Hydrobiologia (2005) 542:77–93
H. Segers & K. Martens (eds), Aquatic Biodiversity II
DOI 10.1007/s10750-004-3951-y

Recovery in diversity of fish and invertebrate communities following remediation of a polluted stream: investigating causal relationships

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Key words: recovery, restoration, fish and macroinvertebrate communities, causal relationships

Abstract

Spatial and temporal responses of biota to anthropogenic disturbance were measured over a 15 year period in a contaminated stream undergoing remediation and recovery. Along the spatial gradient of the stream, levels of contaminants decreased downstream along with improved responses of instream biota at several levels of biological organization. Recovery of the biota in this stream over the 15 year study period is demonstrated by the temporal relationships between levels of decreasing contaminants and the concomitant responses of the periphyton, macroinvertebrate, and fish communities and changes in the various bioindicators of individual fish health. Decreases in contaminants over a temporal scale were followed closely by an improvement in physiological and organismal-level indicators, increases in the diversity of macroinvertebrate and fish communities, and rapid increases in the chlorophyll *a* biomass and photosynthesis rate of the periphyton community. These results emphasize that field studies designed to assess and evaluate the effectiveness of restoration activities on stream recovery should incorporate a variety of response endpoints ranging from sensitive and short-term responses to long-term but ecological relevant indicators of change. The close spatial and temporal relationships observed between changes in physicochemical factors and positive responses in various components of the stream biota over the 15-year study period suggest a strong cause and effect relationship between remediation activities and stream recovery. Understanding causal relationships and the mechanistic processes between environmental stressors, stress responses of biota, and the recovery process is important in the effective management and restoration of aquatic ecosystems.

Introduction

Many aquatic ecosystems are subjected to some level of environmental stress either from natural causes, anthropogenic sources, or both. The aim of restoration ecology and associated recovery studies is to remediate impacted ecosystems to some level of integrity or health that is acceptable to society and to environmental managers. There has been much debate, however, relative to the criteria that defines acceptable states of recovery or restoration in aquatic ecosystems. Recovery has generally been considered as a return to a predisturbance steady-state, termed the nominal state, as a result of the operation of homeostatic control mechanisms operating in the ecosystem. Recovery can also be defined as the rate and manner in which an ecosystem subsequently returns to its unstressed condition or follows a chronological sequence of change that coincides with an unstressed reference condition (Kelly & Harwell, 1990). Studies of recovery in aquatic systems are important for establishing exceedance criteria for water quality standards (Plafkin, 1988), for testing current ecology theory (Resh et al., 1988, Yount & Niemi, 1990), and for evaluating the effectiveness of restoration activities and remedial actions (Detenbeck et al., 1992; Adams et al., 2002).

There are currently no widely accepted approaches or criteria that are used to evaluate when a disturbed aquatic system has 'recovered' to an acceptable state. Conventional approaches for monitoring the status and recovery of aquatic systems include measures of biomass, abundance, species diversity and richness, or biotic indices based on combinations of these parameters (Ford, 1989). The more conventional viewpoint is that structural attributes of the community should be the goals of restoration success while recently a vocal minority has advocated functional endpoints such as ecosystem processes which could serve as indicators of restored ecosystems (Muotka & Laasonen, 2002; Nienhuis et al., 2002). In spite of a well developed theory for recovery and an abundance of studies on the effects and subsequent recovery of aquatic systems, important issues remain to be addressed relative to the dynamics of the recovery process within the framework of restoration ecology (Power, 1999). For example, what are the most important ecosystem parameters for determining ecosystem recovery from stress? Does information on recovery at one level of biological organization reflect or provide reliable predictions of recovery at other levels of organization?

The effects and consequences of stress on aquatic ecosystems may be manifested at many levels of biological organization. Also, the rate and extent of recovery may differ at different levels of biological organization. Subtle changes in community composition, the genotypic make-up of populations, and the extent to which physiological, cellular, and biochemical systems are impaired in organisms following stress may have a profound effect on how a recovered ecosystem responds to subsequent environmental stressors. Ecosystems that are thought to have recovered may, in fact, be more vulnerable than they were originally due to losses in both intraspecific and interspecific biodiversity, such that the ability to adapt to new environments is compromised (Depledge, 1999).

According to Maltby (1999) there is not a 'right' level to study stress and recovery, but different levels of biological organization provide different types of information that, in combination, provide insight into the effects of stress, their mechanistic bases, and their ecological significance. Population and community studies can provide a description of the effects of stress but do not, in themselves, provide information on how effects may be caused (Maltby, 1999). For example, it is relatively straightforward to document that some change has occurred at the population or community level, but identifying the mechanisms or causes of such changes are difficult. Community-level measures, such as biotic and diversity indices, provide useful descriptions of community structure but they are generally insensitive to sublethal levels of stress (Gray et al., 1990; Dawson-Shepherd et al., 1992). In contrast, individual and sub-organismal measures of stress can be used to detect sublethal effects because of their short response times, sensitivity and specificity to stressors, and their potential to provide information on causal agents (Depledge, 1999; Adams et al., 2002). Knowledge of organism-level responses to stress and recovery is essential for understanding how stressors cause adverse effects at higher levels of biological organization, and it follows, therefore, that the effects of stressors on communities could be predicted from knowledge of the effects of stressors at the individual and population level (Maltby, 1999). In this regard, when assessing the effects of stressors on aquatic systems, the effects on the individual should also be understood in order to help explain the ecological relevance of the results (Heinonen et al., 1999).

There are, however, advantages and limitations of using either individual organism metrics or community-level metrics alone for assessing the effects of stressors and the subsequent recovery process of aquatic systems. For example, Detenbeck et al. (1992) reported that autecological factors alone were inadequate for explaining recovery and that recovery of individual species densities was much slower than recovery based on community parameters such as species richness or total density. Community-based metrics are useful for assessing the degree of recovery but these measures provide little insight into the causal mechanisms or underlying processes responsible for recovery (Adams et al., 2002). One of the main concerns in using population and community level responses alone for assessing stress and recovery in aquatic systems is that, by the time an effect is observed using these endpoints, it may be too late to initiate effective environmental management or mitigation. Thus, in addition to these ecologically relevant endpoints, responses at lower levels of biological organization should also be measured to serve as sensitive and early-warning indicators of impending changes in ecosystem health (Adams et al., 2002).

When assessing the effects of environmental stressors on aquatic systems and the recovery of these systems once restoration activities have been initiated, studies should include a variety of response endpoints representing several levels of biological organization. Different endpoints typically represent a variety of specificities, sensitivities, variability, and ecological relevance to environmental stressors (Adams et al., 2002). One of the main reasons that environmental assessment and recovery studies should involve the use of a variety of responses at different levels of biological organization is that disturbance can be unequivocally identified and associated with various specific ecological levels of organization. Disturbance may affect each major level of organization, from the individual to ecosystem and landscape, and the consequences and mechanisms of disturbance are different at each hierarchal level (Rykiel, 1985). Analyses of disturbance at each level of organization are vital to understand the importance of disturbance and the dynamics of the recovery process (Pickett et al., 1989). Given this background, therefore, the principle objectives of this study are (1) assess changes in community diversity following remediation of a contaminated stream, (2) investigate potential causal relationships between changes in community diversity and other abiotic and biotic components of a stream ecosystem, and (3) provide some basic guidance for the design of field studies related to restoration and recovery of stream ecosystems.

