DEFENCE AGAINST PREDATORS BY JUVENILE SIGNAL CRAYFISH (*PACIFASTACUS LENIUSCULUS*, DANA).

A thesis submitted for the degree of
Doctor of Philosophy.

by

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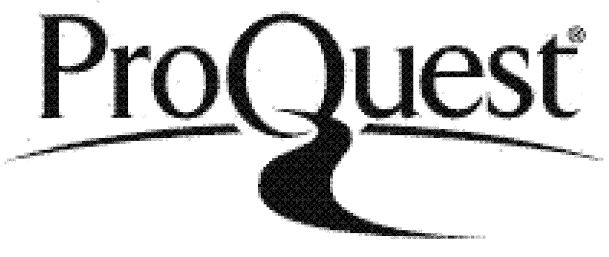

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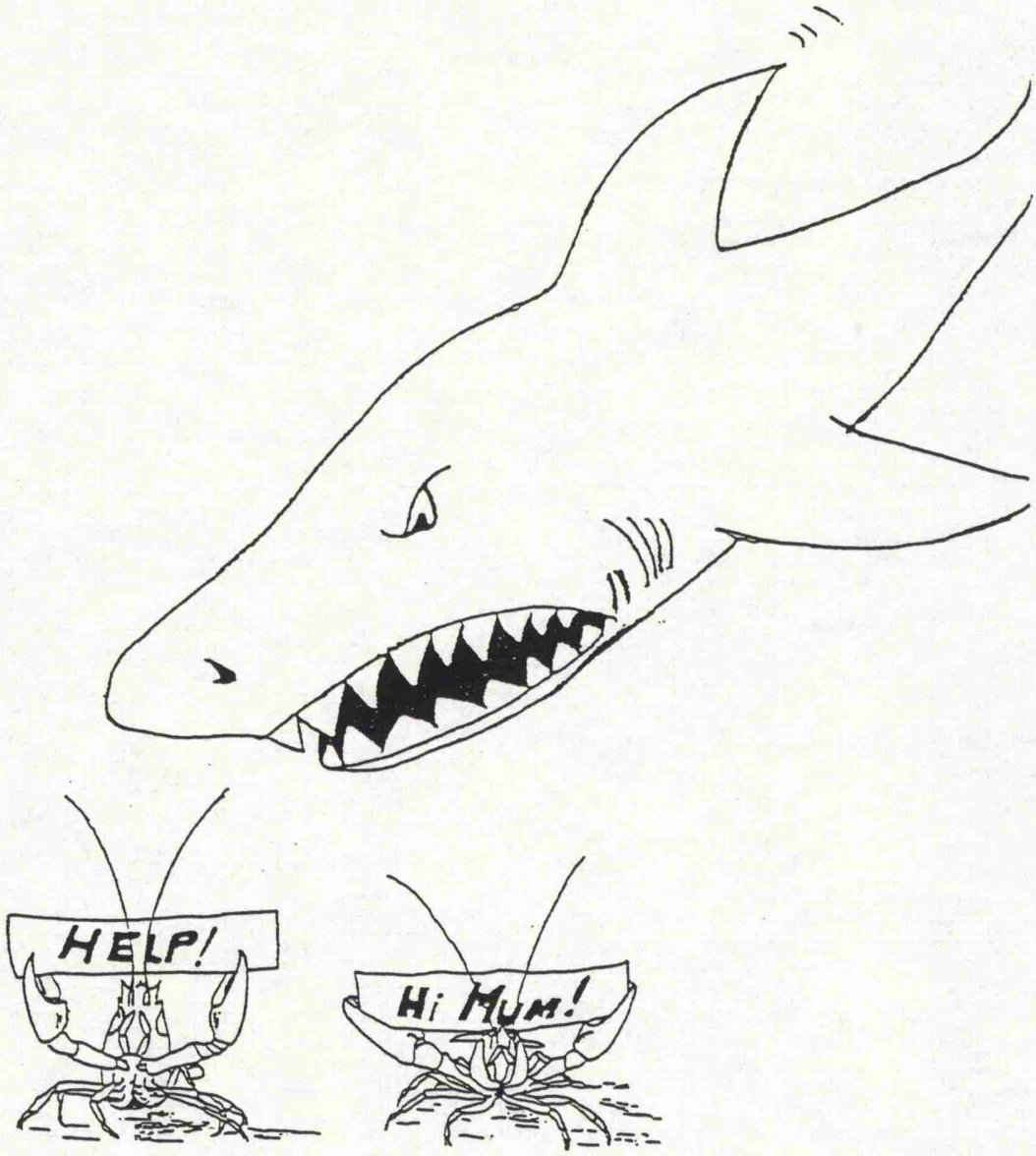
ABSTRACT

This study investigated the defensive responses of juvenile signal crayfish (*Pacifastacus leniusculus*, Dana) to two putative predators, perch (*Perca fluviatilis*, L.) and eel (*Anguilla anguilla*, L.), and attempted to determine the importance of direct and indirect predatory effects on the distribution of newly independent juvenile crayfish in a Swedish pond.

Eels are thought to be more detrimental to crayfish populations than perch. Experiments using juvenile crayfish did not support this assertion. Visual and chemical stimuli elicited crayfish avoidance behaviour. This was most marked when both stimuli were presented together. Both predators elicited similar avoidance behaviour. Crayfish were less active by day, spending more time under shelter. Shelter provided by vegetation and substrata reduced crayfish mortality. Crayfish also avoided small non-predatory fish (*Leucaspius delineatus*, Heckel). It is suggested that these fish indirectly increased crayfish mortality. Adult crayfish increased juvenile crayfish mortality but caused juveniles to be more active by day than at night. These responses illustrate the conflicting demands on crayfish defensive behaviour in multi-predator environments.

Mechanical and visual stimuli elicited evasive behaviour. Crayfish evaded predatory strikes by perch and eels. The response to eels was delayed. Perch chased fleeing crayfish, and caught more crayfish than eels, which never chased prey. Initially, perch preyed on juvenile crayfish more rapidly than eels. Despite having distinct foraging behaviours, perch and eels produced similar crayfish mortalities. If eels are more detrimental than perch to crayfish populations, this may be a result of differences in size selective predation. The initial distribution of newly independent crayfish in a Swedish pond was influenced by the distribution of gravid female crayfish. Perch preyed on juvenile crayfish but were not a major factor determining crayfish distribution. Intraspecific competition and invertebrate predation may have had a greater effect. Crayfish populations may be influenced by perch predation on yearling crayfish.



Individual variability in the assessment of predation risk by juvenile crayfish.

To Mum and Dad,
for being there, whatever the crisis.

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INTRODUCTION

CHAPTER 1.

1.0 INTRODUCTION

The following work describes a study of the impact of fish predation on newly independent juvenile signal crayfish, *Pacifastacus leniusculus* (Dana), and of the mechanisms of defence used by juvenile signal crayfish to avoid predation. Juvenile crayfish were used in this study for two reasons. Firstly, they are the most vulnerable to predation (Momot et al., 1978) and as a result, they should possess the most marked defences against predation. Stein & Magnuson (1976) and Stein (1977) showed that avoidance behaviour was more marked in smaller, more vulnerable age classes of crayfish. Secondly, juvenile crayfish were a managable size and could be used in laboratory interactions with relatively small predators. The study is comprised of four parts: the Introduction, Part I, Part II and the Final Discussion.

Part I investigates 1) the defensive behaviour of juvenile signal crayfish in response to two predators with different foraging strategies, and 2) the mechanisms underlying the detection of different predators. Whilst anti-predator behaviour has been reported extensively in single predator-single prey systems, relatively little work has been done on prey behaviour in response to more than one predator.

Predators can limit prey populations directly through predation, or indirectly by influencing habitat use and growth of prey (Stein, 1979; Sih, 1987). The work in Part II of this thesis was conducted at Simontorp Aquaculture A.B., Sweden (Fig.1.1), and used experimental investigations and field studies to address two questions: 1) how does predation influence crayfish habitat use and thereby growth rates? 2) how significant is predation as a source of crayfish mortality in nature? The field studies were conducted in Rögle pond 3, which contained an exploited population of crayfish, perch and pike, but no eels.

From the literature, perch (*Perca fluviatilis* L.) and eels (*Anguilla anguilla* L.) were indicated to be two of the principal predators of crayfish in Europe (Svärdson, 1972; Kossakowski, 1973; Dehli, 1981; Appelberg, 1987). The scope of Part I of the project was limited to studying the interactions of these two predators with crayfish. Eels are considered to have a greater impact on crayfish populations than perch (Svärdson, 1972; Svärdson et al., 1991), although the evidence is circumstantial. This provided the theoretical basis for Part I of this study. If eels are more successful predators of crayfish than perch, then it should be possible to predict differences in the foraging activity of the predators that might cause this. It was the aim of this part of the study to test the following predictions:

1) Eels might reach a size class that allows them to prey on size classes of crayfish that are larger than those available to perch.

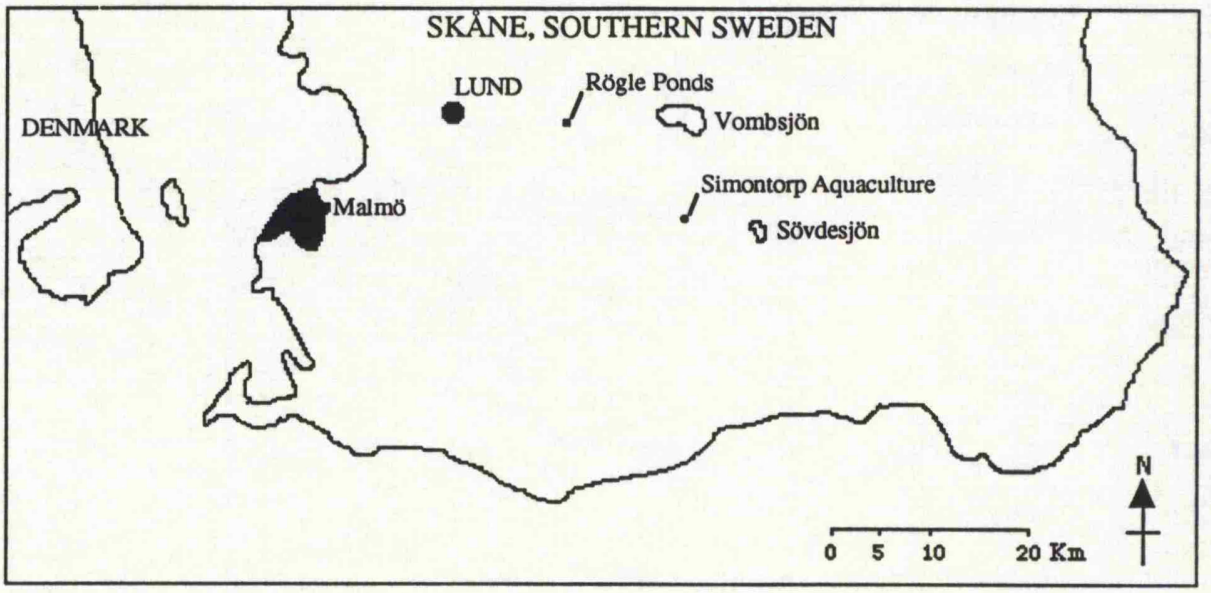


Figure 1.1. A map of Skåne, southern Sweden, showing Simontorp Aquaculture A.B., where experimental work was carried out, and Rögle ponds, where field experiments were conducted. Perch used in the experimental work were caught from two lakes, Vombsjön and Sövdesjön.

2) Eels may prey on larger size classes of crayfish because, unlike perch, eels are not restricted to choosing prey smaller than their gape size.
3) Eels may have a preference for crayfish over other prey.
4) Eels forage principally by chemoreception and this may enable eels to detect prey more easily than perch if the prey are hidden under shelter.
5) Eels may burrow into some substrata and this may enable eels to catch hidden prey more easily.
6) Eels move slowly whilst foraging, thus they may be better able to approach crayfish without detection or without eliciting a response.

