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1 Phenotypic variability of rusty crayfish (*Faxonius rusticus*) at the leading edge

2 of its riverine invasion

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8 Keywords

9 Aquatic invasive species, *Faxonius rusticus*, invasive crayfish, John Day River, range margin

10 Summary

11	1.	Species around the globe are undergoing phenotypic shifts at ecologically relevant
12		timescales as they invade new ecosystems and respond to changing environments.
13		Disentangling the contribution of environmental gradients from the process of range
14		expansion in shaping these changes, and identifying the specific traits undergoing
15		selection, are both critical to anticipate the secondary spread and impact of invasive
16		species.
17	2.	Here, we investigate phenotypic changes in rusty crayfish (Faxonius rusticus), a nuisance
18		invasive species, through an extensive survey of their invasion gradient in multiple
19		tributaries of the John Day River (JDR, Oregon, U.SA.), a major tributary of the
20		Columbia River.
21	3.	Rusty crayfish in the JDR have developed better physiological condition (intrinsic growth
22		and/or reproductive potential measured as RNA/DNA ratio) but less competitive
23		morphology (lighter body and smaller claws) as they spread upstream and downstream
24		from their location of initial introduction. In addition, rusty crayfish in invasion front
25		populations are at a lower trophic level than conspecifics closer to core areas.
26	4.	By accounting for variations in temperature, primary productivity, and prey
27		(macroinvertebrates) biomass throughout the invasion extent of rusty crayfish, our
28		findings suggest that low conspecific densities at the invasion edge and spatial sorting
29		primarily drive these phenotypic changes. The trends observed here are thus likely to
30		intensify over time as rusty crayfish continues to rapidly spread throughout the JDR and
31		reach the mainstem Columbia River.

32	5.	Our study shows that phenotypic shifts can manifest rapidly over environmental
33		gradients experienced during the range expansion of aquatic invasive species. Patterns in
34		both morphological and functional traits documented in the JDR demonstrate the
35		importance of both environmental factors and dispersal processes in shaping these
36		responses in riverine networks.
37		

38 Introduction

39 Species around the globe are exposed to changing selection pressures as they invade new 40 landscapes or shift their range to track environmental change (Moran & Alexander, 2014). When 41 their geographic distribution shifts or expands, the individuals in the vanguard of these 42 populations often face novel environmental conditions, predators, and competitors (Chuang & 43 Peterson, 2016). Mounting evidence suggests that these factors, in combination with low 44 conspecific densities relative to those experienced by core populations, promote rapid changes in 45 species phenotypes at range boundaries (Chuang & Peterson, 2016). 46 Phenotypic changes at the leading edge of invasive populations' range have been observed in 47 many taxonomic groups, including amphibians [e.g. cane toad Rhinella marina (Perkins, 48 Phillips, Baskett, & Hastings, 2013)], insects [e.g. ground beetle Merizodus soledadinus 49 (Laparie, Renault, Lebouvier, & Delattre, 2013)], fish [e.g. round goby Neogobius melanostomus 50 (Brandner, Cerwenka, Schliewen, & Geist, 2013)] and decapods [e.g. signal crayfish Pacifastacus leniusculus (Hudina, Hock, Žganec, & Lucić, 2012)]. These changes have been 51 52 manifested in traits ranging from body length and fecundity to boldness (Chuang & Peterson, 53 2016), and have been associated with accelerated invasion rates (Phillips, Brown, & Shine, 54 2010a; Weiss-Lehman, Hufbauer, & Melbourne, 2017) and increased impacts to recipient 55 ecosystems (Brandner et al., 2013; Iacarella, Dick, & Ricciardi, 2015). Enhanced insight into the 56 processes leading to phenotypic changes in invasive species is thus essential for anticipating their 57 future spread and impact (Phillips, 2015), as well as predicting the outcome of species range 58 shifts in response to climate change (Caplat et al., 2013; Travis et al., 2013). 59 Phenotypic plasticity (Davidson, Jennions, & Nicotra, 2011), natural selection (Brown, 60 Kelehear, & Shine, 2013), and spatial sorting (Shine, Brown, & Phillips, 2011) are the three

61 dominant processes responsible for observed trait variability at range edges, yet their respective 62 contributions are seldom understood. Phenotypic plasticity, the ability for multiple phenotypes to 63 arise from a single genotype in response to changing environmental conditions, is particularly 64 prevalent in invasive species (Davidson et al., 2011). It is crucial in allowing populations to 65 spread and adapt to changing environments faster than would otherwise be possible by evolution 66 through natural selection alone (Chevin, Lande, & Mace, 2010). Furthermore, abiotic and biotic 67 forces at the invasion front can lead to trait evolution by natural selection. Low intra-specific 68 density at the leading edge is most likely to shift selective pressures towards higher growth and 69 reproduction (Phillips, Brown, & Shine, 2010b). Lastly, the range expansion process itself can 70 lead to adaptive changes in traits through spatial sorting, whereby the fastest dispersing 71 individuals at the expanding edge of the population systematically interbreed, resulting in 72 selection for enhanced dispersal ability in their offspring if dispersive traits are heritable (Shine 73 et al., 2011). This runaway process continues in subsequent generations until trade-offs between 74 traits begin to limit the potential for directional selection (Burton, Phillips, & Travis, 2010), 75 although dispersal ability may evolve independently of other life-history traits (Bonte & Dahirel, 76 2017). The traits involved in promoting dispersal and growth at the invasion front are so 77 numerous that natural selection and spatial sorting, when enhancing these traits, can impact 78 morphology, physiology, behavior, immunology, and life-history, among others (Chuang & 79 Peterson, 2016). Understanding the specific traits undergoing selection in invasive species and 80 disentangling the influence of environmental conditions from contemporary evolution therefore 81 requires empirical studies that are specific to the species and systems at hand.

