Comparative biology

of the signal crayfish, *Pacifastacus leniusculus* (Dana), and the narrow-clawed crayfish, *Astacus leptodactylus* Eschscholtz

by

Muzaffer Mustafa Harlıoğlu

A thesis submitted to the

University of Nottingham

for the degree of Doctor of Philosophy.

Department of Life Science

1996

Dedicated to everybody working hard in the way of science.

Acknowledgements

Many thanks are dedicated to my supervisor, Dr. David Holdich for his support and encouragement during the course of this study. Thanks are also dedicated to Firat University, Turkey for providing the financial support and to Prof. P.N.R. Usherwood for providing equipment and laboratory facilities.

I would like to thank Dr. Jim Reader for his technical and statistical guidance. I would also like to thank Dr. David Rogers for his very warm friendship and supplying crayfish during this study.

Very special thanks to Tim Smith for helping me learn to use the scanning electron microscope, to Brain Case for developing the photographs, to Dr. David de Pomerai for helping with the description of the twins.

I am also very grateful to the following friends: Dr. Paula Child, Dr. Dawn Middleton, Dr. Claire Ashley, Dr. Metin Calta, Karen Smith and Babar Javaid for their enthusiastic help in the laboratory.

Finally, I would like to thank my parents and my wife for their support and encouragement during my studies.

Contents

Chapter 9

Chapter 10

Abstract

Some aspects of the biology of *Pacifastacus leniusculus* and *Astacus leptodactylus* have been compared.

The literature survey shows that considerably more studies have been carried out on *P. leniusculus* than *A. leptodactylus*.

Although no major differences have been found in the morphology of appendages and mouthparts of the species, structural differences have been found in the abundance of setae on the second maxilliped, in the number of teeth on the mandibles and the *crista dentata*, and form of the chelipeds.

Studies on the environmental tolerance of the species show that both species are able to survive in saline water for long periods of time but they can only increase in number in low salinities. Both species can survive over a wide range of temperatures, but they cannot tolerate temperatures of 34 °C after stepwise acclimation. Although the results do not show a clear difference in the tolerance of *P. leniusculus* and *A. leptodactylus* to low oxygen, there are some indications that *A. leptodactylus* is more tolerant of decreased oxygen tensions than *P. leniusculus*. By using a non-invasive heart beat monitor on crayfish it has been observed that the frequency of heart beats is extremely variable and can be affected by many factors, such as temperature and salinity.

Juveniles of the two species can have a significant impact on plant and macroinvertebrate communities. The results also show that both species can have a negative effect on the recruitment of fish populations in freshwaters by eating fish eggs.

Competition experiments show that both juveniles and adults of *P. leniusculus* are significantly more aggressive than those of *A. leptodactylus*. The results also show that *A. leptodactylus* would be eliminated by *P. leniusculus* if they met in a wild.

Adults of the species prey on their juveniles, except the brooding females with stage 2s. This predation occurs in the presence of adequate nutrition. Non-predatory behaviour of the brooding females may indicate the presence of pheromones in the species.

Reproductive efficiency of the populations of the species in Britain is as good as any studied elsewhere. In comparison to *A. leptodactylus*, *P. leniusculus* has more eggs, but smaller in size.

Pleopodal egg development of the species can be reduced from seven months to three months with temperature acclimation, but photoperiod is not a factor in reducing pleopodal egg development.

In both species sexual dimorphism was observed between males and females. Males of both species and females of *P. leniusculus* exhibit allometric or isometric growth during their lives but female *A. leptodactylus* exhibits isometric growth throughout.

Comparison of body parameters shows that *P. leniusculus* can be considered as a morphologically better species to adapt to environmental conditions than *A. leptodactylus* because it has large and heavy chelae, and heavy body weight.

Both species grow fast, but because *P. leniusculus* hatches earlier it has an advantage over *A. leptodactylus* and has bigger juveniles by the end of the first summer.

In both species males produce significantly more claw meat than females. Although *A. leptodactylus* produces significantly more tail meat, males of *P. leniusculus* produce significantly more claw and total meat. Significant differences occur in the tail meat yield of female *A. leptodactylus* and in the claw meat yield of female *P. leniusculus*, but males produce similar amount of meat in winter and summer.

The Swedish trappy is very effective method of catching both species over a certain size. Day and night catches show that both species are very active during the day and night.

Chapter 1

General introduction

1.1 Relationships

Crayfish are members of the largest crustacean Order, the Decapoda, which is mainly characterised by having five pairs of legs, of which the first pair is usually formed into a pair of claws (chelipeds), and a carapace covering the head and thorax.

Although Hobbs (1988) states that crayfish are most closely related to the Nephropoidea (e.g. *Nephrops*) and places them in the Infraorder Astacidea, Scholtz and Richter (1995) have erected a new taxon for them, the Astacida, and state that its sister-group remains obscure. Crayfish differ from other decapod taxa in various characters including the form of the carapace, the attachment of the fifth pair of legs and the structure of the gills, and in the fact that they are mainly restricted to freshwater habitats. A unique characteristic of crayfish is that they do not have larvae, all embryonic stages occurring within the egg during development.

There are two Superfamilies of crayfish: the Parastacoidea, with a single Family, the Parastacidae, and the Astacoidea, with two Families, Astacidae and Cambaridae. The main differences between the Families concern gill and reproductive structures.

1.2 Geographical distribution

There are over 500 species of crayfish, most of which occur in America and Australia. Europe by contrast has only five native species. Most species occur in temperate regions. Africa, central Asia, the Indian sub-continent, and some islands have no native crayfish species (Hobbs, 1988), despite the fact that suitable habits exist.

The Parastacidae are restricted to the Southern Hemisphere, whilst the other two families occur in the Northern Hemisphere: the Cambaridae in North America and the Far East, and the Astacidae in North America and Eurasia.

Due to the fact that crayfish are considered a delicacy in many countries (see Section 1.6), some species, which have been found to be fast growing, highly fecund and with a good meat yield, have been introduced outside their natural range (Holdich, 1988). This has been particularly the case in Western Europe, where introduced species are now commoner than the native species in some areas (Lowery & Holdich, 1988). The main introduced species involved are *Orconectes limosus* (Rafinesque), *Pacifastacus leniusculus* (Dana) and *Procambarus clarkii* (Girard), all originating from North America. In addition, *Astacus leptodactylus* Eschscholtz, from Eastern Europe and Turkey have been translocated into Western Europe.

1.3 Ecology and behaviour

Crayfish are the largest, mobile invertebrates inhabiting freshwater environments. Consequently, when they occur they can be a key species (Momot, 1995). They

inhabit a wide variety of freshwater environments from streams and rivers to ponds and lakes. In some countries they have been found inhabiting saline coastal waters (Lowery & Holdich, 1988; Köksal, 1988) and in others are semi-terrestrial (Hobbs, 1988).

The absence of larvae in crayfish is thought to be an adaptation to living in flowing water, where larvae would be carried downstream (Hobbs, 1988).

Crayfish are omnivores consuming a wide range of food items, including plant and animal matter (Goddard, 1988). However, Momot (1995) considers that crayfish obtain most of their protein from animal sources, only reverting to plant material if animals are not available. Other workers have, however, shown that the sudden absence of crayfish can have a marked, positive effect on aquatic vegetation (e.g. Abrahamsson, 1973; Matthews *et al.*, 1993), or alternatively that they can be used to clear nuisance plants (Blake & Laurent, 1982).

Crayfish seem to be naturally aggressive and this is particularly true of those species introduced outside their natural range. Males tend to be territorial, particularly in the breeding season (Hogger, 1988).

1.4 Life history

Following mating females lay a clutch of eggs which become attached to the pleopods. As no larvae are involved, crayfish lay far fewer eggs than their marine relatives, i.e. 50-400 depending on species (Lee & Wickens, 1992). As they are being laid they are

fertilised by sperm released from a spermatophore or a special chamber into which the male deposited his gametes. The fertilised eggs undergo their full development to hatchling stage on the female. The process sometimes takes over six months, although in warm water species it can be considerably shorter. Hatchlings gradually become independent of the mother but, for the first few days at least, they return to her protection. It is believed that a pheromone is involved in this process (Little, 1975, 1976).

Following dependence juvenile crayfish moult frequently but by the time they become sexually mature this process may be reduced to twice a year. The time taken to reach sexual maturity depends on species, but can be from less than a year to more than four years (Lowery, 1988).

1.5 Diseases and parasites

Crayfish suffer from a number of diseases, the most notable being thelohaniasis (porcelain disease), caused by the protozoan, *Thelohania* spp., and crayfish plague caused by the fungus, *Aphanomyces astaci* Schikora (Alderman & Polglase, 1988). Porcelain disease rarely causes large-scale mortalities and infected individuals may live for a number of years. Crayfish plague is a very virulent disease which is carried by North American crayfish species. They are relatively immune to the effects of the fungus, but crayfish from other continents which have been tested have been found to be very susceptible (Unestam, 1975).

Crayfish plague entered Europe in the middle of last century and has had a devastating impact on the populations of native crayfish ever since. However, all the native species still survive and in some countries are abundant, e.g. Ireland and parts of Germany (Holdich, D.M., pers. comm.). The disease did not reach the British Isles and Turkey until the 1980s and this was probably related to the introduction of North American crayfish for aquacultural purposes (Lowery & Holdich, 1988).

1.6 Crayfish as a luxury food for humans

Crayfish are harvested in large quantities from the wild or, alternatively, they are cultured, either by "ranching" in open systems with little management, or in semi-intensive systems with management (Holdich & Rogers, 1992; Holdich, 1993). Few attempts have been made to culture crayfish intensively because of their cannibalistic tenancies and in most species, their relatively slow growth rate compared with prawns and shrimps (Lee & Wickens, 1992). In most countries they can be considered a luxury food which demand a high price (Holdich, 1993). In Western Europe, largely as a result of crayfish plague, demand outstrips supply, and has to be filled by imports.

1.7 Reasons for and aims of the study

The American signal crayfish, *Pacifastacus leniusculus*, and the narrow-clawed crayfish, *Astacus leptodactylus*, originate from western North America and western Asia (e.g. Turkey) respectively. Both these species have been introduced into Britain and, as well as being cultured, are present in large quantities in the wild (Rogers &

Holdich, 1995), where they compete with the native white-clawed crayfish, *Austropotamobius pallipes* (Lereboullet) (Holdich & Domaniewski, 1995). *Astacus leptodactylus* is native to Turkish lakes and was the main species imported by North European countries to try and meet demand until stocks were affected by overfishing and crayfish plague in the 1980s (Köksal, 1988). There is some evidence that *P. leniusculus* has been introduced into Turkey (P. Bagot, pers. comm.). If it has then it is highly likely that it will escape and compete with the native, *A. leptodactylus*, as well as spreading crayfish plague, in a similar situation to what has happened in Britain (Holdich & Reeve, 1991).

Despite the fact that both *P. leniusculus* and *A. leptodactylus* are now so wide-spread, relatively little is known of their biology, and few attempts have been made to compare them. As the author comes from Turkey it seemed that it would be of benefit to undertake a comparative study of various aspects of the biology of the two species, the results of which might be of value to both the British and Turkish crayfish situation. In addition, it was hoped that many of the results would be useful for those culturing crayfish.

Consequently, the following aspects were studied over a three year period, the aim being to produce a comprehensive comparison of those aspects of the biology of both species about which little is known. All the work, other than collecting crayfish for experimental purposes, was carried out in the laboratory and in indoor and outdoor aquaria.

The following aspects were studied in order to assess similarities and differences between the two species:

- A review of the literature pertaining to both species.
- A comparison of the morphology of mouthparts and appendages.
- Their survival under certain environmental conditions, e.g. increased levels of salt.
- The sublethal effects of low oxygen levels, increased temperature and salt levels as measured by the heart beat rate.
- Their environmental impact in mesocosms.
- Inter- and intra-specific competition.
- The impact of adults on the survival of juvenile crayfish.
- Fecundity and the relationship between female size and egg size.
- Influence of light on egg development and reducing hatching time.
- Length-weight relationships.
- Growth of juveniles under different temperature and densities.
- Meat yield of wild populations.
- An evaluation of the Swedish "Trappy".

The literature review was deemed necessary as this had not been undertaken before. As the two crayfish studied are popular experimental animals such a review should prove useful to other workers. Both species under consideration can live in identical habitats. It is not known, however, if they feed on the same or different food in the wild state. If there are differences then this might be reflected in differences in the mouthparts and legs. Consequently, these were examined in detail by means of

scanning electron microscopy.

Although both species live primarily in freshwater there are records of them inhabiting saline conditions in other countries. As both species live in tidal rivers in Britain they may enter the estuarine environment. Experiments were therefore carried out to test their ability to survive saline conditions at different stages of the life cycle. The sublethal and lethal effects of certain other environmental conditions which are likely to be experienced by the crayfish such as low oxygen levels and higher than normal temperatures were tested, along with salinity, using the non-invasive CAPMON technique for measuring heart beat. This is the first time it has been used for such a purpose in crayfish.

One worry with introduced species is that they will have more impact on their new environment than the native species. In order to see which might have the greater, impact experiments involving the two crayfish species in question were set up using known amounts of algae, macrophytes and invertebrates as a food source.

Both species have been introduced into Britain where they have escaped into the wild and have built up large populations in a number of localities (Holdich & Reeve, 1991) but, as yet no mixed populations have been reported, although this is considered to be just a matter of time (Holdich, D.M., pers. comm.). To observe what would happen if *P. leniusculus* and *A. leptodactylus* were to meet, competition experiments were set up with adults and juveniles of the two species. In aquaculture it is important to get the maximum yield for the minimum effort. Monospecific experiments were set up to see what survival would be like for the two species under semi-intensive conditions,

i.e. is the yield better for *P. leniusculus* or for *A. leptodactylus*? In addition, parallel experiments were carried out to examine the predatory activities of adults on stage 2 juveniles.

Recruitement to an expanding population is very important in determining its success. This depends on the fecundity of females and survival of the resultant juveniles. Egg size is one of the main factors on fecundity. Apparently, big egg size brings about less fecundity. In order to compare the fecundity and egg size of *P. leniusculus* and *A. leptodactylus* egg counts were made on females from different sites.

Temperate crayfish are thought to need a cold period during winter followed by a spring rise in temperature to stimulate development and hatching. Under natural conditions the juveniles of *P. leniusculus* hatch and are released from their mother at least four weeks earlier than those of *A. leptodactylus*. From the point of view of culturing crayfish it is important to get juveniles started as early as possible each year. To show whether this temperature sequence is necessary for egg development in *P. leniusculus* and *A. leptodactylus* and whether it is possible to induce early hatching of juveniles under artificial conditions, experiments were carried out with berried females at 5, 13, 17 and 21 °C. In addition, the effect of the light and darkness on egg development was also observed.

In addition to having juveniles as early as possible in the year, the growth rate of juveniles is also an important factor in the management of crayfish. It is known that growth rate is dependent on temperature. A comparison was made on the growth rate of *P. leniusculus* and *A. leptodactylus* by keeping juveniles at diferent temperatures

and densities.

To stock (or restock) freshwaters with crayfish, in addition to adults, crayfish juveniles are also used. Apparently, using relatively large juveniles for stocking increases their chances of survival. As temperature is known to affect growth rate, juvenile crayfish were subjected to increased temperatures to see if their growth rates could be increased so as to produce large juveniles early in the year for restocking.

In addition to the importance of sexual dimorphism in the biology of species, it is also important for aquacultural purposes such as in the determination of a proper cropping strategy. Sexual dimorphism has been studied in a number of crustaceans including crayfish. These studies have been mainly focused on the differences between males and females, and/or within the same sex in terms of length-length or length-weight relations. No studies have been made on the sexual dimorphism of *P. leniusculus* and *A. leptodactylus*. To compare the differences in the length or length-weight between males and females within and between the two species a number of measurements were taken from the samples of the two species.

When considering crayfish for introduction, the meat yield is as important as their ecological adaptability. Although *P. leniusculus* has been cultured in Europe for many years, there is only an estimated value for its meat yield and little reliable data is available for *A. leptodactylus*. To compare the meat yield content of *P. leniusculus* and *A. leptodactylus*, samples of males and females of the two species from different populations were analysed and the yield related to body size.

In many European countries including Britain, the Swedish "Trappy" (referred to subsequently as the trappy) is used to catch crayfish. No study has been carried out on the effectiveness of this trap in catching crayfish. In adition, no comparison has been made on the ability of this trap to catch *P. leniusculus* and *A. leptodactylus*. A study was carried out to evaluate the effectiveness of this type of trap for catching the species under study.

There are many aspects of the biology of the two species which could have been studied, e.g. food preferences and behaviour, but time did not permit such studies to be undertaken.

The crayfish used in this study were mainly obtained from colonies kept in outdoor tanks at the University of Nottingham. When needed fresh supplies were mostly obtained from Boxmoor Fishery (Hemel Hempstead north of London) for *Pacifastacus leniusculus*, and from the Serpentine Lake (Hyde Park, London) or Tykes Water (north of London) for *Astacus leptodactylus*.

Chapter 2

Introduction to *Pacifastacus leniusculus* (Dana) and *Astacus leptodactylus* Eschscholtz

2.1 Comparison of *Pacifastacus leniusculus* and *Astacus leptodactylus*

According to Hobbs (1988) both *Pacifastacus leniusculus* and *Astacus leptodactylus* belong to the crayfish family Astacidae which contains only three genera: *Astacus*, *Austropotamobius* and *Pacifastacus*. Consequently *P. leniusculus* and *A. leptodactylus* are similar in their morphology. The genus *Astacus* contains three species: *astacus*, *leptodactylus* and *pachypus*; *Austropotamobius* contains two species: *pallipes* and *torrentium*; and *Pacifastacus* contains five species: *connectens*, *fortis*, *gambeli*, *leniusculus* and *nigrescens* (Holdich and Lowery, 1988). Only *A. astacus*, *A. leptodactylus*, *A. pallipes* and *P. leniusculus* occur in Britain, but only *A. pallipes* is native, the rest having been introduced for aquacultural and culinary purposes and which have subsequently escaped into the wild (Holdich *et al.*, 1995b).

Astacus leptodactylus is easily identified by its long claws and is commonly named the narrow-clawed or Turkish crayfish. *P. leniusculus* is easily identified by its red colour, and large and robust claws. In addition, a white to turquoise patch on the claw gives the signal crayfish its common name. Table 2.1 lists the main features separating the two species. Figures 2.1-8 illustrates these differences.

2.2 Biology of *Pacifastacus leniusculus*

Pacifastacus leniusculus inhabits lakes, streams and rivers (Lowery and Holdich, 1988). It also occupies saline waters (Kerley and Prichard, 1967; Prichard and Kerley, 1970; Henry and Wheatly, 1988). It is tolerant to environmental extremes such as temperature and various pollutants (Firkins, 1993; Firkins and Holdich, 1993). Because it is able to adapt to a wide range of habitats and conditions, it has been introduced to many waterbodies in Europe (Lowery and Holdich, 1988).

The average egg number of female *P. leniusculus* is higher than that of the other astacids (Lowery, 1988). Because of its high fecundity, it may become more abundant and, because the individuals of *P. leniusculus* are usually bigger in size than the other astacids, it may have more impact on freshwater environments. Compared with many other members of the Astacidae, *P. leniusculus* has a fast growth rate. It is also a vagrant, aggressive species, which escapes easily from captivity.

With respect to the time of breeding, *P. leniusculus* starts to mate from late September onwards in southern and central England. The early breeding of *P. leniusculus* brings about an earlier hatching and release of juveniles (April to May). This gives *P. leniusculus* a clear advantage over other species as it has more time to moult and grow up. As compared with *P. leniusculus*, the eggs of *A. astacus*, *A. pallipes* and *A. leptodactylus* hatch out in late May to June (this might even be July for *A. astacus*) (Cukerzis, 1988; Lowery, 1988 and Köksal, 1988). Thus the juveniles of *P. leniusculus* are significantly larger when other astacid juveniles are released. In addition, it is possible to produce juvenile *P. leniusculus* in December by maintaining berried

females indoors at 16 (± 1 °C) in the early winter months (Chapter 9.1).

Another main aspect of the biology of *P. leniusculus* which is of interest concerns its burrowing ability (Guan, 1994). It is able to survive for 2-3 weeks in burrows when the water level is lowered. It is also reported that *P. leniusculus* can survive for long periods in dried up rivers if there is some moisture under rocks (Holdich *et al.*, 1995b).

2.3 Biology of *Astacus leptodactylus*

Astacus leptodactylus occupies a very similar niche to that of *P. leniusculus*. However, in addition to streams, rivers, lakes and ponds, *A. leptodactylus* also inhabits swamps. Thus, it is sometimes called the swamp crayfish (Kossakowski, 1971; Cherkashina, 1975; Köksal, 1988).

Although it has a wide distribution and is of economic importance the biology of *A. leptodactylus* has been researched very little. Some aspects of its biology have been reviewed by Kossakowski (1971), Cherkashina (1975), Papadopol (1975), Köksal (1988), Firkins and Holdich (1993) and more recently Holdich *et al.* (1995a)

Like *P. leniusculus*, it is also fast-growing and highly fecund (Lowery, 1988; Köksal, 1988). But it is not as cannibalistic as *P. leniusculus* and in mixed cultures is outcompeted by *P. leniusculus* (Chapter 6).

Although the time and length of the reproductive cycle depends on the climatic factors, in its native habitat the first signs of breeding activity (e.g. glair production) appears with the decline in water temperature (7-12 °C) in the autumn and they mate during October and November (Köksal, 1988). Then, the females lay their eggs four to six weeks later (when the water temperature is 6-11°C). The eggs hatch out from late May to the end of June.

The breeding season of *A. leptodactylus* in England is different from that in Turkey. During the 1994 and 1995 breeding seasons it was observed that the first signs of breeding activity (e.g. glair production) did not start before November and that they started to mate in the first week of December. The eggs hatched out from late April to the middle of May even when they were kept in the same conditions as *P. leniusculus* (see Chapter 9.1).

Like *P. leniusculus* eggs, the hatching time of *A. leptodactylus* eggs can be reduced by maintaining berried females indoors at 16 (± 1 °C) in the winter months (Chapter 9.1). But the time of hatching with increased temperature it is not as early as that of *P. leniusculus* eggs.

2.4 Bibliographies on crayfish biology

The most extensive bibliography of freshwater crayfish was produced by Hart and Clark (1987), which covered all references from the time of Aristotle (384 B.C.) through 1985. Holdich (1991a) listed all those papers in the volumes resulting from the International Association of Astacology Symposia (Volumes I-VII), 1971-1988,

and Holdich and Pearce-Higgins (1995) listed all papers for Volume I - X, and included an index.

A selected bibliography of the red swamp crayfish, *Procambarus clarkii* (Girard) and the white river crayfish, *Procambarus acutus acutus* (Girard) has been produced by Spohrer *et al.* (1975). The bibliography consists of general biology, taxonomy, geographical distribution, physiology, toxicology, parasitology and pathology, culture techniques, processing and marketing. Arringon (1979) produced a selected bibliography of the white footed crayfish *Austropotamobius pallipes* and the red footed crayfish *Astacus astacus*. Most of the papers relate to their taxonomy and distribution. The literature published in Finland or by Finnish authors on *A. astacus* and *P. leniusculus* have been recorded by Westman (1979). The record consists of 66 references which are mainly in Finnish. Cukerzis (1983) has reviewed the selected literature on the native and introduced crayfish in Lithuania. Sixty-two percent of the reference are in Russian, 4.6% and 20% are in English or in Russian with English summary respectively. More recently a bibliography of the Australian redclaw crayfish, *Cherax quadricarinatus* has been published by Medley *et al.* (1995). The bibliography contains many papers on the early development and cultivation of this species.

No bibliographies have dealt specifically with *A. leptodactylus* or *P. leniusculus* which is surprising considering their commercial importance and the environmental impact they have when moved outside their natural ranges (Holdich, 1988). As part of the comparative study on *P. leniusculus and A. leptodactylus* a literature review has been carried out using BIDS (Bath Information and Data Services) and manual library searches.

References on *Pacifastacus leniusculus* and *Astacus leptodactylus*

The majority of the papers relating to *A. leptodactylus* concern their physiology and biochemistry (Table 2.1). In comparison to *A. leptodactylus*, more papers have been published on *P. leniusculus*, especially concerning its physiology, farming-culture, immunology, translocation and distribution (Table 2.1). The main reason for this is due to the fact that this species has been introduced into most European countries for aquacultural and wild-stocking purposes (Lowery and Holdich, 1988). In addition, because it is easy to culture and maintain, it has become a popular experimental animal. The majority of the papers relate to its physiology, although there is an increasing number concerning aquaculture and immunology (Table 2.1). Because *P. leniusculus* carries crayfish plague, many investigators have concentrated on its immune system (e.g. Persson *et al.*, 1987; Duvic and Söderhäll 1990; Aspan and Söderhäll 1991; Kopacek *et al.*, 1993) and the implications for susceptible crayfish when this species has been translocated (e.g. Holdich and Reeve, 1991).

Figure 2. Aspects of the comparative morphology of *P. leniusculus* and *A. leptodactylus* (modified from NRA, 1994).

Fig. 2.1 Dorsal view of male *P. leniusculus*

Fig. 2.2 Dorsal view of male *A. leptodactylus*

Fig. 2.3 Ventral view of the dactylus and propodus of the cheliped of male *P. leniusculus*

Fig. 2.4 Ventral view of the dactylus and propodus of the cheliped of male *A. leptodactylus*

Fig. 2.5 Two post orbital spines on the rostrum in *P. leniusculus*

Fig. 2.6 Two post orbital spines on the rostrum in *A. leptodactylus*

Fig. 2.7 Absence of large spines on the side of the carapace in *P. leniusculus*

Fig. 2.8 Presence of large spines on the side of the carapace in *A. leptodactylus*

Fig. 2.1

Fig. 2.2

Fig. 2.3

Fig. 2.4

Fig. 2.5

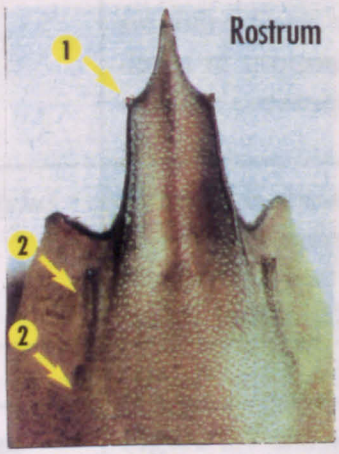


Fig. 2.6

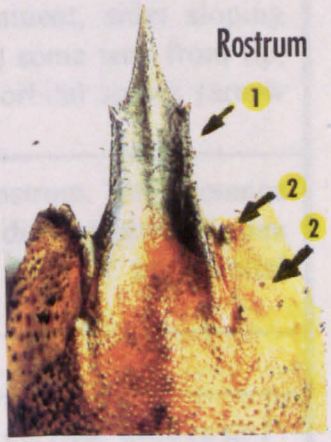


Fig. 2.7

Fig. 2.8

Note: Figures were taken from NRA- A guide to identifying freshwater crayfish in Britain and Ireland (1994).

Table 2.1. Features separating *P. leniusculus* and *A. leptodactylus*

Body	
P. leniusculus	Figure 2.1 shows the dorsal view of male *P. leniusculus*. Body smooth, generally bluish-brown to reddish-brown, may be almost black in colour in some habitats. Chelae large and very robust, dorsally smooth. The arrow shows the prominent white to turquoise patch (gives the signal crayfish its common name) on upperside of the claw at the finger joint.
A. leptodactylus	Figure 2.2 shows the dorsal view of male *A. leptodactylus*. Usually a uniform light yellow to green. Sides of carapace rough. Long and slender chelae.
Claws	
P. leniusculus	Figure 2.3 shows the large, robust and smooth chelae of *P. leniusculus* (ventral).
A. leptodactylus	Figure 2.4 shows the long and narrow chelae of *A. leptodactylus* (ventral).
Rostrum	
P. leniusculus	Figure 2.5 shows the dorsal view of rostrum. Sides of rostrum smooth and more or less parallel. Median ridge smooth. Apex very pointed and prominent, sides sloping down to prominent shoulders (arrow 1) some way from tip, and the presence of two pairs of post-orbital spines (arrow 2).
A. leptodactylus	Figure 2.6 shows the dorsal view of rostrum. The presence of toothed margins on basal part with distinct pointed apex (arrow 1), and the presence of two pairs of post-orbital spines (arrow 2).
Carapace	
P. leniusculus	Figure 2.7 shows no spines behind the cervical groove in *P. leniusculus*.
A. leptodactylus	Figure 2.8 shows the presence of prominent spines behind the cervical groove in *A. leptodactylus* (arrow 3).

Note: Figures were taken from NRA- A guide to identifying freshwater crayfish in Britain and Ireland (1994).

Table 2.2. Number of papers on the biology of *A. leptodactylus* and *P. leniusculus*.

	Number of papers on *A. leptodactylus*	Number of papers on *P. leniusculus*
Astaciculture	7	17
Behaviour	3	20
Bibliographies	5	5
Biochemistry	60	19
Biology	9	5
Conservation	3	3
Disease	22	27
Ecology, Distribution and Population Biology (including trapping)	24	37
Farming and Wild Harvest	4	16
Fine Structure	5	0
Food, Diet and Digestion	3	7
Genetics	2	2
Histology	0	3
Immunology	1	19
Introduction of crayfish	10	25
Life Histories, Growth, Development and Reproduction	20	36
Management	4	5
Physiology	43	60
Taxonomy	12	13
Toxicology	0	2

A subject grouping literature survey on *A. leptodactylus* and *P. leniusculus* mentioned in Table 2.2 is given in the Appendix.

Chapter 3

Morphology of appendages and mouthparts using scanning electron microscopy

3.1 Introduction

As pointed out in Chapter 2 *Pacifastacus leniusculus* and *Astacus leptodactylus* both belong to the family, Astacidae and consequently they share many common features (Hobbs, 1988). Although descriptions of the two species are given in many keys (Curra, 1967; Pennack, 1978; Laurent and Forest, 1979; Brodski, 1983; Gledhill *et al.*, 1993; Vigneux *et al.*, 1993), few studies have highlighted their morphological differences in any detail, e.g. arrangement and variety of setae.

Differences in the morphology and structure of mouthparts and pereopods of crayfish are useful in classifying them for systematic purposes. For example, variations in the rostra, chelae, mandibles and third maxillipeds were used by Hobbs (1987) to classify species of the genus *Astacoides*. Variations in chelae were also considered to determine taxonomic differences between *Orconectes propinquus* and *Orconectes obscurus* by Tierney (1982). Morphological differences of the western North American crayfish species of the genus *Pacifastacus* have been classified into the subgenera *Pacifastacus* and *Hobbsastacus* (Bouchard, 1977).

Scanning electron microscopy has been used to study the external morphology of mouthparts, pereopods and pleopods in a variety of decapod crustaceans. Particularly the fine structure of setae (Thomas, 1971; Farmer, 1974; Budd *et al.*, 1978; Factor, 1978 and 1989; Hindley and Alexander, 1978; Alexander *et al.* 1980; Solon and Cobb,

1980; Tautz and Sandeman, 1980; Tyson and Sullivan 1981; Derby, 1982; Felgenhauer and Abele, 1983; Mittenthal and Trevarrow, 1983; Bauer, 1989; Mura and Caldo, 1993; Loya-Javellana, 1995).

The functional morphology of the mouthparts and pereopods has been observed in the Norway lobster *Nephrops norvegicus* by Farmer (1974) and the shrimp *Atya innocous* by Felgenhauer and Abele (1983); in the prawn *Penaeus merguiensis* by Hindley and Alexander (1978) and Alexander *et al.* (1980); and in the crabs *Pagurus longicarpus* and *Pagurus pollicaris* by Roberts (1968). In addition to these, Mura and Caldo (1993) have studied the structure of the molar surface of the mandibles in the shrimp *Branchinella spinosa.* A similar study has been carried out on brine shrimp by Tyson and Sullivan (1981).

Observations on the morphology and structure of feeding apparatus and distribution of setae in lobsters have been mainly reported for *Homarus americanus* and *Homarus gammarus.* The morphology of the mouthparts and type and distribution of setae in larvae of *H. americanus* have been illustrated by Factor (1978) and Hinton and Corey (1979). Derby (1982) has described the type of setae on the antennules, antennae, mouthparts and pereopods of *H. americanus.* Similarly, Solon and Cobb (1980) have investigated the type of setae on the chelae of *H. americanus.*

The number and structure of the chordotonal organs on the coxopodite and dactylopodite of pereopods and third maxillipeds of *H. gammarus* have been examined by Wales *et al.* (1970). A study has been carried out on the feeding mechanism, structure of the gut, and digestive physiology of *H. gammarus* by Barker and Gibson

(1977). Wales *et al.* (1976) have also explained the mandibular movements and their control in *H. gammarus*.

As compared with prawns, shrimps, crabs and lobsters, more studies have been carried out on the morphology of crayfish bodyparts. The morphology of *Astacus fluviatilis* was detailed by Huxley (1973). Huxley also briefly described the setae of *A. fluviatilis* in his study. The structure of some appendages and mouthparts of the stage one, two and three juveniles of *Pacifastacus leniusculus* and *Orconectes limosus* was drawn by Andrews (1907). Whitehouse and Grove (1947) published the external features of *Astacus* sp. Recently, *P. leniusculus* was chosen as a model in the description of functional morphology of crayfish by Holdich and Reeve (1988). Holdich (1992) also reviewed the early post-embryonic development of astacid, cambarid and parastacid crayfish.

A number of studies have been carried out on the morphology and setae of *Austropotamobius pallipes*. The variety and distribution of setae in adult and juvenile of *A. pallipes* have been documented by Thomas (1970, 1971, 1973, 1978, 1983 and 1986; Thomas and Ingle, 1987). The comparative morphology of the mouthparts and setal variations in different stages of free-living juveniles and adult *Cherax quadricarinatus* have been described by Loya-Javellana (1995). Similarly, setae types on different parts of the body were also observed in *Procambarus clarkii* by Aoki (1965) and Ameyaw-Akumfi (1977); *Orconectes immunis* by Budd *et al.* (1978); and *Cherax destructor* by Tautz and Sandeman (1980). In addition, setal development in the uropod and telson was also investigated in juvenile and adult *A. leptodactylus* by van Herp and Bellon-Humbert (1978). Similarly, Mills and Lake (1975) observed the

setal development in the uropods and telson of *Parastacoides tasmanicus*. Malaczynska-Suchcitz (1956) briefly described the telson, second and third walking legs of stage one juvenile *Potamobius* (*Astacus*) *leptodactylus*.

In the present study, the structural differences between the juveniles of *P. leniusculus* and *A. leptodactylus* are compared under the scanning electron microscope. In addition, the development of mouthparts in stage 1, 2 and 3 juveniles of the two species is also evaluated. The aim of this part of the study was to try and detect differences which may indicate the ability to feed on different food items, both between the juveniles of the two species and within the different stages of the same species.

3.2 Materials and methods

Juveniles of different stages (first, second and third stages and juveniles 12 mm in carapace length) were used. The samples were reared under artificial conditions in clean containers instead of keeping them in concrete tanks which have a muddy floor and would therefore make them dirty and unsuitable for photography. After individuals had moulted, they were killed when their body became hard enough to dissect and before they lost any appendages. To kill the samples, they were placed in a freezer for ten minutes. Then they were preserved in 70 percent alcohol.

Mouthparts and appendages were removed under a light microscope. After dissection, the selected bodyparts of 12 mm length (carapace) juveniles were air dried for approximately 12 hours at room temperature (18 ±1 °C). Because the stage 1, 2 and

3 juveniles were too delicate to apply air drying technique, the critical-point drying technique was applied for them.

Then, the selected body parts were attached to aluminium stubs with silver colloidal paint and coated with gold using a Polaron Sputter Coating Unit E5100. Finally, to view the bodyparts the stubs were set up in a JSM-840 scanning electron microscope operated at either 10, 15 or 25 KV.

The terminology used is taken from Holdich and Reeve (1988). The segments of the appendages are named from attachment point as: coxopodite (coxa), basipodite (basis), ischiopodite (ischium), meropodite (merus), caropodite (carpus), propodite (propodus), dactylopodite (dactylus) with a terminal unguis.

3.3 Observations

To show differences between *P. leniusculus* and *A. leptodactylus*, stage 3 and 12 mm (carapace length) juveniles of the two species were compared. To show differences in the rostrum, carapace, second pereopod, fourth pereopod, third maxilliped, second maxilliped, first maxilliped and mandible between *P. leniusculus* and *A. leptodactylus*, 12 mm (carapace length) juveniles were used. Third stage juveniles were also used to show differences in the first pereopod (cheliped).

The development of stage 1, 2, 3 and 12 mm (CL) juveniles was also evaluated within and between the two species (except the maxilla of stage 1 juveniles). In addition, setae types were also compared for the two species.

Due to the delicate nature of the mouthparts and appendages of juveniles it proved very difficult to prepare them for scanning electron microscopy. Consequently, some of the photographs exhibit charging (brightness). In the description below only the differences which were apparent between the two species are described.

3.3.1 Differences in the morphology of appendages and mouthparts

Rostrum and carapace

In general shape the rostra of the two species are very similar. They taper to a point but near the apex a sharp spine occurs on either side (c.f. Figure 3.3 for *P. leniusculus* and Figure 3.4 for *A. leptodactylus*).

Although both species have two post orbital spines on each side of the rostrum, *A. leptodactylus* also has a large spine on each side of the carapace; this is absent in *P. leniusculus*. (c.f. Figure 3.1 for *P. leniusculus* and Figure 3.2 for *A. leptodactylus*). In the juvenile competition experiments (see Chapter 6), this spine (next to the cervical groove) is the best character by which to distinguish stage 2 juveniles of the two species under the light microscope. This spine is also present in *Austropotamobius pallipes* (but not in stage 2 of *A. pallipes*) (NRA, 1994).

The sides of the rostrum of *A. leptodactylus* are bordered by a regular row of setae. This row of setae on the rostrum of *P. leniusculus* is not as regular as that of *A. leptodactylus*. (c.f. Figure 3.3 for *P. leniusculus* and Figure 3.4 for *A. leptodactylus*).

Cheliped (first pereopod)

The gap between the dactylus and propodus of the cheliped is wide in *A. leptodactylus* and the margins are serrated. The gap is narrower in *P. leniusculus* and the inner edges of the dactylus and propodus are not so serrated. (c.f. Figure 3.5 for *P. leniusculus* and Figure 3.6 for *A. leptodactylus*).

There is a row of setae on the propodus of the cheliped in *A. leptodactylus* (c.f. Figure 3.8), but there is no a row of setae on the cheliped in *P. leniusculus* (Figure 3.7).

The carpus of *P. leniusculus* has setae, but that of *A. leptodactylus* has not. Also, the shape of the spine on the edge of the carpus is different in the two species, that of *A. leptodactylus* being much larger. (c.f. Figure 3.7 for *P. leniusculus* and Figure 3.8 for *A. leptodactylus*).

Second pereopod

A difference was found regarding the propodus and dactylus between the species. Although *A. leptodactylus* has an unguis at the tip of propodus and dactylus, this unguis does not appear in *P. leniusculus*. (c.f. Figure 3.9 for *P. leniusculus* and Figures 3.10 and 3.11 for *A. leptodactylus*).

Fourth pereopod

More abundant setae are present on the ventral side of the propodus of *A. leptodactylus* than for *P. leniusculus* (c.f. Figure 3.12 for *P. leniusculus* and Figure 3.13 for *A. leptodactylus*).

Third maxilliped

A spine is present on the second and third segments of the third maxilliped in *A. leptodactylus* (Figure 3.15). These spines are not seen in *P. leniusculus* (Figure 3.14).

The number and distribution of teeth are also different on the *crista dentata* (first segment) of the two species. There are more teeth on the *crista dentata* of *P. leniusculus*. (c.f. Figure 3.16 for *P. leniusculus* and Figure 3.17 for *A. leptodactylus*).

Second maxilliped

Compared to *A. leptodactylus*, more abundant and very close setae are present on the endopod of *P. leniusculus*. (c.f. Figure 3.24 for *P. leniusculus* and Figure 3.25 for *A. leptodactylus*).

First maxilliped

A difference occurs in the length of setae on the exopod. Bigger setae are present on the exopod of *P. leniusculus*. (c.f. Figure 3.32 for *P. leniusculus* and Figure 3.33 for

A. leptodactylus).

Mandible

There are more teeth on the incisor lobe of the mandible of *P. leniusculus*. (c.f. Figure 3.40 for *P. leniusculus* and Figure 3.41 for *A. leptodactylus*).

Maxillule

The length of setae on the protopod of *P. leniusculus* is greater than that of *A. leptodactylus*. In addition, unlike *P. leniusculus*, in *A. leptodactylus* the tip of the protopod of the maxillule has long setae. (c.f. Figure 3.48 for *P. leniusculus* and Figure 3.49 for *A. leptodactylus*)

Maxilla

Setae on the edge of the protopod lobes are present in *A. leptodactylus*. These setae are not present on the protopod lobes of *P. leniusculus*. (c.f. Figure 3.56 for *P. leniusculus* and Figure 3.57 for *A. leptodactylus*). Figure 3.66 also shows these setae in *A. leptodactylus* at high magnification.

3.3.2 Juvenile development of *Pacifastacus leniusculus* and *Astacus leptodactylus*

In addition to an increase in size, changes in morphology in the feeding apparatus of *P. leniusculus* and *A. leptodactylus* are known to occur between the developmental

stages (stage 1, stage 2, stage 3 and 12 mm (CL) juveniles). Differences between the development stages in the first, second and third maxilliped, mandible, maxillule and maxilla of the two species are shown in Figures 3.16 - 3.61.

Differences mainly occur in the length and abundance of setae on the feeding appendages, and in the number and dimension of teeth on the mandibles and the *crista dentata* of the third maxillipeds.

The increase in the number and dimension of teeth on the mandible of *P. leniusculus* as the crayfish moults are shown in Figures 3.46, 3.44, 3.42; 3.40, and those of *A. leptodactylus* are given in Figure 3.47, 3.45, 3.43; 3.41. Similarly, the increase in the number and dimension of teeth on the *crista dentata* of *P. leniusculus* as the crayfish moults are shown in Figures 3.22, 3.20, 3.18; 3.16, and those of *A. leptodactylus* in Figures 3.23, 3.21, 3.19; 3.17.

It is well known that stage 1 juveniles are not active feeder (Thomas, 1970). Although stage 1 juveniles feed on the yolk the presence of setae were found in the maxillipeds (first, second and third), mandible, maxillule, and maxilla of the two species (Figures 3.38, 3.30, 3.22, 3.46; 3.54 for *P. leniusculus*, and Figures 3.39, 3.31, 3.23, 3.47; 3.55 for *A. leptodactylus* respectively. Although setae were found in the maxilla of stage 1 *P. leniusculus* and *A. leptodactylus*, because of too much charging, they are not shown).

According to Thomas (1970) only hamate (on the epipodites), pappose (on the carapace and on the scaphognathite margins), pappose and plumodenticulate (on the

epipoditic flange) setae occur in the stage 1 juveniles of *A. pallipes*, but in the present study, micro-conate setae were also found in the stage 1 juveniles of *P. leniusculus* and *A. leptodactylus*. This is only shown for *P. leniusculus* in Figures 3.67 and 3.78.

3.3.3 Setae types

Setae have a great importance in the feeding process of crayfish, as they do in all free living Crustacea (Thomas, 1970; Farmer, 1974; Factor, 1978; Holdich and Reeve, 1988).

An increase was observed in the length and variety of setae with the development of juveniles. After the first moult of juveniles a sudden and massive appearance of setae occurs.

Although varieties of setae have been recognized in both the species, the same basic setal types are found in the same location of different individuals. These setae are: tooth, rod, serrate, multiserrate, plumose, acuminate, conate and cuspidate setae.

Figures 3.62 - 3.83 show the basic setal types present in the two species.

Description and location of the setae

In order to describe the groups of setae and their location Thomas's (1970) terminology were used in the present study.

Tooth setae

These are sturdy setae, relatively short (average 0.4 mm in lenght). They are conspicuously oval in cross-section and are situated in well-developed sockets (Figures 3.68 and 3.75). They rapidly increase in number as crayfish grow.

Tooth setae occur on the ventral margins of the dactylopodites of the four pairs of walking legs and on the dorsal margins of the propodite projection of the first two pairs of walking legs. They also occur at the biting edges of the chelae in juveniles (Thomas, 1970).

Rod setae

These are relatively long setae, varying in length from 0.4 to 1 mm. Rod setae become gradually thinner from the socket to the tip (Figures 3.75 and 3.79).

They occur on the dactylopodite of the first and fourth walking legs and on the propodites of the first two walking legs, on the basipodite of the first maxilliped and on the dactylopodite of the second maxilliped (Thomas, 1970).

Serrate setae

Serrate setae have a row of serrations along each side of the shaft or along only one side of the shaft. The size and shape of the serrations are very variable. The best developed serrations are found half way along the postannular portion of the shaft.

These serrations become gradually thinner towards the tip and are within approximately 45° of each other (Figures 3.70; 3.72; 3.74; 3.76).

They are most abundant on the second and third maxillipeds, and on the propodite of the third and fourth walking legs. The largest serrate setae are always found on the outer edge of the dactylopodite of the third maxillipeds (Thomas, 1970).

Multiserrate setae

Multiserrate setae are long and very similar to serrate setae. The difference is that multiserrate setae have a multiplicity of rows of denticulations on the shaft (Figure 3.73).

Multiserrate setae occur on the scaphognathite of the maxilla, on the coxopodites of the maxillules, and on the inner surface of the branchiostegite (Thomas, 1970).

Plumose setae

In general, except on the setobranchs (gills), plumose setae are longer than all other setal types. Almost all of the shaft have setules and the shaft has a smoothly pointed tip (Figure 3.71; 3.77; 3.81; 3.82; 3.84).

They are present on the exopodite edges of the antennules and maxillipeds, on the pleopods, uropods and telson (Thomas, 1970).

Acuminate setae

These setae are smooth, very variable in length and thickness. The tip is smoothly pointed. The preannular part of the shaft looks like a pillar, but the shaft starts to taper at the annulation (3.62; 3.65; 3.72; 3.77).

Acuminate setae are commonly distributed over the flat outer surfaces of the appendages. A much slimmer type of the accumunate seta occurs on the margins of the uropods and telson, and on the exopodite and endopodite of the pleopods (Thomas, 1970).

Conate setae

Conate setae are relatively short and are very variable in size. They are sharply conical in shape and the tip is pointed. Conate setae are not flexible and are attached to the socket very rigidly (Figures 3.63; 3.64; 3.65; 3.66; 3.67; 3.78; 3.80).

Conate setae occur on all appendages except on the antennae, pleopods, uropods and telson (Thomas, 1970).

Cuspidate setae

Cuspidate setae are heavily cuticularized. They are robust and relatively long (0.5-1 mm). They have a smooth surface and the tip is rounded. Like conate setae, they are attached to the socket very rigidly (Figures 3.63 and 3.69).

Cuspidate setae are found on the dactylopodites of the second maxillipeds, propodites of the third and fourth walking legs and on the distal basipodite edge of the maxillules (Thomas, 1970).

3.4 Discussion

From the observations described above it is clear that the increase in the number and dimension of teeth on the mandible and *crista dentata*, and the increase in the length and variety of setae on the mouthpart of juveniles may enable them to cope with different types of food as they get older.

The small differences in the setal armature of mouthparts might lead to differences in the feeding behaviour between *P. leniusculus* and *A. leptodactylus*. It seems that when the same food is provided for the same stage of the species, *P. leniusculus* might have an advantage over *A. leptodactylus* because of its long and abundant setae on the second maxilliped, more teeth on the mandibles and *crista dentata*, and the form of the dactylus and propodus of the chelipeds.

The preference of two sizes of juvenile and an adult *P. leniusculus* in the consumption of aquatic weeds has been studied by Warner and Green (1995). This preference is *Spirogyra* sp., *Ceratophyllum demersum*, *Elodea canadensis* or *Groenlandia densa* respectively for all three crayfish size groups. Warner *et al.* (1995) have also found that when different sizes of snails are offerred although larger *P. leniusculus* (55 and 61 mm CL) show no preference, smaller *P. leniusculus* (16-44 mm CL) prefer to eat some sizes of snails more frequently than larger and smaller sizes, and smaller or

larger snails are not eaten by the smaller *P. leniusculus* until prey abundance declines.

Momot (1995) is of the opinion that many crayfish species are primarily carnivorous. He bases this on the fact that they need animal protein to maintain their lifestyle and relatively fast growth rates in the summer months. He maintains that plant food is taken in secondarily or when animal food is not readily available. Indeed Guan (1995) found a high proportion of animal food items in the guts of *P. leniusculus* in his study of a wild population. Others have shown, however, that this species is effective at clearing nuisance weeds in water bodies (Blake and Laurent, 1982). It seems likely therefore that crayfish need to possess an array of setal types to cope with all eventualities. As *P. leniusculus* and *A. leptodactylus* are closely related and can occupy similar environments it is not surprising that the setal armature of their appendages is very similar.

Differences in mouthparts have also been found in penaeid prawns which feed on a wide range of food. It was observed that eleven species show food preferences out of 31 species (Hindley and Alexander, 1978). Food preferences were observed in the different stages of the same species due to the fact that they possess different mouthpart structures and setal types in their ontogeny (Hindley and Alexander, 1978).

Legends

Fig. 3.1 Showing two post orbital spines on the rostrum and absence of the large spine on the side of the carapace in 12 mm (CL) *P. leniusculus*

Fig. 3.2 Showing two post orbital spines on the rostrum and presence of the large spine on the side of the carapace in 12 mm (CL) *A. leptodactylus*

Fig. 3.3 Setae on the sides of the rostrum in high magnification in 12 mm (CL) *P. leniusculus*

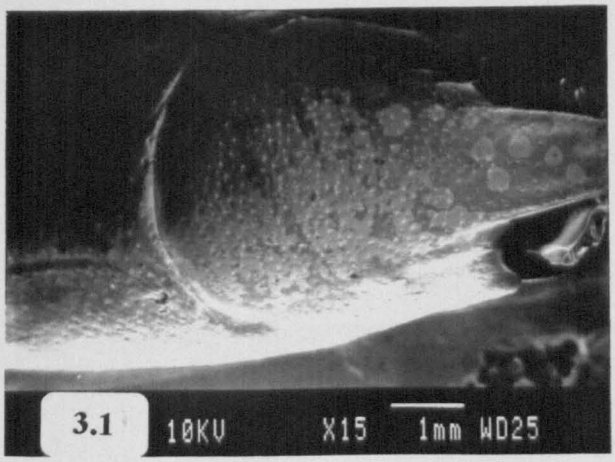
Fig. 3.4 Setae on the sides of the rostrum in high magnification in 12 mm (CL) *A. leptodactylus*

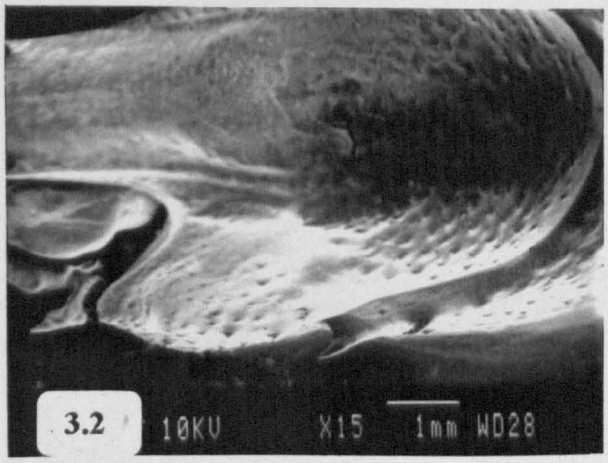
Fig. 3.5 Dactylus and propodus of the first pereopod in stage 3 *P. leniusculus*

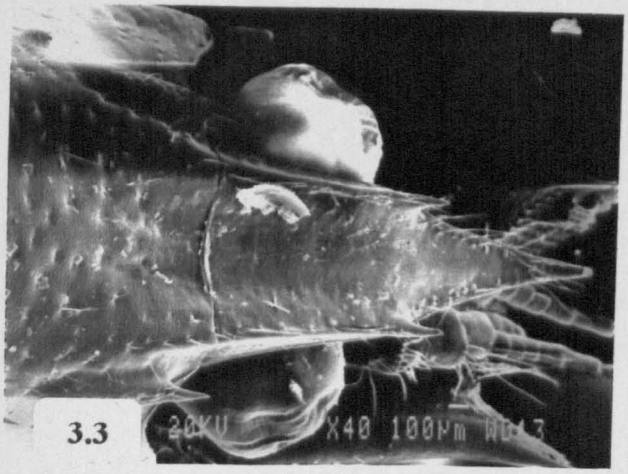
Fig. 3.6 Dactylus and propodus of the first pereopod in stage 3 *A. leptodactylus*

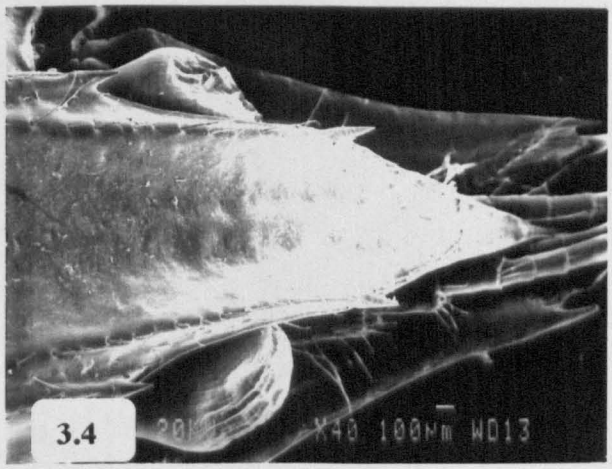
Fig. 3.7 Propodus and carpus of the first pereopod in stage 3 12 mm (CL

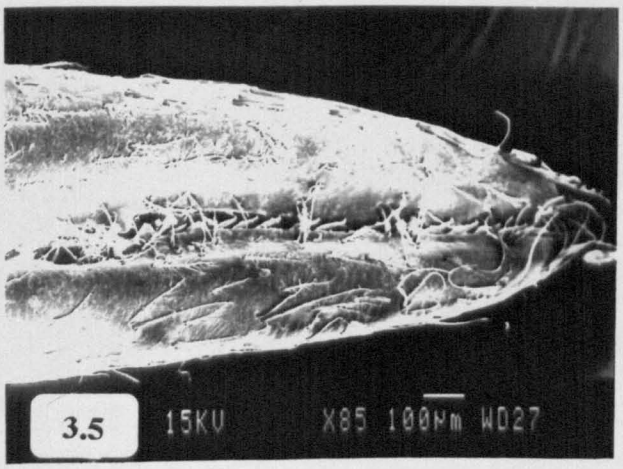
Fig. 3.8 Propodus and carpus of the first pereopod in stage 3 *A. leptodactylus*

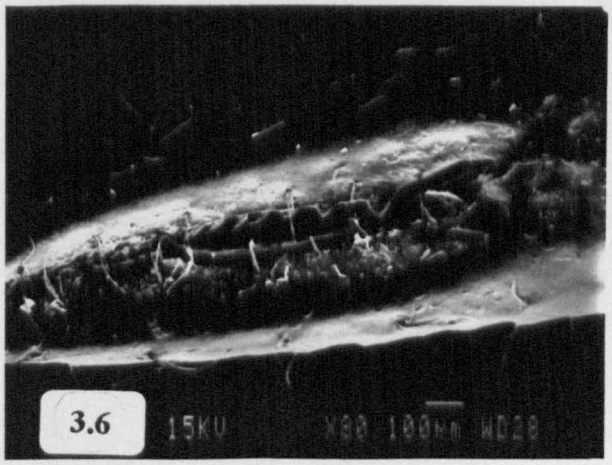

3.1
10KV X15 1mm WD25

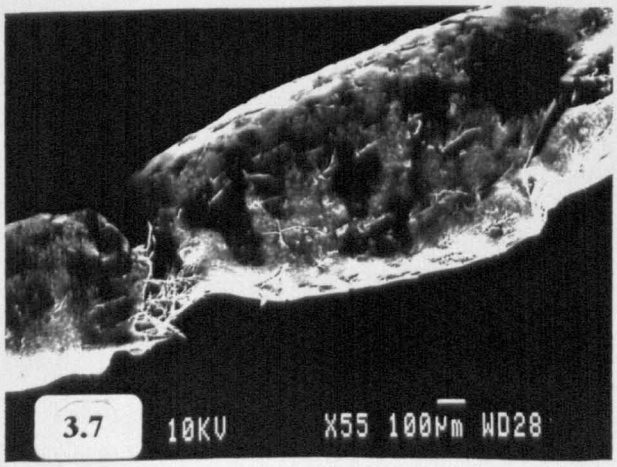

3.2
10KV X15 1mm WD28

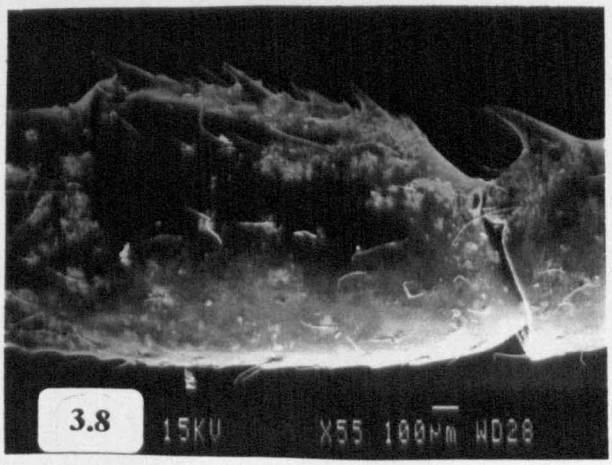

3.3
20KV X40 100µm WD13

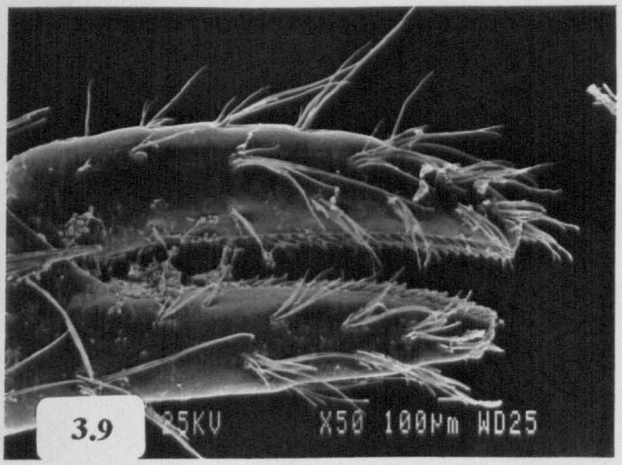

3.4
X40 100µm WD13

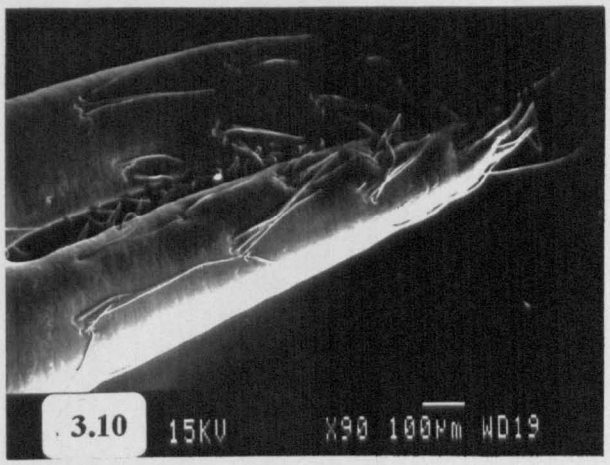

3.5
15KV X85 100µm WD27

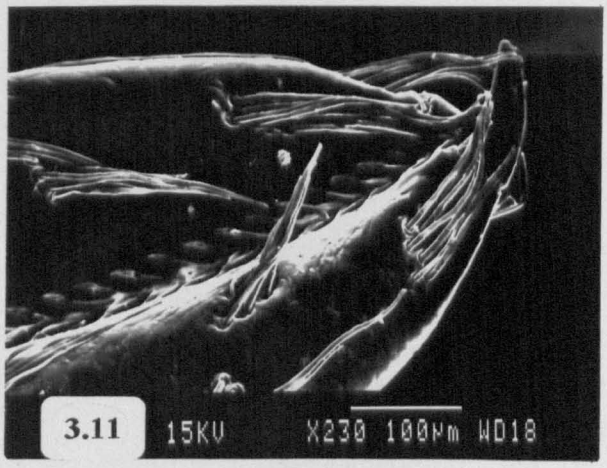

3.6
15KV

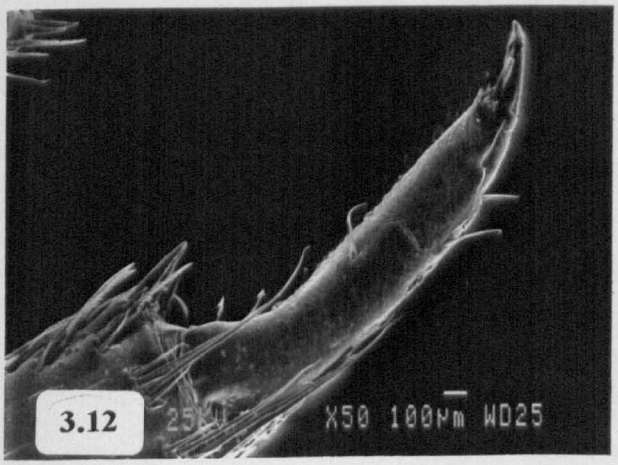

3.7
10KV X55 100µm WD28

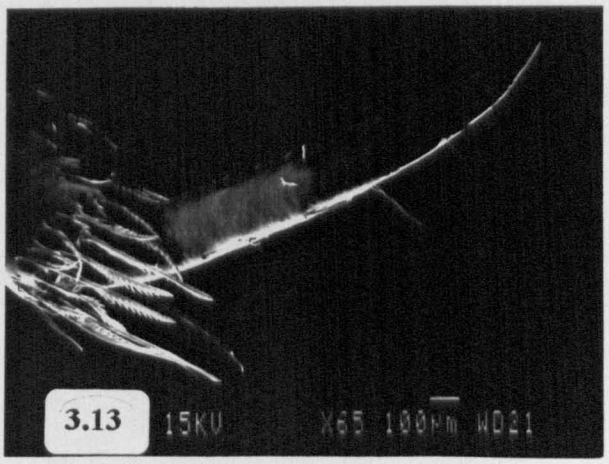

3.8
15KV X55 100µm WD28

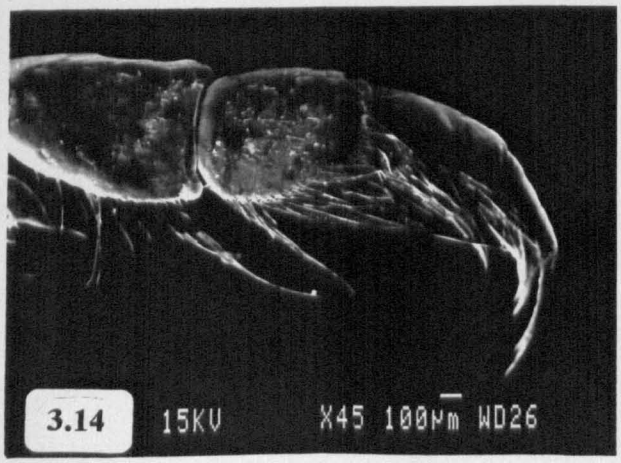

3.9
25KV X50 100µm WD25


3.10
15KV X90 100µm WD19


3.11
15KV X230 100µm WD18


3.12
X50 100µm WD25


3.13
15KV X65 100µm WD21


3.14
15KV X45 100µm WD26

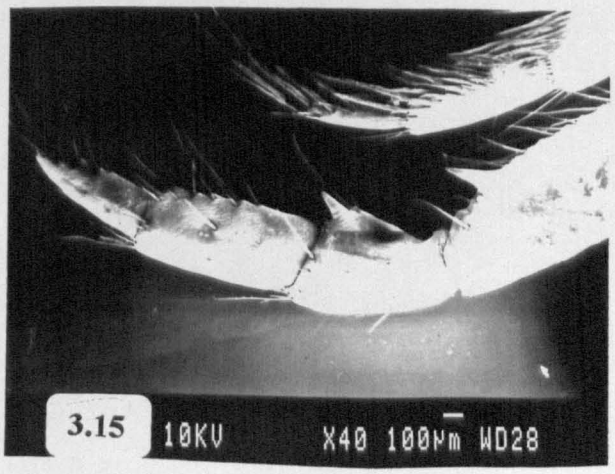

3.15
10KV X40 100µm WD28

Fig. 3.16 First segment (*crista dentata*) of the third maxilliped in 12 mm *P. leniusculus*

Fig. 3.17 First segment (*crista dentata*) of the third maxilliped in 12 mm *A. leptodactylus*

Fig. 3.18 First segment of the third maxilliped in stage 3 *P. leniusculus*

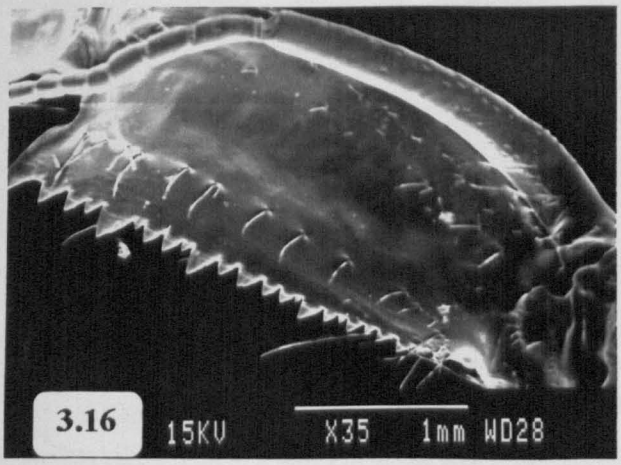
Fig. 3.19 First segment of the third maxilliped in stage 3 *A. leptodactylus*

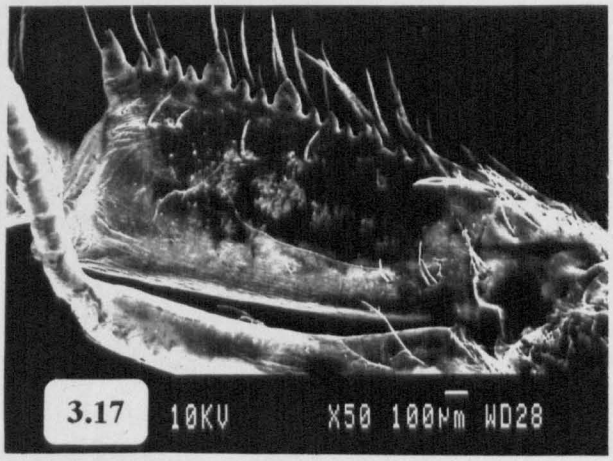
Fig. 3.20 First segment of the third maxilliped in stage 2 *P. leniusculus*

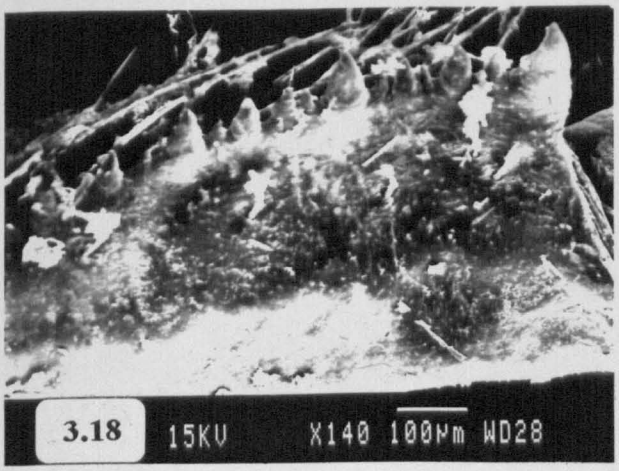
Fig. 3.21 First segment of the third maxilliped in stage 2 *A. leptodactylus*

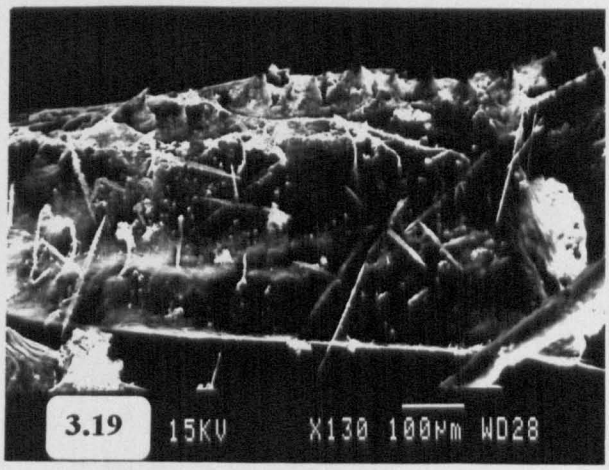
Fig. 3.22 Third maxilliped of stage 1 *P. leniusculus*

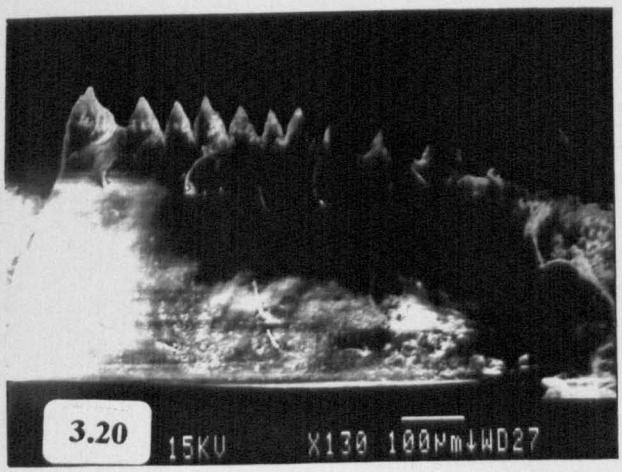
Fig. 3.23 First segment of the third maxilliped in stage 1 *A. leptodactylus*

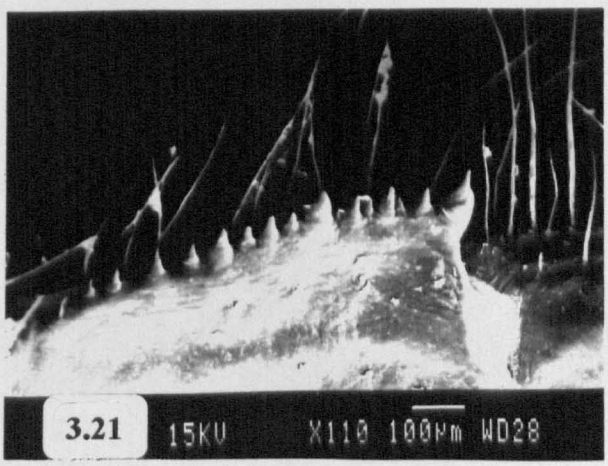

3.16
15KV
X35 1mm WD28

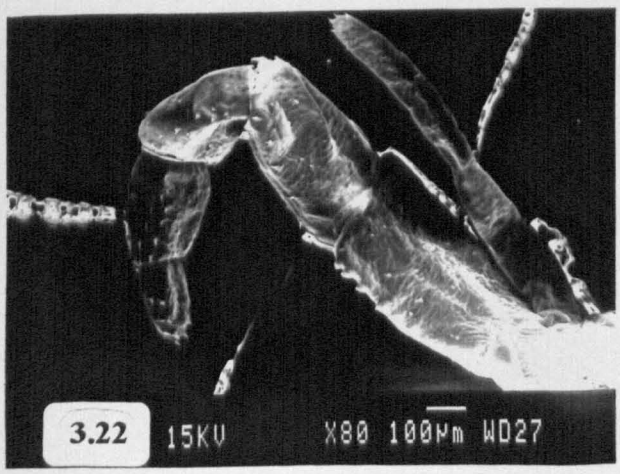

3.17
10KV
X50 100µm WD28

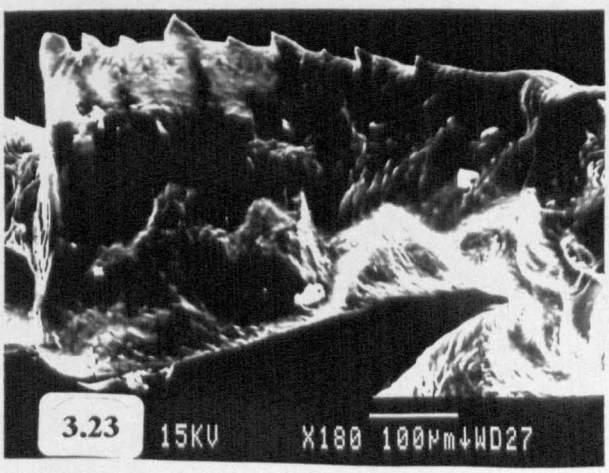

3.18
15KV
X140 100µm WD28

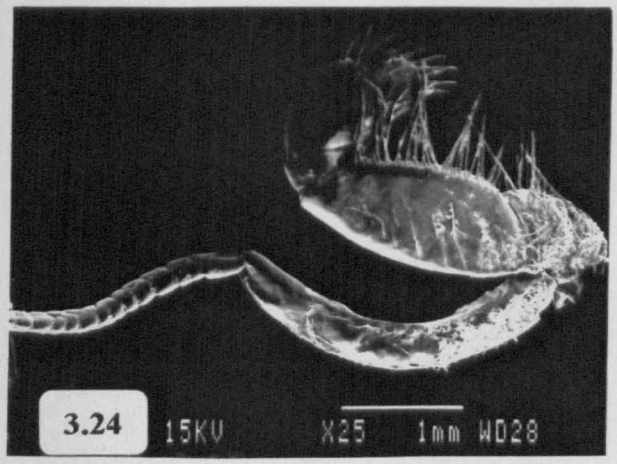

3.19
15KV
X130 100µm WD28

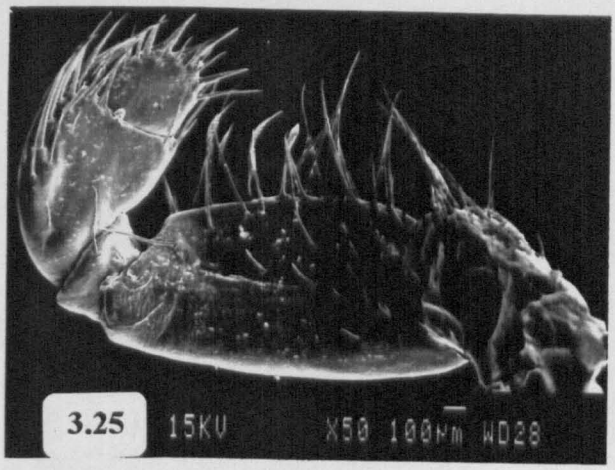

3.20
15KV
X130 100µm↓WD27

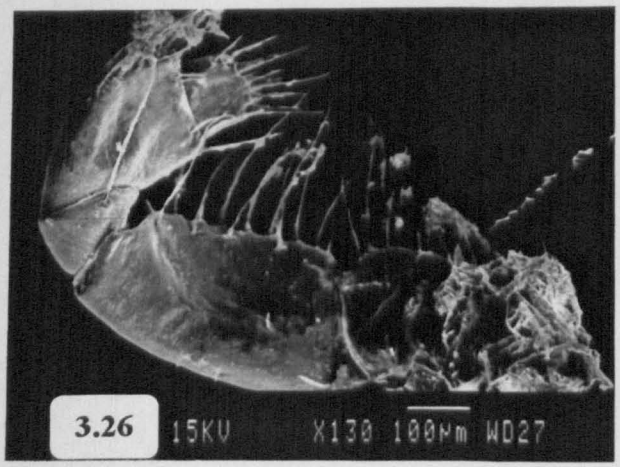

3.21
15KV
X110 100µm WD28

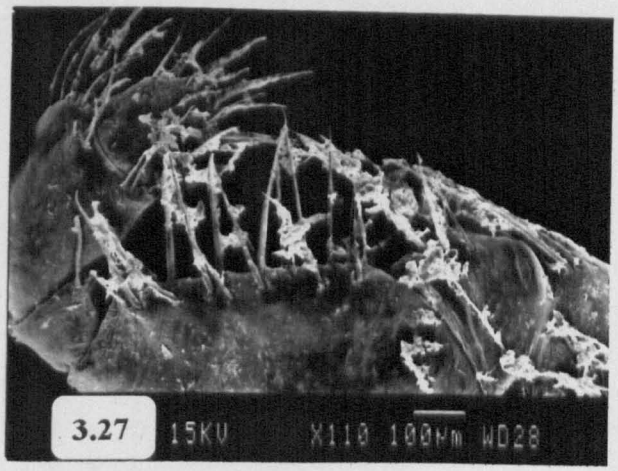

3.22
15KV
X80 100µm WD27

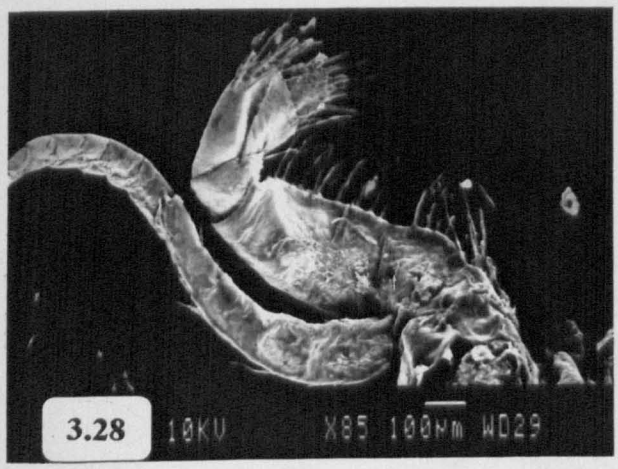

3.23
15KV
X180 100µm↓WD27

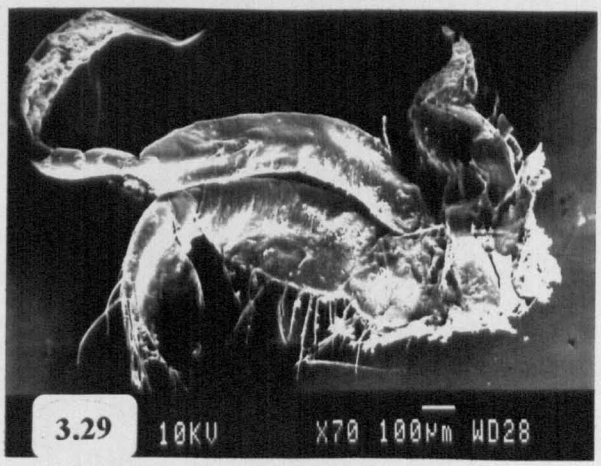

3.24
15KV X25 1mm WD28

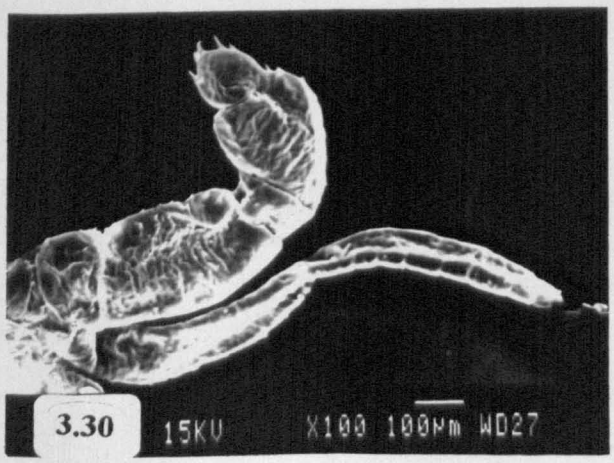

3.25
15KV X50 100µm WD28


3.26
15KV X130 100µm WD27


3.27
15KV X110 100µm WD28


3.28
10KV X85 100µm WD29


3.29
10KV X70 100µm WD28


3.30
15KV X100 100µm WD27

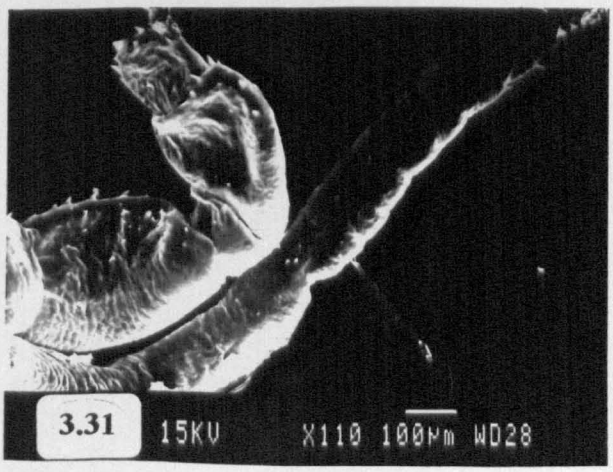

3.31
15KV X110 100µm WD28

Fig. 3.32 First maxilliped of 12 mm (CL) *P. leniusculus*

Fig. 3.33 First maxilliped of 12 mm (CL) *A. leptodactylus*

Fig. 3.34 First maxilliped of stage 3 *P. leniusculus*

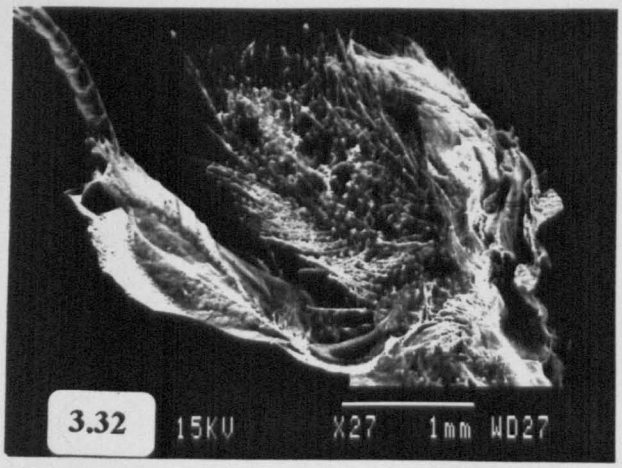
Fig. 3.35 First maxilliped of stage 3 *A. leptodactylus*

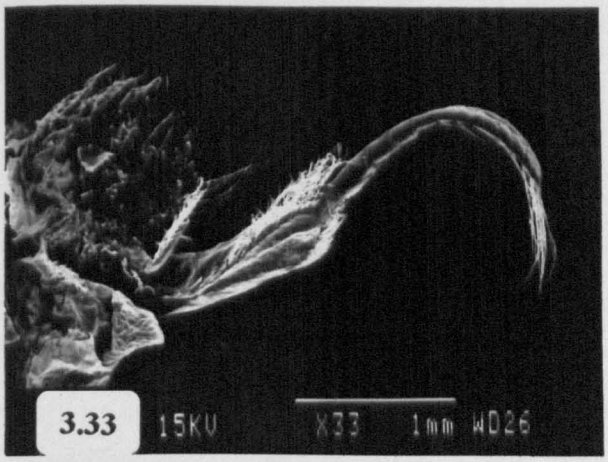
Fig. 3.36 First maxilliped of stage 2 *P. leniusculus*

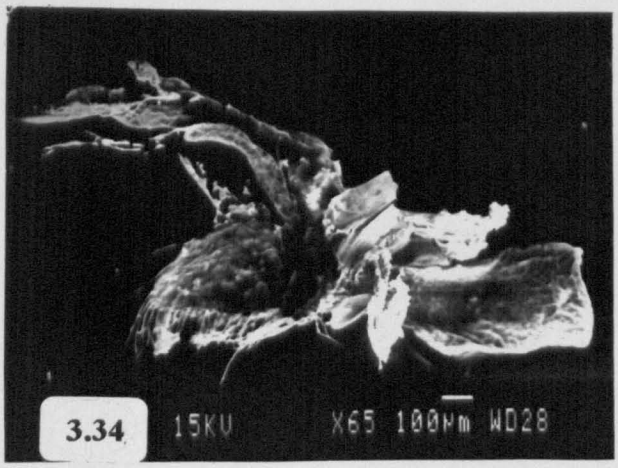
Fig. 3.37 First maxilliped of stage 2 *A. leptodactylus*

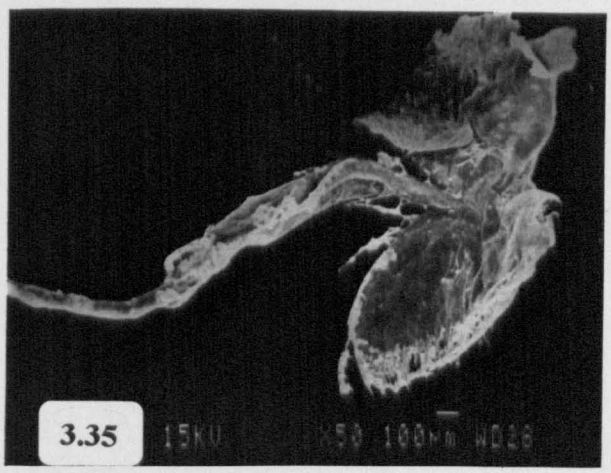
Fig. 3.38 First maxilliped of stage 1 *P. leniusculus*

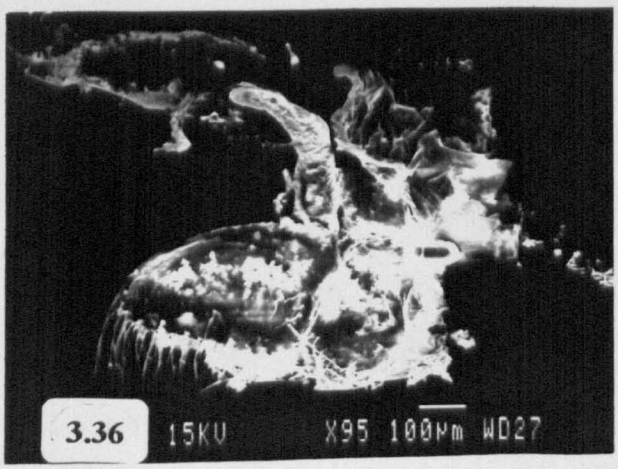
Fig. 3.39 First maxilliped of stage 1 *A. leptodactylus*

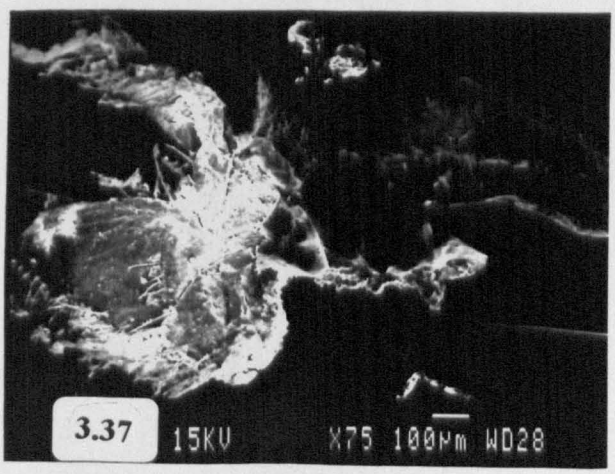

3.32
15KV X27 1mm WD27

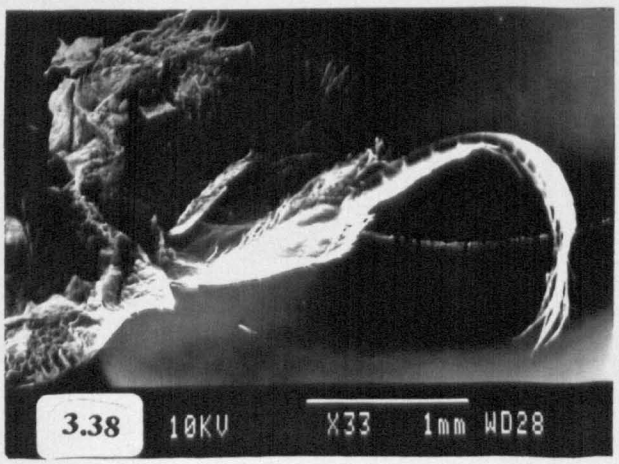

3.33
15KV X33 1mm WD26


3.34
15KV X65 100µm WD28


3.35
15KV X50 100µm WD28


3.36
15KV X95 100µm WD27


3.37
15KV X75 100µm WD28


3.38
10KV X33 1mm WD28

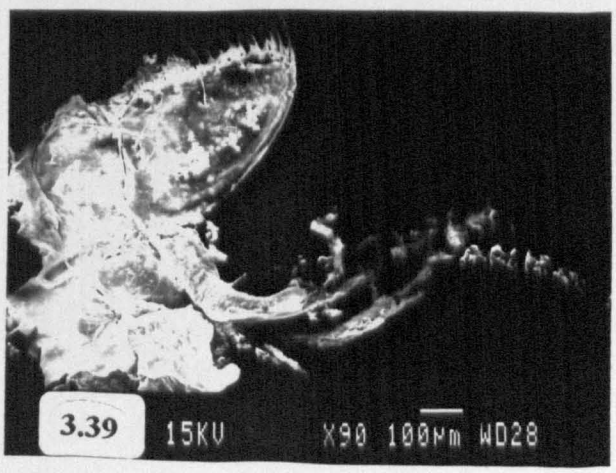

3.39
15KV X90 100µm WD28

Fig. 3.40 Mandible of 12 mm (CL) *P. leniusculus*

Fig. 3.41 Mandible of 12 mm (CL) *A. leptodactylus*

Fig. 3.42 Mandible of stage 3 *P. leniusculus*

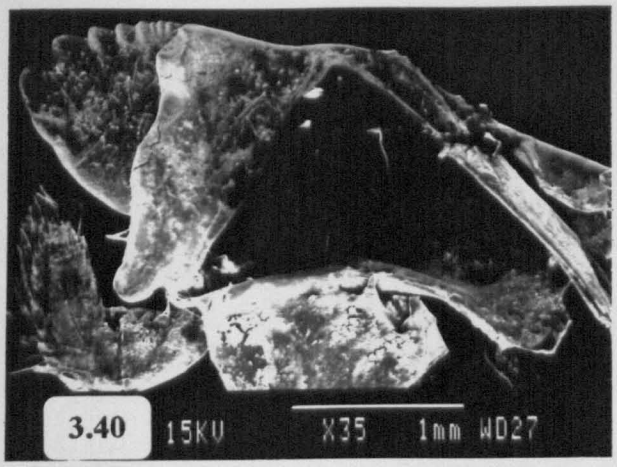
Fig. 3.43 Mandible of stage 3 *A. leptodactylus*

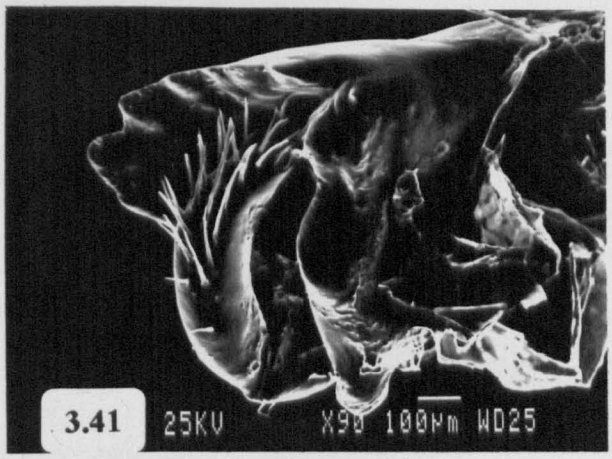
Fig. 3.44 Mandible of stage 2 *P. leniusculus*

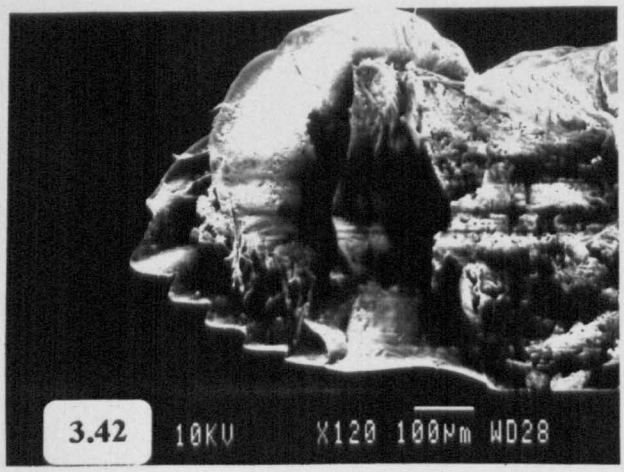
Fig. 3.45 Mandible of stage 2 *A. leptodactylus*

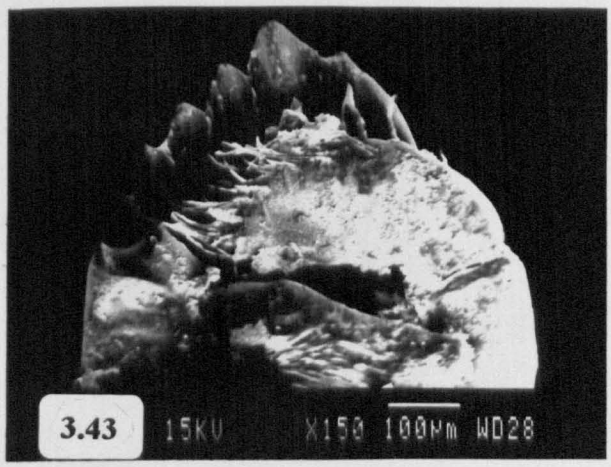
Fig. 3.46 Mandible of stage 1 *P. leniusculus*

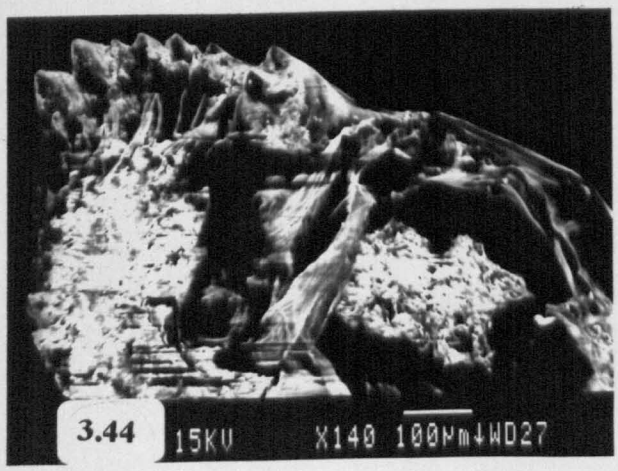
Fig. 3.47 Mandible of stage 1 *A. leptodactylus*

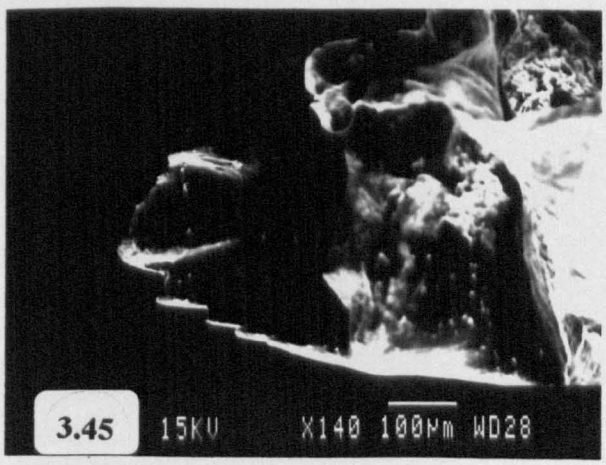

3.40
15KV X35 1mm WD27

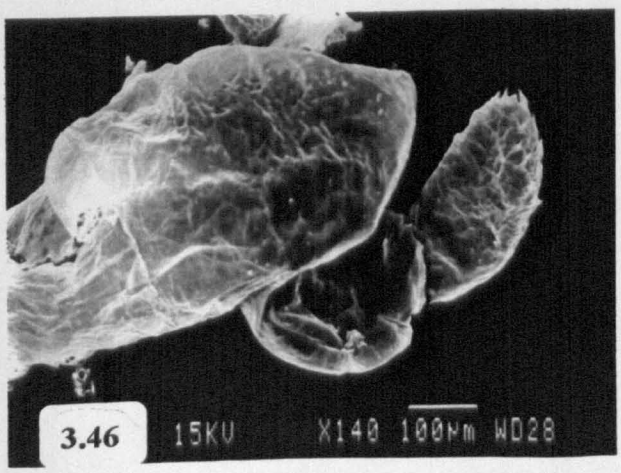

3.41
25KV X90 100µm WD25


3.42
10KV X120 100µm WD28


3.43
15KV X150 100µm WD28


3.44
15KV X140 100µm WD27


3.45
15KV X140 100µm WD28


3.46
15KV X140 100µm WD28

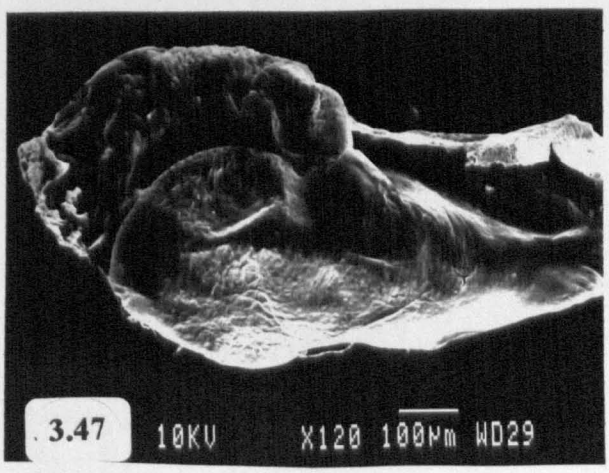

3.47
10KV X120 100µm WD29

Fig. 3.48 Maxillule of 12 mm (CL) *P. leniusculus*

Fig. 3.49 Maxillule of 12 mm (CL) *A. leptodactylus*

Fig. 3.50 Maxillule of stage 3 *P. leniusculus*

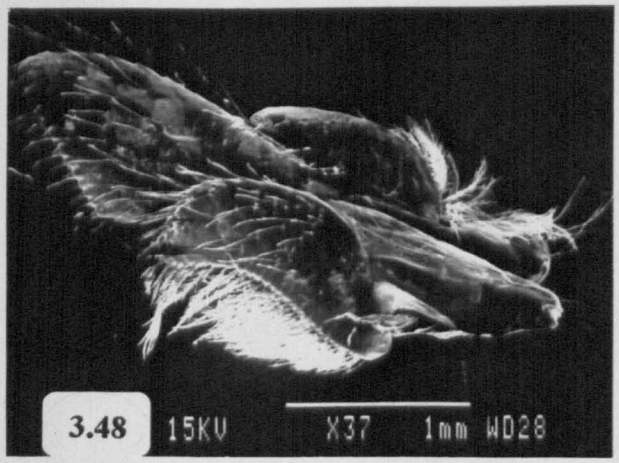
Fig. 3.51 Maxillule of stage 3 *A. leptodactylus*

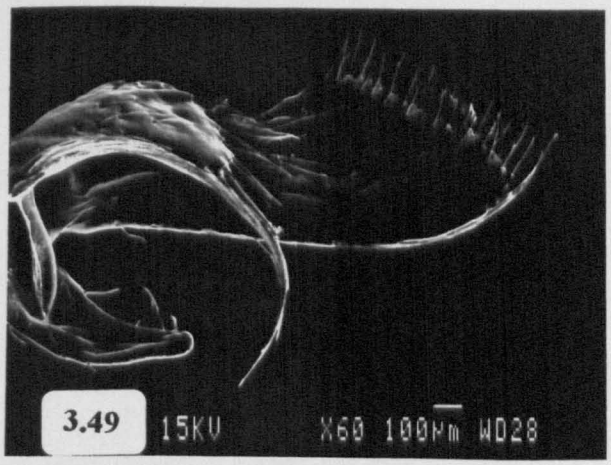
Fig. 3.52 Maxillule of stage 2 *P. leniusculus*

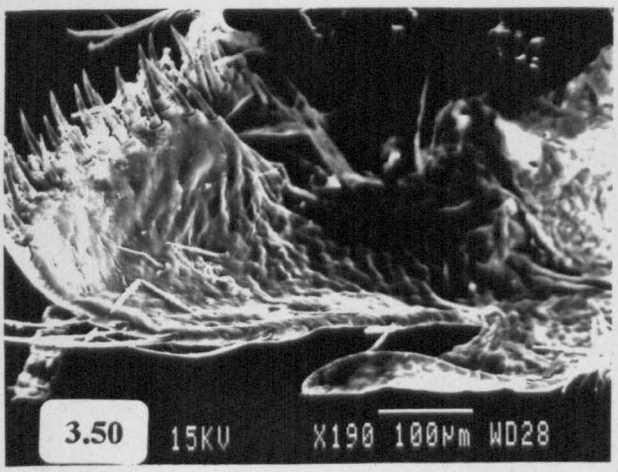
Fig. 3.53 Maxillule of stage 2 *A. leptodactylus*

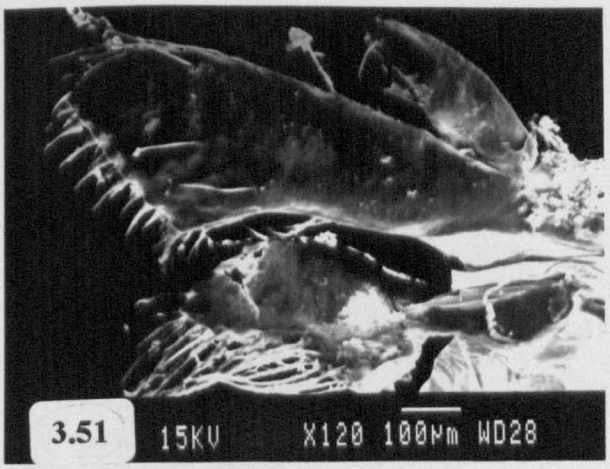
Fig. 3.54 Maxillule of stage 1 *P. leniusculus*

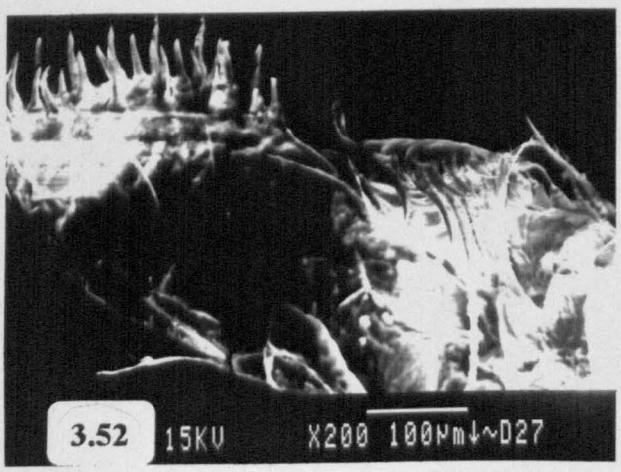
Fig. 3.55 Maxillule of stage 1 *A. leptodactylus*

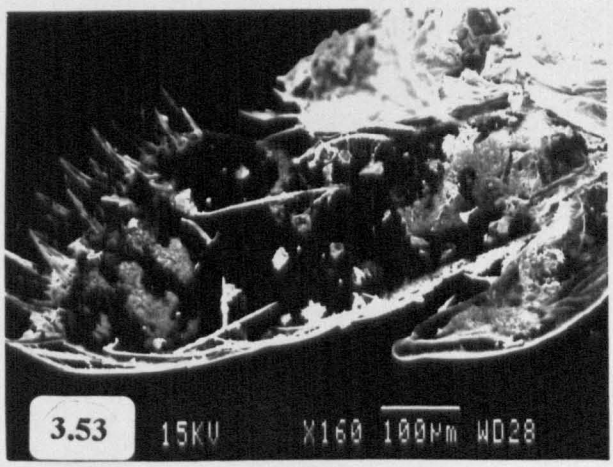

3.48 15KV X37 1mm WD28

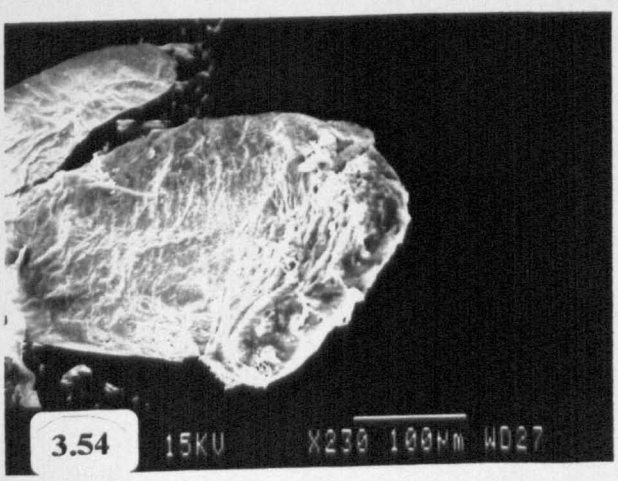

3.49 15KV X60 100µm WD28


3.50 15KV X190 100µm WD28


3.51 15KV X120 100µm WD28


3.52 15KV X200 100µm↓~D27


3.53 15KV X160 100µm WD28


3.54 15KV X230 100µm WD27

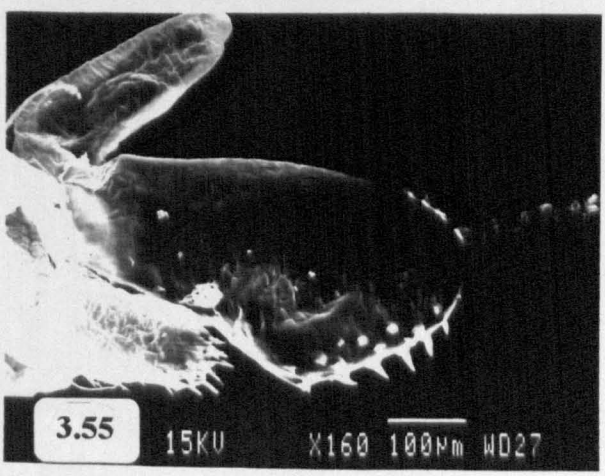

3.55 15KV X160 100µm WD27

Fig. 3.56 Maxilla of 12 mm (CL) *P. leniusculus*

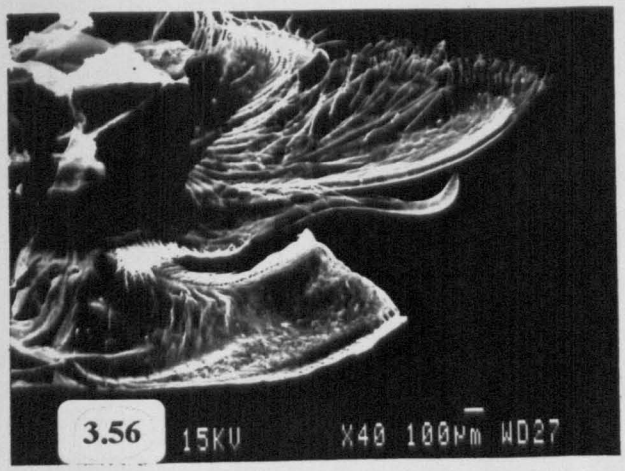
Fig. 3.57 Maxilla of 12 mm (CL) *A. leptodactylus*

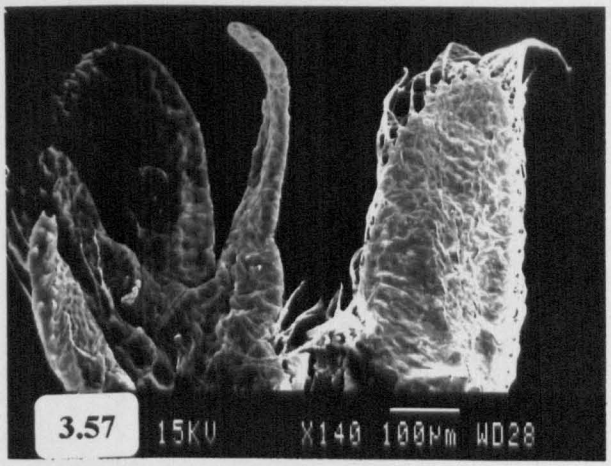
Fig. 3.58 Maxilla of stage 3 *P. leniusculus*

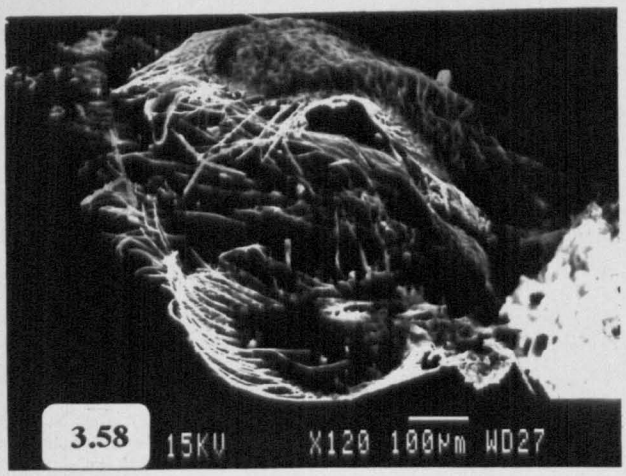
Fig. 3.59 Maxilla of stage 3 *A. leptodactylus*

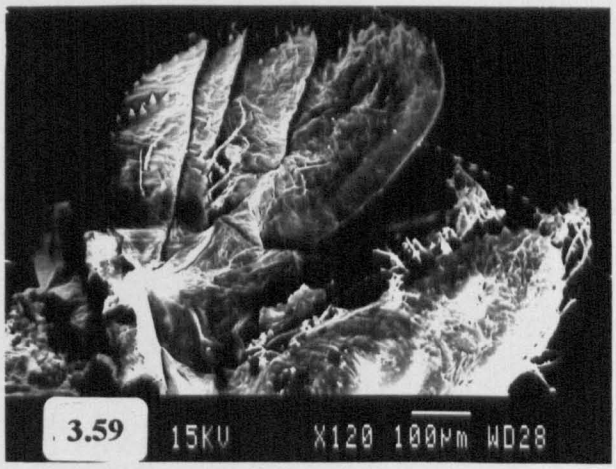
Fig. 3.60 Maxilla of stage 2 *P. leniusculus*

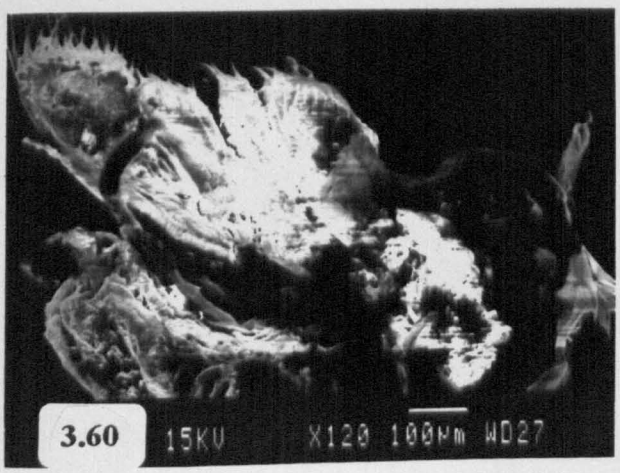
Fig. 3.61 Maxilla of stage 2 *A. leptodactylus*


3.56
15KV X40 100µm WD27


3.57
15KV X140 100µm WD28


3.58
15KV X120 100µm WD27


3.59
15KV X120 100µm WD28


3.60
15KV X120 100µm WD27

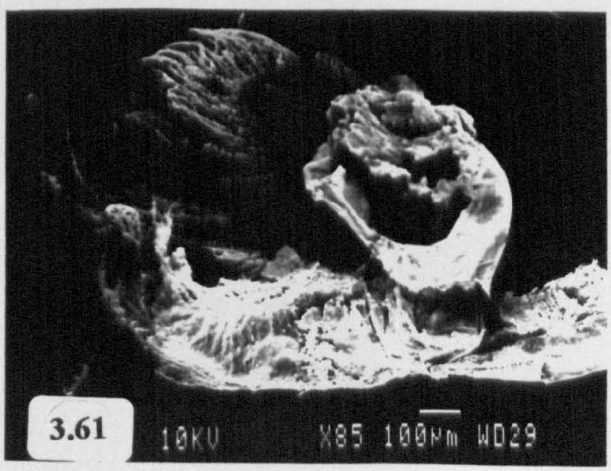

3.61
10KV X85 100µm WD29

Fig. 3.62 Spines (arrow 1) and acuminate setae (arrow 2) on the second and third segment of third maxilliped in stage 2 *A. leptodactylus*