During the early stages of this part of the study, no suitable field sites were located in which eel and perch predation of crayfish could be monitored simultaneously. Also, eels behaved inconsistently in the laboratory. Thus, it was not possible to collect data on the size selectivity of eels for crayfish prey or on the seasonality of eel predation on crayfish. For this reason, the emphasis of this part of the project was shifted towards understanding the mechanisms controlling defence against predators by crayfish. From this knowledge, predictions could be made about the type of predator to which crayfish are most vulnerable (Webb, 1986).

Part I of this thesis consists of Chapters 2 to 4. In Chapter 2, investigations were made into the effect of perch and eels on juvenile crayfish survival and activity, the effect of alternative prey on predation of juvenile crayfish by eels, and the effect of habitat complexity on juvenile crayfish survival and activity in response to the presence of perch and eels. Chapter 3 describes investigations into the stimuli deriving from perch and eels that cause avoidance behaviour in juvenile crayfish. Chapter 4 describes experiments designed to determine the predatory stimuli that cause evasive behaviour in juvenile crayfish, and relates this to predator-prey encounters between crayfish and perch or eels. Thus predictions 3 to 6 are addressed in Part I.

Part II of the thesis consists of Chapters 5 and 6. In chapter 5, juvenile crayfish habitat selection behaviour was examined in response to different water depths and substrata. The habitat preferences were related to juvenile crayfish survival and distribution in response to perch predation in a Swedish pond. The role of gravid females (egg-bearing females) in determining young-of-the-year (YOY) crayfish distribution and survival was also considered. Chapter 6 describes the effect of aquatic vegetation on juvenile crayfish habitat selection, and relates this to the distribution, survival and growth of juvenile crayfish in response to fish and adult crayfish.

The results of Parts I and II are discussed together in Chapter 7, which places the anti-predator behaviour of juvenile *P. leniusculus* in the context of the ecological factors which control juvenile crayfish distribution and survival. The following sections of the introduction give an overview of predation on crayfish populations and of the anti-predator defences used by crayfish.

1.1 PREDATION ON CRAYFISH

Throughout their life span, crayfish are subject to predation by many predators (Hogger, 1988 for review). After their first summers growth, crayfish are no longer available as prey to invertebrate predators or to many species of predacious fish, one of their main defences against predation being a rapid growth rate in their early years (Momot et al., 1978). After this time, of the freshwater fish, only pike (*Esox lucius* L.), perch (*P. fluviatilis*) and eel (*A. anguilla*) are putative crayfish predators in Europe (Svärdson, 1972; Dehli, 1981; McFadden & Fairley, 1984a & b). Other major predators are mink, *Mustela vison* Schreber (Burgess & Bider, 1980; Ward et al., 1986), otters, *Lutra lutra* L. (Mcfadden & Fairley, 1984a), and herons, *Ardea cinerea* L. (Hogger, 1988 for review).

In introduced populations and in natural populations, *P. leniusculus* survival during the two years between independence from the female to maturity is between 10 to 25% (Shimizu & Goldman, 1983; Fürst, 1977 cited by Fjälling & Fürst, 1988). The effects of predation during these two years are uncertain. Momot (1967) showed that in a population of crayfish (*Orconectes virilis* Hagen), first year juvenile mortalities due to brook trout (*Salvelinus fontinalis* Mitchill) predation accounted for only 3% of the total mortality during June to January and 16% during January to May. Trout over 229 mm total length took only first year juveniles. Extrapolations of the results of enclosed predation experiments to the wild indicate that predation rates by aeschnid nymphs could account for up to 75 to 100% of juvenile crayfish (*O. virilis*) mortality in the first weeks of independence (Dye & Jones, 1975). Witzig et al. (1986) indicated that dragonfly nymphs (*Anax junius*) may not be of the right size at the right time to coincide with the hatch of *Procambarus clarkii* (Girard), juveniles in extensively managed populations. Gydemo et al. (1990) showed that dragonfly larvae (*Aeschna grandis*) predation on newly hatched crayfish (*Astacus astacus* L.) was severe, independent of the hatching time.

Despite the apparently high proportion of juvenile crayfish lost as a result of predation by fish and dragonfly nymphs, it was concluded by Momot & Gowing (1977) that these predators "eat crayfish that would die anyway", and that at normal densities, these predators do not control crayfish population size or productivity. In pond experiments, sixty days after hatching, the abundance of young-of-the-year (YOY) *P. leniusculus* did not differ between ponds with and without perch (Appelberg & Odelström, 1988), despite perch being known predators of juvenile crayfish (Jacobsen, 1977; Dehli, 1981).

Momot et al. (1978) state that for a predator to have a negative effect on crayfish population size, predation will be concentrated on the larger size classes and especially on females contributing to the brood stock. Predation by perch was suggested as an important factor preventing the noble crayfish, *A. astacus* population recovering in

Swedish lakes that had been limed to neutralise the effects of acidification (Appelberg, 1987; 1990). The abundance of juvenile crayfish was limited in these lakes, but it was not known whether poor YOY densities were a result of predatory mortality or of negative effects on YOY activity and growth. Perch and roach (*Rutilus rutilus* L.) have been shown to reduce YOY *A. astacus* survival in pond experiments (Svensson, 1992). Also, predation by largemouth bass (*Micropterus salmoides* Lacépède) has been shown to limit crayfish populations in N. America, although the availability of cover and vegetation were also important (Taub, 1972; Rickett, 1974; Saiki & Tash, 1979).

Mean crayfish size may be regulated through predation by trout, pike, perch and eel in the Clare River system in Counties Galway and Mayo (McFadden & Fairley, 1984a). Otter spraints from different parts of the system corresponding to areas dominated by trout, pike and perch, and eel populations contained the remains of crayfish of different mean size, suggesting that otters were preying on different sizes of crayfish in the different areas. It was assumed that the mean size of crayfish in the spraints was an indication of the mean size of crayfish available to otters in the different habitats. The explanation put forward is that fish exert size selective predation on adult crayfish populations giving rise to increased mean crayfish size in certain areas. The mean size of consumed crayfish in the spraints rose from the trout stream habitat to the pike/perch habitats to the principally eel habitat. This would indicate that, whilst pike and perch may take small adult crayfish, eels are able to take larger sizes. The proportion of spraints containing crayfish fell significantly where eels were abundant indicating that crayfish populations were reduced, possibly as a result of eel predation, although this site was at the mouth of the river system where environmental conditions might conceivably produce similar results.

1.2 PREDATION ON CRAYFISH BY EELS

Of the predatory fish species identified as important predators of crayfish populations, eels are suggested to be the most destructive (Svärdson, 1972; Fürst, 1977; Svärdson et al., 1991). The evidence for this has been largely circumstantial. That of Mcfadden & Fairley (1984a & b) has already been discussed.

Further evidence comes from Sweden where Svärdson (1972) analysed lake surveys and fishermen's records for 1,671 lakes with regard to population trends in eels, the native crayfish *A. astacus* and the introduced signal crayfish *P. leniusculus*. He found that there was an historical allopatry for eels and *A. astacus*. Eels were found in the western and crayfish in the eastern parts of southern Sweden. Both crayfish and eels inhabit similar lake types, and when such lakes were analysed for sympatry, this was found to be less frequent than would occur by chance. High yields of either eel or crayfish could only be obtained in cases of allopatry, whereas moderate yields of both could be achieved in cases of sympatry.

It was also suggested that eels were the most important biological limiting factor of *P. lenitusculus* population growth in 44 unsuccessful stocking attempts in Swedish lakes (Fürst, 1977). Data from a study where 1000 juvenile crayfish were released into a 4000 m^2 pond containing dense aquatic vegetation and abundant predators, including perch and eel, suggests that other factors may mitigate the impact of predation on crayfish populations. The pond was drained after one year and 33% of the crayfish were recovered, although survival was probably higher (Brink, 1977). This survival rate is comparable to others where predation is not thought to be a significant problem (Hogger, 1986). Also, there is evidence that crayfish population size increased in an area of Lake Hjälmaren where the eel population also increased (Svärdson et al., 1991).

Predation by eels on crayfish has been demonstrated in several studies. Facey & LaBar (1981) found crayfish (*Orconectes* spp) in 26% of American eel (*Anguilla rostrata* LeSueur) stomachs analysed, compared to 26% for fish and 43% for insects. Insects were more important in terms of volume in smaller eels and fish were more important in larger eels. Crayfish were eaten in equal numbers by all sizes of eel. No information is given on the size classes of crayfish that were eaten, however, there was a significant relationship between the size of the predominant food and eel size. Crayfish (*Austropotamobius pallipes* Lereboullet) were also found in 26% of eel stomachs in a study on English rivers (Hartley, 1948). In general, however, the importance of predatory mortality due to eels compared to other causes of mortality has not been studied experimentally.

1.3 EEL FORAGING BEHAVIOUR

If eels are of major importance as crayfish predators, certain questions arise concerning the nature of this predation. Why are crayfish more vulnerable to eel predation? At what point in the life cycle of crayfish do eels exert their major predatory effect ? Is it directed at the recruitment of juveniles into the brood stock or at the brood stock itself? As mentioned above (Section 1.0), if eels are the most destructive predators of crayfish populations, then testable predictions can be made about the possible advantages that eels have over other predators. These predictions are expanded below.

(1) Size.

If eels grow to sizes that allow them to feed on crayfish of greater sizes than other predators such as perch, they would be able to prey on a greater proportion of the brood stock. The relative jaw morphologies and body sizes of the predators would then be of major importance. Perch are restricted in the size of crayfish they may eat. There is a correlation between size of predator and of prey, but usually perch feed on crayfish less than 70 mm in length, often only taking 70 mm crayfish during their moult when

the carapace is soft (Dehli, 1981). Pike are probably restricted less, as they can take prey fish of up to half of their own body weight (Moriarty, 1978), and can grow to sizes in excess of 50 cm long, plus pike have broad jaws with a large gape (Wheeler, 1978).

Eels typically have two phenotypes with respect to jaw morphology (Deedler, 1970; Tesch, 1977). Thin and broad headed eels from the same habitat have been shown to have different diets, the former principally feeding on small invertebrates and the latter on fish. This was shown in cases where broad headed eels were of greater length than thin headed (Tesch, 1977) and when body lengths were the same for the two types (Lammens & Visser, 1989). The latter study also indicated that the mouth width of individual eels could change in relation to the types of prey available. When small prey items became scarce there was an increase in average jaw width of eels and a corresponding increase in the number of fish eaten. The change in jaw size was thought to occur in individual eels, between seasons, as food availability changed. It therefore seems, that larger eels (with lengths of 40 cm upwards), which are more often broad headed, are better adapted to feeding on larger prey items for a given body length of eel.

(2) No gape limitations when foraging.

Eels can feed on prey larger than can be swallowed whole. This may allow eels to prey on a greater proportion of a crayfish population. Facey & LaBar (1981) found that when all eel size classes were considered, the relative sizes of prey and predator were related. Beumer (1979) did not find this to be so, indicating that eels have the ability to feed on prey items with sizes unrelated to gape. Three methods of feeding in anguillid eels were identified by Helfman and Clark (1986) which allow predation on a greater diversity of food items. These were (a) inertial sucking, (b) breaking soft-bodied items by pulling and shaking, and (c) dismembering firm material by grasping and spinning. Eels have been observed to attack and shake crayfish, causing chelae to be lost before ingestion (Behrendt, 1987) and rotational feeding was observed in a 25 to 30 cm eel when feeding on a recently moulted dead crayfish of over 10 cm total length (pers. obs.). This has also been reported in eels feeding on mitten crabs (*Eriocheir sinensis* Milne-Edwards) on the River Elbe. Crab legs with gill lamellae attached were found in eel stomachs, indicating attacks with rotational feeding. Only eels greater than 40 cm in length appeared to prey on these crabs (Ladiges, 1936 cited by Tesch, 1977).

