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Rapid Communication

Classroom citizen scientists discover red swamp crayfish *Procambarus clarkii* (Girard, 1852) from northern Idaho, United States

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Abstract

Citizen scientists may provide unique opportunities for surveillance and early detection of management-relevant biological invasions. Here, we report results of a classroom citizen science program that detected the first record of the invasive red swamp crayfish *Procambarus clarkii* (Girard, 1852) from northern Idaho, United States. Secondary school students trapping storm water ponds in a public park of Lewiston, Idaho detected an unusual crayfish that they identified as *P. clarkii*. We confirmed this identification by crayfish morphology and mitochondrial DNA barcoding. Management agencies were promptly notified and have initiated monitoring and removal efforts for this population, which directly neighbors a major river in the study region. *Procambarus clarkii* may be well-suited to citizen science surveillance by classroom programs because of its tendency to invade freshwater ecosystems like storm water ponds in urban and suburban landscapes.

Key words: Columbia River; community science; invasive species; mtDNA barcoding; Pacific Northwest; Snake River; Washington State

Introduction

Non-native crayfish species have become increasingly prevalent in the Pacific Northwest (northern California, Idaho, western Montana, Oregon, Washington State) of the United States over recent decades (Larson and Olden 2011; Pearl et al. 2013; Egly and Larson 2018). Many of these non-native crayfishes have subsequently become invasive (Blackburn et al. 2011), spreading rapidly in their non-native ranges (e.g., Messager and Olden 2018) and negatively impacting or harming native species through mechanisms like competition for habitat (e.g., Hanshew and Garcia 2012). Non-native crayfishes have been introduced to the Pacific Northwest through a variety of pathways and vectors, including stocking for sport fish management (Sheldon 1989) and possible releases of laboratory organisms by classroom

students (Larson and Olden 2008). Releases associated with live seafood, the aquarium trade, contaminated fish shipments or stocking, and fishing bait are also possible causes of crayfish invasions in this region (Larson and Olden 2011). Early detection of new crayfish invasions is critical for rapid responses to contain, control, and potentially eradicate these populations before they spread and impact native species or ecosystems (Vander Zanden et al. 2010; Manfrin et al. 2019).

Invasion biologists and managers increasingly recruit and train citizen scientists to facilitate surveillance and early detection of biological invasions (Larson et al. 2020). Primary and secondary school teachers and their students may be particularly well-suited to conduct invasive species surveillance as part of environmental science or other classroom curricula (Delaney et al. 2008; Tarter et al. 2019). We report here a novel instance of classroom citizen scientists discovering a population of the invasive red swamp crayfish *Procambarus clarkii* (Girard, 1852) from northern Idaho, United States. These classroom citizen scientists were participating in The River Mile's Crayfish Study, a program in which schools and their students monitor native and non-native crayfish populations throughout the Pacific Northwest (https://therivermile.org/network-projects/the-river-mile-crayfish-study/). The River Mile, a program of the United States National Park Service and Lake Roosevelt Forum, trains participating schools and students in crayfish sampling, identification, and data management as part of a classroom curriculum. The River Mile Crayfish Study has previously documented spread of non-native virile crayfish *Faxonius virilis* (Hagen, 1870) in the Pacific Northwest (Larson et al. 2020). However, the discovery of *P. clarkii* reported here is the first record of this species from northern Idaho and may represent the only established population in the Snake River watershed, a major tributary of the large, downstream Columbia River (Naiman et al. 2012).

Procambarus clarkii is native to the southern United States and northeastern Mexico, but has become a globally widespread invasive species (Oficialdegui et al. 2019) with well-documented impacts on native species and ecosystems (Matsuzaki et al. 2009; Twardochleb et al. 2013). *Procambarus clarkii* has previously established populations in the Pacific Northwest in western Oregon (Pearl et al. 2013) and western Washington State (Larson and Olden 2008). Conversely, *P. clarkii* has only been documented once from Idaho in 1975 at a location approximately 320 km south of our study (Clark and Wroten 1978; Figure 1). *Procambarus clarkii* found by Clark and Wroten (1978) may have failed to establish a permanent population, as Egly and Larson (2018) did not find this species during extensive sampling throughout southern Idaho in 2016–2017. Other populations of *P. clarkii* in the Pacific Northwest are isolated hundreds of kilometers downstream from our study location by a series of large navigation and hydropower dams on the Columbia and Snake rivers that are likely impassable to crayfish (but see Welsh and Loughman 2015). As such,

