-through conditions. Laboratory water was used as diluent and fed to each trough via peristaltic pumps at a rate of 50 ml/min. Stock solutions of DGH/QUAT were mixed in laboratory water and similarly administered with peristaltic pumps via an injection port located on the diluent inflow. An airstone was situated at the inflow to both aerate and mix the test solution. Stock concentrations and flow rates were adjusted to overcome system demand and obtain measured target levels within the troughs. Asian clams were fed a concentrated di-algal (*Chlamydomonas* and *Chlorella*) mixture administered to each trough at a rate of 2 ml/min, again to achieve a target of a least 800 cells/ml through the test troughs. Algae added to system demand but was overcome as described above. Treatments included an control of diluent only, and molluscicide levels of 0.2, 0.4, 0.8, 1.6, and 3.2 mg/L. The exposures lasted seven days to match the duration of standardized tests with fatheads minnows and the cladoceran, *Ceriodaphnia dubia.*

5.2.4 Water Chemistry

Dissolved oxygen and temperature were measured in each trough on a daily basis, while pH and conductivity were measured on days 1, 4, and 7. Similarly, alkalinity and

hardness were measured in the control and highest molluscicide level tested. Molluscicide levels were measured in each trough at least twice daily.

5.2.5 Biochemical Variables

As the primary energy store of bivalves (Cantelmo-Cristini et al. 1985), glycogen was chosen as a potential indicator of general stress. Whole body tissue water levels were monitored to detect osmotic effects associated with surfactant exposure (Abel 1974).

Whole Body Glycogen

For glycogen determination, clam tissues were dissected, frozen with liquid nitrogen, and stored in liquid nitrogen until analysis which was usually performed within two weeks. The glycogen assay followed a procedure described by Roehrig and Allred (1974). Tissues were first homogenized in 0.05 M phosphate buffer (pH 4.8; 500 ml/g dry tissue) with a Tekmar Ultra-Turrax homogenizer at 15,000 rpm for 15 seconds. Following a 20 min centrifugation at 5,000 x g, the supernatant was drawn off and the remaining tissue pellet was dried at 60 °C for 24 hrs. Duplicate 100 μl aliquots of homogenate were then incubated with 3.5 units of Amyloglucosidase (Sigma Chemical Co.) and phosphate buffer (final volume 0.55 ml) for 2 hrs at 37°C. Glycogen standards (from *Mytilus edulis*) containing 5 to 80 μg glycogen were assayed as described for the samples. Following the enzymatic conversion of glycogen to glucose, 0.5 ml aliquots of sample or standard were incubated with a freshly prepared oxidase-peroxidase dye reagent specific for glucose determination for 30 min at 37°C. Absorbance was then

measured at 450 nm, and total concentrations determined from a glycogen standard curve. Free glucose present in the tissues was determined by incubating supernatant aliquots without amyloglucosidase, and then assaying for glucose as previously described. True glycogen content was determined by subtracting free glucose from the total concentrations, and was reported as percent dry weight.

Tissue Water

Whole body tissue water levels were determined by excising the visceral mass, determining the wet weight to the nearest 0.0001g on a Mettler electronic balance, drying the tissue at 60°C for 24 hrs, and then reweighing. Tissue water was calculated as wet weight - dry weight/wet weight, and then expressed as a percentage.

Asian clams were sampled for glycogen (n=6) and tissue water levels (n=10) on days 1, 4, and 7. A separate group of 20 clams were monitored for mortality on a daily basis. The organisms were considered dead if they exhibited no resistance to a blunt probe inserted between the valves (McMahon et al. 1993).

5.2.6 Statistical Analyses

Whole body glycogen and tissue water levels were analyzed by analysis of variance (ANOVA) following arcsine-squareroot transformation of the data. Differences among the treatment groups were determined by Fisher's Protected LSD using at $\alpha=0.05$. The Spearman-Karber method was used to calculate the LC_{50} (that concentration lethal to 50% of the clams) and associated confidence intervals (Hamilton et al. 1978).

5.3 Results

5.3.1 Mortality

Mortality of the Asian clams was limited to 1.43 and 3.12 mg/L, the highest concentrations of DGH/QUAT tested (Table 5-1). The threshold at which mortality occurred was abrupt. There was no mortality at 0.78 mg/L, but all died at the next highest concentration of 1.43 mg/L. Most mortality occurred between days two and four, and the 96 hr LC_{50} was 1.21 mg DGH/QUAT/L (1.07 - 1.37, 95 % CI).

5.3.2 Biochemical Variables

Tissue water content of control clams remained steady during the bioassay, deviating by only 0.2 % from 85.1 to 84.9 (Table 5-2). The only significant impact on tissue water occurred on day 4 of the study when levels of the variable increased to 88.1 % in individuals from the 1.43 mg/L treatment. This increase coincided with high mortality in that group (Table 5-1), although care was taken to avoid sampling dead or moribund individuals. No effect on tissue water was observed at the 3.12 mg DGH/QUAT/L treatment, probably because all the clams died before the scheduled sampling on day 4.

There were no significant effects on whole body glycogen levels from clams in any of the treatments (Table 5-2). Glycogen levels ranged between 12.7 and 18.2 % dry weight for the group of clams as a whole.