(3) A preference for crayfish over other prey types.

Eels are extremely varied in their choice of diets (Deedler, 1970; Sinha & Jones, 1975). There appears to be an important change from feeding on small invertebrates to larger prey items such as fish, molluscs and Crustacea when eels reach approximately 40 cm in length (Tesch, 1977). The diet of smaller eels varies between different habitats (Sinha & Jones, 1975; Tesch, 1977; Moriarty, 1978). This may be a result of prey abundance. Eel diets often change depending on prey availability and competition between other fish predators (Lammens et al., 1985), although there is also a suggestion of some selectivity (Tesch, 1977). The choice of larger prey items available to eels will be

limited in most habitats. Data on the diet of South African eels showed that 10 to 20 cm long individuals ate nothing but insect larvae (Jubb, 1961 cited by Tesch, 1977). Eels over 20 cm included fish and Crustacea in their diet, especially freshwater crabs (Genus *Potamon*). The proportion of these increased in the diet of 50 to 60 cm eels with the crabs comprising the largest proportion of the diet of 60 to 70 cm eels. This indicates that crayfish may become a preferred prey item in larger eels, although, eel diets are often dictated by prey availability. Eels of all sizes have been known to feed extensively on dense patches of Cladocera (Schiemenz, 1910 cited by Deedler, 1970; Tesch, 1977).

(4) The ability to detect prey using chemoreception.

Eels forage principally by chemoreception (Deedler, 1970), but may also use vision, although the visual system in yellow eels is not well adapted for diurnal vision (Tesch, 1977 for review). Chemoreception would be advantageous in detecting hidden prey. Crayfish have been shown to reduce activity levels when in the presence of visual fish predators (Stein, 1977; Stein & Magnuson, 1976; Hamrin, 1987; Appelberg & Odelström, 1988). Therefore, the ability to find hidden crayfish may increase the impact of eels on crayfish populations. Predatory fish take larger crayfish when the latter are post-moult (Stein, 1977; Dehli, 1981). Chemoreception may enable predators to detect moulting or post-moult crayfish more easily, again this may be of great significance in terms of the number and size of crayfish available to eels.

(5) The ability to burrow into certain substrata to find crayfish.

This is related to chemoreceptive foraging. Foraging eels nose around stones often turning them over when searching for prey. It is also postulated by Moriarty (1978) that eels thrive because they are able to catch food organisms that other more active "round" fish cannot find.

(6) The effect of foraging behaviour.

If the stimuli causing evasive behaviour in crayfish are related to the size and speed of the approaching predator, as shown for fish (Dill, 1974a; Webb, 1982), then crayfish may react less violently to approaching eels by comparison to perch, as eels are smaller in cross-section. Also the approach and strike behaviours of the two predators may differ. Eels in the Severn estuary between 19 and 60 cm long were found to feed principally on the decapod *Crangon vulgaris* (Fabricius) and the mysid *Neomysis integer* (Leach) during spring and summer, whereas flounders *(Platichthys flesus* L.) did not. The inability of flounders to feed on *C. vulgaris* was due to a dash characteristic in their attack invoking a quick evasive reaction. Observations on eels showed that their approach was usually slow and that this rarely caused an escape response, so facilitating capture (Moore & Moore, 1976a & b). Eels also tend to be nocturnal. If crayfish evasive behaviour is less efficient without visual stimuli, then eels may capture prey more easily than perch, which are crepuscular.

1.4 FORAGING BEHAVIOUR IN RESPONSE TO VISUAL AND CHEMICAL STIMULI

A fundamental factor determining the relative risk of crayfish to perch and eel predation, is how the criteria for prey selection differ between visual and chemoreceptive foragers. Generally, rates of predation are dependent upon the vulnerability of the prey. Prey density, size and predator hunger all result in an increase in feeding rate of rainbow trout *Onchorhynchus mykiss* Walbaum (Ware, 1972). In this study, it was concluded that the diets of visually foraging fish were more closely related to the physical and behavioural properties of prey than to prey densities or biomass. Visual foraging is influenced by the complexity of the substratum (Ware, loc. cit.). Other physical properties such as water temperature and turbidity have also been shown to alter predator selectivity (Moore & Moore, 1976b; Crowl, 1989). Ware (1973) concluded that in benthic food chains, the main determinants of the risk of prey to predation by a visual predator were prey activity, exposure, density and size.

Brewer & Warburton (1992) found that the main criteria limiting the availability of benthic prey to a chemoreceptive forager, the golden lined whiting (*Sillago analis* Whiteley), in a complex habitat, were prey accessibility, mobility, morphology and energy content. Prey size did not influence predator selectivity.

Invertebrate predators differ in their ability to prey upon invertebrate prey species (Jeffries, 1988). A similar effect has also been shown between fish which feed visually and fish which use chemoreception (Moore & Moore, 1976a & b). Eels and flounder differed in their abilities to catch *C. vulgaris*. This was due to differences in the foraging behaviour of the two predators and the mobility of the prey. This study also concluded that eels would feed preferentially on benthos rather than fish when high concentrations of benthos were available, due to the relative immobility of the benthic prey.

Chemical stimuli are not specific to individual invertebrate prey species. As a result, bullheads *Ictalurus nebulosus* and *I. natalis* (LeSueur) in Lake Ontario, which fed using chemoreception, were mainly generalist and oppertunistic foragers (Keast, 1985). Certain morphological features were also found to differ between these bullheads and centrarchids from the same lake. Bullheads had smaller eyes and larger mouths relative to their body size, with the exception of the mouth to body size ratio of largemouth bass (*M. salmoides*). Smaller eyes limited visual acuity, but larger mouths gave bullheads an increased surface area with which to detect and acquire prey due to gustatory sensation. This also facilitated the capture of larger prey items. This was demonstrated in older *I. natalis* which selectively fed on fish and crayfish, a diet which also provided a higher average calorific value than diets comprising other benthos. These morphological differences also apply to a comparison of eels and perch. The diet shift of older *I. natalis* resembles that of older/larger eels (Tesch, 1977).

1.5 DEFENCE AGAINST PREDATION BY CRAYFISH

"Behavioural responses of a prey should be a specific, direct function of their vulnerability to a particular predator. And the sensory mechanisms for assessing the degree of potential danger should be well developed for prey which exhibit complex, reactive anti-predator patterns of behaviour" (Stein 1979).

Defence against predation can be viewed in terms of prey interrupting a sequence of six stages of predator behaviour associated with increasing predation risk: encounter, detection, identification, approach, subjugation, and consumption (Endler, 1991). If this behavioural sequence is interrupted early, then the risk of death to the prey and the energetic costs of the defence employed by the prey are reduced. Crayfish possess traits to counter predation at these various stages.

Encounter - crayfish behave so as to appear rare to predators. In response to diurnal fish predators, crayfish show microdistributional habitat shifts and an increase in nocturnal activity (Stein & Magnuson, 1976; Hamrin, 1987; Appelberg & Odelström, 1988). These responses limit crayfish exposure to visually foraging fish but may result in resource enhancement for fish that forage nocturnally. Crayfish also detect and react to the scent of predators and of disturbed prey (Hazlett, 1985; 1990; Appelberg, pers. comm.). This should enable crayfish to detect predators over greater distances than they can be detected by visual predators.

Detection - crayfish have cryptic colouration which matches the prevailing substratum (Köksal, 1988). Young *P. clarkii* can rapidly approximate to their background through physiological colour changes (Beingesser & Copp, 1985). Older *P. clarkii* undergo slower and more permanent colour changes.

Approach - after orientating towards an approaching predator, crayfish may react by rapid escape swimming towards cover or may engage in a defensive chelae display (meral spread), where the body is orientated towards the predator with the anterior of the carapace raised and the chelae spread above the carapace. The display position and the tendancy to display differ between species (Hayes, 1977; Reeve, pers. comm.). The colouration of the underside of the chelae of signal crayfish (*P. leniusculus*) is bright red, which is highly visible in freshwater and therefore startling to predators. The meral spread is easily induced in this species unlike *A. pallipes*, in which the underside of the chelae are less bright (Reeve, pers. comm.).

The tendancy to flee, and the mode of flight change with crayfish body size and the stage of development. Stein (1977) found that young *O. propinquus* swam further in escape and terminated escape swimming by hiding more often than did adults. Adults terminated escape more often by initiating a defensive display. The tendancy to flee rather than display changes with age. Early in the post-embryonic phase, crayfish

evasive behaviour is inflexible, but at a size of 3 to 4 cm the behaviour becomes more plastic (Toler & Fricke, 1985). Lang et al. (1977) showed that differential growth of neurones to the chelae and abdomen caused lobsters to display more and escape less with age.

Subjugation - crayfish possess morphological traits to prevent being successfully handled and consumed by predators. They possess a hard exoskeleton containing spiked protruberances on the carapace and limb segments, particularly the chelae. The limbs are also autotomous (Holdich & Reeve, 1988; Hirvonen, 1992). Although chelae are functional in predator defence, Stein (1976) suggested that large chelae were more likely to have evolved in response to the pressures of agonistic interactions and mating in *O. propinquus*, than in response to predation pressure.

In the following work, defensive behaviour was categorised into avoidance and evasive behaviour (Weihs & Webb, 1984). Avoidance behaviour is the movement of the prey in order to reduce the likelihood of detection and attack by a predator. Evasive behaviour is the movement of the prey in response to an attacking predator. Avoidance responses are generally flexible but evasive behaviour is likely to be more specialised and fixed (Sih, 1987; Endler, 1991). Crayfish avoidance behaviour has been described above. The following section describes evasive behaviour in more detail.

1.6 THE EVASIVE RESPONSE OF CRAYFISH

Evasive behaviour consists of two distinct reactions. Firstly, the startle response and secondly, the escape response (Bennet, 1984). The startle response is highly stereotyped, but both this and the escape response may vary, depending on the strength and abruptness of the detected stimulus. These responses can also be modified by habituation, sensitisation and prey motivation (Wine & Krasne, 1972; Dill, 1974b; Krasne & Wine, 1984). Stein (1977) showed that crayfish evasive behaviour changed with crayfish age, size, and stage of development. These factors affected the vulnerability of crayfish to predation.

The startle response may not necessarily lead to escape. Instead it may be followed by other defensive behaviours such as fin raising in percids or head withdrawal in anguillids (Eaton & Hacket, 1984). Depending on the severity of the stimuli and the degree of danger, startled crayfish may respond by walking backwards away from the threat, by raising their chelae in a defensive posture, or by escape swimming powered by repeated tail-flips (Stein, 1977; Beall et al., 1990).

Crayfish startle responses are mediated by two giant axons, the medial giant axon (MG fibre) and the lateral giant axon (LG fibre), (Wine & Krasne, 1972; Krasne & Wine, 1984). The escape response is mediated by non-giant axons. The giant fibres are

fired in response to abrupt visual or mechanical stimuli, and have a reaction latency of 3-7 ms. Escape movements occur after 10 ms. This allows little time for the evaluation of the nature or location of the threat, making this reaction highly stereotyped (Krasne & Wine, loc. cit.). Stimulation of MG fibres drives crayfish away from an anterior threat. LG fibres move the abdomen up and away from a posterior threat. These fibres do not allow orientated locomotion, and subsequent escape is driven by non-giant fibres, which are triggered by the giant fibres and the threatening stimuli (Bennet, 1984). Non-giant fibre tail-flips are less stereotyped and can be directed to areas of safety (Krasne &Wine, 1984). These responses occur 50 to 500 ms after the initial startle stimulus (Krasne & Wine, loc. cit.), and probably initiate after the flexion of the tail during the giant fibre mediated startle response (Davey & Macmillan, 1991).

