ECOTOXICOLOGICAL IMPLICATIONS OF AQUATIC DISPOSAL OF COAL COMBUSTION RESIDUES IN THE UNITED STATES: A REVIEW*

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Abstract. We provide an overview of research related to environmental effects of disposal of coal combustion residues (CCR) in sites in the United States. Our focus is on aspects of CCR that have the potential to negatively influence aquatic organisms and the health of aquatic ecosystems. We identify major issues of concern, as well as areas in need of further investigation.

Intentional or accidental release of CCR into aquatic systems has generally been associated with deleterious environmental effects. A large number of metals and trace elements are present in CCR, some of which are rapidly accumulated to high concentrations by aquatic organisms. Moreover, a variety of biological responses have been observed in organisms following exposure to and accumulation of CCR-related contaminants. In some vertebrates and invertebrates, CCR exposure has led to numerous histopathological, behavioral, and physiological (reproductive, energetic, and endocrinological) effects. Fish kills and extirpation of some fish species have been associated with CCR release, as have indirect effects on survival and growth of aquatic animals mediated by changes in resource abundance or quality. Recovery of CCR-impacted sites can be extremely slow due to continued cycling of contaminants within the system, even in sites that only received CCR effluents for short periods of time.

The literature synthesis reveals important considerations for future investigations of CCR-impacted sites. Many studies have examined biological responses to CCR with respect to Se concentrations and accumulation because of teratogenic and reproductively toxic effects known to be associated with this element. However, the complex mixture of metals and trace elements characteristic of CCR suggests that biological assessments of many CCR-contaminated habitats should examine a variety of inorganic compounds in sediments, water, and tissues before causation can be linked to individual CCR components. Most evaluations of effects of CCR in aquatic environments have focused on lentic systems and the populations of animals occupying them. Much less is known about CCR effects in lotic systems, in which the contaminants may be transported downstream, diluted or concentrated in downstream areas, and accumulated by more transient species. Although some research has examined accumulation and effects of contaminants on terrestrial and avian species that visit CCR-impacted aquatic sites, more extensive research is also needed in this area. Effects in terrestrial or semiaquatic species range from accumulation and maternal transfer of elements to complete recruitment failure, suggesting that CCR effects need to be examined both within and outside of the aquatic habitats into which CCR is released. Requiring special attention are waterfowl and amphibians that use CCR-contaminated sites during specific seasons or life stages and are highly dependent on aquatic habitat quality during those periods.

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Whether accidentally discharged into natural aquatic systems or present in impoundments that attract wildlife, CCR appears to present significant risks to aquatic and semiaquatic organisms. Effects may be as subtle as changes in physiology or as drastic as extirpation of entire populations. When examined as a whole, research on responses of aquatic organisms to CCR suggests that reducing the use of disposal methods that include an aquatic slurry phase may alleviate some environmental risks associated with the waste products.

Keywords: accumulation, aquatic animals, coal ash, electric power, energy, heavy metals, sublethal effects, trace elements

1. Introduction

Coal is widely recognized as a fuel source associated with substantial environmental impacts. Mining, transport, and storage of coal are associated with habitat degradation and environmental pollution (Dvorak *et al.*, 1977). Large-scale, industrial combustion of coal produces both air-borne and solid wastes, the former having been under stringent regulation by federal and state governments for several decades. In contrast, solid coal combustion residues (hereafter CCR) which account for 90% of fossil fuel combustion wastes in the U.S. (USEPA, 1988) remain only under state regulation, which varies in rigor by jurisdiction. In some states, basic environmental protection standards for CCR disposal sites such as use of groundwater monitoring programs, leachate collection systems, and impermeable impoundment liners are not required. For example, in a national survey of 259 coal utilities having greater than 100 megawatt capacity, nearly 40% reported operating under no standards for groundwater quality (EPRI, 1997).

Federal regulations on CCR disposal remain in exemption following the 1980 Bevill Amendment to the Resource Conservation and Recovery ACT (RCRA; USEPA, 1988). The rationale for the amendment to RCRA was that: 1) the wastes were produced in large volumes, 2) there was little information available on characteristics and environmental behavior of the wastes, and 3) the limited data available suggested that risks posed by the wastes were low (EPRI, 1997). However, research conducted in the past two decades has revealed that CCR is a chemically complex mixture that can pose substantial risks to the environment. In particular, mounting evidence suggests that disposal of CCR in natural and man-made aquatic systems results in environmental degradation and poses health risks to wildlife. The goal of this paper is to review the literature related to environmental risks posed by aquatic disposal of CCR and to make recommendations for future research. Our purpose is not to provide a thorough review of CCR disposal technologies, or chemical and physical properties of CCR. Treatments of these and related issues are available in the literature (Adriano *et al.*, 1980; Roy *et al.*, 1981; EPRI, 1987a and b; Bignoli, 1989; Sharma *et al.*, 1989; Eary *et al.*, 1990; Mattigod *et al.*, 1990; Carlson and Adriano, 1993; Prasad *et al.*, 1996). However, to provide general background on CCR, we provide a brief a summary below.

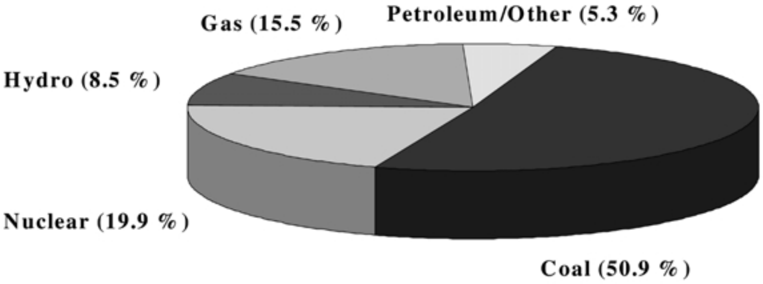

Figure 1. Net electricity generation in the U.S. by fuel source, 1999 (USDOE, 2000).

The organization of the main body of this review follows a typical risk assessment format, beginning with a discussion of sources of exposure to organisms and leading to discussions of accumulation, lethal and sublethal effects on individuals, and ecological (population and community-level) effects. While the tables are meant to provide exhaustive references to pertinent studies as well as provide data in support of the text, not all studies listed in tables are specifically discussed in the text. Rather, the text provides overviews of specific topic areas with reference to information in the tables when necessary. Because several systems have been particularly well-studied with respect to accumulation and/or effects, we include brief case studies based upon these systems within appropriate topic areas. Tables specifically related to the case studies are presented in the Appendix. Throughout the text and tables we refer to study organisms by the common or group names used by the original authors. Scientific names of all organisms discussed are provided in Appendix Table I.

2. Production and Disposal of CCR in the U.S.

With a growing human population, electricity demands continue to increase. Although an increased reliance on other energy sources in the U.S. in recent decades has resulted in a slight decrease in dependence on coal (USDOE, 1999), the largest portion of electric utility capability in the U.S. remains fueled by coal (Figure 1; USDOE, 2000). Reliance on coal for power generation has resulted in a concomitant rise in high- and low-volume waste production, with fly ash being the largest component (see below and Table I). Technologies used to reduce airborne emission of harmful particulates such as fly ash have resulted in large volumes of these wastes being removed from exhaust stacks and the subsequent need for disposal of the particulate materials. Production of fly ash, which makes up approximately 60% of the CCR waste stream, has increased in the U.S. from about 24 million tonne in 1970 to nearly 57 million tonne in 1998 (EPRI, 1997; EPA, 1997; ACAA, 1998; Figure 2).

TABLE I

Characteristics of high and low volume CCR (Van Hook, 1979; Carlson and Adriano, 1992; EPRI, 1987a and b; 1997)

Waste Type	Description	Chemical Constituents
	A. High Volume Wastes	
Fly Ash	Fine particulate residue collected in emission-control devices. Comprises $\sim$ 60% of high volume wastes.	Various elements, including As, Cd, Cr, Cu, Hg, Ni, Pb, Se, Sr, V, Zn. Most enriched in volatile elements (e.g. As, B, Cl, F, S, Se).
Bottom Ash and Slag	Fine and coarse grain residue remaining in the boiler following combustion.	Various trace elements, including As, Cd, Cr, Cu, Hg, Ni, Pb, Se, Sr, V, Zn.
Flue Gas Desulfurization (FGD) Wastes (Scrubber Sludge)	Fine grain residues removed from stack via addition of limestone slurry to the flue stream.	Fly- and bottom ash constituents, often enriched in Ca-S salts and carbonates.
Fluidized Bed Boiler (FBB) Wastes	Residues mixed with ash resulting from mixing limestone and coal in the furnace on an air- fluidized bed.	Ash constituents plus Ca-S salts and carbonates.
Coal Gasification Ash (CGA)	Waste produced from conversion of coal to gaseous and liquid fuels, and is similar to fly ash but contains a higher proportion of coarse particulate material.	Ash constituents, iron sulfides, acids.
	B. Low Volume Wastes	
Air Heater, Precipitator Wash Waters	Effluent generated by high pressure washing of fly ash from air heaters and precipitators.	Ash constituents.
Boiler Chemical Cleaning Wastes	Wastewater produced from descaling boiler tubes.	Ash constituents, solvents and corrosion inhibitors.
Boiler Blowdown	Low purity water resulting from continued recirculation during steam production.	Dissolved minerals, phosphate, hydrazine.
Cooling Tower Blowdown	Low purity water periodically removed from cooling systems.	Dissolved minerals, anti-fouling and anti-fungal compounds.

TABLE I
Continued.

Waste Type	Description	Chemical Constituents
Coal Pile Runoff	Runoff wastestream produced from precipitation on coal pile stores.	Trace elements, PAHs, acids (bituminous coal) or alkaline compounds (subbituminous coal).
Coal Mill Rejects	Solids rejected from milling process.	Rocks, metal fragments, minerals, hard coal, iron and sulfur compounds.
Demineralizer Regenerant and Resins	Acidic and basic solutions from regenerating ion exchange beds.	Acids, bases, mineral salts.
Surface drainage	Collected runoff from floors, yards, and low pressure service water.	Various organic and inorganic materials.

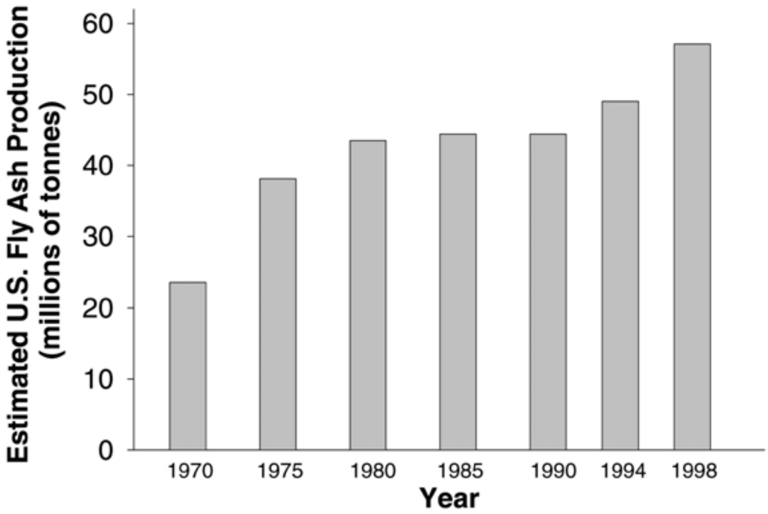

Figure 2. Estimated annual production of fly ash in the U.S., 1970 to 1998 (EPRI, 1997; USEPA, 1997; ACAA, 1998).

Because enormous quantities of wastes are produced from coal combustion, there has been a need for economically efficient disposal systems. An economically-attractive disposal method has been aquatic disposal, which is less labor intensive than land-or mine-filling (Carlson and Adriano, 1993). Typically, aquatic disposal of CCR involves pumping slurried wastes from the production site to constructed basins that, in many cases, ultimately discharge into natural water bodies. Aquatic basins serve as a physical treatment, relying on gravitational settling of particulate material from the slurried waste stream. Approximately 45% of coal-fired power plants rely on aquatic basins for disposal of CCR (EPRI, 1997). In terms of volume disposed, approximately two-thirds of CCR was disposed of using aquatic basins prior to 1980 (EPRI, 1997). Today, aquatic basins still account for disposal of approximately one-third of CCR produced (EPRI, 1997; Figure 3).

3. Composition of CCR

The composition of CCR can be quite variable (Tables I and II), reflecting differences in parent coal composition (Dvorak, 1977, 1978), inclusion of other fuels in the combustion processes, combustion and cleaning technology, and disposal techniques (Carlson and Adriano, 1993). Because coal is itself a concentrated source of many trace elements, oxidation and loss of carbon from the solid substrate during combustion produces a residual ash material that is further concentrated in non-volatile elements. Addition of materials collected from boiler flues and air scrubbing units to the bulk CCR stream can return volatile components to the CCR stream which would otherwise have been lost during combustion. Moreover, waste

TABLE II

Inorganic and organic constituents of fly- and bottom ash components of CCR. Inorganic contents were determined in ash samples derived from 42 coal-fired power plants in the U.S., and are presented as ranges of means (ppm dry mass). Organic analyses are for semi-volatile EPA-priority organic pollutants in methylene chloride concentrates of base/neutral fractions of 6 fly ash and 7 bottom ash samples selected from the 42 power plants providing samples for analyses. Concentrations of organic compounds are presented as ranges of means (ppb in extract). All data from EPRI (1987a). Note that samples were analyzed for a total of 42 organic contaminants; only those two which were detected are included below

Element	Fly	Bottom	Element	Fly	Bottom	Element/Compound	Fly	Bottom
Al	46000–152000	30500–14500	Mo	7–236	3–443	V	< 95–652	< 50–275
As	8–1385	< 5–37	Na	1300–62500	814–41300	U	11–30	< 5–26
Ba	251–10850	150–9360	Ni	23–353	< 10–1067	Zn	27–2880	4–515
Ca	7400–223000	2200–241000	P	1100–10340	< 500–4630	bis(2-ethylhexyl)phthalate	17–286	6–204
Cd	6–17	< 5	Pb	21–2120	5–843	Di-n-octylphthalate	ND - 3.2	ND - 6.2
Cl	180–1190	< 150–2630	S	1300–64400	460–74000			
Cr	37–651	< 40–4710	Sb	11–131	< 10			
Cu	45–1452	27–146	Se	6–47	< 2–10			
Fe	25000–177000	20200–201000	Si	89500–275000	51000–312000			
K	3000–25300	2600–24000	Sn	8–56	< 9–90			
Mg	1600–41800	2500–46000	Sr	204–6820	182–6460			
Mn	44–1332	56–1940	Ti	1310–10100	1540–11300			

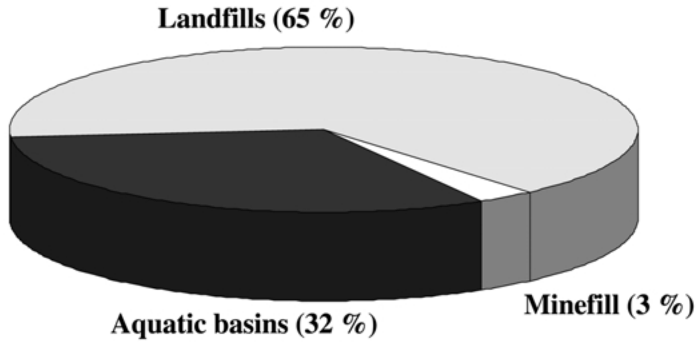

Figure 3. Percentage of CCR disposed of in landfills, aquatic basins, and minefills in the U.S. (EPRI, 1997).

management practices vary among facilities, and may entail combining numerous waste products associated with coal combustion and typical plant operations into a single, chemically complex CCR effluent. Depending upon the site in question, the CCR stream can thus contain a variety of waste types, including fly ash (typically the largest component), bottom ash, flue gas desulfurization (FGD) wastes, fluidized bed boiler (FBB) wastes, coal gasification ash (CGA), and multiple types of low volume comanaged wastes (EPRI, 1997). The result of modern, industrial coal combustion practices is thus a solid CCR waste enriched in numerous elements and compounds, some of which may pose risks of toxicity to organisms that interact with the wastes in natural or man-made habitats (Tables I and II). Of the three commonly employed disposal techniques (landfills, aquatic basins, and minefills), comanagement of multiple waste types is most prevalent at facilities using aquatic basins for disposal. In a survey of 259 disposal facilities, 91% of sites using aquatic basins simultaneously disposed of high and low volume waste types, whereas 70 and 75% of landfills and minefills, respectively, received the mixed effluents (EPRI, 1997).

The largest proportion of CCR is in the form of solids such as ash (USEPA, 1988) that contain a variety of potentially toxic elements and compounds (Tables I and II). Thus, from the standpoint of potential environmental impacts associated with CCR, the solid ash fraction appears to be a component of CCR that requires particular attention. The emphasis of this paper will be on environmental impacts of solid CCR in aquatic environments, with a primary focus on effects on aquatic organisms. Moreover, we will focus on inorganic contaminants associated with CCR disposal in aquatic systems which appear to be much more prevalent than organic contaminants (Table II), and thus have received greater attention from researchers.

