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A Comparison of Fish Health Indices Applied to Freshwater Species of the Chesapeake Bay Watershed

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Abstract

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Josiah Jensen

Fish kills, increased disease prevalence, and endocrine disruption have been observed in multiple freshwater fish populations of the Chesapeake Bay watershed (CBW). Some of these health issues occur in conjunction with declining abundance. A combination of multiple stressors is believed to be weakening sensitive fish species in non-tidal regions of the CBW. Fish health indices such as Deformity, Erosion, Lesion, and Tumor counts (DELTs), and the Health Assessment Index (HAI) are simple tools designed to evaluate the general health of fish populations. Both indices could be widely applied in the watershed because they require limited training and equipment. However, their utility in the CBW needs to be demonstrated. Fish health concerns in the CBW often occur in areas dominated by forested and agricultural land. The research in this thesis evaluates the influences of agricultural land-use, season, seasonal stream discharge, species, age, and sex on DELTs and the HAI. Two studies are included. The first occurred in the Shenandoah Valley of Virginia and West Virginia and applies the DELT index to fish aggregations and the HAI to white sucker and fantail dater in wadable streams. These streams were spread over a gradient of catchment pastureland. The second looked at DELTs and the HAI applied to smallmouth bass at 5 sites, 2 in the Potomac River watershed and 3 in the Susquehanna River watershed, sampled from 2013 to 2020 in the spring and fall. These sites varied in catchment agricultural and forested land with small areas of development. The utility of the two indices in agricultural regions of the CBW was not demonstrated. Both DELTs and HAI scores were correlated with the age and sex of fish sampled, and not with any of the included environmental variables. Future research directions and other concerns surrounding the use of DELTs, and the HAI are discussed.

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Forward

This thesis contains three chapters. The first chapter is a literature review and introduction to research objectives. It will not be published but was included to give a background of fish health issues in the Chesapeake Bay watershed, past research on fish health indices, and why fish health indices might be useful for evaluating and or monitoring freshwater fish species in the Chesapeake Bay watershed.

The second chapter is titled “Two Field Based Fish Health Indices Applied Over a Gradient of Pastureland in the Shenandoah Valley of VA and WV.” This chapter is written as a standalone article. A possible target journal is the Journal of Aquatic Animal Health. This chapter evaluates two simple fish health indices in multiple game and nongame species with comments on macroscopic and microscopic changes associated with a parasite in white sucker. Fish health professionals may find the information in this chapter useful.

The third chapter is titled “Temporal Analysis of a Health Assessment Index (HAI) and Deformity, Erosion, Lesion, and Tumor Counts (DELTs) Applied to Smallmouth Bass in the Chesapeake Bay Watershed.” This chapter is also written as a standalone article. A possible target journal is the North American Journal of Fisheries Management. Smallmouth bass are a popular gamefish, and the fish health indices investigated in this chapter are ones that may be implemented as additions to routine fish population surveys. Fishery managers may benefit from the information contained in this chapter.

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Chapter 1

Literature Review and Introduction

Fish Health Issues in Non-tidal Freshwaters of the Chesapeake Bay Watershed

Certain freshwater fishes of the Chesapeake Bay watershed (CBW) have suffered multiple mortality events in the last 20 years. In the upper Potomac River fish kills affecting smallmouth bass *Micropterus dolmieu* (SMB), redbreast sunfish *Lepomis auritus*, rock bass *Ambloplites rupestris,* northern hog sucker *Hypentelium nigricans* (NHS), and golden redhorse *Moxostoma erthrum* (GR) occurred in the early 2000s. These mortality events typically occurred in the spring and affected adult fish. Kills were first noticed in the South Branch of the Potomac River in 2002. This event affecting a world class SMB fishery garnered the attention of anglers and other stakeholders. Similar events occurred in 2004-2005 in the Shenandoah River, in 2007-2009 in the James River and in 2009 an angler reported lesions and mortality of SMB in the Monocacy River (Blazer et al., 2010). Large obvious kills appear to be less common in more recent years, possibly due to less public interest in reporting events, lower fish populations making events less dramatic, or fish mortality becoming more chronic (Keplinger et al., 2022) . In the Susquehanna River system sporadic mortality of young of the year SMB has occurred in the last 20 years (Smith et al. 2015; Walsh et al.2018; Schall et al., 2020). Disease in age-0 SMB was first noted in 2005 and shortly after there were declines in both adult and juvenile abundance from 2005 to 2011 (Schall et al., 2020). While infectious agents such as Largemouth Bass Virus and various bacteria including *Aeromonas* sp. and *Flavobacterium* sp. have been detected in fish populations of the Potomac and Susquehanna Rivers, none have been identified as the sole cause of these events (Blazer et al., 2010; Schall et al., 2020). Biologic endpoints including testicular oocysts and elevated vitellogenin in male SMB, indicative of exposure to endocrine disruptors, have also been observed in SMB from these areas. The current theory behind health issues in the region is that a combination of parasites, pathogens, and chemical stressors in the upper CBW are leaving sensitive fish populations on the brink between a sustainable healthy condition and a vulnerable condition where any additional stressor triggers disease and mass mortality.

Smallmouth bass were a primary species affected by the fish kills. They are not native to the CBW but were introduced around 150 years ago (Jenkins & Burkhead, 1994). They have become a major sport fishing species, and their population health is important to anglers and the public in general. They are a pelagic apex predator that bioaccumulate certain chemical contaminants such as mercury (Neumann & Ward, 1999; Blazer et al. 2022), and are a sensitive species that may be indicator species, responding before other species in a watershed. After reviewing fish health and population changes in SMB of the Susquehanna River, background fish health screening was recommended in important fisheries along with periodic comprehensive examinations to assess emerging disease and health changes (Schall et al., 2020).

Fish of the Catostomidae family (suckers) such as NHS and GR were involved in Potomac River fish mortality events. Suckers often receive less attention because they are not a sport fish. Suckers are a benthic species that typically feed on invertebrates. Unpublished reports from the West Virginia Department of Natural Resources notes mortality of “suckers or carp” as early as 1989 in the South Branch of the Potomac River, before SMB mortality brought attention to fish health issues in the river (Keplinger et al., 2022). Their involvement in fish kills indicates health issues spanning multiple trophic levels and taxonomic classifications. Monitoring sucker health may also be a priority in certain watersheds. One class of tools that could be used to monitor fish health are fish health indices. Fish health indices are comprised of multiple indicators of health quantitatively aggregated into one index (Mayer, 2008). Two commonly used indices which can be calculated in the field are Deformity, Erosion, Lesion, and Tumor counts (DELTs) (Sanders et al., 1999) and the Health Assessment Index (HAI) (Goede and Barton, 1990; Adams et al., 1993). These are both options for long term background health monitoring that could be applied widely because they require limited funds, training, and equipment. However, their utility in watersheds of the Chesapeake Bay needs to be demonstrated.

The DELTs Index

The DELTs assessment is based entirely on observation of grossly visible external anomalies. The popularity of DELTs is largely due to the Index of Biotic Integrity (IBI). The IBI uses fish community indicators to evaluate the biologic integrity of streams (Karr, 1981). One of the suggested indicators is the proportion of fish with “disease, tumors, fin damage, and other anomalies.” Surveying streams using the IBI typically involves non-lethal methods. Because recording DELT abnormalities is nonlethal and quick, this method has often been used for the fish health portion of the IBI. The IBI quickly gained popularity and by 1991 was implemented by at least 35 U.S. states, several U.S. federal agencies, multiple Canadian providences, and in France (Karr & Dionna, 1991). In 1987 the Ohio EPA included DELT anomalies as part of their stream monitoring program. The Ohio monitoring program is one of the most comprehensive assessments of the DELT index and was used to analyze seven Ohio streams with sampling points spread over large reaches of each waterway (Sanders et al., 1999). The study added to the growing body of research that demonstrated correlation between the proportion of fish with DELT anomalies and various forms of chemical pollution, often from industrial and municipal discharges. Because many of the fish kills in the Chesapeake involved adult fish with lesions on their body surface, it makes intuitive sense to monitor the prevalence of external abnormalities to identify stressed populations.

External Anomalies as Indicators of Fish Health

External anomalies also make up a large portion of the HAI. Since at least the 1960s studies have suggested a relationship between external fish abnormalities and pollution. An early example was a head deformity in carp called “knothead” that was thought to be correlated with areas of increased pollution in the Illinois River (Mills et al., 1966). In more recent years, fish with tumors and other deformities often observed externally have been associated with pollution from legacy compounds such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and heavy metals (Simon & Burskey, 2016). These contaminants can result from heavy industry and have been of concern in the Great Lakes region of the United States and Canada. Tumors and other deformities are listed as a Beneficial Use Impairment (BUI) in many Great Lakes Areas of Concern (AOC) (United States Policy Committee, 2001). The AOCs are ecologically degraded sites that are prioritized for restoration. The Grand Calumet River of southwestern Lake Michigan is listed as an AOC. Sediment samples from the river show high levels of PCBs, PAHs, heavy metals, and other contaminants (MacDonald et al., 2002). Bluntnose minnows *Pimephales notatus* sampled from the Grand Calumet River displayed DELTs on 70.0% of sampled individuals compared to 16.7% at the Little Calumet River, a less impacted reference site (Simon & Burskey, 2016). A study in 1987 found high incidence of skin tumors in brown bullhead *Ameiurus nebulosus* from the industrial Black River, a tributary of Lake Erie located in Ohio (Baumann et al., 1987). The Black River also had high sediment PAH levels. Raised pale lesions on the lips and body surface of white sucker *Catostomus commersonii* (WS) were more prevalent at heavily industrialized tributaries of Lake Ontario in Canada (Smith et al., 1989). These contaminant sources and external fish anomalies are not unique to the Great Lakes. Rocky Fork, a small stream in Mansfield Ohio, is part of the Ohio River Watershed. At the time of the study, the stream received industrial and municipal effluent and was contaminated with heavy metals (Reash & Berra, 1989). Fin erosion prevalence and severity was higher at Rocky Fork compared to the nearby and less impacted Clear Fork. Many species were affected but WS, creek chub *Semotilus atromaculatus*, central stoneroller *Campostoma anomalum* and gizzard shad *Dorosoma cepedianum* displayed the most fin erosion. The DELT prevalence was significantly elevated in a study on the polluted Felix Reservoir of the Ebro River, Spain (Benejam et al., 2010). The reservoir is impacted by a long-standing organochloride industry. The industry has created high sediment concentrations of heavy metals (primarily mercury), PCBs, DDT, and radioactive ^{210}Pb. Common carp *Cyprinus carpio,* roach *Rutilus rutilus,* and pumpkinseed *Lepomis gibbosus* showed high DELT counts in the reservoir. Industrial and urban locations in the CBW have similar contaminant issues and fish displaying associated external anomalies. Adult brown bullhead (>260mm total length) had significantly higher deformities including eroded or clubbed barbels, and skin tumors in the Anacostia River, Washington DC compared to the less impacted Tuckahoe River, Maryland (Pinkney et al., 2004). The land surrounding the Anacostia is highly developed, and biliary PAH–like metabolite levels were elevated in large Brown Bullhead from this site. Mummichogs *Fundulus heteroclitus* collected from Elizabeth River, Virginia showed neoplasms in areas contaminated with PAHs (Hargis et al., 1989). The neoplasms were primarily oral papillomas. The above examples of externally visible anomalies are all associated with urbanization, industry and their associated contaminants – particularly legacy compounds.

Legacy compounds and urban/industrial effluent are an issue in areas of the CBW, but they are not believed to be the only driver behind large scale fish health issues and mortality in non-tidal freshwaters. Many of the rivers with noted health issues are predominantly surrounded by a mixture of forest and agricultural land-use. Endocrine disruption, pesticide contamination, higher stream temperatures, eutrophication and sedimentation can result from agricultural practices and impact fish health. Pesticide contamination resulting from agriculture was suggested as a risk factor for intersex in SMB of the Chesapeake Bay watershed (Iwanowics et al., 2009). Elevated levels of intersex are a sign of endocrine disruption. Endocrine disruptors can also modulate immune function, leaving fish more vulnerable to disease. Industrial pollution inputs are certainly still an issue in areas of the non-tidal CBW. Multiple tributaries of the Shenandoah River have high levels of mercury from historic industrial sources (Eggleston, 2009). These additional pollution sources add to the stress on sensitive fish populations and complicate interpretation of cause behind health issues. Industrial carcinogens such as PAHs and heavy metals may directly cause external tumors (Logan, 2007). Teratogenic compounds may trigger grossly visible deformities early in life. Chemical contaminants interact with disease agents and parasites which may also induce external anomalies (Baumann et al., 1996). Age, sex, reproductive status, environmental and climatic variables influence the interactions of these stressors and their impact on wild fish health (Hamilton et al., 2016). Changes in pH, hardness and alkalinity affect the toxicity of heavy metals (Paquin et al., 2002). Species life history and feeding habits influence exposure to chemical contamination. Vulnerability to various toxicants change with life-stage. Species and inter-species population differences also influence vulnerability to contamination. Chemical pollution affecting wild fish typically involves complex mixtures of contaminants. Even if individual chemical concentrations are well below establish toxicity levels, there may be interactions with other contaminants that increases toxicity. Accounting for all these factors at once is not easily accomplished. Biologic endpoints of stress such as external abnormalities provide a first step of health evaluation that can direct further research.

The HAI

In 1990 Goede and Barton published a necropsy method for rapidly evaluating fish health and condition in the field (Goede & Barton 1990). This method involves recording both internal and external anomalies, and a few blood parameters. Differences within these categories can be compared between groups to evaluate the general health of a population. This method was designed using only parameters that could be evaluated in the field to provide a rapid assessment of fish health allowing timely remedial or corrective action. A few years later, this necropsy method was modified by adding numeric ratings to anomalies allowing easier statistical comparison between populations (Adams et al., 1993). This method was named the Health Assessment Index and has been applied to multiple species in many different systems throughout the world (Coughlan et al., 1994; Sutton et al., 2000; Lohner et al., 2001; Kovacs et al., 2002; Adams et al., 2003; Chaiyapechara et al., 2003; Schleiger, 2004; Hinck et al., 2007; van Dyk et al., 2009; Merten et al., 2010; du Preez & Wepener, 2016; Abraham et al., 2019; Oh et al., 2020). The index is made up of categorized anomalies that are assigned a score based on the relative severity of the abnormality. The more severe an observation, the higher it will be scored in the index. The combined scores of all observations from one fish become the HAI value for that individual, and higher index values indicate a fish with poor health. The distribution of HAI values can then be compared between samples to identify stressed fish populations. HAI scores for largemouth bass *Micropterus salmoides* (LMB) followed a gradient of PCB contamination in the Hartwell Reservoir on the South Carolina – Georgia border (Adams et al., 1993). The LMB HAI scores were also highest in a reservoir with the worst water quality out of 28 reservoirs managed by the Tennessee Valley Authority. LMB HAI scores positively correlated sediment mercury, PCB and arsenic concentrations in the Clinch River and Watts Bar Reservoir of Tennessee (Adams et al., 1999). Recently HAI scores were shown to be correlated with total mercury tissue concentrations in SMB within the CBW (Blazer et al., 2023). Redbreast sunfish in the Pigeon River of Western North Carolina had the highest HAI scores near an area impacted by kraft mill effluent (Adams et al., 1993). Pooled SMB and LMB HAI values were higher in the lower Colorado River compared to the upper river and tributaries (Hinck et al., 2007). The lower Colorado river is down stream of many anthropogenic contaminant sources including agricultural inputs, urban discharges, and mining activity. White sucker had higher HAI values below two pulp and paper mills in the St. Francois River, Quebec, Canada (Kovacs et al., 2002). However, age differences in WS samples were listed as a confounding factor. An artificial stream study exposed WS and SMB to fine sand and agricultural soil loads (Merten et al., 2010). WS tended to have higher HAI values when exposed to both sediment types. The SMB HAI scores did not show a clear relationship with sediment treatments.

The HAI has also been used in aquaculture. The HAI was applied to four carp species (*Catla catla, Labeo rohita, Cirrinus mrigala* and *Labeo bata*) cultured in West Bengal, India (Abraham et al., 2019). HAI values were negatively corelated with temperature for all species with HAI values increasing progressively from May through December. This timeline also corresponded with increasing rain during the monsoon period. There were significantly different HAI scores between rearing ponds and species. Ponds that were managed poorly with reduced water quality had the highest HAI values. A recent study on hatchery rainbow trout *Oncorhynchus mykiss* used a modified health assessment to investigate the health of trout from two different sources (Martinelli et al., 2020). Hatchery staff believed fish from one source were less healthy, with suppressed growth rates, but the health assessment found the fish to be similar. This prompted the discovery of a growth rate calculation error which had deflated the growth rates of trout from that source. Once the calculations were fixed, there was no longer a significant growth rate difference.

The HAI is based on necropsy observations, consequently fish must be sacrificed to calculate scores. This index provides more information than DELT anomaly counts by including internal abnormality observations and blood parameters, but this information comes at the cost of a sample of fish. If the additional information provided is not worth this cost, the DELTs assessment may be the preferred tool. The extra information provided by the HAI is still largely based on gross observations and doesn't allow for diagnosis of disease and etiology. Procedures including histopathologic evaluation are necessary for identifying the cause of the lesion (bacteria, parasite, neoplasia, inflammation etc).

A recent study in the South Branch of the Potomac River used external and gill anomalies to monitor fish health over multiple years and seasons (Keplinger et al., 2022). Smallmouth bass and GR were sampled in all four seasons from 2008 – 2013. This study found gill, body, and fin lesions to be more severe in the summer and fall for both species. Low pH and low discharge were correlated with an increase in raised body lesions on GR, body erosions in SMB and GR, and erosion of gill lamellae in SMB. Large declines in SMB between age 2 and 3 corresponded with high gill abnormalities. The correlations seen in the South Branch of the Potomac River study provide an initial screening of health issues. Future studies can use this information to focus their diagnostic efforts on gill abnormalities in age 2-3 SMB or the mechanisms behind low pH, discharge and associated lesions and erosions, also suggesting that an index beyond just external abnormalities may be more beneficial. Neither DELT counts nor the HAI are intended to be diagnostic, however, they may play an important role in identifying health issues affecting freshwater species of the CBW. The HAI includes abnormalities observed on internal organs and a few blood parameters. This additional information may be necessary to identify stressed populations that do not exhibit their condition externally.

Histopathology

Histopathology, or evaluation of pathological changes at the cellular level, is a powerful tool in the field of fish health. It allows for identification of infectious agents, tissue stress response such as inflammation, and other indicators of fish health issues such as testicular oocytes and macrophage aggregates. Histopathology is frequently used in ecotoxicology. It picks up a broad range of toxic effects. Histopathology can also detect early changes in cell and tissue function before they significantly impact population health. SMB and redbreast sunfish sampled around the time of mortality events in the Potomac River drainage displayed microscopic cellular changes indicating complex etiology behind skin lesions and mortality. Microscopic pathology found skin lesions with inflammation extending as far as the underly musculature. Most often lesions were composed of epidermal sloughing with acute inflammation of the dermis and hypodermis. Many of the lesions had rods consistent with *Aeromonas* sp. and *Flavobacterium* sp. Pale and ulcerative lesions observed grossly were covered with fungal hyphae that penetrated necrotic epidermis and dermis. Raised mucoid lesions were papillomas or areas of epidermal thickening. Approximately 20% of fish sampled during mortality events had signs of systematic *A. salmonicida* infection in the liver, kidney, and spleen. Many parasites including trematodes, acanthocephalans, and myxozoans were found internally. There were also cellular changes associated with chemical contaminant exposure. Many SMB males had testicular oocytes. Smallmouth bass also had focal areas of liver necrosis and regeneration as well as numerous macrophage aggregates in the liver, kidney, and spleen. Bile duct proliferation and increased interrenal tissue in the anterior kidney was present. Gill hyperplasia and hypertrophy affected multiple fish (Blazer et al., 2010). Histologic evaluation of age-0 SMB from the Susquehanna River Basin found various parasitic infections including cestodes, trematodes, and myxozoans (Walsh et al., 2018).

None of these cellular observations would be possible from gross visual examination. Lesions caused by different stressors may appear similar grossly. Others are not visible at all. Parasites may create cysts that look like tumors. Cysts caused by different parasite species may also look the same. A benign growth cannot be differentiated from a malignant tumor without the aid of histopathology. These limitations complicate the determination of causes behind index values based on gross observation. Indices have also been created using metrics derived using histology. Microscopic changes can be rated and compared between sites. Macrophage aggregates (counts per sq. mm.) and testicular oocytes (0-4) are often rated to compare severity along with prevalence in the population (Blazer et al., 2007). Barnet et al. (1999) created a histology-based scoring system designed to systematically compare cellular changes associated with exposer to contamination. This tool generates a “score value” for each cellular anomaly that was then combined with a pathologic “importance factor.” The sum of score values and importance factors could then be calculated for an individual fish or organ and compared between sites. However, the downside of histopathology is that it requires lethal sampling, is time consuming and requires training for collecting and processing the tissues and extensive training and experience to accurately evaluates cellular changes.

