Phenotypic variability of rusty crayfish (Faxonius rusticus) at the leading edge of its riverine invasion

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Abstract
1. Species around the globe are undergoing phenotypic shifts at ecologically relevant timescales as they invade new ecosystems and respond to changing environments. Disentangling the contribution of environmental gradients from the process of range expansion in shaping these changes, and identifying the specific traits undergoing selection, are both critical to anticipate the secondary spread and impact of invasive species.

2. Here, we investigate phenotypic changes in rusty crayfish (Faxonius rusticus), a nuisance invasive species, through an extensive survey of their invasion gradient in multiple tributaries of the John Day River (JDR, Oregon, U.S.A.), a major tributary of the Columbia River.

3. Rusty crayfish in the JDR have developed better physiological condition (intrinsic growth and/or reproductive potential measured as RNA/DNA ratio) but less competitive morphology (lighter body and smaller claws) as they spread upstream and downstream from their location of initial introduction. In addition, rusty crayfish in invasion front populations are at a lower trophic level than conspecifics closer to core areas.

4. By accounting for variations in temperature, primary productivity, and prey (macroinvertebrates) biomass throughout the invasion extent of rusty crayfish, our findings suggest that low conspecific densities at the invasion edge and spatial sorting primarily drive these phenotypic changes. The trends observed here are thus likely to intensify over time as rusty crayfish continues to rapidly spread throughout the JDR and reach the mainstem Columbia River.

5. Our study shows that phenotypic shifts can manifest rapidly over environmental gradients experienced during the range expansion of aquatic invasive species. Patterns in both morphological and functional traits documented in the JDR demonstrate the importance of both environmental factors and dispersal processes in shaping these responses in riverine networks.

Keywords
aquatic invasive species, Faxonius rusticus, invasive crayfish, John Day River, range margin
Species around the globe are exposed to changing selection pressures as they invade new landscapes or shift their range to track environmental change (Moran & Alexander, 2014). When their geographic distribution shifts or expands, the individuals in the vanguard of these populations often face novel environmental conditions, predators, and competitors (Chuang & Peterson, 2016). Mounting evidence suggests that these factors, in combination with low conspecific densities relative to those experienced by core populations, promote rapid changes in species phenotypes at range boundaries (Chuang & Peterson, 2016).

Phenotypic changes at the leading edge of invasive populations’ range have been observed in many taxonomic groups, including amphibians (e.g. cane toad Rhinella marina, Perkins, Phillips, Baskett, & Hastings, 2013), insects (e.g. ground beetle Merizodus soledadinus, Laparie, Renault, Lebouvier, & Delattre, 2013), fish (e.g. round goby Neogobius melanostomus, Brandner, Cerwenka, Schliewen, & Geist, 2013), and decapods (e.g. signal crayfish Pacifastacus leniusculus, Hudina, Hock, Žganec, & Lucić, 2012). These changes have been manifested in traits ranging from body length and fecundity to boldness (Chuang & Peterson, 2016), and have been associated with accelerated invasion rates (Phillips, Brown, & Shine, 2010a; Weiss-Lehman, Huffbauer, & Melbourne, 2017) and increased impacts to recipient ecosystems (Brandner et al., 2013; Iacarella, Dick, & Ricciardi, 2015). Enhanced insight into the processes leading to phenotypic changes in invasive species is thus essential for anticipating their future spread and impact (Phillips, 2015), as well as predicting the outcome of species range shifts in response to climate change (Caplat et al., 2013; Travis et al., 2013).

Phenotypic plasticity (Davidson, Jennions, & Nicotra, 2011), natural selection (Brown, Kelehear, & Shine, 2013), and spatial sorting (Shine, Brown, & Phillips, 2011) are the three dominant processes responsible for observed trait variability at range edges, yet their respective contributions are seldom understood. Phenotypic plasticity, the ability for multiple phenotypes to arise from a single genotype in response to changing environmental conditions, is particularly prevalent in invasive species (Davidson et al., 2011). It is crucial in allowing populations to spread and adapt to changing environments faster than would otherwise be possible by evolution through natural selection alone (Chevin, Lande, & Mace, 2010). Furthermore, abiotic and biotic forces at the invasion front can lead to trait evolution by natural selection. Low intra-specific density at the leading edge is most likely to shift selective pressures towards higher growth and reproduction (Phillips, Brown, & Shine, 2010b). Lastly, the range expansion process itself can lead to adaptive changes in traits through spatial sorting, whereby the fastest dispersing individuals at the expanding edge of the population systematically interbreed, resulting in selection for enhanced dispersal ability in their offspring if dispersive traits are heritable (Shine et al., 2011). This runaway process continues in subsequent generations until trade-offs between traits begin to limit the potential for directional selection (Burton, Phillips, & Travis, 2010), although dispersal ability may evolve independently of other life-history traits (Bonte & Dahirel, 2017). The traits involved in promoting dispersal and growth at the invasion front are so numerous that natural selection and spatial sorting, when enhancing these traits, can impact morphology, physiology, behaviour, immunology, and life history, among others (Chuang & Peterson, 2016). Understanding the specific traits undergoing selection in invasive species and disentangling the influence of environmental conditions from contemporary evolution therefore requires empirical studies that are specific to the species and systems at hand.