Fig. 3.63 Conate (arrow 1) and cuspidate setae (arrow 2) on the first pereopod of stage 1 *P. leniusculus*, also showing terminal hooks at the end of the pereopod

Fig. 3.64 Conate setae on the endopod of second maxilliped in stage 1 *P. leniusculus*

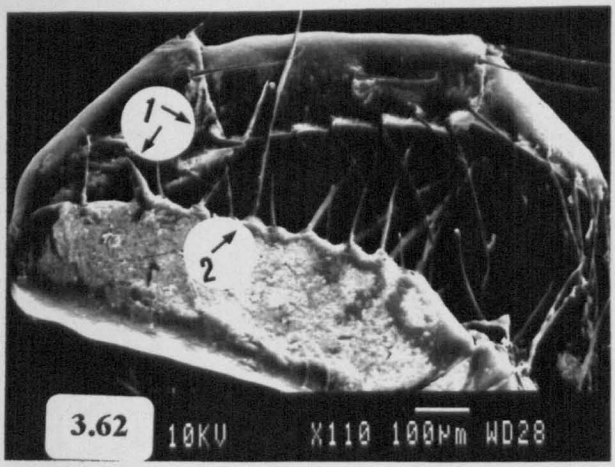
Fig. 3.65 Conate (arrow 1) and plumose setae (arrow 2) on the maxilla of 12 mm (CL) *A. leptodactylus* (to show three different setae)

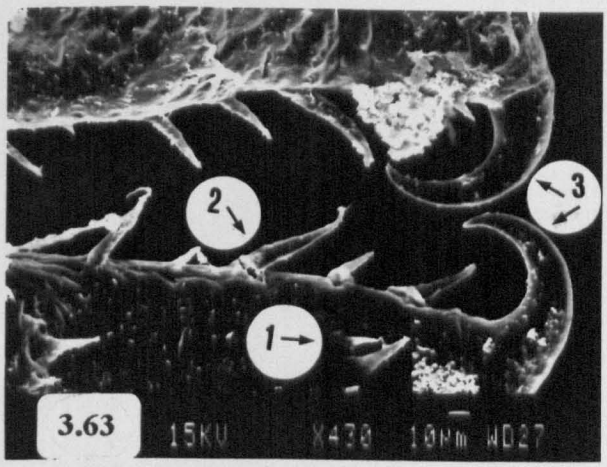
Fig. 3.66 Conate setae on the endopod of second maxilliped in stage 1 *P. leniusculus* (high magnification)

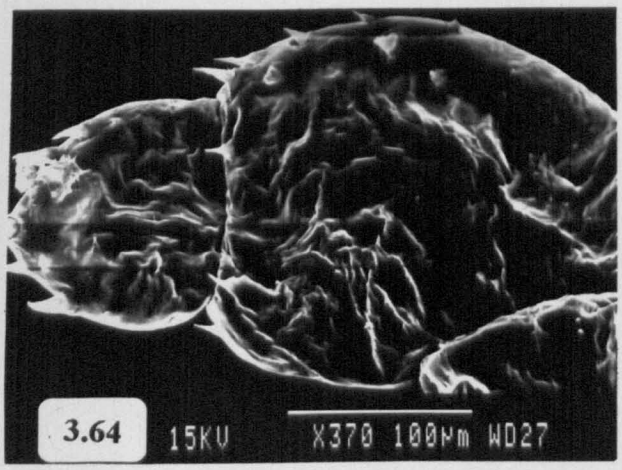
Fig. 3.67 Ventral view of mandible of stage 1 *P. leniusculus* and showing conate setae on the palp

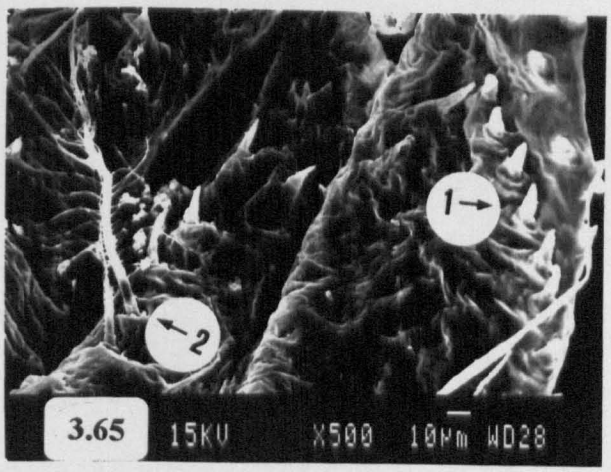
Fig. 3.68 Tooth setae on the dactylus of fourth pereopod in high magnification in 12 mm (CL) *A.leptodactylus*

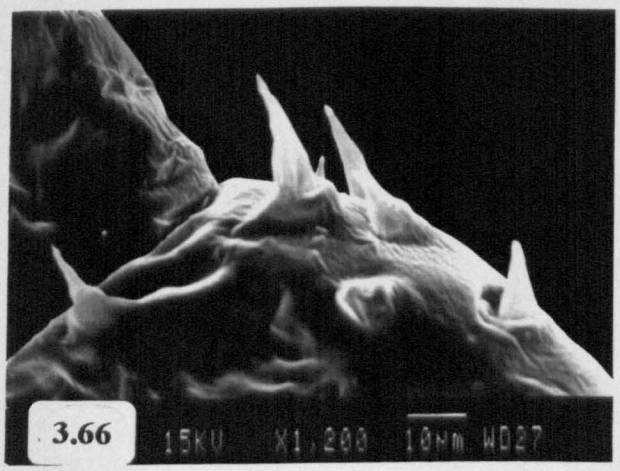
Fig. 3.69 Cuspidate setae on the protopod of maxillule in high magnification in 12 mm (CL) *A. leptodactylus*

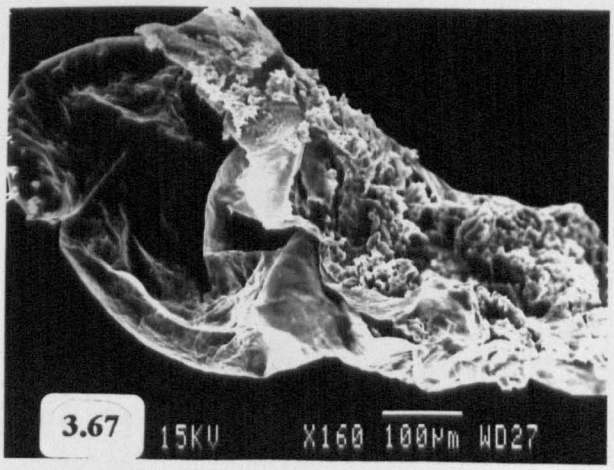

1
2
3.62
10KV X110 100µm WD28

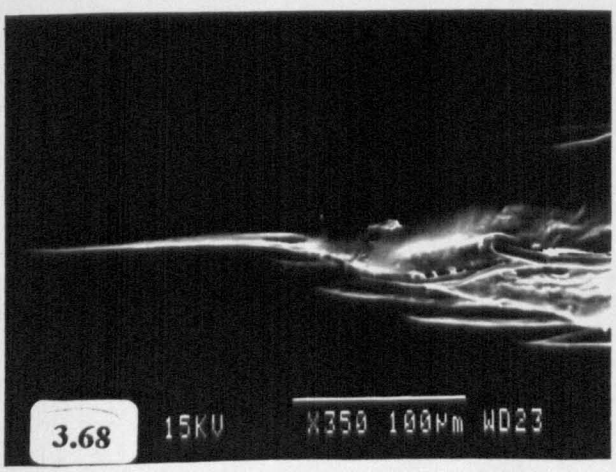

2
3
1
3.63
15KV X430 10µm WD27


3.64
15KV X370 100µm WD27


1
2
3.65
15KV X500 10µm WD28


3.66
15KV X1,200 10µm WD27


3.67
15KV X160 100µm WD27


3.68
15KV X350 100µm WD23

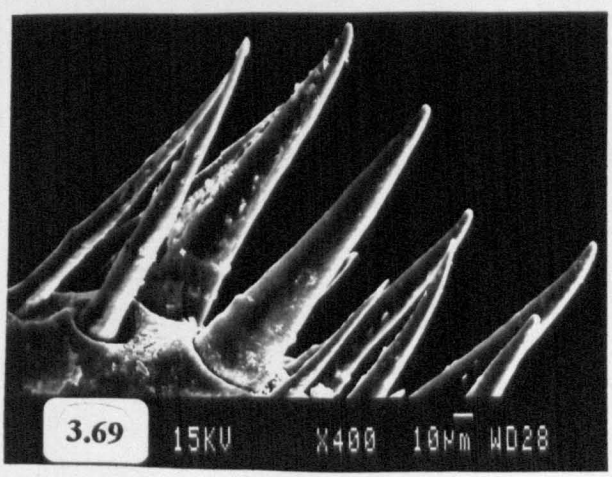

3.69
15KV X400 10µm WD28

Fig. 3.70 Serrate setae on the third segment of third maxilliped in high magnification in stage 2 *A. leptodactylus*

Fig. 3.71 Plumose setae and end of exopode of the first maxilliped in 12 mm (CL) *P. leniusculus*

Fig. 3.72 Serrate (arrow 1) and acuminate setae (arrow 2) on the telson of 12 mm (CL) *P. leniusculus* in high magnification

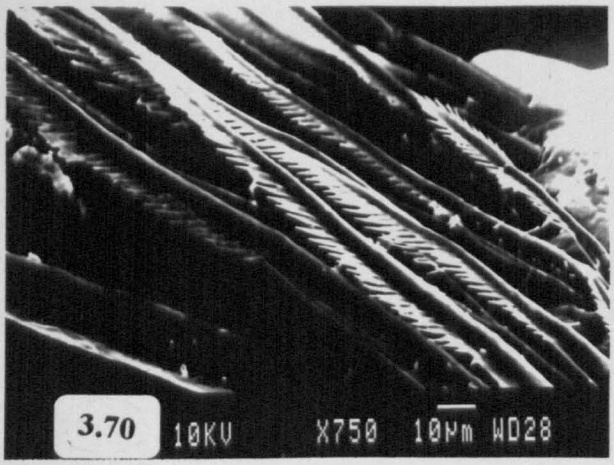
Fig. 3.73 Multiserrate setae on the protopod of maxillule in 12 mm (CL) *P. leniusculus*

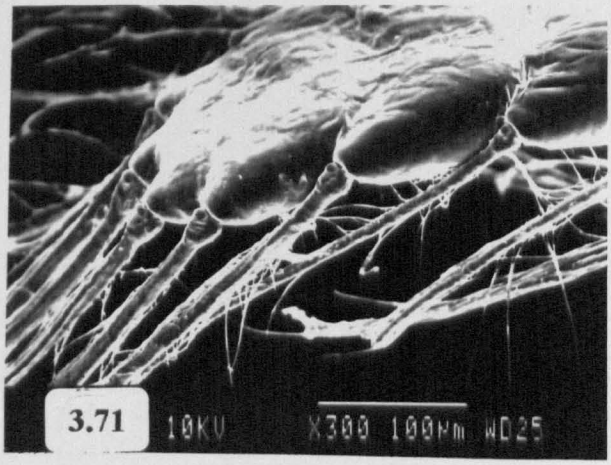
Fig. 3.74 Serrate setae on the telson of 12 mm (CL) *A. leptodactylus*

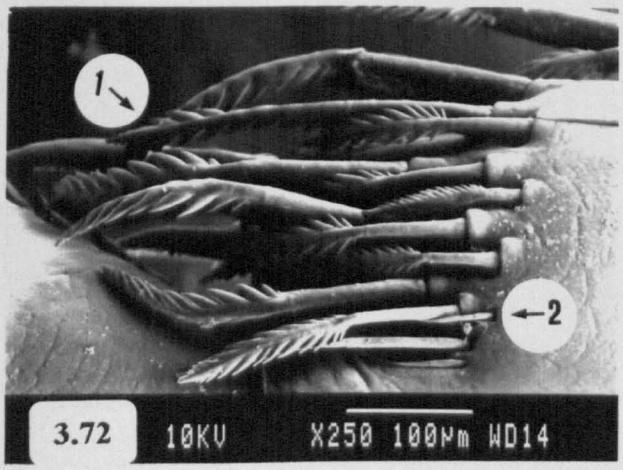
Fig. 3.75 Rod (arrow 1) and tooth setae (arrow 2) on second pereopod of 12 mm (CL) *A. leptodactylus*

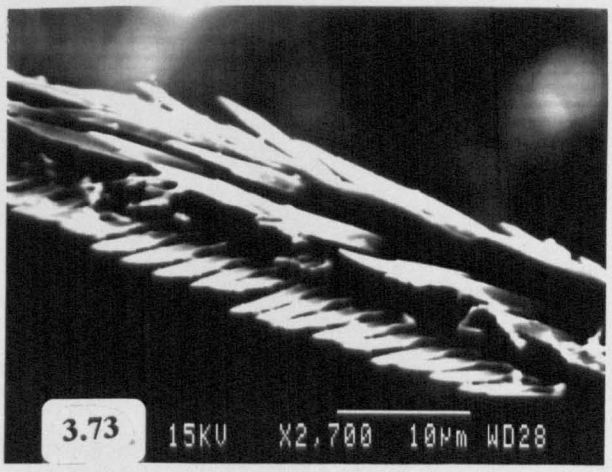
Fig. 3.76 Serrate setae in high magnification on the telson of 12 mm (CL) *P. leniusculus*

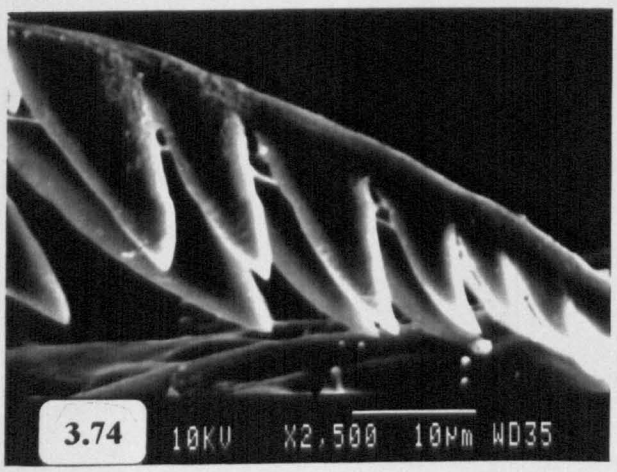
Fig. 3.77 Plumose (arrow 1) and acuminate setae (arrow 2) on the telson of 12 mm (CL) *A. leptodactylus*

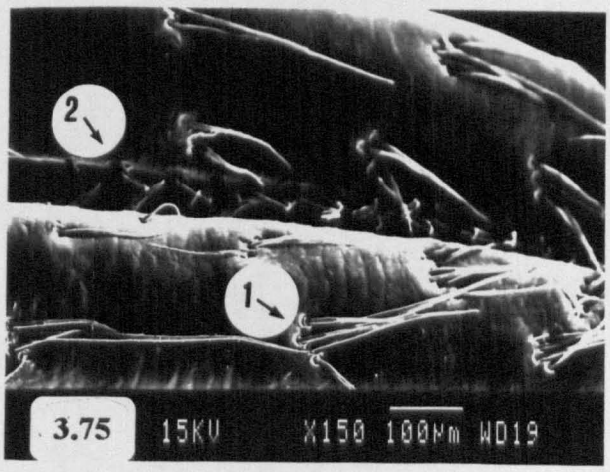

3.70 10KV X750 10µm WD28

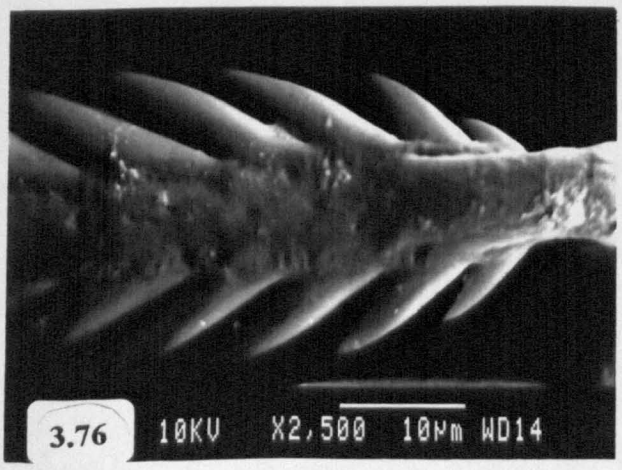

3.71 10KV X300 100µm WD25


1
2
3.72 10KV X250 100µm WD14


3.73 15KV X2,700 10µm WD28


3.74 10KV X2,500 10µm WD35


2
1
3.75 15KV X150 100µm WD19


3.76 10KV X2,500 10µm WD14

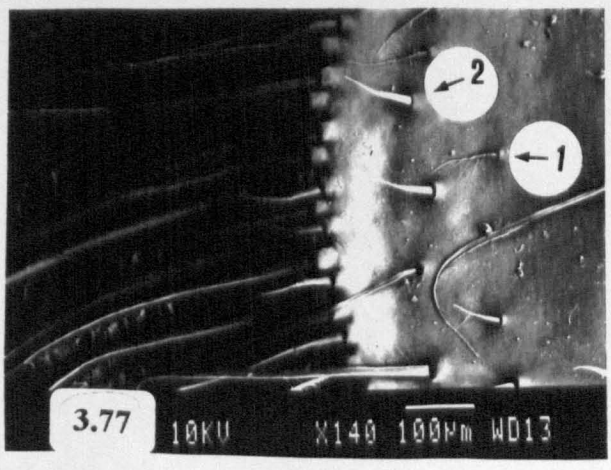

2
1
3.77 10KV X140 100µm WD13

Fig. 3.78 Conate setae on the palp of mandible in stage 1 *P. leniusculus*

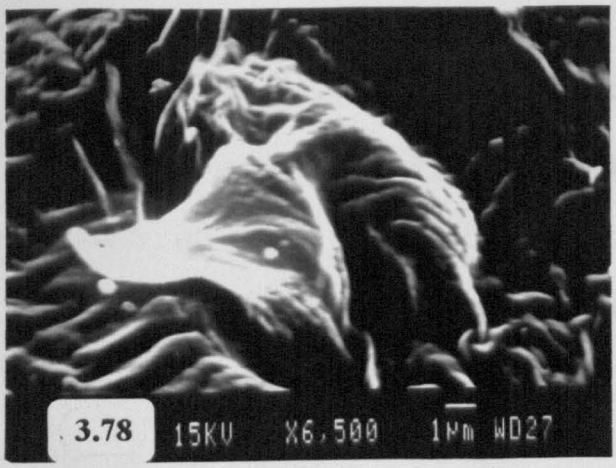
Fig. 3.79 Rod setae on the protopod of first maxilliped in 12 mm (CL) *A. leptodactylus*

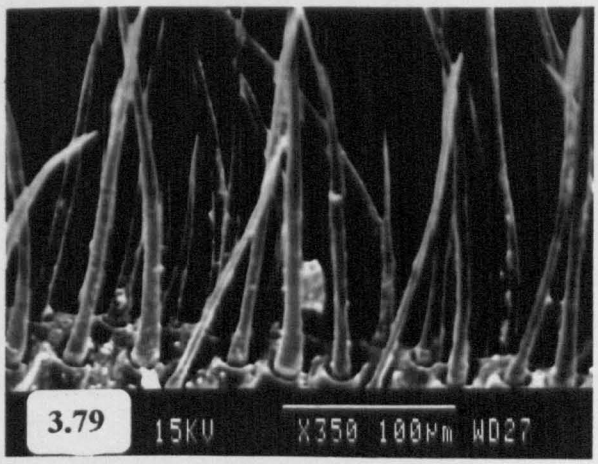
Fig. 3.80 Conate setae on the fourth segment of third maxilliped in stage 1 *P. leniusculus*

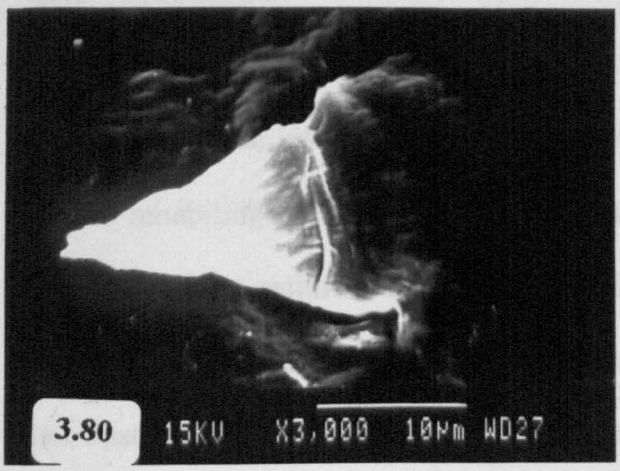
Fig. 3.81 Plumose (arrow 1) and serrulate setae (arrow 2) on the protopod of first maxilliped in 12 mm (CL) *A. leptodactylus*

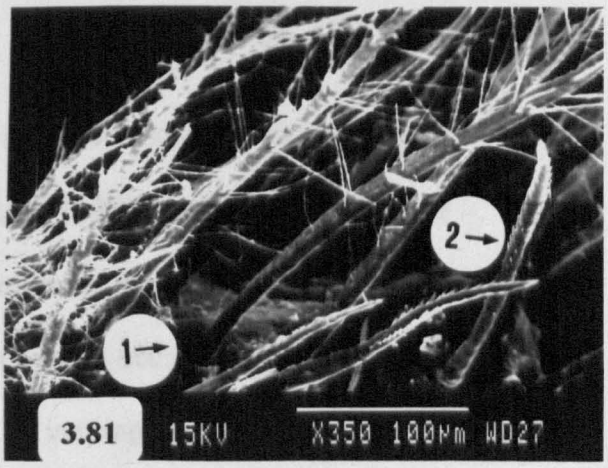
Fig. 3.82 Plumose setae on the cheliped of 12 mm (CL) *P. leniusculus*

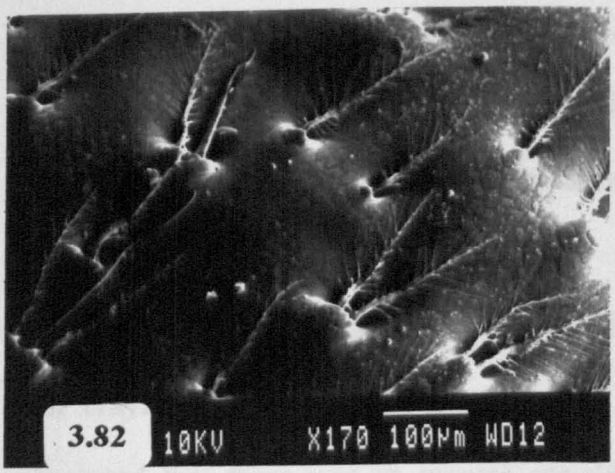
Fig. 3.83 Plumose setae in high magnification on the cheliped of in 12 mm (CL) *P. leniusculus*

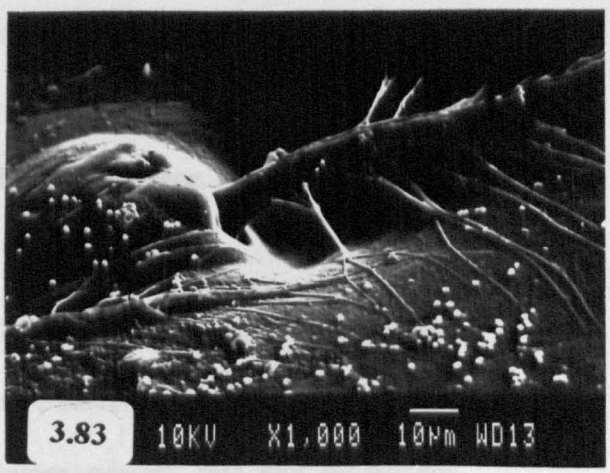

3.78
15KV X6,500 1μm WD27


3.79
15KV X350 100μm WD27


3.80
15KV X3,000 10μm WD27


1
2
3.81
15KV X350 100μm WD27


3.82
10KV X170 100μm WD12


3.83
10KV X1,000 10μm WD13

Chapter 4

Environmental tolerance

4.1 Salinity tolerance and changes in blood parameters at different salinity concentrations

4.1.1 Introduction

Although crayfish inhabit freshwater systems there are also records of some crayfish species inhabiting brackish waters. Burt and McAlister (1959) reported *Pacifastacus leniusculus* from salinities around 15-20‰ in the Colombia River at Astoria, Oregon and Rundquist and Goldman (1978) reported that this species occupied the Sacramento San Juaquin Delta in California, an area subject to varying salinity. Cherkashina (1975) stated that *Astacus leptodactylus* occurred in salinities around 5-14‰ and *Astacus pachypus* around 12-14‰ in the open part of the Caspian Sea. Therefore, as both species live in tidal rivers in Britain they may enter the estuarine environment and perhaps compete with the resident animals for food and space.

In addition to the presence of crayfish species in brackish waters, there some evidence that crayfish can thrive and grow in low salinities. Sharfstein and Chafin (1979) suggested that there was an inverse correlation between growth rate and increased salinity (over the range 0, 3, 6, 9 and 12‰) in *Procambarus clarkii*, which is able to survive and grow at salinities as high as 12‰. In another study on *P. clarkii*, Loyacano (in Jones, 1995) suggested that there was a reduction in the growth rate at salinities of 10 and 20‰. Similarly, the survival ability of crayfish in low salinity and

the reduction of crayfish growth with the increasing salinity was reported for *P. leniusculus* by Rundquist and Goldman (1978) and for *Cherax destructor* by Brian and Geddes (1995).

Short-term laboratory experiments have also shown that crayfish can tolerate high salinity concentrations. *C. destructor* tolerates 100% seawater for at least 48 hours (Frost, 1975). *P. leniusculus* was able to survive in 70% seawater for at least 21 days (Kerley and Pritchard, 1967). Wheatly and McMahon (1983) also found that *P. leniusculus* tolerated 75% seawater for at least 48 hours. Kendall and Schwarz (1964) observed that 50% of *Orconectes virilis* could survive in 75% seawater for at least 72 hours and 50% of *Cambarus bartonii* could survive at this salinity for at least 96 hours. Jones (1995) also observed that although no significant mortalities were observed in *Cherax quadricarinatus* at 18 and 24‰ seawater during the first two weeks of the treatment period, significant mortalities occurred during the last seven days.

These results show the varying ability of different crayfish species to survive in different salinity concentrations. The changes in blood osmotic and ionic concentrations when crayfish were exposed to different seawater concentrations have been studied by many workers (Herrmann, 1931; Kerley and Pritchard, 1967; Pritchard and Kerley, 1970; Wong and Freeman, 1976; Mills and Geddes, 1980; Wheatly and McMahon, 1982a and 1983; McMahon, 1986; Wheatly and Henry, 1987; Henry and Wheatly, 1988; Firkins, 1993; Jones, 1995). In addition, the response of the tissue to salinity stress in *Orconectes rusticus* was studied by Barkman (1970).

Many of these experiments involved only relatively short time periods. For example, in order to observe changes in blood osmotic and ionic concentrations in different salinity concentrations (20-100% by increments of 20%) experiments were carried out by Firkins (1993). *P. leniusculus*, *A. leptodactylus* and *Austropotamobius pallipes* were stepwise acclimated to different salinity concentrations and blood samples were taken after 48 hours (for each acclimation). *P. leniusculus* was also acclimated to 20 and 60% seawater and blood samples were taken after two days, after seven days and after 14 days (for each seawater concentration) (Firkins, 1993). In another study the blood samples were taken from crayfish acclimated to 20, 40, 60, 70, 80 and 100% seawater for two days by Kerley and Pritchard (1967). Similarly, Pritchard and Kerley (1970) and Wheatly and McMahon (1982b) took the blood samples after only two days acclimation. Wheatly and Henry (1987) extended the study period slightly longer by taking blood samples from the crayfish after three weeks.

In this study, a number of experiments were carried out in order to observe the lethal and sublethal effects of increased salinity on juvenile and adult *P. leniusculus* and *A. leptodactylus*, the effects of increased salinity on the osmotic and ionic composition of adult crayfish blood and the response of berried females to different salinity concentrations over longer time periods than had previously been studied.

4.1.2 Materials and methods

Survival experiments with adults and juveniles were carried out in a constant temperature room at 13 ± 1 °C. *Cladophora* was given as food during the experiment. In all experiments, crayfish were acclimated to freshwater for two days.

Seawater solutions were made up from "Instant Ocean" stock. In order to make one litre of 100% seawater 33.333 g "Instant Ocean" salt were dissolved in fresh (tap) water.

4.1.2.1 Survival experiments with juveniles in different salinity concentrations

Forty eight juveniles of *P. leniusculus* and 48 juveniles *A. leptodactylus* were used. The juveniles of the two species were 15 ± 1 mm in carapace length.

Eight juveniles for each species were set up in 20% seawater. Each juvenile was put in one plastic container (55 mm x 105 mm x 45 mm) containing 150 ml of 20% seawater. This procedure was repeated with freshwater (as control), 40, 60, 80 and 100% seawater.

A small stone was placed in each container to provide a sheltered area. Water was changed and mortality was recorded every three days.

4.1.2.2 Survival experiments with adults in different salinity concentrations

Twenty four adults of *P. leniusculus* (size range 43-69 mm in carapace length) and 24 adults of *A. leptodactylus* (size range 45-66 mm in carapace length) were used. Four *P. leniusculus* were put in one container (380 mm x 230 mm x 110 mm) containing six litres of 20% seawater and four *A. leptodactylus* were put in another container at the same concentration. This procedure was repeated with freshwater (as control), 40, 60, 80 and 100% seawater for the two species.

A plastic tube (160 mm in length and 60 mm in diameter) was provided for each adult as a hide. Water was aerated and was changed every week. Mortality was recorded every two days.

After 63 days no mortality was observed in the control, or in 20, 40, 60% seawater. Subsequently 12 crayfish were exposed to 20, 40 and 60% seawater, two of which acted as controls for each concentrations of seawater, and two of the animals kept in 20% seawater were transferred to 40% seawater, two animals were transferred from 40 to 60% seawater and two animals transferred from 60 to 80% seawater.

4.1.2.3 Osmoregulatory experiments with adults in different salinity concentrations

In the second experiment the results revealed that both species were able to survive for a long time (more than nine weeks) in 20, 40 and 60% seawater but were not able to survive when they were transferred to 60 from 40 and to 80 from 60% seawater. Therefore, to observe changes in blood parameters (such as osmolality, chloride and sodium concentrations) in relation to the length of exposure and the effect of transferring crayfish between salinities, long term acclimation experiments were carried out. Additional survival (with more replicates) and reverse acclimation experiments were carried out by removing crayfish from 60% seawater to 40% seawater, from 40% seawater to 20% seawater and from 20% seawater to freshwater. In these experiments four crayfish were put in one container (380 mm x 230 mm x 110 mm) containing six litres of freshwater (which acted as a control) or one of the seawater solutions. Water was aerated and was changed every week.

i) Long term acclimation

Twenty adults of *P. leniusculus* and 20 adults of *A. leptodactylus* were used.

Eight crayfish were set up in 20% seawater and eight crayfish were set up in 40% seawater, and four crayfish were exposed to freshwater as a control for the two species.

After three weeks, blood samples were taken from four individuals of the two crayfish species from each concentration and the remaining crayfish were sampled after six weeks.

ii) Stepwise long term acclimation

Eight adults of *P. leniusculus* and eight adults of *A. leptodactylus* were used.

Four adults of the two species were put in 20% seawater and the other four crayfish were put in 40% seawater. After three weeks, crayfish were then transferred from 20 to 40 and from 40 to 60% seawater.

Blood samples were taken from all crayfish three weeks after the transfer to higher seawater concentration.

To observe changes in osmotic and ionic (Na^+ and Cl^-) concentration in blood with the increased salinity concentration approximately 0.7 ml of blood was taken from each

crayfish by inserting an hypodermic needle through the junction of the carapace and the abdomen membrane into the sinus surrounding the heart.

Before this procedure needles and Eppendorf centrifuge tubes were kept on ice to prevent clotting of the blood. After taking blood samples they were also kept on ice until they were centrifuged.

To remove blood cells, haemolymph samples were spun for 15 minutes at 16 000 RPM in an Eppendorf 5415C Centrifuge. Serum was pipetted off and was put into clean tubes. Then serum samples were kept in -20 °C until the osmotic and ionic concentrations were measured.

For each blood sample both osmotic and ionic concentrations were determined as well as for the different concentrations of seawater. A Wescor 5500 Vapour Pressure Osmometer was used to measure osmotic concentration. The results of osmotic concentration measurements were expressed as mOsmol kg^{-1} (=osmolality). Analysis of chloride ion concentrations was carried out by using a Jenway PCLN3 Chloride Meter. A Pye Unicam SP9 Atomic Absorption Spectrophotometer was used to measure sodium ion concentration. In order to analyze sodium ion concentration blood samples were diluted 1/2500 with deionised water. Chloride and sodium ion concentrations were expressed as mmol l^{-1}.

iii) Reverse acclimation

Sixteen adults of *P. leniusculus* and 16 adults of *A. leptodactylus* were used.

Four adults of *P. leniusculus* and four adults of *A. leptodactylus* were put in each seawater concentration (20, 40 and 60%). The remaining four crayfish were kept in freshwater as a control. After eight weeks all crayfish were transferred to freshwater from the seawater solutions.

iv) Survival experiment in 60% seawater transferred from 40% seawater

Sixteen adults of *P. leniusculus* and sixteen adults of *A. leptodactylus* were set up in 40% seawater.

After three weeks all crayfish were transferred to 60% seawater. The mortality of the two species was recorded every two days.

4.1.2.4 Experiment with berried females in different salinity concentrations

In order to observe whether crayfish eggs hatch in different salinity concentrations berried females of *P. leniusculus* and *A. leptodactylus* were put in freshwater (control), 20, 40 and 60% seawater.

The females of *A. leptodactylus* were set up on 15.03.1995 and the females of *P. leniusculus* were set up on 26.04.1995.

Four berried females of the two species were used for each concentrations. Two females were put in one circular plastic container (250 mm diameter) containing four litres of freshwater (control) or one of the seawater solutions (20, 40 or 60%).

Because adults of the two species could not survive in 80 and 100% seawater more than three weeks as shown by previous experiments, berried females were not put in 80 and 100% seawater.

The experiments were carried out in a constant temperature room at 15 ± 1 °C. *Cladophora* and Minced Morsels (Town and Country petfoods Ltd.) were given as food. Water was aerated and was changed every week.

4.1.3 Results

4.1.3.1 Survival experiments with juveniles in different salinity concentrations

In *P. leniusculus*, during nine weeks, although no mortality was noted in 40 and 60% seawater and in the freshwater (control), and only one mortality was observed in 20% seawater, there were mortalities when the crayfish were exposed to 80 and 100% seawater. In 100% seawater, mortality was 62.5% after one week and 75% after two weeks. In 80% seawater, mortality was 12.5% after one week, 25% after six weeks and 37.5% after eight weeks.

In *A. leptodactylus*, during nine weeks, although no mortality was observed in 20% seawater and in the freshwater (control), and only one mortality was observed in 40%

seawater, mortality increased when crayfish were exposed to 60, 80 and 100% seawater. In 60 and 80% seawater there was 50% mortality after nine weeks. In 100% seawater, mortality was 12.5% after one week, 87.5% after two weeks and 100% after five weeks.

During the nine weeks, numbers of *P. leniusculus* and *A. leptodactylus* moulted in freshwater (control) 20, 40 and 60% seawater and all crayfish were able to harden the shell after moulting.

Comparison of *P. leniusculus* and *A. leptodactylus*:

The results show that there are differences in mortality between the juvenile of *P. leniusculus* and *A. leptodactylus*. Mortality in different seawater concentrations for the two species is given in Table 4.1.1.

Although there were no significant difference in mortality between the juvenile of *P. leniusculus* and *A. leptodactylus* during nine weeks in 80% seawater, significantly more ($P<0.05$, Chi square test) juvenile *A. leptodactylus* died in 60% seawater during the nine weeks. A comparison of mortality versus time between the juvenile of *P. leniusculus* and *A. leptodactylus* in 60, 80 and 100% is given in Figure 4.1.1.

4.1.3.2 Survival experiments with adults in different salinity concentrations

In both *P. leniusculus* and *A. leptodactylus* no mortality was observed in the freshwater (control), 20, 40 and 60% seawater during the nine weeks. However, 100%

mortality in the two species was observed in 80 and 100% seawater during first three weeks.

After transferring *P. leniusculus* and *A. leptodactylus* to 40, 60 and 80% seawater from 20%, 40% and 60% respectively there were no mortalities in 40% but 100% mortality in 60% and 80%. During the six week period no mortalities occurred in the 40%, 60% and 80% controls.

4.1.3.3 Osmoregulatory experiments with adults in different salinity concentrations

i) Long term acclimation

Mean osmotic and ionic concentrations of *P. leniusculus* and *A. leptodactylus* acclimated to freshwater, 20 and 40% seawater for three weeks and for six weeks are given in Table 4.1.2.

After three weeks:

Mean osmotic and ionic concentrations of *P. leniusculus* and *A. leptodactylus* acclimated to freshwater, 20 and 40% seawater for three weeks were plotted against the mean osmotic and ionic concentrations of the medium (freshwater, 20 and 40% seawater) and are given in Figure 4.1.3. The results show that there is a significant increase in osmotic concentration as the salinity of the medium increases in *P. leniusculus* ($r= 0.666$, $df=11$, $P<0.05$) and in *A. leptodactylus* ($r= 0.857$, $df=11$, $P<0.01$). A significant increase was also observed in sodium concentration in both *P.*

leniusculus (r= 0.852, df=11, P<0.01) and *A. leptodactylus* (r= 0.820, df=11, P<0.01). Although no significant difference was observed in chloride concentration in *A. leptodactylus* (r= 0.539, df=11, P>0.05), a slight significant difference was observed in *P. leniusculus* (r= 0.600, df=11, P<0.05).

However, comparison of regression lines showed that there was no significant difference (P>0.05) in the osmotic and ionic (sodium and chloride) response between *P. leniusculus* and *A. leptodactylus* (Table 4.1.3).

After six weeks:

Mean osmotic and ionic concentrations of *P. leniusculus* and *A. leptodactylus* acclimated to freshwater, 20 and 40% seawater for six weeks were plotted against the mean osmotic and ionic concentrations of the medium (freshwater, 20 and 40% seawater) are shown in Figure 4.1.3. As the salinity of the medium increased both osmotic and ionic concentrations also increased significantly after six weeks in *P. leniusculus* and *A. leptodactylus*. The significance of the degree of increase in the two species is shown in Table 4.1.2. These increases are higher (more significant) for both *P. leniusculus* and *A. leptodactylus* than after three weeks of exposure. Comparison of regression lines showed that there was not a significant difference (P>0.05) in the osmotic concentration or in the sodium concentration between *P. leniusculus* and *A. leptodactylus*. However, the chloride concentration of *A. leptodactylus* was significantly higher than that of *P. leniusculus* (P<0.01) and their regression lines were significantly different (Table 4.1.3).

For both species in 20% seawater, there was not a significant increase in osmolality, chloride and sodium concentration ($P>0.05$ in all cases) between crayfish acclimated for three weeks and crayfish acclimated for six weeks (Table 4.1.5). For *P. leniusculus* in 40% seawater, there was a significant increase ($P<0.05$) in sodium concentration between crayfish acclimated for three weeks and those acclimated for six weeks. In *A. leptodactylus* significant increases were observed in osmolality, chloride and sodium concentration ($P<0.01$ in all cases) between crayfish acclimated for three weeks and those acclimated for six weeks (Table 4.1.6).

The diagonal line in Figure 4.1.3 represents the isosmotic line which shows the boundary between hyper-regulation (if values above or to the left of the line) and hypo-regulation (if values below or to the right of the line). It can be seen in Figure 4.1.3 that both species are still able to hyper-regulate in freshwater, 20% and 40% seawater after three weeks and six weeks.

ii) Stepwise long term acclimation

Mean osmotic and ionic concentrations of *P. leniusculus* and *A. leptodactylus* stepwise acclimated to increased salinity (from 20 to 40% seawater and from 40 to 60% seawater) are given in Table 4.1.4.

There was a significant increase in osmotic and ionic concentration in both *P. leniusculus* and *A. leptodactylus* after the transfer of crayfish to 60% from 40% seawater. The degree of significance in osmolality, chloride and sodium concentration between six weeks acclimation to 40% seawater and three weeks acclimation to 40%

leptodactylus is given in Table 4.1.8.

The results revealed that after the six weeks of exposure in both 40% seawater and 60% (transferred from 40% after three weeks) seawater the blood osmotic and ionic concentrations of the crayfish were found to be higher than the medium in both species (Figure 4.1.4). In addition, although no mortality was observed in the survival experiment with adults in 40% seawater and transferred to 40% from 20% seawater (Section 4.1.3.2), very high mortality was observed in the survival experiment with adults in 60% seawater (transferred from 40% seawater) (See below Survival experiment on crayfish in 60% seawater transferred from 40% seawater).

The osmotic and ionic concentrations of the experimental salinities and those of *P. leniusculus* and *A. leptodactylus* acclimated to 40% seawater, transferred to 40 from 20% seawater, and transferred to 60 from 40% seawater are given in Figure 4.1.4.

In *A. leptodactylus*, there was a significant difference ($P<0.01$) in osmolality, chloride and sodium concentration between acclimated to 40% seawater and transferred to 40% seawater (from 20% sea water). The increases in osmotic and ionic concentrations were higher in crayfish acclimated to 40% seawater than in those crayfish transferred to 40% seawater (from 20%). However, only the sodium concentration was significantly higher ($P<0.01$) in *P. leniusculus* acclimated to 40% seawater.

The degree of significance in osmolality, chloride and sodium concentration between six weeks acclimated to 40% seawater and three weeks acclimated to 20% seawater and then transferred to 40% seawater for three weeks in *P. leniusculus* and *A. leptodactylus* is given in Table 4.1.7.

iii) Reverse acclimation

In both *P. leniusculus* and *A. leptodactylus* no mortality was observed in any of the seawater concentrations after nine weeks when this experiment was terminated.

iv) Survival experiment on crayfish in 60% seawater transferred from 40% seawater

No mortality occurred in 40% seawater during three weeks. After transfer to the higher salinity concentration (to 60% from 40%) no mortality was observed in both *P. leniusculus* and *A. leptodactylus* during the first two weeks, but 25% of *P. leniusculus* and 12.5% of *A. leptodactylus* died in the third week. Subsequently, 100% mortality was recorded for *A. leptodactylus* after five weeks and for *P. leniusculus* after seven weeks. A comparison of mortality versus time between *P. leniusculus* and *A. leptodactylus* in 60% seawater (transferred from 40% seawater) is given in Figure 4.1.2.

4.1.3.4 Experiment with berried females in different salinity concentrations

In *A. leptodactylus* egg development continued during the incubation period in all seawater concentrations and in the control. All eggs of four females in the control and all eggs of three females in 20% seawater hatched successfully, the fourth had only a few stage 1 juveniles. In addition, stage 2 juveniles were observed in all replicates of the control and 20 % seawater (Table 4.1.10). These were still alive when the experiments were terminated. However, approximately one week before hatching abnormalities in egg development were observed in three out of four females in 60%

seawater and in two out of four females in 40% seawater. Abnormalities took the form of discoloration and bloating of the eggs. Subsequently, a few juveniles hatched out from only one female (one out of four) in 60% seawater and from two females (two out of four) in 40% seawater. These juveniles died after a few hours of hatching in both 40 and 60% seawater.

Because *P. leniusculus* was not available at the beginning of the breeding season their early egg development was not observed in different seawater concentrations. However, an experiment was carried out with berried females at the end of the breeding season of *P. leniusculus* before their eggs hatched.

As observed in *A. leptodactylus*, the eggs of four females hatched out and developed into stage 2 juveniles in the control and 20% seawater. Although three females (three out of four) in 40% seawater and two females (two out of four) in 60% seawater had a few stage 1 juveniles these died a few hours after hatching (Table 4.1.9).

It is interesting to note that the time of hatching in experimental and control crayfish occurred over a similar time period in both species, i.e. salinity did not delay development.

4.1.4 Discussion and conclusions

Estuarine animals are classified into a number of categories from conformers to regulators on the basis of their physiological responses to changes in external salinity. In conformers, the osmotic and ionic concentrations of the body fluids closely follows that of external medium, whereas in regulators a relatively constant body fluid composition is sustained independent of the environment (Firkins, 1993; Péqueux, 1995).

Although crayfish are freshwater animals the osmotic and ionic concentrations of their body fluids are usually above those of the external medium. Therefore, they are called osmo- or ionic-regulators (Wheatly and McMahon, 1982a). Although their carapace provides a good obstruction to diffusion, because of the fact that the gills are used in the exchange of gas and ions (Bergmiller and Bielawski, 1970; Bielawski, 1971; Ehrenfield, 1974; Avenet and Lignon, 1985) they are not totally impermeable. As a result of this, crayfish gain a constant influx of water by osmosis and lose ions by diffusion. To regulate this system crayfish use their renal organs (antennary or green glands) to discharge water influx and produce plentiful urine, and intake salts from the gut and gills and reduce membrane permeability (Shaw, 1960; McMahon, 1986; Firkins, 1993).

The present study has shown that in long-term experiments using *P. leniusculus* and *A. leptodactylus* the blood osmotic and ionic concentrations increased significantly as the salinity of the medium increased in both species when acclimated to freshwater, 20% and 40% seawater. There was not a significant difference between *P. leniusculus*

and *A. leptodactylus* when the elevation of regression lines were compared for osmotic and ionic concentrations after three weeks. However, although their regression lines were not significantly different in relation to the osmolality and sodium concentrations after six weeks, because *A. leptodactylus* regulated more chloride than *P. leniusculus* there was a significant difference in the elevation of the regression lines for chloride between the two species after six weeks.

Similar results were observed by Firkins (1993) for *P. leniusculus*, *A. leptodactylus* and *Austropotamobius pallipes*. Firkins (1993) found that although these three species maintained the osmotic and ionic concentration of the blood significantly above those of the medium in low salinities, there were significant increases in the osmotic and ionic concentrations of the blood in all three species as the salinity of the medium increased up to 40% seawater. Firkins (1993) also found that there were no significant difference in the response to increasing salinity between the three species. The increase of blood osmotic and ionic concentrations with increased salinity concentrations were also observed in *Cherax destructor* by Mills and Geddes (1980) and in *Cherax quadricarinatus* by Jones (1995).

The ability of *P. leniusculus* to hypo-regulate at high salinities has been observed by Kerley and Pritchard (1967), Rundquist and Goldman (1978), Wheatly and McMahon (1983) and McMahon (1986). Firkins (1993) also observed that as osmotic and ionic concentration of the blood were lower than the medium, *P. leniusculus*, *A. leptodactylus* and *A. pallipes* showed some degree of hypo-regulation at a concentration between 40 and 60% seawater and it was concluded that crayfish could convert from hyper-regulation to hypo-regulation. In addition, Firkins (1993) found

that in long-term experiments, when *P. leniusculus* was exposed to a hyper-osmotic medium (60% seawater), it was initially hypo-osmotic to the medium, but after two weeks it was roughly isosmotic to the medium.

Similar results were found for *P. leniusculus* after three weeks in the present study. In *P. leniusculus* transferred to 60% seawater (from 40%) the osmotic and chloride ion concentrations were roughly isosmotic to the external medium three weeks after the transfer, but in *A. leptodactylus* after three weeks osmotic and chloride ion concentrations were significantly higher than the external medium (Figure 4.1.4). The present study also shows that although both species are able to survive for a long time at 60% seawater, if they start at 40% seawater and are then moved to 60% mortality commences two weeks after the transfer.

The present study shows that both *P. leniusculus* and *A. leptodactylus* are able to tolerate low salinity fluctuations. In the survival experiments with adults, although no mortality was observed in both species in 20, 40 and 60% seawater and in stepwise acclimation to 40% from 20% seawater, 100% mortality was observed in *A. leptodactylus* (after five weeks) and in *P. leniusculus* (after seven weeks) transferred to 60% from 40% seawater. These results suggested that although blood osmotic and ionic concentrations increased significantly with the time of exposure and as the salinity of the medium increased in the long term experiment, and similar increases occurred in animals stepwise acclimated to 40% from 20% seawater, both species could tolerate low salinity fluctuations and were osmo- or iono-regulators in these low salinities (20% seawater = 6.6‰, and 40% seawater = 13.2‰).

An increase in the osmotic and ionic concentrations of the external medium has numerous detrimental effects on this regulatory system. These effects are reviewed by McMahon (1986) as potential osmotic shock (when animals are placed in water more concentrated than the body fluid) which results in loss of water through the gills by osmosis, and salt accumulation in the body. In addition to above, changes in environmental salinity also affect blood acid-base balance. For example, acidosis was observed in *P. leniusculus* as it was exposed to 75% seawater (Wheatly and McMahon, 1983).

There is also evidence that a hyper-osmotic medium also causes abnormalities in the oxygen carrying capacity of crayfish. Haemocyanin, the oxygen transport molecule and the major protein in the blood was found to be broken down as *P. leniusculus* was acclimated to 75% seawater (Wheatly and McMahon, 1982a).

It seems that these negative factors on the metabolism of crayfish in hyper-saline media prevent them surviving long term in such environments. In order to reduce the effects of the detrimental factors of hyper-saline mediums, the following compensatory adjustments occur:

i) sodium and chloride are efficiently reabsorbed by the antennal gland (Pritchard and Kerley, 1970; Bergmiller and Bielawski, 1970; Wheatly and McMahon, 1982b; Wheatly and Gannon, 1995),

ii) the production of primary urine is reduced by antennal gland (Pritchard and Kerley, 1970; Wheatly and Henry, 1987),

iii) ion permeability is reduced and this provides a long time period of equilibration between the blood and external medium to allow crayfish to carry out hypo-osmotic regulation (Ehrenfield, 1974; McMahon, 1986).

Most studies on crayfish osmoregulation were carried out on adult forms and only few studies were made on juveniles. The salinity tolerances of juveniles and adults were investigated in *Cherax destructor* by Mills and Geddes (1980) and in *Cherax tenuimanus* by Morrissy (in Jones 1995). Juveniles were found to be slightly less tolerant of increased salinity than adults in both species.

Different results were observed in the present study. In *P. leniusculus* although no remarkable differences were observed between adults and juveniles in the tolerance of low salinities, juveniles were found to be more tolerant of 80% seawater than adults. Similarly in *A. leptodactylus* no significant differences were observed between adults and juveniles in the tolerance of low salinities. However, although no mortality was observed in adults in 60% seawater, 50% of juveniles died in 60% seawater after nine weeks. In contrast to 60% seawater, although 100% of mortality was observed in adults after three weeks in 80% seawater, 50% of juveniles could tolerate 80% seawater at least nine weeks.

Regarding the salinity tolerance of berried females, the results show that both species are able to produce juveniles only in 20% seawater. Therefore, it can be concluded that although both juvenile and adult forms of the two species can survive in high salinity concentrations they are unable to reproduce successfully at high salinities. Unfortunately, there is no study available on the reproductive potential of crayfish in

different seawater concentrations to compare the experimental results observed in the present study. It would also be interesting to observe the mating and spawning behaviour of *P. leniusculus* and *A. leptodactylus* and to observe their egg development from spawning to hatching in different salinity concentrations. According to Jones (1995) one female *Cherax quadricarinatus* in 18‰ seawater and two females *C. quadricarinatus* in 6‰ seawater spawned and carried fertilized eggs.

In conclusion, it is clear that both species of crayfish can survive in saline water for long periods of time but they can only breed in low salinities.

In low salinities they are hyper osmotic and hyper ionic to the external medium. In higher salinities they gradually become osmo and iono conformers but they cannot survive for a long time in more than 60% seawater.

It is highly likely that both species could survive in the estuarine environment in Britain but it is unlikely that they will be able to increase their numbers to a sufficient level to cause a problem to the other organisms there.

Table 4.1.1. Mortality in juvenile *P. leniusculus* and *A. leptodactylus* in different seawater concentrations

	Freshwater (control)		20% seawater		40% seawater		60% seawater		80% seawater		100% seawater	
Time (weeks)	*P. leniusculus*	*A. leptodactylus*	*P. leniusculus*	*A. leptodactylus*	*P. leniusculus*	*A. leptodactylus*	*P. leniusculus*	*A. leptodactylus*	*P. leniusculus*	*A. leptodactylus*	*P. leniusculus*	*A. leptodactylus*
1	0	0	0	0	0	0	0	0	12.5	0	62.5	12.5
2	0	0	0	0	0	0	0	0	12.5	12.5	75	87.5
3	0	0	0	0	0	0	0	0	12.5	12.5	75	87.5
4	0	0	0	0	0	0	0	0	12.5	12.5	75	87.5
5	0	0	0	0	0	0	0	0	12.5	12.5	75	100
6	0	0	12.5	0	0	0	0	12.5	25	12.5	87.5	
7	0	0	12.5	0	0	12.5	0	37.5	25	37.5	100	
8	0	0	12.5	0	0	12.5	0	37.5	37.5	37.5		
9	0	0	12.5	0	0	12.5	0	50	37.5	50		

Table 4.1.2. Blood osmotic and ion (chloride and sodium) concentrations of crayfish acclimated to freshwater, 20 and 40% seawater for three weeks and six weeks. Values are means with standard errors (n=4).

	Freshwater (control)	20 % seawater	40 % seawater	Significance degree of increase
a) Osmotic Concentration (mOsmol kg^{-1})				
P. leniusculus 3 weeks 6 weeks	431.5 (2.35)	436 (8.99) 437 (4.99)	479 (14.40) 508 (11.19)	P<0.05 P<0.01
A. leptodactylus 3 weeks 6 weeks	414.5 (3.40)	437 (5.08) 432 (5.31)	455 (6.14) 554 (6.13)	P<0.01 P<0.001
Control (tank's water)	75 (0.58)	204 (0.62)	395 (0.71)	
b) Chloride Concentration (mmol l^{-1})				
P. leniusculus 3 weeks 6 weeks	203 (4.64)	209 (1.36) 211 (2.03)	218 (5.22) 237 (8.67)	P<0.05 P<0.01
A. leptodactylus 3 weeks 6 weeks	210 (2.57)	214 (3.25) 211 (3.25)	218 (2.16) 288 (4.49)	P>0.05 P<0.01
Control (tank's water)	1.5 (0.5)	95.6 (1.3)	186.1 (1.8)	
c) Sodium Concentration (mmol l^{-1})				
P. leniusculus 3 weeks 6 weeks	179.4 (9.27)	225.5 (7.60) 207.2 (2.16)	249.2 (7.20) 281.9 (6.23)	P<0.01 P<0.001
A. leptodactylus 3 weeks 6 weeks	171.4 (10.15)	198.3 (11.73) 196.4 (4.47)	241.30 (7.15) 298.5 (1.82)	P<0.01 P<0.001
Control (tank's water)	1.2 (0.21)	89.62 (0.9)	189.12 (1.4)	

Table 4.1.3. Comparison of regression lines of osmotic and ionic response between *P. leniusculus* and *A. leptodactylus* after three weeks and after six weeks (exposed to freshwater, 20 and 40% seawater) (Ancova test)

	After 3 weeks	After 6 weeks
Osmolality	P>0.05 (NS)	P>0.05 (NS)
Chloride	P>0.05 (NS)	P<0.01
Sodium	P>0.05 (NS)	P>0.05 (NS)

Table 4.1.4. Blood osmotic and ion (chloride and sodium) concentrations of *P. leniusculus* and *A. leptodactylus* stepwise acclimated to increasing salinity. Values are means with standard errors (n=4).

	40% from 20% seawater	60% from 40% seawater
a) Osmotic Concentration (mOsmol kg^{-1})		
Pacifastacus leniusculus	485 (19.42)	591 (8.69)
Astacus leptodactylus	506 (2.45)	672 (13.44)
b) Chloride Concentration (mmol l^{-1})		
Pacifastacus leniusculus	246 (8.72)	284 (3.24)
Astacus leptodactylus	252 (0.35)	391.6 (6.71)
c) Sodium Concentration (mmol l^{-1})		
Pacifastacus leniusculus	239.3 (2.72)	339.2 (8.73)
Astacus leptodactylus	257 (2.83)	389 (4.56)

Table 4.1.5. Degree of significance in osmolality, chloride and sodium concentration between crayfish acclimated to 20% seawater for three weeks and those acclimated for six weeks in *P. leniusculus* and *A. leptodactylus* (2 Sample t test).

	P. leniusculus	*A. leptodactylus*
Osmolality	P>0.05 (NS)	P>0.05 (NS)
Chloride	P>0.05 (NS)	P>0.05 (NS)
Sodium	P>0.05 (NS)	P>0.05 (NS)

Table 4.1.6. Degree of significance in osmolality, chloride and sodium concentration between crayfish acclimated to 40% seawater for three weeks and those acclimated for six weeks in *P. leniusculus* and *A. leptodactylus* (2 Sample t test).

	P. leniusculus	*A. leptodactylus*
Osmolality	P>0.05 (NS)	P<0.01
Chloride	P>0.05 (NS)	P<0.01
Sodium	P<0.05	P<0.01

Table 4.1.7. Degree of significance in osmolality, chloride and sodium concentration between crayfish acclimated to 40% seawater for six weeks and those acclimated to 40% seawater for three weeks and then transferred to 60% seawater for three weeks in *P. leniusculus* and *A. leptodactylus* (2 Sample t-test).

	P. leniusculus	*A. leptodactylus*
Osmolality	P<0.01	P<0.01
Chloride	P<0.05	P<0.001
Sodium	P<0.01	P<0.01

Table 4.1.8. Degree of significance in osmolality, chloride and sodium concentration between crayfish acclimated to 40% seawater for six weeks and those acclimated to 20% seawater for three weeks and then transferred to 40% seawater for three weeks in *P. leniusculus* and *A. leptodactylus* (2 Sample t-test).

	P. leniusculus	*A. leptodactylus*
Osmolality	P>0.05 (NS)	P<0.01
Chloride	P>0.05 (NS)	P<0.01
Sodium	P<0.01	P<0.01

Table 4.1.9. The response of berried *P. leniusculus* to different salinity concentrations.

	Stock 26.04.95	30.04.95 - 06.05.95	06.05.95 - 17.05.95
	No of female	hatch (female with stage one juvenile)	stage two juvenile
Freshwater (control)	4	4	4
20% seawater	4	3 and 1 (a few juv.)	3 (some dead stage one with a female)
40% seawater	4	3 (only a few juv.) and dead embryo	0 (dead stage one)
60% seawater	4	2 (very few juv.) and dead embryo	0 (dead stage one)

Table 4.1.10. The response of berried *A. leptodactylus* to different salinity concentrations.

	Stock 15.03.95	15.03.95 - 05.04.95	05.04.95 - 15.04.95	15.04.95 - 23.04.95	23.04.95 - 30.04.95	30.04.95 - 07.05.95	07.05.95 - 17.05.95
Output	No of female	development	development and changes in egg colour	development	abnormalities in egg development and dead embryo	hatch (female with stage one juveniles)	stage two juveniles
Freshwater (control)	4	4	4	4	0	4	4
20% seawater	4	4	4	4	0	3 (and 1 female with a few juveniles)	3 (and 1 female with some dead stage one juveniles)
40% seawater	4	4	4	4	2	2 (only a few juv. and dead eggs)	0 (dead stage one)
60% seawater	4	4	4	4	3	1 (very few juveniles and dead eggs)	0 (dead eggs)

Figure 4.1.1 Percentage mortality in juvenile *P. leniusculus* and *A. leptodactylus* in 60, 80 and 100% seawater

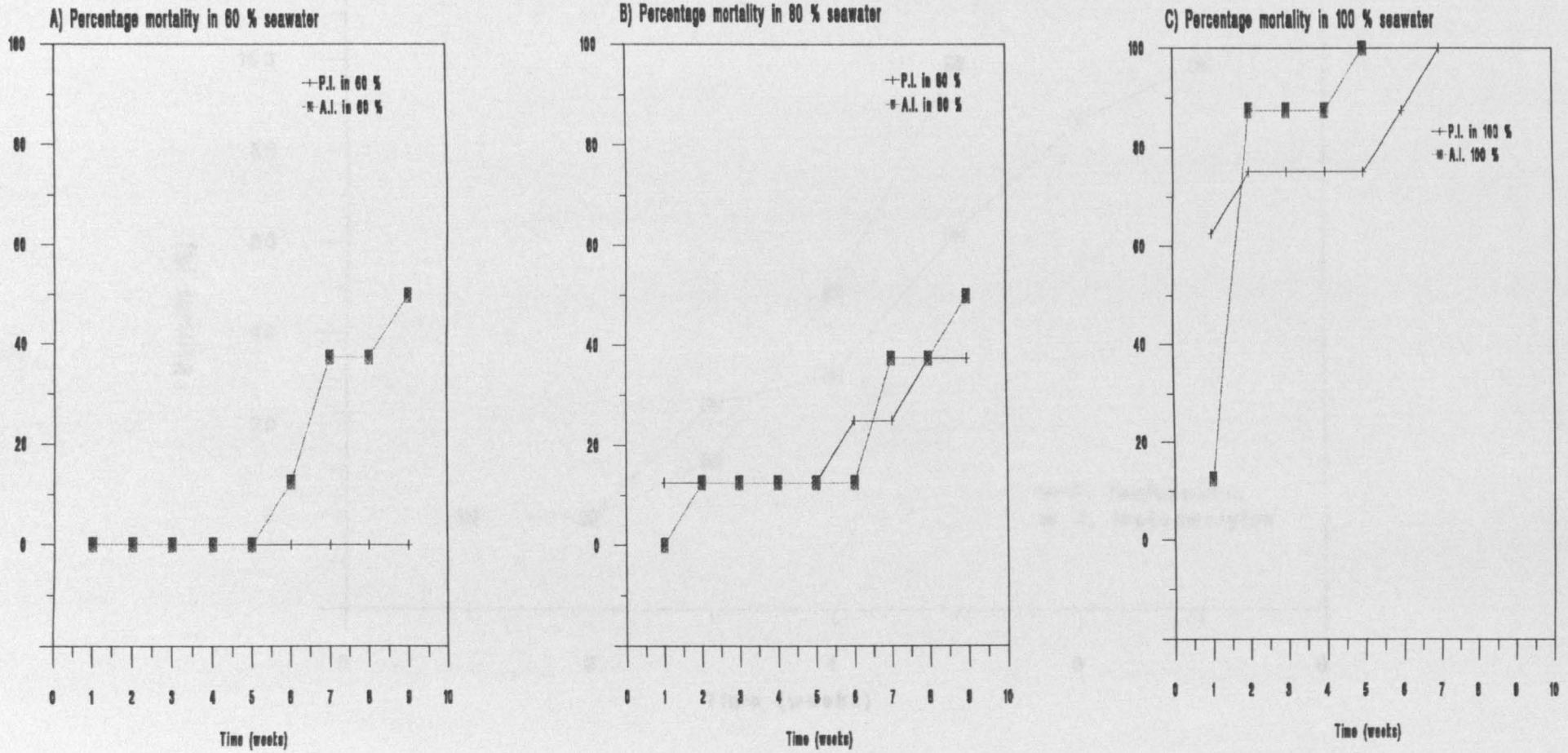


Figure 4.1.2 Percentage mortality in *P. leniusculus* and *A. leptodactylus* in 60% seawater after transfer from 40% seawater

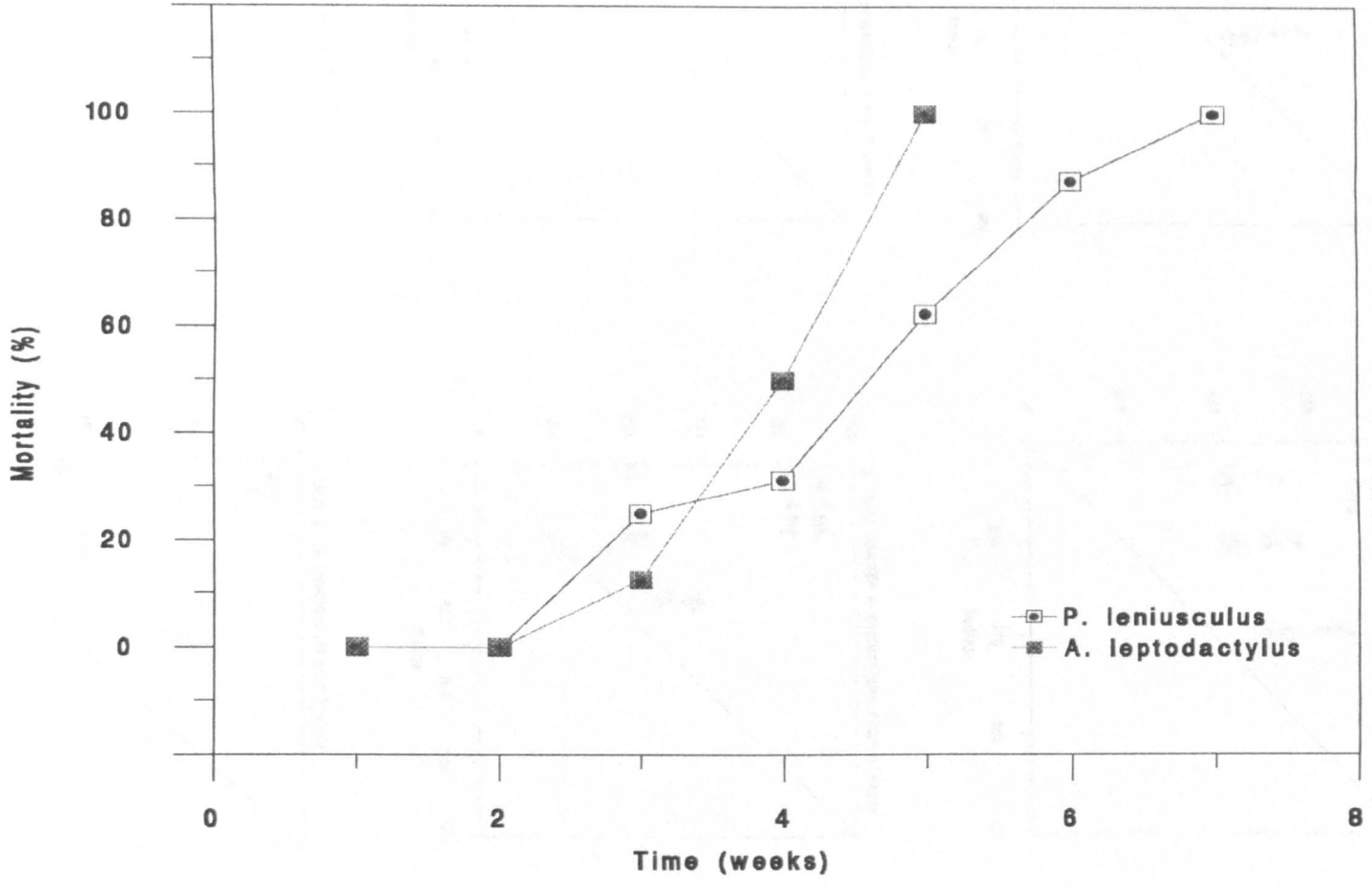
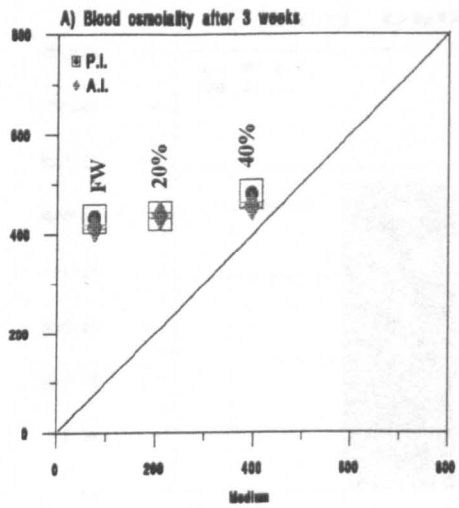
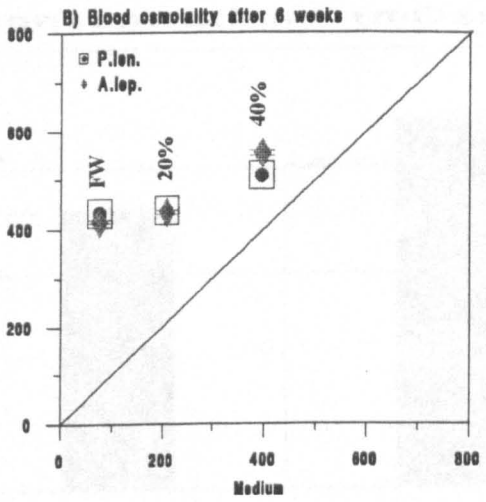
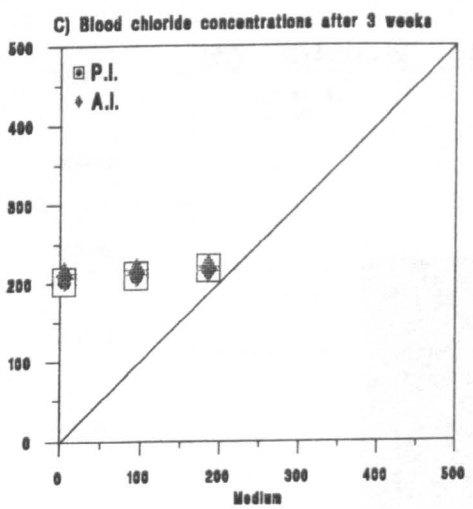
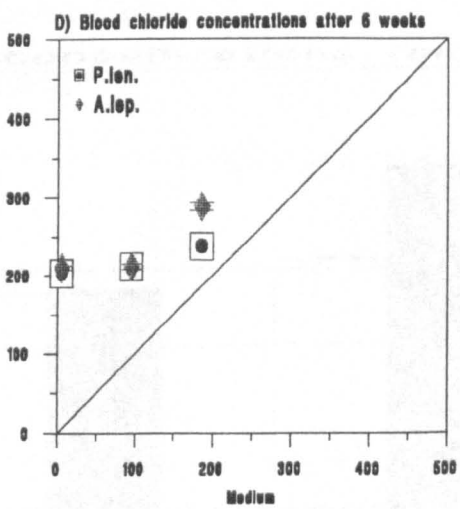


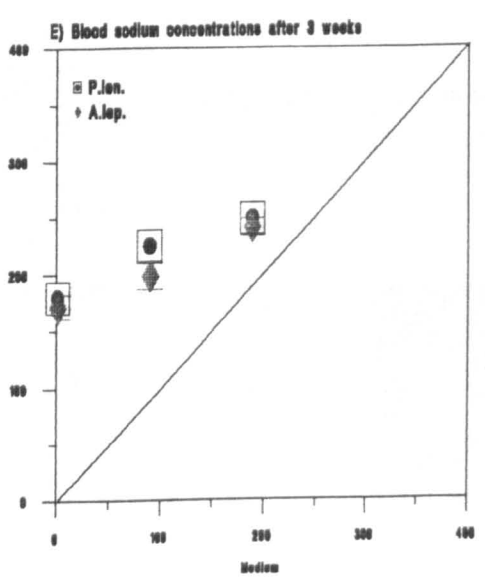
Figure 4.1.3 Blood osmotic (mOsmol/kg) and ionic (chloride and sodium) concentrations (mmol/l) after three weeks and after six weeks in *P. leniusculus* and *A. leptodactylus* acclimated to freshwater (control), 20 and 40% seawater. Values are means with standard errors (n=4).











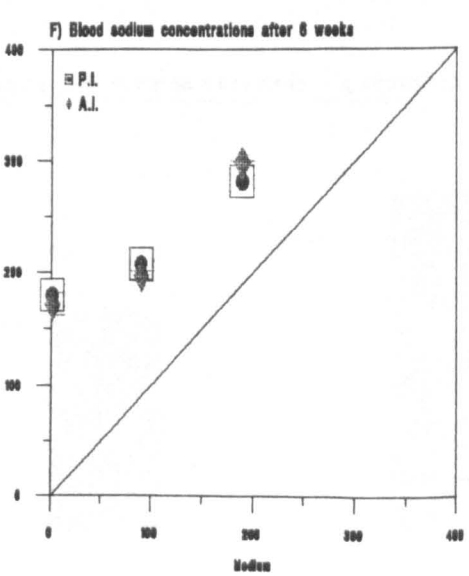
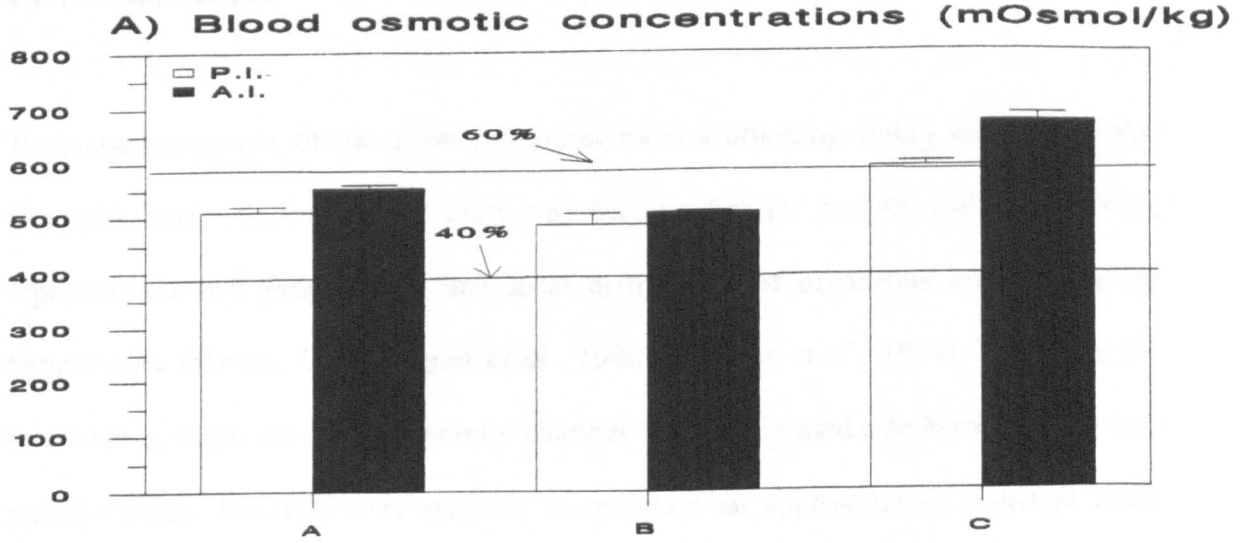
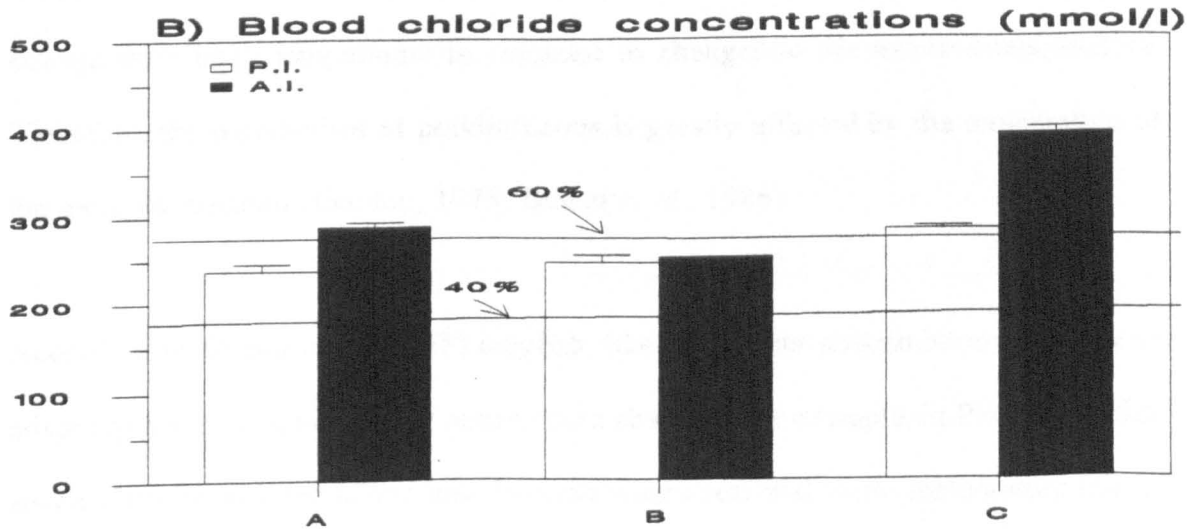
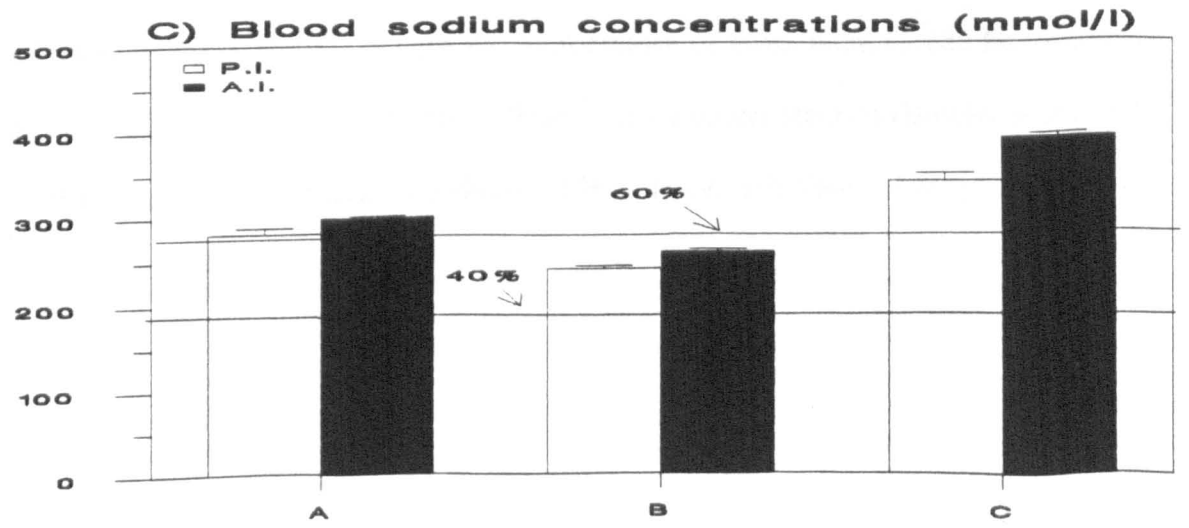


Figure 4.1.4 Blood osmotic and ionic (chloride and sodium) concentrations in *P. leniusculus* and *A. leptodactylus* acclimated to 40% seawater, stepwise acclimated to 40% seawater (from 20%), and stepwise acclimated to 60% seawater (from 40%). On the x axis "A" represents animals in 40% seawater; "B" represents animals in 40% seawater transferred from 20%; "C" represents animals in 60% seawater transferred from 40%. Values are means with standard errors (n=4).







4.2 Tolerance of crayfish to high temperatures

4.2.1 Introduction

Temperature is one of the most important factors affecting living organisms. For example, survival, behaviour, food and feeding habits, growth and metabolism, reproduction and geographical and local distribution of organisms are affected by temperature (Hynes, 1970; Begon *et al.*, 1986; Ramirez *et al.*, 1994). Based on the tolerance of organisms to temperature changes they are divided into homeotherms and poikilotherms. Homeotherms are able to maintain an approximately constant body temperature as the external temperature varies, while poikilotherms significantly change their body temperature in response to changes in the external temperature. Therefore, the metabolism of poikilotherms is greatly affected by the temperature of the external medium (Gordon, 1975; Begon *et al.*, 1986).

According to Becker *et al.* (1975) crayfish, like most other poikilotherms, are able to adapt rapidly to a wide range of temperature changes. For example, in Britain crayfish species live in both lentic and lotic habitats where seasonal water temperature ranges from near 0 °C in winter to over 20 °C in summer (Firkins and Holdich, 1993). In addition to the seasonal changes the temperature of these habitats can be influenced by man for example by the thermal effluents from power stations (Bowler *et al.*, 1973; Hall *et al.*, 1980; Cox and Beauchamp, 1982; Toole and Toole, 1988).

It has been reported that in Britain thermal effluents from power stations give rise to a constant increase in the water temperature downstream of discharge points. Firkins (1993) mentions that the downstream influence of Castle Donington power station on the River Trent causes an increase in water temperature by approximately 7 °C. As a result of this, during the summer the downstream temperatures of the station varied between 25-30 °C. Bowler *et al.* (1973) also stated that thermal effluents cause an increase in water temperature downstream of the discharge point up to 32 °C in summer in some localities in Britain.

In a study on the physiological condition of *Astacus astacus* in warm waste waters of a steel works in northwest Finland no unusual mortalities were observed due to high temperature, 30 °C in summer, but there was a decrease in food consumption and reduced activity in the warmer part of the lagoon at Kuljunlahti (Viikinkonski *et al.*, 1995).

In order to protect aquatic organisms from the harmful effects of pollutants such as the cooling water of power stations legislation has been established by regulatory authorities (Childs, 1981). In addition, the thermal preference of a number of aquatic organisms has also been determined (Hall *et al.*, 1978). Although the temperature preference of more than 100 fish species are known (Coutant in Hall *et al.*, 1978) there are only a few data on freshwater decapods (Hall *et al.*, 1978).

In general, most organisms cannot tolerate a temperature of more than 40 °C (Holdich, 1981). Carp are able to tolerate 35-38 °C, whereas trout cannot tolerate temperatures above 25 °C and their eggs are not able to hatch at temperatures above 14.5 °C.

A number of studies have been carried out to observe the tolerances of crayfish species (mainly North American crayfish species) to high temperatures (Spoor, 1955; Becker *et al.*, 1975; Bovbjerg in Becker *et al.*, 1975; Crawshaw in Hall *et al.*, 1978; Hall *et al.*, 1978; Caine in Claussen, 1980; Claussen, 1980; Mathur *et al.*, 1982; Mirenda and Dimock, 1985; Mundahl and Benton, 1990; Firkins and Holdich, 1993). In general, stream dwelling crayfish species (such as *Orconectes propinquus* and *Orconectes rusticus*) have a lower tolerance limit to elevated temperatures than lake dwellers (such as *Orconectes virilis, Cambarus fodiens* and *Cambarus diogenes*) (Hobbs and Edward, 1974). Similarly, crayfish acclimated to warmer temperatures usually exhibit higher thermal preferences than those acclimated to cooler temperatures (Bowler *et al.*, 1973; Holdich, 1981; Layne *et al.*, 1987; Mobberly in Layne *et al.*, 1987; Mundahl and Benton, 1990; Firkins and Holdich, 1993).

However, because different methods (such as initial acclimation temperatures, the increments of temperature acclimation) have been used by different workers it is difficult to make a comparison between the tolerance of crayfish species to high temperatures except for a few studies.

Caine (in Claussen 1980) found that the maximum thermal tolerance of *Procambarus horsti* was 28.6 °C and that of *Procambarus leonensis* was 38.9 °C. In another study, 50% mortality was observed after 14 hours in *O. propinquus* and after 18 hours in *Cambarus fodiens* exposed to 35 °C from 18-28 °C by (Bovbjerg in Becker *et al.*, 1975). According to Bowler (1963a) when *Austropotamobius pallipes* is initially acclimated to 25 °C for three weeks and the temperature of water is then increased approximately 1°C per minute it can tolerate as high as 37 °C. The tolerance of

Orconectes rusticus was 39.5 °C when it was tested similarly (Claussen, 1980). In one experiment, stage 2 juveniles of *Astacus leptodactylus*, *Austropotamobius pallipes* and *Pacifastacus leniusculus* were initially acclimated to 15 °C then the water temperature was continuously increased from 15 °C and the lethal temperature for each individual crayfish recorded. In that study two rates of heating were used; 0.5 °C $hour^{-1}$ and 2 °C $hour^{-1}$ by Firkins (1993). He found that *A. leptodactylus*, *A. pallipes* and *P. leniusculus* survived in both regimes up to 33 °C, but 100% mortality was observed in *A. pallipes* for temperature between 34-34.25 °C. That of *P. leniusculus* and *A. leptodactylus* was 36-37 °C and 37-37.75 °C respectively. The average lethal temperature for each species was also calculated. This was 33.7 °C for *A. pallipes*, 35.3 °C for *P. leniusculus* and 36.4 °C for *A. leptodactylus*.

In addition to these comparative studies, some studies have focused on the effect of temperature shock on crayfish survival (Spoor, 1955; Bowler 1963a; Mirenda in Cox and Beauchamp, 1982). Fifty percent mortality were observed after eight minutes in *A. pallipes* transferred to 34 from 8 °C (Bowler, 1963a). *Orconectes rusticus* were initially acclimated at temperatures between 22 °C and 26 °C for one to five weeks and were then transferred to 37 °C by Spoor (1955). It was found that *O. rusticus* was not able to survive more than six hours at 37 °C.

In another study, the juveniles of *Cambarus bartoni* were acclimated to 20 °C for at least one week and then transferred to 32 and 33 °C. A 24 hour exposure of juvenile *C. bartoni* to 33 °C resulted in 100% mortality, while exposure to 32 °C for an equal time resulted in 90% survival (Mirenda in Cox and Beauchamp, 1982).

In the present study, the effect of elevated temperature on the survival of the juvenile and adult of *P. leniusculus* and *A. leptodactylus* has been investigated. In addition, a relatively long term survival experiment with juveniles has been carried out to observe the survival ability of the juveniles of *P. leniusculus* and *A. leptodactylus* at constant high temperatures.

4.2.2 Materials and methods

Prior to the experiments crayfish were acclimated to 24 °C for two weeks in water baths with a lid to prevent escape. *Cladophora* and Minced Morsels (Town and Country Petfoods Ltd.) were given as food and a hide was provided for each crayfish.

Survival experiments with adults and juveniles

Ten adults (size range: 53-68 mm CL for *P. leniusculus*; 48-64 mm CL for *A. leptodactylus*) and ten juveniles (14 ±1 mm CL for the two species) of each species were used.

Adults or juveniles of each species were put in a water-bath (70 x 31 x 27 cm) containing 40 litres of water and then stepwise acclimated from 24 to 36 °C by increments of 2 °C. The water bath was heated at a rate of approximately 0.8 °C per minute and crayfish were exposed for 150 minutes to each experimental temperature. Mortality was recorded at the end of the time period (150 minutes) of each acclimation temperature. Specimens were considered to be dead when they failed to respond to prodding with a blunt instrument.

Water was aerated to maintain oxygen at saturation level and no food was given during the experiment.

A relatively long term survival experiment with juveniles at constant high temperatures

Thirty juvenile *P. leniusculus* and 30 juvenile *A. leptodactylus* were used (size range: 14 ±1 mm CL for the two species). Ten of each species were acclimated to 28, 30 and 32 °C.

Temperature was increased from 24 °C to the acclimation temperature (28, 30 or 32 °C) by 2 °C every 90 minutes.

Crayfish were kept under a 12h L: 12h D photoperiod. Water was aerated and minced morsels were given as food.

Mortality was recorded daily during the 7-day experiment.

4.2.3 Results

Survival experiments with adults and juveniles

No mortality was observed in either adults or juveniles of the two species stepwise acclimated to 26 from 24 °C, to 28 from 26 °C, to 30 from 28 °C, to 32 from 30 °C.

Although no mortality was also observed in both species acclimated to 34 from 32 °C all crayfish were very sluggish at the end of the acclimation period. Finally, 100% mortality was observed in both species acclimated to 36 from 34 °C after 150 minutes.

A relatively long term survival experiment with juveniles at constant high temperatures

No mortality was observed in both species acclimated to 28 and 30 °C after seven days and the experiment was terminated. However, in both species acclimated to 32 °C mortality commenced one day after the start of acclimation, and 100% mortality was observed in both species after three days.

Crayfish acclimated to 28 and 30 °C were able to feed on minced morsels; no abnormalities were observed in their behaviour at these temperatures.

4.2.4 Discussion and conclusions

Firkins and Holdich (1993) found that when *P. leniusculus* and *A. leptodactylus* were acclimated to a range of temperatures (5, 10, 20 and 25 °C), with the temperature of water being increased at a rate of approximately 0.8 °C per minute, *P. leniusculus* had a significantly higher thermal tolerance than *A. leptodactylus*. However, the present study revealed no difference between *P. leniusculus* and *A. leptodactylus* when they were stepwise acclimated to high temperatures.

The experimental results showed that both adult and juvenile of *P. leniusculus* and *A. leptodactylus* possess a very wide ability to adapt to elevated temperatures up to 32

°C (from 24 °C by increments of 2 °C and 150 minutes of acclimation for each experimental temperature). In addition to the elevated temperatures, the juveniles of the two species are also able to survive at least seven days at temperatures of 28 and 30 °C, and approximately 24 hours at 32 °C.

The experimental results also showed that there was no difference between adults and juveniles in both species in their survival tolerance of high temperatures. Different results were found by Mundahl and Benton (1990) for the juveniles and adults of *Orconectes rusticus*. They found that the thermal tolerance of adult *O. rusticus* was significantly lower than that of juveniles. They concluded that the difference between adults and juveniles may result from their ontogenetic differences in heat tolerance. Becker *et al.* (1975) also suggested that there may be physiological differences between species and even geographically separated populations of the same species due to long term adaptation to regional climatic factors.

Bowler (1963b) and Bowler *et al.* (1973) observed that mortalities at high temperatures are brought about by a loss of nervous co-ordination which is the result of the instability occurring in blood sodium and potassium concentrations. In addition to mortality, high temperature may also cause an adverse effect on the reproductive and moulting success of crayfish. For example, there was no evidence of successful reproduction in *Astacus astacus* in the warmer part of the lagoon at Kuljunlahti (Finland) where temperatures varied between 28-30 °C (Viikinkonski *et al.*, 1995). In another study, Lahti and Ikaheimo (in Huner, 1994) found that in June the critical thermal maximum for berried *A. astacus* was about 21 °C and mortalities occurred between 23-24 °C. In addition, as part of the study on reducing hatching time (see

Chapter 9) it was found that the berried females of *P. leniusculus* and *A. leptodactylus* lost their eggs two days after acclimation at 21 °C.

In conclusion, from the experiments carried out in the present study it is clear that although both *P. leniusculus* and *A. leptodactylus* survive over a wide range of temperatures they are not able to tolerate temperatures of 34 °C after stepwise acclimation. It is also clear that sudden alterations (increases or decreases) in temperature will adversely affect their life history (such as their reproduction) and 100% mortality will be seen in both species as the temperature is increased from 34 to 36 °C.

4.3 The tolerance of crayfish to low oxygen levels

4.3.1 Introduction

The oxygen concentration of waters is dependent on temperature, there being more dissolved in colder water. Other factors such as pollution, particularly industrial discharges can also affect oxygen levels (Firkins, 1993; Foster and Turner, 1993; Horne and Goldman, 1994). In addition to these, the depletion of dissolved oxygen concentration by plant communities during respiratory activities at night causes a reduction in the oxygen concentration of water (Richards, 1983; Horne and Goldman, 1994).

Low dissolved oxygen concentration and sudden increases in oxygen concentrations are one of the main problems in crayfish culture (Huner, 1988). For example, low oxygen content of water is one of the limiting factors on crayfish growth. In general, crayfish reduce feeding and growing when they are maintained under continuous low oxygen concentrations (Chien and Avault, 1983). Avault *et al.* (1975) and Day and Avault (1986) found that the production of *Procambarus clarkii* is greatly affected by low dissolved oxygen.

In order to observe the tolerance of crayfish species as the dissolved oxygen level of water decreased a number of experiments have been carried out. Huner (1988) stated that abnormalities in crayfish behaviour commenced at dissolved oxygen levels below 3 mg l^{-1} and mortality occured at concentrations below 1 mg l^{-1}. *Cherax destructor* is

able to remain active in less than 0.1 mgO_2 l^{-1} and is able to survive up to 14 hours in this low oxygen concentrations (Fradd in Foster and Turner, 1993).

When the stream-dwellering crayfish, *Orconectes propinquus*, and the pond-dwelling crayfish, *Fallicambarus fodiens*, were exposed to water having an initial oxygen concentration of near 0.50 mg l^{-1} mortality varied from 110 to 312 minutes in *O. propinquus* and from 365 to 1328 minutes in *F. fodiens* (Bovbjerg in Hobbs and Edward, 1974). Maloeuf (in Florey and Kriebel, 1974) reported that the crayfish *Cambarus bartoni* tolerated anaerobic conditions for up to three hours. Bovbjerg (in Hobbs and Edward, 1974) found that when *Orconectes virilis* and *Orconectes immunis* were exposed to water in which the oxygen concentration was less than 1 ppm, the mean survival time for *O. virilis* was 4.2 ± 0.40 hours and that of *O. immunis* was 1.6 ± 0.83 hours.

In another study, *Orconectes neglectus* and *Cambarus setotus* were exposed to a mean oxygen concentration of 4.07 mg l^{-1}. The comparison of mean mortality time showed that *C. setotus* is more tolerant of reduced oxygen tensions than *O. neglectus* (Burbank *et al.*, 1948). The mean mortality time was 892.9 (± 35.0 minutes) in the former and was 272.3 (± 21.5 minutes) in the latter.

Woodland (in Foster and Turner, 1993) found that *Cherax albidus* is able to survive when dissolved oxygen levels fall to 0.4 mg l^{-1}. In another study mortality occurred in *Procambarus spiculifer* at concentrations between 1.3 and 2.1 mgO_2 l^{-1} and in *Procambarus paeninsulanus* at 1.7 to 2.3 mgO_2 l^{-1} (Hobbs and Edward, 1974).

Wheatly and Taylor (1981) found that *Austropotamobius pallipes* is very tolerant of low oxygen levels but tended to emerge from water into air when conditions approached anoxia. They also found that this crayfish can switch to anaerobic metabolism for short periods.

No studies have been carried out to observe the tolerance of *Pacifastacus leniusculus* and *Astacus leptodactylus* to low oxygen levels. Therefore, the present study focused on the survival ability of the two species in low oxygen concentrations.

4.3.2 Materials and Methods

Prior to the experiments crayfish were acclimated to 20 °C for four days and experiments were carried out at 20 °C . During the acclimation water was aerated and *Cladophora* sp. was given as food.

During the experiments the crayfish were confined in a plastic tube and were not allowed to emerge from the water.

Size range was between 51 and 53 mm (carapace length) for both *P. leniusculus* and *A. leptodactylus*.

Experiment 1

Eight adult *P. leniusculus* and eight adult *A. leptodactylus* were used.

Four *P. leniusculus* were put in a glass container (31 cm x 17 cm x 18 cm) with one replicate and four *A. leptodactylus* were put in another glass container with one replicate. Crayfish were then allowed to consume the dissolved oxygen of six litres of water in each container.

At the beginning of the experiment, when the air stones were removed the water contained approximately 4.5 mg O_2 l^{-1} in all containers.

Oxygen concentration of the water was measured by means of a Jenway-9015 Oxygen Meter and a Horiba Model U-10 probe.

Experiment 2

The same procedure used for Experiment 1 was repeated but the crayfish were exposed directly to 0.00-0.05 mg O_2 l^{-1} (the water used was that available at the end of Experiment 1).

4.3.3 Results

Experiment 1

After four hours, the oxygen concentration of the water was between 0.00-0.10 mg/l in both the *P. leniusculus* and *A. leptodactylus* containers. After this time mortalities started to occur.

Figure 4.3.1 shows the mortality of both species with the time of exposure. Individuals of both species were able to survive for a long time in very low oxygen concentration (0.00 -0.05 mg O_2 l^{-1}). During the first six hours no mortality was observed in both *P. leniusculus* and *A. leptodactylus*, but after seven hours mortality was 37.5% for *P. leniusculus* and 12.5% for *A. leptodactylus*. Mortality was 100% for *P. leniusculus* after 21 hours and for *A. leptodactylus* after 19 hours.

Although similar sizes of crayfish were used there was a big difference in the mortality of individuals with the time of exposure in both *P. leniusculus* and *A. leptodactylus*. Individuals of *P. leniusculus* survived from seven hours to 21 hours in low oxygen concentrations. This range was between seven hours and 19 hours in *A. leptodactylus*.

To test their ability to survive in low oxygen concentrations a comparison was made between *P. leniusculus* and *A. leptodactylus* in relation to the mean mortality time of the two species. *A. leptodactylus* was better able to survive in slightly lower oxygen concentrations than *P. leniusculus*. The mean mortality time of *A. leptodactylus* was 13.25 hours (s.e.= 1.29), whereas that of *P. leniusculus* was 12 hours (s.e.=. 1.87).

Experiment 2

Figure 4.3.2 shows the mortality of both species with the time of exposure. During the first four hours no mortality was observed in both species. After five hours mortality was 25 % for *P. leniusculus* and 12.5 % for *A. leptodactylus*.

As was observed in the first experiment, there was a big difference in the mortality of individuals with the time of exposure in both species.

The result showed that *A. leptodactylus* were able to survive better than *P. leniusculus* when they were exposed to 0.00-0.05 mg O_2 l^{-1}. After eleven hours, although 50% mortality were observed for *A. leptodactylus*, 100% mortality was observed for *P. leniusculus*. In addition, the mean mortality time of *A. leptodactylus* was 12 hours (s.e.= 1.36) but that of *P. leniusculus* was 7.5 hours (s.e.= 0.68).

4.3.4 Discussion

Differences in the tolerance of crayfish species to low oxygen levels have been found by some workers. In general, crayfish species inhabiting poorly oxygenated waters are more tolerant of low oxygen than crayfish inhabiting well-oxygenated waters. The stream dwelling crayfish species *O. propinquus*, *O. immunis* and *O. neglectus* are less tolerant of low oxygen concentrations than the pond-dwelling crayfish *F. fodiens*, *O. virilis* and *C. setotus* (Burbank *et al.*, 1948; Bovbjerg in Hobbs and Edward, 1974).

In the present study experimental results do not show a clear difference in the tolerance of *P. leniusculus* and *A. leptodactylus* to low oxygen levels. This may be due to the fact that both species had been maintained in similar conditions prior to the experiments for many weeks.

However, there are some indications that *A. leptodactylus* is more tolerant of decreased oxygen tensions than *P. leniusculus*. In both experiments, when crayfish

were exposed to water having an initial oxygen concentration of approximately 4.50 mg O_2 l^{-1} in the first experiment and when they were exposed to 0.00-0.05 mg O_2 l^{-1} in the second experiment, the mean survival time was longer in *A. leptodactylus* than *P. leniusculus*.

Although *A. leptodactylus* is a cold-water crayfish species it is also known as the swamp or pond crayfish (Köksal, 1988). Evidently, in Britain, *A. leptodactylus* lives in eutrophic conditions, such as lakes, canals and stagnant waters, and in the Serpentine, London, living in contact with anoxic mud (Angersbach and Decker, 1978; Köksal, 1988; Firkins, 1993). *P. leniusculus* is also a cold-water crayfish species. It originates from the cold water lakes and streams of the Sierra and Cascade mountain ranges of the western United States. It also lives in low temperatures and high oxygen concentrations in Lake Tahoe (Nevada) (Moshiri *et al.*, 1970). On the other hand, according to Foster and Turner (1993), in Britain, *P. leniusculus* is able to occupy eutrophic water having as low as 1.2 mg l^{-1} of dissolved oxygen. It seems that although both species originate from cold-waters having high oxygen content both species are very capable of adapting to changing environmental conditions, such as low oxygen concentrations in their new environments.

Decaying vegetation can reduce oxygen levels significantly. In the crayfish ponds in Louisiana constant aeration is needed as the rice forage decomposes, even though *Procambarus clarkii* is very tolerant of low oxygen levels (Huner and Barr, 1991). The same problem is known to occur on crayfish farms in Britain where there is a high level of vegetation and little water movement (Rogers, W.D., pers. comm.).

For a comparative purpose, it would be interesting to know what the tolerance of *Austropotamobius pallipes*, the native crayfish species of Britain, to low oxygen concentrations is in order to evaluate the ability of *P. leniusculus* and *A. leptodactylus*, which were introduced into Britain, to populate unoccupied waters by *A. pallipes*. Although a number of studies have been published on the effect of progressive hypoxia and on the effect of long-term aerial exposure on heart rate, ventilation, respiration gas exchange and acid-base status in *Austropotamobius pallipes* (Wheatly and Taylor, 1981; Taylor and Wheatly, 1981), no data are available on the survival ability of *A. pallipes* in low oxygen concentrations to compare with *P. leniusculus* and *A. leptodactylus*.

As Wheatly and Taylor (1981) found, crayfish may emerge into air if hypoxic conditions develop. *A. leptodactylus* have been known to do this in England when the oxygen levels of their environment are reduced by high temperatures (Holdich, D.M., pers. comm.).

Figure 4.3.1. Mortality of P. leniusculus and A. leptodactylus in Experiment 1.

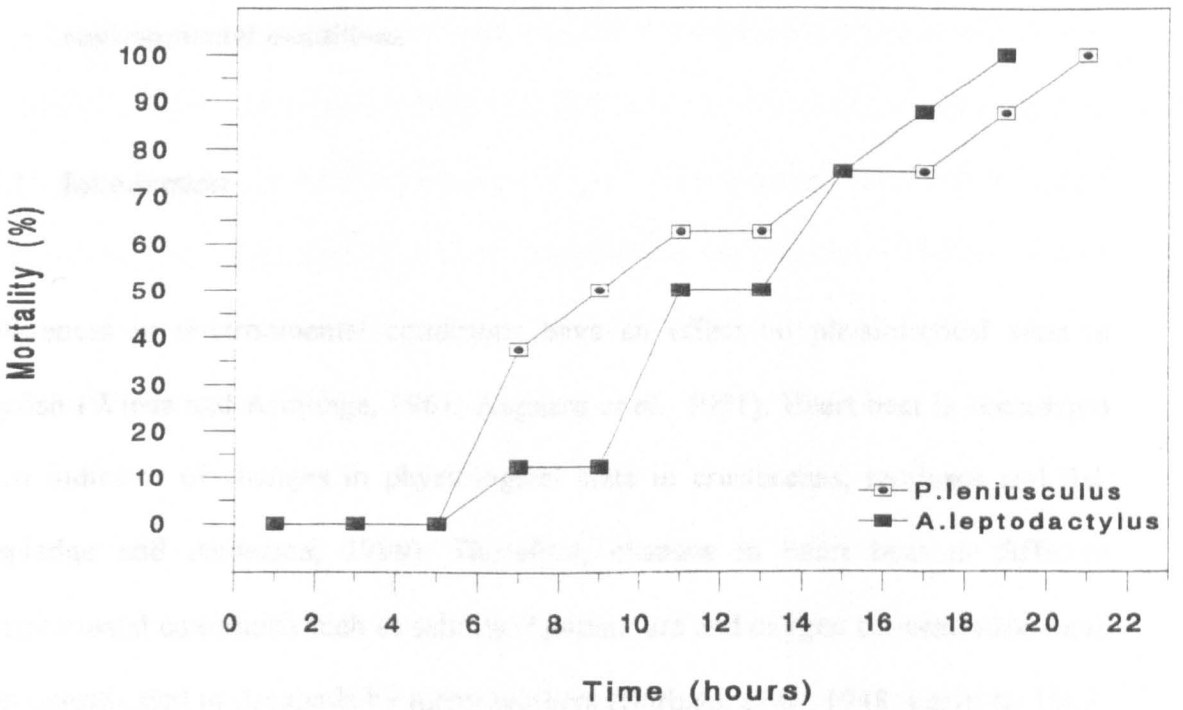
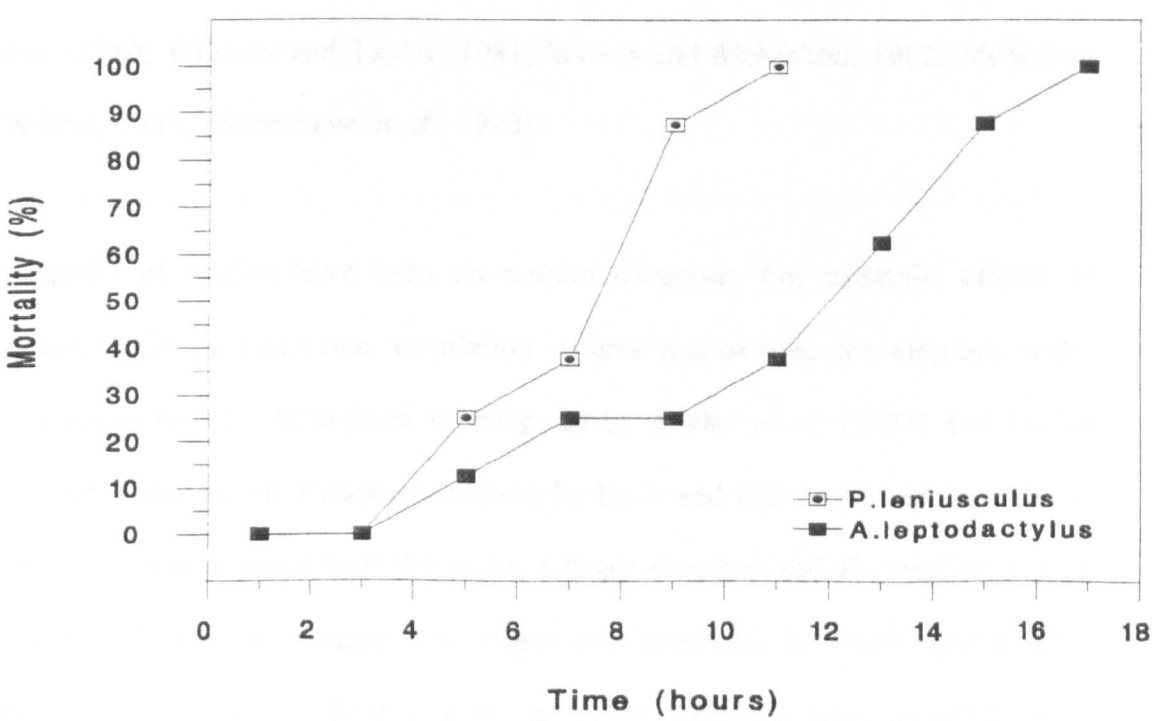


Figure 4.3.2. Mortality of P. leniusculus and A. leptodactylus in Experiment 2.



4.4 The use of changes in heart beat as a measure of the effect of changing environmental conditions

4.4.1 Introduction

Differences in environmental conditions have an effect on physiological state in crayfish (Wiens and Armitage, 1961; Aagaard *et al.*, 1991). Heart beat is considered as an indicator of changes in physiological state in crustaceans, molluscs and fish (Depledge and Andersen, 1990). Therefore, changes in heart beat in different environmental conditions such as salinity, temperature and oxygen concentration have been investigated in decapods by many workers (Burbank *et al.*, 1948; Larimer, 1962; Flindt and Kahrmann, 1972; Spaargaren, 1973; Taylor *et al.*, 1973; Froley and Kriebel, 1974; McMahon *et al.*, 1974; Taylor, 1977; Taylor *et al.*, 1977; Butler *et al.*, 1978; Harri and Florey, 1979; deFur and Magnum, 1979; Dyer and Uglow, 1980; Taylor and Wheatly, 1980; Wheatly and Taylor, 1981; Wilkes and McMahon, 1982; McMahon and Wilkes, 1983a; Styrishave *et al.*, 1995).

The majority of studies have been on marine decapods. For example, effects of decreased salinity on heart rate, ventilation volume and oxygen consumption in the crab, *Carcinus maenas*, have been investigated by Taylor *et al.* (1977) and Taylor (1977), and in the prawn, *Crangon crangon*, by Dyer and Uglow (1980). In order to determine changes in heart beat the crabs, *Canger magister* and *C. productus* were exposed to different temperature and oxygen concentrations by Florey and Kriebel (1974). Similarly, changes in heart beat in *Carcinus maenas* have been recorded during

environmental hypoxia at different temperatures by Taylor *et al.* (1973). A similar experiment has been carried out by Butler *et al.* (1978) on the lobster (*Homarus vulgaris*). Heart beat numbers have been compared between crabs which were buried in sediment or not (Dyer and Uglow, 1978). Similarly, the heart beat numbers and scaphognathite activities of *Carcinus maenas* and *Cancer pagurus* have been compared before, during and after digging activity (Cumberlidge and Uglow, 1978). In another study, in order to evaluate energy consumption in shrimps, changes in heart beat were studied by Spaargaren (1973).

Amongst freshwater decapods most studies have been carried out on crayfish. For example, variations in heart beat in low oxygen concentrations have been reported for *Procambarus simulans* by Larimer (1962), for *Astacus leptodactylus* and *Cambarus affinis* by Flindt and Kahrmann (1972), for *Orconectes virilis* by McMahon *et al.* (1974) and for *Orconectes rusticus* by Wilkes and McMahon (1982). Similarly, the effect of different oxygen concentrations on the heart rate, ventilation and respiratory gas exchange in *Austropotamobius pallipes* has been observed by Taylor and Wheatly (1980) and Wheatly and Taylor (1981). Harri and Florey (1979) have also observed the effects of acclimation temperature on the neuromuscular system of *A. leptodactylus*.

A number of methods have been used to measure heart beats. In some studies a part of carapace was removed over the heart and a piece of wire was connected through the hypodermis (Larimer, 1962; Taylor, 1970). Taylor and Wheatly (1980) inserted wires through the carapace above the heart. They were careful not to penetrate the hypodermis which would have resulted in bleeding. A similar method was used by

McDonald *et al.* (1977). In order to insert the wires or electrodes to the region of the heart, small holes were drilled in the carapace above the heart by Ansell (1973), Taylor *et al.* (1973), Florey and Kriebel (1974), Field and Larimer (1975a), Angersbach and Decker (1978), Watson III and Wyse (1978) and deFur and Magnum (1979). An electrode implantation technique was used to monitor the activity of the heart and both scaphognathites in crustaceans by Dyer and Uglow (1977, 1978, 1980), Cumberlidge and Uglow (1977, 1978) and Hagerman and Uglow (1979). In this technique, small electrodes were connected to the heart and scaphognathites.

It can be seen, therefore, that most of the methods used to measure heart beat have involved invasive techniques despite the fact that this might cause physiological disturbance (Aagaard *et al.*, 1991).

Consequently, a non-invasive, long-term, continuous recording computer-aided technique (CAPMON) was developed by Depledge and Andersen (1990) in order to monitor cardiac activity in crustaceans and molluscs. The CAPMON technique involves glueing an infrared sensor/detector unit onto the carapace above the heart. The translucent properties of the carapace allow the signal to pass in and out and any changes in shape of the heart are detected. This technique was used to investigate the heart beat in the freshwater bivalve, *Anodonta* (Depledge and Andersen, 1990) and to measure simultaneously heart rate, oxygen consumption and locomotor activity in *Carcinus maenas* by Aagaard *et al.* (1991). Recently, the effect of different mercury concentrations on the circadian heart rate rhythms in the freshwater crab, *Potamon potamios* (Styrishave *et al.*, 1995; Styrishave and Depledge, unpublished) and the crayfish, *Astacus astacus* was measured by using this method (Styrishave *et al.*, 1995;

Styrishave and Depledge, unpublished).

As the overall aim of the work presented in this thesis is to compare the biology of two species of crayfish (*Pacifastacus leniusculus* and *Astacus leptodactylus*), the CAPMON technique appeared to have a lot of potential for measuring the sublethal effects of different environmental conditions. As survival experiments had been carried out in relation to salinity, temperature and low oxygen levels (see Chapter 4.1, 4.2 and 4.3) it was decided to use heart beat as a comparative measure of the response of the two species to these environmental variables. In addition, for comparative purposes, two other species, *Procambarus clarkii* and *Astacus astacus*, were also used for some of the experiments.

4.4.2 Materials and methods

The number of crayfish heart beats was monitored continuously by using the CAPMON technique. The detector of the system was attached to the carapace over the heart to record the changes in the shape of the heart. To attach the detector a circular plastic clamp was stuck to the carapace with super glue. A lap-top computer was connected via a control box to the detector to display the data (and heart beat rhythms in graph form) on the monitor screen and to store the data on a floppy disk. In order to prevent the crayfish from cutting the cable it was sheathed in plastic tubing.

