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River bank burrowing is innate in native and invasive signal crayfish (*Pacifastacus leniusculus*) and is driven by biotic and abiotic cues

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Abstract

The behavior of animals can change when they become invasive. Whilst many species demonstrate exaggerations of existing behaviors, signal crayfish (*Pacifastacus leniusculus*) display a novel burrowing activity in some invaded rivers. Understanding if burrowing is learned or innate is important for modelling the geomorphological effects of invasion into new territories. Mesocosm experiments were undertaken with signal crayfish to investigate the effects of population density, shelter availability, and population provenance on their likelihood to burrow. Crayfish were collected within their native range in the USA; a recently invaded site in the USA; and two well-established invasive populations in the UK – one where burrowing in the field was present, and one population where burrowing in the field was absent. Crayfish from all populations constructed burrows in laboratory experiments. Population density and shelter availability were significant drivers of burrowing. There was no difference in burrowing between the invasive UK populations and the US native population, suggesting that burrowing is an innate, rather than learned. Therefore, crayfish have the capacity to affect geomorphic processes in any river that they invade, regardless of the source population. However, crayfish from the recently invaded USA river excavated more sediment than crayfish from their native range. These results demonstrate high plasticity of signal crayfish activities and show that innate behavioral strategies not seen in the native range can be activated at invaded sites.

Key words: behavioral plasticity, crayfish, zoogeomorphology, invasive species, burrowing

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1. Introduction

The behavior and activities of animals can change when they become invasive (Reznick and Ghalambor 2001; Wright *et al.* 2010; Sol and Weis 2019); they may cognitively adapt (behavioral flexibility) or express innate responses to new external stimuli (behavioral plasticity) leading to differences in the strength of a behavior or level of activity between native and invasive populations (e.g. Magurran *et al.* 1992; Holway and Suarez 1999; Sol and Lefebvre 2000; Jones and DiRienzo 2018; Mowery *et al.* 2021). The ability of an animal to modify its behavior may be important for determining its capacity to become invasive (Sol and Lefebvre 2000; Sakai *et al.* 2001; Sol *et al.* 2002; Pavlov *et al.* 2006). Behavioral flexibility and plasticity may benefit invasive species through stronger avoidance of predators (Levri *et al.* 2019), feeding on new / novel prey items (Martin and Fitzgerald 2005; Green *et al.* 2011), and increased migration to colonise and occupy new habitats and niches (Phillips *et al.* 2006). Numerous studies have focussed on the ecological impacts of invasions such as direct predator-prey and parasite-host interactions (Pavlov *et al.* 2006; Sol and Weis 2019), but the activities of animals can also directly and indirectly alter the physical environment via ecosystem engineering (Jones *et al.* 1994; Wright and Jones 2006; Hastings *et al.* 2007; Emery-Butcher *et al.* 2020) and zoogeomorphology (Viles 1988; Butler 1995; Philips 2009; Statzner 2012; Mason and Sanders 2021). Indeed, invasive species are often particularly effective ecosystem engineers because the long-term development of the landscape has occurred in the absence of the invader, meaning that new activities and behavoirs within the landscape can bring about abrupt change (Crooks 2002; Harvey *et al*. 2011, Fei *et al.* 2014, Mason and Sanders 2021; Sanders et al. 2022). Understanding how species change their behavior and modify their activities upon becoming invasive is therefore important for understanding their impact on community dynamics but also their impacts on habitat structure and geomorphology.

Many studies have investigated the exaggeration or adaptation of animal behaviors during invasion (e.g. Magurran et al. 1992; Holway and Suarez 1999; Phillips et al. 2006; Pintor and Sih 2009; Gruber et al. 2017; Jones and DiRienzo 2018; Mowery et al. 2021). However, some animals are also able to develop entirely new behaviors upon invasion, termed ‘innovation’ (Reader and Laland 2003). The development of novel behaviors has been associated with cognition levels and social learning (Lefebvre *et al.* 2004; Arbilly and Laland 2017; van Schaik *et al.* 2017), but little is known about specific environmental and genetic factors associated with the emergence of these behaviors, in part because patterns are mixed and different studies provide support for different hypotheses (see Reader and Laland 2003; Amici *et al.* 2019). Thus, examination of the behavioral changes of specific invasive species are required to address and test existing hypotheses.

An example of the expression of a novel behavior is riverbank burrowing by signal crayfish (*Pacifastacus leniusculus*). Burrowing is evident in some, but not all, invaded rivers in Great Britain (Figure 1a; Guan 1994; Harvey *et al.* 2011; Harvey *et al.* 2014; Faller *et al.* 2016) with burrows up to 0.87 m deep (Sanders 2020) and at densities of up to 21 burrows m^{-1} of riverbank (Guan and Wiles 1997). However, burrowing has not been reported in their endemic, North American range and has not been documented in all invaded territories, or in some British rivers where signal crayfish are present. Crayfish have displayed behavioral flexibility when exposed to novel flow regimes and predation cues (Blake and Hart 1993; Hazlett *et al*. 2002; Acquistapace *et al*. 2003; Pintor and Sih 2009; Ramalho and Anastacio 2011; Ion *et al.* 2020), but the specific biotic and abiotic drivers of burrowing behavior have not been investigated.

Invasive signal crayfish in the UK provide an opportunity to conduct an ‘unintended experiment’ (Suarez and Cassey 2016) to compare behavioral responses to biotic and abiotic stimuli between invasive populations, and between native and invasive populations. Further, because crayfish burrowing has been recorded in some, but not all, invaded British rivers, they present an opportunity to compare the role of biotic and abiotic stimuli in driving crayfish burrowing activity between populations with prior behavioral experience of burrowing (from an invaded UK river where crayfish burrows are present) and populations where no prior burrowing has been recorded (from an invaded UK river where crayfish burrows are absent). As such, whilst the aim of this research is to understand the specific drivers of river bank burrowing by signal crayfish, such change across populations allows for signal crayfish invasion to be evaluated within the context of behavioral flexibility, plasticity, and innovation frameworks.

Figure 1: Burrows constructed by signal crayfish in the UK. (a) Burrows in Gaddesby Brook, Leicestershire, UK, and (b) bank collapse facilitated by crayfish burrows on the River Bain, Lincolnshire, UK. Visible burrows in (a) are highlighted by the red and white arrows.

Quantifying the burrowing response of crayfish to external cues has important geomorphological applications. Signal crayfish are important drivers of fine sediment dynamics in some rivers (Harvey *et al*. 2014; Rice *et al*., 2016), and can supply up to 24.5 t km^{-1} a^{-1} of floodplain sediments to river channels by accelerating riverbank retreat (Sanders *et al*. 2021; Figure 1b). The excess delivery of fine sediment can have deleterious effects on water chemistry (Bai and Lung 2005) and aquatic ecology (Bilotta & Brazier, 2008; Jones et al., 2012a; 2012b; Kemp et al., 2011) and can increase flood risk (Lane et al., 2007; Lisle & Church, 2002; Marston et al., 1995; Sidorchuk & Golosov, 2003). To better understand the biotic and abiotic conditions associated with signal crayfish burrowing activity and therefore sediment supply, field observations from multiple sites and populations have been used to produce predictive models of burrow distributions and sediment supply based on ecological, hydrological, and geomorphological stream characteristics (Sanders 2020). These highlight the importance of crayfish density and shelter availability as strong covariates of burrowing activity.

However, crayfish have displayed capacity for learning (Acquistapace *et al.* 2003; Ion *et al.* 2020), and so the behavioral response of different crayfish populations to external cues may differ. Therefore, quantifying the responses of signal crayfish from different populations to the variables that were significant in constructing predictive models of crayfish burrowing across British populations (shelter availability and crayfish density) is necessary to understand if these variables were direct drivers of burrowing activity, or covariates of system wide processes. Further, understanding if burrowing is restricted to exclusively British populations (with only anecdotal evidence to suggest burrowing activity in rivers outside of the UK) is required to aid in understanding the potential effects of future invasions. Therefore, an experimental study was undertaken to investigate the importance of population provenance on the crayfish burrowing when exposed to alterations in biotic and abiotic variables previously associated with burrowing. Using laboratory experiments, this study aimed to investigate:

1. How does shelter availability affect crayfish burrowing?
2. How does crayfish density affect crayfish burrowing?
3. How does burrowing differ between native, recently invaded, and established invasive populations?
4. How does burrowing differ between populations from invaded rivers where burrows are present and from rivers where burrows are absent?

2. Methods

2.1 Study Species

There are an estimated 323 species of crustaceans which are considered invasive (Bojko *et al.* 2021), with crustacean taxa accounting for 53% of invasive species in European freshwater systems (Karatyev *et al.* 2009). In particular, crayfish are some of the most successful invasive species worldwide (Gherardi 2013; Kouba *et al.* 2014), with 46% of all crayfish species considered as invasive (Vila *et al.* 2010). In particular, the American signal crayfish (*Pacifastacus leniusculus*), native to the Pacific coast of North America (Johnsen and Taugbol 2010; Larson and Olden 2011), is now present in at least 29 territories (Kouba *et al.* 2014; Petrusek *et al.* 2017). Signal crayfish were introduced to Europe in the 1960s for aquaculture and became established as a result of escaping from farms, aided through deliberate introductions. Signal crayfish are present in 60% of English river catchments, and the number of affected catchments is expanding at a rate of 1.6% per year (Chadwick 2019).

Signal crayfish are the largest freshwater macroinvertebrate in the UK, and exist in densities of up to 20 adults m^{-2} (Bubb *et al.* 2004), and up to 110 m^{-2} when the full population (including juveniles) is considered (Chadwick *et al.* 2021). As such, signal crayfish facilitate substantial ecological and geomorphic disturbance. As well as affecting geomorphic processes, signal crayfish have substantial deleterious effects on aquatic ecology directly via predation (e.g. fish, Findlay *et al.* 2015; macroinvertebrates, Mathers *et al.* 2020; Sanders and Mills 2022; amphibians, Nystrom *et al.* 1997) as well as indirectly acting as a vector of disease (e.g. Holdich and Reeve 1991; James *et al.* 2017).

