# Portland's Keystone Crustacean: Signal Crayfish Behavior, Health, & Habitat in the Tryon Creek Watershed

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## Abstract

In the Portland Metro Area, the signal crayfish (*Pacifastacus leniusculus*) is a keystone species. Signal crayfish consume inaccessible plant matter and detritus, feed various game fish, engineer riverbeds, and are even harvested commercially for human consumption. Recognizing signal crayfishes' multifaceted ecological and social significance, the purpose of this study was to assess the habitat factors that impact signal crayfish health and behavior in the Tryon Creek Watershed. Data collection revealed that overall, Tryon Creek crayfish were disproportionately likely to be observed in locations with high infrastructure presence levels; substrates composed of silt/sand or a combination of boulders and cobbles; culverts, runs, and pools; and water depths between 10 and 39 cm. Meanwhile, juvenile crayfish were disproportionately likely to be observed in locations with low infrastructure presence levels, substrates composed of silt/sand and a combination of cobbles and gravel, and water depths between 0 and 19 cm. Similarly, unhealthy crayfish — specimens that were deceased, immobile, or struggling, or that consisted of severed appendages — were disproportionately likely to be observed in locations with culverts and riffles and water depths between 0 and 19 cm. The study found no evidence for an established population of invasive crayfish in Tryon Creek, although further monitoring (particularly in Tryon Cove) are necessary to ensure the watershed's continued protection. The analysis also suggested that human-caused ecosystem disruptions can seriously decrease the health and wellness of signal crayfish populations, pointing to a need for better waterway designs that can benefit fish and crustaceans alike.

## Keywords

signal crayfish, native species, ecological restoration, population monitoring, Tryon Creek, Willamette River

## Introduction

Aquatic ecosystems are a longstanding pillar of the unique cultural, economic, and natural environment of Oregon's Portland Metro Area. Nearly 70 percent of Oregonians live within 33 kilometers of the Willamette River, a 300-kilometer waterway which bisects the Portland Metro Area and comprises a key piece of the region's sociocultural connectedness, economic activity, and vital natural resources (U.S. Army Engineer Institute for Water Resources, 2022). The Willamette River, along with its many tributaries, provides resources for countless communities and attracts hikers, swimmers, fishers, and wildlife enthusiasts from every corner of the state.

To this end, one of the Willamette River basin's most significant features is the expansive stock of game fish that call its waters home: salmon, trout, bass, bluegill, bullhead, and crappies (Oregon Department of Fish and Wildlife, 2022). The state's fishing industry garnered $152 million in onshore landings in 2020 (Knoder, 2021) — and all before factoring in the additional revenue from non-industrial/recreational fishing and ecotourism.

Due to the important economic and sociocultural roles that these game fish play, they tend to be at the center of the state's aquatic research, monitoring, and management efforts. Game fish populations are carefully monitored by experts across the region, and environmental agencies and organizations regularly undertake projects to enhance piscine health and habitat. In fact, as of January 2022, 100 percent of the publicly-available projects implemented through the Oregon Department of Fish and Wildlife's Corvallis Research Lab either partially or exclusively featured salmonids (Oregon Department of Fish and Wildlife, 2022).

Funnelling already-scarce funding and resources to support species with high market and social value is not, on its face, an unreasonable endeavor. However, this piscine-centric approach has, in many cases, led to aquatic species that are perhaps less charismatic, but just as ecologically vital, being overlooked and underreaserched. In the case of certain aquatic organisms, the epistemological imbalance is particularly pronounced. Here, I aim to widen the lens through which ecologists can understand and manage local ecosystems by examining a keystone species that is seldom brought to the forefront of the Portland Metro Area's ecological community: the signal crayfish (*Pacifastacus leniusculus*).

Before beginning my study, I first conducted a literature review to establish the current body of knowledge surrounding signal crayfishes' ecological role in the Portland Metro Area. This literature review facilitated a better understanding of both the strengths and limitations of general and regionally-specific signal crayfish research. Next, I collected and analyzed my own field data; I used these results to theorize about the state of signal crayfish behavior, health, and habitat in the Tryon Creek watershed, a subwatershed of the Portland Metro Area's broader Willamette River basin.

## Literature Review

The signal crayfish is the only crayfish species native to the Willamette River basin. Named for the distinguishing light patch found over the hinge of each claw (Riegel, 1959; Larson & Olden, 2011), signal crayfish can be found in various freshwater bodies across the Columbia River Basin and its tributaries, which span much of Oregon, Washington, Idaho, and British Columbia (Larson et al., 2012). Signal crayfish are benthic and can adapt to life amongst various types of substrate, though they are known to prefer boulders, cobbles, and woody debris (Lowery & Holdich, 1988). The coloration of signal crayfish ranges from bright red to brown to blue, and their claws and carapace are fairly smooth, lacking the pronounced bumps found on most other species of crayfish (Riegel, 1959; Larson & Olden, 2011).

In waterways throughout the Portland Metro Area, the signal crayfish makes a common meal for many types of local game fish. In the lower Willamette River, signal crayfish are a primary or significant food source for northern pikeminnow (*Ptychocheilus oregonensis*), largemouth bass (*Micropterus salmoides*), smallmouth bass (*Micropterus dolomieu*), yellow perch (*Perca flavescens*), and other predatory species (Friesen, 2005).[1] Signal crayfish also play a parallel role in human food chains, where they are commercially harvested for human consumption. Oregon's crayfish industry has been on the rise in recent years, bringing in $376,000 in 2020 even as commerce plummeted statewide (Knoder, 2021). As in the case of fishing, these numbers are conservative; they do not account for the additional value that harvests provide to non-industrial/recreational crayfishers.

For their own part, signal crayfish are omnivorous generalists, consuming plant matter, detritus, small fish, frogs, and even other crayfish. These varied dietary preferences allow signal crayfish to fulfill multiple important ecosystem services: they both control aquatic plant proliferation (Goldman, 1973) and link otherwise inaccessible energy sources (e.g., decomposing organic matter) with larger aquatic predators (Larson & Olden 2011). Through this process, crayfish bolster species abundance and diversity by acting as "conduit[s] of energy" (Reynolds et. al, 2013).

Signal crayfishes' undeniable impacts on trophic networks are not the only mechanism by which they influence their surrounding environment. Signal crayfish are ecosystem engineers, a title earned for their ostensibly inconsequential habit of burrowing into the riverbeds where they reside. While signal crayfish may be small, these habitual sediment-influencing practices can dramatically impact an aquatic ecosystem's structural composition (Clifford et al., 2013).

However, signal crayfish populations in the Portland Metro Area are under threat, placing this species and the valuable ecosystem services that they provide in jeopardy. Invasive red swamp crayfish (*Procambarus clarkii*) have been sighted in bodies of water throughout the Tualatin River Basin — including the Portland Metro Area's Summer Creek, Terrace Lake, and Commonwealth Lake — and ringed, rusty, and virile crayfish (*Faxonius neglectus*, *Faxonius rusticus*, and *Faxonius virilis*) have been reported elsewhere across the state, as demonstrated in Fig. 1 (iMapInvasives, 2022).

