Received: 2 May 2023 | Revised: 25 April 2024 | Accepted: 13 May 2024
DOI: 10.1111/fwb.14308

ORIGINAL ARTICLE

Freshwater Biology WILEY

Complex selection processes on invasive crayfish phenotype at the invasion front of the Zambezi floodplains ecoregion

Nawa Nawa[1,2,3] | **Josie South**[4,5] | **Bruce R. Ellender**[4] | **Josephine Pegg**[4,6] | **Takudzwa C. Madzivanzira**[7] | **Ryan J. Wasserman**[1,4]

[1]Department of Zoology and Entomology, Rhodes University, Makhanda, South Africa

[2]DSI/NRF Research Chair in Inland Fisheries and Freshwater Ecology, South African Institute for Aquatic Biodiversity (SAIAB), Makhanda, South Africa

[3]School of Natural and Applied Sciences, Department of Chemistry and Biology, Mulungushi University, Kabwe, Zambia

[4]South African Institute for Aquatic Biodiversity, Makhanda, South Africa

[5]Water@Leeds, School of Biology, Faculty of Biological Sciences, University of Leeds, Leeds, UK

[6]Department of Ichthyology and Fisheries Science, Rhodes University, Makhanda, South Africa

[7]School of Biology and Environmental Sciences, Aquatic Systems Research Group, University of Mpumalanga, Nelspruit, South Africa

Correspondence
Ryan J. Wasserman, Department of Zoology and Entomology, Rhodes University, Makhanda, South Africa.
Email: r.wasserman@ru.ac.za

Abstract

1. Understanding the eco-evolutionary processes which drive phenotypic variability in non-native species is crucial to predicting impact and informing spatially explicit management models. Invasive redclaw crayfish, *Cherax quadricarinatus*, are spreading rapidly through the ecologically and economically important Upper and Middle Zambezi system in south-central Africa.
2. We tested whether there was an invasion front effect in the Barotse floodplain and disentangle whether these effects were related to environmental filtering, spatial sorting, or density-dependent processes along a nonlinear 200 km invasion gradient over seasonal hydrological periods.
3. Crayfish spread up- and down-stream indicated an expanding population compared to the former invasion front in 2019, facilitated by both sexes. Wet season detection probability and relative abundance were lower than in the dry season. No signals of environmental filtering were detected as a result of high connectivity of habitats in the wet season.
4. The invasion front population was characterised by lower relative abundance, lower body condition, larger overall body and chelae size, and longer front leg length. There was no effect on sex ratio. This indicated that spatial sorting, density-dependent processes and predation pressure are acting as selective forces on phenotypic variability across the invasion gradient.
5. Better understanding of the effects of flood regime and trophic interactions on phenotypic variability and dispersal is urgently needed. Population suppression to reduce spatial sorting and conspecific competition could be a viable management option at the invasion front.

KEYWORDS
Cherax quadricarinatus, eco-evolutionary selection, environmental filtering, intra-specific variation, invasion gradient, redclaw crayfish, spatial sorting, traits

---

Josie South and Ryan J. Wasserman indicates shared senior authorship.

1 | INTRODUCTION

Non-native invasive species are a major driver of biodiversity decline and economic burden globally (Kouba et al., 2022; Tickner et al., 2020). For an introduced population to become invasive it must transition through the invasion process by repeatedly overcoming multiple abiotic and biotic barriers (Blackburn et al., 2011). The leading-edge hypothesis indicates that a non-random subset of individuals is likely to be driving invasion success and progress, where physical, metabolic and behavioural trait values are different at the invasion front to those at the invasion core (Briski et al., 2018; Chapple et al., 2012; Myles-Gonzalez et al., 2015). This may be facilitated by selection for certain traits and expressing intra-specific trait plasticity which promotes persistence, reproduction and dispersal in a rapidly expanding population (Chapple et al., 2012). This may be driven by non-random processes such as environmental filtering, spatial sorting and intra-specific density dependence (Chuang & Peterson, 2016). Understanding the processes which drive phenotypic trait variability is crucial to predicting ecological impact and developing appropriate models of dispersal dynamics for conservation initiatives (Lang et al., 2021; Závorka et al., 2020).

Environmental filtering influences the assemblage of communities suggesting that the abiotic environment acts as a trait filter which selects for individuals that are not able to tolerate local conditions as the population disperses and encounters new environmental invasion barriers (Blackburn et al., 2011; Kraft et al., 2015; Poff, 1997). This may be in response to habitat type, predation pressure or temperature (Akmal et al., 2021; Carboni et al., 2016; Lang et al., 2021; Laparie et al., 2010). Spatial sorting regards filtering of genotypes across space, rather than time (i.e., natural selection), whereupon assortative mating occurs because individuals with dispersal-adapted traits are likely to reach the range front and reproduce together, thus increasing trait expression (Phillips et al., 2008; Shine et al., 2011; Travis & Dytham, 2002). Thereby, rapid generational shifts lead to novel phenotypes adapted to dispersal (Chuang & Peterson, 2016). A notorious example is the cane toad *Rhinella marina* invasion in Australia where individuals at the front of the population had longer legs which facilitated rapid expansion (Phillips et al., 2006). Density-dependent processes are induced by an intra-specific competition gradient that is high at the invasion core and low at the invasion front, which may result in selection for increased competitive ability and reproduction at the expense of dispersal ability (Burton et al., 2010). However, low population density at the invasion front may select for higher reproduction and dispersal instead of increased investment in competitive traits such as weapons (Messager & Olden, 2019; Tarkan et al., 2021).

Freshwater crayfish are a highly successful group of invasive species as a consequence of their broad phenotypic plasticity, high fecundity and omnivory (Lodge et al., 2012; Twardochleb et al., 2013). Continental Africa has no native crayfish species; therefore, crayfish invasions in Africa provide a unique opportunity to understand how a phylogenetically unique species adapts to a novel environment (Madzivanzira et al., 2020). The redclaw crayfish (*Cherax quadricarinatus*), native to northern Australia and Papua New Guinea, was first reported to be introduced into the Upper Zambezi system in 2001 in the Kafue Flats and Siavonga on the Zambian side of Lake Kariba in 2002 (Douthwaite et al., 2018). In 2014, *C. quadricarinatus* was reported from the Barotse floodplain, the largest wetland in the Upper Zambezi (Nunes et al., 2017). Establishment, distribution and abundance were confirmed and quantified in the three invasion cores noting an invasion gradient of larger mass and body size in the older invasions, and more female and intersex individuals in the recent invasion in the Barotse floodplain (Madzivanzira, South, Ellender, et al., 2021).

African floodplains are highly heterogenous and stoichastic ecosystems which support diverse aquatic and terrestrial biota, as well as providing integral ecosystem services to riparian human communities (Tweddle, 2010). In tropical regions, dry–wet seasonal variation drive changes in physiology, morphology and behaviour of organisms (William et al., 2017). Impacts of introduced species on floodplains are dependent on ecological matching of traits to highly variable hydrological regimes (Catford et al., 2011). Flood pulse dynamics may either promote or impede successful invasion by non-native species, depending on the magnitude and timing of flood regimes (Catford et al., 2011; Thomaz, 2021). Habitat heterogeneity and hydrological connectivity on floodplains may enhance the spread of invasive species through active dispersal in river channels and passive movement by flood transport (Höfle et al., 2014; Thomaz, 2021). Further spread, initially estimated at 49 km/year downstream and 12 km/year upstream, of invasive *C. quadricarinatus* through the Zambezi basin threatens ecological structure and fish recruitment, and confers cost to artisanal fishers (Madzivanzira, South, Ellender, et al., 2021; Madzivanzira, South, Nhiwatiwa, et al., 2021; South et al., 2020). Determining spread, assessing invasion processes and trait plasticity in the Upper Zambezi is of the utmost priority as the invasion threatens two UNESCO World Heritage sites. The Okavango Delta, which is connected through the seasonally flooded Selinda Spillway (Nunes et al., 2017), and Lake Malawi which may ultimately be at risk via the Shire River tributaries connecting to the invaded Middle and Upper Zambezi (Madzivanzira, South, Ellender, et al., 2021).

As both the introduction site and date of *C. quadricarinatus* is known for the Barotse floodplain (2012 Lealui, near Mongu, Zambia [Figure 1]; Madzivanzira et al., 2020) using a series of complementary field-based investigations, we aim to test a series of hypotheses regarding *C. quadricarinatus* invasion dynamics and drivers of phenotypic variation along the invasion gradient in a floodplain ecosystem. An extensive seasonal survey was carried out to assess changes in invasion status from the initial 2019 survey, and traits were measured across the invasion gradient to test for signals of environmental filtering, spatial sorting and density-dependent processes. We expected that there would be further dispersal up- and downstream and that there will be a core-front invasion gradient of declining relative abundance, and differing sex ratios across the gradient. Owing to the habitat

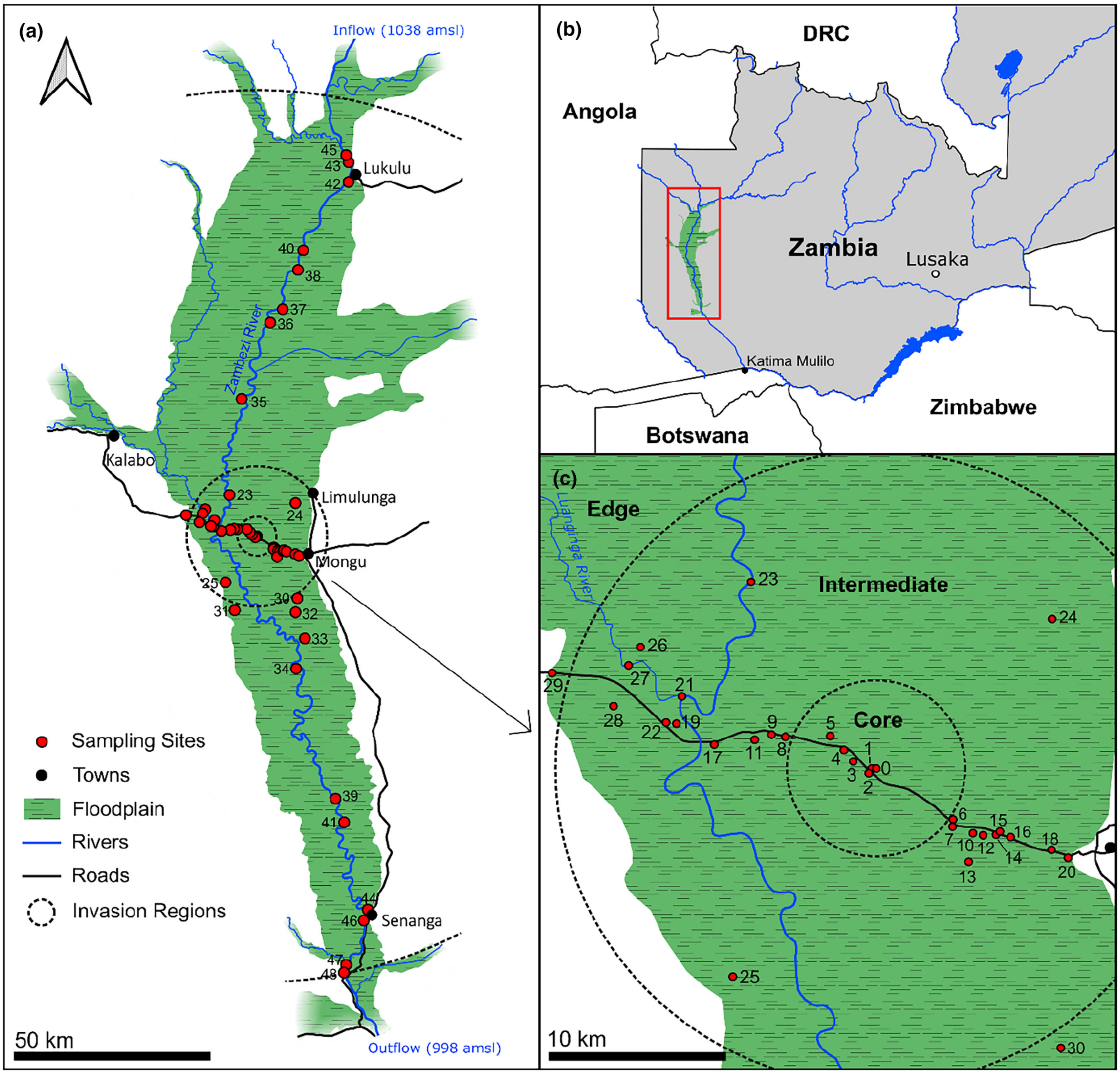

FIGURE 1 Distribution (a) of 48 sampling sites across the three invasion regions (core, intermediate and front) on the Barotse floodplain of the (b) Upper Zambezi System in Zambia; (c) shows sampling sites around the central section of the Barotse floodplain. Sites are numbered in the order of increasing distance from the point of introduction in Lealui (Site 0).