Crayfish are among the most widely introduced freshwater animals worldwide (Lodge et al., 2012). Following their introduction, non-native crayfish can cause severe ecological impacts across entire food webs to a greater extent than native crayfish because, like their native counterparts, they have polytrophic feeding habits, but also often reach much greater densities and heightened levels of foraging activity (Hansen et al., 2013; Pintor & Sih, 2009; Twardochleb, Olden, & Larson, 2013). In invaded ecosystems, native crayfish species can be displaced within a few years, and populations of macrophytes, insects, snails, and fish often decline (Bobełdyk & Lambert, 2010; McCarthy, Hein, Olden, & Vander Zanden, 2006; Olden, McCarthy, Maxted, Fetzer, & Vander Zanden, 2006; Rosenthal, Stevens, & Lodge, 2006). Changes in population structure, behaviour, morphology, and physiology have already been reported between core and edge populations in several ongoing river invasions by crayfish (Hudina, Žganec, & Hock, 2015; Hudina et al., 2012; Pârvulescu, Pirvu, Moroșan, & Zaharia, 2015; Rebrina, Skejo, Lucić, & Hudina, 2015). At the biogeographical level, differences in growth, survival, feeding habits, and behaviour are also common among crayfish congeners between their native and non-native range, further demonstrating the potential phenotypic changes wrought by the invasion process (Glön, Resinger, & Pintor, 2018; Pintor & Sih, 2009; Resinger, Elgin, Towlce, Chan, & Lodge, 2017; Sargent & Lodge, 2014). Even though the consequences of these changes on invaded ecosystems often remain unexplored, increased invasion rates alone could challenge our ability to respond to new and ongoing crayfish invasions. In addition, given that the impact of an invasive species on the recipient ecosystem is not only a function of its range size but also of its abundance, per-capita effect, and other factors (Thomsen, Olden, Wernberg, Griffin, & Silliman, 2011), changes in its somatic and reproductive growth rates or trophic niche could have severe consequences for native communities. Knowledge of the potential phenotypic shifts occurring at the front of crayfish invasions could thus shed light on both the selection pressures exerted upon dispersing populations and on the future impact of these invasions.

In this study, we investigated changes in rusty crayfish (Faxonius rusticus, Girard 1852, previously Orconectes rusticus, Crandall & De Grave, 2017) traits across their invasion gradient in the John Day River (JDR), the only known occurrence of this species west of the North American continental divide (Olden, Adams, & Larson, 2009) and where secondary spread is ongoing (Messager & Olden, 2018).
Our objective was two-fold. We first assessed whether rusty crayfish individuals displayed phenotypic differences progressing from established core populations near the initial location of their introduction to recently colonised invasion fronts. We used a riverscape survey to analyse rusty crayfish population structure, morphology, physiological condition, and trophic position across its range in the mainstem of the JDR and its main tributaries. We hypothesised that phenotypic changes occurred across rusty crayfish generations as they dispersed from their location of introduction to their present invasion fronts in the JDR. We expected that low conspecific densities in newly invaded river sections would lead to increased access to resources and relative consumption of growth-inducing food like macroinvertebrates (Hill, Sinars, & Lodge, 1993). We thus expected that rusty crayfish would exhibit better physiological condition and higher trophic positions toward the invasion front and, as a result, larger carapace length and higher weight (Brown et al., 2013). We also posited that two additional mechanisms could affect crayfish trait values during range expansion. If larger, faster growing, and more competitive crayfish are better dispersers, as has been reported in multiple invasions (Chuang & Peterson, 2016), we expected to observe an increase in crayfish body size, relative chela length, and physiological condition towards the invasion front. By contrast, if trade-offs exist among crayfish traits, then selection for high population growth rates and faster dispersal at the invasion leading edge could lead to unexpected changes in other trait values (e.g., decreased chela length) towards the invasion front (Phillips et al., 2010b).

We then evaluated whether these phenotypic changes in populations towards the invasion front were caused by plasticity to environmental factors or selection due to range expansion. We sought to disentangle these two sets of processes by studying rusty crayfish subpopulations both upstream and downstream of their initial location of introduction, and by simultaneously accounting at each site for the distance from the invasion core (reflecting the invasion stage at that location), gradients in environmental conditions, and the availability of food resources. We hypothesised that if selection due to the range expansion process was driving changes in rusty crayfish trait values as they spread throughout the JDR, then

FIGURE 1 Regional map of the John Day River basin (JDR, inset) and relative densities of rusty crayfish along the mainstem, North Fork, and South Fork of the JDR (circle size), with the putative location of initial introduction (crayfish symbol). CPUE, catch per unit effort.
respectively, from a generalised additive model. Vertical dashed lines represent the predicted mean CPUE and 95% Bayesian credible interval, respectively, from a generalised additive model. Vertical dashed lines represent the predicted mean CPUE and 95% confidence interval (bars) of the catch per unit effort (CPUE). The smooth solid line and shaded region represent (points) and 95% confidence interval (bars) of the catch per unit effort (CPUE). The smooth solid line and shaded region represent
these phenotypic differences would become larger in sites further from the core and be greatest at the invasion fronts, regardless of whether populations spread upstream or downstream and the local gradient in environmental conditions.

2 | METHODS

2.1 | Study area

The JDR originates in the Blue Mountains of north-eastern Oregon (U.S.A.) and runs undammed for 457 river km until its confluence with the Columbia River just upstream from the Columbia River Gorges (Figure 1). One of the largest free-flowing rivers in the U.S.A. with a drainage area of 21,000 km², the JDR is of high conservation importance as it supports several fish species of significant cultural and economic value, including endangered spring Chinook salmon, Oncorhynchus tshawytscha, and threatened steelhead, O. mykiss.

Native to the Ohio River basin, rusty crayfish is a habitat generalist; it can inhabit all substrates but prefers cobble habitat, thrives both in areas of high flow and standing water, can withstand temperatures ranging from close to 0°C up to 35°C with an optimum near 22°C, and opportunistically consumes a variety of aquatic plants, benthic invertebrates, detritus, periphyton, fish eggs, and small fish (Lorman, 1980; Mundahl & Benton, 1990). Mature rusty crayfish mate in late summer, early fall, or early spring, and achieve high growth rates (Berrill & Arsenault, 1984; Lorman, 1980).

