Toxicological, Physiological, and Behavioral Responses of the Asiatic Clam, Corbicula sp., to Biocidal and Copper Perturbations

by

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(ABSTRACT)

Experiments were conducted on the effectiveness of exposure to simultaneous temperature shock with chlorination, monochloramine, and ammonia as control agents of the Asiatic clam, *Corbicula* sp.. Control procedures were evaluated based on lethal and sublethal responses (e.g., glycogen, tissue water, dry weight index, and siphoning activity) of clams during 30-day laboratory artificial stream studies. Studies also were conducted comparing sublethal responses (e.g., glycogen, tissue water, soluble protein, and siphoning activity) of clams to copper, a component of power plant effluents, during 30-day laboratory, site-specific, and in-situ copper exposures. This was done to evaluate the use of the Asiatic clam as a biomonitoring organism of copper contamination.

Regarding temperature and chlorine interactions, it was demonstrated that an increase of 10° C was needed to increase significantly adult and juvenile mortality in the presence of chlorine (0.30 mg/l TRC) during winter and summer. Naturally high temperatures also increased adult mortality during in-plant chlorination procedures, with the highest mortality occuring during the spring. Significant decreases in the dry weight condition index were observed for adults chlorinated at 5° increases during winter and at 10° C increases for control (non-chlorinated) clams during both winter and summer. Similarly, glycogen content responded with a temperature-dependent decrease in both control and chlorinated clams during the summer. In addition, exposure to increased

temperatures significantly increased the siphoning activity of control adults during summer and juveniles during winter. Chlorinated clams experienced near total inhibition of siphoning activity at all temperatures tested, except for adults exposed at 33° C. Increased siphoning activity, decreased glycogen content, and possibly ammonia accumulation in the mantle cavity were believed to be responsible for the increased mortality of clams chlorinated at higher temperatures.

Total residual chlorine, with < 90% as monochloramine, was found to be equally toxic to adults and more toxic to juveniles compared to total residual chlorine containing higher amounts of free residual chlorine. Since free residual chlorine is considered to be more toxic than combined residual chlorine (e.g., monochloramine), questions were raised as to which form of chlorine was actually exposed to the tissues of adducted Asiatic clams. Ammonia was considerably less toxic to adults but more toxic to juveniles compared to chlorine. Both monochloramine and ammonia caused significant reductions in clam glycogen content and siphoning activity. The siphoning activity of clams exposed to ammonia, although significantly reduced, was considerably higher than siphoning activities observed for monochlorinated and chlorinated clams. Clam tissue water content decreased in the presence of ammonia but remained unaffected in the presence of monochloramine. Ammonia toxicity to adult clams was highly pH dependent but may be useful in controlling larval stages of Asiatic clams. More definitive research is needed to evaluate fully the potential of monochlorination as a biofouling control agent.

Clams were more sensitive to copper exposures, with respect to glycogen content, in field-located (i.e. site-specific) artificial streams than in laboratory artificial streams. Specifically, the "no observable effect concentration" was between 5.5 and 8.4 μg Cu/l during the 30-day site-specific studies compared to 17.2-32.1 μg Cu/l in the laboratory. Copper significantly increased clam tissue water content during the Clinch River and

June site-specific studies. However, clam soluble protein content demonstrated no consistant dose-dependent response during the laboratory or site-specific studies. Glycogen and tissue water content, although subject to some seasonal influences, are recommended for use in *Corbicula* for future site-specific and in-situ long-term toxicity experiments.

Acknowledgements

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I also thank and for their help in the studies included in this thesis. I realize that graduate school is not all work and no play, and my colleagues and friends, , , , , and reminded me of that on numerous occasions. Thanks, guys.

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Thanks also go to Dr. Anne McNabb for keeping me "on track" with the graduate school, and to the Biology department for their offering of tuition wavers at various points during my graduate school career. This research was supported through grants from American Electric Power Company and the Union Carbide Corporation.

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PROLOGUE

The Asiatic clam, *Corbicula* sp., was first discovered in North America in the Columbia River of Washington State in 1938 (Burch, 1944; Sinclair and Isom, 1963; McMahon, 1977). Following its introduction into this continent, *Corbicula* rapidly spread both south and eastward having invaded the Sacramento River at Pittsburg, California in 1945 (Hanna, 1966) and the Salt River at Phoenix, Arizona in 1956 (Dundee and Dundee, 1958). *Corbicula* was discovered subsequently in the Ohio River at Paducah, Kentucky in 1957 and the Tennessee River in 1959 (Sinclair and Isom, 1963), which was hypothesized to have been a result of a second, human mediated introduction (McMahon, 1982; Counts, 1986). Soon after its discovery in the Tennessee River, Thomas and Mackenthun (1964) reported the colonization of the upper Ohio and Kanawah River drainage system by *Corbicula* at Charleston, West Virginia. *Corbicula* continued its southeastwardly migration, having been discovered in the Savannah River, Georgia in 1972 (Fuller and Powell, 1973) and in the New River at Applachian Power Company's Glen Lyn Plant in 1975 (Rodgers et al., 1977). Henceforth, Rodgers et al. (1977) calculated its upstream migration rate in the New River to be approximately 15 km per year. *Corbicula* has recently extended its northern extensions to Lake Erie

(Clarke, 1981) and the Susquehanna River at the Conawingo Dam (Nicholes and Domermuth, 1981). Thus, in less than 50 years, *Corbicula* sp. has successfully colonized most major water ways in the United States (for reviews see Cherry et al., 1980; McMahon, 1982; Counts, 1986).

After initial colonization, populations of *Corbicula* are known to increase rapidly in density in both lentic and lotic habitats. For example, population densities of *Corbicula* in the New River rapidly increased from 94/m^2 in February 1978 to 2,286/m^2 in November 1978 at thermally uninfluenced areas at Glen Lyn, Virginia (Cherry et al., 1980). A massive late summer spawn 15 km upstream at the Celanese Fibers Corporation pumphouse in Narrows, Virginia caused *Corbicula* densities to rise from 2,529/m^2 in July, 1981 to a New River record 269,105/m^2 in September 1981 (Cherry et al., 1986). In lentic habitats, Abbott (1979) reported that *Corbicula* densities in the Dale Hollow Reservoir, Tennessee reached a maximum of 1215.9/m^2 in August, 1973.

Reasons for the Asiatic clam's ability to achieve such high densities include its lack of ecological restraints, interactions with man and industry, and high reproductive capacity. It is hypothesized that *Corbicula* was introduced in North America by Chinese immigrants and intitially dispersed by its use as fish bait and through bilge flushings of merchant ships and barges (Hanna, 1966; Fox, 1971; Morton, 1973, Isom, 1986). Once initially dispersed, the Asiatic clam possessed a competitive advantage over endemic bivalves because of its lack of native ecological restraints such as predation, parasitism, and competition (Morton, 1979; Kraemer, 1979). Also important in maintaining high population densities is *Corbicula's* rather opportunistic ability to colonize heated industrial discharge areas during winter months. In fact, the relative intolerance of *Corbicula* to cold temperatures (e.g. lower incipient lethal temperature of 2°C) seems to be the only major limitation on its dispersal in North America (Mattice and Dye, 1976). Therefore, Graney et al. (1980) suggests that thermally influenced areas provide a "stepping stone"

for increased clam survival through the winter. Specifically, in February 1978, Graney et al. (1980) reported *Corbicula* densities in thermally influenced and uninfluenced areas of the New River of 11,522/m^2 and 94/m^2, respectively. Clearly then, colonization of thermal discharges by *Corbicula* during winter enable a large proportion of clams to reach sexual maturity and greatly increases their ability to survive to the following year.

Relative to its reproductive capacity, *Corbicula* has been classified as both an "r-strategist" and a "k-strategist" (Boozer and Mirks, 1979). As an r-strategist, *Corbicula* rapidly attains sexual maturity (i.e. within 6 months) and produces several thousand larvae per adult clam (Kraemer and Lott, 1977; Britton and Morton, 1979; Britton and Morton, 1982). Kraemer et al. (1986) presented evidence that in addition to cross fertilization, *Corbicula* may possess the ability to self-fertilize by means of transporting both male and female gametes through a common duct. As a k-strategist, *Corbicula* retains its embryos on its inner demibranch through the trochophore stage until they develop into veligers, which possess both valves, a hinge, and a fully developed foot, prior to release (Sinclair and Isom, 1963; Britton and Morton, 1979; Kraemer et al., 1986). This gives *Corbicula* veligers an advantage over other bivalve larvae which are not as developed upon release (i.e. released as swimming trochophores) or are released as glochidia which rely specifically on fish hosts for survival and dispersal. Characteristically, *Corbicula* exhibits further k-selection in slow-flow or "stable" systems, such as lakes and slow flowing rivers, as evident from its large size (e.g. 46 mm in Lake Arlington, Texas) and occasionally, reduced fecundity upon aging (Aldridge and McMahon, 1978; Sickel, 1979). In addition, to r and k-selective advantages, *Corbicula* larvae are known to produce mucous strands which extend through the exhalent siphon and are thought to aid their downstream dispersal in lotic habitats (Prezant and Chalernwat, 1984).

Because of the reproductive advantage of *Corbicula* over endemic bivalves, dense populations of *Corbicula* become established which cause considerable economic and ecological impacts in North America. Ecologically, the Asiatic clam is thought to be responsible for the displacement of endemic bivalves in the Savannah River, Georgia (Sickel, 1973). Sickel suggests that because of the superior filtering rate of *Corbicula*, "native" bivalves may not be able to successfully compete for the available food supply. Cohen et al. (1984) have also documented high filtering rates of dense populations of *Corbicula* which they hypothesize to be partly responsible for a phytoplankton overturn in the Potomac River, Maryland. In addition, Fuller and Richardson (1977) suggest that the displacement of Unionidae in the Altamaha River, South Carolina is achieved through burrowing activities of *Corbicula*, which tend to uproot native Unionid bivalves.

Economically, the Asiatic clam has caused serious fouling problems with aquaducts, industrial condensor systems, and even concrete manufacturing industries (Morton, 1979; Cherry et al., 1986; Isom et al., 1986). With respect to aquaducts, densities of 10,000-20,000/m^2 caused severe reductions in the delivery capacity of the Delta-Mendota Canal of California and necessitated expensive dewatering and dredging procedures (Prokopovich, 1969, Prokopovich, 1973; Eng, 1979). *Corbicula* also has caused structural weaknesses in concrete manufactured from dredged river beds infested with high clam densities (Sinclair and Isom, 1963). Most importantly, *Corbicula* has caused fouling of condensor systems of industries using raw river or lake water for cooling purposes. Severe reductions in cooling capacity result when large numbers of clams become lodged in condensor pipes and restrict the water flow through the condensor system. In steam generating electric power plants, reductions in cooling capacity cause decreases in the efficiency of electrical generation and may increase the cost of electricity to the consumer. More importantly, *Corbicula* fouling has been reported in condensor and emer-

gency cooling systems of nuclear power plants, which could precipitate a nuclear accident (Parsons, 1980; Henager et al., 1985).

The extent of *Corbicula* fouling depends greatly on the size and design of the industrial plant, which in turn, affects the type of control procedure used. For example, large plants with high cooling demands often require cooling water volumes too great to use effective (< 0.2 mm mesh size) intake screens which impede larval intrusions into the cooling water system. Once in the system, larval growth and subsequent fouling is enhanced further in areas of reduced flow in the cooling water system. These "dead spaces" provide an ideal environment for the attachment and growth of *Corbicula* larvae, which may achieve fouling sizes within 6 months of their introduction into the cooling water system (Aldridge and McMahon, 1978; Johnson et al., 1986). Although numerous chemicals such as bromine chloride, triphenyl lead acetate (Goss et al., 1979), tributyl tin floride (Mussalli et al., 1986) and a brominated phenol (Doherty et al., 1986) have been investigated as control agents, industries designed with "once-through" cooling water systems are limited in the type of chemical control method used due to the chemical's adverse impact upon the aquatic community. Some of these industries have used periodic chlorination of cooling water effectively to control clam fouling (Isom et al., 1986; Cherry et al., 1986). However, restrictions on the frequency of chlorine discharge and reductions in the ambient water quality criteria for chlorine have further complicated the use of chlorination as a biofouling control procedure (USEPA, 1974; 1985a). Therefore, the need for effective and environmentally sound biofouling control procedures becomes clear.

Despite the adverse economic impact of the Asiatic clam, some beneficial uses of *Corbicula* do exist. For example, populations of *Corbicula* at densities of 8 million clams per hectare are cultivated as a food source in Asia (Chen, 1976). In North America, Fox (1970) reported revenues in excess of $230,000 from clams sold as fish bait in California

from 1963 to 1968. *Corbicula* was also considered a valuable component of the fish bait industry in the lower Tennessee River prior to massive clam mortalities during the summer of 1977 (Sickel et al., 1980). Another beneficial use of *Corbicula* that is not widely recognized is its use as a freshwater biomonitoring organism. The wide North American distribution, relative immobility, and filter feeding life style of *Corbicula* have resulted in its use as a biomonitor for numerous heavy metal, mineral, and organic contaminants (Rodgers et al., 1977; Leard, 1980; Cairns and Cherry, 1983; Graney et al., 1983; Belanger et al., 1987). In fact, Foe and Knight (1986b) have developed a sublethal biomonitoring protocol specifically designed for *Corbicula* as the test organism. The relatively small size and ease of sampling of *Corbicula* have resulted in its use in numerous laboratory studies of the uptake and bioaccumulation of heavy metals such as zinc, cadmium, and copper (Rodgers et al., 1980; Graney et al., 1984; Belanger et al., 1986a; Farris, 1986). In addition, the existance and response of metallothionein-like binding proteins to cadmium and zinc in *Corbicula* was recently documented (Doherty et al., 1987).

The purpose of this research was to determine the effectiveness of several biofouling control strategies in controlling *Corbicula* and to determine the potential use of *Corbicula* as a biomonitor of copper, a power plant effluent constituent. Specifically, it was hypothesized that both seasonal and temperature effects could be exploited to increase the sensitivity of the Asiatic clam to chlorine, thereby reducing the amount of chlorine required for adequate *Corbicula* control. This hypothesis is addressed in Chapter 1 through the examination of the toxicological, physiological, and behavioral responses of *Corbicula* to combined temperature shock with chlorination during winter and summer laboratory studies and during in-plant chlorination studies in the spring and fall. Likewise, it was hypothesized that the use of biocides with similar properties of chlorine (e.g. rapid volatilization, ease of application, high toxicity), but which were

thought to be relatively undetectable to *Corbicula*, would be an effective alternative to the continuous chlorination control method. This hypothesis is addressed in Chapter 2 in which the effectiveness of monochloramine and ammonia on Asiatic clam control is examined. Finally, the hypothesis that *Corbicula* would be a successful candidate for the biomonitoring of copper contamination is addressed in Chapter 3. This chapter specifically examines several physiological responses of *Corbicula* to copper in laboratory, site-specific, and in-situ long-term studies.

1.0 TEMPERATURE AND CHLORINE

1.1 INTRODUCTION

The economic impact of the Asiatic clam, *Corbicula* sp., upon manufacturing and power generating industries has been substantial since its discovery in North America approximately 50 years ago (Burch, 1944). These impacts result mostly from the clogging of cooling pipes by *Corbicula* in industries using raw river water for cooling purposes (Sinclair and Isom, 1963; Goss et al., 1979; Henager et al., 1985; Cherry et al., 1986). It has been estimated that over 1 billion dollars is lost annually in the United States due to reductions in production efficiency, plant shutdowns, and control costs resulting from *Corbicula* fouling (Isom, 1986). Because of this degree of economic impact, numerous studies have been conducted which examine the efficient control of *Corbicula.* Mechanical control procedures range from the installation of sophisticated self-cleaning strainers on intake pipes (Goss et al., 1979; Mussalli et al., 1986), removal of clam infested sediment (Cherry et al., 1986), and partial plant shutdowns for subsequent dewatering and clam removal (Potter and Liden, 1986). Chemical control proce-

dures include (but are not limited to) the use of chlorine, bromine chloride, triphenyl lead acetate (Goss et al., 1979), copper (Cherry et al., 1980), tributyl tin floride (Mussalli et al., 1986), and a brominated phenol (Doherty et al., 1986).

Of the control procedures in use, chlorination has been used most extensively for Asiatic clam control because of its relatively low cost, ease of application, rapid dissipation, and high toxicity. Recommended control strategies of *Corbicula* with chlorine consist of continuous chlorination of raw intake river water to total residual chlorine (TRC) levels of 0.5 to 1.0 mg/l for three to four week intervals either before, during, and/or after clam spawning periods (Isom et al., 1986; Cherry et al., 1986). This control procedure results in the eradication of spawning adults and released veligers in the cooling water system and has been effectively used in the past for controlling Asiatic clam fouling in both the nuclear power generating and manufacturing industries (Isom et al., 1986; Cherry et al., 1986).

Despite the effectiveness of the chlorination biofouling control procedure, the Environmental Protection Agency has restricted the continual discharge of chlorine from steam generating power plants into natural waters to no longer than two hours per discharging unit, at a concentration of 0.2 mg/l TRC (USEPA, 1974). While this level of chlorination limits algal and fungal growth in condensor systems, it is inadequate in controlling *Corbicula* (Mattice et al., 1982). Furthermore, recent revisions of EPA water criteria for chlorine specify a four-day average of 0.011 mg/l and a one-hour maximum of 0.019 mg/l are not to be exceeded more than once every three years for the adequate protection of aquatic life (USEPA, 1985a). Although special variances allowing for continuous chlorine discharge for Asiatic clam control are available on a case-by-case basis, these too are subject to severe restrictions, as examplified by recent discharge limitations and dechlorination requirements placed on the Celanese Fibers Corporation in Narrows, Virginia (Cherry, pers. comm.).

As a result of these EPA restrictions on chlorine discharges, recent studies have focused on the effectiveness of variations to the continuous chlorination procedure employed for Asiatic clam control. For example, Doherty et al. (1986) examined the effectiveness of "low" (0.25 mg/l TRC) two-week exposures followed by "high" (0.5-1.0 mg/l TRC) two-week exposures on Asiatic clam survivorship following observations that the onset of clam mortality (usually after 10 to 14 days) was independent of chlorine concentrations ranging from 0.25 to 1.0 mg/l TRC. They found that clam mortality following 14 days of exposure to "low" chlorine doses immediately followed by 14 days of "high" chlorine exposure was comparable to clam mortality during 28-day "high" chlorine exposures. Doherty et al. (1986) further noted that clams collected during the winter and acclimated to summer temperatures had significantly lower LT50 (lethal time for the onset of 50% mortality) values than clams collected and exposed during the summer at comparable chlorine concentrations. This was attributed to the reduced visceral mass (i.e., poor body condition) of clams following winter and the increased metabolic demands which were placed on the organisms. Another study examining temperature and chlorine interactions on *Corbicula* survivorship was conducted by Mattice et al. (1982). In this study, Asiatic clams were subjected to temperature increases and chlorination up to 10 mg/l for 30 minute durations with no resultant mortality. The chlorine exposures used in this study, although in accordance with standard EPA chlorine discharge limits with respect to time span, are not environmentally realistic and do not represent recommended Asiatic clam control protocol (Isom et al., 1986; Page et al., 1986).

Therefore, the primary objective of this portion of research was to examine both seasonal and temperature influences on the effectiveness of previously recommended continuous chlorination procedures in order to reduce the amount of chlorine needed to control Asiatic clams. It was hypothesized that continuous chlorination at reduced lev-

els, in combination with temperature and seasonal influences, would result in adequate control of Asiatic clam populations. Both lethal (survivorship) and sublethal (body condition, glycogen content, and siphoning activity) responses were examined in order to fully address the response of *Corbicula* to thermal and chlorine perturbations. Since *Corbicula* characteristically exhibits a "clamming up" response to chlorine, it was hypothesized that increased temperature during chlorination would reduce clam body condition and energy reserves as a result of increased metabolic demands being placed on the clam. It was further hypothesized that the decreased energy reserve content in combination with increased clam metabolic rate, would result in increased siphoning activity and subsequent chlorine exposure. Therefore, clam body condition (e.g., dry tissue weight / shell volume) was examined because of its indication of tissue growth, ease of measurement, and its widespread examination in bivalves (Medcof and Needler, 1941; Whyte and Englar, 1982). Glycogen content was examined because it is the primary energy reserve of bivalves and has previously been shown to decrease in response to toxicant exposure in *Corbicula* (Cantelmo et al., 1985). Siphoning activity was addressed because it has been proposed as a measure of sublethal effects on *Corbicula* (Belanger et al., 1986b). In addition, siphoning activity indirectly reflects the valve closure behavior of *Corbicula*, which is instrumental in maintaining its defense to toxicants.