2.2 Experimental Study

We used mesocosm experiments to compare how burrowing activity was affected by crayfish density and shelter availability for signal crayfish collected from four locations: from within their native range in Montana, USA (Clark Fork and Bitterroot Rivers); from a recently invaded site outside of their native range, following introduction by humans in Montana, USA (East Gallatin River); and from two well-established invasive populations in the UK – one where burrowing in the field was present (Gaddesby Brook), and one population where burrowing in the field was absent (River Etherow; Table I). Crayfish burrows have not been reported in the USA and were not recorded by us at the two sites in the USA where we collected signal crayfish. We used catch per unit effort (CPUE) overnight trapping for ~18 hours using Swedish 'trappy' traps (see Fjalling 1995; trap dimensions 510 mm x 210 mm, entrance diameter 50 mm, mesh size 30 mm x 20 mm) to estimate population density at the three invaded sites, as a surrogate measure for the severity of invasion (UK Environment Agency Trapper Number EW071-E-738). Trapping at the native sites was not possible, but hand searching indicated that signal crayfish were more abundant than at the East Gallatin River, Montana, and comparable to some invaded UK sites where burrows have been recorded (Sanders 2020), although this was not quantified. No crayfish were trapped at the East Gallatin River, Montana, indicating that population densities were too low to be detected by trapping. Hand searching at the East Gallatin River confirmed signal crayfish presence but at low population densities compared to the other tested sites. Population details of the animals collected are detailed in Table II.

Crayfish Group	River	Coordinates	Burrow observations	Collection date	Notes	Abbreviation
Native, USA	*Clark Fork & Bitterroot Rivers, Montana*	*46.868, -113.996* *46.853, -114.099*	*Absent*	*October 2018*	*Native population (Larson and Olden 2011)*	*NX; Native, No Burrows*
Recent Invasion, USA	*East Gallatin River, Montana*	*45.782, -111.113*	*Absent*	*October 2018*	*Human introduction. One reported sighting in five years prior to collection (Montana Field Guide 2019)*	*IRX; Invasive Recent, No Burrows*
Established Invasion, UK	*River Etherow, Greater Manchester*	*53.454, -1.987*	*Absent*	*Mid-October 2017,* *Mid-Septermber 2018*	*Human introduction*	*IEX; Invasive Established, No Burrows*
Established Invasion, UK	*Gaddesby Brook, Leicestershire*	*52.708, -0.975*	*Present, 102 burrows per 100 m of riverbank*	*Mid-October 2017,* *Mid-Septermber 2018*	*Human introduction*	*IEB; Invasive Established, Burrows Present*

Table I: Details of crayfish collection locations.

Crayfish population	Years since introduction	Estimated population density (CPUE)	Number collected	Male / Female	Carapace length range (mm)	Mean carapace length (mm)
NX	*-*	*-*	*31*	*15/16*	*30-58*	*38.4*
IRX	*4*	*0*	*15*	*10/5*	*30-64*	*47.4*
IEX	*20*	*2.8*	*44*	*18/26*	*37-66*	*49.3*
IEB	*17*	*9.3*	*37*	*16/21*	*40-60*	*49.4*

Table II: Population details of crayfish collected from the four locations. Population identifiers are detailed in Table I. Trapping was not undertaken at the NX site. CPUE = 0 from trapping at the IRX site does not indicate an absence of crayfish, but that population densities were too low to be detected by this method.

2.3 Laboratory Setup

Experiments using the two UK populations (IEX, IEB) were completed between January and March in 2018 and 2019 for both populations in mesocosms at

Loughborough University. We trapped crayfish for use in the study in mid-October 2017 and mid-September 2018. We kept these crayfish in two separate indoor circular holding tanks (1.2 m x 1.2 m x 1.4 m), containing 450 l of dechlorinated tap water, which were aerated, filtered, filled to a depth of 0.4 m, and maintained at an ambient laboratory temperature (~15 °C), commensurate with summer and autumn temperatures at both UK sites (Environment Agency 2022), when signal crayfish burrowing has been hypothesised to most commonly occur (Sanders 2020). A maximum of 25 crayfish were held in each holding tank. We placed appropriate shelters in the form of plastic pipes (exceeding the number of crayfish) in the tanks. Holding tanks were illuminated for 12 h (07:00 – 19:00) using laboratory lighting.

We constructed a 0.2 m thick bentonite clay bank, the maximum depth recorded in laboratory conditions for a crayfish burrow (Stanton 2004), at one end of each of 14 identical opaque mesocosms (0.53 m x 0.33 m x 0.29 m; Figure 2). To create the banks, we expanded bentonite clay pellets in tap water (1:1.5 pellet to water volume ratio) and compacted into the ends of the mesocosms using a shovel. Identical banks were constructed with an angle of approximately 70° to replicate the steep profiles where burrows are typically located in rivers in the UK (Faller *et al.* 2016; Sanders 2020). We smoothed the banks prior to the start of the experiment, and filled the mesocosms with dechlorinated tap water, which was kept at an ambient temperature. Mesocosms were illuminated for 12 h (07:00 – 19:00) by natural range LED lights (6,500 K white) suspended 0.65 m above the mesocosms, and air stones were used to oxygenate the water.

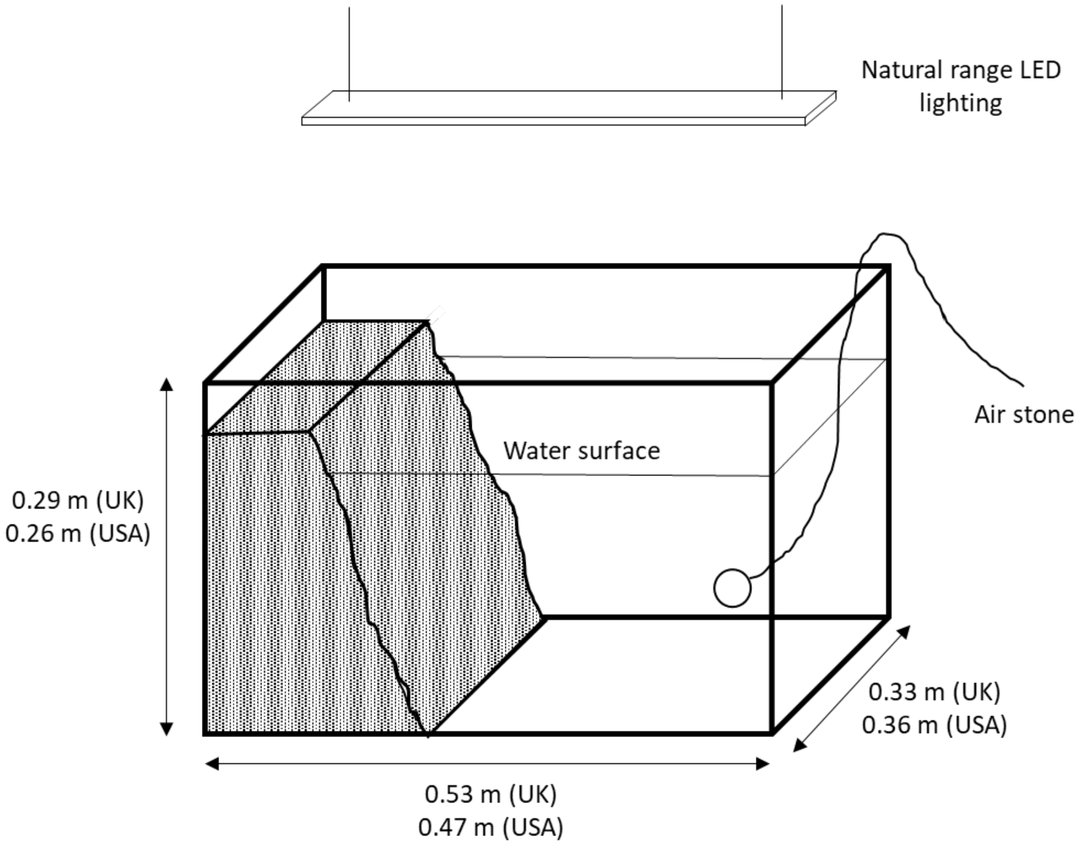

Figure 2: Schematic diagram of experimental setup. Measurements are given for the mesocosms used in both the UK and the USA, which had minor differences in dimensions (see main text).

Experiments using the two American populations (NX; IRX) were completed at Montana State University, USA between September and November in 2018, using 16 near-identical mesocosms (0.47 m x 0.36 m x 0.26 m) with the same lighting regime (6,500 K white). The two US populations were kept in two separate holding tanks (1.3 m x 0.7 m x 0.6 m), containing 180 l of dechlorinated tap water, which were aerated, filtered, filled to a depth of 0.2 m, and maintained at ambient laboratory temperature. Experiments involved the same treatments, measurements and processes as were applied to the UK populations.

2.4 Experimental Treatments

Burrowing activities were compared for each of the four populations across three levels of crayfish density and between two or three different shelter types. Low (1 crayfish per mesocosm; 5.5 crayfish m^{-2}), medium (2 crayfish per mesocosm; 11 crayfish m^{-2}) and high (4 crayfish per mesocosm; 22 crayfish m^{-2}) densities were tested, where the high level is similar to the highest densities of adult signal crayfish recorded in British streams (20 m^{-2}; Bubb *et al.* 2004). The lowest density burrowing was compared using three shelter types (no shelter; a single large rock; deep unconsolidated fine sediment [herein ‘deep fine substrate’]). These alternatives represent comparable shelter types widely available in British streams where burrows exist. For the medium and high levels of crayfish density, laboratory time and space restrictions meant that only two shelter types could be compared, so the large rock and no shelter situations were tested. The large rock shelter was selected due to the stronger prior evidence of its use by crayfish in rivers (Peay and Rogers 1999). There were therefore 7 different treatments for each of the four populations.

For the deep fine substrate treatment, a layer of loose bentonite clay covered the base of the mesocosms to a depth of 0.05 m. This was created by expanding bentonite clay pellets in an excess of water (1:5 pellet to water volume ratio), and manually disaggregating the loose clay structure evenly across the base of the mesocosm. The loose bentonite was allowed to settle to form a uniform substrate before any experiments commenced. Each rock used as a shelter in experiments was a cobble (b-axis 128 mm to 180 mm) and rounded or sub-rounded on the Wentworth (1922) scale. One rock was used per mesocosm, with rocks being deliberately selected to be consistent in both shape and size.

2.5 *Experimental Procedure*

For each run, we selected crayfish at random from their holding tank. We recorded crayfish size and sex, and used only crayfish that were not in moult, were sexually mature (larger than 30 mm carapace length (CL); Johnsen and Taugbol 2010), and had intact legs, antennae and chelae. We placed crayfish into mesocosms, and experiments ran for 84 hours, after which we removed the crayfish and returned them to holding tanks. Crayfish were fed for at least three days between experimental runs on carrot sticks and sinking catfish pellets but were not fed during the experiments. Experimental treatments were randomly assigned across mesocosms and through time.