4. Environmental Impacts of CCR in Aquatic Systems

4.1. Exposure to contaminants

4.1.1. *Sources of Contaminants to Biota*

Disposal of CCR into aquatic systems can physically and chemically alter habitat conditions via sedimentation and changes to sediment particle size distribution, turbidity, pH, conductivity, and inputs of contaminants (Theis, 1975; Carlson and Adriano, 1993; Dvorak 1977, 1978). Numerous aquatic systems have been studied with respect to these habitat modifications, the focus primarily being on inorganic contaminants associated with CCR. Concentrations of several trace elements (primarily As, Cd, Cr, Cu, Pb, and Se) have been particularly well characterized in several CCR-impacted systems because of the abundance of these elements in CCR and/or concerns associated with the known toxicological actions of these elements. Whereas in some systems the focus of chemical screening was primarily on dissolved fractions of one or a few trace elements in water, surveys in other systems suggest that numerous trace elements are elevated in CCR-impacted systems not only in water, but also in suspended solids and sediments (Table III).

The results of chemical surveys presented in Table III reflect the elevated concentrations of contaminants associated with CCR in dissolved and particle-associated forms. However, to examine the potential risks that elevated CCR-derived contaminants in aquatic systems may pose for wildlife, the propensity for contaminants to be accumulated from the environment must be examined, as must the biological responses associated with contaminant accumulation. These topics are treated in the following sections of this document.

4.1.2. *Trace Element Accumulation by Biota*

There is a large amount of data demonstrating that plants and animals inhabiting CCR-contaminated sites or chronically exposed to CCR in laboratory or field-based experiments accumulate trace elements, sometimes to very high concentrations (Table IV). Accumulation of trace elements from water and sediments by vascular and non-vascular plants suggests the potential for trophic transfer of bioaccumulative elements to grazers. For example, in the D-Area facility, SC, numerous types of producers accumulated trace elements from sediments and/or water, themselves apparently serving as vectors of the contaminants to several grazing invertebrates (Table IV; Cherry and Guthrie, 1976, 1977; Guthrie and Cherry, 1979). Occurrence of some trace elements at very high concentrations in micro- and macroinvertebrates also suggests that predatory vertebrates may accumulate some trace elements to levels that may ultimately result in lethal or sublethal effects (Hopkins, 2001). In Stingy Run, OH, high tissue burdens of some contaminants in odonates may have been a source of contaminants to several species of fish which accumulated trace elements in numerous tissues (Table IV; Lohner and Reash, 1999; Reash *et al.*, 1999). Such relationships between tissue trace element

TABLE III

Mean or ranges of trace element concentrations in water (ppb), suspended solids (ppm dry mass), and sediments (ppm dry mass except where noted) in aquatic sites contaminated by CCR. NR = not reported, BDL = below detection limits. Decimal places reflect those presented by the original authors

Site	Description	As	Cd	Cr	Cu	Pb	Se	Reference
				Water (ppb)				
Belews Lake, NC	Prior to ash effluent discharge	BDL	NR	NR	NR	NR	BDL	Olmsted *et al*., 1986
Belews Lake, NC	Ash effluent entering lake	190–253	NR	NR	NR	NR	157–218	Cumbie, 1978
Belews Lake, NC	Lake water, 2 yr following initial ash effluent discharge	4–10	NR	NR	NR	NR	7–14	Cumbie, 1978
Belews Lake, NC	Lake water, 2 yr following initial ash effluent discharge	6.6	NR	NR	NR	NR	12.6	Olmsted *et al*., 1986
Belews Lake, NC	Lake water, 5 yr following initial ash effluent discharge	4.3	NR	NR	NR	NR	9.5	Olmsted *et al*., 1986
Belews Lake, NC	Lake water, 8 yr following initial ash effluent discharge	3.1	NR	NR	NR	NR	8.8	Olmsted *et al*., 1986
Belews Lake, NC	Lake water, 22 yr following initial ash effluent discharge, 11 yr after discharge had ceased	NR	NR	NR	NR	NR	< 1.0	Lemly, 1997
Martin Creek Reservoir, TX	Fly ash ponds discharging into reservoir	NR	NR	NR	NR	NR	2,200–2,700	Garrett and Inman, 1984
Columbia Generating Station, WI	Drainage from ash pit entering Rocky Run Creek	NR	2.4–2.9	35–65	4–43	NR	NR	Magnuson *et al*., 1980

TABLE III
Continued.

Site	Description	As	Cd	Cr	Cu	Pb	Se	Reference
Fruitland, NM	Ash pond surface water	33	1	3	2	NR	60	Dreesen *et al*., 1977
Fruitland, NM	Ash pond effluent water	27	1	2	3	NR	57	Dreesen *et al*., 1977
Lansing, NY	Farm pond receiving airborne drift of coal ash	NR	NR	NR	NR	NR	0.35	Gutenmann *et al*., 1976
Harrodsburg, KY	Ash settling pond	NR	0.46	NR	4.38	NR	NR	Benson and Birge, 1985
Roger's Quarry fly ash reservoir, Oak Ridge, TN	During period of active use	NR	NR	NR	NR	NR	25	Southworth *et al*., 1994
Roger's Quarry fly ash reservoir, Oak Ridge, TN	After cessation of discharge	NR	NR	NR	NR	NR	< 2	Southworth *et al*., 1994
Stingy Run, OH	Stream draining ash reservoir, measurements prior to ash effluent inputs	BDL	NR	BDL	NR	NR	BDL	Reash *et al*., 1988
Stingy Run, OH	Stream draining ash reservoir; measurements following ash effluent inputs[a]	21–24	NR	62–129	NR	NR	19–33	Reash *et al*., 1988
Stingy Run, OH	Stream draining ash reservoir [b]	< 4–14.3	0.7–0.8	1.6–29.7	2.9–6.2	< 2–2.1	3.2–11.8	Lohner and Reash, 1999; Lohner *et al*., 2001
Little Scary Creek, WV	Stream drainage ash reservoir	64	NR	NR	13	NR	32	Reash *et al*., 1999
Glen Lyn, VA	Ash basin input [c]	NR	30–43	NR	270–2,880	NR	NR	Cairns and Cherry, 1983
Glen Lyn, VA	Ash basin outfall [c]	NR	2–150	NR	5–20	NR	NR	Cairns and Cherry, 1983

TABLE III
Continued.

Site	Description	As	Cd	Cr	Cu	Pb	Se	Reference
D-Area Power Facility, Savannah River Site, SC	Multiple portions of drainage system (1973–1979)	58–100	100–123	160–200	390–660	NR	100–110	Cherry *et al*., 1976, 1979 a and b; Guthrie and Cherry, 1976, 1979; Cherry and Guthrie, 1977
D-Area Power Facility, Savannah River Site, SC	Secondary settling basin, drainage swamp, and swamp outflow combined	46.0	0.3	0.4	2.6	NR	NR	Alberts *et al*., 1985
D-Area Power Facility, Savannah River Site, SC	Beaver Dam Creek, 0.3 to 1 km below drainage swamp outflow	2.4	0.2	0.4	20.0	NR	NR	Alberts *et al*., 1985
D-Area Power Facility, Savannah River Site, SC	Primary settling basin	17.17	0.11	0.44	2.53	0.08	7.0	Rowe, 1998
			Suspended solids (ppm dry mass)					
D-Area Power Facility, Savannah River Site, SC	Secondary settling basin, drainage swamp, and swamp outflow combined	762	9.6	73	207	NR	NR	Alberts *et al*. (1985)
D-Area Power Facility, Savannah River Site, SC	Beaver Dam Creek, 0.3 to 1 km below drainage swamp outflow	28	0.9	52	406	NR	NR	Alberts *et al*. (1985)

TABLE III
Continued.

Site	Description	As	Cd	Cr	Cu	Pb	Se	Reference
D-Area Power Facility, Savannah River Site, SC	Beaver Dam Creek	NR	1.9	70	149	80	NR	Evans and Giesy (1978)
Sediment (ppm dry mass)								
Belews Lake, NC	2 yr after discharge of ash effluent had begun	31.2–59.8	NR	NR	NR	NR	6.08–8.93	Cumbie, 1978
Belews Lake, NC	22 yr following initial ash effluent discharge, 11 yr after discharge had ceased	NR	NR	NR	NR	NR	1–4	Lemly, 1997
Hyco Reservoir, NC	Cooling reservoir receiving CCR effluent	1.8–13.3	NR	24–197	15–104	NR	0.68–5.50	CPL, 1979
Lansing, NY	Farm pond receiving airborne drift of coal ash	103	NR	142	298	NR	14	Furr *et al.*, 1979
Stingy Run, OH	Stream draining ash reservoir [d]	27.6–58	1–1.9	45.4–132	40.6–57	19.8–30	5–20	Lohner and Reash, 1999
Little Scary Creek, WV	Stream drainage ash reservoir [e]	68–107	7–35	83–92	105–110	27–29	9–14	Lohner and Reash, 1999
D-Area Power Facility, Savannah River Site, SC	Multiple portions of drainage system (prior to 1976; ppm wet mass)	19.7–47.9	1.7	38–38.4	52–81	NR	5.6–6.1	Cherry *et al.*, 1976, 1979 a and b; Guthrie and Cherry, 1976, 1979; Cherry and Guthrie, 1977
D-Area Power Facility, Savannah River Site, SC	Outflow from drainage swamp	0.95–1.69	0.05–0.06	0.57–0.62	0.65-0.96	NR	0.15–0.19	McCloskey and Newman, 1995

TABLE III
Continued.

Site	Description	As	Cd	Cr	Cu	Pb	Se	Reference
D-Area Power Facility, Savannah River Site, SC	Outflow from drainage swamp	2.48	0.12	0.77	2.09	NR	0.24	McCloskey *et al*., 1995
D-Area Power Facility, Savannah River Site, SC	Primary settling basin	70.8	0.57	NR[f]	71.8	45.2	6.21	Rowe *et al*., 1996
D-Area Power Facility, Savannah River Site, SC	Drainage swamp	116.6	2.32	NR[f]	147.5	66.2	7.78	Rowe *et al*., 1996
D-Area Power Facility, Savannah River Site, SC	Terrestrial margins of primary settling basin	39.638	0.252	10.869	18.386	6.457	4.383	Hopkins *et al*., 1998
D-Area Power Facility, Savannah River Site, SC	Secondary settling basin	49.39	0.72	23.85	84.72	NR	6.11	Hopkins *et al*., 2000a
D-Area Power Facility, Savannah River Site, SC	Drainage swamp	28.94	1.38	22.04	43.50	NR	7.11	Hopkins *et al*., 2000a

[a] Values are ranges of median values reported 1974–1986. [b] Values are ranges of means reported 1993–1995. [c] Values are ranges of means reported 1979–1980. [d] Values are ranges of means reported 1992, 1994, 1997. [e] Values are ranges of means reported 1996–1997. [f] Cr concentrations reported in original publication were incorrect.

TABLE IV

Means or ranges of trace element burdens (ppm dry mass 'DM' or wet mass 'WM') in organisms collected from CCR-contaminated sites or experimentally exposed to CCR. For experimentally exposed organisms, methods are noted. If tissue burdens were associated with biological effects, or were measured in sites treated in case histories, results are presented in Tables V to VII, and Appendix Tables II to V. NR = not reported. BDL = below detection limit. Decimal places reflect those presented by the original authors. Scientific names for all species examined are provided in Appendix Table I

Species; exposure methods, if applicable	As	Cd	Cr	Cu	Pb	Se	Site (reference)
				Plants			
Sago pondweed (DM)	NR	NR	NR	NR	NR	3.7	Lansing, NY, farm pond receiving airborne drift of coal ash (Gutenmann *et al.*, 1976)
Sago pondweed (DM)	84	NR	8.4	BDL	NR	3.7	Lansing, NY, farm pond receiving airborne drift of coal ash (Furr *et al.*, 1979)
Algae (DM)	NR	NR	NR	NR	NR	0.9	Lansing, NY, farm pond receiving airborne drift of coal ash (Gutenmann *et al.*, 1976)
Algae (DM)	9.6	NR	22	BDL	NR	0.9	Lansing, NY, farm pond receiving airborne drift of coal ash (Furr *et al.*, 1979)
Plants (averages from measurements of 35 species; DM)	1.0	2.8	3.8	NR	NR	10.3	Monroe County, MI, ash slurry pond (Brieger *et al.*, 1992)
Plants (pooled samples of 6 species; WM)	4.2–5.3	0.9–1.5	2.9–5.7	7.2–14	NR	1.8–5	D-Area facility, SC (Cherry and Guthrie, 1976, 1977)
Plants (pooled samples of 5 species; WM)	NR	0.4–4.7	0.9–4.2	2–34	NR	1.8–5.7	D-Area facility, SC (Guthrie and Cherry, 1979)
Algae (WM)	NR	1.3-1.9	4–4.5	7–9.9	NR	1.3–1.4	D-Area facility, SC (Guthrie and Cherry, 1979)
Periphyton (DM)	NR	1.7	28	144	33	NR	D-Area facility, SC (Evans and Giesy, 1978)
Black willow, leaves (DM)	NR	0.36	0.55	6.0	1.9	NR	D-Area facility, SC (Evans and Giesy, 1978)
Black willow, stems (DM)	NR	0.35	0.24	5.4	2.6	NR	D-Area facility, SC (Evans and Giesy, 1978)
Arrowhead, stems and leaves (DM)	NR	1.00	5.7	24.6	17.9	NR	D-Area facility, SC (Evans and Giesy, 1978)
Cattail, stems and leaves (DM)	NR	1.57	2.4	11.8	6.4	NR	D-Area facility, SC (Evans and Giesy, 1978)

TABLE IV
Continued.

Species; exposure methods, if applicable	As	Cd	Cr	Cu	Pb	Se	Site (reference)
				Invertebrates			
Plankton (DM)	3.1–11.3	NR	NR	NR	NR	41.3–97.0	Belews Lake, NC (Cumbie, 1978)
Mayfly (WM)	3.05	NR	NR	NR	NR	8.36	Belews Lake, NC (Olmsted *et al.*, 1986)
Mayfly (WM)	NR	NR	NR	NR	NR	13.6	Belews Lake, NC (Finley, 1985)
Mayfly (DM)	NR	NR	NR	NR	NR	14.8	Martin Creek Lake, TX (USDI, 1988)
Caddisflies, whole body (DM).	102	3.6	19	43.1	0.76	18.5	Stingy Run, OH, (Lohner and Reash, 1999)
Caddisflies, whole body (DM).	18	4.1	NR	85	11	31	Little Scary Creek, WV (Reash *et al.*, 1999)
Hellgrammites, whole body (DM).	56.2	4.6	10.2	135.2	3.9	38.8	Little Scary Creek, WV (Lohner and Reash, 1999)
Chironomids (WM)[a]	NR	1.2	38	50	NR	0.7	D-Area facility, SC (Guthrie and Cherry, 1979)
Odonates (WM)[a]	NR	1–1.2	3.4–4.5	20–27	NR	2.5–2.6	D-Area facility, SC (Guthrie and Cherry, 1979)
Multiple species of insects, molluscs, and crustaceans, pooled (WM)	2.1–60	2.5–4	3.5–9.7	31–67	NR	2.6–6.5	D-Area facility, SC (Cherry and Guthrie, 1976, 1977)
Asiatic clams, flesh (DM)	13.22	4.02	5.63	64.87	NR	8.69	D-Area facility, SC (Nagle *et al.*, 2001)
Crayfish, whole body (DM)	8.71	2.78	2.46	158.52	NR	14.92	D-Area facility, SC (Nagle *et al.*, 2001)
Crayfish (WM)[a]	NR	16	7.7	19	NR	7.2	D-Area facility, SC (Guthrie and Cherry, 1979)
Dragonfly nymphs, whole body (DM)	NR	NR	NR	NR	NR	4.1	Lansing, NY, farm pond receiving airborne drift of coal ash (Gutenmann *et al.*, 1976)
Dragonfly nymphs, whole body (DM)	BDL	NR	1.9	86	NR	4.1	Lansing, NY, farm pond receiving airborne drift of coal ash (Furr *et al.*, 1979)
Cricket, whole body (DM)	1.1	< 3.0	12.6	NR	NR	11.6	Monroe County, MI, from vicinity of ash slurry pond (Brieger *et al.*, 1992)
Grasshopper, whole body (DM)	< 1.3	< 3.8	1.2	NR	NR	9.7	Monroe County, MI, from vicinity of ash slurry pond (Brieger *et al.*, 1992)

TABLE IV

Continued.

Species; exposure methods, if applicable	As	Cd	Cr	Cu	Pb	Se	Site (reference)
Earthworm, whole body (DM), gut not voided	53.7	< 5.0	51.7	NR	NR	79.5	Monroe County, MI, from vicinity of ash slurry pond (Brieger *et al.*, 1992)
Pond snail, whole body (DM)	11.5	< 2.0	6.4	NR	NR	26.2	Monroe County, MI, from vicinity of ash slurry pond (Brieger *et al.*, 1992)
Fish							
Spotted gar, muscle (WM)	NR	NR	NR	NR	NR	2.0–3.0	Martin Creek Lake, TX (Garrett and Inman, 1984)
Catfish, skeletal muscle (WM)	< 0.1–0.34	NR	0.21–0.27	NR	NR	7.96–11.3	Belews Lake, NC (Cumbie, 1978)
Sunfish, skeletal muscle (WM)	< 0.1–2.65	NR	0.05–1.69	NR	NR	10.6–22.3	Belews Lake, NC (Cumbie, 1978)
Largemouth bass, muscle (WM)	NR	NR	NR	NR	NR	2.2–3.3	Roger's Quarry, Oak Ridge, TN, coal ash disposal reservoir (Southworth *et al.*, 1994)
Brown bullhead, 5 cm long, flesh (DM)	NR	NR	NR	NR	NR	5.2	Lansing, NY, farm pond receiving airborne drift of coal ash (Furr *et al.*, 1979)
Brown bullhead, 12.5 cm long, flesh (DM)	NR	NR	NR	NR	NR	3.4	Lansing, NY, farm pond receiving airborne drift of coal ash (Furr *et al.*, 1979)
Brown bullhead, 22.5 cm long adult, flesh (DM)	NR	NR	NR	NR	NR	1.9	Lansing, NY, farm pond receiving airborne drift of coal ash (Furr *et al.*, 1979)
Brown bullhead, 22.5 cm long, liver (DM)	NR	NR	NR	NR	NR	9.2	Lansing, NY, farm pond receiving airborne drift of coal ash (Furr *et al.*, 1979)
Brown bullhead, 30 cm long, flesh (DM)	NR	NR	NR	NR	NR	1.7	Lansing, NY, farm pond receiving airborne drift of coal ash (Furr *et al.*, 1979)
Brown bullhead, 30 cm long, liver (DM)	0.4	NR	3.3	BDL	NR	8.2	Lansing, NY, farm pond receiving airborne drift of coal ash (Furr *et al.*, 1979)
Brown bullhead, 20 cm adult, liver (DM)	NR	NR	NR	NR	NR	9.0	Lansing, NY, farm pond receiving airborne drift of coal ash (Gutenmann *et al.*, 1976)
Bluegill, whole body (DM).	0.58	0.18	2.29	4.24	1.85	17.30	Stingy Run, OH, (Lohner and Reash, 1999)

TABLE IV
Continued.