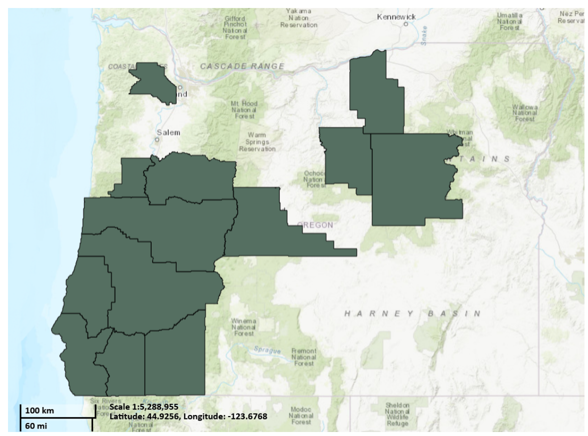



*Figure 1. Oregon counties with reported sightings of invasive crayfish species (iMapInvasives, 2022 © 2022 NatureServe).*

While the Portland Metro Area has not yet seen a comprehensive investigation on the localized impacts of invasive crayfish, studies of other aquatic environments paint a foreboding picture: historically, nonnative crayfish species have led to a plethora of environmental, cultural, and economic issues as they proliferate. One study found that the presence of red swamp crayfish was correlated with decreases in native crayfish harvest, crop production, genetic diversity of native ecosystems, primary production, nutrient cycling, macrophytes, larval amphibians, low-trophic-level native species, shelters, and traditional fishing practices and recipes.

Conversely, it was correlated with increases in erosion, predation on invertebrates, benthic algae, wildlife disease spread, human pathogen spread, structural damage to banks, and turbidity (Manenti et al., 2019). Further, non-native crayfish directly harm native crayfish poplations through competition and pathogen introduction (Hanshew & Garcia, 2012; Sorenson, 2012), reducing native crayfish numbers and, on occasion, resulting in local extinction events (Manenti et al., 2019).

These impacts translate to real-world costs, many of which are borne by local communities. In Vilas County, Wisconsin, for instance, rusty crayfish cost the sport-fishing industry $1.5 million in damage every year. Once invasive populations become established, there is no cheap or easy fix. Removing rusty crayfish from five small bodies of water in Wyoming cost the state over $34,000 (Oregon Sea Grant, 2011), and despite these efforts, the species is once again making a comeback in the area (Newman, 2021).[2] These empirical cases suggest that monitoring and other measures to prevent emerging nonnative species from becoming established are key.

Yet despite the numerous reports of nonnative crayfish sightings in Oregon, little research has been done on behavior, health, or habitat of crayfish in the Portland Metro Area, much less on the presence of nonnative species. While no crayfish data for the region were publicly available, I was able to access two unpublished datasets through correspondence with the City of Portland's Bureau of Environmental Services. The first dataset contained general location and identification information for 138 signal crayfish samples that were collected across the Portland Metro Area over a span of six years. The second displayed a couple of simple, cumulative totals, demonstrating that 279 signal crayfish samples were collected on July 28 and September 28 of 2020 at the Southwest Boones Ferry Road Bridge construction site (City of Portland Bureau of Environmental Services, personal communication, 2021). While these data provided tentative evidence against the existence of a widespread, well-established invasive crayfish population in the Portland Metro Area, the dozens of regional invasive crayfish sightings reported by other sources could not be discounted, either. In addition, the data lacked specificity in certain areas of interest (e.g., crayfish behavior).

In the realm of publicly-accessible information on local crayfish patterns, I was only able to find one relevant database: the River Mile Network's Crayfish Project. Notably, the Crayfish Project database indicated that a virile crayfish had been found at Oxbow Regional Park in February of 2022; if confirmed, this would mark the first publicly-known virile crayfish sighting in Oregon. In 2020 and 2021, meanwhile, numerous virile crayfish were found in the Columbia River (The River Mile Network, 2022), which is confluent with the Willamette River and could theoretically allow virile crayfish to cross into the Willamette River basin. However, featuring only a small handful of data from the Portland Metro Area and relying heavily upon difficult-to-confirm submissions from community volunteers, the River Mile Network's Crayfish Study initiative is still limited in scope and cannot be used to draw firm scientific conclusions.

Thus, in light of the insubstantial body of published research on the Portland Metro Area's native and invasive crayfish populations, and in order to increase public knowledge and awareness about this keystone species, the central goals of this study were to:

(1) Create a database of crayfish and habitat observations across several diverse aquatic sites, including:
   (a) photographs and coordinates of each site
   (b) identications, quantifications, and qualifications of crayfish species

through a low-impact (no-take, photography-based) documentation process

(c) documentation of habitat factors (infrastructure presence; vegetation nativity; substrate composition; habitat type; geographical location; and water temperature, pH level, alkalinity level, and depth) surrounding each crayfish observation

(2) Determine whether there was a link between habitat factors and the presence of a healthy crayfish population in the study area

(3) Establish whether there was an established population of invasive crayfish in the study area

## Materials and Methods

### Site selection

My site-selection process was centered around two main parameters. Firstly, the stretch of aquatic habitat I sampled from could not be surrounded by privately-owned land: not only would the involvement of private landowners greatly complicate the data collection process, it would also make any findings significantly less actionable. Secondly, the stretch had to be diverse: I wanted my data to be representative of crayfish populations across a variety of habitat types. With these two parameters in mind, I determined Tryon Creek State Natural Area (Fig. 3) to be an optimal location for data collection because of both its publicness and its multiformity within a small geographic range (650 acres). Surrounded mostly by state- and city-owned land, Tryon Creek weaves through pedestrian and road bridges, beaver dams, wetlands, and culverts, working its way through a wide variety of substrates before ultimately joining its course with the Willamette River.

In order to attain a diverse set of data within the Tryon Creek watershed, I selected eight field sites that represented various habitat types, substrate compositions, vegetation types, geographical locations, and levels of infrastructure presence. Six of the eight sites fell within the official boundaries of Tryon Creek State Natural Area. The remaining two sites were located just upstream and just downstream of Tryon Creek State Natural Area; both fell within 300 meters of the park's boundaries. The relative locations of these sites are pinpointed in Fig. 2.


