

The Pennsylvania State University

The Graduate School

Intercollege Program in Ecology

**A MULTIDISCIPLINARY APPROACH TO EVALUATING POPULATION STATUS
AND DISEASE DYNAMICS OF SMALLMOUTH BASS**

A Dissertation in

Ecology

by

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ABSTRACT

Fish and wildlife populations are impacted by various stressors including overexploitation, habitat destruction, introduced species, pathogens, contaminants, and climate change. These stressors can have effects at multiple levels of biological organization, from cellular to ecosystem-level impacts. Smallmouth bass (*Micropterus dolomieu*) are an important socioeconomic and ecological species of conservation interest throughout the United States including in the Susquehanna River Basin, Pennsylvania. Within the past 10-15 years, there have been concerns about the overall health of smallmouth bass in the Susquehanna River Basin, which has manifested itself in declines in the abundance of adult fish, mortality events of young fish, evidence of endocrine disruption, and clinical signs of disease. Potential contributing factors include bacterial and viral pathogens, parasites, degraded water quality conditions, and contaminants.

Elucidating which potential contributing factors are important is complicated by the fact that the Susquehanna River Basin is comprised of river-tributary networks that vary in habitat type, habitat quality, and therefore the potential for exposure to different stressors. In riverine systems, there is a large amount of variability in how much a smallmouth bass might move over its lifespan, with some evidence for long range movements. Therefore, understanding the movement ecology and gene flow of smallmouth bass will directly help efforts to identify and understand ecological drivers of smallmouth bass populations, and is necessary to help inform management decisions (e.g., fishing regulations), the design of ecological field studies, and conservation of management units. In addition, despite the fact that declines in smallmouth bass catch

rates have been noted, trends in relative abundance have not been quantified or put within a regional context to see if observed patterns in the Susquehanna River differ from other large river systems in the mid-Atlantic region that also support smallmouth bass fisheries. I evaluated population trends, individual fish movement, and population genetic structure of smallmouth bass in Chapters 1-3. Chapter 4 looks specifically at a potential stressor of smallmouth bass by focusing on the disease dynamics of a myxozoan parasite infecting juvenile smallmouth bass. This parasite has been found across the Susquehanna River Basin, but how its prevalence varies across the landscape and is influenced by anthropogenic land use practices is not understood.

In Chapter 1 of my dissertation, I evaluated trends in adult smallmouth bass catch per effort data (CPE) from the Susquehanna River Basin and compared trends to out of basin sites both within and outside the state of Pennsylvania. Dynamic linear models, as opposed to traditional linear models, permitted investigation of short term non-monotonic changes in fish CPE, which is important in order to detect short-term perturbations and nonlinear responses of populations to stressors. When compared with other rivers, the Susquehanna River Basin had the largest magnitude and probability of decline in smallmouth bass CPE. The declines began in the late 1990s prior to the first documentation of disease, but the general patterns of decline and trend towards recovery at the end of the study was not consistent across the Susquehanna River Basin. This chapter provided a foundational level of understanding on smallmouth bass CPE temporal dynamics in the study system over important time periods relevant to ongoing disease and mortality events.

Chapters 2 and 3 examine population genetics and spatial movement ecology of adult smallmouth bass in the system. Both chapters were largely motivated by observations of adult smallmouth bass using both river and tributary habitats across the Susquehanna River Basin. This led to questions regarding whether fish utilizing tributaries and rivers were separate ecological and genetic groups. In Chapter 2, population genetics structure of smallmouth bass was evaluated across 24 sites (both river and tributary sites) in the Susquehanna River Basin and compared to one out of basin site. Using twelve polymorphic microsatellite loci, limited genetic differentiation (overall mean pairwise $F_{ST} = 0.012$) and lack of population structure ($k = 3$ admixed population clusters) was found across the Susquehanna River Basin. Chapter 2 provided important information for the future conservation of smallmouth bass including the fact that there was little genetic support for the use of separate management units within this system.

Chapter 2 quantified gene flow across a large spatial extent; however, there was still uncertainty about finer-scale seasonal movement dynamics of smallmouth bass. Understanding how individual smallmouth bass were using rivers and tributaries could help inform the mechanisms contributing to genetic similarity across the basin. Knowledge of the movement of smallmouth bass in the system could also be important for understanding the range of stressors fish could be exposed to, especially if river and tributary habitats are both utilized. To evaluate the spatial movement ecology of smallmouth bass in the Susquehanna River Basin, I completed a radio-telemetry study in connected river-tributary habitat in the Susquehanna River Basin. I evaluated movement dynamics and river-tributary usage as well as investigated environmental drivers (i.e., temperature and flow) of fish movement. In general, fish movement was variable with

some fish moving large distances ($n = 76$ fish, average = 27.2 ± 25.9 km, range = 0.2 to 118 km). Movement between rivers and tributaries was common. Using a generalized additive mixed model, I found varied seasonal effects of temperature and flow on fish movement. These findings indicate the importance for maintaining fish movement corridors for smallmouth bass, including connectivity between river and tributaries. Additionally, long distance movement supports the possibility for gene flow and lack of genetic differentiation demonstrated in Chapter 2.

Chapter 4 focuses on the prevalence of a myxozoan parasite infecting juvenile smallmouth bass. This research investigated temporal and spatial factors, including land use, that were hypothesized to influence parasite prevalence. For this chapter, I used a hierarchical logistic regression model to evaluate spatial and temporal variability in myxozoan parasite prevalence across four years and at 31 sites. There was little temporal variability in myxozoan prevalence (posterior mean annual prevalence ranged from 0.42-0.55); however, prevalence varied substantially among sites (posterior mean site-level prevalence ranged from 0.08-0.82). Myxozoan prevalence had a negative relationship with urban land use and a positive relationship with agricultural land use. The effects of both urban and agricultural land use were stronger at the local scale when compared to the accumulated catchment scale. The relationship between prevalence and land use indicates that land use practices could be mediating parasite-host relationship, including invertebrate host density and parasite abundance, and contributing to infection rates in smallmouth bass. This chapter provided insight into land use relationships that may be important for myxozoan parasites in the Susquehanna River Basin.

My dissertation research crosses multiple sub-disciplines of ecology to provide valuable information that can be used to help understand smallmouth bass ecology, health, and can inform management of this important fishery. For example, fishing regulations, which extend a half mile into tributaries in sections of the Susquehanna River Basin, may need to consider larger distances of protection into tributaries given smallmouth bass movement in the system. Additionally, smallmouth bass movement across a large area could indicate exposure to stressors across a larger gradient. Land use impacts may differ at local and watershed scales as demonstrated for the myxozoan parasite investigated. Thus, in the context of fish health, future research efforts should integrate finer-scale information with large-scale habitat conditions and processes and consider the potential for cross-scale interactions.

In a larger context, it is important to consider why we as humans care about smallmouth bass health. The fish health issues observed in the Susquehanna River is more than a smallmouth bass problem. Smallmouth bass may be more sensitive to specific stressors than other organisms, but there could also be larger human health and ecosystem implications. The Susquehanna River provides drinking water to millions of inhabitants located along the river and also major cities outside of the basin. The Susquehanna River Basin is also part of the larger Chesapeake Bay Watershed and provides around half of the freshwater to the bay. The Susquehanna River also provides an important role in ecosystem functioning and a source for recreating across the region. Recent impairment of portions of the river for coliform bacteria and fish consumption advisories by Pennsylvania Department of Environmental Protection have led to concerns not only for the health of smallmouth bass, but also for the overall impact the health of

the river could have on humans. Understanding smallmouth bass ecology, an important indicator of ecosystem health, could provide a better understanding of the health of the river and therefore the health of the many people and economies that depend on this valuable resource.

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Chapter 1

Temporal dynamics of riverine Smallmouth Bass catch per effort data in Mid-Atlantic States

Chapter 1 has been submitted for peer review to the *North American Journal of Fisheries Management*. This chapter was completed with collaboration of several coauthors including my advisor, Tyler Wagner, my committee member, Vicki Blazer, Robert Lorantas (Pennsylvania Fish and Boat Commission), Geoffrey Smith (Pennsylvania Fish and Boat Commission), John Mullican (Maryland Department of Natural Resources), and Brandon Keplinger (West Virginia Division of Natural Resources). The manuscript is being provided below as it was submitted to the journal for review.

Temporal dynamics of riverine Smallmouth Bass catch per effort data in Mid-Atlantic States

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<A> Abstract

Smallmouth Bass *Micropterus dolomieu* populations inhabiting rivers in Mid-Atlantic States provide important recreational resources. To evaluate regional differences in temporal dynamics of Smallmouth Bass catch per unit effort (CPE), dynamic linear models (DLMs) were used to quantify trends and annual probabilities of decline in CPE from major rivers in the Mid-Atlantic States. Not unexpectedly, periods of declines were present in almost all rivers, but were greatest within rivers and river sections of the Susquehanna River Basin, Pennsylvania. Within the Susquehanna River Basin, declines began in the late 1990s and CPE began to stabilize towards the end of the time series (2011). In contrast, many of the other rivers investigated, including both within and outside of the state of Pennsylvania, did not have the same magnitude or duration of decline in CPE. Even within the Susquehanna River Basin, rivers were highly variable in CPE trends which could provide insight into management needs in different rivers. Quantification of annual dynamics in CPE across a region, in contrast to examining long-term average conditions, can provide insight to management agencies on the dynamic characteristics of fish populations and associated fisheries.

<A> Introduction

Recreational freshwater fish species face a myriad of threats that can have direct and indirect effects on fish communities and their habitats (Geist 2011; Dudgeon et al. 2006). Some of the pressures being exerted on freshwater fisheries include changes in climate and land use, disease, and invasive species – each of which can operate across large spatial and temporal extents and at multiple spatial scales (Hughes 2015). These

stressors, therefore, have the potential to influence the temporal dynamics of fish populations across broad scales. In addition, there is an increasing emphasis on understanding and managing aquatic systems across large spatial extents, as opposed to single lake or watershed management (Midway et al. 2016). For example, Bethke and Staples (2015) performed a broad scale analysis of trends in fish populations in Minnesota lakes in an effort to put locally observed changes into a larger context, and to assist managers in preparing for future large-scale management of important fishery resources. Such broad regional analyses of fish populations are likely to become more common and necessary to make inferences about regional status, trends, and temporal dynamics of important fish species within the context of global change, and also to help evaluate local temporal dynamics by placing them within a broader regional context (Midway et al. 2016; Paukert et al. 2016).

Quantifying temporal trends in fish abundance (or indices of abundance), in particular, is used to help inform a variety of fisheries management and conservation priorities (Hansen et al. 2015; Wagner et al. 2013; Dauwalter et al. 2009). However, as highlighted by Wagner et al. (2013), the use of linear models for quantifying temporal trends may fail to detect short term changes and nonlinear temporal patterns. The use of such models may reduce the ability to elucidate ecologically and managerially relevant temporal trends. Therefore, the ability to make inferences about regional dynamics of economically and recreationally important fish species may be restricted. Focusing on long-term monotonic changes through the use of traditional linear models may obscure the ability to detect and make inferences about ecologically important population dynamics that operate over shorter temporal scales. Disease outbreaks, effects of fishing

regulations, environment stochasticity, or changes in angler behavior may contribute to short-term (e.g., 5 years), management-relevant changes in fish abundance (Hansen et al. 2015; Hughes et al. 2015; Johnston et al. 2013). In addition, many fish populations are tightly linked to annual variations in habitat conditions (Nunn et al. 2007; Rogers and Milner 1997), which makes it unlikely that these populations would demonstrate linear (or monotonic) temporal dynamics. Thus, the ability to make inferences about the nonlinear nature and year-to-year changes (e.g., the probability of annual declines) in fisheries indices will likely play an increasingly important role within the context of global change (Wagner et al. 2013).

As a nationally important recreational fish species, the Smallmouth Bass *Micropterus dolomieu* is a species of management interest due to recreational appeal and its role as an apex predator (Noble 2002). Black bass, which include Smallmouth Bass, are among the most preferred sportfish targeted by anglers in several Mid-Atlantic States including Maryland, Pennsylvania, and West Virginia (U.S. Department of the Interior, U.S. Fish and Wildlife Service, and U.S. Department of Commerce, U.S. Census Bureau 2011). Recently there have been concerns about how Smallmouth Bass populations have changed over time in some rivers in the Mid-Atlantic States where fish health issues have also been documented (Smith et al. 2015; Arway and Smith 2013; Blazer et al. 2010). Management agencies have also raised concerns about declines in assessment catch rates in some rivers (e.g., the Susquehanna and Juniata rivers) over the past 10-15 years, but not in others (e.g., Delaware River). However, it is unclear how the temporal dynamics of riverine Smallmouth Bass populations vary across this region as a whole. Therefore, the objective of this research was to quantify temporal dynamics in catch per unit effort

(CPE) and the probability of annual declines in riverine Smallmouth Bass from rivers in several Mid-Atlantic States.

<A>Sampling Methods

Riverine Smallmouth Bass catch data were collected by state agencies in Maryland, Pennsylvania, and West Virginia (Figure 1). Sampling methods varied among states, but were standardized within each respective state. Rivers were selected based on several criteria including the: (1) availability of long-term datasets (9 years of data was the minimum used), (2) adherence to standardized sampling over the time series, and (3) utility of multiple sites/observations throughout the sampling duration.

 Pennsylvania –Age 1 and older Smallmouth Bass were sampled by the Pennsylvania Fish and Boat Commission Fisheries Management Division (PFBC) from 1990 - 2011 using single-pass nighttime boat electrofishing. Age 1 fish were generally greater than 125-150mm in length and were verified with age specific data. Sampling took place during late summer and fall and catch per unit effort (CPE) was reported as catch per hour of electrofishing. Six rivers were selected for trend analysis including the Allegheny, Delaware, Juniata, Susquehanna (below confluence with West Branch Susquehanna), North Branch Susquehanna (Susquehanna River above confluence with West Branch Susquehanna), and West Branch Susquehanna rivers (Figure 1). The Susquehanna River Basin included the West Branch Susquehanna, North Branch Susquehanna, Susquehanna River (main stem), and Juniata River. Rivers outside of the Susquehanna River Basin, included the Allegheny and Delaware rivers. The Susquehanna River and Juniata River were divided into upper and lower sections based on sampling

divisions previously determined by PFBC (Figure 1). Separating these rivers into lower and upper sections resulted in a total of eight separate rivers and/or river sections. Not all rivers were sampled in every year and the number of years with no catch data varied between rivers and river sections (Table 1). The majority of the missing data were between 1998 and 2005. Surveys were directed at quantifying total CPE (age 1 and older) and CPE of larger fish ≥ 375 mm in total length (TL).

 Maryland.– Stock size and larger (>178 mm TL; Gabelhouse 1984)

Smallmouth Bass were sampled from the mainstem Potomac River (Figure 1) by the Maryland Department of Natural Resources (MD DNR) from 1988 through 2014. Sampling consisted of standardized single-pass daytime boat electrofishing at 24 possible fixed mainstem sites from Pawpaw, WV downstream to Edwards Ferry, MD during the fall when water temperatures were below 18.3°C. Sampling targeted all Smallmouth Bass with CPE reported as catch per hour of electrofishing. In addition to quantifying stock size and larger CPE, the management was interested in quantifying CPE of quality -size (>279 mm TL; Gabelhouse 1984) fish.

 West Virginia.– Smallmouth Bass were sampled by the West Virginia

Division of Natural Resources (WV DNR) in the South Branch Potomac River (Figure 1). The South Branch of the Potomac River is a tributary of the Potomac River. Standardized surveys were completed during the fall using nighttime boat electrofishing at five fixed sites from 2005 to 2014. Sample sites were distributed between the North Fork South Branch Potomac River and the confluence with the Potomac River mainstem. Sampling targeted all Smallmouth Bass and CPE was reported as catch per hour of

electrofishing. In addition to all catch, larger size targets were based on proportional stock density similar to Maryland (Gabelhouse 1984).

<A>Statistical Methods

Dynamic linear models (DLMs) have temporally varying parameters which circumvent the limitations of traditional linear models when making inferences about ecologically relevant temporal dynamics. DLMs have been used in a variety of ecological trend detection applications (e.g., trends in chlordane in fish; Azim et al. 2011), and have been suggested as a useful tool for quantifying temporal dynamics in fisheries (Wagner et al. 2013). Dynamic linear models were used for investigating annual trends in Smallmouth Bass CPE and fitted using Bayesian estimation, which allowed for probabilistic statements about annual changes in CPE (Wagner et al. 2013). The DLM consisted of an observation and system level equation, following the methodologies outlined in Wagner et al. (2013). The following model was fitted to each river or river section separately:

Observation Level:

(1)

$$\log_e(CPE + 1)_{ti} = level_t + \Psi_{ti}, \Psi_{ti} \sim N(0, \Psi_t)$$

Systems Level:

(2)

$$level_t = level_{t-1} + rate_t + \omega_{t1}, \omega_{t1} \sim N(0, \Omega_{t1})$$

(3)

$$rate_t = rate_{t-1} + \omega_{t2}, \omega_{t2} \sim N(0, \Omega_{t2})$$

Where $\log_e(CPE + 1)_{ti}$ is the \log_e of CPE with 1 added for scaling of zero catches for observation i in year t ; $level_t$ is the mean \log_e of CPE for year t ; $rate_t$ is the mean change in \log_e of CPE for each year t , and Ψ_{ti} , ω_{t1} , ω_{t2} , are error terms and are assumed to be normally distributed with a mean of zero and variance Ψ_t , Ω_{t1} , Ω_{t2} , respectively. See Wagner et al. (2013) for details on prior specification. All models were fitted for each river separately using the program JAGS and the R2jags package (Su and Yajima 2012) called from Program R (R Core Team 2013). Three separate chains were run, each for 70,000 iterations with 10,000 iterations removed as burn-in during chain convergence. Every third sample was retained for a total of 60,000 samples used to summarize the posterior distribution. Model convergence was assessed using \hat{R} , a convergence statistic, and visual inspection of trace plots.

Posterior distributions were summarized to obtain estimates of annual mean CPE estimates and rates of change between years, along with 95% credible intervals. The probability of an annual decline in CPE (i.e., the probability that the annual slope was < 0) was also calculated. A probability of decline greater or less than 50% could be viewed as not due to chance alone. Although we report these probability values, we do not suggest a specific threshold that is more or less important – as thresholds would need to be identified by managers and stakeholders on a case-specific basis. Models were fitted to the most comprehensive data (include all target size classes) from each state as well as to catch data of longer length classes (PA rivers ≥ 375 mm TL and MD and WV rivers > 279 mm TL). The longer size classes selected were based on discussion with fisheries managers regarding size structure in the system over the time series and were deemed management-relevant by the respective agencies. For example, the longer size classes

represent an important part of the population recreationally because they are generally the most appealing for anglers.

<A>Results

Pennsylvania

The total number of CPE observations per river ranged from 33 for the Upper Juniata River section to 166 in the Delaware River, and within a given year the number of observations ranged from 1 in the Lower Juniata River to 15 in the Upper Susquehanna River (Table 1). Mean CPE over the entire time series (1990-2011) was highest in the Upper Juniata River (116.7 ± 79.6 fish/h, mean \pm s.d.; Table 1) and lowest in the West Branch Susquehanna River (39.6 ± 64.5 fish/h; Table 1). There was also high variability in the catch data for each river and river section. For instance, CPE at the Allegheny River ranged from 10 fish per hour to the highest CPE across all sites of 687.7 fish per hour (Table 1).

Temporal dynamics in Smallmouth Bass total catch varied across regions and rivers (Figure 2). At various points in time, all rivers, except for the Delaware River, had consecutive years of declining catch rates, where the probability of an annual decline was greater than 50% (Figure 3). The Susquehanna River sections and its tributaries all had extended periods of declining catch rates, but the time periods and magnitude of the declines varied. The Upper Juniata River and West Branch Susquehanna River had a decline in catch rates throughout most of the time series (Figure 2), with an annual probability of decline ranging between 50-75% (Figure 3). In contrast, the Lower Juniata River, Lower Susquehanna River, and Upper Susquehanna River had more defined

periods of decline and probabilities of annual declines that exceeded 90% (Figure 3). The North Branch Susquehanna River also had a more defined period of decline with the probability of decline reaching near 80%. The highest annual probability of decline across all rivers was 97.7% in 2002 in the Lower Susquehanna River. The time periods during which the declines in catch rates occurred also varied. For example, the Lower Susquehanna and Upper Susquehanna sections had the largest probability of decline in catch rates (annual declines > 80% probability) between 1999 and 2004; whereas, the Lower Juniata River had the largest probability of decline between 1993 and 1999 (Figure 3). Towards the end of the time series, a majority of the rivers in Pennsylvania (five out of eight) also had an increasing or stable trend in catch rates, except for the West Branch Susquehanna River, Upper Juniata River, and Allegheny River (Figure 2).

In contrast to periods of declines in total catch for most rivers, when the analysis was restricted to fish longer than or equal to 375 mm TL, there was a general trend toward increasing or stable catch rates observed across all rivers (Figure S1). In addition, probability of decline rarely exceeded 50% for more than a few years and only in a few of the rivers (Figures S2). For example, the Upper Juniata River had a period of decline for fish longer than or equal to 375 mm TL between 1990 and 1995, where probability of an annual decline ranged between 58-83% (Figure S2).

Maryland

In the Potomac River, there were a total of 439 observations collected between 1988 and 2014. In a given year, the total number of observations ranged from 4 to 65. Total CPE, including stock length fish or longer, ranged from 5 to 397 fish per hour (mean = 77.5 ± 57.0 , Table 1). Annual trends in Smallmouth Bass total CPE were

relatively stable over time, except for a few short periods of decline, with the largest occurring from 1997 to 2001. During this time period, the probability of an annual decline ranged from 52.8% to 76.5% (Figure 4). The highest annual probability of decline occurred in 2000. When the analysis was restricted to quality length and longer fish, the temporal patterns in CPE was variable, but was generally increasing or stable (i.e., probability of annual declines < 50% throughout the time series; Figure S3)

West Virginia

There were a total of 143 CPE observations between 2005 and 2014 in the South Branch Potomac River. The number of observations per year ranged from 10 to 19 and CPE ranged from 36 to 978 fish per hour for all Smallmouth Bass collected (mean CPE = 249.2 ± 182.9 fish per hour; Table 1). Annual trends in catch rates were relatively stable throughout the time series (Figure 5). There was a slight decline in catch rates during the last three years (2012-2014), with a probability of decline just above 50% (55.3-58.3%; Figure 5). When the analysis was restricted to fish that were quality length and longer (>279mm TL), the declining trend in catch rates during the last three years of the time series was more apparent, with probability of annual declines ranging between 59.7 and 74.2% (Figure S4).

<A> Discussion

We found that temporal dynamics of Smallmouth Bass CPE varied both within and across rivers or river sections in several Mid-Atlantic States. Several rivers and river sections had multiple years of declining catch rates, high probabilities of annual declines or declining trends in catch rates throughout the length of their time series. The most

notable declines using total catch data (duration and magnitude) in this study occurred within the Susquehanna River Basin, which also had a recent (within the last 10-15 years) history of clinical signs of disease and mortality of young-of-year Smallmouth Bass (Smith et al. 2015; Blazer et al. 2014; Arway and Smith 2013). Variable river characteristics including fish disease and recovery from acid mine drainage impairment were present in the study area and could have influenced the temporal dynamics of CPE. Other factors that may have contributed to temporal changes in fish populations include annual variability in flow, water temperature, and harvest rates (Alos et al. 2014, Miller et al. 2014, Nunn et al. 2003). Unfortunately, these factors could not be quantified for this analysis. Regardless of the drivers of annual changes in CPE, our analysis and methods provide detailed insight (i.e., at the annual time-step) into temporal trends and a metric (annual probability of decline) that is easily communicated with stakeholders. It should be noted that the annual probabilities could be summarized in a variety of ways depending on management objectives. For instance, one could calculate the probability of an annual increase or decrease that exceeds some threshold identified as management or ecologically relevant.

Trends in CPE may be used for a wide range of management purposes. Some of the uses of temporal trends in CPE include the following: understanding invasive species colonization (i.e., Northern Snakehead *Channa argus*, Odenkirk and Isel 2016), management of sportfish (Bethke and Staples 2015), and evaluating changes in fish community structure (Riley et al. 2008, Bethke and Staples 2015). In Virginia, CPE trends were used to assess abundance of invasive Northern Snakeheads and provided insight regarding the trends in abundance after colonization (i.e., stabilizing or declining

rate of increase in abundance; Odenkirk and Isel 2016). In Lake Huron, CPE was used to show declines in several deep water fish species over a time period of great ecological change (i.e., invasive species introductions, stocking of fish, disease; Riley et al. 2008). Whether trends in CPE are focused on a single species of management interest or changes in community structure, abundance indices provide valuable information for managers (Bethke and Staples 2015).

Managing important Smallmouth Bass fisheries throughout the Mid-Atlantic Region is a priority for many state agencies. Our analysis and methods, by quantifying annual changes in CPE, may provide useful information to help guide management decisions over management-relevant timeframes (Wagner et al. 2013) and helps provide a regional perspective on temporal trends in riverine Smallmouth Bass populations. Although quantifying temporal trends does not directly give an indication of what may be driving temporal dynamics, it does allow management agencies to (1) determine whether or not management thresholds may be crossed that might trigger management actions, (2) consider if there are stressors that may need to be investigated in more detail, and (3) evaluate management actions to curtail declines in the quality of the recreational fisheries. For example, the Susquehanna River Basin is under a five-year management plan from the PFBC (Pennsylvania Fish and Boat Commission 2011, internal report Susquehanna River Management Plan). Temporal changes in fish populations within a five year time period will provide insight for the next management plan. This is an example where a framework that includes methods capable of quantifying short-term patterns in CPE, such as DLMs, may provide useful information that can help guide management strategies. Regional, multi-system investigations that also capture short-term

(i.e., annual) variability in fish populations will likely become more important to help inform management strategies for addressing many contemporary environmental stressors related to global change, including climate, and land use change.

<A> Acknowledgements

We would like to thank all of the state agency personnel from Pennsylvania Fish and Boat Commission (PFBC), Maryland Department of Natural Resources (MD DNR), and West Virginia Division of Natural Resources (WV DNR) that participated in collecting and providing Smallmouth Bass survey data for this analysis. Special thanks are given to MD DNR personnel Mark Toms and Josh Henesy for their assistance with Smallmouth Bass collections on the Potomac River and WV DNR biologist Jim Hedrick for assistance on the South Branch of the Potomac. Use of trade names is for identification purposes only and does not imply endorsement by the U.S. government. Data used in this study can be publicly available through each respective management agency.

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Table I: Descriptive information for rivers used for trend analysis of Smallmouth Bass catch per effort (CPE), including range of time series, years with missing data, total number of observations (Obs) and range, mean and standard deviation (SD) of CPE across the time series Rivers were sampled in the states of Pennsylvania, Maryland, and West Virginia.

State	River	Data Range	Missing data	Total Years	Number of Sites	Number of obs.	CPE (min-max)	Mean CPE (\pm SD)
PA	Allegheny River	1990-2011	1998-2005	14	3	66	10.0-687.7	103.7 (98.5)
PA	Delaware River	1990-2011	2001-2004	18	8	166	8.4-253.8	52.4(40.8)
PA	Lower Juniata River	1990-2011	1997,1999-2004	15	2	48	1.7-508.9	69.2 (79.2)
PA	Upper Juniata River	1990-2011	1996-1997,1999-2004	14	2	33	50.0-430.0	116.7 (79.6)
PA	North Branch Susquehanna River	1990-2011	2002-2004	19	6	93	12.5-564.5	105.0 (90.0)
PA	West Branch Susquehanna River	1990-2011	1997,1999-2004	15	3	71	1.4-492.0	39.6 (64.5)
PA	Lower Susquehanna River	1990-2011	2002-2004	19	3	69	1.0-299.0	67.2 (65.6)
PA	Upper Susquehanna River	1990-2010	1996-1997, 1999-2000,2002-	14	4	86	2.0-361.6	80.0 (81.9)

			2004					
MD	Potomac River	1988-2014	1989, 1991, 2003	24	24	439	5.0-397.0	77.5 (57.0)
WV	South Branch Potomac River	2005-2014	2007	9	5	143	36.0-978.0	249.2 (182.9)

Figure 1: Map of study region showing the rivers used in Smallmouth Bass trend analysis. Rivers were located in Maryland, Pennsylvania, and West Virginia. Two rivers, the Susquehanna River and Juniata Rivers in Pennsylvania were divided into upper and lower sections prior to analysis. The Susquehanna River was separated by the York Haven Dam (black square) and the Juniata River was separated by the town of Lewistown (black circle) into upper and lower sections.

