

Invasion of the shelter snatchers: behavioural plasticity in invasive red swamp crayfish, *Procambarus clarkii*

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SUMMARY

1. In many freshwater systems, competition for shelter plays an important role in determining the persistence of both native and alien species. The red swamp crayfish, *Procambarus clarkii*, is currently invading the native habitat of the signal crayfish, *Pacifastacus leniusculus*, in southern Oregon, and interspecific competition for shelter may be driving the species replacement in this region.

2. We designed a 2×3 factorial mesocosm experiment, with shelter density and species combination as factors, to investigate shelter occupancy and resource competition. Contrary to our predictions, the two crayfish species are equal competitors for shelter. Further, the invasive *P. clarkii* modified its shelter occupancy behaviour in the presence of the native *P. leniusculus* and has broader microhabitat preferences.

3. Specifically, we found that *P. clarkii* alters shelter occupancy and space use patterns when the two species occurred together, such that shelter use was identical between *P. clarkii* and *P. leniusculus* in mixed-species treatments. In such treatments, both species increased their use of shelters when shelter density increased. When *P. clarkii* was alone, however, individuals did not alter shelter use as a function of shelter density, whereas *P. leniusculus* exhibited similar density-dependent behaviour in both mixed- and single-species treatments.

4. In a complementary field survey, we employed an ‘epicentre-based’ design to sample two field sites. We observed patterns of microhabitat use and breadth for each species similar to those in our mesocosm experiment: the invasive *P. clarkii* was more abundant across different habitats and used a broader range of microhabitats than the native *P. leniusculus*. As such, we found that *P. clarkii* was more abundant across both field sites than the *P. leniusculus*, occupying microhabitats within and beyond the preferred range of *P. leniusculus*. Both field sites were affected by urban development and agriculture.

5. The use of microhabitats by both species was similar in the laboratory and the field. This study confirms that *P. clarkii* individuals can, and do, successfully occupy microhabitats preferred by *P. leniusculus* in the Willamette Valley. The results from our study may be relevant to other freshwater systems inhabited by *P. clarkii* and contribute to the understanding of ‘niche opportunity’, a concept which defines the environmental conditions that promote biological invasions.

Keywords: behavioural ecology, biological invasions, interspecific competition, *Pacifastacus leniusculus*, *Procambarus clarkii*

Introduction

Freshwater systems are the most heavily degraded on earth and are increasingly stressed by anthropogenic activity (Vitousek *et al.*, 1997). Intense land and

hydrological modifications, climate change and transport of species into novel habitats are promoting biological homogenisation (McKinney & Lockwood, 1999; Olden *et al.*, 2004). Second only to habitat loss, the introduction of non-native species poses the greatest

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threat to freshwater ecosystems (With, 2002). Further, species introductions can interact with the effects of habitat loss (Agrawal *et al.*, 2007), enhancing the invasiveness of alien species (Crooks, 2002; Brook, Sodhi & Bradshaw, 2008).

Invasive species often have broad abiotic tolerances and thrive in degraded habitats (Moyle & Light, 1996), allowing them to compete strongly with native species for resources (Mooney & Cleland, 2001; Snyder & Evans, 2006; White, Wilson & Clarke, 2006). Accordingly, a key driver of the decline of native species is competition with invaders (Shea & Chesson, 2002; Sakai *et al.*, 2003). In many freshwater systems, competition for shelter plays an important role in determining the persistence of both native and alien species (Gherardi & Cioni, 2004; Matsuzaki *et al.*, 2012). For example, many crayfish occupy and defend shelters (families Cambaridae, Astacidae, Parastacidae; Holdich, 2002; Gherardi & Daniels, 2004; Alonso & Martínez, 2006). Shelters provide refuge from extreme fluctuations in abiotic conditions and reduce predation risk (Garvey, Stein & Thomas, 1994; Lodge & Hill, 1994). Therefore, competition with alien crayfish species for shelters could significantly affect the fitness of native crayfish species.

In this study, we explored the effect of resource limitation (shelter density) on the interaction between the native Oregon signal crayfish [*Pacifastacus leniusculus* (Dana, 1852)] and the invasive red swamp crayfish [*Procambarus clarkii* (Girard, 1852)]. Our main objectives were to: (i) quantify potential competition for shelter between the two species in an experimental setting and (ii) explore the distribution, co-occurrence and microhabitat associations of signal and red swamp crayfish in the field. To assess microhabitat use under controlled and natural conditions, we quantified shelter use and microhabitat use in single- and mixed-species combinations in a large-scale mesocosm experiment and a field survey.

Outside of their native ranges, both species are considered highly successful invaders (Holdich, 2002). Currently, the red swamp crayfish is invading habitats occupied by native signal crayfish in the Northwestern United States. We hypothesised that invasive red swamp crayfish individuals would exclude native signal crayfish from shelter in mixed-species treatments and that red swamp crayfish would occupy a broader range of microhabitats than signal crayfish both in the mesocosms and in the field. The coexistence of these two presumably even-matched competitors provides us with an ideal study system with which to address our research questions.

Methods

Mesocosm experiment

We performed a 2 × 3 fully factorial mesocosm experiment with manipulation of shelter density and species combinations. We used two levels of shelter availability (low, high) for each of three species combinations (red swamp crayfish alone, signal crayfish alone and both species together). We filled black Rubbermaid polyethylene stock tanks (1,135 L volume; 175 × 160 × 63.5 cm; Model no. 424700BLA) with well water. The tanks were placed on a slope, thus creating a depth profile within each one. Each shelter consisted of a 15-cm length of white PVC pipe (4 cm internal diameter) fixed to a 10 × 10 cm tile, which prevented crayfish from moving shelters around on the bottom of the tank. In the low shelter availability treatment, there was a single shelter in each of the shallow, medium and deep sections of the water column (25, 30 and 35 cm, respectively). In the high shelter availability treatment, there was a single shelter in the shallow and deep sections, with four shelters in the middle depth (30 cm). Additionally, the drain for each tank served as an unintentional, albeit effective, shelter, resulting in a total shelter number of four in the low shelter availability treatment and seven in the high shelter availability treatment.

