Burrowing Behaviour and Movements of the Signal Crayfish *Pacifastacus leniusculus* (Dana)

A thesis submitted for the degree of Doctor of Philosophy

By

Jeama Amanda Stanton
Department of Biology
University of Leicester

2004

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

Abstract

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The major burrowing characteristics leading to, during and after burrow construction are described. Burrow initiation was significantly correlated to crayfish size; smaller individuals beginning construction more quickly. Field burrow morphologies, examined using an optic cable video camera, showed 92% to be simple with only a single opening (Length range 3.5 – 79.0 cm).

Significant associations were found between the clay/sand content of stream bank sediments and crayfish burrow densities. Substrate selection experiments indicated a significant preference for artificial shelter over burrowing in clay for adult crayfish, and a significant preference for clay and artificial shelter over mud or gravel in juveniles.

The rate of range extension of *P. leniusculus* along the Gaddesby Brook shows polynomial expansion i.e. the speed of new habitat colonisation is increasing each year. Juveniles, on the basis of burrow sizes and movements made by adult crayfish, are mainly responsible for this colonisation.

Measurements of burrow water O_2, CO_2, ammonia and pH were made and showed slightly hypoxic conditions and elevated levels of ammonia in occupied burrows. Burrow irrigation rates were examined with average turnover rates being 14.8 l h^{-1} for adults (mass range = 31.7 - 117 g).

Crayfish movements were monitored by means of radio tracking. Results indicated that activity was greatest during and immediately following dusk and that crayfish activity was significantly less in winter than summer. Most individuals were position-fixed at the same burrow/shelter for the duration of radio tracking, a few made occasional large movements between stationary phases of between 2-8 days. The maximum distance recorded by any individual in one night was 89.6 m. During two flood events, all tagged animals maintained their pre-flood positions. Abdominal tags used to measure longer-term movements (over 2 years) gave an overall recapture rate of 19.0% (51 from 268) and generally showed that adult *P. leniusculus* remained in the same vicinity for in excess of 2 years.

Hopefully this study will help in the control and management of *P. leniusculus*. For example, forecasts on preferred sites for population expansion and identification of sites vulnerable to bank damage can be deduced from substrate preferences. Furthermore, information on burrow construction, behaviour and irrigation rates may be relevant in biocide application and assessing the effects on bank stability. A knowledge of crayfish movement and activity is important for predicting time scale of spread and colonisation of new habitats.

Acknowledgements

Thank you to Dr. Bob Harris for his vast experience, advice and unerring support on fieldwork, laboratory work and the writing of this thesis. My grateful thanks must also go to all those in the Biology Department of the University of Leicester who have helped with this work, especially Maurice Andrews.

Thanks also to NERC for their support over the years and to Peter Sibley of the Environment Agency for his help, support and the provision of a radio receiver and transmitters for animal tracking.

To Mr. and Mrs. Smith and Professor and Mrs. Lamming, thank you, for granting access to your land in order to carry out fieldwork (even at the most unsocial hours!)

Final thanks must go to my family and friends for their understanding, forbearance and unstinting support, without which this thesis could not have been realised.

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1.1 General Introduction

Crayfish are arthropod crustaceans of the class Malacostraca and Order Decapoda. They inhabit mostly freshwater habitats, for example, rivers, lakes and ponds. Higher salinity is tolerated by some species, but a real marine environment is never inhabited (Scholtz, 1999).

There are more than 540 recognised crayfish species (Holdich, 2002b), divided into two superfamilies:

1. Astacoidea, which occur in the Northern Hemisphere and consist of two families, the Astacidae and Cambaridae.
2. Parastacoidea, which occur in the Southern Hemisphere and consist of one family, the Parastacidae.

Crayfish are naturally absent from the Antarctic and African continents (except Madagascar), the Indian sub-continent and the northern parts of central Asia (Holdich, 2002b). In these regions, the predominant freshwater decapod crustaceans are brachyuran crabs. Crayfish have been frequently transported by man, a common practice from as early as the Middle Ages (Laurent, 1988), and it was found that certain crayfish species when moved outside their home range, quickly established themselves in new areas (Holdich and Gherardi, 1999).

The only species native to the British Isles is the white-clawed crayfish, *Austropotamobius pallipes* (Holdich *et al.*, 1990), which is widespread in England and parts of lowland Wales. It prefers either running or standing clean water and is absent in areas with naturally acidic water or weather-resistant rocks, for example, Cornwall and the Lake District (Laurent, 1988).

In the 1970's, *Pacifastacus leniusculus*, the signal crayfish, was introduced into Britain for culinary and aquacultural purposes (Lowery and Holdich, 1988; Richards, 1983).This species originated from north-western North America between the Pacific Ocean and the Rocky Mountains (Hobbs, 1988; Lowery and Holdich, 1988). Unlike A. *pallipes*, *P. leniusculus* was found to be more suitable for commercial farming, as it grew rapidly, reached a larger fully-grown size and could sometimes reach a marketable size in two years (Alderman and Wickins, 1996).

There were other advantages for farming *P. leniusculus.* It was highly adaptable. It could occupy both clean and brackish water, survive high salinity as much as 20 ppt (Rundquist and Goldman, 1978) and acclimatise itself to a wide range of temperatures up to 33^0C (Becker *et al.*, 1975).

By 1983, there were over two hundred and fifty commercial crayfish farms of varying sizes throughout the country. The industry proved profitable; there was a virtually guaranteed market, with the additional advantages that the breeding stock could be kept out-of-doors with minimum outlay and low maintenance costs (Marren, 1986).

By the mid 1980's, however, the crayfish market collapsed in Britain and ponds and lakes containing signal crayfish stocks were left neglected. Many of them relied on a water source from small streams or springs (Harris, 1999). Not surprisingly, these very mobile animals, which are capable of climbing obstacles and walking large distances out of water, were able to escape into nearby waters. Once in the rivers, it was found that they altered aquatic ecosystems by destroying plant life, invertebrate communities and fish populations and by burrowing and weakening riverbanks (Alderman and Wickins, 1996).

After *P. leniusculus* was introduced into Britain it was also discovered that it was the vector for crayfish plague (Alderman, 1993). This is caused by the fungus *Aphanomyces astaci,* which signal crayfish carry naturally in their tissues, but are immune to its effects unless under extreme stress (Smith and Soderhall, 1986). Britain's native crayfish *A. pallipes* is, however, susceptible to the fungus.

The fungal hyphae grow through the shell to invade the animal's muscles, causing a melanisation of the exoskeleton. Infected crayfish show abnormal behaviour. For example, although normally nocturnal, they wander about the stream in broad daylight and appear disorientated. Later, dying animals are found lying on their backs with brown patches on their carapace, indicative of the presence of the plague (Marren, 1986).

The fungal spores were found to be transmittable through water (Cerenius *et al.*, 1988). The first confirmed outbreak of the plague in Britain was in 1981, but it was not until 1983, that the then Ministry of Agriculture, Fisheries and Food (MAFF, now DEFRA), identified the fungus, which has since devastated populations of native crayfish, *A. pallipes,* particularly in southern England (Marren, 1986).

In the 1980's, three Acts of Parliament were passed, which were intended to prevent or contain the spread of crayfish plague in Britain. These were, *The Import of Live Fish Act 1980, The Animal Health Act 1981* and *The Diseases of Fish Act 1983.* At the time, there was a reluctance to enforce these laws, and, by 1984, it was considered that any restrictions imposed to prohibit imports of live crayfish or to control their movements would be too disruptive to trade (Marren, 1986).

Since then, efforts have been made to try and prevent the further spread of signal crayfish in an attempt to protect *A. pallipes.* In 1985, for example, an order was made that required crayfish farmers to register with the fishery boards, giving details of location, stocks and facilities. Farmers were also required to keep records, which were open to inspection, of all movements of live crayfish to and from their farms. In March 1992, legislation was introduced listing *P. leniusculus, Astacus astacus* and *Astacus leptodactylus* under the Part 1 of Schedule 9 of the *Wildlife and Countryside Act* (1981) (Guan, 1995). These species became subject to controls under Schedule 14 (1) where it is an offence (except under licence) to release into the wild 'any animal of a species not ordinarily in resident in, or not a regular visitor to, Great Britain in the wild state' (Holdich and Rogers, 1992). This was then followed by *The Prohibition of Keeping Live Fish (Crayfish) Order 1996*, making unlicensed keeping of non-native crayfish an offence with the exception of the species *P. leniusculus* kept within certain postcode areas of England and Wales.

1.2 Habitat selection and the use of refuges and burrows

Crayfish can live in lentic and lotic waters, in environments that are subterranean and semi-terrestrial, as well as brackish waters. Both physiologically and behaviourally, they are very adaptable (Holdich, 2002b).

All freshwater crayfish show burrowing behaviour to some extent, given the need to do so, that is, when there are no natural refuges present (Berrill and Chenoweth, 1982; Horwitz *et al.*, 1985a). Crayfish do, however, require a suitable substrate in which to find refuge or to burrow. Some species, such as, *P. leniusculus* and *A. pallipes* tend to favour slower-flowing waters coupled with adequate conditions for shelter, like tree roots, stones or sediment banks in which to burrow (Nystrom, 2002; Peay and Rogers, 1999). Other species, such as, *Cambarus diogenes diogenes* and *Engaeus leptorhyncus,* live in burrows which are not directly connected to any surface water body. Instead the burrows extend to below the water table (Grow and Merchant, 1979; Horwitz *et al.*, 1985b).

The most vulnerable periods for the crayfish, regarding predation, are, the juvenile stage, particularly between hatching and finding a suitable 'safe' refuge (Blake and Hart, 1993) and the immediate post-moult period, particularly when food is scarce, which can affect the frequency of cannibalism (Dong and Polis, 1992).

In the study by Alberstadt *et al.* (1995), on cover-seeking behaviour of the juvenile and adult crayfish, *Orconectes rusticus*, thigmotactic cues (tactile stimulation) and the effect of darkness were found to be of prime importance. This was supported by Antonelli *et al.*, (1999), who observed that for both juvenile and adult *Procambarus clarkii,* darkness appeared to be the controlling factor in their cover seeking-behaviour.

Crayfish distribution can also be related to temperature (Claussen, 1980; Crawshaw, 1974; Kivivuori, 1977). *Orconectes causeyi*, for example, was found to actively seek shelter, but only within optimum thermal areas. This species was sensitive to a range of temperatures, establishing a thermal selection index (14 – 29°C) (Loring and Hill, 1976).

Thus, there is evidence that, in some instances, choice of habitat does occur (Partridge, 1978), but because some species are extremely invasive and competitive (Holdich and Gherardi, 1999) some individuals, due to either interspecific or intraspecific agonistic encounters and/or the effects of predation, are excluded from preferred habitats and thus forced to live in less suitable areas (Gherardi, 2002).

To fully understand the patterns of distribution and spread of the species *P. leniusculus*, it is important to have an understanding of their substrate preferences. Although, work has been carried out to determine substrate choices in crayfish (Klosterman and Goldman, 1983; Vorburger and Ribi, 1998), research has been limited, and has not examined how preferences of habitat may be affected when animals are in competition with one another.

Many species of crayfish have been observed to burrow, particularly in the family Cambaridae (Abbott, 1884; Berrill and Chenoweth, 1982; Bouchard and Etnier, 1979; Capelli, 1980; Creaser, 1931; Girard, 1852; Grow, 1982; Hasiotis, 1993a, 1993b; Huner, 2002; McManus, 1960; Payne and Price, 1981; Rogers and Huner, 1985; Tarr, 1884; Williams *et al.*, 1974). In addition, the families Astacidae, (Guan, 1994; Holdich, 2002a; Stanton and Harris, 2003) and Parastacidae (Horwitz and Richardson, 1986; Horwitz et al.,

1985a; Horwitz et al., 1985b; Richardson, 1983; Richardson and Swain, 1980) have been reported to show burrowing behaviour.

Although there is a positive correlation found between the abundance of non-burrowing crayfish and the abundance of refuges (Lodge and Hill, 1994), it is interesting to note that non-burrowing species will occasionally construct burrows when necessary. For example, Berrill and Chenoweth (1982), observed that burrowing occurred when river or lake waters disappeared and Guan (1994) found that in the British Isles, when appropriate substrate was available, the species *P. leniusculus,* a non-burrowing species in its native habitat, burrowed extensively.

For most burrowing animals the primary role of a burrow is for protection, be it from predators, competitive rivals or severe weather conditions. However, some burrows can serve more than one purpose. For example, Karnofsky and Price (1989), observed that burrows of *Homarus americanus*, the common lobster, not only offered protection, but were also used for food storage and to aid food harvesting. Horwitz *et al.*, (1985a), found that *Engaeus leptorhyncus*, constructed burrows with large chambers and that the juvenile crayfish grew within the parental burrow. Unlike other crayfish the juveniles were unable to move at an early age to a new burrow, as they could not make their way easily into a nearby water body.

North American burrowing species of crayfish have been classified into three categories (Hobbs, 1981):

1. Primary burrowers – for example, *Procambarus hagenianus*. Crayfish that spend almost their entire life in complex burrow systems, below the surface of the ground, rarely linked with open water.
2. Secondary burrowers – for example, *Procambarus clarkii*. Crayfish that excavate simple burrows, which they remain in for most of their life. However, they frequently move to open water when the water table rises, usually in the rainy season.
3. Tertiary burrowers – for example, *Orconectes causeyi.* Crayfish that only burrow in winter, during drought conditions and in some cases during the breeding season. The burrows are usually simple tubes extending 1-2 m into the substrate with one opening.

The burrows of Australian crayfish have also been divided into three types (Horwitz and Richardson, 1986). Type 1 burrows are found in, or directly connected to, open water; Type 2

burrows are connected to the water table and Type 3 burrows are independent of the water table.

Burrow morphology is usually determined using a variety of casting methods, including foam, plaster and concrete, and can be excavated by either digging or by the use of high pressured water (Guan, 1995; Lawrence *et al.*, 2002). The burrows of some crayfish species, particularly those present in stream banks such as *P. leniusculus* and *Orconectes immunis,* can cause serious erosion. Burrows, in the first instance, weaken the bank and if this is followed by a small amount of water table fluctuation, whole stream or river banks can be undermined (Hasiotis, 1993a).

Holdich and Rogers, (1992), found that in a river in Buckinghamshire, banks had collapsed under the weight of grazing cattle, which may have been the direct result of the burrows of *P. leniusculus*. The extent of the damage caused by burrowing has not as yet been quantified. The gathering of these data is important for, as the species *P. leniusculus* spreads, it may eventually reach areas at risk of flooding and its burrowing could not only escalate erosion, but also detrimentally affect any defence systems in place.

Irrigation is also an important feature of burrow dwelling crustaceans. This is the process of creating water circulation between burrow water and external water, with the purpose of exchanging potentially toxic water (e.g. hypoxic or high in ammonia) with more favourable water. A common and well-studied initial reaction to compensate for hypoxic conditions in the environment, is to increase ventilation rate (McMahon, 2001; Taylor and Wheatly, 1980). This behaviour in crustaceans creates a water current in burrowing species which helps to exchange burrow water with external water. Normal ventilation achieves the same results but to a lesser extent.

Another method is passive irrigation. This can either be due to induced water flow over burrows with two openings at different levels, as employed by a thalassinidean shrimp, *Jaxea nocturna* (Pervesler and Dworschak, 1985), or by water exchange as a result of externally flowing water. A less investigated process of irrigation is that created by the beating of abdominal pleopods or swimmerets. The current produced allows water with higher oxygen levels, to reach the ventilating animal (Gerhardt and Baden, 1998).

It is not known whether the species *P. leniusculus* irrigates its burrows, but if it does, it would be interesting to understand when and how burrow irrigation takes place. The process of irrigation may well influence its behaviour due to possible energy cost implications.

1.3 Movements and Activity Patterns

Crayfish movements and activity patterns are crucial in the understanding of habitat requirements, colonisation of new areas and use of essential resources, such as, food, shelter and accessible mates.

Crayfish are capable of making substantial active movements. They can live on land for several days and have the ability to walk forwards, backwards and sideways both on land and in water (Pond, 1975). Their powerful tails also help to propel them backwards in water. These 'tail flips' (repeated abdominal contractions), are used predominantly as an escape mechanism (McMahon, 2002).

Crayfish movements and activity can be affected by temperature, light, food and the presence of predators. Gherardi *et al.* (2002a), found that a decrease in air temperature, water level and day length, increased burrow occupancy, thus reducing locomotory speed of *Procambaras clarkii.*

Both the signal and noble crayfish were more active during the night (Abrahamsson, 1983). Merkle (1969), found that *Orconectes juvenalis* seemed to be more active on dark nights, cloudy days or in muddy water. In some species, movement was related to seasonal events. For example, *P. leniusculus* was observed to reside in shallow water in Lake Tahoe during summer and autumn, while in late autumn moved to deeper waters, possibly to avoid winter storms (Flint, 1977). Abrahamsson (1983), found that dense populations of crayfish were more active and foraged during the day.

Overcrowding, limited shelters, poor environmental conditions, predation and interspecific and intraspecific agonistic encounters can cause crayfish to colonise new areas (Westman, 1973). These have been noted for *O. rusticus* where predation affected its distribution (Hill and Lodge, 1994), *A. astacus* where distribution was limited by acidification, predation and climate in lakes and streams in Sweden (Furst and Eriksson, 1973), and *O. virilis* where

distribution was affected by agonistic behaviour of conspecifics (Levenbach and Hazlett, 1996). Peay and Rogers (1999), reported that the expansion of *P. leniusculus* was intermittent rather than a uniform spread. The reason for this was a reluctance to move into and beyond unfavourable habitats, thus delaying spread until full population capacity was reached. At this point, some individuals were forced to seek new more favourable sites.

Home range is an area in which an individual normally travels for food, shelter and mates. Some crayfish species such as *O. juvenalis,* have been observed to have a home range (Merkle, 1969). However, many crayfish species such as *P. leniusculus* (Bubb *et al.*, 2002a), *A. pallipes* (Gherardi *et al.*, 1998; Robinson *et al.*, 2000), and *P. clarkii* (Ilheu *et al.*, 2003), have been observed to have an ephemeral home range, that is, they live in one place for only a short time and make occasional movements to new locations.

Homing behaviour is when an animal, if displaced, can return to its original place of origin. There is little research in this area and most studies carried out suggested that crayfish species displayed no homing behaviour. For example, *P. clarkii* was shown to have no homing behaviour in either a stream south of the Iberian Peninsula (Ilheu *et al.*, 2003), or in a stream in southern Portugal (Gherardi *et al.*, 2002b). Similarly, *A, pallipes* displayed no homing behaviour in Dalton Beck, North Yorkshire (Robinson *et al.*, 2000).

Various methods of tagging have been tried to investigate movement, territoriality and homing of crayfish, some external and some internal in nature.

Examples of external tags, include, metal labels, glued on discs (Penn, 1943) and streamer tags (attached through the animal). The use of paint and dye applied to the carapace, mutilating the surface of the animal by clipping or punching holes in the telson or uropods (Abrahamsson, 1965; George, 1957, 1958) or branding with a soldering iron (Pratten, 1980), have also been used.

There are a number of limitations to external tagging. The projections were found to restrict shelter-seeking behaviour (Bubb *et al.*, 2002b), there was evidence to suggest that the crayfish were more prone to predators (Weingartner, 1982), and the tags proved only to be semi-permanent as they became indistinct or were lost over time due to moulting (Bubb *et al.*, 2002b). A study by Guan (1997), also suggested that mutilating the surface of the crayfish restricted growth and affected behaviour.

However, there are a number of advantages of external tagging. They are, for example, usually quick and easy to attach and relatively cheap, enabling large numbers of animals to be tagged. Nevertheless, retrieval of tagged individuals is reliant on recapture by hand and traps.

Internal tags are usually inserted into crayfish tissues or body cavities by means of hypodermic needles. The advantages of this technique are, that the tags do not protrude and they are more permanent because they are not affected by moulting. Examples of internal tags include, binary coded tags (Isely and Eversole, 1998), visual implant elastomer (VLE) and visual alphanumeric (Vialpha) tags (Jerry *et al.*, 2001), radio-active tags (Merkle, 1969), colour coded flexible nylon rods (Weingartner, 1982), and PIT tags (passive integrated transponders) (Bubb *et al.*, 2002b).

More recently, there has been an increased use of external radiotelemetry tags. Crayfish can be fitted with individual radio-transmitters, which transmit signals at a unique frequency. This means that individual animals can be differentiated.

The United Kingdom originally had a protected frequency allocation for radio tracking at 102 MHz, but this was later shifted to 104.6-105.0 MHz. Another band was available at 173.20-173.35 MHz. Unfortunately for many biologists, the lower band is being lost to radio broadcasting. In compensation, a further band has been allocated at 173.70-174.00 (Kenward, 1987).

For use on crayfish, these transmitters are waterproof, light in weight and can be attached to either the cephalthorax or chelae with epoxy resin. The animal on release is then able to move freely, dig shelters and hide inside natural refuges (Gherardi *et al.*, 2000).

Not only can crayfish movements be assessed using this method, but also activity levels can be monitored, as alignment of the receiving antenna with the whip antenna of the tag transmitter, results in changes of signal amplitude (Nams, 1989). However, one major disadvantage is the cost involved, usually resulting in a reduced sample number. This is outweighed, however, by the fact that individual crayfish can be observed continuously without being disturbed (Bohl, 1999; Gherardi *et al.*, 2000).

This method is ideal for monitoring short-term movements of crayfish and is capable of providing valuable information of microhabitat preferences, activity levels and travelling distances of *P. leniusculus.*

1.4 Population densities and species invasions

It is difficult to ascertain crayfish population density because unless the whole population can be captured, the researcher has to rely on sampling. Sampling is when crayfish are caught, marked and recaptured. The frequency of recapture is then analysed in order to estimate population density of any given area. Any sampling must be representative of all stages of the life cycle (Brown and Brewis, 1978).

In order to make valid estimates of population density using the mark-recapture method, certain assumptions have to be upheld. One basic assumption, albeit difficult to test under natural conditions, is, that all members of the population are equally likely to be captured (Southwood, 1978).

There are a number of methods of taking samples from crayfish populations, but the most common is the use of baited traps. Unlike the other methods, which include, the use of drop nets, hand collection with or without scuba, electro-fishing and dip netting, baited traps do not rely on the nature and conditions of the water body (Brown and Brewis, 1978).

The use of baited traps, however, can produce unreliable results (Brown and Brewis, 1978). Research suggests that unequal catchability may be the result of innate or learned behavioural responses to traps (Cormack, 1969), and trapping may select the hungrier or more active segments of the population. It has also been suggested that adult male crayfish may be 'trap happy' and females 'trap shy', resulting in an under-estimation of population density and sex ratio (Brown and Brewis, 1978). However, for comparative purposes, trapping is still a useful technique to use.

Exotic species of crayfish introduced into freshwater communities have proved a major threat to native species, in fact, several species have been driven to local extinction through interactions with exotic crayfishes and/or their diseases. Examples of these include, the replacement of *A. astacus*, a European native crayfish by the introduced species *P. leniusculus*

in a small enclosed Finnish lake (Westman *et al.*, 2002) and a Swedish lake (Soderback, 1995), the replacement of *O. virilis* (the native) and *O. propinqus* (a previous invader) by *Orconectes rusticus* in northern Wisconsin Lakes (Hill and Lodge, 1994), and the replacement of *A. papilles* in British streams by *P. leniusculus.* The mechanisms of these invasions include, crayfish plague, which can wipe out populations of native crayfish, and predation which can also play an important role in species replacement (Butler and Stein, 1985). Higher growth rates, early sexual maturity and a higher capita egg production have also been thought to aid the predominance of a species (Soderback, 1995).

Garvey and Stein (1993), found that chela size was also an important factor in the replacement of species. Chela size was advantageous in aggressive encounters and for successful mating (Garvey and Stein, 1993). Agonistic interspecific encounters occur between species. It was observed, for example, that *P. leniusculus* had fewer chela injuries than *A. astacus* (Westman *et al.*, 2002), as the former was much more competitive. This resulted in *A. astacus* being displaced from preferred food sources and safer shelters, leaving them more at risk of predation (Garvey and Stein, 1993). However, another crucial factor in the decline of *A. astacus* was the cessation of successful reproduction due to reproductive interference (Soderback, 1995; Westman *et al.*, 2002).

1.5 Social interactions

Territory can be defined as a 'fixed portion of an individual's or group's range in which it has priority of access to one or more critical resources over others', and territoriality can be defined as a form of social dominance (Kaufmann, 1983).

Dominance/subordinance is a relationship where one individual defers to the other. Reasons for this may vary, for example, size or age. However, compromises are made in each relationship and are dependent on the circumstances, each animal weighing up the costs and benefits of either deferring to the other animal of not (Kaufmann, 1983). Dominance can be absolute or relative depending on the species and the situation. However, high population density and a decrease in available resources can shift relative dominance towards absolute dominance (Kaufmann, 1983).

Important characteristics such as rate of growth, maturation and reproduction are important for the fitness and survival of any crayfish species (Guan and Wiles, 1999). It has been suggested that deteriorating living conditions due to increased crayfish population density may be one of the main factors affecting poor growth and decreased fertility (Guan and Wiles, 1999).

Reasons for agonistic behaviour vary. Competition for suitable refuges, reduced availability of food (Stein and Magnuson, 1976) and inappropriate mate selection (Butler and Stein, 1985) have all been suggested.

Agonistic behaviour in crayfish can be either interspecific, that is when they defend against individuals of other species for example *Cambarus bartonii* and *C. robustus* (Guiasu and Dunham, 1999) or intraspecific where they defend against members of their own species, such as *C. robustus* (Guiasu and Dunham, 1998). Juvenile species were found to be less aggressive than adult ones, especially those in a larger body size groups (Soderback, 1990). However, in accordance with theoretical models of animal conflicts it was found that interactions between equally sized contestants were more severe than between crayfish of different size (Vorburger and Ribi, 1999).

Karnofsky *et al.* (1989), observed that in *Homarus americanus,* high-level aggression was displayed by mature males which were establishing a mating shelter area and that low-level aggression was generally related to premoult increase in activity.

Research has been carried out to examine the sensory cues involved in crayfish agonistic behaviour. It was found that during combative encounters crayfish used both vision and taction (Bruski and Dunham, 1987). Bruski and Dunham (1987), also investigated the importance of vision in *Orconectes rusticas* and found that the efficiency of communication diminished as it became darker and that combative behaviour changed from visual cues like, 'Lunge and Follow' to more tactile encounters like 'Antenna Tap', 'Chelae Strike' and 'Push'.

Some species demonstrate maternal aggression such as *P. clarkii* (Figler *et al.*, 1997). This is when a female carrying eggs or offspring may well have a dominant advantage in an agonistic encounter against conspecific males or non-maternal females.

1.6 Predation and feeding behaviour

Crayfish are abundant omnivores (Capelli and Hamilton, 1984; Chambers *et al.*, 1990; Chambers *et al.*, 1991; Guan and Wiles, 1998; Nystrom *et al.*, 1996), and are opportunistic in nature (Gherardi, 2002). Cannibalism is common, but they also feed on aquatic invertebrates such as stoneflies and mayflies (Keller and Ruman, 1998), as well as grazing on macrophytes (Nystrom and Strand, 1996), vegetable detritus and moss, important sources for protein and energy.

As crayfish graze and scavenge, they keep waters free of carrion and quantities of algae and vegetation (Richards and Fluke, 1977). The five main diet items consumed by *P. leniusculus* in the River Great Ouse were vascular plant detritus, filamentous green algae *Cladophora*, crayfish fragments (cannibalism), *Chironomidae*, and *Ephemeroptera*. This was apparently similar for all ages, seasons and gender (Guan and Wiles, 1998).

Research suggests that high population density and poor availability of food affect the frequency of cannibalism in crayfish (Dong and Polis, 1992). Crayfish were found to prey on their own species as well as on other crayfish species and there is evidence to support the notion that aggressive species may be partly responsible for the demise or decline of less aggressive species (Holdich and Domaniewski, 1995).

Several studies have shown that crayfish can have a negative impact on macrophyte biomass (Flint and Goldman, 1975) and invertebrates (Chambers *et al.*, 1991; Keller and Ruman, 1998), thus disturbing the aquatic ecosystem. Crayfish can reduce or eliminate aquatic vegetation from many lakes and rivers and have the ability to modify macro invertebrates and ultimately affect the fish community (Chambers *et al.*, 1991; Nystrom and Strand, 1996).

Plant species, crayfish sex and activity and the amount of alternative food sources affect macrophyte growth (Chambers *et al.*, 1991). It was also noted that the impact of crayfish grazing on aquatic macrophytes was dependent on the stage of development of the macrophyte when grazed. For example, younger plants and seedlings were more affected than older more established plants (Chambers *et al.*, 1990; Lorman and Magnuson, 1978).

Crayfish have a number of predators, including fish, aquatic invertebrates, reptiles, birds, amphibians and mammals (Marren, 1986; Nystrom, 2002). Freshwater crayfish are more at risk from predatory fish, for example, perch and bass. Larger crayfish have more chance of survival (Elvira *et al.*, 1996; Stein and Magnuson, 1976), in fact, it was found that bass had no affect on the survival rate of adult crayfish (Stein and Magnuson, 1976). Juveniles were however, more at risk, because of their size and their less rigid exoskeleton (Stein, 1977).

It was also observed that newly independent juvenile *P. leniusculus* on average comprised 22% of the prey items in the diets of the perch in which they were found. Yearling juveniles on average constituted 48% of the total number of prey items per stomach in which they were found, but in terms of volume they constituted the majority of the diet (Blake and Hart, 1993).

Appelberg and Odelström (1988), observed that the presence of perch strongly reduced the activity and growth of *P. leniusculus* young of the year.

Predators impact on crayfish behaviour. It was observed that crayfish growth rate was slower when the abundance of predators was high (Appelberg and Odelström, 1988), and that crayfish nocturnal activity patterns were possibly an adaptation from visual predators (Hamrin, 1987; Stein, 1977).

1.7 Management and exploitation

For those who harvest, farm or sell crayfish, the introduction of alien species has been relatively successful (Holdich *et al.*, 1999), a view not shared by conservationists who have serious concerns about the adverse affects of alien crayfish species on native species and on freshwater environments. It is believed that introduced crayfish alter ecological balance (Holdich *et al.*, 1999), for example, by causing shifts in species diversity (Hobbs *et al.*, 1989).

Legislation aimed at controlling the spread of alien crayfish and conserving the native species is in place in many countries in Europe, for example, Britain (as described at the beginning of the chapter), Austria (Pockl, 1999), Germany (Lukowicz, 1999) and Sweden (Holdich *et al.*, 1999), to name but a few. These laws, however, are proving to have only a limited impact due to lowered trade barriers between countries and a reluctance by authorities to prosecute (Holdich *et al.*, 1999).

It has been suggested that a unified approach within Europe is needed in order to preserve and conserve native crayfish like *A. papilles*, *A. astacas* and *A.torrentium* and to manage the spread of alien crayfish populations. A way forward is perhaps to set up a European database, like the one in Britain, which holds information on species details and distribution and which is regularly updated (Holdich *et al.*, 1999). Also, maybe there is a need to develop crayfish management plans as described by (Skurdal *et al.*, 1999). These would culminate in co-ordinated action plans, bearing in mind the need to protect, restore and enhance native populations, protect the freshwater environment as well as sustain the exploitation of the crayfish populations for aquaculture and culinary purposes.

1.8 Study aims and objectives

Thus it is clear from the literature that there are areas of the biology, behaviour and ecology of *P. leniusculus,* which require further research. The intention of this study, therefore, will be to look into aspects of substrate selection, burrowing behaviour and movements by *P. leniusculus* as these areas are useful in terms of predicting population spread, ascertaining colonisation and the impact it has on stream environments. The gathering of the above data will aid future recommendations to manage and control the spread of the species and provide a basis for decisions to this end.

Investigation of substrate preference of *P. leniusculus* for either natural refuges or burrow construction will be useful in managing population spread by identifying those stretches of river that are most likely to be at risk of bank erosion in the future and perhaps introduce the possibility of channel modification which would discourage extensive burrowing. Further information on burrow morphology, construction and environment will provide an indication of the level of burrow damage and help to predict the likelihood of bank collapse. A knowledge of burrow water chemistry could be useful in identifying reliable indicators of burrow occupancy and may provide data on burrow irrigation levels and water turnover.

The possibility of water exchange between burrow and stream will be of significance in providing vital information for those researching into the use of biocides to eradicate crayfish. If no or very slow water exchange is taking place then these methods would be relatively ineffective.

Finally, the investigation of short and long-term movements of adult crayfish will be helpful in establishing distances travelled, providing information about any home range and identifying those life stages responsible for range extension. It should then be possible to predict future spread of this alien invader.

Chapter 2

Materials and Methods

Chapter 2: Materials and Methods

Collection and maintenance of animals

A licence authorising the keeping of *Pacifastacus leniusculus* (Dana) was obtained from the Ministry of Agriculture, Fisheries and Food (now DEFRA) (Appendix I). Before the commencement of any practical or fieldwork, a risk assessment for laboratory and solo fieldwork procedures was completed (Appendix II). This incorporated a buddy system, a log detailing locations and times of visits, a mobile phone and a buoyancy aid, all of which adhered to Health and Safety requirements set by the University.

Crayfish used in the following experiments were collected from the Gaddesby/Twyford Brook using Swedish 'Trappys' baited with 'Safeway Savers' cat food. The cans of cat food were cut in half and each half was placed in a trap and replaced daily. Traps were set in the afternoon and lifted the following day. Any captured animals were placed in lidded buckets with a little water and a few handfuls of damp grass to transport them back to the laboratory by car. Whilst conducting fieldwork, a letter outlining the project (from the Environment Agency) was carried, which was shown to landowners, when seeking permission for access to their land (Appendix III).

All traps and equipment were thoroughly washed and scrubbed to remove mud and silt before use at different locations in the stream. They were then soaked in 11% industrial sodium hypochlorite (Chloros) to destroy any biofilm present. The equipment was left to air dry before rinsing in tap water to remove any residual disinfectant (Harris and Lawrence, 1999).

Captured crayfish were kept in opaque-lidded polythene tanks in copper-free re-circulated filtered water (System "Tropical Marine System 2500/5000 Freshwater Filtration Unit") at a temperature of 15 ± 1°C. Short lengths of plastic pipe of sizes ranging from 4-7 cm diameter and 10-20 cm in length, were provided for use as artificial refuges. A maximum of 6 adult animals were held in each 0.6 x 0.4 x 0.5 m tank, with a combination of single and mixed sexed tanks. The lighting regime was set on a 12 h light and dark cycle. Crayfish were fed weekly with 'HiLife Complete Moist Menus' dog pellets. Any ovigerous females were placed in individual tanks until their young were hatched. After hatching they were removed and

placed in shared holding tanks. The newly hatched crayfish were left until they grew to a size where it was judged that they required more space per individual. They were then re-allocated to other tanks. Animal size was determined as Carapace Length (CL; mm to the nearest 0.1 mm); measured from the rostral apex to the posterior median edge of the cephalothorax by means of Vernier callipers.

2.1 Field Studies

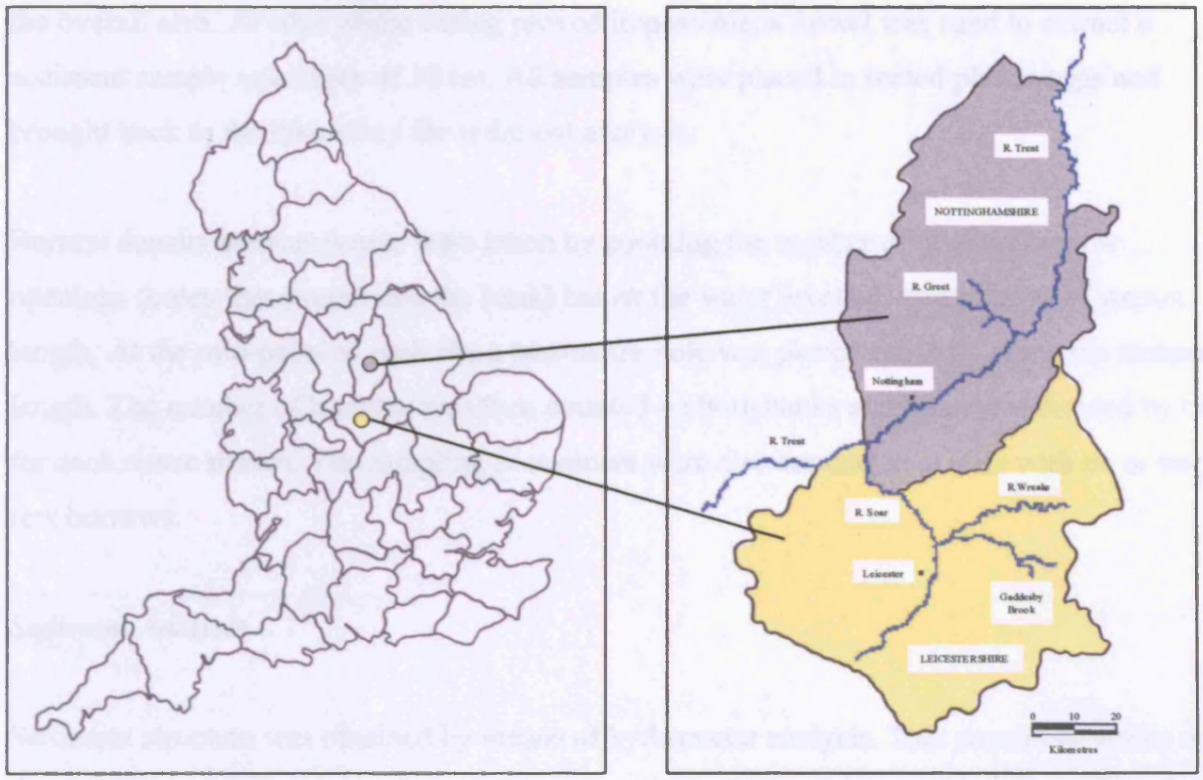
The main field research site was the Gaddesby Brook in Leicestershire (SK 792 082 to SK 627 133). However, a small comparison study was conducted on the River Greet in Nottinghamshire (SK 667 574 to SK 743 515) to establish whether a different population of *P. leniusculus* would behave similarly. These sites were chosen because of known introductions of signal crayfish.

The Gaddesby Brook runs through mainly rural areas and farmland in a northwesterly direction. It is approximately 15 km long and joins with the Queniborough Brook shortly before running into the River Wreake at East Goscote (Figure 2.1.1). The bank substrate varies from clay to sand-based sediments and the stream bed ranges from fine silt to boulders. The depth of the Brook can be from as little as a few centimetres to as much as several metres; the width also varies considerably along its course. Water velocities are also very variable and change rapidly in response to rainfall with the result that large changes in depth can occur.

The River Greet also runs through mainly rural landscape and is approximately 10 km in length. It flows in a southeasterly direction and enters the River Trent at Fiskerton, south of Nottingham (Figure 2.1.1). Its bank and bed substrate shows a variability similar to that of the Gaddesby Brook.

During the majority of field based research, and whilst making general observations of crayfish behaviour in their natural environment, it was necessary to enter the stream water equipped with a dry suit, mask and snorkel. This was because, at times, animals or burrows were inaccessible from the stream bank. It was particularly important in winter since the stream water temperature would otherwise have prevented long term exposure.

Figure 2.1.1 Location of Gaddesby Brook and River Greet



2.1.1 Burrow densities in relation to substrate type

Sediment collection and burrow recording

A series of pools along the Gaddesby Brook between Owston and Gaddesby were marked out using numbered wooden stakes. Although only alternate pools were staked, every pool was described in terms of position and size, with key landmarks recorded. The riffle areas between each pool were also allocated numbers. Using Minitab, 40 riffle sites and 40 pool sites were selected at random, thus providing a randomly stratified sample set (which would most appropriately represent the whole stream) for substrate sampling and burrow density estimates. These samples were collected at approximately weekly intervals throughout the period of January to April 2000.

On each sampling occasion the time, date and weather conditions were recorded. The mid-point of each pool or riffle was then established and measurements of water depth, water current velocity (using a Marsh McBirney 2000 cm Flow-Mate current meter with an Em3000 standard wading wand), water temperature, water pH and burrow density were recorded.

Seven sediment core samples were collected, at the established mid-point in a 'W' formation, with a corer to a depth of 10 cm. These cores were then bulk sampled in order to best describe the overall area. At sites where coring proved impossible, a trowel was used to extract a sediment sample to a depth of 10 cm. All samples were placed in sealed plastic bags and brought back to the laboratory for sediment analysis.

Burrow density measurements were taken by counting the number of obvious burrow openings (holes that extend into the bank) below the water level per linear metre of stream length. At the mid-point of each site a two-metre pole was placed centrally along the stream's length. The number of burrows was then counted on both banks and this value divided by two for each metre stretch. The sampling procedures were also conducted at sites with no or very few burrows.

Sediment Analysis

Sediment structure was obtained by means of hydrometer analysis. This procedure works on the principle of Stoke's Law: the velocity of a particle falling through a viscous medium is directly proportional to the diameter of the particle. However, as individual particles are hard to monitor, this technique measures the change in density of the suspension over time, thus heavier particles such as sand will settle first leaving the remaining suspension less dense. From this, the particle size can be extrapolated, along with the relative proportions of each size in the sample.