Species; exposure methods, if applicable	As	Cd	Cr	Cu	Pb	Se	Site (reference)
Bullhead minnow, whole body (DM).	6.64	1.84	4.98	14.8	0.47	44.5	Stingy Run, OH, (Lohner and Reash, 1999)
Bluegill, liver (DM) [b]	1.7–4.7	0.8–3.9	0.9–2.7	4.6–33.0	0.7–11.5	20.9–57.3	Stingy Run, OH, (Lohner and Reash, 1999; Lohner *et al*., 2001)
Bluegill, ovary (DM) [b]	1.00–1.70	0.13–0.24	1.07–1.47	3.98–7.21	1.99–2.66	11.50–32.50	Stingy Run, OH, (Lohner and Reash, 1999)
Bluegill, testes (DM) [b]	0.80–4.27	0.08–0.40	1.36–3.60	6.81–6.94	1.29–3.15	4.03–37.00	Stingy Run, OH, (Lohner and Reash, 1999; Lohner *et al*., 2001)
Green sunfish, liver (DM)[c]	0.5–1.7	0.5–4.9	0.3–4.8	1.8–19.7	0.3–2.1	4.8–21.6	Stingy Run, OH, (Lohner and Reash, 1999)
Green sunfish, ovary (DM)[c]	5.77	0.52	10.70	7.97	7.53	15.00	Stingy Run, OH, (Lohner and Reash, 1999)
Green sunfish, testes (DM)[c]	2.40–7.40	0.20–0.91	2.90–14.90	0.89–6.50	0.90–10.25	5.40–9.75	Stingy Run, OH, (Lohner and Reash, 1999)
Largemouth bass, ovary (WM) [d]	NR	NR	NR	NR	NR	4.4	Catfish Reservoir, NC (Baumann Gillespie, 1986)
Largemouth bass, ovary-free carcass (WM) [d]	NR	NR	NR	NR	NR	3.9	Catfish Reservoir, NC (Baumann and Gillespie, 1986)
Largemouth bass, testes (WM) [d]	NR	NR	NR	NR	NR	3.3	Catfish Reservoir, NC (Baumann and Gillespie, 1986)
Largemouth bass, testes-free carcass (WM) [d]	NR	NR	NR	NR	NR	3.5	Catfish Reservoir, NC (Baumann and Gillespie, 1986)
Bluegill, ovary (WM)[d]	NR	NR	NR	NR	NR	5.4	Catfish Reservoir, NC (Baumann and Gillespie, 1986)
Bluegill, ovary-free carcass (WM) [d]	NR	NR	NR	NR	NR	3.2	Catfish Reservoir, NC (Baumann and Gillespie, 1986)
Bluegill, testes (WM) [d]	NR	NR	NR	NR	NR	3.7	Catfish Reservoir, NC (Baumann and Gillespie, 1986)
Bluegill, testes-free carcass (WM) [d]	NR	NR	NR	NR	NR	3.2	Catfish Reservoir, NC (Baumann and Gillespie, 1986)

TABLE IV
Continued.

Species; exposure methods, if applicable	As	Cd	Cr	Cu	Pb	Se	Site (reference)
Sunfish (DM)	NR	NR	NR	NR	NR	16.9	Martin Creek Lake, TX (USDI, 1988)
Largemouth bass (DM)	NR	NR	NR	NR	NR	39.0	Martin Creek Lake, TX (USDI, 1988)
Black crappie (WM)	NR	NR	NR	NR	NR	5.4–6.8	Martin Creek Lake, TX (Garrett and Inman, 1984)
Gizzard shad (DM)	NR	NR	NR	NR	NR	32.3	Martin Creek Lake, TX (USDI, 1988)
Mosquitofish, caudal peduncle muscle (WM)	0.50	1.30	2.76	8.45	NR	9.40	D-Area facility, SC (Cherry *et al*., 1976)
Mosquitofish, whole body (WM)	0.5	1.3	2.8	6.9	NR	9.4	D-Area facility, SC (Guthrie and Cherry, 1976, 1979)
Mosquitofish, whole body (DM)	2.89	0.32	1.56	4.97	NR	14.28	D-Area facility, SC (Hopkins *et al*., 1999a)
Bluegill, whole body (DM)	2.61	0.75	2.38	1.02	NR	19.52	D-Area facility, SC (Hopkins *et al*., 1999a)
Largemouth bass, whole body (DM)	1.92	0.31	1.27	4.20	NR	18.32	D-Area facility, SC (Hopkins *et al*., 1999a)
				Amphibians			
Green frog, larvae, whole body (DM)	NR	NR	NR	NR	NR	4.7	Lansing, NY, farm pond receiving airborne drift of coal ash (Gutenmann *et al*., 1976)
Green frog, larvae, whole body (DM)	BDL	NR	25	0.9	NR	4.7	Lansing, NY, farm pond receiving airborne drift of coal ash (Furr *et al*., 1979)
Red spotted newt, whole body (DM)	0.6	NR	2.5	BDL	NR	4.2	Lansing, NY, farm pond receiving airborne drift of coal ash (Furr *et al*., 1979)
Frog larvae (WM)[a]	NR	0.8	0.6	13	NR	6.6	D-Area facility, SC (Guthrie and Cherry, 1979)
Bullfrogs, recent metamorphs, whole body (DM)	15.55	0.80	1.58	13.79	NR	26.85	D-Area facility, SC (Hopkins *et al*., 1999a)
Southern toads, adults, whole body (DM)	1.58	0.27	1.87	29.50	0.70	17.40	D-Area facility, SC (Hopkins *et al*., 1998)
Green treefrogs, adults, whole body (DM)	1.01	0.28	7.86	19.82	NR	9.82	D-Area facility, SC (Hopkins *et al*., 1999a)

TABLE IV
Continued.

Species; exposure methods, if applicable	As	Cd	Cr	Cu	Pb	Se	Site (reference)
Reptiles							
Banded water snake, adult, liver (DM)	134.3	0.5	2.0	82.7	NR	141.9	D-Area facility, SC (Hopkins *et al*., 1999a)
Softshell turtle, adult, muscle (DM)	18.3	4.9	2.2	41.4	0.7	21.9	D-Area facility, SC (Hopkins, Rowe, Congdon, unpublished)
Slider turtle, adult, liver (DM)	9.56	3.57	6.19	102.23	NR	37.18	D-Area facility, SC (Nagle *et al*., 2001)
Banded water snake, liver (DM); fed fish collected from CCR-contaminated site for 13.5 mo.	0.86	1.07	NR	35.07	NR	22.63	D-Area facility, SC (Hopkins *et al*., 2001)
Banded water snake, kidney (DM); fed fish collected from CCR-contaminated site for 13.5 mo.	0.35	0.44	NR	7.78	NR	23.20	D-Area facility, SC (Hopkins *et al*., 2001)
Banded water snake, gonad (DM); fed fish collected from CCR-contaminated site for 13.5 mo.	0.15	BDL	NR	7.55	NR	15.34	D-Area facility, SC (Hopkins *et al*., 2001)
Banded water snake, liver (DM); fed fish collected from CCR-contaminated site for 2 yr	1.851–2.010	1.625–1.718	NR	27.822–60.475	NR	24.076–24.220	D-Area facility, SC (Hopkins *et al*., 2002a)

TABLE IV
Continued.

Species; exposure methods, if applicable	As	Cd	Cr	Cu	Pb	Se	Site (reference)
Banded water snake, liver (DM); fed alternating diet of uncontaminated and CCR-contaminated fish for 2 yr	0.585–0.623	0.695–0.723	NR	29.567–39.164	NR	10.798–11.630	D-Area facility, SC (Hopkins *et al.*, 2002a)
Banded water snake, kidney (DM); fed fish collected from CCR-contaminated site for 2 yr	0.817–1.055	0.234–0.573	NR	6.475–6.777	NR	25.379–32.036	D-Area facility, SC (Hopkins *et al.*, 2002a)
Banded water snake, kidney (DM); fed alternating diet of uncontaminated and CCR-contaminated fish for 2 yr	0.401–0.615	0.169–0.398	NR	7.269–7.768	NR	16.006–21.055	D-Area facility, SC (Hopkins *et al.*, 2002a)
Banded water snake, gonad (DM); fed fish collected from CCR-contaminated site for 2 yr	0.335–0.520	0.055–0.059	NR	5.299–5.570	NR	17.642–19.060	D-Area facility, SC (Hopkins *et al.*, 2002a)
Banded water snake, gonad (DM); fed alternating diet of uncontaminated and CCR-contaminated fish for 2 yr	0.197–0.415	0.026–0.041	NR	4.695–5.400	NR	9.534–9.972	D-Area facility, SC (Hopkins *et al.*, 2002a)

TABLE IV
Continued.

Species; exposure methods, if applicable	As	Cd	Cr	Cu	Pb	Se	Site (reference)
Birds							
Barn swallow, eggs (DM)	NR	NR	NR	NR	NR	2.8	Martin Creek Lake, TX (King *et al*., 1994)
Barn swallow, liver and kidney (DM)	NR	NR	NR	NR	NR	14	Martin Creek Lake, TX (King *et al*., 1994)
Red wing blackbird, kidney (DM)	NR	NR	NR	NR	NR	33.1	Martin Creek Lake, TX (USDI, 1988)
Red wing blackbird, stomach contents (DM)	NR	NR	NR	NR	NR	1.3	Martin Creek Lake, TX (USDI, 1988)
Red wing blackbird, egg (DM)	NR	NR	NR	NR	NR	11.1	Martin Creek Lake, TX (USDI, 1988)
Barn swallow, kidney (DM)	NR	NR	NR	NR	NR	14.7	Martin Creek Lake, TX (USDI, 1988)
Barn swallow, egg (DM)	NR	NR	NR	NR	NR	3.3	Martin Creek Lake, TX (USDI, 1988)
American coot, eggs (estimated by author from liver concentrations)	NR	NR	NR	NR	NR	2–5	Belews Lake, NC, 1996, 22 years following initial ash effluent discharge, 11 yr after discharge had ceased (Lemly, 1997)
American coot, liver (back-calculated by author from egg concentration estimates)	NR	NR	NR	NR	NR	6–15	Belews Lake, NC, 1996, 22 yr following initial ash effluent discharge, 11 yr after discharge had ceased (Lemly, 1997)
Mammals							
Muskrat, adult, liver (DM)	NR	NR	NR	NR	NR	2.8	Lansing, NY, farm pond receiving airborne drift of coal ash (Gutenmann *et al*., 1976)
Muskrat, adult, liver (DM)	BDL	NR	1.8	BDL	NR	2.3–2.8	Lansing, NY, farm pond receiving airborne drift of coal ash (Furr *et al*., 1979)
Muskrat, adult, kidney (DM)	NR	NR	NR	NR	NR	4.5–4.9	Lansing, NY, farm pond receiving airborne drift of coal ash (Furr *et al*., 1979)

[a] Values are estimates from bar graphs in Guthrie and Cherry (1979). [b] Values are ranges of means reported 1993–1995. [c] Values are ranges of means reported 1995, 1997. [d] Values are estimates from bar graphs in Baumann and Gillespie (1986).

concentrations in fish and accumulation by invertebrate prey species were apparent in several systems in which biotic samples were surveyed (Table IV). Note that some authors have reported body burdens in concentrations per unit wet mass, whereas others have reported concentrations relative to dry mass. We indicate in the tables the different ways in which concentrations were presented by the original authors.

The importance of trophic vectors for trace element accumulation by vertebrates in CCR-contaminated systems was demonstrated by a recent series of experiments on the lake chubsucker, a benthic fish. Exposure to CCR-contaminated sediments alone (with uncontaminated water and food provided) resulted in rapid accumulation of trace elements (Table IV; Hopkins *et al.*, 2000b). When the same species of fish was exposed to CCR under semi-natural mesocosms conditions (water, sediments, and prey collected from the CCR disposal site), trace element accumulation was much greater than in fish previously exposed to sediments alone (Table IV; Hopkins, 2001), and effects on growth and survival were greatly exacerbated. Trace element accumulation by invertebrates was likely the most important factor influencing accumulation by fish, and led to body burdens in fish more than an order of magnitude higher than burdens found in fish exposed to contaminated sediments alone (i.e., provided with uncontaminated water and food; Table IV).

Amphibians, reptiles, birds, and mammals also accumulate contaminants from CCR-contaminated sites as a result of their feeding niche/trophic status, and/or long life spans which expose them to contaminants over exceptionally long periods of time (Table IV). For example, the banded water snake is a relatively long-lived predator with high trophic status (preying upon other vertebrates such as fish and amphibians). Banded water snakes collected from a CCR-contaminated system have the highest hepatic concentrations of Se and As yet reported in a reptile (Table IV; Hopkins *et al.*, 1999a). In addition, a series of laboratory studies with the banded water snake demonstrated the importance of ingestion of contaminated prey items in accumulation of contaminants. Adult and juvenile snakes were fed contaminated prey items (fish) collected from a CCR-contaminated swamp (D-area site, SC) for up to two years. Resulting accumulation was pronounced, with particularly high concentrations of Se accumulating in liver, gonads, and kidney (Table IV; Hopkins *et al.*, 2001; Hopkins *et al.*, 2002a). Concentrations of Se greatly exceeded concentrations known to induce reproductive failure in birds and fish (Lemly, 1993, 1996). Moreover, snakes fed alternating diets of contaminated and uncontaminated prey (Hopkins *et al.*, 2002a) also accumulated Se burdens above the reproductive toxicity thresholds proposed by Lemly (1993, 1996). Results from these studies suggest that even periodic feeding on prey items derived from CCR-contaminated sites can result in high tissue burdens in predatory vertebrates. Therefore, terrestrial vertebrates inhabiting nearby habitats could accumulate trace elements from prey items dispersing from the contaminated sites, even if the remaining portion of a predator's diet consists of prey items with no history of contaminant exposure.

A particularly well-studied system with respect to trace element accumulation in aquatic vertebrates as a result of CCR contamination is Hyco Reservoir, NC. Investigators have examined several tissues in numerous species of fish to quantify Se accumulation. Hyco Reservoir is thus examined more thoroughly in the case study to follow.

4.1.3. *A Case Study of Selenium Accumulation by Fish: Hyco Reservoir, NC*
Hyco Reservoir is a 1764 ha cooling reservoir serving a 2495 MW coal-fired power plant in Roxboro, North Carolina. As well as heated water discharge, the reservoir also received effluents from coal fly ash basins (CPL, 1981). Fish declines and a fish kill in autumn of 1980 (CPL, 1981) prompted several investigations to examine coal-related contaminants and potential effects on the aquatic community within the reservoir. Here we provide an overview of Se accumulation by fish in Hyco Reservoir, because of the large number of species examined in that system. Biological responses to Se accumulation in Hyco Reservoir are presented elsewhere in this document where sublethal and ecological effects of CCR are considered (Sections 4.2 and 4.3).

Water chemistry surveys in Hyco Reservoir found that dissolved Se concentrations were quite high (Table III), whereas waterborne concentrations of other CCR-derived trace elements did not appear to be elevated (CPL, 1981). Measurements of organic contaminants (PAHs, PCBs, pesticides, herbicides) showed no elevations above detection limits (CPL, 1981). Sampling of fish tissues revealed similar patterns as did the water chemistry surveys: fish inhabiting Hyco Reservoir experienced significant tissue burdens of Se, while other trace elements (Hg, As, Cu, Cr, Zn) were not elevated above normal (Appendix Table II; CPL, 1981). Tissue levels of organic contaminants (PAHs, PCBs, pesticides, herbicides) were below detection limits, except for DDD and DDE which were detectible but within normal background concentrations (CPL, 1981). Because of the predominance of Se in water and tissues, subsequent investigations of the Hyco system focused primarily on Se accumulation and its effects on aquatic organisms (Appendix Table II).

Selenium accumulation was observed in several trophic groups in Hyco Reservoir. Accumulation of Se by plankton may have been a source of Se accumulation to planktivorous and ultimately higher-level predatory fish (Appendix Table II). Selenium accumulation varied among fish species. Muscle Se concentrations were generally highest for bluegill and several other sunfish, and lowest for catfish (Appendix Table II). Liver Se concentrations in bluegill collected from Hyco Reservoir were about 50 times greater than liver concentrations in reference fish (Sager and Cofield, 1984), and were considerably higher than liver Se concentrations of other species (Appendix Table II). Gonadal Se concentrations also appeared higher for bluegill sunfish than other species and there were sex-specific differences in Se concentrations in gonads; ovarian Se concentrations were about twice the concentrations observed in testes (Appendix Table II; Sager and Cofield, 1984; Baumann and Gillespie, 1986). Moreover, bioaccumulation led to Se concentrations in ovaries of bluegills about 1000 times above ambient water concentrations (Baumann and Gillespie, 1986).