Figure 2: Temporal trends in \log_e -transformed Smallmouth Bass catch per effort (CPE) in eight river or river sections in Pennsylvania. The solid circles are observed data, black lines represent the posterior mean fitted lines and the gray shaded area represents the 95% credible regions. The river and river sections are as follows: (A): Allegheny River, (B): Delaware River, (C): Lower Juniata River, (D): Upper Juniata River, (E): North Branch Susquehanna River, (F): West Branch Susquehanna River, (G): Lower Susquehanna River, and (H) Upper Susquehanna River.

Figure 3: Probability of annual declines in \log_e -catch per effort (CPE) of Smallmouth Bass in eight river or river sections in Pennsylvania. The solid black line represents the probability of annual decline for each year and the horizontal dotted line represents a 50% probability of decline for reference. The river and river sections are as follows: (A): Allegheny River, (B): Delaware River, (C): Lower Juniata River, (D): Upper Juniata River, (E): North Branch Susquehanna River, (F): West Branch Susquehanna River, (G): Lower Susquehanna River, and (H) Upper Susquehanna River.

Figure 4: Temporal trends in \log_e -transformed Smallmouth Bass catch per effort (CPE) (A) and probability of annual declines in \log_e -catch per effort (CPE) (B) Smallmouth Bass catch data from the Potomac River in Maryland. In plot (A), the solid circles are observed data, black lines represent the posterior mean fitted lines and the gray shaded area represents the 95% credible regions. In plot (B), the solid line represents the annual probability of decline and the dashed horizontal line represents a 50% probability of decline for comparative purposes.

Figure 5: Temporal trends in \log_e -transformed Smallmouth Bass catch per effort (CPE) (A) and probability of annual declines in \log_e -catch per effort (CPE) (B) in Smallmouth Bass catch data from the South Branch of the Potomac River in West Virginia. In plot (A), the solid circles are observed data, black lines represent the posterior mean fitted lines and the gray shaded area represents the 95% credible regions. In plot (B), the solid line represents the annual probability of decline and the dashed horizontal line represents a 50% probability of decline for comparative purpose.

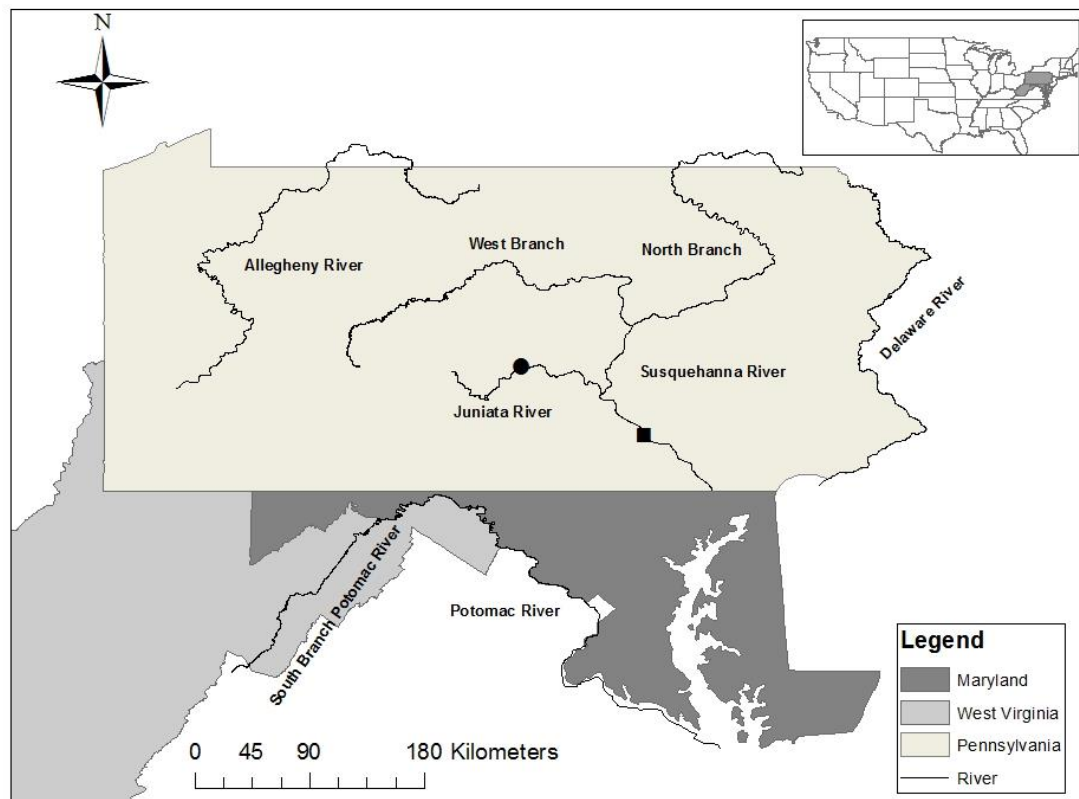


Figure 1: Schall et al.

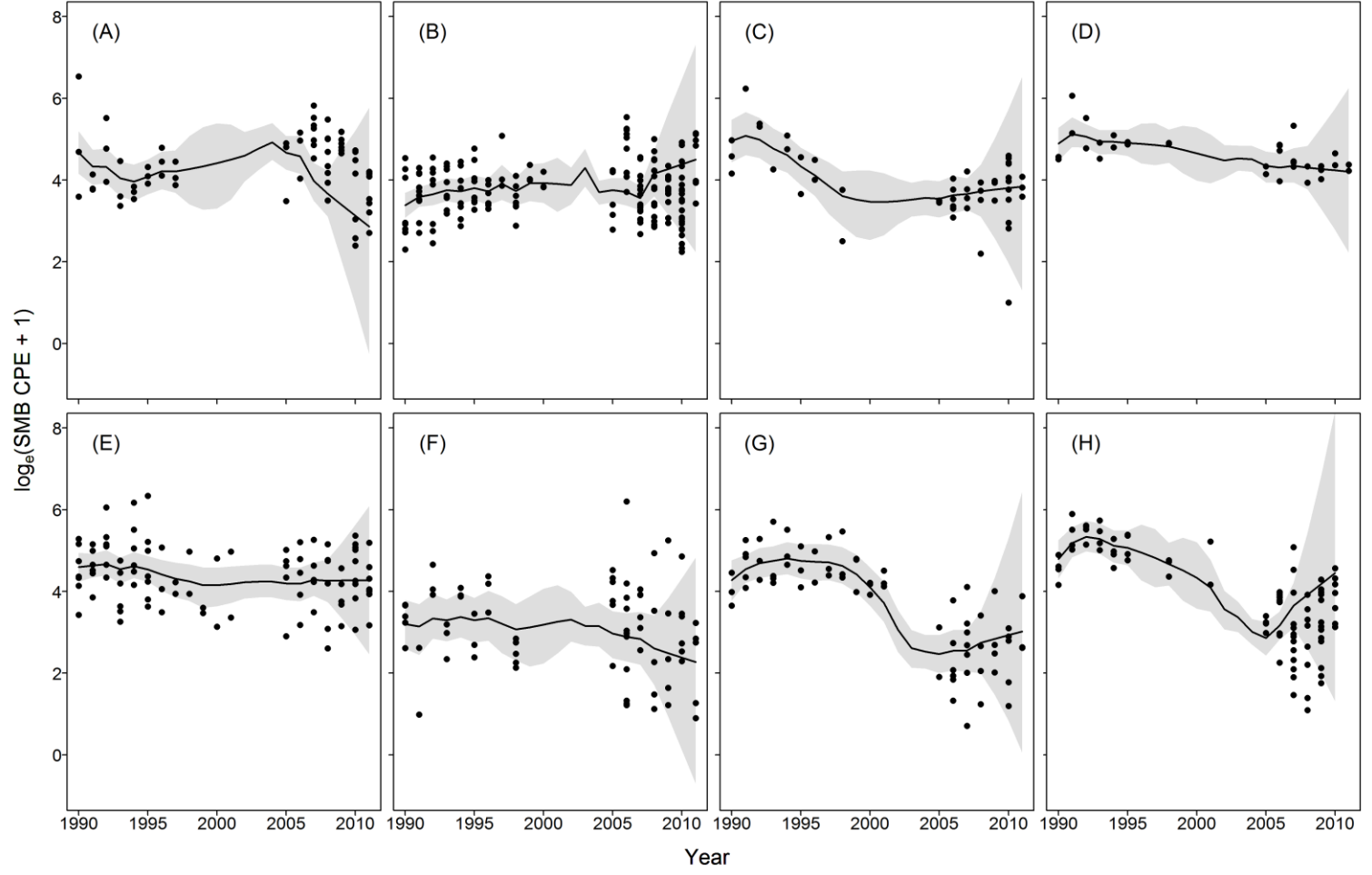


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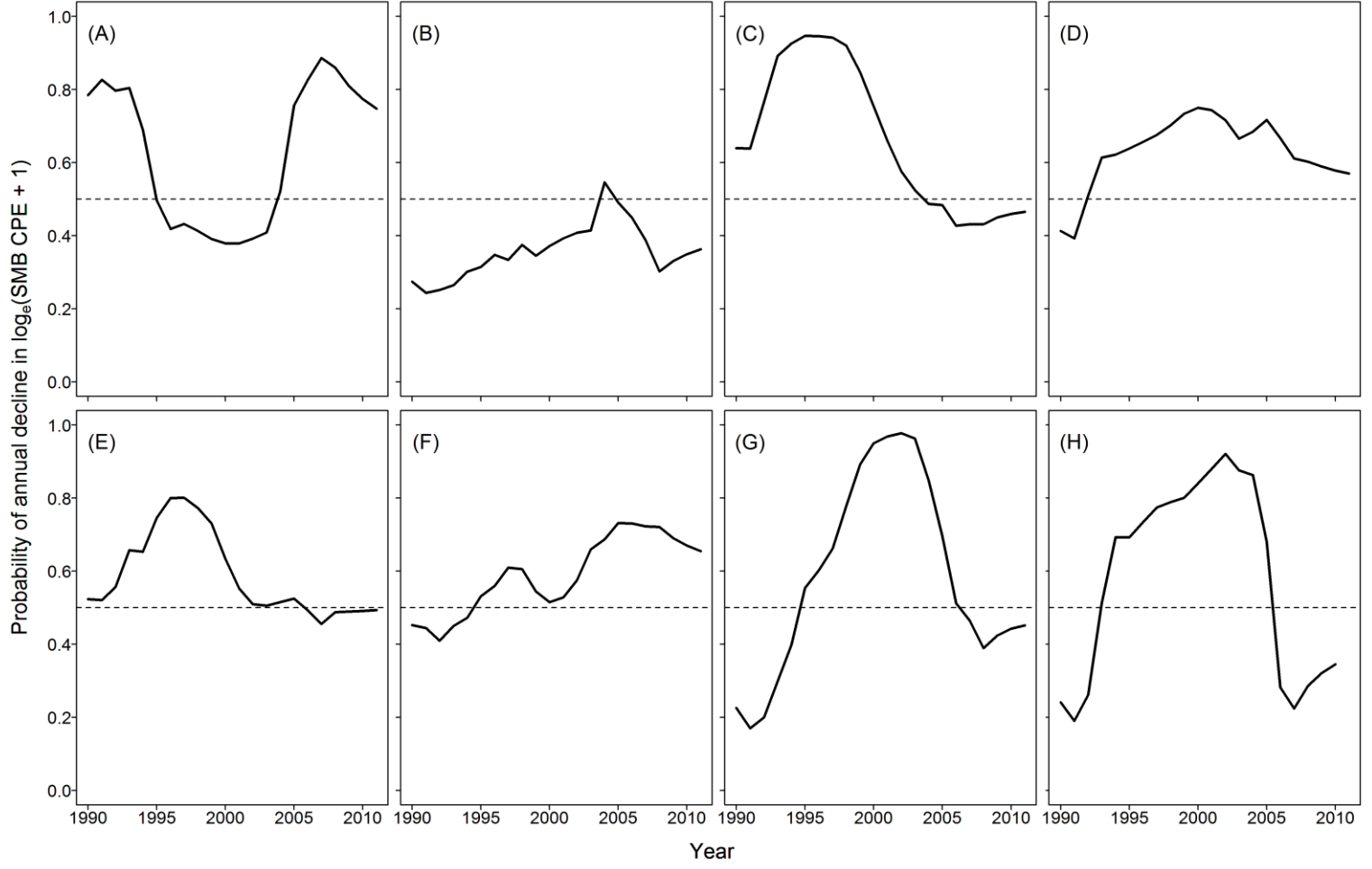


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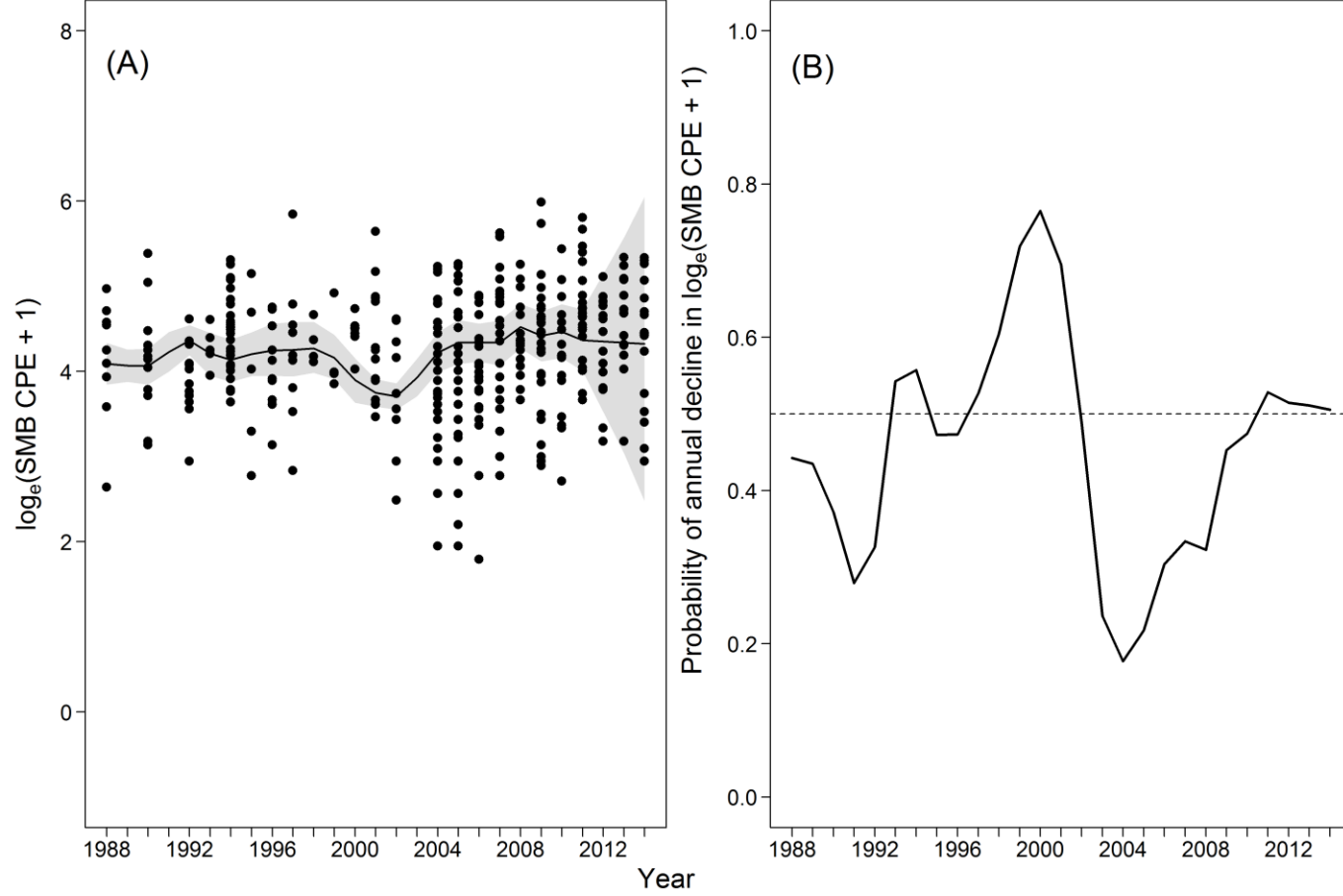


Figure 4: Schall et al.

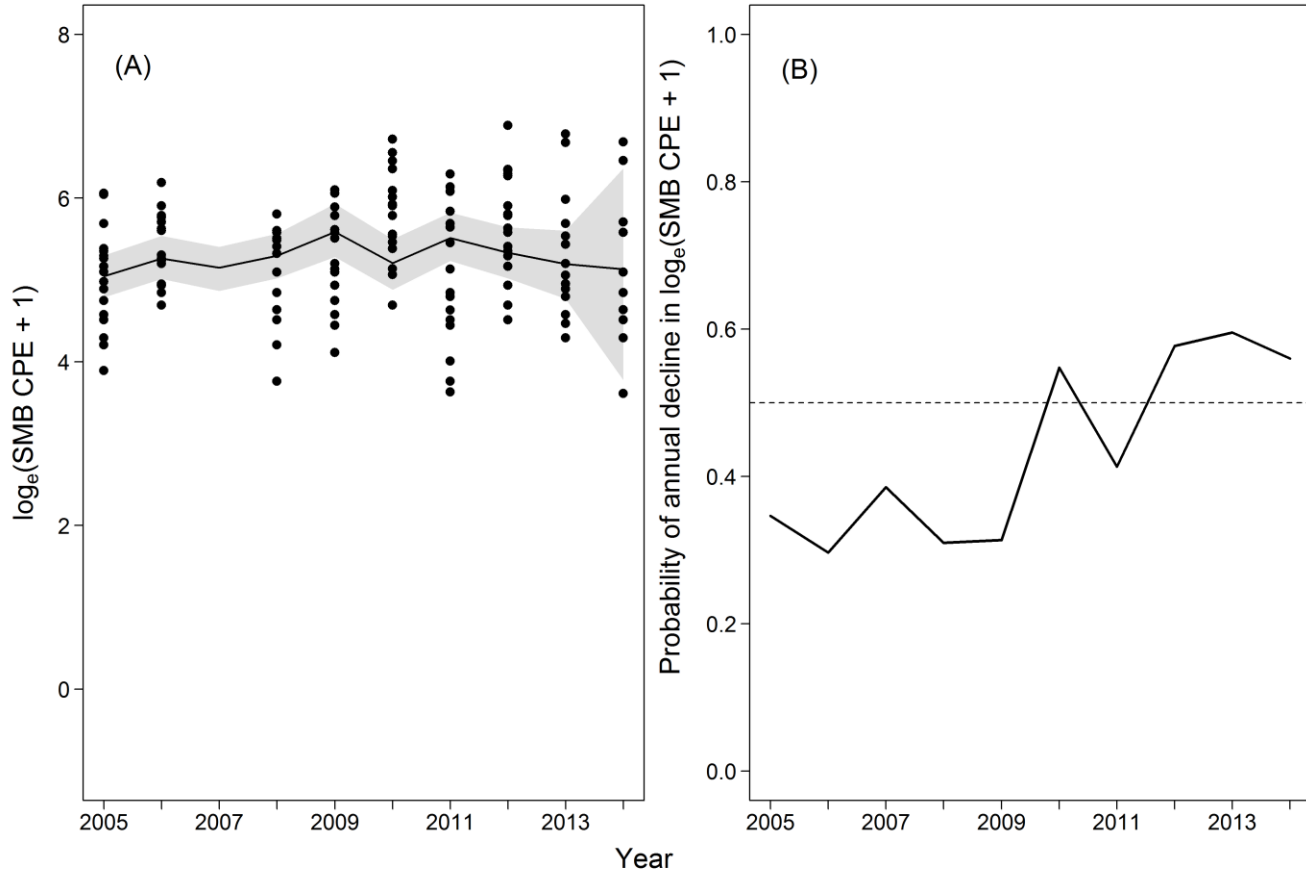


Figure 5: Schall et al.

Schall_et_al_Supplemental Figures

Figure S1: Temporal trends in \log_e -transformed Smallmouth Bass (> 375 mm) catch per effort (CPE) in eight river or river sections in Pennsylvania. The solid circles are observed data, black lines represent the posterior mean fitted lines and the gray shaded area represents the 95% credible regions. The river and river sections are as follows: (A): Allegheny River, (B): Delaware River, (C): Lower Juniata River, (D): Upper Juniata River, (E): North Branch Susquehanna River, (F): West Branch Susquehanna River, (G): Lower Susquehanna River, and (H) Upper Susquehanna River.

Figure S2: Probability of annual declines in \log_e -catch per effort (CPE) of Smallmouth Bass >375mm in eight river or river sections in Pennsylvania. The solid black line represents the probability of annual decline for each year and the horizontal dotted line represents a 50% probability of decline for reference. The river and river sections are as follows: (A): Allegheny River, (B): Delaware River, (C): Lower Juniata River, (D): Upper Juniata River, (E): North Branch Susquehanna River, (F): West Branch Susquehanna River, (G): Lower Susquehanna River, and (H) Upper Susquehanna River.

Figure S3: Temporal trends in \log_e -transformed Smallmouth Bass (>279mm) catch per effort (CPE) (A) and probability of annual declines in \log_e -catch per effort (CPE) (B) of Smallmouth Bass > 279mm from the Potomac River in Maryland. In plot (A), the solid circles are observed data, black lines represent the posterior mean fitted lines and the gray shaded area represents the 95% credible regions. In plot (B), the solid line represents the

annual probability of decline and the dashed horizontal line represents a 50% probability of decline for comparative purposes.

Figure S4: Temporal trends in \log_e -transformed Smallmouth Bass catch per effort (CPE) $>279\text{mm}$ (A) and probability of annual declines in \log_e -catch per effort (CPE) (B) in Smallmouth Bass catch data ($>279\text{mm}$) from the South Branch of the Potomac River in West Virginia. In plot (A), the solid circles are observed data, black lines represent the posterior mean fitted lines and the gray shaded area represents the 95% credible regions. In plot (B), the solid line represents the annual probability of decline and the dashed horizontal line represents a 50% probability of decline for comparative purposes.

Supplemental Figures

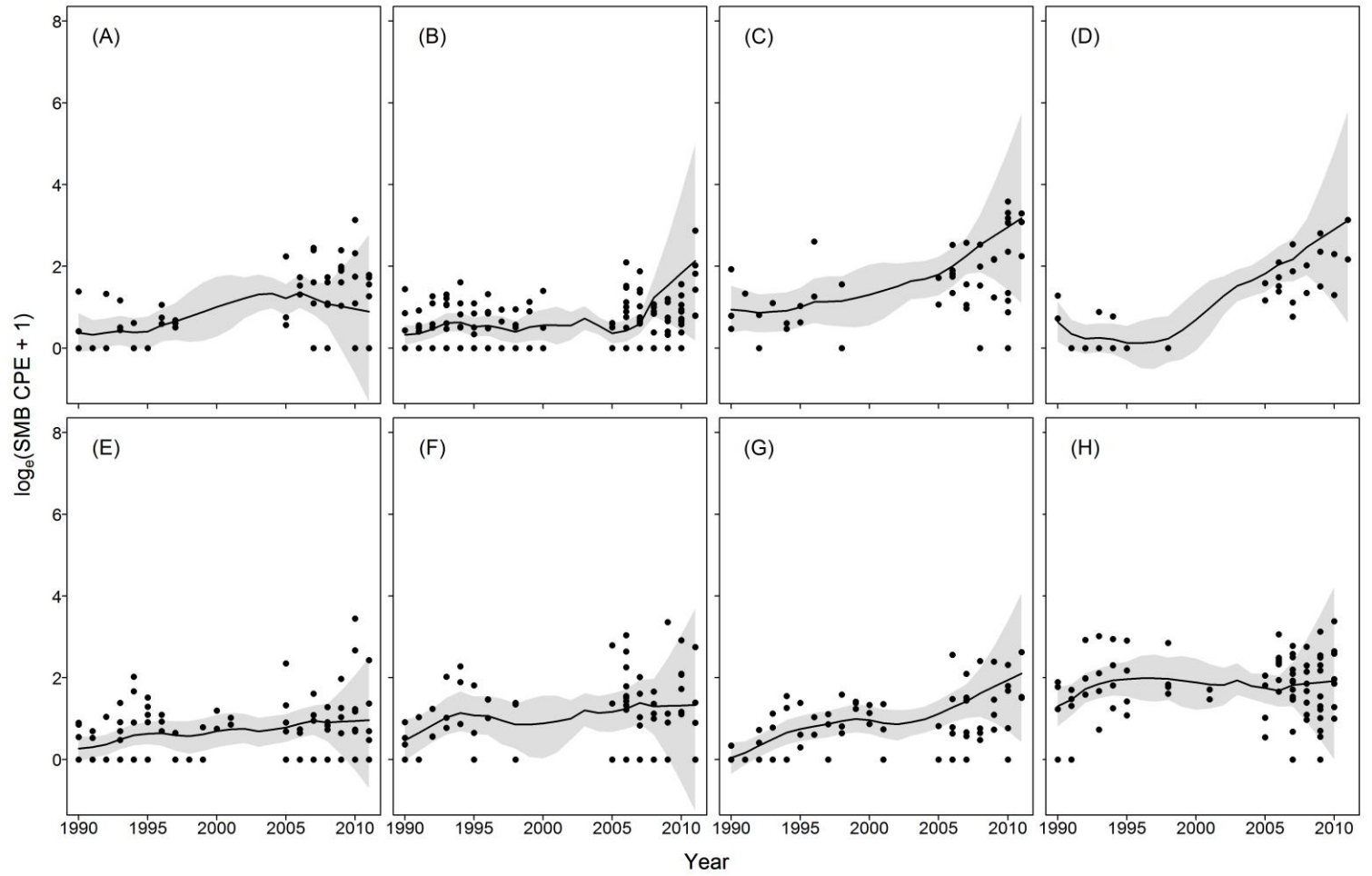


Figure S1: Schall et al.

