An Ecotoxicological Recovery Assessment of the Clinch River Following Coal Industry-related Disturbances in Carbo, Virginia (USA): 1967-2002

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Abstract: American Electric Power's (AEP) coal-fired Clinch River Plant, a power-generating facility in Carbo, Russell County, Virginia (USA), has impaired Clinch River biota through toxic spills in 1967 and 1970, and effluent copper (Cu) concentrations that were reported to have exceeded water quality criteria from 1985-1989. These impacts have provided impetus for many research projects addressing the absence of bivalves, including federally protected species of native mussels (Unionoidea), from sites influenced by CRP effluent. Modifications in CRP effluent during 1987 and 1993 drastically reduced Cu levels and warranted the present study, which assessed long-term biological recovery in Clinch River biota near the CRP. In 2000-2001, surveys of benthic macroinvertebrate communities and instantaneous measures of effluent toxicity did not foretell significant reductions in survivorship and growth of field-caged Asian clams (*Corbicula fluminea*) at sites downstream of the CRP. More importantly, these results indicated renewed toxicity in CRP effluent. Additional transplant studies using two enclosure types were conducted to isolate effects attributable to CRP effluent from the potentially confounding effects of substrate variability among study sites. While it was found that mean growth of clams was greatest in the enclosure that minimized substrate variability (p=0.0157), both enclosure types clearly distinguished significant impairment of survivorship and growth at sites downstream of the CRP discharge, and strengthened the association between impairment and CRP effluent. An intensive field investigation was undertaken to determine whether impairment observed in transplant studies extended to resident bivalves. During 2001-2002, densities and age structures of *C. fluminea* and distributions of mussels suggested that impairment indeed extended to resident bivalves for a distance of 0.5 to 0.6 km downstream of the CRP discharge. Impairment of bivalves was less evident below (1) a fly ash landfill and (2) coal mining activities and low-volume leachate from a bottom ash settling pond. With respect to long-term recovery, modifications in CRP effluent treatment have reduced Cu concentrations from an average of 436 μg/L in 1985-1989 to 13 μg/L in 1991-2002. Subsequently, Cu body burdens of Asian clams (*Corbicula fluminea*) transplanted within CRP influence have decreased from 442% of levels accumulated at reference sites in 1986, to 163% of these levels in 2002. The reduction in effluent Cu largely explains recovery of most benthic macroinvertebrate community parameters (e.g., richness, diversity) at influenced sites from levels that were typically less than 70% of reference levels, to levels that frequently range from 80 to greater than 100% of reference levels. Nevertheless, bivalves remain impaired downstream of the CRP; survivorship and growth of *C. fluminea* transplanted to CRP-influenced sites have typically been less than 40 and 20% of reference values, respectively. Furthermore, *C. fluminea* has seldom been encountered within CRP influence for nearly two decades. Likewise, native mussels remain absent within CRP influence, but recent surveys suggest their downstream distributions are more proximate to the CRP discharge than has been reported previously. A preliminary assessment of factors potentially contributing to toxicity revealed that (1) water reclaimed from settling basins for discharge with CRP effluent significantly impaired fecundity of ceriodaphnids at concentrations of 50%, (2) LC_{50} values for industrial treatment chemicals were misrepresented on Material Safety Data Sheets and consequently, were subject to misapplication by operators, (3) Cu concentrations of 96 μg/L significantly impaired growth of Asian clams in artificial stream testing, and (4) effluent Al exceeded acute and chronic water quality criteria, suggesting this ion should receive further consideration in future studies.

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Overview

The Clinch River, a major tributary of the upper Tennessee River system (along with the Holston and Powell Rivers), contains an assemblage of freshwater mussel fauna that is among the most diverse in the world. Native ranges for many of these mussel species are restricted to a specific area of the southern Appalachian Mountains known as the Cumberland Plateau Region, and have therefore been previously referred to as the "Cumberlandian fauna" (Ortmann, 1918). Sampling performed in 1979, 1983, 1988, and 1994, found a total of 39 mussel species, six of which were endangered and 14 of which were endemic to the Cumberland Plateau, at selected sites within the Clinch River (Ahlstedt and Tuberville, 1997). Increasing pressures on the limited habitat of these imperiled mussel species prompted the 1978 Symposium on Endangered and Threatened Plants and Animals of Virginia to name 54 freshwater mussel species (most of which reside in the southwestern Virginia section of the upper Tennessee drainage) as endangered (Neves, 1991). Priority has been given to the protection of these organisms, as many mussel species remain in low abundance while the incidence of others becomes increasingly rare (Ahlstedt and Tuberville, 1997).

American Electric Power's (AEP) Clinch River Plant (CRP), located in Carbo, Virginia, has historically impacted Clinch River biota. In 1967, a fly ash spill at the CRP resulted in an alkaline pH excursion (>11) that was reported to have impacted fish and benthic fauna downstream (Cairns et al., 1971, 1973; Crossman et al., 1973; Kaesler, 1974). Aquatic insects were eliminated from 5-6 km below the plant, while snail and mussel populations were reportedly eliminated for a distance of 18 km (Anonymous, 1967). In general, benthic macroinvertebrate density and diversity recovered by 1969, but molluscan populations remained impaired within 30 km of the insult. Recovery from this accident was interrupted in 1970, when a more isolated sulfuric acid spill occurred at the CRP (Crossman, 1973). Most benthos recovered within six weeks but according to some accounts (e.g., Cairns et al., 1971; Crossman et al., 1973) molluscan fauna failed to recolonize the stream sections damaged by the spills. Since assessments of mussel fauna made prior to the plant's initial operation in 1958 (e.g., Ortmann, 1918) were understandably not conducted at the resolution necessary to track small-scale changes in unionoid assemblages, it is unclear if unionoids ever existed in the stream section of interest. Results from an intensive survey of unionoid fauna in the vicinity of the CRP were reported by Stansbery et al. (1986), and indicated that unionoids were non-existent within stream sections influenced by CRP effluent. Although these surveys were conducted after the spills of 1967 and 1970, they established a baseline to which future evaluations of Clinch River mussel fauna could be compared.

In addition to the acutely toxic spills of 1967 and 1970, the CRP effluent has discharged in its effluent, high concentrations of metals, most notably Cu, that have been associated with biological impairment. Van Hassel and Gaulke (1986) reported that CRP effluent structured Ephemeroptera composition downstream. Clements et al. (1988) linked increased Cu concentrations to shifts in aquatic insect communities from pollution sensitive EPT (Ephemeroptera Plecoptera Trichoptera) to more tolerant Chironomidae. In studies conducted using the bivalve *Corbicula fluminea* (the Asian clam), Farris et al.

(1988) reported that CRP-associated Cu concentrations impaired enzymatic activity of clams transplanted downstream. Similarly, Belanger et al. (1990) reported that survivorship and growth of transplanted *C. fluminea*, and densities of naturally occurring *C. fluminea* were significantly reduced downstream of the CRP discharge, and attributed these reductions to elevated Cu concentrations.

The findings of these researchers provided impetus for modifications at the CRP to reduce effluent Cu levels. Replacement of cooling tower piping was completed in 1987, and wastewater treatment modifications were made in 1993 to reduce effluent metals concentrations. Consequently, CRP effluent Cu levels fell from an average of 436 μg/L in 1985-1989 to 12 μg/L in 1991-1995. Following these modifications, Cherry et al. documented recovery of Asian clam densities from 1994 to 1995.

Nearly a decade has passed since the last intensive studies were conducted near the CRP, and heightened awareness of disturbing declines in indigenous mussel fauna throughout North America has prompted bioassessments of areas identified as critical habitat for these imperiled organisms (Parmalee and Bogan, 1998). The present study was undertaken to investigate more recent trends in biological recovery downstream of the CRP discharge, with specific concern for bivalves. Chapter One presents results of qualitative surveys of benthic macroinvertebrate communities and in situ field bioassays with *C. fluminea*. This chapter emphasizes the importance of integrative bioassessment and demonstrates a situation in which impairment at lower levels of biological organization (i.e., impaired survivorship and growth of transplanted *C. fluminea*) can be masked by apparent health at higher levels (i.e., benthic macroinvertebrate communities). Furthermore, Chapter One suggests a renewed source of toxicological impairment downstream of the CRP. Because bivalves maintain intimate ecological contact with stream substrates, it is possible that substrate variations among study sites could potentially confound bioassessment results and falsely associate impairment with a potential source. This is addressed in Chapter Two, which presents results that strengthened the association between CRP effluent and the impairment of transplanted bivalves by using variable caging techniques to minimize site-specific substrate variations. Chapter Three extends the impairment we observed for transplanted bivalves to bivalves that naturally occur in the Clinch River. Furthermore, this chapter separates effects attributable to CRP effluent from potential effects from Dump's Creek, a Clinch River tributary influenced by various coal industry-related activities, and a CRP-owned fly ash landfill located downstream of the CRP discharge. This was accomplished by integrating results of Asian clam field bioassays with (1) density sampling of resident *C. fluminea*, (2) surveys of indigenous mussels, and (3) field bioassays with the rainbow mussel, *Villosa iris*. Chapter Four synthesizes historic bioassessment findings with the results of our more recent studies to approximate chemical and biological recovery trajectories for (1) effluent Cu concentrations, (2) Cu body burdens of transplanted bivalves, (3) survivorship and growth of transplanted bivalves, (4) benthic macroinvertebrate communities, (5) Asian clam densities, and (6) mussel distributions. Finally, Chapter Five provides results of preliminary studies that were conducted in effort to identify the source of CRP effluent toxicity to bivalves.

CHAPTER 1. Comparison of Asian clam field bioassays and benthic community surveys in quantifying effects of a coal-fired power plant effluent on Clinch River biota

Abstract: Survival and growth of Asian clams may be more sensitive endpoints than benthic macroinvertebrate community richness parameters at distinguishing biotic impairment attributable to complex effluents from coal-burning utilities. We conducted (1) field bioassays with the Asian clam (*Corbicula fluminea*) during 2000-02 and (2) rapid bioassessments of benthic macroinvertebrate communities during 2000-01 at sites upstream and downstream of American Electric Power's (AEP) Clinch River Plant (CRP) in Russell County, Virginia (U.S.A). Survival and growth of transplanted *C. fluminea* were significantly impaired within the CRP effluent plume (averages of 35% and 0.21 mm, respectively) relative to all other study sites within the Clinch River (averages of 89% and 1.58 mm). Conversely, richness metrics for Ephemeroptera, Ephemeroptera-Plecoptera-Trichoptera (EPT), and total taxa were not reduced downstream of the CRP. However, relative abundance metrics for Ephemeroptera and EPT were minimally reduced at the CRP-influenced site during 2000-01. More importantly, our results suggest that richness metrics for benthic macroinvertebrate communities may be inadequate for assessing the effects of complex industrial effluents on *C. fluminea*. These findings have implications for bioassessment techniques employed to monitor streams inhabited by imperiled freshwater mussels because (1) *C. fluminea* and Unionoidea are ecologically similar and (2) recent findings suggest certain genera of Unionidae may be more sensitive than *C. fluminea*.

1.1. Introduction

Few river systems remain where conditions are suitable for a speciose freshwater mussel fauna (Unionoidea), but the Clinch River, a major tributary of the upper Tennessee River drainage system, contains a rich assemblage of Cumberlandian species that is among the most diverse in the world (Ortmann, 1918; Ahlstedt, 1984; Stansbery et al., 1986; Ahlstedt and Tuberville, 1997; Chaplin et al., 2000). During recent decades, the abundance and diversity of freshwater unionoids have declined drastically in rivers throughout North America; presently 213 of 297 described species are considered endangered, threatened, or of special concern (Williams et al., 1993). As sensitive members of benthic communities, unionoids are among the first species lost to degrading environmental conditions; factors ranging from proximity to mined lands (Ahlstedt and Tuberville, 1997) to industrial point-source discharges (U.S. Environmental Protection Agency, 1994) have been linked to their decline.

By-products of coal-fired electric power generation from American Electric Power's (AEP) Clinch River Plant (CRP) in Carbo, Russell County, Virginia (USA) have adversely influenced Clinch River biota (Cairns et al., 1971; Clements et al., 1988; Farris et al., 1988; Reed-Judkins et al., 1998). Farris et al. (1988) reported elevated copper (Cu) and zinc (Zn) concentrations impaired enzymatic activity of *C. fluminea* transplanted below the CRP. Clements et al. (1988) reported that relatively low levels of Cu and Zn significantly reduced abundance and taxa richness of benthic macroinvertebrate assemblages and contributed to shifts in community composition from mayflies to chironomids during experimental and field studies at the CRP. Recent surveys indicate that natural colonization of mussels below Carbo has been limited to the side of the river opposite the CRP effluent discharge (Stansbery et al., 1986). Furthermore, efforts to translocate mussels to these reaches have been unsuccessful (Sheehan et al., 1989). Consequently, state and federal agencies (e.g., U.S. Fish and Wildlife Service) have prioritized efforts to protect the diverse Clinch River mussel fauna.

Field bioassays with Asian clams (*Corbicula fluminea* [Müller]) may offer a practical tool for determining the potential effects of environmental contaminants on imperiled Unionoidea. Despite obvious differences in life history (see Parmalee and Bogan, 1998), Corbiculidae and Unionoidea are ecologically similar as benthic filter/deposit feeders (Pennak, 1989; Parmalee and Bogan, 1998; Vaughn and Hakenkamp, 2001) with an affinity for bioaccumulating sediment-associated and dissolved trace metals (Adams et al., 1981; Graney et al., 1983; Muncaster et al., 1990; ASTM, 2001). The effectiveness of transplanted *C. fluminea* to rapidly detect contaminant gradients associated with point-source discharges in general and coal-fired power plant effluents in particular has been well documented (e.g., Foe and Knight, 1987; Doherty, 1990). Farris et al. (1988) reported that impairment of *C. fluminea* exo- and endocellulase activity was evident within 14 d of exposure to CRP effluent with elevated concentrations of Cu and Zn. Belanger et al (1986) reported that within 10 d, growth of field-caged *Corbicula* sp. was sensitive to Zn stress at levels considered by the US EPA to be protective of aquatic life. Freshwater mussels have been similarly employed as sentinel organisms (e.g., Adams et al., 1981; Aldridge et al., 1987; Schmitt et al., 1987). However, current assay procedures for freshwater mussels may not sufficiently encompass the unionoid life cycle, which can exceed 50 years (Naimo, 1995). Thus, while direct extrapolation of results from *C. fluminea* to native Unionoidea is inherently limited, field bioassays employing caged *C. fluminea* may still serve as important tools for efficient detection of pollutant effects on bivalves.

Measuring biological responses at multiple levels of organization facilitates mechanistic understanding of environmental contaminant effects, while simultaneously maintaining ecological relevance (Clements, 2000). Field bioassays with bivalves expose test organisms to the same factors influencing indigenous biota (Doherty and Cherry, 1988; Doherty, 1990; Soucek et al., 2001), and thus, provide mechanistic understanding of contaminant effects at lower levels, which may precede population- and community-level effects (Farris et al., 1988). Augmenting *in situ* tests with surveys of benthos provides ecologically significant information about indigenous benthic macroinvertebrate communities and can indicate impairment attributable to human activities (Rosenberg et al., 1986; Lenat and Barbour, 1993; Resh et al., 1995; Wallace et al., 1996; Smith and Beauchamp, 2000). Nonetheless, few studies have related the responses of transplanted bivalves and benthic macroinvertebrate communities in an effort to quantify the effects of environmental contaminants (Farris et al., 1988; Soucek et al., 2001; Smith and Beauchamp, 2000). The present study examines the responses of field-caged Asian clams and benthic macroinvertebrate communities to CRP effluent and a fly ash landfill, and has implications for bioassessments within streams inhabited by freshwater mussels.

1.2. Materials and Methods

1.2.1. Study site

The Clinch River originates in Tazewell County, Virginia, and flows for 611.6 river km through southwestern Virginia and eastern Tennessee (Goudreau et al., 1993). In Carbo, Virginia (Russell County), between river km 429.7 and 431.3, the river receives the effluent discharge of the CRP's wastewater treatment plant. Six sites in the Clinch River mainstem were used to assess biological effects attributable to the CRP effluent and a nearby fly ash landfill (Fig. 1.1). Study sites were similar with respect to general habitat characteristics and had similar scores (data not shown) for habitat assessments conducted in accordance with Barbour et al. (1999). Benthic substrate and cover were optimal with little evidence of sedimentation, and permitted colonization by a wide variety of benthic taxa at all sites. Riffle and run habitats were dominant although other habitats, including shallow to moderately deep pools (~1 m), woody debris, root exposed areas, and submerged macrophytes were sufficiently present to warrant their sampling during surveys. Under base-flow conditions, water depths ranged from 0.30 m in the shallowest riffles to 0.70 m in the deepest pools. Current velocities ranged from 0.23 m/sec in slow-moving pools to 0.58 m/sec in the fastest riffles. Riparian zones and vegetative cover on stream banks were well established with minimal evidence of human influence.

The first upstream reference site located on the Clinch River (CRUR1) was 4.8 km upstream of the CRP in Cleveland, Virginia (Fig. 1.1). A second reference site (CRUR2) was less than 1.0 km upstream of the discharge, near the CRP cooling water intake. The first site downstream of the CRP was located ~400 m below the effluent discharge. This site (CREFF) was divided into as many as three separate sites (CREFF1, CREFF2, and CREFF3) for Asian clam field bioassays (CREFF1-2 during 2000 and 2001 tests; CREFF 1-3 during 2002 tests), but was maintained as a single site of ~100 m in length (400-500 m below discharge) for benthic macroinvertebrate surveys. Another study site (CROE) was located near the bank opposite of CREFF but outside of the effluent's influence. An additional site was located adjacent to the coal fly ash landfill (CRFA), ~3.0 km below the CRP, on the opposite bank of the effluent's influence. This site was least similar to the others being located in a channel of the Clinch River with higher shading, greater water depth, and reduced current velocity. The farthest downstream site (CRDR1) was located more than 4.0 km downstream of the CRP, on the same bank as the effluent discharge. We used this site to assess downstream recovery from the influence of the effluent.

1.2.2. Physico-chemical characteristics of study sites

Water samples were collected from all study sites during surveys and field bioassays in clean 1-L Nalgene® bottles. Samples were taken from just below the surface of the water and bottles were completely filled to minimize agitation during transport. Bottles were immediately placed on ice for storage at ~4°C until further analysis. Measures of pH, conductivity, temperature, and dissolved oxygen (DO) were obtained in the field; alkalinity and hardness values were determined in the laboratory. We used an Accumet® (Fisher Scientific, Pittsburgh, PA, USA) pH meter with an Accumet gel-filled combination electrode (accuracy $< \pm 0.05$ pH at 25°C) to measure pH. Dissolved oxygen was measured using a Yellow Springs model 54A meter calibrated for elevation (Yellow Springs, Yellow Springs, OH, USA). A Yellow Springs model 30 conductivity meter® (Yellow Springs, Yellow Springs, OH, USA) was used to measure specific conductivity (accuracy $\pm$ 0.5%). Total hardness and alkalinity (as mg/L $CaCO_3$) were measured through colorimetric titrations in accordance with APHA et al. (1998).

*1.2.3. Field bioassays with Asian clams**

In 2000 and 2001, *C. fluminea* were obtained using clam rakes from the New River, near Ripplemead, Virginia, because of its thriving Asian clam population (Soucek et al., 2000, 2001). In 2002, *C. fluminea* were obtained from an upstream reference site on the Clinch River in Pounding Mill, VA. Organisms were transported to the laboratory on ice where they were held in Living Streams® (Toledo, OH) until needed for testing; transport duration never exceeded 2-h. Clams measuring between 9.0 mm and 12.0 mm were selected as test organisms because this size range (1) represented a fraction of the most frequently encountered size class within the Clinch River, (2) was readily available for testing purposes, and (3) could be measured and marked most efficiently.

For each site, five clams were uniquely marked with a slim-taper file, measured from umbo to ventral margin to the nearest 0.01 mm using Max-Cal® digital calipers, and placed into each of five replicate nylon mesh bags (18 cm wide by 36 cm long; mesh size was ~0.5 cm^2) and/or plastic, substrate-filled cages (hereafter referred to as bioboxes). A detailed description of the biobox testing procedure and a direct comparison of the biobox and mesh bag cage designs is provided elsewhere (Hull et al., in review). Test chambers were transported in ice-filled coolers (minimizes metabolic activity and the accumulation of harmful waste products) to the study sites and staked to the river bottom. During September 2000, test organisms remained at study sites for ~31 d; in May 2001 and May 2002, organisms remained at study sites for 96 d, with growth and survival being determined in the field at ~31-d intervals. Table 1.1 provides a summary of the experimental design for Asian clam field bioassays.

* Asian clams are an invasive species that should only be used to monitor systems where their populations have been previously established. *Corbicula fluminea* had invaded the Clinch River prior to this study.

At the end of the test period, the clams were retrieved to determine survival and growth. Clams were considered dead if valves were separated or if they were easily teased apart. Mean values of percent survival and growth (± SE mean) were calculated for each site. Differences in means were determined using ToxStat® software (West and Gulley, 1996) and the non-parametric Kruskal-Wallis test followed by Dunn's test (α = 0.05 level) as data were typically either non-normally distributed, heteroscedastic, or unequally replicated (due to vandalism, siltation, or other form of loss). One exception was for 2000 survival data when Fisher's test (α = 0.05 level) was used with CRUR1 as a control, because no replicates were lost and this procedure was more sensitive than the Kruskal-Wallis/Dunn's approach.

1.2.4. Benthic macroinvertebrate community analysis

Qualitative assessments of benthic macroinvertebrate communities were conducted during September 2000 and May 2001 using the U.S. Environmental Protection Agency's (US EPA) Rapid Bioassessment Protocols (RBP) (Barbour et al., 1999). Preference for qualitative rather than quantitative sampling was due to the habitat specificity and additional processing costs usually associated with quantitative sampling (e.g., Lenat, 1988). Four samples were collected at each site using a standard D-frame dip-net (800-µm mesh size) to survey riffle, run, pool, and shoreline (root exposed) habitats in approximate proportion to their representation at a given site. Samples were field-preserved in 70% ethanol, and the organisms were identified to the lowest practical taxon (usually genus; Chironomidae were identified as Tanypodinae or non-Tanypodinae) using standard keys (Pennak, 1989; Merritt and Cummins, 1996).

We selected US EPA "best candidate metrics" (Barbour et al., 1999) from the richness, composition, and feeding categories primarily based on their demonstrated sensitivity to heavy metals (Winner et al., 1980; Carlisle and Clements, 1999). The justification for their selection is that elevated metals concentrations have been frequently associated with the CRP effluent (e.g., Graney et al., 1983; Farris et al., 1988). Additional metrics were used to determine overall integrity of the river system. Richness measures included total number of taxa, Ephemeroptera-Plecoptera-Trichoptera (EPT) taxa, and Ephemeroptera taxa. Composition measures included percent EPT, Chironomidae-to-EPT ratio, and percent Ephemeroptera. Percent collector-filterers, percent scrapers, and percent predators were included as functional feeding metrics. The Shannon-Wiener index was used to assess community-level diversity. Percent Heptageniidae, percent predacious Plecoptera, and percent Hydropsychidae were among the additional metrics analyzed. Data were normally distributed and means for each parameter (±SD) were compared between sites by ANOVA, using JMP IN® software (Sall and Lehman, 1996). For pairwise analysis at an $\alpha = 0.05$ level, the Tukey-Kramer honestly significant post-hoc test in JMP IN® was used. Additionally, parameter means were compared using paired t-tests to determine whether community-level responses differed significantly ($\alpha = 0.05$) by season.

1.3. Results

1.3.1. Physico-chemical characteristics of study sites

Differences in the physico-chemical parameters measured were minimal between sites (Table 1.2). Temperatures were slightly elevated below the CRP, but differences among sites were less than 1.5 °C. Mean conductivity values increased only slightly from the uppermost reference site to the farthest downstream site; the same trend was observed for median pH values. Variations in values for alkalinity and hardness were minor among all sites. Alkalinity ranged from 141 ± 10 mg/L at CRUR1 to 171 ± 8 mg/L at CRDR1. Hardness was lowest at CRUR2 (142 ± 8 mg/L) and highest at CREFF (177 ± 30 mg/L).

1.3.2. Survival and growth of field-caged Asian clams

Clam survival and growth were reduced downstream of the CRP during the 30-d test in September 2000 (Fig. 1.2). Values for mean survival were 44 ± 10% and 40 ± 10% at CREFF1 and CREFF2, respectively, significantly lower than at all other sites. The negative mean growth values for CROE (-0.02 ± 0.02 mm), CREFF1 (-0.02 ± 0.01 mm) and CREFF2 (-0.02 ± 0.01 mm) indicated de-growth (a reduction in shell growth which can occur during periods of environmental stress) at these sites and were significantly lower than at CRUR2. Survival and growth were not significantly reduced adjacent to the fly ash landfill and appeared to recover by CRDR1.

In situ bioassays conducted for 96-d in May-August 2001 provided less variable results than 30-d tests in September (Fig. 1.2). Survival of clams was significantly reduced at CREFF1 and CREFF2 for both caging procedures. For mesh bag cages, survival at these two sites was 36 ± 10% and 24 ± 9%, respectively. Survival averaged 90% in mesh bag cages at all other sites. Similar reductions in survival were observed for biobox cages (Fig. 1.2). Survival was 52 ±10% and 20 ± 9% for CREFF1 and CREFF2, respectively. Survival averaged nearly 90% for all other sites despite the loss of three replicates at CROE to vandalism. For mesh bags, mean growth values of 0.28 ± 0.04 at CREFF1 and 0.35 ± 0.08 at CREFF2 were significantly reduced relative to upstream and adjacent reference sites, which averaged nearly 1.70 mm (Fig. 1.2). Overall, growth was greater for biobox cages relative to mesh bags and values at CREFF1 (0.41 ± 0.05 mm) and CREFF2 (0.24 ± 0.20 mm) were similarly impaired relative to upstream and adjacent reference sites, which averaged nearly 2.50 mm (Fig. 1.2). Once again, survival was not significantly impaired at CRFA and CRDR1, and despite minimal reductions in growth relative to upstream sites, recovery from CRP effluent influence was evident for both cage types.

In situ bioassays conducted during 2002 employing organisms from the Clinch River, strongly supported the findings of the previous two seasons, which used organisms from the New River (Fig. 1.2). Furthermore, the addition of a third CRP-influenced site indicated the effluent contaminant gradient extended nearly 50 m farther downstream than observed during 2000 and 2001. Survival was 36 ±10% at CREFF1, 29 ± 9% at CREFF2, and 36 ± 15% at CREFF3. Survival averaged 96% at all other sites. Growth was once again a sensitive endpoint for discerning contaminant effects downstream and despite averaging nearly 2.60 mm at all other sites, growth at CREFF1, CREFF2, and CREFF3 was 0.18 ± 0.01, 0.23 ± 0.03, and 0.22 ± 0.01, respectively. Similar to bioassays conducted during 2000 and 2001, survival was not significantly reduced at CRFA and CRDR1. Growth once again indicated recovery from CRP effluent-influence despite being minimally reduced relative to upstream reference sites.

1.3.3. Benthic macroinvertebrate communities

The metrics used to assess benthic macroinvertebrate communities provided little indication of the significant reductions we observed for survival and growth of Asian clams below the CRP. No significant differences were detected for any of the richness metrics (total taxa richness, EPT taxa richness, and Ephemeroptera taxa richness) during both seasons, and functional-feeding groups appeared to be structured more by subtle habitat variations than by CRP effluent (Table 1.3). Specific compositional metrics were more sensitive than richness metrics and related better to overall trends for survival and growth of clams (Table 1.4). Relative abundances of Ephemeroptera and EPT were reduced at CREFF relative to uninfluenced sites during both seasons of bioassessment. The relative abundance of collector-filterers was reduced at CREFF during 2000 but not 2001. Three metrics, in addition to relative abundances for Ephemeroptera and EPT, suggested impairment at CREFF during 2001. The Shannon-Wiener diversity index was significantly lower at both CREFF (0.96 ± 0.06) and CROE (0.92 ± 0.09). A conspicuous reduction in relative predator abundance was also observed at CREFF and CROE relative to other sites. The Chironomidae-to-EPT ratio increased at CREFF (0.86 ± 0.30) and the adjacent CROE site (1.15 ± 0.60). Scraper composition was significantly reduced at CRFA during both surveys.

1.3.4. Relating benthic macroinvertebrate communities and field-caged Asian clams

In 2000, clam survivorship at CREFF averaged 42%, significantly less than at all other sites (Fig. 1.2). At this same site, Ephemeroptera and EPT composition was 12.9 and 16.8%, respectively, compared to averages of 16.1 and 23.4% at all other sites (Table 1.4). This trend was observed again in 2001, but the differences between CREFF and all other sites were greater. In 2001, clam survivorship and growth (which was a more effective endpoint during 2001 relative to 2000) averaged 33% and 0.32 mm, respectively, compared to averages of nearly 90% and 1.64 mm for all other sites (Fig. 1.2). Ephemeroptera composition was 15.5% at CREFF compared to an average of

26.1% among the remaining sites. Similarly, EPT composition was 29.2% at CREFF versus an average of 43.1% at all other sites (Table 1.4)

It is important to note that at one site (CROE), certain community-level metrics were impaired while survival and growth of Asian clams were not. During 2001, significant reductions in Ephemeroptera and predator composition, the Shannon-Wiener diversity index and significant increases in Chironomidae-to-EPT ratio at CROE (Table 1.4) were not accompanied by similar reductions in survival or growth of clams (Fig. 1.2). The mean value for relative Ephemeroptera abundance at CROE (17.2 ± 2.6) was significantly lower than CRUR2 (32.2 ± 4.3) and CRDR1 (33.5 ± 5.2). Predators comprised 5.2% of the community at CROE while they averaged nearly 12% at upstream reference and downstream recovery sites. The Shannon-Wiener diversity index was lowest of all sites at CROE (0.9 versus 1.1 average for remaining sites) while the Chironomidae-to-EPT ratio of 1.15 at CROE was highest of all sites. In contrast to the community-level metrics, mean values of survival and growth for clams caged in mesh bags at CROE were 90 ± 7% and 1.75 ± 0.07 mm respectively, among the highest observed at all sites during 2001 (biobox data were excluded from this comparison due to loss of three replicates at CROE).

1.3.5. Seasonal influence on clam growth and benthic macroinvertebrate communities

Although survival of clams was similar between seasons, mean growth values for the first 30 d of Asian clam field bioassays were significantly greater during May 2001 than in September 2000 (Fig. 1.2). In addition, mean values for nearly all community-level metrics followed this same pattern, suggesting that communities were more diverse and individuals more abundant during the May 2001 survey (Tables 1.3 and 1.4). A significant deviation from this trend was that relative abundance of scrapers was highest during the September 2000 bioassessment. Mean values for three other community-level metrics (percent collector-filterers, percent Heptageniidae, and percent Hydropsychidae) did not vary significantly with season.

1.4. Discussion

Our results show that field-caged *C. fluminea* had impaired survival and growth below the CRP in Carbo, Virginia. Survival was consistently lower within direct influence of the CRP effluent during three seasons, and although growth was low and variable during the September 2000 test, the May-August 2001 and 2002 studies demonstrated impairment downstream of the CRP. Richness measures for benthic macroinvertebrate communities (total, EPT, and Ephemeroptera) did not show evidence of impairment. Other investigators have shown that richness of benthic macroinvertebrate communities is highly sensitive to metals and various other ecosystem stressors (e.g., Carlisle and Clements, 1999) and the effectiveness of EPT richness to differentiate between influenced and recovering freshwater ecosystem processes was demonstrated previously (Barbour et al., 1992; Wallace et al., 1996; Hickey and Clements, 1998). Smith and Beauchamp (2000) reported good general agreement between quantitative assessments of benthic macroinvertebrate communities and bioassays employing the fingernail clam (*Sphaerium fabale*) to monitor the influence of an industrial discharge on a headwater stream. Nonetheless, our results show that significant ecotoxicological impairment to field-caged Asian clams may not correspond to reductions in community-level richness metrics for total, EPT, and Ephemeroptera taxa. These findings suggest that (1) reliance upon benthic macroinvertebrate community metrics to evaluate stream health may not adequately protect bivalves and (2) field bioassays with a suitable bivalve species should be included with traditional bioassessments of benthic macroinvertebrate communities to more adequately discern the impacts of contaminants on freshwater bivalves.

During 2000 and 2001, significant reductions in *C. fluminea* survivorship and growth downstream of the CRP were similar to reductions in the relative abundances of Ephemeroptera and EPT. Van Hassel and Gaulke (1986) reported that reductions in Ephemeroptera abundance could be used to establish on-site water quality criteria in the Clinch River, and EPT organisms, particularly Ephemeroptera, have been frequently employed as indicators of ecosystem health (Clements et al., 1990; Diamond et al., 1993; Wallace et al., 1996; Hickey and Clements, 1998). Furthermore, Soucek et al. (2001) reported a significant positive correlation between relative abundance of Ephemeroptera and survival of field-caged *C. fluminea* in headwater streams impacted by acid-mine drainage. Thus, the relationship between survival of Asian clams and relative abundance of Ephemeroptera may convey information about a variety of watersheds and pollutant influences at levels permissive of both mechanistic understanding and ecological relevance.

Decreases in relative predator and collector-filterer abundance, the Shannon-Weiner diversity index and an increase in the Chironomidae-to-EPT ratio were similar to reductions in clam survivorship and growth. However, their occurrence during only one season of sampling and considerable variability among study sites attenuates their potential application to bioassessments. Nevertheless, they may provide intriguing direction for future research. Relationships between clam survivorship and growth and functional feeding groups such as predators or collector-filterers might exist under specific conditions. Clam growth has been previously related to the relative abundance of collector-filterers by Soucek et al. (2001) who reported a significant, positive correlation between collector-filterers and growth of field-caged *C. fluminea* in headwater tributaries influenced by dilute nutrient inputs. The association between clam survival and the Shannon-Wiener diversity index suggested that clam mortality was similar to variations in benthic macroinvertebrate community diversity. However, factors other than toxic influences may structure diversity index values (Hughes, 1978), and while the ecological relevance of diversity indices has been questioned (e.g., Rosenberg and Resh, 1996), researchers have found this particular index to be both effective (e.g., Jhingran et al., 1989) and ineffective (e.g., García-Criado et al., 1999) at detecting pollutant influence. The negative association between clam survival and Chironomidae-to-EPT ratio suggests that decreases in clam survival coincided with shifts in community-level composition from sensitive to more tolerant benthic macroinvertebrate taxa. Increases in relative abundance of chironomids tend to coincide with increases in environmental contaminants such as heavy metals (e.g., Savage and Rabe, 1973; Armitage, 1980, Winner et al., 1980; Clements et al., 1988).