To measure the size of individual burrows that crayfish constructed, the total mass of sediment excavated, and the speed at which burrows were constructed, we recorded burrowing activity each morning after lights were switched on between 0700-0900 hrs, and each evening immediately prior to the lights being switched off between 1700-1900 hrs. This approach allowed us to determine the rate at which burrows were constructed. We deemed burrows 'usable' at a depth of 70 mm, as this was the smallest burrow depth we recorded where crayfish inhabited and defended a shelter.

We measured burrow depths and the width and height of burrow entrances using a ruler to the nearest 5 mm. We measured burrow depths to the centre of the burrow height, due to the sloping bank face. We then calculated the volume of sediment excavated by treating the burrow shape as an elliptical cylinder (as in Faller *et al.* 2016);

$$V_B = \pi\ (W/2\ H/2)\ L \qquad (\textit{Equation 1})$$

where V_B is burrow volume, W is the burrow entrance width; H is the entrance height, and L is the length of the burrow.

In the event of multiple burrows being excavated, we summed the volumes of the burrows together to report the total volume of sediment excavated in a single experiment, which is reported as 'total sediment excavated' herein. We also analysed differences in individual burrows between treatments, and reported as 'burrow size'. This distinction is important to consider, as the same mass of sediment could be excavated by digging one large burrow constructed for retreat purposes or multiple smaller burrows as temporary refuges. A separate variable was also calculated by dividing the total mass of sediment excavated by the number of crayfish present in the mesocosm to calculate the total mass of sediment moved per crayfish.

The bentonite clay used in these experiments had a bulk density of 1.41 g cm^{-3}, and so all volumes were converted to sediment mass for analysis;

$$M_B = \gamma V_B \qquad (\textit{Equation 2})$$

where M_B is burrow mass, and γ is the calculated bulk density of the excavated sediment, which here is 1.41 g cm^{-3}.

We siphoned out any sediment excavated from the no-shelter and rock treatments of the mesocosms at 12-hour intervals, to avoid recreating the deep fine substrate treatment.

We replicated each of the 7 treatments seven times, yielding 49 experimental runs for each population, with the order of treatments randomised throughout the experimental period. However, fewer replications were achieved with the IRX population (low density: no shelter (7), rock shelter (7), silt shelter (4); medium density: rock shelter (4), yielding 22 experimental runs. Four experimental runs from the NX population (high density, no shelter) were excluded due to the death of one crayfish during the experiment. In total, this yielded 165 experimental runs (IEB 49; IEX 49; NX 45; IRX 22).

The 49 tested runs for each population accounted to 105 separate crayfish uses per population. However, fewer than 105 crayfish per population were collected (Table II). Therefore, some crayfish were reused in experiments. However, as crayfish were randomly assigned for experimental selection, and treatments were randomly assigned to mesocosms, the reuse (and influence of differences in burrowing capacity by specific individual crayfish) was minimised due to their random distribution across the experimental treatments.

By randomly selecting crayfish for a random order of experimental treatments, we use a cohort design which subsequently allowed us to approximate crayfish behavioral plasticity. Whilst we did not directly measure the reaction norms of signal crayfish, the native range of signal crayfish covers diverse environmental, geographical, and ecological regions (Larson and Olden 2011), with no burrows having been recorded *in situ*. Therefore, the reaction norm of signal crayfish to a gradient of environmental, geographical, and ecological variables can be inferred to be not constructing a burrow. This therefore allowed for the interpretation of the results in relation to the framework of behavioral plasticity.

2.6 Data Analysis

Burrow measurements (the size of individual burrows constructed, total sediment excavated, total sediment excavated per crayfish and the speed at which burrows were constructed) were tested for normality by inspecting density plots, Q-Q plots, and using the Shapiro-Wilk test, and none were normally distributed ($p < 0.001$ in all cases). After $\log_{10}$ transformation, the size of individual burrows constructed were normally distributed, and met the assumptions required for linear mixed effects modelling (LME). Log and square root data transformations were not possible for the total mass of sediment excavated, due to the presence of a high number of zeros in the dataset, which were true zeros (crayfish did not burrow) as opposed to an absence of data. Similarly, the speed at which burrows were constructed could not be normalised due to the recordings taking place over 12-hour intervals. Therefore, non-parametric statistical techniques (Kruskal-Wallis (*H*) tests with Dunn's post-hoc test for differences between groups, and Wilcoxon rank sum test (W) for differences between pairs) were used to analyse the mass of sediment excavated and the speed at which burrows were constructed. All data were analysed using R version 4.2.2 (R Core Team 2022) in R Studio.

To consider how shelter availability affected crayfish burrowing (Q1), Kruskal-Wallis tests of total sediment excavated, total sediment excavated per crayfish, and the time taken to construct a burrow were undertaken between shelter treatments. LME (with the experimental year, crayfish sex, and crayfish size as random effects) with Satterthwaite approximation within the R package lme4 (Bates et al. 2015) were used to examine differences in burrow size, with differences within groups examined via least-square means using the package lsmeans (Lenth 2016). The effect of shelter was considered independently at low, medium, and high crayfish densities.

To consider how crayfish density affected crayfish burrowing (Q2), Kruskal-Wallis tests of total sediment excavated, total sediment excavated per crayfish, and the time taken to construct a burrow were undertaken between low, medium and high crayfish densities, in the presence and absence of a shelter. LME was used to consider the effect of crayfish density on individual burrow size. NX, IEX, and IEB data were pooled for shelter and crayfish density analyses, as all populations completed all experimental runs, and no significant differences were observed between populations. IRX crayfish were excluded, as not all treatments were completed.

To consider how burrowing differed between native, recently invaded, and established invasive populations (Q3), Kruskal-Wallis tests of total sediment excavated, total sediment excavated per crayfish, and the time taken to construct a burrow were undertaken between crayfish populations, at low, medium, and high crayfish densities, considering runs where a shelter was present, and runs where a shelter was absent. LME was used to consider the effect of crayfish population provenance on individual burrow size. Comparisons between populations from the medium and high-density treatments did not include analysis of the IRX populations, as not all treatments were completed. Between population analysis included comparisons between the IEB and IEX populations to allow consideration of how burrowing differed between populations from rivers where burrows are present and from rivers where burrows are absent (Q4).

3. Results

3.1 General Results

Overall, 108 burrows were constructed in 81 of the 165 experimental runs (Figure 3). Crayfish burrowed significantly more at night than during the day ($W(N_{day} = 108, N_{night} = 108) = 297.5$; $p < 0.001$), with 97.9% of sediment excavation occurring when the lights were off. Across all experiments, LME indicated that, controlling for all other variables, crayfish sex ($p = 0.529$) and crayfish size ($p = 0.529$) did not affect the size of burrows constructed.

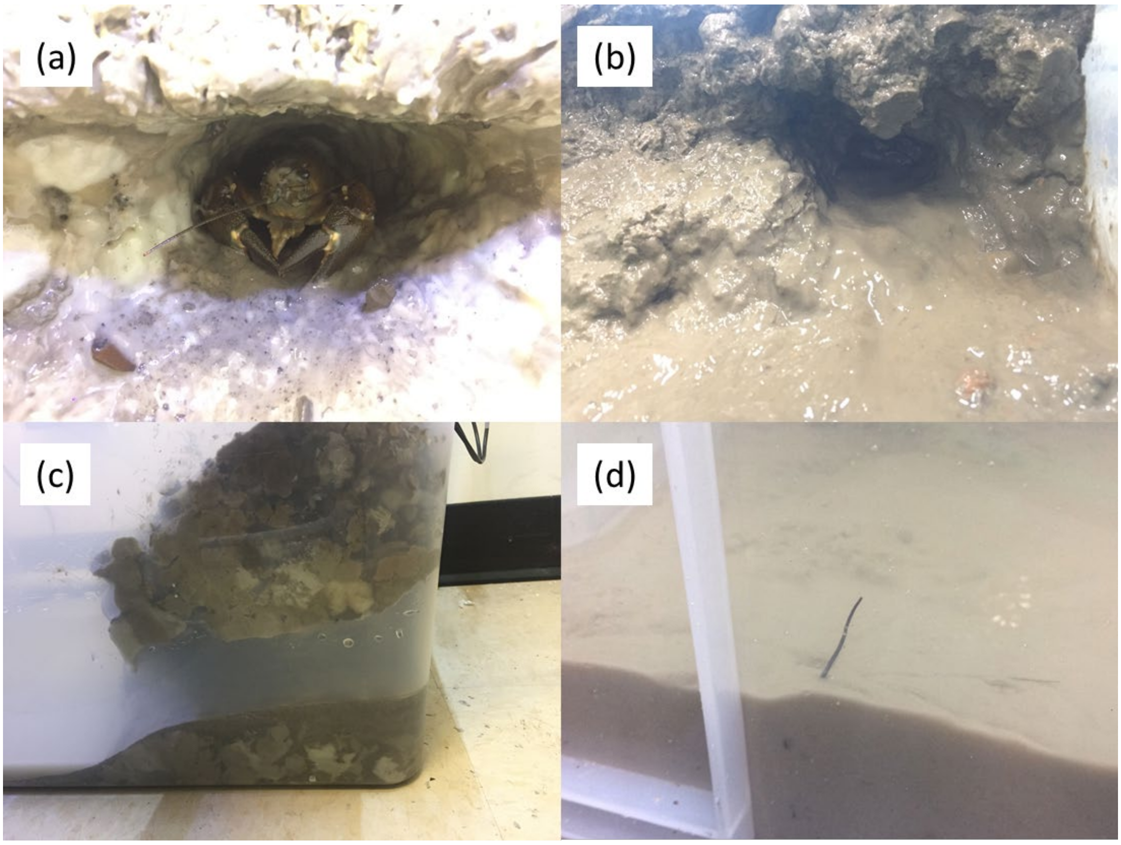

Figure 3: *(a-c) Burrows constructed by crayfish during the experiments, and (d) a crayfish hiding in a layer of deep fine sediment.*

3.2 Shelter Availability

Shelter availability was a significant driver of burrowing. In the low-density treatment, total sediment excavated by burrowing differed between shelter availability ($H_{(2,\ 63)}$ = 14.438, $p < 0.001$). Crayfish constructed burrows in the no shelter (median = 438.3 g) and deep fine substrate shelter (median = 522.0 g) treatments, but no burrows were constructed when a large rock shelter was available. Dunn's pairwise comparisons indicated that the mass of sediment excavated in the presence of no shelter was significantly greater than in the deep fine sediment treatment ($p = 0.016$), and the large rock shelter ($p < 0.001$), and there was no difference between the deep fine sediment and large rock shelter ($p = 0.246$; Table III). As only one crayfish was present in these experiments, this was also true for the sediment mass per crayfish excavated. When burrows were constructed, the time taken to construct a functioning burrow did not differ between treatments ($p = 0.687$). LME controlling for the experimental year, crayfish size and crayfish sex, indicated that burrow size also did not differ between shelter treatments ($p = 0.590$; Table III).