1.7 GENERAL STATISTICAL METHODS

In the following work (Chapters 2 to 6), the majority of the statistical analyses are nonparametric. Due to the small sample sizes, the majority of the data did not meet the criteria justifying the use of parametric tests. The Student's T-test and One-way and Two-way ANOVAS were used to test for differences in the size and weight distributions of two, or more than two crayfish samples respectively. These data fulfilled the criteria allowing parametric analysis (Siegel & Castellan, 1988).

The following tests were used when data were ordinal or interval and could not be tested using parametric methods. Mann-Whitney and Kruskal-Wallis tests were used to test for differences between two or more than two independent samples respectively. The Wilcoxon and Friedman tests were used to test for differences between two or more than two dependent samples respectively. The Spearman rank-order correlation coefficient was used to test for associations between two independent samples. Fisher exact and Chi-square tests were used to test between two independent groups when the data was measured using discrete categories (frequencies; Siegel & Castellan, loc. cit.). Equivalent tests drawn from Meddis (1984) were also employed. In addition to these, 2 x 2 factorial analyses were used to test the simultaneous effects of two independent variables and their mutually interactive effect upon a dependent variable (Meddis, loc. cit.). Nonspecific (two-tailed) alternative hypotheses were used in all the tests.

PART I

CHAPTER 2

2.0 EEL AND PERCH PREDATION ON JUVENILE *P. LENIUSCULUS*.

2.1 SUMMARY

Experiments were conducted to compare the impact of eel (*A. anguilla*) and perch (*P. fluviatilis*) on juvenile signal crayfish (*P. leniusculus*) mortality in habitats of differing complexity. The effect of alternative prey on juvenile crayfish mortality due to eel predation was also investigated.

Juvenile crayfish activity decreased in response to shelter availability and fish predators. Juveniles were most active at night. Actively feeding perch and eels caused similar behavioural changes in juvenile crayfish. It is suggested that stimuli characteristic of foraging activity affected this, as crayfish behaviour did not change in response to eels that did not feed.

It was hypothesised that crayfish would be more vulnerable to eel than to perch predation as a result of the ability of eels to use scent to detect prey, and their ability to burrow for hidden prey. This was not the case. Perch and eels increased juvenile crayfish mortality to a similar extent over a two week period, although perch reduced juvenile crayfish numbers more rapidly. Perch predation was least successful on substrata which provided the most shelter. Eels did not feed on *Gammarus* (spp) in preference to juvenile crayfish

The impact of eel and perch predation on the survival of juvenile crayfish is discussed. It is suggested that perch may have a stronger impact than eels on the mortality of newly independent juvenile crayfish.

2.2 GENERAL INTRODUCTION

Both eel and perch prey on crayfish (Svärdson, 1972; Dehli, 1981), but their foraging strategies are markedly different. Eels usually forage using chemoreception and are primarily nocturnal (Deedler, 1970). Perch are visual foragers and are mainly crepuscular (Disler & Smirnov, 1977; Hamrin, 1987). Fish predators have been shown to modify the activity and substrata selection of crayfish prey (Stein & Magnuson, 1976; Stein, 1977). Perch have been shown to reduce *A. astacus* and *P. leniusculus* activity (Hamrin, loc. cit.; Appelberg & Odelström, 1988). Defensive behaviour is stimulated by distinct predatory cues which may be exhibited to differing degrees in the foraging behaviour of different predators (Webb, 1982). Perch and eels may produce different defensive behaviour in crayfish prey, and as a result, may be more or less successful at capturing crayfish.

Predators differ in their ability to catch certain species of prey (Moore & Moore, 1974a & b; Jeffries, 1988). In the former studies, eels were more successful than flounder at capturing *C. vulgaris* because of their slower approach speed. Juvenile crayfish survival improves in habitats with good shelter availability, even in the absence of predators (Mason, 1979). More complex habitats reduce predation by rainbow trout (*O. mykiss*) on invertebrate prey (Ware, 1973) and also reduce predation by smallmouth bass (*Micropterus dolomieui* Lacépède) on juvenile crayfish, *Orconectes propinquus* Girard (Stein & Magnuson, 1976). In a situation where shelter is readily available to crayfish prey, predators with differing predation strategies may be more or less efficient at capturing crayfish. As shelter becomes more complex, differences in predatory success may become more apparent. This was demonstrated by Diehl (1988). Increased vegetation cover reduced the predatory success of perch feeding on chironomid larvae but reduced the success of roach and bream to a greater extent.

Predatory fish such as smallmouth bass, reduce crayfish activity (Stein & Magnuson, 1976; Hamrin, 1987; Appelberg & Odelström, 1988). Crayfish spend more time hidden under shelter. If eels cause a similar reaction in crayfish, then the ability of eels to forage using chemoreception may be advantageous in capturing crayfish hidden under shelter. Such crayfish would be largely inaccessible to visually foraging perch. The following experiments investigated the impact of eel predation on juvenile (0+) crayfish in comparison to perch predation. Tests were made on the effect of substratum complexity on juvenile crayfish activity and mortality due to both predators (Experiment 2.4, 2.5 & 2.6). It was hypothesised that the ability of eels to react to scent would allow them to feed on a greater number of juvenile crayfish than perch could.

The relative vulnerability of prey species is often a factor governing apparent predator preferences (Peckarsky, 1984). In the presence of an alternative more vulnerable prey, predation on a less vulnerable prey can be reduced (Jeffries, 1988). Eels

showed no preference for *Asellus* (spp) or juvenile *P. leniusculus* when they were presented together, although eel feeding behaviour was limited (Hart et al. unpublished). Behavioural observations suggested that *Asellus* were less responsive to an approaching eel and were, therefore, more vulnerable to eel predation. Populations of *Gammarus* (spp) and juvenile *P. leniusculus* co-exist in the littoral margins of ponds which are extensively managed by Simontorp Aquaculture A.B. Thus *Gammarus* are a potential alternative prey to *P. leniusculus* juveniles for predatory fish in these ponds. The effect of *Gammarus* on juvenile *P. leniusculus* mortality due to eel predation was investigated in Experiment 2.4 below.

2.3 GENERAL MATERIALS AND METHODS

Experimental animals

Perch and eels were caught in nets by fisherman from Vombsjön and Sövdesjön, two lakes in Skåne, southern Sweden (Fig 1.1), and were stored, prior to the experiments, in tanks at temperatures of between 11 and 12 °C. In Experiment 2.5, fish were placed in the experimental tanks without food, one week prior to the introduction of crayfish. Fish used in Experiments 2.4 and 2.6 were kept in the holding tanks for 7 to 10 days, before being placed in the experimental tanks at the start of the experiments. Fish used in Experiment 2.6 were fish recovered from Experiment 2.4 which had actively fed on juvenile crayfish. Throughout the course of these experiments, problems were encountered with fungal infections of the perch, in particular, but also of the eels. Fish were only used if they appeared healthy on visual inspection. When experimental fish became infected they were immediately replaced from the holding fish stocks. Eels were between 33.6 and 45.1 cm total length (mean 39.3 cm) and perch were between 13.3 and 19.2 cm fork lengths (mean 15.9 cm).

Newly independent juvenile signal crayfish (*P. leniusculus*) were available from an indoor hatchery at Simontorp Aquaculture A.B. from May to July 1990. A representative sample of crayfish used in Experiments 2.4 and 2.5 measured between 8.0 and 10.7 mm total length (from the tail to the rostrum tip, mean=9.7, S.D.=0.6, n=18). Crayfish used in Experiment 2.6 were taken from the survivors of Experiment 2.5. A representative sample of crayfish used in Experiment 2.6 measured between 11.1 to 17.2 mm total length (mean=13.4, S.D.=1.6, n=48). *Gammarus* used in Experiment 2.4 were collected from ponds and a stream at Simontorp Aquaculture A.B. A representative sample of these *Gammarus* were between 7.4 and 12.9 mm total length (tail to head, mean= 10.3, S.D.=1.3, n=51). Vernier callipers were used for all length measurements.

Crayfish and *Gammarus* were fed a standard quantity of *Artemia* and algal suspension every other day during the experiments. One extra feed of either shredded potato or fish was given at the weekends. All tanks were situated indoors under artificial lighting. A 12:12 light:dark regime was used with no simulation of dawn or dusk. Lights came on at 07.00 hours and off at 19.00 hours.

Experiment 2.4: ACTIVITY AND SURVIVAL IN RESPONSE TO EELS AND PERCH, AND THE INFLUENCE OF AN ALTERNATIVE PREY SPECIES

2.4.1 INTRODUCTION

This experiment was a preliminary investigation into predation of juvenile crayfish by eels and perch. The main objective was to establish criteria for further experiments of this type. The further specific objectives of this experiment were 1) to compare the predatory mortality of crayfish due to eel and perch predation, in the short term (i.e. 14 days), in a laboratory situation, 2) to determine whether eels affect crayfish activity in the same way as has been demonstrated for perch, and 3) To determine whether the presence of an alternative prey species alters the impact of eels on crayfish mortality.

2.4.2 MATERIALS AND METHODS

Four replicates of four treatments were run simultaneously in 16 tanks. The tanks were constructed from concrete channels lined by black plastic sheets. Four lines of four tanks were used, each 1.5 x 5 m, filled to a depth of 30 cm. In the first three treatments, 400 newly independent crayfish were placed in each tank with either 1) no predator, 2) one perch or 3) one eel. In the fourth treatment 200 crayfish and 200 *Gammarus* were placed in each tank with one eel. This approximated to 53 prey/m^2 of tank floor. Prey were placed in the tanks eight days before the predators were introducted. Tanks had separate inflows and outflows and contained one centrally placed fish shelter made from a length of plastic drainpipe (Fig. 2.1). Two 0.25-m^2 quadrats and two artificial crayfish shelters were placed at 0.5 m and at 1.5 m respectively, either side of the fish shelters. Quadrats were formed from plastic frames 1 cm wide, laid on the tank floor. Crayfish shelters were constructed using 50 corrugated plastic cylinders (5 cm diameter by 3 cm long) contained in a 50 x 25 cm plastic mesh sack (mesh size 6 x 4 mm). All food was added to the tanks over the quadrats. Tanks were supplied from the same ground water source. The water was mainly recirculated with a small additional inflow. The water temperature was maintained by controlling the air temperature in the experimental hall, and varied between 13.1 to 13.9 °C.