heterogeneity of the floodplain, we hypothesised that environmental filtering may be seen where specific morphotypes would be found in each habitat. Similar to the cane toad invasion, we expected that if spatial sorting was acting on the population, crayfish at the periphery of the invasion would have longer front legs as the walking legs are used for active dispersal across the benthos (Pond, 1995). Density-dependent trait plasticity was expected to be seen where individuals at the invasion front would have better body condition as a consequence of to less competition, whereas individuals at the core would be larger as a consequence of longer establishment and with larger chelae as competition increases weapon investment (Messager & Olden, 2019; Moorhouse & Macdonald, 2011).

2 | METHODS

2.1 | Study area

The study was conducted on the Barotse floodplain of the Upper Zambezi River system located in Western Zambia in the Zambezi Floodplains Ecoregion (Abell et al., 2008). Covering an area of approximately 5,500 km^2 (Turpie et al., 1999), the floodplain is an extensive lowland area that stretches from upstream at the confluence of Zambezi and Kabompo rivers in Lukulu to downstream at Senanga near the Ngonye falls (Cai et al., 2017). The landscape is characterised by Kalahari sands which are predominantly covered by grassland

(Timberlake, 1997). The floodplain undergoes seasonal inundation resulting from increased discharge of the Zambezi River caused by rainfall of over 1,400 mm experienced over the headwater catchment areas in south-eastern Angola and north-western Zambia (Cai et al., 2017). The minimum and maximum inundated area is approximately 2,500 and 5,520 km^2, respectively (Cai et al., 2017). The flood period generally occurs between February and April before receding between May and July (Zimba et al., 2018). The Barotse floodplain comprises the main river channel, several small rivers, streams, lagoons, swamps, ponds and man-made canals which support diverse flora and fauna (Turpie et al., 1999). Aquatic habitats range from rocky wooded substrate to sand habitats which support a high fish diversity (Tweddle et al., 2004). During the flood period various water bodies become interconnected into a large single expanse of water for about 3 months (Figure S1); as the floods recede the many water bodies become disconnected and isolated once again.

2.2 | Field sampling

The study covered the entire longitudinal extent (≈250 km) of the Barotse floodplain (upstream 14.343366S, 23.222265E; downstream 16.235489S, 23.236797E) (Figure 1). Sampling was conducted in 2021 during both wet season/high water period (April–May) and dry season/low water period (October–November) approximately 2 years on from the initial Upper Zambezi survey in 2019 (Madzivanzira, South, Ellender, et al., 2021). The main river channel, tributaries, backwaters, lagoons, canals and flooded plain were sampled. Three distributional regions along the invasion range were identified (Table 1; Figure 1): (1) the invasion core established around the central section of the floodplain (Madzivanzira, South, Ellender, et al., 2021) covering a radius of less than 5 km from the point of introduction, (2) the intermediate range between 5 to 18 km along the radius and (3) the invasion front (mainly upstream and downstream area) covering a radius ranging between 18 to 114 km from the point of introduction. A total of 48 sites were sampled from the invasion core to the invasion front (Table 1; Figure 1) and the coordinates for each sampling site were also recorded using a Garmin GPSMAP 78s. The sites were chosen by focusing on areas where *C. quadricarinatus* was suspected to be present based the previous survey (Madzivanzira, South, Ellender, et al., 2021) as well as local reports from fishers.

The number of sampling sites in each location ranged from one to three depending on the availability of water bodies. In localities with more than five water bodies (i.e., ponds), two or three water bodies with surface area of at least 400 m^2 were sampled. The distance between sampling sites varied between 0.3 and 1 km for sites within the same locality, and between 2 and 26 km for sites in different localities across the invasion range.

Collapsible Promar traps (610 mm × 460 mm × 200 mm: mesh size 10 mm) were used, baited with 100 g dry dog food (Madzivanzira, South, Nhiwatiwa, et al., 2021). Between 18 and 20 traps were deployed at each site (17:00–18:00 hr) with approximately 15–20 m between traps. The traps were left and retrieved the following morning (06:00–08:00 hr) with a soak time of 13–14 hr. All crayfish caught were counted, sexed and weighed (g). Individuals that exhibited both male and female genitals were recorded as intersex (Madzivanzira, South, Nhiwatiwa, et al., 2021). From each specimen, five morphometric variables were measured using a digital Vernier calliper: chelae length (CL), chelae width (CW), carapace length (CPL), carapace width (CPW), front leg length (FLL) and total length (TL).

2.3 | Data analyses

All statistical analyses were conducted within the R statistical software environment (R Core Team, 2021). Mapping was completed in QGIS 4.13 (QGIS Development Team, 2021).

2.3.1 | Presence/absence and spread rate

Detection probability (P_{capture}), expressed as a fraction of traps that caught at least one crayfish (Madzivanzira, South, Nhiwatiwa, et al., 2021), was determined for each of the three invasion regions. Differences in P_{capture} across the invasion range and between invasion regions was analysed using the χ^2 test of independence via 2 × 3 and 2 × 2 contingency tables, respectively. Spread rate was calculated using straight line distance (km) from the 2019 invasion front (Madzivanzira, South, Ellender, et al., 2021) to the furthest sites where crayfish were detected (both upstream and downstream) using Google Earth divided by the number of years since the last survey (i.e., 2 years).

TABLE 1 Sites sampled during the wet and dry season across the three distributional regions (core, intermediate and front) on the Barotse floodplain.

Invasion region	Sampling radius around point of introduction (km)	Sites sampled			Total no. of sites
		Wet & dry season	Wet season	Dry season	
Core	<5	5	1	–	6
Intermediate	≥5 < 18	16	6	1	23
Front	≥18 < 114	7	–	13	20

2.3.2 | Relative abundance

Catch per unit effort (CPUE) expressed as mean number of individuals caught per trap per night was used as a proxy for relative abundance (Madzivanzira, South, Nhiwatiwa, et al., 2021). The CPUE data were zero-inflated, so a delta-X transformation was applied to correct standard errors (Ellender et al., 2010). A generalised additive model (GAM) was used to model CPUE trends along the invasion gradient with respect to season. Changes in sex ratios across the invasion range were analysed using a 2 × 3 contingency table.

2.3.3 | Environmental filtering

All measured traits were firstly corrected and standardised to account for allometry by applying Mosimann corrections, as this provides the most accurate way of identifying shape differences without losing information, as opposed to using residuals from linear regressions (Jungers et al., 1995). The framework developed by Mosimann for extracting ratios and size variations in multivariate morphological analyses is flexible, powerful and compatible with other frameworks developed for similar purposes (reviewed and tested extensively in Jungers et al., 1995; Klingenberg, 2016). To do this, geometric mean (GM) was calculated and then individual trait values were divided by GM to produce size-corrected values. Then GM was used as a new trait variable to represent overall body size instead of the correlated variables of mass, TL and CPL (Figure S2). Therefore it can be understood as a conglomerate metric indicating overall size/shape, while not losing information from log-transformation or multivariate regression of residuals (Jungers et al., 1995; Klingenberg, 2016).

Using GM and remaining measured traits (CL, CW, CPW, FLL), a principal components analysis (PCA) was completed on the correlation matrix to determine individual overlap in morphospace with respect to habitat type and invaded range (i.e., core-front). We calculated the total ellipse areas and performed an analysis of multivariate homogeneity of group dispersions (i.e., variances) via"betadisper" in *vegan* for both habitat type and invaded range, if significant a permutation-based test of multivariate homogeneity of group dispersions was implemented through"permutest" in *vegan* with 99 permutations, to derive *post hoc* differences between groups (Oksanen et al., 2019). We would expect that group dispersion and ellipse size would differ significantly between factor levels if there was environmental filtering.

2.3.4 | Spatial sorting

Front leg length

A generalised linear model (GLM) with an inverse Gaussian error distribution was modelled to assess relationship with distance, sex and season on FLL).

2.3.5 | Density dependence

Overall body size

A GLM with Gamma error distribution and a log-link function were used to model whether GM has a relationship with distance, sex and season.

Chelae length

A GLM with a Gamma error distribution and identity link function was also fitted to determine the effect of distance, sex and season on CL.

Body condition

Body condition was calculated for each individual using Fulton's Condition Factor:

$$K = 100 \times (W / L^3)$$

where W is wet weight (g) and L is total length (mm) (Froese, 2006). A GLM with Gamma error distribution and a log-link function was used to determine the effect of range and season on body condition. Given that body condition is sex-dependent (Streissl & Hold, 2002), only same-sex comparisons were performed within analyses. All crayfish with missing appendages were excluded from the analyses.

All model assumptions were checked firstly using the R package *DHARMa* (Florian, 2022) by simulating and comparing predicted and actual residual distributions. A model with full interaction terms was initially fitted and then simplified stepwise, starting with the interaction terms. Variables were compared using analysis of deviance tables and Wald χ^2 tests (Florian, 2022). The marginal estimated means were then plotted using the R package *emmeans* (Lenth, 2022).

3 | RESULTS

3.1 | Presence/absence and spread rate

Cherax quadricarinatus was detected in all the three invasion regions (core, intermediate and front ranges) across the Barotse floodplain with a total of 1,310 crayfish caught. Crayfish were caught in 19% of all traps set and were present at 33 of the 48 sites sampled (Table S1). Using only the sites where *C. quadricarinatus* were detected, the P_{capture} across the three invasion regions was significantly different ($\chi^2{}_2 = 730.87$, $p < 0.01$; Table S1). Detection probability at the invasion core ($P_{\text{capture}} = 0.41$) was significantly higher than the intermediate range ($P_{\text{capture}} = 0.21$: $\chi^2{}_2 = 45.58$, $p < 0.01$) and invasion front ($P_{\text{capture}} = 0.02$: $\chi^2{}_2 = 538.15$, $p < 0.01$) (Table S1). Detection probability for intermediate range was also significantly higher than the front ($\chi^2{}_2 = 418.49$, $p < 0.01$) (Table S1).

The furthest distance on the invasion front where *C. quadricarinatus* was detected from the point of introduction was 113.96 km downstream in Senanga and 54.80 km upstream in Lukulu. A crayfish carcass was also observed further upstream (93.70 km) on the banks of the Zambezi River in Lukulu. Using the former invasion front estimation from 2019 and trap data from the present survey, the current minimum spread rate is approximately at 53.92 km/year downstream and 27.4 km/year upstream, both faster than the previous estimates.