Methods and approach

Study sites

Both spatial and temporal responses of a stream ecosystem to environmental contamination were examined in a disturbed stream undergoing remediation and restoration over a 15 year period. Levels of contaminants in water and biota along with various measures of periphyton, benthic macroinvertebrate, individual fish, and fish community health were measured in East Fork Poplar Creek (EFPC), a third order stream in East Tennessee whose headwaters historically have received point-source discharges of various contaminants from a nuclear weapons production facility. This system is typical of most ridge and valley streams in East Tennessee which are characterized by alternating pool and riffle areas, riparian vegetation along both banks, silt and sand substrates, and annual mean flows generally ranging between 5 and 15 cfs. The headwaters of EFPC originate a few hundred meters upstream of this facility and then flows through the city of Oak Ridge and the Oak Ridge Department of Energy reservation before confluence with Poplar Creek 24 km downstream (Fig. 1). The reference stream, Brushy Fork Creek, which is located in a nearby watershed (Fig. 1), was selected for its hydrological and physicochemical similarity to EFPC except this stream has no measurable levels of contaminants or other anthropogenic disturbances. From the mid-1940s until the late 1980s, EFPC received discharges of relatively high levels of various contaminants including heavy metals, chlorinated organics, PAHs, and residual chlorine. During this 50 year period the stream was characterized by a distinct longitudinal gradient in contaminant loading as evidenced by decreasing downstream concentrations of Polychlorineted biphenyl (PCBs) and mercury in fish, sediment, and water (Southworth, 1990; Peterson et al., 1994; LMES, 1997). In addition to point-source contaminant discharges, this stream has also been subjected to multiple disturbances such as sediment inputs, altered hydrodynamic flows, thermal loading, and habitat modification. For the past 15 years, however, EFPC has experienced extensive remediation primarily through water treatment processes and abatement of contaminant sources at the site facility along with the physical removal of contaminated sediment from various areas within the floodplain.

To monitor and assess spatial patterns and temporal changes in stream recovery during and following remediation activity, sampling for physicochemical parameters and instream biota including periphyton, benthos, and fish was

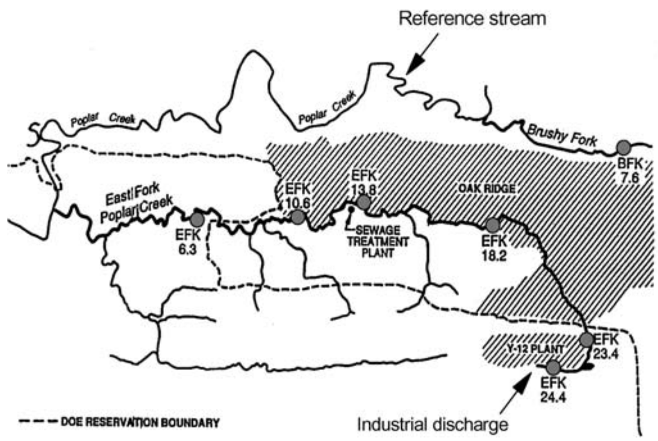

Figure 1. Location of sample sites at the contaminated stream, EFPC, and at the reference stream, Brushy Fork. Sample site numbers represent the distance in kilometers from the stream mouth with EFK 24 located closest to the industrial discharge.

conducted annually from 1988 through 2002 at three sites in EFPC and at the reference stream. Assessment of periphyton, benthic macroinvertebrate communities, and fish communities along with individual bioindicators of fish health were conducted in the spring from April to June of each year. Sample sites in the contaminated stream were located at East Fork Kilometer (EFK) 24 and 23 in the upper reaches of the stream near the sources of industrial discharge and at EFK 14, located 9 km below the industrial discharge (Fig. 1). The reference site, Brushy Fork Creek (BFC), is located in an adjacent watershed and it was sampled each year for the same chemical and biological parameters as were measured for the contaminated stream.

Contaminant analysis

Contaminants were measured in EFPC and in the reference stream for mercury in water and PCBs and mercury in fish tissue. In addition, heavy metals were analyzed annually in periphyton samples collected from the two upper EFPC sites and the reference site. For metals in periphyton, two to three composite samples were collected from each site with each sample consisting of periphyton harvested from 5 to 10 large cobbles. Samples were dried to constant mass at 60 °C before being extracted with hydrochloric and nitric acid and analyzed by ICP or ICP/MS.

To evaluate spatial and temporal trends in mercury and PCB contamination in fish, redbreast sunfish, *Lepomis auritus*, were collected twice annually from three sites in EFPC and the reference area from 1988 to 2002. Samples were collected and processed according to project-specific standardized technical procedures developed by the Biological Monitoring and Abatement Program (BMAP) at this laboratory to ensure sample quality and integrity (Peterson & Phipps, 1995). In general, 6–8 adult (50–150 g) sunfish were collected by electrofishing from each site at each sample period and placed on ice for transport to the processing laboratory. Total mercury analysis on filets was conducted by cold vapor atomic adsorption spectrometry (AA). PCBs were quantified against standard commercial mixtures (Aroclor 1254 and 1260) by capillary column gas chromatography using electron capture detection (EPA, 1980; EPA, 1984).

Periphyton analysis

Biomass of periphyton (chlorophyll *a*) and photosynthesis were measured seasonally (four times a year) at three sites in EFPC and at one reference site in Brushy Fork Creek. Sampling occurred at EFK 24 and 23 and the reference site from 1988 to 2002 and at EFK 14 during 1988–1995. Four rocks per site were collected at each sampling time and taken to the laboratory where photosynthesis was measured by ^{14}C uptake in glass chambers with recirculating flow (Hill & Boston, 1991). Uptake occurred at photosaturating irradiance (300–400 μmol quanta m^{-2} s^{-1}) and at ambient stream temperature. Rocks were removed from the chambers, rinsed in stream water, and placed overnight in individual jars containing DMSO which extracted both chlorophyll *a* and ^{14}C-labeled photosynthate (Palumbo et al., 1987).

Benthic macroinvertebrates

Five randomly selected benthic samples were collected during October each year from designated riffles at each of the three sites in EFPC and from the reference stream with a Hess stream bottom sampler (0.1 m^2) fitted with a 363-μm-mesh collection net. In the field, samples were placed in polyurethane coated glass jars, preserved with 95% ethanol, and returned to the laboratory for processing. In the laboratory, samples were placed in a US Standard No. 60-mesh (250-μm-mesh) sieve and washed with tap water. Organisms were sorted from the sample debris in a white tray at a magnification of 2×, identified to the lowest practical taxon (usually genus), and enumerated. Annelids were identified to class and chironomids were identified to subfamily or tribe. Additional details of the field and laboratory procedures are provided in Smith & Smith (1995).

