Hydrobiologia (2006) 556:341–355
DOI 10.1007/s10750-005-9111-1

Primary Research Paper

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

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Received 15 December 2004; in revised form 27 July 2005; accepted 31 July 2005

Key words: Asian clam, Clinch River, coal ash, coal mining, Corbicula, bivalves, mussels, power plant effluent, Unionoidea

Abstract

Previously, we reported that survivorship and growth of field-caged Asian clams (*Corbicula fluminea* [Müller]) were significantly reduced downstream of the wastewater effluent discharge of the Clinch River Plant (CRP), a coal-fired electric power-generating facility in Russell County, VA (USA). These findings warranted the present study, which investigated whether reduced survivorship and growth of transplanted *C. fluminea* were predictive of adverse effects on resident bivalves – most notably the Clinch River's (CR's) federally protected freshwater mussel fauna (Unionoidea). Thus, surveys of unionoid assemblages and *C. fluminea* population densities and age distributions were conducted to assess ecotoxicological effects on bivalve assemblages naturally occurring in the CR. Results of these surveys suggested that field bioassays

the CR including (i) the wastewater effluent discharge of the coal-fired, Clinch River Plant (CRP), (ii) the confluence with a low order tributary, Dump's Creek (DC), which is influenced by active mining, coal-processing effluent, and coal ash settling pond leachate, and (iii) a coal fly ash landfill (FA). 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 & Canton, 1983), coal ash settling basins (e.g., Cherry et al., 1979), and trace metals associated with coal FAs (Cherry & Guthrie, 1977) have been documented previously.

Distinguishing the specific effects of multiple-source perturbations 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 & Clements, 1998) and typically involve concurrence between controlled, laboratory experiments and field biomonitoring data (e.g., Kimball & Levin, 1985; Hickey & Clements, 1998). Most of these studies have integrated laboratory toxicity tests with responses of benthic macroinvertebrate communities, primarily aquatic insect assemblages (Cairns & Pratt, 1993; Hickey & 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 & Kiffney, 1994).

Bivalves have been used as sentinel organisms to monitor the effects of environmental contaminants on aquatic ecosystems (e.g., Haynes & Toohey, 1998; Gunther et al., 1999; Cattani et al., 1999; Hull et al., 2002, 2004). 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 used in experimental procedures to integrate exposure effects over time (ASTM, 2001), and to permit variable sublethal response measurements such as growth (Belanger et al., 1990), enzymatic activity (Farris et al., 1988), natality (Smith & Beauchamp, 2000) and damage to DNA strands (Black et al., 1996). Despite their documented suitability for biomonitoring, relatively few studies have integrated the responses of transplanted and naturally occurring bivalves to assess ecotoxicological condition of streams (Phillips, 1976; Belanger et al., 1990). Rather, most studies have related the responses of field-caged bivalves with surveys of benthic macroinvertebrate communities (Smith & Beauchamp, 2000; Soucek et al., 2001; Hull et al., 2002).

Hull et al. (2002) reported that surveys of CR benthic macroinvertebrate communities 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 CRP effluent 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 & Waller, 2000). Thus, the field assessments described in this manuscript were necessary to determine whether impairment observed during *C. fluminea* transplant studies extended to resident bivalves, particularly imperiled unionoid fauna, and whether transplanted and resident bivalves could be used to quantify effects of multiple coal mining and combustion related activities in the CR watershed. To accomplish this, we conducted field-bioassays using transplanted *C. fluminea* and *Villosa iris*, and related these data to in-stream density sampling of *C. fluminea* and occurrence of native mussels.

Materials and methods

Site descriptions

Study sites were selected based on their location relative to coal processing and combustion-related activities in the CR mainstem, the DC tributary to CR, and the Hurricane Fork (HF) tributary to DC (Fig. 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