Sediment samples were dried overnight at 60 °C in an oven and weighed on an Oertling OB152 balance every half hour until three consecutive measurements were the same to ± 0.1 g. They were then broken up using a pestle and mortar and sieved using an Endecotts 2000 microns (mesh No. 8). Next 100 ml of polymetaphosphate solution (50 g in a litre) was placed into a stainless steel blender cup. To this, was added 40 g of the sieved oven-dried sediment sample and then the cup was partially filled with distilled water. This mixture was blended using a Prima PDM002 blender on setting No. 1 for 5 min. The solution was poured into a litre-measuring cylinder and filled to the one litre mark with more distilled water. This was mixed with a plunger and on the point of the plunger's removal a stopwatch was started. A hydrometer was immediately placed into the measuring cylinder and a reading taken after 30 sec. Further readings were taken at 1, 3, 10 min and 17 h. Values of θ (sedimentation

parameter) for each hydrometer reading were obtained from Day (1956) and particle size was then calculated using the following equation:

$$\text{Particle size } (\mu) = \frac{\theta}{\sqrt{t\ (\text{mins})}}$$

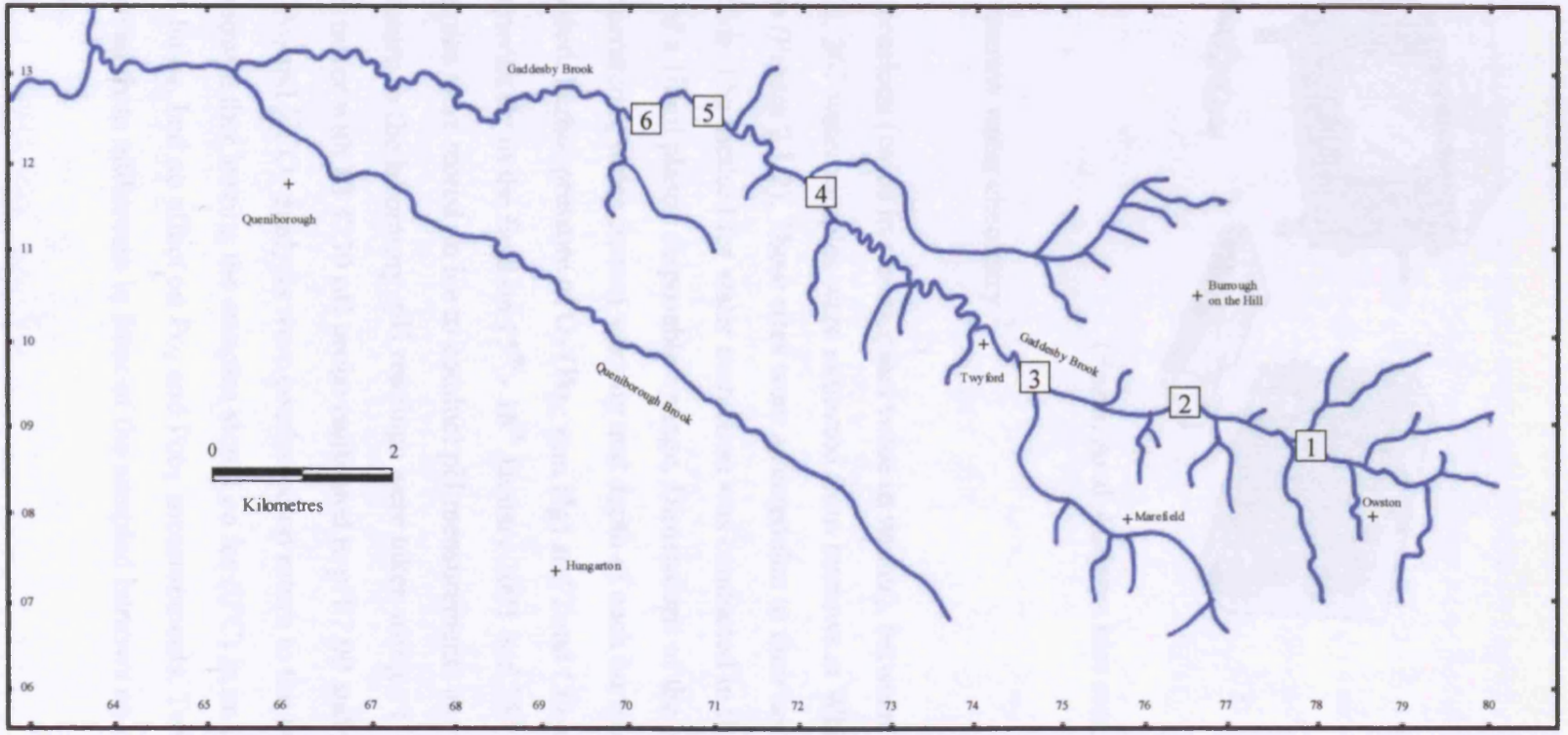
The % of sediment was plotted against calculated particle size on 90 Divisions (2 millimetres) 5th, 10th Accent by 2 Cycle Semi-Log graph paper. At particle sizes of $<2\ \mu$, $2\ \mu<20\ \mu$, $20\ \mu\leq63\ \mu$ and $>63\ \mu$, the percentages of clay, fine silt, coarse silt and sand respectively were extrapolated. This established the composition of each sediment type (British Standard 1377: 1975).

2.1.2 Internal burrow features

In the period 21st - 26th June 2001, one hundred burrows were examined at two sites, Newbold Farm (SK 766 091) and White House Farm (SK 753 089) (Figure 2.1.2), using an optic fibre VS6 mini TV camera with infrared lighting hired from Acal Auriema Ltd. This camera was 12.5 mm in diameter and was normally used for the internal examination of small pipes of diameters in the range of 15-100 mm (Figure 2.1.3). The optic cable with camera head was inserted slowly into the burrows and images were observed on the stream bank on a portable monitor and recorded onto videotape. The length of cable inserted once the camera was touching the back wall of the burrow determined burrow length. Information was also recorded on:

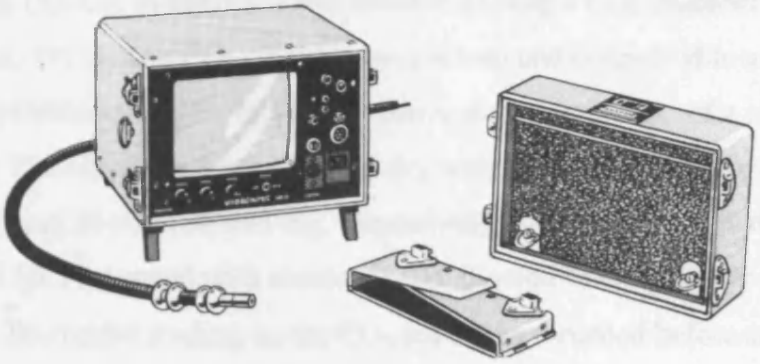
1. Number of entrances in each burrow.
2. Number of tunnels in each burrow.
3. Angle at which the burrow entered the stream bank.
4. Approximate shape of the burrow and whether it was tapered.
5. Presence of plant matter.
6. Percentage occupancy of burrows examined.

Figure 2.1.2 Numbered squares indicate locations for studies on: short and long term movements; population densities; burrow morphologies; burrow water chemistry analysis.



1 Newbold Farm
2 White House Farm
3 Lowenva Lodge
4 Ashby Folville
5 Mill Farm
6 Gaddesby

Figure 2.1.3: An optic fibre VS6 mini TV camera with infra-red lighting.



(From: Acal Auriema hire catalogue)

2.1.3 The internal burrow water chemistry

On four separate occasions (twice in summer and twice in winter), between 15th January 2001 and 4th March 2002, 267 water samples were extracted from burrows at White House Farm and Newbold Farm (Figure 2.1.2). These sites were selected due to their accessibility and high burrow densities. The method for water extraction was conducted in the same manner at all sites by means of a 10 ml plastic disposable syringe. Dimensions of the burrow openings, stream and air temperatures, water current velocity and depth of each burrow from the water surface, were recorded. Partial pressure of O_2 (Po_2; mm Hg) and Total CO_2 ($\sum CO_2$;mequiv l^{-1}) were measured immediately in the field on 15th - 18th January 2001 and 25th - 26th June 2001 and remaining samples were stored on ice to conduct pH measurements and ammonia determination on return to the laboratory. pH readings were taken using a Hannah HI 9024 microcomputer pH meter with HI 1230 pH probe calibrated to pH 7.00 and pH 9.18. On subsequent visits, Po_2 and $\sum CO_2$ analysis were conducted on return to the laboratory after preliminary tests showed that leaving the samples stored on ice (0°C) in an insulated box for approximately two hours, had no effect on Po_2 and Pco_2 measurements. Two samples of stream water, removed from midstream in front of the sampled burrows on each occasion, were also analysed.

Determination of water CO_2 (ΣCO_2)

Total CO_2 (ΣCO_2; m equiv l^{-1}) was measured using a CO_2 chamber and electrode method (Cameron, 1971). The CO_2 electrode was set-up and connected to a 'Radiometer Copenhagen PHM73 pH/Blood Gas Monitor' and thermostatted by means of a circulating water bath (set at 30°C). The electrode was calibrated dry with 1% and 5% CO_2 in air mixture (BOC special gases) to read 20 and 100 mm Hg, respectively. The chamber was then filled with a solution of 0.01 N HCl saturated with n-octanol and allowed to temperature equilibrate for five minutes. The initial reading on the CO_2 scale was recorded before adding 10 µl of $NaHCO_3$ standard (30 m moles l^{-1}) using a Hamilton microlitre syringe. On mixing with dilute HCl, the dissolved CO_2 released from HCO_3^- and CO_3^{--} was measured by the CO_2 electrode. As soon as the meter reading stabilised it was noted, and the process repeated twice. Fifty microlitres of stream and burrow water were treated similarly.

After 7-8 samples the 0.01 N HCl n-octanol chamber fluid was replaced and the process was repeated until all water samples were measured. The meter readings were converted to find total CO_2 (m equiv l^{-1}) corrected for stream temperature (Appendix IV), which in turn was used to calculate Pco_2 and [HCO_3^-] by means of the Henderson-Hasselbalch equation (Appendix V),

Determination of water PO_2

Po_2 was measured directly using a Po_2 electrode connected to a Radiometer Copenhagen PHM73 pH/Blood Gas Monitor. The electrode was surrounded by a water jacket connected to a thermostatically controlled circulating water bath. The water bath was set at the same temperature as the stream water for that day. The electrode was zeroed with zero Po_2 solution and calibrated with air-saturated stream water. The expected Po_2 value of air-equilibrated water for that day was calculated by:

$$Po_2 = \frac{(Pa - Vp) \times 20.9}{100}$$

Pa = Barometric pressure (mm Hg), Vp = saturated water vapour pressure at stream temperature (mm Hg)

The sample chamber was emptied by suction and then injected with a 200 µl volume of the sample water directly from the syringe. After a couple of minutes, a further 100 µl volume of sample was injected and a reading was noted after 30 seconds. In between samples, the chamber was flushed with distilled water and emptied by suction.

The unit mm Hg was used for comparative purposes, 1 mm Hg (also called Torr) equates to 0.133 kPa (kilopascals) the SI unit of partial pressure.

Ammonia Determination

A 1 M ammonia stock solution was prepared with NH_4NO_3 (Analar) in deionised water. The stock was subsequently diluted to give standards in the range of 10-100 µmoles l^{-1}. 1 ml of water samples and standards were then added to 1 ml each of salicylate and cyanurate reagent, shaken and allowed to develop (blue colour) for 30 minutes. The standards were then read in a 1 ml cuvette at 655 nm by a Pye Unicam SP6-400 UV spectrophotometer. A calibration curve was then plotted and fitted with a linear regression line. The equation of the line was then re-arranged to find unknown ammonia concentrations from the absorbance values of water samples prepared in the same way (Harris and Andrews, 1985). The lowest limit of detection for this method was 1µmoles l^{-1}.

2.1.4 Population densities, range extension and burrow erosion damage

Population density

In August 2000, 2001 and 2002, population density measurements were carried out in the Gaddesby Brook. Five sites were selected for their accessibility and positioning along the length of the stream (Figure 2.1.2):

1) SK 774 085 (Newbold Farm)
2) SK 753 093 (White House Farm)
3) SK 737 094 (Lowenva Lodge)
4) SK 710 118 (Ashby Folville, at start of public footpath)
5) SK 693 127 (Gaddesby, Under Ashby Road bridge)

Five Swedish 'Trappys' with cable ties attached at the funnel entrances to reduce escapees were deployed 3 m apart at each site and baited (See page 18). Captured animals were weighed, measured, sexed and numbered with quick drying red nail varnish before being re-released; a laboratory trial showed this to have no behavioural effect and the marks lasted for weeks after application. Trapping continued for 3 consecutive nights and all newly captured animals were marked, whilst a record was made of any recaptures.

It was assumed, as with all capture-mark-release (CMR) experiments, that tagging and repeat capture did not affect the 'catchability' of individuals and that the population was sampled at random (Southwood, 1978).

Population sizes at each site in all three years were calculated using two methods:

Bailey's Triple catch method, with a correction for small numbers of recaptures (Southwood, 1978):

$$N_2 = a_2(n_2+1)r_{31} / (r_{21}+1)(r_{32}+1)$$

N_2 = estimate of the number of individuals in the population sampled.
a_2 = number of newly marked individuals released on day 2.
n_2 = total number of animals captured on day 2.
r = recaptures, with 1st subscript representing day of capture and 2nd subscript the day of marking.

A standard Peterson (Lincoln) method with Bailey's correction for small samples (Southwood, 1978):

$$N = a(n+1) / r+1$$

a = total number of marked animals
n = total number of individuals in second sample
r = total number of recaptures.

Range Extension

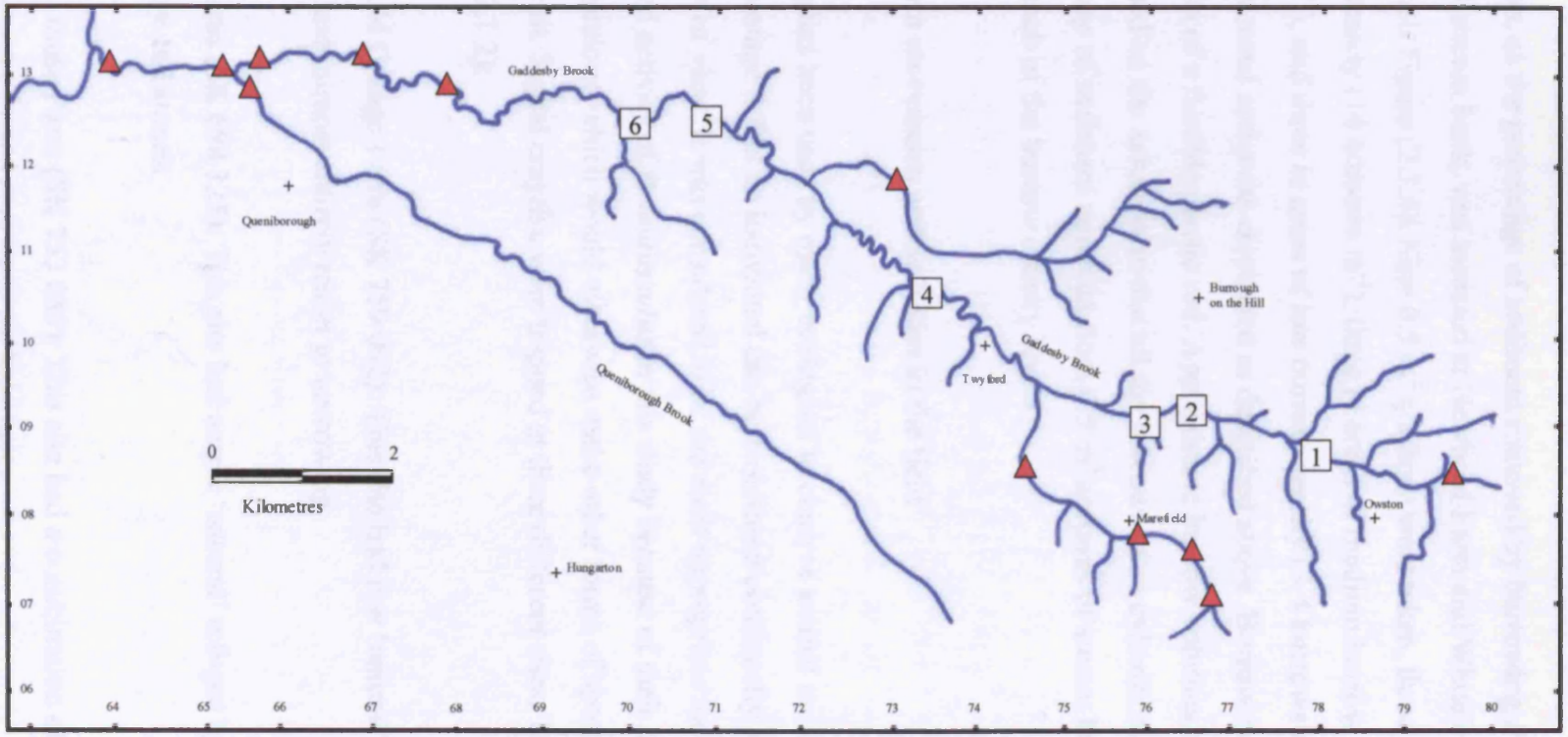
Previous studies of the Gaddesby Brook, the most recent conducted in 2000 and reported by Sibley (2001), showed how far up and down the stream the crayfish had reached since their introduction in 1985. In order to ascertain the present situation, traps were placed upstream and downstream of the last known place of occurrence (Figure 2.1.4). These traps were baited, left for a week and checked daily. If trapping failed to locate crayfish, the Brook was searched by hand at these sites, turning boulders and netting to see if animals could be located. Presence or absence could then be plotted onto maps and any range extension determined by comparison with previous records.

The sizes of burrow openings were measured at progressive sites from the area of *P. leniusculus* introduction to the lower most site where burrows had been observed. This was to ascertain which size range of individuals were occupying the newest areas of stream habitat, thereby indicating how range extension was occurring. Six sites were selected (Figure 2.1.4). These were:

1. Newbold Farm (SK 766 091)
2. Newbold Grange Farm (SK 759 092)
3. White House Farm (SK 753 089)
4. Twyford Lodge (SK 725 105)
5. Ashby Folville (SK 703 124)
6. Gaddesby (SK 686 127)

At each site a minimum of 35 burrow entrances were measured (equating to the maximum number present at Gaddesby), height (mm) and width (mm). These were then converted to areas assuming the burrow entrance was elliptical, using the formula: (0.5 x height) x (0.5 x width) x π.

Figure 2.1.4 Map of the Gaddesby Brook: the red triangles indicate trap locations for range extension assessment and the numbered squares indicate sites where burrow size measurements were taken.



1 Newbold Farm
2 Newbold Grange Farm
3 White House Farm
4 Twyford
5 Ashby Folville
6 Gaddesby

Burrow erosion damage

Burrow erosion, as the percentage of sediment removed by burrowing *P. leniusculus* from 0.5 m^3 sections of stream bank, was assessed at Newbold Farm and White House Farm on the Gaddesby Brook Figure (2.1.4). Nine 0.5 m^2 quadrats were taken, three randomly in areas of high burrow density (14 burrows m^{-1}), three in areas of medium burrow density (7 burrows m^{-1}), and three in areas of low burrow density (3-4 burrows m^{-1}). Burrow entrance sizes were measured and areas calculated as described above. Burrow depths were determined by the insertion of a flexible plastic rod. Approximate burrow volumes could then be calculated based on the assumption that all the burrows were cylindrical. This enabled the mean percentage of sediment removed from 0.5 m^3 sections of stream bank by crayfish to be calculated at each of the burrow density levels.

2.1.5 Short term movements and activities in the field

Radio-tagging has been used by many ecologists to observe animal movements and activities. The main advantage is that an individual can be monitored continually without disturbance, even when out of view. It was considered to be the most appropriate method to determine the movements and activity of *P. leniusculus* in this study because of their nocturnal and burrowing behaviour, which would otherwise make other forms of short term tagging and tracking difficult. Signal crayfish were trapped at three different sites. The three sites chosen were (Figure 2.1.2):

1) Newbold Grange Farm (SK 759 092): This site had few 'natural' refuges and individuals almost entirely resort to burrowing.

2) Mill Farm (SK 698 125): This site had ample 'natural' refuges by way of broken masonry and stones.

3) White House Farm (SK 753 089): This site had a combination of both 'natural' refuges and suitable clay bank for burrowing.

These sites were selected because of dense populations of signal crayfish, accessibility, permission from land owners and because they were well away from crayfish clearance

projects being conducted during this period by the Environment Agency.

A total of 44 Crayfish were radio tagged between May 2000 and August 2002, 18 at Mill Farm, 20 at Newbold Grange Farm and 6 relocated animals at White House Farm.

Animals selected for tagging met the following criteria:

1) Large enough to be fitted with transmitter (ratio of mass of transmitter to mass of animal generally not exceeding 1:10)(mass range 15.5 to 97.4 g).
2) Healthy, i.e. no signs of moulting or loss of limbs;
3) A balance of sexes to allow 1:1 ratio of radio-tagged males and females at each site.

The selected captured crayfish were dried, measured, sexed, weighed and fitted with TW-4 (392Ag cell) radio transmitter (Biotrack Ltd) (Figure 2.1.5). These were attached dorso-laterally with the aerial pointing posteriorly, by means of Superglue and a covering of Araldite 5-minute quick setting epoxy resin (Plate 2.1.1). Each tag transmitted on a unique radio frequency (between a 173.200 – 173.400 MHz range) allowing individuals to be identified in the field and at night. The transmitters weighed 1.5 g, excluding the epoxy resin covering.

Figure 2.1.5: TW-4 (392Ag cell) radio transmitter

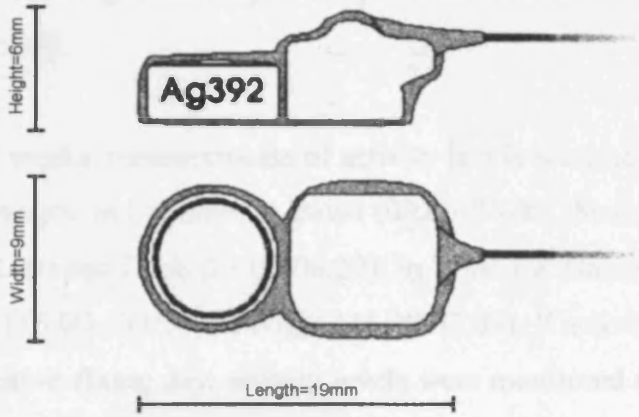


Plate 2.1.1: Photograph of a TW-4 (392Ag cell) radio transmitter and plastidipped Ibutton temperature data logger glued to a Signal Crayfish.

The stream at Mill Farm and Newbold Grange Farm was then divided up into 5 m lengths marked by stakes driven into the bank to enable accurate position fixing. Animals were released at their exact point of capture and were tracked using a Mariner-57 Biotag receiver with Yagi antenna every other day for four weeks. The position of each crayfish was obtained by wading into the stream and pinpointing the burrows or natural refuges where they were hidden (point of maximum signal strength determined). The accuracy of the position fixing was approximately ± 15 cm, but this decreased with depth. It was sometimes possible to see the animals, which confirmed the readings obtained. On pinpointing the crayfish, its position was then transcribed onto a gridded map of the particular stretch of the Brook, which had been prepared previously.

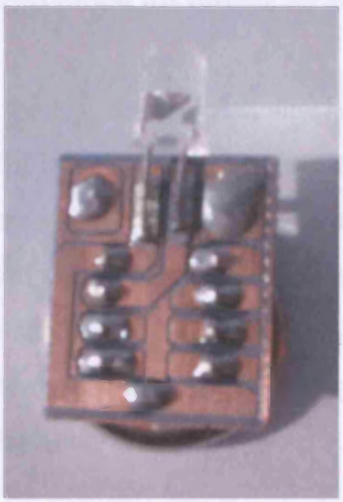
Over the course of 4 weeks, measurements of activity levels were obtained using changes in transmitter signal strength; in Summer, at Dawn (03.00-06.00), Morning (09.00-12.00), Afternoon (15.00-18.00) and Dusk (21.00-00.00); in Winter at Dawn (05.00-08.00), Day (11.00-14.00), Dusk (16.00-19.00) and Night (23.00-02.00). If activity measurements coincided with a position-fixing day, activity levels were monitored first so that water disturbances were not the cause of increased activity. Activity was gauged by monitoring the changes in signal strength emitted by the tags of individual crayfish for 10 minutes. Activity was categorised in terms of movement; level 0: no movement, level 1: 1 or 2 movements,

level 2: 3 or 4 movements and level 3: more than 4 movements (Robinson *et al.*, 2000). When the tag's signal strength rose or fell by more than 2 on the receiver scale it was considered as one movement. It was very important that when monitoring the signal strength the receiver and antenna remained stationary and that there was no movement or obstruction between the antenna and the crayfish transmitter. In laboratory preliminary tests, crayfish movements were found to be correlated with directionality of antennas (strongest signal when antennae aligned).

During radio-tracking, stream and burrow/refuge water temperatures were continually recorded by means of TinyTalk or Ibutton temperature data loggers. Three crayfish were also fitted with Ibutton temperature data loggers coated with Plastidip to make them less conspicuous (Plate 2.1.2). Four crayfish were fitted with battery powered red light L.E.D. units, designed in conjunction with the company 'EMP Designs Ltd' with a seven-day life span to confirm movement patterns and, in areas with high burrow densities, exact burrow habitation. These units weighed approximately 4.0 g (Plate 2.1.2a and b). The red light emitted was only visible at low light levels (dusk to dawn).

Plate 2.1.2: a) L.E.D circuit before coating of plastidip
b) Crayfish fitted with a red light L.E.D. unit.

a)



1 cm

b)

At White House Farm, a small relocation project was conducted with the last six retrieved radio-tags. Six animals were collected by hand from their refuges. They were treated in the same way as above, except that 3 crayfish were released 50 m upstream and 3 crayfish were released 50 m downstream of their initial point of capture. Their positions were determined every other day for 4 weeks to see if they attempted to return to their place of capture.

2.1.6 Long term movements and tagging

Long term tracking of crayfish presents many problems due to the fact that crayfish moult. It was necessary to design a tag, enabling the identification of individual crayfish, which would not hinder moulting and would stay in after several moults. It had to be lightweight, cheap, not be highly visible to predators, able to be attached reasonably easily and still be readable after 2 or more years. As previously discussed in the Introduction (Section 1.3), there have been many attempts at long-term tagging of crustaceans, some more effective than others. This tag overcame all the problems except one; it was not suitable for crayfish less than 15 g in weight. The concept of streamer tags is not new, but the design of this tag allowed moulting to occur more easily. The tags consisted of a length of nylon fishing line (Team Daiwa, monofilament = 0.32 mm), a 7 mm transparent plastic bead and a yellow plastic half-disc imprinted with a 4-digit identity number (Plate 2.1.3). The tags were prepared for use by drilling a small hole in the numbered disk, threading and knotting a length of nylon thread and then Super-gluing it for further security.

To attach the tag, the crayfish was held securely with elastic bands to a polystyrene block with a V cut in it. Using a fine needle, the tag was threaded through the ventral abdominal musculature in front of the first set of pleopods. Care was taken to ensure the line passed dorsal to the ventral nerve cord. After this the bead was knotted on and glued. A small laboratory study was conducted and this showed that the tag could be retained for 2+ years. The animals tested moulted normally and showed normal behaviour, including tail-flipping escape behaviour.

Tagging was carried out in August 2000 and 2001 at Newbold Farm, White House Farm and Lowenva Lodge (Figure 2.1.2). These sites were selected because of known large crayfish populations. Trapped animals were weighed, measured, sexed, tagged and replaced at the point they were captured.

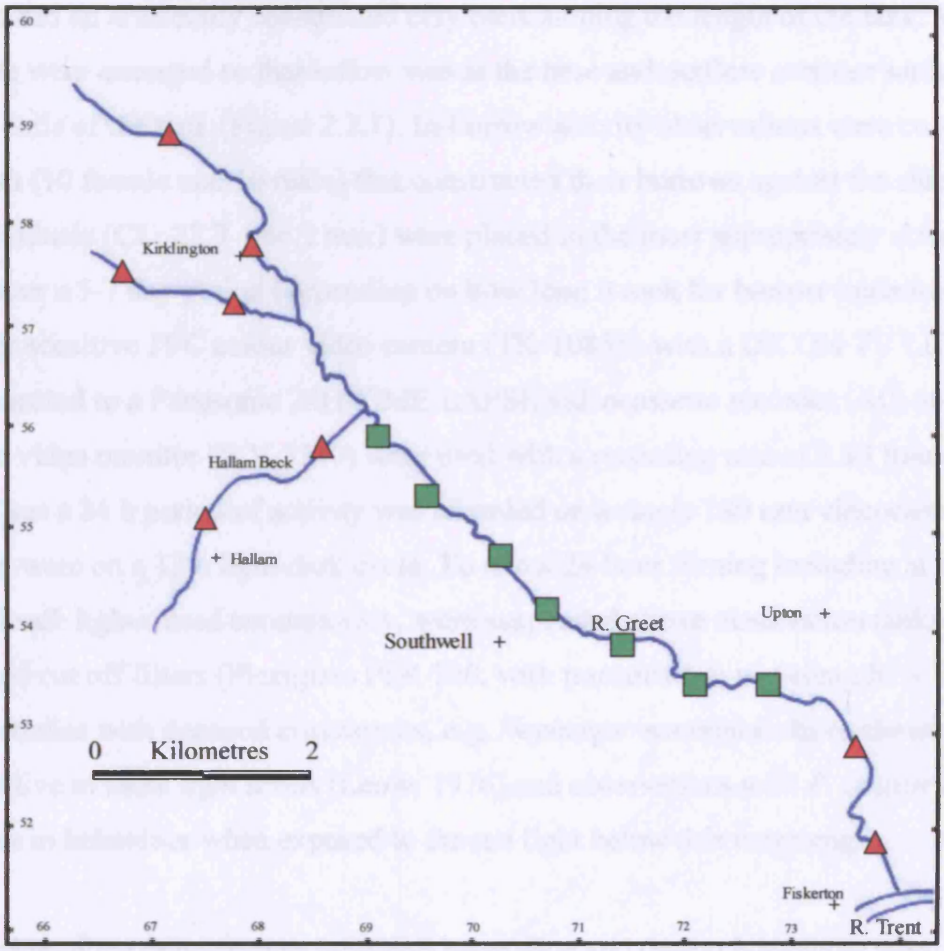
In 2001 and 2002, when retrieval of animals was attempted, as many as 20 traps were placed at each site spanning 150 m up and down stream of their initial release points. Dates and positions of all recaptures were recorded.

Plate 2.1.3 Crayfish tagged with an individually numbered and permanent streamer tag

2.1.7 Comparisons with the River Greet

Burrow densities in relation to substrate type and population range extension studies were conducted at the River Greet for comparison with the Gaddesby Brook. The methods were identical to those described in sections 2.1.1 and 2.1.4. The 7 sites chosen for study of burrow densities in relation to substrate type (Figure 2.1.6) were selected because of known well-established populations of signal crayfish (Harris, 1999). Range extension was assessed at 8 sites which previously had none or very few crayfish present (Harris,1999).

Figure 2.1.6 Map of the River Greet: the red triangles indicate trap locations for range extension assessment and the green squares indicate sites where burrow densities were compared with substrate type



2.2 Observations of burrowing and associated behaviour in the laboratory

The 'clay' used in burrowing and substrate choice was collected from an excavated site situated alongside the Gaddesby Brook (SK 753 089). Its composition was 62% clay, 13.5% fine silt, 10.5% coarse silt and 14% sand. From the completed field substrate experiments (section 3.1.1), this was considered to be a typical representation of stream bank sediment in which natural burrows were seen. The 'mud' used here consisted of 51% sand, 17% coarse silt, 15% fine silt and 17% clay, it was collected close to Lowenva Lodge (SK 738 091) and was considered to be unsuitable for burrow excavation. The 'gravel' substrate used was proprietary potting gravel with a size range of 3-5 mm.

2.2.1 Crayfish behaviour before, during and after burrowing

Three sizes of glass tanks ranging from 1.22 x 0.38 x 0.45 to 0.3 x 0.2 x 0.22 m were set-up. Each tank had an artificially constructed clay bank sloping the length of the tank. Water inlets and outlets were arranged so that inflow was at the base and outflow at water surface levels at opposing ends of the tank (Figure 2.2.1). In-burrow activity observations were conducted on 20 crayfish (10 female and 10 male) that constructed their burrows against the side of the tank. Individuals (CL; 22.3 – 66.2 mm) were placed in the most appropriately sized tank and videoed over a 5-7 day period (depending on how long it took for burrow initiation to occur). A red light sensitive JVC colour video camera (TK-1085E) with a DICON TV LENS (8 mm F1.3) connected to a Panasonic 24H TIME LAPSE videocassette recorder (AG-6024) and Panasonic video monitor (WV-5340) were used with a recording rate of 8.33 frames per second. Thus a 24 h period of activity was recorded on a single 180 mm videocassette. Light conditions were on a 12 h light-dark cycle. To allow 24 hour filming including at night, two additional safe lights, used continuously, were suspended above observation tanks and fitted with far-red cut off filters (Plexiglass PFR 700, with transmission wavelengths > 700 nm). Previous studies with decapod crustaceans, e.g. *Nephrops norvegicus* have shown that they are insensitive to these light levels (Leow, 1976) and observations with *P. leniusculus* showed no changes in behaviour when exposed to far-red light below this wavelength.

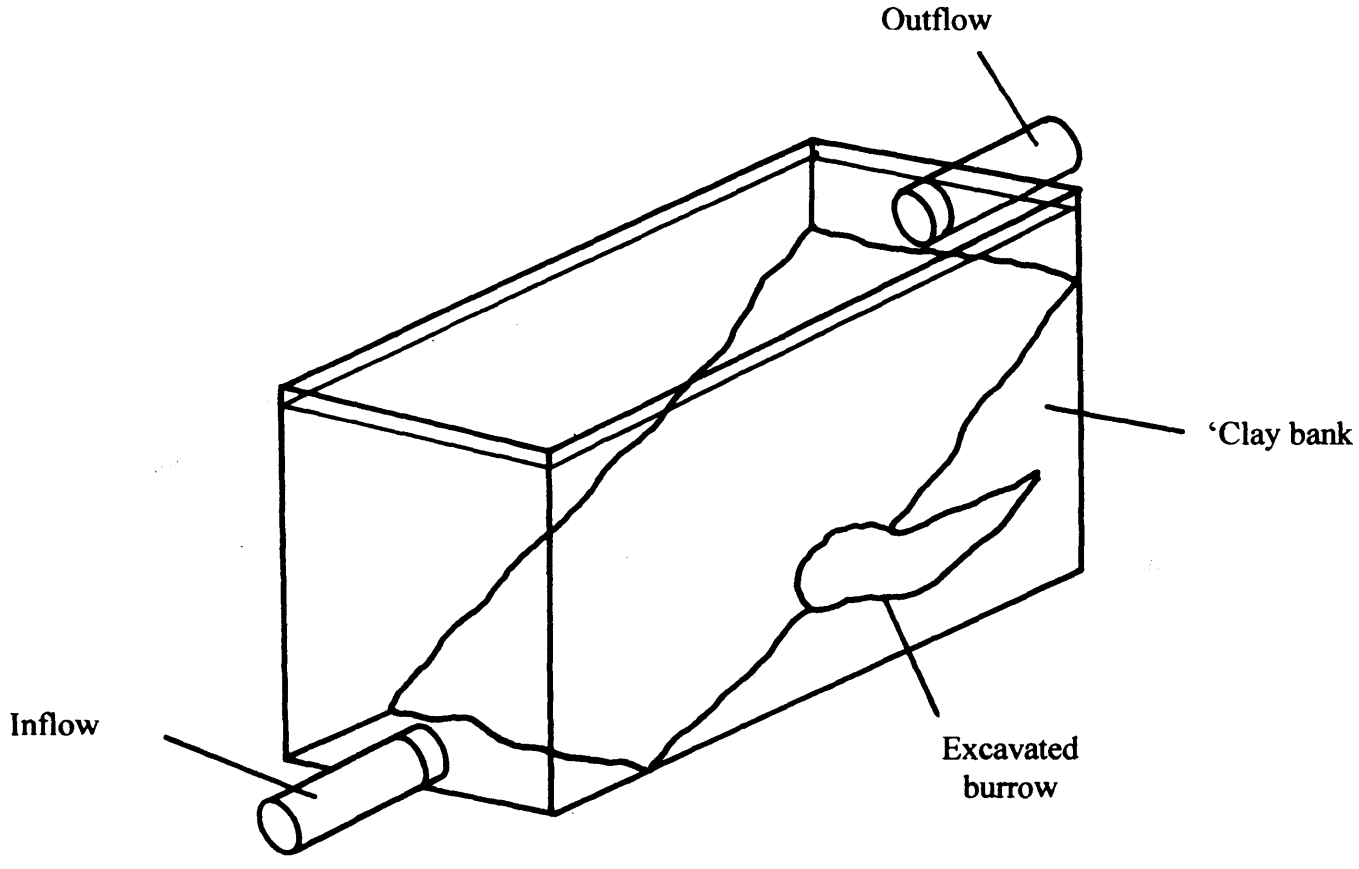
A description of the major events initiating burrowing and during burrowing were recorded, along with qualitative data on the following activities:

1) Time to initiate burrowing after being placed in the tank (h : min)
2) Total time to build burrow (h) (when repetitive periods of extended digging cease)
3) Length of burrow (mm)
4) Area of burrow opening (mm^2)
5) Number of burrow openings
6) Time spent on "burrow maintenance" (h : min day^{-1})
7) Time spent in burrow (h : min day^{-1})
8) Time spent out of burrow (h : min day^{-1})

Timings of these activities were taken from the tapes using the timer display and were accurate to ± 5min except in the case of the time to build burrow as this was more subjective

and was approximated to the nearest hour. Of the twenty animals observed, five did not burrow against the side of the tank, but were filmed nevertheless. However, timings were not obtained for activities 2, 3 and 7.

Figure 2.2.1: Tank set-up for burrowing behaviour observations. Crayfish were able to construct a burrow in the artificial clay bank. Water flow removes disturbed sediment to enable videoing.



2.2.2 Substrate Choice Experiments

Individual choices

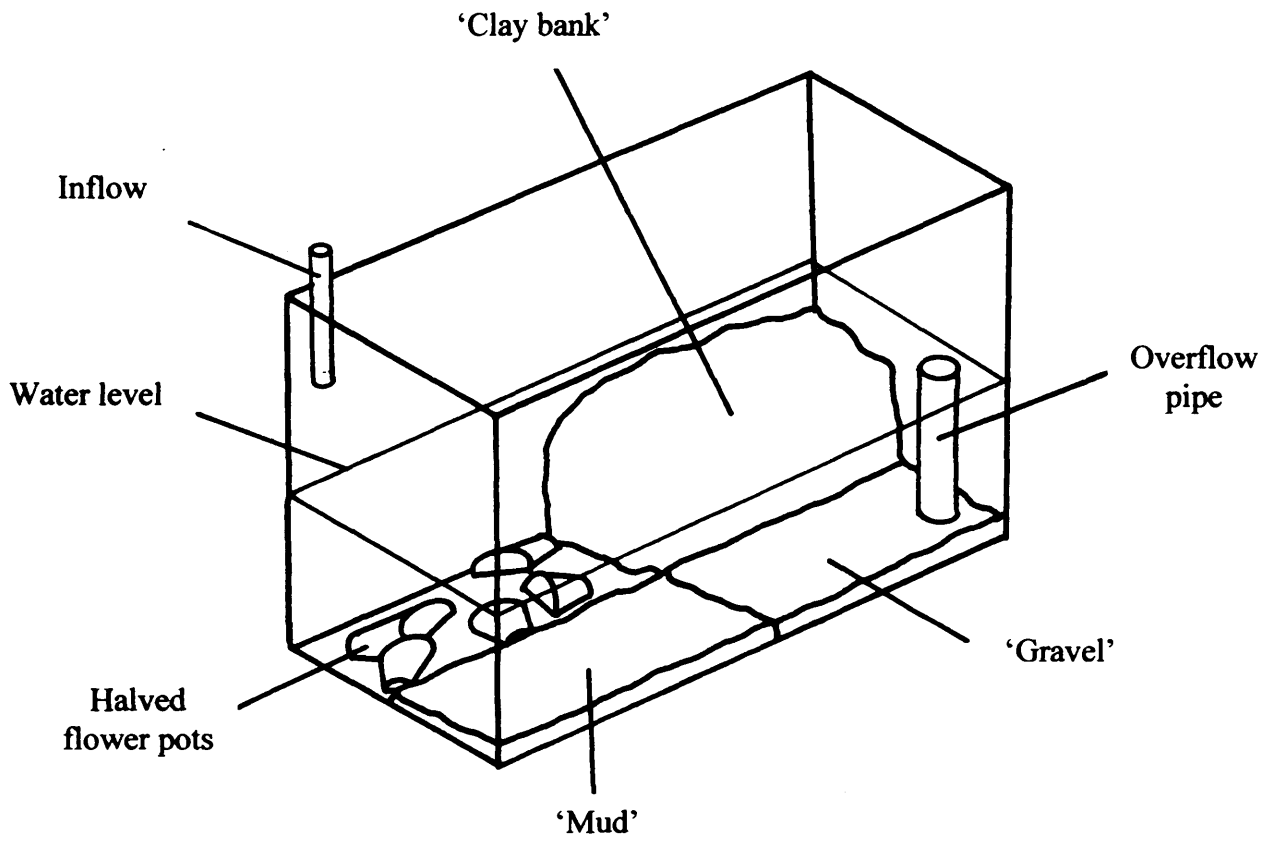
Twelve black opaque tanks (0.44 x 0.30 x 0.30 m (Merlin C4 polypropylene feed and expansion cistern) with lids, were set up containing copper-free Leicester tap water at 15°C, each with a single airline located centrally. The airlines were set-up from the same multi-way air-tap using tubing of equal length to ensure the rate of airflow was similar in all tanks. A

choice of substrate was provided in each half of the tank. These were 'gravel' verses 'mud', 'mud' verses 'clay' bank and 'clay' bank verses 'artificial shelter' (halved ceramic flower pots). Forty-eight animals were tested for each substrate combination, including the control tanks, which were prepared in the same way except for a uniform 'gravel' substrate. The carapace length of each individual was measured before it was placed centrally in each tank and left for a 24 h period with the lid on. After this, for the following 5 h, the position of the crayfish was then noted hourly (End A or B).