Although shelter availability was a significant driver of burrowing in the low crayfish density experiments, this outcome was not observed in the medium and high crayfish density treatments. There was no significant difference in the mass of sediment excavated in the presence or absence of a rock shelter in the medium ($p = 0.370$) or high-density treatments ($p = 0.321$). This was also observed when the mass of sediment per crayfish was considered (medium density: $p = 0.370$; high density: $p = 0.321$).

There was no significant difference in the time that it took crayfish to construct burrows when a rock shelter was present or absent for the medium ($p = 0.441$) or high ($p = 0.081$) crayfish density treatments. LMEs, accounting for interaction and controlling for other variables, also indicated that there was also no difference in the size of individual burrows that were constructed in the presence and absence of a rock shelter in the medium and low-density treatments ($p = 0.154$).

	Shelter Availability	**Crayfish Density**	**Mass of Sediment Excavated (g)**					**Burrow Size (g)**				
			IEB	*IEX*	*IRX*	*NX*	*All*	*IEB*	*IEX*	*IRX*	*NX*	*All*
Mean	*No Shelter*	*Low*	*70.4*	*612.9*	*998.0*	*204.7*	*296.0*	*246.6*	*858.0*	*1023.4*	*358.2*	565.1
		Medium	*97.3*	*144.4*	-	*380.1*	*207.3*	*170.3*	*202.1*	-	*332.6*	256.0
		High	*211.9*	*1170.7*	-	*308.8*	*623.8*	*296.6*	*910.5*	-	*308.8*	623.8
	Large Rock	*Low*	*0.0*	*0.0*	*53.7*	*0.0*	*0.0*	-	-	*375.9*	-	-
		Medium	*151.4*	*448.6*	*323.1*	*375.1*	*325.1*	*323.8*	*314.0*	*646.3*	*437.7*	341.3
		High	*530.3*	*845.0*	-	*512.9*	*629.4*	*464.0*	*492.9*	-	*598.4*	508.4
	Deep Silt	*Low*	*132.8*	*41.7*	*1394.0*	*74.6*	*83.0*	*929.8*	*292.2*	*1394.0*	*522.0*	581.3
		All Runs	*88.7*	*275.8*	*646.8*	*163.6*		*348.3*	*543.9*	*1016.4*	*419.9*	
Standard Error	*No Shelter*	*Low*	*61.8*	310.7	*231.2*	*83.6*	*115.7*	*191.8*	262.4	155.1	78.3	145.4
		Medium	*48.6*	*77.5*	-	*119.0*	*54.9*	*46.3*	58.4	-	92.8	49.5
		High	*147.8*	*531.5*	-	*157.2*	*246.4*	*79.9*	308.0	-	80.8	177.5
	Large Rock	*Low*	-	-	-	-	-	-	-	-	-	-
		Medium	*54.8*	*166.8*	-	*207.8*	*90.5*	*21.6*	82.9	417.1	113.9	78.8
		High	*185.8*	*271.7*	-	*128.8*	*116.9*	*144.3*	131.5	-	234.4	77.5
	Deep Silt	*Low*	-	-	*462.5*	-	*50.6*	-	-	462.5	-	186.4
		All Runs	*44.9*	*110.4*	*162.3*	*51.1*		*60.7*	*90.4*	*168.7*	*62.5*	
Median	*No Shelter*	*Low*	*0.0*	*265.6*	*946.4*	*222.7*	*0.0*	*246.6*	*650.8*	*946.4*	*312.8*	438.3
		Medium	*0.0*	*0.0*	-	*197.6*	*149.4*	*138.6*	*149.4*	-	*183.5*	157.7
		High	*0.0*	*534.6*	-	*411.7*	*411.7*	*307.2*	*531.3*	-	*365.3*	411.7
	Large Rock	*Low*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	-	-	*375.9*	-	-
		Medium	*221.4*	*432.2*	*0.0*	*159.4*	*199.2*	*257.3*	*216.1*	*646.3*	*169.6*	220.3
		High	*503.6*	*647.5*	-	*514.7*	*514.7*	*393.8*	*309.9*	-	*668.3*	350.1
	Deep Silt	*Low*	*0.0*	*0.0*	*1604.4*	*0.0*	*0.0*	*929.8*	*292.2*	*1604.4*	*522.0*	522.0
		All Runs	*204.2*	*517.0*	*761.1*	*328.2*		*274.8*	*309.9*	*959.4*	*333.3*	
Burrow Frequency	*No Shelter*	*Low*	*2/7*	*4/7*	*6/7*	*4/7*	*10/21*	2/2	5/4	8/6	4/4	11/10
		Medium	*3/7*	*3/7*	-	*7/7*	*13/21*	4/3	5/3	-	8/7	17/13
		High	*3/7*	*6/7*	-	*2/3*	*11/17*	5/3	9/6	-	3/2	17/11
	Large Rock	*Low*	*0/7*	*0/7*	*1/7*	*0/7*	*0/21*	-	-	1/1	-	-
		Medium	*4/7*	*5/7*	*1/7*	*6/7*	*15/21*	4/4	10/5	2/1	6/6	20/15
		High	*5/7*	*6/7*	-	*6/7*	*17/21*	8/5	12/6	-	6/6	26/17
	Deep Silt	*Low*	*1/7*	*1/7*	*4/4*	*1/7*	*3/21*	1/1	1/1	4/4	1/1	3/3
		All Runs	*18/49*	*25/49*	*12/22*	*26/45*		24/18	42/25	14/12	28/26	

Table III: Mean, standard error, and median values of the mass of total sediment excavated and burrow size in experiments. Burrow frequency (the number of mesocosms where burrowing occurred / the total number of experiments, and the number of burrows constructed / the number of mesocosms where burrowing occurred) is also shown. 'All Population' data excludes IRX crayfish, as these did not complete all treatments, to allow for equal comparisons.

3.3 *Crayfish Density*

Crayfish density was a significant driver of burrowing. When a rock shelter was present, the mass of total sediment excavated differed with crayfish density ($H_{(2,\,63)}$ = 30.333, p < 0.001; Figure 4a). Dunn's pairwise tests indicated that both two crayfish (p < 0.001) and four crayfish (p < 0.001) excavated significantly more sediment than one crayfish, but there was no difference in the mass of excavated sediment between the medium and high-density treatments ($p = 0.079$). These results were also observed for the mass of sediment per crayfish (high > low, $p < 0.001$; medium > low, $p < 0.001$; high = medium, $p = 0.490$).

The median size of the burrows constructed appeared to increase with crayfish density (low: 0 g due to no burrows; medium: 220.3 g; high: 350.1 g), but LME indicated that the difference between the medium and high density treatments was not significant ($p = 0.119$). Burrows were constructed significantly more quickly in the high crayfish density treatment than the medium density treatment ($W(N_{medium} = 20, N_{high} = 26) = 113$; $p = 0.014$).

When no rock shelter was present, there was no association between excavated sediment and crayfish density (Figure 4b), with no significant difference in the mass of excavated sediment between population densities ($p = 0.277$). This was also observed when the mass of sediment per crayfish was considered ($p = 0.892$). LME indicated that whilst burrows constructed appeared smallest in the medium density treatment (median = low: 438.3 g; medium: 157.7 g; high: 411.7 g), these were not significantly different across densities ($p > 0.05$). The time to construct these burrows did not differ between density treatments ($p = 0.095$).

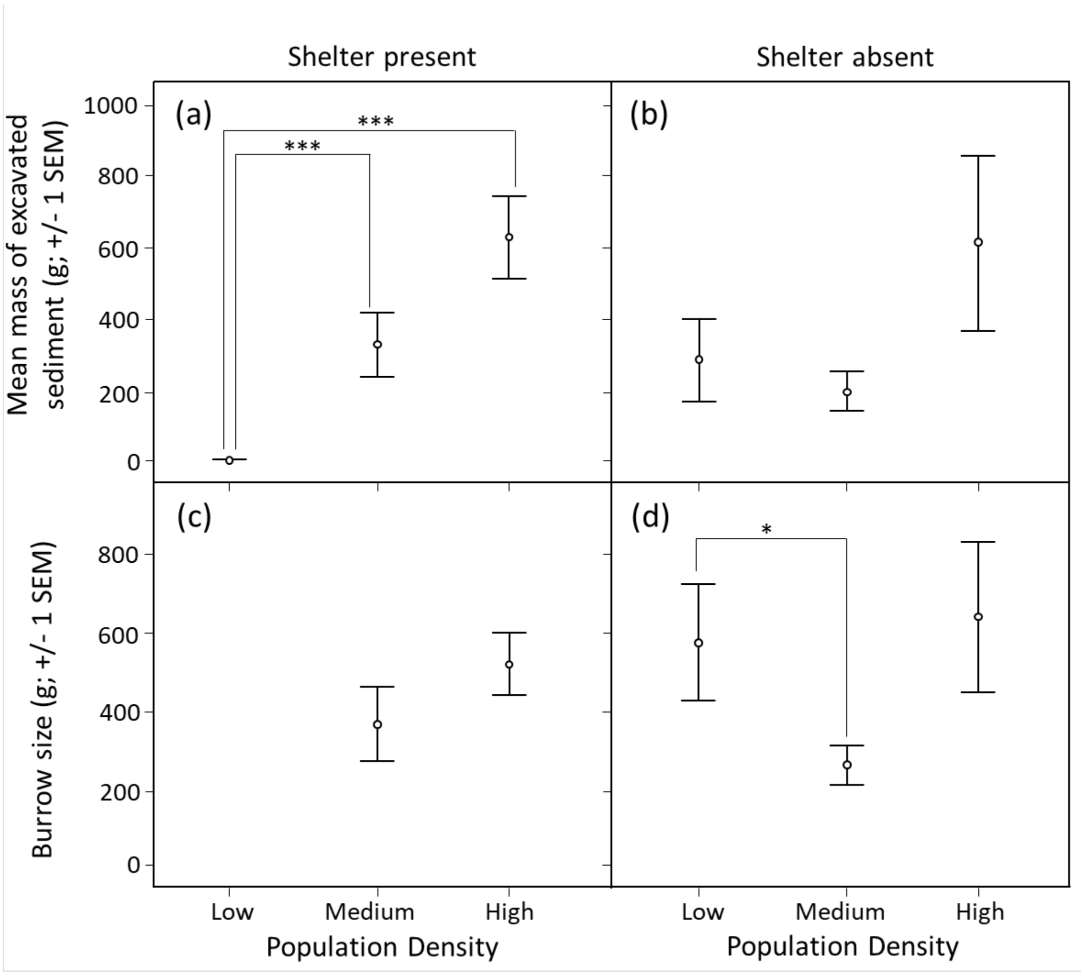

Figure 4: The effect of crayfish density on (a and b) mass of total sediment excavated and (c and d) burrow size when a large rock shelter was (a and c) present and (b and d) absent, considering mean values, +/- 1 standard error (SEM). Asterisks indicate significant pairwise differences (< 0.05; ** < 0.01; *** < 0.001).*