*Figure 2. Precise locations of eight field sites (Google, n.d.).*

*Figures 4A, 4B and 4C. Three different vantage points of Site I.*

Site I — the Southwest Boones Ferry Bridge site (45°26'49.0"N, 122°41'14.3"W) — was one of two research sites intersected by a well-trafficked road (Fig. 4A). Site I was recently subject to a year-long construction project in which the City of Portland replaced a section of Southwest Boones Ferry Road (which, at the time, rested atop a culvert) with a new, 38-meter bridge. The project concluded in April 2021 (City of Portland, 2021) and included the removal of the road foundation and the reconstruction of a riparian habitat, which entailed placing boulders, engineering pools, replanting native vegetation, and installing large woody debris (Fig. 4B, Fig. 4C).

*Figures 5A, 5B, and 5C. Three different vantage points of Site II.*

Site II — the North Creek Beaver Dam site (45°26'40.8"N, 122°41'01.5"W) — was the only site not in close proximity to a pedestrian bridge, road bridge, or culvert. It was primarily characterized by the presence of two beaver dams (Fig. 5A, Fig. 5B), which caused significant pooling in multiple spots along the narrow run (Fig. 5C).

*Figures 6A, 6B, and 6C. Three different vantage points of Site III.*

Site III — the High Bridge site (45°26'29.9"N, 122°40'50.4"W) — was distinguished by its abundance of log jams (Fig. 6C) and thickets of vegetation that jutted out over the streambank (Fig. 6A). Site III was home to the natural area's most-traversed pedestrian bridge (Fig. 6B).

*Figures 7A, 7B, and 7C. Three different vantage points of Site IV.*

Site IV — the Highway 43 Culvert site (45°25'26.5"N, 122°39'39.0"W) — consisted of the culvert running beneath Highway 43 (Fig. 7A, Fig. 7B) and the habitat directly upstream of the culvert (Fig. 7C). The site was located near sewage infrastructure, the odor of which was evident. The culvert contained recently-retrofitted baffles installed to assist with fish passage (City of Portland Bureau of Environmental Services, 2022).

*Figures 8A, 8B, and 8C. Three different vantage points of Site V.*

Site V — the Nettle Creek Bridge site (45°25'41.1"N, 122°40'44.4"W) — was home to the natural area's southernmost pedestrian bridge (Fig. 8A). Site V was also the only site located along Nettle Creek, one of Tryon Creek's tributaries. This section of Nettle Creek was characterized by a slight dropoff just downstream of the bridge (Fig. 8B) and dense English ivy (*Hedera helix*) (Fig. 8C).

*Figures 9A, 9B, and 9C. Three different vantage points of Site VI.*

Site VI — the Iron Mountain Bridge site (45°25'53.0"N, 122°40'21.5"W) — was located just upstream of the confluence of Tryon Creek and Nettle Creek. Site VI contained the natural area's easternmost pedestrian bridge and an abundance of grasses and low-lying vegetation (Fig. 9B, Fig. 9C). The run was particularly wide and fairly shallow throughout (Fig. 9A).

*Figures 10A, 10B, and 10C. Three different vantage points of Site VII.*

Site VII — the Beaver Bridge site (45°26'20.5"N, 122°40'47.5"W) — was unique in the amount of plant matter debris that pervaded its section of the creek (Fig. 10C). Branches, leaves, and logs cluttered the water and shoreline (Fig. 10A). Site VII was intersected by one of the natural area's central-most footbridges (Fig. 10B).

*Figures 11A, 11B, and 11C. Three different vantage points of Site VIII.*

Site VIII — the Tryon Cove site (45°25'26.2"N, 122°39'39.0"W) — was located immediately beneath the Highway 43 culvert (Fig. 11C). At the end of the culvert, the creek spilled into a deep, still pool (Fig. 11A), which then ran downstream through a field of boulders to reach the Willamette River (Fig. 11B).

**Data collection**

From September 11 through September 18 of 2021, I collected habitat data and conducted crayfish counts at the eight field sites. I studied one site per night for a duration of 35 minutes each, beginning at the start of nautical twilight and concluding at the end of nautical twilight. Sites were centered around a set of coordinates (the "entry point" for the survey), and surveying was constrained by temporal rather than metric boundaries.

During these study periods, I collected two different levels of data: site-level data (i.e., measurements taken once per site) and individual-level data (i.e., measurements taken individually for each unique crayfish observation). Site-level data consisted of geographical location (coordinates); infrastructure presence; and water temperature, pH level, and alkalinity level. Individual-level data accounted for vegetation nativity (around the observation), substrate composition, habitat type, water depth, crayfish species, crayfish health, crayfish maturity (juvenile or adult), and crayfish behavior. My methodology for collecting each data type can be found below, numbered 1-14.

(1) I photographed sites and crayfish using a Fujifilm FinePix HS10 and an iPhone 11 (Fig. 4-12).
(2) I scored infrastructure presence by assigning each site a number between 1 and 3. “3” indicated that a site contained/was crossed by a road bridge or a culvert, “2” indicated that a site contained/was crossed by a wooden pedestrian bridge, and “1” indicated that a site contained/was crossed by no significant man-made structures.
(3) I scored vegetation nativity by assigning the clusters of vegetation found on the riverbanks parallel to each specimen a number between 1 and 5. “5” indicated that the vegetation was entirely/almost entirely native ($r > \sim 0.8$ native), “4” indicated that the vegetation was mostly native ($\sim 0.6 < r < \sim 0.8$ native), “3” indicated that the vegetation was about equal parts native and invasive ($\sim 0.4 < r < \sim 0.6$ native), “2” indicated that the vegetation was mostly invasive ($\sim 0.2 < r < \sim 0.6$ native), and “1” indicated that the vegetation was entirely/almost entirely invasive ($r < \sim 0.2$ native). These assessments were made onsite based on a visual survey of creekside vegetation.
(4) I identified substrate composition (which I defined as the substrate found directly beneath each specimen) in accordance with the “Streambed Substrate Characterization Guide” (Leverich, 2021). I assigned each substrate type a number corresponding with its size: bedrock was “5,” boulders were “4,” cobbles were “3,” gravel was “2,” and silt and sand were “1.” If the substrate where a specimen was found consisted of multiple material sizes, I took the average of those numbers.
(5) I identified the habitat type where each specimen was found per the “Stream Biology” guide (Cave, 1998). I recognized four different types of habitat: pools, runs, riffles, and culverts.
(6) I measured water depth alongside the specimen with a standard tape measure.
(7) I measured water temperature via a standard digital thermometer.
(8) I measured water pH level via an AquaChek test strip.
(9) I measured water alkalinity level via an AquaChek test strip.
(10) I collected location data with the “My GPS Coordinates” mobile application (Neal, 2018). The signal accuracy of the readings ranged between 4-13 meters.
(11) I classified crayfish specimens that were deceased, immobile, or struggling, or that consisted of severed appendages, as “unhealthy.”
(12) I classified crayfish maturity broadly, assigning individuals as either “juveniles” ($L < \sim 3$ cm) or “adults” ($L > \sim 3$ cm). I did not find any adult crayfish just above (or anywhere) near the 3-cm threshold; all adults found were at least 100 percent larger than all juveniles found.
(13) I recorded notable or unusual behavior under the guidance of “Behavior of Crayfish” (Jurcak-Detter et. al, 2016).
(14) I determined the species of each specimen with help from the “Crayfish Found in Oregon” booklet (Oregon State University, 2011).