Table 5-1. Mortality of Asian clams (n= 20/treatment) during a 7-day exposure to the surfactant-based molluscicide DGH/QUAT.

DGH/QUAT (mg/L)		% Mortality / Day						
Nominal	Avg. Measured	1	2	3	4	5	6	7
0 - 0.80 (see Table 2 for measured levels)		No mortality						
1.60	1.43	0	0	20	80	95	100	---
3.20	3.12	0	10	85	100	---	---	---

Table 5-2. Average tissue water content (TW) and whole body glycogen levels (Gly) of Asian clams during a 7-day exposure to the surfactant-based molluscicide DGH/QUAT. Numbers in parentheses represent ± 1 SEM and the dagger (†) indicates significant difference from control at $\alpha = 0.05$.

DGH/QUAT (mg/L)		Day 1	Day 4	Day 7
Nominal	Avg. Measured	TW \ Gly	TW \ Gly	TW \ Gly
0	0	85.1 (0.2) \ 12.7 (0.5)	85.0 (0.3) \ 14.9 (1.2)	84.9 (0.2) \ 13.4 (1.5)
0.20	0.22	85.3 (0.3) \ 14.3 (1.2)	84.7 (0.3) \ 14.3 (1.2)	84.6 (0.2) \ 11.7 (2.0)
0.40	0.42	85.0 (0.3) \ 13.9 (1.6)	85.3 (0.2) \ 17.8 (2.0)	84.3 (0.3) \ 18.1 (2.2)
0.80	0.78	85.3 (0.3) \ 15.5 (1.0)	85.1 (0.3) \ 14.5 (2.5)	85.6 (0.3) \ 18.0 (1.3)
1.60	1.43	85.3 (0.2) \ 17.1 (1.6)	88.1 (0.5)† \ *	CM
3.20	3.12	85.7 (0.3) \ 18.2 (1.4)	CM	CM

* = Insufficient numbers for sampling due to mortality
CM = Complete mortality of test organisms

5.4 Discussion

Free levels of cationic surfactants are commonly short-lived in receiving systems because of a propensity to bind with naturally occurring suspended matter in the water (Lewis and Wee 1983; Cooper 1988). In turn, this binding can reduce bioavailability and toxicity of the chemicals (Lewis and Wee 1983; Huber 1984). In Chapter 4, it was established that free levels of DGH/QUAT are inversely related to total suspended solids in natural water. In addition, application of the molluscicide for macrofouling control commonly includes a 24-hr dosing period with addition of bentonite clay to bind the surfactant actives before dosed effluent is released to a receiving system (Lyons et al. 1990; Cherry et al. 1990). During dosing, "detoxified" effluent is monitored for free levels of the molluscicide to determine if sufficient levels of clay are being added (Cherry et al. 1990). If free molluscicide was inadvertently released to a receiving system, the event would probably be short-lived and the concentrations low due to analytical monitoring of the effluent and the adsorptive potential of suspended matter in the receiving system. Such an event would still be of concern, since the molluscicide is acutely toxic to invertebrate species at low levels. If the Asian clam were to be used as a monitor of potential impact during dosing, its response would have to be sufficiently rapid to detect acute exposure to low levels of molluscicide.

The 96 hr LC_{50} of 1.21 mg DGH/QUAT/L generated for Asian clams ranks 6 out of 9 invertebrate species (clams included) previously tested in acute exposures to the molluscicide (Table 5-3), and is comparable to an unpublished 72 hr LC_{50} of 2.7 mg/L for the clams (J. Petrille, Betz Labs., pers. comm.). Asian clams were an order of

Table 5-3. Summary of acute data previously generated for the surfactant-based molluscicide DGH/QUAT. Dashed line transecting the table indicates where the 96-hr LC_{50} generated for the Asian clam falls.

Species	Duration	LC_{50} (mg/L)	95 % Confidence Interval	Reference
Amphipod, (*Hyallela azteca*)	96 hr	0.12	0.13 - 0.26	Chapter 4
Cladoceran, (*Ceriodaphnia dubia*)	48 hr	0.14	0.12 - 0.16	Chapter 4
Cladoceran, (*Daphnia magna*)	48 hr	0.20	0.48 - 0.54	Betz (1993)
Marine Shrimp, (*Mysidopsis bahia*)	96 hr	0.34	0.28 - 0.41	Chapter 4
Zebra mussel, (*Dreissena polymorpha*)	48 and 72 hr	0.88	*	J. Petrille, Betz Labs., D.L. Waller, USFWS, Pers. Comm.
Midge, (*Chironomus riparius*)	48 hr	6.49	5.33 - 8.11	Chapter 4
Unionid Mussel, (*Obliquaria reflexa*)	48 hr	7.70	6.62 - 8.94	D.L. Waller, USFWS, Pers. Comm.
Snail, (*Goniobasis*) sp.	96 hrs	11.0	10.3 - 11.9	Chapter 4

* Combined confidence intervals not available

magnitude more resistent to the molluscicide than the amphipod, *H. azteca*, and the cladoceran, *C. dubia*. The lowest observed effect concentration (LOEC), or the lowest concentration at which significant mortality occurred was 1.43 mg/l. In 7-day chronic tests with fathead minnows or *C. dubia* exposed to DGH/QUAT, LOECs were 0.10 and 0.057 mg/L, respectively.