3.4 Population Provenance

Crayfish from all populations burrowed during the experiments. In the low-density treatments, there was a significant difference in the mass of sediment excavated between populations ($H_{(3, 81)}$ 14.729, $p = 0.002$). Dunn's pairwise comparison indicated that there was no difference in the mass of total sediment excavated by the IEB, IEX or NX crayfish (mean = 67.8 g, 218.2 g and 258.0 g, and median = 0.0 g, 0.0 g and 0.0 g, respectively). However, the IRX crayfish excavated significantly more sediment than crayfish from any other population (mean = 718.8 g; median = 561.5 g; pairwise: $p = 0.002$ IEB; $p = 0.009$ IEX; $p = 0.008$ NX; Figure 5a). The size of burrows constructed by IRX crayfish in experiments trended towards being larger than those constructed by other populations (Figure 5b), but LME indicated that mean burrow size was not significantly different to other populations ($p = 0.125$). IRX crayfish took the shortest time to constructed burrows, with Dunn's pairwise test indicating IRX crayfish were significantly faster than IEX crayfish ($p = 0.023$).

In the medium density treatment, there was no difference between populations in the mass of total sediment excavated ($p = 0.204$), or in the size of burrows constructed ($p = 0.893$). In the high-density treatment, there was no difference in the total mass of sediment excavated ($p = 0.096$) or the size of burrows constructed ($p = 0.162$) between populations.

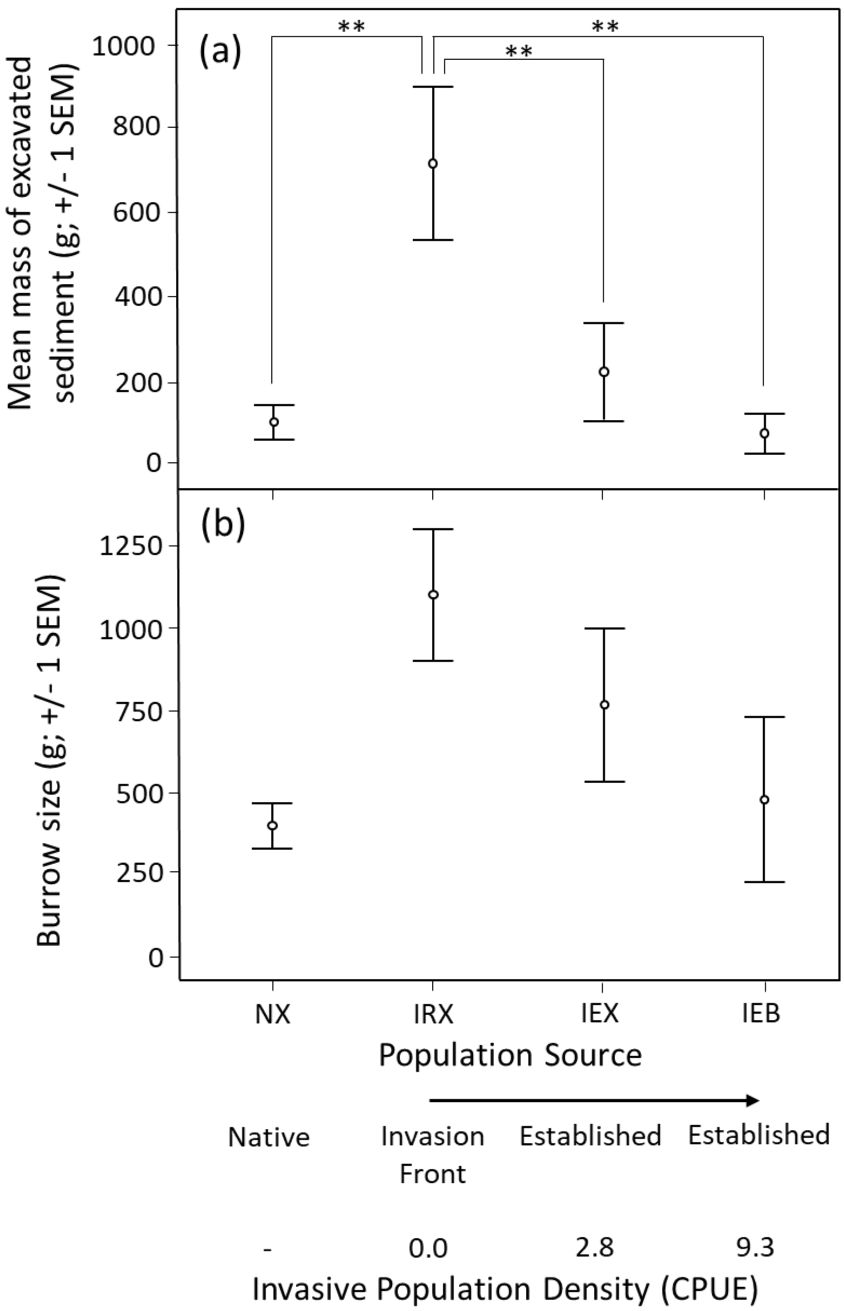

Figure 5: *Differences between populations considering (a) mass of total sediment excavated, and (b) burrow size in the low crayfish density treatments, considering mean values, +/- 1 standard error (SEM). NX = Native population, no burrows; IRX = Invasive (recent) population, no burrows; IEX = Invasive (established) population, no burrows; IEB = Invasive*

(established), burrows present. Asterisks indicate significant pairwise differences (< 0.05; ** < 0.01; *** < 0.001).*

4. Discussion

While previous studies have demonstrated changes in the frequency and intensity of an animal's behavior upon becoming invasive, this study shows that innate behavioral strategies not seen in the native range can be activated under specific conditions, such as at invaded sites in the UK, where burrowing by signal crayfish has been extensively documented (e.g. Guan 1994; Faller et al. 2016; Sanders 2020; Sanders et al. 2021). Crayfish from all populations burrowed in the experiments, but the intensity of expression varied, suggesting a difference in behavioral expression between native and invasive populations that has not previously been quantified.

4.1 Crayfish Density and Shelter Availability

A significant increase in burrowing activity with increasing crayfish density was observed. Previous studies have not considered crayfish density as a driving factor (Faller *et al*. 2016) or not observed an association between burrow densities and crayfish population density (Guan 1994), which has been attributed to the poor relationship between crayfish population density and trapping catch per unit effort (Guan 1994; Chadwick *et al.* 2021). The experiments reported here demonstrate that crayfish density plays a significant role in driving the burrowing behavior of signal crayfish.

In the low crayfish density treatments, significant differences in crayfish burrowing activity were recorded depending on shelter availability. Most striking was the reduction in burrowing in the large rock treatment compared to the no shelter treatment, suggesting that large rocks provide alternative shelter that can preclude burrowing; a preference that may reflect an energy saving strategy (see Meysman *et al*. 2006). This effect was not as strong with two or four crayfish. Nevertheless, when a rock shelter was present, a positive association in excavated sediment was observed with increasing density, which was not observed when the rock was absent. In all cases, the rock was occupied by a single crayfish that defended its shelter, which resulted in other crayfish burrowing. Therefore, burrowing increased when the crayfish density exceeded the availability of shelter. This finding suggests that riverbank burrowing is directly related to the size of the crayfish population relative to in-stream shelter availability and is consistent with field survey data from 30 UK rivers, which shows that burrowing increased with crayfish density and decreased with the abundance of coarse bed sediment (Sanders 2020).

When a shelter was absent, constructed burrows were significantly smaller when two crayfish were present compared to when one crayfish was present. Signal crayfish are highly aggressive (Houghton *et al*. 2017), and shelter limitations are a significant driver of agonistic interactions in crayfish (Bergman and Moore 2003, Capelli and Hamilton 1984). Therefore, this result may reflect aggressive interactions between the crayfish, where time and energy was spent interacting with other crayfish rather than constructing burrows. Alternately, these smaller burrows may be made as temporary refuges from antagonistic interactions, or less energy may have been expended in creating a shelter, reserving energy to strongly defend the ownership of the shelter from others (Ranta and Lindstrom 1993; Guan 1994; Bergman and Moore 2003). These results contradict Statzner and Peltret’s (2006) observation that limiting shelter space, which led to more interactions, did not reduce the engineering activity of crayfish, but are consistent with other studies that found no association between increased crayfish density and increased sediment transport (Rice et al. 2012; Albertson and Daniels 2018).

4.2 *Population Provenance*

Many studies have investigated the exaggeration or adaptation of animal activities and behaviors during invasion (e.g. Magurran *et al.* 1992; Holway and Suarez 1999; Phillips *et al.* 2006; Pintor and Sih 2009; Gruber *et al.* 2017; Jones and DiRienzo 2018), but to our knowledge, this is the first time that the expression of a novel behavior that has not been recorded in the native range has been quantified for both native and invasive populations. Burrowing was recorded by crayfish in all populations examined, including those that do not express a burrowing behavior in the rivers they inhabit. This finding suggests that should signal crayfish spread or be introduced to a new river, they have the capacity to burrow regardless of the source population.