Regarding seasonal influences on the effectiveness of continuous chlorination procedures, it was hypothesized that combined temperature increases with chlorination during winter would result in greater clam mortality relative to comparable summer control procedures due to the poorer body condition of clams during winter. This hypothesis was tested by comparing clam mortality and body condition responses to combined temperature shock and chlorination during winter and summer artificial stream studies. Furthermore, seasonal influences on the effectiveness of industrial chlorination

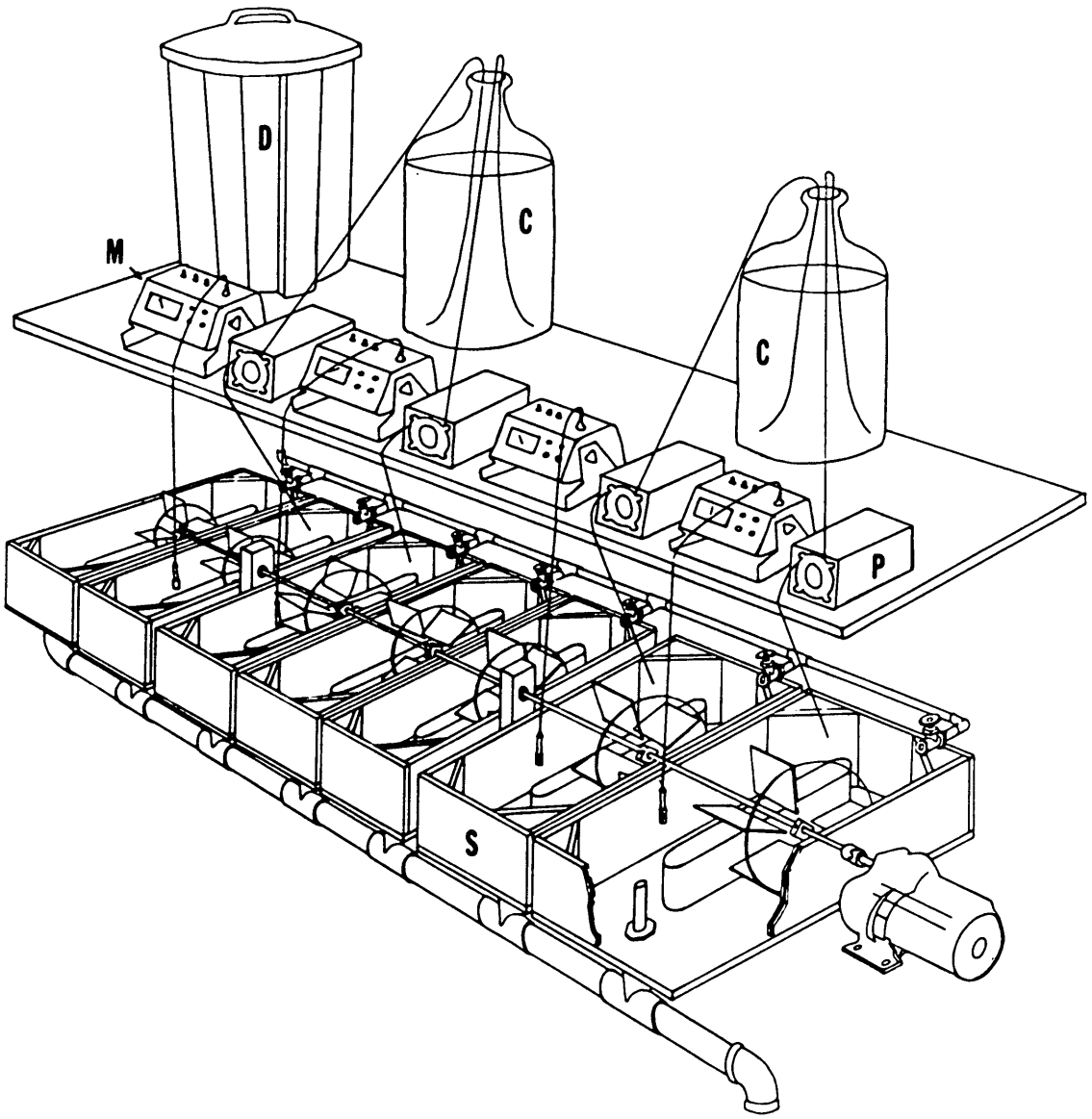
control procedures were examined through the comparison of clam survivorship responses during spring and fall in-plant chlorination control studies.

1.2 METHODS

1.2.1 WINTER TEMPERATURE SHOCK STUDY

Adult (15-21 mm shell length [SL]) and juvenile (5-10 mm SL) Asiatic clams were collected from the New River at Pearisburg, Virginia at 7°C and placed in flow-through, constant circulating artificial streams (Farris, 1986) at 7°C for 2 days in Virginia Tech's Ecosystem Simulation Laboratory (Figure 1-1). Groups of 30 clams were then placed in nylon baskets in a series of 6 streams at 7°, 12°, and 17°C with no prior acclimation for 30 days. Stream temperatures were maintained to $\pm 0.5°$ of target values with a Haake model A82 external circulating chiller system. A daily feeding regime was maintained consisting of the addition of 500 mls of concentrated *Chlamydomonas reinhardti* culture stock to each stream. One stream at each temperature served as a control, while the treated streams received a constant dosage of $Ca(OCl)_2$ via peristaltic pumps to achieve a target TRC concentration of 0.30 mg/l. Stock chlorine solutions were prepared once every 2 days by dissolving $Ca(OCl)_2$ in dechlorinated tap water in 20-l glass carboys placed on stir plates above the streams. Toxicant homogencity was maintained in the stock solutions by constant slow mixing with a magnetic stir bar. Stock and diluent flow rate were 6 and 12 ml/min., respectively, which allowed ~1 overturn (18 l) a day. Constant water movement (~10 cm/sec) was maintained via revolving paddle wheels above the streams.

Figure 1-1. Artificial stream apparatus used for exposing Asiatic clams with selected toxicants in Virginia Tech's Ecosystem Simulation Laboratory. Continuous water circulation was maintained with revolving paddle wheels in each stream. D= dechlorinator, C= carboy, P= peristaltic pump, M= pH meter, S= stream.


D
C
C
M
P
S

Standard water chemistry (e.g., pH, alkalinity as $CaCO_3$, hardness as $CaCO_3$, and conductivity), was measured on days 0 and 30 and showed little deviation and is summarized in Appendix A (USEPA, 1983). Stream temperature, TRC, and FRC (free residual chlorine) were measured a minimum of twice daily, while pH, siphoning activity, and mortality were recorded once daily. TRC and FRC were measured using a Wallace and Tiernan amperometric titrator accurate to ± 0.02 mg/l. Deviations in TRC concentrations greater than $\pm 20\%$ of the target value were corrected for by the addition of concentrated $Ca(OCl)_2$ solution or diluent followed by TRC and FRC remeasurement. Siphoning activity was determined by recording the proportion of clams with extended siphons and was expressed as percent of individuals siphoning. Clam mortality was determined by the presence of valve gaping and the lack of response to gentle proding of the mantle edges.

Following a 10-day recovery period to check for latent mortality, the total volume of 10 clams from each treatment was measured by displacement and the dry weight determined by lyophylization. The dry weight condition index was calculated as:

dry wt. (mg) / clam volume (ml).

1.2.2 SUMMER TEMPERATURE SHOCK STUDY

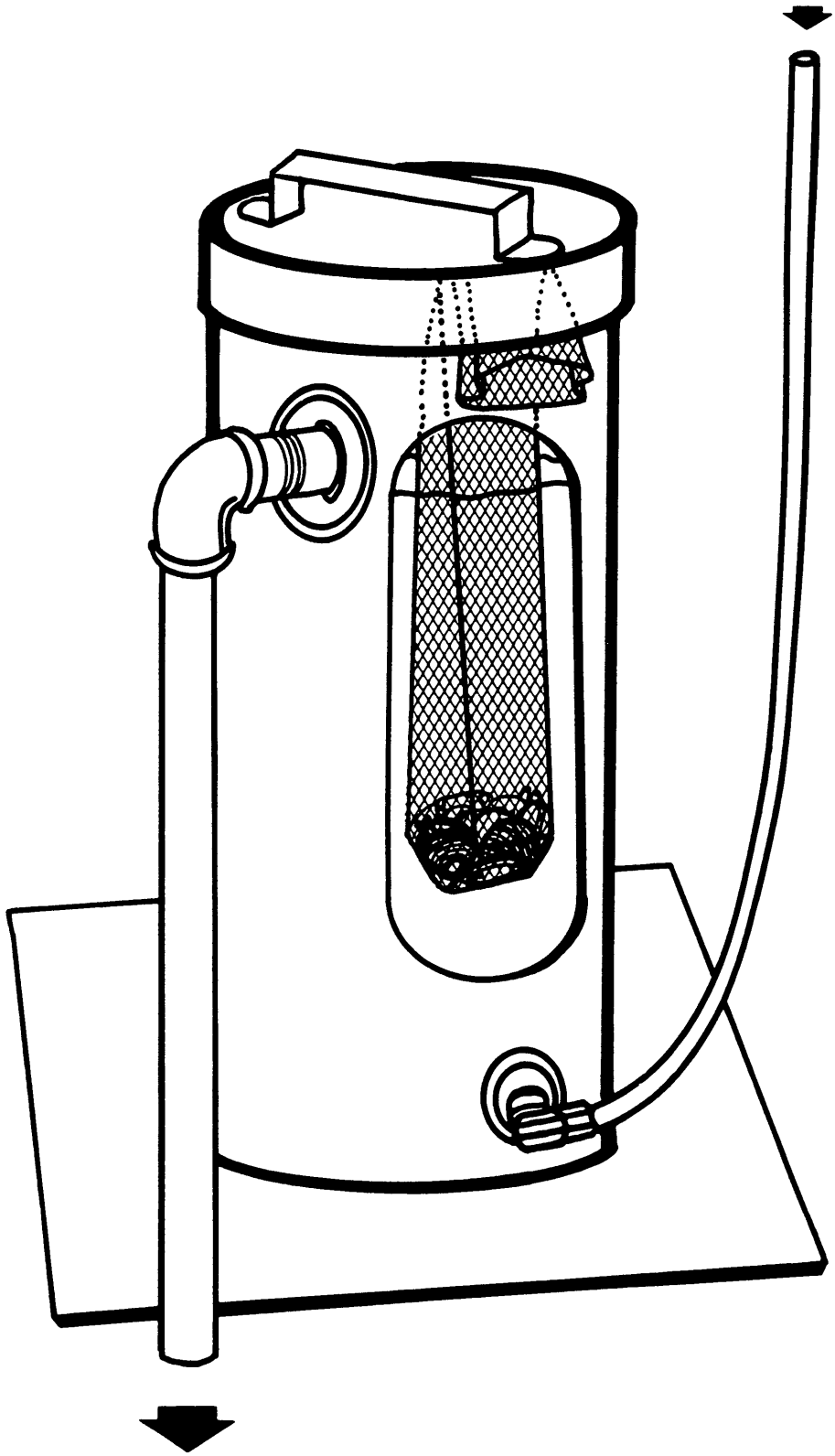
Adult (14-18 mm SL) and juvenile (5-9 mm SL) Asiatic clams were collected from the aforementioned location at 23°C followed by a 2-day 23°C holding period. Groups of 30 were then placed in a series of 6 artificial streams at 23°, 28°, and 33°C. Identical experimental protocol was followed as previously described except for the deletion of the 10-day recovery period and the doubling of the feeding regime to 1000 mls /stream/day. In addition, the *C. reinhardti* was filtered and resuspended in distilled water prior to its addition to each stream to remove excess medium.

In addition to the determination of the dry weight condition index on day 30, whole body glycogen content was determined on days 0, 5, 10, 15, 25, and 30, using slight modifications of the enzymatic methods of Roerig and Alred (1974). Prior to glycogen determination, clam tissues (n = 6) were dissected, quick frozen with liquid nitrogen, and then lyophylized. Their dry weights were determined, and the tissues stored frozen (< -10°C) and dessicated until analysis. For glycogen analysis, clam tissues were homogenized in 0.05M phosphate buffer (pH 4.8; 500 ml/g dry tissue) with a Tekmar Ultra-Turrax homogenizer at 15,000 rpm for 15 seconds. Triplicate 100 µl aliquots of homogenate were then incubated with 3.5 units of Amyloglucosidase (Sigma Chemical Co.) and phosphate buffer (final vol. 0.5 ml) for 2 hrs. at 37°C. Aliquots (100 µl) of glycogen standards (from the Sea mussel, *Mytilus edulis*) containing 5 to 80 µg glycogen were assayed identically to the samples described. Following the conversion of glycogen to glucose, 0.5 ml of phosphate buffer was added to each sample, the samples centrifuged for 10 min to remove fine particulates, and 0.5 ml aliquots were incubated with a freshly prepared glucose oxidase-peroxidase dye reagent specific for glucose determination (Roerig and Alred, 1974) for 30 min at 37°C. Absorbance was then measured at 500 nm and concentrations determined from a glycogen standard curve. Free glucose present in the clam tissues was determined as previously described on clam supernatents, following a 15 min centrifugation at 15,000 rpm, and was subtracted from the previous glycogen determination to yield true glycogen content.

1.2.3 SPRING AND FALL IN-PLANT CHLORINATION STUDIES

In May and September 1986, adult Asiatic clams were collected from the New River, VA at the Celanese Fibers Corporation's Celco Plant pumphouse and placed in nylon sleeves suspended in stainless steel containers receiving chlorinated river cooling water for 28 days (Figure 1.2). The containers were placed at 3 locations in the plant

Figure 1-2. Stainless steel holding chamber used for exposing Asiatic clams (seen here suspended in nylon sleeves) to chlorinated river water during the spring and fall in-plant chlorination studies. Arrows indicate inflow and outflow of chlorinated river water.



and TRC, FRC, temperature, and mortality were measured on a biweekly basis. In addition, glycogen content was measured on days 0 and 32 (following a four-day recovery period) as described previously.

1.2.4 STATISTICAL ANALYSES

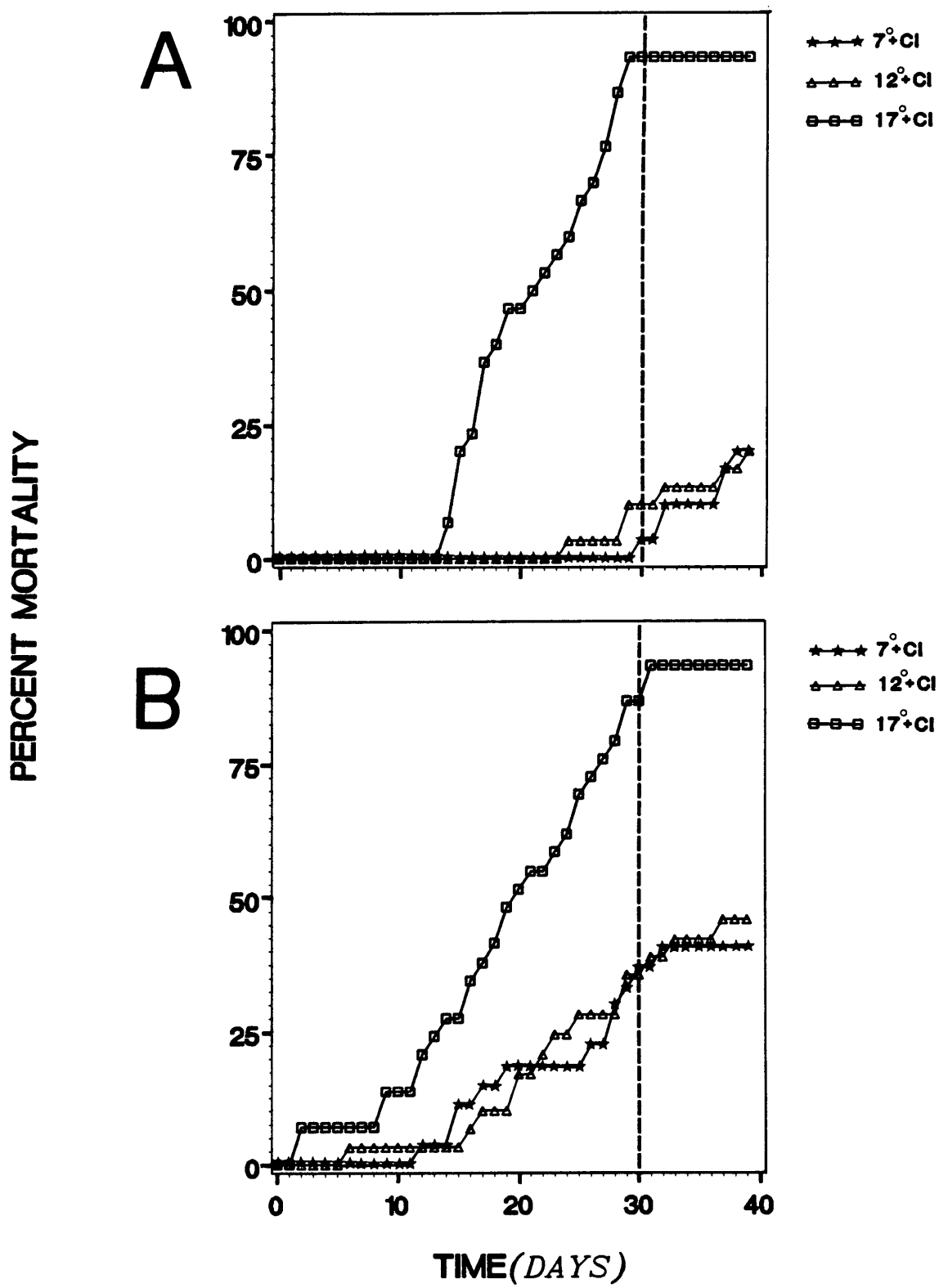
LT50 values of clam mortality were calculated when applicable using Probit analysis and significant differences inferred if LT50 values had non-overlapping fiducial limits. Glycogen content (percent dry weight) and siphoning activity (percent siphoning) were transformed using the arcsine-square root transformation to remove any non-normality which may have resulted from their expression on a percent scale. The transformed data were analyzed using a two-way (winter and summer studies) or one-way analysis of variance (ANOVA) procedure (spring and fall studies) on the Statistical Analysis System ® (SAS, 1985) and significant differences determined using the Duncan's New Multiple Range Test or Studentized t-Test ($\alpha = .05$).

1.3 RESULTS

1.3.1 MORTALITY

Laboratory Studies: Mortality responses of adult and juvenile Asiatic clams exposed to 0.30 mg/l TRC during the winter temperature shock study revealed significant effects of temperature on the sensitivity of *Corbicula* to chlorine (Figure 1-3). Specifically, the time required for the onset of adult mortality decreased with increasing temperature in the chlorinated streams and occurred on days 30, 24, and 14 at 7°, 12°, and 17°C, re-

Figure 1-3. Percent mortality (n = 30) for adult (A) and juvenile (B) Asiatic clams exposed to 0.30 mg/l TRC at 7°, 12°, and 17° C for 30 days during the winter temperature shock study. The beginning of the 10-day recovery period is indicated by a vertical dashed line.


A
B
100
75
50
25
0
7°+Cl
12°+Cl
17°+Cl
PERCENT MORTALITY
0
10
20
30
40
TIME(DAYS)

spectively. Likewise, the onset of juvenile mortality occurred on days 12, 6, and 3 at 7°, 12°, and 17° C, respectively. Adult and juvenile LT50 values give a more precise representation of overall clam mortality and are expressed in Table 1-1, in addition to mean stream TRC, FRC, and temperature. Adult and juvenile LT50's for clams chlorinated at 17°C (e.g. 21.8 and 20.9 days, respectively) were not significantly different, while adults and juveniles dosed at 7° and 12°C experienced mortality too low (i.e. less than 50%) for the calculation of LT50 values. Control mortality was 0% for adults and < 11% for juveniles and, therefore, was not included in Figure 1-3.