Rusty crayfish was first found in the JDR in 2005, marking its first recorded occurrence west of the North American continental divide (Olden et al., 2009). Evidence suggests that rusty crayfish were first released in the late 1990s in the mainstem JDR, about 380 km upstream from its confluence with the Columbia River, near the town of Mount Vernon, Oregon, by a teacher and students of a nearby school (Olden et al., 2009). In the c. 20 years since their presumed date of introduction, rusty crayfish have rapidly spread throughout the JDR catchment at rates exceeding 15 km/year, raising concerns that the mainstem of the Columbia River may soon be reached (Messager & Olden, 2018). Only the native signal crayfish Pacifastacus leniusculus was known to be present in the catchment prior to the introduction of rusty crayfish in the JDR (Larson & Olden, 2011). Previous studies and records from rotary screw traps operated by the U.S. Forest Service show that signal crayfish were widespread throughout the JDR catchment despite low densities prior to the introduction of rusty crayfish (Sorenson, 2012; David Wooster, Oregon State University personal communications August 2013; Keith Dehart, U.S. Forest Service, personal communications March 2016).

2.2 | Field data collection

We implemented a spatially extensive survey of rusty crayfish densities, phenotypes, and environmental conditions throughout its invasion range to capture gradients in these variables from core to leading-edge populations. We used predictions of rusty crayfish distribution in August 2016 from a spatially explicit individual-based model (Messager & Olden, 2018) to distribute 60 sampling sites every 5–10 km along the main stem and primary tributaries of the JDR, encompassing the invasion extent of rusty crayfish. Sampling was conducted 1–22 August 2016, late enough in the summer so that females would not be in berry, young-of-the-year would be large enough to be sampled, and almost all mature males would have changed to a reproductive form (form I) with larger chelae in preparation for fall mating (Butler & Stein, 1985; Hamr, 1999; Prins, 1968).

To assess the relative density of rusty crayfish across the catchment, area-standardised kick-seining was performed in six locations across a 50-m long reach at each surveyed site. One person disturbed 1 m² of substrate upstream of a seine net held by another team member to flush crayfish downstream, yielding a mean and standard deviation of crayfish density at each site. To ensure consistency in our measure of relative density, we exclusively sampled in runs (i.e. rather than pools or riffles) when possible, because runs provide the water velocity and depth needed for this sampling method to be most effective (Larson & Olden, 2016). To avoid false absences, snorkelling, hand-netting, and baited traps were also used when rusty crayfish were not detected using seining.

Where rusty crayfish were found, the sex, carapace length (mm), chela length (mm), mass (g), missing chelae (yes/no), and moulting condition (yes/no) of captured crayfish were measured at every site, while two tissue samples (abdominal white muscle) from 14 rusty crayfish were taken at every other site. Regenerating chelae, soft-shelled, or visibly smaller than the other chela, were not measured. When our standard sampling protocol yielded <14 crayfish, additional specimens were caught by hand-netting so that these measurements and tissue samples could be taken—although these individuals were not included.
in our estimates of relative density. When crayfish density was high, morphological measurements were recorded for a random subsample of 30 of the crayfish that were caught by kick-seining. The first tissue sample was immediately stored in non-iodised salt for subsequent $\delta^{15}N$ stable isotope analysis to determine the trophic position of rusty crayfish at that site—the energy-weighted number of trophic energy transfers from primary producer to crayfish (Vander Zanden & Rasmussen, 1999). We also collected 12-20 mayfly nymphs (Ephemeroptera: Heptageniidae) in runs and riffles at each study site where crayfish tissues were sampled to characterise the baseline $\delta^{15}N$ values of primary consumers throughout the JDR (Anderson & Cabana, 2007). The second tissue sample, preserved in RNAlater®, was used to quantify the relative concentration of RNA and DNA in rusty crayfish cells. While the amount of DNA remains mostly constant in cells regardless of conditions, the amount of RNA positively correlates with the amount of protein synthesis (anabolic activity). Therefore, the ratio of the amount of RNA to that of DNA in a cell is an effective eco-physiological indicator of condition (hereafter physiological condition) that reflects the organism’s potential investment in somatic growth and gamete production (i.e. fertility) under a given set of environmental conditions (Koop, Winkelmann, Becker, Hellmann, & Ortmann, 2011).

Environmental conditions at each site were characterised by measuring water depth, temperature, and velocity, as well as benthic chlorophyll a concentration of green algae and diatoms at 10 points along a transect perpendicular to the river banks. The benthic concentration of chlorophyll a is a proxy of benthic algal biomass (Dodds, Smith, & Lohman, 2002) measured using a Benthotorch (Kahlert & Mckie, 2014). The biomass of macroinvertebrates was also quantified at all sites where crayfish tissue samples were taken. The abundance of macroinvertebrates was assessed by taking three 0.09-m$^2$ standardised samples in runs and riffles with a D-frame kick net. All macroinvertebrate samples were then washed through 0.5 mm sieves and preserved in 70% ethanol.

### 2.3 Stable isotope analysis

Stable isotope analysis was conducted on rusty crayfish tissues and mayfly whole specimens to assess differences in crayfish feeding patterns throughout their invasion gradient. All samples were prepared for isotope analysis using standard protocols, with the exception of the salt-based preservation, a field-appropriate method that results in minimal and directionally uniform effects on $\delta^{13}C$ and $\delta^{15}N$ (Arrington & Winemiller, 2002). Prior to processing, all crayfish muscle tissues and mayfly whole bodies were rinsed with distilled water until the salt was dissolved. Samples were then dried at 60°C for 24 hr, ground to powder, and sent for nitrogen isotope analysis to the University of California Davis Stable Isotope Facility. The trophic position of each crayfish at site (S) was estimated according to:

$$\text{Trophic position}_{\text{crayfish,S}} = 2 + \left( \frac{\sum_{\text{mayfly,S}} \delta^{15}N_{\text{mayfly,S}}}{2.54} \right) / 3$$

where 2.54 is the rusty crayfish discrimination factor or fractionation factor ($\Delta$) representing the absolute difference in $\delta^{15}N$ between rusty crayfish and its diet, determined in laboratory based on an algae diet (Glon, Larson, & Pangle, 2015). We applied a single fractionation factor to all trophic links of the food web between primary consumers and crayfish and did not account for fractionation differences among crayfish diets due to a lack of more specific reference values.