It is clear from studies to date that, when CCR is discharged into aquatic systems, some potentially toxic trace elements in water, sediments, and suspended solids (Table III) are accumulated by biota and further transferred through the food web (Table IV; Appendix Table II). Biological responses resulting from exposure and accumulation would thus be predicted. For example, the propensity for Se to accumulate in fish from Hyco Reservoir, especially within ovarian tissues, suggests that some species in this system may have been at risk of reproductive impairment. Demonstrated lethal and sublethal responses of biota to CCR-derived contaminants will be the subject of the following sections.

4.2. Effects of CCR on individuals

4.2.1. *Lethal Effects*

Lethality of CCR to aquatic organisms has been observed in laboratory and field studies (Table V). For example, comparative studies by Birge (1982) showed that CCR effluent was acutely toxic to embryonic fish and amphibians in the laboratory (Table V). Birge (1982) also conducted laboratory bioassays to examine relative toxicities of 22 individual CCR-related elements to goldfish, rainbow trout, and narrow-mouth toads. Based upon comparisons of 7 and 28 d LC_{50} values, narrow mouth toads were found to be the most sensitive species to 17 of the elements (in order of decreasing toxicity: Hg, Zn, Cr, Cu, Cd, As, Pb, Co, Ge, Al, Sn, Se, Tl, Sr, Sb, Mn, W), whereas rainbow trout were most sensitive to 5 elements (Ag, La, Ni, V, Mo). Acute laboratory studies on other vertebrates and invertebrates have also demonstrated lethality responses by several species when exposed to water, sediments, or suspended solids from CCR-contaminated sites (Table V).

Field and outdoor mesocosm studies also suggest that for some species, acute or chronic exposure to CCR can ultimately be lethal (Table V). For example, in a 5 d field-caging study, shrimp, darters, and salamanders were extremely sensitive to conditions in a CCR-contaminated site, whereas other invertebrates and fish experienced much lower mortality rates (Table V; Guthrie and Cherry, 1976). A recent exposure of benthic fish in outdoor mesocosms for 45 days indicated that prolonged exposures to CCR, as would occur in contaminated habitats, may result in extremely high mortality (75%; Hopkins, 2001).

As a whole, results of field- and laboratory-based lethality studies (Table V) suggest that, if lethality is to be used as an endpoint for examining ecological risks of CCR, numerous species must be simultaneously examined due to extreme species-specific differences in sensitivity. Particular attention should be devoted to the duration and conditions of exposure; a recent study indicates that reductions in resource abundance during chronic exposure to CCR increases the sensitivity of fish to CCR (Hopkins *et al.*, 2002a). Moreover, the absence of a lethal response by

TABLE V

Results of studies of lethality of CCR to aquatic animals. Tissue trace element concentrations were usually unmeasured or unreported in these studies. When tissue burdens were measured, the reference is denoted by '[a]' and concentrations are included in Appendix Table IV

Species	Exposure method	Exposure duration	Observed effect(s)	Reference
		Invertebrates		
Amphipod	Laboratory exposure to water from ashpit drainage ditch	4 d	Low survival of early instars compared to adults	Magnuson *et al.*, 1981
Shrimp	Caged *in situ* at drainage basin outflow	5 d	100% mortality	Guthrie and Cherry, 1976
Shrimp	Caged *in situ* in drainage basin outflow ditch	5 d	100% mortality	Guthrie and Cherry, 1976
Shrimp	Caged *in situ* at confluence of outflow ditch and a creek	5 d	45% mortality	Guthrie and Cherry, 1976
Odonates	Caged *in situ* at drainage basin outflow	5 d	50% mortality	Guthrie and Cherry, 1976
Odonates	Caged *in situ* in drainage basin outflow ditch	5 d	No mortality	Guthrie and Cherry, 1976
Odonates	Caged *in situ* at confluence of outflow ditch and a creek	5 d	No mortality	Guthrie and Cherry, 1976
Crayfish	Caged *in situ* at drainage basin outflow	5 d	No mortality	Guthrie and Cherry, 1976
Crayfish	Caged *in situ* in drainage basin outflow ditch	5 d	No mortality	Guthrie and Cherry, 1976
Crayfish	Caged *in situ* at confluence of outflow ditch and a creek	5 d	No mortality	Guthrie and Cherry, 1976
		Fish		
Channel catfish	Caged *in situ* at drainage basin outflow	5 d	No mortality	Guthrie and Cherry, 1976
Channel catfish	Caged *in situ* in drainage basin outflow ditch	5 d	No mortality	Guthrie and Cherry, 1976
Channel catfish	Caged *in situ* at confluence of outflow ditch and a creek	5 d	No mortality	Guthrie and Cherry, 1976
Mosquitofish	Caged *in situ* at drainage basin outflow	5 d	40% mortality	Guthrie and Cherry, 1976
Mosquitofish	Caged *in situ* in drainage basin outflow ditch	5 d	No mortality	Guthrie and Cherry, 1976
Mosquitofish	Caged *in situ* at confluence of outflow ditch and a creek	5 d	No mortality	Guthrie and Cherry, 1976
Largemouth bass	Caged *in situ* at drainage basin outflow	5 d	20% mortality	Guthrie and Cherry, 1976
Largemouth bass	Caged *in situ* in drainage basin outflow ditch	5 d	No mortality	Guthrie and Cherry, 1976

TABLE V
Continued.

Species	Exposure method	Exposure duration	Observed effect(s)	Reference
Largemouth bass	Caged *in situ* at confluence of outflow ditch and a creek	5 d	No mortality	Guthrie and Cherry, 1976
Darters	Caged *in situ* at drainage basin outflow	5 d	100% mortality	Guthrie and Cherry, 1976
Darters	Caged *in situ* in drainage basin outflow ditch	5 d	100% mortality	Guthrie and Cherry, 1976
Darters	Caged *in situ* at confluence of outflow ditch and a creek	5 d	33% mortality	Guthrie and Cherry, 1976
Largemouth bass, fingerlings	Stocking of isolated coves of reservoir receiving coal ash effluent with 200,000 fingerlings.	7 d	100% mortality	Olmsted *et al*., 1986
Channel catfish, juveniles	Caged *in situ* for exposure to acidic seepage from a coal ash pond	2 wk	Secretion of protective mucus; 100% mortality	Coutant *et al*., 1978
Rainbow trout	Exposure to different concentrations of suspended ash in static systems	96 hr	Mortality at some concentrations; no discernible pattern	Cairns and Cherry, 1983
Bluegill sunfish	Exposure to different concentrations of suspended ash in static systems	96 hr	Mortality of 30 to 80% of individuals at 1800–6000 ppm Total Suspended Solids	Cairns and Cherry, 1983
Banded sculpin	Released into coal ash-impacted stream 2–3 yrs after cessation of discharge into stream	Multi-year	No effects detected	Carrico and Ryan, 1996
Red ear sunfish, embryos	Laboratory exposure to fly ash effluent dilutions (water only)	3 d	100% mortality in undiluted effluent; 58% mortality in effluent dilted to 10%	Birge, 1978
Goldfish, embryos	Laboratory exposure to fly ash effluent dilutions (water only)	3 d	43% mortality in undiluted effluent; 24% mortality in effluent dilted to 10%	Bridge, 1978
Lake chubsuckers, juveniles	Laboratory exposure to sediments from a CCR impacted site (uncontaminated water and food provided)	124 d	25% mortality	Hopkins *et al*., 2000b[a]

TABLE V
Continued.

Species	Exposure method	Exposure duration	Observed effect(s)	Reference
Lake chubsuckers, juveniles	Laboratory exposure to sediments from a CCR impacted site (uncontaminated water and food provided); Three ration levels provided	78 d	10% mortality in fish provided with medium and high rations; 60% mortality in fish provided with low rations	Hopkins *et al.*, 2002b[a]
Lake chubsuckers, juveniles	Laboratory exposure to sediments from a CCR impacted site (uncontaminated water and food provided)	100 d	17% mortality	Hopkins, 2001[a]
Lake chubsuckers, juveniles	Outdoor mesocosm exposure to sediments, water, and food from a CCR impacted site	45 d	75% mortality	Hopkins, 2001[a]
		Amphibians		
Leopard frogs, embryos	Laboratory exposure to fly ash effluent (water only)	2.5 d	100% mortality in undiluted effluent	Birge, 1978
Fowler's toad, embryos	Laboratory exposure to fly ash effluent (water only)	1.5 d	54% mortality in undiluted effluent	Birge, 1978
Salamanders	Caged *in situ* at drainage basin outflow	5 d	100% mortality	Guthrie and Cherry, 1976
Salamanders	Caged *in situ* in drainage basin outflow ditch	5 d	100% mortality	Guthrie and Cherry, 1976
Salamanders	Caged *in situ* at confluence of outflow ditch and a creek	5 d	80% mortality	Guthrie and Cherry, 1976
Southern toads, larvae	Caged *in situ* in CCR-ash settling basin	Entire larval period (> 60 d)	100% mortality	Rowe *et al.*, 2001a
Bullfrogs, embryos	Laboratory exposure to sediment and water collected from CCR-ash settling basin	Embryonic period (4 d)	32% mortality (10% mortality in controls)	Rowe, unpublished
Bullfrogs, embryos	Laboratory exposure to sediment and water collected from drainage swamp receiving effluent from CCR-ash settling basin	Embryonic period (4 d)	18% mortality (10% mortality in controls)	Rowe, unpublished

TABLE V
Continued.

Species	Exposure method	Exposure duration	Observed effect(s)	Reference
Bullfrogs, embryos/larvae	Laboratory exposure to sediment and water collected from CCR-ash settling basin	Embryonic period and portion of larval period (34 d total)	87% mortality (46% mortality in controls)	Rowe, unpublished
Bullfrogs, embryos/larvae	Laboratory exposure to sediment and water collected drainage swamp receiving effluent from CCR-ash settling basin	Embryonic period and portion of larval period (34 d total)	75% mortality (46% mortality in controls)	Rowe, unpublished
		Reptiles		
Banded water snakes, adults	Fed fish collected from CCR-contaminated site	2 yr	No mortality	Hopkins *et al.*, 2002a
Banded water snakes, juveniles	Fed fish collected from CCR-contaminated site	13.5 mo	No mortality	Hopkins *et al.*, 2001

organisms in acute or chronic tests should not be interpreted as lack of significant biological effects of CCR. Individuals of many species interacting with CCR in natural and artificial systems have been shown to respond sublethally, often in ways in which individual fitness may ultimately be compromised.

4.2.2. *Sublethal Effects of CCR*

Sublethal effects of CCR have been observed in numerous invertebrates and vertebrates in sites in the U.S. (Table VI, Appendix Tables III and IV). Studies have shown that several invertebrates experience changes in dispersal and metabolic processes (Table VI). Fish have been shown to exhibit numerous sublethal responses upon exposure to CCR and accumulation of trace elements. In Little Scary Creek, WV, a system receiving outflow from a CCR retention basin, bluegill sunfish experienced decreased liver weight and white blood cell counts, and elevated serum levels of sodium, potassium, and chloride, although condition factors and general morphology appeared normal (Table VI; Reash *et al*., 1999). Perhaps the most frequently observed sublethal effects in fish exposed to CCR, however, are abnormalities in developing larvae and histopathological changes in adults. Bluegill sunfish in Hyco Reservoir that were shown to accumulate Se in ovarian tissues (Appendix Table II) produced edamatous larvae which eventually died (Table VI; Gillespie and Baumann, 1986). Also in Belews Lake, NC and other systems, fish have been observed to produce edamatous larvae, as well as to experience numerous histopathological changes (Table VI). In some cases, abnormalities in larvae were associated with reproductive failure and population declines (Section 4.3). In one CCR-contaminated system in particular (Martin Creek, TX), thorough histopathological surveys have revealed widespread changes in native fish associated with accumulation of Se. An overview of findings from histopathological studies in the Martin Creek system is presented in the following case study. In a CCR disposal site on the Savannah River Site, SC, numerous taxa have been shown to respond sublethally to multiple trace elements accumulated from CCR-contaminated sediments, water, and food. The Savannah River site is the subject of a second case study regarding sublethal responses to CCR.

4.2.3. *A Case Study of Selenium Accumulation from CCR and Sublethal Responses by Fish: Martin Creek, TX*

Martin Creek Reservoir is a 2000 ha cooling water reservoir used by a coal-fueled power plant operated by the Texas Utilities Generating Co. The reservoir, constructed in 1974, is located on Martin Creek, Texas, a tributary of the Sabine River. In September, 1978 the utility company began discharging effluents from two fly ash settling ponds into the reservoir (Sorensen *et al*., 1982a). Shortly thereafter, fish kills in the reservoir were observed (Garrett and Inman, 1984). In May, 1979, approximately 8 months after effluent release had begun, discharge of the effluents into Martin Creek Reservoir ceased. The Martin Creek site provided a unique opportunity to examine the magnitude of biological changes that can occur following

TABLE VI

Sublethal effects of CCR associated with trace element body burdens in animals collected from CCR-contaminated sites or experimentally exposed to CCR. For experimentally exposed organisms, exposure methods are noted. Trace element concentrations are means or ranges expressed as ppm dry mass 'DM' or wet mass 'WM'. Additional information on sublethal effects is compiled in Appendix Tables III to V for systems in which case histories are presented. If known, the specific tissue(s) in which trace elelments were measured are provided. NR = not reported. BDL = below detection limit. Decimal places reflect those presented by the original authors. Scientific names for species examined are provided in Appendix Table I

Species, tissue analyzed for contaminants; protocol	As	Cd	Cr	Cu	Pb	Se	Observed effect(s)	Site (reference)
							Invertebrates	
Amphipods; held for 2d in laboratory streams containing CCR	NR	NR	NR	NR	NR	NR	Reduced downstream movements	Rocky Run Creek, WI (Webster *et al.*, 1981)
Isopods; held for 2d in laboratory streams containing CCR	NR	NR	NR	NR	NR	NR	Reduced downstream movements	Rocky Run Creek, WI (Webster *et al.*, 1981)
Crayfish, muscle (DM); caged for 62 d in ashpit drainage ditch	NR	NR	0.6–1.8	NR	NR	0.4	Reduced metabolic rate	Rocky Run Creek, WI (Magnuson *et al.*, 1981; Forbes *et al.*, 1981)
Crayfish, hepatopancreas (DM); caged for 62 d in ashpit drainage ditch	NR	NR	5.6–6.2	NR	NR	3.6–32.5	Reduced metabolic rate	Rocky Run Creek, WI (Magnuson *et al.*, 1981; Forbes *et al.*, 1981)
Crayfish, muscle (DM); caged for 62 d in creek receiving effluent from ashpit drainage ditch	NR	NR	0.5–0.8	NR	NR	0.2–0.4	Reduced metabolic rate	Rocky Run Creek, WI (Magnuson *et al.*, 1981; Forbes *et al.*, 1981)

TABLE VI
Continued.

Species, tissue analyzed for contaminants; protocol	As	Cd	Cr	Cu	Pb	Se	Observed effect(s)	Site (reference)
Crayfish, hepatopancreas (DM); caged for 62 d in creek receiving effluent from ashpit drainage ditch	NR	NR	2.8–12.6	NR	NR	2.9–12.1	Reduced metabolic rate	Rocky Run Creek, WI (Magnuson *et al*., 1981; Forbes *et al*., 1981)
Fish								
Green sunfish, skeletal muscle (WM); field collected	NR	NR	NR	NR	NR	12.9	Decreased hematocrit, increased condition factor and hepatopancreas-to-bodyweight ratio due to edema, histological abnormalities (liver, kidney, gill, heart, ovary)	Belews Lake, NC (Sorensen *et al*., 1984)
Green sunfish, liver (WM); field collected	NR	NR	NR	NR	NR	21.4	Decreased hematocrit, increased condition factor and hepatopancreas-to-bodyweight ratio due to edema, histological abnormalities (liver, kidney, gill, heart, ovary)	Belews Lake, NC (Sorensen *et al*., 1984)
Fathead minnow, whole body (WM); field collected	NR	0.2	NR	0.6	NR	NR	Decreased sensitivity to metals in acute exposures	Harrodsburg, KY, ash settling pond (Benson and Birge, 1985)
Fathead minnow, internal organs (WM); field collected	NR	0.7	NR	1.9	NR	NR	Decreased sensitivity to metals in acute exposures, perhaps due to metallothionein production	Harrodsburg, KY, ash settling pond (Benson and Birge, 1985)

TABLE VI
Continued.

