Impacts of Nonnative and Invasive Fish Pathogens to North Carolina's Trout Resources and its Managers

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Abstract—Three parasitic species that affect salmonid populations were discovered recently in North Carolina: the parasitic copepods (aka "gill lice") *Salmincola edwardsii* (infections in Brook Trout *Salvelinus fontinalis*; 2014) and *Salmincola californiensis* (infections in Rainbow Trout *Oncorhynchus mykiss*; 2015) as well as the causative agent of whirling disease, *Myxobolus cerebralis* (infections in Rainbow Trout, Brown Trout *Salmo trutta*, and Brook Trout; 2015 and 2016). None of these pathogens previously had been diagnosed taxonomically from North Carolina or from the southeastern United States, but all can exert deleterious population-level effects on salmonids elsewhere in North America and abroad. As such, these pathogens fall within a geographic area where potential biological threats to coldwater resources (including the State's only native salmonid: Brook Trout) are indeterminate. Such knowledge gaps make it difficult to achieve informed decisions on behalf of resource managers. To address this issue, personnel of the North Carolina Wildlife Resources Commission (NCWRC), in collaboration with Auburn University's Southeastern Cooperative Fish Parasite and Disease Laboratory, have obtained and disseminated pathogen-specific information in a step-wise fashion to (1) inform management decisions within the state, (2) disseminate scientific research data to adjacent resource managers likewise concerned with these salmonid pathogens, (3) hasten inter- and intrastate biosecurity measures, and (4) engage in public outreach that targets anglers and other citizens. Although much remains to be investigated, progress has been made as a result of focused studies on disease diagnostics, parasite identification, pathological effects, pathogen temporal and spatial distribution, parasite life cycles, and parasite-host relationships. Research efforts will continue to expand in order to inform management decisions and improve knowledge on the health of North Carolina's salmonids.

Introduction

As with many of the coldwater resources across the United States, trout are a significant biological, cultural, and economic entity for North Carolina. As the State's only native salmonid, the Brook Trout *Salvelinus fontinalis* is a species of conservation importance (NCWRC 2013; 2015) and one that has served as a local fishery for generations of anglers. In addition to self-sustaining populations of Brook Trout, the NCWRC also manages stocked Brook Trout and self-sustaining and stocked Brown Trout *Salmo trutta* and Rainbow Trout *Oncorhynchus mykiss* fisheries through its Public Mountain Trout Waters program. These diverse resources are popular with anglers; approximately 76% of anglers expressed satisfaction with their trout fishing in North Carolina (Responsive Management 2015a). Furthermore, nearly 149,000 anglers fished in Public Mountain Trout Waters during 2014 and had a total effect of approximately $383 million to the State's economy (Responsive Management 2015b). Trout also serve as an important agricultural commodity for North Carolina. At approximately $8.5 million, North Carolina was second to Idaho in total value of trout sold by state in 2016 (USDA 2017).

Unfortunately, there are threats to these important resources that could impact their well-being immediately and into the future. Not limited to North Carolina, fisheries managers in the United States and abroad are dealing with a growing set of challenges associated with introduced species (Li and Moyle 1999; Smith et al. 2008; Thomas et al. 2009; ANSTF 2012; Chapman et al. 2016; Gallardo et al. 2016; Hulme 2017). These species can have varied, but often significant, effects on resident aquatic communities, and when these interactions are proven disruptive, these introduced species are often labeled as nuisances.

Aquatic nuisance species (ANSs) are diverse and include representatives from many animal and plant phyla, including free-living and parasitic species. Free-living ANSs can disrupt food webs and alter substrata within an ecological landscape. For example, the macroalga *Cladophora glomerata* caused nuisance algal blooms in Lake Ontario (Higgins et al. 2008; Kuczynski et al. 2016), and blooms of *Didymosphenia geminata* (an ANS recently discovered in North Carolina; Bowman et al. 2016) have been reported globally (Spaulding and Elwell 2007). While algae like these can alter aquatic ecosystems and inconvenience anglers, exotic Silver Carp *Hypophthalmicthys molitrix* continue to expand their range in North America and can even pose physical risks to boaters (Vetter et al. 2017). Manifestations of ANSs resulting in algal mats or leaping fish resonate with resource users because they interfere with recreation.