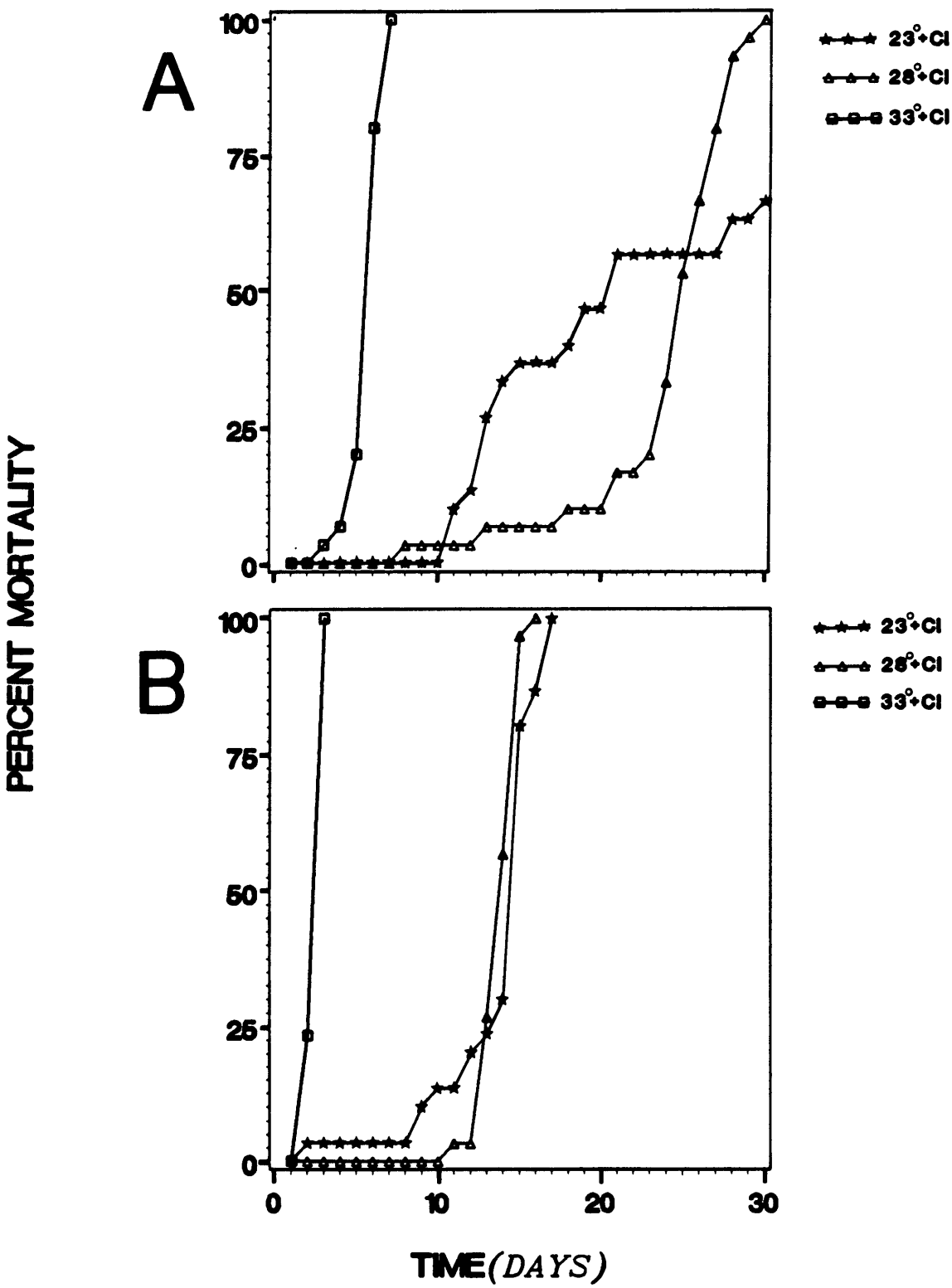
Mortality responses of adults and juveniles chlorinated at identical (5°C) temperature increments during the summer temperature shock study were overall, greater than those observed during the winter study (Figure 1-4). For example, initial adult mortality occurred as soon as day 11, 8, and 3 when chlorinated at 23°, 28°, and 33°C, respectively. Juveniles responded differently than adults by exhibiting initial mortality when chlorinated at 23°, 28°, and 33° on days 2, 11, and 2, respectively. Examination of adult LT50 values indicate that significant reductions in LT50's due to increased temperature occurred only for clams chlorinated at 33°C (Table 1-1). At this temperature, the adult LT50 occurred at 5.6 days, nearly a four-fold reduction from adults dosed at 23° and 28°C. Similarily, significant reductions in juvenile LT50's occurred only when juveniles were chlorinated at 33°C. The juvenile LT50 at this temperature occurred at 2.2 days, with 100% mortality occuring by day 3. Overall, juvenile mortality was greater than adult mortality during the summer as evident by their significantly lower LT50 values at all exposure temperatures. Surprisingly, both control adults and juveniles experienced no mortality even following 30 days at 33°C.

In-Plant Chlorination Studies: Mortality responses of adult Asiatic clams during the spring and fall in-plant chlorination studies contrasted sharply from the spring to the fall

TABLE 1-1 Mean temperature, TRC, FRC (± SE, n = 30), and LT50 values for adult and juvenile Asiatic clams exposed to chlorine at ambient temperature, 5°, and 10°C increases for 30 days during the winter and summer temperature shock studies (n = 30 ± SE). Significant differences between LT50 values for clams exposed at ambient and increased temperatures are indicated by an asterisk (*). Significant differences in LT50 values between adults and juveniles are indicated by a dagger (†). Confident limits (95%) surrounding LT50 measurements are shown in brackets.

Temp. (°C)	TRC (mg/l)	FRC (mg/l)	ADULT LT50 (days)	JUVENILE LT50 (days)
Winter 1986				
7.0° ± .1	0.29 ± .01	0.24 ± .01	> 30	> 30
11.5° ± .2	0.30 ± .01	0.27 ± .01	> 30	> 30
16.8° ± .2	0.30 ± .01	0.22 ± .01	21.8 [20.6-23.1] *	20.9 [19.8-22.0] *
Summer 1986				
23.0° ± .1	0.29 ± .01	0.13 ± .02	23.3 [21.3-26.0]	14.4 [13.6-14.9] †
28.1° ± .1	0.28 ± .01	0.14 ± .01	25.2 [24.7-25.7]	13.8 [13.0-14.4] †
33.2° ± .1	0.26 ± .01	0.02 ± .01	5.6 [5.3-5.8] *	2.2 [2.0-2.4] * †

Figure 1-4. Percent mortality (n = 30) for adult (A) and juvenile (B) Asiatic clams exposed to 0.30 mg/l TRC at 23°, 28°, and 33° C for 30 days during the summer temperature shock study.


A
B
100
75
50
25
0
0
10
20
30
23°+Cl
28°+Cl
33°+Cl
PERCENT MORTALITY
TIME(DAYS)

(Figure 1-5). Despite the higher TRC concentration of the fall study, clam mortality was markedly reduced during the fall and reached a maximum of 3.3% compared to 100% during the spring study. LT50 values calculated for clams exposed during the spring ranged from 20.6 to 23.9 days and did not differ significantly between building location (Table 1-2). These LT50 values are comparable to LT50's generated for clams exposed at 23° C during the summer temperature shock study. LT50 values could not be calculated for clams chlorinated during the fall due to the extremely low mortality responses observed during that study. Exposure temperature differed greatly between the spring and fall studies with the mean spring exposure temperature (e.g. 26° C) being over twice the temperature recorded during the fall (e.g. 12.4° C). This two-fold difference in exposure temperature is thought to contribute greatly to the contrasting mortality responses observed between the spring and fall.

1.3.2. PHYSIOLOGY

Laboratory Studies: Dry weight condition indices of control and chlorinated clams following the winter and summer temperature shock studies showed similar temperature-dependent reductions in both studies (Table 1-3). The influence of temperature on clam tissue mass was significant in both chlorinated and control streams during the winter study. Specifically, significant reductions in body condition were evident at 17° for control clams and at 12°C for chlorinated clams. Clams chlorinated at 17°C experienced high mortality and too few specimens remained for an adequate determination of the dry weight condition index. The effect of chlorine on clam body condition at a given temperature was not significant at 7° but was nearly significant ($p < .07$) at 12°C. Results of body condition analyses following the summer study paralleled the winter study having shown significant reductions due to temperature only for control clams held at 33°C.

Figure 1-5. Percent mortality for adult Asiatic clams exposed to chlorine for 28 days during the spring (n = 40) and fall (n = 30) in-plant chlorination studies. Clams were exposed to 0.24, 0.23, and 0.17 mg/l TRC during the spring and 0.31, 0.30, and 0.40 mg/l TRC during the fall at buildings 1, 7 and 10, respectively. The beginning of the 4-day recovery period is indicated by a vertical dashed line.

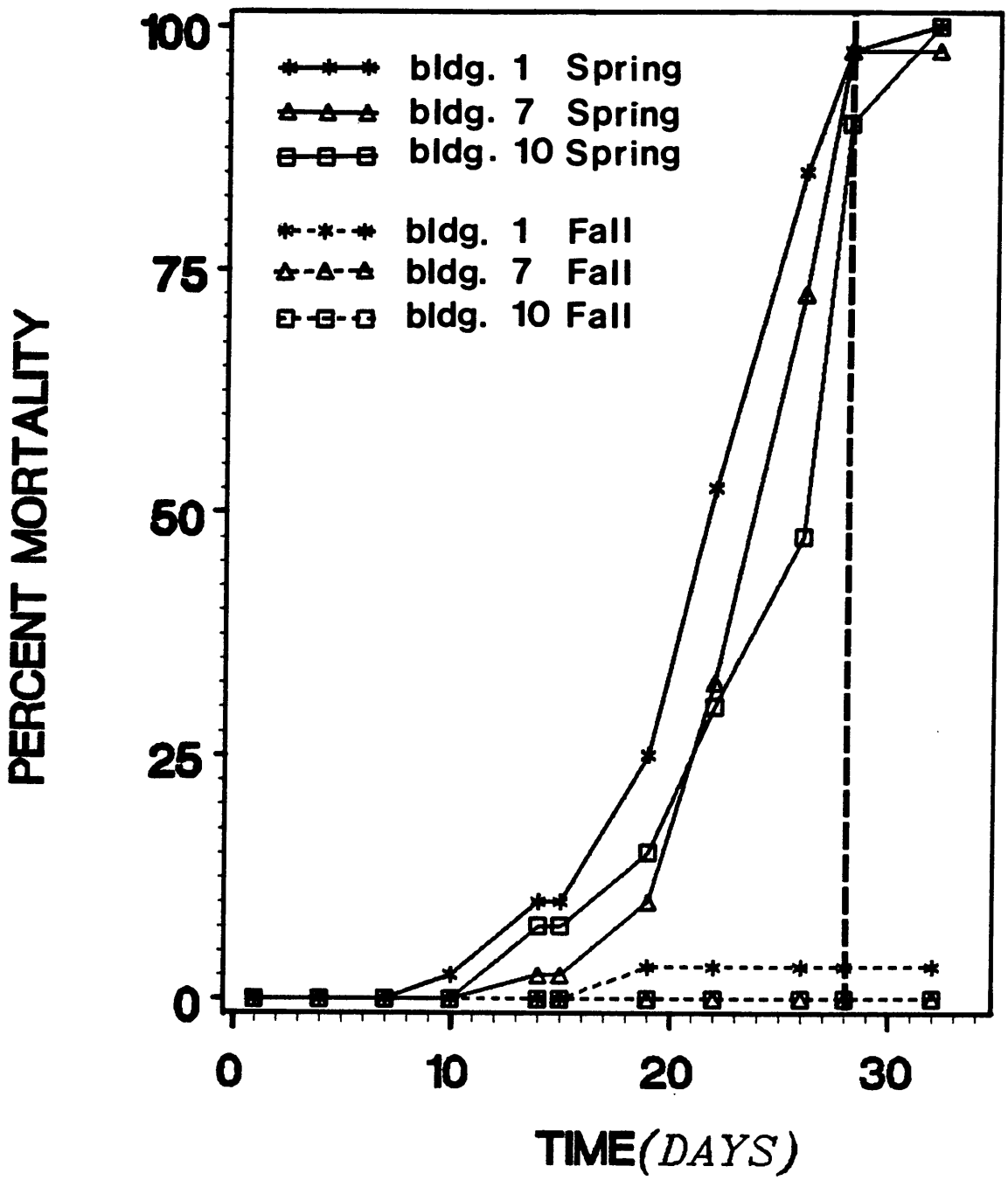

100
75
50
25
0
PERCENT MORTALITY
bldg. 1 Spring
bldg. 7 Spring
bldg. 10 Spring
bldg. 1 Fall
bldg. 7 Fall
bldg. 10 Fall
0
10
20
30
TIME(DAYS)

TABLE 1-2 Mean temperature, TRC[1], and LT50 values for adult Asiatic clams exposed to chlorine during the spring and fall in-plant chlorination studies. Percent mortality following 28 days of chlorination and 4 days of recovery is reported for the spring (n = 40) and fall (n = 30) studies. Confident limits (95%) surrounding LT50 measurements are shown in brackets.

Bldg. (#)	Temp. (°C)	TRC (mg/l)	Mort. (%)	LT50 (days)
Spring 1986				
1	25.9°	0.24	100.0	20.6 [18.9-22.3]
7	26.2°	0.23	97.5	22.5 [18.9-25.2]
10	26.1°	0.17	100.0	23.9 [18.6-28.7]
Fall 1986				
1	12.0°	0.31	3.3	> 30
7	12.2°	0.30	0.0	> 30
10	12.9°	0.40	0.0	> 30

[1] mean TRC concentrations were calculated by multiplying the proportion of time clams were chlorinated at particular chlorination rates (e.g. 250-500 lbs/day) by measured TRC concentrations at these rates. These products were then summed to yield the mean TRC concentrations reported.

TABLE 1-3. Mean dry weight index (± SE, n = 6) for control and chlorinated (0.30 mg/l TRC) adult Asiatic clams at ambient temperature, 5°, and 10°C increases during the winter and summer temperature shock studies. Means with different letters indicate significant differences between ambient and increased temperature clams within a treatment ($\alpha = .05$, Duncan's New Multiple Range Test or Studentized t-Test).

	Target Temp. (°C)	CONTROL Dry Wt. Index (mg/ml)	CHLORINATED Dry Wt. Index (mg/ml)
Winter 1986			
	7°	23.8 ± 0.5 A	25.3 ± 1.0 A
	12°	23.3 ± 1.0 A	20.3 ± 1.2 B
	17°	17.9 ± 0.8 B	(a)
Summer 1986			
	23°	31.6 ± 1.4 A	30.7 ± 0.9
	28°	28.4 ± 1.0 A	(a)
	33°	22.7 ± 0.8 B	(a)

(a) too few specimens (< 5) remained for adequate determination of the dry weight condition index.

Likewise, no significant chlorine effect was observed at the ambient, 23° exposed clams, while 100% mortality responses for adults exposed at 28° and 33°C resulted in no body condition analysis for clams at these treatments. Overall, clams tested during the summer had greater tissue mass relative to shell volume than clams tested during the winter, which suggests the "poorer" body condition of winter clams.

Analysis of clam glycogen content during the summer temperature shock study provided a much clearer picture of both temperature and chlorine influences on adult Asiatic clams (Figure 1-6). Specifically, the onset of significant reductions in glycogen content due to chlorine exposure occurred sooner with increasing temperature. For example, clams chlorinated at 33° experienced significant reductions in glycogen content compared to control 33°C clams by day 5. Significant reductions in the glycogen content of clams chlorinated at 28° and 23° C (relative to control 28° and 23° C clams) did not occur until day 15 and 25, respectively. Temperature influences on the glycogen content of chlorinated clams were evident only at 33° on day 5, at which clams experienced a 34% reduction in glycogen content compared to ambient (23° C) chlorinated clams (Table 1-4). By day 25, temperature influences on control clams were evident only at 33° C, as indicated by the 23% drop in glycogen relative to 23° C held clams.

Fall In-Plant Chlorination Study: Glycogen content analyses of adult Asiatic clams taken prior to and following the fall chlorination study revealed no significant reductions following 28 days of chlorination and 4 days of recovery at any of the plant locations tested (Table 1-5). These results are in accordance with the low (0-3.3%) mortality observed following this study. Overall, clam glycogen content was markedly reduced from summer levels with day 0 (fall) clams containing less than 50% of day 0 clams analyzed during the summer. This suggests that season and perhaps spawning activity influenced Asiatic clam glycogen content.

Figure 1-6. Mean glycogen concentration (% dry wt., ± SE) of control and chlorinated (0.30 mg/l TRC) Asiatic clams exposed to 23°, 28°, and 33° C for 30 days during the summer temperature shock study (n = 6). Significant reductions in the glycogen content of chlorinated clams from controls on a given day is indicated with an asterisk (*) (Studentized t-Test, $\alpha = .05$).

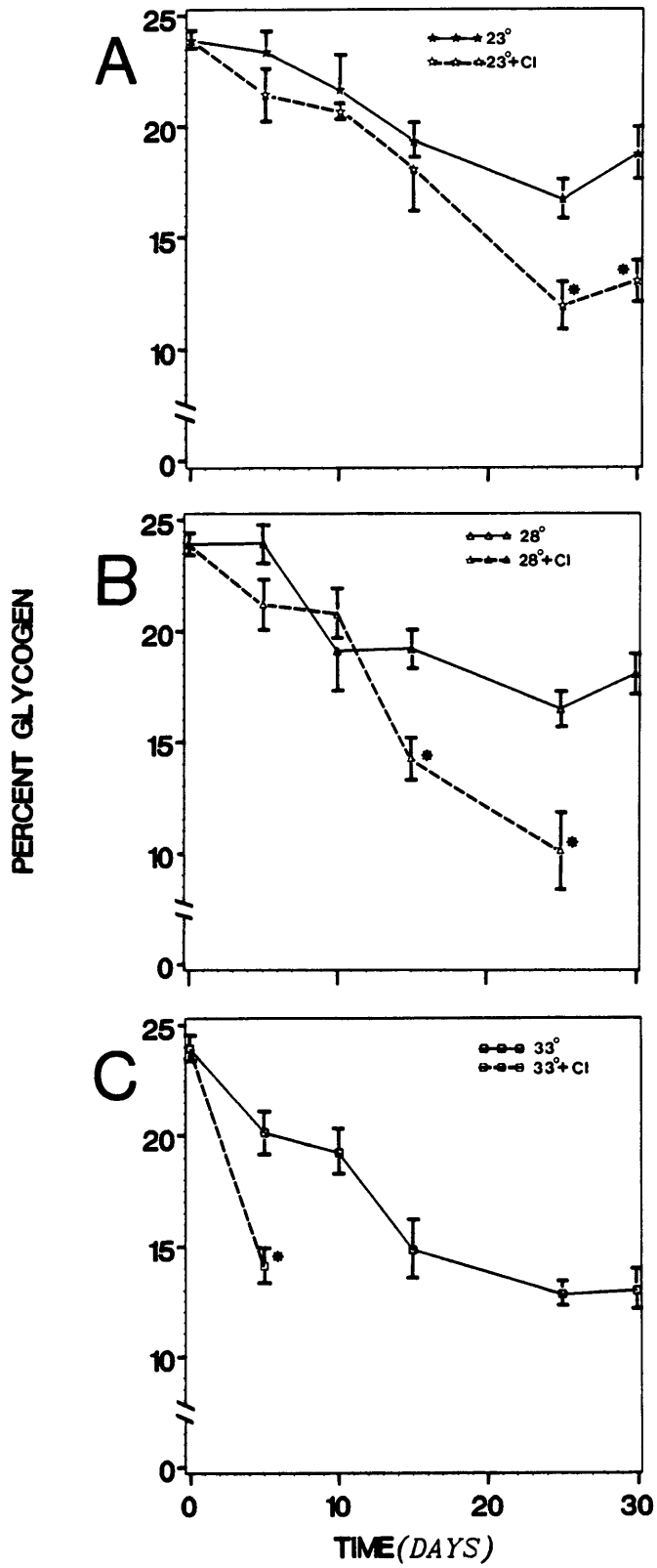

A
B
C
23°
23°+Cl
28°
28°+Cl
33°
33°+Cl
25
20
15
10
0
0
10
20
30
PERCENT GLYCOGEN
TIME (DAYS)

TABLE 1-4. Mean glycogen content (± SE, n = 6) for control and chlorinated (0.30 mg/l TRC) adult Asiatic clams at ambient temperature, 5°, and 10°C increases during the summer temperature shock study. Means with different letters indicate significant differences between ambient and increased temperature clams within a treatment ($\alpha = .05$, Duncan's New Multiple Range Test or Studentized t-Test). Significant differences between control and chlorinated clams (at a given temperature) are indicated by a double asterisk (**) ($\alpha = .05$, Studentized t-Test).

Day	Target Temp. (°C)	CONTROL Glycogen (% dry wt.)	CHLORINATED Glycogen (% dry wt.)
0	23°	23.9 ± 0.8	n/a
5	23°	23.4 ± 1.0	21.4 ± 1.2 A
	28°	24.0 ± 1.2	21.2 ± 1.1 A
	33°	20.1 ± 1.4	14.1 ± 1.0 B **
10	23°	21.7 ± 1.9	20.7 ± 0.8
	28°	19.1 ± 2.0	20.7 ± 1.0
	33°	19.2 ± 1.3	(a)
15	23°	19.4 ± 1.1	18.1 ± 2.1
	28°	19.2 ± 1.1	14.3 ± 1.3 **
	33°	14.8 ± 1.9	(a)
25	23°	16.7 ± 1.3 A	12.0 ± 1.0 **
	28°	16.5 ± 1.0 A	10.1 ± 1.9 **
	33°	12.8 ± 0.7 B	(a)
30	23°	18.8 ± 1.3 A	13.1 ± 1.2 **
	28°	18.0 ± 1.4 A	(a)
	33°	13.0 ± 1.6 B	(a)

(a) too few specimens (< 5) remained for adequate determination of clam glycogen content.

TABLE 1-5. Mean glycogen content (± SE, n = 6) for control (pumphouse) and chlorinated adult Asiatic clams prior to and following the fall in-plant chlorination study.

Day	TRC (mg/l)	Building (#)	Glycogen (% dry wt.)
0	control	pumphouse	10.3 ± 1.2
32	control	pumphouse	10.9 ± 0.8
32	0.31	1	10.6 ± 1.0
32	0.30	7	12.5 ± 1.1
32	0.40	10	11.1 ± 1.3

1.3.3 SIPHONING ACTIVITY

Winter Temperature Shock Study: The presence of chlorine at 0.30 mg/l significantly reduced clam siphoning activity to 0% at all temperatures for both adults and juveniles during the winter (Figure 1-7). Consequently, the effect of increased temperature on clam siphoning activity was seen only in the control streams, where juvenile siphoning activity increased nearly 2-fold to 69.9% and 67.0% at 12° and 17° C, respectively. No significant increase in adult siphoning activity was observed in response to temperature in the control streams.

Summer Temperature Shock Study: As observed during the winter, all clams exposed to 0.30 mg/l TRC during the summer showed significant reductions in siphoning activity approaching 0% (Figure 1-8). However, adult siphoning activity increased significantly to approximately 5% at 33° C , while juveniles chlorinated at 33° C surprisingly exhibited no significant increase in siphoning activity, despite their higher mortality at 33° C. Control adults of the summer study responded similarily to adults of the winter study by exhibiting no significant temperature-dependent increase in siphoning activity. Juveniles, however, showed no temperature-dependent increase in the control streams, which was contrary to the winter study. Regarding adult and juvenile comparisons, juvenile siphoning activity was approximately twice that of adults in the control streams at all temperatures tested. In addition, control adults showed little or no increase in siphoning activity from the winter to the summer, while juveniles experienced as high as a two-fold increase during the summer.