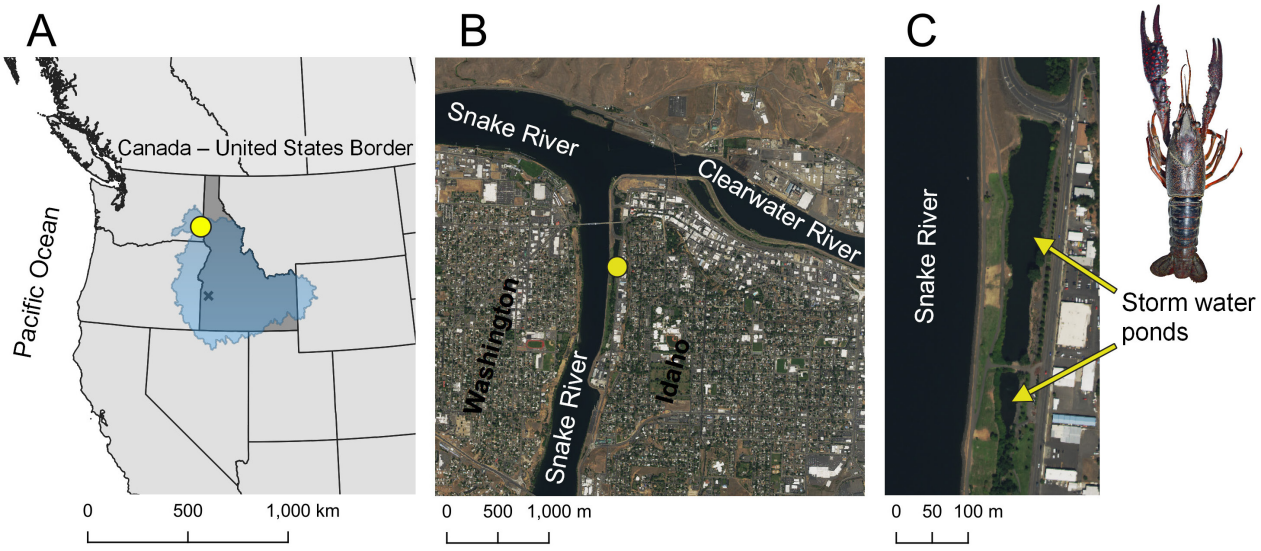

Figure 1. *Procambarus clarkii* detection (yellow) in Idaho (dark gray) within the Snake River watershed (blue) relative to previous Clark and Wroten (1978) detection of *P. clarkii* (black x) from southern Idaho (A). Kiwanis Park storm water ponds relative to the Snake and Clearwater rivers (B, C). The Snake and Clearwater rivers are impounded at this location by Lower Granite Dam approximately 50 km downstream, but structures visible in imagery are highway and railroad bridges that are passable to freshwater organisms. 2019 imagery data from United States Department of Agriculture Farm Service Agency Aerial Photography Field Office. Maps created using QGIS 3.14.0-Pi.

discovery of non-native *P. clarkii* from northern Idaho is a novel threat to the culturally, economically, and ecologically important Snake River (Naiman et al. 2012), including its three native crayfish species of the genus *Pacifastacus* (Larson and Olden 2011). Further, the entire *Procambarus* genus is on Idaho's prohibited species list, which makes commerce, possession, or transportation of *P. clarkii* illegal locally (Idaho Administrative Procedures Act 02.06.09, 2022).

Given the ecological and regulatory consequences of *P. clarkii* discovery in northern Idaho and the Snake River watershed, we sought to confirm our morphological identification with molecular sequencing, which is atypical for The River Mile's Crayfish Study. This molecular sequencing also allowed us to compare *P. clarkii* from Lewiston, Idaho to other non-native populations of the species from a recent global phylogeographic study (Oficialdegui et al. 2019). Our study represents a notable range expansion of *P. clarkii* into a new region, while simultaneously demonstrating the potential for classroom citizen scientists to detect invasive species like *P. clarkii* (Larson et al. 2020).

