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RESEARCH NOTE

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Detection of Salmonid Pathogen eDNA in the Malheur River Basin, Oregon

Running footer: Salmonid parasite eDNA in Malheur River

1 table, 1 figure, 1 Suppl. table

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Abstract

The Malheur River Basin (MRB) in eastern Oregon is an historically important salmonid habitat that faces ecological challenges from dam construction, habitat fragmentation, and invasive species. This study aimed to determine the presence and distribution in the basin of three salmonid pathogens, *Ceratonova shasta, Parvicapsula minibicornis*, and *Myxobolus cerebralis*, to inform fish health management and restoration efforts led by the Burns Paiute Tribe and Oregon Department of Fish and Wildlife. Water samples were collected from 22 sites across the MRB in summer (July) and fall (October) 2024. Samples were filtered, and concentrated environmental DNA was extracted and analyzed using species-specific quantitative PCR assays targeting each parasite. *C. shasta* DNA was detected in multiple tributaries during both sampling periods, with recurring detections at one site each in the Middle Fork and Willow Creek. In October, *C. shasta* was also detected in the mainstem. Neither *P. minibicornis* nor *M. cerebralis* was detected in any sample. This study provides the first environmental DNA-based evidence of *C. shasta* in the Malheur River Basin and establishes a baseline for future pathogen surveillance. These findings will help guide ongoing fish health monitoring and restoration planning for native salmonid populations in the region.

Key Points

The Malheur River Basin is an important salmonid habitat, yet baseline pathogen distribution and abundance are unknown.

We tested environmental DNA for three salmonid parasites, *Ceratonova shasta, Parvicapsula minibicornis*, and *Myxobolus cerebralis*; only *C. shasta* was detected.

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This approach informs fish health management and restoration efforts led by the Burns Paiute Tribe and Oregon Department of Fish & Wildlife.

Keywords: *Ceratonova shasta, Myxobolus cerebralis, Parvicapsula minibicornis, Bull Trout (Salvelinus confluentus), Redband Trout (Oncorhynchus mykiss gairdneri)*

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Introduction

The Malheur River, a vital waterway in the arid landscape of eastern Oregon, has long been an essential component of the region's ecosystems and history. Historically, the river supported abundant populations of salmonid species. However, the construction of dams, including the Agency Valley Dam, Bully Creek Dam, and Warm Springs Dam, drastically altered the river's hydrology and thermal profiles and contributed to habitat fragmentation and degradation. These modifications disrupted spawning and rearing habitats and blocked upstream migration of anadromous fishes, contributing to the extirpation of Chinook salmon *Oncorhynchus tshawytscha* and other anadromous species from the Malheur River Basin (MRB, Malheur Watershed Council et al. 2010). The extirpation of salmon from the basin represents a significant ecological and cultural loss as a recreational fishery and for native communities who relied on these fish for subsistence and ceremonial practices (Malheur Watershed Council et al. 2010).

The ecological significance of remaining resident salmonids, such as Bull Trout *Salvelinus confluentus* and Redband Trout *Oncorhynchus mykiss gairdneri*, extends beyond their roles as focus species. For the Burns Paiute Tribe (BPT), these fishes hold immense cultural and subsistence importance. Traditionally, the tribe relied on these populations for food, economic stability, and spiritual practices. However, habitat degradation, reduced water quality, and the introduction of invasive species have disrupted the population dynamics of these fishes, threatening their long-term survival (Beschta 1997); Bull Trout are federally listed as endangered and Redband Trout are state listed as sensitive (ODFW 2021).

The Upper Malheur River Watershed has been designated as critical habitat for Bull Trout, encompassing spawning, rearing, and migratory areas (U.S. Fish and Wildlife Service 2010).

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Critical habitat is defined as areas containing physical and biological features essential to Bull Trout conservation, which may require special management or protection (U.S. Fish and Wildlife Service 2015). However, this habitat and the Bull Trout populations it supports face significant challenges, including thermal barriers, habitat fragmentation, and competition with invasive species. These issues further emphasize the need for effective conservation measures to maintain and restore ecological balance within the basin.

