Final Report

Investigation into Smallmouth Bass Mortality in Virginia's Rivers

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Executive Summary

Background

Episodic fish mortality began within the Shenandoah basin in 2004 and has since spread to upper James River basin. The reasons for this are unknown although water-borne contaminants, stormwater runoff, levels of parasitism, and intersex are considered important factors in these outbreaks. Utilizing an integrated research approach, we proposed to identify the principal factors associated with the fish kills and use this information to begin to identify mitigation and management options for securing the health of these waterways and the fish communities that live there. Our study design for 2008 included four sites that have experienced chronic spring-time fish kills and three sites that have not experienced fish kills. We have collected data on spatial and temporal dynamics of fish kills and examined invertebrate host species for trematode parasites, fish health metrics and parasitism, and land use and contaminant loads in a broad-scale study design in order to identify important variables associated with outbreaks of spring-time fish mortality and potiential long-term monitoring approaches. We collected over 20 smallmouth bass at each of seven sites during pre-kill period and repeated this during typical kill periods.

Our primary objective was to investigate the spatial and temporal dynamics of fish kills in relation to select contaminants, fish health metrics, fish parasitism, land use, and population characteristics of snails in order to identify important variables associated with outbreaks of fish mortality in the system and candidate monitoring approaches. Specific aims 1. Classify watersheds to design a stratified random sampling procedure for examining land use, effect of suspected contaminants including suspended solids, dissolved nutrients, benthic chlorophyll a, periphyton biomass, estrogenic activity, and other organic contaminants. 2. Assess the health of wild, free-living smallmouth bass populations in sample sites based upon physiological markers of stress, key chemical stressors, macroparasite prevalence, diversity and intensity of infection; gross and histological assessment of tissue and organ pathology including pathogenic bacterial infection. 3. Using the data collected in (2) develop a novel health assessment index (HAI) to allow assessment of fish health by watershed classification and other key variables. 4. Measure snail population density, sex ratios, and measure trematode prevalence, diversity and intensity of infection. 5. Develop detailed maps of land use and stressor sources and statistically assess land use and stressor relationships to items (2)-(4) above. 6. Provide preliminary identification and ranking of primary variables associated with fish kills in the Shenandoah basin and rank watersheds by levels of risk.

Findings are based on one-year of field studies and are addressed here in terms of frequently asked questions

Are these rivers fundamentally unhealthy for aquatic life?

Assessing aquatic ecosystem health is a complex undertaking but essential if we are to manage our Virginia waterways effectively. Fish are sensitive bioindicators of aquatic health and thus the continued occurrence of fish kill events signals that the system is undergoing change and experiencing negative impacts. Differences among sites detected in this study suggest that different levels and/or types of stressors are acting on these systems. In particular, the North Fork Shenandoah River appears be the most stressed system of those sampled.

At the landscape level our initial analysis of contaminants in the Shenandoah watershed was based on the hypothesis of a host parasite system mediated by stressors, in the absence of which the host and parasite would coexist. A GIS-based kriging model based on Euclidean distances predicted the concentrations of arsenic (As), chromium (Cr), and mercury (Hg). Multiple Poisson regressions relating each of the three metals' predicted concentration across the Shenandoah basin to the reported fish kill intensity in 2005 revealed a significant correlation between Cr concentrations and fish kill intensity. The kriging produced grids that showed distinct spatial patterns for the three metals. Arsenic was highest toward the north, Cr was highest toward the center, and Hg was highest toward the south of the watershed. We find the significance of Cr an interesting preliminary result as there has been relatively less interest in chromium as a stressor in the fishkill investigations compared to As.

This metals analysis has led to results that would be useful in further investigation of fish stressors in Virginia rivers. In particular, improvements and adaptation of the approach used here to include other watersheds, further refining the kriging and regressions with flow distances, creating a more rigorous coding of kill intensities, and inclusion of random sample of sites (especially tributaries where no fish kills are usually monitored) will create the foundation for a more broadly applicable predictive model of fish kills in the future. This model is a small part of the hypothesized process leading to fish kills. Many factors that may be related to stressor pathways were not included and spatial focus was limited.

Changes in fish parasite species richness have previously been linked to pollution impacts on invertebrate communities as many parasites possess complex life cycles involving multiple species for successful completion of their life cycle (McVicar, 1997). In this study, fish gut parasite studies reveal significant differences between reference sites and fish kill sites with species richness and intensity of infection significantly higher in reference sample sites. This preliminary data will need to be further examined but suggests declines in invertebrate biodiversity related to environmental stressors such as pollution and/or other disturbances that might be acting at fish kill affected sites.