The season of bioassessment may affect the degree to which metrics for benthic macroinvertebrate communities relate to survival and growth of Asian clams. This may be partially due to the increases in taxonomic composition of benthic macroinvertebrate communities that we observed during the Spring 2001 bioassessment. Benthic macroinvertebrate communities vary seasonally due to such factors as changes in water temperatures, dissolved oxygen level, photoperiod, flow regime, nutrient availability and individual life history patterns (Resh and Rosenberg, 1984). Asian clam responses also vary seasonally (Mattice and Wright, 1986), and year-to-year variations in life span, growth, and population dynamics (e.g., natality) can be remarkable (McMahon and Williams, 1986; Williams and McMahon, 1986). Smith and Beauchamp (2000) reported a seasonal effect upon growth of field-caged *Sphaerium fabale*; relative to tests conducted in the summer months, differences in growth between study sites were generally less during the fall. In our study, seasonal effects (e.g., variable thermal regimes, changes in nutrient dynamics) were likely contributors to, for example, the differences in clam growth at CROE between September 2000 and May 2001, although verification of this claim requires further research over multiple seasons. Nevertheless, attempts to extrapolate biological responses from one level of organization to another should be cautious of inherent seasonal variability and its effects upon lotic, freshwater ecosystems.

Many of the confounding variables inherent to surveys of benthic macroinvertebrate communities can be effectively circumvented by *in situ* studies with field-caged test organisms (Rosenberg and Resh, 1996; Smith and Beauchamp, 2000). Transplanted organisms remain caged at specific study sites, and unlike certain members of indigenous benthic macroinvertebrate communities (e.g, some mayflies, stoneflies, and caddisflies), are unable to avoid continuous or intermittent influence. Even though bivalves can temporarily avoid influences by closing their valves during periods of environmental stress (McMahon and Williams, 1986), the consequences of doing so may result in impaired sublethal endpoints such as growth or enzymatic activity. Effects caused by environmental contaminants vary across levels of biological organization and while individual or species level responses (e.g., Asian clam survival and growth) can be moderately controlled by experimental manipulation, community-level responses are structured by a myriad of potentially confounding variables such as habitat, drift, nutrient availability, and predator-prey interactions. Thus, factors other than environmental contaminants may well have caused, for example, the reductions in Ephemeroptera composition and community diversity at CROE and reduced scraper composition at CRFA. Alternatively, the sublethal responses (e.g., growth) of bivalves may produce false positive results when organisms are transplanted to stream reaches where their resource needs are not adequately met (e.g., Smith and Beauchamp, 2000; Soucek et al., 2001). In the present study, care was taken to assess sites of generally similar habitat (e.g., current velocity, substrate) within the mainstem of the Clinch River. Thus, habitat-related reductions in growth of *C. fluminea*, such as those reported by Smith and Beauchamp (2000) to have occurred in small, oligotrophic headwater streams, were unlikely.

Our findings necessitate further research to determine if impairment of transplanted *C. fluminea* extends to resident bivalves, particularly the Clinch River's diverse freshwater mussel fauna. Because *C. fluminea* and Unionoidea are generally filter and deposit feeders within Class Bivalvia, *in situ* toxicity tests with Asian clams may be particularly applicable to potential contaminant effects on indigenous unionoids. Furthermore, recent research by Cherry et al. (2002) reported that of eight genera of Unionidae tested from the Clinch River, all were more sensitive than *C. fluminea* to copper during acute exposures. Obvious physiological differences exist between bivalve families (e.g., Byrne and Dietz, 1997; Zheng and Dietz, 1998; Fraysse et al., 2000) and the comparative limitations of our study design do not permit direct conclusions regarding the current status of Unionoidea below the CRP. However, previous studies have reported (1) an absence of living mussel fauna for nearly 5 km below the CRP despite suitable habitat (Stansbery et al., 1986) and (2) that mortality of mussels translocated to the Clinch River were highest below the CRP (Sheehan et al., 1989). Thus, our results with *C. fluminea* may provide experimental evidence of impairment to bivalves downstream of the CRP effluent discharge.

The effects of environmental contaminants are manifested through complex biological responses (Farris et al., 1988; Clements, 2000), and those associated with dynamic industrial effluents can be especially difficult to detect because species-level impairment (e.g., impaired survival of *C. fluminea*) may be masked by apparent community-level health (e.g., similar richness of EPT taxa among reference and influenced sites). Researchers have previously noted the difficulties associated with determining the biological ramifications of complex effluents stemming from operational variability and the interaction of effluent with the biotic and abiotic components of receiving systems (Dickson and Rodgers, 1986; Fetterolf et al., 1986; Farris et al., 1988). Because richness metrics for benthic macroinvertebrate communities did not agree well with the results of our field bioassays, these metrics may not be adequate for assessing impairment to bivalves. However, the linkages between field bioassays and taxonomic composition metrics, such as relative abundance of Ephemeroptera, should be investigated further as they may provide evidence of subtle contaminant effects at more ecologically relevant levels of organization.

Because similar studies (e.g., Smith and Beauchamp, 2000; Soucek et al., 2001) have been primarily limited to anthropogenic impacts on benthic macroinvertebrate communities and transplanted bivalves in headwater streams, they may lack relevance to larger systems that are less severely degraded. For example, Smith and Beauchamp (2000) reported "good general agreement" between field bioassays with sphaerid clams and quantitative assessments of benthic macroinvertebrate communities in a headwater stream influenced by an industrial discharge and extensive alteration of physical habitat. In contrast, our study assessing Clinch River mainstem sites upstream and downstream of a complex industrial effluent does not indicate good general agreement between qualitative surveys of benthic macroinvertebrate communities and field bioassays with Asian clams. Thus, bioassays with field-caged bivalves may not only serve as additional tools for assessing stream health, but their use may be critical in detecting impairment not sufficiently demonstrated by resilient benthic macroinvertebrate communities. Furthermore, the recent standardization of field bioassay procedures employing bivalves (ASTM, 2001) and the widespread distribution of *C. fluminea* has made incorporating this tool into traditional bioassessments more feasible. Future research should (1) determine whether impairment to *C. fluminea* transplanted below the CRP extends to resident bivalves, and (2) examine the implications of these findings to bioassessments in similar systems.

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Table 1.1. Summary of experimental design for field bioassays with Asian clams (adapted from Smith and Beauchamp, 2000). Initial lengths for all clams were between 9.0 and 12.0 mm. Survival and growth were determined in the field at ~31-d intervals.

Season (duration)	Sites	Cage type	No. of replicates[a]	No. of clams/replicate (no. of clams/site)
Fall 2000 (Sept.-Oct.)	CRUR1, CRUR2, CROE, CREFF1, CREFF2, CRFA, CRDR1	Mesh bags	5	5 (25)
Spring 2001 (May-Aug.)	CRUR1, CRUR2, CROE, CREFF1, CREFF2, CRFA, CRDR1	Mesh bags	5	5 (25)
	CRUR1, CRUR2, CROE, CREFF1, CREFF2, CRFA, CRDR1	Bioboxes	5	5 (25)
Spring 2002 (May-Aug.)	CRUR1, CRUR2, CROE, CREFF1, CREFF2, CREFF3, CRFA, CRDR1	Bioboxes	5	5 (25)

[a] Some replicates were excluded due to vandalism, excessive siltation, or other form of loss

Table 1.2. Mean values (± SD) for physico-chemical parameters at Clinch River sites upstream and downstream of the CRP in Carbo, Virginia (USA). Values were obtained during *in situ* testing and sampling of benthic macroinvertebrate communities in 2000 and 2001 (n = 4).

Site Name/Designation (***GPS coordinates***)	**Temp. (°C)**	**DO (mg/L)**	**pH[a] (su)**	**Cond. (μmhos)**	**Alk. (mg/L as $CaCO_3$)**	**Hard. (mg/L as $CaCO_3$)**
CRUR1/Upstream Reference *(36°56'26"N, 82°09'72"W)*	22 ± 2	7.3 ± 0.6	8.2	338 ± 20	141 ± 10	151 ± 20
CRUR2/Upstream Reference *(36°55'86"N, 82°11'75"W)*	22 ± 2	7.4 ± 0.5	8.2	336 ± 19	147 ± 11	142 ± 8
CROE/Opposite Effluent *(36°55'70"N, 82°12'05"W)*	23 ± 1	8.0 ± 0.6	8.3	375 ± 39	153 ± 13	163 ± 18
CREFF/Effluent Influenced *(36°55'73"N, 82°12'04"W)*	23 ± 2	7.9 ± 0.5	8.3	363 ± 35	155 ± 11	177 ± 30
CRFA/Fly Ash Landfill *(36°55'69"N, 82°12'56"W)*	23 ± 1	7.6 ± 0.8	8.3	370 ± 39	158 ± 11	164 ± 20
CRDR1/Downstream Recovery *(36°55'46"N, 82°12'89"W)*	23 ± 1	7.4 ± 0.4	8.3	372 ± 39	171 ± 8	153 ± 13

[a] pH reported in median values

Table 1.3. Mean values (± SD) for benthic macroinvertebrate community metrics that indicated little difference between sites upstream and downstream of the CRP. Metrics are separated according to sampling period (September 2000 or May 2001). Means with the same letter are not significantly different ($\alpha = 0.05$).

September 2000

Parameter	*CRUR1*	*CRUR2*	*CROE*	*CREFF*	*CRFA*	*CRDR1*
Taxa richness[a]	A 18.5±1.7	A 15.8± 2.5	A 18.0±4.2	A 16.3±2.5	A 19.0±4.7	A 14.8±5.0
EPT richness[a]	A 7.5±1.7	A 6.3±1.5	A 6.0±2.8	A 6.8±1.7	A 6.8±2.1	A 6.0±2.9
Ephemeroptera richness[a]	A 4.0±0.8	A 4.3±1.5	A 3.9±1.9	A 4.3±1.0	A 4.3±1.0	A 3.3±1.3
Midge/EPT ratio	A 0.1±0.1	A 0.4±0.6	A 0.2±0.0	A 0.2±0.1	A 0.3±0.2	A 0.3±0.2
Shannon-Wiener	A 0.8±0.0	A 0.8±0.1	A 0.9±0.1	A 0.8±0.1	A 0.9±0.0	A 0.7±0.2
% Predators	A 6.1±2.2	A 2.4±1.1	A 4.8±2.5	A 4.6±2.5	A 4.4±2.3	A 3.5±1.2
% Scrapers[a]	A 65.3±10.6	A 73.3±4.2	A 62.3±9.2	A 77.4±3.3	A 51.8±8.4	A 75.0±13.8

May 2001

Parameter	*CRUR1*	*CRUR2*	*CROE*	*CREFF*	*CRFA*	*CRDR1*
Taxa richness[a]	A 29.0±2.4	A 27.0± 1.8	A 23.8±3.2	A 23±3.6	A 28.5±3.1	A 24.3±4.7
EPT richness[a]	A 15.0±2.0	A 13.5±1.7	A 14.0±1.2	A 12.8±2.2	A 15.5±1.3	A 14.3±3.0
Ephemeroptera richness[a]	A 8.0±0.8	A 7.3±1.0	A 6.5±0.6	A 6.5±1.3	A 7.8±0.5	A 7.0±1.2
% Filterers[a]	A 7.4±3.8	A 8.0±2.4	A 11.8±2.5	A 12.4±5.8	A 16.8±15.4	A 7.1±6.0
% Scrapers[a]	A 28.7±7.5	A 40.1±10.0	A 36.2±6.4	A 44.7±8.3	B 23.0±11.3	A 30.2±9.2

[a] Denotes US EPA best-candidate metric according to Barbour et al., (1999)

Table 1.4. Mean values (± SD) for benthic macroinvertebrate community metrics that indicated considerable and/or statistically significant differences between sites upstream and downstream of the CRP. Metrics are separated according to sampling period (September 2000 or May 2001). Means with the same letter are not significantly different ($\alpha = 0.05$).

September 2000

Parameter	*CRUR1*	*CRUR2*	*CROE*	*CREFF*	*CRFA*	*CRDR1*
% Ephemeroptera[a]	A	AB	AB	B	AB	B
	23.9±5.5	15.4±7.7	17.9±4.0	12.9±3.2	14.7±2.6	8.7±3.3
% EPT[a]	A	B	A	B	A	B
	27.9±4.8	18.0±7.5	24.2±2.2	16.8±3.3	30.7±8.7	16.2±9.6
% Filterers[a]	AB	B	AB	B	A	B
	21.6±6.8	10.2±6.4	12.5±7.0	7.2±1.9	26.5±5.7	11.3±9.1

May 2001

Parameter	*CRUR1*	*CRUR2*	*CROE*	*CREFF*	*CRFA*	*CRDR1*
% Ephemeroptera[a]	AB	A	B	B	AB	A
	23.5±6.5	32.2±4.3	17.2±2.6	15.5±7.4	24.2±10.6	33.5±5.2
% EPT[a]	ABC	ABC	BC	C	AB	A
	39.5±4.9	43.9±2.7	30.7±5.8	29.2±3.4	47.9±14.3	53.3±9.6
Midge/EPT ratio	AB	AB	A	AB	AB	B
	0.7±0.3	0.4±0.2	1.15±0.6	0.9±0.3	0.7±0.3	0.4±0.1
Shannon-Wiener	A	AB	C	BC	ABC	A
	1.1±0.0	1.1±0.1	0.9±0.1	1.0±0.1	1.0±0.14	1.2±0.1
% Predators	A	AB	B	B	B	AB
	19.1±8.0	9.3±1.6	5.2±1.9	5.3±4.3	8.0±2.0	10.9±5.9

[a] Denotes US EPA best-candidate metric according to Barbour et al., (1999)

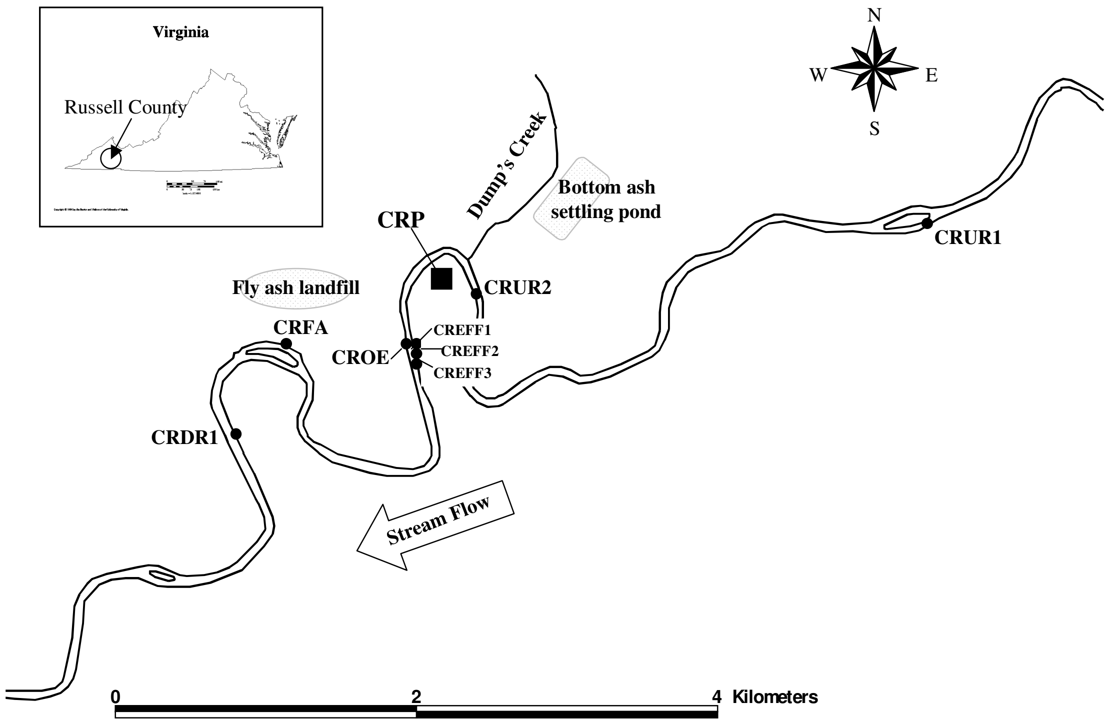

Fig. 1.1. Map of study sites used to assess biological integrity upstream and downstream of a coal-fired power plant discharge and fly ash landfill in the Clinch River watershed, Carbo, Russell County, Virginia (USA). The GPS coordinates for study sites are provided in Table 1.2.

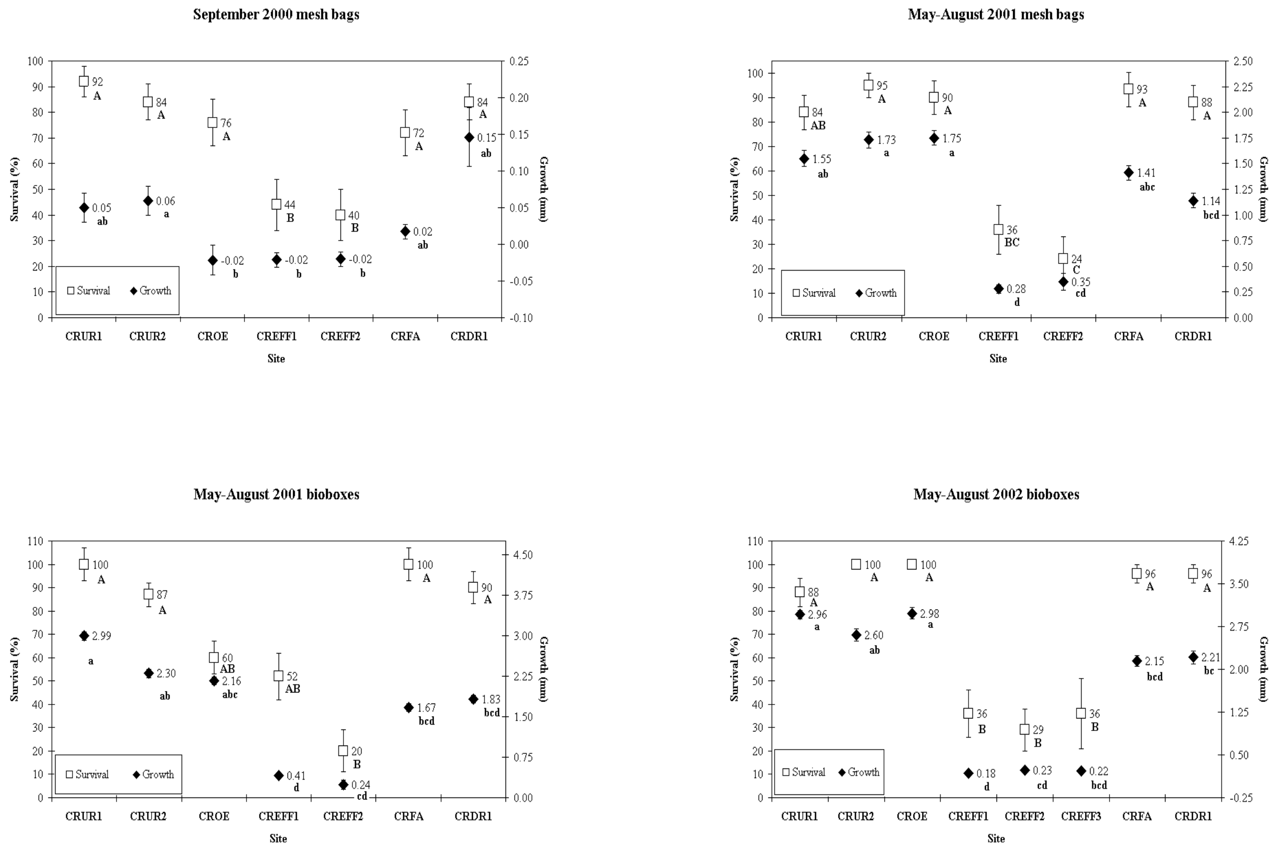

Fig. 1.2. Mean values (± SE mean) for survival and growth during *C. fluminea* field bioassays conducted in September 2000, May-August 2001, May-August 2002. Means with the same letter are not significantly different ($\alpha = 0.05$). Uppercase letters represent survival data whereas lowercase letters represent growth data.

CHAPTER 2. Effect of cage design on growth of transplanted Asian clams: implications for assessing bivalve responses in streams

Abstract. This study was designed to determine whether survival and growth of Asian clams (*Corbicula fluminea* [Müller]) differed significantly between two types of enclosures. Enclosures consisted of either flexible mesh bags or rigid cages (hereto after referred as bioboxes) designed to homogenize substrate among study sites and more effectively accommodate Asian clam feeding mechanisms. For 96-d, cages remained at 12 Clinch River (CR), Hurricane Fork (HF), and Dump's Creek (DC) sites upstream and downstream of a coal-fired power plant discharge, coal mining effluent, and coal-combustion related disposal facilities in Carbo, Virginia. Although survival was not significantly different between caging devices, mean growth of clams in bioboxes was significantly greater overall (p=0.0157). Despite the difference in growth between the two cages, both confirmed significant impairment of survival and growth directly below the power plant discharge. Additionally, coefficient of variation values for biobox growth data were reduced at 8 of 12 study sites (averages of 14.5% for bioboxes versus 23% for mesh bags), suggesting that measurements of growth were more precise from bioboxes at most study sites. Our results have implications toward strengthening weight-of-evidence approaches used to link impairment of transplanted bivalves to environmental contaminants.

2.1. Introduction

In situ toxicity tests with field-caged Asian clams (*Corbicula fluminea* [Müller]) have been effectively used to detect various sources of ecotoxicological impairment (e.g., Belanger et al., 1990; Soucek et al., 2001). Measured endpoints vary from mortality to sensitive sub-lethal endpoints such as growth (ASTM, 2001), cellulolytic activity (Farris et al., 1988), valve-movement behavior (Allen et al., 1996), and damage to DNA strands (Black et al., 1996, 1998). The sub-lethal endpoint of growth integrates all physiological processes that occur in an organism (Sheehan, 1984), has been linked to ecotoxicological impairment of bivalve populations (Bayne et al., 1985; Belanger et al., 1990), and is more sensitive than mortality (Foe and Knight, 1985; ASTM, 2001). However, as a sensitive sub-lethal endpoint, growth is more dramatically influenced by the inherent variability of natural factors (i.e., nutrients, substrates, current velocities) within and among study sites, which can result in highly variable or confounded bioassessment data (ASTM, 2001).

A suitable cage design for field bioassays with bivalves must accommodate specific aspects of bivalve ecology to accurately quantify contaminant effects. In 2001, procedures for conducting *in situ* field bioassays with marine, estuarine, and freshwater bivalves to characterize “chemical exposure and associated biological effects in the same organism under environmentally realistic conditions" were standardized by the American Society for Testing and Materials (ASTM, 2001). This document provides recommendations for the design of bivalve-containing field enclosures, which primarily relate to (1) maximization of mesh size to increase water flow to test organisms, (2) compartmentalization of chambers to permit tracking of individuals, (3) sufficient space provisions to accommodate growth throughout test duration, and (4) access of test organisms to substrate. A variety of test chambers has been used to conduct field bioassays with caged bivalves (e.g., Foe and Knight, 1987; Weber, 1988; Muncaster et al., 1990). Consequently, it is expected that each cage design will differ in the degree to which it accommodates specific aspects of bivalve ecology, such as feeding mechanisms. Asian clams have generally been considered suspension feeders, consuming primarily water column phytoplankton and detritus (Cherry et al., 1980; Reid et al., 1992). In addition, the importance of their pedal-feeding mechanism to obtain organic matter from sediments has been documented (Hakenkamp and Palmer, 1999) and may be more frequently employed to obtain nutrients than previously thought (Vaughn and Hakenkamp, 2001).

The ASTM (2001) suggests that, "comparisons of results obtained using modified and unmodified versions of [standard *in situ*] procedures might provide useful information concerning new concepts and procedures for conducting field bioassays with bivalves." Although factors other than environmental contaminants may influence sub-lethal endpoints for *in situ* test organisms, few studies have investigated their effects on bioassessment results. We were concerned that the degree of natural influence on growth of Asian clam test organisms would vary according to caging procedure and, consequently lead to discrepancies in pairwise comparisons among study sites. Specifically, we aimed to strengthen the association between impairment of field-caged *C. fluminea* and anthropogenic influences by reducing the effects of substrate on survival and growth. We conducted *in situ* tests with *C. fluminea* transplanted to sites upstream and downstream of (1) the effluent discharge of a coal-burning utility, (2) a coal processing effluent, (3) active coal-mining, (4) alkaline bottom ash leachate, and (5) a fly ash landfill, and determined whether survival and growth were significantly different among organisms field-caged in either flexible mesh bags or rigid, substrate-filled cages (hereto after referred as 'bioboxes'). Bioboxes were designed to (1) homogenize coarse substrate among study sites and (2) be less restrictive to bivalve feeding mechanisms.

2.2. Materials and Methods

2.2.1. Study area

The Clinch River originates in Tazewell County, Virginia, and flows 611.6 river km through southwestern Virginia into eastern Tennessee (Goudreau et al., 1993). Between river kms 426.5 and 431.3, the river is susceptible to environmental contaminants associated with the by-products of coal-fired, electric power generation, and active coal mining and processing within the Dump's Creek sub-watershed. Our study area contained twelve stream sites (Fig. 2.1), located between Cleveland and Carbo, Russell County, Virginia, and were located within each of three stream systems described below.

2.2.1.1. Clinch River

Eight study sites were located in the Clinch River (CR) mainstem and were used to assess ecological integrity downstream of the wastewater discharge and associated coal ash disposal facilities of American Electric Power's (AEP) coal-fired, Clinch River Plant (CRP). At these sites, the CR is a fourth-order stream of approximately 60 m in width and 0.5 to 0.8 m deep (Clements, 1988), with abundant nutrients (Parmalee and Bogan, 1998), and a substrate dominated by large pebble to intermediate cobble (Clements, 1988).

2.2.1.2. Dump's Creek and Hurricane Fork

Dump's Creek (DC) is a third-order tributary joining the Clinch River (CR) approximately 50 m above and on the opposite bank of the CRP effluent discharge. Three sites were located along this tributary to assess the influences of (1) active mining and (2) alkaline leachate from a bottom ash settling pond on downstream biota. The entire DC sub-watershed is actively mined, thus making all three of our DC study sites subject to this influence. Sites were located above, adjacent to, and below a bottom ash settling pond to assess additive toxicity attributable to this influence. Because the entire length of the DC sub-watershed is actively mined, a single reference site, free of influence from this stressor, was located in the Hurricane Fork (HF) first-order tributary of DC. Growth of Asian clams in nutrient-limited, headwater tributaries is minimal, relative to larger downstream locations (Soucek et al., 2001), so this site was primarily used to investigate effects on survival.

2.2.2. Cage construction

Figures 2.2a and 2.2b depict the two cage designs used to conduct *in situ* toxicity tests with Asian clams. One of the enclosures we evaluated has been used previously (see Soucek et al., 2000, 2001) and consisted of nylon mesh bags measuring 18 cm wide by 36 cm long, with a mesh size of ~0.5 cm^2 (see Soucek et al., 2000, 2001). Biobox enclosures were designed to be more permissive of *C. fluminea* feeding mechanisms and minimize substrate variability among study sites. Bioboxes were flow-through chambers that were constructed of rigid Sterilite® (Townsend, MA) plastic baskets (15.9 cm long by 12.7 cm wide by 5.4 cm high), and were lined with Quick Count® (Waunakee, WI) plastic mesh (2.5 mm^2 mesh size). Each biobox enclosure was filled with a ~2 cm layer of coarse, aquarium-grade substrate. To facilitate placement, bioboxes were contained within mesh bags identical to those described for the mesh bag procedure.

2.2.3. Collection and in situ deployment of Asian clam test organisms

Corbicula fluminea [Müller] served as test organisms and were collected from a thriving population of Asian clams in the New River, near Ripplemead, Virginia. Clam rakes were used to collect the clams, which were transported (~25 min) to the Virginia Tech Ecosystem Simulation Laboratory (ESL), Blacksburg, Virginia, in coolers filled with site water. Clams with a shell height (umbo to ventral margin) of 9.0 mm to 12.0 mm were selected as test organisms.

For each of the two cage designs, 25 clams (5 clams per each of 5 replicate cages) were measured for height to the nearest 0.01 mm using Max-Cal® digital calipers and uniquely marked with a slim-taper file. During May 2001, *C. fluminea* were transported to the 12 sampling locations in ice-filled coolers to deploy 10 cages (5 cages per method) with 50 test organisms at each site.

Cages were placed within an area ~2 m in radius, and comprised of uniform substrate and flow, and tied to five stakes driven into the river substratum. One mesh bag and one biobox cage was fastened to each stake and oriented so that both were in contact with the natural substrate and could receive similar flow (Fig. 2.2c). We used nearby cobble to secure cages to the streambed, and care was taken to prevent crushing the organisms during placement. Test organisms were checked for survival and growth at ~31-d intervals for a total of 96 d (May 10, 2001 to August 14, 2001). Clams were recorded as 'dead' if valves were separated or if they were easily teased apart. After monthly survival and growth examinations, cages were randomly returned to stakes to minimize the likelihood that a single cage would be exposed to the same microhabitat conditions for the duration of the experiment.

2.2.4. Statistical analyses

Mean values for percent survival and growth of clams were calculated for each site. On occasion, individual test chambers were either (1) lost completely or (2) found buried from excessive sediment deposition resulting in 100% mortality of enclosed clams. In these situations, affected replicates were excluded from analysis.

To determine whether statistically significant differences between study sites varied according to cage type, normally distributed transformations of survival and growth means for mesh bags and bioboxes were compared separately (i.e., mean values for bioboxes were never compared directly to mean values for mesh bags during this procedure), using analysis of variance (ANOVA) and JMP IN® software (Sall and Lehman, 1996).

For pairwise analysis, the Tukey-Kramer honestly significant post-hoc test (HSD) was used with significance determined at $\alpha = 0.05$ level. Analysis of variance calculations and HSD tests were performed separately for CR, DC, HF sites to prevent the comparison of lower order DC and HF sites to higher order CR sites. For direct comparisons between mean values for bioboxes and mesh bags, a paired t-test in JMP IN® software was used with $\alpha = 0.05$.

2.3. Results

2.3.1. Asian clam responses between caging procedures

Survival of Asian clams did not vary significantly between cage types in either stream, and the only significant reduction for this endpoint, directly below the CRP effluent discharge, was evident for both enclosure types. Therefore, results presented in this section are limited to the more sensitive growth endpoint. Throughout the duration of the 96-d experiment, growth of Asian clams caged in bioboxes was consistently higher than that in mesh bags (Fig. 2.3), and upon test conclusion, these differences were significant at CR ($p = 0.0015$), DC ($p = 0.0387$), and HF ($p = 0.0068$) sites.

2.3.2. Statistically significant differences among sites

Pairwise comparisons among CR sites were markedly different for the two caging procedures (Fig. 2.4a). For bioboxes, there were three levels of statistically significant differences for growth of Asian clams. Growth at the farthest upstream CR reference, CRUR1 (2.99 ± 0.21mm) was significantly greater than that at all other sites. Conversely, mean growth at CREFF (0.35 ± 0.12mm) was significantly lower than that at all other CR sites. Growth at the farthest downstream recovery site, CRDR2 (2.37 ± 0.38mm) was significantly greater than at

CRCF (1.71 ± 0.18mm) and CRFA (1.67 ± 0.26mm), but similar to that at CRUR2 (2.29 ± 0.15mm), CROE (2.31 ± 0.61mm), and CRDR1 (1.82 ± 0.21mm).

In contrast to the biobox cages, there were two levels of statistically significant differences for mesh bag enclosures at CR sites. Values for mean growth of clams at CRUR2 (1.74 ± 0.14mm), CRCF (1.86 ± 0.52mm), and CROE (1.77 ± 0.17mm) were significantly greater than those at CREFF (0.32 ± 0.13mm) and CRDR1 (1.14 ± 0.25mm), but were statistically similar to CRUR1 (1.58 ± 0.25mm), CRFA (1.40 ± 0.13mm), and CRDR2 (1.45 ± 0.24mm). Despite the dissimilarities, the site where clams were most severely impaired (CREFF) was clearly distinguished by both mesh bag and biobox enclosures.

Pairwise comparisons for the HF site and three DC sites were similar for the two enclosure types with one notable difference (Fig. 2.4b). Mean growth for DCADJ bioboxes (2.80±0.09) was significantly greater than those of all other HF and DC biobox sites, whereas mean growth for DCADJ mesh bags (2.06±0.33) was significantly greater than all but one HF and DC site, DCBL (2.09±0.09). Mean growth at HFUR1 was significantly lower than that at all other DC sites for bioboxes (0.68±0.09mm) and mesh bags (0.41±0.09mm).

2.4. Discussion

Use of field bioassays with caged bivalves to determine effects of environmental contaminants may benefit from the use of modified caging devices, specifically those providing uniform substrate to all test organisms, to separate the confounding effects of natural variables. Adams et al. (1981) suggested that cage design might indirectly affect bivalve accumulation of environmental contaminants by altering filtration rates. More recently, researchers demonstrated that enclosure type had a minimal effect on contaminant uptake (Muncaster et al., 1990). Our findings suggest that modifying *in situ* field-caging devices to be less restrictive of bivalve feeding mechanisms, while simultaneously homogenizing coarse substrate among field cages, may (1) increase organism growth, (2) reduce variability in growth data, and (3) enhance associations between environmental contaminants and sub-lethal responses of bivalves. Conversely, survival was far less variable between enclosure types than the more sensitive growth endpoint.

The increased growth of Asian clams in bioboxes relative to mesh bags may have been attributable to enhanced accommodation of bivalve feeding mechanisms in the rigid biobox structures. Unlike flexible mesh bag enclosures, bioboxes can easily support overlying mesh material and accumulated objects (e.g., cobble, debris) that may restrict siphoning activity of test organisms. Removal of suspended particulate matter through filtration is a primary component of bivalve ecology (Vaughn and Hakenkamp, 2001) and can drastically reduce water column phytoplankton and other suspended particulates (Kasprzak, 1986; Ulanowicz and Tuttle, 1992; Strayer et al., 1999). Factors influencing filtration rates of freshwater bivalves include temperature (e.g., Lauritsen, 1986), particle concentration (e.g., Paterson, 1984) and perhaps current velocity in lotic systems (Englund and Heino, 1996). Because growth of Asian clams is closely related to siphoning behavior (Haines, 1979; Foe and Knight, 1985), a field-caging device that inhibits the removal of nutrients from the water column by filter-feeding bivalves could reduce siphoning efficiency and subsequent growth.

In addition to improving conditions for siphon-feeding, bioboxes continuously facilitated the pelagic-feeding mechanisms that are facultatively used by *C. fluminea* to obtain nutrients by (1) filtering interstitial water and (2) sediment deposit-feeding (McMahon, 1991). The 2 mm^2 plastic mesh that lined bioboxes easily contained the coarse aquarium substrate for the duration of the experiment while permitting infiltration of the finer particulates preferred by Asian clams

(Belanger et al., 1985). The deposition of local particulate matter provides the only source of substrate for mesh bag enclosures and, even so, it is uncertain whether such sediment provisions are adequate for bivalves given the potential for mesh to act as an organism-to-substrate barrier. Hakenkamp and Palmer (1999) reported that *C. fluminea* "used pedal-feeding on benthic organic material to grow at a faster rate than that possible by filter-feeding alone." Other researchers have reached similar conclusions through feeding studies involving other bivalves (Yeager et al., 1994; Gatenby et al., 1996), and Raikow and Hamilton (2000) reported that deposited material represented 80% of total consumption by unionids in a nutrient-enriched, headwater stream.

