ANGLIA RUSKIN UNIVERSITY

# SAMPLING UK *PACIFASTACUS LENIUSCULUS* (DANA, 1852): THE EFFECT OF TRAPPING ON POPULATION STRUCTURE

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ABSTRACT

ANGLIA RUSKIN UNIVERSITY

FACULTY OF SCIENCE AND TECHNOLOGY

MASTER OF PHILOSOPHY

# SAMPLING UK *PACIFASTACUS LENIUSCULUS* (DANA, 1852): THE EFFECT OF TRAPPING ON POPULATION STRUCTURE

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Populations of non-native signal crayfish, *Pacifastacus leniusculus*, are damaging to UK native species and habitats though their populations are expanding with no coherent framework in place for their control. This is partly the result of a literature gap on the effect of trapping on non-native crayfish population structure which this thesis will explore in order to add to the European literature.

Population size structure analysis has been facilitated via the creation of novel samplers and an in-depth analysis of the effect of aperture on the size/life stage of crayfish sampled. Smaller trap apertures, the addition of refuge material and novel samplers increased the catch of juvenile crayfish. Sex was indeterminable for up to 50% of juvenile crayfish, with juvenile sex ratios potentially biased towards females. Conditions on the River Lark did not limit populations, though temperature varied significantly between sites whilst substrata, pH and biological oxygen demand did not.

Three years of trapping and juvenile sampling enabled population analysis at a site level. The population at Lark Head (professionally trapped), had a consistent size structure from 2010 to 2012, whilst individuals at Barton Mills (community trapped) and the Plough (untrapped), showed size decreases over time. The proportions of adult to juvenile individuals, and males to females, were similar at all three sites in 2011 & 2012. Catch per unit effort, decreased at all three sites with the greatest reductions at trapped sites. There is no evidence that catch sizes, or the proportion of juveniles, increased with trapping in spite of one site being trapped by the community since 2001 and another trapped by professionals since 2005. This refutes inferences that trapping causes an increase in biomass due to a reduction in the number of cannibalistic and dominant large males, with size and sex bias in traps also not corroborated.

Keywords: NICS; juveniles; trap; aperture; stunting; control.

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# Chapter 1: Introduction

## 1.1 Context

Human-mediated biological invasions are occurring with ever increasing regularity (Elton, 1958). This is illustrated by 'exponential' literature growth since the 1980s (Davis, 2009; Chisholm, 2010) (though may also signify increasing awareness) with at least four dedicated journals launched (e.g. Biological Invasions and NeoBiota in 1999, Aquatic Invasions in 2006 and Management of Biological Invasions in 2010). Declines in freshwater biodiversity exceed those of terrestrial ecosystems with "invasions by exotic species" considered a major threat (Sala et al., 2000). Global crayfish movements are responsible for catastrophic biodiversity declines and disrupted ecosystems, though non-indigenous crayfish species (NICS) have been intentionally introduced as a high value food product for humans and for the pet/ aquarium trade (Chucholl, 2013). Around the world (e.g. France, Spain, Sweden, Madagascar) indigenous crayfish species are consumed whilst also being revered for their inherent aesthetic and biodiversity value (Hanson et al., 1994; Ackefors, 2000; Füreder et al., 2003; Jones et al., 2006). In the UK there is no national history of native crayfish consumption (which is now illegal) whilst in contrast to the rest of Europe the consumption of non-native crayfish is contentious.

The status of European native crayfish will now be outlined together with the threat posed by NICS, in particular *P. leniusculus* (Dana, 1852/ Astacidae, Decapoda). The characteristics that make *P. leniusculus* (North American signal crayfish) successful invaders are described, and the differing consequences for native species and habitats of NICS presence and abundance are elucidated. The threat posed to native crayfish and freshwater habitats and species are outlined. The use of exploitation as a management tool is explored followed by an overview of the aims of data chapters 3, 4 & 5 (pages 11-13).

## 1.2 European native crayfish species

*Austropotamobius pallipes* (Lereboullet, 1858) (white-clawed crayfish) is the only native crayfish species found in the UK though it is recorded in a total of 18 countries across Europe. In England, Ireland and Wales historical introductions account for its presence (Holdich et al., 1995; Pöckl et al., 2006), with introductions in the 1940's into Scotland (Gladman et al., 2009). Though an introduced species itself *A. pallipes* is considered a valued UK native. Across Europe there are five crayfish species (Lindqvist, 1987; Holdich, 2002a; Holdich and Sibley, 2003; Machino and Holdich, 2006), all of which belong to the family Astacidae (Appendix A). Globally native crayfish are subject to overfishing, poaching, predation, habitat alteration and pollution, together with threats from crayfish plague and the deliberate or accidental introduction of NICS (Lodge et al., 2000).

In the UK *A. pallipes* population declines were noted prior to the government sponsored *Pacifastacus leniusculus* introductions (for human consumption) in the 1970s and '80s (Shardlow et al., 2002). However, the introduction of NICS has certainly exacerbated the decline of *A. pallipes* which is increasingly imperilled (Sibley et al., 2011) with extinction predicted by 2033 (Holdich et al., 2004). *P. leniusculus*, and other introduced North American crayfish species, are carriers of *Aphanomyces astaci* (Schikora, 1903) (fungal crayfish plague) which extirpates native crayfish populations. As well as carrying crayfish plague NICS reduce the abundance and diversity of other aquatic biota, damage ecosystems via burrowing and habitat perturbation and out-compete native crayfish species (Holdich et al., 2009).

**1.3 Non-indigenous crayfish species (NICS): Introductions into Europe**

From 1907 Swedish *A. astacus* populations (the native noble crayfish) were negatively affected by outbreaks of crayfish plague (Souty-Grosset et al., 2006), with concomitant disruption to commercial fisheries and national traditions. A successful campaign to bolster stocks of crayfish in Sweden was fought with 'plague-resistant' crayfish from North America (namely *P. leniusculus*) promoted as an 'ecological and gastronomic homologue' of the native *A. astacus* (Abrahamsson and Goldman, 1970). The UK had only a limited tradition of local native crayfish consumption yet the Ministry of Agriculture, Fisheries and Food (MAFF) decided to copy Sweden and commenced the introduction of *P. leniusculus* (for subsequent sale as a human foodstuff) against scientific advice (Bowler, 1979; Holdich and Whisson, 2004). In the UK crayfish "farm-diversification" enterprises were incentivised via generous subsidies (Alderman et al., 1990), with marketing and distribution support offered via the newly formed British Crayfish Marketing Association (BCMA; Reynolds and Gherardi, 2012).

In the UK over 300 'implants' of juvenile *P. leniusculus* had taken place by 1992 (Rogers and Watson, 2011), with 110 new crayfish farms registered (David Rogers Associates, 2012). However, new "crayfish farmers" reported slow growth and low yields and the dissolution of the BCMA followed in 1990, with ponds abandoned and stock left to grow-on unchecked. Meanwhile in Sweden it became apparent that North American crayfish species were carriers of crayfish plague (Alderman et al., 1990), further exacerbating the decline of Swedish *A. astacus* stocks. *P. leniusculus* now occurs in 24 European countries making it the most widely distributed NICS in Europe with population growth, movement and accidental & deliberate introductions contributing to its increasing distribution in the UK and elsewhere (Figure 1.1).

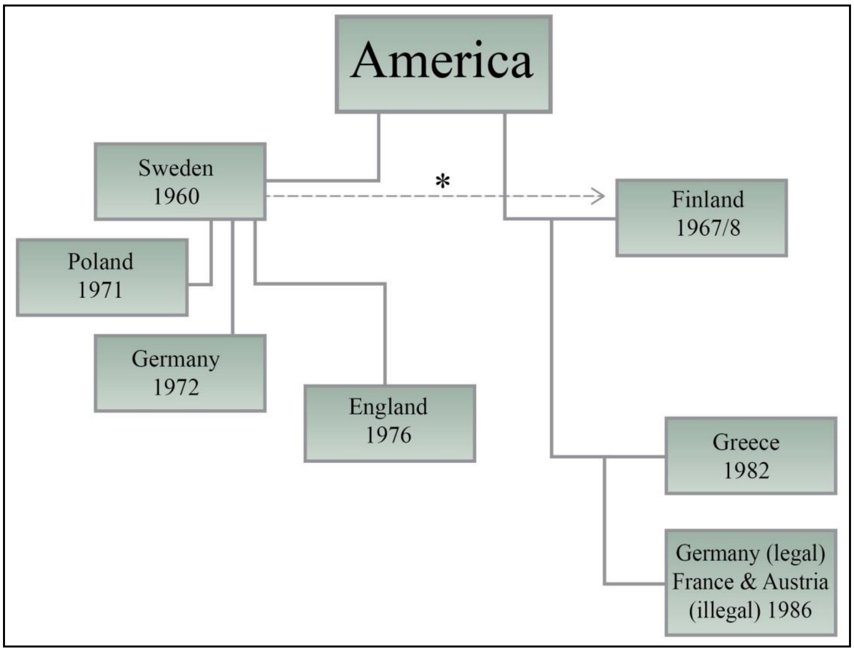



Figure 1.1. Geographical source, and dates, of primary introductions of *P. leniusculus* from rivers and lakes in America, with secondary introductions from Sweden then implanted into Poland, Germany and England, plus juvenile implants (*) from Swedish hatcheries to Finland (Information after Lewis, 2001; Reeve, 2004; Souty-Grosset et al., 2006).

## **<u>1.4 The signal crayfish: A successful invader</u>**

Adaptability is a feature of NICS, with their success as invaders demonstrated by the ubiquitous and increasing distribution of *P. leniusculus* which is a large, long-lived, fecund, aggressive, polytrophic, cannibalistic crustacean with wide-ranging environmental tolerances rendering it a tenacious and dominant invader. *P. leniusculus* can breed successfully in brackish waters (Holdich et al., 1997), fluctuating thermal conditions (Rutledge and Pritchard, 1981; Firkins and Holdich, 1993), varying water qualities and significant heavy metal concentrations (Antón et al., 2000). *P. leniusculus* can survive on land for up to three months in damp conditions and can travel overland (as well as through watercourses), their excellent climbing abilities allowing them to circumnavigate weirs and other obstacles (Holdich, 1991). It is not known how far a crayfish can walk over land (Holdich et al., 2004), though spread through rivers has been estimated at 2.4 km $yr^{-1}$ downstream (Bubb et al., 2005).

An additional threat is present in the form of movement of juvenile or adult crayfish by predators including *Mustela vison* (mink), *Lutra lutra* (European otter), *Ardea cinerea*, (grey heron) and *Anas platrhynchos* (mallard) in the UK (Holdich et al., 2004; Banha and Anastácio, 2011; Capinha et al., 2013). NICS movement by predators could result in false recording of presence data generated from NICS remains or even the spread of live individuals between catchments.

**1.5 Presence and abundance**

*P. leniusculus* poses a threat via its presence, abundance and its ability to act as a crayfish plague vector. Native crayfish populations tend to be in equilibrium with the other components of the aquatic environment, whereas NICS often achieve very high densities that are "out of balance" (Momot, 1995; Guan and Wiles, 1997). A clear link between crayfish plague outbreaks and *P. leniusculus* has been identified in a DEFRA commissioned report (Figure 1.2), which concluded that NICS control was key to limiting the spread of crayfish plague, following the reasoning that increased population size leads to increased spread (Rogers and Watson, 2011). A comprehensive overview of research into control methodology is provided in an additional Department of the Environment, Food and Rural Affairs (DEFRA) commissioned report (Stebbing et al., 2012).

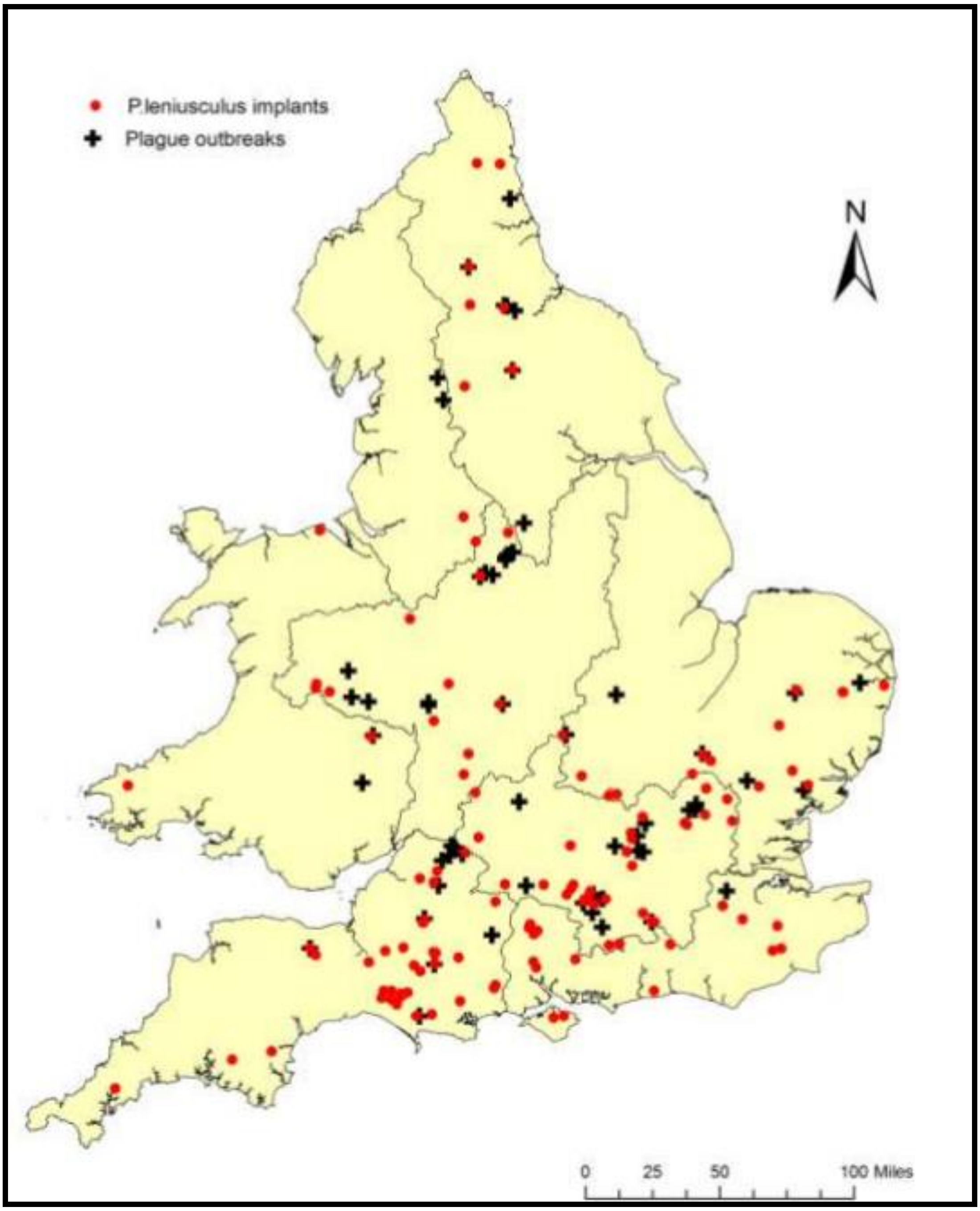



Figure 1.2. The location of *P. leniusculus* implants in the 1970s and 1980s (red dots), and UK crayfish plague outbreaks subsequently (black crosses) (From: Rogers and Watson, 2011).

## 1.6 Inter-specific competition

*P. leniusculus* out-competes the native *A. pallipes*, as it is larger (maximum total length 15 cm vs 10 cm; Gherardi and Holdich, 1999) and more aggressive (Pintor et al., 2008). In addition *P. leniusculus* is more fecund, producing 110 to 201 eggs at a time (Abrahamsson and Goldman, 1970), compared with *A. pallipes*' 20 to 150 eggs (Holdich and Reeve, 1991). Juvenile *P. leniusculus* mature faster than juvenile *A. pallipes* whilst adults live longer (Lewis, 2001). Competitive mating (male *P. leniusculus* and female *A. pallipes*), has been reported with aggressive encounters between the two species resulting in *A. pallipes* being killed or wounded (Holdich and Domaniewski, 2005).

## 1.7 Ecosystem consequences

NICS pose a significant threat to freshwater systems (Sala et al., 2000; Perrings, 2002; Park, 2004; Genovesi, 2005; Rogers et al., 2005) and are keystone species acting as both predator and prey (Hogger, 1988; Nyström et al., 2000; Freeman et al., 2010). Crayfish are considered 'polytrophic' (Momot, 1995), with their role as plant grazers, predators and detrivores now considered though, as Huxley (1880) pointed out …

*"…few things in the way of food are amiss to the crayfish."*

Crayfish are grazers of periphyton and macroalgae with large plant growth increases noted when native crayfish have been extirpated by plague (Abrahamsson, 1966; Mathews and Reynolds, 1992), to the extent that watercourses become choked. As voracious consumers NICS were often successfully introduced to control aquatic vegetation (Laurent and Vey, 1986; Larson and Magoulick, 2008).

Crayfish preferentially predate slow moving aquatic species (Abrahamsson, 1966) with Gastropoda, Amphibia, Hirundinea, Chironomidae (Diptera) and Gammaridae (Amphipodae) particularly affected (Stenroth and Nyström, 2003; Rogers and Watson, 2005a; Crawford et al., 2006; Gherardi, 2007). However, the interplay between NICS consumption of both vegetation and herbivorous invertebrates affects complex aquatic food webs in ways that are hard to predict or fully interpret (Ficetola et al., 2012).

Fish species that share their habitat with NICS have their eggs, fry and adults predated (Guan, 1997; Guan and Wiles, 1998) and breeding and spawning areas compromised (Svensson, 1993; Guan, 1997; Ribbens and Graham, 2004; Everard et al., 2009; Freeman et al., 2010). Predatory crayfish also modify the behaviour of prey species with competition for refuges resulting in fish becoming vulnerable to other predators due to refuge loss (Griffiths et al., 2004; Peay et al., 2010). The ability of NICS to degrade habitats and reduce biodiversity reduces ecosystem resilience, leading to a cascade of negative environmental impacts as one invasive species facilitates the establishment of the next (Simberloff and Von Holle, 1999), prompting “invasional meltdown” (Ricciardi, 2001). Control of non-native crayfish, and other invasive species, is considered a UK priority (e.g. DEFRA, 2008), although a practical management framework for UK NICS remains conspicuously absent.

**1.8 Exploitation as management**

Humans have a well-established history of natural resource exploitation leading to population declines and extinctions. Fisheries for marine prawns, crabs and lobsters are regulated in the hope of providing a 'sustainable' harvest (Barnes, 1987), though fisheries management practices are fraught with uncertainty. *Homarus americanus* (American lobster) populations off the coasts of New England and Canada are considered heavily overfished (Barnes, 1987; Ingle, 1997), with efforts being made to rear *H. gammarus* (European lobster) juveniles in captivity (Anon., 1995). In Australia, consumption of the crayfish *Astacopsis gouldii* (Giant Tasmanian lobster; Taylor, 2002; Appendix A), locally valued for its meat, has contributed to extinctions and declines throughout its range. Similarly, the range reduction of three species of *Euastacus* in Australia is attributed to fishing mortality (Horwitz, 1990).

The received wisdom in the UK is based on the premise that trapping NICS exacerbates the problem of NIC populations, with websites and the grey literature providing statements without citation. For example in 2012 the Environment Agency website notes…

*"Often traps catch the larger crayfish, leaving the smaller ones to breed prolifically. This can result in a population explosion as more space and food becomes available and competition is reduced."*

Whilst Buglife's website (2012), urged the public to understand that…

*"Trapping can do more harm than good! …trapping large crayfish can actually help to boost the future population – this is because large crayfish eat a lot of the smaller ones, so removing large crayfish lets more young crayfish survive."*

Though the statements made on the previous two organisational websites are now diluted, information on the Scottish Environmental Protection Authority (SEPA) website continues to echo these sentiments in 2014…

*"Trapping trials have concluded that although numbers may be reduced during the short-term, traps may favour the capture of larger individuals. An unintended consequence of selective harvesting is the increased growth and earlier maturation of juvenile crayfish, which can cause the population to increase. It is not, therefore a sustainable long-term solution"*

These statements may be attributed, at least in part, to the misreporting by Peay (2001) of Keller's (1999 a, b) studies. Keller aimed to quantify the maximum sustainable harvest of *A. astacus* from stocked ponds and to this end used 0+ to 1+ individuals (thereby reducing the impact of juvenile mortality) to stock ponds whilst excluding predators (obviating natural predation). Overcrowding occurred as juveniles became adults, and stunting (attributed to density dependence/ resource competition by Keller) followed. However, the stunting prompted in this study was inappropriately attributed to 'trapping' with this study's findings unsuitable for extrapolation to wild NIC populations in ponds, lakes or rivers.

Globally, and in a European context, native and non-native crayfish are harvested for food, so trapped populations are substantial (or trapping would not be considered worthwhile), rendering population studies inconclusive. Density dependence and/ or trapping may both prompt stunting when both are present. As natural mortality is high amongst juveniles, and *P. leniusculus* have a long life span (up to 16 years; Belchier et al., 1998), reduction of the reproductively active population is key. If the impact of trapping is being examined then quantification of trapping effort is vital. Commercial/ professional harvest will always exceed that of recreational/ scientific endeavours (Darimont et al., 2009), though the two are rarely examined in tandem. Niche availability is considered the only potential limiting factor for wild NICS populations (Hill et al., 1993; Söderbäck, 1993). Crayfish movement/ migration may therefore be motivated by a desire to locate vacant niches (Moorhouse and Macdonald, 2010; 2011), with the potential movement of a growing crayfish population in a river perhaps depicted as a 'travelling wave' (Williamson, 1996; Figure 1.3).

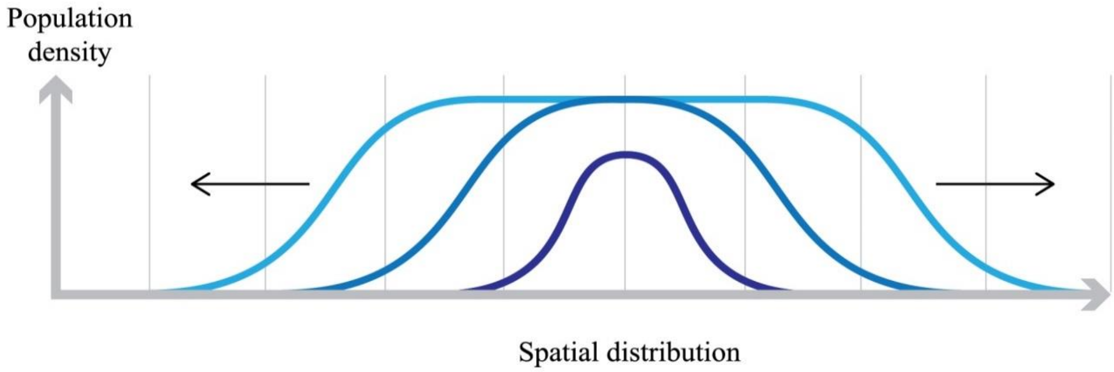



Figure 1.3. The travelling wave model of population distribution with successive distribution fronts spreading away from the centre (From: Williamson, 1996).

*P. leniusculus* easily spread through rivers, with movement potentially prompted as the population increases. It is therefore not only the presence of NICS, but also their abundance, that is of importance. The famed tolerances and climbing abilities of *P. leniusculus* mean that abundance increases can affect both the immediate area and any nearby waterbodies.

## 1.9 THE RESEARCH AIMS OF DATA CHAPTERS 3, 4 AND 5

A paucity of data on how trapping affects the size and sex structure of populations of non-native *P. leniusculus* led to this study. Areas of the River Lark have been trapped by the 'community' since 2001 whilst others have been trapped by professionals since 2005. Further sites have remained untrapped to avoid disturbance to a native trout fly fishery. This offered the opportunity to study the effect of trapping, if any, over a period of years. In order to consider the effect of trapping one must first evaluate any site based differences (water chemistry and channel characteristics that comprise the aquatic habitat that the crayfish inhabit; Chapter 3) as variations between sites could account for any differences in the number, size or sex structure of the non-native crayfish populations.

To compare sites that differ in their management/ trapping history, one must be able to comprehensively sample the populations. As trapping is considered a size and sex selective removal method which may skew population structure over time initially sampling methods needed to be evaluated. Research was also carried out into methods and equipment that could effectively sample juvenile crayfish (Chapter 4). In order to compare the size and sex structure of populations on the River Lark the relative proportions of different sizes of crayfish will be considered vis percentage contribution of males/ females and the different size classes using proportions and numerical techniques (Principal Components Analysis and Bhattacharya plots). The identification of potential cohorts will then facilitate between site comparisons. Finally catch per unit effort (CPUE) is compared between years and sites in relation to trapped and untrapped sites on the River Lark (Chapter 5). The implications of the research for sampling and management are considered in Chapter 6.

## Chapter 3: Does site-based variation influence River Lark non-native *P. leniusculus* populations?

**Aim:** To determine if study sites on the River Lark vary in their water chemistry or channel characteristics and how these factors might affect non-native crayfish populations.

**Hypothesis:** That non-native crayfish populations will not vary in their size or sex structure in relation to the characteristics of the water/ river channel environment.
**Hypothesis:** That channel characteristics may have an impact on the non-native crayfish population carrying capacity of the various reaches.

## Chapter 4: The effect of aperture diameter, life history stage and behaviour on the size and sex of crayfish sampled

**Aim:** To determine suitable equipment and methods to sample all sexes/ sizes and life stages of non-native crayfish.

**Juveniles**

**Hypothesis:** Juvenile crayfish sampling does not differ from adult crayfish sampling.
**Hypothesis:** Juvenile crayfish may be successfully sampled using refuge media that offers aperture sizes appropriate to their dimensions/ behaviour.

**Adults**

**Hypothesis:** Trapping is not size selective, aperture size is the key determinant of the size of non-native crayfish sampled. Trapping does not only capture male crayfish.

## Chapter 5: Variation in size and sex structure of three River Lark *P. leniusculus* populations

**Aim:** To represent non-native crayfish populations (size and sex structure) fully and comprehensively and to compare the populations at the three study sites on the River Lark with reference to their crayfish management/ trapping history. To investigate changes in CPUE over time at trapped and untrapped sites on the River Lark.

**Hypotheses:** Trapping will reduce CPUE over time but does not affect the size or sex structure of non-native crayfish populations.
**Hypothesis:** Population size structure will vary on the River Lark independently of whether a site is trapped or untrapped.

# CHAPTER 2: GENERAL METHODS

## 2.1 Overview

The following methods have been designed to enable the comprehensive assessment of the size and sex structure of a NIC population and its comparison across three sites on a UK river. Existing equipment and techniques have been utilised to provide baselines and to facilitate comparison with the published literature. Novel methods have been developed (particularly in relation to juvenile crayfish sampling) to meet the stated aims of comprehensively assessing NIC population size and sex structure and thus to facilitate research into the effect of trapping and add to the literature.

### 2.1.1 The River Lark

The River Lark, which lies in the Brecks Natural Area, is a tributary of the Cam-Ely-Ouse catchment which covers c.3600 $km^2$ of East Anglia (Figure 2.1). In common with many UK rivers, the Lark has been substantially modified with locks, staunches, re-directions, dredging, straightening and flood defence works. In the Cam-Ely-Ouse catchment, many of the monitored rivers (including the Lark) are naturally slow flowing with heavy sediment loads. Low rainfall (a major issue in East Anglia) contributes to high sediment loads as increasing water abstraction and soil erosion are experienced (Environment Agency, 2009). The River Lark now contains large populations of *P. leniusculus*, possibly derived from escapes from a crayfish farm at Hengrave (Figure 2.2), in the late 1980s or early 1990s. *P. leniusculus* was first recorded in the River Lark at Icklingham in 1995 (West, 2010). The known management history of NICS on the River Lark makes it an ideal location for studies on the effect of long-term management on *P. leniusculus* population structure.

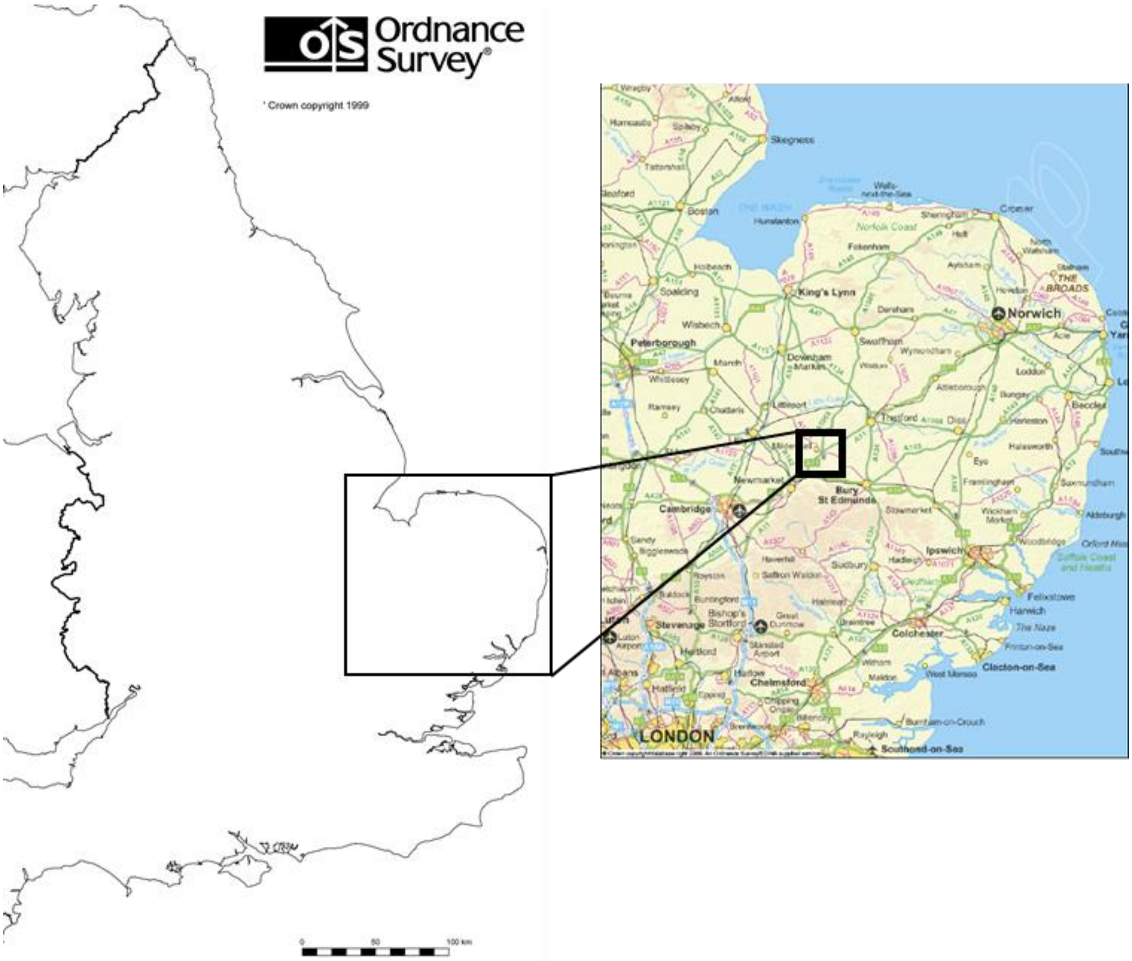



Figure 2.1. Location of study sites on the River Lark, Suffolk, in the context of the UK region of East Anglia.

### 2.1.2 Study site selection

Three sites on the River Lark were selected on the basis of their trapping histories, ease of access, permissions and low interference potential (Figure 2.2). All Barton Mills sampling locations were in private gardens, with access agreed with householders. Additional permission was sought from the Elveden Estate, Forest Enterprise, the Environment Agency (EA), Natural England (NE) and the Lark Angling and Preservation Society (LAPS). The River Lark lies within the Breckland natural area (Norfolk/ Suffolk) in East Anglia where the underlying geology is defined as Cretaceous cuesta with chalk, mixed with flint, overlain by thin sands and gravels (Straw and Clayton, 1979; Suffolk County Council, 2010).

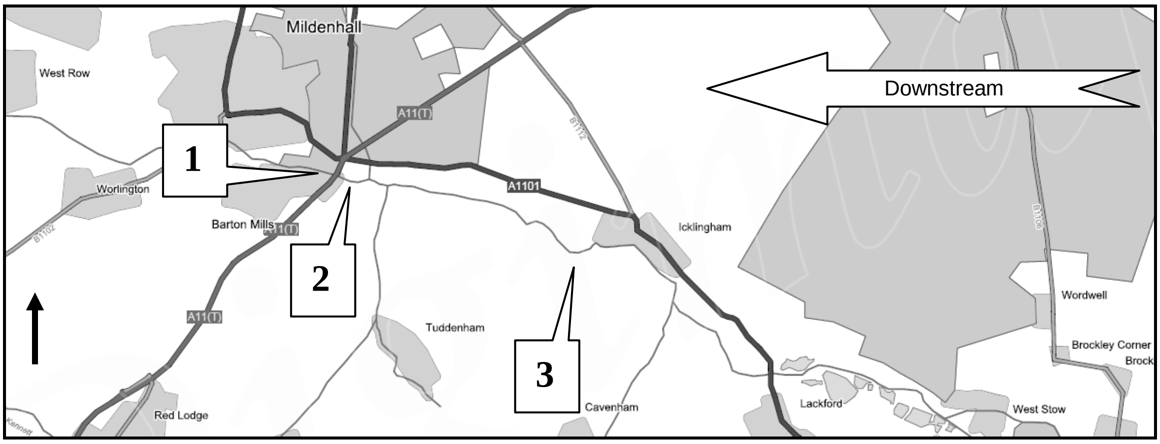



Figure 2.2. Study locations on the River Lark with differing management histories. [©Crown copyright An Ordnance Survey/EDINA supplied service 1:50,000)]

Key:
1. Barton Mils - Community trapping from 2001 onwards
2. Lark Head - Professional trapping from 2005 onwards
3. The Plough - Control: No trapping allowed (LAPS native brown trout section)

### 2.1.3 Barton Mills

The Barton Mills study site lies between the A11 road-bridge at Barton Mills and Mildenhall town (Figure 2.2) with this reach bounded at each end by sluice gates. The midpoint of the sampling area is NGTL 719740 (Figure 2.3). Formerly extensive vegetation has been depleted since the first signs of *P. leniusculus* were observed in 1999, though beds of streamer weed (*Potamogeton* spp.) remain. Trapping by LAPS, in addition to trapping by landowners and public 'crayfishing' days at this site, began in response to the burgeoning *P. leniusculus* population in 2001. Increased removal efforts and a more scientific approach to data collection began in 2004 (West, 2010).

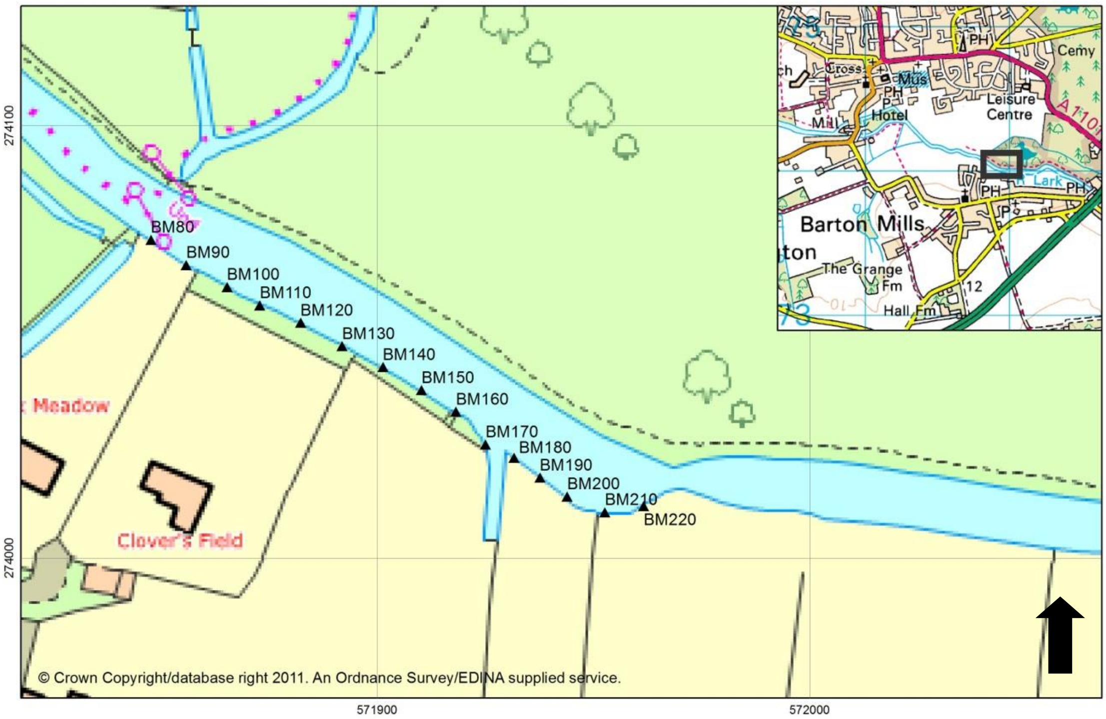



Figure 2.3. Barton Mills (Site 1) (Scale: Grid lines are 1 km apart). BM80 - BM220 labels depict the location of sampling points (15 in total) at 10 metre intervals.
[Inset picture shows the location of the sampling points within the village of Barton Mills].

### 2.1.4 Lark Head

Lark Head has high, steep banks (a legacy of past flood defence work) where the river has been straightened and dredged. The mid-point of the sampling area is NGTL 732737 (Figure 2.4). The area is owned and managed by the EA (with public access, but not angling, permitted), with vehicle gates in place and a secure compound on site which contains a sluice gate to control flow from the cut-off channel (dug as a flood relief intervention) into the River Lark. Crayfish at Lark Head have been trapped professionally since 2005 (West, 2008).