Fish communities

For assessment of community-related metrics, quantitative sampling of fish was conducted by electrofishing in April through May each year at each of the EFPC sites and at the reference site. Information obtained from this quantitative sampling was used to estimated population size, calculate fish biomass per unit area, determine species richness, and evaluate community composition characteristics, such as the abundance of sensitive species (Karr et al., 1986), All sampling reaches were approximately 100 m in length and were isolated at the top and bottom of each reach by block-nets to prevent fish movement in or out of the area during sampling. A sampling event consisted of 5–8 investigators using two to three backpack electrofishers making three upstream passes, and collecting all stunned fish. Data obtained from this progressive removal method was use to calculate fish community structure at each stream site (Zippin, 1956). Following collection, fish were anesthetized, identified, measured (total length), and weighed on site. After processing, fish were allowed to fully recover and returned to the stream. Species population estimates were calculated using the method of Carle & Strub (1978). Biomass was estimated by multiplying the population estimate by the mean weight per individual per size class. To calculate biomass per unit area, total numbers and biomass were divided by the surface area (m^2) of each study reach. For each sampling date, surface area was estimated by multiplying the length of the reach by the mean width based on measurements taken at 5-m intervals.

Individual fish health

A suite of bioindicators reflecting individual fish health were measured for redbreast sunfish (*Lepomis auritus*), the dominant higher trophic level fish species collected at the EFPC sites and the reference stream during the spring of each year. At each site, 14–17 adult sunfish of each sex were collected by electrofishing techniques for assessing biochemical, physiological, and overall condition. Immediately after collection, a blood sample was taken from each fish by puncture of the caudal vessels, centrifuged immediately to obtain serum, and frozen in liquid nitrogen for subsequent analysis of biochemical and physiological parameters. Total length (mm) and mass (g) were recorded for each fish, and the liver and spleen removed for additional analysis. The liver (LSI) and spleno-somatic (SS1) indices were calculated as the mass of these respective organs divided by total body mass. Condition factor was calculated as $K = 100(W/L^3)$, where W = body mass (g) and L = total length (cm). Blood samples from each fish were analyzed for indicators of organ dysfunction and nutritional status. Creatinine (Rock et al., 1986) was used as an indicator of kidney damage and serum triglycerides (Bucolo & David 1973) as a short-term indicator of feeding or nutritional status. Growth and age structure of the redbreast sunfish population at each site were determined by analyzing scales taken from fish of all sizes. Scales were measured and annulus marks identified following the procedures of Jearld

(1983). Data from the scale analysis were used to assign fish to age groups. Growth for each age-class was estimated by back-calculation of size at age (Bagenal & Tesch, 1978).

Statistical procedures

Temporal patterns in chemical and biological parameters were tested with a least squares regression analysis (SAS, 1996) to determine significance of long-term changes in the data. Our assumption was that long-term trends in the various biological metrics at the reference site would show neither a persistent increase or decrease while at the impacted sites there would be a significant increase or decrease in metric values through time as demonstrated by significant ($p \leq 0.05$) deviations of the regression slope from zero. Data were first checked for heteroscadasticity using Levene's test (Johnson & Wichern, 1992). In some cases data were log transformed to meet the heterosadasticity assumption. Each regression analysis utilized all the individual observation points within a temporal data set which represented a sample size range of from 20 to 25 in the case of some chemical measurements up to 250–300 observations in a data set for some of the biological parameters. Fish community data were compiled and analyzed by a comprehensive Fortran 77 program developed by Railsback et al. (1989). The benthic macroinvertebrate data were transformed using a square root transformation (Elliott, 1977), and temporal changes in various aspects of the benthic invertebrate data such as EPT richness were analyzed with regression analysis.

Results

Spatial patterns

Distinct spatial gradients in contaminants and various biological components were observed in EFPC. Concentrations of PCBs in sediment and fish, levels of mercury in water, and heavy metal loading in periphyton displayed a distinct downstream gradient from the upper to lower reaches of EFPC (Fig. 2). The downstream pattern in various components of the stream biota also tracked the spatial gradient in contaminants within the stream (Fig. 3). Biomass of periphyton was highest upstream and species richness of the benthic macroinvertebrate and fish communities where lowest upstream but increased downstream (Fig. 3).

Temporal patterns

In addition to distinct spatial patterns of contaminants and biota in EFPC, there were also clear temporal patterns in contaminant levels and in the temporal response of the periphyton, macroinvertebrate, and fish communities over the 15 year study period.

Contaminants

Mercury concentrations in sunfish gradually declined from an annual mean peak of about 1.2 mg/1 in 1993–1994 at the upper sections of EFPC to annual mean values approaching 0.5 mg/l in the late 1990s (Fig. 4a). Mercury levels were not only higher for fish at the upper sites of EFPC compared to downstream, they also demonstrated a more dramatic decline in the upper sites over the study period. Among all three EFPC sites, there were no evident temporal patterns in PCB levels in fish even though there were occasional high spikes in body burden levels particularly in 1994 and 1996 at the upper sites. There was also a tendency for PCB levels in fish to be lower in the downstream reaches compared to upstream areas which were closer to the contaminant source (Peterson et al., 1994). Levels of PCBs in reference fish were typically below 0.1 mg/kg and exhibited no temporal variation (Peterson et al., 1994). In the upper reaches of EFPC, total mercury concentrations in water steadily declined from high values of about 1.7 μg/l in 1989–1990 to current levels of about 0.5 μg/l (Fig. 4a). This significant temporal decrease in water concentrations of mercury (significance of regression line slope, $p < 0.05$) generally followed that of reductions in levels of mercury in fish tissue in upper EFPC after 1994.