Overall, I collected data on 93 crayfish specimens, 91 of which I went on to include in my analysis. I elected to exclude the data I collected at the Tryon Cove site because I had sampled from the previous seven sites during a dry period (September 11 through September 17), but a heavy rainfall occurred between the evening of September 17 and the evening of September 18. The rainfall significantly increased the creek’s turbidity, reducing in-water visibility and inhibiting my photography-based documentation methods. Furthermore, summer rainfall and other abrupt changes in weather can affect crayfish behavior (Brown et. al, 2020). Given this unexpected summertime rain event's potential to distort the data I collected from Site VIII — and thus, skew the overall dataset — I did not include these datapoints in my computations.

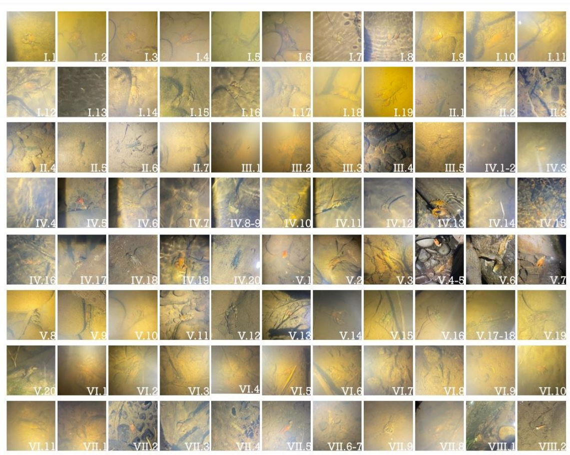


*Figure 12. Crayfish photo-documentation gallery.*


*Figure 13. Excerpt from crayfish photo-documentation gallery.*

**Data analysis**

I analyzed overall crayfish counts in terms of infrastructure presence; vegetation nativity; substrate composition; habitat type; geographical location; and water temperature, pH level, alkalinity level, and depth. My initial analysis allowed me to determine which habitat factors were most likely to influence overall population size. I then tested smaller population subsets (determined by crayfish health and maturity) against these same habitat factors.

In order to calculate the test statistics and p-values, I used Microsoft Excel. For the categorical data (infrastructure presence, vegetation nativity, substrate composition, habitat type, and water depth), I ran a chi-square test of independence:

$$\chi^2 = \sum (((0 - E)^2) / (E))$$

For the continuous data (water temperature, pH level, and alkalinity level), I ran a Pearson correlation test:

$$r = (\sum(x_i - \bar{x})(y_i - \bar{y})) / ((\sum(x_i - \bar{x})^2 \sum(y_i - \bar{y})^2)^{.5}).$$

For both analyses, I used the standard scientific alpha-value of 0.05.

For the chi-square tests of independence, my methodology for finding the expected frequencies/null hypotheses varied based on the metric I was analyzing. Water depth and substrate composition were the only categorical variables that I assumed to be distributed relatively evenly across all of the research sites; this judgment was based upon preliminary surveying of the sites and my understanding of the creeks' characteristics and dynamics. I also assumed habitat type to be distributed relatively evenly, with one exception: culverts. Culverts are not naturally-occuring habitats like pools, runs, and riffles, and because there was only one culvert habitat that was featured at one field site out of the seven I analyzed in my study, I manipulated the expected frequencies to reflect that I could expect to find more crayfish overall in pool, run, and riffle habitats (an equal amount in each) than in culvert habitats.

However, per the preliminary surveying and my understanding of botany, vegetation nativity could *not* be assumed to be evenly distributed across all sites. To get an accurate picture of how different types of vegetation might be distributed, I reviewed the photographs I had taken of each site and used them to assess the nativity of each site's vegetation; I cross-referenced these with the individual-level data I collected on vegetation for accuracy. Based on these estimations, I was able to quantify the approximate proportion of overall sites that fell into each of the five nativity categories. From there, I calculated the overall number of crayfish I could expect to find if this null hypothesis were true.

Finally, since infrastructure presence was a site-level variable, the null expectation was that the overall number of crayfish found at each level of infrastructure presence would be directly proportional to the number of sites displaying each level of infrastructure presence.

Once these analyses of the overall crayfish population were complete, I was able to use them as a framework for calculating the null proportions for subsets of the overall population (such as unhealthy or juvenile crayfish). I used the actual overall number of crayfish associated with each type of habitat factor as a baseline for the expected frequencies of unhealthy and juvenile populations. This method allowed me to determine whether subsets of the population were found in disproportionate abundance relative to the actual overall crayfish abundance in each category.

To generate a geographical representation of crayfish distribution throughout the watershed, I ran the "Tryon-Creek-Watershed-Heatmap" code in R (Aghdaei, 2021). I used Visme to create all other graphs.

## Results

### Infrastructure presence

Disproportionately fewer juvenile crayfish observations were recorded in areas with high to moderate infrastructure presence, and more were recorded in areas with low infrastructure presence ($\chi^2$ = 0.9997, df = 2, p = 0.0005). However, disproportionately more overall crayfish observations were recorded in areas with high infrastructure presence, and less were recorded in areas with moderate to low infrastructure presence ($\chi^2$ = 0.9970, df = 2, p = 0.0061). Observations of unhealthy crayfish were not significantly disproportionate by infrastructure presence level. These distributions are reflected in Fig. 14.

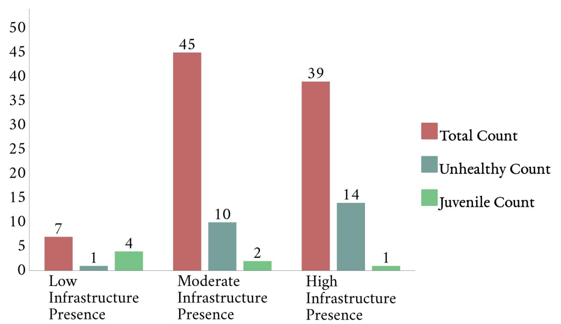



*Figure 14. Effect of infrastructure presence on overall crayfish observations, unhealthy crayfish observations, and juvenile crayfish observations.*

### Vegetation nativity

Overall, unhealthy, and juvenile crayfish observations were not significantly disproportionate among levels of vegetation nativity. These distributions are reflected in Fig. 15.