Nutrients in the affected systems were high and are indicative of changes in system ecology and stress from eutrophication. Estrogenic activity in water samples was measurable at all Shenandoah River sites in spring and fall with estrogenic activity occurring at levels high enough to cause biological effects in fish. Robertson et al. (2009) have identified a link between endocrine disruption and immune suppression in fishes. Endocrine disruption has caused reproductive failure in a short-lived species (Kidd 2007).

The identification of the fish bacterial pathogen *Aeromonas salmonicida* on some affected fish by scientists with the USGS signifies an important finding. It is unclear, however, to what extent this fish pathogen is responsible for observed mortality in affected rivers and to what extent invasion of this pathogen is a consequence of larger changes in the health of the system and fish populations under study. If the latter is true, we can expect other fish pathogens to emerge or co-occur in compromised fish populations.

In conclusion, our data from this study and that of others suggest strongly that these rivers are experiencing a significant change in health and stability. Further studies should be focused on the examination of sources of multiple contaminants, fate in affected fish species, presumed immune suppression, and spring-time fish health.

What are the effects of nutrient and contaminant runoff on pleurocerid snails, which are potential intermediate hosts for fish parasites?

Based on previous studies of benthic macroinvertebrates in 2006 and 2007, we saw strong influence of nutrient concentrations on some metrics describing macroinvertebrate communities. In this study we developed the working hypothesis that nutrient enrichment increases populations of vectors of fish parasites, potentially leading to increased parasitic infection. These parasites could indirectly weaken fish immune systems and increase their susceptibility to other pathogens or introduce pathogens directly during penetration of the integument. The pleurocerid snail, *Leptoxis carinata*, is widely distributed in the Shenandoah River watershed and high population densities are supported in the Shenandoah River. Snails are primary intermediate hosts for trematode parasites. Miracidia hatch from trematode eggs and either penetrate or are ingested by the snail host. Once inside the snail, the miracidia metamorphose into sporocysts and replicate asexually to form daughter sporocysts or redia, in which cercariae develop. Cercariae leave the snail and are briefly free living before they penetrate or are ingested by the next (secondary) host. Encysted trematode cercariae (metacercariae) have been found in the organs of Shenandoah River fish species affected by the fish kills (present study). Therefore, processes controlling availability of cercariae to fish in the Shenandoah River are potentially relevant to the fish kills.

Only one species of snail, *Leptoxis carinata*, was collected from all Shenandoah River and tributary sampling sites and the sampling sites located on the Cowpasture and James rivers. *Leptoxis carinata* was collected at the Rappahannock River sampling site, but a second species of snail in the family Pleuroceridae, *Elimia virginica*, was also present. *Leptoxis dilatata* was the only species of snail collected from the New River. Two species of pleurocerid snails were collected from the North Fork Holston River, *Leptoxis praerosa* and *Pleurocera uncialis*. Three types of identified cercariae have the potential to infect fish, including those in families Opecoelidae, Cyathocotylidae, and Opisthorchiidae. Cercariae in the family Opecoelidae were collected on at least one date from every site except New River and South Fork Shenandoah. Generally, trematodes in this family encyst in either arthropods or small fish and the definitive host is a fish. Reported definitive hosts include pikeminnows and smallmouth bass (genus *Nezpercella* spp.), and eel, sculpin and hogsucker (*Plagioporus* spp. and *Nicolla* spp.). We did not find any support for the general hypothesis that higher population densities of pleurocerid snails results in a greater proportion of snails infected with trematodes. However, number of species of snails were limited to one at all fish-kill affected sites.

The abundance of periphyton, as measured by chlorophyll *a* concentration, was very high at river and tributary sites, with many values indicating eutrophication in the Shenandoah River watershed. The abundance of periphyton was significantly different among groupings of river sites, but there were no significant seasonal differences. Snail population densities, trematode infection rates, and proportions of female snails were not correlated with periphyton abundance.

Estrogenic activity was measurable in water samples at all Shenandoah River sites in spring and fall and was significantly higher at these sites than reference sites. Likewise, estrogenic activity was measurable at most of the tributary sites. Estrogenic activity occurred at levels high enough to cause biological effects in fish at several river and tributary sites. Studies by Hinck et al. (2009) and Blazer et al. (2007) have documented the occurrence of intersex (presence of testicular oocytes) in the rivers affected by fish kills as well as other river basins in the United States.