Group choice

Two tanks of size 1.25 x 0.65 x 0.8 m were set-up with water through-flow, using copper-free Leicester tap water. A 12 h light/dark lighting regime was used. One tank was a control and had a uniform gravel substrate and the second tank was divided into four quarters with a choice of 'mud', 'gravel', 'clay' and 'artificial shelters'. These were halved ceramic flowerpots placed inside downwards (Figure 2.2.2). Eight animals were introduced into the centre of each tank for each experiment. 4 male and 4 female crayfish were used in each experiment to simulate wild population sex ratios in a high-density environment. Typical densities recorded by (Harris, 1999) for the Gaddesby Brook were in the range of 1.2 to 12.3 m^{-2}, with sex ratios in the range of 0.8: 1 to 5.0: 1 (Male: female) depending on time of year. However, the higher male ratios have been attributed to their increased trappability in October/November when searching for a mate. Animals used were in the size range of 34.9 – 65.1 mm and counts of crayfish on each substrate type were recorded at 24 h intervals over 5 days to establish preferred daytime refuges. This experiment was conducted 5 times with a total of 40 individuals.

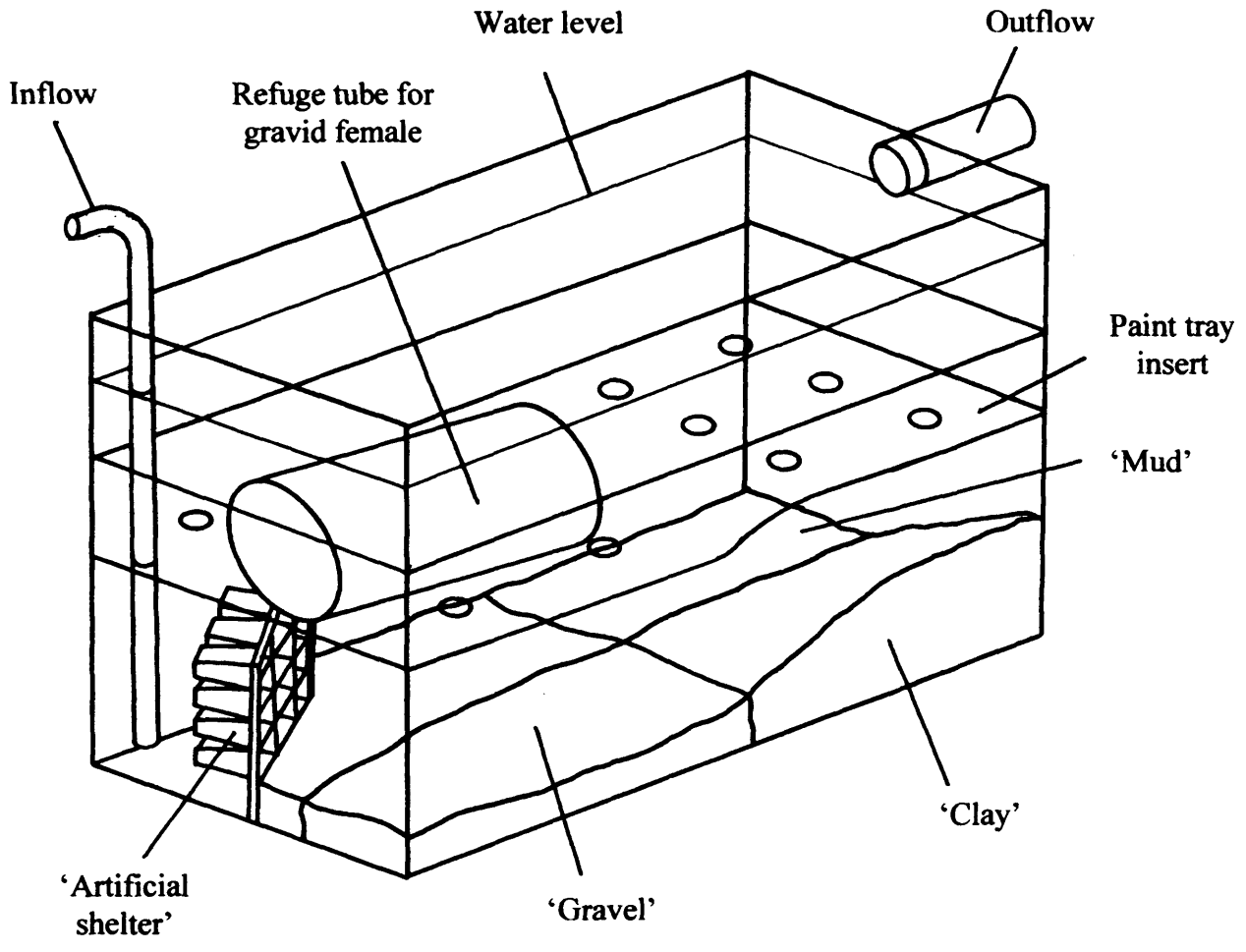
Figure 2.2.2: Tank set-up for substrate choice experiments involving groups of animals.



Juvenile Choice Experiment

Seven transparent acrylic tanks of dimensions 0.28 x 0.42 x 0.3 m were set-up with continuous water-through flow with a 12 h light/dark cycle. Each tank was divided into quarters, each with a different substrate type, 'mud', 'clay', 'gravel' and 'artificial shelter'. Here, the shelters were black plastic plant propagation trays with 'plugs' of dimensions 2 x 2 x 3.4 cm. Above the substrates were fitted Harris™ paint tray inserts which had 1cm diameter holes cut into them at 10 cm intervals (12 holes in total) to allow juvenile animals to drop through onto the different substrates. On the tray an adult gravid female was placed with an opaque plastic pipe provided as a refuge (Figure 2.2.3). The tanks were monitored daily and, on the release of the last juvenile crayfish from the females abdomen, counts were made of the number of individuals on each substrate type each day over 5 days. Controls were conducted in the same way but with an all-gravel substrate.

Figure 2.2.3: Tank set-up for juvenile crayfish substrate choice experiments.



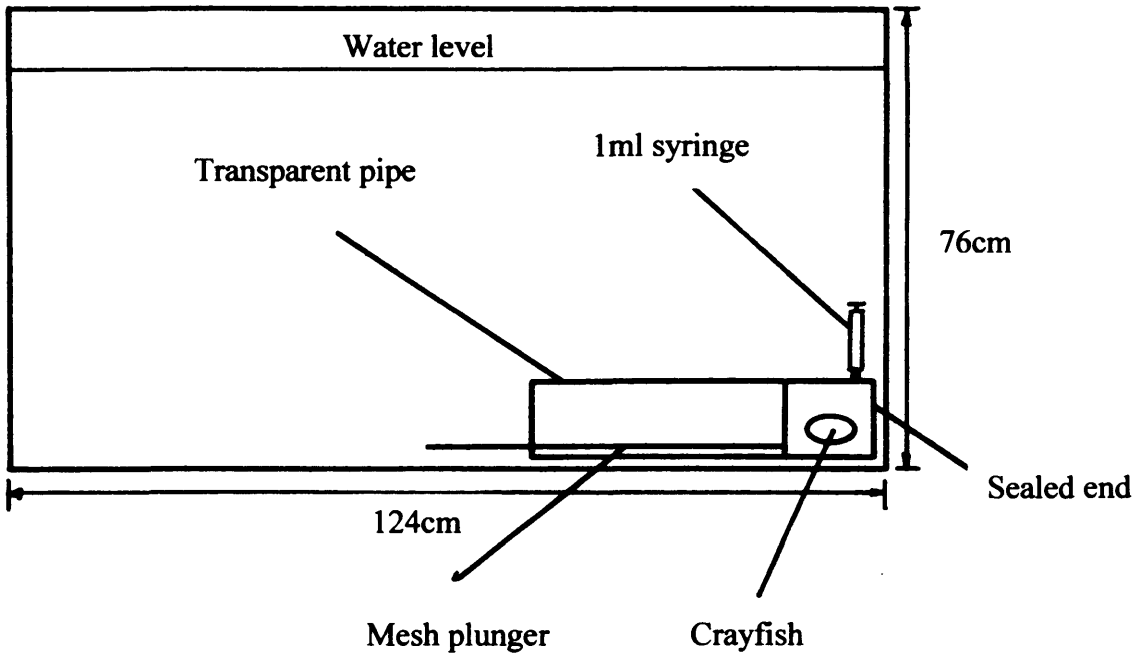
2.2.3 Rates of burrow irrigation by crayfish and passive irrigation

Rates of burrow irrigation by crayfish

This experiment was designed to establish if *P. leniusculus* irrigate their burrows and if so by what means and how rapidly. A transparent Perspex pipe (diameter 7.5 cm and length of 45 cm) was sealed at one end with a piece of acrylic sheet. A hole was drilled into it on the upper-most surface, 1 cm from the sealed end into which the Luer fitting of a plastic disposable 1ml syringe fitted tightly. In a lidded tank, N_2 was pumped through copper-free Leicester tap water to make it hypoxic down to a Po_2 in the range of 40-70 mm Hg. This water was then poured into the transparent pipe and covered with a square of acrylic. The pipe was then submerged in a filled static glass tank of dimensions 1.22 x 3.8 x 4.5 m and the

acrylic cover removed. Immediately a 0.5 ml sample of water was extracted by the syringe, removed and replaced by a second syringe. A crayfish was then introduced into the tube, pushed gently to the end and restrained by a mesh (1 cm holes) plunger (Figure 2.2.4). 0.5 ml water samples were then extracted at intervals of: 0, 0.5, 1, 1.5, 2, 2.5, 5, 10, 20,30, 60, 90 and 120 minutes. (pre-determined by preliminary tests). The Po_2 levels of these samples were then determined using the Radiometer oxygen electrode as described above. Controls were also carried out in exactly the same way, except with no animals present. Graphs of Po_2 verses time were then plotted and fitted using the function $Y=A-Bexp[kt]$ where y = Po_2 of burrow water (mm Hg), A = curve asymptote, B = asymptote to y intersect value, k = constant h^{-1}, t = time (h) (Crowe, 1969). From ascertaining the constant k h^{-1}, it was possible to deduce a water turnover rate in l h^{-1} by multiplying by the cylinder volume (litres), and relate this to each animal mass and carapace length.

Figure 2.2.4: Tank set-up for measuring crayfish irrigation rates

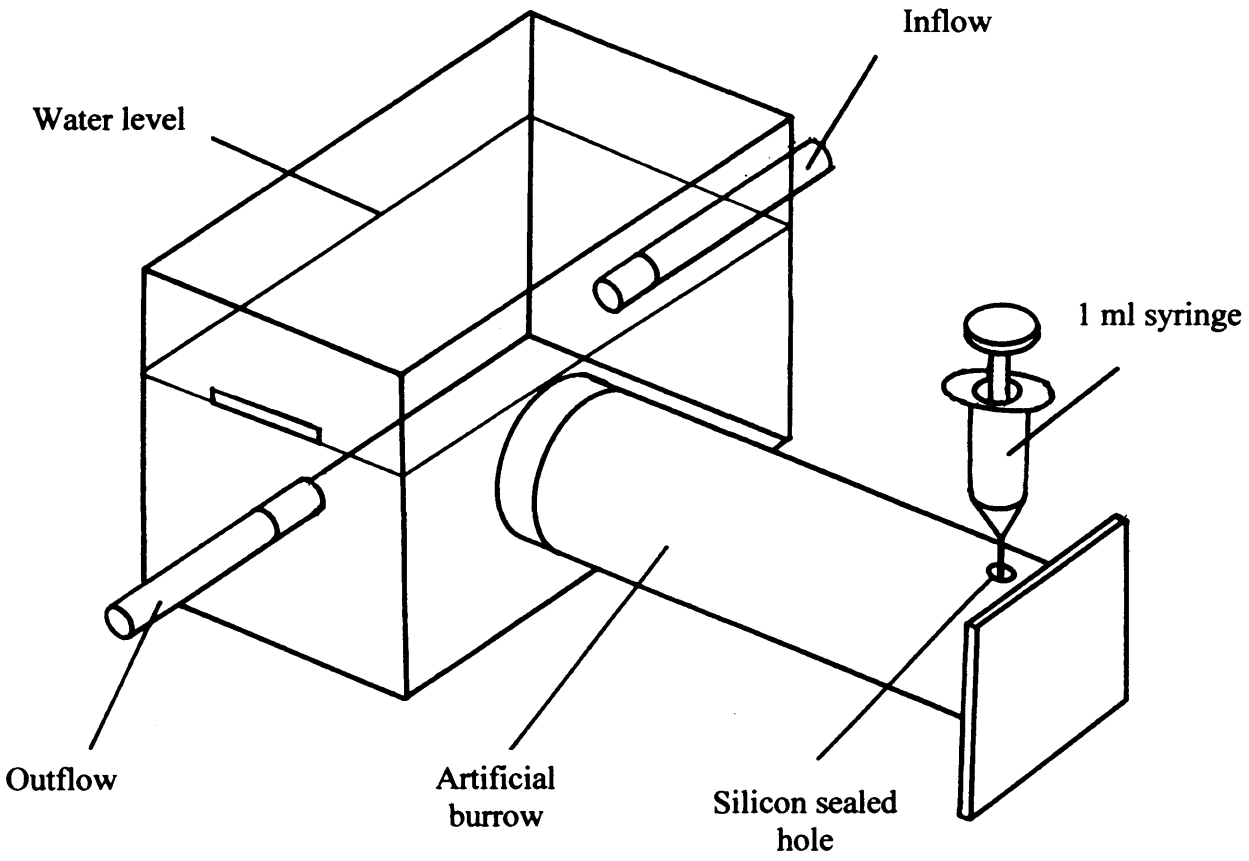


Passive irrigation

In order to interpret data on burrow water chemistry and possible crayfish irrigation, it was necessary to determine the effect, if any, that stream flow had on water exchange within *P.leniusculus* burrows. A tank of dimensions 0.44 x 0.30 x 0.30 m with inflow and outflow

tubes (over-flow level set at 0.3 m) was fitted with a transparent Perspex tube set-up at right angles to the direction of water flow. The pipe had a diameter of 7.5 cm and length of 45 cm and was sealed at one end (as described above). In order to ensure a water tight seal between the pipe and tank, Plumbers mate™ was used. This set-up thereby simulated stream flow past a crayfish burrow entrance. Flow rates of 0 to 0.6 m sec^{-1} were generated by adjustments of tap pressure and measured by a Marsh McBirney 2000 cm Flow-Mate current meter. The pipe was filled with hypoxic water and sealed whilst the tank was filled and adjusted to the desired flow rate. Next the seal was removed and 0.5 ml water samples were extracted with a 1 ml hypodermic syringe through the silicon-sealed hole at the end of the pipe every 5 minutes for 1 h (Figure 2.2.5). Changes in Po_2 were recorded and plotted against time as described above.

Figure 2.2.5: Tank set-up for measuring burrow water exchange with increasing external water flow.



Statistics

Statistical analysis was carried out using two software packages, Minitab and TexaSoft's 'WINKS 4.651' program. All data was checked for normality and equal variance before the

application of appropriate parametric or non-parametric tests. The TexaSoft's 'WINKS 4.651' program was used because it could perform ranked Tukey multiple comparisons on a non-parametric Kruskal-Wallis analysis.

Chapter 3

Results

Chapter 3: Results

3.1 Field Studies

3.1.1 Burrow densities in relation to substrate type

The Gaddesby Brook runs through mainly rural areas and farmland in a northwesterly direction in North Leicestershire. It is approximately 15 km long and joins the Queniborough Brook shortly before running into the R.Wreake at East Gosgote. The difference in altitude between its source and joining the R.Wreake is ~125 m.

Distribution of substrate type varied, but consisted in the most part of clay/mud banks with silt or gravel streambeds. The remaining sites predominantly consisted of large rocks, boulders and masonry.

Aim: To establish that wild populations of *P. leniusculus* select a particular substrate type for burrowing.

Hypothesis: There will be a relationship between the burrow construction sites of wild populations of *P. leniusculus* and substrate composition.

During the sampling period, the water depth range was 0.08 m – 1.2 m. The bank substrate varied considerably, with clay and sand being the predominant components combined with smaller proportions of fine and coarse silt. Large stretches of the stream were surrounded by dense vegetation in the summer particularly, such as *Crataegus monogyna*, *C. laevigata*, *Alnus glutinosa*, *Urtica dioica*, *Lamium album*, *Cirsium arvense* and *Galium aparine*. In these areas, the bank substrate was bound by ramifying shrub and tree roots. Stream water current velocities ranged from $-0.14 ms^{-1}$ to $1.3 ms^{-1}$ (some pools having small back eddies), changing rapidly in response to rainfall. The width and depth of the brook varied considerably along its length and was as little as a few centimetres to several metres. The stream pH ranged from 7.4 to 8.6 (Table 3.1.1).

Table 3.1.1 Mean physical stream data with standard errors (SE) and ranges. Data collected at 78 randomly selected sites between January and April 2000.

	Mean ± SE	Range	
		Min	Max
Stream depth (m)	0.36 ± 0.03	0.08	1.2
Stream current velocity (ms^{-1})	0.26 ± 0.03	-0.14	1.3
Stream temperature (°C)	6.38 ± 0.34	1	14.5
Stream pH	8.03 ± 0.03	7.4	8.6
Burrow density (No. burrows m^{-1} bank)	2.39 ± 0.32	0	14

Burrow densities of *Pacifastacus leniusculus,* a known burrowing crayfish species in the UK (Holdich *et al,* 1995), were first investigated by Harris and Young at the Gaddesby Brook in 1996 (Figure 3.1.1). At this time, burrows were identified from Owston (SK 778 083) to just before Ashby Folville (SK 711 115). The situation in the year 2000, saw burrows present as far downstream as the Gaddesby sewage works (SK 685 127), with an overall increase in burrow densities (Figure 3.1.2).

Figure 3.1.1 Map of the distribution and densities of signal crayfish burrows in the Gaddesby Brook in 1996 as recorded by Harris and Young.

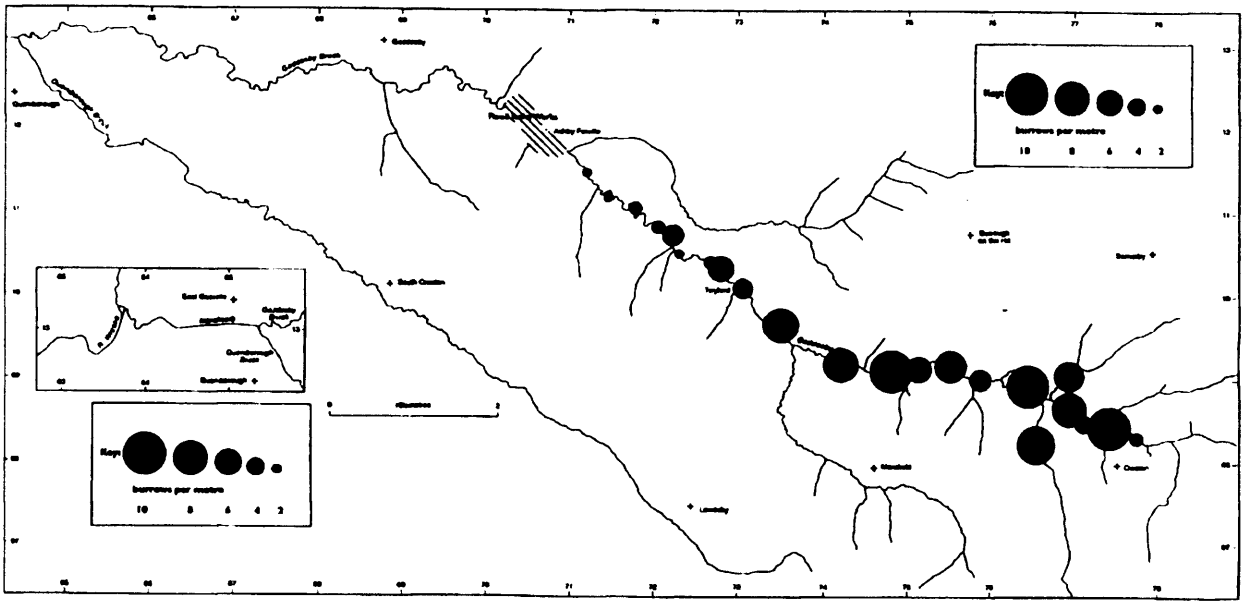
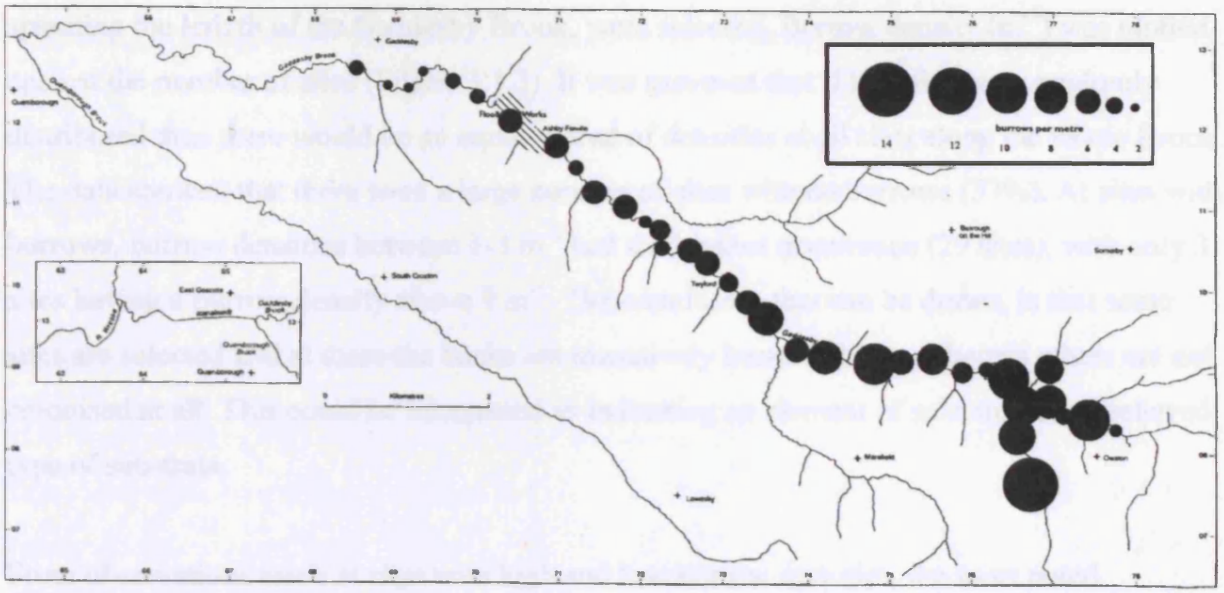
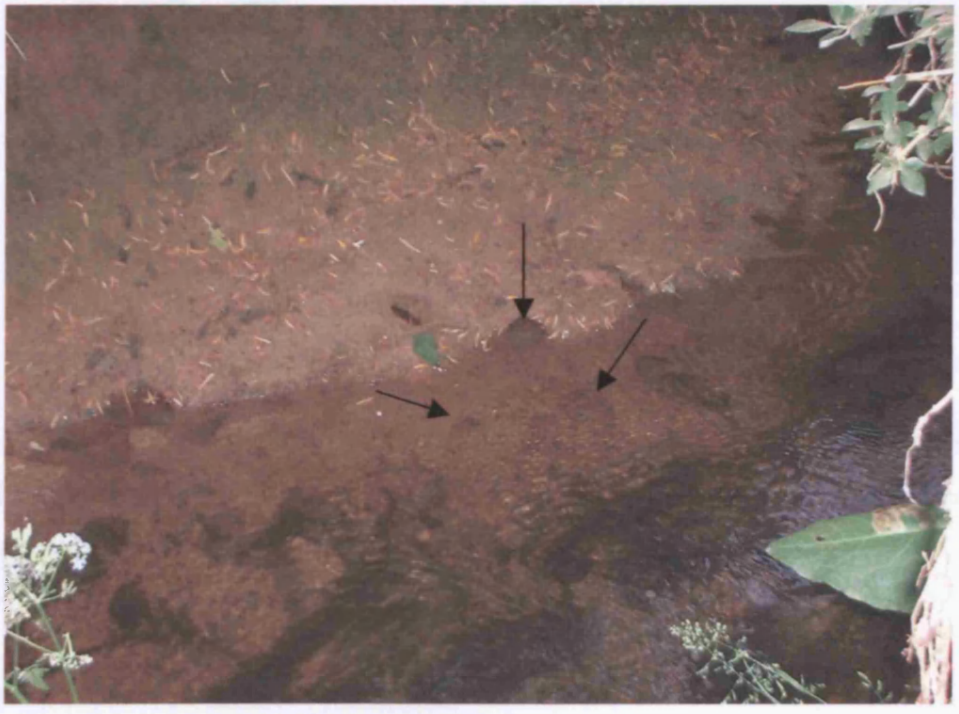


Figure 3.1.2 Map of the distribution and densities of signal crayfish burrows in the Gaddesby Brook in 2000.



Preliminary observations of the stream revealed large numbers of oval burrow openings of different sizes, located at or below the water level (Plate 3.1.1). It appeared that larger numbers of these openings were present in sections of stream bank with a clay-like texture.

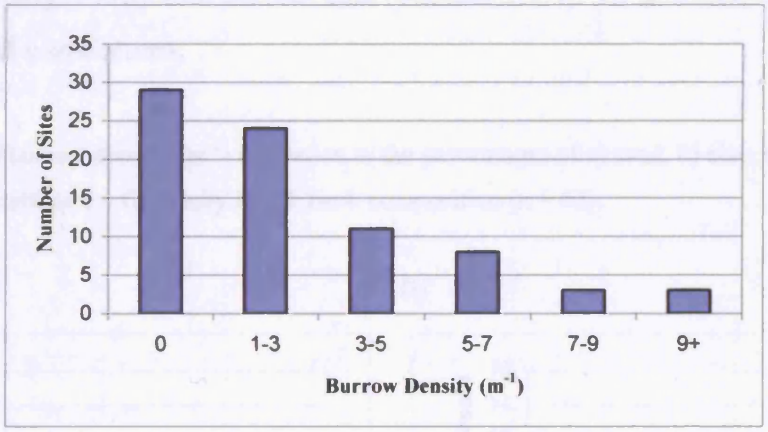
Plate 3.1.1 Photograph of a section of stream bank with numerous burrow openings (indicated by arrows).



To establish whether the burrows of *P. leniusculus* were distributed randomly or whether there was a degree of burrow aggregation at particular sites, seventy-eight random sites, spanning the length of the Gaddesby Brook, were selected. Burrow density (m^{-1}) was plotted against the number of sites (Figure 3.1.3). It was assumed that if burrows were randomly distributed then there would be an equal spread of densities at all sites along the whole Brook. The data showed that there were a large number of sites with no burrows (37%). At sites with burrows, burrow densities between 1-3 m^{-1} had the highest occurrence (29 sites), with only 3 sites having a burrow density above 9 m^{-1}. The conclusion that can be drawn, is that some sites are selected and at these the banks are intensively burrowed into, whereas others are not colonised at all. This could be interpreted as indicating an element of selection of a preferred type of substrate.

From observations made at sites with high and low burrow densities, the most noted difference was the sediment composition of bank substrate. Making comparisons of substrate composition with burrow densities would test the hypothesis above.

Figure 3.1.3 The numbers of sites with different burrow densities (m^{-1}) from the Gaddesby Brook. (n = 78).



The composition of the stream bank substrate was represented as percentages of sand, coarse silt, fine silt and clay at each of the 78 sites (Appendix VI). Clay and sand accounted for the largest proportions of bank substrate composition (Table 3.1.2).

Table 3.1.2 Mean % composition and ranges of stream bank substrate at 78 randomised sites along the Gaddesby Brook.

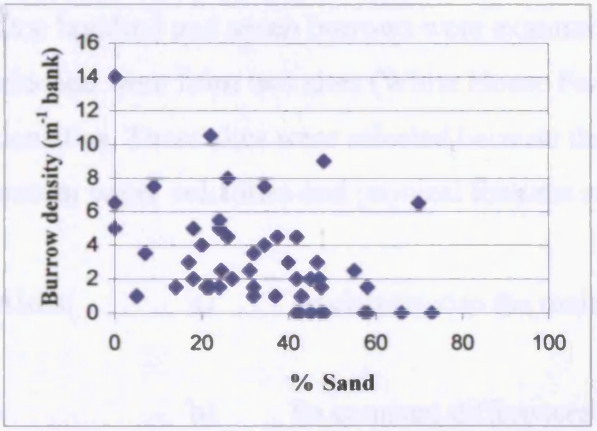
	% composition (Mean ± SE)	Range Min	Max
% clay	39.03 ± 1.48	16	90
% sand	34.36 ± 2.09	0	73
% fine silt	16.51 ± 0.79	1	35
% coarse silt	10.10 ± 0.52	0	23

The mean value of clay content in the bank substrate at the 78 sites sampled was 39%. This value was used to compare counts of burrow densities above and below this threshold. The result showed a significantly higher burrow density in bank substrates with more than 40% clay (Mann-Whitney, W = 1471.0, P = 0.002).

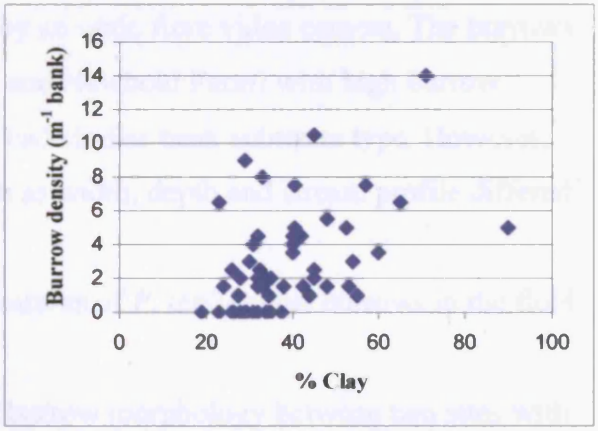
Correlations were carried out on burrow densities with percentages of sand, clay, fine silt and coarse silt. Data on sites downstream of Lowenva Lodge were omitted as these sites were judged, not to have attained a maximum stable crayfish density, having compared historical data (Harris & Young, 1995) with current data (Section 3.1.4). To this end, 68 sites were used for these statistical correlations.

Figure 3.1.4 Plots of burrow density (m^{-1}) in relation to the percentages of a) sand, b) clay, c) fine silt and d) coarse silt, which constitute the Gaddesby Brook bank composition (n = 62).

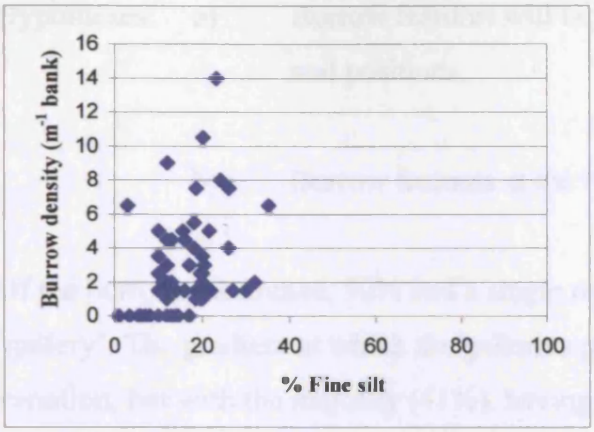
a)



b)



c)



d)

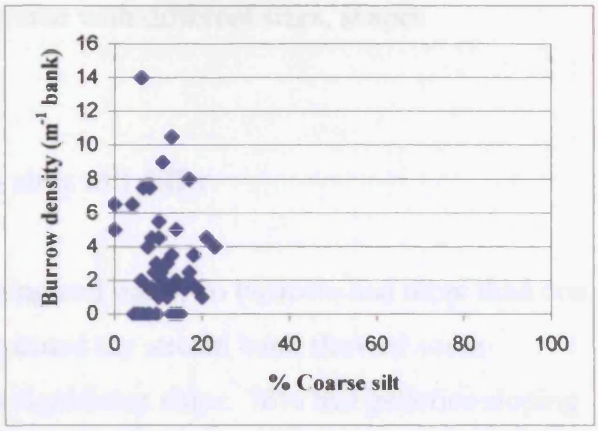


Figure 3.1.4a shows a significant negative correlation (Spearman Rank Correlation, $t = -0.349$, $df = 60$, $p<0.01$), with a greater sand content at sites with a lower burrow density. A larger proportion of sand in the stream bank would render it less stable and less desirable for burrow construction. This hypothesis was supported by the positive correlation between % clay in the bank substrate and burrow density (Figure 3.1.4b). Higher clay content was significantly related to a higher burrow density ($t = 2.35$, $df = 60$, $p<0.05$). Neither the fine nor coarse silts alone (Figure 3.1.4c & d) were significant in determining preferred substrate for burrow construction (Fine silt, $t = 1.97$, $df = 60$, $p>0.05$) (Coarse silt, $t = 1.4$, $df = 60$, $P>0.05$). However, when clay was combined with fine silt and sand was combined with coarse silt, this led to a strengthening of the association between burrow density and bank substrate (Spearman Rank $t = 3.05$, $n=62$, $p=<0.01$ and $t = -2.97$, $n = 62$, $p = <0.01$ respectively).

3.1.2 Internal burrow features

One hundred and seven burrows were examined by an optic fibre video camera. The burrows videoed were from two sites (White House Farm and Newbold Farm) with high burrow densities. These sites were selected because they had similar bank substrate type. However, stream water velocities and physical features such as width, depth and stream profile differed.

Aims:

a) To characterise the main features of *P. leniusculus* burrows in the field.

b) To compare differences in burrow morphology between two sites with different water flow and physical features.

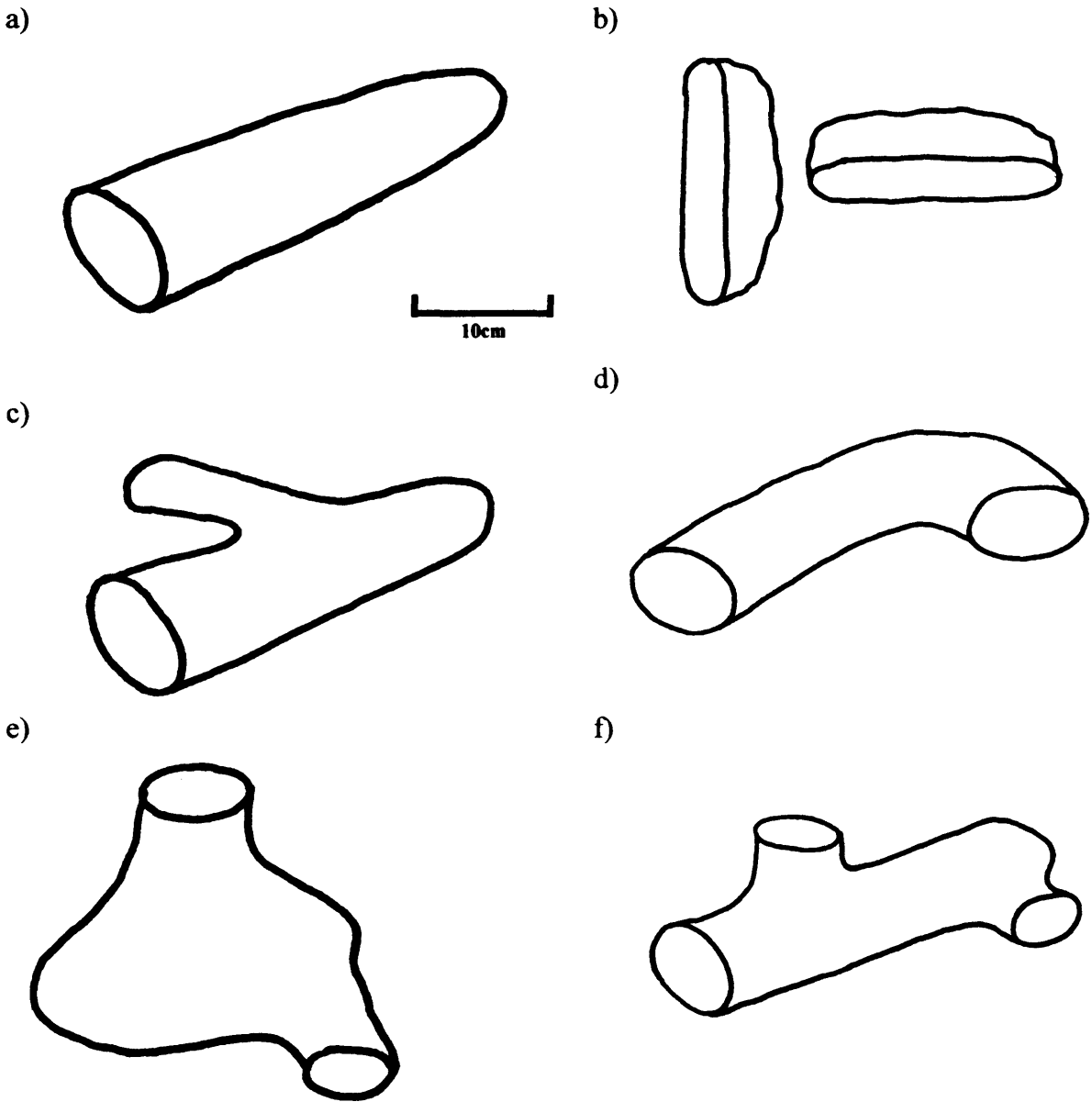
Hypotheses: a) Burrow features will be diverse with different sizes, shapes and positions.

b) Burrow features at the two sites will differ.

Of the burrows examined, 92% had a single opening and only two burrows had more than one 'gallery'. The gradient at which the galleries penetrated the stream bank showed some variation, but with the majority (41%), having no significant slope. 38% had galleries sloping in an upward direction, with only 21% having galleries sloping in a downward direction.

Six different burrow shapes were identified, the most common being those with a single entrance and tapered gallery (77% of sample) (Plate 3.1.2a). Nine per cent of burrows were crevice-shaped (the crayfish had excavated further into a naturally created shelf) (Plate 3.1.2b). Six per cent of the burrows were cylindrical with an additional branching gallery (Plate 3.1.2c). A few burrows were cylindrical with two entrances (5%) (Plate 3.1.2d). Other shapes were chambered with two entrances (1%) (Plate 3.1.2e) and cylindrical with three entrances (1%) (Plate 3.1.2f). Only 3% of burrows had plant matter growing within them (such as the penetrating roots of *Urtica dioica*), although some burrows had plant roots concealing the burrow entrance (tree roots such as *Alnus glutinosa*).

Plate 3.1.2 The six identified burrow shapes a) single entrance cylinder b) crevice-shaped burrows c) branched cylinder burrow, d) two-ended cylindrical burrow, e) chambered burrow and f) three-ended cylindrical burrow.



From Table 3.1.3, it can be seen that of the 107 burrows examined only 43% were occupied. This could mean one of several things. It is possible that at the time of observation occupants were out foraging or had died (from predation, age or disease). Some may have abandoned their burrows or possibly had constructed and were utilising more than one burrow. Newbold Farm was found to have a higher burrow occupancy rate than that of White House Farm.

Table 3.1.3 Burrow dimensions, depths and occupancy at two sites on Gaddesby Brook. Burrow depth refers to the depth below water surface of burrow entrances. Means ± SE (range).

Burrow features	Site		
	Newbold Farm	White House Farm	Combined
Total No. burrows sampled	57	50	107
% of occupied burrows	51%	34%	43%
Entrance depth from water surface (cm)	10.7 ± 1.5 (0 – 46)	3.7 ± 0.8 (0 - 18)	7.6 ± 0.9 (0 – 46)
Burrow entrance height (cm)	5.9 ± 0.4 (1.5 – 16)	5.6 ± 0.3 (2 – 11)	5.7 ± 0.3 (1.5 – 16)
Burrow entrance width (cm)	5.2 ± 0.3 (1.5 – 14)	7.0 ± 0.6 (1.5 – 22)	6.0 ± 0.3 (1.5 – 22)
Burrow length (cm)	14.6 ± 1.1 (3.5 – 40)	21.5 ± 2.2 (6 – 79)	17.7 ± 1.2 (3.5 – 79)

Some burrows extended up to 79 cm into the stream bank, others were very short and not much more than the body length of an averaged sized *P. leniusculus*. Some openings were wide (showing signs of erosion of the edges). Others were more defined oval/circular openings.

There were significant differences between the burrow depths as measured from the stream surface. The mean burrow depth was significantly greater at Newbold Farm ($W = 3504.0$, $p = <0.001$) than at White House Farm. This may have been due to the stream being deeper, but it appeared that crayfish were prepared to construct burrows at different water depths within the stream bank. Burrow length and width also varied between the two sites. Burrow length was significantly greater at White House Farm ($W = 2540.0$, $p = 0.003$), as was width ($W = 2583.5$, $p = 0.008$).

3.1.3 The internal burrow water chemistry

Water samples were extracted by large hypodermic syringe from 267 burrows and analysed for selected water chemistry components. This data was compared with that of the external flowing stream water also collected on each sampling occasion (Table 3.1.4).

