OVERLAND MOVEMENT AND ACTIVITY OF THE ENDANGERED WHITE-CLAWED CRAYFISH *AUSTROPOTAMOBIUS PALLIPES*.

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Abstract

The UK's only native crayfish, the white-clawed crayfish *Austropotamobius pallipes,* is endangered and in decline across its range in Europe. The principle threat derives from the introduction, accidental and deliberate, of invasive crayfish species, which lead to losses of native crayfish through transmission of crayfish plague *Aphanomyces astaci* and competitive exclusion. Conservation programmes for white-clawed crayfish include captive breeding and conservation translocations.

Crayfish are able to move terrestrially and this thesis examined the potential of white-clawed crayfish to make terrestrial movements within an experimental arena. Results were compared with experiments on two UK invasive crayfish species, the signal crayfish *Pacifastacus leniusculus* and the red swamp crayfish *Procambarus clarkii*. White-clawed crayfish proved surprisingly willing to move onto a terrestrial environment, with 65% of animals tested making an exit from the water. Fewer signal crayfish and red swamp crayfish exited, but those that did, exited more frequently and spent longer in the terrestrial environment than white-clawed crayfish. An immediate and important outcome of these findings is to review captive enclosure design.

Many more white-clawed crayfish made exits onto the terrestrial bridge when tested singly, than when in groups of four. This suggests a negative density-dependence in which crayfish are assessing habitat quality through the presence of conspecifics, or that when alone they make different behavioural decisions than when kept with conspecifics.

White-clawed crayfish were tested in groups of four at varying sex ratios to investigate changes in activity level and interactions. Three metrics were captured: number of crayfish active in a group, how active the focal animal was, and number of interactions between the focal animal and its conspecifics. Size proved significant across the metrics, with larger animals showing less activity, taking part in fewer interactions, and a large size range corresponding to less activity in a group. This demonstrates the importance of using size in models of behaviour within crayfish, even when using size-matched animals as seen here.

Activity levels within the experimental tank corresponded with sex ratios. Having a higher percentage of males corresponded with more activity in the tank and female only trials were the least active groups. This is hypothesised to be due to the more agonistic nature of male crayfish and is despite the finding that overall males were slightly less active than females. Both sexes responded to the sex ratio of the system which consisted of four experimental tanks running through the same water system. Both sexes were more active when there were more males in the system. The finding that crayfish activity levels corresponded with sex ratios of animals they were only in aquatic contact with indicates they are responding to pheromones, which is relevant to aquarists and researchers alike.

Overall this thesis found interesting differences in native and invasive species behaviours, and some thought-provoking intrinsic and extrinsic factors relating to activity levels.

Declaration

I declare that the work in this thesis was carried out in accordance with the regulations of the University of Gloucestershire and is original except where indicated by specific reference in the text. No part of the thesis has been submitted as part of any other academic award. The thesis has not been presented to any other education institution in the United Kingdom or overseas.

Any views expressed in the thesis are those of the author and in no way represent those of the University.

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1 Introduction

1.1 Conservation and behaviour

Biodiversity losses have occurred on so vast a scale that it is widely considered the planet is undergoing a ‘sixth extinction’ (Wilson, 1992; Kolbert, 2014), with a rate of extinction 1,000 times higher than the background rate (De Vos *et al.,* 2014). This and other significant alterations to the earth’s biosphere have culminated in the proposition of new era, ‘the Anthropocene’ (Crutzen, 2002).

Freshwater environments make up only approximately 0.8% of the Earth’s surface, yet support 6% of described species (Dudgeon *et al*., 2006). Biodiversity in freshwater ecosystems is disproportionally threatened in comparison with terrestrial environments (Sala *et al*., 2000) and freshwater habitats are particularly vulnerable to invasive species (Johnson *et al*., 2008). Crayfish are no exception to global diversity loss patterns, with extensive losses of indigenous crayfish and multiple invasions of non-indigenous species across the globe.

Twenty years ago, calls were made to bring the field of behavioural ecology to work with conservation biology in order to facilitate more effective conservation practices (Curio, 1996; Sutherland, 1998; Caro, 1999). However, the connection between the fields remains largely untapped (Linklater, 2004; Angeloni *et al.,* 2008) despite the paradox that with declining animal populations, the discipline of behavioural ecology is itself threatened (Caro & Sherman, 2011).

Berger-Tal *et al.,* (2011) propose a model to identify links where the fields can work together (Figure 1-1), with three main areas where behavioural ecology can support conservation: anthropogenic impacts on animal behaviour, behavioural indicators and behaviour based management. The model also identifies aspects of behavioural ecology that are of particular import to conservation: movement and spatial use, foraging and vigilance, and social organisation and reproductive behaviour.

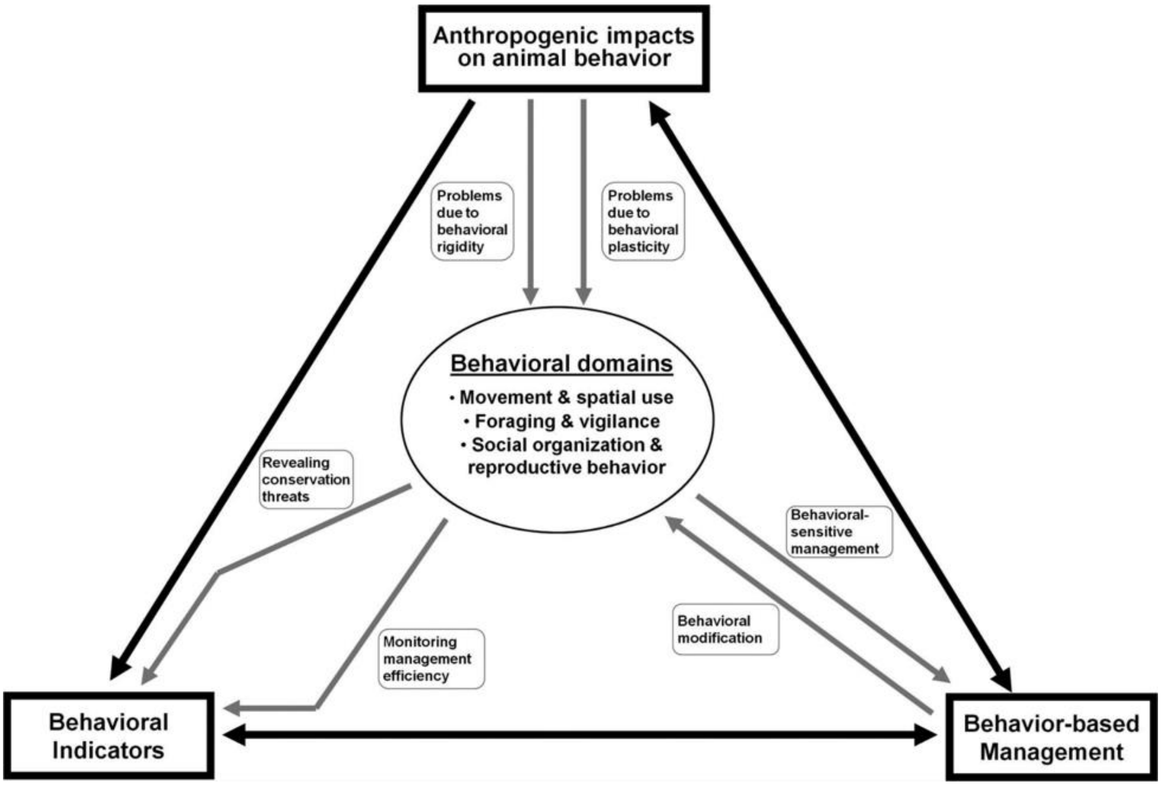

Figure 1-1. "The conservation behaviour framework" (Berger-Tal *et al*., 2011)

This thesis aims to investigate movement decisions of crayfish with a view to informing in situ and ex situ management of the endangered white-clawed crayfish *Austropotamobius pallipes* (Lereboullet, 1858). Referring to Berger-Tal *et al.,* (2011)'s model, the research position is within the behavioural domains of movement and spatial use, with some reference to social organisation through experimental manipulation of sex ratios. This in turn can inform Behaviour-based Management and could provide some useful Behavioural Indicators for conservation practitioners.

1.2 White-clawed crayfish

The white-clawed crayfish *A. pallipes* is the UK's only native crayfish and has suffered local extinctions across much of the UK, with a range status considered "Unfavourable-Bad and Deteriorating" (Joint Nature Conservation Committee, 2007). Eight Special Areas of Conservation (SACs) have been designated in the UK for the presence of *A.*

pallipes under the European Habitats Directive, as the UK population represents a significant proportion of the European Union resource (JNCC, 2018). White-clawed crayfish *A. pallipes* have been a protected species in the UK since 1981 under Provision 9, Schedule 5 of the Wildlife and Countryside Act 1981, meaning it is an offence to take, kill, hold or sell animals. They are listed as globally Endangered in the IUCN Red List (Füreder *et al.,* 2010).

A. pallipes are considered indigenous to the UK (Holdich *et al*., 2009), following the IUCN definition that species that colonize a country without anthropogenic help, or that were introduced prior to 1800AD, can be assessed as indigenous (IUCN, 2012). Kouba *et al.,* (2014) take an alternate view in their review of the distribution of crayfish in Europe, finding that the evidence for human-mediated transport is more compelling than a natural colonisation, and that *A. pallipes* are an introduced species in the UK.

1.2.1 Crayfish taxonomy

A. pallipes are under threat from invasive crayfish species (See 1.3.1 Impacts of invasive species), and species comparisons can provide both interesting and useful results. The evolutionary divergence of crayfish families provides important information about relatedness between *A. pallipes* and invasive species.

Crayfish belong to the order Decapoda, which also includes marine species such as lobsters and shrimps. Crayfish form the infraorder Astacidea (Latreille 1802), further diverging into two superfamilies, the Astacoidea and the Parastacoidea (Souty-Grosset & Fetzner Jr, 2016) (Figure 1-2).

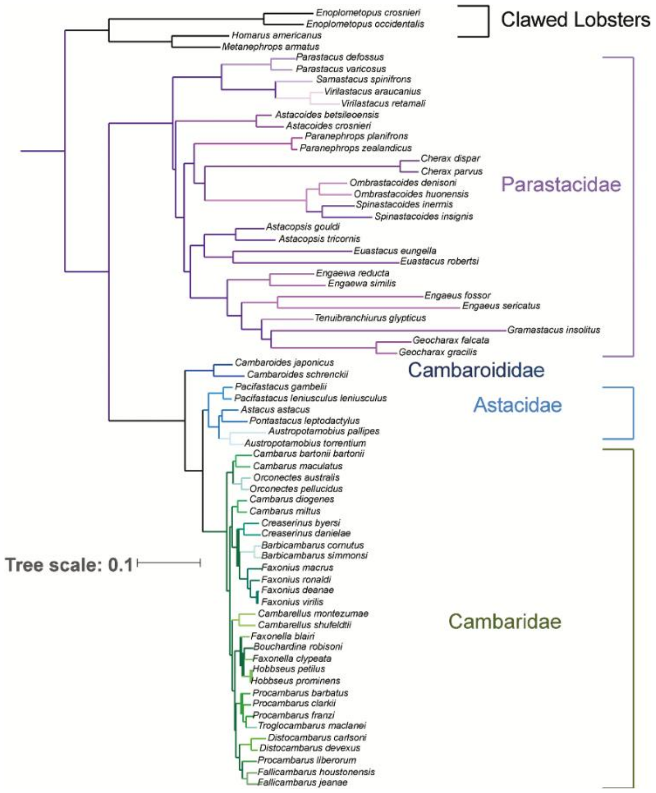

Figure 1-2 Classification of freshwater crayfishes (Crandall & De Grave, 2017)

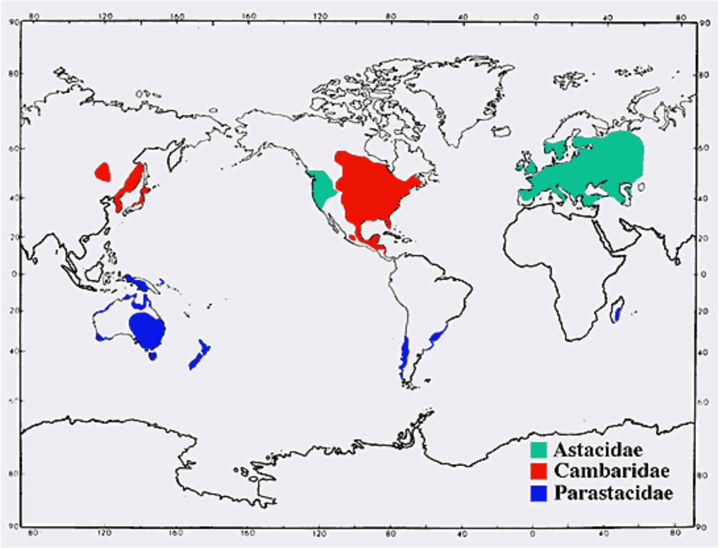

Figure 1-3 Global distribution of freshwater crayfish (Crandall & Fetzner Jr, 2010)

This thesis uses behavioural data from three species of crayfish, principally *A. pallipes* but also the American signal crayfish *Pacifastacus leniusculus* (Dana, 1852) and the red swamp crayfish *Procambarus clarkii* (Girard, 1852).

The white-clawed crayfish *A. pallipes* often refers to a species complex, as sub-species taxonomy is still subject to some debate. This species ranges from Scotland in the North to Spain in the South and is postulated to have a diversity centre in Italy (Fratini *et al.,* 2005). The UK population has low levels of morphological and genetic diversity (Grandjean & Souty-Grosset, 2000). It may eventually be described as belonging to a subspecies *A. pallipes pallipes* but for clarity, this thesis will continue to refer to the UK population of white-clawed crayfish as *A. pallipes*.

The American signal crayfish *P. leniusculus* has been introduced into the UK and across Europe, and it is likely that the invasive populations descend from *P. leniusculus leniusculus*, one of three subspecies present in their home range of eastern North America. In an analysis of cytochrome C oxidase subunit I (COI) gene fragments from *P. leniusculus* from 17 European countries, Filipová, (2012) found only haplotypes related to *P. leniusculus leniusculus.* This thesis will continue to use *P. leniusculus* for clarity.

Red swamp crayfish *P. clarkii* originate from northern Mexico, and southern and south-eastern United States. They have been introduced around the world, and much interest has been given to their invasion routes and history (e.g. Souty-Grosset, 2016); however, there is low genetic variation within both native and invasive stocks and no suggestion of subspecies within this taxon (Souty-Grosset, 2016).

1.3 Crayfish conservation

1.3.1 Impacts of invasive species

Non-indigenous Crayfish Species (NICS) are having a negative effect on indigenous species, and many fit all definitions, criteria and impacts of invasive species biology.

As seen in Table 1-1, seven NICS have been recorded in the wild in the UK. According to the 'tens rule' (Williamson, 1996) only one in ten introduced non-native species is likely to overcome the first invasion barrier and become widespread and self-sustaining. In Europe, of ten known introduced crayfish species, nine have achieved this level (Holdich, Reynolds, *et al.,* 2009) indicating that crayfish have exceptionally high invasion potential. The 'tens rule' also goes on to state that of those non-natives that can settle and spread, only one in ten will become problematic enough to warrant the term 'invasive'.

Of the 26 animals on the EU list of invasive species of Union concern, EU Regulation 1143/2014 (EU, 2014), five are crayfish, demonstrating their high disposition to successfully invade an ecosystem and have significant ecological and economic impacts as defined by European law. In the UK, the most widespread and invasive crayfish is the American signal crayfish *P. leniusculus.*

P. leniusculus as an invasive species is having irrefutable impacts on the aquatic ecosystem in the UK, through trophic effects and burrowing behaviour in addition to the displacement of the native white-clawed crayfish *A. pallipes*. Using Lockwood *et al.,* (2007)'s review of invasion ecology we can describe impacts of invasive species within six categories: genetic impacts, individual impacts, population impacts, community impacts, ecosystem impacts and landscape/global impacts.

Genetic impacts such as hybridisation are not currently seen within the UK crayfish invasion picture. In Italian mountain streams, a close relative of *A. pallipes, A. italicus,* has not hybridized when the two are naturally co-occurring in Italy (Santucci *et al.,* 1997), although the possibility remains that newly introduced crayfish species can hybridize with indigenous species. In North America, hybridisation between introduced and indigenous *Orconectes* species is one factor in the decline of the native (Perry *et al.,* 2001).

Table 1-1: Non-indigenous Crayfish Species (NICS) identified as present in the UK

Species	Common Name	Distribution	Level of establishment	Reference
Austropotamobius pallipes (Lereboullet, 1858)	White-clawed (or Atlantic stream) crayfish	Across England, Wales, Ireland. Absent from most of Scotland, West Wales and Cornwall	Indigenous but receding	(Holdich, Palmer, *et al.,* 2009)
Pacifastacus leniusculus (Dana, 1852)	Signal crayfish	Across England, Scotland, Wales. Not Ireland	Expanding range	(Holdich *et al.,* 2014)
Procambarus clarkii (Girard, 1852)	Red swamp crayfish	Two sites in London	Evidence of some expansion	(Ellis *et al.,* 2012)
Orconectes limosus (Rafinesque, 1817)	Spiny-cheek crayfish	Several locations	Evidence of expansion	(Aldridge, 2016a)
Astacus astacus (Linnaeus, 1758)	Noble crayfish	One known location	Range not thought to be expanding	(Aldridge, 2016b)
Orconectes virilis (Hagen, 1870)	Virile crayfish	Two locations	Evidence of expansion	(Ahern *et al.,* 2008)
Astacus leptodactylus (Eschscholtz, 1823)	Narrow clawed crayfish	Widespread	Likely deteriorating due to plague	2011) (Almeida *et al.,* 2014)
Procambarus cf acutus (Girard, 1852)	White river crayfish	One location	High potential to expand	(Stebbing, 2015)

A potentially new crayfish species, the marbled crayfish *P. fallax f. virginalis* (Martin *et al.,* 2010) is a parthogenic species, possibly a mutation occurring in captivity that is most closely related to the Georgia crayfish *Procambarus fallax* (Scholtz *et al.,* 2003). The species started to be detected living wild, almost certainly as a result of release/escape from the aquaria trade, for example in Italy (Marzano *et al.,* 2009). To date, this species has not been detected in the UK, but its ability to reproduce from one female alone increases its capability as an invasive species and it has been risk assessed as high risk (Holdich, 2011). It is also a carrier of *Aphanomyces astaci* and hence could displace native species through disease transfer alone.

Individual impacts on the behaviour or morphology of individuals within a species from introduced species can be seen in crayfish. Crayfish can be plastic to environmental conditions, for example increasing carapace size within four months when moved from a lotic to a lentic site, perhaps to maximise oxygen uptake in the less oxygenated environment (Haddaway *et al*., 2012). *Orconectes virilis* which had been exposed to the invasive *Orconectes rusticus* for more than 30 years were more aggressive to *O. rusticus* and maintained higher growth rates in the presence of *O. rusticus* than naïve *O. virilise* (Hayes *et al.,* 2009). These could be a result of evolutionary pressures or plasticity within individuals, but nonetheless shows that invasive species can impact upon indigenous species in subtle ways. *A. pallipes* have been found to be smaller in mixed populations with *P. leniusculus,* likely due to competitive exclusion from refugia causing increased predation of larger individuals (Dunn *et al.,* 2009). This phenomenon could be used as an indicator stage in the extirpation of native populations by conservation managers. Individual level impacts can then multiply up to population level effects.

Population level impacts on native species can be quite subtle in early invasion stages, but multiply up to cause measurable population declines. Eventually, competition with and predation from *P. leniusculus*, alongside the transmission of the lethal plague pathogen *A. astaci,* have profound population impacts on *A. pallipes,* leading to extinction within three to seven years in all documented cases in the UK (Holdich, 2003a; Bubb *et al*., 2005, 2006a).