### 2.4 RNA/DNA analysis

The procedure for the extraction and quantitation of nucleic acids in rusty crayfish tissues was adapted from Berdalet, Roldán, Olivar, and Lysnes (2005) and Vrede, Persson, and Aronsen (2002) using fluorochromes that indiscriminately bind to DNA and RNA. We provide a brief description below but refer the reader to Supporting Information Appendix S1 for a more detailed protocol. Berdalet, Roldán, and Olivar (2005) recommend using three separate aliquots of each sample to compute the quantity of RNA and DNA in crustacean tissues: the first assay measures RNA after DNA digestion; the second measures DNA after RNA digestion; and the third measures residual fluorescence after digestion of both DNA and RNA. Four nucleic acid standard curves with six concentrations each were thus run for every batch of samples: RNA + DNase, RNA + RNase, DNA + RNase, and DNA + DNase. To quantify RNA and DNA fluorescence, 50 µl of diluted (1:200) Quant-IT™ RiboGreen® reagent was added to each plate well. The slopes of the standard curves were then estimated using linear regression and the quantity of RNA ($\mu$g RNA/ml assay), DNA ($\mu$g DNA/ml assay), and their ratio in each sample was calculated using the equations provided by Berdalet, Roldán, and Olivar (2005).

### 2.5 Macroinvertebrate biomass

Macroinvertebrate ash-free dry weight (AFDW) was estimated at every other site along the length of the invasion gradient to reflect the prey biomass available for consumption by rusty crayfish, one of the main environmental drivers of crayfish trophic position (Olsson et al., 2008). Macroinvertebrates were sorted using a stereo microscope and separated from other material found in the samples. Sorted macroinvertebrates were then rinsed with distilled water and dried in an oven at 60°C for 48 hr, weighed, combusted in a muffle furnace at 550°C for 4 hr (Mason, Lewis, & Weber, 1983), cooled down to room temperature in a desiccator for 6 hr, and reweighed. Ash mass (after combustion in furnace) was then subtracted from dry mass (before combustion) to obtain AFDW.

### 2.6 Data analysis

The goal of this study was to assess whether a phenotypic shift has occurred along the invasion gradient of rusty crayfish, and whether this shift is best explained by the range expansion process or by longitudinal gradients in environmental conditions.
Environmental conditions and the speed of rusty crayfish spread differed among tributaries, so each invasion leading edge was analysed separately. In total, four leading-edge populations were analysed, one downstream edge in the mainstem JDR and three upstream edges—in the mainstem, South Fork, and North Fork JDR (Figure 1).

Six traits were analysed throughout the invasion extent of rusty crayfish: carapace length, chela length, weight, trophic position, physiological condition, and sex ratio (the proportion of males at a site). Only data for crayfish caught by kick-seining, hand-netting, and snorkelling were included in the analysis, due to the known size and sex bias of trapping for large males (Larson & Olden, 2016). To control for the strong relationship between body size, crayfish weight, and chela length due to allometric growth, residuals from carapace length-weight and carapace length-chela length non-linear regression models, developed separately for each sex, were used as response variables in the models (hereafter relative weight and relative chela length).

A subset of the variables measured at each site was selected as potential environmental predictors of crayfish trait values: the estimated number of degree days from August 2015 to July 2016 (°C), macroinvertebrate AFDW (mg), and chlorophyll a concentration from benthic green algae and diatoms (µg Chl-a/cm²). Degree days were computed based on water temperature estimated from a multiple regression model using satellite-measured daily land-surface temperature, calendar day, catchment area, and elevation as predictor variables (Messager & Olden, 2018). In situ temperature measurements were not used in this analysis, as diel temperature variations were of the same order of magnitude as differences between upstream and downstream sites. Velocity and depth measurements were not included in the analysis either due to their high spatial variability at base flow in the JDR. Only the sites for which all variables had been measured were included in the analysis for a total of 18 sites, 14 sites in the mainstem JDR, two in the South Fork JDR, and two in the North Fork JDR.

In the South Fork and North Fork JDR, where tissue samples were taken from crayfish in only two sites, differences in trait values among sites were tested using two-sample t-tests and differences in sex ratio were assessed with Yate’s χ² test. In the mainstem JDR, generalised additive models (GAMs) were developed to analyse the drivers of crayfish morphology, physiological condition, and trophic position. GAMs were built separately for two main categories of predictor variables. A first category of models was developed to account for the role of the range expansion process in driving phenotypic changes by using each site’s distance from the initial location of rusty crayfish introduction as the predictor variable. The second model category was based on environmental variables that might influence trait values. Additional models were also built using distance from the initial introduction location together with crayfish density and sex ratio as predictor variables, or combining multiple environmental variables. The significance and fit of candidate models (Akaike information criterion, the p-value of the coefficients, and the adjusted $R^2$, see Supporting Information Table S3.2) were then compared among invasion fronts to determine whether consistent patterns arose.