Mean growth of clams caged in mesh bags exceeded that of bioboxes at only two of the 12 stream sites where field bioassays were conducted. These two sites, CRCF and DCBL, were located <20 m below and <20 m above the Clinch River and Dump's Creek confluence, respectively. At both sites, cages were repeatedly found buried by substantial accumulations of sediment. At CRCF, this accumulation was primarily from decaying plant matter while that at DCBL was from upstream nonpoint source runoff. Despite relatively unsuitable conditions at these two sites, assessing contaminant effects at these locations was essential to meeting our primary objective, and the biobox enclosure was used in an attempt to minimize the potentially confounding effects of unsuitable habitat on growth of clams. Although advantages associated with biobox cages were compromised at these two sites, mean growth of clams caged in bioboxes was greater at most study sites.

Values for coefficient of variation (CV) were reduced in biobox cages (Table 2.1). At 8 of 12 stream sites, CV values for bioboxes were reduced relative to mesh bags (average of 14.5% for bioboxes versus 23% for mesh bags). Although sub-lethal endpoints (e.g., growth) are generally more sensitive than survival, they are also more susceptible to confounding factors.

For example, stream sediments can be "highly variable on a small spatial scale" (Brumbaugh et al., 1994), and because Asian clams have demonstrated preference for specific types of sediment (Belanger et al., 1985), such variability may contribute error to sub-lethal values within a given site and confound the detection of environmental contaminant effects among study sites. The addition of a stable, uniform substrate to all cages not only benefited the deposit-feeding mechanism of Asian clams, but also unified substrate conditions within and among study sites. This afforded additional control over the inherent variability of naturally occurring substrates, yet permitted environmentally-relevant deposition of site-specific, fine particulate matter.

In situ experiments with field-caged Asian clams effectively link biotic responses to causes of ecotoxicological impairment, but in some instances, natural factors may confound results and ultimately weaken the association between such causes and their accompanying biological effects. Statistically significant impairment of Asian clams below the CRP was clearly discerned by both enclosure types, yet pair-wise comparisons among the remaining sites varied for each. For example, growth of clams in mesh bags at CRFA was statistically similar to mesh-bag clams at uppermost reference and farthest downstream recovery sites (e.g., CRUR1 and CRDR2 respectively). For bioboxes, however, clam growth at CRFA was significantly reduced relative to other biobox-caged organisms at these two sites. Such discrepancies may have implications for strengthening associations between environmental contaminants and biological impairment.

Because endpoints from *in situ* tests can be influenced by a myriad of natural variables (ASTM, 2001), controlled experiments are necessary to distinguish between alternative explanations for biological responses (Levin, 1992; Clements, 2000). Although increased experimental control over *in situ* experiments will inherently reduce environmental realism, biobox cages afford both additional experimental control (by minimizing substrate variability among study sites) and increased environmental realism (by creating more realistic microhabitat conditions). The addition of a uniform substrate to rigid field bioassay chambers not only permitted a degree of control over site-specific substrate conditions, but also created a more realistic microcosm in which the ecological processes of test organisms were uninhibited by the potential limitations of flexible mesh bags. Clams were similarly, significantly impaired below the CRP for both enclosure types suggesting that poor survival and growth of clams at this site were most likely unrelated to substrate.

In summary, the present study has demonstrated that enclosure type has a significant effect on growth of *C. fluminea*. Clams grew better in enclosures designed to accommodate specific aspects of bivalve ecology. In addition, the noticeable reduction in CV values for growth in biobox cages at most study sites suggests this enclosure type may reduce substrate variability among study sites. Survival and growth of test organisms located directly below the CRP effluent discharge were significantly impaired for both enclosure types, suggesting that impairment at this site functions independently of substrate conditions.

2.5. Acknowledgements

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Table 2.1. Coefficient of variation values (CV) for growth in biobox and mesh bag enclosures.

Site	Biobox CV (%)	Mesh bag CV (%)	Difference[a]
CRUR1	12	21	-9
CRUR2	11	21	-10
CRCF	21	9	+12
CROE	9	17	-8
CREFF	13	35	-22
CRFA	23	15	+8
CRDR1	17	23	-6
CRDR2	20	22	-2
HFUR1	18	29	-11
DCAB	22	16	+6
DCADJ	16	16	0
DCBL	15	7	+8

[a]Negative values (-) denote sites where CV values were lower for bioboxes than for mesh bags and positive values (+) denote sites where CV values were higher for bioboxes relative to mesh bags.

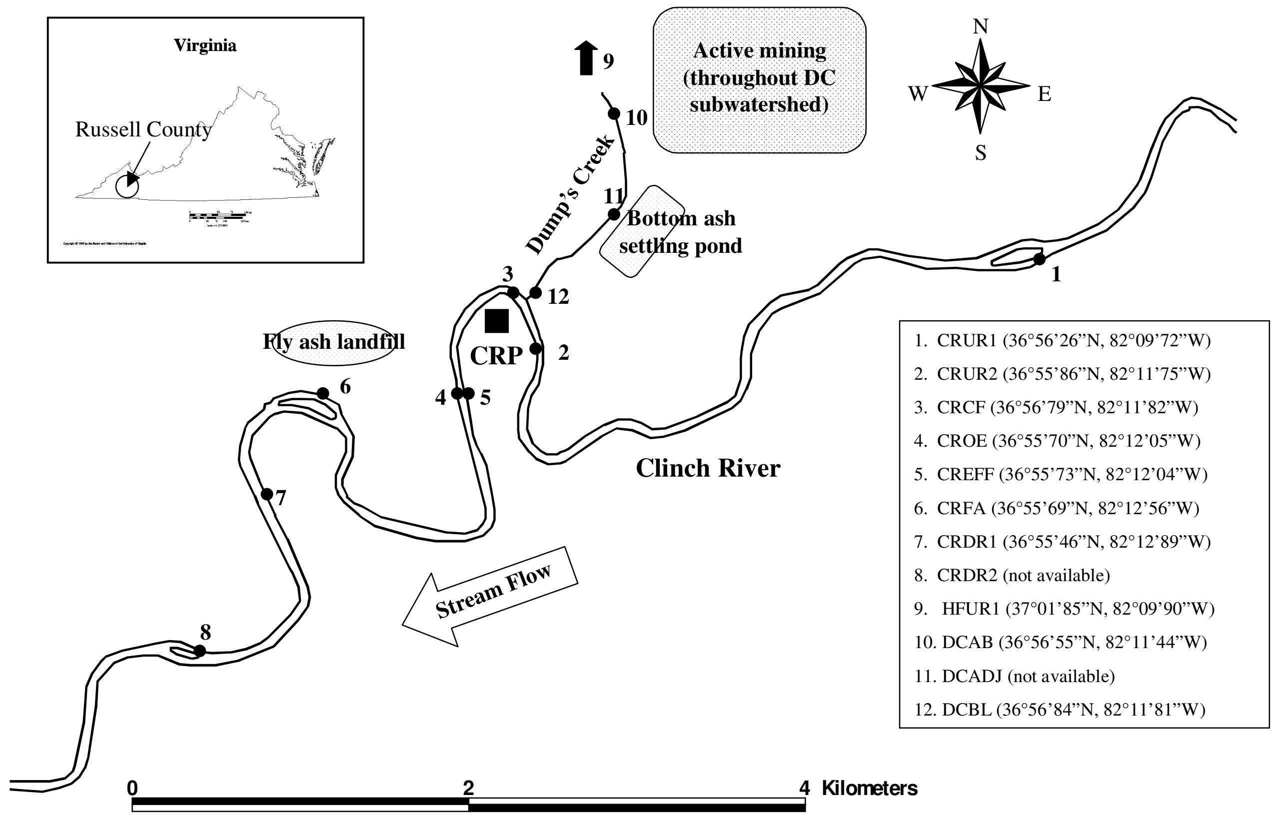


Fig. 2.1. Study area located in the Clinch River watershed, Carbo (Russell County), Virginia, USA.

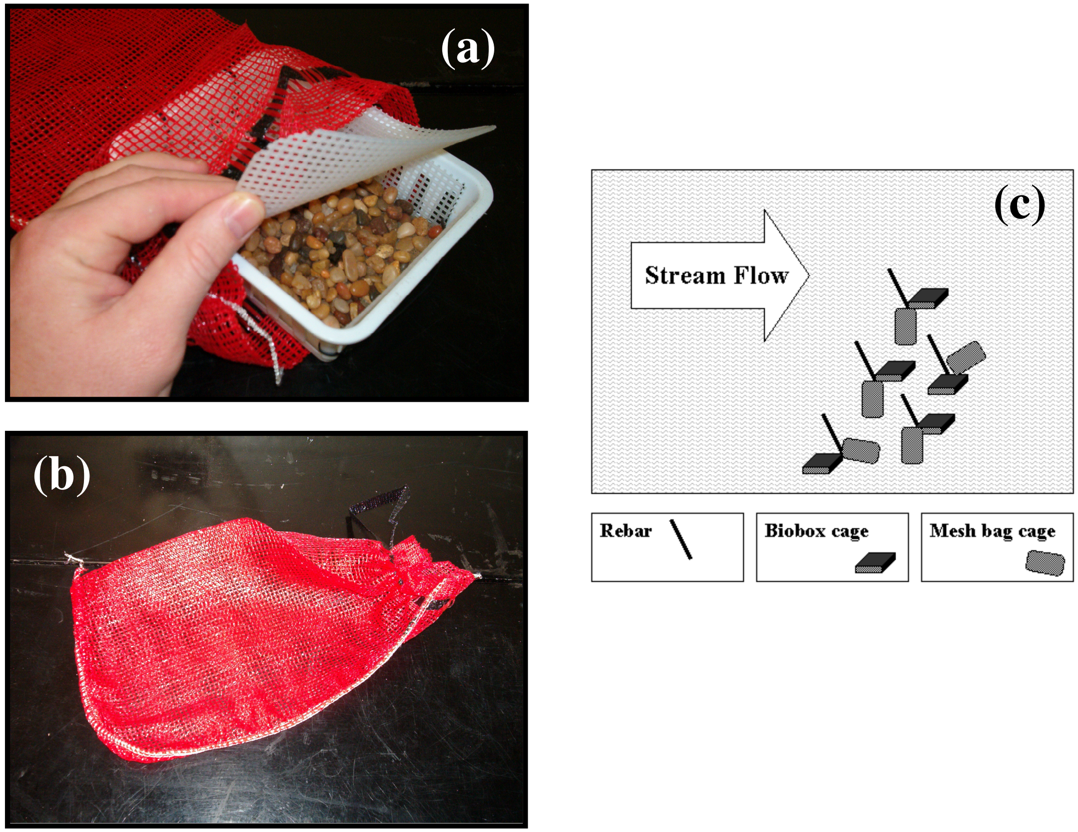


Fig. 2.2. Schematic design for (a) rigid, substrate-filled biobox cages and (b) flexible mesh bag enclosures. Panel (c) shows the method of placement for field bioassays and orientation relative to stream flow at each site.

(a)

(b)

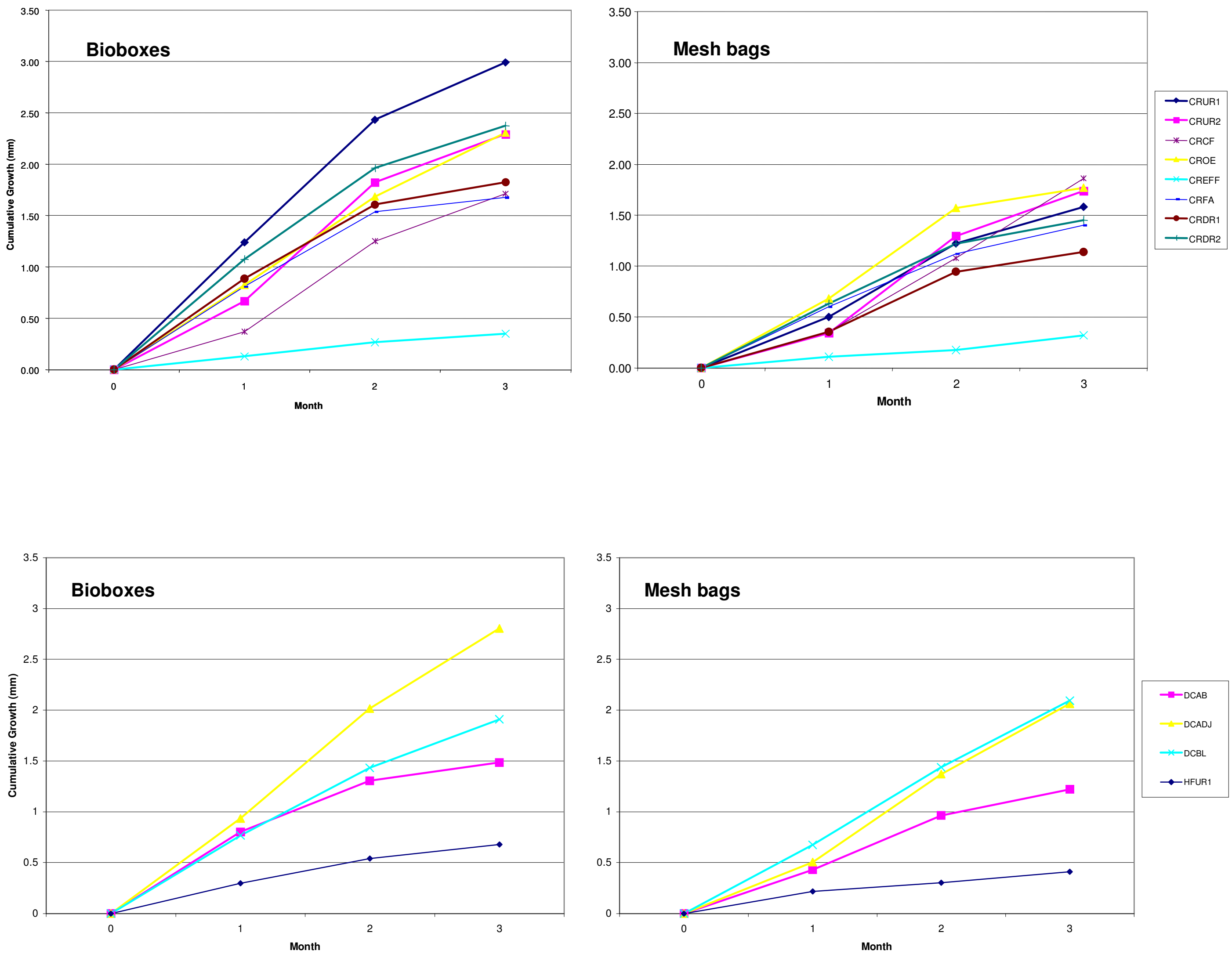
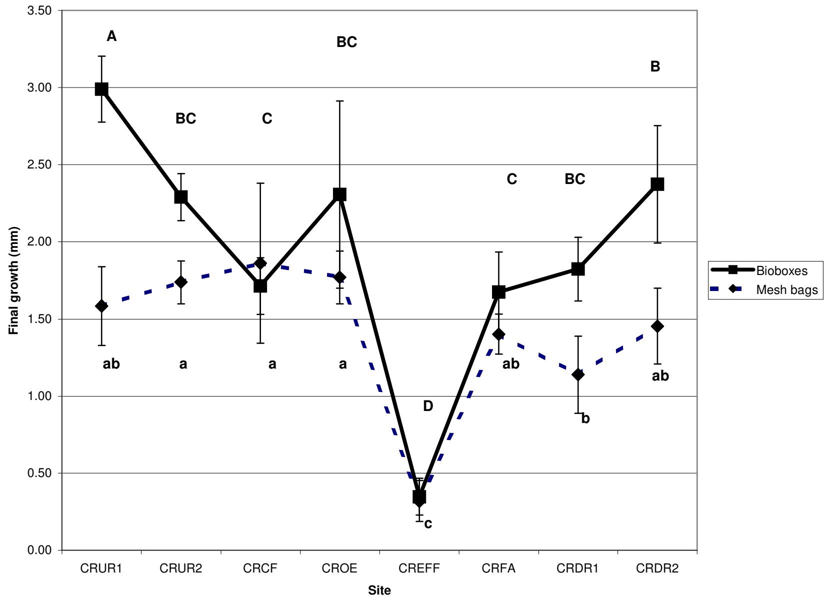


Fig. 2.3. Cumulative growth means for Asian clams contained in bioboxes and mesh bags throughout a 96-d field bioassay conducted during May-August 2001 at (a) eight Clinch River sites and (b) four Dump's Creek and Hurricane Fork tributary sites.

(a)



(b)

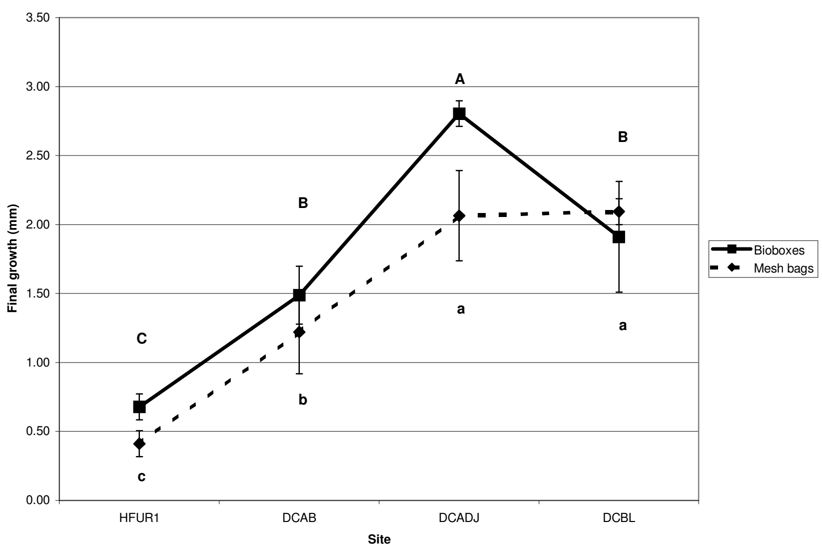


Fig. 2.4. Pairwise analyses of mean growth values for Asian clams after 96-d exposures in two types of enclosures used to assess (a) Clinch River sites and (b) Hurricane Fork and Dump's Creek sites, using Tukey's HSD with $\alpha = 0.05$. Means with the same capital or lowercase letter are not significantly different for bioboxes and mesh bags, respectively.

CHAPTER 3. Use of bivalve metrics to quantify influences of coal-related activities in the Clinch River watershed, Virginia

Abstract: In 2000-2001, surveys of benthic macroinvertebrate communities and instantaneous measures of effluent toxicity were not indicative of significant reductions in survivorship and growth of field-caged Asian clams (*Corbicula fluminea*) transplanted downstream of the coal-fired Clinch River Plant (CRP), a power-generating facility in Russell County, VA (USA). This necessitated an intensive field investigation to quantify the extent of impairment to resident bivalves, which included an indigenous assemblage of mussels (Unionoidea) identified as a national conservation priority. During 2001-2002, densities and age class structures of resident *C. fluminea* and distributions of freshwater mussels suggested that impairment extended to resident bivalves downstream of the CRP effluent for a distance of 0.5 to 0.6 km. Asian clams were virtually absent directly below the CRP, and were encountered only once during 2000-2002, when a mean of 4 clams/m^2 from one year class of recently spawned organisms (size class: 5.0 to 9.9 mm) was found. Surveys to determine the presence or absence of unionoids yielded no living specimens within 0.6 km below the CRP discharge. In 2002, field-caged rainbow mussels (*Villosa iris*), used to augment *C. fluminea* assays, had significantly reduced growth after 96 d at a site influenced by CRP effluent. Growth impairment of transplanted and resident bivalves was less evident below (1) a fly ash landfill and (2) coal mining activities and low-volume leachate from a bottom ash settling pond within the Dump's Creek tributary to the Clinch River. Our findings indicate that studies of transplanted and resident bivalves may be used, in conjunction with standard bioassessment procedures, to characterize the effects of complex effluents on receiving system biota, and prioritize source-reduction efforts in similar watersheds.

3.1. Introduction

Freshwater biota are frequently affected by environmental contaminants associated with the byproducts of coal processing and combustion (e.g., Cherry et al., 1979, 1984; Specht et al., 1984; Van Hassel et al., 1988). Negative effects have been especially evident in the Clinch River watershed near Carbo, Russell County, VA (USA), where multiple processes associated with coal mining and coal-fired electric power generation are proximate to one of the world's most diverse assemblages of native mussels (Unionoidea) (Ortmann, 1918; Ahlstedt and Tuberville, 1997; Chaplin et al., 2000). Presently, this section of the river (1) receives effluent discharged from American Electric Power's coal-fired, Clinch River Plant (CRP), (2) is joined by Dump's Creek, where active mining, coal-processing effluent, and low-volume alkaline leachate (pH>11.0) from a coal ash settling pond occur, and (3) is proximate to a coal fly ash landfill. Adverse biological effects attributable to the CRP effluent (Farris et al., 1988; Belanger et al., 1990), coal mining (García-Criado et al., 1999; Bonta, 2000), coal mine drainages (Chadwick and Canton, 1983), coal ash settling basins (e.g., Cherry et al., 1979), and trace metals associated with fly ash (Cherry and Guthrie, 1977) have been documented previously.

Distinguishing the specific effects of multiple-source perturbations, such as those described above, is often necessary to devise appropriate management strategies, and can be achieved through more integrative bioassessment techniques (Van Hassel et al., 1988). Integrated approaches to bioassessment have been favored by many researchers (e.g., Van Hassel et al., 1988; Hickey and Clements, 1998) and typically involve concurrence between controlled, laboratory experimentation and field biomonitoring data (e.g., Kimball and Levin, 1985; Hickey and Clements, 1998). Most of these studies have integrated laboratory toxicity tests with responses of benthic macroinvertebrate communities, primarily aquatic insect assemblages (Cairns and Pratt, 1993; Hickey and Clements, 1998) and have been met with varying levels of success (e.g., Pontasch et al., 1989; Eagleson et al., 1990; Dickson et al., 1992; Clements and Kiffney, 1994).

Bivalves have been used as sentinel organisms to monitor the effects of environmental contaminants on aquatic ecosystems (e.g., Haynes and Toohey, 1998; Gunther et al., 1999; Cattani et al., 1999; Hull et al., In press). Gunther et al. (1999) noted that "transplanted or resident bivalves can provide an indication of temporally and spatially averaged concentrations of bioavailable contaminants in aquatic ecosystems, thereby providing an integrated picture, for example, of the success of source reduction efforts in a watershed". As effective bioaccumulators of environmental contaminants (Graney et al., 1983), bivalves can be efficiently used in experimental procedures to integrate exposure effects over time (ASTM, 2001), and permit variable sublethal response measurements such as growth (Belanger et al., 1990), enzymatic activity (Farris et al., 1988), natality (Smith and Beauchamp, 2000) and damage to DNA strands (Black et al., 1996). Despite their documented suitability for biomonitoring in lotic freshwater ecosystems, relatively few studies have integrated the responses of transplanted and naturally occurring bivalves to environmental contaminants (Phillips, 1976; Belanger et al., 1990). Rather, most studies have related the responses of field-caged bivalves with surveys of benthic macroinvertebrate communities (Smith and Beauchamp, 2000; Soucek et al., 2001; Hull et al., In press).

Hull et al. (In press) reported that surveys of benthic macroinvertebrate communities in the Clinch River failed to reflect the severity of in-stream impairment indicated by Asian clams (*Corbicula fluminea*) transplanted downstream of the CRP effluent discharge. Similarly, laboratory toxicity tests exposing US EPA-recommended test organisms to instantaneously-collected effluent samples provided no indication of adverse effects. This is not surprising given that such procedures are not meant for direct measures of ecosystem responses (Waller et al., 1996), especially when assessing effects of moderately toxic effluents (LaPoint and Waller, 2000). Thus, the field assessments described in this manuscript were necessary to determine (1) whether impairment observed during *C. fluminea* transplant studies extended to resident bivalves, particularly imperiled unionoid fauna, and (2) whether transplanted and resident bivalves could be used to quantify effects of multiple coal mining and combustion related activities in the Clinch River watershed. To accomplish this, we conducted field-bioassays using transplanted *C. fluminea* and *V. iris*, and related these data to instream density sampling of *C. fluminea* and occurrence of native mussels.

3.2. Methods

3.2.1. Site descriptions

Study sites were selected based on their location relative to coal processing and combustion-related activities in the Clinch River (CR) mainstem, the Dump's Creek (DC) tributary to CR, and the Hurricane Fork (HF) tributary to DC (Fig. 3.1). Hurricane Fork was designated as a reference stream for DC since coal-related influences occur throughout the entire length of DC. Two mainstem CR sites, Clinch River Upstream References 1 (CRUR1) and 2 (CRUR2), and the HF sites (HFUR1 and HFUR2) were located upstream of identified CR and DC influences. Proceeding downstream along the CR mainstem from CRUR1 and CRUR2, a study site was located ~10 m downstream of the DC confluence with CR and designated as CRCF (Clinch River Confluence). Within DC, three sites were located upstream (DCUP), adjacent to (DCADJ), and downstream (DCDN) of a bottom ash settling pond that leaches low volumes (~0.034 MGD) of alkaline wastewater (pH>11.0).

From the right descending bank of the CR mainstem, DC enters ~100 m upstream and on the opposite river bank of the CRP effluent discharge. Two sites were located on opposing sides of the river ~0.5 km downstream of the CR-DC confluence and the CRP effluent discharge, to separately assess the downstream effects of DC and the CRP effluent on the CR mainstem. The first site influenced by the CRP effluent (CREFF1) is located near the left bank and is the first of three CRP-influenced sites (CREFF1, CREFF2, and CREFF3) within a ~100 m section of the river. Located opposite the effluent (unaffected by CRP effluent based on previous research) was the second CR site influenced by DC, designated as CROE (Clinch River Opposite Effluent). In 2001, two additional sites were used to separate the potential toxic effects of DC and CRP effluent. These sites, CRPUP and CRPDN (Clinch River Plant Upstream and Downstream), were located across the river from the CR-DC confluence and ~100 m upstream and ~15 m downstream of the CRP effluent discharge, respectively. The next downstream CR site was located ~3.0 km below the CRP, adjacent to a fly ash landfill situated within the CR drainage basin, and was referred to as CRFA (Clinch River Fly Ash landfill). Three sites were used to investigate downstream recovery, CRDR1, CRDR2, and CRDR3 (Clinch

River Downstream Recovery 1-3), and were located from ~1.3 to ~13.3 km below the last identified, coal-related influence.

3.2.2. Physico-chemical parameters

Methods used to measure basic water chemistry parameters at all study sites have been previously described by Hull et al. (In press). Current velocities were determined at each site using a Flo-Mate® (Marsh-McBirney Inc., Frederick, MD, USA), Model 2000, portable flow meter. A single transect was established at each site, and approximately ten measurements (m/s) were made at equidistant intervals. At stream depths below 1 m, a single measurement was taken at 60% depth. When depths exceeded 1 m, two measurements were taken at 20 and 80% depth and averaged.

Particle size distributions were determined by oven-drying sediments at 50ºC for 16 h. Sediments were then sieved and weighed to obtain the percent dry weight (%w/w) for a given size class of sediment particles. Size classes were designated as <0.045 mm, 0.045-0.150 mm, 0.150-0.850 mm, and >0.850 mm, and the percentage of particles in each class was determined. Total Organic Carbon (TOC) was measured according to US EPA Method 415.1 in *Methods for the Chemical Analysis of Water and Wastes* (US EPA, 1979).

3.2.3. Density sampling of resident Asian clams

From 1986 to 1995, the invasion of *Corbicula fluminea* [Müller] into the CR was documented by Cherry et al. (1996) and by 1987, *C. fluminea* had fully colonized the study area. Asian clams are hermaphroditic and produce juveniles annually during mid-spring through mid-summer, and late summer through late fall (McMahon and Williams, 1986). Typically, Asian clams thrive in streams with slow to moderate current (Parmalee and Bogan, 1998) and prefer substrates of mixed sand-silt-mud (Belanger, 1985; Parmalee and Bogan, 1998).

During September 2001 (post late fall-spawn), July 2002 (post late spring-spawn), and October-November 2002 (post late fall-spawn) densities of resident Asian clams were determined at most CR and DC sites using a 0.5 m^2 modified Surber Bottom Sampler. The intent of these sampling events was to determine the presence of reproducing Asian clam populations at study sites. An additional sampling event was conducted in October 2001 to determine the status of a recent year-class of *C. fluminea* (5.0-9.9 mm) discovered below the CRP during the September 2001 survey. At each site, the sampler was placed firmly on the stream bottom in four locations with flow and substrate conducive to the habitation of *C. fluminea* (McMahon, 1983; Belanger et al., 1990). The sampler consisted of a metal frame and mesh plankton netting (0.05 mm) which was held open by the current (see Rodgers et al., 1980; Belanger et al., 1990). Within the sampler perimeter, large debris was cleared by hand and sediments were vigorously disturbed using a hand-held garden tool until either hardpan or a depth of ~10 cm was reached. All materials dislodged in this manner were directed into the fine-mesh net and later sieved through 1mm^2 wire mesh. *Corbicula fluminea* were separated from substrate and placed into plastic freezer bags for transport on ice to the Ecosystem Simulation Laboratory (ESL) at Virginia Tech, Blacksburg, VA. Organisms were stored on ice for less than 24 h until living or fresh-dead specimens could be enumerated according to size classes of 0-4.9 mm, 5.0–9.9 mm, 10.0-14.9 mm, 15-20mm, and > 20.0 mm. Total densities and the average number of individuals in each size class were estimated for a 1.0 m^2 area of streambed.

3.2.4. Surveys of native mussels

Because Clinch River unionoids have been extensively surveyed (e.g., Bates and Dennis, 1978; Ahlstedt, 1984; TVA, 1988) and particularly in the vicinity of the CRP (Stansbery, 1986), our surveys were not intended to intensively re-sample and unnecessarily disturb CR mussels. Our objective was to provide more recent data with respect to the presence or absence of unionoid bivalves in relation to each of the aforementioned influences. To accomplish this, seven mainstem CR sites were surveyed during Fall 2001 (CRUR1, CRUR2, CROE, CREFF, CRFA, CRDR1, and CRDR3). At each site, two researchers used snorkel and mask to search for living mussels in stream sections with moderate to fast current and gravel-sand dominated substrates, which is preferred by mussels (see Neves and Widlak, 1987; Parmalee and Bogan, 1998). In addition to snorkel surveys, 8-10 excavations of 0.5 m^2 plots were performed at all but the CRUR1 site for the purpose of collecting subsurface mussels. Excavated samples were sieved through a ~1 mm^2 mesh screen to search for juveniles as evidence of recent recruitment.

Survey duration varied among sites and was dictated by (1) number of living mussels found, and (2) amount of time required to sufficiently survey available habitats. Surveys were never less than 2 man-hours in duration, but as many as 7 man-hours were required to sufficiently sample CRUR1, a site protected by The Nature Conservancy (TNC) for its diverse assemblage of unionoids. Mussels were removed from the substrate, photographed and identified to species, measured for shell length (anterior to posterior), and returned to the streambed at the approximate location of collection.

3.2.5. In situ testing with transplanted C. fluminea *and* V. iris

Detailed descriptions of the procedures used for in situ tests with field-caged *C. fluminea* are found in Hull et al. (In press, In review A). *Corbicula* were obtained from either the New River, near Ripplemead, VA (2000-2001), or from a site upstream in the Clinch River, near Pounding Mill, VA (2002). Clams measuring between 9.0 and 12.0 mm were selected as test organisms, uniquely marked with a slim-taper file, and placed into each of five replicate test chambers. Two types of test chambers were used, mesh bags and substrate-filled plastic cages, and have been described elsewhere (Hull et al., In review A). Test chambers were transported in ice-filled coolers to site locations (described above) and secured to the river bottom where they remained for 30 d in 2000 and 96 d in 2001 and 2002. Survival and growth were determined in the field after 30 d in 2000 and at ~31-d intervals during 2001 and 2002. Statistical analysis for in situ toxicity tests with *C. fluminea* was similar to those previously described by Hull et al. (In press, In review A).

During 2002, in situ bioassays with *C. fluminea* were augmented at CRUR1, CROE, CREFF2, CRFA, and CRDR1, using a similar assay procedure with *V. iris*. Juveniles of *V. iris* (age was ~12 mo) of 3.5 to 6.5 mm in height (umbo to ventral margin) were obtained from the Virginia Tech Aquaculture Center in May 2002. Tests were limited to five key study sites due to the limited number of cultured *V. iris* available for testing. Prior to test initiation, organisms were examined for viability (observed activity after several minutes in water), and measured for height using digital calipers. Although malacologists typically measure anterior to posterior length of mussels, we modified the procedure so that it would be similar for both *C. fluminea* and *V. iris*. Furthermore, *V.*

iris used in our study were small enough to demonstrate a measurable change in height over 96 d that could be accurately measured. Four juvenile mussels were placed into each of five replicate cages. Cages were similar to those described for *C. fluminea* biobox assays (Hull et al., In press, In review A), except that they were partitioned into four separate sections to facilitate tracking of individuals. Juvenile rainbow mussels were too fragile to undergo the marking procedure used for *C. fluminea*.

Bivalves transplanted in 2002 were immediately placed into ice-filled coolers and transported to the laboratory for tissue digestion and spectrochemical analysis (ICP-MS) of total recoverable metals according to US EPA Method 200.3 in *Methods for the Determination of Metals in Environmental Samples* (US EPA, 1991). The procedure was modified to accommodate dry tissue weights rather than wet tissue weights. Metals selected for analysis were Al, Cu, Fe, and Zn, as researchers have previously associated the accumulation of some of these metals with coal-related activities (e.g., Belanger et al., 1990).

3.3. Results

3.3.1. Physico-chemical parameters

Water chemistry parameters were generally similar among reference and influenced sites (Table 3.1a). Exceptions occurred within the DC tributary, where values for specific conductivity were more than twice those of the CR mainstem (averages of 792 μmhos and 395 μmhos for DC and CR sites, respectively). Mean water temperatures varied from 17 ± 1 °C in the headwaters of HF to 23 ± 3 °C throughout most of the CR. Dissolved oxygen levels were at saturation for all stream sites. Median pH values ranged from 8.2 to 8.5 throughout CR and DC sites, while those recorded for HF sites were somewhat reduced at approximately 7.6.