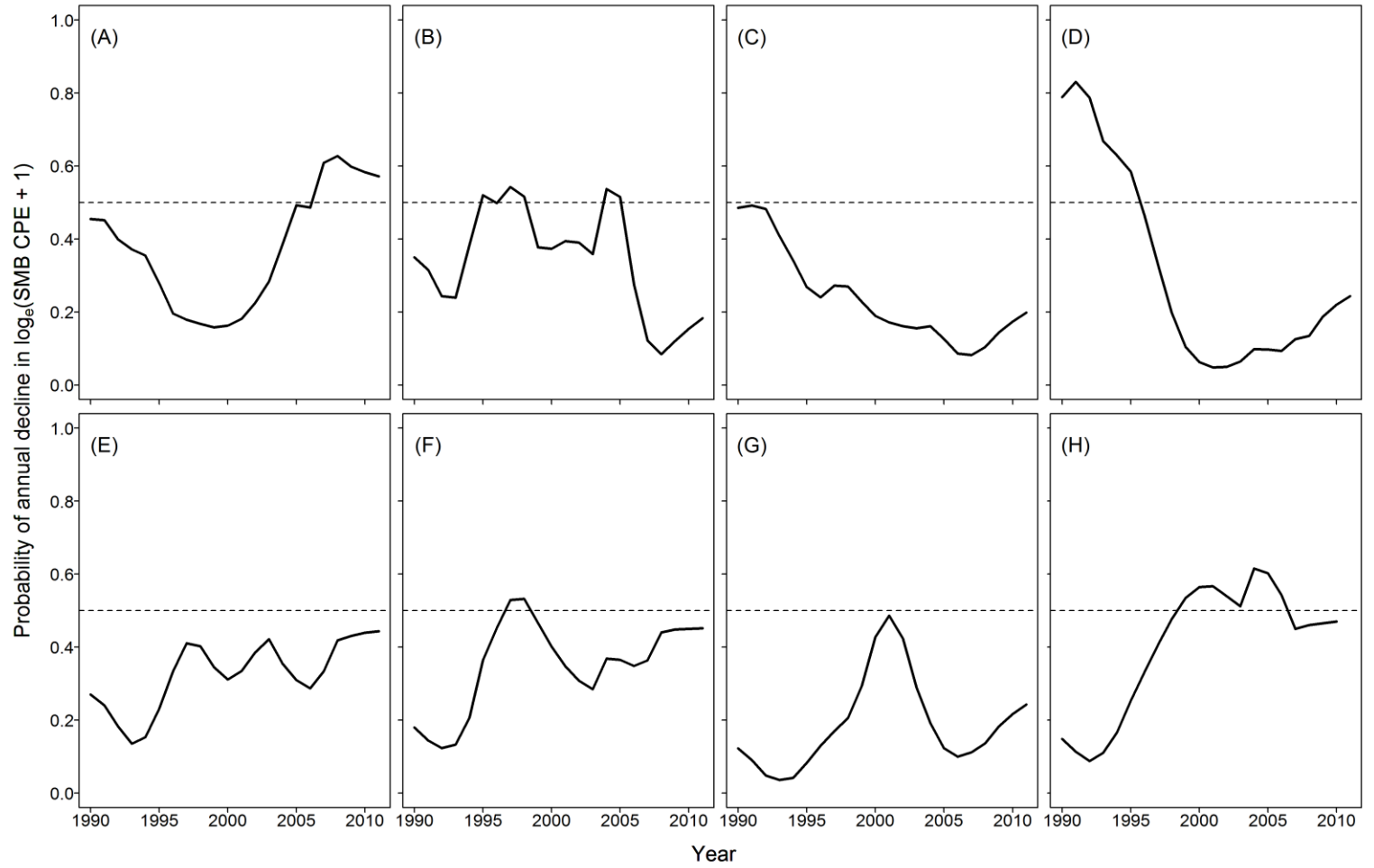


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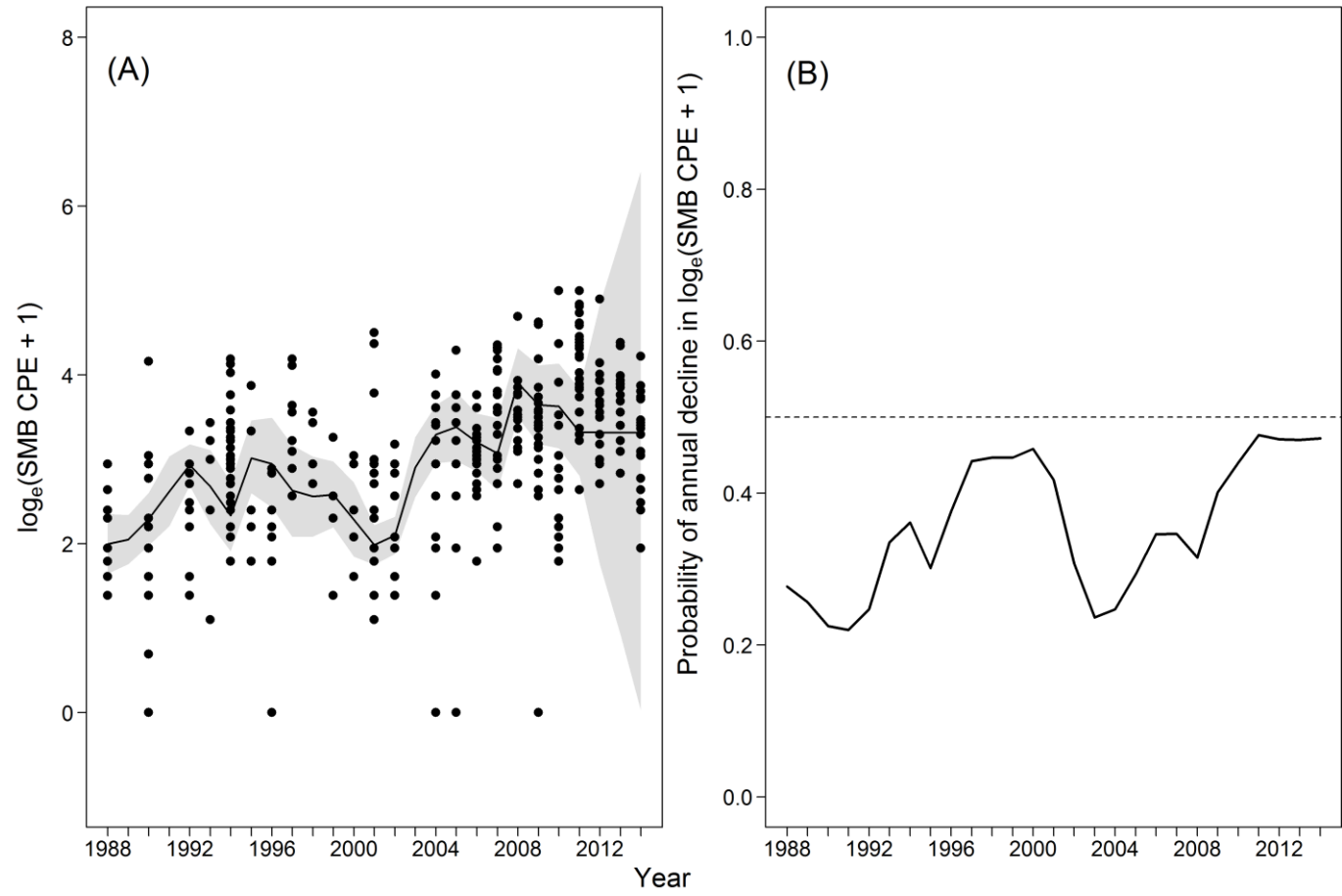


Figure S3: Schall et al.

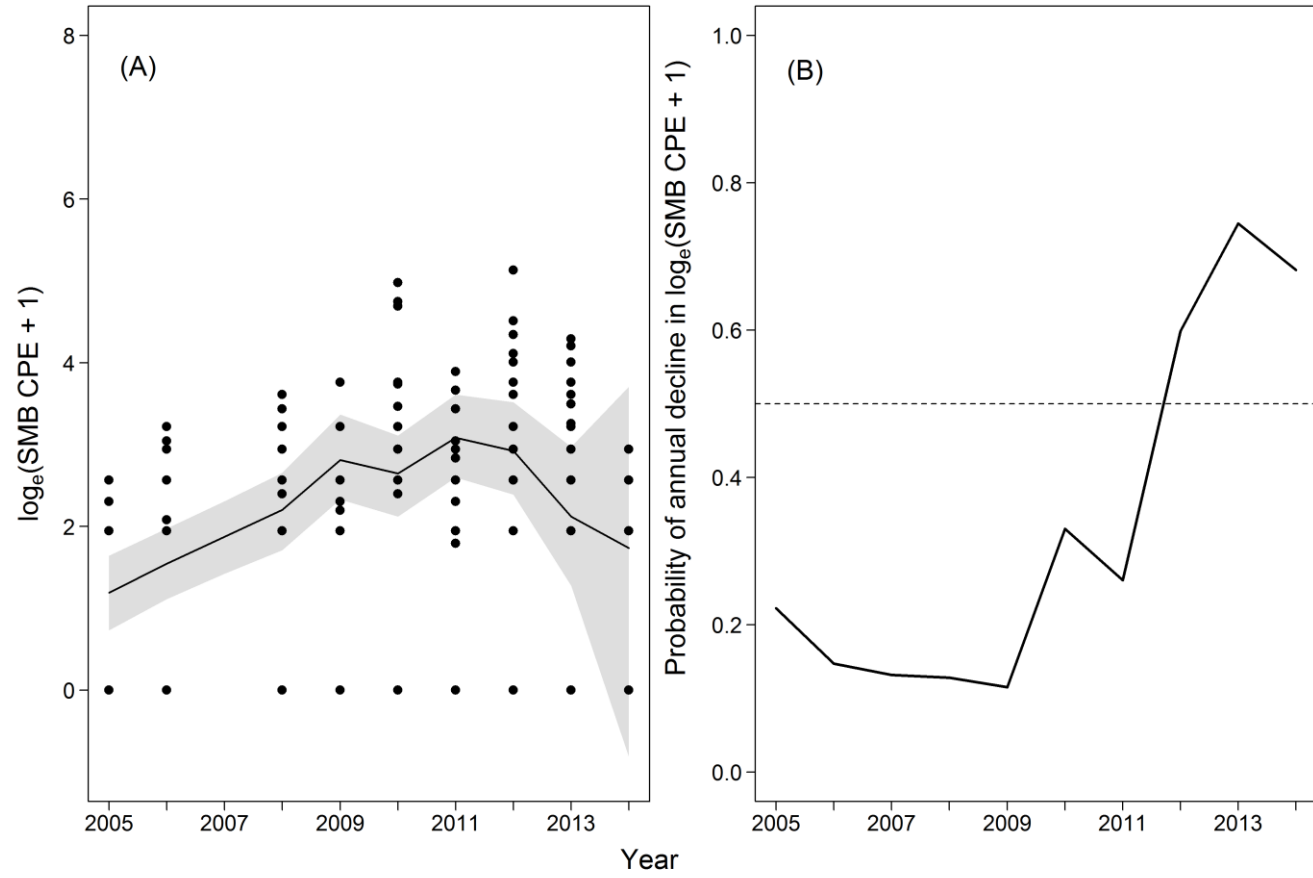


Figure S4: Schall et al.

Chapter 2

Evaluation of genetic population structure of Smallmouth Bass in the Susquehanna River Basin, Pennsylvania

Chapter 2 was published in the *North American Journal of Fisheries Management* and is included below in the published manuscript form.



ARTICLE

Evaluation of Genetic Population Structure of Smallmouth Bass in the Susquehanna River Basin, Pennsylvania

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Abstract

The Smallmouth Bass *Micropterus dolomieu* was introduced into the Susquehanna River basin, Pennsylvania, nearly 150 years ago. Since introduction, it has become an economically and ecologically important species that supports popular recreational fisheries. It is also one of the most abundant top predators in the system. Currently, there is no information on the level of genetic diversity or genetic structuring that may have occurred since introduction. An understanding of genetic diversity is important for the delineation of management units and investigation of gene flow at various management scales. The goals of this research were to investigate population genetic structure of Smallmouth Bass at sites within the Susquehanna River basin and to assess genetic differentiation relative to Smallmouth Bass at an out-of-basin site (Allegheny River, Pennsylvania) located within the species' native range. During spring 2015, fin clips ($n = 1,034$) were collected from adults at 11 river sites and 13 tributary sites in the Susquehanna River basin and at one site on the Allegheny River. Fin clips were genotyped at 12 polymorphic microsatellite loci. Based on our results, adults sampled throughout the Susquehanna River basin did not represent separate genetic populations. There were only subtle differences in genetic diversity among sites (mean pairwise genetic differentiation index $F_{ST} = 0.012$), and there was an overall lack of population differentiation ($K = 3$ admixed populations). The greatest genetic differentiation was observed between fish collected from the

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out-of-basin site and those from the Susquehanna River basin sites. Knowledge that separate genetic populations of Smallmouth Bass do not exist in the Susquehanna River basin is valuable information for fisheries management in addition to providing baseline genetic data on an introduced sport fish population.

The Smallmouth Bass *Micropterus dolomieu* is a prized sport fish and an apex predator that inhabits a variety of warmwater lotic and lentic systems. This species is native to Midwestern river systems (e.g., the Mississippi River) and the Laurentian Great Lakes. Recreational appeal has led to extensive stocking of Smallmouth Bass both within and outside of its native range in the United States (Jackson 2002) and has resulted in popular introduced sport fisheries throughout the Chesapeake Bay watershed (e.g., Potomac and Susquehanna River basins). Smallmouth Bass were first introduced into the Susquehanna River basin, Pennsylvania, in approximately 1870 by using stock from the Potomac River in West Virginia (Milner 1874; Stillwell et al. 1895). The initial stocking event was followed by multiple supplemental introductions shortly after, along with widespread stocking across the basin by local anglers (Milner 1874; Stillwell et al. 1895). Smallmouth Bass were also not native to the Potomac River but were introduced into the region from their native range in the Ohio River basin (Wheeling, West Virginia; Smith 1907; Goldsborough and Clark 1908). After its introduction, the Smallmouth Bass became a popular sport fish in the Susquehanna River; however, declines in catch rates of adult fish occurred throughout the 2000s (Arway and Smith 2013). High frequencies of clinical signs of disease (i.e., melanistic spots and lesions) and intersex conditions (i.e., testicular oocytes; Blazer et al. 2014) were also observed during that period. Such observations have raised concerns regarding the overall health of Smallmouth Bass in the Susquehanna River and have led to questions about genetic population structure. Since their introduction, there has been no evaluation of population genetic structure and genetic diversity of Smallmouth Bass within the Susquehanna River basin. In fact, the current knowledge of Smallmouth Bass population genetics in riverine systems is limited to a few studies within the native range of the species, including the Interior Highlands (Stark and Echelle 1998) and the Laurentian Great Lakes region (Borden and Stepien 2006; Stepien et al. 2007; Borden and Krebs 2009). One study examined the population genetics of both native and introduced populations of Smallmouth Bass in Virginia (Hallerman et al. 2015). Information on the population genetic structure of Susquehanna River Smallmouth Bass could provide management-relevant information about gene flow and demographic connectivity among sites within the system.

Management strategies often include both ecological and evolutionary considerations, such as the physical space that organisms utilize (“the ecological population”) and the degree to which gene flow occurs (“the genetic population”; Waples and Gaggiotti 2006). Information about population genetic structure, colonization histories, ecological interactions, and mechanisms of

genetic isolation may be assessed to help define management units of interest. Stocking and translocation histories of introduced species also affect geographic patterning of genetic variation (Jeschke and Strayer 2006; Hänfling 2007). For example, gene flow between introduced and native Smallmouth Bass populations was inferred in the New and James rivers, Virginia (Hallerman et al. 2015). The strength of selection pressures, including the degree of geographical or behavioral isolation, may play a role in genetic differentiation and the separation of fish into genetically distinct populations and subsequent management units.

Within the Susquehanna River basin, both river main-stem (West Branch Susquehanna River, North Branch Susquehanna River, and the Susquehanna River proper) and tributary habitats provide suitable warmwater spawning areas. Cohabitation of juvenile Smallmouth Bass spawned in tributaries and rivers has been evaluated previously, with tributary-spawned fish moving to the river during early life and potentially remaining in this habitat for an extended period (Humston et al. 2010). How fish in tributaries and main-stem rivers interact to affect population genetic structure is not known, but an understanding of this is important because fish in tributaries and main-stem rivers are often managed under different fishing regulations. Characterizing and quantifying gene flow between tributary and main-stem systems may provide important information for delineating management units and evaluating management strategies (e.g., setting fishing regulations). Therefore, the objectives of this research were to (1) investigate whether Smallmouth Bass spawning in river and tributary habitats at nearby sites were genetically distinct (local scale); and (2) evaluate whether Smallmouth Bass collected at sites across the Susquehanna River basin were separate genetic populations (regional scale).

METHODS

Study sites and fish sampling.—Smallmouth Bass were collected during the prespawn period (April–June 2015) by using towboat and boat electrofishing from 24 sites within the Susquehanna River basin (11 river sites and 13 tributary sites) and at one out-of-basin site (the Allegheny River near Franklin [ARF], Pennsylvania; Figure 1). Most neighboring river main-stem and tributary sites lacked barriers to movement. Samples were collected in four of the six Susquehanna River subbasins, including the West Branch Susquehanna River (10 sites), Middle Susquehanna River (4 sites), Lower Susquehanna River (8 sites), and Juniata River

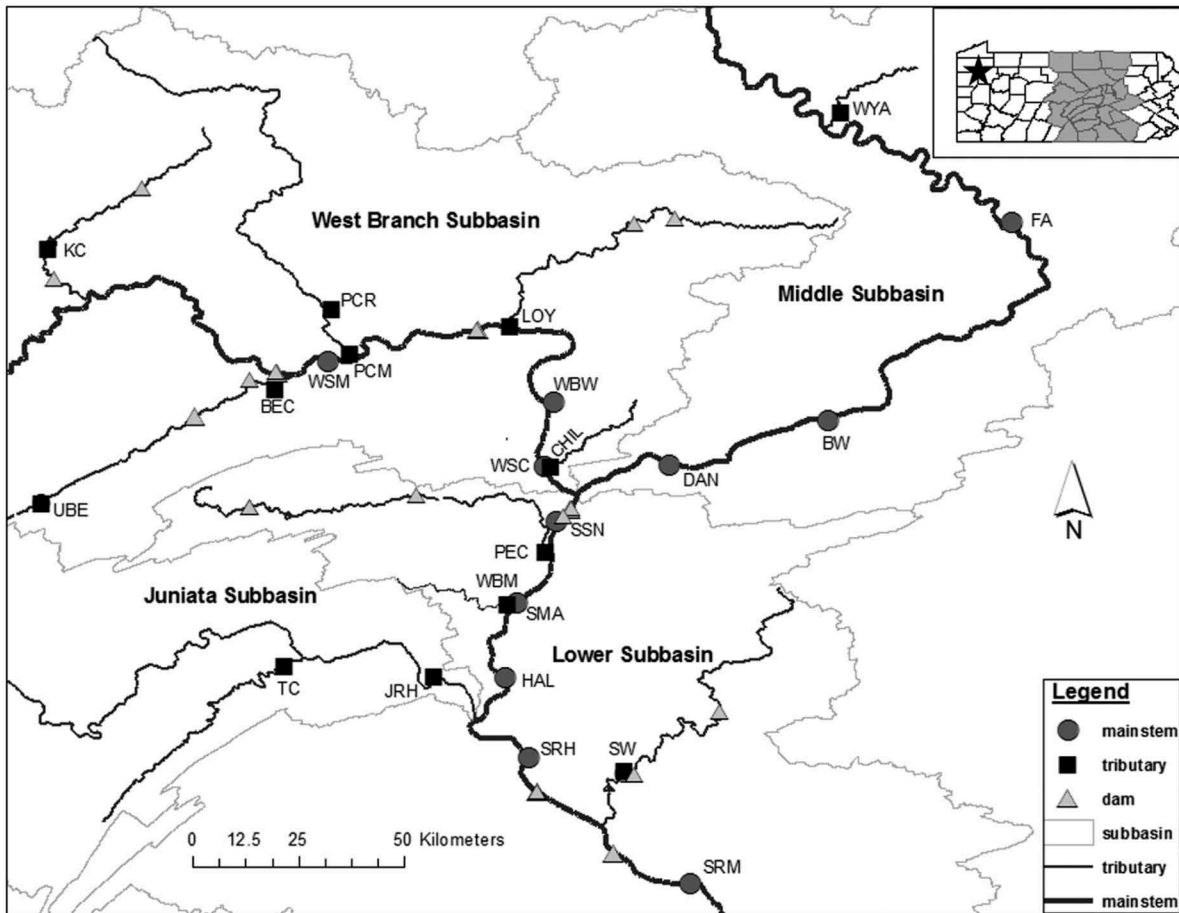


FIGURE 1. Study area in the Susquehanna River basin, Pennsylvania, where the population genetic structure of Smallmouth Bass was assessed. The out-of-basin site (Allegheny River [ARF]; black star on inset map) is in the northwestern part of the state. Samples from the West Branch Susquehanna River at Watstown (WBW; $n = 3$ fish), West Branch Susquehanna River near Chillisquaque (WSC; $n = 16$ fish), and Kettle Creek (KC; $n = 17$ fish) were excluded due to low sample sizes. All other site abbreviations are defined in Table 2.

(2 sites). The two subbasins that were not sampled were the Chemung and Upper Susquehanna rivers, which are located primarily in New York State. A sample size of 50 Smallmouth Bass was targeted at each sample site, and a 9–25-mm² tissue sample was removed from the upper caudal fin and placed in 95% nondenatured ethanol for preservation.

Genetic analysis.—Genetic analyses were completed at the U.S. Fish and Wildlife Service (USFWS) Northeast Fishery Center in Lamar, Pennsylvania. Genomic DNA was extracted from caudal fin samples in accordance with protocols for the MagBind Tissue DNA Kit KF (Omega Biotek, Norcross, Georgia) and the KingFisher Flex Magnetic Particle Processor (Thermo-Fisher Scientific, Waltham, Massachusetts). Extracted samples were diluted 70:30 or 60:40 (reagent-grade water : eluted DNA product) depending on preliminary spectrophotometry (Gene Spec 1; Hitachi Solutions America) of DNA to reach a target of 5–30 ng/ μ L of diluted DNA. Ten microsatellite markers previously used to genotype Smallmouth

Bass were used in this study: *Mdo1*, *Mdo2*, *Mdo3*, *Mdo5*, *Mdo7*, *Mdo8*, and *Mdo10* (Malloy et al. 2000); *RB7* (DeWoody et al. 1998); and *MSI9* (DeWoody et al. 2000). Two additional microsatellite markers, *Mdo12* (KY056662) and *Mdo14* (KY056263) were identified from 454 GS-FLX sequencing (Roche, Branford, Connecticut) of the Smallmouth Bass transcriptome (SRX156704) using MSATCOMMANDER (Faircloth 2008). The *Mdo12* marker was derived from a 281-base-pair (bp) sequence for transcription elongation factor B, polypeptide 3, which contains a hexanucleotide repeat (AGCCTG). The marker *Mdo14* was derived from a 1,792-bp sequence for vitamin-K-dependent gamma-carboxylase, which contains a tetranucleotide repeat (AAAC). Primers for these loci were designed using PRIMER3 (Untergasser et al. 2012) and are presented in Table 1. Forward primers of microsatellite markers were fluorescently labeled with GeneScan LIZ size standard (Applied Biosystems, Thermo-Fisher Scientific) compatible dyes and multiplexed into four groups for polymerase chain reactions (Table 1). Multiplex polymerase

TABLE 1. Microsatellite markers used to investigate the genetic diversity of Smallmouth Bass in the Susquehanna River basin, Pennsylvania.

Marker	Size range (base pairs)	Number of alleles	Multiplex group	Source	Primer sequence
<i>RB7</i>	110–138	6	1	DeWoody et al. 1998	
<i>MS19</i>	104–132	13	1	DeWoody et al. 2000	
<i>Mdo1</i>	195–213	8	2	Malloy et al. 2000	
<i>Mdo2</i>	182–202	6	2	Malloy et al. 2000	
<i>Mdo3</i>	106–138	10	4	Malloy et al. 2000	
<i>Mdo5</i>	191–205	5	4	Malloy et al. 2000	
<i>Mdo7</i>	163–171	5	4	Malloy et al. 2000	
<i>Mdo8</i>	207–229	11	3	Malloy et al. 2000	
<i>Mdo10</i>	104–108	3	2	Malloy et al. 2000	
<i>Mdo11</i>	171–177	4	3	Malloy et al. 2000	
<i>Mdo12</i>	226–238	3	3	This study	Forward: GAGAGAGGCTATCACACGAAC Reverse: GACAGAAACAGGGACAGAGAC
<i>Mdo14</i>	351–355	2	2	This study	Forward: ACGGCATTTTCAGATAATTTCC Reverse: CGTATCAACTTTCAAGCTAACC

chain reactions occurred in 15- μ L volumes and included 1.5 \times GoTaq Flexi buffer, 3.75-mM MgCl₂, 0.3175-mM deoxynucleotide triphosphates, 0.12–0.2 μ M of each forward and reverse primer, 0.08 units/ μ L of GoTaq Flexi DNA polymerase (Promega, Madison, Wisconsin), and 1.5 μ L of diluted DNA template. Annealing temperature for polymerase chain reaction was 53°C for all markers except *RB7* and *MS19*, which had an annealing temperature of 48°C. Programs for polymerase chain reaction included denaturation at 94°C for 2 min; followed by 35 cycles of 94°C for 45 s, annealing at 53°C or 48°C for 45 s, and 72°C for 2 min; and a final step of 72°C for 5 min. The polymerase chain reaction products were diluted 1:35 (polymerase chain reaction product : distilled water), and fragment analysis was completed on an Applied Biosystems 3130XL Genetic Analyzer (Thermo-Fisher Scientific). Genotypes were scored by two separate individuals using GeneMapper version 5 (Thermo-Fisher Scientific). Quality controls were completed by selecting 96 samples (9.3% of the samples analyzed) and extracting and genotyping those samples again to compare results for consistency.

Site descriptive statistics.—Site-level analysis included testing of all markers and marker pairs for deviations from Hardy–Weinberg equilibrium and linkage disequilibrium using Fisher’s exact test in Genetic Data Analysis software (Lewis and Zaykin 2001), with a Bonferroni correction for multiple comparisons (Rice 1989). Descriptive site-level statistics included the average number of alleles per locus (A), allelic richness (A_r), expected heterozygosity (H_e), observed heterozygosity (H_o), and number of unique alleles (n_u). Effective population size (N_e) was estimated using the linkage disequilibrium method in N_e Estimator version 2.01

(Do et al. 2014), with 95% confidence intervals (CIs) derived from jackknifing on loci with the lowest allele frequency set to 0.01.

Site comparisons.—A global genetic differentiation index F_{ST} was computed by using FSTAT (Goudet 2002) and the methods of Weir and Cockerham (1984), with 1,000 bootstrap replicates used to derive 95% CIs. Site comparisons employed pairwise F_{ST} (Weir and Cockerham 1984) to investigate genetic differentiation between sites using the program FSTAT, and Bonferroni correction was used for multiple comparisons of significant differences. To assess the degree of hierarchical structuring across Smallmouth Bass sites, analysis of molecular variance (AMOVA) was used to quantify genetic variability in relation to geographic distances among subbasins, among sites within subbasins, and within sites. Subbasins included four in the Susquehanna River basin (West Branch Susquehanna, Middle Susquehanna, Lower Susquehanna, and Juniata rivers) and the Allegheny River as an out-of-basin site for comparison. Sites were also grouped with respect to dam locations, and a second AMOVA was completed with only the Susquehanna River basin sites (i.e., excluding the Allegheny River site) to test for genetic variability in relation to dams (see Figure 1). Dam locations separated the study area into six regions as follows: (1) UBE; (2) BEC, WSM, PCM, and PCR; (3) LOY, CHIL, DAN, BW, FA, and WYA; (4) SSN, PEC, WBM, SMA, HAL, JRH, TC, and SRH; (5) SW; and (6) SRM (site abbreviations are defined in Table 2). We used ARLEQUIN version 3.5.2 (Excoffier and Lischer 2010) to compute a standard AMOVA with F_{ST} values and 1,000 permutations.

Site relationships and population structure.—To examine genetic relationships and genetic distances, PHYLIP version 3.695 (Felsenstein 2005) and associated programs were used to generate 1,000 replicate data sets, to calculate Cavalli-

TABLE 2. Descriptive and genetic characteristics of Susquehanna River basin sites where samples of Smallmouth Bass were collected (site = site name and location in Pennsylvania [NB = North Branch; WB = West Branch]; Abbrev = site abbreviation; Subbasin = Susquehanna River subbasin; n = number of individuals sampled; A = average number of alleles per locus; A_r = allelic richness at the lowest population size [$n = 25$]; H_e = expected heterozygosity; H_o = observed heterozygosity; N_e = effective population size [Inf = infinite]; 95% CI = 95% confidence interval for N_e [∞ = infinity]).