Materials and methods

Secondary school students at Lewiston High School, Idaho began participating in The River Mile's Crayfish Study in 2019. Lewiston High School students sample for crayfish using traps (cylindrical steel mesh traps of 43 cm length and 23 cm diameter with two 5 cm openings) baited with commercial canned cat food or canned corn. Traps are set overnight and recovered the following day (approximately 24 hours). Lewiston High School students

Figure 2. First *Procambarus clarkii* individual trapped by Lewiston High School students at Kiwanis Park storm water ponds subsequently barcoded by mtDNA. Scale bar (black) is 1 cm. Photograph by Kimberly K. Holzer.

sample for crayfish at Kiwanis Park in Lewiston, Idaho and the adjacent Snake River (Figure 1) using a maximum of five traps per night. Lewiston High School students collected only native signal crayfish *Pacifastacus leniusculus* (Dana, 1852) from the Snake River and Kiwanis Park storm water ponds on September 26th 2019, as well as only *P. leniusculus* from the Snake River on October 21st 2020 and May 12th 2021. On October 22nd 2021, Lewiston High School students recovered five crayfish traps from the Kiwanis Park storm water ponds, collecting three *P. leniusculus* and one male, 40 mm total carapace length crayfish they identified as *P. clarkii* from the smaller, south Kiwanis Park storm water pond (Latitude 46.4158, Longitude −117.0333). This identification was supported by morphological traits including red tubercules on the chelae, a triangular rostrum with accessory spines on its margins, a nearly absent areola, and a black V-shaped marking on the dorsal abdomen (Figure 2). A portion of the third walking leg of this individual was preserved in 95% ethanol and shipped to University of Illinois for mitochondrial DNA (mtDNA) barcoding to further confirm taxonomic identity (Filipová et al. 2011). Continued trapping of the Kiwanis Park storm water ponds in autumn 2021 collected six additional *P. clarkii* specimens, including three males ranging from 40 to 50 mm total carapace length and three females ranging from 40 to 90 mm total carapace length.

At the University of Illinois, DNA was extracted from the *P. clarkii* walking leg using a DNeasy Bloody and Tissue Kit (Qiagen, Hilden, Germany) following manufacturer instructions, which was then amplified for the cytochrome c oxidase subunit I (COI) mitochondrial gene on a PTC-100 thermal cycler using 0.5 µl each of forward and reverse primers from Folmer et al. (1994), 8.5 µl of water, 12.5 µl of GoTaq G2 DNA Polymerase Master Mix (Promega Corporation, Madison, Wisconsin, United States), and 3 µl of template DNA. The reaction was amplified through 35 cycles at

