Analysis of Regional Scale Risk of Whirling Disease in Populations of Colorado and Rio Grande Cutthroat Trout Using a Bayesian Belief Network Model

Kimberley Kolb Ayre, Colleen A. Caldwell, Jonah Stinson, and Wayne G. Landis

Introduction and spread of the parasite *Myxobolus cerebralis*, the causative agent of whirling disease, has contributed to the collapse of wild trout populations throughout the intermountain west. Of concern is the risk the disease may have on conservation and recovery of native cutthroat trout. We employed a Bayesian belief network to assess probability of whirling disease in Colorado River and Rio Grande cutthroat trout (*Oncorhynchus clarkii pleuriticus* and *Oncorhynchus clarkii virginalis*, respectively) within their current ranges in the southwest United States. Available habitat (as defined by gradient and elevation) for intermediate oligochaete worm host, *Tubifex tubifex*, exerted the greatest influence on the likelihood of infection, yet prevalence of stream barriers also affected the risk outcome. Management areas that had the highest likelihood of infected Colorado River cutthroat trout were in the eastern portion of their range, although the probability of infection was highest for populations in the southern, San Juan subbasin. Rio Grande cutthroat trout had a relatively low likelihood of infection, with populations in the southernmost Pecos management area predicted to be at greatest risk. The Bayesian risk assessment model predicted the likelihood of whirling disease infection from its principal transmission vector, fish movement, and suggested that barriers may be effective in reducing risk of exposure to native trout populations. Data gaps, especially with regard to location of spawning, highlighted the importance in developing monitoring plans that support future risk assessments and adaptive management for subspecies of cutthroat trout.

KEY WORDS: Bayesian belief network; ecological risk; emerging diseases

1. INTRODUCTION

The introduction and epizootic spread of the aquatic parasite *Myxobolus cerebralis*, causative agent of whirling disease, has been implicated in the decline of wild trout populations throughout the intermountain west.\(^1,2\) Prevalence of the disease is the result of a series of complex events related to the spatial distributions and life histories of the parasite and its two obligate hosts (an aquatic oligochaete *Tubifex tubifex* and a salmonid fish).\(^3-6\) The parasite produces myxospores inside infected fish that are released into the sediment upon death of the fish. Myxospores are ingested by *T. tubifex* worms, and the second infective stage, the actinospore, develops in the gut epithelium of the worm, and is released into the water column as a triactinomyxon (TAM).\(^7\) Upon contact with young salmonid fish, the TAM attaches and the infectious agent is injected...
into cells of the epidermis, buccal cavity, or gills.\(^{(8,9)}\) From the injection site, the parasite moves along the nervous system feeding on cartilage, eventually resulting in skeletal deformities and tail-chasing swimming behavior, which is a classic symptom of diseased fish. The parasite replicates inside the infected fish, eventually producing myxospores that are released into the environment when the fish dies.

Whirling disease infection rates in wild fish depend on the occurrence and range in susceptibility of *T. tubifex* lineages,\(^{(10,11)}\) and varied susceptibility of salmonids.\(^{(12–16)}\) Whirling disease has been implicated in major declines of wild trout populations in Colorado,\(^{(17)}\) Montana,\(^{(18)}\) Utah,\(^{(19)}\) and in Yellowstone National Park, Wyoming.\(^{(20)}\) Not only can population declines in wild trout lead to changes in community composition favoring less susceptible species, but whirling disease may result in economic losses because of negative impacts on recreational fishing industry and aquaculture.\(^{(21)}\) Given the unpredictable nature of the disease in the wild, considerable interest has been focused on identifying “hot spots” for the disease because environmental variables affect the timing of exposure between hosts and two infective stages (the myxospore and the parasite). Although removal and elimination of the parasite is not practical, it may be possible to mitigate the risk of exposure for populations in areas where the disease is not yet established. This requires a decision support tool within a structured process to aid managers in estimating the likelihood of salmonid exposure *M. cerebralis* and spread of the parasite within a stream system following introduction.