Pathogens present additional challenges for salmonid populations in North America. Freshwater myxozoan parasites, including *Ceratonova shasta*, *Parvicapsula minibicornis*, and *Myxobolus cerebralis*, can cause enteronecrosis, glomerulosis, and whirling disease, respectively. In other Pacific Northwest river systems, fish infected with these parasites can experience high morbidity and mortality, exacerbating existing population declines (e.g. *P. minibicornis* in sockeye salmon in British Columbia (Bradford et al. 2010), *C. shasta* and *P. minibicornis* in Chinook salmon in Oregon (Hallett et al. 2012, Voss et al. 2018), *M. cerebralis* in rainbow trout in California (Malm et al. 2012, Hallett et al. 2026)). Environmental stressors such as elevated water temperature, altered flow conditions, and degraded habitat quality may increase susceptibility of salmonids to parasitic infection and associated disease, particularly during vulnerable juvenile life stages when fish experience prolonged freshwater exposure (Bradford et al. 2010, Hallett et al. 2012). These myxozoan parasites have complex life cycles that require passage through an invertebrate (annelid) host. Hydrologic alteration and habitat fragmentation may therefore also influence aquatic pathogen ecology by modifying water temperature, sediment deposition, flow conditions, and habitat suitability for invertebrate hosts involved in these parasite life cycles (Alexander et al. 2014, Bartholomew et al. 2023). Environmental variability among tributaries within the Malheur Basin likely influences spatial patterns of pathogen occurrence and persistence.

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Our study builds upon the cooperative agreement between the Oregon Department of Fish and Wildlife (ODFW) and the BPT, which emphasizes a phased approach to the restoration and conservation of fish populations in the Malheur River Basin (Burns Paiute Tribe et al. 2022). A central component of this agreement involves conducting pathogen assessments and identifying strategies to reduce the risks these pose. Previous pathogen monitoring focused directly on fish - Redband Trout populations in the basin - with the BPT collecting and analyzing 77 individuals across multiple tributaries during the 2017 spawning season. The trout were tested (method not specified) for four pathogens: *M. cerebralis, C. shasta*, *Renibacterium salmoninarum* and infectious hematopoietic necrosis virus (IHNV); only *R. salmoninarum,* the causative agent of bacterial kidney disease, was detected (Haslick 2018).

Myxozoan parasites are transmitted via waterborne spore stages. This feature makes them ideal candidates for direct sampling in river water and avoids the need to collect invertebrate or vertebrate hosts (Hallett and Bartholomew 2009, Hallett et al. 2012, Richey et al. 2018). We selected water sampling combined with molecular analysis as our sole monitoring method due to several key advantages. First, it is a cost-effective approach compared to traditional sentinel fish exposures, which are more labor-intensive and time-consuming (Hallett and Bartholomew 2006). Secondly, water sampling allows for rapid assessment of pathogen presence and abundance, with results available within days rather than the weeks required for fish exposure studies (Hallett et al. 2012). Additionally, this method is non-lethal, eliminating concerns about impacts on vulnerable fish populations, such as Bull Trout, during monitoring activities. Finally, this approach had not been implemented in the Malheur River but has become a core fish health monitoring tool in other systems (Hallett et al. 2012).

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Integrating traditional ecological knowledge from the BPT (e.g. salmonid life histories) with current Western scientific research (e.g. environmental DNA analyses (eDNA)), our study aims to inform culturally sensitive and scientifically robust conservation practices. The overarching goal is to fill knowledge gaps that will contribute to the recovery of salmonid populations in the Malheur River, ensuring their sustainability for future generations of both humans and wildlife that depend on this vital ecosystem. Specifically, this study aimed to establish a baseline understanding of the presence and distribution of three salmonid pathogens within the MRB using eDNA surveillance techniques to support future fish health monitoring, restoration and reintroduction planning efforts.