Cairns (1990) stated that information generated from biological monitoring can be used as "an early warning system of impending change" or to examine long-term trends due to either natural or more chronic anthropogenic effects. Obviously, when implementing an early warning system the response examined should be sufficiently sensitive to insure avoidance of deleterious effects. Based on comparison of acute toxicity data, mortality of Asian clams as a response parameter lacks sufficient sensitivity, and would not provide an effective early warning of impact associated with applications of DGH/QUAT.

As compared to mortality, biochemical indices may provide a more rapid indication of hazard since the toxic effects of chemicals are often biochemical in origin, and so changes in biochemical variables may precede whole animal responses (Giesy et al 1983; Larsson et al 1985; Heath 1987). It was therefore predicted that changes in tissue water or glycogen levels of the clams would provide more sensitive indicators of molluscicide exposure than mortality.

In Chapter 3 increases in the tissue water content of Asian clams were observed within hours of exposure to DGH/QUAT, and these changes preceded death of the organisms. Impacts on gill permeability often occur during the initial stages of surfactant

exposure (Jackson and Fromm 1977), so the effect was attributed to water uptake associated with the impact of the molluscicide on the clam's gill. In the present study, significant increases in tissue water levels were not observed until day 4 sampling of individuals in the 1.43 mg DGH/QUAT/L exposure. This effect coincided with high mortality of the clams. In all likelihood, changes in this variable occurred prior to the onset of mortality, but were not detected due to the sampling strategy used (sampling on days 1, 4, and 7). Similarly, no tissue water effects were observed at the 3.12 mg/L treatment because high mortality occurred before the clams were sampled. In contrast to the present data, tissue water levels of Asian clams exposed to DGH/QUAT in the studies discussed in Chapter 3 began to increase within 6 hr of dosing at 15 mg/L. Heath (1987) discussed the importance of factors such as temperature, exposure duration, and toxicant concentration on observed physiological effects, and these factors clearly explain the difference between the studies.

In retrospect, it would have been advantageous to monitor tissue water of the Asian clams on a daily basis rather than on days 1, 4, and 7. Although, based on comparison of effects levels generated for other invertebrates, the utility of Asian clam tissue water levels as an indicator of DGH/QUAT in an effluent is questionable.

Depletion of glycogen has been used to indicate energy demands associated with chemical-induced stress in both vertebrate and invertebrate species (Cantelmo-Cristini et al. 1985; Mayer et al. 1992), and is considered part of a general adaptive response as an organism attempts to maintain homeostasis during the stress (Selye 1976; Mazeud et al. 1977). The speed at which a biochemical response occurs is dependent on the magnitude

of the stimulus causing the stress (in this case concentration of molluscicide) (Heath 1987).

In Chapter 3, exposure to 15 mg DGH/QUAT/L reduced whole body glycogen levels of Asian clams within 24 hr at 15 or 25 °C. In contrast, no impact on glycogen levels were observed at any treatment in the present study. This apparent lack of response may have been due to the sampling strategy used. That is, too many clams died before the scheduled sampling times. Still, as with tissue water, glycogen levels of the Asian clam appear to be affected at levels of molluscicide higher than those acutely toxic to other invertebrates, and so would not provide an effective "early warning" parameter to monitor molluscicide applications.

Biochemical or physiological indices, including ciliary action of excised gill tissue, free amino acid levels, oxygen consumption, whole body lipid, glycogen, and water concentrations, adenylate energy charge, and activity of cellulolytic enzymes have been used to successfully detect stress in Asian clams exposed to such toxicants as ammonia, chlorine, complex effluents, and heavy metals (Anderson 1978; Giesy et al 1983; Cantelmo-Cristini et al 1985; Farris 1986; Sappington 1987; Graney and Giesy 1988; Farris et al 1988). Farris et al. (1991) found that effects levels for cellulolytic enzyme activity in clams exposed to copper were among the lowest of invertebrates tested, including the cladoceran, *C. dubia*. In addition, the response of the clams was comparable to that of selected native mussel species.

The common denominator in the majority of these studies is a focus on chronic effects with exposure durations lasting an average of 30 days. Because of the ability to

reduce exposure to toxicants through valve closure, the use of bivalves in acute testing has been questioned (Farris et al. 1988). Interestingly enough, while valve closure may affect the use of certain response parameters in acute monitoring, studies with the zebra mussel have successfully used the closure response itself in water quality monitoring (Sloof et al. 1983; Jenner et al. 1989). Asian clams probably do not actively avoid non-oxidizing biocides as they would an oxidizing agent such as chlorine (McMahon and Lutey 1988), but natural rhythms in valve closure would serve to reduce toxicant exposure under acute conditions.

In Chapter 3, it was suggested that differences in sensitivity to DGH/QUAT observed between the zebra mussel and the Asian clam may have been due to a difference in physiological tolerance of the two bivalves. Specifically, during short-term exposures to the molluscicide, Asian clams appeared able to regulate tissue water levels while no regulation was observed in zebra mussels. Previous studies have demonstrated that the Asian clam has an enhanced capacity for osmoregulation as compared to other freshwater bivalves (Gainey 1978a; 1978b), possibly due to retention of physiological capabilities stemming from its brackish ancestry (McMahon 1983). Unfortunately, most work in this area has focused on the clam's ability to tolerate elevated salinity so the extent to which this conveys an ability to tolerate water influx is unclear. Such an ability would obviously make the clam a poor biomonitoring organism of toxicants which are primarily osmoregulatory stressors.