82 Crayfish are among the most widely introduced freshwater animals worldwide (Lodge et 83 al., 2012). Following their introduction, nonnative crayfish can cause severe ecological impacts

84 across entire food webs to a greater extent than native crayfish because, like their native 85 counterparts, they have polytrophic feeding habits, but also often reach much greater densities 86 and heightened levels of foraging activity (Hansen et al., 2013; Pintor, Sih, & Kerby, 2009; 87 Twardochleb, Olden, & Larson, 2013). In invaded ecosystems, native crayfish species can be 88 displaced within a few years, and populations of macrophytes, insects, snails, and fish often 89 decline (Bobeldyk & Lamberti, 2010; McCarthy, Hein, Olden, & Vander Zanden, 2006; Olden, 90 McCarthy, Maxted, Fetzer, & Vander Zanden, 2006; Rosenthal, Stevens, & Lodge, 2006). 91 Changes in population structure, behavior, morphology, and physiology have already been 92 reported between core and edge populations in several ongoing river invasions by crayfish 93 (Hudina et al., 2012; Hudina, Zganec, & Hock, 2015; Pârvulescu, Pîrvu, Moroşan, & Zaharia, 94 2015; Rebrina, Skejo, Lucić, & Hudina, 2015). At the biogeographical level, differences in 95 growth, survival, feeding habits, and behavior are also common among crayfish congeners 96 between their native and non-native range, further demonstrating the potential phenotypic 97 changes wrought by the invasion process (Glon, Reisinger, & Pintor, 2018; Pintor & Sih, 2009; 98 Reisinger, Elgin, Towle, Chan, & Lodge, 2017; Sargent & Lodge, 2014). Even though the 99 consequences of these changes on invaded ecosystems often remain unexplored, increased 100 invasion rates alone could challenge our ability to respond to new and ongoing crayfish 101 invasions. In addition, given that the impact of an invasive species on the recipient ecosystem is 102 not only a function of its range size but also of its abundance, per-capita effect, and other factors 103 (Thomsen, Olden, Wernberg, Griffin, & Silliman, 2011), changes in its somatic and reproductive 104 growth rates or trophic niche could have severe consequences for native communities. 105 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 thefuture 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).

113 Our objective was twofold. We first assessed whether rusty crayfish individuals displayed 114 phenotypic differences progressing from established core populations near the initial location of 115 their introduction to recently colonized invasion fronts. We used a riverscape survey to analyze 116 rusty crayfish population structure, morphology, physiological condition, and trophic position 117 across its range in the mainstem of the JDR and its main tributaries. We hypothesized that 118 phenotypic changes occurred across rusty crayfish generations as they dispersed from their 119 location of introduction to their present invasion fronts in the JDR. We expected that low 120 conspecific densities in newly invaded river sections would lead to increased access to resources 121 and relative consumption of growth-inducing food like macroinvertebrates (Hill, Sinars, & 122 Lodge, 1993). We thus expected that rusty crayfish would exhibit better physiological condition 123 and higher trophic positions toward the invasion front and, as a result, larger carapace length and 124 higher weight (Brown et al., 2013). We also posited that two additional mechanisms could affect 125 crayfish trait values during range expansion. If larger, faster growing, and more competitive 126 crayfish are better dispersers, as has been reported in multiple invasions (Chuang & Peterson, 127 2016), we expected to observe an increase in crayfish body size, relative chela length, and 128 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).

132 We then evaluated whether these phenotypic changes in populations towards the invasion 133 front were caused by plasticity to environmental factors or selection due to range expansion. We 134 sought to disentangle these two sets of processes by studying rusty crayfish subpopulations both 135 upstream and downstream of their initial location of introduction, and by simultaneously 136 accounting at each site for the distance from the invasion core (reflecting the invasion stage at 137 that location), gradients in environmental conditions, and the availability of food resources. We 138 hypothesized that if selection due to the range expansion process was driving changes in rusty 139 crayfish trait values as they spread throughout the JDR, then these phenotypic differences would 140 become larger in sites further from the core and be greatest at the invasion fronts, regardless of 141 whether populations spread upstream or downstream and the local gradient in environmental 142 conditions.

143 Methods

144 Study area

The JDR originates in the Blue Mountains of northeastern Oregon (U.S.A) and runs undammed for 457 river kilometers (hereafter 'km') until its confluence with the Columbia River just upstream from the Columbia River Gorges (Fig. 1). One of the largest free-flowing rivers in the United States 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*.

152 Native to the Ohio River basin, rusty crayfish is a habitat generalist; it can inhabit all 153 substrates but prefers cobble habitat, thrives both in areas of high flow and standing water, can 154 withstand temperatures ranging from close to 0°C up to 35°C with an optimum near 22°C, and 155 opportunistically consumes a variety of aquatic plants, benthic invertebrates, detritus, periphyton, 156 fish eggs, and small fish (Lorman, 1980; Mundahl & Benton, 1990). Mature rusty crayfish mate 157 in late summer, early fall, or early spring, and achieve high growth rates (Berrill & Arsenault, 158 1984; Lorman, 1980).

159 Rusty crayfish was first found in the JDR in 2005, marking its first recorded occurrence 160 west of the North American continental divide (Olden et al., 2009). Evidence suggests that rusty 161 crayfish were first released in the late 1990s in the mainstem JDR, about 380 km upstream from 162 its confluence with the Columbia River, near the town of Mount Vernon, Oregon, by a teacher 163 and students of a nearby school (Olden et al., 2009). In the \sim 20 years since their presumed date 164 of introduction, rusty crayfish have rapidly spread throughout the JDR watershed at rates 165 exceeding 15 km/yr, raising concerns that the mainstem of the Columbia River may soon be 166 reached (Messager & Olden, 2018). Only the native signal crayfish *Pacifastacus leniusculus* was 167 known to be present in the watershed prior to the introduction of rusty crayfish in the JDR 168 (Larson & Olden, 2011). Previous studies and records from rotary screw traps operated by the 169 United States Forest Service (USFS) show that signal crayfish were widespread throughout the 170 JDR watershed despite low densities prior to the introduction of rusty crayfish (Sorenson., 2012; 171 David Wooster, Oregon State University personal communications August 2013; Keith Dehart,

172 USFS, *personal communications* March 2016).

173 Field data collection

174 We implemented a spatially extensive survey of rusty crayfish densities, phenotypes, and 175 environmental conditions throughout its invasion range to capture gradients in these variables 176 from core to leading-edge populations. We used predictions of rusty crayfish distribution in 177 August 2016 from a spatially explicit individual-based model (Messager & Olden, 2018) to 178 distribute 60 sampling sites every 5-10 km along the main stem and primary tributaries of the 179 JDR, encompassing the invasion extent of rusty crayfish. Sampling was conducted August 1-22, 180 2016, late enough in the summer so that females would not be in berry, young-of-the-year would 181 be large enough to be sampled and almost all mature males would have changed to a 182 reproductive form (form I) with larger chelae in preparation for fall mating (Butler & Stein, 183 1985; Hamr, 1999; Prins, 1968).