Figure 1-7. Mean Siphoning activity (percent siphoning, ± SE) of adult (A) and juvenile (B) Asiatic clams exposed to 0.30 mg/l TRC at 7°, 12°, and 17° C for 30 days during the winter temperature shock study (n = 30). Means with different letters (upper case = controls; lower case = chlorinated) are significantly different within a treatment (Duncan's Multiple Range Test, $\alpha = .05$). Significant differences between mean siphoning activity of control and chlorinated clams are indicated with a star.

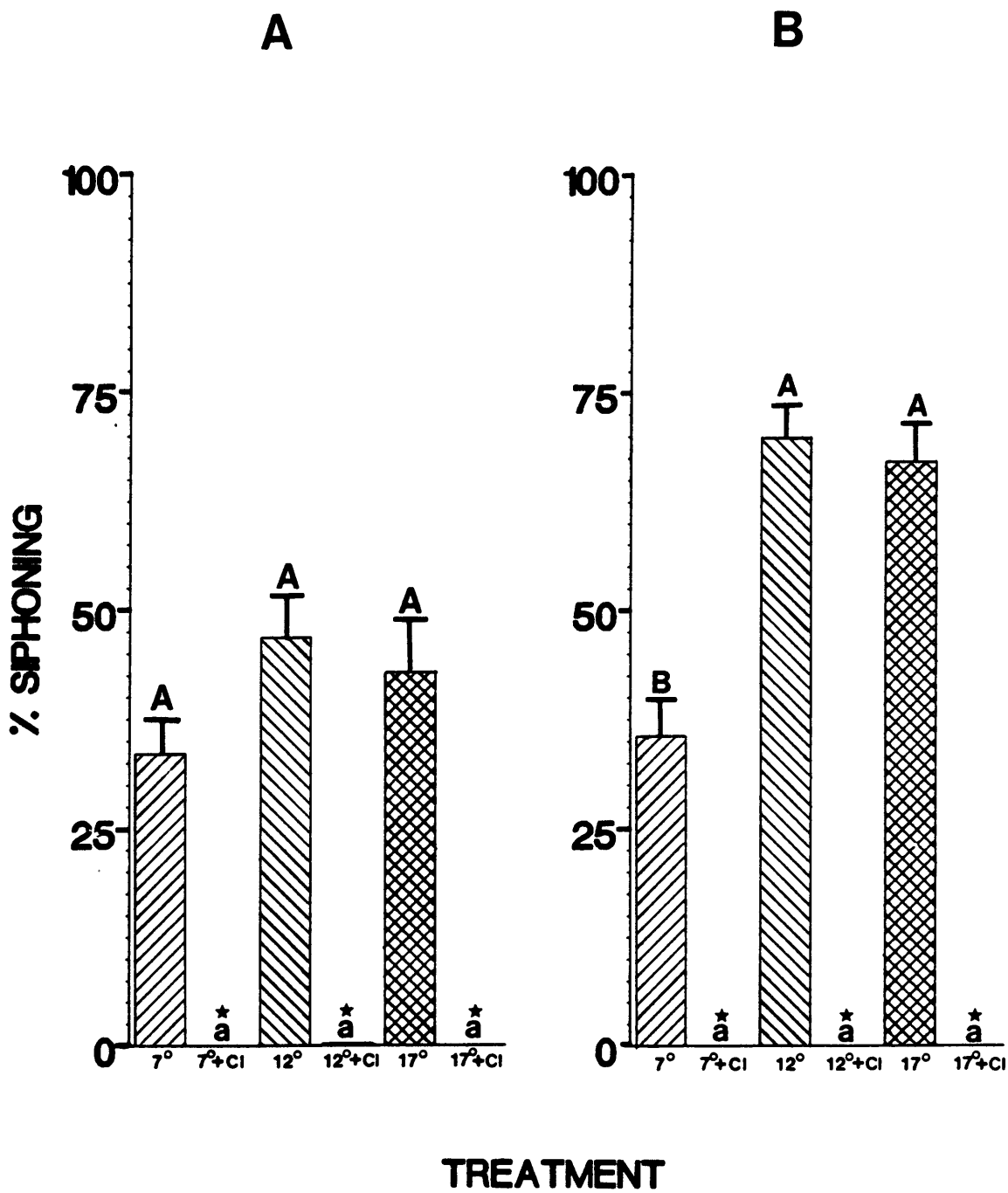
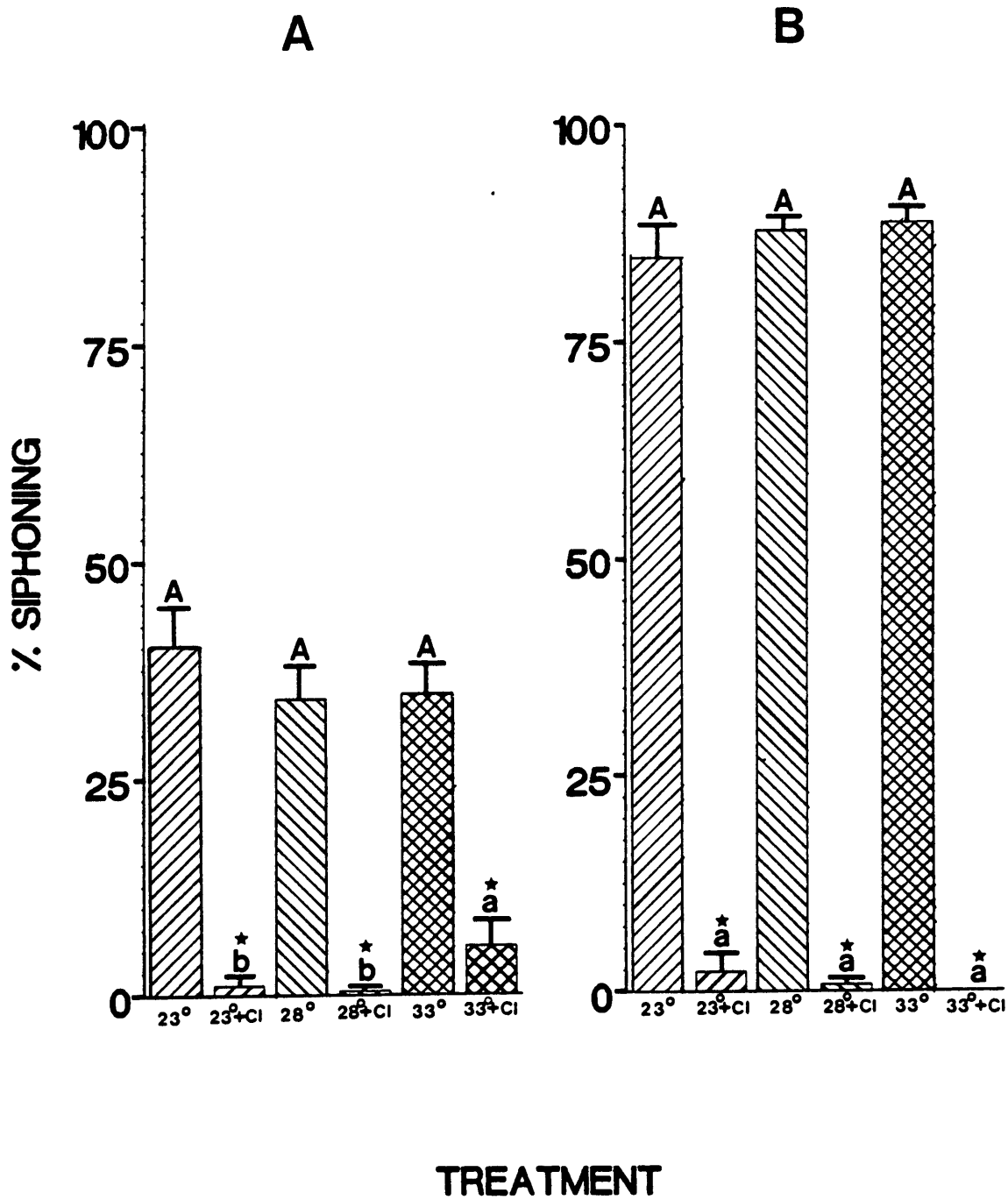

A
B
100
75
50
25
0
% SIPHONING
A
A
A
B
A
A
a
a
a
a
a
a
7°
7°+Cl
12°
12°+Cl
17°
17°+Cl
TREATMENT

Figure 1-8. Mean Siphoning activity (percent siphoning, ± SE) of adult (A) and juvenile (B) Asiatic clams exposed to 0.30 mg/l TRC at 23°, 28°, and 33° C for 30 days during the summer temperature shock study (n = 30). Means with different letters (upper case = controls; lower case = chlorinated) are significantly different within a treatment (Duncan's Multiple Range Test, $\alpha = .05$). Significant differences between mean siphoning activity of control and chlorinated clams are indicated with a star.


A
B
100
75
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25
0
% SIPHONING
A
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a
23°
23°+Cl
28°
28°+Cl
33°
33°+Cl
TREATMENT

1.4 DISCUSSION

1.4.1 LABORATORY STUDIES

Previous laboratory artificial stream studies which examined *Corbicula* survivorship responses to constant chlorination revealed that exposure to concentrations ranging from 0.29 to 1.02 mg/l at 20° C resulted in LT50's no less than 18.3 days (Doherty et al., 1986). It has been demonstrated in this study, with adult LT50's occurring as soon as 5.6 days, that both temperature and season can be exploited to significantly increase Asiatic clam suseptability to constant, "low level" (0.30 mg/l) chlorination. In the laboratory, it was found that temperature increases of at least 10° C were needed to significantly increase clam mortality during both the winter and summer. Despite the weakened body condition of clams during the winter, the most dramatic influence of increased temperature upon clam survivorship occurred during the summer, whereby adults and juveniles chlorinated at 33° C were completely eradicated within 7 and 3 days, respectively. Complete (100%) mortality was never attained for adults or juveniles chlorinated at identical temperature increases during the winter. Therefore, it seems that the overall higher exposure temperatures during the summer were more significant in increasing clam susceptibility to chlorine than the reduced body condition of clams during the winter. Likewise, size class became a significant factor contributing to the toxicity of chlorine to clams during the summer, with juveniles experiencing significantly higher mortality at all exposure temperatures tested. Control juveniles also experienced significantly higher siphoning activity than adults during the summer, which suggests that juveniles were responding to a greater metabolic demand than adults at summer temperatures. The inverse relationship between body size and metabolic rate which ex-

ists for many species has been clearly documented in *Corbicula* (Britton and Morton, 1982).

Despite the reported upper incipient lethal temperature for *Corbicula* of 34° C (Mattice and Dye, 1976), no adult or juvenile mortality occurred at 33° C. This is contrary to other reports in the literature of near 100% mortality of juveniles at 32° C (Foe and Knight, 1986a; Busch, 1974). However, numerous studies examining the optimal growth temperature of *Corbicula* have also differed greatly, with optimal growth temperature ranging from 20° (Foe and Knight, 1986a), to 25° (Mattice and Wright, 1986), and even significant growth reported at 36° C (McMahon and Williams, 1986). It can be expected that with such variation in optimal growth rates, which is attributed largely to zoogeographic variation in thermal life histories, differences in lethality between clams of different geographic locations at increased temperatures, would also occur. In a recent laboratory study, Cherry et al. (in preparation) reported clams inhabiting a thermal discharge area to be significantly more resistant to monochloramine exposures than thermally uninfluenced clams at increased temperatures. Clams collected for the winter and summer temperature shock studies were exposed in their natural habitat to temperatures ranging from approximately 2° during the winter to 25° C during the summer. Therefore, inferences based on these results to clams occupying differing thermal life histories is cautioned.

Analyses of the sublethal impacts of temperature and chlorine on *Corbicula* help clarify the causes of clam mortality previously discussed. Regarding body condition, adult clams exposed to chlorine experienced a significant loss of tissue mass relative to shell volume at 5° C increases during the winter. Lethality was too high at 10° increases during the winter and both 5° and 10° C increases during the summer for the dry weight index to be performed. Interestingly, at a given temperature, (where enough specimens survived), the effect of chlorine on tissue mass was neglegible despite near zero siphoning

activity of clams exposed during the winter and summer (Table 1-3, Figure 1-7, Figure 1-8). This may have been expected during the winter, since *Corbicula* has been reported to experience zero growth at temperatures below 12° C (Foe and Knight, 1986a; Joy, 1985). In addition, it has been reported that during anaerobosis, bivalves may utilize only 5% of the ATP required during aerobic metabolism (De Zwaan and Wijsman, 1976). Thus, differences between the tissue mass of control clams (which would presumably not have grown) and chlorinated clams would be minimal despite the lack of siphoning for clams in the presence of chlorine. However, at 23° C, which is near the optimal growth temperature for *Corbicula* (Foe and Knight, 1986a; Mattice and Wright, 1986), one would expect differences between siphoning (control) and nonsiphoning (chlorinated) clams within 30 days with respect to tissue mass. One possible explanation for the lack of significant tissue mass reduction is that the removal and subsequent analysis of living specimens following 30 days of chlorination selects for the remaining "hardy" clams, which in turn makes estimates of tissue mass loss due to chlorine conservative by nature. The relatively small sample size (e.g. $n = 6$) may also contribute to the lack of sensitivity of this index. Therefore, the use of the dry weight index, although sensitive to temperature influences, is not recommended for determining sublethal effects of chlorine on *Corbicula*.

Glycogen content was more sensitive to chlorine and temperature perturbations and consequently, provided a clearer picture of the effect of temperature and chlorine on Asiatic clam physiology. Since glycogen is the primary energy reserve in bivalves and comprises up to 30% of the dry weight in *Corbicula* (Cantelmo et al., 1985; Sappington, unpublished data), analysis of glycogen content should give some indication of the available energy remaining for metabolism and valve closure maintenance. During the summer, it was found that the time required for a significant reduction in glycogen content due to chlorine was reduced with increasing temperature (Figure 1-6). It is be-

lieved that because chlorinated clams experience severely reduced siphoning activity (Figure 1-8), reductions in glycogen content result from both decreases in food intake and increases in metabolic rate resulting from increased temperature. Therefore, clams chlorinated at higher temperatures may have experienced greater mortality due to a reduced ability to maintain valve closure resulting from a severe reduction in glycogen content. Siphoning activity data for adult clams chlorinated during the summer support this hypothesis with significant increases observed for adults chlorinated at 33° C, compared to those chlorinated at 23° and 28° C.

Another factor that is potentially important in influencing clam survivorship in the presence of chlorine may be the accumulation of ammonia within the mantle cavity. Ammonia accumulation has been reported in the mantle cavity of other freshwater and estuarine bivalves in response to aerial exposure (Akberali et al., 1977; De Vooys and De Zwaan, 1978) and has recently been documented in response to both air and chlorine exposure in *Corbicula* (Farris et al., in preparation). Ammonia exposure has been reported to cause gill ultrastructural damage in fish (Smart 1976; Redner and Stickney, 1979) and therefore, may result in direct toxicity to *Corbicula* or indirect toxicity through the increased chlorine exposure resulting from parted valves during ammonia excretion. Incidently, exposed mantle edges were observed by the author on numerous occasions preceding clam mortality, which may be indicative of ammonia excretion.

Significant reductions in glycogen content of control clams at increased temperatures were also observed (Table 1-4). These reductions may have resulted from temperature influences on algal assimilation efficiency and clam metabolic rate. For example, Foe and Knight (1986a) reported that the algal assimilation efficiency in *Corbicula* was reduced from 51% at 20° C to 13% at 30° C . At the same time, clam filtration rates increased from 3.85 ml/mg-hr at 20 ° to 13.39 ml/mg-hr at 30° C Thus, even though the feeding regime was doubled to 1000 mls/day/stream, clams may have

experienced significant losses of glycogen at 28° and 33° C due to a combination of decreased assimilation efficiences and increased filtration rates, and not necessarily food availability.

1.4.2. IN-PLANT CHLORINATION STUDIES

Clams exposed to chlorine in the field, (although not manipulated with respect to temperature increases), showed a similar temperature-dependent toxicity trend as observed in the laboratory. For example, of the 120 clams tested during the spring, all *died* except for one following 28 days of exposure to 0.17-0.24 mg/l TRC. In contrast, all but one of the 90 clams exposed to 0.30-0.40 mg/l TRC *survived* during the fall. These huge differences in mortality are thought to be due largely to a 2-fold reduction in water temperature which occurred from the spring to the fall. However, another perhaps important difference between the spring and fall study was the differing gravid condition of the the clams tested. For example, Cherry (1987) reported the gravid condition of clams during the peak spring spawning period to be 60% gorged (i.e. > 300 pediveligers per gill). Clams tested during the fall were reported to be 5% gorged at their peak spawning period. Therefore, clams chlorinated during the spring may have experienced higher mortality as a result of their greater burden of incubating larvae. Significant reductions in mantle glycogen content have been associated with gametogenesis in the Sea Mussel, *Mytilus edulis* (Bayne et al., 1982) and may also occur during gametogenesis and larval incubation in *Corbicula.* In a recent study, Cantelmo et al. (1985) attributed reductions in total body glycogen content of *Corbicula* during the spring and fall to gametogenesis and larval incubation, although no conclusive evidence was reported.

In summary then, it has been demonstrated in this study that exposure temperature can be exploited to significantly increase the susceptibility of the Asiatic clam to con-

tinuous chlorination control procedures. Regarding sublethal effects, it was found that increased temperatures significantly reduced the tissue mass, and perhaps more importantly, the glycogen content of chlorinated and control clams. In addition, chlorinated adults responded with significantly higher siphoning activity at 33° C during the summer, which is indicative of the increased metabolic demand being placed on the organism. Regarding toxicological responses, it was found that a temperature increase of 10° C during the summer resulted in the greatest mortality responses from chlorinated adults and juveniles. Whether industries could maintain thermal increases of 10° C during the summer and a TRC level of 0.30 mg/l in their cooling lines upwards to a week is questionable. The feasability of such an operation depends greatly on the plant design, plant size, and the economic costs of reduced cooling efficiency experienced during the thermal control procedure. In addition, this control procedure is not designed to prevent *Corbicula* invasion into an industry's cooling water system. However, if such an operation was economically feasible, it could be used effectively to kill a resident population of Asiatic clams at reduced chlorine levels and may reduce, or even eliminate, the need for dewatering and manual removal of such a population from the cooling lines.

2.0 MONOCHLORAMINE AND AMMONIA

2.1 INTRODUCTION

The economic impacts resulting from Asiatic clam fouling of industrial cooling water systems currently is estimated to total over 1 billion dollars annually in the United States alone (Isom, 1986). From this cost, it is not surprising that much research has been directed towards methods of controlling *Corbicula* fouling. Some studies have focused on the development of retaining screens and self-cleaning filters designed to trap *Corbicula* larvae in effort to prevent clam intrusion into, and subsequent colonization of, cooling water systems (Goss et al., 1979; Mussalli et al., 1986). However, the use of screens with mesh sizes < 0.2 mm (i.e. the average shell length of *Corbicula* larvae) may not be feasible for industries which require rapid flow at their intake sources (Mussalli et al., 1986). The majority of studies addressing *Corbicula* control have focused on physical and chemical methods designed to eradicate clam populations already established in cooling water systems. Physical control methods consist of thermal backflushing or more commonly, condensor dewatering and manual removal, both of which may

be costly from decreases in cooling efficiency or entire plant shutdowns. Chemical control procedures include the use of chlorine (Cherry et al., 1986; Isom et al., 1986), tributyl tin fluoride (Mussalli et al., 1986), triphenyl lead acetate (Goss et. al., 1979), and bromine (Doherty et al., 1986), all of which have achieved varying degrees of use.

The selection, and the extent of application of a particular chemical control method is often limited by several factors which include: the size and design of the industrial plant; the toxicological and physical properties of the biocide; and the regulations surrounding release of such a compound into the environment. Regarding plant design, industries that employ "once through" cooling water systems are often at a disadvantage to those that use "closed loop" systems because of the loss in cooling water retention time usually needed for the dissipation or pretreatment of compounds prior to their release into the environment. Important physical and toxicological properties that influence the use of a biocide include its volatilization rate, water solubility, corrosive potential, toxicity to *Corbicula* and other aquatic life, and its ability to accumulate in the aquatic biota. Ultimately, however, it is the environmental regulations governing the release of a compound into natural waters which determine its use. For example, continuous chlorination of cooling water for three to four weeks before, during, and/or after clam spawning periods has been used effectively for preventing clam fouling and is currently recommended for Asiatic clam control in power plants (Isom et al., 1986; Cherry et al., 1986). Although chlorine rapidly dissipates in water and has no known bioaccumulative properties, its high toxicity to aquatic life has prompted EPA restrictions on the level, duration, and dechlorination of chlorine discharges. This has complicated the use of continuous chlorination as an economically feasible control method (USEPA, 1974; 1985a; Cherry, pers. comm.). Therefore, the need to identify compounds that are toxic to Asiatic clams at an environmentally acceptable concentration becomes clear.