Research Objectives

The goal of this research was to evaluate the DELTs and HAI indices as potential fish health monitoring tools in non-tidal tributaries of the Chesapeake Bay. To that end data will be utilized from two projects. Chapter 2 evaluates the two indices applied to multiple species in 33 small tributaries of the Shenandoah River. These sites are spread over a gradient of upstream pastureland and agricultural Best Management Practice (BMP) implementation. BMPs are conservation practices developed by U.S. Department of Agriculture Natural Resource Conservation Service to reduce sediment and nutrient flow into the Chesapeake Bay (Hively et al., 2018). Some may also reduce the overland flow of certain pesticides (Smalling et al., 2021). Goals of the Shenandoah study are to: (1) investigate potential relationship between indices, agricultural land-use, and BMP application; (2) compare the DELT index application on fish aggregation samples with the HAI use on white sucker *Catostomus commersonii*, and fantail darter *Etheostoma flabellare* and (3) use histopathology to describe cellular changes behind unusual anomalies observed grossly. Chapter 3 will focus on SMB from 2 sites in the Upper Potomac River Basin and 3 sites in the Susquehanna River Basin spread over a gradient of agricultural land-use. Sites were sampled in the spring and fall from 2013 – 2020. The goal of this study is to evaluate temporal relationships between the HAI, DELTs, age, sex, season, discharge, and site.

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Chapter 2

Two Field Based Fish Health Indices Applied in Streams with a of Gradient of Catchment Pastureland in the Shenandoah Valley of VA and WV

Abstract

Fish kills, increased disease prevalence, and endocrine disruption have been observed in multiple freshwater fish populations of the Chesapeake Bay watershed. In addition to fish kills, some of these health issues occurred in conjunction with declining abundance. A combination of multiple stressors is believed to be weakening sensitive fish species in non-tidal waters of the Chesapeake. Agriculture is practiced heavily in these watersheds. Poor agricultural practices can increase stress on sensitive fish species through changing stream temperature, increasing suspended solids, nutrient loading, pesticide contamination, and hormone pollution. The impact of pastureland on fish health was evaluated in this study using a Health Assessment Index (HAI) and Deformity, Erosion, Lesion, and Tumor counts (DELTs). The impact of Best Management Practices (BMPs) meant to reduce the environmental impact of agriculture was also investigated. DELTs were calculated for fish aggregations in 33 wadable streams with a gradient of catchment pastureland (primarily cattle) in the Shenandoah Valley of Virginia and West Virginia. HAIs were calculated for white sucker *Catostomus commersonii* and fantail darter *Etheostoma flabellare* at a subset of those sites (n = 15 and n = 20, respectively). Tissue samples were evaluated histologically from fish with unusual DELTs. Neither the HAI nor DELTs correlated with land-use variables. HAI values for white sucker were best predicted by age. Incidence of DELTs in fish aggregations was low and species dependent. An effect of pastureland and BMP implementation was not seen in this study. Future research directions and limitations of the HAI and DELTs are discussed.

Introduction

The health of certain fishes in the non-tidal Chesapeake Bay watershed (CBW) has come into question in the last 20 years. Fish mortality events in the Potomac River and surrounding watersheds occurred frequently in the early 2000s. These events involved large numbers of adult fishes sometimes displaying external lesions and occurred most often in the spring. Species involved in the Potomac mortality events included smallmouth bass *Micropterus dolomieu*, redbreast sunfish *Lepomis auritus*, rock bass *Ambloplites rupestris*, northern hog sucker *Hypentelium nigricans*, and golden redhorses *Moxostoma erythrurum* (Blazer et al., 2010) In the Susquehanna River drainage mortality of age-0 smallmouth bass was noted beginning in 2005 (Smith et al., 2015) and population declines were also documented (Schall et al., 2020). Research on the cause(s) of morbidity and mortality in effected rivers points to a complex etiology. In both adults and young bass, multiple infectious agents including several bacteria species (*Aromonas hydrophila, A. salmonicida, and Flavobacterium columnare*), Largemouth Bass Virus, and various parasites such as trematodes and myxozoans were isolated from fish in these areas (Starliper et al. 2013; Walsh et al., 2018). In addition to signs of infectious disease, signs of endocrine disruption in the form of testicular oocytes and high levels of vitellogenin in male SMB were also documented (Blazer et al., 2012).

Fish in these rivers are exposed to a myriad of pollutants from various sources that may be impacting their health. Toxicity of contaminants may be influenced by water chemistry which is in turn influenced by land-use, weather events, and climatic trends. The watersheds surrounding these rivers are primarily covered in mixed forested and agricultural land with smaller areas of development. One of the common agricultural land-uses is pastureland which can be a source of phosphorous and nitrogen (Vasconcelos et al., 2007) as well as hormones

(Johnson et al., 2006). Nutrients (ammonia, nitrite) can be directly toxic to fish at high levels or indirectly impact fish health by triggering stream eutrophication. Eutrophication may result in toxic algae blooms or larger populations of fish parasite vectors (Marcogliese et al., 2021). Pastureland can also increase the temperature and sediment load in streams impacting fish physiology (Kemp et al., 2011; Little et al., 2020). All these factors interact with potential impacts on the health of sensitive fish species.

U.S. Department of Agriculture Natural Resource Conservation Service developed best management practices (BMPs) designed to reduce the flow of sediment and nutrients into the CBW (Hively et al., 2018). They can also reduce the flow of other contaminants such as pesticides into streams of the watershed (Dellaha, 1990). However, BMPs are not mandatory and therefore are not universally applied. They were also not designed specifically to protect fish health.

A study was initiated by the U.S. Geological Survey and collaborators to assess effects of pastureland and associated BMPs on stream health within the Shenandoah Valley (James and Shenandoah/Potomac watersheds) as part of the USGS Chesapeake Bay Land Management program. One aspect of this study was to document potential effects of pastureland and BMPs on fish communities and fish health. Fish health indices are a popular class of tools used to monitor fish health. They are created by quantitively aggregating multiple health indicators to produce a single value for each fish. Indices can vary from simple counts of external abnormalities to in depth assessments at multiple levels of organization from the organismal to the molecular. Resulting values can then be used as a proxy for the health of that individual, population, or community depending on the design of the study. Two methods based on gross visual observation are DELTs (Deformities, Erosions, Lesions, and Tumors) and HAI (Health

Assessment Index). DELTs are one of the simplest fish health indices. Counts of these anomalies can be recorded during routine population surveys. They are observed externally making them nonlethal, counting them requires no additional equipment, and identification of the anomalies is easily learned. In some species and sites these types of anomalies have been correlated with various sources of pollution and stream degradation, most often from urban or industrial activity (Sanders et al., 1999; Simon & Burskey, 2016). Studies investigating DELTs response to agricultural land-use influences are not available. The Health Assessment Index (HAI) first described by Goede and Barton (1990) and modified by Adams et al., (1993) is a more detailed fish health index that is based on a fish necropsy procedure. It has been used throughout the world in many wild and cultured fish populations (Coughlan et al., 1994; Raymond & Shaw, 1997; Sutton et al., 2000; Lohner et al., 2001; McKinney et al., 2001; Kovacs et al., 2002; Adams et al., 2003; Chaiyapechara et al., 2003; Schleiger, 2004; Hinck et al., 2007; van Dyk et al., 2009; Merten et al., 2010; du Preez & Wepener, 2016; Abraham et al., 2019; Oh et al., 2020; Blazer et al. 2023). The metrics in the HAI are based on gross observation of tissue anomalies along with a few blood parameters. Both the HAI and DELTs indices are simple procedures that can be performed in the field. If either or both indices effectively evaluate the health of wild fish populations in the region, they could be more widely applied. However, they both need to be validated in the freshwaters of the CBW. This study evaluates the HAI and DELTs indices for use in the CBW by evaluating their utility in mixed forest and pastureland settings with varying levels of BMP implementation.

Materials and Methods

Site Selection and Fish Collection

Fish communities in 33 wadable streams of the Shenandoah Valley, Virginia and West Virginia were sampled (Figure 1). Sites were selected along a gradient of upstream proportion pastureland and BMP Ecological Impact Intensity - a metric based on the extents of ecologically relevant BMPs in the watershed. Upstream proportion pastureland is based on National Land Cover Database (NLCD) code 81 (Pasture/Hay) within the drainage area of the sampled stream COMID (Dewitz & U.S. Geological Survey, 2021). The COMID is the unique code identifying each stream in the National Hydrography Data Set Plus (NHDS+) (Wieczorek et al., 2018). The BMP Ecological Impact Intensity metric is the sum of extent in square kilometers of each ecologically relevant BMP in the COMID divided by the area of agricultural land-use (NLCD codes 81 and 82) (Olivia Devereaux of Devereux Consulting, Inc. & Matthew Cashman of the U.S. Geological Survey, personal communication). NLCD code 82 indicates cultivated crops. Because multiple ecologically relevant BMPs can be practiced on the same section of land, the BMP Ecological Impact Intensity metric can be greater than 1. BMPs were determined to be ecologically relevant if they affected one of these categories: herbicide reduction, infiltration, phosphorus reduction, sediment reduction, runoff control, and temperature reduction (Table 1). Some other BMPs not in the listed categories were added individually as deemed necessary, such as fencing. Fish collection utilized 2 pass backpack electrofishing with block nets when a natural barrier was unavailable. At each site two stream segments were sampled. Stream segments were a minimum of 20 channel widths long with at least two pool-riffle sequences.

A subset of the 33 sites were selected for calculation of HAI for fantail darter *Etheostoma flabellare* and white sucker *Catostomus commersonii*. HAI calculation was performed on white sucker at 15 sites with proportion pastureland ranging from 0.08 to 0.58 and BMP Ecological Impact Intensity ranging from 0.19 to 5.02. HAI calculation was performed on fantail darter at

20 sites with proportion pastureland ranging from 0.08 to 0.64 and BMP Ecological Impact Intensity ranging from 0.19 to 5.02. Both species were concurrently evaluated at 8 sites. See Table 2 for a summary of where each health evaluation occurred.

Field Fish Health Evaluations

All fish observed during community sampling were evaluated for DELTs while identifying and enumerating species. The count of each anomaly type (deformity, erosion, lesion, or tumor) was recorded by species at each site. Anomalies were defined according to the system in Sanders et al., (1999). Deformities include any deformed structure: spine, fin ray, head etc. Erosions include erosions of fins, operculum, or barbels. Lesions include sores, exposed tissue, or ulcerations. Discolored areas that visually appeared pathologic, such as mucoid areas, were also considered lesions. Tumors were defined as any grossly visible raised area. Anomaly types were counted for each species at each site. If a fish had more than one anomaly, both anomalies were counted for that species at that site. Fish with particularly unusual anomalies were vouchered for histopathologic evaluation. If a site had enough white sucker or fantail darter, a wild fish necropsy was performed (Blazer et al., 2018). All fantail darter samples consisted of 20 individuals. White sucker sample sizes range from 14-20. Observations from the necropsy were used to calculate HAIs. A minimum size for white sucker HAI calculation was set at 125mm total length. A few fish sampled early in the study were below this limit. This reduced the range of sample sizes per site for fish with HAI calculation to 11-20 with an outlier site with only 3 fish within the size range. The necropsy procedure and HAI was modified for each species. HAI for white sucker included 13 of the original 14 variables from Adams et al., (1993). Thymus observations included in the original index were removed. The fantail darter HAI included 7 of the original parameters. Internal organs in the fantail darter were too small to reliably observe grossly. For this reason, they were removed from the HAI. The included parameters for fantail darter are fins, skin, eyes, gills, parasites, hematocrit and leukocrit. For a summary and descriptions of categories included in HAIs for both species see Table 3.

Both species were euthanized with an overdose of MS-222 before beginning necropsy procedure. External and gill abnormalities were noted prior to obtaining a blood sample. For fantail darter, the caudal peduncle was cut to expose the caudal vein. A heparinized microcapillary tube was used to collect blood at the site of the laceration. Darters were then cut along the ventral surface of the abdomen to expose the body cavity for proper fixation. The whole body was preserved in Z-Fix (Anatech Ltd, Battle Creek, MI). White sucker blood was collected from the caudal vein using a 23-gauge needle and 3 cc syringe and placed in heparinized microcapillary tubes. When possible, two tubes of blood were collected. The body cavity was then exposed, and grossly visible anomalies were recorded for the spleen, kidney, liver, pseudobranch and hindgut. Sections of the gills, spleen, anterior kidney, liver, and any anomalies were placed in Z-Fix for histopathologic evaluation. Lapillus otoliths were removed from white sucker for aging.

Microcapillary tubes for both species were centrifuged in the field with a battery powered micro-hematocrit centrifuge (EKF Diagnostics' HemaStat II, Cardiff, United Kingdom). Hematocrit percentages were recorded using the sliding reader on the front of the centrifuge. Leukocrits, when visible, were read using a hematocrit card. Fantail darter blood samples were small, typically filling 1/8 the length of the microhematocrit tube. The small blood sample prevented analysis of fantail darter plasma protein. Plasma protein was analyzed for white sucker. After reading white sucker hematocrit and leukocrit, the microcapillary tube was snapped above the packed erythrocyte portion of the tube to obtain plasma. Plasma protein was initially read using an electronic refractometer (Fisherbrand Handheld Digital Brix/RI Refractometer, Waltham, Massachusetts), but an analog refractometer was used for most sites because it was better suited for field use. All blood parameters for white sucker were sampled twice when possible and the average of the two replicates was used in calculation of the HAI. The small blood samples from fantail darters prevented replication. Normal ranges for white sucker hematocrit, leukocrit and plasma protein were based on a previous study using an HAI for white sucker (Kovacs et al., 2002). Normal hematocrit range was not available for fantail darter and was therefore estimated from the sample using methods described in Kovacs et al., (2002). Normal ranges are based on 2 standard deviations above and below the mean.

Laboratory Processing

Lapillus otoliths from white sucker were aged using a process described in Koch and Quist (2007). The process was optimized for white sucker otoliths in Blazer et al., (2019). Otoliths were imbedded in epoxy and sectioned using a low-speed saw (Buehler IsoMet Low Speed Saw, Lake Bluff, Illinois). Sections were then aged by two independent readers under light microscopy. Any disagreements were revaluated by the readers and a consensus age was determined. Fantail darter were not aged because of their short life span of typically 3-4 years (Roberts & Angermeier, 2007). Sex of fantail darter was determined microscopically from fish with histologic evaluation. White sucker sex was determined in the field and validated by histologic evaluation when available.

Fixed tissue from both species was routinely processed, mounted in paraffin, sectioned at 6 µm, and stained with hematoxylin and eosin (Luna 1992). Sections of fantail darter included the head cut along the medial plane. The rest of the body was sectioned in transverse cuts until all internal organs were sectioned. Additional cuts were made of any grossly visible lesions not in one of the sections. Samples of each type of tissue saved from white sucker were sectioned prioritizing grossly abnormal areas within the tissue. Tissue was analyzed with light microscopy by an experienced fish histopathologist for sex verification and etiology behind select anomalies.

Quantitative Analysis

DELT anomalies were organized by species. The number of sites with a DELT anomaly for each species was recorded. The number of DELTs compared to the count of each species was recorded as a percentage. Total counts of anomalies for each species were also noted. Statistical analysis of DELTs data was not performed because of low incidence and species dependance.

The original HAI from Adams et al., (1993) rated severity of an anomaly within each category from 0 to 30 in increments of 10. The index in this paper was modified to list anomalies in increasing severity from 0 to 3 in increments of 1. This change was made to better reflect the categorical and ordered nature of the data. Ordinal logistic regression was selected to analyze HAI data (Gelman & Hill, 2006). Model selection was performed using Akaike Information Criterion (AIC). Candidate models for white sucker HAI values included all combinations of proportion pastureland, BMP Ecological Impact Intensity, age and sex as predictors. Fantail darter HAI candidate models included all combinations of proportion pastureland, BMP Ecological Impact Intensity, length, and sex as predictors. Length was used as a proxy for age in fantail darter. Ordinal logistic regressions with random effects were fit using “clmm()” function in package “Ordinal” (Christensen, 2022) in the program R version 4.1.1 (R Core Team, 2021). All models were run with a random site effect. This was done to account for repeated measures at each site. Parametric bootstrapping was used with 1000 iterations to generate prediction intervals (PIs) surrounding model estimates. An alpha of 0.05 was used to test for significance in all analyses.

Results

DELTs

DELTs were recorded for 39,151 individuals from 11 different families and 47 species not including hybrids. There were 2 sunfish (*Centrarchidae*) hybrids and one minnow (*Leuciscidae*) hybrid. There were 24 species not including hybrids that showed no DELT anomalies (Table 4). Overall incidence of DELTs was low and species dependent. For all species the percentage of fish with DELT anomalies was 0.29%. Note that some fish had multiple DELT anomalies. Because data were recorded in aggregate for each species at each site, multiple anomalies on one fish could not be differentiated from anomalies on multiple separate fish. A single fish with multiple anomalies was extremely rare except at one site where multiple common carp *Cyprinus carpio* had more than one DELT anomaly. Many of the species encountered in this study were small minnows in the family *Leuciscidae* and darters (*Percidae*). Common carp were only observed at one site. At this site every carp had a DELT anomaly, and many had multiple anomalies. Nine common carp were captured with 13 DELTs yielding a mean DELTs per fish of 1.44. DELTs on carp from this site made up 11.4% of all DELTs observed in this study despite comprising 0.02% of the total fish sample. The species with the next highest percentage of fish with DELTs were brown bullhead *Ameiurus nebulosus* at 33.33% with 3 fish captured with 1 DELT anomaly total. Of the 18 minnow species captured, 8 had no DELTs. The 10 minnow species with DELT anomalies ranged in percent DELTs per fish from 0.06% to 0.68%. Of the 6 darter species captured, 2 displayed DELT anomalies. Fantail darter and longfin darter *Etheostoma longimanum* displayed 0.11% and 3.13% of fish with DELTs, respectively. All 3 species of sculpin (*Cottidae*) displayed DELT anomalies, but incidence was low ranging from 0.05% and 0.20% of fish. Lesions were the most recorded anomaly with a count of 50, followed by erosions with a count of 40. There were 15 deformities recorded and 9 tumors. Most tumors were found on white sucker (4) and common carp (2), with 1 tumor each found on checkered sculpin *Cottus n.sp.* (formerly *Cottus cognatus*), rosyside dace *Clinostomus funduloides*, and yellow bullhead *Ameiurus natalis*. See Table 5 for a summary of DELT observations by species.

HAI

White sucker HAI scores (n=240) were best explained by a model with age as the only predictor. The age range of white sucker in this study was 1 to 8 with a median of 2. White sucker HAI scores ranged from 0 to 16 with a median of 2. See Figure 2 for box plots of HAI scores at each site. Based on the selected model, the probability of an age-1 white sucker having a low HAI value of 0 or 1 was 0.42 (95% PI: 0.17 - 0.71). An age-8 fish had a 0.10 (95% PI: 0.03 – 0.24) probability of displaying an HAI value of 0 or 1. On the other end of the HAI spectrum, an age-8 white sucker had a 0.20 (95% PI: 0.05 - 0.47) probability of having an HAI value greater than 9. An age-1 fish had a 0.04 (95% PI: 0.01 - 0.11) probability of having an HAI value greater than 9. White sucker with intermediate ages followed the same pattern of high HAI values becoming more probable in older fish. See Figure 3 for a visualization of white sucker HAI model predictions. Young age-1 and age-2 white sucker were very common making up 77% of individuals with an HAI value. The study contained 6 sites with white suckers of at least age-4. Of those 6 sites, 5 showed a positive linear relationship between age and HAI value (see Figure 4).

Sex was the only predictor of fantail darter HAI value in the best model indicated using AIC model selection. However, the 95% confidence interval on the coefficient for sex overlapped with 0. The fantail darter sample was made up of 64% males and 46% females. Fantail darter HAI values ranged from 0 to 7 with a median of 0. Sex data were only available for fish that were sampled for histologic evaluation and could not be determined if gonad tissue was absent from tissue cross sections. This limited sex data to 165 fish with an HAI score.