In a preliminary experiment variations in the number of heart beats was compared between mobile (free in the tank) and restricted crayfish. Because more variation in the number of heart beats was observed in mobile crayfish the experimental animals

had their movements restricted by placing them in plastic tubes similar to their natural hides (160 mm in length and 60 mm in diameter).

The crayfish were restricted in all experiments except the mobile crayfish in the first experiment. Both mobile and restricted crayfish were conditioned one week earlier before starting the experiment and were fed with *Cladophora* and Minced Morsels during conditioning. After conditioning, no food was given as long as physiological responses of heart beats were being recorded.

In each experiment four crayfish were put in a glass aquarium (30 x 18 x 17 cm) with 14 cm of water for heart beat recording (except the mobile crayfish in the first experiment where they were kept in individual tanks). Water was aerated during the experiments except the seventh experiment. No crayfish moulted during the experiments.

Procambarus clarkii were obtained from breeding stock in the University and *Astacus astacus* from a population in the Mendips (Holdich *et al.*, 1995b), individuals of which were kept in the outside concrete tanks at the University.

The aims of the experiments were to observe:

Variations in heart beat in mobile and relatively immobile crayfish at different temperatures

Before the experiments all crayfish were kept at 14 °C (±1) for two weeks. The first experiment was carried out at 8 °C. The specimens of *P. leniusculus* were 49 mm in CL and 39.3 g, 39.8 g, 45.0 g and 40.0 g respectively in weight for the four replicates. All *A. leptodactylus* specimens were also 49 mm in CL and 36.3 g, 34.0 g, 34.9 g and 35.9 g respectively.

The experiment was repeated at 19 °C using the same crayfish. Crayfish were conditioned to 19 °C for one week prior to the experiments.

In addition, two adult *P. clarkii* and two adult *A. astacus* were used for this experiment at 19 °C. Both species had their movements restricted.

Variability in heart beat in the same crayfish on different days at 19 °C

The variations in heart beat in two female *P. leniusculus* and two female *A. leptodactylus* were recorded for nine days. Half of the aquarium water was changed every three days. Before the experiments all crayfish had been kept at 14 °C (±1) for two weeks.

The relationship between heart beat, length and weight of the crayfish

Forty male *P. leniusculus* and twenty-four male *A. leptodactylus* were used. Recordings were taken for 1440 minutes at 19 °C. Before the experiments crayfish were acclimated to 19 °C for approximately three weeks.

Differences in heart beat between males and females of the two species

The heart beat of four female *P. leniusculus* and four female *A. leptodactylus* were recorded for 1440 minutes at 19 °C to compare to the heart beat of similar sized male which were observed in a third experiment. Before the experiments crayfish were acclimated to 19 °C for approximately three weeks.

The effect of stepwise changes in water temperature

The heart beats of seven male *P. leniusculus* and eight male *A. leptodactylus* were recorded for 360 minutes at each temperature.

This experiment was initially started at 14 °C in which the specimens were kept for approximately ten hours before the experiment. A glass aquarium (30 x 18 x 17 cm) was set up in a temperature-controlled water bath (67 x 30 x 34 cm) and the water temperature was decreased with a cooling coil. Then a series of experiments were carried out at 11, 8 and 5 °C. After taking the recording at 5 °C, the water of the experiment was changed and the specimens were set up again in 5 °C water. After that the experiment was left in a 17 °C room for two days. Finally, after taking the records at 17 °C the water bath was raised to 20 °C and then 23 °C. Temperature was increased and was decreased 1°C in approximately two minutes.

Between each experiment a four hour period of acclimation was provided for the crayfish.

In order to facilitate analysis and to allow for a settling down period after the experiments had started only the middle 180 minutes of the 360 minute run was considered.

The effect of changing temperatures on four species of crayfish

Two adult male *P. leniusculus* and two adult male *A. leptodactylus* were kept at 18 (±1) °C for a week. Then, the temperature was increased to 25 °C from 18 °C (1 °C in approximately two minutes) and the specimens were exposed to 25 °C for 180 minutes. After that, water temperature was decreased gradually to 18 °C approximately within 12 hours in an 18 °C room.

This experiment was repeated three times by using the same specimens. In addition, two adult *P. clarkii* and two adult *A. astacus* were subjected to this experiment. This experiment was repeated twice for *P. clarkii* and *A. astacus*.

The effect of decreased oxygen concentrations

Two similar sized (50-52 mm C.L.), adult male *P. leniusculus* and two adult male *A. leptodactylus* were kept at 18 °C in the glass aquarium (30 x 18 x 17 cm with 14 cm water level). The oxygen concentration was maintained at 6.2 mg O_2 l^{-1} by providing aeration. Then the air stone was removed from the water and the decrease in oxygen concentration was recorded every three hours by means of an oxygen meter (Jenway 9015 dissolved oxygen meter). The oxygen concentration was 6.0 mg l^{-1} when the air stone was removed, 4.2 mg l^{-1} after three hours, 2.4 mg l^{-1} after six hours, 1.6 mg l^{-1}

after nine hours, 0.8 mg l^{-1} after 12 hours and 0.5 mg l^{-1} after 15 hours.

The effect of exposure to 20 and 40% sea water (100% seawater = 33.3‰)

Four adult male *P. leniusculus* and four adult male *A. leptodactylus* were used.

The heart beats were recorded when the specimens were in freshwater as a control then the recordings were taken when the specimens were exposed to 20 and 40% sea water respectively for 24 hours for each concentration at 14 °C.

4.4.3 Results

Variations in heart beat in mobile and relatively immobile crayfish at different temperatures

A time period (750 minutes) of the recordings from the middle of the whole record (1440 minutes) was taken to compare variations in heart beat in mobile and restricted crayfish at different temperatures.

Although the same sized (49 mm in C.L.) specimens were used the variations in heart beat in mobile and restricted *P. leniusculus* and *A. leptodactylus* were very variable within the species and even within the same crayfish. Similar variations were observed for restricted *P. clarkii* and *A. astacus* at 19 °C. Because of these variations within species and within the same crayfish the variations in heart beat were not compared between species. The variations in heart beat with SD values in mobile and restricted

crayfish at 8 °C and 19 °C are given in Table 4.4.1.

The variations in heart beats in *P. leniusculus* and *A. leptodactylus* were higher in mobile crayfish at 8 °C and 19 °C. In addition, variations in SD values were higher at 19 °C.

The variations in heart beats with SD values in mobile and immobile *P. leniusculus* and *A. leptodactylus* at 8 °C are given in Figures 4.4.1, 4.4. 2, 4.4.3 and 4.4.4.

Variability in heart beat in the same crayfish on different days at 19 °C

The mean number of heart beats was very variable on different days in the two specimens of the two species. Daily variations in the number of heart beats with SD values in *P. leniusculus* and *A. leptodactylus* are given in Figures 4.4.5, 4.4.6, 4.4.7 and 4.4.8.

The relationship between heart beat, length and weight of the crayfish

A time period (750 minutes) of the recordings from the middle of the whole record (1440 minutes) was taken to observe the relationship in restricted crayfish.

No relationship was found between the body length and the frequency of heart beat, and the body weight and the frequency of heart beat in the two species. As a result of simple regression analyses the following results were observed:

$r^2_{P.leniusculus}$ = 0.34 (heart beat versus length),

$r^2_{P.leniusculus}$ = 0.30 (heart beat versus weight),

$r^2_{A.leptodactylus}$= 0.02 (heart beat versus length),

$r^2_{A.leptodactylus}$= 0.05 (heart beat versus weight),

As was observed in the first and in the second experiment, variations in heart beat within the same specimen were very variable (Figures 4.4.9 and 4.4.10) in both species. Although it was not observed in all cases, the small animals of the two species had higher heart beat values with bigger SD values than the large animals.

Differences in heart beat between males and females of the two species

The mean number of heart beats of similar sized males and females are given in Table 4.4.2. The heart beats of both sexes were found to be very variable within the same specimen.

The effect of stepwise changes in water temperature

In the two species, the alterations in the heart beat at different temperatures were similar. The number of heart beats of the specimens was relatively high in high temperatures and low in cold temperatures.

At the first glance it seems that there is a strong positive relationship (r^2= 0.96 for *P. leniusculus* and r^2= 0.92 for *A. leptodactylus*) between temperature and heart beat in the two species when the mean of the heart beat of all specimens is considered (Figure

4.4.11). In fact, it is possible to observe more heart beats in cold temperature than those in higher temperatures in the same specimen in the two species. For example, as can be seen in Table 4.4.3, in *P. leniusculus*, the mean heart beat of the second specimen was 79 (per/min) at 11 °C and 73 (per/min) at 14 °C. The third specimen had 65 heart beats (per/min) at 8 °C and 46 heart beat at 11 °C. Similarly, in *A. leptodactylus*, the sixth specimen had 96 heart beats (per/min) at 14 °C and 80 heart beats at 17 °C. The first specimen had 86 heart beat (per/min) at 17 °C and 102 heart beat at 14 °C.

The variations in heart beat number were higher at 17 and 20 °C in the two species. In addition, this variation was least at the lowest (5 °C) and at the highest (23 °C) temperatures (Figure 4.4.12 for *P. leniusculus* and Figure 4.4.13 for *A. leptodactylus*).

The effect of changing temperatures on four species of crayfish

The mean heart beat of the specimens in the last three hours at 18 °C was taken to compare to the mean heart beat of the specimens at 25 °C.

Similar results were observed in the variations of the heart beat in the three replicates in *P. leniusculus* and *A. leptodactylus*. There was a significant increase ($P<0.001$, 2 sample t-test) in the number of heart beats when the specimens were kept at 25 °C as compared to 18 °C.

The heart beat of *P. clarkii* and *A. astacus* also increased significantly ($P<0.001$) when the water temperature was increased to 25 °C. But the increase in the number of heart

beats and variations in heart beat in *P. clarkii* were lower than the other species when temperature was increased to 25 °C from 18 °C. In the first hour at 25 °C, the mean heart beat of *P. clarkii* increased to 103 (±10.5) from 85 (±9.0) for the first replicate and to 115 (±8.5) from 98 (±8.0) for the second replicate, whereas the mean heart beat of *A. astacus* increased to 152 (±20.0) from 118 (±7.4) for the first replicate and to 139 (±20.4) from 103 (±4.4) for the second replicate. In addition to this, at 25 °C during 180 minutes, the specimens of *P. clarkii* had the lowest heart beat. The first replicate had a mean of 111 (±7.3) heart beats and the second replicate had 128 (±4.8). Those of *A. astacus* were 164 (±4.4) and 168 (±2.6) respectively.

The mean heart beat of the specimens in the last three hours at 18 °C, the increase in the number of heart beat at 25 °C, and the decrease in the number of heart beat when temperature goes down to 18 °C are given in Figures 4.4.14 and 4.4.15 for *A. leptodactylus* and Figures 4.4.16 and 4.4.17 for *P. leniusculus.*

The effect of decreased oxygen concentrations

There was initially an irregular increase and then an irregular decrease in the number of heart beats in the two replicates of *A. leptodactylus* (Figures 4.4.18 and 4.4.19).

The number of heart beat decreased gradually with declining oxygen concentration in the two replicates of *P. leniusculus* (Figures 4.4.20 and 4.4.21)

The effect of exposure to 20 and 40% sea water (100% seawater = 33.3‰)

The heart beat frequency was less variable in the controls (freshwater) of each species as compared to the heart beat number when the specimens were exposed to 20 and 40% sea water (Figures 4.4.22, 4.4.23 and 4.4.24 for *P. leniusculus* and Figures 4.4.25, 4.4.26 and 4.4.27 for *A. leptodactylus*).

In addition, the mean heart beat of the four specimens was lower in freshwater than 20% sea water, and there was an increase in the number of heart beats with the increased salinity (from 20% to 40%) especially during the first 15 hours in *A. leptodactylus* and the first eight hours in *P. leniusculus*.

4.4.4 Discussion and conclusions

In the present study, before the experiments were set up during the prior experimental work, it was observed that handling and disturbance of the crayfish caused irregular and generally high heart beats for some time period (from ten minutes to two hours). This has also been reported by other investigators (Hagerman and Uglow, 1979; Taylor and Wheatly, 1980). Because of these observations the records of the first hour after handling were not considered and the crayfish were not disturbed during recordings.

The differences in heart beat between mobile and restricted crayfish have been reported by some workers. Because animal movements caused variations in heart beat the specimens were immobilised by Larimer (1962) and Taylor (1977). Ansell (1973)

also reported that the activity caused an increase in heart beat in crabs. In the present study in order to evaluate the heart beat when the crayfish were in resting condition they were restricted to plastic tubes which were similar to those occupied in their outdoor holding facilities.

The study revealed that heart beats within an individual crayfish or between crayfish which were kept under constant conditions were very variable. This has also been found in similar studies. According to Cumberlidge and Uglow (1977) in some crustaceans the variations in heart beat per minute within an individual were as wide as 15-150. deFur and Magnum (1979) found that this was as much as 40 in the blue crab *Callinectes sapidus*, 35 in the spider crab *Libinia emarginata*, and 20 in the horseshoe crab, *Limulus polyphemus*. In the crab, *Cancer magister*, considerable variations in heart beat were recorded between the specimens (McDonald *et al.*, 1977). In addition to these, considerable variations in heart beat were observed in the crayfish *Austropotamobius pallipes* by Taylor (1970) and in *Limulus* by Watson and Wyse (1978). Cumberlidge and Uglow (1977) and Tonapi and Varghese (1987) concluded that the variation in heart beat was depended on the physiological state of the animal.

In a study on cardio-physiological responses of the freshwater cladocerans, *Daphnia carinata*, *Moina restirostris* and *Simocephalus exspinosus*, to three common pollutants, it was reported that there was no significant difference in the number of heart beats between males and females in all three species before they were exposed to pollutants (Tonapi and Varghese, 1987). In the present study, similar heart beats (with high SD values within the same specimen) were recorded in males and females of *P. leniusculus* and *A. leptodactylus*. Because variations within the same specimen were

very high, therefore, no statistical comparison was carried out between males and females within the species and between the species.

The relationship between heart beat and body size weight has been the subject of a number of studies. A correlation has been found between body weight and heart rate in arthropods but no relation was found between body size and heart rate in molluscs (r=0.19) (deFur and Magnum, 1979). Angersbach and Decker (1978) stated that in general there was an inverse relationship between weight and heart rate in crayfish. Similarly, in another study, within three specimens, the heaviest *A. leptodactylus* had the lowest heart beat (Flindt and Kahrmann, 1972).

In the present study the results showed that there was no correlation between body weight and heart rate, and between body size (C.L.) and heart rate in *P. leniusculus* and *A. leptodactylus*. Because a different experimental method (such as recording technique, temperature range) was used in this experiment the data cannot be compared to Angersbach and Decker's (1978), and Flindt and Kahrmann's (1972) results.

A considerable number of experiments have been carried out to observe the effect of environmental changes (such as salinity, oxygen and temperature) on heart beat in invertebrates. In the literature, different results have been reported on the effect of salinity changes on heart beat in arthropods. There was a decline (an average of 25%) in the number of heart beat in *Limulus polyphemus* when they were transferred from 30 to 20‰ sea water (deFur and Magnum 1979). Spaargaren (1973) found that the heart beat rhythm of osmoregulating and osmoconforming shrimps was salinity-

dependent. The number of heart beats in *Lysmata seticaudata* decreased with increased salinity. A slight increase in heart beat was observed in *Palaemon serratus* when it was exposed to hypo- and hyper-normal salinities. On the other hand, it was observed that the heart beat rhythms of the shrimp, *Palaemon adspersus* (Hagerman and Uglow 1979), the crab, *Carcinus means* (Taylor *et al.* 1977), and the shrimp, *Crangon crangon* (Dyer and Uglow 1980) were not salinity-dependent.

In the present study a sudden increase was observed in the number of heart beats with increased salinity then this increase started to drop down after some hours (Figures 4.4.24 and 4.4.27). Similar results were found in some other studies. Hagerman and Uglow (1979) concluded that although changes in salinity had an effect on heart rate this effect was not a long term one. Similarly, changes in salinity did not cause changes in the heart beat of *Limulus polyphemus* after three days (deFur and Magnum, 1979).

Styrishave *et al.* (1995) found that an increase in salinity appeared to upset the circadian rhythmicity in the heart rate of *Astacus astacus* by reducing heart beat at night.

The changes in heart beat in invertebrates exposed to different oxygen concentrations have also been investigated. According to Larimer (1962) and deFur and Magnum (1979) in fish, decapod crustaceans, bivalve molluscs, gastropod molluscs and diving animals the common reaction of the heart to lowered oxygen concentration (hypoxia) was a reduction in heart beat (bradycardia). This was reported for the shrimp, *Palaemon adspersus*, by Hagerman and Uglow (1979); for the crabs, *Carcinus maenas*,

by Taylor *et al.* (1973), *Cancer pagurus* by Ansell (1973), *Cancer magister* and *Cancer productus* by Florey and Kriebel (1974); and for the horseshoe crab, *Limulus polyphemus*, by Watson and Wyse (1978). Similarly, reduction in the number of heart beats with decreased oxygen concentration was also observed in the crayfish, *Procambarus simulans*, by Larimer (1962), *Cambarus affinis* and *Astacus leptodactylus* by Flindt and Kahrmann (1972), *Orconectes virilis* by McMahon *et al.* (1974), and *Austropotamobius pallipes* by Wheatly and Taylor (1981). Although a small but significant decrease and irregular heart beat was seen in the lobster, *Homarus vulgaris*, in the beginning of the experiments, an investigation of the respiratory and circulatory changes during long-term exposure to moderate hypoxia, indicated that no more irregular and decreased heart beat occurred subsequently (Butler *et al.* 1978). In addition to a decrease in heart beat with reduced oxygen, different patterns of alterations in heart beat were observed during hypoxia in *Palaemon adspersus* (Hagerman and Uglow, 1979). Similar results were observed in the present study in *P. leniusculus* and *A. leptodactylus*. Both increases and decreases and irregular heart beats were recorded during reduced oxygen concentration.

In decapods, the reaction of the heart to changes in oxygen concentration may be dependent on where the animals naturally live. In their natural habitat *P. leniusculus* and *A. leptodactylus* live in streams, rivers and lakes (Lowery and Holdich, 1988; Huner, 1994). *A. leptodactylus* also inhabits swamps (Kossakowski, 1971; Köksal, 1988). Therefore, an investigation was carried out to see whether there was a difference in the response of heart activity to reduced oxygen between *P. leniusculus* and *A. leptodactylus*. However, no marked differences were found. This may be due to the fact that both species had been acclimated to similar conditions prior to the

experiments for many weeks.

In an experiment on the effects of temperature, anoxia and sensory stimulation on the heart rate of unrestrained crabs it was found that the heart was still active in the absence of external oxygen, and when they were exposed to anoxic conditions no symptoms of anoxic conditions were observed on heart beat (Florey and Kriebel, 1974). In another study, the crayfish, *Cambarus bartoni*, survived in anaerobic conditions for up to three hours (Maloeuf in Florey and Kriebel, 1974). In the present study on the tolerance of *P. leniusculus* and *A. leptodactylus* to hypoxic and anoxic conditions it was found that these species could tolerate more than three hours when they were exposed to anoxic conditions (Chapter 4.3).

According to Reiber (1995), crayfish are able to detect dissolved oxygen concentrations in water with oxygen sensitive receptors which are situated on the gills or on the branchiocardiac veins. Larimer (1962) has suggested that this response is governed by the cardioregulatory nerves. It was thought that during this adaptation (or regulation) process the heart rhythm of *P. leniusculus* and *A. leptodactylus* showed irregular and regular activity when oxygen level decreased in the present study.

An increase in the number of heart beats in *Limulus polyphemus* was found when it was transferred to normoxic water from anoxic water (Watson and Wyse, 1978). This increase was also found in *P. leniusculus* and *A. leptodactylus*.

Another environmental condition which has an effect on heart beat is temperature. An increase in temperature has been found to bring about an increase in heart beat in

crabs (*Carcinus maenas* by Taylor *et al.*, 1973; *Cancer magister* and *Cancer productus* by Florey and Kriebel, 1974), and in crayfish (*Astacus fluviatilis, A. leptodactylus* and *Cambarus affinis* by Dauscher and Flindt, 1969; *A. leptodactylus* by Flindt and Kahrmann, 1972 and *P. leniusculus* by Rutledge, 1981). The present study revealed that a sudden increase in temperature (from 18 to 25 °C) caused a significant increase in heart beat in *P. leniusculus* and *A. leptodactylus*. In addition, although an increase in heart beat occurs with increased temperature this increase does not always occur in the same crayfish (see experiment on the effect of stepwise changes in water temperature). This might be evidence of other factors which affect heart rhythm in crayfish rather than the effects of temperature.

In addition to the effect of environmental conditions on the physiological state in crayfish, because of the importance of the neurogenic structure of the crayfish heart, it has been subjected to a considerable number of investigations. In crayfish, blood is circulated by contractions of a neurogenic heart (Wiersma and Novitski, 1942; Huxley, 1973; Holdich and Reeve, 1988; Wilkens, 1995). Its contractions are regulated by the central nervous system with one inhibitory and two acceleratory nerves (Florey, 1960; Larimer, 1962; Holdich and Reeve, 1988). The mechanism of the nervous regulation has been described for *Procambarus clarkii* by Wiersma and Novitski (1942), for *Procambarus simulans* by Larimer (1964) and for *Austropotamobius pallipes* by Taylor (1970).

The activity of the inhibitory and acceleratory nerves is controlled by cardiac command fibres (interneurones) which cause three different heart movements (i) strong inhibitors which cause cardiac arrest, (ii) weak inhibitors which cause bradycardia, and

(iii) accelerators which cause tachycardia (Field and Larimer, 1975 b; McMahon and Wilkens, 1983 a). In addition, heart movements are modified by hormones or other chemical changes in blood (Larimer, 1962 and 1964; Gordon, 1976; deFur and Magnum, 1979; McMahon and Wilkens, 1983 b; Wilkens, 1995). Atwood and Nguyen (1995) concluded that these changes enable crayfish to adapt to changes in environmental conditions.

In a study on the effects of environmental variables on the heart rates of invertebrates, heart rates were found to be more variable in the horseshoe crab, *Limulus polyphemus*, than those of molluscs and annelids. Sudden increases in the heart rate of *L. polyphemus* were associated with spontaneous increases in the regulatory mechanism or with external stimuli (deFur and Magnum, 1979). Mercier and Russenes (1992) investigated the effects of FMRFamide-related peptides on the crayfish heart. It was observed that two lobster peptides TNRNFLRFamide and SDRNFLRFamide caused an increase in the rate and amplitude of heart beat of *P. clarkii*. More recently Wilkens (1995) found a number of FLRFamide-related peptides in the pericardial organs of crayfish and lobsters which caused an increase in heart rate in *P. clarkii*.

From the account given above it is clear that the frequency of heart beats in crayfish and other arthropods is extremely variable and can be affected by a wide range of factors. Even when conditions appear identical replicates can give different results.

The CAPMON system, being non-invasive, would appear to overcome some of the variation which might be introduced by stress but variation do still occurs. It is not clear why this should be the case and more work is required to make the system more effective.

Table 4.4.1. Variations in heart beat per minute in mobile and restrained crayfish at different temperatures

	r	length, mm (CL)	weight (g)	restrained at 8 °C	mobile at 8 °C	restrained at 19 °C	mobile at 19 °C
P.leniusculus	1	49	39.35	55 (8.0)	77 (17.1)	71 (15.4)	85 (32.1)
	2	49	39.84	53 (4.2)	81 (24.3)	75 (12.3)	96 (28.7)
	3	49	45.00	47 (7.7)	73 (14.4)	69 (9.8)	91 (19.9)
	4	49	40.05	57 (9.2)	50 (12.4)	78 (16.1)	87 (35.0)
A.leptodactylus	1	49	36.39	62 (5.0)	86 (16.1)	72 (17.3)	92 (24.3)
	2	49	34.00	54 (7.1)	69 (15.8)	69 (9.3)	87 (31.7)
	3	49	34.91	59 (8.3)	72 (13.7)	86 (14.8)	102 (12.1)
	4	49	35.98	63 (6.0)	80 (18.0)	79 (12.2)	97 (20.0)
P.clarkii	1	55	45.96			85 (30.9)	
	2	55	43.57			95 (15.2)	
A.astacus	1	49	39.99			108 (20.9)	
	2	47	28.84			124 (19.4)	

Note: r= replicates, values in ( ): SD

Table 4.4.2. Mean heart beat per minute in male and female *P.leniusculus* and *A.leptodactylus*

	r	length, mm (CL)	weight (g)	mean heart beat
in male				
P.leniusculus	1	51	45.70	128 (10.6)
	2	51	49.33	131 (14.7)
	3	53	53.97	125 (9.52)
	4	53	61.61	114 (26.6)
mean				124 (6.4)
in female				
P.leniusculus	1	50	43.96	128 (23.0)
	2	52	47.02	123 (18.9)
	3	52	45.84	111 (12.3)
	4	53	49.12	96 (8.21)
mean				114 (12.3)
in male				
A.leptodactylus	1	50	35.36	86 (20.3)
	2	51	37.49	121 (15.1)
	3	52	44.77	100 (15.9)
	4	52	37.74	114 (11.0)
mean				105 (13.4)
in female				
A.leptodactylus	1	50	38.63	76 (16.0)
	2	51	32.93	95 (9.11)
	3	52	33.72	115 (12.9)
	4	53	37.25	103 (15.0)
mean				97 (14.1)

Table 4.4.3. Number of heart beats per minute in male *P.leniusculus* and male *A.leptodactylus* at different temperatures

	r	length, mm (CL)	weight (g)	5 °C	8 °C	11 °C	14 °C	17 °C	20 °C	23 °C
P.leniusculus	1	50	45.91	33 (7.6)	34 (4.2)	63 (6.9)	63 (8.3)	87 (14.7)	88 (8.5)	114 (11.3)
	2	51	45.76	42 (28.9)	42 (20.1)	79 (35.1)	73 (20.3)	102 (31.0)	117 (36.5)	128 (14.0)
	3	52	48.52	62 (8.8)	65 (3.9)	46 (16.2)	62 (4.5)	85 (13.1)	95 (9.6)	123 (15.3)
	4	53	53.97	48 (5.8)	53 (5.7)	74 (7.8)	91 (6.5)	97 (8.9)	116 (11.2)	132 (5.6)
	5	55	61.96	65 (5.4)	69 (7.3)	75 (6.5)	82 (15.0)	101 (17.3)	129 (6.4)	124 (25.4)
	6	56	62.51	46 (5.6)	65 (9.9)	65 (19.0)	70 (9.3)	130 (11.0)	110 (14.8)	129 (13.6)
	7	57	82.05	57 (7.7)	57 (5.4)	80 (14.6)	61 (7.2)	65 (9.4)	124 (14.0)	128 (15.6)
Mean				50.5 (10.6)	55.0 (12.0)	68.8 (11.1)	71.7 (10.4)	95.2 (18.4)	111.2 (13.8)	125.4 (5.4)
A.leptodactylus	1	50	35.36	49 (10.3)	76 (14.6)	83 (23.0)	102 (11.0)	86 (9.4)	100 (13.5)	120 (11.1)
	2	51	37.49	58 (5.4)	67 (9.2)	66 (6.5)	83 (10.3)	108 (9.2)	112 (7.4)	120 (6.3)
	3	52	44.77	63 (8.8)	76 (8.6)	71 (8.1)	88 (11.9)	72 (8.5)	93 (5.3)	115 (13.7)
	4	52	37.74	56 (3.5)	60 (9.4)	74 (10.3)	71 (10.3)	92 (6.6)	79 (11.7)	113 (10.6)
	5	54	45.77	51 (4.7)	59 (2.7)	65 (8.6)	89 (9.2)	102 (12.0)	107 (19.1)	114 (21.3)
	6	54	42.36	59 (7.0)	84 (7.6)	82 (16.0)	92 (32.5)	102 (16.9)	114 (12.4)	114 (14.9)
	7	57	48.61	59 (5.7)	84 (24.7)	92 (16.2)	96 (22.2)	80 (13.0)	93 (9.6)	102 (18.2)
	8	57	51.13	56 (7.6)	73 (13.7)	74 (10.6)	73 (6.9)	80 (23.1)	86 (5.2)	102 (15.4)
Mean				56.3 (4.2)	72.3 (9.0)	75.8 (8.6)	86.7 (10.0)	90.2 (12.0)	98 (11.6)	112.5 (6.5)

Note: r= replicates, values in ( ): SD

Figure 4.4.1. Mean heart beat of restrained P. leniusculus in a hide at 8 °C.

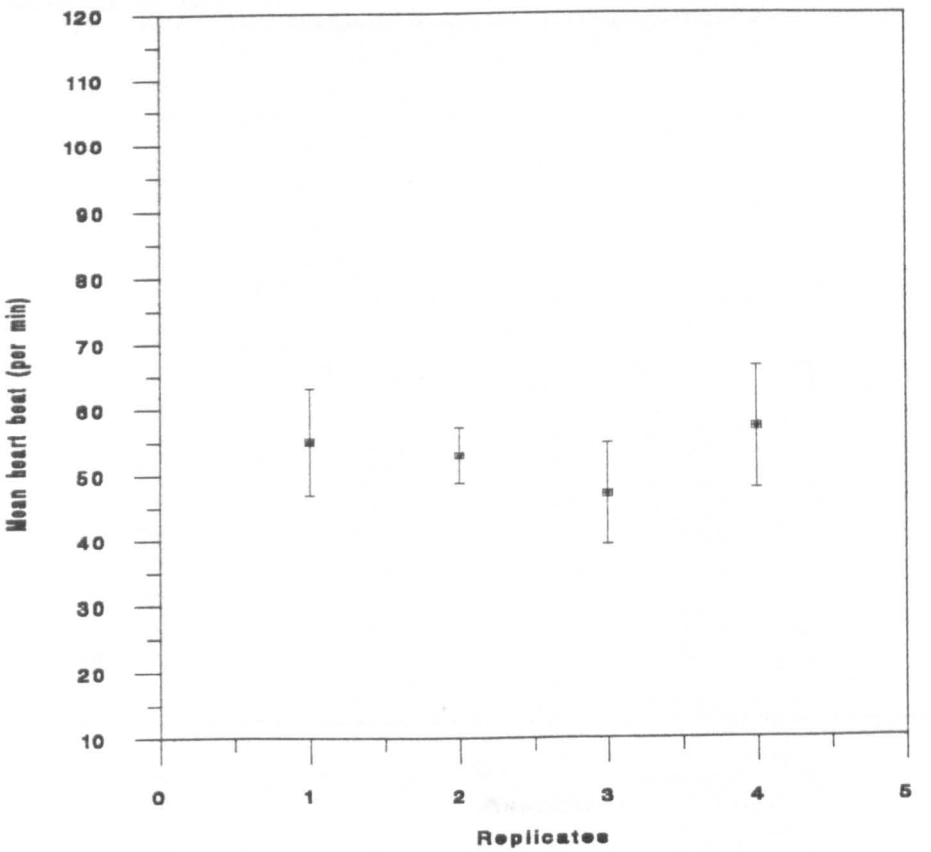
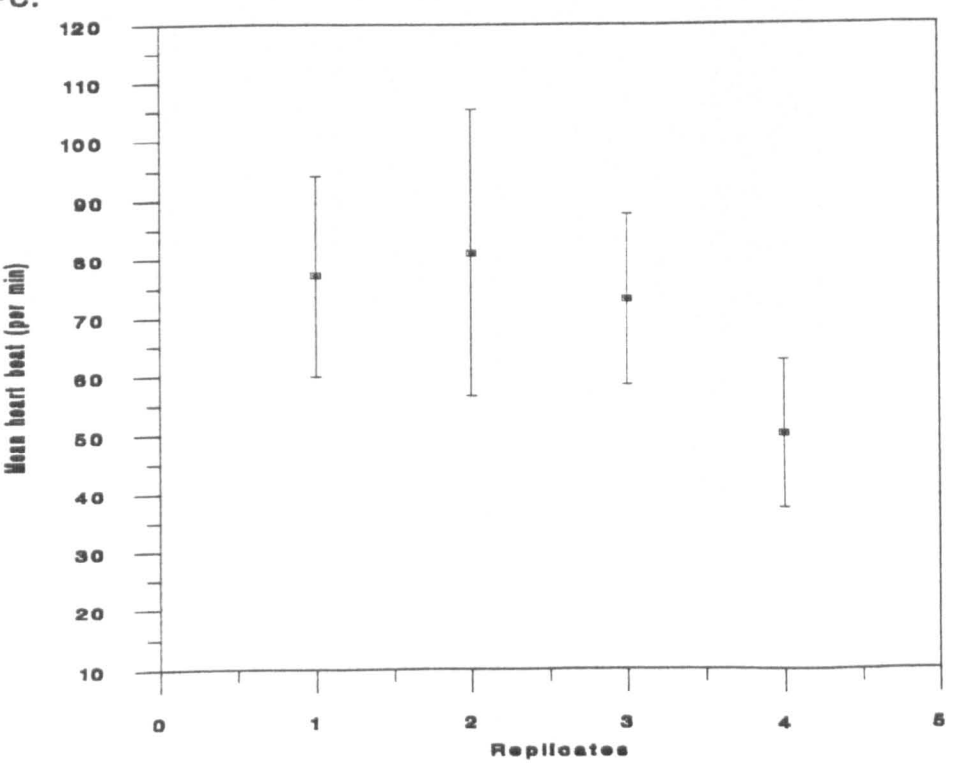


Figure 4.4.2. Mean heart beat of mobile P. leniusculus at 8 °C.



Note: Values are means with standard deviations.

Figure 4.4.3. Mean heart beat of restrained A. leptodactylus in a hide at 8 °C.

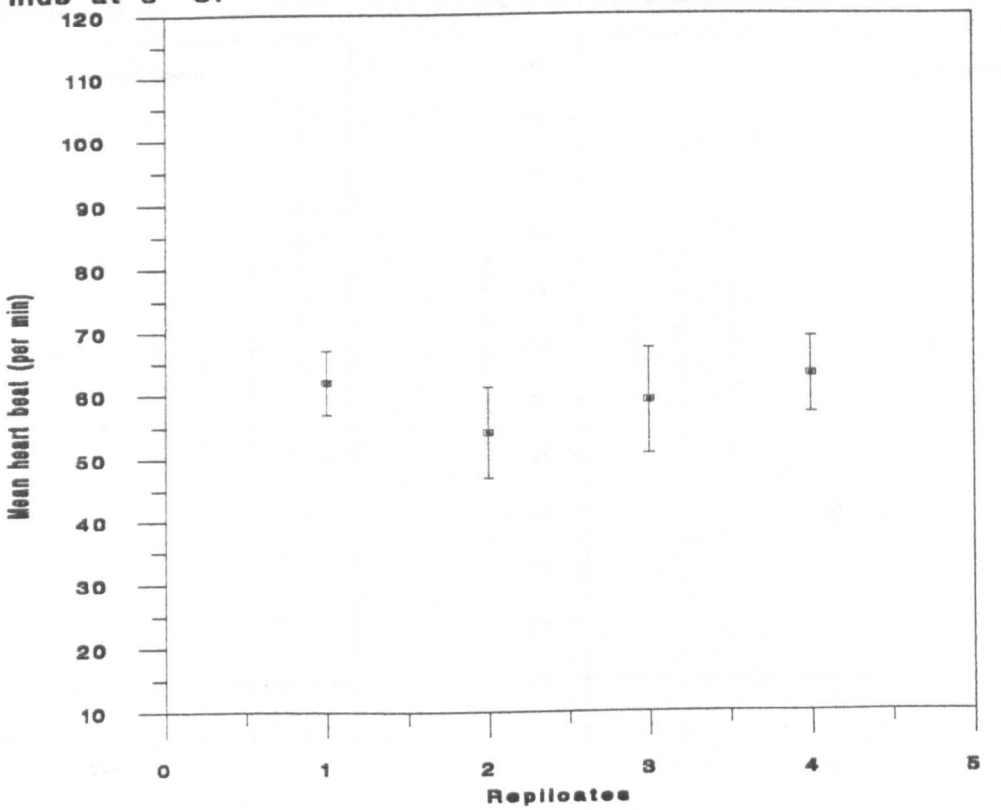
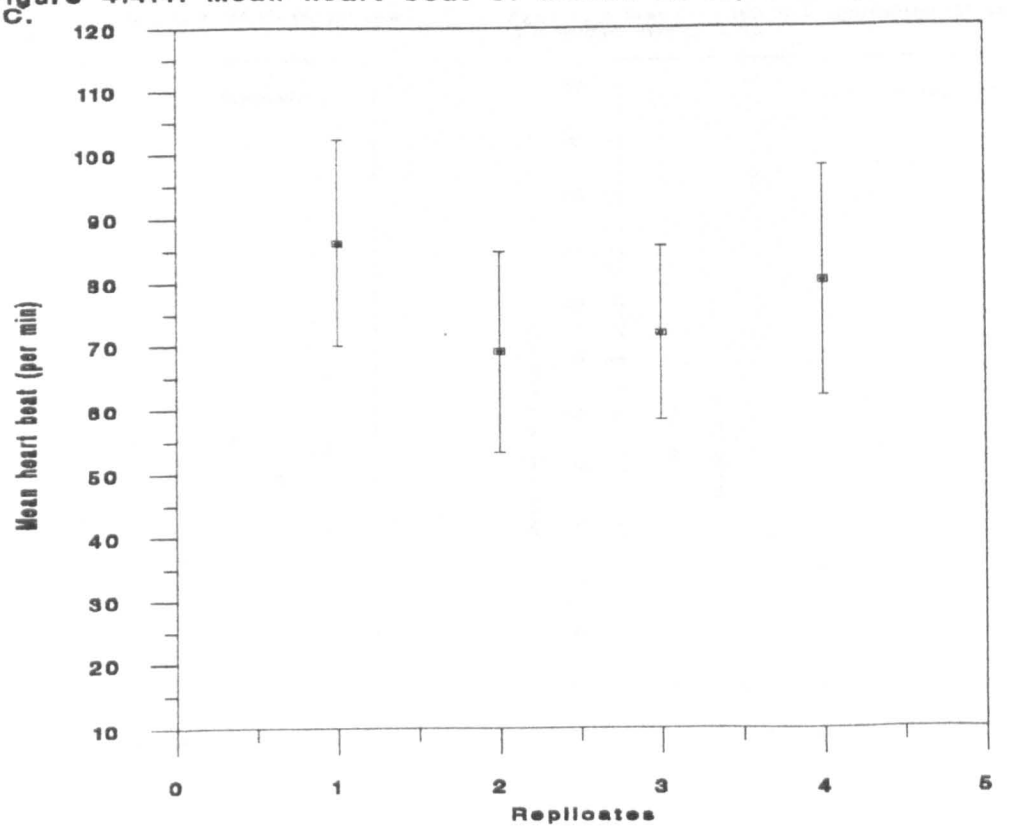


Figure 4.4.4. Mean heart beat of mobile A. leptodactylus at 8 °C.



Note: Values are means with standard deviations.

Figure 4.4.5. Mean heart beat of female P. leniusculus (50 mm CL and 43.9 g) in different days at 19 °C.

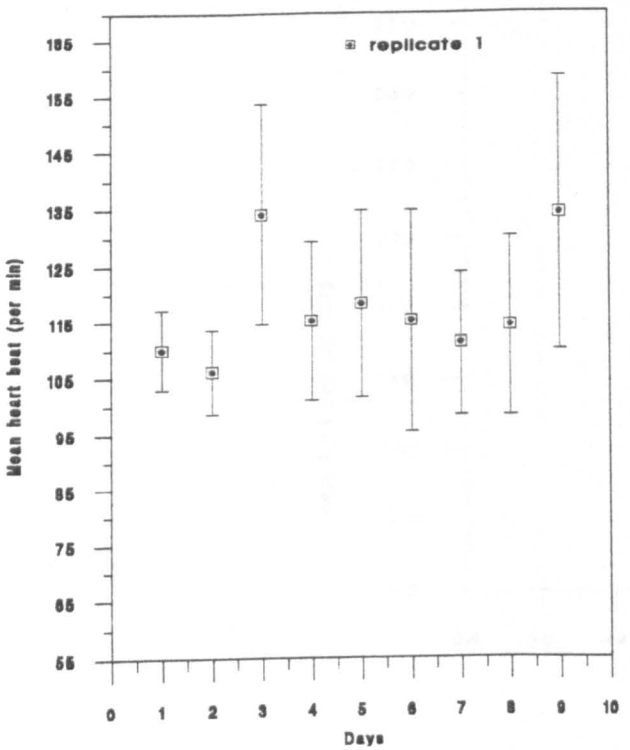


Figure 4.4.6. Mean heart beat of female P. leniusculus (52 mm CL and 47.0 g) in different days at 19 C.

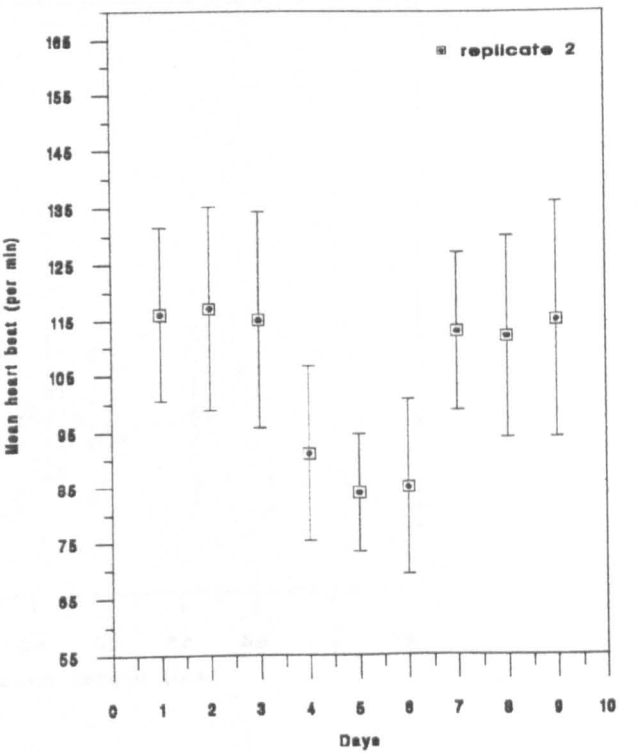


Figure 4.4.7. Mean heart beat of A. leptodactylus (50 mm CL and 38.6 g) in different days at 19 °C.

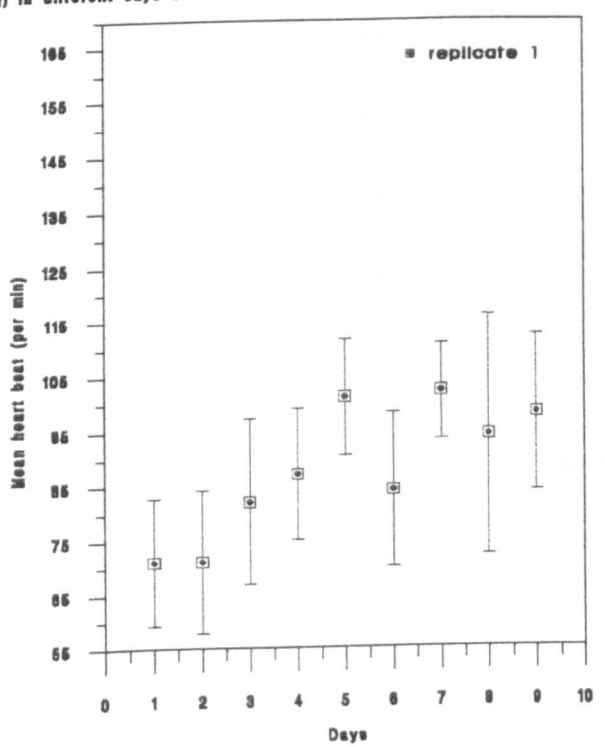
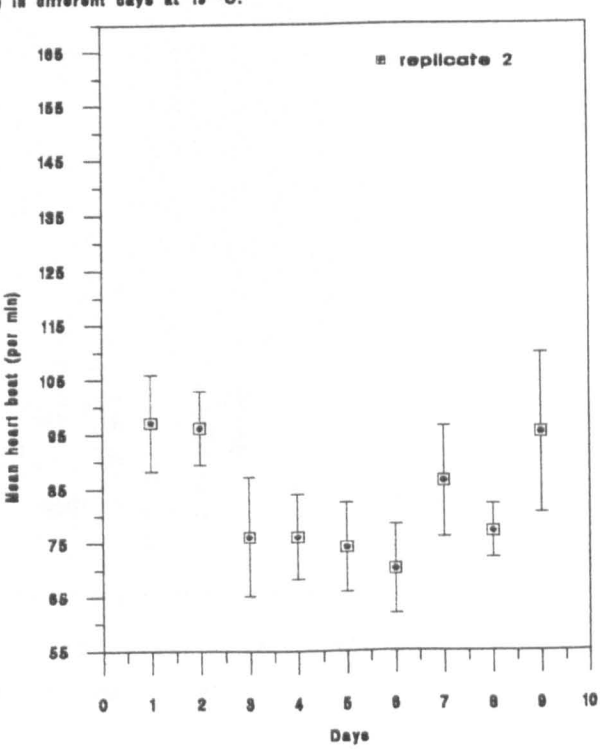


Figure 4.4.8. Mean heart beat of A. leptodactylus (51 mm CL and 32.9 g) in different days at 19 °C.



Note: Values are means with standard deviations.

Figure 4.4.9. The relationship between carapace length and mean heart beat in male P. leniusculus at 19 °C (n= 40).

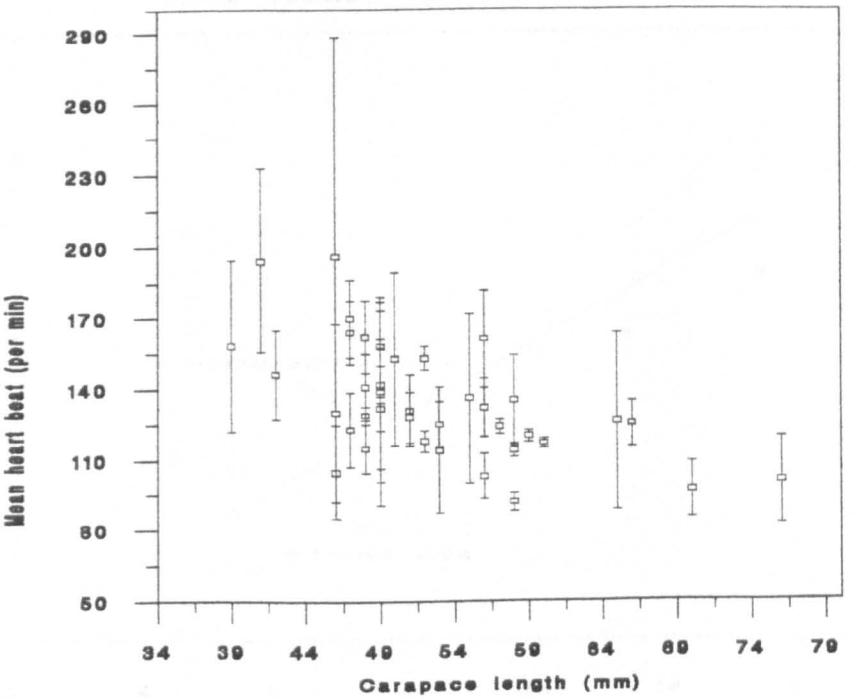
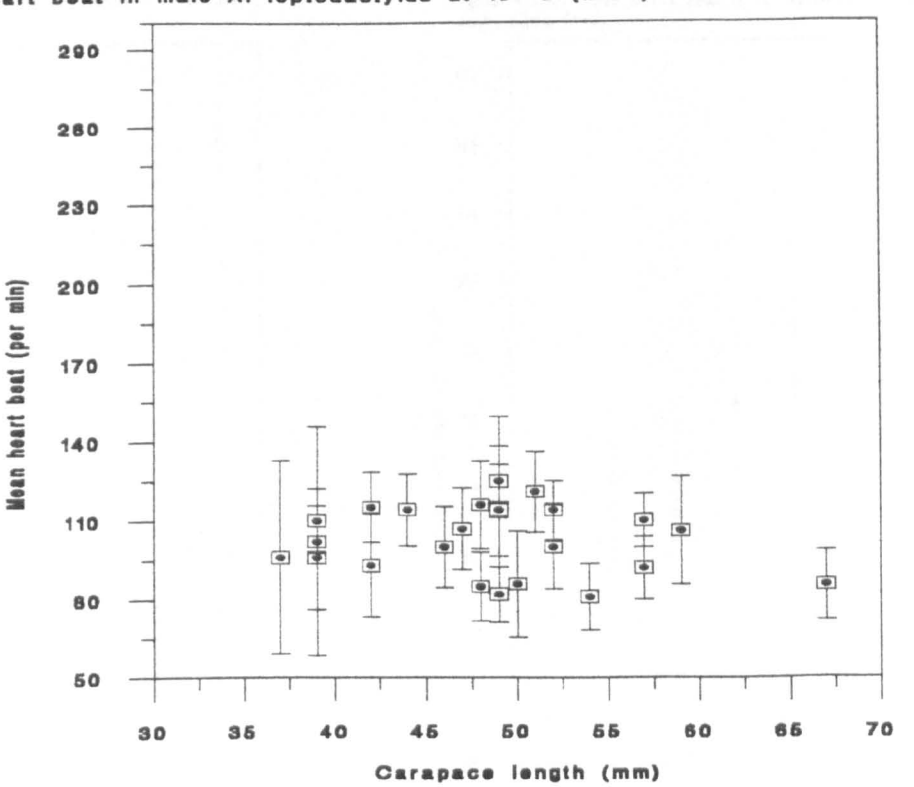


Figure 4.4.10. The relationship between carapace length and mean heart beat in male A. leptodactylus at 19 °C (n=24).



Note: Values are means with standard deviations.

Figure 4.4.11. Simple regression analysis of mean heart beat versus different temperatures in A. leptodactylus and P. leniusculus.

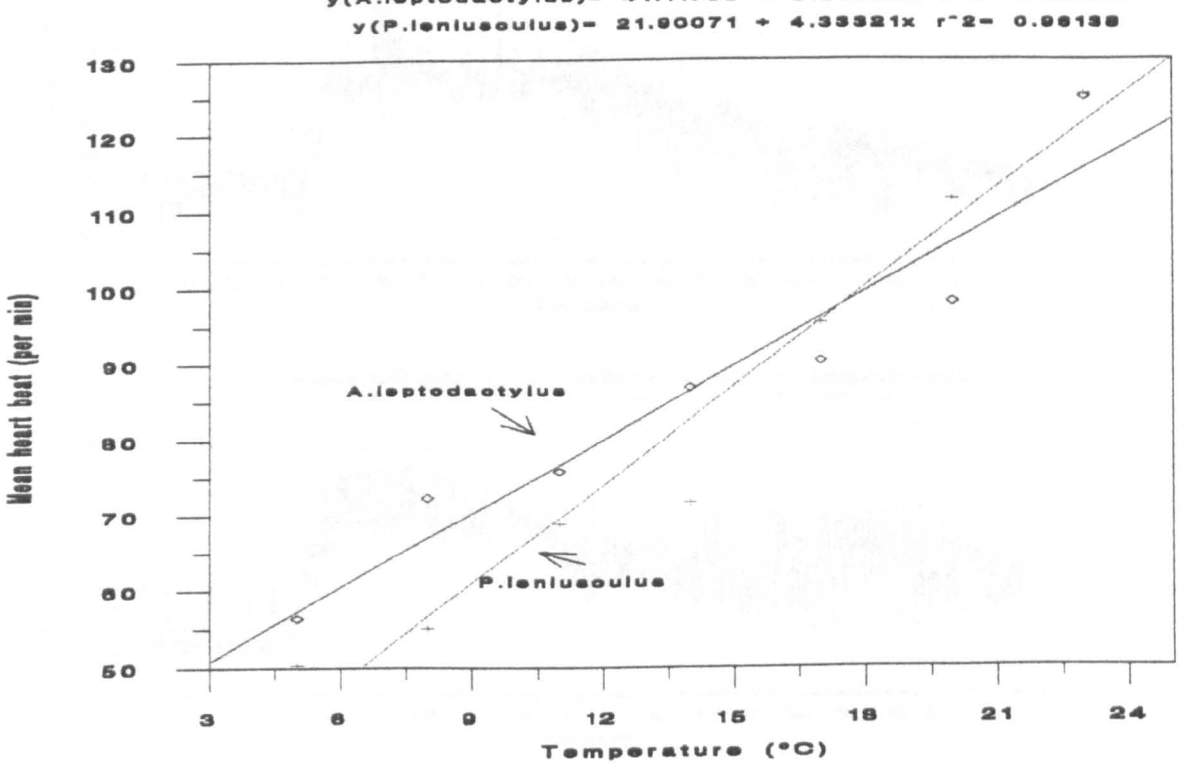


Figure 4.4.12. Mean heart beat in P. leniusculus at different temperature (n=7).

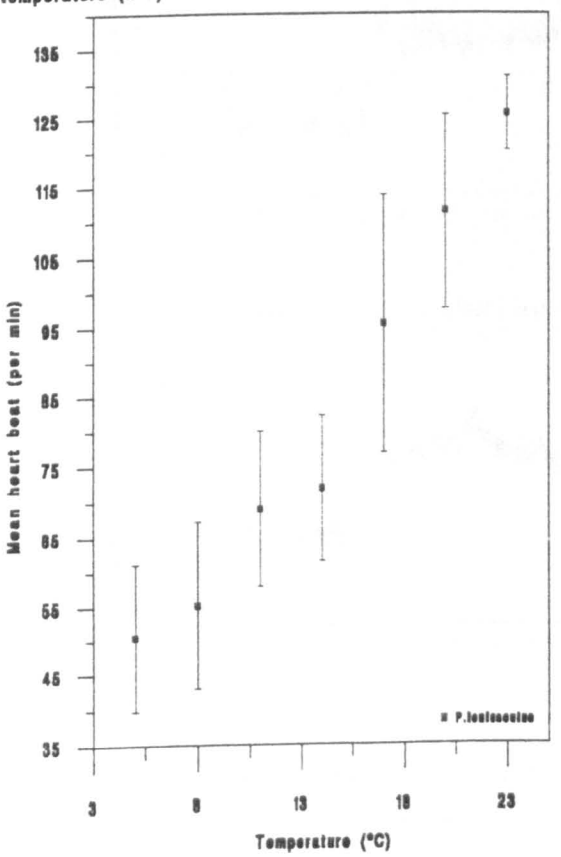
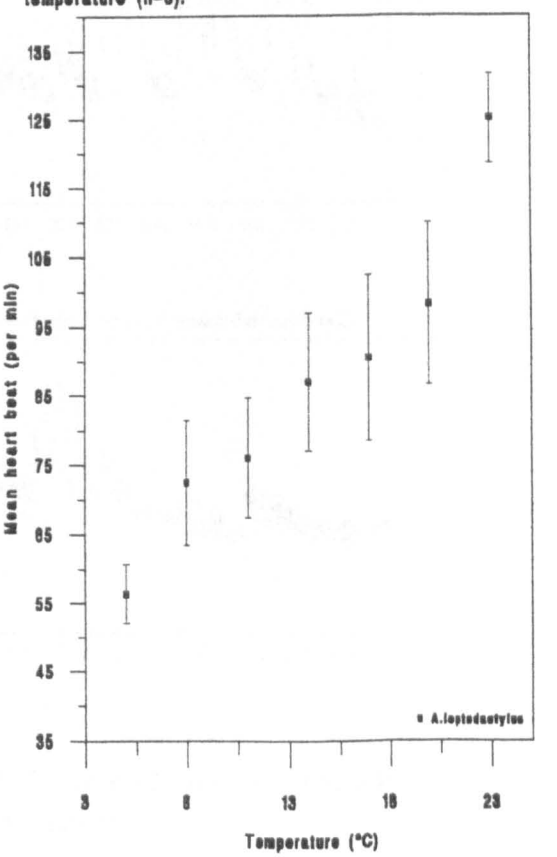


Figure 4.4.13. Mean heart beat in A. leptodactylus at different temperature (n=8).



Note: Values are means with standard deviations.

Figure 4.4.14. Variations in heart beat at 18 °C, 25 °C and decreased temperature in A. leptodactylus (replicate 1).

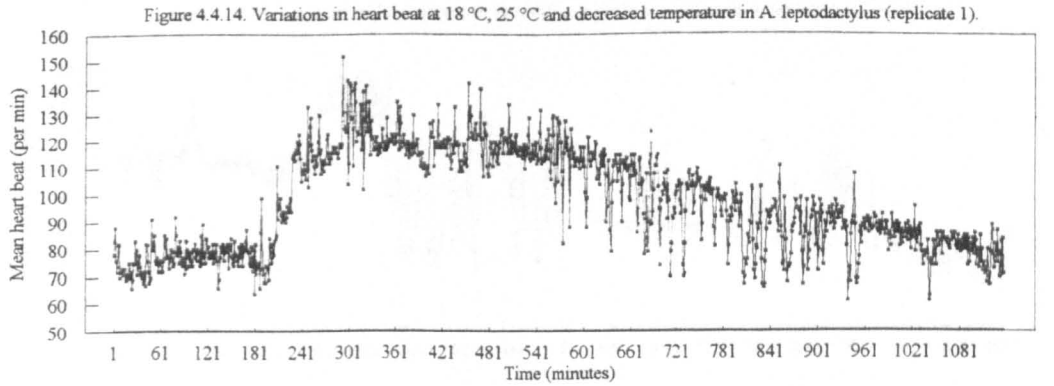


Figure 4.4.15. Variations in heart beat at 18 °C, 25 °C and decreased temperature in A. leptodactylus (replicate 2).

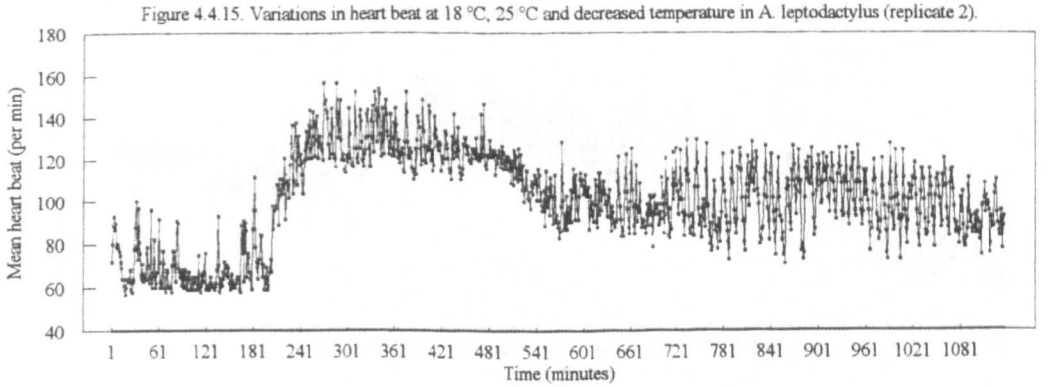


Figure 4.4.16. Variations in heart beat at 18 °C, 25 °C and decreased temperature in P. leniusculus (replicate 1).

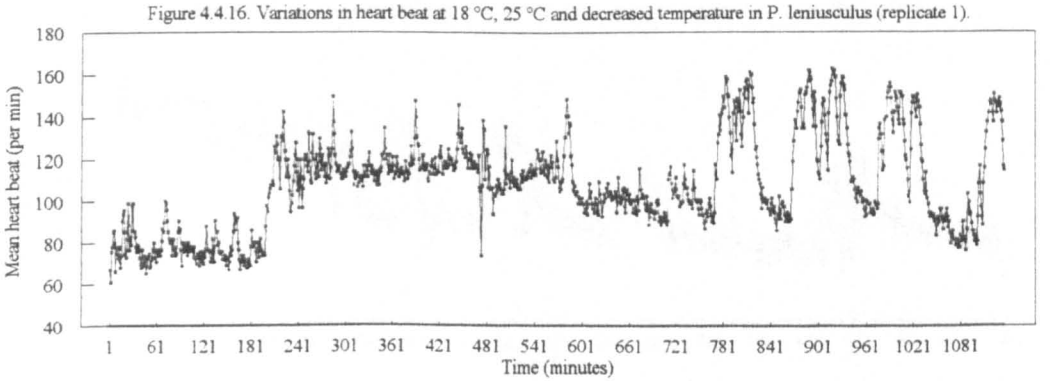
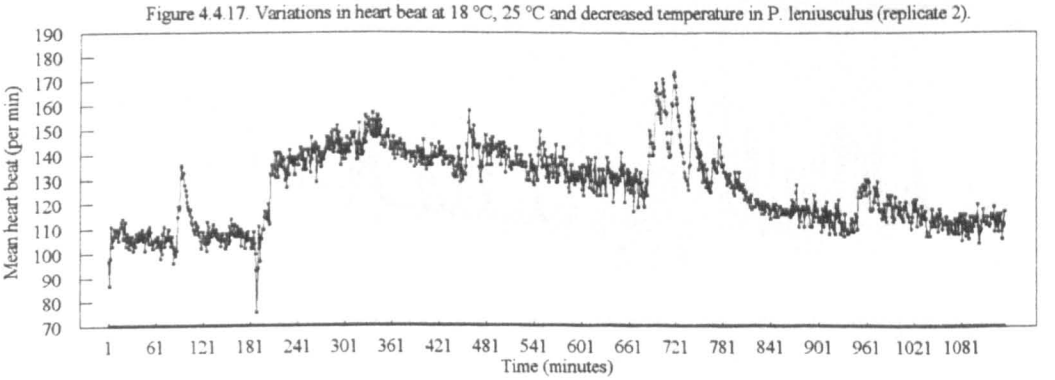


Figure 4.4.17. Variations in heart beat at 18 °C, 25 °C and decreased temperature in P. leniusculus (replicate 2).



Note: Temperature was increased to 25 from 18 °C after 180 minutes for 180 minutes, than was decreased gradually to 18 °C within 12 hours.

Figure 4.4.18. Variations in heart beat with decreased oxygen concentration in A. leptodactylus (replicate 1).

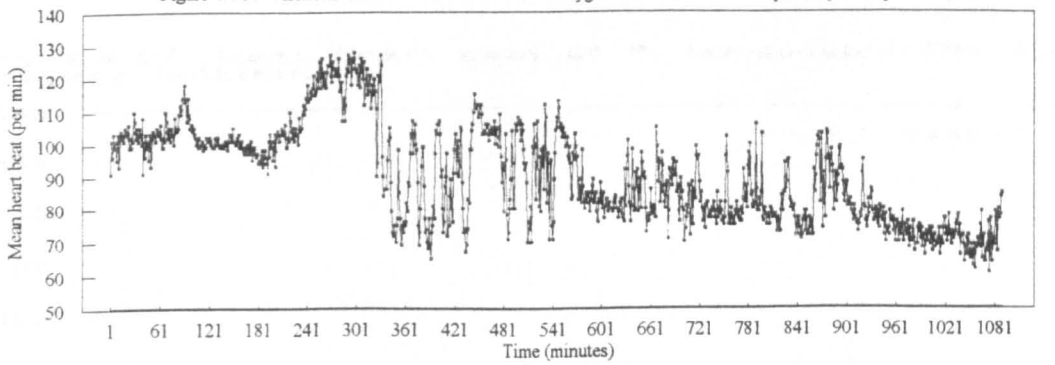


Figure 4.4.19. Variations in heart beat with decreased oxygen concentration in A. leptodactylus (replicate 2).

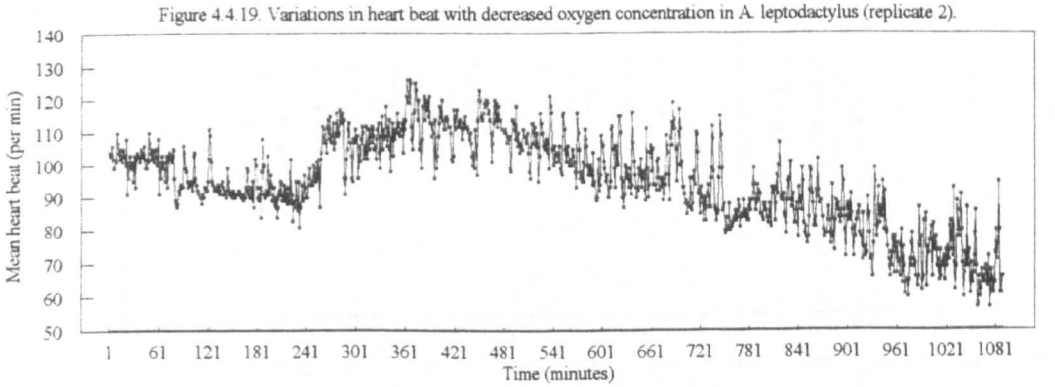


Figure 4.4.20. Variations in hear beat with decreased oxygen concentration in P. leniusculus (replicate 1).

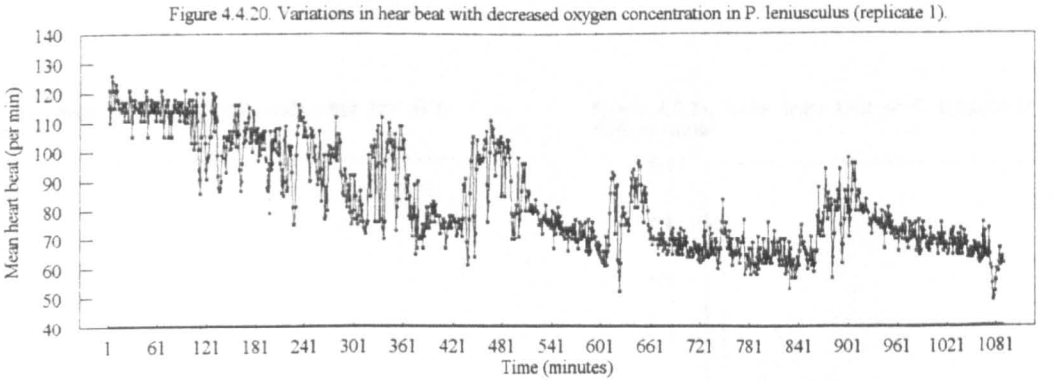


Figure 4.4.21. Variations in heart beat with decreased oxygen concentration in P. leniusculus (replicate 2).

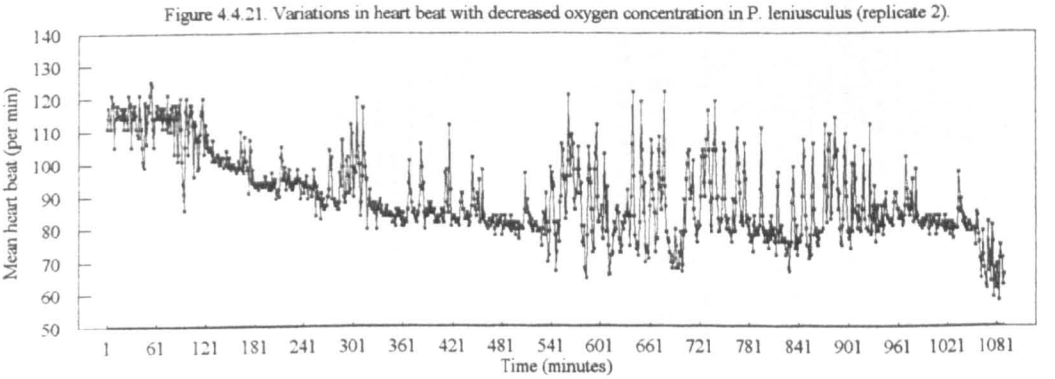


Figure 4.4.22. Mean heart beat of P. leniusculus (N= 4)in freshwater (control).

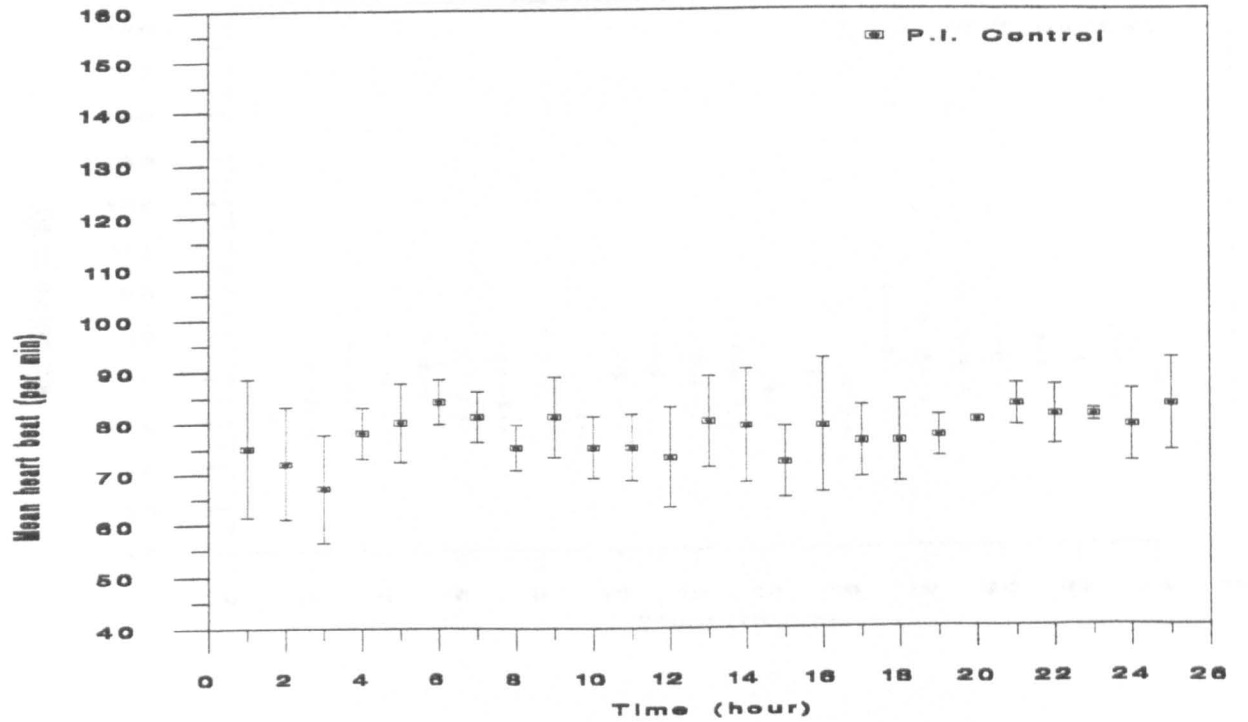


Figure 4.4.23. Mean heart beat of P. leniusculus (N= 4) in 20% seawater.

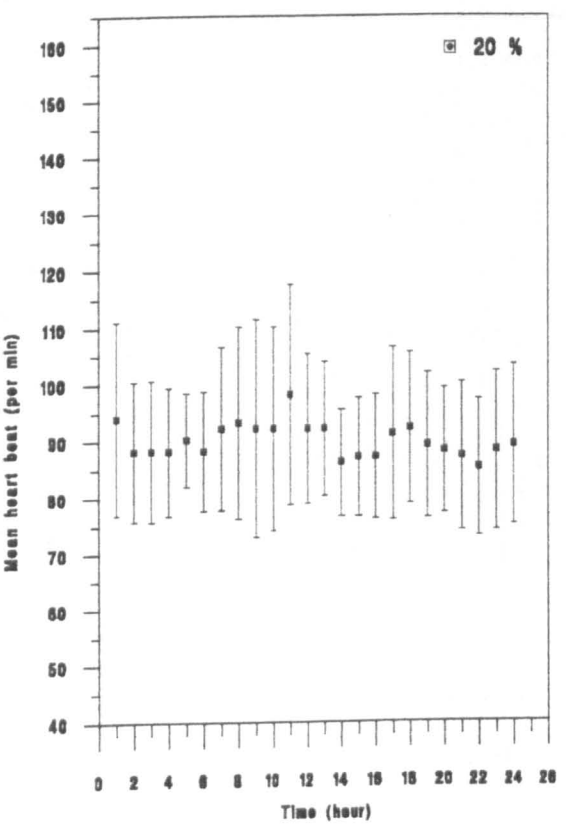
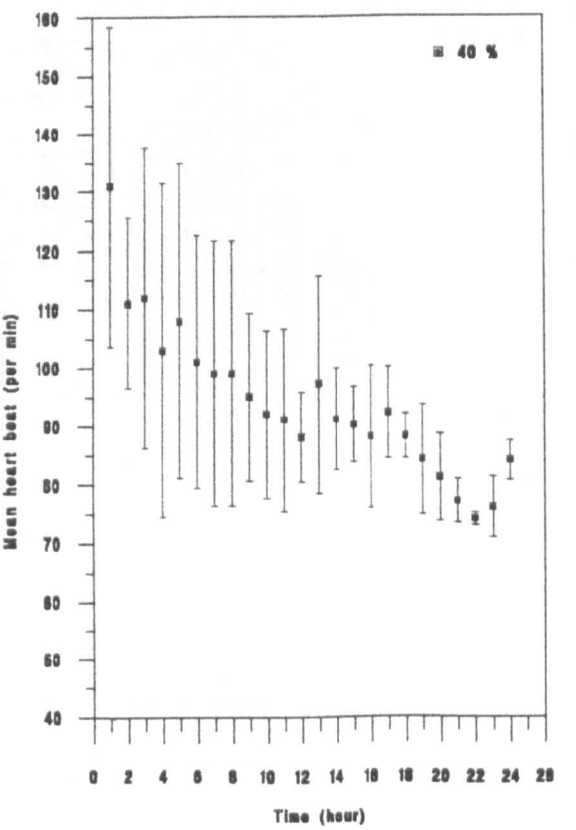


Figure 4.4.24. Mean heart beat of P. leniusculus (N= 4) in 40% seawater.



Note: Values are means with standard deviations.

Figure 4.4.25. Mean heart beat of A. leptodactylus (N=4) in freshwater (control)

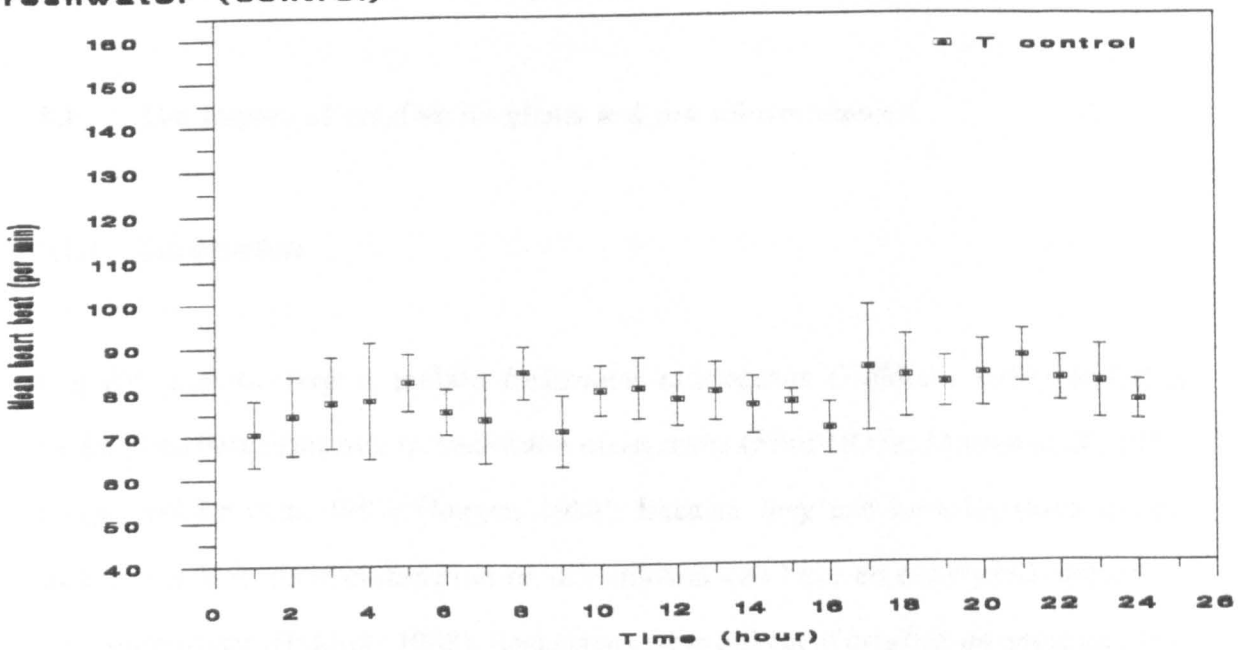


Figure 4.4.26. Mean heart beat of A. leptodactylus (N=4) in 20% seawater

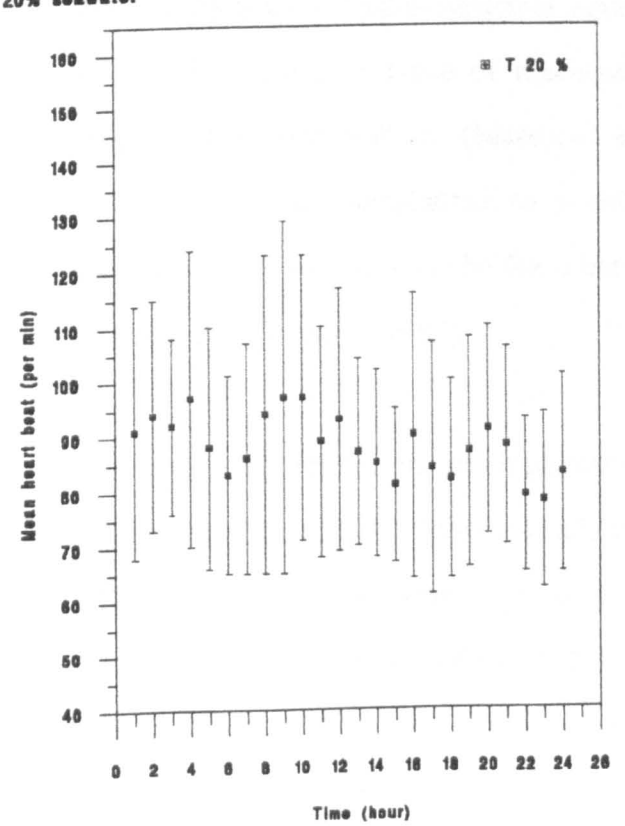
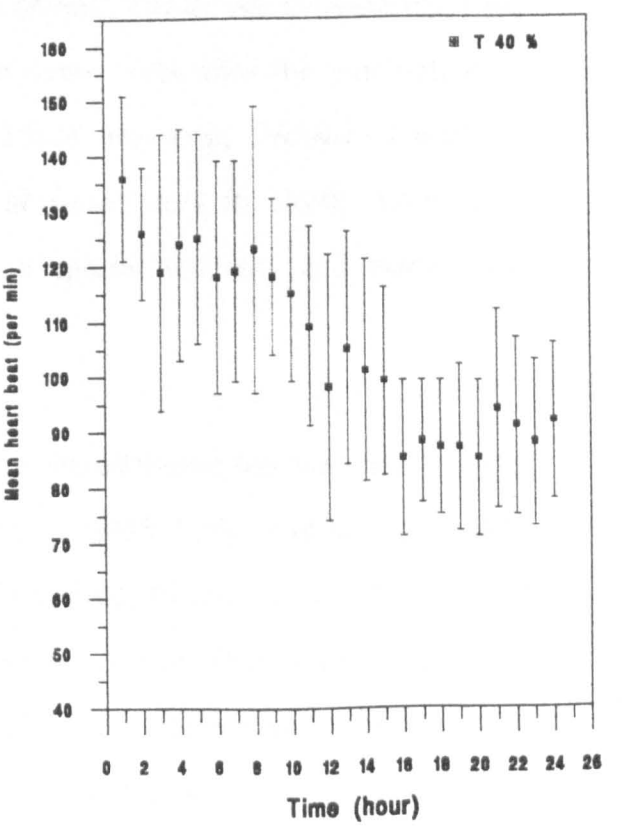


Figure 4.4.27. Mean heart beat of A. leptodactylus (N=4)in 40% seawater



Note: Values are means with standard deviations.

Chapter 5

The impact of crayfish on aquatic communities

5.1 The impact of crayfish on plants and macroinvertebrates

5.1.1 Introduction

Crayfish are the largest mobile freshwater crustaceans (Holdich, 1988) and they perform an important role in freshwater ecosystems (Flint 1977a; Momot *et al.*, 1978; Lodge and Lorman, 1987; Hogger, 1988). Because they can be a keystone species where they live, their elimination or introduction can have an observable impact on the environment (Holdich, 1988). Besides negative effects of crayfish on environments, positive effects of crayfish have also been determined. *Procambarus clarkii* has been used in the control of mosquito numbers in freshwater marshes (Feminella and Resh, 1986). *Orconectes limosus* has been used to clear the zebra mussel, *Dreissena polymorpha*, from hydro-electrical equipment (Piesik, 1974). An increase has been observed in the abundance of macrophytes in some lakes after the elimination of *Austropotamobius pallipes* (Matthews *et al.*, 1993). However, *Orconectes rusticus*, which has been transplanted to a number of freshwaters in North America is considered to be responsible for alterations in species diversity and macrophyte composition (Capelli, 1982).

The impact of crayfish on macrophytes and macroinvertebrates has been investigated in America (Stein, 1977; Momot *et al.*, 1978; France, 1985; Lodge and Lorman, 1987; Chambers *et al.*, 1990; Hanson *et al.*, 1990; Hart, 1992; Elser *et al.*, 1994). Most of the papers on the impact of crayfish have been focused on *Orconectes virilis*. The impact of this crayfish on aquatic macrophytes has been studied by Chambers *et al.* (1990), and on macroinvertabretes by Hanson *et al.* (1990). Momot *et al.* (1978) have

dealt with the dynamics of *O. virilis* and their role in ecosystems. In addition to these, the relationships between *O. virilis* growth, population abundance and system productivity in small oligotrophic lakes in Northwestern Ontario have been investigated by France (1985).

Like *O. virilis*, the impact of *Orconectes propinquus* has been observed in some studies. The interaction between *Orconectes propinquus* and *Cladophora* has been studied by Hart (1992). Stein (1977) reviewed the selective predation, optimal foraging, and predator-prey interaction between fish (*Micropterus dolomieui*) and *O. propinquus*. Similarly, the reductions in submerged macrophyte biomass by *Orconectes rusticus* have been investigated by Lodge and Lorman (1987). In exclosure and enclosure experiments, the effect of *Pacifastacus leniusculus* on macrophyte species in Castle Lake, in California has been observed by Elser *et al.* (1994).

However, in comparison to America, the majority of researches on the impact of crayfish in Europe has been focused on the impact of alien crayfish on native crayfish populations (Abrahamsson, 1973; Westman, 1973a; Unestam, 1975; Fürst, 1977; Holdich, 1988; Holdich and Domaniewski, 1995; Holdich *et al.*, 1995a). Söderback (1995) has also recently published a paper on the replacement of *A stacus astacus* by the introduced species *Pacifastacus leniusculus* in a Swedish lake.

In recent years there has been an increase in the number of introduced crayfish populations in Britain and they can reach much greater densities in freshwater environments than the native species of Britain, because they are fast-growing, more fecund, and more adaptable to environmental conditions (Holdich *et al.*, 1995b). *Pacifastacus leniusculus* and *A stacus leptodactylus* are both introduced crayfish species in England. Although there has been an increase in the number of *P. leniusculus* and *A. leptodactylus* populations in the wild, particularly in southern England (Holdich and

Rogers 1992), there has been no study of the impact of *P. leniusculus* and *A. leptodactylus* on macrophyte and macroinvertebrate communities except that of Holdich and Reeve (in Holdich *et al.*, 1995b) and Guan (1995). However, their results were obtained from the field and proved rather inconclusive due to a lack of comparable sites.

The introduction of alien crayfish species such as *P. leniusculus* and *A. leptodactylus* may cause a deterioration to the macrophyte and macroinvertebrate communities. The present study was carried out in order to investigate whether *P. leniusculus* and *A. leptodactylus* can cause an adverse effect to macrophyte and macroinvertebrate communities, using tanks with an established biota.

5.1.2 Materials and methods

Experiment 1

In order to assess the impact of juvenile *P. leniusculus* in an established tank of biota over a relatively long time 75 g (drained wet mass) of a mixture of *Callitriche* (water starwort), *Elodea*, *Lemna* and *Cladophora* were planted into each tank (380 mm x 230 mm x 110 mm) in the presence of macroinvertebrates (Table 5.1.1) in equal quantities of substrate which was a composite of silt from a local stream and gravel. In order to allow growth of flora and fauna, the tanks were kept under shelter for five weeks in outside conditions. Then, in late June, five (57 m^{-2}) and 20 juvenile (229 m^{-2}) *P. leniusculus* of stage 4 (10 ±1 mm CL) were set up with two replicates into the tanks, and the other two tanks were left as controls for 14 weeks. No additional food was given as long as the experiment continued. The water loss as a result of evaporation was maintained accordingly.

Experiment 2

In order to compare the impact of juvenile *P. leniusculus* and *A. leptodactylus* (14 ±1 mm in carapace length) on plant and macroinvertebrates within species and between species a known mass of macrophytes (*Cladophora*) and a known number of macroinvertebrates (*Gammarus*, *Asellus*, *Planorbis*, *Sphaerium*, *Lymnaea*) were set up with a density of five juvenile (57 m^{-2}) per tank with two replicates. A layer of fine stones was placed on the floor of each tank (380 mm x 230 mm x 110 mm) and hides were provided for crayfish. The experiment was carried out in a constant temperature room (13 °C) between 25.11.93 and 12.01.94. *Cladophora* was used as the plant and approximately 18 grams (drained wet mass) were placed in the tanks. Water was added as it was necessary.

Experiment 3

To assess the impact of crayfish on different combinations of invertebrates and algae 28 *P. leniusculus* and *A. leptodactylus* (14♂, 14♀ and of 12 or 13 mm in carapace length) were used between 28.09.94 and 19.10.94 in a constant temperature room (13 °C). In order to standardize the state of feeding, juveniles were initially maintained on a diet of algae until 07.10.94. Water was changed every two days.

Each dish (55 mm x 105 mm x 45 mm) consisted of one juvenile. The juveniles were put into dishes containing one of the following combinations with two replicates on 07.10.94. The experiment was dismantled after two days and was repeated on 09.10.94. Similarly the experiment was dismantled after two days but the dishes with snails in were kept until 19.10.94 and final observation was taken on 19.10.94.

4 snails (*Planorbis contortus*),

4 *Asellus*,

Cladophora (approximately 0.200 g - drained wet weight) + 3 snails (*Planorbis*

contortus) + 3 *A sellus*,

3 snails (*Planorbis contortus*) + 3 *A sellus*,

Cladophora (approximately 0.200 g - drained wet weight) + 3 *A sellus*,

Cladophora (approximately 0.200 g - drained wet weight) + 3 snails,

Starvation (no food in the dish) dishes with two replicates.

Snail size used in the experiments varied between 5 to 8 mm in height.

In order to compare the impact of different sizes of *P. leniusculus* and *A. leptodactylus* on plant and macroinvertebrate the same procedure was followed using 25 or 27 mm carapace length juveniles. The juveniles were set up in dishes (100 mm x 150 mm x 70 mm) on 19.10.94, and similarly the results were observed after two days (21.10.94). The juveniles were initially kept on a diet of algae between 11.10.94 and 19.10.94.

Experiment 4

This experiment was designed to see if maggots could be used as a source of crayfish food and, if so, at what rate the two species fed on them. Eight male *P. leniusculus* and *A. leptodactylus* juveniles of 25-27 mm in carapace length (sub-adults) were used on 22.10.94. In order to standardize the state of feeding juveniles were initially maintained on a diet of algae until 25.10.94. Each dish consisted (10 cm x 15 cm x 7 cm) of one juvenile and one maggot (*Calliphora* sp), one juvenile and two maggots, one juvenile and four maggots, and one juvenile and six maggots with two replicates. In order to observe which species fed faster on maggots, observations were made every 90 minutes between 10.00 h and 17.30 h, on 25.10.94, and the final reading was taken in the morning (09.00 h) on 26.10.94. The experiment was repeated on 31.10.94. Between 26.10.94 and 31.10.94 *Cladophora* was given as food for the juveniles. Water was changed every day. The experiment was carried out in a constant temperature room (13 °C).

Experiment 5

An experiment was set up to compare the impact of variously sized juveniles (stage 2 juveniles and those of 18 or 20 mm carapace length) on *Gammarus* and *Asellus* in the absence of plant material (*Cladophora*). Twelve stage two juveniles and 18 or 20 mm carapace length juveniles were used between 06.05.94 and 14.05.94 in a constant temperature room (15 °C). Algae was given in order to standardize the state of feeding until 13.05.94. On 13.05.94 the juveniles were put into dishes (55 mm x 105 mm x 45 mm) containing one of the following combinations:

2 *Asellus* (alive) + 2 *Asellus* (frozen),

4 *Asellus* (alive),

4 *Gammarus* (frozen),

2 *Gammarus* (alive) + 2 *Asellus* (alive),

4 *Gammarus* (alive),

4 *Asellus* (frozen).

In order to evaluate the results Chi-squared test was used for all experiments.

5.1.3 Results

Experiment 1

As can be seen in Table 5.1.1, the juvenile *P. leniusculus* had an adverse effect on the growth and development of plants and macroinvertebrates as compared to the control. Statistical analysis showed that except for the reduction in the number of molluscs at the density of five juveniles per tank (57 m^{-2}), the reduction in the total wet mass of plants and in the number of macroinvertebrates by juvenile *P. leniusculus* at the density of 20 juvenile per tank (229 m^{-2}) and/or even at the density of five juvenile per tank was highly significant ($P<0.001$) compared to the control.

Experiment 2

Table 5.1.2 gives a break-down of the results within and between species. The reduction in the number of *Asellus*, *Gammarus* and *Lymnaea peregra* by the juveniles of the species was significant (P<0.001). Although the reduction in the number of *Sphaerium* by the juvenile *P. leniusculus* was not significant, the juveniles *A. leptodactylus* fed significantly on *Sphaerium*.

Within species

The decrease in the number of *Asellus* and *Gammarus* is significant (P<0.001) but there is no difference (NS>0.05) in the number of *Planorbis* and *Lymnaea stagnalis* eaten by either juvenile *P. leniusculus* or *A. leptodactylus* compared to the control, although there was for *Lymnaea peregra*.

Between species

There is no significant difference between juvenile *P. leniusculus* and *A. leptodactylus* in the reduction of *Asellus*, *Gammarus*, and *Lymnaea stagnalis*.

Juvenile *A. leptodactylus* consumed more *Planorbis* and *Sphaerium/Pisidium*. There is a significant difference (P<0.05) between juvenile *P. leniusculus* and *A. leptodactylus* in the reduction of *Planorbis* and *Sphaerium/Pisidium*. Juvenile *P. leniusculus* depleted significantly more *Lymnaea peregra* than *A. leptodactylus* (P<0.001).

The reduction in the wet mass of the alga was not statistically compared to the control because it was too difficult to keep the weed alive in this experiment. It was thought that the reason for this was that to prevent crayfish and macroinvertebrates escaping from the tanks a relatively opaque cover was placed on each tank. As a result of this, weed did not have enough light to grow.

Experiment 3

During the experiment no dead crayfish were observed in any dishes including the starvation dishes.

Within species (12 or 13 mm CL)

Twelve or 13 mm (CL) juveniles of the two species had an adverse effect on *Cladophora* and *A sellus* ($P<0.001$), and *Planorbis contortus* numbers. However, the impact of the juveniles on the snail species (*P. contortus*) was not as fast as was observed on *Cladophora* and *A sellus* (Table 5.1.3, 5.1.4, 5.1.5, 5.1.6). There was no significant difference between females and males feeding on *Cladophora* and *A sellus*. However, it was really difficult to make a decision between males and females on their impact on the snail. In *A. leptodactylus*, the impact of females on the snail was more significant than that of the male in the presence of *Cladophora*, whereas the male *A. leptodactylus* affected the snail number more significantly than the female *A. leptodactylus* in the presence of *Cladophora* and *A sellus*.

Similarly, although the impact of male *P. leniusculus* on the snail in the presence of *A sellus* and in the presence of *Cladophora* was significant ($P<0.05$) in the first reading (after 48 h), it was not significant in the second reading (after 96 h). In addition to these, the female *P. leniusculus* fed significantly better on the snails in the presence of *Cladophora* than the male *P. leniusculus* and the male *P. leniusculus* was significantly better eating snails in the presence of *A sellus* in the final reading (after 288 h).

Between species (12 or 13 mm CL)

There was no significant difference between *P. leniusculus* and *A. leptodactylus* on the reduction of *A sellus* and *Cladophora*.

Within species (25 or 27 mm CL)

After 48 hours, as can be seen in Table 5.1.7, the juveniles of the two species had a detrimental effect on *Asellus*, snails and *Cladophora*. In the both female and male of the two species' dishes almost all of the *Asellus* and *Planorbis contortus* were eaten in the presence of *Cladophora*. There was no significant difference between females and males on the reduction of *Asellus* and *Cladophora*.

Between species (25 or 27 mm CL)

There was no significant difference between the species on the reduction of *Asellus*, snails and *Cladophora* except the difference in the snail + *Asellus* dishes ($P<0.01$) (Table 5.1.7). The juveniles of *P. leniusculus* consumed more snails than those of *A. leptodactylus*.

To compare 12 or 13 mm CL and 25 or 27 mm CL juveniles

There was no significant difference between 12 or 13 mm CL and 25 or 27 mm CL juveniles of *A. leptodactylus* and *P. leniusculus* in the reduction of *Asellus* and *Cladophora*. However, 25 or 27 mm CL juveniles of both species had a significantly greater impact on the reduction of the snail ($P<0.001$).

Experiment 4

The reduction in the number of maggots by the juvenile *P. leniusculus* and *A. leptodactylus* was highly significant. At the end of the experiments, 57.7% of the maggots in the first experiment ($P<0.001$), and 63.5% of the maggots in the second experiment ($P<0.001$) were consumed by the juvenile *P. leniusculus*, whereas the juveniles of *A. leptodactylus* ate 26.9% and 30.8% respectively ($P<0.01$).

In the two experiment, the juvenile *P. leniusculus* was faster at feeding on maggots than those of *A. leptodactylus* (Table 5.1.8, 5.1.9, 5.1.10, 5.1.11). There was a

significant difference ($P<0.05$) between *P. leniusculus* and *A. leptodactylus* regarding the numbers of maggots eaten in the first 90 minutes. Although 9.5 maggots in the first experiment and 4.5 maggots in the second experiment were eaten by the juvenile *P. leniusculus*, only 3 maggots in the first experiment and 0.5 maggots in the second experiment were eaten by the juvenile *A. leptodactylus* after 90 minutes.

In addition to the number of maggots consumed, the juveniles of the two species attempted to eat the other maggots in the dishes, but they did not completely eat all maggots and these were reported as "killed maggots" in the tables.

Experiment 5

As it was reported in the other experiments, in this experiment 18 or 20 mm (CL) juveniles of the two species significantly affected the number of *Gammarus* and *Asellus* (alive or/and frozen) in the absence of *Cladophora* (Table 5.1.12). Although the stage 2 juveniles of the two species ate *Gammarus* and *Asellus* (alive or/and frozen) this was not significantly important ($P>0.05$) (Table 5.1.12).

5.1.4 Discussion and conclusions

The effects of crayfish on plant and macroinvertebrate communities have been investigated by many workers. It was claimed that naturally occurring crayfish reduce or eliminate vegetation from lakes (Momot *et al.*, 1978). This was supported by Piesik (1974) for *O. limosus*, Seroll and Coler (1975) for *O. immunis*, Magnuson *et al.* (1975) for *O. causeyi*, Capelli (1980) for *O. propinquus*, Lodge and Lorman (1987) for *O. rusticus*, Chambers *et al.* (1991) for *O. virilis*, Hart (1992) for *O. propinquus* and Matthews *et al.* (1993) for *A. pallipes*. In addition, *P. leniusculus* was found to significantly affect the biomass of *Chara* in Castle Lake (USA) when enclosures were used (Elser *et al.*, 1994), and has been used to clear weed from a lake in France

(Blake and Laurent, 1982). Likewise, *Myriophyllum* sp. was controlled by the same species (Flint and Goldman, 1977). Flint (1975) found that an increase in the number of *P. leniusculus* had a greater impact on the littoral zone and this has also been reported for the same species by Elser *et al.* (1994) and for *O. rusticus* by Lodge *et al.* (1985).

The choice and consumption of aquatic weeds by *P. leniusculus* were investigated by Warner and Green (1995). *Spirogyra* sp., *Ceratophyllum demersum*, *Elodea canadensis* and *Groenlandia densa* were provided for two sizes of juvenile *P. leniusculus* (2-4 g and 6-8 g) and for an adult *P. leniusculus* (32 g). Warner and Green (1995) found that the amount of weed was reduced significantly by crayfish and there were no differences between the sizes of crayfish in the amount of weed consumed as a proportion of the body weight, nor in the order of weed choice which was *Spirogyra* sp. > *C. demersum* > *E. canadensis* = *G. densa*. Warner and Green (1995) also found that the range of weed consumption by *P. leniusculus* was 13-26% of the body weight in 24 hours. However, in another study on the food consumption and growth of *Astacus astacus*, Söderback *et al.* (1988) found that the rate of food consumption is lower than 10% of the body weight in 24 hours. Therefore, Warner and Green (1995) stated that the food consumption of *P. leniusculus* is higher than that of *A. astacus*.

The impact of crayfish on macroinvertebrate communities has been mentioned by Piesik (1974) for *O. limosus*, Lodge and Lorman (1987) for *O. rusticus*, Hanson *et al.* (1990) for *O. virilis* and Saffran and Barton (1993) for *O. propinquus*. Warner *et al.* (1995) has also studied the feeding behaviour of different sizes of *P. leniusculus* (16-61 mm CL) on different sizes of snails (3-35 mm shell length), *Lymnaea peregra* and *Lymnaea stagnalis*. It has been found that the thin shell of these snails brings about a significant crayfish predation.

The results presented here show that both juvenile *P. leniusculus* and *A. leptodactylus* can have a dramatic impact on plant and macroinvertebrate communities over a long time period (Experiments 1 and 2) as well as over a short time period (Experiments 3, 4 and 5). For example, 12 or 13 mm (CL) juveniles of the two species had a detrimental impact on *Cladophora* and *Asellus*, and even *Planorbis contortus* number. However, the impact of the 12 or 13 mm (CL) juveniles on the number of *P. contortus* was not as fast as was observed on *Cladophora* and *Asellus*. In comparison to the impact of 12 or 13 mm (CL) juveniles on the snail species, the impact of 25 or 27 mm (CL) juveniles of the two species was more significant.

No significant differences were observed between juvenile *P. leniusculus* and *A. leptodactylus* in the reduction of *Cladophora*, *Asellus*, and *Gammarus*. In order to compare the impact of juvenile *P. leniusculus* and *A. leptodactylus* a relatively soft-bodied food, maggots (*Calliphora* sp), was chosen (experiment 4). At the end of the experiment it was observed that the impact of juvenile *P. leniusculus* on maggot numbers was significantly different than that of juvenile *A. leptodactylus*. In addition to this, juvenile *P. leniusculus* were significantly quicker at feeding on maggots.

According to Lund (1944) the food choice of different sizes of crayfish is not the same. For example, plant materials, algae and diatoms, but especially larvae of *Chironomus* are preferred by the smallest crayfish. Moreover, in addition, plant materials improve juvenile growth rate, they also offer a shelter facility for crayfish against predators (Blake *et al.*, 1994). Accordingly, in this study it was found that although stage 2 juveniles feed on macroinvertebrates this feeding was not significant. This shows the importance of plant material in their early stages.

Some studies have shown that even low densities of crayfish can have a negative effect on environments. Chambers *et al.* (1990) suggested that low densities of *O.*

virilis can have a negative consequence on the growth of submerged aquatic plants. In a similar study, Hanson *et al.* (1990) concluded that the low densities of the same species can affect the number of macroinvertebrates. Similarly, the low densities of *O. rusticus* can dramatically reduce macroinvertebrate and macrophyte communities (Lodge and Lorman, 1987). It can be concluded from this study that even low densities of juvenile *P. leniusculus* and *A. leptodactylus* can have an adverse effect on plant and macroinvertebrate communities (Experiments 1 and 2).

The negative impact of introduced crayfish on their new environment has been often reported (Abrahamsson, 1966; Capelli, 1982; Momot, 1984; Butler and Stein 1985; Lodge and Lorman 1987; Hepworth and Duffield 1987; Hobbs *et al.* 1989). Capelli (1982) suggested that the introduced crayfish, *O. rusticus*, significantly affected the species diversity and macrophyte composition in North America. This was reported for *O. rusticus* in a similar study by Lodge and Lorman (1987). In another study it was observed that the adverse effects of crayfish would be seen if *O. virilis* had been introduced into some Canadian lakes (Chambers *et al.*, 1990; Hanson *et al.*, 1990).

It is clear from the experiments described and those of other workers that crayfish can have a significant impact on the freshwater environment. This should be born in mind when consideration is being given to introducing crayfish for stocking purposes. Crayfish can quickly become a key stone species in a waterbody, both as a predator and as a grazer of plants as well providing a source of food for other animals (Flint, 1975, 1977a; Momot *et al.*, 1978). Despite the fact that many workers consider crayfish to be primarily herbivorous, Momot (1995) considers that they are primarily carnivores, only eating plant material secondarily or when animal food is limited. In addition, Guan (1995) states that crayfish feed on a wide range of food items. In a study over four seasons, 22 food groups (mainly vascular detritus, filaments of green alga *Cladophora*, crayfish fragments, Chironomidae and Ephemeroptera) have been

classified from the gut contents of *P. leniusculus* by Guan (1995). He also observed that the juveniles of *P. leniusculus* consumed more animals (mainly benthic invertebrates), but the adults of *P. leniusculus* consumed more vascular detritus and fish. Consequently, both juvenile and adult crayfish may play a more important role in secondary productivity than previously thought.

Table 5.1.1 Survival rate of juvenile *P. leniusculus*, total wet mass of macrophytes, and number of macroinvertebrates at the end of the experiment

	Survival of crayfish	Macrophytes[1] and alga (total drained wet mass, g)	Number of Mollusca[2]	Number of Crustacea[3]	Others[4] (number)
Control 1 (with no crayfish)		203	90	11	30
Control 2 (with no crayfish)		124	20	60	30
Five juvenile *P. leniusculus* replicate 1	2	160	90	3	10
Five juvenile *P. leniusculus* replicate 2	4	110	20	0	0
Twenty juvenile *P. leniusculus* replicate 1	16	94	0	0	0
Twenty juvenile P. leniusculus replicate 2	17	84	0	0	0

Note:
[1]*Callitriche, Elodea, Lemna, Cladophora*
[2]*Limnaea* and Planorbidae (Gastropoda)
[3]*Asellus* (Isopoda) and *Crangonyx* (Amphipoda)
[4]Turbellaria, Oligochaeta, Hirudinea and Insecta
Total drained wet mass of macrophytes: after 3 good squeezes

Table 5.1.2 Stocking number of juveniles, number of macroinvertebrates, and wet mass of *Cladophora* at the beginning of the experiment and degree of significance in their reduction at the end of the experiment

	r	control before / after	within species *P. leniusculus* before / after	within species *A.leptodactylus* before / after	between species
Number of crayfish	1 2	- / - - / -	5 / 4 5 / 5	5 / 5 5 / 5	
Gammarus	1 2	25 / 20 25 / 17	25 / 2 25 / 0 ***	25 / 3 25 / 2 ***	NS
Asellus	1 2	25 / 9 25 / 14	25 / 0 25 / 0 ***	25 / 0 25 / 0 ***	NS
Planorbis	1 2	3 / 2 3 / 3	3 / 3 3 / 3 NS	3 / 1 3 / 1 NS	*
Sphaerium/Pisidium	1 2	3 / 2 3 / 3	3 / 2 3 / 1 NS	3 / 0 3 / 0 ***	*
Lymnaea peregra	1 2	10 / 10 10 / 3	10 / 0 10 / 1 ***	10 / 6 10 / 5 ***	***
Lymnea stagnalis	1 2	3 / 2 3 / 3	3 / 3 3 / 0 NS	3 / 3 3 / 0 NS	NS
Cladophora	1 2	18.10 g / 14.19 g	18.16 / 11.38	18.23 / 11.17	

Note: NS: $P>0.05$, ***: $P<0.001$, r: replicates

Table 5.1.3 The degree of significance in the decrease of *Asellus*, snails (*P. contortus*) and *Cladophora* by 12 or 13 mm (CL) juvenile female *A. leptodactylus* (two replicates of each combination were pooled)

	09.10.94 (after 48 h)		11.10.94 (after 48 h)		19.10.94 (after 240 h)	
Combinations	No of macroinvertebrate or amount of weed Before /After	Degree of significance	No of macroinvertebrate or amount of weed Before /After	Degree of significance	No of macroinvertebrate or amount of weed Before /After	Degree of significance
4 snails	8 / 7	NS	8 / 7	NS	8 / 6	NS
4 *Asellus*	8 / 0	***	8 / 0	***		
Cladophora + 3 snails + 3 *Asellus*	400 g / 65 g 6 / 6 6 / 0	*** NS ***	400 g / 40 g 6 / 6 6 / 0	*** NS ***	6 / 5	NS
3 snails + 3 *Asellus*	6 / 5 6 / 0	NS ***	6 / 6 6 / 0	NS ***	6 / 3	*
Cladophora + 3 *Asellus*	400 g / 50 g 6 / 0	*** ***	400 g / 0 g 6 / 0	*** ***		
Cladophora + 3 snails	400 g / 0 g 6 / 6	*** NS	400 g / 80 g 6 / 6	*** NS	6 / 1	**

Note: NS: $P>0.05$; *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$

Table 5.1.4 The degree of significance in the decrease of *Asellus*, snails (*P. contortus*) and *Cladophora* by 12 or 13 mm (CL) juvenile male *A. leptodactylus* (two replicates of each combination were pooled)

	09.10.94 (after 48 h)		11.10.94 (after 48 h)		19.10.94 (after 240 h)	
	No of macroinvertebrate or amount of weed Before /After	Degree of significance	No of macroinvertebrate or amount of weed Before /After	Degree of significance	No of macroinvertebrate or amount of weed Before /After	Degree of significance
4 snails	8 / 8	NS	8 / 8	NS	8 / 7	NS
4 *Asellus*	8 / 0	***	8 / 0	***		
Cladophora + 3 snails + 3 *Asellus*	400 g / 0 g 6 / 6 6 / 0	*** NS ***	400g / 70 g 6 / 6 6 / 0	*** NS ***	6 / 5	NS
3 snails + 3 *Asellus*	6 / 6 6 / 0	NS ***	6 / 6 6 / 0	NS ***	6 / 3	*
Cladophora + 3 *Asellus*	400 g / 45 g 6 / 0	*** ***	400 g / 0 g 6 / 0	*** ***		
Cladophora + 3 snails	400 g / 80 g 6 / 5	*** NS	400 g / 55 g 6 / 6	*** NS	6 / 1	**

Note: NS: $P>0.05$; *: $P<0.05$; ***: $P<0.001$

Table 5.1.5 The degree of significance degree in the decrease of *Asellus*, snails (*P. contortus*) and *Cladophora* by 12 or 13 mm (CL) juvenile female *P. leniusculus* (two replicates of each combination were pooled)

	09.10.94 (after 48 h)		11.10.94 (after 48 h)		19.10.94 (after 240 h)	
	No of macroinvertebrate or amount of weed Before /After	Degree of significance	No of macroinvertebrate or amount of weed Before /After	Degree of significance	No of macroinvertebrate or amount of weed Before /After	Degree of significance
4 snails	8 / 8	NS	8 / 8	NS	8 / 7	NS
4 *Asellus*	8 / 0	***	8 / 0	***		
Cladophora + 3 snails + 3 *Asellus*	400 g / 30 g 6 / 6 6 / 0	*** NS ***	400 g / 0 g 6 / 5 6 / 0	*** NS ***	5 / 5	NS
3 snails + 3 *Asellus*	6 / 5 6 / 0	NS ***	6 / 6 6 / 0	NS ***	6 / 5	NS
Cladophora + 3 *Asellus*	400 g / 0 g 6 / 0	*** ***	400 g / 45 g 6 / 0	*** ***		
Cladophora + 3 snails	400 g / 65 6 / 5	*** NS	400 g / 30 g 6 / 6	*** NS	6 / 3	*

Note: NS: P>0.05; *: P<0.05; ***: P<0.001

Table 5.1.6 The degree of significance in the decrease of *Asellus*, snails (*P. contortus*) and *Cladophora* by 12 or 13 mm (CL) juvenile male *P. leniusculus* (two replicates of each combination were pooled)

	09.10.94 (after 48 h)		11.10.94 (after 48 h)		19.10.94 (after 240 h)	
	No of macroinvertebrate or amount of weed Before /After	Degree of significance	No of macroinvertebrate or amount of weed Before /After	Degree of significance	No of macroinvertebrate or amount of weed Before /After	Degree of significance
4 snails	8 / 7	NS	8 / 8	NS	8 / 8	NS
4 *Asellus*	8 / 0	***	8 / 0	***		
Cladophora + 3 snails + 3 *Asellus*	400 g / 0 g 6 / 6	*** NS ***	400 g / 60 g 6 / 6	*** NS ***	6 / 5	NS
3 snails + 3 *Asellus*	6 / 3 6 / 0	* ***	6 / 6 6 / 0	NS ***	6 / 1	**
Cladophora + 3 *Asellus*	400 g / 50 g 6 / 0	*** ***	400 g / 0 g 6 / 0	*** ***		
Cladophora + 3 snails	400 g / 0 6 / 3	*** *	400 g / 40 g 6 / 6	*** NS	6 / 6	NS

Note: NS: $P>0.05$; *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$

Table 5.1.7 The degree of significance in the decrease of *Asellus*, snails(*P. contortus*) and *Cladophora* by 25 or 27 mm (CL) juvenile *P. leniusculus* and *A. leptodactylus* after 48 hours (two replicates of each combination were pooled)

	female *P.leniusculus* Before/After	male *P.leniusculus* Before/After	female *A. leptodactylus* Before/After	male *A.leptodactylus* Before/After	Between species
4 snails	8 / 1 (***)	8 / 3 (**)	8 / 1 (***)	8 / 0 (***)	NS
4 *Asellus*	8 / 0 (***)	8 / 0 (***)	8 / 0 (***)	8 / 1 (***)	NS
Cladophora +	400/20 g (***)	400/0 g (***)	400/0 g (***)	400/ 30 g (***)	NS
3 snails +	6 / 0 (***)	6 / 0 (***)	6 / 0 (***)	6 / 0 (***)	NS
3 *Asellus*	6 / 0 (***)	6 / 0 (***)	6 / 0 (***)	6 / 0 (***)	NS
3 snails +	6 / 0 (***)	6 / 0 (***)	6 / 3 (*)	6 / 3 (*)	**
3 *Asellus*	6 / 0 (***)	6 / 0 (***)	6 / 0 (***)	6 / 0 (***)	NS
Cladophora +	400/0 g (***)	400/35 g (***)	400/30 g (***)	400/0 g (***)	NS
3 *Asellus*	6 / 0 (***)	6 / 0 (***)	6 / 0 (***)	6 / 0 (***)	NS
Cladophora +	400/40 g (***)	400/20 g (***)	400/0 g (***)	400/40 g (***)	NS
3 snails +	6 / 3 (*)	6 / 1 (***)	6 / 3 (*)	6 / 1 (**)	NS

Note: NS: P>0.05; *: P<0.05; **: P<0.01; ***: P<0.001

Table 5.1.8 Number of maggots eaten by juvenile *P. leniusculus* in experiment 1.

No of maggots0 min (stock)	r	90 min	180 min	270 min	360 min	450 min	overnight (26.10.94 09.00 h)
1	1 2	1/2 1	-	1/2			
2	1 2	2 3/4, 1/4	-	1/4, 3/4			
4	1 2	2 -	- -	1 -	- -	- -	1 4 k
6	1 2	1 2	- -	- 1	- -	- 1	1, 3 k 2 k

Note: r= replicates, k= killed by juvenile, 3/4= if a maggot is divided for 4 equal part, 3/4 is equal with three parts of a maggot were eaten.