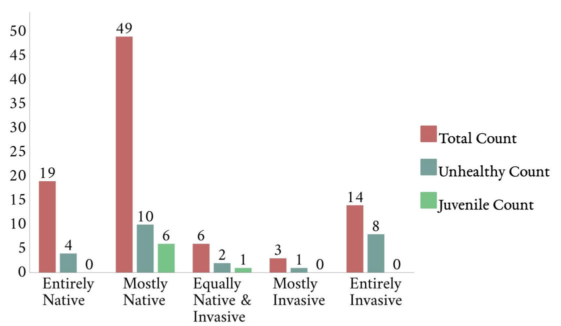



*Figure 15. Effect of vegetation nativity on overall crayfish observations, unhealthy crayfish observations, and juvenile crayfish observations.*

### Substrate composition

Disproportionately more juvenile crayfish observations were recorded in substrates composed of silt/sand and a combination of cobbles and gravel, and fewer were recorded in substrates composed of boulders, a combination of boulders and cobbles, cobbles, gravel, and a combination of gravel and silt/sand ($\chi^2$ > 0.9999, df = 6, p = 0.0219). Meanwhile, disproportionately more overall crayfish observations were recorded in substrates composed of silt/sand and a combination of boulders and cobbles, and fewer were recorded in substrates composed of boulders, cobbles, a combination of cobbles and gravel, gravel, and a combination of gravel and silt/sand ($\chi^2$ > 0.9999, df = 6, p < 0.0001). Unhealthy crayfish observations were not significantly disproportionate across substrate types. Notably, no crayfish observations were recorded in areas composed of bedrock or a combination of boulders and bedrock. These distributions are reflected in Fig. 16.

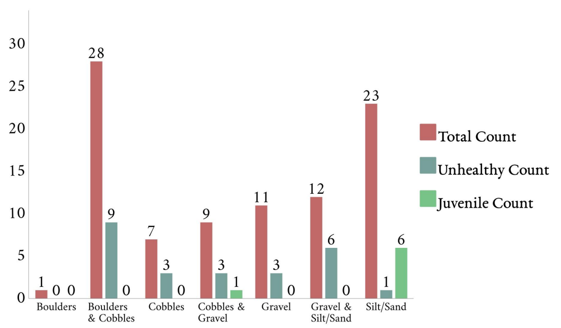



*Figure 16. Effect of substrate composition on overall crayfish observations, unhealthy crayfish observations, and juvenile crayfish observations.*

## Habitat type

Disproportionately more unhealthy crayfish observations were recorded in culvert and riffle habitats, and fewer were recorded in pool and run habitats ($\chi^2$ > 0.9999, df = 3, p = 0.0003). Further, disproportionately more overall crayfish observations were recorded in culvert, pool, and run habitats, and fewer were recorded in riffle habitats ($\chi^2$ > 0.9999, df = 3, p < 0.0001). Juvenile crayfish observations were not significantly disproportionate across habitat types. These distributions are reflected in Fig. 17.

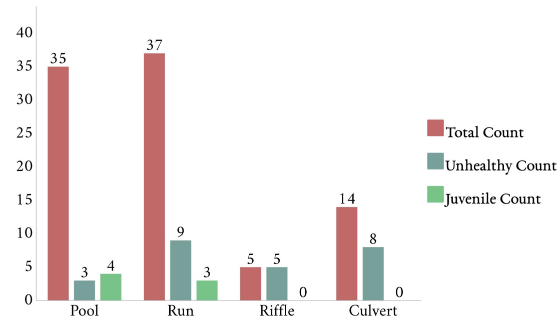



*Figure 17. Effect of habitat type on overall crayfish observations, unhealthy crayfish observations, and juvenile crayfish observations.*

## Water depth

Disproportionately more overall crayfish observations were recorded in water depths between 10 and 39 cm, and fewer were recorded in water depths between 0 and 9 cm and 40 and 59 cm ($\chi^2$ > 0.9999, df = 5, p < 0.0001). Additionally, disproportionately more unhealthy and juvenile crayfish observations were recorded in water depths between 0 and 19 cm, and fewer were recorded in water depths between 20 and 59 cm ($\chi^2$ > 0.9999, df = 5, p < 0.0001 and $\chi^2$ > 0.9999, df = 5, p = 0.0273). These distributions are reflected in Fig. 18.

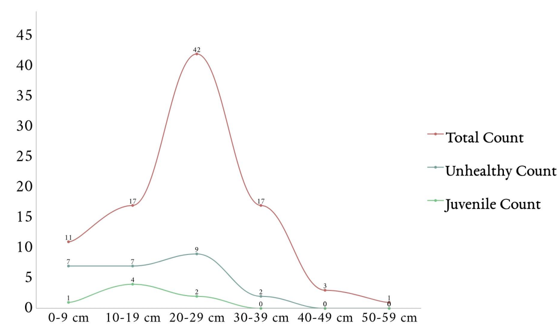



*Figure 18. Effect of water depth on overall crayfish observations, unhealthy crayfish observations, and juvenile crayfish observations.*

## Water temperature

Overall, unhealthy, and juvenile crayfish observations were not correlated with water temperature. These distributions are reflected in Fig. 19.

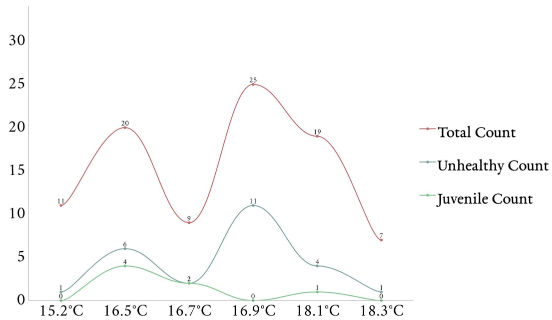



*Figure 19. Effect of water temperature on overall crayfish observations, unhealthy crayfish observations, and juvenile crayfish observations.*

**Water pH level**

Overall, unhealthy, and juvenile crayfish observations were not correlated with water pH level. These distributions are reflected in Fig. 20.

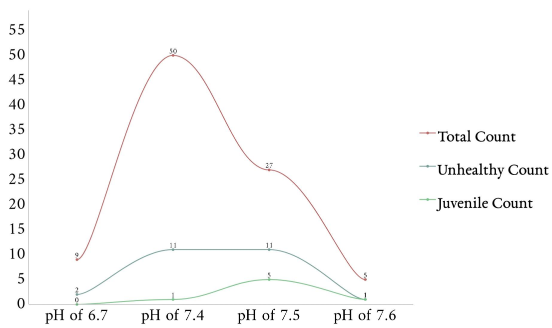



*Figure 20. Effect of water pH level on overall crayfish observations, unhealthy crayfish observations, and juvenile crayfish observations.*

**Water alkalinity level**

Overall, unhealthy, and juvenile crayfish observations were not correlated with water alkalinity level. These distributions are reflected in Fig. 21.