Each tank was stocked with eight crayfish, and we controlled for size and sex ratio in each tank. All eight crayfish within a tank were within 4.8 mm of the average post-orbital carapace length (POCL; SD 2.7 mm), to minimise the competitive advantage of large individuals over small, and the average crayfish size across all mesocosms was 28.3 mm (SD 4.8 mm). Similarly, the sex ratio was balanced (50 : 50 male:non-breeding female), as male crayfish tend to be more aggressive than non-breeding females (Figler *et al.*, 1995, Figler *et al.* 2005). Experimental density was based on estimates of crayfish density at the collection site (4.4 crayfish m⁻², personal observation). Each tank was covered with 20% light transmission shade cloth to exclude predators and minimise temperature fluctuations. Water temperature ranged from 17 to 25 °C with a daily mean of 22 °C. When necessary, we kept maximum water temperature below 25 °C by adding cold water. With a total of 12 tanks, we ran two blocks with two replicates per treatment in each block (total of four replicates per treatment). Food was provided in the form of four Wardley® Premium Algae (Hartz, Seacucus, NJ, U.S.A.) Discs per tank each night. The experiment was run out-of-doors at the Oregon State University Fish Research Laboratory, located within 13

miles of the crayfish collection site. The experiment was ended after 7 days.

The response variables were shelter occupancy and position inside the tank. We made two observations per day (midday and dusk) for a total of 14 observations per tank. During each observation, we recorded spatial information for each crayfish: for individuals occupying a shelter, we simply recorded the specific shelter occupied. For individuals not occupying a shelter, we recorded microhabitat data as depth in the water column and position in tank. We scored mesocosm space use to calculate our main matrix for mesocosm ordination. Scores for shelter (occupied = 10, unoccupied = 0), depth (shallow = 10, medium = 20, deep = 30) and location of individual crayfish (open = 1, side = 2, corner = 3) were summed for each individual at each observation and served as a categorical surrogate value for 3-D location inside the mesocosm tank.

We collected both species of crayfish from Waverly Lake, Albany, Oregon, on two separate occasions (6 and 7 and 14 and 15 August 2008) corresponding to the two time blocks of the experiment (14–20 and 22–28 August 2008). Crayfish were transported to Oregon State University, Corvallis, Oregon, where we immediately recorded, for each individual, species, POCL (mm), right and left chela length (CL, mm), mass (g.) and sex. We assigned each individual an identification number and marked it on the carapace using non-toxic, white DecoColor® paint pen (Marvy Uchida, Torrance, CA, U.S.A.) (L. Pintor, pers. comm.). During processing, crayfish were held in a controlled environmental chamber on a 12 h : 12 h photoperiod at 20 °C (the mean temperature at the collection site). The crayfish were segregated by species to avoid exposure to heterospecific cues and housed in 227 L tanks at a maximum density of 48 individuals per tank. Within each tank, crayfish were held individually in 13 × 13 × 16 cm plexiglass, stackable containment units to limit aggressive interactions. The tanks were filled with filtered Corvallis tap water and treated with AmQuel® (Kordon LLC, Hayward, CA, U.S.A.), a non-toxic water conditioner which removes chlorine, chloramines and ammonia. Tank water was aerated with four 15-cm long air stones and filtered using 1,325 Lph Marineland® Bio-Wheel Power Filters (Spectrum Brands, Madison, WI, U.S.A.).

Field survey

Using *a priori* information on the distribution of the red swamp crayfish in the Willamette Valley, we selected two regional sites for our field survey (Fig. 1; S.V. Gregory,

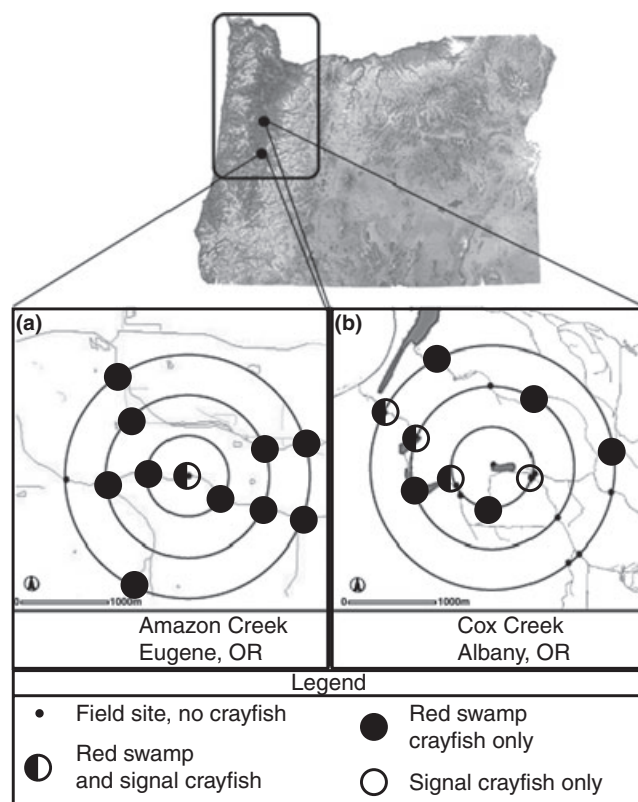


Fig. 1 Diagrammatic map demonstrating epicentre sampling design at sampling sites in Eugene, Oregon and Albany, Oregon, and sites indicated on the outline of Oregon State. Species presence and absence at sample sites is indicated.

pers. comm.). The first field site was Amazon Creek ($44^{\circ} 3' 0.288''$ N, $123^{\circ} 9' 45.435''$ W), an urban stream near the city of Eugene, OR, (altitude: 130 m) that drains into the Fern Ridge reservoir. This site has little riparian canopy, armoured sections of streambed beneath road crossings and a path adjacent to the channel. The second field site was Cox Creek ($44^{\circ} 38' 16.358''$ N, $123^{\circ} 3' 24.216''$ W), a second-order tributary of the Willamette River in Albany, OR (altitude: 64 m), with headwaters draining land used for commercial grass seed production.

