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**Evaluation of the capacity of pheromones for control of
invasive non-native crayfish: part 2**

Part 2 of 2

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Acknowledgements

Executive summary

Background

Signal crayfish (*Pacifastacus leniusculus*) is an invasive species that has been introduced into Britain and has spread throughout many watercourses. The species has posed significant environmental problems by destabilising riverbanks by burrowing and decimating aquatic plants and invertebrates through grazing and predation. Signal crayfish pose a particularly acute threat to the native crayfish (*Austropotamobius pallipes*) directly through predation and competition for resources and indirectly through the fungus *Aphanomyces astaci*, which is carried by signal crayfish but causes lethal crayfish plague in *A. pallipes*. Current estimates suggest that native crayfish could become extinct in 30 years (Sibley and others, 2002).

Various methods to control the spread of signal crayfish have been tested, such as trapping, biological control and pesticides, but none appears to offer any practical long-term solution to the problem. Furthermore, the use of certain biological or chemical controls may be deemed unacceptable in terms of general environmental impacts. One possible alternative is the use of pheromone traps. Pheromone traps have been used in the past to control terrestrial insect pests, although this technique has never been applied to the aquatic environment.

The main objective of the project was to examine the potential use of pheromones as a method of controlling *P. leniusculus*. The main subjects that were examined as potential control mechanisms are sex pheromones, predator odours (expanded to include crayfish stress and alarm odours), feeding stimulants, feeding deterrents and cannibalism inducing compounds. Pheromone trials were conducted both in the laboratory and field. This is the second part of a two-part report.

Effects on white-clawed crayfish

Water conditioned by *P. leniusculus* was tested on *A. pallipes*. It was found that *A. pallipes* was repelled by the chemical presence of both male and female *P. leniusculus* with juvenile *A. pallipes* showing the stronger response. Although a certain degree of acclimatisation was found this was over a short period of exposure time. When *A. pallipes* water was exposed to *P. leniusculus, A pallipes* was slightly attracted to the water. Neither of the populations that the crayfish were taken from had prior experience of the other species.

Purification and identification

Boiling, freeze drying, ultra-filtration and HPLC was carried out to attempt to isolate and identify the pheromone. Despite difficulties with handling the pheromone, which caused problems with the identification, it was found that the pheromone was possibly peptide based, smaller than 10, 000 Dalton's and consisted of at least two parts.

Case study

A large-scale field trial was carried out using the traps developed as explained in part 1. The sex pheromone traps used were standard 'trappy' traps that were baited with a slow release gel matrix that had partially concentrated female conditioned water containing the sex pheromone imbedded into it. The data reiterated what was already found in the preliminary

trials, with the sex pheromone traps only attracting adult males during the breeding season. The traps were found to be less effective than food baited traps. Habitat analysis found that *P. leniusculus* males and females preferred similar habitats, avoiding finer sediment and preferring coarser materials were the habitat was more complex. However, sites which allowed for burrowing, such as with the presence of compacted clay were used despite the presence of fine sand or silt, suggesting that the main restricting factor in crayfish habitat preference is the requirement of shelter.

Conclusion

The sex pheromone of *P. leniusculus* was found to be repellent to the native *A. pallipes*. Meaning that traps baited with the sex pheromone would not attract the native species.

Attempts at purification and identification of the sex pheromone have proven difficult but have indicated that the sex pheromone consists of more than one active compounds that may be peptide based, that may possibly not be released by the female outside of the breeding season.

Sex pheromone baited traps could potentially be used to control populations of *P. leniusculus*. By further purification of the sex pheromone and the development of a specific releaser matrix that traps could possibly be made more effective than the food baited traps. Extensive trapping using the pheromone traps during the breeding season could potentially remove a large proportion of the adult males and therefore reduce the levels of breeding in the population. Even though the removal of large number of alpha males could potentially induce juvenile males to mature at a younger age this would mean that they would also become receptive to the pheromone traps.

Much progress has been made into the understanding of chemical communication in *P. leniusculus* within this contract as well as taking the first steps in the development of a viable control mechanism on a relatively small budget.

Contents

1. Introduction

1.1 Background

There are six species of crayfish that are known to have breeding populations in the U.K., only one of these, *Austropotamobius pallipes*, being indigenous (Holdich, 2002). Of the five invasive species, the North American signal crayfish (*Pacifastacus leniusculus*) is the most widely distributed (Sibley and others, 2002). Since the deliberate introduction of *P. leniusculus* into Britain during the 1970s, the species has spread significantly throughout waterways (Holdich and others, 1999; Sibley and others, 2002), with extensive populations now being found as far north as the River Clyde in Scotland (Maitland, 1996). *P. leniusculus* occurs at high densities due to a lack of predator control outside of its natural range and reproducing at high numbers. It has been found to burrow extensively causing considerable damage to riparian verges (Holdich, 1999), as well as significantly impacting other resident species, often denuding entire river reaches of their native fauna and flora (Guan & Wiles, 1998).

One of the many species that *P. leniusculus* has been shown to have a profound impact on is Britain's native crayfish, *A. pallipes*. *A. pallipes* is listed on Annexes 2 and 5 of the European Union Habitats Directive and protected under the Wildlife and Countryside Act 1981, and a priority species under the U.K. Biodiversity Action Plan. *P. leniusculus* is the larger, more aggressive and more fecund of the species, and shares the shame niche range as *A. pallipes*, therefore it is out competed for resources as well as being predated on by *P. leniusculus*. To compound the situation further *A. pallipes* is highly susceptible to a fungal infection *Aphanomyces astaci* (crayfish plague) which *P. leniusculus* is a vector of, but which is lethal to *A. pallipes*.

Despite various legislation aimed at reducing the spread of alien crayfish in Britain, such as the set up of 'no-go' areas by the Ministry of Agriculture, Fisheries and Food (M.A.F.F.) in 1996, the illegal and accidental introduction of alien crayfish, has to date proven almost impossible to control (Holdich and others, 1999). The feasibility of the eradication or control of non-native crayfish populations has been discussed in a number of studies (Holdich and others, 1999; Howard, 2000; Kemp, 2000; Peay, 2001; Sibley & Nöel, 2002). The use of traps, barriers, pesticides and biological control have all been examined and, of the currently available control methods biocides are the only means with potential, although this option does carry a range of adverse side effects that are detrimental to more than just the crayfish (Kemp, 2000; Sibley & Nöel, 2002).

At present there are no environmentally friendly management tools that have been fully tested in the field available for controlling invasive species of crayfish. A method that has been mentioned by Holdich and others, (1999), Kemp (2000) and Sibley & Nöel, (2002), as a potential control mechanism is the use of pheromones. Past experiences of the use of pheromones in the control of terrestrial crop pests have been successful in many cases and meet with the IUCN's (IUCN 2000) guidelines on environmentally friendly control of invasive species, but had previously not been examined as a control method for crayfish. The Environment Agency and English Nature have therefore funded a two-year R&D project (with extended funding for 1 year) examining pheromones as potential aids in controlling populations of *P. leniusculus*.

This is the 2nd part of a 2 part report on the project and will aim to cover the work in the proposal not covered in part 1 and to present a case study of the field trials carried out as part of the proposal.

1.2 Aims and objectives

The overall aim of the project is to ascertain if pheromones can potentially be used as a form of control for invasive crayfish species, with a view to developing a viable control strategy for crayfish. The specific objectives of the work are:

1. To determine the behavioural effects of the following on the signal crayfish (*P. leniusculus*) in a laboratory environment:

   - Sex pheromones
   - Predator odours (expanded to include stress and alarm odours)
   - Feeding stimulants
   - Feeding deterrents and cannibalism inducing compounds

2. Determine the species specificity of the aforementioned pheromone(s) (in particular in relation to *Austropotamobius pallipes*).

3. Undertake chemical analysis of the bioactive compounds determined above

4. Undertake limited field trials with synthetic/natural compounds

5. In light of the above results, discuss the overall feasibility of the use of chemical signals to interfere with introduced crayfish and propose a strategy/method for its potential practical application.

1.3 Progress in relation to objectives

Part 1 of the reports covered all of point 1 (see section 1.2 above) and parts of points 4 and 5. This report will cover points 2, 3 as well as covering in more detail points 4 and 5. Points 4 and 5 will be presented as a case study of a 17 month trial of sex pheromone baited traps the aim of which was to evaluate their effect relative to normal food baited traps and to the habitat that the traps are placed in.

1.4 Recap of findings of part 1

1.4.1 Sex pheromone

Adult female *P. leniusculus* released a sex pheromone during the breeding season that attracts adult males but not immature males. Males showed mating behaviour in response to the pheromone only during the breeding season, although an increase of activity was noted outside of breeding season. The main source of this pheromone was determined to be the urine.

1.4.2   Predator odours

The odours of predatory fish (perch and eels) were tested against different life stages of
P. leniusculus. Perch did not elicit a response in any of the life stages tested whereas eels
caused a significant response in juvenile *P. leniusculus,* and a smaller response in adults.

1.4.3   Stress and alarm pheromones

Water from stressed and alarmed adult *P. leniusculus* was tested against different conspecific
life stages. Both adult and juvenile *P. leniusculus* showed a response to both of the
pheromones, with juveniles showing the most significant and extreme response, and adults
showing a lower level of response.

1.4.4   Food preference

Various food types were tested for preference, these were: potato, fish muscle from perch,
smoked mackerel, and trout, tined ham, cat food, carrageenan, Phytagel, and crushed *P.
leniusculus*. Despite being omnivorous *P. leniusculus* showed a preference for protein based
food types, with smoked mackerel being the most attractive food type tested.

1.4.5   Releaser mechanisms

Various gels were tested as a medium for incorporating pheromones, in order that these can
be deployed to facilitate slow release of the pheromones. The pheromones tested (sex alarm
and stress) were partially concentrated and placed into the gels; their effect on adult *P.
leniusculus* was examined in a flow-through environment over a 24-hour period. Phytagel
was found to be the most suitable gel tested. Both sex and stress pheromone release from the
gel attracted animals, whereas alarm pheromones repelled the animals albeit only a short
period of time.

1.4.6   Field trials

Pheromone impregnated gels were placed into standard cylindrical Swedish crayfish traps
and placed out in the field for 24-hours. This was carried out both inside and outside breeding
season. Stress and alarm pheromone baited traps attracted the same number of animals as
normal food baited traps whereas sex pheromone traps attracted less animals than the food-
baited traps, however they only attracted males.

1.5   Amendments to part 1

In part 1 of the report figures 28 and 29 (pages 30 and 31 respectively) should be swapped
around.

2.   Sex pheromone purification and isolation

2.1   Introduction

In part 1 of the report it was ascertained that adult female *P. leniusculus* released a sex
pheromone during the breeding season that attracts adult males but not immature males. This
pheromone induces courtship behaviour in adult males. Males showed mating behaviour in

response to the pheromone only during the breeding season, although an increase of activity was noted outside of breeding season. The main source of this pheromone was determined to be the urine. The work reported in part 1 had been carried out using conditioned water (see section 2.3 in part 1 for further details) and urine collected from the crayfish (see section 2.4, part 1). By further purification and eventual identification of the sex pheromone it is hypothesised that the effectiveness of the traps will be improved, making them more effective at catching crayfish. It would also allow for the production of a chemical specific releaser mechanism to be developed allowing closer control of the rate and concentration of the release of the chemical. This would also reduce production time and potentially cost.

The identification of pheromones is not an easy process, and in some cases such as with the common shore crab (*Carcinus maenas*) the identification has taken over 9 years (see Hardege and others, 2002 for the latest details on this work). However, the development of specific releaser mechanisms and field trials using *C. maenas* sex pheromone traps are well progressed (per. com. Hardege).

There are several tests that can be carried out on the conditioned water and urine that have been positively tested for activity and so considered to be containing the unidentified pheromone(s). These tests will help in purification, isolation and characterisation of the chemical(s) involved. This will aid in the eventual identification of the pheromone(s). This section will describe the processes used to try and purify and isolate the pheromone and the conclusions drawn from them.