Table 5.1.9 Number of maggots eaten by juvenile *P. leniusculus* in experiment 2.

No of maggots0 min (stock)	r	90 min	180 min	270 min	360 min	450 min	overnight (26.10.94 09.00 h)
1	1 2	1 -	-	1			
2	1 2	1 1/2	1 1	1/2			
4	1 2	1 -	- -	- -	- -	- -	1 3
6	1 2	- 1	- -	- 1	- -	1 -	1 1, 1/2

Note: r= replicates, k= killed by juvenile, 3/4= if a maggot is divided for 4 equal part, 3/4 is equal with three parts of a maggot were eaten.

Table 5.1.10 Number of maggots eaten by juvenile *A. leptodactylus* in experiment 1.

No of maggots0 min (stock)	r	90 min	180 min	270 min	360 min	450 min	overnight (26.10.94 09.00 h)
1	1 2	1 -	-	-	1		
2	1 2	1 -	- -	- 1/2	- -	- -	1 1/2
4	1 2	- -	- -	1 -	- -	- -	2 k 3 k
6	1 2	- 1	- -	- -	- -	- -	4 k 1, 4 k

Note: r= replicates, k= killed by juvenile, 3/4= if a maggot is divided for 4 equal part, 3/4 is equal with three parts of a maggot were eaten.

Table 5.1.11 Number of maggots eaten by juvenile *A. leptodactylus* in experiment 2.

No of maggots0 min (stock)	r	90 min	180 min	270 min	360 min	450 min	overnight (26.10.94 09.00 h)
1	1 2	- -	- -	- -	- -	- -	1 half
2	1 2	- -	- -	- -	- -	- -	1, 1/2 -
4	1 2	- 1/2	- -	- -	- 1	- -	- 2, 1/2
6	1 2	- -	- -	- -	- -	- -	- 1

Note: r= replicates, k= killed by juvenile, 3/4= if a maggot is divided for 4 equal part, 3/4 is equal with three parts of a maggot were eaten.

Table 5.1.12 The degree of significance in the decrease of *Asellus* (alive or frozen) and *Gammarus* (alive or frozen) by stage 2 juveniles and 18 or 20 mm (CL) juveniles (two replicates of each species were pooled)

	stage 2 *P. leniusculus* Before/After	18 or 20 mm (CL) *P. leniusculus* Before/After	stage 2 *A. leptodactylus* Before/After	18 or 20 mm (CL) *A. leptodactylus* Before/After
2 *Asellus* (alive) + 2 *Asellus* (frozen)	4 / 3 (NS) 4 / 3 (NS)	4 / 0 (***) 4 / 0 (***)	4 / 3 (NS) 4 / 3 (NS)	4 / 0 (***) 4 / 0 (***)
4 *Asellus* (alive)	8 / 6 (NS)	8 / 0 (***)	8 / 7 (NS)	8 / 1 (***)
4 *Gammarus* (frozen)	8 / 7 (NS)	8 / 1 (***)	8 / 7 (NS)	8 / 0 (***)
2 *Gammarus* (alive) + 2 *Asellus* (alive)	4 / 4 (NS) 4 / 3 (NS)	4 / 0 (***) 4 / 0 (***)	4 / 3 (NS) 4 / 4 (NS)	4 / 0 (***) 4 / 0 (***)
4 *Gammarus* (alive)	8 / 6 (NS)	8 / 0 (***)	8 / 6 (NS)	8 / 1 (***)
4 *Asellus* (frozen)	8 / 6 (NS)	8 / 1 (***)	8 / 6 (NS)	8 / 0 (***)

Note: NS: $P>0.05$; ***: $P<0.001$

5.2 The impact of crayfish on fish eggs

5.2.1 Introduction

The majority of reports relating to crayfish-fish interactions have focused on the feeding ability of fish on crayfish (Momot, 1967; Svardson, 1972; Rickett, 1974; Jacobsen, 1977; Stein, 1977; Saiki and Tash, 1979; Momot, 1984; France, 1985; Hepworth and Duffield, 1987; Appelberg and Odelstrom, 1988; Svardson *et al.*, 1991; Mather and Stein, 1993; Blake and Hart, 1993; Blake *et al.*, 1994; Blake and Hart, 1995; Elvira *et al.*, 1996), or the feeding ability of crayfish on fish (Lund, 1944; Minckley and Craddock, 1961; Capelli, 1980; Rahel and Stein, 1988; Guan, 1995; Xinya, 1995).

The predation of rainbow trout on crayfish has been observed by Goldman and Rundquist (1977), Momot (1984) and Hepworth and Duffield (1987). Stein (1977) and France (1985) determined that *Orconectes propinquus* and *Orconectes virilis* were consumed by fish. Both the young-of-the-year *Micropterus salmoides* and adult black bullheads (*Ictalurus melas*) destroyed *Orconectes nais* in experimental ponds (Rickett, 1974). The abundance of *Orconectes causeyi* in Parker Canyon Lake was reduced by largemouth bass (*M. salmoides*) (Saiki and Tash, 1979). In addition to these studies, Mather and Stein (1993) found that fish (*Micropterus dolomieui*) fed on *Orconectes rusticus* and *Orconectes sanborni* and that there was a reduction in the activity of juveniles in the presence of *M. dolomieui* in laboratory and field studies. Similarly, the activity and survival of juvenile *Pacifastacus leniusculus* were reduced in the presence of fish (white aspe) (Blake *et al.*, 1994).

On the other hand, the active predation of *O. nais* and *Cambarus bartoni* on the fish *Catostomus commersoni* and *Rhinichthys atratulus* has been observed by Minckley and Craddock (1961). In another study, the reduced numbers of benthic fish (bullheads and stone loach) were thought to be correlated to the presence of *P. leniusculus* (Guan, 1995). Additionally, Rahel and Stein (1988) found that the number of small fish (*Etheostoma nigrum*) was significantly reduced in the presence of crayfish (*O. rusticus*) and fish (*M. dolomieui*).

However, little literature is available regarding the impact of crayfish on fish eggs. It was found that crayfish *Orconectes limosus* (Magnuson *et al.*, 1975), *O. rusticus* (Lodge *et al.*, 1986) and *O. propinquus* (Hobbs *et al.*, 1989) consume fish eggs. Therefore, the present study was undertaken to observe the impact of different sizes of *Pacifastacus leniusculus* and *Astacus leptodactylus* on fish eggs under laboratory conditions. The native crayfish species of Britain, *Austropotamobius pallipes*, was also used for a comparative purpose on the impact of these three species on trout eggs. The aims of the experiments were to determine if these three species do indeed eat fish eggs and to see if there was any differences between the species.

5.2.2 Materials and methods

Experiments with chub and carp eggs

Eggs of chub (*Leuciscus cephalus*) and crucian carp (*Carassius carassius*) were used. Both species lay their eggs on aquatic vegetation or stones (Wheeler, 1969; Philips and Rix, 1985) where crayfish may feed.

Adults (size range: 51-62 mm carapace length) and three sizes of juvenile *P. leniusculus* (15 ±1, 30 ±1, and 40 ±1 mm carapace length) and adults (size range: 47-58 mm carapace length) and two sizes of juvenile *A. leptodactylus* (15 ±1 and 30 ±1

mm carapace length) were used.

In order to standardize the state of feeding, juveniles were initially maintained on a diet of *Asellus*, *Crangonyx*, *Cladophora* and *Callitriche* until 14.05.95. Six chub eggs (*Leuciscus cephalus*) for each juvenile and 30 fish eggs for each adult were provided in the presence of *Cladophora* and *Callitriche* on 14.05.95. In addition, control dishes were set up in the absence of crayfish. The experiment was carried out in a constant temperature room (15 °C).

For juveniles each dish (10 cm x 15 cm x 7 cm) consisted of one individual with eight replicates for each size of juvenile for each species. For adults each container (38 cm x 23 cm x 11 cm) consisted of one individual with four replicates for each species. A small stone for each juvenile and a plastic tube for each adult was provided as a hide.

This experiment was repeated by using another benthic fish, crucian carp (*Carassius carassius*), eggs on 12.06.95.

All eggs which were used in the experiments were healthy and the eggs in the control dishes hatched out into fish larvae. Chub and carp eggs were provided from Calverton Fish Farm, Nottingham.

Experiment with brown trout eggs

Five adults of *P. leniusculus*, *A. leptodactylus* and *A. pallipes* (size range: 44-52 mm carapace length for the three species), and five of two sizes of juvenile *P. leniusculus* and *A. leptodactylus* (20 ±1 and 28 ±1 mm in carapace length) were used.

Each crayfish was put in a dish (10 cm x 15 cm x 7 cm). A layer of pebbles (approximately 1.5 cm depth) were laid over the floor of each dish. The pebbles were approximately 5 mm in diameter.

In order to standardize the state of feeding, juveniles were initially maintained on a diet of *Callitriche* between 19.12.1995 and 20.12.1995. Experiments were set up on 20.12.1995. Brown trout eggs were provided from Trent Fish Culture (Mercaston, Derbyshire).

In nature, eggs of brown trout are buried between 2 and 23 cm below the substrate surface in redds (Grost *et al.*, 1991). In order to provide semi-natural conditions of their natural redds ten brown trout eggs (*Salmo trutta*) were hidden in the pebbles in each dish. In addition, *Callitriche* and ten *Crangonyx* were also given for crayfish as a food choice. Control dishes were set up in the absence of crayfish. Numbers of eggs and *Crangonyx* were recounted after 24 hours.

In addition, an adult of each species with five replicates was also set up with ten brown trout eggs and *Callitriche* in the absence of *Crangonyx*. The Chi-square test was used to evaluate the results of the experiments.

5.2.3 Results

Experiment with chub eggs:

At the end of the experiment the reduction in the number of chub eggs by the adults and juveniles of *P. leniusculus* and *A. leptodactylus* was highly significant ($P<0.001$) as compared to the control. Number of chub eggs eaten by the adults and juveniles of the species is given in Tables 5.2.1 and 5.2.2 respectively.

There were no significant differences (P>0.05) between different sized juveniles within *P. leniusculus* and *A. leptodactylus*. Between species, 15 mm (CL) and 30 mm (CL) juveniles of *A. leptodactylus* consumed significantly more eggs (P<0.001 and P<0.01 respectively) than those of *P. leniusculus*. (In order to compare the different sized juveniles, eight replicates of each size were pooled).

Experiment with carp eggs:

At the end of the experiment the reduction in the number of carp eggs by the adults and juveniles of *P. leniusculus* and *A. leptodactylus* was highly significant (P<0.001) as compared to the control. Number of carp eggs eaten by the adults and juveniles of the species is given in Tables 5.2.1 and 5.2.2 respectively.

All eggs were eaten after 24 hours in all dishes by the juveniles of *A. leptodactylus*. There was no significant difference between 15 mm and 30 mm juveniles of *A. leptodactylus*. In *P. leniusculus*, significantly more eggs were eaten by 15 mm juveniles than 30 mm and 40 mm juveniles (P<0.05).

Between species, although there was not a significant difference between 15 mm juveniles of the two species, 30 mm juvenile *A. leptodactylus* consumed significantly (P<0.05) more eggs than 30 mm juvenile *P. leniusculus*.

Experiment with brown trout eggs

The reduction in the number of brown trout eggs in the presence of *Crangonyx* by adult *P. leniusculus*, *A. leptodactylus* and *A. pallipes* is given Table 5.2.3. Table 5.2.5 also shows the reduction in the number of brown trout eggs in the absence of *Crangonyx* by adult *P. leniusculus*, *A. leptodactylus* and *A. pallipes*. After 24 hours, the reduction in the number of *Crangonyx* by the adults of *P. leniusculus*, *A. leptodactylus* and *A. pallipes*, and the juveniles of *P. leniusculus* and *A. leptodactylus*

was highly significant (P<0.001) as compared to the control. In addition, the adults of the three species also consumed significantly more trout eggs (P<0.001) both in the presence of *Crangonyx* and in the absence of *Crangonyx*. There were differences between species in the number of trout eggs eaten. The adults of *P. leniusculus* and *A. leptodactylus* ate significantly more juveniles than those of *A. pallipes* when *Crangonyx* was given as an additional food choice (P<0.01).

The reduction in the number of brown trout eggs and *Crangonyx* by two sizes of *P. leniusculus* and *A. leptodactylus* (20 and 28 ±1) is given in Table 5.2.4. With regard to 28 mm juveniles of *P. leniusculus* and *A. leptodactylus*, there was a significant reduction in the number of *Crangonyx* and trout eggs (P<0.001) after 24 hours. There was a slight difference between the species (P<0.05). The juveniles of *A. leptodactylus* consumed more eggs than those of *P. leniusculus*.

Concerning 20 mm juveniles of *P. leniusculus* and *A. leptodactylus*, there was a significant reduction in the number of *Crangonyx* (P<0.001) and trout eggs consumed by both species (P<0.01) as compared to the control.

5.2.4 Discussion and conclusions

The results show that both juvenile and adult *P. leniusculus* and *A. leptodactylus*, and adult *A. pallipes* consumed fish eggs at a high rate. Although there was no significant difference between adults of *P. leniusculus* and *A. leptodactylus*, the consumption rate of carp eggs and brown trout eggs by juvenile *A. leptodactylus* was significantly higher than that of juvenile *P. leniusculus* in some cases. In addition, when *Crangonyx* was given as a food choice the consumption rate of brown trout eggs by *A. pallipes* was not as significant as the consumption rate of *P. leniusculus* and *A. leptodactylus*.

The predation of crayfish on fish eggs has been observed in some studies. Stomach analysis showed that *O. limosus* consumed *Rutilus rutilus* eggs (Magnuson *et al.*, 1975). According to Lodge *et al.* (1986) *O. rusticus* fed on lake trout (*Salvelinus namaycus*) eggs. Hobbs *et al.* (1989) pointed out that *O. propinquus* ate *S. namaycus* and *Coregonus clupeaformis* eggs. Therefore, it was concluded that *O. rusticus*, *O. propinquus*, *O. limosus* and *O. virilis* were significant predators on fish eggs (Magnuson *et al.*, 1975; Lodge *et al.*, 1986; Hobbs *et al.*, 1989). The present study showed that both *P. leniusculus* and *A. leptodactylus* are also predators on fish eggs.

In addition to the direct effect of crayfish on fish eggs, some crayfish species have been linked to detrimental changes in fish populations (Lodge *et al.*, 1985; Lodge *et al.*, 1986; Holdich, 1988; Hanson *et al.*, 1990). The translocation of *O. rusticus* into Wisconsin lakes caused negative effects on fish populations. Same crayfish species resulted in a reduction in the recruitment of walleye (*Stizostedion vitreum*) and some sport fish species (Lodge *et al.*, 1985 and Lodge *et al.*, 1986). The introduction of *O. virilis* into Newcastle Reservoir, Utah gave rise to competition for food between rainbow trout (*Salmo gairdneri*) and *O. virilis*. This resulted in a slow growth and poor condition of *S. gairdneri* (Hepworth and Duffield 1987).

Another aspect of the impact of crayfish on fish populations is that of competition for food between crayfish and fish. Both Huner *et al.* (1983) and Hobbs *et al.* (1989) point out there is competition between crayfish and fish for food and Hanson *et al.* (1990) concluded that an unexploited population of crayfish may cause a decline in fish growth. Similarly, as a result of field experiments, Guan (1995) found that there was a negative correlation between the number of *P. leniusculus* and the number and biomass of benthic fish (bullheads and stone loach) in a stream.

In addition, the impact of crayfish on fish populations via their grazing of macrophytes may be important. According to Cattaneo (1983) grazers are one of the most important factors in the decrease of epiphyte abundance in lakes. Because crayfish graze on aquatic plants which provide food, space to hide and spawning for fish (Holdich, 1994), this grazing on primary production of crayfish may have a dramatic effect on freshwater systems including fish communities (Momot *et al.*, 1978; Capelli, 1980; Elser *et al.*, 1994).

Introduced crayfish species in Britain, such as *P. leniusculus* and *A. leptodactylus*, as was found in this study may have a negative effect on the recruitment of fish populations in freshwaters by eating fish eggs. Yet this negative effect has not been attributed to the native crayfish *A. pallipes* (Holdich, D.M., pers. comm.).

5.2.1 The reduction in the number of chub and carp eggs at the end of the experiment by adult *P. leniusculus* and *A. leptodactylus*

	No of chub eggs Before / After	No of carp eggs Before / After
P. leniusculus replicates		
1	30 / 12	30 / 14
2	30 / 8	30 / 11
3	30 / 13	30 / 0
4	30 / 6	30 / 9
A. leptodactylus replicates		
1	30 / 11	30 / 4
2	30 / 7	30 / 13
3	30 / 14	30 / 9
4	30 / 10	30 / 14

5.2.2 The reduction in the number of chub and carp eggs at the end of the experiment by three sizes of juvenile *P. leniusculus* (15 ±1, 30 ±1, and 40 ±1 mm CL length) and two sizes of juvenile *A. leptodactylus* (15 ±1 and 30 ±1 mm CL)

	No of chub eggs Before / After	No of carp eggs Before / After
15 mm (±1) *P. leniusculus* replicates		
1	6 / 0	6 / 0
2	6 / 3	6 / 0
3	6 / 4	6 / 0
4	6 / 2	6 / 0
5	6 / 0	6 / 0
6	6 / 1	6 / 0
7	6 / 1	6 / 0
8	6 / 0	6 / 0
30 mm (±1) *P. leniusculus*		
1	6 / 0	6 / 0
2	6 / 0	6 / 0
3	6 / 6	6 / 0
4	6 / 1	6 / 0
5	6 / 0	6 / 1
6	6 / 0	6 / 0
7	6 / 5	6 / 0
8	6 / 0	6 / 4
40 mm (±1) *P. leniusculus*		
1	6 / 0	6 / 0
2	6 / 0	6 / 1
3	6 / 0	6 / 0
4	6 / 0	6 / 0
5	6 / 6	6 / 0
6	6 / 6	6 / 0
7	6 / 0	6 / 0
8	6 / 0	6 / 3
15 mm (±1) *A. leptodactylus* replicates		
1	6 / 0	6 / 0
2	6 / 0	6 / 0
3	6 / 0	6 / 0
4	6 / 0	6 / 0
5	6 / 0	6 / 0
6	6 / 0	6 / 0
7	6 / 0	6 / 0
8	6 / 0	6 / 0
30 mm (±1) *A. leptodactylus*		
1	6 / 1	6 / 0
2	6 / 0	6 / 0
3	6 / 0	6 / 0
4	6 / 0	6 / 0
5	6 / 0	6 / 0
6	6 / 0	6 / 0
7	6 / 0	6 / 0
8	6 / 0	6 / 0

Table 5.2.3 The reduction in the number of brown trout eggs and *Crangonyx* at the end of the experiment by adult *P. leniusculus*, *A. leptodactylus* and *A. pallipes*

	No of brown trout eggs Before / After	No of *Crangonyx* Before / After
P. leniusculus		
replicates		
1	10 / 1	10 / 6
2	10 / 2	10 / 3
3	10 / 4	10 / 7
4	10 / 1	10 / 4
5	10 / 3	10 / 5
A. leptodactylus		
replicates		
1	10 / 3	10 / 4
2	10 / 4	10 / 6
3	10 / 1	10 / 7
4	10 / 1	10 / 7
5	10 / 3	10 / 3
A. pallipes		
replicates		
1	10 / 7	10 / 8
2	10 / 5	10 / 4
3	10 / 4	10 / 6
4	10 / 6	10 / 2
5	10 / 3	10 / 5

Table 5.2.4 The reduction in the number of brown trout eggs and *Crangonyx* at the end of the experiment by two sizes of *P. leniusculus* and *A. leptodactylus* (20 and 28 ±1)

	No of brown trout eggs Before / After	No of *Crangonyx* Before / After
20 mm (±1) *P. leniusculus*		
replicates		
1	10 / 8	10 / 5
2	10 / 6	10 / 7
3	10 / 3	10 / 6
4	10 / 6	10 / 8
5	10 / 7	10 / 7
28 mm (±1) *P. leniusculus*		
replicates		
1	10 / 4	10 / 7
2	10 / 5	10 / 7
3	10 / 7	10 / 6
4	10 / 7	10 / 4
5	10 / 7	10 / 3
20 mm (±1) *A. leptodactylus*		
replicates		
1	10 / 6	10 / 6
2	10 / 4	10 / 6
3	10 / 7	10 / 8
4	10 / 8	10 / 5
5	10 / 5	10 / 7
28 mm (±1) *A. leptodactylus*		
replicates		
1	10 / 1	10 / 4
2	10 / 4	10 / 4
3	10 / 7	10 / 7
4	10 / 3	10 / 5
5	10 / 5	10 / 3

Table 5.2.5 The reduction in the number of brown trout eggs in the absence of *Crangonyx* by adult *P. leniusculus*, *A. leptodactylus* and *A. pallipes*

	No of brown trout eggs Before / After
P. leniusculus	
replicates	
1	10 / 3
2	10 / 5
3	10 / 5
4	10 / 2
5	10 / 6
A. leptodactylus	
replicates	
1	10 / 6
2	10 / 2
3	10 / 4
4	10 / 7
5	10 / 2
A. pallipes	
replicates	
1	10 / 5
2	10 / 3
3	10 / 7
4	10 / 4
5	10 / 6

Chapter 6

Inter- and intra-specific competition

6.1 Introduction

Freshwater crayfish are used extensively in aquaculture throughout the world (Holdich, 1993). Some species growth faster and to a larger size than others (Lowery, 1988). Consequently, there has been a trend towards moving such species to new areas. This has often led to the displacement of native crayfish in countries to which they have been introduced (Holdich, 1988).

Both *Pacifastacus leniusculus* and *Astacus leptodactylus* have been widely introduced in European waters. Both are invasive species which build up large populations in a short time period (Holdich, 1988). Although *P. leniusculus* can act as a vector of crayfish plague to which *A. leptodactylus* is susceptible (Alderman & Polglase, 1988), mixed populations do occur occasionally when *P. leniusculus* are disease free (Tsukerzis, 1976). However, little is known about the interaction of the two species under such conditions. Both species have also been introduced into Britain, where they have escaped into the wild and formed large populations (Holdich & Reeve, 1991) but, as yet no mixed populations of the two species have been reported, although this is considered to be just a matter of time (Holdich, D.M., pers. comm.). However, mixed populations of disease-free *P. leniusculus* and *Austropotamobius pallipes* have been reported in Britain. In all cases *P. leniusculus* has come to dominate *A. pallipes* and then to eliminate it (Holdich and Domaniewski, 1995).

In aquaculture it is important to get the maximum yield for the minimum effort, and the monospecific experiments reported on here give some indication of what survival would be like for the two species under semi-intensive conditions, i.e. is the yield

better for *P. leniusculus* or for *A. leptodactylus*? A number of competition experiments have been carried out with the juveniles and adults of the two species.

6.2 Materials and methods

Competition experiments with adults

Pacifastacus leniusculus (31-75 mm CL) were obtained from Boxmoor Fishery north of London, *A. leptodactylus* (33-71 mm CL) were caught with fyke nets from Tykes Water north of London. Each species was kept in separate tanks during the early part of 1993 and only complete (i.e. with all appendages), apparently healthy specimens were used for the experiment. The individuals used reflected the trappable, and therefore mobile, part of each population. As the experiments were started in April the majority of females were carrying eggs as they would be in the field.

Six outdoor tanks of two sizes (3.43 m^2 and 1.7 m^2) and water depths of 80 cm and 50 cm respectively, were set up in April 1993 with initial crayfish densities of 14 m^{-2}. The tanks had either *P. leniusculus* or *A. leptodactylus* on their own (one large and one small tank per species), or two of the species together in equal numbers (one large and one small tank per combination). In all cases the sex ratio was 3:1 in favour of males - this was determined by the availability of specimens. The tanks were drained and examined every month until September 1993 when the experiments were terminated. Each tank contained an excess of hides in the form of short lengths of plastic drainpipe or bricks with three holes. Food in the form the alga, *Cladophora* sp., and associated macroinvertebrates, e.g. *Asellus aquaticus*, *Crangonyx pseudogracilis* and *Bithynia tentaculata*, were supplied to the tanks as needed. The alga grew profusely in the summer providing additional shelter. All tanks had running mains water and consequently temperatures remained a few degrees above ambient in the spring and autumn and a few degree lower in the summer. The majority of animals

moulted at least once during the course of experiment and berried females released their juveniles.

Competition experiments with juveniles

One to one competition experiments were carried out with different sizes [stage 2 juveniles (mean CL= 5.2 mm for *P. leniusculus* and 5.8 for *A. leptodactylus*), stage 3 juveniles (mean CL= 5.9 mm for *P. leniusculus* and 6.5 mm for *A. leptodactylus*), and 15 mm CL juveniles] of *P. leniusculus* and *A. leptodactylus*. In addition, 16 (±1) mm (CL) juveniles of the two species were also used for a large scale competition experiment. In the one to one competition experiments with stage 2, stage 3 and 15 mm CL juveniles, *Cladophora* was given as a food source and a stone was put in each container (55 mm x 105 mm x 45 mm) to provide hides. The water of the containers was changed and number of cannibalism and/or predation were recorded every two days. Experiments were carried out at 15 °C.

One to one competition experiments with stage 2 (approx. 5.5 mm CL) and stage 3 juveniles (approx. 7 mm CL)

Experiments with stage 2 juveniles were carried out between 10.05.93 and 17.05.93, and this experiment was repeated with different stage 2 juveniles between 18.05.93 and 25.05.93. Experiments with stage 3 juveniles were carried out between 10.06.93 and 17.06.93, and it was repeated with different stage 3 juveniles between 17.06.93 and 24.06.93. For the single species experiment, two juveniles were put into each container (55 mm x 110 mm x 40 mm with 30 mm water depth) with 20 replicates, and for the mixed species experiment one individual of each species was put into each mixed container with 20 replicates.

One to one competition experiments with 15 (±1) mm CL juveniles

For the single species experiment, two juveniles were put into each container (55 mm

x 110 mm x 40 mm with 30 mm water depth) with 15 replicates, and for the mixed species experiment one individual of each species was put into each mixed container with 15 replicates. The experiment was started on 22.09.93 and terminated on 10.10.93.

Competition experiments with 16 (±1) mm CL juveniles on a large scale

Four outdoor tanks of 3.43 m^2 with 80 cm water depths were set up in May 1995 with initial crayfish densities of 58 m^{-2}. The tanks had either juvenile *P. leniusculus* or juvenile *A. leptodactylus* on their own, or two of the species together. They were drained and examined in October 1995. In addition to alga and macroinvertebrates (as was provided for the adults) the juveniles were also fed with minced morsels, fish meat and mussels. A hide was provided for each juvenile. Statistical analysis of the results was carried out using Chi-squared test.

6.3 Results

Competition experiments with adults

The results of the adult competition experiment are given in Table 6.1 for mixed and monospecific tanks, 1.7 m^2, and Table 6.2 for mixed and monospecific tanks, 3.43 m^2. Statistical analysis showed that there was no significant difference between the results obtained in the two sizes of tank thus the results were combined (Table 6.3).

All tanks showed a decline in numbers from the start to the end of the experiments. This is thought to have been due to cannibalism in the monospecific tanks (2, 3, 5 and 6), in addition, in the mixed tanks (1 and 4), to predation and cannibalism. No dead crayfish were found in the tanks during the experiment. In nearby tanks used for stocking crayfish it was noted that crayfish dying of natural causes were not eaten by other crayfish.

In the tanks containing *P. leniusculus* and *A. leptodactylus* there was a significant difference between numbers of crayfish at the start compared with the finish. When the survival of *P. leniusculus* alone is compared with their survival when they are with *A. leptodactylus*, then the probability of the null hypothesis is close to the 0.05 limit. However, when the survival of *A. leptodactylus* alone is compared with when they are with *P. leniusculus*, then the results are significant at the P< 0.001 level (Table 6.4).

Cannibalism and predation occurred particularly during the moulting time of crayfish. It was noticed that there was a rapid decline in *A. leptodactylus* numbers from 75% to 33% between 22.06.93 and 22.07.93 in the mixed tanks (Figure 6.1). Similarly, in the monospecific signal tanks, cannibalism was less before the moulting started. As far as the monospecific tanks are considered, the survival was higher in the *A. leptodactylus* tanks than the *P. leniusculus* tanks. Sixty-seven percent of the *A. leptodactylus* were able to survive to the end of the experiment, whereas that of *P. leniusculus* was 42% (Figure 6.1).

Competition experiments with juveniles

In the one to one interspecific competition experiments with stage 2, stage 3 and 15 mm CL juveniles, the predation on *A. leptodactylus* by *P. leniusculus* was significant at the end of the experiments (P<0.001), but the predation on *P. leniusculus* by *A. leptodactylus* was not significant (P>0.05).

Although in the container containing juvenile *A. leptodactylus* (intraspecific competition) there were no significant differences between numbers of crayfish at the start compared with the finish (P>0.05), in the container containing juvenile *P. leniusculus* (intraspecific competition) there were significant differences between numbers of crayfish at the start compared with the finish (P<0.001).

The degree of significance in the number of eaten crayfish (cannibalism in *P. leniusculus* and in *A. leptodactylus*, and predation between the species) is given in Table 6.5 for the one to one competition experiment with stage 2, in Table 6.6 for stage 3, and Table 6.7 for 15 mm CL juveniles.

Competition experiments with 16 (±1) mm CL juveniles on a large scale

No abnormal mortalities were observed during the experiment. Therefore, the reductions in the number of crayfish were thought to be due to cannibalism in the monospecific tanks, and cannibalism and/or predation in the mixed tanks.

The reduction in the number of *A. leptodactylus* by *P. leniusculus* in two replicates was highly significant ($P<0.001$) at the end of the experiment in comparison with the start. Only 9% of *A. leptodactylus* in the first replicate and 34% of *A. leptodactylus* in the second replicate survived. This reduction was also significant ($P<0.001$) in comparison with the reduction in the monospecific tank. The reduction in the number of *P. leniusculus* in the monospecific tank was higher in comparison to that of *A. leptodactylus*. The reduction of *P. leniusculus* and *A. leptodactylus* was 33.5% and 17% respectively.

6.4 Discussion and conclusions

There are many factors affecting fighting success or competitive interactions in crayfish. According to Bovbjerg (1970) and Edsman & Jonsson (1991) these factors are body weight and body size, sex and behaviour. Apart from these, crayfish have heavy and big claws which give rise to a competitive advantage in fighting (Edsman and Jonsson, 1991; Garvey and Stein, 1993). *Pacifastacus leniusculus* has heavy claws in comparison with most species (Goldman *et al.*, 1975). Thus, it may dominate crayfish having small or light claws in the same size ranges. By contrast, *A.*

leptodactylus is known as the narrow-clawed crayfish (Köksal, 1988).

In the present study, the results showed that *A. leptodactylus* could be eliminated by *P. leniusculus* if they met in a wild. The main reason for this is that *P. leniusculus* and *A. leptodactylus* differ in body weight and chelae size. Besides heavy body weight and large claws, aggressive behaviour of *P. leniusculus* is responsible for predation on *A. leptodactylus*. In addition, Edsman and Jonsson (1991) and Garvey and Stein (1993) have suggested that large and heavy claws resulting in fighting success are related to its muscle mass. *Pacifastacus leniusculus* has significantly more muscle in its claws than *A. leptodactylus* (see Chapter 12). It seems that this gives *P. leniusculus* an advantage over *A. leptodactylus* during fighting.

Crayfish are naturally aggressive, cannibalistic and predatory, although the degree to which they are carnivores depends on the stage of the life cycle, the time of year, and the species (Hogger, 1988). Moulting is a key event in the life of crayfish, and they are particularly vulnerable to predation at this time (Lowery, 1988). This was also observed in the present study. For example, a dramatic reduction in the number of *A. leptodactylus* was observed during the moulting time, from the end of June to July in the mixed tanks (held with *P. leniusculus*).

There have been many reports on the aggressive interactions of crayfish species in North America. Momot and Leering (1986) have carried out experiments on the aggressive interaction between *P. leniusculus* and *Orconectes virilis*. The adults and juveniles of the two species were held under laboratory conditions. As a result of the study, it was suggested that if *P. leniusculus* were introduced into eastern Canada, it would probably predominate over the indigenous species *O. virilis*. The growth rates, aggressive differences, food consumption and reproductive activities of *O. rusticus and O. sanbornii* have been compared under laboratory and field conditions (Butler and

Stein, 1985; Payne, 1986). They stated that if they are stocked together, *O. rusticus* will outcompete *O. sanbornii*. In another study on the aggressive interactions for shelter and various substrate types of *O. rusticus*, *O. virilis* and *O. propinquus*, it was observed that *O. rusticus* is more aggressive than the other two species (Capelli and Munjal, 1982; Capelli and Magnuson, 1983; Magnuson and Beckel, 1985). Both laboratory and field observations showed that *Orconectes virilis* is more aggressive than *Orconectes immunis* (Bovbjerg, 1970). However, it was observed that the aggressive behaviour of *O. rusticus* can be reduced by providing more shelter and food (Payne 1986).

Interactions between introduced and indigenous crayfish in Europe have been relatively little studied. Tsukerzis (1976) has briefly dealt with the aggressive interactions between *Astacus astacus*, *P. leniusculus* and *A. leptodactylus*. He stated that *P. leniusculus* is more aggressive than the others. According to Holdich and Domaniewski (1995), *Austropotamobius pallipes* can survive with disease free *P. leniusculus* but is eventually eliminated. Interactions between *A. pallipes* and *P. leniusculus* were studied by Holdich *et al.* (1995a). In the competition experiments, it was found that the reduction in the number of *A. pallipes* by *P. leniusculus* was highly significant. Söderbäck (1994 and 1995) also found that the native crayfish, *A. astacus*, of a Swedish Lake has been replaced by the introduced North American crayfish *P. leniusculus*. Predation of *P. leniusculus* on *A. astacus* was given as the main reason for the elimination of *A. astacus* by Söderbäck.

With regard to the survival of the two species in the tanks, because of the fact that *A. leptodactylus* has less aggressive behaviour than that of *P. leniusculus*, it may have more potential than *P. leniusculus* for aquaculture. In addition, Köksal (1988) has claimed that *A. leptodactylus* is the best crayfish species among *P. leniusculus*, *A. astacus* and *A. pallipes* with respect to rearing in ponds for commercial purposes.

Table 6.1 Results of adult competition experiments to show numbers at the start and end of experiment in mixed and monospecific tanks, 1.7 m^2

Tank no	1	2	3
Species	T / S	T	S
Start (27.04.93)	9♂+3♀ / 9♂+3♀	18♂+6♀	18♂+6♀
Finish (22.09.93)	0 / 7♂+2♀	10♂+6♀	4♂+2♀

S= *Pacifastacus leniusculus* and T= *Astacus leptodactylus*

Table 6.2 Results of adult competition experiments to show numbers at the start and end of experiment in mixed and monospecific tanks, 3.43 m^2

Tank no	4	5	6
Species	T / S	T	S
Start (27.04.93)	18♂+6♀ / 18♂+6♀	36♂+12♀	36♂+12♀
Finish (22.09.93)	2♂ / 14♂+1♀	27♂+5♀	20♂+4♀

Table 6.3 Combined results of adult competition experiments to show numbers at the start and end of experiment in mixed and monospecific tanks

Combined tank no	1 + 4	2 + 5	3 + 6
Species	T / S	T	S
Start (27.04.93)	27♂+9♀ / 27♂+9♀	54♂+18♀	54♂+18♀
Finish (22.09.93)	2♂ / 21♂+3♀	37♂+11♀	24♂+6♀

Table 6.4 Statistical analysis of data in Table 6.1, 6.2 and 6.3: Chi-squared test

Comparison	Tank size: 1.7 m^2	Tank size: 3.43 m^2	Combined: 1.7 + 3.43 (m^2)
S vs T mixed	***	***	***
S alone vs S mixed with T	*	NS	*
T alone vs T mixed with S	**	***	***

NS: P> 0.05, *: P< 0.05, **: P< 0.01, ***: P< 0.001

Table 6.5 Degree of significance in cannibalism in monospecific containers, and predation in mixed containers in experiments with stage 2 juveniles

	between 10.05.93 and 17.05.93	between 18.05.93 and 25.05.93
A. leptodactylus		
Cannibalism occurred	3	3
No cannibalism	17	17
Degree of significance	NS	NS
P. leniusculus		
Cannibalism occurred	8	10
No cannibalism	12	10
Degree of significance	**	***
Predation in mixed containers		
Predation on *A. leptodactylus* by *P. leniusculus*	9 out of 20	11 out of 20
Degree of significance	***	***
Predation on *P. leniusculus* by *A. leptodactylus*	3 out of 20	2 out of 20
Degree of significance	NS	NS

NS: P> 0.05, **: P< 0.01, ***: P< 0.001

Table 6.6 Degree of significance in cannibalism in monospecific containers, and predation in mixed containers in experiments with stage 3 juveniles

	between 10.06.93 and 17.06.93	between 17.06.93 and 24.06.93
A. leptodactylus		
Cannibalism occurred	2	3
No cannibalism	18	17
Degree of significance	NS	NS
P. leniusculus		
Cannibalism occurred	10	9
No cannibalism	10	11
Degree of significance	***	***
Predation in mixed containers		
Predation on *A. leptodactylus* by *P. leniusculus*	10 out of 20	12 out of 20
Degree of significance	***	***
Predation on *P. leniusculus* by *A. leptodactylus*	3 out of 20	3 out of 20
Degree of significance	NS	NS

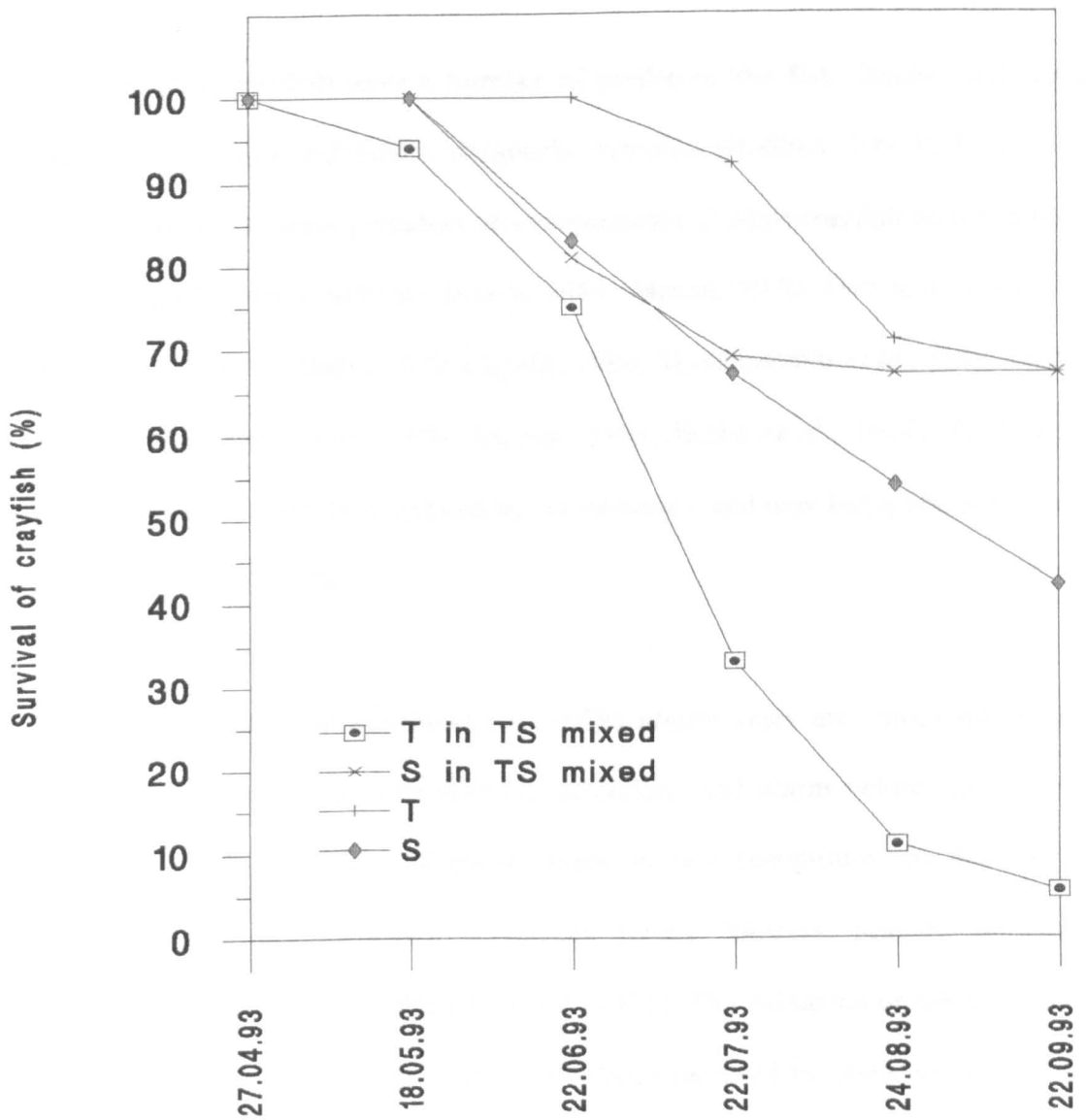
NS: P> 0.05, ***: P< 0.001

Table 6.7 Degree of significance in cannibalism in monospecific containers, and predation in mixed containers in experiments with 15 mm CL juveniles

	between 22.09.93 and 10.10.93
A. leptodactylus	
Cannibalism occurred	3
No cannibalism	12
Degree of significance	NS
P. leniusculus	
Cannibalism occurred	9
No cannibalism	6
Degree of significance	***
Predation in mixed containers	
Predation on *A. leptodactylus* by *P. leniusculus*	6 out of 15
Degree of significance	**
Predation on *P. leniusculus* by *A. leptodactylus*	0 out of 15
Degree of significance	NS

NS: P> 0.05, **: P< 0.01, ***: P< 0.001

Figure 6.1 Percentage survival in adult competition experiments from the start to the end of the experiment in the monospecific and mixed tanks (T= A. leptodactylus; S= P. leniusculus)



Chapter 7

Predation and cannibalism of adult crayfish on juveniles

7.1 Introduction

The juveniles of crayfish have a number of predators like fish, leeches and aquatic insects (beetle larvae and adults, dragonfly nymphs) (Holdich, 1991b; Blake *et al.*, 1994). In addition to these predators, the cannibalism of adult crayfish on juveniles has been reported by some workers (Lund, 1944; Mason, 1970; Dye and Jones, 1975; Little, 1975 and 1976; Ingle, 1979; Capelli, 1980; Munkhammar *et al.*, 1989; Gydemo *et al.*, 1990; Hanson *et al.*, 1990; Momot, 1993; Blake *et al.*, 1994). Predation on juveniles by adults, may be regulated by pheromones, and may bring about a decrease in recruitment of crayfish.

According to Thorp and Ammermann (1978) pheromones are important in social regulation and recognition, reproductive behaviour and alarm behaviour of aquatic invertebrates. The evidence of pheromones in sex recognition in echinoderms, annelids, Echiuroidea, molluscs, Crustacea (crabs, lobsters, prawns, amphipods, isopods, copepods) has been listed by Dahl (1975). The existence of sex pheromones and chemical communication in crayfish has been reported by many workers (Piesik, 1974; Ameyaw-Akumfi and Hazlet 1975; Thorp and Ammermann 1978; Ingle, 1979; Itagaki and Thorp 1981; Rose 1982; Tierney and Dunham 1982; Burba, 1983; Rose 1984; Hazlett, 1985; Bechler *et al.*, 1988). For example, the presence of a sex pheromone has been found in *Procambarus clarkii* (Ameyaw-Akumfi and Hazlet 1975). Tierney and Dunham (1982) have investigated the use of chemical signals in

species recognition in *Orconectes propinquus* and *Orconectes virilis*. Ingle (1979) has suggested that mating is governed by a pheromone in *A. pallipes*. More recently Bechler (1995) has published a review of sexual and interspecific pheromonal communication in crayfish.

Pheromonal communication between brood and adults in crayfish has also been reported (Little, 1975 and 1976; Burba, 1983; Munkhammar *et al.*, 1989). Little (1975, 1976) found that the juveniles of *P. clarkii* were able to discriminate their own mother and avoid other adult crayfish. According to Burba (1983) and Munkhammar *et al.* (1989) the juveniles of *A. astacus* are attracted to brooding females because of a chemical signal. In the present study, a number of experiments were carried out under laboratory conditions to investigate inter- and intraspecific behaviour of *P. leniusculus* and *A. leptodactylus* between juveniles and adults. In addition, the cannibalistic behaviour of *Austropotamobius pallipes* between adults and juveniles, and the impact of introduced crayfish species *P. leniusculus* and *A. leptodactylus* on the native crayfish juveniles (*A. pallipes*) were also investigated.

7.2 Materials and Methods

In all experiments, *Callitriche* sp. and *Cladophora* sp. were provided as food for crayfish. A plastic tube (16 cm in length and 6 cm in diameter) was provided as a hide for adults in each container (380 mm x 230 mm x 110 mm). Before the experiments were set up, adult crayfish had been fed with a mixture of *Cladophora* sp., *Callitriche* sp., *Asellus aquaticus*, *Crangonyx pseudogracilis* and minced morsels.

Experiments were carried out during the 1994 and 1995 breeding season of the two species. In addition, in order to have stage 2 juveniles of *P. leniusculus* and *A. leptodactylus* at the same time for some combinations, the egg development of *A. leptodactylus* was accelerated by keeping the brooding females at a temperature of 15 °C from February to May (see Chapter 9.1).

Experiment 1

To observe whether adults of *P. leniusculus* and *A. leptodactylus* are cannibalistic and/or predators on stage 2 juveniles: 40 stage 2 *P. leniusculus* and 40 stage 2 *A. leptodactylus* were set up in the presence of an adult male or female in one of the following combinations. Each combination was set up with four replicates.

Signal juveniles with:

1. Signal ♀ own mother
2. Signal ♀ not their own mother (her eggs hatched out on the same day as the stage 2 which were used as prey in the experiment and her own stage 2 juveniles were removed)
3. Signal ♀ (did not have juveniles, lost her eggs or did not have eggs)
4. Signal ♀ with stage 1 juveniles
5. Signal ♀ with eggs
6. Signal ♂
7. Turkish ♀ (her eggs hatched out on the same day as the stage 2 which were used as prey in the experiment and her own stage 2 juveniles were removed)
8. Turkish ♀ (did not have juveniles, lost her eggs or did not have eggs)

9. Turkish ♀ with stage 1 juveniles

10. Turkish ♀ with eggs

11. Turkish ♂

12. Signal juveniles on their own (as a control).

Turkish juveniles with:

1. Turkish ♀ own mother

2. Turkish ♀ not their own mother (her eggs hatched out on the same day as the stage 2 which were used as prey in the experiment and her own stage 2 juveniles were removed)

3. Turkish ♀ (did not have juveniles, lost her eggs or did not have eggs)

4. Turkish ♀ with stage 1 juveniles

5. Turkish ♀ with eggs

6. Turkish ♂

7. Signal ♀ (her eggs hatched out on the same day as the stage 2 which were used as prey in the experiment and her own stage 2 juveniles were removed)

8. Signal ♀ (did not have juveniles, lost her eggs or did not have eggs)

9. Signal ♀ with stage 1 juveniles

10. Signal ♀ with eggs

11. Signal ♂

12. Turkish juveniles on their own (as a control).

The experiment was checked and the number of juveniles was counted every two days. The water temperature varied between 15 °C and 18 °C during the experiment.

Experiment 2

To observe whether mother crayfish which have stage 2 juveniles release a chemical into the water which prevents any female or male from eating stage 2 juveniles:

Each container was divided into two compartments with a mesh. A mother crayfish with stage 2 juveniles was set up in the upper compartment. After two days, 40 stage 2 juveniles were removed from the mother and were set up in the second compartment in the presence of a female (which did not have juveniles, lost her eggs or did not have eggs) or a male. This process was repeated for another female and another male for each species.

The experiment was carried out in a 15 °C room. The number of juveniles was counted after 24 hours.

Experiment 3

To observe whether females with stage 1 of *P. leniusculus* and *A. leptodactylus* cannibalise their own stage 1 juveniles, three females with stage 1 of *P. leniusculus* and three females with stage 1 of *A. leptodactylus* were used. Forty stage 1 juveniles were supplied to their own mother.

The experiment was carried out in a 15 °C room. The number of juveniles was counted daily.

Experiment 4

To see whether juveniles go to (i) specific mother, because of special type of secretion in the form of pheromone produced by the mother, or (ii) go to any female, because of general type of secretion in the form of pheromone produced by the mother.

Eighty stage 2 juveniles of *P. leniusculus* and 80 stage 2 juveniles of *A. leptodactylus* were set up with one of the following combinations. Each combination was set up with three replicates for each species.

Signal juveniles with:

1. Signal ♀ (did not have juveniles, lost her eggs or did not have eggs)
2. Signal ♀ (did not have juveniles, lost her eggs or did not have eggs) plus their own mother

Turkish juveniles with:

1. Turkish ♀ (did not have juveniles, lost her eggs or did not have eggs)
2. Turkish ♀ (did not have juveniles, lost her eggs or did not have eggs) plus their own mother

The water used in this experiment was taken from the initial container of the stage 2s. The experiment was carried out in a 15 °C room. The number of juveniles which attached to the female was counted every hour. The experiment was terminated after five hours.

Experiment 5

To observe (i) what would happen if adult *A. leptodactylus* or *P. leniusculus* had been introduced in a native crayfish population where the juveniles of the native crayfish, *A. pallipes*, had been released from their mother, and (ii) whether adult native crayfish were cannibalistic on stage 2 juveniles.

Twenty-five stage 2 native crayfish were set up in each container in one of the following combinations with three replicates.

1. Signal ♂
2. Signal ♀ (did not have juveniles, lost her eggs or did not have eggs)
3. Turkish ♂
4. Turkish ♀ (did not have juveniles, lost her eggs or did not have eggs)
5. Native ♂
6. Native ♀ own mother
7. Native juveniles on their own (as a control)

The experiment was carried out at 16.5 °C (±1.5) water temperature.

The Chi-square test was used in order to evaluate the results in the experiments.

7.3 Results

Experiment 1

After 48 hours, except for stage 2 juveniles with their own mother and stage 2 juveniles which were not in their own mother containers, the reduction in the number of stage 2 juveniles by both *P. leniusculus* and *A. leptodactylus* was highly significant in all containers ($P<0.001$) compared to that of the control (stage 2 juveniles on their own).

The decrease in the number of stage 2 juveniles by their own mother and not their own mother of *P. leniusculus* and *A. leptodactylus* was significant ($P<0.001$) compared with that of the control (stage 2 juveniles on their own) after 16-21 days.

The decrease in the number of stage 2 juveniles is given in Tables 7.1 (for *P. leniusculus*) and 7.2 (for *A. leptodactylus*). (Because there is no significant difference between the four replicates of each combination they were pooled). In addition, the decrease in the number of stage 2 juveniles by male and female (own mother) is given in Figure 7.1 for *P. leniusculus* and in Figure 7.2 for *A. leptodactylus*.

Experiment 2

After one day, the reduction in the number of stage 2 juveniles by adults was highly significant ($P<0.001$).

The number of stage 2 juveniles after 24 hours is given in Table 7.3.

Experiment 3

After two days, the cannibalism of females with stage 1 of both *P. leniusculus* and *A. leptodactylus* on their stage 1 juveniles was highly significant ($P<0.001$). The number of stage 1 juveniles after 48 hours is given in Table 7.4.

Experiment 4

The stage 2 juveniles of both *P. leniusculus* and *A. leptodactylus* did not show any mother-dependent behaviour. There was no significant difference between the number of stage 2 attached to their own mother and in the number of stage 2 attached to a non-brooding female in all replicates (Table 7.6). In addition, in the stage 2 juveniles with non-brooding female container, almost all stage 2 juveniles attached to the non-brooding female.

Experiment 5

After 48 hours, the reduction in the number of stage 2 *A. pallipes* by *P. leniusculus*, *A. leptodactylus* and male *A. pallipes* was highly significant ($P<0.001$). The cannibalism of the female *A. pallipes* on their own stage 2 was significant after 16-21 days (Table 7.5). Figure 7.3 also shows the decrease in the number of stage 2 *A. pallipes* by male and female (own mother) *A. pallipes*.

7.4 Discussion and conclusions

The results showed that except the brooding females with stage 2s, the adults of *P. leniusculus* and *A. leptodactylus* were highly predatory on juveniles. This predation was not due to inadequate nutrition and starvation but exclusively because of predation on juveniles.

Predation on juveniles has been reviewed by a number of workers. In a laboratory experiment a reduction in the number of juvenile *P. leniusculus* was observed when the juveniles were kept with an adult in no weed and plastic weed habitats (Blake *et al.*, 1994). Cannibalism of adult *Orconectes virilis* on its juveniles was reported in laboratory experiments (Dye and Jones, 1975 and Hanson *et al.*, 1990) and in a field experiment (Momot, 1993). Similarly, cannibalism of intermoult juveniles was found in field populations of *O. propinquus* (Capelli, 1980). Non-brooding *Pacifastacus trowbridgi* (Mason, 1970), *Procambarus clarkii* (Little, 1975, 1976), *Austropotamobius pallipes* (Ingle, 1979), and *Astacus astacus* (Munkhammar *et al.*, 1989) have been reported to consume juvenile crayfish in laboratory experiments.

In this study it was observed that the females with stage 1 juveniles of *P. leniusculus* and *A. leptodactylus* ate their own stage 1 juveniles. Similar results were found by Ingle (1979), in which the female of *A. pallipes* fed on its stage 1s as they dropped down. However, Little (1975) suggested that when the females of *Orconectes sanborni*, *Cambarus virilis* and *P. clarkii* laid their eggs, they stopped eating juveniles. Whereas the present study indicated that the females with eggs of *P. leniusculus* and *A. leptodactylus* also preyed on juveniles.

The results revealed that the females with stage 2 juveniles of *P. leniusculus* and *A. leptodactylus* did not release a chemical into the water to prevent any other female or male from eating her stage 2 juveniles. In addition, the juveniles of both species did not show any preference for their own mother. Different results were observed for the brooding female of *Orconectes sanborni*, *Cambarus virilis* and *P. clarkii* by Little (1975). He concluded that because a chemical product was released into the water by the mother, the stage 3 juveniles of *O. sanborni*, *C. virilis* and *P. clarkii* demonstrated a significant choice for own mother.

The study also revealed that the stage 2 juveniles of *P. leniusculus* and *A. leptodactylus* are not mother specific as was reported for *A. astacus* by Munkhammar *et al.* (1989). They are attracted to any mother irrespective of where they are born. In addition, in the first experiment, it was observed that in the *P. leniusculus* and *A. leptodactylus* stage 2 juveniles with adult male containers, the juveniles of the two species become attached to the abdomen of male, even to the abdomen of the opposite species' male. On the basis of these observations, it can be hypothesized that a general type of pheromones is produced by the mother which attracts the juveniles and prevents the mother from eating stage 2s during the first 16-21 days after the first moult of juveniles.

It has also been observed that when any adult (male or female) tried to eat a juvenile, the juveniles still moved towards them instead of escaping. It may be that there is a general attractant produced by adult crayfish which encourages juveniles to move towards them. The chemical composition of these secretions or pheromones has yet to be discovered. Therefore there is a need for further research.

The non-predatory behaviour of females on their brood has been observed in some crayfish species. In a study on the copulatory and maternal-offspring behaviour in *Orconectes inermis inermis* and *Orconectes pellucidus*, the female did not feed on juveniles for 40 days after hatching (Bechler, 1981). In another study, an increase was found in the number of juveniles eaten by their own mother and not their own mother 18 days after hatching (at 18-21 °C of water temperature). This was attributed to the presence of pheromones which were produced by the female (Munkhammar *et al.*, 1989).

With regard to cannibalism on juveniles by female with stage 2s, this study shows that the female with stage 2 of *P. leniusculus*, *A. leptodactylus* and *A. pallipes* have a different behaviour than other adults. In addition, the females with stage 2 of *P. leniusculus* and *A. leptodactylus* are not predators against the opposite species' stage 2 juveniles for a specific time. *P. leniusculus* and *A. leptodactylus* are known as closely related crayfish species. According to Karlson and Luscher (1959) pheromones act within species and between closely related species. Therefore, it can be concluded that there is evidence of the presence of pheromones in these closely related crayfish species in the breeding season for 16-21 days at 15-18 °C. However, the study also showed that the adult of *P. leniusculus* and *A. leptodactylus* would have a dramatic impact on the juveniles of *A. pallipes* if they had been introduced in a native crayfish population where the juveniles of the native crayfish had released from their mother.

Table 7.1 The decrease in the number of stage 2 *Pacifastacus leniusculus* (The number of 160 represents four replicates combined).

	Combinations											
Counts	1	2	3	4	5	6	7	8	9	10	11	12
No of juven.	160	160	160	160	160	160	160	160	160	160	160	160
After 2 days	160/160	160/160	79/160	84/160	81/160	53/160	160/160	95/160	69/160	79/160	67/160	160/160
After 4 days	160/160	160/160					160/160					160/160
After 6 days	160/160	160/160					160/160					160/160
After 8 days	160/160	158/160					160/160					160/160
After 10 days	158/160	157/158					160/160					158/160
After 12 days	155/158	157/157					159/160					158/158
After 14 days	151/155	154/157					154/159					156/158
After 16 days	151/151	96/154					154/154					153/156
After 17 days	148/151	69/96					149/154					151/153
After 18 days	97/141	45/69					146/149					150/151
After 19 days	61/97						119/146					147/150
After 20 days	40/61						69/119					143/147
After 21 days							34/69					139/143
After 22 days												134/139

Note: Number before the diagonal line (to the left of the diagonal line, /) represents number of juveniles after checking, and number after the diagonal line (to the right of the diagonal line, /) represents number of juveniles before checking. Blanks indicate experiment terminated.

Combinations

Signal juveniles with:

1. Signal ♀ own mother
2. Signal ♀ not their own mother (her eggs hatched out on the same day as the stage 2 which were used as prey in the experiment and her own stage 2 juveniles were removed)
3. Signal ♀ (did not have juveniles, lost her eggs or did not have eggs)
4. Signal ♀ with stage 1 juveniles
5. Signal ♀ with eggs
6. Signal ♂
7. Turkish♀ (her eggs hatched out on the same day as the stage 2 which were used as prey in the experiment and her own stage 2 juveniles were removed)
8. Turkish♀ (did not have juveniles, lost her eggs or did not have eggs)
9. Turkish ♀ with stage 1 juveniles
10. Turkish ♀ with eggs
11. Turkish ♂
12. Signal juveniles on their own (as a control).

Table 7.2 The decrease in the number of stage 2 *Astacus leptodactylus* (The number of 160 represents four replicates combined).

	Combinations											
Counts	1	2	3	4	5	6	7	8	9	10	11	12
No of juven.	160	160	160	160	160	160	160	160	160	160	160	160
After 2 days	160/160	159/160	66/160	78/160	55/160	88/160	160/160	71/160	59/160	63/160	79/160	160/160
After 4 days	160/160	159/159					160/160					159/160
After 6 days	160/160	158/159					160/160					159/159
After 8 days	160/160	158/158					160/160					159/159
After 10 days	159/160	158/158					160/160					159/159
After 12 days	159/159	156/158					157/160					158/159
After 14 days	158/159	155/156					155/157					158/158
After 16 days	126/158	154/155					154/155					158/158
After 17 days	98/126	113/154					154/154					158/158
After 18 days	92/98	69/113					113/154					154/158
After 19 days	81/92	64/69					82/113					154/154
After 20 days	66/81	62/64					54/82					152/154
After 21 days		41/62					39/54					149/152
After 22 days												144/149

Note: Number before the diagonal line (to the left of the diagonal line, /) represents number of juveniles after checking, and number after the diagonal line (to the right of the diagonal line, /) represents number of juveniles before checking. Blanks indicate experiment terminated.

Combinations

Turkish juveniles with:

1. Turkish ♀ own mother
2. Turkish ♀ not their own mother (her eggs hatched out on the same day as the stage 2 which were used as prey in the experiment and her own stage 2 juveniles were removed)
3. Turkish ♀ (did not have juveniles, lost her eggs or did not have eggs)
4. Turkish ♀ with stage 1 juveniles
5. Turkish ♀ with eggs
6. Turkish ♂
7. Signal ♀ (her eggs hatched out on the same day as the stage 2 which were used as prey in the experiment and her own stage 2 juveniles were removed)
8. Signal ♀ (did not have juveniles, lost her eggs or did not have eggs)
9. Signal ♀ with stage 1 juveniles
10. Signal ♀ with eggs
11. Signal ♂
12. Turkish juveniles on their own (as a control).

Table 7.3 The number of stage 2 juveniles after 24 hours

	Stock (before)	After 24 hours
Stage 2 *P. leniusculus* + a ♀ (replicate 1)	40	18/40
Stage 2 *P. leniusculus* + a ♀ (replicate 2)	40	26/40
Stage 2 *P. leniusculus* + a ♂ (replicate 1)	40	15/40
Stage 2 *P. leniusculus* + a ♂ (replicate 2)	40	9/40
Stage 2 *A. leptodactylus* + a ♀ (replicate 1)	40	19/40
Stage 2 *A. leptodactylus* + a ♀ (replicate 2)	40	23/40
Stage 2 *A. leptodactylus* + a ♂ (replicate 1)	40	11/40
Stage 2 *A. leptodactylus* + a ♂ (replicate 2)	40	21/40

Table 7.4 The number of stage 1 juveniles after 48 hours

	Stock (before)	After 48 hours
Female with stage 1 of *P. leniusculus*		
replicate 1	40	18/40
replicate 1	40	13/40
replicate 1	40	27/40
Female with stage 1 of *A. leptodactylus*		
replicate 1	40	24/40
replicate 1	40	18/40
replicate 1	40	11/40

Table 7.5 The decrease in the number of stage 2 *Austropotamobius pallipes* (The number of 75 represents three replicates combined).

	Combinations						
Counts	1	2	3	4	5	6	7
No of juven.	75	75	75	75	75	75	75
After 2 days	18/75	27/75	24/75	32/35	29/35	75/75	75/75
After 4 days						75/75	75/75
After 6 days						74/75	75/75
After 8 days						74/74	75/75
After 10 days						74/74	75/75
After 12 days						72/74	75/75
After 14 days						72/72	73/75
After 16 days						59/72	73/73
After 17 days						58/59	71/73
After 18 days						43/58	70/71
After 19 days						41/43	70/70
After 20 days						39/41	69/70
After 21 days						21/39	69/69
After 22 days							68/69

Note: Number before the diagonal line (to the left of the diagonal line, /) represents number of juveniles after checking, and number after the diagonal line (to the right of the diagonal line, /) represents number of juveniles before checking. Blanks indicate experiment terminated.

Combinations

1. Signal ♂
2. Signal ♀ (did not have juveniles, lost her eggs or did not have eggs)
3. Turkish ♂
4. Turkish ♀ (did not have juveniles, lost her eggs or did not have eggs)
5. Native ♂
6. Native ♀ own mother
7. Native juveniles on their own (as a control)

Table 7.6 Number of stage 2 juveniles attached to a female or their own mother

	Time (hours)									
	1		2		3		4		5	
	own mother	a female	own mother	a female	own mother	a female	own mother	a female	own mother	a female
Stage 2 *P.leniusculus*										
replicate 1	32	39	42	36	35	24	34	46	45	33
replicate 2	38	42	37	29	32	48	38	42	28	34
replicate 3	39	29	29	34	42	35	33	47	37	43
Stage 2 *A. leptodactylus*										
replicate 1	41	39	36	41	39	33	34	39	42	36
replicate 2	34	41	33	26	25	37	41	34	30	41
replicate 3	29	36	32	39	32	44	29	35	35	38

Figure 7.1 Decrease in the number of stage 2 P. leniusculus by male P. leniusculus and their own mother

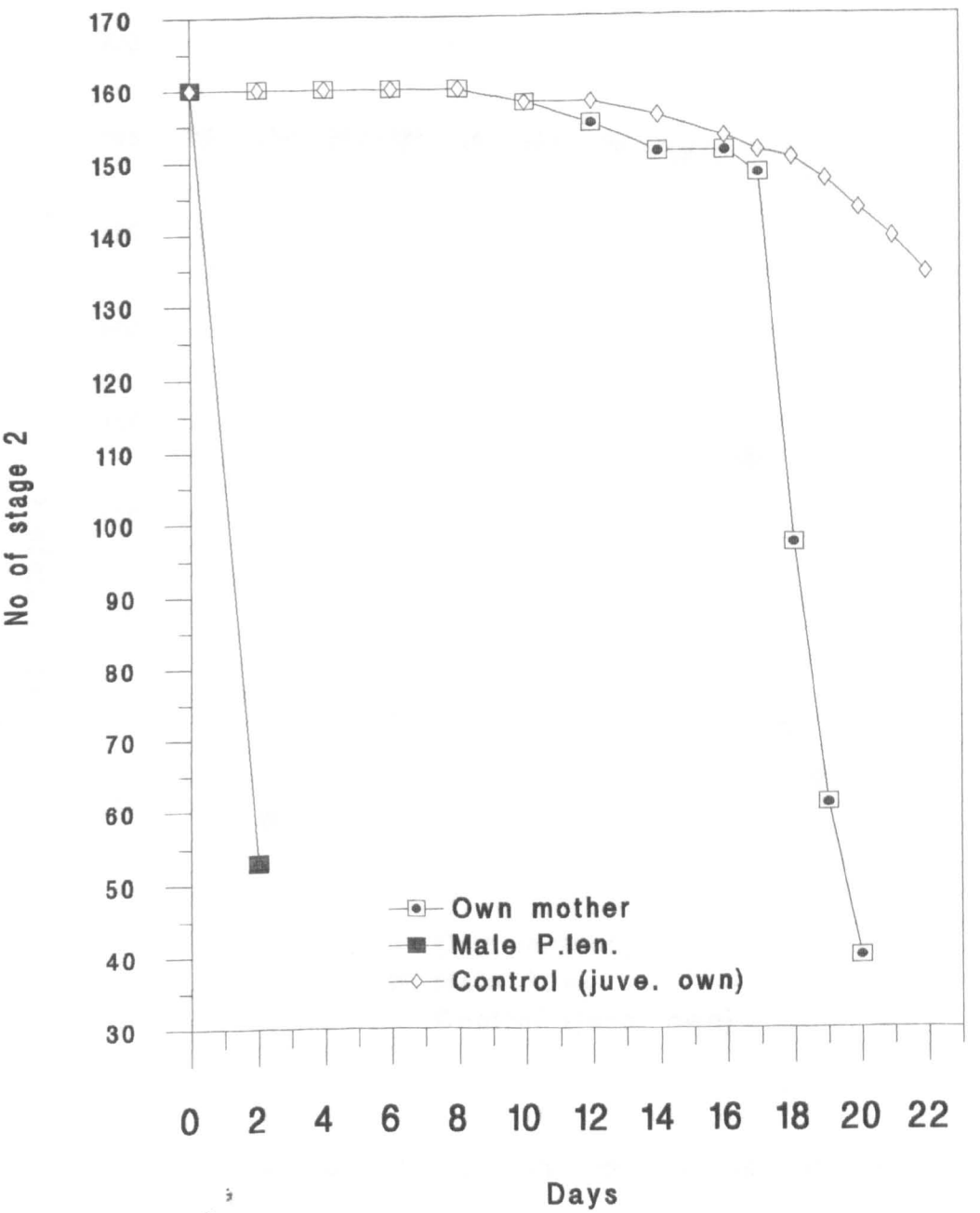


Figure 7.2 Decrease in the number of stage 2 A. leptodactylus by male A. leptodactylus and their own mother

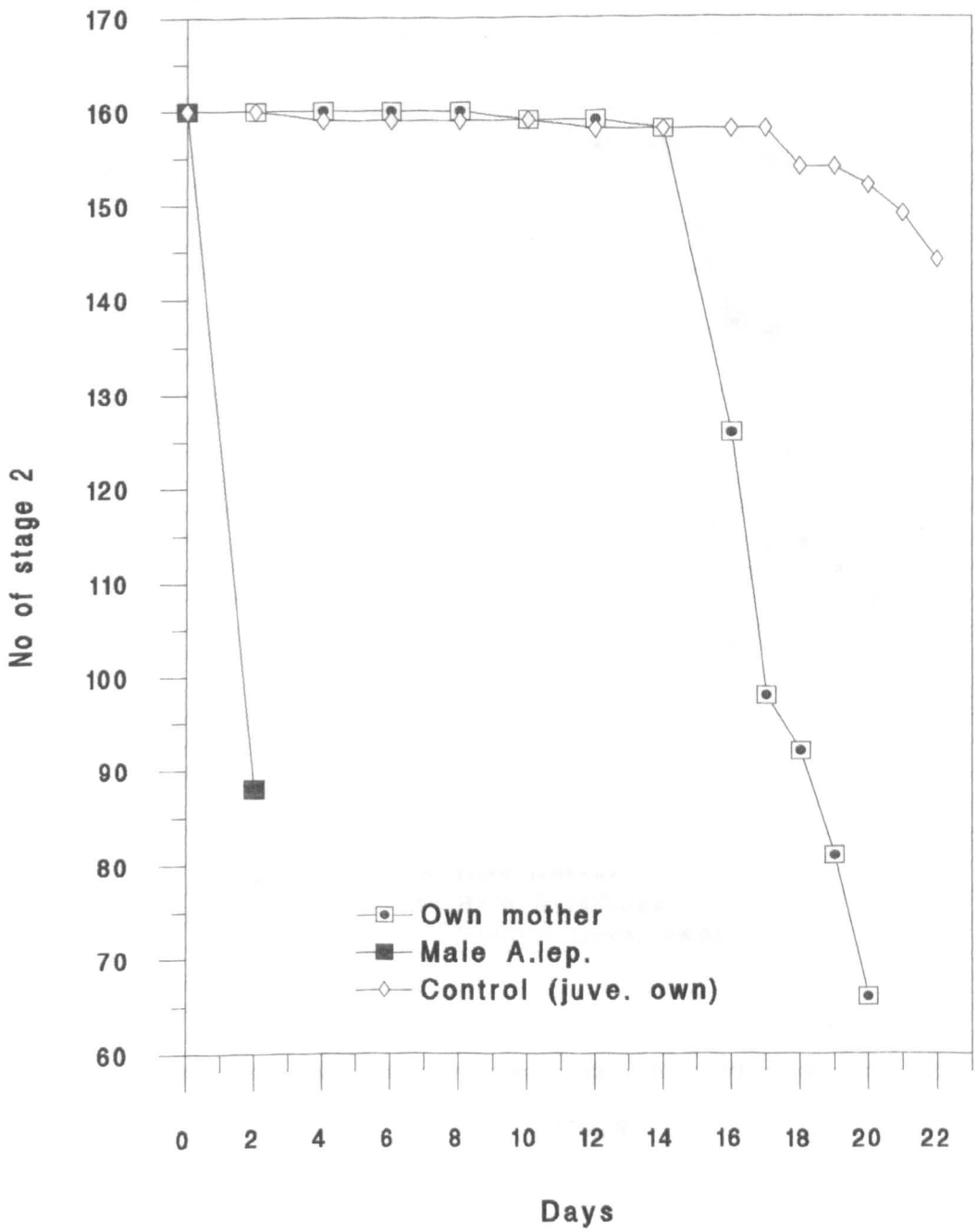
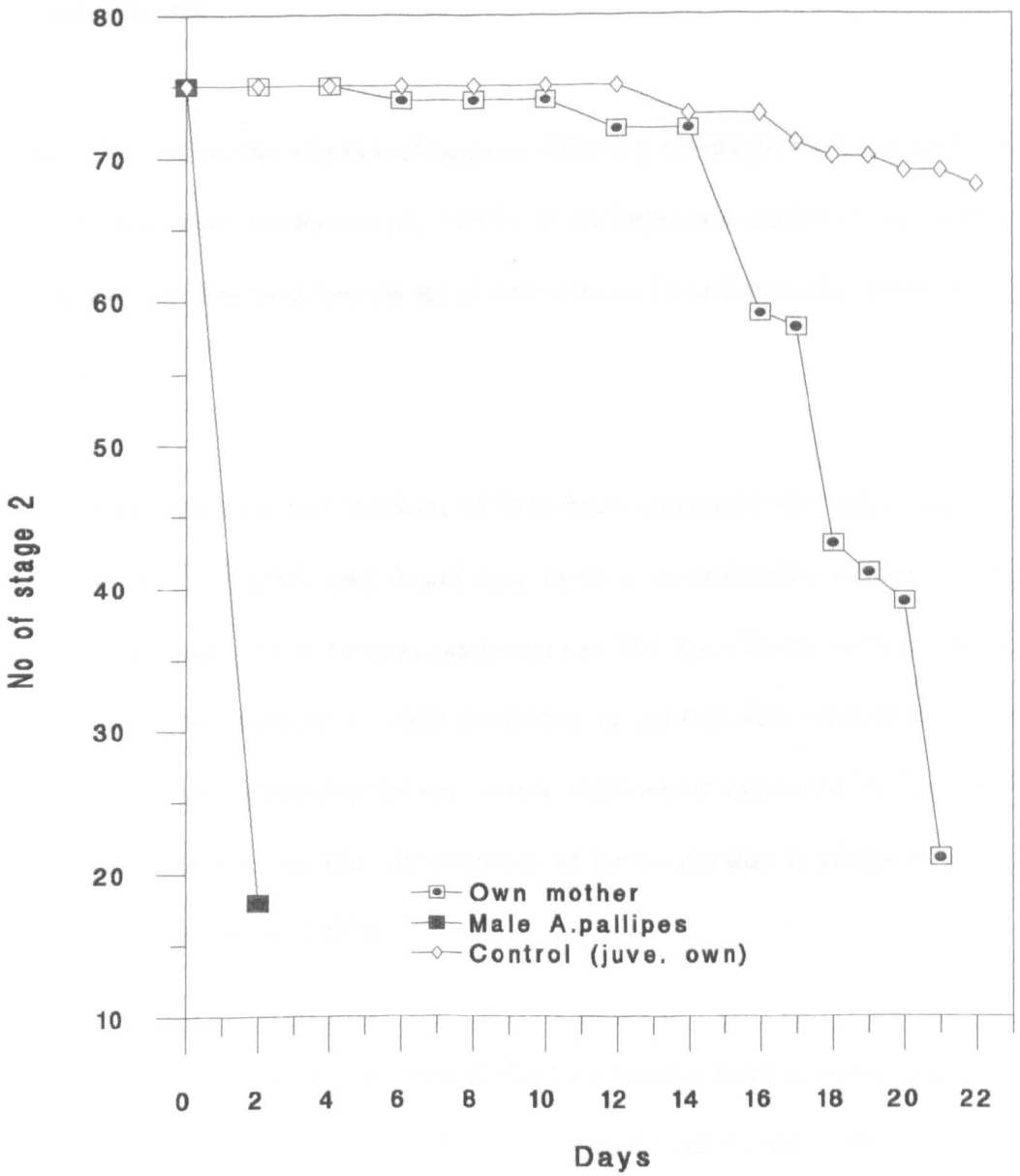


Figure 7.3 Decrease in the number of stage 2 A. pallipes by male A. pallipes and their own mother



Chapter 8

Fecundity and egg size

8.1 Fecundity

8.1.1 Introduction

Fecundity, defined as the number of eggs or offspring produced by a female (Lincoln *et al.*, 1982 and Abercrombie *et al.*, 1992), is an important aspect of the biology to study when comparing two species as, if one is more fecund than the other, it may be more successful.

Lowery (1988) and Lee and Wickins (1992) have compared the egg production of various species of crayfish and found that there is considerable variation between species ranging from 5 (in *Astacus pachypus*) to 960 (in *Cherax destructor*) (Table 8.1.2). Even so the number of eggs produced is considerably less than for those decapod crustaceans possessing larvae, where millions of eggs may be laid in order that a few juveniles survive after the majority of larvae die due to predation and other causes (Lee and Wickins, 1992).

Between the crayfish families in general the Cambaridae have a greater egg number than the Astacidae (Lowery, 1988). For example, *Procambarus clarkii* has between 100-700 eggs, whereas *Astacus leptodactylus* has between 200-400 eggs (Köksal, 1988; Lee and Wickins, 1992). In comparison to astacid crayfish, some parastacid crayfish also have more eggs, e.g. *Cherax tenuimanus* and *Cherax destructor* have 200-

800 and 124-960 respectively (Sokol, 1988; Lee and Wickins, 1992). The pleopodal egg number of several crayfish species is given in Table 8.1.2.

It has also been found that within a species the number of eggs carried by females can vary between sites. This may be due to the prevailing environmental conditions or be genetically based (Abercrombie *et al.*, 1992).

Although there have been many studies on fecundity for *Pacifastacus leniusculus* and *Astacus leptodactylus* none have been carried out on British populations. The aim of this part of the study was to compare fecundity between different sites for different sized individuals and between the two species.

8.1.2 Materials and methods

To compare the fecundity of the two species in the beginning and at the end of the breeding season, crayfish samples were taken in early winter and at the end of spring for *P. leniusculus* from Dinesens' crayfish farm in Hampshire (22 females for early winter and 56 females for end of spring) and for *A. leptodactylus* from the Serpentine lake in Hyde Park (40 females for early winter and 63 females for end of spring).

To compare the number of stage 2 juveniles of *P. leniusculus* (from Dinesens' crayfish farm) and *A. leptodactylus* (from the Serpentine), before the eggs hatched each female was set up in an individual container (41 cm x 27 cm x 11 cm). The number of stage 2 juveniles was counted on 23-26.05.94 for *P. leniusculus* and on 20-23.06.94 for *A. leptodactylus*.

In addition, to compare the fecundity of different populations of *P. leniusculus* and *A. leptodactylus* within and between species, 29 females from Boxmoor Fishery (Hemel Hempstead) and eight females from Gaddesby Brook (Leicester) for *P. leniusculus* and 91 females from Tykes Water (north of London) for *A. leptodactylus* were also used in this study.

In order to observe which pair of pleopods had the largest egg number, they were counted separately for *A. leptodactylus* collected from the Serpentine.

8.1.3 Results

In the present study fecundity was related to carapace length, although the actual number of eggs may be related to the ventral volume of the abdomen this is difficult to calculate.

The results reveal that female size (C.L.) is not a factor affecting fecundity in both *P. leniusculus* and *A. leptodactylus*, because the slopes of log C.L. versus log egg number are smaller than 3 (Table 8.1.1) (see Section 8.1.4 Discussion for explanation).

The relationship between female size (carapace length) and egg number is given for *P. leniusculus* collected from Dinesens in Figure 8.1.1, from Boxmoor in Figure 8.1.2, from Gaddesby Brook (Leicester) in Figure 8.1.3, for *A. leptodactylus* collected from the Serpentine in Figure 8.1.4 and Tykes Water in Figure 8.1.5. In addition, the formulas of regression analyses and their r^2 values (coefficient of determination) are given in Table 8.1.1.

Although the third pair of pleopods had the maximum number of eggs in general, the second pair of pleopods had more eggs in nine females out of 31 than the third pair of pleopods had in *A. leptodactylus*. Numbers of eggs carried out by the first, second, third and fourth pleopods of females *A. leptodactylus* are given in Table 8.1.4.

Differences between sites

For *P. leniusculus*: the females collected from Dinesens' crayfish farm were significantly more fecund ($P<0.001$, 2-Sample t test) than those collected from Boxmoor and Gaddesby Brook. The females collected from Dinesens' crayfish farm had mean 278 pleopodal eggs but those from Boxmoor and Gaddesby Brook had 195 and 203 respectively (Table 8.1.1).

For *A. leptodactylus*: the females collected from Tykes Water were significantly more fecund ($P<0.001$, 2-Sample t test) than those collected from the Serpentine. The females collected from Tykes Water had a mean of 221 eggs but those collected from the Serpentine had only 144 (Table 8.1.1).

Differences between species

The results show that the females of *P. leniusculus* collected from Dinesens' laid significantly more eggs ($P<0.001$, 2-Sample t test) than those of *A. leptodactylus* collected from the Serpentine. Although the females of *P. leniusculus* (collected from Dinesens') laid a mean of 278 pleopodal eggs those of *A. leptodactylus* (collected from the Serpentine) laid only 144 eggs (Table 8.1.1).

A comparison was also made on the number of stage 2 juveniles of the two species. Significantly more juveniles ($P<0.001$) hatched out from the females of *P. leniusculus* (from Dinesens') than those of *A. leptodactylus* (from Serpentine). The mean number of stage 2 was 195 for *P. leniusculus* and 141 for *A. leptodactylus*. In both species a logaritmic relationship ($r^2 = 0.24$ for *P. leniusculus* and $r^2 = 0.06$ for *A. leptodactylus*) was observed between the number of stage 2 juveniles and female size (Figure 8.1.6).

Because the samples were taken at different times of the year, the pleopodal egg number of *A. leptodactylus* collected from Tykes Water and *P. leniusculus* collected from Dinesens', Boxmoor and Gaddesby Brook were not compared.

8.1.4 Discussion and conclusions

Both ovarian and pleopodal egg number are used to describe crayfish fecundity. It has been observed that ovarian egg numbers may be more than 50 % higher than pleopodal egg numbers (Cukerzis, 1988; Corey, 1991 and Huner and Lindqvist, 1991). The reasons for this are:

(i) some females may lose a number of eggs between spawning and hatching (Abrahamsson, 1971; Mason, 1975 and Payne, 1978),

(ii) some females do not lay all of their eggs at spawning (Skurdal and Qvenild, 1986).

The fecundity of *Pacifastacus leniusculus* has been well studied (Abrahamsson and Goldman, 1970; Abrahamsson, 1971; Mason, 1975, 1977a and 1978; Brinck, 1977; McGriff, 1983; Shimizu and Goldman, 1983; Reynolds *et al.*, 1992). The reproductive

potential of *P. leniusculus* in Lake Tahoe, California was researched by Abrahamsson and Goldman (1970). They found that the mean pleopodal egg number of the females was 110 (size range: 76-103 mm in total length). In order to establish a management plan for the Delta populations of *P. leniusculus* in California, the life history parameters and fecundity of *P. leniusculus* were studied between 1975 and 1979 by McGriff (1983). She compared the ovarian egg number of *P. leniusculus* from Lake Tahoe, Berry Creek and the Delta. The mean ovarian egg number of these populations was 144, 175 and 161 respectively (size range: 80-90 mm total length for all populations). McGriff (1983) also stated that the females of *P. leniusculus* from Rogle Pond (in Sweden) and from the Delta had a mean of 164 and 224 pleopodal eggs respectively (size range 100 and 105 mm total length for the two populations). According to Abrahamsson (1971) *P. leniusculus* had 90 % more pleopodal eggs than *A. astacus* had in an isolated pond. Therefore, it was concluded that *P. leniusculus* is one of the most fecund astacid species and it is considered as an r-selected crayfish species (Brinck, 1977; Mason, 1977a; Lindqvist, 1988). Its high fecundity was also one of the mean reasons for introducing *P. leniusculus* into Scandinavian and other European countries. The present study also shows that the females of *P. leniusculus* collected from Dinesens' are more productive than those of *A. leptodactylus* collected from the Serpentine. The females of *P. leniusculus* had significantly more pleopodal eggs (278 against 144) and stage 2 juveniles (195 against 141) than those of *A. leptodactylus*.

Compared with *P. leniusculus*, fewer studies have been carried out on the fecundity of *Astacus leptodacylus* (Cherkashina, 1970; Kossakowski, 1971; Papadopol, 1975; Stypinska, 1979; Köksal, 1988). The fecundities of *A. leptodacylus* from six lakes in

Danube Delta were investigated by Papadopol (1975). He found that the ovarian and pleopodal egg numbers of females of 96-135 mm in total length from Lake Rosulet were 390 and 269 respectively. The fecundity of *A. leptodacylus*, *Orconectes limosus* and *A. astacus* was studied in Mazurian Lakeland by Stypinska (1979). The ovarian egg numbers of these species were 379, 447 and 242 respectively. Stypinska also found that (in Köksal, 1988) the mean ovarian egg number of the female *A. leptodacylus* collected from Lake Dluzek in Poland varied between 210 and 410 (size range: 95-135 mm total length). Köksal (1988) worked on the fecundity of *A. leptodacylus* in Turkey and found that the mean number of ovarian and pleopodal eggs was 210 and 183 respectively in Lake Egridir.

Although ovarian egg counts do not provide a more accurate estimation than pleopodal counts, the fecundity of many crayfish species has been calculated by the former method (Prins, 1968; Papadopol, 1975; Stypinska, 1979; Rhodes and Holdich, 1982; McGriff, 1983; Huner and Lindqvist, 1986; Huner, 1988; Taugbol *et al.*, 1988; Köksal, 1988; Skurdal *et al.*, 1993). In order to have more accurate estimations on the fecundity of *P. leniusculus* and *A. leptodactylus* for their different populations, pleopodal egg numbers and stage 2 juvenile numbers of females were counted in the present study.

In crustaceans, a number of regression models from simple linear regressions to complex asymptotic curves have been used to analyze the relationship between female size and egg number (Somers, 1991). However, in fisheries biology, the relation between log fecundity and log size has commonly been used in the description of data, in order to stabilize the variance in fecundity. This relationship employs the power

function or allometric model based on the equation log Y = log a + b [log X]. The relationship between fecundity and size (length) differs by a factor of three. Consequently, if the slope is > 3 or < 3, fecundity increases or decreases with body size and implies the absence of a simple volumetric relationship (Somers, 1991).

This model has also been used for crayfish. In the comparison of the fecundity of *Orconectes virilis*, *O. rusticus*, *O. propinguus* and *Cambarus robutus*, the slopes were found to be 13.53, 12.51, 8.83 and 4.98 respectively when comparing actual values of size and fecundity (i.e. not logged) (Corey, 1987). Thus, it was concluded that there was a positive linear relationship between the increase in the number of eggs and the increase in the size of females in *O. rusticus*, *O. propinguus* and *C. robutus* (Corey, 1987). This linear relationship between egg number and female size was also reported for *P. leniusculus* by Abrahamsson (1971); for *A. leptodactylus* by Köksal (1988); and for *Procambarus clarkii* by Huner (1988). The present study using the allometric model (Somers, 1991) showed that fecundity is not a function of female size in both *P. leniusculus* and *A. leptodactlus*: because, the slopes (C.L. versus egg number) were smaller than 3 (Table 8.1.1).

The simple regression analyses show that r^2 (carapace versus egg number) values are very low in *P. leniusculus* and *A. leptodactylus* (Table 8.1.1). This was attributed to the high variability of individual fecundity in the two species. For example, very variable mean egg numbers were observed for three 54 mm (CL) females of *P. leniusculus* (collected from Dinesens') and *A. leptodactylus* (collected from Tykes Water). These numbers were 188, 215 and 280 for *P. leniusculus* and 205, 280 and

349 for *A. leptodactylus*.

In crayfish, larger females produce more eggs than smaller females (Huner and Lindqvist, 1991). This was reviewed for *P. leniusculus* by Mason (1975a), for *A. leptodactlus* by Köksal (1988), for *Orconectes rusticus*, *O. kentuckiensis* and *Procambarus hayi* by Corey (1991). In addition, McGriff (1983) found that 2+ female of *P. leniusculus* had significantly less eggs than 3+. Similar results were found in the present study. To compare the fecundity of different sizes, a comparison was made between the different size ranges of crayfish (crayfish smaller than 51 mm, between 51-60 mm and bigger than 60 mm in carapace length) within the same species. In general, the larger females were more fecund than the smaller females in both *P. leniusculus* and *A. leptodactlus*, except the females of *P. leniusculus* collected from Dinesens' on 26.05.1994.

In *P. leniusculus* collected from Dinesens' on 26.05.94, although 51-60 mm crayfish had significantly more stage 2 juveniles ($P<0.001$) than the crayfish smaller than 50 mm, crayfish bigger than 60 mm in CL had a smaller number of stage two juveniles than those of 51-60 mm had. In *A. leptodactylus* collected from the Serpentine on 23.06.1993, although there was an increase in the number of stage 2 juveniles with the increasing size range, this increase was not significant between the different sizes ($P>0.05$). The mean egg numbers for the given size ranges for the other populations of the two species examined in this study are given in Table 8.1.3.

According to Mason (1978a) the third pair of pleopods in *P. leniusculus* have the maximum egg number. In this study, different results were observed for *A.*

leptodactylus from the Serpentine. Although the third pair of pleopods had the maximum eggs in general, the second pair of pleopods also had the largest egg number in some cases.

In crayfish, after mating females lay their eggs which are attached to the female's pleopods (Fitter and Manuel, 1986). After this, eggs undergo their full development to hatching stage on the female. In general this egg development takes over six months (this is considerably shorter in warm water species). During this process crayfish may lose a considerable number of eggs (Abrahamsson, 1971 and Mason, 1977a). In the present study this was observed in both *P. leniusculus* (collected from Dinesens') and *A. leptodactylus* (collected from Tykes Water). In *P. leniusculus*, the mean egg number of the females was 278 in the beginning of breeding season (in October), but the mean number of stage 2 juveniles was only 195 for each female at the end of breeding season (in May). In *A. leptodactylus*, as can be seen in Figure 8.1.7, some females lost considerable numbers of eggs at the end of the incubation period (in May) or laid relatively a few eggs after the mating (in December).

Many factors have an effect on the reproductive efficiency of crayfish such as: food (quality and quantity), water quality (temperature, oxygen, pollution), egg size, shelter, density, internal parasites, infertile eggs, unsuccessful attachment of eggs to the pleopods, the structure of the abdomen to protect the eggs during incubation (Rhodes and Holdich, 1982; Lowery, 1988; Botsford, 1991; Corey, 1991; Huner and Lindqvist, 1991).

The egg production of *Orconectes virilis* has been found to be higher in high nutrient lakes than in low nutrient lakes. Similarly, the egg production of *Cherax tenuimanus* was negatively affected by poor nutrition in pond culture studies (Huner and Lindqvist, 1991). Regarding the effects of density on egg production, Morgan and Momot (1988) found that females produce smaller and fewer eggs in the unharvested populations of *Orconectes virilis* than the harvested populations of *O. virilis*. With regard to the effects of temperature on egg production, the fecundity of *Austropotamobius pallipes* was 40 % higher in the warm mesotrophic White Lake than in the cool oligotrophic Lisheens stream (Reynolds *et al.*, 1992). Egg size is also another factor affecting fecundity. This is studied in more detail in Chapter 8.2.

Differences in fecundity for the different populations of the same crayfish species have been reported by some workers (Papadopol 1975; Rhodes and Holdich, 1982; McGriff, 1983; Taugbol *et al.*, 1988). In a comparative study on the reproductive potential of some populations of *Astacus leptodactylus* in the Danube Delta, Papadopol (1975) found that females from the Matita Danube Delta produced significantly more ovarian eggs (mean 482, range 287-680) than females from the Rosulet Danube Delta (mean 390, range 220-602). Similarly, the number of ovarian eggs varied greatly between the six populations of *A. astacus* (Taugbol *et al.*, 1988). Pleopodal egg number was lower in a Northumberland population (northern England) of *A. pallipes* than that of the River Darent (southern England) (Rhodes and Holdich, 1982).

In addition, differences in the fecundity of *A. astacus* (Taugbol *et al.*, 1988) and *P. leniusculus* (McGriff, 1983) were also reported between years. Moreover, changes in the number of *A. pallipes* eggs between years in White Lake were observed by

Reynolds *et al.* (1992).

Similarly, differences in the egg number for the different populations of *P. leniusculus* and *A. leptodactylus* were also observed in the present study. The females of *P. leniusculus* from Dinesens' were more fecund (at the beginning of the breeding season) than those from Boxmoor. Mean egg numbers were 278 and 195 respectively. In *A. leptodactylus*, the females from Tykes Water were more fecund (at the end of the breeding season) than those from the Serpentine. Mean egg numbers were 221 and 144 respectively. It seems that the differences in the reproductive efficiency between *P. leniusculus* and *A. leptodactylus*, and within the same species for different populations, may be due to the one or more of the prevailing environmental conditions as mentioned above or are genetically based. In conclusion, it is clear that populations of *P. leniusculus* and *A. leptodactylus* in British waters are as fecund as any studied elsewhere. It would appear that females of *P. leniusculus* are more fecund than those of *A. leptodactylus* and consequently they may be more successful in establishing themselves in new waters.

Table 8.1.1 A comparison of the fecundity between and within *Pacifastacus leniusculus* and *Astacus leptodactylus* from different populations

	P. leniusculus (Dinesens')	*P. leniusculus* (Dinesens')	*P. leniusculus* (Boxmoor)	*P. leniusculus* (Leicester)	*A. leptodactylus* (Serpentine)	*A. leptodactylus* (Serpentine)	*A. leptodactylus* (Tykes)
Date	26.05.1994	14.10.1993	15.02.1993	06.12.93	23.06.1994	11.01.1995	05.05.1993
Number of crayfish	56	22	29	8	63	40	91
CL range Mean CL SD	40-68 52.5 7.25	41-68 53.09 6.65	43-61 52 4.22	37-52 44 4.47	40-74 54.3 6.19	37-68 49.7 7.23	37-68 51.72 7.48
Mean egg SD	195[a] 74.07	278 56.17	195 55.92	203 60.32	141[a] 59.36	144 72.87	221 78.52
reg. formu. (log y) r^2	-0.45530+ 1.57822*logx 0.241	0.96179+ 0.85564*logx 0.234	-2.23355+ 2.62723*logx 0.452	-2.34472+ 2.82364*logx 0.861	0.55694+ 0.89971*logx 0.066	-2.69595+ 2.82777*logx 0.405	-1.25721+ 2.09095*lox 0.636

Note: [a]= number of stage 2 juveniles

Table 8.1.2 Pleopodal egg number of several crayfish species.

Species	Mean egg no.	Range	Source
O. virilis	139	20-310	Corey (1987)
		86-415	Momot (1988)
	214	115-320	Weagle *et al.* in Corey (1987)
O. propinguus	75	21-249	Corey (1987)
	60		Capelli and Magnuson (1975)
O. rusticus	161	75-351	Corey, 1987
		150-276	Momot (1988)
		42-231	Prins (1968)
O. immunis		84-195	Momot (1988)
	84	4-170	Tack in Corey (1987)
O. limosus	163		Momot (1988)
	163	57-396	Smith in Corey (1987)
C. destructor		300-400	Frost (1975)
	323	?-438	Woodland in Sokol (1988)
		124-498	Johnson in Sokol (1988)
		618-960	Reynolds in Sokol (1988)
C. tenuimanus		200-800	Wickins (1982)
C. quadricarinatus		60-600	Jones in Lee and Wickins (1992)
C. robutus	64	25-128	Corey (1987)
A. pallipes	64	18-220	Reynolds (1992)
	59		Brown and Bowler (1977)
		50-60	Cuellar and Coll (1978)
		?-130	Lowery and Holdich (1988)
		25-105	Rhodes and Holdich (1982)
		15-115	Rhodes and Holdich (1982)
A. leptodactylus	210		Köksal (1988)
		200-400	Hofmann in Köksal (1988)
	269		Papadopol (1975)
A. caspius	115	48-267	Cherkashina (1970)
A. kessleri	152	37-320	Cherkashina (1970)
A. pachypus	27	5-52	Cherkashina (1970)
A. torrentium		40-70	Laurent (1988)
A. astacus		100-150	Cukerzis (1988)
P. leniusculus	110	65-160	Abrahamsson and Goldman (1970)
		70-260	Lee and Wickins (1992)
	152	120-179	McGriff (1983)
	164	80-242	McGriff (1983)
	224	41-340	McGriff (1983)
P. clarkii		100-700	Lee and Wickins (1992)
	246	130-480	Penn in Corey (1987)

Table 8.1.3 A comparison of the fecundity between different size ranges in *P. leniusculus* and *A. leptodactylus*

	Sampling date	size range < 51 mm	size range 51-60 mm	size range > 60
P. leniusculus (Dinesens')	26.05.94			
Mean number of stage 2		147	242	199
SD		59.0	62.1	66.9
Sample No		24	24	8
P. leniusculus (Dinesens')	14.10.93			
Mean egg no		258	282	324
SD		61.2	49.6	59.7
Sample No		9	10	3
P. leniusculus (Boxmoor)	15.02.93			
Mean egg no		153	216	
SD		39.6	49.1	
Sample No		11	17	
A. leptodactylus (Serpentine)	23.06.94			
Mean number of stage 2		126	140	178
SD		54.1	59.4	65.6
Sample No		17	38	8
A. leptodactylus (Serpentine)	11.01.95			
Mean egg no		119	190	204
SD		57.1	77.3	101
Sample No		27	9	4
A. leptodactylus (Tykes Water)	05.05.93			
Mean egg no		164	250	310
SD		46.6	58.5	72.5
Sample No		41	35	15

Table 8.1.4 Numbers of eggs carried out by the pleopods of female *A. leptodactylus*

	Pleopods (left)				Pleopods (right)			
crayfish	1	2	3	4	1	2	3	4
1	28	63	61	32	16	41	64	18
2	19	35	30	23	20	28	23	22
3	10	19	25	12	8	12	21	12
4	12	22	21	8	8	16	19	5
5	7	6	10	7	11	19	20	10
6	39	37	36	18	25	43	47	28
7	16	25	31	21	10	24	31	14
8	18	21	24	14	23	20	30	23
9	4	22	16	16	5	20	23	15
10	7	25	27	14	9	16	25	16
11	6	14	25	14	9	23	21	8
12	13	27	23	19	15	20	41	12
13	18	32	17	12	24	41	36	15
14	14	16	19	7	13	21	24	14
15	1	12	21	10	1	16	25	13
16	11	15	16	10	11	20	17	13
17	9	11	11	5	10	18	13	11
18	24	17	26	15	14	22	21	10
19	16	28	36	17	22	25	38	23
20	11	5	7	3	15	11	18	5
21	13	14	12	4	15	16	13	7
22	31	34	27	11	31	40	43	15
23	15	25	33	19	17	21	37	26
24	5	10	15	8	10	19	20	8
25	15	34	25	26	15	41	17	13
26	22	29	36	22	16	27	31	22
27	5	8	16	6	7	14	12	9
28	11	23	8	18	10	18	23	17
29	23	34	39	30	22	33	39	30
30	13	16	18	14	15	13	29	23
31	21	38	14	5	20	25	16	2

Figure 8.1.1 The relationship between carapace length and egg number in P.lenlusculus collected from Dinesens'

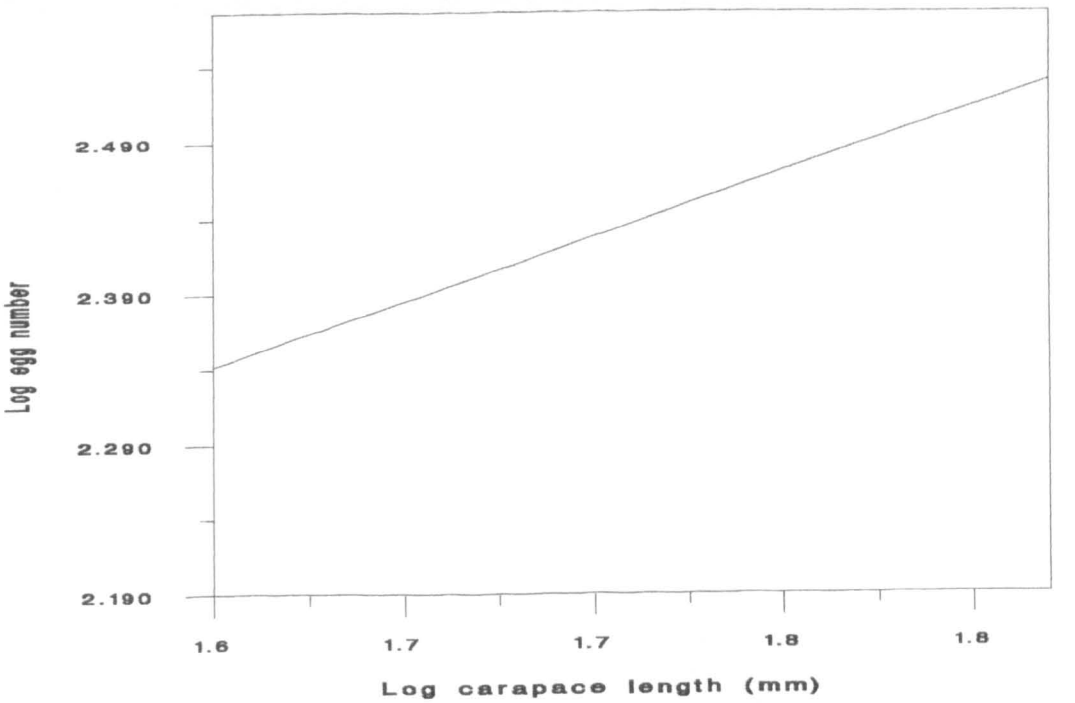


Figure 8.1.2 The relationship between carapace length and egg number in P.lenlusculus collected from Boxmoor.

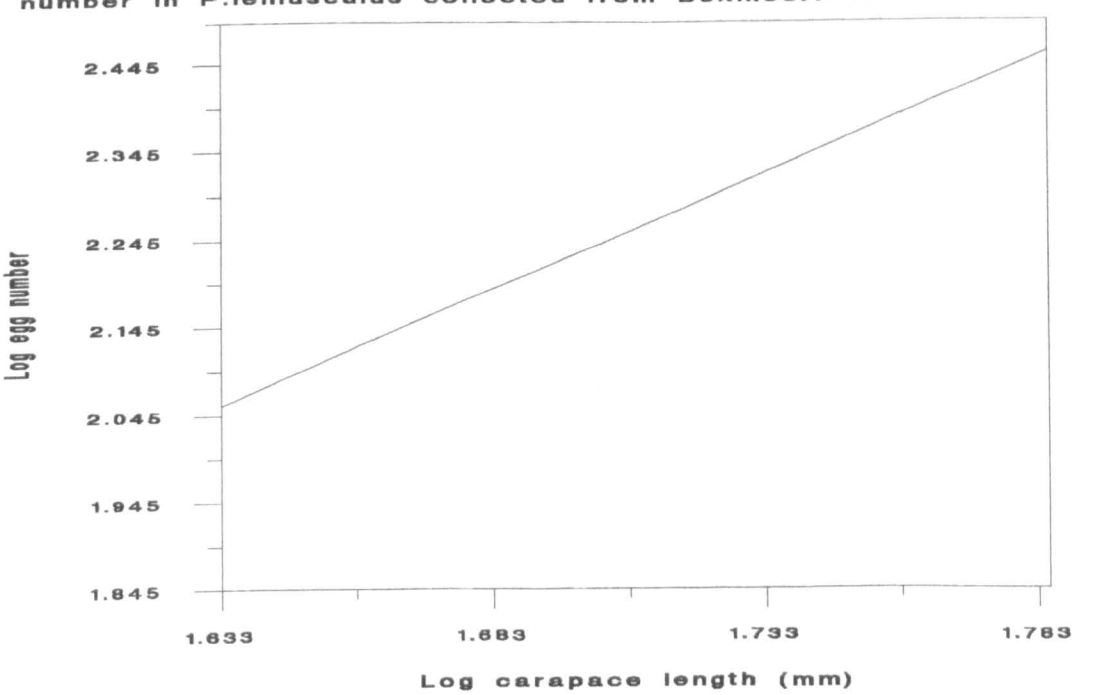


Figure 8.1.3 The relationship between carapace length and egg number in P.lenlusculus collected from Goddesby Brook

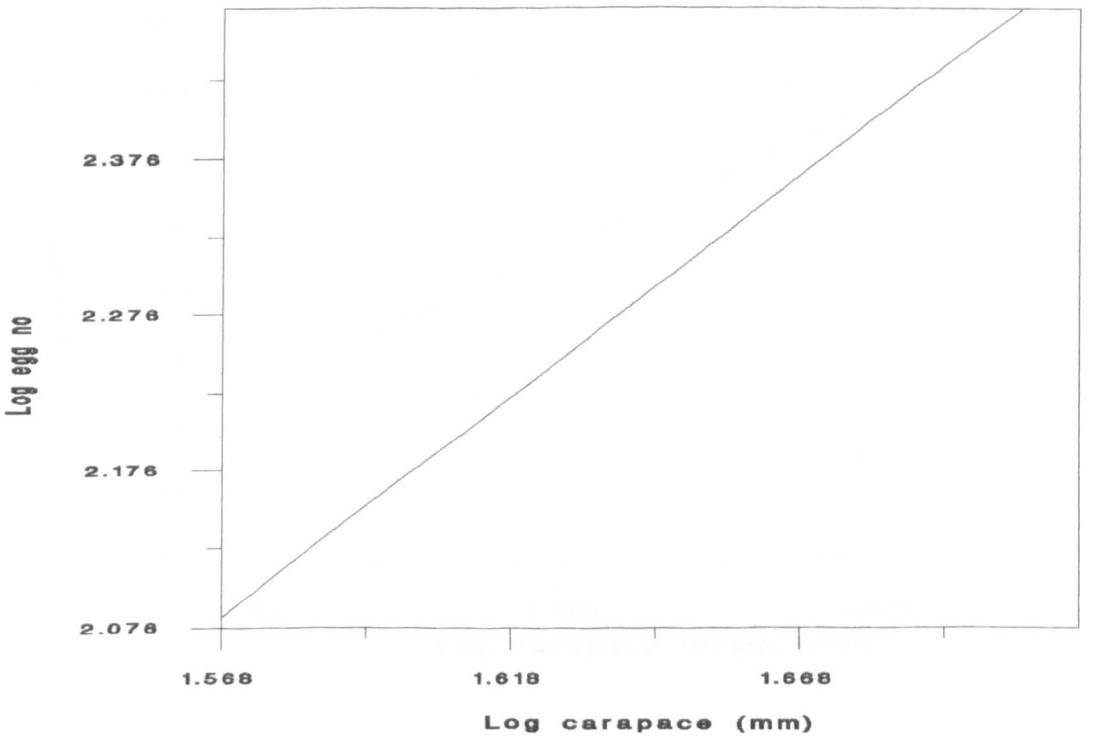


Figure 8.1.4 The relationship between carapace length and egg number in A.leptodactylus collected from the Serpentine

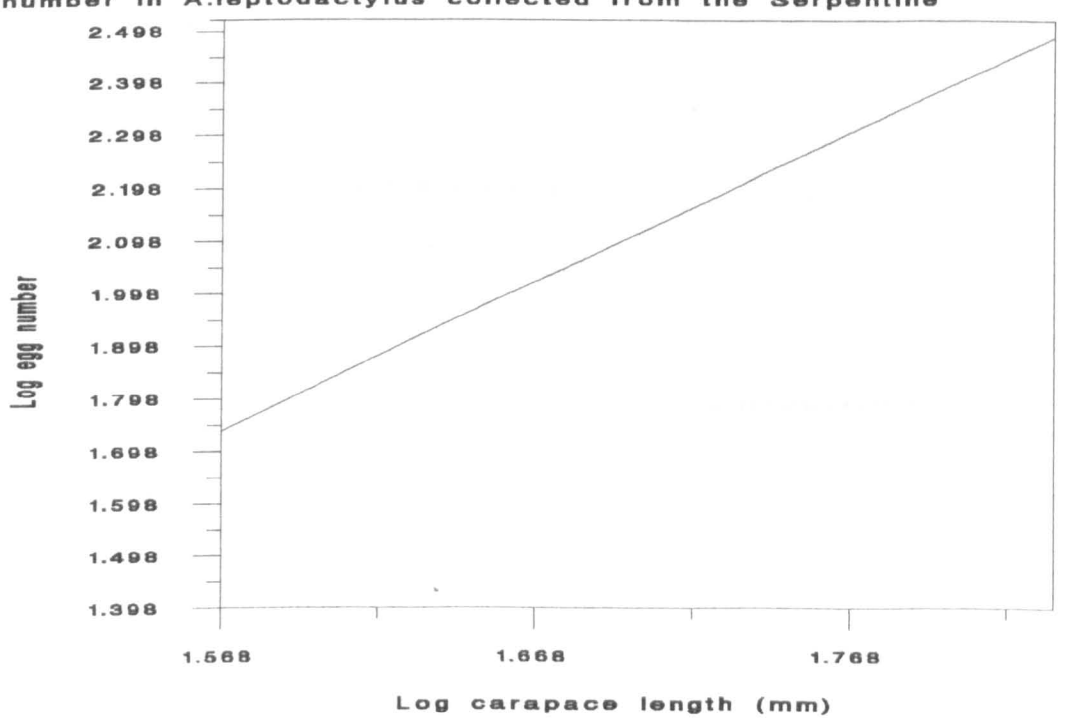


Figure 8.1.5 The relationship between carapace length and egg number in A.leptodactylus collected from Tykes Water

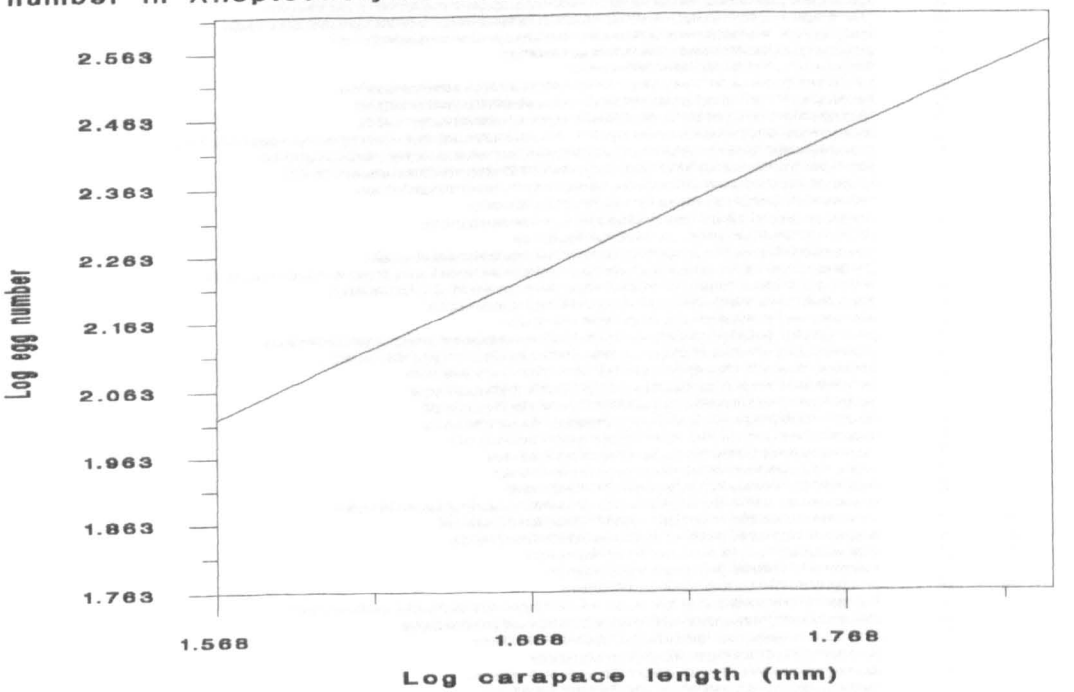


Figure 8.1.6 The relationship between carapace length and number of stage 2 juveniles in P.leniusculus and A.leptodactylus (collected from Dinesens' and Serpentine respectively)

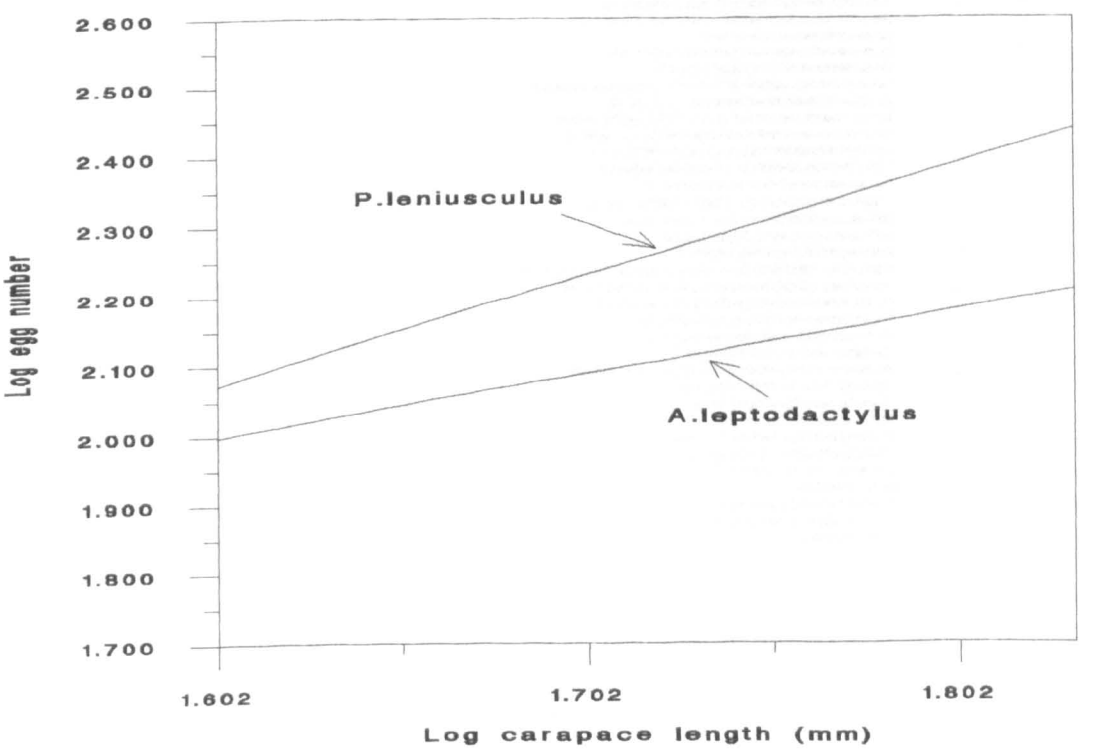
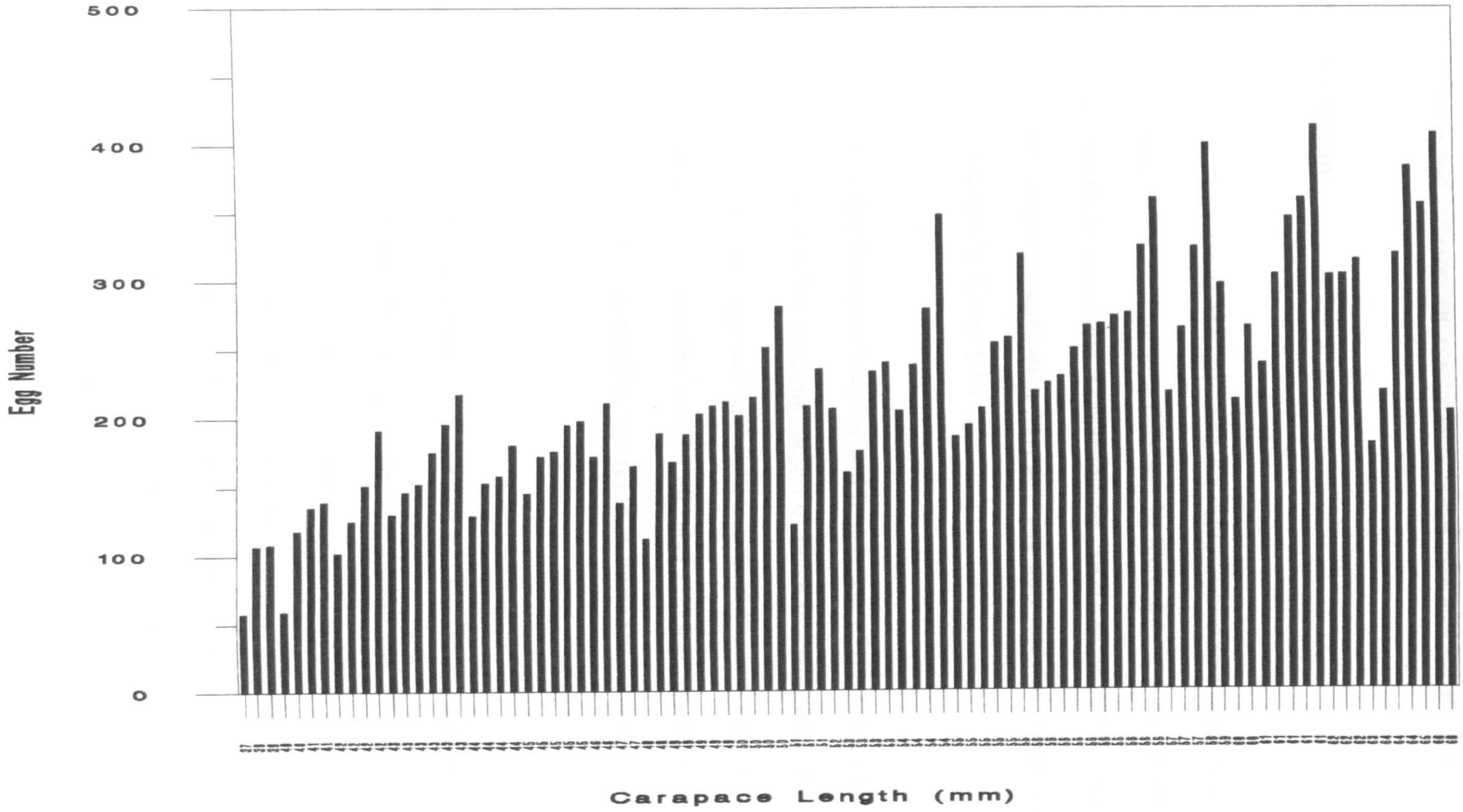


Figure 8.1.7 The number of eggs in A. leptodactylus collected from Tykes Water at the end of the breeding season



8.2 An intra- and interspecific comparison of egg size and female size

8.2.1 Introduction

Egg size is one of the main factors affecting the reproductive efficiency of crayfish. An increase in egg size gives rise to a decrease in fecundity in fish, gammaridean amphipods and similarly in crayfish (Abrahamsson, 1971; Ware, 1975; Steele and Steele, 1991). The negative correlation between egg size and fecundity has been reviewed for crustaceans by Corey (1991).