Methods

Study System and Sites

Originating in the Blue Mountains, the Malheur River flows generally southeast for approximately 190 miles (305 kilometers) before joining the Snake River near Ontario, Oregon (Figure 1). It drains a watershed of about 4,700 square miles (12,173 km²), encompassing diverse upland and lowland habitats (Oregon Department of Environmental Quality, 2009; U.S. Forest Service, 2010). The MRB encompasses a diverse range of aquatic habitats, including high-gradient tributaries, lower-gradient depositional reaches, and regulated river sections with varying thermal and flow characteristics. Habitat conditions throughout the basin vary substantially with respect to water temperature, substrate composition, flow regime, and sediment deposition, all of which may influence pathogen persistence and eDNA transport.

We collected water samples from 22 sites across the MRB for parasite assessment, as mandated by the cooperative agreement between the ODFW and the BPT (Burns Paiute Tribe et al. 2022).

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The sites were selected strategically based on their long-term accessibility and habitat type. To provide a comprehensive overview of the river system, the sites represent a diverse range of aquatic habitats which should enable us to capture potential variations in pathogen prevalence and associated eDNA signals. Particular emphasis was placed on capturing environmental variability among tributaries, including habitats associated with Bull Trout critical habitat, cold-water refugia, and depositional reaches that may support annelid host habitat. Accessibility considerations prioritized public lands and areas with established access to ensure efficient logistics and compliance with land-use regulations. The sample sites were then divided to represent the five major tributaries: Willow Creek (Will), Bully Creek (Bully), North Fork (North), South Fork (South), Middle Fork (Mid) and main stem of the Malheur River (Malh) (Figure 1, Supplementary Table 1).

To assess temporal variation in pathogen presence, water samples were collected in summer (July) and fall (October) 2024. All water samples for each sampling period were collected within a single day to maintain consistency in temporal representation and sample integrity. The sampling effort was carried out by coordinated field teams from the BPT, the ODFW, and Oregon State University's Parasite-Host Interactions Laboratory (PHIn Lab). The watershed was divided into sections, and teams sampled assigned sections in a downstream-to-upstream sequence where feasible, facilitating timely sample collection and minimizing variability in environmental conditions. All teams convened in Burns to deliver the samples, which were then transported on ice to the PHIn Lab in Corvallis for processing.

Water sample collection

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Sampling bottles were rinsed at each site using source water. From the riverbank, three 1-L samples (each bottle equals one sample) were taken approximately 1 ft (30 cm) below the water surface, then placed on ice. Sampling from the riverbank was conducted to maintain consistency among sites while minimizing disturbance to stream substrates and reducing the potential for sediment resuspension that could influence eDNA concentration. *DNA capture and extraction*

In the laboratory, all samples were filtered within 36 hours to minimize spore degradation. Each water sample underwent vacuum filtration to isolate eDNA on a sterile 145 mm diameter mixed cellulose ester 0.2 µm filter membrane (Sterlitech). The membranes were dried using a SpeedVac for 1–2 h at medium heat and stored at -20°C until DNA extraction. Each sample was subjected to an acetone treatment to dissolve the filter, and DNA was extracted using a Qiagen DNeasy blood and tissue kit, as per Hallett et al. (2012). The resultant purified DNA was stored at -20°C.

Parasite detection

Quantitative PCR (qPCR) assays were used to detect three aquatic pathogens: *C. shasta, P. minibicornis,* and *M. cerebralis* (Kelley et al. 2004, Hallett and Bartholomew 2006, Hallett and Bartholomew 2009, respectively). Extracted eDNA from water samples was diluted 1:10 with AE buffer (Qiagen). Samples were assayed in duplicate (i.e., in two wells). The *C. shasta* qPCR was a duplex assay that included an internal positive control (IPC; Applied Biosystems) to assess inhibition and was run first. Each assay included positive reference controls (DNA extracted from known numbers of parasite spores) and negative controls (molecular-grade water).

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Any sample that tested positive for *C. shasta* with a quantity equivalent to one spore (= waterborne infectious stage) underwent separate PCR and Sanger sequencing at OSU's Center for Quantitative Life Sciences to determine the specific genotype present (Atkinson et al. 2018).