2.2   Boiling

The process of boiling can be used to concentrate samples of conditioned water by removing the water leaving behind the pheromone. However, boiling can also break down certain chemicals and compounds such as proteins and polypeptides. Therefore by boiling a sample of conditioned water certain characteristics of the pheromone can be ascertained.

10 1L samples of active conditioned water were boiled for 10 minutes and then tested for activity as described in part 1 (section 3). No activity was found in any of the samples after the boiling process. This suggests that the pheromone was broken down during the boiling process. Therefore the pheromone may be polypeptide based, as proteins are denatured at high temperature.

2.3   Ultra-filtration

Ultra filtration is a process of concentrating protein solutions that make use of membranes of well defined pore size that selectively pass solvent and solutes below a critical molecular weight. The protein solution is forced through the membrane by the application of pressure. Solvents and solutes pass through the membrane while larger protein molecules are retained. By this process it is possible to ascertain a weight range of the active molecule by passing an active sample through a membrane of a known pore size and then testing the filtrate substances which have passed through the filter) and the precipitate (substances which have been retained on the filter).

Samples (N= 14) where run through a membrane with a pore size of 10,000 Da (one Dalton, or Da, corresponds to 1Mw) after being tested for activity. All samples passed through the filter (the filtrate) were active. This indicates that the molecule(s) in question are smaller than

10,000 Da. This process was repeated with a membrane with a pore size of 5,000 Da. The samples were tested for activity before filtering however these samples had to be passed through the 10,000 Da filter first to remove large unwanted molecules that would of otherwise blocked the smaller pore filter. However, there was a loss of activity of both the filtrate and the precipitate. This may have been due to the active part of the sample being lost due to its volatile nature and the length of time taken to filter it such that the active molecule(s) were no longer present. Alternatively/additionally it may have been due to the molecule(s) denaturing, despite every measure being taken to assure that the samples remain as cold as possible to prevent denaturising of the sample it is sometimes difficult to prevent.

2.4　High performance liquid chromatography (HPLC)

There are many different HPLC processes used in analytical chemistry, the process selected for use here was reverse phase HPLC. In this process proteins are separated on the basis of their strength of hydrophobic interaction with the hydrophobic surface of the stationary phase (the filling of the column that the protein solution is passed through). Proteins can then be removed from the column by increasing the amount of a non-polar organic solvent being passed through the column (the mobile phase). The sample is then run through a UV detector that will record the absorbance of the proteins at a set wavelength as they are passed through. This will allow a plot of the protein structure of the sample to be created. Each peak indicates the strength of the hydrophobic interaction with the stationary phase, as those that interact weakly with the stationary phase will elute sooner than those with a stronger interaction. The peak will also indicate an approximate quantity of the protein or proteins that constitute the peak. Peaks can be isolated by collecting the elution at set times. This was tried with active samples taken during the breeding season; the separated peaks were then tested for activity, but none of the individual peaks were active. However if the peaks were recombined then the sample was again active. This suggests that there is more than one active component to the pheromone, and a combination of different peaks (re: proteins) are required to stimulate a response in the animal.

3.　Pheromone communication between native and signal crayfish

3.1　Introduction

It has long been recognised that crayfish can communicate intra-specifically using pheromones (Ameyaw-Akumfi & Hazlett, 1975). It was not until 1982, however, that Tierney and Dunham examined not only intra-specific communication in crayfish, but also inter-specific communication among crayfish species. They found that several *Orconectes* species could distinguish between each other (Tierney & Dunham, 1982, 1984), which had implications for the mechanisms behind the dynamic changes taking place in the distribution of *Orconectes* species around the Great Lakes in the U.S.A at that time (see Tierney & Dunham, 1982 and 1984). The findings suggested that the pre-conception that pheromones where highly species specific could well-be wrong, and could even account for hybridisation in *Orconectes*. Pheromones may not always function as a highly reliable mechanism for maintaining species isolation, especially if one of the species is an invasive.

Previous studies on the interaction of introduced and native crayfish species have focused either on the dynamics of mixed populations (Bovbjerg, 1970; Capelli, 1982; Hill and others,

1993; Holdich & Domaniewski, 1995; Söderbäck, 1995; Westman, 2002), or on direct inter-specific aggressive interactions and competition between individuals for limited resources, such as shelter or food (Söderbäck, 1991; Elvey *et. al*, 1997; Guiaşu & Dunham, 1998; Vorburger & Ribi, 1999; Tierney and others, 2000; Usio, 2001). So far, the reasons for the successful displacement of one crayfish species by another have been attributed to superior competitive abilities of the invasive species for limited resources as they are intrinsically more aggressive (Söderbäck, 1991), have larger mean body size and are quicker growing than native species (Vorburger & Ribib, 1999). These characteristics also enable the invasive species to avoid predation more successfully and from a younger relative age than native species and also means that native crayfish are often predated by invasive species. Invasive crayfish tend also to be more fecund, and have been noted to interfere with the reproduction of native species causing severe falls in the reproductive success of entire populations of native crayfish (Westman, 2002). Invasive crayfish are also vectors for various diseases, to which native species of crayfish are often susceptible (Holdich and others, 1995).

It has been well documented that crayfish communicate using pheromones (see Bechler, 1995 for an overview). Pheromones have been demonstrated to be used to attract or repel individuals or to control mating behaviour of individuals of the same species (Dunham, 1978; Stebbing and others, 2003). There are several possible effects that the sex pheromone(s) of an invasive species could exert on a native species. The native species could be attracted to the pheromone in the same manner as conspecifics of the invasive species. This could facilitate the chances of breeding interference and cannibalism, both of which are well documented in native-invasive crayfish interactions. Alternatively, the pheromone may have no effect, not even being detected by the native species. The third possibility is that the pheromone induces an alarm response in the native individual, as chemicals that prey respond to often originate as intraspecific cues (eg a sex pheromone) released by the predator, (Chivers & Smith, 1998). It has also been suggested that invasive crayfish use a broader range of chemical signals than native crayfish (Hazlett, 2000).

To further assess the possible usefulness of *P. leniusculus* pheromone baited traps it is important to ascertain their effect on native biota especially the native crayfish *A. pallipes*.

3.2   Methods and materials

Austropotamobius pallipes were collected from the river Wansbeck at Kirkwhelpington, Northumberland, U.K. (OS grid reference NY 968836). *Pacifastacus leniusculus* were collected from Lartington ponds, near to Barnard Castle in Teesdale, UK (OS grid reference NZ 003162). Both were mono-species populations with no prior experience of other crayfish species. All animals were collected and kept in the laboratory under appropriate licensed conditions. Animals were maintained at ambient temperature and photoperiod, in gender- and age-specific tanks. A quarantine tank was set up for *A. pallipes* that had been exposed to water conditioned by *P. leniusculus*. *A. pallipes* that had been exposed to *P. leniusculus* were placed into the quarantine tank rather than back into the main holding tanks and kept there for 5 weeks. Despite the *P. leniusculus* population showing no external signs of crayfish plague this was still a necessary precaution against the potential spread of plague.

Water was conditioned by placing 4 *P. leniusculus* of the same gender-age group into a tank containing 2L of distilled water at ambient temperature over a period of 24-hours. Water was conditioned using:

- Mature female *P. leniusculus* during the breeding season.
- Mature female *P. leniusculus* outside of the breeding season
- Mature male *P. leniusculus* outside of the breeding season

Water that was conditioned using mature females during the breeding season was tested for activity as described by Stebbing and others. (2003). All water that was conditioned by female *P. leniusculus* during the breeding season used in this experiment had been shown to induce mating behaviour in male *P. leniusculus*. The control for the experiment was the use of 'self-water' (water taken from the experimental tank during the acclimatisation period as outlined by Rose (1986)).

Individual *Austropotamobius pallipes* were placed into a glass tank (34cm x 21cm x 19cm) containing 6.4L of distilled water with three blacked out sides in an ambient temperature dark room under red illumination. The animal was left to acclimatise until the animal had adopted a neutral position. Once the animal was acclimatised 20ml of 'self-water' was taken from the tank containing the crayfish, the animal was then left to acclimatise again if it was disturbed during this process. The previously collected 'self-water' was tested on the individual *A. pallipes* by holding a 20ml syringe approximately 1-3cm above the head of the crayfish and its entire contents (20ml) then released at an even rate (taking no longer than 15s). The behavioural response of the animal was classified into one of four categories depending on the extent of the reaction to the water:

0= Neutral position. Animal stationary. The natural resting position of crayfish, with abdomen tucked under the cephalothorax, chelae held close to body, chelae, abdomen and cephalothorax touching the substratum.
1= Alert position. Animal stationary. Cephalothorax and abdomen raised above substratum, but with telson still touching the substratum. Chelae extended but not raised.
2= Alert moving. As above, but with the animal moving slowly backwards.
3= Retreat response. As above, but with the animal moving quickly backwards using its legs, with chelae often raised in an aggressive manner.
4= Escape response. The animal showing a tail flip to carry it away from the area of introduction.

Once the animal had been tested using 'self-water' it was again left to acclimatise and adopt the neutral position (0). Water that had been conditioned by *P. leniusculus* was then tested on the animal in the same manner as with the 'self-water' and the behaviour recorded. Each type of water was tested on both mature and juvenile male and female *A. pallipes* both inside and outside of the breeding season. This was repeated 10 times for each age, sex and season combination for each of the waters tested.

A second experiment was run to examine the effect of *A. pallipes* conditioned water on *P. leniusculus*. The water was conditioned using:

- Mature female *A. pallipes* during the breeding season.
- Mature male *A. pallipes* outside of the breeding season

The water was conditioned in the same manner as with *P. leniusculus*, but the mature female water conditioned during the breeding season was not tested for activity before being used. The *A. pallipes* conditioned water was tested only on mature male and female inside and outside of the breeding season. However, preliminary trials using water conditioned by

mature female *A. pallipes* during the breeding season showed that *P. leniusculus* were attracted to *A. pallipes* conditioned water therefore a different behavioural scale was observed as follows:

0= Neutral position. Animal stationary. The natural resting position of crayfish, with abdomen tucked under the cephalothorax, chelae held close to body, chelae, abdomen and cephalothorax touching the substratum.
1= Alert position. Animal stationary. Cephalothorax and abdomen raised above substratum, but with telson still touching the substratum. Chelae extended but not raised.
2= Moving. As above, but with the animal moving slowly forwards.
3= Alert and moving. As above, but with the animal moving quickly forwards, often around the point of introduction using its legs. Cephalothorax raised above the level of the abdomen, with chelae often raised moving around in the water in front of the animal sometimes moving to the animals mouth.

A further experiment was also carried out to examine the effects of repeated applications of *P. leniusculus* conditioned water on *A. pallipes*. The experiment was set up the same as with the previous trials of *P. leniusculus* water on *A. pallipes*, but the application of the conditioned water was repeated with the same type of water as before, but the application was repeated three times with 30-minute intervals between each application. This was repeated 10 times on each sex, age and season combination for each water type tested.

All of the data for all experiments were ranked and analysed using a general linear model with a pair-wise comparison, at the 95% significance level (Conover and Iman, 1981).

3.3   Results
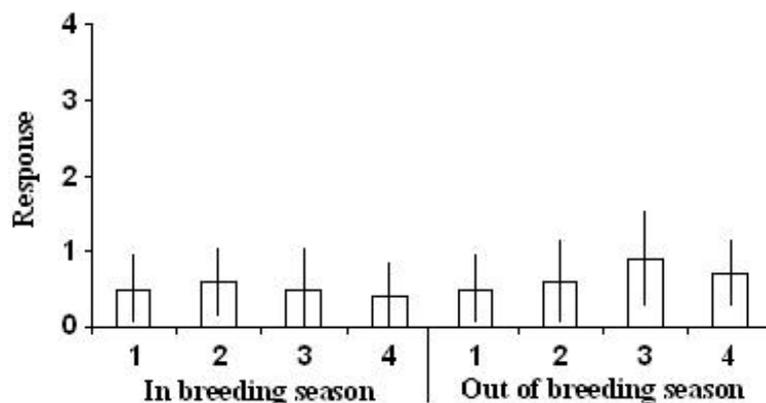
Figure 1. Mean response of (1) juvenile male, (2) adult male, (3) juvenile female and (4) adult female *Austropotamobius pallipes* in and outside of the breeding season to self-water (N=10 per sex/age group), with 95% C.I.