In spatial comparisons among tributary sites, all agricultural variables were significantly correlated with estrogenic activity, concentrations of dissolved nutrients, and specific conductivity. Within land cover classes, pasture/hay and the total density of animal feeding operations were generally the strongest predictors of water quality. Both estrogenicity and nutrients increased with agricultural land uses, especially during periods of high runoff.

Snail population sex ratios had a lack of female-bias at all reference basin sites compared to female-bias at North Fork and James River sites and the high prevalence of female-bias at tributary sites suggests that an environmental stressor may be affecting snail populations.

In conclusion, while nutrients are likely not causative agents of the fish kills, high concentrations are indicative of a larger environmental problem. Furthermore, the estrogenic activity measured at fish kill sites, were high enough to cause biological effects in fish and contributions of multiple sources must be determined. Further work at multiple spatial and biological scales should be initiated to explain sources of estrogenicity, severity of intersex, and linkages to immune system in affected fish species.

How do we precisely and consistently measure fish health and monitor other fish populations before a fish kill or disease outbreak occurs?

A major barrier to understanding fish kill phenomena has been the lack of standardized long-term data and related river and landscape attributes. Observations of dead fish in aquatic system signal, in most cases, the end of a longer path of environmental change, not the beginning of a new problem. Thus, it is essential that any aquatic management activity have longitudinal monitoring activities, which will allow early detection of health perturbations in the system. Complex systems require monitoring approaches which engage this complexity across both the scale of space and time in a manner, which provides robust information that managers can use to detect system change before fish kill events become extreme, generating public concern. This requires a detailed knowledge of system under normal circumstances and monitoring activity, which allows detection of change. This monitoring framework must be constructed such that it is implementable over time and within the human and financial resources of the agency required to implement. So, given this complexity what do you measure? This study dedicated a significant amount of time to piloting and refining monitoring approaches, which would allow consistent collection of appropriate long-term data as well as an assessment of the scale of operation necessary to capture true system changes.

Motivated by the needs of this study we have stratified Virginia watersheds into similar types based on land use/land cover as predicted by physiographic and climatic variables of the watersheds. This classification is named physiographically constrained land use/land cover regionalization (PCLR). There are five major watershed types (PCLRs) identified through the model, of which three are well represented in Virgina. It is expected that processes such as hydrologic and contaminant loading changes associated with land uses like agriculture and urbanization will affect rivers differently in these watershed types. Thus, the PCLRs provide a sound basis for stratification and sampling design. The basic units used for the spatial framework are the USGS hydrologic landscape regions, approximating 200km^2 in average area. These units are perfect size for bridging sampling unit definitions for segments of larger rivers as well as entire watersheds of streams that are fourth-order and smaller ('tributaries'). These spatial units can be used to randomly sample Virginia flowing waters in a more robust research and monitoring design that could also include the existing monitoring sites.

Many fish parasites have complex life cycles that require the presence of an invertebrate host to complete their life cycle. In this study, we focused on the inventory of gut parasites found in the Small mouth bass. Significant differences were noted between reference and fish kill affected sites although further refinement of this analysis is still required. Both parasite intensity and richness were higher in reference sites. This is the first survey of its kind in Virginia rivers to our knowledge. This data suggests that macroparasites might provide an important bio-indictor of the stability and health of a system and components. Previous studies have identified the utility of this approach in system monitoring particularily pollution affects to benthic invertebrate biodiversity (D'Amelio 2003; Dzikowski et al. 2003). However, the responses of individual taxa and functional groups to specific contaminants and degree of effect have yet to be assessed quantitatively. This is an important area of future work. There is also evidence to suggest the parasites such as acanthocephalans (*Pomphorhynchus laevis*) can have a protective effect on host exposure to contaminants as they may bioaccumulate heavy metals lowering host exposure. One such study identified mean concentrations of lead and cadmium in the acanthocephalan, which were 2700 and 400 times higher than in the tissue of the host, the chub (*Squalius cephalus*) and 11,000 and 27,000 times higher than in the water, respectively (Sures et al 1994). They may also and serve an important biomonitoring function for exposure (Sure, 2008). Thus, fish parasite monitoring identifies multiple areas of potential assessment. This is an extremely promising area of future research.