Histopathology

Microscopic analysis was used to evaluate certain visual abnormalities. Several white suckers at Long Glade Creek (LGC) had external growths which were recorded as “tumors” according to the DELTs definition. One fish had 6 to 7 of these growths causing significant disfigurement on both sides. Under microscopic evaluation the cysts were related to a myxozoan skin infection causing inflammation (Figure 5). They were therefore not caused by neoplasia – the traditional definition of a tumor.

Discussion:

DELTs were uncommon in this study. Only 0.29% of fish displayed DELTs. This is likely due to a combination of the types of pollution impacting the streams investigated, and the species present. One of the most comprehensive evaluations of DELTs evaluated their utility in 7 streams of Ohio (Sanders et al., 1999). This study found DELTs in 2.6% of fish examined. The stream segments in that study ranged in upstream catchment from 6,838 hectares in a small tributary creek to 19,515,560 hectares in a section of the Ohio River. Sources of pollution in these streams and rivers included wastewater operations, industrial activity, and highly developed areas. The inclusion of larger order streams in different watersheds influences species presence and abundance (Paller, 1994). Our research in the Shenandoah Valley occurred entirely in small, wadable streams without major industrial or developed areas upstream. Common carp contributed heavily to the total DELTs in both studies. Common carp comprised 28% of fish with DELTs in the Ohio study despite representing only 2.6% of the total catch. In the current study, common carp represented 11.4% of DELTs while representing 0.02% of the catch. The larger catch of common carp explains much of the higher rate of DELTs in the Ohio study. The study in Ohio noted long-lived pollution tolerant species were more likely to display DELTs. More long-lived pollution tolerant species were captured in that study, and they contributed highly to DELTs. Two examples include channel catfish (*Ictalurus punctatus*) and green sunfish (*Lepomis cyanellus*) which contributed 8.9% and 6.8% of total DELTs, respectively. Channel catfish were absent from our study and green sunfish made up less of the sample compared to the Ohio study (1.12% versus 2.67%, respectively). These observations highlight the necessity of having similar species compositions between streams if comparisons of DELTs are to be meaningful. DELTs may be more useful in large surveys of one species, particularly if the species frequently displays DELTs. DELTs may not be the best for evaluating fish health in small streams with many short-lived species such as darters and small minnows. If these species are sensitive to pollution from pastureland, they may perish or leave the area before displaying grossly visible DELTs. It is also possible that the gradient of pastureland was not large enough to observe a relationship with DELTs. Agricultural pollutants such as pesticides and endocrine disrupting compounds may also be less likely to trigger grossly visible DELTs. Bioindicators of endocrine disruption such as testicular oocytes and elevated vitellogenin levels in males are not visible externally (Blazer et al., 2012). While risk factors eliciting responses such as intersex or endocrine disruption may not frequently trigger DELTs they may influence fish populations through reproductive impairment. If DELTs were used as the only fish health evaluation in regions of the CBW, important fish health concerns would be missed.

The HAI is a more comprehensive assessment as it includes external and internal abnormalities. Other studies have noted the influence of age on HAI score (Coughlan et al., 1994; Kovacs et al., 2002). Older fish may display more abnormalities possibly due to longer exposure to chemical stressors, more time to have sustained a physical injury, longer exposure to pathogens, and a changing immune system with advanced age (Manning & Tatner, 2014). A study looking at paper and pulp mill effluents in the Francois River, Quebec, Canada noted difference in HAI values between species along a gradient of age (Kovacs et al., 2002). Tessellated darters (*Etheostoma olmstedi*) in the study ranged in age from 1.9 to 2.8 years old and showed the lowest HAI values. Smallmouth bass were in the middle with an age range of 3.5 to 6.0 years with corresponding moderate HAI scores. White sucker had the highest age range from 4.3 to 7.6 and had the highest HAI values. Authors of that study mentioned that some of the difference could be related to life habits of the species. White suckers are a benthic species which are more likely to encounter the stream bed, exposing them to physical damage and any pollutants settled on the bottom. Smallmouth bass are a pelagic species with less exposure to the stream bed. Aside from typical age and life history variation, species also have different tolerances for pathogens, pollutants, and stream conditions. This limits the utility of comparing any health metric or index between species. HAIs in the current study were separately modified for fantail darter and white sucker making a direct comparison between the species impossible. Age does appear to be an important factor within species as observed in the analysis of white sucker HAIs. An age-8 white sucker, the oldest white sucker encountered, was predicted to have a 20% chance of an HAI value greater than 9. That was higher than the predicted 4% chance for an age-1 white sucker. This age dependance can be an issue when trying to identify unhealthy fish populations if age is nor accounted for in study design or model structure. Kovacs et al., (2002) identified the same issue within the Francois River, Quebec, Canada. One of the mill sites investigated had higher white sucker HAI values downstream, but there was a 2-year age difference between the samples. It is hard to know if the higher HAI values are due to degraded health related to the mill effluent, or an artifact from sampling fish with different ages. White sucker are not the only species where HAI values tend to be higher in older individuals. A study on largemouth bass (*Micropterus salmoides*) in the Catawba River in NC and SC found the HAI values were significantly and positively correlated with age (Coughlan et al., 1994).

While the relationship was not significant, sex was the best predictor of fantail HAI value according to AIC model selection. This is another relationship that could bias comparison of HAI values between two fish samples if the sex ratio is notably different. Age differences between fish samples could be reduced by creating a slot limit for sampled fish. This was recommended in the largemouth bass study on the Catawba River (Coughlan et al., 1994).

Limiting the size range of fish sampled would be easy in areas with a large fish population to draw from. However, in this study it was challenging to sample enough white sucker within designated reaches. Adding a length slot would increase the difficulty of finding sites with an adequate sample size. It could also be biased by sites with significantly different growth rates. Fish sex is hard to identify externally in most circumstances. Fish typically need to be euthanized and sexed internally. This makes achieving an even sex ratio between samples challenging. However, the relationship between HAI values and sex was not significant in fantail darters and was not included in the model that best explained white sucker HAI values. Ignoring sex between samples may not bias results as much as ignoring age, at least in white sucker and fantail darter sampled in the summer. Fantail darter and white sucker both spawn in the spring. Spring and fall are important periods in the reproductive cycle of fish that spawn in the spring. Spawning in the spring may cause differential stress between sexes. Sex dependent stress levels could also occur in the fall during recrudescence. If fantail darter or white sucker were sampled during those seasons, sex may become an important predictor of HAI value. Especially if fish in the region are experiencing endocrine disruption which is likely to impact male and female fish in different ways.

A recent study in the South Branch of the Potomac River, WV evaluated temporal trends in macroscopic indicators of fish health (Keplinger et al., 2022). Golden redhorse and smallmouth bass were evaluated for external and gill anomalies during seasonal surveys conducted from 2008 – 2013. The study found species, seasonal, annual, and life-stage differences in occurrence and severity of grossly observed lesions. Sex was not evaluated in the study. Adult smallmouth bass tended to have worse health metrics compared to juveniles. Golden redhorse adults typically had more body lesions than juveniles of the same species.

However, juvenile redhorses were more likely to have gill lesions. Body erosion scores were higher for both smallmouth bass and golden redhorse in 2010 and 2013, the two years fish kills occurred during the study. For both species gill, body, and fin lesions were the most severe in the summer and fall. Many of the fish kill events in the upper Potomac occurred in the spring during or just after the spawning timeframe for both smallmouth bass and golden redhorse. Grossly visible lesions may not occur immediately during the period of highest stress. Fish that are physiologically stressed during spawning may exhibit macroscopic lesions after the event. This may be the reason smallmouth bass and golden redhorse showed the most severe lesions in the summer and fall. Both fantail darter and white sucker spawn in the spring. This means summer may be the best time for health assessments based on grossly visible lesions such the HAI and DELTs. However, seasonal sample timing should be evaluated for each species specifically. Additionally, this trend may not hold up in different watersheds experiencing different fish health concerns. While sex was not evaluated in this study, seasonal, annual, life-stage and species differences were observed. All these factors need to be considered when using the HAI or DELTs to investigate or monitor fish health.

HAI values did not correlate with proportion pastureland or BMP Ecological Impact Intensity for either species. This could mean that pastureland and BMP implementations are not impacting the health of fantail darter and white sucker, the two land-use variables do not accurately represent the impact of pastureland or level of remediation achieved with BMP implementation, sample sizes were too small, or the HAIs for fantail darter and white sucker are not good tools for monitoring fish health impacts in a pastureland setting. Few past studies have used HAIs to evaluate the impact of agricultural pollution sources. An artificial stream study in Minnesota saw a weak relationship in white suckers exposed to agricultural soil loads with higher HAI values compared to controls (Merten et al., 2010). However, the relationship was not statistically significant. Most studies using HAIs have focused on pollution resulting from urbanization and industrial activity (Adams et al., 1993; Raymond & Shaw, 1997; Adams et al., 1999; Lohner et al., 2001; Kovacs et al., 2002; du Preez & Wepener, 2016). Pollutants in these studies include heavy metals, such as mercury and arsenic (Adams et al., 1999), and legacy compounds such as Polychlorinated Biphenyls (PCBs) (Adams et al., 1993). These types of pollutants may be more likely to accumulate and cause grossly visible abnormalities such as those included in an HAI.

One of the health concerns in a pastureland setting is endocrine disruption (Wang et al., 2004). Typical indicators for endocrine disruption in fish include testicular oocytes in males and elevated vitellogenin levels. More advanced methods include genomic techniques looking at transcripts associated with endocrine disruption (Walsh et al., 2022). None of these indicators are included in the HAI. It is possible that calculating HAIs at more sites could elucidate a relationship with proportion pastureland. However, evidence justifying a greater HAI sampling effort is lacking. As with DELTs, if HAI scores were relied on to monitor fish health in pastureland settings which are common in the CBW, important health concerns may be missed.

In this study histopathology was used to identify certain visible abnormalities. Tissue from multiple externally identified “tumors”, multiple large lumps on the sides of a white sucker, were identified as fluid filled cysts resulting from inflammation associated with a myxozoan infection (Figure 5). Another “tumor” involved a checkered sculpin with a large lump on its abdomen. When vouchering the sculpin for histologic evaluation, the lump was identified as a consumed fish significantly disfiguring the sculpin’s abdomen (Figure 6). The “tumor” and other DELTs categories can be potentially problematic, subjective, and lead to misunderstandings. The term tumor is defined as a raised area or swelling, caused by abnormal growth of tissue and most often is understood to be benign or malignant neoplasia. Many abnormalities identified as “tumors” when recording DELTs may not be neoplastic. Also, the terminology surrounding lesions and erosions may be problematic. The study in Ohio performed by Sanders et al., (1999) defined lesions as: open sores, exposed tissue, or ulcerations. Limiting “lesions” to open sores, exposed tissue, or ulcerations may cause confusion. Also, erosions, exposed tissue, and ulcerations typically all refer to the same thing – epidermis and sometimes epidermis missing (Roberts, 2012). Limiting “erosions” to eroded tissue on fins, gill covers, or barbels could be miss leading as well. While DELTs are not meant to be diagnostic, the “tumor”, “erosion”, and “lesion” definitions may cause confusion when interpreting results from a study using DELTs. Care must be taken when interpreting and communicating results from a DELTs study, making a clear distinction between the DELTs definitions and those typical to the field of fish health.

Several studies have developed more complex indices that include detailed histopathology or changes at the cellular level (Bernet et al., 1999; Zimmerli et al., 2007; McHugh et al., 2011; Liebel et al., 2013; Raskovic et al., 2013). However, as with all indices these index systems can be misused (Wolf & Wheeler, 2018). It is likely that a combination of indicators is needed at the organism, organ, cellular, subcellular, and molecular to fully evaluate health and the risk factors associated with adverse effects at specific sites.

Conclusions

An effect of pastureland or BMP implementation on DELTs and the HAI was not observed in this study. DELTs were rare and species dependent. White sucker HAI values were best explained by age. Age structure of fish samples for HAI calculation need to be similar for accurate site comparisons. Care must be taken when communicating the DELTs definitions for “erosion”, “lesion”, and “tumor” as they differ from the definitions typically used in the fish health field. Other health assessment methods including evaluations at the organism, organismal, cellular, subcellular, and molecular level are likely important for identifying fish health concerns - particularly in a pastureland setting.

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Tables

Table 1

Table lists ecologically relevant BMPs. Any of the below BMPs which are applied over the landscape are included in the BMP Ecological Impact Intensity metric. Data from internal U.S. Geological Survey report by Walker et al. (Not Published).

Practice Name	Practice Code
Agrichemical Handling Facility	309
Waste Management System	312
Waste Storage System	313
Animal Mortality Facility	316
Composting Facility	317
Channel Bank Vegetation	322
Deep Tillage	324
Conservation Cover	327
Conservation Crop Rotation	328
Residue and Tillage Management	329
Contour Farming	330
Contour Orchard and Other Perennial Crops	331
Contour Buffer Strips	332
Amending Soil Properties with Gypsum Products	333
Critical Area Planting	342
Residue Management, Seasonal	344
Residue and Tillage Management, Reduced Till	345
Residue and Tillage Management, Ridge Till	346
Sediment Basin	350
Waste Treatment Lagoon	359
Anaerobic Digestor, Ambient Temperature	365
Anaerobic Digester	366
Roofs and Covers	367
Windbreak/Shelterbelt Establishment	380
Fence	382
Field Border	386
Riparian Herbaceous Cover	390
Riparian Forest Cover	391
Filter Strip	393
Stream Habitat Improvement Management	395
Bivalve Aquaculture Gear and Biofouling Control	400
Grade Stabilization Structure	410
Grassed Waterway	412
Hedgerow Planting	422
Access Control	472

Table 1 (continued)

Practice Name	Practice Code
Mulching	484
Forage and Biomass Planting	512
Prescribed Grazing	528
Drainage Water Management	554
Roof Runoff Structure	558
Access Road	560
Heavy Use Area Protection	561
Trails and Walkways	568
Stormwater Runoff Control	570
Trails and Walkways	575
Stream Crossing	578
Streambank and Shoreline Protection	580
Channel Bed Stabilization	584
Structure for Water Control	587
Nutrient Management	590
Amendments for Treatment of Agricultural Waste	591
Feed Management	592
Vegetative Barrier	601
Herbaceous Wind Barriers	603
Saturated Buffer	604
Constructed Wetland	656
Wetland Restoration	657
Wetland Creation	658
Wetland Enhancement	659
Tree/Shrub Planting	660
Forest Stand Improvement	666
Agrichemical Mixing Facility	702
Barnyard Runoff Management	707
Agricultural Secondary Containment Facility	710
Stream Crossing	728
Cross Slope Farming	733
Denitrifying Reactor	747
Livestock Use Area Protection	757
Riparian Buffers - Vegetative	759
Alum Treatment of Poultry Litter	786
Transition to Organic Production	789
Residue Management, No-Till/Strip Till	329A
Residue Management, Mulch Till	329B
Residue Management, Ridge Till	329C
Prescribed Grazing	528A
Injecting or Incorporating Manure	AIR01
Nitrogen Stabilizers for Air Emissions Control	AIR02

Table 1 (continued)

Practice Name	Practice Code
Use Drift Reducing Nozzles, Low Pressure, Lower Boom Height and Adjuvants to reduce Pesticide Drift	AIR04
GPS, Targeted Spray Application (SmartSprayer), or Other Chemical Application Electronic Control Technology	AIR07
Nitrification Inhibitors or Urease Inhibitors	AIR08
Nitrification Inhibitors or Urease Inhibitors	AIR09
Extend Existing Filter Strips for Water Quality Protection and Wildlife Habitat	ANM04
Extend Riparian Forest Buffer for Water Quality Protection and Wildlife Habitat	ANM05
Extend Existing Riparian Herbaceous Cover for Water Quality Protection and Wildlife Habitat	ANM06
Extend Existing Field Boarders for Water Quality Protection and Wildlife Habitat	ANM07
Improve the Plant Diversity and Structure of Non-Cropped Areas for Wildlife Food and Habitat	ANM08
Grazing Management to Improve Wildlife Habitat	ANM09
Non-Forested Riparian Zone Enhancement for Fish and Wildlife	ANM13
Riparian Forest Buffer, Terrestrial and Aquatic Wildlife Habitat	ANM14
Multi-Species Native Perennials for Biomass/Wildlife Habitat	ANM23
Extend Existing Filter Strips or Riparian Herbaceous Cover	ANM32
Riparian Buffer, Terrestrial and Aquatic Wildlife Habitat	ANM33
Prescription Grazing Management System for Grazed Lands	ANM37
Forest Stand Improvement for Wildlife Habitat	ANM42
Creation and Retention of Snags, Den Trees and Course Woody Debris for Wildlife Habitat	ANM55
Removal of All Threats to Sensitive Wildlife Species in the Operation	ANM57
Crop Bundle#3 - Soil Health Rotation, No Till	B000CPL3
Crop Technology Bundle #1	BCR01
Crop Technology Bundle #2	BCR02
Crop Technology Bundle #4	BCR04
Crop Technology #6 (Improves Nutrient and Pesticide Application Techniques and Widen Buffers)	BCR06
Crop Technology Bundle #9 (Addresses Orchard and Vineyard Resource Concerns	BCR09
SE Pine Forest Bundle #1	BFO01
Forest Bundle #2	BFO02
Forest Bundle #3	BFO03
Forest Bundle #6 (Improves Wildlife Habitat and Soil Quality)	BFO06
Forest Bundle #8 (Improves Wildlife Habitat in Hardwood of Mixed Forests	BFO08
Pasture Grazing Bundle #1	BPA01
Pasture Bundle #4	BPA04
Pasture Grazing Bundle #7 (Improves Forage Utilization)	BPA07
Pasture Grazing Bundle #9 (Addresses Multiple Resource Concerns)	BPA09
Resource-Conserving Crop Rotation	CCR99
Conservation Cover to Provide Food Habitat for Pollinators and Beneficial Insects	E327136Z1

Table 1 (continued)

Practice Name	Practice Code
Conservation Cover and Shelter Habitat for Pollinators and Beneficial Insects	E327137Z
Conservation Cover to Provide Habitat Continuity for Pollinators and Beneficial Insects	E327139Z
Resource Conserving Crop Rotation to Reduce Water Erosion	E328101R
Resource Conserving Crop Rotation for Soil Organic Matter Improvement	E328106R
Soil Health Crop Rotation	E328106Z1
Modifications to Improve Soil Health and Increase Organic Matter	E328106Z2
Improved Resource Conserving Crop Rotation to Improve Soil Compaction	E328107I
Resource Conserving Crop Rotation to Improve Soli Compaction	E328107R
No Till to Reduce Water Erosion	E329101Z
No Till System to Increase Soil health and Soil Organic Matter Content	E329106Z
No Till to Increase Plant-Available Moisture: Moisture Management	E329115Z
Controlled Traffic Farming to Reduce Compaction	E334107Z
Intensive Cover Cropping to Increase Soil Health and Soil Organic Matter Content	E340106Z1
Use of Multi-Species Cover Crops to Improve Soil Health	E340106Z2
Use of SHA to Assist with Development of Cover Crop Mix to improve Soil health and Increase SOM	E340106Z4
Reduced Tillage to Reduce Water Erosion	E345101Z
Reduced Tillage to Increase Soil Health and Soil Organic Matter Content	E345106Z
Enhanced Field Boarders to Reduce Water Induced Erosion Along the Edges(s) of the Field	E386101Z
Enhanced Field Boarders to Increase Carbon Storage Along the Edges of the Field	E386106Z
Enhanced field border to provide wildlife food for pollinators along the edge(s) of a field	E386136Z
Enhanced field border to provide wildlife cover or shelter along the edge(s) of a field	E386137Z
Enhanced field border to provide wildlife habitat continuity along the edge(s) of a field	E386139Z
Increase riparian herbaceous cover width to reduce sediment loading	E390126Z
Increase riparian forest buffer width to enhance wildlife habitat	E391136Z
Manage livestock access to streams/ditches/other waterbodies to reduce nutrients in surface water	E472118Z
Manage livestock access to streams/ditches/other waterbodies to reduce pathogens in surface water	E472122Z
Mulching to improve soil health	E484106Z
Cropland conversion to grass-based agriculture to reduce water erosion	E512101Z1
Forage and biomass planting for water erosion to improve soil health	E512101Z2
Cropland conversion to grass-based agriculture for soil organic matter improvement	E512106Z1
Cropland conversion to grass-based agriculture to reduce sediment loading	E512126Z
Native grasses or legumes in forage base to improve plant community structure and composition	E512133Z1
Prescribed grazing that improves or maintains riparian and watershed function-erosion	E528105Z

Table 1 (continued)