Due to the limited information available on distribution of the red swamp crayfish in Oregon, we used an epicentre-based survey design at each of the regional study sites (Fig. 1). An epicentre design uses a site with *a priori* qualifications as a focus, while additional survey sites are selected at intersections of potentially suitable habitat and concentric circles, which radiate from the focus. This design is useful in providing a standardised protocol for sampling locations when data for species distributions or habitat preferences are sparse and can be used to establish rates of spread for invasive species. For the purpose of our study, we chose two sites that were

known to have coexisting populations of both red swamp and signal crayfish, and we sampled all aquatic sites (lentic and lotic) that intersected with our concentric circles. We used ArcGIS (vers. 9.2, ESRI) to identify the intersections of our circles with all waterbodies 500, 1000 and 1500 m from the designated foci. We did not survey only lentic or lotic waters, as both species are capable of inhabiting both habitat types (Holdich, 2002). We identified 24 sites on Cox Creek and 14 sites on Amazon Creek as candidate survey sites using GPS to within 2-m accuracy. We eliminated 10 sites on Cox Creek and three on Amazon Creek due to lack of water, lack of water clarity and lack of access.

At each lotic site, we performed a visual survey on a 10-m reach of stream. At each lentic site, we performed visual surveys bounded by a 10-m reach of shoreline, parallel lines that were approximately perpendicular to the shoreline, and the maximum depth of visibility in the water column for a length of 10 m between the two parallel boundaries (i.e. an irregular polygon with two parallel sides, 10 m in length). We recorded water temperature and categorised visibility conditions (1–5, where five is perfect clarity). We crossed the channel (or designated survey area at lentic sites) in the upstream direction using a zigzag course parallel to the stream banks, overturning or disturbing all moveable objects. Two observers were present during the surveys, each surveying half of the channel. In waterbodies deep enough to survey via snorkel (lentic and lotic), one observer would search while the other observer would record data and prevent the double counting of individuals. Each time a crayfish was detected, we identified the species, life stage, habitat feature the individual was associated with, dominant substratum, per cent of the crayfish's body that was in refuge, depth in the water column, maximum depth of the water column, per cent canopy cover above the water column and, in lotic sites, position relative to the active channel. After an individual was recorded, it was removed to a location downstream of the transect to prevent double counting.

Statistical methods

Mesocosm experiment – univariate analysis. Using univariate analyses, we evaluated the subset of data for 5 days following the establishment of a social dominance hierarchy among the crayfish, which we determined *post hoc* by analysing total activity with analysis of variance (ANOVA). After the second day, there was no effect of 'date' on total activity, which we interpreted as a stable social rank having been established. We performed a factorial

ANOVA, with three levels of species combination (red swamp crayfish alone, signal crayfish alone, both species together) and two levels of shelter density (low and high shelter availability). We blocked by tank and assessed variation within tanks as random effects. We performed pairwise comparisons using Tukey HSD linear contrasts to isolate factors contributing to the differences between means. We performed all univariate analyses using the packages *glht* and *multcomp* in the program R (R Development Core Team, 2008).

Mesocosm experiment – multivariate analysis. We performed a non-metric multidimensional scaling (NMS) ordination on the entirety of our mesocosm data (day and dusk observations for each of the 7 days) to evaluate patterns of space use throughout the experiment. We assigned categorical, numerical values to microhabitats at three levels according to shelter availability, shelter depth and position if outside of shelter, such that all shelter locations would be scored higher than non-shelter locations (scores ≥ 100 and scores ≤ 50 , respectively), with enough matrix distance between the two categories to allow for potentially mutually exclusive patterns of individual space use. We evaluated the data profile to ensure that the range of distances in the main matrix was appropriate for the ordination. Thus, each point in the ordination represents the average pattern of space use of an individual crayfish throughout the course of the experiment. All multivariate analyses were performed using PC-ORD 6.0 (McCune & Mefford, 2006).

Our main matrix was 186 rows (individual crayfish) by 14 columns (observations), with values in each cell representing the specific location of a crayfish at a certain observation. Our second matrix was 186 rows by 13 columns and contained design and overlay factors, such as treatment, species and biometric data. We evaluated the profile of the data for outliers by assessing the coefficient of variation for rows and columns in the main matrix (desired coefficient of variation ≤ 50). We deleted all six individuals (rows) that did not survive the duration of the experiment, yielding main and second matrices of 186 rather than the intended 192 rows. The data set did not need further transformation. We ran an NMS ordination using the method of Mather (1976) and Kruskal (1964a,b). We assessed one- through six-dimensional solutions with 250 runs each of real and randomised data and random starting configurations (McCune & Mefford, 2006). The data set had a moderate degree of variability after transformation; therefore, we used Sorensen distances in our calculations to retain sensitivity (McCune, Grace & Urban, 2002). We assessed the dimensionality of

the final solution by determining the inflection point in the stress scree plot. We plotted individuals according to species (red swamp or signal) and species combination (single or mixed species) and interpreted the axes based on the strongest gradients of microhabitat preferences.