Crayfish activity was measured in two ways: 1) the number of exposed crayfish were counted at 09.30 and 21.30 hours, in a 4.5 m^2 area of tank floor between the crayfish hides (excluding the quadrats), 2) the number of crayfish that were in or on the artificial hides at 09.00 and 21.00 h were counted . All measurements were made at

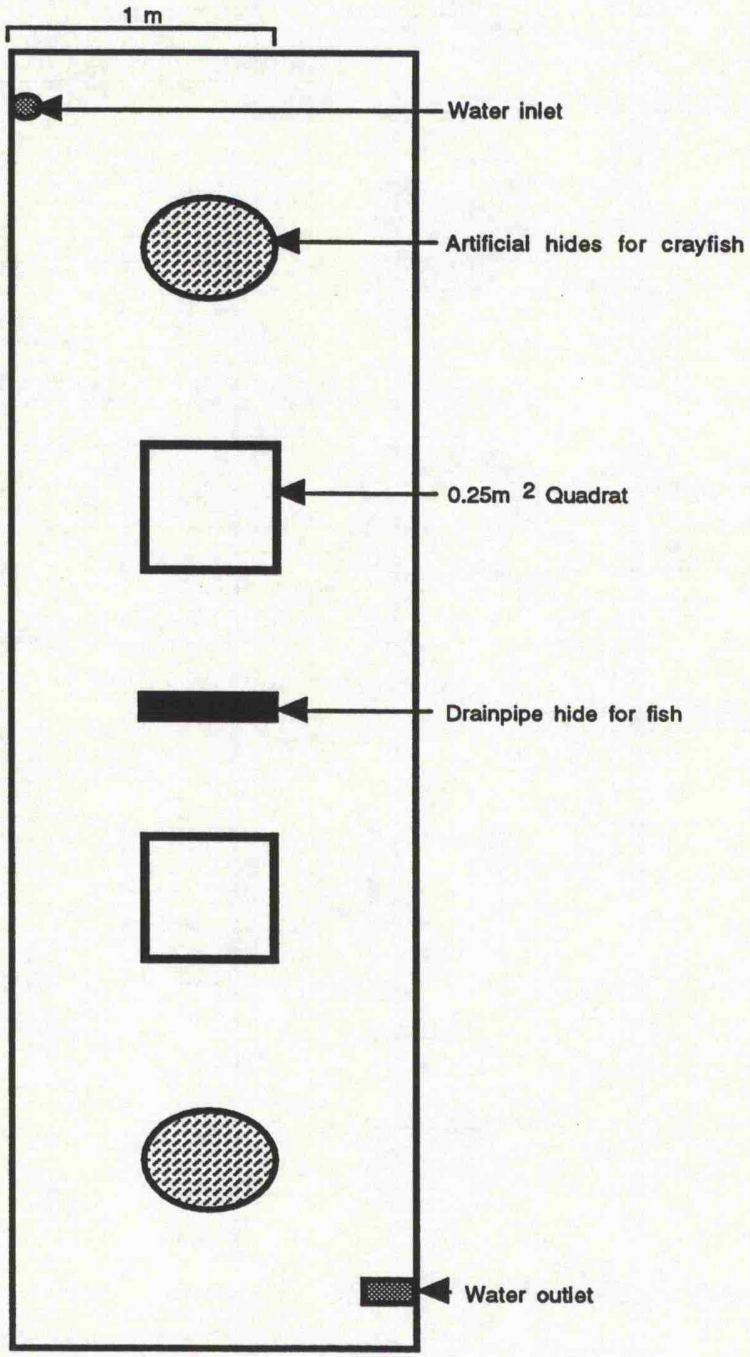


Figure 2.1. Tank design used in Experiment 2.4.

intervals throughout the eight days prior to the addition of the fish and the subsequent 14 days of the experiment.

At the end of the 14 days, during which crayfish were exposed to predatory fish (except in controls), fish were removed and the crayfish remaining in each tank were counted. Throughout the 14 days of the experiment, observations were made on the activity of each fish, prior to each count of crayfish activity. Fish faeces were collected and analysed for remains of crayfish and *Gammarus*.

2.4.3 RESULTS

In the last week of the experiment, one control tank began to leak. It was not possible to repair this tank and it is assumed that crayfish escaped and that this was the cause of the apparently poor 'survival' of crayfish from this tank (Table 2.1). As a result of this, the data from this tank were not included in the following analyses, despite the fact that prior to the leak there appeared to be no difference in crayfish behaviour between this and the other control tanks.

Table 2.1. Numbers (and percentages) of crayfish and *Gammarus* surviving in each treatment replicate in Experiment 2.4.

Predator treatment	Number of treatment replicates			
	1	2	3	4
Control (no predator)	†18 (4.5)	226 (56.5)	125 (31.2)	238 (59.5)
Perch	23 (5.7)	25 (6.2)	44 (11.0)	16 (4.0)
Eel	11 (2.7)	230 (57.5)	32 (8.0)	151 (37.7)
Eel (with alternative prey)				
Crayfish surviving	74 (37.0)	10 (5.0)	134 (67.0)	6 (3.0)
Gammarus surviving	6 (3.0)	0 (0.0)	114 (57.0)	2 (1.0)
Total prey surviving	80 (20.0)	10 (2.5)	244 (61.0)	8 (2.0)

† tank leaked and was excluded from the analyses.

At the end of the experiment, it was also evident that some of the eels had not fed to a great extent and one had not fed at all. This was indicated by the numbers of surviving juveniles in these tanks and was verified by the faecal analyses (Table 2.2). As a result, it was not possible to statistically test the effects of eels on crayfish survival and behaviour. Instead comparisons have been made from visual inspections of the data.

Table 2.2. Cumulative counts of the number of juvenile crayfish estimated to have been eaten by perch and eels. Estimates were made from the remains of crayfish eyestalks and chelae found in the fish faeces collected from each tank.

Treatment (Predator)	Day of experiment on which faeces were collected							Total surviving
	2	4	6	8	10	12	End	prey
Perch	0	54	96	113	118	121	121	23
	0	51	65	85	91	91	91	25
	0	37	66	66	75	81	87	44
	0	36	82	90	90	90	96	16
Eel	2	23	37	51	86	116	116	11
	0	0	0	0	0	0	10	230
	0	0	16	28	54	73	101	32
	0	0	1	1	10	23	35	151
Eel	0	17	23	23	23	23	23	74
(+ alternative	0	29	55	76	78	81	81	10
prey)	0	0	0	0	0	0	0	134
	0	18	41	73	86	90	97	6

Crayfish Survival

Throughout the experiment, counts were made of the numbers of crayfish and *Gammarus* exposed on 4.5 m^2 of the tank floor and of the numbers using the shelters. These counts were made over periods of 24 hours. The data from these counts were summed for each 24-hour period to estimate prey survival during the experiment (Fig. 2.2).

Due to the variability in eel feeding behaviour, neither eel predation, nor an alternative prey were shown to affect juvenile crayfish survival. When eels did feed, juvenile crayfish survival was similar when crayfish were the sole prey and when *Gammarus* were present (Table 2.1). *Gammarus* were rarely seen in the open tank or in the shelters. The estimates of survival in the tank where the eel did not feed underestimated the actual survival (Fig. 2.2). The estimates of survival in tanks where eels did feed were more accurate.

The following analyses concern only the three treatments without *Gammarus* as an alternative prey. In an overall comparison, crayfish survival did not differ between treatments. When data from the eel tanks were removed from the analysis, perch were shown to reduce the final crayfish survival by comparison to controls (Wilcoxon-Mann-Whitney test, $W_x=18$, $m=3$, $n=4$, $P<0.05$; Fig. 2.3).

A comparison of the estimates of juvenile survival made on the last day of the experiment with the final counts (i.e. the number of crayfish that were found in the whole tank at the end of the experiment) indicate that these estimates were likely to

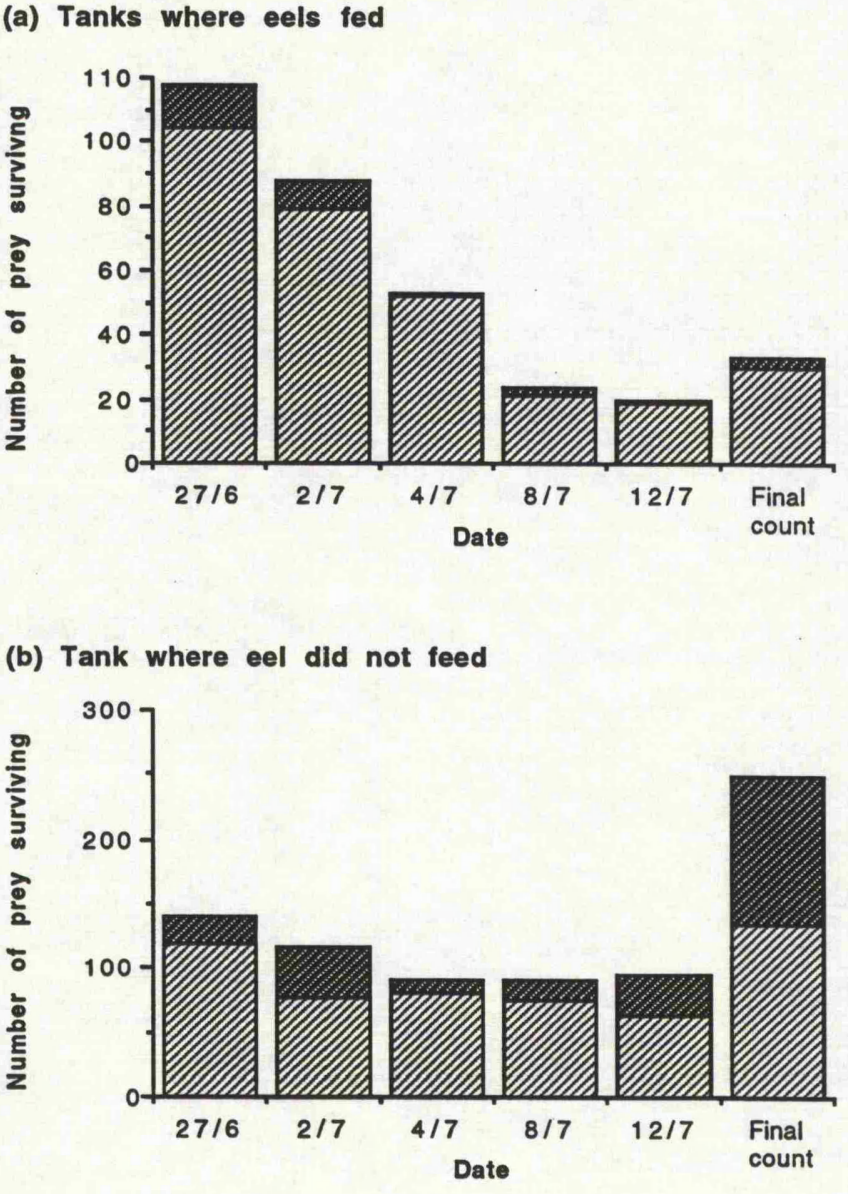


Figure 2.2. Mean numbers of juvenile crayfish (light cross-hatching) and *Gammarus* (dark cross-hatching) surviving on successive days of Experiment 2.4, a) when eels fed (n=3), and b) when eels did not feed (n=1). Survival estimates were calculated from the number of juveniles exposed on 4.5 m^2 of tank floor and in shelters at 09.00 h on consecutive days. Final counts were the number of prey found in the whole tank at the end of the experiment.

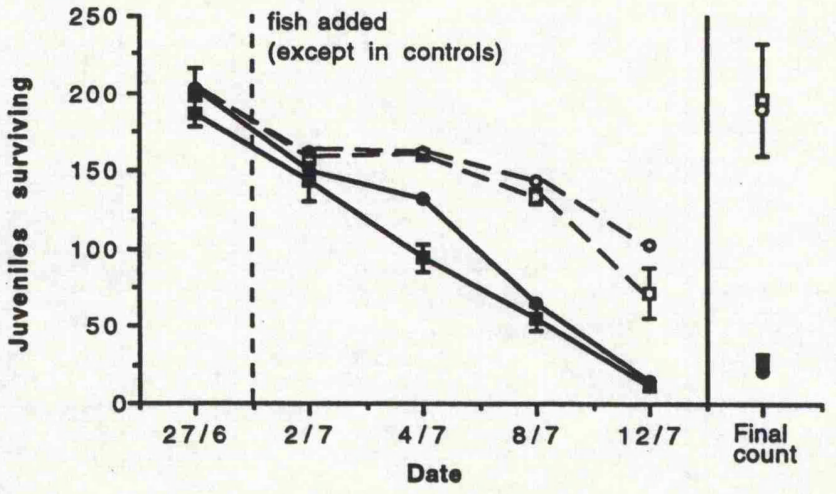


Figure 2.3. Mean numbers of crayfish (± 1 S.E.) surviving on successive days in control tanks –□– (n=3), with perch –■– (n=4), with eels that fed –●– (n=2), and with eels that hardly fed –○– (n=2). Survival estimates are calculated from the number of juveniles exposed on 4.5 m^2 of tank floor and in shelters at 09.00 h on consecutive days. Final counts were the number of crayfish found in the whole tank at the end of the experiment.