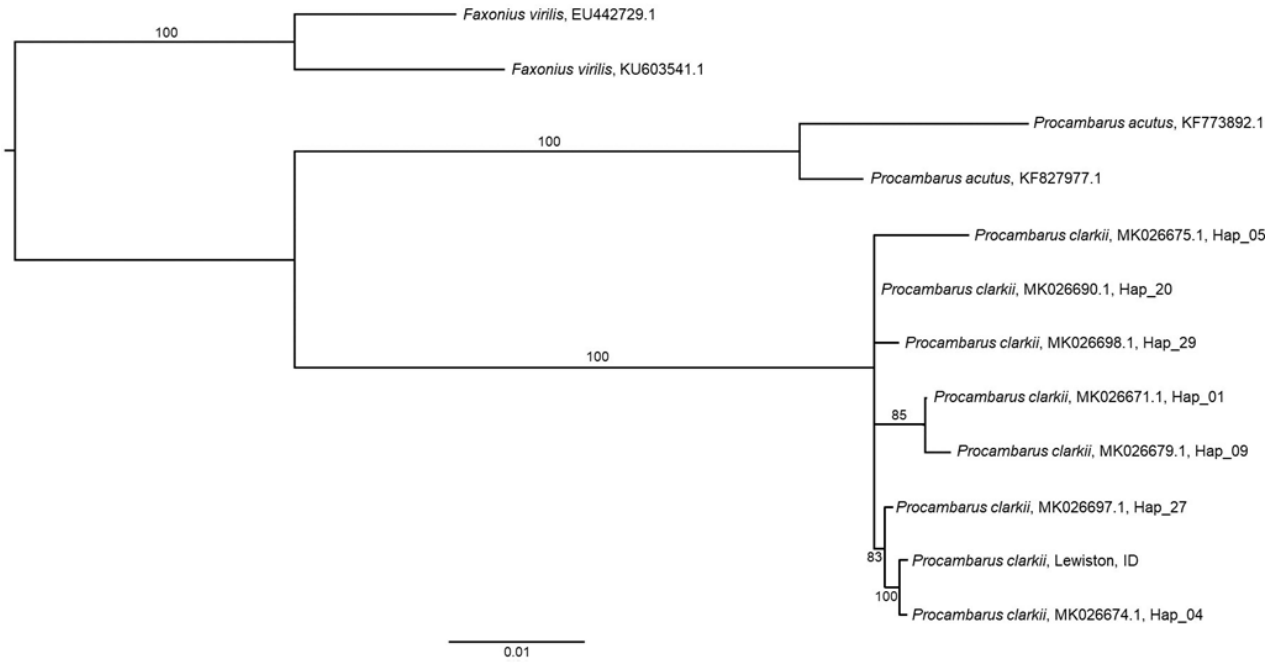

Figure 3. Neighbor joining tree with percent consensus support (branch numbers) showing *Procambarus clarkii* from Lewiston, Idaho (ID) relative to GenBank accessions for within-species sequences and outgroups within (*Procambarus acutus*) and outside of the genus (*Faxonius virilis*), along with *P. clarkii* haplotype numbers (Hap_XX) from Oficialdegui et al. (2019). Branch lengths are substitutions per site of the sequence alignment. The GenBank accession for our *P. clarkii* individual from Lewiston, Idaho is OP495632.

95 °C for 1 min, 40 °C for 1 min, and 72 °C for 1 min 30 sec followed by a final extension step at 72 °C for 7 min. Amplification was confirmed by gel electrophoresis and cleaned using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). Sanger sequencing was conducted by the University of Illinois Core Sequencing Facility (Urbana, Illinois, United States). Forward and reverse sequences were aligned and edited using Geneious Prime 2020.2.2 (Biomatters, Auckland, New Zealand) to obtain the final COI sequence. We constructed a bootstrapped (1000 iterations) neighbor joining tree using Geneious Prime on the resulting COI sequence, which included two sequences of *F. virilis* as an out-of-genus outgroup, two sequences of the white river crayfish *Procambarus acutus* (Girard, 1852) as a within-genus outgroup, and seven sequences of *P. clarkii* from Oficialdegui et al.'s (2019) global phylogeographic study of this species. These sequences were the most common, global haplotypes of *P. clarkii* from Oficialdegui et al. (2019), including common haplotypes documented from populations of *P. clarkii* in western Oregon (Pearl et al. 2013) and western Washington State (Larson and Olden 2008). We acquired sequences for the neighbor joining tree from GenBank (National Center for Biotechnology Information, Bethesda, Maryland, United States).

Results and discussion

Our COI sequencing confirmed the crayfish collected from the Kiwanis Park storm water pond as *P. clarkii* (Figure 3), with a 100% match to the most common haplotype (Hap_04) documented from populations in

western Washington State per Oficialdegui et al. (2019). This suggests that *P. clarkii* might have been introduced to the Kiwanis Park storm water ponds from a population in western Washington State, or that it may originate from similar source populations, vectors, and pathways as these other Pacific Northwest invasions (e.g., biological supply per Larson and Olden 2008). This is the first record of *P. clarkii* from northern Idaho, although species distribution modeling has consistently predicted that *P. clarkii* could establish populations at lower elevation locations in Idaho and the adjacent Pacific Northwest (Capinha et al. 2011; Larson and Olden 2012; Zhang et al. 2020), and the species has previously been demonstrated to overwinter in colder climates than its native range (Veselý et al. 2015; Smith et al. 2018; O'Shaughnessey et al. 2021). Proximity of the Kiwanis Park storm water ponds to the Snake River, a major Columbia River tributary, threatens freshwater ecosystems and native species of the Pacific Northwest with subsequent spread of *P. clarkii* (Naiman et al. 2012).