We used Multi-Response Permutation Procedures (MRPP) to test for differences in patterns of shelter and space use between different groups. We used MRPP instead of multivariate analysis of variance (MANOVA) or discriminant analysis (DA) because MRPP avoids assumptions about distributions (regarding multivariate normality and homogeneity of variances), which is necessary considering the nature of the data and the differing numbers of individuals in each of the four species groups (McCune & Mefford, 2006). We analysed the data set of 186 individuals by 14 observations with MRPP (McCune *et al.*, 2002) on the transformed data set, using species combinations (four total – red swamp crayfish alone, signal crayfish alone, red swamp crayfish in mixed-species treatments, signal crayfish in mixed-species treatments) and Sorensen distance measures to test for species and treatment effects in mesocosm space use. MRPP constructs a distance matrix for the data set (as was calculated prior to the ordination), calculates the average within-group distance for each group and the weighted mean within-group distance (δ), then determines the probability of a δ this small or smaller obtained by chance with a randomisation test. Several statistics describe the difference between groups. The statistic P simply notes the likelihood of the difference between groups occurring by chance and is estimated as:

$$P = \frac{1 + \text{number of smaller } \delta}{\text{total number of possible comparisons}}$$

The test statistic T describes the strength of separation between groups and is estimated as:

$$T = \frac{\delta_{\text{observed}} - \delta_{\text{expected}}}{SD\delta_{\text{expected}}}$$

The more negative T is, the more different the groups are. The effect size, A (range, $-1 \leq A \leq 1$), is the chance-corrected within-group agreement, with positive values of A corresponding to greater homogeneity within groups than expected by chance.

Field survey – multivariate analysis. All analyses of field survey data were performed using PC-ORD 6.0 (McCune

& Mefford, 2006). Our main matrix initially consisted of 407 individuals (rows) by 24 microhabitat variables, representing dominant substratum, substratum on which the crayfish was observed, per cent of body covered, per cent canopy cover and channel position (Table 3). We performed a Beals smoothing transformation for individuals (rows). We performed an outlier analysis and deleted one individual. We performed a NMS ordination and used Sorensen distances to explore one to six dimensional solutions, with 250 runs each of real and randomised (Monte Carlo test) data (PC-ORD Autopilot, Slow and Thorough setting). We interpreted the top two axes, as the 2-D solution was parsimonious for explaining the variability in the data. We coded individuals by species groups (red swamp crayfish alone, signal crayfish alone, red swamp crayfish mixed, signal crayfish mixed) and interpreted axes based on correlations with microhabitats.

We analysed the transformed data set with MRPP (McCune *et al.*, 2002), using both species and crayfish assemblage from the second matrix as grouping variables and Sorensen distance measures. Since MRPP is a nonparametric test of no difference between two or more groups, if there is a species difference in microhabitat association then the test should yield significant differences between species groups. Similarly, if there are differences in microhabitat associations in sites with one species of crayfish and sites with both species of crayfish, the test should yield differences between crayfish assemblages.

Results

Mesocosm experiment – univariate analysis

There were distinct patterns in shelter occupancy as a result of species composition and shelter density (Table 1). There were no statistically significant differences in activity rates between the species in either diel period in which the data were collected (single-species treatments, Welch's t -test, $P > 0.05$ for diurnal and crepuscular observation windows). However, we did observe a diel effect within species for *P. clarkii*, which was more active during the day (Welch's $t = -2.9099$, $P < 0.05$). There was no difference in activity between diurnal and crepuscular observations within species for *P. leniusculus* (Welch's $t = 0.0903$, $P > 0.10$). As such, the two diel periods were analysed separately (Table 1). The Tukey HSD multiple comparisons separated the factors contributing to significant effects in the factorial ANOVA. In single-species tanks, species differed in shelter use. Shelter use did not differ between low and high shelter

Table 1 Factorial ANOVA results for mesocosm experiment. The responses are total shelter occupancy for diurnal and crepuscular observation periods, respectively, with three factors for species combination and two factors for shelter density blocked by tank (random effect)

Observation period	Source of variation	df	SS (Type I)	MS	F	P
Day	Species combination	2	156.817	78.408	31.716	0.0001***
	Shelter density	1	86.700	86.700	35.070	0.0001***
	Species combination × shelter density	2	31.550	15.775	6.3809	0.01**
	Error (tank)	18	44.500	2.472		
	Residuals	96	107.600	1.121		
Dusk	Species combination	2	82.917	41.458	10.181	0.001**
	Shelter density	1	36.300	36.300	8.914	0.007**
	Species combination × shelter density	2	29.450	14.725	3.616	0.047*
	Error (tank)	18	73.300	4.072		
	Residuals	96	139.2	1.450		

P-values <0.05*, <0.01** and <0.001***.

densities in red swamp crayfish. Signal crayfish occupied shelters more frequently at high than at low shelter density (Fig. 2; Table 2). In mixed-species treatments, both species occupied shelters to a similar degree, increasing their use when shelter density was high (Fig. 2; Table 2). In other words, when the species were mixed and shelter density was high, the red swamp crayfish increased its use of shelters. At low shelter densities, occupancy was similar between mixed- and single-species treatments.

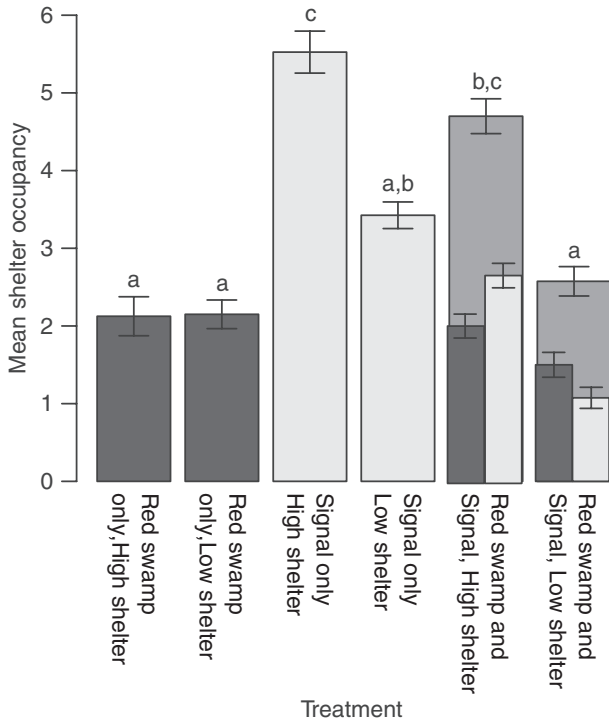


Fig. 2 Mean (±SE) mesocosm shelter occupancy by treatment. Bars with the same lower case letters have statistically similar means (from Tukey HSD multiple comparison tests).