Species, tissue analyzed for contaminants; protocol	As	Cd	Cr	Cu	Pb	Se	Observed effect(s)	Site (reference)
Fathead minnow, gills (WM); field collected	NR	0.4	NR	1.7	NR	NR	Decreased sensitivity to metals in acute exposures, perhaps due to metallothionein production	Harrodsburg, KY, ash settling pond (Benson and Birge, 1985)
Bluegill, liver (DM); field collected	5.4	4.2	3.5	33.5	NR	53.8	Leukopenia, elevated serum salts, decreased liver mass	Little Scary Creek, WV (Reash *et al*., 1999)
Bluegill, ovary (DM); field collected	0.6	0.1	2.3	5.8	NR	23.4	Leukopenia, elevated serum salts, decreased liver mass	Little Scary Creek, WV (Reash *et al*., 1999)
Bluegill, testes (DM); field collected	3.1	0.6	8.3	7.8	NR	24.5	Leukopenia, elevated serum salts, decreased liver mass	Little Scary Creek, WV (Reash *et al*., 1999)
Bluegill, carcass (WM); field collected	0.05–0.11	0.007–0.01	NR	0.36–0.99	0.05–0.26	6.90–7.20	Reproductive failure	Hyco Reservoir, NC (Gillespie and Baumann, 1986)
Bluegill larvae, whole body (DM); larvae derived from crosses of adults from CCR-contaminated site	NR	NR	NR	NR	NR	28.20	Edema and reduced larval survival	Hyco reservoir, NC (Gillespie and Baumann, 1986)
Bluegill fingerlings, muscle (WM); caged for 8 d in lake receiving CCR	< 0.01–0.03	NR	NR	NR	NR	0.6–3.4	Erratic swimming, exophthalmia, abdominal distention	Belews Lake, NC (Olmsted *et al*., 1986)
Bluegill fingerlings, viscera (WM); caged for 8 d in lake receiving CCR	< 0.02–0.20	NR	NR	NR	NR	3.6–7.5	Erratic swimming, exophthalmia, abdominal distention	Belews Lake, NC (Olmsted *et al*., 1986)

TABLE VI
Continued.

Species, tissue analyzed for contaminants; protocol	As	Cd	Cr	Cu	Pb	Se	Observed effect(s)	Site (reference)
Bluegill juveniles, muscle (WM); Fed invertebrates collected from CCR-contaminated lake for 44 d	NR	NR	NR	NR	NR	7.503–7.936	Edema, food avoidance, histopathological changes	Belews Lake, NC (Finley, 1985)
Bluegill juveniles, liver (WM); Fed invertebrates collected from CCR-contaminated lake for 44 d	NR	NR	NR	NR	NR	69–86	Edema, food avoidance, histopathological changes	Belews Lake, NC (Finley, 1985)
Striped bass[a] muscle (WM); fed fish collected from CCR contaminated lake	NR	NR	NR	NR	NR	3.8	Food avoidance, decreased growth and condition factor, histopathological changes	(Coughlan and Velte, 1989)
Birds								
Red wing blackbirds, eggs (DM)	NR	NR	NR	NR	NR	11.1	Reduced hatching success	Martin Creek Reservoir, TX (USDI, 1988)

[a] Tissue concentration reported for animals having died from day 12 to day 78, and therefore reflect a range in actual exposure durations.

a rather brief period of CCR inputs (8 mo), and the ensuing recovery period. In this case study, we will focus on examinations of sublethal, histopathological changes observed in fish in Martin Creek Reservoir. Population-level studies in this system will be addressed in Section 4.3.

In 1977, one year prior to ash effluent discharges into the reservoir, fish sampling efforts were initiated by the Texas Parks and Wildlife Department and sampling recurred for three years after the discharge of coal ash effluents (1979–1981; Garrett and Inman, 1984). During the same month that discharges began, dissolved Se concentrations of Martin Creek Reservoir reached 2.2 to 2.7 ppm (Table III; Garrett and Inman, 1984). Associated with Se in water were high tissue Se concentrations and a variety of histopathological abnormalities in fish (Table IV, Appendix Table III). Livers of fish sampled from Martin Creek Reservoir in 1979 exhibited a number of alterations typical of Se poisoning (Appendix Table III). Such hepatic alterations included focal necrosis, granular cytoplasm, abnormally high densities of Kupffer cells, and general disorganization of the hepatic architecture (Sorensen *et al.*, 1983a). Kidneys of green sunfish also showed necrotic cells in the convoluted tubules, proliferative glomerulonephritis, and hematuria (Sorensen *et al.*, 1982a). Because of high concentrations of Se and observations that other measured trace elements (Zn, Cu, Hg, Ag, Mg, Cr) were not elevated in tissues, the authors concluded that Se was the likely cause for observed histopathological changes (Sorensen *et al.*, 1983a; Garrett and Inman, 1984).

Studies conducted in 1980 and 1981, two to three years after the discharge of CCR effluent into Martin Creek Reservoir had ceased, revealed that histopathological changes persisted in numerous organs in sunfish. Although there were no abnormalities found in stomach, spleen, gill, or heart of red ear sunfish, the kidneys, liver, and gonads were characterized by a number of abnormalities similar to those previously reported for green sunfish (Sorensen *et al.*, 1983b). Livers having approximately 20 ppm Se (wet mass) were necrotic, displayed reductions in rough endoplasmic reticulum and glycogen particles, and had increased densities of lysosomes (Sorensen *et al.*, 1983b). Red ear sunfish also displayed proliferative glomerulonephritis in kidneys and hypertrophy of pancreatic tissue (Sorensen *et al.*, 1983b). Ovaries of several red ear sunfish exhibited an abnormally high incidence of atretic follicles, but testicular abnormalities were not observed (Sorensen *et al.*, 1982b). Green sunfish exhibited similar abnormalities in liver, kidneys, and ovaries, and additional abnormalities in myocardium and gills. Dramatic increases were observed in inflammatory cells in cardiac tissue. Gills were heavily vacuolated and had lamellae up to six-times thicker than those of reference fish (Sorensen *et al.*, 1982b).

Whereas the discharge of CCR effluents into Martin Creek Reservoir lasted only about 8 months, recovery of the system took several years. One year following effluent discharge, gizzard shad had muscle Se concentrations as high as 7.3 ppm (wet mass), which declined to about 2.9 ppm by the third year after discharge (Garrett and Inman, 1984). From 1978 to 1982, other species such as common carp and largemouth bass exhibited similar decreases in muscle Se concentrations (from 9.1 to 3.6 for carp and 8.3 to 3.8 ppm for bass; Garrett and Inman, 1984). However, some species retained high tissue Se concentrations over time, despite the cessation of CCR inputs to the system. For example, red ear sunfish sampled in 1986 (7 years after CCR input had ceased) still had hepatic Se concentrations of 7.6 ppm wet mass, exhibited lower condition factors than reference fish, and continued to exhibit histological alterations in the hepatic architecture similar to those observed in earlier surveys (Appendix Table III; Sorensen, 1988). In addition, mature red ear sunfish showed histopathological abnormalities in ovaries suggestive of overall reproductive impairment. Sorensen (1988) concluded that overall health of red ear sunfish in Martin Creek Reservoir remained poor, even 8 years following a brief (8 month) release of CCR into the system.

Studies of the fish assemblage in the Martin Creek system demonstrated severe and widespread changes in tissue morphology which appeared to be primarily related to availability and accumulation of high concentrations of Se derived from CCR inputs. However, the complex chemical nature of CCR suggests that in many systems, a single contaminant such as Se may not be responsible for biological changes (e.g. Tables II to IV). Rather, the combined effects of multiple accumulated elements may lead to numerous changes in individuals that could compromise individual fitness or health (Rowe *et al.*, 2001c). The following case study summarizes research conducted to examine sublethal responses of biota upon exposure to, and accumulation of, multiple trace elements derived from CCR.

4.2.4. *A Case Study of Accumulation of Numerous Trace Elements from CCR and Sublethal Responses by Numerous Taxa: D-Area Facility, Savannah River Site, SC*

Perhaps the most studied site in the U.S. with respect to aquatic CCR is the disposal system associated with the D-Area Power Facility on the U.S. Department of Energy's Savannah River Site near Aiken, South Carolina. Beginning in the 1970s and continuing today, investigators have studied chemical, physical, and biological features of the aquatic environments in the D-Area CCR disposal basins and downstream habitats. At the D-Area site a 70 MW, coal-fired power plant discharges sluiced fly and bottom ash into a series of open settling basins. The configuration of the system since the late 1970s has entailed use of two settling basins and a drainage swamp. Sluiced ash is pumped into a receiving ditch which empties into primary (15 ha) and secondary (6 ha) settling basins. A continuous flow of surface water exits the secondary basin where it enters a 2 ha swamp. Discharge from the swamp enters Beaver Dam Creek, a tributary of the nearby Savannah River. Sediments throughout the disposal system are elevated in numerous CCR-related trace elements (Table III). In addition to the elements presented in Table III, water, sediments, and biota in the D-Area site have elevated concentrations of Al, Ba, Fe, Hg, Mn, Sr, V, and Zn (Cherry *et al.*, 1979a and b; Guthrie and Cherry 1979;

Alberts *et al*., 1985; McCloskey and Newman 1995; McCloskey *et al*., 1995; Rowe *et al*., 1996; Hopkins *et al*., 1998).

Plants and animals inhabiting the basins, drainage swamp, and Beaver Dam Creek accumulate high concentrations of trace elements such as As, Cd, Cr, Cu, and Se (Table IV). Particularly elevated in amphibians, reptiles, and invertebrates are As and Se, considered to be among the most toxic trace elements to developing organisms (e.g. amphibians; Herfenist *et al*., 1989). For example, larval bullfrogs developing in D-area and those individuals that successfully metamorphosed and dispersed from the site had whole body concentrations of As and Se that were 8–20 times the concentrations found in larvae from reference sites (Table IV; Rowe *et al*., 1996; Hopkins *et al*., 1999a). Banded water snakes, which feed on contaminated fish and amphibians in D-area, accumulated the highest tissue concentrations of As and Se yet reported for a reptile (Table IV; Hopkins *et al*., 1999a). Moreover, accumulation of trace elements was not limited to aquatic and semi-aquatic species. The southern toad, a terrestrial amphibian that congregates at the contaminated aquatic habitat seasonally for reproduction, has also been found to rapidly accumulate As and Se from the polluted habitat (Table IV; Hopkins *et al*., 1998).

While several studies have shown population-level changes in invertebrates in the D-Area system (Section 4.3), several invertebrates have been examined for specific sublethal effects of CCR exposure on physiology and growth. Grass shrimp caged *in situ* in the D-Area settling basin for 8 mo experienced standard metabolic rates 51% higher than shrimp caged in an unpolluted pond (Appendix Table IV). Such increases in metabolic expenditures may reflect energetically costly processes invoked in response to contaminant exposure and accumulation, and are predicted to ultimately detract from portions of the energy budget associated with production (e.g. energy storage, growth, or reproduction). The relationship between standard metabolic costs and production was examined in another crustacean, a crayfish, exposed chronically to CCR. Crayfish captured in D-Area had much higher standard metabolic rates than did crayfish collected from an unpolluted site. Crayfish collected from unpolluted sites and exposed for 50 d to sediments and food collected from D-Area also experienced initial increases in metabolic rate, and over the duration of the experiment, suffered reduced growth rates compared to controls (Rowe *et al*., 2001b; Appendix Table IV). Results from this laboratory study are consistent with the prediction that CCR-derived elevations in metabolic expenditures may ultimately be responsible for reductions in production processes such as growth. Interestingly, the phenomenon of abnormally high metabolic rates in response to chronic exposure to CCR in the D-Area site has been observed in two vertebrates as well, suggesting that similar physiological responses to CCR are invoked by several, taxonomically distant species (Appendix Table IV; Rowe *et al*., 2001b).

Several species of fish have been shown to accumulate contaminants from the D-Area site (Table IV; Appendix Table IV). However, only the lake chubsucker has been extensively examined with respect to sublethal changes in physiology or performance (Appendix Table IV). Recent work on lake chubsuckers indicated that critical swimming speed (U_{crit}) and burst swimming speeds were greatly reduced in fish experimentally exposed to CCR (Appendix Table IV). After 3 months of exposure to CCR under conservative laboratory conditions, fish exposed to the contaminated sediments exhibited a 50% reduction in mean U_{crit} values (from 47.91 to 24.02 cm sec^{-1}; Hopkins *et al.*, 2003). Moreover, the typical relationship between U_{crit} and body mass was reversed in fish exposed to CCR. Instead of larger fish having higher U_{crit}, the smallest CCR-exposed fish actually performed best, suggesting that exposure to CCR induced tradeoffs between growth and performance. Burst swimming speeds were also affected by CCR exposure, with reductions becoming exacerbated as sprint distance increased (Hopkins *et al.*, 2003). Additional experimental exposures of chubsuckers to CCR indicate that growth, fin morphology, lipid storage, and metabolic rates can be adversely affected by CCR depending on the duration and conditions of exposure (Hopkins *et al.*, 2000, 2002b; Hopkins, 2001; Appendix Table IV).

Much research on sublethal responses of animals to CCR in the D-Area site has been conducted on amphibians. Numerous sublethal effects have been reported in amphibians inhabiting, or chronically exposed experimentally to, conditions in the D-Area site, including changes in morphology, behavior, energetics, and endocrinology (Appendix Table IV).

Studies conducted recently in the D-Area site have demonstrated frequent occurrence of morphological abnormalities in larval bullfrogs (Appendix Table IV). Up to 96% of larval bullfrogs captured in D-Area exhibited abnormalities of the oral structures, including absence of grazing teeth or entire tooth rows and abnormal morphology of labial papillae (Rowe *et al.*, 1996). When embryos were transplanted from a reference site into the D-Area settling basin and held for 80 d post-hatching, over 97% of larvae expressed oral abnormalities, compared to less than 1% in an unpolluted site (Rowe *et al.*, 1998a). Oral abnormalities changed the feeding ecology of the affected individuals, limiting their feeding niche and subsequently reducing growth rate when heterogeneous sources of food were unavailable (Rowe *et al.*, 1996). Axial malformations in the tail region (scoliosis) have also been observed in larval bullfrogs in the D-Area site (Appendix Table IV). Thirty seven percent of bullfrog larvae captured in D-Area exhibited scoliosis of the tail, whereas such malformations were rare in nearby unpolluted reference sites (< 3% overall; Hopkins *et al.*, 2000a).

Abnormal swimming behaviors by larval bullfrogs have been observed in animals captured from the D-Area site (Raimondo *et al.*, 1998; Hopkins *et al.*, 2000a). In larval bullfrogs experiencing scoliosis, swimming speeds were reduced compared to animals from the same site which lacked the spinal malformations (Hopkins *et al.*, 2000a). Moreover, larval bullfrogs from D-Area that did not have scoliosis had decreased swimming speeds and were less responsive to physical stimuli when compared to larvae from an unpolluted reference site (Raimondo *et al.*, 1998). In experimental mesocosms, larval bullfrogs from D-Area were more frequently preyed upon than were bullfrogs from an unpolluted site (Raimondo *et al*., 1998), suggesting a relationship between altered swimming behaviors and predation risk.

Aberrant behaviors were also observed in adult southern toads exposed to coal ash (Hopkins *et al*., 1997). Male southern toads inhabiting the margins of a coal ash settling basin displayed breeding behaviors (vocalizations, posturing, selection of conspicuous microhabitats) for over one month beyond the typical breeding period, during which time females were unresponsive to male advertisements. These disrupted breeding cycles, which coincided with modified circulating hormone levels that regulate male reproductive behaviors (discussed below), were not observed in other local populations of toads (see below; Hopkins *et al.*, 1997).

Energetic changes similar to those observed in grass shrimp and crayfish were also observed in larval bullfrogs in D-Area. Larval bullfrogs captured from D-Area had metabolic rates from 30 to > 100% higher than did bullfrogs in uncontaminated sites. A transplant experiment with embryos from different populations indicated that increased metabolic rates were induced by environmental conditions in D-Area, but were unrelated to the population from which embryos were derived (Appendix Table IV; Rowe *et al*., 1998b). As with crayfish which experienced reduced production of tissue (i.e., growth rates) when metabolic rate was elevated (Rowe *et al*., 2001b), bullfrogs from D-Area appear to have lower production of lipid reserves at metamorphosis, perhaps a result of elevated metabolic expenditures due to CCR exposure (Appendix Table IV; Rowe and Hopkins, unpublished). However, controlled experimental work is required to verify the relationship between lipid reserves and metabolic rates in D-Area bullfrogs.

In adult southern toads in D-Area, changes in endocrinological traits have been observed. Adult male toads inhabiting the site exhibited increased circulating levels of adrenal stress hormones and androgens (Hopkins *et al*., 1997). Circulating hormone levels were elevated under seasonal and behavioral circumstances in which hormones should have been at baseline levels, coinciding with aberrant calling behaviors discussed previously. In addition, adult toads collected from a reference site and transplanted to D-Area exhibited a pronounced adrenal stress response (Hopkins *et al*., 1997; Hopkins *et al*., 1999b). Toads chronically exposed to CCR in D-Area were less efficient at responding hormonally to direct additional stimulus of the corticosteroid producing axis (Hopkins *et al*., 1999b). The observed inability to respond to the stimulus indicates that the normal stress response might be disrupted and that appropriate responses to additional environmental stressors may be impaired (Hontela, 1998).

Although much research in the D-Area site has focused on sublethal responses of animals to CCR, lethality has also been observed, reflecting either direct toxicity of CCR to the study species, or indirect effects that led to mortality via CCR effects on resources. Southern toads transplanted as embryos into the D-Area site and an unpolluted area had no differences in survival through the embryonic period; yet exposure to coal ash during the ensuing larval period resulted in mortality of 100% of study organisms prior to metamorphosis (Table VII; Rowe *et al*., 2001a).

Larval mortality was associated with extremely low resource abundance in D-Area, and very high trace element concentrations in available resources. It thus appears that effects of CCR on D-Area toads probably reflected a combination of direct toxic action and limitation of resources (Rowe *et al.*, 2001a). Moreover, the low recruitment of toads in D-Area suggests that the adult breeding population is made up of immigrants from nearby uncontaminated sites. In such a way, this CCR-contaminated site may act as a population sink, attracting migrants from nearby populations that use the site for breeding, resulting in reproductive failure (Rowe *et al.*, 2001a).