Perhaps less obvious to resources users but potentially more impactful to the resource itself, parasitic ANSs can cause infectious diseases among endemic fishes and invertebrates. While free-living ANSs are worrisome because they can have deleterious effects on the behavior and ecology of endemic free-living species, parasitic ANSs are worrisome because they can outright kill endemic free-living species or cause them to be more susceptible to endemic opportunistic pathogens that, under normal conditions, may be benign. In addition, once invasive, the parasitic ANS may persist irrevocably in a particular aquatic ecosystem. Further challenging is that the introduction event is seldom detected immediately nor even years later such that population-level changes are observed before the manager is aware that an invasive pathogen is even present. In this way, ANSs that are parasitic and cause disease can be insidious in aquatic ecosystems.

Recent Discoveries

Three parasite species known to exert deleterious population-level effects on salmonid populations were newly discovered in North Carolina. The parasitic copepods (aka "gill lice") *Salmincola edwardsii* (infections in Brook Trout) and *Salmincola californiensis* (infections in Rainbow Trout) were identified in 2014 and 2015, respectively (Ruiz et al. 2017). In 2015 and 2016, the causative agent of whirling disease, *Myxobolus cerebralis* (*Mc*; infections in Rainbow Trout, Brown Trout, and Brook Trout) was confirmed (Ruiz et al., in review). These observations represent the first time each species has been documented taxonomically in North Carolina and their most southeastern distribution in North America.

Gill lice.—Elsewhere within the United States, *S. edwardsii* and *S. californiensis* are known to infect salmonids of the genera of *Salvelinus* and *Oncorhynchus*, respectively. Taxonomic and molecular analyses of copepods confirmed the identity of both species in the State (Ruiz et al. 2017). To date, *S. californiensis* has been documented on Rainbow Trout only; however, this copepod also has the potential to affect the State's only Kokanee Salmon *O. nerka* population within Nantahala Reservoir. Unfortunately, little is known about the ecology of copepods of the genus *Salmincola* and their impacts to salmonids in the wild (Black et al. 1983; Amundsen et al. 1997; Hargis et al. 2014).

Given the uncertainty associated with the copepods' impacts, NCWRC biologists are concerned about what effect these copepods could have on wild trout populations (especially, native Brook Trout populations). As the State's only native salmonid, Brook Trout are an important ecological and cultural resource: their distribution and populations are reduced from historic levels, and any additional stressor is of concern. Heavy infections of gill lice damage the gill (Ruiz et al. 2017) and can impair fish performance (Conley and Curtis 1993; Amundsen et al. 1997; Alteen 2009; Hargis et al. 2014; Mitro et al. 2014), but low-level infections may be benign (Amundsen et al. 1997). While possible that populations suffering from other stressors (drought, elevated water temperatures, nonnative species) and heavy gill lice infections could experience mortality (Mitro et al. 2014), empirical evidence of these hypothetical interactions is lacking because it is exceedingly difficult to collect and analyze in space and time.

Many additional questions remain. For example, the bulk of the State's Brook Trout populations are at low density and isolated above 914 m, so what are the characteristics (e.g., rate and mechanisms) associated with the spread of gill lice? Moreover, infection intensity (= the number of gill lice on an individual trout) is proportional to fish body size (Black 1982; Amundsen et al. 1997), so how is this impacting the larger, sexually mature individuals and ultimately, the demography of a population? The examples noted above are a small portion of the questions that could be examined to allow the NCWRC to understand the short- and long-term impacts of gill lice to salmonids in the state and abroad.