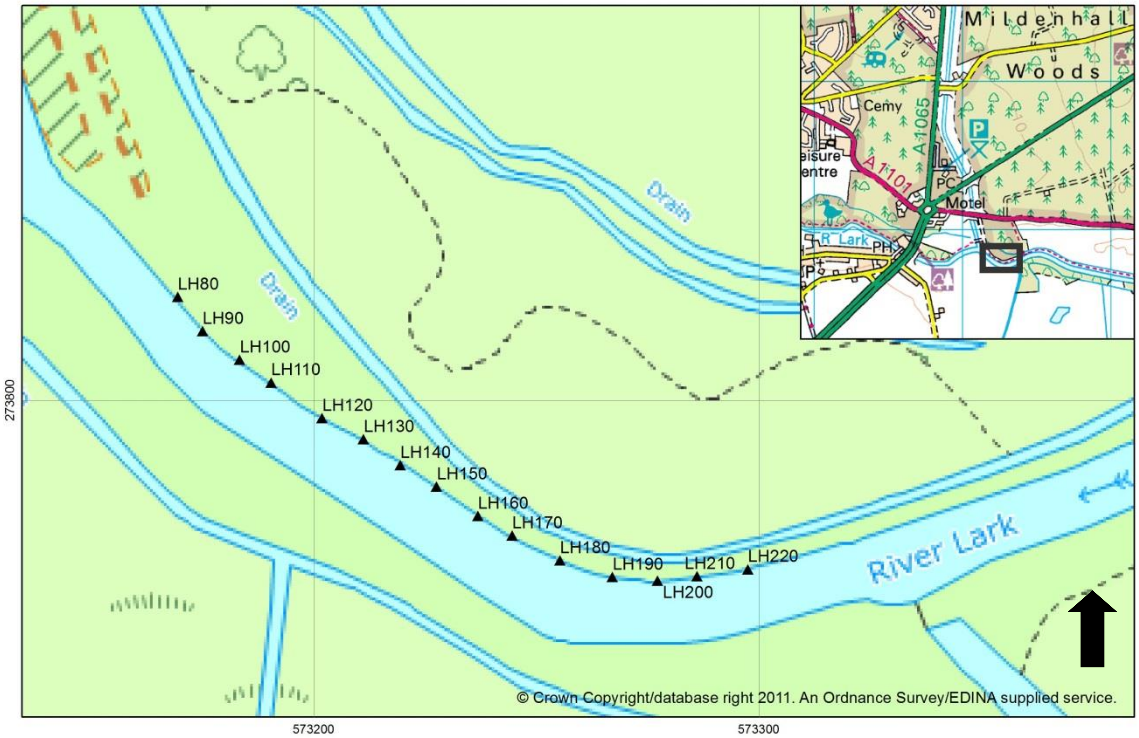



Figure 2.4. Lark Head (Site 2) (Scale: Grid lines 1 km apart). LH80 - LH220 labels depict the location of sampling points (15 in total) at 10 metre intervals.
[Inset picture shows the location of the sampling points in the Fiveways roundabout area].

### 2.1.5 Plough

The Plough site lies in a wooded, rural/ agricultural area surrounded by fields. This stretch of the River Lark is known as the LAPS "trout section" because of its native *Salmo trutta* (brown trout) population with habitat restoration undertaken by the club (West, 2008). Natural sedimentation has reduced the width of the river upstream of this study site. The midpoint of the sampling area is NGTL 774724 (Figure 2.5). This site has not been trapped and is here considered as a control.

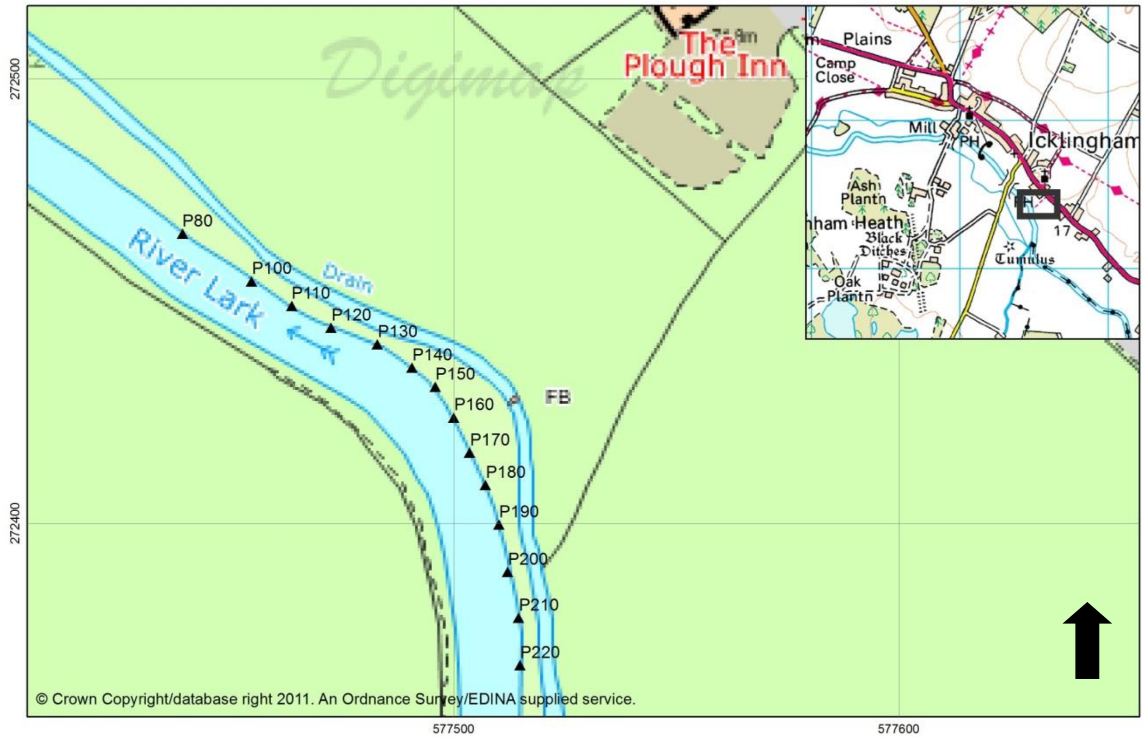



Figure 2.5. Plough (Site 3) (Scale: Grid lines are 1 km apart). P80 - P220 labels depict the location of sampling points (15 in total) at 10 metre intervals.
[Inset picture shows the location of the sampling points within the village of Icklingham].

## 2.2 Method

### 2.2.1 Passive sampling: Traps, trapping and bait

Eight different trap designs were used between 2010 and 2012 (Table II.I) in order to test commercially available designs and consider the impact of both design and aperture dimensions and construction. Traps were attached to the bank via black 2 mm ⌀ nylon twine (Appendix A), weighted to reduce detection of, and disturbance to equipment. Traps were all deployed from one accessible bank and left to self-orientate. The twine was attached to short wooden posts driven into the undergrowth and labelled with looped cable ties with numbered PVC wire markers and site locations recorded using a portable Garmin® GPS 60Cx GPS unit.

Table II.I. Trap name, aperture shape, dimensions and number of entrances.

| **Trap name** | **Aperture shape, size in mm (no. of entrances)** |
|---|---|
| **Minnow small** | circular: 20 (2) |
| **Minnow smallmedium** | circular: 30 (2) |
| **Minnow medium** | circular: 40 (2) |
| **Minnow large** | circular: 50 (2) |
| **Pirat** | hexagonal: 90 x 50 (2) |
| **Professional** | triangular: 110 x 70 (2) |
| **Trapman** | circular (long spikes): 60 (1) |
| **LiNi** | round: 60 (2) |

NB: Swedish 'Trappy' ™ traps were not used in this study. They were tested against expanded minnow traps (⌀ 40 mm; MX) during long-term monitoring studies on the River Lark (unpublished data) and were found to be inefficient in both capture and retention potentially as a result of their large flexible yellow entrance funnels.

Trap types were chosen on the basis of utility and widespread use. The Gees™ minnow trap and modifications to this trap were used to experimentally verify the function of all the other trap types. Minnow traps are sold as standard with small apertures (minnow small; 20 mm; Figure 2.6) or large (crawfish™ trap; minnow large; 50 mm; Figure 2.7). LAPS, in common with other agencies and researchers, adapted standard minnow traps by increasing the aperture size to 40 mm which are termed minnow medium in this study (Figure 2.7; West 2008, 2010). Additionally a minnow 'smallmedium' trap was created with an aperture of 30 mm.

Figure 2.6. Standard Gees ™ minnow trap (small; 20 mm ø), (Scale bar = 100 mm).

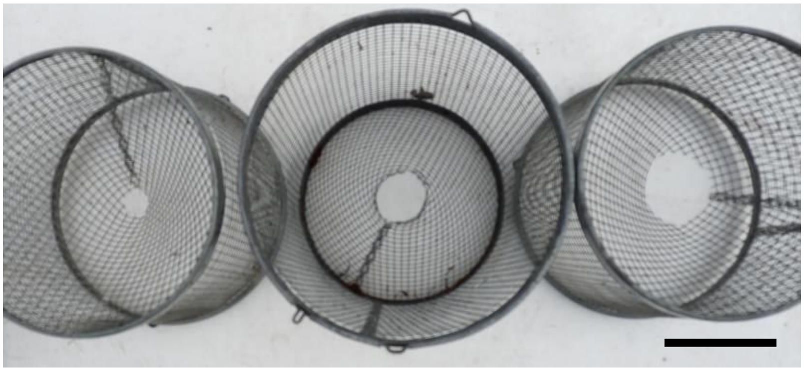


Figure 2.7. Minnow traps with differing aperture sizes (left to right) small (20 mm Ø), medium (40 mm Ø), and large (50 mm Ø), (Scale bar = 100 mm). NB: The minnow smallmedium (30 mm Ø) trap aperture is not depicted.

Minnow medium trap and minnow smallmedium (developed in 2012), trap apertures were measured and cut to ensure a consistent size. The custom made ‘professional’ trap (GT Products, UK; Figure 2.8), has been used by professional trappers on the River Lark. The UK ‘Trapman’ ™ (Figure 2.9), and Finnish ‘Pirat’ ™ (Figure 2.10), traps were used in 2011 to study catch bias and increase the potential capture of larger individuals. ‘LiNi’ ™ traps (Figure 2.11), were used by professional trappers in 2012 as part of a bulk removal exercise, baited with fresh fish and set from a boat in contrast to all the other traps types used. A variety of trap types were utilised in order to both assess their individual utility and to increase the amount of equipment available for sampling.

Figure 2.8. Professional trap (x2) (Scale bar = 100 mm).

During all experimental work traps were baited with fish-flavoured cat food (Felix™ ‘Purina tuna in jelly’). Composition: meat derivatives, fish derivatives (tuna 4%), vegetable derivatives, minerals, various sugars, moisture 82.5% , protein 7.5% , fat 4.5% , crude ash 3% , crude fibres 0.1% and linoleic acid (omega 6 fatty acids), 0.6%. As the bait was provided fresh and water soluble, a refractory period was deemed unnecessary.

Figure 2.9. Trapman ™ trap (Scale bar = 100 mm).

Figure 2.10. Finnish 'Pirat'™ trap (scale bar = 100 mm)

Cat food was chosen as it provides easily reproducible, accessible, low cost bait (Rach and Bills, 1989; Litvan et al., 2010). Trappy™ bait boxes (60 x 40 x 40 mm with 5 x 5 mm mesh each containing c.50g of cat food) were utilised, except with Pirat traps (as the aperture design permitted bait box loss), where cylindrical tins (74 mm diameter x 107 mm high) were used.

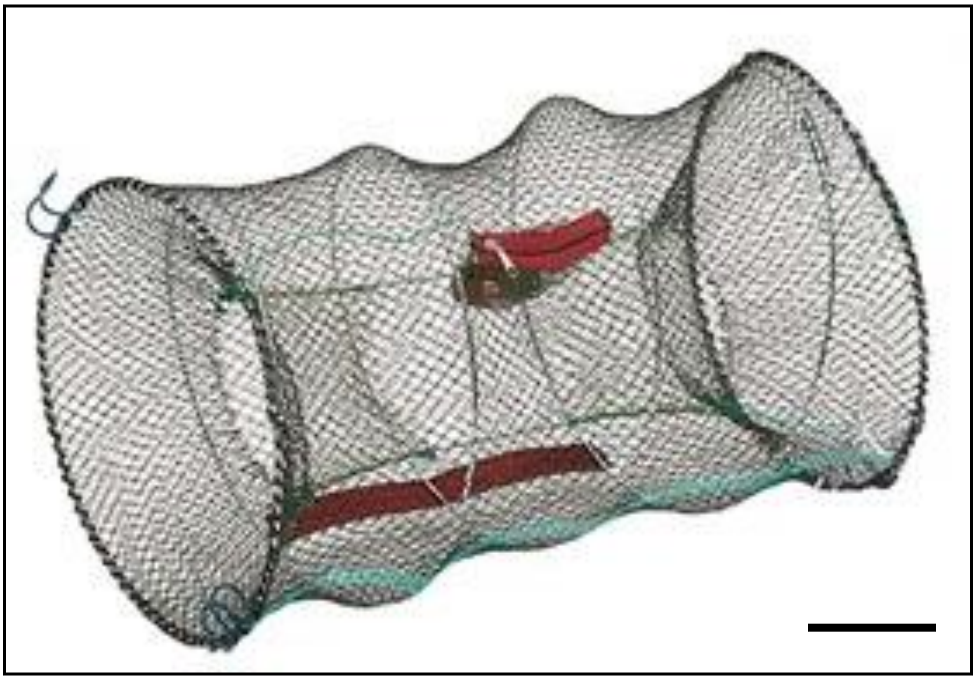


Figure 2.11. 'LiNi' ™ trap (Scale bar = 100 mm).

Four trap types (minnow small, medium and large and professional) were used consistently and were deployed in the same two locations (100 m & 200 m; eight traps in total at each site comprising a 'standard set') from one accessible bank at each site (Figures 2.3, 2.4 & 2.5) from 2010 to 2012. Soak time varied between years with traps left in the water for 24 hrs (± 5) in 2010 and 2012 and 48 hrs (± 5) in 2011. Acosta and Perry (2000) found that catches only declined after the soak time exceeded 72 hours. Soak time variation between years is not considered to affect catch as it did not exceed 72 hours in 2010, 2011 or 2012.

### 2.2.2 Trap variation, and the number of traps used in 2010 & 2011

In 2010 minnow small traps were set in arrays of four with the addition of hay as a refuge material (termed minnow small extras) (eight at each site). These were located away from traps with larger apertures, in shallow marginal areas with high habitat complexity. In 2011 standard trap arrays were supplemented with alternate pairs of traps (one minnow medium and a Trapman or Pirat), covering 150 m of riverbank in total. Trapman/ Pirat trap location were alternated between sampling sessions. Twenty six traps were used at each site on each trapping occasion with an additional eight traps at each site (2 x 'standard set' p.24) giving a total of 34 traps at each site on each trapping occasion. Traps were retrieved from the river via the post and twine and individually emptied into plastic trugs (37 litre capacity), containing labels to ensure accurate recording (waterproof paper/ permanent marker pen) for preliminary observation. The trugs had steep, smooth sides to offer no purchase to even large numbers of crayfish whilst the bright pink/ light blue colouration of the trugs enhanced visibility in low ambient light conditions.

### 2.2.3 Crayfish measurement definitions and sex recording

*P. leniusculus* were sampled between 2010 and 2012. All measurements were taken on the right hand side (viewing the crayfish dorsally) to ensure equipment orientation consistency (*Wiha* Dial*Max* vernier calipers accurate to ± 0.1 mm).

The following measurements of crayfish were taken (Figure 2.12):

- CL - Carapace length (rostral apex to the posterior median edge)
- TL - Total length (rostral apex to terminal telson end)
- POCL - Post orbital carapace length
  (posterior eye socket to posterior carapace notch)
- AW - Abdominal width (maximum width of 2$^{nd}$ tail segment).

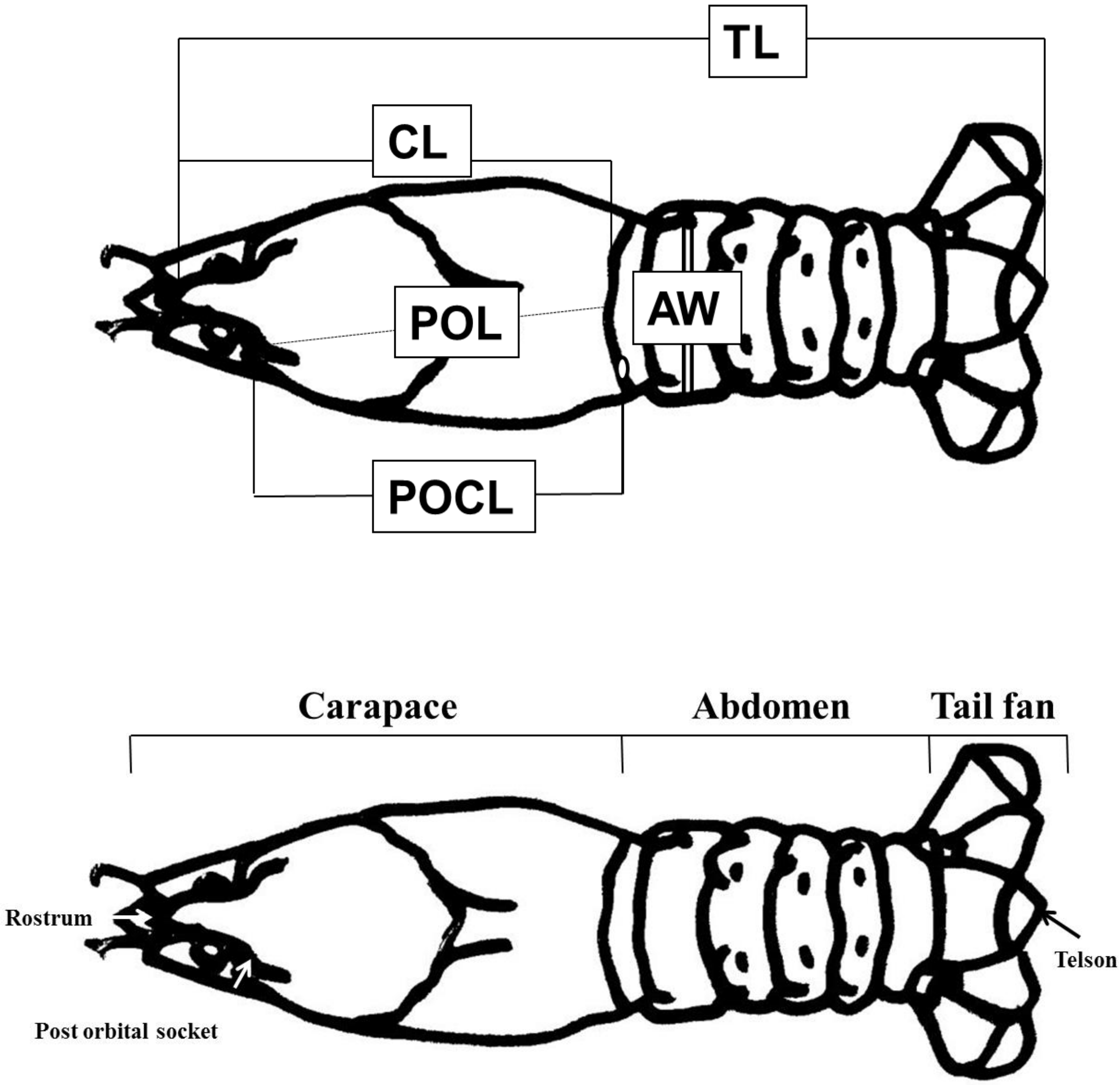



Figure 2.12. Dorsal side of a generalised crayfish (chelae omitted), illustrating the key morphometrical parameters used in this study (Total Length; TL/ Carapace Length; CL/ Post Orbital Carapace Length; POCL/ Abdominal Width; AW) and in the literature (Post Orbital Length; POL/ top diagram) and the terms used to describe the various parts (bottom diagram). NB: The abdomen is sometimes erroneously referred to as the tail, as in “tail meat”. From: Fitzpatrick, 1977.

*P. leniusculus* are sexed with reference to their 1st and 2nd ventral pleopods (Figure 2.13). In males these are lengthened into sub-tubular structures for the transfer of spermatophore (copulatory pleopods; Figure 2.14). Male or female status can only be determined once development has proceeded past a certain size (Holdich, 2002a) as some female crayfish retain vestigial (do not extend to first walking legs) copulatory pleopods.

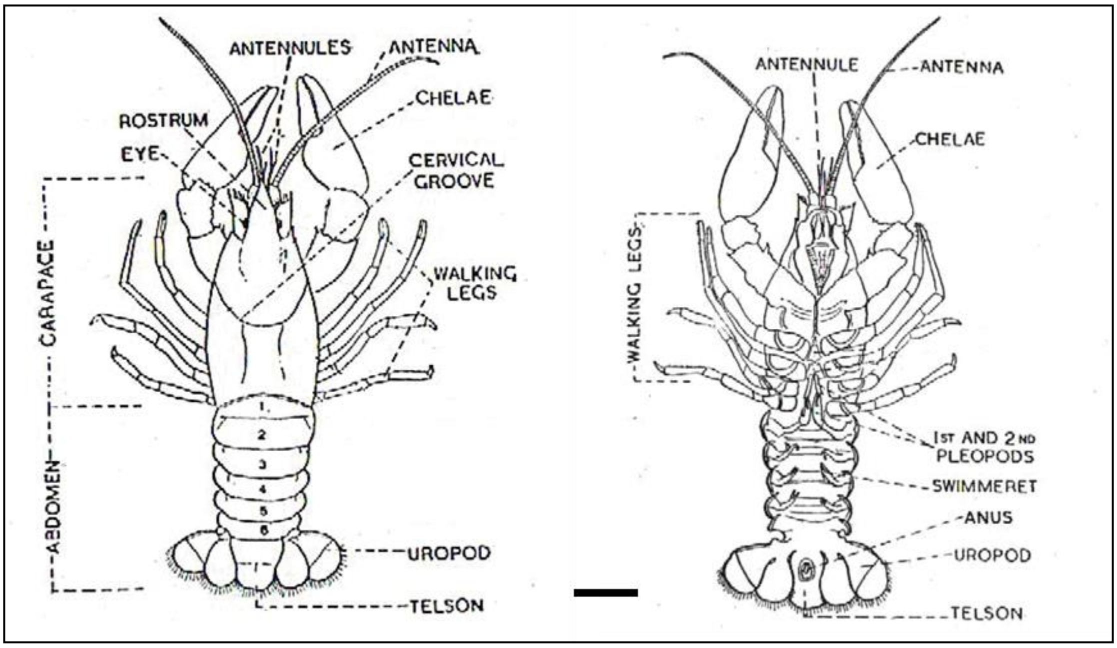



Figure 2.13. Generalised crayfish diagram showing the dorsal (left) and ventral surfaces (right), with male copulatory pleopods (1st and 2nd pleopods) depicted on the right (Scale bar = 20 mm). From: Whitehouse and Grove, 1947.

In this study individuals were classified/ numerically coded as follows:

- Male/ 1 - Copulatory pleopods past first walking legs (Figure 2.14)
- Female/ 2 - No copulatory pleopods (all juveniles recorded as female had no copulatory pleopods (Figure 2.15) or vestigial (recorded as female for adults).
- Unsexed/ 3 - Copulatory pleopods present but insufficiently developed for them to be recorded as male or female.
- Not recorded/ 0 - Individuals whose sex was not recorded.


Figure 2.14. Juvenile male crayfish with copulatory pleopods extending to the first walking legs (arrow depicts terminal limit of copulatory pleopods) defined as male in this study. [Coin = 17 mm ø].


Figure 2.15. Juvenile female crayfish (absence of copulatory pleopods). [Coin = 17 mm ø].

### 2.2.4 Seine netting in September 2012: An active sampling method

Crayfish capture data from seine netting have been included as an example of an unselective active sampling method (no aperture dimension restrictions). Seine netting was carried out by an experienced contractor, with the catch contributing to 'bulk removal' efforts at the end of the study (September 2012). Four sweeps were undertaken at each site working in a downstream direction and covering the 150 m sampling area.

### 2.2.5 Perforated bricks as juvenile refuges: A passive sampling method

In 2011 pairs of perforated bricks one with 18 apertures (P18) and one with 24 apertures (P24) were suspended from cable ties and twine (Figure 2.16) and deployed < 1 metre from the riverbank at 20 m intervals. P18s have a mean aperture diameter of 9.83 ± 1.74 mm; P24 apertures have a mean aperture diameter of 17.24 ± 1.04 mm. Bricks were left for an initial soak time of two weeks with monthly checks subsequently. In 2012, the number of pairs of bricks was doubled, with sets deployed every 10 m and all sampled juveniles tagged and released. Pilot studies using perforated bricks as refuge samplers demonstrated that loss of individuals on removal was unusual as juveniles withdrew into the refuge. Two way access to refugial spaces allowed the bricks to present a number of options to juvenile crayfish regardless or orientation or disruption with horizontal and vertical refuges occupied when observed 'in situ' during snorkelling at Lark Head in 2012.

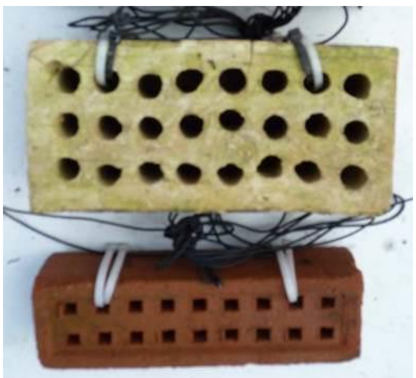


Figure 2.16. Two perforated brick types with differing aperture dimensions used for passive juvenile refuge sampling in 2011 & 2012. Top - P24 (230 x 68 x 110 mm); Bottom - P18 (220 x 50 x 70 mm).

### 2.2.6 Quadrat sampler arrays for juvenile/ adult sampling (passive)

Plastic coated wire quadrats (0.5 $m^2$) were used to support passive juvenile sampling media i.e. pairs of perforated bricks (one P18 & one P24; Figure 2.17a), squares of PVC roofing material (Figure 2.17c, d), a three unit invertebrate colonisation sampler and straw bundle (Figure 2.17a), with the quadrat covered in mesh netting (Figure 2.17b). Supplier details: Appendix B.

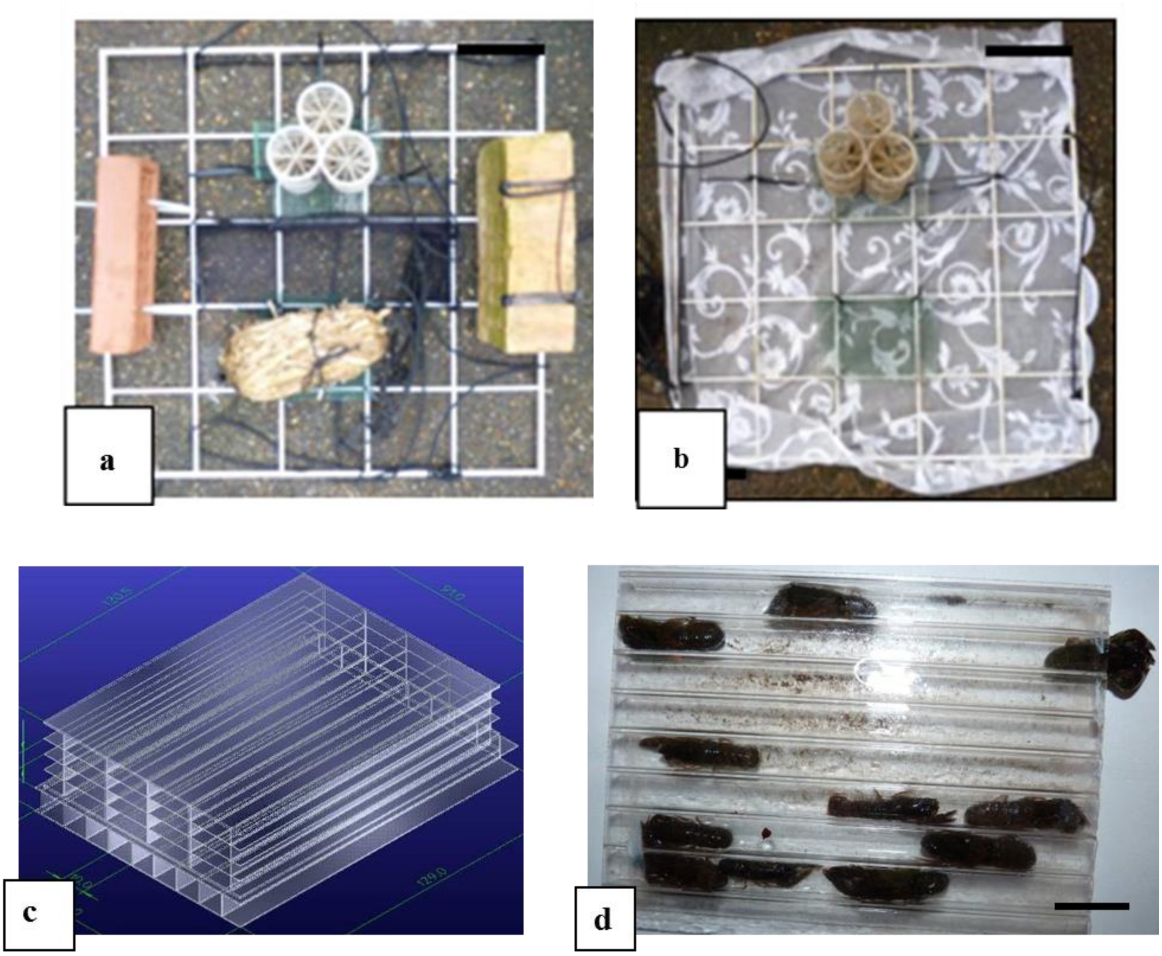



Figure 2.17. Quadrat sampler layouts (Scale bar = 10 mm) – passive sampling media.
a. P18 (left) and P24 (right) including a three unit invertebrate colonisation sampler (top) and straw bundle (bottom).
b. Quadrat with full mesh (500 mm square quadrats).
c. Diagram of squares of roofing material in alternating stacks of four. All sheets 95 x 129 mm with square voids (8.9 x 8.9 mm) and rectangular voids (four layers 6.1 x 8.2 mm).
d. Juvenile *P. leniusculus* (eleven individuals depicted) in square horticultural insulated roofing material during a pilot study.

Quadrat samplers were suspended from twine and placed into shallow areas (< 50 cm deep), with high structural complexity at each site. Samplers were emptied by first placing the apparatus on a light coloured waterproof surface (to aid the detection of escapees), with a team member designated to watch as each quadrat sampler was removed. Individual items of equipment were then detached and placed within separate labelled high sided containers prior to recording. Animals were recorded separately for each item of equipment, with the individuals retained on the mesh recorded as ‘quadrat area’.

### 2.2.7 Sampling methods and equipment in 2010, 2011 & 2012

Juvenile samplers were created in 2011 and used in 2011 & 2012. Juvenile and adult sampling effort was increased in 2012 in order to maximise data collection and potential benefits to the study area. The life stages targeted, and equipment used are summarised (Table II.II).

**Table II.II. Sampling protocols for juvenile and adult crayfish 2010 - 2012.**

<table>
<tr><th rowspan="2"></th><th rowspan="2">Aim</th><th colspan="12">Barton Mills, Lark Head, Plough</th></tr>
<tr><th>Jan</th><th>Feb</th><th>Mar</th><th>Apr</th><th>May</th><th>Jun</th><th>Jul</th><th>Aug</th><th>Sep</th><th>Oct</th><th>Nov</th><th>Dec</th></tr>
<tr><td rowspan="2">2010</td><td>Adults</td><td></td><td></td><td></td><td></td><td></td><td colspan="7">MM, MX, MC, PT</td></tr>
<tr><td>Juveniles</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td colspan="5">MM extras</td></tr>
<tr><td rowspan="2">2011</td><td>Adults</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td colspan="2">MM, MX, MC, PT</td><td></td><td></td><td></td></tr>
<tr><td>Juveniles</td><td></td><td></td><td></td><td colspan="3">Quadrat trials</td><td></td><td colspan="5">Perforated bricks</td></tr>
<tr><td rowspan="2">2012</td><td>Removal</td><td></td><td></td><td></td><td></td><td></td><td colspan="3">MM, MX, MC, PT</td><td>Bulk removal</td><td></td><td></td><td></td></tr>
<tr><td>Juveniles</td><td colspan="6">Quadrats/ Perforated bricks</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
</table>

**Key:** MM = Minnow minnow trap; MX = Expanded minnow trap; MC = Minnow crayfish trap; PT = Professional trap (together these constitute a ‘standard set of traps’ in this study). Bulk removal involved volunteer and professional trappers with data collected contributing to Chapter 4 on differing trap designs and catch.

## 2.3 An introduction to the multivariate statistical techniques used for the analysis of substratum data (Chapter 3) and population size structure data (Chapter 5)

Multivariate statistical techniques are used to identify patterns in data where variables are reduced to a set of derived and condensed values, which are plotted within a multi-dimensional matrix or space (Beaugrand et al., 2003). Ordination and clustering are the principal multivariate techniques both of which are non-parametric and thus make no assumptions about the distribution of data. PCA (Pearson, 1901 cited in Jolliffe, 1986, p.7), is the earliest, and most utlised, ordination technique where variables are reduced to eigenvalues representing either the range of values or variance within the data, which are then plotted on axes respresenting the principal components of signal extraction (Palmer, 2008). Groupings within the data are then identified from scatterplots which can be validated in terms of net signal extraction. Cluster analyses group samples in terms of either shared or not-shared characteristics (Shaw, 2003; Ridley et al., 2010) with the results (displayed as a dendrogram), depicting similar samples closer together/ clustered (Beaugrand et al., 2003). Clustering and ordination techniques have been used together to consider metal contamination in *Procambarus clarkii* (Bugnot and López Greco, 2009), the nutritional condition of fish larvae (Cunha et al., 2003), breeding characteristics in crayfish (Galleotti et al., 2006), cellular analysis (Pinheiro et al., 2003) and the distribution of Antarctic endemics (Pugh and Convey, 2008). Individual multivariate techniques have also been applied to crayfish research, including the impact of abiotic and biotic factors on *Austropotamobius pallipes* management in France (Trouilhe et al., 2003), aggression and serotonin levels in *Astacus astacus* (Huber and Delago, 1998) and the relationship between chelae size and sex in Australian *Cherax dispar* (Wilson et al., 2009), as well as allometry and size in *Cambarus bartonii* (Somers, 1986). Principal components and cluster analyses will be used to analyse substratum samples in Chapter 3, and to complement the analyses of cohort/ age-size classes in Chapter 5.

## 2.4 Legislation and Welfare

It is illegal under Section 14 (1) of The Wildlife and Countryside Act (1981) to "release or to allow to escape into the wild, any animal which is not ordinarily resident in Great Britain", whilst The Import of Live Fish (England and Wales) Act 1980 prohibits the keeping of non-native crayfish in captivity (with some exceptions). The researcher held a current EA trapping of NICS licence, an NE licence allowing tagging and release of NICS (NNR/2010/0016), and an NE native crayfish handling licence (CLS02750). The following welfare protocols were designed to minimise experimental animal stress, to guard against escape (e.g. accidental re-introduction), and to ensure biosecurity (particularly in relation to crayfish plague). The crayfish holding vessels were kept in the shade and covered with black fabric. Splashes of cool water were applied during very hot weather to maintain humidity levels and plastic hides were provided for shelter, and to reduce aggressive interactions (Antonelli, Steele and Skinner, 1999). All equipment was sterilised with Virkon S before and after use as a biosecurity measure in relation to crayfish plague. Virkon S was found to be the most effective of four proprietary agents tested by Jussila et al. (2014).

# CHAPTER 3: DOES SITE-BASED VARIATION INFLUENCE RIVER LARK NON-NATIVE *P. LENIUSCULUS* POPULATIONS?

## 3.1 Introduction

Crayfish thrive in a wide variety of habitats; though need a suitable substratum for burrowing/ refuge (Flint, 1975) with habitat availability potentially the only limiting factor (Holdich et al., 1995). *P. leniusculus* population size and structure on the River Lark may vary between sites as a result of differences in substratum, channel characteristics (flow and depth) or water chemistry. Native crayfish are more sensitive to their environment than invasive non-native species in the UK (Holdich, 2003) and across Europe (Pârvulescu et al., 2011; Svobodová et al., 2012) with gill clogging by fine sediments a particular issue (e.g. Shimizu and Goldman, 1983; Kirjavainen and Westman, 1999; Rosewarne et al., 2014) giving NICS a further competitive edge. However, native crayfish have been demonstrated to have some tolerance to muddy, sandy and/ or silty conditions in the UK (Brickland et al., 2006).

Information on river depth profiles and substratum type may inform observations on crayfish habitat use with adults favouring refuges in riverbanks or amongst submerged tree roots whilst shallow waters with high structural complexity offer potential habitat for vulnerable juveniles (Kutka et al., 1992; Smith et al., 1996; Englund and Krupa, 2000). Such size segregation may help to minimise cannibalism, particularly during moulting/ ecdysis (Ackefors, 1999). The underlying geology of an area and river bed composition have been considered by a number of authors in relation to burrow formation (Grow, 1982; Guan, 2010; Barbaresi et al., 2004), though more recent work has focused on the impact of *P. leniusculus* on habitat rather than crayfish habitat preferences *per se* (Harvey et al., 2011; Roberts, 2011). An individual's microhabitat encompasses depth, substratum and habitat structure together with the available food resources.

Juvenile crayfish show a preference for shallow margins with high levels of organic matter/ structural complexity and vegetation (Momot, 1967; Gherardi et al., 2001), with other decapod crustaceans (e.g. juvenile *Panulirus argus*/ spiny lobsters) also favouring refuges with food value (Marx and Herrnkind, 1985). Juvenile NICS in the UK are not noted for their burrowing though adult crayfish activity alters habitat structure (Shimizu and Goldman, 1983; Hogger, 1986; Guan, 2010; Figure 3.1). In their native North America *P. leniusculus* as a species are not described as 'burrowers'. However, in the UK, their need to find and/ or excavate burrows results in high levels of erosion when populations are large. Substrata mobilised by burrowing NICS affects riverine sediment dynamics (Harvey et al., 2011; Roberts, 2011) with a range of crayfish movements (e.g. tail flipping/ fighting) having an influence on sediment distribution (Statzner, Gore and Resh, 1988; Holdich, 2002b; Zulandt et al., 2008; Parkyn et al., 2011). Foraging activity similarly increases the availability of fine sediment (Creed and Reed, 2004) leading to increased water turbidity and reduced macrophyte primary production (Wood and Armitage, 1997; Owens et al., 2005). The range of interactions taking place in rivers occupied by abundant *P. leniusculus* populations were described by Harvey et al. in 2011 (Figure 3.1).

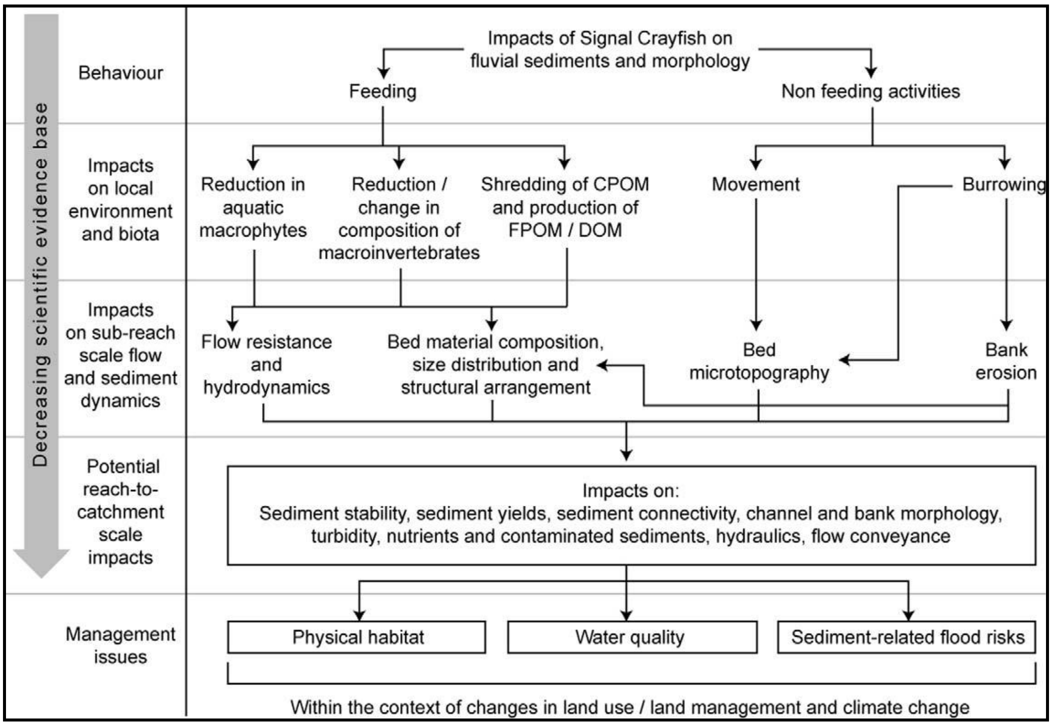



Figure 3.1. Conceptual model of the impacts of crayfish on the physical structure of river systems from the micro scale to the catchment scale (From: Harvey et al., 2011).
Key: CPOM/ FPOM/ DOM - Coarse/ Fine and Dissolved organic matter.

