Evaluating the population control of invasive crayfish using removals and male sterilisation

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Abstract

This research tests the control of invasive American signal crayfish *Pacifastacus leniusculus* using a combination of intensive trapping (baited traps and artificial refuge traps: ARTs) and the mechanical sterilisation and release of large males (Sterile Male Release Technique: SMRT) using both field studies (on the River Barle, Devon, Southern England) and laboratory-based experiments in controlled conditions. In the laboratory experiments, no differences were found in the ability of sterilised males to win dominance contests and compete for mates. Sterilised and non-sterilised males were equally likely to guard their mates post copulation, and females had low levels of promiscuity. When testing the functionality of the sterilisation technique, sterilised males were found to deposit significantly less spermatophore on females and it was placed less accurately. Full gonopod regeneration took at least two years and the re-trimmed gonopods were frequently deformed. However, the resultant brood sizes from these males did not decrease significantly from non-sterilised males in either the laboratory experiments or in the field study. Sterilised male recapture rates in the field were low and its low effectiveness was potentially influenced negatively by male age and migration.

The field studies on the River Barle compared the efficacy of the two trap types and the population responses of male sterilisation and intensive trapping. Artificial refuge traps (ARTs) had a significantly higher catch per unit effort (CPUE) and were an effective way of capturing females and small individuals when compared with baited funnel traps. However, after six years of the application of a management programme involving the

combined use of removals with ARTs and SMRT, the overall CPUE of the crayfish population was not significantly reduced. However, by year 6, the CPUE of the smallest crayfish size class (≤ 24 mm CL) had decreased significantly, along with some shifts in the size and sex structure of the population and with some evidence of reduced reproductive efficiency. A number of potential reasons for the lack of more substantive population responses were identified, including sterilised male survival and low trapping effort, and will be the subject of ongoing studies. Overall, these results highlight the difficulty of managing invasive crayfish in open lotic systems.

Contents

List of figures

List of tables

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Author's declaration

I confirm that the work presented in this thesis is my own work, although as an integrated thesis my supervisory team have collaborated on Chapters 2 – 5 for publication.

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Chapter 1. Introduction

1.1 Overview

This first chapter outlines the main themes of the thesis, invasive crayfish, their impacts and their management followed by the research rationale and aims and objectives. The thesis is presented in an integrated format, whereby material is incorporated in a style suitable for submission and publication in a peer-reviewed journal. Thus, the data chapters (Chapters 2 to 5) are each presented as original and complete pieces of research, either as the actual, published paper or as a manuscript suitable for submission to a relevant scientific, peer review journal. This format has been selected as the research focuses on the practical application of an applied technique where dissemination of the findings to practitioners is of high importance. Finally, Chapter 6 discusses the implications of the research and concludes the thesis. A complete list of references is provided at the end, in order to avoid their replication in the chapters and to improve readability.

1.2 Invasive Alien Species

Invasive non-native species (INNS) are organisms that have been introduced outside their natural range that threaten to damage other species, ecosystems or habitats (NNSS 2020). The term 'invasive' implies that the species has not only been introduced but that it has also established a sustainable population and has dispersed from the introduction site (Colautti and MacIsaac 2004). Of the many species that are introduced outside their natural range, roughly ten percent become established. Most are benign but 10-15% spread to the point of negatively impacting native ecosystems and causing economic and cultural impacts (Twardochleb et al. 2013). They are regarded as being the second greatest contributor to global loss of biodiversity after habitat destruction (Pejchar and Mooney

2009). Examples in the UK include grey squirrel *Sciurus carolinensis* which has adversely affected the native red squirrel *Sciurus vulgaris* in the UK (NNSS 2020); the plant Japanese knotweed *Reynoutria Japonica* which shades native vegetation and damages infrastructure across Europe and the USA (Bailey 2012); and lionfish *Pterois volitans* which is degrading ecosystems along the Atlantic coast of north America through out-competition (USDA 2021).

1.3 Overview of invasive non-native crayfish and their management

Freshwater crayfish belong to the subphylum Crustacea, class Malacostraca and order Decapoda along with lobsters, prawns and crabs. Together with lobsters they belong to the infraorder Astacidae which is divided into two superfamilies, Astacoidea (northern hemisphere: Astacidae and Cambaridae families) and Parastacoidea (southern hemisphere: Parastacidae family). The highest diversity of crayfish are found in south-eastern USA, where some 80% of the cambarid species can be found, and south-eastern Australia which supports the majority of parastacid species (McCormack, 2012).

Non-native crayfish are globally recognised as highly successful invaders (Capinha et al. 2011). Invasive crayfish tend to be faster growing, more aggressive, have longer life spans, are highly fecund, utilise a wide range of feeding habitats and are tolerant to a wider range of environmental conditions than their native counterparts (Doledec and Statzner 2008). Species originating from North America also carry the oomycete pathogen *Aphanomyces astaci* that causes 'crayfish plague', which is generally fatal to Astacid crayfish species outside that continent (Svoboda et al. 2017). As omnivores, invasive crayfish have capacity to disrupt food webs at multiple trophic levels, and they can also alter the hydrology, biochemical recycling and biotic composition of invaded ecosystems (Reynolds and Souty-Grousset

2012). Their impacts include the extirpation of native crayfish species through inter-specific competition and disease (as crayfish plague), declines in fish and macro-invertebrate diversity and abundance, and altered geomorphology of the riparian zone caused by burrowing and bioturbation (Holdich et al. 2014; Gherardi et al.2011; Manfrin et al.2018). These impacts vary with the species of crayfish and the nature of the recipient environment; for example, the red-swamp crayfish *Procambarus clarkii* tends to cause major declines in macrophytes (Harper 1992), while rusty crayfish *Faxonius rusticus* invasions are more likely to affect benthic invertebrate communities (Twardlocheb et al. 2013). The most widespread and impacting invasive crayfish in Great Britain is the American signal crayfish *Pacifastacus leniusculus*, which has been responsible for an 80% decline in the native White-clawed crayfish *Austropotamobius pallipes* since the 1980s (Holdich et al. 2014).

Given the wide-ranging ecological impacts of invasive crayfish, their populations have been subjected to numerous methods for control, containment and eradication (Stebbing et al. 2014; Manfrin et al. 2018). A definitive management method has yet to be found, however, and although there have been some successes in population control and eradication, frequent failures have led to pessimism and subsequent inaction (Gherardi et al. 2011; Stebbing et al. 2014). Reasons for this include removal methods usually being sex or size-biased and thus failing to target all life-stages present in the population (Bomford and O'Brien 1995). Density-dependent compensatory responses, where reduced competition for resources at low population density engenders population growth and spatial expansion, have also been widely reported following removals (e.g. Holdich et al. 2009; Freeman et al. 2010). The research presented in this thesis seeks to address these issues by applying an integrated pest management (IPM) approach to *P. leniusculus* control using three methods which, in combination, aim to target all life stages of crayfish and inhibit density-dependent compensatory effects. Research into the efficacy of the

approach will incorporate a combination of laboratory experiments and long-term field studies on the River Barle, an upland river on Exmoor in south-west England.

1.4 American signal crayfish Pacifastacus leniusculus

The American signal crayfish, from the Astacidae family (Dana 1852) is among the world's most widespread invasive non-native species (Holdich and Reeve 1991; Holdich et al. 2014). Their dorsal surface is typically brownish-tan in colour, although this can be highly variable depending on locality (Larson and Olden 2011). The surface of the carapace and claws are smooth, lacking the pronounced bumps that are typical of other non-native crayfish (Larson and Olden 2011). As with crayfish generally, they have powerful, multi-functional chelae (Figure 1.1).

Figure 1.1 Dorsal a) and ventral b) views of signal crayfish in the River Barle, south-west England. Photos N. Green.

The natural range of *P. leniusculus* is north-western America (Holdich et al. 2014). Its initial introduction into Europe was into Sweden in 1959, from where it has spread to at least 27 European countries (Holdich et al. 2009). It was introduced into Britain from Sweden in the mid-1970s for aquaculture, but rapidly escaped from holding facilities into the wild (Edsman 2004). Through a combination of natural colonisation and accidental

and deliberate introductions it is now widespread in most British river catchments (Holdich et al. 2014; Figure 1.2). It can tolerate a wide range of habitat conditions, including brackish and acidic waters, and those contaminated with heavy metals (Holdich et al. 2014). It can survive out of water and will readily leave watercourses to access new habitats or negotiate barriers to expansion (Krieg et al. 2020).

Figure 1.2 Distribution of *P. Leniusculus* in Great Britain in 2020, where filled circles indicate a record of the species (www.nbnatlas.org)

1.5 Biology of P. leniusculus in England and Wales

Signal crayfish are a fast growing, long lived, early maturing crayfish, reaching sizes of up to 180mm total length and living for up to 16 years (Belchier et al. 1998). Typical sex ratios are 1:1 M:F and they reach sexual maturity within two years, producing on average 150 ova per female per year (Guan and Wiles 1999). They can achieve high densities, with up to 110 individuals per m^2 being reported in British waters (Chadwick et al. 2020). Density can be influenced by calcium and other nutrient levels, food availability, temperature and flow levels (in lotic systems; Holdich et al. 2014) as well as invasion stage (Hudina et al. 2012). However, accurate population estimates are difficult to achieve (Nowicki et al. 2008). Signal crayfish occupy a wide range of habitats in England and Wales (Holdich et al. 2014), achieving the highest densities in lowland mesotrophic rivers and ponds, though they can also reach high densities in upland streams and reservoirs in limestone systems (Chadwick et al. 2020). Where natural shelters are limited, they readily burrow into the banks of waterbodies, behaviour not recorded in their native range (Lewis 2002), and utilise man-made structures such as gabions, pipes and stonework (A. Belloni pers.comm.).

Signal crayfish are generally nocturnal in order to avoid predation (Johnson et al. 2014), with different life stages utilising different habitats. Larger animals, being more resistant to predation by gape-limited predatory fish, select deeper water in order to avoid bird and mammalian predators (Harrison et al. 2006). Conversely, smaller crayfish inhabit shallower waters where they avoid both predation by fish and encounters with larger crayfish (Harrison et al. 2006) but are vulnerable to semi-aquatic predators (Johnson et al. 2014). Juveniles are at the highest risk of predation, resulting in an estimated mortality rate of 90% in their first year of life (Shimizu and Goldman 1983). They take refuge in fine

sands, gravels and dense macrophyte cover (Harrison et al. 2006). Predators of crayfish in British waters include Eurasian otter *Lutra lutra,* grey heron *Ardea cinerea,* cormorant *Phalacrocorax carbo* and numerous fish species including Atlantic salmon *Salmo salar,* brown trout *Salmo trutta,* European eel *Anguilla anguilla* and European perch *Perca fluviatilis* (Blake and Hart 1995; Aquiloni et al. 2010; Nyström et al. 1999). Despite these potentially high numbers of predators, they have not provided sufficient biological resistance to prevent the invasion of British waters by *P. leniusculus* (Reynolds 2011).

Signal crayfish are considered to be omnivorous with the ability to adapt their diet to maximise the exploitation of available resources (Olsson et al. 2009). Guan and Wiles (1998) studied a population in the River Great Ouse in eastern England and revealed that the five most frequently consumed foods were vascular detritus, green algae *Cladophora,* crayfish fragments, *Chironomidae* and *Ephemeroptera*. In total, 22 food groups were utilised including fish, water mites, *Tricoptera, Coleoptera, Plecoptera, Mollusca, Diptera, Asellus, Gammarus, Odonata, Oligochaeta* and insect eggs. There were seasonal and size differences in the ratios of these items consumed, indicating their dietary adaptability (Guan and Wiles 1998). They are also cannibalistic, with the incidence of cannibalism increasing with crayfish size (Houghton et al. 2017). Food consumption, together with habitat utilisation and mating behaviour, is influenced by the presence of dominance hierarchies within populations (Goessmann et al. 2000); these form from agonistic encounters where the 'winners' obtain greater access to food, refuges and mates (Tricario 2016). Size is the main influencing factor on dominance, with larger animals tending to win fights (Bergmann and Moore 2003). These hierarchies influence the behaviour of both dominant and subordinate individuals, with subordinates avoiding

dominant individuals where possible, resulting in these individuals being less able to acquire resources (Ahvenharju and Ruohonen 2007).

Mating occurs between mid-September and late October in Britain, with males exuding spermatophores: tough, protein-based capsules containing spermatozoa (McCormack 2012), via the *vas deferens* and two pairs of modified walking legs known as gonopods. These appendages are used to position the spermatophore on the ventral surface of the female close to the *annus ventralis* (gonopore, Figure 1.3B).

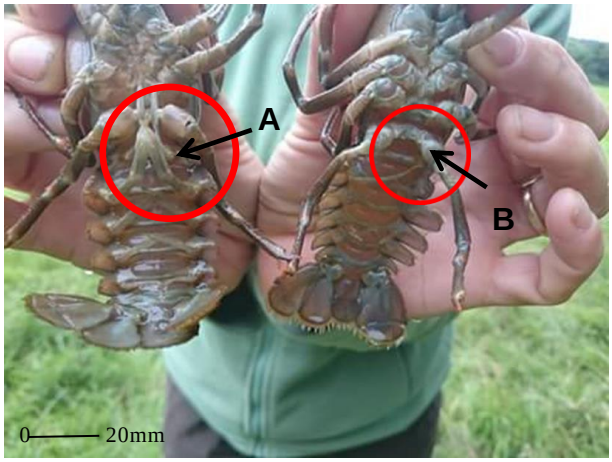


Figure 1.3 Signal crayfish reproductive anatomy: male gonopods (A) and female gonopore (B). Photo N. Green.

The female exudes between 50 and 470 ova (McLay and van den Brink 2015) into a tent of mucous-like material ('glair') within 48 hours of mating (Figure 1.4a). A substance within the glair dissolves the spermatophore wall, allowing the sperm to fertilise the ova (McLay and van den Brink 2015). The developing embryos are then attached to pleopods on the underside of the female abdomen (Figure 1.4b) and incubated throughout the British winter (Holdich et al. 2014; Figure 1.4b). Any unfertilised ova die and drop off within three months of mating and embryos can also be lost due to stress, disease,

fluctuating water temperature and/or flow conditions (Guan and Wiles 1999). Hatching occurs between late March and late July; juveniles are immobile (Figure 1.4c) and remain attached to the mother's pleopods for two moults until they become fully formed up to 14 days later (Figure 1.4d). They then become increasingly independent and leave their mother after a further 1 to 2 weeks. In general, juveniles reach sexual maturity within two years, potentially one year in productive systems, with males growing faster than females (Guan and Wiles 1999). Growth is achieved via moulting, which initially occurs at relatively high frequency (e.g. up to 11 moults/year at 0+ years) but decreases with age (e.g. once per year at 4+ years; McLay and van den Brink 2015).

Figure 1.4 Egg and juvenile development in *P. leniusculus*: a) ova in glair tent shortly after spawning; b) developing embryos; c) Stage 1 juveniles; d) Stage 2 juveniles. Photos N. Green.

Signal crayfish move predominantly by walking on the benthic substrate of waterbodies but are capable of swimming short distances via backward movement ('tail flip'; Bergmann and Moore 2003). Expansion of populations is density-dependent (Holdich et

al. 2014) and occurs in both up- and down-stream directions, though is more pronounced downstream in watercourses with higher gradient and flow (Bubb 2004). Rates of expansion vary widely, from 1km y^{-1} reported in Britain (Holdich et al. 2014), 7 km y^{-1}in Austria and up to 24 km y^{-1} in Croatia (Hudina et al. 2011). Many studies have found that the invasion front comprises mainly large adults, particularly males (e.g. Wutz and Geist 2013; Hudina et al. 2012). Their chelae are used for fighting, defence, capturing and disassembling prey, mating, burrowing, climbing and winnowing sediments in search of food (Holdich et al. 2014). Chelae, legs and other body parts regularly lost, including during agonistic encounters with other crayfish and are regenerated within three to four moults (Dunoyer 2020).

1.5 Environmental and ecological consequences of invasive P. leniusculus

The most pronounced consequence of *P. leniusculus* in invaded freshwaters has been the extirpation of native crayfish species, such as the European white-clawed crayfish (Reynolds and Souty-Grousset 2012). The main driver is crayfish plague, for which *P. leniusculus* is a 'healthy' carrier, transmitting the pathogen to native European crayfish that are then infected, resulting in very high mortality (Edgerton et al. 2004). They can also displace native crayfish through a combination of increased competition for food and shelter (Holdich et al. 2014), predation, and habitat degradation caused by their ecosystem engineering activities (Rice et al. 2014, Harvey et al. 2011).

Multiple negative effects on ecosystem structure and function can also result from *P. leniusculus* invasions, such as their omnivory causing detrimental effects across multiple trophic levels (Guan and Wiles 1998). Crawford et al. (2006) revealed that in sections of the River Clyde, Scotland, *P. leniusculus* reduced macro-invertebrate populations by 40%

and also reduced their diversity. Mathers et al. (2016) also noted significant and persistent temporal shifts in invertebrate community composition following *P. leniusculus* invasion. They can also have negative effects on freshwater fishes, including juvenile salmonids, mainly through predation and competition for refugia (Griffiths et al. 2004; Peay et al. 2009; Galib et al. 2020), with Edmonds et al. (2011) also recording their predation of emerging *S. salar* fry in laboratory experiments. However, studies on impacts of crayfish predation on salmonid eggs and inter-gravel embryos have generally been equivocal, with Gladman et al. (2012) and Edmonds et al. (2011) finding *P. leniusculus* were unable to detect *S. salar* eggs buried in artificial redds, but with Setzer et al. (2011) reporting the loss of 80% of Arctic charr *Salvelinus alpinus* eggs in Lake Vattern, Sweden due to predation.

Signal crayfish can also cause multiple indirect impacts on river ecosystems through burrowing and bioturbation (Rice et al. 2014). Burrowing increases sediment deposition and leads to bank collapses, altering channel morphology, reducing flows and increasing flood risk (Harvey et al. 2011). Their nocturnal movements have been shown to increase suspended sediment loads by at least 20% (Rice et al. 2014). These combined effects can lead to adverse effects on a range of biota, including salmonid fishes, macrophytes and benthic invertebrates, including white-clawed crayfish (Rice et al. 2014). Other effects include the mobilisation of nutrients and contaminants, reduced habitat and water quality (Harvey et al. 2011).

1.6 Management interventions to control populations of Pacifastacus leniusculus

1.6.1 Principles of invasive crayfish control

The eradication of populations of invasive crayfish has rarely been achieved unless biocides have been used (Peay et al.2019). However, biocides are not widely used owing to their actions being non-species specific and the consequent risks to non-target organisms and the environment (Peay et al. 2019). As biocides can thus rarely be used, in most situations population control is used as an alternative that attempts aim to reduce populations below an environmental impact threshold (Stebbing et al. 2014). Moorhouse et al. (2014) revealed that the abundance and diversity of macroinvertebrates increased after intensive removal of *P. leniusculus* from two tributaries of the river Thames, England, with these increases inversely correlated to crayfish densities. However, where control attempts succeed, long term maintenance management is then necessary to inhibit population recovery (Simberloff 2020). For invasive species control programmes to be successful, Bomford and O'Brien (1995) suggest the following criteria have to be met:

1) The rate of removal exceeds the rate of increase at all population densities; i.e. compensatory responses through immigration and/or reproduction must be avoided;
2) immigration should be prevented or reduced;
3) all reproductive animals must be at risk of capture; and
4) animals must be able to be detected at low population densities.

In crayfish, meeting these criteria of Bomford and O'Brien (1995) is considered problematic, especially as most widely used capture (and so removal) methods are biased towards capturing large adults, especially males (Kozack and Policar 2003). Due to the hierarchical structure of crayfish populations and the cannibalistic tendencies of adults

(Houghton et al. 2017), this can lead to compensatory responses through decreased density of dominant males and increased juvenile survival via reduced cannibalism (Houghton et al. 2017). Preventing immigration can be achieved in closed lentic systems but is problematic in open lotic systems. Migration into lower density areas fuels range expansion (Hudina et al. 2011), although there is no firm evidence that crayfish will migrate into areas where trapping has been applied (Moorhouse and McDonald 2011a). The chosen control methods must at least also be capable of monitoring immigration (Moorhouse and McDonald 2011a). Due the relatively rapid growth of *P. leniusculus* and their ability to become sexually mature by the age of two years, all size and sex classes should be at risk of capture (Stebbing et al.2014). Juvenile and young adult crayfish (<35 mm carapace length) comprise up to 80% of population abundance (Chadwick et al. 2020), yet these life stages are particularly difficult to capture using conventional methods (Houghton et al. 2017). Additionally, many conventional control methods also fail to capture individuals when their populations are at low density (Stebbing et al. 2014).

The most successful control attempts (excluding biocide use) are those that have been applied over extended time periods, with substantial reductions in population abundances (but not extirpation) recorded that have enabled some recovery of the impacted native biota, which can help suppress population recovery (e.g. Dana et al. 2010; Hein et al. 2007). However, such methods can require substantial effort and commitment, especially as crayfish density reduces, meaning the surviving individuals become harder to catch due to the density-dependent relationship between catch and effort (Stebbing et al. 2014). Here, the utilisation of natural processes such as predation (Hein et al. 2007), or the assistance of citizen scientists to extend the control period, could contribute towards success (Simberloff 2009). Thus, to be successful, an invasive crayfish control

programme must be able to target all life stages of the population, most likely requiring a multi-method, 'integrated pest management' approach (Manfrin et al. 2019). It must avoid and/or overcome density-dependent responses in surviving individuals and be sustainable in the long-term, potentially utilising citizen scientists and/or incorporating natural processes (Simberloff 2009; Stebbing et al. 2014).

1.6.2 Invasive crayfish policy and legislation

The INNS Strategy for Great Britain (2015) adopts a three-stage hierarchical approach in line with that of the Convention for Biological Diversity: - firstly prevention, through awareness raising such as the Environment Agency's 'Check, Clean Dry' campaign and the development of risk analyses and pathway action plans for activities such as angling (NNSS 2008). Second is early detection, surveillance and rapid response in order to eradicate newly established populations and the third level is mitigation, control and eradication (NNSS 2015). As *P. leniusculus* is well established, the focus is on prevention of new introductions and the development of successful control strategies. Other crayfish species introduced to the UK include narrow-clawed crayfish *Astacus leptodactylus,* red swamp crayfish *Procambarus clarkii* and noble crayfish *Astacus astacus* though none have become widely established, potentially as a result of this strategy.

The legislative basis of controlling invasive crayfish in England and Wales is the Wildlife and Countryside Act (1981 as amended) that prohibits the release of *P. leniusculus* into the wild. The Import of Live Fish Act 1980 (ILFA) Prohibition of keeping of Live Fish (Crayfish) Order 1996 then prohibits the keeping of *P. leniusculus* in captivity except under licence (excluding large areas where the species has become established). It is still

possible to trap *P. leniusculus* both for personal and commercial consumption (subject to Environment Agency consent) and this policy has arguably led to increased introductions of *P. leniusculus* and crayfish plague through the escape of captured animals and illegal introductions, either deliberately or accidentally (Edsman 2004). The EU Regulation (1143/2014) on Invasive Alien Species of Union Concern (2015) imposed restrictions on a list of species of Union Concern which member countries were required to implement. In 2019, the response for England and Wales was The Invasive Alien Species (enforcement and permitting) Order which makes it an offence to release; transport; place on the market (sell); use; exchange or breed live specimens of 5 species of non-native crayfish, including *P. leniusculus*, unless in accordance with a licence or permit (NE 2019).

1.6.3 Crayfish removal methods

Mechanical control

Mechanical control is the removal of crayfish via their direct capture and utilises traps of various designs, nets, electrofishing and manual removal (Stebbing et al. 2014). The most widely used trap is the funnel trap, of which there are several versions available (Figure 1.5) that utilise a bait attractant. Other types of traps are habitat-based attractants, such as the Artificial Refuge Trap (ART; Figure 1.6), nest trap, bracken bundles (Kusabs and Quinn 2009) and microhabitat traps (Parkyn et al. 2011). Manual removal methods include electrofishing and manual search methods, such as kick sampling (Houghton et al. 2017) and the use of quadrats and Surber samplers (DiStefano et al. 2003).

Funnel traps tend to be deployed in deeper, slow flowing waters predominantly inhabited by adult crayfish, creating a bias towards the capture of adults, particularly males (Kozak

and Policar 2003). Once inside, larger males will actively defend a trap against entry by subordinates (Ogle and Kret 2011). Moreover, the relatively large mesh sizes of these traps usually preclude the entrapment of juveniles. Long-term studies have indicated the suppression of populations through this method, but have failed to eradicate the target population (West 2011). Stebbing et al. (2016) found that the likelihood of effective control using funnel traps is influenced by crayfish density and trapping intensity, and recommended year-round trapping at high density for several years. Attempts to improve the efficacy of these traps, such as by reducing the size of the entrance (Stuecheli 1999) and mesh size (Peay and Hiley 2001) in order to capture smaller crayfish, have been attempted with mixed success (Stebbing et al. 2016*)*. Indeed, crayfish can escape from traps even when the trap has been modified to try and prevent this (Kozak and Policar 2003). Funnel traps also tend to require a high resource input, with current regulatory requirements in England requiring their lifting and emptying every 24 hours (Environment Agency 2021).

The use of ARTs has increased in recent years due to the tendency of funnel traps to misrepresent population structure in their capture (Stebbing et al. 2014), resulting in experimentation with alternative methods designed to capture smaller crayfish, such as micro-habitat traps (Kusabs and Quinn 2010; Parkyn 2011) and enclosure traps (Engdahl 2013). In Britain, widely available materials, such as perforated bricks and PVC roofing material, have been trialled (Stancliffe-Vaughan 2015). Habitat-based attractants can also be more cost effective than baited traps, as they can be left *in situ* for longer periods and do not require baiting (Stancliffe-Vaughan 2015).

In general, ARTs consist of a series of tubes on a metal base that are designed to mimic natural refuges (Green, 2016; Figure 1.6). They require securing to the substratum, such as by weighing down with stones, meaning they cannot be easily set or retrieved in waters deeper than 0.5 m (Green 2016). Consequently, they are currently used to target the shallower waters inhabited by sub-adult and juvenile crayfish (Harrison et al. 2006). As they are also not considered a trap until lifted then they are not subject to animal welfare legislation (in England at least) and can be left *in situ* over extended periods without regular checks (O'Connor et al. 2018). Initial pilot trials have suggested ARTs are more efficient than both baited traps and manual searches at detecting low-density crayfish populations in lotic systems (Scott 2012; O'Connor et al. 2018). ARTs also attract more vulnerable classes of individuals that are rarely encountered in baited traps, such as ovigerous females, juveniles and those undergoing ecdysis (Green et al. 2018). The differences between the ability of baited traps and ARTs to capture different size classes of crayfish suggest that their use might be highly complementary, with considerable potential for their combined use to be more effective in the control of *P. leniusculus* than when applied in isolation.

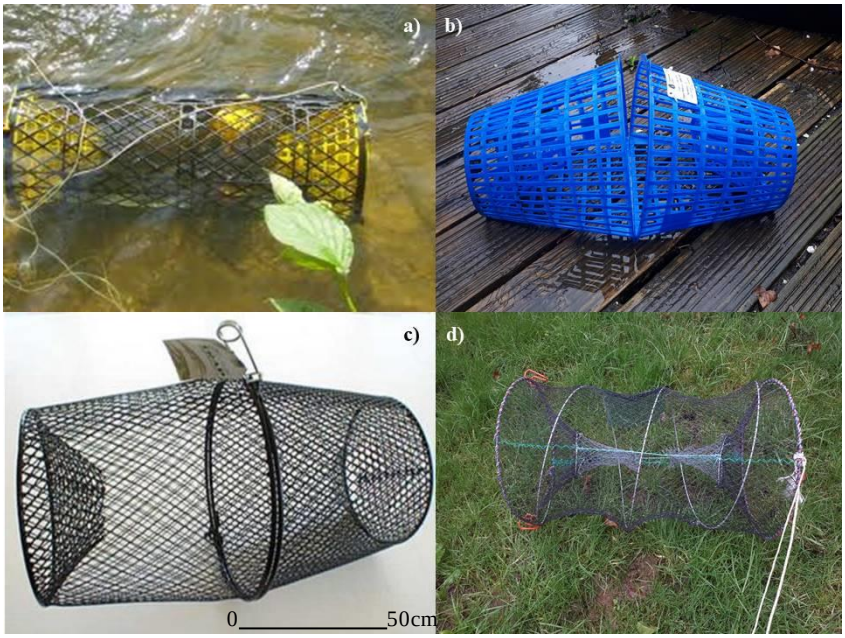


Figure 1.5 Types of funnel trap: a) Trappy trap; b) Fishkit Swedish trap; c) minnow trap; d) collapsible mesh trap. Photos N. Green.

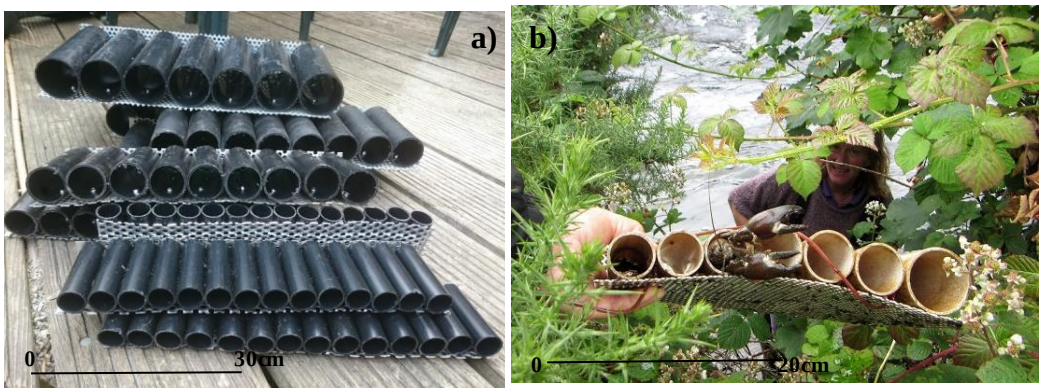


Figure 1.6 a) different sized ARTs used to attract specific size classes and b) Artificial refuge trap lifted from the River Barle, south-west England. Photos N. Green.