3 | RESULTS

3.1 | Crayfish distribution and habitat conditions

In total, 1,266 crayfish were captured across the 18 sites analysed in this study, of which 299 were sampled for morphological traits, 259 for physiological condition, and 254 for trophic position. Our survey, combined with historical distribution records and a model of rusty crayfish spread in the JDR (Messager & Olden, 2018), showed that rusty crayfish spread at an accelerating rate since its introduction and occupied at least 705 km of river across the JDR catchment in August 2016. By that summer, it had spread nearly 30 km upstream in the North Fork and South Fork JDR, and colonised the mainstem along a 250 km stretch downstream of its introduction point (Figure 1). In contrast to its extensive spread downstream, the upstream spread of rusty crayfish in the mainstem had been temporarily halted at the time of the survey due to a low-head dam 12 km upstream of the putative site of crayfish introduction. Densities downstream of the dam were similar to those found at the core of their range. Therefore, the upstream mainstem invasion leading edge was not included in this analysis. In addition, the precise location of the downstream mainstem leading edge could not be determined due to limited access to the river; thus, the downstream-most surveyed site where rusty crayfish was found in the mainstem was treated as the downstream edge of their range in this study.

There was a consistent decrease in rusty crayfish densities among the sampled tributaries from their initial location of introduction to their invasion fronts. Rusty crayfish densities (measured as kick-seining catch per unit effort, Figure 2) were highest in both the mainstem and South Fork JDR (>30 crayfish/m²) 40–75 km downstream of the initial site of rusty crayfish establishment, but rapidly dropped by an order of magnitude beyond 60 km in the South Fork JDR and beyond 80 km in the mainstem and North Fork JDR. Native signal crayfish were found in sympathy with rusty crayfish in only a few sites at the upstream invasion fronts where rusty crayfish were found at lower densities (in the South Fork, mainstem, and other smaller side tributaries of the JDR). Where native signal crayfish were present, they occurred at very low densities (<2 crayfish/m²) including in sites without rusty crayfish. These findings, together with past records (2013) of signal crayfish occurrence in sympathy with rusty crayfish at sites where signal crayfish were absent during our 2016 survey, suggest that signal crayfish is rapidly excluded from sites invaded by rusty crayfish as it spreads across the JDR catchment. Therefore, interspecific competition was not considered a significant mechanism influencing the traits investigated in this study.

Temperature and macroinvertebrate biomass followed similar longitudinal gradients from upstream to downstream between the mainstem, South Fork, and North Fork JDR, whereas benthic biomass of green algae and diatom were highly variable among tributaries (Supporting Information Figure S2.1). Degree days increased
monotonically downstream while macroinvertebrate biomass decreased downstream. There was also considerable variability in macroinvertebrate biomass among adjacent sites, including a sudden increase in biomass downstream from the confluence of the mainstem and the North Fork JDR where low crayfish densities prevailed. Green algae were sparse to absent in all tributaries. The biomass of diatoms, on the other hand, was highest in the South Fork and upper mainstem JDR (up to 4.5 μg Chl-a/cm²) and low in the lower mainstem and North Fork JDR, with inconsistent longitudinal gradients among tributaries.

3.2 | Morphology

There were consistent trends in rusty crayfish morphology from core to leading-edge populations in all tributaries (Figure 4, Supporting Information Appendix S3). The relative chela length of rusty crayfish in leading-edge populations was significantly smaller than those behind the front in all three tributaries (Mainstem: GAM, \( p = 0.03 \), \( R^2\)-adjusted = 0.28, \( n = 14 \); North Fork: \( t = 2.17, df = 29, p = 0.04 \); South Fork: \( t = 3.03, df = 42, p < 0.01 \); Figures 3 and 4, Supporting Information Tables S3.1 and S3.2). Crayfish density was also strongly and positively associated with chela length across the JDR (Mainstem: GAM, \( p = 0.01 \), \( R^2\)-adjusted = 0.37, \( n = 14 \); Figure 4, Supporting Information Table S3.2). No consistent difference was found in mean carapace length between core and invasion front populations of the JDR or between male and female crayfish; however, there was a consistent decrease in carapace length variance in the direction of the invasion in both upstream and downstream dispersing populations (Figure 4, Supporting Information Appendix S3). Crayfish relative weight significantly decreased towards the invasion front both downstream in the mainstem (GAM, \( p = 0.04 \), \( R^2\)-adjusted = 0.25, \( n = 14 \)) and upstream in the North Fork (\( W = 177, p = 0.01 \)) and South Fork (\( W = 328, p = 0.01 \); Supporting Information Figure S4.1); along with decreasing crayfish density (Mainstem: GAM, \( p = 0.02 \), \( R^2\)-adjusted = 0.33, \( n = 14 \); Figure 4, Supporting Information Table S3.2). Lastly, there was no significant trend in the proportion of males towards the fronts of the invasion despite a slight increase in male dominance in both upstream and downstream leading edges, when considering all sites where >10 crayfish were captured (Supporting Information Figure S4.2).

Environmental conditions were not strong predictors of crayfish morphology throughout their invasion gradient. Degree days did not correlate consistently across invasion fronts with any morphological trait values (Figure 4, Supporting Information Table S3.2). For instance, while decreasing upstream temperatures were positively correlated to relative weight in the South Fork and North Fork JDR, temperature and relative weight were negatively correlated in the mainstem. Similarly, inconsistent patterns were observed between temperature and both relative chela length and carapace length. Macroinvertebrate biomass (AFDW) was negatively but not significantly correlated with relative chela length and weight across tributaries and was not consistently or significantly associated with shifts in carapace length across tributaries (Figure 4, Supporting Information Tables S3.1 and S3.2). Finally, while green algae biomass was not consistently correlated with any morphological trait values, diatom biomass was positively correlated with relative chela length and weight throughout the JDR (Figure 4, Supporting Information Table S3.2).
3.3 | Trophic position

The trophic position of rusty crayfish was consistently lower for individuals at invasion fronts than in populations closer to the core, despite wide variations among sites throughout the catchment (Figures 3b and 4, Supporting Information Appendix S3). In the mainstem, rusty crayfish diet first increased downstream from that typical of a secondary consumer or omnivore (trophic position of c. 3) to that of a top carnivore (trophic position of c. 4) and then decreased towards the front of the invasion down to that of a primary consumer. 