One factor which greatly influences the amount of time and money needed to effectively control *Corbicula* is its ability to detect and subsequently avoid toxicants by maintaining extended periods of valve closure. This valve closure response has been documented extensively for clams exposed to various compounds which include chlorine, copper, cadmium, zinc, bromine, and even asbestos (Doherty, 1986; Sappington et al., 1986; 1987; Belanger et al., 1986a; 1986b). Specifically, it has been demonstrated that because of *Corbicula's* valve closure behavior, initial clam mortality often occurs after two weeks of exposure to chlorine (Doherty et al., 1986; Sappington et al., 1986). In fact, this valve closure response is so effective that, depending on relative humidity, adult clams can withstand up to 27 days of exposure to air (McMahon, 1979). Therefore, biocides that are relatively undetectable to *Corbicula* may be useful as an alternative to chlorine for controlling Asiatic clam fouling.

Ammonia may be such a candidate for controlling *Corbicula.* Ammonia is highly water soluble, dissipates rapidly in water, has no known bioaccumulative properties, and perhaps most importantly, may be relatively undetectable by *Corbicula.* Evidence of the low detectability of ammonia by *Corbicula* was obtained from Union Carbide's Seadrift, Texas plant when an ammonia spill killed nearly all of the resident clams within 3 days. It seems plausible that such immediate toxicity, which is uncharacteristic of typical chlorination control procedures, may have resulted from the inability of *Corbicula* to detect, and therefore avoid, ammonia. Unfortunately, the concentration of ammonia in the cooling system was not known and estimates are speculative at best. Of the few studies examining ammonia toxicity to *Corbicula*, Horne and McIntosh (1979) reported that 38% of the adults died following 7 days of exposure to 5.0 mg/l total ammonia. Based on pH and temperature data from their paper, this concentration represents approximately 0.26 mg/l un-ionized ammonia (NH_3), which is considered to be over 50 times more toxic that the ionized (NH_4^+) form (Armstrong et al., 1978). Although a

daily representation of clam mortality was not reported, the final mortality (38%) is still generally higher than mortalities observed following 7 days of exposure to comparable concentrations of chlorine. This further supports the hypothesized inability of *Corbicula* to detect ammonia.

Another compound which may be effective in controlling *Corbicula* is monochloramine (NH_2Cl). The formation of monochloramine occurs readily in the environment when water containing ammonia (or other organics) is chlorinated in the following manner:

$$NH_3 + HOCl \rightarrow NH_2Cl + H_2O$$

Thus, monochloramine (and other chloramines) are classifed as *combined residual chlorine* while hypochlorous acid (HOCl) and the hypochlorite ion (OCl^-), which are considered to be more toxic, are termed *free residual chlorine*. Together, combined residual chlorine (CRC) and free residual chlorine (FRC) comprise what is termed *total residual chlorine* (TRC). Evidence supporting the biocidal properties of monochloramine for Asiatic clam control stem from observations of greater clam mortality during in-plant chlorination procedures, (where monochloramine was a major TRC constituent), than that observed during laboratory chlorination procedures, (where monochloramine was a minor TRC constituent). Specifically, it was observed that 0.25 mg/l TRC was needed during in-plant control procedures to eradicate 90% of adult Asiatic clams in 30 days, while 0.65 mg/l TRC was needed to acheive comparable mortalities in the laboratory (Doherty et al., 1986). It is thought that the higher mortalities observed in the field may be due to a reduced ability of clams to detect monochloramine and remain closed in its presence.

Therefore, the primary goal of the research presented in this chapter was to determine the potential of ammonia and monochloramine as alternatives to chlorine for Asiatic clam control. It was hypothesized that *Corbicula* would be less able to detect

ammonia and monochloramine than chlorine, and therefore, toxicity would occur sooner in the presence of monochloramine and ammonia, compared to chlorine under similar test conditions. The toxicological aspect of this hypothesis was addressed during the fall monochloramine-ammonia study, whereby adult and juvenile mortalities were compared in the presence of monochloramine and ammonia at exposure concentrations comparable to those used during previous chlorination studies. In subsequent studies, the siphoning activity of *Corbicula* was also monitored in the presence of monochloramine and ammonia in order to document the ability of *Corbicula* to detect these compounds. In addition to characterizing *Corbicula's* toxicological and behavioral responses to monochloramine and ammonia, sublethal responses (e.g. glycogen and tissue water content) of *Corbicula* also were examined. It was hypothesized that reductions in glycogen content would be less severe in the presence of monochloramine and ammonia, than observed previously in the presence of chlorine, because clams presumably would remain open and therefore, would be feeding. Tissue water content was examined because it is indicative of osmoregulatory function, which may be affected since gill ultrastructural damage has been reported in fish exposed to ammonia and monochloramine (Smart, 1976; Redner and Stickney, 1979).

2.2 METHODS

2.2.1 FALL MONOCHLORAMINE-AMMONIA STUDY

Adult (14-17mm SL) and juvenile (5-8mm SL) Asiatic clams, used to document the toxicological responses of *Corbicula* to ammonia and monochloramine, were collected from the New River at Narrows, VA and placed in constant circulating artificial streams

for 2 days at the collection temperature of 20° C (Figure 1-1). Groups of 30 adults and juveniles were then placed for 30 days into nylon baskets in a series of 3 streams designated as control, 0.25 mg/l monochloramine (NH_2Cl), and 0.25 mg/l un-ionized ammonia (NH_3) streams. Clams were maintained on a daily feeding regime consisting of the addition of 300 mls of tri-algal stock (*Chlamydomonas rheinhardti* + *Chlorella vulgaris* + *Ankistrodesmus sp.*) to each stream. Stock solutions of NH_2Cl were prepared in 20-l carboys by dissolving $Ca(OCl)_2$ and NH_4Cl at a 1.1:1 molar ratio of chlorine to nitrogen in dechlorinated tap water and adjusting pH to 8.3 with NaOH. Stock solutions of ammonia were prepared in 20-l glass carboys by dissolving NH_4Cl in dechlorinated tap water. Both stock solutions were delivered at a rate of 6 mls/min. via peristaltic pumps while diluent (dechlorinated tap water) was delivered at 15 mls/min. These flow rates maintained approximately 1.75 overturns (i.e. 30 l) a day.

Standard water chemistry (pH, alkalinity, hardness, conductivity) was measured prior to and following the study and deviated little within a treatment over time (Appendix A). Stream temperature, pH, and clam mortality were measured once daily for all streams, while TRC, FRC, and monochloramine were measured a minimum of twice daily in the monochloramine stream with a Wallace and Tiernan amperometric titrator accurate to ± 0.02 mg/l. Samples for ammonia analysis taken every other day from all streams were preserved with concentrated H_2SO_4 until analysis by the Phenate method for total ammonia determination (APHA et al., 1980). The un-ionized species of ammonia (NH_3) was calculated for each sample by multiplying the total ammonia concentration by the percent of un-ionized ammonia present under the appropriate temperature and pH conditions (USEPA, 1979). For the purposes of this study, un-ionized ammonia will be expressed as mg/l NH_3 and total ammonia expressed as mg/l NH_3-N.

2.2.2 WINTER AMMONIA STUDY

Clams used to test the effect of ammonia on the toxicological, physiological, and siphoning responses of *Corbicula* during the winter, 1987 were collected from cooling water ponds of Union Carbide Corporation's Seadrift, TX plant at a mean water temperature approximating 20° C. Clams were transported in polyethylene bags (without water) to the Ecosystem Simulation Laboratory and placed in artificial streams at 20° C within 48 hours. Following a 3-day acclimation period, two groups of adult clams (18-24 mm SL, n = 20) were placed in nylon mesh baskets and distributed into a control and a 0.50 mg/l un-ionized ammonia stream for 30 days. One basket in each stream contained clams to be used for mortality and siphoning activity monitoring; the other basket contained clams to be used for glycogen and tissue water analyses. The dosing of ammonia, standard water chemistry (Appendix A), and the feeding regime were conducted as described previously for the ammonia portion of the fall monochloramine-ammonia study. Stream water samples for ammonia determination for this and all subsequent studies were preserved by freezing at < -10° C and analyzed as previously described within 96 hours. Clam mortality and percent of clams siphoning were recorded once daily, while glycogen and tissue water content were determined on days 0 and 30 for control and ammonia exposed clams (n = 6) as described for the summer heat shock study.

2.2.3 SPRING MONOCHLORAMINE-AMMONIA STUDY

Adult (15-18 mm SL) Asiatic clams used to document the toxicological, physiological, and siphoning responses to "high level" monochloramine and ammonia exposures during the spring, 1987 were collected from a heated zone of the New River at Glen Lyn, VA (2 week mean water temperature of 17.6° C). Clams were immediately transfered to artificial streams at the Ecosystem Simulation Laboratory and acclimated to 20° C over a period of 2 days. Clams (n = 20) were divided into two groups of nylon baskets; of

which one was designated for the monitoring of mortality and siphoning activity, and the other designated for the determination of glycogen and tissue water content. One basket from each group was placed into a control, 0.75 mg/l NH_2Cl, and 0.50 mg/l NH_3 stream. Toxicant preparation and dosing strateies were identical to those followed for the fall monochloramine-ammonia study. All other experimental protocols (e.g. standard water chemistry, toxicant delivery, etc.) were identical to the fall study, except for the deletion of the adjustment of the monochloramine stream to pH 8.3 and the H_2SO_4 preservation of ammonia samples. Clam mortality and percent siphoning were recorded once daily, while samples (n = 6) for glycogen and tissue water analysis were taken on days 0, 4 and 7, and assayed as described for the summer heat shock study.

2.2.4 STATISTICAL ANALYSES

The lethal time for 50% mortality (LT50) was calculated when applicable using Probit analysis (SAS, 1985) and significant differences determined between LT50's with non-overlapping confidence limits. Tissue water (percent wet weight), glycogen content (percent dry weight), and siphoning activity (percent siphoning) were transformed prior to analysis by using the arcsine-square root transformation to remove non-normality which may have occurred from their expression on a percent scale. The transformed data were then analyzed using a one-way ANOVA procedure, and significant differences were determined for analysis of variance procedures with p-values $< .05$ using Duncan's New Multiple Range Test or by the Studentized t-Test.

2.3 RESULTS

2.3.1 MORTALITY

Fall Monochloramine-Ammonia Study: Examination of the mortality of adult and juvenile Asiatic clams exposed to monochloramine, ammonia, and chlorine (with high FRC during the summer temperature shock study) revealed varying degrees of toxicity of these compounds at comparable concentrations and experimental conditions (Figure 2-1, Table 2-1). For example, the onset of adult mortality occurred on days 9, 11, and 22 in the monochloramine, chlorine, and ammonia exposed streams, respectively. Subsequently, during the first three weeks of exposure, mortality was highest for adults exposed to chlorine, followed by monochloramine and lastly, ammonia, with 0% mortality by day 21. During the last week of exposure, adult mortality was generally highest in the presence of monochloramine and reached a maximum of 93%, followed by chlorine and ammonia, with maximum mortalities of 67% and 6.7%, respectively. Adult LT50 values indicate no significant difference between monochloramine and chlorine exposed adults, while LT50 values for adult clams exposed to ammonia could not be calculated due to their low (< 7%) mortality at the conclusion of the test. Control mortality did not occur during any of the experiments and, therefore, was not included in Figure 2-1 or Table 2-1.

Overall, juveniles were more sensitive than adults, as indicated by their quicker onset of mortality which occurred on days 2, 4, and 4 in the presence of chlorine, monochloramine, and ammonia, respectively (Figure 2-1 B). Likewise, juvenile LT50 values were significantly lower than adult LT50 s at all treatments (Table 2-1). Furthermore, during the first two weeks of exposure, juvenile mortalities in the

Figure 2-1. Comparison of mortality responses of adult (A) and juvenile (B) Asiatic clams (n = 30) exposed to monochloramine and ammonia at 20° C (during the fall monochloramine-ammonia study) and chlorine at 23° C (during the summer temperature shock study) for 30 days.

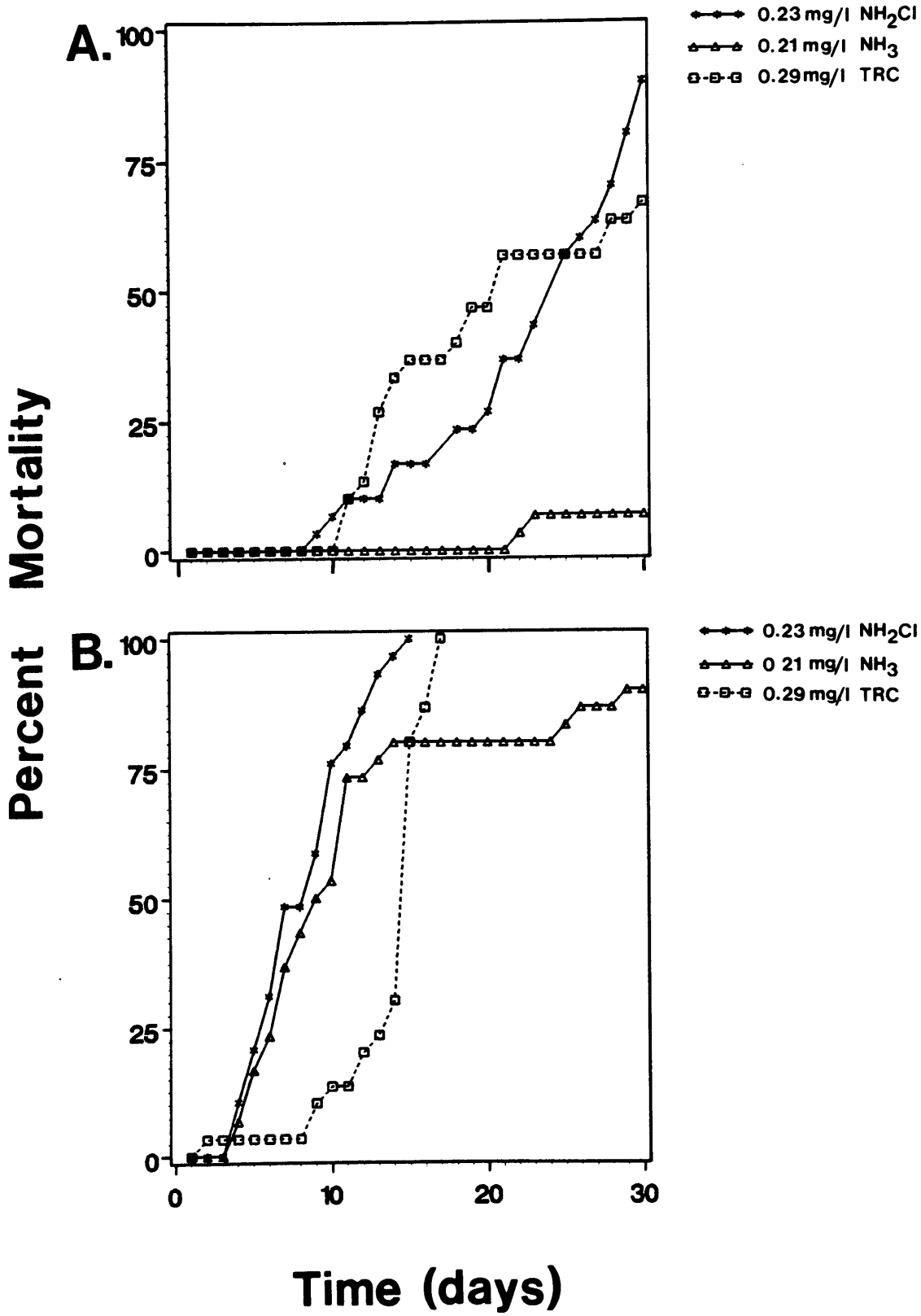

A.
B.
Percent Mortality
Time (days)
0.23 mg/l NH2Cl
0.21 mg/l NH3
0.29 mg/l TRC
0.23 mg/l NH2Cl
0 21 mg/l NH3
0.29 mg/l TRC
0
25
50
75
100
0
10
20
30

TABLE 2-1. Comparison of LT50 values (lethal time for the onset of 50% mortality) of adult and juvenile Asiatic clams collected from Narrows, VA and exposed to monochloramine, ammonia, and chlorine (with a high FRC) for 30 days at comparable exposure concentrations and temperatures. Total and un-ionized ammonia are represented by NH_3-N and NH_3, respectively. Confident limits (95%) surrounding LT50 measurements are shown in brackets.

Toxicant	Conc. (mg/l)	Temp. (°C)	pH	ADULT LT50 (days)	JUVENILE LT50 (days)
NH_2Cl[1]	0.23 ± 0.01	19.0° ± 0.2	8.29 ± 0.02	23.1 [21.9-24.4]	7.4 [6.9-7.9]
NH_3 (NH_3-N)	0.211 ± 0.003 (4.68 ± 0.07)	21.1° ± 0.2	8.07 ± 0.01	> 28	8.9 [7.7-9.9]
TRC[2]	0.29 ± 0.01	23.0° ± 0.1	8.06 ± 0.02	23.3 (21.3-26.0)	14.4 [13.6-14.9]

[1] The overall TRC was 0.27 ± 0.02 mg/l and FRC < 0.01 mg/l, respectively.

[2] The measured FRC was 0.13 ± 0.02 mg/l.

monochloramine and ammonia streams were nearly identical and occurred sooner (as hypothesized) than mortality observed for juveniles exposed to chlorine. Likewise, juvenile LT50 values (e.g. 7.4 and 8.9 days) were not significantly different in the presence of monochloramine and ammonia, respectively, but were both significantly lower than the 14.4 day LT50 calculated for chlorine exposed juveniles.

Winter Ammonia Study: Adult clams collected from Texas exhibited no mortality in the presence of 0.29 mg/l NH_3 for 30 days (Table 2-2). The mean pH in both treatments was considerably lower than the pH during the fall study (e.g. 7.57 vs. 8.07) and, therefore, much more total ammonia (e.g. 16.5 mg/l NH_3-N) was needed to achieve the target (0.50 mg/l NH_3) concentration. Despite the high total ammonia concentration, only a mean concentration of 0.29 mg/l un-ionized ammonia was reached.

Spring Monochloramine-Ammonia Study: Adult (Glen Lyn) clams exposed to 0.73 mg/l NH_2Cl and 0.54 mg/l NH_3 experienced mortality so severe that the remaining clams designated for the monitoring of mortality had to be used for the physiological (glycogen and tissue water) analyses (Table 2-3). Clams exposed to 0.54 mg/l NH_3 experienced mortality sooner and in greater magnitude than clams exposed to 0.73 mg/l NH_2Cl. Values of LT50 s calculated for clams exposed to ammonia and monochloramine (Table 2-4) occured at 3.6 and 6.5 days, respectively. Because significant mortality ($> 3\%$) occurred only on two days in both treatments, LT50 confidence limits could not be calculated using Probit analysis. Therefore, significant differences between LT50 values of clams exposed to ammonia and monochloramine could not be determined. It should be noted that because the pH was relatively low in the ammonia-dosed stream (e.g. 7.46), a massive dose of total ammonia (e.g. 47.63 mg/l) was needed to achieve the desired concentration of 0.50 mg/l un-ionized ammonia (Table 2-4). Also evident from

TABLE 2-2. Mean water temperature, pH (± SE, n = 30) and percent mortality (n = 30) for adult Asiatic clams collected from Seadrift, TX and exposed to ammonia, (mean ± SE, n = 15) in laboratory artificial streams. The mean concentrations of total and un-ionized ammonia are represented by NH_3-N and NH_3, respectively.

Treatment	Conc. (mg/l)	Temp. (°C)	pH	Mortality (%)
control	BDL[1]	20.2° ± 0.1	7.60 ± 0.07	0.0
NH_3 (NH_3-N)	0.29 ± 0.04 (16.50 ± 1.09)	20.2° ± 0.1	7.57 ± 0.05	0.0

[1] below detectable limit.

TABLE 2-3. Cumulative percent mortality (n = 20) for adult Asiatic clams collected from Glen Lyn, VA and exposed to monochloramine and ammonia at 20° C in laboratory artificial streams. Ammonia is expressed as mg/l un-ionized ammonia (NH_3).

Day	NH_2Cl (0.73 ± .09 mg/l) Mortality (%)	NH_3 (0.54 ± .03 mg/l) Mortality (%)
1	0	0
2	0	0
3	0	20
4	0	70 †
5	0	
6	20	
7	75 †	

† Test for mortality was suspended in order to sacrifice remaining clams for glycogen and tissue water analyses.

TABLE 2-4. LT50 values (lethal time for the onset of 50% mortality) for adult Asiatic clams collected from Glen Lyn, VA and exposed to monochloramine (mean ± SE, n = 7) or ammonia (mean ± SE, n = 4) in laboratory artificial streams. The mean concentrations of total and un-ionized ammonia are represented by NH_3-N and NH_3, respectively.

Treatment	Conc. (mg/l)	Temp. (°C)	pH	LT50 (days)
control	BDL[1]	20.1° ± 0.3	7.70 ± 0.06	n/a[3]
NH_2Cl[2]	0.73 ± 0.09	19.8° ± 0.2	7.60 ± 0.05	6.5
(NH_3-N)	(0.60 ± 0.14)			
(NH_3)	(0.007 ± 0.001)			
NH_3	0.54 ± 0.03	20.3° ± 0.1	7.46 ± 0.02	3.6
(NH_3-N)	(47.63 ± 2.37)			

[1] below detectable limit.

[2] The mean TRC and FRC were 0.94 ± 0.09 mg/l and 0.17 ± 0.04 mg/l, respectively.

[3] Not applicable.

Table 2-4 is the presence of 0.60 mg/l total ammonia in the monochloramine stream. This may have been due to the accumulation of ammonia from numerous clam mortalities, or the decay of monochloramine to ammonia and hypochlorous acid.