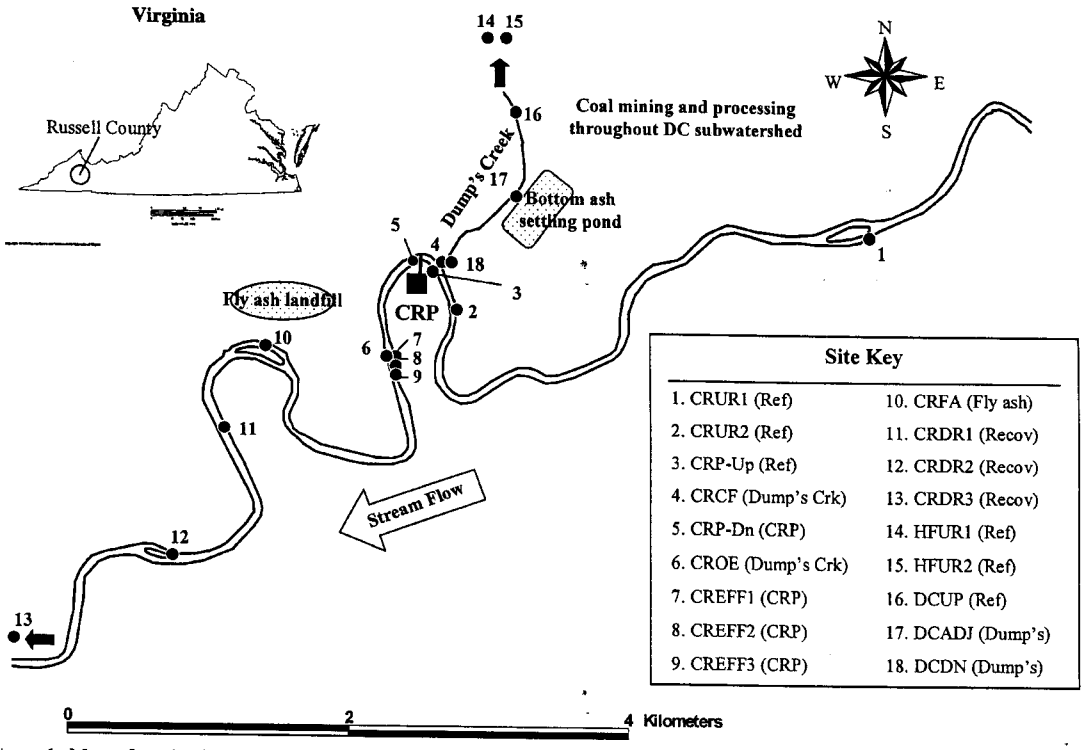

Figure 1. Map of study sites used to quantify relative effects of coal-related activities on transplanted and resident bivalves in the Clinch River watershed, Carbo, Virginia, USA.

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 Clinch River Confluence (CRCF). 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 descending 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 Clinch River Opposite Effluent (CROE). In 2001, two additional sites were used to separate the potential toxic effects of DC and CRP effluent. These sites, Clinch River Plant Upstream and Downstream (CRPUP and CRPDN), 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 (FA) landfill situated within the CR drainage basin, and was referred to as Clinch River Fly Ash landfill (CRFA). 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.

Physico-chemical parameters

Methods used to measure basic water chemistry parameters at all study sites have been described elsewhere (Hull et al., 2002). 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 50 °C 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).

Density sampling of resident Asian clams

From 1986 to 1995, the invasion of *C. 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 & Williams, 1986). Typically, Asian clams thrive in streams with slow to moderate current (Parmalee & Bogan, 1998) and prefer substrates of mixed sand–silt–mud (Belanger, 1985; Parmalee & 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. Additional sampling was performed 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 perimeter of the sampler, large cobble and accumulated debris were 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 1 mm^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, 5.0–9.9, 10.0–14.9, 15–20, and >20.0 mm (umbo to ventral margin). Total densities and the average number of individuals in each size class were estimated for a 1.0 m^2 area of streambed.

Surveys of native mussels

Because CR unionoids have been surveyed extensively (e.g., Bates & 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. Rather, 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 freshwater mussels (see Neves & Widlak, 1987; Parmalee & 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 number of living mussels found, and 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.

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. (2002, 2004). *Corbicula* were obtained from either the New River, near Ripplemead, VA (during 2000–2001), or from a site upstream in the CR, near Pounding Mill, VA (during 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, nylon mesh bags and substrate-filled plastic cages, and have been described elsewhere (Hull et al., 2004). Test chambers were transported in ice-filled coolers to site locations 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 analyses of *in situ* toxicity tests with *C. fluminea* have been described previously by Hull et al. (2002, 2004).

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* (~12 mo in age) of 3.5–6.5 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., 2002, 2004), except that they were partitioned into four separate sections to facilitate tracking of individuals. The shells of juvenile rainbow mussels are typically 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 previously have associated the accumulation of some of these metals with coal-related activities (e.g., Belanger et al., 1990).

Results

Physico-chemical parameters

Water chemistry parameters were generally similar among reference and influenced sites (data available upon request). 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 7.6.

Water depths averaged 0.5 m throughout the CR mainstem, 0.3 m in DC and 0.1 m in HF. 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/s at CR sites, compared to 0.18 m/s within DC and 0.15 m/s in HF. Total organic carbon 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, 67% 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.