Mesocosm experiment – multivariate analysis

The ordination of data on individual space use reflected the pattern of shelter use. The three-dimensional NMS solution was significant (Monte Carlo $P = 0.0040$) and explained 88.0% of the variation in the distance matrix, and all three axes of the ordination solution were highly orthogonal (range, 79.3–95.9%). We interpreted the 3D solution with two plots, one for axis 1 versus 2 and one for axis 1 versus 3 (Fig. 3). Shelter occupancy could not be explained by one axis alone, so it was necessary to include both plots for proper interpretation. Stress was reduced from an average of 49.741 in the original configuration to 14.741 in the 3-D solution. We overlaid species combination (red swamp crayfish alone, signal crayfish alone, red

Table 2 Tukey linear contrasts for differences between treatments in shelter occupancy

Test	Estimate	Standard error	Z value	P
RL-RH	0.0250	0.4983	0.050	1.000
SH-RH	3.4000	0.4983	6.833	0.001***
SL-RH	1.3000	0.4983	2.609	0.095
TH-RH	2.5750	0.4983	3.167	0.001***
TL-RH	0.4500	0.4983	0.903	0.946
SH-RL	3.3750	0.4983	6.773	0.001***
SL-RL	1.2750	0.4983	2.339	0.108
TH-RL	2.5500	0.4983	3.117	0.001***
TL-RL	0.4250	0.4983	0.853	0.957
SL-SH	-2.1000	0.4983	-4.214	0.001***
TH-SH	-0.8250	0.4983	-1.656	0.561
TL-SH	-2.9500	0.4983	-3.920	0.001***
TH-SL	1.2750	0.4983	2.559	0.108
TL-SL	-0.8500	0.4983	-1.706	0.528
TL-TH	-2.1250	0.4983	-4.264	0.001***

R, red swamp crayfish alone; S, signal crayfish alone; T, mixed species; L, low shelter density; H, high shelter density.

All P-values were adjusted for multiple comparisons.

P-values <0.001***.

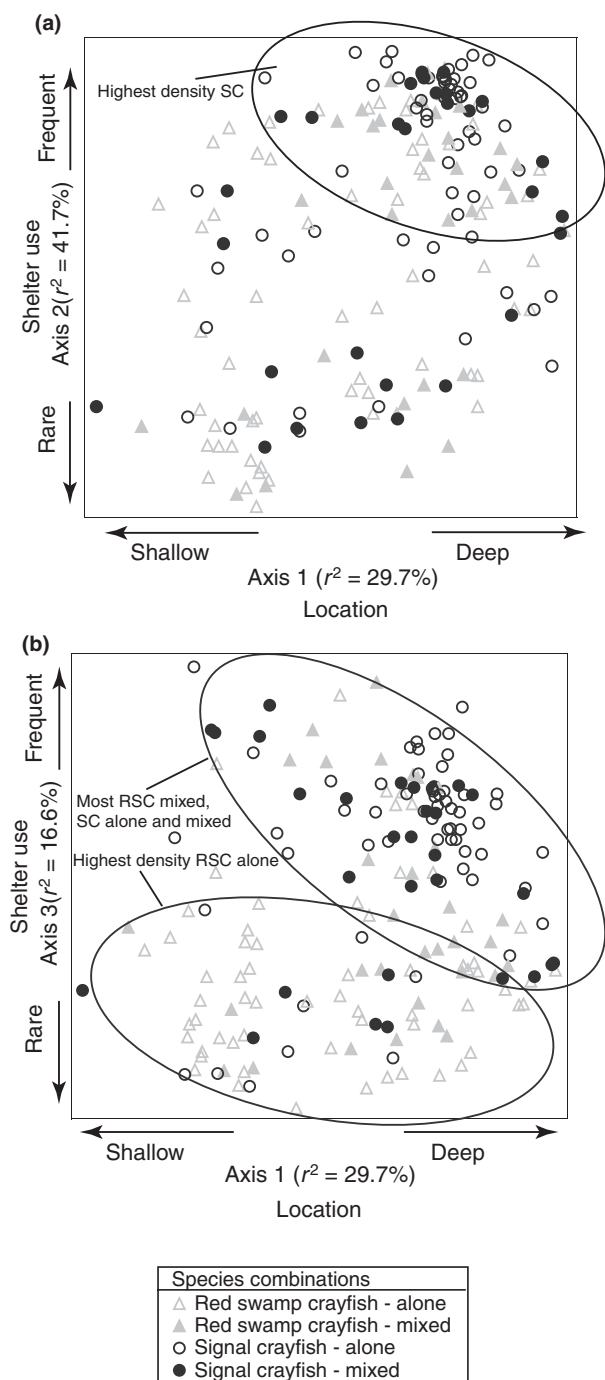


Fig. 3 Two-dimensional non-metric multidimensional scaling solutions for mesocosm space use. The species-treatment overlay indicates the space use patterns for individuals in single-species and mixed-species treatments and the appropriate species identification. Ovals approximate group membership for signal crayfish (SC) and red swamp crayfish (RSC). The vertical axes (2 and 3) best represent shelter use, while the horizontal axis best represents defensibility. R^2 values for each axis are provided. Intermediate positions along the 'Shelter Use' axis indicate inconsistent shelter use. Intermediate positions along the 'Location' axis indicate different spatial preferences within the tank, according to depth and position.

swamp crayfish mixed, signal crayfish mixed) on the ordination.