5.5 Summary

Tissue water and whole body glycogen levels were not effective response parameters for early warning of the presence of free levels of DGH/QUAT. However, biochemical variables such as these have been used in chronic studies with the clam, with sensitivity levels comparable to that of test species such as cladocerans or unionid mussels. As a biomonitoring organism, the Asian clam may best be suited for those studies seeking to characterize the condition of receiving systems. The potential for physiological differences to alter the response of the Asian clam should be examined, as should long-term exposures to surfactants in order to further assess the utility of the organism in biological monitoring of these chemicals.

6.0 SUMMARY

The primary results of each study are as follows:

Chapter 2

1) Intermittent (2 - 4 hr/day) treatments with chlorine and bromine at levels of 0.5 and 1.0 mg/L TRO caused significant reductions in settling of zebra mussel postveligers, with an effect that was both dose and time dependent. Density reductions as high as 91 % from control values were observed.

2) None of the treatments were completely effective at inhibiting larval settling, and the final density of mussels that accumulated in the treatment tanks was dependent on larval densities in the water (higher densities in the water led to higher numbers settled).

3) Growth comparisons indicated that mussels which remained settled within the exposure system were able to grow comparably to control individuals.

4) The intermittent treatments had no effect on survival of adult zebra mussels or Asian clams, and all showed positive growth over the course of the studies.

5) Adult mussels and clams which received a 4-hr treatment with 0.5 mg/L chlorine, did not grow as much as control animals, probably due to avoidance of the oxidants and in turn a reduction in feeding.

6) No impact on glycogen levels of selected groups of adult bivalves was observed, indicating that under the exposure conditions used, the halogen treatments had no effect on energy reserves.

Chapter 3

1) Cumulative mortality of zebra mussels and Asian clams was comparable after a 24-hr exposure to a surfactant-based molluscicide, DGH/QUAT, at levels up to 20 mg/L, although the rate at which this mortality occurred was significantly greater for the zebra mussel.

2) Differences in sensitivity to the molluscicide were evident in short-term exposures. At temperatures of 20 - 25 °C, 6-hr exposures to 10 or 15 mg/L resulted in 100 % mortality of the zebra mussel but only induced 43 % mortality in Asian clams.

3) Biochemical responses to DGH/QUAT were similar in both organisms, with whole body tissue water increasing while whole body glycogen decreased. These effects were attributed to gill tissue damage and general stress associated with exposure to the molluscicide.

4) In general, the zebra mussel exhibited greater sensitivity to DGH/QUAT than Asian clams, possibly due to differences in the time the two bivalves spent siphoning. Differences in tolerance to the biochemical effects induced by the molluscicide may also be a factor.

Chapter 4

1) Like the majority of other available "molluscicides", DGH/QUAT was toxic to nontarget organisms with acute LC_{50} values ranging from 0.12 - 11.0 mg/L, and chronic lowest observed effects levels ranging between 0.03 and 2.81 mg/L.

2) Free levels of the surfactant-based molluscicide dropped in the presence of bentonite clay, naturally suspended solids, or sediment. The adsorptive potential of the actives lead to differences in effects levels generated with static or flow-through test systems.

3) In laboratory bioassays, mixing DGH/QUAT with bentonite clay could detoxify the molluscicide, although the clay itself had a negative impact on some test organisms.

4) Microbial conditioning of leaf material as determined by penetrance measurements was affected by dosing with full strength mixtures of DGH/QUAT and clay, although it is not clear if this was due to toxicity or use of the surfactants as an alternate carbon source to the leaf material.

5) In a field application of the molluscicide, impairment of test organisms was apparent at 50 % effluent, indicating that application of this biocide may not be compatible with systems having low levels of effluent dilution.

Chapter 5

1) Tissue water and whole body glycogen levels of Asian clams were not effective response parameters for early warning of the presence of free levels of DGH/QUAT. The lowest effect level for both mortality and the biochemical variables was 1.43 mg/L in a 7-day exposure of the clams, as compared to acute LC_{50} values of 0.12 to 0.14 mg/L for species such as the amphipod, *Hyallela azteca*, and the cladocerans, *Ceriodaphnia dubia*, and *Daphnia magna*.

The major conclusions of these various studies were as follows:

1) Intermittent (2 - 4 hr) exposure regimes to levels of bromine and chlorine between 0.5 and 1.0 mg/L can reduce, but will not eliminate, zebra mussel settling. Mussels which do settle are largely unaffected by the treatments, so additional application of a more effective biocide would be necessary to avoid eventual fouling problems.

2) Differences in sensitivities to chemicals exist between the zebra mussel and Asian clam. These differences should be considered when evaluating or optimizing biofouling control strategies for either organism.

3) The propensity for adsorption onto naturally occurring solids in the receiving system indicates that free levels of DGH/QUAT will be minimized. As a result, field effluent monitoring rather than the derivation of numerical criteria may be the most appropriate strategy for regulation of the molluscicide, or other surfactant-based chemicals.