Density sampling of resident Asian clams

During 2001, living *C. fluminea* were found at all 11 CR and DC sites surveyed. 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 FA 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 (data available upon request). 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. 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.

Surveys of native mussels

Live mussels of 17 species were collected from 5 of the 7 sites surveyed (Table 1). The assemblage inhabiting the uppermost reference site, CRUR1,

Table 1. Results of qualitative mussel surveys conducted in Fall 2001 at CR sites downstream of Dump's Creek (DC), the Clinch River Plant effluent discharge (CRP), and the coal fly ash landfill (FA) in Russell County, VA

Species	Upstream Ref.		Below DC	Below CRP	Below FA	Downstream Recovery	
	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	–	–	–	–	5	–
Villosa iris	2	–	4	–	–		
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

Results for upstream reference and downstream recovery sites also are shown. [SC] = Species of special concern.[F] = Federally endangered species.[TEV] = Species threatened and endangered in the state of Virginia (USA).

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 2 federally listed species (*Fusconaia cor* and *Quadrula c. strigillata*), 1 species threatened and endangered in Virginia (*Lexingtonia dolabelloides*), and 4 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 FA, 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.

Transplant studies using C. fluminea and V. iris

Hull et al. (2002, 2004) 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. At sites downstream of the DC tributary and the FA, however, survival and growth were 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. 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. 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. 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

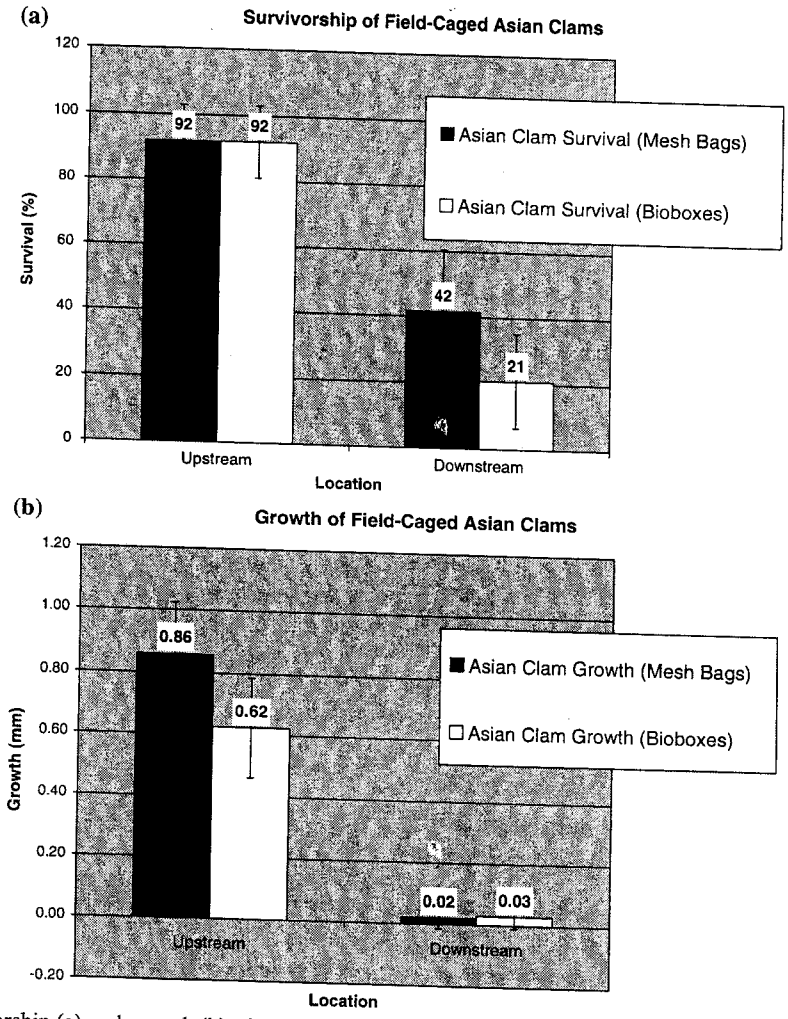

Figure 2. Survivorship (a) and growth (b) of Asian clams in two types of field enclosures, transplanted ~100 m up- and ~15 m down-stream of the CRP effluent discharge. Differences between upstream and downstream values for both enclosure types were significant at $\alpha = 0.05$.