Practice Name	Practice Code
Improved grazing management for soil compaction through monitoring activities	E528107Z1
Prescribed grazing that maintains/improves riparian/watershed function impairment from nutrients	E528118Z1
Grazing management that protects sensitive areas-ground water from nutrients	E528119Z
Prescribed grazing that maintains/improves riparian/watershed function-pathogens/chemicals	E528122Z
Prescribed grazing that maintains/improves riparian/watershed function-min sediment in surface water	E528126Z
Reduce risks of nutrient losses to surface water by utilizing precision ag technologies	E590118X
Improving nutrient uptake efficiency and reducing risk of nutrient losses to surface water	E590118Z
Improving nutrient uptake efficiency and reducing risk of nutrient losses to groundwater	E590119Z
Improving nutrient uptake efficiency and reducing risks to air quality - emissions of GHGs	E590130Z
Reduce risk of pesticides in surface water by utilizing precision pesticide application techniques	E595116X
Reduce risk of pesticides in surface water by utilizing IPM PAMS techniques	E595116Z
Reduce ozone precursor emissions related to pesticides by utilizing IPM PAMS techniques	E595129Z
Establishing tree/shrub species to restore native plant communities	E612132Z
Tree/shrub planting for wildlife food	E612136Z
Tree/shrub planting for wildlife cover	E612137Z
Maintaining and improving forest soil quality	E666106Z2
Maintaining and improving forest soil quality by limiting compaction	E666107Z
Forest Stand Improvement to rehabilitate degraded hardwood stands	E666133X
Snags, den trees, and coarse woody debris for wildlife habitat	E666137Z1
Enhancement - Grazing Management	EGM
Enhancement - Nutrient Management	ENM
Recycle 100% of farm lubricants	ENR04
Using nitrogen provided by legumes, animal manure and compost to supply 100% of the nitrogen needs	ENR08
Using N provided by legumes, animal manure and compost to supply 90 to 100% of the N needs	ENR10
Use of legume cover crops as a nitrogen source	ENR12
Enhancement - Soil Management	ESM
Monitor key grazing areas to improve grazing management	PLT02
Habitat Development for Beneficial Insects for Pest Management	PLT08
Intensive Management of Rotational Grazing	PLT10
Forest Stand Improvement for Wildfire Reduction	PLT13
Intensive rotational grazing	PLT16
Herbicide resistant weed management	PLT19

Table 1 (continued)

Practice Name	Practice Code
Prune low density pine or hardwood trees to improve tree quality and wildlife habitat	PLT25
Continuous no till with high residue	SOE01
Continuous No Till Organic System	SOE03
Continuous No Till	SOE04
Intensive no-till (Organic or Non-organic systems)	SOE05
Controlled traffic system	SQL01
Use of Cover Crop Mixes	SQL04
Use deep rooted crops to breakup soil compaction	SQL05
Conversion of cropped land to grass-based agriculture	SQL06
Forest Stand Improvement for Soil Quality	SQL07
Conversion of cropped land to grass-based agriculture	SQL09
Forest stand improvement for soil health	SQL13
Biological suppression and other non-chemical techniques to manage brush, weeds and invasive species	WQL01
Biological suppression and other non-chemical techniques to manage herbaceous weeds invasive species	WQL02
Rotation of supplement and feeding areas	WQL03
Apply nutrients no more than 30 days prior to planned planting date	WQL05
Apply controlled release nitrogen fertilizer	WQL06
Split nitrogen applications 50% after crop/pasture emergence/green up	WQL07
Apply split applications of nitrogen based on a pre-sidedress nitrogen test on cropland	WQL08
Apply phosphorus fertilizer below soil surface	WQL09
Precision application technology to apply nutrients	WQL11
Managing livestock access to water bodies/courses	WQL12

Table 2

Sample sites BMP and proportion upstream pastureland gradients with fish health data available. All sites listed have DELTs data. FD = fantail darter, WS = white sucker. Listed in increasing proportion pastureland.

Site Name	Site Code	COMID	Pasture Proportion	BMP ECO Impact Intensity	HAI Available
Tumbling Run	TUM	8440765	NA	NA	none
Pine Run	PIR	5909197	0.078	0.19	WS, FD*
War Branch West	WBW	5908077	0.12	0.669	WS*
Thorny Branch	TR	5908951	0.141	0.203	WS*
Beaver Creek	BEC	5908925	0.192	3.236	WS*, FD*
Middle Creek	MDC	5895342	0.216	0.841	none
Toms Brook	TB	8440797	0.241	0.229	FD
Tuscarora Creek	TC	5895158	0.247	0.247	FD*
Muddy Creek	MUC	5908085	0.247	0.817	none
Mill Creek, Berkeley County	MCB	5895450	0.254	0.377	WS*, FD*
Narrow Passage Creek	NPC	8440899	0.256	1.17	WS
Bullskin Run	BR	8445006	0.292	1.057	FD*
Smith Creek	SC	8441323	0.293	0.975	WS*, FD*
West Run	WR		0.296	0.374	FD
Harlan Run	HR	5891598	0.297	0.357	WS*, FD*
Mill Creek, Rockbridge County	MCRB	8539525	0.298	2.013	FD
West Fork Linville Creek†	WFLC	8441103	0.31	0.889	WS
Mill Creek, Rockingham County	MCRH	5908289	0.315	0.956	none
Pughs Run	PUR	8441259	0.315	2.092	WS*, FD*
Folly Mills Creek	FMC	5909047	0.375	0.697	none
Naked Creek	NC	5908297	0.422	1.348	none
Mossy Creek	MOC	5908973	0.423	5.017	WS*, FD
Meadow Run	MR	5908429	0.43	2.101	FD
Holmans Creek	HC	8441001	0.432	0.786	FD
Poague Run	POR	8539869	0.446	3.517	FD*
South River	SOR	5909079	0.447	1.105	FD
Long Glade Creek	LGC	5908967	0.476	1.676	WS*

Table 2 (continued)

Site Name	Site Code	COMID	Pasture Proportion	BMP ECO Impact Intensity	HAI Available
Cedar Grove Branch	CGR	8539129	0.484	4.195	none
Mill Creek, Page County	MCP	5907059	0.497	0.666	WS*, FD*
Pisgah Branch	PB	8538701	0.52	1.492	FD*
Spout Run	SPR	8445434	0.529	0.2	WS*
Eidson Creek	EC	5909041	0.578	0.523	WS*, FD*
Back Creek	BAC	5909061	0.641	2.032	FD

*histologic observations available for this sample

†not actual COMID, sampled reach outside of NHD$^+$V2 network

Table 3

HAI index description. Index is applied per individual fish but estimates of index score distribution in a population is of primary interest. Within each category, only one anomaly is selected for rating. This means the highest value a category can hold is 3. All categories are used in calculating white sucker HAI those not used for fantail darters are noted. Hematocrit, leukocrit, and plasma protein are modified for species based on a study out of Ottawa, Canada (Kovacs et al., 2002)

Category	Condition	Value
Fins	no active erosion	0
	Light active erosion	1
	Moderate hemorrhage	2
	Severe active erosion	3
Spleen*	Normal; black or dark red	0
	Normal; granular rough appearance	0
	Nodular; containing fistulas or nodules	3
	Enlarged; noticeably enlarged	3
	Other; abnormality not fitting above categories	3
Kidney*	Normal; firm dark red color, lying along vertebral column	0
	Swollen; swollen or enlarged wholly or in part	3
	Mottled; gray discoloration	3
	Granular; granular appearance and texture	3
	Urolithiasis or nephrocalcinosis; white, hard material in kidney tubules	3
	Other; abnormality not fitting above categories	3

Table 3 (cont.)

Category	Condition	Value
Skin	Normal; no aberrations	0
	Mild skin aberrations	1
	Moderate skin aberrations	2
	Severe skin aberrations	3
Liver*	Normal; solid red or light red color	0
	Tan/Pale; “fatty” liver, coffee with cream color	3
	Nodular; cysts or nodules	3
	Focal discoloration; distinct local color changes	3
	General discoloration; whole liver color change	3
	Other; liver abnormality not fitting above categories	3
Eyes	No aberrations	0
	Opaque eye; one or both	3
	Swollen/exophthalmic; protruding eye, one or both	3
	Hemorrhaging or bleeding eye; one or both	3
	Missing; one or both	3
	Other; abnormality not listed above	3
Gills	Normal; no apparent aberrations	0
	Frayed; erosion of the tips of gill lamellae	3
	Clubbed; swelling of the tips of gill lamellae	3

Table 3 (cont.)

Category	Condition	Value
	Marginate; gills with light discolored margin along tips of lamellae	3
	Pale; abnormally light colored	3
	Other; any abnormality not listed above	3
Parasites	No observed parasites	0
	Few observed parasites	1
	Moderate parasite infestation	2
	Numerous parasites	3
Pseudobranch*	Normal; flat, containing no aberrations	0
	Swollen; concave	3
	Lithic; mineral deposits	3
	Swollen & Lithic	3
	Inflamed; redness, hemorrhage, other	3
	Other; anomaly not listed above	3
Hindgut*	Normal; no inflammation or reddening	0
	Slight inflammation or reddening	1
	Moderate inflammation or reddening	2
	Severe inflammation or reddening	3
Hematocrit	White sucker: Normal range; 24-50%	0
	Fantail darter: Normal range; 24-56%	

Table 3 (cont.)

Category	Condition	Value
	White sucker: Above normal range; > 50%	1
	Fantail darter: Above normal range; >56%	
	White Sucker: Slightly below normal range; 14-24%	2
	Fantail darter: Slightly below normal range; 14-24%	
	White Sucker: Well below normal range; <14%	3
	Fantail darter: Well below normal range; <14%	
Leukocrit	White Sucker: Ranged defined as normal; <2%	0
	Fantail darter: NA[†]	
	White Sucker: Outside normal range; ≥2%	3
	Fantail darter: NA[†]	
Plasma protein*	White Sucker: Normal range; 4.3-8 mg/dL	0
	Fantail darter: NA	
	White Sucker: Above normal range; ≥8 mg/dL	1
	Fantail darter: NA	
	White Sucker: Below normal range; ≤4.3 mg/dL	3
	Fantail darter: NA	
Total Possible Score	The highest index score possible.	39

[*] Not used for fantail darter

[†] No leukocrit observed in fantail darter – could not estimate normal

Table 4

This table contains fish species encountered without any DELTs along with the number of individual fish and number of sites where the species was observed.

Family	Species	n	n sites
Anguillidae			
	Anguilla rostrata (american eel)	3	2
Catostomidae			
	Hypentelium nigicans (northern hog sucker)	17	4
	(sucker spp.)	36	2
Centrarchidae			
	Ambloplites rupestris (rock bass)	116	14
	Lepomis gibbosus (pumpkinseed)	1	1
	Lepomis macrochirus (bluegill)	137	11
	Lepomis microlophus (redear sunfish)	1	1
	Lepomis spp. (bluegill*rebreast sunfish)	1	1
	Lepomis spp. (bluegill*green sunfish)	3	3
	Micropterus dolomieu (smallmouth bass)	43	6
	Micropterus salmoides (largemouth bass)	31	8
	(sunfish spp.)	11	3
Fundulidae			
	Fundulus diaphanus (banded killifish)	12	2

Table 4 (continued)

Family	Species	n	n sites
Leuciscidae			
	Exoglossum maxillingua (cutlip minnow)	85	5
	Hybognathus regius (eastern silvery minnow)	2	1
	Lythrurus ardens (rosefin shiner)	14	2
	Notropis hudsonius (spottail shiner)	17	5
	Notropis procne (swallowtail shiner)	27	3
	Notropis rubellus (rosyface shiner)	3	3
	Pimephales promelas (fathead minnow)	1	1
	Semotilus corporalis (Fallfish)	84	7
	Chrosomus oreas X Rhinichthys atratulus (mountain redbelly X blacknose dace)	1	1
	(shiner spp.)	1	1
	(minnow spp.)	552	17
Percidae			
	Etheostoma nigrum (johnny darter)	210	3
	Etheostoma blennioides (greenside darter)	26	4

Table 4 (continued)

Family	Species	n	n sites
	Etheostoma olmstedi (tessellated darter)	1	1
	(darter spp.)	3	1
Poeciliidae			
	Gambusia holbrooki (eastern mosquitofish)	4	2
Salmonidae			
	Oncorhynchus mykiss (rainbow trout)	25	5
	Salmo trutta (brown trout)	10	3
	Salvelinus fontinalis (brook trout)	3	1

Table 5

This table contains DELT anomaly counts and percentages for each species where DELTs were observed during fish aggregation sampling.

Family	Species	n	n sites	n sites with DELT	Total DELT Count	Total DELT %
Catostomidae						
	Catostomus commersonii (white sucker)	1495	29	8	11	0.74
	Thoburnia rhothoeca (torrent sucker)	1178	8	1	1	0.08
Centrarchidae						
	Lepomis auratus (redbreast sunfish)	156	13	2	2	1.28
	Lepomis cyanellus (green sunfish)	437	22	2	2	0.46
Cottidae						
	Cottus caeruleomentum (blue ridge sculpin)	1702	12	2	2	0.12
	Cottus n.sp. (checkered sculpin)	3569	7	4	7	0.2
	Cottus girardi (potomac sculpin)	1946	13	1	1	0.05
	(sculpin spp.)	777	17	1	1	0.13
Cyprinidae						
	Cyprinus carpio (common carp)	9	1	1	13	144.44*
Ictaluridae						
	Ameiurus natalis (yellow bullhead)	74	11	1	1	1.35
	Ameiurus nebulosus	3	3	1	1	33.33

Table 5 (continued)

Family	Species	n	n sites	n sites with DELT	Total DELT Count	Total DELT %
	(brown bullhead)					
	Noturus insignis (margined madtom)	90	11	3	5	5.56
Leuciscidae						
	Campostoma anomalum (central stoneroller)	863	14	4	4	0.46
	Chrosomus oreas (mountian redbelly dace)	2779	13	4	8	0.29
	Clinostomus funduloides (rosyside dace)	1320	15	2	6	0.45
	Luxilus cornutus (common shiner)	2854	22	6	8	0.28
	Margariscus margarita (allegheny pearl dace)	602	10	1	1	0.17
	Nocomis leptocephalus (bluehead chub)	3514	23	10	19	0.54
	Pimephales notatus (bluntnose minnow)	851	18	1	1	0.12
	Rhinichthys atratulus (eastern blacknose dace)	6277	32	4	4	0.06
	Rhinichthys cataractae (longnose dace)	1348	22	6	7	0.52
	Semotilus atromaculatus (creek chub)	293	15	2	2	0.68
Percidae						
	Etheostoma flabellare (fantail darter)	5501	28	5	6	0.11

Table 5 (continued)

Family	Species	n	n sites	n sites with DELT	Total DELT Count	Total DELT %
	Etheostoma longimanum (longfin darter)	32	4	1	1	3.13
Totals[†]		39151	33		114	0.29

*Percentage over 100 because multiple DELTs observed per fish

[†] Totals are for all fish encountered including species without DELTs

Table 6

DELT anomaly index descriptions. All observations are made externally and recorded as presence or absence per individual fish. Percentage of fish with anomalies are then calculated for the sample. Modified from Sanders et al., 1999.

Type of Anomaly (Field Code)	**Example**
Deformities (D)	Deformed fin, head, vertebrae
Erosions (E)	Eroded fin, or operculum
Lesions (L)	Open sore, ulceration, exposed tissue
Tumors (T)	Abnormal growth anywhere

Figures

Figure 1

Map showing fish sampling locations and relevant watersheds.

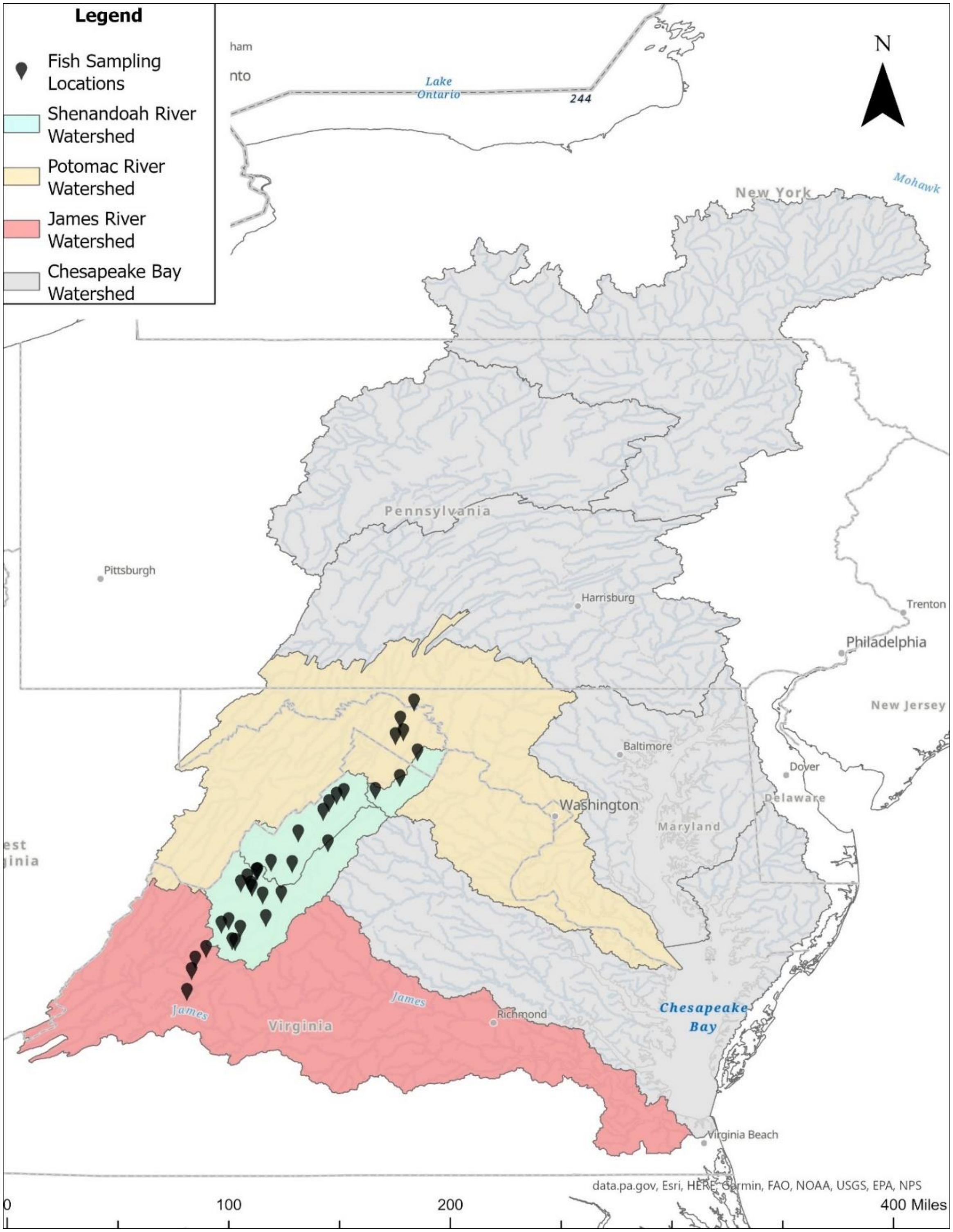

Figure 2

This figure shows box plots of HAI score (Y axis) for both fantail darter and white sucker in order of increasing upstream proportion pasture (X axis). Site codes are included in parentheses. See Table 2 for site code key. Note the difference in y-scales between charts.