184 To assess the relative density of rusty crayfish across the watershed, area-standardized 185 kick-seining was performed in six locations across a 50-m long reach at each surveyed site. One 186 person disturbed one square meter of substrate upstream of a seine net held by another team 187 member to flush crayfish downstream, yielding a mean and standard deviation of crayfish 188 density at each site. To ensure consistency in our measure of relative density, we exclusively 189 sampled in runs (i.e., rather than pools or riffles) when possible, because runs provide the water 190 velocity and depth needed for this sampling method to be most effective (Larson & Olden, 191 2016). To avoid false absences, snorkeling, hand-netting, and baited traps were also used when 192 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 molting condition (yes/no) of captured crayfish were measured at every site, while two tissue samples (abdominal white muscle) from 14 rusty

196 crayfish were taken at every other site. Regenerating chelae, soft-shelled or visibly smaller than 197 the other chela, were not measured. When our standard sampling protocol yielded less than 14 198 crayfish, additional specimens were caught by hand-netting so that these measurements and 199 tissue samples could be taken — though these individuals were not included in our estimates of 200 relative density. When crayfish density was high, morphological measurements were recorded 201 for a random subsample of 30 of the crayfish that were caught by kick-seining. The first tissue 202 sample was immediately stored in non-iodized salt for subsequent $\delta^{15}N$ stable isotope analysis to 203 determine the trophic position of rusty crayfish at that site — the energy-weighted number of 204 trophic energy transfers from primary producer to crayfish (Vander Zanden & Rasmussen, 205 1999). We also collected 12–20 mayfly nymphs (Ephemeroptera: Heptageniidae) in runs and riffles at each study site where crayfish tissues were sampled to characterize the baseline $\delta^{15}N$ 206 207 values of primary consumers throughout the JDR (Anderson & Cabana, 2007). The second tissue sample, preserved in RNA*later*[®], was used to quantify the relative concentration of RNA and 208 209 DNA in rusty crayfish cells. While the amount of DNA remains mostly constant in cells 210 regardless of conditions, the amount of RNA positively correlates with the amount of protein 211 synthesis (anabolic activity). Therefore, the ratio of the amount of RNA to that of DNA in a cell 212 is an effective eco-physiological indicator of condition (hereafter 'physiological condition') that 213 reflects the organism's potential investment in somatic growth and gamete production (i.e. 214 fertility) under a given set of environmental conditions (Koop, Winkelmann, Becker, Hellmann, 215 & Ortmann, 2011).

Environmental conditions at each site were characterized 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-m² (1-ft²) standardized 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.

225 Stable isotope analysis

226 Stable isotope analysis was conducted on rusty crayfish tissues and mayfly whole 227 specimens to assess differences in crayfish feeding patterns throughout their invasion gradient. 228 All samples were prepared for isotope analysis using standard protocols, with the exception of 229 the salt-based preservation, a field-appropriate method that results in minimal and directionally uniform effects on δ^{13} C and δ^{15} N (Arrington & Winemiller, 2002). Prior to processing, all 230 231 crayfish muscle tissues and mayfly whole bodies were rinsed with distilled water until the salt 232 was dissolved. Samples were then dried at 60°C for 24 h, ground to powder, and sent for 233 nitrogen isotope analysis to the University of California Davis Stable Isotope Facility. The 234 trophic position of each crayfish at site (S) was estimated according to:

235 Trophic position_{crayfish,S} = 2 +
$$\frac{\delta^{15}N_{crayfish,S} - (\sum_{mayfly=1}^{3} \delta^{15}N_{mayfly,S})/3}{2.54}$$
, (1)

where 2.54 is the rusty crayfish discrimination factor or fractionation factor (Δ) representing the absolute difference in δ^{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.

242 The procedure for the extraction and quantitation of nucleic acids in rusty crayfish tissues 243 was adapted from Berdalet, Roldán, Olivar, and Lysnes (2005) and Vrede, Persson, and Aronsen 244 (2002) using fluorochromes that indiscriminately bind to DNA and RNA. We provide a brief 245 description below but refer the reader to Appendix 1 in the Supporting Information for a more 246 detailed protocol. Berdalet, Roldán, and Olivar (2005) recommend using three separate aliquots 247 of each sample to compute the quantity of RNA and DNA in crustacean tissues: the first assay 248 measures RNA after DNA digestion; the second measures DNA after RNA digestion; and the 249 third measures residual fluorescence after digestion of both DNA and RNA. Four nucleic acid 250 standard curves with six concentrations each were thus run for every batch of samples: 251 RNA+DNase, RNA+RNase, DNA+RNase, and DNA+DNase. To quantify RNA and DNA 252 fluorescence, 50 µl of diluted (1:200) Quant-iT[™] RiboGreen® reagent was added to each plate 253 well. The slopes of the standard curves were then estimated using linear regression and the 254 quantity of RNA [µg RNA/ml assay], DNA [µg DNA/ml assay], and their ratio in each sample 255 was calculated using the equations provided by Berdalet, Roldán, and Olivar (2005).

256 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 h, weighed, combusted in a muffle furnace at 550°C for four hours (Mason 1983), cooled down to room temperature in a desiccator for 6 h, and reweighed. Ash

264 mass (after combustion in furnace) was then subtracted from dry mass (before combustion) to265 obtain AFDW.

266 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 analyzed separately. In total, four leading-edge populations were analyzed, one downstream edge in the mainstem JDR and three upstream edges — in the mainstem, South Fork, and North Fork JDR (Fig. 1).

