2007 Findings
Virginia Fish Kill Investigations
Shenandoah, James, and Cowpasture Rivers
(April 24, 2008 compilation)

The Virginia Department of Environmental Quality (DEQ) and Department of Game & Inland Fisheries (DGIF) coordinated investigations into fish mortality in the Shenandoah, James, and Cowpasture Rivers in 2007. Those investigations continue into 2008. Findings from 2007 are summarized below.

Water and Sediment Chemistry

- **Conventional pollutants.** DEQ monitored weekly for conventional pollutants from March through August 2007 at twelve sites. Parameters included nitrate, nitrite, ammonia, TKN, total and dissolved phosphorous, and suspended solids. All results were within "normal" ranges or, where water quality standards exist, they were below those threshold values.
- **Continuous monitoring**. DEQ recorded pH, temperature, dissolved oxygen, and conductivity at six sites every 15 minutes from the end of March through August 2007. Results showed high variability in daily pH measurements, with the largest swings in daily pH ranging from about 7.5 to 9.3 pH units. This is a large swing, but similar pH shifts have been seen in other Virginia waters with no associated fish kills; also some sites where fish kills occurred had relatively small daily swings of less than 1 pH unit. Therefore, pH changes do not appear to be related to the fish kills.
  Dissolved oxygen does not seem to be a factor in the fish kills. Levels were acceptable at all sites during the spring period (when fish kills occurred). Low dissolved oxygen levels occurred in the North Fork Shenandoah River at Cootes Store when the water flows dropped and temperatures increased in July and August, but there were no fish kills at that time.
  Water temperature seems to be related to timing of fish kills. Problems seemed to begin once river temperatures reached about 15 ˚ C (59˚ F), then seemed to stop when river temperatures reached about 25˚ C (75˚ F).
- **Passive Samplers**. From March – May, 2007, DEQ and the Friends of the North Fork Shenandoah deployed passive samplers - devices designed to collect polar and non-polar organic chemicals. These units are typically submerged in the river for 6-8 weeks and are particularly effective at measuring chemicals that are normally at concentrations too low to be quantified through conventional sampling methods. The samplers were placed at 12 locations including: the Shenandoah River, North Fork Shenandoah River, South Fork Shenandoah River, Linville Creek, Cedar Creek, North River, South River, Cowpasture River, and Maury River. Extracts from the passive samplers were analyzed by a USGS laboratory for a list of 199 organic chemicals including PAHs, PCBs, herbicides, pesticides, waste indicators, pharmaceuticals, and hormones. While laboratory results are still preliminary, and data analysis is ongoing, some initial findings have emerged. As with many rivers that have been sampled using this technology, the fish kill areas yielded a diverse listing of compounds. The chemicals detected and the levels present were largely indicative of an agricultural area. Chemicals such as PCBs, pesticides, PAHs, and pharmaceutical compounds were found, but results were not inconsistent with other developed areas that have not experienced fish kills. No individual chemical compounds were found at levels that by themselves would indicate a clear cause of observed fish mortality.
- **Ambient Toxicity Testing.** Water samples were collected from 4 sites in the Shenandoah River drainage for 7-day chronic toxicity testing by the EPA Region III Office of Analytical Services and Quality in Wheeling, WV. Water taken from all four sites during the week of March 19, 2007 had significant effects on survival, relative to a control, or reference, sample. EPA scientists suspected that a pathogen may have been interfering with the tests, so sampling was repeated during the week of May 14. The South Fork Shenandoah River water did not have any significant effect on survival or growth in the follow-up tests. Water from the remaining three sites all had significant effects on survival. When water from North Fork Shenandoah River and Cedar Creek was disinfected with ultraviolet radiation, however, there was no significant effect on survival or biomass. It appears that the outcome of these tests could have been more strongly influenced by a biological pathogen than chemical constituents in the water.

- **Sediment Metals Concentrations**. Sediments were sampled at North Fork and South Fork Shenandoah River sites, and sites in the South, North, Maury, and Cowpasture Rivers. The sediments were analyzed for heavy metals and arsenic. No samples had concentrations in excess of levels that would be expected to have effects on growth or survival in fish. Metals tested for included arsenic, copper, cadmium, nickel, zinc, lead, and mercury.
- **Volunteer Storm Monitoring**. Citizen volunteers and DEQ personnel continued to collect samples for ammonia and other nutrients during storm events. Samples from several storms were collected during the period from March 2007 to June 2007. Results of the analyses indicated that levels of ammonia were below either chronic or acute toxic concentrations for fish. These levels were also below Water Quality Criteria for Virginia freshwaters.
- **Water Column Metals Analyses**. Several sampling events were conducted during the spring of 2007. Water samples were analyzed for metals to determine whether water column concentrations exceeded chronic or acute concentrations. Results of the analyses were that all metals were detected below levels expected to cause death or sub-lethal effects in fish.