Figure 1 shows the mean response of (1) juvenile male, (2) adult male, (3) juvenile female and (4) adult female *A. pallipes* for both in and outside of the breeding season, with 95% confidence intervals, to 'self-water' that was used as a control. The mean value for each age, sex and seasonal combination is less than 1. The combined mean behavioural response of *A. pallipes* to the control water was <0.6 suggesting that there was little or no effect of the mechanical introduction of the water. There were also no significant differences in the application of the control water with age, sex or season. There was a significant difference in

the response of all *A. pallipes* sex/age/season combinations in the control (self water) to waters conditioned by *P. leniusculus* in all cases (P= 0.0001).
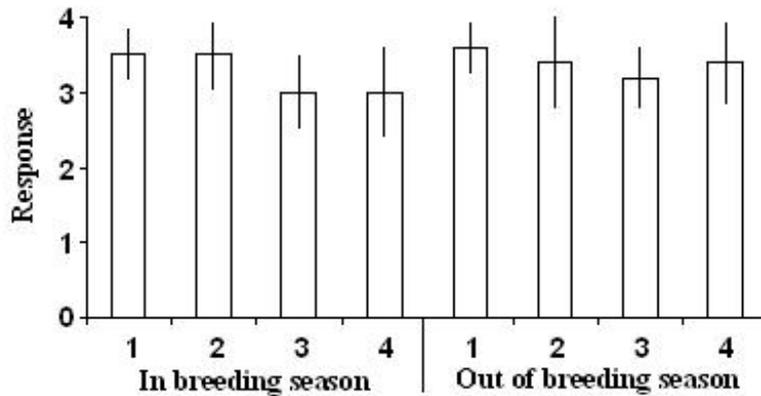
Figure 2. Mean response of (1) juvenile male, (2) adult male, (3) juvenile female and (4) adult female *Austropotamobius pallipes* in and outside of the breeding season, to water conditioned by mature female *Pacifastacus leniusculus* during the breeding season (N=10 per sex/age group), with 95% C.I.

Figure 2 shows the mean response of (1) juvenile male, (2) adult male, (3) juvenile female and (4) adult female *A. pallipes* for both in and outside of the breeding season, with 95% confidence intervals to water conditioned by mature female *P. leniusculus* during the breeding season. The figure illustrates that there were no significant differences between sex, age or season for this treatment. There were also no significant differences between any of the sex, age and season combinations of this treatment and that of any other treatment of water conditioned by *P. leniusculus* and tested on *A. pallipes*.
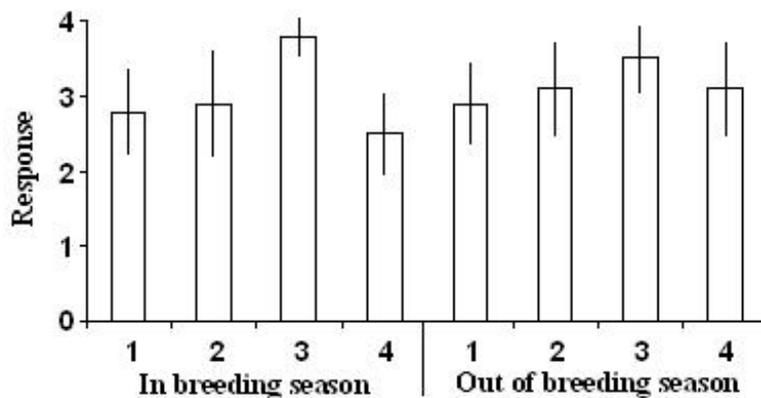
Figure 3. Mean response of (1) juvenile male, (2) adult male, (3) juvenile female and (4) adult female *Austropotamobius pallipes* in and outside of the breeding season, to water conditioned by mature female *Pacifastacus leniusculus* outside of the breeding season (N=10 per sex/age group), with 95% C.I.

Figure 3 shows the mean response of (1) juvenile male, (2) adult male, (3) juvenile female and (4) adult female *A. pallipes* for both in and outside of the breeding season, with 95% confidence intervals to water conditioned by mature female *P. leniusculus* outside of the breeding season. The only significant difference was between adult and juvenile female *A. pallipes* tested during the breeding season (T –3.900, P 0.0327), with the juveniles showing a higher response than the adults.
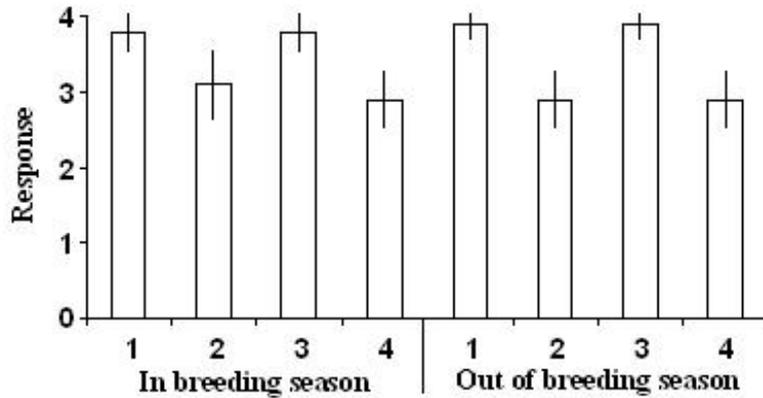
Figure 4. Mean response of (1) juvenile male, (2) adult male, (3) juvenile female and (4) adult female *Austropotamobius pallipes* in and outside of the breeding season, to water conditioned by mature male *Pacifastacus leniusculus* (N=10 per sex/age group), with 95% C.I.

Figure 4 shows the mean (+/- 95% confidence intervals) response of (1) juvenile male, (2) adult male, (3) juvenile female and (4) adult female *A. pallipes* both inside and outside of the breeding season, to water conditioned by mature male *P. leniusculus*. Season had no effect on the response of *A. pallipes* to male *P. leniusculus* conditioned water. There was also no significant difference between sexes. Age showed a significant difference between adults and juveniles (P 0.000) in all cases with juveniles showing greater response than the adults.
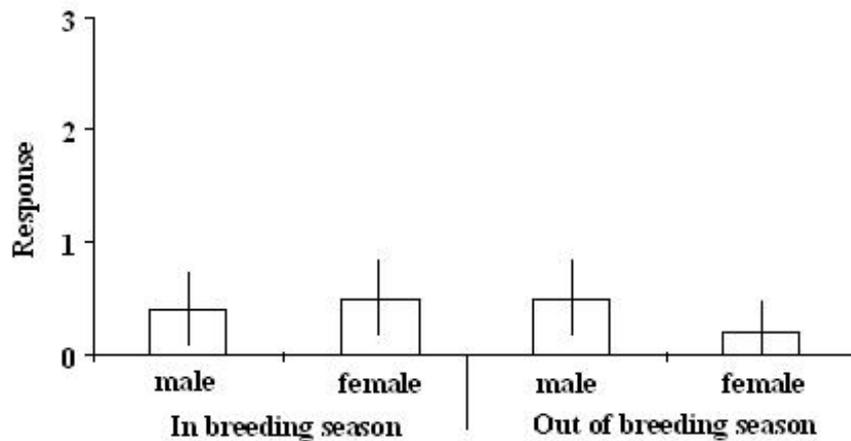
Figure 5. Mean response of mature male and female *Pacifastacus leniusculus* in and outside of the breeding season, to self-water (N=10 per sex/season group), with 95% C.I.

As was the case with *A. pallipes* when presented with self-water, *P. leniusculus* showed little or no response to the mechanical introduction of the water (see figure 5), the mean response being 0.4. There were no significant differences in the application if self-water between sex, age and season. In all cases there was no significant difference between the water conditioned by *A. pallipes* and the blank control for all sex, age and season combination, save for water conditioned by mature female *A. pallipes* during the breeding season.
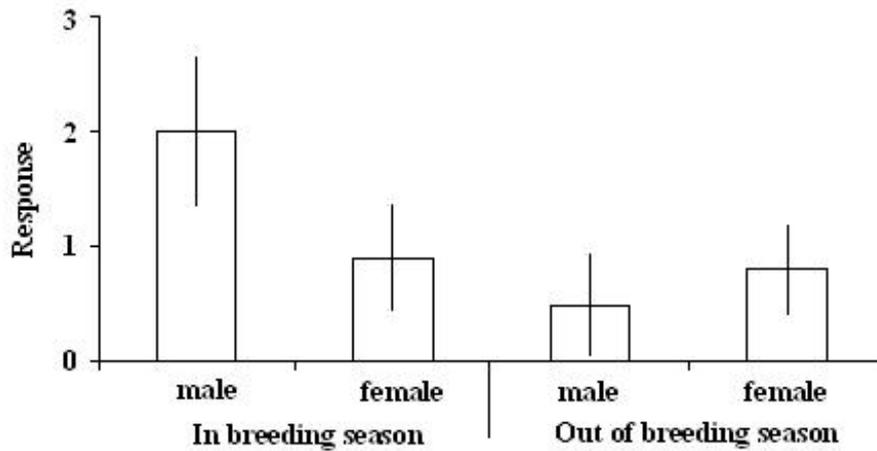
Figure 6. Mean response of mature male and female *Pacifastacus leniusculus* in and outside of the breeding season, to water conditioned by mature female *Austropotamobius pallipes* during the breeding season (N=10 per sex/season group), with 95% C.I.

Figure 6 shows the mean response of mature male and female *P. leniusculus* to the introduction of mature female *A. pallipes* water conditioned during the breeding season (+/- 95% confidence intervals). There was a significant difference between the response of males and females (T-3.511, P 0.0304) tested during the breeding season. There was also a significant difference between males tested during the breeding season and males (T-4.788, P 0.0004) and females (T-3.830, P 0.0111) tested outside of the breeding season. Males tested during the breeding season showing the greater response in all cases.
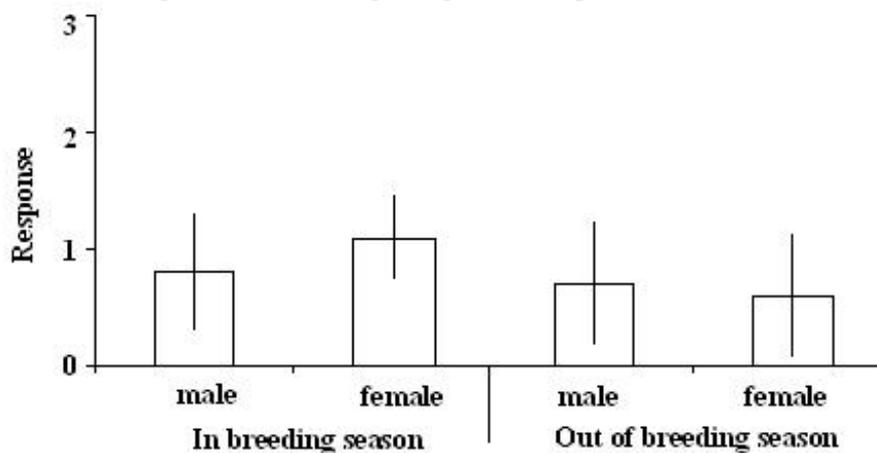
Figure 7. Mean response of mature male and female *Pacifastacus leniusculus* in and outside of the breeding season, to water conditioned by mature male *Austropotamobius pallipes* during the breeding season (N=10 per sex/season group), with 95% C.I.

Figure 7 shows the mean response (+/- 95% confidence intervals) of mature male and female *P. leniusculus* tested both inside and outside of the breeding season with water conditioned by mature male *A. pallipes* during the breeding season. There were no significant differences between the males tested or females tested inside or outside of the breeding season.