Shelter use was highly correlated with axes 2 and 3 (Fig. 3). Individuals that occupied shelter frequently are located in the upper right corner of the ordination space, while individuals that rarely occupied shelter are located in the lower half of the ordination space. Intermediate positions along the 'Shelter Use' axis indicate inconsistent shelter use. Intermediate positions along the 'Location' axis indicate different spatial distributions within the tank, according to level of protection. Individuals that occupied shallow, open areas trended towards the left-hand side of the ordination, while individuals occupying deep, protected areas (side and corner locations) trended towards the right-hand side of the ordination (Fig. 3).

In general, signal crayfish were more consistent and occupied a more restricted portion of the ordination space, with most individuals occupying shelter and relatively protected areas relative to open space (Fig. 3). Microhabitat use in red swamp crayfish differed from that of signal crayfish; they occupied shelters less frequently than signals, as indicated by a large effect size (T) and significant P -value (Supporting Information). Red swamp crayfish were more variable and thus more evenly distributed throughout the ordination space (Fig. 3).

Signal crayfish occupied a relatively narrow group of microhabitats (Fig. 3; see Supporting Information Table S1). In mixed-species treatments, however, patterns of individual space use by red swamp crayfish resembled those of signal crayfish (Fig. 3; Table S1). Between single-species and mixed-species treatments, red swamp crayfish differed in individual space use, going from broader to narrower microhabitat use. Signal crayfish had similar patterns of space use between single- and mixed-species treatments and thus have a smaller within-group distance and did not differ in space use patterns (Fig. 3). These specific space use patterns reflected the general shelter occupancy patterns observed with univariate analysis, indicating that certain individuals occupied shelter more frequently than others within treatments, and species-level patterns in microhabitat use.

Field survey

We detected crayfish in 19 of the initial 25 sample sites across both regional sites. In total, we detected signal crayfish alone at one site, red swamp crayfish alone at 14 sites and both together at four sites. In Amazon Creek, we found signal crayfish at only one site (the epicentre) where they coexisted with red swamp crayfish (Fig. 1). All other locations in Amazon Creek contained only red swamp

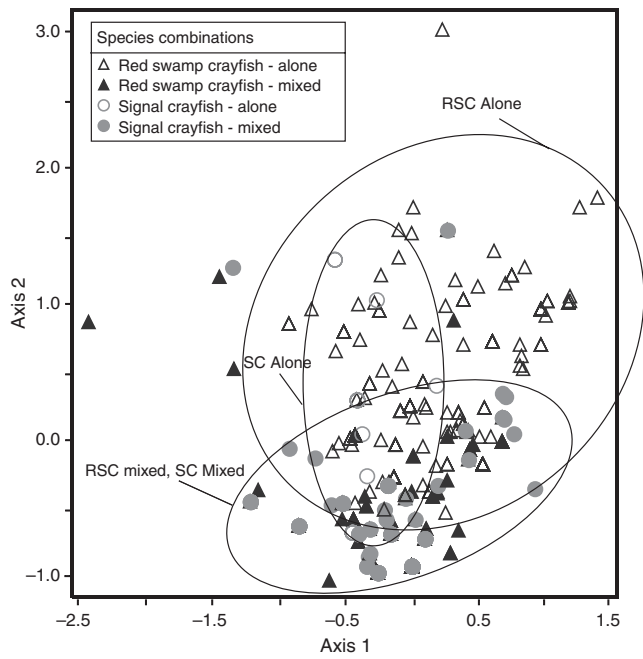


Fig. 4 Two-dimensional non-metric multidimensional scaling ordination solution on Beals smoothed data, representing individual microhabitat preferences in the field survey for red swamp and signal crayfish. The species–species combination overlay indicates the microhabitat preferences for individuals in relation to the crayfish assemblage to which they belong. Ovals approximate group membership for signal crayfish (SC) and red swamp crayfish (RSC). Points closer together represent individuals with similar microhabitat preferences than points father away. See Table 4 for interpreting the ordination axes.

crayfish. In Cox Creek, we detected signal crayfish at four sites, three of which all had red swamp crayfish. We detected red swamp crayfish only at the remaining five locations in Cox Creek (Fig. 1). The NMS ordination that we performed represents individual microhabitat preferences for red swamp and signal crayfish (Fig. 4). Points represent the microhabitat preferences for individual crayfish, and the distance between points approximates similarity in microhabitat use: points that are close together represent microhabitats that are more similar than those that are far apart (Fig. 4). When alone, red swamp crayfish had a wide distribution throughout the ordination space, but a restricted distribution in the presence of signal crayfish. When signal crayfish were alone, they had a relatively narrow distribution that was completely enveloped by red swamp crayfish. When red swamp and signal crayfish coexist, they had similar microhabitat preferences. The group of points for red swamp crayfish and signal crayfish found together also overlaps heavily with the groups for each species found alone (Fig. 4).

Table 3 Correlations of microhabitat classifications with species for 2D non-metric multidimensional scaling ordination of field survey data. Correlation sign (\pm), strength (r) and fit (r^2) are provided for each ordination axis. Species relative abundances that are positively correlated with microhabitat features will have the same sign and similar strengths, while negative correlations will have opposite sign and similar magnitudes. Correlation strengths >0.3 for both signs have been highlighted