Alternative removal methods include nets, although there are few studies outlining their efficacy on crayfish capture and removal. However, the use of nets has shown some potential, with a recent study by Garcia-de-Lomas et al. (2020) revealing that horizontally

hauled sweep netting in pools within streams were selective towards capturing young-of-year (YOY) crayfish and had a higher catch efficiency than funnel traps. Fyke nets are reputedly effective at capturing crayfish in stillwater fisheries in England, although their use is controlled by legislation, requiring consents to be gained before their use; they also require experienced operators which may constrain their use in control programmes (A. Booker pers. comm.).

Other manual capture methods include electrofishing and assorted hand capture techniques, such as searching beneath suitable sized rocks in the substrate, kick sampling and the use of quadrat samplers (Larsen and Olden 2015). Peay et al. (2015) applied high intensity electric shocks (96KW, DC current) to a population of *P. leniusculus* in a headwater stream in north Yorkshire, England in two-minute cycles for a total of 98 minutes over 72 hours at one site, resulting in 86 % mortality. At a second site further 15 - minute shocks were delivered over a total of 308 minutes, causing 97 % mortality (Peay et al. 2015). Although this trial was highly successful, it demonstrated that eradication could not be achieved due to a proportion of the population being inaccessible in burrows (Peay et al. 2015). While there is scope to improve the method, its use would be restricted to small, shallow watercourses and there are inherent issues with non-target organisms that need consideration, given the potential for the electricity to adversely affect fish if applied incorrectly (Beaumont 2016). Gladman et al. (2010) compared hand searching, Surber sampling, kick sampling and single pass electrofishing in riffles in the River Clyde, Scotland, and found no technique to be 100% successful at detecting *P. leniusculus,* with kick sampling considered to be the only effective method. Although these techniques can be useful at detecting and possibly monitoring crayfish, their labour

intensity, potential environmental risks and restriction to shallow, slow moving waters excludes them as widespread control methods (Stebbing et al. 2014).

1.6.4 Physical control

Physical means of controlling *P. leniusculus* and other invasive crayfish include the drainage of ponds, dewatering rivers and the creation of barriers to expansion. Dewatering methods are hindered by the ability of *P. leniusculus* to survive out of water and within burrows, with Kozack and Policar (2003) finding live *P. leniusculus* still present in an experimental pond that had been drained and left dry for three months, despite air temperatures lowering to minus 20°C. Dewatering will encourage crayfish to exit otherwise inaccessible burrows and could be more effective when repeated and/or combined with electrofishing (Peay and Dunn, 2014). Chadwick et al. (2020) found that a triple drawdown (dewatering) method captured between 72.5 and 99.6 % of crayfish at four stream sites, although logistical issues, labour intensity and risks to non-target organisms mean these methods are restricted to small ponds or short stretches of small watercourses. On a larger scale, manipulating the water levels of waterbodies subject to other methods of control (e.g., trapping, predatory fish), could increase removal rates through increased exposure to these controls (pers. obs.).

There are few studies outlining how barriers, such as weirs, culverts, dams and waterfalls, prevent the upstream movement of *P. leniusculus.* Frings et al. (2013) tested different barrier designs and found flow velocity, slope and surface roughness determined the extent to which crayfish could pass the barrier. Zenker et al. (2019) produced guidance in Switzerland on how existing barriers can be augmented to increase their resistance to crayfish passage whilst allowing fish passage (Krieg et al. 2020). With the prevalent view

in England and Wales supporting the removal of barriers that hinder fish passage (Kitchen et al. 2016), it is unlikely that the widespread installation of crayfish barriers is a feasible option.

1.6.5 Biological control

Biological control is defined as interventions based on the natural enemies of the invader (Gherardi et al. 2011). For *P. leniusculus* and other invasive crayfish, these enemies are predators, disease-causing organisms and microbes, such as bacteria that produce toxins (Gherardi et al. 2011). Effective predators of *P. leniusculus* include European eel, perch, northern pike *Esox lucius* and brown trout (Blake and Hart 1995; Aquiloni et al. 2010; Nyström et al. 1999). However, all are restricted to preying on small (< 35 mm CL) crayfish due to their limited gape size and maintaining introduced fish within open lotic systems is problematic (S. Rice, pers. comm.). Some species, such as European eel, have low consumption rates and require high density stocking in order to be effective (Musseau et al. 2014), whilst overall effectiveness is influenced by crayfish habitat complexity and shelter availability (Blake and Hart 1995). The presence of fish that do not predate on crayfish can still adversely affect the behaviour and feeding of *P. leniusculus,* reducing their growth rates (Nyström 2005). Clearly, the use of predatory fish within control programmes has potential to be beneficial, especially if used in combination with other control methods, although risks to host ecosystems and non-target species must be avoided (Hansen et al. 2013).

Other biological control methods under consideration include natural pathogens such as the *P. leniusculus* bacilliform virus (*Pl*BV), which has been recorded in Britain (Longshaw et al. 2012), and the *Spiroplasma* pathogen, though there has been little

progress to date (Manfrin et al. 2019). Recent research into *A. astaci* shows potential for genetic manipulation to make *P. leniusculus* more vulnerable to this pathogen (Martin-Torrijos 2019).

1.6.6 Biocidal control

Biocides are the only current method to have achieved the eradication of *P. leniusculus* populations, such as from the island of Gotland, Sweden, in the mid-2000s (Sandodden and Johnsen 2010). A number of compounds have been used, including organo-phosphates, isoflavones (e.g. rotenone), and synthetic and natural pyrethroids (Peay et al. 2019). The main issues are toxicity to non-target taxa and the environment and many UK organisations consider the use of such chemicals as being inappropriate for use (S. Ford, pers.comm.). Other considerations include the need to target crayfish in burrows and prevent the overland escape of individuals (Peay 2010). Peay et al. (2019) reviewed eleven biocide treatments using either natural or synthetic pyrethroids in the UK, Sweden and Norway between 2004 and 2012, with eight successful at eradicating *P. leniusculus*. Factors influencing success included site size, habitat complexity and environmental risk, and applying the requisite dosage rates to achieve crayfish mortality in all habitats (including marginal habitats, burrows and open water; Peay et al. 2019). Recent research efforts have focussed on mechanisms to deliver the biocide directly to the crayfish, such as bait stations (Solari et al. 2018) and matrices (P. Stebbing pers. comm.) to minimise leaching into the water column and avoid effects on non-target organisms.

1.6.7 Autocidal control

Autocidal control can be defined as control through manipulation of the biology of an invading organism (Gherardi et al. 2011). Methods trialled for crayfish include sexual

attractants, genetic and hormonal manipulation, and the sterile male release technique (SMRT; Stebbing et al. 2014). Sexual attractants are based on pheromone signals released by female *P. leniusculus* during mating (Stebbing et al. 2003; Berry and Breithaupt 2010). The structures of these compounds have yet to be assessed, but they have potential to be used to disrupt reproductive success in *P. leniusculus* (Manfrin et al. 2018). Opportunities for hormone manipulation include interference with the androgenic gland hormone, which causes sex change leading to mono-sex populations (Ventura and Sagi 2012), and the silencing of key hormones that influence moult cycles, reproductive behaviour and immune defence (Manfrin et al.2015).

1.6.8 Sterile male release technique

SMRT is the practice of introducing large numbers of sterile males into a population in order to generate non-viable offspring and provoke a subsequent decline in recruitment (Stebbing and Rimmer 2014). Traditionally, SMRT methods raise a population of the target species in captivity, with male sterility achieved via exposure to ionising irradiation (Klassen and Curtis 2005). These individuals are subsequently released into the wild to enable their mating with females, resulting in the production of non-viable progeny. It has been applied successfully to invertebrates, such as the screw-worm *Callitroga hominivorax* (Knipling, 1959) and especially the Mediterranean fruit fly *Ceratitis capitata* (Cheikh et al. 1975; Wong et al. 1992; Harris et al. 1996). The tendency for crayfish to form population hierarchies results in dominant males potentially exerting a controlling influence on population growth, including the control of reproductive activities (Gherardi et al. 2011). Consequently, it has been hypothesised that the application of a sterile male release technique (SMRT) to crayfish has the potential to be a successful control method (Aquiloni et al. 2009). It has the benefits of being an inversely

density dependent method that targets juveniles which are difficult to capture using other methods, and limited trials on crayfish have produced promising results (Aquiloni et al. 2009; Stebbing and Rimmer 2014; Johović et al. 2019). In Aquiloni et al. (2009), wild caught male *Procambarus clarkii* were sterilised by irradiation, but the cost and effort of capturing then transporting them to an irradiation facility prior to release was considered unlikely to be practical when applied to real-world control programmes. A potentially more practical and cost effective method was suggested by Stebbing and Rimmer (2014), involving the functional sterilisation of the animals via mechanical removal of the first and second pairs of pleopods (gonopods), theoretically rendering them incapable of mating effectively by reducing the accuracy of spermatophore placement on the females' ventral surface. This was trialled on red swamp crayfish *P. clarkii* by Johović et al. (2019), where females mated with sterilised males failed to produce any offspring. However, *P. clarkii* have internal fertilisation so the effects are not directly transferable to *P. leniusculus* (Johović et al. 2019).

In invasive crayfish populations, it remains uncertain whether male sterilisation would alter interactions between males and affect reproductive behaviours in both sexes. The effectiveness of SMRT is reliant on sterilised males being capable of competing with non-sterilised males for food, shelter and mates (Gherardi et al. 2011). They must also be equally likely to be chosen by females and sterilised in sufficient proportions to overcome any potential promiscuous behaviour amongst females (Aquiloni and Gherardi 2009). Stebbing and Rimmer (2014) reported no differences between the agonistic behaviours of sterilised and non-sterilised males, implying male-male interactions would not be affected by sterilisation. Studies examining female choice and its relationship to male dominance have produced mixed results. For example, Aquiloni et al. (2008) found

females unable to recognise dominant males unless allowed to 'eavesdrop' on agonistic reactions and Fero et al. (2007) found no relationship between social status and mating in *Faxonius rusticus.* However, Aquiloni and Gherardi (2008) reported that females of some invasive crayfish species did prefer larger males. Other questions relating to the functionality of the mechanical technique suggested by Stebbing and Rimmer (2014), such as brood sizes, procedure survival and gonopod regeneration rates, still require investigation. Consequently, there is a considerable knowledge gap in the application of SMRT for the management of invasive crayfish in the wild (Stebbing and Rimmer 2014).

1.6.9 Use of citizen scientists in crayfish management

The use of citizen science, which involves the intentional involvement of volunteers in the scientific process, has increased rapidly over the last twenty years due to its acceptance as a cost-effective method for monitoring and research (Pocock et al. 2017). The benefits are that substantial datasets can be generated at relatively low cost, making large scale or long-term projects feasible when they would otherwise be prohibitively expensive (Schuttler et al. 2018). In addition, citizen science promotes public engagement with the scientific process and leads to benefits for the natural world (Theobald et al. 2015), as well as to the volunteers themselves (Schuttler et al. 2018).

Since invasive crayfish control experiments require high labour input over the long term, the potentially high costs can act as barriers to implementation and subsequent development of knowledge (Peay 2004). Involvement of citizen scientists in crayfish research and monitoring can therefore enable long term and adaptive management projects to take place, though is not without its challenges. As crayfish management can require volunteers to work in or near water and over rough ground, health and safety and individual fitness must be considered; the methods employed must be scientifically robust

but also straightforward and managers must provide ongoing training, support and motivation (Green 2015). The use of citizen scientists in crayfish projects in Britain is increasing and initial projects have been successful, albeit not without limitations (Green 2015; Stebbing et al. 2016).

1.7 Research rationale

It has been outlined that all of the crayfish control methods currently available have limitations that inhibit their efficacy. Therefore, a multi-method, integrated approach is more likely to have success at population control than methods used in isolation (Manfrin et al. 2018). For any population control programme to be successful, all animals must be at risk of capture (Bomford and O'Brien, 1995). Consequently, this research is designed to target animals of all size classes that are present at differing densities and cover all physical habitats that may support the animals whilst aiming to avoid density dependent compensatory effects. The overall aim of the research is thus to identify how a long-term IPM-based control programme can influence the population abundance and structure of an invasive crayfish population, whilst evaluating the efficacy of individual elements of the programme and investigating any density-dependent responses that arise. It will be completed through field studies based on the River Barle, Exmoor, Somerset, assisted by citizen scientists, and complemented by experimental trials conducted under controlled conditions.

The success of mechanical sterilisation is dependent on the behavioural responses of both male and female *P. leniusculus* not being altered by the procedure (Gherardi et al. 2011). Chapter 2 uses laboratory studies in controlled conditions to determine whether sterilised males can achieve positions of dominance and successfully compete for mates with non-

sterilised males. The responses of females, when presented with a choice of potential mate, are then tested, assessing the effects of both sterilisation and dominance on their choices. Furthermore, the incidence of guarding behaviour by males and promiscuity amongst females, which may also affect the success of the technique, are investigated.

The purpose of SMRT is to reduce the production of progeny in the target organism, which in crayfish entails reducing the number of fertilised eggs produced by females via reduced quantity and placement accuracy of male spermatophore during mating (Stebbing and Rimmer 2014). In addition, for the technique to be successful, treated males must survive the procedure of gonopod removal and the removed appendages must not regenerate rapidly Only one study has attempted to quantify gonopod regrowth and with limited success due to a small sample size (Stebbing and Rimmer 2014). In Chapter 3, a combination of field and laboratory studies examine male gonopod regeneration rates and the influence of SMRT on female brood sizes. Furthermore, matings under controlled conditions evaluate the cover and accuracy of spermatophore placement of sterilised males of differing gonopod removal and regeneration status versus non-sterilised males.

Mechanical removal via funnel trapping is a widely used control method with known biases that has rarely achieved sustainable population reductions (Section 1.5.3; Freeman et al. 2010). Funnel traps are predominantly used in still waters and slow moving, deep rivers (Larson and Oldham 2016). The study site on the River Barle has an average depth of 0.5m and is considered a typical upland spate river (catchment area 128 km^2, mean velocity 5.16 m^3/sec), though it does contain deeper, slower moving sections. The ART is more suited to the shallow, fast flowing waters of this river, and is hypothesised to capture more animals of equal sex ratios and a wider size range than funnel traps (Scott

2012). There are no known published studies on the efficacy of funnel traps on *P. leniusculus* in habitats similar to the River Barle or in comparison to ARTs. Chapter 4 of this thesis aims to address this knowledge gap and determine whether the use of the two traps in combination will achieve the aim of targeting all size classes and habitats within the river.

The more successful crayfish control attempts have taken place over the long term and in still waters (Stebbing et al. 2016). The study site on the River Barle consists of a 1.5 km stretch of an open lotic system, where the *P. leniusculus* population extends for at least 3km both upstream and downstream. There have been few long-term control attempts on upland rivers, and changes to population structure, as well as abundance and the occurrence of density dependant compensatory effects, have not been studied in detail. Moreover, the use of mechanical sterilisation and the release of sterilised large males to reduce recruitment and maintain the influence of dominance hierarchies and cannibalism, has not been trialled. Chapter 5 thus provides a detailed investigation into the effects of this combined approach using capture data of *P. leniusculus* at the study site over a six year period.

The objectives (O) of the research are thus to:

O1. Determine female reproductive behaviour and mate choice in relation to the presence of sterilised and non-sterilised males (Chapter 2).

O2. Assess the long-term efficacy of mechanical male sterilisation in relation to persistence and functionality (Chapter 3).

O3. Compare the efficacy and selectivity of baited versus artificial refuge traps for invasive crayfish (Chapter 4).

O4. Evaluate how trapping and male sterilisation can reduce invasive crayfish abundance (Chapter 5).

Chapter 2. Dominance, reproductive behaviours and female mate choice in sterilised versus non-sterilised invasive male crayfish

Abstract

Many methods of controlling invasive crayfishes have limited success because they fail to target all life stages of the population, notably by capturing only large adults that can result in increased juvenile recruitment by removing intraspecific predation. An alternative approach uses the sterile male release technique (SMRT) that involves the mass release of sterile males into the environment, which then mate with fertile females, resulting in unfertilised eggs and, ultimately, reduced juvenile recruitment. This does, however, rely on the sterilised males exhibiting behaviours similar to non-sterilised (entire) males and remaining attractive to females during mate choice. Post-copulatory male guarding behaviour and female promiscuity might also be affected by male sterilisation. To test for the presence of normal reproductive behaviours in sterilised male American signal crayfish *Pacifastacus leniusculus*, a two-stage experiment examined how sterilisation affects female mate choice and promiscuity, male hierarchical status (relative dominance) and post-copulation guarding. Sterilised males showed similar reproductive behaviours to entire males and remained as attractive to females, with no differences in relative dominance. Post-copulation, guarding behaviours were also unaffected. Females did not display promiscuous behaviour and this was unaffected by whether males were entire or sterilised. The results demonstrated that sterilised males were equally as capable as entire males of achieving dominance and winning mates. In combination, these findings suggest that male sterilisation could be an effective control technique to help reduce juvenile recruitment in wild *P. leniusculus* populations by reducing reproductive success.

2.1 Introduction

Biological invasions are recognised as a major threat to global biodiversity, with the capacity to disrupt ecosystem functioning (Simberloff et al. 2013; Gallardo et al. 2016). In fresh waters, alien crayfishes are among the most invasive taxa globally, having been spread around the world for reasons including aquaculture, human consumption and the aquarium trade (Capinha et al. 2011). They are also highly invasive, impacting on entire ecosystems through the extirpation of native crayfishes via transmission of novel fungal pathogens, increased predation pressure on fishes and macro-invertebrates and physical changes to habitats (Jackson et al. 2016; Lodge et al. 2012; Twardochleb et al. 2013).

Populations of invasive crayfishes are thus subjected to regular management control programmes, where the methods used include mechanical and physical removal, biological control and biocide application (Gherardi et al. 2011; Stebbing et al. 2014). These control methods have the capacity to reduce crayfish abundance and enable the recovery of impacted fauna but are usually effective only when applied over extended periods (Dana et al. 2010; Hein et al. 2007). Commonly-used control methods, such as trapping, are frequently used over short time periods and tend to be size and/or sex-biased, resulting in only a proportion of the population being targeted and removed (e.g. Freeman et al. 2010; Stebbing et al. 2014). As a result, there remains a requirement for the development of new methods that can inhibit the invasion of alien crayfishes and reduce their impacts without incurring high management costs and impacting non-target species (Hansen et al. 2013).

Autocidal approaches, including the sterile male release technique (SMRT), potentially provide effective control methods for invasive crayfishes (Gherardi et al. 2011; Stebbing et al. 2014). The SMRT involves the mass release of sterile males into the environment, which then mate with fertile females, resulting in low or negligible fertilisation rates, or the production of non-viable progeny (Knipling 1959). It has been used successfully on other taxa, such as in the control of insect pests (Takken et al. 1986) and invasive sea lamprey *Petromyzon marinus*. When applied to the latter in tributaries of Lake Superior between 1991 and 1999, a 59% - 86% reduction in reproduction was achieved (Twohey et al. 2003). These methods generally require the use of either genetic manipulation or gamma irradiation to generate sterilised males.

The SMRT is considered to have potential for use on crayfish as their tendency to form population hierarchies results in dominant males potentially exerting a controlling influence on population growth, including the control of reproductive activities. It has the additional benefits of being an inversely density-dependent method that aims to reduce the number of juveniles, a life-stage that is difficult to capture using other methods (Stebbing et al. 2014). Aquiloni et al. (2009) sterilised male *Procambarus clarkii* collected from the wild using irradiation then returned them to the wild. Although successful in reducing the number of progeny by 43%, this compared unfavourably to results achieved for insect species such as the tsetse fly *Glossina palpalis* in Nigeria, where the introduction of sterile males to a depleted population caused eradication (Takken et al. 1986). The irradiation procedure also has resource implications given the time and expense required to capture, transport, irradiate and return the crayfish, comparing unfavourably with control methods such as the use of biocides (Peay et al. 2018), although is likely to be more effective than trapping alone (Stebbing et al. 2014).

A potentially more practical and cost-effective method, as suggested by Stebbing and Rimmer (2014), is the functional sterilisation of the individuals via mechanical removal of the first and second pairs of pleopods (gonopods), theoretically rendering them incapable of mating effectively.

The effectiveness of SMRT is reliant on sterilised males exhibiting similar behaviours to non-sterilised males (Gherardi et al. 2011) and remaining attractive to females during mate choice. Regarding male behaviours, Stebbing and Rimmer (2014) reported no differences between the agonistic behaviours of sterilised and non-sterilised males, implying that male-male interactions would not be affected by sterilisation. Similarly, Johović et al. (2019) found the removal of gonopods in *Procambarus clarkii* did not affect their ability to compete with untreated males for mates. Regarding mate choice, whilst females of some crayfish species prefer larger males (Aquiloni and Gherardi 2008), studies examining male dominance and female choice have produced mixed results. For example, Fero et al. (2007) detected no relationship between social status and mating in *Faxonius rusticus*, whereas Aquiloni et al. (2008) found females unable to recognise dominant males unless allowed to 'eavesdrop' on agonistic reactions. In addition, although crayfishes are assumed to have promiscuous mating systems (Kubec et al. 2018), female promiscuity has only been demonstrated in some species (e.g. Walker et al. 2002; Yue et al. 2010), and not in American signal crayfish *Pacifastacus leniusculus*, the subject of this study. Stebbing et al. (2003) provided evidence for female *P. leniusculus* promiscuity, reporting that after mating, 45 % of female *P. leniusculus* would move away from males that attempted to 'guard' them. Johović et al. (2019) found sterilised male *P. clarkii* had to expend more effort to persuade promiscuous females to mate with them. Furthermore, in this study, copulations were shorter and more difficult to achieve by sterilised males,

with this likely to relate to *P. clarkii* using internal fertilisation, the lack of gonopod-*annulus ventralis* contact being potentially problematic. Both of these findings suggest males sterilised via gonopod removal are less competitive than entire males (Johović et al. 2019). Consequently, across invasive crayfish populations, there remains high uncertainty whether male sterilisation would alter their interactions with other males and affect reproductive behaviours in both sexes. This knowledge gap remains a major constraint in the application of SMRT for the management of invasive crayfishes in the wild.

The present study is a trial of mechanical sterilisation via the physical removal of the adult male gonopods in *P. leniusculus*. Mechanical sterilisation reduces the ability of the male to accurately place his spermatophore on the ventral surface of the female, thereby reducing the number of ova that can be fertilised. As there is little knowledge on the effects of this change to the physical state of the animal in relation to reproductive and hierarchical behaviour in *P. leniusculus*, the aim here was to overcome this by experimentally testing, in *ex-situ* conditions, how male sterilisation affects their reproduction through testing its effect on male hierarchical status, female mate choice and promiscuity, and then post-copulation guarding. The null hypothesis tested was that reproductive behaviours of both sexes, and male hierarchical status, were not altered by male sterilisation.

2.2 Methods

Using *P. leniusculus,* two sets of experiments were completed; the first set tested male dominance and guarding, and female promiscuity, and the second set tested female mate choice. Dominance, guarding and promiscuity experiments took place in September and October 2018, whilst female choice experiments were carried out in 2017 and 2018.

2.2.1 Experimental animals

Adult male and female *P. leniusculus,* for use in the experiments, were collected using baited funnel traps from two adjacent fishing ponds located in Southern England in September 2017 and September 2018. In each year, 200 crayfish (100 M and 100 F) were collected. The size (carapace length) of males ranged from 40 mm to 56 mm, and 38 mm to 46 mm for females. In both years, ≈ 50 % of males were sterilised upon capture via removal of the gonopods by either cutting them off with scissors (Stebbing and Rimmer 2014) or pulling them out using a pair of tweezers, with the sterilised/non-sterilised groups being size matched to ensure the groups were of similar carapace lengths. Only crayfish with both chelae intact were kept, although owing to a shortage of adult males, some did have unevenly sized or relatively small chelae in relation to their body size.

On arrival at the laboratory, all crayfish were placed individually into one of five sections within 90 × 30 × 30 cm (80 L) glass tanks. Each section was divided using an opaque plastic partition with a small grille of 2 mm wire mesh at low level (25 mm from bottom of tank) to allow water circulation. Water temperature was initially maintained at 14 °C (to match the field site) with light on a 12:12 h light: dark cycle. Each section (16 L) contained a 20 mm layer of gravel and a shelter made from 50 mm diameter plastic pipe.

The divided tanks were set up in three-tiered flow-through filtration systems, with each system housing 15 crayfish (five per tank). Water was pumped to the topmost tier then circulated down through the two lower tanks and finally through a filter at ground level. Sterilised males, non-sterilised males and females were housed in different systems thereby preventing accidental physical contact or semiochemical interaction between individuals prior to the experiments. The crayfish were fed a diet of fresh carrot twice weekly.

Following a four-day acclimation period in the laboratory, all crayfish carapace lengths were recorded and, for males, chelae length was measured and any abnormalities recorded. Each individual was given an identifying number (via permanent marker pen on the carapace) that was colour-coded in accordance with sex and sterilisation status. In 2017, temperature was reduced from (± SE) 14°C to 11°C ±0.5 °C, the temperature at which *P. leniusculus* mate in the UK, in 1°C daily increments over a period of three days. In 2018, temperature was maintained at ambient levels (14°C ± 0.5°C) due to the chillers no longer being able to reduce the temperatures any further. For the female choice experiments, males were fitted with a tethering loop a minimum of 24 hours prior to being used in an experiment. For this, a hole was made through the central uropod using a sterile needle then a short length of light-gauge fishing line was threaded through and tied into a loop approximately 10mm diameter. Sterilised and non-sterilised males were captured from separate ponds in order to prevent the pairing of males with prior contact experience and therefore potential hierarchical effects.

For experiments, sterilised and non-sterilised males were paired according to carapace and chelae length, with a maximum difference of 2 mm in either characteristic to

minimise any size effects. Crayfish with unevenly or unusually sized claws were matched with males with similar attributes in order to prevent any competitive advantage. Females were selected to be a similar size or slightly smaller than the males. Animals in moult or that did not appear to be in good condition were not used.

All subsequent analyses on experimental data were completed using SPSS v.23.0 (IBM, 2017). Where error values are presented around means, they represent standard error unless stated. Significance is reported as exact two-tailed unless stated. All data were non-parametric.

2.2.2 Experimental design, data capture and analysis

The set of experiments initially compared sterilised and non-sterilised males' ability to achieve dominance in an agonistic encounter and to generate dominant and subordinate individuals for the subsequent female choice experiments (2018 only). Here, a female was allowed to choose between a sterilised and non-sterilised male. When mating occurred, guarding and promiscuity were tested via the introduction of a new male, post-copulation.

All experiments were conducted in a 900 × 300 mm tank on a flow-through system (as described above). The water temperature in the experimental tanks also differed between the two years (11.0 ± 0.5 °C in 2017; 14.0 ± 0.5 °C in 2018). All experiments were completed in darkness (between 19.00 and 00.00 hours), the time when crayfish are normally most active (Franke and Hörstgen-Schwark 2015). The nature of the experiments meant that the males used in individual dominance trials were then re-used in female choice trials; the latter took place a minimum of 24 hours and a maximum of

17 days (mean = 12.3 ± 5.8 days (SD)) after the dominance trial. The only illumination was above the tanks to allow filming; this comprised two battery operated 'stick on' lights positioned 30 cm above the tank in 2017 and LED aquarium lights in 2018. Filming was conducted using a Go-Pro Hero 3 video camera suspended 30 cm above the tank.

The basis for the analysis of the dominance, guarding and promiscuity experiments was a fight ethogram adapted from Bruski and Dunham (1987) by Bergman and Moore (2003). This categorised the different aspects of agonistic behaviour (Table 1) and enabled each animal to be scored by multiplying the length of time (s) spent displaying each behaviour with the score for that behavioural category. For guarding and promiscuity the ethogram was modified to include relevant behaviours such as sexual activity.

Table 2.1 The fight and behavioural ethogram codes used in the experiments, as devised by Bruski & Dunham (1987) and adapted by Bergman & Moore (2003).

Intensity level	**Fight behaviour description**	**Intensity level**	**Guarding behaviour description**
-2	Tailflip away from opponent or fast retreat	-2	Tailflip away from opponent or fast retreat
-1	Slowly back away from opponent	-1	Slowly back away from opponent
0	Ignore opponent with no response or threat display	0	Ignore opponent with no response or threat display
1	Approach without a threat display	1	Approach without a threat display/mate positions himself within one body length of female
2	Approach with threat display using meral spread and/or antennal whip	2	Approach with threat display using meral spread and/or antennal whip
3	Initial claw use by boxing, pushing or touching with closed claws	3	Initial claw use by boxing, pushing or touching with closed claws
4	Active claw use by grabbing opponent with open claws	4	Active claw use by grabbing opponent with open claws
5	Unrestrained fighting by grabbing opponents claws or appendages	5	Intervention: mate actively attempts to remove challenger from contact with female

2.2.3 Male dominance

There were 26 male dominance trials completed and analysed. One sterilised male and one non-sterilised male were placed at each end of the tank, being separated by an opaque plastic partition placed half way along its length and extending to the top of the tank. Following an acclimatisation period (10 min), the partition was removed and the behaviour of both males filmed (15 min). The starting position in the tank (left/ right) was alternated for the two categories of male to avoid positional bias. The males were not tethered during this experiment and the fitted tethering loop was considered unlikely to interfere with normal behaviour. In the analyses, an encounter was deemed to have started when one crayfish approached another and ended when the crayfish moved more than one body length away and reverted to behavioural intensity level 0 (Table 1). The frequency and intensity of each behaviour was multiplied together to give each male a dominance score in accordance with the fight ethogram. These data were then tested between using a Mann-Whitney U test using the dominance score as the test variable and sterilised or non-sterilised (S or M) as the grouping variable. Position in arena (left or right side) was tested in the same way in order to ascertain positional bias. The distribution of sterilised and non-sterilised males as dominance contest winners was tested for goodness of fit using a Chi-square test (χ^2).

