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# Evaluation of the capacity of pheromones for control of invasive non-native crayfish

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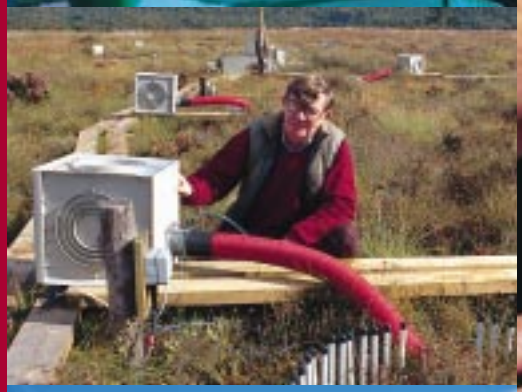

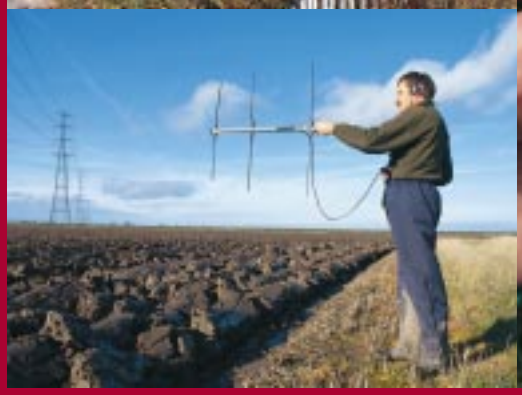

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**Number 578**

# Evaluation of the capacity of pheromones for control of invasive non-native crayfish

Part 1 of a 2 part report. Part 2 available later in 2004

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# *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, such as destabilising river banks by burrowing, and decimating aquatic plants and invertebrates through predation. Signal crayfish pose a particularly acute threat to the native white-clawed crayfish (*Austropotamobius pallipes*), directly through predation and competition for resources, and indirectly through being a vector of the fungus *Aphanomyces astaci*, which causes lethal plague in *A. pallipes*.

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, certain biological or chemical controls are unacceptable in terms of general environmental impacts. One possible alternative is the use of pheromone traps. These 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 this project was to examine the potential use of pheromones as a method of controlling *P. leniusculus*. The main subjects examined as potential control mechanisms were sex pheromones, predator odours (as well as crayfish stress and alarm odours), feeding stimulants and deterrents, and cannibalism-inducing compounds. Pheromone trials were conducted both in the laboratory and field.

**Sex pheromone**

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

**Predator odours**

The odours of predatory fish (perch and eel) 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.

**Stress and alarm pheromones**

Water from stressed and alarmed adult *P. leniusculus* (determined by experimentation) was tested with different conspecific life stages. Both adult and juvenile *P. leniusculus* responded to both stress and alarm pheromones, with juveniles showing the most significant and extreme response, and adults showing
a lower level of response.

**Food preference**

Various food types were tested for preference – potato, perch muscle, smoked mackerel, trout, tinned 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.

**Releaser mechanisms**

Various gels were tested as a medium for incorporating pheromones and facilitating their slow release into the environment. The pheromones tested (sex, alarm and stress) were placed into the gels and their effect on adult *P. leniusculus* 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 pheromones released from the gel attracted crayfish, while alarm pheromones repelled them (if only for a short time).

**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 in and outside the breeding season. Stress and alarm pheromone-baited traps attracted the same number of signal crayfish as normal food-baited traps, whereas sex pheromone traps attracted fewer animals than those baited with food and only attracted males.

**Conclusion**

Sex pheromone-baited traps could potentially be used to control populations of *P. leniusculus*. This could be achieved if the traps were made more effective by purifying the sex pheromone and developing a specific releaser matrix. Extensive trapping using the pheromone traps during the breeding season could potentially remove a large proportion of adult male signal crayfish, consequently reducing the levels of breeding in the population.

## Contents

# 1. Introduction

## 1.1 Background

The considerable increase in world trade over the past century has also seen a dramatic rise in invasive species, particularly in the aquatic environment. A reliable, environmentally friendly method of control is required. Certain species of North American crayfish have become established outside their natural range, and due to having few natural predators and no competition, have become pests.

Six species of crayfish are known to have breeding populations in the UK, 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 *et al.*, 2002). Since the deliberate introduction of *P. leniusculus* into Britain during the 1970s, the species has spread significantly throughout waterways (Holdich *et al.*, 1999; Sibley *et al.*, 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 its natural range and reproducing at high numbers. It burrows 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 and Wiles, 1998).

*P. leniusculus* has been shown to have a profound impact on Britain's native crayfish, *A. pallipes*, a protected species listed in annexes II and V of the European Union Habitats Directive and the UK Wildlife and Countryside Act 1981, and a priority species under the UK Biodiversity Action Plan. *P. leniusculus* shares the same niche range as *A. pallipes* but is larger, more aggressive and more fecund, outcompeting it for resources. It also preys on *A. pallipes.* To compound the situation, *P. leniusculus* is a vector of the fungal infection *Aphanomyces astaci* (crayfish plague), which is lethal to *A. pallipes*.

Despite 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 (MAFF) in 1996, the illegal and accidental introduction of alien crayfish has to date proven almost impossible to control (Holdich *et al.*, 1999). The feasibility of the eradication or control of non-native crayfish populations has been discussed in a number of studies (Holdich *et al.*, 1999; Howard, 2000; Kemp, 2000; Sibley & Nöel, 2002). The use of traps, barriers, pesticides and biological control have all been examined. Of the currently available control methods, biocides are the only means with potential, but this option carries a range of adverse side effects that are detrimental to other species, as well as *P. leniusculus* (Kemp, 2000; Sibley & Nöel, 2002).

At present, no environmentally friendly management tools for controlling invasive species of crayfish have been fully tested in the field A method mentioned by Holdich *et al.*, (1999), Kemp (2000) and Sibley & Nöel, (2002) as a potential control mechanism is the use of pheromones. In many cases this has been successful in the control of terrestrial crop pests and meets with the I.U.C.N.'s guidelines on environmentally friendly control of invasive species(I.U.C.N. 2000). However, pheromones had previously not been examined as a control method for crayfish.

The Environment Agency and English Nature therefore funded a two-year R&D project (with extended funding for one year) examining the potential of pheromones as a potential aid in controlling populations of *P. leniusculus*.

## 1.2 Aims and objectives

The overall aim of the project was to ascertain if pheromones can be used to control invasive crayfish species, with a view to developing a viable control strategy that is applicable to other aquatic pest species. The specific objectives of the work were:

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

   Sex pheromones
   Predator odours (along with stress and alarm odours)
   Feeding stimulants
   Feeding deterrents and cannibalism-inducing compounds

2. To determine the species-specificity of the aforementioned pheromone(s) (in particular in relation to *A. pallipes*).
3. To undertake chemical analysis of the bioactive compounds determined above.
4. To undertake limited field trials with synthetic/natural compounds.
5. To prepare a report on the 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

Due to the lack of literature on feeding deterrents and cannibalism-inducing compounds, and the abundance of literature relevant to the other research areas, it was decided that efforts should concentrate on the objectives that have already been studied, in order to improve knowledge about them, rather than delving into new areas of research.

With the need to develop and complete key objectives and finish experiments before the release of the data, certain aspects of the work have not been fully addressed in Part 1 of the report, but will be included in Part 2.

# 2. General methods

## 2.1 Introduction

The following section briefly discusses how crayfish were maintained during the experimentation period. It also explains certain techniques used in the experiments, such as the conditioning of water and the catheterization of the crayfish.

## 2.2 Crayfish cultures

Signal crayfish were maintained in tanks within self-contained incubation cabinets in accordance with Crayfish Order License 20 (MAFF). Animals were kept in recirculating

systems containing activated charcoal filters and calcium aquaria blocks to maintain calcium concentrations. The cabinets were maintained at ambient photoperiod and temperature. Approximately 50-60 animals were kept in each tank with an assortment of shelter sizes. Age classes were not separated, although males were kept apart from females. Crayfish were fed daily with potato and once a week with either tinned ham or smoked mackerel

Eels (*Anguilla anguilla*) and perch (*Perca fluviatilis*), which are predators of crayfish, were maintained in constantly aerated tanks in the laboratory for as brief a time as necessary, then returned to their natural habitat.

## 2.3 Conditioning of water with crayfish

Crayfish release pheromones and other chemical signals into their surrounding water, and these are then distributed either by natural water currents or by the animals' own fan organ movement (Breithaupt, 2001). The sex pheromone is released by females during the breeding season, while the stress, alarm and avoidance chemicals require stimulation, either from exposure to a predator or from a damaged or undamaged conspecific. The water containing the pheromone canthen be tested in a bioassay (see Section 3.2), or used for chemical analysis.