Further, there was no significant difference observed in the burrowing response of the two UK populations (one from a burrowed river, and one from a river with no evidence of burrowing) and the native population. This is interesting because burrowing has never been reported in riverbanks within the native range. This outcome suggests that the native population has an innate capacity to burrow and does so in response to biotic and abiotic drivers, such as a lack of shelter, or high crayfish density, as examined in the current study. Lack of burrowing in the field may therefore reflect environmental conditions which preclude the need or ability to burrow, rather than biological capability. These conditions include typically coarse bed material size, which may preclude burrowing by providing alternative, less energy expensive shelters to use, and shallow, rocky banks, which cannot be excavated, because signal crayfish require steep, cohesive banks for burrow construction (Faller *et al.* 2016; Sanders 2020).

However, signal crayfish from the recently invaded East Gallatin River supplied significantly more sediment through burrowing than any of the three other populations in the low-density experiments. This was true, even though the morphological characteristics of the East Gallatin were not substantially different from the UK rivers. The riverbank profiles of the East Gallatin were qualitatively consistent with those of the UK IEB study site, consisting of cohesive bank material that formed a steep bank, with many crayfish being collected that were walking on the riverbank, but no burrows were found when surveyed. This may be the result of a low crayfish density (the catch per unit effort from trapping was zero). The population in the East Gallatin was only recently detected, and both visual and hand searching confirmed the very low crayfish density of signal crayfish relative to the other study sites. It may be that the population density of signal crayfish at the East Gallatin does not yet exceed the availability of alternative shelters, and so in the field, crayfish have not yet resorted to burrowing.

However, this hypothesis does not explain why the IRX crayfish burrowed significantly more than the other populations in the experiments. It may be that the magnitude to which burrowing behavior exhibited varies during the process of invasion. The variability of learned behavioral variants exhibited throughout the different stages of invasion is well understood (see adaptive flexibility hypothesis; Wright et al. 2010), and it may be that the magnitude to which innate behaviors are expressed are also strongest during the introductory stages of invasion. Whilst the populations tested here are independent of each other, and do not give the opportunity to observe the magnitude of burrowing over time in a single population, population density estimates (CPUE) can be used as a surrogate measure for the stage of invasion. As such, an increase in the expression of the burrowing behavior could be associated with the recent introduction of the IRX population, with the expression of the burrowing behavior reducing with population establishment (IEX and IEB; Figure 5).

The strength of the expression and the situational requirement for burrowing together combine to result in the total mass of sediment excavated from riverbanks in streams. For example, two crayfish may have the same strength of the expression of burrowing behavior in a given situation (e.g. IEX and IEB measured in experiments), but they may be subject to different environmental conditions (e.g. difference in the number of alternative shelters available). Alternately, they may experience similar environmental conditions, but have a different strength of crayfish burrowing behavior, resulting in differing burrowing rates in the field, when similar burrowing rates have been observed in laboratory experiments. Thus, understanding both the strength of crayfish burrowing behavior and the environmental requirement for them to burrow is required to understand why the presence and rates of burrowing differs in the field.

The current experiments did not provide an opportunity for crayfish to socially learn due to the lack of social interaction in low density treatments, which is a key component of behavioral flexibility (Wright *et al*. 2010; Lea *et al*. 2020). Nevertheless, crayfish from all locations, including those with no prior observation of burrowing to learn from, burrowed in these experiments. Signal crayfish have previously shown a capacity for learning (Acquistapace *et al.* 2003; Ion *et al.* 2020), and exposing animals to novel environments that require a specific behavioral solution is a common methodology to examine animal innovation (Griffin and Guez 2014). However, it is unlikely that the burrowing behavior observed in these experiments is a form of innovation. This is because, when faced with the novel environment, crayfish from the three independent populations where burrowing is not known to occur, each independently ‘innovated’ the same solution, expressed to the same magnitude, within in the same timeframe. Therefore, due to the consistency of the response shown, it is unlikely that burrowing by signal crayfish is innovation. Rather, it appears more likely that burrowing is an innate response that reflects behavioral plasticity (Mery and Burns 2010).

The extent and magnitude of the behavioral plasticity shown here by signal crayfish is particularly worthy of further investigation. Rather than adjusting a gradient response, such as a dispersal rate (c.f. Phillips *et al.* 2006; Mowery *et al.* 2021), an anti-predator behavior (c.f. Magurran *et al.* 1992), foraging ability (c.f. Pintor and Sih 2009), or variation in voracity (c.f. Jones and DiRienzo 2018), which is observed to a greater or lesser degree in response in novel environmental conditions, signal crayfish exhibit an entirely new behavior in constructing burrows, which is not observed in the field, in the native range. Future work could extend the experiments presented here by undertaking similar experiments with crayfish along an invasion gradient to identify the specific stages of an invasion where the magnitude of burrowing deviates from that of the response of the native population. If future work directly tests and finds support for the ideas presented in this discussion, then these patterns may be analogous to Wright *et al.*'s (2010) hypothesis of adaptive flexibility.

The behavioral plasticity of burrowing was present – if not consistent – across all populations. Indeed, Berrill and Chenoweth (1982) suggest that all crayfish species may have the ability to construct burrows under the required environmental cues, and so this hypothesis may be applicable to species invasions beyond signal crayfish. These experiments further demonstrate that the behavior of animals can change when they become invasive, and extend Wright *et al.*’s (2010) model in demonstrating that the modification of innate as well as learned behaviors can dynamically change throughout the process of invasion. Behavioral plasticity has been suggested to be an important factor in the success of crustacean invasions (Weis 2010), and this extreme behavioral plasticity may have contributed to the global success of global signal crayfish invasions.

5. Summary

Previous studies have demonstrated exaggerations of existing behaviors by invasive animals. The experiments reported here have quantified, for the first time, the expression of a behavior assumed to be unique to some invasive populations. In particular, they quantified the expression of this behavior in non-invasive (endemic) and invasive populations (one exhibiting burrowing behavior, and two where it was absent). Both biotic (crayfish density) and abiotic drivers (shelter availability) were significant in driving signal crayfish burrowing, which was displayed by all populations in the experiments, suggesting any signal crayfish population has the capacity to burrow under appropriate environmental conditions. The availability of shelters relative to population size is critical because as shelters become scarce, due to occupation by defensive crayfish, those crayfish excluded from shelters are more likely to burrow in order to create a shelter. Crayfish from the most recently invaded site burrowed significantly more than any other population, which was attributed to the stage of invasion. As all crayfish burrowed in the experiments, the novel case of burrowing in the UK by signal crayfish is not ‘innovation’, or ‘behavioral flexibility’, but rather that signal crayfish possess extreme behavioral plasticity, which may aid future invasions. These experiments demonstrate that invasive species may possess behaviors currently unrecorded due to the absence of biotic or abiotic drivers in their native or currently invaded range, which presents an added and unknown cost to future biological invasions.

Data Availability Statement

Data are attached as supplementary materials.

References

Acquistapace, P., Hazlett, B.A. and Gherardi, F. (2003). Unsuccessful predation and learning of predator cues by crayfish. *Journal of Crustacean Biology*, 23(2): 364-370. https://doi.org/10.1163/20021975-99990346

Albertson, L.K. and Daniels, M.D. (2018). Crayfish ecosystem engineering effects on riverbed disturbance and topography are mediated by size and behavior. *Freshwater Science*, 37(4): 836-844. https://doi.org/10.1086/700884

Amici, F., Widdig, A., Lehmann, J. and Majolo, B. (2019). A meta-analysis of interindividual differences in innovation. *Animal Behavior*, 155: 257-268. https://doi.org/10.1016/j.anbehav.2019.07.008

Arbilly, M. and Laland, K.N. (2017). The magnitude of innovation and its evolution in social animals. *Proceedings of the Royal Society B*, 284(1848): 20162385. https://doi.org/10.1098/rspb.2016.2385

Bai, S. and Lung, W.S. (2005). Modelling sediment impact on the transport of fecal bacteria. *Water Research*, 39(20): 5232-5240. https://doi.org/10.1016/j.watres.2005.10.013

Barrett, S.C.H. and Richardson, B. (1986). Genetic attributes of invading species. In R. Groves and J. Burdon (Eds.) Ecology of Biological Invasions: An Australian Perspective. Australian Academy of Sciences: Canberra. pp.21-33.

Bates, D., Maechler, M., Bolker, B. and Walker, S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67(1), 1-48. doi:10.18637/jss.v067.i01.

Bergman, D.A. and Moore, P.A. (2003). Field observations of intraspecific agonistic behavior of two crayfish species, *Orconectes rusticus* and *Orconectes virilis*, in different habitats. *The Biological Bulletin*, 205(1): 26-35.

Berrill, M. and Chenoweth, B. (1982). The burrowing ability of nonburrowing crayfish. *American Midland Naturalist*, 199-201. https://doi.org/10.2307/2425310

Bilotta, G.S. and Brazier, R.E. (2008). Understanding the influence of suspended solids on water quality and aquatic biota. *Water Research*, 42: 2849-2861. https://doi.org/10.1016/j.watres.2008.03.018

Blake, M.A. and Hart, P.J.B. (1993). The behavioral responses of juvenile signal crayfish *Pacifastacus leniusculus* to stimuli from perch and eels. *Freshwater Biology*, 29: 89-97. https://doi.org/10.1111/j.1365-2427.1993.tb00747.x

Bojko, J., Burgess, A. L., Baker, A. G., & Orr, C. H. (2021). Invasive non-native crustacean symbionts: diversity and impact. *Journal of Invertebrate pathology*, *186*, 107482.

Bubb, D.H., Thom, T.J. and Lucas, M.C. (2004) Movement and dispersal of the invasive signal crayfish Pacifastacus leniusculus in upland rivers. *Freshwater Biology*, *49*(3), 357-368.

Bubb, D.H., Thom, T.J. and Lucas, M.C. (2004). Movement and dispersal of the invasive signal crayfish *Pacifastacus leniusculus* in upland rivers. *Freshwater Biology*, 49(3): 357-368. https://doi.org/10.1111/j.1365-2426.2003.01178.x

Butler, D.R. (1995). Zoogeomorphology: Animals as Geomorphic Agents. Cambridge: Cambridge University Press.