21
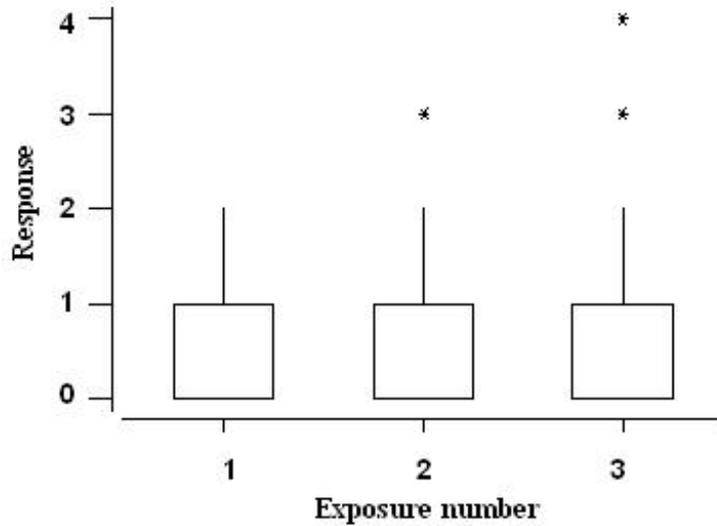
Figure 8. A box-plot of the combined response of *Austropotamobius pallipes* to 3 sequential exposures to 'self-water' (N=40 per exposure).

Figure 8 shows the combined response of *A. pallipes* to 3 sequential exposures to 'self-water'. As can be seen from the figure there was no significant difference between each exposure.
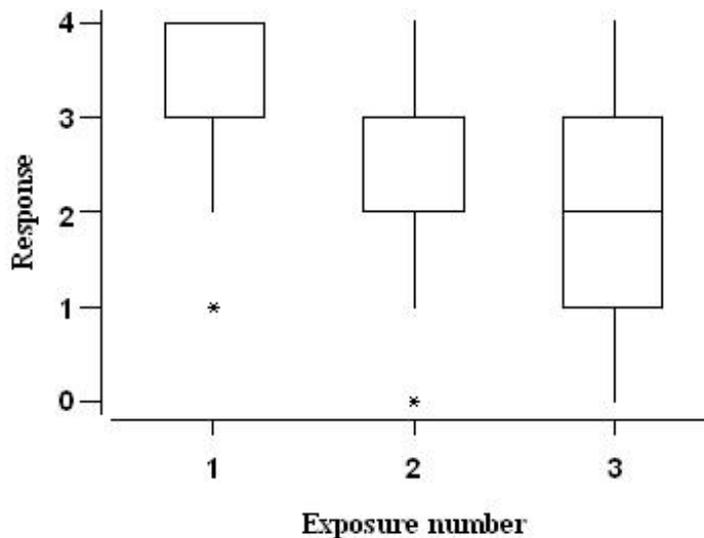
Figure 9. A box-plot of the combined response of *Austropotamobius pallipes* to 3 sequential exposures to water conditioned by mature female *Pacifastacus leniusculus* during the breeding season (N=40 per exposure).

Figure 9 shows the combined response of *A. pallipes* to 3 sequential exposures to water conditioned by mature female *P. leniusculus* during the breeding season. There was a significant difference between each of the exposures (exposure 1 vs. 2, T –4.953, P 0.0000; exposure 1 vs. 3, T –8.454, P 0.0000; exposure 2 vs. 3, T –3.501 P 0.0016) with a decrease in response being seen with each subsequent exposure.
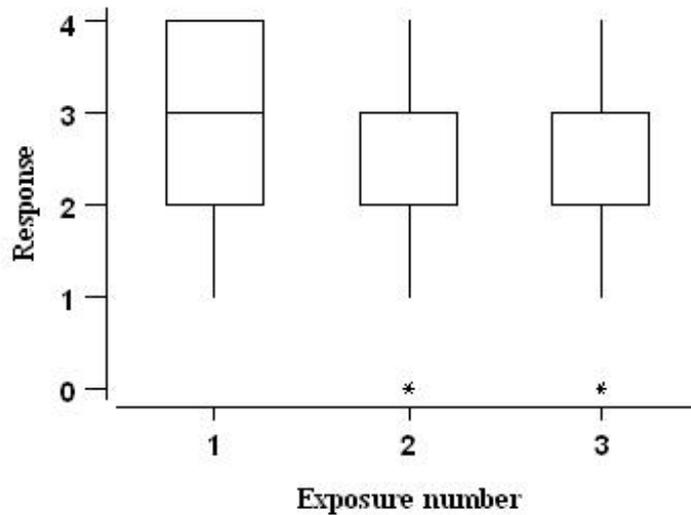
Figure 10. A box-plot of the combined response of *Austropotamobius pallipes* to 3 sequential exposures to water conditioned by mature female *Pacifastacus leniusculus* outside of the breeding season (N=40 per exposure).

Figure 10 shows the combined response of *A. pallipes* to 3 sequential exposures to water conditioned by mature female *P. leniusculus* outside of the breeding season. A significant difference was found between the 1st exposure and the 2nd (T –3.097, P0.0062) and the 3rd exposure (T –3.732, P 0.0007). However, there was no significant difference between the 2nd and 3rd exposure (T –0.6352, P 0.8009).
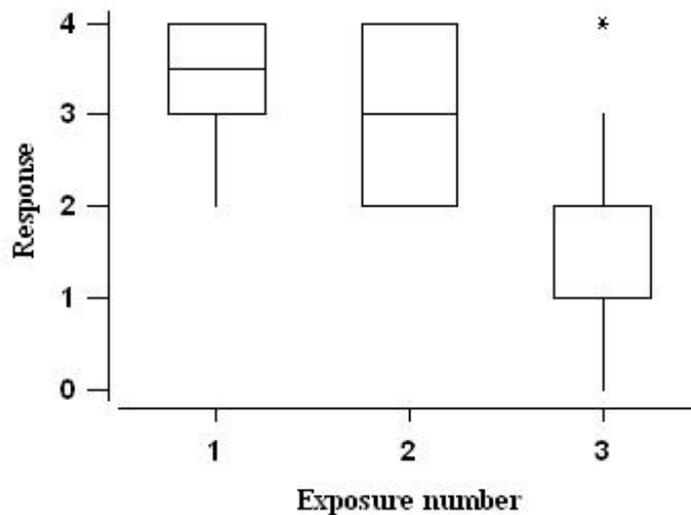
Figure 11. A box-plot of the combined response of *Austropotamobius pallipes* to 3 sequential exposures to water conditioned by mature male *Pacifastacus leniusculus* (N=40 per exposure).

Figure 11 shows the combined response of *A. pallipes* to 3 sequential exposures to water conditioned by mature male *P. leniusculus*. As with the case of water conditioned by mature female *P. leniusculus* during the breeding season there was a significant difference between each exposure (1st vs. 2nd T –3.22, P 0.0042; 1st vs. 3rd T –12.77, P 0.0000; 2nd vs. 3rd T –9.544, P 0.0000).

3.4   Discussion

The results demonstrate that the chemical presence of *Pacifastacus leniusculus* adults repels mature and juvenile *Austropotamobius pallipes* of both sexes. Whilst sex, age and season seemed to have no particular effect on the outcome of trials using water conditioned by mature female *P. leniusculus* inside or outside of the breeding season (see figs 2& 3), juvenile *A. pallipes* were seen to respond to a higher degree to adult male *P. leniusculus* conditioned water than the adult *A. pallipes*.

Water conditioned by mature female *P. leniusculus* during the breeding season was tested for the presence of the sex pheromone on mature male *P. leniusculus* before being tested on *A. pallipes* (see Stebbing and others, 2003). The presence of the pheromone did not affect *A. pallipes* any differently to water conditioned by mature female *P. leniusculus* outside of the breeding season (ie the pheromone was either not present or of too low a concentration to be detectable in the bioassay). This suggests that the response stimulated in *A. pallipes* was not from the pheromone *per se* but rather from another chemical component released by *P. leniusculus* during the conditioning period. It has been suggested that alarm responses in prey animals can be stimulated by a wide variety of sources from the predatory animal including exuviae, eggs, excreta, marking and sex pheromones or any other products of the predator (Dicke & Grostal, 2001). The fact that the specimens of *A. pallipes* used during the trails had experienced no prior contact with *P. leniusculus* suggests that the chemical that stimulated the response was either unfamiliar and was therefore avoided, or it was similar to a known predator odour. The *P. leniusculus* used in the trials had been fed a varied diet of potatoes and flesh from varies fishes, there was also a degree of cannibalism in the holding tanks. Protein metabolites from the carnivorous diet present in the excreta, specifically from the predation of conspecifics, present in the *P. leniusculus* conditioned water may be responsible for the induced response observed in *A. pallipes.* This does not explain, however, why juvenile *A. pallipes* showed a greater response to male *P. leniusculus* conditioned water than to either of the female conditioned waters. This maybe due to male *P. leniusculus* generally being of a larger size than females and may, therefore produce more of the chemical that *A. pallipes* is responding to than the females either inside or outside of the breeding season. Males may also produce a chemical that juveniles respond to that females may not produce at all. It maybe due to the fact that adult male *P. leniusculus* are perceived as more of a threat to juvenile *A. pallipes* than the female *P. leniusculus*, but with no prior knowledge of the species it is not clear how juveniles respond more than the adults. Juveniles may show a greater response generally to perceived threats due to them being more vulnerable to predation than adults, but more significance would be expected between juveniles and adults with the other water tested if this was the case.

The ability to respond to the odours of predators has been documented for a number of aquatic prey species including Protozoa, Arthropoda, fishes and Amphibia (for overview see Kats & Dill, 1998). The study of alarm responses in crayfish to date has focused on the response of individuals to damaged or stressed conspecifics (Hazlett, 1985; 1989; 1990; 1994; 2000; 2003). Hazlett (1989; 1990) demonstrated that crayfish (*Orconectes spp.*) showed alarm response to animals of different taxa. *Orconectes spp.* responded to chemical cues from stressed leech (*Macrobdella decora*), the darter (*Etheostoma exile*) and rock bass (*Ambloplites rupestris*), the newt (*Notopthalamus viridescens*) and the catfish (*Ictalurus natalis*); two of which are natural predators, the catfish and the rock bass. Hazelett (2000) demonstrated that invasive crayfish respond more to alarm signals that native species did; this

paper, however, gives evidence supporting the fact that native species may also respond to chemical signals of which they have no prior experience.

When the waters conditioned by *A. pallipes* were tested on *P. leniusculus*, at low levels of response the animal adopted a more alert stance, but with increased response individuals moved towards the source of the water. In extreme cases the animal would wave its chelae, often moving them to their maxillae as if eating or 'tasting' the water, a similar feeding motion or 'tasting' has been observed during mating in *P. leniusculus* (pers. obs.). It was not clear whether this was a feeding response or a possible sexual response, this degree of response was only seen with water conditioned by mature female *A. pallipes* during the breeding season supports the idea that it may have been a sexual response, although the conditioned water was not tested for the presence of a sex pheromone as was the case with water conditioned by mature female *P. leniusculus* during the breeding season. Even though the utilisation of a sex pheromone by *A. pallipes* has not yet been confirmed (Villanelli & Gherardi, 1998) the increased response of male *P. leniusculus* to water conditioned by mature female *A. pallipes* during the breeding season suggests that there is an increase in chemical output by *A. pallipes* during this period.

The results of the present study suggest that chemical odours from invasive *P. leniusculus* may represent a possible mechanism for the displacement of *A. pallipes*. If the same response is seen in the field, then simply the chemical presence of *P. leniusculus* would have a detrimental effect on populations of *A. pallipes* possibly causing animals to spend more time in their burrows thereby limiting feeding and reproductive opportunities and hence limiting population growth. It could also mean that *P. leniusculus* may displace *A. pallipes* from of an area of prime habitat to an area of less suitable habitat. It would also mean that populations of *P. leniusculus* may have a broader range of effect than just the physical extent of the population.

The fact the *A. pallipes* water is attractive to *P. leniusculus*, especially to males during the breeding season, also helps to explain the displacement process. Inter-specific mating has been suggested to be one of the main contributing factors in crayfish species displacement; young adult male *P. leniusculus* may mate with female *A. pallipes* to avoid the more aggressive intraspecific male-male competition. However, if the observed response was feeding, then the increased attractiveness of adult female *A. pallipes* during the breeding season may lead to an increase of predation.