A traditional risk assessment framework involves defining the scope of the problem in question, analyzing exposure and effects, and risk characterizing.\(^{(22)}\) Bartholomew *et al.*\(^{(23)}\) used the first two steps in the process to synthesize existing information on the factors that contribute to the introduction and establishment of the *M. cerebralis* parasite and the development of whirling disease in salmonids. However, qualitative risk analysis approaches like the ones they recommended are limited to circumstances when stressors are few, receptors are clearly identified, and effects are clearly defined. These simple models are not applicable to broad ecological landscapes or regions with diverse regional geographies.\(^{(24,25)}\) As summarized by Landis,\(^{(26)}\) ecological risk assessment has been used primarily to estimate risk because of contaminants, yet it need not be limited to such cases. The utility of ecological risk assessment has been successfully demonstrated for forestry management,\(^{(27,28)}\) and in this study, we show how an ecological risk assessment approach can be applied to evaluate the risk of disease transmission for populations of threatened and endangered species.

Our approach was to use a Bayesian belief network (BBN) to assess the probability of whirling disease infection across the distribution ranges of two native cutthroat trout taking into account factors that may affect prevalence of the parasite and exposure. Bayesian approaches are well suited to environmental risk assessments across large spatial scales because model predictions and expert judgment can be utilized when empirical or site-specific data are scarce.\(^{(29,30)}\) Uncertainty is intrinsically incorporated into the risk assessment because input parameters, relationships between parameters, and model outcomes are all expressed as probability distributions.\(^{(31)}\) Sensitivity analyses of model outcomes are straightforward and provide information on the factors that exert the strongest influence on the likelihood of risk for a given endpoint. Prior studies have used BBNs to predict emerging infectious diseases,\(^{(29,32,33)}\) identify causal pathways of invasive fish species,\(^{(34)}\) establish decision paths to restore aquatic ecosystems,\(^{(30)}\) and explore management alternatives for salmonid fisheries at the ecosystem level.\(^{(35,36)}\) When spatially explicit data are incorporated into a BBN, it can be used as a tool for identifying the likelihood of risk and the factors that influence risk for individual management areas within a landscape or region. A Bayesian risk assessment model can also be used to evaluate different management or environmental scenarios, and analyze the conditions needed for a desired outcome.

The primary goal of this research was to estimate the likelihood of *M. cerebralis* exposure, spread, and establishment throughout the current range of two southwest native trout (Colorado River cutthroat trout *Oncorhynchus clarkii pleuriticus* and Rio Grande cutthroat trout *Oncorhynchus clarkii virginalis*). We developed a spatially explicit BBN to assess the probability of whirling disease infection within watersheds containing native cutthroat trout to aid in the identification of critical factors that, if mitigated, might reduce risk of disease. Through discussions with experts and review of the relevant literature, we constructed a conceptual model that described the pathways that link exposure of the two hosts (*T. tubifex* and two native cutthroat subspecies) to the infective stages of the parasite (myxospore...
and TAM). The conceptual model became the basis for the BBN structure. Although dispersion by avian piscivores\(^{(37)}\) and felt soles of fishing waders\(^{(38)}\) can contribute to the spread of the disease between watersheds, our risk analysis focused on fish exposure to the parasite after its introduction into a river system.

The spatial extent for our assessment of whirling disease infection risk extended from southwestern Wyoming to northern Arizona and New Mexico (Fig. 1). The range of Colorado River cutthroat trout encompasses portions of Colorado, Utah, and Wyoming as well as northern Arizona and New Mexico.\(^{(39)}\) Colorado River cutthroat trout have been designated as a species of special concern by Colorado and Wyoming and a sensitive species in Utah. Although the U.S. Fish and Wildlife Service has not listed the subspecies, a systematic management plan was developed to assure the long-term viability throughout its historical range.\(^{(39,40)}\) Rio Grande cutthroat trout are the southern-most subspecies of cutthroat trout\(^{(41)}\) and endemic to three river basins in Colorado and New Mexico (Fig. 1). When Rio Grande cutthroat trout was added as a candidate for protection under the Endangered Species Act of 1973,\(^{(42)}\) whirling disease was listed as a potential threat to its persistence.

2. METHODS

2.1. Study Area

Resource managers for Colorado River and Rio Grande cutthroat trout had divided the geographic range of each subspecies into discrete conservation areas, referred to as geographic management units (GMUs). These GMUs were used as risk regions and the probability of whirling disease infection in populations of cutthroat trout was assessed for each one. Most of the risk regions (GMUs) corresponded to hydrologic subbasins, with eight regions for Colorado River cutthroat and four for Rio Grande (delineated in Fig. 1). For each risk region, we combined hydrological data with spatial analysis of barriers and the locations and results of disease testing in wild fishes.