3.2 | Relative abundance

The CPUE of *C. quadricarinatus* changed significantly across the invasion range ($p < 0.05$), declining from the core to the front for both dry and wet season (Figure 2). In the dry season CPUE was significantly higher ($p < 0.05$) than in the wet season (Figures 2 and 3). The combined seasonal average CPUE at the invasion core and intermediate range was 1.471 ± 0.685 and 0.695 ± 0.338 ind./trap/night, respectively. Crayfish abundance at the invasion front was lower at 0.027 ± 0.002 ind./trap/night.

The GAM showed that both distance and season had a significant effect on CPUE ($p < 0.05$), where CPUE declined at the range front compared to the core regardless of season but the dry season showed a comparatively higher CPUE (Figures 3 and 4). The average CPUE in the dry season was higher (0.789 ± 0.518 ind./trap/night) than in the wet season (0.331 ± 0.138 ind./trap/night). When compared to the 2019 survey there was both an increase in crayfish relative abundance at three of the 6 sampled sites and a decrease at the three other sites (Figure 3).

3.3 | Sex ratio

Overall, male-to-female sex ratio did not differ significantly between the three sampled regions across the invasion range ($\chi^2_2 = 1.434$, df = 2, $p = 0.489$; Table S1). Crayfish caught at the invasion core consisted of 35.1% males, 54.8% females and 10.1% intersex, whereas the intermediate range comprised 34.1% male, 55.5% female and 10.4% intersex (Table S1). The sex ratio at the invasion front comprised 53.3% males and 46.7% females, with no intersex individuals sampled from this region (Table S1).

3.4 | Environmental filtering

The PCA explained 87.4% of the variance along the first and second axes. Principal component 1 (PC 1) of the PCA accounted for 63.2% (eigenvalue = 3.17) and was characterised by GM, while principal component 2 (PC 2) accounted for 24.2% (eigenvalue = 1.21). There was no effect of habitat type on morphological variance ($F_{4,1279} = 1.17$,

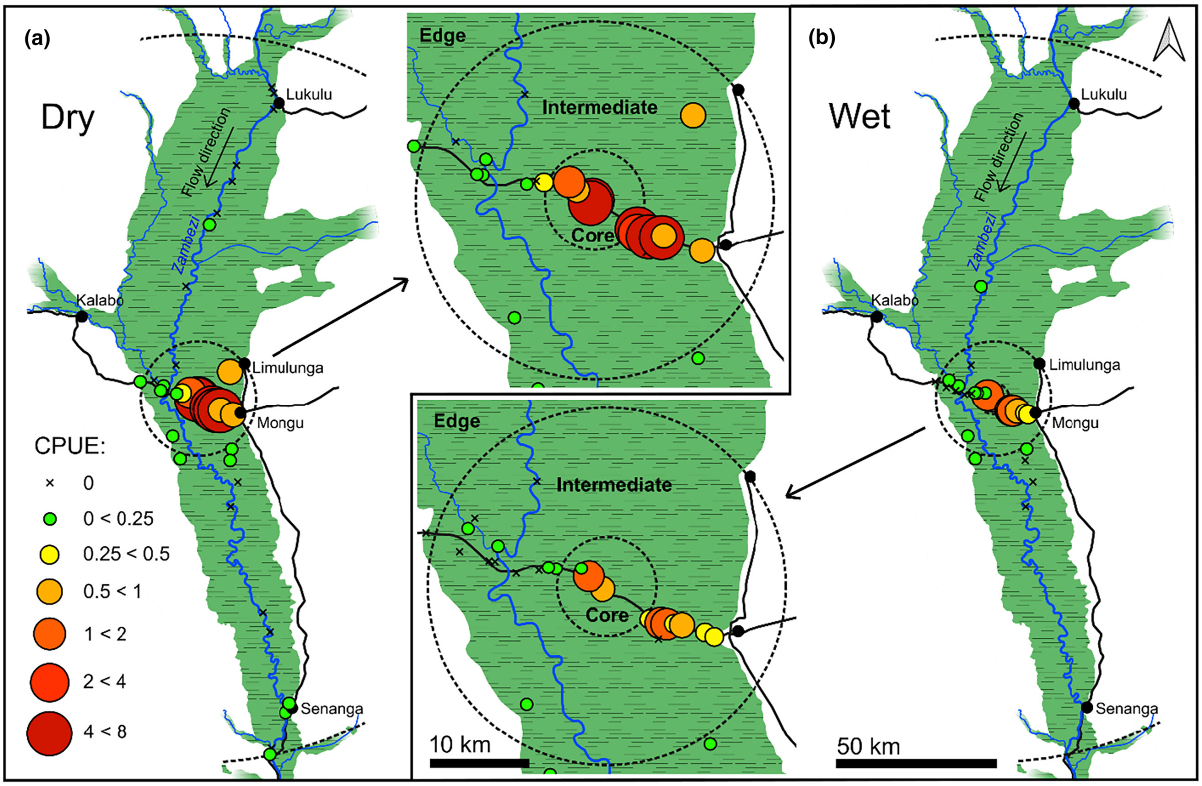

FIGURE 2 Relative abundance and spatial distribution of *Cherax quadricarinatus* in (a) dry and (b) wet season across the three distributional regions (core, intermediate and front) on the Barotse floodplain. Catch per unit effort (CPUE) is represented by circles and absence of crayfish is indicated by open squares.

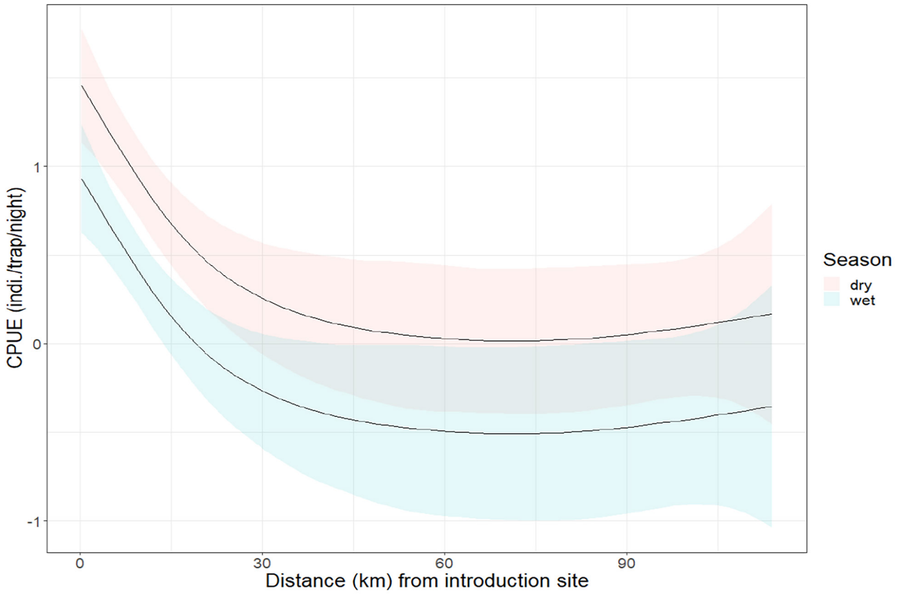

FIGURE 3 Seasonal change in catch per unit effort (CPUE) of *Cherax quadricarinatus* across the invasion range on the Barotse floodplain, Upper Zambezi River, Zambia. Zero (0) is the core and distance is outward from the core to the front. Wet and dry season CPUE are comparable at the front.

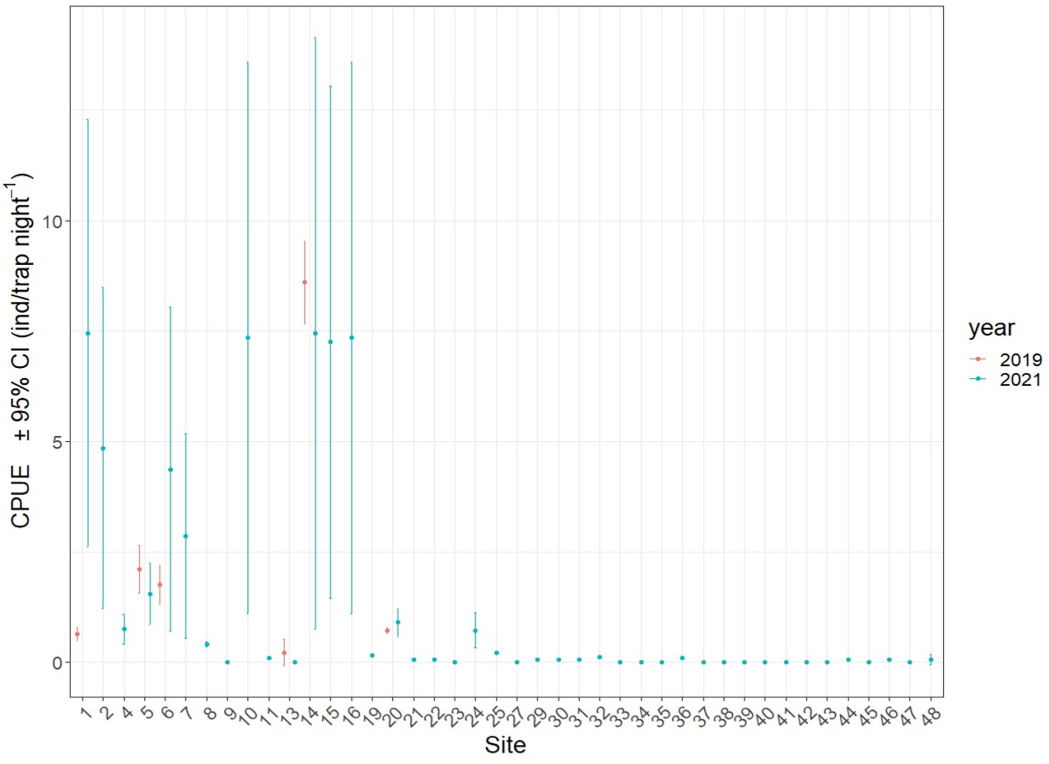

FIGURE 4 Dry season catch per unit effort (CPUE) of *Cherax quadricarinatus* for sites sampled in the year 2019 and 2021 on the Barotse floodplain, Upper Zambezi River, Zambia. Sites arranged in the order of increasing distance from the point of introduction. Plot of means showing 95% confidence interval.