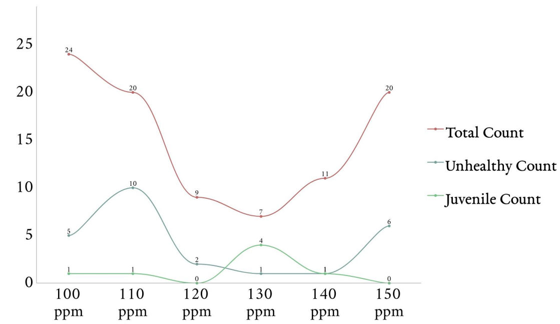



*Figure 21. Effect of water alkalinity level on overall crayfish observations, unhealthy crayfish observations, and juvenile crayfish observations.*

**Geographical location**

Fig. 22 provides a graphical depiction of crayfish distribution throughout the Tryon Creek watershed. Because the geographical range of this study was fairly small (the most polar sites were less than four kilometers apart), precise geographical location was not considered an independently influential habitat factor for the purposes of this study and was not analyzed for statistical significance in Excel. However, the hypothesis that geographical location is a driver of crayfish patterns cannot be rejected, and thus, geography is still a variable to consider, even if its effects cannot be tested within this study.

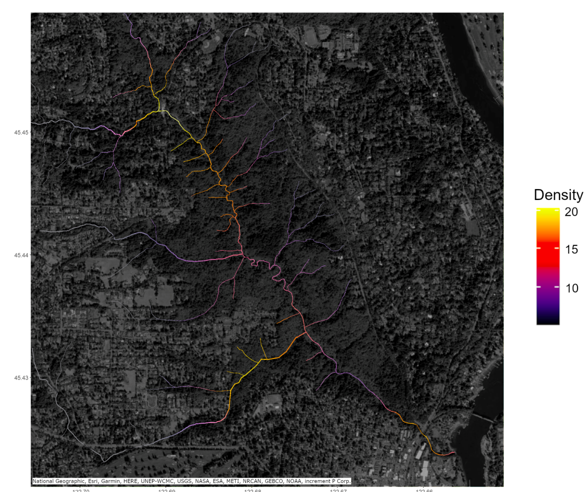
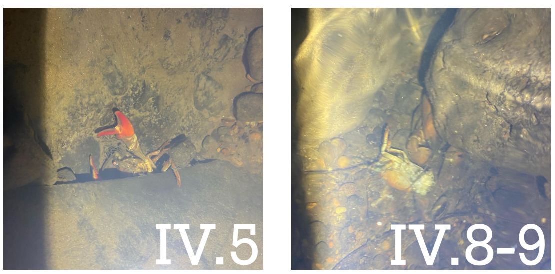



*Figure 22. Geographical distribution of crayfish density in Tryon Creek watershed.*

**Crayfish behavior**

Crayfish behavior was not analyzed for statistical significance due to the low number of crayfish that were observed demonstrating each notable or unusual behavior type. In all, 13 crayfish behaved notably or unusually (14 percent of the overall count). Amongst these 13 crayfish, documented behaviors including burrowing (four), approaching or aggressing a human (three), feeding (two; one was feeding on another crayfish (Fig. 23B) and the other was feeding on an unidentified piece of flesh), struggling to escape the crevice between a baffle and culvert wall (two (Fig. 23A)), crawling out of the water along the edge of a baffle (one), and attacking a feeding crayfish (one).




*Figures 23A and 23B. Crayfish struggling to escape crevice between baffle and culvert wall (23A) and crayfish feeding upon another crayfish (23B); both documented inside culvert.*

Even without statistical analysis, a few key consistencies can be noted. Every crayfish exhibiting burrowing tendencies was found in a run and was digging into a substrate composed of silt/sand or a combination of silt/sand and gravel. Crayfish also tended to burrow in shallower water; while overall crayfish specimens were found in water depths up to nearly 60 cm, burrowing specimens were only found in water 8.89 to 25.40 cm deep. Burrowing behavior was exhibited by both adults and juveniles.

In addition, every crayfish approaching or aggressing a human, feeding on another crayfish, struggling to escape the crevice between a baffle and culvert wall, and crawling out of the water along the edge of a baffle were found in the culvert habitat.

**Crayfish species**

Unlike the categorizations of "unhealthy" and "juvenile," "species" was not ultimately considered to be a defining crayfish population subset because no crayfish was positively identified as belonging to an invasive species of crayfish (red swamp, ringed, rusty, or virile). 53 crayfish observations were positively identified as signal crayfish. The remaining 38 observations could not be definitively categorized as belonging to any of the five species — signal, red swamp, ringed, rusty, or virile.

## Discussion

**Limitations**

The sampling for this study took place from September 11 to September 18 of 2021; eight sites (of which seven were ultimately deemed appropriate for comparative analysis) were sampled from for a duration of 35 minutes each. Therefore, while more than 90 individual crayfish specimens were included in this study, the data do not depict seasonal or temporal variation. Rather, they provide a snapshot of the state of crayfish populations in the Tryon Creek watershed in September of 2021.

For these reasons, this study should only be used to substantiate *preliminary* hypotheses about invasive species presence, natural habitat preference, and impacts of human-caused ecosystem disturbances. Its results should be interpreted in the context of the in-stream survey's limited duration and sampling methodlogy; in other words, the potential for sampling error or inaccurate representation of a site due to chance should not be overlooked. While the sample size was fairly high, data collection was not replicated across a significant period of time.

Further limitations arose from the no-take approach I used: I could not definitively identify the species of all of the specimens I observed, and I also struggled with visibility in certain substrate compositions and water depths. To address these shortcomings, future research utilizing catch-and-release methods would be a welcome addition to the body of scientific literature on signal crayfish in the Tryon Creek watershed. Physically handling the crayfish, though a more intrusive procedure, would help ensure the accuracy of the data and open up new avenues for analysis; these catch-and-release methods would allow for specimens to be measured, weighed, definitively identified, and photographed with higher clarity.

Finally, the data I collected from Tryon Cove, which I excluded from my analysis due to weather inconsistencies, may have had significant implications for this study if conditions had been different. Tryon Cove was selected as a key research site because it marks the confluence of Tryon Creek and the Willamette River. It is unknown whether invasive crayfish species currently reside in the Willamette River proper, but if they do, Tryon Cove would be the sole natural avenue through which they could enter the Tryon Creek watershed. Thus, future monitoring in Tryon Cove is crucial to help determine whether or not this invasive takeover may be beginning to occur.

**Presence of invasive species**

One of the central questions this study sought to answer was whether or not there was an established invasive crayfish population in the Tryon Creek watershed. Of the crayfish specimens I observed throughout the duration of this study, none was positively identified as belonging to an invasive crayfish species. While these results are by no means conclusive — due to my no-take approach, I could not definitively confirm the species of every single crayfish I observed — they do provide some level of evidence against the presence of invasive crayfish species in Tryon Creek and underscore the importance of continuing to closely monitor the area. As outlined in the introduction, prevention is crucial, and it is much easier to obstruct an invasive species from becoming established in the first place than it is to attempt to eradicate an established invasive species post hoc.