Water depths averaged 0.5 m throughout the mainstem CR, 0.3 m in DC and 0.1 m in HF (Table 3.1b). Stream width varied from an average of 33.9 m in the CR to 6.4 m in DC and 0.3 m in HF. Current velocities averaged 0.38 m/sec at CR sites, compared to 0.18 m/sec within DC and 0.15 m/sec in HF. Total organic carbon (TOC) levels were on average, higher for CR sites (3.7%) than for DC sites (2.2%), and varied from a high of 7.3% at CROE to a low of 1.3% at CRCF. Substrate particle-size distributions indicated that on average, the majority (66.9%) of sediment particles for all sites were within the 0.150-0.850 mm range followed by the 0.045-0.150 mm range (20.7%). Less than 15% of sediment particles comprised the <0.045 and >0.850 mm size classes combined. Notable exceptions to these patterns included CRUR1, where an exceptionally high percentage (20.3%) of sediment particles comprised the <0.045 mm size class. Conversely, at CRCF, the <0.045 mm size class was only 0.01% of sediment particles.

3.3.2. Density sampling of resident Asian clams

During 2001, living *C. fluminea* were found at all 11 CR and DC sites surveyed (Table 3.2). The two upstream CR reference sites contained an average of 52.5 ± 22 individuals from four size classes (0-4.9, 5-9.9, 10-14.9, and 15-20 mm), and 137 ± 30 individuals from four size classes (5-9.9, 10-14.9, 15-20, and >20 mm)/m^2, respectively. An average density of 129.5 ± 75 organisms/m^2 from four size classes (0-4.9, 5-9.9, 10-14.9, and 15-20 mm) occurred at DCUP. The average of 232.5 ± 116 individuals/m^2 from all five size classes recorded at CRCF, downstream of DC coal-related activities, was the greatest density of all CR sites sampled. The greatest overall density occurred at

DCDN, with an average of 430.5 ± 227 individuals/m^2, and all five size classes. Downstream at CROE, densities were moderately reduced with 23.5 ± 5 individuals/m^2 representing three size classes (5-9.9, 10-14.9, and 15-20 mm). On the opposite bank at CREFF1-3, directly below CRP's effluent discharge, *C. fluminea* densities were the lowest surveyed at 3.5 ± 3 individuals/m^2 with a single size class (5-9.9 mm). Adjacent to the fly ash landfill at CRFA, densities were moderately reduced and averaged 13 ± 12 organisms/m^2 with three size classes (5-9.9, 10-14.9, and 15-20 mm). Reductions in density also were observed at CRDR1, where samples yielded 5.5 ± 9 individuals/m^2 of two size classes (5-9.9 and 10-14.9 mm). Densities increased with greater distance downstream, from an average of 38.5 ± 19 individuals/m^2 from three size classes (5-9.9, 10-14.9, and 15-20 mm) at CRDR2, to an average of 191.5 ± 62 individuals/m^2 of four size classes (5-9.9, 10-14.9, 15-20, and >20 mm) at CRDR3.

Densities generally decreased at most sites during July 2002 compared to September 2001 (Table 3.2). Exceptions to this trend were CRUR1 (73.5 ± 51) and CRDR1 (16.5 ± 17), which increased in 2002, and DCUP (136.5 ± 84), CRFA (9.5 ± 8), and CRDR2 (36 ± 20), which remained relatively similar during both years. Reductions occurred at CRUR2 (31.5 ± 18), DCDN (28 ± 26), CRCF (9 ± 3), and CRDR3 (45 ± 17). No living clams were found at CREFF1-3 or CROE. At DCADJ (not sampled during 2001), average clam density was 45.5 ± 27, with four size classes. The number of size classes at each site in 2002 remained relatively similar to those recorded in 2001.

Densities increased from July 2002 at most study sites during the October-November 2002 sampling period (Table 3.2). The number of cohorts present at each site remained similar to those encountered during the previous two sampling periods. Once again, however, no living clams were found at CREFF1-3. An important distinction between the July and October-November 2002 sampling events occurred at CROE. No clams were present at this site during the July survey, whereas sampling in October-November 2002 indicated densities had increased to 23 ± 7 clams/m^2 of three cohorts. This density was nearly identical to that encountered at CROE during September 2001 (23.5 ± 5 clams/m^2). At the CR-DC confluence area, densities were 111 ± 30 clams/m^2 of three cohorts at DCDN and 64 ± 25 clams/m^2 of four cohorts at CRCF, substantially greater than in July 2002. Densities at CRFA increased to 23 ± 11 clams/m^2 of two cohorts.

3.3.3. Surveys of native mussels

Live mussels of 17 species were collected from five of the seven sites surveyed (Table 3.3). The assemblage inhabiting the uppermost reference site, CRUR1, was diverse and abundant relative to other sites surveyed (Shannon-Weiner diversity = 2.05). A total of 93 mussels from 15 species were collected at this site, including two federally listed species (*Fusconaia cor* and *Quadrula c. strigillata*), one species threatened and endangered in Virginia (*Lexingtonia dolabelloides*), and four species of special concern (*Actinonaias pectorosa*, *Fusconaia barnesiana*, *Medionidus conradicus*, and *Ptychobranchus subtentum*).

Eight mussels of two species were found just above the CRP at CRUR2 (Shannon-Weiner diversity = 0.69). Proceeding downstream to the first site influenced by DC (CROE), five mussels of two species were collected (Shannon-Weiner diversity = 0.50), one of which (*Pleurobema oviforme*) is currently listed as a species of special concern (Williams et al., 1993). Across the river at the stream sites influenced by CRP effluent, CREFF1-3, no live mussels were found. Adjacent to the fly ash landfill, CRFA, four individuals of four species were collected (Shannon Weiner diversity = 1.39). We collected 18 individuals of four species at CRDR1, the first downstream recovery site (Shannon-Weiner diversity = 1.32). No live mussels were collected at CRDR3.

3.3.4. Transplant studies using C. fluminea *and* V. iris

Hull et al. (In press, In review A) reported that during 96-d exposures in 2001-2002, survival and growth of transplanted *C. fluminea* were significantly reduced at sites directly influenced by the CRP effluent discharge (Table 3.4). At sites downstream of the DC tributary and the fly ash landfill, however, survival and growth were more similar to those of upstream reference sites. Results for HF and DC study sites indicated that growth of *C. fluminea* increased with distance, from the headwaters at HFUR1 to the lower reaches of DC and the CR-DC confluence at CRCF. Across the river from the CR-DC confluence, at the site located ~15 m below the CRP discharge (CRPDN), mean survivorship and growth were significantly reduced relative to CRPUP (Fig. 3.2).

Differences among sites were minimal for survivorship of juvenile *V. iris*, which averaged 95% at all sites, but growth after 96 d was significantly reduced at CREFF2 (Fig. 3.3a). After ~31 d, growth at CREFF2 was highest of all sites but steadily diminished with test duration, and was more than 1.0 mm less than the average for all other study sites after the 96-d exposure period. Although *V. iris* growth was greater at all study sites compared to *C. fluminea*, growth for both species was lowest at CREFF (Fig. 3.3b).

Levels of Al, Cu, Fe, and Zn accumulated in soft tissues of *C. fluminea* were highest at sites located downstream of the CRP (CREFF1-3, CRFA, CRDR1, and

CRDR2), yet no clear relationship was established between metals levels and reductions in survival or growth. The most intriguing trend was observed for Cu, which was more than twofold greater downstream of the plant than at upstream sites (Fig. 3.3c). Body burdens at CREFF1-3 and CRFA were 47 and 53 mg Cu/kg tissue, respectively. These levels decreased to 36 mg Cu/kg tissue at CRDR1, and returned to a level approximating upstream burdens by CRDR2 (24 mg Cu/kg tissue). Similarly, Cu burdens for *V. iris* were elevated downstream of the CRP. A key difference between *V. iris* and *C. fluminea* burdens however, was that the Cu levels accumulated by *V. iris* at CREFF1-3 (82 mg Cu/kg tissue) was more than three times greater than levels accumulated at all other sites, including those located further downstream (<23 mg Cu/kg tissue).

3.4. Discussion

Previous studies have elucidated the limitations of "snap-shot" (i.e., one point grab) and 24-h composite sampling of complex effluents (Waller et al., 1996; La Point and Waller, 2000). When effluent toxicity is moderate, or varies temporally, chemical and toxicological measures of instantaneously collected effluent samples may be insufficient for predicting effluent toxicity to aquatic ecosystems (La Point and Waller, 2000). Under these circumstances, bioassessments incorporating field-caged test organisms, surveys of indigenous biota, or mesocosms can be used to directly measure effluent effects on receiving system biota (La Point and Waller, 2000; Culp et al., 2000). Hull et al. (In press, In review A) reported that survival and growth of *C. fluminea* transplanted less than 0.5-0.6 km downstream of the CRP effluent discharge were significantly reduced, when compared to that at reference sites. Conversely, instantaneous and 24-h grab sampling of CRP effluent, followed by laboratory toxicity testing with US EPA test organisms provided no indication of biotic impairment (Hull, unpublished data). Similarly, qualitative assessments of macroinvertebrate communities, primarily composed of resilient aquatic insects, provided little evidence of ecotoxicological impairment below the CRP effluent discharge (Hull et al., In press). These conflicting results necessitated efforts to determine whether impairment of transplanted *C. fluminea* extended to native bivalves, most notably the imperiled species.

Population densities and age distributions for resident Asian clams supported the findings of the 2000-2002 transplant studies with *C. fluminea*. Live *C. fluminea* from multiple size classes were collected at most study sites, indicating reproducing populations of Asian clams within the CR. Downstream of the CRP wastewater discharge, however, live *C. fluminea* were encountered only once. Following the late-summer spawn in 2001, a density of ~4 clams/m^2 at CREFF1-3 from a single size class (5-9.9 mm), suggested that cohort was attempting to colonize this site. Intensive re-sampling performed ~30 d later to document the fate of these clams yielded no live specimens and indicated a failed attempt to colonize CREFF1-3.

Belanger et al. (1990) reported that CRP effluent (1) significantly reduced mortality and growth of transplanted *C. fluminea*, and (2) substantially reduced the densities of naturally occurring *C. fluminea*. A key difference between the environmental conditions reported by Belanger et al. (1990) and the present study is that in the former, Cu concentrations at impacted Clinch River sites were measured at 47.4 to 104.8 μg/L, and were directly linked to the impairment of transplanted and resident *C. fluminea*. Copper body burdens in transplanted *C. fluminea* were measured at 205 mg/kg within 0.025 km downstream, and were 161.5 mg/kg at 0.45 km. Significantly elevated Cu body burdens were recorded as far downstream as 5.5 km, but reductions of survival and growth were not evident in these reaches (Belanger et al., 1990). In the present study, monthly monitoring data for CRP wastewater indicated that during 2000-2002, effluent Cu averaged only 14.5 μg/L, and instream Cu concentrations were below detection limits at influenced sites in the CR (Hull, unpublished data). Downstream of the CRP discharge, *C. fluminea* bioaccumulated Cu to levels of 53 mg/kg at CRFA and 47 mg/kg at CREFF1-3. *Villosa* transplanted at CREFF2 had a Cu body burden of 82 mg/kg, nearly four times the levels measured in mussels transplanted to all other stream sites. While elevated Cu body burdens did not necessarily result in the significant impairment of organism fitness (e.g., as observed for *C. fluminea* transplanted to CRFA), the recurrence of elevated Cu levels and bivalve impairment downstream of the CRP suggests that this linkage warrants further attention. However, our findings reiterate the conclusion formulated by Belanger et al. (1990), which emphasizes caution when the body burdens of transplanted bivalves are used to assess stream sites potentially contaminated by metals. Further studies should (1) determine whether effluent Cu concentrations could chronically or intermittently impair bivalves at their presently reduced levels or (2) determine whether a different effluent component is responsible for bivalve impairment. In either case, further testing is warranted to identify and reduce CRP effluent toxicity to resident mollusks.

Results of qualitative surveys of native mussels further supported the conclusions derived from our Asian clam field bioassays and density sampling. Unionoids were absent downstream of the CRP effluent discharge for a distance of ~0.6 km. Across the river from CREFF1-3, five live mussels of two species were found at CROE where previous research confirmed the absence of CRP effluent effects. These findings were consistent with those of a more intensive mussel survey conducted in the vicinity of the CRP during 1985. That study reported a complete absence of mussels from the CRP-influenced side of the river for a distance of 0.6 km downstream, despite physical habitat characteristics that were similar to the opposite side of the river, where 145 live mussels of 21 species were found (Stansbery et al., 1986). In contrast to the absence of mussels below the CRP, four mussels of four species were found at CRFA during the present study. Approximately 4.5 km downstream of the CRP, recovery from effluent influence was evident as 18 live mussels of five different species were collected.

Discrepancies between *C. fluminea* densities and mussel abundance and richness at specific sampling locations (e.g., CRDR1, CRDR3) may be attributable to (1) interactive effects between Corbiculidae and Unionoidea and (2) physiological and ecological differences between these two groups of bivalves. With respect to the proliferation of *C. fluminea* into non-native habitats, Cherry et al. (1980) reported "the overall effect upon indigenous mollusks and other benthic populations may result in the competitive exclusion of the other naturally occurring mollusk populations". This displacement of indigenous mollusks may explain the incredibly high density of *C. fluminea* and absence of native mussels at CRDR3. Conversely at CRDR1, mussels were clearly the dominant bivalves, while *C. fluminea* densities at this site were markedly reduced. Different physiological and ecological considerations between these two groups of bivalves could further contribute to the discrepancies we observed. Corbiculidae demonstrate a remarkable ability to rapidly disperse and colonize stream reaches within North American drainage systems (McMahon, 1982, 1983). In addition, "its reduced age and size at maturity, high growth rates, elevated fecundity, short generation times, abbreviated life cycles and hermaphroditic reproductive schesis make this species highly adapted for reproduction and survival in disturbed, highly variable, lotic freshwater habitats" (McMahon and Williams, 1986). Conversely, Unionoidea are far less well adapted for rapid dispersal and colonization of lotic freshwater systems. This is a direct consequence of their extended pre-reproductive stage, primarily dioecious reproductive strategies, dependence upon an intermediate fish host during the larval stage, slow growth rates, and extended life cycles (Neves, 1993).

Sheehan et al. (1989) found that during the early to mid-1980s, efforts to translocate mussels below the CRP effluent discharge were unsuccessful. In the present study, survival and growth of field-caged *V. iris* were not as severely impacted below the CRP effluent discharge as was observed for field-caged *C. fluminea*. Nevertheless, growth of *V. iris* declined appreciably throughout the 96-d exposure, implying that cumulative effects might have worsened with a longer exposure period. This hypothesis could be tested using field bioassays of >96-d duration with *V. iris*. The differences in survival and growth of *C. fluminea* and *V. iris* may be due to several factors. The *C. fluminea* life cycle is markedly abbreviated, as this species typically lives less than four years (McMahon and Williams, 1986), whereas the life spans of freshwater mussels range from 10-50 years (Naimo, 1995). When compared to other freshwater organisms, Asian clams have a remarkably high filtration rate (Buttner and Heidinger, 1982; Foe and Knight, 1987) and ion transport capacity (Zheng and Dietz, 1998). Furthermore, Yeager et al. (1994) demonstrated that juvenile *V. iris* burrowed into sediments where they might find refuge from water column contaminants (Naimo, 1995). Thus, during a given exposure period, *C. fluminea* would likely (1) be exposed to a contaminant for a greater portion of its life cycle and (2) encounter an environmental contaminant more frequently than *V. iris* through its robust feeding activities. These considerations would not only explain the differences we observed for survival and growth between the two species, but might also favor the use of *C. fluminea* over long-lived and comparatively less active freshwater mussels when time-efficient biomonitoring applications are desired. The latter point, however, raises the issue of inter-specific differences in sensitivity that would require conjunctive toxicity testing with both species under controlled laboratory conditions.

Measures of habitat variability among sites did not sufficiently explain (1) significant reductions in survival and growth of field-caged Asian clams and growth of field-caged *V. iris*, (2) the virtual absence of naturally occurring *C. fluminea*, and (3) the complete absence of native mussels within 0.6 km downstream of the CRP effluent discharge. While variations in site-to-site habitat provisions could structure the distributions of naturally occurring bivalves, the effects of habitat on in situ tests are generally minimal (LaPoint and Waller, 2000). Furthermore, Hull et al. (In review, A) reported that survival and growth of *C. fluminea* transplanted downstream of the CRP were significantly reduced in two enclosure types, one of which minimized substrate variability among study sites. Habitat characteristics at CREFF1-3 were generally similar to other mainstem sites where survival and growth of transplanted bivalves were high, live mussels of multiple species were present, and *C. fluminea* populations were well established. Stansbery et al. (1986) also noted similarities in habitat between the CRP influenced section of the CR where surveyors found no live mussels, and uninfluenced stream sites where mussels were found.

Burton et al. (1996) noted, "significant advancements in understanding ecotoxicological processes and in conducting site assessments will come from the creative use of laboratory, in situ testing, and community survey approaches together". This concept has met with considerable agreement by the scientific community (e.g., Clements, 2000; Culp et al., 2000). Collectively, the bivalve metrics we used to evaluate the condition of receiving system biota (1) facilitated comparisons among stream sites potentially influenced by various coal mining and combustion-related activities in the CR watershed, and (2) indicated an adverse effect upon transplanted and resident bivalves downstream of the CRP effluent discharge. This point is clearly illustrated in Figure 3.4, where metrics measured during 2001-2002 have been normalized according to overall ranks, and sites have been grouped according to their designation as either reference, influenced, or downstream recovery sites.

Researchers have claimed that bivalves (Belanger et al., 1990) and in situ toxicity tests (LaPoint and Waller, 2000) have been under-used in monitoring the quality of aquatic resources, and our study demonstrates the importance of incorporating these techniques into traditional risk assessments. A combination of in situ toxicity testing and field density and distribution assessments with transplanted and resident bivalves, respectively, may be used to delineate the potential effects of multiple anthropogenic influences, specifically those associated with the coal industry, on a relatively small spatial scale. Furthermore, in riverine systems where the influences of complex, point-source discharges are difficult to discern using instantaneous measures of effluent toxicity, a field assessment approach incorporating transplanted and resident bivalves may be a feasible alternative for predicting effluent toxicity on receiving system biota and directing resources for source-reduction efforts.

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Table 3.1a. Mean values for water-column chemistry (± SD) at Clinch River, Dump's Creek, and Hurricane Fork study sites during 2001-2002 (N=9).

Site	Temp.	pH	DO_2	Conduct.	Alk.	Hard.
CRUR1	23±3	8.2	7.5±0.9	345±19	141±10	151±20
CRUR2	23±3	8.2	7.8±0.4	343±20	147±11	142±8
CRCF	23±3	8.4	8.3±0.7	480±155	205±32	141±1
CROE	23±3	8.4	8.8±0.8	383±36	153±13	163±18
CREFF	24±3	8.4	8.8±0.7	411±76	155±11	163±18
CRFA	24±3	8.4	8.3±0.9	393±55	158±11	164±20
CRDR1	24±3	8.4	8.4±1.1	394±53	171±8	153±13
CRDR2	24±3	8.3	8.2±0.9	394±58	156±0	-
CRDR3	23±0	8.3	9.1±0.0	410±0	-	-
HFUR1	17±1	7.6	8.2±0.4	412±9	103±28	161±1
HFUR2	20±3	7.6	7.2±1.5	729±57	-	-
DCUP	22±3	8.6	8.3±0.8	831±198	275±79	130±0
DCADJ	23±2	8.5	8.4±0.5	801±206	318±0	-
DCDN	22±3	8.6	8.9±1.0	744±222	296±71	137±14

Table 3.1b. Physical characteristics of Clinch River, Dump's Creek, and Hurricane Fork study sites during 2001-2002.

Site	Depth (m)	Width (m)	TOC (%)	Current Velocity (m/sec)	Particle Size Dist. (%)			
					<0.045mm	*0.045-0.15mm*	*0.15-0.85mm*	*>0.85mm*
CRUR1	0.3	15.4	3.1	0.54	20.3	30.5	47.0	2.2
CRUR2	0.5	41.8	1.5	0.46	3.1	16.4	75.9	4.6
CRCF	0.9	41.4	1.3	0.17	<0.1	0. 8	88. 9	10.3
CROE	0.3	46.9	7.3	0.46	7.0	29. 5	48.2	15.4
CREFF	0.4	46.9	2.1	0.58	0.9	11.8	85.8	1.4
CRFA	0.7	18.3	5.2	0.23	3.3	28.7	65.3	2.7
CRDR1	0.6	43.9	6.5	0.28	6.5	31.9	55.6	6.1
CRDR2	0.4	16.5	2.6	0.32	2.6	15.9	68.4	13.0
CRDR3	-	-	-	-	-	-	-	-
HFUR1	<0.1	0.1	-	0.15	-	-	-	-
DCUP	0.3	4.3	3.1	0.17	0.8	8.4	78.2	12.6
DCADJ	0.2	4.9	1.9	0.27	6.7	37.6	42.4	13.3
DCDN	0.3	10.1	1.6	0.10	1.1	16.0	80.1	2.8

Table 3.2. Average total densities (± SD) of *C. fluminea*/m^2 and *C. fluminea*/size class (mm) in September 2001, July 2002, and October-November 2002 at Clinch River and Dump's Creek sites upstream and downstream of activities associated with the coal industry in Russell County, VA (N=4).

	September 2001						July 2002					
Site	0-4.9	5-9.9	10-14.9	15-20	>20	Total	0-4.9	5-9.9	10-14.9	15-20	>20	Total
CRUR1	0.5	7	38	7	0	52.5±22	0	9	32	30.5	2	73.5±51
CRUR2	0	37	92	7.5	0.5	137±30	0.5	2.5	12	16.5	0	31.5±18
DCUP	3.5	34.5	82.5	9	0	129.5±75	1	28	67	40.5	0	136.5±84
DCADJ	-	-	-	-	-	-	8	19	13.5	5	0	45.5±27
DCDN	0.5	53	338.5	38	0.5	430.5±227	3	6	11.5	7	0.5	28±26
CRCF	0.5	23	188.5	20	0.5	232.5±116	0.5	4.5	2.5	1.5	0	9±3
CROE	0	11.5	11	1	0	23.5±5	0	0	0	0	0	0±0
CREFF1-3	0	3.5[A]	0	0	0	3.5±3	0	0	0	0	0	0±0
CRFA	0	7	1.5	4.5	0	13±12	0	9	0.5	0	0	9.5±8
CRDR1	0	4.5	1	0	0	5.5±9	0.5	15	1	0	0	16.5±17
CRDR2	0	14	22.5	2	0	38.5±19	4.5	29.5	1	1	0	36±20
CRDR3	0	12.5	110.5	64	4.5	191.5±62	0	5.5	5.5	34	0	45±17

[A] Population resampled 10/25/2001 and no living clams were found

	October-November 2002					
Site	0-4.9	5-9.9	10-14.9	15-20	>20	Total
CRUR1	30	65	31	23	2	150±61
CRUR2	10	47	50	31	1	138±94
DCUP	7	94	145	19	1	265±206
DCADJ	5	22	22	5	0	54±15
DCDN	7	40	5	0	0	52±25
CRCF	2	50	7	5	0	64±25
CROE	4	14	5	0	0	23±7
CREFF1-3	0	0	0	0	0	0±0
CRFA	0	19	4	0	0	23±11
CRDR1	0	37	15	0	0	52±31
CRDR2	1	64	47	0	0	111±30
CRDR3	-	-	-	-	-	-

Table 3.3. Results of qualitative mussel surveys conducted in Fall 2001 at CR sites above and below coal related activities in Russell County, VA.

	Sites						
Species	CRUR1	CRUR2	CROE	CREFF	CRFA	CRDR1	CRDR3
Actinonaias ligamentina	1	-	-	-	-	-	-
Actinonaias pectorosa SC	30	-	-	-	-	5	-
Alasmidonta marginata	1	-	-	-	-	-	-
Amblema plicata	5	-	-	-	1	-	-
Elliptio dilatata	10	4	-	-	1	-	-
Fusconaia barnesiana SC	1	-	-	-	-	-	-
Fusconaia cor F	1	-	-	-	-	-	-
Lampsilis fasciola	7	-	-	-	1	-	-
Lampsilis ovata	-	-	-	-	-	2	-
Lasmigona costata	5	-	-	-	-	-	-
Lexingtonia dolabelloides TEV	1	-	-	-	-	-	-
Medionidus conradicus SC	19	4	-	-	1	6	-
Pleurobema oviforme SC	-	-	1	-	-	-	-
Ptychobranchus fasciolaris	8	-	-	-	-	-	-
Ptychobranchus subtentum SC	1	-	-	-	-	-	-
Quadrula c. strigillata F	1	-	-	-	-	-	-
Villosa iris	2	-	4	-	-	5	-
Total no. of species	15	2	2	0	4	4	0
Total no. of mussels	93	8	5	0	4	18	0
Shannon-Weiner Diversity	2.05	0.69	0.50	0	1.39	1.32	0

SC = Species of special concern
F = Federally endangered species
TEV = Species threatened and endangered in the state of Virginia (USA)

Table 3.4. Mean values (± SE mean) for survival and growth of *C. fluminea* transplanted to sites within Hurricane Fork (HF), Dump's Creek (DC), and the Clinch River (CR). Tests were conducted for 96 d during May-August of 2001 using mesh bags and bioboxes, and during May-August 2002 using bioboxes only (for each test method N=5). Mean values followed by the same letter are not significantly different. Site-wise comparisons were conducted separately for lower order HF and DC sites (lowercase letters) and higher order CR sites (capital letters).

	May-August 2001				**May-August 2002**	
	Mesh bags		*Bioboxes*		*Bioboxes*	
Site	Survival	Growth	Survival	Growth	Survival	Growth
CRUR1	80±16 AB	1.58±0.11 AB	100±0 A	2.99±0.10 A	88±5 A	2.96±0.06 A
CRUR2	95±5 A	1.74±0.07 A	87±7 AB	2.29±0.09 BC	100±0 A	2.60±0.02 B
HFUR1	100±0 a	0.41±0.04 c	96±4 a	0.65±0.05 c	-	-
HFUR2	-	-	-	-	88±12 a	1.31±0.22 bc
DCUP	79±6 b	1.35±0.16 b	95±5 a	1.49±0.11 b	84±7 a	0.86±0.05 c
DCADJ	95±5 ab	2.07±0.16 a	92±5 a	2.80±0.04 a	100±0 a	2.02±0.13 a
DCDN	89±13 ab	2.10±0.07 a	100±0 a	1.91±0.23 b	96±4 a	1.84±0.13 ab
CRCF	[A] 47±27 AB	1.86±0.30 A	87±13 AB	1.71±0.11 BC	88±13 A	2.16±0.19 B
CROE	87±13 AB	1.77±0.09 A	[B] 60±20 ABC	2.31±0.43 ABC	100±0 A	2.98±0.11 A
CREFF1	36±16 AB	0.29±0.03 C	52±18 BC	0.46±0.10 D	36±18 B	0.16±0.03 C
CREFF2	24±15 B	0.35±0.16 C	20±8 C	0.24±0.02 D	23±7 B	0.24±0.02 C
CREFF3	-	-	-	-	27±13 B	0.23±0.02 C
CRFA	93±7 A	1.40±0.07 AB	100±0 AB	1.68±0.15 C	96±4 A	2.15±0.11 B
CRDR1	88±5 A	1.13±0.10 B	90±6 AB	1.83±0.10 BC	96±4 A	2.20±0.09 B
CRDR2	94±6 A	1.45±0.12 AB	96±4 AB	2.39±0.18 B	100±0 A	2.68±0.08 A

[A] Enclosures repeatedly found buried in anoxic sediment
[B] Three replicates lost to vandalism

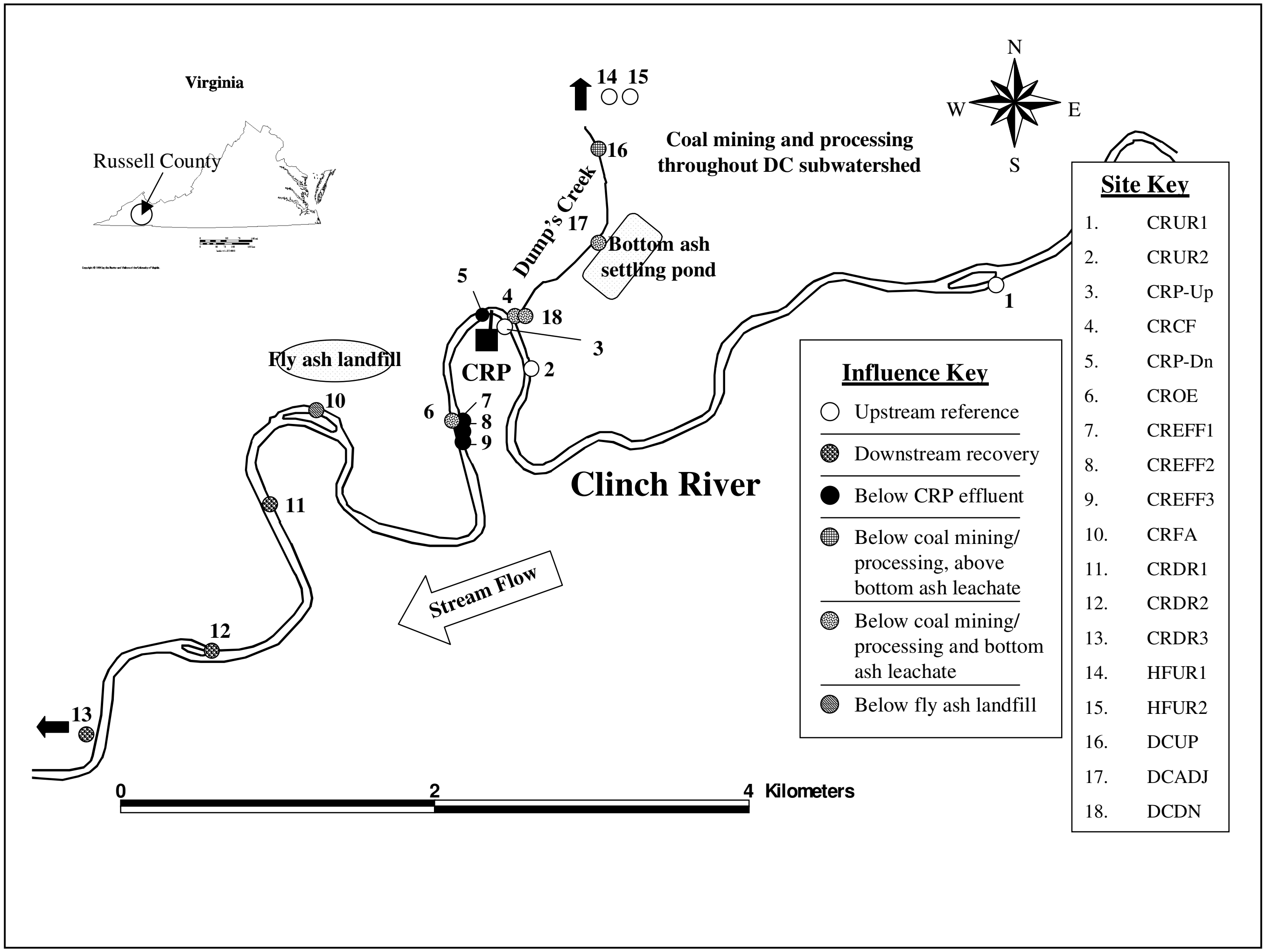
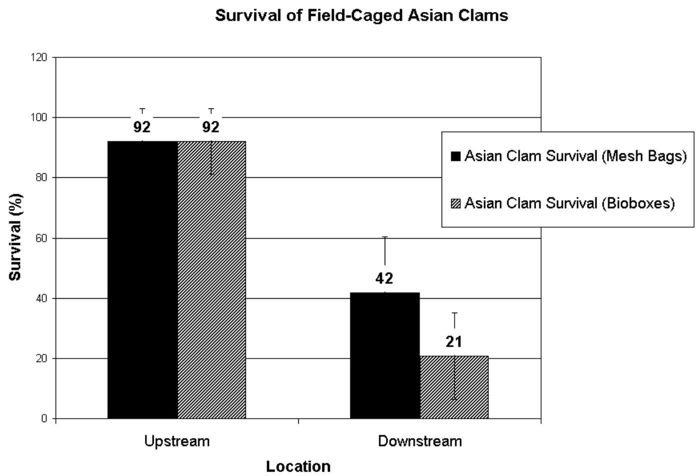


Fig. 3.1. Map of study sites used to quantify relative effects of coal-related activities on transplanted and resident bivalves in the Clinch River watershed, Carbo, Virginia, USA.

(a)



(b)

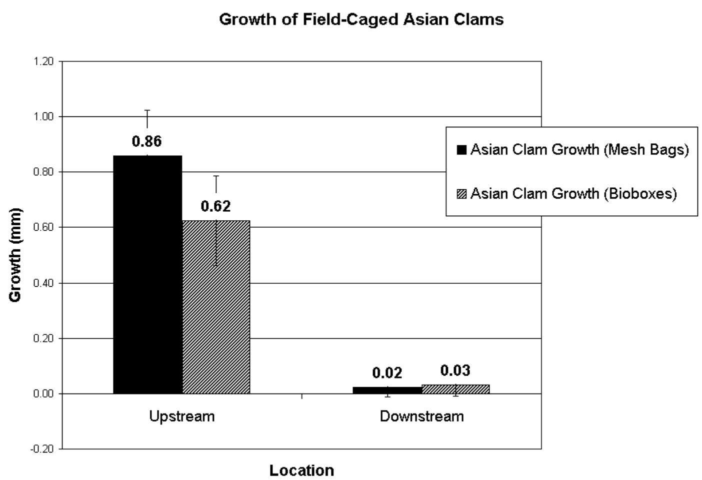


Fig. 3.2. Survival (a) and growth (b) of Asian clams in two types of field enclosures, transplanted ~100 m upstream and ~15 m downstream of the CRP effluent discharge. Sites were located on the opposing bank of the Clinch River-Dump's Creek confluence, uninfluenced by the Dump's Creek tributary. Differences between upstream and downstream values for both enclosure types were significant at $\alpha = 0.05$.