The findings of this study also have implications on the development of pheromone traps for the control of *P. leniusculus* (see Stebbing and others, 2004 for overview). Traps containing the female *P. leniusculus* sex pheromone that could potentially be deployed in populations of mixed species would repel *A. pallipes* meaning that they would not enter the traps. As a result, the catch would not have to be carefully sorted to remove native crayfish and that *A. pallipes* would not be threatened by aggressive interaction with *P. leniusculus* whilst in the trap.

The response of *A. pallipes* to repeated exposure to water conditioned by *P. leniusculus* does show a degree of habituation. However, the experiments were carried out over a relatively short period of time ($1\frac{1}{2}$ hours). This may reflect a saturation of the water in the test tank with the chemical that stimulated the response in *A. pallipes*, due to the complete saturation to which the animal stopped responding. It may also be due to blockage of the chemoreceptors on the test animal with the chemical. Even though these experiments give

evidence for a degree of short term habituation this is not a realistic model of what may occur in a natural environment with *A. pallipes* possibly not coming into contact with *P. leniusculus* for several days at a time. These experiments will need to be repeated but with days rather than minutes between subsequent exposures.

A more detailed study of some of the processes described in this paper are required before a clearer picture of the role that chemical communication plays in species displacement in crayfish is understood. Work is in progress to examine the potential habituation of *A. pallipes* to *P. leniusculus* and vice versa, and to ascertain whether the chemical attraction of *P. leniusculus* to *A. pallipes* is a feeding or sexual response.

4.   Case study: field trials

4.1   Introduction

Field trials of the sex pheromone baited traps were carried out over a year to ascertain when the traps would be most effective, and in which type of habitat the traps would be best placed. The field trials were carried out at ponds in, North Yorkshire. A lentic system was chosen for the case study to remove the complexities of a more turbulent environment from the equation.

The pond used for the field trials is filled by land drainage from a large catchment area and empties into the River Tees via a small stream; the pond covers an area of 36,710m^2. The pond was originally built at the turn of the 18th/19th century to supply the then recently built steam railway system with water, which took quarried rock from the dales down to the coast. The pond was created by damming the east side of the shallow marshy hollow. The pond is shallow (deepest point 1.54m) and lined with clay with coniferous woodland on the north and east and deciduous on the south bank; the west side is marshy and dominated by reeds.

After the closure of the railway system the ponds were used as a coarse fishery. The pond was stocked with *P. leniusculus* circa 1980 to supply the restaurant trade in London. At that time 30 cairns (large wire bags filled with rocks) where placed 4-5m off the banks around the circumference of the pond. Despite regular harvesting by trapping the crayfish population has continued to increase. Fishermen that regularly use the pond often complain of bait being taken by crayfish, and fish being attacked in keep nets. *P. leniusculus* have recently been found in the River Tees, Lartington Ponds are the expected source of the new population as this is the only potential source in the vicinity and has an easy route of introduction via natural colonisation or internal transfer.

4.2   Methods and materials

4.2.1   Population analysis

Capture-recapture was carried out over a three-day period (04/07/03-06/07/03) to determine the population density and the degree of movement within the pond. Standard Swedish crayfish traps were placed at 20 sites along the east and south banks (see figure 12) of the pond, each baited with approximately 25g of smoked mackerel and left for 24 hours.
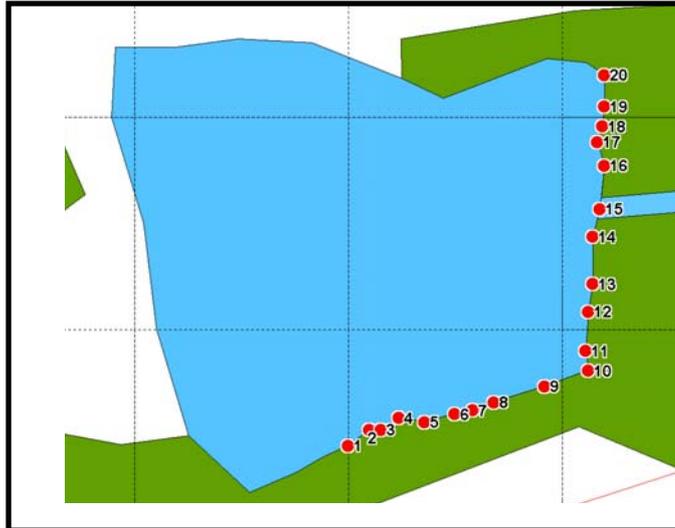
Figure 12. A more detailed view of Lartington pond and the surrounding area, showing site location and number (1 square= 100m).

The traps were emptied and re-baited after the first 24 hours period; each individual crayfish caught was sexed, measured (carapace length in mm) and marked. The crayfish were marked by removing combinations of pleura and/or telson tips (see fig 13) as described by Slack (1995) using a pair of nail clippers. This is a method of marking that last for 2-4 moults meaning that individuals would not lose their mark during the trapping period. The process of trapping and marking animals was repeated for 2 days, while on the 3rd day animals were not marked. This process allowed for the distinction of which animal came from which site and on which day.

Figure 13

The 28 marking positions on telson and pleura
(Slack, 1995)
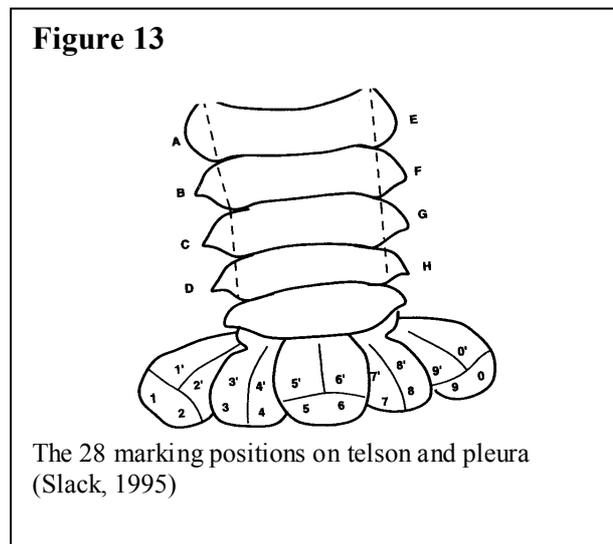
To estimate population size the Bailey's triple capture method was used, this assumes that:

- The population is closed ie there is no immigration of emigration from the population
- That all individuals in the population have an equal chance of being caught
- There is no input into the population during the sampling period (ie no natality or immigration)

- The markings do not alter an individuals behaviour, make them more likely to emigrate or be preyed upon or influence their likelihood of being recaptured
- Mortality must be equal for marked and unmarked individuals

The equations for the Bailey's triple capture method are:

$$M = \frac{S_2 \times R_{1,3}}{R_{2,3}} + R_{1,2}$$

$$N = \frac{M \times S_2}{R_{1,2}}$$

Where:
M= the estimate of the number of individuals marked in the population.
N= total population estimate.
S_1= the number of individuals caught, marked and released in the 1st sample.
$R_{1,2}$= the number of individuals that were marked in the 1st sample and recaptured in the 2nd sample.
S_2= the total number of individuals caught in the 2nd sample.
$R_{2,3}$= the number of individuals caught in the 2nd sample and recaptured in the 3rd.
$R_{1,3}$= the number of individuals marked in the 1st sample and recaptured in the 3rd.

A standard iterative method was also used, presuming the same assumptions as with Bailey's triple capture the equation used was:

$$N = \frac{n_1^2 + (n_1 \times n_2)}{n_1 - n_3}$$

Where:
N= total population estimate
n_1= total number of animals captured on day 1
n_2= total number of animals captured on day 2
n_3 = total number of animals captured on day 3

4.2.2 Habitat analysis

Habitat features were recorded that have been previously associated with the absence or presence of crayfish. Each site consisted of a 5m^2 area parallel with the bank side. For each site substrate and bank side features were recorded as a percentage of cover. This could equal more than 100% to account for the over lap of certain features that were being recorded. Features recorded were:

Substrate

- Bed rock/artificial
- Boulders (>25cm)
- Cobbles (<25cm)
- Gravel/course sand (0.2-6cm)

- Fine sand/silt (<0.2cm)
- Compacted clay

Bank side

- Bed rock/artificial
- Boulders (>25cm)
- Cobbles (<25cm)
- Gravel/course sand (0.2-6cm)
- Fine sand/silt (<0.2cm)
- Compacted clay
- Tree roots
- Under cut banks

The possible effect of habitat was analysed using a best subset regression to fine the best predictors and then a standard regression for further analysis. The data for the habitat scores collected throughout the trials period were averaged and tested against data collected using food-baited traps only. Males and females were analysed separately.

4.2.3 Field trials

Field trials were carried out in a similar manner as outline in section 8 of part one of the report. Preliminary trials of the stress and alarm pheromones in the field showed that there was no significant difference of the effect of the traps when compared to normal food baited traps (see the first report, and Stebbing and others, 2004). Therefore no further field trials were carried out for this study, which continued to focus on the sex pheromone bait. The field trials presented in this study focuses on the effect of sex pheromone baited traps compared to normal food baited traps which were deployed over a 24-hour period through out the year at the pond. A trap was placed approximately in the middle of each $5m^2$ site (see fig. 12) for month from August 2002 to December 2003.

For the majority of months there was two trapping events (see fig. 14) On the first event traps were placed out with alternating food/sex pheromone baits. The second event of the month the traps were placed out with alternating sex pheromone/food bait. This meant that for each site data was collected for each bait type for almost each month. Months where sex pheromone baited traps were not deployed only food-baited traps were used. On these occasions there was only one trapping event, this was done to obtain data of the number and sex ratio of crayfish trappable (re: 'active') at that particular time of year.

Month	1st trapping	2nd trapping	Habitat data
August '02	Food	-	Yes
September '02	Food/sex	Sex/food	No
October '02	Sex/food	Food/sex	No
November '02	Food/sex	Sex/food	Yes
December '02	Sex/food	Food/sex	No

Month	1st trapping	2nd trapping	Habitat data
January '03	Food/sex	Sex/food	No
February '03	Food	-	No
March '03	Sex/food	Food/sex	No
April '03	Food/sex	Sex/food	Yes
May '03	Sex/food	Food/sex	No
June '03	Food	-	No
July '03	Food/sex	Sex/food	No
August '03	Sex/food	Food/sex	No
September '03	Food	-	No
October '03	Food/sex	Sex/food	No
November '03	Sex/food	Food/sex	Yes

Figure 14. Table showing the number of trapping trials each month, bait used and if habitat data was collected.

To aid with analysis the data were grouped from September to December, January to April and May to August. These correspond to the breeding season (September to December), the period when females are carrying eggs and live young (January to April) and the summer months where the animals are most active (May to August). The data was ranked and analysed using a MANCOVA with a pair wise comparison at the 95% confidence interval, with habitat as the correlate. Males and females were analysed separately.

4.3   Results

4.3.1   Population analysis

For Bailey's triple capture method the whole population N was estimated at 7,982.55. For the standard iterative method $N= 92, 016$. The population was assumed to be fully mixed, this supported by the fact that after 24 hours on average males moved 30.96m while females moved 73.35m; however, after 24 hours males moved 54.81m and females 32.36m. The maximum distance moved was by an individual was 236.5m by a female over 24 hours.

4.3.2 Habitat analysis

Figure 15. Map showing the substrate and bank side habitat variability, shown as PCA scores for the combined results.

Figure 15 shows the habitat variables for the substrate and bank side features as combined PCA scores. This places the habitat scores on to a scale, which, for the substrate relates to a scale from silt/fine sand (+4) depicted in red to bedrock –4 (depicted in blue). For the bank side scores the scale goes from bedrock (red) to fine sand and silt (yellow), the blue is the combined effect of the sediment type with the under cut banks and tree roots.