Site	Abbrev	Subbasin	n	A	A_r	H_e	H_o	N_e	95% CI
Allegheny River, Franklin	ARF	Out of basin	43	5.17	4.72	0.52	0.53	Inf	683.4, ∞
Bald Eagle Creek, Castanea	BEC	WB	46	3.92	3.72	0.52	0.51	114.2	58.0, 566.9
NB Susquehanna River, Berwick	BW	Middle	49	3.50	3.28	0.55	0.53	Inf	204.2, ∞
Chillisquaque Creek	CHIL	WB	46	3.50	3.28	0.54	0.56	Inf	164.2, ∞
NB Susquehanna River, Danville	DAN	Middle	50	3.25	3.10	0.51	0.48	1,221.1	82.2, ∞
NB Susquehanna River, Falls	FA	Middle	50	3.50	3.29	0.54	0.55	Inf	247.5, ∞
Susquehanna River, Halifax	HAL	Lower	50	3.50	3.30	0.53	0.54	Inf	225.7, ∞
Juniata River, Howe Township	JRH	Juniata	50	3.58	3.38	0.51	0.51	546.8	88.2, ∞
Loyalsock Creek	LOY	WB	35	3.42	3.29	0.54	0.54	1,040.0	58.6, ∞
Pine Creek Mouth	PCM	WB	37	3.33	3.26	0.51	0.49	60.9	27.1, 667.7
Pine Creek, Ramsey	PCR	WB	63	3.58	3.26	0.50	0.52	Inf	114.4, ∞
Penns Creek	PEC	Lower	48	3.42	3.21	0.53	0.51	Inf	231.6, ∞
Susquehanna River, Mahantango	SMA	Lower	36	3.50	3.34	0.53	0.52	Inf	95.5, ∞
Susquehanna River, Harrisburg	SRH	Lower	50	3.33	3.19	0.55	0.55	335.4	61.0, ∞
Susquehanna River, Marietta	SRM	Lower	50	4.00	3.52	0.55	0.58	45.0	23.9, 121.7
Susquehanna River, Shady Nook	SSN	Lower	49	3.50	3.31	0.54	0.53	Inf	135.2, ∞
Swatara Creek	SW	Lower	35	3.75	3.57	0.55	0.56	59.0	29.4, 264.0
Tuscarora Creek	TC	Juniata	42	3.50	3.30	0.54	0.57	Inf	70.8, ∞
Upper Bald Eagle Creek	UBE	WB	25	3.33	3.33	0.54	0.56	110.2	28.1, ∞
WB Mahantango Creek	WBM	Lower	50	3.33	3.14	0.51	0.51	342.1	68.8, ∞
WB Susquehanna River, McElhattan	WSM	WB	41	3.83	3.57	0.53	0.53	59.0	34.3, 141.3
Wyalusing Creek	WYA1	Middle	45	3.33	3.21	0.52	0.51	152.9	49.3, ∞

Sforza and Edwards (1967) chord distances, and to draw an unrooted neighbor-joining tree. TreeView (Page 1996) was used to create the final neighbor-joining dendrogram. A Mantel test and corresponding correlogram were used to evaluate the relationship between genetic distance and geographic distance. The package “ecodist” (Goslee and Urban 2007) was used to compute the Mantel test in R software (R Core Team 2013), with 10,000 permutations and 1,000 bootstrap replicates. Genetic distance was measured as $F_{ST}/(1 - F_{ST})$, and geographic distance (river kilometers [rkm] between sites) was \log_e transformed as recommended by Rousset (1997). In the correlogram, site comparisons were used to determine the number of distance classes. The out-of-basin site (ARF) was omitted from this analysis because network distance was not measurable.

Reassignment of individual multilocus genotypes to original populations was tested using GeneClass2 (Piry et al. 2004) with the Bayesian reassignment algorithm (Rannala and Mountain 1997). Reassignment tests assign each individual to a predefined population based on allele frequency. Genetically unique populations show a higher proportion of correct assignments than genetically homogeneous populations. In contrast, Bayesian clustering methods group individuals based on minimization of (1) deviations from Hardy–Weinberg equilibrium and (2) linkage

disequilibrium. The Bayesian clustering software STRUCTURE version 2.3 (Pritchard et al. 2000) was used to identify the most likely number of genetic clusters (i.e., populations; K) found among sample sites ($K = 1–25$) with an admixture model. In total, 20 runs were completed at each K -value, with 50,000 iterations used for burn-in and 50,000 iterations used for analysis. Bayesian clustering results were extracted with STRUCTURE HARVESTER (Earl and vonHoldt 2012), and the likelihood of K ($L[K]$) and ΔK (Evanno et al. 2005) were evaluated to determine the most likely number of populations. The program CLUMPP (Jakobsson and Rosenberg 2007) was used to combine separate STRUCTURE runs via the GREEDY algorithm with 1,000 permutations, and final plots were created and visualized with DISTRUCT (Rosenberg 2004).

RESULTS

Site-Level Descriptive Statistics

Eight of the sampled Smallmouth Bass (from a total of 1,034) were omitted because of missing tissue samples, poor DNA quality, or allele sizes that were outside the range expected based on the published literature. Three sites (Figure 1) were removed from all analyses due to low sample sizes, including the

West Branch Susquehanna River at Watsonstown (WBW; $n = 3$ fish), West Branch Susquehanna River near Chillisquaque (WSC; $n = 16$ fish), and Kettle Creek (KC; $n = 17$ fish). At the remaining 22 sites, the average number of individuals collected was 45 (range = 25–63; Table 2).

In tests of Hardy–Weinberg equilibrium and linkage disequilibrium, no markers significantly deviated from equilibrium ($P > 0.0002$), but two marker pairs (*Mdo12* and *Mdo5*; *Mdo11* and *Mdo2*) significantly ($P < 0.0002$) deviated from expectations at one sample site per locus pair. The average A and A_r were greatest for the out-of-basin site (ARF; Table 2). In the Susquehanna River basin, A ranged from 3.25 (DAN) to 4.0 (SRM). Allelic richness adjusted for low sample size ($n = 25$) ranged from 3.10 (DAN) to 4.72 (ARF; Table 2). The ARF site also had the largest n_u ($n_u = 8$), and four sites in the Susquehanna River basin (BEC, BW, JRH, and SRM) had 1 n_u each. Estimates of H_o were between 0.50 and 0.55 (Table 2); deviations between H_o and H_e were greatest at SRM, DAN, TC, and UBE (most [77%] deviations were less than 0.02).

Finite N_e values were estimated for 12 of the 22 sites; seven sites had N_e estimates that could not be bound at the upper limit (upper 95% confidence limit was equal to infinity; Table 2). The lowest N_e estimates with fully bounded CIs occurred at SRM, WSM, SW, and LOY, with mean estimates between 45.0 (95% CI = 23.9–121.7) and 60.9 (95% CI = 27.1–667.7) individuals. The greatest N_e estimates of over 1,000 individuals occurred at DAN and LOY. The inability to estimate and determine upper confidence limits for N_e at several sites may be a result of too few alleles observed for the loci used in this analysis. This inability to estimate an upper limit on N_e has been documented elsewhere when population sizes were large (Waples and Do 2010).

Site Comparisons

The mean pairwise F_{ST} across sample sites was 0.016 (95% CI = 0.012–0.021). Pairwise comparisons of F_{ST} resulted in significant differences ($P < 0.0002$) between several sites (35% of all pairwise site comparisons), including the out-of-basin site ARF, which was significantly different from all Susquehanna River basin sites (mean $F_{ST} = 0.072$; Table 3). Overall, pairwise F_{ST} estimates within the Susquehanna River basin indicated genetic similarity among most of the sites (mean pairwise F_{ST} across Susquehanna River sites = 0.012). Three sites in the West Branch Susquehanna River basin (UBE, BEC, and PCR) had the greatest number of significant pairwise differences relative to other sites, with 20 significant differences for UBE (mean pairwise $F_{ST} = 0.050$), 16 differences for BEC (mean pairwise $F_{ST} = 0.023$), and 15 differences for PCR (mean pairwise $F_{ST} = 0.022$). Collectively, these three sites represented 48 (78.9%) of the 61 significant pairwise differences in F_{ST} between Susquehanna River basin sites.

An AMOVA was used to partition total genetic variation among subbasins, among sites within subbasins, and within sites. Results of the AMOVA indicated that 98% of the

genetic variability was found within sites ($df = 1,958$). Only 1% of the variability was explained by among-subbasin groupings ($df = 4$), and 0.86% of the variability was explained by within-subbasin groupings ($df = 17$). When sites were organized based on separation by dams, 98.7% of the genetic variability was found within sites ($df = 1,873$). Only a small percentage of variability was explained by separation by dams (1%; $df = 5$) or within-dam-reach groupings (0.3%; $df = 15$), suggesting that dams had little influence on the overall partitioning of genetic variation among sample sites.

Site Relationships and Population Structure

The unrooted neighbor-joining tree further supported the observation of genetic similarity among many sites in the Susquehanna River basin, with only six nodes occurring in over 50% of the permutations (Figure 2). Only two sites, ARF and UBE, were separated from other sites at greater than 0.01 substitutions per base unit. Some groupings appeared to be associated with geographic distances between the respective sites, including the branching of the two Pine Creek sites (PCM and PCR) and the separation of the West Branch Susquehanna River subbasin sites (BEC and WSM) from several of the other Susquehanna River sites. However, evaluation of genetic distance and geographic distance resulted in weak relationships. The Mantel test and correlogram resulted in significant correlation scores ($\alpha = 0.05$) for three of the six log-transformed distance classes. The magnitudes of those corresponding scores were 0.26 (distance class 1: mean \log_e [rkm] = 1.9), -0.20 (distance class 5: mean \log_e [rkm] = 5.3), and -0.34 (distance class 6: mean \log_e [rkm] = 5.7). Mantel correlation scores ranged from -1 to 1 ; although these scores were statistically significant, they were on the lower end of the range of possible scores, thus demonstrating that distance between sites was not necessarily an indication of the degree of genetic differentiation.

Although some statistically significant genetic differences (pairwise F_{ST}) were present among sites, it is uncertain whether the differences correspond to genetically separate populations. GeneClass2 (Piry et al. 2004) assignment tests, in which individuals were assigned to empirically derived clusters based on multilocus genotypes, resulted in only one site (ARF) with a large percentage (74%) of individuals that were reassigned correctly to the site of origin. Within the Susquehanna River basin, UBE was the only site to have greater than 50% reassignment. The remaining Susquehanna River basin sites had less than 35% reassignment (mean = 11%; range = 0–34%). Results from Bayesian clustering analysis using STRUCTURE (Pritchard et al. 2000) identified three genetically distinct population clusters (i.e., $K = 3$) based on both $L(K)$ and the ΔK value (Figure 3). Although individuals at the respective sites had different proportions of population clusters found within them, none of the sites

TABLE 3. Pairwise comparisons of the genetic differentiation index F_{ST} between all Smallmouth Bass sample sites within the Susquehanna River basin, Pennsylvania, and one out-of-basin site (ARF). Site abbreviations are defined in Table 2; site locations are shown in Figure 1. Bold italic values represent significant site comparisons from pairwise testing after Bonferroni correction ($\alpha = 0.0002$).

Site	ARF	BEC	BW	CHIL	DAN	FA	HAL	JRH	LOY	PCM	PCR	PEC	SMA	SRH	SRM	SSN	SW	TC	UBE	WBM	WSM	WYA	
ARF	0																						
BEC	<i>0.047</i>	0																					
BW	<i>0.077</i>	<i>0.023</i>	0																				
CHIL	<i>0.076</i>	<i>0.025</i>	0.000	0																			
DAN	<i>0.077</i>	<i>0.020</i>	0.002	0.004	0																		
FA	<i>0.071</i>	<i>0.018</i>	0.001	0.005	0.001	0																	
HAL	<i>0.064</i>	<i>0.009</i>	0.000	0.002	0.000	0.002	0																
JRH	<i>0.077</i>	<i>0.023</i>	0.007	0.011	0.009	0.008	0.009	0															
LOY	<i>0.056</i>	<i>0.007</i>	0.000	0.000	0.000	0.001	0.000	0.005	0														
PCM	<i>0.062</i>	<i>0.000</i>	0.012	0.012	0.004	0.010	0.003	0.010	0.000	0													
PCR	<i>0.077</i>	<i>0.011</i>	<i>0.024</i>	<i>0.024</i>	<i>0.016</i>	<i>0.022</i>	<i>0.016</i>	<i>0.019</i>	0.003	0.000	0												
PEC	<i>0.064</i>	<i>0.020</i>	0.000	0.004	0.000	0.002	0.000	0.000	0.000	0.007	<i>0.019</i>	0											
SMA	<i>0.079</i>	0.015	0.001	0.010	0.003	0.000	0.004	0.000	0.003	0.010	0.001	0.001	0										
SRH	<i>0.070</i>	<i>0.016</i>	0.000	0.001	0.004	0.005	0.000	0.003	0.000	0.008	<i>0.021</i>	0.000	0.003	0									
SRM	<i>0.074</i>	<i>0.018</i>	0.000	0.001	0.004	0.007	0.002	0.003	0.000	0.002	<i>0.009</i>	0.000	0.004	0.000	0								
SSN	<i>0.071</i>	<i>0.025</i>	0.000	0.003	0.000	0.004	0.001	0.000	0.000	0.010	<i>0.020</i>	0.000	0.002	0.000	0.000	0							
SW	<i>0.070</i>	<i>0.016</i>	0.020	<i>0.026</i>	<i>0.032</i>	<i>0.026</i>	<i>0.020</i>	<i>0.020</i>	0.010	0.010	0.017	0.018	0.018	0.012	0.011	<i>0.028</i>	0						
TC	<i>0.079</i>	<i>0.026</i>	0.006	0.000	0.010	0.015	0.008	0.012	0.004	<i>0.018</i>	<i>0.028</i>	0.006	0.018	0.001	0.004	0.005	<i>0.023</i>	0					
UBE	<i>0.115</i>	<i>0.063</i>	<i>0.053</i>	<i>0.054</i>	<i>0.058</i>	<i>0.052</i>	<i>0.049</i>	<i>0.048</i>	<i>0.044</i>	<i>0.051</i>	<i>0.063</i>	<i>0.038</i>	<i>0.059</i>	<i>0.042</i>	<i>0.036</i>	<i>0.053</i>	<i>0.029</i>	<i>0.037</i>	0				
WBM	<i>0.071</i>	<i>0.014</i>	0.001	0.005	0.003	0.007	0.003	0.000	0.000	0.001	0.012	0.000	0.003	0.002	0.000	0.000	0.021	0.014	<i>0.064</i>	0			
WSM	<i>0.064</i>	0.001	0.006	<i>0.015</i>	0.009	0.010	0.003	0.012	0.000	0.002	<i>0.012</i>	<i>0.010</i>	0.008	0.006	0.006	0.011	0.010	0.014	<i>0.044</i>	0.007	0		
WYA	<i>0.069</i>	<i>0.028</i>	0.005	0.007	0.008	0.006	0.005	0.019	0.009	<i>0.027</i>	<i>0.044</i>	0.009	0.019	0.012	0.018	0.013	<i>0.038</i>	0.012	<i>0.058</i>	0.015	<i>0.016</i>	0	

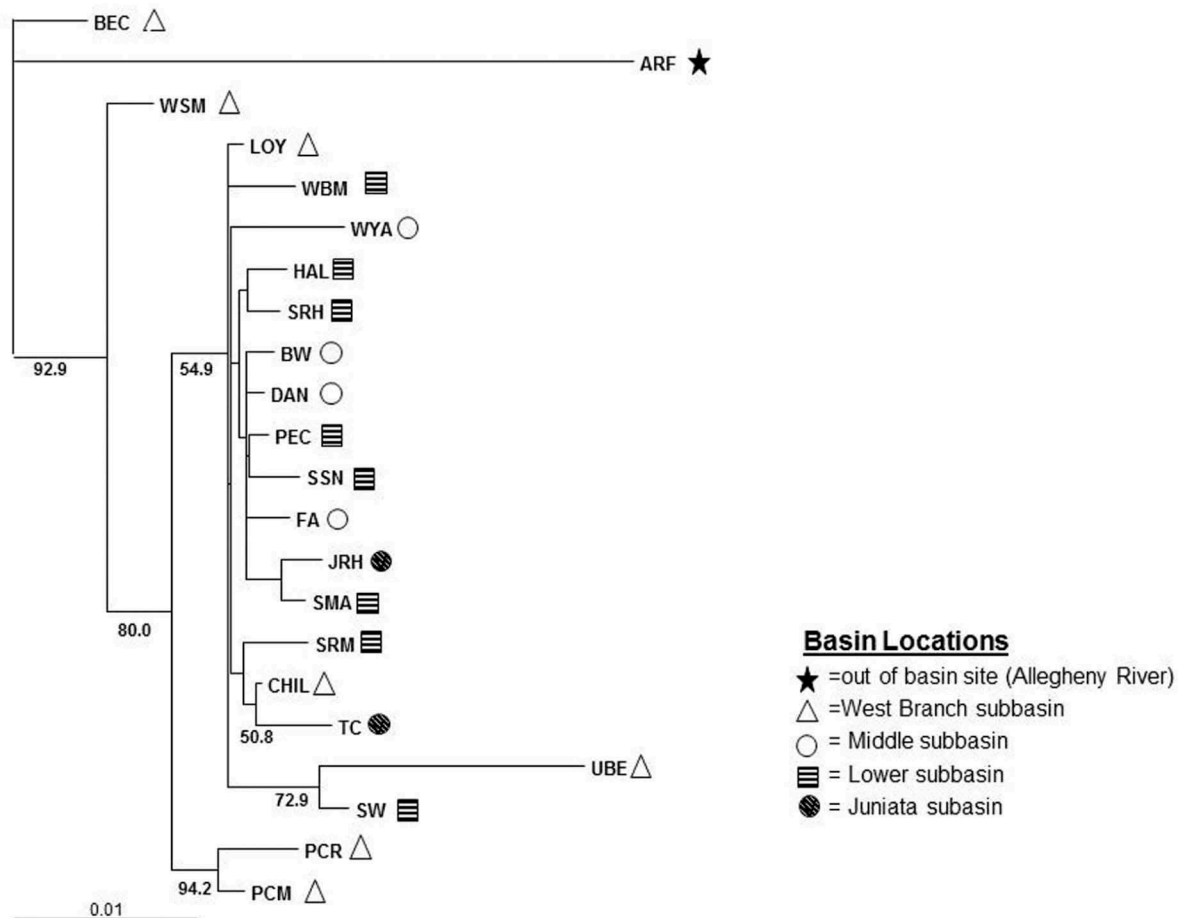


FIGURE 2. Neighbor-joining relationships based on Cavalli-Sforza and Edwards (1967) chord distances for Smallmouth Bass sampled from sites within the Susquehanna River basin (site abbreviations are defined in Table 2) and from one out-of-basin site (Allegheny River [ARF]). Symbols next to each site code correspond to basin location. Branches that occurred in over 50% of the runs include percentages associated with the branch point (scale = 0.01 substitutions per site).

appeared to have genetically distinct populations. The ARF site was the most distinct from the other sites.

DISCUSSION

We examined genetic differentiation in Smallmouth Bass among 21 sites in the Susquehanna River basin, where this species was introduced approximately 150 years ago. Overall, we found a lack of genetic differentiation among Smallmouth Bass in the Susquehanna River basin by using a combination of microsatellite markers that have successfully identified distinct populations in other studies (Stepien et al. 2007; Hallerman et al. 2015). This low genetic variability among sites was contrary to much of the previous genetic work on Smallmouth Bass population structure. Native riverine Smallmouth Bass in the Interior Highlands showed high levels of population divergence, which was consistent with historic lineages, and the fish were partitioned into separate subspecies (northern and southern forms) by using allozymes (Stark and Echelle 1998). Similarly, Stepien

et al. (2007) found intermediate genetic differentiation within and among Smallmouth Bass populations sampled from the Laurentian Great Lakes region, with an overall F_{ST} estimated at 0.147. The geographic scale evaluated by Stepien et al. (2007) was much larger than the Susquehanna River, yet genetic dissimilarity (64% of F_{ST} comparisons between sites were significant after standard Bonferroni correction) was also identified within a single basin (i.e., Lake Erie). In contrast, the findings identified in the present study indicate more recent differentiation or considerable gene flow among sites in the Susquehanna River.

Smallmouth Bass populations in the Susquehanna River were founded through introductions from the same source population (Stillwell et al. 1895). These introductions do raise concern for decreased genetic diversity, although our estimates of heterozygosity (average $H_o = 0.531$) were similar to those of Laurentian Great Lakes populations (average $H_o = 0.522$; Stepien et al. 2007). Qualitative comparisons indicated lower genetic variability in the Susquehanna River basin (12 markers, 67 alleles, and 5.58 alleles/locus) than in the Laurentian Great Lakes (8 markers, 66 alleles,

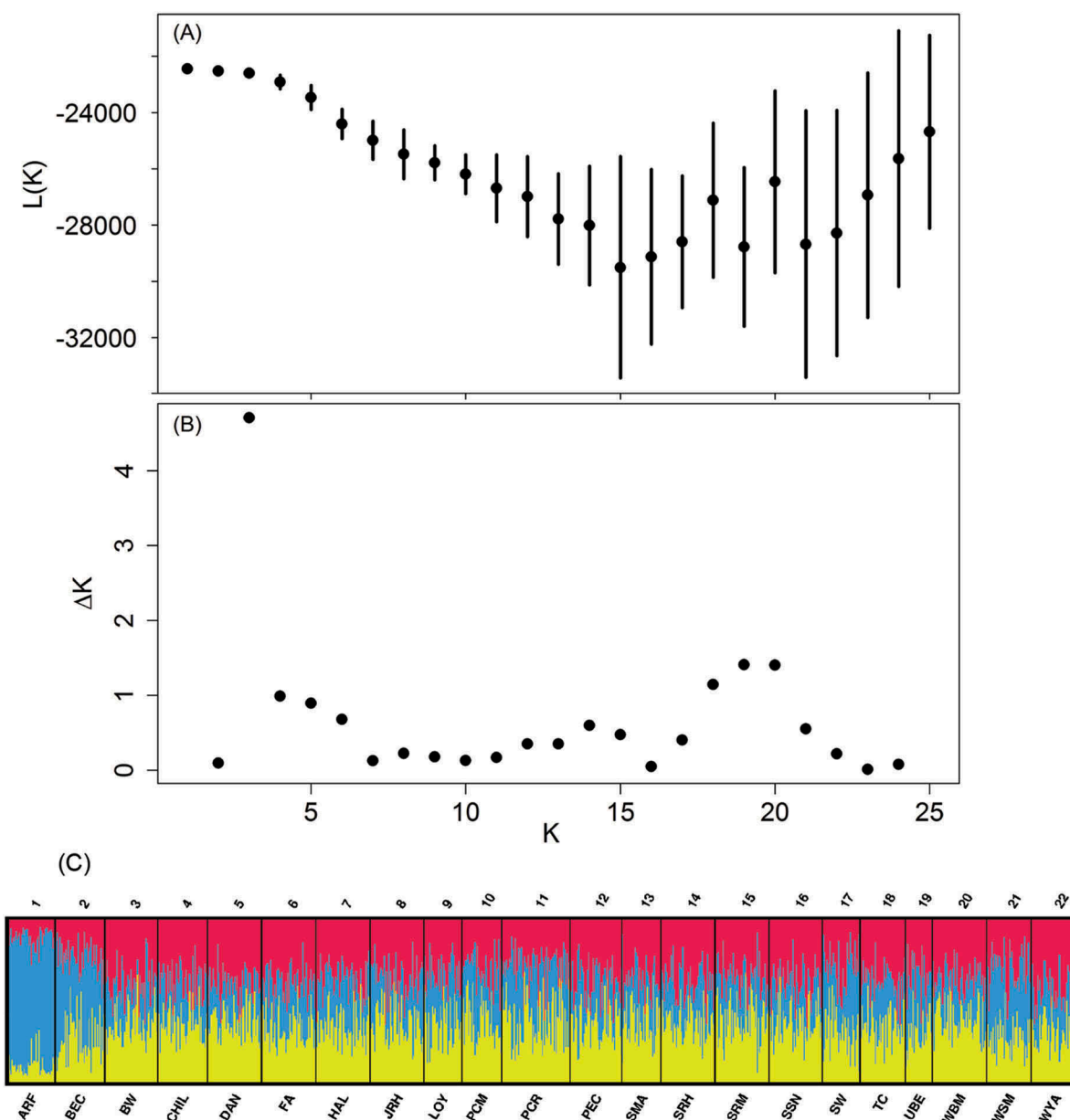


FIGURE 3. Investigation of Smallmouth Bass population structure through cluster analysis across a range of possible numbers of populations ($K = 1-25$) in the Susquehanna River basin and the out-of-basin site (ARF) and a graphical representation of population structure based on likely K -values (site abbreviations are defined in Table 2). Relationships between K and (A) the mean (\pm SD) likelihood of K ($L[K]$) or (B) ΔK (from the Evanno et al. 2005 method) were used to determine the most probable number of population clusters. (C) Graphical representation of three population clusters is presented (K -value of 3 was selected based on the ΔK and $L[K]$ relationships).

and 8.25 alleles/locus; Stepien et al. 2007). A similar pattern was evident when comparing the Susquehanna River sites to the out-of-basin ARF site, with heterozygosity being similar and A_r being lower. This pattern may indicate a loss of alleles over time, resulting from genetic drift. Genetic drift attributed to random loss of alleles generally affects A_r more so than heterozygosity (Allendorf et al. 2013). Loss of genetic diversity may not be an immediate concern given our estimates of genetic diversity, but introductions

from the same source population may have contributed to the observed genetic similarity between sites and the limited population genetic structure. Lack of population genetic structure may also contribute to higher, unbounded estimates of N_e if populations are not genetically distinct.

Since their initial introduction into the Susquehanna River, Smallmouth Bass have not become genetically differentiated into clearly distinguishable populations. Some other stocked nonnative

fish species have diverged and formed genetically distinguishable populations in less than a century after initial introduction (e.g., Chinook Salmon *Oncorhynchus tshawytscha*; Quinn et al. 2000). The degree of genetic differentiation on shorter time scales may vary depending on the system and specific characteristics of the species of interest (i.e., the degree to which behavioral and geographical isolation occurs).

The greatest genetic differences identified for sites in this study were between the Susquehanna River sites and the out-of-basin site. This was not surprising since the Allegheny and Susquehanna rivers are not connected. Subsequent analyses, including Bayesian clustering, demonstrated the possibility for shared genetic cluster(s) with the ARF site. A historical connection is possible since Susquehanna River Smallmouth Bass were thought to have originated from the Ohio River basin (Smith 1907), the drainage into which the Allegheny River flows. Multiple genetic signatures and admixing were apparent in our cluster analysis. Black bass were historically translocated with the development of canal and railway systems (Milner 1874; Henshall 1881; Goldsborough and Clark 1908). There are reports of black bass entering into rivers through the Erie Canal system (Henshall 1881), and at some point during the 1800s, there may have been a connection between the Laurentian Great Lakes region and the Susquehanna River via the Chenango Canal (Beyer 1957). Whether Smallmouth Bass moved through this canal system into the Susquehanna River is unknown. Additionally, the known source population for the Potomac River came from the Ohio River basin near Wheeling, West Virginia, which is not proximate to the ARF site (Milner 1874; Goldsborough and Clark 1908). Even in the presence of historical connections, some of the differences between the out-of-basin site and the Susquehanna River sites may be attributable to differences in founding or initial stocks. Comparisons of genetic composition among more sites in the Laurentian Great Lakes, the Ohio River basin, and the Potomac River basin may better elucidate sources for historical stocking of the Susquehanna River and may identify the origin of the additional genetic cluster(s).