Aim: To establish what affect the presence of *P. leniusculus* has on internal burrow water chemistry.

Hypothesis: The internal burrow water chemistry of occupied burrows will differ significantly from unoccupied burrows and external stream water.

From Table 3.1.4, it can be seen that the total ammonia (T_{amm}) levels within burrows were higher than the external stream water at both sites during summer and winter. However, the differences were significantly greater in summer compared to that of winter (Mann-Whitney, W = 15568.0, P = 0.008). This may be linked to increased activity levels shown by *P. leniusculus* during this season. The P_{O_2} levels within the burrows during winter at both sites were quite similar to that of the stream level. During summer, however, they were significantly higher (Mann-Whitney, W = 13441.5, P = 0.036), demonstrating slight hypoxic conditions. In general, levels of $\sum CO_2$ were higher within the burrows showing slight hypercapnic conditions, however, there were no significant differences between summer and winter levels (Mann-Whitney, W = 15687.0, P = 0.123). The pH levels remained fairly consistent, with no substantial differences between sites or within burrows.

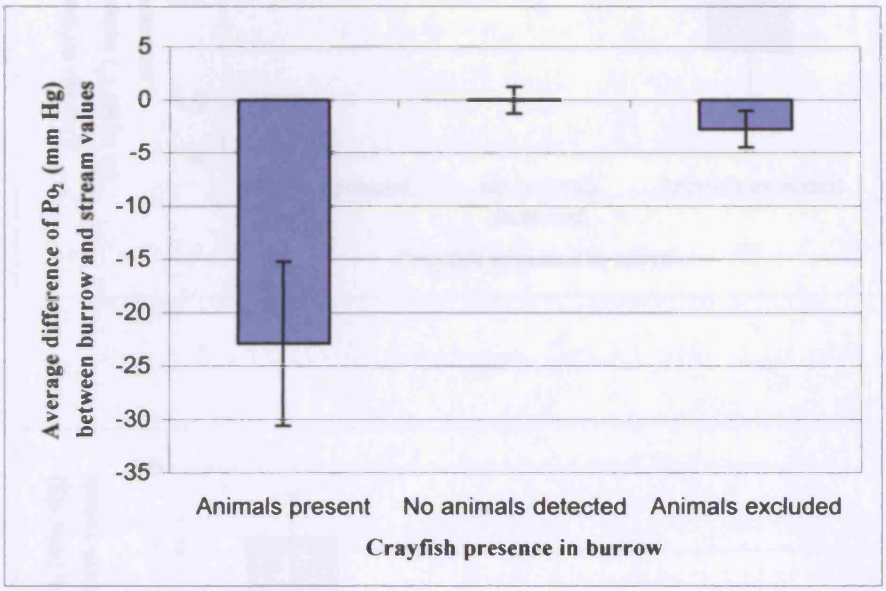
Table 3.1.4 Ammonia concentration (T_{amm} μmoles l^{-1}), Po_2 (mmHg), ΣCO_2 (m equiv l^{-1}) and pH in water samples extracted from 131 burrows at Newbold Farm and 125 burrows at White House Farm. (In bold: Stream values sampled at each site). Samples taken in winter and summer are also shown. (Mean ± SE).

Site	Mean T_{amm} (μmoles l^{-1}) (Stream level)	Mean Po_2 (mmHg) (Stream level)	Mean ΣCO_2 (m equiv l^{-1}) (Stream level)	Mean pH (Stream level)
Newbold Farm	14.08 ± 0.77	149.51 ± 0.80	5.83 ± 0.27	8.18 ± 0.01
(Winter)	**(7.24 ± 0.35)**	**(150.50 ± 5.5)**	**(5.09 ± 1.31)**	**(8.21 ± 0.01)**
Newbold Farm	28.78 ± 3.99	125.91 ± 3.79	8.57 ± 0.33	8.18 ± 0.03
(Summer)	**(15.57 ± 9.57)**	**(142.70 ± 11.70)**	**(7.07 ± 1.87)**	**(8.01 ± 0.5)**
White House	13.01 ± 1.25	151.32 ± 0.78	5.38 ± 0.14	8.05 ± 0.01
Farm (Winter)	**(6.75 ± 0.35)**	**(148.20 ± 3.79)**	**(7.58 ± 3.30)**	**(8.16 ± 0.15)**
White House	12.56 ± 1.53	132.29 ± 3.92	7.73 ± 0.38	8.01 ± 0.01
Farm (Summer)	**(4.10 ± 2.37)**	**(134.55 ± 2.46)**	**(7.47 ± 2.19)**	**(8.11 ± 0.65)**

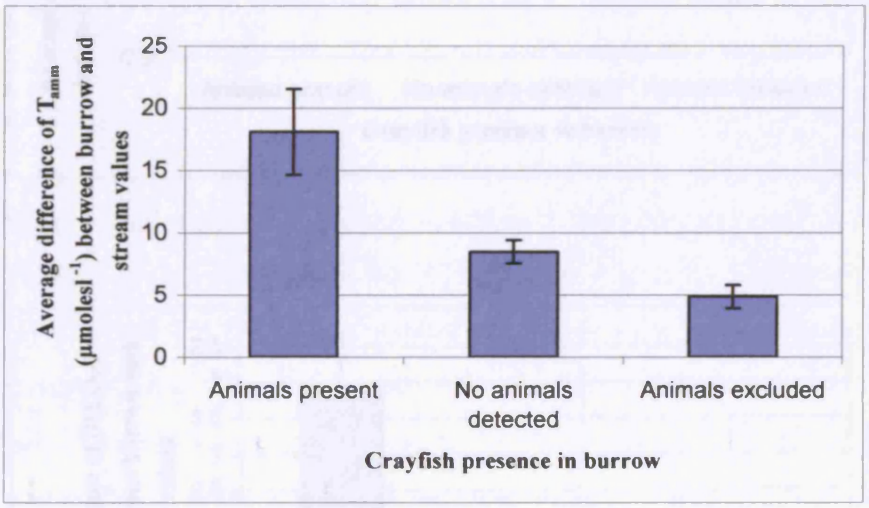
Having deducted the stream values from each corresponding burrow water chemistry measurement, it was possible to compare the effect *P. leniusculus* had on internal burrow water chemistry when occupying it. Also a group of burrows were sealed to prevent animals entering (having ensured none were within, initially) while allowing water exchange between them and the stream (Figure 3.1.5).

Figure 3.1.5 Mean differences between internal burrow water chemistry of a) Po_2 (mm Hg), b) T_{amm} (μmoles l^{-1}), c) $\Sigma CO2$ (m equiv l^{-1}), d) Pco_2 (mm Hg), e) HCO_3^- (m equiv l^{-1}) and external stream water values. Shown for animals present, not detected and excluded from burrows (± SE).

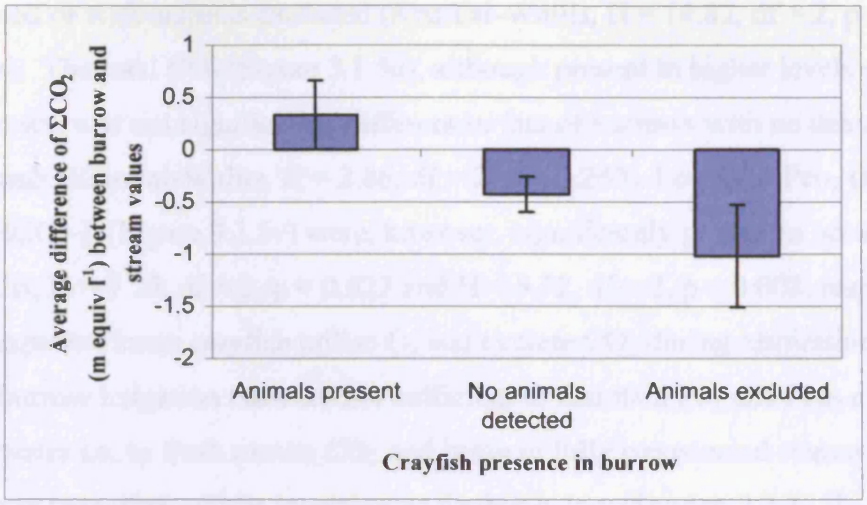
a)



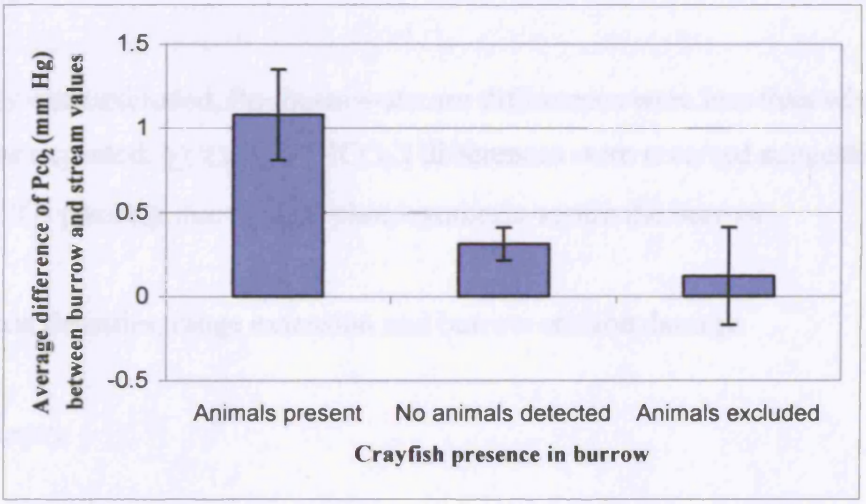
b)



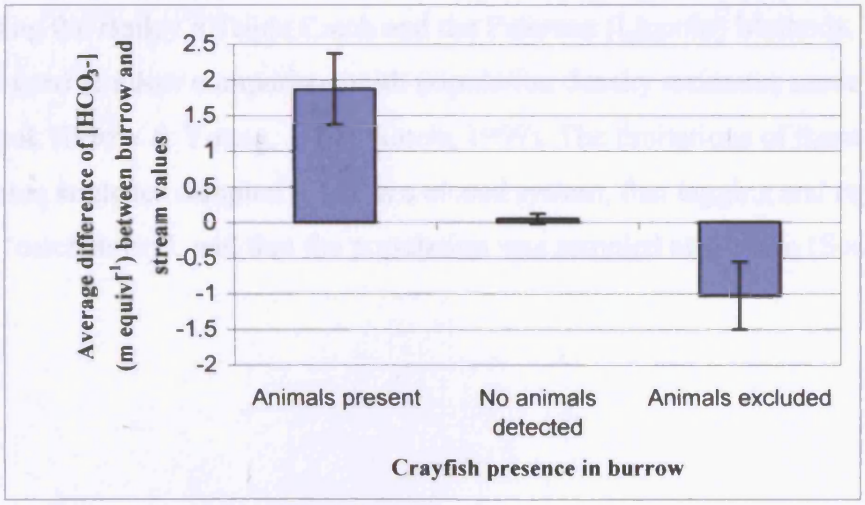
c)


Average difference of ΣCO2 (m equiv l-1) between burrow and stream values
1
0.5
0
-0.5
-1
-1.5
-2
Animals present
No animals detected
Animals excluded
Crayfish presence in burrow

d)


Average difference of Pco2 (mm Hg) between burrow and stream values
1.5
1
0.5
0
-0.5
Animals present
No animals detected
Animals excluded
Crayfish presence in burrow

e)


Average difference of [HCO3-] (m equivl-1) between burrow and stream values
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
Animals present
No animals detected
Animals excluded
Crayfish presence in burrow

Po_2 levels within occupied burrows were significantly lower than within burrows with no animals detected or with animals excluded (Kruskal-Wallis, H = 14.82, df = 2, p = 0.001) (Figure 3.1.5a). The total CO_2 (Figure 3.1.5c), although present in higher levels within occupied burrows, was not significantly different to that of burrows with no detected or excluded animals (Kruskal-Wallis, H = 2.86, df = 2, p = 0.240). Levels of Pco_2 (Figure 3.1.5d) and $[HCO_3^-]$ (Figure 3.1.5e) were, however, significantly greater in occupied burrows (Kruskal-Wallis, H = 7.23, df = 2, p = 0.027 and H = 9.72, df = 2, p = 0.008, respectively). This is to be expected since crayfish utilise O_2 and excrete CO_2 during respiration. It also suggests that burrow irrigation rates are not sufficient to maintain Po_2 and Pco_2 at levels equal to the stream water i.e. to flush excess CO_2 and bring in fully oxygenated stream water. This aspect of burrow irrigation will be investigated further below (Section 3.2.3). T_{amm} levels were also significantly greater in occupied burrows (Kruskal-Wallis, H = 11.56, df = 2, p = 0.003) (Figure 3.1.5b).

Where animals were excluded, Po_2 burrow-stream differences were less than when animals were present as expected. $\sum CO_2$ and $[HCO_3^-]$ differences were reversed suggesting a utilisation of CO_2 possibly due to algal photosynthesis within the burrow.

3.1.4 Population densities, range extension and burrow erosion damage

Population density

Population density measurements were conducted over three years at five sites between Owston (SK776 095) and Gaddesby (SK 686 127) (M&M Figure 2.1.2). Population estimates were made using the Bailey's Triple Catch and the Peterson (Lincoln) Methods. These methods were used to allow comparison with population density estimates made in the Gaddesby Brook (Harris & Young, 1995; Harris, 1999). The limitations of these methods are that, they assume stretches sampled acted as a closed system, that tagging and repeat capture did not affect 'catchability', and that the population was sampled at random (Southwood, 1978).

Aim: To monitor the change in population densities at five sites along the Gaddesby Brook over a three-year period.

Hypothesis: Crayfish population density at any one site will increase until all available substrate and resources have been used.

Table 3.1.5 provides data on changes in population density at the five consecutive sites over three years. Newbold Farm was the site nearest to the point of the first introduction of *P. leniusculus* in the Gaddesby Brook. Gaddesby (village) was the site furthest away. The site with the highest overall population density (m^{-2}) was White House Farm. Although this site had a low catch per unit effort (CPUE), when corrections for length of bank and area of streambed were made (m^{-2}) it proved to have the greatest population density. The site at Gaddesby had the lowest population densities and with such low CPUE's it was not possible to calculate the densities using the Bailey's Triple Catch method. The population densities at all sites showed fluctuation from year to year but, in general, at the first three sites, densities remained relatively constant throughout. The final two sites, however, showed in some cases, more than a two-fold increase in population densities over the three years.

Table 3.1.5 Calculated population densities of signal crayfish at five sites in the Gaddesby Brook.

Site	Year	Average CPUE (1)	Density m^{-1} (2)	Density m^{-1} (3)	Density m^{-2} (4)	Density m^{-2} (5)
Newbold	2000	5.0	12.0	11.3	8.0	7.5
Farm	2001	5.3	13.0	12.2	8.7	8.1
(SK774-085)	2002	1.4	12.0	4.7	8.0	3.1
White House	2000	2.1	10.2	12.8	8.5	10.7
Farm	2001	4.2	13.6	6.7	11.3	5.6
(SK753-093)	2002	2.1	7.2	4.1	6.0	3.4
Lowenva	2000	3.3	22.0	9.2	5.2	2.2
Lodge Farm	2001	8.5	29.1	32.3	6.9	7.7
(SK737-094)	2002	4.0	18.1	34.0	4.3	8.1
Ashby Folville	2000	0.3	0.0	0.8	0.0	0.1
(SK710-118)	2001	3.7	6.1	17.1	1.1	3.1
	2002	3.9	13.3	12.2	2.4	2.2
Gaddesby	2000	0.5	-	0.6	-	0.3
(SK693-127)	2001	0.4	-	0.8	-	0.4
	2002	0.6	-	0.9	-	0.4

Legend

(1) CPUE (catch per unit effort) calculated during overall sampling period.

(2) Estimates by Bailey's Triple Catch expressed m^{-1} stream bank.

(3) Estimates by Peterson (Lincoln) Method expressed m^{-1} stream bank.

(4) Estimates as (2) but expressed m^{-2} stream bed.

(5) Estimates as (3) but expressed m^{-2} stream bed.

Range extension

Trapping and hand searching ascertained the current extent of *P. leniusculus* in the Gaddesby Brook. This was carried out upstream and downstream of the sites where they were last observed (Sibley, 2001).

Aim: To calculate the rate of range extension by *P. leniusculus* along the Gaddesby Brook.

Hypothesis: The rate of range extension will increase with population growth.

Figure 3.1.6 The red sections of the stream course in the maps below indicate the range extension of *P. leniusculus* during the period of 1992 through to 2002. The red dot indicates the site of introduction in 1985.

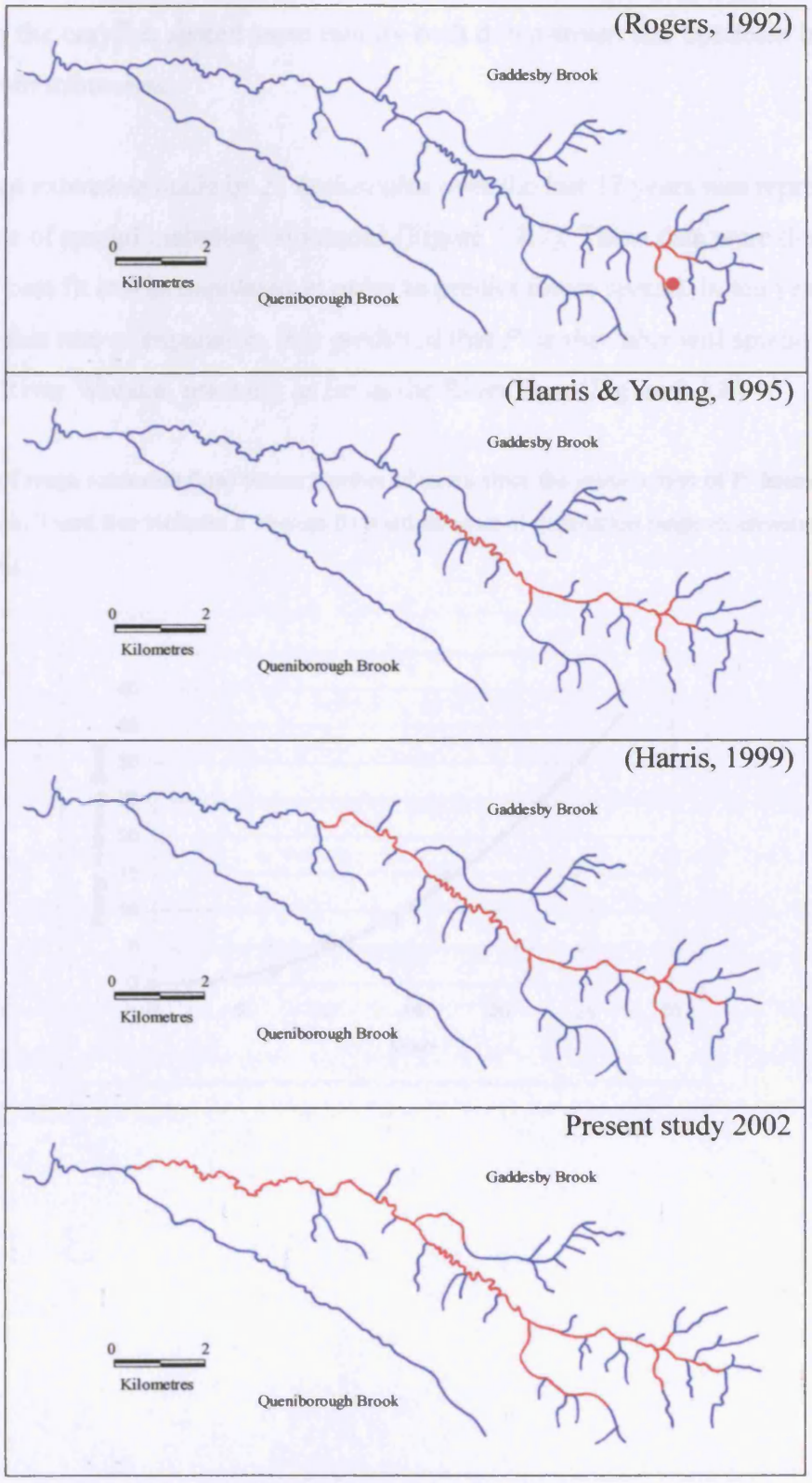


Figure 3.1.6 shows the range extension of *P. leniusculus* over the last 17 years from its first introduction in 1985. It can be seen that in the first 7 years, up to 1992, the crayfish had only established themselves in a small section of the stream. Over the course of the proceeding 10 years, however, the crayfish spread more rapidly both downstream and upstream into some of the smaller stream tributaries.

The rate of range extension made by *P. leniusculus* over the last 17 years was represented as the total distance of spread including tributaries (Figure 3.1.7). These data were then fitted with a curve of best fit and extrapolated in order to predict future spread. In ten years time, in 2012, based on this rate of expansion, it is predicted that *P. leniusculus* will spread a further 22 km into the River Wreake, reaching as far as the River Soar (Figure 3.1.8).

Figure 3.1.7 Plot of range extension (km) verses number of years since the introduction of *P. leniusculus* into the Gaddesby Brook. Trend line includes a 10-year forward forecast of population range extension ($y = 0.0528x^2 - 0.0687x - 0.0016$).

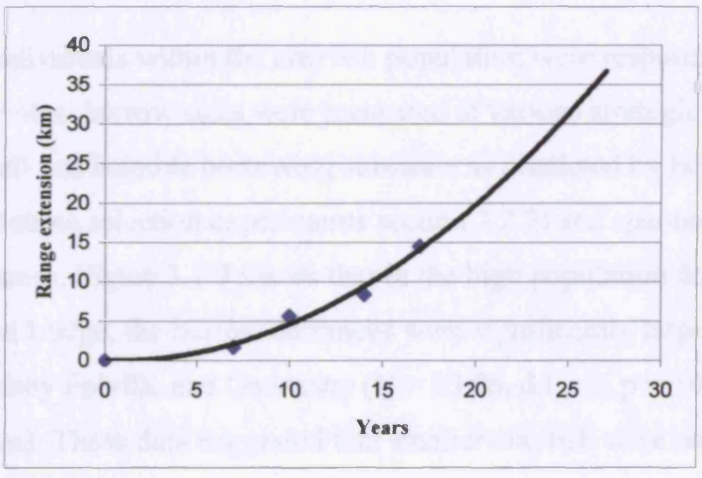
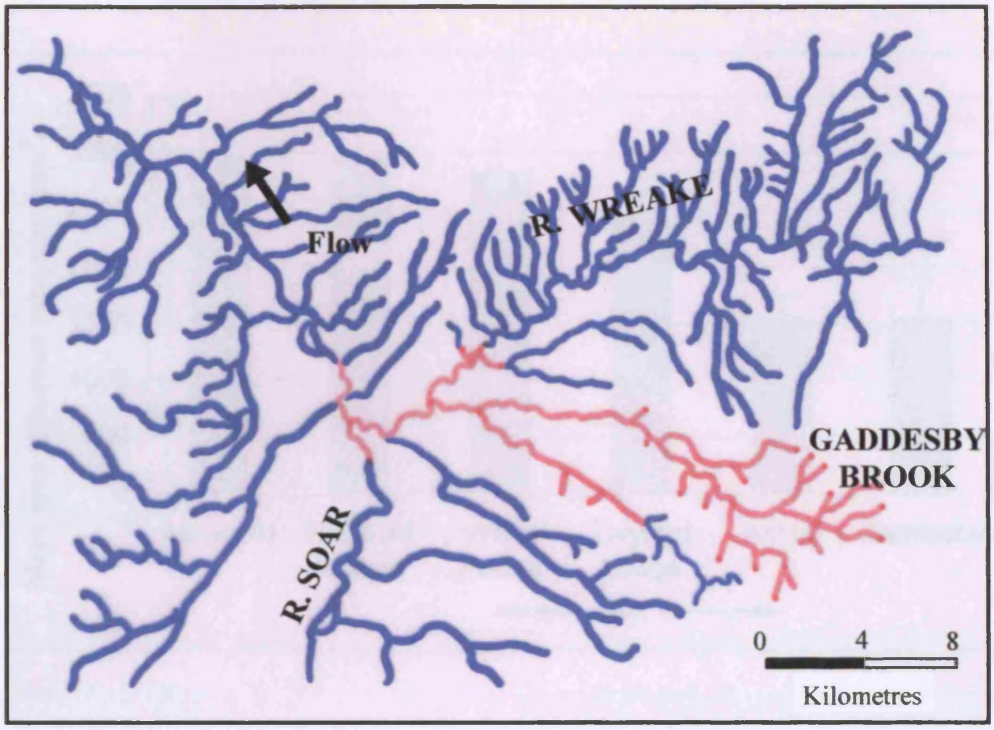
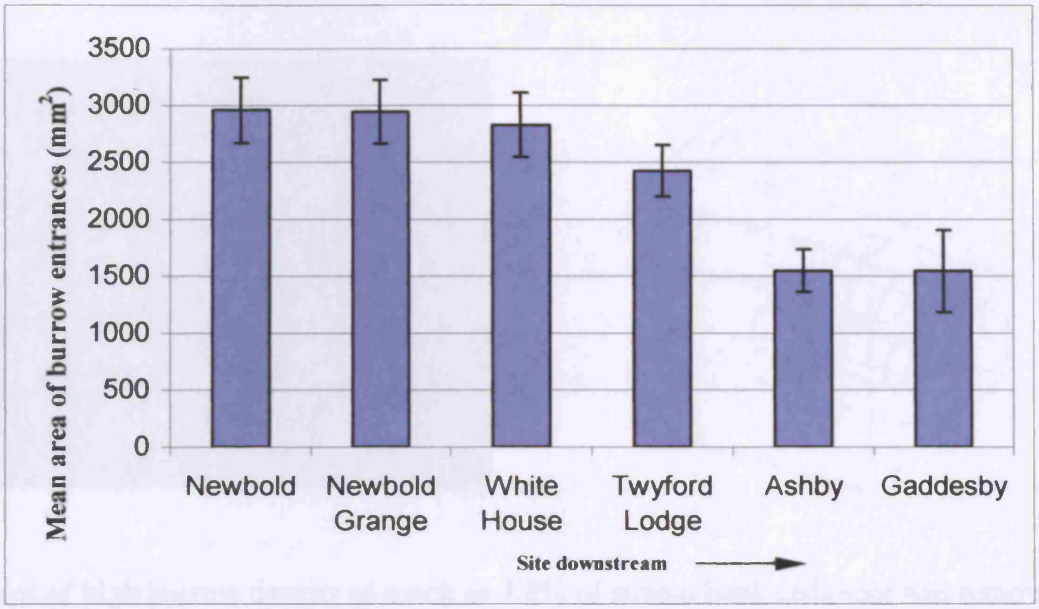


Figure 3.1.8 Predicted range extension of *P. leniusculus* by 2012.



To identify which individuals within the crayfish population were responsible for the initial colonisation of new sites, burrow sizes were measured at various strategic points along the stream. These sites all had suitable burrowing substrate as predicted by burrow and sediment surveys (and the substrate selection experiments section 3.2.2) and spanned from high to low population density areas. Figure 3.1.9 shows that in the high population density areas, Newbold to Twyford Lodge, the burrow entrances were significantly larger than those at the low-density sites Ashby Folville and Gaddesby ($H = 33.06$, $d.f = 5$, $p = <0.001$ with Tukey multiple comparisons). These data suggested that smaller crayfish were responsible for the initial colonisation of new sites.

Figure 3.1.9 Mean area of burrow entrances (mm^2) at six consecutive locations downstream along the Gaddesby Brook (Standard error bars displayed).



Burrow erosion damage

Burrow erosion damage was assessed by calculating percentage volume of sediment removed from 0.5 m^3 sections of stream bank at different burrow densities at different sites.

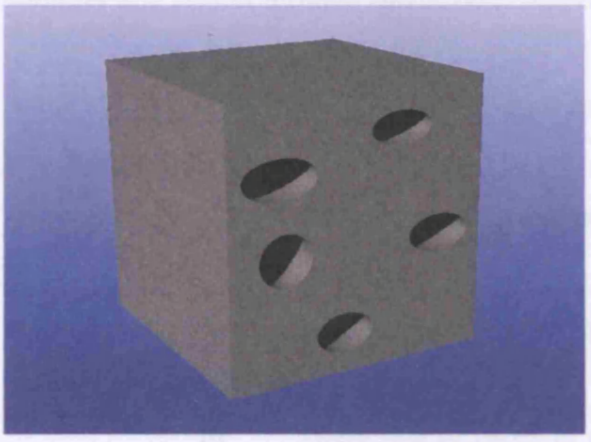
Aim: To estimate the proportion of bank volume removed by burrowing crayfish at different burrow densities.

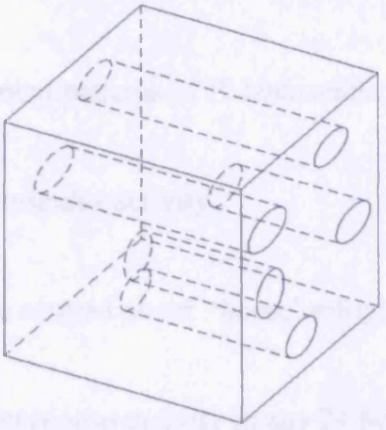
Hypothesis: Stream banks will be potentially unstable at sites with a higher burrow density.

A typical example of a 0.5 m^3 section of stream bank with a high burrow density is shown in Plate 3.1.3. The most vulnerable portion of the bank section was the area directly exposed to the flowing stream water.

Plate 3.1.3 Representation of a 0.5 m^3 section of stream bank with burrow excavations, a) front view, b) interior view.

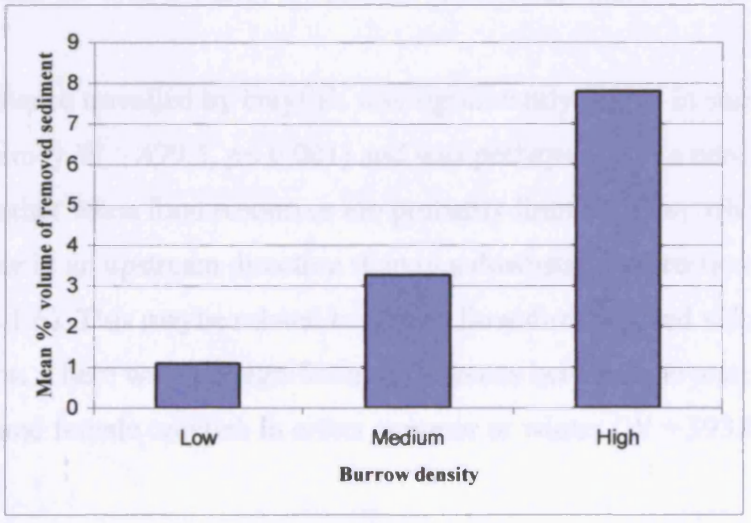
a) b)





In an area of high burrow density as much as 7.8% of stream bank sediment was removed by burrowing crayfish (Figure 3.1.10). This would have implications on bank stability and thus speed of erosion.

Figure 3.1.10 Plot of Mean % volume of sediment removed from a 0.5 m^3 section of stream bank at low, medium and high burrow densities.



In the first 10 cm of the stream bank section, in a high density area, the percentage of removed sediment was as much as 12 %. This percentage was almost double that of the entire section, making it high risk to erosion from stream flow and from animals such as cattle drinking at the stream edge.

3.1.5 Short term movements and activities in the field

A total of 38 crayfish were radio-tracked at two sites for periods of between 16 and 30 days in summer and winter months 2001/2002 (Appendix VII).

Aims: a) To monitor the short term movement patterns of *P. leniusculus.*

b) To ascertain times of peak *P. leniusculus* activity.

Hypotheses: a) *P. leniusculus* movements will be centred about 'home' refugia.

b) There will be times of peak *P. leniusculus* activity in any 24 hour period.

There were large variations in distances travelled by the radio-tracked crayfish on a daily basis (Figures 3.1.11 & 3.1.12). The maximum cumulative distance made by any one individual during the period of tracking was 340.4 m, with the maximum distance travelled in a single day being 89.6 m (Animal 38). The minimum cumulative distance travelled by an individual was 0 m. There was no significant association between the size of an animal (carapace length) and the total distance it travelled (Spearman Rank, $t = 1.90$, $n = 38$, $p<0.05$).

The mean total distance travelled by crayfish was significantly higher in summer than in winter (Mann-Whitney $W = 499.5$, $p< 0.001$) and was perhaps due to a need to conserve energy in cold weather when food resources are probably limiting. Crayfish moved significantly further in an upstream direction than in a downstream direction ($W = 1929.0$, $p<0.001$) (Table 3.1.6). This maybe related to stream flow direction and velocity and the need to maintain position. There were no significant differences between the total distances travelled by male and female crayfish in either summer or winter ($W = 393.0$, $p=0.52$).

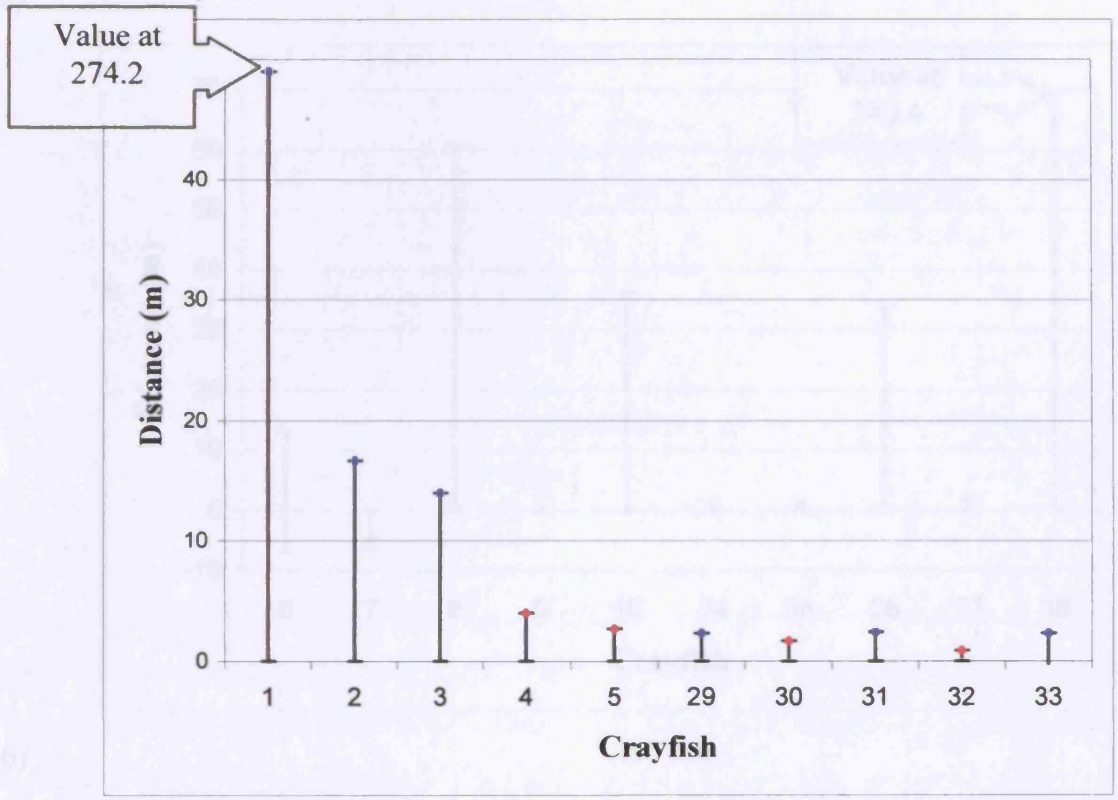
Table 3.1.6 Table of mean distances travelled (m) (± Standard errors) of 38 radio-tracked male and female crayfish monitored during summer and winter periods.

Season	Sex	Mean Total Distance (m)	Mean Upstream Distance (m)	Mean Downstream Distance (m)
Summer	Male	39.2 ± 26.4	37.5 ± 26.5	1.7 ± 0.9
	Female	48.2 ± 33.4	45.2 ± 33.4	3.1 ± 3.0
	Combined	43.7 ± 20.7	41.3 ± 20.8	2.4 ± 1.5
Winter	Male	2.9 ± 1.4	2.9 ± 1.4	0.0 ± 0.0
	Female	2.6 ± 1.3	2.6 ± 1.3	0.0 ± 0.0
	Combined	2.7 ±0.9	2.7 ± 0.9	0.0 ± 0.0
Combined Summer and Winter	Male	22.0 ± 14.2	21.1 ± 14.2	0.9 ± 0.5
	Female	26.6 ± 18.0	25.0 ± 17.9	1.6 ± 1.6
	Combined	24.3 ±11.3	23.0 ± 11.3	1.3 ± 0.8

The two sites used for radio-tracking were chosen because at Newbold Grange Farm the crayfish predominantly lived in burrows, which required energy expenditure to build, whereas at Mill Farm the crayfish lived under pieces of existing masonry i.e. using existing refugia. The data showed that in fact there were no significant differences in the total distances moved between the two sites ($W = 331.5$, $p = 0.58$). This did not support the notion that crayfish that did not commit resources to burrowing would travel more extensively and possibly change refuge. It was observed that a small number of crayfish remained in one location for several days before travelling some distance to new locations (Figure 3.1.13).