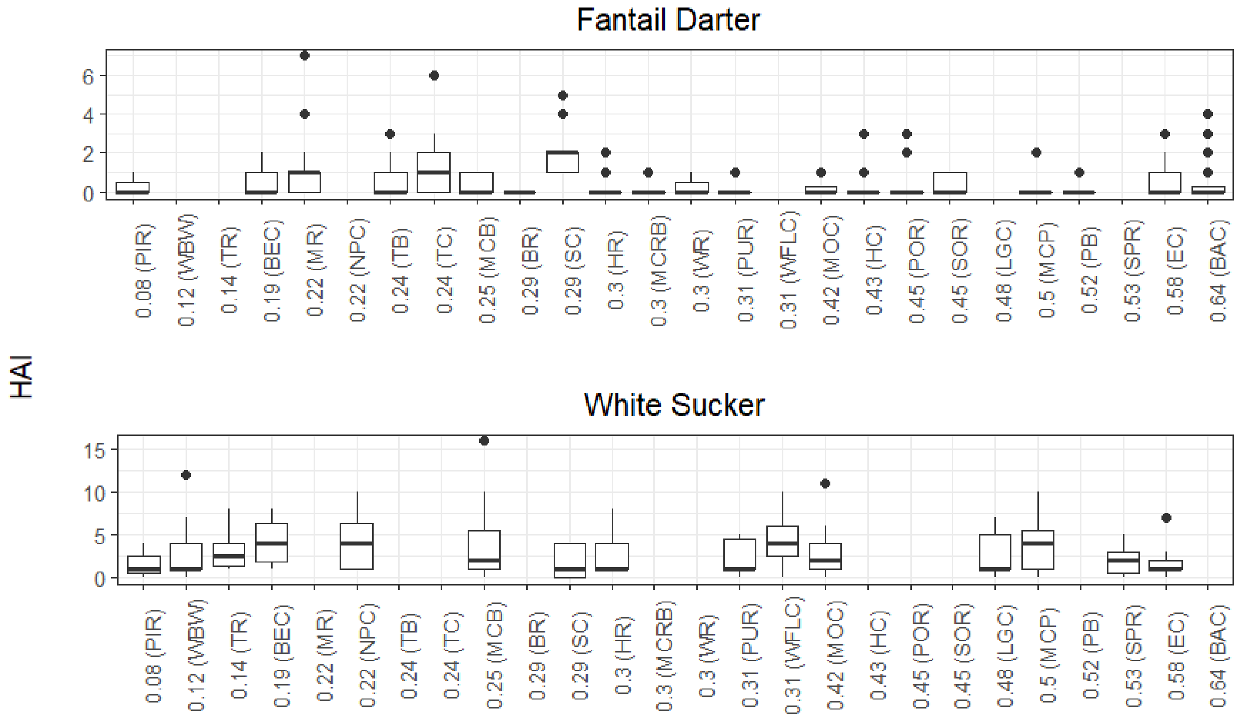

Figure 3

This figure shows the bootstrapped predictions from a mixed effects ordinal logistic regression model of white sucker HAI values by age. Bars represent 95% prediction intervals.

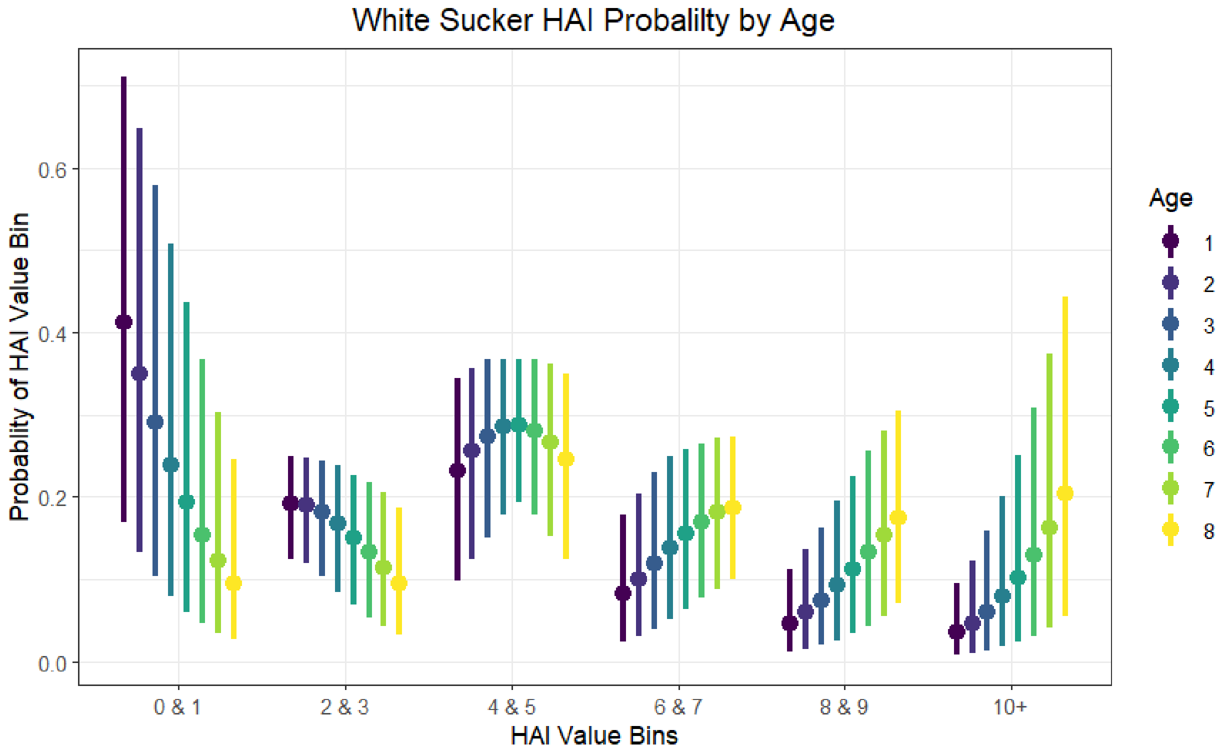

Figure 4

This figure shows white sucker HAI values over age at each site. Plots are listed from left to right in order of upstream proportion pasture. Site codes listed in parentheses. See Table 2 for a site code key. Note that all but 1 instance where 4 or more age classes of fish exist there is a positive relationship between age and HAI value.

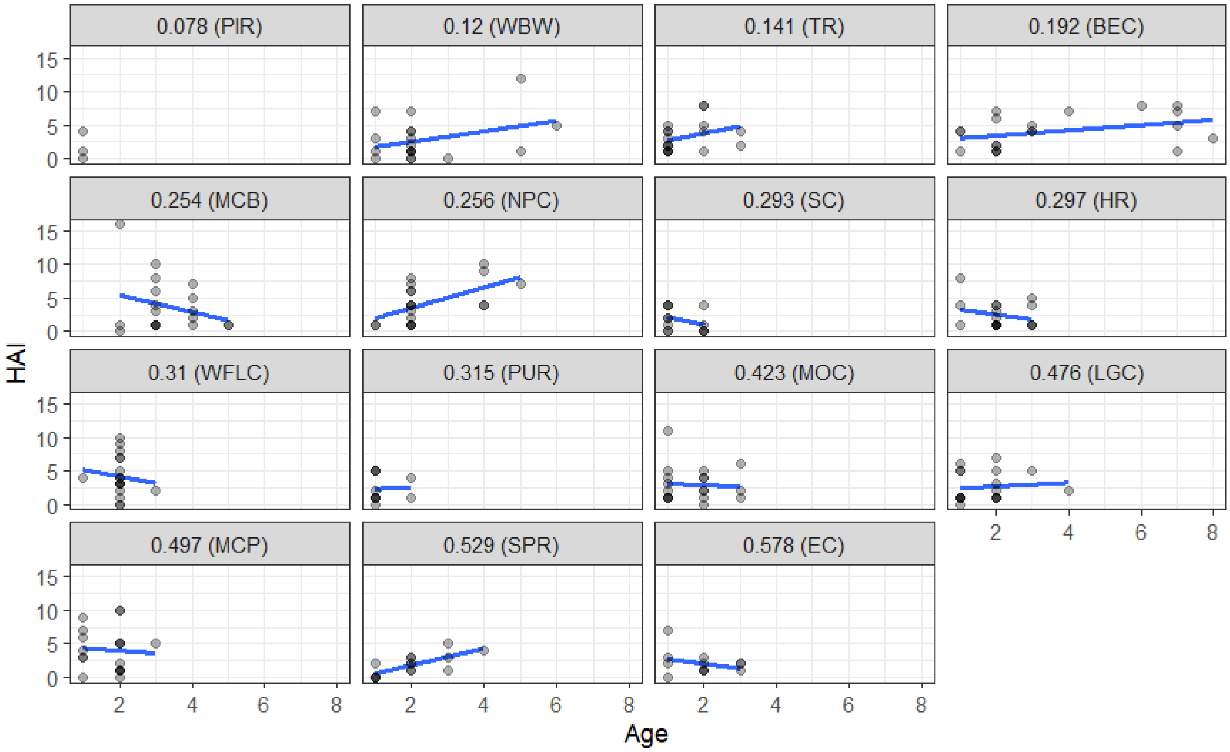

Figure 5

Panel (A) shows a white sucker from Long Glade Creek, VA with numerous protrusions that would be considered “tumors” when counting DELTs. Panel (B) shows tissue preserved from one of the protrusions which appear to result from a myxozoan infection. The stars indicate myxzospores and the arrow points to an area of inflammation.

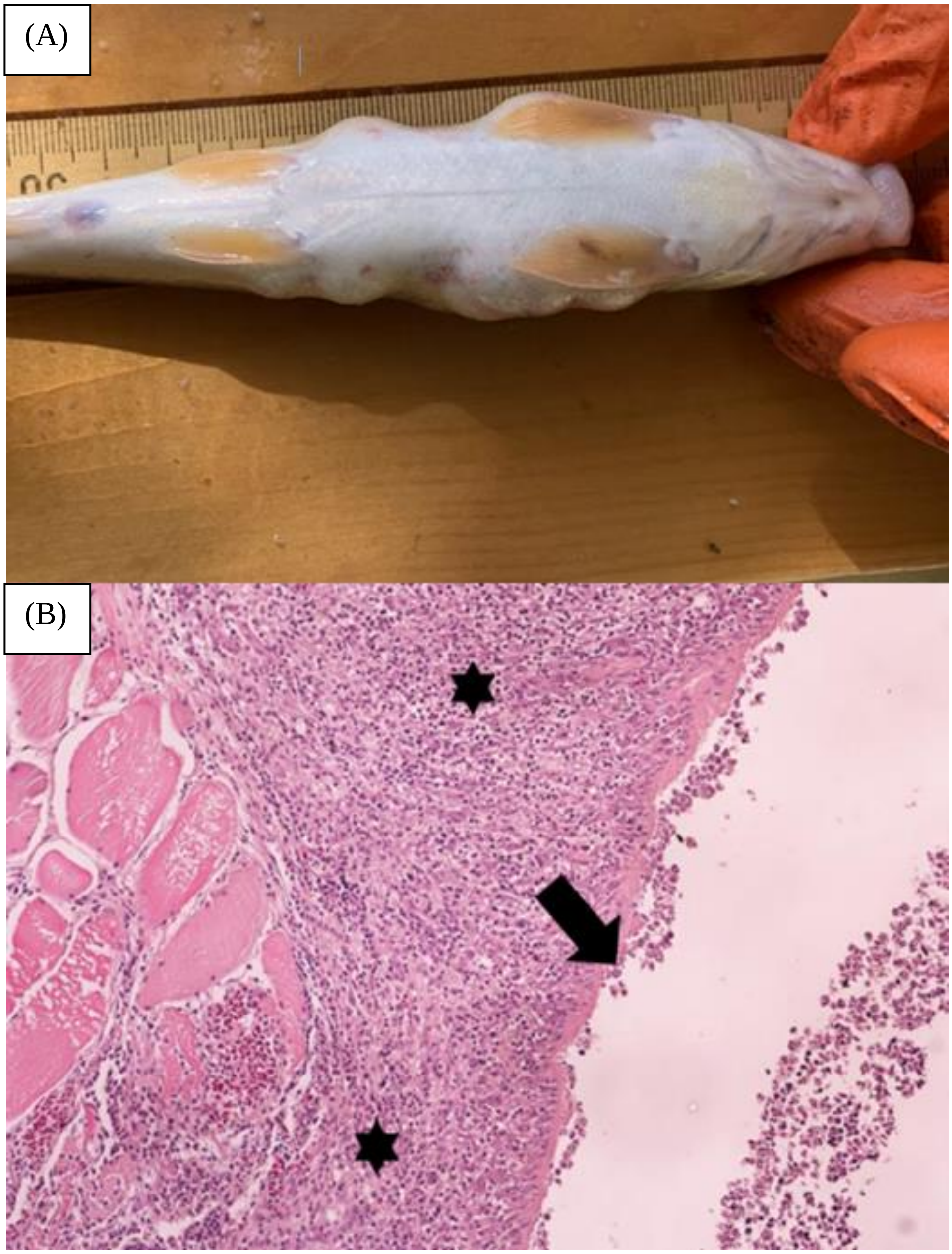

Figure 6

Picture of sculpin (Cottus spp.) with ingested prey. Externally the protrusion looked like an abnormal growth and would have been recorded as a tumor when counting DELTs without internal observation.

Chapter 3

Title Page

Temporal Analysis of a Health Assessment Index (HAI) and Deformity, Erosion, Lesion, and
Tumor Counts (DELTs) Applied to Smallmouth Bass in the Chesapeake Bay Watershed

Abstract

Fish kills, endocrine disruption, increased disease prevalence, and periods of declining abundance have been observed in smallmouth bass (SMB) populations of the Chesapeake Bay watershed. A single cause behind SMB health issues has not been identified in the watershed. It is rather believed that multiple anthropogenic and natural stressors are working in concert to reduce the health of SMB and increase susceptibility to various pathogens and parasites. Fish health indices such as the Health Assessment Index (HAI) and Deformity, Erosion, Lesion, and Tumor counts (DELTs) are simple tools designed to monitor the general health of fish populations or communities. This study applies a HAI, and DELTs to SMB necropsied at 5 sites in the Chesapeake Bay watershed over an 8-year period from 2013 to 2020 yielding a total sample size of 889 bass. Sites varied in catchment land-use with a mixture of primarily forested and agricultural land and smaller areas of development. The influence of site, sampling season (spring or fall), seasonal flow statistics, sex and age of SMB on the indices was analyzed. Ordinal logistic regression with mixed effects was used to analyze HAI scores and logistic regression with mixed effects and a logit link was used to analyze DELT data. Only age and sex were found to be important predictors of HAI scores and DELT presence. Past studies using the HAI and DELTs have noted the correlation with age, but few explicitly accounted for age when using index values to identify unhealthy fish populations. Future studies should incorporate

controls to keep fish sample ages and sex ratios similar. Other concerns surrounding HAI and DELTs application in Chesapeake Bay watershed are discussed.

Introduction

Beginning in the early 2000s fish kills impacting smallmouth bass *Micropterus dolomieu* (SMB) were observed in the upper Potomac, Monocacy and Shenandoah Rivers. Mortality typically occurred in the spring and impacted fish often displayed external lesions. These events gained the attention of anglers and other stakeholders in the region. Multiple state and federal agencies, nonprofits, and universities began investigating the cause(s) of this fish mortality. Multiple bacterial, parasitic, and viral pathogens were found in SMB from the affected rivers (Blazer et al., 2010). Bacteria species isolated include *Aeromonas hydrophila, Aeromonas salmonicida* and *Flavobacterium columnare*. Some of the most common parasites identified were trematodes, cestodes and myxozoans. Largemouth Bass Virus (LMBV) was also detected. Signs of endocrine disruption including testicular oocytes and elevated vitellogenin in male SMB were also documented. In the Susquehanna River, increased disease prevalence in young of the year SMB and grossly visible lesions on adult SMB were first observed in the mid-2000s immediately before declines in juvenile and adult abundance were noted (Schall et al., 2020). Pathogens like those found in the Potomac River watershed were found in age-0 SMB from the Susquehanna River watershed. Bacteria such as *Aeromonas spp.* and *Flavobacterium columnare* were also isolated. Parasites including trematodes, cestodes, and the myxozoan *Myxoblus inornatus* were observed, as was LMBV. Many of these pathogens occurred as co-infections. Chemical contaminants were also detected in tissue including polychlorinated biphenyl (PCB) congeners, organochlorines, and current use pesticides (Walsh et al., 2018). Biological endpoints of endocrine disruption including testicular oocytes and plasma vitellogenin in adult males were also identified in the Susquehanna River watershed (Blazer et al., 2014). None of these stressors were determined to the primary cause of SMB mortality and population declines in the

Chesapeake Bay watershed. It is rather believed that all these stressors work together to impact the health of SMB, and in some cases overwhelm the fish causing mortality.

Counts of external deformities, erosions, lesions, and tumors (DELTs) and the health assessment index (HAI) are both fish health indices intended to evaluate the general health of a fish population or community (Adams et al., 1993; Sanders et al., 1999). Both combine multiple anomaly observations to create a single numeric indicator of fish health. The idea behind DELTs is simple. More fish with external deformities, erosions, lesions, and tumors, indicate a less healthy fish population or community. DELTs can be calculated without sacrificing any fish because anomalies are only observed externally. However, a fish health assessment limited to external observations can miss important health information. Table 1 contains a description of what is recorded in DELTs. The HAI requires a necropsy because it includes observations of external and internal visual anomalies. The HAI may also include a few simple blood parameters. Observations recorded for the HAI are rated in categories according to their severity. Categorical values are combined to create a score for each individual fish. Higher scores suggest fish are less healthy. Table 2 describes what is included in the HAI and how it is calculated. Both indices are designed to be simple and rapid, requiring limited tools and training. If either of the indices effectively evaluates SMB health in the Chesapeake Bay watershed, they could be applied widely with limited cost.

Stressors impacting SMB in the Chesapeake Bay watershed interact with fish reproductive cycles and changing stream conditions. Fish kills in the upper Potomac and its tributaries often occurred in the spring (late March through June) and some were associated with high discharge events (Blazer et al., 2010). Morbidity and mortality occurred after high discharge in the North Fork of the Shenandoah River in 2004 and in the South Fork in 2005. A kill of SMB in the Monocacy River also occurred after a high discharge event. Conversely, a recent study in the South Branch of the Potomac River found a correlation between low seasonal discharge and an increase in body erosions and erosions of gill lamellae in SMB (Keplinger et al., 2022). Past studies on DELTs and the HAI typically focus on one or two years of sampling (Adams et al., 1993; Sanders et al., 1999; Schleiger et al., 2004; Simon & Burskey 2016). These studies also evaluated urban and industrial pollution sources. SMB mortality events, increased disease prevalence, endocrine disruption, and population declines occurred in areas of low development where agricultural land-use is common. Few studies have looked at DELTs and HAI score responses to agricultural pollution. One study found white sucker *Catostomus commersonii* HAI scores tended to be higher after exposure to agricultural soil loads in an artificial stream study (Merten et al., 2010). The research presented below evaluates DELTs and HAI applied over 8 years at 5 sites in the Chesapeake Bay watershed. Sites were spread over a gradient of agricultural and forested land with smaller areas of development. The impact of site, season (spring and fall), seasonal discharge statistics, age, and sex on SMB HAI scores and DELTs were investigated.

Materials and Methods

Sites and Sampling

SMB were sampled at five sites, two in the Potomac River watershed and three in the Susquehanna River watershed as part of a long-term U.S. Geological Survey monitoring and assessment program directed at determining risk factors for reproductive endocrine disruption. A map of sampling locations in relation to the Chesapeake Bay watershed is available in Figure 1.

Sites were sampled from 2013 to 2020 in the spring and fall. Spring sampling occurred between late March (earliest sample March 29th) and late May (latest sample May 28th). Fall sampling occurred between August (earliest sample August 8th) and early November (latest sample November 12th). The two sites in the Potomac River watershed were the South Branch of the Potomac near Moorefield, WV and Antietam Creek at its confluence with the Potomac River near Sharpsburg, MD. The South Branch of the Potomac River drains 315,057 hectares of land covered with a mixture of primarily forested (81% of catchment) and agricultural (14% of catchment) land. Antietam Creek drains 72,934 hectares of land covered by primarily agricultural land (49% of catchment) followed by forested land (32% of catchment) with varying levels of developed land covering 17% of the catchment. The three sites in the Susquehanna River watershed are Pine Creek, West Branch Mahantango Creek, and Chillisquaque Creek. Pine Creek was sampled near the confluence with the Susquehanna River near Waterville, PA and drains 243,681 hectares of primarily forested land (84% of catchment) with small areas of agricultural land (8% of catchment). West Branch Mahantango Creek was sampled near its confluence with the Susquehanna River and drains 21,849 hectares of land covered in forest (60% of catchment) and agricultural land (32% of catchment). Chillisquaque Creek was also sampled near the confluence with the Susquehanna River near Chillisquaque, PA and drains 28,953 hectares of land covered in primarily agricultural land (59% of catchment) and forest (31% of catchment). All land cover values are based on 2016 data from the National Land Cover Database (Dewitz, 2019).

Field Necropsy Procedure and Index Calculation

Fish were sampled using a wild fish health necropsy procedure (Blazer et al., 2018). Adult SMB were collected using boat electrofishing and held in a live well until returned to the bank where they were held in an aerated bin filled with local water until processed. A minimum size of 200mm total length was used limiting sampling to sexually mature fish. Processing began by euthanizing fish with a lethal dose of MS-222. As soon as mortality was confirmed, fish were bled using a heparinized 23-gauge needle and 3 cc syringe. Fish were then observed for any external or gill anomalies and obvious parasites. After external and gill observations were recorded, the body cavity was exposed using a longitudinal incision along the abdomen from the bottom of the opercular cavity to the anus. The cut was then continued dorsally and anteriorly until the top of the opercular cavity is reached. A final cut along the posterior edge of the opercular cavity fully exposed the body cavity. Any grossly visible anomalies and parasites in the body cavity and organs were recorded. Pieces of liver, spleen, kidney, gonad, gill and any other tissue with visible abnormalities were removed and placed in Z-fix to preserve for histopathology. Sagittal otoliths were removed from each fish and saved for aging using the crack and burn method with two independent readers (Christensen, 1964).