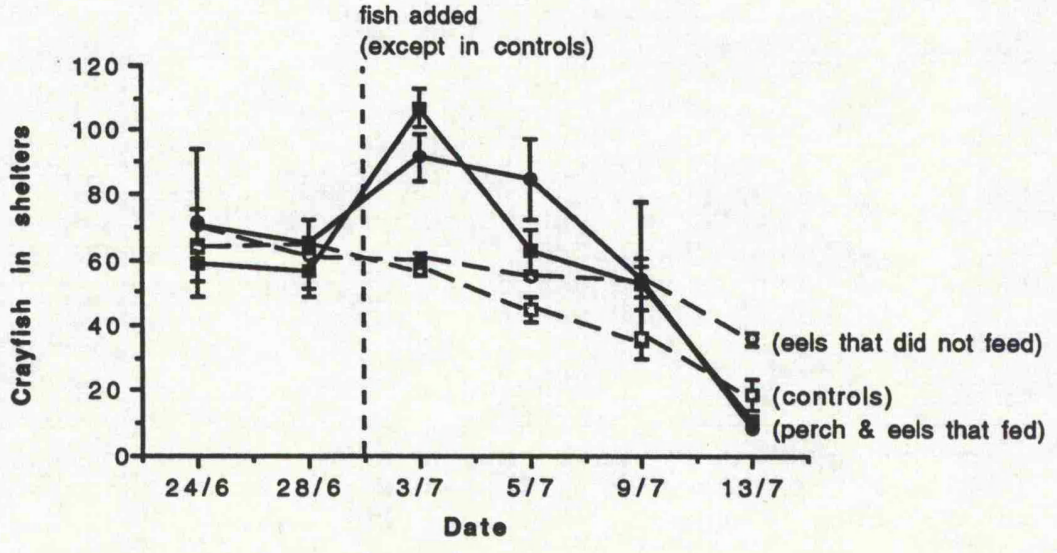


Figure 2.4. Mean number (± 1 S.E.) of crayfish in shelters at 09.00 hours throughout Experiment 2.4 in control tanks –□– (n=3), with perch –■– (n=4), with eels that fed –●– (n=2), and with eels that hardly fed –○– (n=2).

underestimate the actual survival of juvenile crayfish in control tanks and in tanks where eels were not feeding. This is likely, as part of the tank floor was not used for the calculation. The estimate of crayfish survival was more accurate in tanks where fish fed. Although the actual survival of juveniles was likely to be greater than the estimates made from the 24-hour counts, the data do highlight trends in prey survival under the different predator treatments. Perch reduced the survival of juvenile crayfish, as did eels when they fed well. Similar numbers of crayfish survived in the controls and in tanks where eels did not feed well.

Crayfish Activity

Crayfish tended to show a preference for the shelters nearest the water inlet. This effect was shown by Klosterman & Goldman (1983). For the purposes of the present experiment, the numbers of crayfish in the two shelters per tank were pooled. The total number of crayfish using shelters at 09.00 hours differed throughout the experiment, within perch tanks (Kruskal-Wallis test, $H=16.5$, $df=4$, $n=20$, $p<0.01$), and within the controls (Kruskal-Wallis test, $H=12.8$, $df=4$, $n=15$, $p<0.025$; Fig 2.4). The total number of crayfish per shelter increased in response to the addition of perch between 28 June and 3 July (Wilcoxon-Mann-Whitney test; $W_X=10$, $m=4$, $n=4$, $p<0.025$), and was greater in perch tanks than in control tanks on the 3 July (Wilcoxon-Mann-Whitney test; $W_X=6$, $m=3$, $n=4$, $p<0.05$). Shelter use did not increase in control tanks. After the initial increase on the 3 July, the total number of juveniles in the shelters in tanks containing perch declined throughout the experiment. The number of crayfish in the shelters in the control tanks also declined with time.

Prior to the addition of perch, there was no difference in the proportion of the estimated number of surviving crayfish that were using shelters between treatments at 09.00 or 21.00 hours (Fig. 2.5a & b). Two days after crayfish were exposed to perch, proportionally more crayfish were found in the shelters in these tanks than in the control tanks without perch (Wilcoxon-Mann-Whitney test; 09.00 h, $W_X=6$, $m=3$, $n=4$, $p<0.05$; 21.00 h, $W_X=6$, $m=3$, $n=4$, $p<0.05$). These differences persisted throughout the experiment. A comparative study of crayfish behaviour in response to eels showed that when eels fed, crayfish behaviour mirrored the responses shown to perch. When eels did not feed, crayfish behaviour resembled that shown in the controls.

Fish Activity

Both perch and eels were more active at night (Fig. 2.6). Perch were more active than eels just before dawn and during the day. Eels retreated into their shelters as dawn approached whereas perch were at their most active at this time. Eels were most active earlier in the night.

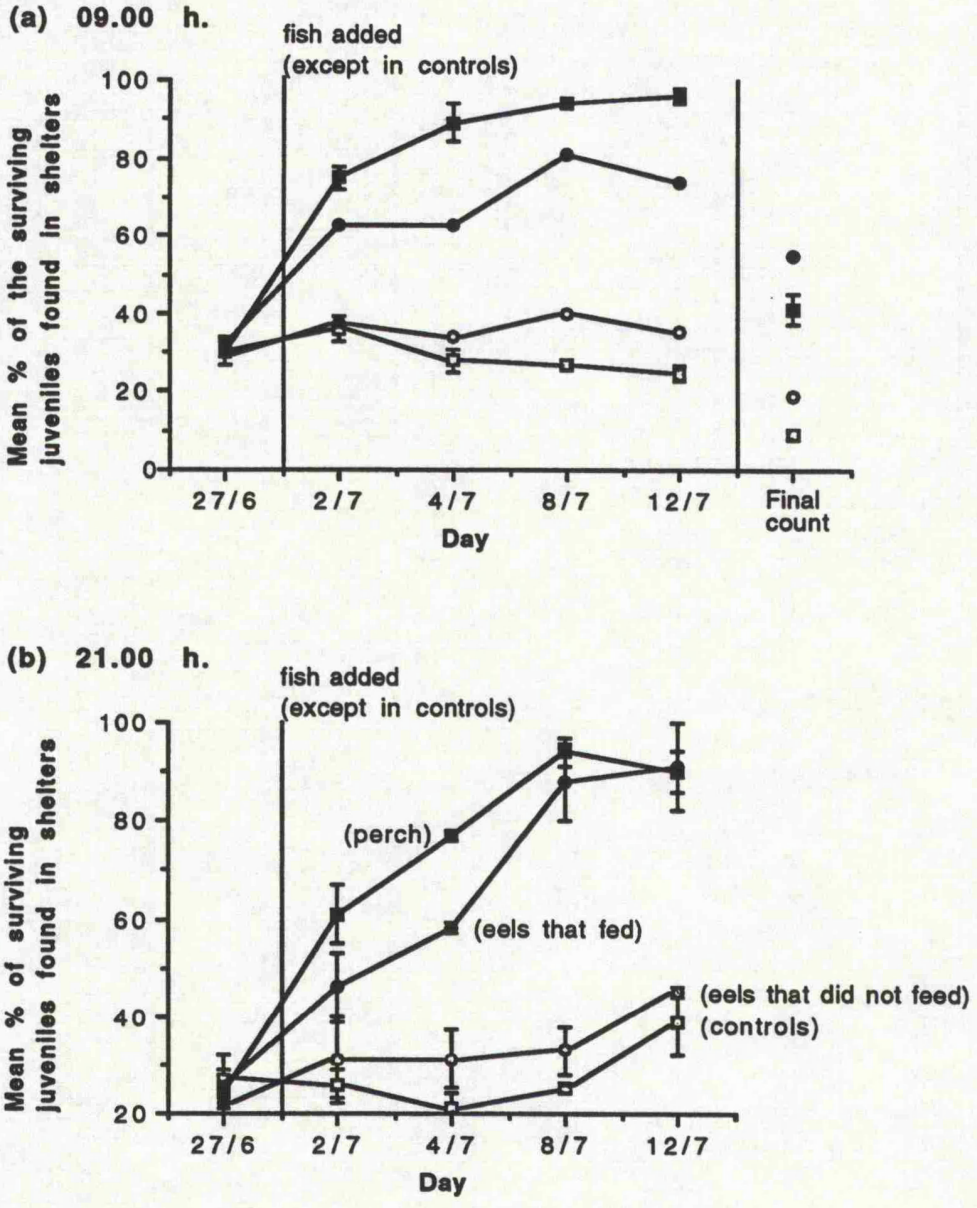


Figure 2.5. Mean percentage (± 1 S.E.) of the estimated number of surviving crayfish that were found in shelters a) at 09.00 h, and b) at 21.00 h in control tanks —□— (n=3), with perch —■— (n=4), with eels that fed —●— (n=2), and with eels that hardly fed —○— (n=2). Survival estimates were calculated from the number of juveniles exposed on 4.5 m^2 of tank floor and in shelters at 09.00 h and 21.00 h on consecutive days. Final counts were calculated from the number of prey found in the whole tank at the end of the experiment.

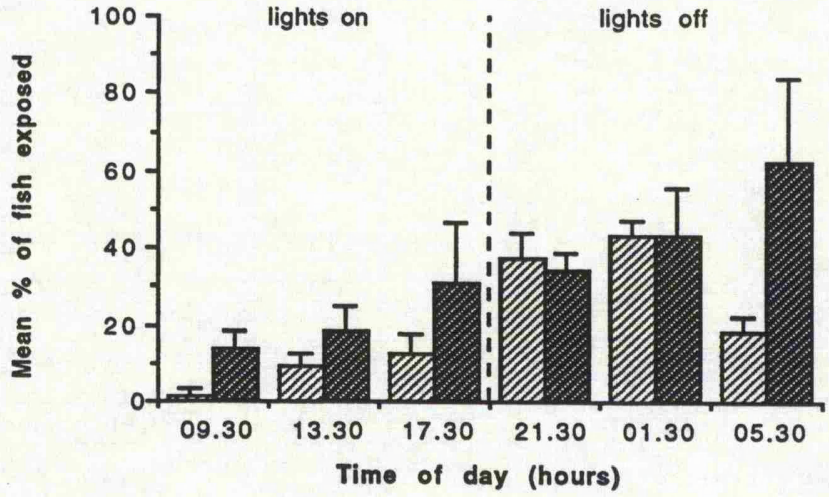


Figure 2.6. Mean percentage (± 1 S.E.) of eels (light cross-hatching) and perch (dark cross-hatching) exposed at each time period, counted on eight days at 09.00 h and 21.00 h and on four days for the other time periods, throughout Experiment 2.4.

Experiment 2.5. THE EFFECT OF SHELTER AVAILABILITY ON THE SURVIVAL AND ACTIVITY OF JUVENILE CRAYFISH EXPOSED TO PERCH AND EELS.

2.5.1 INTRODUCTION

In Experiment 2.4, both perch and eels preyed upon juvenile crayfish, but no difference in predatory mortality of juvenile crayfish was detected. The following experiment was designed firstly, to test whether predation on juvenile crayfish by perch and eels differed with respect to substrata of differing complexity, and secondly, to study more closely the behaviour of juvenile crayfish in response to these predators. It was hypothesised that eels would cause greater juvenile crayfish mortality than perch when crayfish had access to movable shelter. Unlike perch, eels should be able to detect and capture hidden crayfish by using chemical cues and burrowing behaviour.

2.5.2 MATERIALS AND METHODS

A 3 x 3 factorial test design was used involving three predator treatments and three substratum treatments (Table 2.3).

Table 2.3 Combinations of predator and substrata treatments in Experiment 2.5.