4) For biomonitoring purposes, examination of biochemical variables in the Asian clam may best be suited for those studies seeking to characterize the condition of receiving systems, rather than for early warning indicator impact. Physiological differences, and the potential for differential response to contaminants, between this introduced species and native freshwater organisms must be characterized in order to further evaluate the use of the clams in biomonitoring. These same questions must be addressed as the zebra mussel is used in a similar role.

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APPENDIX I.

CONTAINMENT PROTOCOL FOR ZEBRA MUSSELS

Protocol for Conducting Research with the Zebra Mussel, *Dreissena polymorpha*, in Laboratory Facilities

Joseph R. Bidwell
Department of Biology
Virginia Tech
Blacksburg, VA 24061

I. Information to be Included in Initial Research Proposal

A. General Description of Laboratory and Experimental Facilities

1. Location

a. distance of laboratory from nearest body of water

b. distance from nearest zebra mussel population (consult distribution maps from USF&WS Gainesville, or NY Sea Grant)

2. Use

a. summary of all activities (including those not related to zebra mussel work) for which the laboratory is used

b. total number of personnel using the facility

3. Diagram of Laboratory include:

a. location of primary testing/work areas

b. location of sink and floor drains

c. location of access points: doors, windows

II. Laboratory Protocol

A. Security

1. Access to the facility should be limited to those personnel authorized by the investigator

2. Doors shall be kept locked when the facility is empty

3. The laboratory will be clearly marked as a restricted or biohazard area

4. Post the names and telephone numbers of at least three persons to contact in the event of an emergency

B. Holding

1. Holding tanks and experimental chambers must be:

    a. static or closed recirculation

    b. surrounded by a secondary catch basin to contain drips or leaks

2. to prevent unregulated spawning of mussels, the primary holding chambers should be fitted with a chiller to maintain water temperature below 10 °C

3. All floor drains in the experimental area will be sealed to prevent accidental release through the laboratory drainage system. Sinks should have stoppered standpipes

C. Equipment

1. Frequently used in project (nets, siphon tubing, selected glassware or buckets)

    a. marked for zebra mussel use only, stored separately from general equipment.

2. Infrequent use (bioassay containers, items which must remain available for general laboratory use)

    a. disinfect (soak in 10 mg/L chlorine for 24 hrs)

    b. wash with general laboratory glassware

3. All equipment, holding tanks, and experimental chambers should be disinfected at the end of the project

D. Handling and Disposal of Mussels

1. During handling, small mussels may adhere to the hand and go unseen. Disposable gloves should be used during contact with mussels or associated wastewater. After use, the gloves may be discarded in the trash.

2. Dead or unwanted mussels should be bagged and stored for disposal. The investigator should follow institutional guidelines for the disposal of animal carcasses.

E. Disposal of Wastewater

1. All water which has been in contact with live mussels should first be passed through a 1 mm mesh screen (to remove any juveniles or adults) then transferred to a waste tank for treatment before release.

2. Wastewater treatment should follow an acceptable disinfection method such as establishing a 10 mg/L chlorine concentration within the waste tank. This can be accomplished through the addition of chlorine bleach or powdered pool chlorine. The water should be heated to 40 °C or held for 24 hr prior to release.

F. Spill Containment (Procedures to be displayed in facility)

1. Wipe up with paper towels or mop

2. Ring excess water into waste tank

3. Discard towels in trash, treat mop with appropriate disinfectant

G. Emergency Termination (Procedure to be displayed in facility)

1. If the integrity of the research facility is threatened (e.g. flood, hurricane, etc.), experiments must be terminated and all specimens destroyed with appropriate disinfection.

Curriculum Vita

Joseph R. Bidwell

September, 1993

Present Address

University Center for Environmental and Hazardous Materials Studies
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Virginia Polytechnic Institute and State University
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Birthdate and Place

5/29/63. Plainview, NY.

Education

Ph.D in Zoology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, 24061. 1993. Dissertation title: Control Strategies for the Zebra Mussel, *Dreissena polymorpha*, and the Asian Clam, *Corbicula fluminea*: Comparative Stress Responses and Nontarget Impact.

M.S. in Zoology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061. September, 1988. Thesis title: The Effect of Mercury Exposure and Season on the Physiological Status of Field Collected Rock Bass.

B.S. in Biology, Siena College, Loudonville, NY 12211. June 1985 (Magna Cum Laude).

Employment

Fall 1984
Research Assistant, Dept. of Biology, Siena College.

1984-1985

General Biology Laboratory Coordinator, Dept. of Biology, Siena College.

1985

Laboratory Technician, D.M. Graham Pharmaceutical Laboratories, Hobart, NY.

1986-1989

Graduate Teaching Assistant, Dept. of Biology, VPI&SU.

1989-1993

Graduate Research Assistant, Dept. of Biology, VPI&SU.

1993

Research Associate and Lecturer in General Biology, VPI&SU.

Honors

Tuition Scholarship, VPI&SU, Fall Qtr., 1987, Fall Sem., 1988, 1990, Academic Year 1991-1992, Fall 1993
Presidential Scholar, Siena College
New York State Regents Scholarship

Honor Societies

Sigma Xi
Phi Kappa Phi

Professional Organizations

Society of Environmental Toxicology and Chemistry
Virginia Academy of Science
North American Benthological Society

Research Grants

1987

Sigma Xi Grant-in-Aid of Research: Physiological effects of chronic mercury exposure on wild fish. $250.