Periphyton are known to efficiently sequester metals from the water, thus analysis of metals in periphyton provides an informative method to evaluate the dynamics of metal loading in aquatic systems. Zinc, cadmium, and chromium concen-

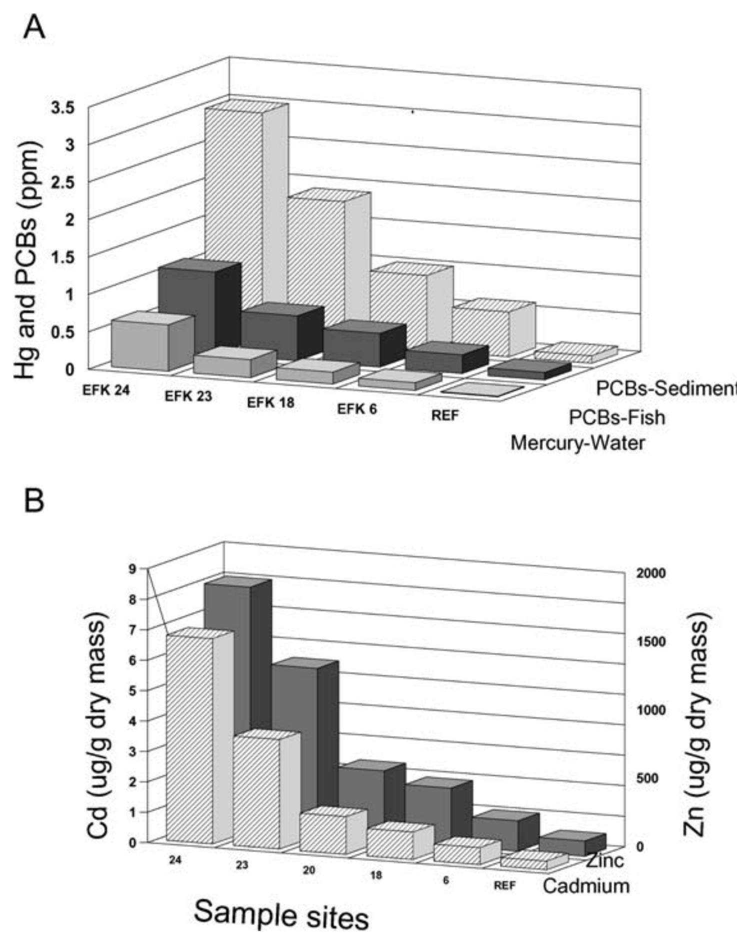

Figure 2. Spatial gradient in the concentration of PCBs in sediment and fish tissue and levels of mercury in water (A) and heavy metals in periphyton (B) at four sites in EFPC and in the reference stream.

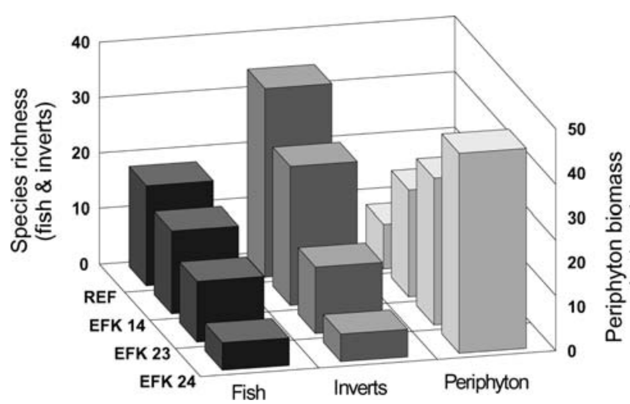

Figure 3. Spatial gradient in species richness of the fish and invertebrate communities and biomass (chl *a*) of the periphyton community at three sites in EFPC and at the reference site.

trations in periphyton generally decreased over time in upper EFPC (Fig. 4b). From 1996 to 2002, chromium levels in the upper reach of the stream declined approximately 50% , cadmium about 80%, and zinc 90% over this time period. Zinc and cadmium declined rapidly from 1996 to 1998–1999

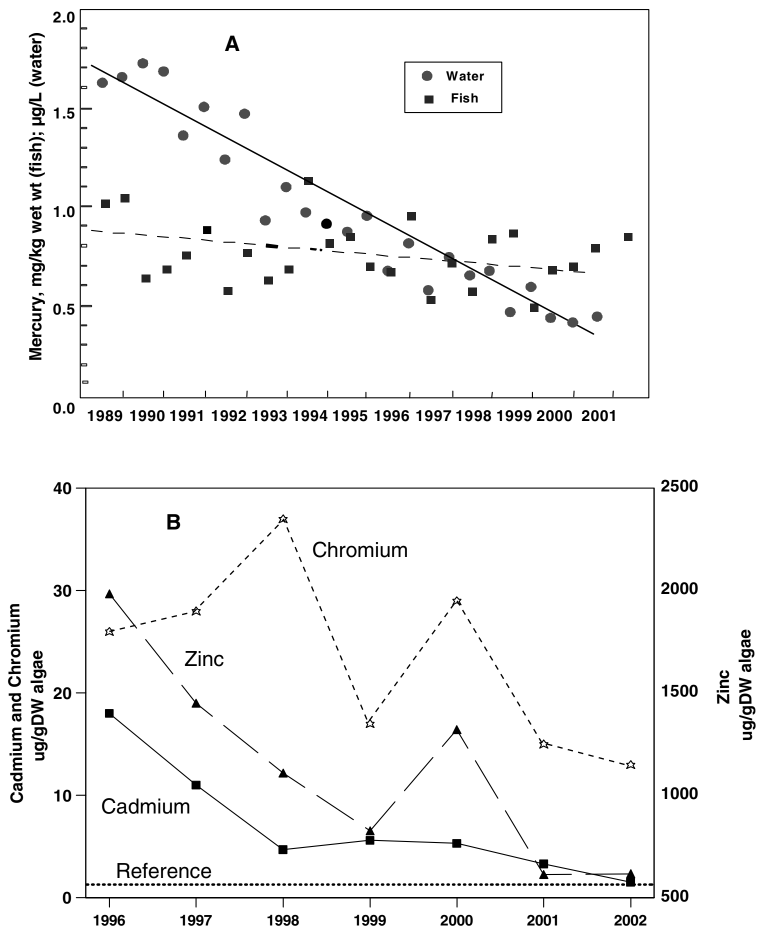

Figure 4. Temporal changes of contaminants in the upper sections of EFPC for mercury in water and in fish tissue (A) and three heavy metals in periphyton (B) over the 15 year study period.

and then exhibited slower decreases over time. Over the period of the study, concentrations of metals in reference site periphyton ranged from 2 to 10 times lower for cadmium and chromium and 2–3 times lower for zinc than levels in periphyton from EFPC sites, In addition, no temporal trends were evident for metals in periphyton from the reference site.

Periphyton

Biomass of periphyton as reflected by chlorophyll (chl) *a* increased over time in upper EFPC (Fig. 5) (regression; $p < 0.05$). Chlorophyll *a* levels increased from lows around 15 $\mu g/cm^2$ of rock surface in the late 1980s to peaks of 45–50 $\mu g/cm^2$ in the late 1990s, a threefold increase (Fig. 5). No significant temporal trends in chl *a* levels were apparent at the lower EFPC site (EFK 14) and at the reference stream. In the upper reaches of EFPC, photosynthesis generally tracked the temporal trend in algal biomass with the lowest levels occurring at initiation of the remediation period (1988–1990), after which photosynthesis increased rapidly during the early and mid-1990s, reaching maximum values by 1997 (regression: $p < 0.05$). Photosynthesis was similar to reference values during the first 3 years of the study but increased 3–4 times over the reference by the late 1990s.