## 2.4 Collection of urine

Urine has been suggested to be the source of many pheromones in Crustacea (Atema & Cowan, 1986; Hazlett, 1990; Breithaupt *et al.*, 1999; Zultandt Schneider & Moore, 2000). Collection of urine directly from the animal, instead of the more common method of conditioning water, allows for more concentrated samples of the pheromone(s) to be collected. Catheterization of crayfish involved the placing of catchment tubes, which led to a collection tank attached to the animal's carapace, over the animal's nephropores (the execretory gland, situated just below the eyes). As the animal excreted urine containing the pheromone(s) from its nephropores, the urine passed into the collection tank. A small hole in the collection tank allowed pressure built up in the system to be released. Only larger crayfish could effectively be catheterized due the intricate nature of the catheterization process.

# 3. Sex pheromones

## 3.1 Introduction

Sex pheromones are used by a broad range of animals in a variety of habitats (see Agosta, 1990, for a broad overview). Several studies have shown that crayfish use sex pheromones (see Bechler, 1995, for an overview, and Stebbing *et al*., 2003, for specific details on *P. leniusculus*). The consensus is that many species utilise a female-released sex pheromone that attract males and stimulates them to start the mating process. Sex pheromones have been used in the past to control terrestrial insect pests by removing large numbers of the attracted gender from the breeding population. A trap baited with crayfish sex pheromone could potentially be used in a similar manner. However, the presence of such a pheromone in *P. leniusculus* needed to be established and aspects of its nature investigated. Therefore, the aims of this study were to:

- develop a bioassay to test for the presence of a sex pheromone in crayfish;
- determine if female *P. leniusculus* release a sex pheromone during the breeding season that affects the behaviour of male conspecifics;
- determine which life stage of the female releases the pheromone(s);
- identify the source of the pheromone(s) (i.e. point of release);
- determine when males respond to the pheromone(s);
- determine which life stages of the male respond to the pheromone(s).

## 3.2 Mating behaviour analysis

The best way to test for the presence of sex pheromones (which are, at this time, chemicals of unknown size and structure) in water is to use a male crayfish as an indicator. This technique, commonly called a bioassay, involves using behavioural responses typical of the animal when it comes into contact with the chemical being tested for. To develop a reliable bioassay, the behaviour to be used – in this case the very distinctive mating behaviour – needs to be well documented.

Mating behaviour of *P. leniusculus* was recorded from 20 matings and divided into categories. The mating behaviour was categorised into seven basic steps, described below. Photographs of the behaviour are shown in Figures 1-7. Even though the behaviour was divided into distinct categories for the sake of the experiment, in reality, the behaviour represents a continuum.

1. **Orientation.** The male orientated and moved towards the female. The female tended to remain stationary until the male approached.
2. **Contact.** Initial contact was aggressive in 13 out of the 20 observed pairings. The animals met with chelae raised, but the female soon became submissive. The larger the male (relative to the female) the more rapidly the female became submissive. Mating contact differed from non-mating contact because the female would not attempt to swim away when grasped by the male, which is characteristic of the subordinate in non-mating contact of both male-male and male-female interactions. In the other seven encounters there was no aggressive interaction and the male exhibited behaviour of Stage 3 directly.
3. **Seizure.** Once the female had become submissive, the male grasped the female by her chelipeds, antennae or rostrum. Eleven males used both chelipeds to grasp the female; the others used only one, although they would often change the grip on the female to gain a more suitable hold. The female remained stationary throughout this period, while the male climbed onto her back.
4. **Turning.** Once on top of the female, the male began to turn the female over onto her back, often adjusting his grip on her. In all cases the male used his pereopods, and his already established hold(s), to roll the female over while she was held beneath him. In 18 cases the male ended up with his ventral surface parallel with the underside of the female's abdomen, while in the other two cases, the ventral surfaces were already parallel.
5. **Mounting.** The male would then move along the female's body until their ventral surfaces were opposed. During this entire period the male still had a firm hold of the female with his chelipeds, with his other pereopods around her cephalothorax.

6. **Spermatophore deposition.** The male then proceeded to deposit spermatophores onto the female's ventral surface, while maintaining his hold on her. This deposition behaviour was characterised by arching and depression, in quick succession, of the male's abdomen at an average rate of one full cycle every 10 seconds (1 Hz). The male would sometimes rest during this process for up to 30 seconds between bouts.

7. **Dismounting.** After deposition, half the females struggled and turned over, effectively throwing the males off, while the males moved off the other females. The female then moved away from the male. On 12 occasions the male tried to remount the female. During this stage the female constantly tried to move away – this often involved aggressive interaction. On nine occasions the male ended up 'guarding' the female, holding her close to him with his chelipeds and not allowing her to move, despite her efforts to move away. This tended to be the case only with the larger males (relative to the female).


1. Orientation


2. Contact


3. Seizure


4. Turning


5. Mounting


6. Spermatophore deposition


7. Dismounting

**Figures 1-7.** The seven stages of mating behaviour of *P. leniusculus.*

## 3.3 Methods

Two litres of constantly aerated fresh water were 'conditioned' (as described in Section 2.3) in a tank over a 24-hour period both inside and outside the crayfish breeding season using either four mature females, four females carrying eggs, four females that had had their eggs removed, or four immature females. Urine from catheterized (Section 2.3) mature females was also collected over a 24-hour period in the breeding season. Females were catheterized outside the breeding season as well, but the amount of urine produced over a 24-hour period was negligible and so could not be used in a comparable study. Water was also conditioned by mature females with their nephropores blocked with cyanoacrylate glue (this is a reversible process). The control used for all the tests.was fresh water aerated over a 24-hour period with no crayfish present.

### 3.3.1 Bioassay experimental design

Individual males were placed in visually isolated tanks. Males were left to acclimatise for one hour before the start of the experiment. The males' behaviour was then recorded for 15 minutes before the treatment began. Thirty minutes into the acclimatisation period a blue cylindrical aquarium air-stone 2 cm in length was placed at the end of the tank furthest away from the test animal. A syringe containing 20 ml of the female-conditioned water was attached to the air-stone with 15 cm of 5 mm-diameter silicone tubing. The males' behaviour was recorded for 15 minutes after the introduction of the air-stone using a video camera mounted above the test tank.

Due to the small volume of urine collected in catheters over the 24-hour period, the bioassay testing the urine was slightly different. The air-stone was not placed into the tank during the acclimatisation period, and instead of the urine being passed through the air-stone, the stone was dipped into the urine and then placed into the tank. The recording period started as soon as the air-stone was placed into the tank.

All experiments were run between 10.00 h and 15.00 h (the period of the day when the animals were least active). Experiments in the breeding season were run between mid-September and mid-November, while experiments outside the breeding season were run from May to August. The experiments were as follows:

1. Water conditioned by mature females during the breeding season was tested on mature males during the breeding season (N=32).
2. Water conditioned by mature females during the breeding season was tested on immature males during the breeding season (N=16).
3. Water conditioned by immature females during the breeding season was tested on mature males during the breeding season (N=16).
4. Water conditioned by females carrying eggs was tested on mature males during the breeding season (N=16).
5. Water conditioned by females that had had their eggs removed was tested on mature males during the breeding season (n=16).
6. Water conditioned by mature females with their nephropores blocked was tested on mature males during the breeding season (N=16),

7. Urine collected from mature females during the breeding season was tested on mature males during the breeding season (N=16),
8. Water conditioned by mature females during the breeding season was tested on mature males outside the breeding season (N=16),
9. Water conditioned by mature females outside the breeding season was tested on mature males during the breeding season (N=16),
10. Water conditioned by mature females outside the breeding season was tested on mature males outside the breeding season (N=16).

### 3.3.2 Data collection and analysis of bioassay

For the bioassay, three behavioural categories were recorded based on the observed mating behaviour: 'quiescent', in which the crayfish remained motionless; 'motile', in which the animal was moving around the tank; and 'handling', in which the animal was making contact with, seizing, and mounting the air-stone. Contact, seizing, turning and mounting were not recorded as separate behaviours as it was difficult to distinguish them as distinct categories without the interaction between a male and female. Spermatophore deposition happened rarely so was only noted.

The time that the animals spent in each behavioural category, before and after the treatment, was recorded. The data were ranked and analysed using a general linear model with a pair-wise comparison at the 95% significance level (Conover and Iman, 1981).

## 3.4 Results

Figure 8 shows the average time that mature males spent during the breeding season exhibiting each behaviour before (white), and after (grey) the addition of blank, non-conditioned water (N=32). The data showed that there was no significant difference in the time spent quiescent (T= -0.34, P 0.9860), motile (T=0.832, P 0.8999), or handling (T=1.011, P 1.0000) for before and after the treatment. This demonstrates that the physical process of injection had no effect on the animals' behaviour.