2.2.4 Female choice experiment

In this experiment, one sterilised and one non-sterilised male was tethered and placed into equal sized arenas delineated by a clear Perspex partition (enabling the female to have sight of both males) secured half way across the width of the tank (at 150 mm), extending for 300 mm into the tank and being 300 mm high (Fig. 1). In 2018, the same pairs used in the previous dominance experiments were selected, meaning the relative dominance

status of each male was known prior to the experiment. Each male was tethered by attaching the loop through the uropod to a 500 g fishing weight via a metal clip (in 2017) or a safety pin (in 2018) and length of fishing line, the length of line being sufficient to maintain them within the area delineated by the partition and prevent them from interacting with each other. The relative position of the two categories of male was alternated between experiments in order to prevent left-right bias.

The males were allowed to acclimatise (10 min) prior to the introduction of the female at the opposite end of the tank (Fig. 1). Interactions between the three individuals were filmed either until mating took place or for 30 min in the absence of mating. Videos where mating did not occur within 30 min were discarded. A nominal 'territory' for each male covering two-thirds of their half of the tank was devised for video analysis purposes (Fig.1). The videos were subsequently analysed to record the amount of time the female spent in the 'territory' of each male (Fig. 1), recorded as starting when half of the female's carapace crossed the line. To ensure that the female had the opportunity to make a choice, only videos where the female had sight of both males prior to copulation were analysed. A total of 50 trials were completed, of which 28 resulted in copulation between the female and one of the males. However, of these 28 copulations, 9 were considered as not being appropriate for analysis as the female appeared not to have sight of both males prior to the start of copulation or the video was not of sufficient quality for analysis, reducing the sample size to 19 (9 in 2017, 10 in 2018).

The total time spent by the female in the territory of each male was expressed as the percentage of total interaction time, i.e. of total time spent in their territory. Owing to the differences in temperature between 2017 and 2018, the data were tested for difference

between years using a Mann-Whitney 'U' Test. These differences were not significant (U = 180.0; P = 1.0) so the data were combined to enable a single test. The mate choice by the female (sterilised or non-sterilised), and (in 2018) his dominance status (dominant or subordinate), were tested using a chi-square goodness of fit test.

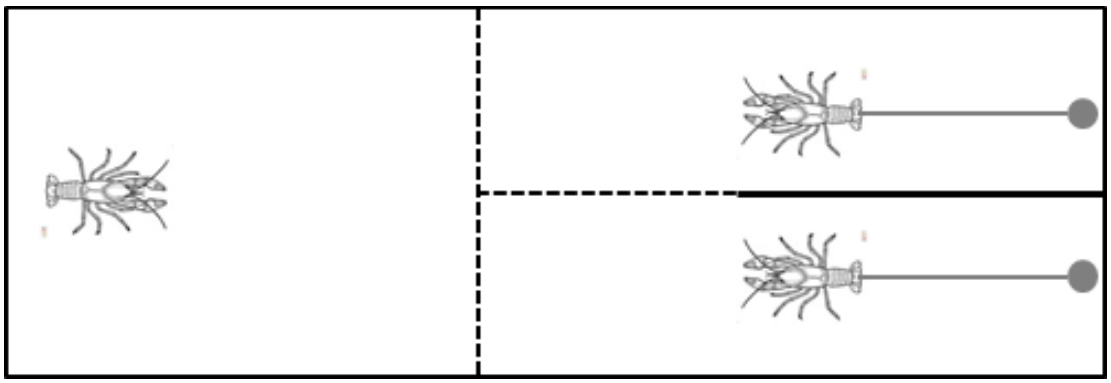


Figure 2.1. Design of experimental arena: Right: tethered males, left: position of female on introduction. Dashed lines represent the 'territory' of each male as used in video analysis.

2.2.5 Post-copulatory guarding experiment

When copulation occurred in the previous experiment, the mated pair were moved post-copulation to a separate 900 × 300 m tank containing two shelters to minimise stress. After 5 min, a new male of haphazardly-chosen size and sterilisation status was introduced and the interaction between the three animals filmed for up to 10 min. The original mate was classified as the 'mate' whilst the new male was the 'challenger'. The videos generated were analysed by categorising the different aspects of agonistic and guarding behaviour using the modified version of the fight ethogram (Table 1). Due to video recording issues, a total of 11 trials were analysed for post-copulatory guarding.

The frequency (as time (s)) and intensity of each recorded behaviour was multiplied together for mate and challenger, giving them a 'guarding' score. As the resulting data had high variance, they were log transformed then tested for differences between 'mate' and 'challenger' scores using a Mann-Whitney U Test. Additionally the animals with the highest scores in each bout were tested against their sterilisation status using Mann Whitney U and chi-square goodness of fit tests.

2.2.6 Promiscuity experiment

At the end of the guarding experiment, the original mate was removed and interactions between the female and male challenger were filmed for up to 10 min. Ten experiments were completed, one being discontinued as the male's attempts to copulate risked harming the female. The videos were analysed using the 'willingness ethogram' modified from the fight ethogram used in the dominance and guarding experiments (Table 2, modifications marked with *). The frequency and intensity of each behaviour was multiplied together to generate a 'willingness score' for each male and female. The difference in willingness scores between males (challengers) and females, and between sterilised and non-sterilised males, were tested using Mann Whitney U and chi-square goodness of fit tests.

Table 2.2. Willingness ethogram codes adapted from Bruski & Dunham (1987) by Bergman & Moore, (2003). Modifications marked with an asterisk.

Intensity Level	Description
-5	Female resists copulation by tucking tail into abdomen*
-2	Tailflip away from opponent or fast retreat
-1	Slowly back away from opponent
0	Ignore opponent with no response or threat display
1	Approach without a threat display
2	Approach with threat display using meral spread and/or antennal whip
3	Initial claw use by boxing, pushing or touching with closed claws
4	Turning of female by male/female allows turning*
5	Mating*

2.3 Results

There were no significant differences in dominance scores between sterilised and non-sterilised males, with the mean dominance score of sterilised males (n = 26) being 912.0 ± 819.3 and non-sterilised males (n = 26) being 952.8 ± 797.2 (Mann-Whitney: U = 327.0, P = 0.84). Differences between dominance score and starting position of the crayfish in the tank were also not significant (Mann-Whitney: U = 335.0, P = 0.96).

Of the 19 copulations analysed for female mate choice, 10 were with non-sterilised males and 9 with sterilised males, with the difference not being significant ($\chi^2 = 0.11$; $P = 0.75$). In 2018, where male dominance was also quantified (n = 10), female mate choice was not significantly related to male dominance status ($\chi^2 = 0.11$; $P = 0.74$). Regarding post-copulatory guarding, mates were found to have higher mean guarding scores than challengers (1106 ± 609 vs. 792 ± 473), but with these differences not significant (Mann Whitney: U = 44.0; $P = 0.29$). Additionally, there were no differences between the guarding scores of sterilised and non-sterilised males (Mann Whitney: U = 44.0; $P = 0.34$).

For promiscuity, the data revealed males had higher mean willingness scores than females (875 ± 1058 vs. 256 ± 856), with the difference between the sexes being significant (Mann Whitney: U = 17.0; $P = 0.01$), implying that females were unwilling to mate a second time. No significant differences between the willingness scores of sterilised and non-sterilised males (U = 8.0; $P = 0.44$) and of females mated with sterilised or non-sterilised males (U = 4.0; $P = 0.14$) were found.

2.4 Discussion

The results infer that sterilised male *P. leniusculus* do not differ from non-sterilised males in their ability to achieve dominance and successfully compete for mates, thus rejecting the null hypothesis. This provides evidence that the use of sterilised males as a *P. leniusculus* management technique could lead to a measurable decrease in juvenile recruitment, leading to reductions in population abundance.

The female choice experiments suggested that mate choice was not related to either sterilisation or dominance status. The actual mechanisms that influence female mate choice within these experiments are unclear. As dominance status was pre-determined, it was possible that *P. leniusculus* females were unable to recognise dominance status without eavesdropping on contests (in concurrence with Aquiloni et al. 2008), bearing in mind that the males were tethered and therefore unable to display dominant/submissive behaviour. The female choice experiments assumed a requirement for visual recognition (i.e. the female having sight of both males) prior to a female being considered as 'choosing' a mate, but the role of semiochemicals in the choice process was not considered (Berry and Breithaupt 2010). Aquiloni et al. (2008) found female *P. clarkii* unable to recognise dominance by sight or smell, however others have found that crayfishes can recognise dominance status semiochemically (e.g. Zulandt-Schneider et al. 1999; Bergman et al. 2003). During our study, when dominance experiments were repeated, males that had previously encountered each other then avoided contact. This suggests that dominant/ subordinate status was determined in their previous encounter, implying that each individual recognised the other via semiochemical signals (Kubec et al. 2018). The role of semiochemicals may also affect the copulation process. For example, Johović et al. (2019) found that sterilised males engaged in longer and more frequent pre-copulatory agonistic interactions with females than untreated males, and speculated that gonopod removal caused a higher aggressive state where treated males released higher levels of urine-borne semiochemicals. In a wild situation, females would be likely to avoid such males in order to prevent injury (Berry 2008). These experiments took place in tanks with a circulating water supply that would have contained semiochemicals from several males and females. Therefore, it is likely both females and males would have difficulties attributing any semiochemicals released to a specific

individual under such conditions, but their agonistic and copulatory behaviours may have been affected by the presence of such chemicals (Johović et al. 2019). It would have been beneficial to change the water between experiments in order to allow further investigations of semiochemicals related influences on copulation (Berry and Breithaupt 2010; Stebbing et al. 2003) and their relevance to the application of male sterilisation.

Fewer copulations occurred in 2018 (14 of 30) compared to 2017 (14 of 20); conditions in the collection and maintenance of animals as well as experimental design were identical for both years, other than some differences in ambient water temperature and light intensity. Although both of these factors could have influenced the results, there were no significant behavioural differences in the experimental results across both years. The females could not be acclimatised in the experimental arena prior to the experiment and could have been stressed by the higher light levels; additionally as *P. leniusculus* are reported to mate between 10°C and 12°C (Guan & Wiles 1999), the higher temperature in 2018 (14 ± 0.5°C *vs.* 11 ± 0.5°C) could have affected their behaviour. It is therefore recommended that future work is performed at the lower temperatures and also uses infra-red lighting/camera systems where possible (e.g. Fero et al. 2006) to reduce light-related stress.

Although not supported statistically, direct observations suggest that male *P. leniusculus* will readily guard the female following copulation, with the lack of significant differences detected in experiments potentially being an artefact of the ethogram design and/or the relatively small sample size (N = 11). In addition, post-copulatory guarding could be less likely to occur in a wild situation as the mate, challenger and female all have greater opportunities to avoid the type of relatively intense encounters that occurred under the

experimental conditions (Bergmann and Moore 2003). Males may be more successful at guarding females post-copulation without the intrusion of a challenger, whereas females could be more able to escape the attentions of her mate and avoid being guarded.

The results also suggest that female *P. leniusculus* did not display promiscuous behaviour, and this behaviour was not influenced by the sterilisation status of the male. Although the experiments took place only a short time (10 min) after the original mating, it is possible that females would seek to mate again hours or possibly days later. However, this is considered unlikely because spawning in *P. leniusculus* generally takes place soon after mating (Vogt 2016). The lack of promiscuous behaviour in *P. leniusculus* supports the potential efficacy of the male sterilisation technique because it implies that wild females are unlikely to seek multiple partners, and as such, following mating with a sterile male, females are considered unlikely to subsequently mate with an entire male. It also implies that females did not find copulation with a sterilised male sufficiently unsatisfactory to then seek new mates. In *P. clarkii,* which is known to be promiscuous (Yue et al. 2010), gonopod removal resulted in shorter and less efficient copulations (Johović et al. 2019), with speculation that both sexes would be aware of such inefficiencies due to their internal mating system, but only 1 of 14 females compensated by mating for a second time. Although our experimental sample size was relatively small (N = 10), the observed behaviours were exhibited under conditions when the females were unable to escape the attentions of the male. In a wild situation, avoidance of additional suitors by the female is more likely to be achievable given that the opportunities for the female to take shelter and avoid such encounters would be higher (Bergmann and Moore 2003). Consequently, in a population control scenario, decreasing fecundity could be initiated once a relatively small proportion of the adult male population has been sterilised (Stebbing and Rimmer

2014). The technique thus has the potential to compare favourably with other methods in terms of resource use, as it would shorten the period of time where intensive trapping would be required. For example, Stebbing et al. (2016) used a population model to simulate various control scenarios and found eradication would be achieved quicker when combining trapping and sterilisation than with trapping alone. The method also compares favourably with techniques such as biocide application which have high environmental as well as financial costs (Peay et al. 2019), and dewatering which is not a feasible method in many waterbodies (Peay & Dunn 2014).

To conclude, the tests undertaken in the present study demonstrated that sterilised males are equally capable of achieving dominance and winning mates as entire males. Furthermore, this research has found further evidence that *P. leniusculus* males, regardless of their sterilisation status, will guard their mates post-copulation, and that females are generally not promiscuous. It is possible that the behaviours observed in the present laboratory study are likely to be magnified in the wild situation due to the ability of individual crayfish to disperse away from agonistic encounters, guarding scenarios and pursuing suitors. The combination of these findings suggests that male sterilisation has potential to be a successful technique in helping control invasive populations of *P. leniusculus* through reducing juvenile recruitment.

Chapter 3. The efficacy of male sterilisation in invasive signal crayfish *Pacifastacus leniusculus*: persistence and functionality in captive and wild conditions

In this chapter, data for ovigerous female CPUE, percentage total females captured and brood size has also been used in Chapter 5.

Aaron Hart assisted with the spermatophore placement experiments and the (controlled) counts of brood size (including sampling and husbandry).

Abstract

Management control methods for invasive crayfish remain of limited effectiveness, resulting in ongoing invasions of high ecological impact.

As management programmes integrating methods to limit juvenile recruitment could reduce population abundances, the efficacy of a sterile male release technique (SMRT) based on the manual removal of male gonopods was tested here in captive and wild conditions by comparing the survival, gonopod regeneration rates and a range of reproductive metrics of sterilised versus non-sterilised males.

Sterilised male survival was high, with their removed gonopods regenerating at sizes that were always smaller than those of non-sterilised males. In captive trials, while sterilised males showed significantly lower areas of spermatophore cover than non-sterilised, and less accuracy in placement, subsequent female brood size did not differ significantly between the two male groups. The number of females retaining their clutches also did not also differ significantly between these groups. Over a seven-year period in the wild, there was no evidence suggesting SMRT significantly reduced female brood sizes and clutch retention rates.

Although mechanical SMRT altered the size and delivery accuracy of sterilised male gonopods, female reproductive success of invasive crayfish was unaffected. Several potential reasons for this failure of the technique were identified and require further research.

3.1 Introduction

Numerous freshwater species have been introduced outside their native range, with their introductions and subsequent invasions in new regions being a powerful driver of native biodiversity loss in inland waters (Gherardi et al. 2011). Crayfish, being omnivorous, mobile, long-lived and resistant to desiccation, have proved to be highly invasive around the world due to these traits enabling their rapid establishment following their introduction into new ecosystems (Nyström et al. 1999). One of the most ecologically damaging invasive crayfish species is the North American signal crayfish *Pacifastacus leniusculus,* which is now widespread across Europe after being introduced for aquaculture in the 1970s (Holdich et al. 2014; Mathers et al. 2016). Their impacts include causing substantial declines in native crayfish populations through a combination of inter-specific competitive interactions and the transmission of crayfish plague *Aphanomyces astaci* (Holdich and Reeve, 1991).

A major challenge with invasive crayfish is in their management, there being no definitive method for controlling or eradicating their populations (Peay 2006; Hein et al. 2007; Gherardi et al. 2011, Stebbing et al. 2014). While baited funnel traps are frequently employed in their population control, these tend primarily to capture larger individuals, predominantly males. Artificial refuge traps can capture a wider size range of crayfish that comprises a much higher proportions of females, but are relatively ineffective at capturing juveniles (Green et al. 2018). Consequently, for a control programme to succeed all size classes of a population need targeting, something most likely achieved via an integrated, multi-method approach (Stebbing et al. 2014). As even the use of a range of trapping methods have not eradicated invasive crayfish populations, they could

be complemented by the application of more novel methods, such as the sterile male release technique (SMRT) (Aquiloni et al. 2009).

Sterile male release techniques are considered a relatively successful method for eradicating invertebrate pest species (Knipling, 1959; Klassen and Curtis, 2005), especially as the methods are inversely density-dependent and species-specific (Stebbing et al. 2014). Previous trials on invasive red swamp crayfish *Procambarus clarkii* involved male sterilisation using irradiation, which significantly reduced male testes size and juvenile production and survival (Aquiloni *et al.,* 2009). However, irradiation-induced gonadal damage in *P. clarkii* was subsequently shown to be repaired within 193 days of treatment (Manfrin *et al.,* 2021). Moreover, the widespread application of this method will be limited due to the costly process of capturing, irradiating and then returning males to the population (Aquiloni *et al.,* 2009). Correspondingly, it has been posited that the mechanical removal of the gonopods could result in a more cost effective and efficient male sterilisation method that reduces the extent and accuracy of spermatophore placement (Stebbing and Rimmer, 2014). This is because during copulation, male crayfish use their gonopods to place extruded spermatophores onto the ventral surface of the female. Crayfish spermatozoa are non-motile so it is assumed that spermatophores closest to the gonopore have the highest chance of fertilising ova (McLay and Van den Brink, 2016), although spermatozoa can be circulated during the secretion of glair, a highly viscous gel secreted by the female into which the ova are deposited (Yazicioglu *et al.*, 2016), and through movement of the female's pleopods (Niksirat *et al.*, 2014).

Initial testing of the effectiveness of gonopod removal as a SMRT on *P. leniusculus* resulted in only one copulation from 20 pairings with sterilised males, which failed to

deposit spermatophores anywhere on the female abdomen (Stebbing and Rimmer, 2014). It was also fully effective in female *P. clarkii*, with sterilised males initiating mating as frequently as non-sterilised, but having to invest more effort in dominating the female and having shortened copulation times (Johović *et al.*, 2019). However *P. clarkii* have internal fertilisation so this study is not directly comparable to *P. leniusculus*. No study to date has followed this mechanical SMRT through to the brood hatch stage. Moreover, there remain considerable knowledge gaps on the persistence of gonopod removal. Stebbing and Rimmer (2014) suggested mechanical removal would be effective for approximately three years due to adults moulting annually, but moulting studies suggest this period will be shorter as adult crayfish can moult twice per year (Abrahamsson, 1971), especially at smaller sizes (Westman and Savolainen, 2002; Guan and Wiles, 1999). Sterilised male *P. clarkii* moulted more frequently than non-sterilised, with most regenerating all their gonopods after their first moult post-sterilisation, although many of these were malformed (Johović *et al.*, 2019). Stebbing and Rimmer (2014) also detected increased mortality rates of sterilised crayfish.

Given the uncertainties that remain in the long-term effectiveness of gonopod removal as a SMRT, especially in relation to its ability to reduce population abundances, this study aimed to understand how male *P. leniusculus* respond physically and functionally to gonopod removal through comparing relevant reproductive metrics with non-sterilised males and then testing the effects on female reproductive success. Experimental trials in captive and wild conditions assessed post-sterilisation survival and gonopod regeneration rates, copulation effectiveness (as accuracy of spermatophore placement), and frequency of ovigerous females and the resultant brood sizes produced between sterilised versus non-sterilised males. We posit: (1) regenerated gonopod lengths are significantly smaller

in sterilised versus non-sterilised males; (2) the smaller (and potentially deformed) gonopods significantly reduce copulation effectiveness in sterilised males; and (3) application of SMRT in the wild will result in reduced frequencies of ovigerous females and significantly lower brood sizes.

3.2 Methods

3.2.1 Sterilisation trials in captive conditions

Samples of *P. leniusculus* for use in laboratory trials were collected each September between 2016 and 2019. Animals were captured from a lake fishery in Dorset (50°49′49″N, 001°56′17″W) in the south of England using baited funnel traps set overnight, with captured animals transferred to the laboratory where they were sorted by sex, with males being selected for sterilisation. Male crayfish collected between 2016 and 2018 were used in pilot studies to determine an effective sterilisation procedure. The basis of the sterilisation procedure was the removal of the gonopods by excising with scissors or pulling them out with tweezers (Green *et al.*, 2020). Work was also completed to successfully determine whether the crayfish survived trimming the regenerated gonopods on one and two occasions. This work then enabled the use of five groups of male crayfish to be used experimentally in 2019: a control group (not sterilised) and then four groups comprising males sterilised by a range of methods (Table 3.1).

The crayfish to be used in the trials were initially held in a secure outdoor area where, following sterilisation of males, they were housed in separate treatment/sex groups in filtered and aerated 200 litre black tanks. Each tank had a gravel substrate c. 30mm deep with lengths of PVC pipe (L: 150 mm, D: 50 mm) added (> 1:1 ratio of pipe to

individuals) to act as refuges. To reduce the likelihood of intra-specific conflict, a maximum of twelve individuals were housed in each tank. The animals were fed on raw carrot every two days and the tanks cleaned weekly by syphoning the gravel. Each tank was covered with netting and secured with timber along the edges in order to prevent crayfish egress.

Table 3.1 Sterilisation methods for male signal crayfish *Pacifastacus leniusculus* in captive conditions and the mean lengths of both males and females used subsequently in their copulation trials.

				Mean carapace length in trials (mm)	
Group	**Sterilisation method**	**n**	**Sampling date(s)**	**Female**	**Male**
1	Not sterilised (control/CTRL)	24	Sept. 2019	42.2 ± 2.6	43.5 ± 3.0
2	Cutting whole gonopods (cut/CWG)	24	Sept. 2019	41.8 ± 1.4	42.8 ± 1.6
3	Pulling whole gonopods (pulled/PWG)	24	Sept. 2019	41.8 ± 1.0	42.5 ± 1.0
4	One year regeneration (trimmed once/R1T1)	12	Sept. 2018	43.8 ± 3.7	49.5 ± 1.2
5	One year regeneration (trimmed twice/R1T2)	7	Sept. 2016 (n=2) Sept. 2017 (n=5)	45.8 ± 8.5	51.7 ± 9.1

3.2.2 Procedural survival and gonopod regeneration

The survival rates of sterilised versus non-sterilised males in controlled conditions were determined using the 2019 samples, where the survival of males to be used in experiments (CTRL, CWG and PWG; N = 72: sterilised, n = 48, control: n = 24) were monitored between 24th September (date of collection/sterilisation) and 25th October 2019 (conclusion of experiments). To then assess gonopod regeneration, all males used in the mating experiments were euthanised post copulation, and the gonopods for groups CTRL, R1T1 and R1T2 removed and photographed (DSLR camera on a horizontal mount with a ruler in frame). These gonopod areas were then measured using ImageJ (Rueden *et al.,* 2017) to establish rates of regeneration as accurately as possible, and test these versus the control group. To test these differences in gonopod regeneration between the groups (Tab. 1), the extent of regeneration was standardised to the carapace length (CL) of each individual as adjusted gonopod area (mean area mm^2 / CL). As the data were normally distributed (Shapiro Wilk P = 0.65), differences between the groups were tested using ANOVA (adjusted post-hoc with Tukey's HSD).

Copulation effectiveness as defined by spermatophore placement

The first trial on copulation effectiveness tested whether male sterilisation reduced the extent and accuracy of the spermatophore placement. Females were selected for experiments based on their glair (mating receptivity) status, i.e., blueish/whitish colouration caused by the formation of glair glands. The trials were completed between 17th and 25th October 2019 in nine clear plastic tanks (900 x 300 x 250 mm) located outdoors in ambient conditions and covered in black HDPE (high density polyethylene) sheeting that maintained dark conditions, as crayfish are generally more active and, therefore, more likely to copulate at night (Franke and Hörstgen-Schwark, 2015). Each tank was half-filled with dechlorinated tap water and allowed to settle to ambient temperature (12 to 14°C). One female in glair was placed into each

tank and allowed to acclimatise for five minutes. A male was then introduced and the animals were left together until either copulation was concluded or for a maximum of 30 minutes. Across the trial, pairs were sized matched (as carapace length, CL) where possible (Tab. 1). The number of experiments per group varied, the aim being for every male held prior to 2019 to copulate, with the number of copulations from the 2019 groups being commensurate with that. Only nine of twelve males from the largest pre-2019 group (R1T1: 2018 trimmed once; Tab. 1) copulated whilst in Group 5 (R1T2: 2016 and 2017 trimmed twice; Tab 1), all five of the 2017 but neither of the 2016 males copulated, resulting in nine copulations for all groups except for R1T2 which had five copulations. Some copulations were discarded where the female avoided spermatophore placement on her ventral surface by curling her abdomen up tightly, particularly when the male was larger than the female.

Where copulation had occurred, the male and female were separated and the males euthanised. The mated female was then marked with a reference number on her carapace for identification. The location of spermatophore placement on each female was then measured by immobilising the crayfish beneath a camera using straps placed across the abdomen and holding the chelae down with small magnets, with an image then captured (Fig. 3.1C). The females were then placed in 200 L brood tanks specific to each male group (Tab. 1) and held until February 2020. Where copulation did not occur, the male and female were returned to stock tanks for a minimum of 24 hours before re-use.

Two metrics were used to measure the location of spermatophore placement: total cover (expenditure) and distribution (accuracy), and were measured in three placement areas: (1) between the 2nd and 3rd pair of walking legs adjacent to the ovipore; (2) area covered by the

first and fourth pairs of walking legs; and (3) area of the first two abdominal sections (Fig. 3.1 C).

All areas extended to the full width of the crayfish. In processing, all images were made black and white to display the spermatophores more fully, with the cover and distribution of spermatophores then measured (as the total area and then for each of the three placement areas) in ImageJ. To standardise measurements across different individuals, spermatophore cover was expressed as the proportion of the placement area covered. As the data were not normally distributed then differences between the treatment groups were tested using a Kruskal Wallis test; differences in the data were then also tested as two groups ('sterilised' versus 'non-sterilised') in a Mann Whitney U test.

Figure 3.1 a) Typical spermatophore cover of (a) sterilised and (b) control male, where in (b) the majority of spermatophores are deposited around the egg pore (between the middle pairs of walking legs). C) Spermatophore placement areas 1 (green), 2 (yellow) and 3 (red) used for image analysis. Photos (a) and (b) N. Green, (c) A. Hart.

Clutch retention and brood size

This trial used the females from the spermatophore placement experiments, held in the 200 L brood tanks post mating according to their male sterilisation group at a density of nine per tank. As crayfish density can affect female brood size (Celada *et al.,* 2005), four un-mated females (status marked by removal of one uropod) were added to Group 5, where only five copulations occurred, to provide consistent densities. These crayfish were then held in the tanks until February 2020, with this providing sufficient time for the loss of any unfertilised eggs (Guan and Wiles, 1999; Celada *et al.,* 2005). During this period, feeding and tank cleaning was undertaken weekly. Then, all individuals from each group were placed into their own tank (900 x 300 x 250mm), with their embryos then removed using tweezers and placed into individual plastic pots for counting. This procedure was completed on the same day for all crayfish to minimise embryo loss due to stress. The number of females failing to retain their clutch at this point was also recorded. All the females were then euthanised. In analyses, brood size (as number of embryos) was standardised to account for size differences between females (brood size / CL). Testing for differences in standardised brood size and clutch retention used Kruskal Wallis test, with the five groups were then also tested as two groups ('sterilised' versus 'non-sterilised') in Mann Whitney U and Chi-squared tests.

3.2.3 Sterilisation trials in field conditions: mark-recapture, gonopod regeneration and brood size

The trial to investigate the efficacy of male sterilisation in field conditions was completed in a specific study area of the River Barle, Somerset (51°060 24.200N; 3°390 32.200W; Green *et al.,* 2018). Trapping was undertaken between April and October of 2015 (Year 1) to 2021 (Year 7) using baited or artificial refuge traps that were being deployed on the river on a weekly basis (Green *et al.,* 2018). Throughout this period all males ≥ 40 mm CL were sterilised by either

cutting or pulling of gonopods then returned to the river. In 2021, all males ≥ 30 mm CL were sterilised. All female crayfish and males < 40 mm CL (30 mm in 2021) were euthanised. A total of 3832 (3055 Years 1 - 6; 777 Year 7) males were sterilised and returned to the river close (within 5 m) to their capture location over the study period.

The data for the gonopod regeneration experiments was based on a mark-recapture exercise carried out between September of Year 3 (2017) and October of Year 4 (2018), when all sterilised males were tagged at point of capture with a uniquely coded passive integrated transponder (PIT) tag (FDX-B; 7 x 1.35 mm; Loligo Systems, Denmark). Tags were inserted ventrally between the 2nd and 4th abdominal segments using a PIT tag implanter (Nightingale *et al.,* 2017). Carapace length was then measured (nearest mm) using Vernier callipers and information on moult stage, damage status and capture location recorded. Damage status was categorised as: 1 = none or little damage (e.g., damage to < 2 walking legs, damaged antennae); 2 = moderate damage (e.g., damage to 1 chela, 3 to 4 walking legs); and 3 = major damage (e.g., to 2 chelae, > 4 walking legs). In Year 3, all tagged males (n = 75) were sterilised by pulling the gonopods off with a pair of tweezers; in Year 4 (n = 301), sterilisation involved a mixture of pulling and excising with a pair of scissors, with some (n = 247) subject to the use of both methods per individual by cutting the gonopods on one side and by pulling on the other. Subsequent crayfish trapping events in the study stretch resulted in the recapture of the sterilised males. Each trapped male crayfish was scanned for PIT tag presence and, following identification as a tagged recapture, the data recorded were their carapace length, damage and moult status, and capture location, plus the length of each gonopod if regeneration had occurred (using Vernier calipers). Logistical constrains in the field meant that measures of gonopod area could not be completed as per the captive trial and instead, gonopod length was used as the measure of their regeneration. Mean total gonopod regeneration (total gonopod length/4) for

all gonopods, and total length of only the anterior gonopods and posterior gonopods, were determined for each animal. For the damage status, an additional metric was included (damage increment on recapture: 0 = no change; 1 = new or increased damage).