Category	Ordination axis 1		Ordination axis 2	
	r	r^2	r	r^2
Relative abundance				
Red swamp crayfish	0.339	0.115	0.372	0.139
Signal crayfish	-0.339	0.115	-0.372	0.139
Dominant substratum (within 1.5 body lengths)				
Open*	0.543	0.711	0.240	0.058
Silt	-0.202	0.041	0.130	0.017
Gravel	-0.171	0.029	-0.758	0.373
Cobble	-0.235	0.055	-0.938	0.880
Organic [†]	-0.232	0.054	-0.014	0.000
Immediate substratum (substratum on which crayfish was observed)				
Silt	0.086	0.007	-0.788	0.620
Sand	-0.594	0.353	-0.818	0.668
Clay	0.536	0.287	0.670	0.449
Gravel	-0.322	0.103	-0.848	0.319
Cobble	-0.270	0.031	-0.597	0.446
Organic [†]	-0.240	0.037	0.003	0.014
% Body in shelter				
0%	0.776	0.602	0.194	0.038
1–25%	0.329	0.109	-0.359	0.129
26–50%	0.257	0.066	-0.182	0.033
51–75%	-0.049	0.002	-0.399	0.160
76–100%	-0.267	0.071	-0.926	0.857
Canopy				
0%	-0.485	0.235	-0.084	0.007
1–25%	0.336	0.113	0.328	0.108
26–50%	0.651	0.423	0.397	0.158
51–75%	0.756	0.572	0.366	0.134
76–100%	-0.043	0.002	-0.904	0.818
Channel position				
Center	-0.133	0.018	-0.855	0.731
Side	0.309	0.096	-0.282	0.080
Lentic/no flow	-0.593	0.352	-0.606	0.367
Water Temperature	-0.221	0.049	0.063	0.004
Visibility	0.271	0.073	-0.315	0.099

*'Open' class of microhabitat was assigned when individuals were less than two body lengths from potential cover habitat features.

[†]'Organic' microhabitats and substrata include macrophytes, macroalgae and wood.

To look at the correlation between species abundances and microhabitat variables, and therefore microhabitat preferences, we must compare the signs (direction, + or -) and magnitudes (strength, or r) between individual species and microhabitat variables for both ordination axes (Table 3). Thus, we can interpret species and microhabitat qualities that are similar in sign and

magnitude as positively correlated, and species and microhabitat qualities that differ in sign and magnitude as negatively correlated. Red swamp crayfish relative abundance was positively correlated with both ordination axes, whereas signal crayfish relative abundance was negatively correlated with the ordination axes; thus, the abundances of the two species were negatively correlated (Table 3). The absolute value of correlations of both species on both axes was above 0.300, which is the cut-off for interpretation of correlations recommended by McCune, Grace and Urban (2002) for ecological data. The relative abundance for red swamp crayfish was highest in areas without signal crayfish. For microhabitat, red swamp crayfish abundance was most strongly correlated with open locations, a clay substratum, lack of shelter, intermediate values of canopy cover and the presence of side-channel microhabitats. The abundance of signal crayfish was strongly correlated with gravel and cobble locations, interstitial spaces, extreme values of canopy cover and lentic habitats (Table 3).

We found negative correlations between the relative abundance of each species in the 2-D ordination solution (Fig. 4). The two-dimensional solution was significant (Monte Carlo $P = 0.0040$), explained 86.1% of the variation in the distance matrix, and the two axes were 91.8% orthogonal. Stress was reduced from an average of 45.145 in the starting configuration to 17.867 in the final solution. We overlaid species and species combination (i.e. single or mixed species) on the ordination. Axis 1 explained 38.3% of the distance, and Axis 2 explained 47.8% of the distance in the matrix for a cumulative R^2 of 86.1%. The axes were 91.8% orthogonal, and R^2 values were corrected for any lack of orthogonality.

Multi-response permutation procedures

Pairwise comparison of *a priori* groups from our field survey with MRPP provided quantitative tests for the differences in microhabitat preferences observed with the NMS ordination. Overall, red swamp crayfish occupied a broader suite of microhabitats than signal crayfish (Table 4, mean within-group distances 0.818 versus 0.692). When alone, red swamp crayfish occupied a broader suite of microhabitats than they did in the presence of signal crayfish (Table 4). The breadth of signal crayfish microhabitats did not differ when alone or in the presence of red swamp crayfish; however, the two groups were not identical, as the total within-group distance was greater (at 0.738) than both the alone (0.692) or mixed (0.685) within-group distances. When found together, red swamp crayfish and signal crayfish had identical within-group distances (Table 4).

Discussion

The mesocosm experiment demonstrated that in mixed-species treatments, invasive red swamp crayfish changed their pattern of shelter occupancy, which then matched that of the coexisting signal crayfish. Red swamp crayfish had consistently low shelter occupancy in single-species treatments, regardless of shelter density, which suggests that shelter was relatively unimportant. Signal crayfish used shelters more often than red swamp crayfish, and this use increased with increased shelter density. We conclude that shelter is relatively important to signal crayfish. When both species were held together, however, red swamp crayfish increased shelter use, equalling that

Table 4 Multi-response permutation procedure scores for multivariate tests between groups of individual microhabitat associations from field survey data. Within-group distance (range, 0–1) indicates the degree of similarity or dissimilarity, where lower distances indicate groups that are more similar than those with higher distances. The *A* statistic is an estimate of the proportion of the distances explained by group identity, while *T* is the standardised test statistic. Group codes for species and crayfish beta diversity for the multivariate multiple comparisons are R – all red swamp crayfish, S – all signal crayfish. Single-species groups indicate all mesocosm tubs with one species of crayfish present, and mixed-species groups indicate tubs with both species of crayfish. *N* indicates sample size for each group

Multiple comparisons – field survey							
Group	Observed δ	Expected δ	Variance ($\times 10^{-5}$)	Skewness	<i>T</i>	<i>P</i>	<i>A</i>
R versus S	0.861	0.902	0.131	–2.188	–35.895	0.000	0.045
Single species versus mixed species	0.745	0.902	0.131	–2.190	–137.609	0.000	0.174
Species							
	Red swamp crayfish			Signal crayfish			
	Alone ($n = 142$)	Mixed ($n = 129$)	Total ($n = 271$)	Alone ($n = 13$)	Mixed ($n = 122$)	Total ($n = 135$)	
Average within-group distance	0.818	0.684	0.922	0.692	0.685	0.735	

of the co-occurring signals. This behavioural plasticity did not stop at general shelter occupancy patterns: patterns of space use, inside and outside of shelters, were identical between red swamp crayfish and signal crayfish in mixed-species treatments. The results from our NMS ordination provide a graphical interpretation of the differences and similarities between groups, while the pairwise comparisons with MRPP provide quantitative support to the differences and similarities observed in the ordination. Furthermore, the behavioural patterns in the mesocosm experiment appear to agree with the microhabitat associations observed in the field.