Data from necropsies was used to create an HAI and DELTs for each SMB. The necropsy procedure for this project was not originally designed for HAI calculation. Hence, some of the observations included in the original index from Adams et al., (1993) were not available. Some observations were also not useful for SMB, such as thymus and pseudobranch anomalies. The HAI used in this research includes observations in eight categories: fins, spleen, kidney, skin, liver, eyes, gills, and parasites. Recorded anomaly and parasite observations from necropsies were rated according to descriptions in Adams et al., (1993) with a slight modification. The original HAI rated anomalies with increasing severity from 0 to 30 in increments of 10. The HAI in this paper was modified to list anomalies in increasing severity from 0 to 3 in increments of 1. This change was made to better reflect the categorical and ordered nature of the data. Ratings within each category are then combined to create the score for that fish. The highest score a fish can have is 24, which results from anomalies in all 8 categories being given a severity of 3 – the highest anomaly severity. All observations were available for DELTs.

Quantitative Analysis

To evaluate the impact of discharge on HAI scores and DELTs, data were obtained from U.S. Geological Survey (USGS) stream gauges close to each of the SMB sampling locations. Local gauges were available in 4 of the 5 watersheds. West Branch Mahantango Creek did not have a gauge within its watershed. Discharge in West Branch Mahantango Creek was estimated using a formula based on a gauge in the nearby Penn's Creek (Williams, 2019). The formula is as follows: West Branch Mahantango Creek Discharge = $10^{-1.08419+1.1303*\log(\text{Penn's Creek Discharge})}$

Coordinates of SMB sampling locations and associated USGS gauges are available in Table 3.

Seasonal Coefficient of Variation, the ratio of standard deviation to the mean, for discharge at each site was calculated with seasons defined as follows: winter (December – February), spring (March – May), summer (June – August), and fall (September – November). Season definitions follow those described in a recent temporal fish health study on the South Branch of the Potomac River (Keplinger et al., 2022). The discharge from all sites was then centered (mean of discharge from 2013-2020 subtracted from each value) and scaled (each value divided by standard deviation of discharge from 2013-2020) to allow comparison between sites with different base flows. The seasonal means of centered and scaled discharge was then calculated for each site and year. Centering and scaling of data was not necessary before calculating Coefficient of Variation because it is a unitless statistic.

Ordinal logistic regression was used to evaluate HAI data (Gelman & Hill, 2006). Model selection was performed using Akaike Information Criterion (AIC). Logistic regression with a logit link was used to predict the presence or absence of a DELT anomaly also using AIC for model selection. Candidate models for predicting both DELTs presence and HAI scores included 56 combinations of sex, age, season (spring or fall), mean centered and scaled seasonal discharge, Coefficient of Variation seasonal discharge, and site predicting HAI value. A random effect was included for sampling event to account for repeated measures. Ordinal logistic regressions with random effects were fit using “clmm()” function from the package “Ordinal” (Christensen, 2022) in the program R version 4.1.1 (R Core Team, 2021). Logistic regression models with a random effect were fit using the “glmer” function in the package “lme4” (Bates et al., 2015) also in the program R. Parametric bootstrapping was used with 1000 iterations to generate prediction intervals (PIs) surrounding ordinal logistic model predictions. An alpha of 0.05 was used to test for significance in all analyses.

Results

Sample Timing and Discharge Levels

Mean discharge from 2013-2020 in sampled streams ranged from 97 cubic feet per second (cfs) at West Branch Mahantango Creek to 1499 cfs at Pine Creek. Figures 2 – 6 graph discharge with fish sampling events marked. The SMB were sampled in the spring and fall at most sites except for Chillisquaque Creek which was only sampled in the spring. All other sites were sampled at least twice in the fall. Three SMB samples lumped into the fall category were sampled in August, which puts them before the start of fall discharge data. All spring SMB samples were within the spring discharge data timeframe. Efforts were made to collect 20 adult SMB for each sample, but that was not always possible. Total sample size and the sex distribution at each site is available in Table 4 along with a summary of stream discharge and land cover.

HAI

The median HAI score for all SMB was 3 with a minimum of 0 and a maximum of 13. A total of 17 SMB had an HAI score of 0. SMB bass with HAI scores of 0 were found at 4 of the 5 sites (all except South Branch of the Potomac River) and were mostly sampled in the spring. Of these SMB, 8 were male and 9 were female with a median age of 4, a minimum age of 3 and a maximum age of 9. Only one SMB had the highest HAI score of 13. This fish was sampled in the South Branch of the Potomac on May 6th 2019, was an age-4 female that weighed 808 gm and had a total length of 395 mm. Anomalies contributing to this fish's high HAI score were eroded areas with fungal infections along with red raised areas on the skin (skin rated 3), focal discoloration of the liver (liver rated 3), eroded gills (gills rated 3), small white spots that were assumed to be encysted parasites in the liver, spleen and kidney along with small black spots (not melanistic areas) assumed to be encysted parasites in the skin and fins (parasites rated 3), and mild fin erosion (fins rated 1).

Chillisquaque Creek was the site with the highest median HAI score at 4. All other sites had median HAI scores of 3. The highest median HAI score for any sampling event was 7 and occurred at Chillisquaque Creek on May 12th 2015 (n=20). High HAI scores in this sample were primarily driven by eye abnormalities. Every fish had at least one opaque eye, and 2 fish had skin grown over one of their eyes. Fish in this sample also had liver anomalies with 8 fish having focally discolored livers. Anomalies in the skin and gills were also present to a lesser extent along with moderate parasite loads. The lowest median HAI score for any sampling event was 1 and occurred at West Branch Mahantango Creek on May 7th 2018 (n=20). Figure 7 shows box plots of HAI scores for each sampling event. The SMB sampled in the spring had a median HAI score of 3 which is the same for fish sampled in the fall. Likewise, male and female SMB each had median HAI scores of 3.

The parasite category of the HAI contributed the most to HAI scores making up 47% of all HAI values. Liver was the next highest contributor making up 18% of all HAI values. Gills, eyes, and skin categories were the next most influential making up 12%, 10%, and 9% of all HAI values, respectively. Fins, kidney, and spleen anomalies were the least influential making up 1%, 1%, and <0.5% of all HAI values respectively.

Model selection using AIC indicated the best ordinal logistic regression model for HAI score predicted HAI scores based on age and sex. The next best model according to AIC (Delta AIC: 1.27) included Coefficient of Variation seasonal discharge as an additional predictor. However, the 95% confidence interval surrounding the coefficient on this predictor overlapped with 0. This model was therefore ignored, along with any models with higher delta AIC. The model with SMB age and sex as the only predictors was used in all further analysis.

Modeling predicted male fish were more likely to have higher HAI scores. Prediction intervals overlap between sexes for all HAI value bins. The coefficient on sex was significant, so there appears to be a relationship between sex and HAI value that is not strong enough to predict

HAI score at a given age. Figure 8 visualizes the probability of HAI scores by sex for median aged fish (age 4) along with 95% PIs.

There was a strong relationship between HAI score and age. Older SMB were predicted to have higher HAI values more frequently. According to the OLR model, age-2 female SMB have a 16% (95% PI 5-29%) chance of scoring of 0 or 1 in their HAI, compared to age-15 female SMB which have a <1% (95% PI 0-2%) chance of having a HAI score of 0 or 1. At the other end of the HAI spectrum, age-2 female SMB have a <1% (95% PI both ends <1%) chance of a HAI score of 12 or greater. Age-15 female SMB have a 1% (95% PI <1-4%) chance of a HAI score of 12 or greater. While the chance of an age-15 female SMB having a HAI score of 12 or greater is still low, it is more probable than an age-2 female SMB having a similar score. This relationship holds up with intermediate ages and intermediate HAI values. Figure 9 displays a graph of HAI model predictions over the range of SMB ages found in this research, along with 95% PIs. The graph displays values for female SMB. Male SMB would show the same relationship between ages shifted towards higher HAI values.

DELTs

The mean percent of SMB with a DELT anomaly per sampling event was 22%. The highest percentage of SMB with DELTs in any sampling event was 55% and occurred at Chillisquaque Creek on May 12th 2015 (n=20, 11 fish with DELTs). The most common anomaly was lesions on 7 SMB including reddened areas on the skin, a small lesion on the lip, and an eroded area under the jaw (not considered an erosion by the Sanders et al., 1999 definition). The next most common anomaly was deformities on 4 SMB including skin grown over an eye on two fish, scoliosis, and a deformed caudal fin. One fish had an eroded operculum. This was the same sampling event that had the highest median HAI value. The same site having high DELTs and HAI scores is unsurprising because the anomalies recorded in DELTs are also recorded in the HAI. Two sampling events had no SMB with DELTs. These samples came from West Branch Mahantango Creek on April 20th 2017 (n=20) and South Branch of the Potomac River on May 7th 2014 (n=20). Figure 10 shows percentage of fish with DELTs at each sample event.

The most common DELT anomaly was lesions impacting 17% of all SMB. Erosions were the next most common DELT anomaly impacting 4% of all SMB. Deformities impacted 2% of all SMB and there was only one tumor. The only tumor occurred on a SMB at Chillisquaque Creek sampled on May 3rd 2013. It was described as a small lump on the skin.

Chillisquaque Creek had the highest percentage of fish with DELT anomalies occurring on 30% of all SMB (n=100). West Branch Mahantango Creek was next at 25% of all SMB (n=179) with DELTs. South Branch of the Potomac River was next at 21% of SMB (n=285) with DELTs, followed by Antietam Creek with 19% of SMB (n=151) with DELTs and Pine Creek with 17% of SMB (n=174) with DELTs. DELTs were more common in the spring, occurring on 23% of SMB (n=606) sampled in the spring. DELTs occurred on 19% of SMB (n=283) sampled in the fall.

Model selection using AIC indicated the best model for predicting presence of a DELT anomaly included age and sex as the only predictor. The next best model (Delta AIC: 0.43) added Coefficient of Variation seasonal discharge as an additional predictor. However, the 95% confidence interval surrounding the coefficient on this predictor overlapped with 0. This model was therefore ignored, along with any models with higher delta AIC. The model with SMB age and sex as the predictors of DELT anomaly presence was the only model used.

Modeling predicts 63% (95% CI 54-71%) higher odds of a male fish having a DELT anomaly when age and sampling event are held constant. There are also 55% (95% CI 53-57%) higher odds of a fish having a DELT anomaly with each additional year of age when holding sex and sampling event constant. See Figure 11 for a graph of predictions based on sex at each age sampled.

Discussion

The HAI

HAI scores were best predicted by age and sex of the sampled SMB. The dependance of HAI score on age of fish sampled has been noted in past studies (Coughlan et al., 1994; Kovacs et al., 2002). A study on largemouth bass *Micropterus salmoides* in the Catawba River in NC and SC found the HAI values were significantly and positively correlated with age (Coughlan et al., 1994). Another study looking at paper and pulp mill effluents in the Francois River, Quebec, Canada noted differences in HAI values between species along a gradient of age (Kovacs et al., 2002). Tessellated darters *Etheostoma olmstedi* in the study ranged in age from 1.9 to 2.8 years old and showed the lowest HAI values. Smallmouth bass were in the middle with an age range of 3.5 to 6.0 years with corresponding moderate HAI scores. White sucker had the highest age range from 4.3 to 7.6 and had the highest HAI scores. However, some of the HAI score differences could be due to varying life habits of the species exposing them to different pollutants, or species-specific tolerances for pathogens, pollutants, and stream conditions. Other studies have not noted sex differences in HAI scores. Accounting for the relationships between age and SMB HAI score is important for accurate fish health evaluations. Creating a length slot for sampled fish would help keep size ranges similar between samples. This was suggested in Coughlan et al., 1994. However, slot limited samples could still bias results if population growth rates are different. The relationship between sex and HAI score is harder to control for because SMB sex cannot be reliably determined externally. A larger sample size may increase the likelihood of an even sex ratio but comes at the cost of sacrificing more SMB. Accounting for sex ratio between samples may not be as important as age because the relationship between sex and HAI score was weaker. Large variation in sex ratio is still cause for concern.

A recent study on tissue mercury in smallmouth bass of the Chesapeake Bay watershed found a positive correlation between a modified version of the HAI and total tissue mercury (Blazer et al., 2023). This same study also found higher tissue mercury levels with an increase in smallmouth bass length and in female smallmouth bass. Higher mercury levels in older and longer smallmouth bass may contribute to the higher HAI scores predicted in older bass from our research. However, the correlation between tissue mercury and higher HAI scores found in the mercury paper may also be related to age. Mercury bioaccumulates in larger and older fish (Qian et al., 2001). It is likely that HAI scores are related to both age and tissue mercury levels, which are often correlated, complicating interpretation of cause behind elevated HAI scores.

The HAI utilized in this research included less anomaly categories compared to the original HAI from Adams et al., (1993). Including all anomaly categories from the original index could reduce the relationship between age and HAI scores. However, the relationship between age and HAI was still present in the full HAI applied to largemouth bass in the Catawba River in NC and SC (Coughlan et al., 1994). It is unlikely that adding a few more variables would eradicate the relationship between age and HAI score in SMB. It is also possible that adding more categories would reduce the relationship between sex and HAI value. Once again, adding more anomaly categories is also unlikely to reduce the importance of even sex ratios when comparing SMB HAI scores.

DELTs

Like HAI score, DELT anomaly presence was best predicted by age and sex of SMB. It has been suggested that longer lived species are more likely to display DELT anomalies (Sanders et al., 1999). A recent study on the South Branch of the Potomac River found severity scores of macroscopic body lesions were always higher in adult SMB compared to juveniles (Keplinger 2022). Older fish may be more likely to display external anomalies due to longer exposure to chemical stressors, more time to accumulate physically injuries, longer exposure to pathogens, and changes in immune system with advanced age. A study on Ohio's DELTs monitoring program recommended a minimum size limit to avoid juvenile fish (Sanders et al., 1999). The SMB sampled in the Chesapeake Bay watershed were sexually mature adults, but still showed a relationship between age and DELT presence. Comparing DELTs in length slots may reduce age bias in DELTs presence. Increasing the number of SMB sampled for DELTs could limit uneven sex ratios if enough fish are available. Because recording DELTs is nonlethal and quick, larger samples are more feasible for DELTs compared to large HAI samples if enough fish are available.

Sample Timing and Stream Conditions

Neither Coefficient of Variability seasonal discharge nor mean centered and scaled seasonal discharge were selected as good predictors of HAI score or DELT anomaly presence.

Lack of a relationship between seasonal discharge statistics and fish health indices could be explained by no influence of discharge on SMB health, HAI scores and DELTs failing to capture discharge related impacts to SMB health, or seasonal discharge statistics failing to represent discharge events or trends that impact fish health. Changes in discharge do impact SMB health in the Chesapeake Bay watershed. Multiple mortality events in the Potomac River watershed occurring in the early 2000s appeared to follow high discharge events (Blazer et al., 2010). Changes in discharge alter water chemistry and expose SMB to different pollutants. The streams sampled in this research are primarily covered in a mixture of agricultural and forested land. High discharge typically comes from elevated surface runoff. In an agricultural setting, this runoff can carry nutrients, pesticides, and endocrine disrupting compounds (Kuivila & Foe 1995; Magnien et al., 1995; Gadd et al., 2010). Changes in discharge also alter water chemistry including turbidity, pH, dissolved oxygen, and temperature. These variables all interact and influence fish physiology and health. Vitellogenin prevalence in male SMB, a bioindicator of endocrine disruption, was found to increase with higher runoff levels at sites in the Chesapeake Bay watershed (Blazer et al., 2021). A recent study on the South Branch of the Potomac River found correlation between low seasonal discharge and an increase in body erosion and erosion of gill lamellae in SMB (Keplinger et al., 2022). These observations demonstrate a correlation between fish health indicators in SMB and discharge data. Gill erosions are included in calculating HAI scores, and body erosions are included in DELTs and HAI scores. Correlations between body and gill erosions, and discharge data may be masked by lumping anomaly observations into one index system. Trends may also be masked by looking at seasonal discharge statistics. Peak discharge following storm events can expose SMB to different or higher concentration of chemical contaminants. Concentrations of the herbicide Atrazine often increase during high surface runoff following storm events (Frank & Sirons, 1979). Coefficient of Variation should pick up increased discharge variability, but important peak discharge events could still be masked by calculating the Coefficient of Variation over an entire season. SMB were also sampled earlier and later in a season between sites and years. Seasonal discharge statistics may be a more accurate description of the conditions experienced by a SMB sampled at the end of the season. Analyzing each anomaly observation separately could unmask important SMB health trends. The absence of trends between discharge data and the two fish health indices are likely due to limitations of DELTs and HAI scores as indicators of SMB health or seasonal discharge statistics failing to represent important peak discharge events.

Season of sampling was not selected as a useful predictor of HAI score or DELTs presence. Only spring and fall samples were evaluated in this research, and there were fewer SMB samples in the fall. Past studies on the two fish health indices focus typically on one or two years of sampling (Adams et al., 1993; Sanders et al., 1999; Schleiger et al., 2004; Simon & Burskey, 2016). Mean LMB HAI scores tended to be higher in the winter compared to the previous summer in 4 Georgia Reservoirs sampled from summer 2000 to winter 2002 (Schleiger et al., 2004). However, the summer of 2001 had higher mean LMB HAI scores compared to the previous winter. Past studies on seasonal variation in DELTs are not available. Research on macroscopic indicators of fish health in the South Branch of the Potomac River found more severe skin, body and gill lesions on SMB and golden redhorse sampled in the summer and fall (Keplinger et al., 2022). Important seasonal trends in specific anomalies may be missed when they are grouped in an index system. Future studies evaluating seasonality in DELTs and HAI scores should include all 4 seasons.

Site of sampling was not selected as a good predictor of HAI score or DELTs presence. The Ohio DELTs monitoring program includes full fish community samples (Sanders et al., 1999). Research in this program found the highest percentage of fish with DELT anomalies at the most degraded sites according to index of biotic integrity (IBI) and chemical pollution levels. The IBI uses fish community indicators to evaluate the biologic integrity of streams (Karr, 1981). Environmental degradation primarily resulted from rivers flowing through urban and industrial areas in Lima and Youngstown, Ohio. Another study looked at DELTs on bluntnose minnows (*Pimephales notatus*) in the industrial Grand Calumet River, Indiana (Simon & Burskey, 2016). This study found higher DELTs occurrence near an area contaminated with high levels of polychlorinated biphenyls (PCBs), oils, polycyclic aromatic hydrocarbons (PAHs), and heavy metals. The SMB sampled in the Chesapeake Bay watershed were primarily in a mixed forest and agriculture setting except for some development around Antietam Creek near Hagerstown, Maryland (17% of catchment area). DELTs may not be as useful in picking up fish health impacts from agricultural pollution sources. Previous HAI studies also focus on industrial and urban sources of pollution (Adams et al., 1993; Adams et al., 1999; Hinck et al., 2007). HAI scores for largemouth bass *Micropterus salmoides* (LMB) followed a gradient of PCB contamination in the Hartwell reservoir on the South Carolina – Georgia border (Adams et al., 1993). LMB HAI scores positively correlated sediment mercury, PCB and arsenic concentrations in the Clinch River and Watts Bar Reservoir of Tennessee (Adams et al., 1999). Pooled SMB and LMB HAI scores were higher in the lower Colorado River compared to the upper river and tributaries (Hinck et al., 2007). The lower Colorado River is down stream of many anthropogenic contaminant sources including agricultural inputs, urban discharges, and mining activity. While the lower Colorado River was impacted by agriculture, it is impossible to say if agriculture was driving higher HAI scores. A recent study on tissue mercury in SMB from 8 sites in the Chesapeake Bay watershed found a positive correlation between a modified HAI and total tissue mercury (Blazer et al., 2023). The modified HAI gave every anomaly encountered a severity score instead of rating severity of anomalies within categories. Mercury is a contaminant that is ubiquitous in the aquatic environment, but mercury levels were found to be high in watersheds of the United States dominated by forested and agricultural land (Brumbaugh et al., 2001). One study exposed SMB and white sucker to agricultural soil loads in an artificial stream (Merten et al., 2010). White sucker HAI scores tended to be higher when exposed to agricultural soil loads, but no relationship was evident between SMB HAI scores and agricultural soil. SMB HAI scores, like DELTs, may not be useful for detecting fish health impacts from agricultural sources of pollution common in the Chesapeake Bay watershed. However, there was a positive correlation between tissue mercury and a modified version of the HAI (Blazer et al., 2023). Further refinement of the HAI may increase its utility in agricultural regions of the Chesapeake Bay watershed.