Macroinvertebrate community diversity

Both total taxonomic richness and EPT (Ephemeroptera, Plecoptera, Trichoptera) richness

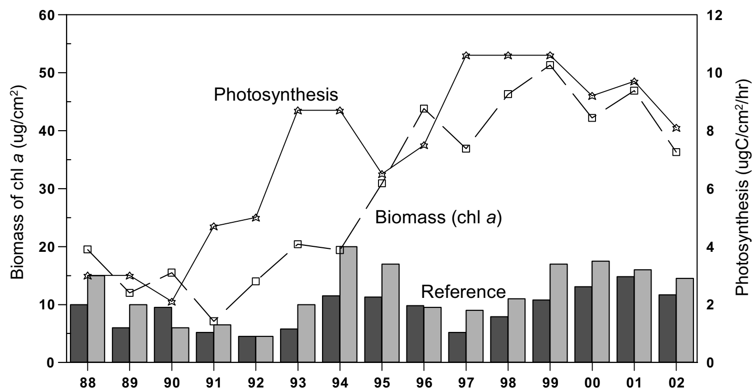

Figure 5. Temporal changes in biomass (chlorophyll *a*) and photosynthesis of the periphyton community in the upper sections of EFPC over the 15 year study period. Darker histograms are reference values for chlorophyll *a* and lighter histograms are reference levels for photosynthesis.

were depressed at the upper two sites in EFPC (EFK 24 and 23) even though a significant positive trend (regression: $p < 0.05$) in temporal recovery was indicated over the 15 year study period (Fig. 6). Total number of taxa per sample increased from a low of 5 and 8 species in 1988 to 21 and 21 species in 2002 at EFK 24 and 23, respectively. In general, EFK 14 and the reference site had similar values for total richness over the study period and both of these sites had 2–3 times the total number of taxa than the upper two EFPC sites. Even though there was no significant temporal increase in total richness at EFK 14 (regression: $p > 0.05$) from 1988 to 2002, there was a higher inter-annual variability at this site than was evident for total taxa at the upper two EFPC sites. Total EPT richness increased from less than 1 taxa at EFK 24 and 23 in 1988 to 5 taxa at both sites in 2002 and did not demonstrate the significant temporal increase in taxa as did total richness (Fig. 6).

A comparison of temporal changes in the major taxonomic groups of benthic macroinvertebrates at the upper two EFPC sites indicates a substantial increase in EPT taxa after the early 1990s, particularly at EFK 23, and a gradual reduction of the pollution-tolerant Chironomidae over the same time period (Fig. 7). The Oligochaeta and other taxa, however, do not appear to change in a consistent pattern over this period at any of the sites. There were no apparent temporal trends in any of the major taxonomic groups at EFK 14 or the reference site. Both of these sites are characterized by a relatively large portion (10–60% at EFK 14 and 10–25% at the reference) of EPT taxa.

Fish community diversity

Compared to the benthic macrobenthos, the total number of fish species increased very little in the upper two EFPC sites over the 15 year study period (Fig. 8a). Before 1988, no fish were present at EFK 24 and the total number of species at this site increased to only 3–4 by 2002. There was, however, a significant temporal trend in recovery at EFK 23 with the number of species increasing significantly (regression: $p < 0.05$) from a low of 7 during 1986–1989 to 14–15 in the early 2000s (Fig. 8a). Temporal species trends at EFK 14, however, exhibited more inter-annual variability, peaking at highs of 23 species in 1995 and 20 in 1997. The species most responsible for recolonization at the EFPC sites were northern hogsucker (*Hypentelium nigricans*), rock bass (*Ambloplites rupestris*), and snubnose darter (*Etheostoma simoterum*).

In contrast to total taxonomic richness, the biomass of sensitive fish species in upper EFPC experienced a rather rapid explosion (Fig. 8b). Biomass of sensitive species (i.e., northern hogsucker, snubnose darter, rock bass) was generally higher at the reference sites than in EFPC (Fig. 8b). In fact 50–60% of the total biomass at the reference site consists of sensitive species whereas in EFPC only 5–10% and 10–20% of the biomass is composed of sensitive species at the upper two sites and at EFK 14, respectively.

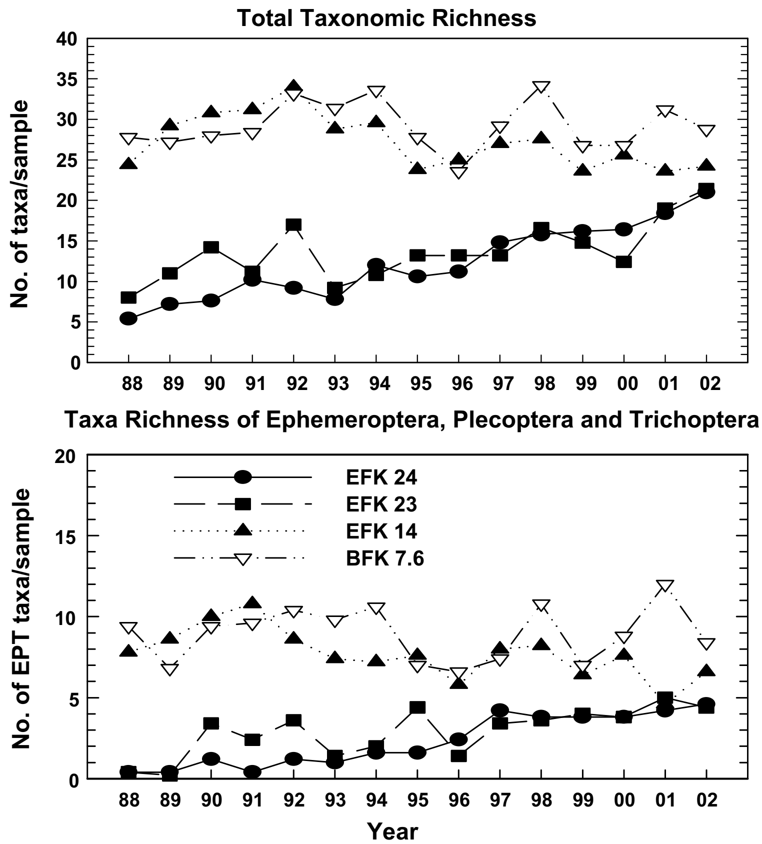

Figure 6. Total species richness (A) and total EPT (Ephemeroptera, Plecoptera, Trichoptera) richness (B) of the benthic macroinvertebrate community at three sites in EFPC and at the reference site.

Individual fish health

Indicators of physiological and bioenergetic status were also measured to determine the health and condition of individual fish in EFPC. Physiological indicators consisted of creatinine (an indicator of kidney dysfunction) and serum triglycerides, an indicator of short-term nutritional and bioenergetic status. Individual health condition indices were represented by the SSI, (general indicator of immune system status) (Goede & Barton, 1990), the LSI (energy storage and metabolism), and individual fish growth (tissue elaboration).