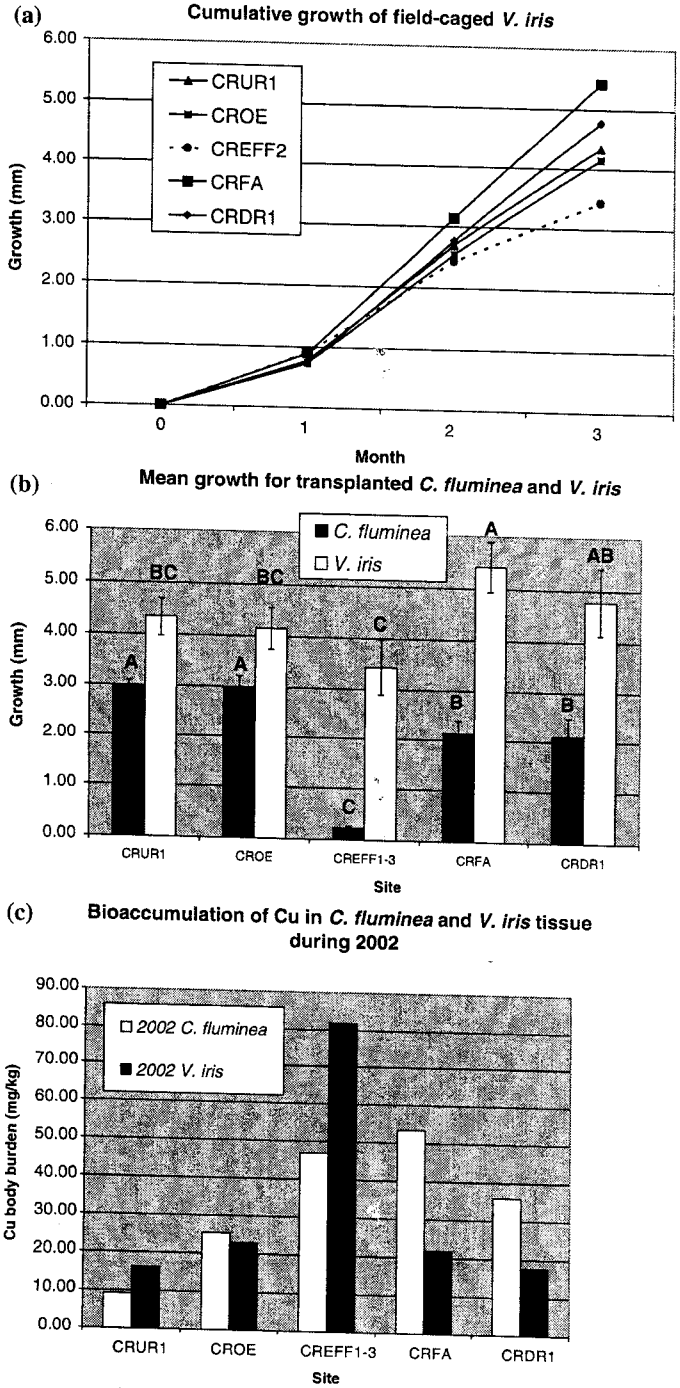

Figure 3. (a) Cumulative growth of field-caged *V. iris* transplanted to mainstem CR sites for 96 d during 2002 (N = 5), (b) a comparison of *V. iris* and *C. fluminea* final growth at the same five sites after the 96-d experiment, and (c) Cu levels (ppb) accumulated in transplanted bivalve tissues during 2002.

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. 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 level of Cu 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).

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 & 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 & 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 & Waller, 2000; Culp et al., 2000). Hull et al. (2002, 2004) 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, relative to 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., 2002). These conflicting results necessitated efforts to determine whether impairment of transplanted *C. fluminea* extended to native bivalves, most notably the federally protected unionoid 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 during multiple sampling seasons, 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, when we observed a density of ~4 clams/m^2 at CREFF1-3 from a single size class (5–9.9 mm). Intensive re-sampling performed ~30 d later to document the fate of these clams yielded no live specimens and may have indicated a failed attempt by *C. fluminea* to effectively colonize CREFF1-3.

Belanger et al. (1990) reported that CRP effluent (i) significantly reduced mortality and growth of transplanted *C. fluminea*, and (ii) 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 CR sites were measured at 47.4–104.8 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 in-stream 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 55 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 be conducted to (i) determine whether effluent Cu concentrations could chronically or intermittently impair bivalves at their presently reduced levels or (ii) 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 bivalves.