Figure 3.1.11 The upstream movement of radio-tracked *P. leniusculus* at Newbold Grange Farm during a) summer and b) winter. Diamonds indicate final position on retrieval, with colour representing sex of crayfish (Blue = male, Red = female). Release location is at zero on the y-axis and the bars represent the maximum distance moved by an individual.

a)



b)

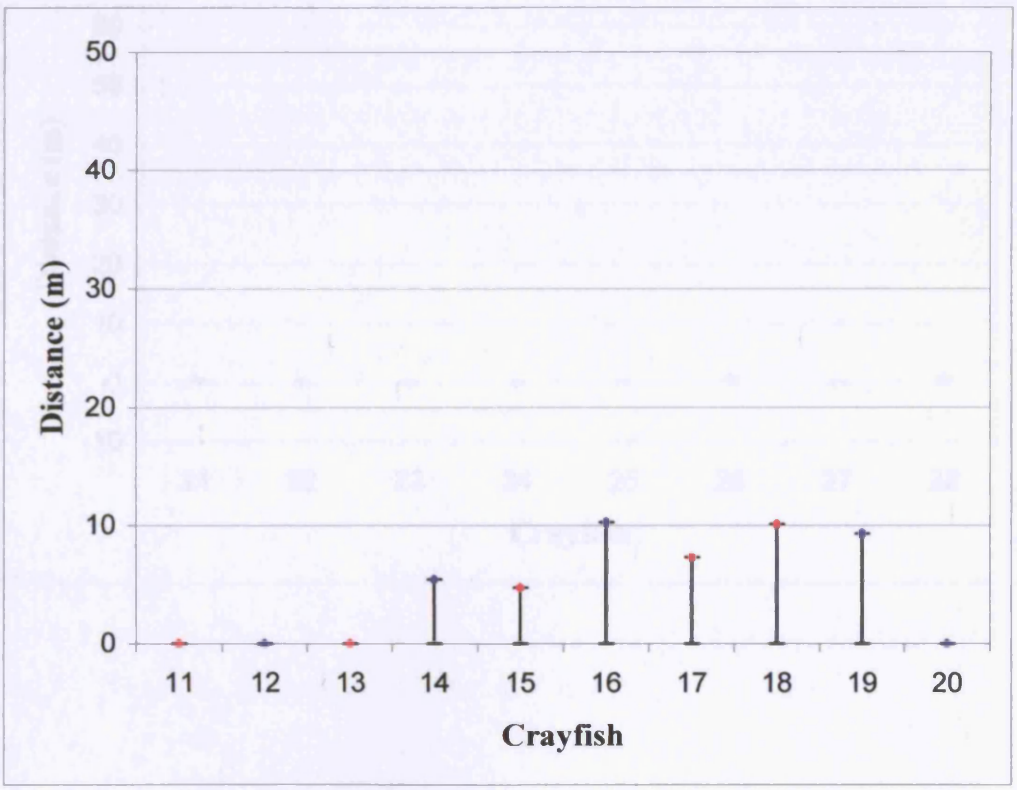
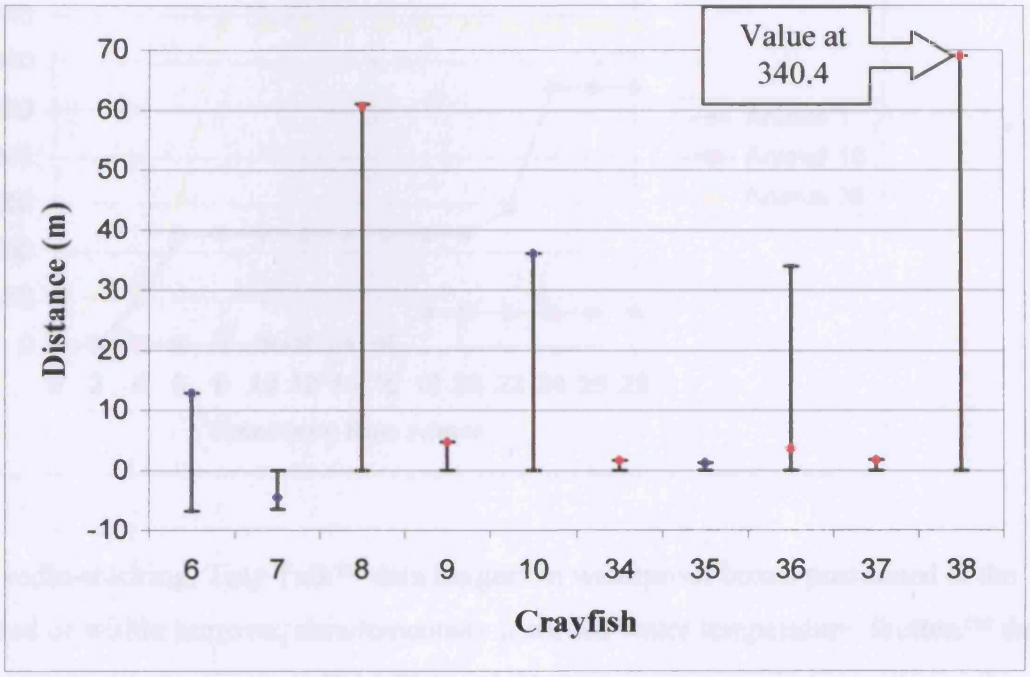


Figure 3.1.12 The upstream (+ve distances) and downstream (-ve distances) movement of radio-tracked *P. leniusculus* at Mill Farm during a) summer and b) winter. Diamonds indicate final position on retrieval, with colour representing sex of crayfish (Blue = male, Red = female). Release location is at zero on the y-axis and the maximum distance moved up and downstream by an individual is represented by the bars.

a)



b)

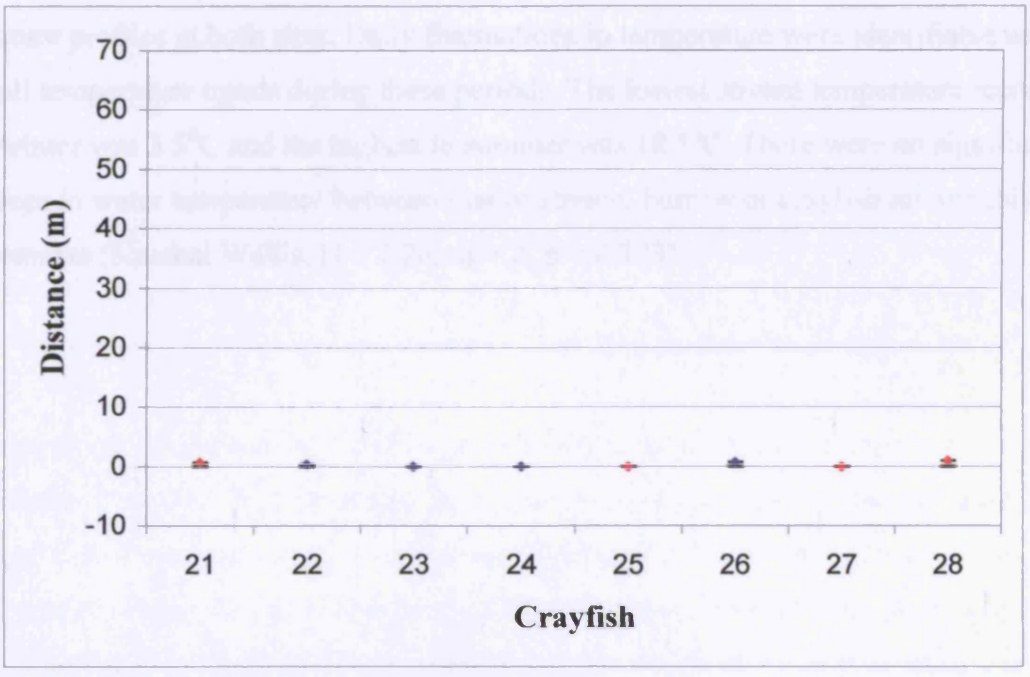
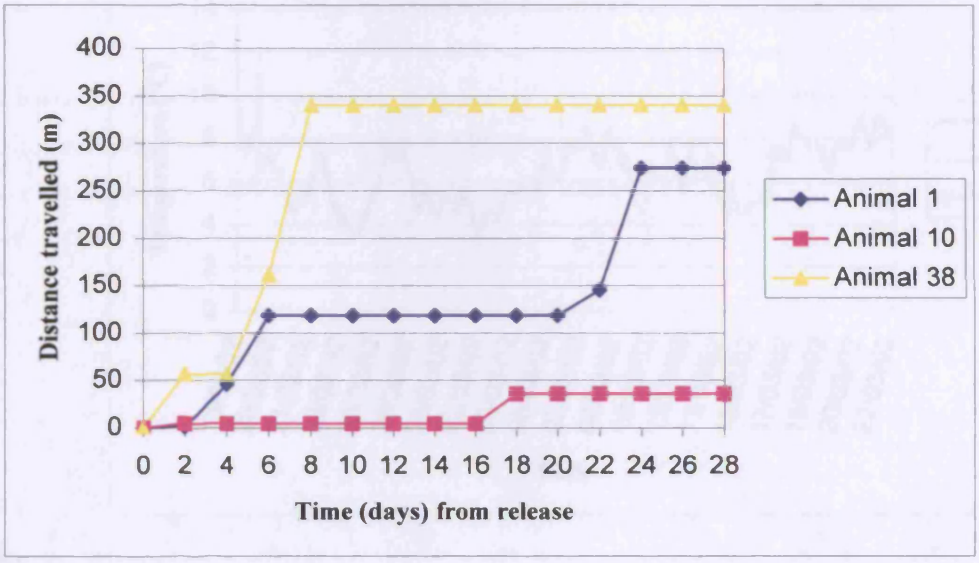


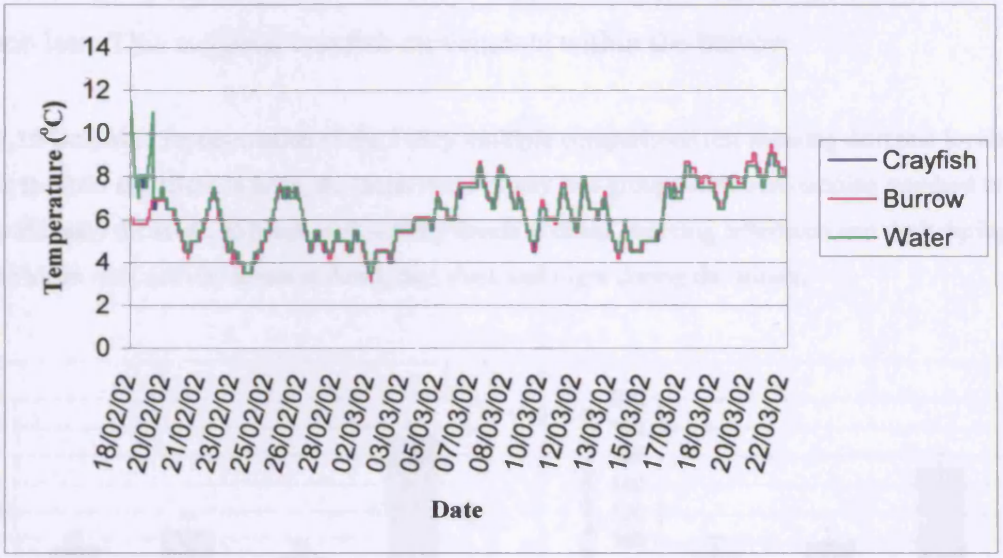
Figure 3.1.13 Distance travelled by three crayfish over a 28-day tracking period. These individuals tended to remain in one location for a number of days and made occasional large movements to new locations. (Animal 1 was tracked at Newbold Grange Farm and Animals 10 and 38 were tracked at Mill Farm).



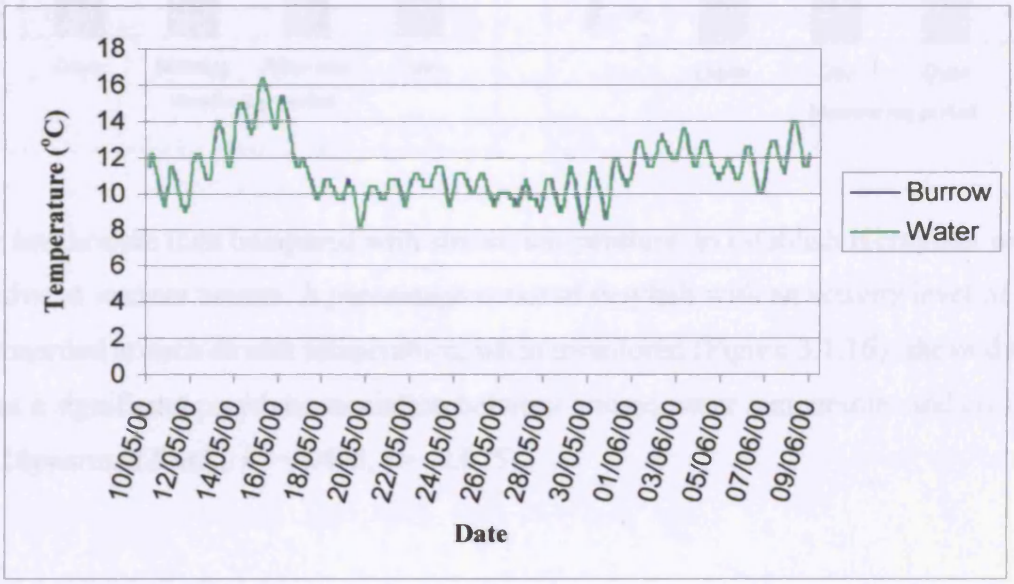
During radio-tracking, Tiny Talk™ data loggers in waterproof boxes positioned at the streambed or within burrows, simultaneously recorded water temperature. ibutton™ data loggers attached to 3 animals (M&M Plate 2.1.1) on separate occasions, continuously measured the water temperature of their microhabitat. Figure 3.1.14 shows examples of temperature profiles at both sites. Daily fluctuations in temperature were identifiable as well as overall temperature trends during these periods. The lowest stream temperature recorded during winter was 3.5°C and the highest in summer was 18.5°C. There were no significant differences in water temperature between that of stream, burrow or crayfish microhabitat measurements (Kruskal Wallis, $H = 2.26$, $df = 2$, $p = 0.323$).

Figure 3.1.14 Typical examples of stream water, burrow and crayfish temperature profiles at a) Mill Farm in winter 2002 and b) Newbold Grange Farm summer in 2000 (temperature profiles overlay identically).

a)



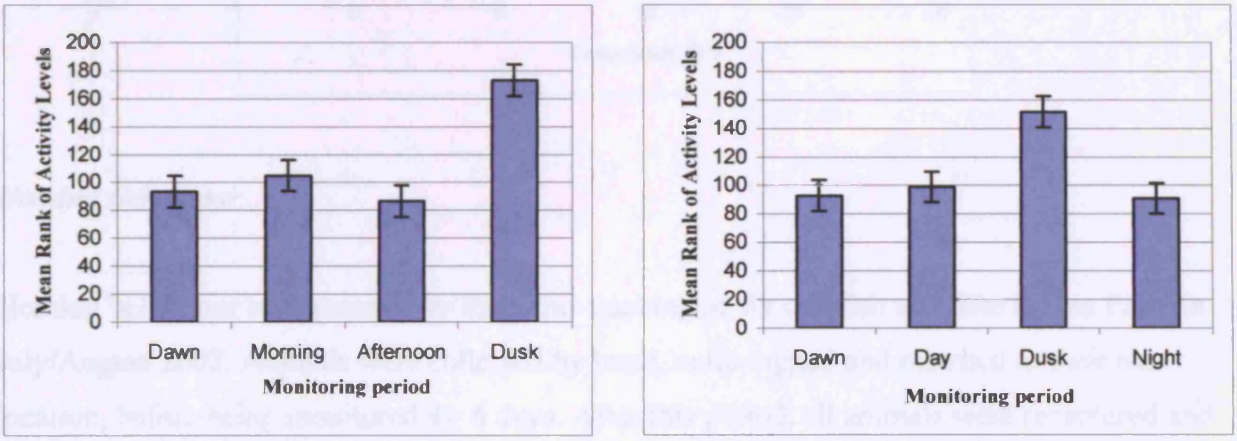
b)



Pacifastacus leniusculus activity levels were recorded during set time frames to ascertain peak periods of activity. Activity levels were taken from changes in transmitter signal strength and scored 0-3 (0 = no activity, 3 = very active)(Section 2.1.5). In both summer and winter, crayfish activity levels during dusk were significantly greater than all other times of day (Kruskal-Wallis, $H = 74.46$, $df = 3$, $p = <0.001$ and $H = 42.29$, $df = 3$, $p = <0.001$, respectively) (Figure 3.2.15). During dusk, crayfish activity was significantly greater during summer than winter (Mann-Whitney, $W = 3584.0$, $p = 0.021$). Overall, there was no significant difference in activity levels between summer and winter at either Mill Farm or

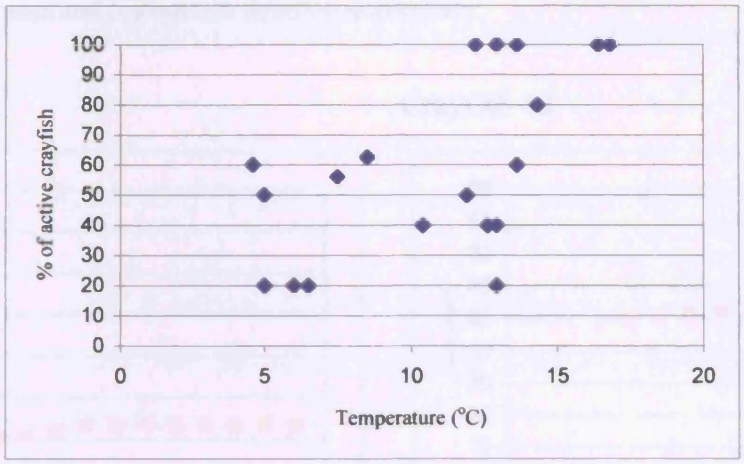
Newbold Grange Farm (Kruskal Wallis, H = 2.71, df = 3, p = 0.438). There were also no significant differences in the activity levels of male of female crayfish during summer or winter periods (Kruskal Wallis, H = 2.71, df = 3, p = 0.438), even though winter movements were much less. This suggests crayfish movements within the burrow.

Figure 3.1.15 Graphical representation of the Tukey multiple comparisons test showing different levels of activity. At the 0.05 significance level, the mean ranks of any two groups with over-lapping standard error bars are not significantly different. a) Mean rank activity levels at dawn, morning, afternoon and dusk during the summer, b) Mean rank activity levels at dawn, day, dusk and night during the winter.



Activity levels were then compared with stream temperature, to establish if crayfish were more active in warmer waters. A percentage count of crayfish with an activity level of 2 and above, recorded at each stream temperature, when monitored (Figure 3.1.16), showed that there was a significant positive association between stream water temperature and crayfish activity (Spearman Rank, $r_s = 0.495$, $p=<0.025$).

Figure 3.1.16 Relationship between recorded stream water temperature and the percentage of crayfish showing activity level 2 and above monitored at dusk during summer and winter.

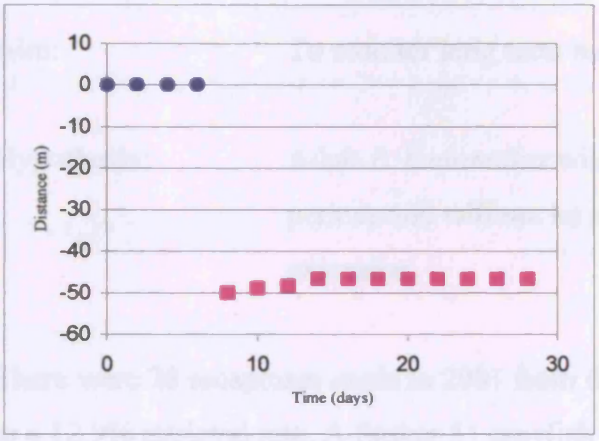


Homing Behaviour

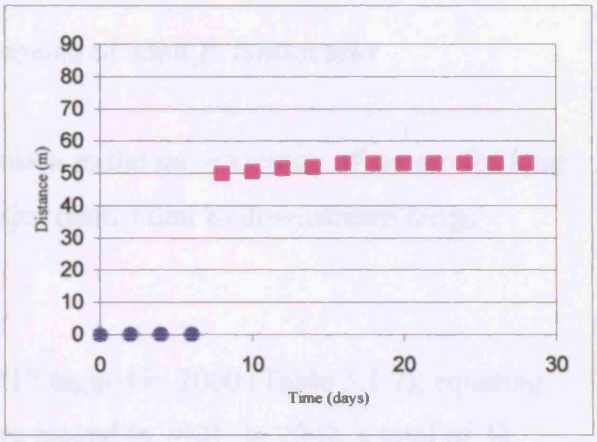
Homing behaviour was assessed by the radio-tracking of six crayfish at White House Farm in July/August 2002. Animals were collected by hand, radio-tagged and returned to their exact location, before being monitored for 6 days. After this period, all animals were recaptured and three displaced 50 m upstream and three animals 50 m downstream of their original positions. For homing behaviour to be demonstrated, it would be expected that the majority of crayfish would make their first movements in the direction of their original position. The crayfish, however, showed no tendency to 'home' back to their original location. All crayfish began movements in an upstream direction (Figure 3.1.17) and within 4 and 16 days of displacement, had found and stayed in a new refuge for a minimum of 8 consecutive days. None of the displaced crayfish returned to their former refugia.

Figure 3.1.17 Distances moved by crayfish before and after displacement up and downstream, from their original site of capture. Blue circles show movements before displacement; pink squares show movement after displacement. Zero on y-axis represents initial release positions with positive and negative figures representing movements in an upstream and downstream direction respectively.

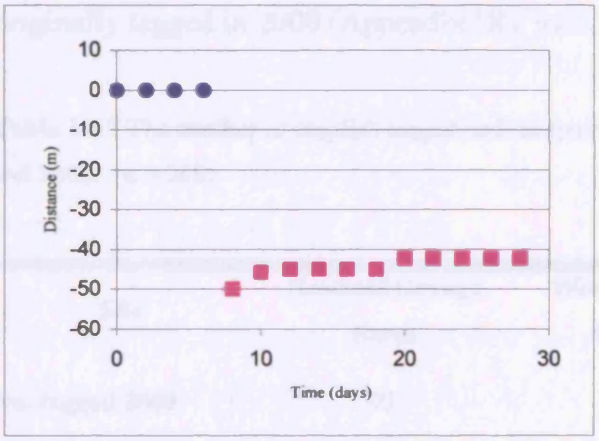
Crayfish 39



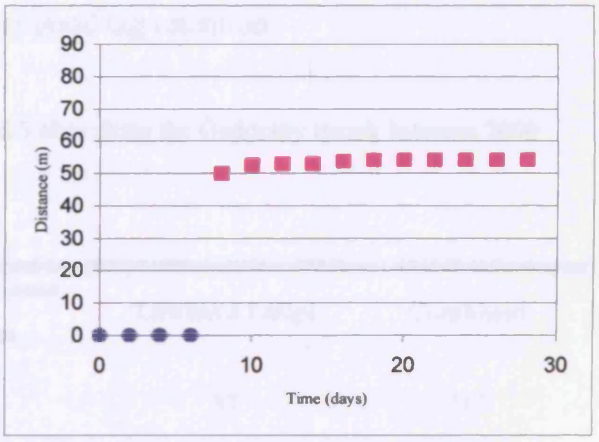
Crayfish 40



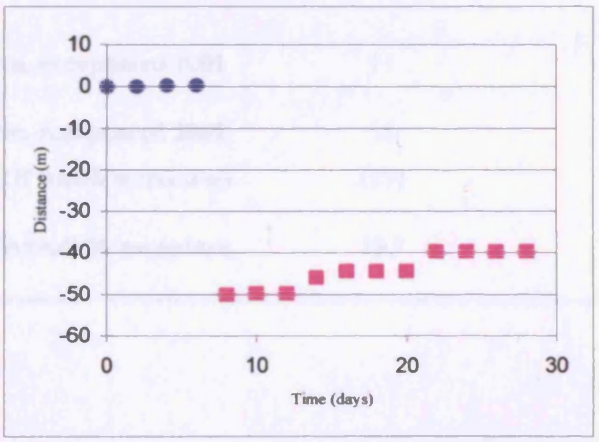
Crayfish 42



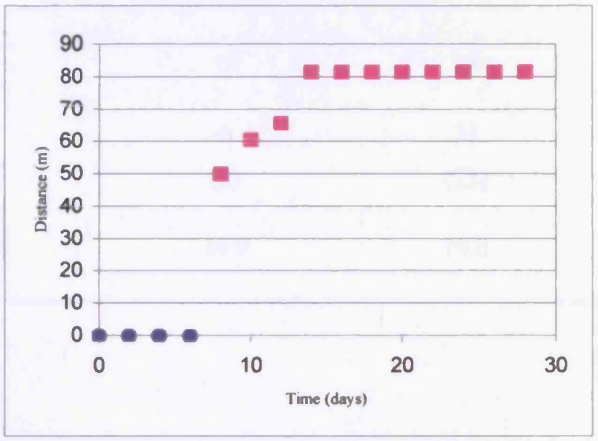
Crayfish 41



Crayfish 43



Crayfish 44



3.1.6 Long term movements and tagging

In the summer of 2000 and 2001 at Newbold Farm, White House Farm and Lowenva Lodge, a total of 268 crayfish were tagged with individually identifiable streamer tags inserted in the abdomen (Appendix VIII).

Aim: To monitor long term movements of adult *P. leniusculus*

Hypothesis: Adult *P. leniusculus* will remain in the same vicinity of stream for long periods and will not be a major contributor to downstream range extension.

There were 28 recaptures made in 2001 from the 217 tagged in 2000 (Table 3.1.7), equating to a 12.9% retrieval rate. A further 51 crayfish were tagged in 2001. In 2002, a total of 33 animals were recaptured, 10 of which were originally tagged in 2001 and 23 of which were originally tagged in 2000 (Appendix IX), indicating good tag retention.

Table 3.1.7 The number of crayfish tagged and recaptured at 3 sites along the Gaddesby Brook between 2000 and 2002. (n = 268).

Site	Newbold Grange Farm	White House Farm	Lowenva Lodge	Combined
No. tagged 2000	93	37	87	217
No. tagged 2001	39	12	0	51
No. recaptured 2001	11	7	10	28
No. recaptured 2002	18	9	6	33
(Of which were new)	(15)	(5)	(3)	(23)
Overall % recapture	19.7	24.5	14.9	19.0

The mean mass and carapace length of tagged crayfish was similar to that of recaptured individuals, demonstrating that recapture was not biased by animal size or, most importantly, that the tag did not unduly affect a particular size class of crayfish (Table 3.1.8).

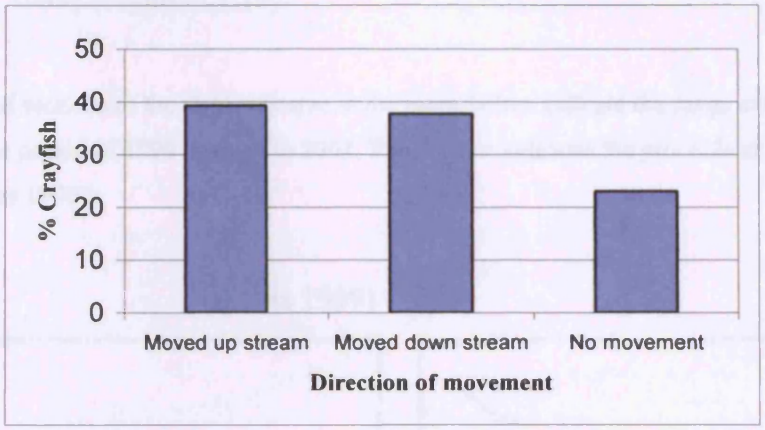
Table 3.1.8 Mean mass (g) and carapace length (mm) of all tagged crayfish (n = 268) and recaptured crayfish (n = 51) at the Gaddesby Brook between 2000 and 2002. Means ± SE (range).

	Mass (g)	**Carapace length (mm)**
All tagged crayfish	39.0 ± 1.1 (9.8 – 121.8)	49.5 ± 0.5 (29.5 – 74.8)
Recaptured crayfish	38.2 ± 2.9 (12.5 – 121.8)	48.4 ± 1.0 (34.8 – 74.8)

On initial capture, the position and number of trap, in which each individual was captured, was recorded. This procedure was repeated for any recaptures, thus any changes in position were recorded. Of all the recaptured crayfish, 23% were caught in the same trap and 60% were caught within 2 traps of their former locations (equating to no more than 7 m). The furthest recorded distance travelled by any recaptured individual from its point of release was 103 m over 2 years (Tag No. 3605).

Overall 39.3% of the recaptured crayfish were located upstream and 37.7% were located downstream of their release point (Figure 3.1.18). All recaptures were made at the same site of release (for example, no animals caught at Newbold were consequently re-caught at White House Farm). Over the 2 years, the overall recapture rate was 19.0% (Table 3.1.7). This suggested that *P. leniusculus* stayed within the same vicinity for long periods. Mortality and predation may also be important factors affecting the overall recapture rates.

Figure 3.1.18 Percentage of crayfish recaptured at the same site, or at sites upstream and downstream, of initial capture.



3.1.7 Comparisons with the River Greet

It is not known when *P. leniusculus* were first introduced into the stream at the R.Greet or indeed the exact location of their original introduction. However, in a study by Harris (1999), the highest population densities of *P. leniusculus* were found at the Maythorn Mill (SK 697 557) and this was thought to be the probable site of first introduction. Based on this information, and the fact that most crayfish introductions in Britain were made in the late 1980's, it is possible to compare population range extension in the R. Greet with that of the Gaddesby Brook.

Aims:

a) To calculate the rate of range extension by *P. leniusculus* along the R.Greet.

b) To establish if wild populations of *P. leniusculus* at the R.Greet select a particular substrate type for burrowing.

Hypotheses:

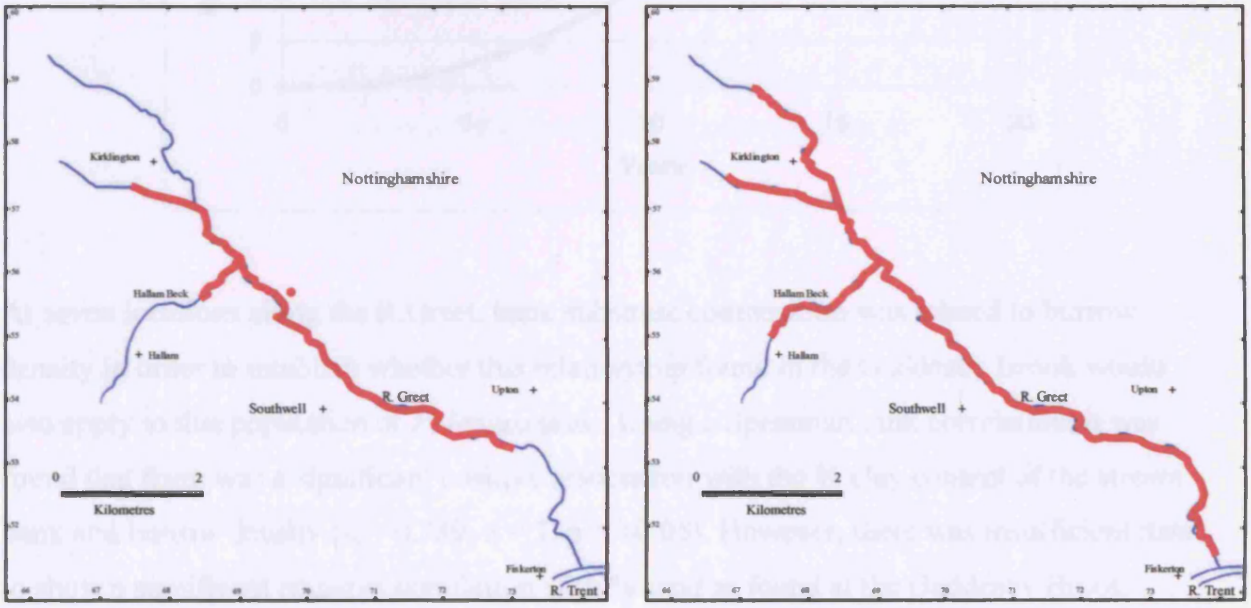
a) The rate of range extension by *P. leniusculus* will be similar to that of the Gaddesby Brook.

b) There will be a relationship between the burrow construction sites of wild populations of *P. leniusculus* and substrate composition, similar to that shown at the Gaddesby Brook.

Trapping and hand searching ascertained the current distribution of *P. leniusculus* in the R. Greet. This was carried out upstream and downstream of the sites where they were last observed (Harris, 1999) (Figure 3.1.19).

Figure 3.1.19 The red sections of the stream course in the maps below indicate the range extension of *P. leniusculus* during the period of 1999 through to 2002. The red dot indicates the probable site of first introduction in the late 1980's.

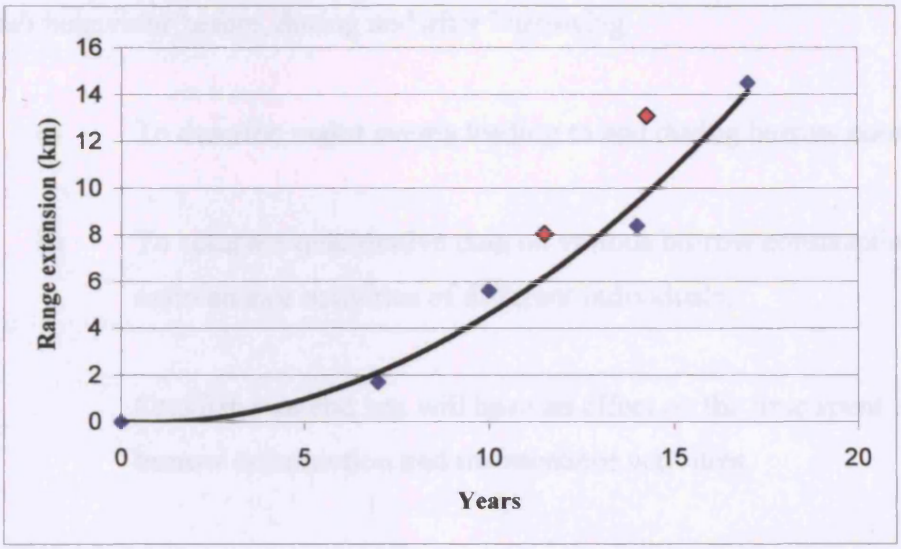
a) (Harris 1999) b) Present study 2002



It is clear that *P. leniusculus* had extended its range in the R. Greet since 1999 in both downstream and upstream directions. The population appeared sparse however, at sites approaching the R. Trent. Hand-searching for the presence of crayfish in the R. Trent at the mouth of the R. Greet proved unsuccessful and talks with local fishermen indicated that *P. leniusculus* had not yet populated the R. Trent.

From these data, it was possible to plot the range extension made by *P. leniusculus* since 1999 to the present day and overlay this with the rate of range extension that occurred at the Gaddesby Brook (Figure 3.1.20). From this it would appear that the range extension made by the population of *P. leniusculus* in the R. Greet is of a similar order to that of the population in the Gaddesby Brook.

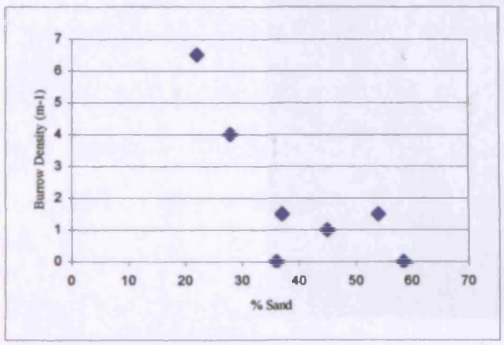
Figure 3.1.20 Comparison of range extension (km) verses number of years since the introduction of *P. leniusculus* in both the R. Greet (red) and the Gaddesby Brook (blue). ($y = 0.0671x^2 - 0.0108x - 1E\text{-}14$).



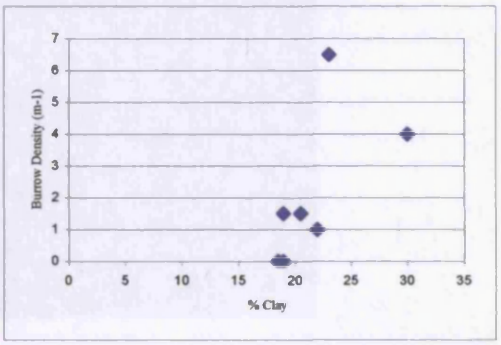
At seven locations along the R.Greet, bank substrate composition was related to burrow density in order to establish whether this relationship found in the Gaddesby Brook would also apply to this population of *P. leniusculus*. Using a Spearman rank correlation, it was found that there was a significant positive association with the % clay content of the stream bank and burrow density ($r_s = 0.789$, $n = 7$, $p = <0.05$). However, there was insufficient data to show a significant negative correlation with % sand as found at the Gaddesby Brook (Figure 3.1.21).

Figure 3.1.21 Plots of burrow density (m^{-1}) in relation to the percentages of a) sand and b) clay, which constitute the R. Greet bank substrate composition. ($n = 7$).

a)



b)



3.2. Observations of burrowing and associated behaviour in the laboratory

3.2.1. Crayfish behaviour before, during and after burrowing

Aims: a) To describe major events leading to and during burrow construction.

b) To compare quantitative data on various burrow construction and maintenance activities of different individuals.

Hypothesis: Crayfish size and sex will have an effect on the time spent on various burrow construction and maintenance activities.

Behaviour before burrowing

All of the twenty crayfish videoed appeared to explore their tank thoroughly after being placed in it. This usually consisted of a sequence of movements backwards and forwards along the margins of the tank, with some attempts at climbing the walls. During this period, the crayfish rapidly moved their antennae, using them to 'explore' water depth (by putting antennae up to the water-air interface), tank corners, surfaces and the substrate (Plate 3.2.1).

Plate 3.2.1 Photograph of crayfish 'exploring' laboratory tank.

After this initial period, the crayfish then started to cross the tank interior, probing the substrate with its 3rd maxillipeds and its 2nd and 3rd pereopods (walking legs) (as classified in Holdich, 2002). This period was interspersed with periods of rest and cleaning of appendages by larger crayfish (CD-ROM File 1).

Burrow initiation

Having probed the substrate, the crayfish then began burrow construction. In all cases, this started with short bursts of digging, using the chelae initially, followed by the additional use of the 2nd and 3rd pereopods (CD-ROM File 2). The digging was interspersed with rest, cleaning or roaming periods.

During burrowing

Below are described the two main methods of behaviour occurring during burrow excavation. These were identified as common activities amongst the crayfish observed.

Method 1

After having excavated a shallow depression, the crayfish entered the new burrow headfirst (Plate 3.2.2a) and used both closed chelae to chisel away at the sidewalls and end of the burrow (CD-ROM File 3). The 3rd and 4th pereopods were used for support, whilst the 1st and 2nd pereopods were used to snip at the loosened sediment dislodged by the chelae and to push it back towards the abdomen. This fine sediment was then expelled from the burrow, suspended in a water current created by the synchronous beating movements of the pleopods, and emerged at the opening from beneath the raised tail (Plate 3.2.2b) (CD-ROM File 4). Three slight variations to this main method of excavation were: a) forcing an open chelae into the wall of the burrow and then closing it to dislodge sediment (Plate 3.2.2d). b) utilising a bent chelae to drag sediment under the body (Plate 3.2.2c). c) inverting the body so enabling more effective excavation at the bottom, leading edge of the burrow. (Plate 3.2.2d).

Plate 3.2.2 Video frames of some stages observed during Method 1 excavation. (Male crayfish, CL = 22.3 mm). a) entering burrow in a forward direction, b) using closed chelae to dislodge sediment (note raised tail position), c) bent chelae being used to drag sediment backwards, d) crayfish inverts its body to allow better access to leading edge of burrow (also showing use of chelae action to cut material from wall of burrow).

a)

b)

c)

d)

Method 2

The crayfish entered the burrow abdomen first and manoeuvred to the end of the burrow (Plate 3.2.3a). It then flexed its tail upwards and with extended uropods pushed its tail into the fine sediment (Plate 3.2.3b). It then scooped up a mass of sediment and held it in its curved-up abdomen (Plate 3.2.3c) (CD-ROM File 5). The crayfish then walked to the burrow entrance (Plate 3.2.3d), turned around (Plate 3.2.3e) and just before it re-entered the burrow to

start digging again, it uncurled its tail, flexed it upwards and discharged the sediment out backwards by generating a water current using its beating pleopods (Plate 3.2.3f).

Plate 3.2.3 Sequential video frames of Method 2 excavation technique (Male crayfish, CL = 22.3 mm). a) reversing into burrow, b) upward flex of tail, c) tail scooping up sediment, d) moves forward towards entrance, e) Rotates 180° at entrance, f) uncurling tail, flexing it upwards and discharging sediment.

a)

b)

c)

d)

e)

f)

Quantitative data on construction and maintenance

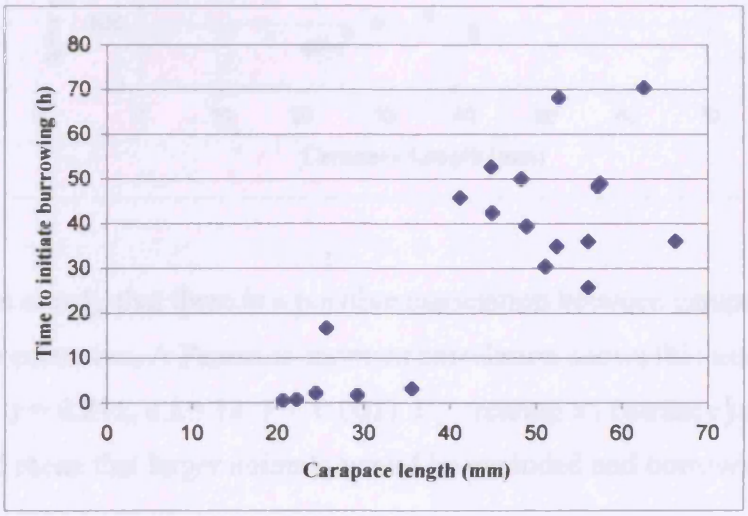
'Maintenance' was defined as being small modifications made to the interior of the burrow following construction and the removal of any fallen substrate. Table 3.2.1 shows the average burrowing data of 20 crayfish, each videoed for up to seven days. From this data a number of significant size and sex related differences in burrow dimensions and construction were identified.

Table 3.2.1 Average data for various burrow construction and maintenance activities. (n = 20; 10 males and 10 females). Means ± SE and ranges given. * indicates a significant difference between means.

Construction and maintenance activities	Male	Female	Combined
Carapace length (mm)	48.3 ± 3.6 (22.3 - 62.6)	41.6 ± 3.1 (20.7 - 66.2)	44.9 ± 3.1
Time taken to initiate burrowing (h)	45.8 ± 6.2* (0.8 - 70.3)	19.7 ± 5.2* (1.8 - 41.8)	32.7 ± 5.0
Total time to construct burrow (h)	50.0 ± 7.4 (31.0 - 72.0)	45.6 ± 3.8 (27 - 64)	47.3 ± 3.6
Length of burrow (mm)	85.7 ± 9.5 (40 - 134)	87.4 ± 14.5 (50 - 200)	86.6 ± 8.4
Area of burrow opening (mm^2)	1250.9 ± 226.7 (325.2 - 2529.0)	1117.5 ± 266.8 (432.0 - 2473.2)	1184.2 ± 171.1
Number of burrow openings	1.0 ± 0.0 (1.0 - 1.0)	1.1 ± 0.1 (1 - 2)	1.1 ± 0.1
Time spent on 'burrow maintenance' ($h\ day^{-1}$)	2.2 ± 0.6 (0.0 - 4.1)	2.6 ± 0.4 (0.9 - 4.3)	2.4 ± 0.31
Time spent in burrow ($h\ day^{-1}$)	13.6 ± 0.4 (12.1 - 16.2)	14.1 ± 0.6 (12.1 - 16.9)	13.9 ± 0.4*
Time spent out of burrow ($h\ day^{-1}$)	10.4 ± 0.4 (7.8 - 11.9)	9.9 ± 0.6 (6.7 - 11.9)	10.1 ± 0.4*

Firstly, the time taken to initiate burrowing was compared with crayfish size, which was determined by carapace length. It was thought that smaller animals which would be more susceptible to predation and would be required to expend proportionally more energy in maintaining their position in flowing water, would begin burrow excavation more rapidly after introduction into the observation tank.

Figure 3.2.1 Time elapsed (h) since the introduction of a *P. leniusculus* onto a new substrate and the start of excavation plotted against carapace length (mm).



From Figure 3.2.1 it can be seen that the time taken to initiate burrowing was less in smaller crayfish compared to larger individuals. A Pearsons-moment correlation indicates a significant positive association between carapace length and time to initiate burrowing ($r = 0.778$, d.f. = 18, $P < 0.001$).

Differences between male and female crayfish in the time taken to initiate burrowing were also analysed. In order to take into account the variation in size between the male and female crayfish tested, a General Linear Model (GML) test was performed incorporating size as a covariant. From this it was found that females started burrowing significantly earlier than male crayfish after transfer (GLM, $F = 14.5$, $P = 0.001$) (Table 3.2.1).

Crayfish tended to excavate burrow entrances sufficiently large enough to allow them to enter and defend efficiently. It is reasonable to assume that smaller animals would not want to expend more energy than was necessary (a larger entrance would require more energy expenditure in excavation).

Figure 3.2.2 Areas of crayfish burrow entrances (mm^2) plotted against carapace length (mm), male and female data combined (n=20).