Missed Fish Health Observations

Fish health observations included in DELTs and the HAI used in this research are entirely based on macroscopically visible abnormalities. Many fish health concerns cannot be observed macroscopically. SMB in the Chesapeake Bay watershed show signs of endocrine disruption. Indicators of endocrine disruption observed in the watershed include the presence vitellogenin (an egg yolk precursor protein) and testicular oocytes in male SMB (Blazer et al., 2010). Neither of these bioindicators can be observable macroscopically. Vitellogenin levels in blood plasma are measured using a direct enzyme-linked immunosorbent assay (ELISA) (Blazer et al., 2014).

Testicular oocytes are detected microscopically. Microscopic pathology is frequently used in ecotoxicology. Cellular changes, such as necrosis, proliferation, inflammation can be used to identify toxic effects as well as the presence of infectious agents such as bacteria and microscopic parasites. SMB and redbreast sunfish sampled around the time of mortality events in the Potomac River drainage displayed microscopic cellular changes indicating complex etiology behind skin lesions and mortality (Blazer at al., 2010). Histologic evaluations of age-0 SMB after increased mortality in the early 2000s found multiple parasites including trematodes, cestodes, and myxozoans, bacteria including *Aeromonas spp.* and *Flavobacterium columnare* (Walsh et al., 2018). Largemouth Bass Virus (LMBV) was also detected in the age-0 SMB (Walsh et al., 2018). Several studies have developed more complex indices that include detailed histopathology or changes at the cellular level (Bernet et al., 1999; Zimmerli et al., 2007; McHugh et al., 2011; Liebel et al., 2013; Raskovic et al., 2013). However, as with all indices these index systems can be misused (Wolf, 2018). It is likely that a combination of indicators is needed at the organism, organ, cellular, subcellular and molecular to fully evaluate health and the risk factors associated with adverse effects at different sites in the Chesapeake Bay watershed.

Terminology Concerns

The definition of “tumor” and other DELTs categories are potentially problematic, subjective, and lead to misunderstandings. The DELTs definition for “tumor” is “abnormal growth anywhere” (Sanders et al., 1999) which could be a “growth” due to a parasite cyst, an infection or actual neoplasia. While the term tumor is defined as a raised area, for many it indicates a benign or malignant neoplasia. However, raised areas can be abnormal growth of tissue in response to infectious agents or other factors. Also, the terminology surrounding lesions and erosions may be problematic. The DELTs monitoring program in Ohio defined lesions as: open sores, exposed tissue, or ulcerations (Sanders et al., 1999). For health professionals a lesion is any abnormality, while erosions, exposed tissue, and ulcerations refer to similar lesions with varying severity. An erosion is more superficial and involves loss of the epidermis, while an ulceration involves both the epidermis and dermis – leading to exposure of the underlying muscle (Roberts, 2012). Limiting “erosions” to eroded tissue on fins, gill covers, or barbels misses body surface lesions. While DELTs are not meant to be diagnostic, the “tumor”, “erosion”, and “lesion” definitions may cause confusion when interpreting results from a study using DELTs, as well as reduce comparability among groups and/or studies. Care must be taken when interpreting and communicating results from a DELTs study, making a clear distinction between the DELTs definitions and those typical to the field of fish health.

Conclusions

DELTs and the HAI did not detect any relationships between fish health and site, season of sampling (spring or fall), or seasonal discharge statistics. Both the HAI and DELTs were best predicted by age and sex. Future studies using these indices should attempt to control age and sex either through study design, or accounting for age and sex in modeling efforts. Impact of age on DELTs and the HAI could be limited by sampling fish within a length slot, as has been suggest for the HAI in the past (Coughlan et al., 1994). Indices can hide important trends, looking at individual anomalies may be more useful. However, refining the HAI may increase its utility. Important health observations may be missed if DELTs and the HAI are relied on to monitor SMB health in the Chesapeake Bay watershed. A combination of multiple indicators at the organism, organ, cellular, subcellular, and molecular level are likely necessary to characterize health risks and associated factors at sites in the Chesapeake Bay watershed. Care must be taken when interpreting and communicating “tumors”, “erosions”, and “lesions” as defined in DELTs.

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Tables

Table 1

DELT anomaly index description. All observations are made externally and recorded as presence or absence per individual fish. Percentage of fish with anomalies are then calculated for the sample. Modified from Sanders et al., 1999.

Type of Anomaly (Field Code)	**Example**
Deformities (D)	Deformed fin, head, vertebrae.
Erosions (E)	Eroded fin, or operculum
Lesions (L)	Open sore, ulceration, exposed tissue
Tumors (T)	Abnormal growth anywhere

Table 2

HAI index description. Index is applied per individual fish but estimates of index score distribution in a population is of primary interest. Within each category, only one anomaly is selected for rating. This means the highest value a category can hold is 3. Modified from Adams et. al., 1993.

Category	Condition	Value
Fins	no active erosion	0
	Light active erosion	1
	Moderate hemorrhage	2
	Severe active erosion	3
Spleen	Normal; black or dark red	0
	Normal; granular rough appearance	0
	Nodular; containing fistulas or nodules	3
	Enlarged; noticeably enlarged	3
	Other; abnormality not fitting above categories	3
Kidney	Normal; firm dark red color, lying along vertebral column	0
	Swollen; swollen or enlarged wholly or in part	3
	Mottled; gray discoloration	3
	Granular; granular appearance and texture	3
	Urolithiasis or nephrocalcinosis; white or cream-colored material in kidney tubules	3
	Other; abnormality not fitting above categories	3

Table 2 (cont.)

Category	Condition	Value
Skin	Normal; no aberrations	0
	Mild skin aberrations	1
	Moderate skin aberrations	2
	Severe skin aberrations	3
Liver	Normal; solid red or light red color	0
	Tan/Pale; “fatty” liver, coffee with cream color	3
	Nodular; cysts or nodules	3
	Focal discoloration; distinct local color changes	3
	General Discoloration; whole liver color change	3
	Other; liver abnormality not fitting above categories	3
Eyes	No aberrations	0
	Opaque eye; one or both	3
	Swollen/exophthalomic; protruding eye, one or both	3
	Hemorrhaging or bleeding eye; one or both	3
	Missing; one or both	3
	Other; abnormality not listed above	3
Gills	Normal; no apparent aberrations	0

Table 2 (cont.)

Category	Condition	Value
	Frayed; erosion of the tips of gill lamellae	3
	Clubbed; swelling of the tips of gill lamellae	3
	Marginate; gills with light discolored margin along tips of lamellae	3
	Pale; abnormally light colored	3
	Other; any abnormality not listed above	3
Parasites	No observed parasites	0
	Few observed parasites	1
	Moderate parasite infestation	2
	Numerous parasites	3
Total Possible Score	The highest index score possible.	24

Table 3

The table below shows smallmouth bass sampling location coordinates and U.S. Geological Survey (USGS) gauge coordinates associated with each sampling location.

Site	Sampling Location		USGS Gauge #	Gauge Location	
	latitude	longitude		latitude	longitude
Chillisquaque Creek	40.941630	-76.85001	1553850	40.97444	-76.8000
Antietam Creek	39.414342	-77.74620	1619500	39.44978	-77.7302
West Branch Mahantango Creek*	40.647800	-76.94296	1555000	40.86667	-77.0486
South Branch of the Potomac River	39.103706	-78.95945	1608000	39.01233	-78.9561
Pine Creek	41.282964	-77.32149	1549700	41.27361	-77.3244

*No gauge on West Branch Mahantango Creek, discharge comes from a formula estimating flow based on nearby Penn’s Creek gauge (USGS gauge # 01555000). Formula from Williams et al., 2020.

Table 4

The first column in the table below shows the site listed in order of increasing forested land in catchment. The second column shows the mean discharge from 2013 – 2020 in cubic feet per second (CFS) for each site along with the standard deviation (SD) in parenthesis. The next columns show the catchment size, percent of the sampled stream catchment covered in forested land, the percent of catchment covered in agricultural land, the smallmouth bass (SMB) sample size, SMB median age, and percent of sample that was female SMB.

Site	Mean Discharge CFS (SD)	Catchment Size (Hectares)	Catchment % Forested	Catchment % Agricultural	SMB Sample Size (n)	SMB Median Age (Range)	SMB % Female
Chillisquaque Creek	142 (314)	28,953	31	59	100	5 (3-11)	51
Antietam Creek	338 (283)	72,934	32	49	151	4 (2-9)	46
West Branch Mahantango Creek	97 (142)*	21,849	60	32	179	4 (2-10)	42
South Branch of the Potomac River	265 (515)	315,057	81	14	285	4 (2-12)	53
Pine Creek	1499 (1884)	243,681	84	8	174	4 (2-15)	41
Total	NA	NA	NA	NA	889	4 (2-15)	47

*No gauge on WB Mahantango Creek, discharge comes from a formula estimating flow based on nearby Penn’s Creek gauge (USGS gauge # 01555000). Formula from Williams et al., 2020.

Figures

Figure 1

Map showing sampled watersheds, smallmouth bass collection sites, and stream gauge locations in relation to the Chesapeake Bay watershed. Sampled watersheds in order from north to south are Pine Creek, Chillisquque Creek, West Branch Mahantango Creek, Antietam Creek, and South Branch of the Potomac River. Note the location of one stream gauge outside of the sampled watershed (West Branch Mahantango Creek). Discharge comes from a formula estimating discharge based on the nearby Penn's Creek gauge (USGS gauge# 01555000) marked on the map. Formula for estimating flow at West Branch Mahantango Creek from Williams et al., 2020.

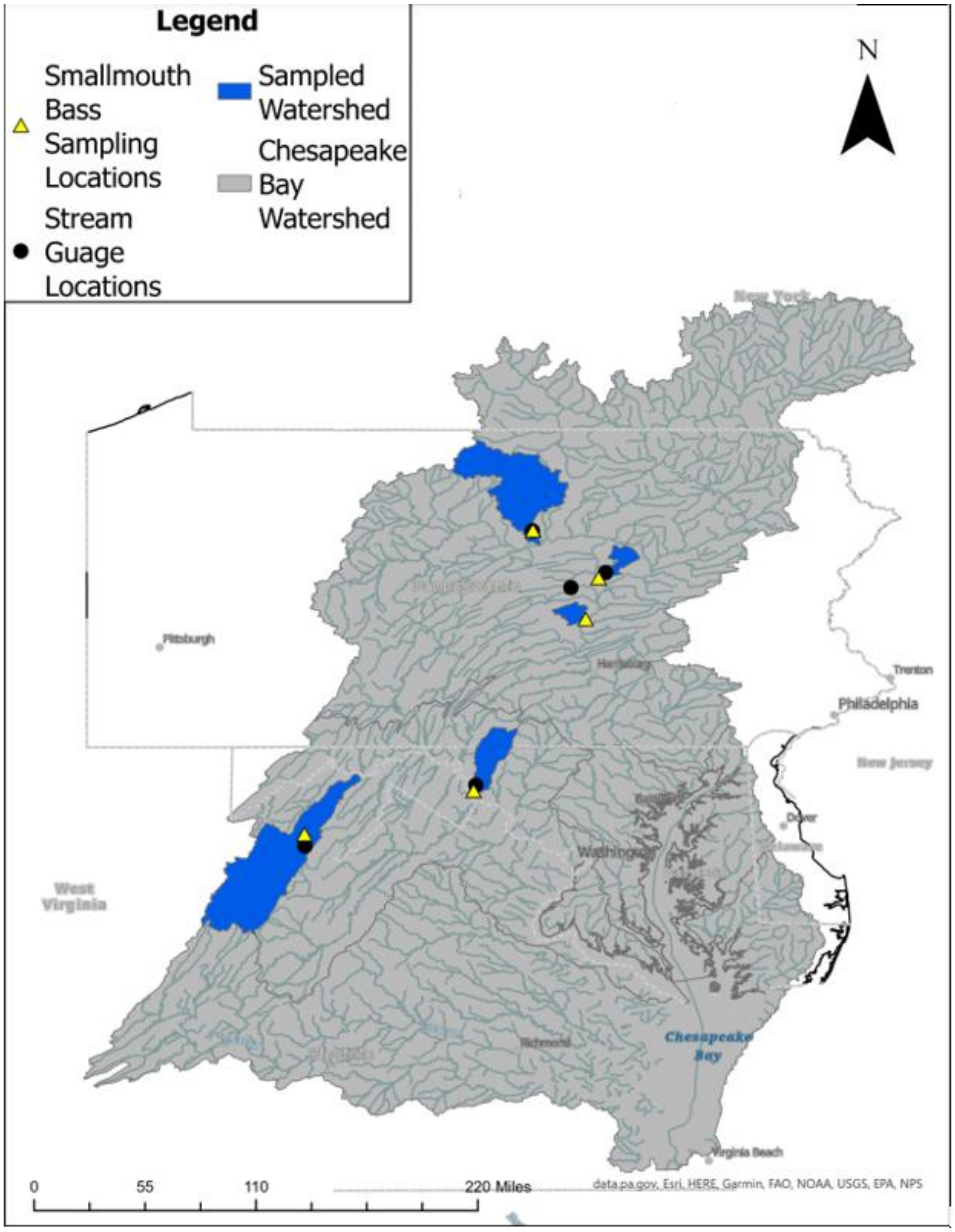

Figure 2

Graph showing discharge (black line) and fish sampling events (dashed vertical blue lines) at Chillisquaque Creek, PA. Discharge is from USGS gauge #1553850.

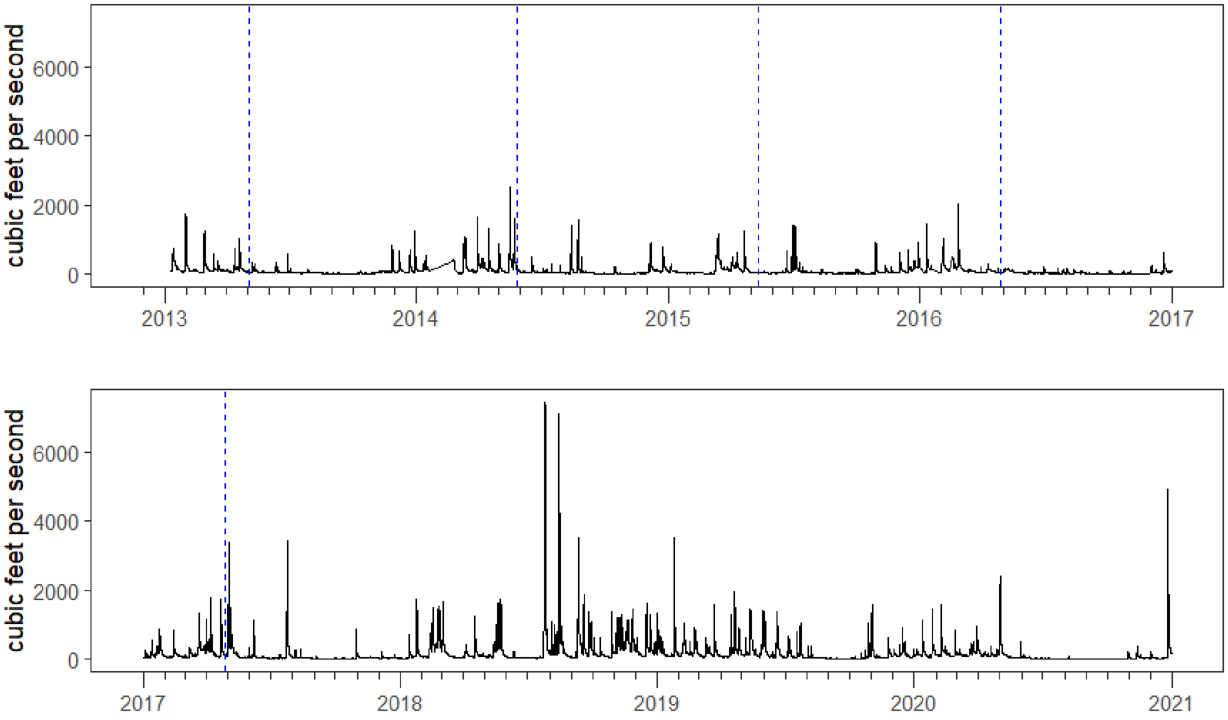

Figure 3

Graph showing discharge (black line) and fish sampling events (dashed vertical blue lines) at the confluence of Antietam Creek and the Potomac River. Discharge is from USGS gauge #1619500.

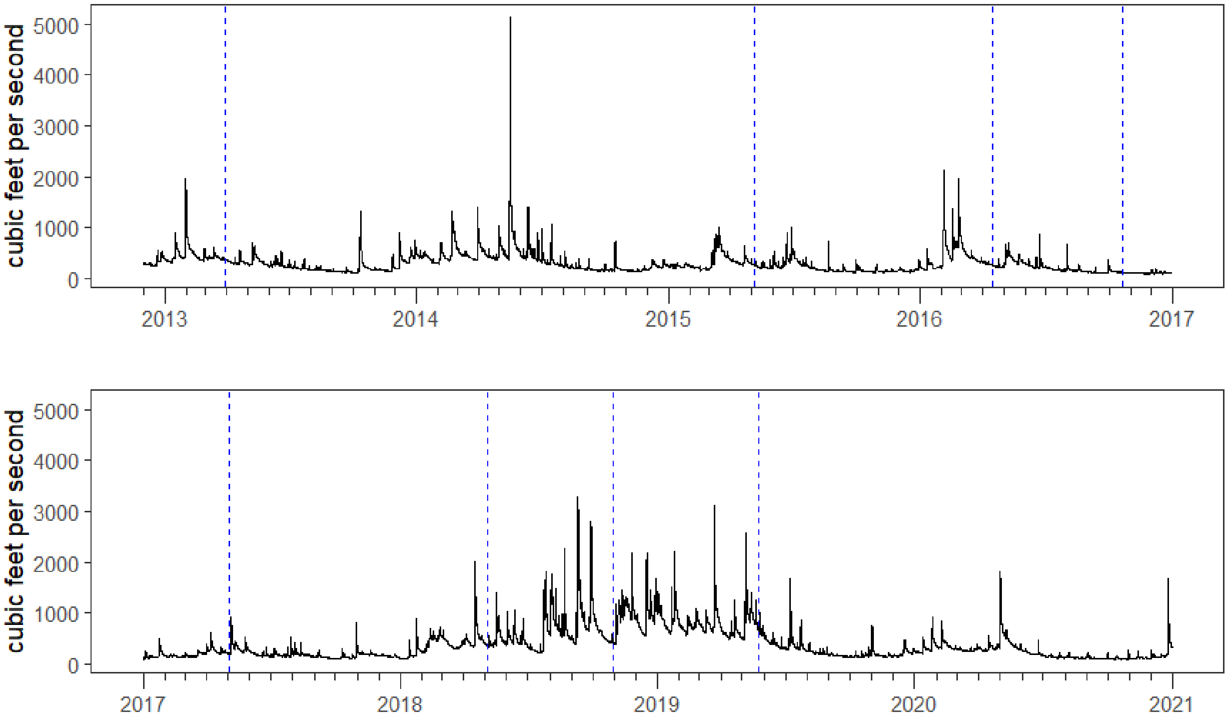

Figure 4

Graph showing discharge (black line) and fish sampling events (dashed vertical blue lines) at West Branch Mahantango Creek, PA. Discharge is from USGS gauge #1555000 on nearby Penn’s Creek modeled to estimate discharge at West Branch Mahantango Creek (Williams et al., 2020).