The greatest crayfish impact on sedimentation load in dense populations is via burrowing. Abundant NICS burrows can lead to extensive erosion, sediment mobilisation and habitat modification (Guan and Wiles, 1997). Burrows can range from simple voids to complex interconnected matrices up to 2 m deep, with crayfish often excavating more than one burrow (Freeman et al., 2010; Guan, 2010). Burrow densities of up to twenty excavations per linear metre have been recorded, which can result in honeycombed networks that feel ‘spongy’ underfoot when saturated (Harvey et al., 2011). If other stressors are added bank collapses can follow (Sibley, 2000).

Crayfish can also mobilise sediment in rivers through their feeding behaviour. By removing algal cover they mobilise sediment (Statzner et al., 2006). This can exacerbate plant smothering effects (Wood and Armitage, 1997), degrading fish breeding and spawning areas (Peay and Hiley, 2004; Harvey et al., 2011) and affecting fish embryo survival (Newcombe and Macdonald, 1991; Rehg et al., 2005). Increasing sediment loads can also increase pollutant concentrations leading to eutrophication and raised algal production resulting in further ecosystem degradation (Likens et al., 1971; Owens et al., 2005).

Flow/ velocity will affect crayfish movement as they must work against any current to maintain their position. Downstream crayfish movement was considered to exceed that of upstream travel (Peay and Rogers, 1999), with passive movements unusual though extreme flood events may cause individuals to be washed out of their refuges/ burrows. However, hypotheses expounded in the last twenty years focus on 'home range' (Guan, 1997), with large scale movements (both upstream and downstream) recorded (Bubb et al., 2002; Bubb et al., 2006). The effect of flow/ velocity over time can be examined via the analysis of bottom sediment/ substratum which provides a historical record of flows prior to the last flood event (Cutler and Malmqvist, 1998; Davie, 2003). Water temperature determines crayfish activity levels, breeding behaviour and growth (Somers and Green, 1993; Litvan et al., 2010), with activity ceasing when water temperatures drop below 8˚C (Harris, 1999).

High water temperatures decrease the availability of oxygen whilst increasing biological oxygen demand (BOD). As *P. leniusculus* have a comparatively high demand for oxygen low levels can lead to heavy mortalities (Avault et al., 1975; Ackefors, 1998). Oxygen availability may be limiting in some situations though crayfish species (including *P. leniusculus*) are able to assimilate atmospheric oxygen so can move onto land if, and when, conditions deteriorate (Holdich et al., 2014). There is a correlation between sluggish rivers, high organic matter and low oxygen, as minimum turbulence offers low oxygen replacement whilst bacteria acting on the organic matter consume oxygen during respiration. Conversely, fast flowing water, preferred by these large invertebrates (Extence et al., 2011), offers greater turbulence and higher oxygen content.

Calcium, in the form of carbonate and bicarbonate salts, is essential for crayfish exoskeleton development and replacement and therefore moulting and growth, with pH affecting calcium availability (Borgstrom and Hendrey, 1976 cited in Haddaway et al., 2013, p.54). The optimal pH range is 6.5 to 8 (Ackefors, 2000), with cuticle thickness affected by pH in *A. pallipes* (Haddaway et al., 2013). Soluble calcium is absorbed from the aquatic environment by crayfish and stored in gastroliths (paired temporary bodies located in the abdomen) to be mobilised post-moult. This storage process is unaffected by size, sex (or sexual maturity), photoperiod, or the presence of branchiobdellids/ crayfish worms (Adegboye et al., 1978a, b). Calcium concentrations were not found to affect distribution in a Europe wide study on native and non-native crayfish (Svobodová et al., 2012). The existence of large populations of *P. leniusculus* in the River Lark suggests that calcium concentrations, and therefore pH levels, are not limiting. In this study pH is measured as its level can be accurately determined in the field and will provide an index of the variability encountered at the three River Lark study sites.

## 3.2 Method

Information relating to the measurement of environmental variables is contained within this section with more general site information included in Chapter 2: General Methods. Three sampling sites on the River Lark were studied to determine the influence, if any, of bottom substratum composition, channel characteristics, BOD, pH, conductivity and temperature on crayfish distribution.

### 3.2.1 Substratum sampling

Substratum samples were collected (as described below) from the bankside and from river transects (Figure 3.2) at three sites on the River Lark in June 2013.

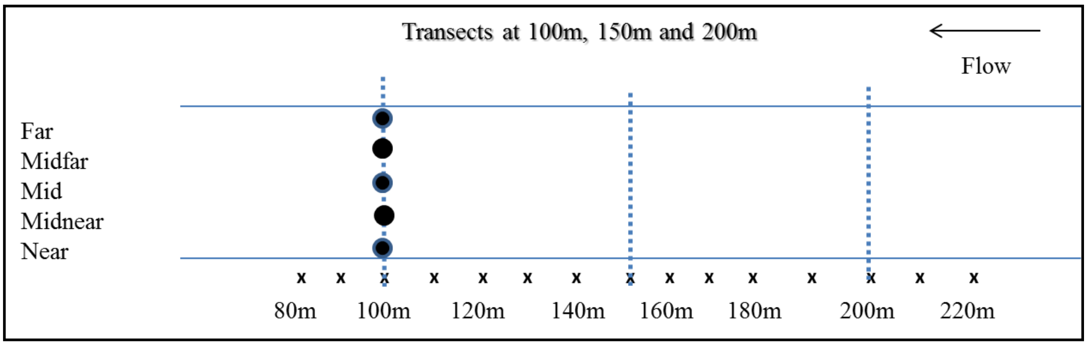



Figure 3.2. Diagrammatic representation of the location of the three equidistant transects ● (100, 150 & 200 m), and bankside samples = x at ten metre intervals (80 to 220 m). The nomenclature used to describe transect locations is listed on the left of the diagram.

### 3.2.2 Bankside substratum cores

Cores were taken using a stainless steel 'angler's' bait pump (48 mm diameter) from close to the water's edge to a depth of c.750 mm. Cores were subdivided to obtain samples from the substrata/ river interface that *P. leniusculus* inhabits, which were transferred directly into screw-topped plastic cylinders (50 mm diameter, 100 mm height/ c.200 $cm^3$ volume). In the laboratory (within < 4 hours) samples were deposited into individually weighed metal tins (Denver Instruments TP-1502, accuracy ± 0.0 g), and oven dried at 105 °C for 20 hours.

### 3.2.3 Transect samples

Three transects were carried out at each site (at 100, 150 and 200 m) using an Ekman dredge (Duncan and Associates, Cumbria; Figure 3.3). Five grab samples per transect (referred to as near, nearmid, mid, midfar and far to denote proximity to the sampling bank; Figure 3.2) were collected using a boat operated by two professional trappers.

Figure 3.3. Ekman dredge (grab elements primed). Release of the grab elements to collect a substratum sample is achieved via the rapid deployment of a brass 'messenger' (weight) along the shot line (protruding from the top of the dredge). Total apparatus ca. 0.15 $m^2$.

Five substratum grabs were taken across each transect at each of the three study sites (Figure 3.2). Samples were placed in a plastic trug for visual analysis, with a bait pump then used to remove a sub-sample, which was placed into a screw-topped plastic cylinder to be processed alongside bank substratum cores.

### 3.2.4 Substratum grain analysis

After oven drying samples were agitated with glass spheres (15 mm diameter), then rolled with a 15 mm cylindrical bar to break up aggregations, with care taken not break up the particles themselves. Phi (Φ) describes the mesh size (and therefore particle size) that retains a particular sediment fraction ($\Phi = -\log_2$ mesh in millimetres), and forms the basis for the Wentworth scale of sediment classification (Wentworth, 1922; Table III.I).

Table III.I. Sieve mesh diameter and equivalent Phi Φ values.

| Mesh diameter | Phi (Φ) |
|---|---|
| 4.00 mm | -2 |
| 3.35 mm | -1.675 |
| 2.00 mm | -1 |
| 1.00 mm | 0 |
| 500 µm | 1 |
| 250 µm | 2 |
| 180 µm | 2.47 |
| 63 µm | 4 |
| pan | 7 |

Samples were placed in an Endecott test sieve series comprising 1 mm, 500 µm, 250 µm, 180 µm, 63 µm sieves and a bottom pan. The test sieve series were placed on a mechanical sieve shaker for 10 minutes. The sample retained by the top 1mm sieve fraction was then re-sieved using 3.35 mm & 2 mm & 1 mm sieves. Any small pebbles (< 4 mm) and gross sample characteristics (plant material, shell etc.) or aggregations were recorded.

NB: A 180 µm sieve was used instead of a 125 µm sieve in these analyses due to equipment availability. As a comparison of substratum was the aim the lack of a precisely stepped sieved series for the smaller particle sizes was not considered an issue.

The following calculations and analyses follow the method of Fernandes and Tett (2001):

Cumulative percentage mass: The mass of each sieve fraction was divided by the total mass and expressed as a percentage to determine $\Phi_{50}$ (median phi value).

The degree of scatter describes the uniformity or homogeneity of the sediment and is calculated using the equation:

$$\sigma = (\Phi_{84} - \Phi_{16})/4 + (\Phi_{95} – \Phi_{5})/6.6$$

The degree of symmetry/ skew in each sample describes the tendency towards fine or coarse particles with a normal distribution termed ‘symmetrical’. Skewness is calculated using the equation Sk = A + B where:

$$A = (\Phi_{16} – \Phi_{84} -2\Phi_{50})/ 2(\Phi_{84} – \Phi_{16}); B = (\Phi_{5} + \Phi_{95} -2\Phi_{50})/ (2(\Phi_{95} – \Phi_{5})$$

Kurtosis is considered in relation to the percentage frequency distribution of particle sizes and the degree of variation from a normal/ mesokurtic distribution with platykurtic (very flattened; low KG) or leptokurtic (very peaked; high KG) distributions determined via KG values calculated using the equation:

$$KG = (\Phi_{90} – \Phi_{5}/ 2.44 (\Phi_{75} – \Phi_{25}) \text{ brackets}$$

[Phi values were obtained by plotting cumulative frequency curves (Figures 3.6 & 3.7) and reading off cumulative percentages from the y-axis. Interpretive tables are included in Appendix C]

### 3.2.5 Depth profiles

Depth profiles were generated by the EA using WinRiver II software, with data collected using a Stream Pro boat (July 2013; Figure 3.4).

### 3.2.6 Vegetation assessments

A species list of bankside and emergent vegetation was recorded to further compare sampling sites with plant identification confirmed by EA personnel and field guides e.g. “River Plants: The macrophytic vegetation of waterways” (Haslem, 1978) and “Vegetational communities of British Rivers” (Holmes et al., 1999).

### 3.2.7 The measurement of river channel characteristics

Measurements of velocity, discharge rate and channel dimensions were taken by the EA Hydrometry and Telemetry team using a Teledyne RD Instruments Stream Pro Acoustic Doppler Current Profiler (ADCP; 27.3.2013), manually pulled across transects at 100m, 150m and 200m at each site.  The Stream Pro (Figure 3.4) estimates velocity by splitting the water into vertical columns and combining depth and channel width dimensions to calculate velocity in ‘bins’ for each vertical strip.  The depth range for this instrument is between 0.1 and 7 metres (1% accuracy in depth calculation in uniform temperature profiles), with water velocity calculated to ± 2 mm/s.  Accuracy is increased via the use of repeat runs and trained experienced operators with equipment costs necessitating the inclusion of EA personnel though the researcher organised and oversaw all data collection.

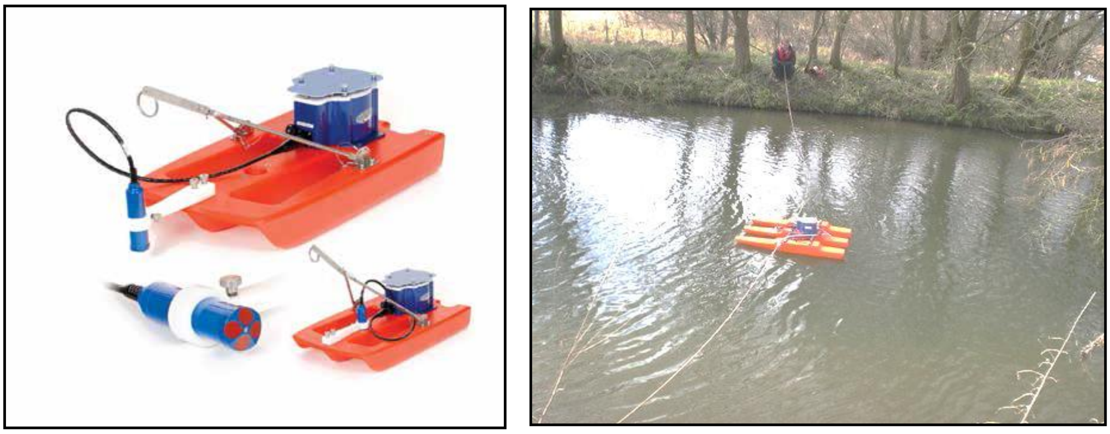


Figure 3.4. Stream Pro boat diagram (left), and on the River Lark (right).

A portable YSI 556 Multi Probe System (MPS on loan from the EA; Figure 3.5) recorded pH, water temperature and biological oxygen demand (BOD) on 23 January 2013. Readings were taken at 10 m intervals (from 80 - 220 m) at each sampling site (Figures 2.2, 2.3 & 2.4). The instrument was calibrated using the manufacturer's reference solutions.  Accuracy: Temperature ± 0.15 °C / pH ± 0.2 units / BOD to ± 2 % of the reading or air saturation.

Figure 3.5. Portable YSI 556 Multi probe system (MPS).

### 3.2.8 Substratum analyses – Wentworth scale

Substratum analyses were conducted using the multivariate techniques (Section 2.3) of ordination (Principal Components Analysis: PCA) and classification (Cluster analysis), with scatterplots and dendrograms plotted to aid analysis (Tabachnick and Fidell, 2007). River channel characteristics were compared within each site via histograms and standard deviations.  Due to the small sample size (n = 3 at each site) within site analysis were inappropriate, though between site comparisons (n = 15) were made via Kruskal-Wallis ANOVA for river channel characteristics and the mean abiotic variables of pH, temperature and BOD.  Wentworth scale analyses that follow rely on equations derived by Fernandes and Tett (2001) which are included in Appendix C.

## 3.3 Results

### 3.3.1 Site based variation in bankside and in channel substrata

Bankside and transect samples (15 of each at each site) were collected at Barton Mills, Lark Head and the Plough. All were sand based (medium to coarse/ very coarse) and poorly or very poorly sorted, with a skew towards fine particles at Barton Mills/ Plough, whilst Lark Head samples were either fine or coarse skewed. All samples (except Barton Mills transect) had a very platykurtic/ flattened particle size distribution (Table III.II).

Table III.II. Sediment grain analysis (bankside, 80-220 mm/ transect samples), for three River Lark sites detailing median phi, sediment type, degree of scatter/ sorting (top table), and symmetry and kurtosis (bottom table).

| Site | Sampling location | Median $\phi_{50}$ | Sediment type | Degree of scatter/ sorting | |
|---|---|---|---|---|---|
| **Barton Mills** | **80-220 m** | 0.90 | coarse sand | 1.43 | poorly sorted |
| | **Transects** | -0.50 | very coarse sand | 1.12 | poorly sorted |
| **Lark Head** | **80-220 m** | 1.35 | medium sand | 2.06 | very poorly sorted |
| | **Transects** | 1.15 | medium sand | 2.08 | very poorly sorted |
| **Plough** | **80-220 m** | 0.25 | coarse sand | 1.68 | poorly sorted |
| | **Transects** | 0.80 | coarse sand | 1.55 | poorly sorted |

| Site | Sampling location | Measure of degree of symmetry | | Kurtosis | |
|---|---|---|---|---|---|
| **Barton Mills** | **80-220 m** | 1.43 | strongly skewed towards fine particles | 0.45 | very platykurtic |
| | **Transects** | 1.12 | strongly skewed towards fine particles | 0.92 | mesokurtic |
| **Lark Head** | **80-220 m** | 2.06 | fine skewed | 0.57 | very platykurtic |
| | **Transects** | 2.08 | coarse skewed | 0.57 | very platykurtic |
| **Plough** | **80-220 m** | 1.68 | strongly skewed towards fine particles | 0.50 | very platykurtic |
| | **Transects** | 1.55 | strongly skewed towards fine particles | 0.52 | very platykurtic |

### 3.3.2 Particle size distribution

The distributions of particle sizes are similar at each site, though cumulative frequency curves for bankside (Figure 3.6) and transect (Figure 3.7) samples demonstrate that Barton Mills has the largest proportion of the two biggest particle sizes (granules). Both Barton Mills and the Plough had substrata that varied across the sampling area, with some sampling points on cobble/ granule, whilst others consisted of sand/ fine silt. Overall there was less substratum variation at Lark Head, suggesting a more homogeneous substratum type at this site.

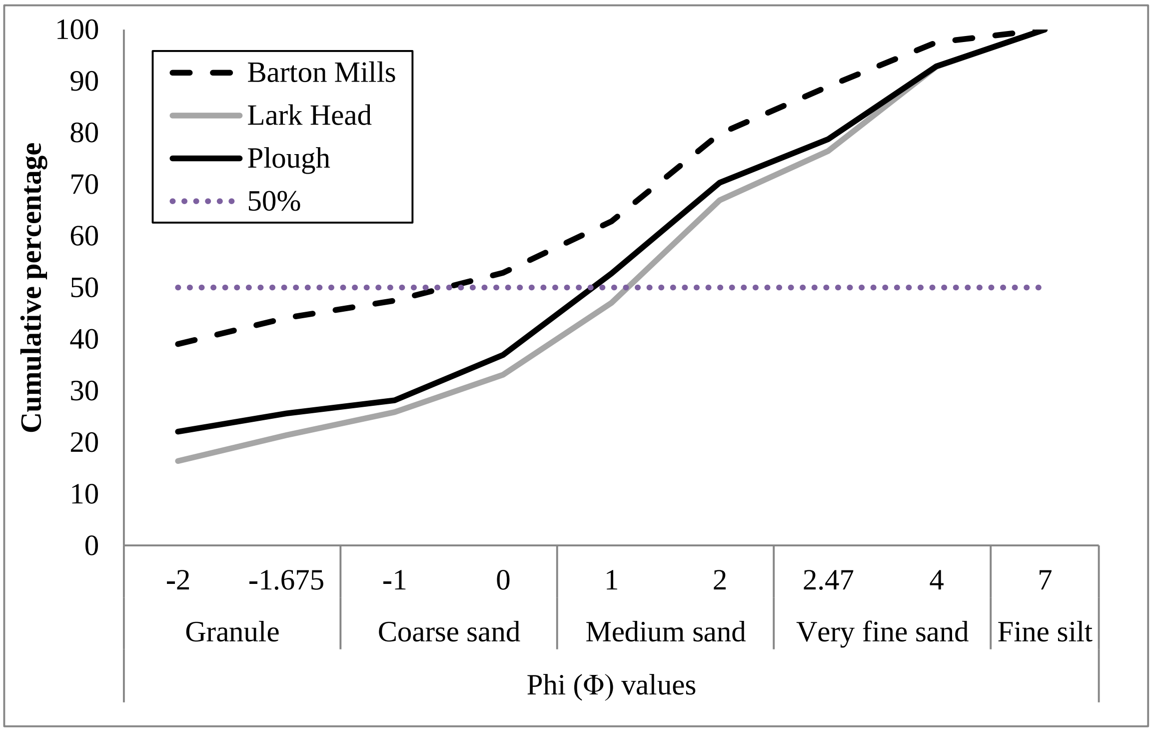



Figure 3.6. Sediment analysis - Bankside samples: Cumulative percentages of phi elements at three sites on the River Lark with median phi ($\Phi_{50}$/ 50 % values) marked.

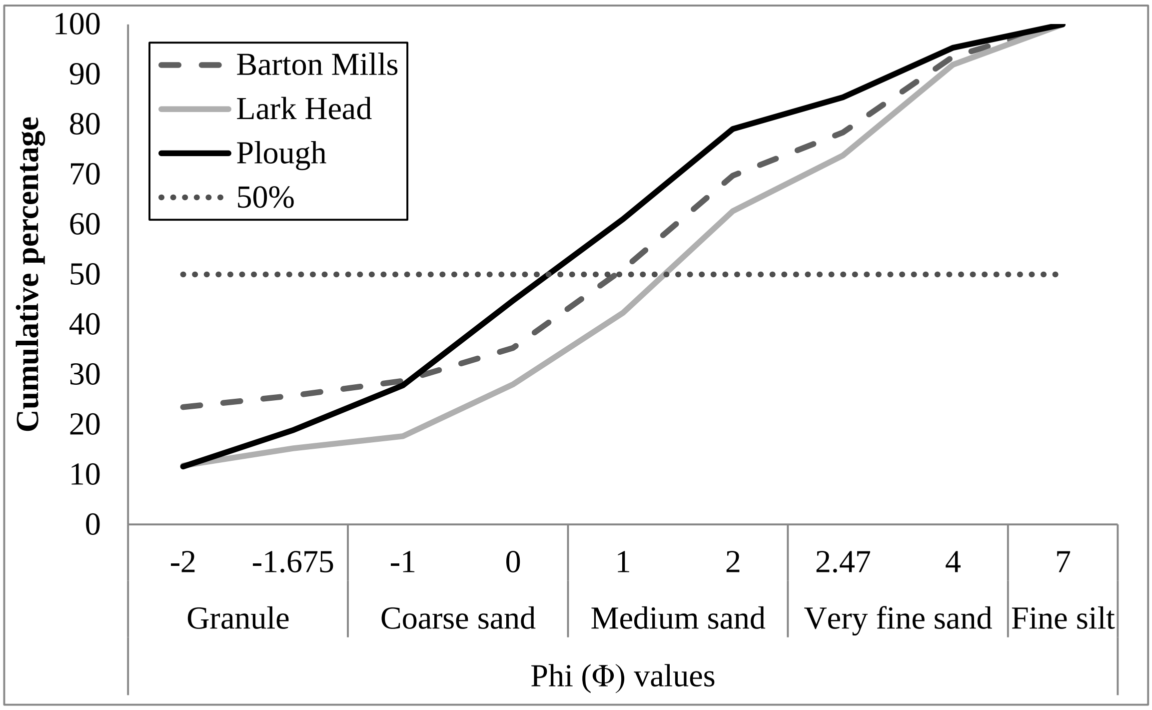



Figure 3.7. Sediment analysis-Transect samples: Cumulative percentages of phi elements at three sites on the River Lark with ($\Phi_{50}$/ 50 % values) marked.

### 3.3.3 Multivariate analysis of substratum samples

Principal Components Analysis (PCA) of bankside and transect sample data showed no substantial grouping of site substratum samples for either 'bankside' (Figures 3.8) or 'transect' samples (Figure 3.9). Data was constrained across three axes (though figures only depict the two principal axes). Bankside samples (Figure 3.8) showed some site similarity with two sampling points at Barton Mills (80/ 90 m) and six at the Plough (80/ 110/ 120/ 130/ 190/ 210 m), showing some correlation/ co-variance. Transect samples (Figure 3.9) showed no appreciable co-variance with analyses performed on standardised data thus eliminating the possibility of an artefact related pattern. Cluster analysis (described later) was also carried out on these data to further check for substratum patterns at each site. Substratum sample variation was described by high eigenvalues on axis 1 in both cases. A large proportion of the variation (bankside c.79 %; transect c.74%) was attributed to axes 1 (Table III.III). Visual analysis of the PCA plots reveals a high level of homogeneity in substratum samples from Lark Head, whilst Barton Mills and the Plough show more variation between sampling points, though all three sites have samples that fit within the same broad range.

Table III.III. Data matrix for PCA of bankside and transect substratum samples.

| **Bankside** | **Axis 1** | **Axis 2** | **Axis 3** | **Axis 4** |
|---|---|---|---|---|
| **Eigenvalues** | 25.40 | 2.82 | 1.80 | NA |
| **Percentage** | 79.41 | 8.81 | 5.62 | NA |
| **Transect** | **Axis 1** | **Axis 2** | **Axis 3** | **Axis 4** |
| **Eigenvalues** | 17.98 | 2.35 | 1.63 | 1.28 |
| **Percentage** | 73.85 | 9.67 | 6.68 | 5.26 |

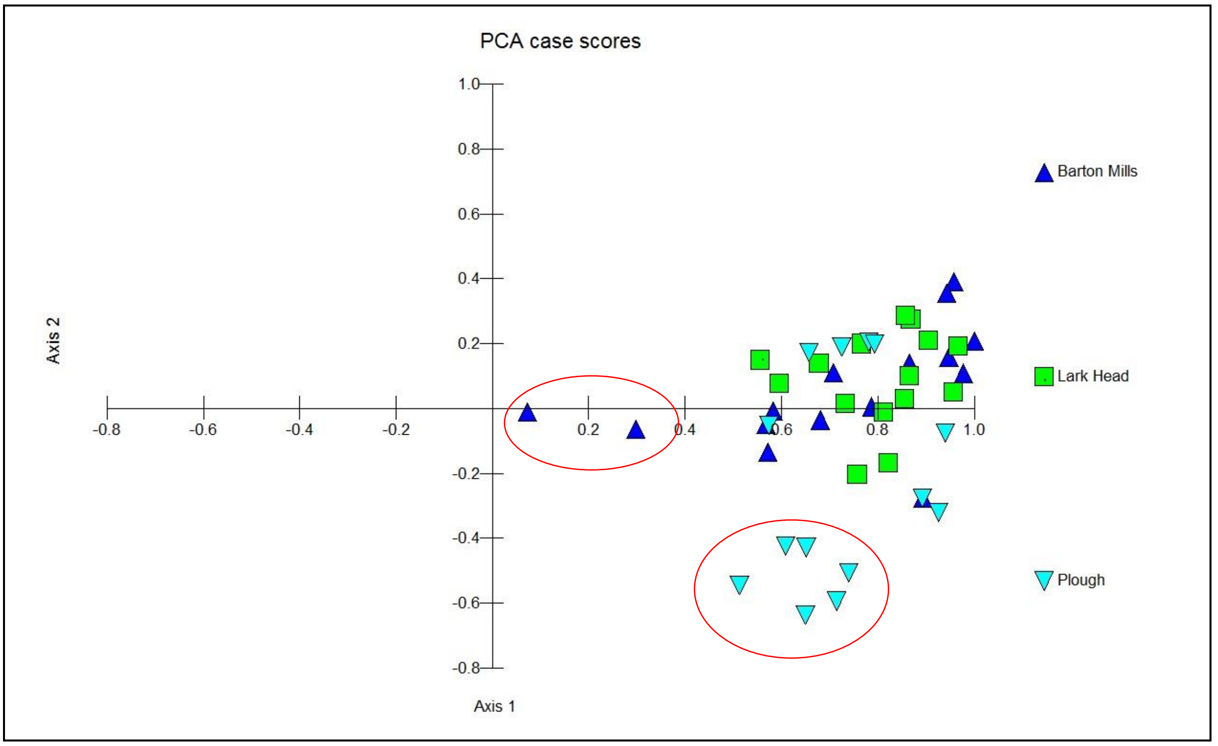



Figure 3.8. Principal Components Analysis of bankside samples at three River Lark sites. NB: Red circles indicate minor groupings in Barton Mills and Plough samples.

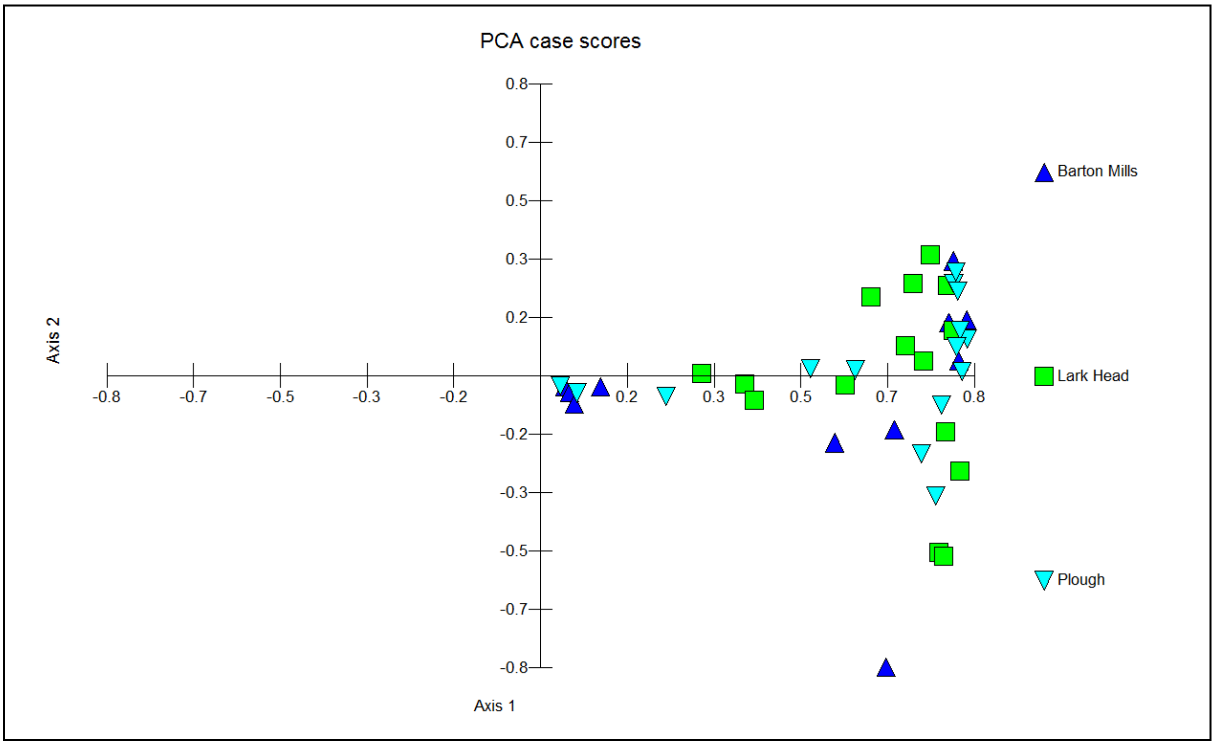



Figure 3.9. Principal Components Analysis of transect samples at three River Lark sites.

The proportion of the different substratum fractions (classified by sieve size; Table 3) for transect samples at each site differed significantly for some particle sizes (Table III.IV).

Table III.IV. Kruskal-Wallis ANOVA results for transect substratum data at three River Lark sites (n = 3 at Barton Mills, Lark Head, Plough).

| | $X^2_2$ = | P = | Sig @ 0.05 |
|---|---|---|---|
| **Pebble/cobble** | 0.22 | 0.897 | ns |
| **35 mm** | 6.15 | 0.046 | * |
| **2 mm** | 3.15 | 0.207 | ns |
| **1 mm** | 7.27 | 0.026 | * |
| **500 µm** | 6.90 | 0.032 | * |
| **250 µm** | 2.34 | 0.311 | ns |
| **180 µm** | 4.34 | 0.114 | ns |
| **63 µm** | 10.33 | 0.006 | * |
| **Pan** | 14.49 | 0.001 | * |

When the data were analysed via clustering and depicted as dendrograms, the three sites did not appear as distinct groups for bankside (Figure 3.10) or transect samples (Figure 3.11). Cluster analyses, in agreement with ordination, demonstrated no clear groupings suggesting that within site variation is similar to between site variation. Dendrograms, in common with PCA plots for banksides, identified two sites at Barton Mills (80/ 90 m; bottom of diagram), and six sites at the Plough (80/110/ 120/ 130/ 190/ 210 m; top of diagram) that were similar. Dendrograms show site similarities via close linkages with only one transect at Barton Mills (100 m transect; Figure 3.11) showing substratum type coherence. When considering substratum at the three sites on the River Lark no overall groupings were apparent.

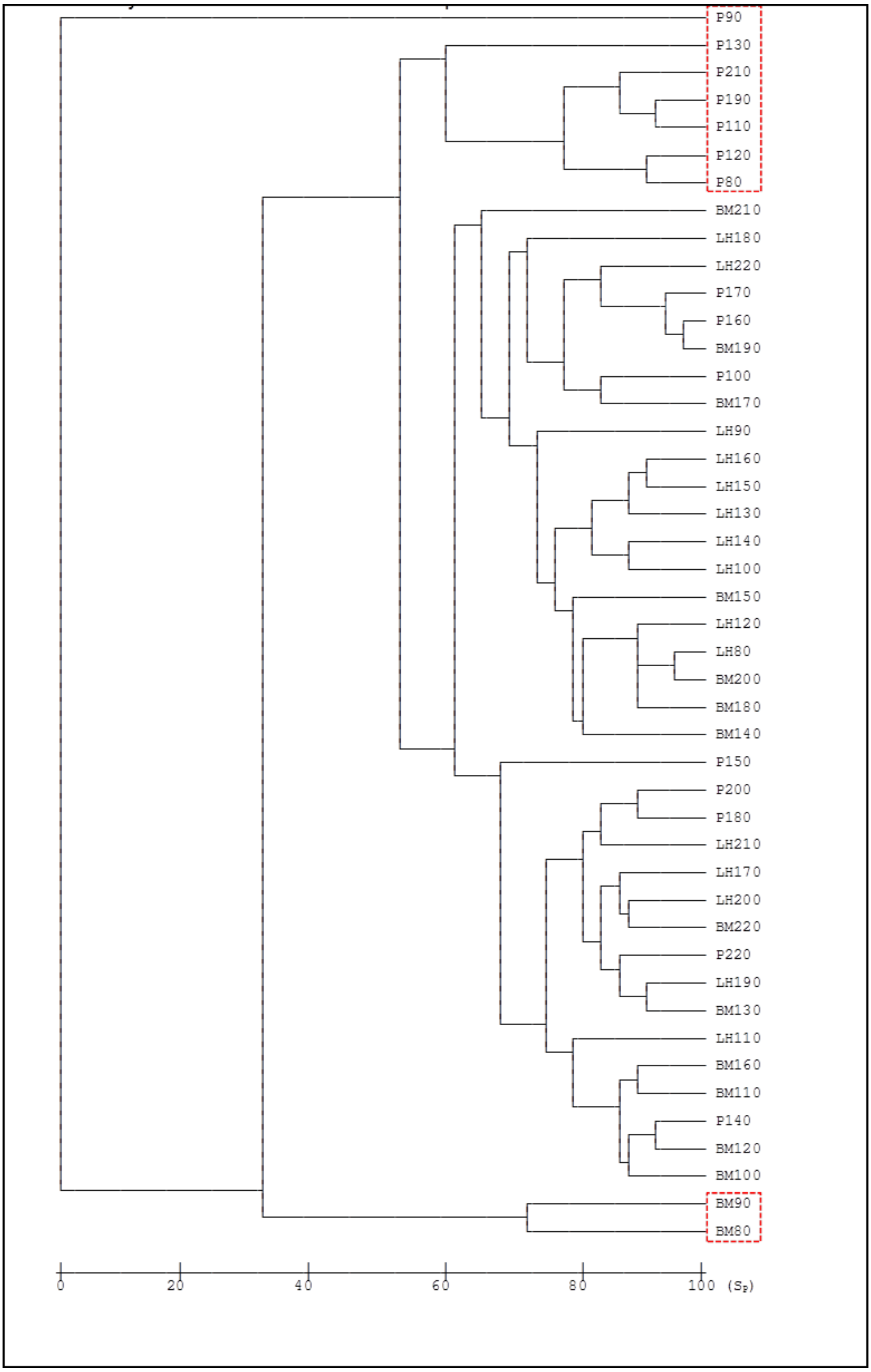



Figure 3.10. Cluster analysis of bankside substratum samples (80 – 220 m) at Barton Mills (BM), Lark Head (LH) and the Plough (P). NB: Red boxes indicate minor groupings of Barton Mills and Plough samples.

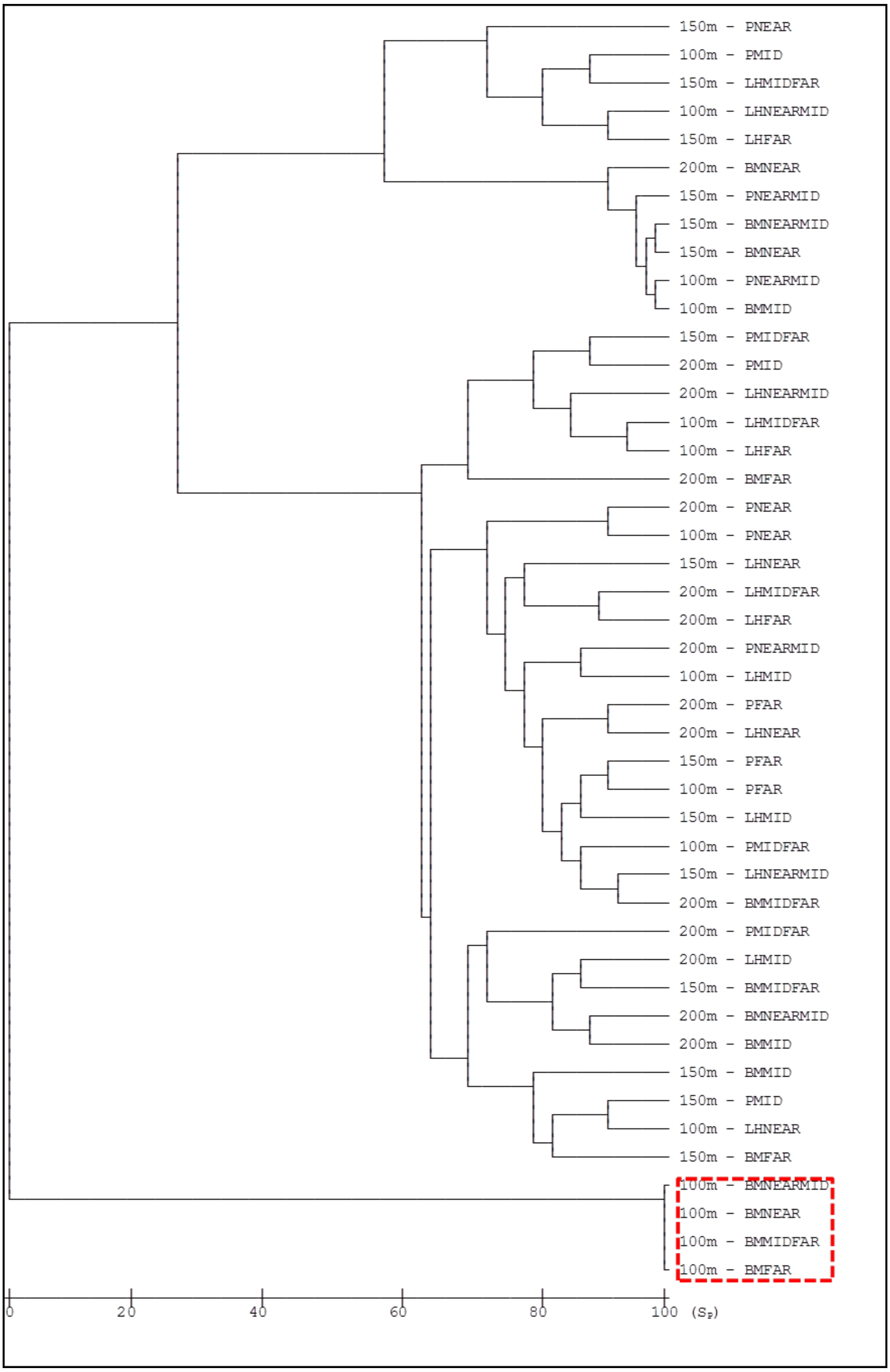



Figure 3.11. Cluster analysis of transect substratum samples at Barton Mills (BM), Lark Head (LH) and the Plough (P).