For male *Pacifastacus leniusculus* the main predicting habitat feature was bank side fine sand and silt (adj. R-sq 9.6%), the regression results show that this had a negative effect on the animals' distribution (T –5.14, P 0.000). Substrate fine sand and silt accounted for 8.5% of the distribution and also had a negative effect (T –4.82, P 0.000). This can be seen in figure 16, where mean numbers of male crayfish are low where the PCA score for substrate is high ie where finer sediment is present, this is clearest at site 1. Bank side boulders accounted for 5.2% of the distribution and was a positive predictor (T 3.77, P 0.000). Substrate cobbles as well as bank side cobbles accounted for 3.6% and 3.4% and both were positive predictors (T 3.14, P 0.002; T 3.06, P 0.002) respectively. This can be seen from sites 11-20 where both bank side and substrate boulders and cobbles are high corresponding with high number of crayfish.
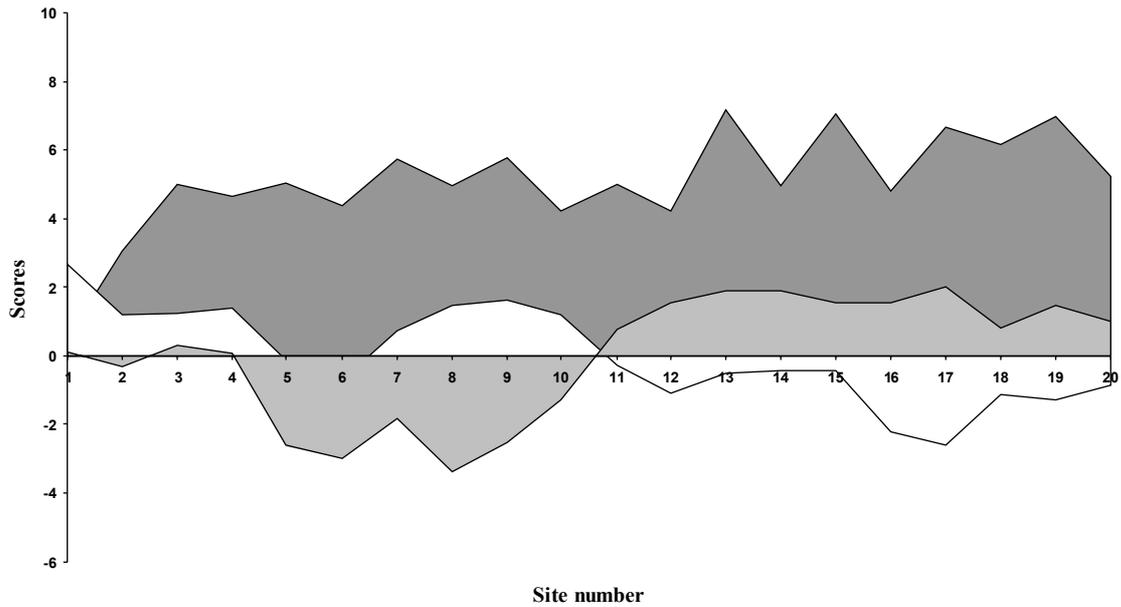
Figure 16. Graph showing the mean number of male *Pacifastacus leniusculus* caught throughout the year for each site (dark grey) along with the PCA scores for substrate (white) and bank side (light grey) habitat variables.

As with male *P. leniusculus* substrate fine sand and silt (4.8%) and bank side fine sand and silt (3.9%) were the two main predictors both negatively effecting distribution (T –3.60, P 0.000; T –3.26, P 0.001). This can be seen in figure 16 where the number of female crayfish are low where there are high levels of silt eg site 1. Substrate cobbles was the next predictor accounting for 2.7%, having a positive effect on distribution (T 2.77, P 0.006), see figure 17, sites11-18. Substrate bedrock was the next predictor (1.5%) having a negative effect (T – 2.13, P 0.034) with bank side cobbles being the 5th predictor (1.3%), which had a positive effect (T 2.03, P 0.043).
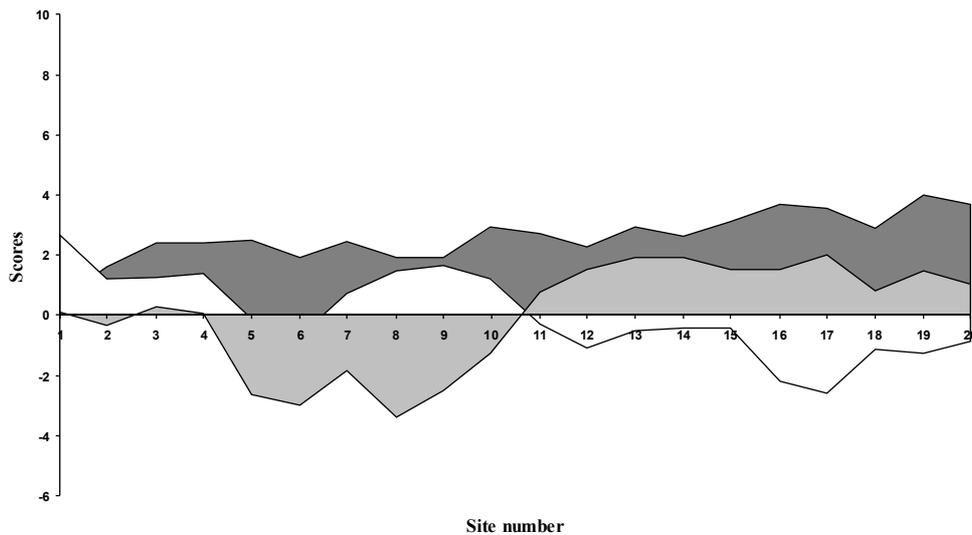
Figure 17. Graph showing the mean number of female *Pacifastacus leniusculus* caught throughout the year for each site (dark grey) along with the PCA scores for substrate (white) and bank side (light grey) habitat variables.

4.3.3   Field trial
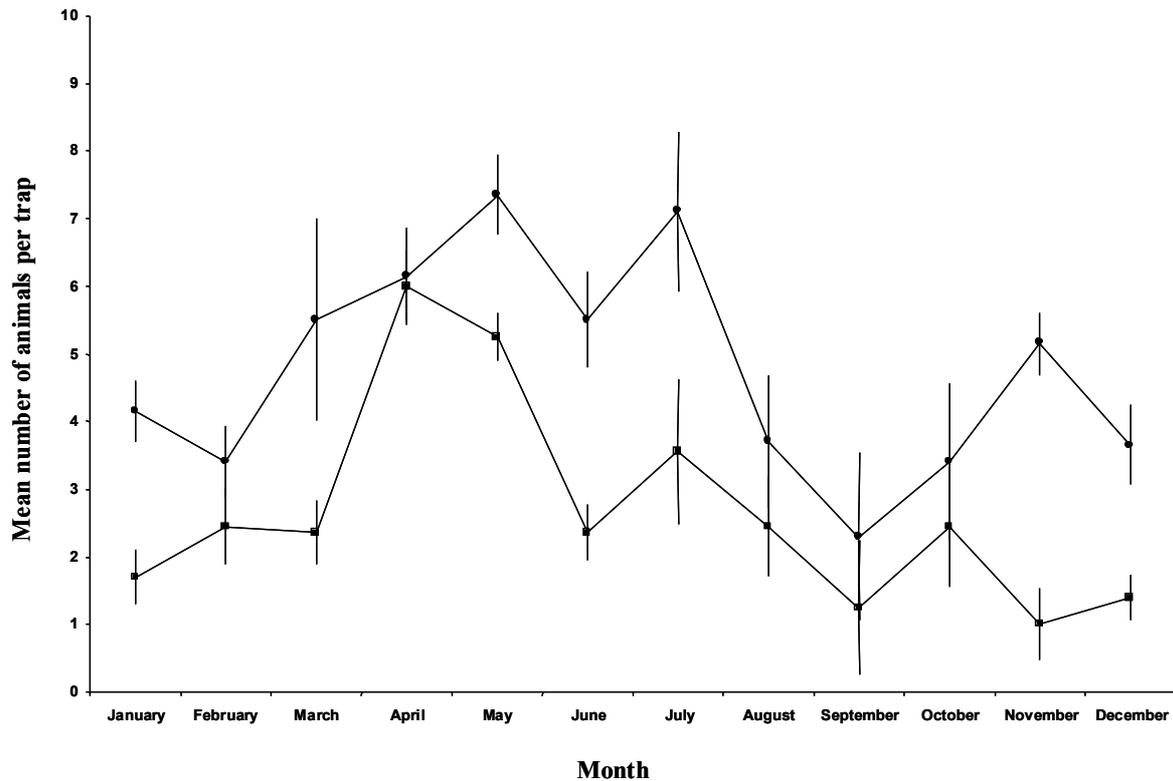
Figure 18. Graph showing the variation with time of year in the mean number of male (•) and female (▪) *Pacifastacus leniusculus* caught using food baited traps with 95% C.I.

Figure 18 shows the variation in the mean number of males and females trapped using food-baited traps throughout the year. This clearly shows the increased number of males and females being trapped with the increase in temperature during the summer months, significantly more animals were caught between April and August than between January and April (T 8.759, P 0.000) and September to December (T –4.55, P 0.0002). The number of animals being trapped can be taken as an indication of the level of activity of the animals. Activity of both males and females decreased towards August-September, but significantly increases again between September and December (T 4.21, P 0.0007) corresponding with the breeding season. The numbers then drop of again with the drop in temperature during the winter months (September to December vs. January to April T –4.55, P 0.0002).
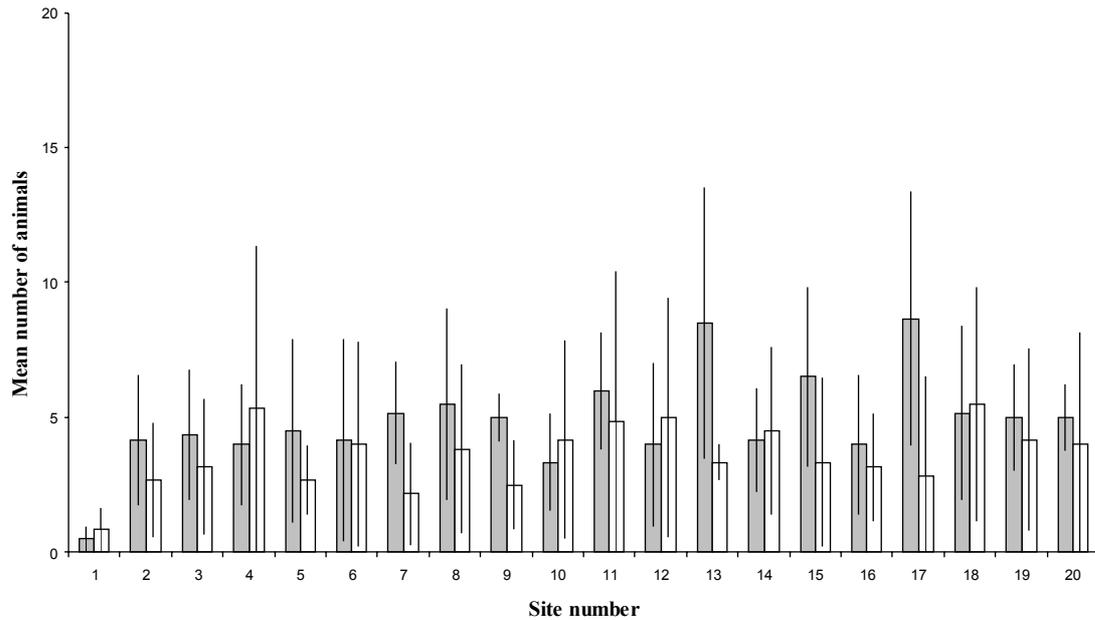
Figure 19. Graph showing the mean number of male *Pacifastacus leniusculus* caught using food baited traps (grey) and sex pheromone baited traps (white) for each site between September and December, with 95% C.I.