Creatinine, and indicator of kidney dysfunction, was elevated above reference values in upper EFPC from 1988 to about 1991 then declined significantly ($p < 0.05$) to within range of reference values before spiking in 1996 and 1997 (due to an episodic input of mercury into upper EFPC), after which levels again declined to reference values (Fig. 9a). In upper EFPC, serum triglycerides increased significantly ($p < 0.05$) over the study period reaching levels 2–3 times the values observed in 1988 (Fig. 9b). Reference fish generally had higher levels of serum triglycerides that did those from EFPC but showed no clear temporal pattern (Fig. 9b). The SSI, a general indicator of disease or immune system competence, declined significantly ($p < 0.05$) over the study period in upper EFPC (Fig. 9c), approximating reference levels by the mid 1990s. Fish in the upper reaches of EFPC displayed a significant increase ($p < 0.05$) in the LSI, or energy storage capacity, over the study period (Fig. 9d). Reference values for the LSI were relatively low until 1993 after which they increased and approximated those from the upper reaches of EFPC. Reduced reference values for the

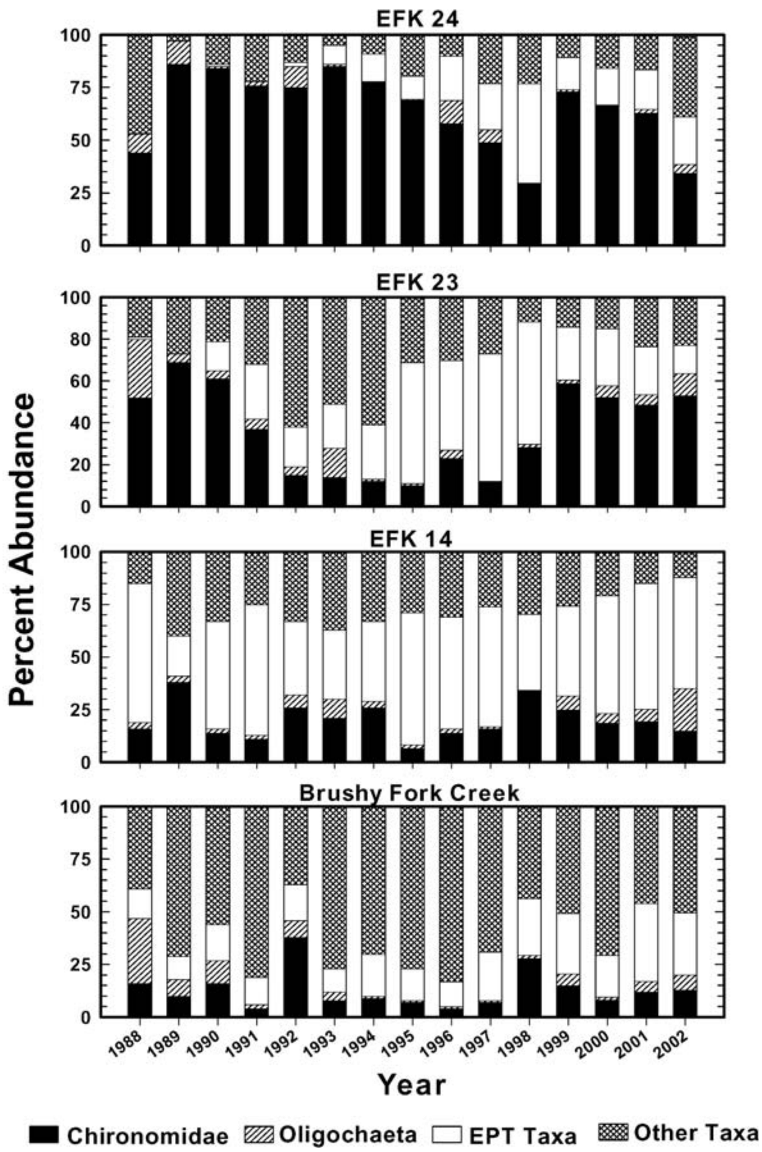

Figure 7. Temporal patterns of change in the percentage of abundance in major benthic macroinvertebrate groups in EFPC and the reference site. EPT = Ephemeroptera, Plecoptera, Trichoptera.

LSI in the first 5–6 years of the study were primarily due to a widespread drought in the Tennessee Valley region which reduced stream flows and consequently available food for growth. Growth rate of the redbreast sunfish population displayed a gradual but significant ($p < 0.05$) temporal reduction over the 15 year study period approaching reference values near the end of the study (Fig. 9e).

Discussion

Spatial responses

Contaminant loading into EFPC had a pronounced effect on the response of instream biota as demonstrated by the spatial patterns in biological components which tracked the downstream gradient in contaminants. The elevated levels of

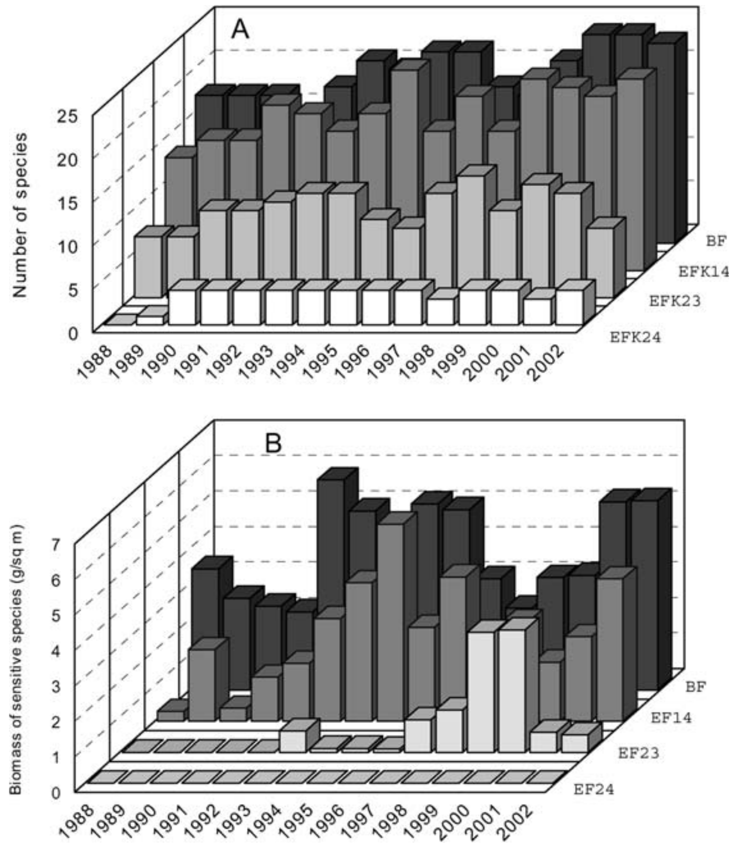

Figure 8. Annual variability in the total species richness (A) and biomass of sensitive species (B) for the fish community at three sites in EFPC and at the reference site.

contaminants in the upper reaches of the stream reflected the highest levels of periphyton biomass, the lowest levels of fish and macroinvertebrate community diversity (species richness), and the poorest condition of individual fish as demonstrated by the various physiological and bioindicators of fish health. The higher levels of periphyton biomass upstream simply reflected the higher nutrient loading upstream which decreased along the downstream gradient of the stream. As the levels of contaminants in the water, sediment, and biota decreased downstream, the health of the individual fish improved in conjunction with diversity (species richness) of the benthic and fish communities.