### 3.3.4 River channel characteristics

Analysis of velocity, depth, discharge, cross-sectional area and width was undertaken to investigate within and between site variations (Figures 3.12 & 3.13). Within sites channel characteristics varied between the three transects, though the standard deviations were small (Table III.V).

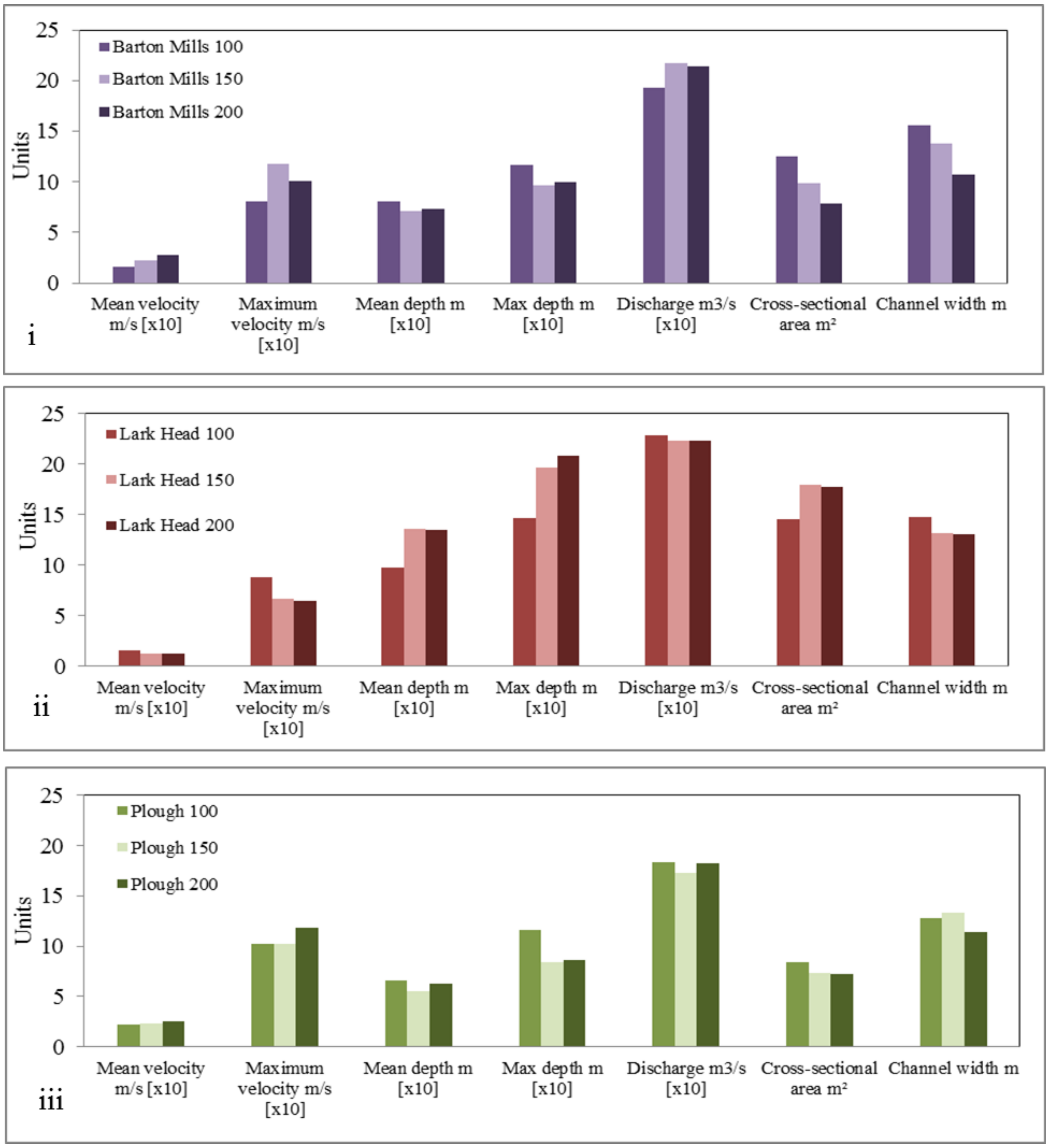



Figure 3.12. River channel/ water characteristics at three locations (velocity, depth, discharge, area/width) at i. Barton Mills, ii. Lark Head and iii. Plough (units multiplied by a factor of 10 where stated).

Table III.V. River channel/ water characteristics (mean and standard deviations) for three transects at each of the three sites on the River Lark, Suffolk.

| Standard deviation of three samples | Mean velocity (m/s) | Max velocity (m/s) | Mean depth (m) | Max depth (m) | Discharge (m3/s) | Cross-sectional area ($m^2$) | Channel width (m) |
|---|---|---|---|---|---|---|---|
| BM | 0.22 ± 0.06 | 1.00 ± 0.02 | 0.75 ± 0.01 | 1.05 ± 0.01 | 2.08 ± 0.01 | 10.07 ± 0.24 | 13.37 ± 0.25 |
| LH | 0.14 ± 0.00 | 0.73 ± 0.01 | 1.23 ± 0.02 | 1.84 ± 0.03 | 2.25 ± 0.00 | 16.70 ± 0.19 | 13.70 ± 0.10 |
| P | 0.24 ± 0.00 | 1.07 ± 0.01 | 0.62 ± 0.01 | 0.96 ± 0.02 | 1.80 ± 0.01 | 7.67 ± 0.06 | 12.50 ± 0.10 |

Kruskal-Wallis ANOVA tests showed no significant variation in velocity, maximum depth or channel width between sites, though mean depth, discharge and cross-sectional area did vary significantly between sites (Table III.VI).

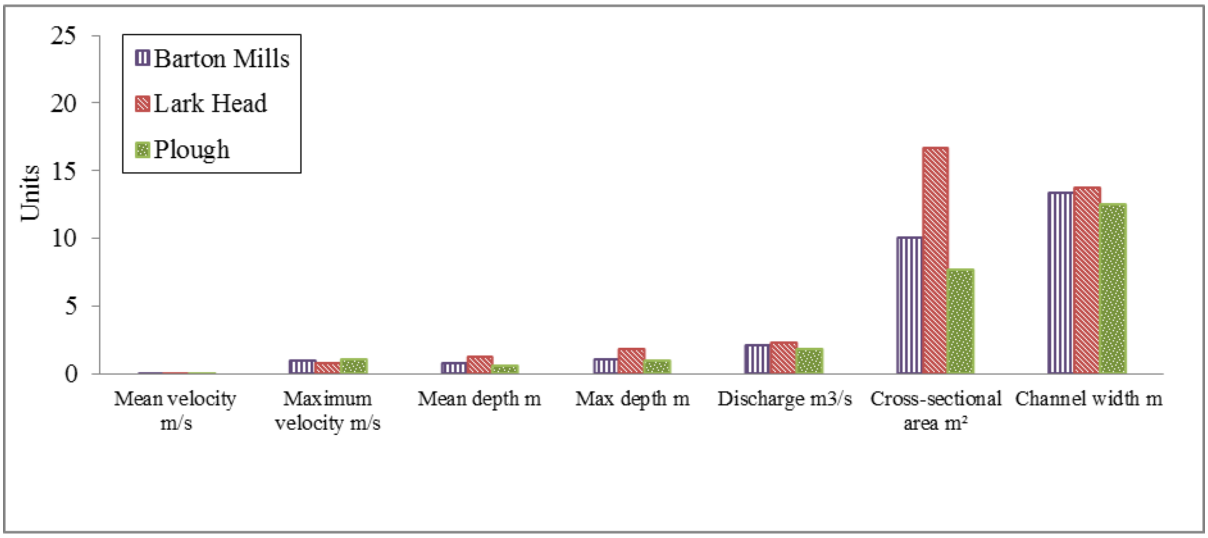



Figure 3.13. Mean river channel/ water characteristics at Barton Mills, Lark Head and the Plough (three transects at each site).

Table III.VI. River characteristics compared via Kruskal Wallis ANOVA (n = 3 for Barton Mills, Lark Head and the Plough).

| | $X^2_2$ = | P = | Sig @ 0.05 |
|---|---|---|---|
| **Mean velocity** | 4.36 | 0.113 | ns |
| **Maximum velocity** | 4.90 | 0.086 | ns |
| **Mean depth** | 7.20 | 0.027 | * |
| **Maximum depth** | 5.96 | 0.051 | ns |
| **Discharge** | 7.26 | 0.027 | * |
| **Cross-sectional area** | 6.49 | 0.039 | * |
| **Channel width** | 1.07 | 0.587 | ns |

### 3.3.5 River depth profiles/ bottom substratum

Depth profiles were generated by the EA using 'WinRiver II' software (Teledyne Instruments) which graphically represent bottom substratum profiles. Data were provided as screenshots and the images manipulated to standardise axial ranges (Figures 3.14, 3.15 & 3.16). Barton Mills has river depths of up to a metre with some variation in the bottom profile (Figure 3.14) though it has a relatively flat profile when compared to Lark Head (Figure 3.15) and the Plough (Figure 3.16). Lark Head has river depths from 1.5 to 2 metres with relatively steep sides and a deep mid channel area (Figure 3.15). The Plough has a maximum depth of one metre with a flattened bottom profile in two of the three transects (Figure 3.16), in common with Barton Mills.

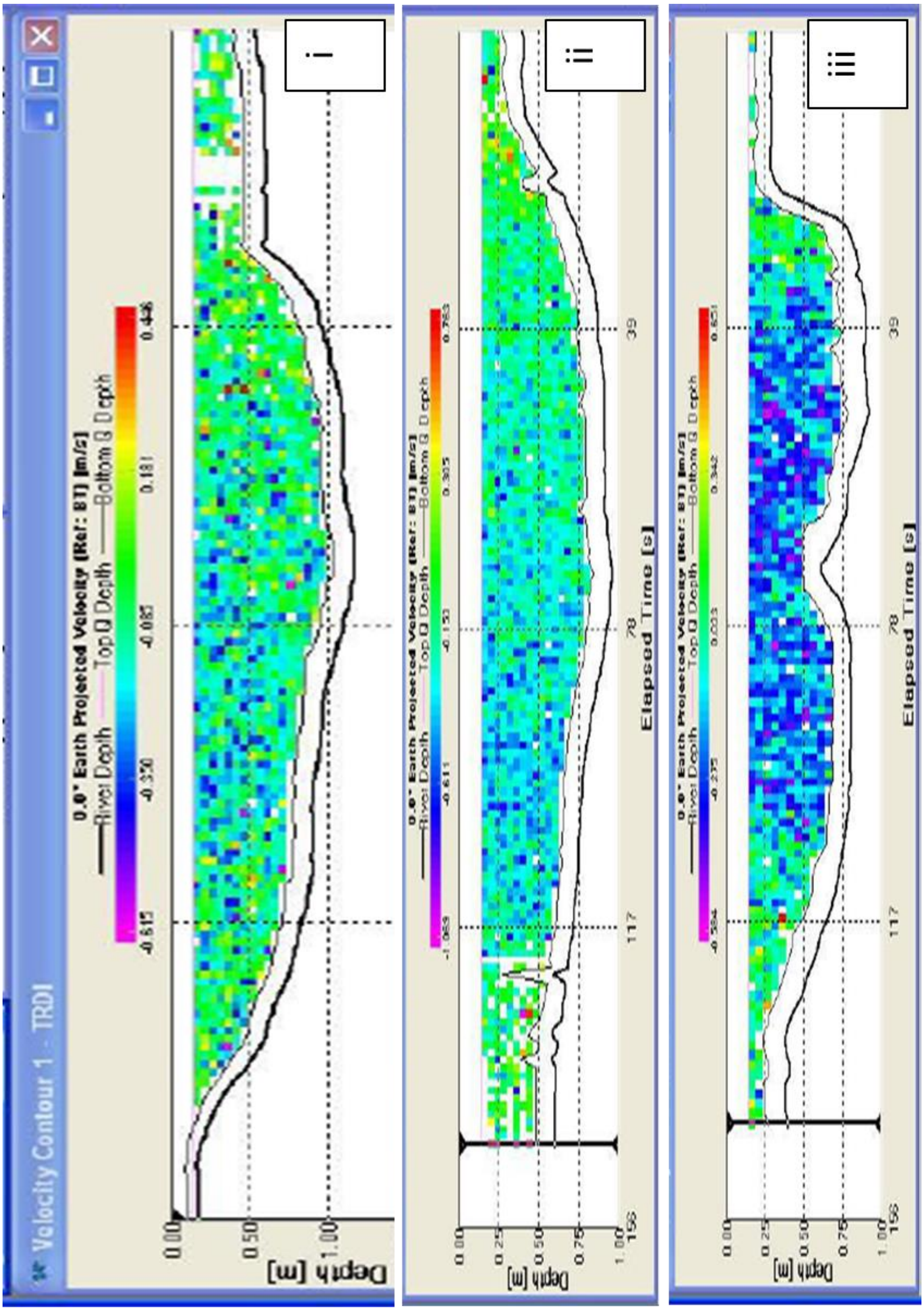



Figure 3.14. Barton Mills river bed profiles (i.100m; ii. 150, iii. 200m).

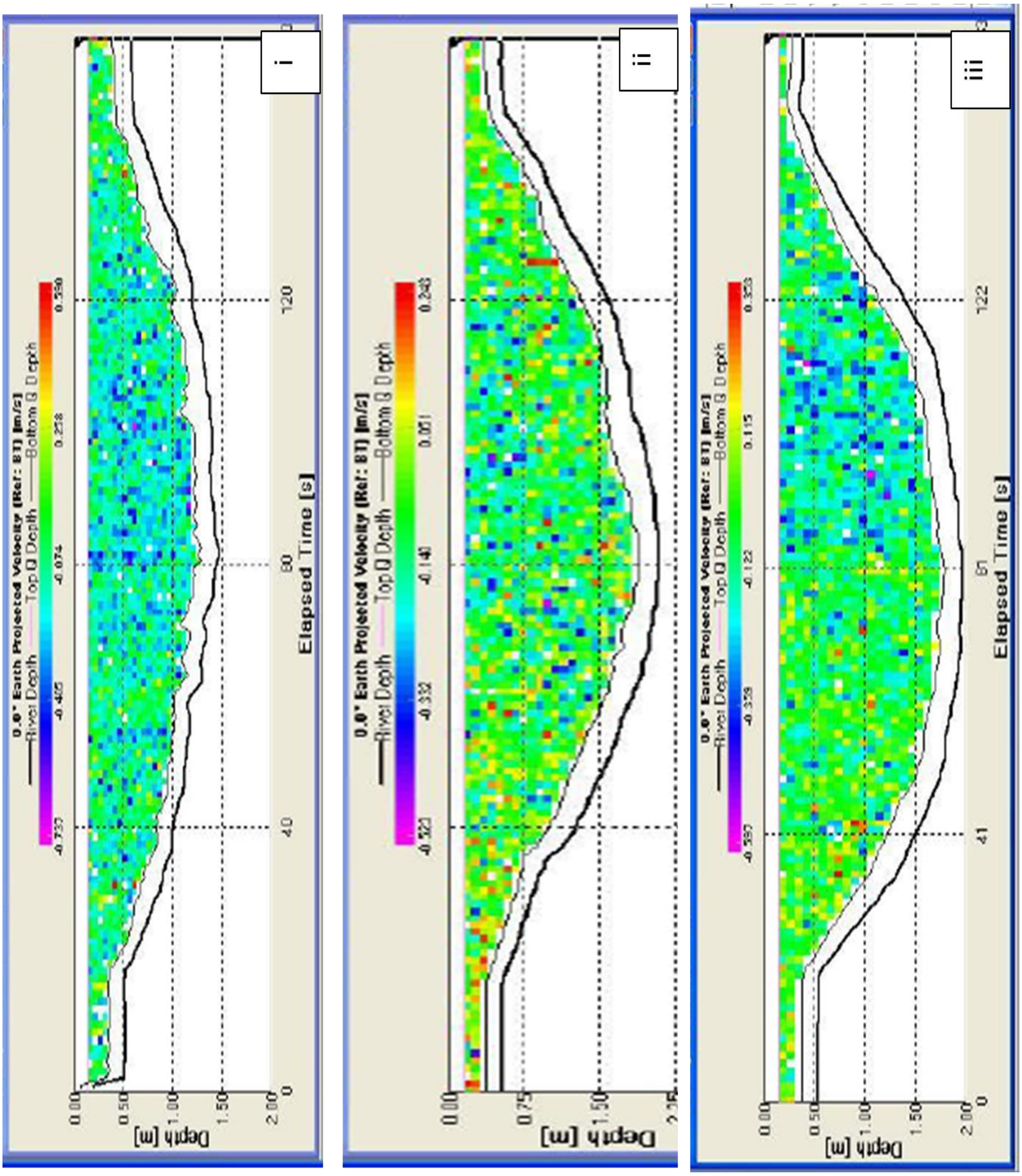



Figure 3.15. Lark Head river bed profiles (i.100m; ii. 150, iii. 200m).

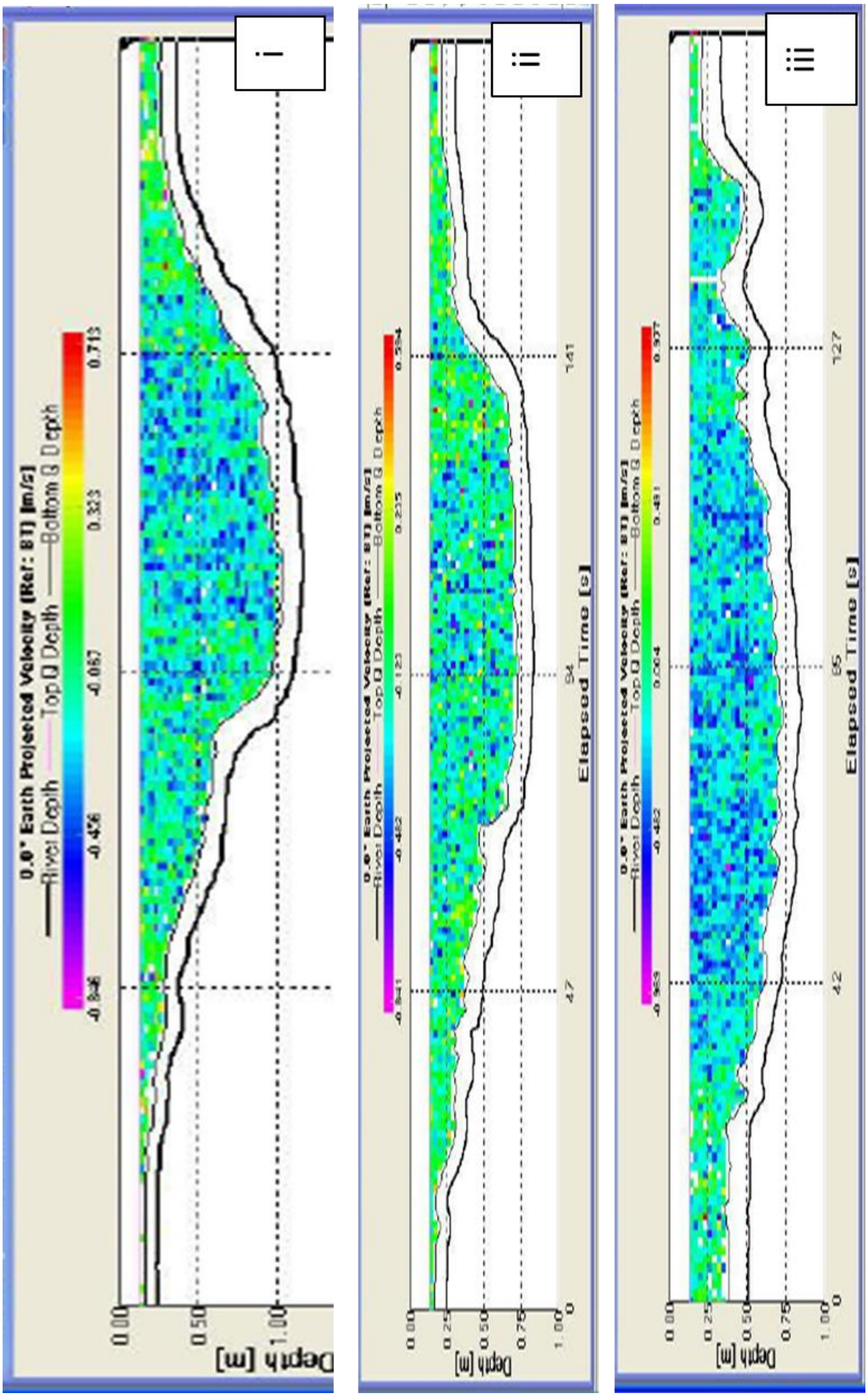



Figure 3.16. Plough river bed profiles (i.100m; ii. 150, iii. 200m).

### 3.3.6 Vegetation and habitat on the River Lark

**Barton Mills:** Bankside vegetation at this site was comprised predominantly of *Lolium* s*pp*. (rye lawn grass; Figure 3.17) with one or two mature trees offering root systems that extend into the shallows. Marginal aquatic vegetation varied with one garden (BM110 to BM130 m) bordered by coir rolls planted with dense riparian vegetation in an attempt to reduce erosion, whereas another property (BM140 to BM160 m) was fringed by dense patches of *Phragmites australis* (common or Norfolk reed) though marginal vegetation was generally sparse in the Barton Mills area (Figure 3.17).

Figure 3.17. A typical Barton Mills garden photographed from the public footpath opposite.

**Lark Head:** Bankside vegetation at this site consisted of rough grassland riverbanks with aquatic margins containing dense stands of *Glyceria maxima* (reed sweet-grass) and *Phalaris arundinacea* (reed canary grass), interspersed with *Typha latifolia* (reedmace), *Clematis vitalba* (old man's beard), *Carex* spp. (sedge) and *Rumex* spp. (dock), with a few mature trees *Alnus glutinosa* (Alder) extending root systems into the water (Figure 3.18).

Figure 3.18. Mature *Alnus glutinosa* (Alder) (left) and marginal vegetation (right) on the bankside at Lark Head with structural complexity in the shallows due to its remains.

**Plough**: Bankside vegetation at the Plough was extensive, with overhanging trees and dense vegetation on one bank, and fields behind and on the opposite bank. The vegetation predominantly consisted of tall grasses, *Urtica dioca* (nettles), *Rubus fructicosus* agg. (bramble), *Rumex* spp. (dock) and *Arctium* spp. (burdock). Marginal vegetation was often dense and included *Glyceria maxima* (reed sweet grass) and *Carex* spp. (sedges) with clumps of *Mentha aquatic* (water mint) and *Myosotis* spp. (water forget-me-not) at the water's edge (Figure 3.19).

Figure 3.19. Plough bankside habitat with fields opposite depicting the rural location.

### 3.3.7 pH, temperature and biological oxygen demand

There was no significant difference between mean pH or biological oxygen demand (BOD) between the three sites studied.  However, January water temperatures (taken c. 500 mm below the surface) did differ significantly between sites (Table III.VII), though this is only a spot value as replicates were ruled out by equipment loan considerations.

Table III.VII. Spot means for pH, temperature and BOD for three sites on the River Lark with * denoting a significant difference between the three sites and n/s denoting a non-significant result via Kruskal – Wallis ANOVA (p = 0.05).

| | **Mean value** | | | **Kruskal - Wallis ANOVA** | | | **P = 0.05** |
|---|---|---|---|---|---|---|---|
| | **Barton Mills** | **Lark Head** | **Plough** | $X_2{}^2 =$ | $n_{1,2,3} =$ | **P =** | ***/ ns** |
| **pH** | 8.01 | 7.99 | 8.05 | 5.50 | 15 | 0.06 | ns |
| **Temp ° C** | 5.70 | 4.62 | 5.39 | 33.43 | 15 | < 0.01 | * |
| **BOD (Mg/ Litre)** | 90.53 | 93.00 | 90.25 | 2.65 | 15 | 0.07 | ns |

## 3.4 Discussion

A high degree of similarity in particle size and abundance at the three sites (bankside and transect) has been demonstrated, with analyses showing little variation within and between sites. The substratum composition of the River Lark, as a whole, is predominantly sand, which is considered 'suboptimal' for large invertebrates (including crayfish) whose gills are sensitive to clogging (Holdich, 2003) and is also an unstable burrowing medium (Field Studies Council, 2012). The effects of bank erosion are pronounced on the River Lark which has been previously reported at Barton Mills (Smith and Stancliffe-Vaughan, 2007; Stancliffe-Vaughan, 2009). Some Barton Mills residents, concerned about the loss of river frontage, have undertaken costly repairs (Morris, 2002) with limited success. Larger landowners (e.g. Elveden Estate) have been forced to re-route footpaths to avoid dangerously eroded riverbanks (Rudderham, 2009). The EA have also undertaken costly breach repairs to mitigate damage caused by *P. leniusculus* on the River Lark (Pryce and Mant, 2008), in addition to maintaining and repairing in-channel structures (e.g. bridges, weirs, staunches; Smith, 2010). 'Gravel cleaning' is now carried out with greater frequency to ameliorate the effect of increased sediment loading (Fielding and Saint, 2013). Whilst erosion in some areas has, in the past, been blamed on fishermen (Murphy and Pearce, 1985), swans, ducks and dogs it is now accepted that *P. leniusculus* have a deleterious impact on riverbanks, with bank retreat/ erosion, weathering, fluvial entrainment and mass failure all interacting (Couper and Maddock, 2001).

The erosional impact of other aquatic invasive species has been noted with *Eriocheir sinensis* (Chinese mitten crab) causing severe bank modification in San Francisco Bay (Rudnick et al., 2003), with rapid UK spread of this species anticipated (Herborg, 2005). *Myocaster coypus* (coypu) still causes widespread bank erosion in France (Breton et al., 2013), though was successfully eradicated in the UK by DEFRA funded professional trappers in the 1980's (Gosling and Baker, 1989). In addition to the increased flood risk *P. leniusculus* burrowing may also affect *Arvicola terrestris* (Northern water vole) habitat (Holdich et al., 2004).

Flows at Lark Head are slow, as demonstrated by substratum variation (bankside fine skewed samples with coarser skews in transects), which is consistent with the greater width/ cross-sectional area at Lark Head compared with Barton Mills and the Plough. Barton Mills transects had a mesokurtic (normal) distribution which may, in part, be an artefact due to large cobbles potentially blocking the Ekman grab mouth during sampling (in two locations), with the potential loss of fine grain particles from samples. Velocity rates did not differ significantly between study sites though depth, discharge and cross-sectional area did vary significantly with a deeper, wider reach at Lark Head. This may facilitate *P. leniusculus* population growth as Lark Head may have a larger carrying capacity. If water depth and habitat structure, including vegetation, influences crayfish distribution the environment at the Plough can be viewed as dissimilar to that at both Barton Mills and Lark Head. However, as Barton Mills and Lark Head have fostered large populations of *P. leniusculus* for over 10 years, whilst the population at the Plough has been less evident, Barton Mills and Lark Head may have been substantially altered by the invaders. The issue is one of 'cause and effect' as non-native crayfish alter the habitats they live in, changing both the environment and the species assemblages over time (Früh et al., 2012) and affecting their suitability for other species.

Deeper areas are sought out by larger crayfish (Rabeni, 1985; Creed, 1994) and fish (Power, 1987; Harvey and Stewart, 1991). Such habitat partitioning in crayfish may be a combination of habitat preference and predator avoidance alongside crayfish dominance hierarchy interactions (Butler and Stein, 1985; Harrison et al., 2006; Jones and Bergey, 2007; Warren et al., 2009). Intraspecific predation avoidance strategies may apply to all sizes of crayfish (Abrahamsson and Goldman, 1970; Englund and Krupa, 2000) and may also relate to interspecific competition with fish predators. The size of individual crayfish has been strongly correlated with habitat use (Clavero et al., 2009; Kusabs and Quinn, 2009) and fish (Harvey and Stewart, 1991). Shallow areas, particularly those lacking structural complexity, have been noted as being avoided by juveniles/ small individuals (Correia, 2001) as part of a predator avoidance strategy. However, terrestrial/ semi-aquatic predators (e.g. *Ardea cinera* – grey heron; *Vulpes vulpes* – fox; *Lutra lutra* – Eurasian otter, in the UK) hunt in the shallows but tend to seek out larger prey.

Water temperature, in relation to depth, is important when considering crayfish distribution, as seasonal life history patterns are mediated by temperature (Momot, 1967; Momot and Gowing, 1972). There was a significant difference in temperature recorded between sites (with Lark Head the lowest) which may be explained by their differing water inputs and width/ depth parameters. Lark Head (Figure 2.4, inset picture) contains input from the cut- off channel (a flood relief cut) and has the largest cross-sectional area with less localised warming possible at this site than the more enclosed Barton Mills and Plough sites. Crayfish abundance/ growth patterns do not appear to be influenced, with smaller individuals recorded at the Plough which had a temperature profile similar to Barton Mills. If growth was affected by these variations in water temperature between the three sites then Lark Head should have smaller crayfish which is not the case.

Kusabs and Quinn (2009) noted that juvenile *Paranephrops planifrons* (koura/ freshwater crayfish), are released by females in the littoral zone, where water temperature is higher and food may be more abundant (Devcich, 1979 cited in Kusabs and Quinn, 2009, p.715). Juvenile *Panulirus argus* (spiny lobsters) have also been found to favour structurally complex habitats that provide refuge and food (Marx and Herrnkind, 1985), thus avoiding conspecific aggression (Figler et al., 1999; Issa, Adamson and Edwards, 1999; Olsson and Nyström, 2009). There was no difference in BOD at the different study sites in January, though this may not fully represent crayfish BOD as they are relatively inactive during the colder months. In hot weather heavy mortalities may be suffered by *P. leniusculus* who have a comparatively high oxygen demand in line with their raised activity levels in the summer months (Ackefors, 1998). However, as all three study sites support substantial *P. leniusculus* populations it can be assumed that BOD levels are not limiting.

Calcium is not considered limiting in the River Lark as there are extensive populations of *P. leniusculus* (Smith, 2014), with similar pH levels at each site indicating that calcium availability should not vary. Moreover, despite similar BOD and pH levels, population sizes varied. This variation in abundance is not easily attributed to the variables studied, though there may be a link to the presence and abundance of the crayfish themselves. The normally wide tolerances of *P. leniusculus* mean that few habitats are inhospitable to NICS, though the size of the non-native crayfish population, and how long it has persisted at each site (number of years from invasion/ introduction), could alter the habitat, significantly affecting its resilience with respect to new invasions.

# CHAPTER 4: THE EFFECT OF APERTURE DIAMETER, LIFE HISTORY STAGE AND BEHAVIOUR ON THE SIZE AND SEX OF CRAYFISH SAMPLED

## 4.1 Introduction: Active and passive crayfish sampling

Globally a variety of equipment is utilised for the capture of Crustacea, including trawls, pots/ creels and hoop nets (Brandt, 1984; Krouse, 1989). Sampling methods can be described as either 'active' or 'passive'. **Active sampling** for crayfish includes hand-searching, night-viewing and underwater census (using quadrats, timed searches, SCUBA or snorkelling, and/or dredge sieving/ suction; Odelström, 1983). Other active methods include seine netting, electrofishing, quadrat and surber sampling, throw nets, dip nets, de-watering and the use of biocides (Hiley and Peay, 2006; Freeman et al., 2010). Manual survey and night-viewing are limited to easily accessible, clear, shallow water (< 1.0 m) (Brown and Bowler, 1978; Peay, 2000, 2001, 2003a, b), although smaller crayfish may be missed (Robinson et al., 2000; Gladman et al., 2009).

**Passive sampling** methods include trapping, which is the most commonly used crayfish harvesting method. Several studies have concluded that traps are biased towards the capture of larger size classes (Brown and Bowler, 1978; Capelli, 1982; Peay, 2000; Kemp et al., 2003), and males (Mason, 1975; Capelli and Magnuson, 1983; Olsen et al., 1991; Peay, 2000) which in the UK has been reported to lead to population explosions of juvenile crayfish (Bills and Marking, 1988; Peay and Hiley, 2004). This is due to the perception that adult males in a crayfish population dominate/ cannibalise juveniles so any selective removal may lead to a perturbation of population structure. This effect has not been reported in the scientific literature, though it appears as 'fact' on websites (Chapter 1). Whilst trap aperture size is considered to have an impact on the size of individuals caught, trap entrance diameters are infrequently reported, and where stated, vary from 33 to 50 mm (e.g. Capelli and Magnuson, 1983; Collins et al., 1983; Somers and Stechey, 1986; Harlioğlu, 2004).

Other passive methods used in crayfish studies are perforated brick refugia (Blake et al., 1994; Griffiths et al., 2004; Peay et al., 2006), and clusters of plastic tubes of different diameters ('pan pipes') (Peay, 2000; Gotteland, 2012). Microhabitat traps consist of bundles of cane, leaf, cedar branches or bracken (Warren et al., 2009). However, they are unsuitable for use in dense macrophyte beds (Parkyn et al., 2011), or on uneven substrata (Fjälling, 2011), with decay, snagging, disturbance and theft an issue (Kusabs and Quinn, 2009). One of the newest designs is an 'enclosure trap' (substratum filled mesh circles) though catches in trials were rather low (Fjälling, 2011).

Trapping is considered "the survey method of last resort" (Kemp et al., 2003; Peay, 2004) due to its purportedly biased catches yet it remains one of the most common methods employed for non-native crayfish research, removal/ harvesting and monitoring. Whilst seine netting and electrofishing (Gladman et al., 2009) are also used, as NICS and habitat degradation are linked, heavily sedimented, turbid deep waters make trapping preferable. Selectivity issues have been reported for all of the sampling methods considered with no single method effective when used in isolation (Freeman et al., 2010).

### 4.1.1 The impact of behaviour, sex and life history stage on sampling

Crayfish are influenced by a suite of environmental, life history and behavioural traits which may interact with both active and passive sampling methods. Crayfish mating and activity is determined by water temperature, and therefore season (Somers and Green, 1993; Litvan et al., 2010). Mating is prompted by a fall in water temperature (usually in late October), with berried females burrow-dependent prior to and during hatching (March/ April; Holdich, 2002b). Activity increases in line with water temperature once temperatures exceed 10°C, with 20 to 25 °C, the maxima for crayfish activity (Firkins and Holdich, 1993). The UK research (and trapping) season for crayfish is from April to September with the interaction between temperature mediated activity levels and seasonal reproductive behaviour potentially resulting in female dominated trap catches from May to September (Capelli and Magnuson, 1983). Once young are independent from the maternal female feeding activity increases to replace reserves lost during young production (Hogger, 1986) resulting in increased opportunity for female catches with traps.

Adult crayfish are predominantly nocturnal (Appelberg and Odelström, 1988; Hogger, 1988; Peay 1997, 2004; Pintor, Sih and Bauer, 2008), when the risk of fish/ bird predation is reduced. However, for juvenile crayfish the risk of cannibalism by adults is increased at night, so they may be more active during daylight hours (Abrahamsson, 1966; Capelli and Munjal, 1982). Juvenile crayfish are also prey for a wide variety of mammals, fish, birds (Svärdson, 1972; Hogger, 1988) and other invertebrates (Dye and Jones, 1975; Hirvonen, 1992) increasing the need for cryptic behaviour. Juvenile crayfish are uniquely vulnerable as a size class as they undergo 8 to 11 moults in their first year (Mason, 1963; Cukerzis, 1986; Burba, 1987), compared to a typical single annual moult in adults (Shimizu and Goldman, 1983). Moulting carries a heightened risk of predation/ cannibalism, activity is reduced during this time and the use of hides/ refuges increased (Abrahamsson, 1973).

A sampling method used overnight (e.g. trapping) may therefore be unsuited to juvenile capture, as it misses their peak activity (i.e. daytime). As juvenile crayfish are extremely vulnerable to both predation and cannibalism, moult frequency may result in refuge being of higher priority than food resources. For adult crayfish behavioural differences between male and female crayfish further complicate sampling and inferences drawn from catches (Stuecheli, 1991).

Male crayfish, for a given age, are larger than females in body and chelae (Garvey and Stein, 1993; Rutherford, Dunham, and Allison, 1995), reducing their vulnerability to gape-limited predatory fish (England and Krupa, 2000). Whilst both sexes of crayfish are affected by the presence of larger fish and crayfish (Blake and Hart, 1993), females show a more pronounced response to predatory fish (Collins et al., 1983). It is theorised that males competitively exclude females from traps (Momot and Gowing, 1977; Lodge, Beckel and Magnuson, 1985; Rach and Bills, 1989). However, sex biased catches may also be a result of seasonal activity patterns (Somers and Green, 1993; Litvan et al., 2010). Both Abrahamsson (1966) and Cormack (1966) proposed that male trap catch may be iterative, with changes over time resulting in initially large catches of males being followed by lower catches of smaller males and more females.

### 4.1.2 Thigmotaxis and the implications for sampling

Crayfish are positively thigmotactic, preferring to have their lateral surfaces touching a surface (Mason, 1979) with the importance of shelter/ burrow use in crayfish and other decapod crustaceans being well documented (e.g. Bovbjerg, 1959; Alberstadt et al., 1995). Adults become less thigmotactic and more flexible in their shelter choices as they grow, whilst juveniles are very shelter size specific (Burba, 1987; Antonelli et al., 1999). This has obvious implications for their catchability. Refuge may therefore be more important than food as a limiting factor for all crayfish age classes (Bovbjerg, 1956; Quinn and Janssen, 1989; Peeke et al., 1995; Figler et al., 1999), though particularly for juveniles (Westin and Gydemo, 1988). In laboratory studies, juvenile crayfish were rarely found in the open (Alberstadt et al., 1995) and were heavily cannibalised in the absence of appropriately sized shelters (Stoeckel et al., 2011).