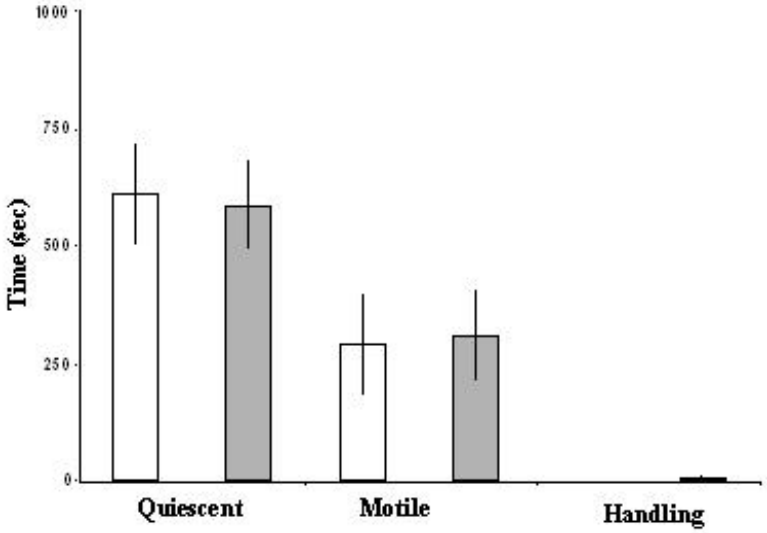


**Figure 8.** Mean time (secs) that mature male *P. leniusculus* spent quiescent, motile or handling before (white) and after (grey) the addition of non-conditioned water during the breeding season (N= 32), with 95% C.I.

Figure 9 shows the average time that mature males spent during the breeding season exhibiting each behaviour before (white), and after (grey) the addition of water conditioned by mature females (N=32). There was a significant decrease in the time spent quiescent after the treatment (T= –6.700, P 0.0001). There was also a slight, but insignificant, increase in the time spent motile after addition of mature female water (T=1.396, P 0.1055). Although there was some handling of the air-stone before the addition of the conditioned water, the handling time increased significantly after the addition of the water (T=5.893, P 0.0001). In addition to the copulatory behaviour observed in the experiments, in two cases males deposited spermatophores onto the surface of the air-stone.

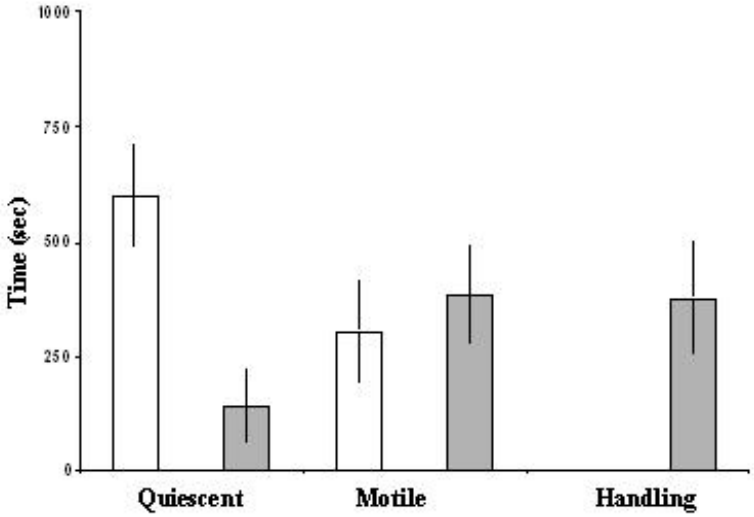


**Figure 9.** Mean time (secs) that mature male *P. leniusculus* spent quiescent, motile or handling during the breeding season before (white) and after (grey) the addition of water conditioned during the breeding season by mature females (N= 32), with 95% C.I.

Figure 10 shows the average time that immature males spent exhibiting each behaviour during the breeding season before (white), and after (grey) the addition of water conditioned during the breeding season by mature females (N= 16). There was no significant difference in the time spent quiescent (T= -0.33, P= 1.0000), motile (T= 0.300, P= 1.0000), or handling (T= 0.0000, P= 1.0000) before or after the treatment.

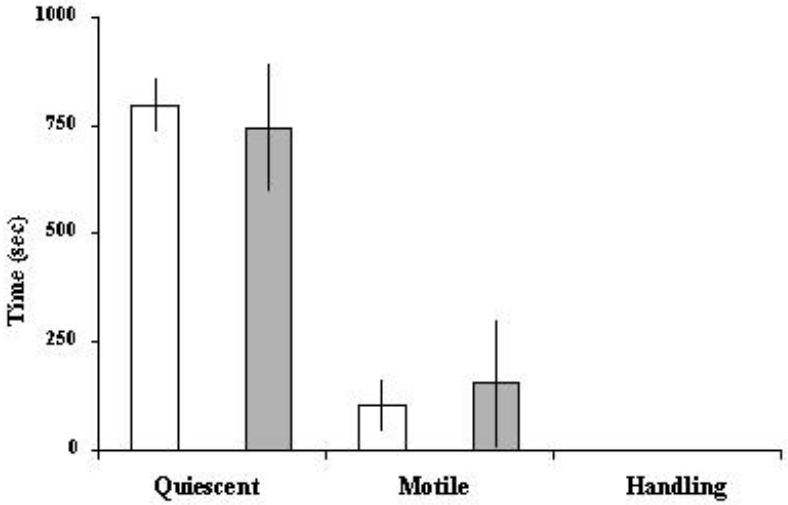


**Figure 10**. Mean time (secs) that immature male *P. leniusculus* spent quiescent, motile or handling during the breeding season before (white) and after (grey) the addition of water conditioned during the breeding season by mature females (N=16), with 95% C.I.

Figure 11 shows the average time that mature males during the breeding season spent exhibiting each behaviour before (white), and after (grey) the addition of water conditioned by immature females during the breeding season (N=16). There were no significant differences in the time spent quiescent (T= -0.49, P 0.9875), motile (T= 0.085, P 0.9999), or handling (T= -0.3513, P 0.9775) before and after introduction of the conditioned water.

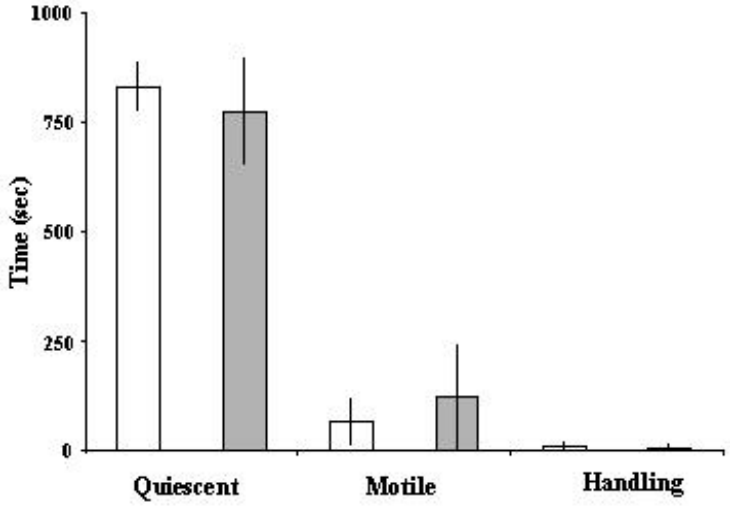


**Figure 11.** Mean time (secs) that mature male *P. leniusculus* during the breeding season spent quiescent, motile or handling before (white) and after (grey) the addition of water conditioned by immature females during the breeding season (N=16), with 95% C.I.

Figure 12 shows the average time that mature males during the breeding season spent exhibiting each behaviour before (white), and after (grey) the addition of water conditioned by females carrying eggs during the breeding season (N=16). There was no significant difference in the time spent quiescent (T= -1.145, P= 1.0000), motile (T= 1.976, P= 0.7689), or handling (T= 0.4628, P= 1.0000).

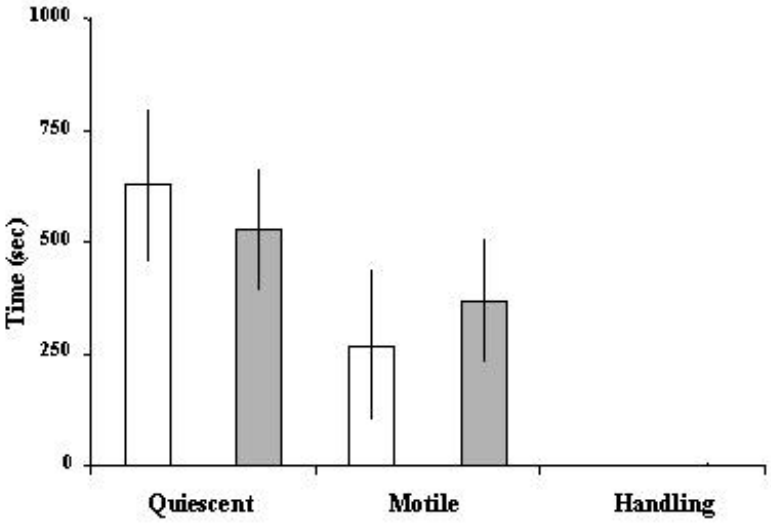


**Figure 12.** Mean time (secs) that mature male *P. leniusculus* during the breeding season spent quiescent, motile or handling before (white) and after (grey) the addition of water conditioned by females carrying eggs during the breeding season (N=16), with 95% C.I.