Although the relationship between fecundity and female size of *Astacus leptodactylus* and *Pacifastacus leniusculus* has been the subject of much investigation (see Chapter 8.1), the relationship between egg size and female size of the two species has been researched very little. This is also the case for all other crayfish species. Only a few studies have been carried out on crayfish species to evaluate the relation between egg size and female size.

Lahti and Lindqvist (1983) found no correlation between female size and egg diameter in *Astacus astacus*. Similarly, in a study on density, growth and reproduction of *A. astacus* and *P. leniusculus* populations in an isolated pond, it was found that there was no relation between female size and egg size in these species (Abrahamsson 1971). In addition to these studies, no correlation was found between the egg weight and female weight of *A. leptodactylus* by Ivanova and Vassilenko (1987). On the other hand, a positive linear relation was found between egg size and carapace size of *P.*

leniusculus and, although individual differences were observed, the largest female *P. leniusculus* produced the largest eggs (Mason 1978b).

The effects of environmental factors (such as food, water quality, predation, parasitism) on egg size have been reported by some workers (Corey, 1991; Huner and Lindqvist, 1991; Wenner *et al.*, 1991). Lower fecundity and small eggs were observed as a result of poor nutrition in the pond culture of *Cherax tenuimanus* (Huner and Lindqvist, 1991). Food availability during the summer when ovarian development occurred and different conditions between populations is thought to have caused variations in egg size in *Astacus astacus* (Lahti and Lindqvist, 1983). Kuris (1991) stated that size structure and density of adult *Orconectes virilis* have an impact on egg size and fecundity. In a long-term study (more than 12 years) on two *O. virilis* populations of northwestern Ontario, Kuris (1991) observed that the females produced greater numbers of smaller eggs in the exploited population than those in the unexploited population.

In order to have an accurate fecundity estimate which is used in the estimation of recruitment, in addition to the relation between egg number and female size, the relation between female size and egg size should also be considered. Therefore, in the present study, the correlation between egg size and female size within *A. leptodactylus* and *P. leniusculus* was studied and the egg size of the species was also compared. In addition, the difference in egg size between approximately four-month-old eggs of two populations of *P. leniusculus* was compared.

8.2.2 Materials and methods

To compare differences in egg size within species and between species approximately ten-day-old, four-month-old, and six-month-old eggs of the two species were measured by use of a light microscope and graticule. Five eggs were removed randomly with forceps from the pleopod of each female. The carapace length of the specimens were also taken. The females of *A. leptodactylus* were collected from Tykes Water (north of London). The females of *P. leniusculus* were collected from Boxmoor Fishery (Hemel Hempstead) and Dinesens' crayfish farm (Hampshire). The number of crayfish used in this experiment is given in Table 8.2.1.

In addition, in order to observe the relation between egg wet weight and female size, five eggs from 66 *A. leptodactylus* were weighed.

The size of the females varied between 39 and 70 mm (carapace length) for the two species.

8.2.3 Results

Differences within species

Regression analysis was carried out on the results. It was found that egg size does not increase with body size in both *P. leniusculus* and *A. leptodactylus* (Table 8.2.1). Figures 8.2.1 and 8.2.2 also show there is no linear relation between the egg size and carapace length of the females in both *P. leniusculus* and *A. leptodactylus*. Polynomial

analyses gave the best relation between the egg size and carapace length of the species (because their r^2 values are still very low the formulae are not given). In addition, no linear relation was found between the egg wet weight and female size in *A. leptodactylus* (Figure 8.2.3). For example, although 11.50 and 11.75 mg mean egg weight were found for the two females 68 mm CL, 15.66 and 16.66 mg mean egg weight were found for those of 46 mm CL.

The eggs of the two species increased significantly in size during the incubation period. There is a significant difference ($P<0.001$, 2 Sample t test) between the egg size of ten-day-old and four-month-old eggs, and between the egg size of four-month-old and six-month-old eggs in both *P. leniusculus* and *A. leptodactylus.* In *P. leniusculus* the mean egg size (diameter) was 2.598 mm for the ten-day-old eggs, 2.807 mm for the four-month-old eggs and 3.034 mm for the six-month-old eggs. Those in *A. leptodactylus* were 2.757, 2.865 mm and 3.333 mm respectively.

No significant difference ($P>0.05$, 2 Sample t test) was found between the egg size of four-month-old eggs of two different populations of *P. leniusculus* (mean egg size= 2.806 mm (SD=0.107) and size range= 2.610-3.074 for Dinesens', and mean egg size= 2.837 mm (SD=0.131) and size range 2.610-3.045 for Boxmoor Fishery respectively.

Differences between species

Astacus leptodactylus has bigger eggs than *P. leniusculus* for ten-day-old, four-month-old, and six-month-old eggs. There is a significant difference between egg size of ten-day-old ($P<0.01$), four-month-old ($P<0.01$), and six-month-old eggs ($P<0.001$, 2 Sample t test) between *P. leniusculus* and *A. leptodactylus.* Mean egg size of the species has been given above, in the comparison of egg size within the species.

8.2.4 Discussion and conclusions

In order to observe whether egg size increases with body size within species, slopes (coefficient value) were investigated. As a result of regression analyses, because slopes are less than three (see Chapter 8.1.4 for explanation) egg size does not increase proportionately with body size in both *P. leniusculus* and *A. leptodactylus*.

This study has shown that there is no relation between female size and egg wet weight of *A. leptodactylus*. This result is similar to that of Ivanova and Vassilenko's (1987).

In contrast to Mason (1978b) no correlation was observed between the egg size and carapace size of *P. leniusculus*, and the largest eggs were not produced by the largest female in the present study. (In the light of this it was decided not to carry out a study of female size and egg wet weight).

The present study showed that the size of *A. leptodactylus* eggs is significantly larger than that of *P. leniusculus*. It has been reported that in crayfish large juveniles which hatch out from larger eggs survive better than small juveniles (Mason, 1978b; Lowery, 1988; Svärdson, 1992). It has also been reported that in fish, benthic marine invertebrates and amphipods, larvae hatching out from large eggs have more adaptive efficiency than those hatching out from small eggs (Steele and Steele, 1975; Ware 1975). It is highly likely that the bigger juveniles of *A. leptodactylus* hatching out from the larger eggs are able to survive better than the smaller juveniles of *P. leniusculus*.

On the other hand, in comparison to the small eggs of *P. leniusculus*, the large eggs of *A. leptodactylus* may bring about a decrease in fecundity. In comparison to the fecundity of *P. leniusculus*, the low fecundity of *A. astacus* due to big eggs was also observed by Abrahamsson (1971) in a study on the density, growth and reproduction of *A. astacus* and *P. leniusculus* in an isolated pond.

In the present study the results showed that in *A. leptodactylus* and *P. leniusculus*, egg size diameter for similar size, even for same sized crayfish is very variable. For example 2.84, 2.90, 3.11, 3.16, 3.33 mm egg size measurements have been seen for *A. leptodactylus* 56 mm in carapace length and 2.75, 2.90 and 2.98 mm egg size measurements for *P. leniusculus* 57 mm in carapace length. Similarly, Huner and Lindqvist (1991) showed that a difference in egg size of two crayfish of the same length causes a difference in their reproductive output. In conclusion, before producing regression models to estimate fecundity for *A. leptodactylus* and *P. leniusculus*, biologists should be aware that there is not a good correlation between female size and egg size in *A. leptodactylus* and *P. leniusculus* due to the fact that the egg size of the two species is very variable, even for the same size crayfish.

The "r" and "K" terms are used in order to characterise differences between species (Price and Payne, 1984). The r-selected crayfish species grows rapidly, produces a high number of small eggs, and has less organic reserves and a short incubation time. For example, *P. clarkii*, which has eggs approximately 2 mm in diameter. A large female can produce more than 600 ovarian eggs. The K-selected species grows slowly, produces a low number of large eggs, has more organic reserves and a long incubation time. For example, *Cambarus robucus*, which has eggs approximately 3 mm in

diameter (Wear, 1974; Brinck, 1977; Huner and Lindqvist 1991; Steele and Steele, 1991). A positive relation has been observed between the egg size and duration of egg development in copepods, barnacles, isopods, cumaceans and decapods. (Steele and Steele, 1975). In this study, it was found that *A. leptodactylus* has larger eggs than *P. leniusculus*. It seems that the large eggs of *A. leptodactylus* need more time to develop than those of *P. leniusculus*. Therefore, *A. leptodactylus* is closer than *P. leniusculus* to be considered as a K-selected species.

Table 8.2.1 Regression, slopes (coefficient value) and polynomial analyses of carapace length plotted against egg size

	Log y	r^2 (simple regression)	slopes	r^2 (polynomial regression)
P. leniusculus six-month-old-eggs n=64	0.18823 + 0.16937(logx)	0.13	0.16	0.23
P. leniusculus four-month-old eggs n=52	0.36625 + 0.04810(logx)	0.02	0.04	0.09
P. leniusculus ten-day-old eggs n=37	-0.20255 + 0.35928(logx)	0.24	0.35	0.31
A. leptodactylus six-month-old-eggs n=65	0.25241 + 0.15476(logx)	0.10	0.15	0.28
A. leptodactylus four-month-old eggs n=61	0.09176 + 0.21179(logx)	0.16	0.21	0.26
A. leptodactylus ten-day-old eggs n=66	0.06996 + 0.21637(logx)	0.17	0.21	0.25

Figure 8.2.1. The relationship between ten-day-old, four-month-old, and six-month-old egg size and female carapace length of A.leptodactylus.

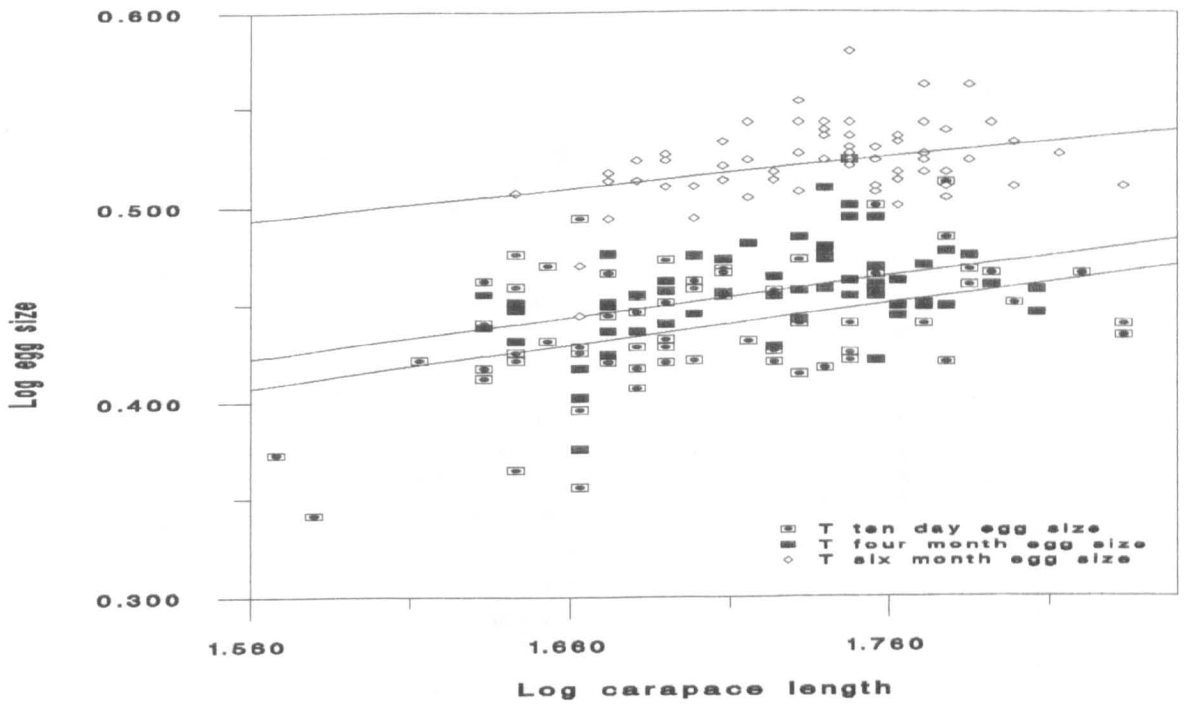


Figure 8.2.2. The relationship between ten-day-old, four-month-old, and six-month-old egg size and female carapace length of P.leniusculus.

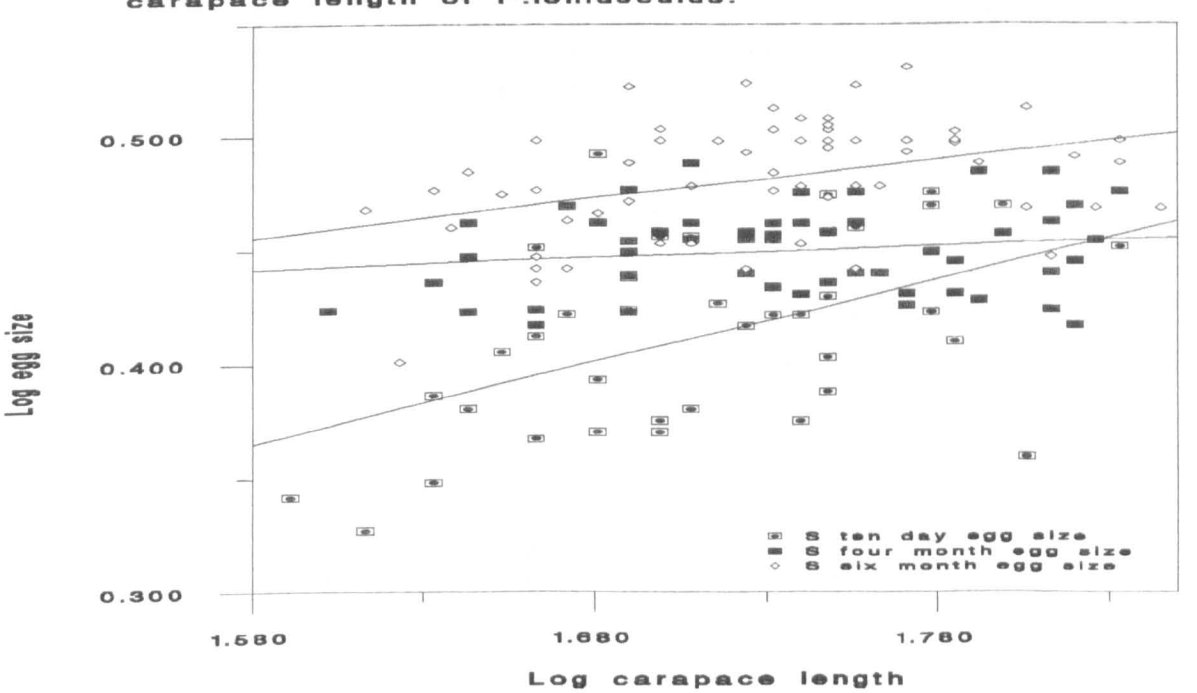
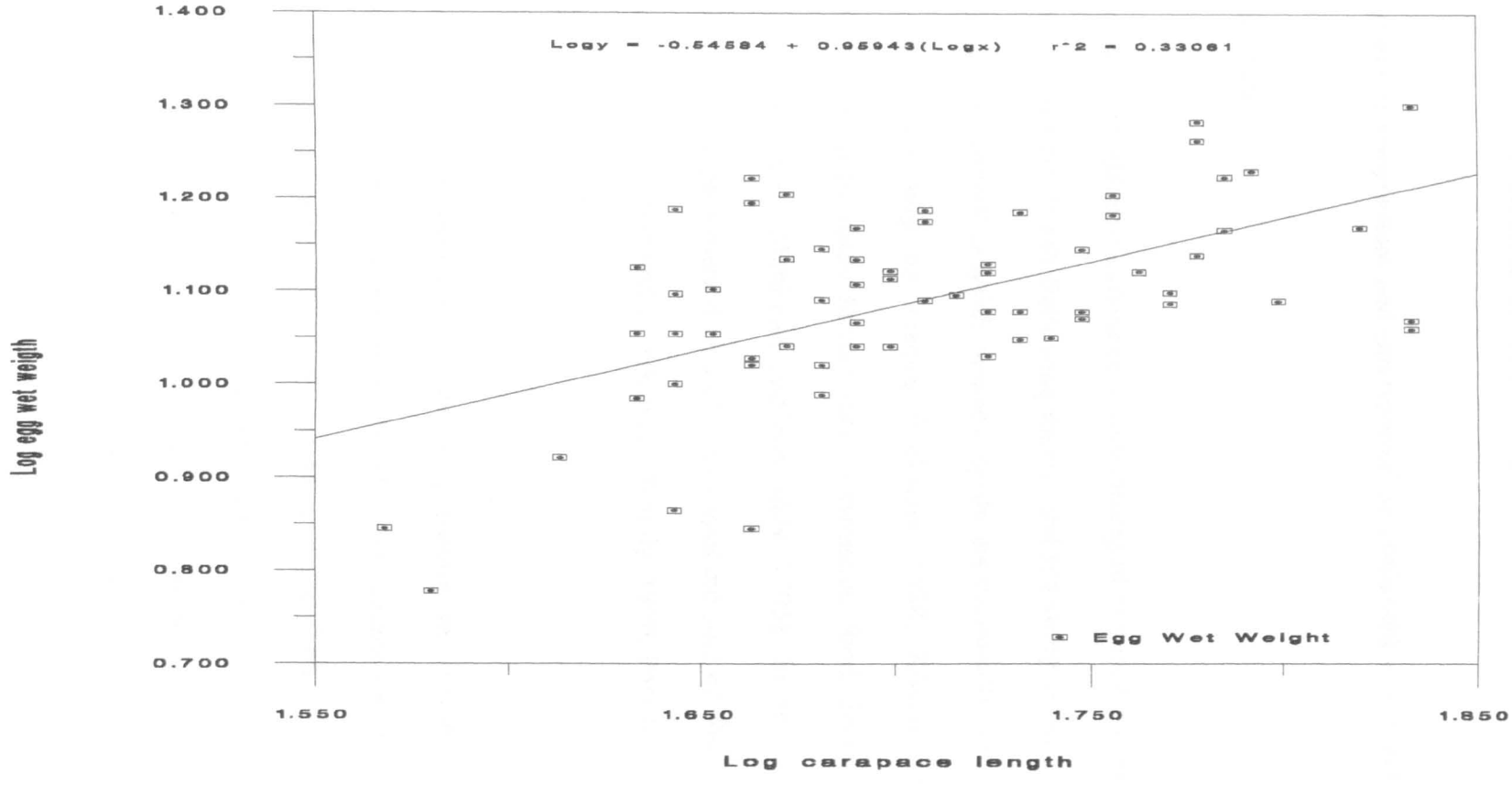


Figure 8.2.3. The relationship between ten-day-old egg wet weight and carapace length of A.leptodactylus



Chapter 9

The effect of temperature and photoperiod on pleopodal egg development and the occurrence of twins in signal crayfish

9.1 The effect of temperature and photoperiod on pleopodal egg development

9.1.1 Introduction

In astacid crayfish reproduction commences with mating of mature females and males in early autumn (Cukerzis, 1988). Following mating and spawning in nature pleopodal egg development of astacid crayfish species needs approximately seven or eight months to hatch depending on species (Cukerzis, 1988; Köksal, 1988). Egg development is affected by many factors such as parasites, food, density, pollution (Corey, 1991; Kuris, 1991; Matthews and Reynolds, 1995). In addition to these factors, temperature and photoperiod also play an important role on the reproductive cycle of crayfish (Aiken, 1969a and b; Aiken and Waddy, 1990; Portalance and Dube, 1995; Yeh and Rouse, 1995).

An increase of water temperature accelerates spawning in *Orconectes immunis* (Momot, 1988), *Orconectes virilis* (Aiken, 1969b), *Orconectes limosus* (Dube and Portlance, 1992) and *Cherax quadricarinatus* (Rouse and Yeh, 1995). However, spawning occurs with the decline of water temperature in astacid crayfish species (Cukerzis, 1988; Köksal, 1988; Laurent, 1988; Lowery and Holdich, 1988). Although spawning was found to be less intensive in *Astacus astacus* exposed to shortened daylength (Huner and Lindqvist, 1985), Provenzano and Handwerker (1995) found that

the highest percentage of *Procambarus clarkii* spawned in constant darkness. In addition, Dube and Portlance (1992) found that a long photoperiod did not promote earlier spawning of *Orconectes limosus*. The effect of photoperiod on the ovarian development of crayfish is also variable between species. No ovarian development occurs in *O. virilis* in constant darkness (Stephens in Fingerman, 1995). Although ovarian development is more rapid at longer daylengths in *Procambarus simulans* (Provenzano and Handwerker, 1995), short days (8.5 L : 15.5 D) cause a more rapid increase of ovarian development in *Orconectes nais* than do long days (15 L: 9 D) (Armitage *et al.* in Provenzano and Handwerker, 1995).

In the management of culturing crayfish it is feasible to produce juveniles earlier than normal in order to stock natural waters at the end of spring with larger individuals (Rhodes, 1981; Westin and Gydemo, 1986; Celada *et al.*, 1988). Because of the fact that crayfish eggs develop faster at higher temperatures (King, 1993; Portelance and Dube, 1995) a number of artificial temperature regimes and incubation techniques have been used to induce early hatching of juveniles (for *Pacifastacus leniusculus* by Mason, 1977b; Carral *et al.*, 1988 & 1992, for *Astacus astacus* by Strempel, 1973; Cukerzis *et al.*, 1978; Westin and Gydemo, 1986; Hessen *et al.*, 1987; Cukerzis, 1988, for *Astacus leptodactylus* by Köksal, 1988; for *Cherax quadricarunatus* by King, 1993; for *Austropotamobius pallipes* by Rhodes, 1981; Matthews and Reynolds, 1995). For example, the incubation period of *A. astacus* has been reduced from seven-eight months to three-four months by Cukerzis *et al.* (1978) and Westin and Gydemo (1986) and reduced to five months by Hessen *et al.* (1987). Similarly, the incubation period of *A. pallipes* has been shortened to early April, approximately two months before hatching occurs in nature (Matthews and Reynolds, 1995). Köksal (1988) states that

a temperature acclimation of 16-18 °C is optimum to have juveniles as early as January or February. The juveniles of *P. leniusculus* have been obtained between late January and early February, approximately four months earlier than in natural conditions (see Cabantous, 1975; Goldman *et al.*, 1975; Mason, 1977a; Strempel, 1975; Westman, 1975; Carral *et al.*, 1992).

According to Cukerzis (1988) there is three or four months of diapause in egg development of *A. astacus* between the formation of the blastoderm and the emergence of the entomesodermal embryo and during this diapause almost no development occurs in the eggs. Cukerzis *et al.* (1978) and King (1993) also state that temperate species may need a cold temperature for proper egg development. In order to see whether this temperature sequence is necessary for egg development in *P. leniusculus* and *A. leptodactylus* and whether it is possible to induce early hatching of juveniles under artificial conditions, a number of experiments have been carried out with berried females at 5, 13, 15, 17 and 21 °C. In addition, the effect of the light and darkness at different temperatures on the pleopodal egg development of the two species has also been observed.

9.1.2 Materials and methods

Prior to the experiments each species was maintained in separate outdoor tanks (3.43 m^2). Water was supplied via a sprinkler from the mains supply. Water temperature of the tanks during the incubation period of the species is given in Figure 9.1.1. Water temperature was recorded by means of a "Tiny Talk" datalogger (Orion Components Ltd.), the readings subsequently being off-loaded into a PC computer. The time taken

for egg development in these was used as a control. For example, the mean monthly water temperatures of the tanks were approximately 13 °C in October, 10 °C in December (939 degree-days), but during these months the experimental temperatures in the first experiment were 17 or 13 °C continuously (1564 or 1196 degree-days respectively). The term "degree-days" means degrees Celsius x days.

Mating and egg-laying occurred during the first ten days of October in *P. leniusculus* and during the first ten days of December in *A. leptodactylus* in the outdoor tanks.

In the experiments each female was put in a glass container (31 cm x 17 cm x 18 cm). Crayfish were fed every two days with minced morsels and *Cladophora*. Water was aerated and was changed weekly. Crayfish were kept under 12 L : 12 D light regime in the first and second experiments.

Effect of ambient temperature on pleopodal egg development

Twenty berried *P. leniusculus* and 20 berried *A. leptodactylus* were used. Five of each species were transferred to 13, 17 and 21 °C in a constant temperature room (on 28.10.93 for *P. leniusculus* and 13.12.93 for *A. leptodactylus*) and five of each species were kept under natural temperature conditions in the outdoor tanks as controls.

Effect of cold shock on pleopodal egg development

Ten berried *P. leniusculus* and ten berried *A. leptodactylus* were used. Five of each species were maintained at 5 and 15 °C in a constant temperature room on 08.02.94

(these crayfish had been kept in the outdoor tanks until 08.02.94 after the mating). After three weeks, crayfish maintained at 5 °C were transferred to 15 °C (on 02.03.94).

Effect of photoperiod on pleopodal egg development

Sixteen berried *P. leniusculus* and 16 berried *A. leptodactylus* were used.

Four of each species were kept under 12 L : 12 D and 0 L : 24 D photoperiod in a constant temperature room, 13 °C. This experiment was also carried out at 15 °C.

Experiments at both temperatures were started with *A. leptodactylus* on 11.01.95 and with *P. leniusculus* on 16.02.95. Before the experiments both species had been kept under natural temperature conditions in the outdoor tanks.

9.1.3 Results

Effect of ambient temperature on pleopodal egg development

In both species transfer to higher than ambient temperatures caused earlier hatching as compared to the control (for outside tanks' temperature conditions see Figure 9.1.1) except crayfish transferred to 21 °C. In that temperature berried females of both *P. leniusculus* and *A. leptodactylus* lost their eggs four days after transfer, but at 13 and 17 °C the time of incubation of the two species was reduced significantly ($P<0.01$, Kruskal-Wallis test).

The time of hatching in *P. leniusculus* and *A. leptodactylus* transferred to 13, 17 °C and natural conditions (control) is given in Table 9.1.1.

Although incubation took 198-205 days (1670-1755 degree-days) in *P. leniusculus* and 182-189 days (1668-1784 degree days) in *A. leptodactylus* under natural conditions, incubation took only 77-90 day (1237-1458 degree-days) at 17 °C and 113-127 (1469-1651) at 13 °C in *P. leniusculus*. Those in *A. leptodactylus* were 85-92 (1416-1535 degree-days) and 114-121 (1465-1556 degree-days) respectively.

A comparison of the incubation period between crayfish transferred to 13, 17 °C and kept under natural conditions in *P. leniusculus* and *A. leptodactylus* is given in Figure 9.1.2.

Effect of cold shock on pleopodal egg development

Similar to the first experiment temperature acclimation caused early hatching in both species as compared to natural conditions (control). As mentioned above although incubation took 198-205 days in *P. leniusculus* and 182-189 days in *A. leptodactylus* under natural conditions, in the present experiment incubation took only 153-156 days (1496-1541 degree-days) in *P. leniusculus* and 129-133 days (1407-1467 degree-days) in *A. leptodactylus* transferred to 15 °C.

Time of hatching in *P. leniusculus* and *A. leptodactylus* transferred to 15 °C, and maintained at 5 °C for three weeks and were transferred to 15 °C is given in Table 9.1.2.

First hatching was observed 32 days after transfer to 15 °C in *P. leniusculus* and 69 days after transfer to 15 °C in *A. leptodactylus*. However, hatching was postponed approximately three weeks in both species transferred to 15 from 5 °C as compared to crayfish maintained directly at 15 °C. The postponement of hatching was highly significant for both species ($P<0.01$, Kruskal-Wallis test).

A comparison of the incubation period between crayfish transferred to 15 °C and transferred to 15 °C from 5 °C, and kept under natural condition in *P. leniusculus* and *A. leptodactylus* is given in Figure 9.1.3. It should be noted that the reason crayfish apparently took longer to develop at 15 °C (see Figure 9.1.2) compared to 13 °C (see Figure 9.1.3) is due to the fact that those at 15 °C were brought in from outside later (i.e. February) than those at 13 °C (i.e. October and December) for *P. leniusculus* and *A. leptodactylus* respectively.

Effect of photoperiod on pleopodal egg development

Light regime did not affect pleopodal egg development of *P. leniusculus* and *A. leptodactylus* at both temperatures. Hatching occurred over a similar time period in both photoperiod regimes in the two species.

The time of hatching in *P. leniusculus* and *A. leptodactylus* kept under 12 L : 12 D and 0 L : 24 D photoperiod at 13 and 15 °C is given in Table 9.1.3.

In *P. leniusculus* hatching occurred between 36-39 days after transfer to 0 L : 24 D and between 37-40 days after transfer to 12 L : 12 D at 13 °C. Those at 15 °C were

between 26-27 and 26-29 days respectively.

In *A. leptodactylus* hatching occurred 100-102 days after transfer to 0 L : 24 D and between 99-104 days after transfer to 12 L : 12 D at 13 °C. Those at 15 °C were between 61 and 68 and between 62-68 respectively.

No mortality was observed in berried females and no abnormality was observed in the hatching of stage 1 juveniles in the three experiments.

9.1.4 Discussion and conclusions

The results reveal that pleopodal egg development of *P. leniusculus* and *A. leptodactylus* can be reduced significantly ($P<0.01$) from seven months to three months when they are transferred to 17 °C at the beginning of the incubation period. The results also reveal that it may be possible to obtain two reproductive cycles of *P. leniusculus* in a year, i.e. in April and December. However, because of the fact that under natural conditions the females of *A. leptodactylus* spawn approximately two months later than those of *P. leniusculus*, the juveniles of *A. leptodactylus* hatch approximately two months later when they are exposed to similar conditions after spawning.

Following mating and spawning, although *P. leniusculus* and *A. leptodactylus* were not subjected to a cold temperature period, the egg development of the two species was normal when they were transferred to 13, 15 and 17 °C. However, low temperatures during the incubation period postponed hatching of juveniles in both

species. After a three week period of cold temperature transfer (5 °C) hatching was postponed by approximately three weeks in both *P. leniusculus* and *A. leptodactylus*. From these experiments it is clear that a cold-warm temperature sequence is not necessary for egg development in *P. leniusculus* and *A. leptodactylus*. The time of egg development in both species decreases as a consequence of shortening the cold temperature period by increasing temperature. Similarly, Suko (1956) found that the winter eggs of *P. clarkii* develop at temperatures above 5.6 °C, but no less than 5.2 °C. Celada *et al.* (1988) also found that a period of low temperature during incubation period improves reproductive efficiency but postpones hatching of juveniles.

The results show that a temperature of 21°C has detrimental effects on pleopodal egg development. The females of *P. leniusculus* and *A. leptodactylus* are not able to keep their eggs when they are exposed to 21 °C. Similarly, temperatures over 30 °C have been found to have a detrimental effect on egg development of *Procambarus* (Provenzano and Handwerker, 1995). In another study, the transfer of *A. pallipes* to 18 °C resulted in complete loss of pleopodal eggs (Rhodes, 1981). He concluded that temperature transfer had an adverse effect on the egg attachment substance or female behaviour.

In addition to the effect of photoperiod on ovarian egg development and spawning, photoperiod also has an effect on moulting of crayfish. According to Patrica and Armitage (1974) constant darkness and constant light inhibit moulting in crayfish. The highest mortality occurred in *Orconectes virilis* exposed to the longest light period (Stephens, 1955). Although Taugbol and Skurdal (1995) and Westin and Gydemo (1986) found that photoperiod has no effect on successful moulting of *A. astacus*,

differences were observed in the number of moults in *Orconectes rusticus* when it was exposed to different photoperiods (Sadewasser and Prins, 1979). In another study, longer daylength caused significantly more moults in *Orconectes nais* (Armitage *et al.*, 1973) and *Orconectes virilis* (Stephens in Patrica and Armitage, 1974).

The present study shows that photoperiod is not a factor affecting pleopodal egg development of *P. leniusculus* and *A. leptodactylus*. Although a number of studies have been carried out on the effect of photoperiod on moulting, ovarian egg development and spawning there is only one study on the effect of photoperiod on pleopodal egg development of crayfish to compare with the result of the present study, i.e. Cukerzis *et al.* (1978) who also found no significant difference in the pleopodal egg development of *A. astacus* maintained at constant dark and light regimes.

Manipulation of incubation in crayfish is carried out in two ways: (i) berried females are transferred to higher temperatures; (ii) eggs are stripped from berried females and are maintained in incubators. The results show that the former method is very feasible when berried *P. leniusculus* and *A. leptodactylus* are exposed to the above ambient experimental temperatures (13, 15 and 17 °C). In those temperatures no egg loss of females was observed in the current experiments. However, a number of eggs have been lost when the eggs were stripped from the females for use in artificial incubators (up to 50% in *A. astacus* by Cukerzis (1988), 10-27% in *P. leniusculus* by Mason (1977b) and 41% by Carral *et al.* (1992), 28% in *A. pallipes* by Rhodes (1981). Therefore, Gonzales *et al.* (1993) stated that eggs should not stripped until advanced phases of egg development. Consequently, this has limited the use of artificial

incubation in crayfish culture.

Although stage 2 juveniles of crayfish have been obtained three or four months earlier than natural conditions in a number of studies, no studies have focused on the maintenance of these juveniles while low temperature and lack of food occur in nature at that time of year. Consequently, in the present study a number of survival and growth experiments have been carried out with such stage 2 juveniles (see Chapter 11).

Table 9.1.1 Time of hatching in *P. leniusculus* and *A. leptodactylus* transferred to 13, 17 °C and kept under natural conditions during the incubation period

	Transfer	Hatching	Degree-day
Crayfish transferred to 13 °C			
P. leniusculus replicates			
1	28.10.93	31.01.94	1469
2	28.10.93	31.01.94	1469
3	28.10.93	03.01.94	1508
4	28.10.93	06.01.94	1547
5	28.10.93	14.02.94	1651
A. leptodactylus replicates			
1	13.12.93	02.04.94	1465
2	13.12.93	03.04.94	1478
3	13.12.93	06.04.94	1517
4	13.12.93	06.04.94	1517
5	13.12.93	09.04.94	1556
Crayfish transferred to 17 °C			
P. leniusculus replicates			
1	28.10.93	26.12.93	1237
2	28.10.93	04.01.94	1390
3	28.10.93	06.01.94	1424
4	28.10.93	07.01.94	1441
5	28.10.93	08.01.94	1458
A. leptodactylus replicates			
1	13.12.93	01.03.94	1416
2	13.12.93	02.03.94	1433
3	13.12.93	06.03.94	1501
4	13.12.93	08.03.94	1535
5	13.12.93	08.03.94	1535
Crayfish kept under natural conditions during the incubation period			
P. leniusculus replicates			
1	10.10.93	26.04.94	1670
2	10.10.93	27.04.94	1681
3	10.10.93	27.04.94	1681
4	10.10.93	29.04.94	1701
5	10.10.93	03.05.94	1755
A. leptodactylus replicates			
1	10.12.93	10.06.94	1668
2	10.12.93	12.06.94	1701
3	10.12.93	12.06.94	1701
4	10.12.93	16.06.94	1767
5	10.12.93	17.06.94	1784

Table 9.1.2 Time of hatching in *P. leniusculus* and *A. leptodactylus* transferred to 15 °C, and maintained at 5 °C for three weeks and were transferred to 15 °C

Crayfish transferred to 15 °C			
	Transfer	Hatching	Degree-day
P. leniusculus replicates			
1	08.02.94	12.03.94	1496
2	08.02.94	12.03.94	1496
3	08.02.94	12.03.94	1496
4	08.02.94	12.03.94	1496
5	08.02.94	15.03.94	1541
A. leptodactylus replicates			
1	08.02.94	19.04.94	1407
2	08.02.94	19.04.94	1407
3	08.02.94	20.04.94	1422
4	08.02.94	22.04.94	1452
5	08.02.94	23.04.94	1467
Crayfish maintained at 5 °C for three weeks and were transferred to 15 °C			
P. leniusculus replicates			
1	08.02.94	04.04.94	1616
2	08.02.94	04.04.94	1616
3	08.02.94	04.04.94	1616
4	08.02.94	04.04.94	1616
5	08.02.94	06.04.94	1646
A. leptodactylus replicates			
1	08.02.94	05.05.94	1437
2	08.02.94	07.05.94	1467
3	08.02.94	08.05.94	1482
4	08.02.94	09.05.94	1497
5	08.02.94	15.05.94	1587

Table 9.1.3 Time of hatching in *P. leniusculus* and *A. leptodactylus* kept under 0 L : 24 D and 12 L : 12 D photoperiod at 13 and 16 °C

		Time of hatching	
		Transfer to 13 °C	
Species	Transfer date	0 L : 24 D	12 L : 12 D
P. leniusculus replicates			
1	16.02.95	23.03.95	24.03.95
2	16.02.95	23.03.95	25.03.95
3	16.02.95	25.03.95	25.03.95
4	16.02.95	26.03.95	27.03.95
A. leptodactylus replicates			
1	11.01.95	21.04.95	19.04.95
2	11.01.95	21.04.95	21.04.95
3	11.01.95	21.04.95	21.04.95
4	11.01.95	22.04.95	24.04.95
		Transfer to 15 °C	
P. leniusculus replicates			
1	16.02.95	13.03.95	13.03.95
2	16.02.95	13.03.95	13.03.95
3	16.02.95	13.03.95	14.03.95
4	16.02.95	14.03.95	16.03.95
A. leptodactylus replicates			
1	11.01.95	13.03.95	14.03.95
2	11.01.95	13.03.95	15.03.95
3	11.01.95	18.03.95	19.03.95
4	11.01.95	20.03.95	20.03.95

Figure 9.1.1 Mean monthly water temperatures of the outside tanks during incubation period of crayfish

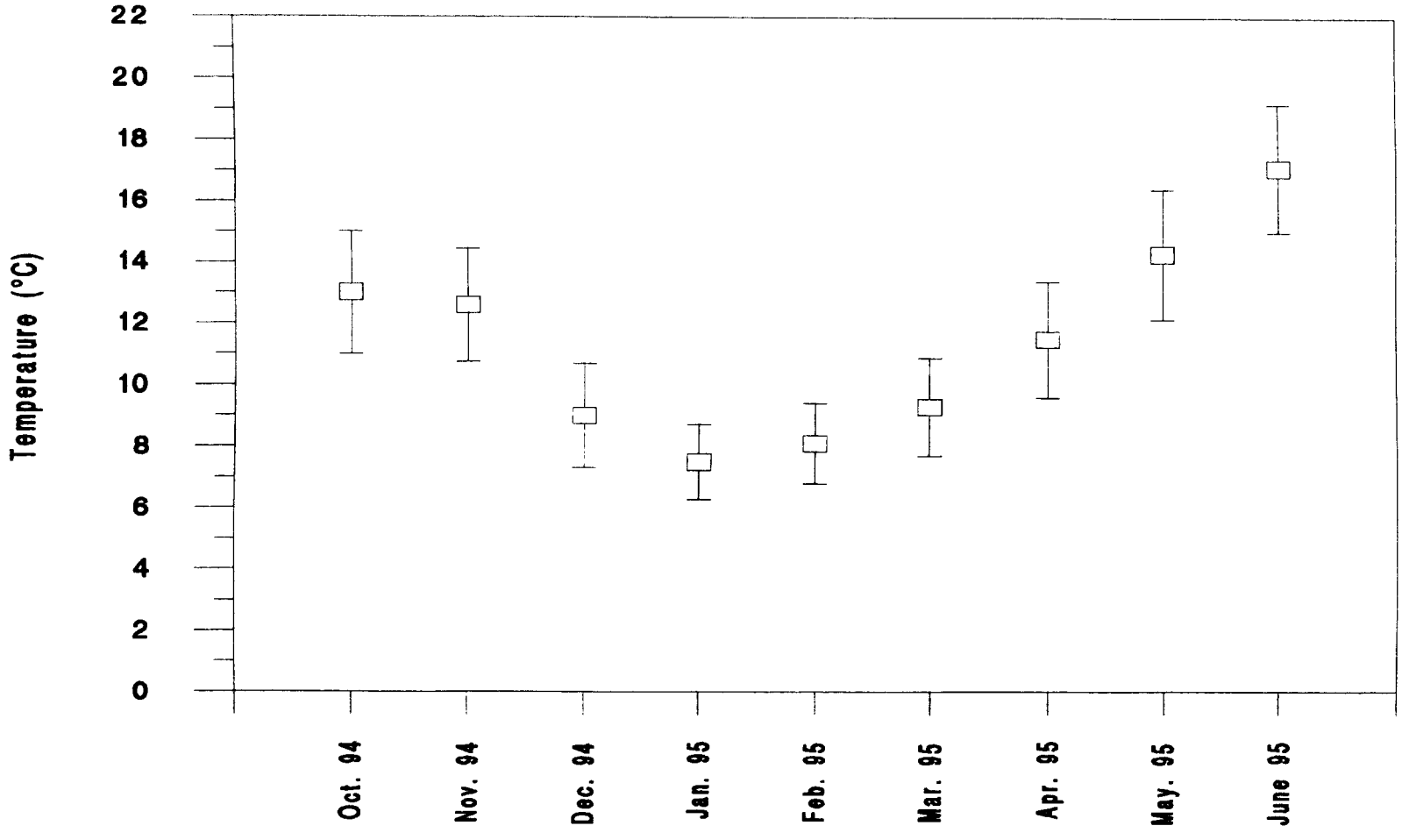


Figure 9.1.2 A comparison of mean incubation period (days) between crayfish transferred to 13, 17 °C and kept under natural conditions in P. leniusculus and A. leptodactylus. Values are means with standard deviations.

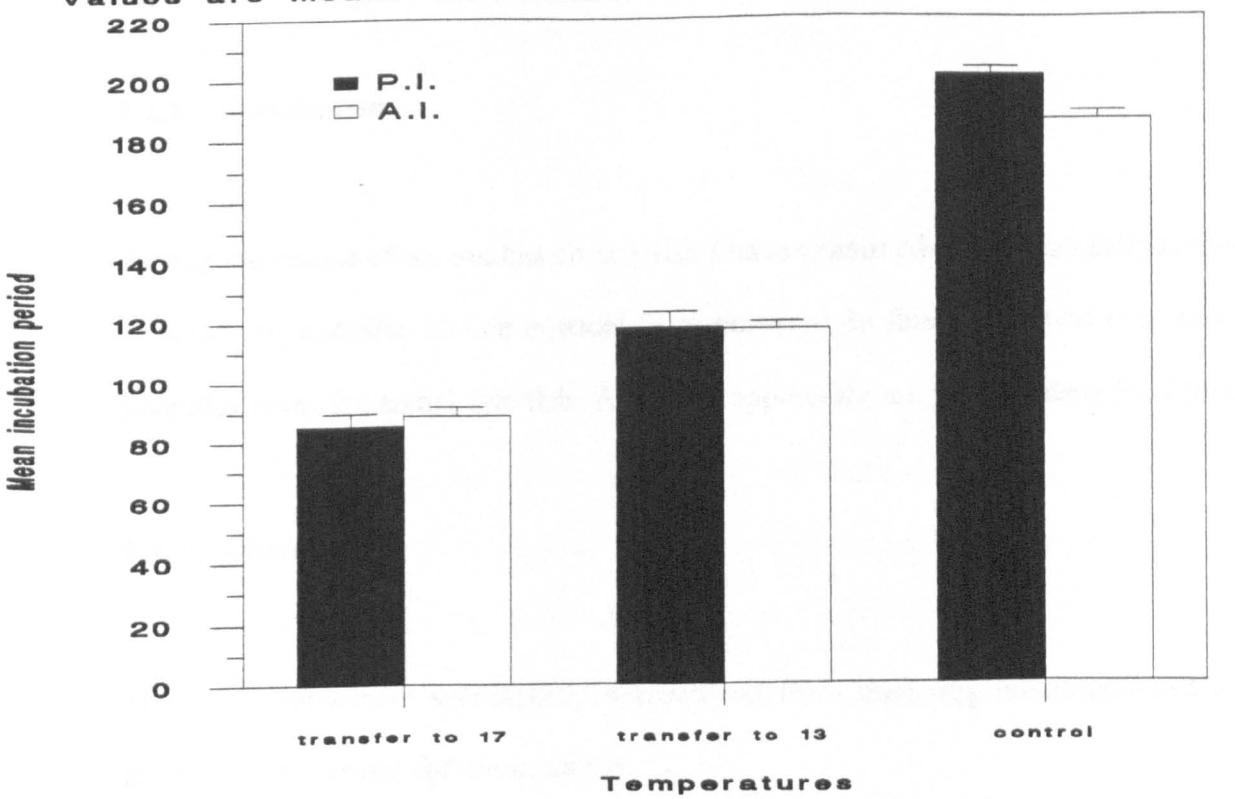
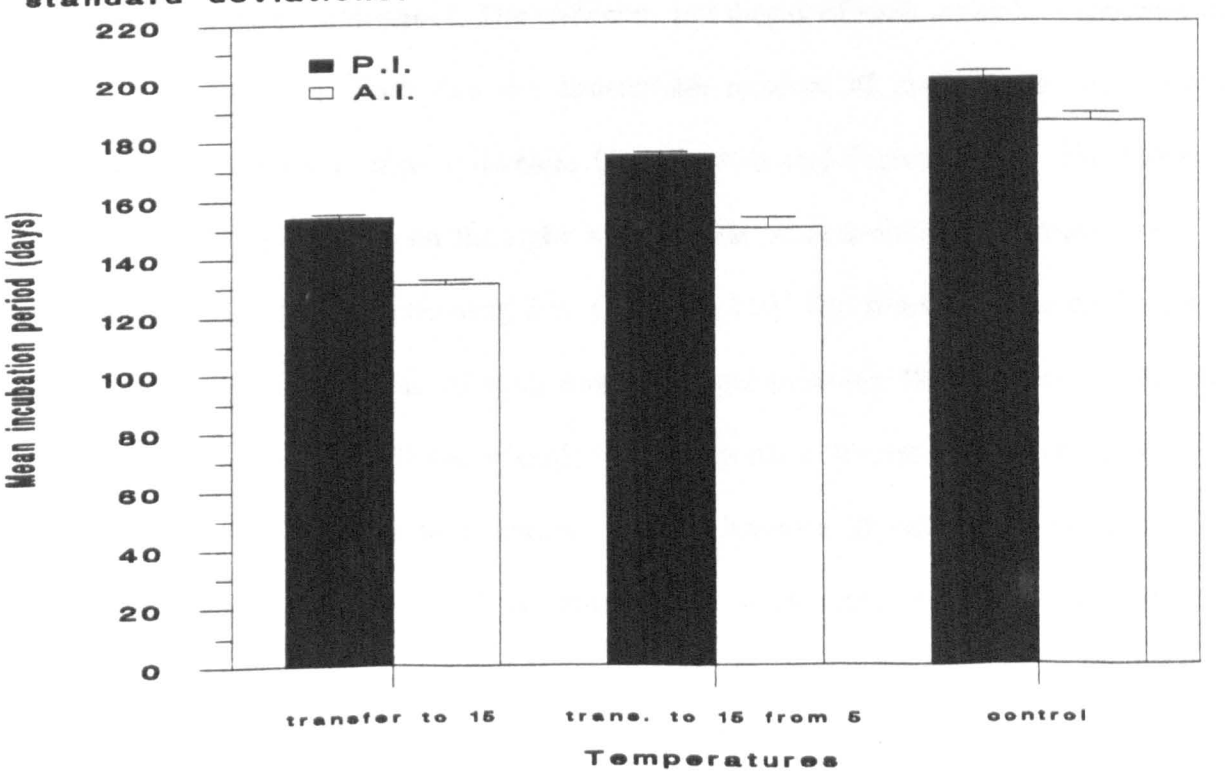


Figure 9.1.3 A comparison of mean incubation period (days) between crayfish transferred to 15 °C and transferred to 15 °C from 5 °C, and kept under natural conditions in P. leniusculus and A. leptodactylus. Values are means with standard deviations.



9.2 The occurrence of twins in *Pacifastacus leniusculus*

9.2.1 Introduction

During the course of my studies on crayfish I have examined many eggs and juveniles. Only on one occasion has an unusual form occurred. In this case it was twin stage 1 juveniles from the signal crayfish. As this is apparently unique it is described below.

9.2.2 Observations

The twins apparently successfully hatched out from their egg membrane and were alive until preserved for examination..

The twins are shown in Figures 9.2.1-9.2.2. They consist of two stage 1 juveniles which are fused at the head. The abdomen and thorax of each juvenile is separate and twisted outwards. Each has the appropriate number of appendages and a telson characteristic of a stage 1 juvenile (see Holdich and Reeve, 1988). The thorax is covered by a carapace on the right and left side of each individual respectively. On their other sides the gills hang free (Figure 9.2.2). The front part is actually single, there being only one pair of eyes, antennules and antennae. However, each individual has a mouth and maxillules, maxillae and three pairs of maxillipeds. The carapace is single and swollen as in a normal stage 1 juvenile. It covers the parts it would normally cover, i.e. the head and thorax down to its junction with the abdomen.

9.2.3 Discussion

No observations such as this one have been located in the literature.

Development in crayfish is epimorphic, i.e. it takes place within the egg and what hatches out is similar to an adult. This is thought to be an adaptation to freshwater in order to stop larvae getting swept downstream (Kaestner, 1970). The formation of the twin must have happened fairly early on in development, i.e. when the abdomo-thorax was forming (see Zender, 1934; Celada *et al.*,1991). However, the formation of the two individuals appears to have been halted by the formation of the carapace which develops dorsally from the mandibular somite (Schmitt, 1973). This corresponds with the fact that up to and including the maxillules all the appendages are double for each individual, but are single after that.

Figure 9.2.1 Dorsal view of the twins

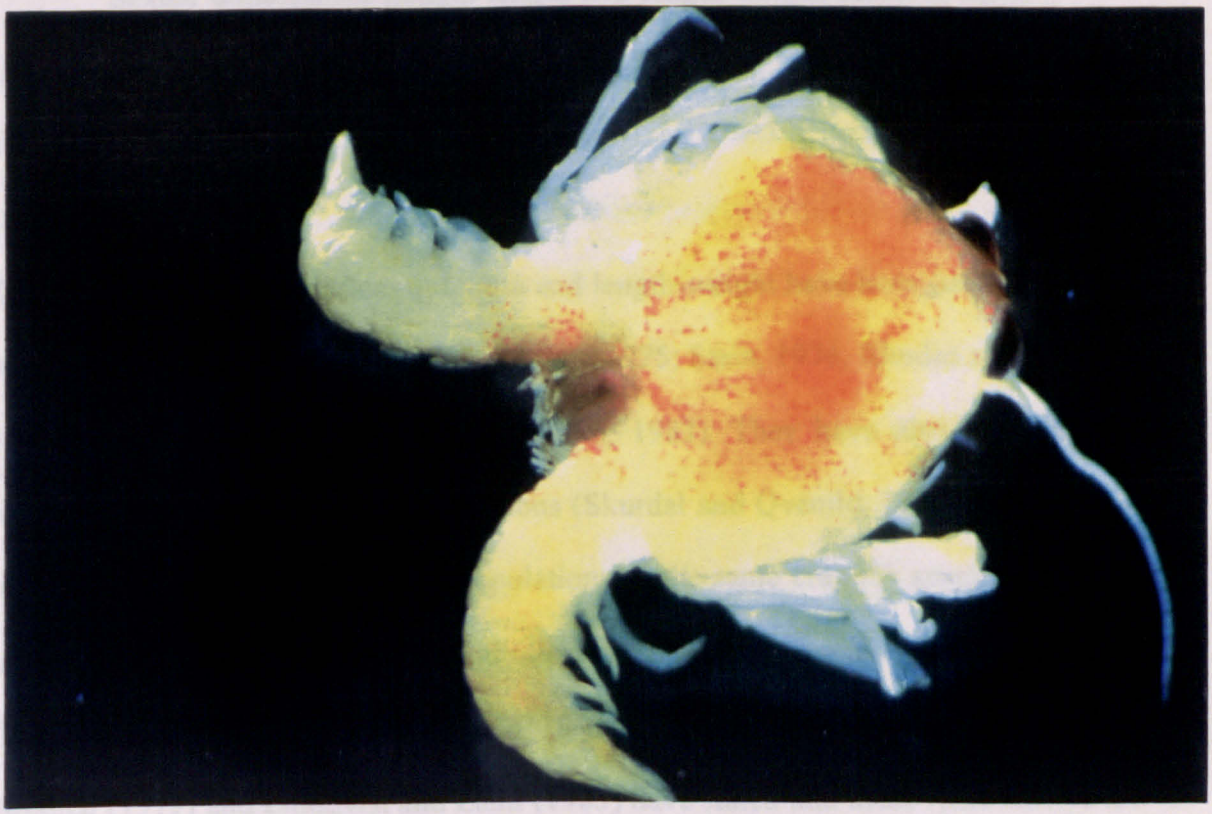
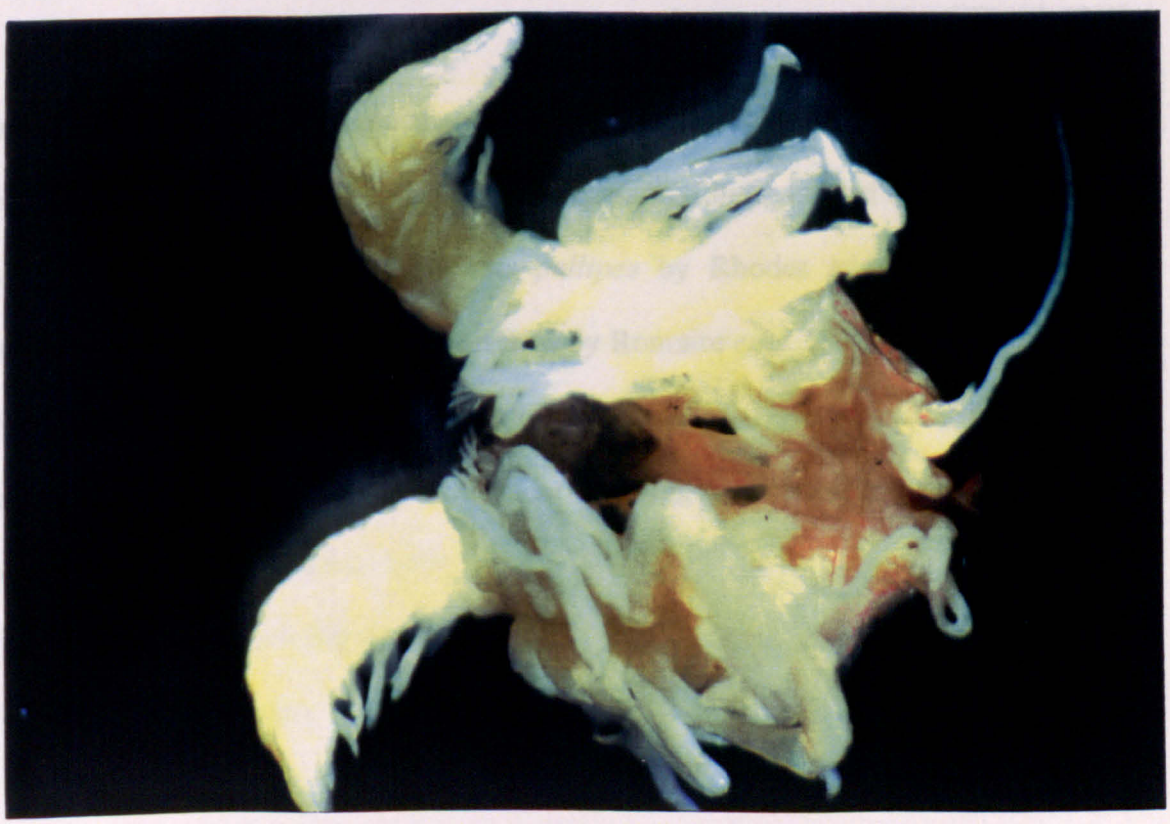


Figure 9.2.2 Ventral view of the twins



Chapter 10

Sexual dimorphism (length-length and length-weight relationships)

10.1 Introduction

The relationships between length-length and length-weight have been used in order to study sexual dimorphism in crayfish (Mason, 1975; Stein, 1976; Rhodes and Holdich, 1979; Adegboye, 1983; Lindqvist and Lahti, 1983). They have also been used to observe the relative growth of populations (Skurdal and Qvenild, 1986; Pursiainen *et al.*, 1988) or to compare different populations of the same crayfish species (Romaire *et al.*, 1977; Correia, 1993; Gillet and Laurent, 1995) or to compare different species (Romaire *et al.*, 1977; Garvey and Stein, 1993; Austin, 1995). In addition, Rhodes and Holdich (1979) and Lindqvist and Lahti (1983) have stated that these relationships are important for aquacultural purposes such as in the determination of a proper cropping strategy.

Studies on the sexual dimorphism of crayfish species have been carried out by a number of workers. For example, *Astacus astacus* by Lindqvist and Lahti (1983) and Huner *et al.* (1991), *Austropotamobius pallipes* by Rhodes and Holdich (1979), *Procambarus clarkii* and *Procambarus acutus* by Romaire *et al.* (1977), and *Orconectes propinquus* by Stein (1976). As a result of these studies it was found that males are heavier than females but females have wider and bigger abdomen especially at larger sizes.

Although the length-weight relationship for *P. leniusculus* and *A. leptodactylus* has been given in some studies (for *P. leniusculus* by Abrahamsson, 1971; Mason, 1975; for *A. leptodactylus* by Köksal, 1988) no studies have been carried out on the sexual dimorphism of *P. leniusculus* and *A. leptodactylus*. To compare the differences in the length-length or length-weight between males and females within and between the two species a number of measurements were taken from the samples of the two species.

10.2 Materials and methods

Length-length and length-weight measurements were made on adult of *P. leniusculus* and *A. leptodactylus*. In addition, length-weight measurements were also made on the juveniles of the two species.

The juveniles of *P. leniusculus* and *A. leptodactylus* were obtained from the Nottingham University crayfish rearing tanks on 25.09.94, 03.05.95 and 13.10.95.

Male and female *P. leniusculus* were collected from Boxmoor Fishery and those of *A. leptodactylus* were collected from Tykes Water.

Measurements of carapace width, abdomen length, abdomen width, chelae length, chelae width and cheliped length were used to examine sexual dimorphism between males and females within *P. leniusculus* and *A. leptodactylus*, and to show differences in the body length parameters between the species. The body length measurement technique was taken from Rhodes and Holdich (1979). Figure 10.1 shows the positions from which measurements were taken.

Body length parameters of each adult individual were measured to the nearest 1 mm and the carapace of juveniles was measured to the nearest 0.5 mm. Body wet weight of crayfish was determined to the nearest 0.001 g.

Length-weight relationships were described by regression analysis of log transformed variables in the form: log y = log (a) + log (b) x. The value of the constant b will be equal to 3.0 when the length-weight relationship (growth) is isometric and this relationship is positively allometric when the value of the constant "b" is bigger than 3.0 (Romaire *et al.*, 1977).

Length-length and length-weight relationships between males and females within species and between species for a given size range were compared by the 2-sample t test.

10.3 Results

Length-weight relationships for immature crayfish

It is important to note that in both species slopes are close to 3.0 in all cases (Table 10.1).

Although both isometric and allometric growth were observed for the female and male *P. leniusculus* and male *A. leptodactylus* in different size classes, isometric growth was observed for the female *A. leptodactylus* in all cases.

Length-weight relationships for mature crayfish

Except for the males of *A. leptodactylus*, isometric length-weight relationships were observed for adults of *P. leniusculus* and *A. leptodactylus*. The slope of body wet weight versus carapace length was 3.25 for male *A. leptodactylus* and 2.74 for female *A. leptodactylus*. Those for male and female *P. leniusculus* were 2.97 and 2.89 respectively. Table 10.2 shows the regression formulae and slopes of body wet weight versus carapace length in mature crayfish.

The relationships between carapace length and body wet weight in male *P. leniusculus* and *A. leptodactylus*, and female *P. leniusculus* and *A. leptodactylus* is given in Figures 10.2 and 10.3 respectively.

Because the females used for the current observations had approximately 5 g of pleopodal eggs (wet weight) no significant differences were found in the body wet weight between males and females within species. However, males and females of *P. leniusculus* were heavier than those of *A. leptodactylus* for a given size range (Figure 10.2 for males and Figure 10.3 for females). Mean body wet weight (g) of male *P. leniusculus* was 47.8 (s.e.= 2.0) whereas that of male *A. leptodactylus* was 31.86 (s.e.= 1.2) for the size range (41-63 mm CL). Females of *P. leniusculus* had a mean of 49.3 (s.e.= 2.8) body wet weight for the size range of 44-63 mm CL whereas that of female *P. leniusculus* was 36.2 (s.e.= 2.3) (Table 10.3).

Sexual dimorphism

In both species there is a good linear relation between carapace length and other parameters, e.g. carapace width and chelae length. In order to compare sexual dimorphism between males and females and between species a comparison of these parameters was carried out using regression analysis. Regression formulae of these parameters versus carapace length are given in Table 10.4 for male *P. leniusculus*, Table 10.5 for female *P. leniusculus*, Table 10.6 for male *A. leptodactylus* and Table 10.7 for female *A. leptodactylus*.

Sexual dimorphism in *Pacifastacus leniusculus*

Sexual dimorphism was observed in the carapace width, abdomen length, abdomen width, chelae length, chelae width and cheliped length of males and females. Males have significantly wider carapace and chelae widths (Figures 10.4 and 10.8 respectively) and longer chelae and chelipeds (Figures 10.7 and 10.9 respectively), whereas females have wider and longer abdomen (Figures 10.6 and 10.5 respectively).

For a given size range (44-63 mm CL), comparison of mean carapace width, abdomen length, abdomen width, chelae length, chelae width and cheliped length between males and females of *P. leniusculus* is given in Table 10.8.

Sexual dimorphism in *Astacus leptodactylus*

Sexual dimorphism was observed in the abdomen length, abdomen width, chelae length, chelae width and cheliped length of males and females. Although females have bigger and wider abdomens (Figures 10.10 and 10.11 respectively) males have longer chelae and cheliped length (Figures 10.12 and 10.14 respectively) and wider chelae width (Figure 10.13).

For a given size range (41-63 mm CL), comparison of mean carapace width, abdomen length, abdomen width, chelae length, chelae width and cheliped length between males and females of *A. leptodactylus* is given in Table 10.9.

Comparison of body length parameters between *Pacifastacus leniusculus* and *Astacus leptodactylus* for a given carapace length

Between males

For a given size range (41-63 mm CL), comparison of mean carapace width, abdomen length, abdomen width, chelae length, chelae width and cheliped length between males of *P. leniusculus* and *A. leptodactylus* is given in Table 10.10.

Although no significant differences were found in the carapace width and abdomen length between male *P. leniusculus* and *A. leptodactylus*, the abdomen width, chelae length, chelae width and cheliped length of male *P. leniusculus* were found to be significantly larger than those of male *A. leptodactylus* (Figures 10.15 - 10.18).

It was also found that although chelae length and cheliped length of male *P. leniusculus* are significantly larger than those of male *A. leptodactylus* for sizes smaller than 58 mm CL, chelae length and cheliped length increased in favour of *A. leptodactylus* for the sizes larger than 58 mm CL.

Between females

For a given size range (44-63 mm CL), comparison of mean carapace width, abdomen length, abdomen width, chelae length, chelae width and cheliped length between females of *P. leniusculus* and *A. leptodactylus* is given in Table 10.11.

Although no significant differences were found in the carapace width, abdomen length and abdomen width between female *P. leniusculus* and *A. leptodactylus*, as observed for males, the chelae length, chelae width and cheliped length of female *P. leniusculus* were found to be larger than those of female *A. leptodactylus* (Figures 10.19 - 10.21).

Relationship between claw wet weight and carapace length in male *Pacifastacus leniusculus* and *Astacus leptodactylus*

As can be seen in Table 10.12 claw wet weight increased allometrically as body size (CL) increased in both species (slope is 3.68 for *P. leniusculus* and 4.56 for *A. leptodactylus*).

A comparison of claw wet weight was made between species for a given size range (48-67 mm CL) (Figure 10.22). Although there was no significant differences in the

carapace length, mean claw wet weight of *P. leniusculus* was found to be significantly heavier than that of *A. leptodactylus* (P<0.001). In addition, the percentage of claw wet weight to body wet weight was found to be higher in *P. leniusculus* than in *A. leptodactylus* (35.85% and 24.76% respectively) (Table 10.13).

10.4 Discussion and conclusions

The results show that both male and female *P. leniusculus* and male *A. leptodactylus* exhibit allometric or isometric growth during their life cycles, but females of *A. leptodactylus* exhibit isometric growth throughout life. Both allometric and isometric growth were also observed for *Cherax destructor* by Sokol (1988). The slope of body wet weight versus carapace length (log-log) in *C. destructor* was found to be 2.83 for the size range of 9.8-15.9 mm CL, 3.45 for the size range of 16-30.9 mm CL, 3.06 for the males (size range 31-51 mm CL) and 3.07 for the females (size range 31-47 mm CL) respectively (Sokol, 1988). Similarly, an isometric growth for the juvenile males and females and an allometric growth for the adults of *Procambarus clarkii* were found by Romaire *et al.* (1977) and Correia (1993).

In general, isometric growth has been observed for female adult crayfish. For example, for *Cherax destructor* [slope (wet weight versus length)= 2.61], *C. quadricarinatus* (slope= 2.92) and for *C. tenuimanus* (slope= 2.76) (Austin, 1995); for *A. leptodactylus* (slope= 2.82) (Köksal, 1988) and for *Pacifastacus trowbridgii* (slope= 3.07) (Mason, 1975). In addition to these studies, in the present study, isometric growth was also observed for adult female *P. leniusculus* (slope= 2.89) and adult female *A. leptodactylus* (slope= 2.74).

In the present study, allometric growth (body wet weight increases faster than the cube of the carapace length) was observed for adult male *A. leptodactylus*. In another study allometric growth (slope= 3.13) was also observed for male *A. leptodactylus* by Köksal (1988). Similarly, allometric growth was observed for male *A. astacus* (slope= 3.83) by Pursiainen *et al.* (1988); for male *C. quadricarinatus* (slope= 3.29) and male *C. destructor* (slope= 3.22) by Austin (1995), and for male *P. trowbridgii* (slope= 3.59) by Mason (1975).

However, isometric growth (body wet weight increases at the same rate as carapace length) was observed for adult male *P. leniusculus*. It is thought that because of the fact that even small sizes of adult male *P. leniusculus* have a heavy body skeleton (shell), body wet weight does not increase faster than the cube of the carapace length for adult males (slope= 2.97). Isometric growth was also observed for male *C. tenuimanus* (slope= 2.73) by Austin (1995). In contrast, because the body skeleton is considerable heavy for bigger sizes than smaller sizes in adult male *A. leptodactylus* an allometric increase occurs in the body wet weight of adult males with the increase in size when the size range of 41-63 mm CL is considered.

According to Stein (1976) the chelae of decapod crustaceans are massive structures that often contribute 35-50% of their dry body weight. For example, the chelae of *Orconectes propinquus* consist of 40% of their dry body weight (Stein, 1976). In the present study, this was found to be 35.85% for *P. leniusculus* and 24.76 for *A. leptodactylus*. It has also been found that in crayfish, cheliped weight increases allometrically as body size increases. This has been observed for *Pacifastacus trowbridgii* by Mason (1975), for *O. propinquus* by Stein (1976) and for *Astacus*

astacus by Skurdal and Qvenild (1986). In the present study, allometric chelae growth has also been observed for male *P. leniusculus* and *A. leptodactylus* (slopes of chelae wet weight versus carapace length= 3.68 for *P. leniusculus* and 4.56 for *A. leptodactylus*).

Crayfish use their chelae mainly in capturing and manipulating food, defence against predators and in reproductive activities (Stein, 1976; Garvey and Stein, 1993). Differences in the chelae length and chelae width for a given size range have been found between crayfish species. Rhodes and Holdich (1979) stated that males of *A. pallipes* have wider chelae than those of *O. virilis* for a given carapace length. Garvey and Stein (1993) also found that the males of *O. rusticus* and *O. propinquus* have larger chelae than equal-sized *O. virilis*. Because of the bigger chelae size of *Orconectes rusticus*, it displaces *O. sanborni* in Ohio Streams (Garvey and Stein, 1993). The present study showed that both males and females of *P. leniusculus* have bigger chelae than those of *A. leptodactylus*. It seems possible therefore that if *P. leniusculus* and *A. leptodactylus* meet in a natural water body then *P. leniusculus* will become dominant over *A. leptodactylus* with its heavy body weight and big claws as it has with *A. pallipes* (Holdich and Domaniewski, 1995). As a part of study to observe the outcome of fighting between *P. leniusculus* and *A. leptodactylus* a number of competition experiments were carried out with the juveniles and adults of the two species (see Chapter 6).

It has been found that males with large chelae are more successful than those with small chelae in copulating with females (Stein, 1976). It is likely that large chelae of male *P. leniusculus* may result in a better mating success.

In addition, as was pointed out by Rhodes and Holdich (1979) bigger chelae size may have a significant importance in the production of meat yield from crayfish. As was found in the present study, significantly bigger chelae of *P. leniusculus* may have significant effects on its meat production. The meat production of the two species from different populations are investigated in Chapter 12.

In conclusion, it seems that *P. leniusculus* possess certain morphological characters, e.g. larger and heavier chelae and heavier body weight, which would give it an advantage over *A. leptodactylus* under natural conditions.

10.1 Regression formulae and slopes (letter "**b**" in the formulae) of body wet weight versus carapace length in immature crayfish

	Date	No of juv.	Size range (CL), mm	Mean CL, mm	log y= log (a) + log **(b)** x	r^2
♂ *P.leniusculus*	25.09.94	250	8.5-18.5	12.73 (1.7)	-3.79298 + **3.14324** x	0.933
♀ *P.leniusculus*	25.09.94	250	8.5-18	12.59 (1.6)	-3.87060 + **3.22098** x	0.946
♂ *A.leptodactylus*	25.09.94	250	9-18	12.35 (1.1)	-3.66394 + **2.95096** x	0.861
♀ *A.leptodactylus*	25.09.94	250	9-18	12.19 (1.1)	-3.47105 + **2.76445** x	0.843
♂ *P.leniusculus*	03.05.95	150	9-24	15.77 (3.6)	-3.89640 + **3.25535** x	0.969
♀ *P.leniusculus*	03.05.95	150	9-24	15.22 (2.8)	-3.51977 + **2.95180** x	0.964
♂ *A.leptodactylus*	03.05.95	150	9-26	13.23 (2.0)	-3.58435 + **2.89274** x	0.945
♀ *A.leptodactylus*	03.05.95	150	9-25	12.82 (2.2)	-3.65834 + **2.96325** x	0.941
♂ *P.leniusculus*	13.10.95	66	20-32	27.28 (0.2)	-3.53143 + **3.04293** x	0.937
♀ *P.leniusculus*	13.10.95	67	20-32	26.00 (0.2)	-3.46684 + **3.00114** x	0.953
♂ *A.leptodactylus*	13.10.95	86	22-42	29.54 (0.2)	-3.63617 + **3.04643** x	0.891
♀ *A.leptodactylus*	13.10.95	76	22-37	29.10 (0.2)	-3.42699 + **2.89854** x	0.881

Note: numbers in ( ): standard error of means

10.2 Regression formulae and slopes (letter "**b**" in the formulae) of body wet weight versus carapace length in adults

	No of crayfish	Size range (CL), mm	Mean CL, mm	log y= log (a) + log **(b)** x	r^2
♂ *P.leniusculus*	77	41-63	51.61 (0.7)	-3.43731 + **2.97553** x	0.90
♀ *P.leniusculus*	36	44-63	52.61 (0.8)	-3.30640 + **2.89509** x	0.79
♂ *A.leptodactylus*	85	41-63	50.14 (0.4)	-4.04584 + **3.25456** x	0.85
♀ *A.leptodactylus*	34	41-63	49.32 (1.1)	-3.14592 + **2.74399** x	0.89

10.3 Comparison of mean body wet weight (g) between males and females within species and between species for a given size range.

	No of crayfish	Size range (CL), mm	Mean CL, mm	Mean body wet wei., g
between				
♂ *P.leniusculus*	68	44-63	52.79 (0.7)	50.4 (2.1)
♀ *P.leniusculus*	36	44-63	52.61 (0.8)	49.3 (2.8)
degree of significance			**NS**	**NS**
between				
♂ *A.leptodactylus*	85	41-63	50.14 (0.4)	31.8 (1.2)
♀ *A.leptodactylus*	34	41-63	49.32 (1.1)	33.0 (2.1)
degree of significance			**NS**	**NS**
between				
♂ *P.leniusculus*	77	41-63	51.61 (0.7)	47.8 (2.0)
♂ *A.leptodactylus*	85	41-63	50.14 (0.4)	31.8 (1.2)
degree of significance			**NS**	***
between				
♀ *P.leniusculus*	36	44-63	52.61 (0.8)	49.3 (2.8)
♀ *A.leptodactylus*	27	44-63	51.22 (1.1)	36.2 (2.3)
degree of significance			**NS**	***

NS: $P>0.05$, ***:$P<0.001$

10.4 Regression formulae of carapace length (CL) versus carapace width (CW), abdomen length (AL), abdomen width (AW), total length (TL), chelae length (ChL), chelae width (ChW) and cheliped length (CheL) in male *P. leniusculus* (size range 41-63 mm CL)

	log y= log (a) + log (b) x	r^2
CL versus CW	-0.30784 + 1.02655 x	0.898
CL versus AL	0.36753 + 0.80003 x	0.915
CL versus AW	-0.25840 + 0.97537 x	0.913
CL versus TL	0.47940 + 0.90393 x	0.961
CL versus ChL	-0.55625 + 1.31440 x	0.839
CL versus ChW	-0.77734 + 1.23938 x	0.798
CL versus CheL	-0.09049 + 1.19900 x	0.898

10.5 Regression formulae of carapace length (CL) versus carapace width (CW), abdomen length (AL), abdomen width (AW), total length (TL), chelae length (ChL), chelae width (ChW) and cheliped length (CheL) in female *P. leniusculus* (size range 44-63 mm CL)

	log y= log (a) + log (b) x	r^2
CL versus CW	-0.35750 + 1.04069 x	0.820
CL versus AL	0.32720 + 0.84658 x	0.901
CL versus AW	-0.51462 + 1.17837 x	0.859
CL versus TL	0.51661 + 0.89418 x	0.971
CL versus ChL	0.00592 + 0.94753 x	0.747
CL versus ChW	-0.84162 + 1.23451 x	0.768
CL versus CheL	0.15915 + 1.02869 x	0.717

10.6 Regression formulae of carapace length (CL) versus carapace width (CW), abdomen length (AL), abdomen width (AW), total length (TL), chelae length (ChL), chelae width (ChW) and cheliped length (CheL) in male *A. leptodactylus* (size range 41-63 mm CL)

	log y= log (a) + log (b) x	r^2
CL versus CW	-0.62944 + 1.21276 x	0.874
CL versus AL	0.37440 + 0.80255 x	0.850
CL versus AW	-0.24375 + 0.94376 x	0.881
CL versus TL	0.47324 + 0.91050 x	0.955
CL versus ChL	-1.71255 + 1.97204 x	0.833
CL versus ChW	-1.62023 + 1.63768 x	0.776
CL versus CheL	-0.66849 + 1.52316 x	0.834

10.7 Regression formulae of carapace length (CL) versus carapace width (CW), abdomen length (AL), abdomen width (AW), total length (TL), chelae length (ChL), chelae width (ChW) and cheliped length (CheL) in female *A. leptodactylus* (size range 41-63 mm CL)

	log y= log (a) + log (b) x	r^2
CL versus CW	-0.18753 + 0.94738 x	0.807
CL versus AL	0.15841 + 0.95267 x	0.960
CL versus AW	-0.48051 + 1.15334 x	0.878
CL versus TL	0.38392 + 0.97523 x	0.988
CL versus ChL	-0.46051 + 1.17954 x	0.827
CL versus ChW	-0.59082 + 1.00426 x	0.690
CL versus CheL	0.01689 + 1.07796 x	0.909

10.8 Comparison of mean carapace width (CW), abdomen length (AL), abdomen width (AW), chelae length (ChL), chelae width (ChW) and cheliped length (CheL) between male *P. leniusculus* (N= 68) and female *P. leniusculus* (N= 36) for a given size (44-63 mm CL for both sex, and mean CL (mm)= 52.79 for males and 52.61 for females)

	Mean length, mm		
	♂ *P. leniusculus*	♀ *P. leniusculus*	Degree of significance
CW	28.85 (0.4)	27.19 (0.5)	*
AL	55.53 (0.6)	60.92 (0.9)	***
AW	26.40 (0.3)	32.78 (0.7)	***
ChL	51.16 (1.0)	43.50 (0.83)	***
ChW	22.82 (0.4)	19.21 (0.4)	***
CheL	94.5 (1.6)	85.50 (1.8)	***

*: P< 0.05, ***: P<0.001

10.9 Comparison of mean carapace width (CW), abdomen length (AL), abdomen width (AW), chelae length (ChL), chelae width (ChW) and cheliped length (CheL) between male *A. leptodactylus* (N= 85) and female *A. leptodactylus* (N= 34) for a given size (41-63 mm CL for both sex, and mean CL (mm)= 50.14 for males and 49.32 for females)

	Mean length, mm		
	♂ *A. leptodactylus*	♀ *A. leptodactylus*	Degree of significance
CW	27.12 (0.3)	26.12 (0.6)	NS
AL	54.81 (0.4)	59.06 (1.2)	**
AW	22.95 (0.2)	29.74 (0.8)	***
ChL	34.53 (0.9)	44.14 (0.9)	***
ChW	14.69 (0.2)	12.91 (0.3)	***
CheL	83.8 (1.4)	69.60 (1.7)	***

NS: P>0.05, **: P<0.01, ***: P<0.001

10.10 Comparison of mean carapace width (CW), abdomen length (AL), abdomen width (AW), chelae length (ChL), chelae width (ChW) and cheliped length (CheL) between male *P. leniusculus* (N= 77) and male *A. leptodactylus* (N= 85) for a given size (41-63 mm CL for both species, and mean CL (mm)= 51.61 for *P. leniusculus* and 50.14 for *A. leptodactylus*

	Mean length or width, mm		
	♂ *P. leniusculus*	♂ *A. leptodactylus*	Degree of significance
CW	28.23 (0.42)	27.12 (0.34)	NS
AL	54.64 (0.63)	54.81 (0.47)	NS
AW	25.84 (0.7)	22.95 (0.6)	***
ChL	49.81 (1.0)	44.14 (0.9)	***
ChW	22.26 (0.4)	14.69 (0.2)	***
CheL	92.1 (1.6)	83.8 (1.4)	***

NS: P>0.05, ***: P>0.001

10.11 Comparison of mean carapace width (CW), abdomen length (AL), abdomen width (AW), chelae length (ChL), chelae width (ChW) and cheliped length (CheL) between female *P. leniusculus* (N= 36) and female *A. leptodactylus* (N= 27) for a given size (44-63 mm CL for both species, and mean CL (mm)= 52.61 for *P. leniusculus* and 51.22 for *A. leptodactylus*

	Mean length, mm		
	♀ *P. leniusculus*	♀ *A. leptodactylus*	Degree of significance
CW	27.19 (0.5)	27.00 (0.6)	NS
AL	60.92 (0.9)	60.02 (0.8)	NS
AW	32.78 (0.7)	30.96 (0.8)	NS
ChL	43.50 (0.8)	36.07 (0.1)	***
ChW	19.31 (0.4)	13.37 (0.3)	***
CheL	85.5 (1.8)	72.37 (1.8)	***

NS: P> 0.05, ***: P< 0.001

10.12 Regression formulae and slopes of carapace length versus claw wet weight in male *P. leniusculus* and *A. leptodactylus*

	No of crayfish	Size range	log y= log (a) + log **(b)** x	r^2
P. leniusculus	51	48-67	-5.15255 + **3.68261** x	0.733
A. leptodactylus	43	48-67	-6.93825 + **4.56027** x	0.808

10.13 Comparison of claw wet weight between male *P. leniusculus* and *A. leptodactylus* and percentage of claw wet weight in body wet weight in male *P. leniusculus* and *A. leptodactylus*

	No of crayfish	Size range	Mean CL, mm	Mean body wet wei., g	Mean claw wet wei., g	Claw wet wei. versus body wet wei (%)
P. leniusculus	43	48-67	55.86 (0.7)	56.4 (2.5)	20.22 (1.2)	35.85
A. leptodactylus	38	48-67	55.74 (0.8)	46.4 (2.4)	11.49 (0.8)	24.76
Degree of signif.			NS	***	***	

NS: P>0.05, ***: P< 0.001

Figure 10.1 A dorsal view of crayfish and its cheliped to show locations from which measurements were taken.

Legend

(a): carapace length, from tip of the rostrum to the posteriomedial edge of the carapace

(b): carapace width, at the widest point of the thorax

(c): abdomen length, from posteriomedial edge of the carapace to the tip of telson (excluding setae)

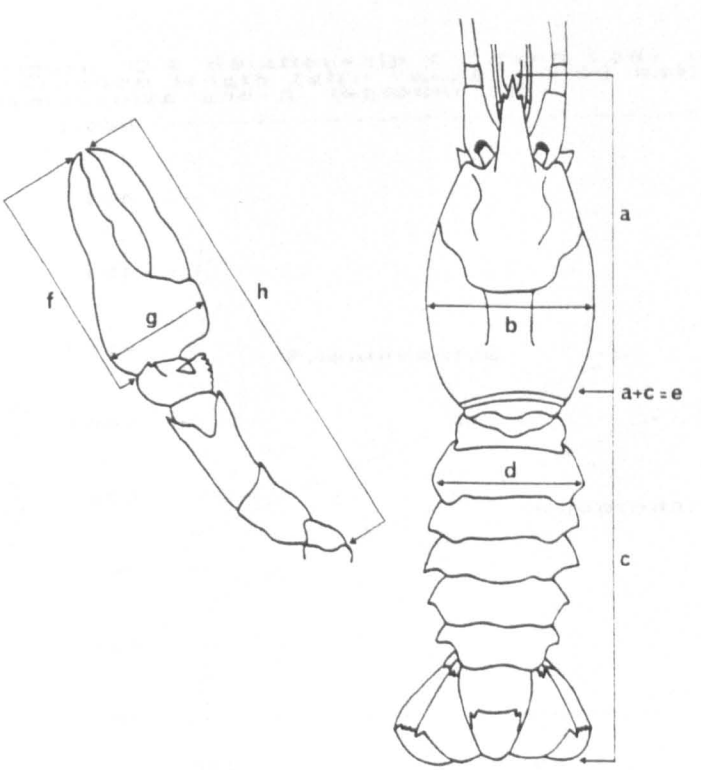
(d): abdomen width, at the widest point of the second segment

(e): total length, from the tip of rostrum to the tip of telson (excluding setae) (carapace length + abdomen length)

(f): chelae length, from carpal joint to the tip of the propodus

(g): chelae width, at the widest point of chelae

(h): cheliped length, from the tip of propodus to basipodite



(adapted from Rhodes and Holdich, 1979)

Figure 10.2 Relationship between body wet weight (g) and carapace length (size range: 41-63 mm) in male P. leniusculus and A. leptodactylus

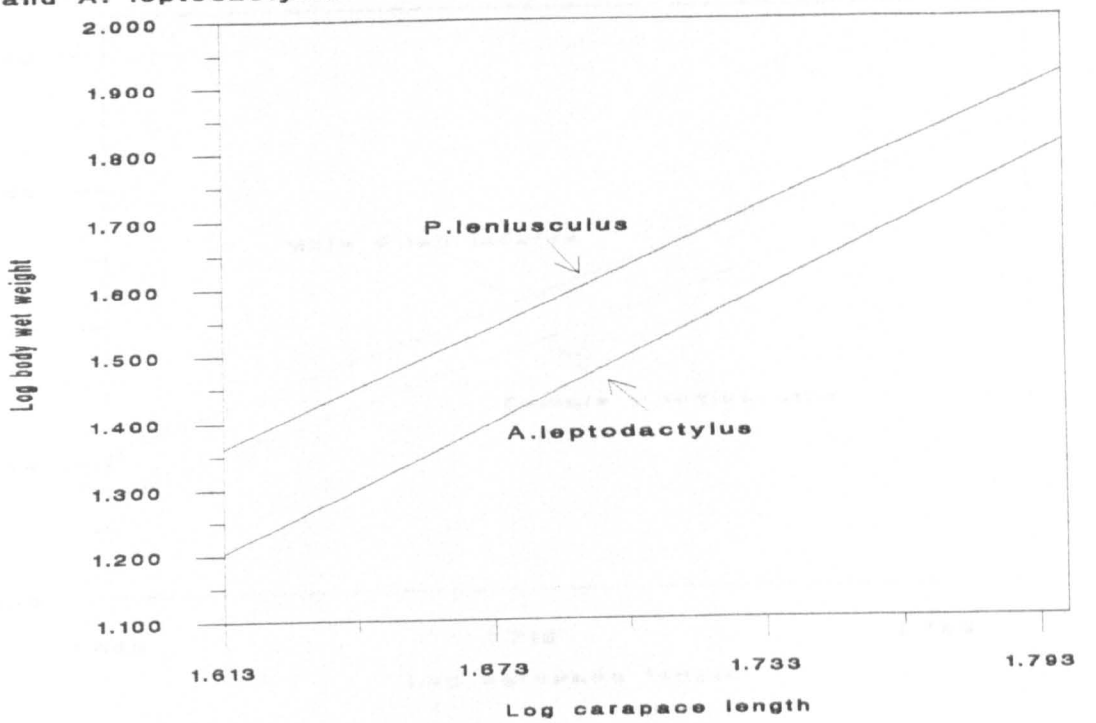


Figure 10.3 Relationship between body wet weight (g) and carapace length (size range: 44-63 mm) in female P. leniusculus and A. leptodactylus

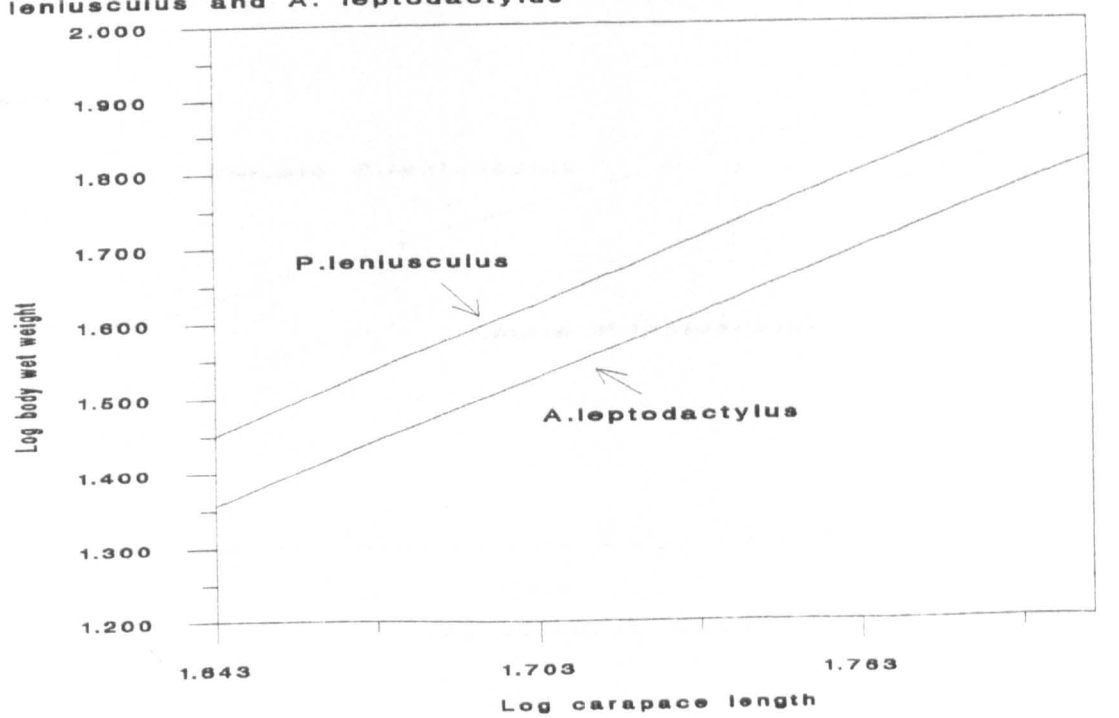


Figure 10.4 Relationship between carapace length (mm) and carapace width (mm) in male and female P. leniusculus

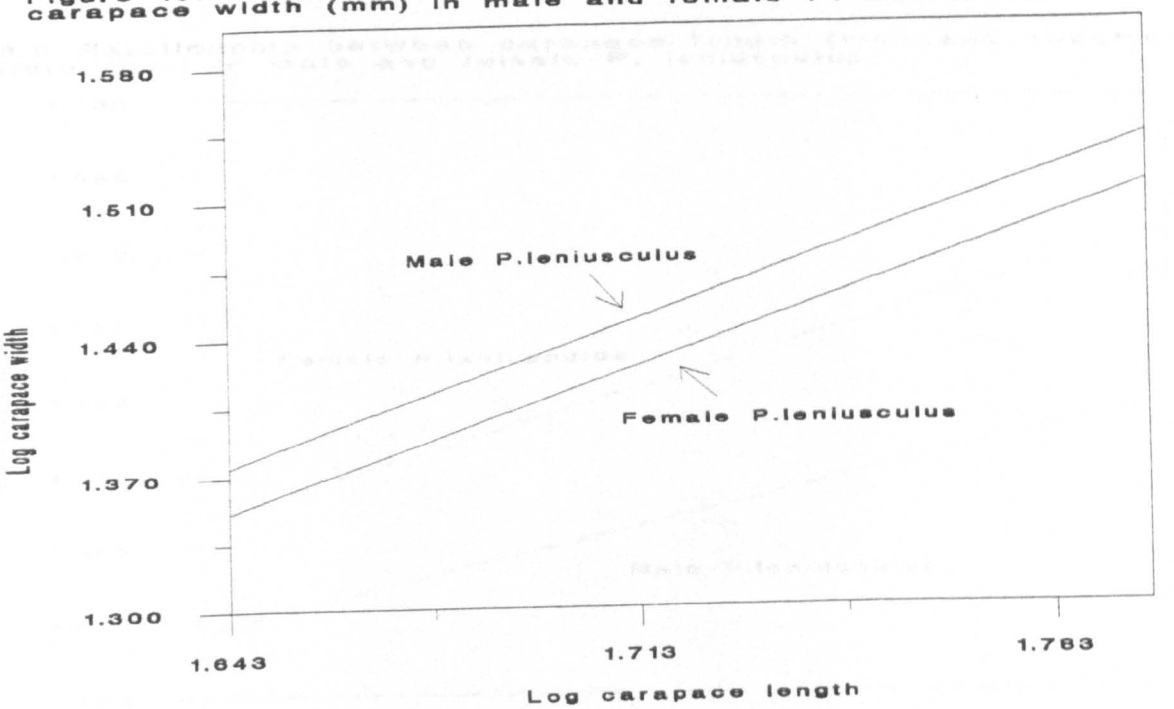
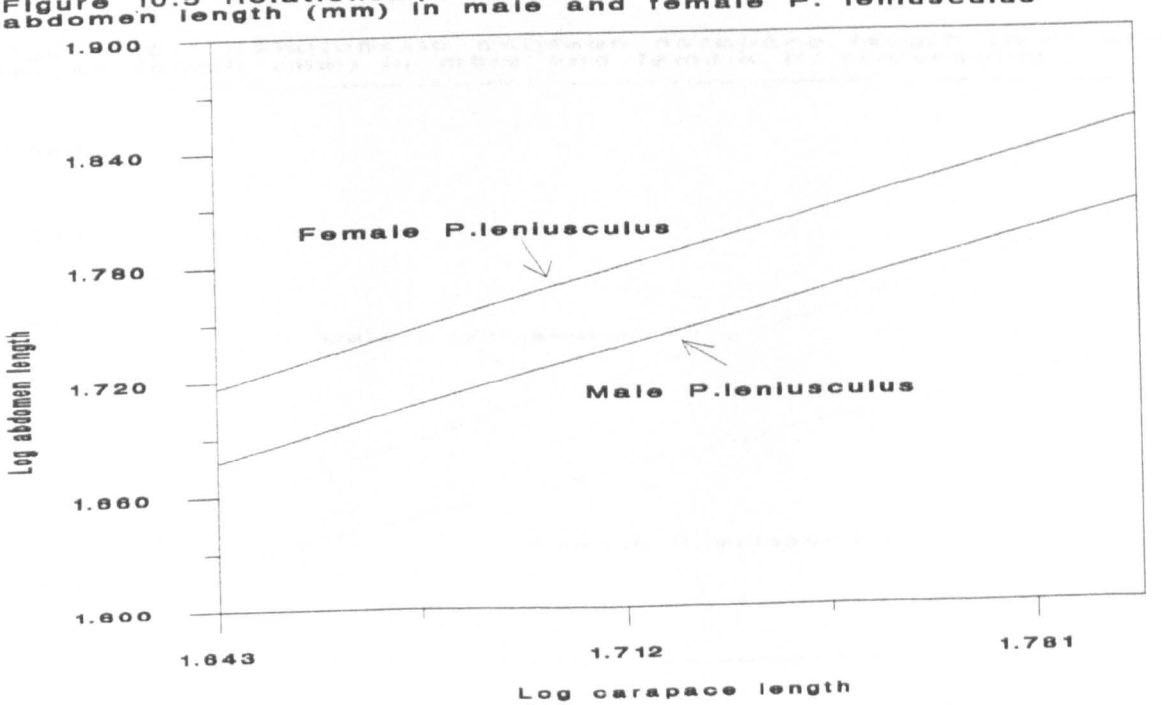


Figure 10.5 Relationship between carapace length (mm) and abdomen length (mm) in male and female P. leniusculus



10.6 Relationship between carapace length (mm) and abdomen width (mm) in male and female P. leniusculus

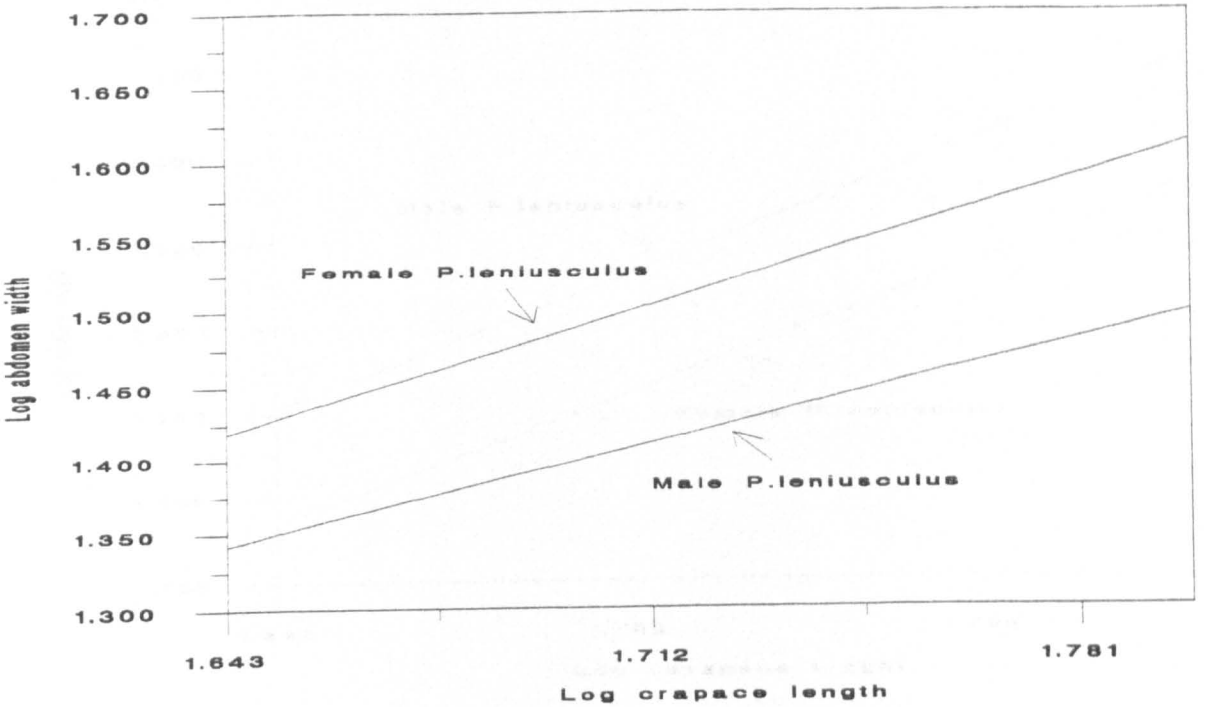


Figure 10.7 Relationship between carapace length (mm) and chelae length (mm) in male and female P. leniusculus

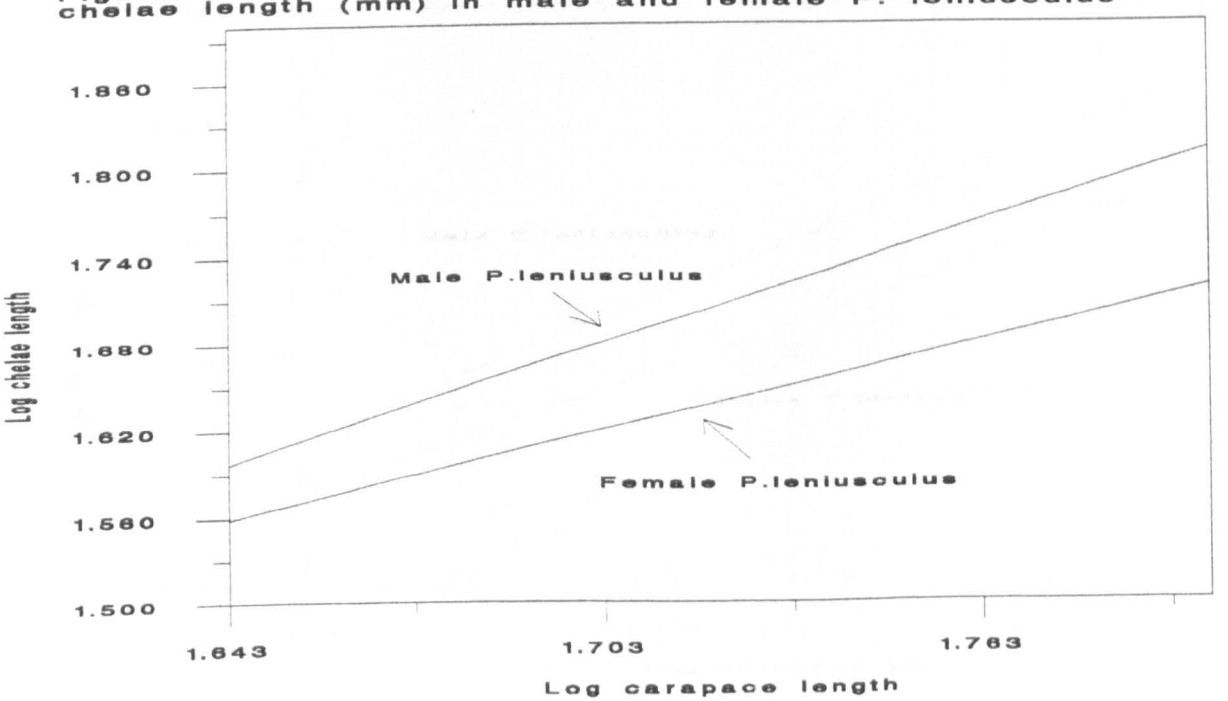


Figure 10.8 Relationship between carapace length (mm) and chelae width (mm) in male and female P. leniusculus

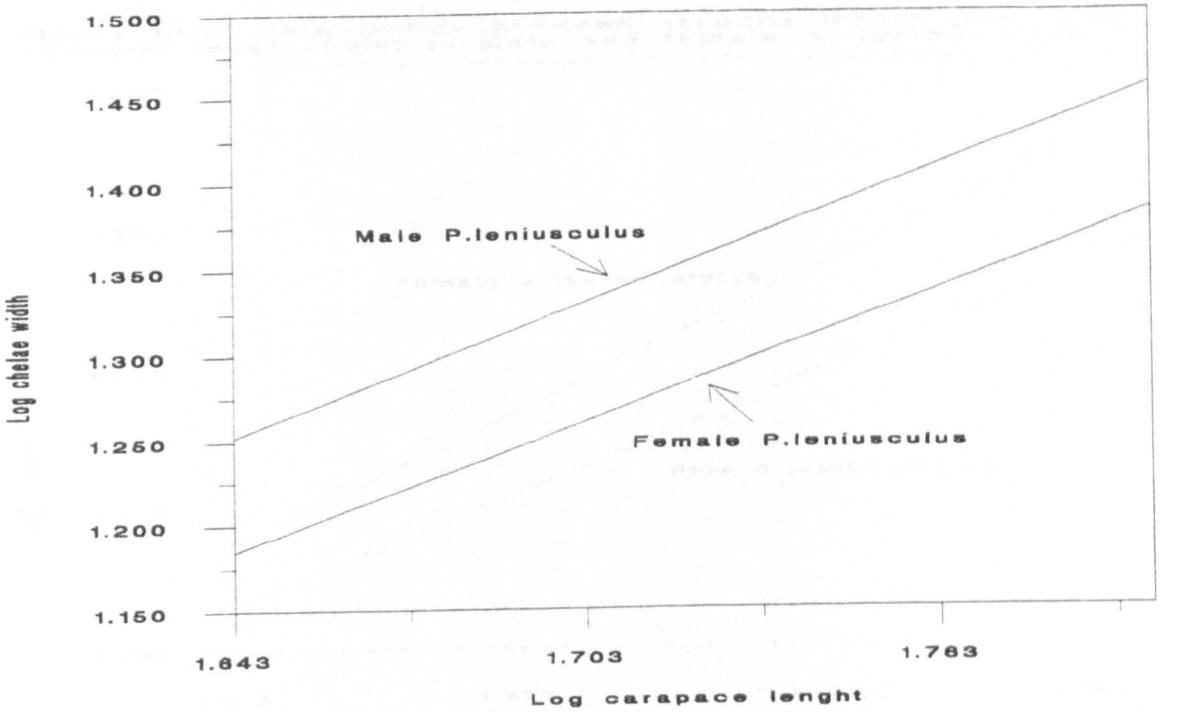


Figure 10.9 Relationship between carapace length (mm) and cheliped length (mm) in male and female P. leniusculus

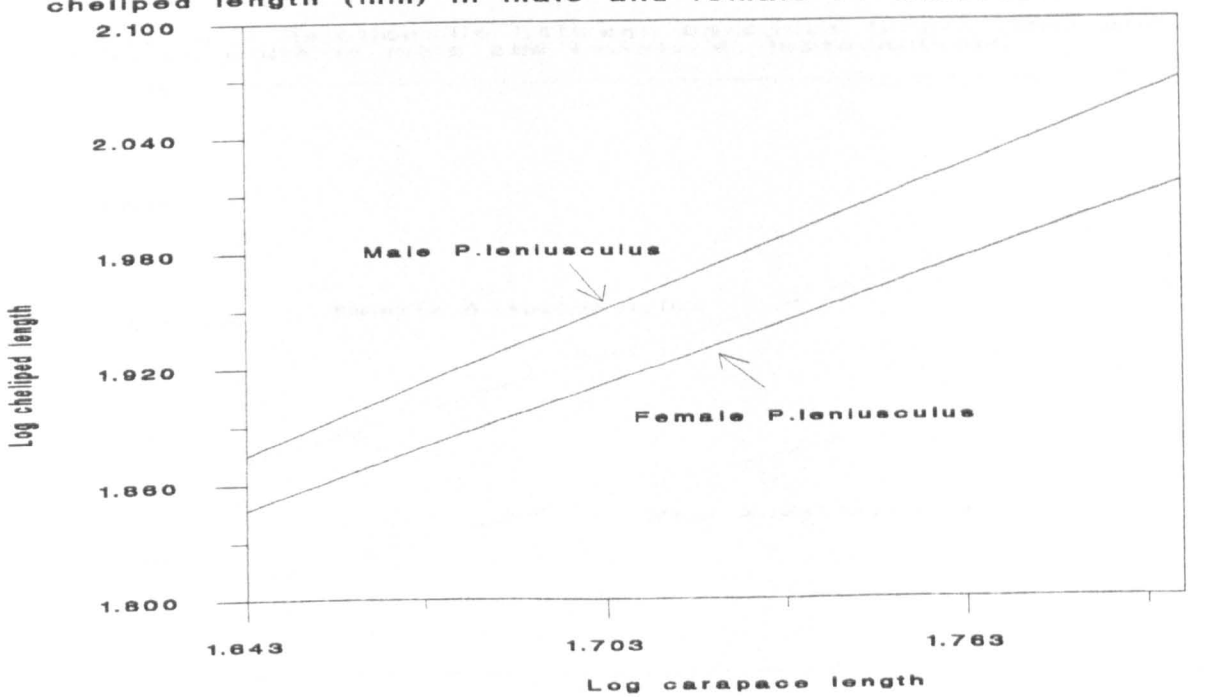


Figure 10.10 Relationship between carpace length (mm) and abdomen length (mm) in male and female A. leptodactylus