Hydrological data for each risk region were obtained from the National Hydrography Dataset Plus (NHD Plus; available: http://www.horizonsystems.com/nhdplus/data.php), which incorporates data from the National Hydrography Dataset, National Elevation Dataset, National Land Cover Dataset, and the Watershed Boundary Dataset. We extracted data for stream length, flow, slope, temperature, elevation, and stream order from the NHD Plus data set (regions 13 and 14) for each risk region. Our analysis focused exclusively on first- or second-order streams because these represented principal habitat of the cutthroat trout subspecies.\(^{(39,43)}\)

2.2. Whirling Disease Testing

Exposure of wild trout to the parasite is a function of many factors, one of which is the proximity of populations to diseased fish. To assess the impact of this element on risk of whirling disease to wild Colorado River and Rio Grande cutthroat trout populations we used a database of whirling disease testing results. The Whirling Disease Initiative, a national research program established by the U.S. Congress in 1997, funded extensive research on whirling disease until 2009, and also served as a clearinghouse for data on the distribution of whirling disease in the United States.\(^{(44)}\) We obtained whirling disease testing data for locations within the distribution ranges for Colorado River cutthroat trout that had been originally collected by state and federal governments in Colorado, New Mexico, Utah, and Wyoming between 2003 and 2006. Whirling disease testing data were collected during the same time period by state and federal agencies in Arizona, Colorado, and New Mexico to survey the extent of the disease and its proximity to populations of Rio Grande cutthroat trout. At each site, the trout community composition was assessed, and fish collected for determination of \(M.\) cerebralis presence or absence. Fish that were less than one year old were sacrificed, and tested for \(M.\) cerebralis genomic DNA using polymerase chain reaction analysis.\(^{(45)}\) The heads from the same fish were macerated, subjected to a pepsin–trypsin digest method, and screened for the presence of myxospores using light microscopy.\(^{(46)}\) No additional large-scale monitoring of whirling disease has been reported since 2006 for populations of Colorado River and Rio Grande cutthroat trout; therefore, the whirling disease testing data used in this assessment represent the current state of knowledge for whirling disease distribution within the study area.

2.3. BBN Model

We constructed a BBN using Netica (Norsys Software Corp., Vancouver, BC, Canada; http://www.norsys.com/netica.html) to evaluate the
impacts of whirling disease exposure pathways such as availability of habitat conducive to TAM production, presence of barriers, diseased fish, spatial overlap with whirling disease sensitive native trout, and availability of spawning areas on the likelihood of whirling disease infection. A conceptual model that described the pathways that link exposure of the two hosts to the infective stages of the parasite, developed in consultation with whirling disease and cutthroat trout experts, was the
basis of the BBN structure (Fig. 2). Input variables that represented potential sources of parasite exposure were represented as the top tier of boxes, referred to as parent nodes. Each of these parameter nodes was quantitatively defined by four discrete states (zero, low, medium, and high) based on established relationships between the parameter and disease exposure. These parameters were described by unconditional, marginal, prior probability distributions. In contrast, child nodes are graphically represented as boxes with arrows leading into them, and were quantified by conditional probabilities.

The BBN had two tiers of child nodes (Fig. 2), aggregate nodes and the terminal node (risk analysis endpoint), each of which had an associated conditional probability table (CPT) containing the conditional probabilities for each combination of parent node states (example shown in Table I). Each child node also had four discrete states (zero, low, medium, and high) that represented the likelihood of impacts of the parent nodes on a relative scale. The probability distribution for the final node, risk of whirling disease infection, expressed the likelihood of each relative risk state for trout populations within a given management area. The number of parent nodes and the number of discrete states for each node determine size of the CPT. Aggregate nodes are used in BBNs to split the model into smaller components, which constrains the size of the associated CPTs by combining the impacts of related exposure parameters.\(^\text{(47,48)}\)

CPTs can be informed by field monitoring data, models, equations derived from peer-reviewed studies, or information elicited from experts or stakeholders.\(^\text{(48)}\) For this study, we conducted an extensive survey of the functional relationships established from peer-reviewed scientific literature, spatial analysis monitoring data, and the input from experts who reviewed preliminary versions of the model to inform the development of CPTs (see Supporting Information). For each combination of parent node states, the most probable outcome for the child node was assigned the highest probability, and least likely outcome was assigned a low probability. Probabilities for the remaining states were assigned so that the overall distribution was reasonable given our current state of knowledge, and uncertainty in the strength of the causal relationships were reflected in the shape of the probability distribution (i.e., flatter distributions reflect greater uncertainty in the child node state).\(^\text{(47,49)}\) Synergistic or antagonistic interactions between variables and the strength of the causal relationships can be incorporated into a CPT, allowing
Table I. The Conditional Probability Table for the Geographical Risk Aggregate Node, a Function of the Conditions of the Two Parent Nodes, TAM Habitat Availability and Stream Temperature