Reptiles and birds in D-Area have also been examined for sublethal effects or maternal transfer of CCR-derived contaminants to offspring. Banded water snakes captured from the D-Area drainage swamp had higher standard metabolic rates and hepatic trace element concentrations than did snakes capture in uncontaminated sites (Appendix Table IV; Hopkins *et al.*, 1999a). Laboratory feeding studies confirm that snakes from the D-area site accumulate much of their trace element burdens from dietary sources. Snakes fed fish collected from D-area for 1–2 years accumulated significant quantities of As, Cd, Se, Sr, and V in target organs (liver, kidneys, and gonads; Hopkins *et al.*, 2001, 2002a). However, trace element concentrations were much lower in laboratory-exposed snakes compared to snakes collected from D-area, suggesting that longer periods of exposure and/or other routes of exposure are encountered by snakes under natural conditions (Hopkins *et al.*, 1999a, 2001, 2002a). Although snakes with lower body burdens of trace elements did not exhibit changes in metabolic rates, approximately one third of the snakes experienced significant tissue damage. Liver fibrosis was the most prevalent pathology, involving proliferation of collagen fibers that resulted in narrowing or occlusion of sinusoids and increasing the mass of the intersinusoidal parenchyma (Rania and Hopkins, unpublished).

Turtle, alligators, and birds inhabiting the vicinity of the D-Area basins and drainage swamp have been found to accumulate several trace elements and transfer some contaminants, primarily Se, to developing offspring (Appendix Table IV). Hatchling slider turtles derived from D-Area females experienced reduced metabolism compared to reference animals, although other traits compared between the groups did not differ. Hatchling alligators from nests constructed by female residents of D-Area have also been found to receive Se via maternal transfer, as have hatchling common grackles. Potential biological ramifications of maternal transfer of Se to hatchling alligators and grackles have not yet been identified.

The observed sublethal effects of CCR in animals in D-Area, Martin Creek, and other systems illustrate that numerous traits in individuals can be substantially modified following chronic exposure to, and accumulation of, contaminants associated with CCR in aquatic systems. However, to examine the potential ecological importance of CCR in aquatic systems, it is necessary to consider the ways that animal populations and inter- and intra-specific interactions among components of natural communities are modified in CCR-contaminated systems. Ecological changes in response to CCR contamination of aquatic habitats will be considered in the following section.

4.3. Ecological effects of CCR

4.3.1. *Population and Community Responses to CCR*

The research summarized thus far was directed primarily at examining sources, accumulation, and effects of CCR-related contaminants on individuals in aquatic systems. However, higher-order, ecological processes have also been found to be modified as a result of CCR discharge into aquatic systems. Here we present overviews of research in which modifications to animal populations, interspecific interactions, and the structure of aquatic communities have been linked to contamination of aquatic habitats by CCR.

Studies in Rocky Run Creek, WI, examined effects of CCR effluents on populations and communities of benthic organisms. Dissolved Cd, Cr, and Cu concentrations were elevated as a result of disposal of CCR in an ashpit draining into the creek. Invertebrates accumulated trace elements and exhibited sublethal changes in metabolism (Table IV). Moreover, effects on invertebrate diversity and abundance were observed (Table VII). Surveys of aquatic invertebrates were conducted prior to and during the period of CCR inputs at sites upstream and downstream of the discharge area. Abundance and diversity of invertebrates within the ashpit drainage decreased after CCR inputs began, and over time a pattern emerged in Rocky Run Creek such that diversity and density of invertebrates were greater as distance increased from the discharge area (Table VII; Forbes *et al.*, 1981; Magnuson *et al.*, 1981). Similar effects of CCR on invertebrate abundance or diversity have been observed elsewhere as well, including the D-Area site in SC, and, in an offshore CCR disposal site in the United Kingdom (Table VII).

By adversely affecting the abundance, diversity, and/or quality of food resources, CCR also has substantial indirect effects in higher trophic level consumers. In the ashpit drainage in the Rocky Run Creek site, fungal decomposition of detritus was extremely limited, reducing the quality of detrital material available to grazing invertebrates, perhaps explaining the reductions in diversity and density of benthic invertebrates in the system (Table VII; Forbes and Magnuson, 1980). Similarly, extremely low periphyton abundance in the D-Area site may have been partially responsible for high larval mortality rates in southern toads (Table VII; Rowe *et al.*, 2001a). Likewise, benthic fish relying on low quality invertebrates from a CCR site exhibit higher mortality rates and greater reductions in growth than fish exposed to CCR with high quality resource provisions (Hopkins, 2001).

Ecological changes as a result of CCR inputs to aquatic system have been most thoroughly studied for populations and communities of fish inhabiting lacustrine systems receiving CCR. Lemly (1985a) suggested that extirpation of largemouth bass in Hyco Reservoir, NC resulted from reproductive effects associated with accumulation of Se (e.g. Appendix Table II). In the same system, severe reductions

TABLE VII

Ecological (population or community) effects of CCR associated with trace element body burdens in animals collected from CCR-contaminated sites or experimentally exposed to CCR. Trace element concentrations are means or ranges expressed as ppm dry mass 'DM' or wet mass 'WM'. Additional information on population effects in the Belews Lake, NC site is provided in Appendix Table V. If known, the specific tissue(s) in which trace elelments were measured are provided. NR = not reported. BDL = below detection limit. Decimal places reflect those presented by the original authors. When possible, scientific names for all species examined are provided in Appendix Table I

Species, tissue analyzed for contaminants; protocol	As	Cd	Cr	Cu	Pb	Se	Observed effect(s)	Site (reference)
Fungi								
Fungi degrading sugar maple leaves; leaf packs placed in ashpit drainage ditch for 96 d	NR	NR	NR	NR	NR	NR	Reduced fungal colonization of leaves and reduced decomposition by detritivorous invertebrates	Rocky Run Creek, WI (Forbes and Magnuson, 1980)
Invertebrates								
Benthic invertebrates; enumerated on artificial substrates	NR	NR	NR	NR	NR	NR	Abundance and diversity increased with distance away from CCR input	Rocky Run Creek, WI (Forbes *et al.*, 1981; Magnuson *et al.*, 1981)
Odonate, muscle (WM); field collected	5.2–6.2	NR	NR	33.8–39.1	NR	4–4.2	Decreased population density	D-Area Facility, SC (Cherry *et al.*, 1979a)
Crayfish, abdominal muscle (WM); field collected	2.1	NR	NR	26.3	NR	4.4	Decreased population density	D-Area Facility, SC (Cherry *et al.*, 1979a)
Gastropod, whole body (WM); field collected	18.2	NR	NR	30.3	NR	1.2	Decreased population density	D-Area Facility, SC (Cherry *et al.*, 1979a)
Chironomid, whole body (WM); field collected	2.9	NR	NR	56.0	NR	NR	Decreased population density	D-Area Facility, SC (Cherry *et al.*, 1979a)
Odonate, muscle (WM); field collected	6.05	1.20	3.43	26.84	NR	2.48	Decreased population density	D-Area Facility, SC (Cherry *et al.*, 1979b)

TABLE VII
Continued.

Species, tissue analyzed for contaminants; protocol	As	Cd	Cr	Cu	Pb	Se	Observed effect(s)	Site (reference)
Odonate, muscle (WM); field collected	1.35	1.00	4.49	20.00	NR	2.50	Decreased population density	D-Area Facility, SC (Cherry *et al.*, 1979b)
Crayfish, abdominal muscle (WM); field collected	1.36	15.63	7.66	19.31	NR	7.20	Decreased population density	D-Area Facility, SC (Cherry *et al.*, 1979b)
Chironomid (WM); field collected	1.93	1.15	38.27	50.00	NR	0.70	Decreased population density	D-Area Facility, SC (Cherry *et al.*, 1979b)
Benthic marine macrofauna; field collected	NR	NR	NR	NR	NR	NR	Decreased abundance and diversity, possibly related to physical characteristics of ash	Northumberland Coast, U.K. (Bamber, 1984)
Fish								
Mosquitofish, caudal peduncle muscle (WM); field collected	2.0	NR	NR	11.5	NR	9.2	Decreased population density	D-Area Facility, SC (Cherry *et al.*, 1979a)
Largemouth bass, adult muscle (WM); field collected	NR	NR	NR	NR	NR	3.8–8.3[a]	Reduced reproductive success and population fluctuations	Martin Creek Reservoir, TX (Garrett and Inman, 1984)
Channel catfish, adult, muscle (WM); field collected	NR	NR	NR	NR	NR	2.7–4.6[a]	Reduced adult biomass	Martin Creek Reservoir, TX (Garrett and Inman, 1984)
Gizzard shad, adult, muscle (WM); field collected	NR	NR	NR	NR	NR	2.9–7.3[a]	Population decline	Martin Creek Reservoir, TX (Garrett and Inman, 1984)

TABLE VII
Continued.

Species, tissue analyzed for contaminants; protocol	As	Cd	Cr	Cu	Pb	Se	Observed effect(s)	Site (reference)
Common carp, adult, muscle (WM); field collected	NR	NR	NR	NR	NR	3.6–9.1[a]	Population decline	Martin Creek Reservoir, TX (Garrett and Inman, 1984)
Long ear sunfish, adult, muscle (WM); field collected	NR	NR	NR	NR	NR	5.1	Population decline	Martin Creek Reservoir, TX (Garrett and Inman, 1984)
Bluegill, adult, muscle (WM); field collected	NR	NR	NR	NR	NR	3.4–6.8[a]	Population decline	Martin Creek Reservoir, TX (Garrett and Inman, 1984)
Red ear sunfish, adult, muscle (WM); field collected	NR	NR	NR	NR	NR	4.4–5.6[a]	Population decline	Martin Creek Reservoir, TX (Garrett and Inman, 1984)
Spottail shiner, adult, whole body (DM); field collected	BDL	BDL	2.5–5.5	NR	NR	1.8–2.1	Decreased fish abundance; Decreased prey abundance	Whiting Power Plant, Western Shore Lake Erie (Hatcher *et al*., 1992)
Brown bullhead, adult, whole body (DM); field collected	BDL	BDL	BDL-1.5	NR	NR	1.0–1.8	Decreased prey abundance	Whiting Power Plant, Western Shore Lake Erie (Hatcher *et al*., 1992)
Brown bullhead, young of the year, whole body (DM); field collected	BDL	BDL	BDL-51.0	NR	NR	1.2–1.6	Decreased prey abundance	Whiting Power Plant, Western Shore Lake Erie (Hatcher *et al*., 1992)
Yellow perch, adult, whole body (DM); field collected	BDL-0.4	BDL	BDL-7.2	NR	NR	1.1–1.4	Decreased prey abundance	Whiting Power Plant, Western (Shore Lake Erie (Hatcher *et al*., 1992)
White bass, yearling, whole body (DM); field collected	BDL	BDL	BDL-1.7	NR	NR	1.3–3.3	Decreased fish abundance; Decreased prey abundance	Whiting Power Plant, Western Shore Lake Erie (Hatcher *et al*., 1992

TABLE VII
Continued.

Species, tissue analyzed for contaminants; protocol	As	Cd	Cr	Cu	Pb	Se	Observed effect(s)	Site (reference)
Amphibians								
Bullfrogs, larvae; raised in CCR settling basin until 60 d old prior to exposure to predators in mesocosms	NR	NR	NR	NR	NR	NR	Increased susceptibility to predation	D-Area Facility, SC (Raimondo *et al.*, 1998)
Southern toads, larvae; hatched and raised in CCR settling basin through metamorphosis	NR	NR	NR	NR	NR	NR	100% mortality associated with severe reductions in resource (periphyton) abundance; potential for contaminated site to act as a sink habitat for local populations	D-Area Facility, SC (Rowe *et al.*, 2001a)

[a] Range in concentrations reflects values obtained one year following an 8 month period of CCR discharge into reservoir (high value; 1980) and values obtained two years later (low value; 1982) to examine recovery of the system.

in populations of the bluegill appear to have resulted from female transfer of Se to offspring, leading to edamatous larvae which were unable to survive the larval period (Table VI; Gillespie and Baumann, 1986).

In Martin Creek Reservoir, TX, populations of several fish were reduced coincident with a relatively brief period of CCR inputs (8 mo; Table VII). The changes in abundance of different species of fish resulted in overall changes to the community structure of the system, which remained for at least three years after CCR inputs had ceased (Garrett and Inman, 1984). Different trophic levels responded differently, with planktivorous and carnivorous fish experiencing severe reductions in total biomass, and omnivorous fish (such as common carp) increasing somewhat in biomass following effluent release. Three years following the effluent release, planktivorous fish populations remained extremely low, whereas carnivores appeared to have nearly recovered (Garrett and Inman, 1984). The effect on planktivorous fish was most notable in the gizzard shad, which experienced an initial reduction in population size from 890 ha^{-1} (1977) to 182 ha^{-1} (1979). Recovery of this species was slow, having attained a population size of only 264 ha^{-1} 1981 (Garett and Inman, 1984). While some carnivorous species appeared to have recovered in biomass by the third year following the effluent release, a striking reduction in small size classes suggested reproductive impairment in surviving adults.

Perhaps the most notable effects of CCR release into an aquatic site on populations of fish were observed in Belews Lake, NC. In this system, surveys of fish populations, as well as incidence of malformations, were conducted during a period of CCR inputs and 7 yr after inputs had ceased. Thus a data set spanning a relatively long time span is available so that population-level effects and recovery can be examined. The fish populations of Belews Lake are examined in the final case study.

4.3.2. *A Case Study of Ecological Effects of CCR on Fish: Belews Lake, NC*

Belews Lake is a 1564 ha cooling reservoir constructed in 1970 by Duke Power Company. Shortly after construction of the reservoir (prior to inputs of CCR), monitoring of the fish populations was initiated. In 1974 to 1975, the two units of the Belews Creek Steam Station went online with a total generating capacity of 2280 MW (Olmsted *et al.*, 1986). In 1974, discharge of CCR effluents into Belews Lake began. During a 12 yr period from 1974 to 1985 selenium-enriched water (150 to 200 ppb; Table III) from a 142 ha coal ash slurry basin was released into the west side of Belews Lake (Lemly, 1993). By 1976 (2 yr after effluent release had begun), Duke Power personnel noted a decline in numbers of large adult fish (Olmsted *et al.*, 1986). Because of community-level changes in Belews Lake caused by the effluent releases (see below), the power station ceased releasing effluent into Belews Lake in 1985, adopting a dry landfilling practice for disposal of coal ash. Because information was available prior to, during, and after release of the effluents, the occurrences at Belews Lake provide a rare opportunity to examine responses and recovery of an aquatic system to CCR contamination (Olmsted *et al.*, 1986).

Release of CCR effluents into Belews Lake brought about rapid and dramatic changes in fish populations. All of the 19 fish species collected in Belews Lake in 1975 (one year after effluent release began) displayed morphological abnormalities, but the centrarchids were the most impacted (Appendix Table V; Lemly, 1993). Morphological abnormalities included lordosis, kyphosis, partial fin loss, edema, cataracts, scoliosis, exopthalmus, and head deformities (Lemly, 1993). Fish population declines were also observed following the onset of discharges into the lake (Appendix Table V); from 1975 to 1976, several species exhibited complete reproductive failure (Cumbie and Van Horn, 1978). By 1978 (four years after release of effluents began), only four species of fish remained in the lake (Appendix Table V; Lemly, 1993). Piscivorous and planktivorous fish were essentially extirpated from the lake. Only omnivorous and very tolerant fish (carp, bullhead, mosquitofish, fathead minnows) remained (Appendix Table V; Lemly, 1993) and only mosquitofish maintained a reproductively viable population (Lemly, 1985a). In 1981, fathead minnows and mosquitofish accounted for 82% of the standing fish stock in Belews Lake (Olmsted *et al.*, 1986). Moreover, the loss of large predatory species from the system appears to have allowed some fish having abnormalities to survive, despite their otherwise high susceptibility to predation (Lemly, 1993).

Initially, several possible causes for the fish declines in Belews Lake were examined, including thermal loading, fluctuating water levels, entrainment, and disease or parasitism (Harrell *et al.*, 1978; Olmsted *et al.*, 1986). When these causes for fish declines were dismissed, the possibility of chemical effects was considered. In 1977, pesticide levels were measured in water from Belews lake, but all compounds assayed were found to be below detection limits (Cumbie, 1978). However, analyses of Belews lake water for inorganic contaminants found elevations in As, Se, and Zn corresponding with the inputs of CCR effluents (Olmsted *et al.*, 1986). Moreover, following the onset of CCR discharge to Belews Lake, accumulation of Se in fish tissues was observed (Cumbie, 1978), and whole-body Se burdens were shown to correlate strongly with morphological abnormalities induced during embryonic and larval development (Lemly, 1993). Plankton samples collected in 1977 revealed high concentrations of Se (40 to 100 ppm dry mass), suggesting that the planktonic community was an important source of Se to the fish in Belews Lake (Cumbie, 1978).

In 1996, 22 yr after effluent release had begun and 11 yr after it had ceased, signs of recovery were evident, but risks to wildlife species had not completely abated. Concentrations of Se in sediments had decreased by 65 to 75%, but remained high enough to pose risks to wildlife via accumulation from ingesting benthic organisms (Lemly, 1997). Concentrations of Se in ovaries of fish (estimated from whole-body concentrations) decreased from 40–159 (prior to 1986) to 3–20 ppm dry mass (in 1996; Lemly, 1997). Despite the reduction in Se concentrations in ovaries with time, Se-induced reproductive anomalies remained abnormally frequent (Lemly, 1997). The long-term studies of Belews Lake illustrated that release of CCR effluents can have rapid and widespread effects on aquatic communities. The studies also demonstrated that recovery of the system was quite slow, possibly due to the long retention time and low sedimentation rates characteristic of the Belews Lake reservoir.