Reference values for mean gonopod length by CL of control males were derived by measuring gonopod length of a minimum of 10 non-sterilised males of each CL (to the nearest mm) that were also captured in traps. Mean values for each of the four gonopods were determined for each CL before combining into categories of all gonopod lengths, total anterior gonopod lengths, and total posterior gonopod lengths. The field regeneration data were combined in the same way, before differences in the gonopod lengths between the control and removal categories were tested in generalised linear models (GLMs). The GLMs used gonopod length as the dependent variable, status (sterilised or control) as the independent variable and CL as the co-variate. The extent of gonopod regeneration between seasons was then tested with a GLM as before, where season was used as the independent variable. In all GLMs, the reported outputs were estimated marginal means of the gonopod lengths of each category or season (± 95 % confidence limits), and the significance of their differences according to linearly independent pairwise comparisons (with Bonferroni adjustment for multiple comparisons). The extent of regeneration from the sterilisation methods (cutting versus pulling) was then tested using a Mann Whitney U test; the damage increments were then tested against the total length of time between sterilisation and recaptures, again using a Mann Whitney U test.

To test the effect of sterilisation on brood size in the field, ovigerous female abundance and brood size data were used from the weekly trapping events. Although the study area covered 1500 m of river, subsequent analyses used only data from the central focal reach (1000m) to reduce the effects of crayfish immigration from adjacent reaches, and only artificial refuge trap

data were used as catches of ovigerous females in baited traps were negligible (Green *et al.*, 2018). The analysis used all female crayfish of ≥ 30mm CL captured within this central reach between the first trapping event each spring (usually mid - late April depending on flow conditions) and the second week of June of each study year. The use of a minimum CL of 30 mm was based on the smallest ovigerous crayfish captured in all samples and the end date was based on the latest date of capture of an ovigerous female during the trial period. Testing of differences in ovigerous female relative abundance (as catch per unit effort (CPUE), weekly catch/ trapping effort) was tested in a generalised linear model and used ovigerous female CPUE as the dependent variable, year as the independent variable and covariates of temperature recorded at 09.30 on day of capture and mean daily flow (m^3/sec, UK Environment Agency data).

Female brood size metrics were calculated using all ovigerous females caught in the spring of each year excluding brood sizes <2 (N = 150), as single ova are frequently a relic after brood release (N. Green, pers. obs.). For each ovigerous female, the embryos/juveniles were removed with a pair of tweezers and counted and brood size standardised to CL consistent with the controlled experiments. Differences in standardised brood sizes were then tested between years using a linear GLM, where brood size was the dependent variable, year was the independent variable, and the covariates were temperature and flow. The reported model outputs included the mean values of the dependent variables (as estimated marginal means (± 95 % confidence limits) adjusted for the effects of the covariates) and the significance of their differences according to linearly independent pairwise comparisons (with Bonferroni adjustment for multiple comparisons). As winter water temperature potentially influences brood size and hatching date, annual winter temperature data were tested between years. As differences

between years were not significant (ANOVA: $F_{3,104} = 1.3$, $P = 0.26$) then winter temperatures were not considered as influencing these data and were not considered further.

All statistical tests on data from the captive and field trials were completed in SPSS v.26 (IBM, 2019); use of parametric tests only followed after testing for normality (Shapiro Wilkes and Kolmogorov-Smirnov tests); non-parametric tests were always used where data were not normally distributed. Where error values are presented around means, they represent standard error unless stated, and results from multiple comparisons were adjusted using Bonferroni correction. Significance is reported as exact two-tailed unless stated and where t-tests are used, Levene's tests assume equal variance unless stated.

3.3 Results

3.3.1 Captive trials

Procedural recovery and gonopod area by sterilisation group

All males survived the sterilisation procedure in the 2019 trial, where the time between sterilisation and their euthanasia was between 23 and 31 days. For gonopod area, there was a significant difference in mean area (adjusted for carapace length) between the control group (mean 40.95 ± 16.9 mm^2) and the sterilised groups (trimmed once: mean 21.93 ± 12.44 mm^2; trimmed twice: mean 17.53 ± 12.68 mm^2) (ANOVA $F_{2,20} = 15.6$, $P < 0.01$). In the test, the significant differences were between the control and both treatment groups (both $P < 0.01$), but not between the two treatment groups ($P = 0.63$).

Copulation effectiveness as defined by spermatophore placement

The groups of sterilised males had lower areas of spermatophore cover than non-sterilised males (mean reduction overall: 49.3 %; mean reduction in cover between the middle two pairs of legs: 43.5 %) (Fig. 3.2). These reductions were all significantly different between the control and sterilised male groups (Kruskal Wallis tests: cut: $H = 3.17$, $P = 0.02$; pulled: $H = 2.9$, $P = 0.04$; trimmed twice: $H = 2.98$, $P = 0.03$). Sterilised males were also less accurate in their spermatophore placement, with an increase in spermatophore cover on the abdomen (46.7 %) and two outer pairs of legs (22.3 %; Fig. 3.2; 3.3). These differences were, however, only significant between the control and the regeneration trimmed twice group ($H = 12.48$, $P = 0.01$). Percentage spermatophore cover on the first two abdominal segments (indicating low placement accuracy) was generally higher than the control in all groups, except the trimmed twice group, with these differences significant ($P < 0.05$; Fig. 3.2). The data on spermatophore cover between the two outer pairs of legs varied between groups and with all differences being non-significant ($P > 0.05$; Fig. 3.3).

When treating the dataset as two groups (non-sterilised versus sterilised), overall percentage spermatophore cover was significantly higher for non-sterilised males (Mann Whitney $U = 26.00$, $P < 0.01$,). The percentage cover between the middle two pairs of legs was also significantly higher for non-sterilised males ($U = 47.00$, $P = 0.01$; Fig. 3.2), but there were no significant differences for cover on the abdomen ($U = 194.5$, $P = 0.11$; Fig. 3.2) or outer two pairs of legs ($U = 172.00$, $P = 0.39$; Fig. 3.3).

Clutch retention and brood size

Brood size was generally higher in the control than all sterilised groups, except the cut gonopod group (Fig 3.4), although the differences were not significant (Kruskal Wallis test: $H (4) =$

5.12, P = 0.28). When treated as two groups (sterilised versus non-sterilised), brood size for sterilised males was again higher than the control, but was not significantly different (Mann Whitney U Test: U = 105.00, P = 0.23; Fig. 3.4). Although the number of females retaining their clutch (i.e. retaining at least one fertile egg through winter until February) was lower in the groups that reproduced with sterilised males than unsterilized (Fig 3.5), the differences were again not significant: X^2 (1, N = 41) = 2.35, P = 0.12.

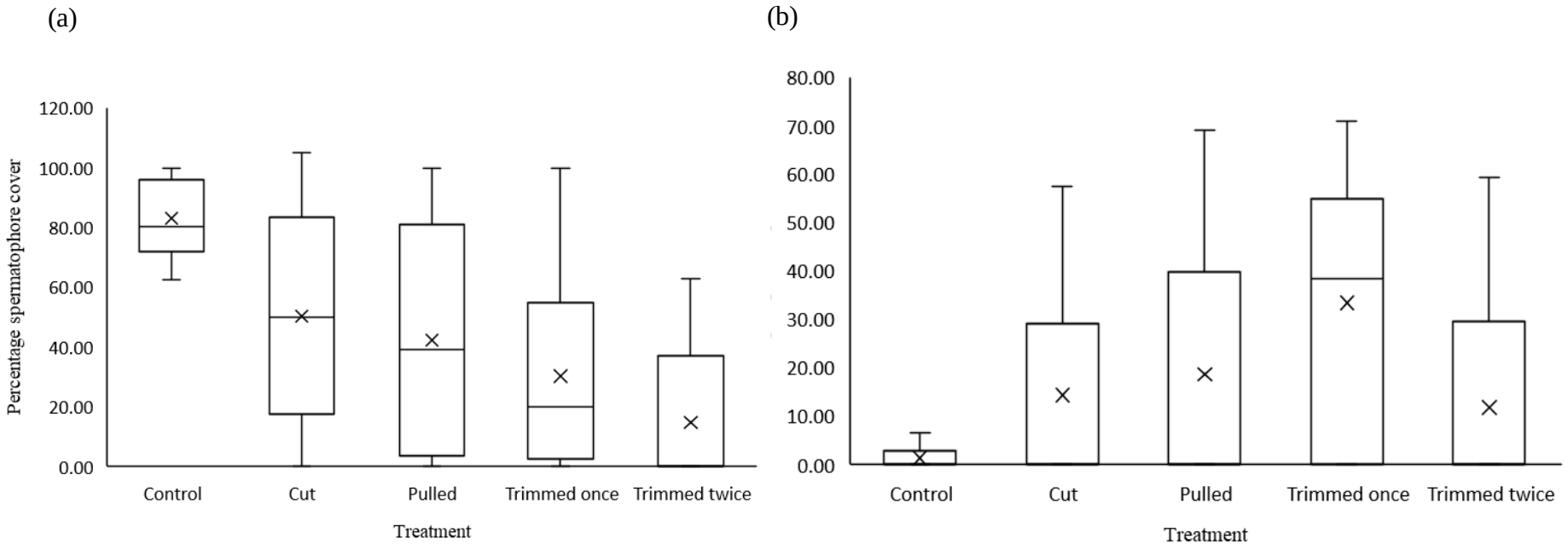


Figure 3.2. Boxplots revealing percentage spermatophore distribution between treatments for the middle (a), and abdomen (b) sections. Horizontal lines mark the 10th, 25th, 50th, 75th and 90th percentiles of the data whilst x is the mean percentage spermatophore cover.

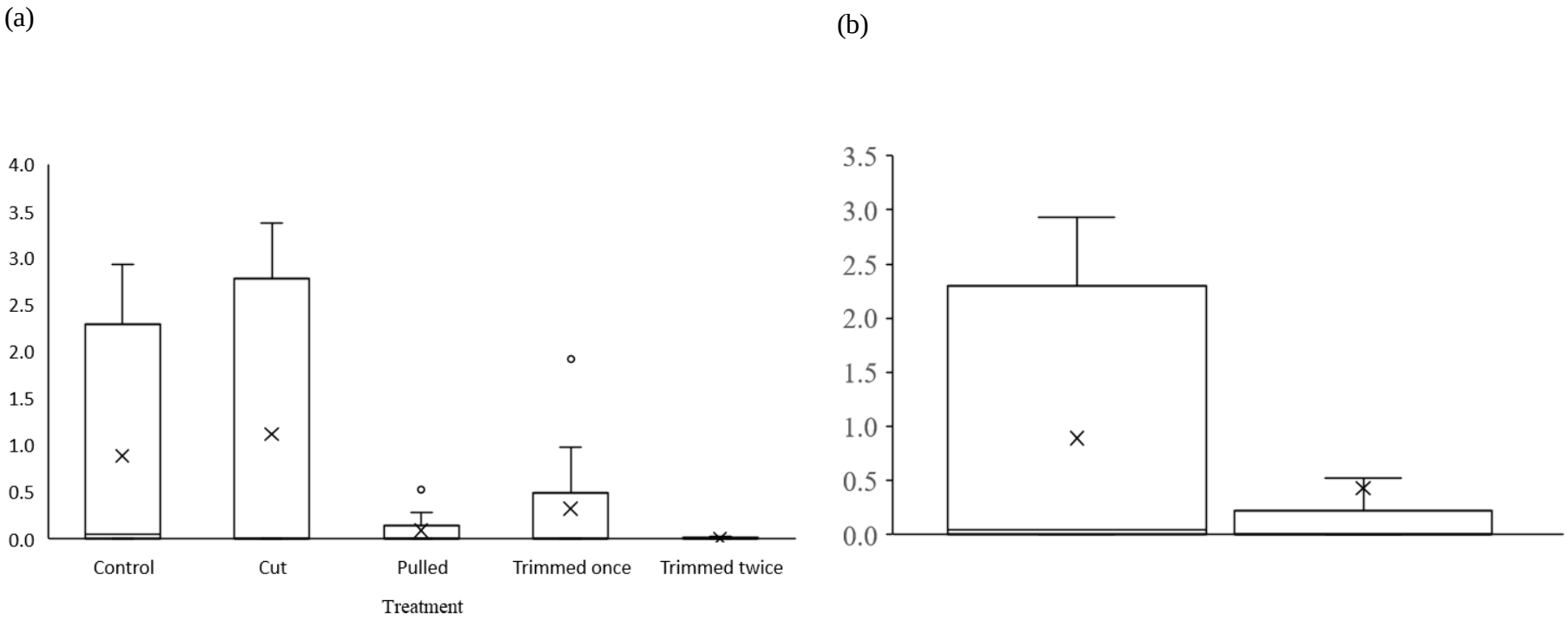


Figure 3.3. Counts of brood size (adjusted for the effect of carapace length) according to the experimental treatments (a) and as sterilised versus non-sterilised (b). Each plot communicates the median (solid line), interquartile range (boxes), 10th and 90th percentiles (error bars), mean (x) and outlier values (circles)

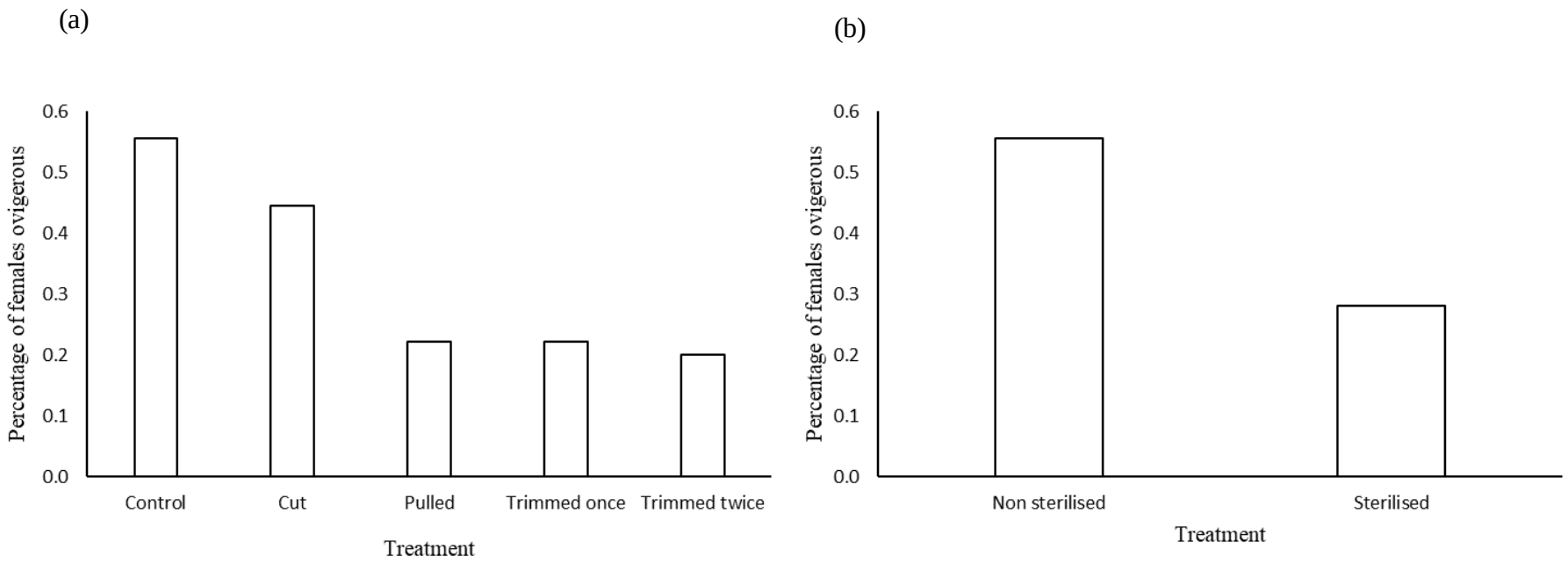


Figure 3.4. Percentage of female crayfish retaining embryos until spring according to (a) the experimental treatments, and (b) as sterilised versus non-sterilised

3.3.2 Trials in field conditions

Recapture rates and intervals

The time interval between the tagging of an individual sterilised male and its final recapture was 11 to 778 days (mean 188 ± 28 days). Due to the seasonality of trapping, the recapture data were split into three groups: 'one season' (11 to 98 days; n = 27), 'two season' (98 to 364 days; n = 17), and 'three season': 410 to 778 days; n = 1). These data indicated that at least 56 % of sterilised males survived the season in which they were tagged, 37 % survived at least one winter and 7 % at least two winters. Only 26.6 % of recaptured tagged males experienced increased damage since sterilisation, with no relationship between increased damage and the time between capture and recapture (Mann Whitney U test = 156, P = 0.58).

Gonopod regeneration

Mean total gonopod lengths of the recaptured sterilised males from all seasons (n = 45) were significantly smaller than reference values for control males (Wald X^2 = 1296.5; P < 0.01; Table 3.2, Figure 3.5). Both mean anterior and posterior gonopod lengths were also significantly larger in control versus sterilised males (anterior: Wald X^2 = 1239.2; posterior: Wald X^2 = 1143.8; P < 0.01 in both cases; Table 3.2). Regeneration lengths were more evenly balanced between anterior and posterior gonopods for the sterilised males, whereas in control males, the posterior gonopods were significantly larger (Table 3.2). Differences in mean gonopod regeneration between one and two seasons were not significant (GLM: Wald X^2 = 1.7, P = 0.20, Fig. 5). There was no suggestion that the sterilisation procedure induced more frequent moulting in males, with little difference between moult rates of males at point of sterilisation (7.4%) and point of recapture (7.0%).

Brood size and ovigerous female abundance in the field

The CPUE of captured females ≥ 30mm that were ovigerous between the start of trapping and the third week of June each year fluctuated between Years (Fig. 3.6), with the GLM indicating that the differences were non-significant (Wald X^2 = 4.8, P = 0.57). Female brood size (standardised to CL) also fluctuated over the study period with the GLM being non-significant (Wald X^2 = 12.0, P < 0.06; Fig. 3.6).

Table 3.2. Comparisons of gonopod lengths (all total, total anterior and total posterior) between non-sterilised and sterilised males in the River Barle study site, with the results of the generalised linear model testing differences in gonopod lengths where the effect of carapace length was a significant covariate in the model.

Gonopod length (mm)	Control (mm)	Sterilised (mm)	Model result
Mean total gonopod length	56.87 ± 5.68	19.33 (± 11.55)	Wald X^2 = 1296.55, P < 0.01
Mean total anterior	25.42 ± 4.75	9.29 (± 5.85)	Wald X^2 = 1229.18, P < 0.01
Mean total posterior	21.32 ± 3.25	10.01 (± 6.61)	Wald X^2 = 1143.77, P < 0.01

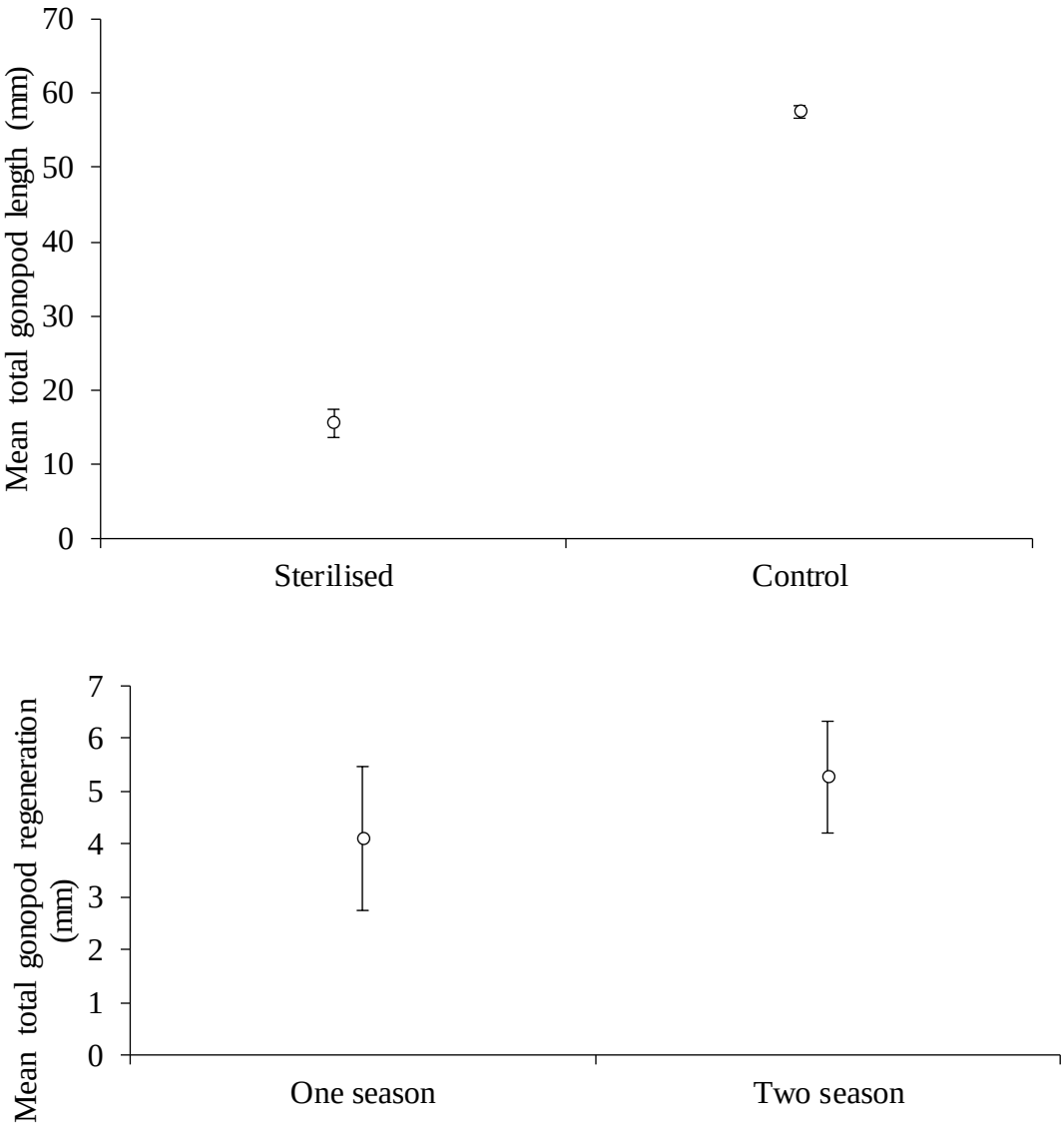


Figure 3.5 Mean gonopod regeneration rates of sterilised males (as estimated marginal means, adjusted for the effects of carapace length) as gonopod length (top), where the comparison is with non-sterilised males, and regeneration between male crayfish recaptured after one and two winters in the River Barle study site (bottom). Error bars represent 95% confidence intervals.

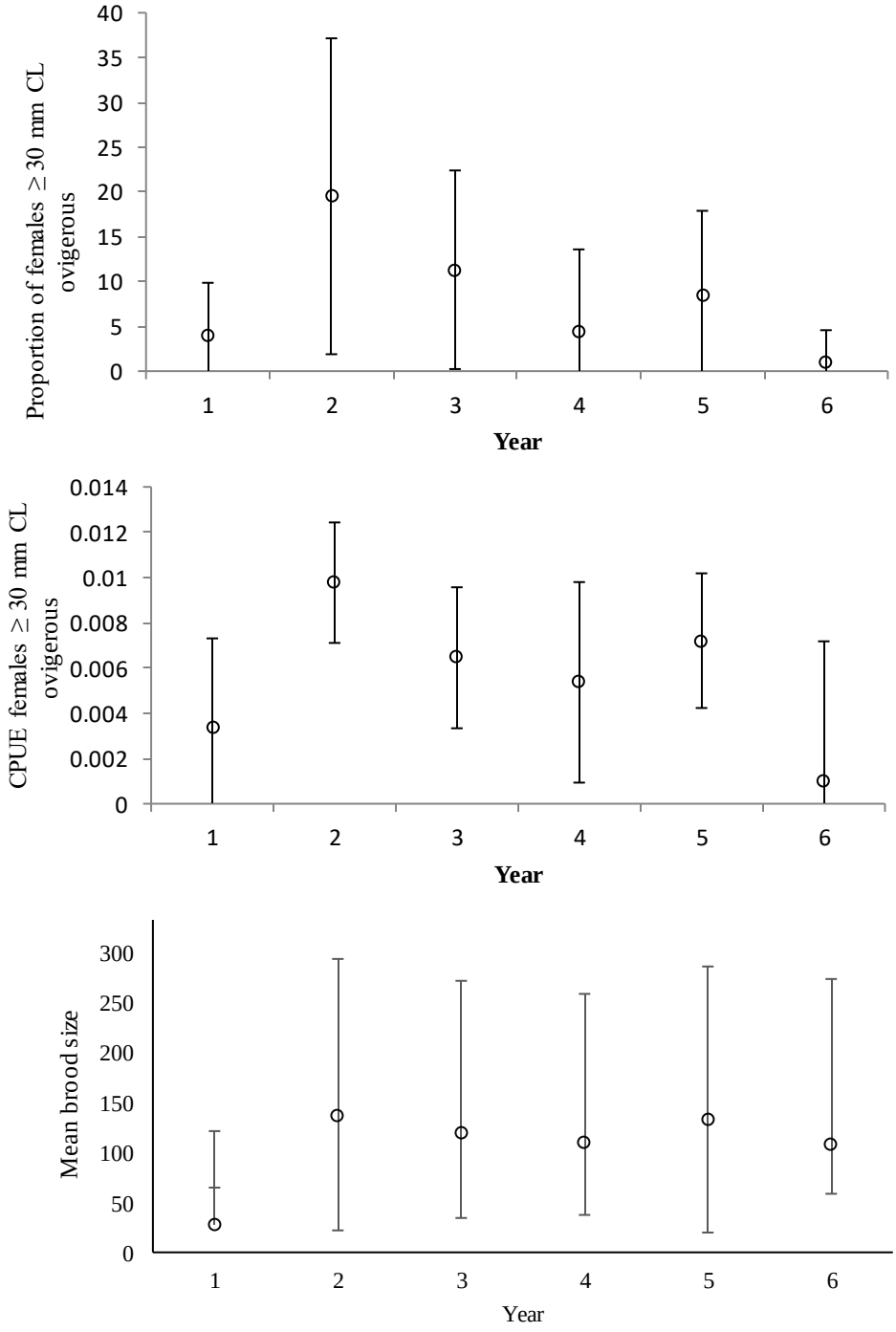


Figure 3.6. Proportion (as percentage) of females captured being ovigerous (as estimated marginal means from the best fitting GLM, top), Mean CPUE (middle) and mean brood size (bottom) at the River Barle study site Years 1 - 6. Error bars represent 95% confidence intervals.

3.4 Discussion

Sterile male release techniques have been posited as providing effective management techniques for reducing the recruitment success of populations of invasive species, especially crayfish (Aquiloni *et al.*, 2009; Stebbing and Rimmer, 2014). Here, investigations into SMRT on *P. leniusculus* enabled testing of its short-term (captive trials) and longer term (field trials) effectiveness. The results revealed that following the manual sterilisation of males, regenerated gonopods were always reduced in area (captive) and length (field), with this consistent with the prediction. In captive trials, sterilised males had significantly lower areas of spermatophore cover than non-sterilised males, and were more inaccurate in their placement, with this again as predicted. However, this did not result in captive females that reproduced with sterilised males having significantly reduced brood sizes compared with those that reproduced with non-sterilised males, with the number of captive females that retained their clutches also not differing significantly between those that reproduced with a sterilised versus non-sterilised male. The field trial data also suggested that SMRT had not significantly reduced female brood sizes and clutch retention rates by the end of the study period, also disagreeing with prediction.

The application of manual sterilisation to male *P. leniusculus* did not appear to reduce their survival rates, with all sterilised males surviving the relatively short experimental periods in captive conditions. Their regenerated gonopod data revealed that when adjusted for the effect of carapace length, gonopod area was reduced, with the extent of the reduction increasing with the number of treatments. Moreover, the regeneration that was observed indicated that gonopod deformation, subsequent spermatophore cover and

resultant brood sizes decreased with the number of treatments. Gonopod regeneration rates amongst recaptured tagged males in the wild also remained substantially and significantly smaller than non-sterilised males. This result concurs with Stebbing and Rimmer (2014), who suggested that complete gonopod regeneration would take up to 3 years. While gonopod regeneration was limited in the field site, a relatively low proportion of sterilised tagged males survived for more than one winter post-sterilisation. Although this might suggest low survival rates due to sterilisation, the trial did not also involve the capture, tagging and release of non-sterilised males, inhibiting assessments of natural versus sterilisation related mortality. The likelihood of tagging related mortality was considered low, as the methods used followed Nightingale *et al.,* (2017) who found no differences in survival or growth between tagged and untagged *Austropotamobius pallipes.* Moreover, the study river is a relatively acidic upland river of low productivity, with it being likely that the crayfish population consists of individuals with relatively limited lifespans and relatively high natural mortality rates. Indeed, while crayfish demographic data from other rivers and lakes indicates life spans of between 6 and 20 years (Belchier *et al.,* 1998; Guan and Wiles, 1999), it is suggested that males in the River Barle rarely attain ages above 6 years old. This is because males are observed from trapping results to reach 'large' size (40 mm CL) at age 3+ years old, with the mean size of that cohort being only 44.9 ± 4.7 mm, and where the maximum length was 64 mm CL, that being representative of the largest and therefore oldest individuals (N. Green, unpublished data). With an average of two moults per year and moult increment of 2 - 4 mm in this size class (N. Green, pers. obs.) then the majority of large males are unlikely to be above six years old.