Figure 13 shows the average time that mature males during the breeding season spent exhibiting each behaviour before (white), and after (grey) the addition of water conditioned by females that have had their eggs removed (N=16). There was a significant decrease in the amount of time spent quiescent after the treatment compared to before the treatment (T= -3.355, P= 0.0174). Despite a slight increase in the amount of time spent motile after the treatment, it was not significant (T= 2.521, P= 0.2020). There was also an increase in the amount of time spent handling, although this was also not significant (T= 2.126, P= 0.5441).

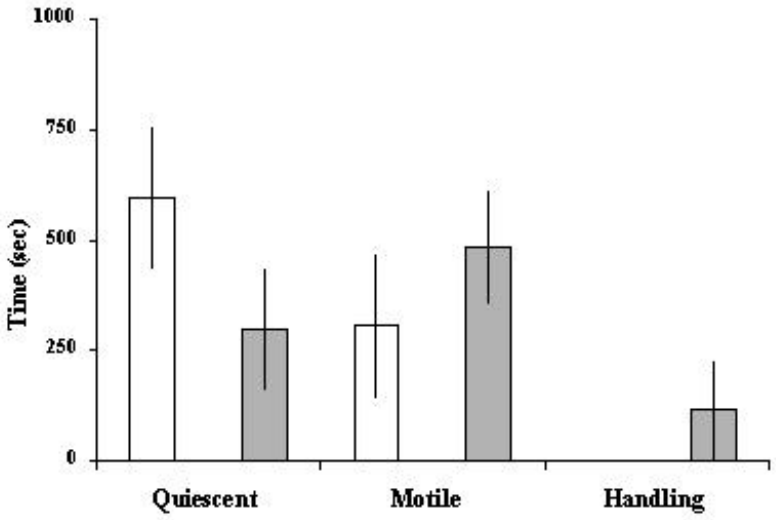


**Figure 13.** Mean time (secs) that mature male *P. leniusculus* during the breeding season spent quiescent, motile or handling before (white) and after (grey) the addition of water conditioned by females that have had their eggs removed during the breeding season (N= 16), with 95% C.I.

Figure 14 shows the average time that mature males during the breeding season spent exhibiting each behaviour before (white), and after (grey) the addition of water conditioned by mature females that have had their nephropores blocked during the breeding season (N=16). There was no significant difference in the time spent quiescent (T= 0.041, P= 1.0000), motile (T= 0.123, P= 0.7689), or handling (T= 0.0000, P= 1.0000).

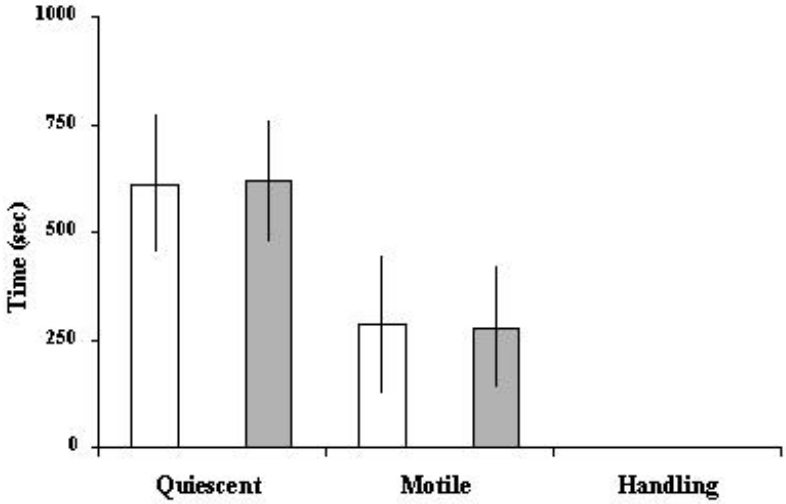


**Figure 14.** Mean time (secs) that mature male *P. leniusculus* during the breeding season spent quiescent, motile or handling before (white) and after (grey) the addition of water conditioned by mature females that have had their nephropores blocked during the breeding season (N= 16), with 95% C.I.

Figure 15 shows the average time that mature males during the breeding season spent exhibiting each behaviour before (white), and after (grey) the addition of urine collected from mature females during the breeding season (N=16). There was a highly significant decrease in the amount of time spent quiescent after the introduction of the treatment (T= -6.556, P= 0.000). There was no significant difference in the amount of time spent motile before and after the treatment (T= -0.288, P= 1.0000), however a highly significant increase was seen in the amount of time spent handling after the treatment (T= 7.037, P= 0.0000).

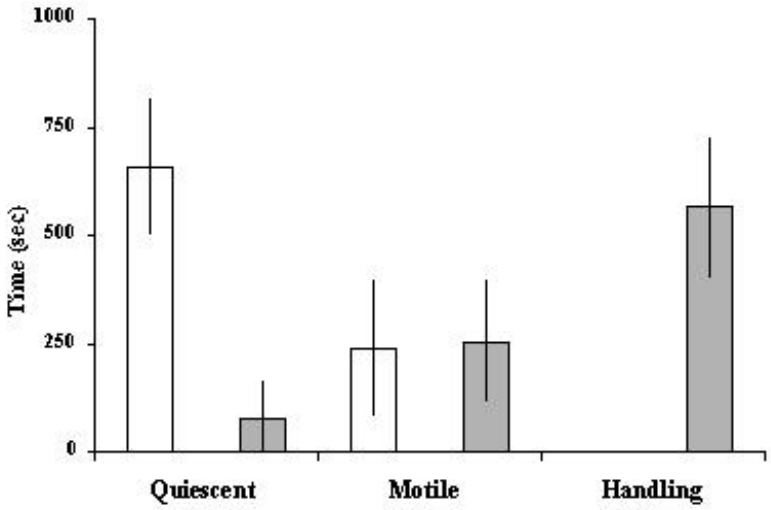


**Figure 15.** Mean time (secs) that mature male *Pacifastacus leniusculus* during the breeding season spent quiescent, motile or handling before (white) and after (grey) the addition of urine collected from mature females during the breeding season (N= 16), with 95% C.I.

Figure 16 shows the average time that mature males outside the breeding season spent exhibiting each behaviour before (white), and after (grey) the addition of water conditioned by mature females during the breeding season (N=16). There was no significant difference in the amount of time spent quiescent before and after the treatment (T= -2.421, P= 0.2619). There was a slight significant increase in the amount of time spent motile before and after the treatment (T= 3.050, P= 0.0451). Despite an increase in the mean time spent handling there was no significant difference (T= 0.2588, P= 1.0000).

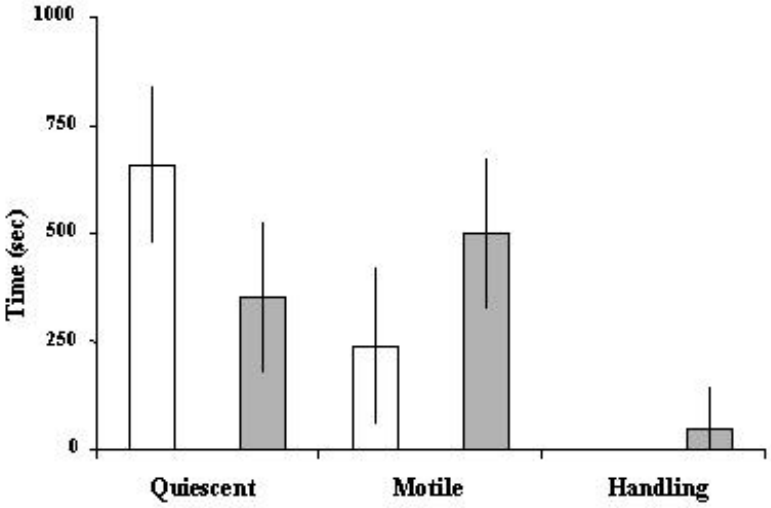


**Figure 16.** Mean time (secs) that mature male *P. leniusculus* outside the breeding season spent quiescent, motile or handling before (white) and after (grey) the addition of water conditioned by mature females during the breeding season (N= 16), with 95% C.I.

Figure 17 shows the average time that mature males during the breeding season spent exhibiting each behaviour before (white), and after (grey) the addition of urine collected from mature females during the breeding season (N=16). There was a significant decrease in the time spent quiescent after the treatment (T= -3.553, P= 0.0091), and a significant increase in the time spent motile after the treatment (T= 4.744, P= 0.0001). However there was no significant difference in the time spent handling after the treatment (T= 4.744, P= 0.0001).

