Pathogens Associated with Trout Populations in Shenandoah National Park and the Relationships to Fish Stocking Practices

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Abstract: Restrictive fish stocking policies in National Parks were developed as early as 1936 in order to preserve native fish assemblages and historic genetic diversity. Despite recent efforts to understand the effects of non-native or exotic fish introductions, park managers have limited information regarding the effects of these introductions on native fish communities. Shenandoah National Park was established in 1936 and brook trout (*Salvelinus fontinalis*) restoration within selected streams in the park began in 1937 in collaboration with the Virginia Department of Game and Inland Fisheries (VDGIF). An analysis of tissue samples from brook, brown (*Salmo trutta*), and rainbow trout (*Oncorhynchus mykiss*) from 29 streams within the park from 1998–2002 revealed the presence of *Renibacterium salmoninarum*, *Yersinia ruckeri*, and infectious pancreatic necrosis virus (IPNv). In order to investigate the relationships of the occurrence of fish pathogens with stocking histories we classified the streams into three categories: 1) streams with no record of stocking, 2) streams that are known to have been stocked historically, and 3) streams that were historically stocked within the park and continue to be stocked downstream of the park boundary. The occurrences of pathogens were summarized relative to this stocking history. *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease, was the most prevalent pathogen found, occurring in all three species and stream stocking categories, and appears to be endemic to the park. Two other pathogens, *Yersinia ruckeri* and infectious pancreatic necrosis virus were also described from brook trout populations within the park. IPNv was only found in brook trout populations in streams with prior stocking histories. *Yersinia ruckeri* was only found in brook trout in steams that have never been stocked and like *R. salmoninarum*, is likely endemic.

Key words: pathogens, trout, stocking

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Threats to native fish populations from stocking non-native species and their associated disease concerns are important natural resource management issues in the U.S. National Parks. Restrictive fish stocking policies in National Parks were developed as early as 1936 in order to preserve native fish assemblages and genetic diversity (Wright 1992). These policies were based largely on the assumptions that fish stocking practices could affect native biological communities and populations. Despite efforts to understand the potential effects of fish introductions, National Park Service biologists and managers have little insight into either the effects of these historic, and in some cases, continued introductions on native fish communities (Panek 1997).

The use of hatchery-reared fish is a common and well accepted fishery management tool. There has been an increasing awareness over the past 30 years or more among fishery managers regarding the potential negative effects of fish stocking on both native fish populations and aquatic biodiversity (Schramm and Piper 1995). Managers have used fish stocking as a tool for the creation of new recreational fisheries, for augmenting existing introduced or native populations, for biological control purposes, or to restore native species including threatened and endangered fishes. Introductions or transfers of fishes have both desirable and undesirable consequences (Coates 1998) and there have been several important symposia resulting in proposed codes of practice for fish stocking (Schramm and Piper 1995, Cowx 1998, Nickum et al. 2004).

Previous studies have shown that infectious diseases of fish and wildlife can be a concern for the conservation or restoration of native species (Lafferty and Gerber 2002, Moffitt et al. 2004). For wildlife in general, there is sufficient evidence to indicate that pathogens can be important factors in population dynamics particularly as they influence mortality rates (Anderson and May 1979). In fish populations the evidence is not as clear largely because dead or moribund fish in open waters go unnoticed, except during epizootics. Moribund fish tend to be quickly removed by scavengers and predators or may not be observed due to the nature of their environment (McCallum and Dobson 1995). Chronic

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disease in fish may be difficult to assess at the population level because of the compensatory nature of fish mortality and population dynamics (Rose et al. 2001).

Shenandoah National Park was established in 1936 and brook trout management within selected streams began in 1937 in collaboration with the Virginia Department of Game and Inland Fisheries (VDGIF). Despite the emphasis on brook trout restoration, exotic brown and rainbow trout were often stocked in some park streams to diversify fishing opportunities or were planted in contiguous stream sections immediately downstream. The only known records associated with park streams stocked during the 1930s were in the narrative accounts in Lambert (1979). Survey reports later completed by the U.S. Fish and Wildlife Service indicate that most accessible streams were annually stocked by the Commonwealth of Virginia with hatchery-reared fingerlings and catchable size trout from 1936 through 1949 (Lennon and Parker 1958). This practice was continued by the U.S. Fish and Wildlife Service through 1955 after which all stocking within the park was terminated. Stocking records for waters outside of the park boundary were provided by the Virginia Department of Game and Inland Fisheries from 1962 through 2004. These data provided the basis for assigning the streams to various sampling categories.