**FIGURE 4** Summary of the relationships between rusty crayfish traits and predictor variables in the John Day River. The direction and colour of the arrows indicate the sign of the relationship (e.g. red upward arrows reflect positive relationships) between the predictor variable (columns) and the trait (rows) for a given invasion leading edge (downstream mainstem, upstream South Fork, or upstream North Fork). Grey arrows show statistically non-significant relationships. Significance and a consistent direction in the relationship between candidate predictors and traits among tributaries suggested the primacy of that predictor in driving observed differences in traits. See Supporting Information Appendix S3 for detailed statistical results. Flat green arrows show variables for which there was no difference among sites in that tributary, and relationships denoted by a – were not tested due to a lack of hypothesised mechanisms relating the variables. †Ash-free dry weight

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<th>Response variable</th>
<th>Edge</th>
<th>Predictor variable</th>
<th>Distance from introduction</th>
<th>Crayfish density</th>
<th>Degree days</th>
<th>Macroinv. AFDW†</th>
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†Ash-free dry weight
(trophic position of c. 2; GAM, p = 0.05, R²-adjusted = 0.44, n = 14; Figure 3b). There was an equivalent drop in crayfish trophic position in the North Fork JDR upstream leading edge (t = 9.66, df = 22, p < 0.001) but no equivalent decrease in the South Fork JDR leading edge. There was no difference in trophic position among male and female crayfish, and although trophic position was weakly correlated with carapace length within sites (carapace length fixed effect 95% CI = 8.0 × 10⁻³ to 1.9 × 10⁻² trophic level/mm in linear mixed effect model with site as random effect), there was no significant correlation between trophic position and mean carapace length across sites along the mainstem. Trophic position was not significantly correlated with relative chela length within sites (relative chela length fixed effect 95% CI = −8.3 × 10⁻³ to 2.3 × 10⁻² trophic level/mm in linear mixed effect model with site as random effect).

Temperature, increasing with downstream distance from the invasion source, was correlated with trophic position only towards the upstream invasion fronts in the North Fork and South Fork JDR (Figure 4, Supporting Information Appendices S2 and S3). None of the other environmental variables significantly covaried with trophic position in a consistent way across tributaries (Figure 4, Supporting Information Appendices S2 and S3).

3.4 | Growth and condition

There was a consistent positive trend in rusty crayfish physiological condition (RNA/DNA ratio) towards invasion fronts along with decreasing crayfish densities (Figures 3c and 4, Supporting Information Appendix S3). Strongest towards the upstream leading edges despite decreasing temperature (t-test, South Fork: W = 46, p-value = 0.01; North Fork: t = −4.91, df = 15.5, p < 0.001), the increase in physiological condition was only marginal in the mainstem [GAM, p = 0.19, R²-adjusted = 0.06, n = 14, mean slope = 6.3 × 10⁻³ (95% CI = −2.7 × 10⁻³ to 1.5 × 10⁻²) [RNA/DNA]/km]. The negative correlation between crayfish physiological condition (RNA/DNA ratio) and crayfish density in the mainstem was also non-significant (GAM, p = 0.43, R²-adjusted = −0.03, n = 14). Lastly, environmental variables (abiotic and biotic) were not consistently and significantly associated with RNA/DNA ratio across tributaries (Figure 4, Supporting Information Appendix S3).

4 | DISCUSSION

This study revealed significant trends in morphological trait values, physiological condition, and trophic position of rusty crayfish from their location of initial introduction to multiple leading edges of invasion in the only known population of this species in western North America. Even though these trends were not consistently significant across tributaries, they suggest that rusty crayfish individuals at the vanguard of the invasion exhibited less competitive morphology (decreased relative weight and chela length) and exploited energetic pathways lower on the food chain, yet were in better physiological condition than individuals located closer to the invasion core. We contend that these phenotypic shifts observed in rusty crayfish are probably due to the range expansion process itself rather than to novel environmental conditions at their range boundaries, and that these trends may intensify as the invasion continues to unfold upstream and downstream towards the main stem of the Columbia River.

The observed decrease in crayfish relative chela length and weight towards the invasion fronts can be attributed to three main reasons—although further research is needed to confirm the respective contribution, if any, of each of these mechanisms. First, the range expansion of rusty crayfish might be driven by the exclusion of subdominant individuals from high-density population centres, therefore leading to the widespread presence of competitively inferior crayfish at the invasion leading edge (Hudina, Hock, & Zganec, 2014). In other words, individual crayfish with less competitive phenotypes may have been forced out and systematically interbred at the invasion front, resulting in the accumulation of relatively light, small-clawed individuals at the leading edge. In support of this mechanism, signal crayfish at the boundary of their invasion range across Croatia display lower levels of aggression and often lose agonistic interactions to individuals from the invasion core, despite being in better physical condition (Hudina et al., 2015). However, a significant increase in relative claw size away from source population was also observed in invasive signal crayfish males of the Mura River, Croatia (Hudina et al., 2012). Second, larger chelae and a stouter body shape may be associated with weaker dispersal ability and thus be selected against through associative mating of the fastest dispersers clustered at the invasion leading edge. Crayfish with shorter chelae and more fusiform body are better able to withstand high water velocities, as suggested by morphologic differences between crayfish found in high-velocity streams compared to those in low-velocity streams and lakes (Perry et al., 2013). Because water velocities in excess of 0.3–0.5 m/s lead to decreased crayfish movement and potentially increased energy expenditure in crayfish (Clark, Kershner, & Holomuzki, 2008; Mather & Stein, 1993; Perry & Jones, 2017), individuals with shorter chelae and more fusiform body may thus be able to disperse for longer periods of the year in the JDR whose discharge is unregulated by dams and flow regime is characterised by spring snow melt. Third, low rusty crayfish conspecific densities at invasion front sites may relax the constraints imposed by competition in high-density areas and thus reduce the requirement for investment in traits associated with competition. Shelter and food limitations are the main drivers of agonistic interactions in crayfish (Bergman & Moore, 2003; Capelli & Hamilton, 1984). When these resources are plentiful in pioneer populations, behavioural phenotypes associated with large claws and weight as part of an aggression syndrome (Hudina et al., 2012) could therefore lead to unnecessary energy expenses and reduced foraging, leading to reduced fitness (Sih, Cote, Evans, Fogarty, & Pruitt, 2012). Thus, a shift in selective pressure may have led to a change in rusty crayfish life history at the edge of the invasion, involving the reallocation of energy from allometric growth of competitive traits to reproduction and dispersal traits not measured here (e.g. walking leg length) (Phillips et al.,
2010b). While some selection for competitive ability in rusty crayfish could be expected as it interacts with signal crayfish at its invasion fronts, the lack of sympatry of these two species in our survey, the low densities of signal crayfish even in uninvaded areas, and the greater rates of somatic growth of rusty crayfish young-of-the-year documented by Sorensen (2012) all suggest a minor impact of interspecific competition on selection in pioneer populations.