<table>
<thead>
<tr>
<th>Parent Node States</th>
<th>Geographical Risk</th>
<th>Probability of Each Node State</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAM Habitat</td>
<td>Stream Temperature</td>
<td>Zero</td>
</tr>
<tr>
<td>Zero</td>
<td>Zero</td>
<td>1.00</td>
</tr>
<tr>
<td>Zero</td>
<td>Low</td>
<td>0.90</td>
</tr>
<tr>
<td>Zero</td>
<td>Medium</td>
<td>0.75</td>
</tr>
<tr>
<td>Zero</td>
<td>High</td>
<td>0.60</td>
</tr>
<tr>
<td>Low</td>
<td>Zero</td>
<td>0.75</td>
</tr>
<tr>
<td>Low</td>
<td>Low</td>
<td>0.50</td>
</tr>
<tr>
<td>Low</td>
<td>Medium</td>
<td>0.25</td>
</tr>
<tr>
<td>Low</td>
<td>High</td>
<td>0.10</td>
</tr>
<tr>
<td>Medium</td>
<td>Zero</td>
<td>0.25</td>
</tr>
<tr>
<td>Medium</td>
<td>Low</td>
<td>0.10</td>
</tr>
<tr>
<td>Medium</td>
<td>Medium</td>
<td>0.05</td>
</tr>
<tr>
<td>Medium</td>
<td>High</td>
<td>0.01</td>
</tr>
<tr>
<td>High</td>
<td>Zero</td>
<td>0.15</td>
</tr>
<tr>
<td>High</td>
<td>Low</td>
<td>0.10</td>
</tr>
<tr>
<td>High</td>
<td>Medium</td>
<td>0.05</td>
</tr>
<tr>
<td>High</td>
<td>High</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Note: The table lists all possible combinations of parent node states, and the conditional probabilities describing the child node.

complex patterns or processes to be included in the model without increasing model complexity.

2.4. Risk from TAM Environment

Whirling disease infection requires that the salmonid host be exposed to the parasite’s infective, TAM stage that is released from the obligate worm host, *T. tubifex*. *T. tubifex* can be found in a variety of freshwater habitats; however, it is most prevalent in reaches that are shallow sloped with slow-moving water and high levels of fine sediments. The most susceptible lineage is *T. tubifex* III, which was the lineage found to be most widely distributed across our study area and predominant in samples collected between 1.800 and 3,300 m. These relationships between stream gradient, elevation, and habitats of TAM-producing, obligate worm hosts were expressed in the model by the TAM Habitat input parameter.

The TAM Habitat node in the BBN model (Fig. 2) characterized the proportion of streams within each management unit with specified gradient and elevation characteristics (Table II) that corresponded to different exposure risk states based on the likelihood of habitat conditions where TAMS may be produced. The four states for this node and all other nodes in the model were zero, low, medium, and high. Streams defined as having high likelihood of risk were at lower elevations (less than the average for the risk region) and a stream gradient ≤2%. Medium- and low-risk streams were defined as having steeper gradients at low or high elevations, respectively. Negligible risk was assigned to streams that had gradients greater than 7% (greater than the habitat preference of the oligochaete host).

Stream temperature was included in the model because of its influence on epidemiological elements of whirling disease. High prevalence of the disease in wild populations of cutthroat trout was associated in streams with water temperature greater than 12 °C because the production of the water-borne infectious TAM stage is optimized between 12 °C and 15 °C. Both subspecies of cutthroat trout examined in this study spawn in the spring in response to snow melt, resulting in the production of young fish at temperatures conducive to optimum TAM production. Furthermore, infectivity of rainbow trout increased linearly with temperature between 6 °C and 12 °C. Based on this evidence, first- and second-order streams with stream temperatures between 12 °C and 15 °C were defined as having a high potential for TAM exposure.