Population divergence in Smallmouth Bass could be shaped by both geographic isolation (Stepien et al. 2007) and behavioral mechanisms (e.g., spawning site fidelity; Gross et al. 1994). Geographic isolation may be attributed to natural events (e.g., glacial retreat in the Laurentian Great Lakes; Stepien et al. 2007) or physical manmade barriers (e.g., dams) that prevent gene flow. Slight genetic differentiation was apparent among a few sites in the Susquehanna River basin, including three tributary sites within the West Branch Susquehanna River subbasin. One of those three sites (UBE) was located above a flood control reservoir and could be experiencing physical isolation from the remainder of the study sites, as evidenced by the significant F_{ST} values, the highest reassignment rate, and the largest chord distance across Susquehanna River basin sites. Other types of dam (e.g., low-head dams and an inflatable dam) are also present in the Susquehanna River basin and may affect gene flow but not to the same degree as for UBE, where Smallmouth Bass

are exhibiting greater genetic differentiation. For the remaining two sites (PCR and BEC), it is unclear whether physical or behavioral isolation (including spawning site fidelity) is the mechanism driving the observed genetic differences.

Variability in movement patterns of Smallmouth Bass further complicates our ability to draw parallels between systems when inferring gene flow and delineation of genetic populations. For example, there may be limited genetic structure across a large spatial extent if Smallmouth Bass are moving long distances. Movement studies on Smallmouth Bass within the species' native range have suggested varied seasonal movement in rivers, with movements ranging from less than 10 km (Todd and Rabeni 1989) to over 100 km (Langhurst and Schoenike 1990) and with homing behavior also documented in both rivers and lakes (Langhurst and Schoenike 1990; Gross et al. 1994).

Straying from natal sites and colonization of new habitat (Keefer and Caudill 2014) could also be factors in the lack of genetic structure we observed. Spawning site fidelity has been documented for Smallmouth Bass in rivers and lakes (Langhurst and Schoenike 1990; Ridgway et al. 1991; Gross et al. 1994; Barthel et al. 2008; Humston et al. 2010), but there are many variables that may influence natal homing and straying (e.g., contaminants, olfactory effects, and competition; Keefer and Caudill 2014). We chose not to estimate the migration rate using its relation to F_{ST} and N_e due to the uncertainty in the N_e estimates (i.e., a large number of unbounded estimates). Although the movement rate of Smallmouth Bass in the Susquehanna River basin has not been estimated, there have been concerns over environmental conditions and fish health within the system (Arway and Smith 2013; Blazer et al. 2014). Whether any of these environmental concerns contribute to movement or whether other mechanisms are responsible for the lack of population genetic structure is currently unknown.

Management and Future Research

Genetic management units are defined as those that exceed a predetermined value of genetic differentiation (i.e., pairwise F_{ST} level; Palsboll et al. 2006). Management decisions rely on defining management units both ecologically and genetically for angling regulations, stocking, and assessment of population status (i.e., if populations are declining; Hawkins et al. 2016). In the James River and New River watersheds, four potential genetic management units were identified for Smallmouth Bass and could be used to guide future management (Hallerman et al. 2015). In contrast, the Susquehanna River basin does not have a genetic basis for separate management units, and currently there is no information on ecologically differentiated groups of Smallmouth Bass.

A primary goal of ongoing research efforts in the Susquehanna River basin is to use genetic and ecological units to provide insights for future management and to better understand underlying mechanisms that could be influencing

fish population dynamics and health. Characterization of the population genetic structure of Smallmouth Bass in this system is one of the first steps toward doing so. Results of the present study indicate a lack of significant genetic structure in Susquehanna River basin Smallmouth Bass, which may be attributed to many factors, including introductions and movement with considerable gene flow. Genetic differentiation among a subset of sites warrants additional investigation, such as evaluation of the mechanisms for differentiation (i.e., variable selection pressure, genetic drift, and subsequent founder events) and additional out-of-basin genetic comparisons.

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Chapter 3

Movement dynamics of Smallmouth Bass (*Micropterus dolomieu*) in a large river-tributary system

Chapter 3 has been submitted for peer review to the *Journal of Fish Biology* and is provided below in the format submitted to the journal. This manuscript was completed in collaboration with my advisor (Tyler Wagner), committee member (Vicki Blazer), and two state agency personnel (Timothy Wertz-Pennsylvania Department of Environmental Protection and Geoffrey Smith-Pennsylvania Fish and Boat Commission).

Movement dynamics of smallmouth bass (*Micropterus dolomieu*) in a large river-tributary system

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Running headline: Fish movement in a river-tributary system

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released by reviewers. Because the manuscript has not yet been approved for publication by the US Geological Survey (USGS), it does not represent any official finding or policy.

Abstract

Smallmouth bass (*Micropterus dolomieu*) movement dynamics in a large river-tributary system were found to vary spatially and temporally both within and among study sites. In general, smallmouth bass moved large distances annually ($n = 76$ fish, average = 27.2 ± 25.9 km, range = 0.2 to 118 km) and had three peak movement periods (pre-spawn, post-spawn, and overwintering). Movement into and out of tributaries was common, with a large proportion of fish that were tagged in tributaries moving out of the tributaries after spawning (22/30 fish). Smallmouth bass movement was influenced by temperature and flow, but these effects varied seasonally. Because of the importance of fish movement patterns on population dynamics, the observed among-individual variability in movement and the potential for long distance movements are important considerations for smallmouth bass conservation and management plans. In addition, river-tributary connectivity appears to play an important role for smallmouth bass during key life history events (i.e., spawning and overwintering). The findings of this study provide valuable information for fishery conservation planning, assessments, and management.

Key Words

Radio-telemetry; spatial movement ecology; recreational sport fish; smallmouth bass

Introduction

Stream connectivity in large river systems plays an important role in the longitudinal movement dynamics and spatial ecology of fishes. In these large systems, tributaries often serve as movement corridors (Fullerton *et al.*, 2010, Koster *et al.*, 2014) that provide fish access to critical habitats. Critical habitat for fishes may include refugia from biophysical disturbances (Sedell *et al.*, 1990), spawning grounds (Fullerton *et al.*, 2010), and rearing habitats for different life stages (Pracheil *et al.*, 2009). As an example, golden perch *Macquaria ambigua* (Richardson 1845) in Australia moved between a tributary and river system for spawning, as well as during times of the year not associated with key life history events (Koster *et al.*, 2014). Tributary and river usage patterns have been documented in a host of riverine fishes, including paddlefish *Polyodon spathula* (Walbaum 1792) (Zigler *et al.*, 2003), barbel *Barbus barbus* (L. 1758) (Lucas and Batley, 1996), and smallmouth bass *Micropterus dolomieu* Lacepède 1802 (Langhurst and Schoenike, 1990). In addition to providing access to critical habitats, large tributaries have the capability to support similar biological communities as those found in river mainstems. Therefore, these tributaries can provide potential source populations for recolonization of mainstem rivers and can represent conservation priorities for ensuring the future persistence of riverine species (Pracheil *et al.*, 2013). Overall, the widespread usage of river networks by a variety of fishes indicates the importance of considering tributaries and river connectivity in management and conservation efforts.

Conservation and management of riverine fish communities requires an understanding of variables influencing spatial movement dynamics of fishes (Cooke *et al.*, 2016). Fish movement in large river systems can be influenced by a range of environmental conditions including flow (Koster *et al.*, 2014) and temperature (Lucas and Batley, 1996). For example, golden perch movement out of tributaries and into the river mainstem was driven by changes in stream flow (Koster *et al.*, 2014). In a river network, temperature was an important predictor of upstream movement for both male and female barbel (Lucas and Batley, 1996). In addition to temperature and flow, fish movement in rivers can also be influenced by habitat requirements (Pauwels *et al.*, 2013), ecological interactions (Castello, 2008), and anthropogenic stressors (Meldgaard *et al.*, 2003). Ecological interactions in a river floodplain system influenced movement of piraracu *Arapaima gigas* (Schinz 1822) into flooded areas where prey were more readily available (Castello, 2008). These studies help to illustrate that fish movement is the result of complex interactions that exist between fish and the environment they inhabit.

This inherent complexity of large rivers systems makes quantifying and understanding drivers of fish movement difficult. However, understanding fish movement ecology is critical for developing fisheries management and conservation plans for riverine species (Cooke *et al.*, 2016), and may be particularly critical for recreational sport fish. For instance, the development and implementation of fishing regulations can be informed by understanding seasonal fish movement and habitat use in the system. For smallmouth bass as an example, it has been recommended that special

regulations consider movement dynamics and resulting habitat occupied (Lyons and Kanehl, 2002).

Throughout the United States, smallmouth bass often inhabit large river systems and are a popular sport fish (Noble, 2002). The movement ecology of smallmouth bass has been investigated in some river systems. These studies have documented a large degree of variability in movement among individual fish and river systems. For example, Todd and Rabeni (1989) found that smallmouth bass moved less than 8 km in a river with deep pools bordered by shallow riffles during low flow; whereas, Langhurst and Schoenike (1990) found fish made movements up to 109 km in a connected river-tributary system. Due to the variability in smallmouth bass movement among river systems, extrapolation of results to other, unstudied systems may not be feasible (Lyons and Kanehl, 2002). Like for other species, smallmouth bass movement has also been shown to vary seasonally and be influenced by environmental factors (Todd and Rabeni, 1989, Hafs *et al.*, 2010), and habitat characteristics (Dauwalter and Fisher, 2008). Seasonal variability in smallmouth bass movement may result in complex environmental relationships that also complicate efforts to make generalizations about movement dynamics among river systems.

The Susquehanna River, located in the state of Pennsylvania, USA, and its tributaries make up a large river-tributary system where smallmouth bass are both a revered sport fish and an apex predator. However, within the past ten to fifteen years, reported declines in catch rates and concerns regarding the overall health of smallmouth

bass populations (e.g., clinical signs of disease and endocrine disruption (i.e., intersex); Blazer *et al.*, 2014) have raised concern from management agencies, the general public, and angling groups (Arway and Smith, 2013). Understanding the movement dynamics and potential drivers of movement is critical for developing conservation and management plans for this species within this system. The objectives of this study were to: (i) quantify smallmouth bass movement and usage of tributary and river habitat in the Susquehanna River Basin and (ii) evaluate movement dynamics in relation to environmental variables that may influence seasonal movement patterns.

Materials and Methods

Study Area and Study Sites

A smallmouth bass radio-telemetry study was conducted in central Pennsylvania, USA and included the West Branch of the Susquehanna River and two tributaries, Bald Eagle and Pine Creek (Figure 1; boundary box (Albers Equal Area): 1532154, 2162169 to 1569066, 2189789). Dams, that represented strong barriers to movement, were present at the upstream and downstream bounds of the study area on the West Branch of the Susquehanna River and Bald Eagle Creek (Figure 1). Fish were tagged spring and fall 2014. A total of seven different tagging locations were used. There were four tagging sites during spring 2014, including three tributary locations: Pine Creek lower and middle (PCL and PCM), Bald Eagle Creek lower (BEL), and one river location: West Branch of the Susquehanna upper (WBU; Figure 1). Four tagging sites were also used fall 2014,

with overlap of one location from spring 2014 tagging efforts (PCM; Figure 1). Fall 2014 tributary locations were farther upstream in the tributaries (Pine Creek upper- PCU and Bald Eagle Creek upper-BEU) and farther downstream on the river (West Branch of the Susquehanna Lower (WBL), Figure 1). Estimated stream widths at the tagging locations ranged from 40 to 90m on the tributaries and 140 to 300m at the river sites.

Tagging and Tracking

Smallmouth bass were collected using boat and towboat electrofishing and angling. Fish were tagged with LOTEK MCFT2-3BM (Lotek Wireless, Newmarket, Canada) temperature and identification radio transmitters weighing 8g. Tags were surgically implanted in the body coelom using the shielded needle technique (Ross and Kleiner, 1982) and had an external antenna trailing outside the body. All procedures and fish handling were conducted in agreement with the Pennsylvania State University Institutional Animal Care and Use Committee (IACUC, study number 42544). Fish were anesthetized using tricaine methanesulfonate (MS-222, Tricaine-S, Western Chemical) during the transmitter implantation procedure. Fish were administered a 70 mg/kg dose of MS-222 through immersion prior to the procedure and a 35 mg/kg maintenance dose during the procedure through a continuous flow of water over the gills. After the procedure, each fish was placed in a recovery net pen located in the stream and held until equilibrium was reestablished and the fish was swimming upright (approximately 15 minutes). Fish were then released back into the river or tributary at the site of capture.

Both fixed stations and manual tracking were used to locate fish (LOTEK SRX-DL3 data logging receivers; LOTEKSRX_400A receivers). Fish were not tracked until at least two days post- surgery. Fixed receivers were placed at the mouth of both tributaries to identify when fish were entering or leaving each tributary. Manual tracking took place throughout the telemetry area by land or water on a set schedule. The tracking schedule was set to target multiple areas weekly. Tracking ceased over the winter due to snow and ice and resumed prior to spring ice melt off. For each manual location, fish identity, temperature, and a GPS location were recorded at the closest proximity possible (estimated within 100-200 meters of the fish location). Fish tracking began May 2014 and the study lasted until August 2015.

Data management

Prior to summarizing fish movement, locations for each fish were plotted using RGoogleMaps (Loecher and Ropkins, 2015) in the program R (R Development Team, 2017) and animations of each fish's movement were created. The movement animations were evaluated to identify erroneous locations and fish movement patterns. Each animation was scored by two independent readers who categorized fish as sedentary (not leaving general tagging location) or dispersing. Dispersing individuals were further evaluated to determine if movement ceased permanently and at that point were identified as possible dropped tags. For example, if a fish moved into overwintering habitat, but did not leave overwintering habitat during spring spawning activity, it was considered a dropped tag after the point that movement ceased. Once a fish was identified as dropping

its tag, locations recorded after the drop date were omitted for future analysis. Fish that had fewer than three locations and that did not move were removed from the dataset. In addition, if multiple locations were made on the same individual within the same day, only one location was retained for analysis. The removal of duplicate locations was performed because repeat locations were most often made on fish near fixed stations, and the spatial and temporal resolution of those data did not match the overall scale of inference for this study (i.e., small movements over short timeframes (i.e., within a day) were not the focus of this study).

Fish Movement Summaries

A stream network dataset (National Hydrology Dataset Plus Version 2, U.S. Geological Survey and U.S. Environmental Protection Agency) was used to obtain a digital map of the telemetry study area (accessed online at: http://www.horizon-systems.com/NHDPlus/NHDPlusV2_02.php). The R package *Secrlinear* (Efford, 2015) was used to create a linear mask over the river section and tributaries in the study area with points spaced at 25 m increments to measure stream network distance between points. Fish locations were plotted over the linear mask with points being referenced to the nearest linear mask point. Distance between each location for all fish was then calculated using the mask points and summed to obtain total distance moved and daily movement rates over the entire time series available for each fish. In addition to calculating total movement, net distance moved to overwintering and/or spawning habitat was also calculated for each fish when applicable (i.e., as long as the fish did not drop a

tag and/or data were collected through the spawning or overwintering period). Distance to overwintering habitat was only calculated for spring tagged fish. Fall tagging occurred around the same time fish were moving into overwintering habitat which prevented overwintering summaries from being completed for fall tagged fish. Fish were considered to be in overwintering habitat after October 16, 2014 when fish were generally no longer moving prior to the onset of winter. Distance to spawning habitat was determined for both spring and fall tagged fish, but only if a location was collected during the spawning time frame, which was the end of April through beginning of June.

Statistical Modeling

A generalized additive mixed model (GAMM) was used to evaluate potential linear and non-linear covariate relationships with fish movement using package *mgcv* (Wood, 2006) in the program R (R Development Team, 2017). This type of model (GAMMs) has been used to analyze fish and wildlife movement data (e.g., bull trout; Martins *et al.*, 2014, polar bears; Zuur *et al.*, 2014) and provides flexibility for investigating complex relationships that often exist between movement data and environmental variables (Zuur *et al.*, 2014). Several predictor variables for movement were evaluated including day of experiment, tagging location, tagging season, fish weight, season, flow, temperature, and interactions between season and flow and season and temperature. Day of experiment corresponded to the day of the study that a fish was located on. Day of experiment started at day 1 on the first day of tagging and continued until the end of the study (day 464). Movement was evaluated as movement rate

(meters/day) to account for time differences between locations. Prior to evaluating fixed effects, the error structure was optimized using a random fish effect to account for repeated measures on individuals. In addition, predictor variables were plotted against the response variable for categorical variables to assess variability in the data and determine if unequal variances needed to be accommodated. Fixed effects were then investigated using backward selection while evaluating the significance of linear and non-linear effects. Non-significant variables ($p > 0.05$) were removed from the model. Thin-plate regression splines with generalized cross validation were used to incorporate nonlinear relationships. Model fit was evaluated by assessing plots of residuals. Movement rate was natural log transformed, with an addition of a constant (i.e., $y + 1$) to accommodate the log transformation of zero movement. Day of experiment was grand mean centered. Flow was included as natural log transformed mean daily flow (ft^3/s) at the date of location and were summarized from a U.S. Geological Survey (USGS) gage station located on the West Branch Susquehanna River (gage 01551500, U.S. Geological Survey, 2016). This river gage was selected because it represented cumulative flow from the study area. Temperature was included as change in mean daily temperature ($^{\circ}\text{C}$) between fish locations. Mean daily stream temperatures were summarized from data sondes and USGS gage stations located within the study area and were based on fish location in the system. Temperature data were missing from the river from 5/14/2014 to 10/30/2014. To accommodate missing data, mean daily temperatures from the tributary sites were averaged and used for fish located on the river when temperature data were absent. Seasons were defined as: spring (March-May), summer (June-August), fall (September-November), and winter (December-February) and locations were categorized into

respective categories. If a time period between locations overlapped multiple seasons it was categorized in the season that it most overlapped with.

Results

Fish Movement Summaries

A total of 85 adult smallmouth bass were tagged – 40 fish tagged spring 2014 and 45 fish tagged fall 2014 (Table I). The 40 fish tagged during spring 2014 weighed an average of 977g (range: 477-1747g) and measured an average of 415 mm in total length (range: 335-505mm). Fish tagged during fall 2014 (45 total) weighed an average of 556g (range: 184-1472g) and measured an average of 330mm in length (range: 220-465mm). A total of 1056 locations were collected with 174 locations from the fixed stations and 882 from manual tracking. After removing locations considered as dropped tags and fish with less than three locations that did not move, 850 locations remained from 76 fish. The duration of time between locations varied among fish, but was sometimes several months, especially during the winter months when manual tracking was not feasible.

Fish movement was variable with some fish remaining close to their tagging location the entire study duration (moving < 1 km), while others moved large distances (greatest total movement = 118 km). Three major movement periods were documented including: post-spawn (summer 2014), prior to overwintering (fall 2014), and pre-spawn (spring 2015). A large proportion of spring 2014 tagged fish from each of the tributary

sites moved into the river after tagging (22 out of 30 fish tagged with four of the remaining eight fish categorized as dropped tags). Additionally, 19 of 40 spring 2014 tagged fish (47.5%) moved greater than 1km between locations (8.6 ± 7.3 km) in the mid to late summer (mid-June through mid-August). Seven of the fish that moved out of the tributaries for overwintering returned to the previous year's tributary spawning ground in spring 2015. Fish that were tagged at the upper river site (WBU) spring 2014 had seasonal movement patterns similar to the tributary tagged fish, but they did not enter either of the tributary sites during the duration of the study.

Fish tagged fall 2015 also demonstrated varied movement patterns and river and tributary usage at each of the sites. Three of five fish tagged at the PCU site (over 20 km upstream from the mouth) left the tributary and moved to the river for overwintering. The PCM site, which was also a tagging site for spring tagged fish, had three of ten tagged fish move out of the tributary and into the river for overwintering. Three of the 15 fish tagged at the BEU site, which was located at a pool created by a dam, left the pool and two fish entered the river. Seven of 15 fish that were tagged at WBL during fall 2014 entered tributaries in spring 2015 and eight fish remained in the river.

Total fish movement averaged 27.2 ± 25.9 km (mean \pm s.d.; range = 0.2 to 118.0 km; n = 76;) with the number of days a fish was tracked averaging 220 ± 111.1 days (range = 13 to 412 days). Total movement and daily movement were variable both within a single site and among sites (Table II). The sites that had the largest daily and total

movement were PCU and BEL. The BEU site (fall tagging), had the lowest average total movement and daily movement.

Movement to overwintering and spawning grounds also varied among sites and for individuals within sites. After omitting dropped tags for spring tagged fish, several individuals moved relatively small distances to overwintering habitat (15% moving <1 km, 4 out of 27 individuals), but a majority of the fish moved large distances to overwintering habitat (67% moved >10km, 18 out of 27 individuals). The maximum distance moved to access overwintering grounds was 46.9 km (mean = 18.3 ± 14.1 km, 27 individuals, Table II). Preferred overwintering locations were impounded sections of the river located in close proximity to the WBL tagging location. During overwintering, greater than 20 fish were located within a 5-7 km stretch of the river. Some of the largest movements documented were from fish tagged at the BEL and WBU sites (>30 km). These two tagging locations were farthest from the overwintering habitats. Similar to movement to overwintering habitats, a majority of fish moved over 10 km to access spawning grounds (21 out of 42 fish or 50% of fish included in the summary) after leaving overwintering habitats.

Modeling Results

The final GAMM model included fixed effects of tagging location, season, day of experiment, and the interactions of change in temperature and flow with season. Day of experiment, change in temperature by season interaction, and flow by season interaction

were nonlinear and incorporated in the model with thin plate regression splines. Variance components included a random fish effect and season-specific residual variances to accommodate the assumption of heterogeneity of variances. The only tagging location that had significantly different movement rates was the BEU site ($p < 0.05$). In general, fish from the BEU site demonstrated the lowest movement rates in comparison to the other tagging sites (Table II). The effects of day of experiment and the interactions between change in temperature and season and flow and season were significant ($p < 0.05$). The nonlinear effect of day of experiment mirrored the movement periods observed in the general movement summaries. Movement rates increased post-spawn (summer) and into the fall for movement into overwintering habitat (Figure 2). Movement rates decreased just before and during winter for overwintering, but increased again prior to spawning (spring). Tracking ceased in August 2015, making it difficult to make any comparisons between years. Movement rates declined at the end of the time series, but tracking efforts also declined at this time as did the number of fish remaining in the study.

The effects of change in temperature and flow on movement rates varied by season. Flow had significant effects on movement rates in spring and summer (Figure 3). These time periods also coincided with a majority of the high flow events that occurred during the study (Figure 4). In general, movement rate increased with increasing flow, except during the summer, which had a decline in movement prior to a nonlinear increase. This nonlinear increase in movement rate could be related to storm events which occurred multiple times during summer 2014 and 2015 (Figure 4). In contrast to

the effect of flow on movement rates, change in temperature had a significant effect on movement rates during the fall only. In the fall, movement rates decreased for large declines in temperatures, which often occurred close to the overwintering period when movement ceased. The movement model explained 25% of the variability in fish movement rates, indicating that a substantial amount of the variability in fish movement was unaccounted for and which could be attributed to variables that were not measured (e.g., physical habitat requirements, fish density).

Discussion

In the Susquehanna River Basin, smallmouth bass utilized both tributary and mainstem river habitats in a large river-tributary system, with many fish moving large distances (>10 km) to reach overwintering and spawning habitats. These findings differed from some of the previous work on riverine smallmouth bass populations, which documented relatively little movement (Funk, 1955; Todd and Rabeni, 1989). For instance, in a river located in Missouri, USA, smallmouth bass were documented to have defined home ranges with maximum total movement of 8 km (Todd and Rabeni, 1989). Yet, Lyons and Kanehl (2002), in a review of movement studies completed on smallmouth bass, expressed concern with studies that documented limited movement because fish could have moved outside of the study area leading to negatively biased movement estimates. In contrast, however, the results in the Susquehanna River Basin are more similar to what has been observed in other studies conducted in river-tributary systems (Langhurst and Schoenike, 1990; Lyons and Kanehl, 2002; Gunderson

VanArnum *et al.*, 2004). For example, in the Wolf River and a tributary (Embarrass River), smallmouth bass moved an average of 77 km downstream and seven of ten tagged fish were documented to have moved between the tributary and river (Langhurst and Schoenike, 1990). In addition to complementing previous studies, this study in the Susquehanna River Basin had a much larger sample size than earlier smallmouth bass movement studies (n=34; Todd and Rabeni, 1989, n=10; Langhurst and Schoenike, 1990, n=15; Lyons and Kanehl, 2002, n=69; Gunderson VanArnum *et al.*, 2004, n=60; Hafs *et al.*, 2010). The large sample size of smallmouth bass used in this study allowed investigation of fish movement variability within a river-tributary system. This included teasing apart variables (e.g., temperature, flow, and distance from preferred habitat) that influenced movements of smallmouth bass throughout the system.

As with other riverine fish species, the tributary-river corridor is likely an important habitat complex for riverine smallmouth bass populations (Langhurst and Schoenike, 1990, Lyons and Kanehl, 2002, Gunderson VanArnum *et al.*, 2004). In the Susquehanna River Basin, tributary use was often associated with pre-spawn and post-spawn movements. Tributary usage for spawning has also been documented in other fishes in river networks. For example, humpback chub *Gila cypha* Miller 1946 in the Colorado River utilized tributaries for spawning habitat (Gorman and Stone, 1999). Similarly, westslope cutthroat trout *Oncorhynchus clarki* (Richardson 1836) in the Blackfoot River, Montana, USA, moved out of the river into smaller tributaries for spawning (Schmetterling, 2001). However, the degree to which fishes utilize tributaries may depend on habitat conditions in the mainstem. For example, in the Susquehanna

River Basin, the degree to which smallmouth bass moved between the river and tributaries, or vice versa, varied spatially. Fish from one river site (WBU) did not move between the river and tributaries. This lack of tributary usage by some river fish could be related to spawning site fidelity, which has been shown to exist in some riverine smallmouth bass populations (Langhurst and Schoenike, 1990; Barthel *et al.*, 2008). If suitable spawning habitat existed in this river section, these fish may not need to use tributaries to fulfill this life history requirement.

Both change in water temperature and increases in flow in this present study were correlated with seasonal movement of smallmouth bass. Langhurst and Schoenike (1990) also documented increased movement of smallmouth bass with increasing flow and in the fall when temperatures dropped below 16°C. Similarly, spring movements of smallmouth bass in a river - tributary system increased with both increasing flow and temperature (Lyons and Kanehl, 2002). The underlying physiochemical characteristics of river systems, including temperature and flow, likely play an important role in determining when and where fish move. For example, large movements prior to overwintering have been documented by smallmouth bass inhabiting rivers with harsh winters and ice cover (Lyons and Kanehl, 2002). It is possible that the large movements by smallmouth bass prior to overwintering are to reach optimal habitat for survival during winter. This aligns with the overwintering movement documented in the Susquehanna River Basin, where large movements (maximum = 46.9km) were observed prior to the onset of winter conditions and the region is often covered by ice during the winter. In fact, some of the sites with the largest longitudinal overwintering movements (BEL, PCU, WBU) were the

farthest away from the preferred overwintering habitat. This indicates that environmental cues, in combination with preferred habitat in the river, may be driving some of the site specific differences in seasonal movement.