We conjecture that a shift in the selection regime experienced by rusty crayfish from core to leading-edge areas is the most probable explanation for the trends in relative chela length and weight observed in this study. However, without knowledge of the heritability of the crayfish traits examined in this study, inference regarding the specific mechanisms in operation is still limited. The accelerating rate of spread of rusty crayfish in the JDR, the low population densities observed within several kilometres of the upstream and downstream invasion fronts, and high physiological fitness in invasion front populations suggest that a pushed invasion excluding subdominant crayfish from higher density areas is unlikely to explain the observed trait differences. Moreover, there is little to no evidence of the influence of chela size or relative body weight on dispersal speed (Kamran & Moore, 2015). Lastly, the difference in relative weight and chela length between invasion core and front populations could also be driven by natural selection in high crayfish density core populations. Historical data from Sorensen (2012), combined with this study, show no difference in chela length (Supporting Information Figure S5.1) but a significant increase in relative weight from 2010 to 2016 at the presumed site of rusty crayfish introduction (Supporting Information Figure S5.2). The changes in trait values observed here are thus likely to be the result of a combination of drivers including reduced fitness of competitive phenotypes at low crayfish density, selection for high relative weight in the population core, and trade-offs between these competitive attributes and other traits associated with higher dispersal ability.

The observed increase in rusty crayfish anabiotic activity, measured as RNA/DNA, in leading-edge populations of the JDR, although weak in the mainstem, indicates that the range expansion process has led to greater somatic growth and/or reproductive potential in pioneer individuals. This pattern matches several documented increases in body condition, growth, and reproductive potential in similar stream invasions, both within a single invasion gradient and between native and non-native populations. Within their non-native range, invasion front signal crayfish in Croatia and female spiny-cheek crayfish in the Danube both displayed greater reproductive potentials than their counterparts in core areas, with the former also being in better condition and energetic status (Pârvulescu et al., 2015; Rebrina et al., 2015). When comparing native and non-native populations of rusty crayfish, both lake and mesocosm experiments found that non-native individuals have higher growth rates and levels of activity than their native congeners (Pintor & Sih, 2009; Sargent & Lodge, 2014). Nonetheless, whether the observed increase in rusty crayfish physiological condition towards the invasion front is associated with greater somatic growth rates or gamete production remains unresolved and warrants further investigation. Indeed, simultaneous increases in condition, growth, and reproductive potential are not universal because selection for dispersal ability during range expansion may lead to unexpected trade-offs. For instance, tadpoles and juveniles in invasion front populations of cane toads in tropical Australia grow up to 31% faster than those from longer established populations (Phillips, 2009), and adults demonstrate higher feeding rates, larger fat stores, and better condition than conspecifics in later invasion stages (Brown et al., 2013). However, lower reproductive rates have also been documented in cane toad invasion front populations (Hudson, Phillips, Brown, & Shine, 2015).

It might seem that higher crayfish growth rates towards the invasion fronts contradict the observed patterns of reduced weight and chela length observed in pioneer crayfish. However, selection for a faster lifestyle could decrease age at maturity and shorten the lifespan of these invasion front crayfish while selecting against allometric growth of competitive morphology. Both crayfish growth rate and fecundity are density dependent (Guan & Wiles, 1999; Momot & Gowing, 1977). Therefore, it is possible that high growth and fecundity phenotypes have arisen from natural selection in the c. 15 generations since their introduction as part of a dispersal syndrome, associating rapid development and high fecundity with dispersive traits (Ronce & Clobert, 2012; Stevens et al., 2013).

Rusty crayfish at the leading edges of their distributional limits appear to be feeding lower in the food web when compared to conspecifics located behind the invasion front. This pattern stands in contradiction with most previous studies of stream invasions. Round gobies at the edge of their expanding range consume more of their favoured prey type than in central populations (Raby, Gutowsky, & Fox, 2010) and have higher δ15N signatures than the previous year front (Brandner et al., 2013). Invasion front bloody red mysid shrimp (Hemimysis anomala) were not more selective in their prey consumption, but showed greater ability to locate and capture zooplankton prey than those shrimp in core populations (lacarella et al., 2015). For crayfish invasions, trophic niche shifts have only been assessed at the biogeographical scale and offer mixed insights. In agreement with our findings, non-native populations of rusty crayfish and virile crayfish (Faxonius virilis) appeared to show greater rates of algae consumption (despite constant macroinvertebrate prey consumption) than did native populations in laboratory assays (Glon et al., 2018). By contrast, an intercontinental stable isotope analysis revealed trophic niche conservatism in signal crayfish between its native and non-native range (Larson, Olden, & Usio, 2010). Trophic flexibility has been shown to be species-dependent even in sympatric native crayfish species (Johnston, Robson, & Fairweather, 2011) and may thus not be a consistent characteristic among invasive omnivores either. It is unlikely that cannibalism, a widespread phenomenon in crayfish populations (Guan & Wiles, 1998), led to the observed increase in trophic position in areas with high densities of crayfish, as conspecific density was not correlated to trophic position in the JDR (Figure 4, Supporting Information Appendix S3). Lastly, it is unlikely that non-crayfish predators and competitors could have driven this downward shift in the trophic position of rusty crayfish as this shift was observed across both the downstream (increasing fish density)
and upstream leading edges (decreasing fish density) of rusty crayfish (in the mainstem and north fork JDR, respectively).