Capelli, G. M. and Hamilton, P. A. (1984). Effects of food and shelter on aggressive activity in the crayfish *Orconectes rusticus* (Girard). *Journal of Crustacean Biology*, 4(2): 252-260. https://doi.org/10.2307/1548022

Chadwick, D. D., Pritchard, E. G., Bradley, P., Sayer, C. D., Chadwick, M. A., Eagle, L. J., & Axmacher, J. C. (2021). A novel 'triple drawdown'method highlights deficiencies in invasive alien crayfish survey and control techniques. *Journal of Applied Ecology*, *58*(2), 316-326.

Chadwick, D.D., Pritchard, E.G., Bradley, P., Sayer, C.D., Chadwick, M.A., Eagle, L.J. and Axmacher, J.C. (2021). A novel 'triple drawdown' method highlights deficiencies in invasive alien crayfish survey and control techniques. *Journal of Applied Ecology*, *58*(2): 316-326. https://doi.org/10.1111/1365-2664.13758

Chadwick, D.D.A. (2019) Invasion of the signal crayfish, *Pacifastcus leniusculus*, in England: implications for the conservation of the white-clawed crayfish, *Austropotamobius pallipes*. *Published PhD Thesis, University College London.*

Crooks J.A. (2002). Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. *Oikos*, 97: 153–166. https://doi.org/10.1034/j.1600-0706.2002.970201.x

Emery-Butcher, H.E., Beatty, S.J. and Robson, B.J. (2020). The impacts of invasive ecosystem engineers in freshwaters: A review. *Freshwater Biology*, 65: 999-1015. https://doi.org/10.1111/fwb.13479

Environment Agency (2022). Environment Agency water quality archive web-site. Available at: environment.data.gov.uk/water-quality. [Access date 19th October 2022].

Faller, M., Harvey, G.L., Henshaw, A.J., Bertoldi, W., Bruno, M.C. and England, J. (2016). River bank burrowing by invasive crayfish: Spatial distribution, biophysical controls and biogemorphic significance. *Science of the Total Environment*, 569-570: 1190-1200. https://doi.org/10.1016/j.scitotenv.2016.06.194

Fei, S., Philips J., Shouse M. (2014). Biogeomorphic Impacts of Invasive Species. *Annual Review of Ecology, Evolution and Systematics*. 45: 69-87. https://doi.org/10.1146/annurev-ecolsys-120213-091928

Findlay, J. D., Riley, W. D., & Lucas, M. C. (2015). Signal crayfish (Pacifastacus leniusculus) predation upon Atlantic salmon (Salmo salar) eggs. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *25*(2), 250-258.

Fjälling, A.B. (1995). Crayfish traps employed in Swedish fisheries. *Freshwater Crayfish*, 8, 201–214.

Gherardi, F. (2013) Crayfish as global invaders: Distribution, impact on ecosystem services and management options. *Freshwater Crayfish*, 19(2), 177-187.

Green, S.J., Atkins, J.L. and Cote, I.M. (2011). Foraging behavior and prey consumption in the Indo-Pacific lionfish on Bahamian coral reefs. *Marine Ecology Progress Series,* 433: 159167. https://doi.org/10.3354/meps09208

Griffin, A.S. and Guez, D., 2014. Innovation and problem solving: a review of common mechanisms. *Behavioural Processes*, *109*, pp.121-134. https://doi.org/10.1016/j.beproc.2014.08.027

Gruber, J., Brown, G., Whiting, M.J. and Shine, R. (2017). Geographic divergence in dispersal-related behavior in cane toads from range-front versus range-core populations in Australia. *Behavioral Ecology and Sociobiology*, 71: 38. https://doi.org/10.1007/s00265-017-2266-8.

Guan, R.Z. (1994). Burrowing behavior of signal crayfish, *Pacifastacus leniusculus* (Dana), in the River Great Ouse, England. *Freshwater Forum*, 4: 155-168.

Guan, R.Z. and Wiles, P.R. (1997). The home range of signal crayfish in a British lowland river. Freshwater Forum, 8, 45-54.

Harvey, G.L., Henshaw, A.J., Moorhouse, T.P., Clifford, N.J., Holah, H., Grey, J. and Macdonald, D.W. (2014). Invasive crayfish as drivers of fine sediment dynamics in rivers: field and laboratory evidence. *Earth Surface Processes and Landforms*, 39: 259-271. https://doi.org/10.1002/esp.3486

Harvey, G.L., Moorhouse, T.P., Clifford, N.J., Henshaw, A.J., Johnson, M.F., Macdonald, D.W., Reid, I. and Rice, S.P. (2011). Evaluating the role of invasive aquatic species as drivers of fine sediment-related river management problems: The case of the signal crayfish (*Pacifastacus leniusculus*). *Progress in Physical Geography*, 35: 517-533. https://doi.org/10.1177%2F0309133311409092

Hastings, A., Byers, J.E., Crooks, J.A., Cuddington, K., Jones, C.G., Lambrinos, J.G., Talley, T.S., and Wilson, W.G. (2007). Ecosystem engineering in space and time. *Ecology Letters*, 10: 153-164. https://doi.org/10.1111/j.1461-0248.2006.00997.x

Hazlett, B.A., Acquitapace, P. and Gherardi, F. (2002). Differences in memory capabilities in invasive and native crayfish. *Journal of Crustacean Biology*, 22(2): 439-448. https://doi.org/10.1163/20021975-99990251

Holdich, D. M., & Reeve, I. D. (1991). Distribution of freshwater crayfish in the British Isles, with particular reference to crayfish plague, alien introductions and water quality. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *1*(2), 139-158.

Holway, D.A. and Suarez, A.V. (1999). Animal behavior: an essential component of invasion biology. *Trends in Ecology and Evolution*, 14(8): 328-330. https://doi.org/10.1016/S0169-5347(99)01636-5

Houghton, R.J., Wood, C. and Lambin, X. (2017). Size-mediated, density-dependent cannibalism in the signal crayfish *Pacifastacus leniusculus* (Dana, 1852) (Decapoda, Astacidea), an invasive crayfish in Britain. *Crustaceana*, 90(4): 417-435. https://doi.org/10.1163/15685403-00003653

Hudina, S., Kutleša, P., Trgovčić, K., & Duplić, A. (2017). Dynamics of range expansion of the signal crayfish (*Pacifastacus leniusculus*) in a recently invaded region in Croatia. *Aquatic Invasions*, *12*(1): 67-75. https://doi.org/10.3391/ai.2017.12.1.07

Hudina, S., Zganec, K. and Hock, K. (2015). Differences in aggressive behavior along the expanding range of an invasive crayfish: an important component of invasion dynamics. *Biological Invasions*, 17(11): 3101-3112. https://doi.org/10.1007/s10530-015-0936-x

Ion, M.C., Puha, A.E., Suciu, T. and Parvulescu, L. (2020). Get a grip: unusual disturbances drive crayfish to improvise. *Behavior*, 157: 101-120. https://doi.org/10.1163/1568539X-00003583

James, J., Nutbeam-Tuffs, S., Cable, J., Mrugała, A., Viñuela-Rodriguez, N., Petrusek, A., & Oidtmann, B. (2017). The prevalence of Aphanomyces astaci in invasive signal crayfish from the UK and implications for native crayfish conservation. *Parasitology*, *144*(4), 411-418.

Johnsen, S.I. and Taugbol, T. (2010) NOBANIS – Invasive Alien Species Fact Sheet – Pacifastacus leniusculus. NOBANIS web-site. Available at: www.nobanis.org [Access date 27 Oct 2016].

Johnsen, S.I. and Taugbol, T. (2010). CABI – Pacifastacus leniusculus (American signal crayfish) datasheet. CABI web-site. Available at: https://www.cabi.org/isc/datasheet/70581#DA5AA83C-A614-422C-9468-AC2D413BCC3B. [Access date 29 May 2021].

Jones, C. and DiRienzo, N. (2018). Behavioral variation post-invasion: Resemblance in some, but not all, behavioral patterns among invasive and native praying mantids. *Behavioral Processes*, 152: 92-99. https://doi.org/10.1016/j.beproc.2018.05.011

Jones, C.G., Lawton, J.H. and Shachak, M. (1994). Organisms as ecosystem engineers. In F. Samson and F. Knopf (Eds.) Ecosystem Management. New York: Springer. pp. 130-147.

Jones, J.I., Collins, A.L., Naden, P.S. and Sear, D.A. (2012a). The relationship between fine sediment and macrophytes in rivers. *River Research and Applications*, 28(7): 1006-1018. https://doi.org/10.1002/rra.1486

Jones, J.I., Murphy, J.F., Collins, A.L., Sear, D.A., Naden, P.S. and Armitage, P.D. (2012b). The impact of fine sediment on macro-invertebrates. *River Research and Applications*, 28: 1055-1071. https://doi.org/10.1002/rra.1516

Karatayev, A.Y., Burlakova, L.E., Padilla, D.K., Mastitsky, S.E. and Olenin, S. (2009) Invaders are not a random selection of species. *Biological Invasions*, 11, 2009-2019.

Kemp, P., Sear, D., Collins, A., Naden, P. and Jones, I. (2011). The impacts of fine sediment on riverine fish. *Hydrological Processes*, 25: 1800-1821. https://doi.org/10.1002/hyp.7940

Kouba, A., Petrusek, A. and Kozak, P. (2014) Continental-wide distribution of crayfish species in Europe: update and maps. *Knowledge and Management of Aquatic Ecosystems*, 413, 5.

Lane, S.N., Tayefi, V., Reid, S.C., Yu, D. and Hardy, R.J. (2007). Interactions between sediment delivery, channel change, climate change and flood risk in a temperate upland environment. *Earth Surface Processes and Landforms*, 32(3): 429–446. https://doi.org/10.1002/esp.1404

Larson, E.R. and Olden, J.D. (2011). The state of crayfish in the Pacific Northwest. *Fisheries*, 36(2): 60-73. https://doi.org/10.1577/03632415.2011.10389069

Lea, S.E., Chow, P.K., Leaver, L.A. and McLaren, I.P., (2020). Behavioral flexibility: A review, a model, and some exploratory tests. *Learning & Behavior*, 48: 173-187. https://doi.org/10.3758/s13420-020-00421-w

Lefebvre, L., Reader, S.M. and Sol, D. (2004). Brains, innovations and evolution in birds and primates. *Brain, Behavior and Evolution*, 63, 233-246. https://doi.org/10.1159/000076784

Lenth, R.V. (2016) Least-Squares Means: The R Package lsmeans. *Journal of Statistical Software*, 69(1), 1-33. doi:10.18637/jss.v069.i01

Levri, E.P., Luft, R. and Xiaosong, L. (2019). Predator detection and a possible dispersal behavior of the invasive New Zealand mud snail, *Potamopyrgus antipodarum* (Gray, 1843). *Aquatic Invasions*, 14(3): 417-432.