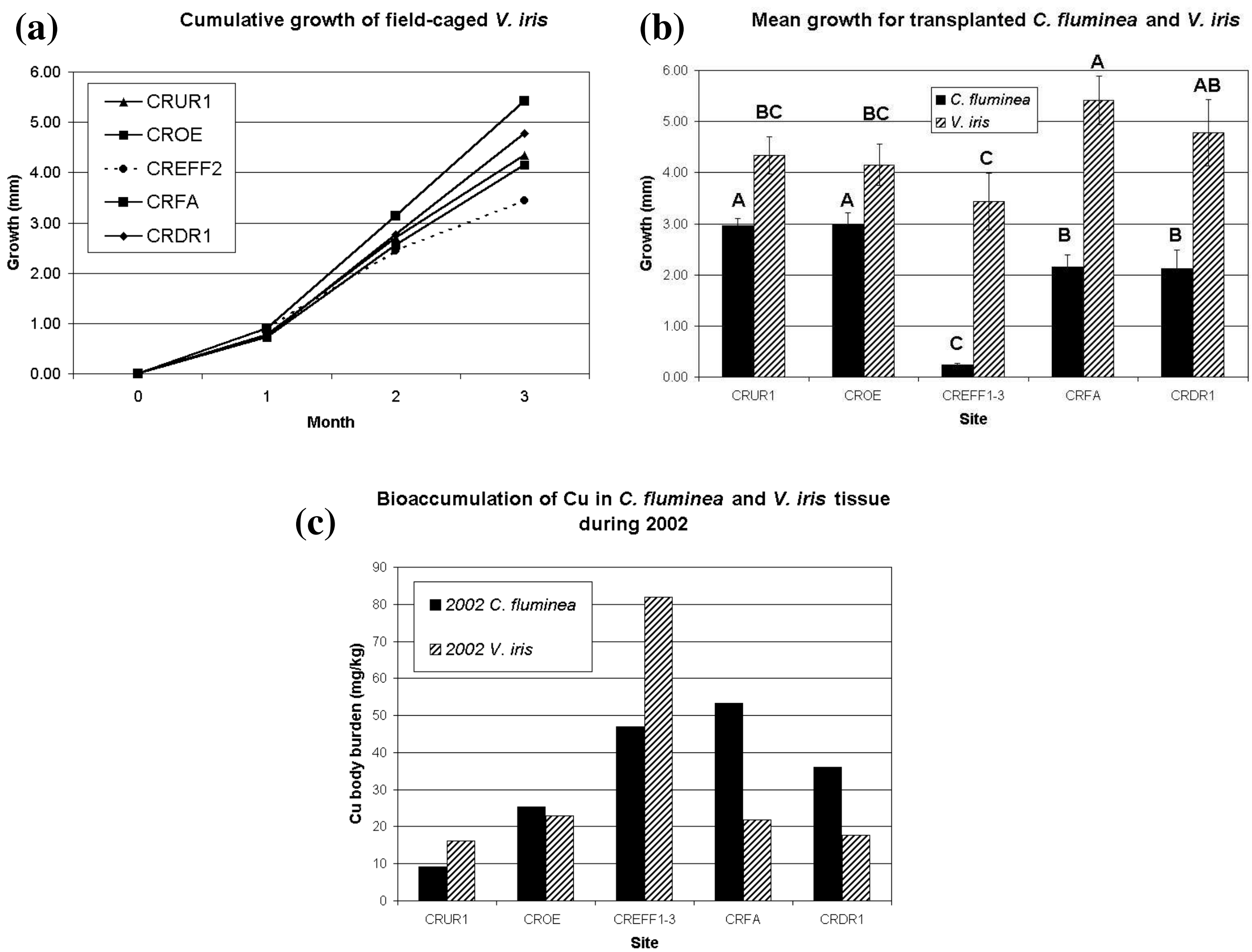


Fig. 3.3. (a) Cumulative growth of field-caged *V. iris* transplanted to mainstem CR sites for 96 d during 2002 (N=5), (b) a comparison of *V. iris* and *C. fluminea* final growth at the same five sites after the 96-d experiment, and (c) Cu levels (ppb) accumulated in transplanted bivalve tissues during 2002.

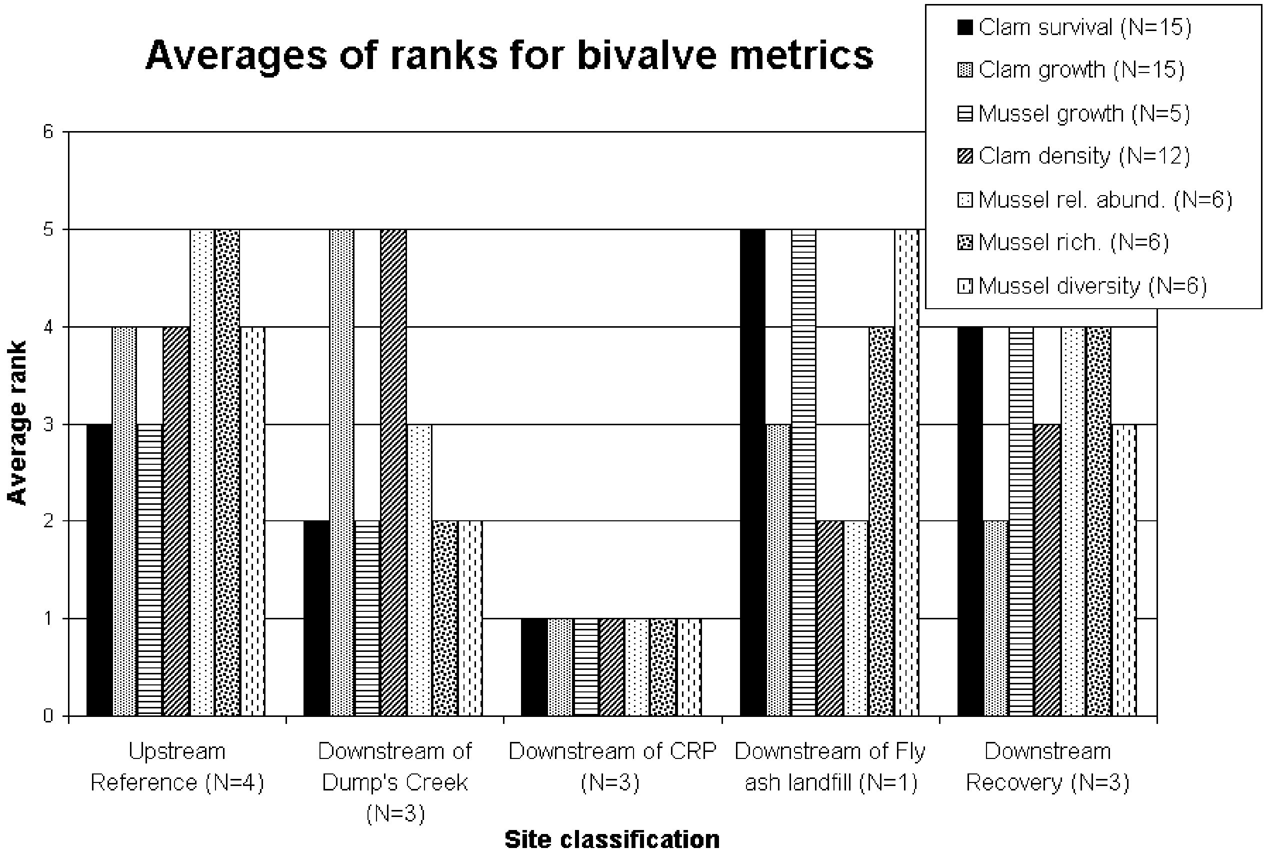


Fig. 3.4. Average ranks for various bivalve metrics used to bioassess five groups of stream sites upstream and downstream of coal-related activities in the Clinch River watershed during 2001-2002. In the legend, 'N' values denote the total number of sites at which a specified parameter was measured. Along the x-axis, 'N' values denote the total number of sites within a designated site class.

CHAPTER 4. Long-term recovery investigation of the Clinch River following coal industry-related disturbances in Carbo, Virginia (USA): 1967-2002

Abstract. Biomonitoring studies performed in Carbo, Virginia, (USA), near American Electric Power's (AEP) coal-fired Clinch River Plant (CRP) spanned more than three decades and offer a seldom encountered opportunity to document long-term biological recovery trajectories in a riverine system. Previous studies investigated spills at the CRP in 1967 (alkaline wastewater from a coal ash settling basin) and 1970 (sulfuric acid), and elevated copper (Cu) concentrations in CRP effluent that were documented from 1985-1989. Following the two spills, recovery of aquatic insect assemblages and fish communities was relatively rapid (< 2 years), but mollusks, which were reportedly decimated downstream, never recovered. The additional stress of elevated Cu concentrations in CRP effluent (documented from 1985-1989) has been linked to various forms of biotic impairment, ranging from depression of enzymatic activity in transplanted clams to shifts in aquatic insect composition from pollution-sensitive EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa to more tolerant Chironomidae. Because Clinch River fauna, primarily its diverse freshwater mussels, has been identified as a national conservation priority, further study was warranted to assess the status of these and other aquatic fauna inhabiting previously disturbed stream reaches. The purpose of the present study was to synthesize the historic and more recent studies quantifying biological integrity near the CRP, to approximate long-term recovery trajectories for aquatic insect assemblages and bivalves. Our results indicate that modifications in CRP effluent treatment have reduced Cu concentrations from an average of 436 μg/L in 1985-1989 to 13 μg/L in 1991-2002. Subsequently, Cu body burdens of Asian clams (*Corbicula fluminea*) transplanted within CRP influence have decreased from 442% of levels accumulated at reference or nominal behavior sites in 1986, to 163% of these levels in

2002. This reduction in effluent Cu largely explains recovery of most benthic macroinvertebrate community parameters (e.g., richness, diversity) at influenced sites from levels that were typically less than 70% of those observed at reference or nominal behavior sites, to levels that typically range from 80 to greater than 100% of nominal behavior. Nevertheless, bivalves remain impaired downstream of the CRP. Survivorship and growth of *C. fluminea* transplanted to CRP-influenced sites have typically been less than 40 and 30% of nominal behavior values, respectively. Furthermore, *C. fluminea* has seldom been encountered within CRP influence for nearly two decades. Likewise, native mussels remain absent within CRP influence, but recent surveys suggest their downstream distributions are more proximate to the CRP discharge than has been reported previously.

4.1. Introduction

Ecotoxicologists are challenged with determining the effectiveness of management and regulatory strategies aimed at restoring the ecological integrity of damaged ecosystems (Depledge, 1999). Nevertheless, few studies, have adequately documented recovery processes in aquatic systems in general (Power, 1999), and of those that have, most have focused on the recovery of low-order streams from natural disturbances such as floods and drought (e.g., Canton et al., 1984; Matthews, 1986). Far fewer studies have specifically addressed the recovery of large rivers from anthropogenic perturbations (Yount and Niemi, 1990). In the present study, we examine long-term biological recovery in the Clinch River, near Carbo, VA (USA), through a review of past and present biomonitoring studies that span more than three decades. These studies investigated the impact of coal industry-related activities, primarily those associated with the by-products of coal-fired electric power generation, on Clinch River biota, and offer a seldom encountered opportunity to observe long-term biological recovery trajectories in a riverine system that has been identified as a conservation priority of national interest (Chaplin et al., 2000; Cherry et al., 2002).

Researchers have previously noted that an adequate framework for quantifying the recovery of disrupted ecosystems has not been established (e.g., Cairns, 1990). Power (1999) points out that this is largely attributable to the complexity of post-disturbance ecosystems stemming from (1) spatial and temporal variability of recovery trajectories (Chapman, 1999), (2) differences in recovery rates at varying levels of biological organization (Depledge, 1999), (3) type and magnitude of disturbance (Gore et al., 1990), (4) the attributes of disturbed communities and their associated habitats (Niemi et al, 1990), (5) the lengthy time-frame and human intervention often necessary for disrupted systems to return to pre-disturbed states (Allan, 1995; Keller et al., 1999), (6) the lack of opportunities to scientifically document ecosystem recovery from a pre-disturbed state (e.g., Wiens and Parker, 1995; Minshall et al., 2001), and (7) the likelihood that, as implied by hystersis, disrupted systems may never return to their pre-disturbed state following removal of the primary disturbance (O'Neill, 1999). To address these inadequacies in the present study, we have taken an approach used by previous researchers (e.g., McDonald and Erickson, 1994) to define recovery as the point at which ecosystem parameters measured at disturbed stream sites reach levels arbitrarily deemed as comparable to reference conditions (i.e., ≥ 80% of reference measurement). We preferred this methodology because it (1) does not assume a return to "undisturbed" conditions which might never occur, and (2) incorporates the natural spatio-temporal variability of ecosystem parameters measured at reference sites, which serve as the best available representation of the stream's typical condition

4.2. Recovery Terminology

Clear definitions of key terms are essential to effectively convey ideas regarding ecosystem recovery. To address this, we have adopted a nomenclature similar to that preferred by Yount and Niemi, (1990) and Stone and Wallace (1998). Thus, "recovery" is defined as it was by Gore (1985), "a return to an ecosystem which closely resembles unstressed surrounding areas", and the term "nominal behavior" (see Gerritsen and Patten, 1985) is preferred to describe the reference conditions to which disrupted stream sites are compared (Yount and Niemi, 1990). We acknowledge that as described by O'Neill (1999), ecosystems may never recover to a pre-disturbed state following the complete removal of a stressor. Therefore, we prefer to gauge the recovery of disrupted stream sites in the context of the stream's nominal behavior, which incorporates natural spatio-temporal variability and provides the best available representation of the characteristics that might have otherwise defined a disrupted section of stream. The terms "disturbance" and "perturbation" will be used synonymously to describe an event that disrupts the "nominal behavior" of a river system, and two types of disturbance, "pulse" and "press", which were proposed by Bender et al. (1984), will be used to designate instantaneous and sustained disturbances, respectively.

4.3. Ecosystem Recovery

In lotic freshwater ecosystems, recovery from anthropogenic influences generally begins when contaminant releases are reduced or eliminated, and influenced biota either adapt to local conditions through accomodation (see Crossman et al., 1973) or are replaced by organisms from unaffected stream reaches (Waters, 1964; Depledge, 1999). Researchers generally agree that recovery is a complex process, varying temporally and across levels of biological organization (Depledge, 1999; O'Neill, 1999). At the cellular level, recovery of biochemical processes may begin immediately following alleviation of toxicant exposure or, as reported by Anderson (1980), cellular damage may remain even after a stressor has been removed (Depledge, 1999). Similarly, at the community level, recolonization of disrupted stream reaches may occur rapidly (frequently less than one year) for fish (Niemi et al., 1990) and the more mobile members of benthic macroinvertebrate communities, such as aquatic insect assemblages (Waters, 1964; MacKay, 1992).

Most investigations of recovery in lotic freshwater ecosystems have occurred in low-order streams subjected to insecticide application, logging activity, general chemical disturbances (Niemi et al., 1990) or natural disturbances such as drought or floods (Yount and Niemi, 1990), and have focused on benthic macroinvertebrates (primarily aquatic insect assemblages) and fish communities (Niemi et al. 1990). For example, Poulton et al. (1997) reported that recolonization of aquatic insect communities inhabiting riffles was rapid following a 3.3-million-L crude oil spill into the Gasconade River. Community diversity, however, remained impaired until the end of the study, 18 months later. In a study evaluating the recovery of a mountain stream from the effects of clear-cut logging, Stone and Wallace (1998) reported differing recovery trajectories for *Baetis* composition and shredder-scraper ratios relative to the NCBI (North Carolina Biotic Index). *Baetis* composition and shredder-scraper ratios recovered within five years, while the NCBI did not indicate recovery until 16 years after the initial disturbance. Schoenthal (1963) used the recolonization rates of Ephemeroptera and Diptera through drift to evaluate recovery of a Montana stream disturbed by an experimental introduction of DDT and reported complete recovery of total density and species composition within 17 months. Edwards et al. (1984) investigated the recovery of smallmouth bass (*Micropterus dolomieu*) and largemouth bass (*Micropterus salmoides*) following habitat mitigation in a channelized stream and reported recovery times of less than five years. One to two years were required for populations of various trout species to recover from DDT application (Warner and Fenderson, 1962). The central finding of such studies is that with the exception of severe press disturbances, recovery rates of most benthic macroinvertebrates and fish have been relatively rapid (Niemi et al., 1990).

Other members of lotic ecosystems, such as freshwater mussels (Unionoidea), have been less frequently incorporated into recovery assessments and may require much longer periods to fully recover if they do so at all (Starrett, 1971; Henley and Neves, 1999; Sietman et al., 2001). In contrast to the results of studies documenting the recovery of aquatic insects and fish, evidence suggests that many indigenous bivalves are far less resilient in their abilities to recover from ecosystem disturbances (e.g., Crossman et al., 1973), and this is likely a direct result of their complex life histories, lengthy generation periods, and general sensitivity to environmental perturbation (Bogan, 1993; Neves, 1993). Because of these factors, certain indigenous bivalves may be used to indicate long-term changes in water quality. In 1995, Henley and Neves (1999) evaluated the recovery of freshwater mussels at North Fork Holston River sites contaminated with mercury during 1950-1972, and reported a distance of ~20 river miles was required before recovery of mussels was evident. Following improvements in water quality and fish communities during the early 1980s, Sietman et al. (2001) documented recolonization of mussel species thought to have been extirpated from sections of the upper Illinois River during the early 20th century. Other bivalves, such as the invasive Asian clam (*Corbicula fluminea* [Müller]), possess certain characteristics (e.g., hermaphroditism, biannual reproductive strategy) that facilitate more rapid recolonization of disturbed stream sites (McMahon and Williams, 1986), and thus may have applications for assessing water quality changes on a more abbreviated time scale (e.g., Cherry et al., 1996). Clearly, however, dissimilarities between the recovery trajectories of various stream components complicate accurate recovery assessments of lotic freshwater ecosystems.

4.4. An Integrated Framework for Assessing Biotic Integrity

To effectively assess ecosystem condition, and ultimately the recovery of ecosystem structure and function, researchers must examine a suite of biological and chemical metrics that can be used to measure a variety of ecosystem components (Kelly and Harwell, 1990). These "suites" of measures have frequently consisted of some combination of laboratory and field bioassessment techniques (e.g., Van Hassel and Gaulke, 1986; Sarakinos and Rasmussen, 1998). Laboratory methods permit more rigorous control of experimental factors than field measures and offer a mechanistic understanding of natural phenomena, but have been criticized for (1) their lack of relevance to complex ecosystems (e.g., Farris et al., 1988) and (2) the inability of laboratory toxicity tests with instantaneously-collected samples to integrate long-term exposure effects (LaPoint and Waller, 2000). To address the criticisms of laboratory-based studies, field techniques heavily dependent on surveys of benthic macroinvertebrate communities have been used to quantify ecosystem disturbances (Rosenberg et al., 1986; Lenat, 1993; Resh et al., 1995; Wallace et al., 1996) and integrate ecologically-relevant exposure effects over time (Rosenberg et al., 1986).

More recently, researchers have advocated the use of (1) in situ studies with transplanted organisms (e.g., LaPoint and Waller, 2000; Smith and Beauchamp, 2000; Hull et al., In review A, C), (2) field-caged and naturally occurring bivalves (e.g., Belanger et al., 1990; Hull et al., In review A, C), and (3) mesocosm studies (e.g., Culp et al., 2000) to augment traditional bioassessment methods. Researchers that have used integrated assessments to measure the effects of anthropogenic stressors on lotic freshwater ecosystems have reported considerable agreement in smaller streams (e.g., Smith and Beauchamp, 2000; Soucek et al., 2001), whereas findings reported from riverine systems have noted contradictory biological responses that can occur across organizational levels (Farris et al., 1988; Hull et al., In review A). This is not surprising since, as previously noted, responses at higher levels of organization may indicate recovery whereas lower level responses, such as biomarkers, can continue to signal impairment (Depledge, 1999; Power, 1999). Therefore, because recovery proceeds differently with respect to spatio-temporal scale and level of biological organization (Depledge, 1999), the integration of multiple biotic and physico-chemical metrics may provide more ecologically relevant measures of ecosystem condition, and ultimately recovery, than either can independently.

4.5. Coal Industry-Related Impacts on Clinch River Biota (1967-1995)

The Clinch River originates in Tazewell County, Virginia, and drains a watershed of approximately 7,600 km^2, before joining with the Powell River at Norris Lake, and ultimately the Tennessee River near Harriman, TN (US EPA, 1997). Clinch River biota are among the most diverse in North America (Ortmann, 1918; Chaplin et al., 2000; Diamond et al., 2002), with at least four fish and 18 freshwater mussel (Unionoidea) species listed as either federally endangered, threatened, or of special concern (US EPA, 1997). Many of these species, particularly the unionoids, occur nowhere else in the world (Ortmann, 1918; Chaplin et al., 2000) and have attracted considerable attention to this otherwise obscure Appalachian stream (Stansbery et al., 1986). As summarized by Church (1997), surveys of the upper Clinch River (above Norris Reservoir) revealed as many as 42 species and subspecies (sp./spp.) of unionoids from 14 sites in the early 1900's (Ortmann, 1918). More recent surveys indicate unionoid richness has declined. Bates and Dennis (1978) reported 38 sp./spp. and Neves (1980) reported 32 sp./spp. from six sites. A comprehensive survey by Ahlstedt (1984) reported as many as 43 sp./spp. from 141 sites, but a follow-up survey in 1988 of 11 sites revealed as few as 35 sp./spp (TVA, 1988). Reported declines in Clinch River mussel fauna may be at least partially attributable to inconsistencies in survey techniques, but the wide-scale demise of freshwater mussels has been well documented (Williams et al., 1993; Ahlstedt and Tuberville, 1997).

Although coal mining has largely sustained the economies of Appalachia, particularly in southwestern Virginia (Eller, 1982), its extraction, subsequent conversion into electrical energy, and the ultimate by-products of this process have left a potentially indelible mark upon Clinch River biota. Of particular interest have been perturbations associated with American Electric Power's (AEP) Clinch River Plant (CRP), a coal-fired, power-generating facility in Carbo, Virginia. Pulse disturbances at the CRP, in the form of a large-scale, alkaline fly ash spill (pH>11) in 1967 and a more isolated sulfuric acid spill in 1970 were reported to have decimated downstream biota. Recovery from the two pulse disturbances was documented by various researchers (Cairns et al., 1971, 1973; Crossman et al., 1973; Kaesler, 1974) and indicated that with the exception of molluscs, most of the fish communities and benthic macroinvertebrate assemblages recovered within two years (Crossman et al., 1973).

In addition to the two major pulse disturbances, a form of press disturbance has occurred as the CRP contributed copper (Cu) concentrations in excess of water quality criteria until the late 1980’s (Appalachian Power Company, 1989; Cherry et al., 1996). Aquatic biota, especially the early life stages of Unionoidea, are sensitive to Cu inputs

(Jacobson et al., 1993, 1997) and researchers have documented reductions in survival, growth (Belanger et al., 1990) and cellulolytic activity (Farris et al., 1988) of transplanted Asian clams (*Corbicula fluminea*), reductions in densities of naturally occurring *C. fluminea* (Belanger et al., 1990; Cherry et al., 1996), shifts in aquatic invertebrate communities from sensitive EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa to more tolerant chironomids (Clements et al., 1988), mortality of transplanted snails, and the absence of naturally occurring snails (Reed-Judkins et al., 1998) that were directly linked to effluent Cu. Stansbery et al. (1986) reported that unionoid mussels were absent for a distance of 0.6 km downstream of the CRP effluent discharge and in doing so, established a baseline to which the results of future studies could be compared. Nevertheless, the extent to which the spills of 1967 and 1970 and the elevated effluent Cu concentrations have impaired indigenous mussels can never be fully known due to an absence of data collected prior to the plant's initial operation in 1958.

Early signs of biological recovery were noted downstream of the CRP (Cherry et al., 1996; Reed-Judkins, 1998) following revisions to the CRP NPDES (National Pollutant Discharge and Elimination System) permit, replacement of much of the CRP's cooling tower piping in 1987 (APCo, 1989), and the application of an iron-based flocculant for metals precipitation in 1993, which led to a significant reduction in effluent Cu concentrations. The long-term ramifications of these actions have yet to be studied and were undertaken by the present study. Table 4.1 summarizes notable studies that have quantified various aspects of physico-chemical and biological integrity in the Clinch River near the CRP.

4.6. Recent (2000-2002) Biomonitoring Studies

4.6.1. Physico-chemical characteristics of study sites

Hull et al. (In review, A) reported that during 2000-2001, differences in physico-chemical parameters were minimal among mainstem CR sites (Hull et al., In review A). This trend continued throughout studies conducted during 2002 (Hull et al., In review C). During sampling periods (typically May-August 2001, 2002), temperatures averaged 23 ± 3 °C at upstream sites and increased slightly (24 ± 3 °C) with distance downstream. Conductivity and pH had a similar trend, generally increasing with distance downstream. Dissolved oxygen levels were at or above saturation for all stream sites. Alkalinity ranged from 141 ± 10 mg/L to 171 ± 8 mg/L. Hardness ranged from 142 ± 8 mg/L to 177 ± 30 mg/L. Since the late 1980's, concentrations of total recoverable Cu measured in CRP effluent have been reduced by more than 97%, falling from an average of 436 μg/L during 1985-1990 to an average of 13 μg/L during 1991-2002 (Fig. 4.1).

Measures of physical habitat characteristics reported by Hull et al. (In review C) indicated that with the exception of sedimentation downstream of the CR confluence with the Dump's Creek tributary, no severe alterations of habitat were evident in the study area. Water depths varied from 0.3 m in shallow riffles to 0.9 m in the deepest pools. Stream width ranged from 15.4 to 46.9 m throughout and current velocities ranged from 0.58 m/sec in fast riffles to 0.17 m/sec in slow moving pools. Particle size distributions indicated sediment composition at all CR sites was dominated by particles within the 0.150-0.850 mm range (average of 66.9%) followed by the 0.045-0.150 mm range (average of 21.5%). Particles in the <0.045 and >0.850 mm size classes combined, accounted for less than 13% of sediment composition at a given study site. Total organic carbon (TOC) levels ranged from 1.3 to 7.3%.

4.6.2. Laboratory toxicity testing

Presently, laboratory toxicity testing using (1) *Ceriodaphnia dubia* and *Daphnia magna* survivorship and fecundity during 48-h to 7-d exposures to instantaneously collected effluent and site-collected water and (2) *C. dubia*, *D. magna*, survivorship and fecundity and *Chironomus tentans* survivorship and growth during 7-d exposures to instantaneously collected effluent and site-collected water and sediments, respectively, have indicated no observable adverse effects to test organisms. These observations may be partially due to the inherent limitations of laboratory toxicity testing procedures that have been criticized for their failure to account for the temporal variability of toxicity associated with complex industrial effluents (LaPoint and Waller, 2000; Farris et al., 1988).

4.6.3. Benthic macroinvertebrate communities

Data collected through quantitative sampling (OEPA, 1987) of Clinch River sites during 1985-1987 (Cherry et al., 1985-1988) indicated that the greatest difference between CRP-influenced sites and sites representing nominal behavior occurred in 1987, when values for richness, abundance, diversity, and Ephemeroptera composition at CRP-influenced sites were 34, 25, 41, and 43% of nominal behavior, respectively (Fig. 4.2a-d). In 1988, following reductions in effluent Cu concentrations, richness recovered to 83% and diversity to 98% of values observed for nominal behavior. The greatest increase, however, was observed for Ephemeroptera composition (Fig. 4.2d), which rose to 246% of the nominal behavior level. Sampling conducted in 2001 indicated that Ephemeroptera composition downstream of the CRP was ~80%. This level is nearly two times higher than was observed from 1985 to 1987 (average of 48%), prior to the major reductions in effluent Cu achieved by the CRP during the late 1980's and early 1990's. Total abundance at CRP-influenced sites was only 58% of nominal behavior. However, the average value for this metric was greatly influenced by remarkably high abundance values at the uppermost reference station, which may have greatly overestimated the river's nominal behavior.

During Fall 2000 and Spring 2001, qualitative sampling (see methods described in Barbour et al., 1999) indicated differences between benthic macroinvertebrate communities at reference, influenced, and recovering stream sites were minimal (Hull et al., In review A). Although historic data for qualitative benthic macroinvertebrate samples were not available, metrics considered by various researchers (e.g., Barbour et al., 1999; Carlisle and Clements, 1999) to be effective at distinguishing disturbed stream sites, particularly those influenced by power plant discharges, were similar between all study sites (Fig. 4.3a-c). No significant differences were detected for total, EPT, and Ephemeroptera richness during both sampling periods. Relative abundance of Ephemeroptera (Fig. 4.3d) was more sensitive than richness metrics, being reduced downstream of the CRP during both seasons of bioassessment (Hull et al., In review A). The most significant impairment of benthic macroinvertebrate communities observed during recent sampling (later than 2000) occurred in the spring of 2001, when Ephemeroptera composition at CRP-influenced sites was 73% of that observed at nominal behavior.

4.6.4. Field-bioassays with transplanted bivalves

Throughout 2000-2002, survivorship and growth of field-caged *C. fluminea* were more sensitive to CRP effluent than any other metric evaluated, and consistently indicated significant reductions within 0.6 km downstream of the CRP discharge (Hull et al., In review

A,B,C). During 2000, values for mean survivorship downstream of the CRP averaged 42 ± 10%, significantly less than at all other sites, but high variability and low overall growth attenuated statistical inferences on these data. Two different bioassay procedures (described in Hull et al., In review B), conducted for 96-d during May-August of 2001, provided less variable results relative to 2000 tests and indicated survivorship and growth of *C. fluminea* were significantly reduced at three sites from ~15 m to ~0.6 km downstream of the CRP discharge. A third season of field-bioassays was conducted in 2002, using *C. fluminea* transplanted from an upstream reference station on the Clinch River (previous studies employed clams from the New River, Giles Co., VA, USA) and yielded similar findings of significantly reduced survivorship and growth at three sites within ~0.6 km of the CRP discharge.

During all studies, recovery of survivorship and growth of transplanted Asian clams was apparent within 3.6 km downstream of the CRP discharge, where values for these endpoints more closely resembled those observed at upstream reference sites. Comparing 2000-2002 data to results reported by Belanger et al. (1990), suggests that since 1986, survivorship of Asian clams transplanted downstream of the CRP has not improved (Fig. 4.4a). In fact, the results of Belanger et al. (1990) indicated that survivorship of clams at CRP-influenced sites was nearly 80% of values observed for nominal behavior, but their tests were conducted for only 30 d and survivorship would likely have declined further after a 96-d exposure period. Studies conducted from 2001 to 2002 by Hull et al. (In review A) indicated survivorship at CRP-influenced sites had fallen to <40% of values observed for nominal behavior.

Relative to survivorship, growth of transplanted *C. fluminea* has been more severely impaired downstream of the CRP effluent discharge (Fig. 4.4b). In 1986, clams exhibited degrowth at influenced sites, a phenomenon observed during periods of environmental stress and reported by other researchers (see McMahon and Williams, 1986), and represented 0% of growth values observed at nominal behavior (Belanger et al., 1990). Positive growth measurements were recorded in the 96-d experiments conducted by Hull et al. (In review A,B,C) during 2001 and 2002, but growth at influenced sites was never more than 20% of nominal behavior.

During the 96-d exposures in 2002, field-bioassays with juvenile rainbow mussels (*Villosa iris*) were used at five study sites to augment experiments with *C. fluminea*. Survivorship of juvenile *V. iris* averaged 95% at all sites and was an insensitive indicator of CRP effluent toxicity (Hull et al., In review C). Upon conclusion of the test, however, growth at a site influenced by CRP effluent, which had declined precipitously after the first 31 d of testing, was significantly reduced relative to other stream sites (Hull et al., In review C), and was 73% of nominal behavior (Fig. 4.4b).

4.6.5. Bioaccumulation studies with transplanted C. fluminea *and* V. iris

According to results reported by Belanger et al. (1990), Cu levels bioaccumulated by transplanted and naturally occurring *C. fluminea* were 442 and 799%, respectively, of levels accumulated at uninfluenced sites (Fig. 4.5). Surviving organisms from the 96-d experiments conducted in 2001 and those described above for *C. fluminea* and *V. iris* were processed so that the soft tissue concentrations of Al, Cu, Fe, and Zn (which have been associated with coal-fired power plant effluents) could be determined spectrochemically. During 2001-2002, all metals were elevated in the tissues of organisms transplanted downstream of the CRP discharge, but Cu was the only metal that accumulated to high levels downstream of the CRP and not at other stream sites (Hull et al., In review C). Copper body burdens at CRP-influenced sites, though dramatically reduced from the levels reported by Belanger et al. (1990), ranged from 162% to 416% of levels at nominal behavior for transplanted *C. fluminea* and *V. iris*, respectively (Fig. 4.5).

Previous studies indicated that elevated Cu concentrations in CRP effluent impaired transplanted freshwater mussels (Cherry et al., 1996). Consequently, site-specific, acute Cu criteria were developed for the Clinch River, and unlike the US EPA database, incorporated freshwater mussels into the derivation process (Cherry et al., 2002). Based upon the findings reported by these researchers, the national CMC (Criterion Maximum Concentration) of 20 μg Cu/L "appears to be protective of the Clinch River biota, including sensitive life stages of freshwater mussels" (Cherry et al., 2002). Because we have observed impairment to transplanted *C. fluminea* and *V. iris* during long-term exposures to CRP effluent Cu concentrations that are typically well below 20 μg/L, it may be necessary for researchers to extend the efforts of Cherry et al. (2002) to include bivalves in the calculation of a site specific CCC (Criterion Chronic Concentration) as well. However, because the efforts of Hull et al. (In review A,B,C) cannot link CRP effluent Cu concentrations to observed impairment indefinitely, further research should examine other potential mechanisms of toxicity in CRP effluent.

4.6.6. Densities and approximate age-structures for naturally occurring C. fluminea

Sampling conducted in Fall 2001, Spring 2002, and Fall 2002 suggested that densities and size distributions of *C. fluminea* populations were structured by CRP effluent (Hull et al., In review C). A brief trend toward recovery of *C. fluminea* populations was observed by

Cherry et al. (1996) from 1993 to 1995 when densities at CRP-influenced sites rose from 23 to 99% of levels representing nominal behavior (Fig. 4.6). More recent research indicated that *C. fluminea* failed to maintain a viable population in the CRP-influenced stream section. In 2001, Hull et al. (In review C) collected only a few newly spawned individuals downstream of the CRP, but further sampling indicated these organisms did not survive. Since their colonization of the study area in 1986 (Cherry et al., 1996), nearly two decades ago, *C. fluminea* have seldom been encountered within ~0.6 km of the CRP effluent discharge while they are consistently found at nearby stream sites.

4.6.7. Surveys of indigenous Unionoidea in the vicinity of the CRP

Based upon the results of surveys conducted by Hull et al. (In review C), conditions remain unsuitable for unionoid habitation for a distance of approximately 0.5 to 0.6 km along the left descending bank, below the CRP (Fig. 4.7). Conditions near the opposite bank, however, were conducive to the habitation of various mussel species. These findings were consistent with those of Stansbery et al. (1986), who reported an absence of unionoid fauna within the same stream section despite the collection of 145 living unionoids along the opposite bank, and encountered very few living mussels for 4.8 km downstream of the CRP. Furthermore, of those few living individuals, all were young specimens (Stansbery et al., 1986). Comparing the surveys by Hull et al. (In review C) in 2001, to those conducted by Stansbery et al. (1986) in 1985, unionoids have recolonized stream sections where they were either not found or found only as a few young specimens in 1985. Hull et al. (In review C) reported the occurrence of 18 mussels of four species at a site 3.6 km downstream of the CRP effluent discharge. Four individuals of four species were found as close as 0.7 km, but these mussels were located in a channel near the right descending bank below the CRP mixing zone, and are believed to be somewhat removed from effluent influence.