Crayfish actively seek shelters that provide maximum protection from fish and other predators (Stein and Magnuson, 1976; Stein, 1977), though the provision of passive refuge media as a sampling/ removal tool has been underutilised to date. In addition to the benefit of methods that avoid by-catch, and the need for bait, refuge provision may enable sampling over longer time periods with large areas covered using less time and effort. Freedom from these constraints may also assist with the tackling of issues such as population growth lag phases and detection thresholds.

### 4.1.3 Lag phase, detection thresholds and the production-attraction debate

Locating and sampling newly established crayfish populations is complicated by the existence of a population increase ‘lag phase’ and ‘detection threshold’. In common with invasive plants (Aikio et al., 2010), invading crayfish populations may only increase their range once a critical density, which prompts resource competition, has been reached (Peay and Rogers, 1999; Peay et al., 2006). This lag phase may last from five to 20 years with the potential for “several years” to elapse between a non-native crayfish species introduction and its detection. The detection threshold may only be reached once populations exceed one individual per 500 $m^2$ (Peay et al., 2011). The use of refuges as a sampling technique may be beneficial though the implications of increasing the available habitat available, particularly for NICS, must first be explored.

In marine lobster fisheries the use of artificial reefs is contentious as it has been unclear whether new habitat produces greater numbers of juvenile lobsters, or merely attracts existing recruits. This is known as the ‘Production Attraction’ debate which is now considered to be a continuum (Pickering and Whitmarsh, 1997; Briones-Fourźan and Lozano-Álvarez, 2001). This dichotomy is moot, as for UK native crayfish, habitat creation is beneficial, whilst for non-native crayfish, as long as any new habitat is frequently emptied, potential damage will be avoided.

## 4.2 Method

CPUE is defined in this study as the number of crayfish caught per equipment item per session (either 24 or 48 hour soak time ±5 hours). See Chapter 2: General methods (p.24).

## 4.3 Results

### 4.3.1 Population structure when all sampling methods are considered

When data from all of the methods used between 2010 and 2012 at all three sites are combined, the size frequency distribution is at least bimodal, and potentially multimodal (Figure 4.1), demonstrating the capture of at least two size classes, with juveniles (POCL < 21 mm), well represented. A mixture of ages of crayfish in a broad range of sizes may be contributing to the 'juvenile' and 'adult' normal population curves.

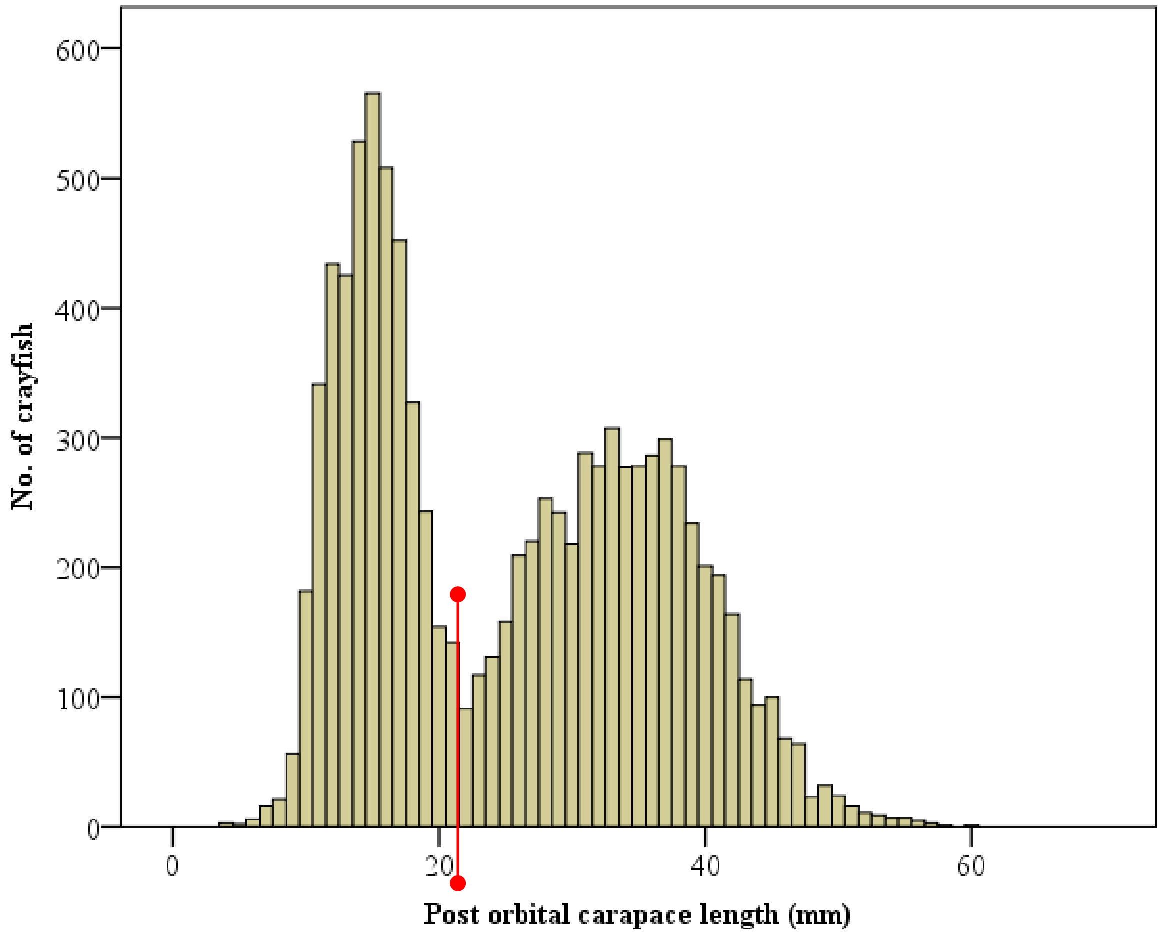



Figure 4.1. Size frequency distribution of crayfish post orbital carapace length (POCL) for all methods, pooled data for three sites 2010-2012 (n = 9707). Red line at ≤ 21 mm POCL delineates juvenile/adult size class separation.

When grouped by sampler category (traps; seines; quadrats; bricks), the means differed significantly (Figure 4.2. Kruskal-Wallis: $X^3_3 = 5828.3$, $n^1 = 936$, $n^2 = 60$, $n^3 = 2959$, $n^4 = 5752$, $P = <0.001$). Traps exhibited the greatest size range, though fewer juvenile crayfish were removed with seines than with bricks or quadrats. Seine netting appeared unselective in the size of crayfish sampled, though the small sample size ($n = 60$), may be masking a normal distribution. Seine netting data is examined in more detail later in this chapter (Figure 4.10).

Quadrats and bricks sampled individuals with a similar size range (Figure 4.3). A Tamhane Post Hoc test for unequal variances showed no significant difference in the means of crayfish caught in bricks or quadrats, though traps and seines differed significantly from all other methods and sampled larger crayfish (Table IV.I).

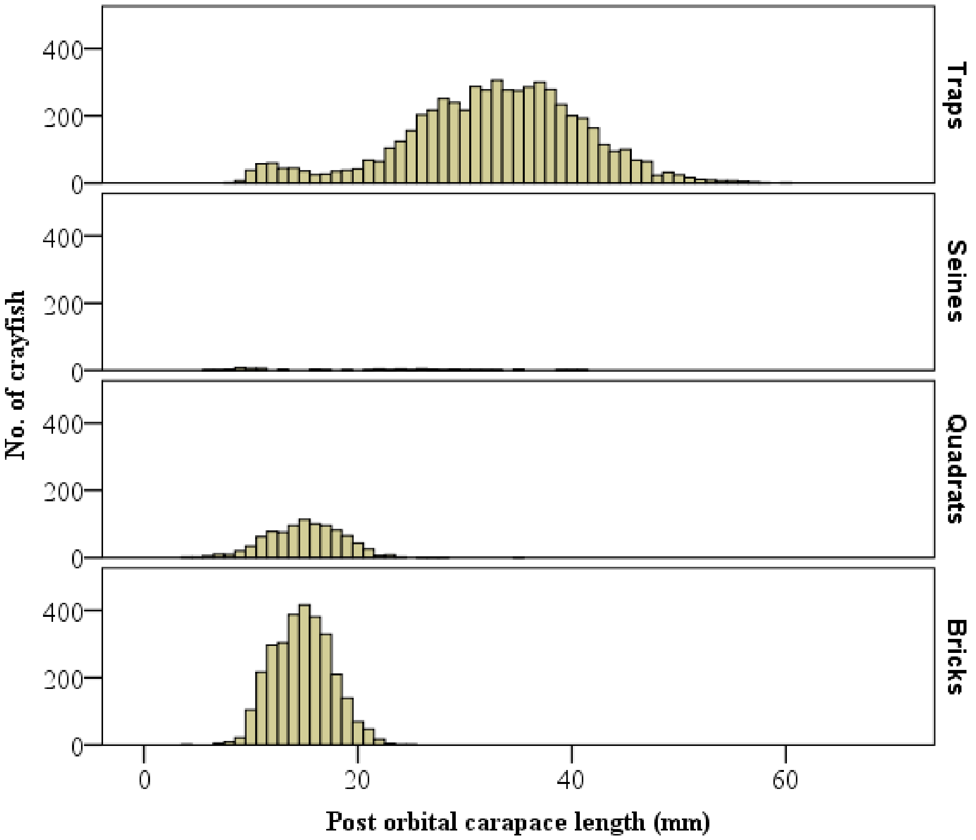



Figure 4.2. Post orbital carapace length (POCL) of crayfish caught using traps (n = 936), seines (n = 60), quadrats (n = 2959) and bricks (n = 5752) at three sites (2010 - 2012).

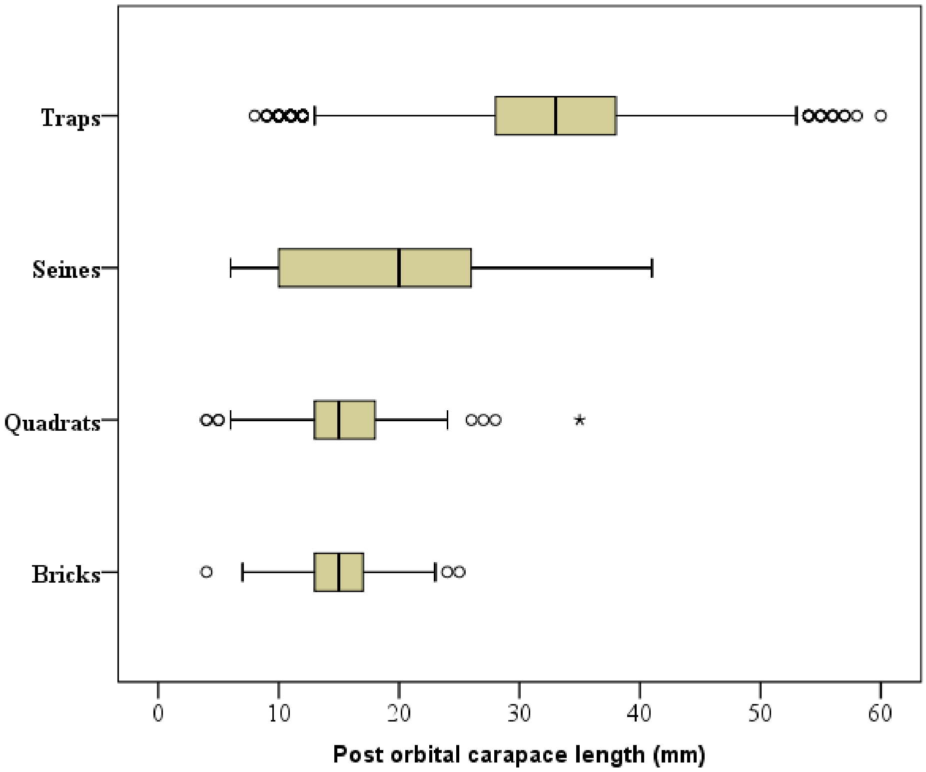



Figure 4.3. The medians and interquartile ranges (including outliers) of crayfish post orbital carapace length (POCL) with data aggregated into equipment category (traps, seines, quadrats and bricks) used from 2010 to 2012 at three sites (n = 9707).

Table IV.I. Tamhane Post Hoc tests (unequal variances) of significant differences between the four categories of sampling equipment (top values are p = x, lower values denote significant (*) or non-significant result (ns)).

| **Post Hoc Tamhane *significant at P = 0.05** | **Traps** | **Seines** | **Quadrats** | **Bricks** |
|---|---|---|---|---|
| **Traps** | | <0.01 | <0.01 | <0.01 |
| **Seines** | * | | <0.01 | <0.01 |
| **Quadrats** | * | * | | 0.17 |
| **Bricks** | * | * | ns | |

### 4.3.2 The implications of trap aperture diameter, design and refuge material in sampling crayfish using nine variants

The size frequency distribution of crayfish collected using nine trap types is bimodal (Figure 4.4). The data cannot be considered parametric or normally distributed due to the inclusion of many individuals < 21mm POCL (Figure 4.4). The mean post orbital length of crayfish caught (Table IV.II; Kruskal-Wallis: $X^8{}_8 = 1326.0$, $n^1 = 340$, $n^2 = 208$, $n^3 = 87$, $n^4 = 1688$, $n^5 = 479$, $n^6 = 51$, $n^7 = 1859$, $n^8 = 137$, $n^9 = 895$, $P = <0.001$) differed significantly between the nine trap types trialled between 2010 and 2012. A Tamhane Post Hoc test (unequal variances; Table IV.III) demonstrated that minnow small extras (with refuge material) caught crayfish that were significantly smaller than minnow small traps, and these two trap types, caught crayfish that were significantly smaller than all the other trap types. There was, however, considerable overlap in the sizes of crayfish caught with trap apertures > 30 mm ø (Table IV.II; minnow smallmedium; medium; large; professional; Pirat, Trapman and LiNi). The specificity of aperture size in relation to the size of the crayfish caught (Figure 4.7) is particularly noteworthy as crayfish traps with large (> 30 mm) apertures failed to represent smaller individuals (that were captured with 20 mm aperture minnow minnow traps/ minnow minnow extra traps with additional refuge material). Appropriatly sized (< 40 mm ø) symmetrical apertures captured more crayfish (higher CPUE) than larger asymmetrical apertures (Table IV.II).

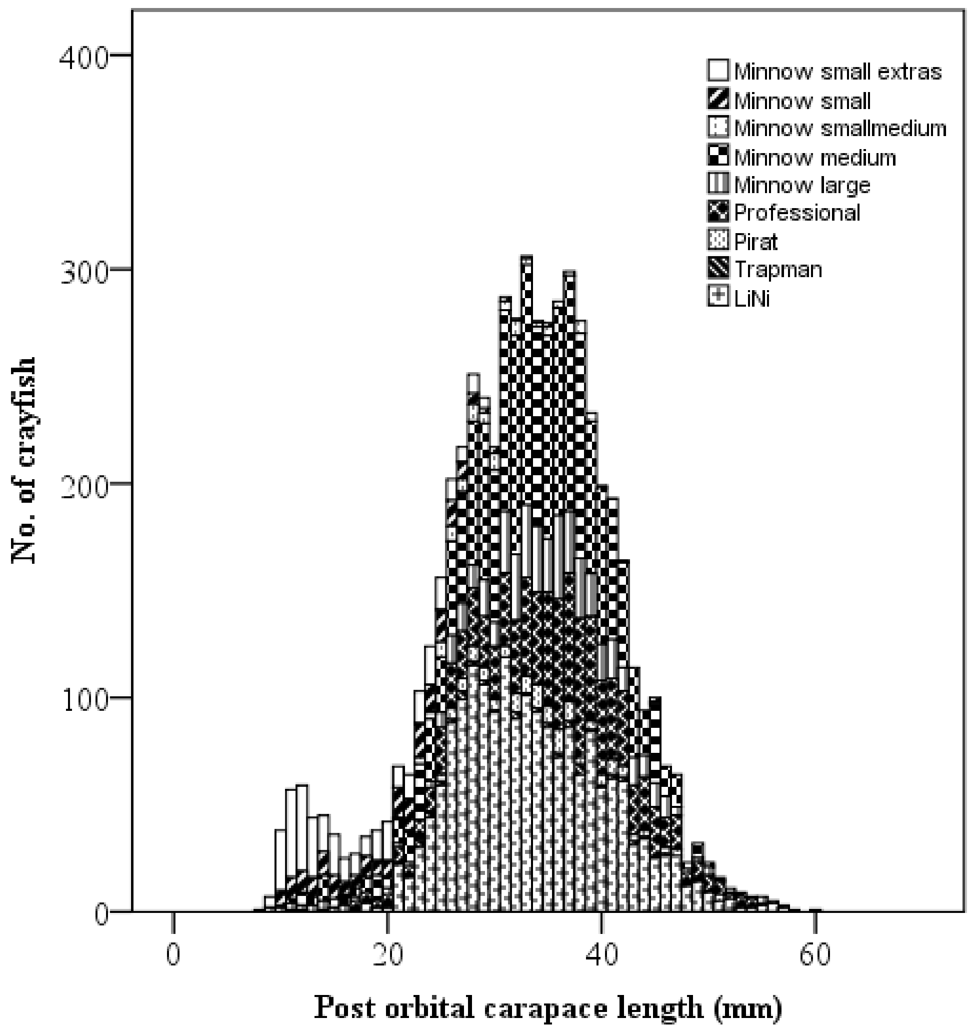



Figure 4.4. The mean post orbital carapace length of crayfish (POCL) caught in different trap types represented by stacked bars (2010 - 2012; n = 5744). Individuals < 21mm POCL are almost entirely represented by the two types of minnow small trap.

The traps used can be divided into two groups, the first representing minnow traps with a range of experimentally altered circular aperture diameters, the second trap group comprising Trapman and LiNi traps (symmetrical apertures), and Pirat/ Professional traps with asymmetrical apertures ('a') (Table II.I & IV.II; Figures 2.6 - 2.11).

Table IV.II. Descriptive statistics of post orbital carapace length of crayfish in traps including aperture and sample size ('a' = asymmetrical apertures/ two measurements).

| Descriptive statistics for POCL (rounded to the nearest mm) for each trap type including sample size and CPUE | Minnow small extras | Minnow small | Minnow smallmedium | Minnow medium | Minnow large | Trapman | LiNi | Pirat | Professional |
|---|---|---|---|---|---|---|---|---|---|
| **Trap aperture diameter** | 20 | 20 | 30 | 40 | 50 | 60 | 60 | 50/ 90[a] | 70/ 110[a] |
| **Sample size, n =** | 340 | 208 | 87 | 1688 | 479 | 51 | 1859 | 137 | 895 |
| **no. of 'trap' sessions** | 48 | 141 | 9 | 219 | 141 | 30 | 120 | 30 | 141 |
| **CPUE** | 7.1 | 1.5 | 9.7 | 7.7 | 3.4 | 1.7 | 15.5 | 4.6 | 6.3 |
| **Mean** | 16.8 | 20.4 | 30.3 | 33.4 | 34.4 | 32.5 | 33.7 | 34.5 | 35.7 |
| **St. deviation** | 5.8 | 6.5 | 5.3 | 6.9 | 7.7 | 8.7 | 6.9 | 6.9 | 7.2 |
| **Median** | 15.0 | 21.0 | 30.0 | 34.0 | 35.0 | 32.5 | 33.0 | 34.0 | 36.0 |
| **Mode** | 11.0 | 22.0 | 28.0 | 33.0 | 36.0 | 38.0 | 31.0 | 34.0 | 38.0 |
| **Range** | 20.0 | 35.0 | 29.0 | 50.0 | 46.0 | 35.0 | 41.0 | 31.0 | 49.0 |
| **Minimum** | 9.0 | 10.0 | 16.0 | 10.0 | 9.0 | 14.0 | 17.0 | 20.0 | 8.0 |
| **Maximum** | 29.0 | 45.0 | 45.0 | 60.0 | 55.0 | 49.0 | 58.0 | 51.0 | 57.0 |

Gees minnow™ traps, with different aperture diameters differed in the mean POCL of crayfish caught. Minnow small and minnow small extra traps (20 mm ø), differed in their setting (with larger aperture traps and without) and in the use of refuge material (minnow extra traps only) though all traps caught crayfish with a POCL < 30 mm (Figure 4.5). Minnow smallmedium traps (30 mm ø), caught crayfish that were slightly larger than minnow small/ small extra traps (Figure 4.5; Table IV.II). Minnow medium (40 mm ø) and minnow large traps (Gees crayfish™; 50 mm ø) caught a similar mean size (30.3 – 34.4 mm) of crayfish, though the range of sizes caught as indicated by the standard deviation was greatest for the trap with the largest aperture (minnow large; Table IV.II). As minnow smallmedium traps (30 mm ø) were created in 2012, there is less power in this analysis due to the small sample size (n = 87).

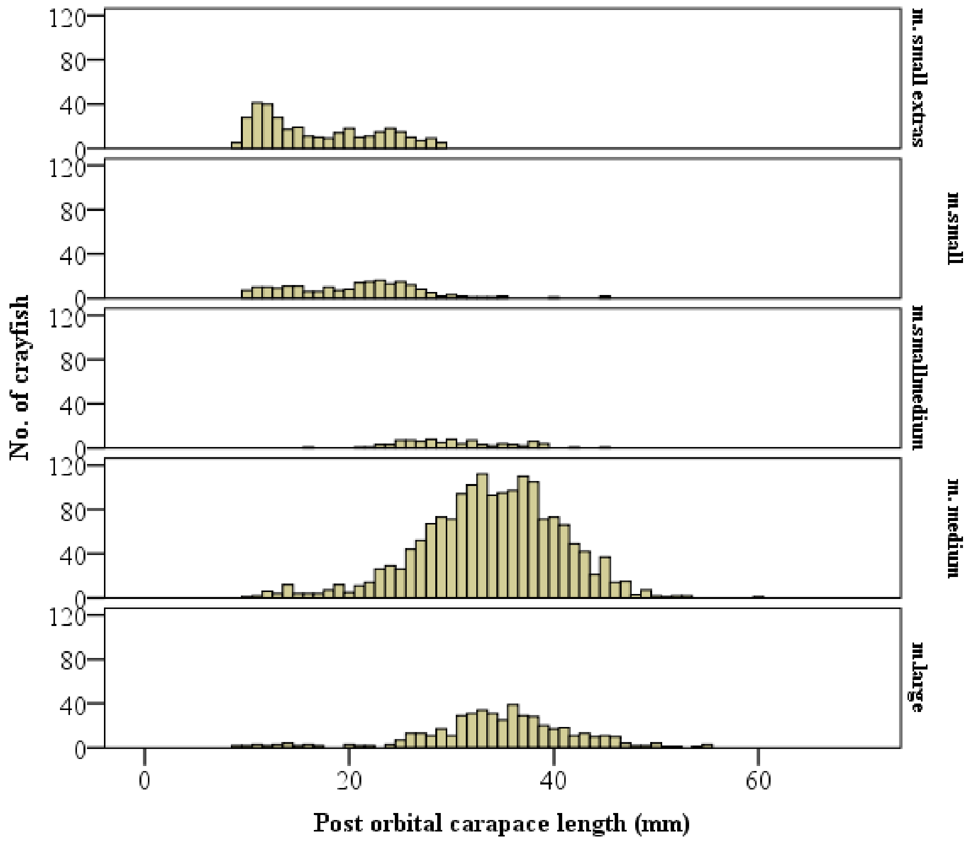



Figure 4.5. Post orbital carapace length (POCL) distribution of crayfish (ranked into 1 mm size classes) for minnow traps with different aperture sizes at three sites, 2010 - 2012 (Minnow small extras n = 340; Minnow small n = 208; Minnow smallmedium n =87; Minnow medium n = 1688; Minnow large n = 479).

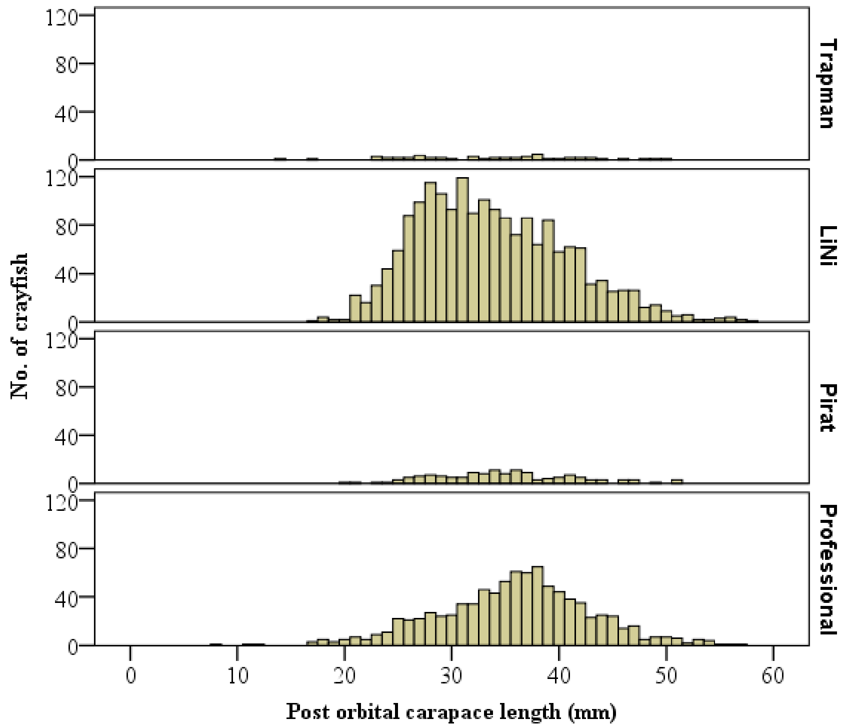



Figure 4.6. Mean post orbital carapace length (POCL) size frequency distribution for traps with symmetrical aperture sizes of 60 mm diameter and asymmetrical apertures up to 70/ 110 mm at three sites, 2010 - 2012 (Trapman n = 51; LiNi n = 1859; Pirat n = 137; Professional n = 895).

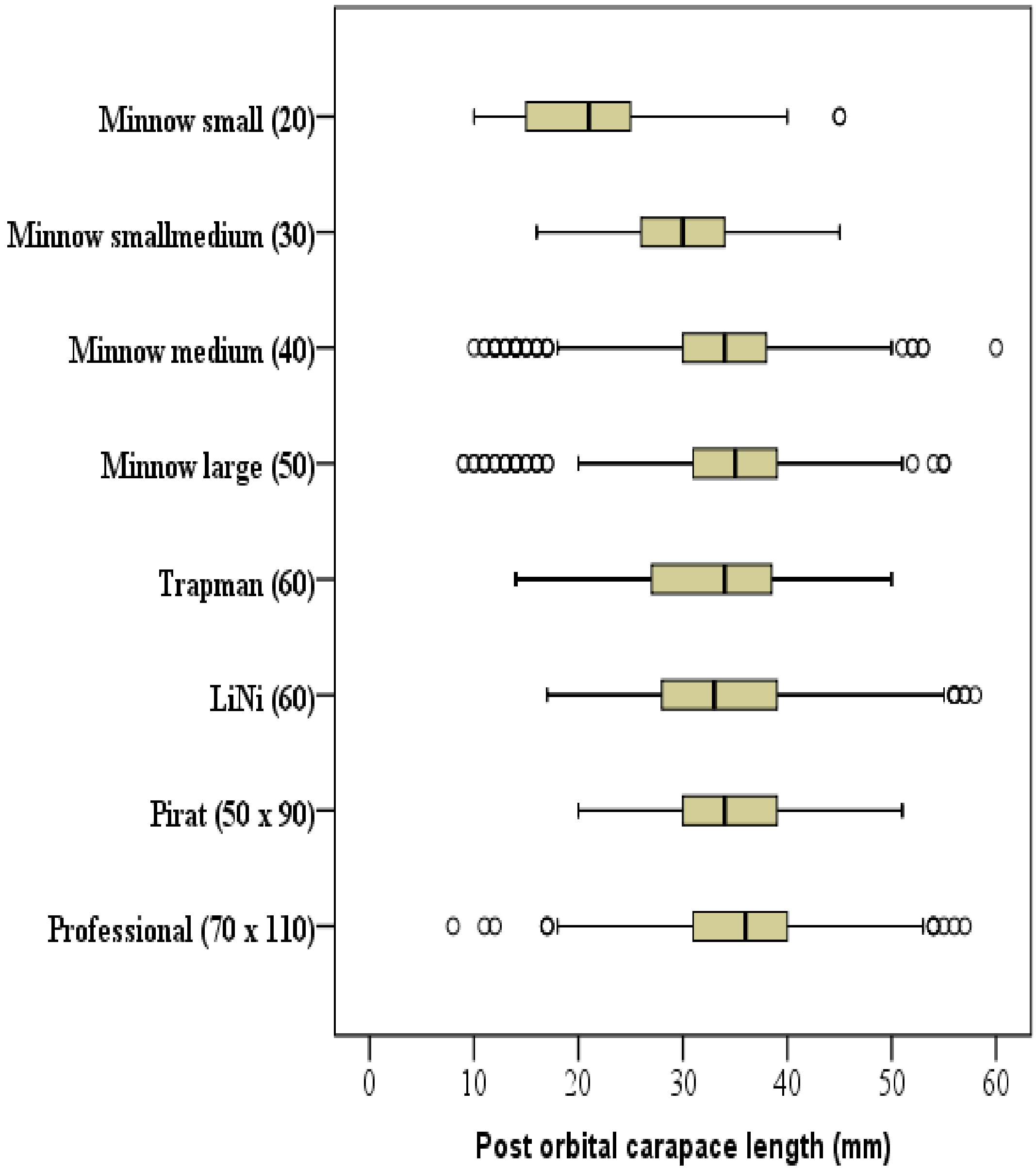



Figure 4.7. The distribution of median POCL measurements (including interquartile ranges and outliers) of crayfish sampled in traps with varying apertures (diameter in mm) and designs (n = 5744).

Trapman, LiNi, Pirat and Professional traps all caught crayfish with a mean POCL similar to the minnow medium and minnow large traps (Table IV.V; Figure 4.7). Large aperture traps (with the exception of the Professional trap), predominantly caught crayfish that exceeded 20 mm POCL (Table IV.VI; Figure 4.7). Catch size distribution and aperture size was similar in traps with apertures > 40 mm ø (Figure 4.8). In contrast, aperture sizes < 40 mm ø (Figure 4.8), showed significant variation in the size of the crayfish sampled depending on the aperture dimensions (Table IV.III).

Table IV.III. Tamhane Post Hoc tests of mean post orbital carapace length (POCL) of crayfish aperture diameter (mm) in brackets after trap name. Table upper value when P = 0.05; Table lower value: significant '*', non-significant 'ns'.

| **Tamhane post hoc tests for nine different trap types. Aperture diameter in brackets (mm) a = asymetrical aperture** | **Minnow small extras** | **Minnow small** | **Minnow smallmedium** | **Minnow medium** | **Minnow large** | **Trapman** | **LiNi** | **Pirat** | **Professional** |
|---|---|---|---|---|---|---|---|---|---|
| **Minnow small extras (20)** | | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| **Minnow small (20)** | * | | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| **Minnow smallmedium (30)** | * | * | | <0.01 | <0.01 | 0.24 | <0.01 | <0.01 | <0.01 |
| **Minnow medium (40)** | * | * | * | | 1.00 | 1.00 | 1.00 | 1.00 | <0.01 |
| **Minnow large (50)** | * | * | * | ns | | 1.00 | 0.82 | 1.00 | 0.02 |
| **Trapman (60)** | * | * | ns | ns | ns | | 1.00 | 1.00 | 0.61 |
| **LiNi (60)** | * | * | * | ns | ns | ns | | 1.00 | <0.01 |
| **Pirat (50/ 90)[a]** | * | * | * | ns | ns | ns | ns | | 1.00 |
| **Professional (70/ 110)[a]** | * | * | * | * | * | ns | * | ns | |

### 4.3.3 Sampling with minnow small traps with and without refuge material

The mean POCL of crayfish caught in the two types of minnow small traps differed significantly (Mann-Whitney U test: $U = 23916.5$, $n_1 = 208$, $n_2 = 340$, $p = < 0.01$) with traps containing refuge material set separately from larger aperture traps retaining/ catching smaller crayfish in larger numbers. The addition of refuge material, and the siting of four minnow small traps as a group, resulted in a greater representation of smaller size classes in minnow small extra traps (< 15 mm POCL; Figure 4.8; Table IV.III) and a larger CPUE (Table IV.II). Crayfish caught in the minnow small traps (no refuge material; traps set alongside larger aperture traps; Figure 4.9), were larger with a lower CPUE (Table IV.II).

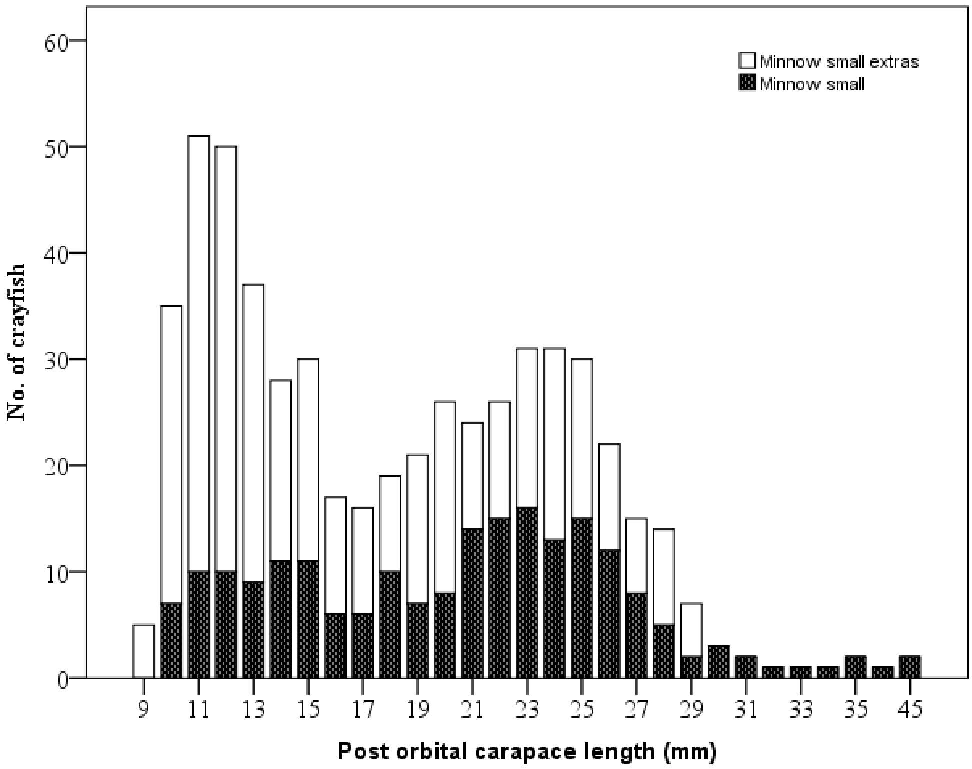



Figure 4.8. Crayfish sampled using minnow small extras traps (clear bars = refuge material), and minnow small traps (shaded bars) (n = 548).

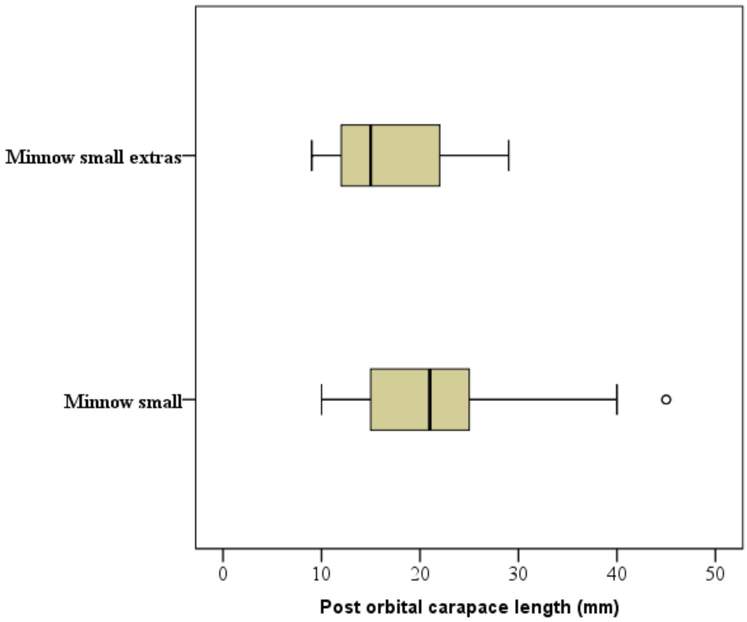



Figure 4.9. The median POCL and interquartile ranges (including outliers) of crayfish sampled using minnow small and minnow small extra traps (n = 548).

### 4.3.4 Seine netting: an active sampling method

Seine netting was undertaken at each site with a mean of five crayfish per ‘pull’ and four pulls undertaken at each site (n = 60). The overall distribution of the catch deviated significantly from normal (Figure 4.10) with at least two size classes represented. The data are included as a reference to the other methods trialled within this study and aid comparison with the literature on sampling. Rigorous analysis is not appropriate because of data paucity as seine netting was only used once at each site during daylight hours.

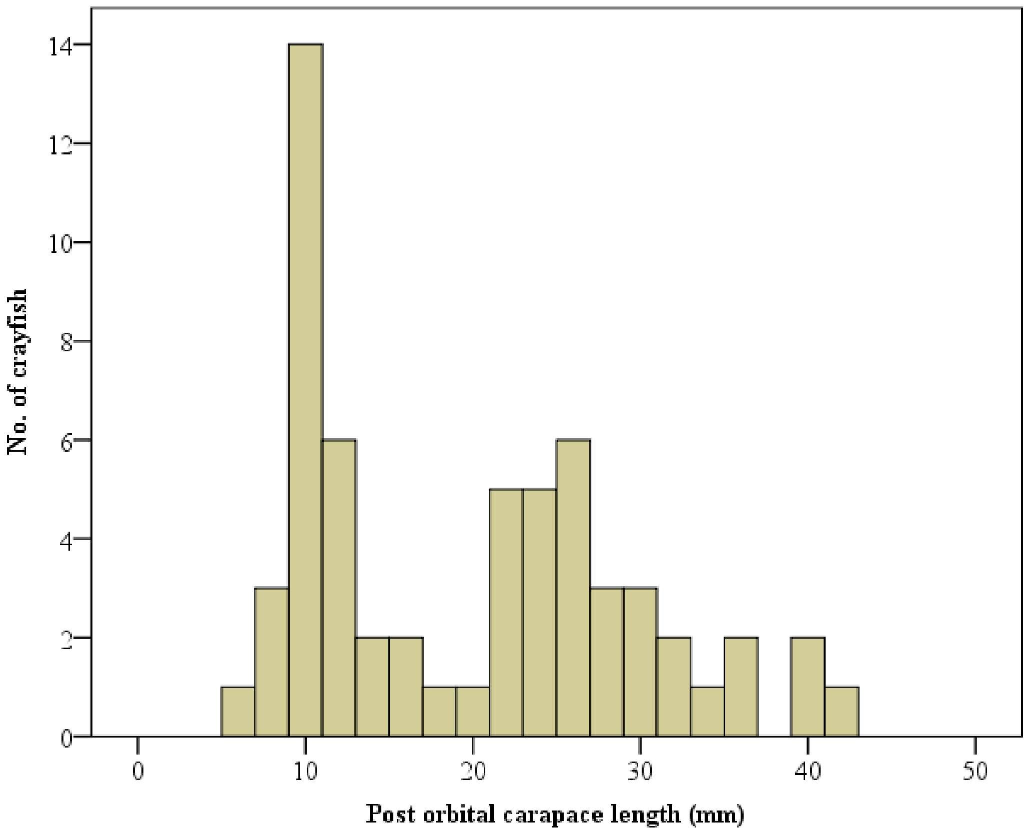



Figure 4.10. Size frequency distribution of post orbital carapace length (POCL) for twelve seine net pulls, four at each of three sites (n = 60).