Community level impacts of *P. leniusculus* have been well documented, as through their faster growth and reproductive rate (Hiley, 2003; Nakata *et al.,* 2004) they consume considerably more than the native *A. pallipes*. Moorhouse *et al.,* (2014) removed *P. leniusculus* from two tributaries of the River Thames and found that lowered crayfish densities correlated with increased macroinvertebrate numbers and taxon richness. Nystrom (1999) described similar negative effects of *P. leniusculus* on macrophyte biomass and richness. Mathers *et al.,* (2016) describe the effect of *P. leniusculus* on the community as persistent with no indication of recovery.

Ecosystem impacts of *P. leniusculus* can derive from their voracious feeding behaviour and burrowing activities; they may form high densities of burrows which can cause bank collapse (Guan, 1994). However, the presence of high densities of prey items can prove a positive ecosystem impact; Tablado *et al.,* (2010) reported the beneficial effects of the introduced *P. clarkii* on predator assemblages in Spanish wetlands, including threatened species. The European otter *Lutra lutra* readily eats the invasive *P. clarkii* in Spain (Barrientos *et al.,* 2014). When crayfish density is high, crayfish form a large percentage of the diet of *L. lutra* populations in the UK (Howells & Slater, 2004), and *L. lutra* readily switch to the invasive *P. leniusculus* (Almeida *et al.,* 2012).

However, the extirpation of native species from watersheds across regions, such as is being seen with crayfish in Europe, is a good example of global impacts and global homogenisation (Clavel *et al.,* 2011), where a few species are ‘winners’ and most are ‘losers’ (McKinney & Lockwood, 1999).

Despite the clear evidence of the negative impact of NICS, invasive species impacts should not always be assumed to be automatically negative and a bias in invasive species ecology towards this view is apparent in the literature (for review see Goodenough, 2010). In Sweden and Spain, there are large economic benefits derived from non-invasive species. The Swedish annual catch of *P. leniusculus* wholesale price is estimated at 30 – 40 million Euros (Bohman & Edsman, 2011). In parts of Africa, introduced crayfish have been used to reduce populations of schistosome-carrying snails (Hofkin *et al.,* 1991).

Clarity on defining impacts of invasive species is being sought (Blackburn *et al.,* 2014; Jeschke *et al.,* 2014; Kumschick *et al.,* 2015). The IUCN invasive species specialist group produces a database of invasive species and many species have been assessed under a local impact mechanism which uses 13 impact types: competition, predation, hybridisation, disease transmission, parasitism, poisoning/toxicity, bio-fouling, grazing/herbivory/browsing, rooting/digging, trampling, flammability, interaction with other invasive species and 'other' (ISSG, 2018a). *P. leniusculus* has not been locally assessed, but *P. clarkii* has been assessed as having a negative impact in Europe in the following areas: competition, disease transmission, ecosystem change, habitat alteration, herbivory and predation (ISSG, 2018b).

1.3.2 Crayfish plague

The principle threat to native crayfish in the UK is the American signal crayfish *P. leniusculus,* which outcompetes and predates upon *A. pallipes* (Holdich *et al.,* 1995), and spreads crayfish plague *Aphanomyces astaci* (Schikora, 1903). Evidence suggests within three to four years of *P. leniusculus* reaching a watercourse with a population of *A. pallipes*, the native *A. pallipes* will become locally extinct through a combination of competition and transmission of *A. astaci* that is lethal to *A. pallipes* (Holdich, 2003; Bubb *et al.,* 2006). North American crayfish species are the primary NICS, have evolved alongside the plague, have varied resistance and can act as hosts.

Although the primary route of transmission of *A. astaci* is through NICS, *A. astaci* can be transferred between waterbodies in their absence, as spores and cysts of the oocyte can move on fomites such as fishing gear and boats. Records of *A. astaci* in Ireland, in the absence of introduced crayfish species, demonstrate that *A. pallipes* suffer catastrophic declines in the presence of the plague alone (Reynolds, 1997). Where host NICS are absent, plague spores eventually die out and restocking of native species can be successful (Reynolds, 1997; Vrålstad *et al.,* 2011). *A. astaci* persists as either mycelia in the body of the host animal or zoospores or cysts when transmitted. In water temperatures of 0-10°C cysts and zoospores can survive at least 14 days, possibly longer as the spores can enter and re-enter a cyst state according to conditions (Oidtmann *et al.,* 2002). Persistence of virile spores in the undigested carapace of crayfish that have passed through fish digestive systems indicates another possible mode of dispersal of the disease (Oidtmann *et al.,* 2002).

In fresh water, *A. astaci* has motile zoospores that are able to locate new host crayfish through weak chemotaxis (Oidtmann *et al.,* 2002). The spores attach to the cuticle and infect the animal. Plague resistance in NICS derives from the ability of these animals to restrict the infection to the cuticle, whereas European native species succumb to the infection throughout their tissues (Svoboda *et al.,* 2016).

1.3.3 Competitive exclusion

Even without transmission of *A. astaci*, indigenous crayfish such as *A. pallipes* in the UK are outcompeted and predated upon by NICS. In the UK, seven invasive crayfish species are recorded as present in watercourses (Table 1-1). Of these, *P. leniusculus* is the most widespread; this species was originally imported for aquaculture as a food item. The import, sale and keeping of crayfish as farmed animals or pets is now restricted in the UK, as understanding of their potential invasive capacity has grown. Nevertheless, the aquarium trade in this species continues and poses a high risk of escape or deliberate release.

By 2010 there were more 10-km squares of potential habitat occupied by invasive species (primarily *P. leniusculus*) than the native *A. pallipes* (JNCC, 2010). In a temporarily co-occurring population, Bubb *et al.,* (2006), found that *P. leniusculus* had a very strong overlap in habitat (85.4%) and refuge preference (79.7%) supporting the competitive exclusion principle. Further evidence of competitive exclusion comes from Dunn *et al.,* (2009)'s study which found that in mixed populations of *A. pallipes* and *P. leniusculus*, *A. pallipes* were smaller than when in single species populations, which they hypothesized was due to exclusion of *A. pallipes* from refuges, causing greater predation of larger individuals.

Many studies have experimentally tested encounter outcomes between NICS and indigenous species (for a review see Gherardi & Cioni, 2004) and find that NICS initiate and win more aggressive encounters, and in some cases predate upon the indigenous species (Holdich *et al.,* 2004).

In addition to competing over habitat, refuge and direct agonism, *P. leniusculus* have life history traits that mean displacement of the indigenous *A. pallipes* is likely. *P.*

leniusculus have faster growth rates than *A. pallipes* (Hiley, 2003), being able to double their carapace length in their second year (Guan & Wiles, 1996). Juvenile *P. leniusculus* have a higher metabolic rate than juvenile *A. pallipes* giving them a competitive advantage in growth and dominance (Rosewarne *et al.,* 2014).

P. leniusculus exist at higher densities than *A. pallipes* (20 signal crayfish m^{-2} vs 2.9 white-clawed crayfish m^{-2}, (Hiley, 2003). Both *P. leniusculus* and *P. clarkii* are capable of producing more eggs than *A. pallipes* - 100-470 (Nakata *et al.,* 2004), 140-595 (Honan & Mitchell, 1995) and 20-165 (Honan & Mitchell, 1995) respectively, so they are able to populate habitats more rapidly. Renai & Gherardi (2004) compared predatory efficiency between *A. italicus* and the invasive *P. clarkii* and found the non-indigenous species was a more voracious predator.

P. leniusculus are thought to be more tolerant of a range of environmental conditions such as short term exposure to the pollutants ammonia and copper, and can survive and breed within a greater thermal range (Firkins, 1993).

P. leniusculus have a great capacity to disperse; through a radio-tracking study, Bubb *et al.,* (2004), found that *P. leniusculus* had potential to expand their range 1.5km/year downstream. Crayfish are able to travel in a terrestrial environment, and the degree to which different species exhibit this behaviour is linked to their native range. Species from swampy ephemeral water bodies, such as the red swamp crayfish *P. clarkii* inhabit an environment that frequently dries out, and adaptations such as burrowing and moving overland enable this species to thrive (Ramalho & Anastácio, 2015). This flexibility of habitat and movement facilitates the dispersal process of NICS; *P. leniusculus* can travel several hundreds of metres overland, and can survive up to three months away from a water body in a humid atmosphere (Hiley, 2003). This is in contrast to *A. pallipes*, which is a species associated with more constant water supplies and although reports exist of terrestrial movement (Pond, 1975, Rushbrook *et al.,* 2012) they are not known for this behaviour.

1.3.4 Other factors

NICS are widely attributed as the cause for indigenous species declines, but their impact is likely to be complicated by other factors such as habitat quality, pollution and climate change. *A. pallipes* are restricted in the UK to areas with low contamination from sewage or run-off (Haddaway *et al.,* 2015). Widespread watershed alterations have occurred across the range of *A. pallipes*, such as canalisation of rivers, dredging activities, and poaching of bankside structure through grazing animals, which all reduce suitability of habitat to the crayfish (Naura *et al.,* 1998).

Climate change is likely to be an emerging factor in the distribution of NICS and indigenous species; Capinha *et al.,* (2013) predict a 19% decrease in suitable habitat for native species based on climate modelling, contrasting with a likely increase in overlap with NICS. Crayfish show an increased activity and dispersal at higher temperatures (Bubb et al., 2004), but there is likely an upper limit of temperature tolerance at which activity reduces, for *A. pallipes* this was found to be over 20 degrees Celsius (Barbaresi & Gherardi, 2001). Invasive species more suited to warmer climates such as red swamp crayfish *Procambarus clarkii* are likely to further dominate invaded temperate climates such as the UK (Gherardi et al., 2013)

1.4 Crayfish Behaviour

1.4.1 General crayfish behavioural traits

Crayfish are polytrophic feeders, occupying different trophic roles within an ecosystem both within and between different life stages and species. Diet varies and includes: plant and animal detritus, snails, benthic macroinvertebrates, macrophytes, small invertebrates (including other crayfish), fish and fish eggs (Jurcak *et al.,* 2016). Crayfish are suggested to undergo an ontogenetic shift from active predation towards opportunistic carnivory and/or herbivory, possibly due to a reduced mobility of chelae with increased size (Reynolds & O’Keeffe, 2005). Crayfish have limited capacity for active predation of mobile prey but successfully employ a ‘sit-and-wait’ strategy which enables predation of amphibian larvae, fry and mobile invertebrates (Renai & Gherardi, 2004). In lentic waterbodies, where conditions are more stable; allowing faster growth in the crayfish and allowing more stable plant and detritus communities to form, a more herbivorous diet may occur. This is in contrast to lotic environments, where carnivory may remain more important as a food source (Reynolds & O’Keeffe, 2005).

Activity periods vary (Davis & Huber, 2007), although largely the group are more active at night and in summer/autumn (Benvenuto *et al*., 2008; Wutz & Geist, 2013a; Englund & Krupa, 2000).

They escape predation through reducing activity, microhabitat use, refuges and burrowing (Gherardi *et al.,* 2001; Benvenuto *et al*., 2008; Holdich, 2003a; Kouba *et al.,* 2016). See Table 1-2.

Table 1-2 General crayfish behavioural traits research

Behaviour	Detail	Reference
Feeding	Investigated *A. pallipes* gut content and food item preference and found that detritus and plant material, especially mosses, were important.	Gherardi *et al.,* 2004
	Crayfish actively selected for finer substrate microhabitats when active as this may provide more food items during foraging trips	Clavero *et al.,* 2009
Anti-predator behaviour	Change in microhabitat use is seen, smaller crayfish using shallow habitat and larger crayfish using deeper water. Fish predation more of a risk to smaller crayfish, terrestrial avian and mammalian predation more of a risk to larger crayfish.	Benvenuto *et al*., 2008; Wutz & Geist, 2013a; Englund & Krupa, 2000
	A. pallipes restricted foraging trips to under one hour as this is when they are most vulnerable.	Gherardi *et al.,* 2001
Burrowing	*A. pallipes* may burrow into suitable substrate, particularly during winter when activity levels drop, but primarily use existing refuges.	Holdich, 2003a
	Indigenous species far less able to create burrows. Most successful burrowing crayfish was *P. clarkii,* which derive	Kouba *et al.,* 2016

	from a marshy habitat, and are able to dig and plug vertical burrows.	
Activity	*A. pallipes* described as largely nocturnal	Holdich, 2003a
	Small rusty crayfish *Orconectes rusticus* were active throughout day and dusk, in shallower parts of the river; medium and large crayfish were more strictly nocturnal, and inhabited deeper parts of the river.	Davis & Huber, 2007

1.4.2 Agonism

Crayfish often exist at high densities (e.g. *A. pallipes* 5m^{-2} (Latham *et al.,* 2016)) and compete for resources with conspecifics and other crayfish species. Food may be pirated (Pintor & Sih, 2009) and patches of detritus may be defended with agonistic behaviour (Bergman & Moore, 2003). Males and females both go through reproductive cycling and are more aggressive and more likely to win fights when in reproductive condition (Martin III & Moore, 2010). Male *A. pallipes* were found to kill females when attempting to mate if there was a large size difference, and can cause females to lose eggs through aggressive encounters, although this was in experimental conditions it reflects the agonistic nature of the species (Woodlock & Reynolds, 1988a).

The resource most commonly contested is the refuge shelter; fight intensity increases in the presence of shelters (Bergman & Moore, 2003) and crayfish fought more intensively if they have previously occupied a shelter than if they had not (Tricarico & Gherardi, 2010). Dunn *et al.,* (2009) propose that one mechanism for the replacement in Britain of *A. pallipes* with *P. leniusculus* is through the eviction of the smaller *A. pallipes* from refuges by the larger *P. leniusculus*, exposing the former to increased predation risk. However, dominant crayfish were found less in shelter than subordinates in a study by Fero *et al.,* (2007) which they attribute to the dominant animal having to maintain position in a hierarchy.

Crayfish are known to avoid excessive agonistic encounters through the maintenance of dominance hierarchies (Herberholz *et al.,* 2001; Fero & Moore, 2008). Tricarico *et al.,* (2005) found that these hierarchies used “winner-loser” effects and status recognition to maintain hierarchies, rather than individual recognition in *A. italicus*. They postulate this is due to the ephemeral territoriality of this species; crayfish were not regularly encountering the same individuals but were able to position themselves correctly within a hierarchy in order to reduce fighting costs and risks. These responses can be different within a species: *P. clarkii* dominant females preferred animals they had met, dominant males showed no preference and subordinate animals of both sexes preferred unfamiliar conspecifics (Tierney *et al.,* (2013).

An aggression syndrome is suggested by Pintor *et al.,* (2008) to explain links found between aggression, boldness and foraging voracity in *P. leniusculus.* More evidence for personality syndromes in crayfish is emerging such as Vainikka *et al.,* (2011) who found repeatability in *A. astacus* boldness as a personality trait, and Thomas, (2018) who found repeatable responses to a terrestrial dispersal option in *P. leniusculus.* Briffa *et al*., (2015) discuss the need to consider personality and behavioural syndromes as themselves plastic within-individuals to prior experience and situation.

This thesis (See chapter 4: Sex Ratio) investigates reactions of crayfish to conspecifics, specifically looking at differences in group sex ratios.

1.4.3 Mate choice

Both males and female crayfish experience reproductive costs; males produce large and limited spermatophores (Aquiloni & Gherardi, 2008) and females extrude and brood eggs, and show parental care for the hatchling crayfish. There therefore exists a mutual mate choice within crayfish. Female crayfish have been found to select for males with large body size (Gherardi *et al.,* 2006; Breithaupt *et al.,* 2016), claw size and symmetry, and dominance (Villanelli & Gherardi, 1998). There may also be flexibility within an individual; *A. italicus* females laid fewer, larger eggs when mated with small sized and large clawed males and the opposite for large sized small clawed males (Galeotti *et al.,* 2006) demonstrating post mating preferences.

Male *P. clarkii* were found to select for larger female size and virginity over mated animals (Aquiloni & Gherardi, 2008) but field observations of *A. pallipes* suggest they mate with any receptive females they encounter when they are in breeding condition (Villanelli & Gherardi, 1998).

Males compete for mating opportunities because there are fewer receptive females than males, as once a female has successfully mated, she is then unreceptive for the remainder of the breeding season. Competition through aggressive encounters is intense in *A. pallipes* during the breeding season (Woodlock & Reynolds, 1988b). In addition sperm competition occurs as male *A. pallipes* removed and ate previous males' spermatophores (Villanelli & Gherardi, 1998).

1.4.4 Crayfish perception

Crayfish use chemoreception to detect information about their environment and it is arguably their most important sense. Chemical cues and signals inform foraging behaviour, predator avoidance, dominance hierarchies and reproductive behaviour.

Crayfish do have well developed compound eyes and extra-ocular photoreceptors (Breithaupt *et al.,* 2016) but as crayfish are largely nocturnal and often are active in turbid environments, they require other sensory organs to navigate around their environment. These include external mechanoreceptors in the form of antennae and sensory hairs, which allow crayfish to detect water currents and vibrations produced by other animals (Breithaupt *et al.,* 2016).

Chemoreceptors allow more detailed social communication; to transmit and receive information on reproductive condition, dominance hierarchy status and body condition. Crayfish exposed to water from a dominant animal demonstrated increased activity and aggression in an experiment by Zulandt Schneider *et al.,* (1999). Crayfish with experimentally obstructed chemoreceptors (Bergman *et al.,* 2003) or blocked urine release (Zulandt Schneider *et al.,* 2001) showed more intense aggression and longer encounters with conspecifics.

1.5 Dispersal

One element of crayfish behaviour investigated in this thesis is the potential for dispersal. The experimental arena (see chapter 2: Methods) set up the option of a terrestrial landscape onto which the crayfish were able to emigrate.

The ideal free dispersal (IFD) (Fretwell & Lucas, 1969) rule states that if current conditions are worse than the average condition, such as an unfavourable density of conspecifics, then an animal moves. This leads to an environmentally-sensitive Evolutionarily Stable Strategy (ESS), as there is no benefit to a replicator to move into an unfavourable condition, nor to stay in one (Tregenza, 1995).

However, IFD rules change when lack of omniscience in the animal, and costs and benefits of the movement are taken into account. Animals can be assumed to be moving into areas about which they have less knowledge than their natal patch (Bowler & Benton, 2005). Some animals exhibit information gathering behaviours prior to dispersal, such as flying squirrels (Selonen & Hanski, 2006) and great tits (Dingemanse *et al.,* 2003), but the effect this has is not always straightforward. In Selonen and Hanski's research on flying squirrels, animals that dispersed greater distances were those that had exhibited less exploratory behaviour (Selonen & Hanski, 2006), whereas Dingemanse *et al* correlated exploratory behaviour with propensity to disperse and found a strong relationship (Dingemanse *et al.,* 2003), which they attributed to personality type.

Dispersing comes with a cost, which can be measured as time lost from other activities, energy costs of moving, and increased risk (of injury, mortality, predation or disease). It may also incur a theoretical cost of lost opportunities, such as through funnelling of resources into dispersal potential rather than growth and reproduction (Bonte *et al.,* 2009).

Crayfish can disperse both within aquatic environments and terrestrially. The costs to an aquatic organism of moving through a terrestrial landscape are manifold. Taylor & Wheatly (1981) found irreversible effects of desiccation on *A. pallipes* at 48 hours, and at 72 hours in air at 70% humidity at 15°C, mortality occurred. In addition, crayfish body weight is equivalent to 400-600% greater in air than in water (Pond, 1975). Larger animals are more powerful, achieving faster walking rates and resist desiccation for longer (Claussen *et al.,* 2000). In addition to energetic and physiological costs, crayfish would expect to incur increased predation risk in a terrestrial landscape; their tail flick reflex escape behaviour (Herberholz *et al.,* 2004) would not function on land, and they are more apparent to predators.