In order to standardize data collection on fish health over time, we developed a novel Health Assessment Index Tool, which would allow standard quantitative approach to monitoring fish health and/or kill events. The development of the HAI Atlas and Guide will allow training and standardized use of the HAI tool. The HAI is further scaled to allow tiered use by the user (external only, public sector). Applying this tool consistently through time will allow fish populations to be monitored more precisely and consistently by different stakeholders including the public who wish to participate. The latter activity identifies an outreach activity, which will improve shared stewardship roles for the public and improved interaction between the public and private sector. This data from all sources, if standardized as outlined here, will be essential as we seek to unravel fish mortality and causation. The prepared database, populated with data from this study will allow repository of all additional data and long-term analysis capacity. The premise that all parasitism is unhealthy and the inclusion of this perimeter in most fish health evaluations is an area requiring further evaluatio, particularly, how it should be handled and weighted in a monitoring scheme. Our results indicate that parasitism is not always associated with ill health; indeed it might signal quite the opposite. Sures (2008) indicated that parasites may mitigate the effects of contaminants in the host fish. More work must be focused on understanding what the results of these data really mean and how to use it appropriately in a HAI tool. Our approach also allows fish to be monitored using only external variable measurements as this was sensitive between fish-kill and non fish-kill periods. This is an important step as we seek to develop approaches which minimize the need for killing older fish classes annually in system monitoring. This also means the other stakeholders can contribute and conduct the HAI without the need to use more refined necropsy skills and tools.

Are fish more stressed in rivers that have experienced annual spring-time fish kills than fish in rivers that have never experienced a similar kill? Is there evidence for pre-disposing conditions that make fish more susceptible to diseases?

This study provides quantitative metrics of organ pathology and parasite load for smallmouth bass in reference rivers. Variance associated with lab measurements were low enough to have statistical power to detect some changes among sites. Pathological alterations that were observed in the spleen and liver included melanomacrophage aggregates, vacuolation of cytoplasm in liver, and pyknotic nuclei in liver, all indicators of organic contaminant exposure. However, we observed no differences in pathology between reference sites and fish kill sites. Based on quantification of histological sections of spleen, liver, kidney, and gill tissue there were differences among sites. Incidence of gill hyperplasia were high, suggestive of contaminants and/or high suspended sediment, and were significantly higher at fish kill sites in the pre-kill sampling period. Smallmouth bass from the North Fork Shenandoah site had the highest incidence of organ pathologies and female smallmouth bass had higher incidence of pathologies than males.

Parasite loads in gut tissue, liver, spleen, and kidney were not significantly higher in fish kill sites. In fact the levels were higher in reference rivers. Smallmouth bass showed significant changes white blood cell differential from pre-kill to kill periods, suggestive of infection. These same fish have more severe skin and fin lesions and ulcerations.

Further studies on fish health should include assays of the hepatic enzymes alkaline phosphatase and alanine transaminase, more sensitive biomarkers of cellular damage. Broeg (2003) concluded that activity of alkaline phosphatase in macrophage aggregates was a reliable and consistent indicator of immunomodulation in different fish species. Further we noted occurrence of pyknotic nuclei in liver, another biomarker of cellular damage.

How can we better use existing data on contaminants in sediments and fish to describe differences in watershed-level exposures? And are these related to fish kill severity?

The kill intensity - metals model has demonstrated in a simple way, the general procedure that could be used to identify potential causal pathways in continuing studies. A comprehensive model would include more variables representing all plausible prevailing and alternative and hypotheses. This ambition is however tempered by the limited number of sites for which the full suite of variables is available or could be compiled in relatively short time.

There should be a concerted effort towards filling the data gaps and expanding the long-term monitoring sites. The most efficient approach here appears to be one that continues on-going monitoring at the existing sites while supplementing these sites with a new set of sites selected by a stratified random scheme. Stratification should be on the basis of fundamental differences among watersheds (e.g., as shown by the PCLR) and patterns of data availability. The former takes care of statistical concerns while the latter is motivated by cost effectiveness. We have compiled and mapped a large amount of the available data. This could be used to characterize areas of the state by data availability/needs; the product would then be input into the stratified sampling procedure to select additional sites for continuing studies and monitoring. Retrospective analysis of archived tissue, for example, would benefit from such spatial analysis of data needs. Similarly, a spatial analysis of data availability/needs is an efficient way for the agency to start building opportunistic data collection into its routine sampling.