Lisle, T.E., and Church, M. (2002). Sediment transport-storage relations for degrading, gravel bed channels. *Water Resources Research*, 38(11): 1219. https://doi.org/10.1029/2001WR001086 .

Magurran, A.E., Seghers, B.H., Carvalho, G.R. and Shaw, P.W. (1992). Behavioral consequences of an artificial introduction of guppies (*Poecilia reticulata*) in N. Trinidad: evidence for the evolution of anti-predator behavior in the wild. *Proceedings of the Royal Society B*, 248(1322): 177-122. https://doi.org/10.1098/rspb.1992.0050

Marston, R.A., Girel, J., Pautou, G., Piegay, H., Bravard, J.P. and Arneson, C. (1995). Channel metamorphosis, floodplain disturbance and vegetation development, Ain River, France. *Geomorphology*, 13: 121–132. https://doi.org/10.1016/0169-555X(95)00066-E

Martin, L.B. and Fitzgerald, L., 2005. A taste for novelty in invading house sparrows, Passer domesticus. *Behavioral Ecology*, *16*(4), pp.702-707.

Mason, R. J. and Sanders, H. (2021). Invertebrate zoogeomorphology: A review and conceptual framework for rivers. *WIREs Water*, 8(5): e1540. https://doi.org/10.1002/wat2.1540

Mathers, K. L., White, J. C., Guareschi, S., Hill, M. J., Heino, J., & Chadd, R. (2020). Invasive crayfish alter the long-term functional biodiversity of lotic macroinvertebrate communities. *Functional Ecology*, *34*(11), 2350-2361.

Mery, F. and Burns, J.G., 2010. Behavioral plasticity: an interaction between evolution and experience. *Evolutionary Ecology*, *24*(3), pp.571-583.

Messager, M.L. and Olden, J.D. (2019). Phenotypic variability of rusty crayfish (*Faxonius rusticus*) at the leading edge of its riverine invasion. *Freshwater Biology*, *64*(6): 1196-1209. https://doi.org/10.1111/fwb.13295

Meysman, F. J., Middelburg, J. J., & Heip, C. H. (2006). Bioturbation: a fresh look at Darwin's last idea. *Trends in Ecology & Evolution*, *21*(12), 688-695.

Montana Field Guide (2019). Signal Crayfish — *Pacifastacus leniusculus*. Available at: http://fieldguide.mt.gov/. [Accessed 29th May 2021].

Mowery, M.A., Vink, C., Mason, A.C. and Andrade, M.C. (2021). Behavioral, morphological, and life history shifts during invasive spread. *Biological Invasions*, 23: 3497-3511. https://doi.org/10.1007/s10530-021-02593-6

Pavlov, D.S., Mikheev, V.N. and Dgebuadze, Y.Y. (2006) Behavioral aspects of biological invasions of alien fish species. *Journal of Ichthyology*, 46: S117-S124.

Peay, S. and Rogers, D. (1999). The peristaltic spread of signal crayfish (*Pacifastacus leniusculus*) in the River Wharfe, Yorkshire, England. *Freshwater Crayfish*, 12: 665-676.

Petrusek, A., Filipová, L., Kozubíková-Balcarová, E. and Grandjean, F. (2017) High genetic variation of invasive signal crayfish in Europe reflects multiple introductions and secondary translocations. *Freshwater Science*, *36*(4), 838-850.

Philips, J.D. (2009) Biological energy in landscape evolution. *American Journal of Science*, 309(3-4): 79-85. https://doi.org/10.1016/j.geomorph.2009.01.007

Phillips, B.L., Brown, G.P., Webb, J.K. and Shine, R. (2006). Invasion and the evolution of speed in toads. *Nature*, *439*(7078): 803-803.

Pintor, L.M. and Sih, A. (2009). Differences in growth and foraging behavior of native and introduced populations of an invasive crayfish. *Biological Invasions*, *11*(8): 1895-1902.

Ramalho, R. O. and Anastacio, P. M. (2011). Crayfish learning abilities: how does familiarization period affect the capture rate of a new prey item?. *Ecological Research*, *26*(1): 53-58. https://doi.org/10.1007/s11284-010-0754-7

Ranta, E. and Lindstrom, K. (1993) Body size and shelter possession in mature signal crayfish, *Pacifastacus leniusculus*. *Annales Zoologici Fennici*, 30, 125-132.

Reader, S. M. and Laland, K. N. (Eds.) (2003). *Animal Innovation* (Vol. 10). Oxford: Oxford University Press.

Rebrina, F., Skejo, J., Lucić, A. and 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*(1): 41-50. http://dx.doi.org/10.3391/ai.2015.10.1.04

Reznick, D.N. and Ghalambor, C.K. (2001). The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. *Genetica*, 112: 183-198. https://doi.org/10.1007/978-94-010-0585-2_12

Rice, S.P., Johnson, M.F. and Reid, I. (2012). Animals and the Geomorphology of Gravel-bed Rivers. In: M. Church, P. Biron and A. Roy (eds.) Gravel Bed Rivers: Tools, Processes, Environments. Wiley-Blackwell. pp. 49-62. https://doi.org/10.1002/9781119952497.ch19

Rice, S.P., Johnson, M.F., Mathers, K., Reeds, J. and Extence, C. (2016). The importance of biotic entrainment for base flow fluvial sediment transport. *Journal of*

Geophysical Research: Earth Surface, 21(5): 890-906. https://doi.org/10.1002/2015JF003726

Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J., With, K.A., Syndallas, B., Cabin, R.J., Cohen, J.E., Ellstrand, N.C., McCauley, D.E., O'Neil, P., Parker, I.M., Thompson, J.N. and Weller, S.G. (2001). The population biology of invasive species. *Annual Review of Ecology and Systematics*, *32*(1): 305-332. https://doi.org/10.1146/annurev.ecolsys.32.081501.114037

Sanders, H. (2020). Biotic and abiotic controls of burrowing by signal crayfish (*Pacifastacus leniusculus*) and the implications for sediment recruitment to rivers. PhD Thesis, Loughborough University.

Sanders, H., & Mills, D. N. (2022). Predation preference of signal crayfish (Pacifastacus leniusculus) on native and invasive bivalve species. *River Research and Applications*, 38(8), 1469-1480.

Sanders, H., Mason, R.J., Mills, D.N. and Rice, S.P., 2022. Stabilization of fluvial bed sediments by invasive quagga mussels (Dreissena bugensis). *Earth Surface Processes and Landforms*, doi.org/10.1002/esp.5455.

Sanders, H., Rice, S.P. and Wood, P.J. (2021). Signal crayfish burrowing, bank retreat and sediment supply to rivers: A biophysical sediment budget. *Earth Surface Processes and Landforms*, *46*(4), 837-852. https://doi.org/10.1002/esp.5070

Sidorchuk, A.Y. and Golosov, V.N. (2003). Erosion and sedimentation on the Russian Plain, II: the history of erosion and sedimentation during the period of intensive agriculture. *Hydrological Processes*, 17: 3347-3358. https://doi.org/10.1002/hyp.1391

Sol, D. and Lefebvre, L. (2000). Behavioral flexibility predicts invasion success in birds introduced to New Zealand. *Oikos*, *90*(3), 599-605. https://doi.org/10.1034/j.1600-0706.2000.900317.x

Sol, D. and Weis, J. S. (2019). Highlights and Insights from" Biological Invasions and Animal Behavior". *Aquatic Invasions*, *14*(3): 551-565. https://doi.org/10.3391/ai.2019.14.3.12

Sol, D., Timmermans, S. and Lefebvre, L. (2002). Behavioral flexibility and invasion success in birds. *Animal Behavior*, *63*(3): 495-502. https://doi.org/10.1006/anbe.2001.1953

IBM Corp. (2020). IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp

Stanton, J.A. (2004). Burrowing behavior and movement of the signal crayfish Pacifastacus leniusculus (Dana). Published PhD Thesis, Department of Biology, University of Leicester.

Statzner, B. (2012). Geomorphic implications of engineering bed sediments by lotic animals. *Geomorphology*, 157-158: 49-65. https://doi.org/10.1016/j.geomorph.2011.03.022

Statzner, B. and Peltret, O. (2006). Assessing potential abiotic and biotic complications of crayfish-induced gravel transport in experimental streams. Geomorphology, 74(1-4): 245-256. https://doi.org/10.1016/j.geomorph.2005.08.007

Suarez, A.V. and Cassey, P. (2016). Introduction. In J.S. Weis and S. Sol (Eds.) Biological Invasions and Animal Behavior. Cambridge University Press. p 1-4.

van Schaik, C., Graber, S., Schuppli, C. and Burkart, J. (2017). The ecology of social learning in animals and its link with intelligence. The Spanish Journal of Psychology, 19(e99): 1-12. doi:10.1017/sjp.2016.100

Vila, M., Basnou, C., Pysek, P., Josefsson, M., Genovesi, P., Gollasch, S., Nentwig, W., Olenin, S., Roques, A., Roy, D., Hulme, P.E. and DAISIE partners (2010) How well do we understand the impacts of alien species on ecosystem services? A pan-European, cross-taxa assessment. *Frontiers in Ecology and the Environment*, 8(3), 135–144.

Viles, H.A. (1988) Biogeomorphology. Blackwell: Oxford.

Weis, J.S. (2010). The role of behavior in the success of invasive crustaceans. *Marine and Freshwater Behavior and Physiology*, 43: 83-98. https://doi.org/10.1080/10236244.2010.480838

Wentworth, C.K., (1922). A scale of grade and class terms for clastic sediments. *The Journal of Geology*, *30*(5): pp.377-392.

Wright, J.P. and Jones, C.G. (2006). The concept of organisms as ecosystem engineers ten years on: Progress, limitations and challenges. *BioScience*, 56(3): 203-209. https://doi.org/10.1641/0006-3568(2006)056[0203:TCOOAE]2.0.CO;2

Wright, T. F., Eberhard, J. R., Hobson, E. A., Avery, M. L. Russello, M. A. (2010) Behavioral flexibility and species invasions: the adaptive flexibility hypothesis. *Ethology Ecology and Evolution,* 22(4): 393-404. https://doi.org/10.1080/03949370.2010.505580