4.7. Summary of Present Findings and Implications to Ecosystem Recovery

Minshall et al. (2001) noted that many studies investigating long-term changes to lotic fauna are primarily based on “two individual surveys completed years apart, with little or no knowledge of what confounding effects may have occurred in the interim;” and in this sense, the present study is unique. Extensive research has been conducted in the Clinch River near Carbo, VA, following the previously described pulse disturbances of 1967 and 1970 (Cairns et al., 1971, 1973; Crossman et al., 1973; Kaesler, 1974) and the press disturbance associated with CRP effluent Cu (Stansbery et al., 1986; Farris et al., 1988; Clements et al., 1988; Belanger et al., 1990; Cherry et al., 1996; Reed-Judkins, 1998). Although methods and site locations inherently vary with time and between individuals, the findings reported in these studies serve to approximate trajectories for the biotic and physico-chemical integrity of disrupted stream sites relative to the Clinch River's nominal behavior.

Dramatic reductions in Cu concentrations have been achieved in CRP effluent through replacement of corrosion-resistant cooling-tower piping in 1987 and the use of an iron-based flocculant (Apco, 1989). Consequently, concentrations of Cu in CRP effluent have fallen from an average of 436 μg/L from 1985-1989 to an average of 13 μg/L from 1991-2002. Nevertheless, recent findings reported by Hull et al. (In review A,B,C) suggest that biotic impairment downstream of the CRP effluent discharge remains an issue of critical importance to Clinch River bivalves. Survivorship and growth of field-caged *C. fluminea* have been consistently impaired during studies in 1985 and 2001-2002 (Belanger et al., 1990; Hull et al., In review A,B,C). Survivorship after 30-d in situ exposures to CRP effluent was

79% of that observed at nominal behavior during 1986. Exposures of 96-d duration during 2001-2002 indicated that survivorship of clams at CRP influenced sites were typically less than 40%. Growth was more sensitive to CRP effluent toxicity, representing 0% of that observed at nominal behavior during 1985, and being no more than 20% in transplant studies conducted from 2001-2002. Survival of transplanted *V. iris* was relatively insensitive to CRP effluent toxicity during 2002, but growth of these organisms at CRP influenced sites was 73% of the values observed at nominal behavior.

Despite the significant reductions in effluent Cu, data from our bioaccumulation studies with *C. fluminea* and *V. iris* suggest that downstream reductions in survival and growth may be partially related to effluent Cu concentrations (Hull et al., In review C). In 1986, when CRP effluent Cu concentrations averaged 540 µg/L, clams transplanted downstream of the CRP discharge had Cu body burdens four times greater than those observed at nominal behavior. Clams naturally occurring downstream of the CRP had Cu body burdens eight times greater than those observed at nominal behavior (Belanger et al., 1990). During 2001-2002 studies, when effluent Cu levels averaged <16 µg/L, clams transplanted downstream of the CRP had Cu body burdens of ~160% of nominal behavior levels, while *V. iris* transplanted downstream of the CRP accumulated Cu to levels four times higher than mussels at nominal behavior sites (Hull et al., C). The results of Hull et al. (In review A,B,C), however, permit only limited causal inference and can not eliminate the possibility that other CRP effluent constituents may be responsible for observed impairment. Because of this, VT and CRP personnel are presently investigating the likelihood that effluent constituents other than Cu could contribute to the observed biological effects, and this will likely remain a research area of high priority. It is clear, however, that transplanted bivalves remain impaired downstream of the CRP effluent discharge.

Contaminant effects attributable to the CRP effluent are not limited to transplanted bivalves. Nearly two decades after *C. fluminea* invaded the Clinch River in 1986 (see Cherry et al., 1996), these organisms have seldom been encountered downstream of the CRP discharge. From 1986 to 1992, *C. fluminea* were never encountered within ~0.6 km of CRP influence downstream (Cherry et al., 1996). Following effluent modifications in early 1993, a short-lived recovery was documented by Cherry et al. (1996) until 1995 when *C. fluminea* densities were at 99% of those encountered at nominal behavior sites. Based upon more recent data, *C. fluminea* populations in the affected stream reach presumably crashed, and as of 2000-2002, had failed to successfully colonize this site. While the eradication of this invasive pest might be perceived as a benefit, the implications of *C. fluminea* absence downstream of the CRP has negative implications to indigenous mollusks.

The fate of indigenous mollusks directly affected by CRP effluent can never be fully ascertained. Because data prior to the CRP's initial operation in 1958 do not exist, it is unclear whether unionoids ever inhabited this section of stream. It is clear however, that since 1985, when Stansbery et al. (1986) intensively surveyed sites in the vicinity of the CRP, that unionoids have been unable to colonize stream reaches influenced by CRP effluent. Recent surveys by Hull et al. (In review C) confirmed that unionoids remained absent for a distance of ~0.6 km below the CRP discharge. A key difference between the surveys conducted in 1985 by Stansbery et al. (1986) and those conducted by Hull et al. (In review C) in 2001, is that the latter study documented the occurrence of a relatively well-established mussel assemblage less than 4 km downstream of the CRP effluent, nearly 1 km farther upstream (and closer to the CRP discharge) than was documented by Stansbery et al. (1986). It is possible that this assemblage was overlooked during the 1985 surveys, but this is unlikely given the breadth and intensity of those surveys and the close proximity of a 1985 sampling location to the site where the assemblage was observed in 2001. Thus, although a degree of speculation remains, this mussel assemblage may represent the early stages of unionoid recolonization into stream reaches believed to have been defaunted by the toxic spills of 1967 and 1970, and the 1985-1990 inputs of elevated Cu in CRP effluent.

Metrics assessing the health of aquatic insect assemblages inhabiting disturbed stream sites continue to indicate recovery at this level of biological organization. Following the pulse disturbances of 1967 and 1970, recovery for most fish species and benthic macroinvertebrate taxa was relatively rapid (~2 years) (Cairns et al., 1971, 1973; Crossman et al., 1973; Kaesler, 1974). This is not surprising given that most fish species and the more mobile members of benthic macroinvertebrate communities can rapidly recover from ecosystem disturbances through (1) their mobility and (2) life histories permissive of relatively short regeneration times. Quantitative sampling from 1985-1988 suggested that following effluent treatment modifications made in 1987, most measures of benthic macroinvertebrate community health rose to levels comparable to or exceeding nominal behavior within one year. This was particularly evident for Ephemeroptera composition at disrupted stream sites which rose to 246% of the levels observed at nominal behavior in 1988. Comparing the 1985-1988 data to quantitative data collected in 2001 and qualitative data collected in 2000-2001, most metrics continue to indicate the presence of relatively healthy assemblages of aquatic insects at disrupted stream sites. Ephemeroptera composition, which according to Van Hassel and Gaulke (1986) could be used to establish site-specific water quality criteria in this watershed, has been periodically reduced. Abundances have been consistently reduced at disrupted stream sites but this metric does not necessarily indicate an adverse biological response to contaminants.

Bivalves have been underused to evaluate the integrity of lotic freshwater ecosystems (Belanger et al., 1990) and consequently, they have seldom been incorporated into recovery assessments. The contrasting ecological and physiological requirements of bivalves relative to assemblages of aquatic insects afford complimentary measures of aquatic ecosystem recovery. The drift adaptations and life histories of many aquatic insect taxa permit relatively rapid recolonization of disturbed stream sites and permit the quantification of recovery on a relatively short time frame. Bivalves, however, are less well-adapted for rapid recolonization of disturbed stream sites and therefore, may be used to measure more long-term changes in water quality. Opportunistic bivalve species such as the invasive Asian clam, recolonize disrupted stream sites much more rapidly than freshwater mussels, which are characterized by their complex life cycle that makes them particularly susceptible to the deleterious effects of ecosystem disturbances. Asian clams are characterized by a much more opportunistic reproductive strategy (McMahon and Williams, 1986) and consequently, are more successful at proliferating in disturbed ecosystems. Nevertheless, the ability of most bivalve species to recover from ecosystem disturbances is markedly reduced relative to aquatic insect assemblages, and long-term improvements in water quality are typically required for most bivalve species to recover from disturbance (Henley and Neves, 1999; Sietman et al., 2001).

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Table 4.1. Summary of notable studies documenting the biological condition of the Clinch River near Carbo, VA.

Investigator(s), Year	Year(s) of study	Study parameter(s)	Principal finding(s)	Probable cause(s) for deviation from or return to nominal state
Anonymous, 1967	1967	Assessment of aquatic fauna	~216,000 sport and rough fish killed in 145 km of CR; benthic organisms eliminated for ~5-6 km; benthic diversity drastically reduced for 124 km; snails and mussels eliminated for 18 km below Carbo, VA	Collapse of dike surrounding fly ash settling pond in June 1967, released 4.9×10^5 m^3 alkaline wastewater (pH>11) into DC tributary to CR
Cairns et al., 1971, 1973; Crossman et al., 1973	1969	Surveys of bottom fauna and fish communities	By 1969, benthic communities indicated linear recovery from 1967 spill; mollusks, however, had not recovered within 29.5 km; minnows and darters had recolonized disrupted sites, but densities remained reduced	Natural dilution, dispersal, and neutralization of alkaline wastewater; recolonization of disrupted stream reaches by unaffected biota; accommodation
	1970	Surveys of bottom fauna and fish communities	Following the 1970 spill, mayfly and mollusk species were completely eliminated for 18.8 km; 60 d after spill, aquatic insect species indicative of nominal state found at all disrupted sites; mollusks not yet recovered	Natural dilution, dispersal, and neutralization of acidic wastewater; recolonization of disrupted stream reaches by unaffected biota; accommodation
	1971	Surveys of bottom fauna and fish communities	Trend toward species homogeneity between nominal state and disrupted sites evident	Recolonization of disrupted stream reaches by unaffected biota; accommodation
Van Hassel and Gaulke, 1986	1981-1984	Benthic surveys and effluent/ambient chemical data	Relative abundance of mayflies was reduced from reference levels at sites influenced by CRP effluent	Increased Cu concentrations in CRP effluent were associated with these reductions
Stansbery et al., 1986	1985	Quantitative and qualitative surveys of unionoid fauna in the vicinity of the CRP	No living unionoids found along the river bank influenced by CRP discharge for a distance of 0.4 km despite habitat similar to the opposite bank where 145 living unionoids were found	A direct link with CRP effluent is weakened because no data exist for this stream section prior to 1958, when the CRP began operation, and it is possible that unionoids never inhabited this reach

Sheehan et al., 1989	1981, 1984, 1985	Mussel translocations	Mortality greatest among mussels translocated downstream of the CRP	CRP effluent suspected but high mortality overall weakened any legitimate association
Farris et al., 1988	1985, 1986	*C. fluminea* field-bioassays	Enzyme activity of clams significantly reduced relative to upstream levels	Reduced enzymatic activity linked to elevated Zn (47-78 ppb) and Cu (80-345 ppb)
	1985, 1986	Quantitative sampling of benthic macroinvertebrate communities	No meaningful differences were found among upstream and downstream sites	Benthic communities indicated a gradation of effects downstream of the CRP discharge that was always consistent with measured metals concentrations
Clements et al., 1988	1986	Colonization studies of benthic communities in substrate-filled trays	Numbers of taxa, individuals, and sensitive Ephemeroptera and Tanytarsini chironomids were significantly reduced at effluent-influenced stations relative to upstream reference stations	Impairment of benthic communities exposed to low levels of Cu and Zn (12 ppb) in experimental streams was similar to Clinch River benthic communities that colonized substrate-filled trays
Belanger et al., 1990	1986	*C. fluminea* field-bioassays, density sampling of resident *C. fluminea*	Survival and growth of transplanted *C. fluminea* (length and weight) and densities of naturally occurring *C. fluminea* were reduced downstream of the CRP effluent discharge	CRP effluent Cu concentrations were directly linked to impairment through bioaccumulation studies of *C. fluminea* tissues and instream Cu concentrations
Cherry et al., 1996	1986-1995	Density sampling of resident *C. fluminea*	Clams remained absent within CRP effluent influence in 1993-1995 when recolonization was apparent	Improvements in effluent treatment process reduced Cu concentration to <12 μg/L
Reed-Judkins et al., 1998	1988	Snail field-bioassays	100% mortality observed for snails exposed to Cu concentrations of 42-89 ppb	Cu accumulated in aufwuchs was significantly higher downstream of the CRP relative to an upstream station
	1988, 1989-1990	Snail density sampling	Snails were absent within CRP effluent influence during 1988, but by 1990, following operational adjustments to reduce CRP effluent Cu from >800 ppb to <150 pp, snail densities recovered to upstream levels	Snail habitat selection was strongly influenced by effluent Cu contributions from the CRP discharge

Hull et al., In review A	2000-2002	*C. fluminea* field-bioassays	Survival and growth of *C. fluminea* transplanted from ~15 m to ~0.6 km downstream of CRP significantly reduced	Further studies necessary to isolate causal mechanism
	2000-2001	Qualitative assessments of benthic macroinvertebrate communities	Total, EPT, and Ephemeroptera richness statistically similar at all study sites, Ephemeroptera composition minimally reduced downstream of CRP	Further studies necessary to isolate causal mechanism
Hull et al., In review C	2002	*V. iris* field-bioassays	Growth of *V. iris* transplanted ~0.6 km downstream of CRP discharge had significantly reduced growth after 96 d; survival >95% at all study sites	Further studies necessary to isolate causal mechanism
	2001-2002	*C. fluminea* density sampling and age structure analysis	*C. fluminea* populations unable to colonize sites within ~0.6 km of CRP discharge	Further studies necessary to isolate causal mechanism
	2001	Unionoid surveys	Live mussels absent for ~0.6 km downstream of CRP	Further studies necessary to isolate causal mechanism

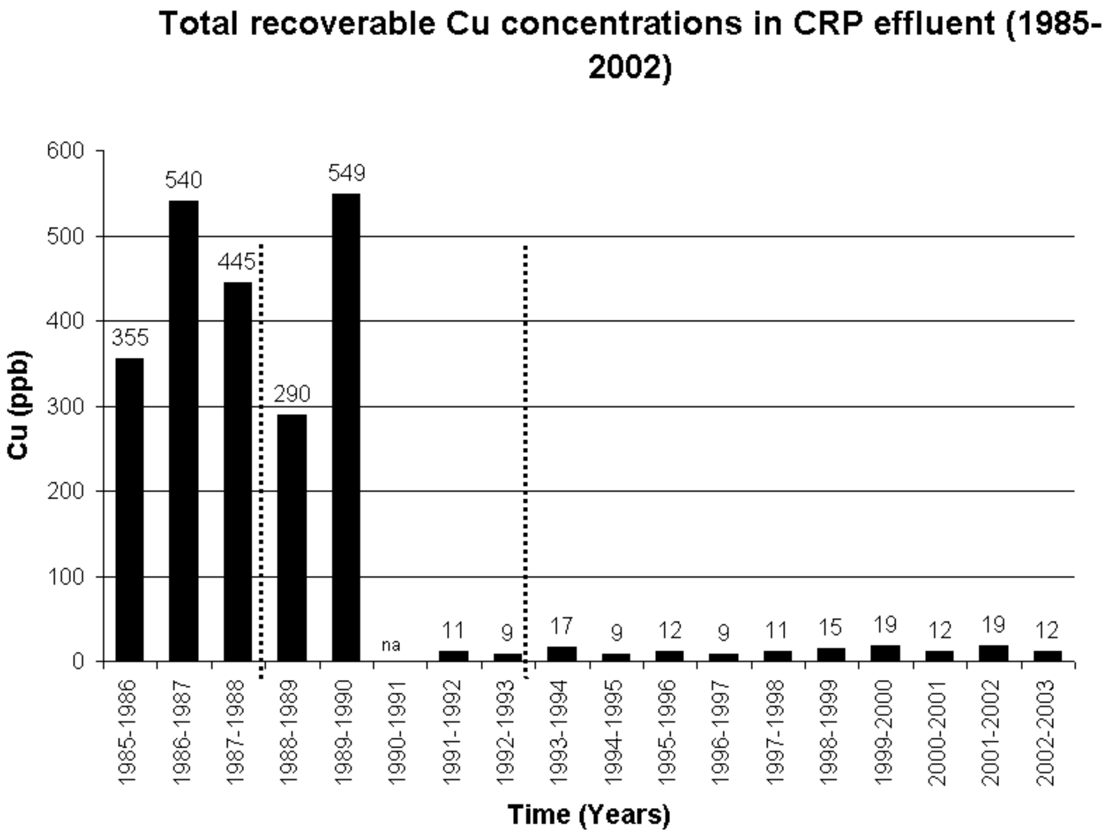

Fig. 4.1. Yearly averages of Cu concentrations measured for CRP effluent in 24-h composite samples collected monthly. Dashed vertical lines indicate points at which significant modifications (e.g., replacement of cooling tower piping in 1987) were made to reduce effluent Cu concentrations.

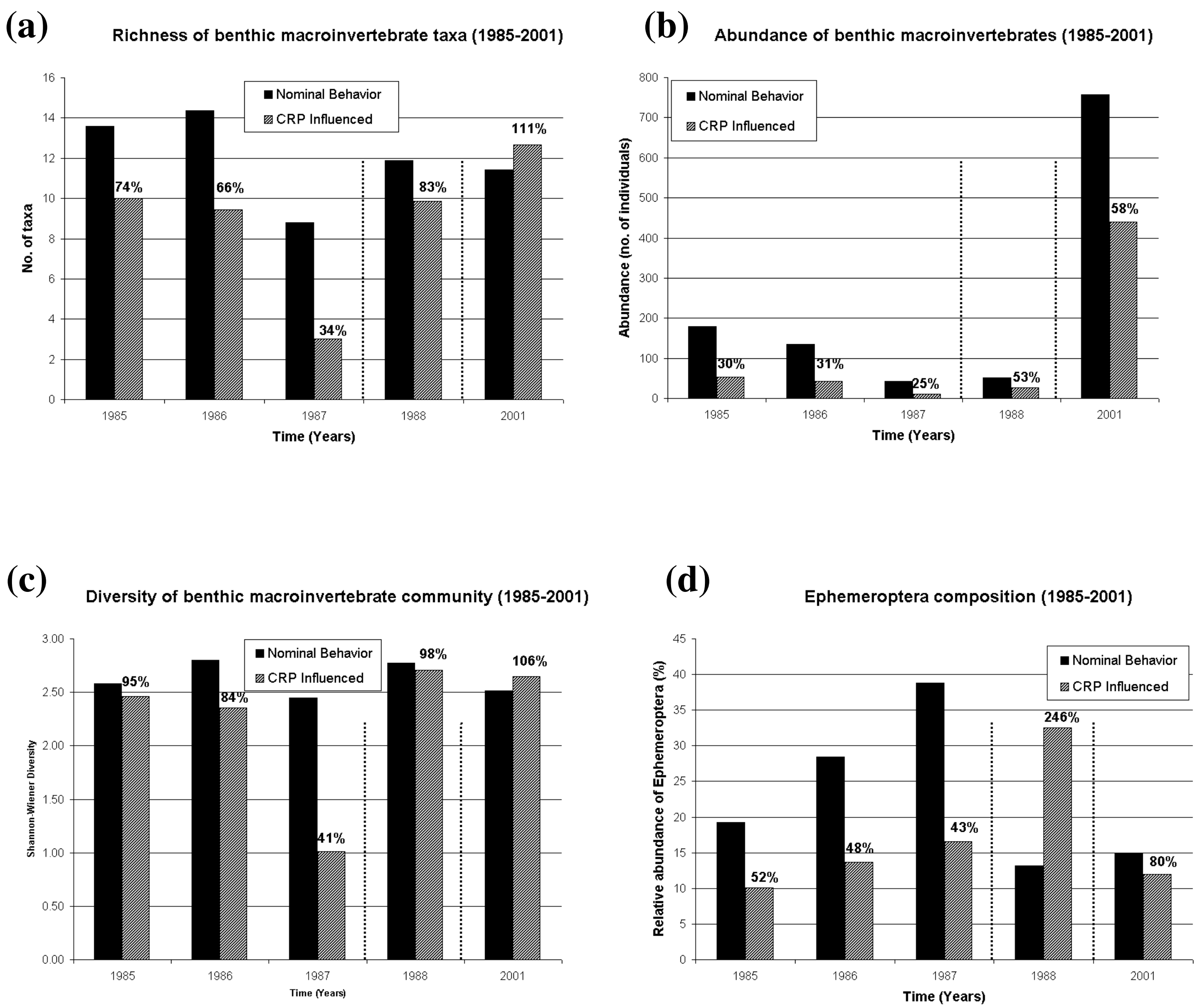

Fig. 4.2. Results of quantitative sampling of benthic macroinvertebrate communities in the Clinch River from 1985-2001. Parameters measured included (a) total taxa richness, (b) total abundance, (c) Shannon-Weiner diversity, and (d) relative abundance of Ephemeroptera. Percentages denote parameter values at CRP-influenced sites relative to values measured at nominal behavior. Dashed vertical lines indicate periods of data collection separated by significant modifications to CRP effluent treatment.

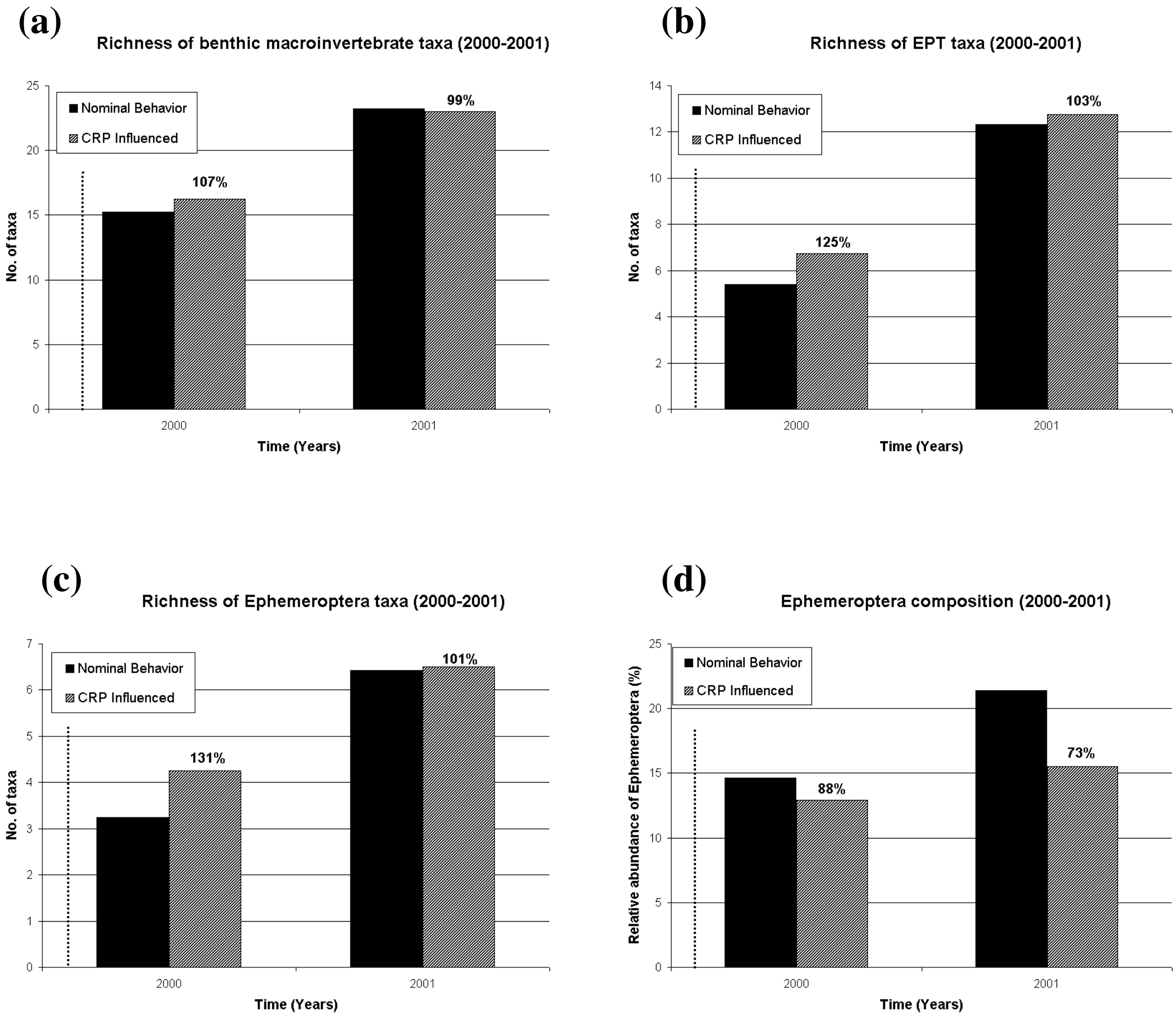

Fig. 4.3. Results of qualitative sampling of benthic macroinvertebrate communities in the Clinch River from 2000-2001. Parameters measured included (a) total taxa richness, (b) richness of EPT taxa, (c) richness of Ephemeroptera taxa, and (d) relative abundance of Ephemeroptera. Percentages denote parameter values at CRP-influenced sites relative to values measured at nominal behavior. Dashed vertical lines indicate periods of data collection separated by significant modifications to CRP effluent treatment.

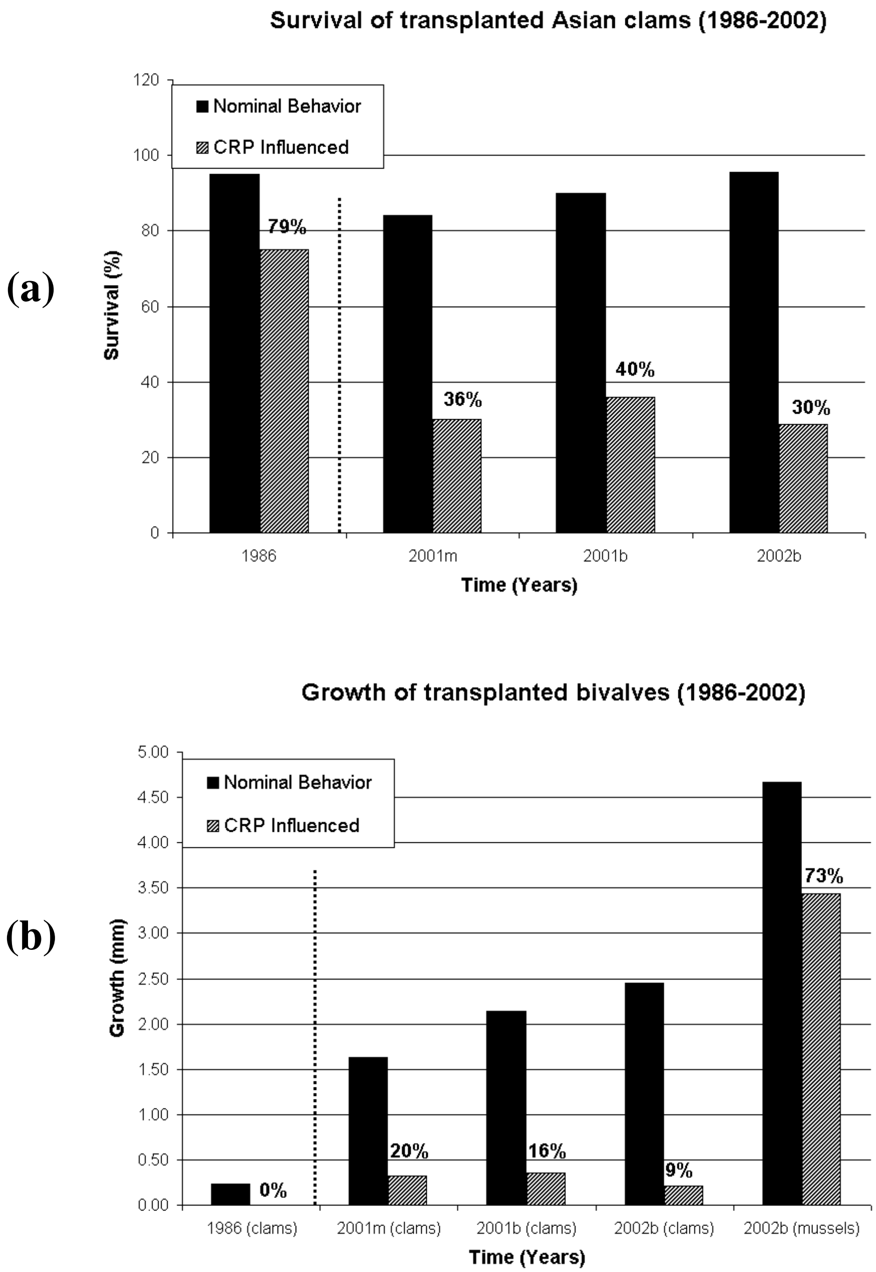

Fig. 4.4. Results of in situ field bioassays using caged bivalves from 1986-2002. Parameters measured included (a) survivorship and (b) growth. Percentages denote parameter values at CRP-influenced sites relative to values measured at nominal behavior. Dashed vertical lines indicate periods of data collection separated by significant modifications to CRP effluent treatment.

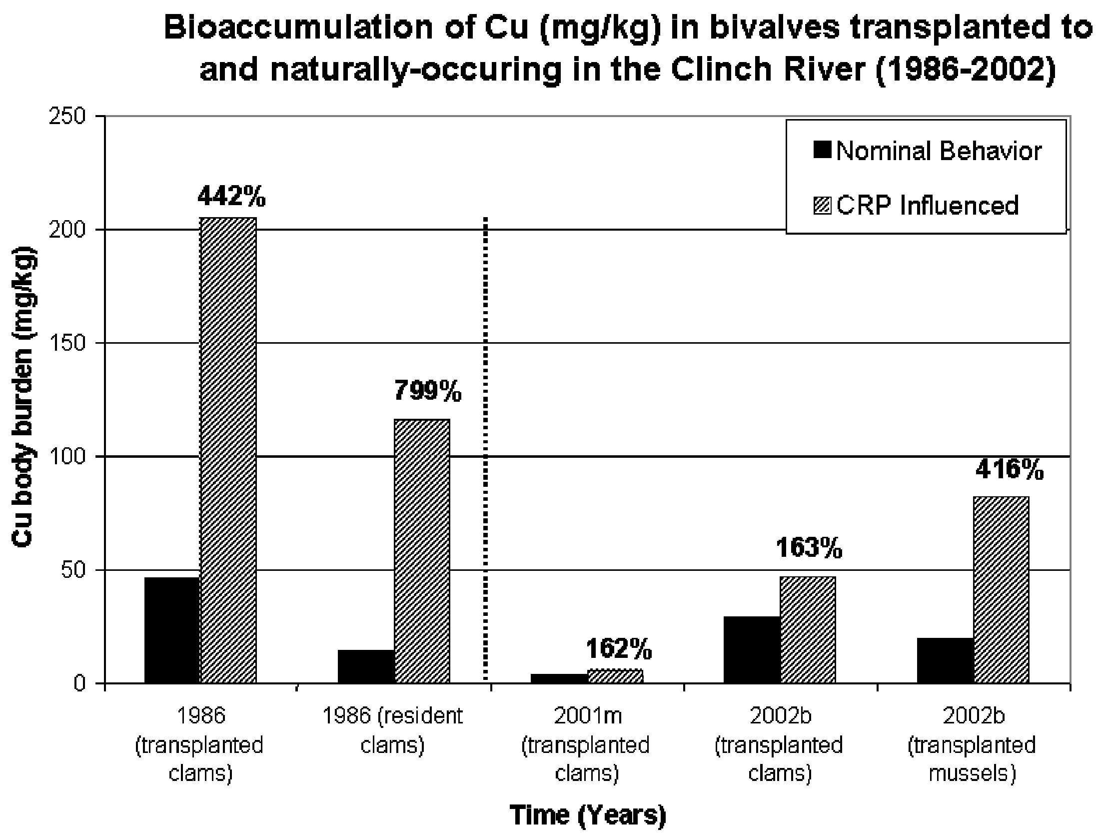

Fig. 4.5. Results of spectrochemical measurement of Cu present in digested tissues from transplanted and resident *C. fluminea* in 1986 (Belanger et al., 1990) and transplanted *C. fluminea* (clams) and *V. iris* (mussels) in 2001-2002. The letters 'm' and 'b' denote cage type used, mesh bag or biobox (see Hull et al., In review B). Percentages denote parameter values at CRP-influenced sites relative to values measured at nominal behavior. Dashed vertical lines indicate periods of data collection separated by significant modifications to CRP effluent treatment.

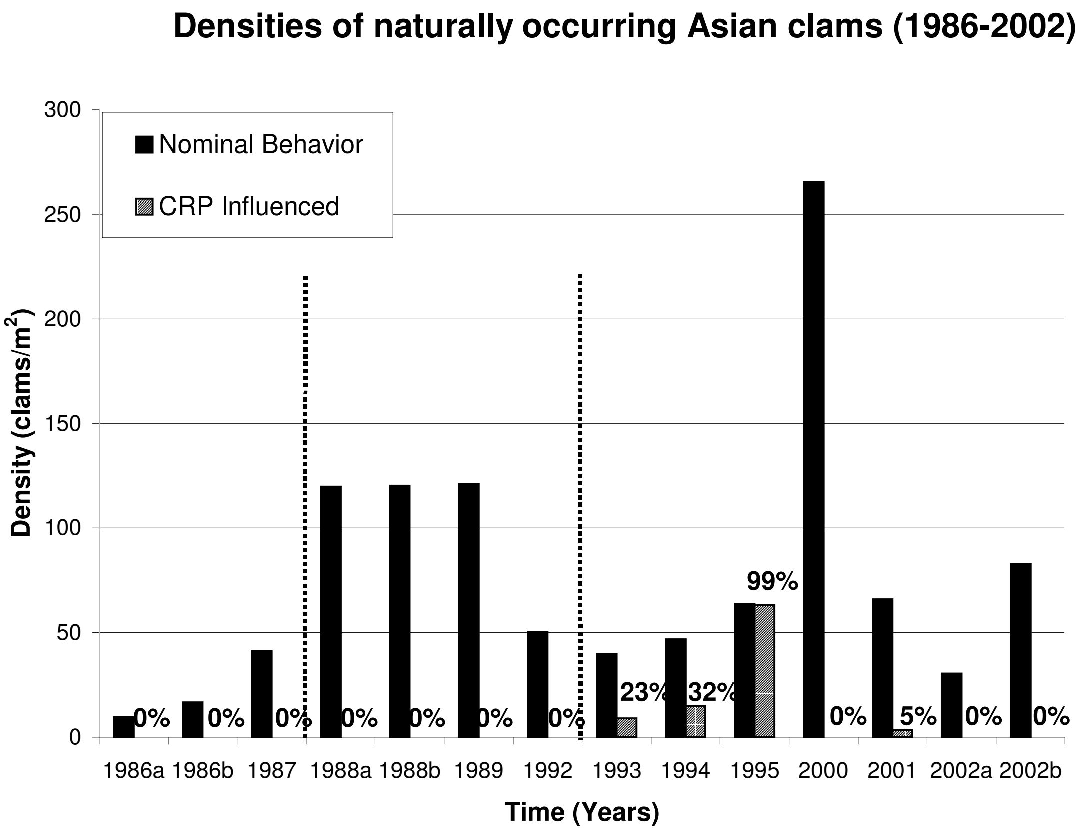

Fig. 4.6. Results of density sampling of *C. fluminea* populations naturally-occurring in the Clinch River from 1986 to 2002. Percentages denote parameter values at CRP-influenced sites relative to values measured at nominal behavior. Dashed vertical lines indicate periods of data collection separated by significant modifications to CRP effluent treatment. Years followed by an 'a' or 'b' denote two separate samples taken within the same year.