The efficiency of SMRT in the field trials could have thus resulted from a relatively high proportion of the sterilised males 'dropping out' of the pool of sterile males within a short time period from natural causes, potentially inhibiting the efficacy of the technique at the population level. In lentic systems and in more productive rivers, signal crayfish have longer life spans and attain much larger sizes (in excess of 90mm CL; Belchier *et al.,* 1998), so the persistence of the sterilisation effects could arguably be greater, increasing its effectiveness in the longer term. Additionally, the proportion of the sterilised males present in the population is likely to be important in determining the efficacy of the technique. For example, Basilico *et al.,* (2013) reported that the proportions of ovigerous females captured following manual sterilisation of male *P. clarkii* in some French streams were related to the proportion of males sterilised. When less than 3% of catches comprised large males that were then sterilised and released, 46% of females were ovigerous the following year; when 20 to 30 % of the catches were sterilised and released males, juvenile catches declined by 90% the following year. As in the River Barle study site the total catch rarely consisted of more than 13 % sterilised males, then this proportion might be insufficient to significantly reduce the presence of ovigerous females in subsequent years.

The failure of SMRT in the field trial to reduce female reproductive success could also relate to the role played by large males in reproduction. Crayfish form dominance hierarchies (Fero *et al.,* 2007; Herberholz and Mc Curdy, 2007; Goessmann *et al.,* 2000) and it is widely assumed that large, dominant males conduct the majority of mating behaviour through preventing smaller males from copulating. Some studies support this, where large males of other crayfish species (*A. pallipes* and *A. italicus*) mated more

frequently than smaller ones (Woodlock and Reynolds, 1988; Galeotti *et al.,* 2006). However, Rubolini *et al.,* (2007) found there was reduced investment in sperm production with increasing male size, suggesting senescence of reproductive performance with age, which is commensurate with studies of other decapods. In addition, Woodlock and Reynolds (1988) found 33 % of large male *A. pallipes* (> 40 mm CL) failed to copulate at all, with copulations taking longer than those of smaller males, and larger males also unable to mate effectively with small females (Woodlock and Reynolds 1988; N. Green, pers. obs.). Consequently, should smaller males be more reproductively active and successful than larger males, the selective application of SMRT here to relatively large (and potentially elderly) males might have inhibited its effectiveness in reducing female reproductive success. This is emphasised by smaller males being at least twice as abundant as larger males in most *P. leniusculus* populations with, for example, Chadwick *et al.* (2020) reporting that that individuals over 35 mm carapace length (CL) comprised between 1 and 5 % of a population versus 4 to 12 % for lengths between 26 and 34 mm CL. In the River Barle study site, trapping data from artificial refuge traps (which are less size biased than conventional funnel traps) from 2015 to 2020, revealed 66 % of captured crayfish were 25 to 39 mm, with only 19 % of 40 mm and above. Although there is no evidence of female *P. leniusculus* being promiscuous (Green *et al.,* 2020), the higher abundance of smaller males creates more mating opportunities for females and an increased likelihood of successful clutches. Furthermore, if the sterilisation of large males leads to increased fatality within that group, females may be more likely to mate with smaller and potentially more productive males, potentially leading to greater reproductive success.

In captive conditions, sterilised males had significantly lower spermatophore cover and placement accuracy than the control groups. The groups where gonopod regeneration had then been trimmed were also the poorest performing in the trials, and gonopod regeneration amongst sterilised males appeared to be malformed, smaller and less functional. These results again infer that sterilisation should be an effective form of population control (Stebbing *et al.,* 2014; Manfrin *et al.,* 2019), but only if reduced spermatophore cover leads to reduced brood sizes. It has been assumed that the 'normal' placement of spermatophore close to the females ovipore is a prerequisite of successful mating (McLay and Van den Brink, 2016) since crayfish spermatozoa are non-motile. However, it is known that spermatozoa can circulate through the female's glair during spawning and through subsequent movement of the female's pleopods (Niksirat *et al.,* 2014; Yazicioglu *et al.,* 2016). The number of spermatozoa produced by *P. leniusculus* is likely to be high: Harlioğlu *et al.* (2012) found the mean sperm number for *Astacus leptodactylus* of 41-56 mm CL ranged from 4×10^8 to 8.5×10^9 sperm/distal vas deferens (DVD) section. Moreover, this study found that spermatophore distribution amongst sterilised males increased on the abdomen, an area in contact with glair and therefore with spermatozoa. Consequently, it is suggested that the sterilisation process still enables widespread fertilisation of the ova due to higher than anticipated levels of sperm circulating through the females' glair.

In captive trials, females that reproduced with sterilised males did produce smaller brood sizes compared with those that reproduced with non-sterilised males, with this consistent with the results of the limited number of studies completed on other crayfish species (Johović*h* *et al.*, 2019; Aquiloni *et al.,* 2009), however the differences were not

significant. Furthermore, reduced brood sizes over time were not evident in the field data. This lack of reduced brood sizes could be an artefact of the constraints of the field sampling. Due to the study site being on an upland river, high flows often restricted access during spring and the ovigerous female/brood size dataset lacked consistency between years, resulting in relatively small sample sizes (CPUE: n = 79; brood size: n = 150). *P. leniusculus* tend to hatch eggs between March and June in England (Guan and Wiles, 1999, Holdich *et al.*, 2014), and inconsistent sampling in April and May could have missed large numbers of ovigerous females.

Another potential explanation for the lack of decline in mean annual brood sizes and the percentages of ovigerous females in the field trials is the role of population compensatory responses. The study population has been subject to weekly trapping between 2015 and 2021, with approximately 20,000 *P. leniusculus* having been removed. Crayfish populations respond to reduced density and greater food availability via increased growth (moulting) and fecundity (brood sizes and incidence of ovigerous females), coupled with migration into lower density areas (Hudina *et al.*, 2012; Westman and Savolainen, 2002; Parvulescu *et al.*, 2015; Moorhouse and McDonald, 2011). It is thus possible that a reduction in reproduction caused by the presence of sterilised males is being confounded by increased female fecundity as they respond to population reductions through trapping by increasing their reproductive investment. Furthermore, sterilised males could have emigrated and non-sterilised males immigrated into the site, given that large crayfish, particularly males, are known to be the most exploratory sex/age class, exhibiting nomadic behaviour (Bubb, 2004) and tending to lead population expansion (Hudina *et al.*, 2012).

To summarise, the captive trials indicated male sterilisation can reduce male reproductive performance through reduced spermatophore placement and placement accuracy. The captive trials also indicated that gonopod regeneration rates were slow and resulted in malformed gonopods, but following reproduction, did not result in lower female brood sizes. While the results on gonopod regeneration were similar in the field data, this also did not result in reduced female brood sizes, with the incidence of ovigerous females also not significantly reducing over time, despite over 3000 male crayfish being sterilised and released over a seven-year period. Potential reasons contributing to this apparent inability of SMRT to reduce female reproductive success were suggested as relating to small sample sizes, the relevance of spermatophore expenditure and accuracy to successful fertilisation, low long-term survival rates of sterilised males, insufficient proportions of sterilised males in the population, low reproductive efficiency in larger versus smaller males, capture efficiency of ovigerous females and / or compensatory responses. Closer investigation of these influences is necessary in order to understand why the technique did not result in reduced female reproductive success, especially as it still has potential to be more effective in more closed, lentic systems, and those of higher productivity where the persistence of sterilised males could be higher and so lead to greater effectiveness of the sterilisation technique.

Chapter 4. Comparing the efficacy and selectivity of baited traps versus novel artificial refuge traps

Abstract

Non-native crayfish can dominate the invertebrate biomass of invaded freshwaters, with their high ecological impacts resulting in their populations being controlled by numerous methods, especially trapping. Although baited funnel traps (BTs) are commonly used, they tend to be selective in mainly catching large-bodied males. Here, the efficacy and selectivity of BTs were tested against an alternative trapping method based on artificial refuges (ARTs) that comprised of a metal base with several tubes (refuges) attached. The target species was signal crayfish *Pacifastacus leniusculus* in an upland river in southwest England. Trapping was completed in April to October over two consecutive years. In total, 5,897 crayfish were captured, with 87 % captured in ARTs. Comparison of the Catch Per Unit Effort (CPUE) between the trapping methods in the same 24 hour periods revealed significantly higher CPUE in ARTs than of BTs. ARTs fished for 6 consecutive days had higher catches than both methods over 24 hours. Whilst catches in BTs were significantly dominated by males (1.49M:1F), the sex ratio of catches in ARTs was 0.99M:1F. The mean carapace length of crayfish was also significantly larger in BTs (43.2 ± 0.6 mm) than in ARTs (33.6 ± 0.2 mm). Thus, ARTs had higher CPUE over 24 hour and 6 day periods versus BTs and also captured a greater proportion of smaller and female individuals. These results indicate that when trapping methods are deployed for managing invasions, the use of ARTs removes substantial numbers of crayfish of both sexes and of varying body sizes.

4.1 Introduction

Biological invasions are a major threat to native biodiversity and result in biotic homogenisation at global scales (Andreou et al., 2011); (Arim, Abades, Neill, Lima, & Marquet, 2006). Non-native crayfish are very successful invaders, with some species having achieved distributions across a number of continents (Capinha et al., 2011). These crayfish frequently dominate the invertebrate biomass of freshwater ecosystems, substantially altering native communities and ecosystem functioning (Jackson M, 2016) Lodge *et al.*, 2012; Twardochleb *et al.*, 2013). Whilst many of their impacts result from trophic interactions with native species (Jackson et al., 2014), they also impact native crayfish through displacement and pathogen transfer (Holdich & Reeve, 1991); Lodge *et al.*, 2012). Their introduction into Great Britain occurred via aquaculture in the 1970s with the introduction of the American signal crayfish *Pacifastacus leniusculus* and has resulted in multiple ecological impacts (e.g.(Holdich et al., 2014); (Mathers et al., 2016), including population declines in native white-clawed crayfish *Austropotomobius pallipes* and increased riverine sediment deposition rates (Holdich et al., 2014; Rice et al., 2014) (Harvey et al., 2011).

Given the wide-ranging ecological impacts of invasive crayfish, their populations have been subjected to numerous methods for control, containment and eradication. These approaches have included mechanical and physical removal, biological control and biocide application, with autocidal methods also proposed (*cf.* Gherardi *et al.*, 2011; Stebbing *et al.*, 2014). Despite management efforts, most mitigation and remediation options remain under-explored (Gherardi *et al.*, 2011). Where control methods have been

applied over extended time periods then substantial reductions in population abundances (but not extirpation) have been recorded, with concomitant recovery in aspects of the impacted native biota (Dana et al., 2010), or it has facilitated their co-existence with native taxa (Kats et al., 2013). A major issue with the application of these management methods is, however, that they require substantial effort and commitment, coupled with the catch composition of many methods, especially trapping and removal, being size- and/or sex-biased, resulting in only a proportion of the population being targeted and removed, with a typical bias towards larger individuals (e.g. Freeman, *et al.*, 2010; Stebbing *et al.*, 2014). In addition, as the crayfish density reduces through removals then the remaining individuals become harder to catch, as many removal methods are ineffective on low-density populations (Stebbing *et al.*, 2014).

For population control programmes to be successful, Bomford and O'Brien (1995) suggested a number of criteria have to be met, including that all reproductive animals must be at risk of capture, with their capture still probable at low population density. For invasive crayfish, an issue is the low rates of capture and removal of juveniles (< 30 mm carapace length), despite them often comprising a high proportion of population abundance (Houghton et al., 2017)). Thus, trapping methods that are biased towards the capture of only mature crayfish tend to result in poor control efficiency due to much of the population remaining unaffected (Peay, 2004). The size-selectivity of trapping tends to be most apparent when conventional funnel or baited traps are used (Fig. 1), with large adults, particularly males, most frequently captured (Freeman *et al.*, 2010; Gherardi *et al.*, 2011). Baited traps are also relatively labour intensive with, for example, them having to be emptied every 24 hours in the UK due to legislative requirements; they are also

more suitable for lentic or deep, slow moving lotic waters. They can also capture non-target species such as water vole *Arvicola amphibious*, whilst smaller crayfish readily escape (Kozak and Policar, 2002). Nevertheless, their use remains commonplace owing to, for example, their availability and known efficacy that enable comparison with data from other studies (Larson & Olden, 2016). Given the issues highlighted with baited traps there remains an outstanding need for a more effective and less selective trapping method for monitoring and/or controlling invasive crayfish populations, with such non-size selective methods then also serving to provide strong data on their populations.

Given these biases of funnel traps, alternative traps have been developed in order to target smaller crayfish, including microhabitat traps (Kusabs and Quinn, 2010; Parkyn *et al.*, 2011), enclosure traps (Engdahl F, 2013) and nest traps made from plastic pipe (Bechler, Hightower, Rousy, & Smith, 2014). Whilst results suggest improved juvenile capture, these designs have not yet been adopted widely or are cited as a potential control method. An alternative is the Artificial Refuge Trap (ART), a series of plastic tubes that mimic natural refugia, such as burrows and crevices beneath stones (Peay, 2004; Green, 2016; Fig. 1). Crayfish will readily utilise ARTs as shelter during inactive periods in the same way they use natural refugia. As they are also not considered a trap until lifted then they are not necessarily subject to animal welfare legislation (in the UK at least) and can be left *in situ* over extended periods without regular checks. Initial pilot trials suggested ARTs are more efficient than both baited traps and manual searches at detecting low-density crayfish populations in lotic systems, with catches being unbiased or female-biased regarding sex, with capture of a wider size range (Scott, 2012; Walter, 2012). Their use has, however, yet to be tested fully versus other trapping methods.

The aim of this study was, therefore, to quantify the ART efficiency versus the most commonly used trap in Europe, the funnel or baited trap (BT) through comparison of catch rates, composition of the catch and the time taken to deploy each type of trap. Given the pilot studies outlined above, it was hypothesised that compared with BTs, ARTs will capture more representative size ranges and sex ratios of invasive crayfish.

4.2 Methods

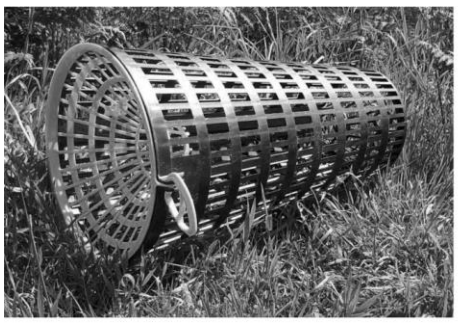
4.2.1 Study site and trapping periods

The trapping and removal of *P. leniusculus* using standard BTs and ARTs took place over two trapping periods, in 2015 and 2016. Trapping during winter periods was not possible due to elevated flow rates at the study site, coupled with crayfish being relatively inactive in winter and thus harder to capture. The trapping site was a 1250 m stretch of the River Barle at Withypool, Exmoor, south-west England (51°06'24.2"N; 3°39'32.2"W; Fig. 2). This river is a typical upland river, having relatively low productivity and variable flows (Q_{95}: 0.63 m^3s^{-1}; Q_{50}: 3.32 m^3s^{-1}; Q_{10}: 11.50 m^3s^{-1}; (CEH, 2017). In the study area, average widths were between 8 to 10 m and depths were generally 0.3 to 0.7 m. Substratum consisted predominantly of a mix of bedrock, boulder and large cobble, with small cobble, gravel and sand/silt towards the banks. The riparian zone was a mix of trees, grassland/scrub and exposed earth, being subject to extensive burrowing by the crayfish. The river has Site of Special Scientific Interest (SSSI) designation for features including its population of Atlantic salmon *Salmo salar* (Natural & England, 2017). The *P. leniusculus* population is well established over a 10 km stretch of the river, with the stretch of river utilised near to the approximate middle of their current distribution.

4.2.2 Trap designs

The artificial refuge trap (ART), also known as the Hutchins trap, pan-pipe trap or multiple tube trap, consists of a series of tubes of 32 to 55 mm diameter and 150 to 250 mm long that are attached to a metal baseplate. The ARTs used in the study comprised of either 7 or 8 tubes of lengths 150 to 170 mm that were attached to a 2 mm thick perforated aluminium base of 300 to 330 mm long (Fig. 1). The tube sizes were a mix of 32, 40, and 50 to 55 mm diameters, with the most frequent (70 % of all traps) combinations being 3 x 32, 3 x 40 and 1 x 50 mm, all 170mm long. A total of 125 ARTs were deployed at 10 m intervals along the 1250 m study site.

The baited traps (or Swedish 'Trappy' Traps) were typically a cylindrical structure constructed of plastic mesh. The BTs (Fig. 1) were the Trappy XL™ type, with entrances at both ends and dimensions 500 x 280 mm, tapering to 180 mm, with diamond shaped mesh of size of 30 x 20 mm (Bubb, Thom, & Lucas, 2004; Trappy, 2017). All trapping was carried out under licence consented by the Environment Agency. The BTs were baited with either cat food or sardines in oil, with their application to specific traps being selected randomly.



(A)

(B)

Figure 4.1. The design of the (A) baited trap and (B) artificial refuge trap as used at the River Barle study site.

4.2.3 Trapping methodology and crayfish collection and movement

Deployment of both traps was conducted between 05/05/2015 and 27/10/2015, and 12/04/2016 and 19/10/2016. During both trapping periods, traps were deployed every 10 m along the study reach. At each of these trapping sites, one ART (weighed down by river substratum) and one BT were deployed (between 0.3 and 3.0 m apart, with the distance dependent on water depth). Both trap types were tied to a wooden stake in the riparian zone. The only exception was that under very low and high flows, BTs could not be deployed at every location due to being exposed (low flow) or displaced (high flow). Whilst ARTs were occasionally washed out during very high flows or dried out when flows were reduced, crayfish were sometimes caught under such conditions, so the total number of 125 ARTs was maintained throughout subsequent data analyses, except when the trap was washed out of the river completely.

The ARTs were left *in situ* throughout both trapping periods, with a brief period of removal each week when the crayfish that had colonised the pipes were removed. In contrast, each BT was deployed once per week, with fishing over a 24-hour period due to extant legislative requirements. When each BT was deployed, the ART was emptied and reset, and when the BT was lifted the following day, the ART was emptied a second time (24-hour soak) and then redeployed (resulting in a 6-day soak to the next trapping day). Due to variability in flows, the day of lifting the ARTs and setting the BTs for their 24-hour soak varied; whilst it was scheduled for every 7 days, occasionally a week had to be missed due to very high flows, resulting in an occasional 7 or 13-day soak for the ARTs.

Data from these 7 or 13-day ART soaks were not included in subsequent data analyses. Consequently, this resulted in a total of 39 trap days over 21 weeks in 2015 and 49 trap days over 27 weeks in 2016. The data from these trapping days were thus the number of crayfish captured per trap over the 24-hour trapping period (BT and ART), and the number of crayfish captured over the 6-day interim period (ART only).

On their removal from the traps, the captured crayfish were counted and held in water-filled containers during processing. For each individual crayfish, its sex and carapace length (CL; nearest mm) were recorded, along with their reproductive state, moult status and any signs of damage or disease. Sex was recorded as male, female or indeterminate for those <12mm CL (where sex could not be determined). In addition, the crayfish were also categorised as small (<21 mm CL; likely to be young-of-the-year), medium (21 to 39 mm CL; likely to be sub-adults and subordinate adults including breeding females), and large (≥ 40 mm CL; likely to be adults and berried females) (Stebbing *et al.*, 2012). Captured crayfish were not returned to the river due to their non-native status, with individuals euthanized by a cut to the carapace. The exception was for some large males that were returned (under licence) to enable a separate experiment to be completed on male sterilisation. Subsequent recaptures of these males (as identified by their sterilisation) were excluded from the dataset. During some sampling occasions, the time taken to deploy and remove an ART and the time to set and collect a BT was recorded to enable comparison of the time taken to use both methods.

4.2.4 Data analysis

The trapping data were used to calculate a catch per unit effort (CPUE) metric that enabled comparison of catch data over time and between trapping method. For each

method, this was determined as the total number of crayfish captured in all traps per sampling occasion divided by the number of traps used. Correspondingly, for each sampling occasion, this provided a single CPUE value for the BTs and three CPUE values for ARTs (one for the 24-hour soak that was directly comparable to the BT data, one for the 6-day soak and a weekly total CPUE value (24-hour soak + 6-day soak)). The latter was calculated as it was considered that the two site visits to set and empty the BTs were commensurate with the effort required to empty the ARTs on days 1 and 6.

Testing whether sex ratios of captured crayfish differed from 1M:1F used Chi-square (goodness of fit). To compare CPUE between BTs and ARTs, two methods were used. The first method considered the data as paired, and thus tested mean CPUE data for BTs versus ARTs when they had been used in the same 24 hour sampling occasion. This was initially tested using a paired t-test, with mean CPUE from BT then plotted against ART and tested using linear regression, where the regression coefficient (*b*) tested the null hypothesis that CPUE was equal between the methods on each trapping occasion. The null hypothesis was rejected when *b* was significantly different to 1.0 and vice-versa, based on its 95 % confidence limits (McDonald, 2014). The second method tested the effects of a range of abiotic and catch variables on the CPUE data within generalized linear models (GLM; family: linear). The initial model tested differences in CPUE only between BTs and ARTs when used for 24 hour periods. In the model, the dependent variable was CPUE per sampling occasion, the independent variable was 'trapping method', and the initial covariates entered into models were water temperature (°C), flow (m^3 s^{-1}) (both taken as their value at 0900 on the day of trapping) and their interaction, plus total cumulative catch prior to each trapping day and sampling year. Temperature

was included as a covariate due to its potential influence on crayfish activity levels and trapping success (Hein *et al.*, 2007). As flow rarely affects the movement of crayfish (Bubb *et al.*, 2004), then it was included as a covariate to account for how elevated flows impacted trap performance. The models were run iteratively, with removal of non-significant covariates and comparisons of AIC to determine the parsimonious model, where the best fitting model was determined by the lowest AIC value. The outputs of the final model were estimated marginal means of CPUE (± 95 % confidence limits) and the significance of their differences according to linearly independent pairwise comparisons (with Bonferroni adjustment for multiple comparisons). A second GLM was then used to test differences between the CPUE of BTs and ARTs, with the latter using data for the periods 24 h, 6 days and 7 days, where for the 6 and 7 day data, CPUE represented the mean number of crayfish captured per trap in that period, rather than per day.

Crayfish size (as CL) was then tested for differences between trap type using a GLM; where CL was the dependent variable, trap type was the independent variable, and temperature, flow, year and cumulative catch were initial co-variates, with the same process used as described for CPUE. To compare the time taken to deploy and remove the BTs and ARTs from the river, the individual time data were compared via means and 95 % confidence limits and then tested for the significance of their differences using ANOVA. All statistics were completed using SPSS v.23.0 (IBM, 2017). Where error is presented around the mean, it represents 95 % confidence limits unless otherwise stated.

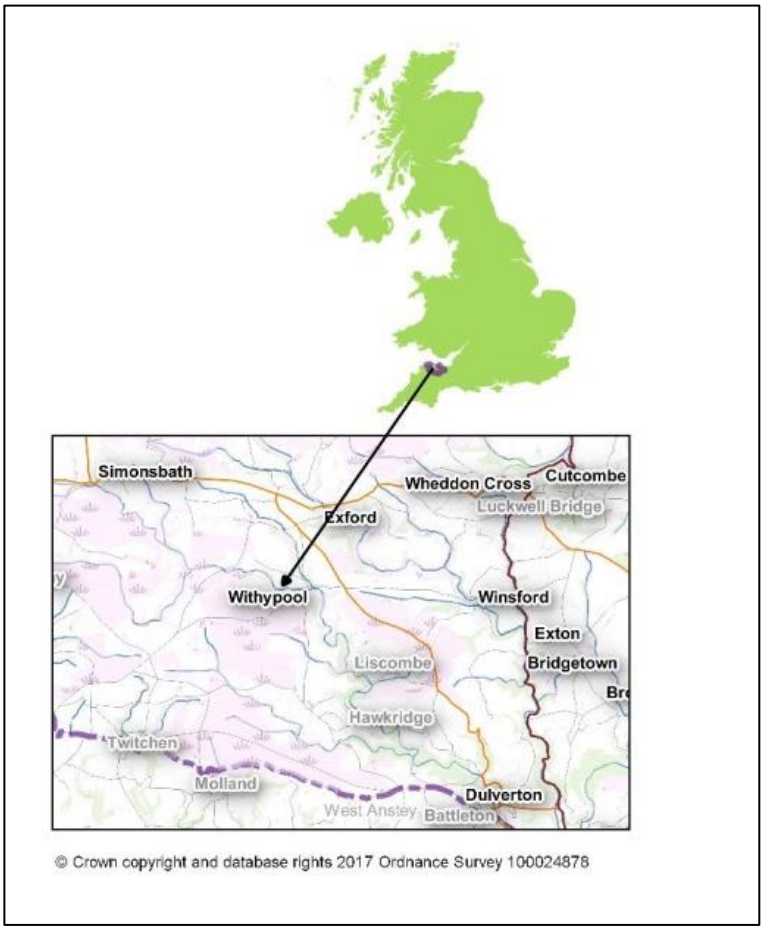


Figure 4.2. Location of study site, River Barle, Withypool, Somerset, England.

4.3 Results

4.3.1 Total catches and catch per unit effort (CPUE)

A total of 5,897 crayfish were captured across the sampling years (Fig. 3A), with 87 % of all crayfish captured in ARTs (Table 1). The cumulative catch of crayfish increased at a linear rate, but with overall mean CPUE declining by 25 % across the entire period (Fig. 3B, C).

Table 4.1 Summary of the total catch data by sex and life-stage. M: male; F: female; I: Indeterminate (< 13mm); YoY: young-of-the-year (<20mm); SA: sub-adult (21-39mm); A: adult (>40mm); BF: berried female.

Trap	N	M	F	I	YoY	SA	A	BF
ART	5131	2494	2576	61	206	3887	1038	105
BT	763	457	305	1	1	183	579	5

Comparison of the paired CPUE data revealed that the 24 h CPUE of ARTs was significantly higher than BTs (mean CPUE 0.47 ± 0.07 vs. 0.22 ± 0.08 n d^{-1}; t = -4.91, P < 0.01; Fig. 4). Linear regression also revealed their relationship deviated significantly from 1:1, rejecting the null hypothesis that CPUE would be similar between the trapping methods on specific trapping days (R^2 = 0.05; $F_{1,27}$ = 1.54, P = 0.23; 95 % confidence interval of *b* = -0.07 to 0.30) (Fig. 3). In GLMs testing differences in CPUE (as independent data) between BTs and ARTs over 24 h, the non-significant covariates of water temperature (P = 0.92), the interaction of temperature and flow (P = 0.62) and cumulative catch (P = 0.47) were removed during model development. In the final model

(AIC: -31.87), the only significant predictor of CPUE was the covariate of flow ($P < 0.01$), with the effect of trapping method and year both non-significant ($P = 0.97$, 0.15 respectively). Mean CPUE was thus not significantly different between the two methods when the data were assessed as independent variables across the entire trapping period (BT: 0.26 ± 0.05, ART: 0.27 ± 0.06 n d^{-1}; Wald $\chi^2 = 0.01$, $P = 0.97$).

The best fitting GLM comparing CPUE from all methods and trapping periods involved all the entered covariates (AIC = -34.31; GLM: Wald $\chi^2 = 283.84$, $P < 0.01$), and with the exception of temperature ($P = 0.13$), the effects of all covariates were significant (flow, year, cumulative catch, $P < 0.01$ in all cases). Mean CPUE values were again not significantly different between the trap types over 24 h ($P = 1.0$), but were significantly different between these data and the ARTs fished for 6 days (0.69 ± 0.07 crayfish per trap over 6 days; $P < 0.01$ in both cases) and 7 days (0.96 ± 0.07 crayfish per trap over 7 days; $P < 0.01$ in both cases).

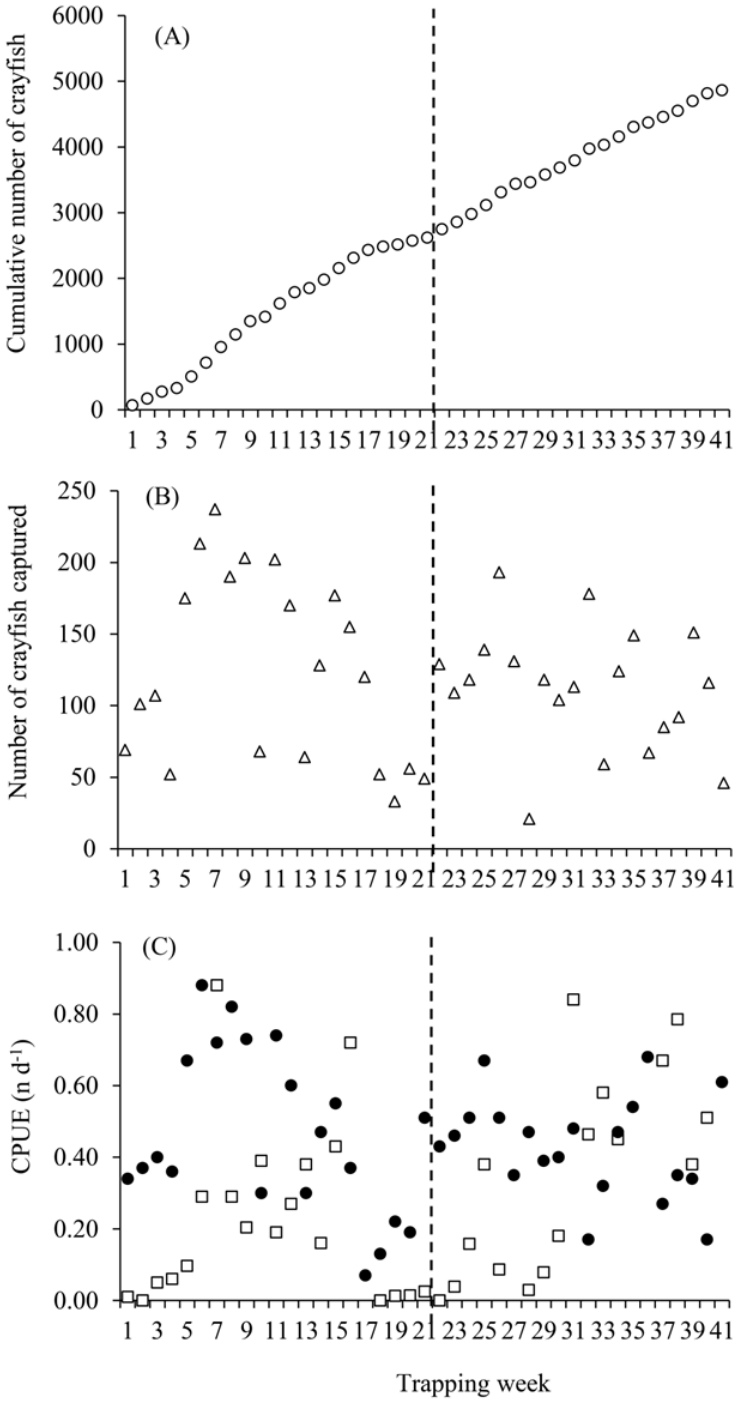


Figure 4.3 (A) Cumulative number of crayfish removed from the site by both trapping methods (B) Total number of crayfish captured during each trapping week (C) Catch per unit effort of crayfish per trapping week (artificial refuge traps filled circles, baited traps

open squares. In all cases the vertical dashed line marks the split between trapping years 2015 and 2016.