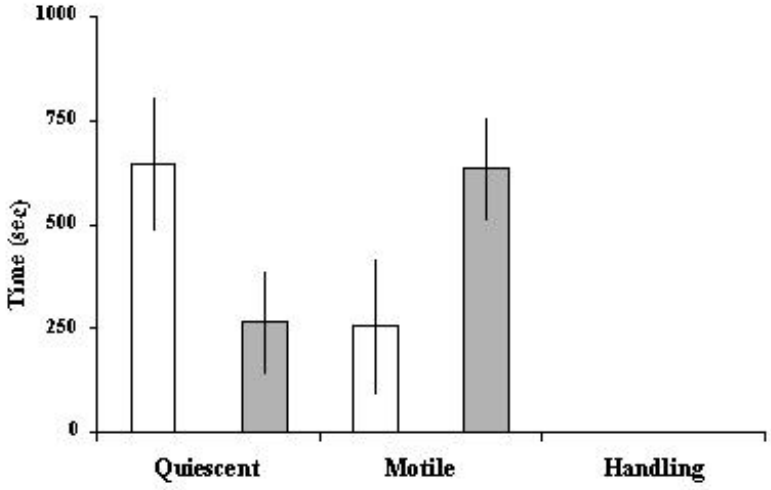


Figure 17. Mean time (secs) that mature male *P. leniusculus* during the breeding season spent quiescent, motile or handling before (white) and after (grey) the addition of water conditioned by mature females outside the breeding season (N=16), with 95% C.I.

Figure 18 shows the average time that mature males outside the breeding season spent exhibiting each behaviour before (white), and after (grey) water conditioned by mature females outside the breeding season (N=16). A significant decrease in the time spent quiescent after the treatment was observed (T=-3.390, P=0.0156), with a significant increase in the time spent motile (T= 3.890, P= 0.0029). However, there was no difference in the time spent handling (T= 0.000, P= 1.0000).

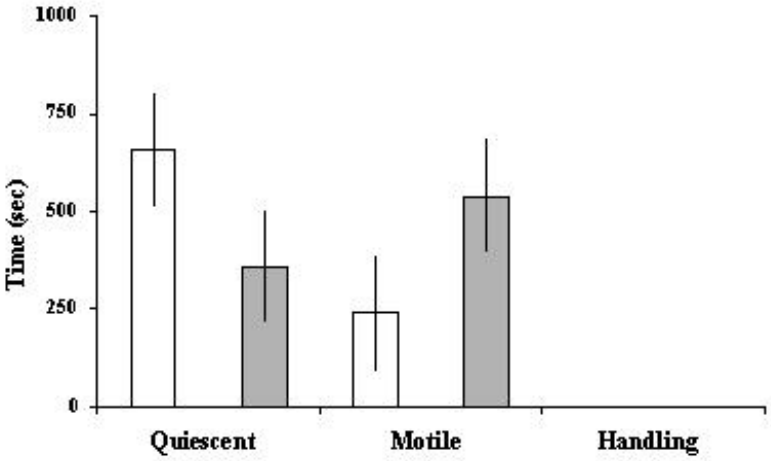


**Figure 18.** Mean time (secs) that mature male *P. leniusculus* outside the breeding season spent quiescent, motile or handling before (white) and after (grey) the addition of water conditioned by mature females outside the breeding season (N= 16), with 95% C.I.

## 3.5 Discussion

It can be concluded from this study that a pheromone was released by mature female *P. leniusculus* during the breeding season that stimulated courtship and mating behaviour in males. This is evident from the increase in the amount of time that the animal spent handling the air-stone after the addition of water conditioned by mature females during the breeding season. As quiescence and motility are non-independent, it can be assumed that the significant decrease in time spent quiescent, and the slight increase in motility after the introduction of the female-conditioned water, can be equated to an increase in activity due to the addition of the mature female-conditioned water. This may be movement towards the air-stone, or at the very least, increased activity due to searching for the source of the attractant. This is supported by the significant increase in the time spent handling the air-stone.

From the data presented in Figure 10 it can be assumed that immature males do not respond to sexually active females during the breeding season. The slight but non-significant increase in the amount of time spent motile suggests that the immature males show some response to sexually mature females but do not show a sexual response. This may be a mechanism to avoid a potential predator.

The lack of significant responses by mature males when tested with water conditioned by immature females during the breeding season (Figure 11) suggests that immature females do not release the pheromone during the breeding season. It also suggests that the behaviour of the males observed with the introduction of mature female water was of a sexual nature.

It can be concluded (Figure 12) that females carrying eggs stop producing the sex pheromone(s) evident in Figure 9. This may be a mechanism to reduce the risk of predation from males on the female or her eggs. However, with the removal of the eggs the mature females appear to begin to release the pheromone again (Figure 13.).

Mature females that have had their nephropores blocked are not sexually attractive to mature males during the breeding season (Figure 14.). This suggests the source of the pheromone(s) was the urine. This is supported by the data presented in Figure 15, showing that urine collected from mature females during the breeding season stimulated a significant increase in the time spent handling after the treatment.

Mature males, when exposed outside the breeding season to water conditioned during the season by mature females showed little response (Figure 16) in comparison to males exposed to the same water during the breeding season (Figure 9). The significant increase in the time spent motile, and the slight increase in the amount of time spent handling (despite not being significant), suggests that there is still some response to the pheromone(s) by mature males outside the breeding season, but to a much-reduced degree.

Figures 17 and 18 both show a similar response by mature males in (Figure 17) and out (Figure 18) of the breeding season to water conditioned by mature females outside the breeding season. This indicates that the production of the pheromone is specific to the breeding season, and is linked to the annual cycle of the female crayfish, and therefore could possibly be linked to the maturation of the eggs.

# 4. Predator odours

## 4.1 Introduction

Several studies have been carried out concerning the effects of predators on crayfish. Evidence of chemically stimulated predator-avoidance behaviour in juvenile *P. leniusculus* has been shown for the eel (*Anguilla anguilla*) and perch (*Perca fluviatillis*) (Blake and Hart, 1993 & 1995; Söderback, 1992). Exposure to water conditioned by the fish caused juvenile *P. leniusculus* to increase the use of shelters and reduced walking and feeding. A similar behaviour has been shown in juvenile *Astacus astacus* for the same fish species (Söderback, 1992). Chemicals released by the fish, if isolated, could potentially be used to keep invasive crayfish confined in certain areas and away from native populations. They could also have detrimental long-term effects on crayfish populations by reducing activity and therefore feeding, breeding and migration.

The main aims of this section of work were:

- to develop a bioassay to test for the presence of chemical(s) released by certain fish species that have a repellent effect on *P. leniusculus;*
- to determine which ages and genders respond to the released chemicals.

## 4.2 Predator avoidance behavioural analysis

The analysis of the behaviour involved in the response of a crayfish to the odour and/or visual presence of a predatory fish was taken from 10 recordings. The level of response was scaled from 0-4 (0 being no response to 4 being the most extreme reponse).

**Resting posture**– only when stationary The animal's antennae and antennules are stationary and pointing down. The animal's chelipeds are touching and parallel with the substrate, tucked into the side of the body. The cephalothorax is lowered and parallel to the substrate with the coxae of the walking legs, and in contact with the substrate). The abdomen is parallel and close to the substrate, with the telson tucked in under the abdomen.

**Neutral posture**. The animal is either stationary or moving. When stationary the animal's antennae are either parallel or touching the substrate and moving, with antennules flicking. The animal's chelipeds are held close and parallel to the substrate and away from the body. The cephalothorax is held close to the substrate and at <30º, with legs slightly extended. The abdomen is close and parallel to the substrate. The telson is held at 45º with the tips touching the substrate. The animal holds this posture when searching and moving about.

**Raised posture.** Only when stationary or moving away (normally from the source of disturbance). The animal's antennae and antennules are both raised and moving. The animal's chelipeds are raised 45º from the substrate. The cephalothorax is also raised up on the animal's legs, although the legs are not fully extended.

**Defensive posture.** When stationary or walking backwards. The animal's antennae and antennules are both raised and moving. The animal's chelipeds are raised 45º from the substrate, but higher than in Stage 2 and in a more aggressive manner (almost a meral spread). The cephalothorax is also raised up on the animal's legs; the legs being more extended than in Stage 3. The abdomen is raised away from the substrate, with the telson curled in underneath.

**Escape response.** Typical tail-flip avoidance behaviour.

## 4.3 Methods

### 4.3.1 Maintenance of fish

Eels and perch were obtained from local fishermen. The two eels obtained were both mature, had been caught in fresh water and measured 74 and 82 cm. The fish were maintained in constantly aerated water in separate tanks in the laboratory. The water was changed twice a week, and the fish were fed with fish pellets once a week. The fish were kept in the laboratory between 4th April and 22nd April, 2002, then returned to the watercourse where they were caught.

### 4.3.2 Bioassay design

A flow-through system was used to test the effects of water conditioned by the fish on the different genders and ages of crayfish. Water flowed from the tank containing the fish via a peristaltic pump into a visually isolated tank containing the test crayfish. This tank then flowed to waste. The crayfish was left to acclimatise in the tank for approximately an hour. The control used was water from a tank that contained no fish.

The behaviour of the crayfish was observed for 10 minutes before the pump was turned on. Water was pumped from the tank containing the fish into the test tank over a 10-minute period, and the reaction of the crayfish was recorded. The behaviour of the crayfish was measured on a 5-point scale (Section 4.2). The animal's behaviour was recorded every minute for the 10-minute period, then averaged for analysis.