Although gill lice have been documented in selected waters, the full spatial extent of their distribution within North Carolina is unknown. Anglers have been asked to report observations of gill lice during recreational outings, and the NCWRC will continue to examine trout populations in conjunction with ongoing efforts to document the distribution and status of Brook Trout, Rainbow Trout, Brown Trout, and Kokanee Salmon populations in North Carolina.

Whirling disease.—Symptoms of whirling disease are well documented and include "whirling" behavior, spinal cord injury, and brain stem compression (infected fishes cannot achieve equilibrium and swim erratically until exhausted or dead; Elwell et al. 2009). Because the causative agent of whirling disease, *Mc*, infects cartilage, young fish are especially vulnerable. Its life cycle requires an oligochaete (reportedly *Tubifex tubifex*) that is common in sediments of coldwater streams throughout the southeastern United States. As such, infected oligochaetes and the translocation of infected trout are primary risk factors in spreading the pathogen and subsequent disease. To date, *Mc* has been documented in four major river basins in the State (Catawba, French Broad, Watauga, and Yadkin rivers; Ruiz et al., in review).

On July 27, 2015, it was confirmed that Rainbow Trout with clinical signs of whirling disease in the Watauga River, North Carolina, were infected with *Mc* (Ruiz et al., in review). Later that year, additional testing confirmed symptomatic, infected trout in the Elk River, North Carolina. Rainbow Trout from additional sites on the Watauga River and a connected private trout farm were also infected. The NCWRC collected oligochaetes (49 total sediment/mud samples collected in Sept 2015; each with 0–53 oligochaetes; a total of 485 individual oligochaetes subjected to nested PCR) from its Delayed Harvest Trout Waters to screen for the presence of *Mc*. Oligochaetes from Mill Creek and Watauga River were infected. In addition, infected oligochaetes were collected from above the NCWRC's Marion State Fish Hatchery, and subsequently, this facility has been renovated to incorporate biosecurity measures (UV filters and drum screens) to treat surface waters. In August 2015 (and annually thereafter), trout also were tested from all NCWRC's trout production facilities; all were negative for infection. Further results and conclusions from these collections are in preparation to be published in peer-reviewed aquatic animal health and parasitology journals.

It was imperative that the NCWRC understand the scale and scope of *Mc* within the State to inform management decisions. A critical short-term, rapid-response research program was initiated in May 2016 to quickly identify infected trout species and *Mc*-positive streams in North Carolina. This project captured "a snapshot in time" of the immediate status of the exotic, invasive pathogen in North Carolina waters. Using AFS Blue Book protocol (MacConnell and Bartholomew 2014), including the specific confirmatory test (nested PCR) for *Mc*, and including approximately 1,500 trout of 3 species from 36 localities in North Carolina, it was discovered that trout from three major river basins were infected with *Mc:* Brook Trout and Brown Trout from Laurel Creek (Yadkin River Basin), Brown Trout from South Toe Creek (French Broad River Basin), Rainbow Trout from Roaring Creek (French Broad River Basin), and Brown Trout from the Boone Fork (Watauga River Basin). Because of budget and logistical limitations to this study, a relatively small sample size of trout representing each species and each locality were collected and analyzed. However, noteworthy is that the pathogen was still detected, suggesting that the prevalence and intensity of infection by the parasite may be underestimated. In addition, this study comprised a single point in time (no locality was sampled more than once) and served to quickly assess the potential invasive geographic distribution of the pathogen only. Although we know more than we did 2 years ago, the NCWRC will continue to work with Auburn University's Southeastern Cooperative Fish Parasite and Disease Laboratory researchers to explore the distribution and life history characteristics of *Mc* in North Carolina.