Area-weighted means of annual stream temperatures were extracted from NHD Plus, and used in the analysis. Stream segments with weighted temperatures between 12 °C and 15 °C were ranked high, whereas a medium rank was assigned for streams that exhibited temperature ±2 °C above and below this range (Table II). Subsequent ranks were designated by increases and decreases of temperature in 5 °C increments, with the zero rank designation for streams below the minimum temperature for TAM production (less than 5 °C or above 20 °C). Collectively, spring temperatures and stream gradient/elevation comprised the submodel that described probability of whirling disease infection because of geographic characteristics that are conducive to TAMS in the environment (Fig. 2, TAM Habitat).
Table II. Sources of Myxobolus cerebralis Exposure that Were Included in the Bayesian Belief Network Model Used to Evaluate the Probability of Whirling Disease Infection for Colorado River and Rio Grande Cutthroat Trout

<table>
<thead>
<tr>
<th>Input Parameter</th>
<th>Definition</th>
<th>States</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAM habitat</td>
<td>Proportion of stream segments that have gradient and elevation characteristics considered ideal for Tubifex and TAM production.</td>
<td>Zero: gradient &gt;7%&lt;br&gt;Low: 2% &lt; gradient ≥ 7% and elevation &gt; region average&lt;br&gt;Med: 2% &lt; gradient ≥ 7% and elevation &lt; region average&lt;br&gt;High: gradient ≤2%</td>
</tr>
<tr>
<td>Stream temperature</td>
<td>Evaluation of geographical distribution of stream weighted temperature with respect to optimal temperature range for TAM production.</td>
<td>Zero: temp ≤ 8 °C or temp ≥ 19 °C&lt;br&gt;Low: 8 °C ≤ temp ≤ 10 °C or 17 °C ≤ temp &lt; 19 °C&lt;br&gt;Med: 10 °C ≤ temp ≤ 12 °C or 15 °C ≤ temp &lt; 17 °C&lt;br&gt;High: 12 °C ≤ temp &gt; 15 °C</td>
</tr>
<tr>
<td>Barriers</td>
<td>Proportion of streams with varying proximities to a barrier.</td>
<td>Zero: complete barrier &lt;1 km&lt;br&gt;Low: partial barrier &lt;1 km or complete barrier &lt;3 km&lt;br&gt;Med: partial barrier &lt;3 km or complete barrier &lt;5 km&lt;br&gt;High: partial barrier &gt;3 km or any type of barrier ≥5 km</td>
</tr>
<tr>
<td>Proximity to diseased fish</td>
<td>Proximity of streams from locations that had diseased fish tested positive for whirling disease.</td>
<td>Zero: &gt;5 km from positive test&lt;br&gt;Low: &lt;5 km from positive test&lt;br&gt;Med: &lt;3 km from positive&lt;br&gt;High: &lt;1 km from positive</td>
</tr>
<tr>
<td>Co-occurrence with rainbow trout</td>
<td>Degree of overlap between the distribution of cutthroat and rainbow trout.</td>
<td>Zero: &gt;5 km from rainbow&lt;br&gt;Low: &lt;5 km from rainbow&lt;br&gt;Med: &lt;3 km from rainbow&lt;br&gt;High: &lt;1 km from rainbow</td>
</tr>
<tr>
<td>Spawning habitats</td>
<td>Proportion of tributaries suitable for spawning and rearing of cutthroat trout.</td>
<td>Even distribution established because no data were available for this parameter.</td>
</tr>
</tbody>
</table>

2.5. Risk from Hydrologic Connectivity

When given the opportunity, adult cutthroat trout may exhibit fluvial life history patterns migrating to tributaries to spawn, and juveniles migrate to areas of suitable habitat to feed and grow.\(^{[53,60]}\) Fish movement is considered the principal vector leading to spread of the parasite within a river system,\(^{[56]}\) and fish that migrate near diseased populations will have a higher risk of exposure to the parasite. Natural and anthropogenic barriers (diversion dams, culverts, bedrock, and waterfalls) reduce the connectivity of streams,\(^{[61]}\) and effectively isolate populations of cutthroat trout.\(^{[62]}\) The presence of barriers, and the resulting impact to stream connectivity, would reduce disease exposure by isolating populations from areas where TAMs may be present. We used barrier point locations collected by state agencies along with classifications of complete or partial based on their impact to hydrologic connectivity. Streams were ranked based on distance from a barrier by mapping 1 km, 3 km, and 5 km buffers around each barrier, and considering the type of barrier present, with complete barriers resulting in a lower level of risk than partial ones (Table II). Exposure-state values were assigned based on the streams present within each buffer distance and for each type of barrier.