If dispersal has a cost, then so too might staying in the natal patch. A plastic dispersal strategy can allow an individual to move away from local conditions which may be unfavourable, such as overcrowding (Matthysen, 2005), or the absence of conspecifics (Roland *et al*., 2000a). Many studies have shown a positive density-dependence on emigration (e.g. Enfjäll & Leimar, 2005) or negative density-dependent dispersal (e.g. Roland *et al.,* 2000). Enfjäll & Leimar (2005) propose that density effects can act at both high and low densities in the same species, and research should acknowledge this possibility.

The functions of dispersing at low density and high density can be different. Animals may leave low density populations, or choose not to immigrate into low density populations, because this density might be the mechanism by which they judge habitat quality (Schuck-Paim & Alonso, 2001). They might need to move to higher densities to find available mates, or to find protection or hunting ability in groups. Such conspecific attraction or allee effects will select for dispersal at low densities (Matthysen, 2012).

High population densities can cause a decrease in an organism's fitness, through exploitative competition and interference competition (Bowler & Benton, 2005). Dispersal at high densities could be driven by interference effects, such as refugia displacement in crayfish (Jurcak *et al.,* 2016), agonistic spatial dominance (Fero & Moore, 2008) and mate harassment issues such as those found in grasshoppers (Bauer *et al.,* 2005).

The theory of density dependent dispersal relies on the animal having a measure of conspecific density. This could be through experience of agonistic encounters, or through the ability to count and measure. Another theory could be body condition-dependent dispersal, in which current body condition could be taken as a measure of the environment and the match of the individual's fitness to the resources. One might expect therefore that a low, or declining body condition would induce an animal to disperse and indeed this has been found in shrews (Hanski *et al.,* 1991). It is also found that animals with high body condition, and corresponding competitive advantage in new environments and ability to survive dispersal costs are those which move (Massot *et al.,* 2002), and move further (Delgado *et al.,* 2010). This has been mathematically supported by Gyllenberg *et al.,* (2008) who demonstrate that both the strongest body condition and weakest body conditions drive dispersal decisions, with the intermediate remaining resident.

State dependent dispersal is also theorised as a mechanism for inducing dispersal. Often one sex disperses while the other stays resident. In sexually dimorphic species, the larger sex may be more suited to dispersal being more able to travel distances and survive hostile matrices (Benton & Bowler, 2012, Salle *et al.,* 2007). Robinson *et al.,* (2000) tracked *A. pallipes* and recorded males moving twice as far as females in an aquatic environment. In crayfish, males have larger claw (Holdich, 2003a), which may make them less likely to move terrestrially; in experiments on *A. pallipes*, Pond (1975) found that claw were held up when walking in water, but were rested on the ground in air, therefore the larger claw of males would make terrestrial movement cumbersome.

Equally often, younger animals disperse while older animals remain, with the older animals having more to lose such as an established territory through an adaptation of the "asset protection principle" (Starrfelt & Kokko, 2012; Clark, 1994). Some invertebrates have different life-stages which are sessile or motile (Benton & Bowler, 2012) and although juvenile crayfish are morphologically similar to adults, they are nonetheless less able to resist flow and passive juvenile dispersal may be a dispersal mechanism for the species.

1.6 Research rationale

First, this thesis seeks to investigate movement behaviour of *A. pallipes* in comparison with invasive species *P. leniusculus* and *P. clarkii*, using an experimental approach. Related species can be expected to have similarities and differences in behaviour, and invasive species in particular can behave differently from both their native range and indigenous species. In improving understanding of similarities and differences between indigenous and invasive species, both theoretical and practical answers may be found for management of invasive species.

Second, this thesis seeks to measure effects of sex ratios on movement and activity in groups of *A. pallipes*. Movement and activity levels can affect fitness through inefficient energy use and increased predation risk. In improving understanding of any interactions between population demographics and movement and activity levels, theoretical and practical answers may be found for management of endangered species.

This thesis aims to deploy behavioural ecology to address conservation goals, enhancing understanding of dispersal of both invasive and indigenous species, to inform optimum conditions for captive management and release demographics.

1.7 Aims

Using an experimental microcosm, this project explores intrinsic and extrinsic factors influencing terrestrial movement and aquatic activity in *A. pallipes*.

Although primarily an aquatic species associated with freshwater environments, nonetheless crayfish species demonstrate overland terrestrial movement (Craddock, 2009, Huner & Llndqvist, 1995, Marques *et al.,* 2015). The extent of terrestrial movement is not well understood in this species and might be negligible.

Terrestrial movement in NICS is a key research area in understanding their dispersal and there is evidence that the commonly invasive crayfish species are proficient at terrestrial movement (e.g. Hiley, 2003; Marques *et al.,* 2015; Ramalho & Anastácio, 2015). Chapter Three investigates terrestrial movement in *A. pallipes* and compares this with terrestrial movement in *P. leniusculus* and *P. clarkii*.

Movement into a hostile landscape can be assumed to be deliberate and has been used to study the emigration stage of dispersal (e.g. Enfjäll & Leimar, 2005, Craddock, 2009). Emigration, interactions and activity levels can all be measured as a response to extrinsic factors, such as population demographics. Chapter Four investigates the impact of conspecifics on terrestrial movement and aquatic activity. Using experimental manipulation of sex ratios, the extent of terrestrial dispersal behaviour, and aquatic behaviour through activity levels and interactions is measured.

1.8 Objectives

- To assess tendency of white-clawed crayfish *Austropotamobius pallipes* to make terrestrial crossings
- To assess frequency and duration of terrestrial crossings of *A. pallipes*
- To compare findings of *A. pallipes* results with results from signal crayfish *Pacifastacus leniusculus* and red swamp crayfish *Procambarus clarkii*
- To assess terrestrial crossings, activity levels and interactions within mixed sex groups of *A. pallipes* with varying sex ratio

2 Methods

2.1 Chapter scope

This chapter considers the methods used in chapter three and four: the ethics, experiment design and practicalities of the studies. Research was carried out in conjunction with Bristol Zoological Society and Cardiff University as part of their white-clawed crayfish *A. pallipes* conservation and research programmes. The initial experimental arena was developed by Rhidian Thomas at Cardiff University Bioscience department (Thomas, 2018) and adapted for *Austropotamobius pallipes* trials in chapter three and multiple animal trials in chapter four. The experiment protocol was developed for this thesis.

2.2 Ethics

Crayfish are invertebrates and as such are without legal protection under the Animal Welfare Act (2006) or Animals (Scientific Procedures) Act 1986. However, proposals have been made to include decapod crustaceans to the legislation protecting animals within the invertebrate additions made for certain cephalopod species (AHAW, 2005) which has subsequently been strengthened due to evidence of pain-detection (Gherardi, 2009, Elwood, 2012) and sentience (Broom, 2007). Aspirational welfare conditions will be adhered to for this study in the absence of specific legislation.

Here I applied the Association for the Study of Animal Behaviour (ASAB) guidelines for working with live animals in research (three principle recommendations; to replace, reduce and refine use of animals)(Buchanan et al., 2006). This experiment required live crayfish, and numbers used were maximised to the facilities available to improve validity of result. The techniques have been refined through previous experiments and trials to ensure best use of animals. As Chapter four is looking at the impact of the potentially aversive stimuli of skewed sex ratios, a potentially mild aversive stimuli will be deliberately present, which therefore requires consideration of ASAB's guidelines (Buchanan *et al*., 2006).

Injuries and mortalities were reported and the captive group of *A. pallipes* was under the care of the BZG veterinary team and experienced aquarists. Many of the risks to which the animals were exposed are identical to any risks in keeping animals captive, such as con-specific aggression and environment-related injuries. This group of animals was part of a conservation breeding programme for reintroduction run by BZG. Animals were protected from risks they would have faced in the wild such as predation or displacement by the invasive species and crayfish plague.

White-clawed crayfish *A. pallipes* are a protected species in the UK under Provision 9, Schedule 5, Wildlife and Countryside Act 1981, meaning it is an offence to take, kill, hold or sell animals. Animals bred in captivity are not covered by this act (WCA 1981). Work for this thesis was carried out under Bristol Zoological Society's Natural England scientific handling license (2016-22248-SCI-SCI-2) and Natural England survey licence (2016-22074-CLS-CLS) to allow the capture of wild individuals.

This thesis has been passed by the Bristol Zoological Society's welfare and research advisory board (WRAB) and the University of Gloucestershire's Ethics Approval Board (See appendix 1).

2.3 Species comparison – *A. pallipes*

This experiment used a group of two year old captive-born white clawed crayfish *A. pallipes*, hatched from wild-caught ovigerous females collected from a local South Gloucestershire stream (exact location confidential) population and reared within aquaculture facilities in Somerset. Crayfish were chosen within a similar size range with carapace length ẋ = 34.3mm ± 2.3, (Figure 2-1); in this experiment claw size was not measured.

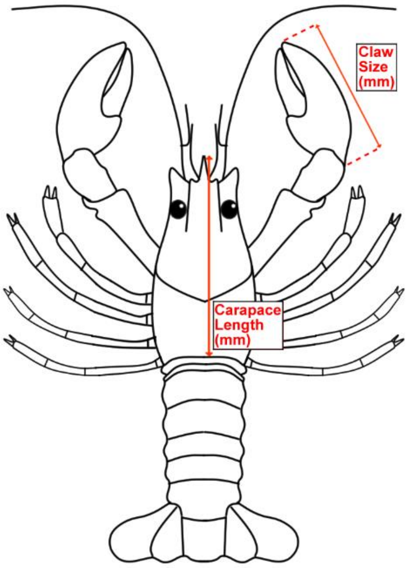

Figure 2-1: Measured morphological parameters for crayfish. Rhi Hunt, University of Cardiff.

Ten males and twelve females were used and held in single sex holding tanks at a density of 23m^{-2}. All animals were PIT tagged (8mm PIT-tags, Trovan ID100A, RFID Systems LTD) following Bubb *et al*., (2002) and Nightingale et al., (2018). In addition animals were marked with Dykem Brite-Mark permanent marker pens following Ramalho *et al*., (2010). Husbandry was undertaken by the Bristol Zoological Gardens aquarium team. Animals were fed a standardized Bristol Zoological Gardens diet (Nightingale, 2017) within holding tanks; no feeding occurred in experimental tanks.

Animals were kept within the BZG holding tanks on their main system with a maintained temperature of 15°C. The research tanks were held within an enclosed outdoor area with a relatively stable ambient temperature (average water temperature 13°C; range 10-15°C). Water was filtered and regular water replacements were undertaken to maintain water quality.

Both tank set ups were held under natural day:night cycles (12hr ± 1hr24min) (Time and Date, 2018). The trials ran between February and April 2017.

Holding tanks consisted of large black storage tubs (189l capacity, 380mmx9100mm, 0.35m²) had an excess of refugia of bricks and PVC tubes and a substrate of 30mm loose gravel. The experiment tank set up consisted of two large black storage tubs connected with a terrestrial bridge (L250cm x H20cm x W20cm) and ramps (L43cm x W29cm; 30° incline) at either end. Both ramps and bridge were wooden with fixed gravel (Figure 2-2).

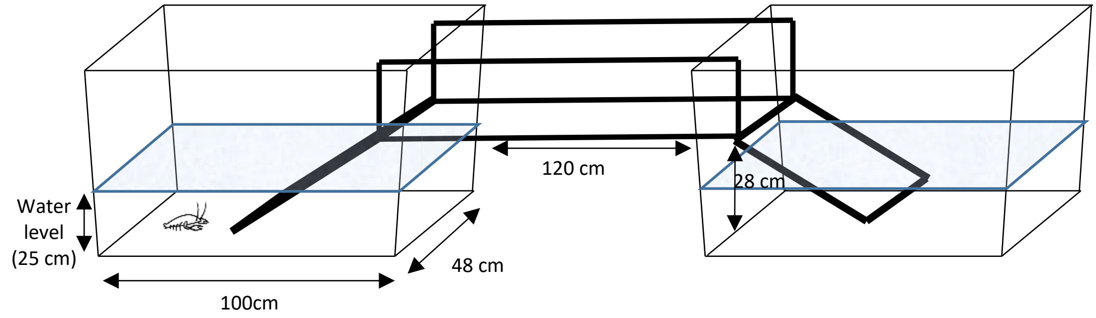

Figure 2-2: Terrestrial arena experimental set up

Individual crayfish were moved into the experiment tanks in the morning and given up to 6 hours to acclimate to the set up. At 17:00 the ramp was placed against the bridge so that the crayfish had access to the terrestrial area. Footage was watched from the point the bridge was in place to 07:00. No crayfish moved terrestrially before 19:00, and in order to compare with the Cardiff experiment, the 12 hour period 19:00-07:00 was used for analysis. The movement of crayfish and set up of the daily runs was carried out by two BZG trained individuals.

Each day the new crayfish started the run in the opposite side of the experiment tanks to the previous set up. This was in order to minimise pheromonal interaction with the previous occupant of the tanks. Males were alternated with females so that the potential effect of seasonal change through the data collection period was spread across males and females. This was considered more important than full pheromonal separation of the sexes.

Infrared CCTV cameras (Swann Pro-Series 615) were suspended above the experiment tanks and recorded to a Digital Video Recorder (Swann DVR8-1525 8 Channel 960H) to monitor crayfish behaviour in all experiments.

At the end of the experiment the crayfish were returned to the aquaculture facilities in Somerset. Five animals were reported as mortalities from the holding tanks. Experienced crayfish aquarists attributed mortality to high densities in holding tanks.

2.4 Species Comparison – *Pacifastacus leniusculus* and *Procambarus clarkii*

Invasive species data from experiments carried out by Rhidian Thomas at Cardiff University (Thomas, 2018) were used for species comparison analysis. *A. pallipes* were never held in the same institution as *P. leniusculus* and *P. clarkii* due to high risk of crayfish plague *Aphanomyces astaci* transmission.

*P. leniusculus*_were trapped from Derw Farm pond (Powys, Wales, OS: SO138375), *P.clarkii* were caught at the Bird Sanctuary pond (Hampstead Heath, London). Both species were caught using cylindrical crayfish traps ('Trappy Traps', Collins Nets Ltd., Dorset, UK) baited with cat food overnight. *P. leniusculus* and *P. clarkii* were transported to the Cardiff University aquarium facility, where they were maintained at 13±1°C under a 12h light: 12h dark cycle (Thomas, 2018).

P. clarkii were much larger than *A. pallipes* (Carapace Length ẋ = 58.8mm ± 6.5), as were *P. leniusculus* (Carapace Length ẋ = 47.9mm ± 6.5). Both invasive species are larger; *P. leniusculus* reach a maximum carapace length of 50-70mm, with a size at maturity ranging between 25mm – 47mm (Lewis, 2002), *P. clarkii* have a wide range of size at maturity of 45mm – 125mm (Huner, 2002). *A. pallipes* in a Northumbrian stream population were found have a size range at two years of 26mm-30.6mm (Brewis & Bowler, 1982).

The invasive crayfish were kept within climate controlled rooms held at 15°C under a 12h light: 12h dark cycle. Holding aquaria used the same large black storage tubs (189l capacity, 380mmx9100mm, 0.35m^2) with an excess of refugia of bricks, plant pots and

PVC tubes and substrate of 2cm gravel. Holding tanks were filtered but experiment tanks were not. Water quality tests were conducted using Salifert Profi test kits (Dijkgraff 13, 6921 RL Duiven, Holland). All crayfish were fed ad libitum on a mix of frozen Tubifex bloodworm (Shirley Aquatics, Solihull, West Midlands, U.K.) and frozen peas (Thomas, 2018). *P. leniusculus* and *P. clarkia* were PIT tagged with 7.5mm PIT-tags (ISO 11784 certified, Loligo Systems).

The experimental tank set up in Cardiff had a longer bridge (L250cm x H20cm x W20cm). At the point of set up at Bristol the bridge length was halved due to concerns for the physiological abilities of the *A. pallipes*, and their high conservation status.

Any movements onto the ramp and bridge were recorded with start and return-to-water time. Bridge crossings and movements were recorded if over half of the body length of the crayfish moved onto the bridge from the ramp. From this, number of overland crossing, time on land and speed on land were calculated.

2.5 Sex ratio experiments

Twenty six females and 26 males were used to create a set of ten focal animals of each sex and 16 treatment animals of each sex. Five animals had to be replaced during the experiment due to mortalities. Half of the animals were wild caught using from a local South Gloucestershire stream population (exact location confidential) under a Natural England survey licence (Jen Nightingale 2016-22074-CLS-CLS) using artificial refuge traps; 13 males and 7 females were brought in for the experiment and 6 females from the same source were already being held in a BZG breeding facility in Somerset. A further 13 male and 13 female two year old captive-born crayfish, hatched from wild-caught ovigerous females from a local South Gloucestershire stream population and reared within aquaculture facilities in Somerset.

Male carapace length ranged from 29.5mm to 34.9mm ($\dot{x}$ = 32.6mm ± 1.4) and claw size (right claw unless this was missing) ranged from 20mm to 29.5mm ($\dot{x}$ = 24.6mm ± 2.6). Female carapace length ranged from 30.5mm to 35.0mm ($\dot{x}$ = 32.7mm ± 1.5) and claw size (right claw unless this was missing) ranged from 18.4mm to 24.8mm ($\dot{x}$ =

21.1mm ± 1.7). Crayfish were only measured at the start of the experiment to reduce handling time. Five animals moulted through the experiment but initial size records were used also to reduce handling time and imposed stress.

All animals were PIT tagged as described above. Females were marked using letters and males were marked using numbers on the carapace. In addition a line (males) or dot (females) was marked on the right claw for focal animals and the left for treatment animals. Marks had to be re-applied occasionally due to wear and moulting.

The experimental set up consisted of four tanks running consecutively. Each tank consisted of a large black storage tub (189l capacity, 38cm x 910cm, 0.35m²) with one ramp (L43cm x W29cm; 30° incline) leading to a half-length terrestrial bridge (L60 cm x H20cm x W20cm). Each tank had a slate and four PVC tube refugia. Bricks were not used as these were found to increase the degradation of markings due to the abrasive refuges rubbing against the carapace of the crayfish.

The water system for all experiment tanks was connected through plumbing pipes running through a communal filter and chiller. This design was intended to standardise the water temperature and quality across the tanks and prevent crayfish reacting to residual pheromones in tanks from prior trials. All animals in the experiment tanks were therefore exposed to the pheromones of the whole system.

Aquatic temperature was maintained through a chiller set at 15°C and monitored using two aquatic data loggers (Tinytag Aquatic 2 TG-4100) in tank 1 and tank 4. The average of the two loggers across the experiment period ran from 15.2°C ±1.1 (maximum 17.3°C, minimum 13.3°C). The average difference between the loggers was 0.53°C.

Water quality was maintained through the biological filter, regular water changes and water quality tests. The experiment tanks were kept outside but under a tarpaulin screen roof to keep light, rain and potential predators from the tanks. The experiment ran under natural day:night cycles with a range in hours of darkness from 8h17min to 11h38min (Time and Date, 2018).

Two holding tanks were maintained for the focal animals and the treatment animals to be kept apart. Mixed sex groups were kept together. The two holding tanks were running on different filters and chillers so were biologically and pheromonally separate from each other and the treatment tanks. Holding tanks were maintained at 15°C and had an excess of PVC tube refuges and bricks. Animals were fed a standardised Bristol Zoological Gardens diet (Nightingale, 2017) within holding tanks, no feeding occurred in experimental tanks.

The holding tanks consisted of a large blue plastic round tank (100cm diameter, 0.78m^2) in which the 20 focal animals were kept, and a large black tub (140l capacity, 70cm x 100cm, 0.7m^2) in which the 26 treatment animals were kept.

Animals were moved into experiment tanks in groups of four (11.4m^{-2}) with varying sex ratios. Each focal animal was trialled in four sex ratios (with some trial failures) of either all the same sex (4:0), all but one the same sex as the focal animal (3:1), equal males to females (2:2) and all but the focal animal a different sex (1:3) (Figure 4-1). The three animals matched to the focal animal came from the treatment groups.