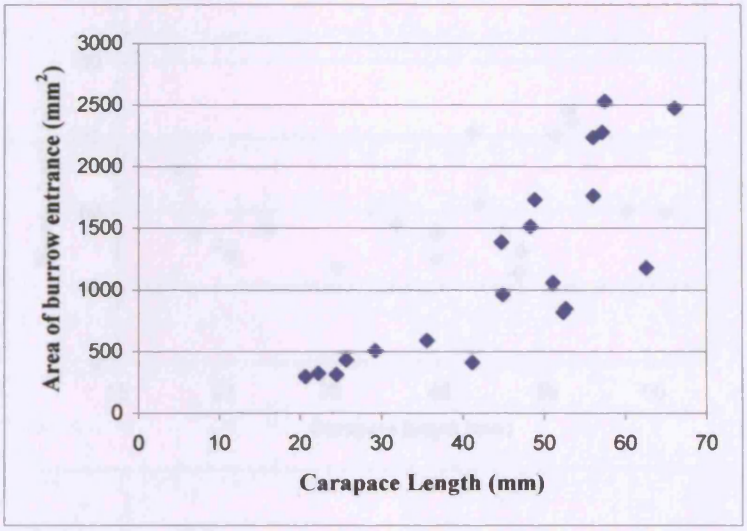
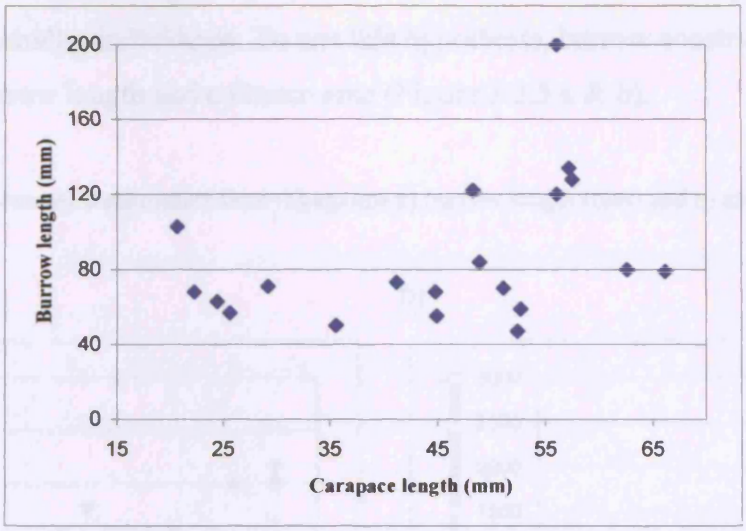


Figure 3.2.2 shows clearly that there is a positive association between carapace length and the area of burrow entrances. A Pearsons-moment correlation shows this association to be highly significant ($r = 0.811$, d.f.= 18, $P < 0.001$). By creating an entrance just big enough for themselves, would mean that larger animals would be excluded and burrow defence would be easier.

It was hypothesised that larger animals would require a longer burrow to conceal themselves fully compared with smaller animals. Although the size of the tank might have been considered a limiting factor, the fact that the burrows of 19 out of the 20 crayfish observed did not reach the walls of the tank on completion, suggested that this analysis was reasonable.

Figure 3.2.3 shows the relationship between burrow length (mm) and carapace length (mm). A Spearman's rank correlation shows this to be a significant association ($Rs = 0.439$, $n = 20$, $\alpha < 0.05$), although the association appears weak and there was considerable individual variation of burrow length within all size classes.

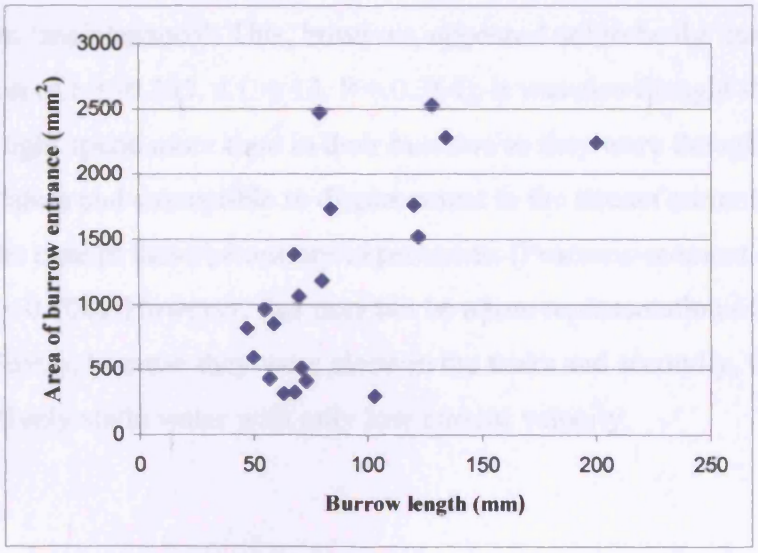
Figure 3.2.3 Burrow length (mm) plotted against carapace length (mm), male and female data combined (n = 20).



A Spearman's rank correlation showed a significant association (Rs = 0.439, n = 20, $\alpha <$ 0.05), between burrow length (mm) and carapace length (mm) (Figure 3.2.3). This association appeared weak however, and was probably due to the considerable individual variation of burrow length within the different size classes.

Figure 3.2.4 shows there was also a significant association between the area of the burrow entrance and the length of the burrow (Spearman's rank correlation, $r_s = 0.590$, n = 20, $\alpha <$ 0.01). This would be expected because crayfish size was positively correlated with both of these burrow dimensions.

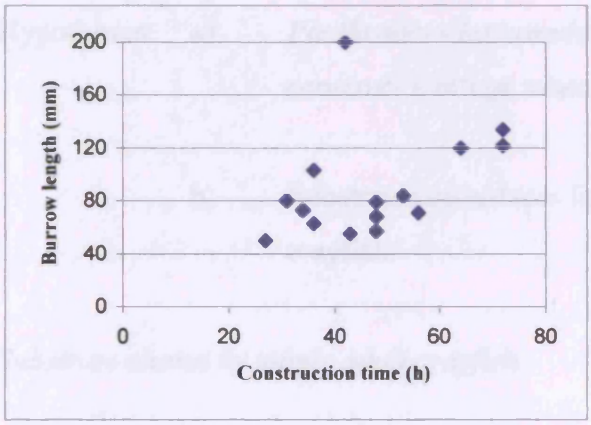
Figure 3.2.4 Burrow length (mm) plotted against area of burrow entrance (mm^2) (n = 20).



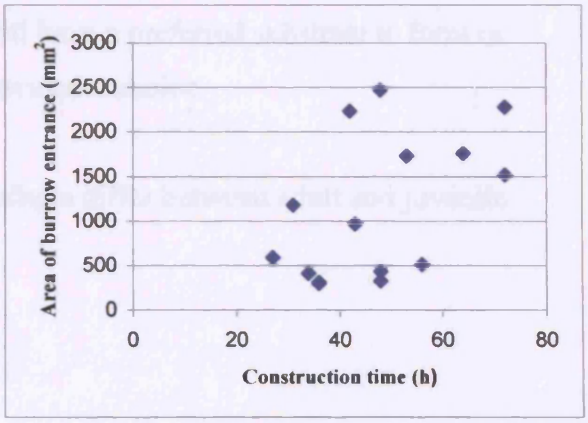
It would be logical to assume that larger burrows took longer to build. Also, with larger animals being less at risk from predation, their need to construct a burrow quickly would not be as great as for smaller individuals. To test this hypothesis, burrow construction time was plotted against burrow length and entrance area (Figure 3.2.5 a & b).

Figure 3.2.5 Plots of burrow construction time (h) against a) burrow length (mm) and b) area of burrow entrance (mm^2).

a)



b)



It can be seen that longer burrows and burrows with larger entrances require greater construction time, as shown by significant positive associations calculated using Spearman's rank correlations ($r_s = 0.449$, $n = 15$, $\alpha < 0.05$ and $r_s = 0.461$, $n = 15$, $\alpha < 0.05$, respectively).

Two other associations relating to burrowing were considered. These were 'maintenance' time and the amount of time spent in and out of the burrow versus carapace length. It was thought that perhaps if animal size reflected the size of the burrow (i.e. larger animals have larger diameter burrows and longer burrows), then perhaps a larger burrow would require more time spent on 'maintenance'. This, however, appeared not to be the case (Pearsons-moment correlation of $r = -0.253$, d.f. = 13, $P = 0.364$). It was also thought that perhaps smaller animals might spend more time in their burrows as they were thought to be more vulnerable to predators and susceptible to displacement in the stream current, but this was shown not to be the case in these laboratory experiments (Pearsons-moment correlation $r = 0.090$, d.f.=13, $P = 0.706$). However, this may not be a true representation of their normal field behaviour. Firstly, because they were alone in the tanks and secondly, they were burrowing in relatively static water with only low current velocity.

3.2.2 Substrate Choice Experiments

Aims: a) To find out if substrate preferences for refugia are shown by adult and juvenile *Pacifastacus leniusculus* or not.

b) To find out if such choices made by individual adult crayfish are influenced in any way by the presence of other adults.

Hypotheses: a) *Pacifastacus leniusculus* will have a preferred substrate to form or construct a refuge when provided a choice.

b) Substrate preferences for refugia differ between adult and juvenile crayfish.

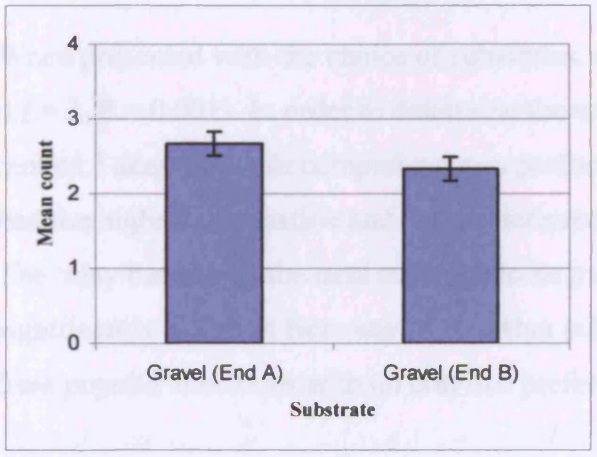
Substrate choice by single adult crayfish

This experiment was designed to verify field observations regarding substrate preference, and location of refugia. It tested whether or not a substrate, which could instantly provide a refuge, would be preferred over that in which the construction of a burrow was necessary.

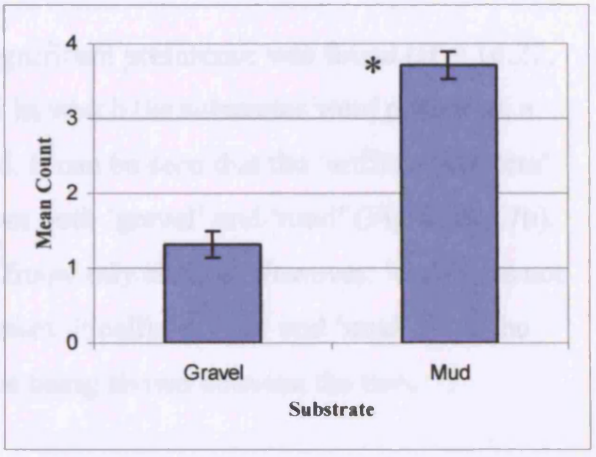
The control experiment (Figure 3.2.6a) demonstrated no significant preference by the crayfish for either end of the experimental tanks (Mann-Whitney, $W = 2532$, $n = 48$, $p < 0.136$). It was assumed, therefore, that selection of a particular tank-end would be due to differences in substrate type. When given a choice between 'gravel' and 'mud' (Figure 3.2.6b), a significant preference was shown towards the 'mud' substrate (Mann-Whitney, $W = 1427$, $n = 48$, $p < 0.001$). When presented with a choice of 'mud' substrate or a 'clay bank' substrate, in a separate experiment, a 'clay bank' substrate was preferred to 'mud' (Fig 3.2.6c Mann-Whitney, $W = 2700$, $n = 48$, $p = 0.0065$). However, the crayfish showed a clear preference for 'artificial shelter' over the 'clay bank' (Mann-Whitney, $W = 1840$, $n = 48$, $p < 0.001$), suggesting that burrowing in the stream banks could be the result of a lack of other suitable refuges (this aspect will be discussed further).There were no differences between males and females in substrate choice.

Figure 3.2.6 Proportional count of crayfish preferring one substrate type to another following 24-hour exposure (each animal was observed five times daily and each trial involved 48 animals) (mean count per trial ± SE). a) control experiment gravel (2-5 mm) vs. gravel (2-5mm), b) gravel (2-5 mm) vs. 'mud', c) 'mud' vs. 'clay', d) 'clay' vs. 'artificial shelter'. (* = Significant difference).

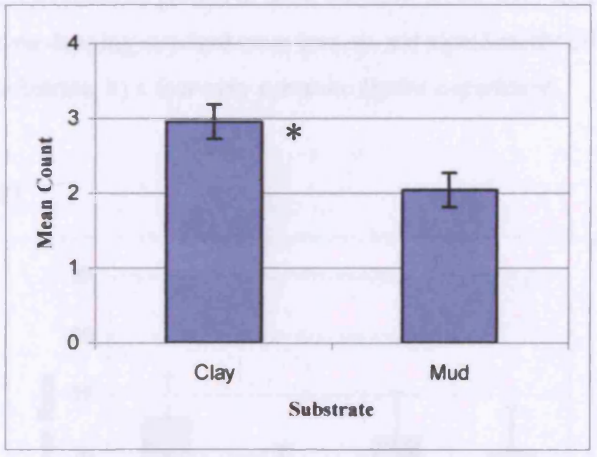
a)



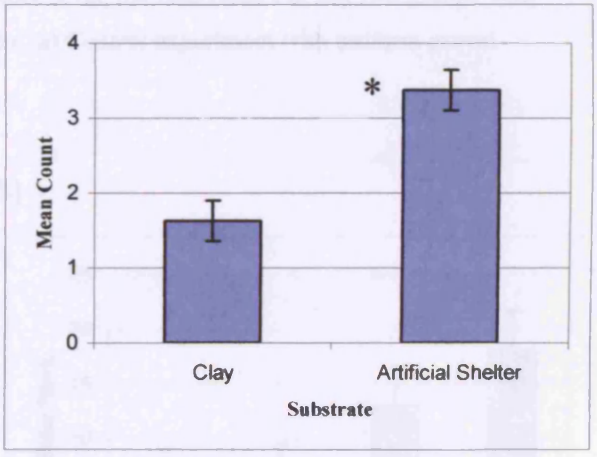
b)



c)



d)



Substrate choice in groups of adult individuals and to investigate the possibility that competition for most favoured substrates for refugia would occur

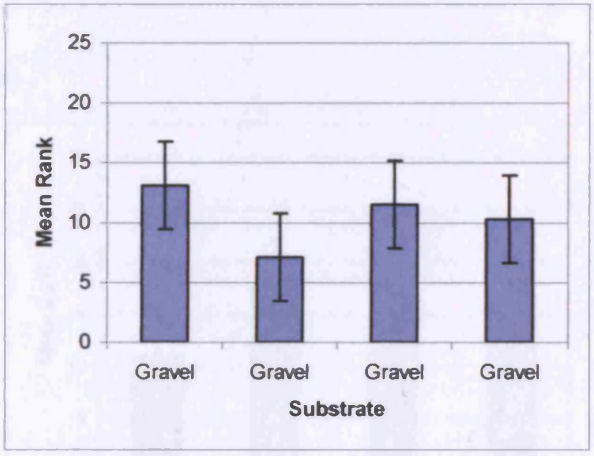
This experiment was designed to show the effect that the presence of other individuals of *P. leniusculus* in an artificially created high-density population (8 individuals m^{-2}) would have on individual crayfish choice. The five groups of eight crayfish tested were given the option of four substrates. These were a) 'gravel', b) 'mud', c) 'clay bank' or d) 'artificial shelters' (of which there were 5). The decision to have only five shelters was deliberate because this would force three animals to either share, or move to an alternative substrate. This would

establish an order of preference. The crayfish were left for 24 hours after introduction to the tanks before counts were made. Control experiments were conducted first with a uniform gravel substrate, to check that no preferences were shown for any particular area of the tank. These showed that there were none (Kruskal-Wallis analysis, H = 2.9, d.f.= 3, P = 0.409) (Figure 3.2.7a).

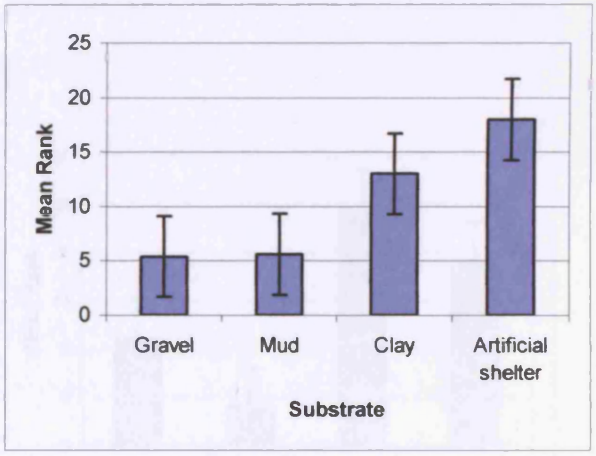
When presented with the choice of substrates, a significant preference was found (H = 16.22, d.f.= 3, P = 0.001). In order to determine the order in which the substrates were preferred, a ranked Tukey Multiple comparison was performed. It can be seen that the 'artificial shelters' had the highest occupation and it was preferred over both 'gravel' and 'mud' (Figure 3.2.7b). The 'clay bank' was the next substrate to be most frequently chosen. However, 'clay' was not significantly different from any of the other substrates. Finally 'gravel' and 'mud' were the least popular substrates with no crayfish preference being shown between the two.

Figure 3.2.7 Graphical representation of the Tukey multiple comparisons test showing different substrate preferences of groups of adult crayfish. At the 0.05 significance level, the mean ranks of any two groups with over-lapping standard error bars are not significantly different. a) Control experiment with uniform gravel substrate, b) a four-way substrate choice experiment.

a)



b)



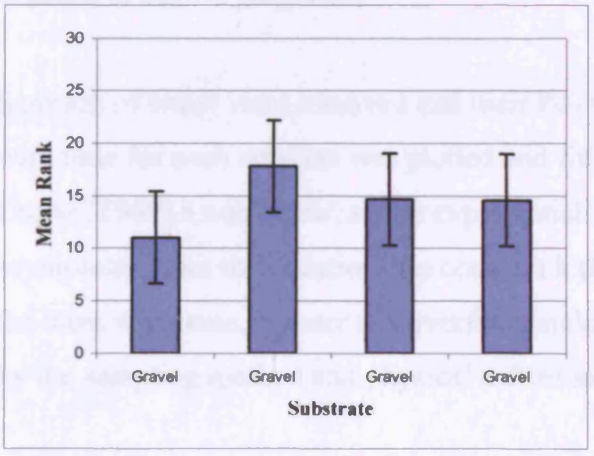
Substrate choice in groups of juvenile crayfish

The purpose of the experiment was to establish whether juvenile crayfish had different substrate preferences to that of adult crayfish. Between 42 and 60 juveniles were released, following hatching, from the pleopods of each of seven gravid female abdomens, using the

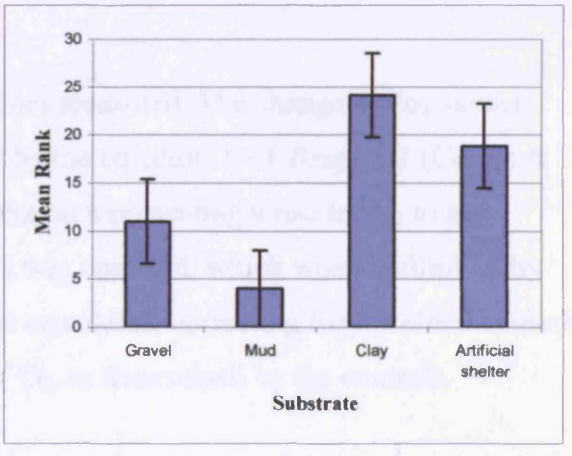
special tanks described in the Materials and Methods. Control trials were carried out using a uniform gravel substrate and these showed that there was no significant preference for any area of the tank (Kruskal-Wallis analysis, H=2.4, d.f. = 3, p=0.495) (Figure 3.2.8a). Therefore, any selection of a particular area was assumed to be due to the differences in substrate type. When presented with a choice of four substrate types, 'gravel', 'mud', 'clay bank' and 'artificial shelter', a significant preference was found (H=24.28, d.f.= 3, p<0.001). To determine in which order the substrates were preferred, a ranked Tukey Multiple comparison was performed. It can be seen that the largest number of juvenile crayfish selected the 'clay bank substrate'. This was preferred by more individuals than either the 'mud' and or the 'gravel' substrates. The second most preferred substrate was that of 'artificial shelter', although not significantly different from that of 'clay bank'. It was however, preferred over the 'mud' substrate. More juvenile crayfish chose the 'gravel' substrate to that of 'mud'. However, the differences in numbers settling on these were not significant. These data showed that there were differences in the order of substrate preferences between adult and juvenile crayfish.

Figure 3.2.8 Graphical representation of the Tukey multiple comparisons test showing different substrate preferences of juvenile crayfish. At the 0.05 significance level, the means of any two groups with over-lapping standard error bars are not significantly different. a) Control experiment with uniform gravel substrate, b) a four-way substrate choice experiment.

a)



b)



3.2.3 Rates of burrow irrigation by crayfish and passive irrigation

Rates of burrow irrigation by crayfish

Aims: a) To establish whether *P. leniusculus* actively irrigate their burrows and the mechanism of irrigation.

b) To find out a range of maximum water turnover rates by different sized individuals.

Hypothesis: Burrow irrigation rates will be related to crayfish size.

Burrow irrigation rates of the twenty-four crayfish (Mass = 30 – 101.3 g) were measured over half hour periods in an artificial burrow. In order to encourage maximum irrigation rates and to be able to ascertain these turnover rates, animals were placed into a Perspex™ burrow filled with hypoxic Leicester copper-free tap water. All crayfish were observed to increase the beat rate of their scaphognathites on entering the burrow. This in turn, would cause an increased respiratory rate generating a water current allowing the exchange of hypoxic water in the burrow, with normoxic water outside (a non-toxic red vegetable dye, amaranth, added to the burrow in preliminary tests showed the extent of this water irrigation). Only 2 of the 24 crayfish appeared to use the beating of pleopods, in addition to the increased ventilation rate, to assist in burrow irrigation.

Samples of water were removed and their Po_2 values measured. The change in Po_2 values with time for each crayfish was plotted and fitted by the equation $Y=A-Bexp[-kt]$ (Crowe & Crowe, 1969) a non-linear, single exponential function representing a rise in Po_2 to an asymptote. From the equation, the constant k (h^{-1}) was obtained, which when multiplied by the burrow volume, a water turnover rate could be calculated, correcting for the effect caused by the sampling method and physical diffusion of O_2, as determined by the controls.

Mean recorded changes in Po_2 of 24 crayfish with time, and the mean recorded Po_2 of the 3 control experiments (with no animals present) are shown below (Figure 3.2.9). Curves generated, using the function $Y=A-Bexp[-kt]$ were overlaid and showed a good degree of fit (Appendix X). Po_2 levels were seen to increase exponentially with time, with the mean rate of

change when animals were present being 15.5 l h^{-1} (litres per hour) compared to that when not (0.69 l h^{-1}). This showed that on average crayfish were capable of a maximum water turnover rate of 14.8 l h^{-1}.

The asymptote of the curves indicating Po_2 levels in burrow water, for both the control and with crayfish, did not reach the external tank water levels within the experimental time period.

Figure 3.2.9 Comparison of Experimental and Fitted Po_2 levels within an artificial burrow over a half hour period. Crayfish mean., n=24. Control mean., n=3 (no animal present). Using the function Y=A-Bexp[-kt], the curves are described as follows:

Crayfish mean: y=121-45.57exp[-7.7634t] & Control mean: y=74.7-14.7exp[-0.3465t]

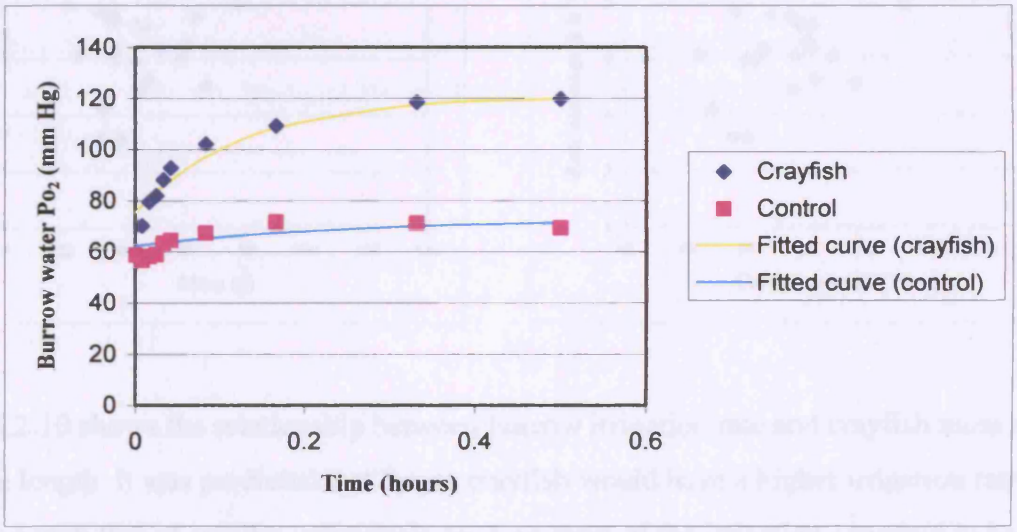


Table 3.2.2 Calculated mean burrow water turnover rates ± standard errors (l h^{-1}). Results are shown for male, female, combined and control experiments.

	n	Mean burrow water turnover rates (l h^{-1}) ± SE
Male	13	15.5 ± 0.9
Female	11	12.3 ± 0.8
Combined	24	13.5 ± 0.6
Control (no animal present)	3	0.69 ± 0.07

The control rate of Po_2 increase, however, was significantly lower than that measured when crayfish were present (Mann-Whitney, W = 370.0, $p < 0.01$) (Table 3.2.2).

There were no significant differences shown between the water turnover rates produced by male or female *P. leniusculus* (Mann-Whitney W = 194.0, p = 0.073).

Figure 3.2.10 The relationship between irrigation rate constant ($k\ h^{-1}$) and a) mass (g) and b) carapace length (mm) of *P. leniusculus* (n = 24).

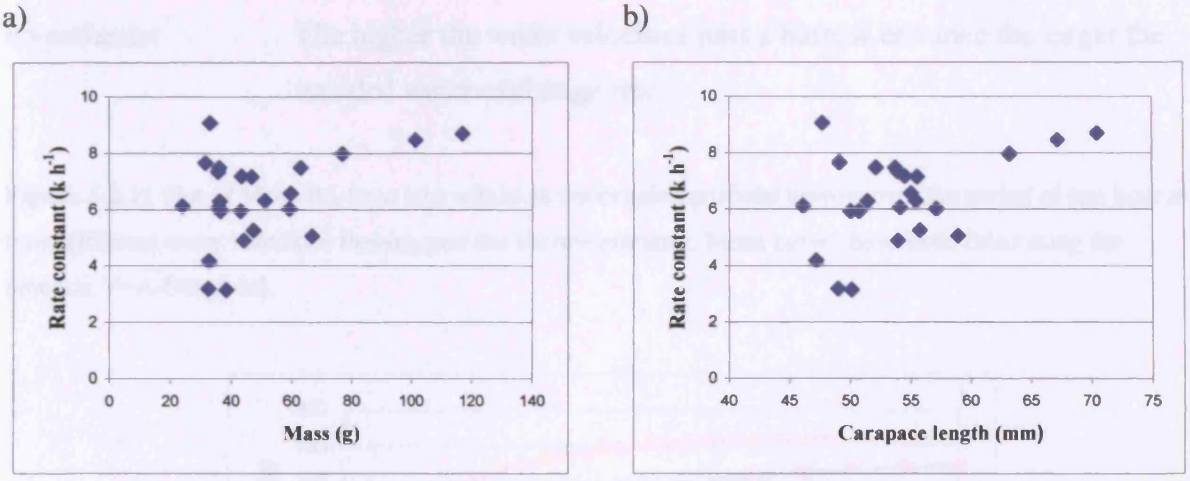


Figure 3.2.10 shows the relationship between burrow irrigation rate and crayfish mass and carapace length. It was predicted that larger crayfish would have a higher irrigation rate compared with that of smaller individuals because most of the irrigation appeared to be carried out by an increased ventilation rate. Thus, bigger animals with presumably larger brachial chambers and scaphognathite stroke volumes, would have the capacity to shift greater volumes of water per unit time and since the same sized artificial burrow was used for all animals it would be expected that that they would produce the most rapid increases in Po_2. This, however, appeared not to be the case. A Spearman's rank correlation showed no associations between k and either crayfish mass or carapace length ($r_s = 0.232$, n = 24, $P > 0.05$ and $r_s = 0.288$, n = 24, $P > 0.05$, respectively). It would appear that there is great variability in turnover and that animals of smaller size are capable of high rates of irrigation.

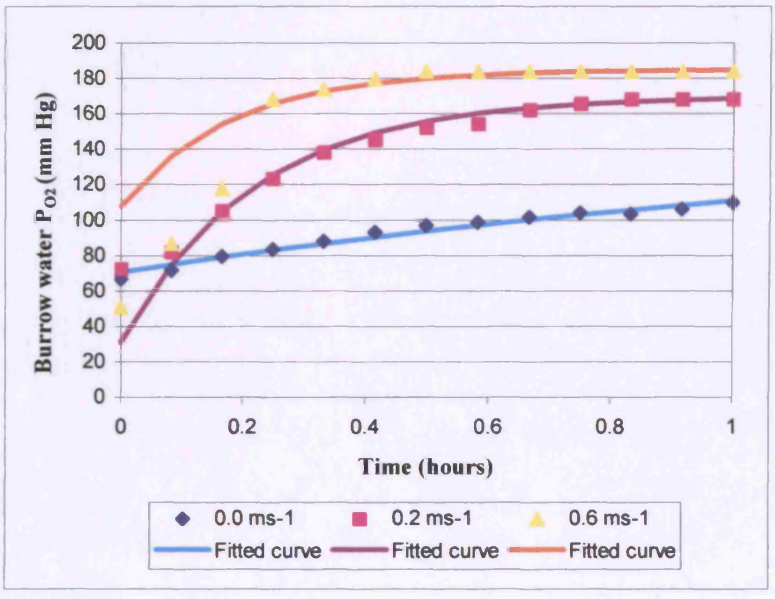
Passive irrigation

Aims: a) To establish whether external water-flow past a burrow entrance would cause some physical water exchange with the external medium, and if so, whether this would increase with increased water velocities.

b) To find out the approximate rates of water exchange with increased water velocities.

Hypothesis: The higher the water velocities past a burrow entrance the larger the unaided water exchange rate.

Figure 3.2.11 Plot of Mean Po_2 (mm Hg) within an unoccupied artificial burrow over the period of one hour at three different water velocities flowing past the burrow entrance. Mean curves have been fitted using the function Y=A-Bexp[-kt].



The fitted curves did not describe the increases in P_{O2} levels well enough for direct comparison with the data in section 3.2.3 or calculation of values of k, as the curve gradients were significantly underestimated. It was thought that there might be more than one exponent needed to describe the curve of the relationship between water flow and burrow turnover rates (Figure 3.2.11). It was clear, however, that with increasing water velocities the exchange of hypoxic internal burrow water with external normoxic water, increased. In a field situation, this would mean that stream flow past the front of burrows would cause water convection, and

thus cause an exchange of burrow water with that of stream water. With increasing stream flow the rate of this exchange would increase.

Chapter 4

Discussion

Chapter 4: Discussion

4.1 Field Studies

4.1.1 Burrow densities in relation to substrate type

Information on population expansion and preference of substrate for burrowing is helpful in the control and management of *P. leniusculus*. Research carried out in this study shows that there has been an increase in burrow densities along the Gaddesby Brook, particularly in areas downstream of Lowenva Lodge (SK 737 094). Burrows are now present a further 3 km downstream, since the data collected by Harris and Young (1996).

Burrow density data collected for *P. leniusculus* in the Gaddesby Brook, ranged between 0 – 14 m^{-1} and were comparable to estimates made by Guan (1995), for *P. leniusculus* in the River Great Ouse (0 to 25.7 m^{-1}), and also, similar to that obtained by Correia and Ferreira (1995), for *Procambarus clarkii* (0.013 to 6.28 m^{-2}), in rice fields, marshes and reservoirs in Portugal.

Adult and juvenile *P. leniusculus* showed definite preferences for particular stream bank substrates when excavating burrows. This was demonstrated by the different burrow densities which occurred along the Gaddesby Brook and which indicated burrow aggregation at particular sites. It was also shown by the significantly higher burrow densities present in areas with a stream bank clay content greater than 39%, and the significant negative correlation between burrow density and sand content. One explanation is that banks with high clay content are more stable and less likely to collapse during and after burrow excavation. This is not necessarily the case for all crayfish species, since Lawrence *et al.* (2002), found that there was no relationship between burrow occurrence and sediment type for *Cherax albidus*.

However, many crayfish species do show preference for burrowing in predominantly clay substrate. For example, Grow (1982), claimed that *Cambarus diogenes diogenes* (Girard) preferred to excavate burrows in fine-grained clays to that of coarse-grained sands and Guan (1995), found that the burrows of *P. leniusculus* had a clumped distribution in clay banks and were absent in predominantly sand and gravel banks. Similarly, Correia and Ferreira (1995),

found burrowing activity of *Procambarus clarkii* occurred only when the ratio of fine particles over coarse particles was higher than 0.1 – 0.2 and Rogers and Huner (1985), observed that the burrows of *Cambarus diogenes* were constructed in soils classified as silty clay loams and silty clays.

There were a large number of sites, spanning the Gaddesby Brook, where no burrows were present, accounting for 37% of sites sampled. There are a number of possible reasons why burrow construction did not occur at these sites. 55% were in areas where a stable population density had not been attained, suggesting that perhaps the available habitat suitable for occupying natural shelters, or for burrowing, had not as yet been utilised. 27% were at sites where substrate type was unsuitable for burrowing or where substrate type was not ideal, combined with the fact that there were plenty of unoccupied 'natural refuges' such as stones, bricks and tree roots, to be used. The final 18% were at sites where there was suitable available bank substrate. The reasons why burrows were not present at these sites could be because, again, there were plenty of 'natural refuges' (therefore no need), or because they were at sites that were shallow, making burrow construction difficult below the waterline, or because some sites had high water velocity (some riffle sites), which may have prevented burrow initiation.

There was sufficient data to suggest that the physical property of the bank substrate appeared to be a major factor in determining burrow density in the Gaddesby Brook. Furthermore, *P. leniusculus* did select a preferred substrate type for burrowing. This finding was concurred by Lodge and Hill (1994), who suggested that substrate type was the most important factor determining habitat choice of freshwater crayfish.

4.1.2 Internal burrow features

Internal burrow features were inspected by use of an infrared optic fibre video camera. This technique was new to crayfish burrow examination and was used because landowners had expressed their concern at bank damage caused by casting. The main advantages of this method were; it was quicker to examine burrows than conventional methods; it did not damage the stream bank; there was minimal disturbance of the crayfish, and finally; it was possible to observe crayfish behaviour in the burrow. However, had the burrows inspected been more complex, with several branched 'galleries', this method would have been less

effective as control of the camera head was limited and videoing could only be conducted in clear waters. Another constraint was that the equipment was expensive to rent.

Although there was a diverse range of burrow features of *P. leniusculus* observed, the most common, accounting for 77% of those examined, was a single entranced burrow with a tapered 'gallery'. This was similar at both White House Farm and Newbold Farm. These burrows were quasi-horizontal to the stream surface and penetrated into the stream bank to a maximum-recorded distance of 79 cm. This burrow type has also been described for several other crayfish species, such as, *Cherax albidus clark*, with a maximum recorded burrow length of 65 cm (Lawrence *et al.*, 2002) and *Procambarus clarkii*, where burrow morphology was generally simple, with a few observed complex burrows and where mean tunnel depths ranged from 0.28 – 0.58 m (Correia and Ferreira, 1995).

This type of simple burrow morphology, lends itself to certain adaptive advantages. Its simplicity means that construction is both quicker and easier, requiring less energy to build and a single entrance probably makes it more easily defendable from conspecifics. The tapered 'gallery' could also have a purpose in defence, because if a larger invader cannot be fended off at the entrance, retreating into a narrower part of the 'gallery' would make it more difficult for an attacker to pursue.

This type of crayfish burrow cannot be classified properly using the North American classification of burrowing crayfish described by (Hobbs, 1981), but is better described using the ecological classification of Australian crayfish burrows (Horwitz and Richardson, 1986). From this *P. leniusculus* can be described as a 'Type 1a' burrowing crayfish, which "lives in permanent bodies of surface water, under rocks, ledges, in rock crevices, in or under logs and in short, unbranched burrows in the substratum".

A number of burrow entrances were observed above the water level in the Gaddesby Brook, all of which, when examined were empty. It was thought that these were a result of changes in water level, and had been constructed when water levels were higher and would have immersed them (in winter). This theory concurred with that of Guan (1995), for *P. leniusculus* in the River Great Ouse.

Burrow lengths were significantly longer at White House Farm to that of Newbold Farm. This may have been due to the higher stream water velocity and the significantly wider burrow entrances present at White House Farm. A higher stream water velocity, combined with a larger entrance width (increased surface area of the stream and burrow water interface), would allow greater water exchange between the stream and burrow waters (see section 4.2.3), so the burrows could be longer and still have sufficient water turnover to maintain favourable internal burrow environments.

There were a surprisingly large number of unoccupied burrows at both White House Farm (66%) and Newbold Farm (49%). This was similar to observations made by Ilheu *et al.* (2003), for *Procambarus clarkii* in a temporary stream of the south of the Iberian Peninsula, in which burrows were mostly found either empty or occupied by a single individual. It was suggested in this case, that the crayfish did not hide exclusively inside excavated burrows, but regularly used natural refuges. However, as later radio tracking data suggests (see section 4.1.5) *P. leniusculus* mainly remained loyal to a particular shelter, thus, some vacant burrows must have been due to other factors, such as, death from predation, age or disease.

The internal burrow surface of most examined burrows of *P. leniusculus* had both scrape marks and 'lumpy' surfaces. These findings were similar at both White House and Newbold Farm. Blank and Figler (1996), suggested that there were three reasons why a rough internal texture for a shelter may have been preferred over a smooth surface. Firstly, it more closely resembled the inside of a burrow or the type of shelter a crayfish would naturally excavate. Secondly, it was easier to defend against an intruder (i.e. provided better leverage during agonistic encounters), and/or, thirdly, it provided an additional food source (i.e., rough shelters appeared to attract algae more rapidly than smooth shelters, perhaps because of the increased surface area).

Through observations of the insides of the burrows of *P. leniusculus,* it seemed that the scrape marks could have been created by the chiselling action of the chelae during excavation and the 'lumpy' surfaces caused by the snipping action of the pereopods both during excavation and whilst making burrow modifications after burrow construction. This was concurred by Hasiotis (1993b), who found scrape marks and knobbly features in internal burrows, in both Triassic and Holocene North American crayfish burrows.

Interestingly, Hasiotis (1993b), also suggested that depths of burrowing, burrow architecture, population densities and distribution of types of crayfish burrowers were highly influenced by environmental factors and that both ancient and modern crayfish were similarly affected despite a difference of 220 million years.

4.1.3 The internal burrow water chemistry

Data on the water chemistry within burrows of aquatic decapod crustaceans is limited and most focus primarily on oxygen levels (Atkinson and Taylor, 1988; Burggren and McMahon, 1983; Forgue *et al.*, 2001; Gerhardt and Baden, 1998; Taylor and Wheatly, 1980). Knowledge of the levels of O_2, CO_2 and ammonia within occupied and non-occupied burrows could be useful indicators of animal presence and possible irrigation behaviours.

Ammonia exists in two forms in the natural environment, NH_3 (unionised) and NH_4^+ (ionised), their relative proportions being dependent primarily on pH, but to a lesser extent on temperature and ionic strength (Lourey and Mitchell, 1995). NH_3 is considered more toxic to aquatic organisms because it is a dissolved gas in water, which can pass unimpeded through gill membranes and, as such, it has been used in a number of acute toxicity experiments (Liu *et al.*, 1995; Lourey and Mitchell, 1995; Rouse and Kastner, 1995). For the purpose of determining internal burrow ammonia concentrations, total ammonia, (T_{amm}), which included both forms, was calculated.

The mean T_{amm} concentrations within the burrows were higher than the external stream water at both Newbold and White House Farm during summer and winter. Crayfish presence was determined to be the main cause of this increased ammonia level, as measurements made in burrows with crayfish excluded, showed significantly lower concentrations. Ammonia is a waste product excreted by all crayfish, including *P. leniusculus* (Harris *et al.*, 2001). The higher ammonia levels recorded in the burrows in summer probably reflect the increased activity levels shown by *P. leniusculus* during this season. This could be due to water temperature, feeding and mating, which would increase metabolic rate. On this basis, the number of burrows, which were indicated to be inhabited by their elevated ammonia concentration, was 33% at White House Farm and 57% at Newbold Farm. This compared with the independent estimates made at each site, determined using an optic camera,

(see section 3.1.2); White House Farm = 34%, Newbold = 51%) which showed that ammonia was a good means of determining burrow occupancy.

The highest mean T_{amm} concentration reached in burrows was at Newbold Farm in summer (28.78 ± 3.99 μmoles l^{-1}), almost 2-fold that of stream levels. It was found that the acute lethal concentration (LC_{50}) for *P. leniusculus,* established by Harris *et al.* (2001), was substantially higher (24 h LC_{50} = 15.0 ± 2.6 $mmol^{-1}$ and 48 h LC_{50} = 4.9 ± 1.1 $mmol^{-1}$(15°C, pH = 8.2)). Thus, *P. leniusculus* could tolerate much higher ammonia concentrations than that found at Newbold Farm.