274 Six traits were analyzed throughout the invasion extent of rusty crayfish: carapace length, 275 chela length, weight, trophic position, physiological condition, and sex ratio (the proportion of 276 males at a site). Only data for crayfish caught by kick-seining, hand-netting, and snorkeling were 277 included in the analysis, due to the known size and sex bias of trapping for large males (Larson 278 & Olden, 2016). To control for the strong relationship between body size, crayfish weight, and 279 chela length due to allometric growth, residuals from carapace length-weight and carapace 280 length-chela length non-linear regression models, developed separately for each sex, were used 281 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 (μ g Chl-*a*/cm²). Degree days were computed based on water temperature

286 estimated from a multiple regression model using satellite-measured daily land-surface 287 temperature, calendar day, watershed area, and elevation as predictor variables (Messager & 288 Olden, 2018). In situ temperature measurements were not used in this analysis, as diel 289 temperature variations were on the same order of magnitude as differences between upstream 290 and downstream sites. Velocity and depth measurements were not included in the analysis either 291 due to their high spatial variability at base flow in the JDR. Only the sites for which all variables 292 had been measured were included in the analysis for a total of 18 sites, 14 sites in the mainstem 293 JDR, 2 in the South Fork JDR, and 2 in the North Fork JDR.

294 In the South Fork and North Fork JDR, where tissue samples were taken from crayfish in 295 only two sites, differences in trait values among sites were tested using two-sample t-tests and 296 differences in sex ratio were assessed with Yate's Chi-square test. In the mainstem JDR, 297 Generalized Additive Models (GAM) were developed to analyze the drivers of crayfish 298 morphology, physiological condition, and trophic position. GAM models were built separately 299 for two main categories of predictor variables. A first category of models was developed to 300 account for the role of the range expansion process in driving phenotypic changes by using each 301 site's distance from the initial location of rusty crayfish introduction as the predictor variable. 302 The second model category was based on environmental variables that might influence trait 303 values. Additional models were also built using distance from the initial introduction location 304 together with crayfish density and sex ratio as predictor variables, or combining multiple 305 environmental variables. The significance and fit of candidate models (Akaike Information 306 Criterion (AIC), the p-value of the coefficients, and the adjusted R^2 , see Table S3.2 in the 307 Supporting Information) were then compared among invasion fronts to determine whether 308 consistent patterns arose.

309 Results

310 Crayfish distribution and habitat conditions

311 In total, 1266 crayfish were captured across the 18 sites analyzed in this study, of which 312 299 were sampled for morphological traits, 259 for physiological condition, and 254 for trophic 313 position. Our survey, combined with historical distribution records and a model of rusty crayfish 314 spread in the JDR (Messager & Olden, 2018), showed that rusty crayfish spread at an 315 accelerating rate since its introduction and occupied at least 705 km of river across the JDR 316 watershed in August 2016. By that summer, it had spread nearly 30 km upstream in the North 317 Fork and South Fork JDR, and colonized the mainstem along a 250 km stretch downstream of its 318 introduction point (Fig. 1). In contrast to its extensive spread downstream, the upstream spread 319 of rusty crayfish in the mainstem had been temporarily halted at the time of the survey due to a 320 low-head dam 12 km upstream of the putative site of crayfish introduction. Densities 321 downstream of the dam were similar to those found at the core of their range. Therefore, the 322 upstream mainstem invasion leading edge was not included in this analysis. In addition, the 323 precise location of the downstream mainstem leading edge could not be determined due to 324 limited access to the river; thus, the downstream-most surveyed site where rusty crayfish was 325 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, Fig. 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

332 in sympatry with rusty crayfish in only a few sites at the upstream invasion fronts where rusty 333 crayfish were found at lower densities (in the South Fork, mainstem, and other smaller side 334 tributaries of the JDR). Where native signal crayfish were present, they occurred at very low 335 densities ($< 2 \operatorname{crayfish/m^2}$) including in sites without rusty crayfish. These findings, together with 336 past records (2013) of signal crayfish occurrence in sympatry with rusty crayfish at sites where 337 signal crayfish were absent during our 2016 survey, suggest that signal crayfish is rapidly 338 excluded from sites invaded by rusty crayfish as it spreads across the JDR watershed. Therefore, 339 interspecific competition was not considered as a significant mechanism influencing the traits 340 investigated in this study.

Temperature and macroinvertebrate biomass followed similar longitudinal gradients from 341 342 upstream to downstream between the mainstem, South Fork, and North Fork JDR, whereas 343 benthic biomass of green algae and diatom were highly variable among tributaries (Fig. S2.1). 344 Degree days increased monotonically downstream while macroinvertebrate biomass decreased 345 downstream. There was also considerable variability in macroinvertebrate biomass among 346 adjacent sites, including a sudden increase in biomass downstream from the confluence of the 347 mainstem and the North Fork JDR where low crayfish densities prevailed. Green algae were 348 sparse to absent in all tributaries. The biomass of diatoms, on the other hand, was highest in the 349 South Fork and upper mainstem JDR (up to 4.5 μ g Chl-*a*/cm²) and low in the lower mainstem 350 and North Fork JDR, with inconsistent longitudinal gradients among tributaries.

351 Morphology

There were consistent trends in rusty crayfish morphology from core to leading edge populations in all tributaries (Table 1, Appendix 3). The relative chela length of rusty crayfish in leading-edge populations was significantly smaller than those behind the front in all three

355	tributaries (Mainstem: GAM, $p = 0.03$, R^2 -adjusted=0.28, n=14; North Fork: t=2.17, df=29, p-
356	value=0.04; South Fork: t=3.03, df=42, p-value < 0.01; Table 1, Fig. 3, Table S3.1 and S3.2).
357	Crayfish density was also strongly and positively associated with chela length across the JDR
358	(Mainstem: GAM, p=0.01, R ² -adjusted =0.37, n=14; Table 1, Table S3.2). No consistent
359	difference was found in mean carapace length between core and invasion front populations of the
360	JDR or between male and female crayfish; however, there was a consistent decrease in carapace
361	length variance in the direction of the invasion in both upstream and downstream dispersing
362	populations (Table 1, Appendix 3). Crayfish relative weight significantly decreased towards the
363	invasion front both downstream in the mainstem (GAM, p=0.04, R ² -adjusted =0.25, n=14) and
364	upstream in the North Fork ($W = 177$, p-value = 0.01) and South Fork ($W = 328$, p-value = 0.01;
365	Fig. S4.1); along with decreasing crayfish density (Mainstem: GAM, p=0.02, R ² -adjusted=0.33,
366	n=14; Table 1, Table S3.2). Lastly, there was no significant trend in the proportion of males
367	towards the fronts of the invasion despite a slight increase in male dominance in both upstream
368	and downstream leading edges, when considering all sites where more than 10 crayfish were
369	captured (Fig. S4.2).