2.3.2 PHYSIOLOGY

Winter Ammonia Study: Adult (Texas) clams exposed to 0.29 mg/l NH_3 for 30 days experienced slight, but significant, reductions in both glycogen and tissue water content (Table 2-5). Specifically, clams exposed to ammonia responded with a 2.2% dry weight loss of glycogen compared to control clams after 30 days. No significant difference in the glycogen content of control clams occurred following 30 days in the laboratory, which may indicate the adequacy of the tri-algal feeding regime for rearing *Corbicula.* Tissue water content of clams exposed to ammonia dropped significantly from controls (e.g. 1.3% wet weight) by day 30. However, it should be noted that the tissue water content of control clams increased significantly after 30 days in the laboratory, and therefore, the drop in tissue water observed for ammonia-exposed clams may only be a function of the increase in tissue water of control clams.

Spring Monochloramine-Ammonia Study: Examination of the physiological responses of adult (Glen Lyn) clams exposed to 0.73 mg/l monochloramine and 0.54 mg/l un-ionized ammonia during the spring indicates that clam glycogen content was significantly reduced in both treatments in just 4 days (Table 2-6). Specifically, the glycogen content of clams exposed to monochloramine and ammonia dropped to 75% and 69% of control clams by day 4, respectively, and comprised 87% of the controls for monochloramine exposed clams by day 7. This apparent increase in glycogen content of monochloramine-exposed clams when expressed as a percentage of controls on day 7 is

TABLE 2-5. Mean (± SE, n = 6) glycogen and tissue water content for adult Asiatic clams collected from Seadrift, TX and exposed to ammonia (mean un-ionized, ± SE, n = 15) for 30 days at 20° C in laboratory artificial streams. Means with different letters are significantly different (Duncan's New Multiple Range Test, $\alpha = .05$).

Day	Treatment	Conc. (mg/l)	Glycogen (% Dry Wt.)	Tissue Water (% Wet Wt.)
0	**control**	BDL[1]	28.9 ± 1.5 AB	79.9 ± 0.5 B
30	**control**	BDL	30.7 ± 0.5 A	81.6 ± 0.3 A
30	NH_3[2]	0.29 ± 0.04	28.5 ± 0.5 B	80.3 ± 0.2 B

[1] below detectable limit.

[2] The measured total ammonia was 16.50 ± 1.09 mg/l.

TABLE 2-6. Mean ($\pm$SE, n=6) glycogen and tissue water content for adult Asiatic clams collected from Glen Lyn, VA and exposed to monochloramine[1] (0.73 $\pm$ .09 mg/l) and ammonia[2] (0.54 $\pm$ .03 mg/l un-ionized) for 7 days at 20° C in laboratory artificial streams. Means with different letters on a given day are significantly different (Duncan's Multiple Range Test or Studentized t-Test, α=.05). Significant differences in control clams from day 0 are indicated with an asterisk (*).

Day	Treatment	Glycogen (% Dry Wt.)	Tissue Water (% Wet Wt.)
0	control	35.4 $\pm$ 1.1	83.6 $\pm$ 0.3
4	control	29.7 $\pm$ 2.2 A*	83.2 $\pm$ 0.3
4	NH_2Cl	22.3 $\pm$ 0.5 B	82.5 $\pm$ 0.5
4	NH_3	20.4 $\pm$ 1.2 B	81.9 $\pm$ 0.8
7	control	23.2 $\pm$ 0.6 A*	83.4 $\pm$ 0.4
7	NH_2Cl	20.4 $\pm$ 0.9 B	82.7 $\pm$ 1.0
7	NH_3	(a)	(a)

[1] The measured TRC and FRC were 0.94 $\pm$ 0.09 mg/l and 0.17 $\pm$ 0.04 mg/l, respectively. The measured total and un-ionized ammonia were 0.60 $\pm$ 0.14 mg/l and $<$0.01 mg/l, respectively.

[2] The measured total was 47.63 $\pm$ 2.37 mg/l.

(a) No clams survived to this day for the determination of glycogen and tissue water content.

actually due to the reduction in control clam glycogen content by day 7. Clam tissue water content was reduced to near significance ($p < .06$) in the presence of ammonia but was not significantly affected ($p < .61$) in the presence of monochloramine by day 4. No significant difference in tissue water content of control and monochloramine exposed clams was evident by day 7.

2.3.3 SIPHONING ACTIVITY

Winter Ammonia Study: Significant reductions in siphoning activity did occur following 30 days of exposure to 0.29 mg/l NH_3 (Table 2-7). Although the siphoning activity of ammonia exposed clams was reduced to 48% of the controls, this reduction is not nearly as severe as the 95% reduction in siphoning activity observed for clams previously exposed to chlorine (Figure 1-7).

Spring Monochloramine-Ammonia Study: Clams from Glen Lyn clams experienced significant reductions in siphoning activity to 35% of control clams during exposure to 0.54 mg/l NH_3 (Table 2-7). During this study, clams were exposed to total ammonia concentrations averaging 47.63 mg/l, which is nearly 4 times the total ammonia concentration of the winter ammonia study. Clams exposed to 0.73 mg/l monochloramine experienced a total inhibition of siphoning activity. This type of response parallels the siphoning activity response observed previously for clams exposed to chlorine containing free residual chlorine. The siphoning activity of Texas and Glen Lyn collected clams was nearly identical in the control streams (e.g., 64.8% and 64.0%, respectively).

TABLE 2-7 Number of clams siphoning (% ± SE) of adult Asiatic clams (n = 20) collected during winter (from Seadrift, TX at 20° C) and spring (from Glen Lyn, VA at 18° C) and exposed to monochloramine and or ammonia at 20° C in laboratory artificial streams. Means with different letters are significantly different ($\alpha = .05$, Duncan's New Multiple Range Test or Studentized t-Test).

	Treatment	Conc. (mg/l)	Siphoning (%)
Winter Study			
(Texas clams)			
	control	BDL*	64.8 ± 5.8 A
	NH_3[1]	0.29 ± 0.04	30.8 ± 4.9 B
Spring Study			
(Glen Lyn clams)			
	control	BDL	64.0 ± 11.2 A
	NH_3[2]	0.54 ± 0.03	22.4 ± 3.3 B
	NH_2Cl[3]	0.73 ± 0.09	0.0 ± 0.0 C

* below detectable limit.

[1] Total ammonia concentration measured was 16.50 ± 1.09 mg/l.

[2] Total ammonia concentration measured was 47.63 ± 2.37 mg/l.

[3] TRC and FRC were 0.94 ± 0.09 mg/l and 0.17 ± 0.04 mg/l, respectively. Total and un-ionized ammmonia concentrations were 0.60 ± 0.14 mg/l and < 0.01 mg/l, respectively.

2.4 DISCUSSION

2.4.1 AMMONIA

Past studies examining the toxicity of ammonia to *Corbicula* have reported 38% mortality for clams (shell length unknown) exposed to 0.32 mg NH_3/l for 7 days (Horne and McIntosh, 1979) and complete inhibition of ciliary beating within one hour of exposure to 0.11 mg NH_3/l (Anderson et al., 1978). Mortality results from this study, although extremely size dependent, did indicate that ammonia toxicity occurred at concentrations as low as 0.21 mg NH_3/l, which is comparable to other reports of ammonia toxicity to *Corbicula* and other freshwater molluscs (Zischke and Arthur, 1987; Horne and McIntosh, 1979; Anderson et al, 1978). Specifically, it was observed that adults experienced only marginal mortality (e.g. 6.7%) following 30 days of exposure to 0.21 mg NH_3/l during the fall, while juveniles experienced 93% mortality during the same study. This size dependent toxicity pattern is not uncommon in *Corbicula* and has been reported to occur in the presence of chlorine (Sappington et al., 1986; Doherty et al., 1986), copper (Belanger et al., in review), and bromine (Doherty et al., 1986). In addition, adults were less sensitive to ammonia compared to chlorine, while juveniles were considerably more sensitive to ammonia than chlorine. Thus, inferences on the relative toxicity of ammonia to *Corbicula* compared to chlorine should be made only within a given size class.

Regarding the onset of mortality, my hypothesis that initial mortality would occur sooner in the presence of ammonia was confirmed for juveniles but rejected for adults. Since adults and juveniles siphoned in the presence of 0.29 mg/l NH_3, it is believed that juveniles may have experienced mortality sooner than adults because of their higher siphoning activity and subsequently, the greater exposure of ammonia to their tissues.

Significantly higher juvenile siphoning activity compared to adults has been documented at 23° C (ambient temperature) during the summer temperature shock study (Figure 1-8).

Mortality responses of adult clams to a slightly higher concentration of ammonia during the winter revealed contrasting results. For example, Texas clams had no mortality following 30 days exposure to 0.29 mg NH_3/l. Since 31% of the clams siphoned in the presence of ammonia at this concentration, factors other than valve closure must have contributed to their apparent resistance to ammonia during this study. One explanation for the lack of mortality observed for Texas clams exposed to ammonia may be their relatively large size (e.g. 18-24 mm SL). Since size class was shown to influence clam mortality in response to ammonia during the fall monochloramine-ammonia study, the larger size of clams used during this study may have contributed to their apparent resistance to ammonia. In addition, it was discovered that clams collected for this study were exposed constantly to a "backround" concentration of total ammonia approximating 0.50 mg/l in the cooling ponds. It is plausible that clams could have developed a tolerance to ammonia in the field, and therefore, were more resistant upon being exposed to ammonia in the laboratory. The ability of fish to aquire a tolerance to ammonia has been documented for *Tilapia aurea* and the cutthroat trout, *Salmo clarki* (Redner and Stickney, 1979; Thurston et al., 1981a).

Finally, when the target 0.50 mg/l NH_3 was reached during the spring monochloramine-ammonia study, adult Glen Lyn clams exhibited acute mortality by experiencing 70% mortality within 4 days exposure to 0.54 mg/l NH_3 (Table 2-3). An average of 22% of the clams siphoned even in the presence of 47.63 mg/l NH_3-N (0.54 mg/l NH_3) during this study. This clearly demonstrates the low detectability of ammonia by *Corbicula.* The high toxicity observed during this study is believed to have resulted from several confounding factors. First, the high concentration of total ammonia,

of which 98.89% was in the NH_4^+ form, almost certainly contributed to the toxicity of NH_3. Likewise, Thurston et al., (1981b) reported lower 96-hour LC50's for rainbow trout exposed to NH_3 at lower pH's and concluded that the NH_4^+ ion or the H^+ ion concentration increases NH_3 toxicity. Toxicity estimates consider the ammonium ion to be 50 times less toxic than the un-ionized ammonia (Anderson et al., 1978). Given this estimate, it can be calculated that clams were actually being exposed to the equivalent of 1.5 mg/l NH_3. In addition, the presence of incubating larvae noticed after several clams were dissected may have increased clam suseptibility to ammonia. Therefore, from the mortality data discussed, although not entirely conclusive, it is suggested that 0.50-1.0 mg/l NH_3 would be effective in controlling adult populations of *Corbicula.* In practice, however, much lower concentrations may be needed to control larval populations during clam spawning periods.

Among sublethal responses to ammonia, it was demonstrated that only a slight reduction in glycogen content occurred following 30 days exposure to 0.29 mg/l NH_3 content (Table 2-6). This lack of a substantial loss in glycogen may in part be due to the ability of clams to siphon and continually feed in the presence of ammonia. However, since no mortality was observed during this study, the lack of a severe reduction in glycogen content is more likely due to the low exposure concentration. When clams were exposed to 0.54 mg/l NH_3, glycogen content decreased severely within 4 days. Therefore, since 22% of the clams exposed to ammonia were siphoning, reductions in glycogen content occurred at least partly, from the direct toxicity of ammonia to *Corbicula.*

Regarding tissue water responses, it was observed that tissue water content dropped significantly during the winter ammonia study, which is opposite of what has been reported for other freshwater organisms during exposure to toxicants (Heath, 1984a; Belanger et al., 1986a). Since the conductivity in the ammonia-dosed stream was nearly

twice that of controls (Appendix A), (presumably due to the influx of NH_4^+ ions), disruptions in the ion exchange capabilities may have caused a significant tissue water loss. Interestingly, control clams experienced a significant increase in tissue water after 30 days in the laboratory. This may reflect their removal from ammonia contaminated ponds in Texas and subsequent acclimation to ammonia free water in the laboratory. However, this same decreasing trend in the tissue water content of clams exposed to ammonia, although not statistically significant, was evident during the spring monochloramine-ammonia study. Therefore, from these data, it is evident that ammonia causes rather unique sublethal responses in *Corbicula* by being relatively undetectable compared to other biocides and by causing significant reductions in clam tissue water content.

2.4.2 MONOCHLORAMINE

Mortality results from the fall monochloramine-ammonia study indicated that Asiatic clams were equally or more sensitive (depending on size) to monochloramine than chlorine (containing FRC) at comparable test conditions and concentrations. Although it was hypothesized that Asiatic clams would not be able to detect monochloramine as well as chlorine, casual observations of siphoning activity during the fall monochloramine-ammonia study and siphoning data taken from the spring monochloramine-ammonia study reveal that *Corbicula* does detect monochloramine, and subsequently, exhibits a valve closure response similar to that observed previously in the presence of chlorine (Table 2-7). Based on LT50 comparisons from the fall monochloramine-ammonia study (Table 2-1), it was demonstrated that compared to chlorine, adult clams were equally sensitive to monochloramine exposures, while juveniles were nearly twice as sensitive when exposed to monochloramine. Since both chlorine and monochloramine elicit similar valve closure responses in *Corbicula*, one

would expect greater clam mortality in the presence of chlorine (containing FRC) than monochloramine because of the reported higher toxicity of free residual chlorine (Heath, 1984b). One explanation for the existence of equal sensitivity of adults to chlorine and monochloramine is that free residual constituents (e.g. HOCl and OCl^-) may be reacting with ammonia accumulated in the mantle cavity of adducted clams to form monochloramine, which then becomes toxic to the clams. Past studies have reported indirectly the accumulation of ammonia within the valves of chlorinated clams by documenting significantly higher excretion rates of ammonia in chlorinated clams compared to controls, when placed in chlorine-free water (Farris et al., in preparation). Since the formation of monochloramine occurs almost instantaneously (e.g. $K = 2.9 \times 10^6$, Gray et al., 1979), clams in the presence of free residual chlorine may actually be exposed to monochloramine within their mantle cavities. Reasons for the increased sensitivity of juveniles to monochloramine compared to chlorine are at this time, unclear.

Asiatic clams exposed to 0.73 mg/l monochloramine during the spring monochloramine-ammonia study experienced unusually high mortality compared to similar chlorine exposures from a previous study (Doherty et al., 1986). Specifically, an adult LT50 of 28.5 days was found for clams exposed to 1.02 mg/l TRC at 23° C, while the LT50 for clams exposed to 0.94 mg/l TRC (with 0.73 mg/l as NH_2Cl) occurred at 6.5 days. It is believed that several confounding factors may have contributed to the toxicity of monochloramine during this study. First, the presence of total ammonia at 0.60 mg/l in the monochloramine stream may have acted synergistically to increase clam mortality in that treatment. In addition, the presence of incubating larvae observed in the gills of clams on day 4 and 7 may have increased clam susceptibility to monochloramine. Gill hyperplasia has been reported to occur from larval encystment in natural populations of *Corbicula* (Morton, 1977) and may occur in spawning adults during periods of valve closure.

On a sublethal level, significant reductions in glycogen content were observed in clams following only 4 days exposure to 0.73 mg/l monochloramine. This rather immediate reduction in glycogen content, which was not observed until day 25 in clams exposed to 0.30 mg/l TRC during the summer, is probably a function of both the high concentration of chlorine (e.g. 0.94 mg/l TRC with 0.73 mg/l as NH_2Cl) and again, the presence of incubating larvae. Interestingly, control clams also experienced a significant reduction in glycogen following 4 days in the laboratory. Since decreases in glycogen content in *Corbicula* have previously been associated with gametogenesis (Cantelmo et al., 1985), it is possible that clams in this study may have experienced similar glycogen reductions. Regarding tissue water responses, monochloramine exposure did not elicit any loss or gain in tissue water content for clams after 7 days.

In conclusion, results from this study indicate that both ammonia and monochloramine are toxic to *Corbicula* at concentrations near those of currently recommended chlorination control procedures (e.g. 0.50-1.0 mg/l). It has been shown that Asiatic clams have a reduced ability to detect ammonia and therefore, siphon at acutely toxic concentrations. However, the concentration of un-ionized ammonia, and subseqent toxicity of total ammonia, declines drastically with decreasing pH and, therefore, may require the use of ammonia concentrations which are economically impractical for the control of adult clam populations. The release of ammonia into natural waters may, in addition to its direct toxic effect, cause eutrophication in ecosystems where nitrogen is a limiting nutrient. Current ambient ammonia criteria at a representative temperature and pH of this study (e.g. 20° C, pH 8.0) state that 0.76 mg/l NH_3-N or 0.05 mg/l NH_3 should not be exceeded more than once every three years for the protection of aquatic life. Although actual discharge limitations generally take into account dilution factors, and therefore, may exceed ambient water criteria concentrations, more research is needed to evaluate the toxicity of ammonia at lower concentrations. This

may lead to the use of ammonia by industries at low levels during clam spawning periods.

Regarding monochloramine, it has been demonstrated that Asiatic clams have the ability to detect and avoid monochloramine as demonstrated in the presence of chlorine. Despite reports of the higher toxicity of free residual chlorine, clams exposed to chlorine, comprised mostly of monochloramine, were equally or more sensitive (depending on size) to comparable concentrations of chlorine containing free residual chlorine. Therefore, questions were raised as to the availablity of free residual chlorine to adducted clams during continuous chlorination control procedures. More studies are needed to address the comparative toxicity of monochloramine and chlorine to adult, juvenile, and larval clams in order to determine the relative costs and benefits of monochlorination.

3.0 FIELD AND LABORATORY RESPONSES TO COPPER

3.1 INTRODUCTION

In the past few decades much research in aquatic toxicology has been directed towards defining and quantifying sublethal effects of pollutants upon aquatic organisms. This research has led to a wealth of toxicologicial data and has been instrumental in the development of ambient water quality criteria by reducing the need for gross extrapolation from traditional "dose and kill" acute toxicity experiments (USEPA, 1985b). In fact, sublethal toxicity tests have established such a foothold in the academic community, that sub-chronic bioassays (e.g. *Ceriodaphnia* fecundity and fathead minnow growth tests) have now been developed for use in the maintenance of effluent standards (USEPA, 1985b). However, the majority of studies addressing sublethal responses of aquatic organisms to pollutants have done so either under strict laboratory protocol or during *in-situ* exposures, with little emphasis on comparing field and laboratory responses in any one study. During laboratory studies, processes such as sorption,

volatilization, transformation, and photolysis, which may reduce or even enhance the toxicity of a compound, are often rendered insignificant. On the other hand, field studies, which take into account the fate processes mentioned previously, often lack the repeatability and standardization needed for the extrapolation of results to other aquatic systems. Therefore, the comparison of laboratory and field toxicity experiments is essential for determining the predictive capability of any toxicological endpoint, whether lethal or sublethal. In addition, there is a need for the identification of sublethal indicators of stress which are both simple to perform and sensitive to toxicants at environmentally realistic concentrations. For example, elaborate molecular indicators such as metallothionein induction or adenylate energy charge, although extremely important for understanding the mode of action of toxicants upon aquatic organisms, are relatively time consuming and probably are not practical for use on a routine or regulatory basis. Therefore, it was the goal of this study to compare several, rather simple, sublethal indicators of stress in response to a reference toxicant, namely copper, in laboratory, site-specific, and *in-situ* exposures. Copper was chosen specifically because it is an effluent constituent of coal-fired power plants, is extremely toxic to aquatic life, and is a contaminant found in river systems throughout the world (Nriagu, 1979; USEPA, 1985c).

One factor important when examining the effect of toxicants at sublethal levels is the test organism(s) chosen for study. The organism used during this study, the freshwater Asiatic clam, *Corbicula* sp., was chosen because it offers many advantages for use in both laboratory and field experimental settings. First, like other bivalve molluscs, *Corbicula* is sedentary and employs a filter feeding lifestyle, which results in its ability to accumulate a wide variety of metals, organics, and minerals (Graney et al., 1983; 1984; Leard et al., 1980; Belanger et al., 1986a; 1986b). Since *Corbicula* is now found in most major river systems in North America, except in the northern-most regions (Counts, 1986), its use in site-specific toxicity experiments is desirable due to its obvious ability

to adapt to a wide variety of aquatic habitats. Finally, Asiatic clams are rather abundant, easily collected, resistant to handling stress, and easily manipulated in the laboratory.

The sublethal indicators examined during this study consisted of whole body glycogen, tissue water, and soluble protein content. These indicators were chosen for study because of their simplicity of measurement, their representation of basic metabolic or osmoregulatory function, and their successful use for toxicity assessment in the past. Specifically, glycogen comprises the major energy reserve of bivalves and has been shown to respond to a wide variety of stressors in freshwater, estuarine, and marine bivalves (Cantelmo et al., 1985; Riley, 1976; Bayne et al., 1982; Sappington et al., 1986). Therefore, since organisms generally experience increased energy costs when undergoing stress, it was hypothesized that increasing copper exposure would reduce clam glycogen content in the laboratory and in the field. Tissue water content is an indicator of osmoregulatory function and has been reported to increase in the bluegill, *Lepomis macrochirus* in response to copper exposure (Heath, 1984b). Significant increases in tissue water content also were reported by Belanger et al. (1986a) for Asiatic clams exposed to zinc in field-located artificial streams. Therefore, it was hypothesized that the tissue water content of clams exposed to copper would increase with increasing copper exposures. Finally, soluble protein content was examined because protein is used as an energy source by bivalves during periods of starvation (Whyte and Englar, 1982; Riley, 1976). In addition, Deaton et al. (1984) hypothesized that protein hydrolysis caused increases in the cellular free amino acid pools of the sea mussel, *Mytilus edulis* during periods of hyperosmotic stress. Therefore, because of the metabolic and possible osmoregulatory role of protein in bivalves, it was hypothesized that soluble protein would decrease in response to laboratory and field copper exposures.