Data Analysis

As described above, three 1-L water samples were collected at each sampling site during each sampling period, and each sample was analyzed in duplicate qPCR wells. qPCR results were evaluated based on the detection of target DNA fluorescence using quantification cycle (Cq) values. Non-fluorescing wells were assigned a Cq value of 44, which is beyond the detection limit of any of the three parasite assays but enables these samples to be included in calculations. Well pairs were averaged for each sample. Samples with an average Cq value <44 were considered positive (note that DNA quantity is inversely proportional to the Cq value). This threshold was selected to maximize sensitivity for early-stage surveillance. Because this study aimed primarily to determine whether target parasites were present within the basin, a conservative detection criterion was used. At this exploratory stage, identifying potential pathogen occurrence was prioritized over strict quantification thresholds.

Average Cq values were converted to spore equivalents by comparison to a spore reference standard run on the same qPCR plate. Positive samples were categorized as whole spore detections (≥1 spore/L) or trace detections (<1 spore/L). Trace detections likely represent partial or degraded DNA and were therefore interpreted with caution. Detection frequency at each site and sampling period were recorded as the number of positive samples out of the three collected. For site-level interpretation, a site was classified as positive if at least one of the three 1-L samples yielded a positive result (average Cq < 44). This approach was selected to increase

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detection sensitivity at low parasite densities, consistent with the study's objective of determining presence or absence during an initial basin-wide assessment.

Results

Molecular analysis of water samples collected throughout the Malheur River Basin for three salmonid pathogens revealed the presence of only one of these, *C. shasta*; neither *M. cerebralis* nor *P. minibicornis* was detected at any site during our sampling periods. Although the primary objective of this study was qualitative (parasite presence), quantitative estimates (spores/L) were determined where possible to provide additional ecological context. In cases where whole spore detections were observed, these values offered insight into relative parasite abundance and helped identify sites that may warrant further targeted investigation. A 'no detection' may reflect absence of the target parasite in the river, absence of the parasite or parasite DNA in the water sample (not captured), or present at a density below the detection limit of the qPCR.

July Sampling

In July 2024, water samples were collected from 21 of 22 designated sites; site Bully 3 was inaccessible due to wildfire-related closures. *C. shasta* was detected at four sites (19% of sites sampled), representing four of five major tributaries: Willow Creek (Will 2), Bully Creek (Bully 4), North Fork (North 1), and Middle Fork (Mid 3) (Figure 2 & Table 1). All four sites with detections had one of three samples (biological replicates) with a detection. The majority of these corresponded to trace detections, whereas the single detection at Willow Creek (one bottle at Will2) represented a whole spore detection (but genotyping was unsuccessful).

October Sampling

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In October 2024, water samples were collected from 21 of 22 designated sites; site South 2 was excluded due to logistical constraints. *C. shasta* was detected at seven sites (33% of sites sampled), representing three major tributaries, Willow Creek (Will 1, Will 2), and North Fork (North 4), Middle Fork (Mid 1, Mid 3), and the mainstem (Malh 1, Malh 2) (Figure 2 & Table 1). There were no detections in the South Fork during either sampling period. All seven sites with detections had one of three samples with a detection. A whole spore detection was observed at North 4, and genotyping revealed that the DNA present was all genotype II.

Discussion

Our study represents the first eDNA-based investigation of *C. shasta*, *P. minibicornis*, and *M. cerebralis* within the Malheur River system of eastern Oregon. To date, there have been no confirmed detections of these salmonid pathogens in the basin, despite their known presence in hydrologically connected systems, including the Columbia River (Hallett et al., 2026). Our detections highlight the sensitivity of water sampling and eDNA analysis as an effective method of pathogen detection, and thus a tool for future monitoring.

We detected *C. shasta* at four sites in July and seven sites in October, indicating a broader spatial distribution in fall. The parasite was detected in four major tributaries (Willow Creek, Bully Creek, North Fork, and Middle Fork) during both periods and in the mainstem (Malh 1 and Malh 2) in October. Recurring detections in Willow Creek and the Middle Fork during both sampling periods indicate temporal consistency in *C. shasta* detection at these locations. The broader spatial distribution observed during October may reflect seasonal environmental conditions influencing parasite transport, persistence, or detectability within the river system. Seasonal

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variation in flow conditions, temperature, and eDNA persistence may contribute to temporal variability in parasite detection throughout the basin.