In this study we investigated the occurrence and prevalence of selected fish pathogens in wild trout populations in Shenandoah National Park. Specifically, we examined the occurrence of a suite of pathogens of national and regional significance collected as part of the U.S. Fish and Wildlife Service's (USFWS) National Wild Fish Health Survey (NWFHS). We correlated these findings with known stream stocking histories to assess the potential linkages of past stocking practices and the occurrence of pathogens.

Table 1. Streams sampled from 1998–2002 for the National Wild Fish Health Survey within Shenandoah National Park (VA) (◆ indicates year sampled).

Stream	1998	1999	2000	2001	2002
Fultz Run	–	–	◆	–	–
Meadow Run	–	–	–	–	◆
North Fork Dry Run	–	–	–	◆	–
One Mile Run	–	–	◆	–	–
Overall Run	◆	–	◆	–	–
Paine Run	◆	–	–	◆	–
Two Mile Run	–	–	◆	–	–
Berry Hollow Run	–	◆	–	–	–
Big Run	–	◆	–	–	–
Broad Hollow Run	–	–	◆	–	–
Cedar Run	–	◆	–	–	–
Doyles River	–	–	◆	–	–
Hannah Run	◆	–	◆	–	–
Hogcamp Brook	◆	–	◆	◆	–
Jeremy's Run	◆	–	◆	◆	–
Madison River	–	–	–	◆	–
N. F. Morman's River	◆	◆	–	–	–
Rapidan River	–	–	◆	◆	–
Staunton River	–	–	◆	–	–
White Oak Canyon Run	–	◆	–	–	–
East Hawksbill Creek	–	–	–	–	◆
Hawksbill Creek	–	–	–	–	◆
Hughes River	◆	–	◆	–	–
Ivy Creek	–	–	◆	–	–
N. F. Thornton River	–	–	◆	–	–
Pass Run	◆	–	–	◆	–
Piney River	–	◆	–	–	–
Rose River	–	–	◆	◆	–
Lower Hawksbill Creek	–	–	–	–	◆

Methods

Fish were collected during summers 1998 through 2002 by joint sampling crews from the U.S. Fish and Wildlife Service and the U.S. National Park Service from three categories of streams supporting wild trout populations: streams with no known stocking history within the park, streams historically stocked within the park, and streams within the park that were historically stocked and are presently stocked downstream of the park boundary (Table 1). Detailed maps of each stream and sampling location can be found in Atkinson (2004). All fish were collected with Smith-Root DC backpack electrofishing equipment according to survey techniques described in the park's fisheries monitoring protocol (Atkinson 2004). Each fish was euthanized by an exposure to a lethal dose of clove oil/ethanol (1:10). After recording the length and weight of each fish, tissue samples were aseptically removed, placed on ice and transported to the laboratory for analysis within 24 h of collection. Tissues harvested including kidney, spleen and cranial cartilage were sampled according to protocols outlined by the NWFHS (Puzach 2006). In streams with low population densities of brook trout, fish collections were often limited to no more than five fish per stream or stream section. Generally, more than 15 fish of each species were collected at each site to provide a minimum of three pooled samples of five fish each for diagnostic analyses.

Targeted pathogens for diagnostic analyses in the NWFHS (Puzach 2006) included: infectious pancreatic necrosis virus (IPNV), infectious hematopoietic necrosis virus (IHNV), viral hemorrhagic septicemia virus (VHSV), *Oncorhynchus masou* virus (OMV), infectious salmon anemia virus (ISAV), *Aeromonas salmonicida* (causative agent of furunculosis), *Yersinia ruckeri* (causative agent of enteric redmouth disease), *Renibacterium salmoninarum* (causative agent of bacterial kidney disease), *Edwardsiella ictaluri* (causative agent of enteric septicemia), and *Myxobolus cerebralis* (causative agent of whirling disease). Procedures for virology and bacteriology followed the techniques outlined in the Fish Health Section Blue Book (AFS 2004) and additional protocols as outlined in (Puzach 2006). The Direct Fluorescent Antibody Technique (DFAT) was the serological method used for confirmatory testing for *A. salmonicida*, *Y. ruckeri*, and *E. ictalui* whereas PCR was used to confirm *R. salmoninarum, M. cerebralis*, and the suite of viral pathogens (Puzach 2006).

Results

Brook, brown, and rainbow trout were collected (*n* =1,401) for fish health analyses from 29 steams in Shenandoah National Park from 1998–2002 (Table 1). Native brook trout was the most commonly collected species (*n* = 1,169), and was collected from all streams that were sampled. Brown trout and rainbow trout are introduced species in the park and were not common; however, they are commonly stocked downstream of the park. Rainbow trout (*n* = 121) were collected in Pass Run and the North Fork Morman's River, whereas brown trout (*n* = 111) were collected from three streams including the N.F. Morman's River, Hughes River, and the Rose River.