Relatively large movements by smallmouth bass in the Susquehanna River Basin during the summer months were in contrast with previously documented findings – which reported limited movement in a majority of the fish during the summer (Lyons and Kanehl, 2002; Hafs *et al.*, 2010). The comparatively large movements during the summer in the Susquehanna River Basin could be attributed to several factors. First, hydrologic events could be influencing smallmouth bass movements, as indicated by the significant effect of flow on summer movement rates. Secondly, smallmouth bass have nesting habits that involve parental care by the male fish after spawning occurs. Parental care by male smallmouth bass in a Canadian lake was found to range from 19 to 45 days after spawning (Ridgway and Friesen, 1992). Post-spawn movements by male fish could have accounted for some of the high movement rates observed in the summer; however, it was not possible to determine the sex of tagged fish, so this was not able to be tested. Additionally, low water levels in the tributaries during the summer months may provide poor habitat, especially for larger fish. Therefore, summer movements may be a result of fish moving out of seasonally unsuitable tributary habitats. This idea is supported by the fact that most of the summer tributary movement by smallmouth bass in the Susquehanna River Basin was out of the tributaries and downstream to the river. A similar phenomenon was observed for smallmouth bass located in a bayou, where fish in areas

that became uninhabitable in the summer made larger movements, on average, compared to fish located in more suitable habitat (Hafs *et al.*, 2010).

An understanding of the spatial and temporal movements of fishes can inform conservation and management efforts (Cooke *et al.*, 2016). However, because the movement by individuals can have demographic effects on populations (Wilson *et al.*, 2004), an understanding of movement ecology may also contribute to our understanding of gene flow that may be occurring as a result of movement. For example, a recent population genetics study on smallmouth bass was conducted using river and tributary sites across the Susquehanna River Basin (Schall *et al.*, 2017). This study documented genetic similarity and admixing of genetic population clusters across the basin. Although it was acknowledged that smallmouth bass were introduced relatively recently into the basin (approximately 150 years ago; Milner, 1874; Stillwell, 1895), one of the suggestions for genetic similarity was gene flow resulting from smallmouth bass moving large distances. Results from this movement study support the assertion that fish movement could be a contributing factor to gene flow in the Susquehanna River Basin. Other factors may also be contributing the lack of genetic differentiation in the Susquehanna River Basin, including the similarity in initial founding populations and the timeframe since introduction (Schall *et al.*, 2017). The combination of the movement and genetics results suggests that the maintenance of river-tributary connectivity may be important to retain the observed genetic structure and access to important habitats.

The importance of understanding fish movement ecology has been well established and has a range of uses for fish conservation and management (Cooke *et al.*, 2013). This movement study conducted on smallmouth bass in a large river-tributary system provided important information that can be used for management of smallmouth bass in the Susquehanna River Basin and other systems with high degrees of connectivity. Consideration of large longitudinal movements, variability in the seasonal use of river-tributary systems that coincide with life history events, and the seasonal drivers of fish movements may be used to inform river-tributary conservation and management strategies for smallmouth bass.

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Table I: Tagging locations and numbers of smallmouth bass *Micropterus dolomieu* Lacepède 1802 tagged for both spring and fall tagging events. Time = season of tagging (spring or fall), Location = river or tributary name, Site= relative location in the respective river or tributary (upper, lower, middle), Abbreviation = site abbreviation, and No. Tags= number of fish tagged at each site.

Time	Location	Site	Abbreviation	No. Tags
Spring 2014	Pine Creek	Lower	PCL	10
	Pine Creek	Middle	PCM	10
	Bald Eagle Creek	Lower	BEL	10
	West Branch of the Susquehanna River	Upper	WBU	10
Fall 2014	Pine Creek	Middle	PCM	10
	Pine Creek	Upper	PCU	5
	Bald Eagle Creek	Upper	BEU	15
	West Branch of the Susquehanna River	Lower	WBL	15
			TOTAL	85

Table II: Movement summaries for smallmouth bass *Micropterus dolomieu* Lacepède 1802 located in the West Branch of the Susquehanna River and two tributaries. Summaries include total movement, daily movement, and net movement to overwintering and spawning habitat. Season = season when tagging occurred (spring or fall) and Site= site abbreviations which were defined in Table I. For all summaries, mean, standard deviation (SD), and range in the data are provided. #Fish represents the number of fish included in the summary after removing dropped tags and fish with limited locations that did not move (see Methods). The number of fish in the summaries did not remain constant due to dropped tags during the study duration. Summaries were only completed if sites had a minimum of three fish remaining.

Season	Site	Total Movement (km)		Daily movement (km/day)		# Fish	Net Movement to Overwintering (km)			Net Movement to Spawning (km)		
		Mean (SD)	Range	Mean	Range		Mean (sd)	Range	# Fish	Mean (sd)	Range	# Fish
Spring	PCL	22.60 (10.80)	(4.61-41.21)	0.16 (0.11)	(0.01-0.39)	10	15.22 (6.67)	(2.16-22.77)	7	5.42 (9.19)	(0.025-16.03)	3
	PCM	19.30 (19.18)	(2.93 - 61.61)	0.20 (0.12)	(0.01-0.41)	10	17.60 (14.98)	(0.35-17.41)	3	-	-	-
	BEL	43.71 (40.59)	(6.46-117.95)	0.27 (0.21)	(0.02-0.89)	10	21.72 (19.44)	(0.33-46.88)	10	19.91 (20.10)	(0.05-44.13)	6
	WBU	36.10 (27.91)	(2.55-73.08)	0.12 (0.10)	(0.01-0.30)	10	18.35 (14.98)	(0.08-35.69)	7	19.44 (15.69)	(0.18-35.66)	6
Fall	PCU	53.29 (37.39)	(0.19-84.98)	0.29 (0.21)	(0.00-0.51)	4	-	-	-	-	-	-
	PCM	16.46 (19.71)	(0.95-49.19)	0.17 (0.28)	(0.00-0.89)	10	-	-	-	3.43 (5.74)	(0.025-12.12)	7
	BEU	10.40 (16.32)	(0.22-43.70)	0.05 (0.08)	(0.00-0.22)	7	-	-	-	5.35 (8.18)	(0.025-18.60)	5
	WBL	26.45 (18.24)	(1.71-58.86)	0.10 (0.06)	(0.01-0.19)	15	-	-	-	17.58 (11.74)	(0.50-29.89)	11
Total						76				27		

Figure 1: Study area and smallmouth bass *Micropterus dolomieu* Lacepède 1802

telemetry tagging locations (triangles) in the West Branch of the Susquehanna River and two tributaries (Pine Creek and Bald Eagle Creek) located in Pennsylvania, USA. Fixed telemetry stations were located at the mouth of both tributaries (squares) and dams (circles) were present at the upper and lower bounds of the study area. Site abbreviations are given on the map with full names listed in Table I.

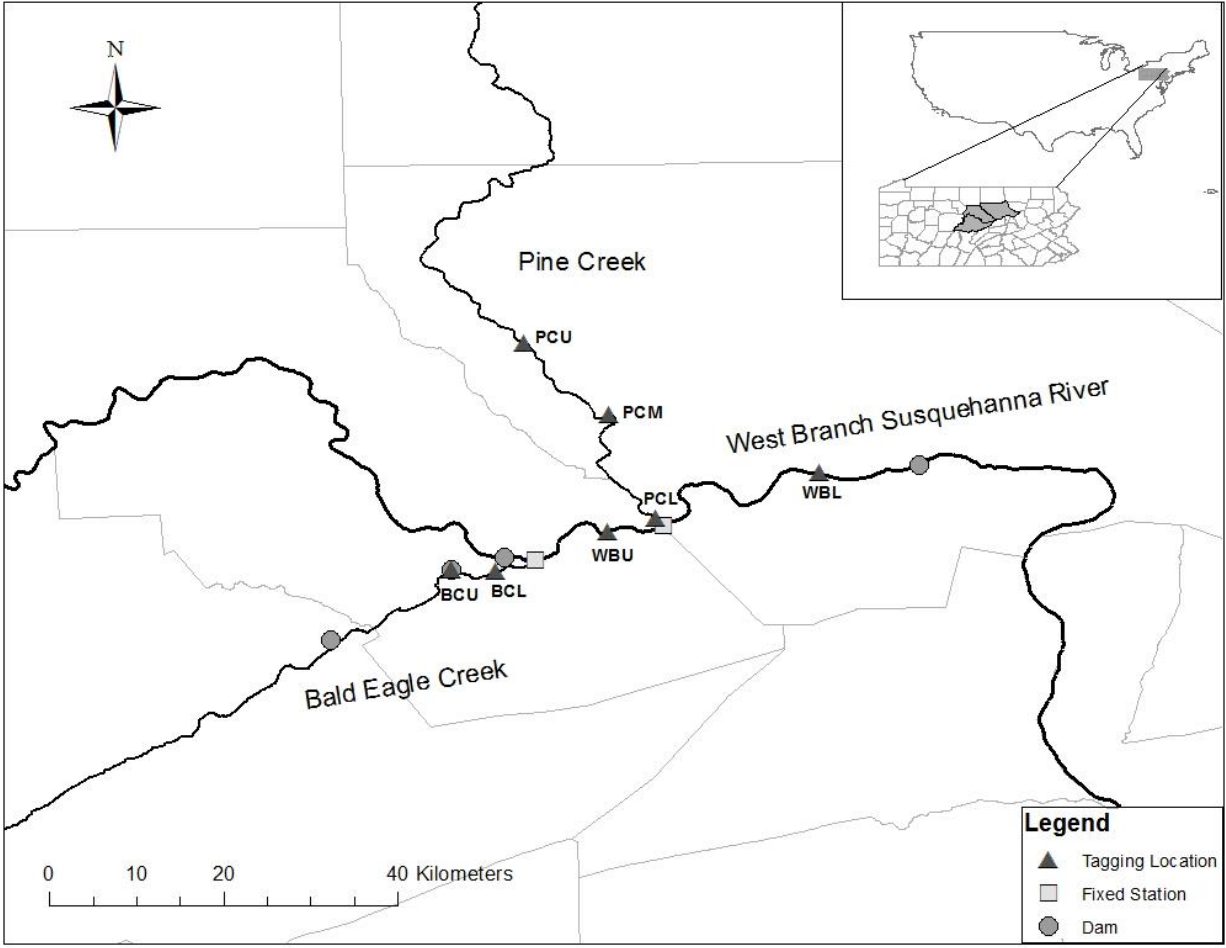
Figure 2: The effect of day of experiment on daily fish movement rates ($\log_e(m/d+1)$).

The solid black line represents the mean estimate from the model and the dashed lines are the 95% confidence intervals. The vertical lines on the x-axis represent the data collected and closely spaced or thickened lines represent amount of data available. The shaded areas represent the seasons where the most movement occurred (from left to right): summer, fall, and spring.

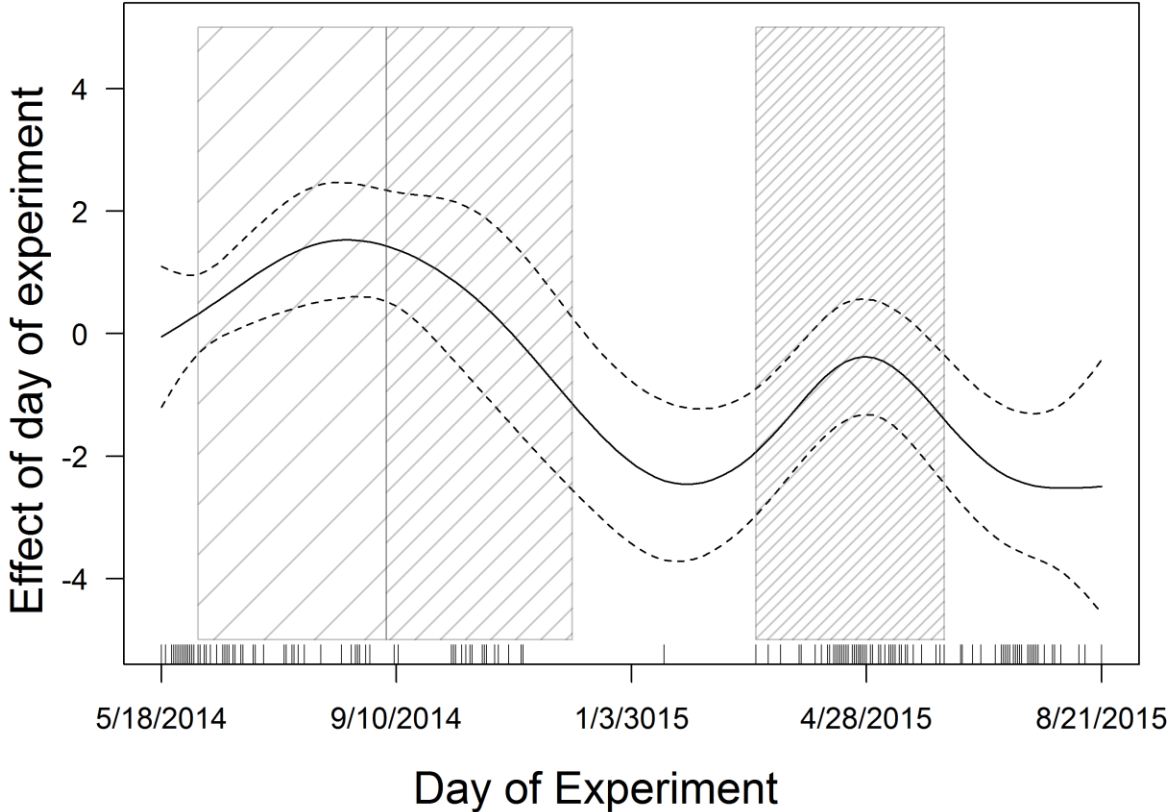
Figure 3: The significant seasonal effects of environmental variables of change in water temperature and flow on fish movement rates ($\log_e(m/d+1)$). Plots (A) and (B) represent the effect of flow ($\log_e(ft^3/s)$) on spring and summer movement rates, respectively. Plot (C) represents the effect of change in temperature ($\Delta^\circ C$) on fish movement rates during the fall. In all plots the black line represents the mean estimates from the GAMM spline and the dashed lines represent the 95% confidence intervals. The vertical lines on the x-axis represent the data collected and increased number of data points can be inferred from closely spaced or thickened lines.

Figure 4: Temporal patterns of flow (ft^3/s) (top) and temperature ($^\circ C$) (bottom) over the smallmouth bass *Micropterus dolomieu* Lacepède 1802 telemetry study duration. Flow is represented as mean daily flow (ft^3/s) from a U.S. Geological Survey (USGS) gaging

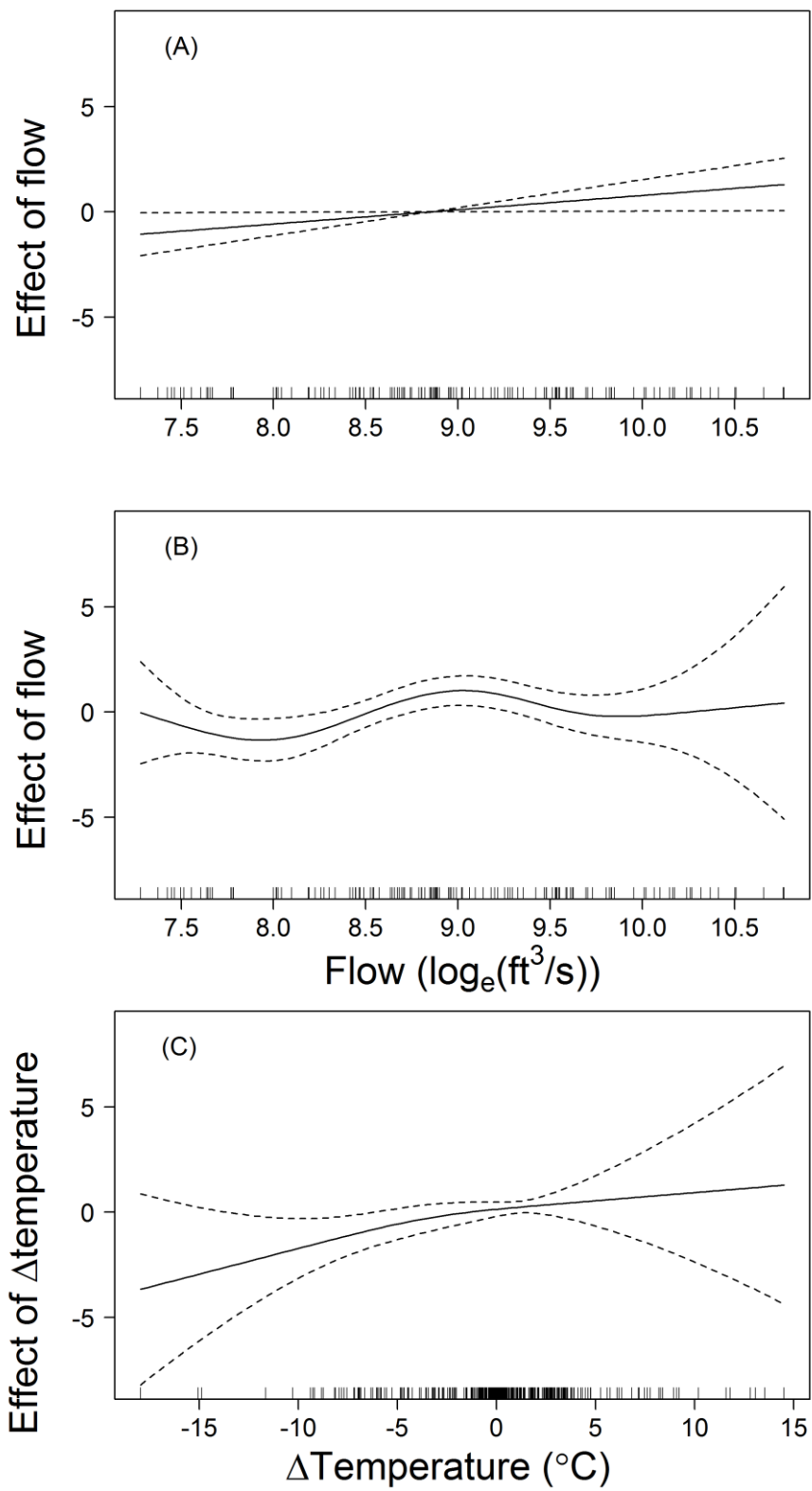
station (see Methods for details). Temperature ($^{\circ}\text{C}$) is represented as mean daily temperature from data sondes and USGS gage stations. Breaks in the plot represent times where data are missing due to ice impacting gage readings. Horizontal dashed lines separate the plots into seasons which are labeled 1 through 4 at the top of the flow plot (season 1 =March-May , season 2 =June-August , season 3 =September-November, season 4 =December-February).



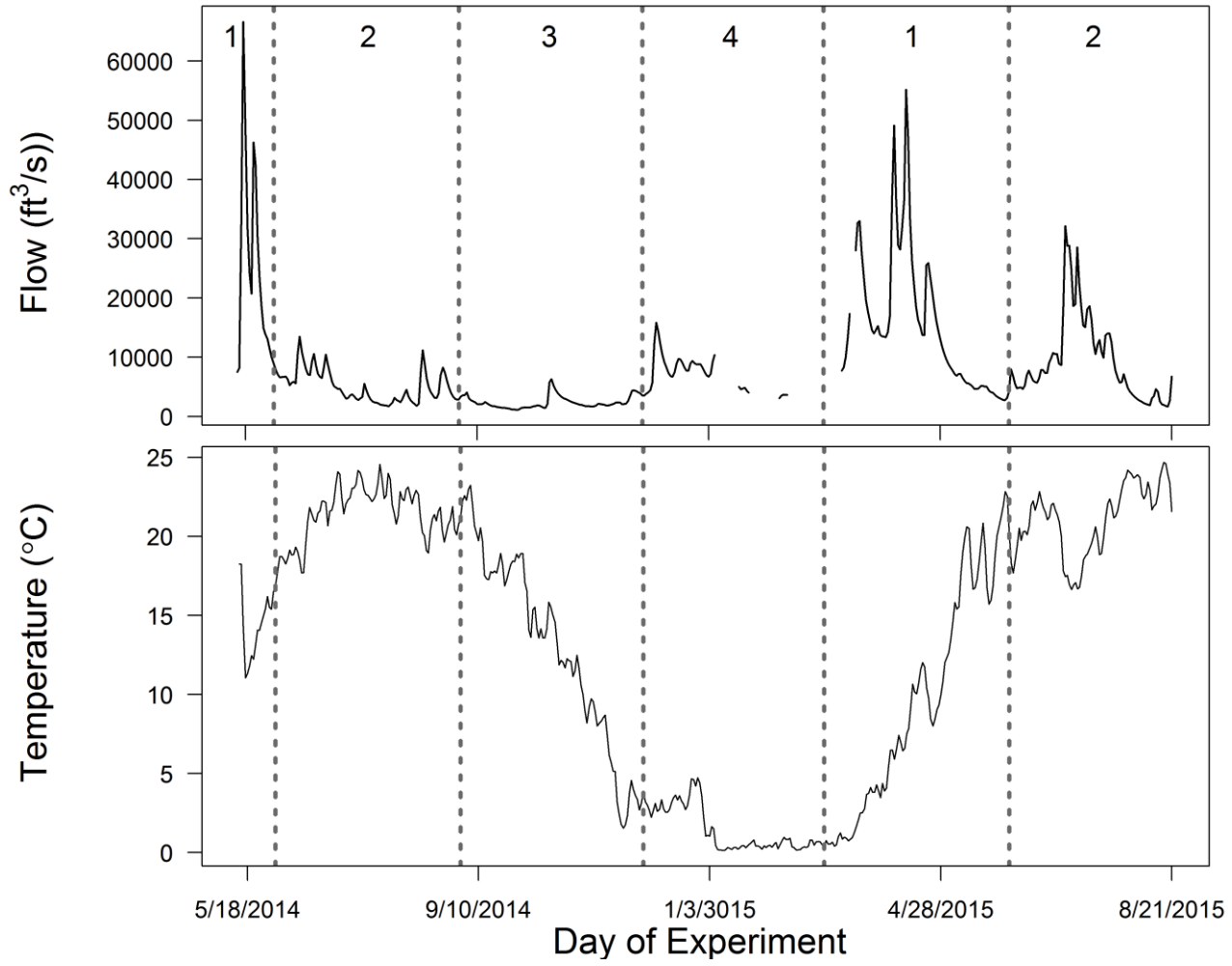
Schall et al. Figure 1



Schall et al. Figure 2



Schall et al. Figure 3



Schall et al. Figure 4

Chapter 4

Spatial and temporal variability of myxozoan parasite prevalence in young of the year smallmouth bass in the Susquehanna River Basin, Pennsylvania

Abstract

Young of the year (YOY) smallmouth bass in the Susquehanna River Basin have been documented to have varying levels of clinical signs of disease and mortality since 2005. A myxozoan parasite, *Myxobolus inornatus*, is one of the disease causative agents that has been identified. To gain a better understanding of the distribution and prevalence of this parasite, we investigated spatial and temporal variability in myxozoan prevalence across the Susquehanna River Basin, Pennsylvania and at several out of basin sites. We also examined potential drivers of *M. inornatus* prevalence, including land use practices that are hypothesized to influence in-stream habitat and parasite dynamics. A total of 1160 YOY smallmouth bass were collected from 31 sites during 2013-2017. Myxozoan parasite infection was documented using histopathology and 41.9% of samples were positive for infection. Site variability in parasite prevalence was greater than temporal variability. The effects of agricultural and developed land uses on myxozoan prevalence were evaluated at both the local and accumulated catchment-scale. The effect of agricultural land use on myxozoan prevalence was positive, and although it was not significant based on 95% credible intervals overlapping zero, it had a high probability of being positively correlated at both spatial scales (probability of positive effect > 0.80). The effect of developed land use on myxozoan prevalence was also not significant, but it

had a relatively high probability of being negatively correlated with prevalence at both spatial scales (probability of negative effect > 0.70). Between the two spatial scales evaluated, the local scale land use had a stronger relationship with myxozoan prevalence, suggesting that local land use conditions may be important in affecting conditions that promote infection. Although quantifying environmental drivers of disease dynamics is difficult, our results suggest that land use practices could be related to infection of smallmouth bass by *M. inornatus* through potentially altering instream habitat mediating parasite - intermediate host dynamics.

Introduction

Myxozoans are a group of fish parasites that have the potential to cause socioeconomic and fish population level impacts (Okamura et al. 2015). Several well-known myxozoan parasites have caused mortality in recreational and commercially important fish species, including rainbow trout *Onchorynchus mykiss* and other salmonids (e.g., *Myxobolus cerebralis*; Hedrick et al. 1998, *Ceratanova shasta*; Ray et al. 2012, *Tetracapsuloides byrosalmonae*; Sterud et al. 2007). As an example, *Myxobolus cerebralis*, the causative agent of whirling disease, has been associated with large scale declines in wild rainbow trout, as well as mortalities of hatchery trout (Hedrick et al. 1998). However, the majority of myxozoan parasites result in more limited adverse effects on the fish host that occur without directly causing mortality (Sindermann 1987). The severity of the effects and prevalence across a stream network, however, is often influenced adjacent land use practices and in-stream habitat conditions (Schmidt-Posthaus and Wahli 2015; Sitja-Bobodilla et al. 2015).

Land use and environmental influences, including temperature (Schisler 2000), the presence of other pathogens (Schisler 2000; Ray et al. 2012), and nutrient pollution (Margogliese and Cone 2001; El-Matbouli and Hoffman 2002), can modulate effects of myxozoan parasites. For example, mortality in juvenile rainbow trout infected with *M. cerebralis* increased significantly with elevated water temperature and the combined effects of water temperature and the bacterial pathogen *Flavobacterium psychrophilum* (Schisler 2000). Environmental conditions may have direct effects on the fish (e.g., thermal stress) or indirect effects through affecting the parasite life cycle, which generally requires an invertebrate host (Sitja-Bobodilla et al. 2015). Anthropogenic land use disturbances, including sewage effluents, have resulted in increased density of aquatic oligochaete invertebrate hosts and as a result were suggested to increase myxozoan parasite prevalence in fish (Margogliese and Cone 2001). Thus, effects of myxozoan parasites on fish hosts are complicated by underlying factors in the environment and landscape which can influence parasite-host dynamics and parasite prevalence.

A myxozoan parasite, *Myxobolus inornatus*, was identified in young of the year (YOY) smallmouth bass in the Susquehanna River Basin (Walsh et al. 2012). *M. inornatus* was first discovered in the Susquehanna River Basin during fish health investigations completed in response to clinical signs of disease and mortality events which have occurred to varying degrees since 2005. Initial findings of *M. inornatus* were in connective tissue near the dorsal and caudal fins. As the parasites develop, the cysts are often visible to the naked eye as small raised areas (Walsh et al. 2012). Subsequent research identified myxozoan spores and cysts with similar tissue tropism, but within

different areas of the fish including the mouth, opercle, and musculature (Walsh et al. *in press*).

The discovery of *M. inornatus* in Susquehanna River YOY smallmouth bass was one of many environmental stressors identified as potential risk factors for YOY disease and mortality (Smith et al. 2015; Walsh et al. *in press*). Multiple pathogens and parasites have been identified including bacteria (e.g., multiple aeromonads, *Flavobacterium columnare*; Starliper et al. 2014), largemouth bass virus (Smith et al. 2015), and trematode parasites (Smith et al. 2015). In addition to pathogens and parasites, water quality concerns include elevated water temperatures, suboptimal dissolved oxygen levels, and contaminants have also been investigated in regards to the overall health of YOY smallmouth bass in the Susquehanna River Basin (Chaplin et al. 2009; Blazer et al. 2014; Smith et al. 2015; Walsh et al. *in press*). Mortality events and low recruitment of YOY smallmouth bass has been a concern overall, as declines in adult smallmouth bass populations have been observed in the Susquehanna River Basin (Arway and Smith 2013; Smith et al. 2015).

Although it is not understood what role *M. inornatus* may be playing in the mortality, it was commonly observed throughout the Susquehanna River Basin between 2007 and 2012 (Walsh et al. *in press*). However, investigation of how myxozoon parasite prevalence varies temporally and across sites in this system has not been evaluated. Quantifying the variability in myxozoan prevalence across the basin will be critical to gain a deeper understanding of the distribution of the parasite and to provide insight on what external variables may be influencing myxozoan prevalence. The objectives of this research were to: 1.) quantify the spatial and temporal variability in myxozoan

prevalence in the Susquehanna River Basin and several out of basin sites, and 2.) to evaluate the effect of potential site-level land use variables on myxozoan parasite prevalence.