The spatial distribution of *C. shasta* detections observed in this study may reflect environmental variability among tributaries within the Malheur River Basin. Hydrologic alteration, thermal variability, substrate composition, and depositional habitat conditions may influence parasite persistence and habitat suitability for annelid alternate hosts. Tributaries containing lower-velocity depositional reaches and fine sediment accumulation may provide more favorable habitat conditions for annelid hosts and parasite maintenance within the system. Recurring detections observed in Willow Creek and the Middle Fork may therefore indicate localized conditions favorable for low-level parasite persistence. Flow regulation and hydrologic alteration within portions of the basin may also influence annelid habitat distribution by modifying sediment transport, depositional habitat availability, and thermal conditions within the river system (Bartholomew et al. 2023).

C. shasta detected in the North Fork in October was sequenced and genotyped as type II. This genotype, though typically associated with Coho Salmon (*Oncorhynchus kisutch*), has been detected in other systems lacking Coho Salmon, suggesting possible associations with resident salmonids (Atkinson et al. 2014, Stinson et al. 2018).

Of the three target myxozoan parasites, neither *M. cerebralis* nor *P. minibicornis* were detected, despite their presence in connected Columbia River tributaries (Bartholomew et al. 2007, Atkinson et al. 2011). This may reflect ecological or geographic barriers to pathogen establishment or temporal/spatial limitations of their detectability in the current study. Their lack of detection here may reflect unsuitable environmental conditions in the Malheur River or some

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host factor. However, *P. minibicornis* and *C. shasta* share a two-host life cycle involving salmonids and the annelid *Manayunkia occidentalis* (Bartholomew et al. 1997, Bartholomew et al. 2006, Atkinson et al. 2020), so conditions that support *C. shasta* transmission are likely to apply to *P. minibicornis.* The distribution and abundance of annelid hosts may strongly influence parasite persistence and distribution within the basin because direct fish-to-fish or annelid-to-annelid transmission does not occur. *M. occidentalis* is commonly associated with depositional habitats containing fine sediment, organic matter accumulation, and relatively low-velocity flow conditions (Alexander et al. 2016), suggesting that habitat variability throughout the Malheur Basin contribute to the patchy spatial distribution of parasite detections observed during this study.

The detection of only *C. shasta* in the present study indicates that although both hosts must be present (these sites are isolated due to multiple barriers to upstream migration and transmission is indirect), *P. minibicornis* was either absent from the basin or missed capture in the summer/fall sampling. In other river systems (e.g. Klamath River; Voss et al. 2018, Bartholomew et al. 2023), *P. minibicornis* is most abundant in early spring, hence future sampling in the Malheur River should include spring sampling to better determine its abundance there.

These results demonstrate the utility of eDNA analysis of river water samples as a screening tool for salmonid pathogens across broad spatial scales. However, detections do not directly indicate infection risk for fish. Environmental DNA detections confirm parasite presence but do not necessarily indicate presence of the fish-infectious stage (eDNA sampling will detect spores shed from fish but these are not transmissible to fish), active infection, disease severity, or population-level impacts within fish populations. Infection risk is likely influenced by multiple interacting

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environmental and biological variables, including water temperature, fish density (infectious units per fish), exposure duration, seasonal flow conditions, and fish life stage. Water quality conditions, including temperature and flow variability, may further influence host stress and susceptibility to infection, particularly during seasonal low-flow periods when environmental conditions become less favorable for salmonids (Bradford et al. 2010, Hallett et al. 2012). For resident salmonids in the Malheur River Basin, exposure may occur across age classes when fish occupy areas where infectious parasite stages are present. Follow-up efforts, including sentinel fish studies, habitat and annelid host assessments, and seasonal time-series sampling, are recommended. Sites with recurring detections (Mid 3, Will 2) and high parasite density (North 4) should be prioritized for further investigation. These findings provide an important baseline for future pathogen surveillance and fish restoration planning within the Malheur River Basin and support continued collaborative monitoring efforts among the BPT, ODFW, and OSU. Understanding environmental conditions associated with pathogen occurrence will become increasingly important as restoration and reintroduction efforts expand within the Malheur River Basin.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Animal Care and Use

No permits or Animal Care Committee compliance were necessary.