Fish samples were assigned to the appropriate stream stocking categories based on stocking records (Lennon and Parker 1958), and results of fish health diagnostics were summarized for fish in streams that have never been stocked, formerly stocked within the park and those streams presently stocked downstream of the park (Table 1). Analyses of the 274 composite samples did not demonstrate the presence of several pathogens routinely sampled for in the National Wild Fish Health Survey including infectious hematopoietic necrosis virus (IHNV), viral hemorrhagic septicemia virus (VHSV), *Oncorhynchus masou* virus (OMV), *Aeromonas salmonicida, Edwardsiella ictaluri*, and *Myxobolus cerebralis*. The most commonly detected pathogen was *Renibacterium salmoninarum* which was present in all three species of trout from most of the streams sampled (Table 2). These data suggest that *R. salmoninarum* is endemic to park streams because it was detected in trout from streams in all three stocking categories. The pathogen tested positive by ELISA in 7.0%–13.5% of the brook trout lots tested, 60.9% of the brown trout lots and in 88.0%–100% of the rainbow trout lots.

A Kruskal-Wallis test for differences in the infection rates of *R. salmoninarum* on trout among the three categories of streams within the park (SYSTAT II 2004)[1] resulted in a rejection of the hypothesis of no difference among stocking categories (K = 61.85, $P < 0.05$, d.f. = 2). Infection rates were highest in presently-stocked brown trout streams (50%) and in brook trout streams that are presently stocked downstream (28.6%). Paine Run, Hannah Run, Hogcamp Brook, Rapidan River, Hughes River, and the Rose River all supported trout populations that tested positive for the pathogen.

Infectious pancreatic necrosis virus was detected only in brook trout populations and in only those populations that were former-

Table 2. Streams and sample lots testing positive for the bacterial pathogen *Renibacterium salmoninarum* for three trout species from streams within Shenandoah National Park managed for public fishing under historically different trout stocking policies (NP-not present)

Stream stocking category	*n* Streams sampled	% Streams positive for pathogen	*n* Fish sampled	*n* Diagnostic lots processed	% Diagnostic lots positive by ELISA	Confirmed positive by PCR
Salvelinus fontinalis						
Never stocked	7	14.3	233	43	7.0	Yes
Formerly stocked	13	23.1	495	96	13.5	Yes
Presently stocked downstream	7	28.6	441	83	9.6	Yes
Salmo trutta						
Never stocked	NP	NP	NP	NP	NP	NP
Formerly stocked	1	0.0	7	3	0.0	No
Presently stocked downstream	2	50.0	104	23	60.9	Yes
Onchorhynchus mykiss						
Never stocked	NP	NP	NP	NP	NP	NP
Formerly stocked	1	100.0	4	1	100.0	Yes
Presently stocked downstream	1	100.0	117	25	88.0	Yes

Table 3. Streams and sample lots testing positive for Infectious Pancreatic Necrosis virus (IPNv) for three trout species from streams within Shenandoah National Park managed for public fishing under historically different trout stocking policies (NP–not present)

Stream stocking category	*n* Streams sampled	% Streams positive for pathogen	*n* Fish sampled	*n* Diagnostic lots processed	% Diagnostic lots positive by EPC/CH	Confirmed positive by PCR
Salvelinus fontinalis						
Never stocked	7	0.0	233	44	0.0	NA
Formerly stocked	13	15.8	480	98	27.6	Yes
Presently stocked downstream	7	14.3	441	87	3.4	Yes
Salmo trutta						
Never stocked	NP	NP	NP	NP	NP	NP
Formerly stocked	1	0.0	7	3	0	No
Presently stocked downstream	1	0.0	104	23	0	No
Onchorhynchus mykiss						
Never stocked	NP	NP	NP	NP	NP	NP
Formerly stocked	0	0	0	0	0	No
Presently stocked downstream	1	0	115	23	0	No

1. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Table 4. Streams and sample lots testing positive for *Yersinia ruckeri* for three species of trout from streams within Shenandoah National Park managed for public fishing under historically different trout stocking policies (NP – not present)