$p = 0.31$; Table S2) and there was no separation along either PC1 or PC2 indicating no clear distinction in morphometric traits of *C. quadricarinatus* sampled from different habitats across the floodplain (Figure 5). Invasion range had a significant effect on morphological variance ($F_{2,1281} = 4.15$, $p < 0.05$; Figure 6; Table S2) whereby variance and ellipse size were higher in the core compared to in the front and

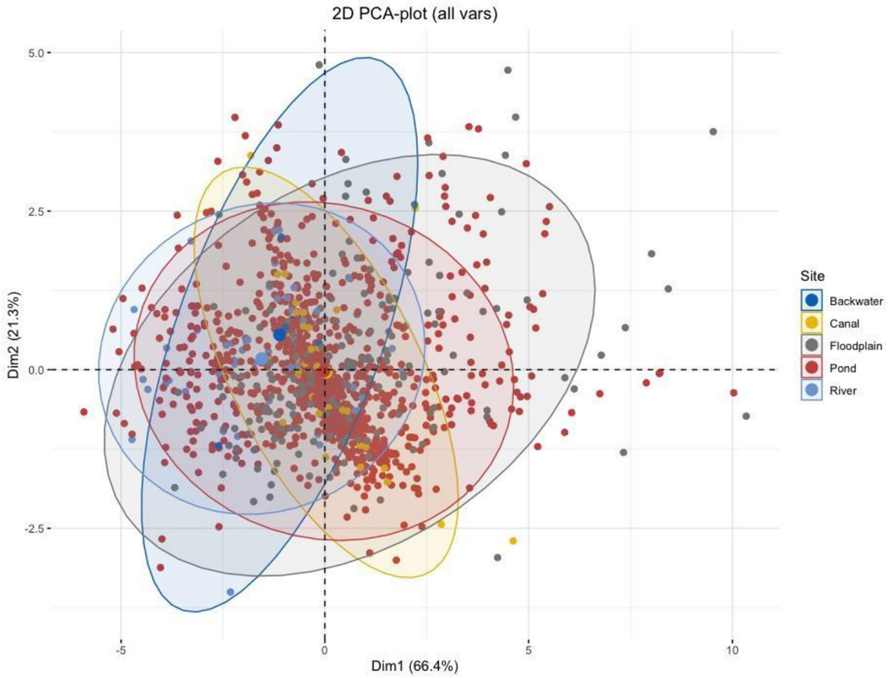

FIGURE 5 A principal components analysis (PCA) plot showing broad overlap in morphometric traits of *Cherax quadricarinatus* along both PC1 and PC2. Crayfish sampled from different aquatic habitats across the Barotse floodplain.

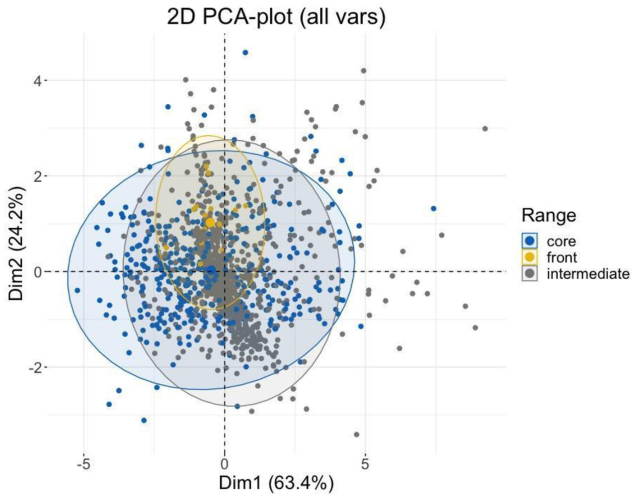

FIGURE 6 A principal components analysis (PCA) plot showing broad overlap in morphometric traits of *Cherax quadricarinatus* along both PC1 and PC2. Crayfish sampled from three regions across the invasion range on the Barotse floodplain.

intermediate ranges, and there was less variance in the front compared to in the core (all permuted $p > 0.05$; Figure 6; Table S2).

3.5 | Spatial sorting

3.5.1 | Front leg length

There was a significant three-way interaction between distance, sex and season (Table 2) whereby male and female FLL were longer towards the front in the wet season (Figure 7). Distance significantly interacted with sex (Table 2) whereby FLL for all sexes got longer towards the front (Figure 7). Sex significantly interacted with season (Table 2) whereby male and female front legs were longer in the wet season, whereas chelae of intersex were longer in the dry season (Figure 7).

TABLE 2 Model terms for all factors from a generalised linear model (GLM) with a gamma distribution used to determine effects on front leg length with regards to factors "distance", "sex" and "season", using Type 3 ANOVA and χ^2 to report the effect size of a factor on the dependent variable.

Model term	χ^2	df	*p*-value
Distance	34.49	1	0.094
Sex	34.47	2	0.729
Season	33.69	1	<0.001
Distance × Sex	33.32	2	<0.001
Distance × Season	33.32	1	0.085
Sex × Season	31.68	2	<0.001
Distance × Sex × Season	31.40	2	<0.05

3.6 | Density dependence

3.6.1 | Geometric mean

There was a significant interaction effect between sex and season on GM, a proxy for overall body size, (Table 3) whereby males and females

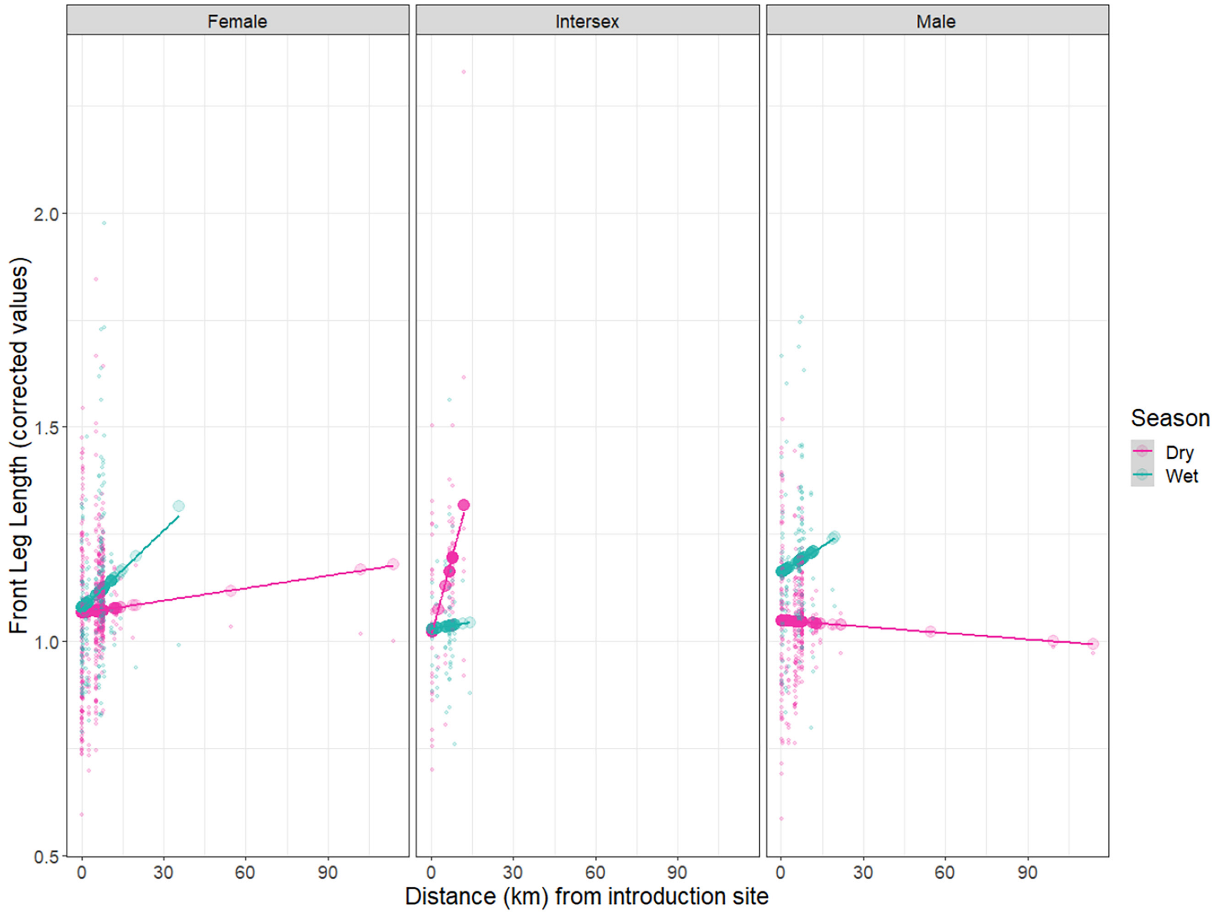

FIGURE 7 Predicted relationship of corrected front leg length (FLL) with distance from introduction site with respect to sex and season. Large points indicate predicted values from the generalised linear model (GLM) and small points indicate individual data points of crayfish FLL.

TABLE 3 Model terms for all factors from a generalised linear model (GLM) with a gamma distribution used to determine effects on geometric mean (GM) with regards to factors "distance", "sex" and "season", using Type 3 ANOVA and χ^2 to report the effect size of a factor on the dependent variable.

Model term	χ^2	df	*p*-value
Distance	69.69	1	0.062
Sex	60.48	2	<0.001
Season	59.44	1	<0.001
Distance × Sex	58.53	2	<0.001
Sex × Season	57.35	2	<0.001

had higher GM than intersex individuals (both, $p<0.01$), whereas males had higher GM than females ($p<0.01$). All sexes were bigger overall in the wet season compared to the dry season ($p<0.01$) (Figure 8). Distance significantly interacted with sex (Table 3) whereby male and female individuals had increased GM at the invasion front whereas intersex individuals had decreased GM (Figure 8).

3.6.2 | Chelae length

There was a significant three-way interaction between distance, sex and season on CL (Table 4) whereby male and female chelae were longer towards the range front in the wet season, whereas chelae of intersex were longer in the dry season (Figure 9).

3.6.3 | Body condition

Invasion range had a significant effect on body condition of both male and female crayfish (Table 5, models A,B). Where individuals in the invasion core were in better condition than at the front (male, $p=0.02$; female, $p<0.001$; Figure 10) but there was no difference in body condition between the core and intermediate range (male, $p=0.706$; female, $p=0.846$; Figure 10). Individuals in the intermediate range had better body condition than those at the front (male, $p=0.033$; female, $p<0.001$; Figure 10). Body condition was better in the wet season for males and females (male, $p<0.001$; female, $p<0.05$; Table 5, models A,B; Figure 10). Neither invasion range nor season affected the body condition of intersex individuals (Table 5, model C).