Data Availability

Data (quantitative PCR and Sanger sequencing) and extracted DNA are available from the authors upon reasonable request.

Author Contributions

Project conceptualized by MZ and SLH. All authors contributed to project design. ZM, DB and BPT performed field collections. ZM and SDA performed lab analyses. ZM, SDA and SLH analysed data and drafted the manuscript. All authors reviewed and edited the document.

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Figure

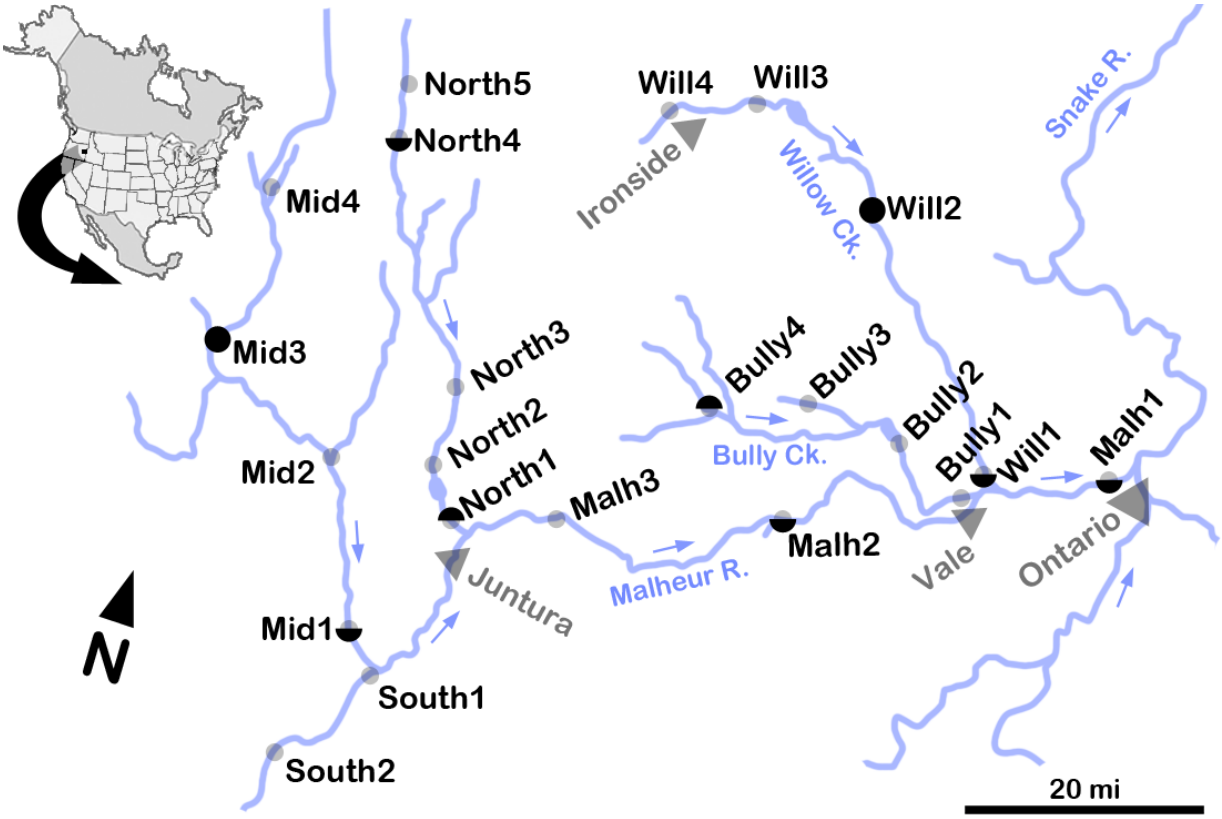


Figure 1. Location of 22 environmental DNA sampling sites in the Malheur River Basin in eastern Oregon, USA, and their pathogen detection status in July and October 2024. The Malheur River and its five primary tributaries, Willow Creek (Will), Bully Creek (Bully), North Fork (North), South Fork (South), and Middle Fork (Mid) are shown with sites labeled sequentially from downstream (1) to upstream. Arrows indicate the general direction of river flow. Major nearby towns (i.e., Ontario, Vale, Juntura, and Ironside) are labeled for geographic reference. Ontario marks the confluence of the Malheur and Snake rivers. The inset map shows the location of the Malheur River Basin within western North America. Upper black semicircle = sites where *Ceratonova shasta* DNA was detected in July, lower black semicircle in October 2024, full black circle = both months, and smaller gray circle = neither. No *Parvicapsula*