5. Future Research Needs

In the past several decades, much information on environmental effects of CCR in aquatic systems has become available. Ecotoxicological studies in many CCR-contaminated sites have been conducted, and in some cases, long term, multi-investigator projects have provided extensive information on biological responses to CCR in specific study sites. Especially in these intensively studied systems, lethal and sublethal effects on individuals and population declines of some species illustrate that release of CCR into aquatic habitats can be environmentally damaging. Despite the large amount of research that has been conducted to date, we have identified several topics which require greater attention when examining this issue in the future.

Because CCR is a chemically complex effluent (Table II), observed biological effects may often be the result of interactive properties of various compounds. In some systems, a single component of CCR has been identified as being primarily responsible for observed biological effects. For example, in the Belews Lake system, Se has been shown to be primarily responsible for effects on fish populations, based upon extensive research that eliminated other potential factors (see Cumbie, 1978; Cumbie and Van Horn, 1978). In other systems (such as the D-Area site), however, it has proven difficult to isolate the effects of any one component of CCR as being responsible for the multiple biological responses observed. Rather, the suite of contaminants potentially interacting agonistically, antagonistically, or additively on biological systems, and differing in bioaccumulation potentials and residence times, precludes identification of a particular contaminant as a primary causal agent. For example, Se and Hg appear to act antagonistically, such that Se accumulation appears to reduce Hg accumulation; during periods of Se input to a lake (via CCR), Hg concentrations in fish flesh remained relatively low, but as Se availability declined after cessation of CCR inputs, Hg concentrations in fish flesh rose concomitantly (Southworth *et al.*, 1994, 2000).

In such chemically-complex systems, biological responses to CCR must be interpreted as overall responses to the mixture of contaminants available to organisms in water, sediments, and food. Among different CCR impacted sites, there may be considerable differences in the suite of trace elements present, their relative concentrations, and their bioavailability. Differences in comanagement practices among facilities can further complicate generalizations due to addition of various organic compounds to the CCR waste stream. The site-specific variability in water and sediment contaminant mixtures and concentrations is problematic when attempting to assess risks associated with CCR-impacted systems overall. Even when ambient contaminant concentrations are consistently elevated, the bioavailability of contaminants may vary on a site-specific basis due to a variety of physical, chemical, and biological parameters (Hamelink *et al*., 1994). Thus, in many systems CCR must be treated as a unique effluent, and thorough chemical surveys should be conducted to characterize the overall chemical environment of CCR-impacted areas. At a minimum, samples from impacted systems should initially be screened for elevated levels of As, Cd, Cr, Cu, Se, Sr, Hg, Zn, Pb, and Ni due to their abundance in some CCR-contaminated sites and their demonstrated effects on organisms. As well as the potentially toxic components of CCR themselves, it is also important to characterize other abiotic aspects (such as pH, hydrodynamics) of the systems that may influence metal speciation and availability, thereby influencing accumulation and toxicity (Soholt *et al*., 1980; EPRI, 1991).

Co-management of various wastes by industry can produce effluents that contain many more types of contaminants than just the inorganics associated with the parent coal. The focus of this report on inorganic contaminants emphasizes the lack of knowledge about the types, quantities, and effects of other compounds that enter aquatic environments as a result of comanagement strategies. Variability in comanagement practices among different CCR producing plants (EPRI, 1997) suggests that in some CCR-contaminated habitats aquatic organisms may be exposed to numerous, potentially harmful organic compounds as well as the mixture of inorganic elements. Comanagement of various waste products is especially common at disposal facilities using aquatic disposal methods. Ninety-one percent of surveyed facilities that use aquatic disposal methods reported comanagement of at least one low-volume waste, and typically more than five low volume wastes are comanaged at such sites (EPRI, 1997). Because of the differences in comanagement practices among disposal sites, each CCR disposal facility may be somewhat unique in its chemical characteristics, presenting unique challenges to aquatic organisms that interact with the effluents within the disposal site or in downstream areas. It is therefore important that comanagement practices in use at the CCR source be identified. Surveys for organic compounds associated with the comanagement practices in use can be used to examine the potential, additional risks to wildlife associated with comanaged wastes.

When characterizing the chemistry of CCR-contaminated sites, it is important that contaminants be quantified in waters, sediments, and tissues. Numerous investigations have focused solely on dissolved contaminants; however, because the metals and trace elements found in CCR are often associated with particles that precipitate from the water column, it is important that sediment chemistry be examined as well. Sediments may act as long term storage sites for CCR-related contaminants, acting as a source of contaminants to organisms and overlying waters for long periods after effluent inputs have ceased. Accumulation of contaminants in sediments can make recovery of aquatic systems following CCR release exceptionally slow. For example, detrital pathways can continue to provide toxic doses of Se to wildlife in CCR-impacted sites even many years after water-borne Se concentrations are below levels of concern (Lemly, 1985a, 1997, 1999). In addition, future studies should regularly include sampling of tissues from biota within CCR-impacted sites, since tissue residues may, in some cases, be better predictors of dose and adverse effects than ambient concentrations alone (Jarvinen and Ankley, 1999). Because of the association of many CCR-related contaminants with sediments, benthic organisms may be particularly informative in tissue sampling regimes.

Locations of aquatic CCR disposal facilities must also be considered when examining potential environmental impacts. Accidental releases of CCR into lentic systems have been shown to have long term effects on individuals and populations entrained within the systems. Such releases have been particularly catastrophic in systems with long water retention times (e.g. Belews Lake, NC; Lemly, 1985b). On the other hand, lotic systems may provide more rapid dilution of CCR effluents and transport from the release site. Lotic systems also may be more quickly recolonized by aquatic organisms, or allow dispersal of some organisms from the most impacted areas. However, location of CCR disposal facilities near lotic systems should not be viewed as a solution to environmental impacts. Very little is known about CCR release and retention within lotic systems. Shallow areas downstream from release sites may become sinks for contaminants in sediments due to reductions in water velocity and settling of suspended materials; these areas would allow continued resuspension of contaminants from the sediments over long periods of time (Lemly, 1998, 1999). Of the trace elements found in CCR, Se may be the contaminant of greatest concern in such shallow, slowly flowing downstream areas because it is readily leached from sediments and is very mobile in the aquatic environment (Lemly, 1985b). Studies conducted in Stingy Run and Little Scary Creek provided mixed results with respect to biological effects, but demonstrated accumulation of several trace elements by fish and invertebrates in creeks downstream of CCR reservoirs (Lohner and Reash, 1999; Reash *et al*., 1999; Lohner *et al*., 2001). Further research in lotic systems such as these would be valuable for evaluating influences of habitat type (e.g. lotic versus lentic) on toxicity of CCR related trace elements.

The potential for groundwater contamination from aquatic basins is an issue that deserves thorough consideration, especially because appropriate monitoring and protection programs continue to be underutilized at CCR disposal sites (EPRI, 1997; EPA, 2000). The EPA's recent report on the regulatory status of comanaged CCR reveals that the percentage of new CCR surface impoundments that use protective controls has increased in recent years (EPA, 2000). However, 62% of the existing surface impoundments do not have groundwater monitoring programs, and 74% of them fail to use protective liners (as of 1995; EPA, 2000). Research focusing on factors that influence leachability of soluble salts and trace elements will be important in clarifying the potential impacts of groundwater contamination on wildlife and human health.

Many studies of biological responses to CCR have focused on specific, sublethal effects on individuals. While such studies are very informative, they are sometimes difficult to interpret with respect to overall relevance to ecological systems (populations, communities). If an understanding of ecological changes in response to CCR disposal is desired, care must be taken in choosing response variables that reflect the operative environment of the individuals (e.g. environmental factors ultimately influencing birth, death, or migration; Congdon *et al.*, 2001; Rowe *et al.*, 2001c). In such a way, observed effects on individuals can be examined within a life history-based perspective, allowing for interpretation within a framework of potential ecological change.

Finally, future studies should evaluate the importance of aquatic CCR disposal sites as habitats that attract wildlife from other habitats. Because operation of such sites usually relies on a high volume water source, they are typically situated near other aquatic habitats. These nearby aquatic sites, as well as surrounding terrestrial habitats, are often inhabited by abundant wildlife that may frequent the contaminated systems. Moreover, areas affected by aquatic disposal of CCR may be utilized by species that rely on them seasonally for critical portions of their life cycle. Examples include amphibians that congregate during seasonal breeding events and waterfowl that may breed or overwinter in CCR-impacted habitats (e.g. USDI, 1988; Hopkins *et al.*, 1998; Lemly, 1997; Rowe *et al.*, 2001a). Because CCR disposal in aquatic systems has been associated with complete reproductive failure in various vertebrate species, consideration should be given to the effects of CCR disposal on population dynamics of seasonally transient species that may experience reduced reproductive success when utilizing such sites. Because these species also eventually leave the contaminated sites, future evaluations should consider their potential as trophic vectors of contaminants not only to other wildlife, but also to humans.

6. Summary

Continued reliance on coal as an energy source, coupled with a growing amount of information on the biological effects of coal combustion residues (CCR), emphasizes a need for greater consideration of the environmental impacts associated with CCR. Coal combustion and associated activities in power generating facilities produce large quantities of wastes. Because the greatest volume of the waste stream produced is in the particulate phase, consisting primarily of ash, disposal of this waste product has proved a significant challenge for industry, and, aside from recycling and use in concrete and other structural materials, has been accomplished primarily in three ways. Use in mine filling has been rarely used, whereas dry land filling and ponding of slurried material have been the predominant methods for disposal. The latter disposal method, currently in use for disposal of roughly one-third of solid CCR produced in the U.S., has received the greatest attention from researchers with respect to potential environmental impacts. This focus by investigators on aquatic (ponding) disposal methods, and thus the basis of this review, reflects the potential for CCR-related contaminants to affect aquatic organisms that interact with the disposal systems and nearby aquatic systems that intentionally or unintentionally receive effluents from the disposal facilities.

Solid CCR has associated with it numerous inorganic elements associated with the parent coal which are highly concentrated as a result of combustion. Many of these elements are of concern due to their toxicological activities, including, but not limited to, As, Cd, Cr, Se, and Zn. Whereas solid CCR (ash) itself does not appear be a large source of available organic compounds, comanagement of multiple industrial wastes by disposal facilities can produce a CCR-based effluent that contains additional organic and inorganic constituents not otherwise associated with coal ash. The use of comanagement practices by a large proportion (> 90%) of facilities employing aquatic CCR disposal methods, and the variability among facilities in the types of comanaged wastes added to the CCR stream, suggests that the composition of CCR entering any specific aquatic system varies considerably among sites (EPRI, 1997).

Because of the abundance of inorganic elements in CCR that are known to have adverse biological effects, most research on CCR-affected aquatic systems has attempted to relate concentrations of inorganic contaminants in water, sediment, and/or food with accumulation and effects on aquatic organisms. Systems receiving CCR have generally been found to be highly elevated in dissolved and sediment-borne concentrations of several, potentially toxic compounds. Water concentrations of As, Cd, Cr, Cu, and Se are frequently elevated above background levels, but are highly variable among sites. One element of particular concern that is found in high concentrations in CCR is Se, an element known to have potent toxicological effects on reproduction and development. In some systems, dissolved Se concentrations in or near CCR aquatic disposal facilities consistently exceed the toxic effects threshold for fish and wildlife (2 ppb) proposed by Lemly (1996), sometimes by more than an order of magnitude. In systems in which Se was identified as the primary agent of toxicity (for example, Belews Lake, NC), severe and long term population level effects on fish have been observed, with the effects sometimes lasting long after CCR release was ceased. Moreover, potential hazards associated with dissolved contaminants are not limited to aquatic wildlife, particularly if groundwater contamination occurs near CCR-impacted sites. Dissolved As concentrations frequently exceed EPA revised drinking water quality criteria (10 ppb) proposed (but recently overturned) for additional protection of human health (USEPA, 2001).

Biological effects observed in animals inhabiting CCR-contaminated aquatic habitats appear to be system-wide, influencing multiple processes in individuals and sometimes bringing about severe ecological changes. Responses to CCR in aquatic habitats include mortality, reproductive failure, developmental abnormalities, and maternal contributions of contaminants to offspring, as well as changes to behavior, endocrinology, and other physiological processes. The most obvious CCR-related effects were the declines in fish populations seen in the Martin Creek, Hyco, and Belews Lake systems. The reductions in fish population sizes and ultimate changes in aquatic community structure likely resulted from direct toxicity to sensitive species and life stages, as well as reproductive impairments resulting from direct actions on reproductive processes and indirect actions via reduced offspring performance. The long period of recovery of resident populations after CCR release ceased (e.g. Martin Creek and Belews Lake) suggests that contaminants can remain in some aquatic systems for long periods of time (particularly in lentic habitats), resulting in continued accumulation by biota at levels high enough to cause residual effects on reproductive health.

While not as immediately obvious as fish population declines, numerous other biological effects of CCR in aquatic systems indicate potential environmental risks. CCR and its components can be acutely or chronically lethal to some aquatic organisms. Sublethal effects on physiology, morphology, and behavior suggest that various biological processes are simultaneously altered in animals chronically exposed to CCR in the aquatic environment, with demonstrated or predicted influences on growth, survival, or reproduction (Rowe *et al.*, 2001c). Maternal transfer of Se to eggs of fish, turtles, alligators, and birds suggests the potential for trans-generational effects, as was seen in fish from Hyco Reservoir. Furthermore, CCR in aquatic systems has been linked to indirect effects on some animals via reductions in resource abundance, diversity, and/or quality to the extent that growth and survival of the consumers are jeopardized. Because terrestrial and semiaquatic organisms utilize some CCR contaminated aquatic habitats for certain activities (breeding, foraging), contaminants and their effects are not necessarily confined to aquatic biota. Rather, transfer of accumulated trace elements from aquatic sites to nearby terrestrial habitats may occur via trophic interactions.

Future research related to aquatic CCR should include exhaustive chemical inventories of the sites of study, to identify the spectrum of elements and compounds to which organisms are be exposed. Complete chemical inventories are particularly important due to the frequency with which multiple industrial wastes are comanaged with solid CCR, resulting in effluents that may be enriched in contaminants not normally associated with coal ash itself. Contaminants derived from CCR may be available to organisms in water, or via sediment or food borne routes. Thus, chemical characterizations should examine all potential sources of uptake by aquatic organisms. When examining potential environmental impacts of aquatic CCR disposal, it is also important that the systems immediately downstream of the disposal site be characterized and examined with respect to chemical, physical, and biological dynamics. The possibility for sediment accumulation and long-term availability of some contaminants in portions of lotic systems as a result of physical processes (Lemly, 1998, 1999) suggests that spatial patterns of contaminant availability should be examined in these systems. Finally, groundwater monitoring programs around aquatic CCR disposal facilities and landfills have not been universally adopted (EPRI, 1997; EPA, 2000), and only about 28% of disposal facilities using aquatic methods employ liners in the basins (from a survey of 259 total facilities; EPRI, 1997). Thus, the potential for leaching of CCR-related contaminants into groundwater requires further examination to determine whether current practices are protective of aquifers.

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Appendix Table I

Scientific names of organismsdiscussed in the text and tables. References are provided when common names or group names were used by different authors to in reference to different organisms. Absence of a reference implies that usage of common and scientific names coincided for all relevant authors

Common or group name	Scientific name	Reference, if applicable
	Plants	
Algae	*Oscillatoria* and *Hydrodictyon* spp.	Guthrie and Cherry, 1979
Algae	*Zygnema* sp.	Gutenmann *et al*., 1976
Arrowhead	*Sagittaria latifolia*	
Sago pondweed	*Potamogeton pectinatus*	
Cattail	*Typha latifolia*	
Black willow	*Salix nigra*	
Sugar maple	*Acer saccharinum*	
	Invertebrates	
Earthworm	*Limbrucus terrestris*	
Asiatic clams	*Corbicula fluminea*	
Pond snail	*Physa integra*	
Gastropod	*Physa sp.*	
Benthic invertebrates	*Gammarus pseudolimnaeus*, *Hyalella azteca, Baetis* spp., *Stenacron interpunctatum*, *Stenonema exiguum, Cheumatopsyche* spp., *Hydropsyche* spp., Chironomidae, Simuliidae	Forbes *et al*., 1981; Magnuson *et al*., 1981

Appendix Table I
Continued.