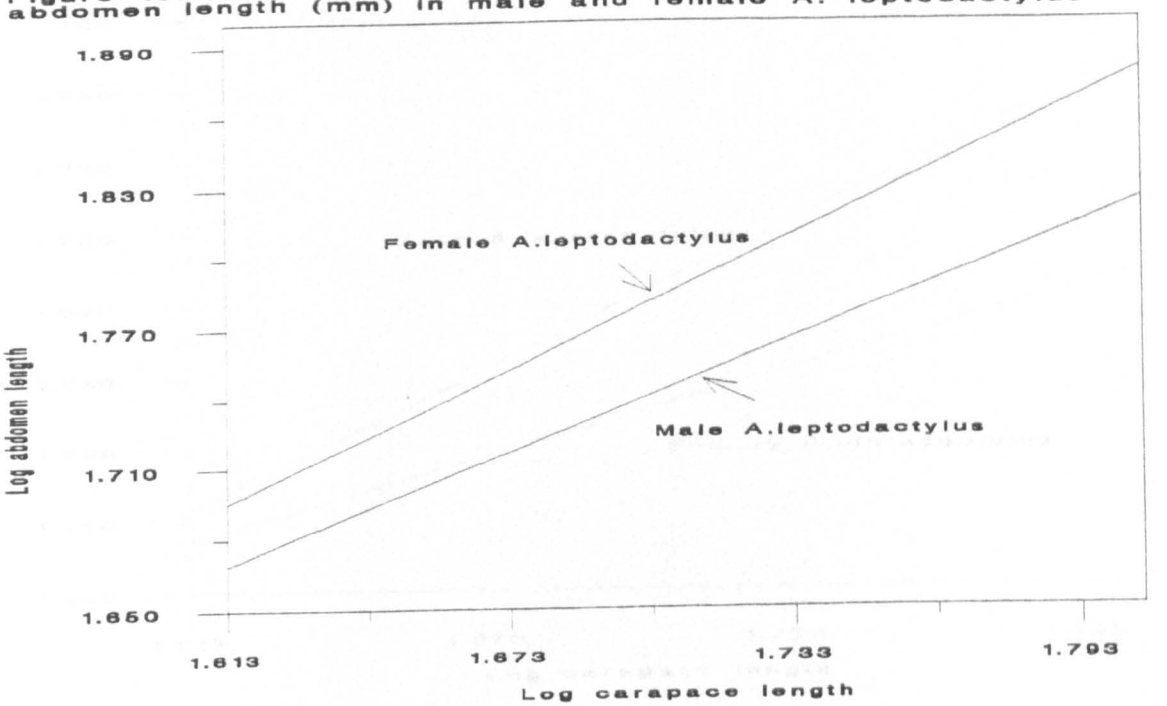


Figure 10.11 Relationship between carapace length (mm) and abdomen width in male and female A. leptodactylus

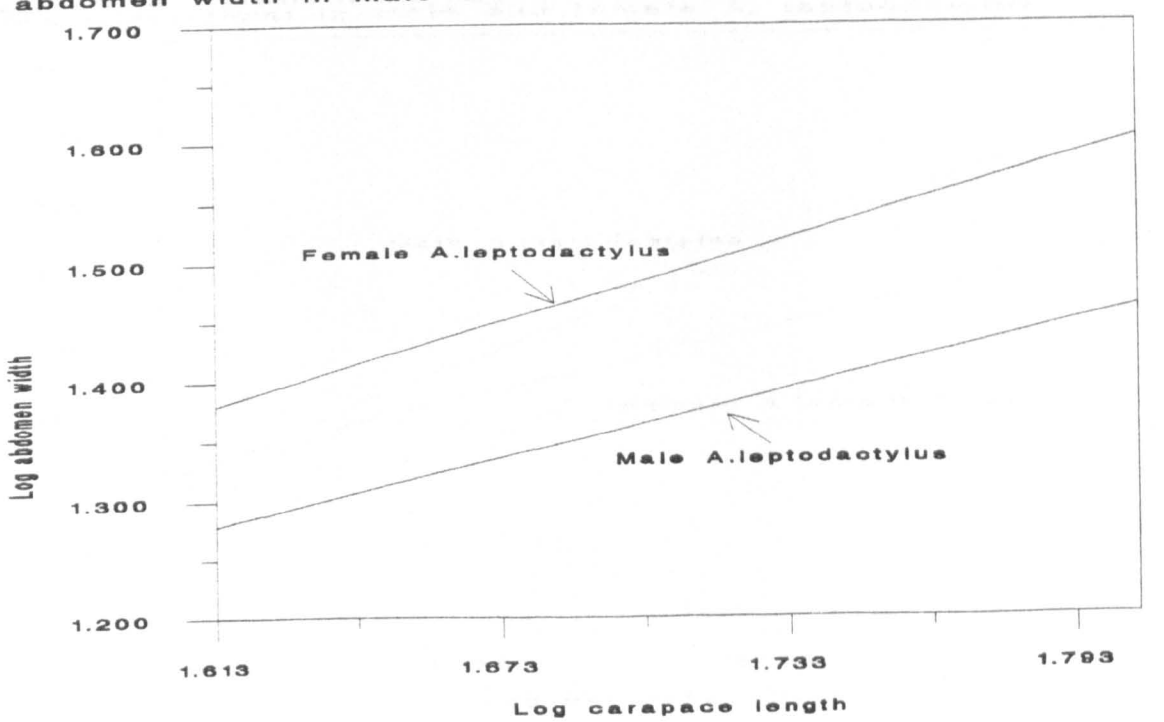


Figure 10.12 Relationship between carapace length (mm) and chelae length (mm) in male and female A. leptodactylus

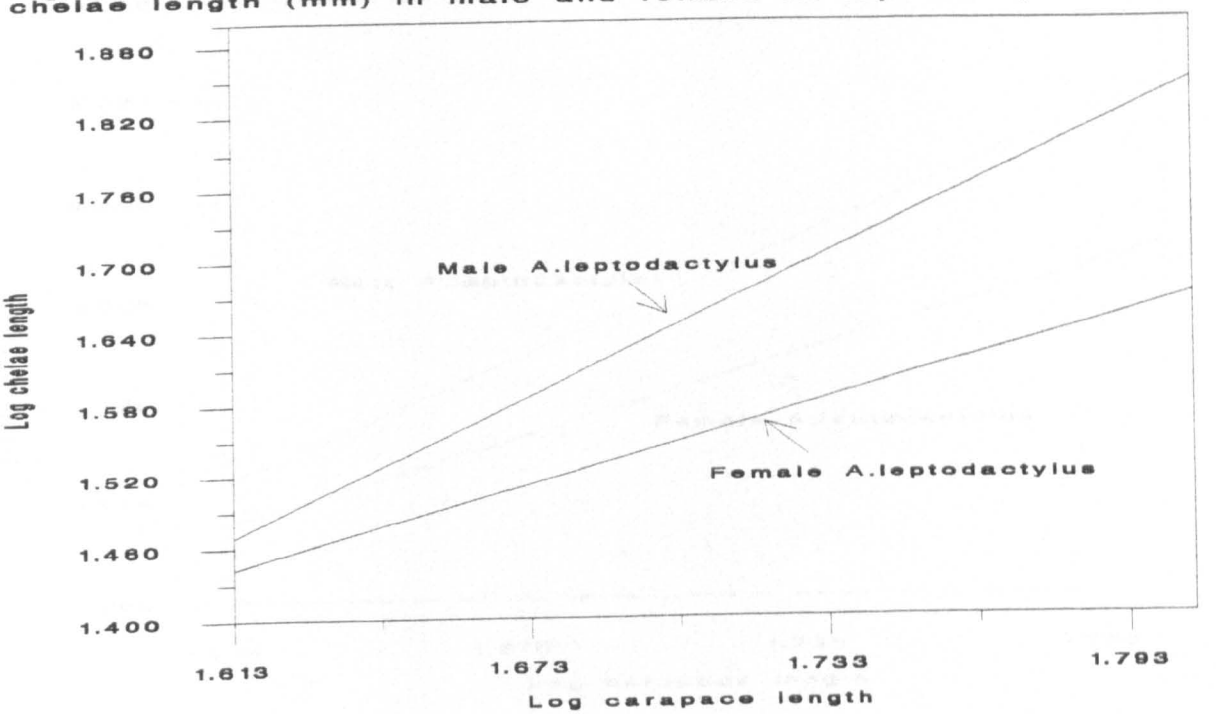


Figure 10.13 Relationship between carapace length (mm) and chelae width (mm) in male and female A. leptodactylus

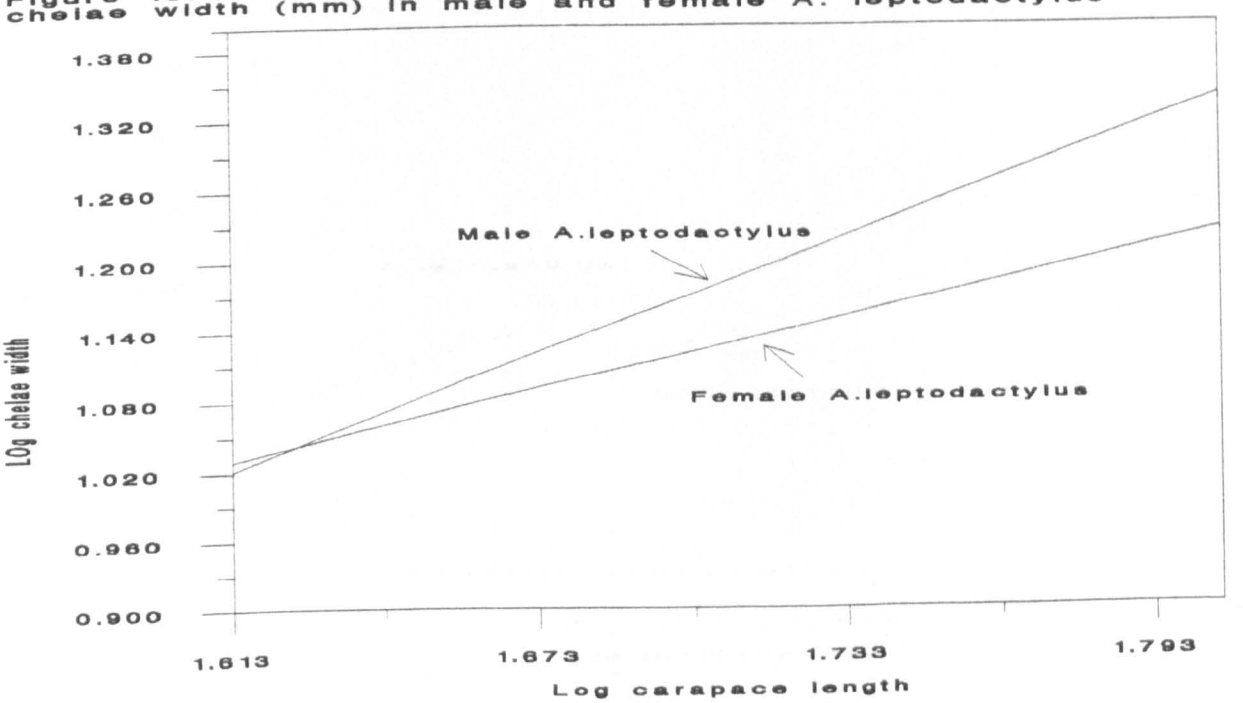


Figure 10.14 Relationship between carapace length (mm) and cheliped length (mm) in male and female A. leptodactylus

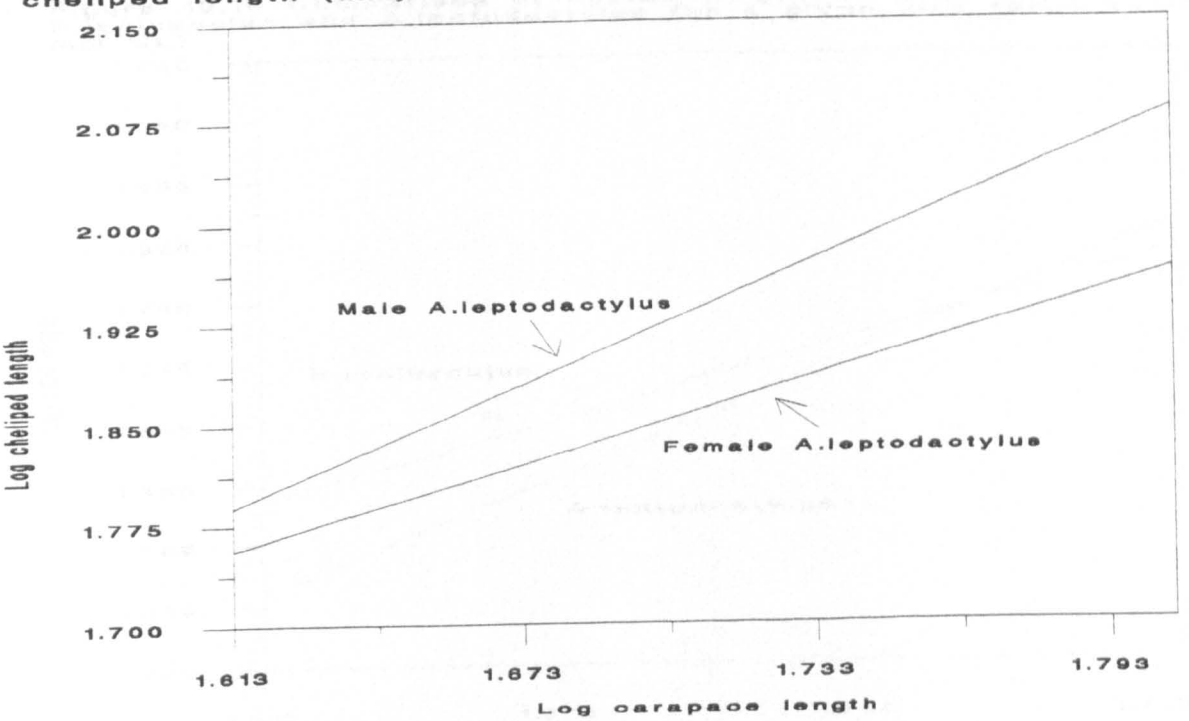


Figure 10.15 Comparison of abdomen width (mm) between male P. leniusculus and A. leptodactylus for a given size range (41-63 mm CL)

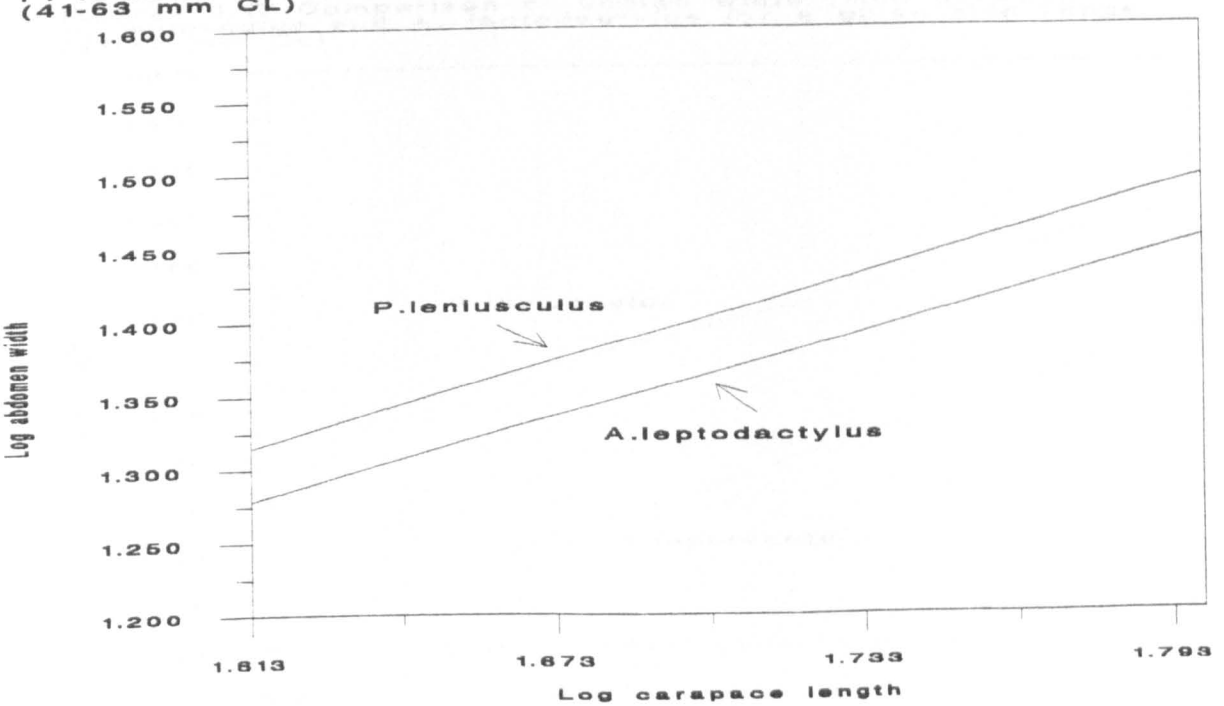


Figure 10.16 Comparison of chelae length (mm) between male P.leniusculus and A.leptodactylus for a given size range (41-63 mm CL)

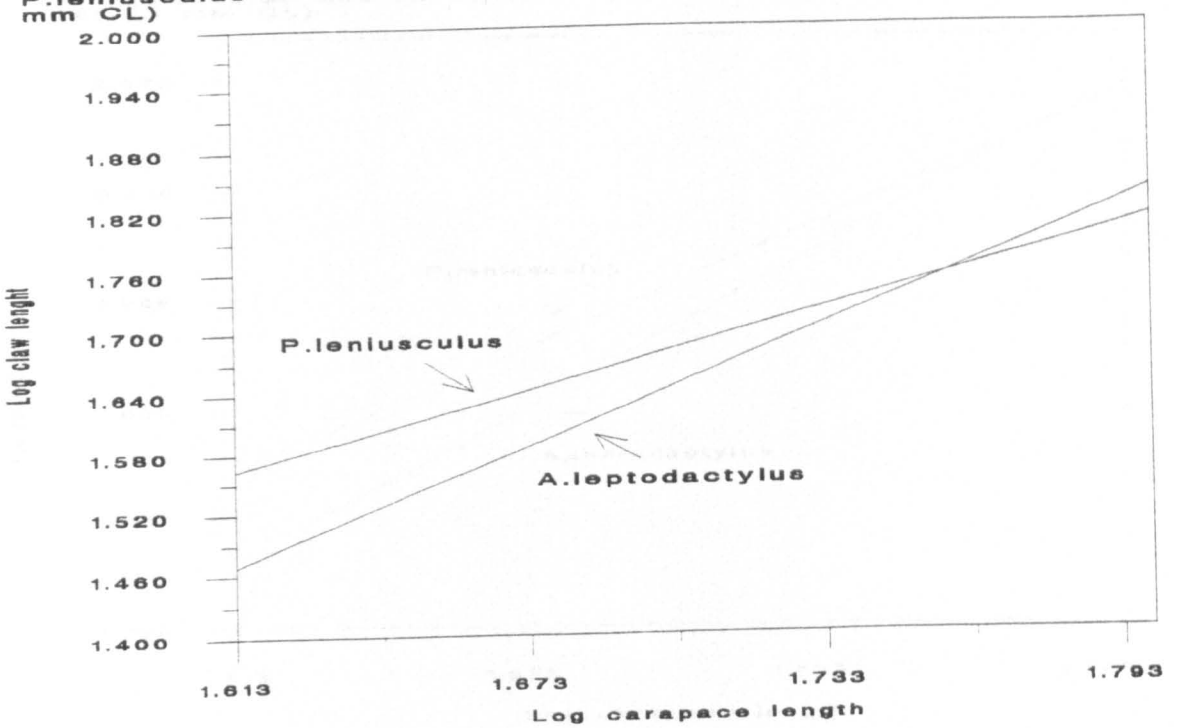


Figure 10.17 Comparison of chelae width (mm) between male P. leniusculus and A. leptodactylus for a given size range (41-63 mm CL)

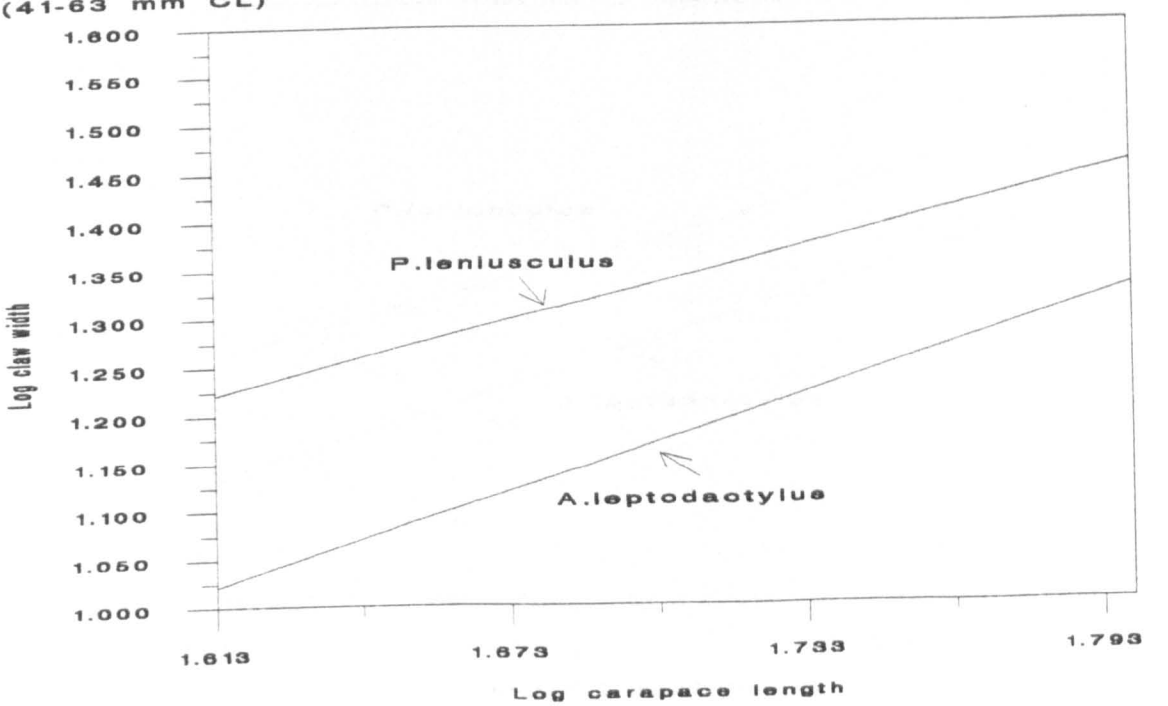


Figure 10.18 Comparison of cheliped length (mm) between male P. leniusculus and A. leptodactylus for a given size range (41-63 mm CL)

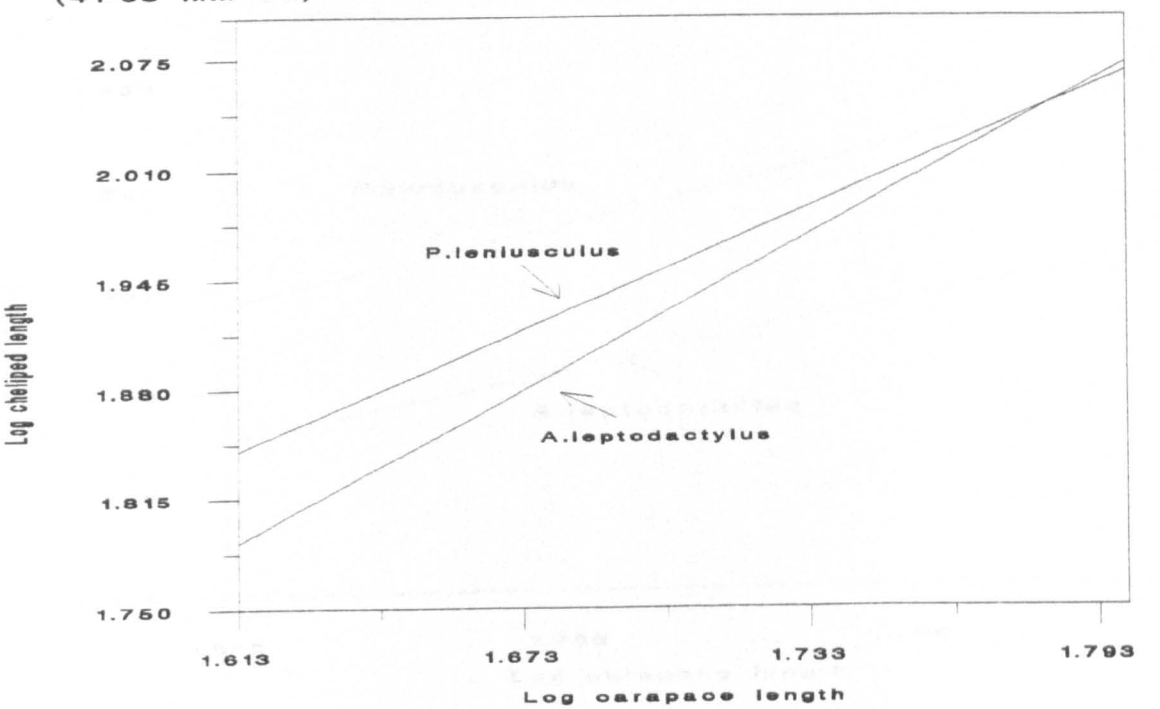


Figure 10.19 Comparison of claw length (mm) between female P. leniusculus and A. leptodactylus for a given size range (44-63 mm CL)

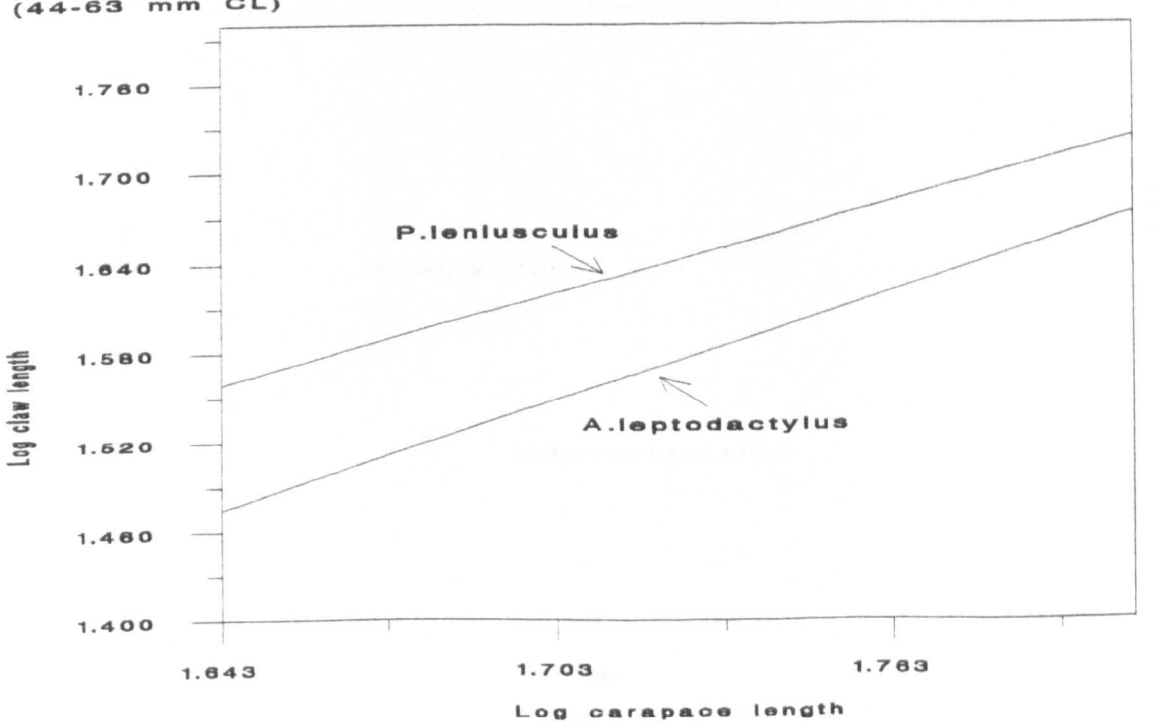


Figure 10.20 Comparison of claw width (mm) between female P. leniusculus and A. leptodactylus for a given size range (44-63 mm CL)

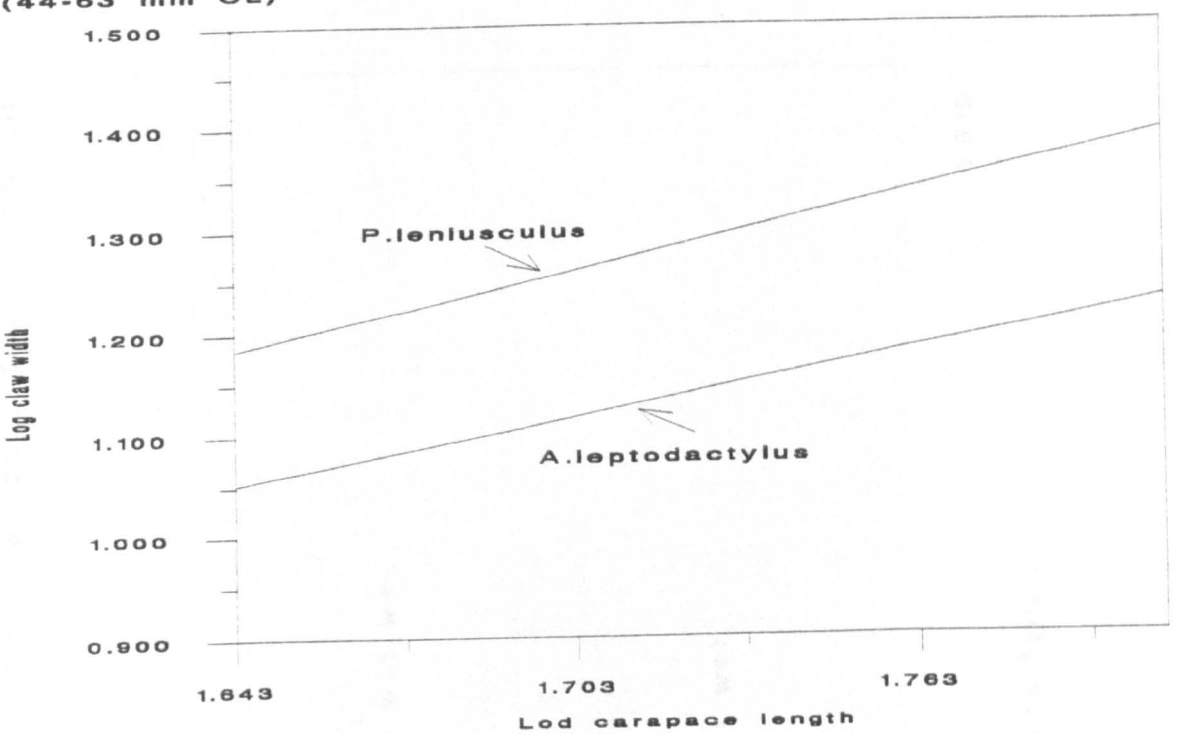


Figure 10.21 Comparison of cheliped length (mm) between female P.leniusculus and A. leptodactylus for a given size range (44-63 mm CL)

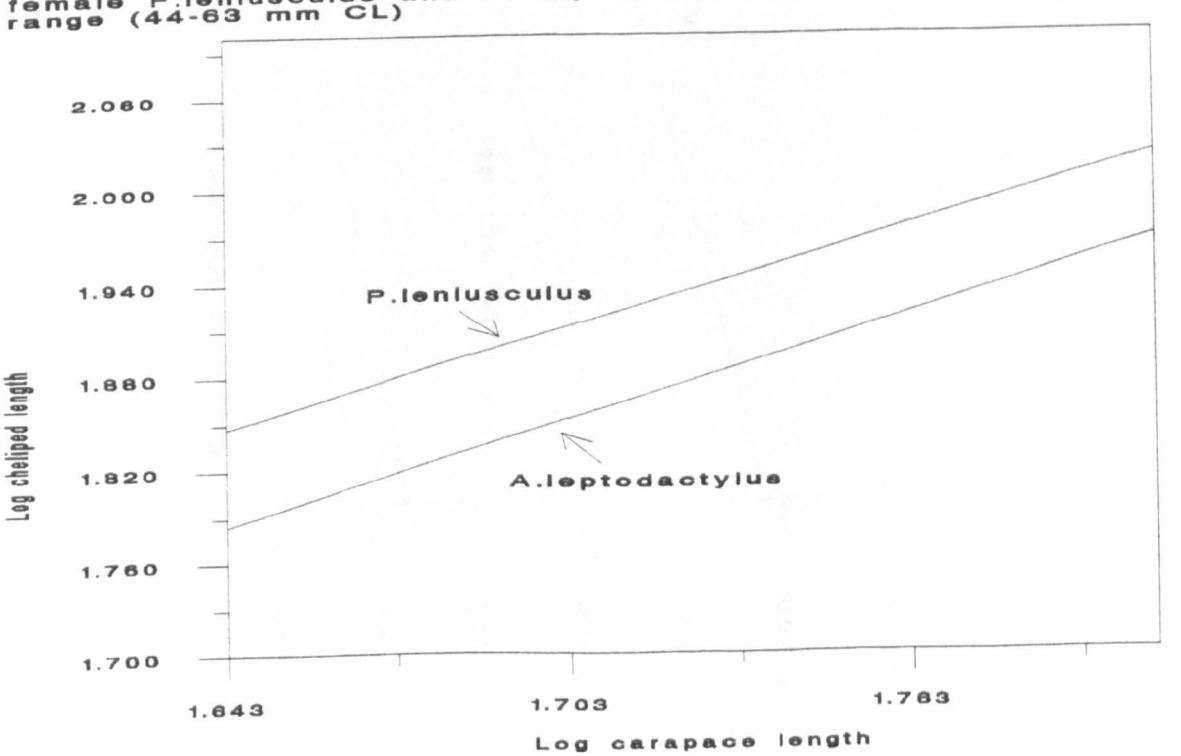
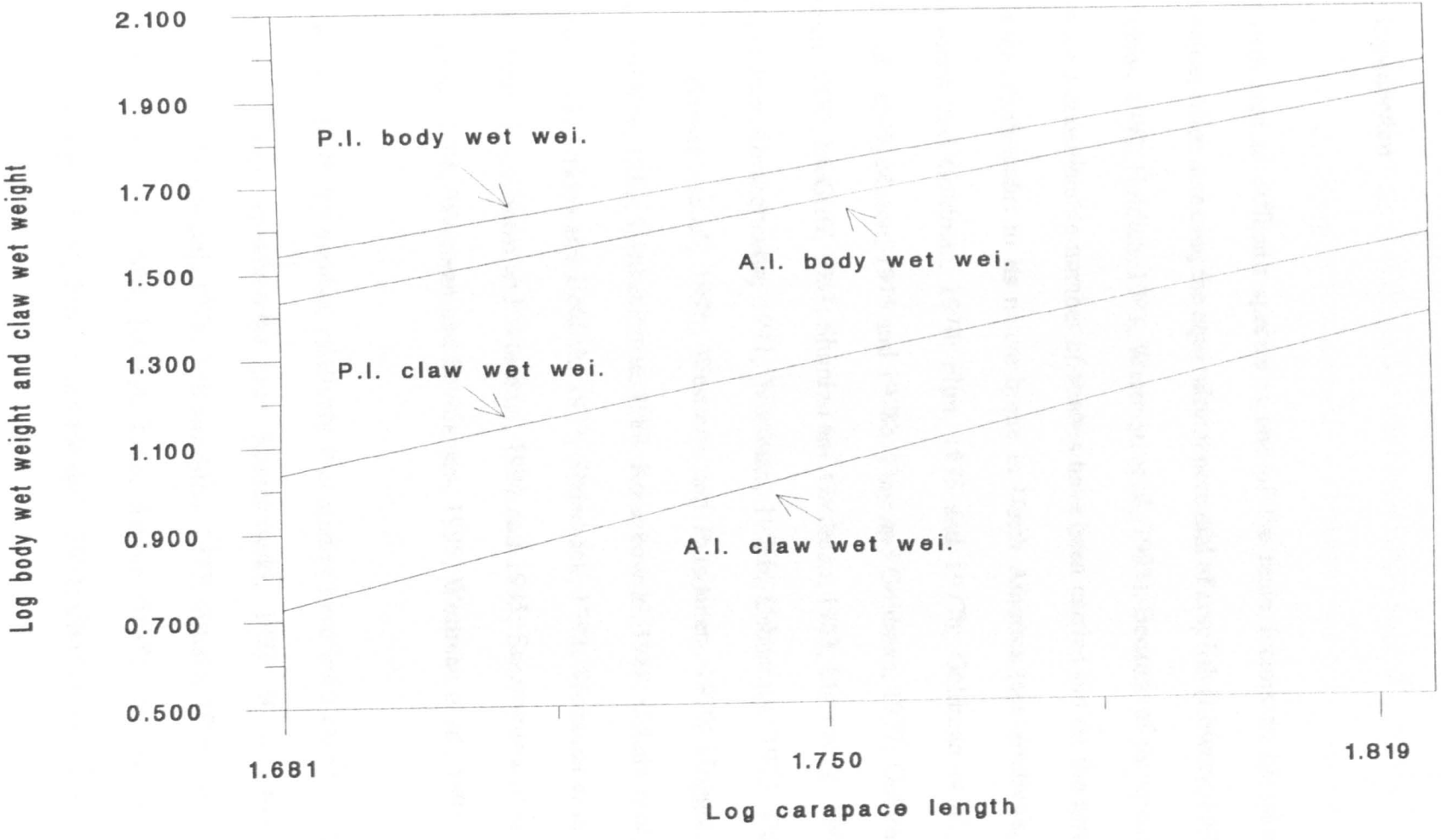


Figure 10.22 Comparison of claw wet weight (g) and body wet weight (g) between male P. leniusculus and A. leptodactylus for a given size range (48-67 mm CL)



Chapter 11

Growth under different temperature and density regimes

11.1 Introduction

The growth rate of different species is one of the main factors to be taken into consideration when assessing the aquacultural potential of crayfish (Lowery, 1988; Lee and Wickins, 1992; Holdich, 1993; Westman *et al.*, 1993). Because of its aquacultural importance a considerable number of studies have been carried out on the growth of *Pacifastacus leniusculus* in its native home in North America (see Andrews, 1907; Abrahamsson and Goldman, 1970; Flint, 1975 and 1977b; Goldman *et al.*, 1975; Huner *et al.*, 1975; Mason, 1975 and 1978b; Flint and Goldman, 1977; Goldman and Rundquist, 1977; McGriff, 1983; Shimizu and Goldman, 1983; Elser *et al.*, 1994) and in Europe (see Abrahamsson, 1971; Westman, 1973b; Cabantous, 1975; Cukerzis, 1978; Kossakowski *et al.*, 1978; Westman and Pursiainen, 1979; Hogger, 1986; Laurent and Vey, 1986; Tamkeviciene, 1988; Kossakowski, 1988; Celada *et al.*, 1989; Arrignon, 1993; Firkins and Holdich, 1993; Söderbäck, 1993; Westman *et al.*, 1993; Jonsson, 1995; Kirjavainen and Westman, 1994 and 1995; Saezroyuela *et al.*, 1995; Tulonen *et al.*, 1995; Westman and Savolainen, 1995; Westman *et al.*, 1995).

In comparison to *P. leniusculus*, relatively few studies have been carried out on the growth of *Astacus leptodactylus* (see Kossakowski, 1971; Rumyantsev, 1973; Arrignon, 1975; Papadopol, 1975; Tcherkashina, 1977; Amato, 1988; Burba, 1988; Karafezlieva-Avramova, 1988; Köksal, 1988; Sevilla, 1988; Tamkeviciene, 1988; Marlet and Roqueplo, 1991; Firkins and Holdich, 1993; Cherkashina, 1995).

As a result of these studies it was concluded that the juveniles of both species grow faster than the other astacids. However, for a comparative purpose, except Firkins and Holdich (1993) no studies have been carried out on the growth and survival of *P. leniusculus* and *A. leptodactylus* under laboratory conditions. Firkins and Holdich (1993) exposed juvenile (O+) *P. leniusculus*, *A. leptodactylus* and *Austropotamobius pallipes* to 15, 20, 25 and 28 °C over a relatively short time period, 30 days, and used only ten juveniles of each species with two replicates for each experimental temperature. In the present study, in order to compare growth and survival of juvenile *P. leniusculus* and *A. leptodactylus* a number of experiments have been carried out under different temperature and density regimes over a relatively long time period.

11.2 Materials and methods

In all experiments crayfish were provided with natural food, i.e. *Cladophora*, *Asellus* and *Crangonyx*. In addition, a mixture of minced morsels, mussels and fish meat was provided twice in a week. In the second experiment, feeding was carried out when it was necessary in the month of January, February and March. Crayfish were provided from the Nottingham University crayfish rearing tanks. Average lengths and weights of stage 2 *P. leniusculus* were 5.31 mm CL (± 0.129) and 0.029 g (± 0.0025). Those of *A. leptodactylus* were 5.9 mm CL (± 0.325) and 0.036 (± 0.0071). (N= 15 for each species).

For each concrete tank (3.43 m^2) running water was provided (5 l min^{-1}, conductivity: 450-500 µS cm^{-1}; pH: 8.0-8.4; hardness 245-255 mg l^{-1} as $CaCO_3$) and water temperature was recorded by means of a "Tiny Talk" datalogger (see Chapter 9.1.2).

Figure 11.1 shows the mean monthly water temperature of the tanks during the experiments.

An excess of bricks with 18 holes and short lengths of plastic drainpipe were provided as hides for each tank.

A two sample t-test was used in order to compare growth rates of the species.

Experiment 1

Six thousand stage 2 *P. leniusculus* and 6,000 stage 2 *A. leptodactylus* were used. Three thousand *P. leniusculus* were put in a tank with two replicates and this was repeated for *A. leptodactylus*.

Experiments were started on 26.05.94 for *P. leniusculus* and on 23.06.94 for *A. leptodactylus*. On 25.09.94, all tanks were drained and the crayfish were counted. After that, only 500 juveniles (250 for each sex) of each species were weighed and measured.

Experiment 2

One thousand six hundred juvenile *P. leniusculus* and 1,600 juvenile *A. leptodactylus* were used. These juveniles were already available at the end of the first experiment and they were selected randomly (size range: 8-25 mm CL for the two species).

Each tank contained 800 *P. leniusculus* or 800 *A. leptodactylus*. The experiment was set up on 26.09.94. All tanks were drained and crayfish were counted on 03.05.95. Only 300 juveniles (150 for each sex) of each species were weighed and measured.

Experiment 3

Four hundred juvenile *P. leniusculus* and 400 juvenile *A. leptodactylus* were used (size range: 15-18 mm CL for the two species). These juveniles were already available at the end of the second experiment.

In order to observe growth and survival of *P. leniusculus* and *A. leptodactylus* when they are kept together, 100 *P. leniusculus* and 100 *A. leptodactylus* were put in a tank with two replicates. Only one monospecific tank for each species was available as a control (200 juveniles of each species were put in a monospecific tank).

The experiment was set up on 04.05.95 and all tanks were drained and all crayfish were weighed and measured on 13.10.95.

Experiment 4

Stage 2 juveniles of *P. leniusculus* and *A. leptodactylus* obtained three or four months earlier than under natural conditions (see Chapter 9.1) were kept at 15 and 25 °C in a constant temperature room with a 12:12 light regime and at different densities (234 m^{-2}, 468 m^{-2} and 937 m^{-2}).

A layer of pebbles of approximately 5 cm depth was placed on the floor of each aquarium (100 cm x 32 cm x 40 cm). Water was aerated and half of the aquarium water was changed with freshwater once every ten days. For both species experiments were started on 25.03.94 and were terminated on 30.05.94. Only 20 crayfish from each replicate were randomly selected to measure and weigh at the end of the experiment.

11.3 Results

Experiment 1, 2 and 3

A comparison of survival in the monospecific tanks of *P. leniusculus* and *A. leptodactylus* showed that in all experiments the survival of *P. leniusculus* was lower than that of *A. leptodactylus* (Table 11.1). For example, in the first experiment, the survival of *P. leniusculus* was 26% in the first monospecific tank and 40.76% in the second monospecific tank. Those of *A. leptodactylus* were 42.3 and 47.7% respectively. Similarly, in the second experiment, survival was 48.5 and 55.3% for *P. leniusculus*, and 65.2 and 68.2% for *A. leptodactylus*.

At the end of the first and second experiments, there were no significant differences in the carapace length, total length and body wet weight between the sexes in *P. leniusculus* and in *A. leptodactylus*. However, carapace length, total length and body wet weight of *P. leniusculus* were significantly greater than those of *A. leptodactylus* at the end of the first and second experiments. Mean carapace length, total length and body wet weight of the species and statistical analysis of data between the species are given in Table 11.2 for the first experiment and in Table 11.3 for the second

experiment. Figures 11.2 (for the first experiment) and 11.3 (for the second experiment) also show a comparison of body wet weight (versus carapace length) between *P. leniusculus* and *A. leptodactylus*.

At the end of the first and second experiments in both species growth rate was highly variable between individual crayfish. Size range was 9 to 18 mm CL for *A. leptodactylus* and 8.5 to 18.5 mm CL for *P. leniusculus* at the end of the first experiment, and 9 to 24 mm CL for *A. leptodactylus* and 9 to 26 mm CL for *P. leniusculus* at the end of the second experiment. Figures 11.5 (for *P. leniusculus*) and 11.6 (for *A. leptodactylus*) show the smallest and largest juveniles at the end of the second experiment.

At the end of the third experiment, in comparison to the monospecific tank, there was a significant reduction in the number of *A. leptodactylus* when they were held with *P. leniusculus* ($P<0.001$). Only 9% *A. leptodactylus* survived in the first replicate of the mixed tanks. There was 34% survival in the second replicate (Table 11.1). The growth rate of *A. leptodactylus* was also depressed by *P. leniusculus*. Although the yield of *P. leniusculus* from the first and second mixed tanks was 835 and 690 g respectively, it was only 30 and 131 g for *A. leptodactylus*. Figure 11.4 shows a comparison of body wet weight between *A. leptodactylus* and *P. leniusculus* when they were kept together (two replicates of the mixed tanks were pooled). In addition, a comparison of total yield of the species from the monospecific tanks showed that there was no significant differences between the species ($P>0.05$). Total yield of *A. leptodactylus* and *P. leniusculus* was 1132 (N= 162) and 897 (N= 133) g respectively.

It is important to note that some female *P. leniusculus* had eggs in their second summer (1+) (at the end of the third experiment). The smallest female *P. leniusculus* with eggs was 66 mm total length and 11.85 g. No *A. leptodactylus* became berried during the experiments.

Experiment 4

In both species growth was significantly better ($P<0.001$) at the higher temperature (25 °C) but survival was lower. As was observed in the first, second and third experiments, the survival of *P. leniusculus* was lower than that of *A. leptodactylus* at 15 and 25 °C. Tables 11.4 (for *P. leniusculus*) and 11.5 (for *A. leptodactylus*) show the survival and growth of the two species at 15 and 25 °C.

In both species at 15 °C growth was not significantly affected by density, but at 25 °C growth was significantly better at the density of 234 m^{-2} than the density of 468 m^{-2}, and better at the density of 468 m^{-2} than the density of 937 m^{-2} ($P<0.01$ and $P<0.001$ respectively).

There were no significant differences between the growth of *A. leptodactylus* and *P. leniusculus* at 15 and 25 °C ($P>0.05$).

11.4 Discussion and conclusion

In a study on the comparison of growth rate between *P. leniusculus*, *A. leptodactylus* and *A. pallipes*, Firkins and Holdich (1993) found that percentage weight increase in

P. leniusculus was significantly greater than *A. leptodactylus* or *A. pallipes*. Although it is important that the conditions must be equal in the comparison of survival and growth of two species (Tulonen *et al.*, 1995), Firkins and Holdich (1993) used O+ juveniles but the initial weight and length of the juveniles were not given and the effect of initial weight on growth rate was not considered. However, Morrissy (1990) stated that growth rate of crayfish depends on initial size. Therefore, in Firkins and Holdich's study, the difference in the percentage weight increase between the species may come from the differences in their initial weight. In the present study, in order to make conditions equal experiments were started with the same stage (stage 2) or same length of the species.

In the present study, under natural conditions, the juveniles of *P. leniusculus* hatched and were released from their mother at least four weeks earlier than those of *A. leptodactylus*. This allowed *P. leniusculus* to have a longer growing period during the summer months. As a result of this, *P. leniusculus* became significantly bigger in size and heavier in weight at the end of the summer. However, no significant difference was observed in the growth of the species when the experiment was started with stage 2 juveniles of the two species on the same day (see Experiment 4).

Size of juvenile crayfish is an important factor when using juvenile crayfish to stock freshwaters. Apparently, as was observed in the present study, under natural conditions, *P. leniusculus* is better than *A. leptodactylus* at producing large juveniles in the first year of stocking with mature females. Similarly, because of its earlier hatching time, *P. leniusculus* is also considered as a better species than *A. astacus* for stocking freshwaters with crayfish by Jonsson (1995), Tulonen *et al.* (1995) and

Westman *et al.* (1993 and 1995).

Many environmental factors influence the growth of crayfish. These are mainly temperature, food supply and population density (Goddard, 1988; Lowery, 1988; Huner and Lindqvist, 1995). In the present study, although crayfish were fed to excess at 15 and 25 °C, those at the higher temperature fed more actively and consumed greater quantities of food, but lower survival was observed at the higher temperature in both species. Survival rate of *P. leniusculus* was lower than that of *A. leptodactylus*. It was thought that aggressive behaviour of *P. leniusculus* resulted in a high rate of cannibalism and consequently low survival.

In the present study, a higher density of crayfish gave higher mortality and a lower growth rate. This is in accordance with results from Mills and McClaud (1983) for *Cherax destructor*, Ackefors *et al.* (1989) for *A. astacus*, Gydemo and Westin (1990) for *A. astacus*, Westman *et al.* (1993) for *A. astacus* and *P. leniusculus*, Brown *et al.* (1995) for *Orconectes virilis*, McClain (1995) for *Procambarus clarkii*, Morrissy *et al.* (1995) for *Cherax tenuimanus* and Whisson (1995) for *Cherax tenuimanus*. As a result of these studies, it was concluded that crayfish exhibit density dependent growth even in situations where sufficient food resources are available.

Although mean wet weight of males was higher than that of females in both species there were no significant differences in growth rate between males and females. This was also observed for *A. astacus* by Pursiainen *et al.* (1983), for *Paranephrops planifrons* by Hopkins (1967) and for *Cherax quadricarinatus* by Karplus *et al.* (1995). In addition, in the present study, the results showed that there was a wide range of

growth rate between individuals in both species. This was also observed for *P. planifrons* by Hopkins (1967), for *A. pallipes* by Pratten (1980), for *Cherax destructor* by Mills and McClaud (1983), for *A. astacus* by Gydemo and Westin (1990), for *C. tenuimanus* by Morrissy (1990), for *C. quadricarinatus* by Curtis and Jones (1995), and De Boulay *et al.* (1993). Mills and McClaud (1983), Gydemo and Westin (1990) and Curtis and Jones (1995) stated that differences in the genetic structure of individuals bring about differences in their growth rate.

Results also show that *P. leniusculus* matures earlier than *A. leptodactylus*. Some female *P. leniusculus* were sexually mature in their second summer (1+). In a study on the development of *P. leniusculus* in the small lake Karisjarvi in central Finland it was observed that female *P. leniusculus* reached maturity in their second summer and the smallest mature female was 60 mm TL (Kirjavainen and Westman, 1995). In the present study, the smallest mature female was 66 mm TL. Tulonen *et al.* (1995) also found that female *P. leniusculus* attained maturity in their second summer. However, Abrahamsson and Goldman (1970) observed that female *P. leniusculus* were sexually mature in their fourth summer (3+), in the sub-alpine Lake Tahoe, Nevada. It seems that the populations of *P. leniusculus* in Britain and in Finland are able to mature earlier than those of *P. leniusculus* matures in its native country i.e. Western North America.

Although Lowery (1988) states that *P. leniusculus* does not grow so fast in cold waters nor in the warm waters of the Sacramento River in North America there is evidence that this species is able to mature in its second summer as was observed in the present study, and by Kirjavainen and Westman (1995) and Tulonen *et al.* (1995). However,

as was mentioned in the comparison of growth rates of different crayfish species by Lowery (1988), *P. leniusculus* is not as fast-growing as the warm water species, *Procambarus clarkii*, which may be cultivated to a marketable size in favourable conditions within 100 days.

In conclusion, it is clear that *P. leniusculus* is a good candidate for aquaculture having a rapid growth rate, early hatching and maturity, but its aggressive behaviour and degree of cannibalism may make it a less attractive proposition than some other species such as *A. leptodactylus*, which is also fast growing but less aggressive. However, *P. leniusculus* has the advantage of earlier hatching and a greater yield of meat as the volume of its claws is much greater than that of *A. leptodactylus* (see Chapter 12).

Table 11.1 The percentage survival of *P. leniusculus* and *A. leptodactylus* in first, second and third experiments

	Number of *P. leniusculus*			Number of *A. leptodactylus*		
	Before	After	Survival (%)	Before	After	Survival (%)
Experiment 1						
Replicate 1	3000	780	26.0	3000	1270	42.3
Replicate 2	3000	1223	40.7	3000	1430	47.6
Experiment 2						
Replicate 1	800	388	48.5	800	522	65.2
Replicate 2	800	442	55.3	800	546	68.2
Experiment 3						
Monospecific tank	200	133	66.5	200	162	81
Mixed tanks						
Replicate 1	100	72	72	100	9	9
Replicate 2	100	61	61	100	34	34

Table 11.2 Mean carapace length, total length and body wet weight of the species and statistical analysis of data at the end of the first experiment

	Mean CL (mm)	Mean TL (mm)	Mean body wet weight (g)
♂ *P. leniusculus*	12.73 (1.77)	26.70 (3.77)	0.513 (0.25)
♂ *A. leptodactylus*	12.35 (1.17)	24.88 (2.36)	0.373 (0.12)
Degree of significance	**	***	***
♀ *P. leniusculus*	12.59 (1.69)	26.49 (3.67)	0.503 (0.23)
♀ *A. leptodactylus*	12.19 (1.10)	24.59 (2.20)	0.348 (0.10)
Degree of significance	**	***	***

Numbers in ( ): SD of means, **: P<0.01, ***: P<0.001

Table 11.3 Mean carapace length, total length and body wet weight of the species and statistical analysis of data at the end of the second experiment

	Mean CL (mm)	Mean TL (mm)	Mean body wet weight (g)
♂ *P. leniusculus*	15.77 (3.06)	33.62 (6.58)	1.167 (0.86)
♂ *A. leptodactylus*	13.23 (2.01)	26.75 (4.15)	0.493 (0.30)
Degree of significance	***	***	***
♀ *P. leniusculus*	15.22 (2.89)	32.61 (6.05)	1.043 (0.65)
♀ *A. leptodactylus*	12.82 (2.22)	26.07 (4.52)	0.466 (0.34)
Degree of significance	***	***	

Numbers in ( ): SD of means, **: P<0.01, ***: P<0.001

Table 11.4 The survival and growth of *P. leniusculus* at 15 and 25 °C at the end of the experiment

Density	No of crayfish before	No of crayfish after	Mean CL (mm)	Mean body wet wei. (g)
Juveniles at 15 °C				
234 juveniles m^{-2}				
replicate 1	75	42	7.82	0.063 (0.011)
replicate 2	75	31	7.50	0.060 (0.010)
468 juveniles m^{-2}				
replicate 1	150	78	7.58	0.072 (0.013)
replicate 2	150	69	7.39	0.061 (0.010)
937 juveniles m^{-2}				
replicate 1	300	129	7.65	0.069 (0.011)
replicate 2	300	103	7.50	0.073 (0.012)
Crayfish at 25 °C				
234 juveniles m^{-2}				
replicate 1	75	30	11.41	0.312 (0.180)
replicate 2	75	27	11.29	0.291 (0.159)
468 juveniles m^{-2}				
replicate 1	150	58	11.25	0.288 (0.171)
replicate 2	150	40	10.97	0.269 (0.159)
937 juveniles m^{-2}				
replicate 1	300	96	10.37	0.220 (0.132)
replicate 2	300	81	10.32	0.233 (0.142)

Numbers in ( ): SD of means

Tables 11.5 The survival and growth of *A. leptodactylus* at 15 and 25 °C at the end of the experiment

Density	No of crayfish before	No of crayfish after	Mean CL (mm)	Mean body wet wei. (g)
Juveniles at 15 °C				
234 juveniles m^{-2}				
replicate 1	75	47	8.06	0.078 (0.012)
replicate 2	75	43	7.99	0.073 (0.010)
468 juveniles m^{-2}				
replicate 1	150	77	8.13	0.081 (0.014)
replicate 2	150	85	8.00	0.085 (0.011)
937 juveniles m^{-2}				
replicate 1	300	126	7.81	0.080 (0.011)
replicate 2	300	133	8.14	0.073 (0.010)
Crayfish at 25 °C				
234 juveniles m^{-2}				
replicate 1	75	33	11.56	0.328 (0.148)
replicate 2	75	41	11.15	0.307 (0.129)
468 juveniles m^{-2}				
replicate 1	150	67	11.27	0.286 (0.171)
replicate 2	150	54	11.03	0.245 (0.110)
937 juveniles m^{-2}				
replicate 1	300	99	10.03	0.239 (0.100)
replicate 2	300	106	10.46	0.225 (0.123)

Numbers in ( ): SD of means

Figure 11.1 Monthly water temperatures of the outside tanks during the first, second and third experiments

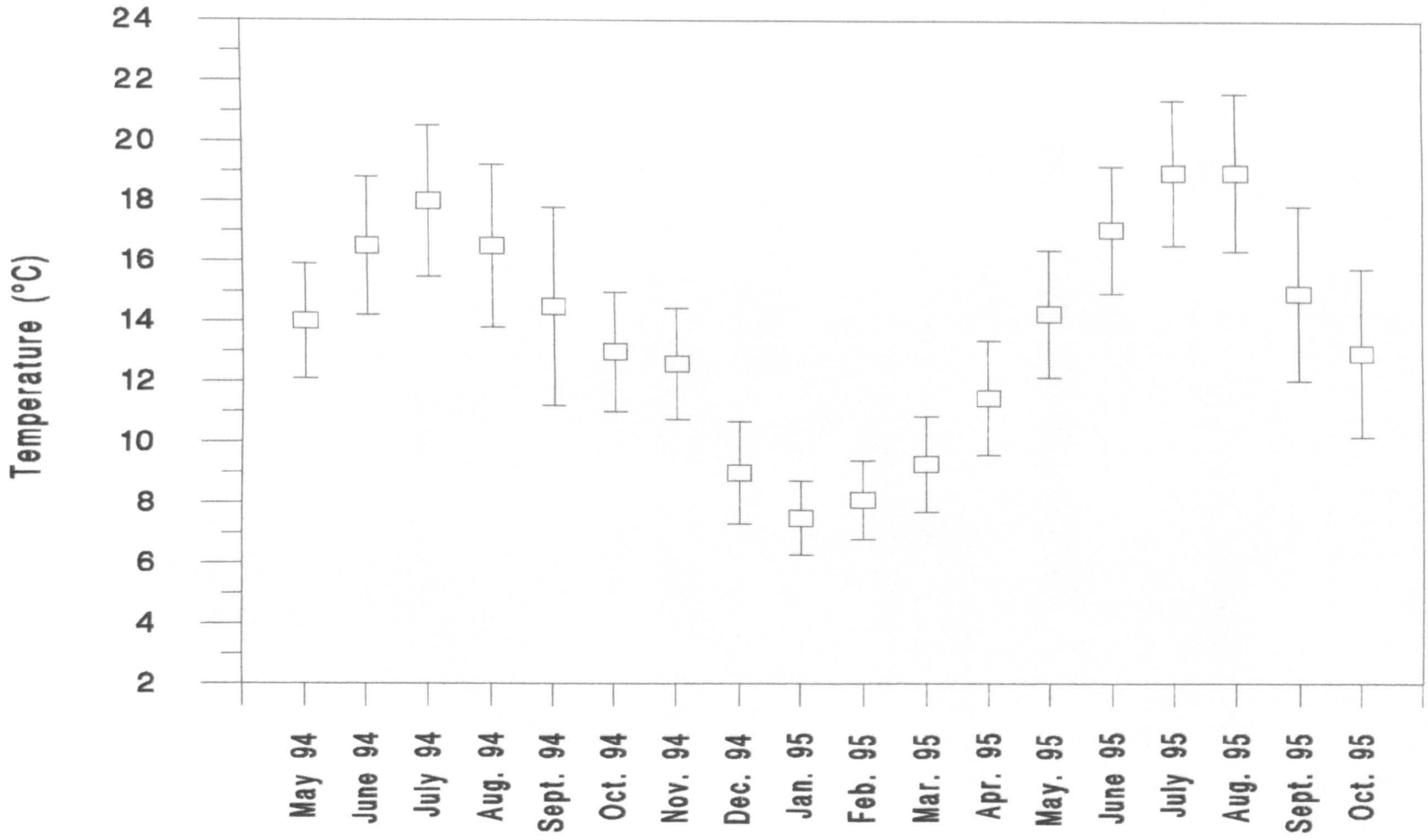


Figure 11.2 A comparison of body wet weight (versus carapace length) between P. leniusculus and A. leptodactylus at the end of the first experiment

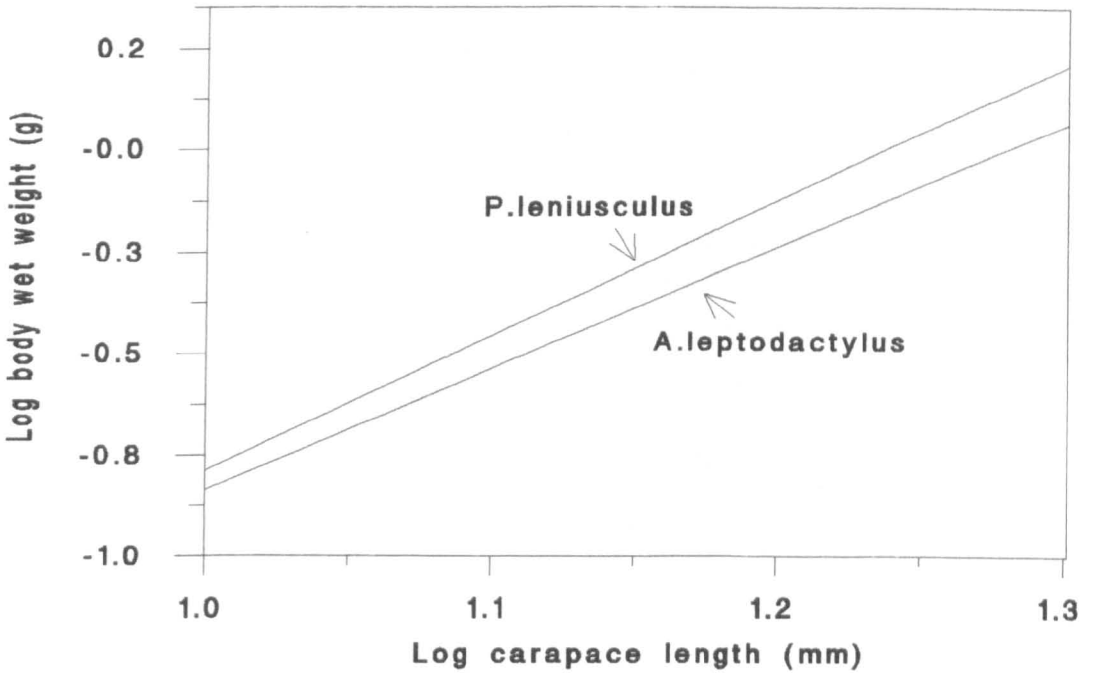


Figure 11.3 A comparison of body wet weight (versus carapace length) between P. leniusculus and A. leptodactylus at the end of the second experiment

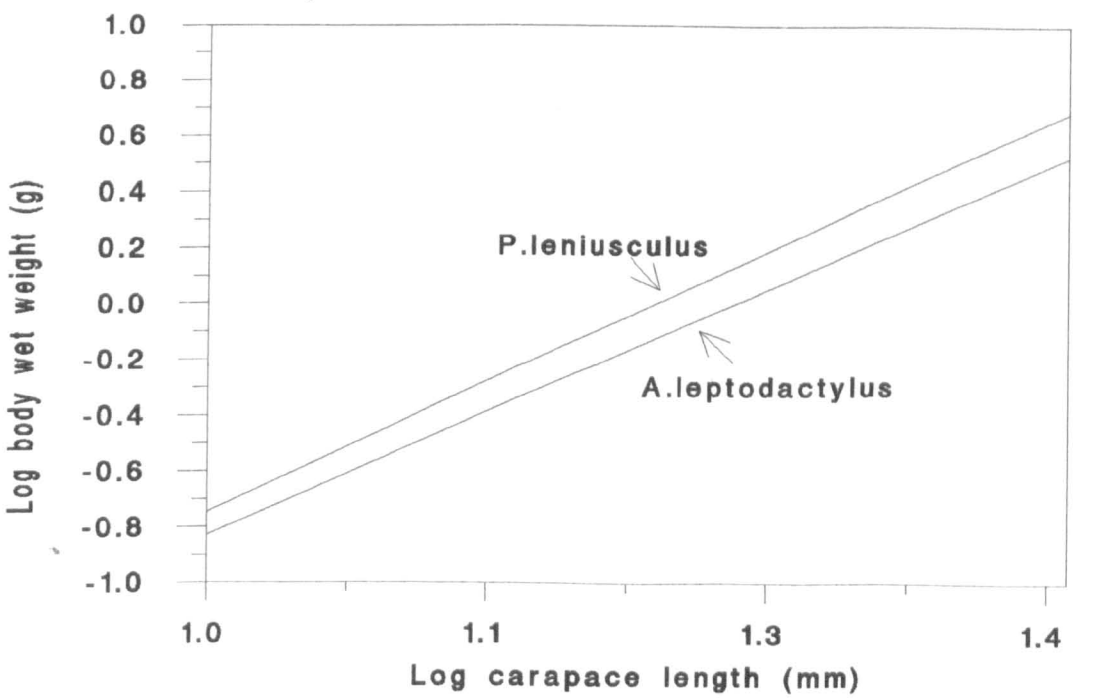


Figure 11.4 A comparison of body wet weight (versus carapace length) between P. leniusculus and A. leptodactylus when they were kept together (third experiment)

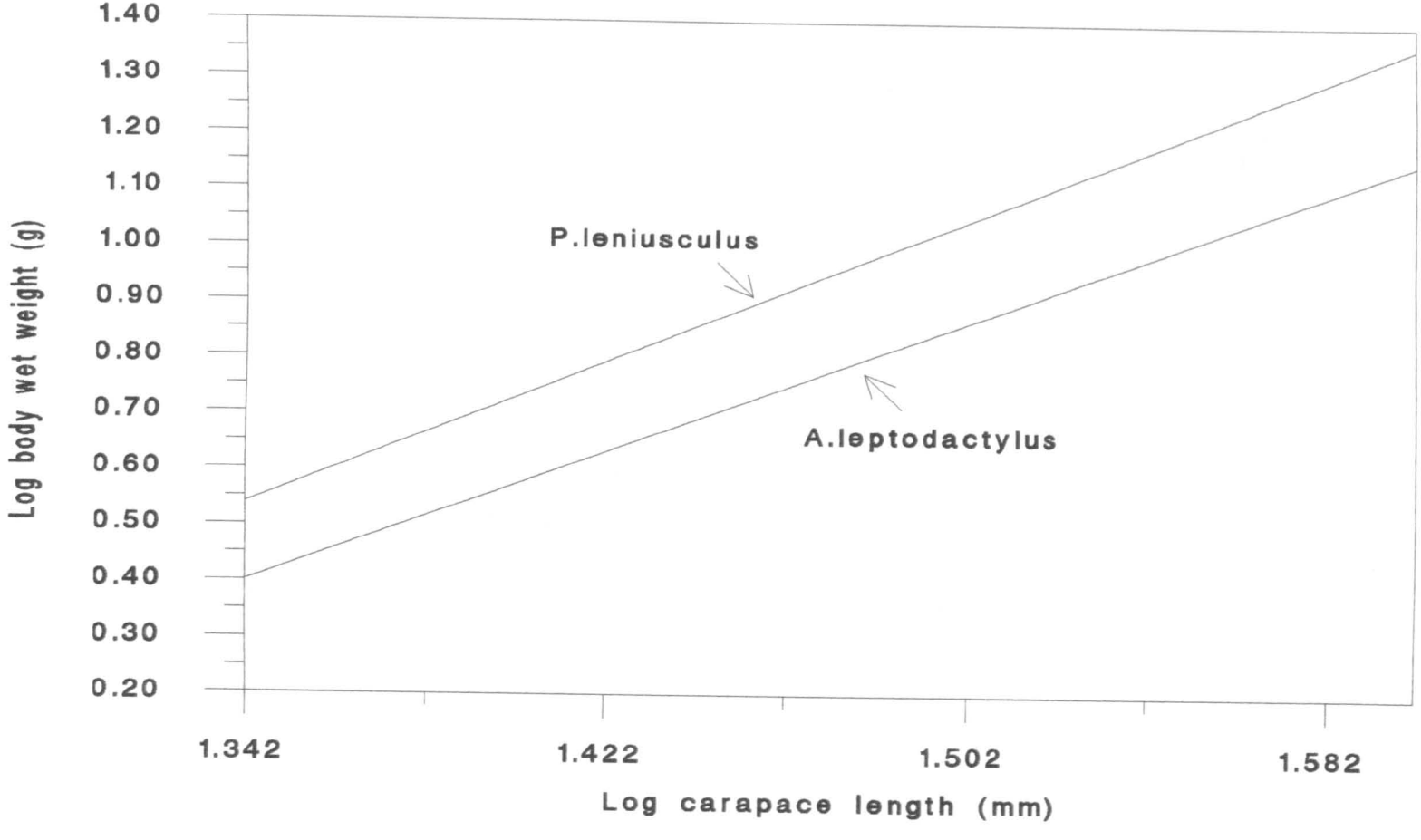


Figure 11.5 Smallest (9 mm CL) and largest (24 mm CL) juvenile *Pacifastacus leniusculus* at the end of the second experiment

Figure 11.6 Smallest (9 mm CL) and largest (24 mm CL) juvenile *Astacus leptodactylus* at the end of the second experiment

Chapter 12

Meat yield of wild populations

12.1 Introduction

In European countries, especially in France, Germany and Sweden, crayfish are consumed as a luxury food item (Goddard, 1988). They fetch high prices, especially in northern Europe. For instance, 1 kg of *Astacus astacus* may fetch US$ 100, and that of *Pacifastacus leniusculus*, *Astacus leptodactylus* and *Procambarus clarkii*, at US$ 70, 20, 11 respectively in Sweden (Holdich, 1993). The consumption of crayfish in that country is approximately 2000 tonnes annually (Huner, 1989).

The North American crayfish species, *P. clarkii* (the red swamp crayfish), comprises approximately 85% of total world crayfish production (70 000-100 000 tonnes in a year) (Huner, 1989; Holdich, 1993). Louisiana, Spain, China and Kenya are the main producers. However, in Europe, all of the native crayfish species especially populations of *Astacus astacus* and *Austropotamobius pallipes* have been diminishing rapidly (Holdich, 1993). Besides overfishing, pollution and waterway management (Holdich, 1988; Köksal, 1988), the main factor destroying crayfish populations in Europe is attributed to the crayfish plague fungus, *Aphanomyces astaci*, which first hit Europe in 1860. Before the crayfish plague appeared in Turkey and Russia, *A. leptodactylus*, the narrow-clawed crayfish, was a valuable commercial species in Europe. Until 1986 it was being exported regularly to Western Europe from Turkey (Baran *et al.*, 1987; Köksal, 1988). The loss of commercially important populations has led to the introduction of a number of alien crayfish species into Europe to

supplement the fishery, including *P. clarkii* and *P. leniusculus*, both of which are relatively immune to crayfish plague but which can act as vectors of the disease (Holdich *et al.*, 1995b).

When considering crayfish for commercial introduction, the quality and quantity of meat yield is as important as their ecological adaptability. Although *P. leniusculus* was introduced into Europe many years ago, there is only an estimated value for its meat yield (Lee and Wickins, 1992). There have also only been a few studies of this nature on *A. leptodactylus*. This present study compares the differences of tail (abdomen) and claw (cheliped) meat yield between males and females of the same species, between *P. leniusculus* and *A. leptodactylus*, the differences in the meat yield in different seasons (winter and summer), and between crayfish from different sites. For comparative purposes, the meat yield of *P. clarkii* was also calculated.

12.2 Materials and methods

Pacifastacus leniusculus and *Astacus leptodactylus* specimens were captured during the winter and summer months of 1993 and 1994 using demersal traps and seine nets. In this way it was hoped to compare the meat yield at the end of their active feeding season and after overwintering.

A total of 100 adult *P. clarkii* (50 males and 50 females were imported from Louisiana), a total of 144 adult male and female *A. leptodactylus* were collected from the Serpentine (43 males in winter and 30 males in summer; 40 females in winter and 31 females in summer) and 88 adult male *A. leptodactylus* were collected from Tykes

Water (40 in winter and 48 in summer). A total of 181 adult males and females were collected from Boxmoor Fishery (63 males in winter and 55 males in summer; 37 females in winter and 26 females in summer) and 55 adult male *P. leniusculus* were collected from Owston Brook (Leicestershire) (in winter).

To compare females and males the specimens of *P. leniusculus* from Boxmoor and the specimens of *A. leptodactylus* from the Serpentine were mainly used because both males and females of the two species were available for winter and summer.

All crayfish were frozen after each had been sorted by sex. For meat analysis, frozen crayfish were cooked for 10 minutes in boiling water (Huner, 1993). After that individuals with all their appendages were dried externally, weighed in grams to two decimal places, and measured (according to Rhodes and Holdich, 1979).

Finally, the claws and tail of the crayfish were cut with scissors and the meat removed with forceps. The tail meat and claw meat were weighed in grams to two decimal places. After weighing the wet meat yield, they were dried at 60 °C for 18 hours and reweighed, and the water content determined (Rhodes & Holdich, 1984).

In order to observe whether meat yields (abdomen, claw and total) increase at a greater rate than the cube of the carapace length in *P. leniusculus*, *A. leptodactylus* and *P. clarkii*, slopes were investigated by applying regression analysis of log transformed variables in the form: $\log y = \log (a) + \log (b) x$. The value of the constant b will be > 3.0 when the meat yields increase at a greater rate than the cube of the carapace length, and this relation is isometric when the constant b is equal to 3.0.

Because there was not a significant difference in the coefficient of determination (r^2) between actual data and log transformed data, only the coefficient of determination (r^2) of the actual data was considered when producing Figures 12.1, 12.2 and 12.3 which compare abdomen, claw and total meat yield of the three species.

12.3 Results

For males, because slopes are > 3, then the total meat and claw meat of the species increases at a greater rate than the cube of the carapace length (Table 12.1). For females, although the abdomen meat yield of *P. leniusculus* and *A. leptodactylus* does not increase at a greater rate than the cube of the carapace length (slopes are < 3), that of *P. clarkii* does (slope is > 3). (Claw meat yield of female *P. clarkii* was not available for comparison). In addition, as was observed for males, the claw meat yield of female *P. leniusculus* and *A. leptodactylus* also increases significantly with size (slopes are > 3) (Table 12.2).

A two sample t-test was used in order to compare abdomen, claw and total meat yield between and within the species. A statistical analysis of the differences in the total, claw and tail wet meat yield between males and females (collected in summer or winter) in the same species is given in Table 12.3, between species in Tables 12.4 (for females) and 12.5 (for males), between seasons for the same sex in Table 12.6, and between sites in Table 12.7. In addition, water content in abdomen meat (%), and abdomen, claw and total meat yield-body wet weight (%) are given in Table 12.8.

Comparison of meat yield between male and female *Pacifastacus leniusculus*

The results show that there is no significant difference in the abdomen meat yield between similar sized males and females ($P>0.05$) collected in winter, but the claw meat yield of male *P. leniusculus* was significantly greater than that of female *P. leniusculus* ($P<0.001$), which results in significantly more total meat yield in males ($P<0.001$). For summer crayfish, as was observed for winter crayfish, significantly more claw meat yield was found in male *P. leniusculus* ($P<0.001$). In addition, slightly more abdomen meat yield was also produced by male *P. leniusculus* collected in summer ($P<0.05$) (Table 12.3).

Comparison of meat yield between male and female *Astacus leptodactylus*

For a given size range ($P>0.05$), abdomen and claw meat yield of male *A. leptodactylus* were significantly greater than those of females collected in winter ($P<0.01$ for abdomen meat and $P<0.001$ for claw meat). However, in summer, the females produced significantly more abdomen meat ($P<0.01$), whilst the males produced significantly more claw meat yield ($P<0.01$). Overall though there was no difference in total meat yield between the sexes in summer.

Comparison of abdomen meat yield between male and female *Procambarus clarkii*

There was a significant difference in abdomen meat yield in favour of females ($P<0.01$) (Table 12.3). For similar sized crayfish (mean CL= 52.24 for females and 51.86 for males), the males had a mean of 3.38 g (s.e.= 0.16) and females had a

mean of 4.12 g (s.e.= 4.12) of abdomen meat.

Differences between seasons for the same sex

A comparison was also made of the differences of abdomen, claw and total meat yield of the same sex between seasons. In males of *P. leniusculus* and *A. leptodactylus* (for a given size range), no significant differences were observed in the abdomen, claw and total meat yield between crayfish collected in summer and those collected in winter ($P>0.05$ for all cases) (Table 12.6).

In females of *P. leniusculus* and *A. leptodactylus* (for a given size range), although there was no significant difference in the abdomen meat yield between female *P. leniusculus* collected in summer and those collected in winter ($P>0.05$), the abdomen meat yield of *A. leptodactylus* collected in summer was significantly higher than that of *A. leptodactylus* collected in winter ($P<0.001$). With regard to claw meat, although there was no significant difference in the claw meat yield between *A. leptodactylus* collected in summer and collected in winter ($P>0.05$), in *P. leniusculus* the claw meat yield was significantly higher for winter animals ($P<0.001$) (Table 12.6). This difference may come from the variability of chelae size of *P. leniusculus*.

Comparison of abdomen and claw meat yield

In order to compare abdomen and claw meat yield, females collected in winter and summer, and males collected in winter and summer were pooled. Results show that female and male *A. leptodactylus* and female *P. leniusculus* have significantly more

meat in the abdomen (P<0.001) in comparison to the meat in the claw, but males of *P. leniusculus* have significantly more claw meat yield (P<0.001) in comparison to their abdomen meat yield. Percentage meat yield per body wet weight of the species is given in Table 12.8.

Comparison of males between species

In the males of *P. leniusculus* and *A. leptodactylus* no significant differences were found in the abdomen, claw and total meat yield between crayfish collected in winter and summer (P>0.05) (Table 12.6), consequently the samples were pooled in order to make a comparison between species.

Although claw and total meat yield of *P. leniusculus* were significantly higher (P<0.001) than those of *A. leptodactylus*, the abdomen meat yield was significantly higher in favour of *A. leptodactylus* (P<0.05). A similar result was also obtained in the comparison of *P. leniusculus* from Boxmoor Fishery and *A. leptodactylus* from Tykes Water (Table 12.5).

A comparison with similar sized males of *P. clarkii*, *P. leniusculus* and *A. leptodactylus* (P>0.05) showed that there was no significant differences in the abdomen, claw and total meat yield between *P. clarkii* and *A. leptodactylus* (P>0.05), but *P. leniusculus* had significantly more claw and total meat yield than *P. clarkii*. Figures 12.1, 12.2 and 12.3 show the differences in the abdomen, claw and total meat yield between *P. clarkii*, *P. leniusculus* and *A. leptodactylus*.

Comparison of females between species

There were no significant differences in the total meat yield between similar sized female *P. leniusculus* and *A. leptodactylus* ($P>0.05$) collected in either winter or summer. However, female *A. leptodactylus* produced significantly more abdomen meat yield in both seasons, particularly in summer ($P<0.01$ for winter and $P<0.001$ for summer) whilst *P. leniusculus* produced significantly more claw meat, particularly in winter ($P<0.01$ for summer and $P<0.001$ for winter).

Only winter crayfish were available for *P. clarkii* to compare with *P. leniusculus* and *A. leptodactylus*. A comparison with similar sized females of *P. leniusculus* and *A. leptodactylus* ($P>0.05$) shows that *P. clarkii* produces more abdominal meat in comparison to *A. leptodactylus* ($P<0.01$), and *P. leniusculus* ($P<0.001$).

Comparison of sites

For *P. leniusculus*, no significant differences were observed in the abdomen, claw and total meat yield between crayfish collected from Boxmoor and Owston Brook ($P>0.05$ for all cases).

For *A. leptodactylus*, although there were no significant differences in the total meat yield between crayfish collected from the Serpentine and Tykes Water ($P>0.05$), abdomen meat yield was significantly greater for crayfish from Tykes Water ($P<0.01$), but claw meat yield was significantly greater in crayfish from the Serpentine ($P<0.05$) (Table 12.7). Overall though there was no significant difference in the total meat yield

between crayfish collected from the Serpentine and Tykes Water.

Water content in abdomen meat yield

There were no significant differences in the water content of abdomen meat yield between males and females in *P. leniusculus* and *A. leptodactylus*. The abdomen meat of female and male *P. leniusculus* consisted of 78.03 and 77.76% water, whilst those in *A. leptodactylus* were 78.37 and 81.04% respectively.

12.4 Discussion and conclusions

The main edible meat product of crayfish is the tail meat (abdominal muscle), the amount depending on maturity and size. That of astacid and cambarid crayfish contributes 10-40% to total body weight (Huner, 1989; Lee and Wickins, 1992). For example, *A. leptodactylus* contains 15-23% meat (Köksal in Lee and Wickins, 1992), 14.4% for females and 16.5% for males (Dabrowski *et al.*, 1966b); *Austropotamobius pallipes* 14.43% for males, 15.73% for females (Rhodes and Holdich, 1984); *Procambarus* spp. 11-25% (Huner, 1993); and *P. leniusculus* 15-25% (an estimated value) (Lee and Wickins, 1992); *A. astacus* 19.6% for males and 17.5% for females (Lindqvist & Louekari, 1975); *Orconectes limosus* 24.3% for females and 24.1% for males (Dabrowski *et al.*, 1966a). In addition, in the parastacids, *Cherax destructor*, *C. tenuimanus* and *C. quadricarinatus*, 7.7-17.4, 22-30 and 22% of total body weight respectively is comprised of meat (Jones in Lee and Wickins, 1992). Moreover, in a different study, according to Sokol (in Lee and Wickins, 1992) the meat yield of *Cherax destructor* is 25% of total body weight. Similarly, Morrissy (in Rhodes and

Holdich, 1984) has pointed out that *Cherax tenuimanus* produces a meat yield of 42% total body weight from the tail and an additional 19% from the cheliped. The latter figures are, however, exaggerated by including the exoskeleton in the weight (Holdich, 1993).

The total meat yield produced by male *P. leniusculus* is significantly higher than that of *A. leptodactylus* or *P. clarkii*. However, the percentage of total meat yield in the body wet weight of male *P. leniusculus* is slightly higher than that of *A. leptodactylus*, but is lower than that of *P. clarkii*. This is because the body weight of *P. leniusculus* is considerably heavier than that of *A. leptodactylus* or *P. clarkii*. For example, a mean of 7.49 g total meat yield of male *P. leniusculus* takes 13.72% of body wet weight, whereas a mean of 4.38 g total meat yield of male *P. clarkii* takes 16.81% of body wet weight. Similarly, a mean of 3.29 g total meat yield of male *A. leptodactylus* takes 11.45% of body wet weight. For the abdomen meat yield of females, a mean of 4.11 g abdomen meat yield for *P. clarkii* takes 14.49% of body wet weight, a mean of 2.91 g abdomen meat yield of *A. leptodactylus* takes 11.15% of body wet weight and a mean of 2.76 g abdomen meat yield of *P. leniusculus* takes 5.46% of body wet weight.

Different results for the amount of muscle per body weight or muscle per body length in the same species from different studies may have resulted from using different methods such as time of cooking and size range of samples. Before the tail meat was removed, the specimens were not cooked by Dabrowski *et al.* (1966 a), Rhodes and Holdich (1984), Huner *et al.* (1988), Lahti (1988) and Gu *et al.* (1994). Lindqvist & Louekari (1975), Köksal (1988) and Lutz and Wolters (1989) cooked the specimens for five minutes, whilst Mikkola (1978) and Huner (1993) cooked the specimens for ten

minutes. In addition, Steward *et al.* (1967) (in Rhodes and Holdich, 1984) claimed that feeding specimens prior to examination can increase the meat yield.

In order to observe the meat yield in *Procambarus clarkii*, Mikkola (1978) examined 36 females (average 90 mm body length) and 28 males (average 79 mm body length). The females had a mean of 4.4 g (±1.6) and males 2.8 g (±1.5) of abdomen meat. Thus, Mikkola (1978) has stated that female *Procambarus clarkii* consist of more tail meat than males. In the present study similar results have been found for females (mean 4.12 g, s.e.= 0.22, for crayfish averaging 52.24 mm CL and 103.98 mm body length), but the abdomen meat yield of the males is higher (mean 3.38 g, s.e.= 0.16 and average 51.86 mm CL and 101.42 mm body length) than Mikkola's results. This is probably due to the fact that bigger males were used in this study.

Sexual dimorphism has been observed in the size of the abdomen and chelipeds (claws) in many crayfish species (see Chapter 10). In general, male crayfish have larger chelipeds and total meat yields than females, and female crayfish have a bigger abdomen, thus producing more tail meat than males (Goddard, 1988). According to Rhodes and Holdich (1984), the cheliped meat yield between male and female *Austropotamobius pallipes* is significantly different. The maximum cheliped meat in males is 5.13 g, but that of females is only 2.52 g. Huner *et al.* (1988) found that the females of *A. astacus* and *P. clarkii* produce more abdominal muscle than the males. This was also observed for *P. clarkii* by D'abramo and Niquette (1991) and for *Cherax quadricarinatus* by Gu *et al.* (1994).

In the present study, the cheliped meat yield between male and female *P. leniusculus* and *A. leptodactylus* was found to be significantly different in favour of males in both seasons. For example, for *P. leniusculus* collected in summer, the males had a mean yield of 4.67 g (s.e.= 0.49) and females 1.28 g (s.e.= 0.12) of cheliped meat for a similar size range. The yield for *A. leptodactylus* was 0.97 g (s.e.= 0.07) for males and 0.67 (s.e.= 0.04) for females.

However, although sexual dimorphism appears in the abdomen length in *Astacus astacus*, the tail meat yield of males and females are similar (Lindqvist and Louekari, 1975). Similarly, according to Köksal (1988) the yield of the tail meat for male and female *A. leptodactylus* is nearly the same. She found that males produced on average 4.25 g of tail meat (minimum 3 g and maximum 12 g from 80 mm to 120 mm total length) and that of females was 4.41 g (minimum 2 g and maximum 12 g, from 80 mm to 132 mm total length). With respect to cheliped meat, for the same size of the males and females as mentioned above, males have 0.8-8.9 g (mean 2.29) and females have 0.63-6 g (mean 1.42) cheliped meat. Males and females of *A. leptodactylus* under 100 mm total length, were found to have similar amounts of cheliped muscle. In the present study, for the summer sample of *A. leptodactylus* similar results were found to Köksal's study. The females of *A. leptodactylus* collected in summer had more abdomen meat but less claw meat than the males. For the size range of 41-54 mm CL, the females had a mean of 2.91 g (s.e.= 0.11) and the males 2.32 g (s.e.= 0.08) of abdomen meat, and the females had a mean of 0.67 g (s.e.= 0.04) and the males 0.97 g (s.e.= 0.07) of claw meat. However, the females of *A. leptodactylus* collected in winter had less abdomen meat than the males for the size range 47-66 mm CL (mean abdomen meat yield for females= 2.53 g (s.e.= 0.13) and for males= 3.26 (s.e.= 0.12)).

The results show that although there is no significant differences in the meat yield (abdomen, claw and total) in male *A. leptodactylus* and in male *P. leniusculus* collected in winter and in summer, and between the males of the same species from different populations, significant differences do occur in the abdomen meat yield of female *A. leptodactylus* and in the claw meat yield of female *P. leniusculus*. The reason for these differences are unknown but could be due to differential growth rates (Lowery, 1988).

A number of studies have also been carried out in order to observe the water content of abdomen meat of crayfish. In *Orconectes limosus* the percentage water content was found to be 79.12% for the males and 81.07 for the females by Dabrowski *et al.* (1966a). In other studies this was found to be 84.23% and 82.54% for the males and females of *A. pallipes* (Rhodes and Holdich, 1984), 83.11% and 83.55% for the males and females of *A. astacus*, and 83.05% and 83.41 for the males and females of *A. leptodactylus* (Dabrowski *et al.*, 1968). In the present study, it was observed that the water content of abdomen meat of *P. clarkii* was lower than that of *A. leptodactylus* and *P. leniusculus*. The percentage water content in abdomen meat was found to be 73.69 and 74.43 for female and male *P. clarkii*, 78.03 and 77.46 for female and male *P. leniusculus*, and 78.37 and 81.04 for female and male *A. leptodactylus*.

Apart from the tail meat, some crayfish species have large claws which may contain as much or more meat than the tail. For example, *P. leniusculus* has heavy claws in comparison with most species (Goldman *et al.*, 1975). In this study, it was observed that male *P. leniusculus* has considerably more cheliped meat yield and total meat yield than *A. leptodactylus* and *P. clarkii*. However, claw meat is much more difficult

to extract than tail meat. Peeling machines for tail meat have been developed (Avault *et al.*, 1975). Extraction methods to recover claw meat have also been used and more than 80% of crayfish meat can be extracted by using the partial extraction method (Meyers, 1985). It seems that *P. leniusculus* is one of the best species to extract cheliped meat from.

Table 12.1 Slopes (constant "b" in the formulae) of abdomen, claw and total meat (log) versus carapace length (log) in male *P. leniusculus*, *A. leptodactylus* and *P. clarkii*

	log y= log (a) + log (b) x	r^2
P. leniusculus		
Abdomen meat	-2.55920 + 1.74930 x	0.648
Claw meat	-6.50034 + 4.13678 x	0.785
Total meat	-4.70679 + 3.23220 x	0.821
A. leptodactylus		
Abdomen meat	-4.19252 + 2.76156 x	0.814
Claw meat	-8.95811 + 5.33225 x	0.761
Total meat	-5.28474 + 3.49370 x	0.844
P. clarkii		
Abdomen meat	-3.05619 + 2.06896 x	0.917
Claw meat	-7.73185 + 4.67098 x	0.531
Total meat	-4.43702 + 3.00033 x	0.775

Table 12.2 Slopes (constant "b" in the formulae) of abdomen, claw and total meat (log) versus carapace length (log) in female *P. leniusculus* and *A. leptodactylus*, and slopes of abdomen (log) versus carapace length (log) in female *P. clarkii*

	log y= log (a) + log (b) x	r^2
P. leniusculus		
Abdomen meat	-3.65506 + 2.36730 x	0.715
Claw meat	-6.53339 + 3.90379 x	0.634
Total meat	-4.24532 + 2.82932 x	0.762
A. leptodactylus		
Abdomen meat	-3.78812 + 2.52528 x	0.855
Claw meat	-5.61903 + 3.23261 x	0.593
Total meat	-3.92235 + 2.65976 x	0.887
P. clarkii		
Abdomen meat	-4.70952 + 3.08892 x	0.842

Table 12.3 A comparison of mean abdomen, claw and total meat yield between males and females in *P. leniusculus*, *A. leptodactylus* and *P. clarkii* collected in summer and winter

	No of crayfish	Size range CL (mm)	Mean length	Abdomen meat yield (g)	Claw meat yield (g)	Total meat yield (g)
Female *P. leniusculus*	37	44-68	53.03 (0.93)	2.76 (0.14)	2.65 (0.16)	5.42 (0.27)
Male *P. leniusculus*	54	44-68	54.24 (0.83)	3.03 (0.10)	4.46 (0.30)	7.50 (0.37)
Degree of significance (collected in winter)			NS	NS	***	***
Female *P. leniusculus*	26	38-63	48.73 (1.2)	2.28 (0.15)	1.28 (0.12)	3.57 (0.25)
Male *P. leniusculus*	29	41-62	51.83 (1.1)	2.75 (0.14)	4.67 (0.49)	7.43 (0.60)
Degree of significance (collected in summer)			NS	*	***	***
Female *A. leptodactylus*	23	47-66	53.00 (1.30)	2.53 (0.13)	1.01 (0.09)	3.54 (0.20)
Male *A. leptodactylus*	37	48-66	55.43 (0.80)	3.26 (0.12)	2.74 (0.26)	6.00 (0.35)
Degree of significance (collected in winter)			NS	**	***	***
Female *A. leptodactylus*	27	41-54	47.96 (0.67)	2.91 (0.11)	0.67 (0.04)	3.59 (0.15)
Male *A. leptodactylus*	27	41-54	46.15 (0.66)	2.32 (0.08)	0.97 (0.07)	3.29 (0.14)
Degree of significance (collected in summer)			NS	**	**	NS
Female *P. clarkii*	50	43-67	52.24 (0.73)	4.12 (0.22)	Data not avai.	Data not avai.
Male *P. clarkii*	50	41-64	51.86 (0.74)	3.38 (0.16)		
Degree of significance (collected in winter)			NS	**		

NS: P>0.05, *: P<0.05, **: P>0.01, ***: P>0.001, and values in ( ): standard error of means

Table 12.4 A comparison of mean abdomen, claw and total meat yield between females of *P. leniusculus*, *A. leptodactylus* and *P. clarkii* collected in summer and winter

	No of crayfish	Size range CL (mm)	Mean length	Mean abdomen meat yield (g)	Mean Claw meat yield (g)	Total meat yield (g)
Female *P. leniusculus* Female *P. clarkii* Degree of significance (collected in winter)	21 48	43-63 43-61	50.71 (1.20) 51.63 (0.61) NS	2.47 (0.15) 3.91 (0.16) ***	Data not avai.	Data not avai.
Female *A. leptodactylus* Female *P. clarkii* Degree of significance (collected in winter)	25 32	45-54 44-54	48.48 (0.62) 49.56 (0.45) NS	2.98 (0.10) 3.41 (0.09) **	Data not avai.	Data not avai.
Female *P. leniusculus* Female *A. leptodactylus* Degree of significance (collected in summer)	22 31	38-55 38-54	46.82 (1.00) 46.77 (0.81) NS	2.08 (0.14) 2.74 (0.14) **	1.17 (0.13) 0.64 (0.04) **	3.26 (0.24) 3.38 (0.16) NS
Female *P. leniusculus* Female *A. leptodactylus* Degree of significance (collected in winter)	26 27	44-64 44-63	51.69 (1.10) 51.22 (1.10) NS	2.61 (0.16) 4.51 (0.11) ***	2.40 (0.17) 1.22 (0.11) ***	5.02 (0.31) 5.73 (0.35) NS

NS: P>0.05, *: P<0.05, **: P>0.01, ***: P>0.001

Table 12.5 A comparison of mean abdomen, claw and total meat yield between males of *P. leniusculus*, *A. leptodactylus* and *P. clarkii*

	No of crayfish	Size range CL (mm)	Mean CL length	Mean abdomen meat yield (g)	Mean claw meat yield (g)	Mean total meat yield (g)
Male *P. leniusculus*	66	41-74	54.15 (1.1)	2.79 (0.11)	5.69 (0.57)	8.49 (0.66)
Male *A. leptodactylus* (from the Serpentine)	65	41-74	54.34 (1.0)	3.21 (0.12)	3.11 (0.44)	6.32 (0.54)
Degree of significance			NS	*	***	**
Male *P. leniusculus*	77	41-63	51.61 (0.7)	2.82 (0.1)	4.24 (0.2)	7.06 (0.3)
Male *A. leptodactylus* (from Tykes Water)	85	41-63	50.14 (0.4)	3.28 (0.1)	1.47 (0.1)	4.76 (0.2)
Degree of significance			NS	***	***	***
Male *P. leniusculus*	54	43-54	48.67 (0.5)	2.48 (0.1)	3.20 (0.1)	5.69 (0.3)
Male *P. clarkii*	12	43-54	48.83 (1.3)	2.76 (0.1)	1.63 (0.2)	4.40 (0.3)
Degree of significance			NS	NS	***	**
Male *A. leptodactylus*	74	43-54	49.03 (0.3)	3.04 (0.1)	1.22 (0.1)	4.27 (0.1)
Male *P. clarkii*	12	43-54	48.83 (1.3)	2.767 (0.1)	1.63 (0.2)	4.40 (0.3)
Degree of significance			NS	NS	NS	NS

NS: P>0.05, *: P<0.05, **: P>0.01, ***: P>0.001

Table 12.6 A comparison of mean abdomen, claw and total meat yield between crayfish collected in winter and summer

	No of crayfish	Size range CL (mm)	Mean CL length	Mean abdomen meat yield (g)	Mean claw meat yield (g)	Mean total meat yield (g)
Female *P. leniusculus* winter	28	44-63	51.64 (1.00)	2.58 (0.15)	2.40 (0.16)	5.00 (0.29)
Female *P. leniusculus* summer	20	44-63	51.10 (1.20)	2.50 (0.16)	1.50 (0.11)	4.01 (0.25)
Degree of significance			NS	NS	***	*
Female *A. leptodactylus* winter	31	39-54	46.13 (0.58)	1.91 (0.09)	0.63 (0.04)	2.54 (0.13)
Female *A. leptodactylus* summer	30	39-54	47.07 (0.78)	2.78 (1.12)	0.65 (0.04)	3.44 (0.15)
Degree of significance			NS	***	NS	***
Male *P. leniusculus* winter	63	39-73	55.65 (0.90)	3.25 (0.11)	6.31 (0.51)	9.56 (0.51)
Male *P. leniusculus* summer	55	39-73	53.85 (1.00)	2.99 (0.10)	5.56 (0.64)	8.55 (0.72)
Degree of significance			NS	NS	NS	NS
Male *A. leptodactylus* winter	40	41-62	50.30 (0.7)	3.27 (0.1)	1.39 (0.1)	4.67 (0.2)
Male *A. leptodactylus* summer	48	38-63	49.50 (0.6)	3.21 (0.1)	1.49 (0.1)	4.70 (0.2)
Degree of significance (collected from Tykes Water)			NS	NS	NS	NS
Male *A. leptodactylus* winter	18	45-62	52.22 (0.79)	2.89 (0.14)	2.01 (0.25)	4.90 (0.36)
Male *A. leptodactylus* summer	19	45-62	49.63 (1.10)	2.67 (0.14)	1.40 (0.19)	4.07 (0.30)
Degree of significance (collected from Serpentine)			NS	NS	NS	NS

NS: $P>0.05$, *: $P<0.05$, **: $P>0.01$, ***: $P>0.001$

12.7 Comparison of mean abdomen, claw and total meat yield between populations

	No of crayfish	Size range CL (mm)	Mean CL length	Mean abdomen meat yield (g)	Mean claw meat yield (g)	Mean total meat yield (g)
for *A. leptodactylus*						
from Tykes Water	87	41-63	50.00 (0.48)	3.26 (0.11)	1.45 (0.09)	4.72 (0.20)
from the Serpentine	64	41-63	51.17 (0.72)	2.83 (0.09)	1.87 (0.16)	4.70 (0.24)
Degree of significance			NS	**	*	NS
for *P. leniusculus*						
from Owston Brook	51	44-71	54.78 (0.87)	3.29 (0.13)	5.70 (0.40)	8.99 (0.50)
from Boxmoor	104	44-71	55.64 (0.66)	3.19 (0.08)	6.07 (0.39)	9.26 (0.45)
Degree of significance			NS	NS	NS	NS

NS: P>0.05, *: P<0.05, **: P>0.01, ***: P>0.001

12.8 Water content in abdomen meat (%), and abdomen, claw and total meat yield-body wet weight (%) in *P. leniusculus*, *A. leptodactylus* and *P. clarkii*

	No of crayfish	Size range CL (mm)	Mean CL length	Mean body (wet) weight (g)	Water in abdomen meat (%)	Abdomen meat-body (wet) weight (%)	Claw meat-body (wet) weight (%)	Total meat-body (wet) weight (%)
Female *P. leniusculus*	37	44-68	53.03	50.66	78.03	5.46	5.23	10.69
Male *P. leniusculus*	54	44-68	54.24	54.62	77.46	5.56	8.16	13.72
Female *A. leptodactylus*	27	41-54	47.96	26.11	78.37	11.15	2.59	13.74
Male *A. leptodactylus*	27	41-54	46.15	28.75	81.04	8.07	3.38	11.45
Female *P. clarkii*	50	43-67	52.24	28.43	73.69	14.49	data not available	data not available
Male *P. clarkii*	50	41-64	51.86	31.13	74.43	10.85		
Male *P. clarkii*	12	43-54	48.83	26.11	74.63	10.57	6.24	16.81

Figure 12.1 Differences in the abdomen meat (wet) yield between male P. leniusculus (from Boxmoor), A. leptodactylus (from Tykes Water) and P. clarkii

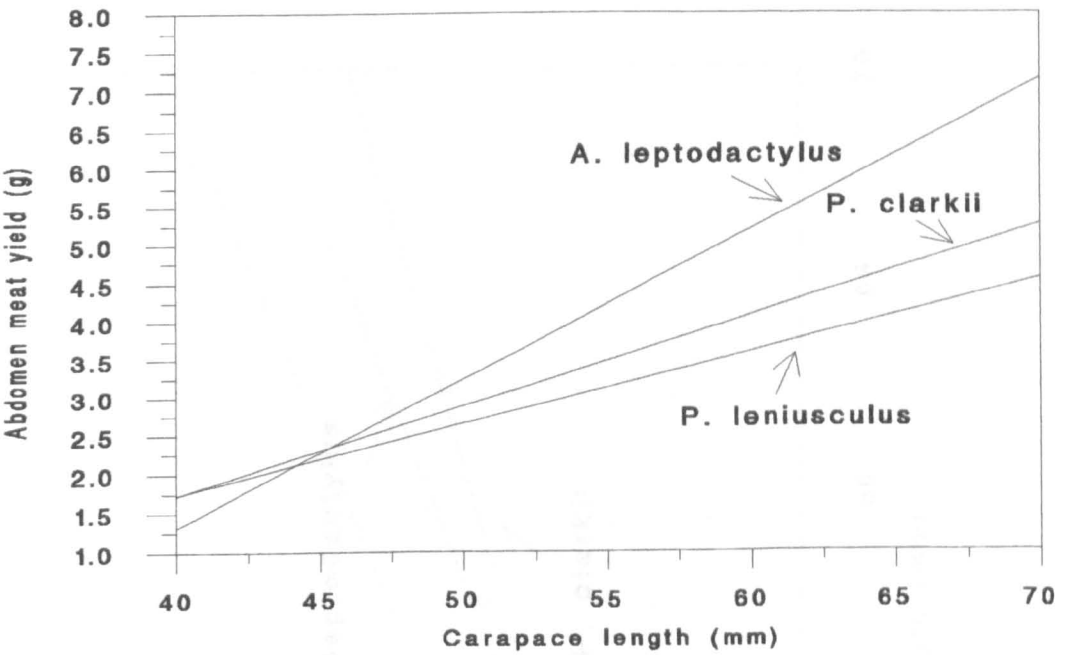


Figure 12.2 Differences in the claw meat (wet) yield between male P. leniusculus (from Boxmoor), A. leptodactylus (from Tykes Water) and P. clarkii

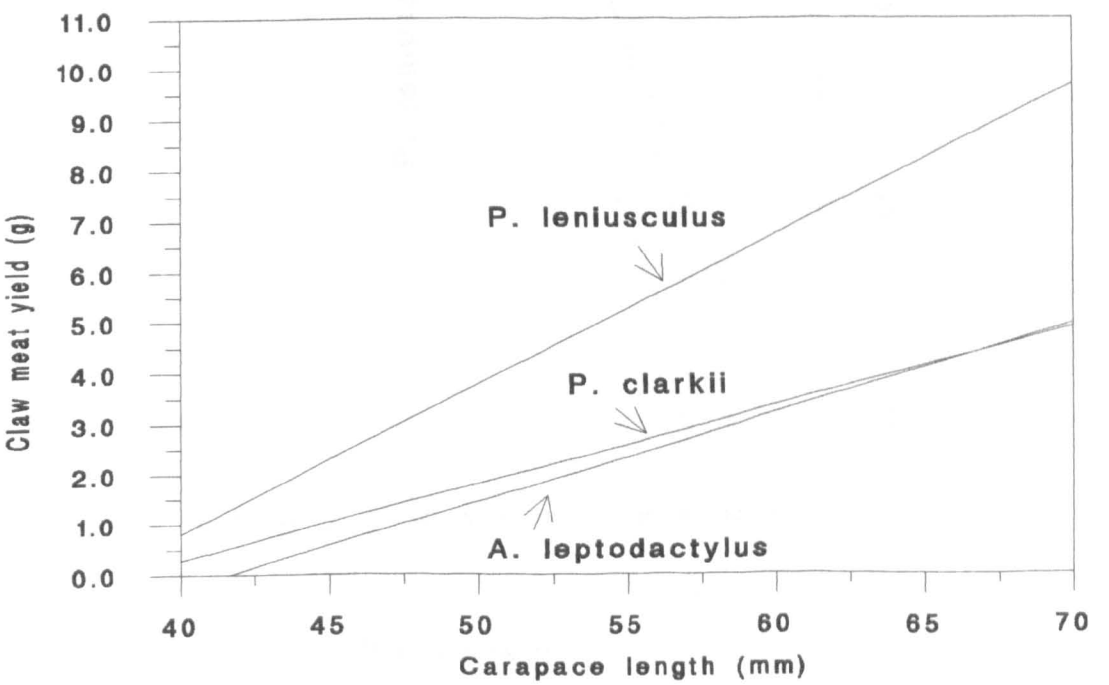
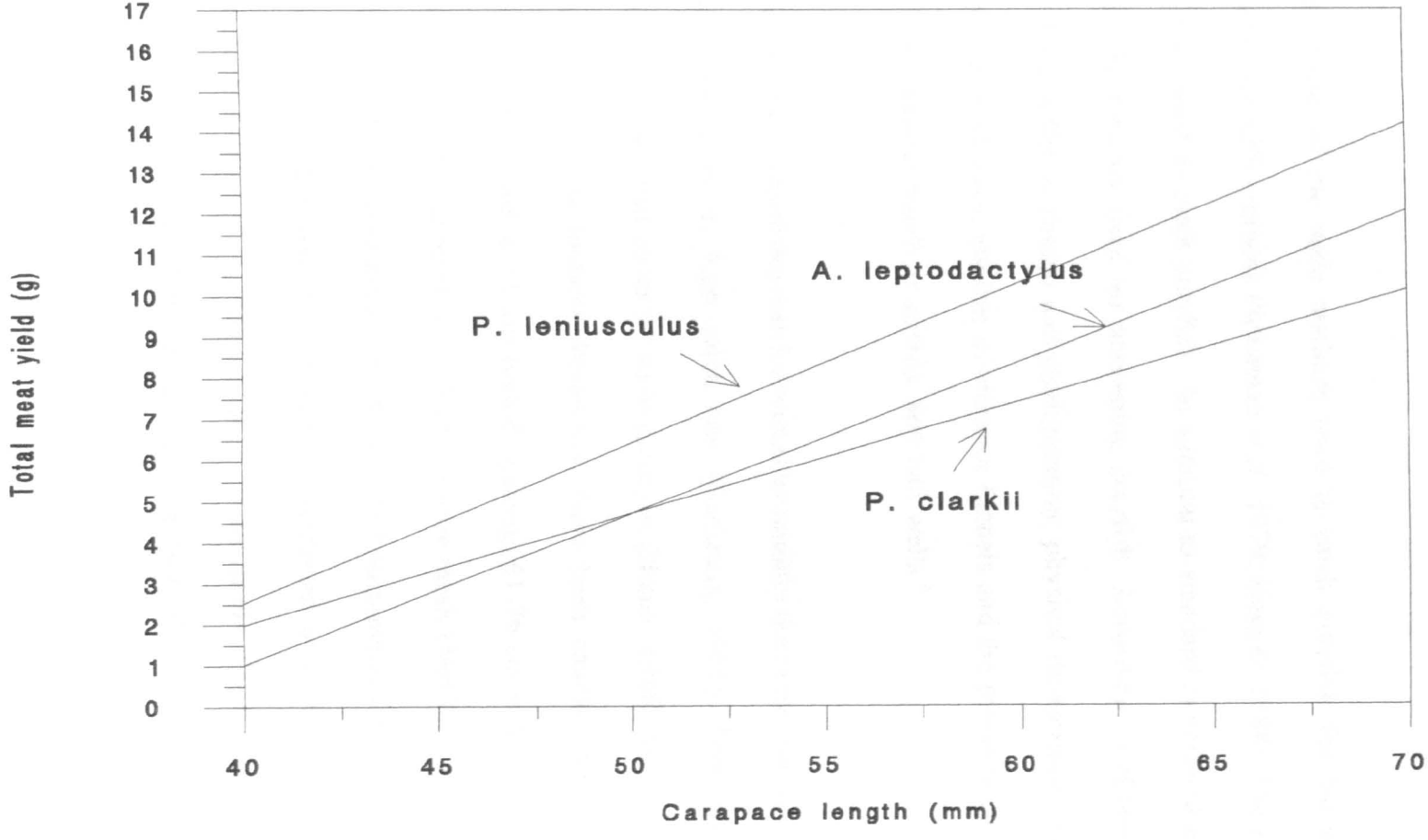


Figure 12.3 Differences in the total meat (wet) yield between male P. leniusculus (from Boxmoor), A. leptodactylus (from Tykes Water) and P. clarkii versus carapace length



Chapter 13

An evaluation of the Swedish trappy for catching crayfish

13.1 Introduction

Trapping is one of the main methods used to catch crayfish for harvesting and sampling for scientific purpose (Westman *et al.*, 1979; Hogger, 1988). There are many types of traps used to catch crayfish. In addition to standard cylindrical traps, seine nets and fyke nets are used for harvesting crayfish. According to Huner and Barr (1991), "They differ in design and configuration; physical dimensions; construction materials and mesh sizes; number of entrance funnels and the presence or absence of support rods, retainer bands or collars, and bait wells."

In the United States, crayfishermen have used essentially the same trap type for around 20 years, because of its high catch rate (Comeaux, 1975). They are generally cylindrical, 1 m long and about 0.5 m in diameter (Huner, 1988). In the Sacramento-San Joaquin Delta, *Pacifastacus leniusculus* have been caught with cylindrically-shaped, baited traps with a 7.5 cm funnel opening, 61-76 cm in length and 30.5 cm in diameter, and constructed of 2.5 x 1.25 cm wire mesh (McGriff, 1981). Baiting is one of the main design components in the trap. In Louisiana, annually, 15 000-30 000 tons of bait are used. Cheap fish species are preferred as a bait. These are mainly *Dorosoma cepedianum* (the gizzard shad), *Cyrinus carpio* (the common carp), *Alosa chrosochloris* (the skip jack herring) (Huner and Barr, 1991).

In the Ukraine, around 20 years ago, before the use of selective crayfish trawls, large unselective fishing nets (gura), were used to catch *Astacus leptodactylus*. After that various types of traps were developed (Brodsky, 1975). In Lithuanian SSR, a few decades ago, a kind of wooden trap (60-80 cm in length and 20-30 cm in width) was being used to catch *Astacus astacus*, although it was not very easily transported. After that, baited folding net traps were chosen to determine the number and density of the populations of *Astacus astacus*. The traps had three metal rings (25 cm in diameter) and were made of rustless wire. The funnel entrances were 5-8 cm at both ends (Cukerzis, 1988). In Turkey, cylindrical net traps with bait and funnel entrances at each end have been used to catch *Astacus leptodactylus* (Köksal, 1988).

One problem with traps is that once the bait loses its attractiveness then the crayfish start to escape (Bean & Huner, 1979; Westman *et al.*, 1979). According to Westman *et al.* (1979) crayfish are skillful at escaping from traps with quite complicated entrances. They designed a number of string-type cyclindrical traps, including the "Evo-trap". This trap had a narrow but flexible slit-like entrance and proved to be better than most other traps tested at preventing escapes, although initial capture was not affected. One trap with a plastic tube entrance was better at retaining crayfish but the catch was reduced. In southern Finland, the "Evo-trap" has been used to catch crayfish in a comparative study of the growth and moulting of the noble crayfish and the signal crayfish (Westman *et al.*, 1993). Another problem with any trapping method is that it is dependent upon both the seasonal and diurnal activity of the crayfish (Westman *et al.*, 1979).

