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Detection of *Yersinia ruckeri* in Pacific Lamprey (*Entosphenus tridentatus*) on the Olympic Peninsula in Washington, USA

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ABSTRACT: Pacific lamprey (*Entosphenus tridentatus*) are important anadromous fish throughout their range in western North America. As conservation programs for lamprey expand, disease surveillance is becoming more prevalent. During routine surveillance, *Yersinia ruckeri* biotype II was isolated from Pacific lamprey. This is the first documented *Y. ruckeri* detection in Pacific lamprey.

Pacific lamprey (*Entosphenus tridentatus*) are anadromous agnathan fish that are native to western North America. Unlike the infamous sea lamprey (*Petromyzon marinus*), which are invasive in eastern North America and pose serious threats to native fish, Pacific lamprey are important for nutrient cycling and as a high-calorie, high-protein food resource for native wildlife (Wang et al. 2020). Among indigenous communities in western North America, lamprey are a prized ceremonial and medicinal resource (Jolley and Lujan 2019).

Pacific lamprey have declined precipitously throughout their native range and have been locally extirpated from some watersheds (US Fish and Wildlife Service 2019; Wang et al. 2020). This population decline has prompted development of the Pacific Lamprey Conservation Initiative, which aims to identify and address threats to lamprey populations, restore native habitats, and increase lamprey numbers in their range. Since 2012, Pacific Lamprey Conservation Initiative member organizations have been propagating Pacific lamprey for release into the wild and translocating lamprey above dams (Moser et al. 2019). Expansion of lamprey conservation efforts highlight the need for research into pathogens of Pacific lamprey and lamprey's role in disease ecology.

Disease outbreaks are rare in lamprey (Jackson et al. 2019). Previous studies have isolated several pathogens from lamprey, including *Aeromonas salmonicida*, *Aeromonas hydrophila*, *Vibrio vulnificus*, and *Renibacterium salmoninarum* (Jackson et al. 2019; Jolley and Lujan 2019). Subsequent challenge experiments with *A. salmonicida*, *R. salmoninarum*, infectious hematopoietic necrosis virus, and viral hemorrhagic septicemia virus failed to induce clinical disease in lamprey (Bell and Traxler 1986; Kurath et al. 2013; Jackson et al. 2019). The role of lamprey as vectors or reservoirs for teleost pathogens is unclear. One study showed that Pacific lamprey ammocoetes larvae did not transmit infectious hematopoietic necrosis virus or viral hemorrhagic septicemia virus to salmon (Kurath et al. 2013). However, another study indicated that river lamprey (*Lampetra ayresis*) density was positively correlated with *R. salmoninarum* infection in sympatric Chinook salmon (*Oncorhynchus tshawytscha*) and postulated that lamprey may be effective horizontal transmitters of *R. salmoninarum* (Rhodes et al. 2011).

We manually collected Pacific lamprey downstream of the weir at a federal hatchery on the Olympic Peninsula, Washington, US (47°24′0.0055″N, 124°5′59.9945″W). Two adult Pacific lamprey and 20 ammocoetes were held in three 5-gallon (19 L) buckets with a fresh flow of surface water from the creek of origin at 9 C at 10 gallons/min (38 L/min) flow for approximately 2 days before sampling. Lamprey were euthanized with tricaine methanesulfonate (750 mg/L; MS-222, Syndel, Ferndale, Washington, USA) buffered 1:1 (m/m) with sodium bicarbonate for 20 min, followed by cervical transection, per American

Veterinary Medical Association humane euthanasia guidelines (AVMA 2020). The ventral skin of each lamprey was disinfected with 95% ethanol before a ventral coeliotomy incision was made with a sterile scalpel blade. The heart and kidney were collected in sterile sampling bags (Whirl-Pak, Nasco, Fort Atkinson, Wisconsin, USA) for bacterial culture; for adults, these organs were pooled for each individual, and for ammocoetes, these tissues were pooled as five animals per sampling bag. A necropsy was performed on each lamprey; no gross lesions were documented.

Swabs from kidney and heart tissue pools were streaked onto Columbia blood plates (Hardy Diagnostics, Springboro, Ohio, USA). Plates were incubated at 20±2 C for 96 h and examined daily for growth. Isolates comprising gram-negative rods were cultured from one 5-ammocoete tissue pool and one adult tissue pool; isolates were suspected to be *Yersinia ruckeri* based on phenotypic characteristics, staining, and biochemical tests. These isolates were investigated with matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (Bruker Reference Library, Billerica, Massachusetts, USA), and both tissue pools were identified as *Y. ruckeri*. An isolate from the ammocoete tissue pool was submitted for molecular diagnosis. From that isolate, DNA was extracted using a commercial kit (QIAamp DNA Mini-Kit, Qiagen, Valencia, California, USA) per manufacturer's instructions. A portion of the 16S rRNA was amplified (LeJeune et al. 2000) and sent to a commercial vendor (Genewiz, South Plainfield, New Jersey, USA) for sequencing. BLAST (National Center for Biotechnology Information 2020) was used to analyze the consensus sequence, and the ammocoete pool was confirmed as *Y. ruckeri*, with 100% sequence identity match with GenBank accession number MK290740. On biochemical testing, this isolate fermented D-sorbitol, classifying the isolate as a type II strain (Welch 2020).

Although primarily a pathogen of salmonids, *Y. ruckeri* has been isolated from bacterial infections in several nonsalmonid fish species, including eel (*Anguilla anguilla*), minnow (*Pimephales promelas*), goldfish (*Carassius auratus*), Nile tilapia (*Oreochromis niloticus*), carp (*Cyprinus carpio*), catfish (*Ictalurus punctatus*), sturgeon (*Acipenser* spp.), perch (*Perca fluviatilis*), and rudd (*Scardinius erythrophthalmus*; Wrobel et al. 2019). *Yersinia ruckeri* is the causative agent of enteric redmouth disease (ERM), which is responsible for large economic losses in salmonid culture worldwide (Tobback et al. 2007). Infection is spread by direct contact with infected animals or by carriers; disease induction in salmonids is more likely under conditions of increased stress. The bacteria is ubiquitous in some freshwater environments, and in some environments a considerable proportion of salmonid populations are subclinical carriers. One study found that up to 25% of rainbow trout (*Oncorhynchus mykiss*) carry *Y. ruckeri* in their intestines without clinical signs (Tobback et al. 2007).

The *Y. ruckeri* isolate detected in this study was identified as a type II strain. This is an emerging biogroup worldwide (Welch et al. 2011). Type II strains are more serologically diverse than the sorbitol-negative Hagerman strain (type I) and include up to five different serotypes (Welch 2020). Characterization beyond the *Y. ruckeri* strain type was not completed for this survey. Vaccination is an important tool for ERM management in salmonid culture; biotype II carries distinct antigenic differences from biotype I, which significantly reduce the success of currently available monovalent ERM vaccines (Tinsley et al. 2011).

Yersinia ruckeri has not previously been detected in lamprey to our knowledge. Although we detected no evidence that *Y. ruckeri* is pathogenic to Pacific lamprey, further study is necessary to investigate the pathogen-host relationship. It also remains unclear what role Pacific lamprey have in transmitting *Y. ruckeri* to susceptible fish species.

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