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, at CROE, five live mussels of two species were found. 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 5 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 (i) interactive effects between Corbiculidae and Unionoidea and (ii) 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 & Williams, 1986). Unionoidea are comparatively 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 4 years (McMahon & Williams, 1986), whereas the life spans of freshwater mussels such as *V. iris* typically range from 10–50 years (Naimo, 1995). When compared to other freshwater organisms, Asian clams have a remarkably high filtration rate (Buttner & Heidinger, 1981; Foe & Knight, 1987) and ion transport capacity (Zheng & 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 be exposed to a contaminant for a greater portion of its life cycle and 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 interspecific 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 (i) significant reductions in survival and growth of field-caged Asian clams and growth of field-caged *V. iris*, (ii) the virtual absence of naturally occurring *C. fluminea*, and (iii) 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 & Waller, 2000). Furthermore, Hull et al. (2004) 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

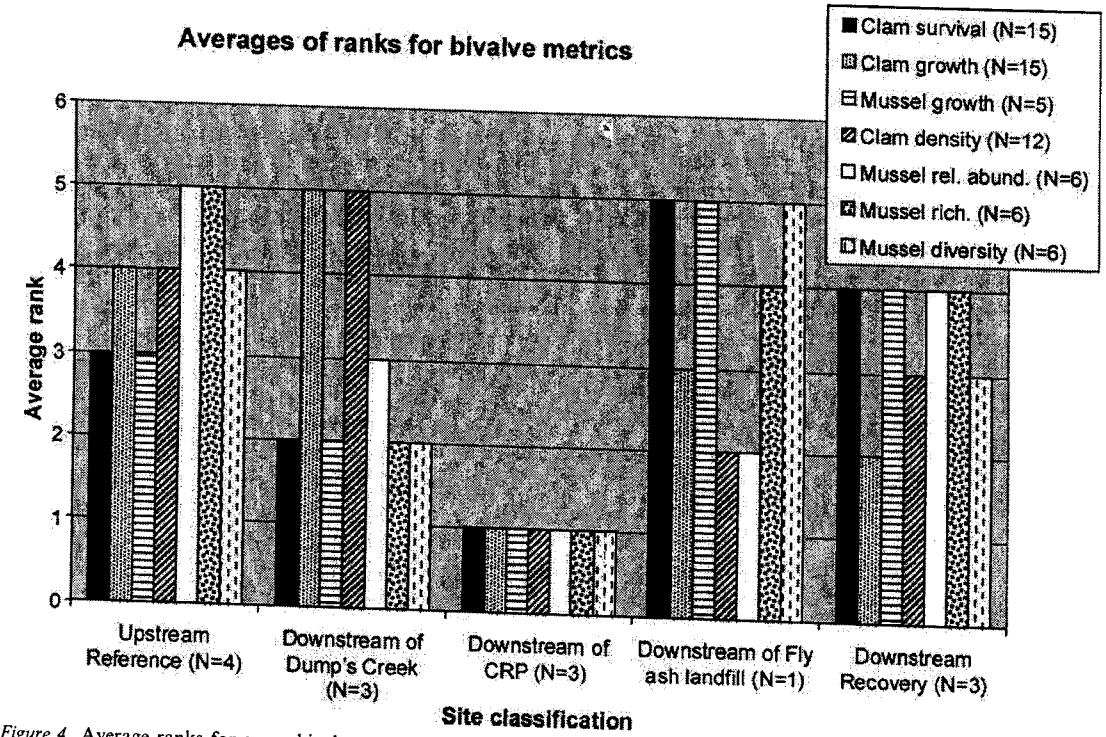

Figure 4. Average ranks for seven bivalve metrics used to bioassess five groups of stream sites upstream and downstream of coal-related activities in the Clinch River watershed during 2001–2002. In the legend, 'N' values denote the total number of sites at which a specified parameter was measured. Along the *x*-axis, 'N' values denote the total number of sites within a designated site class.

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 facilitated comparisons among stream sites potentially influenced by various coal mining and combustion-related activities in the CR watershed, and indicated an adverse effect upon transplanted and resident bivalves downstream of the CRP effluent discharge. This point is clearly illustrated in Figure 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.

Conclusion

Researchers have claimed that bivalves (Belanger et al., 1990) and *in situ* toxicity tests (LaPoint & 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.

Acknowledgements

Funding was provided by American Electric Power (AEP) with assistance from John Van Hassel. A grant from the Virginia Tech Graduate Student Association (GSA) and matching funds from the Virginia Tech Biology Department were also used for this project. Field assistance provided by Chad Merrickes and Braven Beaty, and technical assistance from Virginia Tech Aquaculture Center personnel, particularly Jess Jones, Bill Henley, and Rachel Mair, was instrumental to this research.

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