Many European countries, including Britain, use a trap known as the Swedish trappy. These consist of a plastic mesh sheet which can be folded into a 50 cm long and 20 cm wide cylinder and clipped into place. Funnels fit into each end and have an inner opening 4.5 cm wide. The mesh is diamond-shaped with a size of 2.5 x 3.5 cm. A metal clamp holds bait centrally. No studies of the effectiveness of this trap in catching crayfish have been carried out in Britain and no comparison has been made on their ability to catch signal (*Pacifastacus leniusculus*) and narrow-clawed crayfish (*Astacus leptodactylus*). As crayfish inhabit hides for much of the time but come out to forage periodically, they may view a trap as a hide and enter it anyway, whether or not bait is present.

An experiment was designed to test trap efficiency as part of a long-term monitoring programme on a *P. leniusculus* population (Holdich and Domaniewski, 1995). The results of this led to a second, more controlled experiment, being set up.

13.2 Materials and Methods

There were two parts to this study: 1. a field study involving *P. leniusculus*, and 2. a more controlled study involving both *P. leniusculus* and *A. leptodactylus* separately in concrete tanks at Nottingham University.

13.2.1 Field study

As part of a long-term study on the *P. leniusculus* population at Boxmoor Fishery (Hemel Hempstead) by Nottingham University (Holdich and Domaniewski, 1995) an

exercise was undertaken to test the effectiveness of the trappies. Fifty baited traps were set at 10-metre intervals around the lake in September 1993. They were left down with their catch for three days and checked each morning. Individuals present on each day were given a particular mark for each day using "Tippex".

13.2.2 Concrete tanks study

For this experiment, four concrete tanks (approximately 1.5m x 2.5m x 1 m with 0.5 m water depth and 5 litre/minute water flow) were used for nine days (28.09.93-08.10.93). Thirty crayfish (15 male/15 female) were set in each tank with two replicates for the two species. Adult specimens were chosen randomly. The smallest carapace length was 49 mm for *A. leptodactylus* and 54 mm for *P. leniusculus* .

To provide semi-natural conditions *Cladophora* and hides were placed into the tanks. In each tank there were six groups of 10 hides (bound together by plastic string) and six bricks, each with three holes. The plastic hides were 16 cm in length and 6 cm in diameter.

Five traps were set on the floor of the tank around 18.00 h, on 28.09.93. All traps were checked and crayfish were counted and marked with Tippex each morning (before 09.00 h) and afternoon (around 18.00 h) during the experiment. After counting and marking the specimens, they were kept in the traps overnight and during daytime in order to observe their activity. The main aim was to see if crayfish would move out from the trap and if new crayfish appeared in the traps. Initially, for the first five days (28.09.93-02.10.93), the traps were used without bait and then with bait for

comparison.

To determine trap efficiency with and without bait and crayfish activity in traps, fish meat (sprats) was placed into the traps on 02.10.93 around 09.00 h. The captured crayfish were counted around 18.00 h and were released so as to compare trap efficiency by using bait and no bait and crayfish activity during the day and overnight. After releasing the crayfish from the traps (at 18.00 h on 02.10.93), the traps were not used for one day (03.10.93) and baited traps were set up the following day (04.10.94) around 18.00 h. Bait was changed every morning during the experiment.

Before starting to use traps with bait, all traps were emptied and Tippex marks were cleaned from the carapaces of the specimens.

13.3 Results

13.3.1 Field study

Fifty baited traps were set on 12/09. On 13/09 the first 18 traps yielded 209 signal crayfish (11.6/trap) with a ratio of 1♂:2♀. The crayfish in traps 1-18 were all marked on the exoskeleton over the heart (with Tippex after drying the exoskeleton) and returned to the freshly baited traps. On 14/09 the first 18 traps yielded 142 signal crayfish (7.9/trap) of which 110 were marked, meaning that 99 had escaped and that 32 new individuals had entered the traps. The remaining traps were examined and 293 crayfish (5.86/trap) recovered in total with a sex ratio of 1♂:1.38♀. The exercise was repeated and all crayfish in the traps were marked with a mark on the head (those with

the original mark now had two different marks). On 15/09 all traps were again sampled and 280 signal crayfish (5.6/trap) recovered, 130 being in traps 1-18 (7.2/trap). Of these 130, 61 had two sets of marks, i.e. they had probably stayed in the traps from the initial capture. Thus, 52.6% had remained in the traps over the first 24 h but only 29.2% after 48 h. Therefore, after 24 h the escape rate of the captured crayfish from the traps was significant ($P<0.001$, Chi-square test). Of the 32 crayfish in traps 1-18 that received a head mark only on 14/09, only 11 were remaining on 15/09. However, 19 crayfish in the traps had only a heart mark indicating that they had left the traps after the 13/09 marking but had returned after the 14/09 marking. In addition, some 39 new (unmarked) crayfish had entered the traps.

The results for the 3-day trap effectiveness study are shown in Table 13.1. It was found that there was considerable movement of crayfish in and out of the traps, and that after an initial heavy catch in some traps there was a decline with time. Indeed in traps 1-18 the decline was from 209 crayfish trapped on Day 1 to 142 present on Day 2 to only 130 present on Day 3. However, overall there was little difference in the numbers caught on Day 2 (catch no. 2b) and Day 3 (catch no. 3b).

13.3.2 Concrete tanks study

There was no significant difference in the number of caught crayfish between the two replicates of the two species therefore data were combined to compare the species.

It was observed that the trappy with bait or without bait was very efficient in catching *P. leniusculus* and *A. leptodactylus*. At the end of the experiment 71.6% of the total

crayfish in the *A. leptodactylus* tanks and 60% of the total crayfish in the *P. leniusculus* tanks had been caught (Figures 13.1 and 13.2).

There was no significant difference in the number of captured *P. leniusculus* and *A. leptodactylus*. But there was a significant difference ($P<0.001$) in the case of escapes from the trap and changing the trap (Tables 13.2 and 13.3). In both the morning and afternoon observations it was noticed that the samples of *P. leniusculus* were more active in the tanks and more capable of escaping from the trap than those of *A. leptodactylus*. Accordingly, approximately 12% of the total *P. leniusculus* population were caught between 09.00 h and 18.00 h with the baited traps on 02.10.93, whereas no *A. leptodactylus* was caught (Figures 13.1 and 13.2).

There was a significant difference in the number of crayfish captured between overnight catching and daytime catching of *P. leniusculus* and *A. leptodactylus*. More crayfish were caught during overnight trapping than during daytime trapping.

As far as bait is concerned, there was a significant difference ($P<0.05$) in the number of captured crayfish between baited traps and unbaited traps. The number of the captured crayfish with the baited traps for the two species was higher than the unbaited traps on the first morning checks (29.09.93 for unbaited traps and 05.10.93 for baited traps). The baited traps caught 51.6% of the total crayfish in the *P. leniusculus* tanks on 05.10.93, whereas the unbaited traps caught 38.3% of the total *P. leniusculus*. Similarly, the baited traps caught 53.3% of the total crayfish in the *A. leptodactylus* tanks on 05.10.93, whereas the unbaited traps caught 38.3% of the total *A. leptodactylus*.

13.4 Discussion and conclusions

Crayfishermen from the Sacramento fishery advised that traps should be left for 24 hours in the water (Lowery and Holdich, 1988). In addition, because *Pacifastacus leniusculus* actively feeds at night (like many other crayfish species), the maximum number of crayfish may be caught during dark hours (Lowery and Holdich, 1988). For this reason, the Swedish trappy should be emptied two or three times during night and the opening of the entrances should be reduced for *P. leniusculus*. The field-based trap efficiency exercise reported here also showed that using Swedish trappies there was considerable movement in and out of the traps. It would appear that in order to maximise yield it is better to empty the traps a number of times during night rather than leaving them for a few days which is the current practice. However, the labour involved usually precludes this and emptying traps after one night would seem to be a suitable compromise. The implications for population estimates are clear from the results of traps 1-18 where numbers declined from 209 to 142 to 130 over the 3 day period.

Another factor affecting crayfish catches using traps is where the traps are actually placed. In the Boxmoor study they were placed amongst marginal vegetation where the signal crayfish tended to forage. Traps that were set in deeper water (> 1.5 m) caught very few crayfish (Holdich, D.M., pers. comm.). Kossakowski (1966) has found that in the wild *Astacus leptodactylus* is spread in uniform densities on the offshore region, mainly occupying habitats under plants. In this case, to catch the maximum number of crayfish, traps should be placed as near as possible to their hides and next to vegetation areas (generally at 2 m depth) (Kossakowski, 1966).

Environmental conditions such as light, season and temperature can also have an effect on crayfish trapping. According to Abrahamsson (Westman *et al.*, 1979), crayfish are willing to enter traps in the evening and the early part of the night and they tend to escape from the trap after eating the bait. Similar results have been observed in this experiment in the field study and in the concrete tank study.

According to Arrignon (1993) female *P. leniusculus* are trap-shy from March to late May but after September they can be caught more easily. Despite the fact that egg bearing females are generally trap shy, a large number of egg bearing *Austropotamobius pallipes* can still be caught in traps (Lowery, 1988). In southern Finland, a comparative study of the growth and moulting of *Astacus astacus* and *P. leniusculus* was carried out by trapping in August. It was observed that there was a high activity of females and males in this month (Westman *et al.* 1993). Köksal (1988) reported that the males of *A. leptodactylus* were more active than the females. She also reported that females were inactive during the breeding season (November to June) and the proportion of females per catch ranged from 29-43% from November to the end of June. In this experiment, during the field study the females of *P. leniusculus* showed more active behaviour than the males. The ratio of the male and female captured crayfish was 1♂ : 2♀ in the first catching (for the first 18 traps), 1♂ : 1.38♀ in the second catching (50 traps) and 1♂ : 1.88♀ in the third catching (50 traps).

Apart from environmental conditions, a crucial factor determining the size of catch from crayfish trappings is the type of the trap used. Also, the ability of crayfish to escape from the trap depends on the type of trap used. In addition, size and behaviour of the crayfish population in the catching areas also have an effect on crayfish

catching (Westman *et al.*, 1979). The behaviour of *A. astacus* in response to six different kind of traps have been evaluated by Westman *et al.* (1979). They looked at a standard trap, one-entrance trap, protected-bait trap, narrow entrance trap (Evo-trap), plastic tube-entrance-trap and a bristle-entrance trap. In this study, among the six different type of traps, the greatest number of crayfish were caught and retained with the narrow entrance trap. The narrow opening did not have an adverse effect on the catching of crayfish and in this trap, although crayfish tried to escape from the trap, it was observed that they were unable to escape out. The second most efficient trap was the standard trap with two 7-9 cm circular entrances. The number of crayfish entering was just under the narrow entrance trap's catch. However, approximately two-thirds of the total crayfish caught escaped from the standard trap. None of crayfish could leave from the plastic tube entrance trap which had a 12 cm long and 7 cm diameter black plastic tube at the end of the entrance (Westman *et al.*, 1979). Another factor to consider is the traps' utility. Under normal commercial working conditions, crayfishermen tend to force wire traps causing them to deform or break (Huner and Barr, 1991). Professional crayfishermen have to spend a half of their total expenditure for harvesting crayfish (Bean and Huner, 1979). Several factors are important in the design of economic crayfish traps, (i) they have to have a very good catching efficiency, (ii) they must be easily transferred, (iii) they have to be efficient on all substrates, (iv) they have to be damage resistant (Westman *et al.* 1979). For these reasons, Huner and Barr (1991) suggested that traps made from plastic material should be used, and Kossakowski (1966) suggested that crayfish traps should be easily constructed and be able to be dismantled for transport.

The major advantages of using Swedish trappies are that they are easy and quick to set. Thus, the time required for this exercise is shortened. They are not heavy so the work load is reduced. Thus, a crayfisherman can set many traps in a short time. Further advantages are that they can be placed on different substrates and they can be transported easily (occupying little space). They have a very good catching efficiency (71.6 percentage of the total *A. leptodactylus* were captured (Fig. 13.2) at the end of the experiment). They can be emptied and rebaited quickly. In addition to this, trappies have an advantage over seine nets because they cause less stress during capture.

The efficiency of a crayfish trap may be increased by altering the basic design. Approximately 25% more crayfish were caught with traps made from plastic material than traps made from galvanized wire (Huner and Barr, 1991). In a different study, significantly more crayfish were captured with black plastic coated traps (Huner, 1988). The shape of the traps' entrances are also of great importance in catching and preventing crayfish escaping (Westman *et al.*, 1979). The possibility of escaping from the trap can be reduced by use of funnel entrances (Cukerzis, 1988). Moreover, even if the trap's entrances are narrow, large crayfish are able to push themselves through with little difficulty (Westman *et al.*, 1979). According to Westman *et al.* (1979), trap efficiency can be increased by reducing the diameter of the circular entrances or by setting a pipe or a retainer ring at the end of the funnels to prevent crayfish escaping. In a field study, in the period of 24 hour trapping, funnel traps with circular retainer rings (at the end of the funnels) caught 15 to 20% more crayfish than funnel traps without circular retainer rings (Huner *et al.*, 1991).

In conclusion, it has been shown that the Swedish trappy is very effective at catching both *P. leniusculus* and *A. leptodactylus* over a certain size ($\geq$ 49 mm CL). However, unless the traps are emptied and rebaited frequently much of the catch may escape. Such escapes would have implications for any population study. In addition, very few types of traps catch juvenile crayfish and relatively fine meshed nets are needed to catch these.

Table 13.1 Catch data from Boxmoor Fishery

Catch no.	Date	No. traps	♂ S	♀ S	Total	CPUE
1	13/09/93	18	69	140	209	11.61
2a	14/09/93	18	50	92	142	7.90
2b	14/09/93	50	123	170	293	5.86
3a	15/09/93	18	43	87	130	7.20
3b	15/09/93	50	97	183	280	5.60

S = signal. CPUE = Catch per unit effort

Table 13.2 Total captured crayfish (out of 60 crayfish) for morning and afternoon observations in *P. leniusculus* tanks

Date	captured crayfish **morning** (09.00)	female-male	new crayfish in (f-m)	from another trap (f-m)	out from the trap (f-m)	captured crayfish **afternoon** (18.00)	female-male	new crayfish in (f-m)	from another trap (f-m)	out from the trap (f-m)
29.09.93	23	8-15	8-15			24	9-15	1-0	0-0	0-0
30.09.93	30	9-21	3-4	0-3	2-1	30	9-21	0-0	0-0	0-0
01.10.93	36	11-25	2-4	0-2	0-2	35	11-24	0-0	0-0	0-1
02.10.93	37	15-22	5-1	0-1	1-4	7	4-3			
05.10.93	31	13-18	13-18			33	12-21	0-2	0-1	1-0
06.10.93	31	12-19	0-0	0-0	0-2	32	12-20	0-0	0-1	0-0
07.10.93	33	12-21	0-3	0-0	0-2	33	12-21	0-0	0-0	0-0
08.10.93	36	12-24	0-4	0-1	0-2					

Table 13.3 Total captured crayfish (out of 60 crayfish) for morning and afternoon observations in *A. leptodactylus* tanks

Date	captured crayfish **morning** (09.00)	female-male	new crayfish in (f-m)	from another trap (f-m)	out from the trap (f-m)	captured crayfish **afternoon** (18.00)	female-male	new crayfish in (f-m)	from another trap (f-m)	out from the trap (f-m)
29.09.93	23	14-9	14-9			23	14-9			
30.09.93	29	16-13	2-4	0-1	0-1	29	16-13	0-0	0-0	0-0
01.10.93	34	19-15	3-2	0-0	0-0	33	18-15	0-0	0-0	1-0
02.10.93	36	20-16	2-1	2-0	2-0	0				
05.10.93	32	15-17	8-6			32	15-17	0-0	0-0	0-0
06.10.93	39	21-18	6-2	0-0	0-1	39	21-18	0-0	0-0	0-0
07.10.93	41	22-19	1-2	0-1	0-2	41	22-19	0-0	0-0	0-0
08.10.93	43	22-21	0-1	1-1	1-0					

Figure 13.1 The percentage of the total captured P. leniusculus for morning and afternoon observations

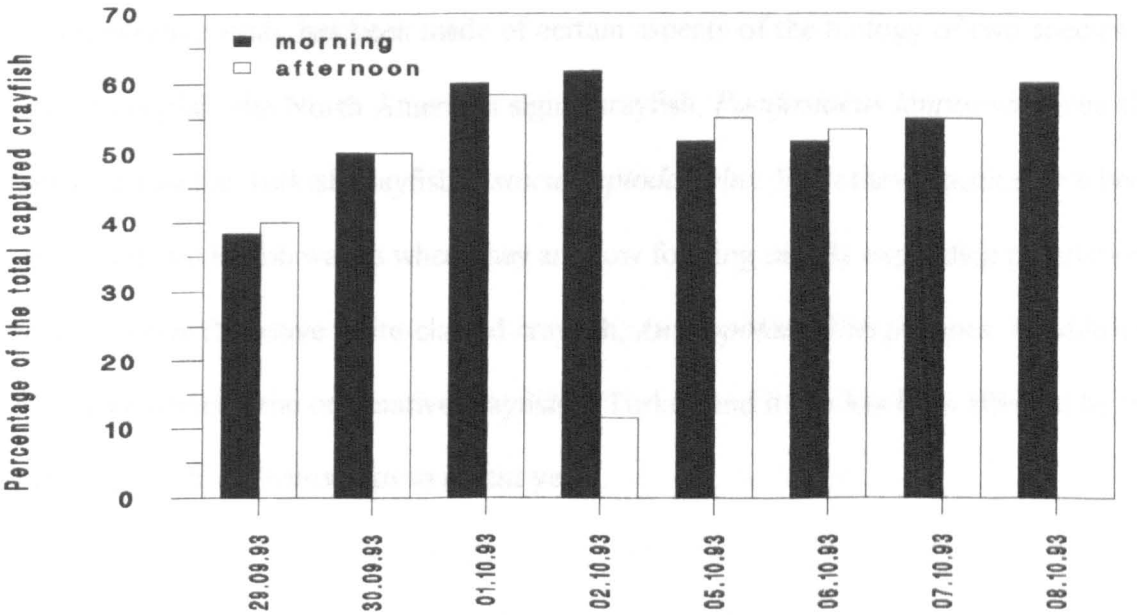
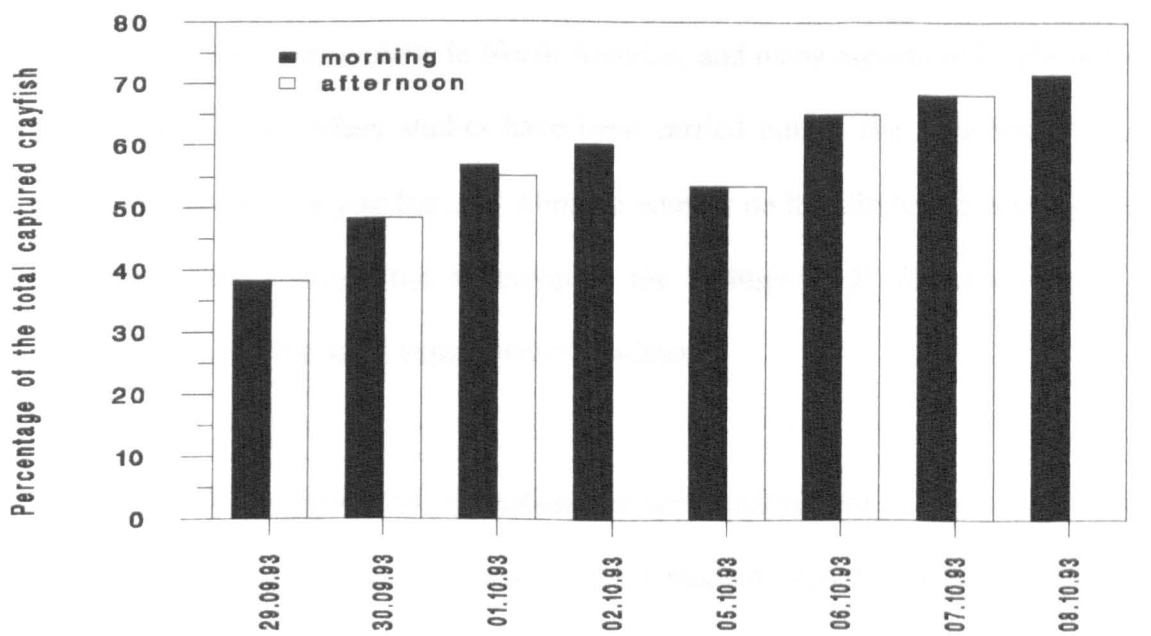


Figure 13.2 Percentage of the total captured A. leptodactylus for morning and afternoon observations



Chapter 14

General conclusions

A comparative study has been made of certain aspects of the biology of two species of astacid crayfish, the North American signal crayfish, *Pacifastacus leniusculus*, and the narrow-clawed or Turkish crayfish, *Astacus leptodactylus*. Both these species have been introduced into British waters where they are now forming rapidly expanding populations which threaten the native white-clawed crayfish, *Austropotamobius pallipes*. In addition, *A. leptodactylus* is the only native crayfish in Turkey and it too has been affected by the introduction of *P. leniusculus* in recent years.

Due to the fact that *P. leniusculus* has been introduced into most European countries for aquacultural and stocking purposes many studies have been carried out on its ecological impact, culture and distribution. Because it acts as one of the main vectors of crayfish plague its immunology has also been extensively studied. It has also become a popular experimental animal, particularly in North America, and many aspects of its physiology are now well known. Many studies have been carried out on the biochemistry and physiology of *A. leptodactylus* but only a limited number on its culture and ecology. Few studies have been carried out to compare the biology of *P. leniusculus* and *A. leptodactylus* under the same experimental conditions.

The present study has shown that the deliberate or accidental release of *P. leniusculus* and *A. leptodactylus* into the wild is likely to have a marked impact on macroinvertebrate populations and plant communities, as well as on fish populations as they have been shown to eat fish eggs. This is not to say that *A. pallipes* does not have an impact but both

P. leniusculus and *A. leptodactylus* are more fecund, have a faster individual and population growth rate and grow to a much larger size. Consequently, they are likely to have a much greater impact on the freshwater environment. In addition, *P. leniusculus* acts as a vector of crayfish plague to which both *A. pallipes* and *A. leptodactylus* are highly susceptible. Due to its more aggressive nature and predatory capabilities, *P. leniusculus* is likely to have a greater environmental impact than *A. leptodactylus*.

The use of scanning electron microscopy has revealed that few differences exist between *P. leniusculus* and *A. leptodactylus* in the structure of their mouthparts, so it is likely that they feed on similar foods. The setal arrays and the number and dimensions of teeth change with age and may enable adults of both species to feed on a wider diet than when they are young.

The environmental tolerances of crayfish are well known but have been rarely compared under identical conditions. The present study has shown that both species are remarkably tolerant of increases in salinity being hyperosmotic and hyperionic regulators in low salinity but, after a brief period when they become hypo-osmotic and hypo-ionic regulators, they become osmoconformers even in 100% seawater. However, early stage juveniles do not survive so well as later stage juveniles and adults in salinities over 20 ppt. Although *A. leptodactylus* has reached the estuarine environment in England and *P. leniusculus* occupies some tidal rivers, their colonisation of the lower reaches may be limited by their ability to produce viable offspring in salty water. Stepwise acclimation to a range of above ambient temperatures has shown that adults and juveniles of both species can survive temperatures up to 34 °C and juveniles of both species can survive at 30 °C for at least seven days. They are both also able to tolerate low oxygen levels in their

aquatic environment, *A. leptodactylus* being more tolerant than *P. leniusculus*. The sublethal effects of changes in salinity, temperature and oxygen levels were assessed using a non-invasive technique which measures heart beat rate. However, due to the extreme amount of variability occurring between individuals few conclusive results were obtained. Their tolerance of saline waters, high temperatures and low oxygen levels may enable them to occupy waters not occupied by *A. pallipes*, including those affected by pollution.

Further evidence for the superior competitive abilities of *P. leniusculus* was obtained from competition experiments utilising both juveniles and adults of both species. In both types of experiment the numbers of *A. leptodactylus* were significantly reduced in the presence of *P. leniusculus*. However, cannibalism was found to occur to a higher level in *P. leniusculus*, both amongst juveniles and adults.

Both *P. leniusculus* and *A. leptodactylus* are much more fecund than *A. pallipes* and consequently recruitment is better. However, stage 2 juveniles of *A. leptodactylus* are larger than those of *P. leniusculus* and so may be better adapted to survive predation. These two introduced species which have become well established in British waters are as fecund as they are in their native countries. From a commercial point of view it might be useful to have juveniles earlier than they would appear normally. These could then be seeded out in spring and the growing season extended. It has proved possible using elevated temperatures to bring forward hatching by some three months. Eggs do not appear to need a diapause period followed by a cold shock to stimulate development as has been suggested by some workers.

An interesting phenomenon exhibited by crayfish is maternal brooding behaviour. This involves the mother acting as a home base for stage 2 juveniles (or stage 3 in cambarid and parastacid crayfish) as they gradually become independent over a 16-21 day period. There is previous evidence to suggest that a pheromone is involved. Present experiments showed that stage 2 juveniles are attracted to other crayfish as well as the mother, even ones of a different species, including males, during this period which suggests that the juveniles respond to any object as a hide, not necessarily the mother so an attractant type pheromone may not necessarily be involved. However, a mother with stage 2 young will not eat stage 2 juveniles even of other species, although other crayfish not carrying broods will. The processes involved in this behaviour are not clear and warrant further study. It has been shown that *P. leniusculus* juveniles may appear in the natural environment some four weeks earlier than those of *A. leptodactylus*. This gives them a head start and they may predate on juveniles of other species appearing later. In addition, the mothers will also have become predatory.

Growth experiments with juveniles at different temperatures and densities highlighted the problem of cannibalism when trying to intensify production of crayfish. However, this proved to be much lower in *A. leptodactylus* suggesting that it might be a better prospect for astaciculture from some points of view. The experiments also highlighted the problem of differential growth with some juveniles hardly growing at all whilst others reached a relatively large size.

A comparison of various body parameters showed that *P. leniusculus* females have a wider abdomen and males larger and heavier claws than *A. leptodactylus*. These features are important when considering a species for aquacultural purposes but *P. leniusculus*

does have some negative features such as its high degree of cannibalism and aggressive nature. Its total meat yield though is higher than that of *A. leptodactylus*, particularly in males. This is because of the meat contained in the robust claws of *P. leniusculus*. In fact *A. leptodactylus* has more meat in its tail than *P. leniusculus*, and this is usually the part which is mainly eaten. Both species compared very favourably with other species in terms of meat yield.

The final part of the study involved an evaluation of the main type of trap used to capture crayfish in Britain - the Swedish trappy. This cylindrical structure with a funnel at either end was shown to be very effective at trapping both species. However, it was shown that if the traps are not emptied regularly then crayfish will readily escape.

Both *P. leniusculus* and *A. leptodactylus* have been shown to be very environmentally tolerant crayfish. Many of the features they possess make them good candidates for astaciculture as well as ensuring their success in wild waters. The fact that the harvest of these two species is now greater from the wild in Britain than from astaciculture (Rogers, W. D., pers. comm.) shows how quickly they can become established. They can swiftly become the keystone species in an environment but often at the expense of other organisms, including crayfish such as *A. pallipes*. It is highly likely that if *P. leniusculus* were to become established in Turkey they would outcompete the native *A. leptodactylus* even if they did not devastate populations through crayfish plague (which they have partially done already). Britain is about to institute a series of large-scale no-go areas where future crayfish farming developments will be banned in an attempt to conserve the remaining populations of native *A. pallipes*. It may be necessary to do the same for the native crayfish in Turkey.

References

Aagaard, A. Andersen, B.B. & Depledge, M.H. (1991). Simultaneous monitoring of physiological and behaviour activity in marine organisms using non-invasive, computer-aided techniques. Marine Ecology Progress Series 73: 277-282.

Abercrombie, M., Hickman, M., Johnson, M.L. & Thain, M. (1992). Dictionary of biology. 596 pp. Penguin Books Ltd. Middlesex, England.

Abrahamsson, S. (1966). Dynamics of an isolated population of the crayfish *A stacus astacus* Linne. Oikos 17: 96-107.

Abrahamsson, S.A.A. & Goldman, C.R. (1970). Distribution, density and production of the crayfish *Pacifastacus leniusculus* Dana in Lake Tahoe, California-Nevada. Oikos: 21: 83-91.

Abrahamsson, S.A.A. (1971). Density, growth and reproduction in populations of *A stacus astacus* and *Pacifastacus leniusculus* in an isolated pond. Oikos 22: 373-380.

Abrahamsson, S. (1973). Methods for restoration of crayfish waters in Europe. The development of an industry for production of young of *Pacifastacus leniusculus* Dana. Freshwater Crayfish 1: 203-210.

Ackefors, H., Gydemo, R., & Westin, L. (1989). Growth and survival of juvenile crayfish, *A stacus astacus* in relation to food and density. In: Aquaculture - A Biotechnology in Progress (N. D. Pauw, Jaspers, E., Ackefors, H., Wilkins, N., eds). pp. 355-373.

Adegboye, D. (1983). The " crayfish condition factor ": a tool in crayfish research. Freshwater Crayfish 5: 154-172.

Aiken, D.E. (1969a). Photoperiod, endocrinology and the crustacean molt cycle. Science 164: 149-154.

Aiken, D.E. (1969b). Ovarian maturation and egg laying in the crayfish *Orconectes virilis*: influence of temperature and photoperiod. Canadian Journal of Zoology 4: 931-935.

Aiken, D.E. & Waddy, S.L. (1990). Winter temperature and spring photoperiod requirements for spawning in the American lobster, *Homarus americanus*, H. Milne Edwards, 1837. Journal of Shellfish Research 9(1): 41-43.

Alderman, D.J. & Polglase, J.L. (1988). Pathogens, parasites and commensals. In: Freshwater crayfish: biology, management and exploitation (Holdich D. M. & Lowery R. S., eds), pp. 167-212. Chapman & Hall, London.

Alexander, C.G., Hindley, J.P.R. & Jones, S.G. (1980). Structure and function of the third maxillipeds of the banana prawn *Penaeus merguiensis*. Marine Biology 58: 245-249.

Amato, G.D. (1988). Freshwater crayfish breeding in Basse-Normandie. Freshwater Crayfish 7: 213-222.

Ameyaw-Akumfi, C. & Hazlett, B.A. (1975). Sex recognition in the crayfish, *Procambarus clarkii*. Science 190: 1225-1226.

Ameyaw-Akumfi, C. (1977). Feeding chemoreceptor sites in the crayfish *Procambarus clarkii* (Girard). Crustaceana 33(3): 259-264.

Andrews, E.A. (1907). The young of the crayfishes *Astacus* (*Pacifastacus leniusculus*) and *Cambarus* (*Orconectes limosus*). 88 pp. Smithsonian Contributions to Knowledge Part of Volume xxxv.

Angersbach, D. & Decker, H. (1978). Oxygen transport in crayfish blood: effect of thermal acclimation, and short-term fluctuations related to ventilation and cardiac performance. Journal of Comparative Physiology 123: 105-112.

Ansell, A.D. (1973). Changes in oxygen consumption, heart rate and ventilation accompanying starvation in the decapod crustacean *Cancer pagurus*. Netherlands Journal of Sea Research 7: 455-475.

Aoki, K. (1965). Responses of sensory hairs of the walking leg of the crayfish, *Procambarus clarkii*. Journal of the Faculty of Science Hikkaido University Series 6. 15: 499-509.

Appelberg, M. & Odelstrom, T. (1988). Interaction between European perch (*Perca fluviatilis*) and juvenile *Pacifastacus leniusculus* (Dana) in a pond experiment. Freshwater Crayfish 7: 37-45.

Armitage, K.B., Buikema, A.L. & Willems, N.J. (1973). The effect of photoperiod on organic constituents and molting of the crayfish *Orconectes nais* (Faxon). Comparative Biochemistry and Physiology 44A: 431-456.

Arrignon, J. (1975). Crayfish farming in France. Freshwater Crayfish 2: 105-116.

Arrignon, J.V.C. (1979). Elements de bibliographie sur le genre *Astacus* en Europe. Freshwater Crayfish 4: 427-436.

Arrignon, J.C.V. (1993). The development of a *Pacifastacus leniusculus* population in a gravel pit in France. Freshwater Crayfish 9: 87-96.

Aspan, A. & Söderhäll, K. (1991). Purification of prophenoloxidase from crayfish blood cells, and its activation by an endogenous serine proteinase. Insect Biochemistry 21(4): 363-373.

Atwood, H.L. & Nguyen, P.V. (1995). Neural adaptation in crayfish. American Zoologist 35: 28-36.

Austin, C.M. (1995). Length-weight relationships of cultured species of Australian freshwater crayfish of the genus *Cherax*. Freshwater Crayfish 10: 410-418.

Avault, J.W.Jr., de la Bretonne, L.W. & Huner, J.V. (1975). Two major problems in culturing crayfish in ponds: oxygen depletion and overcrowding. Freshwater Crayfish 2: 139-145.

Avenet, P. & Lignon, J.M. (1985). Ionic permeabilities of the gill lamina cuticle of the crayfish, *Astacus leptodactylus* (E). Journal of Physiology 363: 377-401.

Baran, I., Timur, M., Oray, I.K., Timur, G., Rahe, R., Soylu, E. (1987). Investigations on a disease causing serious mortality on crayfish (*Astacus leptodactylus*) populations

in Turkey. European Aquaculture Society 6-7.

Barker, P.L. & Gibson, R. (1977). Observations on the feeding mechanism, structure of the gut, and digestive physiology of the European lobster *Homarus gammarus* (L.) (Decapoda: Nephropidae). Journal of Experimental Marine Biology and Ecology 26: 297-324.

Barkman, R.C. (1970). Response of the tissue of *Orconectes rusticus* to salinity stress. Comparative Biochemistry & Physiology 36: 285-290.

Bauer, R.T. (1989). Decapod crustacean grooming: functional morphology, adaptive lavue, and phylogenetic significance. In: functional morphology and grooming in Crustacea. Crustacea Issues 6 (Felgenhauer *et al.* eds) 49-73 pp.

Bean, R.A. & Huner, J.V. 1979. An evaluation of selected crawfish traps and trapping methods. Freshwater Crayfish 4: 141-151.

Bechler, D.L. (1981). Copulatory and maternal-offspring behaviour in the hypogean crayfish, *Orconectes inermis inermis* (Cope) and *Orconectes pellucidus* (Tellkampf) (Decapoda, Astacidea). Crustaceana 40(2): 136-143.

Bechler, D.L., Deng, X. & McDonald, B. (1988). Interspecific pheromonal communication between sympatric crayfish of the genus *Procambarus* (Decapoda, Astacidea) Crustaceana 54(2): 153-161.

Bechler, D.L. (1995). A review and prospectus of sexual and interspecific pheromonal communication in crayfish. Freshwater Crayfish 8: 657-667.

Becker, C.D., Genoway, R.G. & Merrill, J.A. (1975). Resistance of a Northwestern crayfish, *Pacifastacus leniusculus* (Dana), to elevated temperatures. Transactions of the American Fisheries Society 2: 374-387.

Begon, M., Harper, J.L. & Townsend, C.R. (1986). Ecology - individuals, populations and communities. 876 pp. Blackwell Scientific Publications.

Bergmiller, E. & Bielawski, J. (1970). Role of the gills in osmotic regulation in the crayfish *A stacus leptodactylus* Esch. Comparative Biochemistry & Physiology 37: 85-91.

Bielawski, J. (1971). Ultrastructure and ion transport in gill epithelium of the crayfish, *A stacus leptodactylus* Esch. Protoplasma 73: 177-190.

Blake, G. & Laurent, P.J. (1982). Le faucardage par des ecrevisses resultants preliminaires. Extrait du Bulletin Mensuel de la Societe Linneene de Lyon 6: 203-208.

Blake, M. & Hart, P. (1993). The behavioural responses of juvenile signal crayfish, *Pacifastacus leniusculus* (Dana), to stimuli from perch and eels. Freshwater Biology 29: 89-97.

Blake, M., Nystrom, P. & Hart, P. (1994). The effect of weed cover on juvenile signal crayfish (*Pacifastacus leniusculus* Dana) exposed to adult crayfish and non-predatory fish. Annales Zoologici Fennici 31: 297-306.

Blake, M., Nystrom, P. & Hart, P. (1994). The effect of weed cover on juvenile signal crayfish (*Pacifastacus leniusculus* Dana) exposed to adult crayfish and non-predatory

fish. Annales Zoologici Fennici 31: 297-306.

Blake, M. & Hart, P. (1995). The vulnerability of juvenile signal crayfish to perch and eel predation. Freshwater Biology 33: 233-244.

Botsford, L.W. (1991). Crustacean egg production and fisheries management. In: Crustacean egg production (Wenner A. & Kuris A., eds), Crustacean Issues 7: 379-394.

Bourchard, R.W. (1977). Morphology of the mandible in holarctic crayfishes: (Decapoda: Astacidae and CAmbaridae): ecological and phylogenetic implications. Freshwater Crayfish 3: 425-452.

Bovbjerg, R.V. (1970). Ecological isolation and competitive exclusion in two crayfish (*Orconectes virilis* and *Orconectes immunis*). Ecology 51(2): 225-236.

Bowler, K., (1963a). A study of the factors involved in acclimatization to temperature and death at high temperatures in *Austropotamobius pallipes*. I. Experiments on intact animals. Journal of Cellular and Comparative Physiology 62: 119-132.

Bowler, K., (1963b). A study of the factors involved in acclimatization to temperature and death at high temperatures in *Austropotamobius pallipes*. I. Experiments at the tissue level. Journal of Cellular and Comparative Physiology 62: 133-146.

Bowler, K., Gladwell, R.T. & Duncan, C.J. (1973). Acclimatization to temperature and death at high temperatures in the crayfish *Austropotamobius pallipes*. Freshwater Crayfish 1: 122-131.

Brian, J.M. & Geddes, M.C. (1995). Effects of salinity on growth in the freshwater crayfish *Cherax destructor* Clark (Decapoda: Parastacidae) in the laboratory and in ponds. Freshwater Crayfish 8: 410-419.

Brinck, P. (1977). Developing crayfish populations. Freshwater Crayfish 3: 211-228.

Brodsky, S. Ya. (1975). The crayfish situation in Ukraine. Freshwater Crayfish 2: 27-29.

Brodsky, S.Ya., (1983). On the systematics of palaearctic crayfishes (Crustacea, Astacidae). Freshwater Crayfish 5: 464-470.

Brown, D.J. & Bowler, K. (1977). A population study of the British freshwater crayfish *Austropotamobius pallipes* (Lereboullet). Freshwater Crayfish 3: 33-49.

Brown, P.B., Wilson, K.A., Wetzel, J.E. & Hoene, B. (1995). Increased densities result in reduced weight gain of crayfish *Orconectes virilis*. Journal of the World Aquaculture Society 26(2): 165-171.

Budd, T.W., Lewis, J.C. & Tracey, M.L. (1978). The filter-feeding apparatus in crayfish. Canadian Journal of Zoology 56: 695-707.

Burba, A. (1983). Chemical regulation in crayfish behaviour during postembryonic development. Freshwater Crayfish 5: 451-458.

Burba, A. (1988). Exploratory-searching behaviour of *Astacus leptodactylus* Esch. Freshwater Crayfish 7: 335-342.

Burbank, W.D., Edwards, J.P. & Burbank, M.P. (1948). Toleration of lowered oxygen tensions by cave and stream crayfish. Ecology 29: 360-367.

Burt, W.V. & McAlister, W.B. (1959). Recent studies in in the hydrography of Oregon estuaries. Oregon Fish Commission Research Briefs 7(1): 14-27.

Butler, P.J., Taylor, E.W. & McMahon, B.R. (1978). Respiratory and circulatory changes in the lobster (*Homarus vulgaris*) during long-term exposure to moderate hypoxia. Journal of Experimental Biology 73: 131-146.

Butler, M.J. & Stein, R.A. (1985). An analysis of the mechanisms governing species replacements in crayfish. Oecologia (Berlin) 66: 168-177.

Cabantous, M.A. (1975). Introduction and rearing of *Pacifastacus* at the research center of Les Clouzioux. Freshwater Crayfish 2: 49-55.

Capelli, G.M. & Magnuson, J.J. (1975). Reproduction, moulting, and distribution of *Orconectes propinquus* (Girard) in relation to temperature in a Northern Mesotrophic Lake. Freshwater Crayfish 2: 415-428.

Capelli, G.M. (1980). Seasonal variation in the food habits of the crayfish *Orconectes propinquus* (Girard) in Trout Lake, Vilas County, Wisconsin, U.S.A. (Decapoda, Astacidae, Cambaridae). Crustaceana 38(1): 82-86.

Capelli, G.M. (1982). Displacement of northern Wisconsin crayfish by *Orconectes rusticus* (Girard). Limnology and Oceanography 27(4): 741-745.

Capelli, G.M. & Munjal, B.L. (1982). Aggressive interactions and resource competition in relation to species displacement among crayfish of the genus *Orconectes*. Journal of Crustacean Biology 2(4): 486-492.

Capelli, G.M. & Magnuson, J.J. (1983). Morphoedaphic and biogeographic analysis of crayfish distribution in northern Wisconsin. Journal of Crustacean Biology 3(4): 548-564.

Carral, J.M., Celada, J.D., Gaudioso, V.R., Temino, C. & Fernandez, R. (1988). Artificial incubation improvement of crayfish eggs (*Pacifastacus leniusculus* DANA) under low temperatures during embryonic development. Freshwater Crayfish 7: 239-250.

Carral, J.M., Celada, J.D., Gonzalez, J., Gaudioso, V.R., Fernandez, R. & Lopezbaisson, C. (1992). Artificial incubation of crayfish eggs (*Pacifastacus leniusculus* Dana) from early stages of embryonic development. Aquaculture 104(3-4): 261-269.

Cattaneo, A. (1983). Grazing on epiphytes. Limnology and Oceanography 28(1): 124-132.

Celada, J.D., Carral, J.M., Gaudioso, V.R., Temino, C. & Fernandez, R. (1988). Effects of thermic manipulation throughout egg development on the reproductive efficiency of the freshwater crayfish (*Pacifastacus leniusculus* Dana). Aquaculture 72(3-4): 341-348.

Celada, J.D., Carral, J.M., Gaudioso, V.R., Temino, C. & Fernandez, R. (1989). Response of juvenile crayfish (*Pacifastacus leniusculus* Dana) to several and artificially compounded diets. Aquaculture 76(1-2): 67-78.

Celada, J.D. Carral, J.M. & Gonzalez, J. (1991). A study on the identification and chronology of the embryonic stages of the freshwater crayfish *Austropotamobius pallipes* (Lereboullet, 1858). Crustaceana 61: 225-232.

Chambers, P.A., Hanson, J.M., Burke, J.M. & Prepas, E.E. (1990). The impact of the crayfish *Orconectes virilis* on aquatic macrophytes. Freswater Biology 24: 81-91.

Chambers, P.A., Hanson, J.M. & Prepas, E.E. (1991). The effect of aquatic plant chemistry and morphology on feeding selectivity by the crayfish, *Orconectes virilis*. Freswater Biology 25: 339-348.

Cherkashina, N.Ya. (1970). Reproduction of crayfish (Astacidae) along the southeastern coast of the Caspian Sea. Gidrobiology ZH. 6: 83-85.

Cherkashina, N.Ya. (1975). Distribution and biology of crayfishes of genus Astacus (Crustacea, Decapoda, Astacidae) in the Turkmen Waters of the Caspian Sea. Freshwater Crayfish 2: 553-555.

Cherkashina, N.Ya. (1995). Obtaining and rearing of crayfish larvae *Astacus leptodactylus cubanicus* in an installation with controlled conditions. Freshwater Crayfish 8: 307-310.

Chien, Y.C. & Avault, J.W.Jr. (1983). Effects of flooding dates and type of disposal of rice straw on the initial survival and growth of caged juvenile crayfish, *Procambarus clarkii*, in ponds. Freshwater Crayfish 5: 344-350.

Childs, J.G. (1981). Discharge of trade effluent to public sewers. In: Industrial effluent treatment (Volume 1) (J.K. Walters and A. Wint eds.). pp. 21-41. Applied Science Publishers.

Claussen, D.L. (1980). Thermal acclimation in the crayfish *Orconectes rusticus* and *Orconectes virilis*. Comparative Biochemistry and Physiology 66A: 377-384.

Comeaux, M. L. (1975). Historical development of the crayfish industry in the United States. Freshwater Crayfish 2: 609-619.

Corey, S. (1987). Comparative fecundity of four species of crayfish in southwestern Ontario, Canada (Decapoda, Astacidae). Crustaceana 52(3): 276-287.

Corey, S. (1991). Comparative potential reproduction and actual production in several species of North American crayfish. In: Crustacean egg production (Wenner A. & Kuris A., eds), Crustacean Issues 7: 69-76.

Correia, A.M. (1993). Length-weight relationships for two populations of red swamp crayfish, *Procambarus clarkii* (Decapoda, Cambaridae) from Portugal. Freshwater Crayfish 9: 442-450.

Cox, D.K. & Beauchamp, J.J. (1982). Thermal resistance of juvenile crayfish, *Cambarus bartoni* (Fabricus): Experiment and Model. The American Midland Naturalist 108(1): 187-193.

Cuellar, L. & Coll, M. (1978). First essays of controlled breeding of *Astacus pallipes* (Ler.). Freshwater Crayfish 4: 273-276.

Cukerzis, J.M. (1978). On acclimatization of *Pacifastacus leniusculus* in an isolated lake. Freshwater Crayfish 4: 445-450.

Cukerzis, J.M., Sheshtokas, J. & Terentyev, A.L. (1978). Method for accelerated artificial breeding of crayfish juveniles. Freshwater Crayfish 4: 451-458.

Cukerzis, J.M. (1983). Selected literature on the Lithuanian crayfish. Freshwater Crayfish 5: 478-489.

Cukerzis, J.M. (1988). *Astacus astacus* in Europe. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 309-340. Chapman & Hall, London.

Cumberlidge, N. & Uglow, R.F. (1977). Heart and scaphognathite activity in the shore crab, *Carcinus maenas* (L). Journal of Experimental Marine Biology and Ecology 28: 87-107.

Cumberlidge, N. & Uglow, R.F. (1978). Heart and scaphognathite activity during the digging behaviour of the shore crab, *Carcinus maenas* (L). Physiology and Behaviour of Marine Organisms, Proceedings of the European Symposium of Marine Biology

(12th 1977): 23-30.

Curra, R.A. (1967). A key to genera, species and subspecies of Astacinae (Nephropsidea: Astacidae). International Revue der Gesamten Hydrobiologie 52(5): 793-800.

Curtis, M.C. & Jones, C.M. (1995). Overview of redclaw crayfish, *Cherax quadricarinatus*, farming practices in northern Australia. Freshwater Crayfish 10: 447-455.

D'abramo, L.R. & Niquette, D.J. (1991). Seine harvesting and feeding of formulated feeds as new management practices for pond culture of red swamp crayfish, *Procambarus clarkii* (Girard, 1852), and white river crawfish, *P. acutus acutus* (Girard, 1852). Journal of Shellfish Research 10(1): 169-177.

Dabrowski, T., Kolakowski, E., Wawreszuk, H. & Choroszucha, C. (1966a). Studies on chemical composition of American crayfish (*Orconectes limosus*) meat as related to its nutritive value. Journal Fisheries Research Board of Canada 23(11): 1653-1662.

Dabrowski, T., Kolakowski, E. & Sokolowski, E. (1966b). Zusammensetzung und Nahrwert des Krebsfleisches von *Astacus leptodactylus*. Zeitschrift fuer Lebensmittel Untersuchung und Forschung. 129: 337-344.

Dabrowski, T., Kolakowski, E. & Burzynski, J. (1968). Studies on the nitrogen components composition of crayfish (*Astacus astacus* L.) meat as related to its nutritive value. Polskie Archiwum Hydrobiologii 15(28): 145-152.

Dahl, E. (1975). Pheromones in aquatic invertebrates. Offprint from Biological Signals. Published by Kungl. Fysiografiska Sallskapet of Lund, Sweden. pp. 95-113

Dauscher, H. & Flindt, R. (1969). Vergleichende Untersuchungen zur Herztatigkeit bei frei beweglichen dekapoden Krebsen (*Astacus fluviatilis* Fab., *Astacus leptodactylus* Eschscholz und *Cambarus affinis* Say). Zeitschrift fur Vergleichende Physiologie 62: 291-300.

Day, C.H. & Avault, J.W.Jr. (1986). Crayfish *Procambarus clarkii* production in ponds receiving varying amounts of sabayon stubble or rice straw as forage. Freshwater Crayfish 6: 247-265.

De Boulay, A.J.H., Sayer, M.J.D. & Holdich, D.M. (1993). Investigations into intensive culture of the Australian red claw crayfish *Cherax quadricarinatus*. Freshwater Crayfish 9: 70-78.

deFur, P.L. & Magnum, C. (1979). The effects of environmental variables on the heart rates of invertebrates. Comparative Biochemistry and Physiology 62A: 283-294.

Depledge, M.H. & Andersen, B.B. (1990). A computer-aided physiological monitoring system for continuous, long-term recording of cardiac activity in selected invertebrates. Comparative Biochemistry and Physiology 96A: 473-477.

Derby, C.D. (1982). Structure and function of cuticular sensilla of the lobster *Homarus americanus*. Journal of Crustacean Biology 2(1): 1-21.

Dube, P. & Portlance, B. (1992). Temperature and photoperiod effects on ovarian maturation and egg laying of the crayfish, *Orconectes limosus*. Aquaculture 102: 161-168.

Duvic, B. & Söderhäll, K. (1990). Purification and characterization of a beta 1,3 glucan binding protein from plasma of the crayfish *Pacifastacus leniusculus*. Journal of Biological Chemistry 265(16): 9327-9332.

Dye, L. & Jones, P. (1975). The influence of density and invertebrate predation on the survival of young-of-the-year *Orconectes virilis*. Freshwater Crayfish 2: 529-538.

Dyer, M.F. & Uglow, R.F. (1977). On a technique for monitoring heart and scaphognathite activity in natantia. Journal of Experimental Marine Biology and Ecology 27: 117-124.

Dyer, M.F. & Uglow, R.F. (1978). Heart and scaphognathite beat behaviour in laboratory-held *Crangon crangon* (L.). Journal of Experimental Biology and Ecology 32: 209-218.

Dyer, M.F. & Uglow, R.F. (1980). Cardiac and ventilatory changes associated with altered salinity in *Crangon crangon* (L.). Ophelia 19(2): 117-125.

Edsman, L. & Jonsson, A. (1991). The effect of size, injuries and ownership on fighting success in male signal crayfish (*Pacifastacus leniusculus*). National Swedish Board of Fisheries, Institute of Freshwater Research, 19 pp. S-178 91 Drottningholm, Sweden.

Ehrenfeld, J. (1974). Aspects of ionic transport mechanism in crayfish *Astacus leptodactylus*. Comparative Biochemistry & Physiology 61: 57-70.

Elser, J.J., Junge, C. & Goldman, C.R. (1994). Population structure and ecological effects of the crayfish *Pacifastacus leniusculus* in Castle Lake California. Great Basin Naturalist 54(2): 162-169.

Elvira, B., Nicola, G.G. & Almodovar, A. (1996). Pike and red swamp crayfish: a new case on predator-prey relationship between aliens in central Spain. Journal of Fish Biology 48: 437-446.

Factor, J.R. (1978). Morphology of the mouthparts of larval lobsters, *Homarus americanus* (Decapoda: Nephropidae), with special emphasis on their setae. Biological Bulletin 154: 383-408.

Factor, J.R. (1989). Development of the feeding apparatus in decapod crustaceans. In: functional morphology and grooming in Crustacea. Crustacea Issues 6 (Felgenhauer *et al.* eds) 185-203 pp.

Farmer, A.S. (1974). The functional morphology of the moutparts and pereiopods of *Nephrops norvegicus* (L.) (Decapoda: Nephropidae). Journal of Natural History 8: 121-142.

Felgenhauer, B.E. & Abele, L.G. (1983). Ultrastructure and functional morphology of feeding and and associated appendages in the tropical fresh-water shrimp *Atya innocous* (Herbst) with notes on its ecology. Journal of Crustacean Biology 3(3): 336-363.

Feminella, J.W. & Resh, V.H. (1986). Effects of crayfish grazing on mosquito habitat at Coyote Hills Marsh. Proceeding of the California Mosquito and Vector Control Association 54: 101-104.

Field, L.H. & Larimer, J.L. (1975a). The cardioregulatory system of crayfish: Neuroanatomy and physiology. Journal of Experimental Biology 62: 519-530.

Field, L.H. & Larimer, J.L. (1975b). The cardioregulatory system of crayfish: the role of circumoesophageal interneurones. Journal of Experimental Biology 62: 531-543.

Fingerman, M. (1995). Endocrine mechanisms in crayfish, with emphasis on reproduction and neurotransmitter regulation of hormon release. American Zoologist 35: 68-78.

Firkins, I. (1993). Environmental tolerances of three species of freshwater crayfish. PhD Thesis. 288 pp. Nottingham University

Firkins, I., & Holdich, D.M. (1993). Thermal studies with three species of freshwater crayfish. Freshwater Crayfish 9: 241-246.

Fitter, R. & Manuel, R. (1986). Collins Field Guide to Freshwater Life of Britain and North-West Europe. William Collins Sons & Co Ltd. 382 pp.

Flindt, R. & Kahrmann, E.G. (1972). Oxygen-induced variations of heart rates in crayfish. Physiological Biology 45: 119-124.

Flint, R.W. (1975). Growth in a population of the crayfish *Pacifastacus leniusculus* from a subalpine lacustrine environment. Journal of the Fisheries Research Board of Canada 32: 2433-2440.

Flint, R.W. (1977a). Seasonal activity, migration and distribution of the crayfish, *Pacifastacus leniusculus*, in Lake Tahoe. American Midland Naturalist 97: 280-292.

Flint, R.W. (1977b). Crayfish growth in Lake Tahoe: effects of habitat variation. Journal of the Fisheries Research Board of Canada 34: 155-159.

Flint, R.W. & Goldman, C.R. (1977). Crayfish growth in Lake Tahoe: effects of habitat variation. Journal of the Fisheries Research Board of Canada 34: 155-159.

Florey, E. (1960). Studies on the nervous regulation of the heart beat in decapod Crustacea. Journal of General Physiology 43: 1061-1081.

Florey, E. & Kriebel, M.E. (1974). The effects of temperature, anoxia and sensory stimulation on the heart rate of unrestrained crabs. Comparative Biochemistry and Physiology 48A: 285-300.

Foster, J. & Turner, C. (1993). Toxicity of field simulated farm waste episodes to the crayfish *Austropotamobius pallipes* (Lereboullet): elevated ammonia and reduced dissolved oxygen concentrations. Freshwater Crayfish 9: 249-258.

France, R.L. (1985). Relationship of crayfish (*Orconectes virilis*) growth to population abundance and system productivity in small oligotrophic lakes in the experimental Lakes Area, Northwestern Ontario. Canadian Journal of Fisheries and Aquatic Science

42: 1096-1102.

Frost, J.V. (1975). Australian crayfish. Freshwater Crayfish 2: 87-96.

Fürst, M. (1977). Introduction of *Pacifastacus leniusculus* (Dana) into Sweden: methods, results and management. Freshwater Crayfish 3: 229-247.

Garvey, J.E. & Stein, R.A. (1993). Evaluating how chelae size influences the invasion potential of an introduced crayfish (*Orconectes rusticus*). American Midland Naturalist 129: 172-181.

Gillet, C. & Laurent, P.J. (1995). Tail length variations among noble crayfish (*Astacus astacus* (L)) populations. Freshwater Crayfish 10: 31-36

Gledhill, T., Sutcliffe, D.W. & Williams, W.D. (1993). British freshwater Crustacea Malacostraca: a key with ecological notes. 173 pp. Freshwater Biological Association. The Ferry House Far Sawrey, Ambleside, Cumbria LA22 OLP.

Goddard, J.S. (1988). Food and feeding. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery, R.S. eds), pp.145-166. Chapman & Hall, London.

Goldman, C.R., Rundquist, J.C. & Flint, R.W. 1975. Ecological studies of the California crayfish, *Pacifastacus leniusculus*, with emphasis on their growth from recycling waste products. Freshwater Crayfish 2: 481-488.

Goldman, C.R. & Rundquist, J.C. (1977). A comparative ecological study of the California crayfish, *Pacifastacus leniusculus* (Dana), from two subalpine lakes (Lake Tahoe and Lake Donner). Freshwater Crayfish 3: 51-80.

Gonzalez, J., Carral, J.M., Celada, J.D., Saez-Royuela, M., Gaudioso, V.R., Fernandez, R. & Lopez.-Baisson, C. (1993). Management of crayfish eggs (*Pacifastacus leniusculus*) for intensification of juvenile production. Freshwater Crayfish 9: 144-146.

Gordon, M.S. (1976). Zoology. pp. 511. Macmillan Publishing Co. New York.

Grost, R.T., Hubert, W.A. & Wesche, T.A. (1991). Description of brown trout redds in a mountain stream. Transactions of the American Fisheries Society 120(5): 582-588.

Gu, H., Mather, P.B. & Capra, M.F. (1994). The relative growth of chelipeds and abdomen and muscle production in male and female redclaws crayfish, *Cherax quadricarinatus* von Martens. Aquaculture 123: 249-257.

Guan, R.Z. (1994). Burrowing behaviour of signal crayfish, *Pacifastacus leniusculus* (Dana), in the river Great Ouse, England. Freshwater Forum 4: 155-168.

Guan, R.Z. (1995). Ecological studies on the crayfish *Pacifastacus leniusculus* (Dana). Unpublished PhD thesis. pp: 186. The University of Buckingham.

Gydemo, R. & Westin, L. (1990). Growth and survival of *Astacus astacus* L. at optimized water temperature. In: Aquaculture - A Biotechnology in Progress (N. D. Pauw, Jaspers, E., Ackefors, H., Wilkins, N., eds). pp. 383-391.

Gydemo, R., Westin, L. & Nissling, A. (1990). Predation on larvae of the noble crayfish *Astacus astacus* L. Aquaculture 86: 155-161.

Hagerman, L. & Uglow, R.F. (1979). Heart and scaphognathite activity in the shrimp *Palaemon adspersus* Rathke. Ophelia 18(1): 89-96.

Hall, L.W.Jr., Cincotta, D.E., Stauffer, J.R., Jr. & Hocutt, C.H. (1980). Temperature preference of the crayfish *Orconectes obscurus*. Archives of Environmental Contamination and Toxicology 7: 379-383.

Hanson, J.M., Chambers, P.A. & Prepas, E.E. (1990). Selective foraging by the crayfish *Orconectes virilis* and its impact on macroinvertebrates. Freshwater Biology 24: 69-80.

Harri, M. & Florey, E. (1979). The effects of acclimation temperature on a neuromuscular system of the crayfish, *Astacus leptodactylus*. Journal of Experimental Biology 78: 281-293.

Hart, C.W. & Clarck, J. (1987). An Interdisciplinary Bibliography of Freshwater Crayfishes (Astacoidea and Parastacoidea) from Aristotle through 1985. 437 pp. Smithsonian Institution Press, Washington.

Hart, D.D. (1992). Community organization in streams: the importance of species interactions, physicial factors, and chance. Oecologia 91: 220-228.

Hazlett, B.A. (1985). Chemical detection of sex and condition in the crayfish *Orconectes virilis*. Journal of Chemical Ecology 11: 181-189.

Henry, R.P. & Wheatly, M.G. (1988). Dynamics of salinity adaptations in the euryhaline crayfish *Pacifastacus leniusculus*. Physiological Zoology 61(3): 260-271.

Hepworth, D.K. & Duffield, D.J. (1987). Interactions between an exotic crayfish and stocked rainbow trout in Newcastle Reservoir, Utah. North American Journal of Fisheries Management 7: 554-561.

Herrmann, F. (1931). Uber den wasserhaushalt des flusskrebses (*Potamobius astacus* Leach). Zeitschrift fur Vergleichende Physiologie 14: 479-524.

Hessen, D.O., Taugbol, T., Fjeld, E. & Skurdal, J. (1987). Egg development and lifecycle timing in the noble crayfish (*Astacus astacus*). Aquaculture 64: 77-82.

Hindley, J.P.R. & Alexander, C.G. (1978). Structure and function of the chelate pereiopods of the banana prawn *Penaeus merguiensis*. Marine Biology 48: 153-160.

Hinton, D.J. & Corey, S. (1979). The mouthparts and digestive tract in the larval stages of *Homarus americanus*. Canadian Journal of Zoology 57: 1413-1423.

Hobbs, H.H., & Edward, T.H. (1974). Crayfishes (Decapoda: Astacidae). In: Pollution ecology of freshwater invertebrates (C.W. Hart, Samuel L.H. Fuller eds). pp. 195-214. Adacemic Press London.

Hobbs, H.H.Jr. (1987). A review of the crayfish genus *Astacoides* (Decapoda: Parastacidae). Smithsonian Contributions to Zoology No 443. 50 pp.

Hobbs, H.H.Jr. (1988). Crayfish distribution, adaptive radiation and evolution. In: Freshwater crayfish: biology, management and exploitation (Holdich D. M. & Lowery R. S., eds), pp. 11-51. Chapman & Hall, London.

Hobbs, H,H., Jass, J.P. & Huner, J.V. (1989). A review of global crayfish introductions with particular emphasis on two North American species (Decapoda: Cambaridae). Crustaceana 56: 299-316.

Hogger, J.B. (1986). Aspects of the introducing of "signal crayfish", *Pacifastacus leniusculus* (Dana), into the Southern United Kingdom. 1. Growth and survival. Aquaculture 58: 27-44.

Hogger, J. B. (1988). Ecology, Population Biology and Behaviour. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery, R.S. eds), pp. 114-145. Chapman & Hall, London.

Holdich, D.M. (1981). Biological effects. In: Industrial effluent treatment (Volume 1) (J.K. Walters and A. Wint eds.). pp. 91-126. Applied Science Publishers.

Holdich, D.M. (1988). The dangers of inroducing alien animals with particular reference to crayfish. Freshwater Crayfish 7: xv-xxx.

Holdich, D.M. & Lowery, R.S. (1988). Crayfish: an introduction. In: Freshwater crayfish: biology, management and exploitation (Holdich D. M. & Lowery R. S., eds), pp. 1-7. Chapman & Hall, London.

Holdich, D.M. & Reeve, I.D. (1988). Functional morphology and anatomy. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 11-51. Chapman & Hall, London.

Holdich, D.M. (1991a). A bibliography of "Freshwater Crayfish - A journal of Astacology I-VII". Proc. Int. Assoc. Astacology Symp. 1973-1988. Journal of Shellfish Research 10: 341-347.

Holdich, D.M. (1991b). The native crayfish and threats to its existence. British Wildlife 2(3): 141-151.

Holdich, D.M. & Reeve, I.D. (1991). Alien crayfish in British waters. Final report on GR3/6842 for Natural Environment Research Council. 38 pp.

Holdich, D.M. & Rogers, W.D. (1992). Crayfish populations in the British Isles: Farming, legislation, conservation and management. Finnish Fisheries Research 14: 23-32.

Holdich, D.M. (1992). Crayfish nomenclature and terminology: recommondations for uniformity. Finnish Fish Research 14: 149-155.

Holdich, D.M. (1993). A review of astaciculture: freshwater crayfish farming. Aquatic Living Resources, 6: 307-317.

Holdich, D.M. (1994). The British crayfish - a threatened species? Biological Sciences Review 6(5): 12-13.

Holdich, D.M. & Domaniewski, J.J.C. (1995). Studies on a mixed population of *Austropotamobius pallipes* and *Pacifastacus leniusculus* in an English lake. Freshwater Crayfish 10: 37-45.

Holdich, D.M. & Pearce-Higgins, J.W. (1995). A bibliography and taxonomic and subject index to "Freshwater Crayfish I-X", the Proceedings of the International Association of Astacology Symposia 1972-1994. Freshwater Crayfish xvi-lxxix.

Holdich, D.M., Reader, J.P., Rogers, W.D. & Harlıoğlu, M.M. (1995a). Interactions between three species of crayfish (*Austropotamobius pallipes*, *Astacus leptodactylus* and *Pacifastacus leniusculus*. Freshwater Crayfish 10: 46-56.

Holdich, D.M., Reader, J.P. & Rogers, W.D. (1995b). Crayfish conservation final project report to National Rivers Authority, 1995. R&D 378/10/N. 278 pp.

Huxley, T.H. (1973). The crayfish. An introduction to the study of zoology. pp. 371. The MIT Press, Cambridge.

Hopkins, C.L. (1967). Growth rate in a population of the freshwater crayfish, *Paranephrops planifrons* White. New Zealand Journal of Marine and Freshwater Research 1(4): 463-474.

Horne, A.J. & Goldman, C.R. (1994). Limnology. 576 pp. McGraw-Hill International Editions.

Huner, J.V., Meyers, S.P. & Avault, J.W.Jr. (1975). Response and growth of freshwater crawfish to an extruded, water-stable diet. Freshwater Crayfish 2: 149-157.

Huner, J.V., Miltner, M., Avault, Jr.J.W. & Bean, R.A. (1983). Interactions of freshwater prawns, channel catfish fingerlings, and crayfish in earthen ponds. Progressive Fish-Culturist 45(1): 36-40.

Huner, J.V. & Lindqvist, O.V. (1985). Effects of temperature and photoperiods on mating and spawning activities of wild-caught noble crayfish, *Astacus astacus* Linne, (Astacidae, Decapoda). Journal of World Mariculture Society 16: 225-226.

Huner, J.V. & Lindqvist, O.V. (1986). A stunded crayfish *Astacus astacus* population in central Finland. Freshwater Crayfish 6: 156-165.

Huner, J.V. (1988). *Procambarus* in North America and elsewhere. In: Freshwater crayfish: biology, management and exploitation (D. M. Holdich and Lowery R. S. eds). pp. 239-261. Chapman & Hall, London.

Huner J.V., Lindqvist, O.V. & Kononen, H. (1988). Comparison of morphology and edible tissues of two important commercial crayfish, the noble crayfish, *Astacus astacus* Linne, and the red swamp crayfish, *Procambarus clarkii* (Girard) (Decapoda, Astacidae and Cambaridae). Aquaculture 68: 45-57.

Huner, J.V, (1989). Overview of international and domestic freshwater crawfish production. Journal of Shellfish Research 8(1): 259-265.

Huner, J.V. & Barr, J.E. (1991). Red Swamp Crawfish: Biology and Exploitation. 128 p. Louisiana Sea Grant College Program. Center for Wetland Resources Louisiana State University Baton Rouge, Louisiana.

Huner, J.V. & Lindqvist, O.V. (1991). Special problems in freswater crayfish egg production. In: Crustacean egg production (Wenner A. & Kuris A., eds), Crustacean Issues 7: 235-246.

Huner, J.V., Henttoven, P. & Lindqvist, O.V. (1991). Length-length and length-weight characterizations of noble crayfish, *Astacus astacus* L. (Decapoda, Astacidae), from central Finland. Journal of Shellfish Research 10(1): 195-196.

Huner, J.V. (1993). Recovery of edible products from some common North American orconectic and procambarid crayfish (Cambaridae) with emphasis on Procambarus clarkii (Girard) and *Procambarus zonagulus* Hobbs & Hobbs. Freshwater Crayfish 9: 28-37.

Huner, J.V. (1994) (editor). Freshwater crayfish aquaculture in North America, Europe, and Australia: families *Astacidae*, *Cambaridae*, and *Parastacidae*. 312 pp. Haworth Press, Inc.

Huner, J.V. & Lindqvist, O.V. (1995). Physiological adaptations of freshwater crayfishes that permit successful aquacultural enterprises. American Zoologist 35: 12-19.

Huxley, T.H. (1973). The crayfish. An introduction to the study of zoology. pp. 371. The MIT Press, Cambridge.

Hynes, H.B.N. (1970). The ecology of running waters. 555 pp. Liverpool University Press.

Ingle, R.W. (1979). Laboratory and scuba studies on the behaviour of the freshwater crayfish, *Austropotamobius pallipes* (Lereboullet). Report of the Underwater Association 2(NS): 1-16.

Itagaki, H. & Thorp, J.H. (1981). Laboratory experiments to determine if crayfish can communicate chemically in a flow-through system. Journal of Chemical Ecology 7(1): 115-126.

Ivanova, M.B. & Vassilenko, S.V. (1987). Relationship between number of eggs, brood weight, and female body weight in Crustacea. Internationale Revue der Gesamten Hydrobiologie 72(2): 147-169.

Jacobsen, O.J. (1977). Large crayfish, *Astacus astacus*, as food for perch, *Perca fluviatilis*. Fauna 30(2): 98-99.

Jones, C.M. (1995). Salinity tolerance of the tropical freshwater crayfish, *Cherax quadricarinatus* (Von Martens) (Decapoda, Parastacidae). Freshwater Crayfish 8: 399-409.

Jonsson, A. (1995). Life history differences between crayfish *Astacus astacus* and *Pacifastacus leniusculus* in embryonic and juvenile development, laboratory experiences. Freshwater Crayfish 8: 170-178.

Kaestner, A. (1970). Invertebrate Zoology. Vol: III. Interscience Publishers, London. 523 pp.

Karafezlieva-Avramova, R. (1988). The influence of water temperature on the feeding intensity of *Astacus (Pt) leptodactylus* to the age of one year in artificial conditions. Freshwater Crayfish 7: 359-362.

Karlson, P. & Luscher, M. (1959). 'Pheromones': a new term for a class of biologically active substances. Nature 183: 55-56.

Karplus, I., Barki, A., Levi, T., Hulata, G. & Harpaz, S. (1995). Effects of kinship and shelters on growth and survival of juvenile Australian redclaw crayfish (*Cherax quadricarinatus*). Freshwater Crayfish 10: 494-505.

Kendall, A. & Schwartz, F. (1964). Salinity tolerances of two maryland crayfishes. The Ohio Journal of Science 64(6): 403-409.

Kerley, D.E. & Pritchard, A.W. (1967). Osmotic regulation in the crayfish, *Pacifastacus leniusculus*, stepwise acclimated to dilutions of sea water. Comparative Biochemistry and Physiology 20: 101-113.

King, C.R. (1993). Egg development time and storage for redclaws crayfish *Cherax quadricarinatus* von Martens. Aquaculture 109: 275-280.

Kirjavainen, J. & Westman, K. (1994). Comparative growth from length composition and mark-recapture experiments for noble crayfish (*Astacus astacus*) and signal crayfish *Pacifastacus leniusculus* in Finland. Nordic Journal of Freshwater Research 69: 153-161.

Kirjavainen, J. & Westman, K. (1995). Development of an introduced signal crayfish *Pacifastacus leniusculus* (Dana) population in the small lake Karisjarvi in central Finland. Freshwater Crayfish 10: 140-150.

Köksal, G. (1988). *Astacus leptodactylus* in Europe. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 365-400. Chapman & Hall, London.

Kopacek, P., Grubhoffer, L. & Söderhäll, K. (1993). Isolation and characterization of a hemagglutinin with affinity for lipopolysaccharides from plasma of the crayfish *Pacifastacus leniusculus*. Developmental and Comparative Immunology 17(5): 407-418.

Kossakowski, J. (1966). Crayfish (Raki). (Translated by Massey, H.M. in 1971) 163 p. Translated for the National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Department of Commerce and the National Science Foundation, by the Scientific Publications Foreign Cooperation Center of the Central Institute for Scientific Technical and Economic Information, Warsaw, Poland.

Kossakowski, J. (1971). Crayfish (raki). 193 pp. Translated for the National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Department of Commerce and the National Science Foundation, by the Scientific Publications Foreign Cooperation Center of the Central Institute for Scientific Technical and Economic Information, Warsaw, Poland.

Kossakowski, J., Mnich, M. & Kossakowski, G. (1978). The first introduction of the crayfish, *Pacifastacus leniusculus* into Polish waters. Freshwater Crayfish 4: 195.

Kossakowski, J. (1988). Some remarks on the biology of the crayfish *Pacifastacus leniusculus*. Freshwater Crayfish 7: 369-376.

Kuris, A.M. (1991). A review of patterns and causes of crustacean brood mortality. In: Crustacean egg production (Wenner A. & Kuris A., eds), Crustacean Issues 7: 117-141.

Lahti, E. & Lindqvist, O.V. (1983). On the reproductive cycle of the crayfish *Astacus astacus* L. in Finland. Freshwater Crayfish 5: 18-26.

Lahti, E. (1988). On the muscle and hepatopancreas weight in crayfish (*Astacus astacus* L.) in Finland. Freshwater Crayfish 7: 319-325.

Larimer, J.L. (1962). Responses of the crayfish heart during respiratory stress. Physiological Zoölogy 35: 179-186.

Larimer, J.L. (1964). Sensory-induced modifications of ventilation and heart rate in crayfish. Comparative Biochemistry and Physiology 12: 25-36.

Laurent, P.J. & Forest, J. (1979). Données sur les écrevisses qu'on peut rencontrer en France. La Pisciculture Française 56 (15-2): 25-40.

Laurent, P.J. & Vey, A. (1986). The acclimation of *Pacifastacus leniusculus* in Lake Divonne. Freshwater Crayfish 6: 146-155.

Laurent, P.J. (1988). *Austropotamobius pallipes* and *A. torrentium*, with observations on their interactions with other species in Europe. In: Freshwater crayfish: biology,

management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 341-364. Chapman & Hall, London.

Layne, J.R.Jr., Claussen, D.L. & Manis, M.L. (1987). Effects of acclimation temperature, season, and time of day on the critical thermal maxima and minima of the crayfish *Orconectes rusticus*. Journal of Thermal Biology 12(3): 183-187.

Lee, D.O'C. and Wickins, J.F. (1992). Crustacean Farming. 392 pp. Blackwell Scientific Publications.

Lincoln, R.J., Boxshull, G.A. & Clark, P.F. (1982). A dictionary of ecology, evolution and systematics. 298 pp. Cambridge University Press.

Lindqvist, O.V. & Louekari, K. (1975). Muscle and hepatopancreas weight in *A stacus astacus* L. (Crustacea, Astacidae) in the trapping season in Finland. Annales Zoologici Fennici 12: 237-243.

Lindqvist, O.V. & Lahti, E. (1983). On the sexual dimorphism and condition index in the crayfish *A stacus astacus* L. in Finland. Freshwater Crayfish 5: 3-11.

Lindqvist, O.V. (1988). Restoration of native European crayfish stocks. Freshwater Crayfish 7: 6-12.

Little, E.E. (1975). Chemical communication in maternal behaviour of crayfish. Nature 255: 400-401.

Little, E.E. (1976). Ontogeny of maternal behaviour and brood pheromone in crayfish. Journal of Comparative Physiology 112: 133-142.

Lodge, D.M., Beckel, A.L. & Magnuson, J.J. (1985). Lake-bottom Tyrant. Natural History 94(8): 32-37.

Lodge, D.M., Kratz, T.K. & Capelli, G.M. (1986). Long-term dynamics of three crayfish species in trout lake, Wisconsin. Canadian Journal of Fisheries and Aquatic Sciences 43: 993-998.

Lodge, D.M. & Lorman J.G. (1987). Reductions in submersed macrophyte biomass and species richness by the crayfish *Orconectes rusticus*. Canadian Journal of Fisheries and Aquatic Sciences 44: 591-597.

Lowery, R.S. (1988). Growth, moulting and reproduction. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 83-113. Chapman & Hall, London.

Lowery, R.S. & Holdich, D.M. (1988). *Pacifastacus leniusculus* in North America and Europe, with details of the distribution of introduced and native crayfish species in Europe. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 283-308. Chapman & Hall, London.

Loya-Javellana, G.N. (1995). Ontogeny of feeding behaviour, morphology of the mouthparts and foregut, and digestive processes in the redclaw crayfish, *Cherax quadricarinatus*. Unpublished PhD thesis. The University of Queensland.

Lund, M.H. (1944). A study on the food of the crayfish *Potamobius astacus*. Nyt Magazin Natur Vitenskaberne B4: 219-250.

Lutz, C.G. & Wolters, W.R. (1989). Estimation of heritabilities for growth, body size, and processing traits in red swamp crayfish, *Procambarus clarkii* (Girard). Aquaculture 78: 21-33.

Malaczynska-Suchcitz, Z. (1956). Observations on the manner in which newly hatched crayfish (*Potamobius astacus* Leach and *Potamobius leptodactylus* Eschz.) are attached to the pleopods of the female. Bulletin de la Societe Des Amis Des Sciences et des Lettres de Poznan Serie B, Livrasion xiii: 341-351.

Magnuson, J.J., Capelli, G.M., Lorman, J.G. & Stein, R. (1975). Consideration of crayfish for macrophyte control. In: The proceeding of asymposium on water quality management through biological control (Brejanik, P.L. & Fox, J.L. eds). pp. 66-74. University of Florida, Gainesville, Florida.

Magnuson, J.J. & Beckel, A.L. (1985). Exotic species. Wisconsin Academy Review, December: 8-10.

Marlet, B. & Roqueplo, C. (1991). Premier belan des deux station experimentales de production semi-intensive d'ecrevisses *Astacus leptodactylus* en Charente. Compte rendu no 79, 17 pages, prix 40 F (en timbres). CEMA-GREF, Groupement de Bordeaux; B.P. 3, F-33 611 Gazinent cedex, France.

Mason, J.C. (1970). Maternal-offspring behavior of the crayfish, *Pacifastacus trowbridgi* (Stimpson). American Midland Naturalist 84: 463-473.

Mason, J.C. (1975). Crayfish production in a small woodland stream. Freshwater Crayfish 2: 449-479.

Mason, J.C. (1977a). Reproductive efficiency of *Pacifastacus leniusculus* (Dana) in culture. Freshwater Crayfish 3: 101-117.

Mason, J.C. (1977b). Artificial incubation of crayfish eggs (*Pacifastacus leniusculus* (Dana). Freshwater Crayfish 3: 119-132.

Mason, J.C. (1978a). Effects of temperature, photoperiod, substrate, and shelter on survival, growth, and biomass accumulation of juvenile *Pacifastacus leniusculus* in culture. Freshwater Crayfish 4: 73-82.

Mason, J.C. (1978b). Significance of egg size in the freshwater crayfish, *Pacifastacus leniusculus* (Dana). Freshwater Crayfish 4: 83-92.

Mather, M.E. & Stein, R.A. (1993). Direct and indirect effects of fish predation on the replacement of a native crayfish by an invading congener. Canadian Journal of Fisheries and Aquatic Sciences 50: 1279-1288.

Mathur, D., Schutsky, R.M. & Purdy, E.J.Jr. (1982). Temperature preference and avoidance responses of the crayfish *Orconectes obscurus*, and associated statistical problems. Canadian Journal of Fisheries and Aquatic Sciences 39: 548-553.

Matthews, M.A., Reynolds, J.D. & Keatinge, M.J. (1993). Macrophyte reduction and benthic community alteration by the crayfish *Austropotamobius pallipes* (Lereboullet). Freshwater Crayfish 9: 289-299.

Matthews, M. & Reynolds, J.D. (1995). In vitro culture of crayfish eggs using a recirculating airlift incubator. Freshwater Crayfish 8: 300-306.

McClain, W.R. (1995). Investigations of crayfish density and supplemental feeding as factors influencing growth and production of *Procambarus clarkii*. Freshwater Crayfish 10: 512-520.

McDonald, D.C., McMahon, B.R. & Wood, C.M. (1977). Patterns of heart and scaphognathite activity in the crab *Cancer magister*. Journal of Experimental Zoology 202: 33-44.

McGriff, D. 1981. The commercial fishery for *Pacifastacus leniusculus* Dana in the Sacramento-San Joaquin Delta. Freshwater Crayfish 5: 403-417.

McGriff, D. (1983). Growth, maturity, and fecundity of the crayfish, *Pacifastacus leniusculus*, from the Sacramento San Joaquin Delta. California Fish and Game 69(4): 227-242.

McMahon, B.R., Burggren, W.W. & Wilkens, J.L. (1974). Respiratory responses to longterm hypoxic stress in the crayfish *Orconectes virilis*. Journal of Experimental Biology 60: 195-206.

McMahon, B.R. & Wilkes, P.R.H. (1983a). Emergence responses and aerial ventilation in normoxic and hypoxic crayfish *Orconectes rusticus*. Physiological Zoology 56(2): 133-141.

McMahon, B.R. & Wilkens, J.L. (1983b). Ventilation, perfusion and oxygen uptake. In: The biology of Crustacea: Internal anatomy and physiological regulation (Mantel, L.H., eds), Vol: 5, pp. 290-372.

McMahon, B.R. (1986). The adaptable cryfish: mechanisms of physiological adaptation. Freshwater Crayfish 6: 59-74.

Medley, P.B., Jones, C.M. & Avault, J.W. (1995). A bibliography of the Australian redclaw crayfish, *Cherax quadricarinatus* (von Martens 1868) (Decapoda: Parastacidae). Freshwater Crayfish 10: 532-549.

Mercier, A.J. & Russenes, R.T. (1992). Modulation of crayfish hearts by FMRFamide-related peptides. Biological Bulletin 182: 333-340.

Meyers, S.P. 1985. Recovery of meat from crawfish claws. Crawfish Tales, The Official Publication of the Louisiana Crawfish Farmers Assocation. 4(2): 28-32.

Mikkola, H. (1978). Ecological and social problems in the use of the crayfish, *Procambarus clarkii* in Kenya. Freshwater Crayfish 4: 197-206.

Mills, B.J. & Lake, P.S. (1975) Setal development and moult staging in the crayfish *Parastacoides tasmanicus* (Erichson), (Decapoda Parastacidae). Australian Journal of Marine and Freshwater Research 26: 103-107.

Mills, B.J. & Geddes, M.C. (1980). Salinity tolerance and osmoregulation of the Australian freshwater crayfish *Cherax destructor* Clark (Decapoda: Parastacidae). Australian Journal of Marine and Freshwater Research 31: 667-676.

Mills, B.J. & McCloud, P.I. (1983). Effects of stocking and feeding rates on experimental pond production of the crayfish *Cherax destructor* Clark (Decapoda: Parastacidae). Aquaculture 34: 51-72.

Minckley, W.L. & Craddock, J.E. (1961): Active predation of crayfish on fishes. The Progressive Fish-Culturist 123: 120-123.

Mirenda, R.J. & Dimock, R.V.Jr. (1985). Temperature tolerance of the crayfish *Cambarus acuminatus* Faxon, 1884 (Decapoda, Astacidea). Crustaceana 48(3): 249-259.

Mittenthal, J.E. & Trevarrow, W.W. (1983). Intercalary regeneration in legs of crayfish: central segments. Journal of Experimental Zoology 225: 15-31.

Momot, W. T. (1967). Effects of brook trout predation on a crayfish population. Transactions of the American Fisheries Society 96(2): 202-209.

Momot, W.T., Gowing, H. & Jones, P.D. (1978). The dynamics of crayfish and their role in ecosystems. American Midland Naturalist 99(1): 10-35.

Momot, W.T. (1984). Crayfish production: a reflection of community energetics. Journal of Crustacean Biology 4(1): 35-54.

Momot, W.T. & Leering, G.M. (1986). Aggressive interaction between *Pacifastacus leniusculus* and *Orconectes virilis* under laboratory conditions. Freshwater Crayfish 6: 87-93.

Momot, W. (1988). *Orconectes* in North America and elsewhere. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 262-282. Chapman & Hall, London.

Momot, W.T. (1993). The role exploitation in altering the processes regulating crayfish populations. Freshwater Crayfish 9: 101-117.

Momot, W.T. (1995). Redefining the role of crayfish in aquatic ecosystems. Reviews in Fisheries Science 3(1): 33-63.

Morgan, G.E. & Momot, W.T. (1988). Exploitation of *Orconectes virilis* in northern climates: complimentarity of management options with self-regulatory life history strategies. Freshwater Crayfish 7: 69-80.

Morrissy, N.M. (1990). Optimum and favourable temperatures for growth of *Cherax tenuimanus* (Smith 1912) (Decapoda: Parastacidae). Australian Journal of Marine and Freshwater Research 41: 735-746.

Morrissy, N.M., Bird, C. & Cassells, G. (1995). Density-dependent growth of cultured marron, *Cherax tenuimanus* (Smith 1912). Freshwater Crayfish 10: 560-568.

Moshiri, G.A., Goldman, C.R. Godshalk, G.L. & Mull, D.R. (1970). The effects of variations in oxygen tension on certain aspects of respiratory metabolism in *Pacifastacus leniusculus* (Dana). Physiological Zoology 43(1): 23-29.

Mundahl, N.D. & Benton, M.J. (1990). Aspects of the thermal ecology of the rusty crayfish *Orconectes rusticus* (Girard). Oecologia 82: 210-216.

Munkhammar, T., Gydemo, R., Westin, L. & Ackefors, H. (1989). Survival of noble crayfish (*Astacus astacus* L.) larvae alone and in the presence of females. Aquaculture - A Biotechnology in Progress 1989: 409-414.

Mura, G. & Caldo, L.D. (1993). A scanning electron microscope study of the molar surface of the mandibles in *Branchinella spinosa* H. Milne Edwards (Branchiopoda) with some remarks on the taxonomical significance of this character. Crustaceana 64(3): 326-336.

NRA, (1994). A guide to identifiying freshwater crayfish in Britain and Ireland. National Rivers Authority.

Papadopol, M. (1975). Contribution to the study of reproductive potentiality of some populations of *Astacus leptodactylus* from the Danube delta. Travaux du Museum d'Histoire Naturelle "Gr. Antipa," Bucuresti 16: 89-99.

Patrica, R.R. & Armitage, K.B. (1974). The influence of photoperiod on processes associated with molting and reproduction in the crayfish *Orconectes nais* (Faxon). Comparative Biochemistry and Physiology 47A: 243-259.

Payne, J.F. (1978). Aspects of the life histories of selected of North American crayfishes. Fisheries 3(6): 5-8.

Payne, J.F. (1986). Factors influencing patterns of crayfish distribution. Freshwater Crayfish 6: 100-110.

Pennack, R.W. (1978). Fresh-water invertebrates of the United States. 803 p. A Wiley-interscience publication, New York, Chichester, Brisbane, Toronto.

Péqueux, A. (1995). Osmotic regulation in crustaceans. Journal of Crustacean Biology 15(1): 1-60.

Persson, M., Cerenius, L. & Söderhäll, K. (1987). The influence of hemocyte number on the resistance of the freshwater crayfish *Pacifastacus leniusculus* Dana to the parasitic fungus *Aphanomyces astaci*. Journal of Fish Diseases 10(6): 471-477.

Philips, R. & Rix, M. (1985). Freshwater fish of Britain, Ireland and Europe. 144 pp. Printed by Chorley & Pickersgill Ltd. Leeds.

Piesik, Z. (1974). The role of the crayfish *Orconectes limosus* (Raf) in extinction of *Dreissena polymorpha* (Pall) subsisting on steelon-net. Polskie Archiwum Hydrobiologii 21(3/4): 401-410.

Portelance, B. & Dube, P. (1995). Temperature and photoperiod effects on ovarian maturation, ovarian growth, and egg-laying of crayfish *Orconectes virilis*. Freshwater Crayfish 8: 321-330.

Pratten, D.J. (1980). Growth in the crayfish *Austropotamobius pallipes* (Crustacea: Astacidae). Freshwater Biology 10: 401-412.

Price, J.O. & Payne, J.F. (1984). Size, age, and population dynamics in an r-selected population of *Orconectes neglectus chaenodactylus* Williams (Decapoda, Cambaridae). Crustaceana 46(1): 29-38.

Prins, R. (1968). Comparative ecology of the crayfishes *Orconectes rusticus rusticus* and *Cambarus tenebrosus* in Doe Run, Meade Country, Kentucky. International Revue der Gesamten Hydrobiologie 53(5): 667-714.

Pritchard, A.W. & Kerley, D.E. (1970). Kidney function in the North American crayfish *Pacifastacus leniusculus* (Dana) stepwise acclimated to dilutions of sea water. Comparative Biochemistry and Physiology 35: 427-437.

Provenzano, A.J.Jr. & Handwerker, T.S. (1995). Effects of photoperiod on spawning of red swamp crayfish, *Procambarus clarkii*, at elevated temperature. Freshwater Crayfish 8: 311-320.

Pursiainen, M., Jarvenpaa, T. & Westman, K. (1983). A comparative study on the production of crayfish (*Astacus astacus* L.) juveniles in natural food ponds and by feeding in plastic basins. Freshwater Crayfish 5: 392-402.

Pursiainen, M., Saarela, M. & Westman, K. (1988). Moulting and growth of the noble crayfish *Astacus astacus* in an oligotrophic lake. Freshwater Crayfish 7: 155-164.

Rahel, F.J. & Stein, R.A. (1988). Complex predator-prey interactions and predator intimidation among crayfish, piscivorous fish, and small benthic fish. Oecologia (Berlin) 75: 94-98.

Ramirez, L.F.B., Herrera, F.D., Sandoval, F.C., Sevilla, B.B. & Rodriguez, M.H. (1994). Diel thermoregulation of the crawfish *Procambarus clarkii* (Crustacea, Cambaridae). Journal of Thermal Biology 19(6): 419-422.

Reiber, C.L. (1995). Physiological adaptations of crayfish to the hypoxic environment. American Zoologist, 35: 1-11.

Reynolds, J.D., Celada, J.D., Carral, J.M. & Matthews, M.A. (1992). Reproduction of astacid crayfish in captivity - current developments and implications for culture, with special reference to Ireland and Spain. Invertebrate Reproduction and Development 22(1-3): 253-266.

Rhodes, C.P. & Holdich, D.M. (1979). On size and sexual dimorphism in *Austropotamobius pallipes* (Lereboullet) A step in assessing the commercial exploitation potential of the native British freshwater crayfish. Aquaculture 17: 345-358.

Rhodes, C.P. (1981). Artificial incubation of the eggs of the crayfish *Austropotamobius pallipes* (Lereboullet). Aquaculture 25: 129-140.

Rhodes, C.P. & Holdich, D.M. (1982). Observations on the fecundity of the freshwater crayfish, *Austropotamobius pallipes* (Lereboullet) in the British Isles. Hydrobiologia 89: 231-236.

Rhodes, C.P. & Holdich, D.M. (1984). Length-weight relationship, muscle production and proximate composition of the freshwater crayfish *Austropotamobius pallipes* (Lereboullet). Aquaculture 37: 107-123.

Richards, K.J. (1983). The introduction of the signal crayfish into the United Kingdom and its development as a farm Corp. Freshwater Crayfish 5: 557-563.

Rickett, J.D. (1974). Trophic relationships involving crayfish of the genus *Orconectes* in experimental ponds. Progressive Fish-Culturist 34(4): 207-211.

Roberts, M.H.Jr. (1968). Functional morphology of mouth parts of the hermit crabs, *Pagurus longicarpus* and *Pagurus pollicaris.* Chesapeake Science 9(1): 9-20.

Rogers, W.D. & Holdich, D.M. (1995). Conservation and management of crayfish in Britain. Freshwater Crayfish 10: 92-97.

Romaire, R.P., Forester, J.S., Avault, J.V. (1977). Length-weight relationships of two commercially important crayfishes of the genus *Procambarus.* Freshwater Crayfish 3: 463-470.

Rose, R.D. (1982). On the nature of chemical communication by crayfish in a laboratory controlled flow-through system. Journal of Chemical Ecology 8: 1065-1071.

Rose, R.D. (1984). Chemical communication in crayfish: physiological ecology, realism and experimental design. Journal of Chemical Ecology 10(8): 1289-1292.

Rouse & Yeh (1995). Factors influencing spawning of *Cherax quadricarinatus* in indoor hatcheries. Freshwater Crayfish 10: 605-610.

Rumyantsev, V.D. (1973). Growth increment per moult of *Astacus leptodactylus* in the upper delta of the Volga. Gidrobiology ZH 9(4): 65-66.

Rundquist, J.C. & Goldman, C.R. (1978). Growth and food conversion efficiency of juvenile *Pacifastacus leniusculus* along a salinity gradient. Freshwater Crayfish 4: 105-

113.

Rutledge, P.S. (1981). Circulation and oxygen transport during activity in the crayfish, *Pacifastacus leniusculus*. American Journal of Physiology 240: R-99-R105.

Sadewasser, S.G. & Prins, R. (1979). The effects of temperature and photoperiod on molting in seasonal populations of the crayfish *Orconectes rusticus rusticus*. Transactions of the Kentucky Academy of Science 40(3-4): 129-140.

Saezroyuela, J.M., Carral, J.D., Celada & Munoz, C. (1995). Effects of management on survival and growth of stage 2 juvenile freshwater signal crayfish (*Pacifastacus leniusculus* Dana) under laboratory conditions. Aquaculture 133(2): 123-134.

Saffran, K.A. & Barton, D.R. (1993). Trophic ecology of *Orconectes propinquus* (Girard) in Georgian Bay (Ontario, Canada). Freshwater Crayfish 9: 350-358.

Saiki, M.K. & Tash, J.C. (1979). Use of cover and dispersal by crayfish to reduce predation by largemouth bass. North Central Division American Fisheries Society Special Publication 6: 44-48.

Schmitt, W.L. (1973). Crustaceans. David & Charles, Newton Abbot. 204 pp.

Scholtz, G. & Richter, S. (1995). Phylogenetic systematics of the reptantian Decapoda (Crustacea, Malacostraca). Zoological Journal of the Linnean Society 113: 289-328.

Serol, A. & Coler, R.A. (1975). Demonstrated food references of *Orconectes immunis* (Hagen) (Decapoda, Astacidea). Crustaceana 29 (3): 319-320.

Sevilla, C. (1988). Nutrition in intensive crayfish culture with a new dry pelleted diet. Freshwater Crayfish 7: 271-276.

Sharfstein, B.A. & Chafin, C. (1979). Red swamp crayfish: short-term effects of salinity on survival and growth. The Progressive Fish-Culturist 41(3): 156-157.

Shaw, J. (1960). The absorption of sodium ions by the crayfish *Austropotamobius pallipes* Lereboullet. I. The effect of external and internal sodium concentrations. Journal of Experimental Biology 36: 126-144.

Shimizu, S.J. & Goldman, C.R. (1983). *Pacifastacus leniusculus* (Dana) production in the Sacromento River. Freshwater Crayfish 5: 210-228.

Skurdal, J. & Qvenild, T. (1986). Growth, maturity and fecundity of *Astacus astacus* in lake Steinsfjorden, S.E. Norway. Freshwater Crayfish 6: 182-186.

Skurdal, J., Qvenild, T., Taugbol, T. & Gamas, E. (1993). Long term study of exploitation, yield and stock structure of noble crayfish *Astacus astacus* in lake Steinsfjorden, S.E. Norway. Freshwater Crayfish 9: 118-133.

Söderbäck, B., Appleberg, M., Odelstrom, T. & Lindqvist, U. (1988). Food consumption and growth of the crayfish *Astacus astacus* L. in laboratory experiments. Freshwater Crayfish 7: 145-153.

Söderbäck, B. (1993). Population regulation in two co-occurring crayfish species. Acta Universitatis Upsaliensis. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science 434. 29 pp.

Söderbäck, B. (1994). Interactions among juveniles of to freshwater crayfish species and a predatory fish. Oecologia 100: 229-235.

Söderbäck, B. (1995). Replacement of the native crayfish *Astacus astacus* by the introduced species *Pacifastacus leniusculus* in a Swedish lake: possible causes and mechanisms. Freshwater Biology 33: 291-304.

Sokol, A. (1988). The Australian Yabby. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 401-425. Chapman & Hall, London.

Solon, M.H. & Cobb, J.S. (1980). The external morphology and distribution of cuticular hair organs on the claws of the American lobster, *Homarus americanus* (Milne-Edwards). Journal of Experimental Marine Biology and Ecology 48: 205-215.

Somers, K.M. (1991). Characterizing size-specific fecundity in crustaceans. In: Crustacean egg production (Wenner A. & Kuris A., eds), Crustacean Issues 7: 357-378.

Spaargaren, D.H. (1973). The effect of salinity and temperature on the heart rate of osmoregulating and osmoconforming shrimps. Comparative Biochemistry and Physiology 45A: 773-786.

Spohrer, M.L., Williams, J.L. & Avault, J.W.Jr. (1975). A selected bibliography of the red swamp crayfish, *Procambarus clarkii* (Girard) and the white river crayfish, *Procambarus acutus acutus* (Girard). Freshwater Crayfish 2: 637-661.

Spoor, W.A. (1955). Loss and gain of heat-tolerance by the crayfish. Biological Bulletin 108: 77-87.

Steele, D.H. & Steele, V.J. (1975). Egg size and duration of embryonic development in Crustacea. International Revue der Gesamten Hydrobiologie 60(5): 711-715.

Steele, D.H. & Steele, V.J. (1991). Morphological and environmental restraints on egg production in amphipods. In: Crustacean egg production (Wenner A. & Kuris A., eds), Crustacean Issues 7: 157-170.

Stein, R.A. (1976). Sexual dimorphism in crayfish chelae: functional significance linked to reproductive activities. Canadian Journal of Zoology 54: 220-227.

Stein R. A.(1977). Selective Predation, optimal foraging , and the predator-prey interaction between fish and crayfish. Ecology. 58: 1237-1253.

Stephens, G.C. (1955). Induction of molting in the crayfish, *Cambarus*, by modification of daily photoperiod. Biological Bulletin 108: 235-241.

Strempel, K.M. (1973). Edelkrebserbrutung in zuger-glasern und anfutterung der krebsbrut. Freshwater Crayfish 1: 233-237.

Strempel, K.M. (1975). Kunstliche erbrutung von adelkrebsen in zuger-glasern und vergleichende beobachtungen im verhalten und abwachs von edel und signalkrebsen. Freshwater Crayfish 2: 393-403.

Stypnska, M. (1979). Comparison of the species fecundity of crayfishes inhabiting waters of the Mazurian Lakeland. Zoologica Poloniae 27: 279-289.

Styrishave, B., Rasmussen, A.D. & Depledge, M.H. (1995). The influence of bulk and trace metals on the circadian rhythms of heart rates in freshwater crayfish, *Astacus astacus*. Marine Pollution Bulletin 31(1-3): 87-92.

Styrishave, B., & Depledge, M.H. (1995). Evaluation of mercury-induced changes in circadian heart rate rhythms in the freshwater crab, *Potamon potamios* and the crayfish, *Astacus astacus* as an early predictor of mortality (Unpublished).

Suko, T. (1956). Studies on the development of the crayfish. iv. the development of winter eggs. Science reports of the Saitama University Series B, 2(2): 213-219.

Svärdson, G., (1972). The predatory impact of eel (*Anguilla anguilla* L.) on populations of crayfish (*Astacus astacus* L.). Report of the Institute of Freshwater Research, Drottningholm 52: 149-191.

Svärdson, G., Furst, M. & Fjalling, A. (1991). Population resilience of *Pacifastacus leniusculus* in Sweden. Finnish Fisheries Research 12: 165-177.

Svärdson, G. (1992). Ecological co-evolution of the parasitic fungus *Aphanomyces astaci* and its crayfish host. Finnish Fisheries Research 14: 135-143.

Tamkeviciene, E.A. (1988). Growth and development of juveniles of the native and introduced species of freshwater crayfish. Freshwater Crayfish 7: 396-400.

Taugbol, T., Skurdal, J. & Fjeld, E. (1988). Maturity and fecundity of *A stacus astacus* females in Norway. Freshwater Crayfish 7: 107-114.

Taugbol, T. & Skurdal, J. (1995). The significance of a cold water (winter) period for molting, maturation, and fecundity in wild-caught, adult noble crayfish *A stacus astacus* in Norway. Freshwater Crayfish 8: 148-156.

Tautz, J. & Sandeman, D.C. (1980). The detection of waterborne vibration by sensory hairs on the chelae of the crayfish. Journal of Experimental Biology 88: 351-356.

Taylor, E.W. (1970). Spontaneous activity in the cardioaccelerator nerves of the crayfish, *A stacus pallipes lereboullet*. Comparative Biochemistry and Physiology 33: 859-869.

Taylor, E.W., Butler, P.J. & Sherlock, P.J. (1973). The respiratory and cardiovascular changes associated with the emersion response of *Carcinus maenas* (L.) during environmental hypoxia, at three different temperatures. Journal of Comparative Physiology 86: 95-115.

Taylor, A.C. (1977). The respiratory responses of *Carcinus maenas* (L.) to changes in environmental salinity. Journal of Experimental Marine Biology and Ecology 29: 197-210.

Taylor, E.W., Butler, P.J. & Al-Wassia, A. (1977). The effect a decrease in salinity respiration, osmoregulation and activity in the shore crab, *Carcinus maenas* (L.) at different acclimation temperatures. Journal of Comparative Physiology 119: 155-170.

Taylor, E.W. & Wheatly, M.G. (1980). Ventilation, heart rate and respiratory gas exchange in the crayfish *Austropotamobius pallipes* (Lereboullet) submerged in normoxic water and following 3 h exposure in air at 15 °C. Journal of Comparative Physiology B 138: 67-78.

Taylor, E.W. & Wheatly, M.G. (1981). The effect of long-term aerial exposure on heart rate, ventilation, respiratory gas exchange and acid-base status in the crayfish *Austropotamobius pallipes*. Journal of Experimental Biology 92: 109-124.

Tcherkashina, N.Ya. (1977). Survival, growth, and feeding dynamics of juvenile crayfish (*Astacus leptodactylus cubanicus*) in ponds and the River Don. Freshwater Crayfish 3: 95-100.

Thomas, W.J. (1970). The setae of *Austropotamobius pallipes* (Curustacea: Astacidae). Journal of Zoology, London 160: 91-142.

Thomas, W.J. (1971). Electronmicroscope studies of crayfish setae (*Austropotamobius pallipes*). Separatum Experientia (Birkhauser Verlag, Basel): 27: 1454-1455.

Thomas, W.J. (1973). The hatchling setae of *Austropotamobius pallipes* (Lereboullet) (Decapoda, Astacidae). Crustaceana 24: 77-89.

Thomas, W.J. (1978). Aspects of crayfish biology. Freshwater Crayfish 4: 115-122.

Thomas, W.J. (1983). Dimorphism in the British crayfish *Austropotamobius pallipes* (Lereboullet) Freshwater Crayfish 5: 12-17.

Thomas, W.J. (1986). The paragnaths of *Austropotamobius pallipes*. Freshwater Crayfish 6: 42-47.

Thomas, W.J. & Ingle, R.W. (1987). Dimorphism and the life cycle of *Austropotamobius pallipes* (Lereboullet, 1858). Crustaceana 53(2): 170-180.

Thorp, J.H. & Ammerman, K.S. (1978). Chemical communication and agonism in the crayfish, *Procambarus acutus acutus*. The American Midland Naturalist 100(2): 471-474.

Thorp, J.H. & Bergey, E.A. (1981). Field experiments on responses of a freshwater, benthic macroinvertebrate community to vertebrate predators. Ecology 62(2): 365-375.

Tierney, J.A. (1982). Individual variation and taxonomic significance of the seminal receptacle of the crayfish, *Orconectes propinquus* (Girard) (Decapoda Cambaridae). Crustaceana 43(3): 284-295.

Tierney, A.J. & Dunham, D.W. (1982). Chemical communication in the reproductive isolation of the crayfishes *Orconectes propinquus* and *Orconectes virilis* (Decapoda, Cambaridae). Journal of Crustacean Biology 2(4): 544-548.

Tonapi, G.T. & Varghese, G. (1987). Cardio-physiological responses of some selected cladocerans to three common pollutants. Archives of Hydrobiology 110(1): 59-65.

Toole, A.G. & Toole, S.M. (1988). Biology: A-level course companion. 252 pp. Charles Letts & Co Ltd. London.

Tsukerzis, Ya.Ma. (1976). Interspecific competition of closely allied crayfish species (*Astacus astacus* L., *Astacus leptodactylus* Esch., *Pacifastacus leniusculus* Dana, Decapoda Crustacea). Doklady (Proceedings) of the Academy of Sciences of the U.S.S.R. 229(1-6): 283-285.

Tulonen, J., Erkamo, E. & Kirjavainen, J. (1995). Growth rate, survival and reproduction of noble crayfish (*Astacus astacus* (L)), and signal crayfish (*Pacifastacus leniusculus* (Dana)), under similar rearing conditions. Freshwater Crayfish 10: 623-629.

Tyson, G.E. & Sullivan, M.L. (1981). A scanning electron microscopic study of the molar surfaces of the mandibles of the brine shrimp (Cl. Branchiopoda: O. Anostraca). Journal of Morphology 170: 239-251.

Unestam, T. (1975). The dangers of introducing new crayfish species. *Pacifastacus leniusculus* Dana. Freshwater Crayfish 2: 557-561.

van Herp, F. & Bellon-Humbert, C. (1978). Setal development and molt prediction in the larvae and adults of the crayfish, *Astacus leptodactylus* (Nordmann, 1842). Aquaculture 14: 289-301.

Vigneux, E. Keith, P. & Noel, P. (1993). Atlas preliminaire des Crustaces Decapodes d'eau douce de France. Coll. Patrimoines Naturels. Vol: 14, S.F.F., B.I.M.M.-M.N.H.N., C.S.P., Ministere de l'Environnement, Paris, 55 p.

Viikinkonski, T., Henttonen, P., Matinvesi, J.M., Kononen, H. & Suntioinen, S. (1995). The physiological condition and edibility of noble crayfish (*Astacus astacus* (L.)) in warm waste waters of a steel works in northwest Finland. Freshwater Crayfish

10: 304-321.

Wales, W., Clarac, F., Dando, M.R. & Laverack, M.S. (1970). Innervation of the receptors present at the various joints of the pereiopods and third maxilliped of *Homarus gammarus* (L.) and other Macruran decapods (Crustacea). Zeitschrift fur Vergleichende Physiologie 68: 345-384.

Wales, W., Macmillan, D.L. & Laverack, M.S. (1976). Mandibular movements and their control in *Homarus gammarus*. Journal of Comparative Physiology 106: 177-191.

Ware, D.M. (1975). Relation between egg size, growth and natural mortality of larval fish. Journal of Fisheries Research Board of Canada 32: 2503-2512.

Warner, G.F., Wood, J.C. & Orr-Ewing, R.H. (1995). Signal crayfish (*Pacifastacus leniusculus*) feeding on pond snails: optimal foraging. Freshwater Crayfish 8: 352-359.

Warner, G.F. & Green, E.I. (1995). Choice and consumption of aquatic weeds by signal crayfish (*Pacifastacus leniusculus*). Freshwater Crayfish 8: 360-363.

Warner, G.F., Wood, J.C. & Orr-Ewing, R.H. (1995). Signal crayfish (*Pacifastacus leniusculus*) feeding on pond snails: optimal foraging. Freshwater Crayfish 8: 352-359.

Watson III, W.H. & Wyse, G.A. (1978). Coordination of the heart and gill rhythms in *Limulus*. Journal of Comparative Physiology A 124: 267-275.

Wear, R.G. (1974). Incubation in British Decapod Crustacea, and effects of temperature on the rate and success of embryonic development. Journal of the Marine Biological Association, U.K. 54: 745-762.

Wenner, A.M., Dugan, J. & Wells, H. (1991). Estimating egg production in multi-brooding populations. In: Crustacean egg production (Wenner A. & Kuris A., eds), Crustacean Issues 7: 333-355.

Westin, L. & Gydemo, R. (1986). Influence of light and temperature on reproduction and moulting frequency of the crayfish, *Astacus astacus* L. Aquaculture 52: 43-50.

Westman, K. (1973a). The population of the crayfish *Astacus astacus* in Finland and the introduction of the American crayfish *Pacifastacus leniusculus* Dana. Freshwater Crayfish 1: 41-55.

Westman, K. (1973b). Cultivation of the American crayfish *Pacifastacus leniusculus*. Freshwater Crayfish 1: 211-220.

Westman, K. (1975). On crayfish research in Finland. Freshwater Crayfish 2: 65-75.

Westman, K. (1979). Selected Finnish literature on the freshwater crayfish *Astacus astacus* and *Pacifastacus leniusculus*. Freshwater Crayfish 4: 437-443.

Westman, K. & Pursiainen, M. (1979). Development of the European crayfish *Astacus astacus* (L.) and the American crayfish *Pacifastacus leniusculus* (Dana) populations in a small Finnish Lake. Freshwater Crayfish 4: 243-250.

Westman, K., Pursiainen, M. & Vilkman, R. (1979). A new folding trap model which prevents crayfish from escaping. Freshwater Crayfish 4: 235-242.

Westman, K., Savolainen, R. & Pursiainen, M. (1993). A comparative study on the growth and moulting of the noble crayfish, *Astacus astacus* (L), and the signal crayfish, *Pacifastacus leniusculus* (Dana), in a small forest lake in Southern Finland. Freshwater Crayfish 9: 451-465.

Westman, K., Savolainen, R. & Pursiainen, M. (1995). Development of European noble crayfish *Astacus astacus* (L), American signal crayfish *Pacifastacus leniusculus* (Dana) populations in a small Finnish lake - a 20 year study. Freshwater Crayfish 8: 235-248.

Westman, K. & Savolainen, R. (1995). The development of a signal crayfish *Pacifastacus leniusculus* (Dana) population in a small forest lake in central Finland. Freshwater Crayfish 10: 200-208.

Wheatly, M.G. & Taylor, E.W. (1981). The effect of progressive hypoxia on heart rate, ventilation, respiration gas exchange and acid-base status in the crayfish *Austropotamobius pallipes*. Journal of Experimental Biology 92: 125-141.

Wheatly, M.G. & McMahon, B.R. (1982a). Responses to hypersaline exposure in the euryhaline crayfish *Pacifastacus leniusculus* (Dana). I. The interaction between ionic and acid-base regulation. Journal of Experimental Biology 99: 425-445.

Wheatly, M.G. & McMahon, B.R. (1982b). Responses to hypersaline exposure in the euryhaline crayfish *Pacifastacus leniusculus* (Dana). II. Modulation of haemocyanin

oxygen binding in vitro. Journal of Experimental Biology 99: 447-467.

Wheatly, M.G. & McMahon, B.R. (1983). Respiration and ionoregulation in the euryhaline crayfish *Pacifastacus leniusculus* on exposure to high salinity: an overview. Freshwater Crayfish 5: 43-55.

Wheatly, M.G. & Henry, R.P. (1987). Branchial and antennal gland NA^{+}/K^{+} dependent atpase and carbonic anhydrase activity during salinity acclimation of the euryhaline crayfish *Pacifastacus leniusculus*. Journal of Experimental Biology 133 (Nov): 73-86.

Wheatly, M.G. & Gannon, A.T. (1995). Ion regulation in crayfish: freshwater adaptations and the problem of molting. American Zoologist 35: 49-54.

Wheeler, A. (1969). The fishes of the British Isles and North-West Europe. 613 pp. Printed in Great Britain by W&J Mackay & Co Ltd. Chatham.

Whisson, G.J. (1995). Growth and survival as a function of density for marron *Cherax tenuimanus* (Smith)) stocked in a recirculating system. Freshwater Crayfish 10: 630-637.

Whitehouse, R.H & Grove, A.J. (1947). The dissection of the crayfish. 81-83 pp. University Tutorial Press Ltd. London.

Wickins, J.F. (1982). Opportunities for farming crustaceans in western temperate regions. In: Recent advances in aquaculture (Muir, J.F. and Roberts, R.J.) pp. 87-177. Croom Helm, London.

Wiens, A.W. & Armitage, K.B. (1961). The oxygen consumption of the crayfish *Orconectes immunis* and *Orconectes nais* in response to temperature and to oxygen saturation. Physiological Zoölogy 34: 39-54.

Wiersma, C.A.G. & Novitski, E. (1942): The mechanism of the nervous regulation of the crayfish heart. Journal of Experimental Biology 19: 255-265.

Wilkens, J.L. (1995). Regulation of the cardiovascular system in crayfish. American Zoologist 35: 37-48.

Wilkes, P.R.H. & McMahon, B.R. (1982). Effect of maintained hypoxic exposure on the crayfish *Orconectes rusticus.* I. Ventilatory, acid-base and cardiovascular adjustments. Journal of Experimental Biology 98: 119-137.