In addition to these physiological indicators of stress, siphoning activity was also characterized for clams exposed to copper in the laboratory. Clam siphoning activity indirectly reflects the valve closure response exhibited by *Corbicula* during periods of stress, which is its primary defense mechanism against toxicant exposure. In addition, siphoning activity has been shown to decrease in response to a wide variety of stressors which include temperature, chlorine, monochloramine (Sappington et al., 1986), zinc, and even asbestos (Belanger et al., 1986a; 1986b). Therefore it was hypothesized that clams would experience reductions in siphoning activity when exposed to copper in the laboratory.

3.2 METHODS

3.2.1 LABORATORY STUDY

In February, 1987, adult (14-17 mm SL) Asiatic clams were collected from a heated zone of the New River at Glen Lyn, VA and returned to laboratory artificial streams (Figure 1-1) in Va. Tech's Ecosystem Simulation Laboratory (ESL) for a 2-day acclimation period at the collection temperature of 17.2° C. Since acclimation to higher temperatures has been shown to decrease the survivorship of Asiatic clams exposed to chlorine (Doherty et al., 1986), clams used for this study were done so from a zone of heated water (at 17.2° C), as opposed to unheated water at ~ 10° C. This was done to allow comparisons to the site-specific and field experiments. Clams were then divided into groups of 30 and placed into nylon baskets in a series of 5 artificial streams maintained at 0 (control), 6, 12, 25, and 50 µg Cu/l for 30 days. Clams were fed daily with 500 mls of tri-algal stock (*Chlamydomonas reinhardti, Chlorella vulgaris, and*

Ankistrodesmus sp.) to each stream. Copper stock solutions were prepared in 20 l polycarbonate carboys by dissolving $CuSO_4$ (Fisher Scientific) in dechlorinated tap water and were delivered into streams by peristaltic pumps. Stock and diluent flow rates were maintained at 6 and 20 mls/min in order to regulate copper concentrations, stream temperatures, and to prevent rapid algal loss from the streams. Standard water chemistry (pH, alkalinity, hardness, conductivity) were measured on days 0 and 30 (Appendix A), while samples for the determination of acid soluble copper (USEPA, 1983) were analyzed on days 0, 10, 20, and 30 using a Perkin Elmer Model 310 atomic absorption spectrophotometer.

Clam siphoning activity (percent of clams siphoning) was measured daily for clams in each treatment prior to feeding. On days 0 and 30, six clams were sacrificed for glycogen, tissue water, and soluble protein analyses. Glycogen content was analyzed using the methods of Roehrig and Allred (1974) as described previously (Chapter one). Following the centrifugation step of the glycogen assay, clam supernatants were frozen immediately ($< -10°$ C) and stored until analized for soluble protein content. Upon protein determination, samples were thawed at 10° C, and triplicate 100µl aliquots were analyzed for soluble protein content using the Bio-Rad assay for total protein determination (Bio-Rad Tech. Bull. 1051, 1977). Soluble protein concentrations were calculated from a standard curve determined for bovine serum albumin (98% as fraction V, Sigma Chemical Co.) and expressed as percent dry weight. Tissue water content was determined as described in Chapter two and expressed as percent wet weight.

3.2.2 GLEN LYN STUDY #1

In June, 1986, adult (14.5-20.5 mm SL) Asiatic clams were collected from the New River at Narrows, VA and transported to field-located artificial steams receiving New River water at Glen Lyn, VA. Each stream contained approximately 2 cm of coarse

sediment and received New River water at 1.6 l/min. Clams were acclimated to test conditions for 5 days and then randomly distributed (n = 30 per stream) among 12 streams consisting of 3 replicate concentrations of 0, 12, 25, and 50 μg/l copper. Stream copper concentrations were maintained by constant dosage from stock solutions of $CuSO_4$ with peristaltic pumps. Stock solutions were changed and diluent and stock flow rates were monitored every 2 days. On days 0, 5, 10, 20, and 30 acid soluble copper and standard water chemistry were determined as described above. In addition, stream temperatures were recorded twice weekly. On days 0 and 30, clams (n = 6) were returned to the laboratory, dissected, and glycogen, tissue water, and soluble protein determined as outlined in the laboratory study.

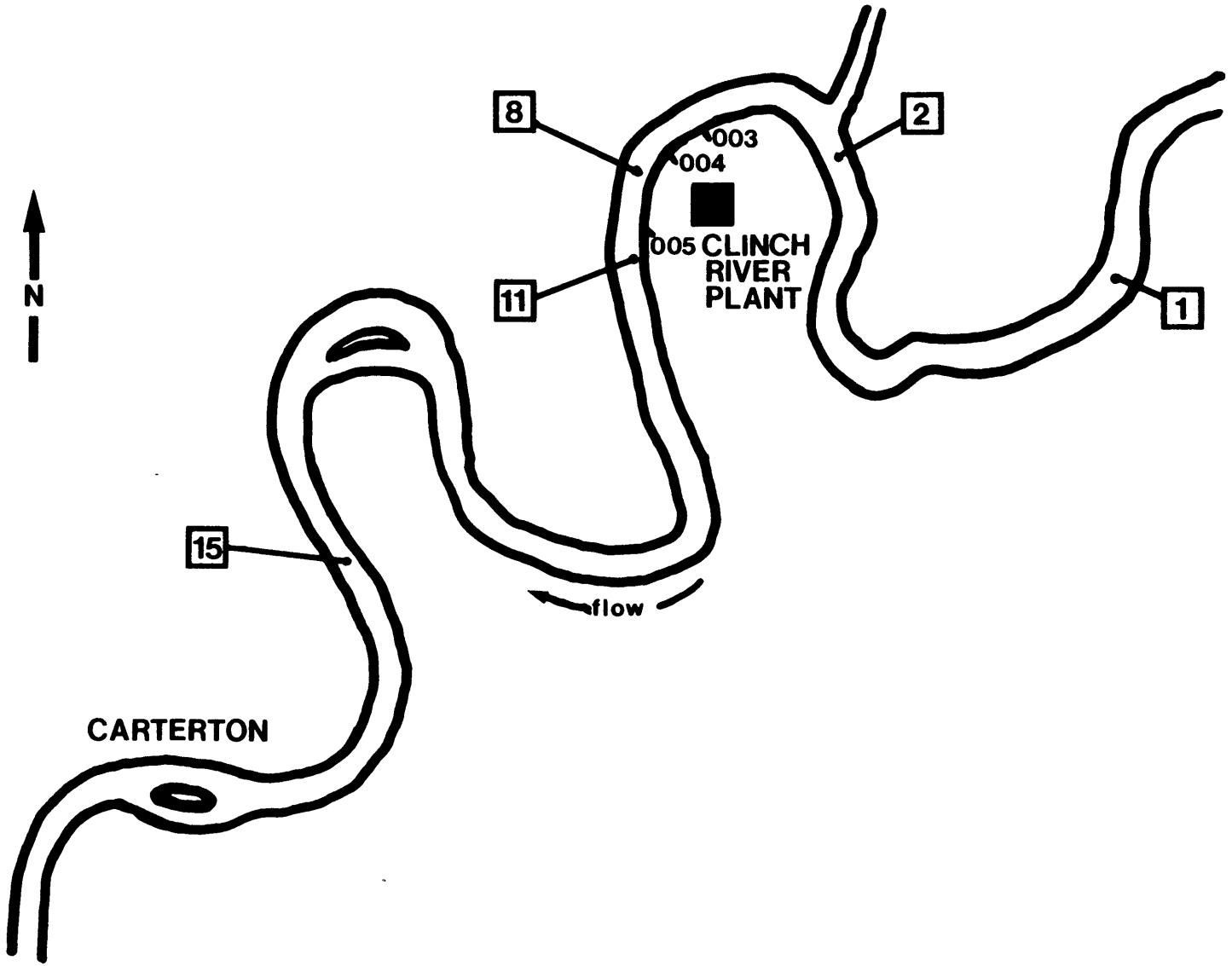
3.2.3 GLEN LYN STUDY #2

Adult clams (14-17 mm SL) were collected in August 1986 and exposed to lower target copper concentrations of 0, 6, 12, and 25 μg Cu/l following a 3-day acclimation period for 30 days. Similar experimental protocol was followed as described for the June Glen Lyn study with respect to the dosing strategy, copper measurements, and determination of clam glycogen, tissue water, and soluble protein content.

3.2.4 CLINCH RIVER STUDY

In September 1986, adult (13.8-17.4 mm SL) Asiatic clams were collected from Narrows, VA and transported 2.5 hours in plastic buckets containing New River water to the Clinch River study site in Russell Co., VA. Following a 36-hour acclimation period at station 1 (an upstream station), clams (n = 30) were placed into nylon baskets with sediment and distributed among 5 stations above and below American Electric Power's Clinch River plant (Figure 3-1). Stations 1 & 2 served as reference stations, while stations 8, 11, and 15 were located downstream from outfall 004, which contained

Figure 3-1. Map of the Clinch River showing plant, discharge, and station locations of caged clams used during the Clinch River monitoring study.


8
2
003
004
005 CLINCH
RIVER
PLANT
11
1
N
15
flow
CARTERTON

copper as its toxic effluent component. In addition to containing copper, this discharge was also found to contain zinc which was present at two of the downstream stations (Appendix A). Outfalls 003 and 005 contained relatively small amounts of municipal wastewater and storm runoff (respectively) and were considered to be of little significance during this study. Standard water chemistry, total recoverable copper, and total recoverable zinc were measured at least once a week during the study. Clam glycogen and tissue water content were measured on days 10 and 30 as previously described.

3.2.5 STATISTICAL ANALYSES

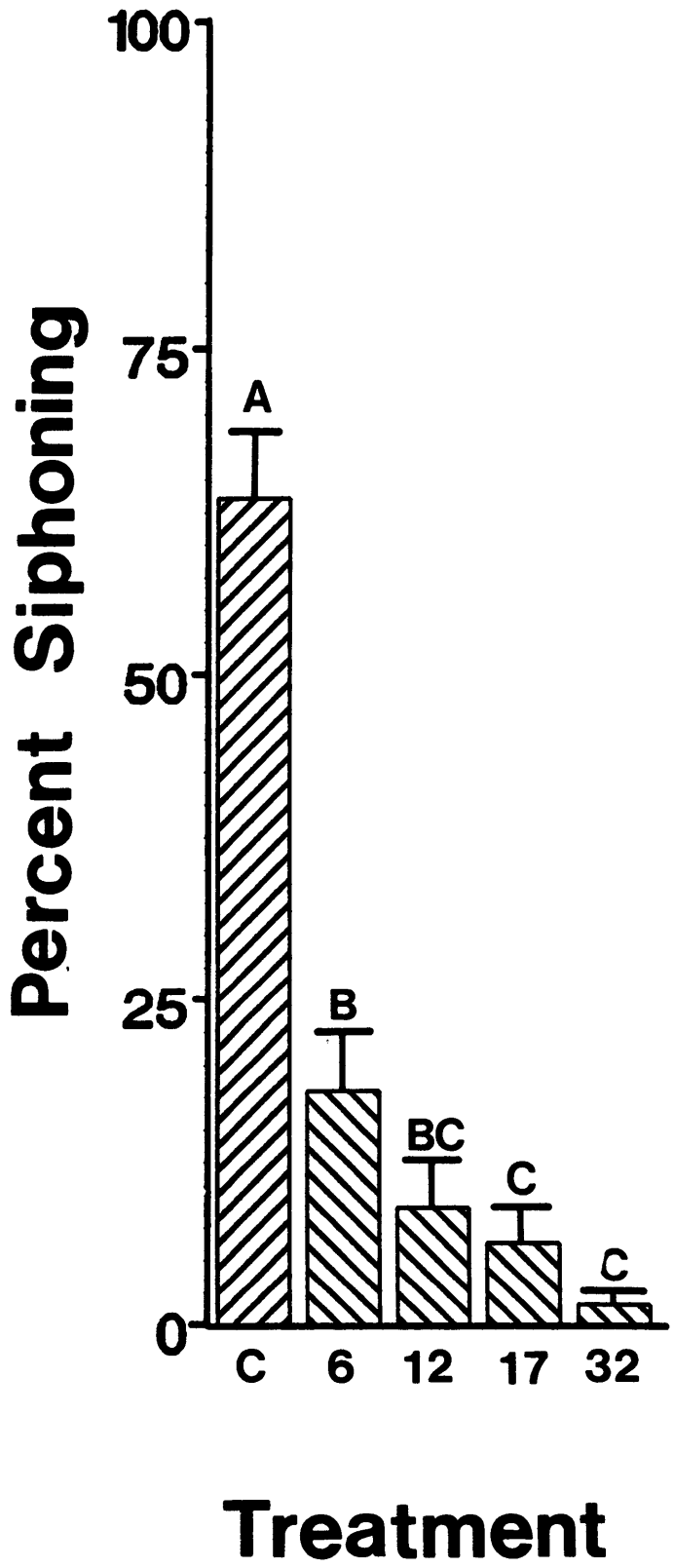
Tissue water, glycogen content, and siphoning activity were all transformed using the arcsine-square root transformation to remove non-normality which may have occurred from their expression on a percent scale. The transformed data were then analyzed using a one-way ANOVA procedure, and significant differences were determined for ANOVA's with p-values $< .05$ using Duncan's New Multiple Range Test or the Studentized t-Test at $\alpha = .05$.

3.3 RESULTS

3.3.1 LABORATORY COPPER STUDY

Clams exposed to copper in the laboratory experienced severe reductions in siphoning activity at concentrations ranging from 5.6-32.1 µg/l (Figure 3-2). Siphoning activity was reduced to 17.9% for clams exposed to 5.6 µg/l, which was the lowest concentration tested. The siphoning activity of control clams was 63.5%, which was within the range of siphoning activities observed previously for adults (Chapters 1 and 2).

Figure 3-2. Percent siphoning activity (mean ± SE) of adult Asiatic clams exposed to copper during the E.S.L. artificial stream study. Copper concentrations are shown below the vertical bars in µg/l and control siphoning activity is represented by the letter "C".


100
75
50
25
0
Percent Siphoning
A
B
BC
C
C
C
6
12
17
32
Treatment

Further reductions were observed at 12.7 and 17.2 µg Cu/l with clams exhibiting siphoning activities of 8.9% and 6.2%, respectively. Clams exposed to 32.1 µg Cu/l experienced almost total inhibition of siphoning activity, which averaged 1.5%.

Contrary to the results observed for siphoning activity, clam glycogen content was significantly reduced only at the highest (32.1 µg/l) copper treatment by day 30 (Table 3-1). This drop (e.g., from 15.9% to 11.1% dry weight) represented a reduction in glycogen content to 66% of that measured for control (day 30) clams. Clams exposed to 12.7 and 17.2 µg/l Cu contained a slightly higher glycogen content than controls, although these increases were not statistically significant. Control clams experienced a 3.9% dry weight drop in glycogen content following 30 days in the laboratory. This may reflect inadequacies in the feeding regime used during this study.

Whole body water content did not change significantly with increasing copper concentrations (Table 3-1). Specifically, clam tissue water content increased from 87.1% in controls to 88.2% in clams exposed to 32.1 µg Cu/l by day 30. In addition, an increase in tissue water of 1.5% was observed for control clams after 30 days holding in the laboratory. Although not statistically significant, this increase in tissue water content was analogous to the slight decrease in glycogen content observed prevously for control clams after 30 days in the laboratory.

The effect of copper on soluble protein content did not follow a dose-dependent trend as expected (Table 3-1). For example, clam soluble protein dropped significantly in clams exposed to 12.7 µg Cu/l by day 30, but significantly increased in clams exposed to 32.1 µg Cu/l. In addition, a significant increase in soluble protein content was evident for control clams held in the laboratory after 30 days.

TABLE 3-1 Mean glycogen , tissue water, and soluble protein content (± SE, n = 6) for adult Asiatic clams exposed to copper in laboratory artificial streams for 30 days. Means with different letters are significantly different (Duncan's New Multiple Range Test, $\alpha = .05$).

LABORATORY STUDY

Day	Target Conc. (µg/l)	Actual Conc. (µg/l)	Glycogen (% dry wt.)	Tissue Water (% wet wt.)	Soluble Protein (% dry wt.)
0	n/a[1]	n/a	19.8 ± 1.3 A	85.7 ± 0.7 C	4.4 ± 0.4 C
30	0	2.5 ± 0.1	15.9 ± 0.5 AB	87.1 ± 0.4 ABC	6.1 ± 0.2 B
30	6	5.6 ± 0.9	14.1 ± 3.8 BC	87.3 ± 0.5 AB	6.3 ± 1.1 B
30	12	12.7 ± 1.7	17.0 ± 1.7 AB	87.6 ± 0.3 AB	4.7 ± 0.2 C
30	25	17.2 ± 2.7	17.9 ± 1.3 AB	86.5 ± 0.5 BC	6.4 ± 0.4 AB
30	50	32.1 ± 2.6	11.1 ± 1.2 C	88.2 ± 0.5 A	7.7 ± 0.3 A

[1] not analyzed.

3.3.2 GLEN LYN STUDY #1

Clams exposed to 16.2 and 21.2 µg Cu/l in field-located artificial streams during June experienced significant reductions in glycogen content to 60% and 66% of controls by day 30, respectively (Table 3-2). Clams exposed to 26.7 µg/l Cu experienced mortality so severe, that by day 30, too few specimens remained for the determination of glycogen, tissue water, or soluble protein content. The glycogen content of control clams was not significantly different after 30 days, and therefore, may indicate the adequacy of New River water for clam diet purposes. Stream temperatures ranged from 22.0-26.3° C during this study.

Clam tissue water responded similarly to copper as glycogen content with significant differences from controls occurring at the lowest concentration tested (Table 3-2). Specifically, clams exposed to 16.2 and 21.1 µg/l Cu contained tissue water contents which were 4.5% and 3.8% higher than controls by day 30. No difference in tissue water content was observed for control clams after 30 days in the streams. As observed during the laboratory study, the variability associated with Asiatic clam tissue water content was quite low within a treatment.

Analyses of clam soluble protein content indicates that clams responded with a dose-dependent decrease in soluble protein (as hypothesized) following exposure to increasing copper concentrations (Table 3-2). However, this response contrasted with soluble protein responses observed previously for clams exposed to similar copper concentrations in the laboratory. For example, clam soluble protein content was reduced significantly following exposure to 16.2 µg/l and 21.1 µg/l at Glen Lyn, while a significant increase in soluble protein content was observed for clams exposed to 17.2 and 32.1 µg/l in the laboratory.

TABLE 3-2 Mean glycogen, tissue water, and soluble protein content (± SE, n = 6) for adult Asiatic clams exposed to copper (n = 6) for 30 days in field-located artificial streams at Glen Lyn, Virginia. Means with different letters are significantly different (Duncan's New Multiple Range Test, $\alpha = .05$).

JUNE 1986

Day	Target Conc. (µg/l)	Actual Conc. (µg/l)	Glycogen (% dry wt.)	Tissue Water (% wet wt.)	Soluble Protein (% dry wt.)
0	0	7.4 ± 1.4	21.8 ± 0.7 A	83.9 ± 0.3 B	5.9 ± 0.2 AB
30	0	5.1 ± 1.0	22.8 ± 1.3 A	83.2 ± 0.9 B	6.6 ± 0.1 A
30	12	16.2 ± 3.0	13.6 ± 2.3 B	87.9 ± 0.4 A	5.0 ± 0.2 BC
30	25	21.1 ± 2.0	15.1 ± 2.1 B	87.0 ± 0.7 A	4.3 ± 0.6 C
30	50	26.7 ± 2.0	(a)	(a)	(a)

(a) too few specimens (< 5) remained for the determination of glycogen, tissue water, or soluble protein content.

3.3.3 GLEN LYN STUDY #2

Asiatic clams exposed to lower copper concentrations in field-located artificial streams in August responded with similar reductions in glycogen content as observed for clams in June (Table 3-3). Clams experienced a significant loss of glycogen after 30 days exposure to 8.4 μg Cu/l, which was the lowest concentration tested. At this concentration, clam glycogen content was reduced to 12.9% dry weight, which represented 71.7% of the glycogen content of control clams on day 30. The glycogen content of clams exposed to 13.9 and 17.7 μg Cu/l was reduced further to 53% and 58% of control clams, respectively. Overall, day 0 clams contained less glycogen during August than in June, which may indicate some seasonal influences on Asiatic clam glycogen content. As observed during the June study, no significant difference in the glycogen content of control clams was evident after 30 days. Temperatures during this study ranged from 23.0 to 26.0° C.

Clam tissue water content did not increase significantly in any of the copper treatments by day 30 (Table 3-3). However, on days 0 and 30, control clams contained greater amounts (~2.5%) of tissue water compared to controls of the June Glen Lyn study. This may indicate the presence of seasonal influences on clam tissue water content.