Substratum	Predator		
	None	Perch	Eel
None(Bare tank)			
Pebbles			
Bricks			

The differing substrata offered crayfish three levels of shelter: no shelter, limited movable shelter (pebbles), and limited immovable shelter (bricks). The pebble substratum consisted of 100 pebbles with irregular surfaces and a mean area of 6.3 cm^2 (range 5.5 to 8.2 cm^2) and a mean height of 1.5 cm (range 0.9 to 2.1 cm; n=25), placed at regular intervals on the tank floor. This represented cover for the crayfish that could be moved by a predator. The brick substratum consisted of 6 building bricks spaced evenly on the tank floor, each with 23 air holes within which crayfish could shelter. This represented limited immovable shelter. (Fig. 2.7).

Eighteen 1-m^2 tanks were used, each with a separate water inlet and outlet. A

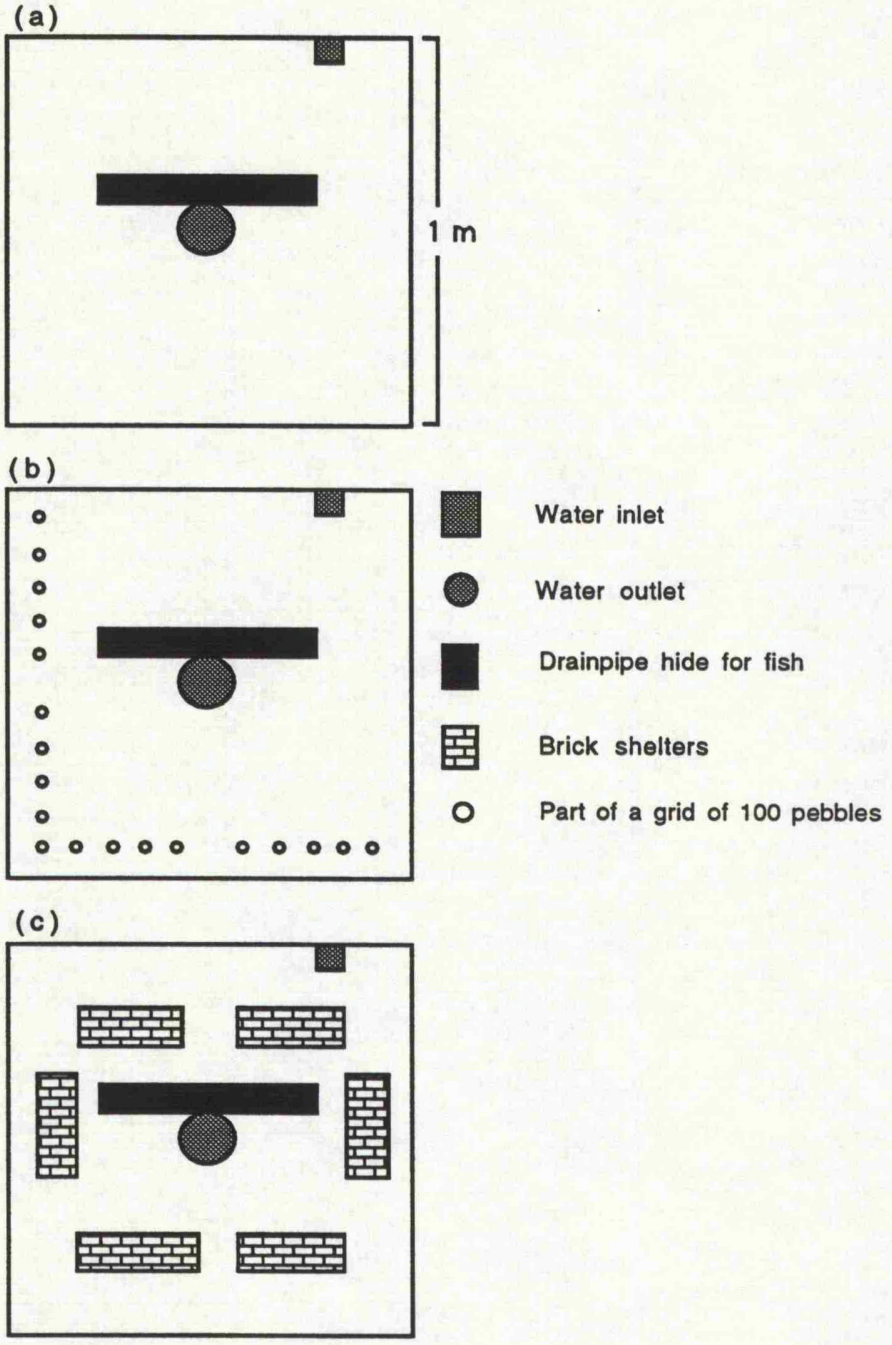


Figure 2.7. Tank designs used in Experiment 2.5 showing substrata treatments: a) no substratum, b) pebbles, and c) bricks.

drainpipe fish shelter was placed by the outlet in each tank. Two adjacent lines of 9 tanks were used. Each line was fed by a separate recirculating water system. The water temperature varied between 15.7 to 17.1 °C. Tanks in line A were filled to a depth of 45 cm and in line B to 30 cm. Each line was used to replicate each of the nine experimental treatments, which ran for 19 days. Tanks were then reset and used for a further 19 days, giving a total of 4 replicates of each treatment. In an attempt to ensure that eels fed, fish were allowed to acclimatise in the tanks for 1 week prior to the introduction of crayfish. One hundred newly independent crayfish were placed in each tank with either one perch, one eel or no predators. This represented a density of 100 individuals/m^2. New crayfish were used in each replicate. Two control treatments had only 75 crayfish per tank. Therefore, survival and activity data are presented as a percentage of the original number of crayfish placed in each tank.

Counts of the number of crayfish exposed (i.e visible) were made every two hours for two 24-hour periods per week, in each week of the experiment. At the end of the experiment, fish were removed and the numbers of surviving crayfish in each tank were counted.

Fish activity was observed at 2-hourly intervals in a 24-hour period, twice a week for the week prior to the introduction of the crayfish, and the two weeks of the experiment. The activities of the fish were classified into 4 categories:
(a) inactive within the shelter
(b) in the shelter with head exposed
(c) exposed, but inactive, on the tank floor
(d) actively swimming.

The classifications were simple and were designed to indicate movement and foraging activity in the test fish. Throughout the experiment, fish faeces were collected and analysed for crayfish remains.

2.5.3 RESULTS

Crayfish Dispersal

Crayfish were introduced at 13.30 hours and had spread to all corners of most of the tanks after 10 minutes. No predatory activity was observed until 18.45 when most of the perch moved out of their hides and became active. This continued after the lights were turned off at 19.00 h. Only two eels showed activity during this time, nosing around their hides and causing tail-flip escape responses in crayfish at a distance of 2 to 3 cm.

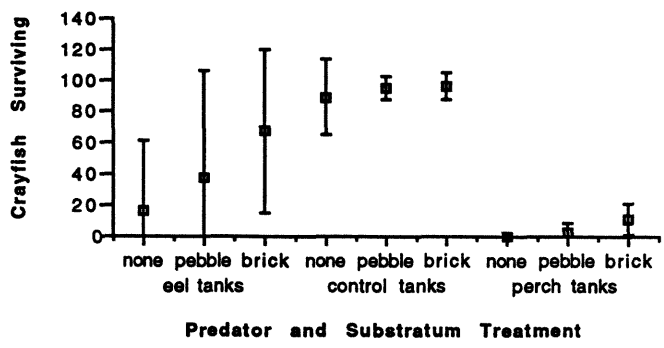


Figure 2.8. Mean numbers (with 95% C.L.) of crayfish surviving in each treatment in Experiment 2.5.

Crayfish Survival

In a two-way nonparametric ANOVA (Meddis, 1984), an overall difference was found in crayfish survival between treatments ($H=28.3$, $df=8$, $n=36$, $p<0.01$; Fig 2.8). Overall, survival was influenced by fish ($H=23.4$ $df=2$, $n=36$, $p<0.001$) but not affected by substratum. Individual pairwise comparisons of crayfish survival in response to perch, eels and controls, indicated that both eels ($p<0.01$) and perch ($p<0.001$) reduced crayfish survival by comparison to controls. In analyses of the effect of substratum on crayfish survival within each predator treatment, there was an indication that survival varied with substratum when perch were present ($H=7.01$, $df=2$, $n=12$, $p<0.05$). Although individual pairwise comparisons failed to show a difference in survival between individual substrata, survival appeared to be improved on the brick substratum by comparison with controls with no substratum (Fig. 2.8).

Faecal analyses indicated that predation by six of the 12 eels was relatively low (Table 2.4). When eels fed well, they produced similar levels of crayfish mortality to those produced by perch. Due to the individual variability in feeding behaviour, the survival of crayfish exposed to eels was more variable than for crayfish exposed to perch or no predators. As a result, no difference in predatory mortality was evident between eels and perch feeding on any substratum, although perch initially reduced crayfish numbers more rapidly than eels (Fig. 2.9).

Table 2.4. The number of crayfish found in faeces and the number surviving in each tank containing perch or eels in Experiment 2.5.

	Number of fish											
	1	2	3	4	5	6	7	8	9	10	11	12
Crayfish in eel faeces	1	2	3	4	6	9	15	18	18	21	37	46
Crayfish surviving in eel tanks	94	81	58	85	68	71	4	1	20	0	2	0
Crayfish in perch faeces	11	17	20	21	21	22	23	23	23	35	38	39
Crayfish surviving in perch tanks	11	0	20	0	8	8	0	5	0	0	1	0

Crayfish Activity

Consecutive readings of crayfish activity with time are not independent sampling points, as the same individuals were being observed at each time period. Therefore, two representative time periods were chosen to statistically analyse differences in crayfish activity; one in the middle of the day at 13.00 hours and the other in the middle of the night at 01.00 hours. The effects of substrata and predators on crayfish activity were analysed at each time period for the first and second week of the experiment, using a two-way nonparametric ANOVA (Meddis, 1984; Table 2.5).

Table 2.5. Results of a two-way ANOVA testing the determinants of crayfish activity for two time periods over the two weeks of Experiment 2.5. Values of H are given in the table. Significance levels are *p<0.05, **p<0.025, ***p<0.01, ****p<0.001.

Time period		Treatment variables		
		Overall effect (df=8)	Effect of Predator (df=2)	Effect of Sustratum (df=2)
Week 1	0115 h (night)	22.9 * * *	17.1 * * * *	5.1 p<0.08
	1315 h (day)	21.4 * *	16.9 * * * *	3.5 ns
Week 2	0115 h (night)	26.7 * * *	16.9 * * * *	1.9 ns
	1315 h (day)	21.3 * *	20.4 * * * *	0.5 ns

Predator treatments significantly affected the numbers of crayfish exposed on the tank floor, in both time periods, over both weeks (Fig. 2.9). In the first week, perch reduced the number of crayfish exposed (Pairwise comparison with controls at night, p<0.001; by day, p<0.01). During the second week of the experiment, both eels and perch reduced the numbers of crayfish exposed at night (Pairwise comparison with controls for perch, p<0.001; for eels, p<0.025) and by day (Pairwise comparison with controls for perch, p<0.001; for eels, p<0.05).

The patterns of crayfish activity in response to eels appeared similar to those produced by perch, although fewer crayfish were exposed on all substrata in response to perch (Fig. 2.9 b & c). This was probably a result of the rapid predation of juvenile crayfish by the perch in the first 24 hours of the experiment, before they had found shelter. By the second week of the experiment, no crayfish were found exposed in tanks with perch. Crayfish showed a distinct preference for nocturnal activity in all treatments, although this pattern was less distinct when bricks were available as shelter (Fig. 2.9). Similar activity patterns existed in control tanks between week 1 and week 2 of the experiment (Fig. 2.10). During the second week, crayfish activity between treatments was less distinct in the eel tanks by comparison to week 1, although the preference for nocturnal activity remained.