Stream stocking category	*n* Streams sampled	*n* Streams positive for pathogen	*n* Fish sampled	*n* Diagnostic lots processed	% Diagnostic lots positive by BHIA	Confirmed positive by PCR
Salvelinus fontinalis						
Never stocked	7	28.6	233	233	<0.5%	Yes
Formerly stocked	13	0	495	472	0	No
Presently stocked downstream	7	0	426	403	0	No
Salmo trutta						
Never stocked	NP	NP	NP	NP	NP	No
Formerly stocked	1	0	7	7	0	No
Presently stocked downstream	2	0	104	103	0	No
Onchorhynchus mykiss						
Never stocked	NP	NP	NP	NP	NP	No
Formerly stocked	0	0	0	0	0	No
Presently stocked downstream	1	0	115	85	0	No

ly stocked within the park or presently stocked downstream of the park. We did not detect IPNv in any of the 111 brown trout or 115 rainbow trout tested (Table 3). Brook trout populations with IPNv were documented from Jeremy's Run, North Fork Morman's River, and in the Rose River. *Yersinia ruckeri* was detected at low prevalence (<0.5%) only in brook trout populations in Paine Run and Two-Mile Run (Table 4). Both of these streams have never been stocked and the pathogen is likely endemic to those systems. We did not detect *Y. ruckeri* in any brown or rainbow trout populations and in no other categories of stocked streams. Sample sizes for IPNv and *Y. ruckeri* were too small for any statistical testing.

Discussion

The stocking of fishes for recreational fisheries development and maintenance, for mitigating the effects of anthropogenic changes to watersheds, for restoration of depleted native stocks, or for the recovery of endangered species has been an important management tool for resource managers for more than 100 years. There has been an on-going debate over the last few decades among fisheries professionals regarding the effectiveness and appropriateness of the use of cultured fish or fish transfers in fisheries management (Radonski and Loftus 1995) which has led to policies on the use of propagated fish in fisheries management (Mudrak and Carmichael 2005). This certainly has been true in the U.S. National Park System where restrictive stocking policies were first developed in 1936 (Wright 1992) and later formalized in management policy (Panek 1997). Concerns over the use of cultured fishes in U.S. National Park waters include impacts to native species resulting from competition, predation, loss of genetic integrity due to introgression, and introduction of diseases into native fish communities.

Infectious diseases can be a concern in conservation biology (Lafferty and Gerber 2002) and in the management of native and introduced fishes (Nickum et al. 2004). Of particular concern for resource managers are the emerging diseases which often involve increased virulence factors or expanding geographic ranges of causative pathogens (Kiesecker et al. 2004). We know from recent reviews that movements of fishes can, in part, be responsible for spreading exotic or emerging pathogens (Moffitt et al. 2004). The spread of whirling disease resulting from the stocking of trout positive for the parasite *Myxobolus cerebralis* is an example of this type of management risk (Bartholomew and Reno 2002). Likewise, Amin (1979) documented a cause and effect relationship between the occurrence of lymphocystis in seven species of fishes in Wisconsin to the stocking of walleye (*Stizostedion vitreum*) for development of recreational fisheries. Genetic introgression of hatchery stocks with wild fish stocks is also a complicating factor known to reduce fitness of fishes, which leads to increased susceptibility to lethal diseases. Currens et al. (1997) found populations of wild rainbow trout with a history of hatchery fish introgression in the Metolius River to be more susceptible to *Ceratomyxa shasta*, an enzootic myxosporean parasite, than wild fish in an adjoining river. In this study we investigated the occurrence of selected fish pathogens in wild trout populations in Shenandoah National Park and compared these findings with assignments of streams to several categories based on stocking histories.

Based on our findings, pathogens and fish diseases are not a significant trout management issue in Shenandoah National Park. Of the ten pathogens of national and regional importance tested during the five years of this investigation (1998–2002) we only documented the occurrence of *Renibacterium salmoninarum*, *Yersinia ruckeri*, and infectious pancreatic necrosis virus (IPNv). Nevertheless, we do have evidence to suggest an increased occurrence and prevalence of pathogens in trout populations in streams that were either historically stocked within the park or are presently stocked to supplement recreational fisheries downstream of the park boundary. *Renibacterium salmoninarum* consistently occurred more often in trout populations within the park when the stream was stocked outside the park, although it appears to be endemic to the park as it was detected in all three categories of streams. Similarly, brook trout that carried IPNv were found in streams that were either historically stocked or are presently stocked downstream. In contrast, IPNv was not detected in any of the 233 fish tested from waters that have never been stocked nor in any of the stocked streams where rainbow and brown were sampled. However, because young-of-the-year brook trout and rainbow trout are highly susceptible to the virus and most likely to exhibit disease (Noga 2000), it was unlikely that we would have observed diseased fish in any of our late summer collections as these fish would have been already removed from the population. *Yersinia ruckeri* was only detected from brook trout populations in Paine Run and Two-Mile Run. These are streams that have never been stocked and we suspect that the pathogen is endemic to these two streams.

Based on our findings, park managers should carefully weigh the advantages and disadvantages of fish stocking for either recreational or restoration purposes within parks. The increased prevalence of pathogens in populations that were previously stocked or within streams that are stocked outside of the park is of a management concern. Any fish stockings should be accompanied by fish health inspections to ensure that hatchery fish or wild fish translocated for restoration purposes are pathogen free.

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