4.3.2 Catch composition by trapping method

Comparison of the catch structure of the trapping methods revealed that the sex ratio of mature crayfish was significantly male biased in the BTs (1.49M:1F; $\chi^2 = 28.60$; $P < 0.01$). In ARTs, of 4720 sexed crayfish captured (Table 1), the sex ratio was 0.99M:1F, with this not significantly different to 1:1 ($\chi^2 = 0.22$; $P = 0.64$). Only one small crayfish was captured in the BTs versus 206 in ARTs (Table 1). ARTs also captured the majority of berried females (95.4 %) and moulting individuals (89.4 %).

The size ranges of crayfish captured across the trapping methods were similar (ARTs 4 to 62 mm; BTs 11 to 64 mm). However, the length distribution within these ranges differed considerably between the trap types, with a general pattern of ARTs capturing smaller sized individuals (Table 1; Fig. 4). The best fitting model testing length (as CL) between methods included all of the covariates being entered into the model except year ($P = 0.61$), with this final model being significant (AIC: -28.91; GLM: Wald $\chi^2 = 1141$, $P < 0.01$). Mean CL of crayfish captured in ARTs was significantly smaller (33.6 ± 0.20 mm) than BTs (43.2 ± 0.55 mm). In this model, the covariates of temperature and cumulative catch were significant ($P < 0.01$), but flow was not ($P = 0.07$).

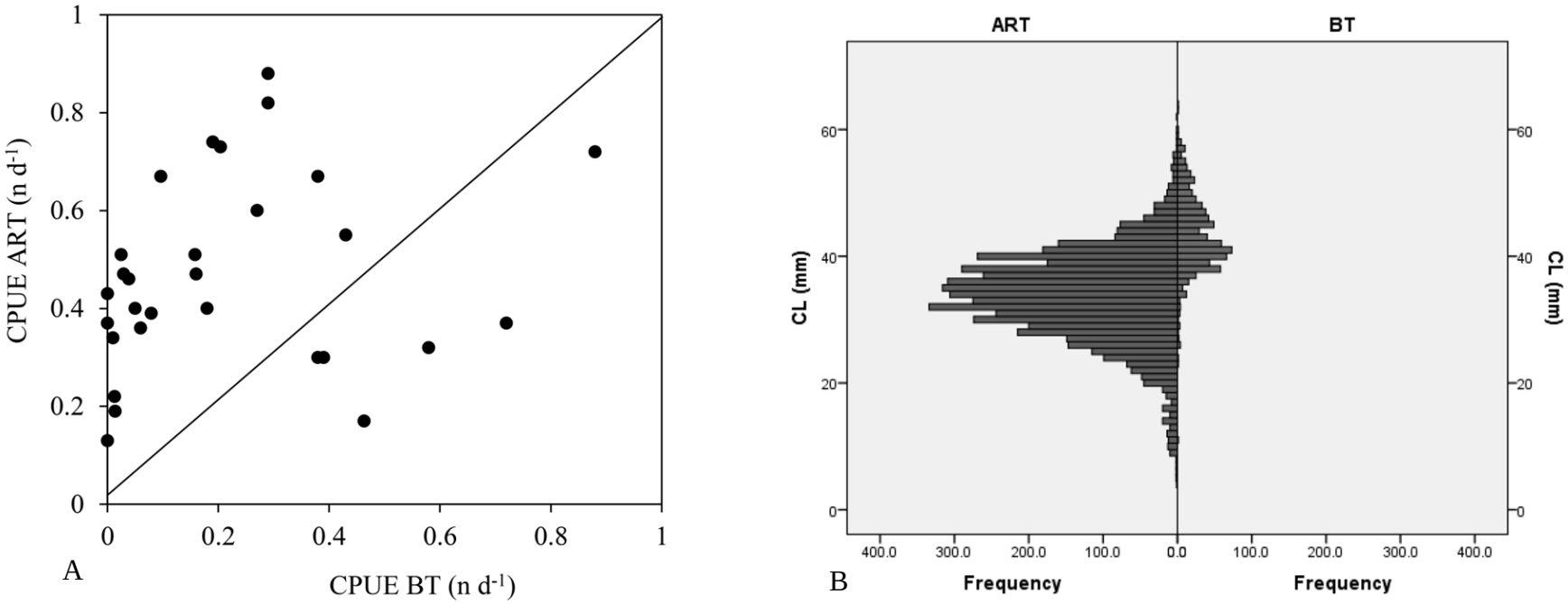


Figure 4.4 A) Catch per unit effort of baited traps versus artificial refuge traps on the same 24 hour soak (n =29). The 45° line represents the 1:1 relationship in the CPUE of the two trapping methods. B) Numbers of crayfish per size class (as their size frequency distribution) of the total of baited traps versus artificial refuge traps.

4.3.3 Time taken for trap deployment/ collection

The mean time taken to bait and deploy then empty and store an individual BT was 87.3 ± 10.4 s versus 33.2 ± 16.4 s to empty and reset the ART, with this difference significant ($F_{1,226} = 965.01$, $P < 0.01$). Note these values exclude the time taken to purchase bait, remove it from and replace it to a storage facility after use.

4.4 Discussion

The trapping of crayfish over this two-year period in the study reach revealed that ARTs had a significantly higher CPUE than BTs when directly compared over 24-hour periods (i.e. as paired data). The ability to leave ARTs to fish for six-day periods, something not possible with BTs, then resulted in them capturing significantly higher numbers of crayfish than both trapping methods fished for 24 hours. In addition to their lower CPUE, BTs generally require more regular management in relation to emptying and re-baiting compared to more passive forms of capture (Gherardi *et al.*, 2011). As ARTs work in a different manner to BTs via their provision of an alternative and heterogeneous habitat for crayfish then it means it can be desirable for them to be left *in situ* for extended periods to enable higher rates of colonisation. When the two trapping methods were compared across the two year sampling period (i.e. not as paired data) then although these indicated the overall differences in CPUE were not significantly different, they did indicate that increased flow rates inhibited the catch efficiency of both methods.

The study reach was located on an upland spate river of relatively low productivity and the crayfish population was estimated as being as of medium abundance (Author, pers. obs.). Thus, leaving the ARTs *in situ* for six-day periods did not result in the artificial

refuges on the traps being saturated with crayfish, thus shortening the time between emptying would not necessarily have increased capture rates. In addition, as crayfish use the ARTs as habitat and are not enclosed within them, the longer the saturation period also does not necessarily mean the greater the catch. If these traps are subsequently applied to populations of higher abundance then work should initially determine if the refuges are rapidly colonised and, if so, then reducing the time between setting and emptying should increase catches. Although work is underway currently to determine the optimum soak length for ARTs on the study site, on a wider scale this is likely to be influenced by context-dependent factors such as population density and habitat quality (e.g. availability of alternative natural refuges).

A further option to increase catch sizes per ART would be to increase the number of refuges (tubes) per trap. As signal crayfish tend to be aggressive and cannibalistic, including antagonistic interactions between individuals that can result in displacement (Graham & Herberholz, 2009), it had been assumed that each tube would only be able to capture an individual, thereby limiting catch size to the number of tubes per trap. This was not the case, however, with multiple crayfish sometimes captured in a single tube. This was interpreted as being due to ARTs capturing smaller individuals than BTs, with higher proportions of females that tend to be less aggressive than large bodied males (Berry & Breithaupt, 2010) and thus more likely to co-habit tubes.

The size distribution of crayfish captured in the ARTs differed to the BTs, with a general pattern of catches comprising individuals of smaller carapace length, with this consistent with the hypothesis. Moreover, the most frequently captured size class in ARTs was 21 to 39mm CL, with individuals of below 30 mm often dominating population size structure

(Houghton *et al.*, 2017), whereas BTs predominantly captured individuals above 40mm CL. This 'medium' size range in the ARTs generally covered the 'sub-adult' and 'subordinate adult' components of the population and it is these individuals that tend to show the density-dependent compensatory responses (e.g. increased growth rates and fecundity) to the removal of larger adults by BTs (Moorhouse and McDonald, 2011, Skurdal and Qvenild, 1986). Therefore, the application of ARTs with BTs potentially reduces the effects of these compensatory responses and thus their combined use could increase the effectiveness of invasive crayfish control attempts when trapping is employed. It should be noted, however, that although ARTs captured crayfish as small as 4 mm CL, small crayfish (i.e. < 20mm CL) were still poorly represented in catches and thus despite their ability to capture a far greater proportion of smaller crayfish than BTs, including an abundance of animals between 21 and 30mm CL, ARTs are also unable to target all life stages of an invasive crayfish population equally.

There were higher proportions of females captured in the ARTs than the BTs; where catches in BTs were significantly male dominated, they were of approximately equal sex ratio in ARTs, although the hypothesis had predicted female dominated catches. Although female crayfish are believed to be less active than males and thus are seen as being less vulnerable to trapping (Gherardi *et al.*, 2011), their frequent capture in the ARTs suggests that they can be as vulnerable as males to some trapping methods. Indeed, the removal of large numbers of females, especially sub-adults, might increase the effectiveness of a trapping programme by removing individuals prior to their first spawning event (Stebbing *et al.*, 2012). In addition, the large numbers of berried females captured and removed could reduce juvenile recruitment substantially. Although not investigated in detail here, the ability of ARTs to capture both moulting animals and berried/brooding females should

also enable further study of their natural behaviours in the wild which could provide insights into traits, such as growth rates and productivity, that could inform and enhance an invasion control programme or, in the case of native crayfish, assist in the development of a conservation strategy (Rogowski, Sitko, & Bonar, 2013).

It has been postulated that traps that are able to remove large numbers of multiple life stages of crayfish are likely to be more effective at eradication or long-term suppression of a population than those that capture only specific size or length classes (Stebbing *et al.*, 2012, Dana *et al.*, 2010). Studies on the management of invasive crayfish also tend to stress the importance of long-term control efforts that aim to not only remove substantial proportions of the population but also prevent their rapid population recovery via compensatory responses (e.g. Gherardi *et al.*, 2011; Moorhouse and McDonald, 2011). Consequently, long-term control methods need to consider the cost of the methods employed in order to ensure the maximum cost-benefit of the approach (Simberloff, 2009). The results reported here suggested that ARTs were more cost effective and precise than BTs in terms of the time per individual crayfish removed and thus long-term crayfish control efforts could have higher feasibility when these are used. However, since BTs capture larger size classes then the most effective trapping technique is likely to be their combined use, ensuring a wider range of life-stages would be removed on each trapping occasion.

It is recommended that future studies also include trials on lentic systems and utilise alternative designs that could potentially capture larger numbers of crayfish and target different size classes, especially young-of-year. For example, tube sizes could be varied to target different size classes, and tubes could be stacked to form bundles. Studies could

also be conducted on the efficacy of control attempts using ARTs alone, with investigation of the optimal time of year to catch different sexes and size classes and the optimum length of soak in relation to the abundance of the target population of crayfish. In summary, the results of this trapping programme on a lotic invasive crayfish population revealed that the application of ARTs provided substantial benefits to population control and the capture of a more representative length range and sex distribution compared with BTs. They also had a higher CPUE than BTs in the same 24 hour period and over longer trapping periods, enabling the capture of substantially higher numbers of crayfish with lower labour input. Thus, ARTs represent a more cost-effective methodology than BTs. Correspondingly, it is recommended that when invasive crayfish populations are being controlled via trapping, a combination of trap types be utilised to ensure that all life-stages are vulnerable to capture and that trapping efficiency is maintained at low population abundance.

Chapter 5. Responses of an invasive riverine crayfish population to multi-method population control

This chapter includes data on ovigerous female abundance and brood size also described in Chapter 3.

Abstract

Invasive crayfish are a major threat to biodiversity and the various control methods applied have been of limited success to date. Conventional trapping tends to be size and sex biased and is therefore of limited effectiveness as it fails to target all life stages of crayfish populations. The sterile male release technique has been suggested as a potentially effective management tool, especially in combination with a trapping technique that removes a greater proportion of females and smaller crayfish. The use of the novel artificial refuge trap in combination with the mechanical sterilisation of large males was tested over a six year period on the River Barle, Somerset, SW England. The results indicated that there was no decrease in overall crayfish CPUE, however the abundance of crayfish sized ≤ 24 mm carapace length had decreased by 70% by the end of trial. Mean crayfish size and the ratio of females to males both increased over the study period. Brood size, CPUE and proportion of ovigerous females all changed over the study period, with decreasing trends from Year 3 although without consistent temporal patterns. Consequently, the study has not shown the expected decreases in crayfish population size although there is some evidence that decreases in reproductive output and CPUE of small crayfish had occurred by its conclusion. The lack of a control site has prevented definitive conclusions from being drawn as to the mechanisms underlying these changes, although it is speculated that low trapping intensity and relatively low proportions of sterilised males present could have impacted on the trial's effectiveness.

5.1 Introduction

Invasive species are a pervasive driver of environmental and biodiversity change (Simberloff et al. 2013). Crayfish are often considered 'keystone' species in freshwater ecosystems, where their ecological engineering activities can have substantial effects on the physical habitat and biota (Reynolds and Souty-Grousset 2012). Consequently, alien crayfish have caused substantial ecological impacts in invaded waters, including shifts in ecosystem functioning (Smart et al. 2002), disruptions to food webs at multiple levels (Jackson et al. 2014), increased levels of river sedimentation (Rice et al. 2014; Mathers et al. 2016), and extirpations of native crayfish via inter-specific competition and transmission of the pathogen that causes crayfish plague, *Aphanomyces astaci* (Freeman and Turnbull 2010; Holdich and Sibley 2009). One of the most impacting of all invasive crayfish is the American signal crayfish *Pacifastacus leniusculus*, introduced into Europe from the north-west USA, firstly into Sweden and then most of Europe in the 1960s and 1970s (Holdich et al. 2014; Mathers et al. 2016).

The substantial ecological impacts caused by signal crayfish have resulted in their populations being targeted regularly for management, where the aim can be eradication, or control and containment (Simberloff 2010; Stebbing et al. 2014). However, the general consensus is that while some management methods can be effective, their success tends to be context-dependent, and there remains no definitive methodology that can eradicate or control invasive crayfish abundance and reduce their ecological impacts (Gherardi et al. 2011; Stebbing et al. 2014). Nevertheless, population control, which generally involves reducing crayfish abundance, has been demonstrated as enabling some recovery of the invaded ecosystem (Moorhouse and McDonald 2011; Hansen et al. 2013). In Britain, for example, reductions of *P. leniusculus* population abundances have resulted in

the increased abundance and diversity of native macro-invertebrate communities (Moorhouse et al. 2014), improved river bank stability (West 2011), and increased abundance of fish populations (West 2011). Population control efforts that target all life stages of a population and extend for a number of years have tended to be more successful at suppressing the abundance of invasive crayfish and enabling some ecosystem recovery (Hein et al. 2007; West 2011). However, the success of management programmes is also influenced by abiotic variables such as habitat complexity, biotic variables (e.g. presence of crayfish predators), and the level of management efforts, such as trapping intensity (Stebbing et al. 2016).

One of the most frequently used removal methods for controlling invasive crayfish populations is the baited funnel trap (BT), whose design tends to result in catches that are biased towards the capture of larger individuals, particularly males (Kozak and Policar 2003). This selective removal of larger males can, however, trigger density-dependent processes that potentially lead to the population compensating for losses through increased reproduction and growth rates (Zipkin et al. 2009; Freeman et al. 2010), and can even result in ecological impacts exceeding those of the original population (Závorka et al. 2020). The artificial refuge trap (ART), a habitat based attractant consisting of a series of tubes attached to a metal base, generally captures crayfish in more equal sex ratios than BTs and across a wider size range (Green et al. 2018; Chapter 2). Correspondingly, their deployment in invaded waters could remove a greater proportion of smaller crayfish and more females than BTs, thus potentially avoiding compensatory responses in the surviving individuals, such as increased ecdysis rates, earlier maturation, increased brood size and higher incidence of ovigerous females (Ramalho and Correia

2008; Freeman et al. 2010; Parvulescu et al. 2015). Other traits associated with lower density crayfish populations include larger mean individual sizes, improved body condition and a higher M:F ratio (Moorhouse and McDonald 2011b; Hudina et al. 2012).

The use of ARTs on invasive crayfish has revealed that despite catching smaller crayfish than BTs, they are still ineffective at capturing juvenile (young of the year) crayfish in substantial numbers, resulting in a proportion of the population still being unaffected by management programmes that are reliant on trapping alone (Green et al. 2018). Consequently, for the juvenile component of the population to be targeted effectively requires an alternative technique to trapping, such as one that aims to reduce reproduction and/or recruitment rates. The Sterile Male Release Technique (SMRT; Knipling 1959) has been proposed as a method to achieve this through the capture, sterilisation and release of large individual males (Aquiloni et al. 2009). Its success is then reliant on the sterilised males retaining their cannibalistic tendencies (Houghton et al. 2017), and maintaining their dominance in the population and especially in reproduction, where the reproduction involving a sterile male is posited to result, ultimately, in reproductive failure via the eggs remaining unfertilised, so lowering the population reproductive success and, subsequently, their recruitment (Stebbing et al. 2014).

The application of SMRT has been successful to some other invertebrate taxa, such as the screw worm *Callitroga hominivorax* (Knipling 1959). The limited trials that have been completed on invasive crayfish have indicated that the method can indeed reduce male reproductive success (Aquiloni et al. 2009; Basilico 2013; Stebbing and Rimmer 2014; Johović et al. 2019). For example, the combined use of mechanical removal of invasive crayfish and SMRT was trialled in red swamp crayfish *Procambarus clarkii* in French

streams (Duperray et al. 2013; Chapter 3). When the proportions of sterilised males were low in the population (e.g. 2 to 3 % of catches being sterilised males) then the proportion of ovigerous females remained high. However, when the proportion of sterilised males was higher (e.g. 20 to 30 % of the total catch), the proportion of juveniles in the population decreased from 20 % to 2 % over three years (Duperray et al. 2013). These results suggest that for SMRT to be successful, there is a requirement for a relatively high proportion of the males present in a population to have been sterilised (Duperray et al. 2013; Chapter 3). There is, however, a paucity of information on how management programmes that combine removals with SMRT could be successful in other invasive crayfish, such as *P. leniusculus*.

The aim of this study was to thus quantify the response of an invasive *P. leniusculus* population to a management control programme that integrated removals (via trapping) with SMRT, using the River Barle, (Somerset, SW England) as the study river over a six year period (2015 to 2020). The objective was to measure the response of the crayfish population to the management programme in relation to their relative population abundance, size and sex structure, reproductive traits and rates of ecdysis. We posit that: (i) the relative population size (measured as catch per unit effort of ARTs) will decline over time and in response to the intensity of removals and the use of SMRT; (ii) as population size declines, the population size structure will shift to larger body sizes (as trapping with ARTs primarily removes smaller individuals) and that sex ratios will favour males (Moorhouse and MacDonald 2011); (iii) due to SMRT, the proportion of ovigerous females and female brood size will reduce over time.

5.2 Methods

5.2.1 Study stretch and reaches

The study was focused on a 1500 m reach of the River Barle at Withypool, Somerset, south-west England (51°06'24.2"N; 3°39'32.2"W). This typical upland river is of relatively low productivity and has highly variable flows that are generally higher in the winter period (Q_{95}: 0.63 m^3s^{-1}; Q_{50}: 3.32 m^3s^{-1}; Q_{10}: 11.50 m^3s^{-1}; CEH, 2017). In the study area, average wetted widths were between 8 to 10 m and depths were generally 0.3 to 0.7 m. Substratum consisted predominantly of a mix of bedrock, boulder and large cobble, with small cobble, gravel and sand/silt towards the banks. The riparian zone was a mix of trees, grassland/scrub and exposed earth, and was subject to extensive burrowing by the crayfish. The river is designated as a Site of Special Scientific Interest (SSSI) for features including its population of Atlantic salmon *Salmo salar* (Natural & England, 2017). The *P. leniusculus* population is now well established over a 10 km stretch of the river, with the stretch of river utilised close to the centre of their current distribution. The study reach was divided into a central focal reach of 1000 m, with a 250 m buffer reach at each end. Although all 1500 m of river was marked with a numbered wooden stake every 10 m on the right-hand bank, with one ART and one BT set in the river at each stake location, subsequent analyses only used data from the central focal reach to reduce the effects of crayfish immigration from adjacent reaches. The left-hand bank was not used due to access issues.

5.2.2 Sampling methods and frequency

The trapping and removal of *P. leniusculus* using BTs and ARTs took place between 2015 and 2020, commencing between mid-April and early May (year-to-year variation in the

start date was due to differences in river conditions), with weekly trapping events being conducted until they were terminated in mid-October as river flows made trapping difficult and low temperatures reduced catches (Table 5.1). The start and finish dates of trapping in 2020 was also impacted by the Covid-19 pandemic that delayed the start date (Table 5.1). Trapping with BTs occurred weekly between 2015 and 2017, but they were used less frequently in 2018 and 2019, where their use focused primarily on trialling the effect of different soak lengths and timings on catch rates (Table 5.1). In 2020, no BTs were deployed. Trapping with ARTs took place twice weekly between 2015 and 2017 then once a week between 2018 and 2020. Trapping and sterilisation activities were assisted by trained citizen scientists (N = 6 to 15 per event) who volunteered their time to the project, and were involved in setting/ lifting traps, measuring, sterilising and euthanising the crayfish and recording data.

Table 5.1. Summary of start and end dates per study year, and the extent of the trapping effort, at the River Barle study site (including buffer zones). Crayfish captured includes killed and sterilised but not recaptures.

Year	Start date	End date	ART events (n)	Crayfish captured (n)	BT events (n)	Crayfish captured (n)
1 (2015)	5th May	20th Oct	38	2919	17	398
2 (2016)	12th April	18th Oct	48	2648	17	417
3 (2017)	11th April	18th Oct	45	2987	15	347
4 (2018)	24th April	16th Oct	25	4029	14	1109
5 (2019)	16th April	15th Oct	21	3762	5	214
6 (2020)	12th May	13th Oct	18	3231	0	0

On each trapping occasion, for each individual crayfish captured, the following details were recorded: trap location (stake no.), trap type (ART / BT), sex (including juvenile), body size (as carapace length (CL) to nearest mm), the presence / absence of ecdysis and females displaying evidence of breeding condition, either via the presence of glair glands ('glair'), eggs (ovigerous) or dependent juveniles. In spring, the number of eggs/juveniles held by the ovigerous females was also recorded. Then, all males ≥ 40mm CL were manually sterilised, removing all four gonopods, either by cutting with scissors or pulling them off with tweezers, before they were released back into the river. When a previously sterilised male was recaptured, any gonopod regeneration was removed prior to their return to the river. Any male crayfish that were considered too damaged (e.g. through loss of both chelae) to compete for mates effectively, were humanely killed using a longitudinal cut through the carapace. All other captured crayfish were humanely killed.

5.2.3 Data and statistical analyses

Due to the inconsistent trapping effort of BTs over the period, only ART data were used in catch analyses to test the effect of the management programme on relative crayfish population abundance. Due to variations in soak lengths between years, ART catches were standardised to a catch per unit effort metric (CPUE, as the number of crayfish captured per trap per day). CPUE was expressed as the overall number of crayfish and then according to three size categories: ≤ 24 mm CL, representing the 0+ age group; 25 to 39 mm CL, representing sub-adults and sexually mature young adults (1+ and 2+ years); and ≥ 40 mm CL (3+). In both sex and size classes, sterilised males were included but recaptures of sterilised males excluded in order to avoid double counting.

Temporal changes in CPUE were tested using generalised linear models (GLMs). The independent variable was year of study, on the basis that the CPUE data had been collected annually between May and October, with each year thus comprising a number of trapping events that resulted in a known number of crayfish being removed from the river (Table 5.1). Therefore, its use as the independent variable in the GLMs enabled testing of the changes in CPUE as the study progressed. The dependent variable (of linear distribution) was CPUE of each sampling occasion (organised by year), with the standard co-variates of mean daily flow and water temperature at 09.30 of each trapping event, with other relevant covariates added to each model where appropriate. The initial model included all variables, with the best-fitting model determined by removing the non-significant covariates and noting the effect of Akaike's Information Criterion (AIC); the final, best fitting model that was reported in the results was the one with the lowest AIC value. The reported results were the mean CPUE of each study year (as estimated marginal means of CPUE (± 95 % confidence limits), adjusted for the effects of retained covariates) and the significance of their differences between years according to linearly independent pairwise comparisons (with Bonferroni adjustment for multiple comparisons). The same model structure and process was then used to test CPUE of each size category over the study period and changes to mean carapace length. Ecdysis was expressed as the CPUE of individuals being in ecdysis at point of capture and tested within GLMs using a linear distribution using the same process and the same covariates, producing models for total CPUE ecdysis and within each size category. For testing changes in sex ratios over time, the three size categories were used (≤ 24 mm CL, 25 to 39 mm CL, ≥ 40 mm CL), but where the smallest size group only used individuals between 13 and 24 mm as individuals of ≤ 12 mm could not be sexed. The changes in sex ratios were tested using Pearson chi-square tests.

Testing the temporal change in CPUE ovigerous females used only data collected in the five week period between the second week of May and the second week of June. The second week of May was used as the start date as it was the latest start date of any study year (Table 5.1). The second week of June was used as the end date as it was the approximate date of the last capture of an ovigerous female in any study year. A minimum CL of 30 mm was used for all as it was the size of the smallest ovigerous female captured across all years. These female-specific metrics were then tested within GLMs using the same model structure and process as outlined for CPUE. The data distribution of ovigerous female CL and brood size (adjusted for CL) was treated as linear, whilst the proportion of ovigerous females used a negative binomial with log link distribution. The adjusted brood size metric used data from all weeks from mid-April to the end of June each year.

All statistical tests were completed in SPSS v.26 (IBM, 2019); use of parametric tests only followed after testing for normality (Shapiro Wilkes and Kolmogorov-Smirnov tests); non-parametric tests were always used where data were not normally distributed. Where error values are presented around means, they represent standard error unless stated, and results from multiple comparisons were adjusted using Bonferroni correction. Significance is reported as exact two-tailed unless stated.

5.3 Results

5.3.1 Relative crayfish population size

A total of 12,245 crayfish were captured by ARTs and then removed from the study site across the six year trapping period, of which 9,617 were of CL between 25 and 39 mm (Table 5.2). A total of 2297 (1,351 in ARTs, 946 in BTs) captured males over 40 mm were sterilised and returned in the focal reach (14% of total catch), with 486 sterilised males recaptured in ARTs (Table 5.2).

The best fitting GLM testing the temporal pattern in overall CPUE (as a measure of relative population size) revealed that although there was some variability over time, the extent of these changes was not significant, with no decrease in 2020 compared to previous years (Figure 1; Table 5.3, S 5.1). Similarly, temporal changes in CPUE of both male (M) and female (F) crayfish were not significant (Table 5.3; S 5.1). Within these models, flow was a significant covariate for male CPUE whilst temperature was significant for female CPUE (Table 5.3, S1). Regarding the crayfish size categories, temporal changes in both 25 to 39 mm and ≥ 40 mm were not significant (Figure 5.2; Table 5.3, S 5.1). Mean CPUE of ≤ 24 mm CL crayfish fluctuated over the study period but was significantly lower in Year 6 compared to Year 1 with neither temp nor flow significant (Figure 5.2; Table 5.3, S 5.1).

Table 5.2. Summary of total crayfish captured in Artificial Refuge Traps from 2015 (Year 1) to 2020 (Year 6) inclusive in the central 1000m of the River Barle study site (as used in data analysis). Sex and size classes include sterilised and recaptured individuals; juveniles are classified as all animals too small to be sexed (< 13 mm CL); berried females are all ovigerous individuals captured between mid-April and mid-July each year. Rejected models are shown in supplementary Table S 5.1 in Appendix 1.

Year	Killed	Sterilised >40 mm CL	Recaptured	≤ 24 mm CL	25 – 39 mm CL	≥ 40 mm CL	Male	Female	Juvenile	Berried female
1	1927	169	32	180	1583	365	1005	1104	19	22
2	1670	261	81	162	1269	582	949	1033	31	48
3	1969	174	94	278	1498	461	1124	1091	22	24
4	2294	273	120	206	1809	674	1342	1315	32	26
5	2481	219	100	246	1972	584	1362	1428	11	48
6	1904	255	59	85	1486	646	1001	1197	19	9
Total	**12245**	**1351**	**486**	**1157**	**9617**	**3312**	**6783**	**7168**	**134**	**177**

Table 5.3. Summary of best fitting generalised models testing the effect of study year on each dependent variable, and where CPUE is catch per unit effort (no. crayfish per trap per day), CL is carapace length measured as mm, AIC is Aikake's information criterion, Wald X^2 is the Wald chi square statistic, P is the significance of the overall model. Temp is temperature recorded at 09.30 on each trapping event and flow is mean daily flow on each trapping event whilst covariate P is the significance of the covariate within the model.