### 4.3.5 Perforated bricks and quadrats for sampling juveniles

When comparing data collected using two novel samplers (quadrats and perforated bricks) there was a statistically significant difference between the mean POCL of the individuals sampled (Kruskal-Wallis: $X^7{}_7$= 1226.1, $n^1$= 111, $n^2$ = 5, $n^3$ = 36, $n^4$ = 67, $n^5$= 134, $n^6$ = 703, $n^7$ = 583, $n^8$ = 2256, P = <0.001). A Tamhane Post Hoc test **(**Table IV.IV), demonstrated no significant difference between the mean POCL of crayfish caught using P18 perforated bricks alone, or in quadrat sampler arrays (Q P18), with P24 bricks and Q P24 bricks similarly showing a consistent range of sizes caught. The data for perforated bricks (set separately and set within quadrats) will be considered separately from other quadrat sampling elements (straw; horticultural insulated roofing; colonisation (3); quadrat area).

Table IV.IV. Comparison of the sizes of crayfish caught using differing types of juvenile sampling equipment via Tamhane Post Hoc tests when P = 0.05 with P values above and significance below.

| **Post Hoc tests:** **Tamhane** ***significant at P = 0.05** | **Quadrat straw** | **Quadrat plastic** | **Colonisation (3)** | **Quadrat area** | **Quadrat P18** | **Quadrat P24** | **P18** | **P24** |
|---|---|---|---|---|---|---|---|---|
| **Quadrat straw** | | 0.01 | <0.01 | <0.01 | 0.01 | 0.01 | <0.01 | 0.03 |
| **Quadrat plastic** | * | | <0.01 | <0.01 | <0.01 | <0.01 | 0.85 | <0.01 |
| **Colonisation (3)** | * | * | | 1.00 | <0.01 | 1.00 | <0.01 | 1.00 |
| **Quadrat area** | * | * | ns | | <0.01 | 0.94 | <0.01 | 1.00 |
| **Quadrat P18** | * | * | * | * | | <0.01 | 0.85 | <0.01 |
| **Quadrat P24** | * | * | ns | ns | * | | <0.01 | 0.07 |
| **P18** | * | ns | * | * | ns | * | | <0.01 |
| **P24** | * | * | ns | ns | * | ns | * | |

**4.3.6 Juvenile sampling with perforated bricks**

Data obtained via sampling with P24 and P18 bricks showed a close to normal distribution for each brick type (Figure 4.11). P18 and P24 perforated bricks sampled crayfish that differed significantly in size (*t*-test: $t_{2932} = 34.689$, P = < 0.01; Figure 4.12) though the range was greater for P24 bricks (Table IV.V). A Bonferroni Post Hoc test (least significant difference) confirmed that there was no significant difference in the mean POCL of crayfish caught when the two perforated bricks were used independently or as part of a quadrat array (Table IV.VI).

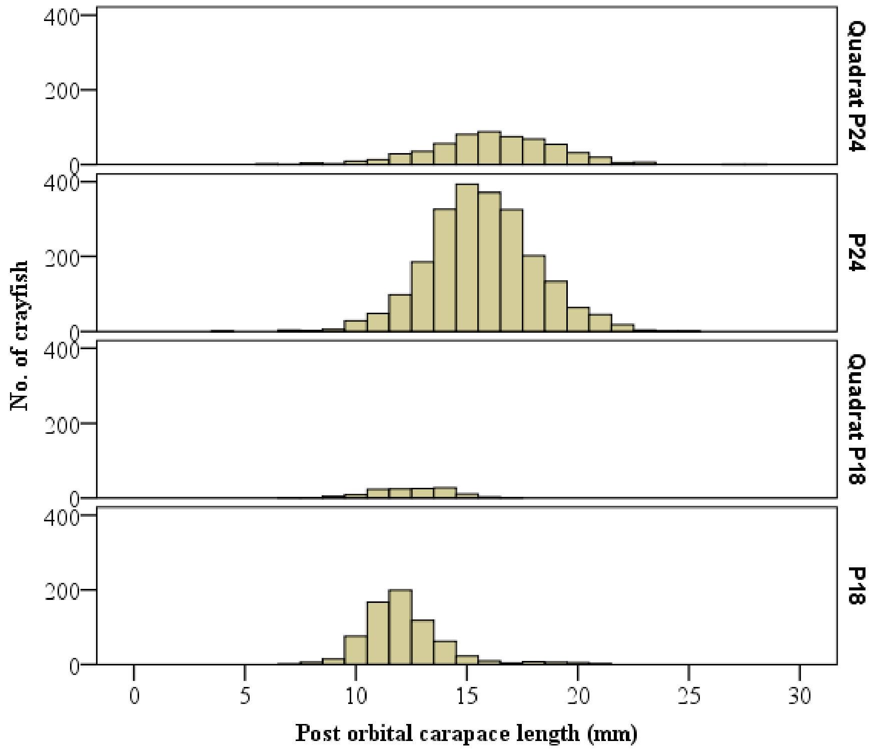



Figure 4.11. Size frequency distribution of brick samplers alone (P18, P24) and as part of quadrat sampler arrays (Quadrat P18, Quadrat P24).

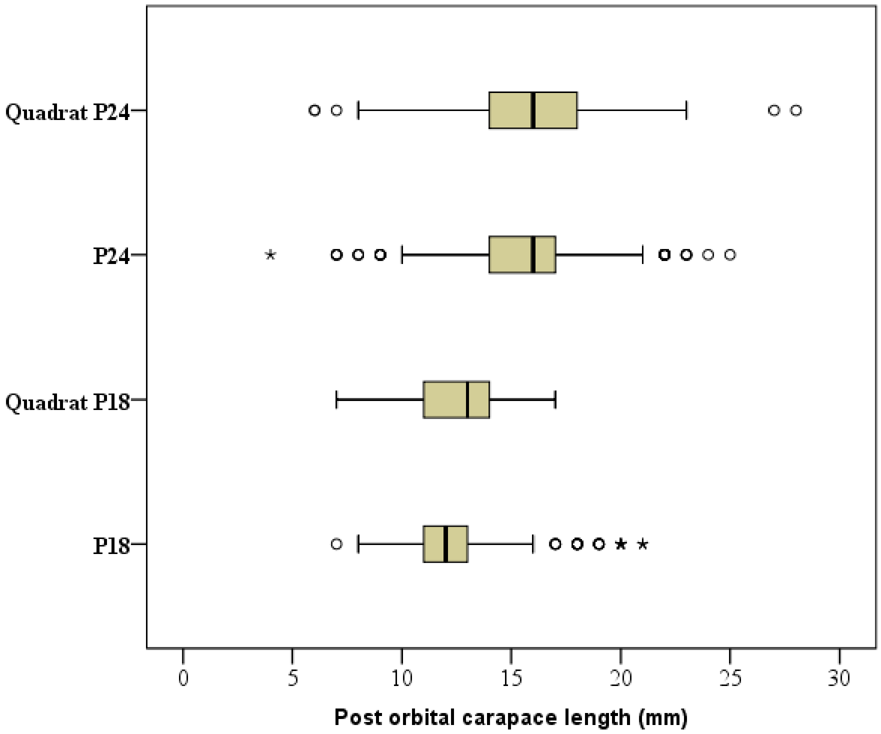



Figure 4.12. Interquartile range of brick samplers and the same bricks included as part of quadrat sampler arrays (Quadrat P18, Quadrat P24).

Table IV.V. Crayfish caught in P18 and P24 bricks alone and on quadrats arrays with aperture and sample size, CPUE and descriptive statistics of catch.

| Perforated bricks | P18 | Q P18 | P24 | Q P24 |
|---|---|---|---|---|
| **Aperture size (mm)** | 10 | | 17 | |
| **Sample size, n =** | 700 | 134 | 2234 | 572 |
| **No. of sessions** | 498 | 95 | 501 | 95 |
| **CPUE** | 1.4 | 1.4 | 4.5 | 6.0 |
| **Mean** | 12.5 | 12.5 | 15.7 | 16.1 |
| **St. deviation** | 1.8 | 1.8 | 2.4 | 2.9 |
| **Median** | 13 | 13 | 16 | 16 |
| **Mode** | 14 | 14 | 15 | 16 |
| **Range** | 10 | 10 | 21 | 22 |
| **Minimum** | 7 | 7 | 4 | 6 |
| **Maximum** | 17 | 17 | 25 | 28 |

Table IV.VI. Bonferroni Post Hoc test of significance for P18 and P24 bricks used separately and within quadrat arrays (Quadrat P18 and Quadrat P24).

| **Post hoc test: Bonferroni *significant at P = 0.05** | **P18** | **Quadrat P18** | **P24** | **Quadrat P24** |
|---|---|---|---|---|
| **P18** | | 0.99 | < 0.01 | < 0.01 |
| **Quadrat P18** | ns | | < 0.01 | < 0.01 |
| **P24** | * | * | | 0.99 |
| **Quadrat P24** | * | * | ns | |

**4.3.7 Straw, plastic, colonisation (3) and quadrat area elements**

Aperture sizes varied amongst the quadrat elements (Table 16). Quadrat samplers sampled individuals with POCL's of 4 to 28 mm with the smallest size classes represented (Table IV.VII). The mean POCL's of crayfish caught using colonisation samplers or on the quadrat area, did not differ significantly, with both items of equipment offering a variety of refuge sizes; Table IV.VII). There were significant differences between the mean POCL's of crayfish caught using straw and roofing plastic. Straw sampled the smallest crayfish (4-7 mm POCL) and offered the smallest interstitial spaces.

Table IV.VII. Crayfish caught in novel quadrat sampler elements with aperture and sample size, CPUE and descriptive statistics of catch (a = multiple modes exist).

| | Q straw | Q plastic | Q colonisation (3) | Q area |
|---|---|---|---|---|
| **Aperture size (mm)** | Small interstices | 6 - 9 mm | 5 - 20 + mm | Various interstices |
| **Sample size, n =** | 5 | 66 | 36 | 111 |
| **No. of sessions** | 92 | 93 | 95 | 94 |
| **CPUE** | 0.1 | 0.7 | 0.4 | 1.2 |
| **Mean** | 5.4 | 10.9 | 16.1 | 15.4 |
| **St. deviation** | 1.5 | 1.4 | 2.8 | 4.9 |
| **Median** | 5.0 | 11.0 | 16.5 | 15.0 |
| **Mode** | 4.0a | 11.0a | 19.0 | 15.0 |
| **Range** | 3.0 | 7.0 | 11.0 | 30.0 |
| **Minimum** | 4.0 | 8.0 | 10.0 | 5.0 |
| **Maximum** | 7.0 | 15.0 | 21.0 | 35.0 |

#### 4.3.8 Seasonality in juvenile crayfish capture using perforated bricks

Juvenile brick samplers (P18 & P24) were used from August 2011 to July 2012 and were emptied monthly. Catches increased during the winter months, with close to full occupancy in some of the bricks (containing either 18 or 24 refuges; Figure 4.13). This may be either due to crayfish availability (due to breeding/ recruitment) or variation in behaviour/ activity between juvenile and adult crayfish. Daytime emptying may also have an influence on catch in juvenile refuge samplers as 'refuges' are not designed to retain crayfish unlike 'traps'.

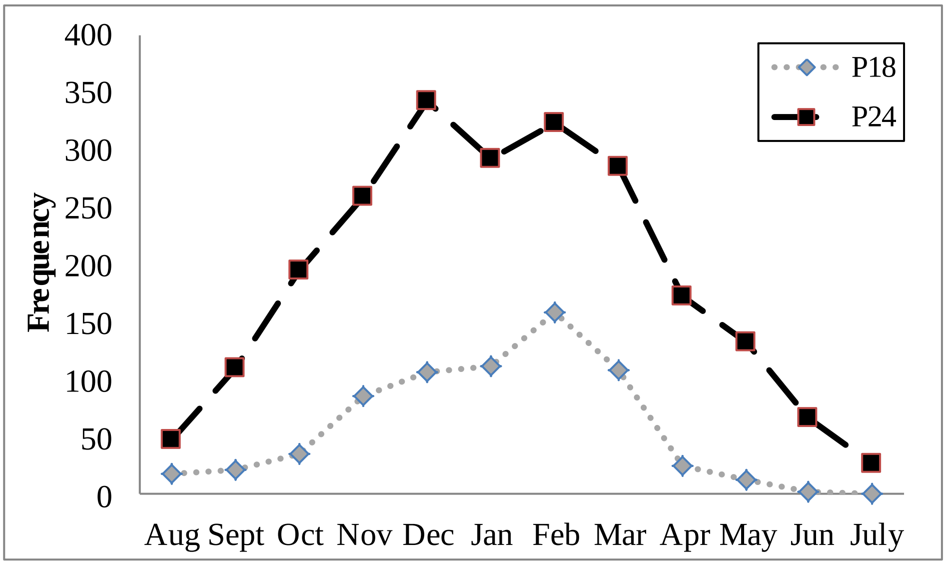



Figure 4.13. Variation in the number of crayfish caught using P18 and P24 perforated bricks with month (2011/ 2012) at three sites on the River Lark (n = 2938).

#### 4.3.9 Sex ratios in juvenile crayfish sampled using perforated bricks

The proportion of male and female crayfish caught using seven different trap types was evaluated for the month of August (2010-2012). The sex ratio (the proportion of males in the total number of males and females caught) was female biased and ranged from 0.37 to 0.49 (Table IV.VIII).

Table IV.VIII. August sex ratios for a range of trap types with differing aperture sizes.

| **Trap type** | **Minnow small** | **Minnow medium** | **Minnow large** | **Professional** | **Pirat** | **Trapman** | **LiNi** |
|---|---|---|---|---|---|---|---|
| **Aperture diameter (mm)** | 20 | 40 | 50 | 50 | 50 | 60 | 60 |
| **Sex ratio** | 0.37 | 0.44 | 0.46 | 0.43 | 0.49 | 0.38 | 0.49 |

The sex ratio of juvenile crayfish caught using P18 (Figure 4.14) and P24 (Figure 4.15) perforated bricks was evaluated over 12 months (August 2011 to July 2012). A high proportion of unsexed individuals were recorded, though the proportion of females exceeds 50% in ten of the twelve months studied for P18 (Figure 4.14). With P24 perforated bricks, the proportion of females exceeded 40% in all months (Figure 4.15). Both brick types showed a higher proportion of females in the summer months. In P18 samplers, females represented over 50% of the sample in August 2011 and May 2012. Similarly over 50 % of the crayfish sampled using P24's were female in August 2011 and June and July 2012.

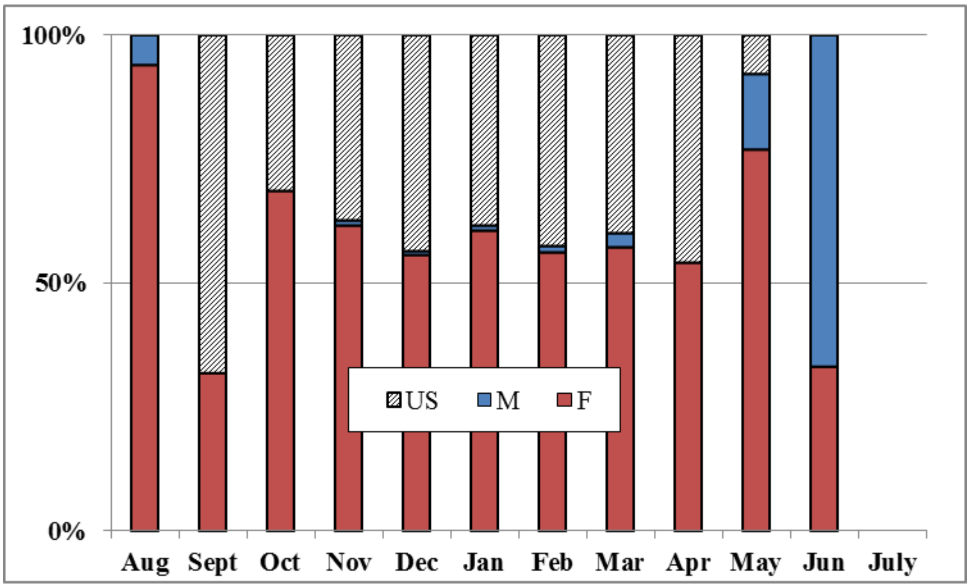



Figure 4.14. The proportion of male, female and unsexed individuals caught at three sites on the River Lark using P18 perforated bricks (Aug 2011 to July 2012; n = 684) No crayfish were caught in July 2012 in P18 samplers. [US = unsexed; M = male; F = female]

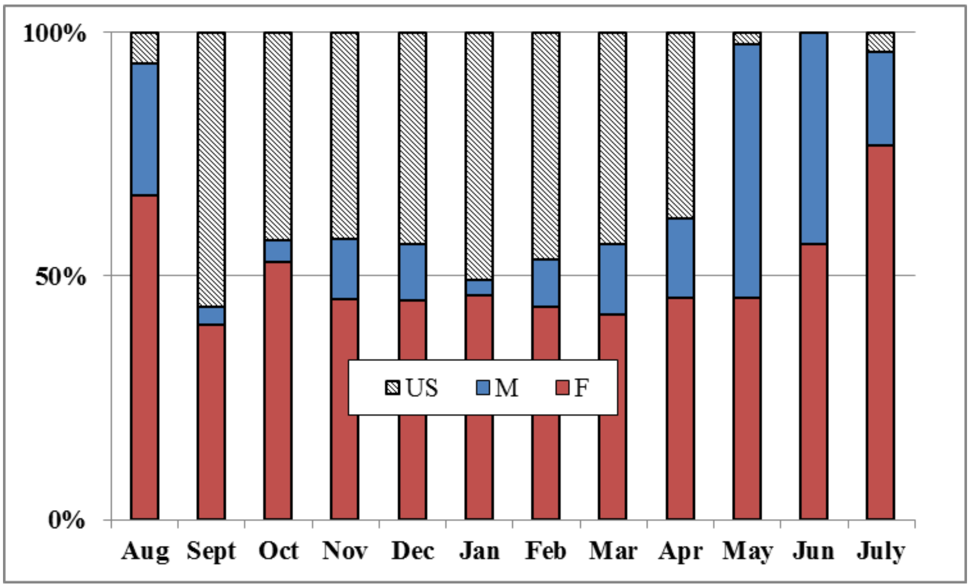



Figure 4.15. The proportion of male, female and unsexed individuals caught at three sites on the River Lark using P24 perforated bricks from Aug 2011 to July 2012 (n = 2254) [US = unsexed; M = male; F = female]

## 4.4 Discussion

There were significant differences in the size of crayfish sampled using equipment with a range of aperture diameters. Traps, with apertures exceeding 20 mm diameter caught fewer juveniles (< 21 mm POCL) than seines, bricks or quadrats. Seines capture unselectively, in that they are an active survey method that relies on operator and equipment suitability, whereas bricks and quadrats offer fixed sizes suitable for juveniles. In addition to occupying the holes with the closest diameter to their own, juvenile crayfish were found in the interstitial spaces on quadrat sampler equipment (hay; horticultural insulated roofing plastic < 10 mm ⌀) and in the spaces inside of attaching cable ties on perforated bricks.

Crayfish are highly thigmotactic (Burba, 1987; Alberstadt et al., 1995; Antonelli et al., 1999) seeking out refuges that match their carapace circumference which will provide maximum protection and defendability. If crayfish occupy the smallest burrow/ refuge possible, they may potentially avoid having to defend it from a larger individual. When trap aperture diameters were altered, particularly in the four entrance sizes of minnow traps studied, the specificity of the sizes caught was significantly different, except for the largest trap apertures (minnow medium and large). *P. leniusculus* will fold their chelae to block an entrance (Ranta and Lindström, 1992a, b) with even one large chelae assisting with the occupation, via offering greater defendability, of a larger burrow. So aperture size becomes less crucial as crayfish size increases, in agreement with the assertion that the thigmotactic response also lessens (Trèpanier and Dunham, 1999; Stoeckel et al., 2011).

Juvenile crayfish are vulnerable to predation by invertebrates, fish and mammals and cannibalism by other juvenile and adult conspecifics (Mason, 1975; Momot and Gowing, 1977; Brewis, 1978; Cukerzis et al., 1978; Hogger, 1986; Westman, Savolainen and Pursiainen, 1990). The type III survivorship curve described by Krebs (1994) depicts losses due to natural mortality (up to 90%) in the first year for invertebrate populations including *Pacifastacus* (Flint, 1975; Tcherkashina, 1977; Shimizu and Goldman, 1983). This implies that, for a short time, there is an abundance of juveniles which current sampling methods fail to detect (Peay, 2004). The novel samplers utilised in this study, however, caught large numbers of juvenile crayfish which points towards differences in behaviour being of consequence.

Juveniles are highly refuge dependent, so incorporating refugial spaces may make equipment attractive to smaller individuals. Juveniles require high habitat complexity (Appelberg and Odelström, 1988; Rabeni et al., 1997; Olsson and Nyström, 2009) and show a preference for cover that provides sustenance (Blake, Nyström and Hart, 1994) though they make consume woody debris/ detritus out of necessity rather than choice (Bondar, Zeron and Richardson, 2006). Interstitial spaces were used by juvenile crayfish in this study (hay bundles and quadrat samplers) in common with microhabitat traps (Kusabs and Quinn, 2009; Warren, Sheldon and Haag, 2009; Parkyn, DiStefano and Imhoff, 2011). The marginal habitats and vegetation do vary at the three sites studied on the River Lark though the impact that this may have on the distribution of juvenile crayfish has not been fully explored in this study.

Perforated bricks offer advantages over other sampling methods as replicates can be obtained in large numbers and they are robust, self-weighted and easy to empty. There is no possibility of by-catch, and incidences of disturbance/ vandalism are reduced. Long-term refuge sampling may help assist with tackling any potential production vs. attraction issues. Brick samplers can capture juvenile crayfish during the winter and can be left in *situ* for long time periods (facilitating sampling during periods of low activity), which may overcome ‘detection’ thresholds. Juvenile crayfish were also caught using minnow small traps, with the combined catch of small aperture traps and perforated bricks moving the ‘under-represented’ size classes from < 10 mm POCL into the 18.5-30.0 mm range. Trap design may bias catch size, mainly via aperture diameter (though mesh size may also be of consequence) with minnow medium traps catching significantly smaller crayfish and the Trapman catching the widest size range, though often the smallest numbers.

Traps are considered inefficient for capturing juvenile crayfish (Peay and Hiley, 2004), though there are a number of reasons why traps may be less appealing to smaller crayfish. The size of traps, and their spacing, means that juvenile crayfish (< 21 mm POCL) need to travel large distances relative to their body size to locate a trap and climb inside. This hypothesis could be tested via the use of traps scaled to suit juveniles deployed in large numbers. Competitive exclusion by large male crayfish (Momot and Gowing, 1977; Lodge, Beckel and Magnuson, 1985; Rach and Bills, 1989) may discourage juveniles from entering traps or may prompt their escape through aperture or mesh (Fjälling, 2011). Traps usually lack internal refuges, which may retard entry, prompt escape, or both. Juvenile crayfish may be cannibalised once inside a trap, though this has not been verified in the field (Qvenild and Skurdal, 1987).

Trapping may be unsuitable for sampling juveniles due to their high moult frequency and 'risk-sensitive' behaviour (Bondar, Zeron and Richardson, 2006) which reduces activity and therefore availability for capture. Trapping is considered financially costly (Peay, 2000) with a high risk of vandalism/ equipment loss and by-catch including fish, otters and water vole (Peay, 2000; Rogers and Watson, 2005b). However, traps are not individually expensive and are robust when compared to seine/ electrofishing methods, and don't require constant effort and surveillance (Westman, Savolainen and Pursiainen, 1990). They are effective in a range of habitats and water depths, with bait acting as an attractant which allows coverage of a larger area (Moorhouse and MacDonald, 2010; 2011).

The bimodal population peaks observed when site data are combined (2010 and 2012) demonstrates a gap in the number of crayfish present ca. 21 mm POCL. This could be an artefact of the various methods employed, so sampling with equipment whose aperture sizes are between 20 and 30 mm should be trialled. Natural crayfish populations are considered to have equal sex ratios (Reynolds et al., 1992; Kirjavainen and Westman, 1999; Reynolds and Gherardi, 2012), though representation within samples varies with season and life history. Trap catches analysed for the month of August have a female biased sex ratio in agreement with the literature (e.g. Capelli and Magnuson, 1983; Hogger, 1986). The sex ratio of juveniles sampled in P18 and P24 bricks varied between months, with large numbers of unsexed individuals caught. August sex ratios in both P18 and P24 bricks were female dominated in common with trapping data. P24 bricks caught fewer unsexed individuals, with differentiation assisted as P24's catch slightly larger crayfish. Sex ratio may be a feature of size class rather than sampler bias, with females perhaps being dominant in juvenile crayfish.

Catch per unit effort varied considerably between traps, with aperture size specificity perhaps being one explanatory factor, though overall design may also have an impact. LiNi traps had the greatest CPUE (15.5) of all the nine trap types studied. Minnow mediums (a commonly used trap on the River Lark) only scored 7.7, whereas minnow 'smallmediums' (aperture diameter 30 mm), had a CPUE of 9.7. Crayfish may be motivated to leave a trap when bait runs out or competition/ aggression within the trap prompts escape. Juvenile catches were higher in minnow small traps containing refuge material perhaps due to the extra protection from cannibalism within the trap, or reduced escapes due to the refugia. As these traps were set in fours, away from traps with larger apertures and in complex habitats suitable for juveniles, robust inferences are not possible. However, minnow small traps set amongst traps designed for larger crayfish had a CPUE of only 1.5. The CPUE of brick samplers was consistent whether bricks were set individually or in quadrat sampler arrays (though the size classes caught on sampler arrays were usually juveniles), with P24 bricks having a CPUE of 4.5 to 6 compared to P18 samplers which had a consistent CPUE of 1.4. This must be a feature of the behaviour of smaller crayfish rather than their distribution/ abundance as logically there must be greater numbers of smaller crayfish for them to grow into larger crayfish. Therefore the numbers of crayfish available for sampling using P18s (which capture smaller crayfish than P24s) must exceed that of the potential number of P24 sized individuals if natural mortality, predation and cannibalism are taken into account.

In conclusion, traps themselves are not selective of larger individuals. It is the aperture diameter that determines individual size and, to a lesser extent, the quantity of catch. The possibility exists that if the researcher is able to provide the right sizes of holes they will be able to sample all sizes of crayfish.

# CHAPTER 5: VARIATION IN SIZE AND SEX STRUCTURE OF THREE RIVER LARK *P. LENIUSCULUS* POPULATIONS

## 5.1 Introduction

The management of NICS, in particular *P. leniusculus*, is of importance in a UK, European and global context due to their deleterious impact on freshwater biodiversity and habitats (Genovesi, 2005; Dudgeon et al., 2006; Strayer, 2010). As limiting factors are lacking (Chapter 3), the growth and spread of *P. leniusculus* populations are unbounded where 'burrowable' habitat exists (Goldman and Rundquist, 1977; Ranta and Lindström, 1992a; Steele et al., 1997; Antonelli, Steele and Skinner, 1999). As crayfish populations increase, movement and range expansion may follow (Figure 1.3), so population reduction may only be mediated via external control/ management practices. However, research into NICS control in the UK is hampered by extrapolating from inferences in the European literature, where sustainable management (and stock production) is the aim.

In Sweden and Finland both native and non-native crayfish are exploited for food with fisheries managed in an attempt to ensure sustainability (Jussila, 1995; Edsman and Söderbäck, 1999; Edsman, 2004; Jussila and Mannonen, 2004), leaving few (if any), dense untrapped populations for comparative study. As *P. leniusculus* has a long lifespan (>10 years; Lindqvist, 1977; Belchier et al., 1998), the formation of static life tables is rarely possible (Vandermeer and Goldberg, 2003), so inferences come from 'snapshots'. These are predominantly drawn from trapped populations, or from research where the focus is not on control or management but the production of juveniles (Keller, 1999a, b) so findings cannot be universally applied (Peay, 2001; Peay and Hiley, 2004). Fortunately, inferences from longer term projects are now available, with Hein, Vander Zanden and Magnuson (2007) reporting on five years of trapping and enhanced fish predation resulting in a population crash of *Orconectes rusticus* (rusty crayfish) in an invaded American lake.

Drastic catch reductions of *P. leniusculus* have been observed in commercially trapped lakes in Sweden (Sandström et al., 2014) and now also in Finland (Jussila et al., 2014). In both studies environmental parameters are cited as being potential causes of population declines with fishing mortality given scant attention, though noted by Sandström et al., (2014) as worthy of further research.

### 5.1.1 Catch per unit effort

Catch per unit effort (CPUE) is an accepted way of estimating relative population abundance, particularly in fisheries management (e.g. Miller, 1990; Westman, Savolainen and Pursiainen, 1990; Olsson et al., 2010; Hudina et al., 2012). Other methods (e.g. capture mark recapture: CMR) offer more precise estimates but are resource-heavy (Nowicki et al., 2008). CPUE may be less sensitive than CMR but is similarly influenced by seasonal water temperature variation (Firkins and Holdich, 1993), with deep-water winter migrations observed in both crayfish (Flint, 1975) and lobster populations (Robichaud and Campbell, 1991). As sampling equipment, effort and deployment may also influence catch (Brown and Brewis, 1978; Dorn, Urgelles and Trexler, 2005), CPUE estimates may only provide relative indices (Zimmerman and Palo, 2011).

### 5.1.2 Size distribution: How size and age vary in crayfish

Crayfish size increase, in common with other shellfish (Waddy and Aiken, 1990) is incremental and facilitated by ecdysis, with growth rate determined by moult increment and frequency. This is in turn affected by population density, dominance hierarchies, resource availability (including food and habitat), photoperiod and the individual's metabolic rate (Jussila and Evans, 1996; Holdich and Lowery, 2002). Size is therefore an unreliable indication of crayfish age as it reveals more about the environment than the passage of time (Reynolds and Gherardi, 2012).

Ageing crayfish by measuring the density of nervous tract lipofuscin granules remains the most reliable technique, with maximum lifespan estimates of 16.7 years for *P. leniusculus* (Lindqvist, 1977; Belchier et al., 1998). Separation of juvenile from adult size classes is problematic as there is considerable size overlap between individuals from year 0+ to four (France, Holmes and Lynch., 1991; Edsman and Jonsson, 1996). Normal distribution curves for cohort analysis have been derived using the following techniques:

- **Peterson interpretation** of the principal peaks in polymodal length-frequency distributions (Grant, Morgan and Olive, 1987).
- **Bhattacharya plots** (Bhattacharya, 1967; Scalici and Gherardi, 2007).
- **Von Bertalanffy/ Bertalanffy growth curves/ ELEFAN analysis** (Fidalgo, Carvalho and Santos, 2001; Scalici and Gherardi, 2007; Dörr and Scalici, 2013).
- **Length frequency distributions** (Westman et al., 1986; Fidalgo, Carvalho and Santos, 2001).

The size structure of crayfish populations may be affected by density or resource competition or both, with ‘stunted’ populations (smaller individuals that breed younger or at a smaller size) potentially the result (Avault, De La Bretonne and Huner, 1975; Huner and Romaire, 1978; Romaire, Foster and Avault, 1978). In the UK trapping is considered to be the main cause of stunting (Peay and Hiley, 2004), with inferences on NICS population structure (and population regulation) widely cited (Chapter 1).

### 5.1.3 Sex ratios and life stages

Sex ratios in crayfish are usually even (e.g. Reynolds and Gherardi, 2012) though trapping is considered to selectively remove large males (Mason, 1975; Capelli and Magnuson, 1983; Olsen, Lodge and Capelli, 1991; Momot, 1995; Peay, 2000). Although seasonal behavioural changes in males and females can vary their trappability (Richards et al., 1996; Alekhnovich et al., 1999; Holdich, Reeve and Rogers, 1995), male trap bias was not observed in this study. The removal of large males from a population is considered problematic as they are postulated to have a regulatory role which decreases/ controls the number of juveniles (Momot, 1995; Ibbotson et al., 1997; Rogers and Holdich, 1998; Sibley, 2000; Smith and Wright, 2000; Wright and Williams, 2000; Reeve, 2004; Ribbens and Graham, 2004; Freeman et al., 2010; Sibley, Holdich and Richman, 2011). As juveniles have in the past been challenging to sample (Chapter 2) inferences on their numbers, and juvenile sex ratios, are relatively unexplored and so left unchallenged (Peay, 2001; Peay and Hiley, 2004). Juvenile sex ratios in this study appeared female biased in common with juvenile lobster (*Homarus americanus*; Robichaud and Campbell, 1991) and fiddler crab (*Uca pugilator*; Johnson, 2003) populations.

In order to evaluate the impact of management strategies population structure must be characterised. The size structure of *P. leniusculus* populations at three River Lark sites were analysed using Principal Components Analysis (PCA: Palmer, 2008), Bhattacharya plots (Bhattacharya, 1967), juvenile to adult ratios and size frequency distributions. Mean smoothed data is considered for adults (2010 to 2012) to examine yearly trends alongside sex ratios and CPUE.

## 5.2 Method

General methods (Chapter 2) are supplemented here with population analysis methods.

**PCA:** Mean smoothed data for each size class (separated into one mm POCL intervals) were analysed with divisions based on three sites studied over three years resulting in nine categories. Data were manipulated so that the percentage contribution of each size class was expressed as a decimal with values totalling 1 for each site and year combination. This contraction of data is necessary for multivariate analysis where data ranges must be simplified and of equal magnitude (e.g. $1<10$, $10<100$, $100<1000$). Bias in the analyses that may arise from unequal catch sizes at the three sites is obviated by use of percentage, mean smoothed data. These analyses are designed to determine differences in population structure by site and year.

**Bhattacharya plots:** These were developed by Bhattacharya (1967), from principles developed by Buchanan-Wollaston and Hodgson (1929). Populations are best represented by normal distribution curves which can be derived from data via the use of natural logs/ equations to linearise the data. In this way ‘pseudo-cohorts’ are derived via the general equation:

$$\text{Bhattacharya coefficient} = \ln (N_{x+1}) - \ln (N_x)$$

Key: ln = natural log and N = number of individuals of a particular size.

## 5.3 Results

### 5.3.1 Juvenile and adult crayfish sampling methods over time

Three sites on the River Lark were studied to gather data on juvenile and adult crayfish. Juvenile methods were developed in 2011 with their use continued in 2012, whilst adult capture data (mostly from traps), was used consistently from 2010 to 2012 (Table V.I).

Table V.I. Main sampling methods referred to in these analyses (2010 to 2012).
Four trap types = 'standard set' (p24, p.25).

| | Aim | Barton Mills, Lark Head, Plough | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
| 2010 | Adults | | | | | | Four traps types; two locations; three sites | | | | | | |
| | Juveniles | | | | | | | | | | | | |
| 2011 | Adults | | | | | | Four traps types; two locations; three sites | | | | | | |
| | Juveniles | | | | Quadrats and perforated bricks | | | | | | | | |
| 2012 | Adults | | | | | | Four traps types; two locations; three sites | | | | | | |
| | Juveniles | Quadrats and perforated bricks | | | | | | | | | | | |

### 5.3.2 Population size as measured by relative CPUE at three sites (2010 & 2012)

Mean CPUE was relatively stable at Barton Mills from June to August in both years, compared to Lark Head, where rapid increases occurred between June and July followed by a slight fall in August (Table V.II). In contrast the Plough showed a gradual increase from June to August in both 2010 and 2012 (Figure 5.1; Table V.II). Seasonal and yearly CPUE values did not differ significantly for Lark Head and the Plough, though large standard deviations in CPUE were discernible at all sites in all years and seasons with the exception of Barton Mills in 2012 (Table V.II).

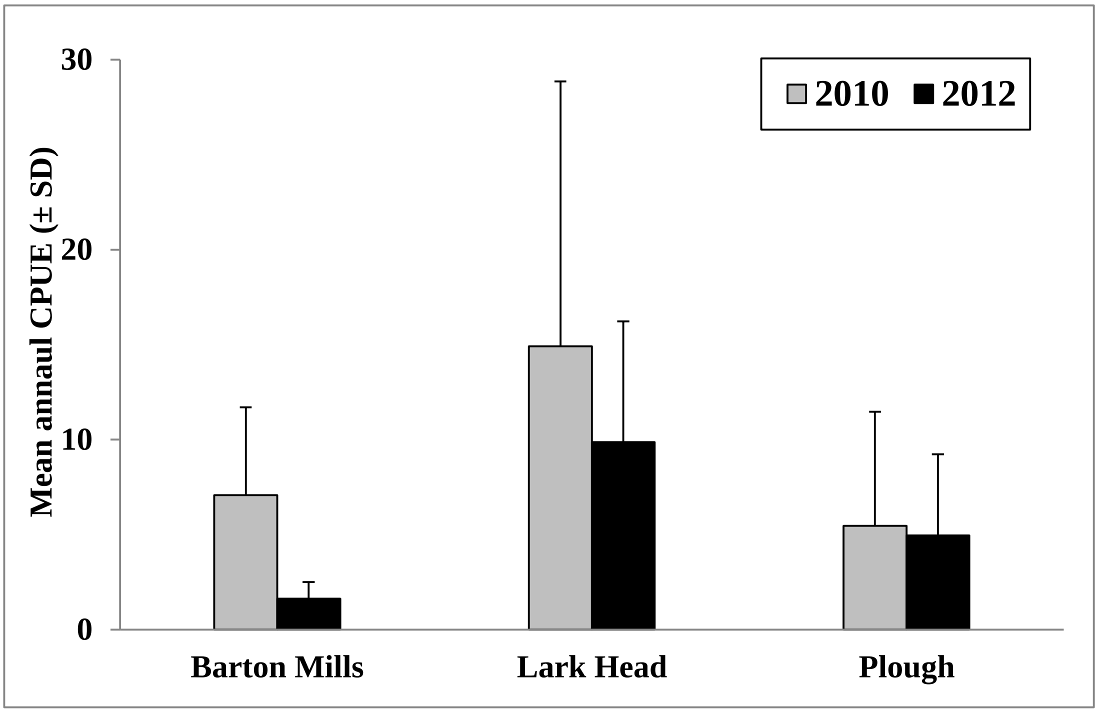



Figure 5.1. Mean annual trap catch per unit effort (with standard deviations) at three sites on the River Lark (June-August 2010 & 2012) [CPUE is defined in this study as the number of crayfish caught per equipment item per session].