Diseased trout represent the largest source of the parasite today; thus, the probability of exposure will be highest when diseased fish are present within the fish population’s migratory route. Five populations of Colorado River cutthroat trout have tested positive for whirling disease\(^{[39]}\) since the disease was first confirmed in Colorado in 1987;\(^{[63]}\) however, diseased populations of other salmonids have been found within the distribution range of Colorado River cutthroat trout.\(^{[55]}\) Wild and captive populations of trout infected with whirling disease have been found in New Mexico since the disease was
discovered in rainbow trout in 1999, however, the disease has not been confirmed in any populations of Rio Grande cutthroat.

We used spatial data of whirling disease testing locations and the presence or absence of diseased fish at those locations to identify disease sources within each management area. Testing sites where the disease was detected were treated as point sources for the disease, with risk of exposure a function of distance from the source. Streams were ranked based on proximity to disease sources by establishing 1 km, 3 km, and 5 km buffers around all sites confirmed for presence of the disease (Table II). The impact of barriers and proximity to diseased populations were summarized in the stream connectivity node that represents the risk of disease exposure during fish migration (Fig. 2).

2.6. Risk Because of Subspecies' Attributes

We quantified risk of exposure to whirling disease from two ecological attributes for each cutthroat trout subspecies: co-occurrence with other sensitive fish species and prevalence of spawning and rearing locations. Spatial overlap of the cutthroat trout with other salmonids susceptible to whirling disease increases the likelihood of exposure to the parasite. Rainbow trout are widely distributed in the intermountain west and highly susceptible to whirling disease; thus, they are another potential source of the disease for native cutthroat trout when their movements within a stream system overlap. We estimated the extent that each subspecies overlaps with identified populations of rainbow trout by creating distance buffers around the stream segments with rainbow trout (Table II). Close proximity between the two trout species will increase the chance of contact and potential for exposure to diseased fish.

The spawning and rearing behavior of cutthroat trout affects whirling disease exposure because susceptibility and severity of the disease are dependent on the age of fish at the time of TAM infection. Young fish have greater susceptibility to the disease; day-old fry were 10 times more susceptible to infection than two-month-old fry. Whirling disease infection rates were highest for sentinel rainbow trout in spawning and rearing locations in a Montana stream where the parasite was previously established. Unfortunately, spawning grounds have not been fully characterized for either subspecies, although spawning time and behavior are likely similar for the two subspecies. Cutthroat trout tend to spawn in low-to-moderate gradient streams similar to the range considered to be ideal for T. tubifex, already included in the BBN. The absence of site-specific data for spawning locations was expressed for the spawning habitat input node by an even probability distribution for the four potential exposure states, reflecting the high uncertainty for this parameter (Table II). Despite the lack of available data, we included spawning habitat in the model to evaluate the importance of this parameter and whether additional information on spawning habitats might improve management of whirling disease prevention.

2.7. Application of the BBN to Management Areas

Risk of whirling disease infection was assessed for the two different cutthroat trout subspecies across a total of 12 different management areas; Colorado River cutthroat trout occur in eight management areas and Rio Grande cutthroat trout in four areas (Fig. 1). Our initial BBN had a hierarchical structure and CPTs that described the relationships between the input parameters and exposure to the disease (Fig. 2). This model was the basis for developing individual, parameterized models for each management area. The model for Colorado River cutthroat trout in the San Juan management area (Fig. 3) was parameterized with data specific to that area, and the model endpoint was the relative risk distribution for whirling disease infection to Colorado cutthroat trout populations within the defined region.

2.8. Comparison of Risk Distributions

To predict the relative risk of whirling disease infection for the two subspecies of cutthroat trout, irrespective of geographical area, we used a Markov chain Monte Carlo (MCMC) analysis to integrate the infection risk distributions from the management area BBN models for a given subspecies into a single estimated risk distribution. The MCMC was conducted using Oracle Crystal Ball software (version 11.1.2.3) as a macro in Microsoft Excel 2010. We ran the Monte Carlo simulation for 10,000 iterations, and derived output distributions for each subspecies of cutthroat trout. The resultant distributions reflected the predicted relative risk of whirling disease infection for Colorado River and Rio Grande cutthroat trout across their respective ranges.
2.9. Model Evaluation

Ideally, models should be tested using additional empirical data; however, additional case data were not available. We evaluated the model by simulating a variety of disease exposure scenarios by systematically changing the values entered for the exposure parameters, and determining whether the resulting probabilities for the terminal node were logical and reasonable. These testing simulations were shared with whirling disease and fisheries experts through reports and informal sharing of the models during various stages of development. Further testing of the model will be possible if additional whirling disease testing and population monitoring takes place in the future and is made publicly available.