The data from before the introduction of the test water were compared to that displayed during the introduction of the water using a Wilcoxon signed-rank test. This determines whether the median of the differences between the observations from before and after the observations are different from zero. Both male and female adults and juveniles were tested. However, no discernable difference was observed in the behaviour of the males and females, so the results were pooled for the different genders. The results from the tests using perch have been omitted as the crayfish showed no response. In all cases the experiment was repeated 20 times.

## 4.4 Results

Figure 24 shows that the juvenile *P. leniusculus* had a significant reaction to water that had contained eels in comparison to before the introduction of the test water (W=0.0, P=0.000), in three cases the juveniles exhibited a tail-flip escape response to the introduction of the water (Category 4). While juveniles show no behavioural response to blank water (W= 4.0, P=0.789). Adults showed a significant difference between before and after the introduction of the test water (W=0.0, P=0.002). However, the maximum behavioural category exhibited during the test was 1. No significant difference was observed in the adult control (W=4.0, P=0.888).

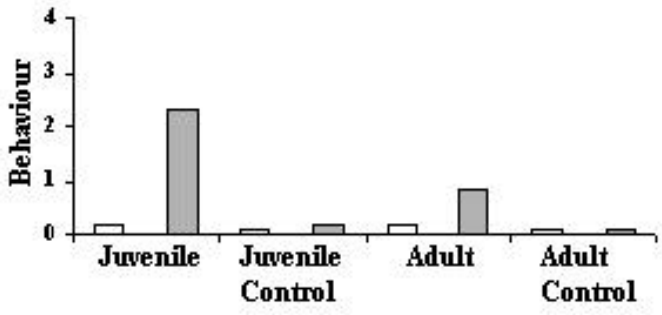


**Figure 24.** Mean behavioural category observed in juvenile and adult *P. leniusculus* before (white) and after (grey) the introduction of water from a tank containing eels ( 'juvenile' and 'adult') or blank water ( 'juvenile control' and 'adult control'). N=20 for each category.

## 4.5 Discussion

Both juvenile and adult *P. leniusculus* responded to the chemical presence of eels, but not to perch. The lack of response to perch may have been due to the size of the fish or their lack of health. The juveniles showed a range of response to the eel test water (from 1 to 4), while the adults only showed the neutral posture (Category 1). In other words, more mature animals than juveniles became alert with the introduction of the test water, while the juveniles showed the range of avoidance behaviour. The results suggest that juveniles show a strong behavioural response to eels due to a potential predation risk that must be avoided, while adults become more alert and adopt the neutral posture. This suggests that eels do not pose a direct predatory risk to adults, but still produce a response.

# 5. Stress and alarm pheromones

## 5.1 Introduction

Zulandt-Schneider and Moore (2000) divided repellent chemical signals into three context-specific categories: chemicals released from a repellent stimulus (avoidance chemicals), chemicals released from damaged conspecifics (alarm chemicals), and chemicals released from stressed but undamaged conspecifics (stress chemicals). Avoidance chemicals, such as those released from a predatory fish, were discussed in Section 4. In this section the potential use of stress and alarm chemicals by *P. leniusculus* is examined.

## 5.2 Methods

The experiments used to test for the presence of stress and alarm pheromones were very similar to those described in Section 4, but with some small differences. Instead of predatory fish being the source of the chemical, adult male crayfish were used. They had been stimulated to release chemicals in two different ways:

1. Ten animals in the source tank were continually agitated by simulated prey chase (the animals were chased around the aquaria using an aquarium net) for the 10-minute period that the water was being introduced into the test tank.

2. Ten animals were stimulated to release alarm chemicals by cheliped ablation at the beginning of the 10-minute introduction period.

The control used in this experiment was blank water. The behaviour of the animal was recorded and analysed using the same scale and in the same manner as in Section 4.

## 5.3 Results

Both adults and juveniles responded to water conditioned with 'stressed' animals (Figure 25). A significant difference was seen in the behaviour of the juveniles from before and after the introduction of the test water (W=0.000, P=0.000), while there was no significant difference in the control (W=28.5, P=0.722). The highest behavioural category exhibited by the juveniles after the introduction of the test water was 4 (escape response), with a mean of 0.155 before the introduction of the test water and 2.02 after. The adults also showed a significant increase in their behavioural-response score with the introduction of the test water (W=0.000, P=0.000), with no difference in the control (W=28.5, P=0.722). The mean behavioural response score before the introduction of the test water was 0.09 and 1.425 after, while the highest behavioural category exhibited by the adults was 3 (defensive posture).

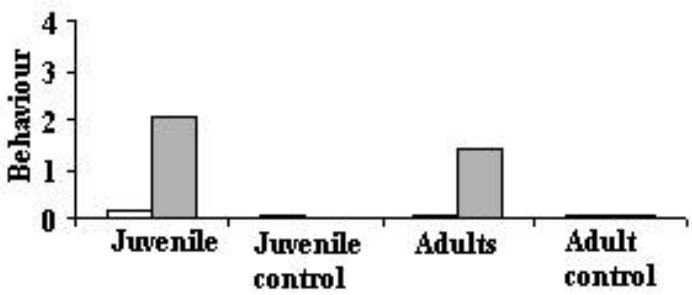


**Figure 25.** Mean behavioural category observed in juvenile and adult *P. leniusculus* before (white) and after (grey) the introduction of water from a tank containing 'stressed' male *P. leniusculus* ( 'juvenile' and 'adult') or blank water ( 'juvenile control' and 'adult control'). N= 20 for each category.

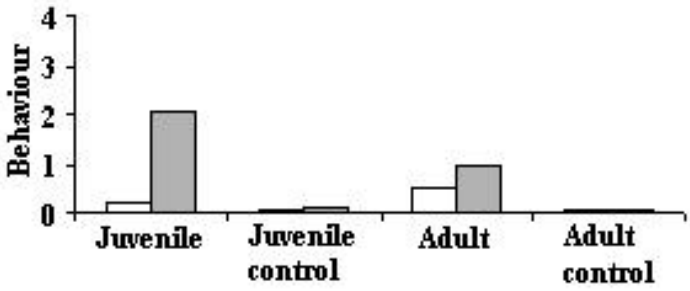


**Figure 26.** Mean behavioural category observed before (white) and after (grey) the introduction of water from a tank containing 'alarmed' male *P. leniusculus* ( 'juvenile' and 'adult') or blank water ( 'juvenile control' and 'adult control'). N= 20 for each category.

Figure 26 shows a similar pattern of response by both juveniles and adults to alarmed crayfish as they did to stressed crayfish. With a significant difference being seen in the response of juveniles (W=0.000, P=0.000) and adults (W=0.000, P=0.000) to the test water. While no significant difference was seen in the response to the control water by either the juveniles (W=21.0, P=0.541) or the adults (W=36, P=0.845). The highest behavioural response of the juveniles was again 4 (with a mean of 2.065) and 3 for the adults (with a mean of 1).

## 5.4 Discussion

Both stressed and alarmed animals stimulated a significant increase in avoidance behaviour in the test animals. In both cases, juveniles showed a stronger response than the adults. This was expected, as juveniles are a lot more vulnerable to predation and other risks than adult *P. leniusculus*.

Although the juveniles did not differentiate between the stress and alarm test water, the adults showed a markedly lower response to the alarm water than to the stress water. This may be because the stress pheromones were continually released by the animals in the source tank (due to continuous agitation), while the release of the alarm pheromone was probably episodic, with the cheliped ablation taking place at the beginning of the 10-minute test period, so therefore not persisting in the water due to the flow-through design of the system.

# 6. Feeding attractants

## 6.1 Introduction

Studies have been carried out to examine how crustacea respond to odours that could signal energy and nutrient properties of food (Zimmer-Faust, 1987). Free amino acids are abundant in living prey, but they diffuse rapidly from carrion, being assimilated by bacteria that degrade them to ammonia. The ratio of amino acids to ammonia decreases with increasing carrion age, which is thought to be an indicator of the nutritional or nitrogen value of the food, and can be detected by an approaching animal (Zimmer-Faust, 1987). To date, the majority of decapods studied have been carnivores, which respond strongly to amino acids and proteins (Mendoza *et al.*, 1997; Wellins *et al.* 1989), but not to sugars, alcohols, starches and fatty acids. Crayfish, however, are omnivorous and so respond to both amino acids and chemicals more usually associated with plant matter (Huber *et al.*, 1997b). In this study whole food items suitable for use in crayfish traps were tested for their level of preference.

## 6.2 Methods

A two-chambered flow-through choice chamber was set up. An adult male crayfish that had been starved for one week was placed into the outer chamber (a different animal was used for each experiment), while two food types were placed one at a time in each choice chamber. The chamber in which the animal was in after 15 minutes was recorded and assumed to contain the preferred food item.

Each food item was tested against 10 other food types, and the results were analysed using a general linear model (see Section 3.2). The apparatus was tested to confirm that there was no preference between chambers by placing the same food item into both chambers, and also alternate testing of the same two food items in different chambers each time. The food items tested were:

- Potato
- Fish (muscle): perch
  smoked mackerel
  trout
- Tinned ham
- Cat food
- Carrageenan
- Phytagel
- Crushed conspecific.