Biology Department, VPI&SU Matching Grant (to Sigma Xi grant). $250.

1989-1993

BETZ Laboratories Inc. (Co-I with D.S. Cherry, J.L. Farris, and J.R. Lauth), Efficacy and Fate and Effects Testing of a Molluscicide Developed for Control of the Asiatic Clam and Zebra Mussel. $105,000.

Duquesne Light Company. (Co-I with D.S. Cherry, J.L. Farris, and J. Cairns Jr), *Corbicula* anti-fouling and molluscicide fate and effects studies regarding nuclear power generation. $93,000.

Published Abstracts and Presentations

Bidwell, J.R. and A.G. Heath. 1986. Development of a field sampling technique to detect environmentally induced stress in fish. VPI & SU Third Annual Graduate Research Symposium Abstracts. (Poster Presented).

Bidwell, J.R. and A.G. Heath. 1987. Determination of the physiological status of wild fish from an area of chronic mercury contamination. Eighth Annual Meeting Abstracts, Society of Environmental Toxicology and Chemistry, 224:470. (Poster Presented).

Bidwell, J.R. and A.G. Heath. 1988. Physiological effects of laboratory mercury exposure on wild fish with a pre-existing mercury body burden. Ninth Annual Meeting Abstracts, Society of Environmental Toxicology and Chemistry, 163:148. (Poster Presented).

Bidwell, J.R., D.S. Cherry, J.L. Farris, J. Cairns Jr., and L.A. Lyons. 1989. Use of selected freshwater invertebrates to evaluate the molluscicide Clam-trol and its detoxification potential. Tenth Annual Meeting Abstracts, Society of Environmental Toxicology and Chemistry, 222:245. (Poster Presented).

Bidwell, J.R., J.L. Farris, D.S. Cherry, J. Cairns Jr., and L.A. Lyons. 1990. A 10-day growth test using artificial substrate with the midge *Chironomus riparius*. Thirty-eighth Annual Meeting Abstracts, North American Benthological Society, 22:26. (Paper Presented).

Bidwell, J.R., J.L. Farris, and D.S Cherry. 1990. Use of introduced bivalves as biological indicators of pollution. Annual meeting of the American Society of Limnology and Oceanography Abstracts, p. 8. (Paper Presented).

Bidwell, J.R., J.L. Farris, D.S. Cherry, and H.E. Kitchell. 1991. Guidelines for laboratory handling and manipulation of the zebra mussel. Virginia Journal of

Science 42(2):166. (Paper presented at 1991 Virginia Academy of Science Meetings).

Bidwell, J.R., D.S. Cherry, J.L. Farris, and L.A. Lyons. 1991. Short-term fate of a surfactant based molluscicide and its effects upon a sediment dwelling organism. Twelfth Annual Meeting Abstracts, Society of Environmental Toxicology and Chemistry, 139: P004. (Poster Presented).

Bidwell, J.R., L.A. Lyons, D.S. Cherry, J.C. Petrille, and M.W. Werner. 1992. Effects of intermittent chlorine and bromine treatments on settling, survival, and growth of the zebra mussel, *Dreissena polymorpha*. J. Shellfish Res. 11(1)218. (Paper presented at Second International Zebra Mussel Research Conference, November 1991, Rochester, NY).

Bidwell, J.R., L.A. Lyons, D.S. Cherry, and J.C. Petrille. 1992. Surveillance of zebra mussel, (*Dreissena polymorpha*), larval densities, settling, and growth at a power plant on Western Lake Erie. J. Shellfish Res. 11(1)218. (Poster Presented at Second International Zebra Mussel Research Conference, November 1991, Rochester, NY).

Petrille, J.C., J.R. Bidwell, L.A. Lyons, D.S. Cherry, and M.W. Werner. 1992. Effectiveness of two treatment strategies to control zebra mussel fouling in once through cooling systems using oxidizing biocides and a non-oxidizing molluscicide. (Poster presented at Second International Zebra Mussel Conference, February 1992, Toronto, Canada).

Bidwell, J.R., D.S. Cherry, and J.L. Farris. 1992. Comparative response of the Asiatic clam, *Corbicula fluminea*,and the zebra mussel, *Dreissena polymorpha* to osmoregulatory stress. VA J. Sci. 43(2)257 (Paper presented at 1992 Virginia Academy of Science Meeting).

Bidwell, J.R., D.S. Cherry, and J.L. Farris. 1992. Biochemical response of the Asiatic clam, *Corbicula fluminea*, and the zebra mussel, *Dreissena polymorpha*, to a surfactant based molluscicide. Thirteenth Annual Meeting Abstracts, Society of Environmental Toxicology and Chemistry, 2299: WA6G10. (Poster Presented).

Cherry, D.S., J.L. Farris, J.R. Bidwell, A. Mikailoff, M.M. Yeager, and S.R. Lynde. 1992. Environmental effects of molluscicide application for *Corbicula* control in a nuclear power plant- a two year study. Thirteenth Annual Meeting Abstracts, Society of Environmental Toxicology and Chemistry, 37:197.