Sensitivity analysis is another layer of model testing that can be used to determine the degree to which variation in the predicted, posterior probability distributions for a given node is affected by findings at other nodes. Netica’s sensitivity analysis procedure for discrete nodes calculates the entropy reduction (i.e., mutual information analysis) between the output node and all other nodes in the BBN, which describes the extent that each input variable is associated with the model output. Entropy reduction was used to evaluate the degree to which each exposure input variable influenced the likelihood of whirling disease infection as entropy reduction values increase with the degree of influence. For BBNs used in risk assessment, the sensitivity analysis can verify whether the model outcome conforms to expectations, and identify priority risk factors or gaps in existing knowledge.

3. RESULTS

The analysis area for Colorado River cutthroat trout spanned eight GMUs across five western states, and generally the likelihood of whirling disease infection was low for streams across the subspecies’ range (Fig. 4). Streams within the Upper Colorado GMU had a 77% likelihood of zero-low risk levels (Fig. 4A) and only an 8% likelihood of a high risk level; in contrast, the streams in the San Juan GMU, at the southern end of the subspecies’ range, had a 62% likelihood of a zero-low risk level and a 15% likelihood of high risk (Fig. 4B). Differences between risk estimates for the Colorado River cutthroat trout GMUs were small, and reflect a relatively low level of risk based on the current exposure scenario.

Similarly, Rio Grande cutthroat trout also had a relatively low level of risk of whirling disease infection, although the distribution patterns were more variable (Fig. 5). The distribution range of
Rio Grande cutthroat trout is smaller than that of Colorado River cutthroat trout, consisting of only four GMUs spanning two states. Generally, populations in the southern portion of the range (Canadian and Pecos GMUs) were estimated to have a higher probability of infection than those in the northern portion of the range. The southern Pecos GMU had the highest risk of infection, with a 44.5% likelihood of medium to high risk levels, whereas the most northern GMU, Rio Grande, had a 75% likelihood of zero-low risk.

The predicted risk of whirling disease infection was similar for the two subspecies (Fig. 6), and the distributions suggest that populations of both Colorado River and Rio Grande cutthroat trout had a relatively low risk of whirling disease infection. The probability distribution for Rio Grande cutthroat trout was slightly broader with a lower likelihood of a low risk, suggesting that these fish may have a slightly higher risk of infection or that there was greater uncertainty in risk estimates across the subspecies’ range.

For both subspecies, the probability of whirling disease infection was most strongly influenced by the TAM habitat exposure parameter (Fig. 7). Although spawning locations ranked second as the most influential parameter for many of the management area models, the lack of available site-specific data represented the principal source of uncertainty in the risk calculations. The third most influential input parameter differed among management regions; however, proximity to barriers exerted a strong influence on the probability of infection for all GMUs. It is notable that barrier parameter exerted the greatest influence on the Upper Colorado and Rio Grande management areas for Colorado cutthroat and Rio
Fig. 5. Probability distributions describing the risk of whirling disease infection for populations of Rio Grande cutthroat trout in each of its four conservation management areas.

Fig. 6. Simulated probability distributions describing the overall risk of whirling disease infection for Colorado River and Rio Grande cutthroat trout (black and gray lines, respectively) across their current ranges.

Grande cutthroat trout, respectively (Fig. 7). These two areas also had more streams with complete and partial barriers than the other management areas (Fig. 8) and the lowest likelihood of high infection risk (Figs. 4 and 5). Our analysis of barriers within each management area characterized streams that were more than 3 km from a partial barrier or 5 km from a complete barrier as contributing a high risk of exposure because of unrestricted fish movement. The majority of streams across the entire study area were in this exposure category (Fig. 8), suggesting that fish can migrate freely within the stream systems.

4. DISCUSSION

Although the predicted risk scores varied slightly among GMUs for both subspecies of cutthroat trout,
all scores corresponded to a relatively low probability of whirling disease infection. This is not to say that risk of the infection is low throughout the range as there may be individual populations at high risk. The uncertainty associated with regional scale data cannot reflect the microtopographic and localized ecological conditions present. The resolution of this assessment is meant for regional risk characterization rather than site-specific extrapolation. In this study, the scale of analysis was based on natural hydrologic subbasins that made up the geographic management entities for each subspecies. Despite the scale of the study area and paucity of empirical data, we were able to generate estimates of risk of infection from this emerging aquatic disease, and relate the estimates of risks to parameters of importance for wild trout management (i.e., presence and integrity of barriers).