CPUE decreased at all sites from 2010 to 2012 (Figure 5.1), with a reduction of 62.5 % at Barton Mills, and 20.4 % at Lark Head. A 4.8 % reduction at the Plough was also demonstrated (Table V.II).

Relative CPUE at the three sites altered from 2010 to 2012 as shown:

2010: **LH** > **BM** > **P**          2012: **LH** > **P** > **BM**

Table V.II. Mean catch per unit effort (CPUE) for June, July and August (2010 and 2012) for three sites on the River Lark with different crayfish management strategies (mean values are reported with standard deviations). Percentage CPUE decrease is the difference between 2010 and 2012 divided by the total of both years.

| | 2010 | 2012 | 2010 | 2012 | 2010 | 2012 |
|---|---|---|---|---|---|---|
| **MEAN CPUE** | **BM (community trapping)** | | **LH (professional trapping)** | | **P (no trapping)** | |
| **mean June** | 6.38 ± 3.77 | 1.13 ± 0.33 | 6.13 ± 4.59 | 8.50 ± 5.63 | 3.13 ± 3.06 | 3.50 ± 2.83 |
| **mean July** | 7.75 ± 5.26 | 1.88 ± 0.93 | 20.50 ± 16.33 | 10.00 ± 5.57 | 3.63 ± 4.18 | 5.13 ± 3.85 |
| **early August** | 7.13 ± 4.28 | 1.88 ± 0.93 | 18.13 ± 12.32 | 11.13 ± 7.04 | 9.63 ± 7.12 | 6.25 ± 5.09 |
| **Yearly mean** | 7.08 ± 4.52 | 1.63 ± 0.86 | 14.92 ± 13.64 | 9.875 ± 6.21 | 5.46 ± 5.88 | 4.96 ± 4.18 |
| **% CPUE decrease** | **62.5%** | | **20.4%** | | **4.8%** | |

### 5.3.3 Size frequency distribution (2010 to 2012) using a range of sampling methods

A distribution that is at least bimodal is apparent when three years data collected using bricks, quadrats and traps are combined for all three sites (See Chapter 4; Figure 4.1). Juvenile crayfish (< 21.00 mm POCL; Chapter 2: General Methods) peak in the range 11 ≤ 19 mm POCL whilst the peak contribution made by adults (≥ 21 mm POCL) was 24 ≤ 40 mm POCL. This implies a distinction between juvenile and adult size classes.

### 5.3.4 The ratio of juvenile to adult crayfish at three sites in 2011 and 2012

A 1:1 ratio of juveniles to adults was evident at Barton Mills and Lark Head (BM: $X^2_{1\ adj}$ = 1.51, n = 1856, P = 0.21; LH: $X^2_{1\ adj}$ = 0.26, n = 3999, P = 0.60) though there were significantly more adults than juveniles (1: 0.88), at the Plough ($X^2_{1\ adj}$ = 9.22, n = 2377, P = <0.01*). Overall the proportion of juvenile and adult crayfish at each site was consistently close to parity (Figure 5.2).

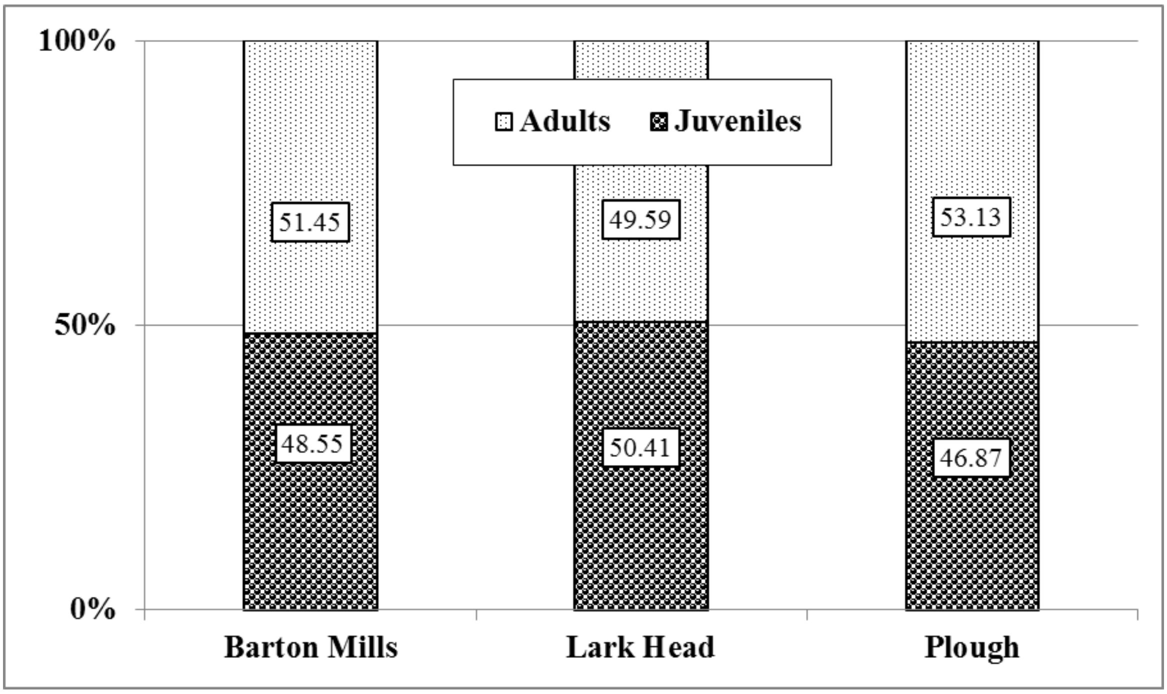



Figure 5.2. Percentage of juvenile (< 21 mm POCL) and adult (≥ 21 mm POCL) crayfish caught (2011 and 2012 combined) using consistent equipment and effort at three sites on the River Lark (juveniles in bricks/quadrats/traps n=4041; adults in traps/ plus all other samplers n=4211).

**5.3.5 Determining size classes using principal components analysis (PCA)**

When trap, quadrat and brick sampling data at all sites were combined (Table V.III) four size groupings were identified (juveniles, small, medium or large adults; Figure 5.3). When the data was constrained across four axes clear groupings could be identified suggesting that POCL measurements could be considered as distinct groups. The smallest group (termed 'One') described crayfish termed 'juvenile' with POCLs ranging from 6-20 mm. Group 'Two' incorporated all those with POCLs of 21-31 mm (small adults) whilst group 'Three' contained larger individuals of 32-40 mm POCL (medium adults) with group 'Four' representing a tight cluster of the largest individuals ranging from 41-57 mm POCL (large adults). No artefact effects were demonstrated (such as linear or horseshoe shaped groupings) which confirms that there were similarities between the grouped points and differences between the different clustered groups (Figure 5.3).

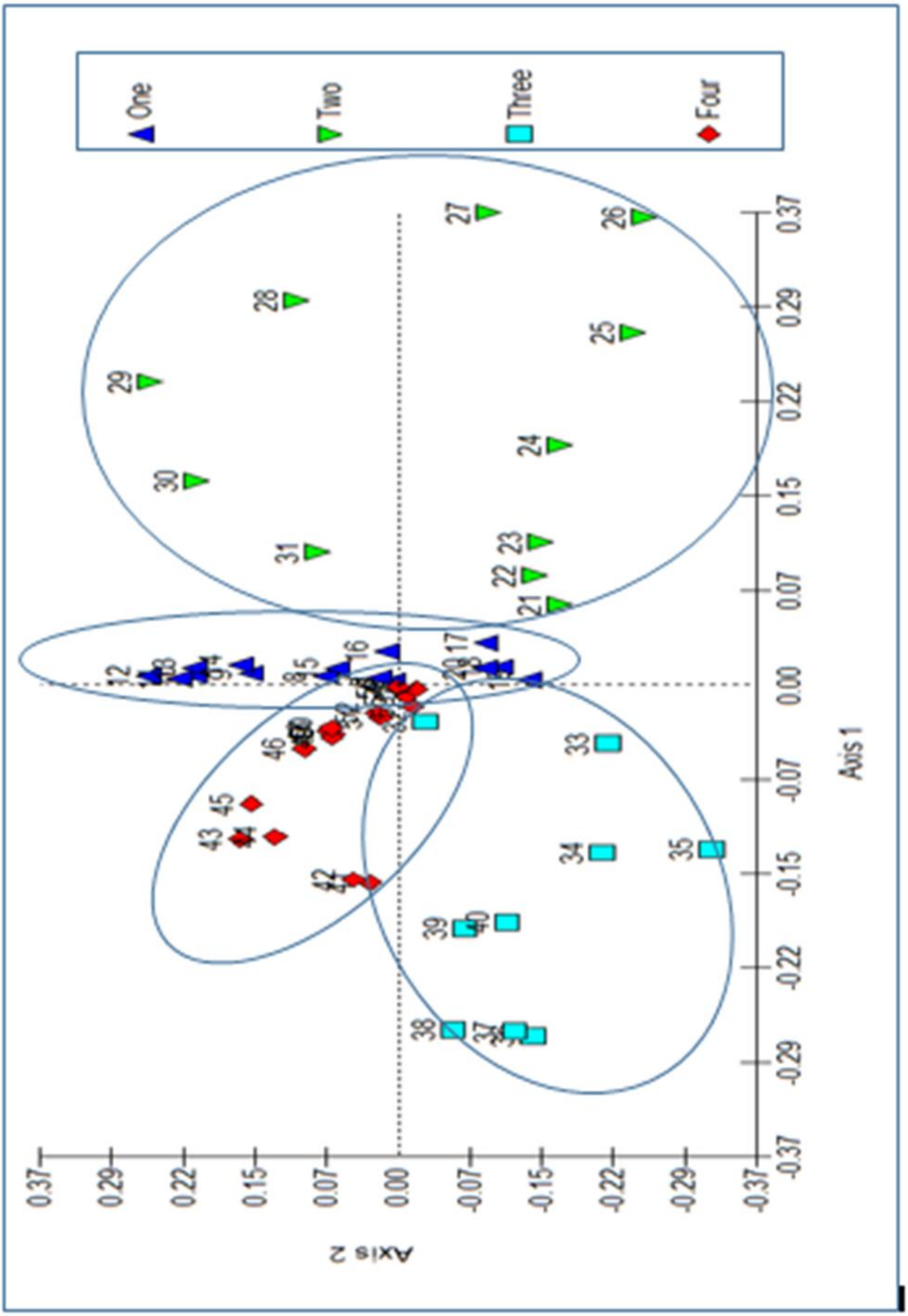



Figure 5.3. Principal Components Analysis (PCA) of size frequency data for all three sites combined (2010 – 2012) with colour coding of symbols to highlight groupings. Key: POCL mm size groupings One (6 - 20), Two (21 - 31), Three (32 - 40), Four (41 - 57).

### 5.3.6 Determining cohort centres using Bhattacharya plots & size frequency distributions

The theoretical basis of Bhattacharya plots was outlined in section 5.2 with the data here presented as Bhattacharya plots superimposed on the smoothed size frequency distributions of trap data (predominantly adults) at each site from 2010 to 2012. The distribution of potential cohort centres varied markedly between sites and years (Figures 5.4 - 5.6) with numerous potential cohort centres identified at some sites in some years whilst others displayed values that rarely crossed the Bhattacharya x-axis. Arrows were added on Bhattacharya plots only when the line crossed the zero on the x-axis going from high to low to reduce the risk of over-estimating cohort centres. However, when the data was pooled and mean values for potential cohort centres were obtained (using PCA groupings as a guide) the use of Bhattacharya plots was instructive providing clarification on potential cohort centres that aided analysis of this large dataset. There was close agreement between cohorts identified using Principal Components Analysis (Figure 5.3) and mean values obtained from pooled Bhattacharya plot mean cohort centre values (Table V.III).

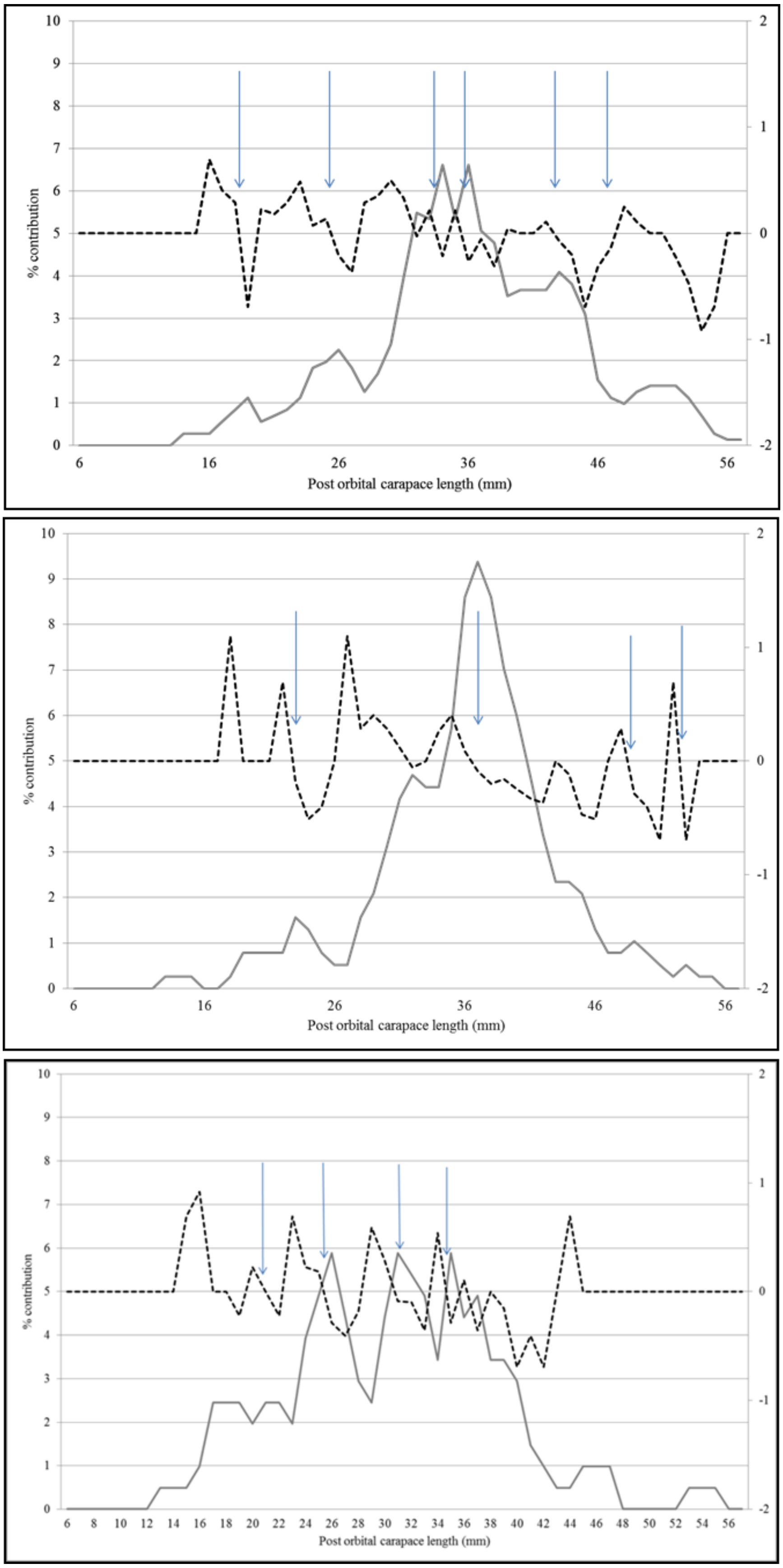



Figure 5.4. Barton Mills Bhattacharya plots/ size frequency distributions (top to bottom 2010, 2011, 2012 trap data only).

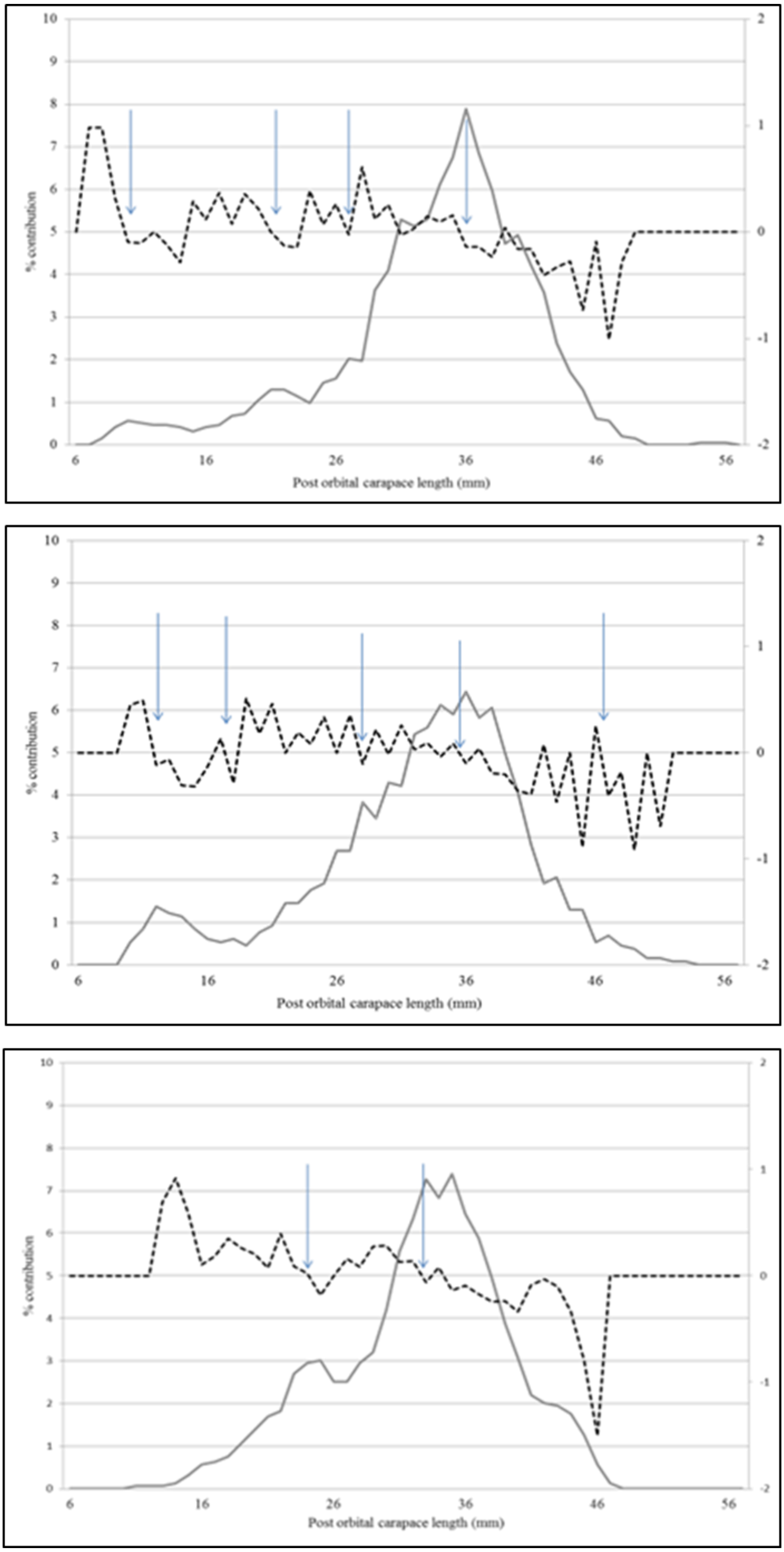



Figure 5.5. Lark Head Bhattacharya plots/ size frequency distributions (top to bottom 2010, 2011, 2012 trap data only).

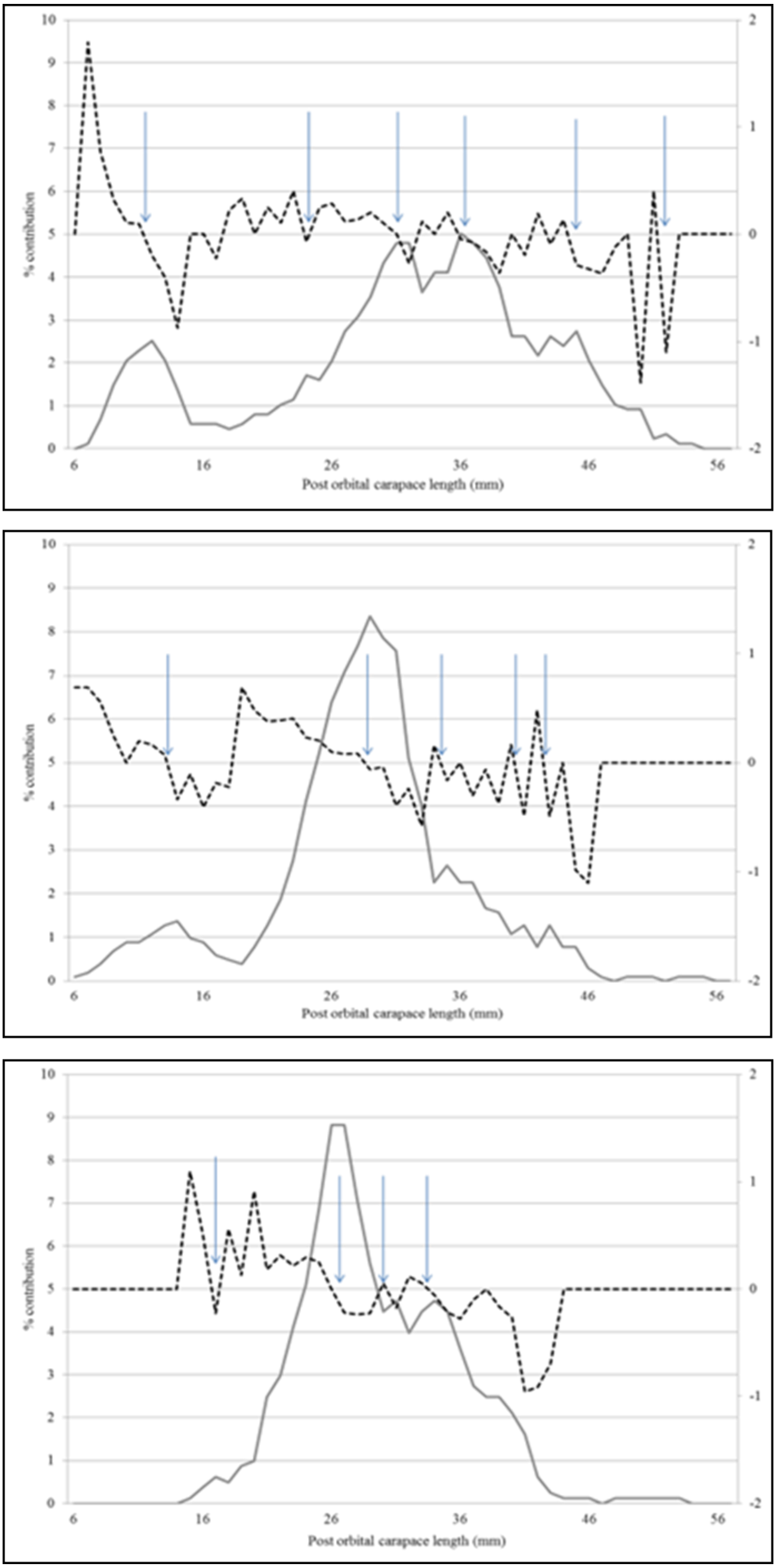



Figure 5.6. Plough Bhattacharya plots/ size frequency distributions (top to bottom 2010, 2011, 2012 trap data only).

PCA divided size frequency data into four distinct categories (juveniles and small, medium or large adults). The number of arrows (and their location) on Bhattacharya plots was determined with potential cohorts grouped according to the four (PCA determined) size categories (Figure 5.3), and the mean value in each category calculated. This mean size category was compared to the mean value of the cohort centre/ size class as determined by Bhattacharya plots. Only actual values were included in the calculation of Bhattacharya means with the difference (±) between the two means tabulated (Table V.III).

Table V.III. Comparison of cohort centres (peak of size distribution) as determined by PCA groupings and arrows on Bhattacharya plots (mean values are compared for both methods).

| | | Post orbital carapace length (POCL) in mm | | | | |
|---|---|---|---|---|---|---|
| | | **PCA groupings** | **2010** | **2011** | **2012** | |
| **B. Mills** | **Juveniles** | 6 to 20.99 | 18 | 0 | 18 | |
| | **Small adults** | 21 - 31.99 | 25 | 23 | 21; 25; 31 | |
| | **Medium adults** | 32 - 40.99 | 33 | 32; 37 | 35; 37 | |
| | **Large adults** | 41 - 57 | 43; 52 | 49; 53 | 0 | |
| **Lark Head** | **Juveniles** | 6 to 20.99 | 10 | 12; 18 | 0 | |
| | **Small adults** | 21 - 31.99 | 21; 27; 31 | 28 | 24 | |
| | **Medium adults** | 32 - 40.99 | 36 | 34; 36; 37 | 33; 35 | |
| | **Large adults** | 41 - 57 | 0 | 43; 47 | 0 | |
| **Plough** | **Juveniles** | 6 to 20.99 | 12 | 14 | 17 | |
| | **Small adults** | 21 - 31.99 | 24 | 29 | 26; 31 | |
| | **Medium adults** | 32 - 40.99 | 31; 36 | 35 | 34 | |
| | **Large adults** | 41 - 57 | 43; 45; 52 | 41; 43 | 0 | **Difference (mm)** |
| **Mean value** | **Juveniles** | **13.50** | **14.88** | | | **± 1.38** |
| | **Small adults** | **26.50** | **26.14** | | | **± 0.35** |
| | **Medium adults** | **36.50** | **34.73** | | | **± 1.76** |
| | **Large adults** | **49.00** | **46.45** | | | **± 2.54** |

The determination of four distinct size categories using PCA, and corroborated by Bhattacharya plots, prompts a second look at the size frequency distribution generated by combining data from traps, bricks and quadrats for the three study sites (2010 to 2012; Figure 4.1). Multi-year cohort estimates will mask the effect of cohort centres shifting year on year, depending on the conditions. Figure 5.7 (below) depicts the size distributions revealed by PCA which are noted on the x-axis to outline potential overlapping cohorts.

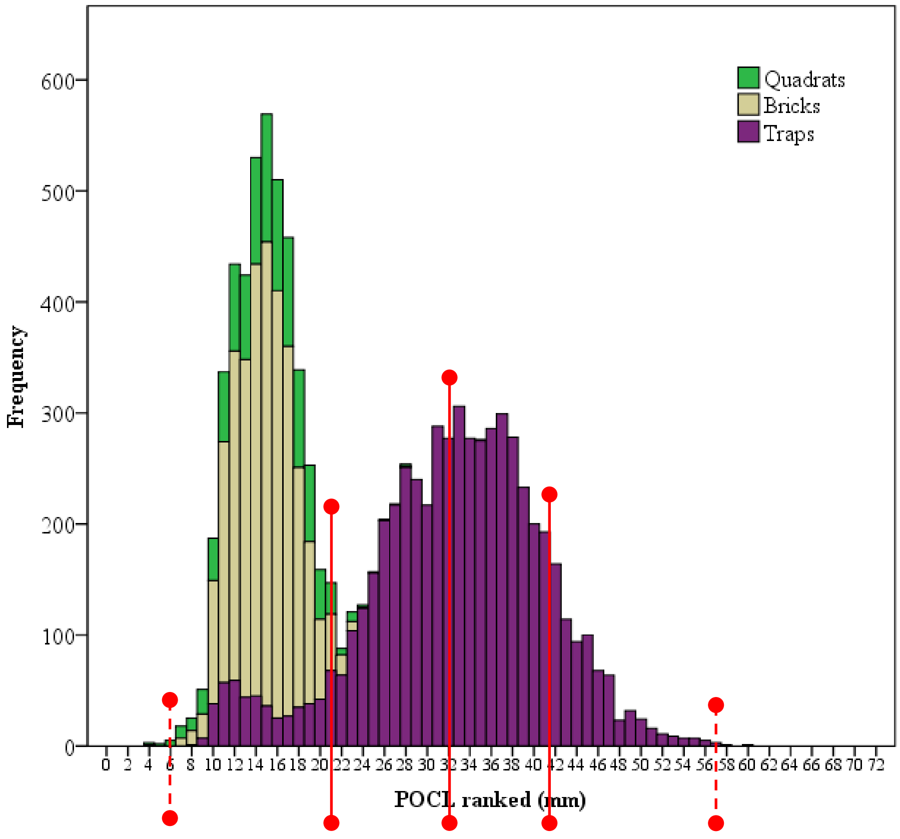



Figure 5.7. Distribution of size classes for all three sites combined (2010 - 2012) using three different types of sampling equipment with POCL ranked into mm size classes (n = 9707) (Red vertical divisions refer to PCA/ Bhattacharya size groupings; Table V.III).

As population size, and therefore catch, varies between sites the data are expressed as the percentage contribution of each size grouping at each site. Juveniles were consistently the largest category contributing 47.7 to 48.8 % at each site. Small adults contributed 26.5 % to the total population at the Plough, but only 16.3 % at Lark Head and 18.5 % at Barton Mills suggesting a larger proportion of small adults at the Plough. Medium adults were well represented at Lark Head (27.3 %) with a lower percentage at the Plough (18.8 %) and a midway value at Barton Mills (22.5 %). The proportion of large adults was highest at Barton Mills at 11.3 % with 9.0% at Lark Head compared to 5.9 % at the Plough (Figure 5.8).

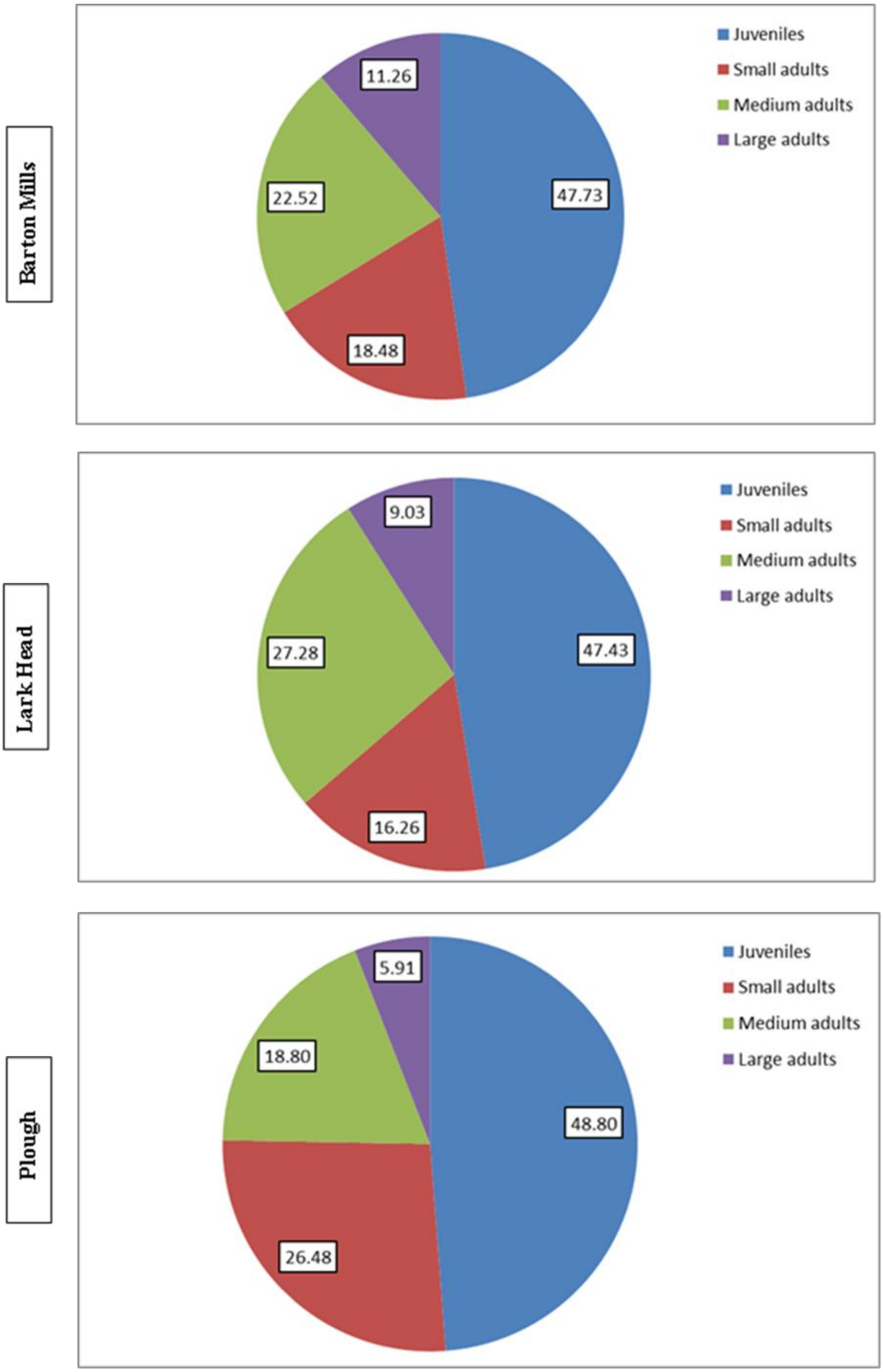



Figure 5.8. Percentage contribution of each of the four size groupings (2010, 2011, 2012) at the three study sites on the River Lark using consistent effort data.

Whilst the proportion of juvenile and adult (small, medium and large) size groupings shows consistency between sites (51 to 52%) the distribution of the three adult crayfish sizes varies (Figure 5.8). These data aid interpretation of size frequency distributions at the three sites (Figure 5.9). At Barton Mills (2010 to 2012) the distribution of adults (≤ 21 mm POCL) is flattened or platykurtic (ca. 18% small; **23**% medium; 11% large). Lark Head, however, has the largest proportion of medium sized adult crayfish demonstrating a normal/ leptokurtic distribution (16; **27**; 9 %), whilst Plough individuals are skewed towards smaller adults (**26**; 19; 6 %; Figures 5.8 & 5.9).

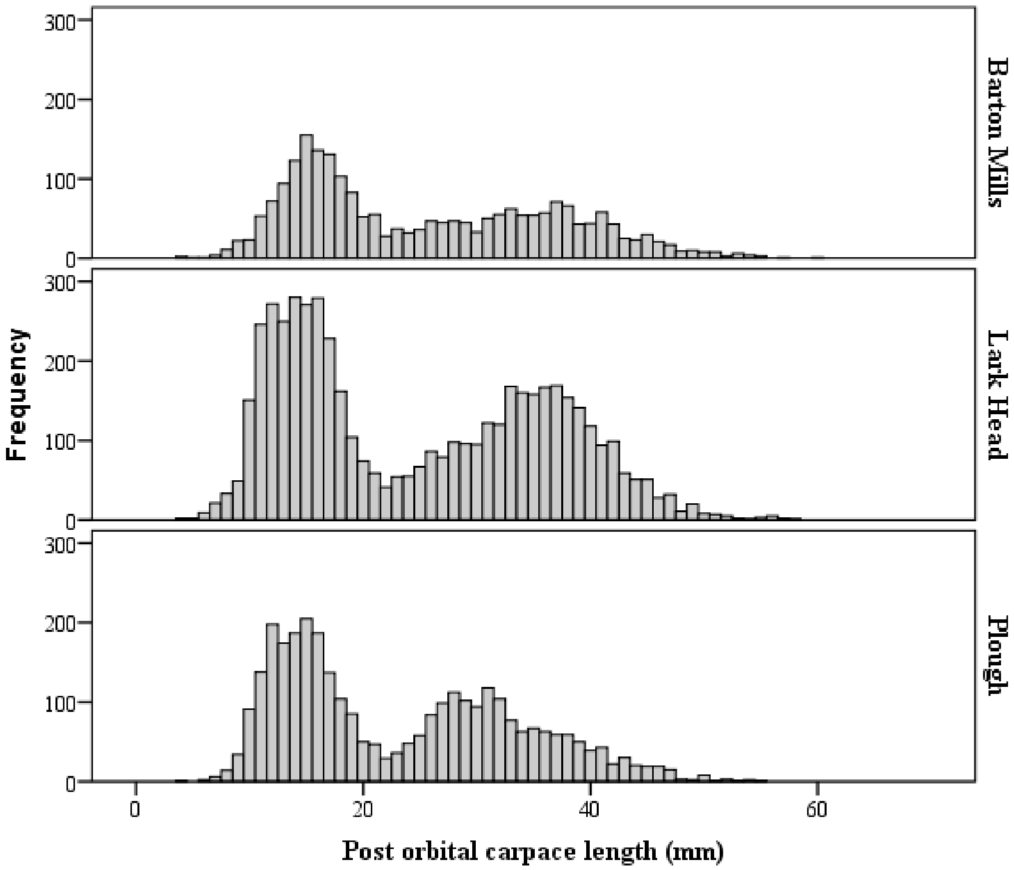
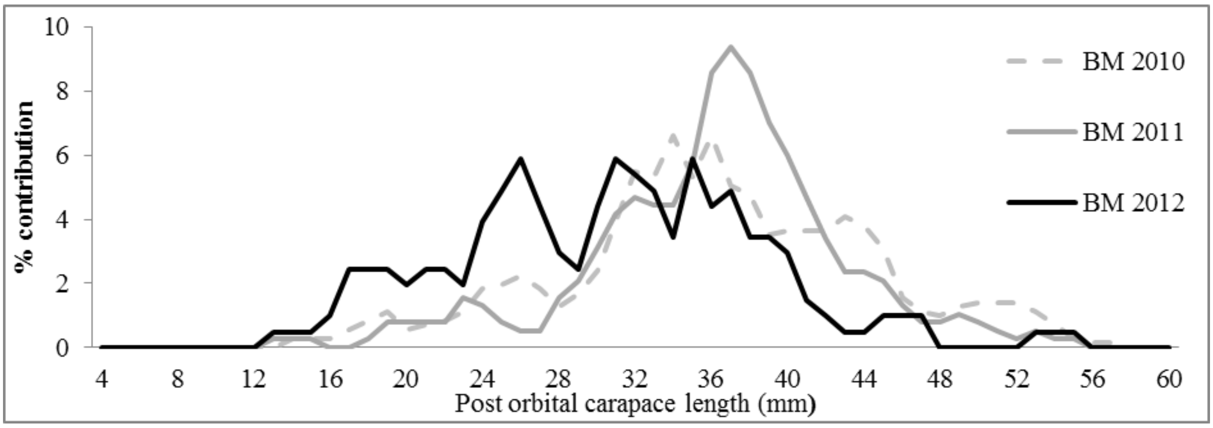
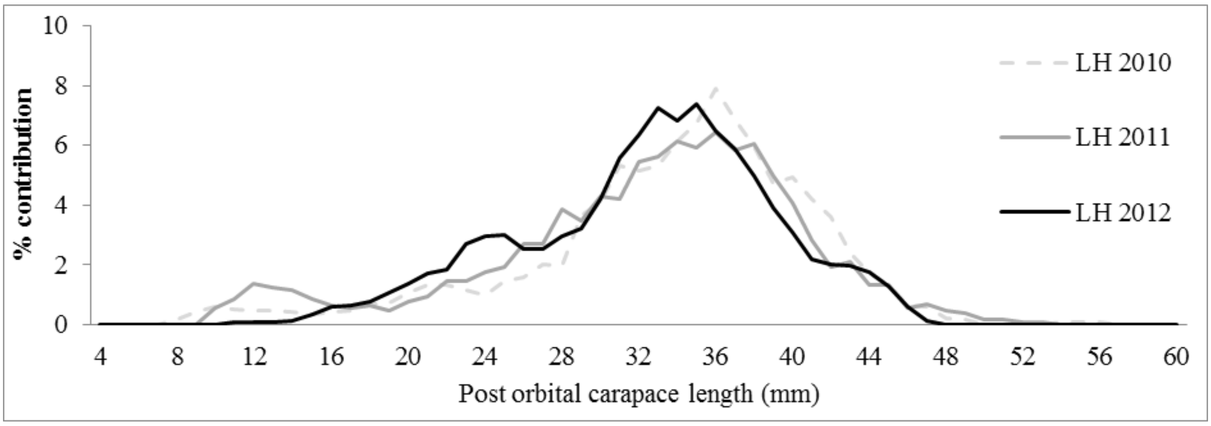



Figure 5.9. Population size distribution for *P. leniusculus* at three sites using all sampler types from 2010 to 2012 (BM n = 2297; LH n = 5119; P n = 3210).