(a)

(b)

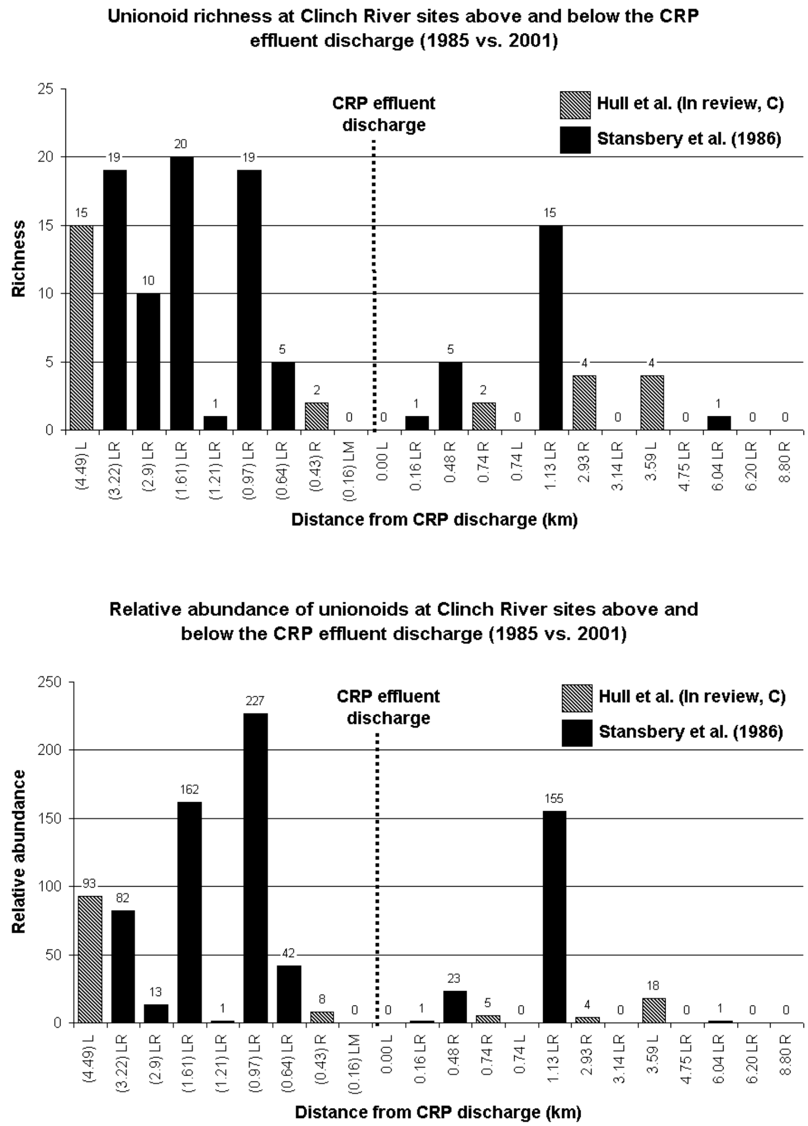

Fig. 4.7. Results of Clinch River mussel surveys conducted by Stansbery et al. (1986) in 1985 and Hull et al. (In review C) in 2001, in the vicinity of the CRP. Dashed vertical lines indicate the point at which the CRP effluent is discharged into the river. Values in parentheses along the x-axis denote distance upstream of the discharge. The letters 'L', 'M', and 'R' indicate the descending section of the stream surveyed, 'Left', 'Middle', or 'Right'.

CHAPTER 5. Preliminary investigation of the toxic constituents of a coal-fired power plant effluent in Carbo, Virginia (USA)

Abstract. A framework for systematically identifying and reducing toxicity associated with point-source discharges has been established through the application of Toxicity Identification and Reduction Evaluations (TI/RE). However, these procedures require that effluent toxicity be measured in effluent samples collected at the discharge pipe. Comparable procedures do not exist for effluents demonstrating instream biotic impairment, but no measurable toxicity in effluent samples. Because of variations in operating procedures (e.g., slug-feeding of biocides), chemical interactions within effluent, and effluent interactions within receiving systems, remarkable potential exists for returning false-negative toxicity results when WETT (Whole Effluent Toxicity Testing) is used to evaluate instantaneously-collected effluent samples. In receiving systems like the Clinch River, where federally endangered mussels occur, corrective action must be taken to minimize instream effects to imperiled species. A collaborative effort with personnel from American Electric Power's (AEP) Clinch River Plant (CRP), a coal-fired, power-generating facility in Carbo, VA (USA), was initiated to identify effluent treatment procedures that threatened downstream biota and establish a basis for further research. Based on this preliminary assessment, guidelines were proposed for (1) the discharge of water reclaimed from a coal ash settling basin, (2) the application of industrial treatment chemicals, and (3) Cu levels that demonstrated chronic toxicity to bivalves. We found that (1) water reclaimed from settling basins significantly impaired survivorship and fecundity of ceriodaphnids at concentrations of 50%, (2) LC_{50} values for industrial treatment chemicals were misrepresented on Material Safety Data Sheets and consequently, subject to misapplication by operators, (3) Cu concentrations of 96 μg/L

significantly impaired growth of Asian clams in artificial stream testing, and (4) effluent Al exceeded acute and chronic water quality criteria, suggesting this ion should receive further consideration in future studies. The present study demonstrates an approach that can be taken to minimize instream effects attributable to complex industrial effluents when traditional TI/RE procedures cannot be undertaken due to insufficient mortality in samples of whole-effluent or ambient water.

5.1. Introduction

Despite the chemical complexity inherent to industrial effluents (the number of chemicals present in an effluent can reach the thousands), adverse biological responses are typically attributed to a small number of especially toxic constituents (Maltby et al., 2000). Nevertheless, isolating and subsequently eliminating effluent toxicity is difficult to achieve. Accurate characterization of effluents that vary temporally with production processes or wastewater treatment efficiency, for example, often necessitate elaborate sampling designs that are both labor-intensive and expensive (Jop et al., 1991; Mersch and Reichard, 1998). Chemical interactions within effluents and effluent interactions within receiving systems further complicate efforts to identify and subsequently reduce sources of effluent toxicity (Dickson and Rodgers, 1986; Fetterolf et al., 1986; Farris et al., 1988).

Whole Effluent Toxicity Tests (WETT) have increasingly been used to predict effluent toxicity on receiving system biota while circumventing the complexity of evaluating specific effluent components individually (Barbour et al., 1996; Maltby et al., 2000). Although the benefits of WETT are numerous, especially in terms of practicality and establishing causality (e.g., Clements and Kiffney, 1996; Sarakinos and Rasmussen, 1998), this approach fails to identify effluent toxicants (Maltby et al., 2000; Sarakinos et al., 2000). To address this, procedures for systematically identifying and reducing effluent toxicity have been established through Toxicity Identification and Reduction Evaluation (TI/RE), and are described at length elsewhere (Durhan et al., 1993; Mount and Norberg-King, 1993). This approach characterizes effluent toxicity by relating physico-chemical manipulations of whole effluent to changes in toxicity as measured using standard test organisms or preferably, the organism that triggered the TIE (Norberg-King et al., 1991). Consequently, a limitation of TI/RE is that the effluent fractionations and manipulations necessary to identify and reduce effluent toxicity can only occur if toxicity has occurred in samples of whole effluent.

Comparable TI/RE procedures do not exist for effluents demonstrating instream effects but no measurable toxicity in instantaneous samples of whole effluent. This situation was reported by Hull et al. (In press, In review B) to have occurred downstream of the Clinch River Plant (CRP) in Carbo, VA (USA), a coal-fired, power-generating facility owned by American Electric Power (AEP). Although adverse effects attributable to CRP effluent were observed in situ (Hull et al., In press, In review A,B), instantaneously-collected and 24-h grab samples of CRP effluent consistently failed to demonstrate measurable toxicity to US EPA test organisms. Therefore an intermittent or otherwise elusive effluent contaminant was suspected, and consequently attenuated the use of traditional TI/RE procedures to cost-effectively identify the source of effluent toxicity.

A practical alternative to traditional TI/RE procedures was to cooperate with CRP personnel to identify potential threats to receiving system biota. Previous studies linked elevated concentrations of Cu in CRP effluent to instream impairment (Van Hassel and Gaulke, 1986; Clements et al., 1988; Farris et al., 1988; Belanger et al., 1990; Reed-Judkins et al., 1998). Although effluent Cu concentrations presently approach or exceed water quality criteria, efforts taken in 1993 dramatically reduced effluent Cu and have largely improved water quality downstream (APCo, 1989; Hull et al., In press, In review C). Nevertheless, stream sediments can remain contaminated long after pollutant discharges have subsided (Salomons et al., 1987), and the potential for residual sediment contamination from historic Cu inputs and chronic water column contamination from present effluent levels necessitated further investigation. Chemicals used for the periodic treatment of CRP wastewater were identified as a concern of high priority because of their intermittent application, which met the criteria of the suspected CRP effluent toxicant, and were evaluated for antagonistic, additive, and synergistic toxicity. An additional area of concern was the CRP's periodic operation under “wet” conditions (not to be confused with WETT), whereby sluice water from electrostatic precipitators is discharged to a series of settling basins to reduce metals concentrations, and then returned to the primary treatment facility for discharge (for description of process see Van Hassel and Wood, 1984). This process minimizes the risk of overflow from settling basins during upset conditions (e.g., high rainfall), but can intermittently raise effluent pH to levels detrimental to aquatic fauna. Therefore, the objectives of the present study were to investigate the potential for these factors to contribute to biotic impairment reported by Hull et al. (In press, In review A,B), to establish management guidelines for factors that constituted a significant biological risk, and to establish a basis for future research.

5.2. Materials and Methods

5.2.1. Culture of test organisms

Ceriodaphnia dubia and *Daphnia magna* used for water column and sediment toxicity tests, and *Chironomus tentans* used for sediment toxicity tests, were cultured by experienced technicians at the Virginia Tech Aquatic Ecotoxicology Laboratory in Blacksburg, VA, 24061. Cladocerans were raised in a combination of EPA Moderately Hard Synthetic (EPA[100], MHS), reconstituted water and filtered, non-toxic water from Sinking Creek, a reference stream in Newport, Virginia. Metals concentrations in Sinking Creek water are typically low to undetectable (1.6 μg Al/L, 14.3 μg Fe/L, and Cu and Zn were below detection limits) and levels for other contaminants were within ranges considered safe for aquatic life. Mean values of pH, conductivity, alkalinity, and hardness for culture water were 8.01 ± 0.10, 225 ± 5.48 μmhos/cm, 131 ± 9.45 mg/L as $CaCO_3$, and 122.6 ± 4.16 mg/L as $CaCO_3$, respectively. Cultures received a daily feeding of 0.18 ml of a *Raphidoselis subcapitata* (formerly *Selenastrum capricornutum*) and Yeast-Cereal Leaves-Trout Chow (YCT) mixture per 30 ml of water. Midge-fly larvae were reared in glass aquariums filled with Sinking Creek water and a layer of sterilized paper bedding for the construction of burrows. Aquariums were continuously aerated and organisms were fed a 50 mL mixture of cereal leaves and tetramin. Fathead minnows (*Pimephales promelas*) used for acute tests with industrial treatment chemicals were obtained from Aquatox, Inc., Hot Springs, AK, 71913.

5.2.2. Evaluation of residual sediment toxicity

Toxic spills in 1967 and 1970, and elevated metals concentrations (primarily Cu) documented from 1985-1989, have impaired biota downstream of the CRP (Cairns et al., 1971; Crossman et al., 1973; Van Hassel and Gaulke, 1986; Clements et al., 1988; Farris et al., 1988; Belanger et al., 1990; Reed-Judkins, 1998). To address the concern that residual contaminants from these perturbations could potentially account for instream impairment observed by Hull et al. (In press, In review A,B) during 2000-2002, sediment toxicity tests were conducted during 2000 using *C. tentans* and *D. magna*, and during 2002 using *C. dubia*. Sediments were processed according to method 200.7 in Methods for the Determination of Metals in Environmental Samples (US EPA, 1991) during 2002 and metals concentrations were determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).

During September 2000, sediments were collected from Clinch River study sites by AEP personnel and held in plastic freezer bags for transport on ice (<4 °C) to Virginia Tech. Proceeding from most upstream to most downstream sites, sediments were collected from two upstream reference sites (CRUR1 and CRUR2- Clinch River Upstream References 1 and 2), one site downstream of the Clinch River confluence with Dump's Creek (CRCF- Clinch River Confluence), a site ~0.6 km downstream of and directly influenced by CRP effluent (CREFF and CREFF dup- Clinch River Effluent and duplicate), a site near the river bank opposite of CREFF and uninfluenced by CRP effluent (CROE- Clinch River Opposite Effluent), a site adjacent to a CRP-owned fly ash landfill (CRFA- Clinch River Fly Ash landfill), and a site used to assess downstream recovery (CRDR1- Clinch River Downstream Recovery 1). Thorough descriptions of study sites can be found elsewhere (Hull et al., In press, In review A,B). Sediment collected from Sinking Creek was used as a sediment control and Sinking Creek water (no sediment) was used as a diluent control for *D. magna*.

During August 2002, sediments were again collected from the Clinch River sites described previously and an additional downstream recovery site (CRDR2- Clinch River Downstream Recovery 2). Tests with *C. tentans* and *D. magna* were conducted in accordance with methods specified by Nebeker *et al*. (1984), U.S. EPA (1994) and the American Society for Testing and Materials (ASTM, 1995). Tests with *C. dubia* were conducted according to methods described by Suedel et al. (1996) with modifications described below.

For tests with *C. dubia*, two ceriodaphnids (age ≤ 24 h) were pipetted into a 50 mL beaker containing ~10 mL of sediment and ~30 mL of overlying EPA water. For each site there were five replicates. Test duration was 7 d with daily water renewal and feeding (0.36 ml of a 1:1 mixture of *Raphidoselis* and YCT/ 30 ml of test water). Mean values of survivorship (%) and fecundity (neonates/ surviving adult) were determined and compared using ANOVA ($\alpha = 0.05$) followed by pairwise analyses, and ToxStat® software (West and Gulley, 1996).

For tests with *D. magna*, one daphnid (age ≤ 24 h) was pipetted into a 50 mL beaker containing ~10 mL of sediment and ~40 mL of overlying Sinking Creek water. For each site there were ten replicates. Test duration was 10 d or until third brood, with daily water renewal and feeding (0.36 ml of a 1:1 mixture of *Raphidoselis* and YCT/ 30 ml of test water). Mean values of survivorship (%) and fecundity (neonates/ surviving adult) were determined and statistically compared as described above for *C. dubia*.

For tests with *C. tentans*, ten chironomid larvae were placed into a 600 mL beaker containing ~100 mL of sediment and ~400 mL of overlying Sinking Creek water. For each site there were five replicates. Test duration was 10 d, with daily water renewal and feeding (1 mL of a mixture of Tetra Min® flake food, TetraWerke, Melle, Germany, and cereal leaves). At the end of the test, mean values of survivorship (%) and growth (weight measured to the nearest 0.001 mg using a Sartorius® microbalance) were determined and statistically compared as described above for *C. dubia* and *D. magna*.

5.2.3. Evaluation of chronic Cu toxicity to bivalves

Effluent Cu concentrations have been dramatically reduced at the CRP, from 436 μg/L during 1985-1989, to 13 μg/L during 1991-2002 (Hull et al., In review C). Nevertheless, instream effects to bivalves transplanted below the CRP discharge corresponded to elevated Cu body burdens (Hull et al., In review B). Although a direct causal relationship was not established, further research was warranted to determine if the reduced Cu concentrations in CRP effluent could impair bivalves chronically.

To test this hypothesis, Asian clams (*Corbicula fluminea*) and rainbow mussels (*Villosa iris*) of two age classes (1-4 months and 12-18 months) were exposed to varying Cu concentrations for 21-d in a recirculating artificial stream system. Five Cu concentrations (6, 12, 24, 48, and 96 μg/L) were made by adding calculated amounts of reagent-grade $CuSO_4$ to fiberglass troughs containing 36 L unfiltered Sinking Creek water (hardness values for Sinking Creek and Clinch River water are ~150 mg/L as $CaCO_3$). Sinking Creek water was used for a control. Four bivalves of each species and age-class were checked for mortality, measured for height (umbo to ventral margin) and length (anterior to posterior) using digital calipers, and placed into each of three replicate test chambers per concentration. Test chambers were plastic ice-trays filled with ~15 mL of sieved and autoclaved sediment (250-450 μm) from a Clinch River reference site near Cleveland, VA, noted for its thriving assemblage of bivalves. Test organisms were fed daily with 200 mL of *Neochloris* sp. to obtain an algal density of ≥25,000 cells/mL (based on algal cell counts), and test water was renewed every 3-4 d. Temperature was 20 ± 1 °C and current velocity, generated by submersible power-heads, was ~0.1 m/sec. To minimize the potential for confounding effects attributable to spatial variations in physicochemical parameters (e.g., current velocity, algal density), a Randomized Complete Block Design (RCBD) was used and bivalves were assigned to test chambers using a random numbers generator in SAS (SAS Institute, Inc., Cary, NC, USA).

5.2.4. Evaluation of acute toxicity, synergism, additivism, and antagonism in industrial chemicals

Seven chemicals (A-G) used to treat CRP wastewater were evaluated for acute toxicity to *D. magna*. For all acute tests with *D. magna*, five daphnids (age ≤ 24 h) were pipetted into a 50 mL beaker containing ~30 mL of test solution. Diluent was EPA water, and five to six exposure concentrations with an EPA control were evaluated. Prior to formal toxicity testing, range-finding tests with *C. dubia* and *D. magna* were conducted to establish a suitable dilution series. Because chemicals exhibited gross toxicity in range-finding tests, frequently resulting in 100% mortality in the lowest test concentrations (e.g., 0.001% toxicant), a 90% dilution series was used for further testing. Although the use of dilution series of >50% have been discouraged, this series consistently produced a desirable range of toxicity (0 to 100% mortality) and provided margin for measurement errors and temporal variations in test organism sensitivity.

For formal toxicity tests, 1 mL of chemical was diluted into 1000 mL of EPA water to create a 0.001% stock solution. The 90% dilution series was then used to make up to six test concentrations with 0.001% being the highest and 1×10^{-8}% being the lowest.

For each test concentration there were four replicates and test duration was 48 h with no feeding or water renewal. Temperature was 25 ± 1 °C. Concentrations were converted to parts per billion (μg/L) using density data provided by the chemical manufacturer in MSDS (Material Safety Data Sheets) and final values for survivorship were used to generate LC_{50} values using the Spearman-Karber method.

Based upon results from *C. dubia* and *D. magna* range-finding tests, the four most toxic chemicals (B, D, E, and F) were identified and used in subsequent formal acute tests with *C. dubia* and *P. promelas* to streamline the assessment and establish a multi-species database of individual chemical toxicity. Tests with *C. dubia* and *P. promelas* were conducted in a similar fashion to those involving *D. magna*, but with the following modifications for *P. promelas*: 10 individuals were added to each of two replicate, 250 mL bioassay jars.

The four most toxic chemicals were further evaluated for antagonistic, additive, or synergistic toxicity. To accomplish this, a factorial treatment structure was derived, whereby test organisms were exposed to all possible combinations of the four chemicals. Because *C. dubia* was more sensitive than both *D. magna* and *P. promelas* during tests with individual chemicals, it was the only species evaluated during this phase of testing. Tests proceeded as described above for individual tests with the appropriate modifications in stock solutions to accommodate the addition of multiple chemicals. Antagonism, additivism, and synergism were determined using Marking's Additive Index (MAI) (Marking, 1977) and the following formula:

(1) $(Am/Ai) + (Bm/Bi) + (Cm/Ci) + (Dm/Di) = S$

where A, B, C, and D are different chemicals, I is the LC_{50} of the individual chemical, and m is the LC_{50} of the chemical in a given mixture. A given index value is made symmetric about zero as follows:

(2) if $S \geq 1.0$, $MAI = -S + 1.0$ (less than additive toxicity or antagonism)

(3) if $S \leq 1.0$, $MAI = (1/S) - 1.0$ (greater than additive toxicity or synergism)

Statistical significance is inferred by deriving 95% confidence intervals as described by Litchfield and Wilcoxon (1949).

5.2.5. Evaluation of settling basin efficiency and residual toxicity in reclaim water

To assess the potential for toxicity attributable to inefficient operation of the CRP settling basin, and the discharge of toxic wastewater under "wet" operating conditions, samples were taken on multiple dates throughout the CRP settling basin. Samples 1A, 1B, and 1C were taken at the sluice water influent pipe. Samples 2 through 7 were taken at various points along the settling gradient, with sample 2 being closest to the influent pipe and sample 7 being closest to the reclaim or polishing pond. Samples 8 and 9 were taken from the reclaim pond.

Samples of influent (1A-C) and reclaimed water (8 and 9) were evaluated for acute toxicity to *C. dubia* on various sampling dates. Five ceriodaphnids (age ≤ 24 h) were pipetted into a 50 mL beaker containing ~30 mL of test solution. Diluent was EPA water, and five test solutions (6.25, 12.5, 25, 50, and 100% reclaimed water) with an EPA control were evaluated. For each test solution there were four replicates. Test duration was 48 h with no feeding or water renewal. Temperature was 25 ± 1 °C. Final values for survivorship were used to generate LC_{50} values using the Spearman-Karber method, which were subsequently used to establish acute safe-limits for the discharge of reclaimed water under "wet" conditions.

Reclaimed water was also evaluated for chronic toxicity to *C. dubia* during 2002. One ceriodaphnid (age ≤ 24 h) was pipetted into a 50 mL beaker containing ~30 mL of test solution. Diluent was EPA water, and five test solutions (6.25, 12.5, 25, 50, and 100% reclaimed water) with an EPA control were evaluated. For each test solution there were ten replicates. Test duration was 7 d with daily water renewal and feeding (0.36 ml of a 1:1 mixture of *Raphidoselis* and YCT/ 30 ml of test water). Temperature was 25 ± 1 °C. Mean values of survivorship (%) and fecundity (neonates/ surviving adult) were determined and compared using ANOVA ($\alpha = 0.05$) followed by pairwise analyses, and ToxStat® software (West and Gulley, 1996). No Observable Adverse Effects Concentrations (NOAEC) and Lowest Observable Adverse Effects Concentrations (LOAEC) were determined and used to establish chronic safe-limits for the discharge of reclaimed water under "wet" conditions.

5.2.6. Water chemistry

Water samples were obtained during initiation and renewal of all tests and immediately analyzed in the laboratory for pH, conductivity, temperature, dissolved oxygen (DO), alkalinity, and hardness. Sub-surface samples of CRP effluent and settling basin water were collected in clean 1-L Nalgene® bottles. Bottles were completely filled to minimize agitation during transport and immediately placed on ice for storage at ≤4 °C. In the laboratory, field-collected samples were warmed to room temperature (~25 °C) and measured for the physico-chemical parameters described previously. We used an Accumet® (Fisher Scientific, Pittsburgh, PA, USA) pH meter with an Accumet gel-filled combination electrode (accuracy < ± 0.05 pH at 25°C) to measure pH. Dissolved oxygen was measured using a Yellow Springs model 54A meter calibrated for elevation (Yellow Springs, Yellow Springs, OH, USA). A Yellow Springs model 30 conductivity meter (Yellow Springs, Yellow Springs, OH, USA) was used to measure specific conductance (accuracy ± 0.5%). Total hardness and alkalinity (as mg/L $CaCO_3$) were measured through colorimetric titrations in accordance with APHA et al. (1998). Samples of CRP effluent, artificial stream water, and the CRP ash settling basin were further processed so that ionic composition could be determined by ICP-MS (US EPA, 1991).

5.3. Results

5.3.1. Chemical analysis of CRP effluent

Effluent temperature, DO, pH, and conductivity averaged 20 ± 8 °C, 8.3 ± 1.0 mg/L, 8.4 ± 0.1, and 993 ± 171 µmhos, respectively, during measurements taken in 2000-2001 (Table 5.1). Alkalinity and hardness were 124 ± 15 and 445 ± 21 mg/L as $CaCO_3$, respectively. Effluent Cu concentrations averaged 14 ± 8 µg/L in monthly composite samples taken from 2000-2002. Concentrations of Zn, Fe, and Al were 7, 14, and 843 µg/L, respectively, in a sample of CRP effluent collected on October 2, 2002. Concentrations of chloride, nitrates as N, sulfate, and phosphate as P were 107, 2, 302, and 0.7 mg/L, respectively. While most parameters were well within water quality criteria, the value for Al exceeded both acute and chronic standards.

5.3.2. Residual sediment toxicity

During 2000, results of sediment toxicity tests with *C. tentans* indicated survivorship and growth for most stream sites were greater than 70% and 3.00 mg, respectively (Fig. 5.1a). Deviations from this trend occurred for survivorship and growth at CREFF (38 ± 35% and 2.44 ± 2.33 mg), and for survival at CRFA (50 ± 24%). The validity of these data were questioned, however, for multiple reasons: (1) survivorship in reference sediment (SC) was less than 60%, (2) a duplicate CREFF site had high survivorship (90 ± 12%) and the highest growth value (4.29 ± 0.66) observed among all sites, and (2) there was high variability in growth at CREFF and CRFA. These factors attenuated statistical inferences on these data.

Fecundity of *D. magna* exposed to Clinch River sediments during 2000 was less variable than survivorship, and indicated no significant differences among stream sites (Fig. 5.1b). The Sinking Creek water control (no sediment) had significantly greater fecundity, and indicated an over-riding effect of sediment addition on daphnid reproduction. Survivorship, as observed for tests with *C. tentans*, was lowest in the Sinking Creek sediment control (63%) and averaged less than 80% at four other sites. Mean values of survivorship and fecundity at CREFF and CREFF duplicate were similar and in most cases, were either comparable to or greater than values at other stream sites.

Survivorship and fecundity of *C. dubia* exposed to Clinch River sediments during 2002 were the least variable endpoints observed for all three species tested during 2000 and 2002 (Fig. 5.1c). Survivorship averaged greater than 80% and fecundity averaged from 29 to 35 neonates/adult at all stream sites. Differences in survivorship and fecundity were not significant ($\alpha = 0.05$).

Metals concentrations measured in Clinch River sediments during 2002 indicated the two metals of greatest concern in previous studies (Cu and Zn) had similar concentrations among all sites (Fig. 5.1d). Copper ranged from 6 μg/L at CROE to approximately 8 µg/L at CRDR1 and CRDR2. Zinc ranged from ~270 µg/L at CREFF and CRFA to ~350 µg/L at CRUR1.

5.3.3. Evaluation of chronic Cu toxicity to bivalves

Measured concentrations of total recoverable Cu in the six artificial streams were 16 µg/L in the control, and 21, 25, 34, 52, and 96 µg/L (Fig. 5.2a). After accounting for background Cu levels in diluent, these measured concentrations compared well with our target concentrations of 0, 6, 12, 24, 48, and 96 µg/L. For all test concentrations, mean values of pH ranged between 7.9 and 8.1. Dissolved oxygen was 7.3 to 7.7 mg/L. Specific conductivity ranged from 360 to 370 µmhos. Alkalinity ranged from 174 mg/L as $CaCO_3$, while hardness ranged from 137 to 155 mg/L as $CaCO_3$. For all streams, chlorides were less than 7 mg/L and ammonia was below detection limits (data not shown).

Survivorship of all organisms was greater than 90%, except for clams age 12-18 months, which had reduced survivorship (75%) in the 96-µg/L concentration. However, this difference was not statistically significant ($\alpha = 0.05$).

Growth of *V. iris* of both age classes was low and variable, and generally did not indicate a dose-dependent response to the increasing Cu concentration (Fig. 5.2b-c). Mussels age 12-18 months had significantly reduced growth in height when exposed to Cu concentrations greater than 16 µg/L, but a contrasting trend was observed for length, where growth increased with increasing Cu concentration. Growth in both height and length of mussels age 1-4 months was low and variable, offering no discernable relationship with increasing Cu concentration.

Growth of *C. fluminea* of both age classes was sufficient to permit statistical comparisons, and indicated a dose-dependent response along the Cu concentration gradient (Fig. 5.2b-c). Clams age 1-4 months outgrew clams age 12-18 months by an average of more than 50% for both height and length. The NOAEC and LOAEC for growth of clams age 1-4 months were 52 and 96 μg Cu/L, respectively, and were the same for growth in both height and length, as these two parameters had a significant positive relationship ($R^2 = 0.89$, $p = 0.005$) using the Spearman Rank Correlation. Similarly, clams age 12-18 months had an NOAEC and LOAEC for growth in height of 52 and 96 μg Cu/L, respectively. A trend similar to that observed for length of clams age 1-4 months occurred for clams age 12-18 months as 96 μg Cu/L noticeably (not significant $\alpha = 0.05$) reduced growth.

5.3.4. Evaluation of acute toxicity, synergism, additivism, and antagonism in industrial chemicals

For industrial chemicals tested individually, LC_{50} values obtained by Virginia Tech researchers were orders of magnitude lower than those reported by the chemical manufacturer on MSDS (Table 5.2). Species sensitivity varied among the different chemicals. For example, *C. dubia* was more sensitive to chemical D (an aluminum hydroxide-based flocculant) than was *D. magna*. Likewise, *P. promelas* was more sensitive to chemical F (a brominated cooling-tower biocide) than was *C. dubia*, but was less sensitive than *C. dubia* to chemical D. Daphnids were most sensitive to chemical B, an anionic polymer, having LC_{50} values of well below 1 ppb for *C. dubia* and *D. magna*. Fathead minnows were most sensitive to chemical F, having an LC_{50} value of only 4 ppb. *Daphnia magna* was least sensitive to chemicals C and G.

Using Marking's Additive Index to evaluate interactive effects between the four chemicals found to be most toxic individually (B, D, E, F), we found only one chemical combination (B-F) had significantly synergistic effects, with an MAI value of 2.44 and positive confidence limits (Table 5.3). When these chemicals were tested in combination, the resulting LC_{50} to *C. dubia* (0.02 ppb for both chemicals) was significantly less than when either of these two chemicals were tested individually (0.07 and 4.22 ppb for chemicals B and F, respectively). All other combinations had antagonistic effects, as indicated by negative MAI values and confidence intervals that overlapped zero. The greatest antagonistic effect observed was for the combination of chemical E, a sulfurous metals precipitant, and chemical F (MAI = -18.39). Individually, LC_{50} values for these two chemicals were 21.14 and 4.22 ppb, respectively. In combination, however, LC_{50} values increased to 63.30 and 69.20 ppb, respectively.

5.3.5. Evaluation of settling basin efficiency and residual toxicity in reclaim water

Measures of pH and specific conductivity decreased with distance from the sluice-water influent to the reclaim pond (Fig. 5.3a-b). Average values for pH ranged from 10.2 near the influent to 9.3 in the reclaim pond. Conductivity decreased from an average of 2400 μmhos at the influent, to little more than 1000 μmhos in the reclaim pond. Mean values of DO and temperature remained consistent throughout, ranging from 6.5 to 8.5 mg/L, and from 20 to 23 °C, respectively (data not shown). With the exception of samples taken at the influent during periods of heavy sluicing (when samples were dark from high ash content), dissolved Cu, Zn, and Fe concentrations either increased or remained constant along the settling gradient (Fig. 5.3c-e). Concentrations of Cu, Zn, and Fe measured near the influent pipe were 3, 2, and 9 μg/L, respectively, but increased to 6 ± 3, 6 ± 3, and 10 ± 2. This was likely attributable to the elevated pH near the influent, which would have decreased the dissolved metals fraction in the water column. Aluminum, however, was reduced along the settling gradient, having its greatest concentration near the influent (1766 μg/L), and decreasing to 1197 ± 498 μg/L in the reclaim pond (Fig. 5.3f).

Settling basin influent was acutely toxic to *C. dubia*, but LC_{50} values ranged from 9.5% in samples collected during periods of heavy sluicing, to 70.7% during periods of minimal to no sluicing (when samples were clear). The settling basin was effective at reducing acute toxicity to *C. dubia*, as LC_{50} values were not calculable due to insufficient mortality in acute tests with reclaimed water. We did, however, notice that body-size and activity of *C. dubia* were reduced in higher concentrations. Consequently, we evaluated the chronic toxicity of reclaim pond water to survivorship and fecundity of *C. dubia*. Significant reductions in these endpoints were measured on two occasions, the most significant of which occurred during June 2002, and resulted in NOAEC and LOAEC values for survivorship of 50 and 100% reclaim water, and NOAEC and LOAEC values for fecundity of 25 and 50% (Fig. 5.4a-b).

5.4. Discussion

Temporal variations in industrial discharge rates and composition, interactions between effluents and receiving systems, and the combination of natural stressors affecting indigenous biota may mask instream effects and complicate causal inference (Dickson and Rodgers, 1986; Fetterolf et al., 1986; Farris et al., 1988). Field assessments may be used to quantify toxic effects to indigenous biota, but can be time-intensive and costly (Jop et al., 1991; Mersch and Reichard, 1998), and are limited by their inability to establish causality (Clements and Kiffney, 1996). In an extensive field assessment of CR sites from Cleveland, VA to Saint Paul, VA, Hull et al. (In press, In review, A, B) reported instream impairment of bivalves, both transplanted and naturally occurring downstream of the CRP effluent discharge during 2001-2002. Because the CR contains a diverse assemblage of world-class yet imperiled mussel fauna (Ahlstedt and Tuberville, 1997; Chaplin et al., 2000), these findings warranted a collaborative research effort with AEP and CRP personnel to identify and reduce CRP effluent toxicity. Although not ideal, this procedure was undertaken because the application of standard TI/RE procedures was attenuated by the lack of toxicity in instantaneously collected effluent samples.

We found no clear evidence of sediment toxicity to US EPA test organisms. Site-collected sediments did not consistently reduce survivorship and growth of *C. tentans* or survivorship and fecundity of *D. magna* and *C. dubia*. Sediment-associated metals concentrations downstream of the CRP were comparable to or less than those measured at stream sites where no adverse biological effects were observed.