Our authorship team alerted state and federal natural resource management agencies with shared jurisdiction in October 2021, and these government agencies have progressed from subsequent monitoring to an ongoing eradication attempt of this apparently established population in 2022. Management agencies and policy makers should continue to be proactive in preventing further *P. clarkii* invasions and secondary spread in the Pacific Northwest, as this invasive crayfish is well-documented to prey on native amphibians (Gamradt and Kats 1996), compete with native crayfishes (Hanshew and Garcia 2012), and trigger shifts in shallow lakes and wetlands from clear water to turbid states through the destruction of aquatic macrophytes (Matsuzaki et al. 2009). Recent work proposes *P. clarkii* may even affect human health by releasing mosquito vectors of disease from other aquatic macroinvertebrate predators that this crayfish consumes (Bucciarelli et al. 2019). Popularity of *P. clarkii* in the live animal trade as food, pets, and laboratory specimens has made the species difficult to manage and regulate (Oficialdegui et al. 2020), but its well-documented impacts as an invasive species should motivate proactive prevention of introduction and secondary spread (Twardochleb et al. 2013).

Non-native crayfishes have been commonly used as live laboratory organisms in primary and secondary schools of the Pacific Northwest, and releases of these organisms by students or teachers have been previously hypothesized as possible sources for crayfish invasions in this region (Larson and Olden 2008; Larson and Olden 2011). Alternatively, we illustrate here a role for primary or secondary schools and their science curricula in providing early warning of new biological invasions. Crayfish trapping by Lewiston High School did not detect *P. clarkii* in the Kiwanis Park storm water ponds in autumn 2019 or the adjacent Snake River in 2020, but detections of multiple *P. clarkii* individuals in autumn 2021 suggests successful early detection of a high priority invasive species population.

Although not a routine part of The River Mile's Crayfish Study, our work also demonstrates a complimentary role for molecular surveillance of biological invasions in citizen science programs (see also Meyer et al. 2021). Biological invasions continue to accumulate at a rate that exceeds the monitoring or management capacity of many government agencies (Mormul et al. 2022). Accordingly, new approaches to the surveillance and early detection of these biological invasions may be needed, including citizen science (Larson et al. 2020).

Invasive crayfishes like *P. clarkii* may be well-suited to surveillance by primary or secondary school students participating in citizen science programs. For example, *P. clarkii* often invades freshwater ecosystems in urban or suburban landscapes like storm water ponds and small lakes (Larson and Olden 2013; Smith et al. 2018; O'Shaughnessey et al. 2021). The storm water ponds of Kiwanis Park in Lewiston, Idaho are publicly accessible freshwater ecosystems that can be conveniently sampled by classroom citizen scientists, while simultaneously serving as important beachheads for subsequent spread into adjacent ecosystems like the Snake River (Rothlisberger and Lodge 2013). Leveraging the potential of classroom citizen scientists to monitor for new biological invasions requires both developing citizen science programs that advance classroom learning objectives (Roche et al. 2020), while simultaneously communicating detections of new biological invasions to organizations with jurisdiction to coordinate rapid management responses (Vander Zanden et al. 2010; Larson et al. 2020).

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Authors' contribution

Research conceptualization, sample design, and data collection were coordinated under The River Mile Crayfish Study, which is overseen in Idaho by the IDAH$_2$O Master Water Steward citizen science water quality monitoring program. JAE and RJR serve as program managers for The River Mile Crayfish Study, assisted by ERL as a scientific adviser. JPE administers the IDAH$_2$O Master Water Steward citizen science water quality monitoring program of University of Idaho Extension's Water Outreach. JLM supervised data collection by Lewiston High School students, including RB and EC, who collected and identified *P. clarkii* from the Kiwanis Park storm water ponds in 2021. KKH provided additional morphological verification of the initial *P. clarkii* identification. JHH performed DNA extractions and phylogenetic tree analysis at the University of Illinois. JLM, KKH, and ERL wrote the first manuscript draft and prepared figures, and all authors contributed to subsequent edits and revisions.

Ethics and permits

Crayfish were collected under Idaho Department of Fish and Game permit F-19-03-21.

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