Lauth, J.R., J.R. Bidwell, and D.S. Cherry. 1992. The chronic toxicity of surfactants to fathead minnows and daphnids in static and flow-through bioassays. Thirteenth

Annual Meeting Abstracts, Society of Environmental Toxicology and Chemistry, 250:WP6A23.

Bidwell, J.R. and D.S. Cherry. 1993. Comparative response of the zebra mussel, *Dreissena polymorpha*, and the Asiatic clam, *Corbicula fluminea*, to the molluscicide CT-1. Third International Zebra Mussel Conference, February 1993, Toronto Canada, Conference Abstracts. (Paper Presented).

Reid, D.F., E. Marsden, J. Nichols, J. Carlton, and J. Bidwell. 1993. Considerations for zebra mussel containment and confinement protocols. Third International Zebra Mussel Conference, February 1993, Toronto Canada, Conference Abstracts. (Poster Presented).

Cherry, D.S., J.L. Farris, M.G. Dobbs, J.R. Bidwell, and E.P. Smith. Laboratory and *in-situ* toxicity of a chemical-municipal waste treatment effluent in the New River, Virginia. Paper to be presented at the fourteenth annual meeting of the Society of Environmental Toxicology and Chemistry (SETAC), Houston TX, November, 1993.

Bidwell, J.R., D.S. Cherry, and M.G. Dobbs. An integrated approach toward determining the impact of a paper mill effluent upon a receiving system. Poster to be presented at the fourteenth annual meeting of the Society of Environmental Toxicology and Chemistry (SETAC), Houston TX, November, 1993.

Bidwell, J.R., D.S. Cherry, J.L. Farris, L.A. Lyons, and J.C. Petrille. Efficacy of intermittent halogen treatments for control of the zebra mussel, *Dreissena polymorpha*. Paper to be presented at the fourteenth annual meeting of the Society of Environmental Toxicology and Chemistry (SETAC), Houston TX, November, 1993.

Publications

Cherry, D.S., J.L. Farris, J.R. Bidwell, A. Mikailoff, R.L. Schema, and J.W. McIntire. 1990. Application of a molluscicide and environmental fate and effects at the Beaver Valley Power Station, Duquesne Light Company. Proceedings of the 1990 EPRI Macrofouling Symposium. Electric Power Research Institute, Palo Alto, CA.

Lyons, L.A., J.R. Bidwell, J.C. Petrille, D.S. Cherry, and M.W. Werner. 1991. A comparative study regarding control of zebra mussels: comparing chlorine,

bromine, and a non-oxidizing molluscicide- an on-site evaluation. Proceedings of the 1991 EPRI Zebra Mussel Research Conference. Electric Power Research Institute, Palo Alto, CA.

Bidwell, J.R. and A.G. Heath. 1993. An *in situ* study of rock bass, *Ambloplites rupestris*, physiology: effect of season and mercury contamination. Hydrobiologia 264:137-152.

Bidwell, J.R., D.S. Cherry, J.L. Farris and L.A. Lyons. (In Press). Comparative response of the zebra mussel, *Dreissena polymorpha*, and the Asiatic clam, *Corbicula fluminea*, to the molluscicide CT-1. Proceedings of the Third International Zebra Mussel Conference, 1993, Toronto, Canada. Electric Power Research Institute, Palo Alto, CA.

Manuscripts in Preparation/Review

Bidwell, J.R., D.S. Cherry, J.L. Farris, and H.E. Kitchell. State guidelines for conducting laboratory research with the zebra mussel, *Dreissena polymorpha*. In preparation for submission to the Journal of Shellfish Research.

Bidwell, J.R., J.L. Farris, and D.S. Cherry. Comparative response of the Asiatic clam, *Corbicula fluminea*, and the Zebra mussel, *Dreissena polymorpha*, to osmoregulatory stress. In preparation for submission to Comparative Biochemistry and Physiology.

Bidwell, J.R., D.S. Cherry, and L.A. Lyons. A study of intermittent halogenation as a control strategy for the zebra mussel, *Dreissena polymorpha*. In preparation as an invited paper for Environmental Pollution.

Manuscript Reviewer

Aquatic Toxicology
Environmental Pollution
Water Research
Environmental Toxicology and Chemistry

Technical Reports

Cherry, D.S., J.L. Farris, J.R. Bidwell, and L.A. Lyons. 1989. Control of *Corbicula* by BETZ Laboratories Chemicals with Environmental Fate and Effects. Final Report Phase I. Submitted to BETZ Laboratories on 27 March 1989. (Prepared Report).

Cherry, D.S., J.L. Farris, J.R. Bidwell, and L.A. Lyons. 1990. Control of *Corbicula* by BETZ Laboratories Chemicals with Environmental Fate and Effects. Final Report Phase II. Submitted to BETZ Laboratories on 8 January 1990. (Prepared Report).

Cherry, D.S., J.L. Farris, J.R. Bidwell, A. Mikailoff, R.L. Schema, and J.W. McIntire. 1990. 1990 *Corbicula* Control Program Environmental Fate and Effects Studies, Baseline and Spring Dosing Studies. Submitted to Duquesne Light on 18 November 1990.

Cherry, D.S., J.L. Farris, J.R. Bidwell, and L.A. Lyons. 1990. Control of *Corbicula* by BETZ Laboratories Chemicals with Environmental Fate and Effects. Final Report Phase III. Submitted to BETZ Laboratories on 20 December 1990. (Prepared Report).