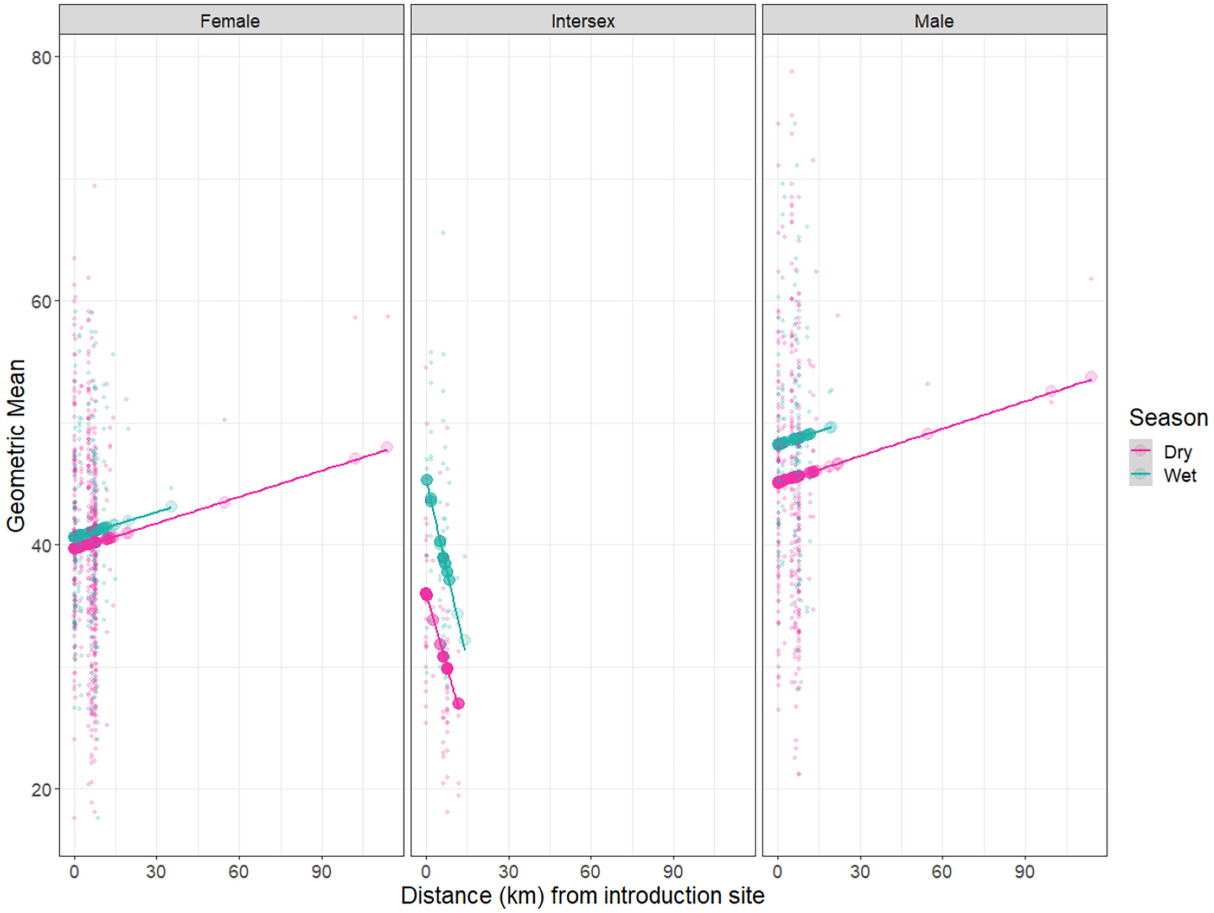

FIGURE 8 Predicted relationship of geometric mean (GM) with distance from introduction site with respect to sex and season. Large points indicate predicted values from the generalised linear model (GLM) and small points indicate individual data points of crayfish GM.

TABLE 4 Model terms for all factors from a generalised linear model (GLM) with a gamma distribution used to determine effects on chelae length mean with regards to factors "distance", "sex" and "season", using Type 3 ANOVA and χ^2 to report the effect size of a factor on the dependent variable.

Model term	χ^2	df	*p* value
Distance	38.82	1	<0.001
Sex	35.58	2	<0.001
Season	33.88	1	<0.001
Distance × Sex	33.65	2	<0.01
Distance × Season	33.32	1	<0.001
Sex × Season	31.68	2	<0.001
Distance × Sex × Season	31.40	2	<0.01

4 | DISCUSSION

Understanding the interactions between the individual, environment and colonisation processes which drive phenotypic variation and dispersal in invasive species is an on-going challenge, yet essential in determining intra-individual responses to environmental change. Our results indicate that the *C. quadricarinatus* invasion is indeed progressing rapidly through the Upper Zambezi system and represents a cause for concern. There was a clear core-front invasion gradient in the population abundance and traits, but there was no evidence of environmental filtering acting on traits of individuals in different habitats. Our hypothesis regarding spatial sorting was confirmed as individuals at the invasion front had longer front legs. Our hypothesis regarding density dependence was not confirmed as individuals at the core were larger overall and had better body condition than those at the front, yet weapons investment (CL) at the front was stronger than at the core, opposing our hypothesis. This indicates that there may be other factors, such as predation pressure, driving trait differences across the invasion gradient.

Within the Barotse floodplain *C. quadricarinatus* has expanded its range approximately 107.84 km downstream and 93.70 km upstream over a period of 2 years (Madzivanzira, South, Ellender, et al., 2021). Detection probability declined at the invasion front and was lower than the detection probability determined in 2019 at the previous front (Madzivanzira, South, Ellender, et al., 2021). This is indicative of a very recently colonised invasion front (Hudina et al., 2012). The current downstream spread

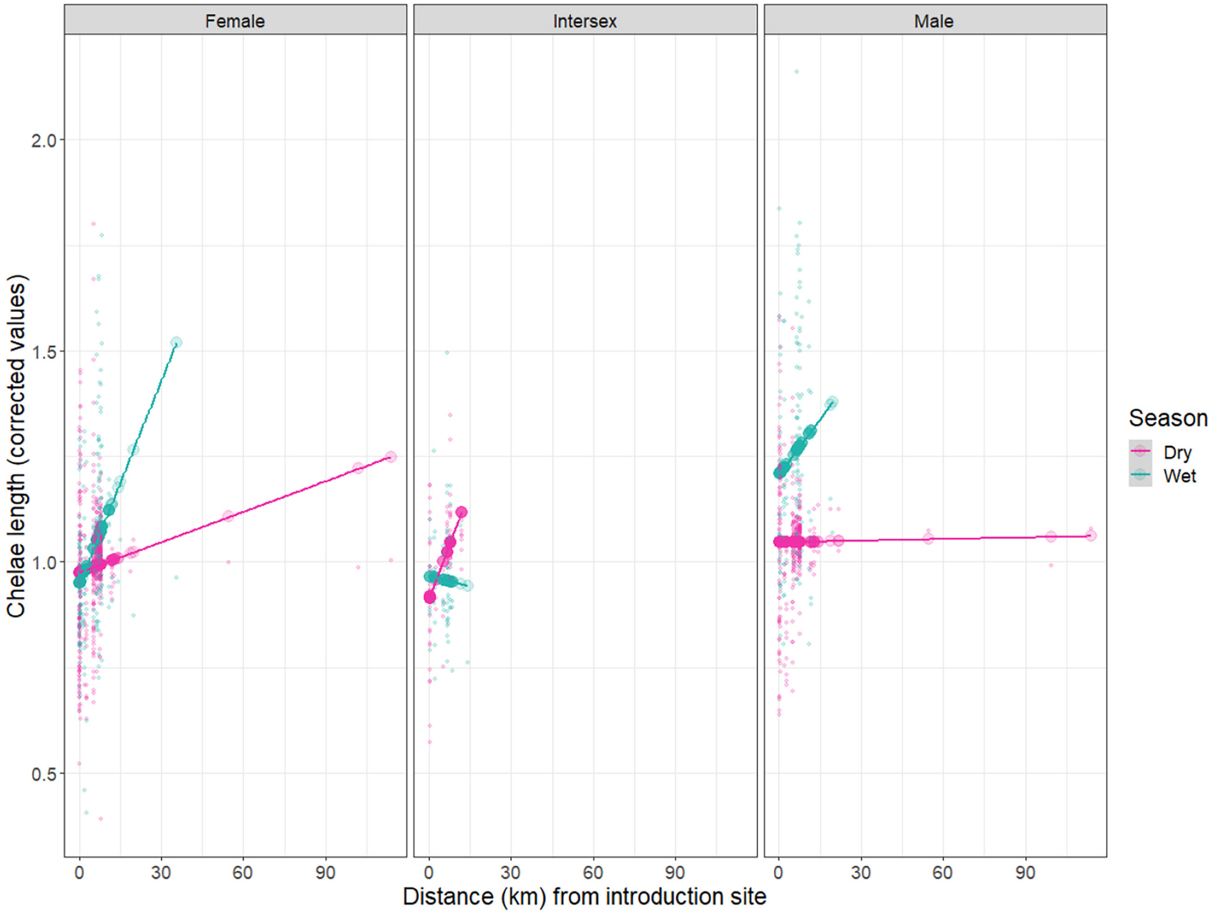

FIGURE 9 Predicted relationship of corrected chelae length (CL) with distance from introduction site with respect to sex and season. Large points indicate predicted values from the generalised linear model (GLM) and small points indicate individual data points of crayfish CL.

TABLE 5 Model terms for all factors from a generalised linear model (GLM) with a gamma distribution used to determine effects on body condition with regards to factors "range" and "season", using Type 2 ANOVA and χ^2 to report the effect size of a factor on the dependent variable.

Model	Model term	χ^2	df	*p* value
A (Male)	Range	7.901	2	0.021
	Season	7.448	1	<0.001
B (Female)	Range	11.672	2	<0.001
	Season	11.599	1	0.024
C (intersex)	Range	2.688	1	0.059
	Season	2.688	1	0.96

Note: Model A for male, Model B for female and Model C for intersex.

rate of *C. quadricarinatus* falls within the earlier estimated range (49 ± 29 km/year) while the upstream spread rate is 2 to 3 times higher than the earlier estimation (12 ± 7 km/year; Madzivanzira, South, Ellender, et al., 2021), both of which are higher than the spread in the Inkomati Basin in South Africa (Nunes et al., 2017) and other non-native crayfish species in Europe (Hudina et al., 2009; Soto et al., 2023). The flood pulse in the Upper Zambezi was very low before sampling in 2019, whereas the flood in 2020 was a record high. Seasonal hydrological variability is likely to contribute to the spread rate as shown by crayfish being found on the floodplain during high water and also in isolated ponds not connected to flowing water channels. Invasion processes in intermittent waterbodies are an emerging and understudied dynamic which would benefit from further research in floodplain ecosystems (Guareschi & South, 2024). Episodic flood events have been associated with transporting other non-native species such as *P. leniusculus* (Bubb et al., 2004), rusty crayfish *Faxonuis rusticus* (Messager & Olden, 2018) and golden mussels *Limnoperna fortunei* (de Amo et al., 2021) to new habitats. Furthermore, climate-induced frequency of flash floods is expected to increase *C. quadricarinatus* dispersal in South Africa (van Wilgen et al., 2022). Seasonal floods may therefore be contributing to the observed nonlinear dispersal pattern of crayfish on the Barotse floodplain. The lack of environmental filtering on morphometric traits is probably a consequence of hydrological connectivity of the population and habitats in the wet season as floods facilitate admixture of seasonally isolated individuals and homogenises environmental conditions (de Amo et al., 2021; Thomaz et al., 2007). Furthermore, in the dry season the ponds distributed across the Barotse floodplain provide refuge

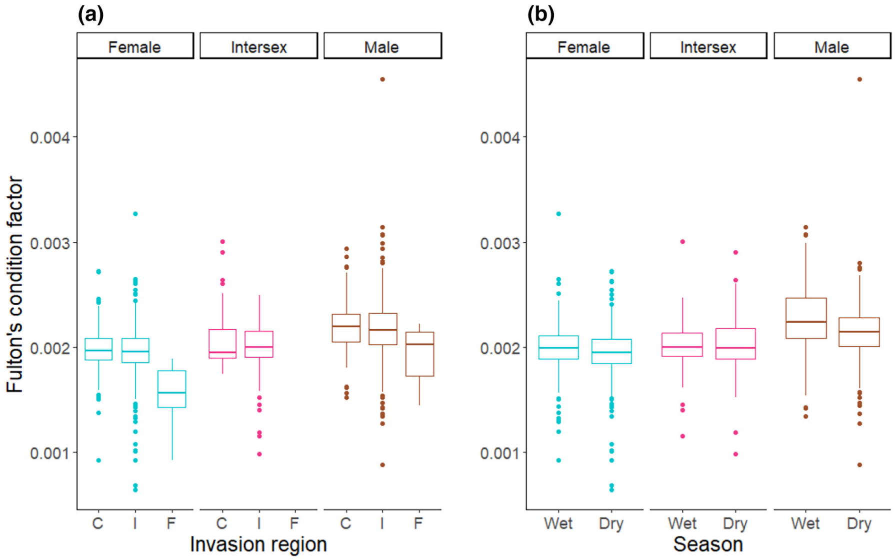

FIGURE 10 Comparison of Fulton's body condition indices of male, female and intersex *Cherax quadricarinatus* (a) between invasion regions (C = Core; I = Intermediate; F = front) and (b) seasons. Boxplots represents median and 25%–75% interquartile range. Points are individual Fulton's condition data.

from the receding water level and are likely to act as source population or stepping-stones for further range expansion (Johnson et al., 2008; Madzivanzira, South, Ellender, et al., 2021; Nunes et al., 2017). Given that crayfish were detected at Kalongola, the furthest sampling sites downstream, it is not surprising that reports of crayfish occurrence much further downstream at Katima Mulilo have recently emerged (Madzivanzira, South, Ellender, et al., 2021; F Jacobs, pers. com). However, it remains to be ascertained whether these reported changes are the result of natural spread or human-mediated translocation.