A random number generator was used to create a trial schedule for the different treatments so that crayfish experienced different sex ratios in a random order. Each morning, the four focal animals from the schedule were selected from the holding tank and placed in buckets. The appropriate numbers of males and females were added from the treatment animals, these were selected at random from the treatment tank using a clockwise searching direction and a random start point. Animals were then moved into the treatment tanks after the previous night's animals were removed. Antennae length, claw health and leg injuries were noted when animals were placed into the experiment tanks and again when removed the following morning. New injuries were noted. Where possible, treatment animals were not used for more than one night consecutively.

The ramp was moved into position in the evening at around 17:00 so that animals had acclimated to the tank before access to the bridge was enabled. An infrared CCTV camera (Swann Pro-Series 615) was suspended above each experiment tank and recorded to a Digital Video Recorder (Swann DVR8-1525 8 Channel 960H). As each tank had only one camera, it was not possible to record the whole base of the tank and the access to the bridge. The percentage of tank base visible varied from 55% to 75%.

All successful trials (correct crayfish used, cameras working, video downloaded, markings identifiable) were watched for any terrestrial activity between 19:00 and 07:00, recording time of emergence, individual emerging and time of return to water.

A randomly generated selection of 16 of the successful trials (20%) were analysed to detect a suitable time period for detailed analysis. Starting at a time of darkness determined by the time the CCTV camera switched to IR recording (LUX ẋ = 6.45 ± 3.1), the number of crayfish visibly active was recorded at five minute intervals until light levels caused the switch back to normal recording. Activity was defined as at least half the body of the crayfish visible and movement seen. Numbers of active crayfish over the night was modelled using a Generalised Additive Mixed Model (GAMM) (Lin & Zhang, 1999), from this analysis an active period between 75mins and 200mins after time of darkness was identified (See 4.5.2). The time period of 100-165 minutes after dark was chosen, representing approximately 10% of the median night length of the sample period (630 minutes). Time of darkness as a starting point was chosen instead of time, as the experiment ran over three months and time of sunset shifted. Crayfish activity is strongly associated with light levels (Bojsen *et al.*, 1998).

During the 65 minute focussed period, more detailed analysis was carried out. The number of crayfish visibly active was recorded at a period of every minute. Location and behaviour of the focal animal was recorded every minute, using an ethogram adapted from Lundberg, (2004) (Table 2-1).

Observer visibility within the system was variable, and detailed observation of movement differences such as between cleaning behaviour and constructive activities were not possible. Therefore a system of recording a crayfish as ‘active’ when movement could be detected that was not locomotory was used. This was given the new ethogram code SM. Active behaviours, non-active behaviours and unknown behaviours were calculated for each focussed period, and used as another measure of crayfish activity.

Table 2-1: Ethogram of crayfish behaviour adapted from (Lundberg, 2004)

Active		Inactive		Not visible	
WA	Walking	FR	Freezing	BU	Behaviour Unknown
CL	Climbing	RO	Resting in the Open		
RS	Repulsive Swimming	RH	Resting in Hiding		
SU	Standing Up				
AF	Acquisition of Food				
CB	Cleaning Behaviour				
CA	Constructive Activities				
FA	Falling				
RW	Reverse Walking				
I	Interacting				
SM*	Stationary Movement				

Interactions were recorded throughout the focussed period noting which individuals were involved, the level of the interaction (adapted from Martin & Moore (2010) (Table 2-2)), the time and duration of the interaction, initiator, winner and loser. Interactions were started when individuals moved between one body length of each other and an interaction followed, and finished when at least one crayfish moved away a full body length. Some interactions occurred in areas of poor visibility and were ignored if the crayfish could not be identified.

Table 2-2 Interaction intensity codes from Martin III & Moore, (2010)

Interaction	Description
-2	Tail-flip away from opponent or a fast retreat
-1	Retreat - slowly back away from opponent
0	No response or threat display
1	Approach without a threat display
2	Approach with threat display - antennal whip, meral spread, or raised posture
3	Initial claw use - boxing, pushing, or touching with closed claws
4	Active claw use - grab opponent with claws or hold other crayfish with claw

3 Terrestrial emigration tendency of UK native crayfish *Austropotamobius pallipes* and two invasive species *Pacifastacus leniusculus* and *Procambarus clarkii*

3.1 Abstract

Understanding differences in behavioural parameters between indigenous and invasive species can prove important in efforts to reduce biodiversity losses. Invasive species often show characteristics that both make them more competitive than indigenous populations, and allow rapid dispersal and establishment. Crayfish are able to move terrestrially which is relevant to both invasive species control and the conservation of indigenous populations.

This chapter compares terrestrial movement between three crayfish species: the UK native white-clawed crayfish *Austropotamobius pallipes,* the invasive signal crayfish *Pacifastacus leniusculus* and the red swamp crayfish *Procambarus clarkii*. In an experimental arena, more *A. pallipes* exited the water than the two invasive species, and were faster than both invasive species. However, of the animals that did use the bridge, both invasive species made more exits indicating the higher tendency of these animals to use a terrestrial environment.

Differences in likelihood and frequency of terrestrial emergences between the species are of importance to aquaculture, invasive species management and native species conservation.

3.2 Introduction

The white-clawed crayfish *Austropotamobius pallipes* (Lereboullet, 1858) is listed as globally Endangered in the IUCN red list (Füreder *et al.,* 2010). It is the UK's only native crayfish and has suffered local extinctions across much of its UK range with a conservation status considered "Unfavourable- Bad and Deteriorating" (JNCC, 2007). Conservation actions for the species include a captive breeding programme and translocation releases into ‘ark sites’ creating new populations isolated from their principle threat; non-indigenous crayfish species (NICS).

This chapter compares movement behaviour in an indigenous species and two non-indigenous species; *A. pallipes*, *Pacifastacus leniusculus* and *Procambarus clarkii* respectively.

3.2.1 Comparisons between invasive and indigenous species

Many studies look at the differences between invasive species and native species, often in an effort to predict impacts and mitigation strategies (see chapter 1, Competitive Exclusion). The observed replacement of indigenous crayfish species by non-indigenous crayfish species across Europe (Holdich, *et al.*, 2009) demonstrates repeated examples of how there are multiple similarities and differences in the two groups. Bubb *et al.*, (2006) found that while *P. leniusculus* shared a very similar habitat preference to *A. pallipes*, they dispersed further around the environment, allowing them to make better use of patchy resources. Dunn *et al.*, (2009) measured crayfish in mixed and single species populations and found in mixed populations of *P. leniusculus* and *A. pallipes,* there were fewer large *A. pallipes*, which they attributed to exclusion from refuges, and subsequent predation, of the *A. pallipes* by the more aggressive *P. leniusculus*.

Ultimately, the difference in resistance to *Aphanomyces astaci* is most frequently cited as the mechanism for the replacement of ICS by NICS (see chapter 1, Crayfish Plague). Indeed, an Irish population of *A. pallipes* went extinct in the absence of NICS, as *A. astaci* had been transmitted via alternative means (Reynolds, 1997). However, in the absence of plague, extinction of the native stocks still appears to be the pattern (Bubb *et al.*, 2005). There is evidence of some European stocks developing resistance to *A. astaci* (Kokko *et al.*, 2012; Martín-Torrijos *et al.*, 2017). However, Gruber *et al.*, (2014) found that resistance to *A. astaci* came with a resource conflict between other energetically ‘expensive’ activities such as exploration behaviour so native stocks may still be compromised in their ability to compete with NICS.

In direct competition, NICS are found to be more dominant and interactions do not form stable hierarchies due to a failure of interspecific status recognition (Gherardi, Cioni, *et al.*, 2004). Pintor *et al.*, (2008) propose an aggression syndrome which incorporates aggression, voracity and boldness, and found that invasive signal crayfish *P. leniusculus* showed higher levels of all three than the native species in the study (the Shasta crayfish *Pacifastacus fortis*). They hypothesise that the linkage of these behaviours in a syndrome explains to some extent why invasive species can exist at high densities and have a disproportionate impact on their novel environment (Pintor *et al.*, 2009).

The invasion potential of NICS such as *P. leniusculus* is increased by their dispersal capacity, which, in addition to boldness/exploratory behaviour, is facilitated by their ability to travel large distances both in water and through terrestrial environments. Bubb *et al.*, (2006) found that *P. leniusculus* dispersed further than *A. pallipes* from a release site, and travelled twice the distance per night. *P. leniusculus* travelled a median maximal distance of 13.5m upstream and 15m downstream in upland rivers, and the maximum distance travelled was 417m (Bubb *et al.*, 2004). This is in contrast to *A. pallipes* with a mean daily movement of 4.6m (males) and 1.5m (females) also in an upland stream (Robinson *et al.*, 2000).

In addition to within stream movements, many crayfish can travel terrestrially. *P. leniusculus* has been observed travelling overland several hundred metres in one night (Hiley, 2003) and *P. clarkii* can move up to 1km overland (Souty-Grosset *et al.*, 2016a). *P. clarkii* and *P. leniusculus* are both able to orient away from higher temperatures when out of water, likely an adaptation for this type of dispersal (Marques *et al.*, 2015). *A. pallipes* have been observed leaving the water to escape dewatering (Latham *et al.*, 2016) and were recorded as moving 1.5cm s^{-1} in a terrestrial environment (Pond, 1975), but they are not known for this behaviour unlike the previous two species.

3.2.2 Invasive species' behaviour

Invasive species may have life history traits that make them successful invaders, and these may be selected for within the invasion process, leading to a skewed population of even more aggressive/bold/voracious animals through a 'selective-filter' (Pintor & Sih, 2009). Chapple *et al*., (2012) propose a scheme that separates the invasion process into five stages: transport uptake, transport transit, introduction, establishment and spread. They propose that different behaviours allow species to survive these filters, and this knowledge can be used to predict success or failure of accidental or deliberate introductions of species. This model also proposes an explanation for the phenomenon that invasive species are often more aggressive, bold, voracious etc., as these are the traits that have enabled survival through the filters.

Although the selective-filter principle would result in a narrower range of behaviours, retaining a behaviourally polymorphic founder population can increase the likelihood of successful invasion. Dispersal rates of cane toads were found to be higher at an invasion front in than in established populations, so that dispersal rate estimates based on established populations are likely to be underestimates of actual potential (Lindström *et al*., 2013). Fogarty *et al*., (2011) describe the process at the invasion front whereby asocial animals seek empty habitats, followed by social animals increasing density, causing asocial animals to disperse further and push the invasion front along.

Dispersal can be separated into its component parts of emigration, inter-patch movement and immigration (Bowler & Benton, 2005). It is also important to distinguish between active and passive dispersal, and it is likely that crayfish show both types during their development, particularly when living in lotic environments. A further distinction can be made between movements that result in dispersal through 'routine movements'; such as foraging, mate location, seeking shelter etc., and those that result from deliberate special dispersal movements (Van Dyck & Baguette, 2005). Special dispersal movements may be faster (Kuras *et al*., 2003) and more directional (Baars, 1979).

The extent to which crayfish will use terrestrial emigration as a dispersal route is not well understood, likely due to difficulties in capturing this behaviour in the field. This thesis seeks to experimentally investigate the propensity of white-clawed crayfish *A. pallipes* to emigrate terrestrially, and look for interspecific differences between *A. pallipes*, *P. leniusculus*, and *P. clarkii.*

3.3 Aims

Terrestrial movement behaviour is compared across a UK indigenous species *A. pallipes* and two invasive species *P. leniusculus* and *P. clarkii*.

The number of partial and full crosses of a terrestrial bridge made by individuals during a one night trial within the experimental arena are compared between species. Speed in the terrestrial environment is estimated from time spent on the bridge by each animal.

3.4 Methods

Crayfish were tested individually in an experimental arena for terrestrial movement.

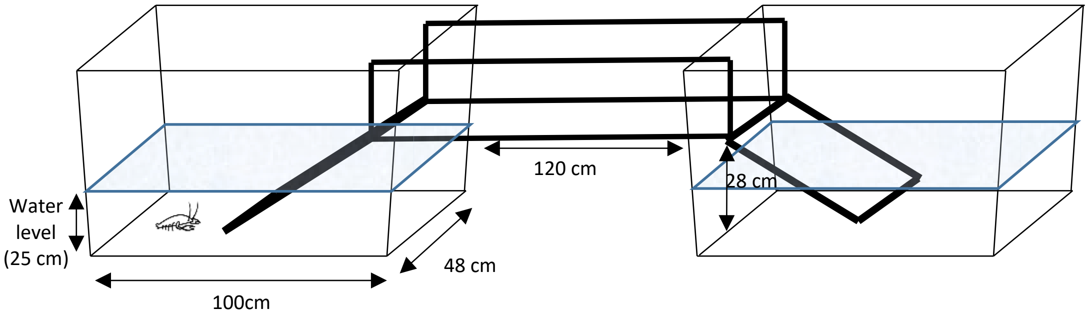

Figure 3-1: Terrestrial arena experimental set up

A group of two year old captive-born white-clawed crayfish *A. pallipes* within a similar size range (carapace length ẋ = 34.3mm ± 2.3) (ten males and twelve females) were tested at Bristol Zoological Gardens and held in single sex holding tanks at a density of 23m^{-2}. Seventeen red swamp crayfish *P. clarkii* (Carapace Length ẋ = 58.8mm ± 6.5) (thirteen males and four females), and fifteen signal crayfish *P. leniusculus* (Carapace Length ẋ = 47.9mm ± 6.5) (five males ten females) were tested at Cardiff University.

All animals were PIT tagged (8 mm PIT-tags [Trovan ID100A, RFID Systems LTD]) following Bubb *et al*., (2002) and marked with Dykem Brite-Mark permanent marker pens following Ramalho *et al*., (2010). Husbandry at Bristol was undertaken by the Bristol Zoological Gardens aquarium team. Animals were fed a standardized Bristol Zoological Gardens diet (Nightingale, 2017) within holding tanks, no feeding occurred in experimental tanks. Husbandry at Cardiff was undertaken by research staff, animals were fed ad libitum on a mix of frozen Tubifex bloodworm (Shirley Aquatics, Solihull, West Midlands, U.K.) and frozen peas (Thomas, 2018) in holding tanks.

White-clawed crayfish tank set ups were held under natural day:night cycles (12hr ± 1hr24min) (Time and Date, 2018). *P. leniusculus* and *P. clarkii* were held inside a secure laboratory with a 12h:12hr night:day cycle.

Individual crayfish were moved into the experiment tanks in the morning and given up to 6 hours to acclimate to the set up. The arena was filmed using infra-red CCTV and the 12 hour period 19:00-07:00 was used for analysis of terrestrial movements.

A. pallipes were never held in the same institution as *P. leniusculus* and *P. clarkii* due to high risk of crayfish plague *A. astaci* transmission. Invasive species experiments at Cardiff University were carried out by Rhidian Thomas (Thomas, 2018) then used for species comparison analysis.

Any movements onto the ramp and bridge were recorded with start and return-to-water time. Bridge crossings and movements were recorded if over half of the body length of the crayfish moved onto the bridge from the ramp. From this, number of overland crossing, time on land and speed on land were calculated.

For a full account of methods see chapter 2 ‘Methods’.

3.4.1 Statistical Analysis

A Generalised Additive Model for Location, Scale and Shape (GAMLSS) (Rigby & Stasinopoulos, 2005) was used to investigate factors affecting terrestrial movement as it is able to simultaneously model both likelihood and frequency of a behaviour occurring (in this case an emergence onto a bridge). The data had a zero-inflated distribution and thus a ZANBI (Zero Adjusted Negative Binomial) distribution was chosen for a better fit to the data. The model was set up to determine whether the explanatory variables of crayfish species, standardised carapace length or sex affected one or both of two parameters; likelihood of terrestrial movement (nu parameter) and/or frequency (mu parameter) of crayfish leaving the water (including times they did not fully cross the bridge).

An ANOVA revealed that the three crayfish species differed in size (white clawed crayfish < signal crayfish < red swamp crayfish; $F = 100.6$, $p < 0.001$) and so carapace length data were mean-centred and standardised within each species, before being included in the models to account for inherent differences in size amongst species. Due to low sample sizes, it was not possible to test a balanced number of male and female red swamp and signal crayfish, and so the overall effect of sex across species was examined in the models.

In addition, walking speed (cm s^{-1}) was tested against crayfish species, standardised carapace length and sex using a GAMLSS model with Normal distribution. Temperature variation during the white-clawed crayfish trials was tested with a further GAMLSS model with ZANBI distribution to check for any association with terrestrial walking behaviour.

GAMLSS model selection and refinement was conducted based on the inclusion of relevant model terms with a family that resulted in the lowest Akaike Information Criterion (AIC) value. Assumptions of normality were confirmed using residual diagnostic plots (See Appendix 2) from the models (Zuur *et al*. 2010).

3.5 Results

The ambient variation in temperature (10-15°C) during the white-clawed crayfish trials had no significant effect on their behaviour ($p > 0.05$).

Table 3-1 Comparative date from the terrestrial movement trials

Species	N	No. crayfish exited water	Mean no. exits ±SD	Mean time out of water per emergence (min) ±SD	Mean crossing speed (cm s^{-1}) ±SD
Red swamp	17	6	8.3 ± 7.4	6 min 26 s (± 1 min 56 s)	0.66 ± 0.07
Signal	15	7	8.3 ± 6.9	8 min 38 s (± 6 min 26 s)	0.51 ± 0.26
White-clawed	20	13	2.8 ± 1.9	2 min 10 s (± 45 s)	0.94 ± 0.22

A higher proportion of white-clawed crayfish (65%) left the water at least once than both signal crayfish (47%) and red swamp crayfish (35%), but of these animals that did move, the non-native species made more movements (Figure 3-2).

Frequency of terrestrial movements differed between species; of individuals that did leave the water, red swamp (mu parameter $t = 2.703$, $df = 47$, $p = 0.09$) and signal crayfish (mu parameter $t = 2.808$, $df = 47$, $p = 0.007$) emerged more frequently (Table 3-1, Figure 3-2, Appendix 2) than white-clawed crayfish.

From individuals making a full cross of 120cm along the bridge, white-clawed crayfish were significantly faster when walking out of water (cm s^{-1}) compared to red swamp and signal crayfish (see Figure 3-3). Sex and standardised carapace length were not significantly associated with walking speed.