Dependent variable	**AIC**	**Overall Wald X^2**	**P**	**Retained covariates**	**Covariate P**
CPUE (total)	-65.26	5.66	0.34	Temp	0.01
CPUE male (excluding recaptures)	-391.61	7.67	0.17	Flow	0.01
CPUE female	-277.42	4.60	0.47	Temp	0.01
CPUE < 25 mm CL	-921.17	18.15	0.046	Flow	<0.01
				CPUE sterilised	<0.01
CPUE 25 – 39 mm CL	-171.1	7.75	0.19	None	
CPUE ≥ 40 mm CL (excluding recaptures)	-747.66	1.98	0.85	Temp	<0.01
				Flow	<0.01

Mean CL total catch	91333.72	262.51	<0.01	Temp	<0.01
				Flow	<0.01
Mean CL (all males)	41516.86	189.7	<0.01	Temp	<0.01
				Flow	<0.01
Mean CL all females	47429.52	137.01	<0.01	Temp	<0.01
				Flow	<0.01
Mean CL ≤ 24 mm CL	6627.86	60.27	<0.01	Flow	0.07
Mean CL 25 – 39 mm CL	52703.78	278.21	<0.01	Temp	<0.01
				Flow	<0.01
Mean CL ≥ 40 mm CL	15011.85	26.06	<0.01	Temp	<0.01
				Flow	0.14
CPUE total catch in ecdysis	-931.86	27.59	<0.01	Temp	0.21
CPUE all males in ecdysis	-839.94	29.49	<0.01	Flow	0.58
				Temp	0.16
CPUE all females in ecdysis	-998.15	31.65	<0.01	Flow	0.06

CPUE total ≤ 24 mm CL in ecdysis	-1316.53	21.78	<0.01	Temp	0.71
				Flow	0.63
CPUE total 25 – 39 mm CL in ecdysis	-859.25	34.11	<0.01	Temp	0.45
CPUE total ≥ 40 mm CL in ecdysis	-1126.6	10.45	0.06	Temp	0.02
CPUE ovigerous females	-294.26	11.74	0.04	Temp	0.44
				Flow	0.47
Proportion of females ≥ 30 mm CL being ovigerous	296.05	21.41	<0.01	Temp	0.01
				Flow	0.05
Brood size	1294.52	12.35	0.03	Temp	<0.01
				Flow	<0.01
				CL	<0.01
Mean CL ovigerous females	661.18	9.12	0.10	Temp	0.39
				Flow	0.72

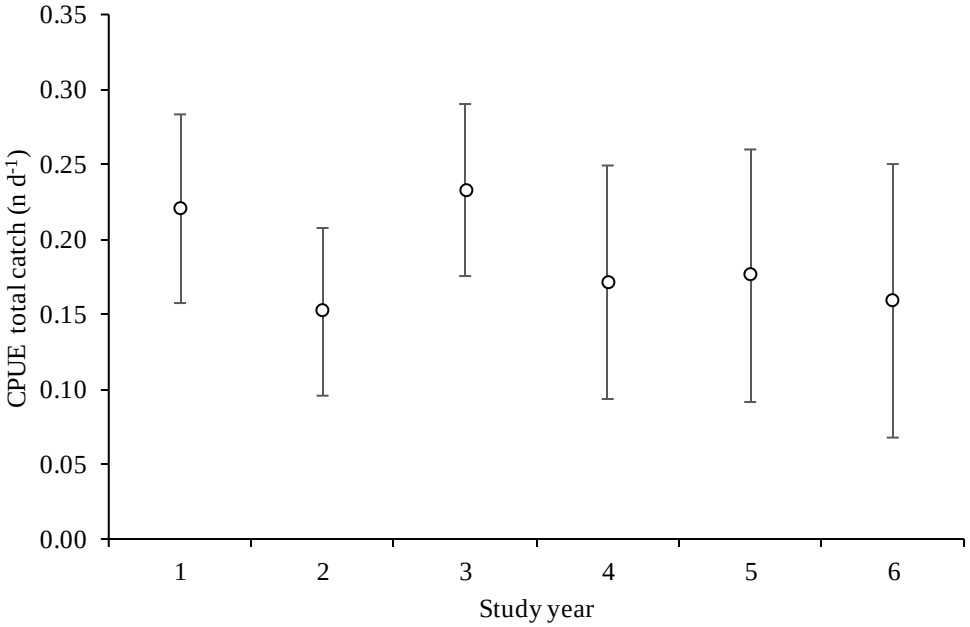


Figure 5.1 Annual mean catch per unit effort (as estimated marginal means from generalised linear model) of invasive crayfish captured in artificial refuge traps from the River Barle study site in study years 1 (2015) to 6 (2020). Error bars represent 95% confidence intervals

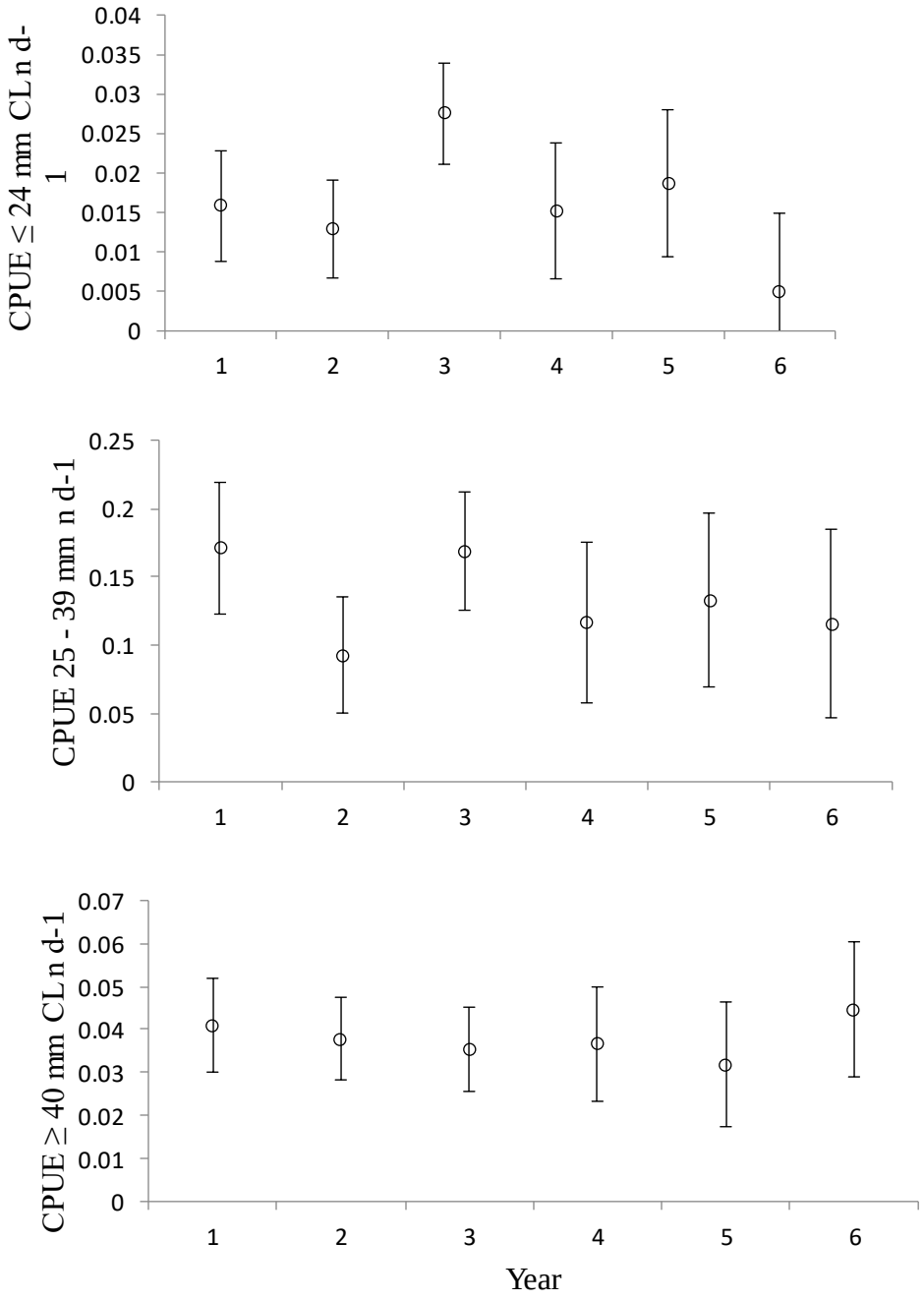


Figure 5.2 Annual mean catch per unit effort (as estimated marginal means from GLM) of invasive crayfish captured in artificial refuge traps: top: ≤ 24 mm CL; middle: 25 - 39 mm CL; and bottom: ≥ 40 mm CL from the River Barle study site in study years 1 (2015) to 6 (2020). Error bars represent 95% confidence intervals. Note difference in CPUE values on the Y axis.

5.3.2 Population size and sex structure

Across the six-year study period, the mean carapace length (CL) of all captured crayfish increased from 34 mm (± 0.2 mm) in Year 1 to 36 mm (± 0.2 mm) in Year 6 (an increase of 9.2 %; Figure 5.3), with this increase being significant (Table 5.3; Figure 5.3). The overall range of CL fluctuated between years but narrowed considerably in Year 6 (Figure 5.3). Patterns between the sexes were similar, with the best fitting models indicating that the CL increase was more pronounced in males than females (Table 5.3). In crayfish ≤ 24 and ≥ 40 mm, there were significant reductions in mean CL, but in the 25 to 39 mm size category there was a significant increase (Figure 5.3, Table 3).

The total M:F sex ratio of catches increased significantly from 1: 1.15 to 1: 1.26 between years 1 and 6 (Pearson X^2 [1, N = 13596] = 28.78, P < 0.01). There was variation between the size classes, where for ≤ 24 mm and 25 to 40 mm, there were significant increases in female dominance (≤ 24 mm: 1: 1.09 to 1: 2.14; X^2 [1, N = 1157] = 13.06, P = 0.02; 25 to 39 mm: 1: 1.19 to 1: 1.34; X^2 [1, N = 9617] = 21.48, P = <0.01; Figure 5.4). The shifts in the ≥ 40 mm CL size class did not change significantly (e.g. Year 1: 1: 0.92; Year 6 1: 1.04; (X^2 [1, N = 3312] = 9.36, P = <0.49, Figure 5.4).

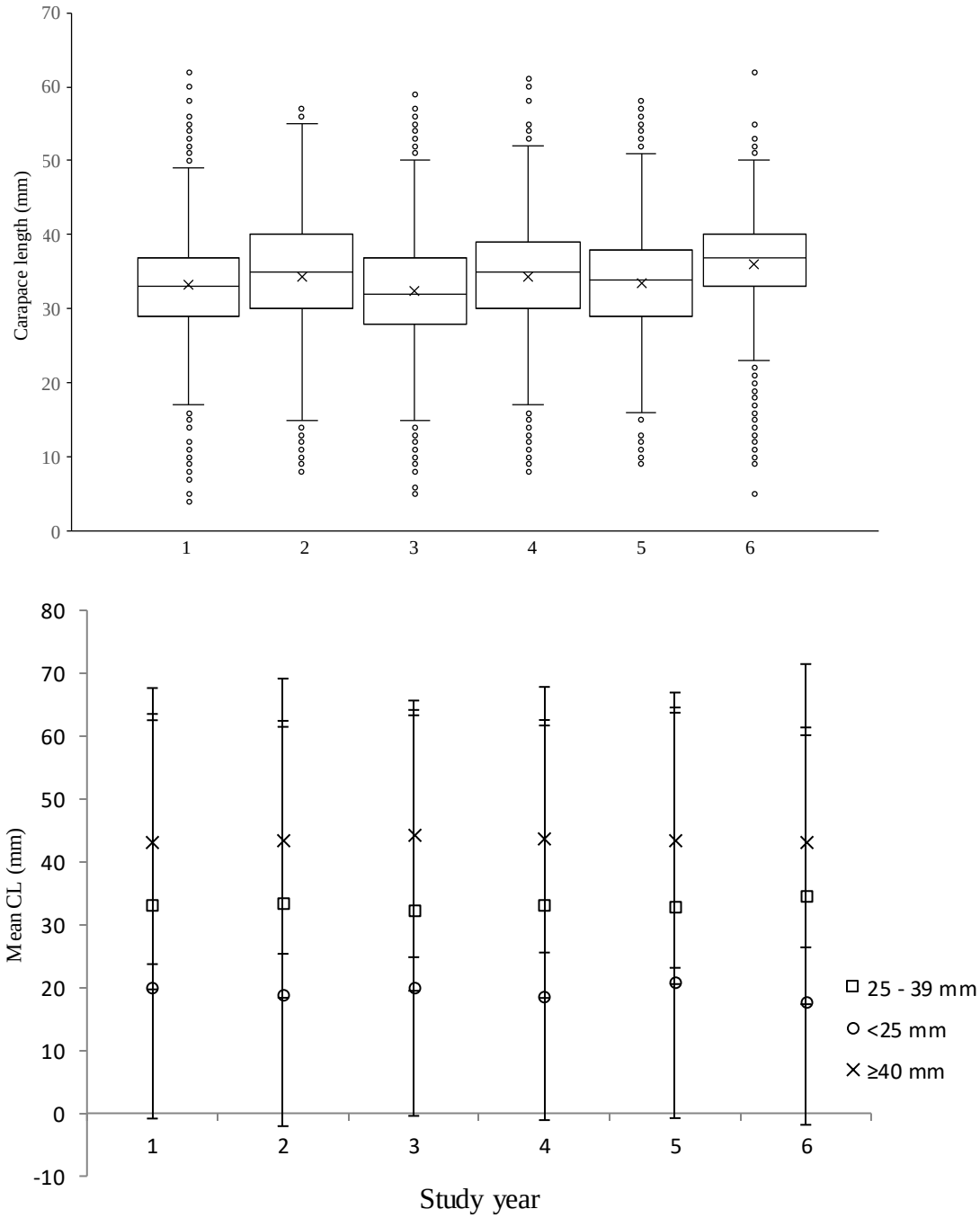


Figure 5.3 Top: box plot revealing the distribution of carapace length data by year of study, where horizontal lines mark the 10th, 25th, 50th, 75th and 90th percentiles of the data, x is the mean carapace length, and clear circles are outlying data points. Bottom: Mean carapace length (as estimated marginal means from the best fitting GLM) of crayfish captured at the River Barle study site, Years 1 – 6. Error bars represent 95% confidence intervals

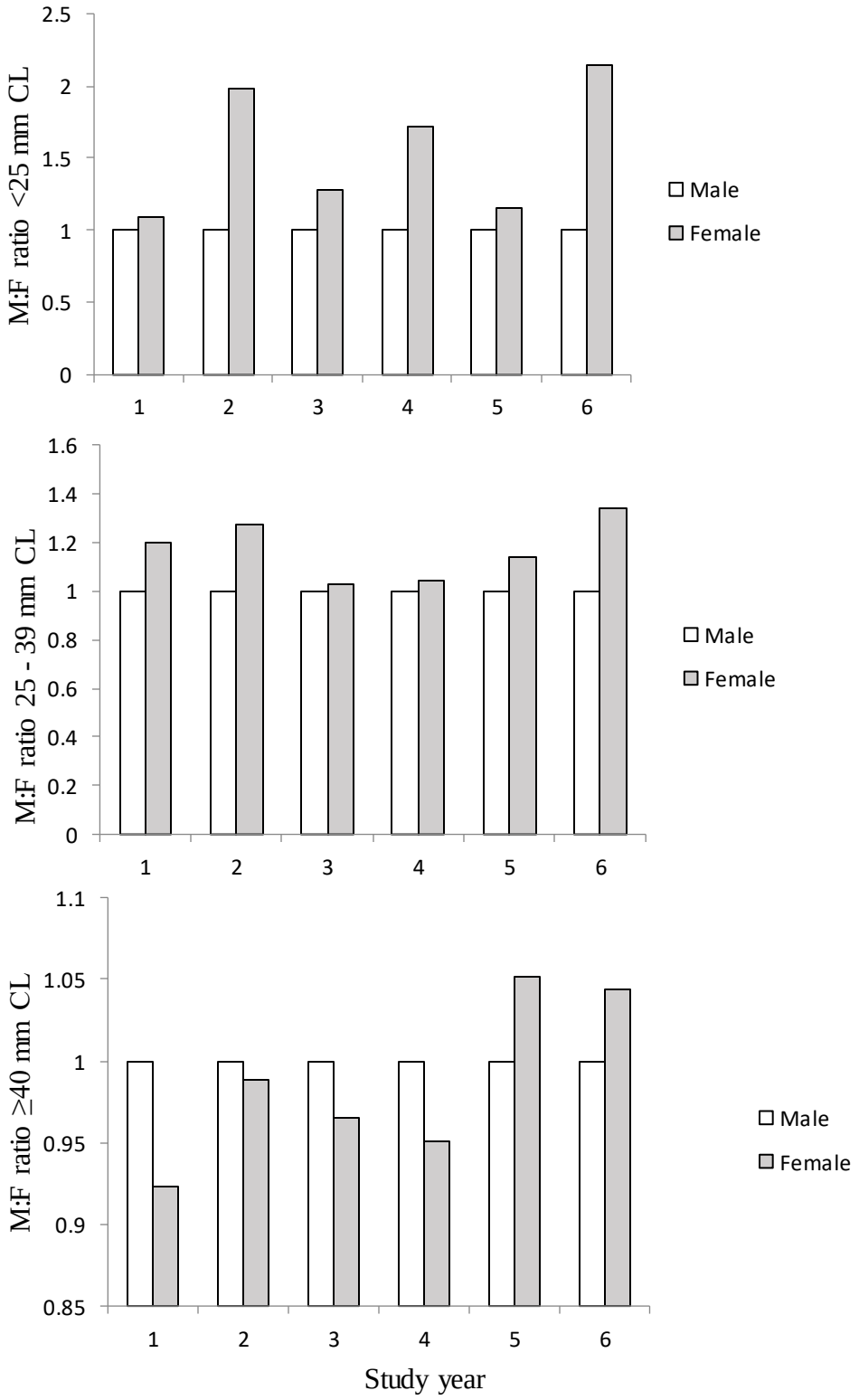


Figure 5.4 Sex ratio of males to females for size small (≤ 24 mm CL, top), medium (25 - 39 mm CL, middle) and large (≥40 mm CL, bottom) captured on the River Barle study site. Note differences in values on the Y axis.

5.3.3 Ecdysis and female reproductive traits

The CPUE of all crayfish (killed + sterilised) in ecdysis at point of capture significantly increased over the study, but with a peak between Years 2 and 3 that decreased thereafter (Table 5.3); ecdysis by sex and within the size classes showed similar patterns (Table 5.3; Figure 5.5). The CPUE of females ≥ 30mm that were ovigerous and as a percentage of all females ≥ 30mm captured over the time period between the second week of May and third week of June each year significantly altered over the study period, with declining trends observed between Years 3 and 6 but no overall temporal pattern (Table 5.3; Figure 5.6). Female brood size changed significantly over the study period, and although the main change was between Year 1 and all other years, pairwise differences between Year 2 and Years 3, 4 and 6 were also significant. (Table 5.3; Figure 5.6). While the mean CL of ovigerous females fluctuated between years, these changes were not significant (Table 5.3).

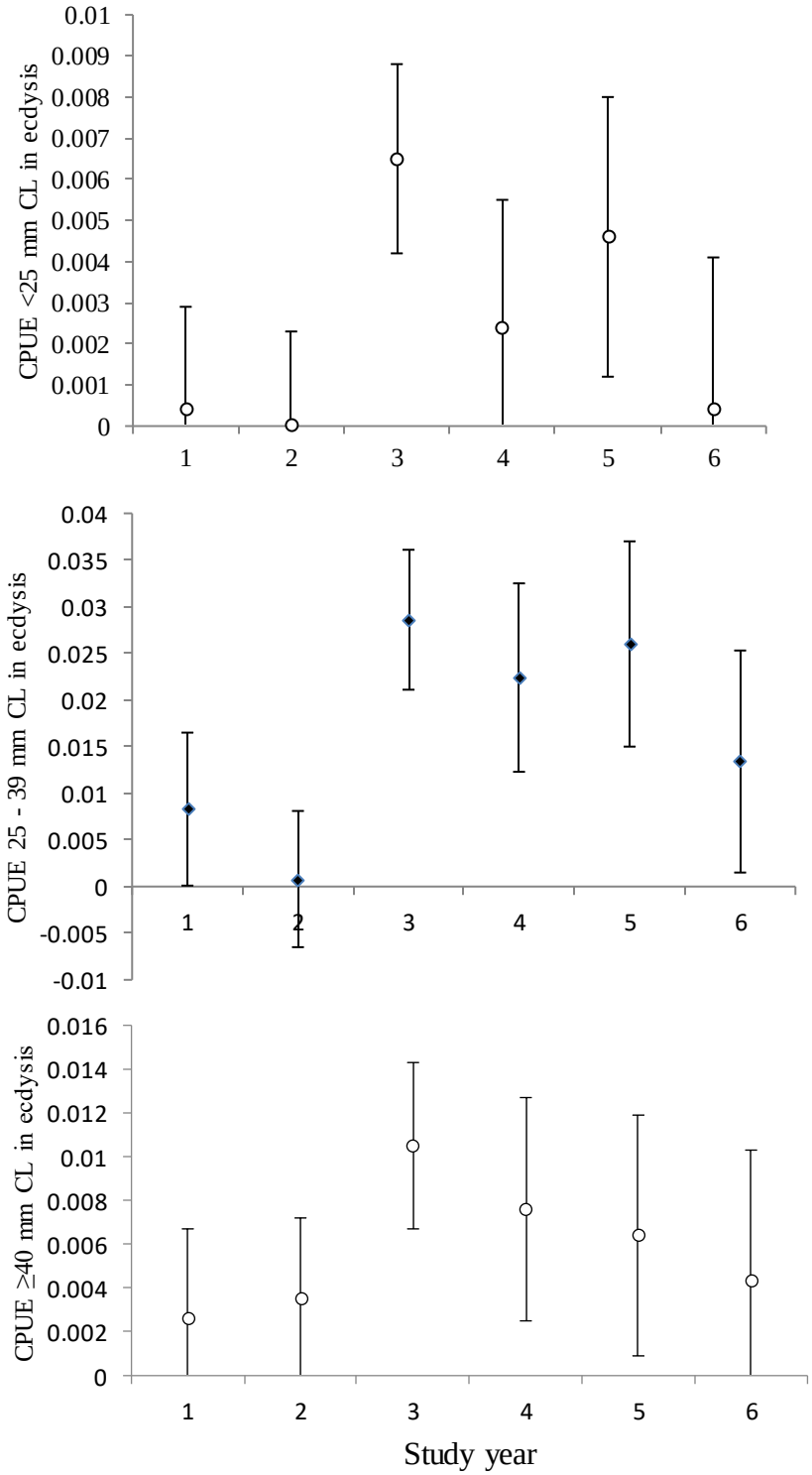


Figure 5.5 Mean CPUE (as estimated marginal means from the best fitting GLM) by size class (small: top; medium: middle; large: bottom) of crayfish in ecdysis captured at the River Barle study site, 2015 - 2020. Error bars represent 95% confidence intervals, note differences to values on the Y axis.

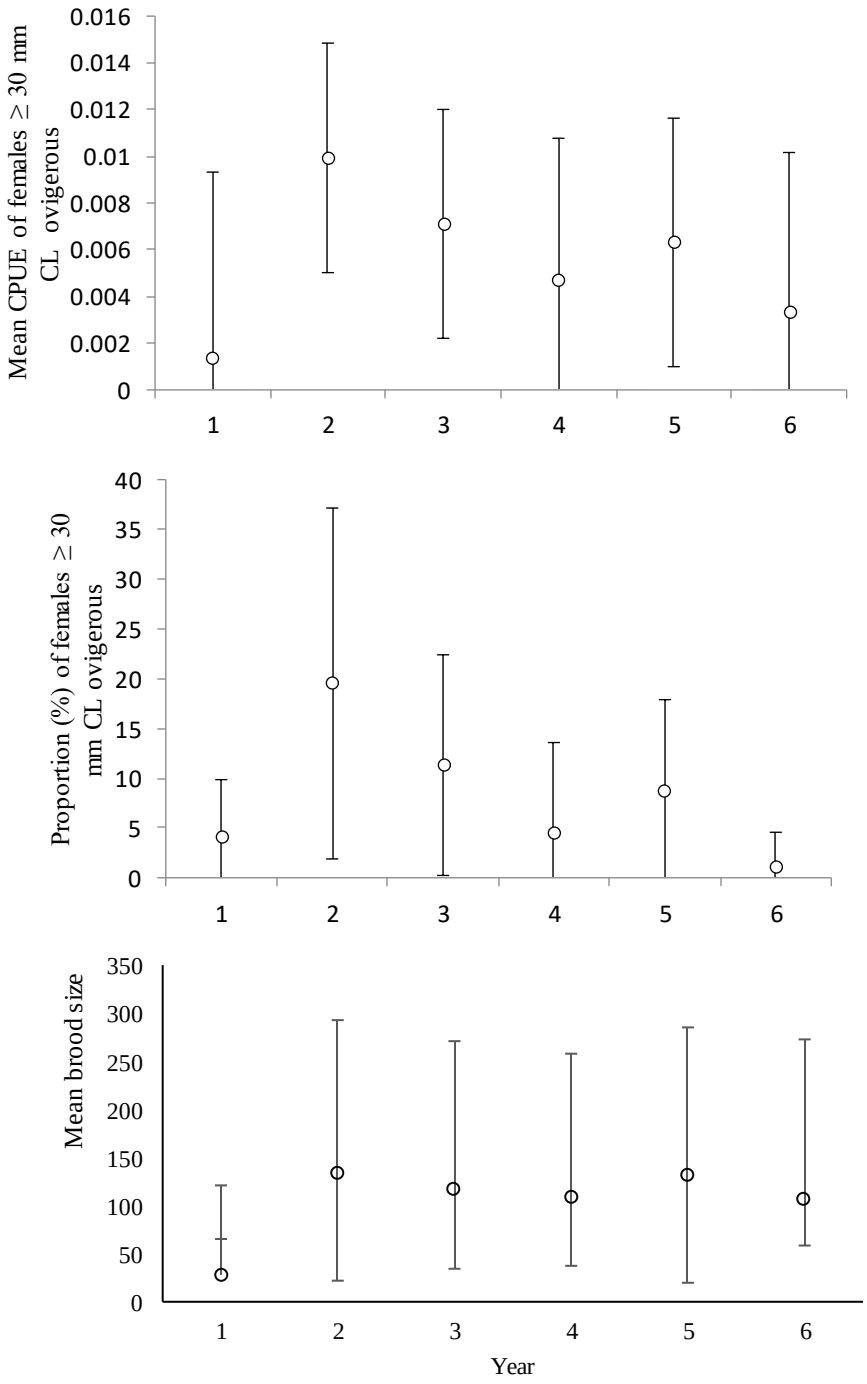


Figure 5.6 Mean CPUE (as estimated marginal means from the best fitting GLM) CPUE ovigerous females (top), proportion (%) of all females captured >30 mm CL (middle) and mean brood size (bottom) at the River Barle study site 2015 – 2020. Error bars represent 95% confidence intervals, note differences to values on the Y axis.

5.4 Discussion

The combination of trapping with ARTs and male sterilisation has been posited as potentially providing an effective control method for invasive crayfish. Investigations here into the efficacy of the combined techniques tested consequences on population size and sex structure and reproductive output over a six-year period revealed that across all crayfish, there was no decrease in overall relative crayfish abundance (as the annual means of CPUE per year over six years), despite 12245 crayfish being removed and 2297 males being sterilised, and with this disagreeing with prediction. The only size class of crayfish that did decline in abundance by the end of the study were those of $\leq$ 24 mm, which decreased by 60% between Years 1 and 6. Mean crayfish size did increase across the study period, as predicted, whilst the ratio of females to males increased over the study period, contrary to prediction. Brood size, CPUE and proportion of ovigerous females all changed over the study period, with decreasing trends from Year 3, though without consistent temporal patterns.

Across the six-year study period, a substantial decline in overall crayfish CPUE was thus not detected, despite the trapping effort and the application of SMRT. This demonstrates the difficulty of reducing the long-term abundances of aquatic invasive species generally (e.g. Rytwinski et al. 2019) and invasive crayfish in particular (Gherardi et al. 2011). The reasons why the management programme was unable to reduce the overall crayfish population size were not necessarily clear, as the study design was unable to maintain a control site to which the results here could have been compared with background, natural fluctuations in crayfish population abundances and dynamics. The population dynamics and trait expression of invasive crayfish can be highly variable and are driven, at least in part, by both intra and inter-specific interactions, and the abiotic environment. For

example, Jackson et al. (2017) revealed that body size, population abundance, and the size and productivity of the aquatic ecosystem was a key driver of the trophic ecology of invasive *P. clarkii*, with Mathers et al. (2020) revealing that riverine invasions of *P. leniusculus* results in functional compositional changes in invertebrate communities. One would expect that, had the combination of trapping and SMRT been highly effective in this relatively small upland river, a marked decline in their population abundances would have been detected by the end of the study period, but this was not evident at the population level. However the most successful control attempts (excluding biocide use) are those that have been applied over extended time periods, such as in the River Lark, where a programme of trapping over 11 years resulted in reduced crayfish density, riverbank stabilisation and a partial recovery of fish stocks (Stancliffe-Vaughan 2015; West 2009), and in Sparkling Lake, Wisconsin, where a programme of trapping combined with the reduced angling of predatory fish resulted in a 95% decrease in *Faxonius rustucus* populations over 11 years (Hansen et al. 2013). Thus, the six-year time frame might not have been of sufficient length to result in the predicted population declines. In addition, with ARTs being relatively inefficient at capturing young of year crayfish, the effects of SMRT would not become apparent until the resultant 1+ progeny were captured two seasons later. Hence, it is speculated that the significant decreases detected in the CPUE of the smallest category of crayfish and in ovigerous females by the end of this six year study could eventually translate into CPUE declines at the population level, with the time-lag within these processes meaning that these decreases have yet to be detected.