370 Environmental conditions were not strong predictors of crayfish morphology throughout 371 their invasion gradient. Degree days did not correlate consistently across invasion fronts with any 372 morphological trait values (Table 1, Table S3.2). For instance, while decreasing upstream 373 temperatures were positively correlated to relative weight in the South Fork and North Fork JDR, 374 temperature and relative weight were negatively correlated in the mainstem. Similarly, 375 inconsistent patterns were observed between temperature and both relative chela length and 376 carapace length. Macroinvertebrate biomass (AFDW) was negatively but not significantly 377 correlated with relative chela length and weight across tributaries and was not consistently or

significantly associated with shifts in carapace length across tributaries (Table 1, Table 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 (Table 1, Table S3.2).

382 *Trophic position*

383 The trophic position of rusty crayfish was consistently lower for individuals at invasion 384 fronts than in populations closer to the core, despite wide variations among sites throughout the 385 watershed (Table 1, Fig. 3B, Appendix 3). In the mainstem, rusty crayfish diet first increased 386 downstream from that typical of a secondary consumer or omnivore (trophic position of \sim 3) to 387 that of a top carnivore (trophic position of ~4) and then decreased towards the front of the 388 invasion down to that of a primary consumer (trophic position of ~ 2 ; ; GAM, p=0.05, R²-389 adjusted =0.44, n=14; Fig. 3B). There was an equivalent drop in crayfish trophic position in the 390 North Fork JDR upstream leading edge (t = 9.66, df = 22, p < 0.001) but no equivalent decrease 391 in the South Fork JDR leading edge. There was no difference in trophic position among male and 392 female crayfish, and although trophic position was weakly correlated with carapace length within sites (carapace length fixed effect 95% CI= $8.0 \cdot 10^{-3}$ to $1.9 \cdot 10^{-2}$ trophic level/mm in linear mixed 393 394 effect model with site as random effect), there was no significant correlation between trophic 395 position and mean carapace length across sites along the mainstem. Trophic position was not 396 significantly correlated with relative chela length within sites (relative chela length fixed effect 95% CI = $-8.3 \cdot 10^{-3}$ to $2.3 \cdot 10^{-2}$ trophic level/mm in linear mixed effect model with site as random 397 398 effect).

399 Temperature, increasing with downstream distance from the invasion source, was400 correlated with trophic position only towards the upstream invasion fronts in the North Fork and

401	South Fork JDR (Table 1, Appendix 2 and 3). None of the other environmental variables
402	significantly covaried with trophic position in a consistent way across tributaries (Table 1,
403	Appendix 2 and 3).
404	Growth and condition
405	There was a consistent positive trend in rusty crayfish physiological condition
406	(RNA/DNA ratio) towards invasion fronts along with decreasing crayfish densities (Fig. 3C,
407	Table 1, Appendix 3). Strongest towards the upstream leading edges despite decreasing
408	temperature (t-test, South Fork: $W = 46$, p-value = 0.01; North Fork: $t = -4.91$, $df = 15.5$, p <
409	0.001), the increase in physiological condition was only marginal in the mainstem [GAM,
410	p=0.19, R ² -adjusted=0.06, n=14, mean slope= $6.3 \cdot 10^{-3}$ (95% CI=-2.7 \cdot 10^{-3} to 1.5 \cdot 10^{-2})
411	(RNA/DNA)/km]. The negative correlation between crayfish physiological condition
412	(RNA/DNA ratio) and crayfish density in the mainstem was also non-significant (GAM, p=0.43,
413	R ² -adjusted=-0.03, n=14). Lastly, environmental variables (abiotic and biotic) were not
414	consistently and significantly associated with RNA/DNA ratio across tributaries (Table 1,

415 Appendix 3).

416 **Discussion**

417 This study revealed significant trends in morphological trait values, physiological 418 condition, and trophic position of rusty crayfish from their location of initial introduction to 419 multiple leading edges of invasion in the only known population of this species in western North 420 America. Even though these trends were not consistently significant across tributaries, they 421 suggest that rusty crayfish individuals at the vanguard of the invasion exhibited less competitive 422 morphology (decreased relative weight and chela length) and exploited energetic pathways lower 423 on the food chain, yet were in better physiological condition than individuals located closer to 424 the invasion core. We contend that these phenotypic shifts observed in rusty crayfish are likely 425 due to the range expansion process itself rather than to novel environmental conditions at their 426 range boundaries, and that these trends may intensify as the invasion continues to unfold 427 upstream and downstream towards the main stem of the Columbia River.