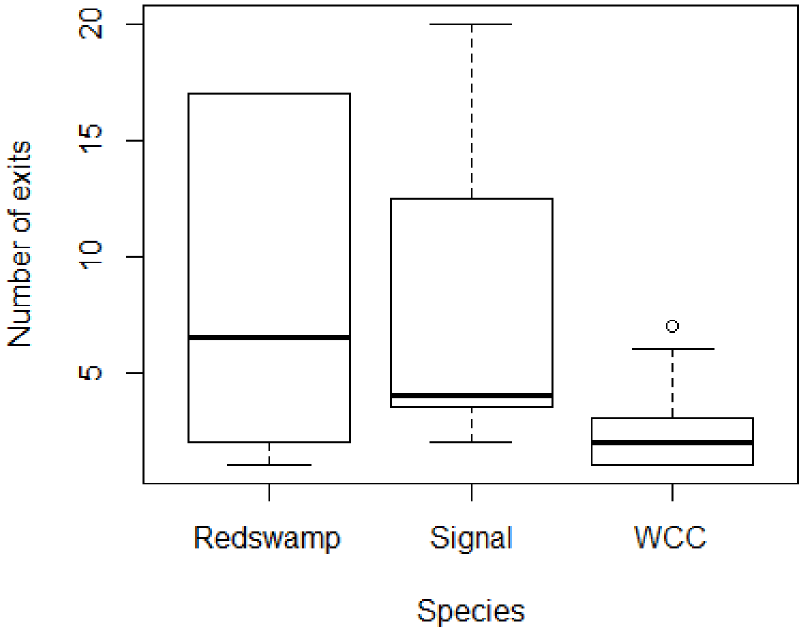

Figure 3-2: Number of exits of crayfish that did exit the water demonstrating a higher frequency of emergence from the invasive red swamp and signal crayfish. Red swamp (mu parameter t = 2.703, df = 47, p = 0.09) signal crayfish (mu parameter t = 2.808, df = 47, p = 0.007)

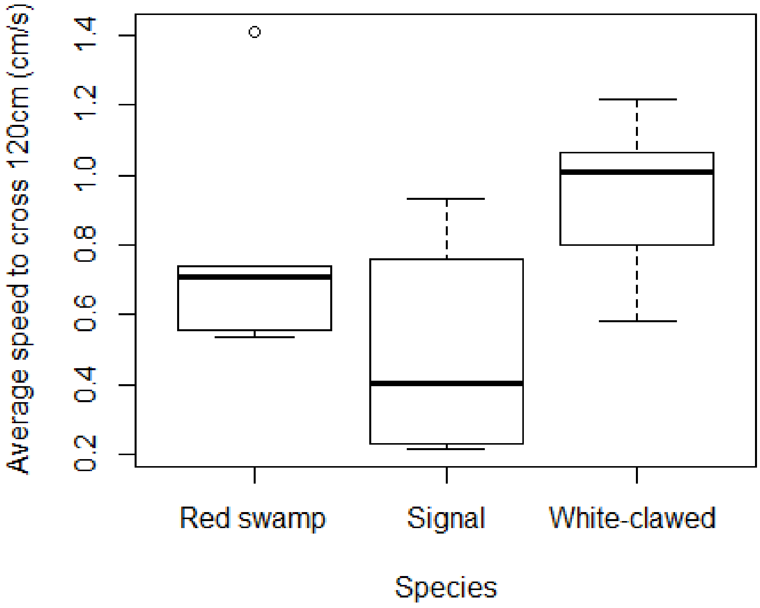

Figure 3-3 Walking speed over first 120cm bridge (cm s^{-1}). White-clawed crayfish significantly faster than red swamp (n = 4; t = -2.452, df = 17, p = 0.025) and signal crayfish (n = 6; t = -4.204, df = 17, p < 0.001)

3.5 Discussion

Of the three crayfish species tested, a higher proportion of indigenous *A. pallipes* exited the water at least once in comparison to the invasive species *P. leniusculus* and *P. clarkii*. This is surprising, given that *A. pallipes* are not known for terrestrial behaviour (Peay & Dunn, 2014), unlike the two invasive species (Cruz & Rebelo, 2007; Banha & Anastacio, 2014). However, of the animals that did leave the water, those of invasive species did so more frequently and for longer. This implies that these invasive species are more tolerant of the terrestrial environment. The factors driving such a high percentage of *A. pallipes* to emigrate from the water remain unknown.

The two invasive species spent longer in the terrestrial environment, and were found to move more slowly than *A. pallipes*. Initially it was hypothesised that the larger species would move faster, due to greater stride length (Claussen *et al*., 2000), but the opposite was shown in this study. This could be explained by the increase in weight experienced by crayfish on land rather than in water (400-600%) (Pond, 1975), which would affect larger crayfish more. In addition, the two invasive species have correspondingly larger claw, which are held aloft when walking terrestrially, increasing the terrestrial burden. Furthermore, the desiccation risk of terrestrial emigration would be higher for the smaller *A. pallipes*, encouraging faster return to water. The result that fewer *A. pallipes* emigrated more than once could be due to some negative impacts of the terrestrial movement such as dehydration, lowered oxygen availability and acidosis (Taylor & Wheatly, 1981).

The two invasive species are also known to be more tolerant to a range of environmental conditions (Firkins, 1993), indicating that there could have been an element of the experimental arena environment that was less suitable to *A. pallipes* than to the invasive *P. leniusculus* and *P. clarkii*, causing the former to attempt to evacuate the tank.

The finding that all three species show a tendency to emigrate terrestrially has important implications for both the control of invasive species and the conservation of native crayfish. Escapes from private and commercial aquaculture facilities for *P.*

leniusculus, and from established populations of both *P. leniusculus* and *P. clarkii* are more likely if the animals are able to walk overland out of facilities. Management practices that treat watercourses such as de-watering, biocide treatment and trapping, are less likely to be effective if animals can escape terrestrially. Finally, the conservation strategy of isolating the native *A. pallipes* can be compromised if these animals walk terrestrially from an 'ark site', and indeed limit the number of sites that are truly isolated from invading *P. leniusculus* populations.

This thesis adds to a growing body of evidence that non-indigenous species implicated in extirpation of indigenous species often have certain behavioural traits that make them more competitive, especially in an anthropogenically altered environment. The invasive zebra mussel *Dreissena polymorpha* are able to disperse more rapidly than native bivalves through a free-swimming larval stage and the tendency to readily attach to motile surfaces (Johnson & Carlton, 1996). Rehage & Sih, (2004) compared invasive and native *Gambusia* spp. and found the invasive species were more likely to disperse and travelled further than native species.

Gurnell *et al*., (2004) found in mixed populations of the European red squirrel *Sciurus vulgaris* (Linnaeus, 1758) and eastern grey squirrels *Sciurus carolinensis* (Gmelin, 1788) red squirrels were smaller and less fecund then when in non-invaded populations. This is directly comparable to Dunn *et al*., (2009) who found smaller *A. pallipes* in mixed populations. Both were due to the non-indigenous species out-competing the indigenous for a resource; for squirrels the resource was cached food; for the crayfish, refuges.

Range expansions of grey squirrels are rapid (17.2km^2/year Bertolino & Genovesi, (2003)) and understanding of habitat features that enhance or reduce range expansion for invasive species is important in management and mitigation of invasive species. Recent work has found an element of the indigenous red squirrels' behaviour that is better adapted to the UK environment than the grey squirrels'; namely their response to the native predator the pine marten *Martes martes* (Sheehy & Lawnton, 2015).

The replacement of indigenous, specialist species by globally abundant, generalist invasive species has been termed 'global homogenisation' (Clavel *et al*., 2011).

Understanding how indigenous species differ in their niche widths from non-indigenous species can be part of a programme to halt and possibly reverse native species' decline.

Comparisons between the three species are useful, but it is relevant to note the differences in experimental set up between the Cardiff non-indigenous species and the Bristol indigenous species. These differences were due to the importance of keeping the indigenous species completely isolated from potential plague vectors at the Cardiff facilities. The facilities and set up at Bristol Zoo were managed to be a close to the Cardiff set up as possible given the limitations of working with a protected endangered species in a conservation collection. The limited numbers of non-indigenous species and to a lesser extent the indigenous species affect the ability to make generalisations about the species as a whole. A priori consideration of the comparison with indigenous species may have improved the experimental validity through tighter control of set-up parameters. However, significant results were detected and refinement of methods may well increase the magnitude of the differences, but are unlikely to change the direction.

This thesis adds to research into movement ecology of crayfish; further work on wild populations and their dispersal behaviour could continue with more in-situ tracking studies. Past and current studies use radio tags (e.g Bubb, Lucas, & Thom, (2002)), which are unlikely to generate finer dispersal detail such as terrestrial movements, or Passive Integrated Transponder (PIT) telemetry (e.g. Bubb *et al.*, (2006b)), which requires hand searching for animals and misses movement details. Live recordings of sections of water course (e.g. Davis & Huber, (2007)) can provide fine detail of activity but fail to capture data out of frame, and can miss identifying individuals. The potential of archival and acoustic tags to capture movement data in crayfish is developing as tag sizes reduce (Cooke *et al.*, 2013) and other aquatic species are tracked (e.g. bull trout, Martins *et al.*, (2014)). Nightingale, (2018) is following released populations of *A. pallipes* with PIT and acoustic tagging to develop a picture of the use of ark sites by introduced animals.

Dispersal behaviour is often related to age and stage (Bowler & Benton, 2005); further research could investigate how different age class crayfish respond to a terrestrial opportunity. Population differences in terrestrial activity may exist and comparing crayfish from different environments, such as lentic or lotic, or geographically separated populations may indicate polymorphism in this behavioural trait.

Links between the tendency to emigrate terrestrially and other behavioural parameters such as aggression or foraging voracity could provide further evidence of behavioural syndromes (Gherardi *et al*., 2012), which could in turn be compared across indigenous and non-indigenous species.

An interesting comparison would be between *P. leniusculus* (or *P. clarkii*) sourced from their native range and *P. leniusculus* (or *P. clarkii*) from an invading population to test if a 'selective filter' had affected this aspect of their behaviour, or if the behaviour extended to their native range. Many exotic species are only studied in an invasive context and important details could be missed through this (Hierro *et al*., 2005). Parker *et al*., (2013) concluded from a meta-study of invasive species that while they do perform better in the invading range, there is considerable variation among taxa. They did not cover any crayfish in the analysis.

More research targeted at understanding movement and therefore spread of non-indigenous species is likely to be a useful programme to mitigate and reduce native species losses.

4 Sex Ratio

4.1 Abstract

Crayfish are often used as a model for activity models, agonistic encounters and dominance hierarchy establishment. Group sex ratio composition can affect behaviour as males and females often behave differently. Their behaviour in groups is of interest to ecologists, aquaculturists and conservationists alike.

This chapter investigated white-clawed crayfish *Austropotamobius pallipes* terrestrial movement, interactions and activity levels under different sex ratios in an experimental arena. Terrestrial movement was minimal when crayfish were in groups of four, in contrast to results from animals tested alone, suggesting a negative density-dependence in emigration decisions.

Larger animals were less active and took part in fewer interactions, and groups with a larger range of carapace lengths showed fewer interactions, suggesting dominance was established quickly when large size differences were present. Males were slightly less active than females.

Activity was affected by sex ratio with groups with more males showing higher activity levels and groups with no males showing lowest activity levels indicating that males cause higher movement in females and males. Activity was also affected by the sex ratio of the system signifying a response in crayfish to an aquatic substance assumed to be a pheromone.

Conservation programmes for *A. pallipes* involve captive breeding and translocations to ark sites and negative impacts of densities and sex ratios can be avoided through a better understanding of these impacts.

4.2 Introduction

Although generally described as a solitary animal, crayfish species can occur at high densities (e.g. *Austropotamobius pallipes* 5m^{-2} (Latham *et al.*, 2016)). Testing behaviour in the presence of conspecifics is likely to be a more valid model of wild populations and behaviours, so improving our understanding of how crayfish react and behave in groups might facilitate better captive and conservation management of the species. Additionally, behavioural differences between males and females and whether sex ratios affect behaviour such as dispersal are important fields of research. This chapter looks at the behaviour of crayfish in groups within the same experimental arena as chapter three. Terrestrial movement was measured in different sex ratios, alongside activity and interaction behaviour within the tank.

4.2.1 Movement, activity and agonistic encounters

Crayfish are largely described as nocturnal (e.g. Barbaresi & Gherardi, 2001), although this could be more complex, with larger crayfish being more strictly nocturnal and small crayfish tending towards a more diurnal/crepuscular pattern (Davis & Huber, 2007). They do not defend specific territories but as they range through suitable habitats they often return to the same refuge, which they may defend. Crayfish can move considerable distances; for example *Pacifastacus leniusculus* travelled 341m in two days (Bubb *et al*., 2006a), but this varies between species; no *A. pallipes* moved more than 70m in the same study.

Differences in movement distances between sexes have not been detected in many studies (Guan *et al*., 1997; Usio & Townsend, 2002; Bubb *et al*., 2006b), however Wutz & Geist, (2013) did find differences in mobility, with a lower (although still high) percentage of females (62.4%) being re-caught in a different trap (defined as mobile) as opposed to males (79.5%).

Larger animals and males have been found to be more likely to be trapped in minnow-type traps (Dorn *et al*., 2005; Price & Welch, 2009), furthermore, Ogle & Kret, (2008) experimentally demonstrated that crayfish avoided entering a trap with a crayfish already inside. This was particularly true for smaller individuals, and particularly females if a male was already inside. Moreover, Aquiloni & Gherardi, (2010) attracted more males to traps with receptive females held within them than females to traps with receptive males held within them. They suggest the males are the mobile sex, using pheromones to locate mates, which is in contrast to females who do not follow pheromone trails, and rely on visual cues to select appropriate mates (Aquiloni *et al*., 2008).

Crayfish frequently engage in agonistic behaviour over resources such as refuge shelters, food and mates. Crayfish breed seasonally and Warren *et al*., (2009) demonstrated that reproductive male *Orconectes quinebaugensis* are more aggressive than reproductive females during the breeding season, but reproductive males are not more aggressive than non-reproductive males or non-reproductive females out of the breeding season. That both male and female crayfish are more aggressive when in their reproductive form, has also been seen in *Orconectes rusticus* where reproductive males won more fights against all partners, and reproductive females won more fights over non-reproductive males (Martin & Moore, 2010).

Where male and female crayfish species are aggressive, during the breeding season females need to have a mechanism to reduce their aggression so that mating activity can occur without injury. Receptive females ‘freeze’ after contact from a receptive male, allowing mating to proceed (Villanelli & Gherardi, 1998). Karplus *et al*., (2003) implanted androgenic glands into female Australian freshwater crayfish *Cherax quadricarinatus* and found they engaged in agonistic behaviour more than non-implanted females, and had lost the submission behaviour. Female aggression rises when they are carrying eggs or juveniles as shown by Figler *et al*., (1995) in *Procambarus clarkii* by shelter defence experiments with maternal and non-maternal females.

Crayfish are often used as a model species to test ideas on agonistic behaviour as they are small and active (Jurcak *et al*., 2016). Many mechanisms are employed by crayfish such as chemical communication and agonistic interactions to form dominance hierarchies (see chapter one). Tierney *et al*., (2013) found differences in status and dominance hierarchy maintenance in males and females – finding that dominant female *P. clarkii* preferred animals they had fought before to unfamiliar animals, whereas males had no preference. Subordinate animals of both sexes preferred unfamiliar animals. Dominance hierarchies were found to be 'transient and revocable' (Graham & Herberholz, 2009) fitting the nature of this mobile, non-territorial animal. Crayfish are well equipped with visual and chemical perception to respond to meeting new animals, which they may avoid through leaving or hiding, or interact with in order to establish hierarchy.

4.2.2 Population density and sex ratio

Holding a higher ratio of females to males is advantageous for aquaculture, including conservation breeding programmes, if males can produce enough spermatophores to fertilise multiple females. Celada *et al*., (2005) found no significant effect on fertilisation and spawning in *P. leniusculus* of a higher sex ratio of one male to four females than the recommended one male to two females. No mortalities were found in densities of $25m^{-2}$ at sex ratios of one male to four females and the researchers achieved 95% and 97% berried (with eggs) females at densities of $5m^{-2}$ and $25m^{-2}$ respectively. Claudia *et al*., (2004) found that higher densities of *Procambarus llamasi* promoted maturation and higher spawning rates. At very low densities they found no females spawned and males revert to non-breeding condition.

However, high densities can also have negative effects on groups of crayfish; Jones & Ruscoe, Ian, (2000) demonstrated reduced growth rates in *Cherax quadricarinatus* at higher densities; *P. leniusculus* showed reduced survival (86.33%, $100m^{-2}$; 39.13%, $1000m^{-2}$) at higher densities and reduced growth rates (González *et al*., 2010); and Ramalho *et al*., (2008) described the effect of density on growth rates as 'profound' with high densities (up to 100 m^{-2}) causing lower growth. Nightingale (2017) advises stocking densities of *A. pallipes* vary according to age class (0+ 100 m^{-2}; 2+ 10 m^{-2}; 1+ 20 m^{-2}; Adults 6 m^{-2}).

The effect of stocking density or sex ratio may change over season, with crayfish primarily interacting over food or refuge availability outside of the breeding season. This thesis looked at crayfish behaviour between July and early September, outside of the normal reproductive season of *A. pallipes* in the UK (Holdich, 2003a). Through this a baseline response to conspecific sex ratio was sought.

Through the description of the extent of terrestrial movement some evidence could have been found to demonstrate sex ratios under which *A. pallipes* make a costly movement decision. In addition, in-tank activity was analysed for changes in levels of activity and numbers of interactions under different sex ratios. Results from the study could be used in captive breeding, restocking and introductions of the species to ark sites in order to maximise conservation benefit.

4.3 Aims

By manipulating sex ratios within groups of *A. pallipes*, this chapter investigates associations between group sex ratio, terrestrial movement and in-tank activity, including interactions.

Focal animal trials within an experimental arena containing randomly selected conspecifics are analysed for any terrestrial movement. Activity of the focal animal, and of the group overall is measured and modelled against sex ratio and intrinsic parameters (sex and size). Number of interactions between the focal animal and conspecifics are also measured and modelled against sex ratio and intrinsic parameters (sex and size).

4.4 Methods

Twenty six female and 26 male white-clawed crayfish were used to create a set of ten focal animals of each sex and 16 treatment animals of each sex. Half of the animals were wild-caught and half were two-year-old captive-born crayfish reared within aquaculture facilities in Somerset. The crayfish were size matched for carapace length, with males measuring 32.6mm ± 1.4 and females 32.7mm ± 1.5. All animals were PIT tagged (8 mm Passive Integrated Transponder tags [Trovan ID100A, RFID Systems LTD]) and marked with Dykem Brite-Mark permanent marker pens (Ramalho *et al.*, 2010).

The experimental set up consisted of four tanks running consecutively. Animals were moved into experiment tanks in groups of four (11.4m^{-2}) with varying sex ratios. Each focal animal was trialled in four different sex ratios (Figure 4-1); the three animals matched to the focal animal came from the treatment group.

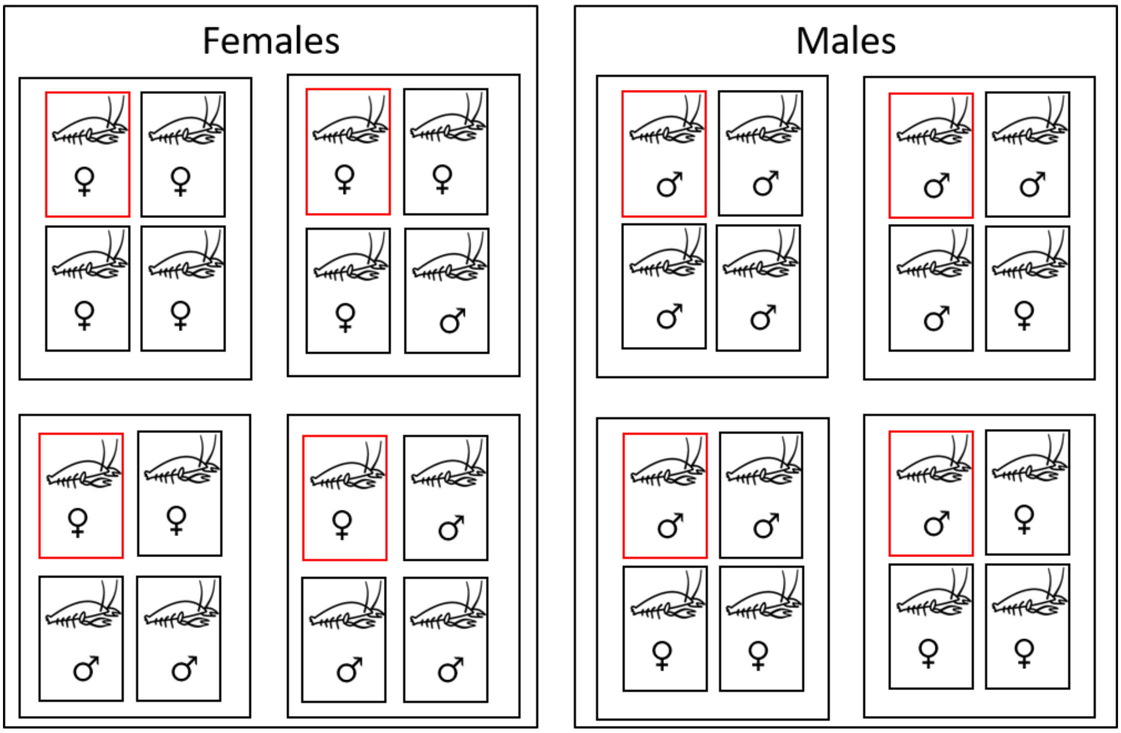

Figure 4-1 Experiment trials. Each focal animal (red) was placed in four trials with sex ratios as shown, in a randomly generated order.