Cutthroat trout are highly susceptible to whirling disease when exposed to the TAM stage of the parasite, resulting in higher morbidity and mortality than most trout species.\(^{10,72}\) The risk from whirling disease is going to be a function of risk of exposure and effects from the disease. We found that the probability of infection was low for Colorado River and Rio Grande cutthroat trout when assessed at the regional scale, yet the risk to the subspecies from the disease may be high if exposed. There are two populations of
Colorado River cutthroat trout that have shown signs of year class failure because of whirling disease infection (R.B. Nehring, personal communication). In 2011, a second survey for whirling disease was conducted that included four Rio Grande cutthroat trout populations, three of which tested positive for the disease (one in New Mexico and two in Colorado). The New Mexico population that tested positive for whirling disease was in the Pecos GMU; however, those data were not included in this analysis. The presence of the disease in populations of Rio Grande cutthroat trout in the Pecos GMU supports our finding that populations in this region had greater predicted risk of infection than in other areas of the subspecies’ range. Although the addition of hydrological barriers to stream systems may be a tangible

Fig. 8. Frequency distribution of barrier types within the study area for the two native cutthroat trout subspecies. Panel A shows the barrier type and frequency within each of the geographic management units for Colorado River cutthroat, whereas Panel B shows the same information for Rio Grande cutthroat management areas.
option to reduce the risk of the disease spread, they do not completely prevent the spread of the disease and tradeoffs should be carefully considered.\(^{(73)}\) Barriers are beneficial in terms of limiting invasion by nonnative species, but only if sufficient quality and size of salmonid habitat is available.\(^{(74)}\)

Although barriers were only one of the input parameters that influenced the likelihood of whirling disease infection in the two subspecies of cutthroat trout, barriers represent one of the few feasible management options for controlling the spread of the disease. Given the limited resources that constrain all management agencies, our recommendation would be to work toward reducing uncertainty associated with risk so that construction or maintenance of barriers could be targeted to protect the populations at highest risk. Recent advances in the use of genetic assays to identify \(T.\) \(tubifex\) lineages that are susceptible or infected with \(M.\) \(cerebralis\) within oligochaete communities\(^{(75)}\) may increase our understanding of how these lineages are distributed in streams where fish are infected with whirling disease. Reduction of uncertainty through additional data collection will call for additional time and expense, yet efforts should include a comprehensive strategy to fill information gaps through a well-designed monitoring initiative designed to refine the risk assessment. BBNs such as the one used in this analysis are well suited toward use in adaptive management.\(^{(76)}\)

Recent decades have witnessed a decline in native fishes as a result of widespread anthropogenic landscape alterations, a general decrease in quality as well as quantity of surface waters, introduction of nonnative species, and disease.\(^{(73,77)}\) The overall benefit of producing a risk assessment for whirling disease targeted at native cutthroat trout has been to identify which populations and exposure pathways should receive more immediate attention by managers. Managing for the long-term persistence of imperiled fishes requires careful consideration of the tradeoffs and unintended consequences associated with conservation and recovery efforts. For example, preventive measures that protect native trout from disease through isolation behind hydrologic barriers may come at the cost of a reduction in genetic diversity.\(^{(73,74,77)}\) In contrast, if the objective is to preserve meta-population dynamics, the risks and cost of efforts to facilitate fish migration and recovery of populations whose size has been reduced in recent years will be increased exposure to disease.

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**REFERENCES**

34. Peterson DP, Fausch KD. Upstream movement by nonnative brook trout (Salvelinus fontinalis) promotes invasion of native cutthroat trout (Oncorhynchus clarkii) habitat. Canadian Journal of Fisheries and Aquatic Sciences, 2003; 60:1502–1516.
45. Cavender WP, Wood JS, Powell MS, Overturf K, Cain KD. Real-time quantitative polymerase chain reaction (QPCR) to identify Myxobolus cerebralis in rainbow trout (Oncorhynchus mykiss). Diseases of Aquatic Organisms, 2004; 60:205–213.


75. Fytilis N, Rizzo DM, Lamb RD, Kerans BL, Stevens L. Using real-time PCR and Bayesian analysis to distinguish susceptible tubificid taxa important in the transmission of...


SUPPORTING INFORMATION

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Appendix. Conditional probability tables for the first tier of aggregate node in the Bayesian network models for whirling disease infection risk to Colorado River and Rio Grande cutthroat trout.