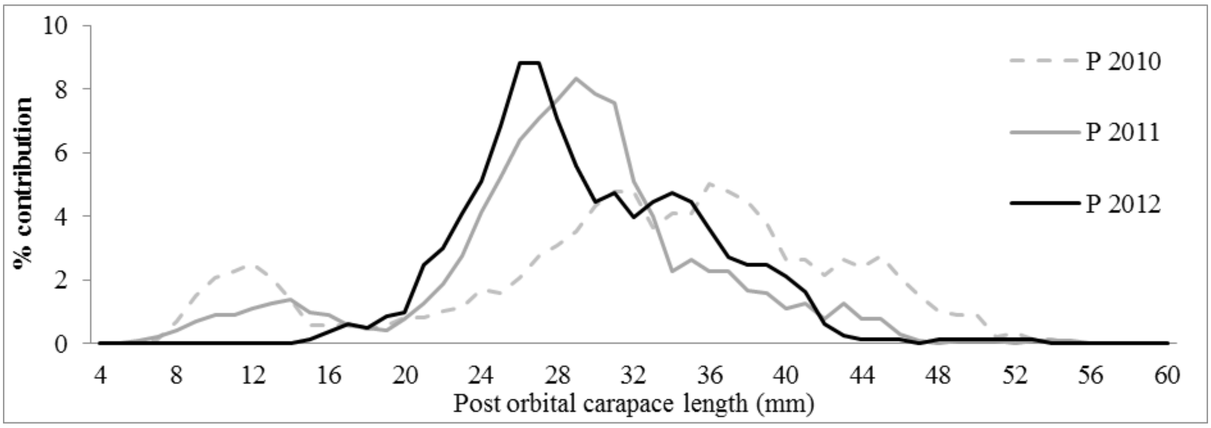
At Barton Mills and the Plough, the size of adults sampled with traps appears to be decreasing over time (Figure 5.10 i. and iii), whilst individual size at Lark Head remains stable (Figure 5.10 ii).




i. Barton Mills




ii. Lark Head




iii. Plough

Figure 5.10. Smoothed (4 value mean) percentage contributions of each mm size class (POCL) in each year of trapping (MM, MX, MC, PT) at i) Barton Mills ii) Lark Head and iii) the Plough.

A significant difference between the mean POCL of adult crayfish (for all sites combined), for 2010, 2011 and 2012 was determined via Kruskal-Wallis Anova ($X_2^2$ = 79.499, $n_1$ = 1171, $n_2$ = 1426, $n_3$ = 2816, P = < 0.01*), with Tamhane post hoc tests demonstrating significant differences between the mean POCL of crayfish caught in 2011 and 2012 (P = 0.23) but not 2010 and 2011 (P = < 0.01*) or 2010 and 2012 (P = < 0.01*). Similarly no significant difference in mean POCL was determined between the three sites via Kruskal-Wallis Anova ($X_2^2$ = 154.770, $n_1$ = 1190, $n_2$ = 2653, $n_3$ = 1570, P = < 0.01*), with Tamhane post hoc tests demonstrating that the mean POCL does not differ significantly between Barton Mills and Lark Head (P = 0.93), though there is a significant difference between the mean POCL at the Plough and Barton Mills and the Plough/ Lark Head (P = < 0.01 *).

### 5.3.7 Sex ratios in River Lark *P. leniusculus* populations

Unsexed individuals recorded during juvenile quadrat and brick sampling, if considered male, generate balanced sex ratios. However, if not all unsexed juveniles develop into males a female biased sex ratio would be apparent (Figure 5.11).


Figure 5.11. The number of male (M), unsexed (U/S) and female (F) crayfish sampled using three different methods (which focus on different life stages with the smallest crayfish captured using bricks) at Barton Mills (top), Lark Head (middle) and the Plough (bottom).

Chi-squared tests (females: unsexed individuals and males) showed no significant difference from a balanced (1:1) sex ratio at Barton Mills/ Plough with traps/ bricks with a female biased sex ratio with quadrats. Lark Head had significantly more females in traps/ quadrats, but not in bricks (Table V.IV).

Table V.IV. Chi-squared test results used to determine deviations from a 1:1 sex ratio comparing different methods and sites (* = significant deviation from 1:1 sex ratio).

| | Traps | Quadrats | Bricks |
|---|---|---|---|
| Barton Mills | $X^2_1$ adj = 0.30, P = 0.56 | $X^2_1$ adj = 4.43, P = 0.03* | $X^2_1$ adj = 0.42, P = 0.49 |
| Lark Head | $X^2_1$ adj = 43.98, P = <0.01* | $X^2_1$ adj = 1.72, P = 0.03* | $X^2_1$ adj = 0.09, P = 0.74 |
| Plough | $X^2_1$ adj = 0.21, P = 0.63 | $X^2_1$ adj = 11.60, P = < 0.01* | $X^2_1$ adj = 1.26, P = 0.25 |

## 5.4 Discussion

The analysis of *P. leniusculus* size and sex structure will be considered in relation to population density, size structure, sex ratios, removal history, density dependence, mortality and control efforts.

### 5.4.1 Population density

In 2010, Barton Mills CPUE was less than half of that at Lark Head, with a reduction from 2010 to 2012 at these two trapped sites. Lark Head had the highest CPUE of all three sites in both 2010 and 2012, whilst the Plough (untrapped), showed a reduction of 6%. Removal efforts at Lark Head and further downstream at the Barton Mills may be reducing CPUE at the Plough, with trapped areas acting as a potential “sink” for a wider area (Moorhouse and MacDonald, 2010). Populations may become more trappable as ‘trap happy’ crayfish become familiar with the equipment (Brown and Brewis, 1978; Erkamo, Kirjavainen and Tulonen, 1992; Fjälling, 1995; Daniels, Petrosky and Wujtewicz, 1997; Harlioğlu, 1999; Campbell and Whisson, 2000; Mangan, Ciliberto and Homewood, 2009; Mangan, Savitiski and Fisher, 2009).

One American lobster study demonstrated over 1000 ‘contacts’ around one trap in a single night (Jury et al., 2001) whilst actual catches < 10. At the Plough, catches increased from June to August each year, suggesting that crayfish are becoming increasingly familiar with traps at this previously untrapped site. If traps, the main harvesting method, initially attract, and/ or retain large male crayfish a reduction in population size may occur, with the movement of individuals also reduced. If population control is the aim, a focus on long-term adult removal is necessary. This study was conducted over three years so can only provide inferences on a small portion of the potential life history of *P. leniusculus*.

### 5.4.2 Population size structure

*P. leniusculus* populations in the River Lark have a bimodal size distribution, with a high proportion of juveniles’ sampled using novel equipment. As equipment and effort were standardised between sites, the proportion of juvenile and adult crayfish can be quantified as being in a ca.1:1 ratio, though there were slightly fewer juveniles proportionally at the Plough. Previous trapping may be responsible for the greater proportion of juvenile crayfish at Barton Mills and Lark Head, though there are alternative explanations. The Plough has a high proportion of favourable juvenile habitat which may be used in preference to the artifical refuges provided. In contrast, the banksides at Barton Mills and Lark Head are steep and eroded, offering excellent burrowing habitat for adults but less of the interstitial spaces needed by juveniles. It is also possible that increases to the populations at the Plough are relatively recent so juvenile production has yet to peak.

Crayfish become increasingly fecund with size (Mason 1963; 1977; 1979; Abrahamsson and Goldman 1970; Momot and Gowing 1977; Westman, Savolainen and Pursiainen, 1990; Söderbäck, 1993; Momot 1998; Celada et al., 2006), so the high proportion of small adults observed at the Plough could result in decreased juvenile production.  A more diverse and abundant range of fish predators, particularly stocked brown trout, may mean an increase in juvenile mortality at the Plough is skewing the population structure.

When PCA was used to analyse population size structure, four separate size categories emerged with these divisions in broad agreement with the literature for *P. leniusculus*.  For example, one Spanish study categorised juveniles as being ca. 15 mm POCL (Dana et al., 2010), while Davis and Huber (2007) categorised *P. leniusculus* as small (c.15-23 mm POCL) and medium sized adults (> 23 mm POCL) with no larger divisions.  Defining juveniles as being < 21 mm POCL incorporates all pre-sexual maturity individuals.

Juvenile crayfish are extremely vulnerable to predation/ cannibalism, whereas large adult crayfish are uniquely invulnerable.  Crayfish exceeding 42 mm POCL are (generally) released from the constraints of dominance hierarchies (Harrison, Hoover and Richardson, 2006; Fero et al., 2007; Martin and Moore, 2008) and are not vulnerable to gape limited fish predators (Didonato and Lodge, 1993; Keller and Moore, 2000; Garvey et al., 2003; Aquiloni et al., 2010).  Whilst dividing population data into only four size categories is undoubtedly an over-simplification, Bhattacharya cohort centres showed good agreement with PCA groupings and these divisions are instructive.

Lark Head adult crayfish are mostly 'medium,' whereas at the Plough adult crayfish are predominantly 'small'. The population at the Plough could be termed "stunted" though as this site is untrapped this cannot be attributed to trapping. It could be that the Plough population is composed of mostly younger/ smaller individuals, or that the lack of trap familiarity at this site is affecting catches (and sizes) so failing to represent the 'true' population. Predators may be selectively targeting larger individuals, though this is unlikely, with the most parsimonious explanation being that competition for resources is resulting in smaller "stunted" individuals as the population is both large and increasing (density dependence hypothesis).

### 5.4.3 Juvenile and adult sex ratios in River Lark *P. leniusculus* populations

All sites demonstrated a 1:1 sex ratio. Though adult sampling sex ratios vary throughout crayfish life history (with adult females become more trappable after brooding ceases in April: Lewis, 1998) juvenile sex ratios should be consistent. Male crayfish are polygamous (Guan and Wiles, 1997) so the number of adult females is limiting thus pointing to an excess of females being desirable. As juvenile mortality is high (Gherardi, 2007) ensuring a large number of potential females is available will assist with ensuring a 1:1 adult sex ratio. As competitive interactions in crayfish are mostly dependant on dominance hierarchies based on size, smaller females will fare worse in such interactions. As Barton Mills and Lark Head have been trapped for some years, potentially reducing the number of males, sampling of females may be facilitated.

### 5.4.4 Trapping as a control method

The study of three different reaches of the same river has facilitated the comparison of crayfish density and removal history, which will add constructively to the debate on the causes of 'stunting'. Throughout mainland Europe, but not the UK, crayfisheries are managed to try and ensure a sustainable yield of large crayfish which command a higher price (Edsman and Söderbäck, 1999). As trapped populations tend to be of significant size (thereby rendering removal cost-effective and/ or necessary), distinguishing between stunting caused by population density and that caused by size-selective removal is confounded. Literature on dense unexploited populations is unavailable so stunting has been attributed to trapping (Avault, De La Bretonne and Huner, 1975; Huner and Romaire, 1978; Romaire, Forester and Avault, 1978), though some authors note that if trapping were increased (Keller, 1999b) stunting would reduce conforming to the 'density promotes stunting' hypothesis.

### 5.4.5 Density dependence

Growth is limited by many factors, including competitive interaction, resource availability, and the incidence of predation and cannibalism (Mason, 1979; Goddard and Hogger, 1986; Keller, 1999a, b). In newly introduced populations, individual growth may initially be high due to reduced intraspecific competition (Hogger, 1986; Guan and Wiles, 1997). However, crayfish growth and size are affected by density, which inversely correlates with substratum area and perimeter when populations are large (Abrahamsson and Goldman, 1970; Romaire and Lutz, 1989; McClain, 1995a, b, c; Huner 1998, 1999; Keller, 1999a, b; McClain and Romaire, 2004), which is further corroborated by laboratory studies (Goyert and Avault, 1978).

Growth rates are slowed by intra-specific competition for food (Svärdson, 1972; Westman, Savolainen and Pursiainen, 1990), with aggression from large males restricting access to food/ shelter for females/ young adults (Rabeni, 1985; Ahvenharju and Ruohonen, 2007; Fero et al., 2007). In the absence of natural regulators (e.g. fish, large crayfish), density increases can result in stunting (Keller, 1999b; Lawrence et al., 2006a, b), though stress can also prompt breeding at a smaller size (Huner and Romaire, 1978; McGriff, 1983a, b). At Barton Mills (trapped) and the Plough (untrapped) the size of individuals reduced from 2010 to 2012, suggesting that density/ resources and/ or removal trapping may be affecting the size structure of the populations. By contrast, Lark Head, which has a greater width/ depth (Chapter 3), and where trapping has also taken place, showed a stable size distribution from 2010 to 2012.

### 5.4.6 Mortality and control/ management

Mortality is high in natural populations (Mason, 1975; Momot and Gowing, 1977; Brewis, 1978; Cukerzis, Sheshokas and Terenkyev, 1978; Hogger, 1986; Westman, Savolainen and Pursiainen, 1990), with the vast majority of the losses (≥ 90%) occurring in the first year of life (Flint, 1975; Shimizu and Goldman, 1983; Westman, Savolainen and Pursiainen, 1990). Lewis (1998), estimated 10% losses at age 2, with a decreased mortality rate subsequently conforming to a type III survivorship curve as described by Krebs (1994) for invertebrates. High levels of natural mortality amongst juveniles suggests that targeting this life stage (which provide food for native species and are the least damaging size class) would have less impact than the removal of sexually mature adults. In this study trapped areas show no proliferation or increased biomass of smaller crayfish, in contrast to the untrapped site that showed the lowest CPUE reduction and the largest proportion of small adults. Barton Mills and the Plough both show a non-significant reduction in the size of individuals over time.

A focus on reducing the size of individuals over time, whilst also reducing the number of sexually reproducing individuals, particularly females, could lead to longer term reductions in productivity.  Even if trapping preferentially removes males, which has not been demonstrated in this study, the continuation of any removal effort, including trapping, could lead to increasing removal of females as control efforts continue.  This study has demonstrated that CPUE has been reduced over time in trapped and untrapped sites, though the % reduction is far greater at trapped sites.  Similarly, the relative catch at the three sites varied with the CPUE at Barton Mills (‘community’ trapped since 2001) decreasing to such an extent that the Plough (untrapped) CPUE exceeded Barton Mills CPUE by the final year of the study.  These are large river reaches containing potentially very high numbers of individual crayfish all capable of range expansion.

# CHAPTER 6: DISCUSSION AND CONCLUSIONS

## 6.1 Discussion

### 6.1.1 Sampling method selection to assess population size structure

To consider the size structure of a NICS population one needs appropriate sampling methods. This study has demonstrated that juvenile (< 21 mm POCL), *P. leniusculus* can be sampled in the field using novel refuge materials, with perforated, 20 mm ⌀, bricks, being the most efficient. Small fixed apertures may appeal to highly thigmotactic juveniles, with hay bundles and PVC roofing squares offering suitable refugia for the smallest individuals. Aperture size is less crucial, though still important, as crayfish size increases in agreement with the assertion that the thigmotactic response lessens as crayfish age. As crayfish grow in size, and their chelae develop, they are more able to defend themselves and are therefore less vulnerable (Trèpanier and Dunham, 1999; Stoeckel, Helm and Cash, 2011). Trap entrance diameters that exceeded 20 mm ⌀ sampled fewer juveniles, with an aperture size of 30 to 40 mm ⌀ suitable for the most abundant adult *P. leniusculus* size classes.

Once trap aperture sizes exceeded 40 mm ⌀ the size specificity of the catch, and CPUE, were reduced for which there are two alternate hypotheses. Firstly crayfish may possess an accurate sense of their own size, as aggressive encounters are predominantly based on size related dominance hierarchies. If crayfish are able to select closely matching entrances, looser fitting diameters may be less appealing, or may precipitate more escapes. Secondly the number of crayfish of a given size within a trap is only representative of the number of crayfish retained, which may be affected by ease of egress as well as other trap occupants/ aggression, bait availability or time of day. For example, LiNi traps had a high CPUE which may be due, in part, to their fabric mesh offering refugial folds and an entrance funnel that closes behind them, which may reduce escapes.

Minnow small traps (20 mm ø), containing refuge material were more attractive to, or retentive of, juveniles. Small aperture size traps caught smaller crayfish with the range of sampling equipment used moving the previously 'under-represented' sizes from < 10 mm to 18.5-30 mm POCL. Specific aperture sizes in this range may be needed to fully represent smaller individuals within the population. However, there may be a gap between juvenile and adult size classes with final moult increment accounting for size discrepancies. It could be inferred that individuals in the range 18.5-30 mm POCL are almost completely absent, though lack of suitably sized apertures is a more testable hypothesis. Traps* are inherently unsuitable for the capture of juvenile crayfish, as they measure crayfish activity (low in vulnerable smaller size classes) and use bait as an attractant (refuge may be more important than food for juveniles). Traps with refuge material had a higher CPUE, which may be the result of greater numbers in the habitats chosen, enhanced retention, reduced cannibalism within the traps, or fewer aggressive encounters in and around the trap.

Traps may be less efficient if larger crayfish or fish predators are reducing activity, or if the size of the trap is out of proportion to the size of the crayfish (an issue of scale). The development of size-scaled traps for juveniles (ca. 10-17 mm ø), may offer an appropriate test in suitable complex marginal habitats. Additionally, juvenile catch may be enhanced by the incorporation of food into refuge traps.

*The term 'trap' describes the commercially available traps intended to capture, but not release, crayfish (Figures 9 – 14), in contrast to 'refuges' which have no barrier to entry or exit.

### 6.1.2 Crayfish behaviour and its effect on sampling

Crayfish behaviour has a number of impacts on sampling and activity patterns, with *P. leniusculus* known to be 'trap happy' (Brown and Brewis, 1978). The majority of trap designs permit both ingress and egress, so are 'leaky', with bait seeking behaviour learnt. If crayfish are able to enter and leave a trap at will, the exact moment of trap lifting determines catch. Catches increased month on month in every year of study at the Plough (untrapped site) which lends weight to this hypothesis. Saturation point, where the CPUE levels off as the number of traps/ trapping events becomes sufficient, may not yet have been reached at the Plough. The two sites that have been regularly trapped (Barton Mills and Lark Head) had catches that decreased from June to August each year.

Juvenile crayfish are more cryptic and 'risk averse,' than adults perhaps as a behavioural response due to their extreme vulnerability. Juveniles preference for complex habitats is well documented (e.g. Bondar, Zeron and Richardson, 2006; Parkyn, DiStefano and Imhoff, 2011), with refugia potentially acting as an attractant in this study.
Juvenile refuge samplers have been successfully trialled in this study, though if left in *situ* and not emptied, they may increase numbers in the longer term, potentially leading to overestimations (production increase). If additional refuges draw juvenile crayfish into an area, then sampling results may provide a snapshot of a wider area (attraction). The sampling radius of a baited trap is estimated to be 4 metres (Acosta and Perry, 2000) though given the small size of juveniles (and the relative distances involved), the effective sampling distance for an un-baited refuge sampler would be consistently smaller. Baited traps (where an odour plume may draw adult crayfish from a larger area), may not be accessible to juveniles due to the relative distances involved (and their low activity levels), though this can be tackled via the use of greater numbers of refugia set closer together.

Refuge samplers may assist with tackling crayfish detection thresholds whilst facilitating extensions to the optimum sampling periods when activity levels are low due to reduced water temperatures. Refuge samplers offer no risk of by-catch (though snagging and entanglement of anglers equipment/ wildlife remains a threat if equipment is tethered), with regular (weekly/ monthly) checking and emptying vital if niche availability for NICS is not to be inadvertently increased.

Refuge preference (artificial vs. natural) is pertinent for juveniles. If sufficient natural refuges are available (high structural complexity with tree roots and woody debris, bankside vegetation etc.), this could increase the number of juveniles which may, or may not, also make use of artificial refuges. Greater numbers of juveniles were found in areas with well vegetated banksides though this was not the focus of this study so there were insufficient replicates to investigate this fully.

### 6.1.3 The influence of environment on River Lark *P. leniusculus* populations

The River Lark has substratum, pH, temperature and BOD within the wide range of *P. leniusculus'* environmental tolerances. No significant difference was found between the three sites in January for BOD and pH spot values, though temperature did vary significantly. Depth, discharge and cross-sectional area differed significantly, with Lark Head being wider and deeper, with a lower temperature and slower flow than Barton Mills and the Plough. Temperature, to some extent, determines growth, though as crayfish were smaller overall at the Plough, which had a slightly higher water temperature, this does not explain size differences between the populations. There were significant differences between some of the substratum fractions (180 µm, 2 mm, pebble/ cobble), though overall substrata types were homogenous.

### 6.1.4 CPUE and carrying capacity

Lark Head, with its larger cross-sectional area and greater depth, may have a larger population carrying capacity than Barton Mills and the Plough. Lark Head had the largest CPUE consistently and has been professionally trapped since 2005. Professional trapping since 2005 has not produced measurable changes to the population size structure at Lark Head, which is consistent from 2010 to 2012 in contrast to Barton Mills (community trapped since 2001), and the Plough (not trapped), where the size of individuals decreased. A reduction in the size of individuals (with reproduction also taking place earlier, at a smaller size), is termed 'stunting' and has been attributed to both density and size selective removal/ trapping in other studies/ reports. If control and management of NICS in the UK is the aim, stunting is a positive, though the inference that trapping leads to individuals becoming smaller and an increase in the proportion of juveniles, is not supported by this study.

### 6.1.5 Trapped and untrapped sites: Is there a difference in population structure?

Smaller individuals may be easier to capture once larger individuals, who may aggressively defend trap entrances, have been removed, though in this study a trapped and untrapped site showed similar size decreases over time. A combination of initial population size, movement, density dependence/ resource depletion/ competition, and size selective removal may be in play. Lark Head adult crayfish were predominantly 'medium' whilst the adult size structure at Barton Mills was 'flattened' (with no adult size category dominating), whilst the adult population at the Plough was predominantly 'small'. A 'normal' population distribution (bell-shaped/ medium sized crayfish in the majority), was evident at Lark Head, the widest, deepest section studied.

The 'stunted' nature of the population at the Plough, in contrast to the population structure elucidated at Lark Head, may be attributed to density dependence in this shallow, narrow untrapped reach. The flattened distribution at Barton Mills may be due to long term trapping in this area with this reach potentially having a lower carrying capacity. Of the three study sites the Plough is in the best ecological condition, with abundant structural complexity and emergent vegetation. However, juvenile *P. leniusculus* were not as well represented during sampling at this site when compared to Barton Mills and Lark Head. The abundance of natural refuges at the Plough may make artifical refuges less necessary (and therefore less attractive) for juvenile *P. leniusculus*. However, there are a number of alternative hypotheses that may explain the smaller proportion of juvenile crayfish observed at the Plough. Increased predation at the Plough (due to stocked brown trout), may be limiting juvenile numbers, with fish predators known to prey on smaller individuals (Power, 1987). As adults are smaller at the Plough, juvenile production may be reduced. Alternatively the population may have yet to peak. The Plough has a large and expanding population (based on this study and reports from LAPS), so it is parsimonious to conclude that this increasing population is now competing for limited food resources resulting in 'stunting' due to density dependence.

### 6.1.6 The influence of management and population density

The Plough (untrapped) and Barton Mills (community trapped since 2001), have a size structure that appears to be decreasing over time, with a fall in the mean size of crayfish from 2010 to 2012. As both sites differ in their management history it cannot be assumed that this is a result of size selective removal altering the population size structure, though both density dependence (Plough), and size selective removal (Barton Mills), may be producing similar broad trends.

Lark Head, which has the largest *P. leniusculus* population (as determined by relative CPUE estimates), and the largest cross-sectional area, appears to have a stable size structure over the three years studied. Professional trapping since 2005 may be having a stabilising effect on the size structure of individuals whilst decreasing CPUE. Removal at Lark Head may also be having an impact on the upstream and downstream sites studied, by acting as a sink. If vacant niches are available this may potentially reduce active spread as the need for range expansion is lessened. In this way active management may reduce damage to biodiversity, habitats and native crayfish populations where this is relevant.

### 6.1.7 The impact of season and sex

Competitive exclusion by large male crayfish (Momot and Gowing, 1977; Lodge, Beckel and Magnusson, 1985; Rach and Bills, 1989) has been largely cited as the cause of male biased sex ratios in trap catches. However, in this study adult crayfish sex ratios were predominantly balanced, though more females were caught in August in agreement with the literature (Capelli and Magnuson, 1983; Hogger, 1986). Juvenile crayfish production may focus on maximising the production of 'potential' females to ensure optimal reproductive capacity. When all unsexed juveniles are considered to be male, sex ratios are equal.

Adult females often have vestigial copulatory pleopods so sex ratios in juveniles are likely to be biased towards females. The timing, or prompts, for juvenile sexual determination are currently unknown. In both aperture sizes of perforated brick a higher proportion of differentiated males were caught between May and August. It may be concluded that sex ratios are a feature of size class, not sampler bias, with females/ unsexed individuals dominating in sampled juveniles.

## 6.2 Conclusions

Population density was highest, relative to the other two sites (as estimated via CPUE), at Lark Head in both 2010 and 2012. CPUE reduction was lowest at the Plough, the untrapped 'control' site. It is possible that crayfish removal at Barton Mills and Lark Head provides a sink for the wider area with removal efforts potentially reducing spread. Assumptions made about population structure, and the effect of size selective removal via trapping, have remained as common currency in part due to an absence of adequate juvenile sampling methods. Targeting juveniles, however, would be an inefficient way of reducing population size due to the high mortality of juveniles. In this study CPUE at the two trapped sites decreased with no discernible increase in juvenile numbers or total biomass observed. Comparing trapped and untrapped reaches revealed that crayfish size varied between sites though this could not be attributed to trapping or to density dependence alone. If size reduction is a response to trapping, which has not been shown in this study, this would not be detrimental to any control/ management initiatives. Smaller crayfish are more vulnerable to predation and cannibalism and are less fecund, producing fewer eggs and smaller young. They are also less damaging to the environment as they make smaller burrows and can utilise natural refuges whilst also having a reduced impact on other flora and fauna.

## 6.3 Recommendations

### 6.3.1 Sampling

Traps are inherently unsuitable for the capture of juvenile crayfish but offer an accessible low cost sampling and removal method for adult NICS in all types of watercourses. The capture of adult crayfish, which represent the true size range of the population, can be achieved with aperture sizes ≤ 40 mm ⌀. Trap aperture construction that utilises fabric tubes, as in LiNi traps, may reduce escapes whilst not impacting on ingress of crayfish and should be investigated further. Refuge traps which provide a range of aperture diameters are important if juvenile sampling is the aim, though there is no justification for targeting juveniles in control/ management programmes.

### 6.3.2 Control and management

*P. leniusculus* control/ management programmes must be undertaken over a number of years (five at least; Hein, Vander Zanden and Magnuson, 2007) due to the longevity of this species. There is evidence to suggest that male catch might exceed female catch initially, though this may reduce as the male population is reduced in a given area. Trap familiarity may increase over time, with the potential that a range of different trap designs in a given area may encourage trap use generally, with catch increasing initially as the population becomes habituated to utilising this new food source. Overall there is evidence to suggest that crayfish size may be reduced, or stabilised, with both the density and size of crayfish having an impact on the area which they inhabit. Control/ management efforts should start as soon as possible as rivers appear to be increasingly impacted depending on the number of crayfish present and the length of time they have been resident. Overall the most important factors affecting a NICS population may be the number of crayfish present and the time since invasion.

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# APPENDIX A: CRAYFISH PHYLOGENY AND GLOBAL DISTRIBUTION

## Crayfish phylogeny

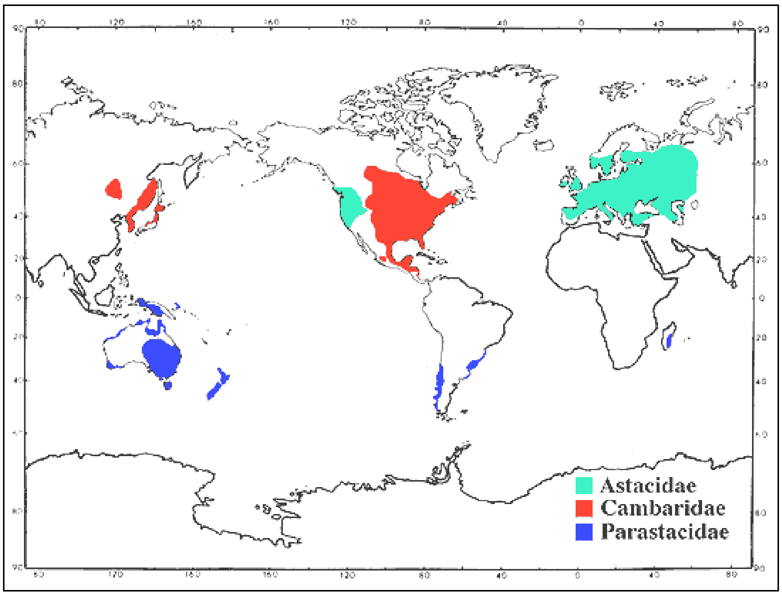
There are close to three quarters of a million species of arthropods, making it the largest phylum (Barnes, 1987), with adults characterised by their segmented body plan, sclerotized integument and jointed appendages (Lincoln and Boxshall, 1987). The subphylum Crustacea contains c. 28 000 mostly marine aquatic species (c. 42 000 species), though there are some freshwater and terrestrial taxa (Barnes, 1987). Decapoda, one of the largest crustacean orders includes shrimp/ prawns, crayfish, crabs and lobsters all of which are important as human food (Gibb and Oseto, 2005). In common with most shrimp/ prawns, crayfish and lobsters are not pelagic but benthic, making use of holes and crevices, or excavating shallow burrows, and rarely swimming. As with other decapods, crayfish exhibit autonomy (the spontaneous expulsion of a limb, usually chelae) as a predator escape mechanism (McVean, 1982), with lost appendages replaced over successive moults (Holdich, 2002a).

## The global distribution of Astacidae: Freshwater crayfish

Crayfish are the most successful of the freshwater decapods, and display a remarkable diversity in terms of size, colour, habitat and life form, with more than 590 species in 30 genera worldwide (Sibley, Holdich and Richman, 2011) in ponds, streams, lakes and caves. Twenty per cent of these species are found in Australia and only 1.5% in Eurasia (Taylor, 2002), with the remainder occurring in North America (Lindqvist, 1987).


Clockwise (i) *A. gouldii* (Giant Tasmanian freshwater lobster, a crayfish) held by Todd Walsh. Photo - The Mercury, Tasmania; (ii) *Engaeus fosser* (adult): Photo Niall Doran ; (iii) *Procambarus falix f. virginalis* (parthenogenetic 'Marmokrebs') Photo: Fabritius-Vilpoux & Harzschiv; (iv) *Orconectes incomptus* (troglobitic blind albino cave crayfish) Photo: Buhay (National Cave Diving Association); (v) *Engaeus martigener* (juvenile) Photo: Niall Doran. Sizes range from a maximum total length of 870 mm for *A. gouldii* (i) to < 5mm for a juvenile *Engaeus martigener* (v).

Crayfish of the family Astacidae are contained within the superfamilies Astacoidea, Cambaridae and Parastacoidea (Scholtz et al., 2003), with two other superfamilies now considered extinct. Indigenous crayfish species occur on all continents except Africa and Antarctica (Hobbs, Jass, and Huner, 1989). The northern hemisphere is home to crayfish of the superfamilies Astacidae and Cambaridae, whilst the Parastacidae are native to the southern hemisphere.




Global crayfish distribution (Tree of Life 2002)

Indigenous crayfish are harvested throughout their range (Jones et al., 2008; Holdich, 1991; Holdich and Lowery, 2002), though native crayfish consumption is considered by local tribes to be ‘fady’ (taboo) in parts of Madagascar, which may have assisted their conservation (Jones et al., 2008)

Crayfish species described by their family, scientific name, authority, date and common name (Pöckl et al., 2005; Souty-Grosset et al., 2006)

| | | |
|---|---|---|
| **Astacidae – European and North American species** | | |
| *Austropotamobius pallipes* | Lereboullet, 1858 | White-clawed crayfish (*UK native) |
| *Austropotamobius torrentium* | Shrank, 1803 | Stone crayfish |
| *Astacus astacus* | Linnaeus, 1758 | Noble crayfish (UK NICS) |
| *Astacus leptodactylus* | Eschscholtz, 1823 | Narrow-clawed/Turkish crayfish (UK NICS) |
| ***Pacifastacus leniusculus*** | **Dana, 1852** | **Signal crayfish (study species: UK NICS)** |
| **Cambaridae – North American** | | |
| *Orconectes limosus* | Rafinesque, 1817 | Spiny-cheek crayfish (UK NICS) |
| *Orconectes immunis* | Hagen, 1870 | Calico crayfish |
| *Orconectes rusticus* | Girard, 1852 | Rusty crayfish |
| *Orconectes virilis* | Hagen, 1870 | Virile crayfish (UK NICS) |
| *Procambarus clarkii* | Girard, 1852 | Red swamp crayfish (UK NICS) |
| *Procambarus falix f. virginalis* | Hagen, 1870 * | Marbled crayfish/ Marmokrebs |

*asexual, parthenogenetic form of the Slough crayfish *Procambarus fallax* (Martin et al., 2010).

| | | |
|---|---|---|
| **Parastacidae – *Cherax spp.* (Southern Hemisphere)** | | |
| *Cherax destructor* | Clark, 1936 | Yabby |
| *Cherax tenuimanus* | Smith, 1912 | Hairy marron |
| *Cherax quadricarinatus* | Von Martens, 1868 | Australian red claw (UK NICS in captivity) |

## APPENDIX B: EQUIPMENT SUPPLIERS.

**Traps**

Professional trap (PT) custom made (Flowers Fisheries) c.2004

www.gtproductseurope.co.uk

Trappy bait boxes: www.collinsnets.co.uk

Gees Minnow/ Finnish 'Pirat': www.mooreandmoorecarp.co.uk

Trapman : www.trap-man.com;

LiNi: www.gooutdoors.co.uk

**Bricks and refuge samplers**

P18 bricks - www.ridgeons.co.uk; P24: www.solopark.co.uk

Horticultural insulated roofing material: www.edplastics.co.uk

**Other**

Vernier Calipers (WilhadialMax): www.wiha.com

Black Nylon Twine: Selsey Fishing Supplies 01243 605289

## APPENDIX C: SUBSTRATUM ANALYSIS TABLES.

Wentworth Scale for Classification of Sediments.

| Sediment Type | Particle Diameter range | | Phi Units range | |
|---|---|---|---|---|
| **Boulder** | 256+ mm | | -8< | |
| **Cobble** | 64 mm | 256 mm | -6 | -8 |
| **Pebble** | 4 | 64 | -2 | -6 |
| **Granule** | 2 | 4 | -1 | -2 |
| **Very Coarse Sand** | 1 | 2 | 0 | -1 |
| **Coarse Sand** | 500 µm | 1 | 1 | 0 |
| **Medium Sand** | 250 | 500 µm | 2 | 1 |
| **Fine Sand** | 125 | 250 | 3 | 2 |
| **Very Fine Sand** | 62 | 125 | 4 | 3 |
| **Coarse Silt** | 31 | 62 | 5 | 4 |
| **Medium Silt** | 15 | 31 | 6 | 5 |
| **Fine Silt** | 8 | 15 | 7 | 6 |
| **Very Fine Silt** | 4 | 8 | 8 | 7 |
| **Coarse Clay** | 2 | 4 | 9 | 8 |
| **Medium Clay** | 1 | 2 | 10 | 9 |

Measure of degree of sorting.

| sI | degree of sorting | sI | degree of sorting |
|---|---|---|---|
| **<0.35** | very well sorted | 0.35-0.50 | well sorted |
| **0.50-0.71** | moderately well sorted | 0.71-1.00 | moderately sorted |
| **1.00-2.00** | poorly sorted | 2.00-4.00 | very poorly sorted |
| **4.00+** | extremely poorly sorted | - | - |

Measure of degree of symmetry.

| $Sk_I$ | skewness |
|---|---|
| 1.00 to 0.30 | strongly skewed towards fine particles |
| 0.30 to 0.10 | fine skewed |
| 0.10 to -0.10 | symmetrical |
| -0.10 to -0.30 | coarse skewed |
| -0.30 to -1.00 | strongly skewed towards coarse particles |

Measure of kurtosis.

| KG | kurtosis | KG | kurtosis |
|---|---|---|---|
| < 0.67 | very platykurtic | 0.67-0.90 | platykurtic |
| 0.90-1.11 | mesokurtic (nearly normal) | - | - |
| 1.11-1.50 | leptokurtic | 1.50 + | very leptokurtic |

After Fernandes and Tett (2001).

## COPYRIGHT DECLARATION

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SAMPLING UK *PACIFASTACUS LENIUSCULUS* (DANA, 1852): THE EFFECT OF TRAPPING ON POPULATION STRUCTURE

ABIGAIL EMMA STANCLIFFE-VAUGHAN

## GLOSSARY

| Symbol/ Acronym/ Abbreviation | Description |
|---|---|
| ⌀ | Diameter |
| Φ | Phi |
| AW | Abdominal width |
| BOD | Biological Oxygen Demand (also referred to as Biochemical Oxygen Demand) |
| CL | Carapace length |
| CMR | Capture-mark-recapture |
| CPUE | Catch per unit effort |
| DEFRA | Department of the Environment Food and Rural Affairs |
| EA | Environment Agency |
| Fi | Finnish trap |
| LAPS | Lark Angling and Preservation Society |
| LiNi | LiNi Swedish trap |
| MC | Minnow large |
| MM | Minnow small |
| MM Extra | Minnow small with refuge material |
| MN | Minnow smallmedium |
| MX | Minnow medium |
| NE | Natural England |
| NICS | Non-indigenous crayfish species |
| PCA | Principle Components Analysis |
| POCL | Post orbital carapace length |
| PT | Professional trap |
| Ra | Rabbit/ 'Trapman' trap |
| TL | Total length |
| y-o-y | Young of the year |