The sex structure of *C. quadricarinatus* was not different across the three invasion regions, suggesting that both males and females are contributing towards range expansion. However, very few individuals were sampled at the front of the invasion range which is characteristic of invasive populations but ultimately limits relationship confidence (Hudina et al., 2012). In linear riverine systems, crayfish species tend to show a more male-biased sex ratio towards the invasion front (Ďuriš et al., 2006; Hudina et al., 2012); a male-biased sex ratio was also found in the invasive freshwater fish round goby *Neogobius melanostomus* (Gutowsky & Fox, 2011). The present study confirmed intersex individuals in the core and intermediate populations, but not in the front. Intersex individuals skew the population to a more female-dominated population as crosses between intersex and normal female tend to produce female-biased progeny (Levy et al., 2020). Intersex individuals may be an ecologically stable strategy to enhance population fitness in highly stochastic environments such as the floodplains where *C. quadricarinatus* evolved and invaded (Levy et al., 2020). Intersex proportional representation across three invasion cores in Southern Africa declined with time since invasion (Madzivanzira, South, Ellender, et al., 2021). Monitoring sex ratios may be an informative index to assess overall population status with respect to identifying boom–bust dynamics along the invasion curve (Haubrock et al., 2022).

We report on the first evidence of spatial sorting in an invasive population of *C. quadricarinatus*. Spatial sorting of dispersal traits has been reported in rusty crayfish *Faxonius rusticus* (Messager & Olden, 2019), signal crayfish (Hudina et al., 2012), cane toads (Brown et al., 2014; Clarke et al., 2019) and African clawed frog *Xenopus laevis* (Kruger et al., 2021). Longer walking legs are a dispersal trait facilitating faster movement across the floodplain (Pond, 1995), while well documented in cane toads (Brown et al., 2014; Phillips et al., 2006, 2008) and the flightless predatory ground beetle, *Merizodus soledadinus* (Laparie et al., 2013), this is the first record of selection for this trait in a crayfish invasion. Longer walking legs may also increase ability to move across terrestrial habitats between water bodies on the floodplain as *C. quadricarinatus* has been found walking overland in the Kafue Flats and the Barotse floodplain (Madzivanzira, South, Ellender, et al., 2021). Overland movement is expected in response to inclement environmental conditions (i.e., poor water quality, overcrowding and predation) which characterises water bodies during the dry season (Light, 2003). Despite this, loss or injury of walking legs does not impede instream dispersal potential in crayfish, but this has not been assessed for overland movement (Galib et al., 2022). During the dry season, the receding water level could be restricting *C. quadricarinatus* to permanent water bodies, which would explain the high observed detection probability. Seasonal changes in phenotype at the invasion front of round goby invasions were also detected with patterns not obvious during the summer period (Gutowsky & Fox, 2011).

We found a trend of increasing overall body size, decreasing body condition and increasing chelae length at the invasion front compared to the core. The clear invasion gradient in terms of density across the length of the floodplain and overall body size reflects the differences identified between invasion cores in Madzivanzira, South, Ellender, et al. (2021). These findings are contrary to the dynamics in rusty crayfish which had lower body mass and smaller chelae at the invasion front (Messager & Olden, 2019), and partially similar to signal crayfish which had smaller sized individuals but larger chelae in males at the invasion front (Hudina et al., 2012). Density, personality (i.e., boldness in an open field test) and habitat were driving factors in riverine dispersal of signal crayfish (Galib et al., 2022). Chelae length was expected to decrease with increased distance from the core because the population was released from density-dependent high intra-specific competition, or because sub-dominant individuals would be leading the invasion front as they were excluded from the core population (Galib et al., 2022; Messager & Olden, 2019). Relative chelae size is associated with resource holding potential and behavioural traits, such as aggression and boldness, which may be selected during dispersal (Hudina et al., 2015; Pintor et al., 2009). Although relative chelae size is important in agonistic interactions (Streissl & Hold, 2002), CL is an unreliable indicator of inter-specific agonistic interaction in *C. quadricarinatus* as closing force is related to body mass (South et al., 2020). In this regard, the individuals at the invasion front would appear to be sub-dominant owing to their lower body condition but instead are dishonest signallers through investment in larger chelae and larger overall body size (i.e., GM) (Graham et al., 2020; South et al., 2020). Another reason for continued investment in weapons may be predation pressure, as fish select crayfish with smaller chelae, or niche competition with native functional analogues potamonautid crabs (South et al., 2020; Stein, 1977). Resolving these dynamics using stable isotope analysis of the aquatic community across the invasion gradient would provide valuable insight into the relationship between the whole food web and invasion processes.

Lower body condition at the invasion front also contrasts with our initial hypothesis, as release from high conspecific competition at the densely populated core sites ought to result in greater resource availability. Crayfish in other systems showed the opposite trend from our results, demonstrating that release from competition at the invasion front increased body condition (Messager & Olden, 2019; Rebrina et al., 2015); however, these studies were completed in riverine environments which may be more resource-restricted than in the Barotse floodplain. Body condition may be a misleading trait in crayfish species, as rusty crayfish had lower relative weight at the invasion front but were in better physiological condition (i.e., anabolic activity) than those at the core (Messager & Olden, 2019). Furthermore, an alternative hypothesis to be considered could be that carapace thickness was not measured, and this could be affecting body condition calculations. Carapace thickness is related to calcium deposition and, thus, mineral concentration in the environment (Ahearn et al. 2004; Wheatly, 1996), and therefore we would expect that the carapace thickness in the Barotse floodplain would be related to calcium concentration. Across the floodplain there is relatively high calcium content which varies by year and decreases somewhat in the dry season (Nyambe et al., 2018). The upper extent of the floodplain (Lukulu–northern invasion front) has a lower calcium content in the dry season compared to the southern portion, but there was a more homogenous calcium content across the floodplain in the wet season (Nyambe et al., 2018). Our results indicate better body condition in the wet season which may be partly to do with increased shell thickness, along with lack of thermal stress, and more resources. However, GM increased at the invasion front which contradicts the pattern of calcium concentration, whereas body condition showed the opposite trend.

These results indicate that the phenotypic variation in the Barotse floodplain invasion by *C. quadricarinatus* can be characterised by complex processes of spatial sorting of dispersal traits related to walking, density-dependent processes pushing out less competitive individuals from the core and phenotypic variation potentially because of predation pressure. All of these processes are further mediated by the flood pulse regime, as body condition was consistently better in the wet season, when resources are plentiful and, thus, competition and physiological stress are reduced. The colonisation and community dynamics of the Barotse floodplain ponds during the dry season are not well-understood, yet drought conditions increasing fishless ponds supports crayfish populations in North American seasonal wetlands (Dorn, 2008). Better understanding of the interplay of the annual flood and hydrological variability with regards to eco-evolutionary drivers of invasions is crucial (Chuang & Peterson, 2016; Perry et al., 2013). Targeted removal and population control in the front populations may reduce density-dependent processes and remove disperser-adapted phenotypes from the gene pool. Implementation of molecular methods, such as single nucleotide polymorphism analysis, may elucidate the population dynamics and trait heritability within this expanding invasion (Chuang & Peterson, 2016).

AUTHOR CONTRIBUTIONS

Conducting the research: Nawa Nawa, Josie South, Takudzwa C. Madzivanzira, Bruce R. Ellender; *Conceptualisation*, *data interpretation*, *data analysis*, *writing*: All authors.

ACKNOWLEDGEMENTS

This project was made possible by the late Prof. Olaf LF Weyl who passed away on 14 November 2020. This study was supported by the Department of Science and Innovation (DSI) through the National Research Fund (NRF)–South African Research Chairs Initiative (Inland Fisheries and Freshwater Ecology, grant no. 110507) and the Worldwide Fund for Nature (WWF) Upper Zambezi Programme that is funded by DOB Ecology. We acknowledge the use of infrastructure and equipment provided by the NRF-SAIAB Research Platform as well as the funding channelled through NRF-SAIAB Institutional Support system. The Zambian Ministry of Fisheries and Livestock, and the Department of Fisheries, Chilanga District are thanked for issuing research permits. The Central Veterinary Research Institute is acknowledged for competence clearance. We also thank Dr M Chomba and Mr K Chinyawezi (WWF-Zambia, Mongu Office) for logistical support during fieldwork. Ethics clearance was acquired through Rhodes University Animal Ethics Committee (ethics approval no. 2020-1444-4697). Any opinion, finding and conclusion or recommendation expressed in this material is that of the authors. The National Research Fund of South Africa does not accept any liability in this regard.

FUNDING INFORMATION

Department of Science and Innovation (DSI), National Research Foundation (NRF) South African Research Chairs Initiative in Inland Fisheries and Freshwater Ecology, grant/award no. 110507; Worldwide Fund for Nature (WWF) Upper Zambezi Programme that is funded by DOB Ecology; UKRI Future Leaders Fellowship grant/award no. MR/X035662/1.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data from this research will be made available on Dryad.

ORCID

Josie South https://orcid.org/0000-0002-6339-4225
Bruce R. Ellender https://orcid.org/0000-0002-4398-9491
Josephine Pegg https://orcid.org/0000-0001-6364-4741
Takudzwa C. Madzivanzira https://orcid.org/0000-0001-9683-5798
Ryan J. Wasserman https://orcid.org/0000-0002-4162-1503

REFERENCES

Abell, R., Thieme, M. L., Revenga, C., Bryer, M., Kottelat, M., Bogutskaya, N., Coad, B., Mandrak, N., Balderas, S. C., Bussing, W., Stiassny, M. L. J., Skelton, P., Allen, G. R., Unmack, P., Naseka, A., Ng, R., Sindorf, N., Robertson, J., Armijo, E., … Petry, P. (2008). Freshwater ecoregions of the world: A new map of biogeographic units for freshwater biodiversity conservation. *Bioscience*, *58*, 403–414. https://doi.org/10.1641/B580507

Ahearn, G. A., Mandal, P. K., & Mandal, A. (2004). Calcium regulation in crustaceans during the molt cycle: A review and update. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *137*(2), 247–257. https://doi.org/10.1016/j.cbpb.2003.10.012

Akmal, S. G., Santoso, A., Yuliana, E., & Patoka, J. (2021). Redclaw crayfish (*Cherax quadricarinatus*): Spatial distribution and dispersal pattern in Java, Indonesia. *Knowledge and Management of Aquatic Ecosystems*, *422*, 16. https://doi.org/10.1051/kmae/2021015

Blackburn, T. M., Pysek, P., Bacher, S., Carlton, J. T., Duncan, R. P., Jarošík, V., Wilson, J. R. U., & Richardson, D. M. (2011). A proposed unified framework for biological invasions. *Trends in Ecology & Evolution*, *26*, 333–339. https://doi.org/10.1016/j.tree.2011.03.023

Briski, E., Chan, F. T., Darling, J. A., Lauringson, V., MacIsaac, H. J., Zhan, A., & Bailey, S. A. (2018). Beyond propagule pressure: Importance of selection during the transport stage of biological invasions. *Frontiers in Ecology and the Environment*, *16*(6), 345–353. https://doi.org/10.1002/fee.1820