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462 *minibicornis* or *Myxobolus cerebralis* DNA was detected at any site during either sampling

463 period.

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Table

Table 1. Detections of *Ceratonova shasta* DNA at sampling sites in the Malheur River Basin, eastern Oregon, during July and October 2024. The table includes only sites with at least one positive detection during either sampling period. At each site and sampling period, three 1-L water samples were collected, and each sample was analyzed in duplicate qPCR wells; the reported average quantification cycle (Cq) values represent the average of the duplicate wells for each sample (non-fluorescing wells assigned Cq = 44). Samples with an average Cq < 44 were considered positive. Average Cq < 44 values were converted to spore equivalents using a spore reference standard run on the same qPCR plate, and detections were categorized as whole spore detections (≥1 spore/L) or trace detections (<1 spore/L). *C. shasta* DNA was detected in only one of three samples of a site cohort (thus Avg Cq and spores/L values in the table pertain to one sample per site). Site codes correspond to tributary and mainstem locations: Willow Creek (Will), Bully Creek (Bully), North Fork (North), Middle Fork (Mid), and mainstem Malheur River (Malh). There were no detections in tributary South Fork (South) during either sample period. No detections of *Parvicapsula minibicornis* or *Myxobolus cerebralis* were recorded during either sampling period.

Sampling Period	Site	Avg Cq	spores/L
July	Will2	33.6	1.9
	Bully4	41.2	trace
	North1	38.6	trace
	Mid3	38.6	trace
October	Will1	39.1	trace
	Will2	37.3	trace

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North4	28.4	37.3
Mid1	37.1	trace
Mid3	42.6	trace
Malh1	40.8	trace
Malh2	38.8	trace

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Supplementary Table 1. Sampling site names and geographic locations (n=22) within the Malheur River Basin, eastern Oregon. Site codes correspond to tributary and mainstem locations, and coordinates are provided in both latitude–longitude (decimal degrees) and Universal Transverse Mercator (UTM) format. Mahl = Malheur River, and its five primary tributaries: Will = Willow Creek, Bully = Bully Creek, North = North Fork, South = South Fork, and Mid = Middle Fork.

Site	Latitude	Longitude	UTM
Mahl1	43.8737	-117.72602	11T 0406906 4850622
Mahl2	44.28607	-120.9589	11T 0389631 4894424
Mahl3	44.37542	-121.0378	11T 0388714 4902077
Will1	44.3245	-121.0395	11T 0407195 4862526
Will2	44.28607	-121.346	11T 0372952 4884707
Will3	44.37542	-121.173	11T 0392798 4488908
Bully1	43.97493	-118.211555	11T 0407195 4862526
Bully2	43.98375	-121.38	11T 0406906 4850622
Bully3	44.209	-121.2432	11T 0388714 4902077
North1	43.57475	-121.346	11T 0389631 4894424
North2	43.7974	-121.38	11T 0406906 4850622
North3	43.9323	-121.0395	11T 0388714 4902077
South1	43.55632	-121.72602	11T 0406906 4850622
South2	43.40818	-121.173	11T 0389631 4894424
South3	43.55632	-121.346	11T 0386901 4884707
South4	43.40818	-121.173	11T 0389631 4894424
Mid1	43.57475	-118.211555	11T 0388714 4902077
Mid2	43.7974	-117.72602	11T 0406906 4850622
Mid3	43.9323	-120.9589	11T 0389631 4894424
Mid4	44.209	-121.0395	11T 0407195 4862526
Bully4	43.98375	-121.72602	11T 0388714 4902077
North4	44.209	-121.0395	11T 0407195 4862526

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