Other environmental factors, in addition to the decreasing contaminants along the gradient of the stream, could also have contributed to the improved condition of the fish and macroinvertebrate communities downstream. The role of confounding and modifying ecological factors such as food availability, competition, and habitat quality on the health and recovery of biological systems is well documented (Yount and Niemi 1990; Dickson et al., 1992; Power, 1999). Along the spatial gradient of EFPC, the physical habitat changes from relatively low quality in the upstream reaches to higher quality downstream. Upstream sections are primarily channelized with portions of the streambank being relatively steep and stabilized by sections of rip-rap. Habitat diversity in upper EFPC is relatively low due to scarcity of submerged physical structures in pool and riffle areas, there is a higher sinuosity in flow, and higher and more variable temperatures regimes generally occur. In contrast, the lower sections are characterized by a higher diversity and quality of habitats and more moderate and less variable temperature and flow regimes. In addition, food resources for secondary consumers

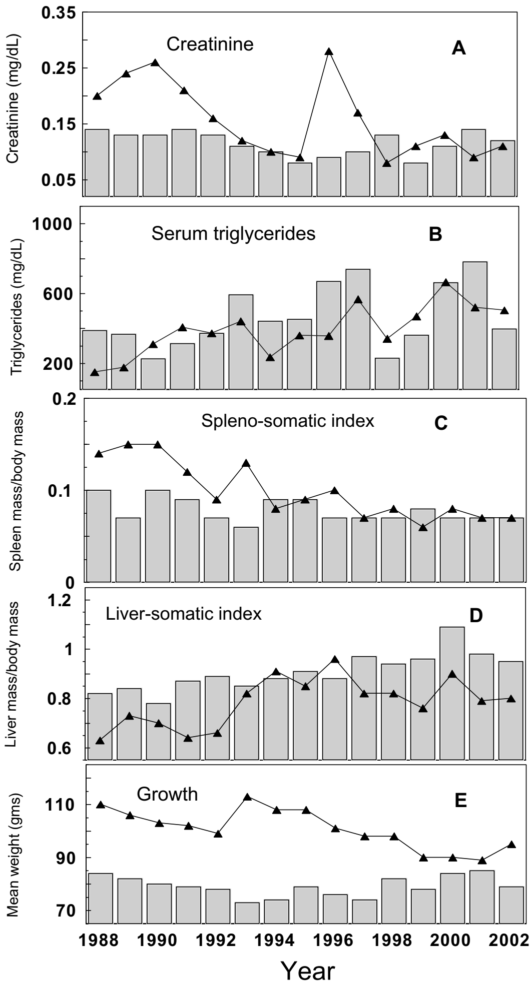

Figure 9. Temporal responses in five bioindicators of individual fish health including creatinine an indicator of kidney dysfunction (A), triglycerides an indicator of bioenergetic status (B), the SSI, a gross indicator of immune system competence (C), the LSI an indicator of metabolic status (D), and growth (mean weight at age 3) which is an overall reflection of individual fish health (E) for redbreast sunfish collected from the upper reaches of East Fork Poplar Creek. Histograms represent temporal responses of sunfish sampled from the reference site.

appears to be more plentiful downstream which would also favor and sustain the higher trophic groups. Episodic variations in physicochemical factors are also more dramatic in the upper sections of the stream which may contribute to the biological responses observed along the spatial gradient of the stream. High variabilities in temperature regimes typically occur during the summer months in upper EFPC. Although these temporal variations in temperature are not at levels which normally cause mortality in fish or other stream biota, nevertheless, these wide variations can cause physiological stress in organisms ultimately resulting in poor condition and fitness of stream biota. In addition, those episodic events which influence the upstream sections of the stream more than at downstream sites can destabilize communities, hinder the ability of populations and communities to realize their maximum recovery potential, and extend recovery time of instream biota (Resh et al., 1988; Death & Winter-bourn, 1995; Brant et al., 1999).

Temporal responses

In general, temporal response patterns in biota tracked the decreases in stream contaminants over time. Contaminants including heavy metals, mercury, and chlorine declined over the 15 year study period particularly in the upper reaches of the stream. Decreases in mercury levels in fish and heavy metals in periphyton followed similar temporal patterns as did the reductions of mercury in stream water. In upper EFPC, increases in chl *a* of periphyton tracked the general temporal decrease of residual chlorine and mercury in water over the study period. Likewise, key metrics of the benthic macroinvertebrate community improved at the upper two EFPC sites which may have been in response to the contaminant decreases in the stream and the increasing trend in chl *a* levels in periphyton. Similar temporal patterns in recovery were found by Watanabe et al. (2000) for a macrobenthic community previously experiencing stress from acid mine drainage. In the upper reaches of EFPC, several bioindicators of individual fish health also demonstrated the same general temporal patterns as did other biological components such as the periphyton, macroinvertebrate, and fish communities.

Recovery of the biota in EFPC over the 15 year study period is demonstrated by the temporal relationships between levels of decreasing contaminants in the stream and the concomitant responses of the various biological components including periphyton, macroinvertebrates, and fish. A summary of these temporal relationships is shown in Fig. 10 for the upper reach of EFPC where recovery was the most dramatic. Decreases