Video of successful trials (correct crayfish used, cameras working, video downloaded, marking identifiable) was observed for any terrestrial activity between 19:00 and 07:00, recording time of emergence, individual emerging and time of return to water.

A randomly-generated selection of 16 of the successful trials (20%) were analysed to detect a suitable time period for detailed analysis. Starting at a time of darkness determined by the time the CCTV camera switched to IR recording (LUX $\dot{x} = 6.45 \pm 3.1$), the number of crayfish visibly active was recorded at five minute intervals until light levels caused the switch back to normal recording. Activity was defined as at least half the body of the crayfish visible and movement seen. Numbers of active crayfish over the night was modelled using a General Additive Mixed Model (GAMM) (Lin & Zhang, 1999), from this analysis an active period between 75mins and 200mins after time of darkness was identified. The time period of 100-165 minutes after dark was selected for further analysis, representing approximately 10% of the median night length of the sample period (630 minutes). Time of darkness as a starting point was selected over time as the experiment ran over three months and time of sunset shifted. Crayfish activity is strongly associated with light levels (Bojsen *et al.*, 1998).

During the 65 minute focussed period, more detailed analysis was carried out. Location and behaviour of the focal animal was recorded every minute, using an ethogram adapted from Lundberg, (2004). The number of crayfish visibly active was also recorded at a period of every minute.

The clarity of visibility of the system was variable and detailed observation of movement differences such as between cleaning behaviour and constructive activities was not accurate. Therefore a system of recording a crayfish as active when movement could be detected that was not locomotory was used. This was given the new ethogram code SM (stationary movement). Active behaviours, non-active behaviours and unknown behaviours of the focal animal were calculated for each focussed period and used as another measure of crayfish activity.

Table 4-1: Ethogram of crayfish behaviour adapted from (Lundberg, 2004) Addition of SM by author.

Active		Inactive		Not visible	
WA	Walking	FR	Freezing	BU	Behaviour Unknown
CL	Climbing	RO	Resting in the Open		
RS	Repulsive Swimming	RH	Resting in Hiding		
SU	Standing Up				
AF	Acquisition of Food				
CB	Cleaning Behaviour				
CA	Constructive Activities				
FA	Falling				
RW	Reverse Walking				
I	Interacting				
SM*	Stationary Movement				

Interactions were recorded throughout the focussed period noting which individuals were involved, the level of the interaction (adapted from Martin & Moore, (2010), the time and duration of the interaction, initiator, winner and loser. Interactions were started when individuals moved between one body length of each other and an interaction of value at least plus or minus one followed, and finished when at least one crayfish moved away a full body length. Some interactions occurred in areas of poor visibility and were ignored if the crayfish could not be identified.

Table 4-2: Interaction intensity codes from Martin III & Moore, (2010)

Interaction	Description
-2	Tail-flip away from opponent or a fast retreat
-1	Retreat - slowly back away from opponent
0	No response or threat display
1	Approach without a threat display
2	Approach with threat display - antennal whip, meral spread, or raised posture
3	Initial claw use - boxing, pushing, or touching with closed claws
4	Active claw use - grab opponent with claws or hold other crayfish with claw

4.4.1 Statistical Analysis

A randomly selected subsection of the trial runs (16 = 20%) were analysed with a score of how many crayfish in the tank were active at a scan point every five minutes. A Generalised Additive Mixed Model (GAMM) (Lin & Zhang, 1999) with a Poisson family and SQRT link (for zero-bounded discrete distributions with equal mean and variance) using trial run as a random factor was used to model nightly activity patterns.

During the 65 minute focussed observation period within each trial run, three behavioural measures were taken; interactions, activity of the focal animal and activity of the group.

The number of interactions between the focal animal and other individuals within the trial group were noted. This was modelled in a GAMLSS model with a ZIP (Zero Inflated Poisson) distribution using the mu parameter for frequency of interactions. Size of focal crayfish (carapace length in mm), range of carapace lengths in the group (in mm), sex of the focal animal and the ratio of the group in the tank that was the same sex as the focal animal and the interaction between sex and sex ratio (the effect of sex ratio on different sexes) were all included as explanatory variables in the model. Focal crayfish was included as a random term in the model. This model was then tested using the ratio of the group of crayfish across the four tanks; the 'system', as these animals were sharing water and through this the possibility of pheromonal detection remained.

The number of active animals was counted at each minute scan of the focussed period. This was modelled in a GAMLSS model with a ZAP (Zero Adjusted Poisson) distribution. The sum of the four crayfish carapace lengths, range of carapace lengths, the ratio of the tank that was male and the date (modelled as number of days since 01/01/2017) were all tested in the model. The trial run was included as a random term in the model.

The focal animal was scored as being active (Y) or not active or not visible (N) at each minute scan. This was modelled in a GAMLSS model with a BI (Binomial) distribution. Size of focal crayfish (carapace length mm), range of carapace lengths(mm) in the group, sex, the ratio of the group in the tank that was the same sex as the focal animal and the interaction between sex and sex ratio (the effect of sex ratio on different sexes) were all included as explanatory variables in the model. The trial run and focal crayfish were included as random terms in the model (See appendix 2).

Model selection and refinement was conducted based on the inclusion of relevant model terms with a family that resulted in the lowest Akaike Information Criterion (AIC). Assumptions of normality were confirmed using residual diagnostic plots from the models (Zuur *et al*. 2010) (See Appendix 2 Model validation).

4.5 Results

4.5.1 Terrestrial Activity

In six of the 72 successful trials, a crayfish moved terrestrially onto the bridge (8.3%). Of those animals that did move terrestrially, only one animal moved more than once, making three exits from the water. Numbers were too low to model against intrinsic or extrinsic parameters.

4.5.2 Nightly activity pattern

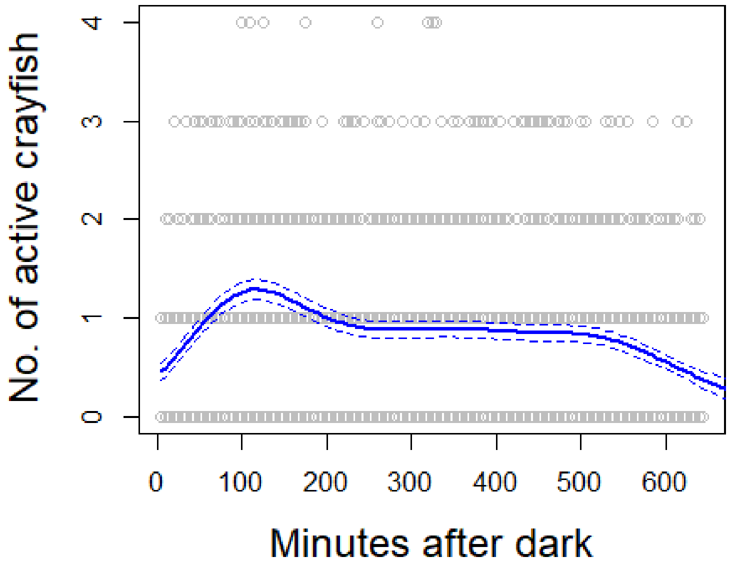

Figure 4-2 Activity data from subsection of trials over whole night, with GAMM model prediction line with smooth moving average and 1SE *confidence interval, run is included as a random factor.*

A time period of 100 minutes after dark to 165 minutes after dark was chosen to represent peak activity (Figure 4-2).

4.5.3 Sex ratio: number of interactions with focal animal

Even though crayfish for this experiment had been selected within a narrow range of sizes (CL range 6.8mm) to avoid strong effects of size on crayfish behaviour, focal animal carapace length was significantly associated with the number of interactions (Figure 4-3), with larger crayfish taking part in fewer interactions. There was no association with sex ratio of either the tank or the system and the number of interactions.

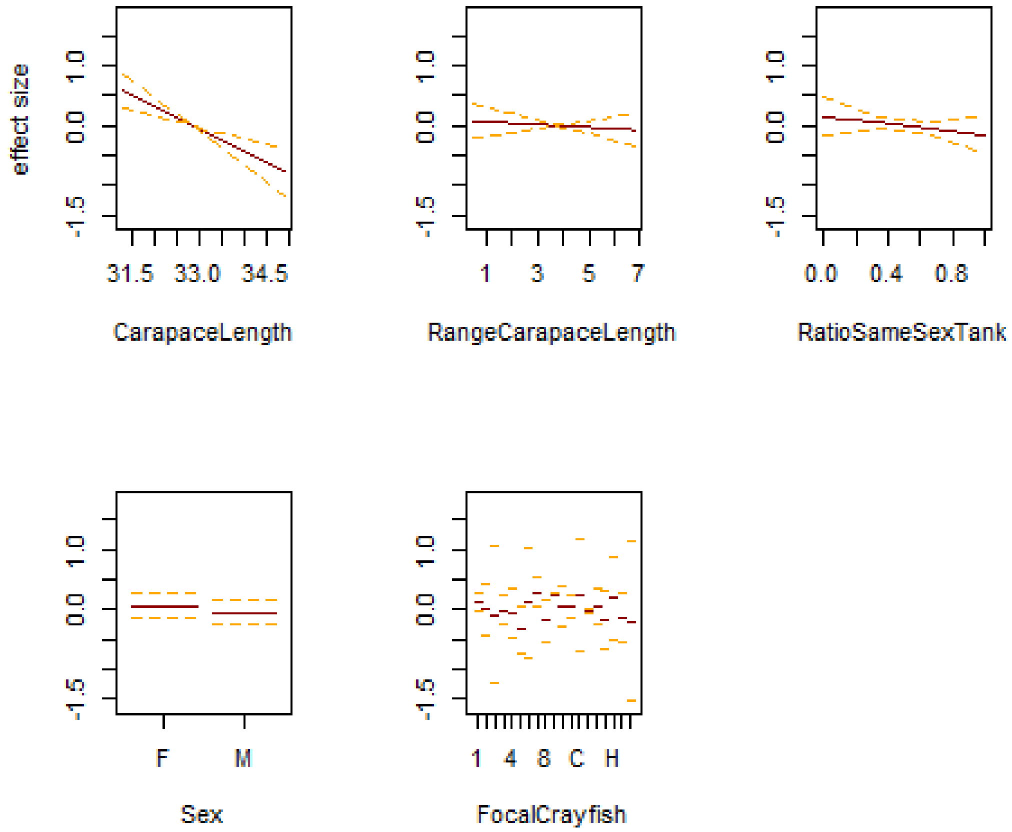

Figure 4-3 Model term effects from focal animal interactions to the sex ratio of the tank. Model interaction terms not included. Carapace length significant (t = -4.534, df = 54 p <0.001).

4.5.4 Sex ratio: group activity

The range of carapace lengths was significantly associated with the number of crayfish active with bigger ranges of carapace lengths in the group showing slightly less activity.

Date as a factor had a very small but significant impact, with very slightly less activity later in the year (or further into experiment) (Figure 4-4).

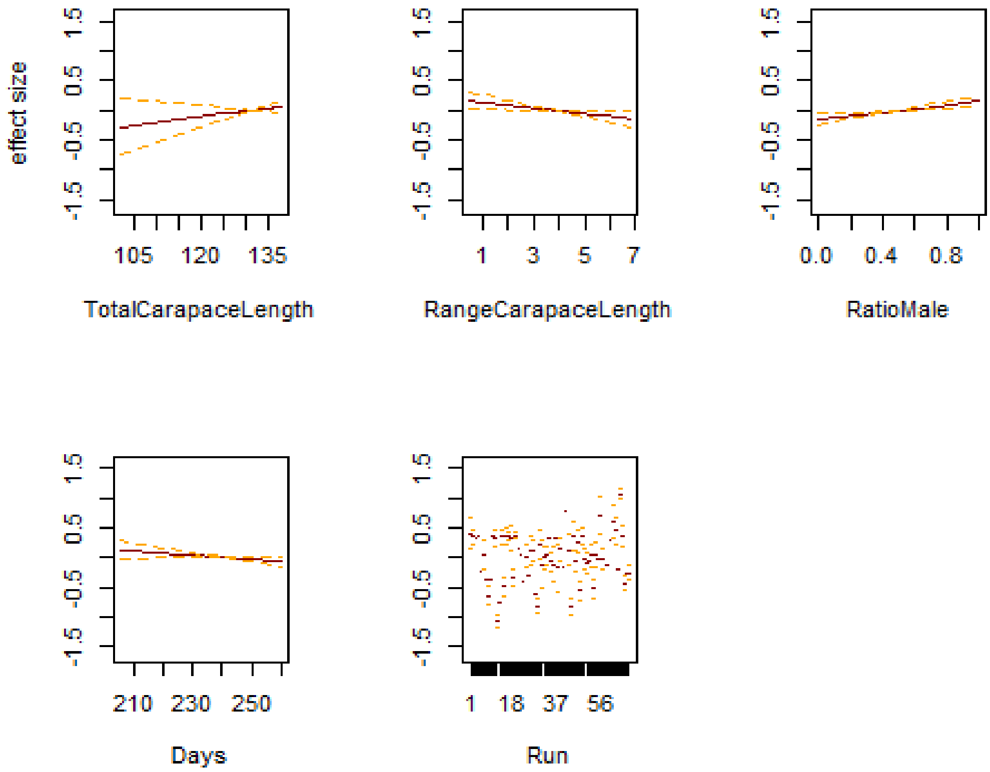

Figure 4-4: Model term effects from group crayfish activity to the sex ratio of the tank. Carapace length range, sex ratio and date significant (t=-2.644, df = 4361, p=0.008) (t=3.750, df = 4361, p<0.001) (t=-2.233, df = 4361, p = 0.026).

The sex ratio of the tank also shows an association with the number of crayfish active; having more males in a tank shows more activity in the tank (Figure 4-4). From Figure 4-5 it is possible to see that this difference arises largely as groups of females without a male are less active than when with a male.

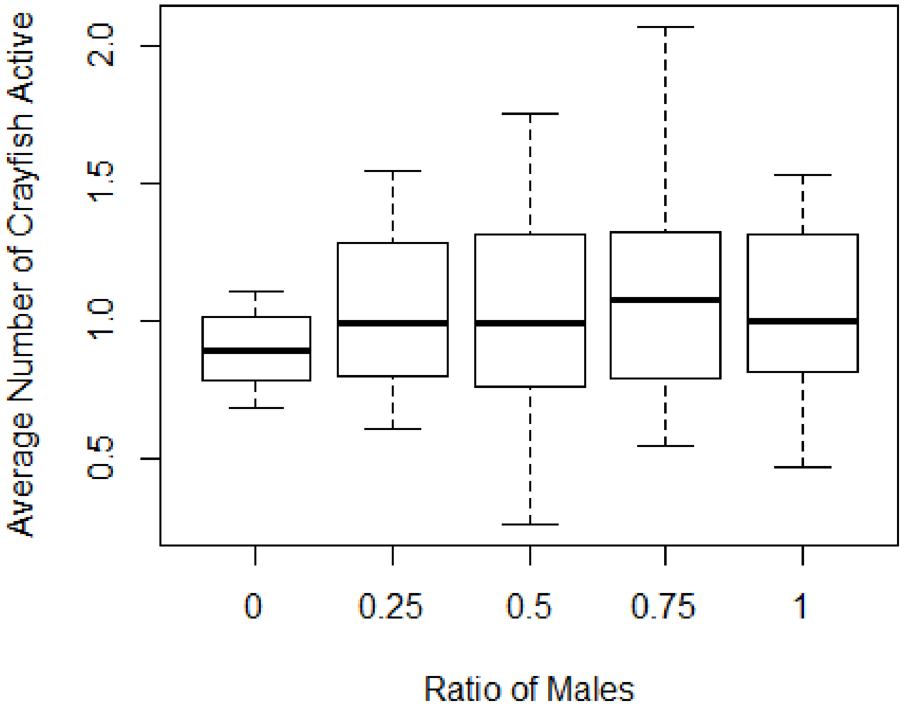

Figure 4-5: Average number of crayfish active under different sex ratios.

4.5.5 Sex ratio: focal animal activity

Focal animal carapace length was significantly associated with activity (t = -10.536, df = 4352 p <0.001) with larger crayfish being less active. There was no significant effect of the sex ratio within the tank.

The interaction between sex of the focal individual and the sex ratio of the system was significantly associated with activity, with males being more active in a sex ratio with more males, and females being less active in a sex ratio with more females (See Figure 4-6). In the system model, focal animal carapace length was still significantly associated with activity, and sex of the focal animal was significantly associated with activity, with males being very slightly less active (Figure 4-7) once other explanatory factors are taken away.

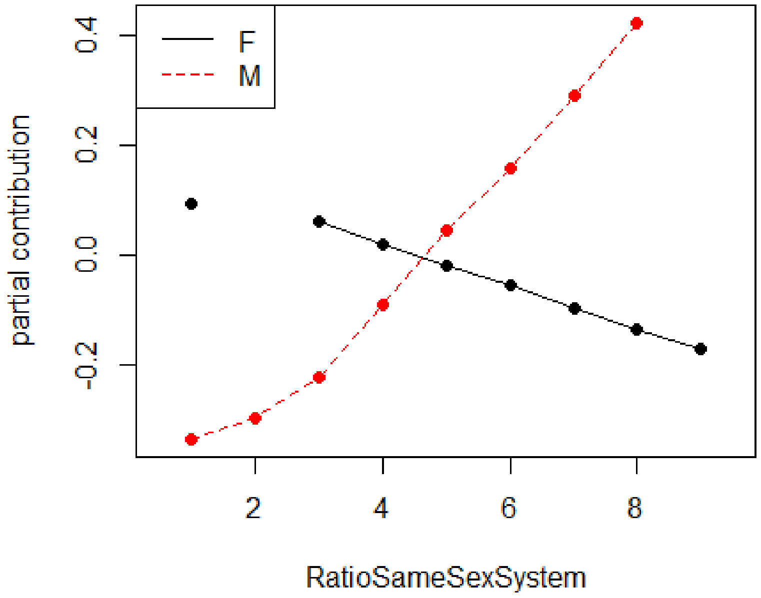

Figure 4-6 Model term effects of the interaction between sex of the focal crayfish and the ratio of the system that were the same sex as the focal crayfish, associated with activity (t=4.162, df = 4352, p < 0.001).

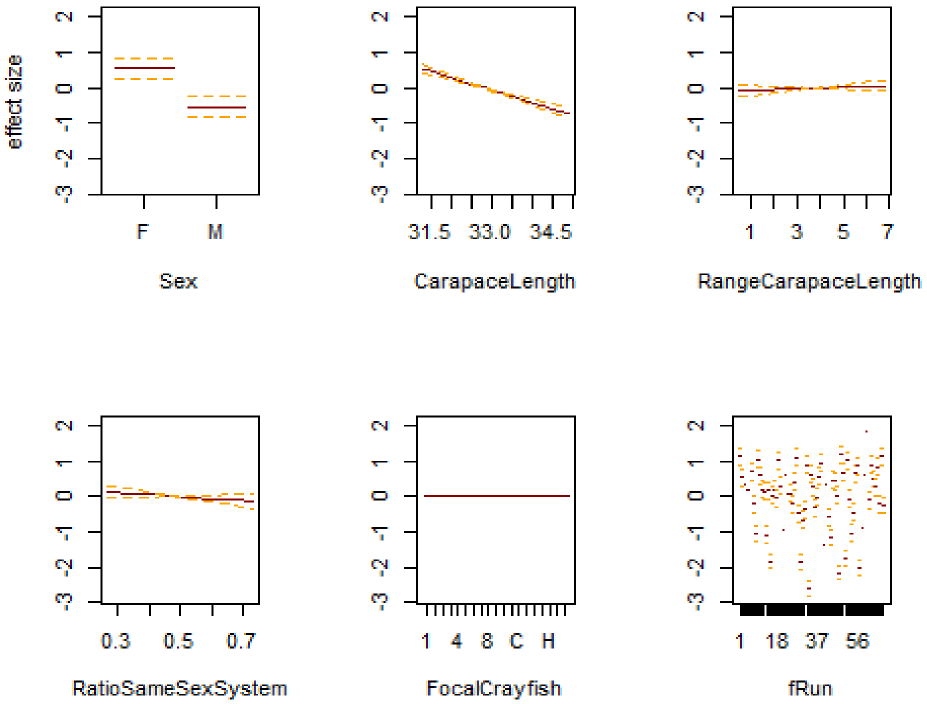

Figure 4-7: Model term effects from focal animal response to the sex ratio of the system. Interaction terms not included. Sex and Carapace length both significant (t=-3.698, df = 4352, p <0.001) (t = -9.347, df = 4352 p <0.001).