Looking Back

The NCWRC employed a step-wise approach to obtain and disseminate pathogen-specific information. Given the uniqueness of *Mc* and *Salmincola* to North Carolina and the southeastern United States, there was a lack of information to inform management decisions. Initial efforts focused on documenting the geographic distribution and level of host specificity of gill lice infections across all sympatric trout populations (Ruiz et al. 2017). Having this baseline allowed us to seek information incrementally. For example, Delayed Harvest Trout Waters were sampled in 2015 for *Mc* in advance of these waters receiving stocked fish and a popular catch-and-release period. This information helped to direct outreach efforts (e.g., on-stream signage). The NCWRC then focused on self-sustaining trout populations the following spring to diversify resources examined and increase spatial coverage. Each effort provided important information and informed the next, and future research is planned for both *Mc* and gill lice that will continue to increase our understanding of these ANSs.

While gathering and planning to collect additional information, the NCWRC communicated effectively with management partners. The issues associated with fish pathogens as well as non-infectious ANSs are not confined to administrative boundaries. This is especially clear in North Carolina, where there are numerous waters and watershed boundaries that are shared between states, municipalities, the Great Smoky Mountains National Park, Eastern Band of Cherokee Indian Reservation, and private aquaculture facilities. With this in mind, the NCWRC kept all aware of the progress with managing these newly-discovered coldwater pathogens.

In the case of both *Mc* and gill lice, the NCWRC has utilized angler reports to identify infected trout populations. As such, increasing public awareness has been an important part of the NCWRC's response to these and other ANSs. Press releases and social media posts conveyed relative findings, while a webpage devoted to whirling disease compiled media and provided additional information. On-stream signage was also placed along waterways where *Mc* was documented. Furthermore, the NCWRC has developed an ANS message (Clean, Drain, Dry, and Never Move) that staff can utilize to promote minimal efforts to prevent the spread of ANS.

The NCWRC has also been working to prevent the spread of ANSs via its review of stocking permit applications. In North Carolina, individuals must obtain a permit from the NCWRC to stock any fish in public waters. During the review process, it must be confirmed that trout slated in stockings are free of infections by *Mc* and gill lice (*Salmincola* spp.). To make this determination, the NCWRC has visited suppliers noted by applicants to collect trout for analyses since 2015. Results are communicated with applicants, private facility contacts, and the North Carolina Department of Agriculture (the State agency with oversight authority over private aquaculture facilities), and if lots are verified to be free of *Mc* and gill lice, the supplier is approved for use.

Although warranted, these efforts are costly. In late August 2015, the NCWRC developed a cost center to better track time and mileage expenditures associated with aquatic nuisance species efforts (primarily activities associated with addressing whirling disease). From 28 August 2015 to 13 July 2017, staff coded 1,247 h and 10,595 mi to that cost center. As one can tell, there was significant amount of effort expended within that approximately 2-year period; much of which was unplanned and all expended at the cost of other activities. Progress has been made as a result of focused studies on parasite species diagnosis, taxonomy and systematics, pathological effects on fishes, pathogen temporal and spatial distribution, and parasite-host relationships. Much remains to be investigated, but research efforts will continue to expand in order to inform management decisions and improve knowledge on the health of North Carolina's salmonids.

Managing in a Parasitic World

Fisheries management is often the manifestation of professional reactions to changing circumstances. Nowhere is this clearer than in the context of adjusting to an exotic ANS while also working to meet biological and socioeconomic goals for fisheries. Can proactive fish health (biosecurity) measures help managers make the best decisions regarding ANSs to reduce impacts to resources and those that manage them? The NCWRC has worked to address newly-discovered fish pathogens and reduce associated biological-knowledge gaps since 2015, and during that time, measures to facilitate such activities were utilized. Yet, there are likely additional opportunities to assist fishery managers in North Carolina and elsewhere. As such, managers should consider the following three focal areas relative to salmonid pathogens within management regimes: research; aquatic animal health planning; and information exchange.