428 The observed decrease in crayfish relative chela length and weight towards the invasion 429 fronts can be attributed to three main reasons – though further research is needed to confirm the 430 respective contribution, if any, of each of these mechanisms. First, the range expansion of rusty 431 crayfish might be driven by the exclusion of sub-dominant individuals from high density 432 population centers, therefore leading to the widespread presence of competitively inferior 433 crayfish at the invasion leading edge (Hudina, Hock, & Zganec, 2014). In other words, 434 individual crayfish with less competitive phenotypes may have been forced out and 435 systematically interbred at the invasion front, resulting in the accumulation of relatively light, 436 small-clawed individuals at the leading edge. In support of this mechanism, signal crayfish at the 437 boundary of their invasion range across Croatia display lower levels of aggression and often lose 438 agonistic interactions to individuals from the invasion core, despite being in better physical 439 condition (Hudina et al., 2015). However, a significant increase in relative claw size away from 440 source population was also observed in invasive signal crayfish males of the Mura River, Croatia 441 (Hudina et al., 2012). Second, larger chelae and a stouter body shape may be associated with 442 weaker dispersal ability and thus be selected against through associative mating of the fastest 443 dispersers clustered at the invasion leading edge. Crayfish with shorter chelae and more fusiform 444 body are better able to withstand high water velocities, as suggested by morphologic differences 445 between crayfish found in high velocity streams compared to those in low velocity streams and

446 lakes (Perry et al., 2013). Because water velocities in excess of 0.3-0.5 m/s lead to decreased 447 crayfish movement and potentially increased energy expenditure in crayfish (Clark, Kershner, & 448 Holomuzki, 2008; Mather & Stein, 1993; William L. Perry & Jones, 2017), individuals with 449 shorter chelae and more fusiform bodies may thus be able to disperse for longer periods of the 450 year in the John Day River whose discharge is unregulated by dams and flow regime is 451 characterized by spring snow melt. Third, low rusty crayfish conspecific densities at invasion 452 front sites may relax the constraints imposed by competition in high density areas and thus 453 reduce the requirement for investment in traits associated with competition. Shelter and food 454 limitations are the main drivers of agonistic interactions in crayfish (Bergman & Moore, 2003; Capelli & Hamilton, 1984). When these resources are plentiful in pioneer populations, 455 456 behavioral phenotypes associated with large claws and weight as part of an aggression syndrome 457 (Hudina et al., 2012) could therefore lead to unnecessary energy expenses and reduced foraging, 458 leading to reduced fitness (Sih, Cote, Evans, Fogarty, & Pruitt, 2012). Thus, a shift in selective 459 pressure may have led to a change in rusty crayfish life history at the edge of the invasion, 460 involving the reallocation of energy from allometric growth of competitive traits to reproduction 461 and dispersal traits not measured here (e.g. walking leg length) (Phillips et al., 2010b). While 462 some selection for competitive ability in rusty crayfish could be expected as it interacts with 463 signal crayfish at its invasion fronts, the lack of sympatry of these two species in our survey, the 464 low densities of signal crayfish even in uninvaded areas, and the greater rates of somatic growth 465 of rusty crayfish young-of-the-year documented by Sorenson (2012) all suggest a minor impact 466 of inter-specific competition on selection in pioneer populations.

We conjecture that a shift in the selection regime experienced by rusty crayfish from coreto leading edge areas is the most likely explanation for the trends in relative chela length and

469 weight observed in this study. However, without knowledge of the heritability of the crayfish 470 traits examined in this study, inference regarding the specific mechanisms in operation is still 471 limited. The accelerating rate of spread of rusty crayfish in the JDR, the low population densities 472 observed within several kilometers of the upstream and downstream invasion fronts, and high 473 physiological fitness in invasion front populations suggest that a pushed invasion excluding 474 subdominant crayfish from higher density areas is unlikely to explain the observed trait 475 differences. Moreover, there is little to no evidence of the influence of chela size or relative body 476 weight on dispersal speed (Kamran & Moore, 2015). Lastly, the difference in relative weight and 477 chela length between invasion core and front populations could also be driven by natural 478 selection in high crayfish density core populations. Historical data from Sorenson (2012), 479 combined with this study, show no difference in chela length (Fig. S5.1) but a significant 480 increase in relative weight from 2010 to 2016 at the presumed site of rusty crayfish introduction 481 (Fig. S5.2). The changes in trait values observed here are thus likely the results of a combination 482 of drivers including reduced fitness of competitive phenotypes at low crayfish density, selection 483 for high relative weight in the population core, and trade-offs between these competitive 484 attributes and other traits associated with higher dispersal ability.

The observed increase in rusty crayfish anabolic 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

492 reproductive potentials than their counterparts in core areas, with the former also being in better 493 condition and energetic status (Pârvulescu et al., 2015, Rebrina et al., 2015). When comparing 494 native and non-native populations of rusty crayfish, both lake and mesocosm experiments found 495 that non-native individuals have higher growth rates and levels of activity than their native 496 congeners (Pintor & Sih, 2009; Sargent & Lodge, 2014). Nonetheless, whether the observed 497 increase in rusty crayfish physiological condition towards the invasion front is associated with 498 greater somatic growth rates or gamete production remains unresolved and warrants further 499 investigation. Indeed, simultaneous increases in condition, growth, and reproductive potential are 500 not universal because selection for dispersal ability during range expansion may lead to 501 unexpected trade-offs. For instance, tadpoles and juveniles in invasion-front populations of cane 502 toads in tropical Australia grow up to 31% faster than those from longer established populations 503 (Phillips, 2009), and adults demonstrate higher feeding rates, larger fat stores, and better 504 condition than conspecifics in later invasion stages (Brown et al., 2013). However, lower 505 reproductive rates have also been documented in cane toad invasion front populations (Hudson, 506 Phillips, Brown, & Shine, 2015).

507 It might seem that higher crayfish growth rates towards the invasion fronts contradict the 508 observed patterns of reduced weight and chela length observed in pioneer crayfish. However, 509 selection for a faster lifestyle could decrease age at maturity and shorten the lifespan of these 510 invasion front crayfish while selecting against allometric growth of competitive morphology. 511 Both crayfish growth rate and fecundity are density dependent (Guan & Wiles, 1999; Momot & 512 Gowing, 1977). Therefore, it is possible that high growth and fecundity phenotypes have arisen 513 from natural selection in the ~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).