Fish and other Aquatic Life - Health and Community Evaluations

- **Benthic Invertebrate Communities.** Virginia Tech has been conducting a multi-year study of benthic invertebrates in fish kill waters and reference streams since 2006. Initial findings confirm that the fish kill areas in the Shenandoah system are indicative of a nutrient-rich environment, a common condition in rivers of this size, but there is no indication of any invertebrate taxa being eliminated due to toxic compounds. More detailed analyses and statistical testing continues. In 2007, field work focusing on local land use influences on invertebrates and water chemistry in tributary streams was initiated, along with responses of selected organisms to laboratory chemical exposures. Data from 2007 projects are now under review. Some of the research topics for 2008 include: quantitative measurements of snail density and rates of parasitic infection, determining relationships of these measurements with environmental stressors/land use, identification of the trematode parasites using the snail as an intermediate host, further investigation of the relationship of observed snail responses and land use, and investigation of the effects of specific stressors on snail respiratory, immune, and reproductive systems.
- **Fish Viruses.** Virus testing was conducted during 2007 by the US Fish & Wildlife Service (Fish Health Center of the FWS Northeast Fishery Center, Lamar, PA) and Cornell University. No known fish viruses were found in numbers sufficient to account for the fish kill events. Testing by Cornell University specifically for Viral Hemorrhagic Septicemia (VHS-v), a virus currently causing lesions and mortality in the Great Lakes and elsewhere, was negative in all samples. A small percentage of Virginia fish kill samples tested positive for Largemouth Bass Virus (LMB-v); the low rate of incidence suggests it is not a cause of the fish kills, although it may further compromise the health of infected fish .
- **Fish Bacteria.** Bacterial assays were conducted by the US Geological Survey (Leetown, WV – National Fish Health Research Lab) and the US Fish & Wildlife Service. A number of indigenous, opportunistic bacteria were cultured from skin, lesions, and gills of fish from both fish kill and non-fish kill sites. These organisms are considered part of the normal bacterial flora, and typically only cause infection in already compromised hosts. In addition to normal bacterial flora, fish from sites experiencing fish kills showed unexpectedly high numbers of two obligate pathogens (*Aeromonas salmonicida and Flavobacterium columnare)*. These pathogens are known to be capable of functioning as primary pathogens, that is, they can be the causative agent of fish disease. These organisms were found at active fish kill sites (in the Shenandoah, Cowpasture, and James Rivers in April and May 2007) and were not found at sites that were not experiencing fish kills (Potomac River, Gauley River, and North Fork Shenandoah prior to fish kill onset in March 2007). A follow-up study on bacteria by USGS is underway in 2008 to better characterize the roles of *A. salmonicida, F. columnare*, and other bacteria..
- **Fish Parasites.** USGS researchers have noted high parasite loads externally and in gills, livers, kidneys and spleens of fish from the fish kill areas. These parasites may weaken fish and increase their susceptibility to disease. Many of these parasites have intermediate hosts, such as snails,

zooplankton, and benthic invertebrates, all of which may be found at higher densities in nutrient enriched waters. This link is being explored further.

- **Fish Pathology, physiology, and histology.** USGS research suggests that smallmouth bass and sunfish are stressed populations which succumb to a variety of pathogens and parasites when stressed beyond their tolerance. Evidence includes:
    - Gill lesions, suggesting that ammonia, diatoms, or other irritants may be leading to respiratory problems..
    - Massive proliferation of internal parasites in bile ducts, leading to destruction of large portions of the liver.
    - Macrophage aggregates in a variety of internal organs – increase in response to both infectious agents and contaminants.
    - Moderate to high incidence of intersex in male smallmouth bass, suggestive of exposure to estrogenic compounds.

Summary

- Extensive water quality and biological sampling was conducted in fish kill and reference areas in 2007.
- Findings seem to rule out the following possible causes:
    - Individual water quality constituents as primary causes of mortality, including pH, ammonia, dissolved oxygen, nutrients, metals and organics
    - Likely fish viruses, including largemouth bass virus (LMB-v) and viral hemmoragic septicemia (VHS-v), along with several opportunistic bacteria
- Areas needing continued or further study include:
    - Effects and relationships of combinations of water quality constituents. Even though no individual constituent appears responsible for the fish kills, how might different combinations of chemicals behave? Are combinations of chemicals additive? Synergistic? Could they have secondary effects as stressors? What differences exist between the rivers experiencing fish kills and rivers with no kills?
    - Biological pathogens. Further work is needed to determine if organisms such as the bacterium *Areomonas salmonicida* are functioning as primary pathogens. Are there other bacteria or viruses that have not yet been identified?
    - Fish health measurements. Additional measurements of tissue responses and histology, blood chemistry, hormones, organ function, and infestation and role of parasites are needed. Sample sizes must be adequate for statistical comparisons between sites, species, age groups, and sex of specimens. Sufficient numbers of specimens need to be collected from reference streams and fish kill areas.
    - Do the fish responses such as intersex have a bearing on fish kills?
    - Are measurable, sublethal effects occurring in other members of the aquatic community, such as benthic macroinvertebrates?

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