As the ammonia levels in burrows at Newbold Farm did not rise to an acute concentration, it could be possible that a process of ammonia removal was occurring. A likely explanation was that some form of burrow irrigation was occurring (discussed further in section 4.2.3). A reason for burrow irrigation could be that, although *P. leniusculus* can survive in high ammonia levels, these conditions are not necessarily favourable. Lourey and Mitchell (1995) found that the growth rate and/or reproduction for *Cherax albidus* were reduced at unionised ammonia levels above 0.096mg l^{-1}.

The PO_2 levels within the burrows during winter at both sites were quite similar to that of the stream level. This may be because *P. leniusculus* were less active during the winter due to reduced water temperature (Bubb *et al.*, 2002a), as such, respiration and metabolic rates would be lower resulting in less oxygen consumption. During summer, the PO_2 levels were significantly lower than that of stream water, demonstrating moderately hypoxic conditions within the burrows. During this season, higher water temperatures meant that *P. leniusculus* were more active within burrows, resulting in an increased metabolic rate, thereby depleting burrow water oxygen levels because of an increase in respiration rate. Grow and Merchant (1979), found that the burrow water of the crayfish *Cambarus diogenes diogenes* showed nearly aneorobic conditions (9.1% of air saturation), and even when in direct contact with highly oxygenated water, resulting from the flooding Potomac River, oxygenation levels remained low.

In general, the levels of $\sum CO_2$ were higher within the burrows at both sites compared to that of stream water, showing slight hypercapnic conditions. Levels of PCO_2 and [$HCO3^-$] were significantly greater in occupied burrows than that of burrows with animals not detected or

excluded, this showed that *P. leniusculus* were responsible for the excretion of CO_2 during respiration.

In burrows where animals were excluded, levels of $\sum CO_2$ and [$HCO3^-$] were reversed, showing lower concentrations than in stream water. This could possibly indicate the process of algal photosynthesis occurring in the burrow, as such, CO_2 would be converted to O_2.

4.1.4 Population densities, range extension and burrow erosion damage

Population densities of *P. leniusculus* at Newbold Farm, White House Farm and Lowenva Lodge remained relatively constant between 2000 and 2002. It is possible that this may have been because the utilisation of all available suitable substrate type had occurred (Mason, 1978; Peay and Rogers, 1999). At Ashby Folville and Gaddesby, however, population densities and CPUE's steadily increased over the two years of this study, indicating that a maximum sustainable density had not yet been reached.

When the data of this study, was compared with that collected by Harris and Young (1996) and Harris (1999), it was found that population density estimates for the sites at Newbold Farm, White House Farm and Lowenva Lodge had increased from 1995 to 1998 and from 1998 to 2000, suggesting perhaps that during these periods, occupancy of all available shelter had not been fully utilised.

Estimates of population densities at sites at Ashby Folville and Gaddesby were not calculated in 1995 or 1998 by Harris and Young and Harris respectively, because there were insufficient numbers of crayfish. However, by 2000, numbers of signal crayfish had increased sufficiently to calculate population density estimates. It was also found at these sites, crayfish density rose between 2000 and 2002. These data, therefore, demonstrate that between 1995 and 2002 there has been a downstream spread of *P. leniusculus*.

To determine the rate of population spread, the current distribution of *P. leniusculus* in the Gaddesby Brook, was ascertained by trapping and hand searches. This information was then compared to data gathered in 1992, 1995 and 1999 by (Harris, 1999; Harris and Young, 1996; Rogers, 1993) respectively. From this it was found that the rate of range extension of *P.*

leniusculus showed polynomial expansion i.e. the speed of new habitat colonisation was increasing each year.

Other research on range extension of crayfish species only tended to quote rate of range extension in terms of average rate in km per year (Guan and Wiles, 1997b; Harris, 1999; Peay and Rogers, 1999; Sibley, 2001), most being ~ 1 km year^{-1}. However, the implications of this polynomial rate of expansion, clearly demonstrates that well-established colonies of *P. leniusculus*, already present in U.K. rivers, will become increasingly more problematic in the future. It is likely that colonisation of this species will be even more rapid than was previously thought.

As burrow entrance size is correlated to crayfish size (Guan, 1995 and see section 3.2.1), the significantly smaller burrows found at the low population density sites of Ashby Folville and Gaddesby compared with those at the high density sites of Newbold Farm and Twyford Lodge. This could suggest that initial colonisation of new sites may be driven by smaller crayfish, as these individuals are less able to defend themselves in a high density population and would therefore need to venture further a field to settle. This view, was supported in part by Reyjol and Roqueplo (2002), for *A. pallipes,* who found that the reduction or disappearance of one habitat, which is particularly favourable to the young of the year, might be compensated by a more active colonisation of some habitats which normally shelter only a few crayfish.

In the knowledge that *P. leniusculus* will continue to colonise new sites, it becomes increasingly important to understand the impact the species will have on stream environments. Much research has been conducted on the impact of invasive crayfish species in relation to the reduction in stream vegetation, impact on benthic fishes and direct and indirect impacts on invertebrate communities (Guan and Wiles, 1997a; Holdich, 1999; Soderback, 1995; Usio, 2002). However, in the U.K., another important factor to be considered are the burrows constructed by *P. leniusculus* and their impact on the structural stability of stream banks.

It was found that in areas of high burrow density as much as 7.8% of stream bank sediment was removed. This had the largest impact at the leading bank edge, which was not only directly exposed to stream water flow, but would be the point at which most weight would be

placed by drinking animals, such as cattle. Several areas were observed to have had recent bank subsidence, where high densities of burrows had been excavated. Guan (1995), also noted, bank collapse from *P. leniusculus* burrows in the River Great Ouse, and, in laboratory experiments by Hasiotis (1993a), it was shown that stream bank collapse did take place because of crayfish burrows as they weakened the banks. It was also suggested that water table fluctuations would accent voids created by the burrows and that these voids would allow the clay in the soil to expand and virtually undermine the whole stream or river bank or that the weight of the bank itself would cause its collapse under the force of gravity (Hasiotis, 1993a).

4.1.5 Short term movements and activities in the field

Radio tracking is a costly method for determining animal movements in their natural environment and as such has meant that only small samples have been observed at any one time. However, the advantage of this method of tracking is that individuals can be continuously monitored without disturbance or having to rely on the tag recapture procedures (Bohl, 1999; Bubb *et al.*, 2002a; Gherardi *et al.*, 2002; Gherardi *et al.*, 2000; Robinson *et al.*, 2000; Schutze *et al.*, 1999). The other major limitation of radio telemetry suggested by Nams (1989), was the inability to determine the behaviour of radio-tagged animals when out of sight (e.g. crayfish in a burrow /refuge or at night).

The use of red L.E.D units solved the problem in darkness and this enabled crayfish movements to be observed directly and be related to radio telemetry data. This technique also enabled the identification of individual burrow use, in areas where burrows/refuges were too closely packed to be differentiated by the radio tracking. The L.E.D. unit enabled verification that individuals were returning to the same burrow on consecutive nights for up to 7 nights, unlike the beta lights used by Robinson (1997) which only lasted for 8 hours.

Initial movements made by *P. leniusculus* after release, did not indicate a 'fright response' as found for *A. pallipes* by Robinson *et al.* (2000). This was in agreement with findings by Bubb *et al.* (2002a), who also observed no 'fright response' for *P. leniusculus*.

Of the 38 radio tracked crayfish, 28 were pinpointed in the same burrow or refuge for the duration of the tracking period. These animals exhibited the behaviour of staying in a 'home'

refuge, with movements centred about it. Robinson *et al.* (2000), also observed high levels of local activity around refuges of *A. pallipes* and suggested it was due to the use of home areas.

Seven crayfish (Animal Nos 1,2,3,6,10,18 and 19), however, made occasional large movements (up to 89.6 m in one day), followed by stationary phases of between 2-8 days. This pattern of behaviour was also observed in *Pacifastacus leniusculus* (Bubb *et al.*, 2002a), *Orconectes rusticus* (Byron and Wilson, 2001), *A. pallipes* (Gherardi *et al.*, 1998; Robinson *et al.*, 2000), and in a freshwater crab *Potamon fluviatile* (Gherardi, 1988). In all these cases, however, the majority of basic movements by individuals consisted of short distances travelled around a shelter. Although it is not known why certain individuals make sudden large-scale movements, it could be attributed to population density pressures (an individual loses its refuge to another crayfish), insufficient food supply, loss of shelter through collapse, or displacement by water flow. Byron and Wilson (2001), have suggested that these large distances could also have important implications in crayfish invasion rates.

In this study, all the long distance movements made by *P. leniusculus* were in an upstream direction. This finding conflicted with those of Bubb *et al.* (2002) who reported movements by *P. leniusculus* being in both directions and Robinson *et al.* (2000) who also found movements were both up and downstream for *A. pallipes*. This suggests that different crayfish populations of the same species exhibit differences in movement traits, which may be dependent on location and habitat environment. In the present study, on no occasion did an individual, after a large-scale movement, return to a previously occupied shelter. This was also reported in *P. leniusculus* (Bubb *et al.*, 2002), *Procambarus clarkii* (Gherardi *et al.*, 2002) and *A. pallipes* (Robinson *et al.*, 2000).

There were no significant differences in the distances travelled by male and female *P. leniusculus*, again, this was likely due to having a fixed refuge. The same findings were also true for *A. pallipes* (Robinson *et al.*, 2000), *Orconectes rusticus* (Byron and Wilson, 2001), *Procambarus clarkii* (Gherardi *et al.*, 2002) and *P. leniusculus* (Bubb *et al.*, 2002b).

As most of the radio-tagged crayfish movements centred about a fixed refuge, it was not surprising that animal size (carapace length), did not affect the overall distances travelled by individuals. This agreed with the findings of Bubb *et al.* (2002a), for *P. leniusculus* and of Byron and Wilson (2001), for *Orconetes rusticus.* However, Robinson *et al.* (2000), found

that there was a positive correlation between distance moved per day and size, for downstream movements made by both male and female crayfish, but not for upstream movements in the species *A. pallipes*. These differences may be related to shelter fidelity, for, if no commitment towards a specific shelter was shown by *A. pallipes*, there would possibly be a greater tendency to roam, with larger animals perhaps covering a greater distance.

No *P. leniusculus* were lost or displaced during the two flood events, which occurred at the Gaddesby Brook during radio tracking. This was similarly the case for *P. leniusculus* in the River Wharfe (Bubb *et al.*, 2002b) and *Orconectes juvenalis* (Merkle, 1969), which contrary to being dislodged, had actually moved upstream. However, Robinson *et al.* (2000), felt that flood events could be catastrophic for *A. pallipes,* because two out of five tracked crayfish were found dead after a high stream-discharge event.

Stream, burrow and crayfish microhabitat temperature data was recorded using Tiny Talk and ibutton data loggers during the radio tracking study. The ibutton data loggers were found to be more reliable because they were not dependent on a rubber sealed casing, which was prone to flooding. However, they were slightly less accurate, recording temperature to only the nearest 0.5°C, rather than that recorded by the Tiny Talks of 0.1°C. Nevertheless, the data obtained showed that there was no significant variation between stream, burrow and crayfish microhabitat temperature. A similar finding was also made by Gherardi and Barbaresi (2000) for *P. clarkii,* in rice fields, where, during the day, both water and crayfish microhabitat temperatures were 22°C and at night there was only a slight difference of 2°C (microhabitat being lower).

Pacifastacus leniusculus was shown to be most active at dusk. This concurred with the findings of Kozak *et al.* (2002), who observed that adult signal crayfish in laboratory experiments showed only one activity peak (at dusk), while there was low activity at dawn. He also noted, that slight activity reduction over the night (at around 2200) was probably caused by the animals processing food already taken into their shelters. Robinson (1997) found that *A. pallipes* were also more active at dusk and that during dawn, morning and afternoon the crayfish remained in their refuges, which would account for their limited activity. This nocturnal behaviour could be considered to be an adaptive advantage as it could reduce the risk of predation from daytime predators, or it could be a synchronising behaviour with prey species (Hamrin, 1987; Stein, 1977).

Distances travelled by *P. leniusculus* were greater in summer than winter. This may be linked to food availability, temperature of the water and energy requirements. In winter, there was less food available generally, the stream water was colder, for example, at times as low as ~ 2°C to as high as ~ 10°C, so there is likely to be more need for *P. leniusculus* to conserve energy.

When water temperature was compared with the activity of *P. leniusculus* in the field, it was found that they were significantly more active in warmer stream temperatures. Temperature is probably a major factor influencing crayfish activity and is substantiated by Flint (1977), who also found peak activity in the warmer summer season and very little activity in winter, and Bubb *et al.* (2002a), who correlated temperature differences in winter with activity for *P. leniusculus*. However, temperature may not be the only factor influencing activity. Day length, food availability, moulting, shelter, predation, stream flow and reproduction are all factors with possible associations to activity levels in crayfish (Bubb *et al.*, 2002a; Byron and Wilson, 2001; Capelli, 1980; Capelli and Hamilton, 1984; Capelli and Munjal, 1982; Cobb, 1971; Collins *et al.*, 1983; Crawshaw, 1974; Doroshenko, 1988; Flint, 1977; Garvey *et al.*, 1994; Gherardi and Barbaresi, 2000; Gherardi *et al.*, 1988; Hazlett *et al.*, 1979; Karnofsky *et al.*, 1989; Kozak *et al.*, 2002; Maude and Williams, 1983; Moller and Naylor, 1980; Williams *et al.*, 2001).

Pacifastacus leniusculus demonstrated no homing behaviour when displaced 50 m up or downstream in the Gaddesby Brook. This was similar to findings for adult *A. pallipes* (Robinson *et al., 2000)* and *Procambarus clarkii* (Ilheu *et al.*, 2003). However, (Gherardi *et al.*, 1998), in a Tuscan stream, found *A. pallipes* to have a weak tendency to return to the 'home' pool when released at a distance of 50 m. This perhaps suggests that they either have poor orientation abilities, or that it is more energy efficient to seek shelter in the new area, especially if shelters are in abundance.

4.1.6 Long term movements and tagging

As discussed earlier, animal movements play a key role in the understanding of habitat requirements, resource utilization and potential colonisation of new areas. Animal tagging and re-capture techniques provide a useful means in which to assess these movements.

The new design of streamer tag used for this study, was successfully retained during moult cycles. This was attributed to the fine monofilament used, which on moulting, effortlessly tore through the softened cuticle, which was being shed. The numbered half disc used to identify individuals became coated with algae after 2 years, however, this was easily scraped away, making all recaptured tags identifiable. The tag was biologically compatible, permanent, easily constructed, cheap and was relatively easy to attach. The only disadvantage was that it was unsuitable for small individuals.

Over the 2 years, recapture of *P. leniusculus* in the Gaddesby Brook, using this tagging method and baited traps for retrieval, was 19.0%. This was higher than other studies such as, Rhodes and Avault (1986), in which only 3.5% of 1602 fluorescent dye marked *P. clarkii* were retrieved (crayfish migration accounting for the low recapture). Robinson (1997), tagged 888 *A. pallipes* and had a 9.3 % retrieval rate over a period of 83 days (this species was suggested to have an 'ephemeral home range' maintained in a restricted area on a daily to weekly time table) and Guan (1995), retrieved only 13.2 % of 8000 marked *P. leniusculus*, the majority of which were recaptured within 100 m of release, 2 months to a year later indicating a possible home range.

The mean mass and carapace length of tagged crayfish were similar to that of re-captured individuals. This demonstrated that the tag did not unduly affect a particular size class of crayfish. There was also no evidence of tag loss, as all re-captured animals, whether they were tagged or not, were checked for potential damage that would have resulted from tag loss.

23% of recaptured animals were re-caught in the same trap, i.e. the exact place of initial capture. 60% of recaptured animals were caught within ~7 m of the initial trap, while 17% of animals were caught up to ~103 m from their original point of release. Overall, these movements equated to 39% of recaptured animals moving in an upstream direction and 37.7% in a downstream direction. However, it must be noted that these distances were small. This agrees with Bubb *et al.* (2002a), who found that there was no significant difference in the movement of *P. leniusculus* in the R. Wharfe, in either an upstream or downstream direction.

Considering that a stream is an open system and that *P leniusculus* are very agile (Harris, 1999), with the ability to make large scale movements, such high recapture rates within the vicinity of release (60% re-caught within 7 m of release) suggests that adult *P. leniusculus*

have a home range and stay within the same vicinity for long periods. It also suggests that adults may only play a small role in the colonisation of new down and upstream sites. This is supported by the data collected on burrow sizes, where significantly larger burrows were found in well-established sites (point of *P. leniusculus* introduction), compared to those, which were being newly colonised.

4.1.7 Comparisons with the River Greet

This small comparison study between the River Greet and the Gaddesby Brook was conducted to ascertain whether a different population of *P. leniusculus* in a different catchment area, would demonstrate similar preferences of substrate for burrow construction and similar rates of range extension.

In 1999, it was observed by Harris, that population densities of *P. leniusculus* in the River Greet, were between 0.1-12.7 m^{-2}, with the highest densities being, 'particularly in the middle stretches'. From this it was suggested, that the site of first introduction of *P. leniusculus* in this river was at Maythorne Mill. At this time, crayfish distribution had been identified to span from Kirklington Mill (the upper most site) to Upton (see Figure 3.1.19a), and burrows had been identified in densities of up to 12.5 m^{-1} comparable to that of the Gaddesby Brook.

Thus the River Greet was similar to that of the Gaddesby Brook in that there was a range of substrates types, predominately consisting of clay/mud banks, with silt and gravel stream beds. Also, a few areas had high numbers of natural refuges, in the form of rubble and masonry. Vegetation on the riverbanks was similar in both water bodies, although access to some of the sites were more difficult on the River Greet.

This study shows that there is a significant positive association between the percentage of clay content of the stream bank and burrow density at the River Greet, similar to that shown at the Gaddesby Brook. This indicates that two separate populations of *P. leniusculus* are selecting the same substrate type in which to burrow.

It was also found that *P. leniusculus* had spread in both an upstream and downstream direction since recorded by Harris in 1999. This was somewhat different than the situation in the Gaddesby Brook, where range extension is predominantly in a downstream direction.

When the range extension data was overlaid with data of the Gaddesby Brook, it could be seen that the rate of range extension appeared to be fairly similar. If this does prove to be the case, that is, when more data is collected over the next few years, it could be that this population of *P. leniusculus* will soon be prevalent in the R. Trent.

4.2. Observations of burrowing and associated behaviour in the laboratory

4.2.1 Crayfish behaviour before, during and after burrowing

Male and female *P. leniusculus* of varying sizes (CL = 20.7 – 66.2 mm) were videoed constructing burrows. The methods used in burrow construction were similar for all sizes and both sexes. This was also reported by Rogers and Huner (1985), for both *Fallicambarus hedgpethi* and *Procambarus planirostris.*

On initial introduction to experimental tanks, all crayfish took time to 'explore' before burrow construction or 'resting'. During this period, there was rapid antennae movement and probing of the substrate by 3rd maxillipeds and 2nd and 3rd pereopods. It could be suggested that this behaviour was to establish whether substrate was suitable for burrowing. Similar tank exploration was noted for adult *P. planirostris*, *P. clarkii*, and *P. a. acutus* (Rogers and Huner, 1985).

A correlation was found between crayfish size and the amount of time taken to begin burrow construction after introduction to the tank. The quickest initiation time recorded was 30 minutes for a small crayfish, with larger crayfish sometimes taking over two days. It could be postulated that this behaviour reflects the need of small individuals to obtain shelter quickly because of a higher risk of predation. Rogers and Huner (1985), investigated the initiation times for burrowing of *P. planirostris, P. clarkii, P. a. acutus. Procambarus planirostris* was found to initiate burrowing after only 6 minutes; *P clarkii* took from between 10 minutes and several hours, whilst all of *P.a.acutus* took several hours. All three species were reported to have completed burrow construction overnight. This was different to that of *P. leniusculus*, as in some instances, it took up to 3 days to complete burrow construction. This supported findings by Guan (1994) who found that adult *P. leniusculus* required periods from several hours to days to complete burrow excavations.

There were two main methods of burrow excavation. These were: Method 1: entering the burrow headfirst and using the chelae to chisel away at the burrow walls. Method 2: entering the burrow abdomen first, and utilising the tail and abdomen to scoop up sediment, before returning to the burrow entrance, turning and releasing the sediment. Both excavation methods, in order to remove fine sediments, generated a current of water by the synchronous beating of the pleopods. This was done, whilst excavating in the burrow for Method 1, and at the burrow entrance, whilst releasing the sediment collected in the abdomen, for Method 2.

Observations on the burrow excavation of *P. leniusculus* were noted by Guan (1994), who described the method of digging using the chelae and walking legs, but made no mention of reversed entrance or extraction of sediment utilising pleopods. However, reversed entrance and use of the abdomen for sediment removal has been observed in the Norwegian lobster *Nephrops norvegicus* (Rice and Chapman, 1971) and extraction of fine sediments utilising vigorous fanning of the pleopods has be observed in the lobster *Homarus americanus* (Cobb, 1971).

From these observations, it was seen that chelae played an important role in burrow construction. However, as demonstrated by Trepanier and Dunham (1999), for some juvenile *Fallicambarus fodiens*, burrow construction still occurred when both major chelipeds were missing. This suggests that chelipeds are not essential for at least some individuals, and that pereopod use is an important part of burrow construction.

Differences between male and female crayfish, in the time taken to initiate burrowing, showed that females started burrowing significantly earlier than male crayfish. This could be due, either to the fact that female crayfish are generally smaller and therefore more vulnerable to predation, or, because burrowing is of greater priority to female crayfish. For example, once a burrow was constructed, less energy would be utilised on defence and position maintenance, so more could be diverted towards reproduction.

A significant association was also found between crayfish size and burrow entrance area. Two possible reasons for this were; constructing a burrow bigger than necessary would require an unnecessary increase in energy expenditure and by having a 'burrow to fit', smaller individuals would be better protected. Burrow defence would be easier because of exclusion from larger conspecifics.

There were significant associations found between burrow length and carapace length, showing that larger individuals constructed longer burrows. There was also a significant association between construction time with that of burrow length and burrow entrance size i.e. larger burrows took longer to build.

4.2.2 Substrate Choice Experiments

Establishing preferred substrate types selected by *P. leniusculus* is important in the understanding of their patterns of distribution and their colonisation of new areas.

A number of laboratory substrate choice experiments have been conducted, but the majority focussed on the most suitable substrate for commercial farming. The purpose of these experiments was to promote good growth and reduced mortality (Klosterman and Goldman, 1983; Kozak *et al.*, 2002; Mason, 1978; Savolainen *et al.*, 2003). As such, limited substrate types were tested and few reflected substrate types that might have been preferred by animals in the natural environment.

Adult *P. leniusculus* were shown to have distinct substrate preferences when provided with a choice. A significant preference for 'mud' was shown over that of 'gravel'. This was also shown by Vorburger and Ribi (1998), where they concluded that mud was preferred over pebbles (8 – 16 mm). This may have been because neither gravel nor pebble substrates offered structural shelter. The mud did allow the crayfish to part conceal their bodies, an advantage in the natural environment, as it would offer camouflage from potential predators. Although this behaviour was not observed in the Gaddesby Brook, it was noted by Guan (1994), for *P. leniusculus* in the River Great Ouse 'crayfish simply buried themselves in the surface mud of the river bottom'.

When *P. leniusculus* was provided with a choice between 'mud' and 'clay bank', a significant preference was shown for 'clay bank'. All crayfish that selected this substrate either constructed a burrow or created a small depression in which to sit, normally against a tank wall. This was supported by Correia and Ferreira (1995), who found that burrowing activity only occurred when the ratio of fine particles over coarser particles was higher than 0.1 – 0.2. Construction of a burrow in the natural environment could possibly offer greater protection from predators than concealment in mud. However, it could also be argued that making a

depression in the clay bank was less effective as a camouflage than concealment in mud. This poses the question of why did *P. leniusculus* select clay? The answer could be because the risk of predation was offset by the disadvantages of loose mud particles, having the potential to reduce gill function due to clogging as found with the lobster *Homarus americanus* (Wilkins, 1972), or perhaps because a depression in clay offers better protection from variations in stream flow.

The most favoured substrate type was that of an 'artificial shelter' which could instantly provide refuge without the need to construct a burrow. This agreed with Shimizu and Goldman (1983), who found that in a river in Sacramento, *P. leniusculus* preferred areas in which there was a rocky substrate, although indications of other substrates available were not mentioned. Klosterman and Goldman (1983), also found that *P. leniusculus* preferred larger mixed rocks, where shelter could be provided, to that of small gravel. Burrowing is considered to be energy expensive (Atkinson and Taylor, 1988), so it is not surprising that if a similarly protective environment is available which negates the need to expend energy in burrow construction, then it would be first choice. However, this is not the case for all crayfish species, for example, adult *Orconectes* species were found to prefer to construct burrows under rocks, cobbles and between stones, rather than to inhabit cracks and crevices between them (Hasiotis, 1993a).

Another important factor governing substrate choice was found to be competition from conspecifics. Although the preferred substrate was 'artificial shelter', when a group of adults was placed in a high-density situation where 'artificial shelters' were limited, it was observed that some individuals were excluded and forced to occupy a less favoured substrate. Thus conspecific competition maybe an important mechanism in habitat colonisation (Levenbach and Hazlett, 1996).

On no occasion was an 'artificial shelter' found to be occupied by more than one individual and on most occasions the shelters were occupied by the largest crayfish. This further supports observations by Ranta and Lindstrom (1993), where it was found that the larger the weight ratio between two opponents competing for shelter, the greater the likelihood of the larger individual 'winning'. However Peeke *et al.* (1995), observed that prior residence could affect shelter possession. In same-sex *P. leniusculus* encounters, 76% of female residents retained shelter possession, as did approximately 70% of male residents. In mixed-sex

encounters of *P. leniusculus*, female residents retained shelter possession against male intruders in 80% of cases, whilst male residents only retained 33% of encounters. This would indicate that size is not solely responsible for crayfish displacements.

From this and individual choice experiments, it was found that the order of preference starting with most preferred first, was 'artificial shelter', 'clay bank', 'mud' and 'gravel'. The relevance of this in the field means that areas with natural refuges would be occupied first, before burrow construction

Juvenile substrate choice experiments were also conducted and showed that juvenile *P. leniusculus* had different substrate preferences to that of adult individuals. There was no significant preference shown between 'clay bank' and 'artificial shelter', and both of these were preferred over 'mud'. 'Clay' was also preferred over gravel. Juveniles that selected 'clay' either, positioned themselves on the clay next to the tank wall, or, hid their bodies in the sediment or burrowed (demonstrating the ability of newly independent *P. leniusculus* to burrowing). Due to there being no significant preference between burrowing or provided shelter, this could imply that in the field, juveniles showed no preference between natural refuges and burrowing unless influenced by other factors, such as, stream velocity, food availability, predation and interspecific encounters (Flinders and Magoulick, 2003; Gherardi *et al.*, 2002b). Blake and Hart (1993), suggested that gravid females may exert a strong influence on the initial distribution of the juvenile *P. leniusculus*.

Another reason why juveniles may choose to burrow or take refuge in 'artifical shelter' may be attributed to the need for darkness. Darkness appears to be a controlling factor in cover seeking behaviour by *Orconectes rusticus* and *Procambarus clarkii* (Alberstadt *et al.*, 1995; Antonelli *et al.*, 1999).

In an experiment by Mason (1978), three substrate conditions were compared, bare floor, pebbles and burrows (artificial). The findings demonstrated that survival of juvenile *P. leniusculus* was highest in burrows, and that an increased usage of burrows was directly dependent on an increase in population density. As the most vulnerable period for the crayfish, regarding predation, is, the juvenile stage, particularly between hatching and finding a suitable 'safe' refuge (Blake and Hart, 1993), the ability to be able to construct a burrow immediately on independence would certainly be an advantage.

4.2.3 Rates of burrow irrigation by crayfish and passive irrigation

Although a burrow-dwelling lifestyle offers many advantages, one disadvantage is that occupants are faced with the problems of reduced oxygen tension (hypoxia) and elevated levels of carbon dioxide and ammonia (Astall *et al.*, 1997; see section 4.1.3). Several burrowing decapods, such as, *Nephrops norvegicus*, *Upogebia stellata* and *Callianassa subterranea* overcome this problem by irrigating their burrows. This was done either by means of an increased ventilation current, pleopod beating, or both (Astall *et al.*, 1997; Gerhardt and Baden, 1998; Stamhuis and Videler, 1998a, 1998b, 1998c).

Studies of burrow irrigation by decapods are limited (Astall *et al.*, 1997; Dworschak, 1981; Gerhardt and Baden, 1998), with only a small number quantifying rates of irrigation, such as, Dworschak (1981), who found the pumping rate of *Upogebia pusilla* to be in the range of 5 to 900 ml/h using over flow apparatus. To date, burrow irrigation by *P.leniusculus* has not been documented, probably because they were only first discovered to be burrowers in Britain in 1995 (Guan, 1995).

The laboratory experiment in this study was deliberately set up to expose each crayfish to artificially created hypoxic burrow waters, in order to produce a rapid irrigation response and a means of calculating a mean maximum irrigation rate (see section 3.2.3). It also insured the use of their main irrigation technique, which was primarily an increased ventilation rate, with only two animals also utilising their pleopods. This was different to the situation found for *N. norvegicus* in which all individuals utilised pleopod activity when placed in a hypoxic water-filled burrow (Gerhardt and Baden, 1998). In initial trials, a few crayfish also had the tendency to move to and from the burrow entrance (until restricted) and this also proved to be an effective means of exchanging internal burrow water with normoxic external water and is probably another adaptive mode of irrigation.

The artificial situation created in the laboratory placed *P. leniusculus* under extreme conditions, which are unlikely to be replicated in the field. Burrow irrigation is energetically expensive (Atkinson and Taylor, 1988), and it is unlikely that signal crayfish will irrigate to the extent described above unless necessary for survival. However, the evidence did suggest that some form of water exchange was taking place because toxic levels of Pco_2, Po_2 and T_{amm} were not reached in occupied burrows in the field (see section 4.2.3).

There are several possibilities for this water exchange. It could be through normal ventilation by the animal, water exchange due to stream flow (passive irrigation) or by active irrigation by the crayfish.

This study investigated the possibility of passive irrigation and found that it did occur. Results showed that with increased water velocity there was an increase in burrow water exchange.

Since the levels of ammonia, Pco_2 and Po_2 were not the same as external stream water and because irrigation is costly in terms of energy expenditure, the most likely explanation is that the majority of water exchange occurs from normal ventilation or passively as a result of stream flow. When necessary, active irrigation could occur if unfavourable levels of ammonia, Pco_2 and Po_2 were reached.

According to Astall *et al.* (1997), the complexity of the burrow architecture affected the spatial distribution of oxygen through the burrow. Thus, the simple burrow with a single entrance constructed by *P. leniusculus*, may be of significance. This shape may make burrow water exchange more effective, reducing the need to actively irrigate.

The mean calculated irrigation rate resulting from an increased ventilation rate was 14.8 l h^{-1}. As this was obtained from changes in Po_2 levels, the picture is slightly more complex as the process of irrigation is in itself oxygen consuming. Thus, the figure of 14.8 h^{-1} maybe a slight over-estimation of actual irrigation rate. Over the duration of the experiment, the internal burrow water Po_2 did not reach that of the external water. This was most likely the result of an equilibrium having been reached between the rate of O_2 consumption and the rate of irrigation.

Burrow irrigation rates did not correlate with animal size, which meant that smaller individuals were managing to create a greater flow of water over their gills. There were also no significant differences in irrigation rates between male or female crayfish. This may be due to smaller crayfish having a faster scaphognthite pumping rate, or as described by Burggren and McMahon (1983), for *O. virilis*, stroke volume was adjustable. This was achieved either by changes in the position or flexibility of the scaphognthites, thereby increasing performance due to increased pressure and sealing, or, by changing the geometry of the scaphognthite channel by raising or depressing the epipodites of the first maxillipeds.

4.2.4 Management prospects for the future

According to Holdich *et al.* (1999), it seems inevitable that alien crayfish species like *P. leniusculus* will remain in our waterways until suitable methods for eradication are found that are specific and environmentally sound. Failing the discovery of such methods the only way forward is damage limitation. This is already occurring in many areas where intense trapping and removals from waterways are being carried out to control alien species like *P. leniusculus*. With a view to preserving native species from the impact of alien crayfish, 'at risk' populations of *A. pallipes* are being transplanted into isolated ponds and lakes in the hope of preserving the genetic variation of the species.

Over the last few years a database has been set up in the U.K., detailing distribution of both native and alien species of crayfish, both regionally and nationally. As this needs to be regularly updated, studies focussing in part on population densities and population spread are important. Hopefully, the relevant information gathered in this thesis will be entered onto the database and the findings will also help with the control and management of *P. leniusculus*.

Data in this study suggest that it may be the juveniles that are mainly responsible for downstream range extension. Evidence to support this theory is threefold.

Firstly, through radio tracking and long-term streamer tagging it was found that most adult *P. leniusculus* remained in the same vicinity for up to two years. Their movements were centred around their refuge/burrow and neither flooding nor increased water velocity dislodged them. And yet, during that time, population expansion occurred and the species continued to extend its range downstream.

Secondly, the burrow opening dimensions measured in the field were found to be significantly smaller towards the leading edge of range extension, suggesting that smaller more vulnerable animals, which include juveniles, dug these burrows.

Thirdly, although not observed in this research, Peay and Rogers (1999), reported that the expansion of *P. leniusculus* was intermittent rather than a uniform spread. It was suggested that the reason for this was a reluctance to move into and beyond unfavourable habitats, thus delaying spread until full population capacity was reached. It could be surmised that smaller

crayfish, including juveniles, may drive the initial colonisation of new sites, as these animals are less able to defend themselves in high-density populations.

Forecasts on preferred sites for population expansion can be deduced by understanding the substrate preferences of *P. leniusculus.* It was found in this study that adult *P. leniusculus* preferred artificial shelters. It may therefore be possible to either slow downstream range extension of the adult species by substrate manipulation. For example, by the introduction of more artificial shelters, which would reduce the need for animals to seek new habitats and contain them within areas where they can be more easily trapped.

Juvenile *P. leniusculus,* however, showed different substrate choices to that of adults, with an equal preference for either clay or artificial shelters. If juveniles are more likely to be responsible for the majority of downstream range extension, as the results suggest, this difference in substrate preference may well be an adaptive advantage, as it enables the juveniles to occupy a greater variety of downstream sites. However, it makes substrate manipulation as an effective strategy to slow downstream range more challenging.

Knowledge of substrate preference enables the identification of sites vulnerable to bank damage. It was found that adults only constructed burrows where there were no available existing shelters and where there were stream banks with sufficient clay content. By either manipulating substrates so that *P. leniusculus* are encouraged to bypass high-risk areas, for example, where bank erosion will cause severe flooding, or by introducing alternative artificial shelters so that burrowing does not occur, some control, although limited, of bank erosion may be possible. This would not be a solution to curtail the activities of the juveniles, but as they are much smaller their burrowing activities have less of an impact on bank erosion and as they grow into adulthood their preference to occupy existing refuges before burrowing will develop.

The above strategies are only short-term solutions. Both would however, either provide a degree of control with regards to downstream range expansion or at the very least delay it, providing some much needed time before more radical or efficient methods of eradication are developed, like suitable biocides or biological control.

Knowledge of water chemistry within the burrow is important because it is possible to predict animal presence, either from the high levels of ammonia or by lower oxygen concentrations. These results mean that it is possible to identify burrows that are inhabited without causing further bank damage. It was also found that *P. leniusculus* burrows were irrigated both passively and actively. With this data, the possible use of biocides or other controls could be successfully used to eradicate *P. leniusculus* if a species specific type could be developed. It should also help in calculations of in-burrow biocide concentrations and persistence.

Furthermore, information on the time span of burrowing, the type of burrows and the mechanics of burrowing may be useful to support predictions on bank erosion. This data would also help decisions regarding the use of biocides.

Finally, the work comparing the range extension of *P. leniusculus* in the River Greet with that of the Gaddesby Brook has proved to be of some importance. It shows that a second separate population of *P. leniusculus* displays a similar pattern of range extension. This suggests that this model could be applied to other populations in the UK in order to predict spread. Also the polynomial expansion recorded shows the importance of dealing with outbreaks sooner rather than later, at the time when the population is still trying to establish itself i.e. less man hours would be needed in terms of clearance work to limit spread.

Appendices

Appendices

Appendix I Licence authorising the keeping of *Pacifastacus leniusculus* (Dana), obtained from the Ministry of Agriculture, Fisheries and Food (now DEFRA)

Ministry of Agriculture, Fisheries and Food
IMPORT OF LIVE FISH (ENGLAND AND WALES) ACT 1980
THE PROHIBITION OF KEEPING OF LIVE FISH (CRAYFISH) ORDER 1996 (as amended)

Licence authorising the keeping of non-native crayfish (crayfish other than the native or white-clawed crayfish (*Austropotamobius pallipes*))

Licence No. 13

Title DR Initials R R

Surname HARRIS

Address Department Of Biology
Adrian Building
University Of Leicester
Leicester
Postcode LE1 7RH

The Minister of Agriculture, Fisheries and Food, in exercise of the powers vested in him under the Import of Live Fish (England and Wales) Act 1980 and of other powers enabling him in this behalf, by virtue of this licence authorises the above-named person or organisation to keep:

Notes for Guidance

1. This licence is not a derogation from, nor does it affect in any way, the need to obtain a licence under the Wildlife and Countryside Act 1981 authorising the release or introduction into the wild of non-native species of crayfish.
2. This licence relates only to the keeping of the species of non-native crayfish and to the location specified below. A further licence will be required to keep other species or in respect of any other location.

1. at (location): DEPARTMENT OF BIOLOGY, ADRIAN BUILDING, UNIVERSITY OF LEICESTER, LEICESTER

2. crayfish of the species: *Pacifastacus Leniusculus*

THIS LICENCE IS GRANTED SUBJECT TO THE FOLLOWING CONDITIONS BEING FULFILLED, BREACH OF ANY OF WHICH SHALL LEAD TO THE LICENCE BEING REVOKED.

1. The licence-holder must inform the Ministry without delay of any proposed changes to the premises specified on the licence, or to the nature, level or management of crayfish stocks held.
2. The facility in which the crayfish are kept must have no direct link to any adjacent watercourse.
3. Effluent water from the facility should be discharged to a soakaway or foul sewer.
4. The facility should be escape-proof for all life history stages of crayfish held.
5. The licence-holder must keep records of all movements to and from the location specified on this licence. These movement records should include the number and species of crayfish involved and the name and address of the supplier/buyer. Details of crayfish mortalities within the facility must be kept as part of these records.
6. On completion of experiments, crayfish not transferred to other persons who hold a licence to keep the same species of crayfish must not be released but should be disposed of in line with current best practice. (Advice may be obtained from CEFAS Weymouth Laboratory, Tel: 01305 206600.)

This licence may be modified or revoked at any time by the Minister of Agriculture, Fisheries and Food.

Signature M. Fimmel (On behalf of the Minister of Agriculture, Fisheries and Food)

Date 26.2.99

Name M FIMMEL

Tel. No. (incl. STD Code) 0171-238 5933

CRAY 1 (10/97) FISHERIES DIVISION II

Appendix II Risk assessment for laboratory and solo fieldwork procedures at the University of Leicester

FACULTY OF MEDICINE & BIOLOGICAL SCIENCES
UNIVERSITY OF LEICESTER
RISK ASSESSMENT FOR LABORATORY AND FIELDWORK PROCEDURES

Person completing form Jeama Stanton.....Position Ph.D. student.....

Date assessment completed 12.10.99.....

To achieve safe working in the laboratory or field, all procedures must be subjected to Risk Assessment when planning experimental work. The first stage in risk assessment requires the identification of the hazards that could be reasonably expected to result in significant harm to the operator or others.

1) Brief description of procedure:

Sampling populations of crayfish in the field using a combination of trapping and netting. Setting of trappy type baited traps overnight and inspecting them the following morning. Clipping uropods of selected animals for capture, mark and recapture experiment. Weighing of animals and measurement of carapace length. Examination of animals for signs of disease. Taking water depth, temperature and velocity measurements. Taking measurements of river bank slopes and Water and sediment samples. Examining burrow morphology by means of resin casting and radio tagging crayfish for home range patterns.

(If all operations cannot be fully described in this space a full protocol must be kept with this form in a Procedures file located in the working area)

2) Location where it will be carried out (e.g. Laboratory no. or experimental site)

Various locations along the Gaddesby/Twyford Brook between Owston (SK776 084) and downstream to Gaddesby village (SK686126).

3) Identified hazards: (e.g. Fire, chemicals, gases, electricity, pressure systems, moving parts of machinery, blades or sharps, drowning, slipping, working in reduced light levels or darkrooms, lone working, working in isolated rooms, potential allergens, u.v. emission, fumes, radioisotopes, explosion, genetic manipulation).

Weils disease through ingestion, inhalation or contact of water on damaged skin. Possibly of drowning or slipping on muddy bank. Minor lesions and stings from waterside plants. Possibility of being 'nipped' by crayfish producing minor lesions.

4) Level of risk:

~~Negligible~~ Low ~~Medium~~ ~~High~~

strike-through as applicable (highlight text, press command shift/)

5) Steps taken to adequately control the risk: (e.g. Use of fume hood, protection from high voltages, electrical safety checks, use of protective clothing, dust mask, goggles, face shield, hard hat, etc., use of lifejackets, training in use of procedures, avoidance of lone working, use of mobile phone, use of sparkproof devices, avoidance of naked flames, preparation of COSHH assessments, proper arrangements for transport of hazardous materials).