The proposed process will ensure data availability at sufficient number of sites to engage in more causal and predictive modeling. While unmonitored watersheds that severely lack data would still be impossible to predict, more and more watersheds can be predicted over time as the set of important variables get narrowed down by the causal modeling process.

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Abstracts of Individual Study Elements

Comparison of white blood cell differentials in smallmouth bass (*Micropterus dolomieui*) affected by fish kill phenomena in Virginia rivers

Episodic fish mortality has occurred in the spring and summer in the Shenandoah River basin of Virginia since 2004, yet no definitive cause of these mortality events has emerged. Immunosuppression has been suggested as a contributing factor to these die-offs. White blood cell differential counts, a simple yet important measurement of circulating immune cells, were determined for smallmouth bass collected from affected sites prior to and during mortality events, as well as from reference sites. This study provides the first data on proportions of circulating leukocytes in wild smallmouth bass. Proportion of granulocytes and lymphocytes were significantly different between pre-affected, affected and reference sites.

These findings suggest immunomodulation may be contributing to these fish kills. The cause of this immunomodulation remains unknown.

Blood variables for wild, free-living smallmouth bass in seven rivers of Virginia

Blood gas, pH, hematocrit, hemoglobin, electrolyte measurements and ion characteristics were measured for twenty individual smallmouth bass from seven rivers in Virginia. We characterized the means, range, and variability for each parameter and compared rivers affected by spring-time fish kills with rivers not affected by kills. Many of the blood parameters were intercorrelated and variability was related to three factors, hematocrit and hemoglobin, pH and plasma bicarbonate, and partial pressures of O2 and CO2. A number of smallmouth bass from fish-kill affected sites that also had moderate or severe skin, fin, or gill erosion exhibited extreme values for hematocrit, hemoglobin, ions, and blood gases.

Macroparasite Evaluation and Smallmouth Bass in Affected and Unaffected Sites in Virginia's Waterways: Preliminary Data

Parasites can be an important factor affecting host health and can identify an important indicator of ecosystem health. However, these interactions are not well understood. Previous histological studies had identified high levels of encysted parasites in organs of clinically ill small mouth bass sampled during outbreaks of fish mortality in Virginia. These findings have lead to the speculation of the role of increased parasitism in the emergence of disease related mortality in these populations. This study attempted to assess the overall gut parasite infection status of small mouth bass between affected and unaffected sites. Our preliminary results indicate that there is a significant increase in the average gut parasite richness and intensity of infection in small mouth bass sampled from reference sites as compared to fish-kill affected sites. Previous studies have identified links between gut parasite richness and stability of aquatic ecosystems and invertebrate species richness. Further evaluation of parasite life history strategies and other perimeters will be necessary in order to explore this potential relationship.

Development of a Health Index Assessment Protocol for Evaluation of Smallmouth Bass in Virginia Rivers

The designed Health Assessment Index (HAI) protocol is effective in detecting changes in fish health. Sampled fish in affected sites were as healthy or more so than reference sites in the early spring. Significant differences were found between kill and non- kill periods in affected sites. The change in the health of fish appears to be a short-term as water temperatures increase. The constructed HAI protocol can be used to identify the onset of fish kill phenomena by various stakeholders and allows a standardized quantitative analysis of fish health. Preliminary assessment of data suggests that external matrices are sufficient to detect disease onset and allow field application of HAI assessments without sacrificing sampled fish. Continued and consistent use of the HAI over time will allow longitudinal assessment of fish health and disease incidence for long-term population monitoring.

Histological Evaluations of Organ Tissues from Smallmouth Bass (*Micropterus dolomieu*) from Selected Sites at Rivers in Virginia