516 Rusty crayfish at the leading edges of their distributional limits appear to be feeding 517 lower in the food web when compared to conspecifics located behind the invasion front. This 518 pattern stands in contradiction with most previous studies of stream invasions. Round gobies at 519 the edge of their expanding range consume more of their favored prey type than in central 520 populations (Raby, Gutowsky, & Fox, 2010) and have higher $\delta^{15}N$ signatures than the previous 521 year front (Brandner et al., 2013). Invasion front bloody red mysid shrimp (*Hemimysis anomala*) 522 were not more selective in their prey consumption, but showed greater ability to locate and 523 capture zooplankton prey than those shrimp in core populations (Iacarella et al., 2015). For 524 crayfish invasions, trophic niche shifts have only been assessed at the biogeographical scale and 525 offer mixed insights. In agreement with our findings, non-native populations of rusty crayfish 526 and virile crayfish (*Faxonius virilis*) appeared to show greater rates of algae consumption 527 (despite constant macroinvertebrate prey consumption) than did native populations in laboratory 528 assays (Glon et al., 2018). By contrast, an intercontinental stable isotope analysis revealed 529 trophic niche conservatism in signal crayfish between its native and non-native range (Larson, 530 Olden, & Usio, 2010). Trophic flexibility has been shown to be species-dependent even in 531 sympatric native crayfish species (Johnston et al. 2011) and may thus not be a consistent 532 characteristic among invasive omnivores either. It is unlikely that cannibalism, a widespread 533 phenomenon in crayfish populations (Guan & Wiles, 1998), led to the observed increase in 534 trophic position in areas with high densities of crayfish, as conspecific density was not correlated 535 to trophic position in the JDR (Table 1, Appendix 3). Lastly, it is unlikely that non-crayfish 536 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).

540 Rusty crayfish had a lower trophic position even when macroinvertebrate prey 541 availability increased towards the invasion front in the North Fork JDR. This pattern contrasted 542 with past evidence of a positive relationship between macroinvertebrate availability and crayfish 543 trophic position (Olsson et al., 2008) and greater assimilation efficiencies of invertebrates than 544 other food items by crayfish (Whitledge & Rabeni, 1997). However, the lack of significant 545 decrease in trophic position towards the invasion front in the South Fork JDR could be due to a 546 counter-effect from increasing macroinvertebrate biomass upstream. Consumption of 547 macroinvertebrates has been linked to increases in weight gain and metabolic rates (Bondar, 548 Bottriell, Zeron, & Richardson, 2005; Hill et al., 1993; McFeeters, Xenopoulos, Spooner, 549 Wagner, & Frost, 2011). Nevertheless, in these same studies, juvenile signal crayfish in a natural 550 setting disproportionately consumed food types that were the opposite of those shown to be of 551 most nutritional value to them (Bondar et al., 2005) and rusty crayfish mortality was higher on a 552 diet based on invertebrate than one on periphyton or detritus (Hill et al., 1993). This suggests that 553 high growth might be associated with greater physiological stress, due to more frequent molting, 554 and higher foraging costs in natural settings. Thus, we hypothesize that energy intake and long-555 term fitness of rusty crayfish associated with periphyton and detritus consumption may be higher 556 than with macroinvertebrate diets despite their lower digestion efficiency of these resources.

557 Trade-offs can also arise between increased dispersal rates at range margins and the 558 functional response of the non-native consumer due to the high cost of dispersal (Fronhofer & 559 Altermatt, 2015). Given their current rate of spread (~20 km/year downstream from 2010 to

560 2016) in the JDR (Messager & Olden, 2018) and a window of activity of 8-9 months (based on 561 water temperatures in the mainstem), rusty crayfish would have to achieve a net downstream 562 spread rate of 80 m per day on average, without considering time dedicated to mating and 563 juvenile parental care. We speculate that this pace could restrict the amount of time available for 564 actively preying on invertebrates, and could select for those crayfish best able to efficiently feed 565 and grow on abundant and accessible basal resources. It has also been hypothesized for stream 566 fishes that those nonnative species that can sustain growth and reproduction on low-quality 567 resources should be best able to become established (Gido & Franssen, 2007). In crayfish, a 568 broader trophic niche that expanded towards lower trophic levels may have afforded competitive 569 advantages to the introduced signal crayfish over the native noble crayfish Astacus astacus in 570 Swedish streams (Olsson, Stenroth, Nystrom, & Graneli, 2009). Therefore, our findings that 571 rusty crayfish at the invasion front act mostly as primary consumers while achieving greater 572 physiological condition might reflect an increase in feeding efficiency on basal resources as a 573 byproduct of greater dispersal ability developed through their range expansion in the JDR. 574 Improved understanding of the distribution and eco-evolutionary drivers of rusty crayfish 575 phenotypes throughout the JDR represents a crucial first step towards developing spatially 576 explicit strategies to control this invasion. If the documented phenotypic shifts from core to 577 invasion front populations are indeed associated with the accelerating rate of spread of rusty 578 crayfish, then targeting those individuals at the invasion leading edge for removal (e.g. by 579 trapping) might constrain the accumulation of dispersive phenotypes in these areas. Accounting 580 for these phenotypic shifts in mechanistic models of invasive spread (e.g. Messager & Olden, 581 2018) could also provide us with a virtual laboratory to test the effectiveness of alternative 582 control strategies in containing the spread of riverine invaders like rusty crayfish.

583 Our results suggest that low conspecific densities and spatial sorting in leading edge 584 populations led to a shift in the phenotype of rusty crayfish towards lower competitive ability, 585 higher intrinsic growth and/or reproduction, and greater foraging efficiency on basal resources, 586 as they spread upstream and downstream in the JDR. Our study design enabled us to link 587 morphological and functional traits to better explore the consequences of this range expansion on 588 invaded ecosystems. We expect that the diminished competitive ability observed in the vanguard 589 of this rusty crayfish invasion might lead to reduced fitness of the invasion front phenotypes 590 once densities in newly colonized areas limit the availability of shelter and intensify competition 591 for mates. The long-term evolutionary implications of these phenotypic shifts might thus be 592 limited (Perkins, Boettiger, & Phillips, 2016). However, the trophic shift observed in invasion 593 front populations could also allow rusty crayfish to reach higher densities in these areas, as they 594 might exploit resources more broadly and efficiently under competitive conditions. The 595 evolutionary forces at play in this invasion have likely interacted with longitudinal gradients in 596 environmental conditions in ways analogous to those experienced by species during their 597 migration towards cooler areas under climate change. Integrating the study of morphological and 598 functional traits with spatial variation in environmental conditions thus provides a robust way to 599 assess whether contemporary evolution is altering the phenotype and ecosystem impacts of 600 species as their range expands through the landscape.