Maintain good hygiene procedures to prevent infection by Weils disease. Use heavy duty gloves to handle traps and animals plus bait tins. Working with adequate waterproof clothing and waders with good grip on soles. Issued with mobile phone and own transport to seek help in emergency.

I have read and understand this risk assessment. I agree to minimise the risks by adopting the steps described under (5) above.

Name	Status	Date
Jeama Stanton.....	Student.....	12.10.99.....
Jeama Stanton.....	.....	12/10/99.
.....	.....	.....
.....	.....	.....

(to be filed in the laboratory)

Signature of Laboratory Safety Supervisor: .....

Appendix III Letter outlining the project (from the Environment Agency), which was shown to landowners, when seeking permission for access to their land

Our ref: PJS/letauth99
Your ref:

Date: 17 December 1999

ENVIRONMENT AGENCY

To whom it may concern

ENVIRONMENT AGENCY: CRAYFISH SURVEY, GADDESBY BK & R.GREET

Previous work has identified the presence of the non-native crayfish, *Pacifastacus leniusculus*, in large numbers within the above watercourses. These populations represent a significant threat to local fauna and flora, including the native crayfish *Austropotamobius pallipes*, which is becoming increasingly threatened across many parts of England and Wales.

The Environment Agency, in partnership with Leicester University, is undertaking to investigate the behaviour and distribution of non-native crayfish in Midlands Region, and reduce established populations where possible.

The person in possession of this letter is working with the Environment Agency to undertake survey work in this area. Your co-operation in allowing them access to parts of the brook or tributaries on your land would be very much appreciated.

Should you require further information regarding this work please contact the Fisheries, Ecology and Recreation section based at our Nottingham office, on 0115 846 3625 and ask for Peter Sibley (biologist).

Yours faithfully

P. Sibley

PETER SIBLEY
Biologist

Environment Agency
Trentside Offices, Scarrington Road, West Bridgford, Nottingham, NG2 5FA
Tel: 0115 945 5722 Fax 0115 981 7743

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Appendix IV Calculations for converting CO_2 electrode meter readings to ΣCO_2 (mequiv l^{-1})

$$\Delta P = P_f - P_i$$

P_i = initial meter reading
P_f = final meter reading
ΔP = change in meter reading

$$P_{cal} = \frac{[Pa - Vp] \times [\%CO_2]}{100}$$

P_{cal} = actual Pco_2 of calibration
Pa = baromic pressure
Vp = saturated water vapour pressure
$\%CO_2$ = CO_2 content of gas mixture

$$\Delta P^1 = \Delta P[P_{cal} / P_{meter}]$$

ΔP = change in meter reading
P_{cal} = actual Pco_2 of calibration
P_{meter} = readings to which calibration mixture was set
ΔP^1 = corrected meter reading

A conversion factor F is calculated as:

$$F = \Delta P^1_{std} / [\text{mM standard injected}]$$

(10 µl of 30mM $NaHCO_3$ = 0.0003mM)

$$\Sigma CO_2 \text{ (m equiv l-1)} = [\Delta P^1 \text{ sample/F}] \times [106/\text{sample vol}(\mu l)]$$

Appendix V Calculating Pco_2 and $[HCO_3^-]$ using Henderson-Hasselbalch equations

$$pK_1 = 0.0001t^2 - 0.0122t + 6.5753$$

pK_1 = Dissociation constant dependent on pH and ionic strength calculated using data from Harned and Davis, (1943)

t = stream temperature (^oC)

$$pH = pK1 + \log [(\Sigma CO_2)/\alpha CO_2 \times Pco_2)-1]$$

αCO_2 = CO_2 solubility of water as a function of temperature (mmol $liter^{-1}$ $torr^{-1}$) (Robert *et al.*, 1984)

$$Pco_2 \text{ (mm Hg)} = [\Sigma CO_2] / ((\text{antilog } (pH - pK_1) + 1) \times \alpha CO_2)$$

$$[HCO_3^-] \text{ (mm equv/ } l^{-1}) = (\text{antilog } pH - pK_1) \times (\alpha CO_2 \times Pco_2)$$

Appendix VI Percentage stream bank substrate composition, burrow density and physical stream properties at the time of sampling at 78 sites along the Gaddesby Brook.

Site No.	% clay	% sand	% fine silt	% coarse silt	Burrow density (m)	water depth (m)	water pH	Water velocity (ms^{-1})	Water Temp (°C)
5P	71	0	23	6	14	0.5	8	0.5	-
12P	44	32	12	12	1	0.15	7.5	0.11	-
19P	32	48	7	13	0	0.19	8	-0.01	-
23P	48	22	14	16	1.5	0.5	8.2	0.04	-
29P	60	7	20	13	3.5	0.5	8.26	0.045	-
4R	29.5	57.5	5.5	7.5	0	0.13	8.3	0.28	6.8
6R	23	70	3	4	6.5	0.22	8.3	0.15	6.8
23R	28	47	11	14	2	0.12	8.36	0.35	6.7
26P	30	46.5	12	11.5	3	0.56	8.36	0.01	6.9
94R	55	5	20	20	1	0.13	8.17	0.47	6.9
95R	54	17	17	12	3	0.14	8.1	0.22	6.9
27R	31	42	12	15	0	0.18	8.16	0.61	4.3
29R	29	47.5	14	9.5	0	0.2	8.3	0.43	4.1
31P	32	47.5	12	8.5	1.5	0.6	8.3	0.03	4.2
31R	45	24.5	20	10.5	2.5	0.2	8.25	0.54	4
34P	34.5	37	18.5	10	1	0.75	8.3	0.02	4
35P	37.5	34	17.5	11	0	0.75	8.3	0.01	4
94P	34	42	13	11	2	0.59	8.4	0.22	5
81P	26.5	58	10	5.5	0	0.5	7.5	0.085	1
82P	90	0	10	0	5	0.75	7.5	0.13	1
87P	45	18	20	17	2	0.4	7.5	0.32	1.4
89P	33	24	30	13	1.5	0.3	7.5	0.2	1.4
93P	40	26	13	21	4.5	0.5	7.5	0.01	1.4
38P	34	47.5	12	6.5	0	0.5	7.4	0.14	3
50R	31	20	26	23	4	0.4	7.72	0.3	3
58P	32	42	16	10	4.5	0.8	8	0.08	3
58R	53	14	22	11	1.5	0.36	8	0.25	3
60R	26	55	10	9	2.5	0.48	8.1	0.21	3
63P	40.5	24	21.5	14	5	0.42	8.2	0.13	3
65R	34	30	21	15	0	0.31	8.2	0.13	3
75P	65	0	35	0	6.5	0.6	8.2	-0.14	3
243P	23	66	6	5	0	0.19	8	0.22	7.7
245P	27.5	43	19.5	10	1	0.26	8	0.045	7.7
246P	32	45	12	11	2	0.22	8	0.14	7.7
247P	26	58	8	8	0	0.3	8	0.07	7.7
248R	24	58	10	8	1.5	0.26	8.04	0.2	7.7
254R	29	48	12	11	8	0.6	8.06	0.5	7.7
260R	52.5	18	15.5	14	5	0.1	8.13	0.31	7.7
101R	35	58	1	6	0	0.19	7.7	0.37	8

Appendix VI (Continued)

Site No.	% clay	% sand	% fine silt	% coarse silt	Burrow density (m)	water depth (m)	water pH	Water velocity (ms^{-1})	Water Temp (°C)
105P	42	37.5	12	8.5	4.5	0.6	7.6	0.032	8
108R	38	32	18.5	11.5	1.5	0.38	7.6	0.092	8
109R	35	42.5	17	5.5	0	0.15	8	0.44	8
116R	35	27	32	6	2	0.11	7.7	0.47	8
119R	34.5	45	14.5	6	0	0.16	8.1	0.14	8
121P	45	22	20	13	10.5	0.12	8.1	0.48	8
146R	57	4	31	8	0	0.52	7.6	0.06	6.1
147P	33	26	24	17	6	1.04	7.6	0.01	6.1
148R	48	24	18	10	5.5	0.25	7.6	0.45	6.1
172R	61	4	25	10	0	0.1	8	0.92	6.1
182R	43	29.5	20	7.5	0	0.12	8.1	0.72	6.1
183P	57	9	26	8	7.5	0.5	8.2	0.02	6.1
187P	30	40	20.5	9.5	3	1.2	8	0.01	6.1
193P	40	32	10	18	3.5	0.5	8.3	0.01	5
211P	40.5	34.5	18.5	6.5	7.5	0.8	8	0.007	5
202R	32.5	31	19.5	17	2.5	0.1	8.1	0.23	5
199R	28	45	13	14	0	0.2	8.2	0.3	5
195R	42.5	32	16	9.5	1.5	0.4	8.4	0.22	5
214R	40	34.5	18	7.5	4	0.4	8.1	0.1	5
227P	29	52.5	21	7.5	0	0.52	8	0.1	7.8
229P	45	16	26	13	5	0.25	8	0.18	7.8
235P	35	38	19	8	6.5	0.55	8	0.2	7.7
235R	37	40	16	7	0	0.24	8	1.3	7.7
236R	47	26	15	12	5.5	0.25	7.9	1.03	7.7
237R	37	40	16	7	0	0.51	7.6	0.32	7.7
262P	54	16.5	18	11.5	0	0.8	7.8	0.03	7.8
263P	21	68	7	4	0	0.24	8.1	0.08	8.1
271P	25.5	67.5	4.5	2.5	0	0.16	8.3	0.25	8.3
272R	52	19	20	9	2	0.18	8.45	0.34	8.45
277P	32	45	16	7	2	0.2	8.4	0.067	8.4
280P	27	45	16	15	1	0.49	8.5	0.07	8.5
281R	28	49	10	13	0	0.18	8.6	0.25	8.6
286R	69	0	30	1	0	0.25	8.6	0.9	8.6
296R	16	68	9	7	0	-	-	-	-
311R	43	30	18	9	3	-	-	-	-
139R	50	27	16	7	0	0.08	8.15	0.58	14.5
129R	33	21	27	19	1.5	0.08	8	0.9	13.4
138R	46	8	24	8	0	0.08	8	0.3	14.5
137R	19	73	3.5	4.5	0	0.09	7.98	0.1	14.2

Appendix VII Site, sex, carapace length, weight, tag frequency and dates of tagging and retrieval of the 44 radio-tracked crayfish.

Animal No.	Site	Sex	Carapace Length (mm)	Weight (g)	Radio frequency (MHz)	Date tagged	Date retrieved
1	Newbold Grange Farm	Male	49.2	37.5	173.320 R1 13	10.5.00	9.6.00
2	Newbold Grange Farm	Male	59.0	62.3	173.325 R1 11.75	10.5.00	9.6.00
3	Newbold Grange Farm	Male	52.1	50.7	173.333 R1 12.75	10.5.00	Not retrieved
4	Newbold Grange Farm	Female	48.7	34.1	173.312 R1 10.75	10.5.00	11.6.00
5	Newbold Grange Farm	Female	48.3	32.7	173.305 R1 10.25	10.5.00	Not retrieved
6	Mill Farm	Male	46.4	56.6	173.339 R1 14	18.5.00	16.6.00
7	Mill Farm	Male	58.2	39.9	173.352 R2 0.25	18.5.00	9.6.00
8	Mill Farm	Female	47.5	27.0	173.361 R2 1.25	18.5.00	16.6.00
9	Mill Farm	Female	45.8	37.4	173.392 R2 4.25	18.5.00	19.6.00
10	Mill Farm	Male	47.8	32.7	173.381 R2 3	18.5.00	19.6.00
11	Newbold Grange Farm	Female	58.8	61.7	173.260 R1 6	13.11.01 Light	Not retrieved
12	Newbold Grange Farm	Male	53.6	51.6	173.381 R2 3	13.11.01	Not retrieved
13	Newbold Grange Farm	Female	63.4	57.8	173.352 R2 0.25	13.11.01	Not retrieved
14	Newbold Grange Farm	Male	61.0	65.9	173.273 R1 7	13.11.01 Temp logger	Not retrieved
15	Newbold Grange Farm	Female	48.5	28.9	173.320 R1 13	13.11.01	Not retrieved
16	Newbold Grange Farm	Male	69.7	66.8	173.325 R1 11.5	13.11.01 Light	15.01.02
17	Newbold Grange Farm	Female	47.1	27.6	173.312 R1 10.75	13.11.01	Not retrieved
18	Newbold Grange Farm	Female	55.7	49.2	173.361 R2 1.25	13.11.01	17.12.01
19	Newbold Grange Farm	Male	66.4	97.4	173.392 R2 4.25	13.11.01 Light	14.01.02
20	Newbold Grange Farm	Male	51.6	62.3	173.339 R1 13.75	13.11.01	17.12.01
21	Mill Farm	Female	50.0	40.7	173.286 R1 8.5	19.02.02 Temp logger	23.05.05
22	Mill Farm	Male	46.7	35.9	173.339 R1 13.75	19.02.02	Not retrieved
23	Mill Farm	Male	47.8	29.0	173.392 R2 4.25	19.02.02	Not retrieved
24	Mill Farm	Male	41.7	21.3	173.361 R2 1.25	19.02.02	Not retrieved
25	Mill Farm	Female	42.9	21.7	173.325 R1 11.75	19.02.02 Light	23.03.02
26	Mill Farm	Male	42.9	20.2	173.216 R1 1.75	19.02.02	Not retrieved
27	Mill Farm	Female	40.1	17.6	173.231 R1 3	19.02.02	23.03.01
28	Mill Farm	Female	40.5	15.5	173.241 R1 4	19.02.02	23.03.02
29	Newbold Grange Farm	Male	56.0	59.0	173.216 R1 1.75	21.05.02	Not retrieved
30	Newbold Grange Farm	Female	52.7	47.9	173.241 R1 4	21.05.02	21.06.02
31	Newbold Grange Farm	Male	52.9	52.2	173.29 R1 12.5	21.05.02 Temp logger	19.06.02
32	Newbold Grange Farm	Female	45.0	27.4	173.325 R1 11.75	21.05.02	Not retrieved
33	Newbold Grange Farm	Male	49.4	38.5	173.264 R1 6.5	21.05.02 Light	19.06.02

Appendix VII (Continued)

Animal No.	Site	Sex	Carapace Length (mm)	Weight (g)	Radio frequency (MHz)	Date tagged	Date retrieved
34	Mill Farm	Female	44.0	22.2	173.231 R1 3	21.05.02	Not retrieved
35	Mill Farm	Male	47.0	29.5	173.222 R1 2.25	21.05.02	Not retrieved
36	Mill Farm	Female	56.4	51.2	173.306 R1 10.4	21.05.02 Temp logger	19.06.02
37	Mill Farm	Female	51.9	46.1	173.294 R1 9.25	21.05.02 Light	21.06.02
38	Mill Farm	Female	49.3	42.2	173.273 R1 L7	21.05.02	Not retrieved
39	White House Farm	Male	47.8	37.3	173.241 R1 4	16.07.02 Down stream	Not retrieved
40	White House Farm	Male	46.9	32.8	173.29 R1 12.5	16.07.02 Up-stream	Not retrieved
41	White House Farm	Female	45.5	40.4	173.286 R1 8.4	16.07.02 Up-stream	21.08.02
42	White House Farm	Male	44.6	34.4	173.306 R1 10.4	16.07.02 Down stream	Not retrieved
43	White House Farm	Female	48.2	31.1	173.294 R1 9.25	16.07.02 Down stream	Not retrieved
44	White House Farm	Female	46.0	27.5	173.264 R1 6.5	16.07.02 Up-stream	Not retrieved

Appendix VIII Site, date and trap number of captured *P. leniusculus* for long term streamer tagging. Also given is the allocated tag number and the sex, weight and carapace length of each individual (n= 268).

Tag No.	Date	Trap No.	Sex	Weight/g	CL (mm)	Site
3719	09.08.00	3	F	31.0	46.0	Newbold Farm
3642	09.08.00	3	F	17.7	39.0	Newbold Farm
3643	09.08.00	1	F	48.2	54.9	Newbold Farm
3645	09.08.00	1	M	63.4	60.5	Newbold Farm
3646	09.08.00	1	F	23.8	42.3	Newbold Farm
3647	09.08.00	1	M	57.4	56.8	Newbold Farm
3648	09.08.00	1	F	51.1	45.4	Newbold Farm
3649	09.08.00	1	F	25.0	45.2	Newbold Farm
3650	09.08.00	1	F	33.3	46.5	Newbold Farm
3651	09.08.00	1	M	32.4	44.2	Newbold Farm
3652	09.08.00	1	F	30.6	45.3	Newbold Farm
3653	09.08.00	1	F	25.6	43.2	Newbold Farm
3655	09.08.00	1	F	25.5	44.3	Newbold Farm
3656	09.08.00	2	F	57.3	60.6	Newbold Farm
3657	09.08.00	2	M	70.0	57.3	Newbold Farm
3658	09.08.00	2	F	44.8	53.6	Newbold Farm
3660	09.08.00	2	M	42.8	50.5	Newbold Farm
3661	09.08.00	2	F	26.9	47.2	Newbold Farm
3662	09.08.00	2	F	45.2	52.9	Newbold Farm
3663	09.08.00	2	F	34.3	49.6	Newbold Farm
3664	09.08.00	2	F	39.7	53.1	Newbold Farm
3667	09.08.00	2	F	39.2	53.6	Newbold Farm
3668	09.08.00	2	F	39.6	51.9	Newbold Farm
3669	09.08.00	2	M	52.1	54.3	Newbold Farm
3670	09.08.00	2	M	28.8	45.9	Newbold Farm
3671	09.08.00	2	M	27.0	45.2	Newbold Farm

Appendix VIII (Continued)

Tag No.	Date	Trap No.	Sex	Weight/g	CL (mm)	Site
3672	09.08.00	2	M	31.3	44.8	Newbold Farm
3673	09.08.00	2	F	25.2	40.2	Newbold Farm
3674	09.08.00	4	M	70.0	56.5	Newbold Farm
3675	09.08.00	4	F	32.7	48.8	Newbold Farm
3677	09.08.00	4	F	16.0	38.6	Newbold Farm
3678	09.08.00	4	F	19.2	39.2	Newbold Farm
3679	09.08.00	5	F	28.4	47.7	Newbold Farm
3680	09.08.00	5	M	61.2	55.2	Newbold Farm
3681	09.08.00	5	F	30.0	46.6	Newbold Farm
3682	09.08.00	5	F	21.2	41.3	Newbold Farm
3683	09.08.00	5	F	42.9	55.9	Newbold Farm
3684	09.08.00	5	F	28.8	43.8	Newbold Farm
3719	10.08.00	4	F	23.6	43.0	Newbold Farm
3720	10.08.00	1	M	92.0	65.7	Newbold Farm
3721	10.08.00	1	F	64.9	62.0	Newbold Farm
3722	10.08.00	1	F	47.3	55.2	Newbold Farm
3724	10.08.00	1	F	37.3	51.8	Newbold Farm
3723	10.08.00	1	M	20.9	39.3	Newbold Farm
3725	10.08.00	1	F	20.0	41.3	Newbold Farm
3726	10.08.00	2	M	24.1	42.8	Newbold Farm
3727	10.08.00	2	F	23.4	43.3	Newbold Farm
3728	10.08.00	2	F	16.0	36.5	Newbold Farm
3729	10.08.00	2	M	32.2	45.2	Newbold Farm
3730	10.08.00	3	F	44.0	55.4	Newbold Farm
3732	10.08.00	3	M	33.2	47.7	Newbold Farm
3731	10.08.00	3	M	22.1	41.1	Newbold Farm
3733	10.08.00	5	F	34.0	50.4	Newbold Farm
3760	11.08.00	1	F	51.4	57	Newbold Farm

Appendix VIII (Continued)

Tag No.	Date	Trap No.	Sex	Weight/g	CL (mm)	Site
3761	11.08.00	1	F	31.1	47.2	Newbold Farm
3762	11.08.00	1	F	32.0	49.2	Newbold Farm
3763	11.08.00	1	F	40.4	51.8	Newbold Farm
3766	11.08.00	1	F	32.1	47.9	Newbold Farm
3767	11.08.00	2	F	24.0	43.8	Newbold Farm
3764	11.08.00	3	F	41.1	52.2	Newbold Farm
3768	11.08.00	3	F	42.0	52.4	Newbold Farm
3769	11.08.00	3	F	47.8	53.8	Newbold Farm
3770	11.08.00	3	M	28.6	44.5	Newbold Farm
3771	11.08.00	3	F	31.3	46.2	Newbold Farm
3772	11.08.00	3	M	14.9	38.4	Newbold Farm
3773	11.08.00	4	F	33.7	49.7	Newbold Farm
3774	11.08.00	4	F	30.2	46.4	Newbold Farm
3775	11.08.00	4	F	18.6	38.6	Newbold Farm
3776	11.08.00	5	F	20.7	41.6	Newbold Farm
3777	11.08.00	5	F	59.0	57.7	Newbold Farm
3778	11.08.00	5	F	35.2	48.9	Newbold Farm
3779	11.08.00	5	F	41.5	52.9	Newbold Farm
3780	11.08.00	5	F	28.9	46.5	Newbold Farm
3781	11.08.00	5	M	43.0	41.2	Newbold Farm
3782	11.08.00	5	F	16.5	38.5	Newbold Farm
4214	12.08.00	1	F	57.9	57.5	Newbold Farm
4216	12.08.00	1	F	29.3	47.3	Newbold Farm
4217	12.08.00	1	F	25.0	44.9	Newbold Farm
4218	12.08.00	1	F	20.4	42.2	Newbold Farm
4219	12.08.00	1	F	29.5	47.1	Newbold Farm
4220	12.08.00	1	F	29.7	48.5	Newbold Farm
4221	12.08.00	2	F	36.6	52.0	Newbold Farm

Appendix VIII (Continued)

Tag No.	Date	Trap No.	Sex	Weight/g	CL (mm)	Site
4222	12.08.00	2	F	33.4	50.5	Newbold Farm
4223	12.08.00	2	M	36.1	46.9	Newbold Farm
4224	12.08.00	2	F	40.5	49.9	Newbold Farm
4225	12.08.00	2	F	37.3	51.4	Newbold Farm
4226	12.08.00	2	F	34.5	48.6	Newbold Farm
4227	12.08.00	2	F	28.7	45.7	Newbold Farm
4228	12.08.00	2	F	18.7	40.4	Newbold Farm
4229	12.08.00	2	F	31.7	46.9	Newbold Farm
4240	12.08.00	3	M	63.0	59.9	Newbold Farm
4241	12.08.00	3	F	33.7	50.9	Newbold Farm
4242	12.08.00	3	F	17.6	39.0	Newbold Farm
4230	29.08.01	1	F	30.1	55.1	Newbold Farm
4231	29.08.01	1	F	23.2	44.3	Newbold Farm
4232	29.08.01	1	M	15.6	42.4	Newbold Farm
4233	29.08.01	2	M	12.5	42.1	Newbold Farm
4234	29.08.01	3	F	27.6	31.5	Newbold Farm
4235	29.08.01	3	F	28.4	44.5	Newbold Farm
4236	29.08.01	3	F	50.2	57.5	Newbold Farm
4237	29.08.01	3	M	75.2	60.2	Newbold Farm
4238	29.08.01	3	M	35.1	46.4	Newbold Farm
4239	29.08.01	4	F	30.3	48.1	Newbold Farm
4261	29.08.01	4	F	75.2	55.9	Newbold Farm
4263	29.08.01	4	F	27.9	43.8	Newbold Farm
4264	29.08.01	4	F	48.8	50.1	Newbold Farm
4265	29.08.01	4	F	31.1	49.0	Newbold Farm
4223	29.08.01	4	M	74.8	59.0	Newbold Farm
4226	29.08.01	4	F	40.0	47.5	Newbold Farm
4268	29.08.01	4	M	18.1	38.0	Newbold Farm

Appendix VIII (Continued)

Tag No.	Date	Trap No.	Sex	Weight/g	CL (mm)	Site
4269	29.08.01	4	M	31.2	51.2	Newbold Farm
4271	29.08.01	4	F	51.9	62.1	Newbold Farm
4270	29.08.01	4	M	25.4	40.8	Newbold Farm
4272	29.08.01	4	F	19.2	39.2	Newbold Farm
4273	29.08.01	4	M	25.1	42.1	Newbold Farm
4274	29.08.01	4	F	25.2	35.8	Newbold Farm
3651	29.08.01	5	M	75.9	55.4	Newbold Farm
4275	29.08.01	5	F	56.6	61.1	Newbold Farm
3781	29.08.01	5	M	74.7	63.0	Newbold Farm
4276	29.08.01	5	F	40.1	48.1	Newbold Farm
4277	29.08.01	5	M	29.0	51.3	Newbold Farm
4278	29.08.01	5	F	45.1	54.1	Newbold Farm
4279	29.08.01	5	M	52.3	48.2	Newbold Farm
4280	29.08.01	5	M	47.3	50.1	Newbold Farm
4281	29.08.01	5	F	25.1	42.4	Newbold Farm
4282	29.08.01	5	F	52.3	56.1	Newbold Farm
4283	29.08.01	5	F	51.1	52.0	Newbold Farm
4284	29.08.01	5	F	49.9	50.3	Newbold Farm
4285	29.08.01	5	F	51.1	53.9	Newbold Farm
4286	29.08.01	5	F	27.5	48.5	Newbold Farm
4287	29.08.01	5	F	26.3	45.3	Newbold Farm
4288	29.08.01	5	F	28.0	46.1	Newbold Farm
3601	08.08.00	1	M	101.0	70.0	White House Farm
3602	08.08.00	2	M	33.1	50.8	White House Farm
3603	08.08.00	2	M	45.7	51.7	White House Farm
3604	08.08.00	2	M	35.1	46.3	White House Farm
3605	08.08.00	2	F	26.2	43.5	White House Farm
3606	08.08.00	2	F	26.3	46.0	White House Farm

Appendix VIII (Continued)

Tag No.	Date	Trap No.	Sex	Weight/g	CL (mm)	Site
3607	08.08.00	2	F	22.8	42.5	White House Farm
3608	08.08.00	2	F	26.1	39.0	White House Farm
3609	08.08.00	2	F	48.6	53.3	White House Farm
3610	08.08.00	2	F	32.6	47.8	White House Farm
3611	08.08.00	3	F	18.0	40.5	White House Farm
3612	08.08.00	4	M	9.8	34.0	White House Farm
3685	09.08.00	1	F	43.1	52.2	White House Farm
3686	09.08.00	2	F	31.8	46.8	White House Farm
3687	09.08.00	2	F	35.5	52.5	White House Farm
3688	09.08.00	2	F	19.5	40.7	White House Farm
3689	09.08.00	2	F	40.4	53.5	White House Farm
3690	09.08.00	2	F	29.9	45.5	White House Farm
3691	09.08.00	2	F	26.0	43.3	White House Farm
3693	09.08.00	2	F	37.7	48.8	White House Farm
3694	09.08.00	2	F	37.0	49.0	White House Farm
3695	09.08.00	2	M	33.4	49.3	White House Farm
3696	09.08.00	2	F	21.1	41.9	White House Farm
3697	09.08.00	2	F	18.5	40.5	White House Farm
3699	09.08.00	2	M	21.4	43.4	White House Farm
3701	09.08.00	3	F	29.8	46.1	White House Farm
3702	09.08.00	3	M	30.0	46.1	White House Farm
3703	09.08.00	4	F	16.7	38.5	White House Farm
3704	09.08.00	4	F	16.2	38.2	White House Farm
3734	10.08.00	2	M	83.9	60.4	White House Farm
3735	10.08.00	2	F	32.0	49.1	White House Farm
3783	11.08.00	1	F	27.1	45.6	White House Farm
3784	11.08.00	2	M	40.9	47.8	White House Farm
3785	11.08.00	2	F	27.7	44.7	White House Farm

Appendix VIII (Continued)

Tag No.	Date	Trap No.	Sex	Weight/g	CL (mm)	Site
3786	11.08.00	5	M	35.9	49.0	White House Farm
3787	11.08.00	5	F	40.9	40.9	White House Farm
3788	11.08.00	5	F	29.5	29.5	White House Farm
4289	29.08.01	1	M	25.1	34.8	White House Farm
3686	29.08.01	1	F	48.2	50.2	White House Farm
4290	29.08.01	1	F	49.9	56.1	White House Farm
4291	29.08.01	1	F	29.8	52.3	White House Farm
4293	29.08.01	1	F	45.3	51.0	White House Farm
4294	29.08.01	1	M	26.2	50.7	White House Farm
4295	29.08.01	1	F	25.1	47.5	White House Farm
4296	29.08.01	1	F	24.0	44.0	White House Farm
4297	29.08.01	2	F	23.2	44.3	White House Farm
4298	29.08.01	2	F	26.5	49.8	White House Farm
4299	29.08.01	2	F	24.1	41.2	White House Farm
4300	29.08.01	2	M	74.7	55.3	White House Farm
4306	29.08.01	2	F	90.6	63.2	White House Farm
3613	08.08.00	1	M	45.9	56.4	Lowenva Lodge
3614	08.08.00	2	F	39.9	52.0	Lowenva Lodge
3615	08.08.00	2	F	16.7	39.5	Lowenva Lodge
3616	08.08.00	2	F	25.8	47.4	Lowenva Lodge
3617	08.08.00	2	F	35.6	51.1	Lowenva Lodge
3618	08.08.00	2	F	19.5	41.6	Lowenva Lodge
3620	08.08.00	4	M	52.6	56.4	Lowenva Lodge
3621	08.08.00	4	F	31.0	47.3	Lowenva Lodge
3622	08.08.00	4	F	19.5	43.5	Lowenva Lodge
3624	08.08.00	4	M	25.4	45.4	Lowenva Lodge
3625	08.08.00	5	M	63.2	63.6	Lowenva Lodge
3626	08.08.00	5	F	57.7	57.4	Lowenva Lodge

Appendix VIII (Continued)

Tag No.	Date	Trap No.	Sex	Weight/g	CL (mm)	Site
3627	08.08.00	5	F	82.4	69.5	Lowenva Lodge
3628	08.08.00	5	M	100.6	65.0	Lowenva Lodge
3629	08.08.00	5	M	59.5	60.0	Lowenva Lodge
3630	08.08.00	5	M	42.5	53.5	Lowenva Lodge
3631	08.08.00	5	F	49.9	59.5	Lowenva Lodge
3632	08.08.00	5	F	54.1	59.0	Lowenva Lodge
3633	08.08.00	5	F	31.3	47.2	Lowenva Lodge
3634	08.08.00	5	F	56.0	59.0	Lowenva Lodge
3705	09.08.00	1	F	26.0	46.4	Lowenva Lodge
3706	09.08.00	2	F	35.5	50.5	Lowenva Lodge
3737	09.08.00	2	M	26.8	45.8	Lowenva Lodge
3708	09.08.00	3	F	35.4	49.6	Lowenva Lodge
3709	09.08.00	5	M	110.0	68.9	Lowenva Lodge
3710	09.08.00	3	M	14.1	46.9	Lowenva Lodge
3711	09.08.00	5	F	20.4	40.0	Lowenva Lodge
3712	09.08.00	5	F	32.7	48.0	Lowenva Lodge
3713	09.08.00	5	M	29.7	48.0	Lowenva Lodge
3714	09.08.00	5	F	30.7	49.0	Lowenva Lodge
3736	10.08.00	2	F	42.0	52.6	Lowenva Lodge
3737	10.08.00	2	F	57.6	56.9	Lowenva Lodge
3738	10.08.00	2	F	46.1	54.9	Lowenva Lodge
3739	10.08.00	2	F	46.0	50.8	Lowenva Lodge
3740	10.08.00	2	F	23.8	43.1	Lowenva Lodge
3741	10.08.00	3	M	113.0	68.8	Lowenva Lodge
3742	10.08.00	3	F	33.8	48.8	Lowenva Lodge
3743	10.08.00	3	F	42.0	52.8	Lowenva Lodge
3745	10.08.00	3	M	21.5	41.4	Lowenva Lodge
3746	10.08.00	3	F	26.3	46.2	Lowenva Lodge

Appendix VIII (Continued)

Tag No.	Date	Trap No.	Sex	Weight/g	CL (mm)	Site
3747	10.08.00	3	F	30.6	48.6	Lowenva Lodge
3748	10.08.00	3	F	42.0	47.3	Lowenva Lodge
3749	10.08.00	3	F	32.9	44.4	Lowenva Lodge
3750	10.08.00	3	M	17.5	40.8	Lowenva Lodge
3751	10.08.00	3	F	39.9	50.3	Lowenva Lodge
3752	10.08.00	4	F	40.7	52.3	Lowenva Lodge
3753	10.08.00	4	F	51.1	56.5	Lowenva Lodge
3754	10.08.00	4	F	41.1	54.5	Lowenva Lodge
3755	10.08.00	4	M	46.9	54.8	Lowenva Lodge
3756	10.08.00	4	F	20.4	40.2	Lowenva Lodge
3789	11.08.00	5	F	55.7	58.9	Lowenva Lodge
3790	11.08.00	5	F	41.1	51.6	Lowenva Lodge
3791	11.08.00	5	F	54.5	58.8	Lowenva Lodge
3792	11.08.00	5	F	36.5	52.8	Lowenva Lodge
3793	11.08.00	5	F	56.6	59.2	Lowenva Lodge
3794	11.08.00	5	F	52.3	56.2	Lowenva Lodge
3795	11.08.00	5	F	45.0	54.5	Lowenva Lodge
3796	11.08.00	5	F	24.9	54.6	Lowenva Lodge
3797	11.08.00	5	F	32.2	47.2	Lowenva Lodge
3798	11.08.00	5	F	27.9	46.4	Lowenva Lodge
4201	11.08.00	4	F	36.2	33.8	Lowenva Lodge
4202	11.08.00	4	F	84.4	69.0	Lowenva Lodge
4203	11.08.00	4	M	26.0	47.2	Lowenva Lodge
4204	11.08.00	4	F	59.8	60.1	Lowenva Lodge
4205	11.08.00	4	F	23.3	44.3	Lowenva Lodge
4206	11.08.00	4	F	36.0	50.9	Lowenva Lodge
4207	11.08.00	4	F	41.8	53.0	Lowenva Lodge
4208	11.08.00	4	M	25.1	44.0	Lowenva Lodge

Appendix VIII (Continued)

Tag No.	Date	Trap No.	Sex	Weight/g	CL (mm)	Site
4209	11.08.00	4	F	32.7	45.7	Lowenva Lodge
4243	12.08.00	5	F	41.3	51.2	Lowenva Lodge
4244	12.08.00	5	M	76.7	60.3	Lowenva Lodge
4245	12.08.00	5	F	35.2	52.6	Lowenva Lodge
4246	12.08.00	5	F	40.8	52.6	Lowenva Lodge
4247	12.08.00	5	F	53.0	54.1	Lowenva Lodge
4248	12.08.00	5	M	89.9	67.2	Lowenva Lodge
4249	12.08.00	5	M	57.4	60.3	Lowenva Lodge
4250	12.08.00	5	F	37.8	51.6	Lowenva Lodge
4251	12.08.00	5	F	43.0	51.6	Lowenva Lodge
4252	12.08.00	4	M	51.3	55.0	Lowenva Lodge
4253	12.08.00	4	F	24.3	40.2	Lowenva Lodge
4254	12.08.00	4	M	90.4	62.4	Lowenva Lodge
4255	12.08.00	4	M	82.7	64.5	Lowenva Lodge
4256	12.08.00	4	M	121.8	74.8	Lowenva Lodge
4257	12.08.00	3	M	30.5	47.8	Lowenva Lodge
4258	12.08.00	3	F	39.6	50.0	Lowenva Lodge
4259	12.08.00	1	F	34.2	49.5	Lowenva Lodge
4260	12.08.00	1	M	53.0	55.6	Lowenva Lodge

Appendix IX The release and capture dates of the 61 recaptured streamer tagged P. leniusculus. Also given are the crayfish tag ID numbers along with the sites and traps in which the crayfish were caught.

Tag Number	Date released	Site	Trap No.	Date recaptured	Site	Trap No.
4226	12.08.00	NBF	2	29.08.01	NBF	3
3651	09.08.00	NBF	1	29.08.01	NBF	5
4223	12.08.00	NBF	2	29.08.01	NBF	2
3731	10.08.00	NBF	3	29.08.01	NBF	2
3719	10.08.00	NBF	4	30.08.01	NBF	3
3681	09.08.00	NBF	5	30.08.01	NBF	4
3781	11.08.00	NBF	5	30.08.01	NBF	5
3646	09.08.00	NBF	1	01.09.01	NBF	1
3768	11.08.00	NBF	3	01.09.01	NBF	5
3725	10.08.00	NBF	1	03.09.01	NBF	3
3774	11.08.00	NBF	4	03.09.01	NBF	5
3686	09.08.00	WHF	2	30.08.01	WHF	1
3695	09.08.00	WHF	2	30.08.01	WHF	2
3605	08.08.00	WHF	2	30.08.01	WHF	3
3609	08.08.00	WHF	2	30.08.01	WHF	2
3611	08.08.00	WHF	3	01.09.01	WHF	3
3703	09.09.00	WHF	4	01.09.01	WHF	7
3734	10.08.00	WHF	2	03.09.01	WHF	2
3754	10.08.00	LL	4	29.08.01	LL	4
3789	11.08.00	LL	5	29.08.01	LL	5
3618	08.08.00	LL	2	30.08.01	LL	5
3626	08.08.00	LL	5	30.08.01	LL	1
3628	08.08.00	LL	5	30.08.01	LL	2
3755	10.08.00	LL	4	30.08.01	LL	2
4244	12.08.00	LL	5	01.09.01	LL	6
3633	08.08.00	LL	5	01.09.01	LL	5
4205	11.08.00	LL	4	02.09.01	LL	8
4246	12.08.00	LL	5	03.09.01	LL	9
4282	29.08.01	NBF	5	20.01.02	NBF	5
3772	11.08.01	NBF	3	20.01.02	NBF	5
3780	11.08.00	NBF	5	26.04.02	NBF	7
4273	29.08.01	NBF	4	26.04.02	NBF	6
3660	09.08.00	NBF	2	02.05.02	NBF	3
3719	10.08.00	NBF	4	14.05.02	NBF	4
4232	29.08.01	NBF	1	21.05.02	NBF	12
3781	11.08.00	NBF	5	21.05.02	NBF	1
4233	29.08.01	NBF	2	24.06.02	NBF	9

Appendix IX (Continued)

Tag Number	Date released	Site	Trap No.	Date recaptured	Site	Trap No.
4280	29.08.01	NBF	5	24.06.02	NBF	12
4229	12.08.00	NBF	2	26.06.02	NBF	10
3771	11.08.00	NBF	3	26.06.02	NBF	4
4240	12.08.00	NBF	2	26.06.02	NBF	2
3768	11.08.00	NBF	3	27.06.02	NBF	11
4237	29.08.01	NBF	3	27.06.02	NBF	12
3767	11.08.00	NBF	2	27.06.02	NBF	13
4285	29.08.01	NBF	5	05.07.02	NBF	7
3663	09.08.00	NBF	2	07.08.02	NBF	5
3605	08.08.00	WHF	2	24.06.02	WHF	14
3604	08.08.00	WHF	2	24.06.02	WHF	8
4299	29.08.01	WHF	2	24.06.02	WHF	7
4295	29.08.01	WHF	1	24.06.02	WHF	7
3611	08.08.00	WHF	3	24.06.02	WHF	7
3784	11.08.00	WHF	2	25.06.02	WHF	4
3609	08.08.00	WHF	2	27.06.02	WHF	10
3686	09.08.00	WHF	3	05.08.02	WHF	3
3685	09.08.00	WHF	1	06.08.02	WHF	2
3681	10.08.00	LL	3	25.06.02	LL	4
4209	11.08.00	LL	4	25.06.02	LL	7
4256	12.08.00	LL	4	26.06.02	LL	10
3754	10.08.00	LL	4	25.07.02	LL	2
4260	12.08.00	LL	1	06.08.02	LL	7
3748	10.08.00	LL	2	07.08.02	LL	11

Appendix X Comparison of Experimental and control PO_2 levels within an artificial burrow over a half hour period. Crayfish mean ± SE., n=24. Control mean ± SE., n=3 (no animal present). The average fitted values were calculated using the function Y=A-Bexp[-kt].

Time (mins)	Burrow PO_2 (crayfish) (mm Hg)	Burrow PO_2 (control) (mm Hg)	Fitted Curve (crayfish) PO_2 (mm Hg)	Fitted Curve (control) PO_2 (mm Hg)
0.0	58.3 ± 3.4	58.8 ± 4.6	69.9 ± 5.0	60.0 ± 3.6
0.5	70.2 ± 5.3	56.6 ± 4.3	74.9 ± 4.9	60.8 ± 3.8
1.0	79.5 ± 5.6	58.1 ± 4.1	79.3 ± 4.9	61.6 ± 3.9
1.5	82.0 ± 5.5	58.9 ± 6.5	83.2 ± 4.9	62.3 ± 4.0
2.0	88.2 ± 5.3	63.3 ± 3.5	86.7 ± 4.9	62.9 ± 4.1
2.5	92.9 ± 4.9	64.5 ± 2.6	89.8 ± 4.9	63.6 ± 4.2
5.0	102.2 ± 4.4	67.3 ± 5.7	101.1 ± 4.7	66.2 ± 4.6
10.0	109.3 ± 4.5	71.7 ± 4.9	112.6 ± 4.4	69.6 ± 4.8
20.0	118.5 ± 3.9	71.2 ± 5.3	120.1 ± 4.0	72.8 ± 4.7
30.0	119.8 ± 3.4	69.3 ± 3.0	121.9 ± 3.8	73.9 ± 4.9

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