There was no significant difference in the number of male *P. leniusculus* found in sex pheromone baited traps when compared to food baited traps from September to December (T –2.85, P 0.0574), as indicated by figure 19. There were however a significantly less males found in the sex pheromone baited traps between January to April (T –7.541, P 0.000) and May to August (T-7.208, P 0.000) than between September and December (see figure 20). However, there was no significant difference in the number of males caught between September and December using sex pheromone baited traps and the number of males caught between January and April using food baited traps (T 0.222, P 0.9999). There was, however, significantly more males found in the food-baited traps than in the sex pheromone baited traps for January to April (T –7.763, P 0.000) and May to August (-13.97, P 0.000) as illustrated in figures 21 and 22 respectively.
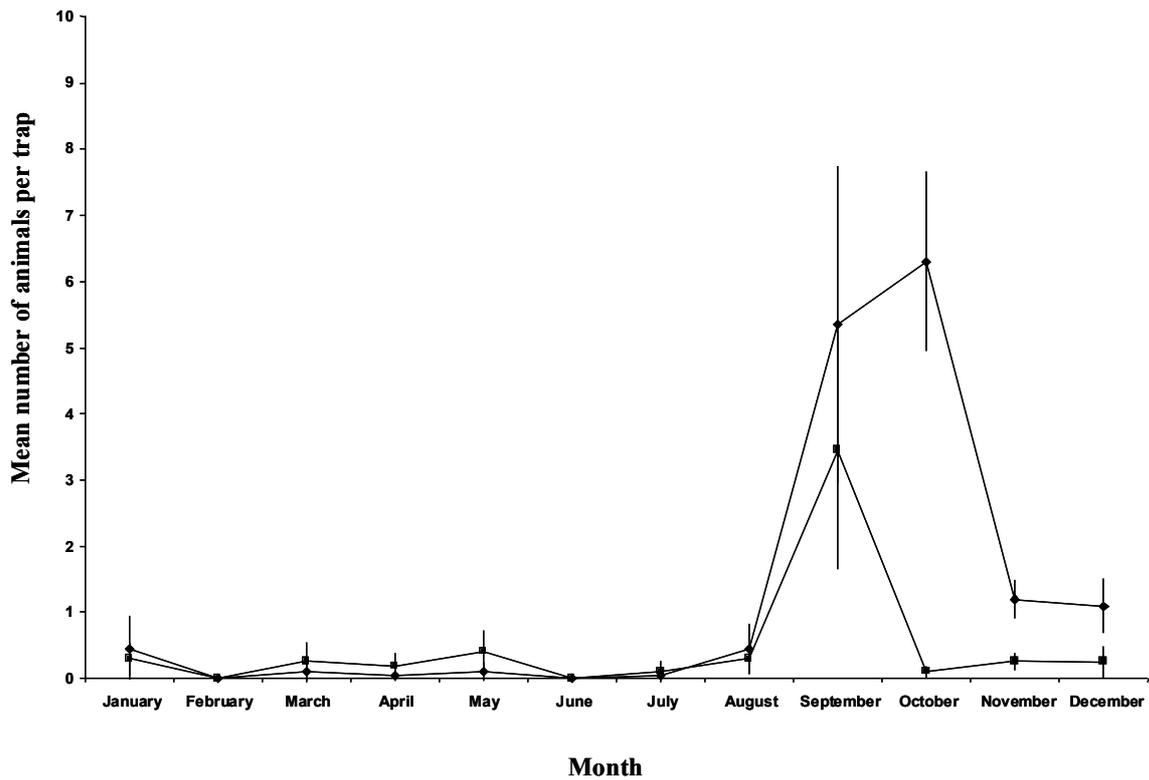
Figure 20. Graph showing the variation with time of year in the mean number of male (•) and female (▪) *Pacifastacus leniusculus* caught using sex pheromone baited traps with 95% C.I.
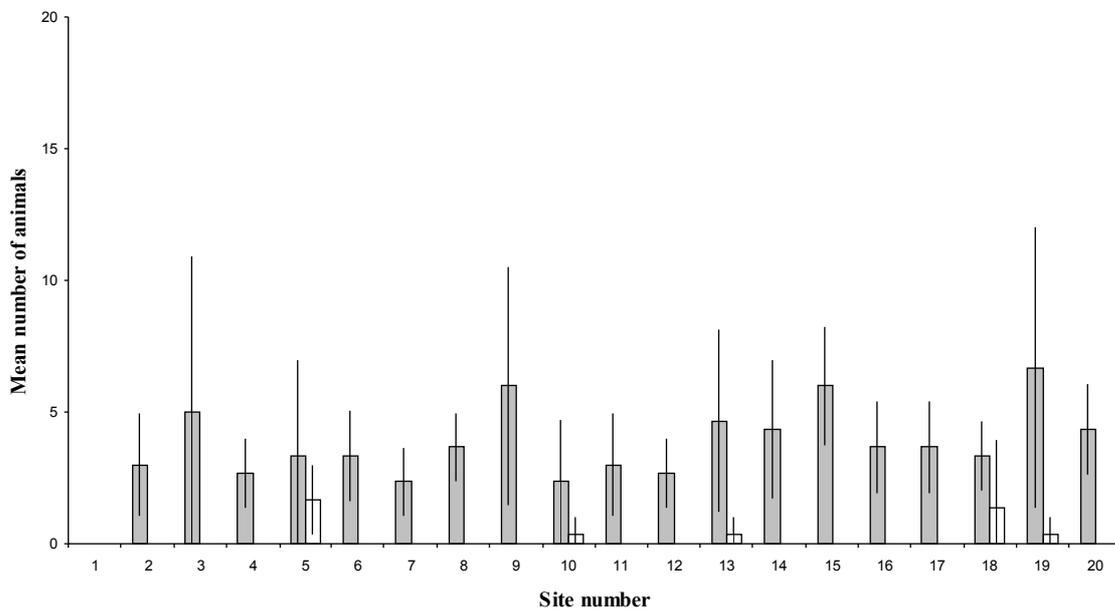
Figure 21. Graph showing the mean number of male *Pacifastacus leniusculus* caught using food baited traps (grey) and sex pheromone baited traps (white) for each site between January and April, with 95% C.I.
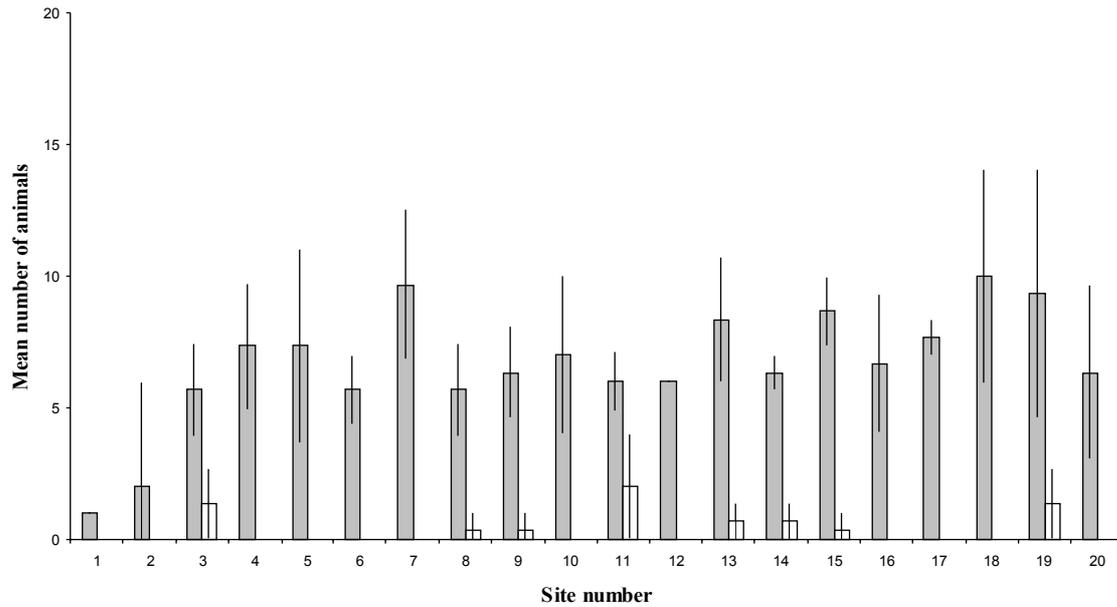
Figure 22. Graph showing the mean number of male *Pacifastacus leniusculus* caught using food baited traps (grey) and sex pheromone baited traps (white) for each site between May and August, with 95% C.I.