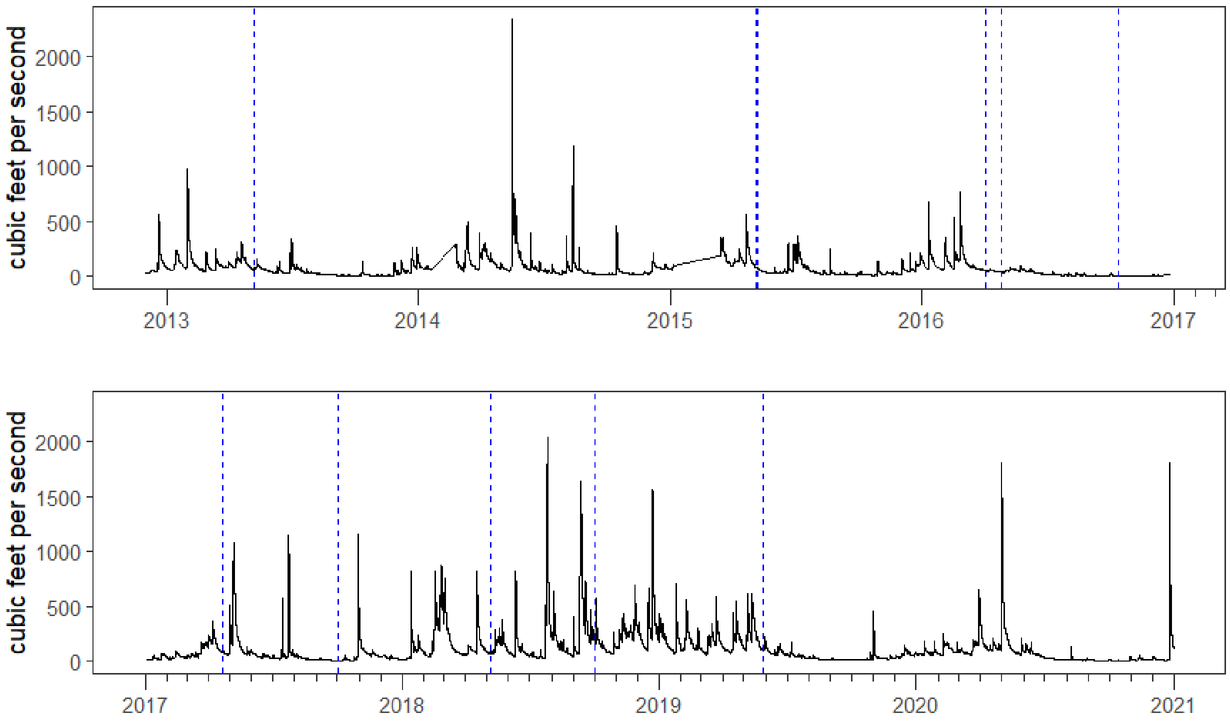

Figure 5

Graph showing discharge (black line) and fish sampling events (dashed vertical blue lines) at the South Branch of the Potomac near Moorefield, WV. Discharge is from USGS gauge #1608000.

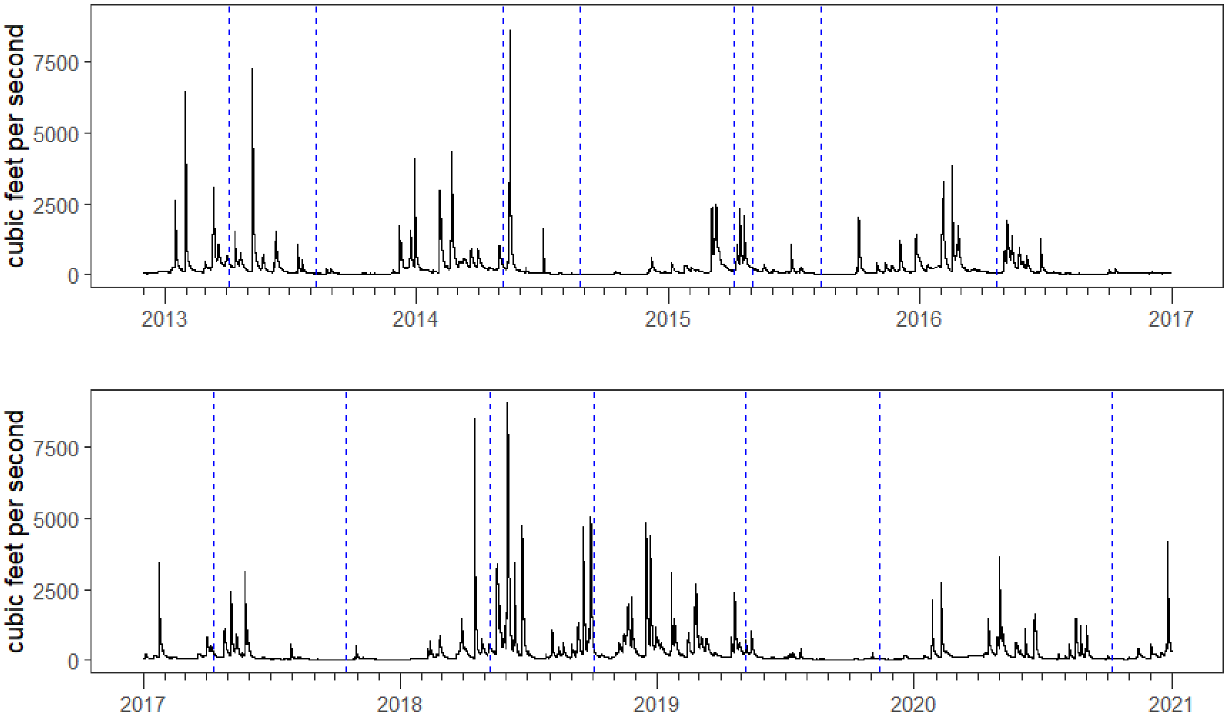

Figure 6

Graph showing discharge (black line) and fish sampling events (dashed vertical blue lines) at Pine Creek, PA. Discharge is from USGS gauge #1549700.

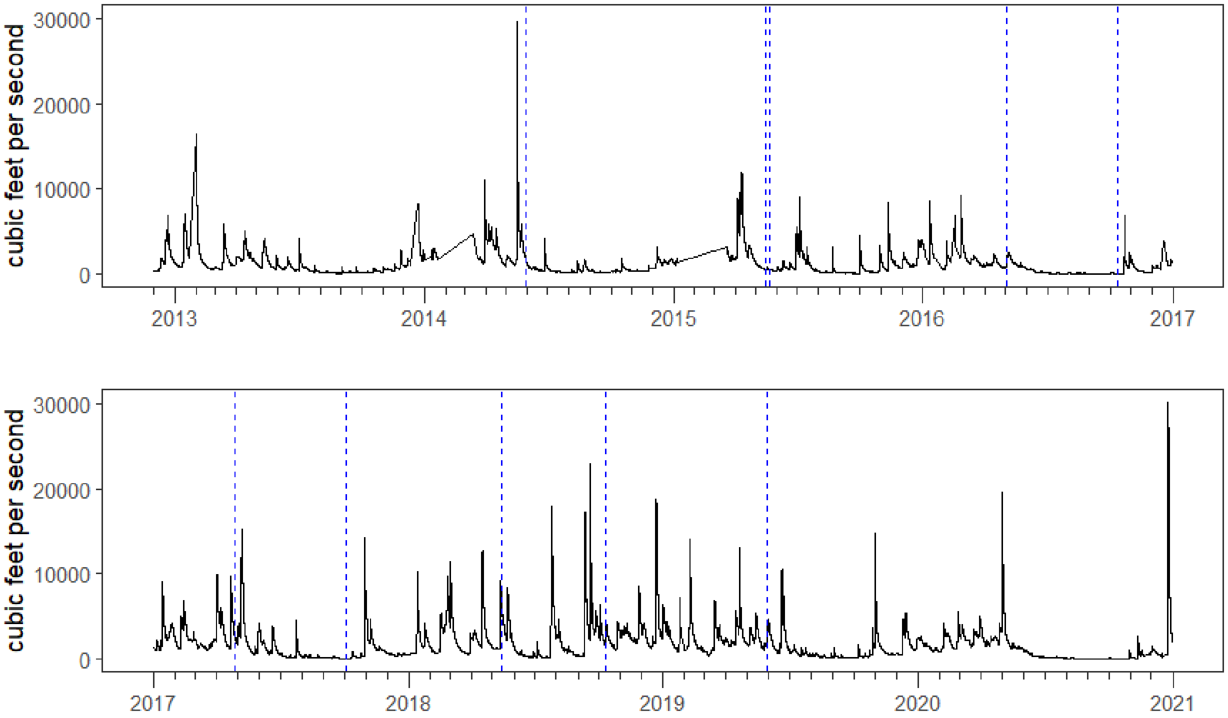

Figure 7

Box charts displaying the distribution of HAI values by site, season, and year.

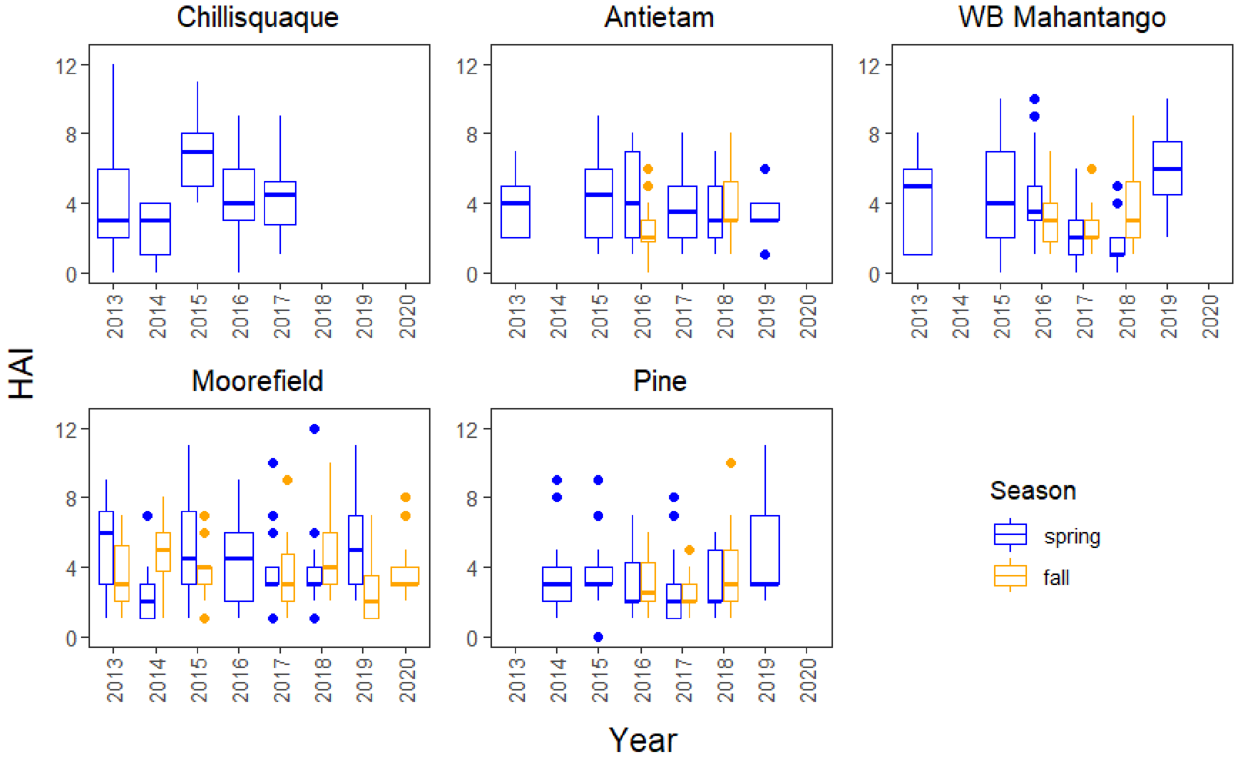

Figure 8

Figure shows the predicted probability of an individual fish being within an HAI value bin based on sex along with 95% prediction interval bars. Predictions are based on OLR with age and sex as the predictors. Age is set to 4 which is the median age of the sample.

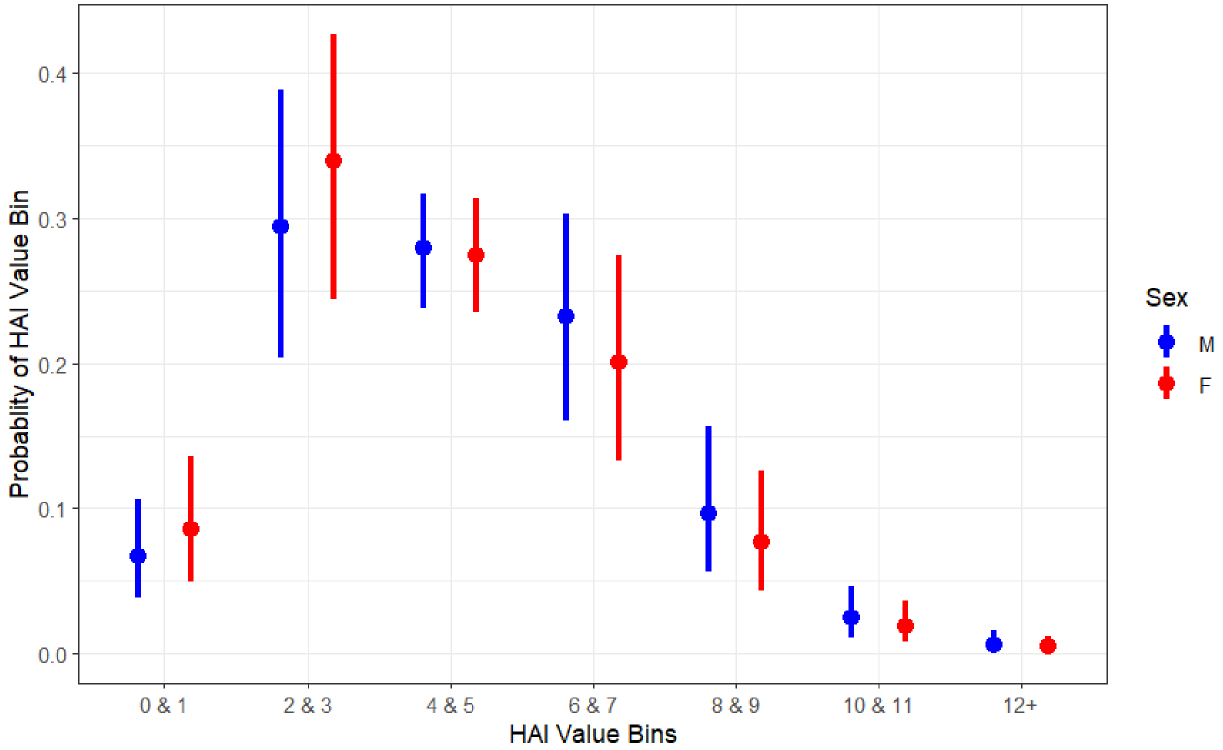

Figure 9

Figure shows the probability of a fish having a given HAI value predicted by age along with 95% prediction intervals. Note the trend of older fish being more likely to have high HAI values and younger fish being more likely to have low HAI continues strongly through medium ages and medium HAI values. Results are based on OLR model with age and sex as predictors. The graph below is for female fish. A graph for male fish would show the same relationship shifted towards higher HAI values.

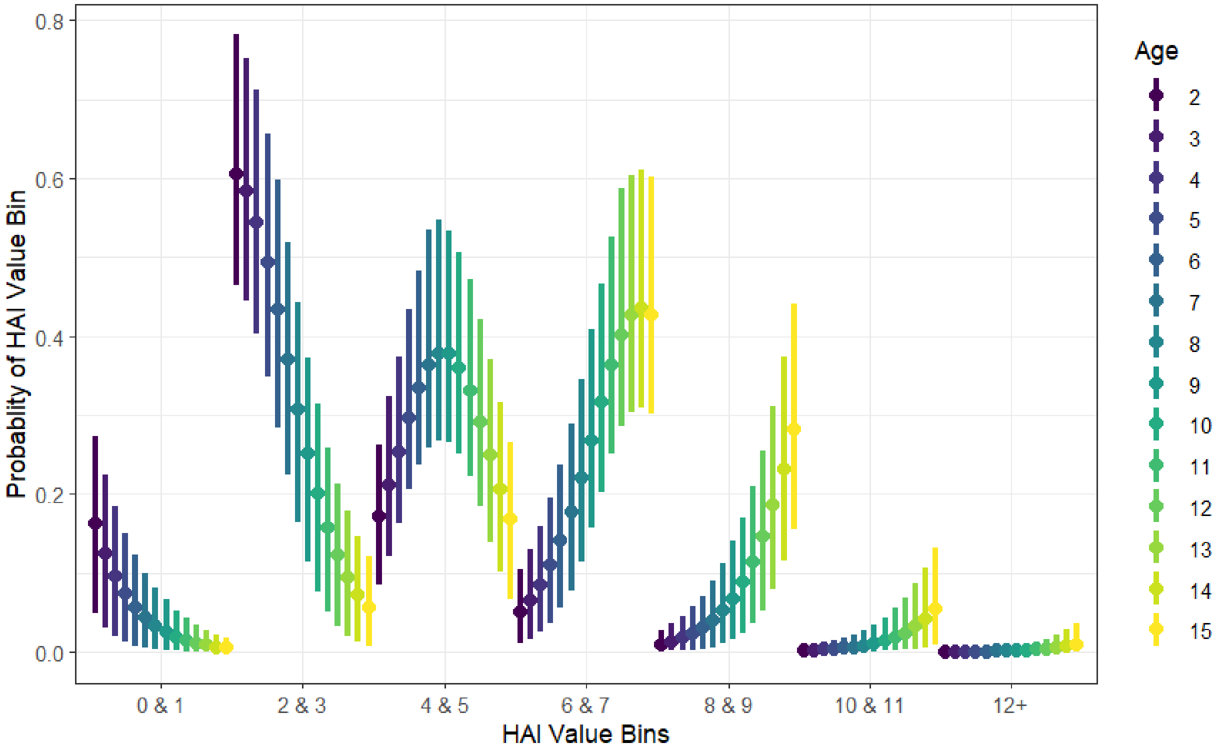

Figure 10

Percentage of fish in a sample with at least one DELT anomaly by site, season, and year. Count of fish with a DELT anomaly in sample is listed on top of each bar.

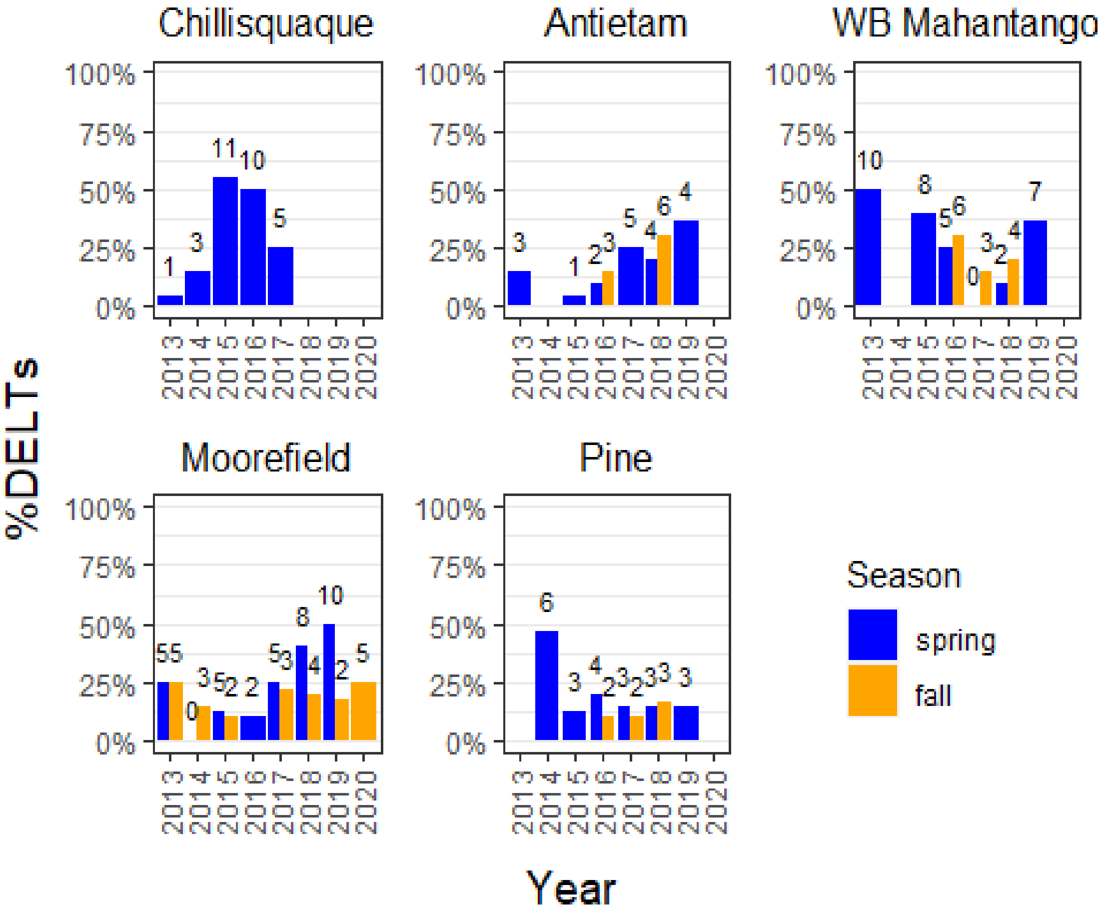

Figure 11

The graphs below show the probability of a DELT occurrence predicted by age and sex of individual fish with 95% confidence intervals. Predictions are based on a generalized linear mixed effects model using a logit link function with age and sex as the only predictors.