Brown, G. P., Phillips, B. L., & Shine, R. (2014). The straight and narrow path: The evolution of straight-line dispersal at a cane toad invasion front. *Proceedings of the Royal Society B: Biological Sciences*, *281*, 20141385. https://doi.org/10.1098/rspb.2014.1385

Bubb, D. H., Thom, T. J., & Lucas, M. C. (2004). Movement and dispersal of the invasive signal crayfish *Pacifastacus leniusculus* in upland rivers. *Freshwater Biology*, *49*, 357–368. https://doi.org/10.1111/j.1365-2426.2003.01178.x

Burton, O. J., Phillips, B. L., & Travis, J. M. J. (2010). Trade-offs and the evolution of life-histories during range expansion. *Ecology Letters*, *13*, 1210–1220. https://doi.org/10.1111/j.1461-0248.2010.01505.x

Cai, X., Haile, A. T., Magidi, J., Mapedza, E., & Nhamo, L. (2017). Living with floods—Household perception and satellite observations in the Barotse floodplain, Zambia. *Physics and Chemistry of the Earth*, *100*, 278–286. https://doi.org/10.1016/j.pce.2016.10.011

Carboni, M., Münkemüller, T., Lavergne, S., Choler, P., Borgy, B., Violle, C., Essl, F., Roquet, C., Munoz, F., DivGrass Consortium, & Thuiller, W. (2016). What it takes to invade grassland ecosystems: Traits, introduction history and filtering processes. *Ecology Letters*, *19*, 219–229. https://doi.org/10.1111/ele.12556

Catford, J. A., Downes, B. J., Gippel, C. J., & Vesk, P. A. (2011). Flow regulation reduces native plant cover and facilitates exotic invasion in riparian wetlands. *Journal of Applied Ecology*, *48*(2), 432–442. https://doi.org/10.1111/j.1365-2664.2010.01945.x

Chapple, D. G., Simmonds, S. M., & Wong, B. B. M. (2012). Can behavioral and personality traits influence the success of unintentional species introductions? *Trends in Ecology & Evolution*, *27*, 57–64. https://doi.org/10.1016/j.tree.2011.09.010

Chuang, A., & Peterson, C. R. (2016). Expanding population edges: Theories, traits, and trade-offs. *Global Change Biology*, *22*(2), 494–512. https://doi.org/10.1111/gcb.13107

Clarke, G. S., Shine, R., & Phillips, B. L. (2019). May the (selective) force be with you: Spatial sorting and natural selection exert opposing forces on limb length in an invasive amphibian. *Journal of Evolutionary Biology*, *32*, 994–1001. https://doi.org/10.1111/jeb.13504

de Amo, V. E., Ernandes-Silva, J., André Moi, D., & Mormul, R. P. (2021). Hydrological connectivity drives the propagule pressure of *Limnoperna fortunei* (Dunker, 1857) in a tropical river–floodplain system. *Hydrobiologia*, *848*, 2043–2053. https://doi.org/10.1007/s10750-021-04543-8

Dorn, N. J. (2008). Colonization and reproduction of large macroinvertebrates are enhanced by drought-related fish reductions. *Hydrobiologia*, *605*, 209–218. https://doi.org/10.1007/s10750-008-9355-7

Douthwaite, R. J., Jones, E. W., Tyser, A. B., & Vrdoljak, S. M. (2018). The introduction, spread and ecology of redclaw crayfish *Cherax quadricarinatus* in the Zambezi catchment. *African Journal of Aquatic Science*, *43*, 353–366. https://doi.org/10.2989/16085914.2018

Ďuriš, Z., Drozd, P., Horká, I., Kozák, P., & Policar, T. (2006). Biometry and demography of the invasive crayfish *Orconectes limosus* in The Czech Republic. *Bulletin Français de la Pêche et de la Pisciculture*, *380–381*, 1215–1228. https://doi.org/10.1051/kmae/20061215

Ellender, B. R., Weyl, O. L. F., Winker, H., & Booth, A. J. (2010). Quantifying the annual fish harvest from South Africa's largest freshwater reservoir. *Water SA*, *36*, 45–52. https://doi.org/10.4314/wsa.v36i1.50906

Florian, H. (2022). DHARMa: Residual diagnostics for hierarchical (multi-level / mixed) regression models. R Package Version 4.1.1. https://CRAN.R-project.org/package=DHARMa

Froese, R. (2006). Cube law, condition factor and weight–length relationships: History, meta-analysis and recommendations. *Journal of Applied Ichthyology*, *22*, 241–253. https://doi.org/10.1111/j.1439-0426.2006.00805.x

Galib, S. M., Sun, J., Twiss, S. D., & Lucas, M. C. (2022). Personality, density and habitat drive the dispersal of invasive crayfish. *Scientific Reports*, *12*, 1114. https://doi.org/10.1038/s41598-021-04228-1

Graham, Z. A., Padilla-Perez, D. J., & Angilletta, M. J., Jr. (2020). Virile crayfish escalate aggression according to body size instead of weapon size. *Animal Behaviour*, *163*, 9–15. https://doi.org/10.1016/j.anbehav.2020.02.014

Guareschi, S., & South, J. (2024). Biological invasions in intermittent rivers and streams: Current knowledge, and future frontiers [Invasiones biológicas en ríos y arroyos intermitentes: Conocimiento actual y fronteras futuras]. *Ecosistemas: Revista Cietifica y Tecnica de Ecologia y Medio Ambiente*, *33*, 2600. https://doi.org/10.7818/ECOS.2600

Gutowsky, L. F. G., & Fox, M. G. (2011). Occupation, body size and sex ratio of round goby (*Neogobius melanostomus*) in established and newly invaded areas of an Ontario River. *Hydrobiologia*, *671*, 27–37. https://doi.org/10.1007/s10750-011-0701-9

Haubrock, P. J., Ahmed, D. A., Cuthbert, R. N., Stubbington, R., Domisch, S., Marquez, J. R. G., Beidas, A., Amatulli, G., Kiesel, J., Shen, L. Q., Soto, I., Angeler, D. G., Bonada, N., Cañedo-Argüelles, M., Csabai, Z., Datry, T., de Eyto, E., Dohet, A., Drohan, E., … Haase, P. (2022). Invasion impacts and dynamics of Europena-wide introduction species. *Global Change Biology*, *22*, 4620–4632.

Höfle, R., Dullinger, S., & Essl, F. (2014). Different factors affect the local distribution, persistence and spread of alien tree species in floodplain forests. *Basic and Applied Ecology*, *15*(5), 426–434. https://doi.org/10.1016/j.baae.2014.07.007

Hudina, S., Faller, M., Lucić, A., Klobučar, G., & Maguire, I. (2009). Distribution and dispersal of two invasive crayfish species in the Drava River basin, Croatia. *Knowledge and Management of Aquatic Ecosystems*, 09, 394–395, 09 https://doi.org/10.1051/KMAE/2009023

Hudina, S., Hock, K., Žganec, K., & Lucić, A. (2012). Changes in population characteristics and structure of the signal crayfish at the edge of its invasive range in a European river. *Annales de Limnologie - International Journal of Limnology*, *48*, 3–11. https://doi.org/10.1051/limn/2011051

Hudina, S., Žganec, K., & Hock, K. (2015). Differences in aggressive behaviour along the expanding range of an invasive crayfish: An important component of invasion dynamics. *Biological Invasions*, *17*, 3101–3112. https://doi.org/10.1007/s10530-015-0936-x

Johnson, P. T. J., Olden, J. D., & Vander Zanden, M. J. (2008). Dam invaders: Impoundments facilitate biological invasions into freshwaters. *Frontiers in Ecology and the Environment*, *6*, 357–363. https://doi.org/10.1890/070156

Jungers, W. L., Falsetti, A. B., & Wall, C. E. (1995). Shape, relative size, and size-adjustments in morphometrics. *American Journal of Physical Anthropology*, *38*, 137e161. https://doi.org/10.1002/ajpa.1330380608

Klingenberg, C. P. (2016). Size, shape, and form: Concepts of allometry in geometric morphometrics. *Development Genes and Evolution*, *226*, 113–137. https://doi.org/10.1007/s00427-016-0539-2

Kouba, A., Oficialdegui, F. J., Cuthbert, R. N., Kourantidou, M., South, J., Tricarico, E., Gozlan, R. E., Courchamp, F., & Haubrock, P. J. (2022). Identifying economic costs and knowledge gaps of invasive aquatic crustaceans. *Science of the Total Environment*, *813*, 152325. https://doi.org/10.1016/j.scitotenv.2021.152325

Kraft, N. J. B., Adler, P. B., Godoy, O., James, E. C., Fuller, S., & Levine, J. M. (2015). Community phylogenetics and ecosystem functioning: Community assembly, coexistence and the environmental filtering metaphor. *Functional Ecology*, *29*, 592–599. https://doi.org/10.1111/1365-2435.12345

Kruger, N., Measey, J., Vimercati, G., Herrel, A., & Secondi, J. (2021). Does the spatial sorting of dispersal traits affect the phenotype of the non-dispersing stages of the invasive frog *Xenopus laevis* through coupling? *Biological Journal of the Linnean Society*, *132*, 257–269. https://doi.org/10.1093/biolinnean/blaa191

Lang, I., Paz-Vinas, I., Cucherousset, J., & Loot, G. (2021). Patterns and determinants of phenotypic variability within two invasive crayfish species. *Freshwater Biology*, *66*, 1782–1798. https://doi.org/10.1111/fwb.13792

Laparie, M., Lebouvier, M., Lalouette, L., & Renault, D. (2010). Variation of morphometric traits in populations of an invasive carabid predator (*Merizodus soledadinus*) within a sub-Antarctic Island. *Biological Invasions*, *12*, 3405–3417. https://doi.org/10.1007/s10530-010-9739-2

Laparie, M., Renault, D., Lebouvier, M., & Delattre, T. (2013). Is dispersal promoted at the invasion front? Morphological analysis of a ground beetle invading the Kerguelen Islands, Merizodus soledadinus (Coleoptera, Carabidae). *Biological Invasions*, *15*, 1641–1648.