The goal of this study was to evaluate histologically prepared tissue sections from vital organs of smallmouth bass (SMB, *Micropterus dolomieu*) collected from die-off (A=affected) and reference (UA=unaffected) sites in selected rivers in Virginia. We collected fish and subsequent organ tissues from these sites before (PRE) and during (DUR) the estimated period of past die-offs. Our specific objectives were: 1) to histologically evaluate vital organ tissues, including spleens, gills, anterior and posterior kidneys, livers, and gonads of SMB from PRE-A, DUR-A, and PRE-UA sites for determination of statistical differences; 2) to evaluate and statistically analyze parasitic infestations in spleens, gills, anterior and posterior kidneys, livers of SMB from these sites; and 3) to compare gender differences in the variables in SMB at the sites. Six smallmouth bass were collected form April 2 to May 22, 2008 from sites on the Cowpasture, James, New, North Fork Holston, North and South forks of the Shenandoah, and Rappahannock rivers in Virginia. The pre- and during-kill (PRE-A and DUR-A) river sites were labeled Walton Tract (PRE-A, Cowpasture), Lynchburg Camp (DUR-A), Buchanan (James), Red Bank (North Fork Shenandoah), and Island Ford (South Fork Shenandoah). The pre-unaffected (PRE-UA) river sites were called Allisonia (New), Saltville (North Fork Holston), and Kelley’s Ford (Rappahannock). Tissues from the organs were fixed and processed for histological evaluation. Dependent variables included fractions of splenic tissues in sections containing pigmented macrophage aggregates (SFPMA), liver hepatocytes that were vacuolated (FCVL), gill lamellae showing hyperplasia (FGHL), and spleen, anterior kidney, posterior kidney, and liver tissue occupied by trematodes (SFTREM, AKFTREM, PKFTREM, and LFTREM, respectively). Fractions of hepatocytes with vacuolation (FCVL), gill lamellae with hyperplasia (FGLH), and anterior kidney containing trematodes (AKFTREM) of PRE-A and DUR-A fish were significantly different. There were differential effects by gender, with PRE-A FCVL, FGLH, and AKFTREM of males significantly less than those of DUR-A males, and PRE-A trematode-based dependent variables (SFTREM, AKFTREM, PKFTREM, and LFTREM) for females were significantly less than females of DUR-A. DUR-A females showed significantly higher SFPMA, FCVL, SFTREM, PKFTREM, and LFTREM means than DUR-A males. Fractions of gill lamellae with hyperplasia (FGLH) and trematodes in examined organs (SFTREM, AKFTREM, PKFTREM, and LFTREM) were significantly greater at PRE-A sites than at PRE-UA sites. Mean fractions of PMA in the spleen (SFPMA) were statistically correlated with fish age and fractions of anterior kidneys, posterior kidneys, and livers containing trematodes (AKFTREM, PKFTREM, and LFTREM)($r=0.26$, 0.25, 0.32, and 0.27, respectively). Means of fractions of all organs containing trematodes were highly correlated with each other (r ranged from 0.71 to 0.81). Based on the histological results, it is reasonable to conclude that fish of the DUR-A sites incurred greater organ-related impacts than fish of the PRE-A and Pre-UA sites, females of the DUR-A sites showed greater organ impacts than males, and fish collected from Red Bank (PRE-A and DUR-A) showed greater impacts than fish from the other A sites.

Relationships between trematode parasites, pleurocerid snails, and environmental variables

Six families of trematodes were identified in snails collected from the 12 river sites, with three of those having the potential to infect fish. One family (Opisthorchiidae) was unique to the Shenandoah River. Five of the six families of trematodes were found in Shenandoah River tributary sites, with two of those having the potential to infect fish. Trematode infection rates in snails ranged from 0.023-0.13 at reference sites, from 0.0-0.07 at James River basin sites, and from 0.016-0.18 at Shenandoah River sites. Similar types of trematodes were found between Shenandoah, James, and reference river basins and the lack of a significant difference in infection rates between snails in these basins suggests that parasites hosted by L. carinata are likely not directly related to fish kills. The trematode parasites identified thus far in L. carinata have not yet been found in smallmouth bass from the