Effluent metals concentrations, particularly Cu, remain a concern at the CRP despite considerable reductions through wastewater treatment modifications in 1993. During monthly monitoring in 2000-2002, effluent Cu levels periodically approached or exceeded water quality criteria (Table 5.1). Transplanted bivalves, *C. fluminea* and *V. iris*, bioaccumulated Cu to its highest levels downstream of the CRP discharge, but a direct causal relationship was not established. The laboratory studies we conducted exposing juvenile and adult *C. fluminea* and *V. iris* to $CuSO_4$ solutions of varying Cu concentration and water hardness similar to that of the Clinch River (~150 mg/L as

$CaCO_3$) suggested that during 21-d exposures, Cu concentrations of 96 μg/L could induce significant reductions in growth of *C. fluminea* age 1-4 and 12-18 months. Growth in height (umbo to ventral margin) of *V. iris* age 12-18 months was significantly reduced at Cu concentrations ≥ 21 μg/L, but a similar reduction for length (anterior to posterior), a more common measure of unionoid growth, did not occur. The sensitivity of mussels age 1-4 months could not be determined due to insufficient growth, perhaps due to sub-optimum sediment particle size (250-450 μm) used in test chambers. While this laboratory study suggests that present CRP Cu levels should not impair bivalves chronically, it cannot exclude potential interactive effects in the receiving environment that could cause lower Cu levels to impair indigenous biota. However, the chronic Cu thresholds of 96 μg/L for growth of *C. fluminea* suggest this species may be far more sensitive to Cu than has been indicated by other researchers (e.g., Cherry et al., 2002). This discrepancy may be due to insufficient exposure durations in previous tests.

Testing with chemicals used by the CRP for the treatment of industrial wastewater indicated dramatic toxicity to *C. dubia*, *D. magna*, and *P. promelas*. Given that the chemicals we tested were in concentrated form and are typically applied in low volumes (i.e., mL to gallons) to treat high volumes of wastewater (i.e., MGD), we expected to observe a high degree of toxicity to test organisms. We were, however, surprised to see that in some instances, LC_{50} values for individual chemicals (e.g., chemical B) were orders of magnitude below those reported by the chemical manufacturer for the same test species under similar test conditions. In combination, synergistic toxicity was observed only once, with all other combinations having antagonistic results. Although our findings emphasize the toxic nature of chemicals used to treat industrial wastewater, we acknowledge that under real-world applications, these chemicals will most likely be (1) diluted to non-toxic levels by high volumes of wastewater, (2) consumed during the treatment process, (3) diluted further by the receiving system, and/or (4) dissipated through physical processes such as agitation and aeration. Combined with their lack of environmental realism, the complexity associated with testing industrial treatment chemicals under laboratory conditions is a time-consuming and expensive process that might not justify the conclusions derived. Nevertheless, the procedures used to determine LC_{50} values for chemicals discharged with industrial wastewater should be reviewed so that more accurate representations of toxicity are conveyed to operators through MSDS.

The CRP wastewater treatment system was not originally designed to treat and discharge reclaimed water from ash settling basins, and this process represents a recent deviation (>1995) from the plant's typical operating procedures. Average pH of the reclaim pond was 9.3, slightly above the range considered safe for aquatic life. Continuous monitoring of CRP effluent indicated that effluent pH has approached levels approximating 9.0. As a result of the elevated pH, most aqueous metals fractions in reclaim pond water were well below water quality criteria. The concentration of Al, however, measured in CRP effluent on October 2, 2002, of 843 μg/L exceeded both acute and chronic water quality criteria. Elevated concentrations of Al were also associated with the CRP reclaim pond, and this metal may warrant further consideration in future studies. Water column samples collected from the reclaim pond on various dates were not acutely toxic to *C. dubia* test organisms during 48-h exposures. However, chronic exposures (7-d duration) significantly reduced survivorship and fecundity of *C. dubia* on two occasions. Based upon our chronic toxicity tests, contributions of reclaimed water to

CRP effluent that exceed 25% of the total effluent could result in measurable sublethal effects (i.e., significantly reduced fecundity of *C. dubia*); contributions exceeding 50% could result in measurable lethal effects (i.e., significantly reduced survivorship of *C. dubia*).

While TI/RE procedures have been particularly effective at identifying and reducing sources of effluent toxicity (e.g., Deanovic et al., 1999; Maltby et al., 2000), their investigative value diminishes when the process cannot be completed quickly and cost efficiently. Hull et al. (In press, In review B) reported that repeated sampling of CRP effluent and ambient waters downstream produced no measurable adverse effects to US EPA test organisms. Despite this, bivalves were consistently impaired downstream of the CRP, with significantly reduced survivorship and growth measured as near as 15 m downstream of the effluent discharge. Survivorship and growth were unimpaired less than 100 m upstream (Hull et al., In review B). Nevertheless, field assessments lack the ability to establish causality (Clements and Kiffney, 1996) and because of this, findings reported by Hull et al., (In press, In review A,B) fail to establish a definitive linkage between CRP effluent and impairment of bivalves.

Sarakinos and Rasmussen (1998) reported that "laboratory bioassays may overestimate field thresholds if measurement endpoints are not sensitive enough." Norberg-King et al. (1991) reported that an essential component in successfully completing a TIE of ambient waters in the Colusa-basin drain, California, was that toxicity could be tracked using the test species that first triggered the request for the TIE. The sensitivity of US EPA test organisms has been frequently questioned, particularly when assessing streams such as the Clinch River where indigenous species are especially sensitive (Cherry et al., 2002) and federally protected. Because of this, future research investigating CRP effluent toxicity should consider the development of a feasible laboratory toxicity testing protocol using multiple bivalve species, particularly *C. fluminea*. Unfortunately, although certain procedures have been proposed for laboratory toxicity testing with freshwater mollusks (e.g., Wade et al., 1989; Jacobson et al., 1989), no standardized toxicity testing protocols presently exist (US EPA, 1990). Consequently, researchers should continue to make this a research area of high priority.

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Table 5.1. Values for various physico-chemical parameters as measured in CRP effluent. Values of temperature, DO, pH, conductivity, alkalinity, and hardness were based on measurement made from November 2000 to September 2001. Values of cations (except for Cu) and anions based on CRP effluent sample collected October 2, 2002. Values of Cu were measured in CRP composite samples taken monthly from January 2000-October 2002. US EPA acute and chronic water quality criteria are provided where available.

Parameter	Mean ± SD (Range)	Water Quality Criteria
Temperature (°C)	20 ± 8 (10-26)	-
Dissolved Oxygen (mg/L)	8.3 ± 1.0 (7.2-9.3)	-
pH	8.4 ± 0.1 (8.3-8.5)	6.0-9.0
Conductivity (μmhos)	993 ± 171(747-1213)	-
Alkalinity (mg/L as $CaCO_3$)	124 ± 15 (110-140)	-
Hardness (mg/L as $CaCO_3$)	445 ± 21 (430-460)	-
Cu (μg/L)	14 ± 8 (5-42)	13 μg/L acute, 9 μg/L chronic
Zn (μg/L)	7	120 μg/L acute, 120 μg/L chronic
Fe (μg/L)	14	1000 μg/L acute, 1000 μg/L chronic
Al (μg/L)	843	750 μg/L acute, 87 μg/L chronic
Chloride (mg/L)	107	860 mg/L acute, 230 mg/L chronic
Nitrate as N (mg/L)	2	-
Sulfate (mg/L)	302	-
Phosphate as P (mg/L)	0.7	-

Table 5.2. Comparison of LC_{50} values obtained by us to those reported by the manufacturer for seven chemicals used to treat industrial wastewater. Chemicals were tested individually. Superscript asterisks denote the four most toxic chemicals as determined by tests conducted at Virginia Tech using *C. dubia*, *D. magna*, and *P. promelas*.

Chemical	**LC_{50} values reported as parts per billion (ppb)**					
	Ceriodaphnia dubia		*Daphnia magna*		*Pimephales promelas*	
	VT	Manufacturer	VT	Manufacturer	VT	Manufacturer
A	-	-	39	76,000	-	-
B$^{*, A}$	0.07	-	<0.01	200,000	74	-
C	-	-	387	>1,000,000	-	-
D*	1.33	-	4	270,000	40	-
E*	21.14	-	154	11,000	39	-
F*	13.36	1600	3	-	4	-
G	-	-	453	520,000	-	-

^{A} Stock solution of 0.1% used by manufacturer to generate LC_{50} values for this chemical

Table 5.3. Results of acute toxicity testing with the four most toxic effluent treatment chemicals in combination, to evaluate the potential for synergistic interactions using Marking's Additive Index (Marking, 1977). Bold font denotes synergistic mixture.

Chemical Comb.	Individual Chemical	LC_{50} (ppb) in comb.	Individual LC_{50} (ppb)	S	MAI	Lower 95% Conf. Limit	Upper 95% Conf. Limit	Add., Syner., or Antag.
B, D, E, F	B	0.20	0.07	3.12	-2.12	0.17	10.67	Antag.
	D	0.25	1.33					
	E	0.24	21.14					
	F	0.26	4.22					
B, D, E	B	0.29	0.07	4.43	-3.43	0.22	10.72	Antag.
	D	0.36	1.33					
	E	0.34	21.14					
B, D, F	B	0.26	0.07	3.81	-2.81	0.20	10.70	Antag.
	D	0.14	1.33					
	F	0.33	4.22					
B, E, F	B	0.11	0.07	1.71	-0.71	0.11	2.17	Antag.
	E	0.30	21.14					
	F	0.14	4.22					
D, E, F	D	4.44	1.33	4.67	-3.67	3.04	12.74	Antag.
	E	4.33	21.14					
	F	4.75	4.22					
B, D	B	0.29	0.07	4.41	-3.41	0.22	0.81	Antag.
	D	0.36	1.33					
B, E	B	0.30	0.07	4.30	-3.30	0.22	10.72	Antag.
	E	0.36	21.14					
B, F	**B**	**0.02**	**0.07**	**0.29**	**2.44**	**0.05**	**2.12**	**Syner.**
	F	**0.02**	**4.22**					
D, E	D	4.46	1.33	3.56	-2.56	2.90	12.73	Antag.
	E	4.32	21.14					
D, F	D	3.96	1.33	3.98	-2.98	2.78	4.09	Antag.
	F	4.23	4.22					
E, F	E	63.30	21.14	19.39	-18.39	36.71	45.17	Antag.
	F	69.20	4.22					

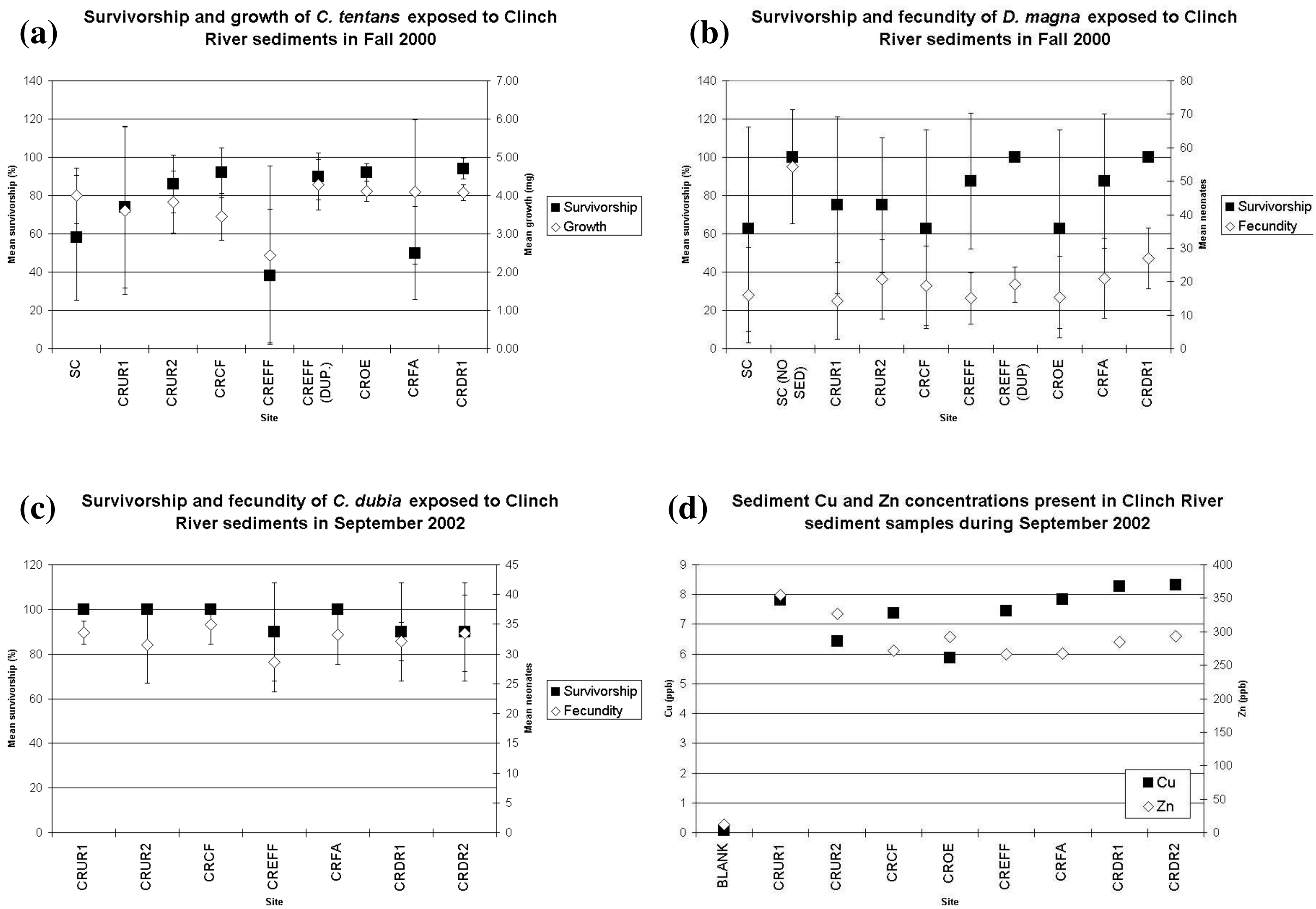

Fig. 5.1. Results of Clinch River sediment toxicity characterization using (a) survivorship and growth of *C. tentans*, (b) survivorship and fecundity of *D. magna*, (c) survivorship and fecundity of *C. dubia*, and (d) the concentrations of Cu and Zn present in digested sediment samples.

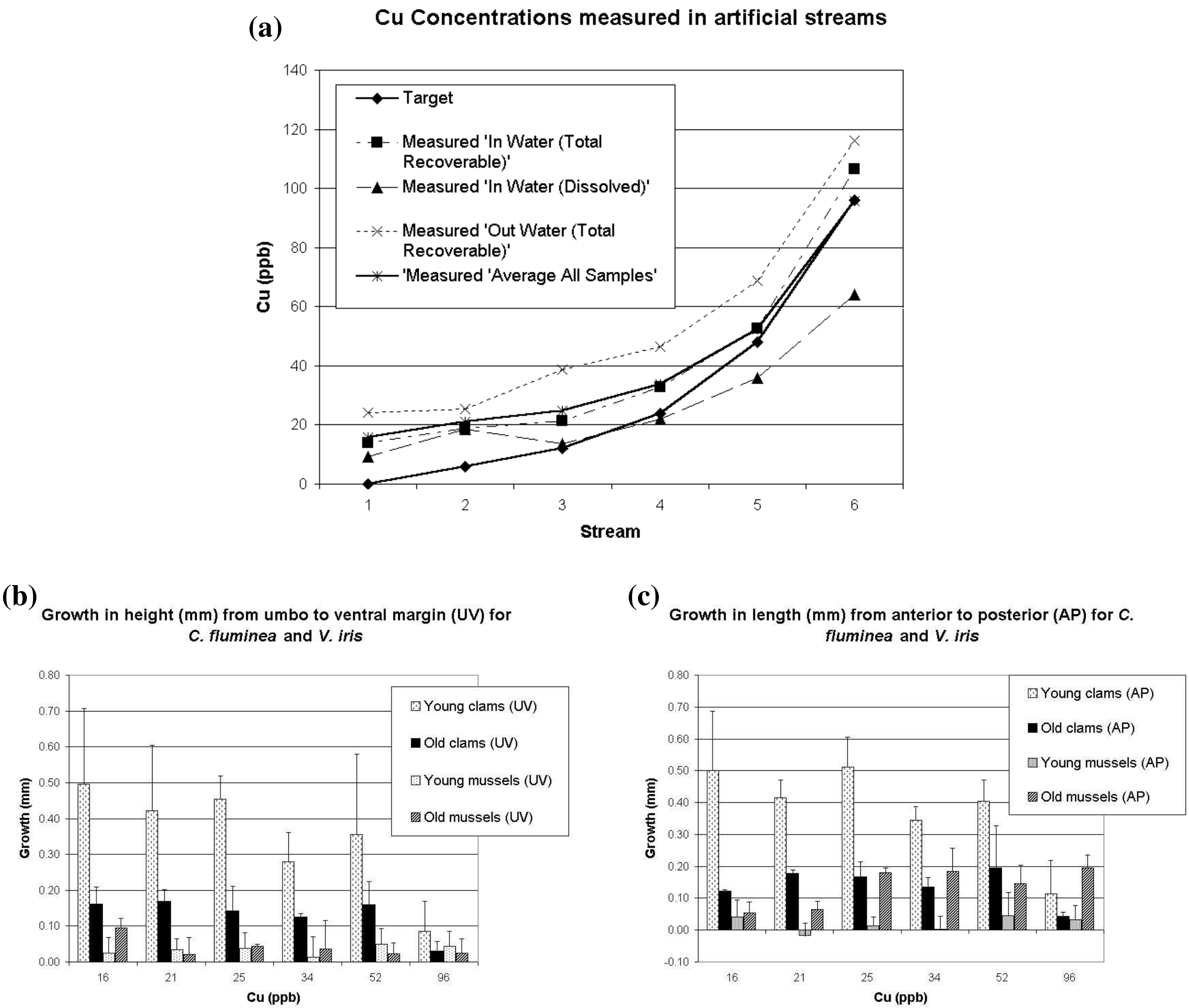

Fig. 5.2. Measured levels of (a) total recoverable and dissolved Cu concentrations are provided. Results for growth in terms of (b) height and (c) length of *C. fluminea* and *V. iris* exposed to Cu during 21-d laboratory exposures.

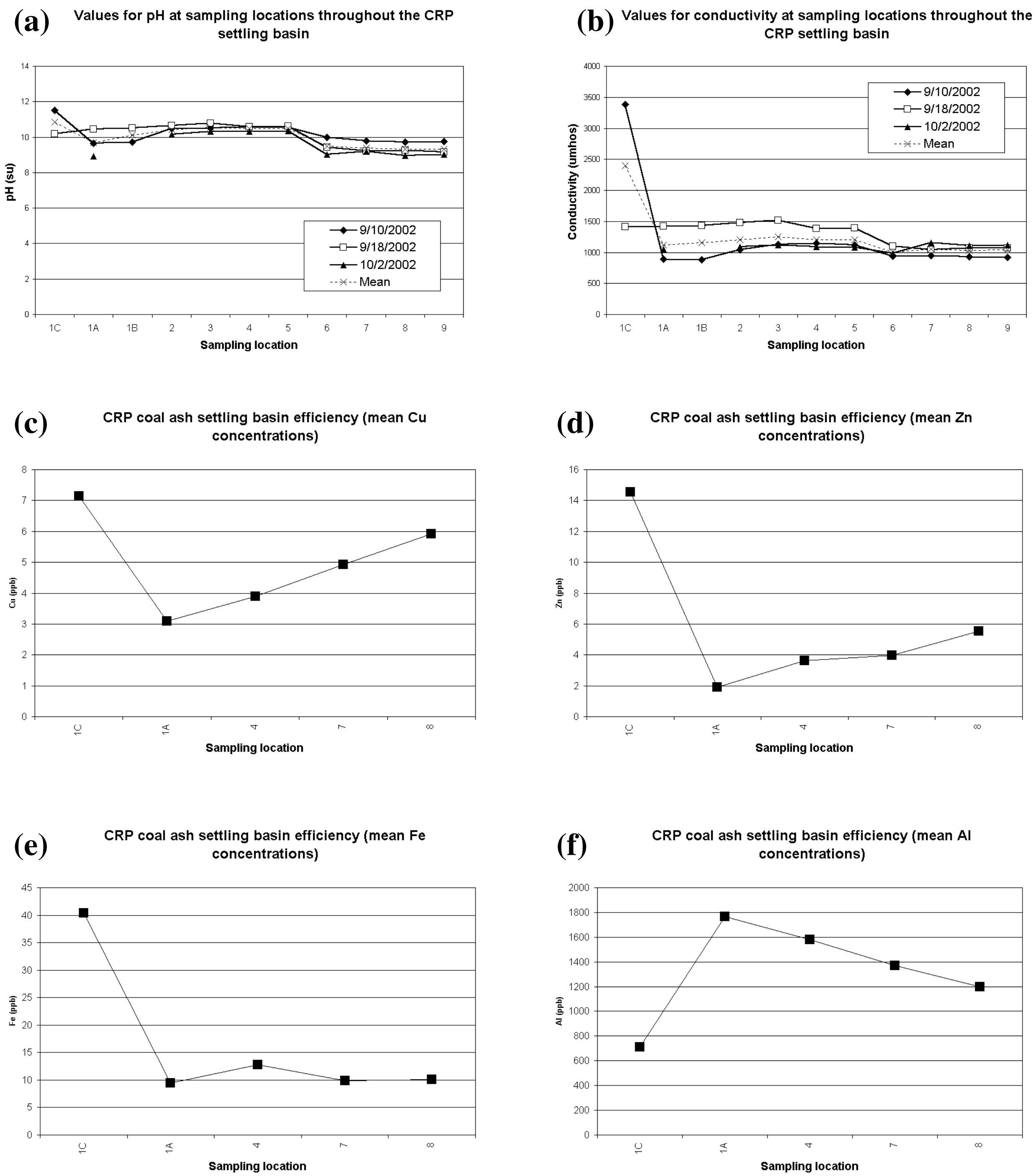

Fig. 5.3. Evaluation of the CRP coal-ash settling basin from influent (location 1C) to polishing pond (location 8), using (a) pH, (b) conductivity, and (c-f) dissolved concentrations of Cu, Zn, Fe, and Al, respectively, as indicators of settling efficiency.

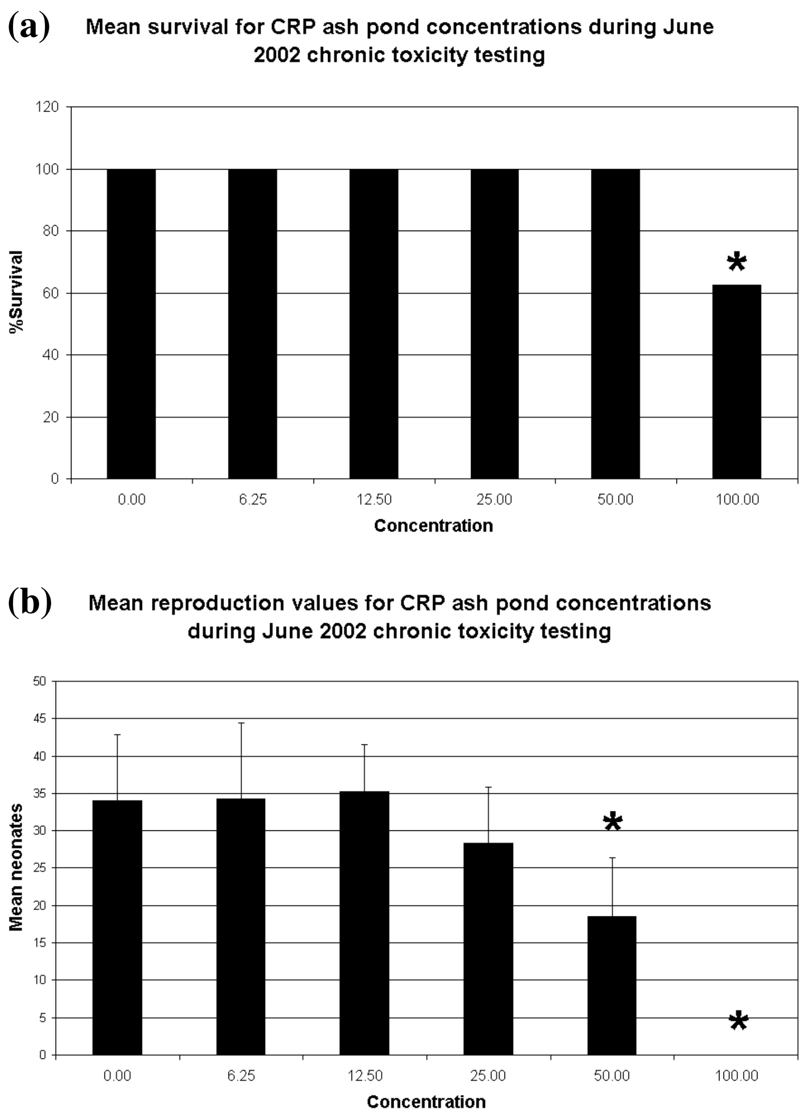

Fig. 5.4. Mean values for (a) survivorship and (b) fecundity of *C. dubia* exposed to water reclaimed from the CRP settling basin for potential discharge with CRP effluent. Asterisks denote significant differences from the control ($\alpha = 0.05$).

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Curriculum Vita

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Date and Place of Birth: 4 May 1978, Pulaski, Virginia.

Present Position and Current Research:

August 2000-Present: Enrolled in Virginia Tech's Master of Science Program (Department of Biology), under the direction of Dr. D.S. Cherry (Aquatic Ecotoxicology). Virginia Polytechnic Institute and State University, Blacksburg, VA. Thesis research focused on assessing the current ecotoxicological health of a power plant influenced section of the Clinch River in Carbo, Virginia. In situ toxicity testing with the Asian clam (*Corbicula fluminea*) and the Rainbow mussel (*Villosa iris*), surveys of native mussels, benthic macroinvertebrate collection and identification, and laboratory toxicity testing with *Daphnia magna*, *Ceriodaphnia dubia*, and *Chironomus tentans* were used to make this assessment. Each chapter of my thesis addresses a specific objective (outlined below) and will be submitted for publication in peer-reviewed journals:

Objective 1: Investigate biotic integrity downstream of power plant effluent discharge
Objective 2: Evaluate performance of caging designs for in situ tests with bivalves
Objective 3: Integrate data obtained from in situ experiments with field surveys
Objective 4: Assess current status of biotic recovery below power plant
Objective 5: Identify probable source of toxicity in power plant effluent discharge

Graduate Education:

May 2000-Present: Nearing completion of Master of Science degree in Biology from Virginia Tech, Blacksburg, VA (terminal defense seminar scheduled for December 2002). Field of Interest – Aquatic Ecotoxicology, GPA 3.44/4.0.

Undergraduate Education:

May 2000: Earned Bachelor of Science degree in Environmental Science from Ferrum College, Ferrum, VA. Major – Environmental Science, Minors – Business and Biology, GPA 3.79/4.0.

Experience:

Present: Research Biologist- Luna Innovations, Blacksburg, VA.
January 2001-December 2002: Research Assistant- Virginia Polytechnic Institute and State University, Blacksburg, VA.
May 2000-December 2000: Teaching Assistant- Virginia Polytechnic Institute and

State University, Blacksburg, VA.
January 2000-May 2000: Conducted Ecotoxicological Campus Assessment- Department of Life Sciences, Ferrum College, Ferrum, VA.
January 2000-May 2000: Peak Creek Water Quality Study (Pulaski)- Department of Life Sciences, Ferrum College, Ferrum, VA.
August 1999-December 1999: Wastewater Treatment Assistant- Ferrum Water and Sewage Authority, Ferrum, VA.
May 1999-August 1999: Industrial Environmental Intern- Department of Environmental Affairs, Volvo Trucks North America Incorporated, Dublin, VA.
January 1998-May 1998: Field Research- Department of Life Sciences, Ferrum College, Ferrum, VA.

Training:

- 40-Hour OSHA HAZWOPPER Certification (1998).
- Acute and chronic toxicity testing using *Daphnia magna*, *Ceriodaphnia dubia* and *Pimephales promelas* for National Pollutant Discharge and Elimination System (NPDES) industrial permits.
- Chronic sediment toxicity testing using *Daphnia magna* and *Chironomus tentans* for all power plant sites in the American Electric Power (AEP) company complex.
- Culture maintenance of *Ceriodaphnia dubia*, *Daphnia magna* and *Chironomus tentans* using U.S. EPA quality assurance/ quality control guidelines.
- YCT and algal food processing for cladoceran feeding.
- In situ toxicity testing using *Corbicula fluminea* and *Villosa iris* for 30 to 120 days to bioassess power plant effluents.
- Basic water chemistry analysis including temperature, pH, dissolved oxygen (DO), conductivity, alkalinity and hardness.
- Preparation of water, sediment, and biological tissue samples for spectrochemical determination of total recoverable elements.
- Benthic macroinvertebrate collection using the U.S. EPA level II Rapid Bioassessment Protocol (RBP) (1989 and 1999) and identification, using standard keys, to the lowest practical taxonomic level (usually genus).

Relevant Course Work:

Environmental Chemistry
Environmental Toxicology
Environmental Engineering
Environmental Planning and Impact
Air and Water Pollution
Aquatic Entomology
OSHA
Natural Resources
Fundamentals of Ecology
Organic Chemistry
Soil Science
Plant Science
Animal Science
Fish and Wildlife Biology
Statistics
Computer Applications
Business Management
Accounting
Statistics in Research

Computer Skills:
Windows, Microsoft Office 1995, 1997, 2000 Suites (Word, Excel, PowerPoint), Internet, Email (Eudora, Outlook, Web mail), JMPIN, Toxstat, and SAS.

Memberships:
Society of Environmental Toxicology and Chemistry (SETAC)
Alpha Chi National Collegiate Honor Society
North American Benthological Society (NABS)
American Malacological Society (AMS)

Honors and Activities:
Youth Academic/Athletic Achievement Award, Pulaski, Virginia, Winter 2000. Presented by Town of Pulaski Department of Recreation.
Environmental Science Scholastic Achievement Award, Ferrum College, Spring 2000. Presented by the Life Sciences Division of Ferrum College.
Dean's List every semester (8) as an Undergraduate, Ferrum College, Fall 1996 – Spring 2000. Presented by Ferrum College.
Four year letterman in Varsity Football, Ferrum College, Fall 1996 – Fall 1999. Presented by Ferrum College Athletic Department.
Academic All Conference, Ferrum College, Fall 1999. Presented by Ferrum College Athletic Department.
Varsity Football Co-Captain, Ferrum College, Fall 1999. Presented by Ferrum College Football Coaching Staff.
Peer Tutor for Statistics and Western Civilizations, Ferrum College, Fall 1997 – Spring 2000.

Funded Grant Proposals:
Hull, M.S. Validation of Laboratory and in situ Ecotoxicological Research Techniques through Instream Density/Distribution Assessments of Resident Bivalves (*C. fluminea* and Unionidae). 2001. Funded by the Virginia Tech Graduate Student Assembly. Amount Requested: $300. Amount Received: $600 ($300 of matching funds provided by Virginia Tech Biology Department.

Cherry, D.S., Currie, R.J., Hull, M.S. 2001. Ecotoxicological Evaluation of the Clinch River Plant 003 Outfall into the Clinch River, Virginia. Funded by American Electric Power (AEP). Amount Requested: $58,212. Amount Received: $58,212.

Manuscripts in Press:
Hull, M.S., Cherry, D.S., Soucek, D.J., Currie, R.J., Neves, R.J. Comparison of Asian clam field bioassays and benthic community surveys in quantifying effects of a coal-fired power plant effluent on Clinch River biota. Journal of Aquatic Ecosystem Stress and Recovery. Accepted November, 2002.

Manuscripts in Review:
Hull, M.S., Cherry, D.S., Soucek, T.C. Growth of transplanted Asian clams varies with cage design in freshwater streams: implications to quantifying sub-lethal bivalve

responses in situ. Submitted to the Environmental Monitoring and Assessment Journal in June, 2002.

Invited Lecturer:

Hull, M.S. An Ecotoxicological Recovery Assessment of a Power Plant Influenced Section of the Clinch River. Presented at Virginia Tech, Blacksburg, Virginia on April 30, 2001.

Hull, M.S. The Role of Ecotoxicological Assessment in the Conservation of Endangered Species: A Case Study Involving the Unionid Fauna of the Clinch River. Presented at Roanoke College, Roanoke, Virginia on October 30, 2001.

Presentation Abstracts:

Hull, M.S., D.S. Cherry, D.J. Soucek, R.J. Currie. Assessing Individual and Community Level Recovery in a River Influenced by the Clinch River Power Plant, Carbo, Virginia. Poster presented at the 2001 National Meeting of the Society of Environmental Toxicology and Chemistry, Baltimore, MD, November 2001.

Hull, M.S., D.S. Cherry, T.C. Merricks. Homogenizing substrate during in situ Asian clam studies strengthens specificity of association between ecotoxicological influences and biological responses. Poster presented at the 2002 National Meeting of the North American Benthological Society, Pittsburgh, PA, June 2002.

Hull, M.S., D.S. Cherry. Surveys of Clinch River bivalves validate findings of Asian clam transplant studies. Poster presented at the 2002 National Meeting of the Society of Environmental Toxicology and Chemistry, Salt Lake City, UT, November 2002.

Technical Reports:

Cherry, D.S., Currie, R.J., Hull, M.S., Kennedy, A.J., and Merricks, T.C. 2001. Evaluation of Potential Acute Toxicity of 003 Effluent from International Business Machines Corporation (IBM), Poughkeepsie, New York.

Cherry, D.S., Currie, R.J., Soucek, D.J., Schmidt, T.S., Hull, M.S., Kennedy, A.J. 2000. Toxicity Test Laboratory Performance Evaluation (DMR-QA Study 20) for Acute and/or Chronic tests.

Cherry, D.S., Currie, R.J., Hull, M.S., Soucek, D.J., Schmidt, T.S., Kennedy, A.J. 2000. Sediment Toxicity Testing of Stream and River Sites Near American Electric Power (AEP) Facilities in Ohio, West Virginia, Virginia, Kentucky and Indiana-Group 4: Glen Lyn and Clinch River Plants.

Cherry, D.S., Currie, R.J., Soucek, D.J., Schmidt, T.S., Kennedy, A.J., Hull, M.S., Merricks, T.C. 2001. Quarterly Chronic Biomonitoring Results of a Water Flea (*Ceriodaphnia dubia*) and Fathead Minnow (*Pimephales promelas*) to Pine Bluff Mill Effluent, International Paper Company, Pine Bluff, Arkansas.

Cherry, D.S., Soucek, D.J., Hull, M.S. 2001. Chronic 21-day Renewal Toxicity Responses with *Daphnia magna* to Biofungicide from Sybron Chemicals, Inc. Submitted to Sybron Chemicals, Inc., 111 Kesler Mill Road, Salem, VA 24153. March 28, 2001.

References:

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