Clam soluble protein responses contrasted those observed previously during the June Glen Lyn study (Table 3-3). For example, both control day 0 and day 30 clams contained nearly half the soluble protein content of control clams examined during the June Glen Lyn study. In addition, exposure to 8.4-17.7 μg/l copper significantly increased the soluble protein content of clams relative to controls on day 30.

TABLE 3-3 Mean glycogen, tissue water, and soluble protein content (± SE, n = 6) for adult Asiatic clams exposed to copper for 30 days in field-located artificial streams at Glen Lyn, Virginia. Means with different letters are significantly different (Duncan's New Multiple Range Test, $\alpha = .05$).

AUGUST 1986

Day	Target Conc. (µg/l)	Actual Conc. (µg/l)	Glycogen (% dry wt.)	Tissue Water (% wet wt.)	Soluble Protein (% dry wt.)
0	0	5.0 ± 0.2	15.8 ± 1.5 AB	86.6 ± 0.7	2.9 ± 0.2 B
30	0	5.5 ± 0.6	18.0 ± 0.6 A	85.9 ± 0.4	3.1 ± 0.4 B
30	6	8.4 ± 1.1	12.9 ± 1.1 BC	86.3 ± 0.5	4.8 ± 0.6 A
30	12	13.9 ± 2.2	9.8 ± 1.5 C	87.1 ± 0.5	4.8 ± 0.4 A
30	25	17.7 ± 2.5	10.5 ± 1.2 C	87.1 ± 0.1	4.9 ± 0.3 A

3.3.4 CLINCH RIVER STUDY

Analysis of water chemistry indicate that clams were exposed to copper concentrations ranging from < 15.0 µg/l (stations 1, 2 and 15) to 104.8 µg/l at station 8. In addition, Zn concentrations ranged from < 20.0 µg/l at stations 1, 2, and 15 to 81.2 µg/l at station 8 (Appendix A). The mean water temperature during this study ranged from 21.8° at station 8 to 22.6°C at stations 2 and 15. Overall, water hardness and conductivity were nearly twice that measured in streams at Glen Lyn and in the laboratory.

Results from glycogen analyses of clams caged above and below the Clinch River power plant indicate significant impacts resulted from effluent exposure below the plant (Table 3-4). Clams caged at station 8 responded with reductions in glycogen content to 38% of the upstream (station 2) clams by day 10. Station 11 clams were impacted less severely, with reductions to 68% of station 2 clams by day 10. Further downstream, no observable effect on glycogen was evident at station 15. Interestingly, station 1 clams contained significantly less glycogen than station 2 clams. This may indicate some habitat deficiencies at station 1, and therefore, downstream stations were compared to station 2 unless otherwise specified. By day 30, the two upstream stations were not significantly different. Clams caged at station 8 experienced an apparent increase in glycogen content from day 10 to day 30 (e.g. 6.2% to 12.5%) and consequently, were not significantly different from either of the upstream stations on day 30. It was later discovered that clams used for day 30 analyses were taken from a neighboring basket designated for growth analyses (Belanger et al., in review) which was located on the margins of the effluent plume. Further downstream, clams caged at station 11 continued to experience significant reductions in glycogen content to 57% of the upstream clams on day 30. As observed on day 10, clams caged at the furthest downstream station (station 15) experienced no significant reductions in glycogen content.

TABLE 3-4 Mean glycogen and tissue water content (± SE, n = 6) for adult Asiatic clams placed at various locations above (stations 1 and 2) and below (stations 8, 11, and 15) a copper containing industrial discharge on the Clinch River, VA. Means with different letters are significantly different (Duncan's New Multiple Range Test, $\alpha = .05$).

		DAY 10		DAY 30	
Station	Conc. (µg/l)	Glycogen (% dry wt.)	Tissue Water (% wet wt.)	Glycogen (% dry wt.)	Tissue Water (% wet wt.)
1	< 15.0	12.8 ± 0.5 B	86.8 ± 0.4 BC	15.8 ± 1.4 A	86.3 ± 0.3 B
2	< 15.0	16.5 ± 0.6 A	86.3 ± 0.1 C	13.6 ± 0.9 A	86.2 ± 0.2 B
8	104.8 ± 18.9	6.2 ± 1.5 C	88.5 ± 0.2 A	12.5 ± 0.8 AB	85.8 ± 0.3 B
11	47.4 ± 11.0	11.2 ± 0.8 B	87.4 ± 0.4 B	7.8 ± 1.2 C	88.3 ± 0.3 A
15	< 15.0	16.5 ± 0.9 A	86.1 ± 0.4 C	13.7 ± 0.9 A	86.0 ± 0.1 B

Clam tissue water responses generally paralleled the impacts observed from the glycogen analyses on day 10 by exhibiting significant increases at downstream stations closest to the discharge. For example, clams caged at station 8 experienced a 2.2% increase in tissue water from upstream (station 2) clams by day 10. Likewise, the tissue water content of station 11 clams significantly increased to 1.1% above the tissue water content of station 2 clams. As observed with the glycogen analyses, clams caged at station 15 exhibited no observable change in tissue water by day 10. No significant difference in the tissue water content of station 1 and 2 clams was evident by day 10, which was contrary to the differences observed from the glycogen analyses. By day 30, clam tissue water content remained increased only for clams caged at station 11. Again, the apparent decrease in the tissue water of station 8 clams from day 10 to day 30 was attributed to the tissue water analyses of clams located on the margins of the effluent plume. No significant difference in tissue water content was evident for clams caged at station 15 relative to the upstream stations.

3.4 DISCUSSION

3.4.1 GLYCOGEN CONTENT

Results for the analyses of Asiatic clam glycogen content indicate that clams were considerably more sensitive to copper when exposed "on site" than in the laboratory. Specifically, clams experienced significant reductions in glycogen content when exposed to 8.4 µg Cu/l at Glen Lyn, compared to 32.1 µg Cu/l in the laboratory (Tables 3-1 and 3-3). Examination of Figure 3-2 indicates that exposure to copper in the laboratory significantly reduced clam siphoning activity at concentrations as low as 5.6 µg/l.

Therefore, based on siphoning activity data, one would have expected significant reductions in clam glycogen content to occur at all copper treatments, presumably due to the reduced food intake of clams experiencing siphoning activity reductions. Since the water hardness of the laboratory and site-specific studies were similar (e.g. 65 mg/l vs 70 mg/l, respectively as $CaCO_3$), changes in copper toxicity from differences in water hardness is not a plausible explanation. Closer examination of Table 3-1 indicates that clams were experiencing a shortage of food as evident from the 3.9% dry weight drop in the glycogen content of controls by day 30. Therefore, since significant differences in clam glycogen content were determined relative to day 30 controls, the reduced sensitivity of glycogen content analysis in the laboratory resulted most likely from the reduction of control glycogen content on day 30. Other long-term laboratory studies examining the effect of zinc on Asiatic clams have attributed the lack of significant differences in growth and cellulolytic responses between control and treated clams to inadequacies in the artificial diet (Belanger et al., 1986a; Farris, 1986). It is also possible that the lower exposure temperature in the laboratory may have contributed some to the resistance of laboratory exposed clams. However, previous studies on the effect of exposure temperature on clam glycogen content did not reveal significant differences between clams held at 23° or 28° C for 30 days (Table 1-4).

Comparisons of the laboratory and site-specific glycogen responses to those observed in the Clinch River study are difficult due to exposure concentrations being either lower (e.g. $<$ 15 µg/l) or higher (e.g. $>$ 47 µg/l) than copper concentrations of the stream studies. However, glycogen content did prove to be valuable in defining the zone of impact during this study. Specifically, the zone of impact stretched from station 8 to between stations 11 and 15 on the Clinch River. In addition, glycogen content proved to be a rapidly responding indicator with significant reductions occurring at stations 8 and 11 in just 10 days. In addition, although not one of the intentions of this study,

subtle differences in the location of clams with respect to the margin of the effluent plume were evident from clam glycogen concentrations. This observation was confirmed with conductivity measurements which have been used previously to document the location of this particular effluent plume (Van Hassel and Gaulke, 1986).

Regarding the adequacy of changes in glycogen content as indicators of copper stress in *Corbicula*, results from these studies indicate that clam glycogen content was sensitive to copper at 8.4 µg/l in field-located streams, which is just above the 6.5 µg/l ambient water quality criteria set for copper in waters with a hardness of 50 mg/l. It should also be noted that a "no observable effect concentration" of copper was not found during the field-located stream studies. In addition, the relative ease of measurement and low variability (with n = 6) also favor the use of glycogen content as a physiological indicator of copper stress. Therefore, glycogen content analysis is recommended for use in site-specific stream or *in-situ* studies in Asiatic clams, but is recommended for use in the laboratory with caution until a more adequate feeding regime is developed.

3.4.2 TISSUE WATER CONTENT

Tissue water analyses indicate that clams were sensitive to copper to different extents in laboratory and field-located artificial streams. For example, clam tissue water content increased significantly following 30 days of exposure to 16.2 µg Cu/l during the June Glen Lyn study, but showed no significant increase at concentrations as high as 32.1 µg/l in the laboratory (Table 3-1 and 3-2). Again, it is believed that the high tissue water content of control clams on day 30 (e.g. 87.1%), which may have resulted from inadequacies in the laboratory diet, was the cause of the apparent resistance of laboratory exposed clams. Likewise, during the August study at Glen Lyn, no significant effect of copper upon clam tissue water content was evident after 30 days. Although the

overall exposure concentrations were lower during the August study compared to the June study, one would have predicted that a significant increase in tissue water would have occurred for clams exposed to 17.7 µg/l Cu in August, based on the significant increase observed for clams exposed to 16.2 µg/l Cu in June. It is believed that the lack of significant increases in tissue water in August may have been a result of seasonal influences on clam tissue water content. Specifically, the tissue water content of control clams increased from 83.2-83.9% in June to 85.9-86.6% in August. This same seasonal increase in tissue water was documented for natural populations of Asiatic clams in the New River, VA from May to August, 1985 (Doherty, 1986). Therefore, it seems plausible that the lack of significant increase in clam tissue water when exposed to copper in August may be due to the naturally high tissue water content of control clams at the beginning of the study.

Regarding the biomonitoring potential of clam tissue water content, results from the Clinch River *in-situ* study support the use of clam tissue water content in future *in-situ* studies of metal contamination. Specifically, it was shown that clam tissue water was sensitive to copper exposures at station 8 and 11 in just 10 days (Table 3-4). Although zinc was also present at 81.2 and 42.4 µg/l at station 8 and 11, recent field-located stream studies of zinc and copper interactions upon Asiatic clam growth and survivorship indicate distinct, overriding effects of copper compared to zinc at comparable concentrations (Farris et al., in preparation). One observable difference between tissue water and glycogen content is the lack of significant difference between stations 1 and 2 on day 10. This may indicate that tissue water is somewhat less sensitive to copper than glycogen content. By day 30, clam tissue water responses were analogous to glycogen content responses with significant increases observed for clams caged at station 11. In addition, clam tissue water content exhibited low variability in the laboratory and field studies

despite the low sample size (n = 6) used. This low sample size enables one to determine tissue water content quick and easily and supports its continued use in the future.

3.4.3 SOLUBLE PROTEIN CONTENT

Conflicting results from the soluble protein analyses of clams exposed to copper during the laboratory and field-located artificial stream studies generally indicate that the use of soluble protein content is insuitable as a measure of copper effects on Asiatic clams. Although the expected decrease in soluble protein concentration in response to copper exposure was observed during the June Glen Lyn study, no identifiable dose-dependent responses were observed in August or in the laboratory study. Since soluble protein is comprised of many different enzymes and was performed on whole body tissues, it is believed that this assay is too general for adequate detection of sublethal effects of copper on Asiatic clams. In addition, strong seasonal differences were observed between control clams from June to August at Glen Lyn, which may detract from the use of soluble protein content as a sublethal indicator of copper contamination.

In summary then, results from these studies indicate that relatively simple physiological indicators of stress can be used for determining effects of copper on Asiatic clams at environmentally realistic concentrations. These results suggest that glycogen content was most sensitive to copper of the indicators examined. This was evident from the significant decrease in glycogen content in clams exposed to 8.4 μg Cu/l in field-located artificial streams. Furthermore, a no observable effect concentration (NOEC) was not found during the field-located stream studies with respect to clam glycogen content. Clam tissue water responses may be more useful than these results suggest, if a larger sample size (perhaps n = 50) is used in future studies. Since the measurement of clam tissue water is extremely easy and quick, an increase in sample size should not detract from its use in further toxicity experiments. Both glycogen and tissue water content were

subject to seasonal influences, so caution is suggested when comparing absolute differences across seasons. Soluble protein responses also were subject to seasonal influences and generally lacked consistency in response to copper in the laboratory and in the field. This lack of consistency may be due, in part, to its interactions with other body constituents (e.g., structural proteins, amino acids pools, and lipids), which were not measured during these experiments. Finally, caution is suggested when examining physiological responses in Asiatic clams during long-term laboratory exposures, until a more adequate diet is developed for rearing *Corbicula*. Poor diet was thought to contribute greatly to the lower sensitivity of these physiological indicators in the laboratory relative to the responses observed in the field.

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Appendix A. Water Chemistry

Table A-1. Selected water chemistry parameters (mean ± SE) for the winter and summer temperature shock artificial streams studies.

Study	Target Concentration (mg/l TRC)	Measured Concentration (mg/l TRC)	Temp. (°C)	pH	Alkalinity (mg/l $CaCO_3$)	Hardness (mg/l $CaCO_3$)	Conductivity (µmhos)
Winter Temperature Shock Study	0.0	< 0.02	6.9 (0.1)	7.92 (0.06)	43.1 (0.6)	65.0 (6.1)	137.0 (9.3)
	0.30	0.29 (0.01)	7.0 (0.1)	7.91 (0.06)	43.6 (1.7)	63.3 (3.3)	143.0 (12.5)
	0.0	< 0.02	11.5 (0.2)	7.96 (0.02)	43.1 (1.9)	62.3 (1.5)	139.1 (14.1)
	0.30	0.30 (0.01)	11.5 (0.2)	7.91 (0.06)	43.7 (1.7)	61.3 (1.7)	147.5 (16.5)
	0.0	< 0.02	16.8 (0.2)	7.99 (0.03)	44.4 (2.8)	63.3 (3.3)	145.7 (15.9)
	0.30	0.30 (0.01)	16.8 (0.2)	8.04 (0.03)	45.4 (2.1)	62.3 (1.7)	150.6 (16.7)
Summer Temperature Shock Study	0.0	< 0.02	22.9 (0.1)	8.07 (0.04)	65.5 (2.3)	77.0 (2.6)	244.0 (11.2)
	0.30	0.29 (0.01)	23.0 (0.1)	8.06 (0.05)	65.0 (3.6)	77.0 (4.9)	226.6 (11.3)

Table A-1 continued.

Study	Target Concentration (mg/l TRC)	Measured Concentration (mg/l TRC)	Temp. (°C)	pH	Alkalinity (mg/l $CaCO_3$)	Hardness (mg/l $CaCO_3$)	Conductivity (µmhos)
	0.0	< 0.02	28.0 (0.1)	8.07 (0.03)	71.8 (2.0)	86.0 (2.9)	241.2 (14.3)
	0.30	0.28 (0.01)	28.1 (0.1)	8.10 (0.03)	73.6 (3.3)	87.5 (2.5)	264.0 (11.3)
	0.0	< 0.02	33.0 (0.1)	8.24 (0.04)	83.5 (7.7)	96.0 (14.6)	301.8 (37.3)
	0.30	0.26 (0.01)	33.2 (0.1)	8.17 (0.15)	80.3 (5.3)	97.5 (2.5)	265.0 (8.0)

Table B-1. Selected water chemistry parameters (mean ± SE) for the monochloramine and ammonia artificial stream studies.

Study	Target Concentration (mg/l)	Measured Concentration (mg/l)	Temp. (°C)	pH	Alkalinity (mg/l $CaCO_3$)	Hardness (mg/l $CaCO_3$)	Conductivity (µmhos)
Fall Mono-chloramine/ Ammonia Study	0.25 NH_2Cl	0.23 (0.01)	19.0 (0.2)	8.29 (0.02)	84.7 (11.6)	70.0 (0.0)	285.5 (18.5)
	0.25 NH_3	0.21 (0.003)	21.1 (0.2)	8.07 (0.01)	40.5 (3.8)	65.0 (0.0)	175.0 (10.2)
	0.30 TRC	0.29 (0.01)	23.0 (0.1)	8.06 (0.05)	65.0 (3.6)	77.0 (4.9)	226.6 (11.3)
	0.0	< 0.02	19.5 (0.2)	8.08 (0.15)	46.9 (0.6)	72.5 (2.3)	152.0 (4.9)
Winter Ammonia Study	0.50 NH_3	0.29 (0.04)	20.2 (0.1)	7.57 (0.05)	36.0 (2.5)	52.0 (1.2)	226.4 (4.3)
	0.0	< 0.03	20.2 (0.1)	7.60 (0.07)	36.3 (1.2)	53.7 (1.3)	123.4 (9.7)
Spring Mono-chloramine/ Ammonia Study	0.75 NH_2Cl	0.73 (0.09)	19.8 (0.2)	7.60 (0.05)	38.8 (1.4)	57.5 (2.5)	152.6 (13.6)
	0.50 NH_3	0.54 (0.03)	20.3 (0.1)	7.46 (0.02)	35.6	55.0	460.0
	0.0	< 0.03	20.1 (0.3)	7.70 (0.06)	35.4 (1.6)	50.0 (0.0)	131.4 (2.4)

Table C-1. Selected water chemistry parameters (mean ± SE) for the laboratory and June, Glen Lyn copper studies.

Study	Target Concentration (mg/l Cu)	Measured Concentration (mg/l Cu)	Temp. (°C)	pH	Alkalinity (mg/l $CaCO_3$)	Hardness (mg/l $CaCO_3$)	Conductivity (µmhos)
Laboratory Study	0	2.5 (0.1)	16.3 (0.2)	7.64 (0.01)	38.5 (1.6)	60.0 (0.0)	124.0 (6.0)
	6	5.6 (0.9)	16.5 (0.2)	7.70 (0.0)	38.4 (0.94)	60.0 (10.0)	120.5 (5.5)
	12	12.7 (1.7)	16.4 (0.2)	7.78 (0.03)	39.1 (0.3)	57.5 (7.5)	121.5 (6.5)
	25	17.2 (2.7)	16.7 (0.2)	7.85 (0.05)	38.1 (0.6)	62.5 (7.5)	122.0 (4.0)
	50	32.1 (2.6)	16.6 (0.2)	7.81 (0.07)	39.1 (0.3)	62.5 (2.5)	124.0 (6.0)
Glen Lyn #1 June 1986	0	5.1 (1.0)	24.7 (0.4)	8.44 (0.03)	71.2 (1.4)	46.5 (0.7)	136.0 (1.3)
	12	16.2 (3.0)	24.5 (0.4)	8.31 (0.03)	70.6 (1.1)	45.3 (0.7)	134.7 (1.4)
	25	21.1 (2.0)	24.5 (0.4)	8.40 (0.04)	71.7 (0.7)	46.4 (0.7)	134.2 (1.5)
	50	26.7 (2.0)	23.9 (0.9)	8.36 (0.03)	70.3 (0.5)	46.9 (0.7)	131.6 (1.5)

Table C-2. Selected water chemistry parameters (mean ± SE) for the August, Glen Lyn copper study.

Study	Target Concentration (mg/l Cu)	Measured Concentration (mg/l Cu)	Temp. (°C)	pH	Alkalinity (mg/l $CaCO_3$)	Hardness (mg/l $CaCO_3$)	Conductivity (µmhos)
Glen Lyn #2 August	0	5.5 (0.6)	24.8 (0.3)	8.21 (0.09)	77.7 (3.6)	60.1 (2.1)	194.2 (9.2)
	6	8.4 (1.1)	24.9 (0.3)	8.21 (0.11)	78.0 (3.1)	59.6 (2.1)	194.9 (8.9)
	12	13.9 (2.2)	24.9 (0.3)	8.36 (0.05)	77.3 (3.3)	60.4 (2.1)	194.8 (9.1)
	25	17.7 (2.5)	24.9 (0.3)	8.18 (0.08)	78.0 (3.1)	60.8 (2.2)	195.3 (9.0)

Table C-3. Selected water chemistry parameters (mean ± SE) for the Clinch River in-situ copper study.

Study	Station	Measured Concentration (µg/l Cu)	Temp. (°C)	pH	Zinc Conc. (µg/l)	Alkalinity (mg/l $CaCO_3$)	Hardness (mg/l $CaCO_3$)	Conductivity (µmhos)
Clinch River Study	1	< 15.0	22.4 (0.6)	8.40 (0.09)	20.0 (7.7)	159.2 (7.1)	144.8 (7.1)	298.5 (5.3)
	2	< 15.0	22.6 (0.8)	8.45 (0.05)	14.2 (2.5)	155.8 (5.4)	156.0 (9.1)	299.9 (7.0)
	8	104.8 (18.9)	21.8 (1.2)	8.36 (0.03)	81.2 (19.6)	186.6 (13.4)	117.0 (5.9)	366.2 (19.5)
	11	47.4 (11.0)	22.3 (1.2)	8.17 (0.21)	42.4 (14.3)	185.0 (8.8)	142.6 (13.3)	360.6 (16.3)
	15	< 15.0	22.6 (0.07)	8.32 (0.16)	12.2 (3.5)	165.8 (4.3)	159.6 (10.6)	323.7 (7.1)

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