In conclusion, this study adds evidence in support of phenotypic changes expected in
nonnative 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 toshifting environmental conditions in the future.

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875 Tables

876 Table 1. Summary of the relationships between rusty crayfish traits and predictor variables in the 877 John Day River. The direction and color of the arrows indicate the sign of the relationship (e.g. 878 red upward arrows reflect positive relationships) between the predictor variable (columns) and 879 the trait (rows) for a given invasion leading edge (downstream mainstem, upstream South Fork, 880 or upstream North Fork). Grey arrows show statistically non-significant relationships. 881 Significance and a consistent direction in the relationship between candidate predictors and traits 882 among tributaries suggested the primacy of that predictor in driving observed differences in 883 traits. See Appendix 3 for detailed statistical results. Flat green arrows show variables for which 884 there was no difference among sites in that tributary, and relationships denoted by a – were not 885 tested due to a lack of hypothesized mechanisms relating the variables.

			Pr	edictor	variable		
Response variable	Edge	Distance from introduction	Crayfish density	Degree days	Macroinv. AFDW [†]	Green algae	Diatom
	Mainstem		∕▼				×
Relative chela length	South Fork		×	×		->	
	North Fork		×	×			_
	Mainstem	\wedge					$\bigvee I$
Trophic position	South Fork		X				
	North Fork		X	∕▼			∕▼
Physiological	Mainstem				>		>
Condition (RNA/DNA)	South Fork				_		
	North Fork	_			_	∕▼	
	Mainstem	\wedge	$\bigvee I$		~		
Carapace length	South Fork	A			~		
	North Fork						
	Mainstem						
Relative weight	South Fork			X			
	North Fork		×	×			_
	Mainstem		-	-	-	-	-
Sex ratio (% males)	South Fork		-	-	-	-	-
	North Fork	X	-	-	_	-	-
	Mainstem			-	_	-	-
Carapace length SD	South Fork			-	_	-	-
	North Fork		×	-	-	-	-

886 [†]Ash-free dry weight

887 Figure captions

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).

891 Figure 2. Density of rusty crayfish (y-axis) as a function of distance from putative location of

892 initial introduction (x-axis) in the mainstem (green), North Fork (orange) and South Fork

893 (purple) John Day River. Crayfish densities are expressed as the mean (points) and 95%

894 confidence interval (bars) of the catch per unit effort (CPUE). The smooth solid line and shaded

region represent the predicted mean CPUE and 95% Bayesian credible interval, respectively,

from a generalized additive model. Vertical dashed lines represent confluences of the South Fork

897 (at 44 km) and North Fork (at 87 km) with the mainstem JDR.

898 Figure 3. Changes in mean crayfish relative chela length (A), trophic position (B), and

899 physiological condition (C; RNA/DNA ratio expressing potential for somatic growth and gamete

900 production) along the invasion gradient of rusty crayfish in the JDR watershed. The smooth solid

901 line and shaded region represent the predicted mean trait value and 95% Bayesian credible

902 interval, respectively, from a generalized additive model and asterisks denote significant

903 differences in mean ('**': $p \le 0.01$, '*': $p \le 0.05$). Vertical dashed lines represent the

904 confluences of the South Fork (at 44 km) and North Fork (at 87 km) with the mainstem JDR.

906 Author's contributions

- 907 M. L. M. and J. D. O. conceived and designed the study, obtained funding, collected data,
- 908 interpreted the data, and prepared the manuscript. M. L. M. performed the laboratory work and
- 909 analyzed data.

910 Data accessibility

- 911 Data available from the figshare project repository at
- 912 https://figshare.com/articles/MessagerOlden2019_Phenotypic_variability_of_rusty_crayfish_JD
- 913 <u>R/7716203</u>. Updated computer codes and data also available from
- 914 <u>https://github.com/messamat/InvasionEdge_rustycrayfish.</u>
- 915

916 **Conflict of interest**

- 917 The authors of this manuscript have no conflict of interest to declare.
- 918

919 Supplementary material

- 920 Appendix 1: RNA/DNA ratio measurement protocol
- Table S1.1. Name and reference of reagents used in RNA and DNA quantification.
- 922 Appendix 2: longitudinal gradients in environmental conditions in the John Day River
- 923 Figure S2.1. Longitudinal variations in degree days (A, y-axis), macroinvertebrate
- biomass (B, y-axis, Ash Free Dry Weight), benthic green algae (C, y-axis) and diatom
- 925 biomass (D, y-axis) with respect to the distance from putative introduction location of
- 926 rusty crayfish downstream in the mainstem (lower panels) and upstream in the South
- 927 Fork and North Fork (upper panels) JDR.

928 Appendix 3: summary tables of model outputs

929	- Table S3.1. Summary of statistical tests for differences in rusty crayfish traits in the					
930	South Fork and North Fork of the John Day River between recently invaded sites at					
931	upstream leading invasion edges and sites where rusty crayfish have been established f	or				
932	a longer period.					
933	- Table S3.2. Summary of outputs from General Additive Models (GAM) of rusty crayfi	sh				
934	traits throughout their invasion range in the mainstem of the John Day River.					
935	Appendix 4: additional trends in rusty crayfish traits along their invasion gradient in the John	1				
936	Day River					
937	- Figure S4.1. Changes in mean crayfish relative carapace length (CL; A), carapace leng	th				
938	standard deviation (B), and relative weight (C) along the invasion gradient of rusty					
939	crayfish in the JDR.					
940	- Figure S4.2. Changes in the proportion of males in rusty crayfish populations along the	ir				
941	invasion gradient in the JDR.					
942	Appendix 5: temporal trend in relative chela length and weight of rusty crayfish from 2010 to					
943	2016 at their initial location of introduction in the John Day River.					
944	- Figure S5.1. Distribution of rusty crayfish relative chela length in 2010, 2011, and 201	6				
945	at their initial location of introduction in the JDR.					
946	- Figure S5.2. Distribution of rusty crayfish relative weight in 2010, 2011, and 2016 at					
947	their initial location of introduction in the John Day River.					