Methods

Study Area

YOY smallmouth bass were collected at sites across the Susquehanna River Basin (Figure 1). Sites were selected based on previous incidence of disease and long term monitoring programs in the state. Sampling sites included both river main stem and tributary sites across four subbasins (Table 1). In addition, YOY smallmouth bass were collected from sites outside of the Susquehanna River Basin, which enabled capturing a larger land use gradient. In all, seven out of basin sites were included; in the Delaware River Basin (Lehigh and Schuylkill rivers) and Ohio River Basin (Allegheny River) within Pennsylvania and the Potomac River Basin (Conococheague Creek and Monocacy River in Maryland, and South Branch Potomac River in West Virginia; Table 1 and Figure 1).

Fish Sampling

YOY smallmouth bass were collected in July and August 2013-2016 using DC backpack and towboat electrofishing. Electrofishing focused primarily on shallow areas near the stream banks, habitat where YOY occupy during this time of year. Bass were approximately two to three months of age at the time of collection. During each sampling event, a sample size of 20 fish was targeted; however, this target was not always achieved. Fish from each site constituted a randomly collected sample. Fish were euthanized using a lethal dose of tricaine methanesulfonate (MS-222, Tricaine-S,

Western Chemical, Ferndale, WA) and whole fish were immediately placed in Z-fix™ (Anatech Ltd, Battle Creek, MI) or PAXGene fixative (PreAnalytiX, Hombrechtikon, Switzerland) for tissue preservation.

Histopathology

Histopathology was completed to evaluate presence of myxozoan parasites in whole fish samples. Following a minimum 24 hour fixation time whole fish were decalcified in a 10% EDTA (ethylenediaminetetraacetic acid , pH 7.2), for 2-4 days, rinsed, routinely processed, paraffin embedded sectioned at 5 µm, and stained using hematoxylin and eosin. Fish samples were cut into longitudinal and lateral cross sections prior to embedding in order to view the optimum amount of tissue. For each fish, microscopic presence and absence of myxozoan parasites was recorded and compared by two readers. A myxozoan positive sample was one that had visible cysts with spores or loose spores documented and confirmed by both readers. Myxozoan cysts or spores having tissue tropism similar to *M. inornatus* were targeted during microscopic review. Cysts in locations other than the near the caudal or dorsal fins were not confirmed molecularly to be *M. inornatus*. In the context of this study we are including myxozoan parasite cysts and/or spores morphologically similar and found in connective or muscle tissue. In addition to documenting parasite presence, location of parasite was also recorded (e.g., near the fin, opercle, mouth, muscle, etc.).

Land use predictor variables

Predictor variables focused on explaining variability in myxozoan prevalence among sites rather than across years. Although environmental variables could influence annual variability in myxozoan prevalence (e.g., temperature and flow), we were unable

to evaluate these variables in the statistical model given the limited number of years ($n = 4$). Site variability focused on evaluating land use relationships that were hypothesized to influence myxozoan prevalence through effects on instream habitat and influences on parasite-intermediate-host dynamics. We only included land use variables that represented anthropogenic disturbances of the landscape including agriculture (sum of pasture/hay and cultivated crops) and developed land uses (sum of low, medium, and high developed). Land use summaries of agricultural and developed land use were derived at two different spatial scales for each sampling location, including the: 1.) local catchment scale, which was defined as the immediate catchment the site was located in, and 2.) accumulated catchment scale, which was the proportion of agricultural or developed land use in all upstream catchments from the sampling location. All land use summaries were from the 2011 National Land Cover Database (NLCD) land use data (Homer et al. 2015) and catchments were based on those contained within the National Hydrography Dataset NHDV2+ (National Hydrology Dataset Plus Version 2, U.S. Geological Survey and U.S. Environmental Protection Agency, accessed online at: http://www.horizon-systems.com/NHDPlus/NHDPlusV2_02.php).

Statistical Modeling

A Bayesian hierarchical logistic regression model was used to evaluate spatial and temporal variability in myxozoan prevalence. Site and year random effects were incorporated to quantify the variability in myxozoan parasite prevalence across sites and years, and to accommodate the lack of statistical independence introduced by having multiple observations from each site and year. In addition, we included the site-level

landscape predictor variables to evaluate potential land use relationships with myxozoan parasite prevalence. The hierarchical logistic regression model was as follows:

Observation-level:

$$Pr(y_i = 1) = \text{logit}^{-1}(\delta_{j[i]} + \varphi_{k[i]}), \text{ for } i \text{ in } 1 \dots, 1160 \text{ observations}$$

(Equation 1)

Site-level:

$$\delta_j \sim N(\beta_0 + \beta_1 * AgNHD_j + \beta_2 * DevNHD_j + \beta_3 * AgAccum_j + \beta_4 * DevAccum_j, \sigma_\delta^2), \text{ for } j \text{ in } 1 \dots, 31 \text{ sites}$$

(Equation 2)

Year-level:

$$\varphi_k \sim N(0, \sigma_\varphi^2), \text{ for } k \text{ in } 1 \dots, 4 \text{ years}$$

(Equation 3)

where $y_i = 1$ if a fish was myxozoan positive and zero otherwise, δ_j and year φ_k are the site and year random effects, respectively. The site-level random effects were modeled as a function of site-level land use characteristics; where β_0 is the intercept and $\beta_{1, \dots, 4}$ are regression coefficients describing the relationship between land use ($AgNHD_j$, $DevNHD_j$, $AgAccum_j$ and $DevNHD_j$) and site-level average myxozoan parasite prevalence and σ_δ^2 is a conditional variance. Land use variables are at two land use scales where NHD = local catchment and Accum = the accumulated upstream catchment. The year random effect φ_k was assumed to be normally distributed with a mean of 0 and

variance σ_{φ}^2 . Land use predictor variables were logit-transformed and standardized prior to analysis.

Models were fitted using Bayesian inference using the jagsUI package (Kellner 2016) in the program R (R Core Team, 2017). The model was fitted by running three parallel Markov chains for 90,000 iterations with 40,000 discarded as burn-in. Every third value, after discarding samples as burn-in, was retained to summarize the posterior distribution. Diffuse uniform priors were used for the variance parameters σ_{δ}^2 and σ_{φ}^2 , while a diffuse normal prior was used for β_0 . To accommodate the correlation of land use predictor variables included in the model, we fitted regression slope parameters ($\beta_{1,\dots,4}$) by employing a Bayesian Lasso (least absolute shrinkage and selection operator; Tibshirani 1996; Hooten and Hobbs 2015). The Bayesian Lasso prior was implemented using a double exponential distribution such that:

$$\beta_{1,\dots,4} \sim dexp(0, \gamma)$$

(Equation 4)

and where $\gamma \sim \exp(10)$. Model convergence was assessed with trace plots and \hat{R} convergence statistics. For each parameter of interest the posterior mean and 95% credible intervals were summarized. In addition, to more fully capture the uncertainty in the posterior distributions of the slope parameters ($\beta_{1,\dots,4}$) estimates, the probability that the respective land use relationship was in the direction of the estimated posterior mean (i.e., either positive or negative) was also calculated. In all results reported below, mean estimates represent the posterior mean summarized from the modeling output and any measure of uncertainty is the 95% credible interval.

Results

Summary statistics

A total of 1160 YOY smallmouth bass were collected from 31 sites over four years of sampling (2013-2016). Twenty-one of the 31 sites were sampled more than once (Table 1, Figure 1). Of 67 total sampling events, five had less than 10 individuals collected. The average sample size was 18 ± 5 individuals (average \pm sd). Myxozoan spores were found in 41.9% of fish samples (486 out of 1160 fish; Table 2). All sites, except two out of basin sites (Schuylkill River Berne and Schuylkill River Port Clinton), had at least one fish positive for myxozoan parasites. Location of spores and encapsulated cysts varied. Of the myxozoan positive fish samples, 43.8% (213 of 486 positive samples) of the spores and/or cysts were found in connective or muscle tissue near the fins (Figure 2, Table 2). Myxozoan spores and/or cysts were also found in the mouth, opercle, esophagus, near the spine, or deep in the musculature in 41.1% of the samples (200 of 486 positive samples; Table 2). In 15.0% (73 out of 486) of the positive fish samples, myxozoan spores/cysts were found both near the fins as well as in one of the alternate locations.

Statistical Modeling

Spatial and Temporal Variability

Myxozoan prevalence varied spatially, with comparatively little variation temporally (e.g., σ_δ and σ_φ ; Table 3). The overall probability for myxozoan infection across all sites and year (β_0) was 0.41 (0.25, 0.60). Estimated mean myxozoan prevalence ranged from a 0.42 (0.26, 0.57) in 2014 to 0.55 (0.39, 0.72) in 2016 (Figure 3).

In contrast to limited temporal variability, myxozoan prevalence estimates were variable across sites within the Susquehanna River Basin and outside of the basin. Myxozoan prevalence estimates ranged from a low of 0.07 (0.02, 0.19) at the Lehigh River site to a high of 0.82 (0.65, 0.93) at the Conococheague Creek site. Sites within the Susquehanna River Basin, in general, had higher estimates of myxozoan prevalence than out of basin sites located within Pennsylvania (Figure 4). In the Susquehanna River Basin, 10 of 24 sites had a mean estimate for myxozoan prevalence greater than 0.50 (Figure 4). Five sites within the Susquehanna River Basin had an estimated mean myxozoan prevalence greater than or equal to 0.60 (Bald Eagle Unionville1, Penns Creek, Susquehanna River Mahantango, West Branch [WB] Susquehanna River Jersey Shore, and WB Susquehanna River Watsontown; Figure 4). There were also sites in the Susquehanna River Basin that had low estimates of myxozoan prevalence (posterior mean estimate ~ 0.20). These sites with relatively low estimates of myxozoan prevalence were Bald Eagle Creek Castanea and WB Susquehanna River McElhattan. The out of basin sites located within Pennsylvania had myxozoan prevalence estimates similar to the sites with the lowest estimates in the Susquehanna River Basin (estimated mean myxozoan prevalence < 0.25). The out of basin sites within the Chesapeake Bay had high myxozoan prevalence estimates at two of the three sites (estimated mean myxozoan prevalence > 0.60 , Conococheague Creek and South Branch (SB) of the Potomac River). In general, however, all sites had a large degree of uncertainty associated with prevalence estimates (Figure 4).

Land Use Effects on Myxozoan Prevalence

The proportion of agricultural land use at the local and accumulated catchment scales range from 0.00-0.72 and from 0.05-0.64, with means of 0.19 and 0.26, respectively. Whereas, the proportion of developed land use at the local and accumulated catchment scales ranged from 0-0.88 and from 0.01-0.20, with means of 0.24 and 0.08, respectively. None of the landscape-based predictors had 95% credible intervals that did not overlap with zero (Table 3). However, three out of four land use relationships had greater than 0.80 probability that the effect was in the direction of the posterior mean (Table 3).

For agriculture at the local NHD scale, there is a relatively high probability (0.87) of a positive correlation between myxozoan prevalence and the proportion of agriculture in the local catchment (Figure 5). Myxozoan prevalence also had a relatively high probability of being positively correlated with the proportion of agricultural land use at the accumulated catchment scale (0.81; Table 3). The relationship was slightly weaker than the local scale with a larger credible interval. The likely positive relationship with agriculture and myxozoan prevalence is also supported by the sites that had high estimates of myxozoan prevalence (> 0.60) both in and out of the Susquehanna River Basin. These sites in general, had higher proportion of agriculture (> 0.35) at either the local or accumulated catchment scale than the average proportion of agriculture across all sample sites included (Table 4). The only site that did not match this pattern was Bald Eagle Unionville1, but it was documented to be near a pig farm.

In contrast to the relationship with agricultural land use, myxozoan prevalence likely declined with increasing proportion of development (Figure 6). At the local catchment scale, there was a 0.92 probability that the relationship between local

development and myxozoan prevalence was negative (Table 3). The relationship between myxozoan prevalence and accumulated catchment development was weaker (0.72) when compared to the local scale (Table 3).

Discussion

Co-infections with bacterial, viral and parasitic pathogens have been suggested to contribute to the mortality of YOY smallmouth bass and consequent declines in abundance (Walsh et al. *in press*). However, the underlying environmental conditions contributing to increased pathogen prevalence/virulence or immunomodulation of the host fish are not fully understood. Previous reports have primarily focused on disease findings in YOY with obvious clinical signs of disease including lesions (Chaplin et al. 2009; Smith et al. 2015; Walsh et al. *in press*). To better understand the factors influencing *M. inornatus* prevalence observed in the Susquehanna River Basin, this study evaluated the prevalence in 1160 random samples collected at 31 sites, including out of basin sites, over a four year period. We found myxozoan prevalence to vary more spatially than temporally. In general, *M. inornatus* was common throughout the Susquehanna River Basin and less common at out of basin sites within the state of Pennsylvania.

The initial description of *M. inornatus* was from juvenile and adult hatchery largemouth bass in Montana. As in this study, raised areas were observed on the caudal peduncle (Fish 1939). *M. inornatus* was later reported from smallmouth bass in Lake Erie during surveys in 1970 to 1975 (Dechtiar and Nepszy 1988). Prior to 2005, there were no concerns regarding YOY smallmouth bass health in the Susquehanna River Basin that would warrant histopathological investigations. However, there were surveys of YOY

that may have led to observations of the visible lesions. Similarly, although sample sizes were small, YOY smallmouth bass from the Potomac were examined microscopically in earlier surveys (2007-2011) and *M. inornatus* was not observed (V. Blazer, personal observation). It is currently not known if this parasite has been introduced to the Susquehanna River Basin and subsequently to the Potomac River Basin; however, findings of the parasite at the Potomac River Basin suggests the spread of the parasite.

Most myxozoan parasites have complex life cycles involving a benthic invertebrate intermediate host such as oligochaetes, polychaetes, and bryozoans. Hence, disease prevalence may be influenced by habitat and environmental factors that affect the fish, the infective stage or intermediate host (Sitja-Bobadilla et al. 2015). Unfortunately the invertebrate host for *M. inornatus* has not yet been identified. Our model indicated a positive relationship between myxozoan prevalence and agricultural land use. Conversely, the percentage of developed land showed a negative relationship with myxozoan prevalence. Agriculture land use is often characterized by inputs of sediment, nutrients, and pesticides into aquatic environments (Allen 2004). Nutrient enrichment and sediment deposition have been shown to affect myxozoan parasite prevalence through multiple mechanisms. An increase in density of the aquatic oligochaete intermediate host for whirling disease, *Tubifex tubifex*, occurred in areas of fecal enrichment near fish production facilities and cattle farming (Kaeser and Sharpe 2006). Density of tubificid aquatic oligochaetes, including *Limnodrilus hoffmeisteri* and *T. tubifex*, was also shown to increase in areas of sediment deposition (Anlauff and Moffitt 2008). The instream habitat produced by agricultural land use inputs could also promote higher release rates of the infective stage for fish (actinospores). For example, *M. cerebralis* actinospores

were released in higher numbers by *T. tubifex* worms in muddy sediment and sand than those in leaf litter (Blazer et al. 2003). In addition, agricultural land use was also associated with pesticide inputs into aquatic environments (Allen 2004). To our knowledge the relationship between pesticides from agriculture and myxozoan prevalence has not been evaluated to date. However, there is ample evidence that pesticides can cause immunomodulation, thus affecting the ability of the host to resist parasite infections (Kreutz et al. 2010, Rohr and McCoy 2010; Dunier and Siwicki 1993). The majority of studies documenting the complexity of the interactive effects of pesticides and parasitic infections involve amphibians and trematode infections (Rohr et al. 2008, Kreutz et al. 2010, Rohr and McCoy 2010; Schotthoeffer et al. 2011). Additional research is needed to understand the role of environmental stressors on diseases caused by parasites with complex life cycles.

In contrast to agricultural land use, a decline in myxozoan prevalence with increased development in the landscape was observed. Certainly wastewater treatment plants associated with development can lead to nutrient enrichment. In spottail shiners *Notropis hudsonius*, myxozoan parasite prevalence and diversity of myxozoans present increased in areas influenced by urban effluents in the St. Lawrence River (Marcogliese and Cone 2001). Subsequently, a known myxozoan host, aquatic oligochaete *L. hoffmeisteri*, was found in higher densities below sewage effluents (Marcogliese et al. 2009). However, non-point nutrient inputs in urban watersheds have been estimated to be 39-66% lower than comparisons to historic agricultural land use estimates in the Northwestern United States (Brett et al. 2005). A similar trend was found in Kentucky where development contributed more to suspended sediments, but agriculture was still

associated with higher nutrient inputs (Coulter et al. 2004). Conversely, development, particularly increases in impervious surface, is related to increases in the amount and variety of chemical contaminants and more erratic hydrology (Allen 2004). The chemical signature in developed land is very different than agricultural land, with increased concentrations of contaminants such as heavy metals, polycyclic aromatic hydrocarbons, polychlorinated biphenyls (Nowell et al. 2013) and different mixtures of pesticides (Nowell et al. 2013; Nowell et al. 2017). Numerous studies have shown benthic invertebrate richness is negatively impacted by development, while agricultural land use often has the highest diversity (Moore and Palmer 2005; Cuffney et al. 2010; Herringshaw et al. 2011).

Land use effects may be occurring differently across scales of inference. In this study, local effects were stronger than accumulated watershed relationships for both development and agriculture. Attention to local factors may be important in future research on myxozoan parasites given the possible relationships discovered in this study. Some localized site-specific factors could also be unaccounted for by general land use groupings. Localized input sources could explain higher prevalence at the Bald Eagle Unionville 1 site which was collected near a farming operation that was not reflected as a major part of the local or accumulated catchment land use. There are likely other sites across the study area that could have similar localized situations that could be investigated further. It is also important to acknowledge that the gradient of development at the accumulated catchment scale also had a relatively limited data range.

The ability to model temporal variability in myxozoan prevalence was limited in our study because of having collected data over only a four year period. Environmental

variables including temperature and flow have been shown to influence myxozoan prevalence and may influence annual variations in prevalence (Hallett and Bartholomew 2008, Okamura et al. 2011, Fontes et al. 2015). In addition, intra-annual temporal variability at these sites in prevalence – in contrast to among year variability across all sites – may be driven by site-specific changes in factors such as flow and temperature. Understanding intra-year prevalence dynamics may help elucidate finer-scale environmental drivers of *M. inornatus*.

Additionally, this myxozoan parasite is only one of many potential stressors identified in the Susquehanna River Basin, thus it will be important for future research to continue to investigate the possible combined effects of stressors. Examining temporal and spatial relationships of other stressors may elucidate common and differing relationships with land use and other factors that could be important across multiple stressors (e.g., other parasites and pathogens). Overall, we found myxozoan prevalence in YOY smallmouth bass to vary more across sites than temporally and spatial patterns of prevalence are likely correlated with agricultural and developed land use patterns. The different relationships with land use may be important for parasite-host dynamics of this myxozoan parasite and warrant future investigation for this parasite and other potential stressors of disease in the Susquehanna River Basin.

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Table 1: Sample sites where YOY smallmouth bass were collected across the Susquehanna River Basin and at out of basin sites. Out of basin sites include those within the state of Pennsylvania and those outside of Pennsylvania, within the Potomac River, Chesapeake Bay. In Stream names, WB = West Branch, Site no. = the site number that is depicted on Figure 1 map. GPS coordinates are provided with site latitude and longitude. Years = the years of data that YOY were collected for each site. For the Susquehanna River Basin, the location (tributary or mainstem) and the subbasin the site is located in is provided.

Susquehanna River Sites		GPS				
Stream name	Site no.	Latitude	Longitude	Years	Location	Subbasin
Bald Eagle Creek Castanea	1	41.12337	-77.4416	2013-2015	Tributary	West Branch
Bald Eagle Unionville1	2	40.91538	-77.8488	2014	Tributary	West Branch
Bald Eagle Unionville2	3	40.90636	-77.8695	2015	Tributary	West Branch
Chillisquaque Creek	4	40.94163	-76.85	2013, 2015	Tributary	West Branch
Kettle Creek	5	41.40506	-77.9219	2015	Tributary	West Branch
Loyalsock Creek	6	41.24099	-76.9377	2013-2015	Tributary	West Branch
Pine Creek Hamilton Bottom	7	41.34864	-77.4019	2013-2015	Tributary	West Branch
Pine Creek Ramsey	8	41.28227	-77.3179	2014-2016	Tributary	West Branch
WB Susquehanna River Jersey Shore	9	41.20223	-77.2527	2014-2015	Main stem	West Branch
WB Susquehanna River Lewisburg	10	40.96633	-76.8778	2015	Main stem	West Branch
WB Susquehanna River McElhattan	11	41.16383	-77.3274	2013-2015	Main stem	West Branch
WB Susquehanna River Watsontown	12	41.07961	-76.8633	2014	Main stem	West Branch
Susquehanna River Danville	13	40.94245	-76.5997	2015	Main stem	Middle
Susquehanna River Falls	14	41.46166	-75.8677	2015	Main stem	Middle

Wyalusing Creek	15	41.69748	-76.2309	2013-2016	Tributary	Middle
Conodoguinet Creek	16	40.25869	-77.0104	2014-2016	Tributary	Lower
Penns Creek	17	40.86672	-77.0497	2013-2015	Tributary	Lower
Susquehanna River Harrisburg	18	40.3186	-76.8988	2013-2016	Main stem	Lower
Susquehanna River Isles of Ques	19	40.78865	-76.8568	2015	Main stem	Lower
Susquehanna River Mahantango	20	40.65133	-76.9232	2013-2014, 2016	Main stem	Lower
Swatara Creek	21	40.21278	-76.721	2013-2016	Tributary	Lower
WB Mahantango Creek	22	40.6478	-76.943	2013-2016	Tributary	Lower
Juniata River Newport	23	40.49193	-77.0968	2014-2015	Tributary	Juniata
Tuscarora Creek	24	40.51553	-77.4195	2014-2015	Tributary	Juniata

Out of basin sites		GPS		
Stream Name	Site No.	Latitude	Longitude	Years
Allegheny River	25	41.382	-79.82	2014-2015
Lehigh River	26	40.60583	-75.4542	2014-2015
Schuylkill River Berne	27	40.52306	-75.9972	2015
Schukyll River Port Clinton	28	40.57722	-76.0253	2013, 2015
Out of state, Potomac River		GPS		
Stream Name	Site No.	Latitude	Longitude	Years
Conococheague Creek	29	39.6251	-77.8176	2013-2014,2016
SB Potomac River	30	39.10375	-78.9591	2014
Monocacy River	31	39.38749	-77.3801	2013-2014

Table 2: Annual summaries of YOY smallmouth bass samples collected in the Susquehanna River Basin and at several out of basin sites. Year = the year of sampling, Number of sampling events = the number of sites where YOY smallmouth bass were collected in a given year, Total samples = sum of all YOY smallmouth bass samples collected in a given year, and Number positive = number of myxozoan positive samples confirmed by histopathology. Myxozoan location in the fish is split up into the following: Traditional = cysts or spores located near the fin where initially described by Walsh et al. 2012, Other = cysts or spores in alternate areas of similar tissue types including the mouth, opercle, and muscle, and both = cysts or spores located near the fins and also one of the alternate locations.

Year	Number sampling events	Total samples	Number positive	Myxozoan location		
				Traditional	Other	Both
2013	13	208	86	45	29	12
2014	21	438	170	77	72	21
2015	24	381	159	65	70	24
2016	8	133	71	26	29	16
Total		1160	486	213	200	73

Table 3: Bayesian hierarchical logistic regression estimates for site and year variability in myxozoan prevalence in YOY smallmouth bass and relationships with land use and myxozoan prevalence across multiple spatial scales (NHD catchment and accumulated [Accum] catchment). Model estimates include posterior mean and 95% credible intervals (CI). Relationships between myxozoan prevalence and land use variables ($\beta_{1,..,4}$) include estimates (posterior mean and 95% CI) as well as probability that the relationship is in the direction of the estimated posterior mean.

Variance components	Posterior mean (95% CI)	
Site (σ_δ)	1.063 (0.721,1.530)	
Year (σ_φ)	0.527 (0.097,1.848)	
Land use variables	Slope posterior mean (95%CI)	Probability
NHD Ag (β_1)	0.239(-0.184,0.661)	0.872
NHD Dev (β_2)	-0.344 (-0.830,0.133)	0.923
Accum Ag (β_3)	0.223 (-0.275,0.765)	0.809
Accum Dev (β_4)	-0.168 (-0.770,0.400)	0.718

Table 4: Sampling sites for YOY smallmouth bass collections and estimated posterior mean myxozoan prevalence greater than 0.6 and associated agriculture land use at the local and accumulated catchment scale. Site location was either in the Susquehanna River Basin (SRB) or out of basin but in the Chesapeake Bay (OBCB). NHD Ag and Accumulated Ag values represent the proportion of agriculture at each of those land use scales. Values that are bold are greater than the average proportion of agriculture in local or accumulated agriculture across all sample sites.

Site	Location	NHD Ag	Accum Ag
Bald Eagle Unionville1	SRB	0.11	0.08
Penns Creek	SRB	0.41	0.22
Susquehanna River Mahantango	SRB	0.11	0.49
WB Susquehanna River Jersey Shore	SRB	0.38	0.09
WB Susquehanna River Watsontown	SRB	0.49	0.11
Conococheague Creek	OBCB	0.57	0.14
SB Potomac River	OBCB	0.72	0.49

Figure headings

Figure 1: Map of the study area where YOY smallmouth bass were collected in Pennsylvania, Maryland, and West Virginia during 2013-2016. Sample sites are numbered 1-31 based on subbasin of origin within the Susquehanna River Basin and out of basin locations. Site names corresponding to number identifiers are found in Table 1. Sampling occurred over four years which is represented by the different sized and shading of circles. The shaded area on the map represents the Susquehanna River Basin.

Figure 2: A. Visible raised areas on a young of year smallmouth bass near the caudal fin (arrow). B. Microscopic appearance of a myxozoan cyst (a) in the caudal area (b) of a smallmouth bass. Raised area illustrating an inflamed and eroded epidermis (arrow). Scale bar equals 50 μm . Hematoxylin and eosin stain. C. Myxozoan cyst with mature spores (a) and immature stages (b). The cyst is surrounded by inflammation (arrows). Scale bar equals 50 μm . Hematoxylin and eosin stain.

Figure 3: Temporal variability in myxozoan prevalence across the four years (2013-2016) when YOY smallmouth bass were collected in the Susquehanna River Basin and several out of basin sites. Points represent the posterior mean myxozoan prevalence for each year of sampling and lines represent 95% credible intervals.

Figure 4: Spatial variability in myxozoan prevalence in YOY smallmouth bass collected in the Susquehanna River Basin and several out of basin sites ($n = 31$ sites). Sites are organized alphabetically in relation to subbasin in the Susquehanna River Basin (same as

Table 1). Points represent the posterior mean myxozoan prevalence for each sample site and lines represent 95% credible intervals. Black points and lines represent sites in the Susquehanna River Basin, dark gray squares and lines represent out of basin sites in Pennsylvania, and, light gray triangles and lines represent out of basin sites in the Chesapeake Bay.

Figure 5: Relationship between myxozoan prevalence (on the logit-scale) and the logit-transformed proportion of local agricultural land use for the 31 sample sites where YOY smallmouth bass were collected. The thick black line represents the estimated hierarchical regression line and gray shaded area is the 95% credible region. Points are estimated posterior means and vertical lines are 95% credible intervals.