Rusty crayfish had a lower trophic position even when macroinvertebrate prey availability increased towards the invasion front in the North Fork JDR. This pattern contrasted with past evidence of a positive relationship between macroinvertebrate availability and crayfish trophic position (Olsson et al., 2008) and greater assimilation efficiencies of invertebrates than other food items by crayfish (Whitledge & Rabeni, 1997). However, the lack of significant decrease in trophic position towards the invasion front in the South Fork JDR could be due to a counter-effect from increasing macroinvertebrate biomass upstream. Consumption of macroinvertebrates has been linked to increases in weight gain and metabolic rates (Bondar, Bottrell, Zeron, & Richardson, 2005; Hill et al., 1993; McFeeters, Xenopoulos, Spooner, Wagner, & Frost, 2011). Nevertheless, in these same studies, juvenile signal crayfish in a natural setting disproportionately consumed food types that were the opposite of those shown to be of most nutritional value to them (Bondar et al., 2005) and rusty crayfish mortality was higher on a diet based on invertebrate than one on periphyton or detritus (Hill et al., 1993). This suggests that high growth might be associated with greater physiological stress, due to more frequent molting, and higher foraging costs in natural settings. Thus, we hypothesise that energy intake and long-term fitness of rusty crayfish associated with periphyton and detritus consumption may be higher than with macroinvertebrate diets despite their lower digestion efficiency of these resources.

Trade-offs can also arise between increased dispersal rates at range margins and the functional response of the non-native consumer due to the high cost of dispersal (Fronhofer & Altermatt, 2015). Given their current rate of spread (c. 20 km/year downstream from 2010 to 2016) in the JDR (Messager & Olden, 2018) and a window of activity of 8–9 months (based on water temperatures in the mainstem), rusty crayfish would have to achieve a net downstream spread rate of 80 m/day on average, without considering time dedicated to mating and juvenile parental care. We speculate that this pace could restrict the amount of time available for actively preying on invertebrates, and could select for those crayfish best able to efficiently feed and grow on abundant and accessible basal resources. It has also been hypothesised for stream fishes that those non-native species that can sustain growth and reproduction on low-quality resources should be best able to become established (Gido & Franssen, 2007). In crayfish, a broader trophic niche that expanded towards lower trophic levels may have afforded competitive advantages to the introduced signal crayfish over the native noble crayfish Astacus astacus in Swedish streams (Olsson, Stenroth, Nystrom, & Granell, 2009). Therefore, our findings that rusty crayfish at the invasion front act mostly as primary consumers while achieving greater physiological condition might reflect an increase in feeding efficiency on basal resources as a by-product of greater dispersal ability developed through their range expansion in the JDR.

Improved understanding of the distribution and eco-evolutionary drivers of rusty crayfish phenotypes throughout the JDR represents a crucial first step towards developing spatially explicit strategies to control this invasion. If the documented phenotypic shifts from core to invasion front populations are indeed associated with the accelerating rate of spread of rusty crayfish, then targeting those individuals at the invasion leading edge for removal (e.g. by trapping) might constrain the accumulation of dispersive phenotypes in these areas. Accounting for these phenotypic shifts in mechanistic models of invasive spread (e.g. Messager & Olden, 2018) could also provide us with a virtual laboratory to test the effectiveness of alternative control strategies in containing the spread of riverine invaders such as rusty crayfish.

Our results suggest that low conspecific densities and spatial sorting in leading-edge populations led to a shift in the phenotype of rusty crayfish towards lower competitive ability, higher intrinsic growth and/or reproduction, and greater foraging efficiency on basal resources, as they spread upstream and downstream in the JDR. Our study design enabled us to link morphological and functional traits to better explore the consequences of this range expansion on invaded ecosystems. We expect that the diminished competitive ability observed in the vanguard of this rusty crayfish invasion might lead to reduced fitness of the invasion front phenotypes once densities in newly colonised areas limit the availability of shelter and intensify competition for mates. The long-term evolutionary implications of these phenotypic shifts might thus be limited (Perkins, Boettiger, & Phillips, 2016). However, the trophic shift observed in invasion front populations could also allow rusty crayfish to reach higher densities in these areas, as they might exploit resources more broadly and efficiently under competitive conditions. The evolutionary forces at play in this invasion have probably interacted with longitudinal gradients in environmental conditions in ways analogous to those experienced by species during their migration towards cooler areas under climate change. Integrating the study of morphological and functional traits with spatial variation in environmental conditions thus provides a robust way to assess whether contemporary evolution is altering the phenotype and ecosystem impacts of species as their range expands through the landscape.

In conclusion, this study adds evidence in support of phenotypic changes expected in non-native organisms exposed to changing selection pressures as they invade new environments. Disentangling the causes of these changes, whether related to environmental factors or selection due to range expansion, remains an important area of investigation as we seek to better understand the ecological impacts of invasive species and how native species will respond to shifting environmental conditions in the future.

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CONFLICT OF INTEREST

The authors of this manuscript have no conflict of interest to declare.

AUTHOR’S CONTRIBUTIONS

M. L. M. and J. D. O. conceived and designed the study, obtained funding, collected data, interpreted the data, and prepared the manuscript. M. L. M. performed the laboratory work and analysed data.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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