## 6.3 Results

**Table 1.** Feeding preference of *P. leniusculus*. The arrows indicate if the food type on the left is preferred (>) over those listed along the top, if the food type along the top is the preferred of the two (<), or if there is no preference for the food types (no diff).

| | **Potato** | **Perch** | **Mackeral** | **Trout** | **Ham** | **Cat food** | **Carrageenan** | **Conspecific** | **Phytagel** |
|---|---|---|---|---|---|---|---|---|---|
| **Potato** | **xxxx** | < | < | < | < | < | no diff | < | no diff |
| **Perch** | > | **xxxx** | no diff | no diff | no diff | no diff | > | no diff | > |
| **Mackeral** | > | no diff | **xxxx** | no diff | no diff | no diff | > | > | > |
| **Trout** | > | no diff | no diff | **xxxx** | no diif | no diff | > | no diff | > |
| **Ham** | > | no diff | no diff | no diff | **xxxx** | no diff | > | no diff | > |
| **Cat food** | > | no diff | no diff | no diff | no diff | **xxxx** | > | no diff | > |
| **Carrageenan** | no diff | < | < | < | < | < | **xxxx** | < | no diff |
| **Conspecific** | > | no diff | < | no diff | no diff | no diff | > | **xxxx** | > |
| **Phytagel** | no diff | < | < | < | < | < | no diff | < | **xxxx** |

From the results above it can be seen that mackeral was the preferred choice. Potato, carrageenan and Phytagel were the least preferred of the food items tested. Ham, trout, cat food and perch were more attractive than potato, carrageenan and Phytagel, but less attractive than mackerel.

## 6.4 Discussion

The fact that the plant-based products (potato, carrageenan and Phytagel) were less attractive than the protein-based products (ham, trout, cat food, perch and mackerel) suggests that the food items that offer the highest energy gain are preferred. Maximising energy gain while minimising energy loss is a key feature of the optimal foraging theory, which can easily be applied to crayfish.

However, the results could be interpreted as the food item that releases the most attractive smell, which disperses the quickest, is the most preferred choice. Mackerel is a very oily fish, and the oil would spread quickly through the water, eliciting a response in the animal before the other food items.

Nevertheless, it is clear that protein-based food items are more attractive, and the more oily they are the better.

# 7. Releaser mechanisms with flume tests

## 7.1 Introduction

Once it had been established that *P. leniusculus* released pheromones, methods of translating the lab-based studies to the field were required. To test the effect of the pheromones on trapping success, a method of releasing the chemicals throughout the trapping period in a natural environment was required. Several gel-based slow-release matrices were considered. The aim of the following experiments was to find a matrix that would release a chemical into water at a steady rate over 24 hours. Because of structure of the pheromones to be released, the matrices were tested in two ways: by using a dye to measure the release rate, and using animals to measure whether the pheromone was being released. Several matrices were tested in the lab for release rate and attractiveness to crayfish (see Section 6).

## 7.2 Methods

### 7.2.1 Gel matrices

Phytagel, carrageenan and alginic acid gels were mixed with 1 ml of black food dye. These were placed in a crystallisation dish with 200 ml of distilled water on a rocker table. After 0, 1, 2, 3, 4, 5, 6, 7, 14 and 28 hours, 100 µm of water was taken from the dish. The optical density of the sample was recorded using a micro-plate reader. This was then compared to a serial dilution of the dye and the percentage release from each gel matrix was recorded for each time. This was repeated 8 times for each gel and the mean value for each time was calculated.

### 7.2.2 Pheromone release

Five hundred ml of water conditioned with pheromone was freeze-dried (this gave approximately 0.1 g of residue). The residue was mixed with Phytagel. The release of the pheromone tested in previous sections was carried out in a flume flowing to waste. The gel containing the pheromone was placed near the inflow of the flume. An actograph, which allows the recording of the position of the animal in the tank via light beams, was used to monitor the position of the animal in the flume relative to the Phytagel containing the

pheromone over a 24-hour period. Sensor 1 was furthest away from the inflow (near where the animal was placed), while Sensor 8 was nearest to the inflow and the gel.

Only adult males were tested in the flume. The flume was run for two hours before the male was introduced into the system, then left for another hour to allow the male to acclimatise before the experiment began. The sex pheromone was tested during the breeding season, while the stress and alarm pheromone(s) were tested outside the breeding season.

## 7.3 Results

Figure 27 shows the release rate of the gels (Phytagel, carrageenan and alginic acid) tested. Alginic acid gel showed a negligible release rate. This is not surprising, as it is normally used to immobilise enzymes. Phytagel and carrageenan both showed almost linear release rates over the test period, although Phytagel released pheromones quicker than the carrageenan. Phytagel also released more over the test period, a total of 13.25% of the dye being released over the 24-hour period, in comparison to 4.55% released by the carrageenan. The carrageenan was also found to be attractive as a food source (Section 6), so not suitable to test the pheromone(s) with.

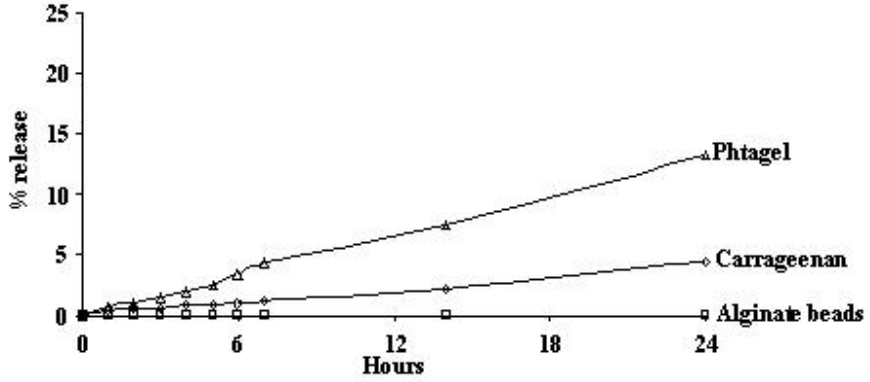


**Figure 27.** Percentage release rate of dye for each gel matrix tested over a 24-hour period.

Figure 28 shows the mean (N=8) recording of the effect of sex pheromone released from Phytagel during the breeding season on a mature male over a 24-hour period. On average the males moved towards the gel within the first hour of testing and remained close to the gel for approximately the first three hours of the testing. After this time the males began to wander away from the gel, but still returned to it. The males spent the majority of the time in the section of the tank closest to the gel.

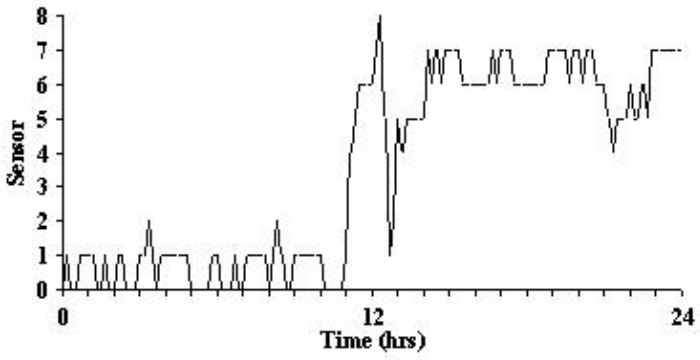


**Figure 28.** Mean response (N=8) of adult males to Phytagel containing freeze-dried sex pheromone. Sensor 1 was where the animal started (at the outflow) and Sensor 8 is where the gel was placed at the inflow. Breaks in the sensor beams were recorded and the movement of the animal in the tank recorded over a 24-hour period.

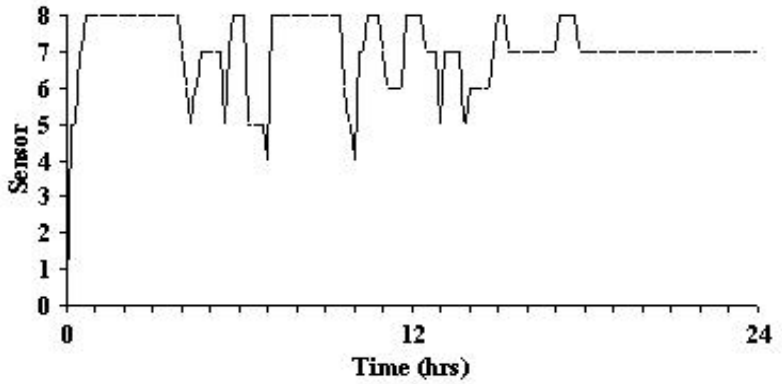


**Figure 29.** Mean response (N=8) of adult males to Phytagel containing freeze-dried stress pheromone. The animal started (at the outflow) at Sensor 1, and the gel was placed at the inflow (Sensor 8). Breaks in the sensor beams were recorded and the movement of the animal in the tank recorded over a 24-hour period.

From Figure 29 it can be seen that the male crayfish remained away from the gel releasing the stress pheromone for almost 12 hours at the start of the experiment. However, after this period the males moved towards the source and remained in that end of the tank for most of the rest of the test period, with occasional movement away from the gel.