4.6 Discussion

There was a notable difference in the numbers of crayfish that exited the water onto a terrestrial bridge when tested in groups of four as opposed to singly in the previous experiment (see Chapter 3.5). The two experiments cannot be directly compared as there were differences in the experiment set up and time of year. However the difference is so stark (8.3% to 65%) it is worth considering implications. The findings mirror those found by (Thomas, 2018) in paired experiments on *P. lenisuculus* whereby 57.1 % left the water when tested individually in contrast to 33.8% when kept in groups.

These results could indicate a density-dependent emigration behaviour, in which animals will leave an area if conspecific density is too low, as seen in the Glanville fritillary *Melitaea cinxia* (Kuussaari *et al*., 1996) and the Rocky Mountain apollo *Parnassius smintheus* (Roland *et al*., 2000). This may occur as conspecific density is seen as an indirect measure of habitat quality, through use of 'public information' where the presence of one or more animal accessing a resource indicates there are opportunities present (Danchin et al., 2014). Conspecific attraction could also occur through allee effects whereby animals need conspecifics for increased fitness, such as reduced predation risk or mating opportunities (Stephens et al., 1999).

An alternative explanation is that when in groups there is less opportunity for exploration as time is spent either in interactions or avoiding them. However activity levels were high in both single and group tests, and the opportunity to escape interactions through the terrestrial bridge was not used. Further exploration of this phenomenon would be worthwhile, including testing for a density that caused emigration to rise again, supporting Enfjäll & Leimar, (2005) who proposed that density-dependent dispersal can occur at both high and low densities in the same species.

Crayfish size proved an important predictor in these experiments, with larger animals taking part in fewer interactions and being less active, and a larger range of carapace lengths corresponding to less activity within the crayfish groups. This suggests dominance hierarchies were quickly established in groups with large animals, and these large animals did not then participate in interactions, nor further explore the arena. Food was not provided within the experiment tanks and the research occurred outside of the breeding season, therefore the resource most likely contested was refuges. Once settled, the larger crayfish perhaps did not need to move or interact. In contrast, smaller crayfish may have needed to participate in interactions to settle dominance. The finding is at odds with Fero *et al.*, (2007) who found larger crayfish were found less in burrows which they postulated was due to the requirement to maintain hierarchies. This experiment used an abundance of refuges, in order to minimise stress on the animals, which may have reduced the pressure to defend burrows. Further research into activity budgets of dominant and subordinate animals under different resource availabilities could add more to understanding on this behaviour.

This study was designed to minimise differences in size, and thus legitimately could have left size out of analyses. However, significant responses of the activity indicators to carapace length demonstrate that experiments can be affected by variability in individuals even when standardisation is attempted. The prior understanding that size was very important in crayfish interactions (Gherardi *et al.*, 2006; Davis & Huber, 2007; Breithaupt *et al.*, 2016), and thus inclusion in the models proved appropriate for this study.

Activity levels did show some response to sex ratio. Having more males in a tank corresponded with more of the four crayfish being active during the observation period. However this difference emerges because of the marked difference in numbers active when there are males *at all*; female-only groups were significantly less active than any of the other groups with a lower range of activity levels (Figure 4-5). From these results it is not shown whether the extra movement is because the females move more in the presence of males or if it is the males moving more causing the higher activity score. The sex ratio with the highest activity was three males to one female, so in this trial males must have been moving too. This could corroborate findings that males are more mobile (Wutz & Geist, 2013b) and could be a measure of higher male agonism (Woodlock & Reynolds, 1988a) as the failure to settle could be due to males competing. The interaction measure did not indicate a significant relationship between sex and number of interactions, although the activity could be a result of animals avoiding encounters.

When looking at the focal animal activity scores across the focussed observation period, once size had been taken into account, males actually proved slightly *less* active than females (Figure 4-7). The focal animal model is a more sensitive measure as it accurately describes which animal is active, unlike the group activity scores which noted solely how many crayfish were active. As the males in this experiment were size matched to the females, applying this result to the previous finding suggests that the presence of males does cause the females to move more and to an extent vice versa.

This tension in the results is interesting and more work is needed to further assess the factors that link to greater activity once size has been taken into account. These results seem to suggest that while size increases activity, being male decreases it, albeit marginally.

Looking at the focal animal alone, activity was not associated with the sex ratio of the crayfish in the tank with the focal animal. It was however associated with an interaction between sex and sex ratio of the crayfish in the whole system; i.e., with those the focal animal was sharing water. Females were less active when there were more females across the system, whereas males were more active when there were more males across the system (Figure 4-6). Therefore, while being male was a predictor of slightly lower activity, having more males within the system led to higher activity, both within females and males.

There is also a somewhat contradictory result here; if sex ratio was important to activity levels, it would seem likely that the conspecifics the focal animal meets directly (i.e., in the tank) would be as important as those the focal animal can detect through waterborne substances (i.e., through the system). However, the effect was very slight, and it could be that the greater range of sex ratios in the system (eight possible ratios in the system compared with four possible ratios in tank) enabled a very small impact to be detected better.

The implications of this finding are important. Experimentally, running aquatic tests on animals that are run through a communal water supply could introduce a source of error as the animals are not isolated as may be assumed. Furthermore, maintaining captive populations with communal water filtering should take into account that the animals are able to detect conspecifics and the implications of this (stress, breeding condition, growth suppression) understood.

The importance of pheromonal communication in crayfish is well understood (Zulandt Schneider *et al*., 1999; Bergman *et al*., 2003). Finding a response in the focal animal to crayfish not within its visual range can only be explained through a waterborne substance, most likely an 'infochemical' (Dicke & Sabelis, 1988). This could be a pheromone deliberately released in order to communicate information to the benefit of both sender and receiver. Crayfish use pheromones in dominance (Zulandt Schneider *et al*., 1999) and sexual communication (Aquiloni *et al*., 2009). Crayfish in neighbouring tanks could have been 'eavesdropping' on pheromones released between tank conspecifics.

Alternatively, the infochemical could represent an 'alarm substance' (Breithaupt *et al*., 2016) released by crayfish in neighbouring tanks in response to interactions occurring in those tanks. Alarm substances have been shown to have an effect on crayfish with animals responding to solutions from injured conspecifics by reducing their activity (e.g. Acquistapace *et al*., 2004) presumably to avoid perceived predation risk. This thesis found an increase in activity in crayfish to an increased ratio of males in the system. A relationship between the number of interactions and sex ratio was not found, which suggests alarm substances were not the cause. In addition, if the increase in males was causing a raised alarm substance concentration you would expect to see a reduced activity, when in fact the opposite was seen.

There was a small but significant impact of date on the number of crayfish active, with crayfish becoming marginally less active throughout the experiment period. This could represent a genuine response to changing seasons, although it could be expected that activity levels would increase as the crayfish got closer to the breeding season (Woodlock & Reynolds, 1988a). An alternative explanation would be that the crayfish had formed familiarity to the tank set up and/or tank conspecifics so there was less exploratory behaviour and/or interactions.

This study demonstrates the influence of surrounding conspecifics to crayfish behaviour, both directly and through waterborne communication. Size proves a very important parameter when considering crayfish activity. The difference in activity levels within different sex ratios, particularly how the different sexes are affected by sex ratio, is of interest for behavioural ecology as an example of how intrinsic and extrinsic factors interact. It also has direct relevance for captive management and conservation translocation protocols. Further research could illuminate in more clarity how these effects are linked to parameters such as sex ratio and density, and differences between sexes. Implications are relevant to both conservation projects and further experimental studies.

5 Discussion

"Every scrap of biological diversity is priceless, to be learned and cherished, and never to be surrendered without a struggle"
E.O. Wilson, (1992)

White-clawed crayfish *Austropotamobius pallipes* are in serious decline in the UK and across their range. Habitat loss, change and pollution are all implicated but it is the invasive crayfish species, such as the signal crayfish *Pacifastacus leniusculus* in the UK, which is the fundamental cause of the decline, through transmission of crayfish plague *Aphanomyces astacus* and competitive exclusion.

Scientists are searching for solutions to biodiversity losses, and improvements in the cross over between practical conservation and scientific evidence are enabling targeted action to be taken. Efficacy of trapping signal crayfish *P. leniusculus* using different trap types (Green *et al*., 2018) and long term identification through tagging of white-clawed crayfish *A. pallipes* (Nightingale *et al*., 2018) are just two examples of current science enabling better crayfish conservation in the UK.

5.1 Key findings

A higher percentage of white-clawed crayfish *A. pallipes* moved onto a terrestrial environment than either signal crayfish *P. leniusculus* or red swamp crayfish *Procambarus clarkii*. Of animals that did move into the terrestrial environment, both signal crayfish *P. leniusculus* and red swamp crayfish *P. clarkii* made more frequent emergences than white-clawed crayfish *A. pallipes*. White-clawed crayfish *A. pallipes* were faster on land than signal crayfish *P. leniusculus* and red swamp crayfish *P. clarkii*. Fewer white-clawed crayfish *A. pallipes* made a terrestrial exit when in groups of four than when tested individually.

White-clawed crayfish *A. pallipes* show a peak of activity at 110 minutes after darkness, which then falls again before plateauing then falling to pre-darkness levels 600 minutes after dark.

Larger white-clawed crayfish *A. pallipes* take part in less interactions with conspecifics, and are less active. Having a larger range of carapace lengths in a group was associated with lower activity levels within the group. Having a higher proportion of males within a tank corresponded with greater activity levels. Both males and females showed higher activity levels in the presence of a higher proportion of males across the experimental system. Males themselves were slightly less active than females.

5.2 Implications

In chapter 3, it was found that *A. pallipes* are far more inclined to use a terrestrial bridge within their environment than was predicted by experienced aquarists and existing research. An immediate and important outcome of this is to review captive enclosure design and ensure lids are in place. More esoteric questions remain: why do crayfish move onto a terrestrial environment? Was the dispersal due to random movement or a deliberate choice to exit the tank? The *A. pallipes* that did move, moved on average 2.8 times, suggesting the movement was not physiologically too challenging for them, but when contrasted with the invasive species potential (signal crayfish *P. leniusculus* 8.3 ± 6.9, red swamp crayfish *Procambarus clarkii* 8.3 ± 7.4) it is clear they are less inclined to use the terrestrial environment frequently. This is almost certainly a reflection of the environment these species evolved to occupy, with *A. pallipes* being suited to permanent water bodies, and the invasive species able to occupy more fluctuating even ephemeral aquatic environments such as swamps.

The ability to move terrestrially has certainly facilitated the escape and spread of *P. leniusculus* populations in the UK and has important implications for future management. Isolation of watercourses may not be sufficient for an ark site when the invading species can walk nearly a kilometre on land (Souty-Grosset *et al.*, 2016b); equally weirs may provide a temporary barrier to upstream dispersal of crayfish (Kerby *et al*., 2005), but when the invasion front reaches high crayfish density it seems likely that sooner or later an invasive crayfish will walk on land to evade the barrier. Equally if native animals are likely to walk from an ark site they are then exposed to risks not necessarily considered in ark site selection. Thus these findings are of importance to conservation management strategies.

Research into differences in behaviour between invasive and indigenous species opens the possibility that a crucial behavioural difference will be discovered that can be used in control programmes. In this case the finding that *A. pallipes* do move terrestrially more than thought removed this difference as one that can be used in control programmes, in fact making control and isolation more difficult.

The finding that crayfish are more likely to move terrestrially when in groups of four than when kept individually (Thomas, 2018) was mirrored in this thesis although not as a deliberate matched experiment. Thomas (2018) concludes that animals are too busy interacting or avoiding each other to find and use the terrestrial option but an equally compelling argument is that the animals are moving in order to find other crayfish. Negative density-dependence can occur when there are advantages to group living, such as reduced predation, or increased feeding opportunities (Matthysen, 2012). Alternatively animals may use the presence of other animals as a measure of habitat quality (Danchin et al., 2014). To further investigate the function behind this reduced movement in groups, an experiment with crayfish that can see and chemically detect each other but not interact (for example in cages within a larger tank) could indicate a density at which terrestrial movement is more likely, or indeed a low and a high density as proposed by Enfjäll & Leimar, (2005).

Whatever the function of the movement, the finding does suggest indigenous crayfish should not be kept singly, nor released in low numbers so that terrestrial exploration occurs. Terrestrial movement carries high risks for crayfish of desiccation and predation which should be minimised to facilitate population expansion.

The differences in terrestrial movement seen between the three species is of interest to conservation and behavioural ecology alike. Another useful avenue of research is to look at whether the animals tested in this experiment, (which were sourced from the invading populations), would behave differently to animals sourced from the native range of these species. Invasive species are often described as being more exploratory, more aggressive, and more successful than when in their native range (Pintor & Sih, 2009). This is often attributed to the 'enemy release hypothesis' (Williamson, 1996) whereby the invasive animal is no longer constrained by the interspecific pressures under which it evolved. An alternative explanation is that of the 'selective filter' (Pintor & Sih, 2009; Chapple *et al*., 2012) whereby the invasion process itself selects for animals with more invasive characteristics. An interesting line of research would be to compare terrestrial movement between crayfish from the native and invasive range, and this capability could be correlated with other invasion-potential characteristics such as aggression or boldness. Comparisons could be made between recent invasions with those that have been inhabiting UK waters since the 1970s.

Activity within the aquatic environment was found to respond to intrinsic factors such as size and sex, and extrinsic factors such as sex ratios in some interesting ways. Larger crayfish moved less, and took part in fewer interactions, and males were slightly less active. Males and females responded differently to a sex ratio with an increased number the same sex as them. Seen another way, both sexes responded with higher activity when there were more males in the tank. Size is important for crayfish (e.g. Ghia *et al*., 2009), and knowing this the carapace length range was minimised and size and range were included in the statistical modelling. Some results were contrary to expectations; in-situ studies find that males are more mobile (Wutz & Geist, 2013b) whereas this study found that males were seen moving slightly less than females.
Females appear to move more in the presence of males, all female groups were less active than either all male groups or mixed groups. If this movement is due to an inability to settle in the presence of males it could be seen as male interference, which could be a cause of reduced fitness in captive crayfish populations. Skewed sex ratios are often recommended in captive breeding or aquaculture programmes (Celada *et al*., 2005) in order to maximise offspring output against resource input. This thesis

occurred outside of the breeding season (October/November) so this background interference is without the breeding impetus of male:male competition or attempted matings. This may have implications for the management of captive populations of *A. pallipes*, or for release demographics to ark sites, when fitness needs to be maximised.

Higher activity per se is not necessarily a negative measure, in fact crayfish have been shown to reduce their activity in the presence of predatory cues (Hazlett & Schoolmaster, 1998) or alarm substances (Acquistapace *et al.*, 2005). Thus the decreased activity in groups of females only could have indicated they were more alert to risk when not in the presence of males. However, in the absence of predators it seems more likely the females were moving more in order to avoid males, as seen in the phenomenon whereby female crayfish are much less likely to enter a trap with a male already present (Ogle & Kret, 2008). The measure of activity used in this thesis is not sensitive enough to state definitively the cause of the inactivity.

This thesis also indirectly considered how the experiment crayfish were responding to aquatic infochemicals in the experiment system set up. Interestingly, males and females showed a response to the sex ratio of the system, indicating that they were detecting pheromones from conspecifics across the experiment. Two important conclusions can be drawn from this. Firstly research on aquatic organisms must take into consideration the possibility of aquatic infochemicals such as pheromones and alarm odours when designing trials. Secondly, aquaculture and conservation breeding programmes should acknowledge that information transfer can occur across water systems which could be to the detriment of productivity if reduced or raised activity causes lower growth and reproduction.

This thesis studied crayfish in an ex-situ environment in order to facilitate the experiment design of replications and variable manipulation. In-situ experiments provide greater ecological validity, and can provide complimentary evidence to ex-situ work (e.g. Davis & Huber, 2007). Intensive monitoring of field sites with *A. pallipes* may yield observations of terrestrial movement, although the difficulties of observing or filming at night and at the interface of the aquatic environment make this a problematic experiment. Population demographics can be studied in a discrete population, such as an ark site through regular trapping and marking of individuals. This could be coupled with underwater filming to determine local population demographics and activity levels. It would be interesting to see if crayfish aggregate into preferred sex ratios in a free-living environment.

5.3 Conclusion

This thesis has explored inter-species differences in terrestrial movement in native *Austropotamobius pallipes* and invasive *Pacifasctacus leniusculus* and *Procambarus clarkii* crayfish and found significant differences in both tendency and frequency of movement onto a terrestrial environment. These findings are relevant to conservation practitioners, through several aspects of conservation strategies, and of interest to behavioural ecologists. The function of terrestrial movement as dispersal is discussed and considered in light of evolutionary and anthropogenic histories.

The thesis further examined terrestrial movement and aquatic activity in *A. pallipes*, finding intrinsic and extrinsic factors that predict activity levels and interaction frequencies. Again, the findings are relevant to managers of captive populations and for conservation translocations, as well as being of interest to behavioural ecologists. Implications of the findings were considered in light of behavioural, physiological and ecological knowledge of the species.

Each of the findings above add a little more to the understanding of *A. pallipes* behaviour. While there are still white-clawed crayfish in streams and breeding programmes, it behoves us as researchers to try to solve the anthropogenic-induced riddle of how to retain the UK native crayfish in the face of its ecological nemesis.

5.4 Recommendations

From the results of this thesis I would make the following recommendations;

High priority

Research;

- Investigate a range of densities at which crayfish make a costly terrestrial dispersal decision, finding minimum and maximum group size to inform release population size.
- Further investigate the impact of sex ratio on activity levels to measure fitness effects (through reduced or increased activity) on males and females from skewed sex ratios with links to activity levels in a wild setting, and over a longer time period to include breeding seasons
- Investigate possibility of an aquatic infochemical that would reduce activity in invasive species without affecting native stocks.

Conservation

- Ensure husbandry guidelines encourage a lidded design on tanks of captive populations
- Ensure release populations are in large groups, with a preference for a higher female to male sex ratio

Medium priority

Research

- Investigate effect of sex ratio on activity contrasting effect of physical contact, visual contact and water sharing. Give females the choice to select different sex ratio environments.
- Follow up release groups to monitor dispersal from release point and any aggregations of males/females indicating local sex ratio preferences

Conservation

- Review ark site selection criteria for barriers to terrestrial dispersal
- Model changes to permanence of crayfish habitat due to climate change and potential for native animals to respond through terrestrial movement.