Common or group name	Scientific name	Reference, if applicable
Amphipod	*Gammarus pseudolimnaeus*	
Isopod	*Asellus racovitzai*	
Crayfish	*Procambarus acutus*	Nagle *et al*., 2001; Rowe
Crayfish	*Orconectes propinquus*	*et al*., 2001b
Crayfish	*Cambarus* sp.	Magnuson *et al*., 1981;
		Forbes *et al*., 1981 Guthrie and Cherry, 1976
Crayfish	*Procambarus sp.*	Cherry *et al*., 1979a and b;
		Guthrie and Cherry, 1979
Shrimp	*Palaemonetes* sp.	
Grass shrimp	*Palaemonetes paludosus*	
Caddisflies	*Hydropsyche* and *Cheumatopsyche* spp.	
Odonates	*Libellula* and *Enallagma* spp.	Guthrie and Cherry, 1979
Odonates	*Plathemis lydia* and *Libellula sp.*	Cherry *et al*., 1979a
Odonates	*Libellula* and *Enallagma* spp.	Cherry *et al*., 1979b
Dragonfly	*Plathemis lydia*	
Mayfly	*Hexagenia limbata*	Finley, 1985
Mayfly	*Hexagenia* sp.	Olmsted *et al*., 1986
Cricket	*Grillus* sp.	
Grasshopper	*Melanoplus* sp.	
	Fish	
Spotted gar	*Lepisosteus oculatus*	
Blueback herring	*Alosa aestivalis*	
Gizzard shad	*Dorosoma cepedianum*	
Threadfin shad	*Dorosoma petenense*	
Goldfish	*Carassius auratus*	
Common carp	*Cyprinus carpio*	
Spottail shiner	*Notropis hudsonius*	
Golden shiner	*Notemigonus crysoleucas*	
Fathead minnow	*Pimephales promelas*	Lemly, 1993
Fathead minnow	*Pimephales notatus*	Benson and Birge, 1985
Bullhead minnow	*Pimephales vigilax*	
Red shiner	*Cyprinella lutrensis*	
Satinfin shiner	*Cyprinella analostana*	
White sucker	*Catostomus commersoni*	
Lake chubsucker	*Erimizon sucetta*	
Catfish	*Ictalurus* sp.	
Brown bullhead	*Ameiurus nebulosus*	
Black bullhead	*Ameiurus melas*	
Flat bullhead	*Ameirus platycephalus*	
Snail bullhead	*Ameirus brunneus*	
White catfish	*Ictalurus catus*	
Channel catfish	*Ictalurus punctatus*	
Rainbow trout	*Oncorhynchus mykiss*	

Appendix Table I
Continued.

Common or group name	Scientific name	Reference, if applicable
Sheepshead minnow	*Cyprinodon variegatus*	
Mosquitofish	*Gambusia* sp.	Lemly, 1993
Mosquitofish	*Gambusia affinis*	Cherry *et al*., 1976, 1979a; Guthrie and Cherry, 1976, 1979; Hopkins *et al*., 1999a
Banded sculpin	*Cottus carolinae*	
White bass	*Morone chrysops*	
White perch	*Morone americana*	
Striped bass	*Morone saxatilis*	
Sunfish	*Lepomis* sp.	Cumbie, 1978; USDI, 1988
Bluegill	*Lepomis macrochirus*	
Green sunfish	*Lepomis cyanellus*	
Red ear sunfish	*Lepomis microlophus*	
Pumpkinseed sunfish	*Lepomis gibbosus*	
Long ear sunfish	*Lepomis megalotis*	
Redbreast sunfish	*Lepomis auritus*	
Warmouth	*Lepomis gulosus*	
Black crappie	*Pomoxis nigromaculatus*	
White crappie	*Pomoxis annularis*	
Largemouth bass	*Micropterus salmoides*	
Darter	*Ethiostoma* sp.	
Yellow perch	*Perca flavescens*	
	Amphibians	
Bullfrog	*Rana catesbeiana*	
Green treefrog	*Hyla cinerea*	
Green frog	*Rana clamitans*	
Leopard frog	*Rana pipiens*	
Frog larvae	*Rana* sp.	
Southern toad	*Bufo terrestris*	
Fowler's toad	*Bufo fowleri*	
Narrow-mouth toad	*Gastrophryne carolinensis*	
Red spotted newt	*Notophthalmus viridescens*	
Salamanders	*Euraycea* sp.	
	Reptiles	
Softshell turtle	*Apalone spinifera*	
Slider turtle	*Trachemys scripta*	
American alligator	*Alligator mississippiensis*	
Banded water snake	*Nerodia fasciata*	
	Birds	
Common grackle	*Quiscalus quiscula*	
Barn swallow	*Hirundo rustica*	
Red wing blackbird	*Agelaius phoeniceus*	
American coot	*Fulica americana*	
	Mammals	
Muskrat	*Ondatra zibethicus*	

Appendix Table II

Selenium accumulation by aquatic organisms in Hyco Reservoir, NC. Values are ppm wet mass. Decimal places reflect those presented by the original authors

Group or Species	Tissue	[Se]	Reference
Plankton	whole body	2.9–5.1	CPL, 1981
Gizzard Shad	muscle	2.0–21.2	CPL, 1979
Gizzard Shad	ovary	3.1	CPL, 1979
Largemouth bass	muscle	0.1–5.2	CPL, 1979
Largemouth bass	ovary	7.3	CPL, 1979
Black crappie	muscle	0.1–10.5	CPL, 1979
Bluegill	muscle	0.2–12.2	CPL, 1979
Channel catfish	muscle	0.1–9.4	CPL, 1979
White catfish	muscle	1.4–2.7	CPL, 1979
Green sunfish	muscle	4.1–15.3	CPL, 1979
Flat bullhead	muscle	0.9–1.9	CPL, 1979
Snail bullhead	muscle	2.9	CPL, 1979
Bluegill	liver	34.0	Sager and Cofield, 1984
Bluegill	muscle	13.0	Sager and Cofield, 1984
Bluegill	ovary	12.1	Sager and Cofield, 1984
Bluegill	testes	5.4	Sager and Cofield, 1984
Largemouth bass	liver	10.2	Sager and Cofield, 1984
Largemouth bass	muscle	6.7	Sager and Cofield, 1984
Largemouth bass	ovary	10.3	Sager and Cofield, 1984
Channel catfish	liver	11.9	Sager and Cofield, 1984
Channel catfish	muscle	8.3	Sager and Cofield, 1984
Channel catfish	ovary	9.9	Sager and Cofield, 1984
Channel catfish	testes	4.4	Sager and Cofield, 1984
White catfish	liver	10.8	Sager and Cofield, 1984
White catfish	muscle	5.4	Sager and Cofield, 1984
White catfish	ovary	8.9	Sager and Cofield, 1984
Largemouth bass[a]	ovary	7.2	Baumann and Gillespie, 1986
Largemouth bass[a]	ovary-free carcass	4.0	Baumann and Gillespie, 1986
Largemouth bass[a]	testes	3.3	Baumann and Gillespie, 1986
Largemouth bass[a]	testes-free carcass	4.1	Baumann and Gillespie, 1986
Bluegill[a]	ovary	11.8	Baumann and Gillespie, 1986
Bluegill[a]	ovary-free carcass	6.9	Baumann and Gillespie, 1986
Bluegill[a]	testes	6.6	Baumann and Gillespie, 1986
Bluegill[a]	testes-free carcass	7.7	Baumann and Gillespie, 1986
Bluegill	testes	4.37	Gillespie and Baumann, 1986
Bluegill	testes-free carcass	7.81	Gillespie and Baumann, 1986
Bluegill	ovary	6.96	Gillespie and Baumann, 1986
Bluegill	ovary-free carcass	5.91	Gillespie and Baumann, 1986

Appendix Table II

Continued.

Group or Species	Tissue	[Se]	Reference
Gizzard shad	muscle	8.7–14.7	CPL, 1981
Gizzard shad	gonad	7.6	CPL, 1981
White crappie	muscle	9.8	CPL, 1981
White crappie	gonad	7.6	CPL, 1981
Black crappie	muscle	12.0	CPL, 1981
Green sunfish	muscle	11.0	CPL, 1981
Channel catfish	muscle	6.8–8.8	CPL, 1981
Channel catfish	liver	9.2	CPL, 1981
Bluegill	muscle	6.7–9.3	CPL, 1981
Brown bullhead	muscle	1.3	CPL, 1981

[a] Values estimated from bar graph in Baumann and Gillespie (1986).

Appendix Table III

Selenium accumulation associated with histopathological effects on fish Martin Creek Reservoir, TX following cessation of CCR inputs. Coal ash effluents were released into the reservoir from Sept., 1978 to May, 1979. All concentrations are in ppm wet mass. NR = not reported. Decimal places reflect those presented by the original authors

Species	Organ	[Se] 1979	[Se] 1980	[Se] 1981	[Se] 1982	[Se] 1986	Effect(s)	Reference
Green sunfish	kidney	11.3	NR	NR	NR	NR	Renal histopathological changes	Sorensen *et al.*, 1982a, 1983a
Green sunfish	heptato-pancreas pancreas	NR	NR	6.05–9.30	NR	NR	Histopathological changes (gill, cardiac, renal, hepatic, ovarian)	Sorensen *et al.*, 1982b
Green sunfish	liver	10.8	NR	NR	NR	NR	Hepatic histopathological changes	Sorensen *et al.*, 1983a
Redear sunfish[a]	liver	NR	20	NR	NR	NR	Decreased condition factor, hepatic, renal, and ovarian histopathological changes	Sorensen *et al.*, 1983b
Redear sunfish	heptato-pancreas pancreas	NR	NR	8.38–11.03	NR	NR	Histopathological changes (renal, hepatic, ovarian)	Sorensen *et al.*, 1982b
Redear sunfish	liver	NR	NR	NR	NR	7.63	Decreased condition factor, hepatic, renal, and ovarian histopathological changes	Sorensen, 1988
Redear sunfish	ovary	NR	NR	NR	NR	4.33	Decreased condition factor, hepatic, renal, and ovarian histopathological changes	Sorensen, 1988

[a] Tissue Se concentration reported as ‘approximate’ value.

Appendix Table IV

Sublethal effects and trace element accumulation in animals captured in the D-Area CCR aquatic disposal facility, Savannah River Site, SC, or experimentally exposed to conditions representative of the site. Data are means of trace element burdens in specific tissues (if known). Concentrations are in ppm dry mass. Ranges presented are the ranges in means for multiple species categorized together. 'NR' = not reported. Decimal places reflect those presented by the original authors

Species, tissues analyzed for contaminants; protocol	As	Cd	Cr	Cu	Pb	Se	Observed effect	Reference
Invertebrates								
Crayfish, whole body; field collected	3.99	4.88	1.37	223.72	NR	14.70	Elevated maintenance costs	Rowe *et al.*, 2001b
Crayfish; exposed to sediments and fed fish collected from D-Area for 50 d	NR	NR	NR	NR	NR	NR	Elevated maintenance costs; reduced growth	Rowe *et al.*, 2001b
Grass shrimp, whole body; caged *in situ* in D-Area for 8 mo	3.154	5.185	3.070	168.501	0.398	11.781	Elevated maintenance costs	Rowe, 1998
Fish								
Lake chubsuckers, juvenile, whole body; laboratory-exposed for 124 d to sediment from D-Area	1.17	0.06	1.46	3.91	NR	5.65	Reduced growth and lipid content, fin erosion	Hopkins *et al.*, 2000b
Lake chubsuckers, juvenile, whole body; laboratory-exposed for 78 d to sediment from D-Area[a]	0.44–0.51	0.07	1.11–1.17	2.08–2.91	NR	2.29–6.55	Reduced growth, fin erosion; effects exacerbated as resource level decreased	Hopkins *et al.*, 2002b
Lake chubsuckers, juvenile, whole body; laboratory-exposed for 100 d to sediment from D-Area	1.45	0.07	2.55	5.37	NR	3.80	Reduced swimming performance	Hopkins *et al.*, 2003
Lake chubsuckers, juvenile, whole body; mesocosm-exposed for 45 d to sediment, water, and food from D-Area	2.18	1.30	1.60	27.15	NR	70.34	Reduced growth and condition factor	Hopkins, 2001

Appendix Table IV
Continued.

Species, tissues analyzed for contaminants; protocol	As	Cd	Cr	Cu	Pb	Se	Observed effect	Reference
Sheepshead minnows, whole body; laboratory-raised for full life cycle ($\sim$ 1 yr) on sediments from D-Area	3.51	0.084	NR	57.70	NR	6.07	Reduced growth, condition factor, lipid content, and egg size	Rowe, 2003
				Amphibians				
Bullfrogs, larvae, whole body	48.9	1.71	17.2	31.4	11.4	25.7	Oral abnormalities	Rowe *et al.*, 1996
Bullfrogs, larvae; caged *in situ* from embryonic stage through 80 d post-hatching	NR	NR	NR	NR	NR	NR	Oral abnormalities in response to environmental conditions, independent of parental population	Rowe *et al.*, 1998a
Bullfrogs, larvae, whole body; caged *in situ* from embryonic stage through 80 d post-hatching	25.95	4.32	27.25	55.12	10.94	25.27	Increased metabolic costs in response to environmental conditions, independent of parental population	Rowe *et al.*, 1998b
Bullfrogs, larvae, whole body; field collected	NR	NR	NR	NR	NR	NR	Increased metabolic costs	Rowe *et al.*, 1998b
Bullfrogs, larvae, whole body; field-collected prior to use in laboratory swimming trials	15.09–33.10	1.59–5.47	3.49–18.25	29.07–116.72	NR	20.25–27.93	Spinal flexures, reduced swimming speed	Hopkins *et al.*, 2000a
Bullfrogs, larvae; field-collected prior to use in laboratory swimming trials	NR	NR	NR	NR	NR	NR	Reduced swimming speed and responsiveness to prodding	Raimondo *et al.*, 1998
Bullfrogs, larvae; raised in contaminated site until 60 d old prior to exposure to predators in mesocosms	NR	NR	NR	NR	NR	NR	Increased susceptibility to predation	Raimondo *et al.*, 1998

Appendix Table IV
Continued.

Species, tissues analyzed for contaminants; protocol	As	Cd	Cr	Cu	Pb	Se	Observed effect	Reference
Bullfrogs, newly metamorphosed juveniles; field collected	NR	NR	NR	NR	NR	NR	5 to 48% lower storage lipid content than reference animals	Rowe and Hopkins, unpublished
Southern toads, adults, whole body; caged at margin of D-Area basin for 12 wk (trace elements measured in free-ranging animals in D-Area)	1.58	0.27	1.87	29.50	0.70	17.40	Abnormal hormone levels, altered stress response	Hopkins *et al.*, 1997, 1998, 1999b
Reptiles								
Banded water snakes, adults, liver; field collected	134.3	0.5	2.0	82.7	NR	141.9	Increased metabolic costs	Hopkins *et al.*, 1999a
Slider turtles, hatchlings, whole body; hatchlings derived from females captured in the site and following incubation of eggs in artificial nests.	0.46–0.49	0.03	0.98–1.05	5.14–5.58	NR	4.45–7.36	Maternal transfer of contaminants to eggs, depressed metabolic rates	Nagle *et al.*, 2001
American alligators, hatchlings derived from field-collected eggs, whole body	0.2	NR	0.32	NR	NR	6.3	Maternal transfer of contaminants	Hopkins, Rowe, Congdon, unpublished
Birds								
Common grackle, eggs; field collected	0.1	0.01	2.4	0.4	NR	4.6	Maternal transfer of contaminants	Bryan *et al.* unpublished

[a] Trace elements reported are the range of values measured in fish fed three food levels.

Appendix Table V

Average Se concentrations (ppm dry mass, whole-body), occurence of abnormalities (spinal malformations, accumulation of body fluids, and abnormalities of fins, eyes, or craniofacial region), and population-level changes in fish in Belews Lake, NC following input of coal ash settling basin effluent from 1974 to 1985. Selenium concentrations provided are means for normal and malformed fish, respectively (e.g. entries appear as: concentration in normal fish, concentration in malformed fish). Data are from Lemly (1993). Dates refer to the following timeline at the Belews Lake site: 1975 – 1 yr after CCR inputs began; 1978 – 4 yr after CCR input began; 1992 – 7 yr after inputs to the lake had ceased. Decimal places reflect those presented by Lemly (1993)

Species	1975 Selenium concentration (Percent of population exhibiting abnormalities)	1978 Selenium concentration (Percent of population exhibiting abnormalities)	1992 Selenium concentration (Percent of population exhibiting abnormalities)
Common carp	62.11, 63.32 (3)	107.92, 112.29 (12)	15.59, 16.20 (7)
Golden shiner	46.54, 48.37 (21)	Extirpated	No recolonization
Black bullhead	57.29, 56.07 (6)	94.18, 103.05 (21)	13.12, 15.76 (8)
Channel catfish	60.91–66.10 (17)	Extirpated	No recolonization
White perch	55.01, 54.63 (33)	Extirpated	No recolonization
Yellow perch	41.87, 44.72 (3)	Extirpated	No recolonization
Mosquitofish	50.61, 52.17 (21)	125.61, 131.87 (27)	18.90, 16.48 (4)
Fathead minnow	Not observed	86.97, 80.13 (34)	21.07, 19.62 (10)
White sucker	42.61–43.70 (23)	Extirpated	No recolonization
Redbreast sunfish	58.36, 56.12 (32)	Extirpated	No recolonization
Green sunfish	66.89, 65.19 (55)	Extirpated	12.40, 14.68 (11)
Pumpkinseed sunfish	46.74, 48.34 (30)	Extirpated	No recolonization
Warmouth	51.22, 54.61 (22)	Extirpated	No recolonization
Bluegill sunfish	53.83, 50.97 (22)	Extirpated	18.40, 19.06 (6)
Redear sunfish	43.13, 41.28 (10)	Extirpated	No recolonization
Largemouth bass	58.4, 59.2 (19)	Extirpated	23.19, 19.72 (5)
White crappie	62.37, 60.21 (32)	Extirpated	No recolonization

Appendix Table V
Continued.

Species	1975 Selenium concentration (Percent of population exhibiting abnormalities)	1978 Selenium concentration (Percent of population exhibiting abnormalities)	1992 Selenium concentration (Percent of population exhibiting abnormalities)
Black crappie	60.83, 61.49 (29)	Extirpated	No recolonization
Blueback herring	54.70, 56.33 (12)	Extirpated	No recolonization
Threadfin shad	39.84, 44.96 (22)	Extirpated	No recolonization
Red shiner	Not observed	Not observed	15.37, 13.28 (6)
Satinfin shiner	Not observed	Not observed	12.39, 11.17 (5)

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