Figure 6: Relationship between myxozoan prevalence (on the logit-scale) and logit-transformed proportion of local development for the 31 sample sites where YOY smallmouth bass were collected. The thick black line represents the estimated hierarchical regression line and gray shaded area is the 95% credible region. Points are estimated posterior means and vertical lines are 95% credible intervals.

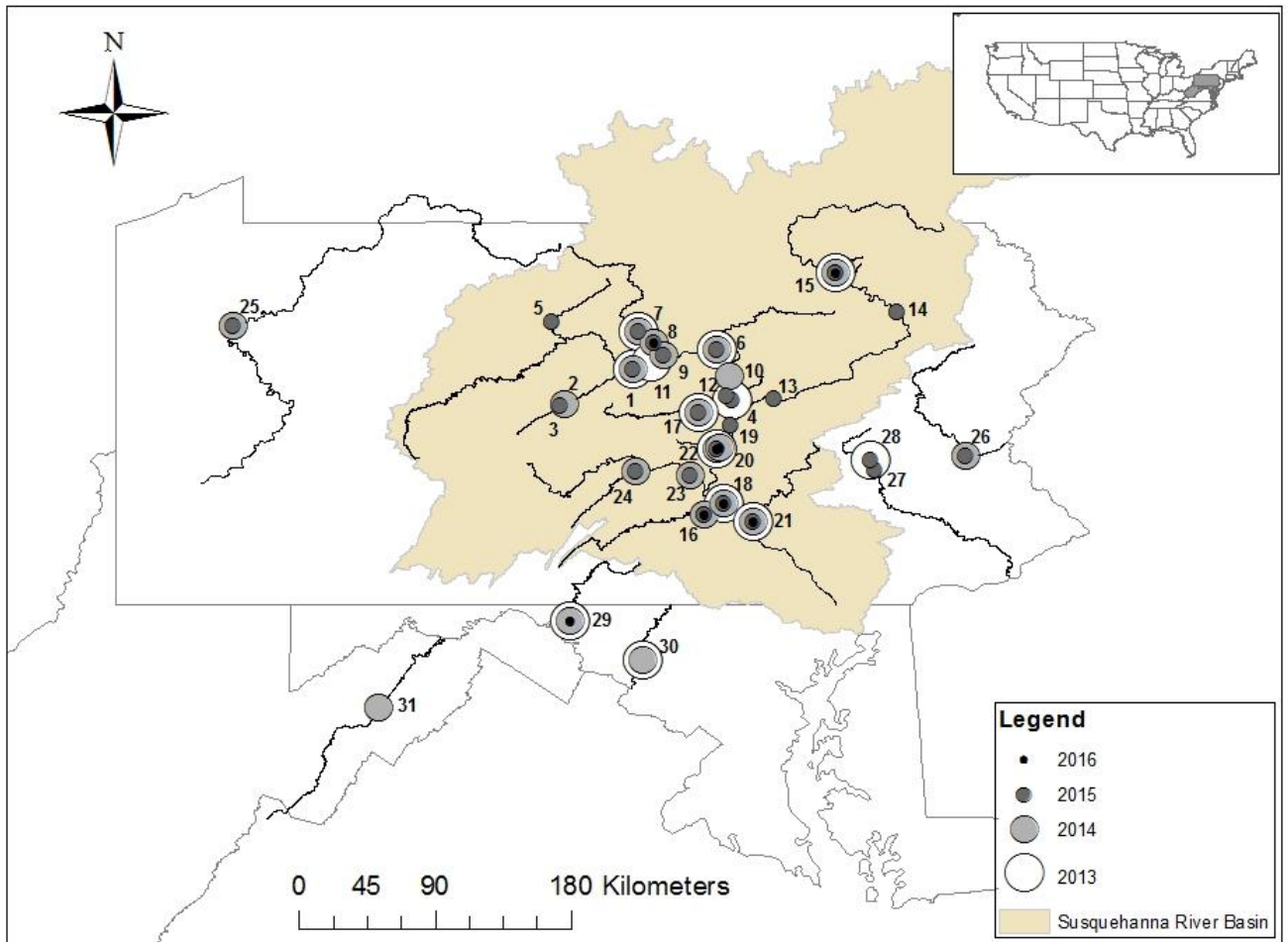


Figure 1: Schall et al.



Figure 2: Schall et al.

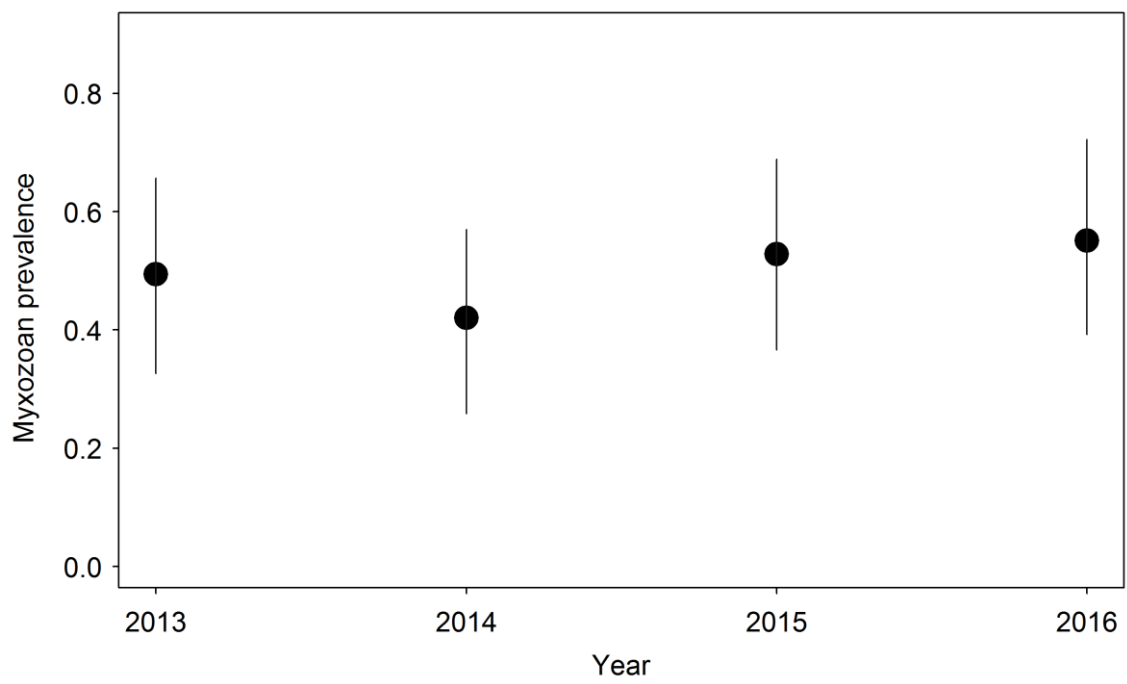


Figure 3: Schall et al.

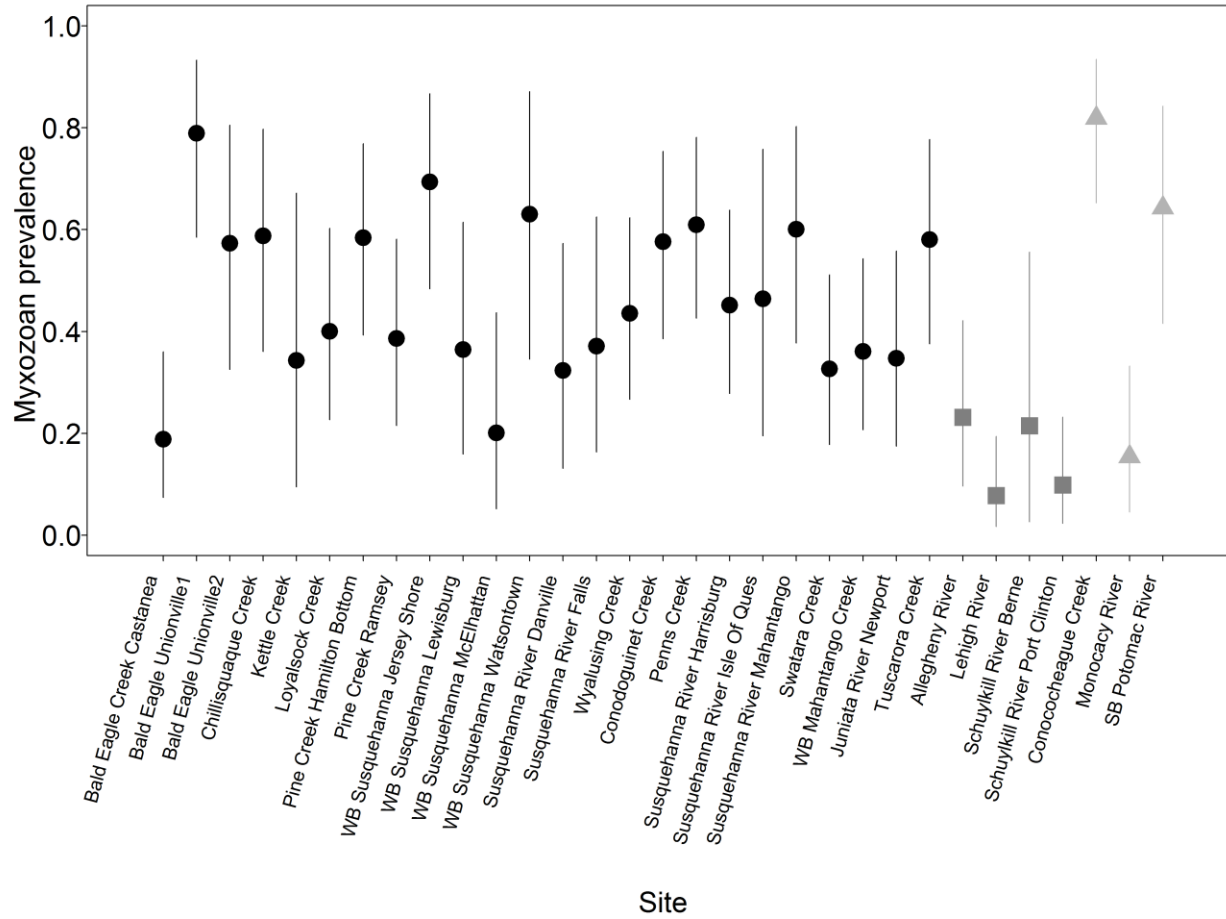


Figure 4: Schall et al.

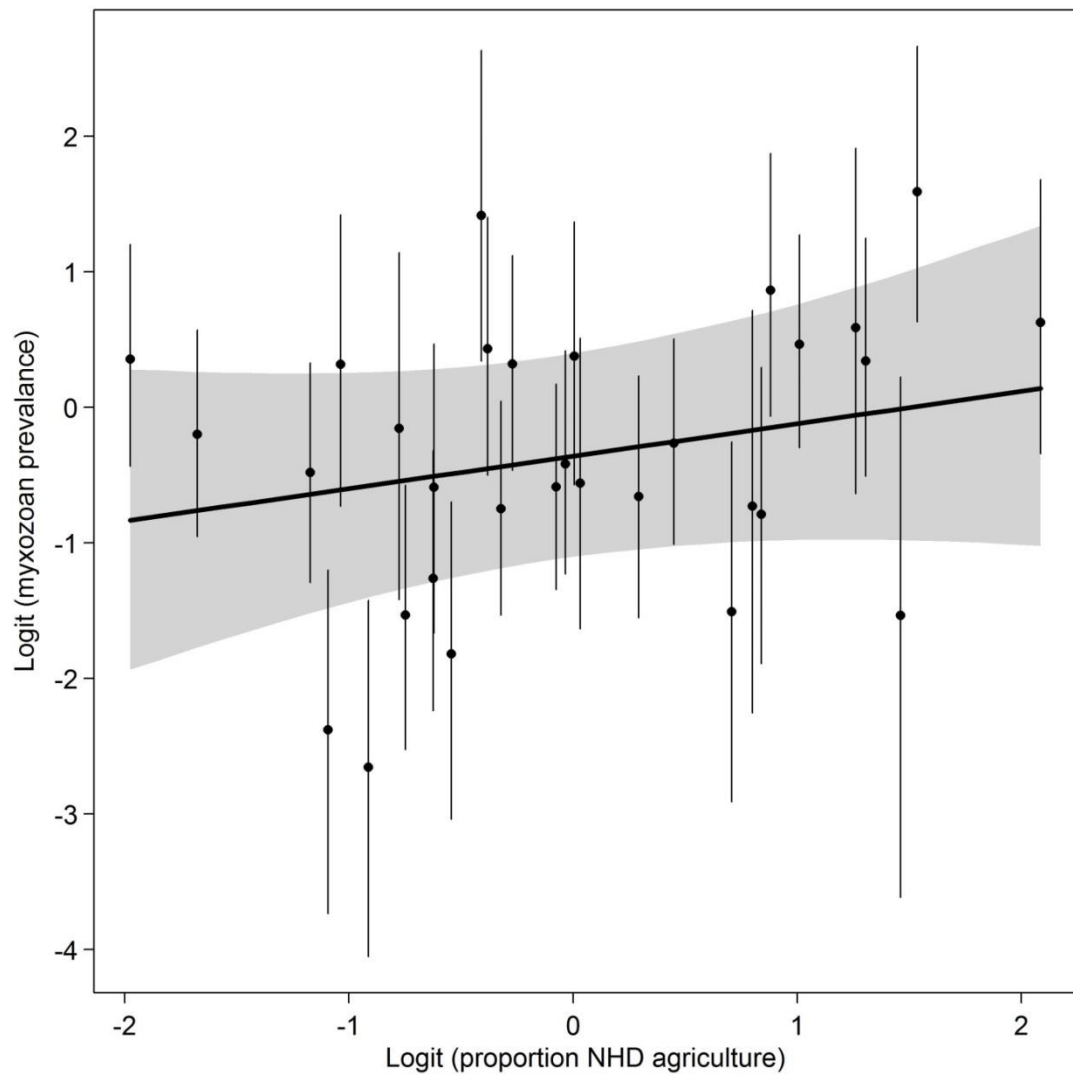


Figure 5: Schall et al.

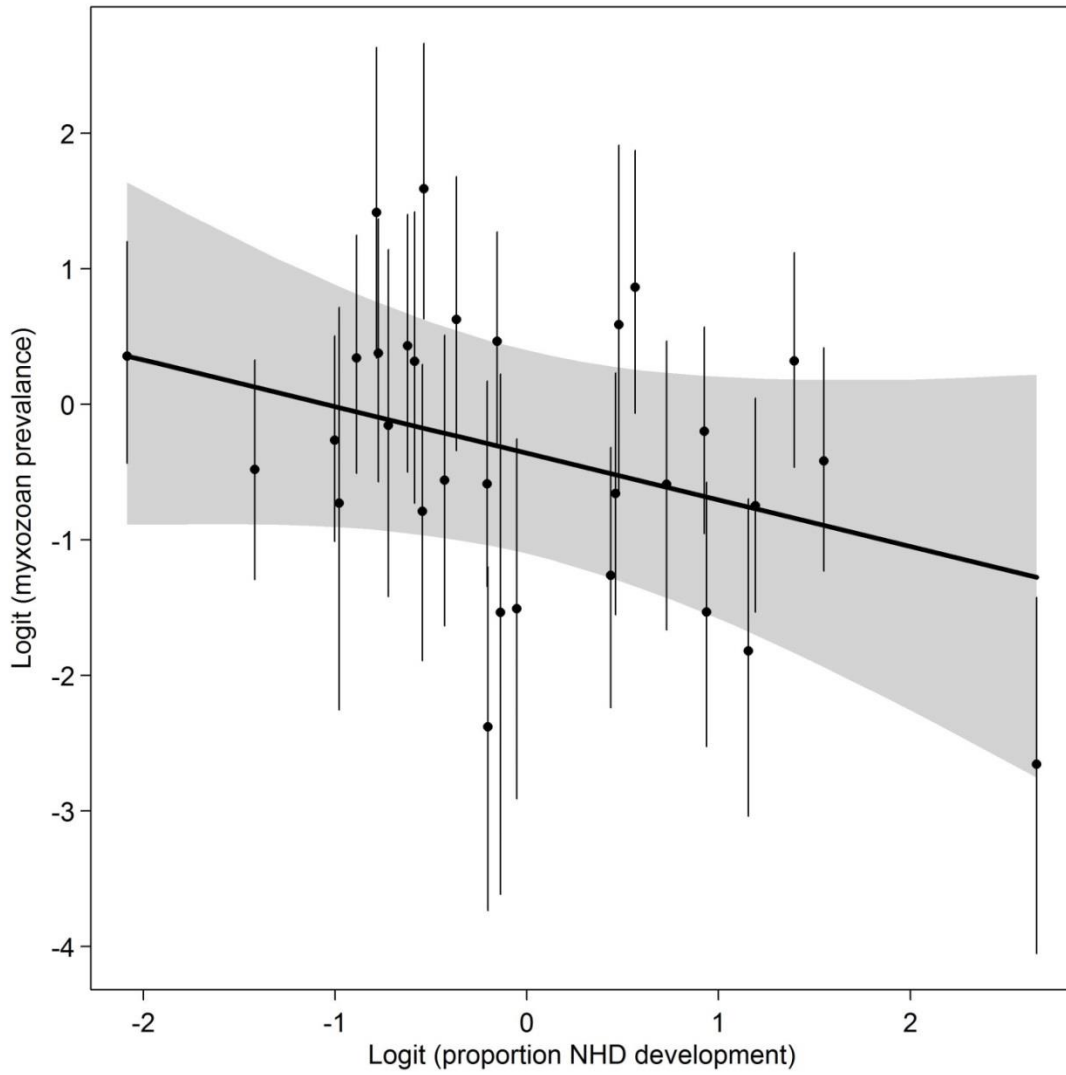


Figure 6: Schall et al.

Chapter 5

Summary and Future Directions

My research addressed four primary questions related to the ecology and health of smallmouth bass, an ecologically and socioeconomically important fish species, in the Susquehanna River Basin. The overall goal was to understand spatial and temporal patterns of abundance, population genetic structure, movement, and myxozoan parasite prevalence. This research integrated across several subdisciplines of ecology and was performed across large spatial extents, larger than have previously been examined for this species and most other freshwater species. These integrative relationships can inform conservation and management strategies for the Susquehanna River Basin, and beyond, because of the connections among the fishery, environmental stressors, and human resource use.

The first step to integrating fish ecology with fish health was to investigate the underlying assumption that there were actual declines in smallmouth bass populations across the Susquehanna River Basin. Declines with high probability of occurrence (>80% probability) were present throughout the Susquehanna River Basin. Of importance, the declines varied in magnitude and duration across the system. Additionally, many of the declines began before recognition of the first overt disease and mortality event in 2005, potentially indicating that stressors in this system were having population-level effects starting in the early 2000s. More recently, population abundance appears to have

stabilized; however, there are still concerns over the overall health of the fishery and rivers in the Susquehanna River basin.

Evidence of variability in the magnitude and duration of declines in smallmouth bass abundance, which may or may not be related to disease distributions across the Susquehanna River Basin, prompted the question of whether genetic differences could be a factor. Identifying differences in genetics among populations together with health indicators and exposure histories could set the stage for prioritization of management actions across local and watershed scales. Population genetic structure and genetic diversity were evaluated to determine if there were discernable levels of genetic differentiation among smallmouth bass populations in the Susquehanna River Basin. Because smallmouth bass were introduced into the system there were questions regarding population connectivity and levels of genetic diversity. In general, a lack of genetic differentiation among sites (pairwise F_{ST} values close to 0) and lack of population structure ($k = 3$ admixed clusters) were found in the Susquehanna River Basin. Although estimates of heterozygosity did not raise immediate concerns regarding levels of genetic diversity, evidence of genetic drift was present when genetic measures of diversity including private alleles were compared to an out-of-basin site (Allegheny River). Some questions remain, however, on the genetic relationships between out-of-basin sites given the limited comparison done in this study. Future out-of-basin sites of interest could include the Potomac and Ohio rivers from which the Susquehanna River population originated. Smallmouth Bass were introduced into the Potomac River Basin from the Ohio River Basin and subsequently introduced from the Potomac River Basin into the

Susquehanna River. At the present time in the Susquehanna River Basin, there is not support for separate management units based on differences in genetic composition. Within the Susquehanna River Basin, there may be several mechanisms contributing to the genetic similarity across the basin including the relatively short timeframe since introductions and fish movement resulting in gene flow.

To determine whether fish movement could contribute to a lack of genetic structure and to understand how fish are using the river and tributary network, a spatial movement ecology study was completed. Smallmouth bass movement was hypothesized to be an important link among fish health, fish ecology, and the environment. From a fish health standpoint, movement of smallmouth bass in tributary and river habitats can provide insight into exposures to possible stressors. Chemical, biological, and environmental stressors varied across the system and could impact fish health endpoints differently depending on movement history. In general, I found that movement by adult smallmouth bass in the Susquehanna River basin varied among individuals with the capability for large longitudinal movements (average movement from spawning to overwintering grounds =18.3 km, range =0.08 to 46.9 km, n=27). Smallmouth bass movement predominantly occurred prior to and after both spawning and overwintering. Movement of fish between river and tributaries was common. Different seasonal ecological groupings of fish during spawning and overwintering could be important factors in the spread of aquatic diseases, which could influence seasonal management needs in order to protect fish populations. Spatial movement ecology of smallmouth bass emphasized the need to maintain fish movement corridors including between rivers and

tributaries. In the Susquehanna River Basin, it will be important to consider how barriers may impact smallmouth bass movement between rivers and tributaries. Additionally, where there are barriers to movement (dams) in the river and tributaries, managers should consider movement of smallmouth bass in the design of new fish ladders as well as the potential modification and maintenance of lifts already in place. Some questions remain, however, including developing methods to determine movement history of individual fish collected for fish health surveys. Otolith microchemistry could be used to distinguish elemental signatures (e.g., strontium to calcium ratios) among rivers and tributaries and determine how long fish are residing in each. Before this could be done, it would be necessary to determine if elemental signatures differ sufficiently throughout the system. Additionally, it would be important to study how divergent or similar biological, chemical, and environmental stressors are across the system to gain a better understanding of the different risks for exposure to stressors in the system. In this system, adult fish making large movements and congregating in different ecological groups could be exposed to a wide range of possible stressors impacting fish health including different inputs or environmental conditions in rivers and tributaries.

Exposure to biological or other stressors in the environment could be different for adults that move throughout the system as compared with young-of-the-year (YOY) collected at spawning sites. To start understanding spatial and temporal relationships between YOY and biological stressors, prevalence of a myxozoan parasite, *Myxobolus inornatus*, was studied. Given the complex life cycle and possible invertebrate host (likely an aquatic oligochaete), it was hypothesized that anthropogenic disturbances on

the landscape including development and agriculture could influence parasite prevalence in the fish host through increasing invertebrate host habitat and thus abundance. For example, agricultural land use has been associated with nutrient and sediment inputs into the system, which could consequently increase invertebrate host populations and as a result influence myxozoan prevalence. Myxozoan prevalence was found to vary more spatially than temporally (mean estimates spatially = 0.07 to 0.82 vs. temporally = 0.42 to 0.55). Examining myxozoan prevalence in relation to land use suggested a positive relationship with agriculture and a negative relationship with development. In this regard, the local catchment scale had stronger effects than the accumulated catchment scale. The relationships among myxozoan prevalence and land use could suggest indirect effects on the myxozoan parasite life cycle including not only increases in invertebrate host abundance but also parasite prevalence from nutrient enrichment associated with agriculture. These findings suggest a need for management at different scales. For this myxozoan parasite, local scales as opposed to accumulated catchment scales may be more important. Additional studies are warranted as the influence of land use at different scales may vary depending on the life stage being studied (i.e., YOY vs. adult) as well as the specific biological, chemical, or environmental stressor being investigated. An adult that moves throughout the system may be exposed to environmental conditions that change at both fine and broad scales. Important questions remain, such as how sample site proximity to non-point-source and point-source pollution may impact myxozoan prevalence. For example, developed land use relationships with myxozoan prevalence may have differed if sample sites had been in close proximity to sewage effluents. Future

research on this parasite may include investigating additional land use relationships (e.g., percent impervious surfaces). Additionally, there are many data gaps with regard to this myxozoan parasite that warrant future investigation. The invertebrate host has not been identified to date. Because of this, laboratory studies have not been completed to investigate variables that could directly and/or indirectly alter the parasite life cycle. Future studies will be important to elucidate some of the unknown relationships among this parasite and other biological, chemical, and environmental stressors in the context of determining what is needed to support improved fish health.

Outside of the specific research conducted in my dissertation, continued research and monitoring will be essential to strengthen the integrative framework at the interface of fish health, fish ecology, and the environment and to inform conservation and management of smallmouth bass health and the health of the river. In the context of ongoing research studies in the system, one large and important need is the integration of contaminant data from sampling of nearby terrestrial environments, water, sediments, foodweb species, adult and YOY smallmouth bass of the Susquehanna River Basin. Results from such research together with health monitoring data could help identify contaminants of concern (e.g., those that account for endocrine disruption) and potential links to possible sources of contaminant exposure in the environment to set the stage for recovery of fish health and sustainability. Fish tissue and environmental samples, including water and sediment, were obtained in the course of these studies in efforts to identify sources and drivers that may undermine ecological and fish health. Unfortunately

however, much of the data on contaminants in fish tissue samples have not been received from the laboratory to date, limiting statistical analyses, conclusions, and inferences.

Fish health studies have been occurring for many years in the Susquehanna River Basin, including during my dissertation research on both YOY and adult smallmouth bass. Similar patterns in relationships are beginning to emerge including agriculture being positively associated with the presence of intersex in adults and myxozoan parasites in YOY. It will be important to evaluate other fish health characteristics to investigate if there are similar or different relationships with land use and other environmental characteristics. The model framework used to evaluate parasite prevalence in YOY smallmouth bass may be extended to other relationships of interest (e.g., presence of other parasites, tissue abnormalities) given that the data collected are often structured similarly and require multiple levels of inference (e.g., spatial and temporal). As the research continues, it will be important to consider how pieces of the fish health puzzle fit together and could be synthesized to inform future research and conservation. To complement field studies, laboratory and mesocosm experiments may help elucidate relationships among chemical, biological, and environmental stressors. Although important questions remain to be investigated in the Susquehanna River Basin with regard to smallmouth bass health, the integrative research studies described in my dissertation and other similar studies have provided a solid foundation of tools, approaches, and diverse general knowledge on smallmouth bass health in the Susquehanna River Basin on which to build needed broad-scale integrative as well as focused studies for the near-term future.

Through linking fish health, fish ecology, and the environment, my dissertation research has identified connections among some of the integrative components needed and knowledge gaps that must be addressed for the larger benefits of conservation and management in the Susquehanna River Basin. Because smallmouth bass can be viewed as an important indicator of environmental health, the ecological and disease relationships can lead to management strategies to protect and improve smallmouth bass and human health. For example, management regulations may need to consider rivers and tributaries more holistically given the movement of smallmouth bass between the two. Continued monitoring of trends in abundance in the system will be needed to gauge factors that drive population differences in the system over time. As fish health endpoints are evaluated for a specific site or area in the basin, consideration should be given to both local and broad landscape factors and how they influence health across life stages (YOY and adults). Ultimately, because the Susquehanna River Basin has such a close tie to humans including drinking water resources and recreational use, protecting the health of smallmouth bass holds promise to benefit humans and the regional economy.

As mentioned at the beginning of my dissertation, the Susquehanna River Basin is impaired by a fish consumption advisory and for recreational use in a section of the river due to bacterial contamination. Such problems in the system impact more than smallmouth bass health. Future studies of chemical, biological and environmental stressors in the Susquehanna Basin that affect fish health should be coordinated with attention to stressors that could impact human health so that they can be identified, prioritized, and avoided through coordinated stewardship efforts. Addressing such

stressors should make it possible to document benefits to ecosystem, fish, and human health. Logical follow-up work should involve collaborations with stakeholders and citizens to ensure recovery of the Susquehanna River for the long-term.

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