Wong, T.M. & Freeman, R.F.H. (1976). Osmotic and ionic regulation in different populations of the New Zealand freshwater crayfish *Paranephrops zealandicus.* Journal of Experimental Biology 64: 645-663.

Xinya, S. (1995). Effect of the crayfish *Procambarus clarkii* on the survival of the fry and fingerlings of fishes cultivated in China. Freshwater Crayfish 8: 528-532.

Yeh, H.S. & Rouse, D.B. (1995). Effects of water temperature, density, and sex ratio on the spawning rate of red claw crayfish *Cherax quadricarinatus* (von Martens). Journal of the World Aquaculture Society 26(2): 160-164.

Zender, H. (1934). Uber die embryonalentwicklung des flusskrebses. Acta Zoologica, Stockholm 15(2)-(3): 261-408.

Appendix

A survey of the literature regarding the biology of *Pacifastacus leniusculus*

Astaciculture

Abrahamsson, S. (1973). Methods for restoration of crayfish waters in Europe. The development of an industry for production of young of *Pacifastacus leniusculus* (Dana). Freshwater Crayfish 1:203-210.

Avault, J.W.Jr. (1983). Crayfish species plan for the United States: aquaculture. Freshwater Crayfish 5: 528-533.

Avault, J.W.Jr. (1993). A review of world crustacean aquaculture with special reference to crayfish. Freshwater Crayfish 9: 1-12.

Cabantous, M.A. (1975). Introduction and rearing of *Pacifastacus* at the research center of Les Clouzioux. Freshwater Crayfish 2: 49-55.

Hogger, J.B. (1986a). A report on some of the first introductions of *Pacifastacus leniusculus* into the UK. Freshwater Crayfish 6: 134-145.

Hogger, J.B. (1986b). Aspects of the introduction of signal crayfish *Pacifastacus leniusculus* (Dana) into the Southern United Kingdom. 1. Growth and survival. Aquaculture 58(1-2): 27-44.

Holdich, D.M. (1993). A review of astaciculture: freshwater crayfish farming. Aquatic Living Resources 6: 307-317.

Huner, J.V. (1978). Exploitation of freshwater crayfishes in North America. Fisheries 3(6): 2-5.

Huner, J.V. (1994) (editor). Freshwater Crayfish Aquaculture in North America, Europe, and Australia: families *Astacidae*, *Cambaridae*, and *Parastacidae*. 312 pp. Haworth Press, Inc.

Huner, J.V. (1995). An overview of the status of freshwater crawfish culture. Journal of Shellfish Research 14(2): 539-543.

Järvenpää, T. & Kirjavainen, J. (1992). Crayfish stockings in Finland. Finnish Fisheries Research 14: 69-75.

Laurent, P.J. (1995). Une decennnie de commerce exterieur des ecrevisses en France - (with English summary- Ten years of crayfish foreign trade in France). Bulletin Public Par l'Association des Astaciculteurs de France 43: 2-6.

Mason, J.C. (1978). Effects of temperature, photoperiod, substrate, and shelter on survival, growth, and biomass accumulation of juvenile *Pacifastacus leniusculus* in culture. Freshwater Crayfish 4: 73-82.

Momot, W.T. (1992). Stocking and exploitation as management methods for European crayfish. Finnish Fisheries Research 14: 145-148.

Strempel, K.M. (1975). Kunstliche erbrutung von adelkrebsen in zuger-glasern und vergleichende beobachtungen im verhalten und abwachs von edel und signalkrebsen. Freshwater Crayfish 2: 393-403.

Vigneux, E. (1978). *Pacifastacus leniusculus* et *Astacus leptodactylus* premier bilan d'exploitation en etang. Freshwater Crayfish 4: 227-234.

Westman, K. (1973). Cultivation of the American crayfish *Pacifastacus leniusculus*. Freshwater Crayfish 1: 211-220.

Behaviour

Appelberg, M. & Odelstrom, T. (1988). Interaction between European perch (*Perca fluviatilis*) and juvenile *Pacifastacus leniusculus* (Dana) in a pond experiment. Freshwater Crayfish 7: 37-45.

Blake, M. A & Hart, P.J.B. (1993). The behavioral responses of juvenile signal crayfish *Pacifastacus leniusculus* to stimuli from perch and eels. Freshwater Biology 29(1): 89-97.

Blake, M., Nystrom, P. & Hart, P. (1994). The effect of weed cover on juvenile signal crayfish (*Pacifastacus leniusculus* Dana) exposed to adult crayfish and non-predatory fish. Annales Zoologici Fennici 31: 297-306.

Blake, M. & Hart, P. (1995). The vulnerability of juvenile signal crayfish to perch and eel predation. Freshwater Biology 33: 233-244.

Burba, A. (1993). Investigation of the effects of anthropogenic factors on crayfish behavioural reactions. Freshwater Crayfish 9: 259-265.

Cukerzis, J.M. (1986). Behaviour of crayfish juveniles during early stages of ontogenesis. Freshwater Crayfish 6: 75-86.

Doroshenko, J. (1988). Socioethological aspects of sexual behaviour of the signal crayfish *Pacifastacus leniusculus* Dana introduced in the Lithuanian SSR. Freshwater Crayfish 7: 351-356.

Edsman, L. & Jonsson, A. (1991). The effect of size, injuries and ownership on fighting success in male signal crayfish (*Pacifastacus leniusculus*). National Swedish Board of Fisheries, Institute of Freshwater Research, 19 pp. S-178 91 Drottningholm, Sweden.

Guan, R.Z. (1994). Burrowing behaviour of signal crayfish, *Pacifastacus leniusculus* (Dana), in the river Great Ouse, England. Freshwater Forum 4: 155-168.

Hirvonen, H. (1992). Effects of backswimmer (*Notonecta*) predation on crayfish (*Pacifastacus*) young: autotomy and behavioural responses. Annales Zoologici Fennici 29(4): 261-271.

Klosterman, B.J. & Goldman, C.R. (1983). Substrate selection behaviour of the crayfish *Pacifastacus leniusculus*. Freshwater Crayfish 5: 254-267.

Momot, W.T. & Leering, G.M. (1986). Aggressive interaction between *Pacifastacus leniusculus* and *Orconectes virilis* under laboratory conditions. Freshwater Crayfish 6: 87-93.

Peek, H.V.S., Sippel, J. & Figler, M.H. (1995). Prior residence effects in shelter defense in adult signal crayfish (*Pacifastacus leniusculus* (Dana)): results in same - and mixed-sex dyads. Crustaceana 68(7): 873-881.

Ranta, E. & Lindstrom, K. (1992). Power to hold sheltering burrows by juveniles of the signal crayfish *Pacifastacus leniusculus*. Ethology 92: 217-226.

Ranta, E. & Lindstrom, K. (1993). Body size and shelter possession in mature signal crayfish, *Pacifastacus leniusculus*. Annales Zoologici Fennici 30: 125-132.

Söderbäck, B. (1991). Interspecific dominance relationship and aggressive interactions in the freshwater crayfishes *Astacus astacus* (L) and *Pacifastacus leniusculus* (Dana). Canadian Journal of Zoology 69(5): 1321-1325.

Söderbäck, B. (1992). Predator avoidance and vulnerability of two cooccurring crayfish species, *Astacus astacus* (L) and *Pacifastacus leniusculus* (Dana). Annales Zoologici Fennici 29(4): 253-259.

Söderbäck, B. (1994). Interactions among juveniles of to freshwater crayfish species and a predatory fish. Oecologia 100: 229-235.

Söderbäck, B. (1995). Replacement of the native crayfish *Astacus astacus* by the introduced species *Pacifastacus leniusculus* in a Swedish lake: possible causes and mechanisms. Freshwater Biology 33: 291-304.

Tsukerzis, Ya. Ma. (1976). Interspecific competition of closely allied crayfish species (*Astacus astacus* L., *Astacus leptodactylus* Esch., *Pacifastacus leniusculus* (Dana) (Crustacea: Decapoda). Doklady Biological Sciences (English translation of Doklady Akademiia NAuk SSSR). Seriya Biologia 119 (1-6): 283-285.

Bibliographies

Arrignon, J. (1979). Elements de bibliographie sur le genre *Astacus* en Europe. Freshwater Crayfish 4: 427-436.

Hart, C.W. & Clarck, J. (1987). An Interdisciplinary Bibliography of Freshwater Crayfishes (Astacoidea and Parastacoidea) from Aristotle through 1985. 437 pp. Smithsonian Institution Press, Washington.

Holdich, D.M. (1991). A bibliography of "Freshwater Crayfish - A journal of Astacology I-VII". Proc. Int. Assoc. Astacology Symp. 1973-1988. Journal of Shellfish Research 10: 341-347.

Holdich, D.M. & Pearce-Higgins, J.W. (1995). A bibliography and taxonomic and subject index to "Freshwater Crayfish I-X", the Proceedings of the International Association of Astacology Symposia 1972-1994. Freshwater Crayfish xvi-lxxix.

Westman, K. (1979). Selected Finnish literature on the freshwater crayfish *Astacus astacus* and *Pacifastacus leniusculus*. Freshwater Crayfish 4: 437-443.

Biochemistry

Aspan, A., Huang, T-s., Cerenuis, L. & Söderhäll, K. (1995). cDNA cloning of prohenoloxidase from the crayfish *Pacifastacus leniusculus* and its activation. Proceedings of the National Academy of Sciences 92: 939-943.

Auvergne, A. (1979). L'élevage des Écrevisses. 87 p. Les éditions du point Vétérinaire 12 rue de Marseille 94700 Maisons-Alfort.

Brinck, C., Carlberg, M. & Elofsson, R. (1989). Identification of L 3,4 dihydroxyphenylalanine in the brain of the crayfish *Pacifastacus leniusculus* (Dana) and in tentacles of the sea *Anemone metridium senile* (L) by use of gas chromatography mass spectrometry. Comparative Biochemistry and Physiology C 92(2): 201-203.

Dabramo, L.R. Wright, J.S., Wright, K.H., Bordner, C.E. & Conklin, D.E. (1985). Sterol requirement of cultured juvenile crayfish *Pacifastacus leniusculus*. Aquaculture 49(3-4): 245-255.

Domnas, A.J. & Warner, S.A. (1991). Biochemical activities of entomophagous fungi. Critical Reviews in Microbiology 18(1): 1-13.

Edsman, L., Jarvi, T. & Niejahr, B. (1994). The RNA concentration as an index of current growth rate in juvenile signal crayfish, *Pacifastacus leniusculus*. Nordic Journal of Freshwater Research 69: 149-152.

Elmanira, A. & Clarac, F. (1991). Gaba mediated presynaptic inhibition in crayfish primary afferents by non a, non b gaba receptors. European Journal of Neuroscience 3(12): 1208-1218.

Elofsson, R., Laxmyr, L., Rosengren, E. & Hansson, C. (1982). Identification and quantitative measurements of biogenic amines and dopa in the central nervous system and hemolymph of the crayfish *Pacifastacus leniusculus* (Crustacea). Comparative Biochemistry and Physiology C 71(2): 195-201.

Garciacarreno, F.L. & Hard, N.F. (1993). Characterization of protein classes in langostilla (*Pleuncodes planipes*) and crayfish (*Pacifastacus leniusculus*) extracts. Journal of Food Biochemistry 17 (2): 97-114.

Hall, M., Söderhäll, K. & Sottrupjensen, L. (1989). Amino acid sequence around the thiolester of alpha 2 macroglobulin from plasma of of the crayfish *Pacifastacus leniusculus*. Febs Letters 254(1-2): 111-114.

Hergenhahn, H.G. & Söderhäll, K. (1985). Alpha 2 macroglobulin like activity in plasma of the crayfish *Pacifastacus leniusculus*. Comparative Biochemistry and Physiology B 81(4): 833-835.

Huner, J.V. & Lindqvist, O.V. (1985). Exoskeleton mineralization in astacid and cambarid crayfishes (Decapoda, Crustacea). Comparative Biochemistry and Physiology A 80(4): 515-521.

Kopacek, P., Hall, M. & Soderhal, K. (1993). Characterization of a clotting protein, isolated from plasma of the freshwater crayfish *Pacifastacus leniusculus*. European Journal of Biochemistry 213: 591-597.

Laxmyr, L. (1985). Tyrosine hydroxylase activity in the central nervous system of the crayfish, *Pacifastacus leniusculus*, (Crustacea, Decapoda). Journal of Comparative Physiology B 155(5): 603-609.

Mackeviciene, G. & Chibisova, N. (1989). Changes of the level of glucocorticoids in the crayfish *Pacifastacus leniusculus* Dana males at separate stages of the intermolt cycle (in Russian). Collection of Scientific Reports of Gosniorch 300: 91-99.

Mackeviciene, G. & Chibisova, N. (1995). Concentrations of steroid hormones at different stages of the intermoult cycle in the crayfish *Astacus astacus* and *Pacifastacus leniusculus*. Freshwater Crayfish 10: 267-273.

Mulloney, B. & Hall, W.M. (1991). Neurons with histamine like immunoreactivity in the segmental and stomatogastric nervous system of the crayfish *Pacifastacus leniusculus* and the lobster *Homarus americanus*. Cell and Tissue Research 266(1): 197-207.

Osterberg, R., Malmensten, B. & Ikai, A. (1991). X ray scattering study of hagfish protease inhibitor, a protein structurally related to complement and alpha 2 macroglobulin. Biochemistry 30(31): 7873-7878.

Wang, M.Y. & Brown, W.D. (1983). Effects of elevated CO_2 atmosphere on storage of freshwater crayfish (*Pacifastacus leniusculus*). Journal of Food Science 48: 158-162.

Biology

Dudenhausen, E.E. & Talbot, P. (1983). An ultrastructural comparison of soft and hardened spermatophores from the crayfish *Pacifastacus leniusculus* Dana. Canadian Journal of Zoology 61: 182-194.

Holdich, D.M. & Lowery, R.S. (1988). Crayfish: an introduction. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 1-7. Chapman & Hall, London.

Holdich, D.M. & Reeve, I.D. (1988). Functional morphology and anatomy. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 11-51. Chapman & Hall, London.

Holdich, D.M. (1992). Crayfish nomenclature and terminology: recommendations for uniformity. Finnish Fisheries Research 14: 149-155.

Huner, J.V. (1990). Biology, fisheries, and cultivation of freshwater crawfishes in the U.S. Reviews in Aquatic Sciences 2(2): 229-254.

Conservation

Holdich, D.M. (1991). The native crayfish and threats to its existence. British Wildlife 2(3): 141-151.

Holdich, D.M., Reader, J.P., Rogers, W.D. (1995). Crayfish conservation. Interm Report for the National Rivers Authority for the Period (January-December) 1993. R&D 378/8/N. 278

pp.

Rogers, W.D. & Holdich, D.M. (1995). Conservation and management of crayfish in Britain. Freshwater Crayfish 10: 92-97.

Disease

Alderman, D.J. & Polglase, J.L. (1988). Pathogens, parasites and commensals. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 167-212. Chapman & Hall, London.

Alderman, D.J., Holdich, D.M. & Reeve, I. (1990). Signal crayfish as vectors in crayfish plague in Britain. Aquaculture 86: 3-6.

Alderman, D.J. (1993). Crayfish plague in Britain, the first twelve years. Freshwater Crayfish 9: 266-272.

Cerenius, L., Söderhäll, K., Persson, M. & Ajaxon, R. (1988). The crayfish plague fungus *Aphanomyces astaci* diagnosis, isolation, and pathobiology. Freshwater Crayfish 7: 131-144.

Cerenius, L. & Söderhäll, K., (1992). Crayfish diseases and crayfish as vectors for important diseases. Finnish Fisheries Research 14: 125-133.

Chinain, M. & Vey, A. (1988a). Comparative studies on strains of *Fusarium solani* parasitic for crayfish or isolated from other hosts. Freshwater Crayfish 7: 179-186.

Chinain, M. & Vey, A. (1988b). Infection caused by *Fusarium solani* in crayfish *Astacus leptodactylus*. Freshwater Crayfish 7: 195-202.

Chinain, M. & Vey, A. (1988c). Experimental study of *Fusarium solani* infections in *Astacus leptodactylus* and *Pacifastacus leniusculus* (Crustacea, Decapoda). Diseases of Aquatic Organisms 5(3): 215-223.

Cuellar, L. & Coll, M. (1983). Epizootiology of the crayfish plague (*Aphanomycosis*) in Spain. Freshwater Crayfish 5: 545-548.

Gydemo, R. (1992). Crayfish diseases and their management - the need for knowledge. Finnish Fisheries Research 14: 119-124.

Jarvenpaa, T., Nylund, V., Railo, E. & Westman, K. (1986). The effects of the crayfish plague fungus *Aphanomyces astaci* on the haemolymph of *Astacus astacus* and *Pacifastacus leniusculus*. Freshwater Crayfish 6: 223-233.

Marren, P. (1986). The lethal harvest of crayfish plague. New Scientist (Jan 1986): 46-50.

McGriff, D. & Modin, J. (1983). *Thelohania contejeani* parasitism of the crayfish, *Pacifastacus leniusculus*, in California. California Fish and Game 69(3): 178-183.

Muller, G. (1978). Studie zur neueinburgerung des gegen die krebspest resistenten signalkrebses (*Pacifastacus leniusculus* Dana). Freshwater Crayfish 4: 381-383.

Nylund, V. & Westman, K. (1983). Frequency of visible symptoms of the crayfish plague fungus (*Aphanomyces astaci*) on the American crayfish (*Pacifastacus leniusculus*) in natural populations in Finland. Freshwater Crayfish 5: 277- 283.

Nylund, V. & Westman, K. (1992). Crayfish diseases and their control in Finland. Finnish Fisheries Research 14: 107-118.

Nylund, V. & Westman, K. (1995). Frequency of visible symptoms of the crayfish plague fungus (*Aphanomyces astaci*) on the signal crayfish (*Pacifastacus leniusculus*) in natural populations in Finland in 1979-1988. Freshwater Crayfish 8: 577- 588.

Persson, M. & Söderhäll, K. (1983). *Pacifastacus leniusculus* Dana and its resistance to the parasitic fungus *Aphanomyces astaci* Schikora. Freshwater Crayfish 5: 292-298.

Smith, V.J. & Söderhäll, K. (1986). Crayfish pathology: an overview. Freshwater Crayfish 6: 199-211.

Söderhäll, K., Svensson E. & Unestam, T. (1977). An inexpensive and effective method for elimination of the crayfish plague: barriers and biological control. Freshwater Crayfish 3: 333-342.

Svärdson, G. (1992). Ecological co-evolution of the parasitic fungus *Aphanomyces astaci* and its crayfish host. Finnish Fisheries Research 14: 135-143.

Unestam, T. (1973). Significance of diseases on freshwater crayfish. Freshwater Crayfish 1:135-150.

Unestam, T. & Söderhäll, K. (1977). Specialization in crayfish defence and fungal aggressiveness upon crayfish plague infection. Freshwater Crayfish 3: 321-331.

Vey, A. (1977). Studies on the pathology of crayfish under rearing conditions. Freshwater Crayfish 3: 311-319.

Vey, A. (1978). recherches sur une maladie des ecrevisses due au parasite *Psorospermium haeckeli* Hilgendrof. Freshwater Crayfish 4: 411-418.

Vey, A. (1983). Susceptibility of *Orconectes limosus* Raff. to the crayfish plague, *Aphanomyces astaci* Schikora. Freshwater Crayfish 5: 284-291.

Vey, A. (1986). Disease problems during aquaculture of freshwater Crustacea. Freshwater Crayfish 6: 212-222.

Ecology, distribution and population biology (including trapping)

Abrahamsson, S.A.A. & Goldman, C.R. (1970). Distribution, density and production of the crayfish *Pacifastacus leniusculus* Dana in Lake Tahoe, California-Nevada. Oikos: 21: 83-91.

Abrahamsson, A. (1983). Trappability, locomotion, and diel pattern of activity of the crayfish *Astacus astacus* and *Pacifastacus leniusculus* Dana. Freshwater Crayfish 5: 239-253.

Arrignon, J.C.V. (1993). The development of a *Pacifastacus leniusculus* population in a gravel pit in France. Freshwater Crayfish 9: 87-96.

Blake, M.A. & Hart, P.J.B. (1993). Habitat preferences and survival of juvenile signal crayfish, *Pacifastacus leniusculus* - the influence of water depth, substratum, predatory fish and gravid female crayfish. Freshwater Crayfish 9: 318-332.

Blake, M., Nystrom, P. & Hart, P. (1994). The effect of weed cover on juvenile signal crayfish *Pacifastacus leniusculus* (Dana) exposed to adult crayfish and non-predatory fish. Annales Zoologici Fennici 31: 297-306.

Burba, A. (1993). Nouvelles de l'ecrevisse en Lithuanie (with English summary - Facts about the crayfish in Lithuanie). Bulletin Publie Par l'Association des Astaciculteurs de France 36: 2-6.

Buttiker, B. (1988). Concerning crayfish in Switzerland. Freshwater Crayfish 7: 2-5.

Daniels, R.A. (1980). Distribution and status of crayfish in the Pit River drainage, California, Crustaceana 38: 131-138.

Ducruet, S., Rojat, G., Nicolas, L. & Laurent, P.J. (1993). A new device for electroction of crayfish. Freshwater Crayfish 9: 154-157.

Elser, J.J., Junge, C. & Goldman C.R. (1994). Population structure and ecological effects of the crayfish *Pacifastacus leniusculus* in Castle Lake, California. Great Basin Naturalist 54(2): 162-169.

Erkamo, E., Kirjavainen, J. & Tulonen, J. (1992). Catching as a management method for crayfish. Finnish Fisheries Research 14: 77-83.

Fjalling, A. (1995). Crayfish traps in Swedish fisheries. Freshwater Crayfish 8: 201-214.

Flint, R.W. (1975). Growth in a population of the crayfish *Pacifastacus leniusculus* from a subalpine lacustrine environment. Journal of the Fisheries Research Board of Canada 32: 2433-2440.

Flint, R.W. & Goldman, C.R. (1977). Crayfish growth in Lake Tahoe: effects of habitat variation. Journal of the Fisheries Research Board of Canada 34: 155-159.

Flint, R.W. (1977). Seasonal activity, migration and distribution of the crayfish *Pacifastacus leniusculus*, in Lake Tahoe. American Midland Naturalist 97: 280-292.

Furst, M. (1995). On the recovery of *Astacus astacus* L. populations after an epizootic of the crayfish plague (*Aphanomyces astaci* Shikora). Freshwater Crayfish 8: 565-576.

Goddard, J.S. & Hogger, J.B. (1986). The current status and distribution of freshwater crayfish in Britain. Field Studies 6: 383-396.

Goldman, C.R. Rundquist, J.C. & Flint, R.W. (1975). Ecological studies of the California crayfish, *Pacifastacus leniusculus*, with emphasis on their growth from recycling waste products. Freshwater Crayfish 2: 481-487.

Goldman, C.R. & Rundquist, J.C. (1977). A comparative ecological study of the California crayfish, *Pacifastacus leniusculus* (Dana), from two subalpine lakes (Lake Tahoe and Lake Donner). Freshwater Crayfish 3: 51-80.

Hobbs, H.H.Jr. (1988). Crayfish distribution, adaptive radiation and evolution. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 52-82. Chapman & Hall, London.

Hogger, J.B. (1988). Ecology, population biology and behaviour. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 114-144. Chapman & Hall, London.

Holdich, D.M., Reader, J.P., Rogers, W.D. & Harlıoğlu, M.M. (1995). Interactions between three species of crayfish (*Austropotamobius pallipes*, *Astacus leptodactylus* and *Pacifastacus leniusculus*. Freshwater Crayfish 10: 46-56.

Holdich, D.M. & Domaniewski, J.C.J. (1995). Studies on a mixed population of *Austropotamobius pallipes* and *Pacifastacus leniusculus* in England. Freshwater Crayfish 10: 37-45.

Laurent, P.J. & Paris, L. (1994). Les mesaventures d'une population de pieds rouges en Morvan. (with English summary - Unfortunate incidents for a noble crayfish population in Morvan). Bulletin Publie Par l'Association des Astaciculteurs de France 41: 6-8.

Lowery, R.S. & Holdich, D.M. (1988). *Pacifastacus leniusculus* in North America and Europe, with details of the distribution of introduced and native crayfish species in Europe. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 283-308. Chapman & Hall, London.

Mahieu, J. & Paris, L. (1995). Ecrevisses et pesticides ne font pas bon menage. (with English summary). Bulletin Publie Par l'Association des Astaciculteurs de France 42: 1-10.

Mason, J.C. (1975). Crayfish production in a small woodland stream. Freshwater Crayfish 2: 449-479.

Momot, W.T. (1984). Crayfish production: A reflection of community energetics. Journal of Crustacean Biology 4(1): 35-54.

Odelstrom, T. (1983). A portable hydraulic diver-operated dredge-sieve for sampling juvenile crayfish. Description and experiences. Freshwater Crayfish 5: 270-274.

Söderbäck, B. (1993). Population regulation in two co-occurring crayfish species. Acta Universitatis Upsaliensis. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science 434. 29 pp.

Shimizu, S.J. & Goldman, C.R. (1983). *Pacifastacus leniusculus* (Dana) production in the Sacramento River. Freshwater Crayfish 5: 210-228.

Slater, F.M. (1995). A simple crayfish trap. Freshwater Crayfish 10: 194-195.

Svardsson, G., Furst, M. & Fjalling, A. (1991). Population resilience of *Pacifastacus leniusculus* in Sweden. Finnish Fisheries Research 12: 165-167.

Troschel, H.J. & Dehus, P. (1993). Distribution of crayfish species in the Federal Republic of Germany, with special reference to *Austropotamobius pallipes*. Freshwater Crayfish 9: 390-398.

Westman, K., Pursiainen, M. & Vilkman, R. (1978). A new folding trap model which prevents crayfish from escaping. Freshwater Crayfish 4: 235-242.

Westman, K. & Pursiainen, M. (1979). Development of the European crayfish *Astacus astacus* (L.) and the American crayfish *Pacifastacus leniusculus* (Dana) populations in a small Finnish Lake. Freshwater Crayfish 4: 243-250.

Wiles, P.R. & Guan, R.Z. (1993). Studies on a new method for permanently tagging crayfish with microchip implants. Freshwater Crayfish 9: 419-425.

Farming and wild harvest

Arrignon, J. (1975). Crayfish farming in France. Freshwater Crayfish 2: 105-116.

Avault, J.W.Jr. (1973). Crayfish farming in the United States. Freshwater Crayfish 1: 239-250.

Behrendt, A. (1979). Export push needed to open market for crayfish. Fish Farmer 2(2): 44-46.

Fjalling, A. Furst, M. (1988). The development of a fishery for the crayfish *Pacifastacus leniusculus* in Sweden 1960-86. Freshwater Crayfish 7: 223-230.

Foex, Y. (1992). Quid de l'astaciculture en France? Point de vue d'un astaciculteur. (with English summary - Facts about crayfish farming in France? Viewpoint of a crayfish farmer). Bulletin Publie Par l'Association des Astaciculteurs de France 33: 8-11.

Holdich, D.M., Jay, D. & Goddard, J.S. (1978). Crayfish in the British Isles. Aquaculture 15: 91-97.

Holdich, D.M. (1990a). Crayfish farming and the house of commons report. Fish Farmer November-December 1990: 41-42.

Holdich, D.M. (1990b). Freshwater crayfish farming: production prospects and environmental implications. Scottish Shellfish Grower 6: 10-11 and 19.

Holdich, D.M. & Rogers, W.D. (1992). Crayfish populations in the British Isles: Farming, legislation, conservation and management. Finnish Fisheries Research 14: 23-32.

Huner, J.V. (1978). Exploitation of freshwater crayfishes in North America. Fisheries 3(6): 2-5.

Kossakowski, J. & Kossakowski, G. (1983). An attempt to raise juvenile crayfish *Pacifastacus leniusculus* Dana. Freshwater Crayfish 5: 555-556.

McGriff, D. (1983). The commercial fishery for *Pacifastacus leniusculus* Dana in the Sacramento-San Joaquin Delta. Freshwater Crayfish 5: 403-417.

Richards, K.J. (1983). The introduction of the signal crayfish into the United Kingdom and its development as a farm crop. Freshwater Crayfish 5: 557-563.

Rogers, W.D. & Holdich, D.M. (1995). Crayfish production in Britain. Freshwater Crayfish 10: 583-595.

Thompson, A.G. (1990). Crayfish: a candidate for culture in British Columbia? World Aquaculture 21(2): 92-94.

Westman, K. (1975). On crayfish research in Finland. Freshwater Crayfish 2: 65-75.

Food, diet and digestion

Goddard, J.S. (1988). Food and feeding. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 145-166. Chapman & Hall, London.

Huner, J.V., Meyers, S.P. & Avault, J.W.Jr. (1975). Response and growth of freshwater crawfish to an extruded, water-stable diet. Freshwater Crayfish 2: 149-157.

Mickeniene, L.M. (1988). Anaerobic microorganisms in the digestive tract of the crayfish *Pacifastacus leniusculus* Dana. Freshwater Crayfish 7: 385-389.

Rundquist, J., Gall, G. & Goldman, C.R. (1977). Watercress-crayfish polyculture as an economic means of stripping nutrients from enriched waters. Freshwater Crayfish 3: 141-159.

Rundquist, J.C. & Goldman, C.R. (1983). Methodological considerations for quantitative determination of consumption for the crayfish *Pacifastacus leniusculus*. Freshwater Crayfish 5: 27-42.

Warner, G.F., Wood, J.C. & Orr-Ewing, R.H. (1995). Signal crayfish (*Pacifastacus leniusculus*) feeding on pond snails: optimal foraging. Freshwater Crayfish 8: 352-359.

Warner, G.F., & Green, E.I. (1995). Choice and consumption of aquatic weeds by signal crayfish (*Pacifastacus leniusculus*). Freshwater Crayfish 8: 360-363.

Genetic

Agerberg, A. (1990). Genetic variation in three species of freshwater crayfish: *Astacus astacus* L., *Astacus leptodactylus* Esch., and *Pacifastacus leniusculus* (Dana), revealed by isozyme electrophoresis. Hereditas 113: 101-108.

Agerberg, A. (1993). Genetic and phenotypic variation in the freshwater crayfish *Pacifastacus leniusculus* (Dana). Acta Universitatis Upsaliensis. Comprehensive summaries of Uppsala dissertations from the Faculty of Science 446. 28 pages.

Histology

Dudenhausen, E.E. & Talbot, P. (1982). An ultrastructural analysis of mature sperm from the crayfish *Pacifastacus leniusculus* Dana. International Journal of Invertebrate Reproduction 5(3):

149-159.

Dudenhausen, E.E. & Talbot, P. (1983). An ultrastructural comparison of soft and hardened spermathophores from the crayfish *Pacifastacus leniusculus* Dana. Canadian Journal of Zoology 61(1): 182-194.

Scheidegger, G. & Scheideggerbloch, D. (1982). Complexly structured cell inclusions in the epidermis of *Pacifastacus leniusculus* (Crustacea, Decapoda). Zoologischer Anzeiger 208(3-4): 237-243.

Immunology

Armstrong, P.B. & Quigley, J.P. (1992). Humoral immunity alpha 2 macroglobulin activity in the plasma of mollusks. Veliger, 35(3): 161-164.

Aspan, A. & Söderhäll, K. (1991). Purification of prophenoloxidase from crayfish blood cells, and its activation by an endogenous serine proteinase. Insect Biochemistry 21(4): 363-373.

Barracco, M.A., Duvic, B. & Söderhäll, K. (1991). The beta 1,3 glucan binding protein from the crayfish *Pacifastacus leniusculus*, when reacted with a beta 1,3 glucan, induces spreading and degranulation of crayfish granular cells. Cell and Tissue Research 266(3): 491-497.

Duvic, B. & Söderhäll, K. (1990). Purification and characterization of a beta 1,3 glucan binding protein from plasma of the crayfish *Pacifastacus leniusculus*. Journal of Biological Chemistry 265(16): 9327-9332.

Duvic, B. & Söderhäll, K. (1992). Purification and partial characterization of a beta 1,3 glucan binding protein membrane receptor from blood cells of the crayfish *Pacifastacus leniusculus*. European Journal of Biochemistry 207(1): 223-228.

Elofsson, R. (1983). Fifth like immunoreactivity in the central nervous system of the crayfish, *Pacifastacus leniusculus*. Cell and Tissue Research 232(1): 221-236.

Fenouil, E., Roch, P. & de Hureaux, D. (1995). Immune defense in *Astacidae* and *Cambaridae*: presence of lysozyme activity. Freshwater Crayfish 8: 614-622.

Hall, M. & Söderhäll, K. (1994). Crayfish alpha macroglobulin as a substrate for transglutaminases. Comparative Biochemistry and Physiology B 108(1): 65-72.

Hergenhann, H.G., Hall, M. & Söderhäll, K. (1988). Purification and characterization of an alpha 2 macroglobuline like proteinase inhibitor from plasma of the crayfish *Pacifastacus leniusculus*. Biochemical Journal 255(3): 801-806.

Johansson, K.U.I. (1991). Identification of different types of serotonin like immunoreactive olfactory interneurons in 4 infraorders of Decapod Crustaceans. Cell and Tissue Research 264(2): 357-362.

Johansson, M.W. & Söderhäll, K. (1993). Intracellular signaling in Arthropod blood cells involvement of protein kinase C and protein tyrosine phosphorylation in the response to the 76 KDA protein or the beta L 3 glucan binding protein in crayfish. Developmental and

Comparative Immunology 17(6): 495-500.

Kopacek, P., Grubhoffer, L. & Söderhäll, K. (1993). Isolation and characterization of a hemagglutinin with affinity for lipopolysaccharides from plasma of the crayfish *Pacifastacus leniusculus*. Developmental and Comparative Immunology 17(5): 407-418.

Liang, Z.C., Lindblad, P., Beauvais, A., Johansson, M.W., Latge, J.P., Hall, M., Cerenius, L. & Söderhäll, K. (1992). Crayfish alpha macroglobulin and 76 KDA protein, their biosynthesis and subcellular localization of the 76 KDA protein. Journal of Insect Physiology 38(12): 987-995.

Persson, M., Cerenius, L. & Söderhäll, K. (1987). The influence of hemocyte number on the resistance of the freshwater crayfish *Pacifastacus leniusculus* Dana to the parasitic fungus *Aphanomyces astaci*. Journal of Fish Diseases 10(6): 471-477.

Quigley, J.P. & Armstrong, P.B. (1994). Invertebrate alpha (2) macroglobulin structure function and the ancient thiol ester bond. Annals of the New York Academy of Sciences 712: 131-145.

Rantamaki, J., Durrant, H., Liang, Z. Ratcliffe, N.A., Duvic, B. & Söderhäll, K. (1991). Isolation of a 90 KDA protein from hemocytes of *Blaberus craniifer* which has similar functional and immunological properties to the 76 KDA protein from crayfish hemocytes. Journal of Insect Physiology 37(8): p.627.

Söderhäll, K., Cerenius, L. & Johansson, M.W. (1994). The prophenoloxidase activating system and its role in invertabrate defense. Annals of the New York Academy of Sciences 712: 155-161.

Thornqvist, P.O. , Johansson, M.W. & Söderhall, K. (1994). Opsonic activity of cell adhesion proteins and beta 1,3 glucan binding proteins from 2 Crustaceans. Developmental and Comparative Immunology 18(1): 3-12.

Watson, A.H.D. & Pfluger, H.J. (1994). Distribution of input synapses from processes exhibiting gaba like or glutamate like immunoreactivity onto terminals of prosternal filiform afferents in the locust. Journal of Comparative Neurology 343(4): 617-629.

Introduction of *Pacifastacus leniusculus*

Abrahamsson, S. (1973). The crayfish *Astacus astacus* in Sweden and the introduction of the American crayfish *Pacifastacus leniusculus*. Freshwater Crayfish 1: 27-40.

Brinck, P. (1975). Crayfish in Sweden. Freshwater Crayfish 2: 77-85.

Brinck, P. (1977). Developing crayfish populations. Freshwater Crayfish 3: 211-228.

Brinck, P. (1983). An ecologist's approach to dealing with the loss of *Astacus astacus*. Freshwater Crayfish 5: xxi-xxxvii.

Carral, J.M., Celada, J.D., Gonzalez, J., Saez-Royuela, M., Gaudioso, V.R., Fernandez, R. & Lopez.-Baisson, C. (1993). Wild freshwater crayfish populations in Spain: current status and perspectives. Freshwater Crayfish 9: 158-162.

Cukerzis, J.M. (1978). On acclimatization of *Pacifastacus leniusculus* in an isolated lake. Freshwater Crayfish 4: 445-450.

Furst, M. (1977). Introduction of *Pacifastacus leniusculus* (Dana) into Sweden: methods, results, and management. Freshwater Crayfish 3: 229-247.

Goldman, C.R. (1973). Ecology and physiology of the California crayfish *Pacifastacus leniusculus* (Dana) in relation to its suitability for introduction into European waters. Freshwater Crayfish 1: 105-120.

Hogger, J.B. (1986). Aspects of the introducing of "signal crayfish", *Pacifastacus leniusculus* (Dana), into the Southern United Kingdom. 1. Growth and survival. Aquaculture 58: 27-44.

Holdich, D.M. (1988). The dangers of introducing alien animals with particular reference to crayfish. Freshwater Crayfish 7: xv-xxx.

Holdich, D.M. & Reeve, I. (1991a). Crayfish in British Waters. Natural Environment Research Council News April 1991: 4-7.

Holdich, D.M. & Reeve, I.D. (1991b). Distribution of freshwater crayfish in the British Isles, with particular reference to crayfish plague, alien introductions and pollution. Aquatic Conservation: Marine and Freshwater Ecosystems 1: 139-158.

Holdich, D.M. & Reeve, I.D. (1991c). Alien crayfish in British waters. Final report on GR3/6842 for Natural Environment Research Council. 38 pp.

Holdich, D.M. (1994). The native crayfish and threats to its existence. British Wildlife 2(3): 141-151.

Holdich, D.M. (1994). *Pacifastacus leniusculus* en Grande Bretagne 18 ans apres son introduction. Bulletin Publie Par l'Association des Astaciculteurs de France 40: 2-5.

Holdich, D.M., Reeve, I.D. & Rogers, W.D. (1995). Introduction and spread of alien crayfish in British Waters - implications for native crayfish populations. Freshwater Crayfish 8: 99-112.

Huner, J.V. (1988). Status of crayfish transplantations. Freshwater Crayfish 7: 29-34.

Kossakowski, J., Mnich, M. & Kossakowski, G. (1978). The first introduction of the crayfish, *Pacifastacus leniusculus* into Polish waters. Freshwater Crayfish 4: 195.

Kossakowski, J. (1988). Some remarks on the biology of the crayfish *Pacifastacus leniusculus*. Freshwater Crayfish 7: 369-376.

Laurent, P.J. & Vey, A. (1986). The acclimation of *Pacifastacus leniusculus* in Lake Divonne. Freshwater Crayfish 6: 146-155.

Lorena, H.A.S. (1978). Present situation of exotic species of crayfish introduced into Spanish continental waters. Freshwater Crayfish 4: 175-184.

Spitzy, R. (1973). Crayfish in Austria, history and actual situation. Freshwater Crayfish 1: 9-14.

Svardson, G. (1995). The early history of signal crayfish introduction into Europe. Freshwater Crayfish 8: 68-77.

Westman, K. (1973). The population of the crayfish *Astacus astacus* in Finland and the introduction of the American crayfish *Pacifastacus leniusculus* Dana. Freshwater Crayfish 1: 41-55.

Westman, K. (1995). Introduction of alien crayfish in the development of crayfish fisheries; experience with signal crayfish *Pacifastacus leniusculus* (Dana) in Finland and the impact on the native noble crayfish (*Astacus astacus* (L)). Freshwater Crayfish 10: 1-17.

Life histories, growth, development and reproduction

Abrahamsson, S.A.A. (1971). Density, growth and reproduction in populations of *Astacus astacus* and *Pacifastacus leniusculus* in an isolated pond. Oikos 22: 373-380.

Agerberg, A. (1988). Multivariate analysis of morphometric variation in three species of freshwater crayfish. Freshwater Crayfish 7: 19-28.

Appelberg, M. (1992). Liming as a measure to restore crayfish populations in acidified lakes. Finnish Fisheries Research 14: 93-105.

Andrews, E.A. (1907). The young of the crayfishes *Astacus* (*Pacifastacus leniusculus*) and *Cambarus* (*Orconectes limosus*). 88 pp. Smithsonian Contributions to Knowledge Part of Volume xxxv.

Carral, J.M., Celada, J.D., Gaudioso, V.R., Temino, C. & Fernandez, R. (1988). Artificial incubation improvement of crayfish eggs (*Pacifastacus leniusculus* DANA) under low temperatures during embryonic development. Freshwater Crayfish 7: 239-250.

Carral, J.M., Celada, J.D., Gonzalez, J., Gaudioso, V.R., Fernandez, R. & Lopezbaisson, C. (1992). Artificial incubation of crayfish eggs (*Pacifastacus leniusculus* Dana) from early stages of embryonic development. Aquaculture 104(3-4): 261-269.

Celada, J.D., Depaz, P., Gaudioso, V.R. & Fernandez, R. (1987). Embryonic development of the freshwater crayfish *Pacifastacus leniusculus*. A scanning electron microscopic study. Anatomical Record 219(3): 304-310.

Celada, J.D., Carral, J.M., Gaudioso, V.R., Temino, C. & Fernandez, R. (1988). Effects of thermic manipulation throughout egg development on the reproductive efficiency of the freshwater crayfish (*Pacifastacus leniusculus* Dana). Aquaculture 72(3-4): 341-348.

Celada, J.D., Carral, J.M., Gaudioso, V.R., Temino, C. & Fernandez, R. (1989). Response of juvenile crayfish (*Pacifastacus leniusculus* Dana) to several and artificially compounded diets. Aquaculture 76(1-2): 67-78.

Flint, R.W. (1975). Growth in a population of the crayfish *Pacifastacus leniusculus* from a subalpine lacustrine environment. Journal of the Fisheries Research Board of Canada 32: 2433-2440.

Flint, R.W. (1977). Crayfish growth in Lake Tahoe: effects of habitat variation. Journal of the Fisheries Research Board of Canada 34: 155-159.

Gonzalez, J., Carral, J.M., Celada, J.D., Saez-Royuela, M., Gaudioso, V.R., Fernandez, R. & Lopez.-Baisson, C. (1993). Management of crayfish eggs (*Pacifastacus leniusculus*) for intensification of juvenile production. Freshwater Crayfish 9: 144-146.

Grunberg, W. & Havelec, L. (1981). Morphometric studies on heterochely in the Californian crayfish *Pacifastacus leniusculus* Dana 1852 (Decapoda, Astacidae). Wiener Tierarztliche Monatsschrift 68(10): 364-376.

Jonsson, A. (1995). Life history differences between crayfish *Astacus astacus* and *Pacifastacus leniusculus* in embryonic and juvenile development, laboratory experiences. Freshwater Crayfish 8: 170-178.

Kirjavainen, J. & Westman, K. (1994). Comparative growth from length composition and mark-recapture experiments for noble crayfish (*Astacus astacus*) and signal crayfish *Pacifastacus leniusculus* in Finland. Nordic Journal of Freshwater Research 69: 153-161.

Kirjavainen, J. & Westman, K. (1995). Development of an introduced signal crayfish *Pacifastacus leniusculus* (Dana) population in the small lake Karisjarvi in central Finland. Freshwater Crayfish 10: 140-150.

Kossman, H. (1973). Haltungs und vermehrungsversuche von susswasserkrebsen im haus. Freshwater Crayfish 1: 221-232.

Lowery, R.S. (1988). Growth, moulting and reproduction. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 83-113. Chapman & Hall, London.

Mason, J.C. (1975). Crayfish production in a small woodland stream. Freshwater Crayfish 2: 449-479.

Mason, J.C. (1977a). Reproductive efficiency of *Pacifastacus leniusculus* (Dana) in culture. Freshwater Crayfish 3: 101-117.

Mason, J.C. (1977b). Artificial incubation of crayfish eggs (*Pacifastacus leniusculus* (Dana). Freshwater Crayfish 3: 119-132.

Mason, J.C. (1978). Significance of egg size in the freshwater crayfish, *Pacifastacus leniusculus* (Dana). Freshwater Crayfish 4: 83-92.

McGriff, D. (1983). Growth, maturity, and fecundity of the crayfish, *Pacifastacus leniusculus*, from the Sacramento San Joaquin Delta. California Fish and Game 69(4): 227-242.

Nystrom, P. (1994). Survival of juvenile signal crayfish (*Pacifastacus leniusculus*) in relation to light intensity and density. Nordic J. Freshwater Research 69: 162-166.

Payne, J.F. (1978). Aspects of the life histories of selected of North American crayfishes. Fisheries 3(6): 5-8.

Reynolds, J.D., Celada, J.D., Carral, J.M. & Matthews, M.A. (1992). Reproduction of astacid crayfish in captivity - current developments and implications for culture, with special reference to Ireland and Spain. Invertebrate Reproduction and Development 22(1-3): 253-266.

Rogers, W.D. & Holdich, D.M. (1995). Crayfish production in Britain. Freshwater Crayfish 10: 583-595.

Saezroyuela, J.M., Carral, J.D., Celada & Munoz, C. (1995). Effects of management on survival and growth of stage 2 juvenile freshwater signal crayfish (*Pacifastacus leniusculus* Dana) under laboratory conditions. Aquaculture 133(2): 123-134.

Shimizu, S.J. & Goldman, C.R. (1983). *Pacifastacus leniusculus* (Dana) production in the Sacromento River. Freshwater Crayfish 5: 210-228.

Söderbäck, B. (1994). Reproductive interference between co-occuring crayfish species, *Astacus astacus* L. and *Pacifastacus leniusculus* (Dana). Nordic Journal of Freshwater Research 69: 137-143.

Tamkeviciene, E.A. (1988). Growth and development of juveniles of the native and introduced species of freshwater crayfish. Freshwater Crayfish 7: 396-400.

Tulonen, J., Erkamo, E. & Kirjavainen, J. (1995). Growth rate, survival and reproduction of noble crayfish (*Astacus astacus* (L)), and signal crayfish (*Pacifastacus leniusculus* (Dana)), under similar rearing conditions. Freshwater Crayfish 10: 623-629.

Westman, K., Savolainen, R. & Pursiainen, M. (1993a). A comparative study on the growth and moulting of the noble crayfish, *Astacus astacus* (L), and the signal crayfish *Pacifastacus leniusculus* (Dana), in a small forest lake in southern Finland. Freshwater Crayfish 9: 451-465.

Westman, K., Savolainen, R. & Pursiainen, M. (1993b). A comparative study on the reproduction of the noble crayfish, *Astacus astacus* (L), and the signal crayfish *Pacifastacus leniusculus* (Dana), in a small lake in southern Finland. Freshwater Crayfish 9: 466-476.

Westman, K., Savolainen, R. & Pursiainen, M. (1995). Development of European noble crayfish *Astacus astacus* (L), American signal crayfish *Pacifastacus leniusculus* (Dana) populations in a small Finnish lake - a 20 year study. Freshwater Crayfish 8: 235-248.

Westman, K. & Savolainen, R. (1995). The development of a signal crayfish *Pacifastacus leniusculus* (Dana) population in a small forest lake in central Finland. Freshwater Crayfish 10: 200-208.

Management

Huner, J.V., Holdich, D.M. & Westman, K. (1992). Crayfish management strategies and management methods in Europe: a summary. Finnish Fisheries Research 14: 157-159.

Lindqvist, O.V. (1988). Restoration of native European crayfish stocks. Freshwater Crayfish 7: 6-12.

Söderbäck, B. (1995). Replacement of the native crayfish *Astacus astacus* by the introduced species *Pacifastacus leniusculus* in a Swedish lake: possible causes and mechanisms.

Freshwater Biology 33(2): 291-304.

Westman, K. (1992). Present status of crayfish management in Europe. Finnish Fisheries Research 14: 1-22.

Westman, K. (1992). Management of the noble crayfish *Astacus astacus* (L.) and the signal crayfish *Pacifastacus leniusculus* (Dana) in Finland. Finnish Fisheries Research 14: 1-22.

Physiology

Appelberg, M. (1985). Changes in hemolymph ion concentrations of *Astacus astacus* L and *Pacifastacus leniusculus* (Dana) after exposure to low pH and aluminum. Hydrobiologia 121(1): 19-25.

Becker, C.D., Genoway, R.T. & Merrill, J.A. (1975). Resistance of a Northwestern crayfish, *Pacifastacus leniusculus* (Dana), to elevated temperatures. Transactions of the American Fisheries Society 104(2): 374-387.

Cattaert, D., Barthe, J.Y., Neil, D.M. & Clarac, F. (1992a). Remote control of the swimmeret central pattern generator in crayfish (*Procambarus clarkii* and *Pacifastacus leniusculus*) effect of a walking leg proprioceptor. Journal of Experimental Biology 169(Aug): 181-206.

Cattaert, D., Elmanira, A. & Clarac, F. (1992b). Direct evidence for presynaptic inhibitory mechanisms in crayfish sensory afferents. Journal of Neurophysiology 67(3): 610-624.

Chrachri, A. (1990). Second thoracic root stimulation affects both the swimmeret and the walking systems of the crayfish *Pacifastacus leniusculus* invitro. Journal of Physiology London (Oct. 1990) Vol: 429.

Chrachri, A. (1995). Ionic currents identified swimmeret motor neurons of the crayfish *Pacifastacus leniusculus*. Journal of Experimental Biology 198(7): 1483-1492.

Cooper, P.D. (1994). Mechanisms of hemolymph acid base regulation in aquatic insects. Physiological Zoology 67(1): 29-53.

Elmanira, A., Dicaprio, R.A., Cattaert, D. & Clarac, F. (1991). Monosynaptic interjoint reflexes and their central modulation during fictive locomotion in crayfish. European Journal of Neuroscience 3(12): 1219-1231.

Elmanira, A. & Clarac, F. (1994). Presynaptic inhibition is mediated by histamine and gaba in the Crustacean escape reaction. Journal of Neurophysiology 71(3): 1088-1095.

Ellis, R.W., Long, J. Leitner, L. & Parsons, J. (1987). Estimation of crude protein, energy, and amino acid digestibilities in freshwater prawns (*Macrobrachium rosenbergii*) and crayfish (*Pacifastacus leniusculus*) with a fecal collection system. The Progressive Fish-Culturist 49: 303-305.

Ellis, R.W., Long, J. (1994). Metabolic flux in fed and fasted crayfish *Pacifastacus leniusculus*. Journal of the World Aquaculture Society 25: 326-332.

Elofsson, R., Nassel, D. & Myhrberg, H.(1977). A catecholaminergic neuron connecting the first two optic neuropiles (lamina ganglionaris and medulla externa) of the crayfish *Pacifastacus leniusculus*. Cell and Tissue Research 182: 287-297.

Elson, R.C., Sillar, K.T. & Bush, B.M.H. (1992). Identified proprioceptive afferents and motor rhythm entrainment in the crayfish walking system. Journal of Neurophysiology 67(3): 530-546.

Florey, E. (1960). Studies on the nervous regulation of the heart beat in decapod Crustacea. Journal of General Physiology 43: 1061-1081.

Fingerman, M. (1995). Endocrine Mechanisms in crayfish, with emphasis on reproduction and neurotransmitter regulation of hormone release. American Zoologist 35: 68-78.

Firkins, I. & Holdich, D.M. (1993). Thermal studies on three species of freshwater crayfish. Freshwater Crayfish 9: 241-248.

Fraser, K. & Heitler, W.J. (1993). Anatomical and physiological identification of inhibitors of the motor giant and segmental giant neurons in the crayfish. Journal of Experimental Biology 180: 55-73.

Gunzel, D., Galler, S. & Rathmayer, W. (1993). Fiber heterogeneity in the closer and opener muscles of crayfish walking legs. Journal of Experimental Biology 175: 267-281.

Harris, R.R. & Coley, S. (1991). The effects of nitrite on chloride regulation in the crayfish *Pacifastacus leniusculus* Dana (Crustacea, Decapoda). Journal of Comparative Physiology B 161(2): 199-206.

Head, S.I. & Bush, B.M.H. (1991). Proprioceptive reflex interactions with central motor rhythms in the isolated thoracic ganglion of the shore crab. Journal of Comparative Physiology A 168(4): 445-459.

Henry, R.P. & Wheatly, M.G. (1988). Dynamics of salinity adaptations in the euryhaline crayfish *Pacifastacus leniusculus*. Physiological Zoology 61(3): 260-271.

Heitler, W.J. & Darrig, S. (1986). The segmental giant neuron of the signal crayfish, *Pacifastacus leniusculus*, and its interactions with abdominal fast flexor and swimmeret motor neurons. Journal of Experimental Biology 121: 55-75.

Heitler, W.J. & Fraser, K. (1993). Thoracic connections between crayfish giant fibers and motor giant neurons reverse abdominal pattern. Journal of Experimental Biology. 181: 329-333.

Kerley, D.E. & Pritchard, A.W. (1967). Osmotic regulation in the crayfish, *Pacifastacus leniusculus*, stepwise acclimated to dilutions of sea water. Comparative Biochemistry and Physiology 20: 101-113.

Kirk, M.D., Prugh, J.I. & Glantz, R.M. (1983). Retinal illumination produces synaptic inhibition of a neurosecretory organ in the crayfish, *Pacifastacus leniusculus* (Dana). Journal of Neurobiology 14(6): 473-480.

Mackeviciene, G. (1993). Respiration during postembryonic development of the freshwater crayfish *Pacifastacus leniusculus* (Dana). Freshwater Crayfish 9:183-188.

McMahon, B.R. (1986). The adaptable crayfish: mechanisms of physiological adaptation. Freshwater Crayfish 6: 59-74.

Morse, H.C., Harris, P.J. & Dornfeld, E.J. (1970). *Pacifastacus leniusculus*: fine structure of the arthrobranch with reference to active ion uptake. Transactions of the American Microscopical Society 89(1): 12-17.

Moshiri, G.A. & Goldman, C.R. (1969). Estimation of assimilation efficiency in the crayfish, *Pacifastacus leniusculus* (Dana) (Crustacea: Decapoda). Archives of Hydrobiology 66(3): 298-306.

Moshiri, G.A., Goldman, C.R. Godshalk, G.L. & Mull, D.R. (1970). The effects of variations in oxygen tension on certain aspects of respiratory metabolism in *Pacifastacus leniusculus* (Dana). Physiological Zoology 43(1): 23-29.

Moshiri, G.A., Goldman, C.R., Mull, D.R., Godshalk, G.L. & Coil, J.A. (1971). Respiratory metabolism in *Pacifastacus leniusculus* (Dana) (Crustacea: Decapoda) as related to its ecology. Hydrobiologia 37(2): 183-195.

Nusbaum, M.P., Weimann, J.M., Golowasch, J. & Marder, E. (1992). Presynaptic control of modulatory fibers by their neural network targets. Journal of Neuroscience 12(7): 2706-2714.

Pritchard, A.W. & Kerley, D.E. (1970). Kidney function in the North American crayfish *Pacifastacus leniusculus* (Dana) stepwise acclimated to dilutions of sea water. Comparative Biochemistry and Physiology 35: 427-437.

Rossidurand, C. (1994). Peripheral proprioceptive modulation in crayfish walking leg by serotonin. Brain Research 632(1-2): 1-15.

Rundquist, J.C. & Goldman, C.R. (1978). Growth and food conversion efficiency of juvenile *Pacifastacus leniusculus* along a salinity gradient. Freshwater Crayfish 4: 105-113.

Rutledge, P.S. & Pritchard, A.W. (1981). Scope for activity in the crayfish *Pacifastacus leniusculus*. American Journal of Physiology 240(1): 87-92.

Rutledge, P.S. (1981a). Circulation and oxygen transport during activity in the crayfish, *Pacifastacus leniusculus*. American Journal of Physiology 240: R-99-R105.

Rutledge, P.S. (1981b). Effects of temperature acclimation on crayfish hemocyanin oxygen binding. American Physiological Society 240(1): 93-98.

Rydqvist, B. & Purali, N. (1993). Transducer properties of the rapidly adapting stretch receptor neuron in the crayfish (*Pacifastacus leniusculus*). Journal of Physiology London 469: 193-211.

Sillar, K.T. & Skorupski, P. (1986). Central input to primary afferent neurons in crayfish, *Pacifastacus leniusculus*, is correlated with rhythmic motor output of thoracic ganglia. Journal of Neurophysiology 55(4): 678-688.

Sillar, K.T., Clarac, F. & Bush, B.M.H. (1987). Intersegmental coordination of central neural oscillators for rhythmic movements of the walking legs in crayfish, *Pacifastacus leniusculus*. Journal of Experimental Biology 131: 245-264.

Skorupski, P. & Sillar, K.T. (1986). Phase dependent reversal of reflexes mediated by the thoracocoxal muscle receptor organ in the crayfish *Pacifastacus leniusculus*. Journal of Neurophysiology 55(4): 689-695.

Skorupski, P. & Bush, B.M.H. (1992a). Parallel reflex and central control of promotor and receptor motor neurons in crayfish. Proceedings of the Royal Society of London Series B Biological Sciences. 249(1324): 7-12.

Skorupski, P. (1992b). Synaptic connections between nonspiking afferent neurons and motor neurons underlying phase dependent reflexes in crayfish. Journal of Neurophysiology 67(3): 664-679.

Skorupski, P. Rawat, B.M. & Bush, B.M.H. (1992c). Heterogeneity and central modulation of feedback reflexes in crayfish motor pool. Journal of Neurophysiology 67(3): 648-663.

Skorupski, P., Vescovi, P.J. & Bush, B.M.H. (1994). Integration of positive and negative feedback loops in a crayfish muscle. Journal of Experimental Biology 187: 305-313.

Söderhäll, K., Johansson, M.W. & Smith, V.J. (1988). Internal defence mechanisms. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 213-235. Chapman & Hall, London.

Syvokiene, J. & Mickeniene, L. (1993). The activity of gut bacteria of the crayfish, *Pacifastacus leniusculus* (Dana), in producing essential free amino acids. Freshwater Crayfish 9: 235-240.

Wang, M.Y. & Brown, W.D. (1983). Effects of elevated CO_2 atmosphere on storage of freshwater crayfish (*Pacifastacus leniusculus*). Journal of Food Science 48(1): 158-162.

Wheatly, M.G. & McMahon, B.R. (1982a). Responses to hypersaline exposure in the euryhaline crayfish *Pacifastacus leniusculus*. 2. Modulation of hemocyanin oxygen binding invitro and invivo. Journal of Experimental Biology 99: 447-467.

Wheatly, M.G. & McMahon, B.R. (1982b). Responses to hypersaline exposure in the euryhaline crayfish *Pacifastacus leniusculus*. 1. The interaction between ionic and acid-base regulation. Journal of Experimental Biology 99: 425-445.

Wheatly, M.G. & McMahon, B.R. (1983). Respiration and ionoregulation in the euryhaline crayfish *Pacifastacus leniusculus* on exposure to high salinity: an overview. Freshwater Crayfish 5: 43-55.

Wheatly, M.G. & Henry, R.P. (1987). Branchial and antennal gland NA^+/K^+ dependent atpase and carbonic anhydrase activity during salinity acclimation of the euryhaline crayfish *Pacifastacus leniusculus*. Journal of Experimental Biology 133: 73-86.

Wheatly, M.G. (1988). Physiological responses of the crayfish *Pacifastacus leniusculus* to environmental hyperoxia: acid-base and ionoregulation. Freshwater Crayfish 7: 203-210.

Wheatly, M.G. (1989a). Physiological responses of the crayfish *Pacifastacus leniusculus* to environmental hyperoxia. 1.Extracellular acid-base and electrolyte status and transbranchial exchange. Journal of Experimental Biology 143: 33-51.

Whealty, M.G. (1989b). Standard rate of oxygen uptake and body size in the crayfish *Pacifastacus leniusculus* (Dana 1852) (Decapoda, Astacidae) intra versus interspecific relations in Crustaceans. Journal of Crustacean Biology 9(2): 212-216.

Wheatly, M.G. & Toop, T. (1989c). Physiological responses of the crayfish *Pacifastacus leniusculus* to environmental hyperoxia. 2. Role of the antennal gland in acid base and ion regulation. Journal of Experimental Biology 143: 53-70.

Wheatly, M.G., Toop, T., Morrison, R.J. & Yow, L.C. (1991). Physiological responses of the crayfish *Pacifastacus leniusculus* (Dana) to environmental hyperoxia. 3. Intracellular acid-base balance. Physiological Zoology 64(1): 323-343.

Wheatly, M.G. & Henry, R.P. (1992). Extracellular and intracellular acid-base regulation in Crustaceans. Journal of Experimental Zoology 263(2): 127-142.

Wheatly, M.G. (1995). An overview of electrolyte regulation in the freshwater crayfish throughout the moulting cycle. Freshwater Crayfish 8: 420-436.

Taxonomy

Albrecht, H. (1982). Das system der europaischen Flußkrebse (Decapoda, Astacidae): Vorschlag und Begründung. Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut 79: 187-210.

Albrecht, H. (1983). Die Protastacidae n. fam., fossile Vorfahren der Flußkrebse? Neues Jahrbuch fuer Geologie und Paleontologie, Monatshefte H.1: 5-15.

Bott, R. (1950). Die Flußkrebse Europas (Decapoda, Astacidae). Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft 483(1-36): 1-36.

Bouchard, R.W. (1977). Distribution, systematic status and ecological notes on five poorly known species of crayfishes in western North America (Decapoda: Astacidae and Cambaridae). Freshwater Crayfish 3: 409-423.

Curra, R.A. (1967). A key to genera, species and subspecies of Astacinae (Nephropsidea: Astacidae). International Revue der Gesamten Hydrobiologie 52(5): 793-800.

Gledhill, T., Sutcliffe, D.W. & Williams, W.D. (1993). British freshwater Crustacea Malacostraca: a key with ecological notes. 173 pp. Freshwater Biological Association. The Ferry House Far Sawrey, Ambleside, Cumbria LA22 OLP.

Hobbs, H.H.Jr. (1988). Crayfish distribution, adaptive radiation and evolution. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 52-82. Chapman & Hall, London.

Hofmann, J. (1971). Die Flußkrebse: biologie, haltung und wirtschaftliche bedeutung. 102 p. Verlag Paul Parey, Hamburg and Berlin.

Karaman, M.S. (1962). Ein beitrag zur systematik der Astacidae (Decapoda). Crustaceana 3: 173-191.

Laurent, P.J. & Forest, J. (1979). Données sur les écrevisses qu'on peut rencontrer en France. La Pisciculture Française 56 (15-2): 25-40.

Pennack, R.W. (1978). Freshwater invertebrates of the United States. 803 p. A Wiley-interscience publication, New York, Chichester, Brisbane, Toronto.

Riegel, J.A. (1959). The systematics and distribution of crayfishes in California. California Fish Game 45: 29-50.

Vigneux, E. Keith, P. & Noel, P. (1993). Atlas preliminaire des Crustaces Decapodes d'eau douce de France. Coll. Patrimoines Naturels. Vol: 14, S.F.F., B.I.M.M.- M.N.H.N., C.S.P., Ministere de l'Environnement, Paris, 55 p.

Toxicology

Dauble, D.D., Bean, R.M. & Hanf, R.W. (1994). Disposition of quinolne in the crayfish *Pacifastacus leniusculus*. Bulletin of Environmental Contamination and Toxicology 53(3): 426-433.

Stinson, M.D. & Eaton, D.L. (1983). Concentrations of lead, cadmium, mercury, and copper in the crayfish (*Pacifastacus leniusculus*) obtained from a lake receiving urban runoff. Archives of Environmental Contamination and Toxicology 12: 693- 700.

A survey of the literature regarding the biology of *Astacus leptodactylus*

Astaciculture

Amato, G.D. (1988). Freshwater crayfish breeding in Basse-Normandie. Freshwater Crayfish 7: 213-222.

Avault, J.W.Jr. (1993). A review of world crustacean aquaculture with special reference to crayfish. Freshwater Crayfish 9: 1-12.

Holdich, D.M. (1993). A review of astaciculture: freshwater crayfish farming. Aquatic Living Resources 6: 307-317.

Huner, J.V. (1994) (editor). Freshwater Crayfish Aquaculture in North America, Europe, and Australia: families *Astacidae*, *Cambaridae*, and *Parastacidae*. 312 pp. Haworth Press, Inc.

Huner, J.V. (1995). An overview of the status of freshwater crawfish culture. Journal of Shellfish Research 14(2): 539-543.

Laurent, P.J. (1992). Le commerce exterieur des ecrevisses depuis une decennie - (with English summary - Crayfish imports and exports since the last ten years). Bulletin Public Par l'Association des Astaciculteurs de France 31: 8-12.

Laurent, P.J. (1995). Une decennnie de commerce exterieur des ecrevisses en France - (with English summary- Ten years of crayfish foreign trade in France). Bulletin Publie Par l'Association des Astaciculteurs de France 43: 2-6.

Behaviour

Belyanin, O.L., Kan, G.S., Stepushkina, T.A. & Moskalev, O.S. (1977). Functional structure of feeding behaviour in the crayfish *Astacus leptodactylus*. Zhurnal Evolution Biokhimi I Fiziologii 13(3): 376-381.

Cukerzis, J.M. (1986). Behaviour of crayfish juveniles during early stages of ontogenesis. Freshwater Crayfish 6: 75-86.

Tsukerzis, Ta.Ma. (1976). Interspecific competition of closely allied crayfish species (*Astacus astacus* L., *Astacus leptodactylus* Esch., *Pacifastacus leptodactylus* Dana, Decapoda Crustacea). Doklady Biological Sciences (English translation of Doklady Akademiia Nauk SSSR). Seriya Biologiya. 119(1-6): 283-285.

Bibliographies

Arrington, J. (1979). Elements de bibliographie sur le genre Astacus en Europe. Freshwater Crayfish 4: 427-436.

Cukerzis, J. (1983). Selected literature on the Lithuanian crayfish. Freshwater Crayfish 5: 478-489.

Hart, C.W. & Clarck, J. (1987). An Interdisciplinary Bibliography of Freshwater Crayfishes (Astacoidea and Parastacoidea) from Aristotle through 1985. 437 pp. Smithsonian Institution Press, Washington.

Holdich, D.M. (1991). A bibliography of "Freshwater Crayfish - A Journal of Astacology I-VII", Proc. Int. Assoc. Astacology Symp. 1973-1988. Journal of Shellfish Research 10: 341-347.

Holdich, D.M. & Pearce-Higgins, J.W. (1995). A bibliography and taxonomic and subject index to "Freshwater Crayfish I-X", the Proceedings of the International Association of Astacology Symposia 1972-1994. Freshwater Crayfish xvi-lxxix.

Biochemistry

Agerberg, A. (1990). Genetic variation in 3 species of fresh water crayfish *Astacus astacus* L, *Astacus leptodactylus* Esch, and *Pacifastacus leniusculus* Dana, revealed by isozyme electrophoresis. Hereditas 113(2): 101-108.

Babula, A. (1975). Histological, histochemical and ultrastructural studies of the mid and hind gut of *Astacus leptodactylus*. Bulletin De La Societe Des Amis Des Sciences Et Des Lettres De Poznan. Serie D 15: 87-98.

Balakhnin, I.A. & Brodsky, S.Y. (1979). Comparative study of hemolymph serum albumens and alkaline phosphatases in crayfishes (*Astacus leptodactylus*) from the Dnieper, Dniesterand Danuberivers. In: Biokhimicheskaja i Populatsionnaja Genetica Ryb., pp. 139-141. (Kirpichnicov, V.S. ed.). Leningrad, Institut Tisitologii Akademy Nauk SSSR (Russian with English summary).

Barbe, L. & Sevilla, C. (1987). Osmotic adaptation in *Astacus leptodactylus*. 1. Evolution of free intracellular amino acids. Biochemical Systematics & Ecology 15(1): 127-132.

Belyanin, O.L., Kan, G.S., Stepushkina, T.A & Moskalev, O.S. (1977). Functional structure of feeding behaviour in the crayfish *Astacus leptodactylus*. Zhurnal Evolyutsionna Biokhimi I Fiziologi Trans by Plenumpubs 13(3): 231-236.

Bielefeld, M., Gellissen, G. & Spindler, K.D. (1986). Protein production and the moulting cycle in the crayfish *Astacus leptodactylus*. 1. Stage specificity of polypeptide patterns in the hypodermis and hepatopancreas. Insect Biochemistry 16(1): 175.

Bock, D., Hinssen, H. & Dhaese, J. (1993). A 100-KDA actin modulator protein with fully reversible actin-bindig properties from the tail muscle of the crayfish *Astacus leptodactylus*. Journal of Muscle Research & Cell Motility 14(2): 268-269.

Dabrowski, T., Kolakowski, E., & Sokolowski, E. (1966). Zusammensetzung und Nahrwet des Krebsfleisches von *Astacus leptodactylus*., Zeitschrift fuer Lebensmittel Untersuchung und Forschung 129: 337-344.

Debethune, B., Cleuter, Y., Marbaix, G. & Preaux, G. (1985). Site of biosynthesis of the hemocyanin in the crayfish *Astacus leptodactylus*. Archives Internationales De Physiologie Et De Biochimie 93(3).

Durliat, M. & Vranckx, R. (1982). Effects of irradiation on the blood factors of *Astacus leptodactylus*. Comptes Rendus Des Seances De L Academie Des Seciences Serie III. Seciences De La Vie 294(2): 119-122.

Durliat, M. & Vranckx, R. (1982a). Proteins of aqueous extracts from the hepatopancreas of *Astacus leptodactylus*. 1.Changes in proteins during the moult cycle. Comparative Biochemistry & Physiology B 71(2): 155-163.

Durliat, M. & Vranckx, R. (1982b). Proteins of aqueous extracts from the hepatopancreas of *Astacus leptodactylus*. 2.Immunological identities of proteins from hepatopancreas and blood. Comparative Biochemistry & Physiology B 71(2): 165-171.

Durliat, M. (1984). Occurrence of plasma proteins in ovary and egg extracts from *Astacus leptodactylus*. Comparative Biochemistry & Physiology B 78(3): 745-753.

Durliat, M. & Vranckx, R. (1986). Cuticular proteins in the crayfish *Astacus leptodactylus* I. Relationships between proteins in plasma soft integument and calcified cuticle. Comparative Biochemistry & Physiology B 85: 267-274.

Durliat, M., Moriniere, M. & Porcheron, P. (1988). Changes in ecdysteroids in *Astacus leptodactylus* during the moulting cycle. Comparative Biochemistry & Physiology A 89(2): 223-229.

Garcia, E., Benitez, A. & Onetti, C.G. (1993). Responsiveness to d-glucose in neurosecretory cells of crustaceans. Journal of Neurophysiology 70(2):758-764.

Gellissen, G.M., Hennecke & Spindler, K.D. (1991). The site of synthesis of hemocyanin in the crayfish *Astacus leptodactylus*. Experentia 47: 194-195.

Gomez, R. Delasrivas, J., Garate, A.M., Barbon, P.G. & Milicua, J.C.G. (1986). Characterization of a blue astaxanthin protein from the carapace of the crayfish *Astacus leptodactylus*. Comparative Biochemistry & Physiology B 83(4): 855-859.

Gomez, R. & Milicua, J.C.G. (1992). Use of borohydride reduction methods to probe carotenoid protein binding. Methods in Enzymology 213: 100-110.

Gorgelskallen, J.L. & Vanherp, F. (1981). Localization of Crustacean hyperglycemic hormone (CHH) in the X organ sinus gland complex in the eyestalk of the crayfish, *Astacus leptodactylus* (Nordmann 1842). Journal of Morphology 170(3): 347-355.

Gorgelskallen, J.L. & Voorter, C.E.M. (1984). Secretory stages of individual CHH producing cells in the eyestalk of the crayfish *Astacus leptodactylus*, determined by means of immunocytochmistry. Cell & Tissue Research 237(2): 291-298.

Gorgelskallen, J.L. & Voorter, C.E.M. (1985a). The secretory dynamics of the CHH producing cell group in the eyestalk of the crayfish, *Astacus leptodactylus*, in the course of the day night cycle. Cell & Tissue Research 241(2): 361-366.

Gorgelskallen, J.L. & Meij, J.T.A. (1985b). Immunocytochemical study of the hyperglycemic hormone (CHH) producing system in the eyestalk of the crayfish *Astacus leptodactylus* during larval and postlarval development. Journal of Morphology 183(2): 155-163.

Hamacher, K. & Stieve, H. (1984). Spectral properties of the rhodopsin system of the crayfish *Astacus leptodactylus*. Photochemistry & Photobiology 39(3): 379-390.

Hennecke, R., Gellissen, G. & Spindler, K.D. (1991). Hemocyanin synthesis in the crayfish, *Astacus leptodactylus*, invitro production by the midgut gland after addition of oxygen binding modlators and exogenous cooper. Zeitschrift Fur Naturforschung C. A Journal of Biosciences 46(1-2): 163-165.

Joureguiadell, J. Wnuk, W. & Cox , J.A. (1987). Amino acid sequence of the sarcoplasmic calcium binding protein-I (SCP-1) from crayfish (*Astacus leptodactylus*). Journal of Muscle Research & Cell Motility 8(1): 92-93.

Kallen, J.L, Reijntjens, F.M.J., Peters, D.J.M. & Vanherp, F. (1986). Biochemical analyses of the Crustacean hyperglycemic hormone of the crayfish *Astacus leptodactylus*. General & Comparative Endocrinology 61(2): 248-259.

Lignon, J., Aubert, A. & Avenet, P. (1987). Delayed CL rectification in the gill lamina cuticle of the crayfish, *Astacus leptodactylus* (E). Comptes Rendus De L Academie Des Sciences Serie III. Sciences De Lavie 304(18): 473-476.

Mackevichiene, G. (1979). Some biochemical differences between two species of freshwater crayfish, *Astacus astacus* and *Astacus leptodactylus* in Lithuania. Freshwater Crayfish 4: 465-470.

Magnus, K.A., Tonthat, H. & Carpenter, J.E. (1994). Recent structural work on the oxygen-transport protein hemocyanin. Chemical Reviews 94(3): 727-735.

Malaczynska-Suchcitz, Z. (1956). The distribution, structure, and fuctions of cement glands in *Potamobius astacus* Leach and *Potamobius leptodactylus* Eschz. Bulletin De La Societe Des Amis Des Sciences Et Des Lettres De Poznan. Serie B -Livrasion XIII: 301-339.

Malaczynska-Suchcitz, Z. & Klepke, A. (1960). Cytophysiological and cytochemical studies of the mid-gut epithelium in *Astacus*. Bulletin De La Societe Des Amis Des Sciences Et Des Lettres De Poznan. Serie D 1: 1-19.

Milicua, J.C.G., Arberas, P.D., Barbon, P.G., Garate, A.M. & Gomez, R. (1986). A yellow carotenoprotein from the carapace of the crayfish *Astacus leptodactylus*. Comparative Biochemistry & Physiology B 85: 615-619.

Nagel, R. & Urich, K. (1981). Elimination and distribution of different substituted phenols by frog (*Rana temporaria*) and crayfish (*Astacus leptodactylus*). Bulletin of Environmental Contamination & Toxiocology 26(3): 289-294.

Obrien, J.J., Kumari, S.S. & Skinner, D.M. (1991). Proteins of crustacean exoskeletons. 1. Similarities and differences among proteins of the 4 exoskeletal layers of 4 Brachyurans. Biological Bulletin 181(3): 427-441.

Ollevier, F. Diederik, H. & Deloof, A. (1984). Identification of nonecdysteroid steroids in the hemolymph of both male and female *Astacus leptodactylus* (Crustacea) by NCI-GC/MS. General & Comparative Endocrinology 53(3): 449-450.

Ollevier, F., Declerck, D., Diederik, H. & Deloof, A. (1986). Identification of nonecdysteroid steroids in hemolymph of both male and female *Astacus leptodactylus* (Crustacea) by gas chromatography mass spectrometry. General & Comparative Endocrinology 61(2): 214-228.

Rivas, J.D.L., Milicua, J.C.G.& Gomez, R. (1988). Furter studies on the blue carotenoprotein from *Astacus leptodactylus*. Comparative Biochemistry & Physiology B 89: 65-68.

Rivera, M.E. & Langer, H. (1983). Enzyme pattern of energy releasing metabolism in eyes, optical ganglia and muscle of the crayfish *Astacus leptodactylus*. Molecular Physiology 3(5-6): 313-329.

Sevilla, C., Linden, E. Baccou, J.C. & Sauvaire, Y. (1993). Biochemical study of the growth of *Astacus leptodactylus* Eschscholtz fed with plant diets by quantitative variations of muscular DNA and proteins. Freshwater Crayfish 9: 226-234.

Skinner, D.M., Kumari, S.S. & Obrien, J.J. (1992). Proteins of the crustacean exoskeleton. American Zoologist 32(3):470-484.

Spindler, K.D., Hennecke, R. & Gellissen. (1992). Protein production and molting cycle in the crayfish *Astacus leptodactylus* (Nordman, 1842). II. Hemocyanin and protein synthesis in th e midgut gland. General & Comparative Endocrinology 85: 248-253.

Strolenberg, G.E.C. & van Helden, H. & van Herp, F. (1977). The ultrastructure of the sinus gland of crayfish *Astacus leptodactylus* (Nordmann). Cell and Tissue Research 180: 203-210.

Strolenberg, G.E.C. & Van Herp, F. (1977). Mise en evidence du phenomene d'exocytose dan la glande du sinus d'*Astacus leptodactylus* (Nordmann) sous l'influence d'injections de serotonine. Comptes Rendu de l'Academie des Sciences, Paris 284 D: 57-60.

Suprunovich, A.V., Kadniuk, R.P., Petkevich, T.A., Stepaniuk, I.A., Lisovskaya, V.I. & Antsupova, L.V. (1983). Biochemical characteristics of the Dniester long-clawed crayfish of Astacidae family. Freshwater Crayfish 5: 490-517.

Tahon, J.P., Vanhoof, D., Vinckier, C., Witters, R., Deley, M. & Lontie. R. (1988). The reaction of nitrite with the hemocyanin of *Astacus leptodactylus*. Biochemical Journal 249(3): 891-896.

Tahon, J.P., Gielens, C., Vinckier, C., Witters, R., Deley, M., Preaux, G. & Lontie, R. (1989). The reaction of nitrogen monoxide with the hemocyanins of the crayfish *Astacus leptodactylus* and the snail *Helix pomatia*. Biochemical Journal 262(1): 253-260.

Tensen, C.P., Kallen, J.L. & Vanherp, F. (1986). Introductory studies on the biosynthesis of neuropeptides in the eyestalk of the crayfish *Astacus leptodactylus* with special attention to the Crustacean hyperglycemic hormone (CHH). Bulletin de la Societe Zoologique de France 111(1-2): 29.

Traub, M., Gellissen, G. & Spindler K-D. (1987). 20 (OH) ecdysone-induced transition from intermoult to premoult biosynthesis patterns in the hypodermis of the crayfish, *Astacus leptodactylus*, in vitro. General & Comparative Endocrinology 65: 469-477.

Valin, A., Castillo, R., Negresadargues, G. & Lenel, R. (1987). Quantitative aspects of carotenoid pigment circadian variations in the crayfish *Astacus leptodactylus*. Biochemical

Systematics & Ecology 15(5): 607-610.

Vandeijnen, J.E., Vek, F. & Vanherp, F. (1985). An immunocytochemical study of the optic ganglia of the crayfish *Astacus leptodactylus* (Nordman 1842) with antisera against biologically active peptides of vertebrates and invertebrates. Cell & Tissue Research 240(1): 175-183.

van Herp, F. (1970). Study of the influence of sinus gland extirpation on the alkaline phosphatese in the hepatopancreas of the crayfish *Astacus leptodactylus*. Comparative Biochemistry & Physiology 34: 439-445.

Vanhoof, D., Witters, R. & Lontie, R. (1988). The reaction of hydroxyurea with oxyhemocyanin and methemocyanin of the crayfish *Astacus leptodactylus* and the snail *Helix pomatia*. Biochemical Journal 254 (2): 605-607.

Vranckx, R. & Durliat, M. (1981). Soluble glycoprotein complexes in the blood and integumental extracts of *Astacus leptodactylus*. Molecular Physiology 1(6): 339-352.

Vranckx, R. & Durliat, M. (1986). Cuticular proteins in the crayfish *Astacus leptodactylus* Main components. Comparative Biochemistry & Physiology 85B: 255-265.

Willibrordus, J., Marrewijk, A.V. & Ravestein, H.J.L. (1974). Amino acid metabolism of *Astacus leptodactylus* (Esch). - I. Composition of the free and protein-bound amino acids in different organs of the crayfish. Comparative Biochemistry & Physiology 47B: 531-542.

Willibrordus, J., Marrewijk, Avan & Zandee, D.I. (1975). Amino acid metabolism of *Astacus leptodactylus* (Esch). II. Biosynthesis of the non-essential amino acids. Comparative Biochemistry & Physiology 50B: 449-455.

Zerbe, T., Klepke, K. & Obuchowicz, L. (1970). Hormonal regulation of metabolism in hepatopancreas mitochondria of crayfish *Astacus leptodactylus* Eschz. Bulletin de la Societe des Sciences et des Lettres de Poznan 11: 45-d51.

Zerbib, C. (1979). Etude ultrastructurale de l'ovocyte en vitellogenese ches les Ecrevisses *Astacus astacus* et *Astacus leptodactylus*. International Journal of Invertebrate Reproduction 1: 289-295.

Zwilling, R., Neurath, H., Ericsson, L.H., & Enfield, D.L. (1975). The amino-terminal sequence of an invertebrate trypsin (crayfish *Astacus leptodactylus*): Homology with other serine proteases. Federation of the European Biochemical Society 60: 247-249.

Biology

Cherkashina, N.Y. & Karnaushenko, I.V. (1982). The pre-embryonic diapause in the crayfish *Astacus leptodactylus cubanicus* bir et win. Zhurnal Obshchei Biologii 43(5): 687-689.

Holdich, D.M. & Lowery, R.S. (1988). Crayfish: an introduction. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 1-7. Chapman & Hall, London.

Holdich, D.M. & Reeve, I.D. (1988). Functional morphology and anatomy. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 11-51. Chapman & Hall, London.

Holdich, D.M. (1992). Crayfish nomenclature and terminology: recommendations for uniformity. Finnish Fish Research 14: 149-155.

Huxley, T.H. (1880). Crayfish. An introduction to the Study of Zoology. The International Scientific Series. 371 pp. D. Appleton & Co. New York.

Kossakowski, J. (1971). Crayfish (raki). 193 pp. Translated for the National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Department of Commerce and the National Science Foundation, by the Scientific Publications Foreign Cooperation Center of the Central Institute for Scientific Technical and Economic Information, Warsaw, Poland.

Köksal, G. (1988). *Astacus leptodactylus* in Europe. In: Freshwater crayfish: biology, management and exploitation (D. M. Holdich and Lowery R. S. eds). pp. 365-400. Chapman & Hall, London.

Laurent, P.J. (1988). *Austropotamobius pallipes* and *A. torrentium*, with observations on their interaction with other species in Europe. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 341-364. Chapman & Hall, London.

Schimkewitsch, W. (1885). Einige bemerkungen uber die entwicklungsgeschichte des flusskrebbses. Zoologischer Anzeiger, Leipzig 8: 303-304.

Conservation

Holdich, D.M. (1991). The native crayfish and threats to its existence. British Wildlife 2(3): 141-151.

Holdich, D.M., Reader, J.P., Rogers, W.D. (1995). Crayfish conservation. Final Project Report to the National Rivers Authority. 1995. R&D 378/10/N. 278 pp.

Rogers, W.D. & Holdich, D.M. (1995). Conservation and management of crayfish in Britain. Freshwater Crayfish 10: 92-97.

Disease

Alderman, D.J., Polglase, J.L., Frayling, M. & Hogger, J. (1984a). Crayfish plague in Britain. Journal of Fish Diseases 7: 401-405.

Alderman, D.J. & Polglase, J.L. (1984b). A comparative investigation of the effects of fungicides on *Saprolegnia parasitica* and *Aphanomyces astaci*. Transactions of the British Mycological Society 83(2): 313-318.

Alderman, D.J. & Polglase, J.L. (1985). Disinfection for crayfish plague. Aquaculture & Fisheries Management 16: 203-205.

Alderman, D.J., Polglase, J.L. & Frayling, M. (1987). *Aphanomyces astaci* pathogenicity under laboratory and field conditions. Journal of Fish Diseases 10: 385-393.

Alderman, D.J. & Polglase, J.L. (1988). Pathogens, parasites and commensals. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 167-212. Chapman & Hall, London.

Chinain, M. & Vey, A. (1988a). Infection caused by *Fusarium solani* in crayfish *Astacus leptodactylus*. Freshwater Crayfish 7: 195-202.

Chinain, M. & Vey, A. (1988b). Experimental study of *Fusarium solani*: infections in *Astacus leptodactylus* and *Pacifastacus leniusculus* (Crustacea, Decapoda). Diseases of Aquatic Organisms 5: 215-223.

Chinain, M. & Vey, A. (1988c). Comparative studies on strains of *Fusarium solani* parasitic for crayfish or isolated from other hosts. Freshwater Crayfish 7: 179-186.

Cerenius, L. & Söderhäll, K., (1992). Crayfish diseases and crayfish as vectors for important diseases. Finnish Fisheries Research 14: 125-133.

Cuellar, L. & Coll, M. (1983). Epizootiology of the crayfish plague (*Aphanomycosis*) in Spain. Freshwater Crayfish 5: 545-548.

Gydemo, R. (1992). Crayfish diseases and their management - the need for knowledge. Finnish Fisheries Research 14: 119-124.

Maestracci, V. & Vey, A. (1988). Fungal infection of gills in crayfish: histological, cytological and physiopathological aspects of the disease. Freshwater Crayfish 7: 187-194.

Oray, I. (1990). The crayfish situation in Turkey. European Aquaculture Society. Special Publication No. 12, Bredene, Belgium.

Persson, M., Vey, A. & Soderhall, K. (1987). Encapsulation of foreign particles by blood cells from crayfish *Astacus leptodactylus*. Cell & Tissue Research 247: 409-415.

Rahe, R. & Soylu, E. (1989). Identification of the pathogenic fungus causing destruction to Turkish crayfish stocks (*Astacus leptodactylus*). Journal of Invertebrate Pathology 54(1): 10-15.

Smith, V.J. (1986). Crayfish pathology: an overview. Freshwater Crayfish 6: 199-211.

Söderhäll, K., Dick, M.V., Clark, G., Furst, M. & Constantinescu, O. (1991). Isolation of *Saprolegnia parasitica* from the crayfish *Astacus leptodactylus*. Aquaculture 92(2-3): 121-125.

Unestam,T. (1969). Resistance to the crayfish plague in some American, Japanese and European crayfishes. Report of the Institate of Freshwater Research Drottningholm 49: 202-209.

Vranckx, R. & Durliat, M. (1981). Encapsulation of *Psorospermium haeckeli* by the hemocytes of *Astacus leptodactylus*. Experientia 37(1): 40-42.

Vey, A. (1977). Studies on the pathology of crayfish under rearing conditions. Freshwater Crayfish 3: 311-319.

Vey, A. (1979). Infections fongiques ches l'ècrevisse *Astacus leptodactylus* Esch. Freshwater Crayfish 4: 403-410.

Vey, A. (1986). Disease problems during aquaculture of freshwater Crustacea. Freshwater Crayfish 6: 212-222.

Ecology, distribution and population biology

Albrecht, H. (1983). Besiedlungsgeschichte und ursprünglich holozäne Verbreitung der europäischen Flußkrebse. Spixiana 6(1): 61-77.

Bonadysenko, A. (1968). Plodovitost riecnych rakov w niekotorych vodoemach Bielorusskogo Poozeria. Limnologia 3(2): Riga.

Brodski, S. Ya. (1974). Crayfish (Crustacea, Astacidae) from the Soviet Union. Communication 2. Distribution of *Astacus leptodactylus*. Ukranian Research Institute for Fisheries 4: 51-59.

Brodsky, S.Ya. (1975). The crayfish situation in Ukraine. Freshwater Crayfish 2: 27-29.

Burba, A. (1993). Nouvelles de l'ecrevisse en Lithuanie (with English summary - Facts about the crayfish in Lithuanie). Bulletin Publie Par l'Association des Astaciculteurs de France 36: 2-6.

Buttiker, B. (1988). Concerning crayfish in Switzerland. Freshwater Crayfish 7: 2-5.

Cherkashina, N.Ya. (1975). Distribution and biology of crayfishes of genus Astacus (Crustacea, Decapoda, Astacidae) in the Turkmen Waters of the Caspian Sea. Freshwater Crayfish 2: 553-555.

Degiorgi, F., Laurent, P.J. & Lumet, J.C. (1994). Des ecrevisses dans un etang etabli sur des residus de mine de charbon. (with English summary - Crayfish in a pond built on charcoal mine refuse). Bulletin Publie Par l'Association des Astaciculteurs de France 41: 2-5.

Erencin, Z. & Köksal, G. (1977). On the crayfish, *Astacus leptodacylus*, in Anatolia. Freshwater Crayfish 3: 187-192.

Goddard, J.S. & Hogger, J.B. (1986). The current status and distribution of freshwater crayfish in Britain. Field Studies 6: 383-396.

Hobbs, H.H.Jr. (1988). Crayfish distribution, adaptive radiation and evolution. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 52-82. Chapman & Hall, London.

Hogger, J.B. (1988). Ecology, population biology and behaviour. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 114-144. Chapman & Hall, London.

Holdich, D.M. & Reeve, I.D. (1991). Distribution of freshwater crayfish in the British Isles, with particular reference to crayfish plague, alien introductions and pollution. Aquatic Conservation: Marine and Freshwater Ecosystems 1: 139-158.

Holdich, D.M., Reader, J.P., Rogers, W.D. & Harlıoğlu, M.M. (1995). Interactions between three species of crayfish (*Austropotamobius pallipes*, *Astacus leptodactylus* and *Pacifastacus leniusculus*. Freshwater Crayfish 10: 46-56.

Kossakowski, J. (1965). Crayfish *Astacus astacus* (L.) and *Astacus leptodactylus* Esch. migrations in lake Loby, Poland. Ekologia Polska, Seria A, Tom XIII, Nr 26: 515-526.

Kossakowski, J. (1973). The freshwater crayfish in Poland. Freshwater Crayfish 1: 17-26.

Lowery, R.S. & Holdich, D.M. (1988). *Pacifastacus leniusculus* in North America and Europe, with details of the distribution of introduced and native crayfish species in Europe. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 283-308. Chapman & Hall, London.

Mahieu, J. & Paris, L. (1995). Ecrevisses et pesticides ne font pas bon menage. (with English summary). Bulletin Publie Par l'Association des Astaciculteurs de France 42: 1-10.

Novoselcev, G.E. (1995). Stocks and prospects of the artificial reproduction of the broad-legged crayfish in Karelian water reservation. Freshwater Crayfish 8: 140-144.

Spitzy, R. (1973). Crayfish in Austria, history and actual situation. Freshwater Crayfish 1: 9-14.

Stypinska, M. (1972). Variability of the fecundity of crayfish (*Astacus leptodactylus* Esch.) in Lake Dluzek depending on the body length and weight. Rocznic Naukowego Rolniczych, H-1: 98-103.

Stypinska, M. (1978). Individual variabilities in absolute fertility of crayfish occurring in the water of Mazurian lake district (in Polish). Rocznic Naukowego Rolniczych H 93-3: 177-203.

Stypinska, M. (1979). Comparison of the species fecundity of crayfishes inhabiting waters of the Mazurian Lakeland. Zoologica Poloniae 27: 279-289.

Troschel, H.J. & Dehus, P. (1993). Distribution of crayfish species in the Federal Republic of Germany, with special reference to *Austropotamobius pallipes*. Freshwater Crayfish 9: 390-398.

Farming

Arrignon, J. (1975). Crayfish farming in France. Freshwater Crayfish 2: 105-116.

Foex, Y. (1992). Quid de l'astaciculture en France? Point de vue d'un astaciculteur. (with English summary - Facts about crayfish farming in France? Viewpoint of a crayfish farmer). Bulletin Publie Par l'Association des Astaciculteurs de France 33: 8-11.

Holdich, D.M. & Rogers, W.D. (1992). Crayfish populations in the British Isles: Farming, legislation, conservation and management. Finnish Fisheries Research 14: 23-32.

Rogers, W.D. & Holdich, D.M. (1995). Crayfish production in Britain. Freshwater Crayfish 10: 583-595.

Fine structure

Adiyodi, R.G. (1985a). Fine structure of the sinus gland of the crayfish, *Pontastacus leptodactylus leptodactylus* (Eschscholtz). Journal of Animal Morphology & Physiology 32: 9-16.

Adiyodi, R.G. (1985b). Ultrastructure of the optic ganglionic-glia of the crayfish, *Pontastacus leptodactylus leptodactylus* (Eschscholtz). Journal of Animal Morphology & Physiology 32: 17-22.

Clarac, F. & Masson, C. (1969). Anatomie comparee des propriocepteurs de la region basi-ischiomeropodite chez certains crustaces decapodes. Zeitschrift fur Vergleichende Physiologie, Berlin 65: 242-273.

Kusakabe, T., Ishii, K. & Ishii, K. (1991). Dense granule containing cells in the wall of the branchio cardiac veins of a fresh-water crayfish (*Astacus leptodactylus*). Anatomy & Embryology 183(6): 553-557.

Payen, G. (1973). Etude descriptive des principales etapes de la morphogenese sexuelle chez un crustace Decapode a developpement condense, l'ecrevisse *Pontastacus leptodactylus leptodactylus* (Eschscholtz, 1823). Annales l'Embryologie et de Morphogenese, Paris 6: 179-206.

Food

Goddard, J.S. (1988). Food and feeding. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 145-166. Chapman & Hall, London.

Karafezlieva-Avramova, R. (1988). The influence of water temperature on the feeding intensity of *Astacus (Pt) leptodactylus* to the age of one year in artificial conditions. Freshwater Crayfish 7: 359-362.

Sevilla, C. (1988). Nutrition in intensive crayfish culture with a new dry pelleted diet. Freshwater Crayfish 7: 271-276.

Genetic

Agerberg, A. (1990). Genetic variation in three species of freshwater crayfish: *Astacus astacus* L., *Astacus leptodactylus* Esch., and *Pacifastacus leniusculus* (Dana), revealed by isozyme electrophoresis. Hereditas 113: 101-108.

Attard, J. & Pasteur, N. (1984). Variabilité et Différenciation Génétiques Chez Cing Espéces d'Ecrevisses Astacidae. Biochemical Systematics and Ecology 12(1): 109-117.

Immunology

Fenouil, E., Roch, P. & de Hureaux, D. (1995). Immune defense in *Astacidae* and *Cambaridae*: presence of lysozyme activity. Freshwater Crayfish 8: 614-622.

Introduction of *Astacus leptodactylus*

Holdich, D.M. (1988). The dangers of introducing alien animals with particular reference to crayfish. Freshwater Crayfish 7: xv-xxx.

Holdich, D.M. & Reeve, I. (1991a). Crayfish in British Waters. Natural Environment Research Council News April 1991: 4-7.

Holdich, D.M. & Reeve, I.D. (1991b). Distribution of freshwater crayfish in the British Isles, with particular reference to crayfish plague, alien introductions and pollution. Aquatic Conservation: Marine and Freshwater Ecosystems 1: 139-158.

Holdich, D.M. & Reeve, I.D. (1991c). Alien crayfish in British waters. Final report on GR3/6842 for Natural Environment Research Council. 38 pp.

Holdich, D.M. (1994). The native crayfish and threats to its existence. British Wildlife 2(3): 141-151.

Holdich, D.M. & Reeve, I.D. & Rogers, W.D. (1995). Introduction and spread of alien crayfish in British waters. Implications for native crayfish populations. Freshwater Crayfish 8: 99-112.

Huner, J.V. (1988). Status of crayfish transplantations. Freshwater Crayfish 7: 29-34.

Ingle, R.W. & Clarke, P.F. (1989). Turkish crayfish thrive in a London canal. The London Naturalist 68: 73-75.

Laurent, P.J. (1993). Les importations d'ecrevisses en France en 1992. (with English summary - Crayfish importations to France in 1992). Bulletin Publie Par l'Association des Astaciculteurs de France 35: 11-18.

Lorena, H.A.S. (1978). Present situation of exotic species of crayfish introduced into Spanish continental waters. Freshwater Crayfish 4: 175-184.

Life histories, growth, development and reproduction

Aiken, D.E. & Waddy, S.L. (1992). The growth process in crayfish. Reviews in Aquatic Science 6: 335-381.

Burba, A. (1988). Exploratory-searching behaviour of *Astacus leptodactylus* Esch. Freshwater Crayfish 7: 335-342.

Cherkashina, N. Ya. (1970). Reproduction of crayfish (Astacidae) along the southeastern coast of the Caspian Sea. Gidrobiology ZH. 6: 83-85.

Cherkashina, N. Ya. (1995). Obtaining and rearing of crayfish larvae *Astacus leptodactylus cubanicus* in an installation with controlled conditions. Freshwater Crayfish 8: 307-310.

Cukerzis, J. (1973). Biologische grundlagen der methode der kunstlichen aufzucht der brut des *Astacus astacus* L. Freshwater Crayfish 1: 187-201

Fedotov, V.P., Rakhmanow, V.R. & Kuznetsow, N.L. (1988). The crayfish stock and the state of crayfish breeding in the north-west of Russia. Freshwater Crayfish 9: 170-173.

Knoepffler, L.Ph. (1979). Essai d'èlevage de l'ècrevisse *Pontastacus leptodactylus leptodactylus* a l'èchelle industrielle. Freshwater Crayfish 4: 299-304.

Kossmann, H. (1973). Haltungs-und vermehrungsversuche von susswasser-krebsen im Haus. Freshwater Crayfish 1: 221-232.

Lindqvist, O.V. (1988). Restoration of native European crayfish stocks. Freshwater Crayfish 7: 6-12.

Lowery, R.S. (1988). Growth, moulting and reproduction. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 83-113. Chapman & Hall, London.

Malaczynska-Suchcitz, Z. (1956). Observations on the manner in which newly hatched crayfish (*Potamobius astacus* Leach and *Potamobius leptodactylus* Eschz.) are attached to the pleopods of the female. Bulletin de la Societe Des Amis Des Sciences et des Lettres de Poznan Serie B, Livrasion xiii: 341-351.

Marlet, B. & Roqueplo, C. (1991). Premier belan des deux station experimentales de production semi-intensive d'ecrevisses *Astacus leptodactylus* en Charente. Compte rendu no 79, 17 pages, prix 40 F (en timbres). CEMA-GREF, Groupement de Bordeaux; B.P. 3, F-33 611 Gazinent cedex, France.

Nefedow, V.N. & Mazanow, N.N. (1973). Determination of the weight of *Astacus leptodactylus* Esch. from its body length. Gidrobiology ZH. 9: 90-93.

Papadopol, M. (1975). Contribution to the study of reproductive potentiality of some populations of *Astacus leptodactylus* from the Danube delta. Travaux du Museum d'Histoire Naturelle "Gr. Antipa," Bucuresti 16: 89-99.

Rogers, W.D. & Holdich, D.M. (1995). Crayfish production in Britain. Freshwater Crayfish 10: 583-595.

Rumyantsev, V.D. (1973). Growth increment per moult of *Astacus leptodactylus* in the upper delta of the Volga. Gidrobiology ZH 9(4): 65-66.

Tamkeviciene, E.A. (1988). Growth and development of juveniles of the native and introduced species of freshwater crayfish. Freshwater Crayfish 7: 396-400.

Tcherkashina, N.Ya. (1977). Survival, growth, and feeding dynamics of juvenile crayfish (*Astacus leptodactylus cubanicus*) in ponds and the River Don. Freshwater Crayfish 3: 95-100.

van Herp, F. & Bellon-Humbert, C. (1978). Setal development and molt prediction in larvae and adults of crayfish *Astacus leptodactylus*. Aquaculture 14: 289-301.

Vigneux, E. (1979). *Pacifastacus leniusculus* et *Astacus leptodactylus*, premier bilan d'exploitation en ètang. Freshwater Crayfish 4: 227-234.

Management

Huner, J.V., Holdich, D.M. & Westman, K. (1992). Crayfish management strategies and management methods in Europe: a summary. Finnish Fisheries Research 14: 157-159.

Laurent, P.J. (1995). Eradication of unwanted crayfish species for astacological management purposes. Freshwater Crayfish 8: 121-133.

Westman, K. (1992a). Present status of crayfish management in Europe. Finnish Fisheries Research 14: 1-22.

Westman, K. (1992b). Management of the noble crayfish *Astacus astacus* (L.) and the signal crayfish *Pacifastacus leniusculus* (Dana) in Finland. Finnish Fisheries Research 14: 1-22.

Physiology

Angersbach, D. & Decker, H. (1978). Oxygen transport in crayfish blood: effect of thermal acclimation, and short-term fluctiations related to ventilation and cardiac performance. Journal of Comparative Physiology 123: 105-112.

Audehm, U., Trube, A. & Dircksen, H. (1993). Patterns and projections of Crustacean cardioactive peptide immunoreactive neurons of the terminal ganglion of crayfish. Cell & Tissue Research 272(3): 473-485.

Avenet, P. & Lignon, J.M. (1985). Ionic permeabilities of the gill lamina cuticle of the crayfish, *Astacus leptodactylus* (E). Journal of Physiology 363: 377-401.

Bergmiller, E. & Bielawski, J. (1970). Role of the gills in osmotic regulation in the crayfish *Astacus leptodactylus* Esch. Comparative Biochemistry & Physiology 37: 85-91.

Bielawski, J. (1964). Chloride transport and water intake into isolated gills of crayfish. Comparative Biochemistry & Physiology 13: 423-432.

Bielawski, J. (1971a). Energetics of ion transport in the gills of the crayfish *Astacus leptodactylus* Esch. Comparative Biochemistry & Physiology 39B : 649-657.

Bielawski, J. (1971b). Ultrastructure and ion transport in gill epithelium of the crayfish, *Astacus leptodactylus* Esch. Protoplasma 73: 177-190.

Burba, A. (1983). Chemical regulation in crayfish behaviour during postembryonic development. Freshwater Crayfish 5: 451-458.

Burtin, B., Massabuau, J.C. & Dejours,P. (1984). Effect of the concentration of NaCl in water on the sanguineous acid-base level in the crawfish *Astacus leptodactylus* hypercapnic activity.

Journal de Physiologie 79(5): 72A.

Dauscher, H. & Flindt, R. (1969). Vergleichende Untersuchungen zur Herztatigkeit bei frei beweglichen dekapoden Krebsen (*Astacus fluviatilis* Fab., *Astacus leptodactylus* Eschscholz und *Cambarus affinis* Say). Zeitschrift fur Vergleichende Physiologie, Berlin 62: 291-300.

Dejours, P. (1983). The hemolymph acid base balance of the crayfish *Astacus leptodactylus* as a function of the ambient water acid base balance and its chloride concentration. Journal of Physiology, London 345: 31.

Durliat, M. & Vranckx, R. (1976). Coagulation in the crayfish, *Astacus leptodactylus*: Attempts to identify a fibrinogen-like factor in the hemolymph. Biological Bulletin (Woods Hole) 151: 467-477.

Ehrenfeld, J. (1974). Aspects of ionic transport mechanism in crayfish *Astacus leptodactylus*.Comparative Biochemistry & Physiology 61: 57-70.

Fedotov, V.P. (1984). Study of impulse responses in higher order interneurons of the crayfish *Astacus leptodactylus*. Journal of Evolutionary Biochemistry & Physiology 20(4): 243 -249.

Flindt, R. & Kahrmann, E.G. (1972). Oxygen-induced variations of heart rates in crayfish. Physiological Biology 45: 119-124.

Fingerman, M. (1995). Endocrine Mechanisms in crayfish, with emphasis on reproduction and neurotransmitter regulation of hormone release. American Zoologist 35: 68-78.

Fischer, L. & Florey E. (1981). Temperature effects on neuro muscular transmission (opener muscle of crayfish, *Astacus leptodactylus).* Journal of Experimental Biology 94: 251-268.

Fischer, L. & Florey, E. (1983). Modulation of synaptic transmission and excitation contraction coupling in the opener muscle of the crayfish, *Astacus leptodactylus*, by 5-hydroxytryptamine and octopamine. Journal of Experimental Biology 102: 187-198.

Firkins, I., & Holdich, D.M. (1993). Thermal studies with three species of freshwater crayfish. Freshwater Crayfish 9: 241-246.

Gorgelskallen, J.L. (1985). Appearance and innervation off CHH producing cells in the eyestalk of the crayfish *Astacus leptodactylus* examinetid after tracing with lucifer yellow. Cell & Tissue Research 240(2): 385-391.

Gunzel, D., Galler, S. & Rathmayer, W. (1993). Fiber heterogeneity in the closer and opener muscles of crayfish walking legs. Journal of Experimental Biology 175: 267-281.

Harri, M. & Florey, E. (1979). The effects of acclimation temperature on a neuromuscular system of the crayfish, *Astacus leptodactylus*. Journal of Experimental Biology 78:281-293.

Hirsch, G.C. & Jacobs, W. (1928). Der arbeitsryhthmus der mitteldarmdruse von *Astacus leptodactylus*. Zeitschrift fur Wissenschaftliche Biologie C. 8: 102-144.

Kunast, V.C. & Renner, M. (1981). Die pharmakologische Beeinflußbarkeit des tagesrhythmischen Aktivitatsverhaltens des Flußkrebses *Astacus leptodactylus*. Zeitschrift fur Angewandte Zoologie 68: 95-108.

Ishii, K., Massabuau, J.C. & Dejours, P. (1989). Oxygen sensitive chemoreceptors in the branchio cardiac veins of the crayfish, *Astacus leptodactylus*. Respiration Physiology 78(1): 73-81.

Lignon, J. & Lenoir, P. (1985). Ionic permeabilities of the gill filament cuticle of the crayfish *Astacus leptodactylus* (E). Comptes Rendus De L Academie Des Sciences Serie III. Sciences De La Vie 301(9): 443-446.

Lignon, J.M. & Pequeux, A. (1988). Transbranchial and transcuticular potentials in the isolated gill flaments of the crayfish, *Astacus leptodactylus* (E). Comparative Biochemistry & Physiology A 90(4): 820.

Malaczynska-Suchcitz, Z. & Obuchowicz, L. (1964). Oxygen consumption during embryonic development of the crayfish *Astacus leptodactylus* Eschz. Bulletin de la Societe des Amis des Sciences et des Lettres de Poznan. Serie D IV: 111-113.

Massabuau, J.C. & Burtin, B. (1984). Regulation of oxygen consumption in the crayfish *Astacus leptodactylus* at different levels of oxygenation: role of peripheral O_2 chemoreception. Journal of Comparative Physiology B 155: 43-49.

Massabuau, J.C. & Burtin, B. (1985). Ventilatory CO_2 reflex response in hypoxic crayfish *Astacus leptodactylus* acclimated to 20 °C. Journal of Comparative Physiology B 156: 115-118.

Muller, U. & Cruse, H. (1991). The contralateral coordination of walking legs in the crayfish *Astacus leptodactylus*. 2.Model calculations. Biological Cybernetics 64(5): 437-446.

Muller, U. & Cruse, H. (1991).The contralateral coordination of walking legs in the crayfish *Astacus leptodactylus*. 1. Experimental results. Biological Cybernetics 64(5): 429-436.

Mullerwilm, U. (1993). A neuron like network with the ability to learn coordinated movement patterns. Biological Cybernetics 68(6): 519-526.

Naynert, V.M. (1975). Studies on the driving system of scaphognathite movements in the decapod crustacea, *Potamobius astacus* Leach and *Astacus leptodactylus* Esch. Zoologische Jahrbucher 79: 77-104.

Popov, I.V. & Atmetchenko, O.A. (1977). Pulse activity in the crayfish abdominal nervous chain. Freshwater Crayfish 3: 275-279.

Puche, J., Barreracalva, E. & Barreramera, B. (1993). Protocerebral deafferentation effects on crayfish glycemic response. A protocerebral circadian pacemaker regulates the hemolymph sugar concentration. Revista Espanola De Fisiologia 49(3): 151-155.

Söderhäll, K., Johansson, M.W. & Smith, V.J. (1988). Internal defence mechanisms. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 213-235. Chapman & Hall, London.

Stepushkina, T.A., Kan, G.S. & Belyanin, O.L. (1977). Role of various structures of the central nervous system of the crayfish *Astacus leptodactylus* in food getting behavior.Journal of Evolutionary Biochemistry & Physiology 13 : 354-356.

Titova, V.A. (1985). Neuropil topograpfy in the cerabral ganglion of the crayfish *Astacus leptodactylus*. Journal of Evolutionary Biochemistry & Physiology 21(4): 256-263.

Tsvileneva, V.A. & Titova, V.A. (1987). Architectonics of neural elements in the supraesophageal ganglion of the crustacean *Astacus leptodactylus*. Journal of Evolutionary Biochemistry & Physiology 23(6): 553-559.

Tsvileneva, V.A. & Titova, V.A. (1987). Descending interneurons in the suprapharyngeal ganglion of the crayfish *Astacus leptodactylus*. Journal of Evolutionary Biochemistery & Physiology 23(4): 379-384.

Van Herp, F., Bellon-Humbert, C. & De Groot, A.J.M. (1979). Presence and development of a sensory formation in the eyestalk of the crayfish, *Astacus leptodactylus* (Nordmann, 1842), during its embryonic, larval and adult life. Journal of Morphology 161: 39-52.

Vanpuymbroeck, S. & Vanderborght, O.L.J. (1986). Effect of Al and humic acids on the AM-241 adsorption on the exoskeleton of the crayfish *Astacus leptodactylus* Eschscholtz. Health Physics 50(2): 277-280.

Taxonomy

Albrecht, H. (1982). Das system der europaischen Flußkrebse (Decapoda, Astacidae): Vorschlag und Begründung. Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut 79: 187-210.

Albrecht, H. (1983). Die Protastacidae n. fam., fossile Vorfahren der Flußkrebse? Neues Jahrbuch fuer Geologie und Paleontologie, Monatshefte H.1: 5-15.

Brodski, S.Ya., (1983). On the systematics of palaearctic crayfishes (Crustacea, Astacidae). Freshwater Crayfish 5: 464-470.

Bott, R. (1950). Die Flußkrebse Europas (Decapoda, Astacidae). Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft 483(1-36): 1-36.

Bott, R. (1972). Besiedlungsgeschichte und systematic der astaciden West-Europas unter besonderer Berucksichtigung der Schweiz. Revue Suisse de Zoologie 13: 387-408.

Curra, R.A. (1967). A key to genera, species and subspecies of Astacinae (Nephropsidea: Astacidae). Internationale Revue der Gesamten Hydrobiologie 52(5): 793-800.

Gledhill, T., Sutcliffe, D.W. & Williams, W.D. (1993). British freshwater Crustacea Malacostraca: a key with ecological notes. 173 pp. Freshwater Biological Association. The Ferry House Far Sawrey, Ambleside, Cumbria LA22 OLP.

Hobbs, H.H.Jr. (1988). Crayfish distribution, adaptiveradiation and evolution. In: Freshwater crayfish: biology, management and exploitation (Holdich D.M. & Lowery R.S., eds), pp. 52-82. Chapman & Hall, London.

Karaman, M.S. (1962). Ein beitrag zur systematik der Astacidae (Decapoda). Crustaceana 3: 173-191.

Laurent, P.J. & Forest, J. (1979). Données sur les écrevisses qu'on peut rencontrer en France. La Pisciculture Française 56 (15-2): 25-40.

Scholtz, G. & Richter, S. (1995). Phylogenetic systematics of the reptantian Decapoda (Crustacea, Malacostraca). Zoological Journal of the Linnean Society 113: 289-328.

Vigneux, E. Keith, P. & Noel, P. (1993). Atlas preliminaire des Crustaces Decapodes d'eau douce de France. Coll. Patrimoines Naturels. Vol: 14, S.F.F., B.I.M.M.- M.N.H.N., C.S.P., Ministere de l'Environnement, Paris, 55 p.