The males responded in a similar fashion to the alarm pheromones as they did to the stress pheromones (Figure 30). Most of the first 12 hours of the test were spent away from the gel, and the second half of the test was spent mainly near the gel or moving about the tank showing little response to the pheromones.

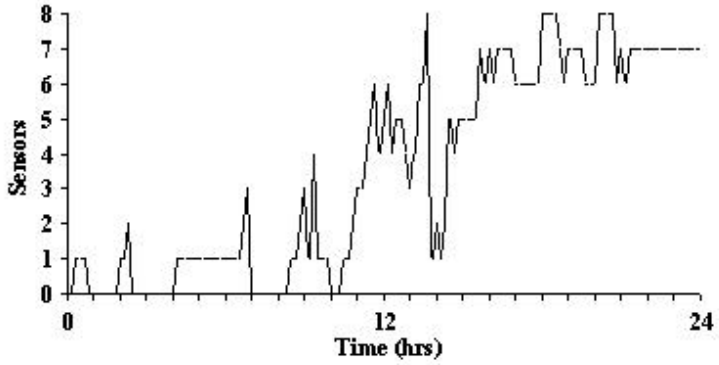


**Figure 30.** Mean response (N=8) of adult males to Phytagel containing freeze-dried alarm pheromone. Sensor 1 was where the animal started (at the outflow), while Sensor 8 was where the gel was placed at the inflow. Breaks in the sensor beams were recorded and the movement of the animal in the tank recorded over a 24-hour period.

## *7.4 Discussion*

The flume trials showed that the Phytagel released all three of the pheromones tested. However, the stress and alarm pheromones only seemed to have had an effect on the test animal for approximately 12 hours. This may have been due to the gel releasing the chemicals quicker than was suggested by the retention times of the Phytagel (Figure 27), or the animals may have become acclimatised to the chemical afterit had broken down. The fact that the animals moved towards the gel after this time may suggest that the reduced concentration of the chemical may have indicated the presence of damaged conspecifics and a possible source of food.

# 8. Field trials

## 8.1 Introduction

The lab-based trials of the pheromones embedded into the Phytagel showed that the animals did respond as was expected. Although the repellent pheromones only seem to function for the first 12 hours of the flume tests (see Section 8) they were still used in the field trials, as there would be comparatively fewer animals in these traps than in the controls.

## 8.2 Methods

Methods of translating the lab-based studies to the field were the next stage in the the project. In the field, crayfish traps are normally left out for a minimum of 24 hours. Pheromone(s) were fixed into Phytagel as described in Section 8 and used on the same day. The pheromone(s) were field-tested using standard Swedish 'trappy' traps, which were left out for 24 hours. Traps were baited with either:

- Sex pheromone water.
- Stress or alarm pheromones with an attractant (food bait), which allowed the testing of the repellents against a known and quantified attractant.
- Food (in most cases approximately 25 g of trout).
- A blank gel matrix (as a control for the sex pheromones and repellents).

Trapping took place all year round (though sex pheromones were only tested during the breeding season) at two field sites; the River Clyde in Scotland and Lartington Ponds in Teesdale, North Yorkshire. These sites were chosen so that both lentic and lotic systems could be tested. Habitat data were collected from the sites to help determine whether differences in available habitat influenced numbers caught. As the data collected so far are only preliminary, the results from the River Clyde and Lartington ponds have been pooled (N=12 for each treatment). The number of males and females in each treatment and the total number of animals in each treatment were compared using a Mann-Whitney U-test at the 95% significance level.

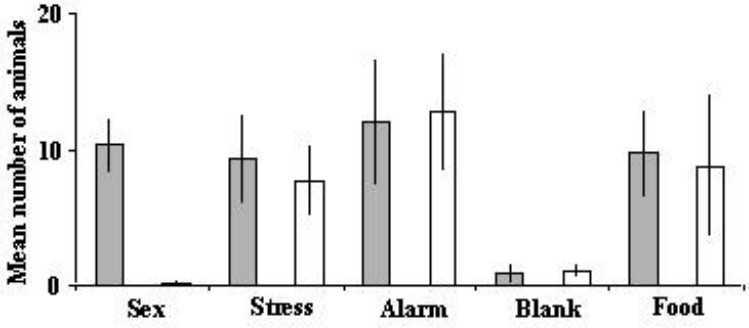


**Figure 31.** Mean number of male (grey) and female (white) *P. leniusculus* caught in each treatment (number of traps deployed for each trial =12), with 95% C.I.

## 8.3 Results

Figure 31 shows that the sex pheromone-baited traps (SPT) caught significantly more males than females (males vs. females, W=222.0, P=0.000), with an average of 10.25 males being found in each trap compared to 0.167 females. There were significantly more animals, in total, in both the stress and alarm traps than in the SPT (sex vs. stress, W=104.5, P=0.0092; sex vs. alarm, W=97, P=0.0024). Significantly more animals were found in the SPT than in the blanks (sex vs. blank, W=222.0, P=0.0000), although no significant difference was seen in the number of animals in the SPT when compared to the food-baited traps (sex vs. food, W=117.0, P=0.0598). There were no significant differences, however, in the number of males in the SPT when compared to the stress-, alarm- or food-baited traps, the significant differences in total numbers of animals being caught in the stress- and alarm-baited traps being due to the number of females.

No significant differences were seen in the number of males compared to females for either the stress- or alarm-baited traps. There was also no significant difference in the number of total animals found in either of the treatments (stress vs. alarm, W 123.0, P 0.1251). This was also the case when the total number of animals found in the stress- or alarm-baited traps were compared with the food-baited traps (stress vs. food, W 150.5, P 1.0000; alarm vs. food, W 175.5, P 0.1482). Significantly more animals were found in both the stress and alarm pheromone-baited traps than in the blank traps.

## 8.4 Discussion

Preliminary results suggest that the sex pheromone-baited traps were effective at trapping male *P. leniusculus* during the breeding season. Although the sex pheromone traps did not appear to be any more effective than food-baited traps, following purification and concentration of the sex pheromone(s), the success rate of the traps could be improved. The only reason for the significant difference in the number of animals caught in the stress- and alarm- (and most food-) baited traps was due to the fact that they were attracting females and males, whereas the SPT did not attract females.

The present design of the traps and level of purification could potentially be used to identify populations of a low density. However, field trials to test the pheromones in this capacity have not been undertaken. Improvements could be made on the releasing mechanism once more is known about the nature of the chemicals involved, and this could also increase the effectiveness of the traps.

The lack of success at repelling animals from traps using the stress or alarm pheromones may be due to the design of the field experiments rather than the chemicals being tested. It is possible that the attractiveness of the food being placed into the traps was a stronger attractant than the repellent was a deterrent, so even though the crayfish may have been detecting the repellent pheromones, the food over-rode their effect. This is supported by the fact that there was no significant difference between the numbers of animals found in the stress- and alarm-baited traps and the food-baited traps.

Field trials are continuing, with more repellents in each trap and less food, but also using shelter traps instead of food-baited traps, the idea being that the shelter is less of an attractant than the food.

It should be noted that the habitat data were not taken into account when analysing the data presented in this paper. With the inclusion of the habitat data into the analyses, a clearer picture could be obtained of the true effectiveness of the pheromones tested.

# 9. Proposed strategy

## 9.1 Proposed strategy

From the work carried out to date it is clear that *P. leniusculus* utilises certain pheromones in a number of circumstances that require conspecific communication. The work so far has demonstrated that mature female *P. leniusculus* release a pheromone during the breeding season that stimulates courtship and mating behaviour in conspecific adult males. Stressed and alarmed adult *P. leniusculus* have also been demonstrated to release pheromones that stimulate avoidance/alarm behaviour in both juvenile conspecifics and adults to a lesser degree.

It has been shown that juvenile (and to a lesser extent adult) *P. leniusculus* demonstrate avoidance behaviour when exposed to water conditioned by adult eels. Certain food types have been seen to be more attractive than others under laboratory conditions. A release mechanism for the pheromones tested has been developed and tested in semi-natural and natural conditions with varying degrees of success.

How can the work to date be translated into a suitable control mechanism? The most revealing data to examine when trying to answer this question are the preliminary field trials (see Section 9.0). An indication is given from these experiments that the pheromones could potentially be used to control populations of *P. leniusculus*. Despite the lack of evidence for the stress and alarm pheromones actually repelling individuals, the sex pheromone tested attracted male individuals. Even though the control traps baited with mackerel trapped more animals than the pheromone traps, with purification of the chemical the traps could potentially be as effective, if not more so. Although the traps are by no means a solution to the current problem of non-native crayfish species, and could not be used for eradication, they still could potentially used in a number of circumstances:

1. The pheromone traps could be used to remove a large proportion of the breeding males in a heterogenous population.

2. The traps could be used to record the presence or absence of a population in a waterway.

3. Only male *P. leniusculus* could be removed from mixed *A. pallipes*/*P. leniusculus* populations.

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