The mesocosm experiment did not test explicitly for a behavioural mechanism to explain the species differences in shelter use. Therefore, we can only postulate that the red swamp crayfish were either mimicking the refuge behaviour of native signal crayfish or responding directly and aggressively to the competitor. Adaptability to a broad suite of microhabitats would be an advantage for an invader; however, the ability of an invader to replicate the microhabitat associations of a native competitor should contribute to the establishment and success of an invader in novel situations. We suggest that this behavioural plasticity potentially increases the establishment of invasive species.

Flexibility in behaviour, whether non-aggressive or agonistic, can promote establishment and is not unique to this system. Pintor, Sih & Kerby (2009) found that aggression and foraging activity were correlated in signal crayfish when it was an invader and comprised an 'aggression syndrome', whereby invasive species were able to sustain high densities without experiencing compensatory effects of intraspecific competition. Sol, Timmermans & Lefebvre (2002) reviewed behavioural flexibility and invasion success for 69 species of birds worldwide and found that species with a relatively large brain, which was considered as a surrogate for behavioural flexibility, established more successfully in new habitats than species with smaller brains. Hazlett, Acquistapace & Gherardi (2002) found that the invasive red swamp and rusty crayfish (*Orconectes rusticus* (Girard, 1852)) retained learned associations regarding alarm and novel odours longer than the native white-clawed (*Austropotamobius pallipes* (Lereboullet, 1858)) and northern (*Orconectes virilis* (Hagen, 1870)) crayfish, respectively.

The mesocosm experiment demonstrated that invasive red swamp crayfish and native signal crayfish differed in their use of shelter when held separately. Contrary to our hypothesis, we did not find that red swamp crayfish explicitly excluded signal crayfish from occupying shelter when held together. Rather, limiting shelter availability

did not induce dominance of one species over the other. This interaction is indicated by the overall increase of shelter occupancy in mixed-species treatments relative to single-species treatments and identical patterns of shelter occupancy between the competing species.

The invasive red swamp crayfish occupied a broader suite of microhabitats in the field, while signal crayfish were found predominately in rocky habitats with interstitial spaces for shelter. Signal crayfish were scarce in the urban Amazon Creek, and while they were more prevalent in the agricultural/urban Cox Creek, they were restricted to a relatively narrow suite of microhabitats. At the population level, the greater breadth of suitable microhabitats for red swamp crayfish could reduce the strength of interspecific competition, as its resource pool is inherently larger than its competitors. Over time, such effects could contribute to positive feedback and affect regional distribution. We acknowledge the potential for sampling bias, as we detected red swamps at 14 sites but only one site with signal crayfish and four sites with both species. This difference in sample size may produce artefacts showing larger distances between site characteristics than really exist.

In terms of microhabitat preferences, signal crayfish are largely dependent on rocky substrata and are more numerous where they can obtain shelter. Though red swamp crayfish were widespread, they were most abundant in areas with silt, clay and organic substrata, areas where signal crayfish were absent. Signal crayfish were more associated with closed canopies than red swamp crayfish and were also found using shelter more often than red swamp crayfish. Similarly, Petren & Case (1998) found that increased topographical structure promoted coexistence and reduced competition in invasive geckos and that adding structure reduced variable advantage between individuals. It appears that a similar mechanism occurs in the streams we surveyed.

The results of the mesocosm experiment and the field survey were remarkably similar. This study supports the observation that red swamp crayfish can, and do, successfully occupy microhabitats preferred by signal crayfish in the Willamette Valley. However, several factors were not accounted for in this study, including indirect effects, temperature, calcium, pH, dissolved oxygen and salinity (Lodge & Hill, 1994). Competitive relationships for shelter may have been influenced by indirect effects, such as time spent foraging (Bergman & Moore, 2003; Corkum & Cronin, 2004). In addition, field sites were highly degraded and would not be expected to have dense populations of signal crayfish, regardless of introduced species. Amazon Creek is an urban stream

with little canopy cover, few cobbles, undercut stream banks, a clay and silt substratum and abundant aquatic macrophytes, all of which are suitable microhabitats for red swamp crayfish but not for signals. Cox Creek is also an urban stream, although it differs from Amazon Creek in several ways, making it better habitat for signal crayfish. It is impounded in several locations that are heavily armoured with cobbles and boulder, and it has reaches with fully closed canopies and gravel substrata.

The relative paucity of signal crayfish in our survey indicates that the native crayfish are more restricted in Oregon than previously acknowledged. Signal crayfish are successful invaders throughout much of the United States, northern Europe and Japan; thus, an invasive and native population comparison may expose interesting differences in habitat requirements and behavioural plasticity (Soderback, 1991; Usio, Konishi & Nakano, 2001; Nakata & Goshima, 2003; Light, 2005). We found that red swamp crayfish are widespread throughout Oregon. Though the sample size and design of our field study does not warrant inference beyond these field sites, the trends are strong enough to encourage further research and inventory work throughout the invasion range in the Northwestern United States.

This study supports the concept of 'niche opportunity' (Shea & Chesson, 2002) as a defining characteristic of invasions. With broad abiotic tolerances and behavioural flexibility, invasive red swamp crayfish can increase invasion potential by enhancing niche opportunity (i.e. acquisition of resources, deterring natural enemies and tolerance to the physical environment). In this case, the niche opportunity for red swamp crayfish is defined by plastic shelter occupancy rates and broader microhabitat tolerances than signal crayfish. As such, the results from our experiment and field survey contribute to the understanding of niche opportunity in the context of red swamp crayfish, an important biological invader in freshwater systems (Sol & Lefebvre, 2000; Rehage, Barnett & Sih, 2005; Gerhardt & Collinge, 2007; Larson, Olden & Usio, 2010).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Multi-response permutation procedure scores for multivariate tests between groups of mesocosm space use.

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