Shenandoah River and the three families using fish as secondary hosts likely infect other fish species. However, the presence of trematodes in the family Opisthorchiidae in only Shenandoah River snails is intriguing. Because of the potential for this trematode to infect humans, its presence in game fish should be investigated. Snail population densities were high, ranging from 65 to 3038 snails/ m^2 at river sites and 32 to 2645 snails/m^2 at tributary sites, with significant differences among site groupings of river sites. These differences were due to high population densities at certain sites within the North and South Fork groupings. We did not find any support for the general hypothesis that higher population densities of pleurocerid snails results in a greater proportion of snails infected with trematodes. Further studies of the factors affecting trematode infection rates in these snails are ongoing. We cannot rule out effects of population densities on infection rates in other snails (i.e. planorbids or physids) with different life histories and habitat utilization. The proportion of female snails varied significantly from the expected ratio of 1:1 at many river and tributary sites, but there was not a seasonal effect. At river sites, ratios were female-biased at North Fork and James River basin sites, but not at reference sites. The proportion of female snails was not correlated with population density of snails or trematode infection rates. While we did not find any correlation of female-biased sex ratios and measured environmental variables, the lack of female-bias at all reference basin sites compared to female-bias at North Fork and James River sites and the high prevalence of female-bias at tributary sites suggests that an environmental stressor may be affecting snail populations. Further investigation of variability of these sex ratios is ongoing in order to assess the utility of this measure in biomonitoring. The abundance of periphyton, as measured by chlorophyll a concentration, was very high at river and tributary sites, with many values indicating eutrophication in the Shenandoah River watershed. The abundance of periphyton was significantly different among groupings of river sites, but there were no significant seasonal differences. Snail population densities, trematode infection rates, and proportions of female snails were not correlated with periphyton abundance. The lack of a relationship between periphyton and snail densities is likely due to 1) lack of food limitation due to very high periphyton biomass and 2) ability of pleurocerid snails to utilize other food sources. Nutrient concentrations were high and showed significant differences spatially and seasonally among river and tributary sites. At river sites, significant differences in nutrient concentrations generally tracked the differences in periphyton abundance. However, there were no significant direct correlations between nutrient concentrations and snail population characteristics. Estrogenic activity was measurable at all Shenandoah River sites in spring and fall and was significantly higher at these sites than reference sites. Likewise, estrogenic activity was measurable at most of the tributary sites. Estrogenic activity occurred at levels high enough to cause biological effects in fish at several river and tributary sites, but was not correlated with the proportion of female snails at river or tributary sites. Land uses involving agriculture were strongly correlated with nutrient concentrations and estrogenic activity, especially during high flows. These analyses demonstrate that the amount of agricultural activity in individual subwatersheds strongly predicts water quality in receiving streams. However, analyses conducted to date have not revealed any predictable responses of snails to agricultural land uses. The differences in concentrations of chlorophyll a, nutrients, and estrogenic compounds between Shenandoah and other river basins were remarkable. Concentrations of nutrients and estrogenic compounds were sustained at low flow, indicating constant sources of these contaminants. Correlations with agricultural land use in Shenandoah River tributaries suggests an overwhelming influence, although the relative contribution of larger WWTPs discharging directly into the river should also be considered. While nutrients and estrogenic compounds are likely not causative agents of the fish kills, high concentrations are indicative of a larger environmental problem.

Kill Intensity–Contaminant Relationship

The preliminary model of kill intensity–contaminant relationship was based on the hypothesis of a host parasite system mediated by stressors, in the absence of which the host and parasite would coexist. The real parasite was assumed rather than known. The model then focused on finding a significant relationship between each metal and intensity of reported kills, assuming that the parasite is ubiquitous at kill sites and need not be modeled explicitly. Our objective was to fit a regression model relating heavy metal concentrations to the intensity of fish kills in different locations in the Shenandoah River watershed. To accomplish this objective, we used the existing DEQ data of metal concentration in fish tissues. Because metals data and fish kill data are not spatially coincident, we first had to predict heavy metal concentrations for all river locations in the Shenandoah River watershed. The fish tissue data for the Shenandoah were sampled in 2005. The 140 records of tissue data reduced to 29 unique values when multiple within site data were averaged. To reduce temporal noises in the data as much as possible, we used the 32 reports of fish kills that had occurred in 2005 in the Shenandoah as the response, ignoring any other records of fish kills in the basin. A GIS-based kriging model based on Euclidean distances were used to predict the concentrations of arsenic (As), chromium (Cr), and mercury (Hg), the three metals that had significant variance within the watershed to allow reasonable spatial prediction models. The kriging was preceded by a test of spatial autocorrelation that showed that metal concentrations were highly correlated in space with each major watershed but the correlations broke down when tested across watershed boundaries. Multiple Poisson regression (a Generalized Linear Model GLM) was employed to relate each of the three metals' predicted concentration across the Shenandoah basin to the reported fish kill intensity in 2005. The raw intensity values were assumed to follow a Poisson instead of a normal distribution because of the discreteness of the scores. The kriging produced grids that showed distinct spatial patterns for the three metals. As was highest toward the north, Cr was highest toward the center, and Hg was highest toward the south. The GLM models found chromium as the only significant metal ($P = 0.0007$). We find the significance of Cr an interesting preliminary result as there has been relatively less interest in chromium as a stressor in the fishkill investigations compared to As. This analysis has led to results that would be useful in further investigation of fish stressors in Virginia rivers. In particular, improvements and adaptation of the approach used here to include other watersheds, further refining the kriging and regressions with flow distances, creating a more rigorous coding of kill intensities, and inclusion of random sample of sites (especially tributaries where no fish kills are usually monitored) will create the foundation for a more broadly applicable predictive model of fish kills in the future. This model is a small part of the hypothesized process leading to fish kills. Many factors that may be related to stressor pathways were not included and spatial focus was limited.