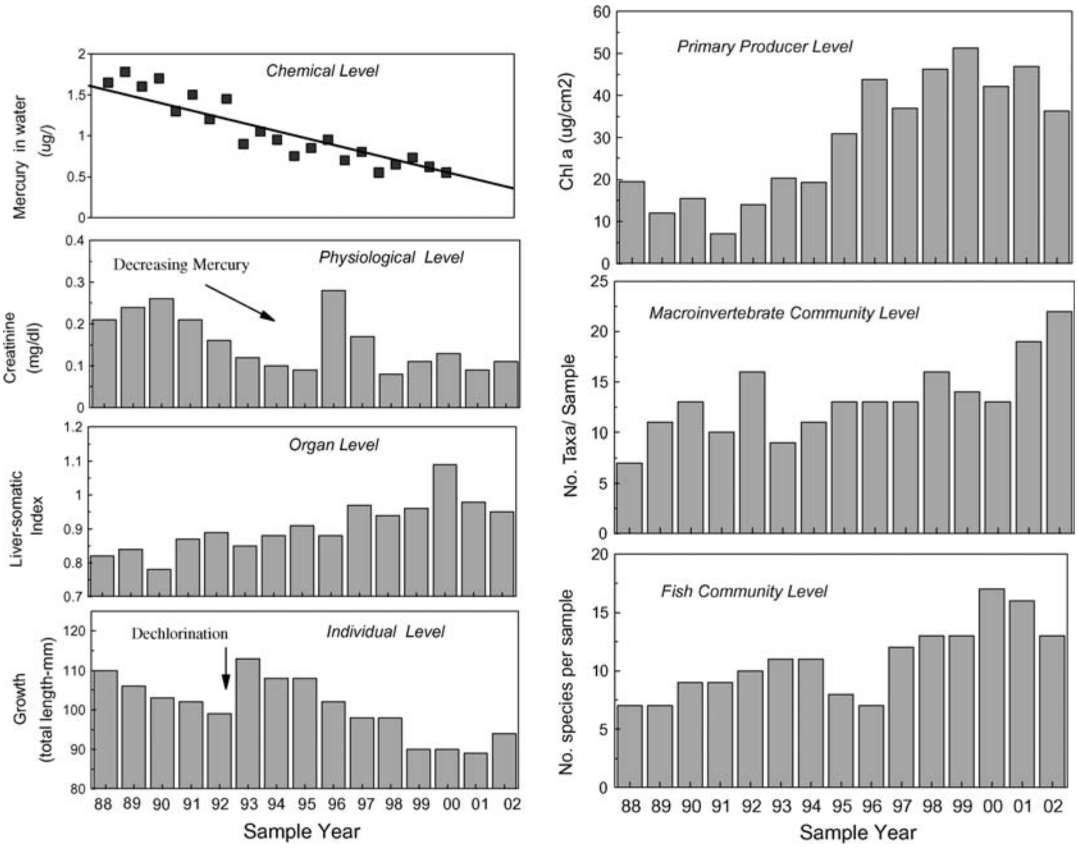

Figure 10. A summary of temporal relationships between remediation of contaminants in EFPC and biological responses at increasing levels of biological organization. The temporal patterns at the higher levels of organization track those temporal changes observed at the lower levels of organization.

in contaminants over a temporal scale was followed closely by an improvement in physiological and organismal-level indicators of individual fish, increases in the diversity of macrobenthic and fish communities, and the rapid response of structural and function properties of the periphyton community. Such responses in the recovery process of stream biota can be due to both direct and indirect factors. In the case of EFPC, direct effects of a stressor such as contaminants can impact organisms by impairing metabolic pathways. Stressors acting through indirect pathways, however, can ultimately affect the health and condition of organisms through bioenergetic pathways via the food chain. For example in EFPC, decreases of chlorine in the late 1980s and early 1990s in EFPC could have resulted in a reduction of toxic effects on the periphyton community, ultimately resulting in increased availability of plant biomass for primary consumers such as benthic macroinvertebrates. Consequently, the fish community would benefit from an increase in the quality (e.g., EPT taxa) and quantity (i.e., biomass) of the macrobenthos (i.e., see Fig. 7b).

Causal relationships

The close spatial and temporal relationships observed between changes in physicochemical factors and positive responses in various components of the stream biota in this study suggest a cause and effect relationship between remediation activities and recovery of this stream ecosystem. When such a relationship appears to exist in an aquatic system, then a credible case for causality between these components can be established (Fox, 1991; Adams, 2003). Results of this study have demonstrated that, on both a spatial and temporal scale, there was a distinct gradient of contaminant levels in EFPC and subsequent responses of biota. Based, therefore, on the causal criteria given by Hill (1965) and Fox (1991), there appears to be a strong relationship between reduction of contaminants and positive responses in the biota of EFPC.

Causality between environmental stressors and biological effects cannot reliably be established, however, based on one or just a few measured response endpoints. Measurement of community-level responses alone such as species richness or biomass would indicate that a change has occurred in an ecologically relevant parameter but the cause(s) of such a change would not be immediately evident. Conversely, if only suborganism or organism-level changes were measured there would be a high fidelity between the cause (stressor) and lower-level effect (i.e., a biomarker response) but the ecological significance of such an observation could not easily be established. In this study, however, we observed concurrent and concomitant changes among the contaminant stressors and several trophic groups (Fig. 10) and also among different levels of biological organization, with changes at the lower levels of organization (physiological, bioenergetic status of individuals) preceding observations at the higher levels (i.e., community diversity) of biological organization. Such relationships provide a 'weight of evidence' approach in establishment of causality in field studies. Establishment of causal relationships are particularly challenging in field studies because of the complex nature of ecological systems, the many biotic and abiotic factors which can influence or modify responses of biological systems to stressors (McCarry & Munkittrick, 1996; Wolfe, 1996), the orders of magnitude involved in extrapolation of effects over different spatial and temporal scales (Holdway, 1996), and compensatory mechanisms operating in populations (Power, 1997).

Understanding causal relationships between environmental stressors and effects on biota is important in the management and restoration of aquatic ecosystems. One of the primary goals of restoration ecology is to help identify the underlying mechanisms or processes responsible for observed changes in biota, and particular those mechanisms that may ultimately influence the status of ecologically-relevant endpoints such as population and community-level parameters. By understanding the factors or mechanisms responsible for responses in biological parameters and the subsequent recovery process of aquatic systems, environmental managers and regulators have a more informed basis for making decisions relative to environmental remediation and restoration activities. Recognizing, however, that efforts to establish relationships between causal factors (i.e., mechanisms) and various levels of biological response may challenge available biomonitoring and assessment resources, the experimental design of field studies should incorporate a selected set of biological responses that represent a wide range of sensitivities, specificities, and response time scales to stressors. Such a design would necessarily include a suite of biological responses represented by different levels of biological organization (Adams et al., 2000). Multiple endpoints are needed in such studies because no single response variable is sufficient to assess the extent of damage to an ecological system or to help in the identification of the factors responsible for damage (Karr, 1993). Therefore, within the framework of this experimental design, the focus of field studies should not only be on the individual organism level but also include (1) a few selected measures at the lower levels of biological organization (represented by high response sensitivity and specificity), and (2) some responses at the higher levels of biological organization (represented by high ecological relevance). The later category would include community-level parameters such as species diversity, richness, biomass of sensitivity species, and integrative community-level indices (e.g., the index of biotic integrity). Inclusion of a variety of biotic response endpoints in environmental effects and restoration studies would lend higher credibility, for example, to the ecological risk assessment process that incorporates community-level attributes as the basis for important decisions relevant to ecological and societal issues.

Acknowledgements

This research was conducted in support of the Y-12 Plant Biological Monitoring and Abatement Program, sponsored by the Environmental Safety and Health Organization, Environmental Compliance

Organization. The Oak Ridge Y-12 Plant is managed by BWXT-Y12 for the U.S. Department of Energy under contract DE-AC05-00-OR22800. The Oak Ridge National Laboratory is managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract DE-AC05-00-OR22725.

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