**Natural habitats**

Overall, crayfish were disproportionately likely to be observed in substrates composed of silt/sand or a combination of boulders and cobbles, in pools or runs, and in water between 10 and 39 cm deep. Meanwhile, juvenile crayfish were disproportionately likely to be observed in substrates composed of silt/sand or a combination of cobbles and gravel and in water between 0 and 19 cm deep. Similarly, unhealthy crayfish were disproportionately likely to be observed in riffles where the water was between 0 and 19 cm deep.

These findings indicate that while Tryon Creek crayfish may be observed across various classes of substrate, they appeared to completely avoid bedrock, which provides minimal shelter and camouflage. This result corroborates the current scientific understanding that crayfish prefer habitats with an abundance of cracks and crevices in which they can hide from predators (Lowery & Holdich, 1988).

Crayfish tended to be observed in fairly shallow water, a trend which was exacerbated for juvenile and unhealthy crayfish in particular. Healthy crayfish were typically found seeking shelter in pools and runs rather than fast-moving, shallow riffles. And while unhealthy crayfish were conversely more likely to be observed in riffle habitats, this phenomenon can most probably be attributed to the stream's deposition of corpses, severed appendages, or weakened crayfish unable to fight the current rather than the notion that sick or dying crayfish made an active choice to migrate to riffles. Further, my no-take approach may have skewed the overall amount of crayfish I observed in deeper water; visibility decreased as depth increased. However, because I modeled my expected frequencies for unhealthy and juvenile crayfish counts based on my real overall counts, statistical significance can still be inferred for the disproportionate distribution of these subsets.

Lastly, while crayfish counts across pH level, alkalinity level, and water temperature were not found to be disproportionately distributed, it should be borne in mind that these ranges did not vary greatly across sites, and thus, broader conclusions should not be drawn about the ecological (un)importance of these factors. For instance, none of the field sites had pH levels lower than 6.7 or higher than 7.6., all testing within what is generally understood to be the "healthy range" for aquatic life (United States Environmental Protection Agency, 2021). Therefore, it should not be assumed that water conditions falling outside the typical "healthy" range for aquatic life would not significantly affect crayfish distribution.

**Human-caused ecosystem disturbances**

Overall, crayfish were disproportionately likely to be observed in areas that had high infrastructure presences and culverts. This finding may initially appear to suggest that the Tryon Creek crayfish population is fairly resilient in the face of human-caused ecosystem disturbances. After all, the crayfish population at Site I — the Southwest Boones Ferry Bridge site — was robust despite their recent removal and relocation for the construction project. However, if ecological preservation had not been a priority in this project, the long-term impacts on the local crayfish population may have looked drastically different. The Southwest Boones Ferry Bridge work involved in-stream isolation, fish salvage, and habitat construction; it also took place just downstream of a major confluence (A. Barton, personal communication, 2022; S. Myers, personal communication, 2022). The crayfish population was able to bounce back quickly from the Boones Ferry disturbance in this instance, but perhaps only because it was well-placed, carefully-managed, and left behind a restored aquatic habitat to return to.

I also found that juvenile crayfish were most likely to be observed in areas that had low infrastructure presence levels. This disproportionate distribution suggests that, while crayfish may be able to tolerate and survive human-caused ecosystem disturbances in the short term, they may prefer to reproduce and raise their young in relatively undisturbed natural areas. A healthy crayfish population is one that reproduces, so the significance of juveniles being primarily found in sections of the creek that were less impacted by humans cannot be overlooked.

On another note, it is important to consider that the large number of crayfish counted in the Highway 43 culvert does not necessarily indicate that the population had a preference for culvert habitats; in fact, quite the opposite may be true. Even after controlling for overall population trends, unhealthy crayfish were disproportionately likely to be observed in culverts. Despite making up less than 10 percent of the habitat area analyzed in this study (only Site IV contained a culvert, and this culvert did not cover the entire site), the Highway 43 culvert contained 32 percent of the total unhealthy crayfish population. In other terms, 57 percent of the crayfish found in the culvert were classified as unhealthy, as opposed to 22 percent in any other habitat type. Overall, crayfish found in culverts were more than 2.5 times as likely to be unhealthy as crayfish found in pools, runs, and riffles.

When examined from a behavioral standpoint, the culvert's suitability as a crayfish habitat continues to deteriorate. Apparent entrapment beneath an object (in these cases, a baffle in the Highway 43 culvert) was a behavioral event that was exclusively observed within the culvert. While the sample size of these occurrences was far too small to draw definitive conclusions, they do support the possibility that crayfishes' disproportionate habitation of culverts may not be fully voluntary. Indeed, for a benthic crustacean, the tiered, waterfalling structure and extensive length of the retrofitted culvert may be difficult to navigate, disorienting, and provide no clear route for escape. In addition, only culvert-dwelling crayfish were witnessed feeding on other crayfish and approaching or attacking humans, providing a potential indication that culvert living conditions increase crayfish aggression. The only visible food sources in the culvert were similarly trapped organisms; there was no vegetation and very few places to hide. Once again, the sample size was small, but the behavioral responses I observed do fall in line with the current scientific understanding of crayfish survival instincts. According to prior research, agonistic behavior in crayfish is influenced by multiple factors, including competition for food and shelter (Bergman & Moore, 2003) and habitat complexity (Corkum & Cronin, 2004).

**Next steps**

These findings suggest that the largest current barrier to a healthy Tryon Creek ecosystem may well be the Highway 43 culvert. The Highway 43 culvert, like many of the outdated culverts of today, was designed and constructed early in the 20th century, when engineering standards for infrastructure were different and scientists did not thoroughly understand the implications of these structures on stream biota and physical processes. In the 1920s when the Highway 43 culvert was built (Macuk, 2017), culverts were sized according to their required flow capacity and did not always take wildlife passage, sediment dynamics, and floodplain systems into account. Modern culverts tend to be at least three times larger than their 20th-century predecessors and incorporate wildlife amenities like native stream backfill and strategically-placed boulders (Washington County Land Use & Transportation, 2022). They also tend to have lower gradients and avoid perched outlets, better allowing for the passage of aquatic organisms.

The effort to remove and replace the Highway 43 culvert with a better crossing has been underway for nearly two decades. However, due to the high cost of the project (forecasted between $11 and $30 million) and the involvement of multiple levels of governmental and private stakeholders, the project remains in the federal appropriations process (A. Barton, personal communication, 2022). In the interim, the City of Portland Bureau of Environmental Services and the Oregon Department of Transportation worked in collaboration to improve piscine access to Tryon Creek by retrofitting the Highway 43 culvert; during the summer of 2008, they installed a new baffle system and raised the water level downstream of the culvert (City of Portland Bureau of Environmental Services, 2022). Between 2005 and 2019, the City of Portland and the United States Fish and Wildlife Service conducted an extensive research report to monitor the restoration response of historically-present fish species to these watershed modifications (Silver et al., 2020; Silver et al., 2017).