Establishment of a Spatial Framework for Virginia Rivers for Fish Kills Investigations

The Virginia Rivers fish kills occur across a large portion of the state composed of watersheds that can vary greatly from one another in natural characteristics and patterns of human activity in the landscape. Due to the variability in the affected watersheds and the desire to designate reference watersheds as control for studying affected watersheds, some type of stratification system is needed to ensure that subsequent studies rigorously account for these differences in the sampling stage. Human land use and land cover alterations have been identified as a major contributor to stream degradation and may play an important role in fish kills as stressor sources. The processes by which such degradations occur depend in part upon physical and climatic, or physiographic, characteristics; the physiographic template in turn determines suitable locations for human land use. Based on this relationship we stratified watersheds by the differences in physiographic characteristics that predict Land Use/Land Cover (LULC). We describe the development of the resulting regional framework– Physiographically Constrained Land Use/Land Cover Regions (PCLRs). The Hydrologic Landscape Regions (HLR) dataset publicly available from the USGS contains information about 13 physiographic variables for all 4th order watersheds throughout the conterminous United States. Because the HLR dataset has a relatively large spatial resolution of 1 km, it does not seem suitable for developing a classification system just for the state of Virginia. The underlying relationships among the variables measured for the landscapes (including outside of Virginia) provided the range needed to capture what relationships are useful in defining Virginia landscapes. Using a subsample of HLRs in the eastern United States, we calculated the percent cover for each of eight classes of land cover from the 2001 National Land Cover Dataset at the watershed extent and within 30 meter riparian buffers for each of the sampled HLR watersheds. We used multivariate regression tree analysis to identify physiographic predictors of LULC and used the decisions conveyed by the tree to create and name classes of watersheds based on similar physical and climatic characteristics that typically have differences in LULC. We also carried out cross tabulation between the watershed and riparian classification systems to determine how closely the two LULC patterns are related to one another. The analysis for watershed LULC resulted in 10 PCLR classes for the entire Eastern United States; 5 of these classes are present in Virginia and 3 are well represented. The identified physiographic predictors include precipitation minus potential evapotranspiration, watershed slope, percent sand in soil, percent total flatland, and average temperature (in order of importance). These natural differences result in differences in the average proportions of forest, wetland, grassland, and agriculture throughout the watersheds. Class 5 watersheds have steep slopes and very little flatland and are characteristically dominated by forest. Class 4 watersheds also have steep slopes but have more total flatland and characteristically have more agricultural land. Class 1 watersheds have low slopes and relatively sandy soils, and are characterized by a mix of forest, wetland and agriculture. The analysis for LULC within a 30 meter riparian buffer resulted in 11 classes for the Eastern United States, with 5 of the 6 classes present in Virginia being well represented. The identified physiographic predictors in order of importance are watershed slope, precipitation minus potential evapotranspiration, minimum elevation, percent sand in soil, and average temperature. Cross tabulation reveals that there are differences in the way that watershed physiographic characteristics predict LULC at the watershed and riparian extents. The PCLR system stratifies watersheds throughout the Eastern United States and Virginia based on similarities in natural characteristics that have been shown to covary with human land use. This regional framework will prove useful in singling out the impacts of human activities on stream systems which may cause fish kills by accounting for some natural differences between watersheds. For example, future study sites may be distributed to represent sites that are similar in each of the three major PCLRs in Virginia, thus giving us greater control over the confounding interaction between LULC and physiography. Furthermore, since the watershed and riparian classification systems vary somewhat, the effects of LULC at different extents can also be determined. Much of the human activity throughout the Shenandoah and James watersheds is strongly related to agricultural and urban land use, and the effects of this activity may well depend upon its proximity to streams and rivers. PCLRs may thus prove to be a useful tool for stratifying the watersheds so that any potential stressors at multiple extents throughout the watersheds can be identified.

Fish Health Assessment Guide and Photo Atlas

This guide is designed to assist with training for field necropsies of smallmouth bass. The purpose is to define all the internal and external features examined during the necropsy. Descriptions and photographs are included describing conditions ranging from normal to severe pathologies. The guide should be used to provide for consistent scoring criteria among field biologists.