Cherry, D.S., J.R. Bidwell, A. Mikailoff, M.M. Yeager, S.R. Lynde, R.L. Schema, and J.W. McIntire. 1991. 1991 *Corbicula* Control Program Environmental Fate and Effects Studies, Summer Dosing Studies. Submitted to Duquesne Light on 23 November 1991.

Cherry, D.S., J.R. Bidwell, J.R. Lauth and L.A. Lyons. 1993. Control of *Corbicula* by BETZ Laboratories Chemicals with Environmental Fate and Effects, Artificial Stream Studies. Final Report Phase VI. Submitted to BETZ Laboratories on 3 February 1993.

Reid, D.F., J.R. Bidwell, J. Carlton, L. Johnson, E. Marsden and S.J. Nichols. 1993. Zebra Mussel Containment Protocols. Submitted to the Research Protocol Committee of the National Aquatic Nuisance Species Task Force.

Professional Activities

1989

Independent study co-advisor for Radford High School student (Michael Murray). Project: A comparison of growth capacity between Asiatic clams *Corbicula fluminea* from the New River and Claytor Lake, VA.

Hoechst Celanese Corporation, Celco Plant, Narrows, VA, Evaluated Asiatic clam, *Corbicula fluminea*, invasion and control strategies.

Hoechst Celanese Corporation, Celriver Plant, Rock Hill, SC, Evaluated Asiatic clam, *Corbicula fluminea*, invasion and control strategies.

1990

VPI & SU, Biology Department, Co-coordinator of zoology seminar series for fall semester

BETZ Laboratories, Trevose, PA, Invited presentation of molluscicide efficacy data.

Virginia Fiber Corporation, Amherst, VA, Benthic macroinvertebrate sampling as part of a project to evaluate effluent mixing zones and dissipation effects upon benthic communities in the James River, VA.

1990-1991

Duquesne Light Company, Beaver Valley, PA, Coordinator of on-site laboratory study to assess fate and effects of a nuclear power plant outfall following application of a molluscicide.

Hoechst Celanese Corporation, Celriver Plant, Development of chlorine minimization plan for control of *Corbicula fluminea*

1991

VPI & SU, Biology Department, Member of graduate student advisory committee to the department chair

Siena College, Albany, NY, Invited Lecturer on zebra mussels

BETZ Laboratories, Trevose, PA, Coordinated *Ceriodaphnia dubia* culturing and testing workshop for BETZ personnel.

BETZ Laboratories, Trevose, PA, Directed summer field study to monitor biofouling and assess control strategies for the zebra mussel, *Dreissena polymorpha*, at a coal fired power plant on western Lake Erie.

1991-1992

Hoechst Celanese Corporation, Celco Plant, Narrows, VA, Benthic macroinvertebrate and fish sampling to evaluate effluent dissipation and potential toxicity of waste treatment effluent in the New River, VA.

1992-1993

Florida Power and Light Company, West Palm Beach, FL, Co-reviewer (with D.S. Cherry) of technical reports concerning toxicity of biocides to control marine biofouling organisms.

Hoechst Celanese Corporation, Celco Plant, Seasonal fish sampling surveys to develop a US EPA 316 "a" variance demonstration on heated effluent dissipation in the New River, VA.

Member, Zebra mussel specific research protocol development committee, National Sea Grant and EPA sponsored committee to establish federal guidelines for laboratory handling and manipulation of the zebra mussel, *Dreissena polymorpha*.

Member, Research Taskgroup, Exotic Species Workgroup, Chesapeake Bay Program

Science Museum of Roanoke, Roanoke, VA, Invited lecturer on zebra mussels.

Sonoco Products Company, Downingtown, PA, Benthic macroinvertebrate sampling to evaluate the impact of a paper mill effluent upon a receiving system.

Workshop Panel Member, Zebra mussel research protocols, Third International Zebra Mussel Research Conference, Toronto Canada.

Reviewer of bioassay data for Quality Control/Quality Assurance between several Canadian environmental testing laboratories, Proctor and Gamble Corporation, Grande Prairie, Edmonton, Alberta.

Research Interests

Research includes the examination of biochemical stress responses in bivalves and the correlation of these responses with data from standardized aquatic toxicity tests, control strategies for introduced bivalves (*Corbicula fluminea*, *Dreissena polymorpha*), response of benthic communities to perturbation, adaptive physiology of introduced species.

Research Experience

Freshwater acute and chronic toxicity testing (static and flow-through)
- *Ceriodaphnia dubia*
- *Pimephales promelas*
- *Daphnia magna*
- *Isonychia bicolor* (mayfly)
- *Goniobasis* sp. (snail)
- *Corbicula fluminea*

Saltwater acute and chronic toxicity testing
- *Mysidopsis bahia*

Sediment toxicity testing
- *Chironomus riparius*
- *Hyallela azteca*

Field effluent monitoring
- Benthic invertebrate sampling
- Fish sampling
- Live box studies

Artificial stream systems
- Dosing and maintenance
- Conducting bioassays to evaluate molluscicide efficacy
- Conducting bioassays to examine fate and effects of a molluscicide

Joseph R. Bidwell