The piscine focus of governmental research and rhetoric noted above suggests that, for at least the last 17 years, the ecological narrative surrounding the Highway 43 culvert has been dominated by fish welfare priorities. However, my findings indicate that adopting a narrow ecological lens can lead to problematic outcomes. While the multidimensional importance of native fish in the Willamette River basin cannot be understated, the doctrine that "what's good for the salmon is good for everyone" may be due for a few revisions. Most notably, the data I collected at the Highway 43 culvert depicts several stark issues with the retrofitted structure. I found that culvert-dwelling crayfish were disproportionately likely to be unhealthy, and violent behaviors such as human-targeted aggression and cannibalism were observed at inordinate levels. Perhaps most striking were the crayfishes' interactions with the baffles themselves: at multiple points in the culvert, crayfish were found wedged in the space between the baffle and the culvert wall, unable to escape the narrow crevice when faced with the strength of the generated current. These baffles, designed with fish passage in mind, seemed to be trapping, sickening, and killing crayfish — a keystone, ecosystem-engineering species, no doubt, but one whose welfare is deprioritized in a piscine framework.

In their pursuit of ensuring holistic ecosystem welfare, ecologists must adapt their practices to accommodate a broader range of species: fish, yes, but other aquatic organisms, too. After all, not only are signal crayfish significant contributors to fish welfare, providing a steady source of food and sculpting habitats for their piscine counterparts, they also deserve to be recognized as an independently-valuable native species in their own right — one that naturalists have much to learn from.

In order to maximize in-stream welfare for all aquatic species (fish, signal crayfish, and beyond), the current culvert should be replaced with a bridge or significantly wider culvert with a natural bottom. The practical function of the baffles should instead be served or bolstered by a natural substrate. Shade-tolerant, native vegetation should be introduced along the riverbank wherever possible, providing a source of food for herbivorous and omnivorous creatures like signal crayfish and helping to keep the creekwater in a clean, healthy balance. If executed in a similar manner to the Southwest Boones Ferry Road project, signal crayfish and fish populations should rebound quickly and ultimately make a full recovery (City of Portland Bureau of Environmental Services, 2021).

My findings indicate that signal crayfish specifically would benefit from the introduction of a variety of non-bedrock substrate types, providing mediums for crayfish of various sizes to seek shelter or burrow within. If stream reconstruction occurs, the resulting waterway should include plenty of mid-depth pools and runs (ranging from 10 to 39 cm, with shallower areas for juveniles) and few extremely-shallow riffle areas that may not provide cover or navigability. These habitat preferences, aggregated with the habitat preferences of other native species, should be optimized to provide a baseline from which to rebuild waterways in Tryon Creek.

However, there is at least one complicating factor that ought to be taken into account during the implementation of such a project. In the status quo, the Highway 43 culvert still inhibits upstream passage for multiple native species of fish, even despite its retrofits. As a result, it most likely shuts out invasive species (such as the rusty, red swamp, ringed, and virile crayfish) as well. Therefore, it should not be overlooked that the installation of a more passable bridge or culvert could also increase the likelihood that invasive species ascend into the park. Replacing the old culvert will provide a net ecological benefit for the Tryon Creek watershed, but only if ecologists are willing to put in the monitoring and prevention work necessary to preempt the introduction and establishment of invasive (crayfish or other) species in the creek.

Of course, the Tryon Creek watershed is not the only site where signal and invasive crayfish can and should be studied further. In my literature review, I also identified potentially-established invasive crayfish populations in the Tualatin River Basin and other watersheds across Oregon. Further, little is known about the specific regional dynamics, services, issues, and needs of the native signal crayfish. Oregon's ecologists still have much to learn about these unique crustaceans, and communities must take coordinated action to ensure their protection from invasive adversaries and human-caused disturbances.

## Conclusion

According to my analysis, there is no current evidence for an established population of invasive crayfish in Tryon Creek. This finding is optimism-inspiring, especially when juxtaposed with the broader, more worrying trend in Oregon: invasive crayfish species are continuing to spread quietly through other parts of the Willamette and Columbia River Basins, largely undetected and unimpeded. For now, though, Tryon Creek State Natural Area still appears to provide a haven where signal crayfish can thrive without threat of outcompetition.

However, my study should only serve to draw preliminary conclusions; it should not be used to unilaterally surmise that there are no invasive crayfish in the watershed or that certain habitat factors are definitively more important than others. For one, my data collection only took place at eight different sites on eight different days in September of 2021. I was also unable to positively identify 38 of the crayfish specimens I found, so it is not unfeasible that one or more of those unidentified specimens belonged to an invasive species of crayfish. Additionally, I excluded my findings downstream of the Highway 43 culvert (the Tryon Cove site) from the overall analysis, yet this site may be amongst the most pivotal in terms of invasive species infiltration into the Tryon Creek watershed. Therefore, I recommend that additional research and monitoring efforts be focused on this confluence and emphasize that further sampling and conservation work are necessary to ensure the watershed's continued protection.

Despite their limitations, however, my findings do suggest that human-caused ecosystem disturbances can seriously decrease the health and wellness of a crayfish population if not managed correctly, pointing to a need for better waterway designs that are safe and usable for fish and crustaceans alike. To this end, I advise that the Highway 43 culvert be replaced by a bridge or a wide culvert with a natural bottom. Once this project is complete, habitat restoration efforts could benefit local crayfish by incorporating some or all of the following habitat factors into their plans:

(1) Substrates composed of silt/sand, a combination of cobbles and gravel, and a combination of boulders and cobbles
(2) A mixture of runs and pools
(3) Water depths between 0 and 39 cm
(4) Healthy, moderate pH levels, alkalinity levels, and water temperatures

All in all, this study highlights the need for additional governmental and organizational investment in monitoring and restoration endeavors surrounding native signal crayfish and their invasive counterparts. As funding and awareness increase, ecologists will become better armed to protect this unique community of keystone crustaceans and the freshwater aquatic habitats that they call home.

## Notes

(1) It should be noted that the largemouth bass, smallmouth bass, and yellow perch populations found in Oregon today originated on the East Coast. Thus, these nonnative species' consumption of signal crayfish does provide a potential limiting factor on the ecological benefit of signal crayfish as prey. However, it should also be acknowledged that bass and perch have been established in the region for well over a century (U.S. Geological Survey, 2022; Lampan, 1946) and that they contribute heavily to Oregon's fishing economy and culture.
(2) Even signal crayfish, for all the benefits they bring to the Portland Metro Area, can cause devastating damage when introduced to areas outside their native range: across Europe, the signal crayfish has cost nations a cumulative $103 million

(Kouba et al., 2022).

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