There was a significant difference between the numbers of female *P. leniusculus* found in the sex pheromone baited traps and food baited traps between September and December (T – 7.921, P 0.000), with significantly more animals being found in the food baited traps (see figure 23). This was also the case for January to April (T -3.561, P 0.007) and May to August (T –12.83, P 0.000), as shown in figure 24 and 25. There was a significant decrease in the number of animals being caught in January to April (T –6.079, P 0.000) when compared to those caught in September to December using food baited traps. There was a slight significant increase in the numbers of females caught during May to August (T 3.132, P 0.0262) when compared to numbers caught between September and December using food-baited traps. There were significantly more females caught during the period May to August than during the period January to April (T 9.210, P 0.000). There were no significant difference is the number of females caught using the sex pheromone baited traps for any of the trapping periods.
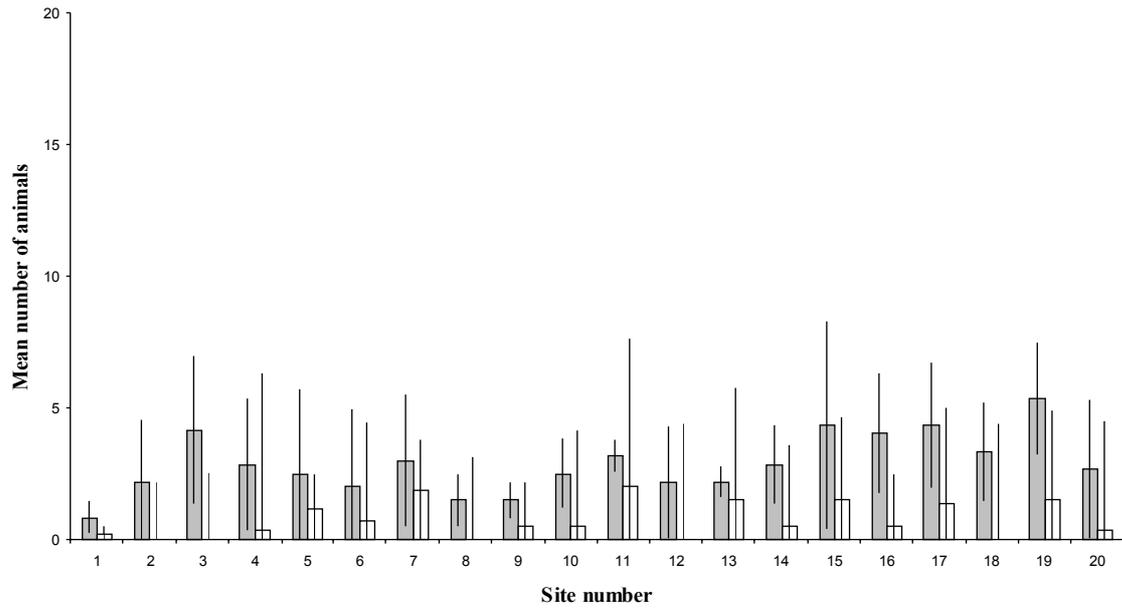
Figure 23. Mean number of female *Pacifastacus leniusculus* caught using food baited traps (grey) and sex pheromone baited traps (white) for each site between September and December, with 95% C.I.
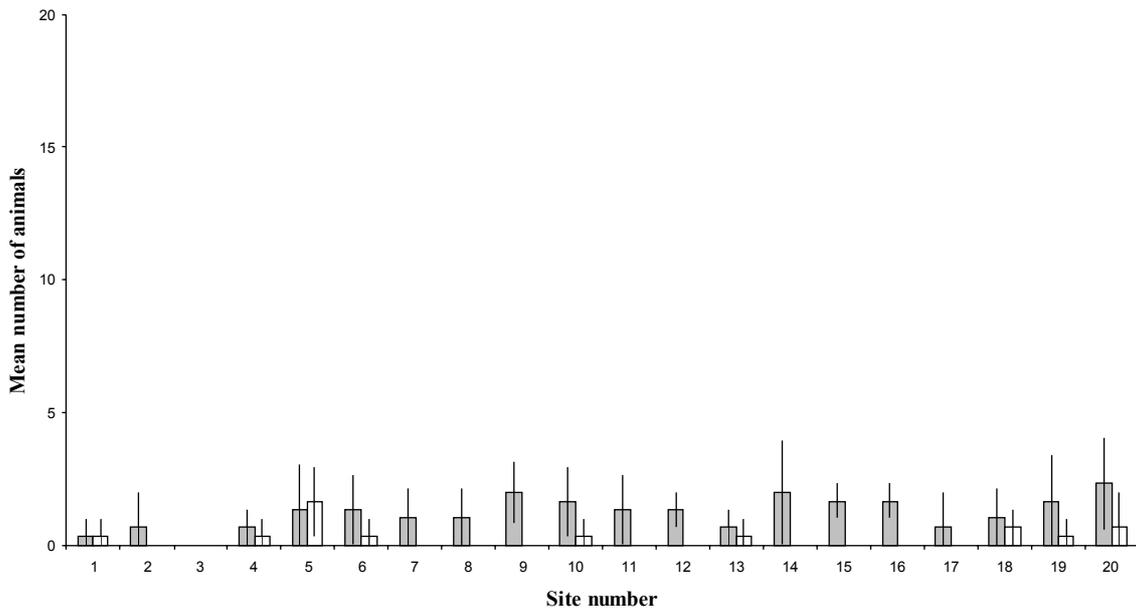
Figure 24. Mean number of female *Pacifastacus leniusculus* caught using food baited traps (grey) and sex pheromone baited traps (white) for each site between January and April, with 95% C.I.
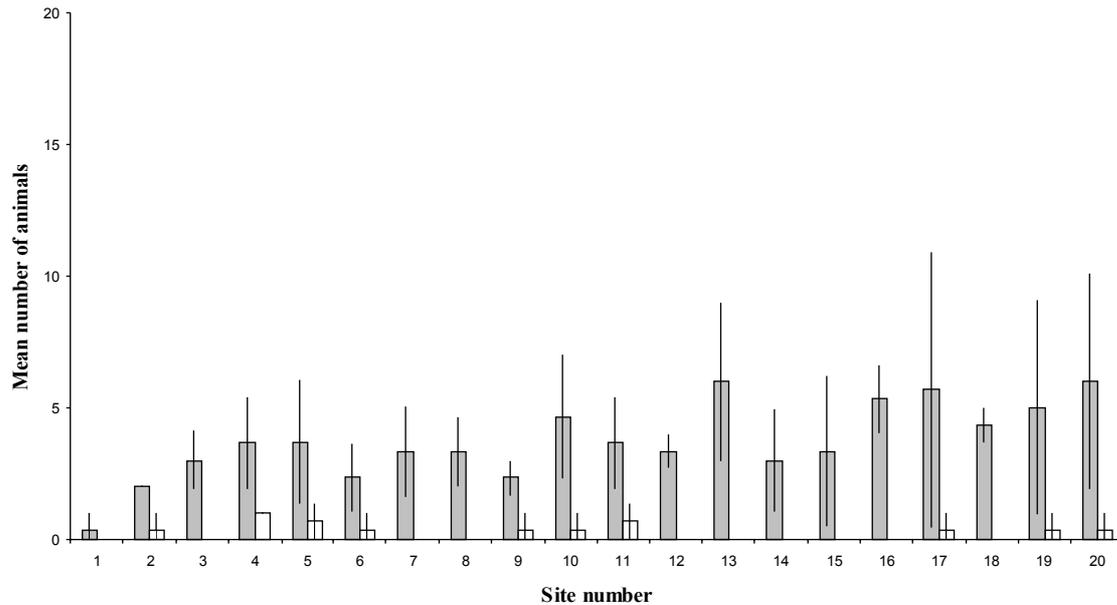
Figure 25. Mean number of female *Pacifastacus leniusculus* caught using food baited traps (grey) and sex pheromone baited traps (white) for each site between May and August, with 95% C.I.

4.4   Discussion

The results from the capture-recapture analysis of the crayfish population at Lartington ponds are ambiguous. The result from the Bailey's triple score indicates a relatively small population, while that of the iterative method suggests a much more extensive population. Given the regular harvesting of the population an estimate as low as 7,982.55 would not be of a large enough size to be sustainable to the extent that the population has been harvested to. During the testing period the author removed at least 4,000 crayfish from the pond, more than half of the estimated population size. Also only part of the population was sampled considering that by removing crayfish via trapping is inherently biased to attracting larger animals and mostly males. Therefore it is felt that the standard iterative methods' estimate of 92, 016 is more accurate. Although there is no quantative evidence to support this, qualitative evidence from the author working at the pond and seeing the large numbers of crayfish in the pond supports the out come of the iterative method. The level of movement indicted by the exercise is also interesting, showing a high level of mobility and confirming the fact that the population is fully mixed.

There was less variation in the habitat as would have been expected. This is due to the fact that the dam along the east side of the pond was constructed from boulders and cobbles, this results in half of the sites being in a very uniform habitat. Even though the south habitat is more natural and therefore shows more variation in the habitat found there it is mainly dominated by finer sediment as it is on the facing the prevalent winds and so therefore has a lot of silt deposited on it from the marshy area west and north-west. Despite this dipolar effect the results still show that the crayfish prefer boulders and cobbles, where it is easy to find shelter due to the complexity of the habitat and there is no need to burrow. In contrast to this the crayfish avoided areas of high fine sand and silt, or course sand and gravel as there would be less shelter available and it would be difficult to burrow. The negative effects of the finer sediment types were off set though by the presence of under cut banks of bank side

compacted clay that allows for burrowing (see figure 26). However, sites which allowed for burrowing, such as with the presence of compacted clay were used despite the presence of fine sand or silt, suggesting that the main restricting factor in crayfish habitat preference is the requirement of shelter. The quantification of habitat variables by observation is subjective and therefore the comparison of habitat data collected in that manner with actual number of animals from the traps may give quasi-realistic conclusions. However, the dipolar nature of Lartington ponds may have aided in giving clearer results due to the lack of variability in the habitat.

Figure 26. Picture showing crayfish burrows at site 6 at Lartington ponds.

The variations found in the number of animals being trapped were as expected, with large males being dominant and with fewer females in general. The annual trends seen with activity peaking during the summer month with the increase in water temperature and a decrease of activity of both sexes during the winter month, but with a secondary peak in activity of the males during the breeding season is was expected.

To aid with the analysis of the data it was required to group the data from several months, although ideally it would have been better to analyse the data on a month-by-month bases to depict trend in more resolution more data for each month would have been required. Designating September to December as the 'breeding season' allowed for the start and end of the season to be sampled for, which showed a steady increase in the number of animals being caught rather than a binary effect. Even though the high number of animals still being caught in the food baited traps during September may have altered the perceived effectiveness of the sex pheromone traps this was off set by the comparatively low numbers caught during December. January to April is the period were crayfish are normally less active due to the low temperature, but also because adult females are carrying eggs during this period and live

young towards the end of this period and therefore stay relatively in active. While May to August is the warmest period were the animals are more active.

As discussed in part 1 of the report the sex pheromone baited traps only attract adult male crayfish during the breeding season. Although on average more males were caught using food baited traps that sex pheromone baited traps there was still no significant difference. The sex pheromone traps attracted as many males during the period of September to December as the normal food baited traps attracted during the period of January to April. There were no or very few females found in the sex pheromone baited traps.

The results from the field trials reiterate what was found in the preliminary trials, which was that the sex pheromone traps were only effective during the breeding season at attracting adult males and were less effective than the food traps (although this was not a significant result).

5. Proposed strategy

5.1 Pheromone traps as a management tool

Sex pheromone baited traps, at this stage of the development, appear to be a less effective management tool than normal food baited traps. The pheromone trapscatch fewer animals than food baited traps, are only effective for a limited window of time and only attract males. However, the traps do not attract native crayfish and may possibly be useful in detecting low densities of *P. leniusculus* as an absent/present test (see Stebbing and others, 2003b for discussion). Eradication of invasive crayfish populations seems an unlikely prospect, but the reduction of the breeding capacity of a population could be possible. In theory the reduction in size of the male breeding population would stimulate animals to mature at an earlier age (Holdich and others, 1999), this would mean that they would become receptive to the pheromone and so more readily trapped at a smaller size (given minor physical modifications to the traps being used). An ultimate goal would be to leave a non-breeding population of females. Sex pheromone baited traps have been used as breeding disruptors in terrestrial pest management with animals that have an equally limited breeding season. The use of sex pheromone baited traps coupled with year round trapping using normal food baited traps removing females and males of both juvenile and adult life stages could potentially restrict the growth rate and even reduce the size of a *Pacifastacus leniusculus* population.

The results of the field trials suggest that pheromone baited traps could have a number of applications. However, it must be stressed that the development of this technique is very much in its infancy and a lot more work is required before this tool will be available (see 5.2 below). Sex pheromone trapping should be viewed as another potential aid in the control of *P. leniusculus* and with further development, pheromone baited traps could be a valuable means by which the threat of *P. leniusculus* to native species of crayfish could be reduced.

5.2 Future work

The possible long-term effects on a crayfish population of shifts in the sex ratio have not been fully investigated; this would be an important process to understand before the use of sex pheromone baited traps are investigated further. The removal of large numbers of adult

males from a population could possibly reduce the intra-specific competition in such a way that it would result in a population explosion, or a reduction in the size of the population.

The field trials reported on above were carried out using partially concentrated female conditioned water. In theory, with further purification, and eventual identification of the sex pheromone(s) the potential effectiveness of the traps will be increased attracting more males during the breeding season. The identification of the pheromone would also allow for a specific releaser matrix to be developed allowing for closer control of the concentration and release rate of the pheromone, making the traps a much more effective management tool. Even though this report shows promise for the potential development of a pheromone based control mechanism for crayfish a lot of work is still required before it can be used as a potential control mechanism. It would be impossible to be able to comment on how long exactly it would take to identify the pheromone. Work to date on the female sex pheromone of *P. lenuisculus* has suggested a compound with more than one active component required to stimulate the males, this could possibly make the identification of the pheromone more complex than that of the common shore crab (*Carcinus meanus*) for example, which took over 10 years.

The field trials have all been carried out in a non-flowing environment (save for part of the development stage which was carried out on the River Clyde). With the further development of the pheromone traps as a management tool it will be required to ascertain how effective the pheromone traps would be at controlling populations in lotic systems. However, after further development of the traps large-scale field trials will have to be carried out to establish the long-term effects of prolonged used of the traps on a population.

The use of the pheromone traps for other uses, such as an absent/present test has not been fully investigated. This could be a very powerful tool as an early warning mechanism allowing for action to be taken before a population becomes established.

Certain aspect of the work could also be useful in isolation. For example, the deployment of sterile male *P. leniusculus* has been proposed as another technique for controlling the species. The bioassay developed for the testing for the presence of the sex pheromone would provide a readily useable method for the development of this technique by determining if sterile males will try to mate with the females in spite of the sterility of the males.

5.3   Conclusion

The work carried out to date from both reports 1 and 2 have investigated a number of different aspects of crayfish ecology, behaviour and chemical communication. Not only has this broadened the understanding of crayfish in general but it has also highlighted a possible control mechanism. Although the sex pheromone traps are still a long way from being developed into a workable management tool, it should be recognised that this project was undertaken on a limited budget, with little prior work in this area having been undertaken. However the work has still managed to demonstrate that with further attention this technique could be developed into a potential control mechanism.

Further progress in this field of research would require the allocation of significant resources and funding. Whether the pursuit of pheromone techniques as opposed to other potential control measures or conservation action is merited is a matter for consideration by conservationists and managers. Whatever the outcome of such discussions, the UK has

international responsibilities to conserve the native crayfish and positive action is required to halt the decline and possible extinction of the species in the UK.

6.   References

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