The aspect of the invasive population that, by year 6, was appearing to respond to the management programme was the smaller crayfish (≤ 24 mm), with a significant decline apparent by the end of the study period. The trapping by ARTs that removed substantial

numbers of crayfish in the 25 to 39 mm CL size class, coupled with the SMRT programme, could have been driving this decline via reducing the numbers of reproductive females and decreasing the reproductive success of the surviving females by increasing their reproductive encounters with sterile males. However, evidence for this from the results is limited, as the temporal pattern in CPUE of crayfish of 25 to 40 mm was largely unchanged over the study period and the sex ratio data indicated an increase in female proportions in the population by the end of the study period. Thus, it was considered unlikely to be related directly to the trapping component of the study. Whilst the temporal data on the abundance and proportions of ovigerous females, and their brood size, showed variability between years, there is currently insufficient evidence to suggest these declines in juvenile recruitment were being driven by changes in female reproductive metrics. While the drivers of this decline in juvenile recruitment could relate more to their abiotic environment, given that aspects of crayfish activity are temperature and water depth related (Johnson et al. 2014), this was also considered unlikely. This was because Bubb et al. (2002) noted that in an upland stream in Northern England, high winter flow events were not a driver of *P. leniusculus* mortality or downstream displacement, and so this can probably be ruled out as a factor in the recent decline of the smaller crayfish here. Again, the absence of control sites prevents further evaluation of whether the decline was related to the management intervention.

Larger individual body sizes and more male dominated populations are associated with lower crayfish density, especially at population expansion fronts, this being at least partially driven by the higher exploratory behaviour of males than females (Moorhouse and MacDonald 2011a). Moreover, higher female to male ratios have been found in expanding populations (Moorhouse and MacDonald 2011b). Here, we detected a shift to

larger mean sizes, where increases in body sizes were tested for the influence of ecdysis rates, given increased rates are expected to support faster growth rates that result from increased resource availability as the population size reduces (Moorhouse and McDonald 2011b). However, despite the larger body sizes detected in the river, ecdysis rates by Year 6 remained highly variable, with substantial increases with study year not apparent. This could suggest that the increase in mean body sizes is driven by changes to population structure due to the reduction in CPUE of ≤ 24 mm individuals, but remains speculative at present. There was also a substantial push to higher proportions of females than males in the population over the study period, where the greatest increase was in the size class of ≤ 24 mm. This was not considered to be due to trap bias, as the sex ratio recorded in Year 1 of this study was 1:1.15 M:F, which is commensurate with sex ratios recorded from drawdown experiments e.g. 1:1.15 M:F recorded by Chadwick et al. (2020). Hudina et al. (2011) reported that expanding and high density populations have a higher proportion of females, although the mechanisms behind this were unclear.

It was considered that the combination of trapping and SMRT had high potential to result in the successful control of invasive crayfish populations. Although the results of the study do not fully support this, there is some evidence that the removal efforts were reducing the CPUE of crayfish ≤ 24 mm CL in the latter part of the study period, although evidence that this was being driven by changes in the female reproductive metrics was equivocal. The role of SMRT as a driver of female reproductive success was likely to have been driven by the survival and persistence of males in the study site post sterilisation (discussed in detail in Chapter 3). Due to the relatively low productivity of the study river, it is argued that the crayfish population consisted of individuals with relatively limited lifespans and relatively high natural mortality rates, which would then

have resulted in a relatively high proportion of the sterilised males 'dropping out' of the pool of sterile males within a short time period, a hypothesis supported by the relatively low number of recaptures (N = 568) over the study period. Moreover, the proportion of the sterilised males present in the population (approximately 14 %) was also lower than the 20 to 30 % reported as causing substantial declines in juvenile catches by Duperray et al. (2013). The lack of clear temporal decline in in female reproductive success across the study period could also relate to the role played by large males in reproduction (Chapter 3). Sterilising smaller males (e.g. > 30 mm CL), which are more abundant (Chadwick et al. 2020) and potentially more reproductively active and successful than larger males (Woodlock & Reynolds 1988), could increase the effects of the SMRT, whereas the focus here was on sterilising males of > 40 mm.

The lack of substantial decreases in CPUE over the six year study period could also relate to an insufficient trapping effort, as in the final years of study this equated to one ART per 100 m^2 of riverbed. Stebbing et al. (2016) recommended the density of baited traps, which have a far higher capacity than ARTs, should be 50 to 100 per acre (i.e. one per 40 to 80 m^2) for removal programmes to be successful. Moreover, the same study predicted that trapping at low density would take over 10 years to reduce population size. Because baited traps are biased toward large males, their initial use during this study contributed 58% of the males that were subsequently sterilised, so their continued use could have substantially increased the proportion of sterilised males in the pool. Increased trap density, including either the use of baited traps, or ARTs designed to capture larger animals (Moser 2017) and with greater capacity, should therefore be considered for use in future removal programmes.

The findings of the study could have been confounded by the effects of it being undertaken within an open river system, subject to migration of individuals. Although only data from the central section of the study site was used in the analysis, migration could have occurred, and since large crayfish, particularly males, are known to be the most exploratory sex/age class (Bubb 2004; Hudina et al. 2012) this could have resulted in the emigration of sterilised males and immigration of non-sterilised males. The River Barle is a high energy upland river and studies on similar systems (e.g. Bubb 2004; Light 2003) have found a general downstream movement of crayfish in response to flow regimes, especially of larger, male crayfish that are more exploratory. Conversely, Moorhouse & Mc Donald (2011a) found that large individuals within areas subject to trapping made longer movements than those in untrapped areas, but the percentage of animals immigrating into the middle of each section (perceived lowest density in the trapped sections) was the same. Here, large-scale immigration of large individuals was considered unlikely to have occurred, since the mean CL of large individuals captured within the study site decreased in the latter three years of the study and, since the largest animals are most likely to immigrate (Hudina et al. 2012), an influx of such animals would be expected to result in increased mean CL. It is possible that the largest animals are not being captured by the ARTs as they target smaller individuals. However, when comparing mean CL of males ≥ 40 mm CL for the study period, mean CL of those captured in BTs (which select large males) was 47 mm (± 2) CL compared to 44 mm (± 1) in ARTs, suggesting a lack of large animals in the population generally.

Although there is some evidence of the combination of SMRT and trapping beginning to have an effect on population size via reductions in the abundance of smaller crayfish, a considerable knowledge gap remains in the relationship between removals by ARTs and

the effects of SMRT. Both have similar effects, the former removing females and smaller individuals that are most vulnerable to predation (Houghton et al. 2018), whilst the latter targets the production of juvenile crayfish. While it is likely that use of both methods together is complementary, their dual use makes it difficult to decouple their individual effects. Consequently, it is recommended that each method is trialled in isolation, ideally in similar lentic systems where migration can be controlled, and incorporating a control site. If trialling ART trapping in isolation, the removal of females only should be considered, as a population with a very high proportion of males is likely to lead to increased agonism and cannibalism (Kubec et al. 2019), whilst fierce competition for relatively small numbers of females is likely to increase copulation related mortality amongst both males and females (Woodlock and Reynolds 1988).

To summarise, this field study has not shown the expected decreases in crayfish population size over a six-year period, but there was some evidence that decreases in the abundance of smaller crayfish did occur by its conclusion. The lack of a control site prevents further conclusions from being drawn on the mechanisms underlying these, although it is speculated that low trapping intensity and relatively low proportions of sterilised males present could have impaired the effectiveness of the programme. Therefore, trapping at higher intensity, combined with sterilising smaller males, is recommended in future applications, which should also attempt to decouple the combined effects of the two methods by testing them in isolation and take place in lentic systems, with control sites, so that abiotic factors and migration can be more tightly controlled.

Chapter 6. General discussion

6.1 Overview of thesis

The American signal crayfish *Pacifastacus leniusculus* is an ecologically damaging invader to aquatic ecosystems (Capinha et al. 2011), estimated to have cost the UK £127 million between 2000 and 2020 (Kouba et al. 2021). The signal crayfish, being one of the most successful global invaders (Holdich et al. 2014), provided a strong model species for the research and the River Barle, an unspoilt, upland SSSI river noted for its salmonid populations (Natural England 2017), exemplified a highly valuable ecosystem vulnerable to the negative impacts of these invaders. There are few examples of successful control or eradication of signal crayfish without the use of biocides (Stebbing et al. 2014) and a successful management method has yet to be found. The aim of this thesis was to conduct a thorough investigation into a specific combination of techniques designed to control invasive crayfish based on the findings of the body of knowledge to date, combined with some understanding of crayfish behaviour and population dynamics.

The chosen method was a combination of the mechanical sterilisation of large males (sterile male release technique: SMRT) with long -term trapping, including the use of a novel habitat attractant style trap. SMRT has been successful on other invertebrate species (Harris et al. 1986) and initial trials on crayfish have shown potential (Aquiloni et al.2009; Stebbing & Rimmer 2014) but no field trials of the technique had been conducted prior to this study. Trapping is the most widespread control method used in both lentic and lotic ecosystems and conventional methods are biased in both size and sex, making them relatively ineffective over the short term (Stebbing et al. 2016). The use of a less selective trap, the ART, again has not been extensively trialled prior to this study. Like conventional trapping, the chosen combination of methods required a high labour input

but this was mitigated somewhat through the deployment of skilled volunteers. It also had low risks of environmental damage, unlike methods such as biocides, electrofishing and dewatering (Manfrin et al. 2019).

Mechanical sterilisation was hypothesised to reduce juvenile production whilst maintaining cannibalism and other competitive mechanisms. The novel Artificial Refuge Trap (ART) was hypothesised to capture equal numbers of males and females and a wide size range of individuals. Moreover, it was posited that the method would be simple to implement and cost effective, thus widely applicable by stakeholder groups and citizen scientists. With regard to the application of SMRT to signal crayfish little information is available. It was therefore necessary to determine the consequences of the treatment on both male and female crayfish behaviour in terms of dominance, competition and female mate choice, and with regard to natural reproductive behaviours, such as female promiscuity and mate guarding. The functional effectiveness of the SMRT, namely mating success in terms of spermatophore placement and brood size, treatment survival and gonopod regeneration rates, also needed to be determined. The efficacy of ARTs compared with conventional baited funnel traps (BTs) with regard to catch rates, size and sex bias was also considered to be a key factor to determine to developing a suitable management programme. Finally, the overall hypothesis was tested by the application of the control effort over a six year period and subsequent analysis of changes to catches, population structure and sex ratios.

Overall, the results indicated that although the chosen combination of control techniques was predicted as potentially being effective at reducing invasive crayfish population abundance, their practical application was less successful than anticipated. In controlled

experiments, no behavioural obstructions were detected to suggest that sterilised males were less able to compete for females (Chapter 2), in concurrence with the only previously known study on *P. leniusculus*, Stebbing and Rimmer (2014), and removed gonopods regenerated slowly and were frequently deformed (Chapter 3). Copulations with sterilised males produced lower spermatophore cover than that of non-sterilised males, again in concurrence with Stebbing and Rimmer (2014), but brood sizes and clutch retention rates did not decrease substantially (Chapter 3). As one of only two known studies on mate choice and copulation effectiveness among *P. leniusculus,* the findings contribute significantly to the body of knowledge on this subject. In the field, ARTs were an effective and unbiased method of capturing females and small individuals (Chapter 4), building on the findings of O'Connor et al. (2018) with a large-scale study. However, six years of application of the combined methods in the wild, only the smallest crayfish size class decreased significantly in abundance, though the size and sex structure of the population shifted and there was some evidence of reduced reproductive efficiency (Chapter 5). Despite this, the lessons learnt have enabled the development of recommendations for adapting the technique which, it is suggested, will improve its efficacy. The use of citizen scientists enabled the field component of the work to run not only for the last six years, but for three additional years to allow some methodological adaptions to be implemented.

Overall, the results of the behavioural experiments suggested that SMRT could be effective when applied to the wild *P. leniusculus* population in the River Barle. While there has been little research on the effects of sterilisation of large, dominant males on signal crayfish hierarchical and reproductive behaviours (Chapter 2), Johović et al. (2019) found no difference in the ability of sterilised male *P. clarkii* to secure mates, and studies

on mate choice amongst other crayfish species have had mixed results (Aquiloni et al. 2008; Aquiloni and Gherardi 2008; Fero et al. 2007; Chapter 2). That there was a lack of significant reductions in brood size in the wild (Chapter 3; Chapter 5) raises questions regarding the role of males in reproduction, where it has been widely assumed that large, dominant males dominate breeding activity (Woodlock & Reynolds 1988; Gherardi et al. 2006). The results here instead tentatively suggest that smaller males may play a more important role in reproduction than previously assumed.

The incidence of post-copulatory guarding and lack of promiscuity that was revealed in Chapter 2 concurs with Stebbing and Rimmer (2014), although evidence for this is mixed for other species (Walker et al. 2002; Yue et al. 2010). Thus, the findings here contribute strongly to the knowledge base of *P. leniusculus* mating behaviours (albeit in experiments with small sample sizes), although there remains a major knowledge gap which constrains the practical application of SMRT to *P. leniusculus* and other invasive crayfishes. The work here on the functionality and persistence of the SMRT technique indicated that despite gonopod regeneration being slow, it did not result in significantly reduced brood sizes (Chapter 3). The only known similar study (Johović et al. 2019) found female *P. clarkii* failed to produce any young after mating with sterilised males, although with *P. clarkii* using internal mating then these results are not directly comparable to *P. leniusculus.* The relationship between spermatophore cover and brood size needs further investigation, since the results suggested both decreased with increasing gonopod deformity (Chapter 3). Survival of sterilised males was high in controlled conditions, but survival in the wild could not be quantified as the tag data could not be compared with non-sterilised males, given that no non-sterilised males were released (Chapter 3), although Nightingale et al. (2017) did detect high survival rates in tagged

Austropotamobius pallipes using similar methods. The research has raised questions relating to the age and size of males relative to their environmental conditions and the reproductive effectiveness of 'old' males (Rubolini et al. 2007; Woodlock & Reynolds 1988), highlighting another knowledge gap.

When comparing the two types of trap deployed in the study, ARTs were found to have higher catch rates overall, equal sex ratios, a smaller mean CL and far wider size range than BTs (Chapter 4). This concurs with O'Connor et al. (2018) who tested ARTs on *A. pallipes* in a smaller trial and contributes substantial new knowledge on trapping biases, and adds to the evidence base regarding the limitations of BTs, which are widely reported to be biased towards large males (Kozak and Policar 2003; Stebbing et al. 2014), for both monitoring and control of crayfish. As there is a clear and widespread need for a simple, reliable and relatively unbiased crayfish capture method, then the results here demonstrate the value of the ART for that purpose (Chapter 4). Moreover, there is considerable scope to further adapt the design of the ARTs to increase their capacity and target specific size classes for both monitoring and control projects.

In the field trial of Chapter 5, the overall catch per unit effort did not show a clear response to the combined use of removals by trapping and SMRT. Although there were some temporal patterns in aspects of the data that suggested some success (e.g. decreasing trends in capture rates of juvenile crayfish, changes to the size and sex structure of the population), the overall results revealed the six year management programme had not been sufficient to significantly reduce the crayfish population size. The reasons for some of the results remain unclear and do not concur with other studies, for instance the increase in overall size structure and lack of evidence of immigration or increased ecdysis

(Chapter 5) suggest that the population is ageing rather than responding to removals as reported by Moorhouse and McDonald (2011b). Similarly, the reasons for an increase in the F:M sex ratio are unclear. Hudina et al. (2011) reported higher proportions of females in expanding populations though there is no other evidence to suggest the study population is expanding. That many of the findings differ between size classes raises additional questions and further research is needed. It was discussed in Chapter 5 that it was not possible to maintain a control site, which would have facilitated the decoupling of what were the population responses due to the removal and SMRT, and what was just natural variability in their populations. Even though the study site was a relatively small upland river, there was sufficient complexity in the population to prevent more definitive conclusions being drawn. For example, with it being an open river system, there were challenges around migration in and out of the site, which was not quantified.

There were, however, several reasons identified as to why there was not a substantial decrease in crayfish abundance in the study site despite the six year management programme. For example, most successful crayfish control attempts have taken at least 10 years of management efforts (Hein et al. 2007; West 2009) and so six years might actually be insufficient to show a strong effect, especially when considering the two year time lag between sterilisation of male crayfish and capture of their progeny at age 1+ (Chapter 5). A further factor could relate to a relatively low trapping effort and low numbers of males sterilised (Chapter 5), although it should also be noted the amount of effort applied still required high effort in terms of time (e.g. weekly trapping events for several months of the year). It is recommended that future work makes more efforts to test whether compensatory responses, including migration, on reproductive efficiency,

enable the crayfish to overcome the effects of the removals and ensure their population remains sustainable.

6.2 Implications for the management of invasive crayfish

6.2.1 The responses of P. leniusculus populations to long-term removals

Attempts to control invasive crayfish in the absence of biocides rarely succeed (Gherardi et al. 2011) and one reason for this is the persistence of the treatment (Stebbing et al. 2015). Due to the biology of *P. leniusculus,* changes resulting from the SMRT were subject to a two year time lag in this study, suggesting that even six years of application might not be sufficient to provide definitive results. The findings differed between sex and size classes, with these differences likely to impact on future population responses, once again emphasising the need for long term studies. Overall, the research has contributed to the evidence that invasive crayfish control requires long term and intensive effort if it is to have a chance to be effective at reducing population abundance.

6.2.2 The importance of trapping effort and adaptive management

Bomford and O'Brien (1995) stated that for invasive species control, methods need to be able to capture individuals at low density once population sizes have been reduced through other means (Chapter 1). Although not addressed in this study, ARTs have been successfully applied to very low density populations of *A. pallipes* (M.R. Lane, J. Nightingale pers. comm.) inferring their potential effectiveness in this respect. Although the long term persistence of the treatment is vital, the study has also highlighted the need for as high a trapping effort as feasible in order to hasten the effects of the control. The

longevity of this study has enabled the identification of areas where adaptations are needed, and due to the flexibility of the ART design such adaptations can be incorporated. The continuation of the control trial will hopefully demonstrate the value of an adaptive management approach to other practitioners.

6.2.3 The value of ARTs for crayfish monitoring and control

The research has shown that ARTs are successful at targeting males and females equally and all sizes of crayfish from age 1+ upwards. They are more efficient than other methods with the same effect such as hand sampling (O'Connor et al. 2018), providing a method that reduces the bias associated with conventional trapping. Moreover, they are a simple and reliable method of capturing ovigerous females and individuals in ecdysis (Chapter 2) as well as detecting crayfish at low density (Lane, M.R. pers. comm). This research has collected and analysed data on brood size and ecdysis (Chapter 5) from crayfish caught using ARTs in quantities not previously attainable without prohibitively large hand sampling efforts. Moreover, the ART has been shown to have high potential as a control tool, with its ability to target reproductive females and small and medium sized crayfish (Chapter 5). Unlike conventional funnel trapping, which targets the largest animals first, mean size decreasing with time, ARTs have a 'bottom up' approach, targeting females and smaller individuals whose removals will have a greater effect on reproduction. Different sized crayfish select different refuge sizes i.e. pipe diameters (Moser 2017), so ARTs can be designed to target certain size classes, enabling research into the effects on population structure of targeting specific size classes. One example would be to target age 1+ and 2+ crayfish, the most abundant size class captured in this study, comprising subadults and immature adults. This size class is posited to be most

vulnerable to predation, reducing the proportion surviving to adulthood (Houghton et al. 2018), so intensive removals would further reduce that proportion reaching adulthood and going on to reproduce. A previous study (Thorne 2019) identified the optimal soak length for an ART to be seven days but the effects of timing, i.e. at optimal capture periods rather than continuously all season, have not been trialled. Variations in both soak length and timing of ART application could also be trialled to determine the most efficient methodology.

In the absence of a crayfish-specific biocides with no risk to non-target organisms, the required longevity of physical removal methods as trialled here have limited potential for large scale control due to the effort required. There remains an ongoing need to make existing approaches as efficient as possible and develop new approaches such as genetic manipulation (Savayer et al. 2020) that can be applied at a landscape scale without compromising ecosystem integrity.

6.3 Research implications of the study

6.3.1 Applied research: development and validation of the control technique

It is intended that the management programme at the River Barle study site will be continued for a further three years (2021 to 2023) in order to further develop the methodologies and identify how the crayfish population abundance might be reduced. The development of these methodologies and their application will cover the following aspects: -

Trapping efficacy, capacity and selectivity

The research identified the potential need for increasing the trapping effort and the most resource effective way of achieving this is to increase the capacity of individual ARTs rather than simply increase the number of traps used. The ART design used in this study incorporated eight pipes per trap whilst new designs using up to 22 pipes will be trialled at the study site and elsewhere. Very little work has been undertaken into the efficacy of ARTs in lentic systems (Chapter 4; Chapter 5), although their use is shown to have potential, having higher catch rates than BTs (J. Nightingale pers. comm; Chapter 5). ARTs of increased capacity and suitability for deployment into lentic waters will be trialled alongside conventional funnel traps (BTs).

The effects of immigration on CPUE

The study did not monitor for immigration into the study site in response to removals (Chapter 5), although other studies have found crayfish will migrate into lower density areas (Hudina et al. 2011; Moorhouse and McDonald 2011b) and that migration is led by larger individuals (Bubb 2004; Hudina et al. 2012). However, of the studies on crayfish movements that have occurred (e.g. Moorhouse and McDonald 2011a; Wutz and Geist 2013), the tagged crayfish were always larger individuals sampled with conventional funnel traps, resulting in a considerable knowledge gap relating to migration with regard to size and sex of individuals. Consequently, it is proposed that up to 300 male and female crayfish as small as 30 mm CL will be (7mm) PIT tagged up to 200m downstream of the study site and monitored for inward migration to the study area. Nightingale et al. (2017) successfully tagged *A pallipes* at 22 mm CL with 7 mm tags so survival is considered unlikely to be compromised by the size of the animal being tagged.

Reproductive effectiveness of sterilised males

A further knowledge gap exists on the mating effectiveness of large males compared to their smaller and potentially more fecund counterparts (Chapter 3; Rubolini et al. 2007; Woodlock and Reynolds 1988). The next phase of the work will thus extend the SMRT treatment to all males >30 mm CL. As smaller individuals moult more frequently (Guan and Wiles 1999), then faster gonopod regeneration is expected (Chapter 3); however frequent trimming of regrowth encourages deformity which infers decreased spermatophore placement and resultant lower brood sizes (Chapter 3). The recapture rate and subsequent removal of regenerated gonopods of such individuals is key to the success of the technique and will be determined by trapping intensity.

Capturing large males for sterilisation

Chapter 3 discussed the effects of trapping intensity and changes to trapping techniques on the relative abundance of sterilised males. In order to increase the capture rate of large males, ARTs that target larger crayfish, by incorporating large pipe diameters, will be trialled at alternative sites in order to avoid conflicts with the effects of sterilising smaller males to be trialled at the study site. As the ART is cheaper and easier to use than conventional BTs, which do target large males (Chapter 4), such a development could be beneficial to stakeholder groups with limited resources.

The need to control for natural variation and decouple SMRT from ART removals

The inability to maintain a control site (Chapter 5) was identified as a shortfall of the field study, as the population dynamics of invasive crayfish can be highly variable and influenced by both biotic and abiotic interactions (Jackson et al. 2017; Mathers et al. 2020). Future research will seek to set up new trials in lentic systems where the two

techniques of removal with ARTs and SMRT can be tested independently alongside a control site. The removal of females only will also be trialled in order to examine its effectiveness in comparison to the other two techniques.

The impact of removals on native biota

An initial aim of the research was to monitor changes to invertebrate and fish diversity and abundance and samples of both through kick sampling (invertebrates) and electrofishing (fish) were taken in the first three years of the project. Due to lack of resources this could not be continued but it is hoped that in future years such sampling can be reinstated. Anecdotal reports (G. Davies 2020 pers. comm.) suggest salmonid numbers have increased but such assumptions need to be quantified.

6.3.2 Research into invasive crayfish biology and population dynamics

In addition to providing valuable insights into how the efficacy of combined application of trapping and SMRT could be improved, there is also the opportunity to explore some aspects of signal crayfish biology and population dynamics for which the research highlighted some important knowledge gaps. These are as follows.

Signal crayfish reproductive behaviours

The contribution of smaller males to the reproductive output of crayfish populations is assumed to be low due to the dominance hierarchies that exist (Goessmann et al. 2000), however this study suggested small males could potentially play a greater role than anticipated (Chapter 3; Chapter 5). Controlled experiments using both copulations

between different sized males and females and within groups representative of typical adult crayfish population structure would provide valuable evidence on the role of small males in reproduction. The results of Chapter 3 also suggested that females are guarded post-copulation by their mates and are not promiscuous, therefore multiple matings, potentially with non-sterilised males, could be considered unlikely. Owing to small sample sizes, additional controlled experiments to confirm the occurrence of these traits, both within *P. leniusculus* and other invasive crayfish species, would inform future control attempts. New studies could include group experiments where the interactions between males and females of different sizes and the impacts of relative size on these behaviours can be observed.

In considering the relationship between spermatophore placement and brood size, both this and the previous study by Stebbing and Rimmer (2014) used comparatively small sample sizes when testing spermatophore cover (Chapter 3). Additional research under controlled conditions with larger sample sizes, in particular investigating the relationship between spermatophore cover and brood size, is recommended. Ideally, experiments should take place in as natural conditions as possible, e.g. mesocosms, in order to increase the number of successful copulations. In addition, the utilisation of a range of different sized individuals in such experiments would also further inform the relationships between mate size and fecundity.

Population level responses to removals

Limited research has been undertaken on the responses of invasive crayfish populations to sustained removals in terms of changes to population structure (e.g. Hansen et al. 2013; West 2009) or compensatory mechanisms (Hudina et al. 2011; Moorhouse and McDonald

2011b) and this small body of literature fails to explain some of the findings in this study. More long-term control studies, especially in lotic systems, are required in order to determine such responses and inform management strategies that can utilise such findings to enhance control treatments. They should incorporate control sites to compare removals with natural fluctuations and ideally monitor for compensatory responses, for example, utilisation of a capture method such as ARTs that attracts ovigerous individuals and those in ecdysis, and the tagging of individuals to test for inward migration.

6.4 Summary

In summary, this thesis reports on a thorough investigation of a specific combination of invasive crayfish control techniques, including behavioural and functional influences on its efficacy. It has conducted research into little known fields of invasive crayfish control such as SMRT and the use of novel traps. While the findings are mixed in relation to reducing crayfish population abundance, they do indicate some promise and have highlighted several knowledge gaps and the value of long term, adaptive management approaches as well as the utility of the ART as a management tool. The continuation and expansion of the management programme on the River Barle should help to fill some of the knowledge gaps identified and determine the efficacy of the treatment, while also providing greater insight into the biology of invasive crayfish.

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Appendix 1. Supplementary table S 5.1

Supplementary Table 5. 1 Summary of rejected generalised linear models as used in Chapter 5, and where CPUE is catch per unit effort (no. crayfish per trap per day), CL is carapace length measured as mm, AIC is Aikake's information criterion, Wald X^2 is the Wald chi square statistic, P is the significance of the overall model. Temp is temperature recorded at 09.30 on each trapping event and flow is mean daily flow on each trapping event whilst covariate P is the significance of the covariate within the model

Model structure	**Retained covariates**	**Covariate sig.**	**Overall Wald X^2**	**Overall sig.**	**AIC**
CPUE total catch/year	Temp	.036	6.45	.265	-66.57
	Flow	.068			
CPUE males/year (excluding recaptures)	Temp	.114	7.84	.165	-392.09
	Flow	.028			
CPUE female/year	Temp	.016	5.252	.386	-277.73
	Flow	.127			
CPUE < 25 mm CL/year	Temp	.052	18.59	.002	-922.91
	Flow	.002			

	CPUE ster.	.000			
CPUE < 25 mm CL/year	Temp	.052	9.023	.002	-922.91
	Flow	.002			
	CPUE ster.	.000			
CPUE 25 – 39 mm CL/year	Temp	.094	9.023	.108	-172.79
	Flow	.083			
CPUE large ≥ 40 mm CL/year	Temp	.012	5.528	.355	-1029.17
(excluding recaptures)	Flow	.571			
	CPUE ster.	.000			
CPUE ≥ 40 mm CL/year	Temp	.009			-1030.85
(excluding recaptures)	CPUE ster.	.000			
Mean CL/year <25 mm	Temp	.201	59.41	<0.01	6628.22
	Flow	.038			
Mean CL/year < 25 mm	None		67.34	<0.01	6629.05
Mean CL/year ≥ 40 mm	Temp	<0.01	26.03	<0.01	15011.98
Mean CL/year ≥ 40 mm	None		34.55	<0.01	15027.72
CPUE total catch in ecdysis	None		30.02	<0.01	-932.3
CPUE total catch in ecdysis	Temp	.368	28.48	<0.01	-932.25
	Flow	.121			
CPUE all males in ecdysis (excluding recaptures	Temp	.382	23.76	<0.01	839.98
CPUE all males in ecdysis (excluding recaptures	None		25.22	<0.01	-841.22
CPUE all females in ecdysis	Temp	.196	28.46	<0.01	-977.81

	Flow	.117			
CPUE all females in ecdysis	Temp	.098	28.89	<0.01	-997.37
CPUE total <25 mm CL in ecdysis	Temp	.23	21.7	<0.01	-1318.3
CPUE total <25 mm CL in ecdysis	None		22.26	<0.01	-1320.07
CPUE total 25 – 39 mm CL in ecdysis	Temp Flow	.697 .1	35.52	<0.01	-859.94
CPUE total 25 – 29 mm CL in ecdysis	None		33.37	<0.01	860.68
CPUE total ≥ 40 mm CL in ecdysis	Temp Flow	.049 .121	10.46	<0.01	-1126.99
CPUE ovigerous	None		11.67	0.04	-297.18