Lenth, R. V. (2022). emmeans: Estimated marginal means, aka least-squares means. R package version 1.7.3. https://CRAN.R-project.org/package=emmeans

Levy, T., Ventura, T., de Leo, G., Grinshpan, N., Abu Abayed, F. A., Manor, R., Savaya, A., Sklarz, M. Y., Chalifa-Caspi, V., Mishmar, D., & Sagi, A. (2020). Two homogametic genotypes–one crayfish: On the consequences of intersexuality. *Iscience*, *23*, 101652. https://doi.org/10.1016/j.isci.2020.101652

Light, T. (2003). Success and failure in a lotic crayfish invasion: The roles of hydrologic variability and habitat alteration. *Freshwater Biology*, *48*, 1886–1897. https://doi.org/10.1046/j.1365-2427.2003.01122.x

Lodge, D. M., Deines, A., Gherardi, F., Yeo, D. C. J., Arcella, T., Baldridge, A. K., Barnes, M. A., Chadderton, W. L., Feder, J. L., Gantz, C. A., Howard, G. W., Jerde, C. L., Peters, B. W., Peters, J. A., Sargent, L. W., Turner, C. R., Wittmann, M. E., & Zeng, Y. (2012). Global introductions of crayfishes: Evaluating the impact of species invasions on ecosystem services. *Annual Review of Ecology, Evolution, and Systematics*, *43*, 449–472. https://doi.org/10.1146/annurev-ecolsys-111511-103919

Madzivanzira, T. C., South, J., Ellender, B. R., Chalmers, R., Chisule, G., Coppinger, C. R., Khaebeb, F. H., Jacobs, F. J., Chomba, M., Musando, B., Mwale, C., Nhiwatiwa, T., Rennie, C. L., Richardson, N., & Weyl, O. L. F. (2021). Distribution and establishment of the alien Australian redclaw crayfish, *Cherax quadricarinatus*, in the Zambezi Basin. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *31*, 3156–3168. https://doi.org/10.1002/aqc.3703

Madzivanzira, T. C., South, J., Nhiwatiwa, T., & Weyl, O. L. F. (2021). Standardisation of alien invasive Australian redclaw crayfish *Cherax quadricarinatus* sampling gear in Africa. *Water SA*, *47*, 380–384. https://doi.org/10.17159/wsa/2021.v47.i3.11866

Madzivanzira, T. C., South, J., Wood, L. E., Nunes, A. L., & Weyl, O. L. F. (2020). A review of freshwater crayfish introductions in Africa. *Reviews in Fisheries Science & Aquaculture*, *29*, 218–241. https://doi.org/10.1080/23308249.2020.1802405

Messager, M. L., & Olden, J. D. (2018). Individual-based models forecast the spread and inform the management of an emerging riverine invader. *Diversity and Distributions*, *24*, 1816–1829. https://doi.org/10.1111/ddi.12829

Messager, M. L., & Olden, J. D. (2019). Phenotypic variability of rusty crayfish (*Faxonius rusticus*) at the leading edge of its riverine invasion. *Freshwater Biology*, *64*, 1196–1209. https://doi.org/10.1111/fwb.13295

Moorhouse, T. P., & Macdonald, D. W. (2011). The effect of removal by trapping on body condition in populations of signal crayfish. *Biological Conservation*, *144*, 1826–1831. https://doi.org/10.1016/j.biocon.2011.03.017

Myles-Gonzalez, E., Burness, G., Yavno, S., Rooke, A., & Fox, M. G. (2015). To boldly go where no goby has gone before: Boldness, dispersal tendency, and metabolism at the invasion front. *Behavioral Ecology*, *26*, 1083–1090. https://doi.org/10.1093/beheco/arv050

Nunes, A. L., Zengeya, T. A., Hoffman, A. C., Measey, G. J., & Weyl, O. L. F. (2017). Distribution and establishment of the alien Australian redclaw crayfish, *Cherax quadricarinatus*, in South Africa and Swaziland. *PeerJ*, *5*, e3135. https://doi.org/10.7717/peerj.3135

Nyambe, I., Chabala, A., Banda, K., Zimba, H., & Phiri, W. (2018). Determinants of spatio-temporal variability of water quality in the Barotse floodplain, western Zambia. In R. Revermann, K. M. Krewenka, U. Schmiedel, J. M. Olwoch, J. Helmschrot, & N. Jürgens (Eds.), *Climate change and adaptive land management in southern Africa—Assessments, changes, challenges, and solutions* (Vol. *6*, pp. 96–105). Biodiversity & Ecology). Klaus Hess Publishers. https://doi.org/10.7809/b-e.00310

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2019). vegan: Community Ecology Package. R package version 2.5–6. https://CRAN.R-project.org/package=vegan

Perry, W. L., Jacks, A. M., Fiorenza, D., Young, M., Kuhnke, R., & Jacquemin, S. J. (2013). Effects of water velocity on the size and shape of rusty crayfish, *Orconectes rusticus*. *Freshwater Science*, *32*, 1398–1409. https://doi.org/10.1899/12-166.2

Phillips, B. L., Brown, G. P., Travis, J. M. J., & Shine, R. (2008). Reid's paradox revisited: The evolution of dispersal kernels during range expansion. *The American Naturalist*, *172*, S34–S48. https://doi.org/10.1086/588255

Phillips, B. L., Brown, G. P., Webb, J. K., & Shine, R. (2006). Invasion and the evolution of speed in toads. *Nature*, *439*, 803. https://doi.org/10.1038/439803a

Pintor, L. M., Sih, A., & Kerby, J. L. (2009). Behavioural correlations provide a mechanism for explaining high invader densities and increased impacts on native prey. *Ecology*, *90*, 581–587. https://doi.org/10.1890/08-0552.1

Poff, N. L. (1997). Landscape filters and species traits: Towards mechanistic understanding and prediction in stream ecology. *Journal of the North American Benthological Society*, *16*, 391–409. https://www.jstor.org/stable/1468026

Pond, C. M. (1995). The role of the 'walking legs' in aquatic and terrestrial locomotion of the crayfish *Austropotamobius pallipes* (Lereboullet). *The Journal of Experimental Biology (1975)*, *62*(2), 447–454. https://doi.org/10.1242/jeb.62.2.447

QGIS Development Team. (2021). *QGIS geographic information system*. Open Source Geospatial Foundation Project. http://qgis.osgeo.org

R Core Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Rebrina, F., Skejo, J., Lucic, A., & Hudina, S. (2015). Trait variability of the signal crayfish (*Pacifastacus leniusculus*) in a recently invaded region reflects potential benefits and trade-offs during dispersal. *Aquatic Invasions*, *10*, 41–50. https://doi.org/10.3391/ai.2015.10.1.04

Shine, R., Brown, G. P., & Phillips, B. L. (2011). An evolutionary process that assembles phenotypes through space rather than through time. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 5708–5711. https://doi.org/10.1073/pnas.1018989108

Soto, I., Ahmed, D. A., Beidas, A., Oficiarldegui, F. J., Tricarico, E., Angeler, D. G., Amatulli, G., Briski, E., Datry, T., Dohet, A., Domisch, S., England, J., Feio, M. J., Forcellini, M., Johnson, R. K., Jones, J. I., Larrañaga, A., L'Hoste, L., Murphy, J. F., … Haubrock, P. J. (2023). Long-term trends in crayfish invasions across European rivers. *Science of the Total Environment*, *867*, 161537. https://doi.org/10.1016/j.scitotenv.2023.161537

South, J., Madzivanzira, T. C., Tshali, N., Measey, J., & Weyl, O. L. F. (2020). In a pinch: Mechanisms behind potential biotic resistance toward two invasive crayfish by native African freshwater crabs. *Frontiers in Ecology and Evolution*, *8*, 72. https://doi.org/10.3389/fevo.2020.00072

Stein, R. A. (1977). Selective predation, optimal foraging, and the predator-prey interaction between fish and crayfish. *Ecology*, *58*, 1237–1253. https://doi.org/10.2307/1935078

Streissl, F., & Hold, W. (2002). Growth, morphometrics, size at maturity, sexual dimorphism and condition index of *Austropotamobius torrentium* Schrank. *Hydrobiologia*, *477*, 201–208. https://doi.org/10.1023/A:1021046426577

Tarkan, A. S., Karakuş, U., Top-Karakuş, N., Keskin, E., Ünal, E. M., & Britton, J. R. (2021). Invasion of pumpkinseed *Lepomis gibbosus* is facilitated by phenotypic plasticity across its invasion gradient. *Biological Invasions*, *23*, 3201–3214. https://doi.org/10.1007/s10530-021-02574-9

Thomaz, S. M. (2021). Propagule pressure and environmental filters related to non-native species success in river-floodplain ecosystems. *Hydrobiologia*, *849*, 3679–3704. https://doi.org/10.1007/s10750-021-04624-8

Thomaz, S. M., Bini, L. M., & Bozelli, R. L. (2007). Floods increase similarity among aquatic habitats in river-floodplain systems. *Hydrobiologia*, *579*, 1–13. https://doi.org/10.1007/s10750-006-0285-y

Tickner, D., Opperman, J. J., Abell, R., Acreman, M., Arthington, A. H., Bunn, S. E., Cooke, S. J., Dalton, J., Darwall, W., Edwards, G., Harrison, I., Hughes, K., Jones, T., Leclère, D., Lynch, A. J., Leonard, P., McClain, M. E., Muruven, D., Olden, J. D., … Young, L. (2020). Bending the curve of global freshwater biodiversity loss: An emergency recovery plan. *Bioscience*, *70*, 330–342. https://doi.org/10.1093/biosci/biaa002

Timberlake, L. (1997). *Biodiversity of the Zambezi Basin wetlands: A review of available information*. Zambezi society and biodiversity Foundation for Africa Report to IUCN – The World Conservation Union Regional Office for Southern Africa.

Travis, J. M. J., & Dytham, C. (2002). Dispersal evolution during invasions. *Evolutionary Ecology Research*, *4*, 1119–1129.

Turpie, J., Smith, B., Emerton, L., & Barnes, B. (1999). *Economic value of the Zambezi Basin wetlands* (p. 346). IUCN Regional Office in Southern Africa.

Twardochleb, L. A., Olden, J. D., & Larson, E. R. (2013). A global meta-analysis of the ecological impacts of nonnative crayfish. *Freshwater Science*, *32*, 1367–1382. https://doi.org/10.1899/12-203.1

Tweddle, D. (2010). Overview of the Zambezi River system: Its history, fish fauna, fisheries, and conservation. *Aquatic Ecosystem Health & Management*, *13*, 224–240. https://doi.org/10.1080/14634988.2010.507035

Tweddle, D., Skelton, P. H., dervan Waal, B. C. W., Bills, I. R., Chilala, A., & Lekoko, O. T. (2004). Aquatic biodiversity survey for the "four corners" transboundary natural resources management area. Report for African Wildlife Foundation, SAIAB Investigational Report, 71, xviii+202 pp.

van Wilgen, N. J., Faulkner, K. T., Robinson, T. B., South, J., Beckett, H., Janion-Scheepers, C., Measey, J., Midgley, G. F., & Richardson, D. M. (2022). Climate change and biological invasions in South Africa. In L. H. Ziska (Eds.), *Invasive species and global climate change* (pp. 158–187). CABI. https://doi.org/10.1079/9781800621459.0009

Wheatly, M. G. (1996). An overview of calcium balance in crustaceans. *Physiological Zoology*, *69*(2), 351–382.

William, C. M., Ragland, G. J., Betini, G., Buckley, L. B., Cheviron, Z. A., Donohue, K., Hereford, J., Humphries, M. M., Lisovski, S., Marshall, K. E., Schmidt, P. S., Sheldon, K. S., Varpe, Ø., & Visser, M. E. (2017). Understanding evolutionary impacts of seasonality: An introduction to the symposium. *Integrative and Comparative Biology*, *57*, 921–933. https://doi.org/10.1093/icb/icx122

Závorka, L., Lassus, R., Britton, R., & Cucherousset, J. (2020). Phenotypic responses of invasive species to removals affect ecosystem functioning. *Global Change Biology*, *26*(10), 5693–5704. https://doi.org/10.1111/gcb.15271

Zimba, H., Kawawa, B., Chabala, A., Phiri, W., Selsam, P., Meinhardt, M., & Nyambe, I. (2018). Assessment of trends in inundation extent in the Barotse floodplain, upper Zambezi River Basin: A remote sensing-based approach. *Journal of Hydrology: Regional Studies*, *15*, 149–170. https://doi.org/10.1016/j.ejrh.2018.01.00

SUPPORTING INFORMATION

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

How to cite this article: Nawa, N., South, J., Ellender, B. R., Pegg, J., Madzivanzira, T. C., & Wasserman, R. J. (2024). Complex selection processes on invasive crayfish phenotype at the invasion front of the Zambezi floodplains ecoregion. *Freshwater Biology*, *69*, 1322–1337. https://doi.org/10.1111/fwb.14308