Research.—As noted previously, the NCWRC's stepwise approach has narrowed data gaps regarding *Mc* and gill lice; however, more research is needed. Of paramount importance moving forward are (1) determining the spatial and temporal (seasonal) distributions of these pathogens in North Carolina trout waters and among its various trout species; and (2) elucidation of the fine details of the life cycles, incidence of disease, and pathological effects of these ANSs on trout. Although much has been published on these various attributes elsewhere, much more evidence is needed for southeastern trout and ecosystems.

Salmincola and *Mc* exemplify salmonid pathogens and are species of focus by the NCWRC, but the need to understand current and potential organisms is consistent across the field of ANS. As pathogens and other ANSs expand their ranges ultimate ramifications to resources are unknown. However, familiarity of ANS identification, ecology, and pathology can provide insight into potential clinical signs of infection, transfer vectors, spatial distribution, information needs, and overall risks of unwanted species; all of which can serve as "warning signs" and foundations to build upon to further knowledge.

Aquatic animal health plans.—All 50 state natural resource agencies make online references to ANSs. While such awareness is encouraging, the amount of species-specific information presented (e.g., handout to formal ANS plan) varied among agencies. Regional differences exist regarding comprehensive, multi-agency aquatic animal health plans. For example, the Northeast Fish Health Committee (NFHC) developed fish health guidelines for member agencies to assist with the importation and transfer of fish, communication, and development of management strategies (NFHC 2015). Although compliance is non-mandatory, the NFHC's guidelines provide wide-ranging objectives and action items that can be implemented and evaluated. Southeastern fisheries managers face the same challenges of shared waters and fish transfers (including those in the private sector) as their northeastern counter parts but lack a codified fish health plan.

In addition, most fish health guidelines and policies focus on captive, cultured stocks in ponds and raceways. While these are important, there is also the need to develop protocols and best management practices that cater to health monitoring of wild fish populations. If done in conjunction, evaluation of both wild and captive aquatic animals provides a comprehensive approach – something of great importance given each stock's ability to influence the other.

The topic of health planning brings to mind the old adage regarding an ounce of prevention being better than a dose of the cure. Taking steps to prevent introductions and proliferations of ANSs will result in infrastructure demands (temporal and financial), but those measures of prevention must be weighed against the biological, social, and economic costs of the long-term outcomes of an introduced fish pathogen. Unfortunately, if we revisit the adage, there are no "doses" for the majority of these species once introduced into the wild. Introductions are seldom detected immediately and may be insidious or catastrophic, depending on the interaction among the pathogen, host, and environment (Combes 2001).

Information exchange.—Increased understanding of pathogens and biosecurity measures are important but efforts will not be successful without effective exchange of information regarding all facets of ANSs. The NCWRC will continue to work with anglers (e.g., angler reports and production of educational materials) as their awareness is important to address ANS (Anderson et al. 2014). Furthermore, continued communication with intra- and interstate managers and agencies is critical to ensure the health of wild and captive trout stocks. Salmonid pathogens like *Salmincola* and *Mc* are no longer potential invaders of North Carolina's trout resources; they have invaded. Obtaining and sharing accurate information (e.g., taxonomic identification, pathological effects, and distributions) regarding these pathogens will be critical to help prevent their spread and to reduce the risk of other ANS.

Conclusion.—In the face of limited time and budgets, it is imperative that fisheries managers focus on strategic measures to achieve management objectives. Unfortunately, unforeseen events arise that challenge the best crafted plans. In the case of fish pathogens, challenges can influence salmonid stocks significantly. Although it may not be possible to be completely prepared, an understanding of potential threats should help improve salmonid health by (1) informing management decisions, (2) disseminating scientific research findings to adjacent resource managers who are likewise concerned with these salmonid pathogens, (3) hastening inter- and intrastate biosecurity measures, and (4) engaging in public outreach that targets anglers and other conservation-minded citizens.

Acknowledgements

We are grateful to all NCWRC and Auburn University personnel that assisted with collection and examination of specimens. We also wish to thank D. Besler and D. Deaton for their reviews of draft copies of this manuscript.

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