Low priority

Research

- Investigate size class differences in terrestrial movement – is there a size at which this starts and is there a size at which this becomes less likely?
- Investigate interactions within *A. pallipes* conspecifics looking for predictors of initiator and outcome

Conservation

- Model effect of artificial selection on captive breeding programmes minimising effects on survivability upon release

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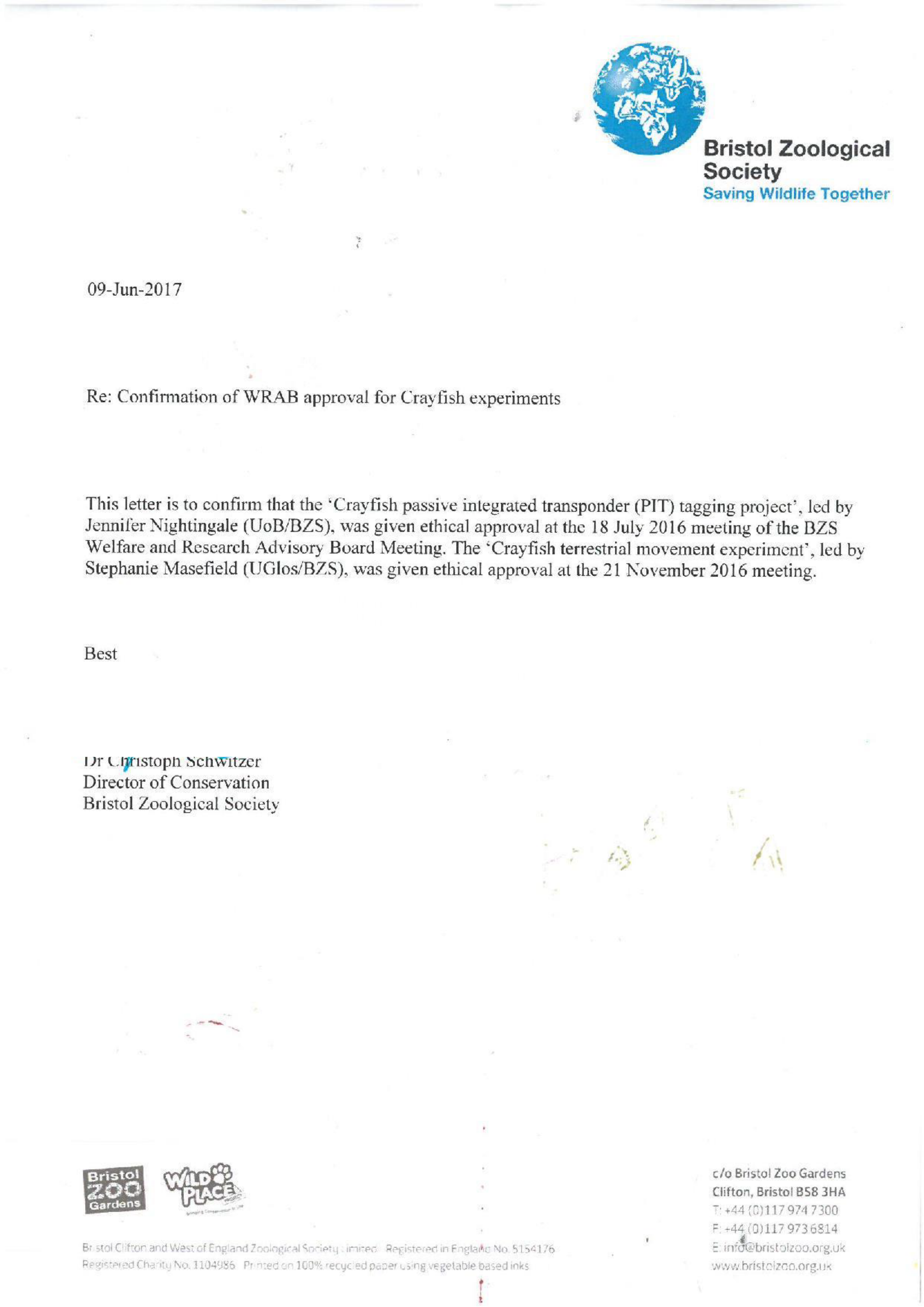
Appendices

Appendix 1 Ethics approval



Bristol Zoological Society
Saving Wildlife Together

09-Jun-2017

Re: Confirmation of WRAB approval for Crayfish experiments

This letter is to confirm that the 'Crayfish passive integrated transponder (PIT) tagging project', led by Jennifer Nightingale (UoB/BZS), was given ethical approval at the 18 July 2016 meeting of the BZS Welfare and Research Advisory Board Meeting. The 'Crayfish terrestrial movement experiment', led by Stephanie Masefield (UGlos/BZS), was given ethical approval at the 21 November 2016 meeting.

Best

Dr Christoph Schwitzer
Director of Conservation
Bristol Zoological Society

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REC.17.71.3 **[MASEFIELD, Stephanie] (REC/27/17)**
Factor affecting overland movement of the endangered white-clawed crayfish Austropotamobius pallipes

Noted: the Researcher was not present at the meeting but the Chair outlined clarification that had been received in relation to the research ethics approval form, prior to the Committee. It was confirmed that:

the application was previously approved by the Bristol Zoological Society (BZS) Welfare and Research Advisory Board (WRAB) on 1 November 2016;

BZS operated within the profession's organisational and ethical guidelines as well as the relevant legal frameworks for animal research that did not require scrutiny of projects involving invertebrates; BZS's consideration of the project through its ethical oversight provisions was therefore in excess of legal requirements;

although a free-standing commercial body, the BZS WRAB maintained operational links with Bristol University;

the project was a collaborative project involving other universities, including Cardiff;

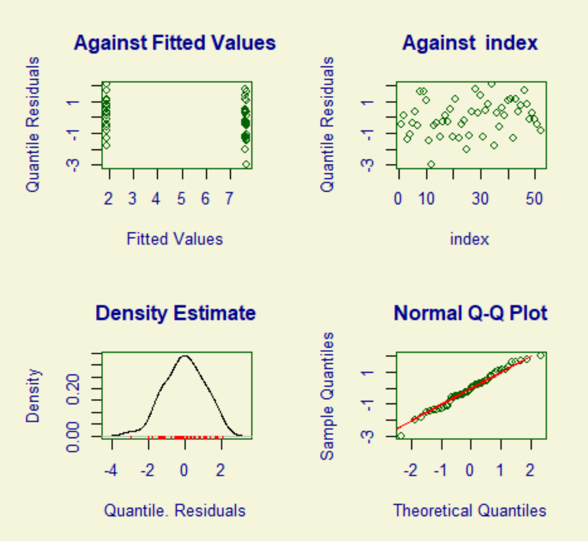
the University's policy was that where another university or recognised REC had approved a project, that that approval was taken as sufficient. The Chair was therefore satisfied that BZS WRAB approval of the project fell within the University's policy that approval by another suitable REC meant that the project could be classified as approved.

REC.17.71.3.2 Resolved: REC noted approval of the project.

Appendix 2 Model validation

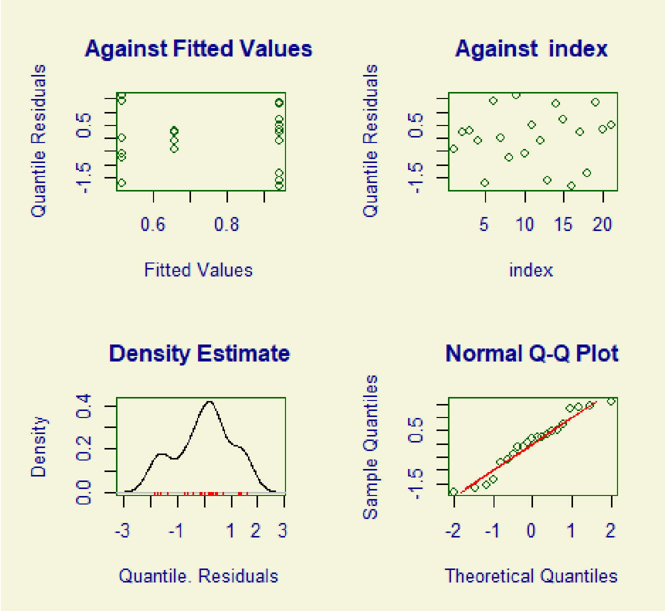
GAMLSS model for effects of size, sex and species on likelihood and frequency of terrestrial emergences

```
Family:  c("ZANBI", "Zero altered negative binomial type I")

Call:  gamlss(formula = Totalexits ~ Species, nu.formula = ~1,
    family = ZANBI(), data = na.omit(dframe1), mu.formula = ~Species +
        Sex + sCL, trace = FALSE)


Mu link function:  log
Mu Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)       0.6462     0.3630   1.780  0.08151 .
SpeciesRedswamp   1.3871     0.5133   2.703  0.00954 **
SpeciesSignal     1.3808     0.4917   2.808  0.00724 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

------------------------------------------------------------------
Sigma link function:  log
Sigma Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  -0.2657     0.5977  -0.445    0.659

------------------------------------------------------------------
Nu link function:  logit
Nu Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   0.0000     0.2774       0        1

------------------------------------------------------------------
No. of observations in the fit:  52
Degrees of Freedom for the fit:  5
      Residual Deg. of Freedom:  47
                      at cycle:  7

Global Deviance:     198.4273
            AIC:     208.4273
            SBC:     218.1835
```



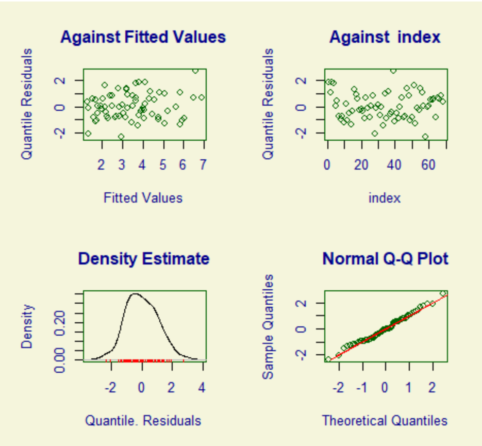
GAMLSS Walking speed

```
Family:  c("NO", "Normal")

Call:  gamlss(formula = Walking.speed ~ Species, family = NO(),
    data = na.omit(dframe1), mu.formula = ~Species +          Sex + sC
L, trace = FALSE)

Fitting method: RS()

-------------------------------------------------------------------
Mu link function:  identity
Mu Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)      0.94203    0.06002  15.695 1.51e-11 ***
SpeciesRedswamp -0.28502    0.11623  -2.452 0.025303 *
SpeciesSignal   -0.42474    0.10103  -4.204 0.000597 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

-------------------------------------------------------------------
Sigma link function:  log
Sigma Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  -1.6141     0.1543  -10.46 7.97e-09 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

-------------------------------------------------------------------
No. of observations in the fit:  21
Degrees of Freedom for the fit:  4
      Residual Deg. of Freedom:  17
                      at cycle:  2

Global Deviance:     -8.196073
            AIC:     -0.1960732
            SBC:     3.982017
```



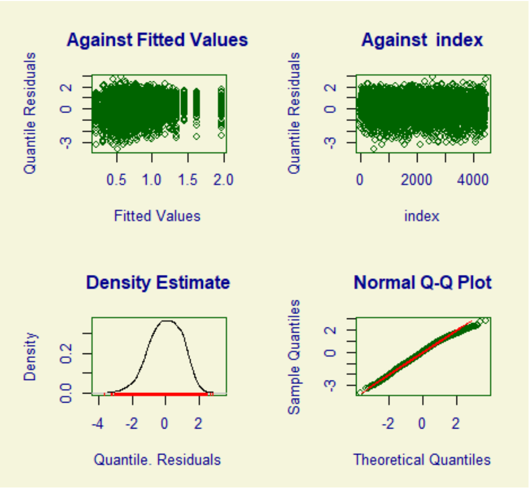
GALMSS sex ratio: number of interactions

```
Family:  c("ZIP", "Poisson Zero Inflated")

Call:  gamlss(formula = NumberofInteractionswithFocalAnimal ~
    CarapaceLength + RangeCarapaceLength + RatioSameSexTank +
        Sex + Sex:RatioSameSexTank + random(FocalCrayfish),      famil
y = ZIP(), data = dframe1)

Fitting method: RS()

-------------------------------------------------------------------
Mu link function:  log
Mu Coefficients:
                      Estimate Std. Error t value Pr(>|t|)
(Intercept)           13.71561    2.70605   5.068 4.96e-06 ***
CarapaceLength        -0.37735    0.08322  -4.534 3.22e-05 ***
RangeCarapaceLength   -0.02343    0.05071  -0.462    0.646
RatioSameSexTank      -0.28942    0.33623  -0.861    0.393
SexM                  -0.11045    0.24417  -0.452    0.653
RatioSameSexTank:SexM  0.48314    0.41931   1.152    0.254
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

-------------------------------------------------------------------
Sigma link function:  logit
Sigma Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  -1.6589     0.3956  -4.193 0.000102 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

-------------------------------------------------------------------
NOTE: Additive smoothing terms exist in the formulas:
 i) Std. Error for smoothers are for the linear effect only.
ii) Std. Error for the linear terms maybe are not accurate.
-------------------------------------------------------------------
No. of observations in the fit:  68
Degrees of Freedom for the fit:  13.6444
      Residual Deg. of Freedom:  54.3556
                      at cycle:  7

Global Deviance:     281.7976
            AIC:     309.0864
            SBC:     339.3703
*******************************************************************
```



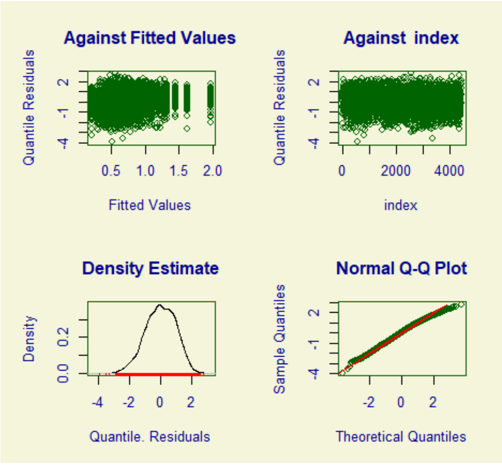
GAMLSS sex ratio: group activity (tank)

```
Family:  c("ZAP", "Poisson Zero Inflated")

Call:  gamlss(formula = NumberofCrayfishActiveinFocussedHour ~
    TotalCarapaceLength + RangeCarapaceLength + RatioMale +
        Days + random(Run), family = ZAP(), data = dframe1)

Fitting method: RS()

-------------------------------------------------------------------
Mu link function:  log
Mu Coefficients:
                     Estimate Std. Error t value Pr(>|t|)
(Intercept)         -0.681687   1.014151  -0.672 0.501508
TotalCarapaceLength  0.009571   0.006662   1.437 0.150917
RangeCarapaceLength -0.047492   0.017960  -2.644 0.008214 **
RatioMale            0.308038   0.082146   3.750 0.000179 ***
Days                -0.003689   0.001652  -2.233 0.025609 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

-------------------------------------------------------------------
Sigma link function:  logit
Sigma Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) -0.87765    0.03303  -26.57   <2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

-------------------------------------------------------------------
NOTE: Additive smoothing terms exist in the formulas:
 i) Std. Error for smoothers are for the linear effect only.
ii) Std. Error for the linear terms maybe are not accurate.
-------------------------------------------------------------------
No. of observations in the fit:  4420
Degrees of Freedom for the fit:  58.60217
      Residual Deg. of Freedom:  4361.398
                      at cycle:  10

Global Deviance:     10613.2
            AIC:     10730.41
            SBC:     11105.1
*******************************************************************
```



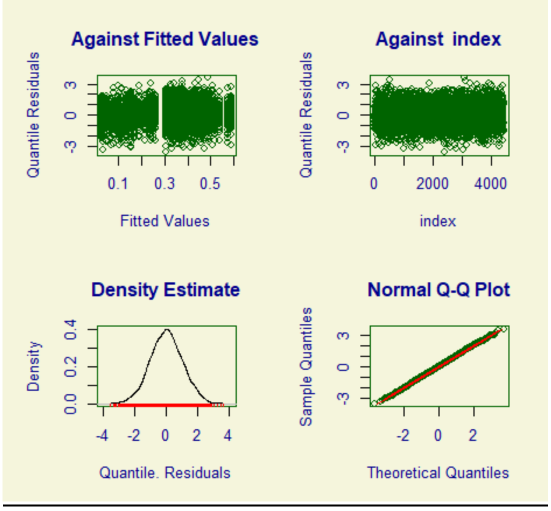
GAMLSS sex ratio: group activity (system)

```
Family:  c("ZAP", "Poisson Zero Inflated")

Call:  gamlss(formula = NumberofCrayfishActiveinFocussedHour ~
    TotalCarapaceLength + RangeCarapaceLength + RatioMaleSystem +
        Days + random(Run), family = ZAP(), data = dframe1)

Fitting method: RS()

------------------------------------------------------------------
Mu link function:  log
Mu Coefficients:
                     Estimate Std. Error t value Pr(>|t|)
(Intercept)         -0.253159   1.050623  -0.241   0.8096
TotalCarapaceLength  0.007959   0.006807   1.169   0.2424
RangeCarapaceLength -0.039200   0.018217  -2.152   0.0315 *
RatioMaleSystem      0.067087   0.244742   0.274   0.7840
Days                -0.004211   0.001656  -2.544   0.0110 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

------------------------------------------------------------------
Sigma link function:  logit
Sigma Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) -0.87765    0.03303  -26.57   <2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

------------------------------------------------------------------
NOTE: Additive smoothing terms exist in the formulas:
 i) Std. Error for smoothers are for the linear effect only.
ii) Std. Error for the linear terms maybe are not accurate.
------------------------------------------------------------------
No. of observations in the fit:  4420
Degrees of Freedom for the fit:  59.09215
      Residual Deg. of Freedom:  4360.908
                      at cycle:  10

Global Deviance:     10612.72
            AIC:     10730.91
            SBC:     11108.74
******************************************************************
```



GAMLSS sex ratio: focal animal activity (tank)

```
Family:  c("BI", "Binomial")

Call:  gamlss(formula = YN ~ Sex + CarapaceLength + RangeCarapaceLengt
h +
    RatioSameSexTank + Sex:RatioSameSexTank + random(FocalCrayfish) +
    random(fRun), family = BI(), data = dframe1)

Fitting method: RS()

-------------------------------------------------------------------
Mu link function:  logit
Mu Coefficients:
                        Estimate Std. Error t value Pr(>|t|)
(Intercept)           11.7250803  1.2059618   9.723   <2e-16 ***
SexM                  -0.0583311  0.1126621  -0.518    0.605
CarapaceLength        -0.3861053  0.0366451 -10.536   <2e-16 ***
RangeCarapaceLength   -0.0006297  0.0244992  -0.026    0.979
RatioSameSexTank      -0.1394448  0.1372636  -1.016    0.310
SexM:RatioSameSexTank  0.2772092  0.1837093   1.509    0.131
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

-------------------------------------------------------------------
NOTE: Additive smoothing terms exist in the formulas:
 i) Std. Error for smoothers are for the linear effect only.
ii) Std. Error for the linear terms maybe are not accurate.
-------------------------------------------------------------------
No. of observations in the fit:  4420
Degrees of Freedom for the fit:  67.39547
      Residual Deg. of Freedom:  4352.605
                      at cycle:  3

Global Deviance:     4825.303
            AIC:     4960.094
            SBC:     5391.013
*******************************************************************
```



GAMLSS sex ratio: focal animal activity (system)

```
Call:  gamlss(formula = YN ~ Sex + CarapaceLength + RangeCarapaceLengt
h +
    RatioSameSexSystem + Sex:RatioSameSexSystem + random(FocalCrayfish
) +
    random(fRun), family = BI(), data = dframe1)

Fitting method: RS()

-------------------------------------------------------------------
Mu link function:  logit
Mu Coefficients:
                        Estimate Std. Error t value Pr(>|t|)
(Intercept)             10.77874    1.30249   8.276  < 2e-16 ***
SexM                    -1.09707    0.29665  -3.698  0.00022 ***
CarapaceLength          -0.35315    0.03778  -9.347  < 2e-16 ***
RangeCarapaceLength      0.02200    0.02528   0.870  0.38424
RatioSameSexSystem      -0.57589    0.42358  -1.360  0.17403
SexM:RatioSameSexSystem  2.47133    0.59385   4.162 3.22e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

-------------------------------------------------------------------
NOTE: Additive smoothing terms exist in the formulas:
 i) Std. Error for smoothers are for the linear effect only.
ii) Std. Error for the linear terms maybe are not accurate.
-------------------------------------------------------------------
No. of observations in the fit:  4420
Degrees of Freedom for the fit:  67.27485
      Residual Deg. of Freedom:  4352.725
                      at cycle:  3

Global Deviance:     4825.176
            AIC:     4959.726
            SBC:     5389.874
*******************************************************************
```