Comparisons of crayfish activity between substrata treatments, but within each predator treatment, indicated that in control tanks (no predators) at 01.00 hours, activity differed with respect to substratum (week 1, H=7.01, df=2, n=12, p<0.05; week 2, H=8.00, df=2, n=12, p<0.025; Fig 2.9a & 2,10a). Fewer crayfish were exposed when bricks formed the substratum than when there was no substratum (pairwise comparisons between brick and no shelter; week 1, p<0.05, week 2, p<0.025). Crayfish activity was

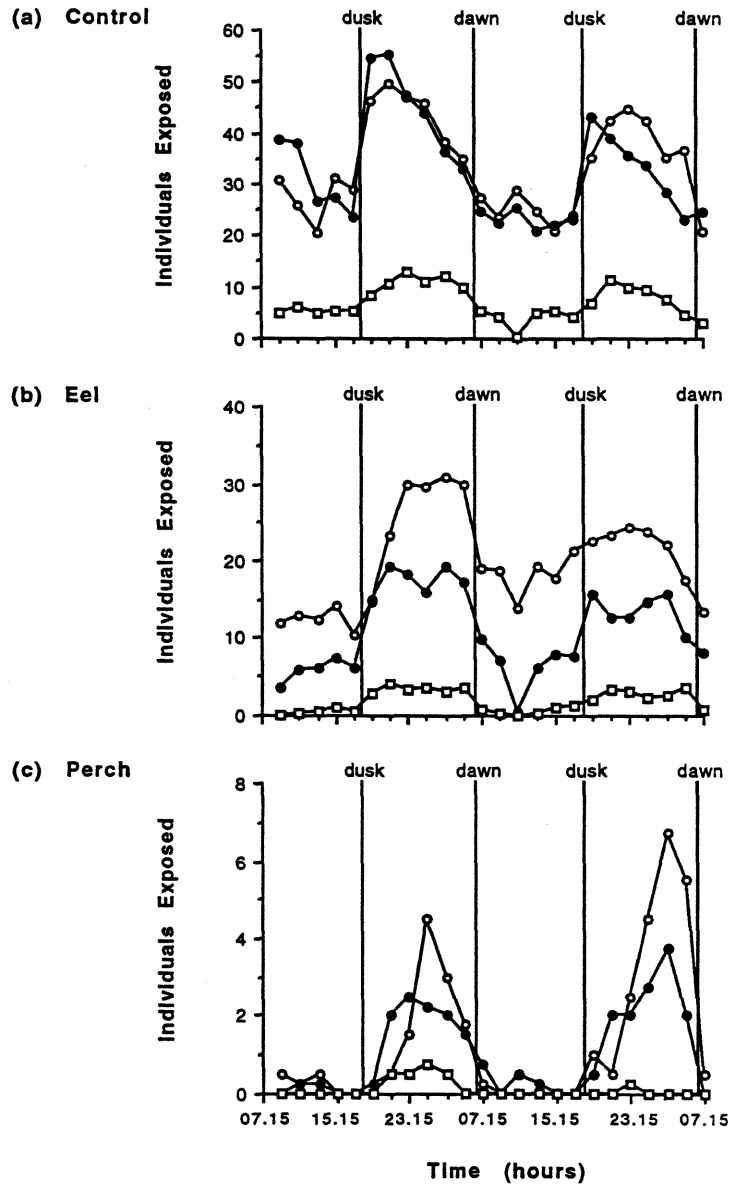


Figure 2.9. Mean number of crayfish exposed on the tank floors in Experiment 2.5 in: a) control tanks, b) eel tanks, and c) perch tanks, with no shelter ○ , with pebbles ● , and with bricks □ . Counts were made every two hours on days two and three of the experiment, starting at 09.15 hours on day two.

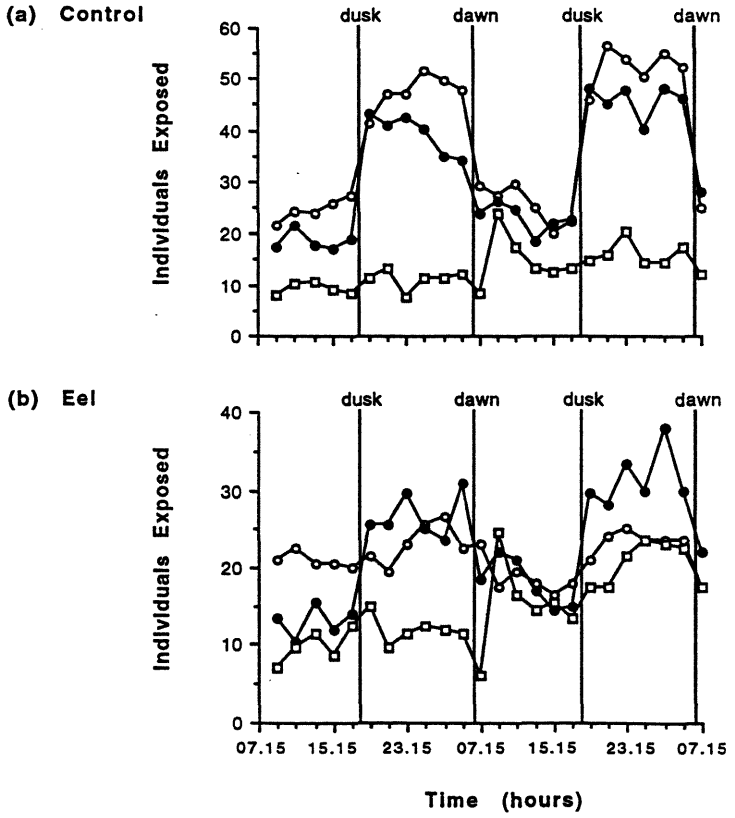


Figure 2.10. Mean number of crayfish exposed on the tank floors in Experiment 2.5 in: a) control tanks, b) eel tanks, with no shelter ○, with pebbles ●, and with bricks □. Counts were made every two hours on days nine and ten of the experiment, starting at 09.15 hours on day nine.

similar when no shelter and pebble substrata were available. The relatively low number of crayfish visible in the control tanks with no substratum, suggests that these crayfish were sheltering under the drainpipe fish shelter. Fish shelters were placed in control tanks to control for the effect of these shelters in tanks containing fish. No differences in crayfish activity were found in control tanks at 13.00 hours or in perch or eel tanks for either time period.

Fish Activity

The activity of perch and eels differed in type and magnitude, although the diel patterns were similar. Both were more active at night (Fig. 2.11). Perch were more active than eels over all time periods. Eels exposed at night were likely to have been foraging. However, eels were often in their shelters with their heads exposed, a position from which they were also seen to feed. Perch foraging was not observed but was only likely to occur in the open water. It was difficult to determine whether perch were actively swimming during the night or whether they were resting before being disturbed by the observer. The fact that perch were apparently nocturnal is probably a result of light escaping from an algal culture present in the tank room. It was not possible to mask this totally.

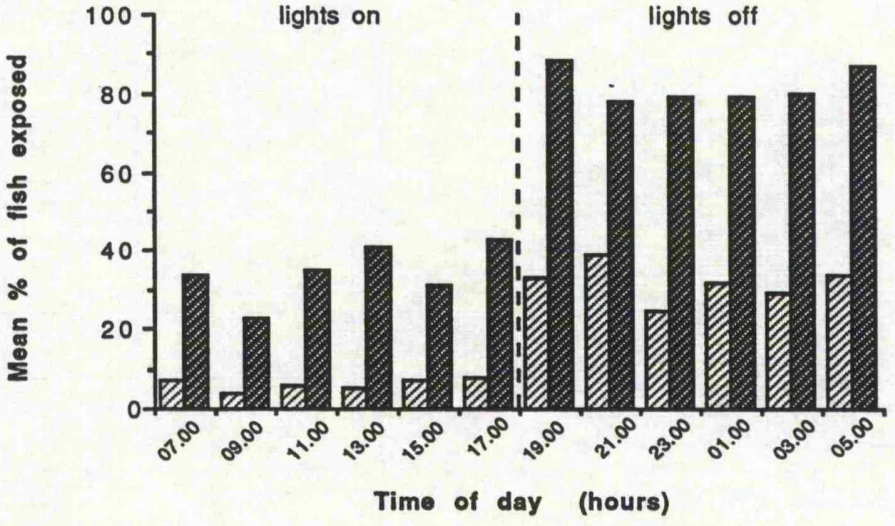


Figure 2.11. Average percentage of a) eels (light cross-hatching) and b) perch (dark cross-hatching) exposed in the tanks at each time period, measured from 6 counts made over the 3 weeks of Experiment 2.5

Experiment 2.6. JUVENILE CRAYFISH MORTALITY DUE TO EEL AND PERCH PREDATION.

2.6.1 INTRODUCTION

Crayfish mortality as a result of eel and perch predation did not differ in Experiment 2.5. Thus the hypothesis that eels would be more successful predators of juvenile crayfish than perch was not supported. This hypothesis was based on the fact that eels can forage using chemoreception and can also burrow for prey, and that this may have allowed eels to catch crayfish which were inaccessible to perch. Thus, either eels were not using scent to detect crayfish, or if they were, this did not give them an advantage when preying on crayfish. Three factors within the design of Experiment 2.5 may have masked a benificial effect of chemoreceptive foraging if such an effect existed:

1) crayfish may have been highly visible to perch due to the paucity of cover afforded by the pebble substratum,
2) the high predation rate by perch on crayfish in the first 24 hours of the experiment, before they had found shelter, and
3) the individual variability in eel feeding behaviour.

In the following experiment, the above hypothesis was re-tested using an experimental design which attempted to remove the factors described above. A more substantial pebble substratum was provided, crayfish were allowed to settle in the tanks prior to the introduction of the fish, and eels were only used if they had previously fed well in Experiments 2.4 and 2.5.

2.6.2 MATERIALS AND METHODS

Twelve 1-m^2 tanks were used to give four replicates of three treatments. Nine tanks were on one recirculating water system, the other three were on another. Tanks had their own inlets and outlets and were filled to a depth of 30 cm. The water temperature was 12.8 to 14.8 °C. Tanks had one half of the bottom area covered to a depth of approximately 3 cm by pebbles, of mean area 5.6 cm^2 (range 4.4 to 7.1 cm^2) and mean height 1.4 cm (range 0.8 to 2.0 cm; n=25). A drainpipe fish shelter was placed by each tank outlet. Forty juvenile crayfish were placed in each tank. After five days either one perch, one eel or no predatory fish were placed in the respective tanks.

Crayfish activity was measured by counting the number of crayfish exposed in the tank at 06.00, 08.00, 12.00, 18.00, 20.00 and 24.00 hours on three occasions: 1) before the introduction of the fish (day 0), 2) one day after the introduction of the fish (day 1), and 3) at the end of the experiment (day 6). Crayfish present in each tank at the

end of the experiment were counted.

Observations on the activity of the fish were made before the crayfish activity counts and the activities were classified as in Experiment 2.5.

2.6.3 RESULTS

Crayfish Survival

Crayfish survival differed between treatments (Kruskal-Wallis Test, H=8.06, df=2, n=12, $p<0.025$; Fig. 2.12). Crayfish survival was reduced by perch (pairwise comparison between perch and control tanks, $p<0.05$). Although eels appeared to reduce crayfish survival, no difference was found between crayfish survival in eel tanks and either perch or control tanks.

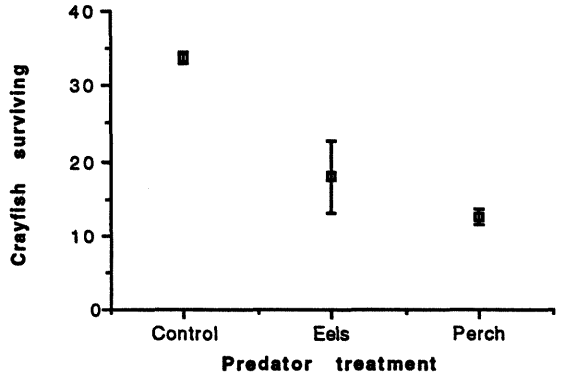


Figure 2.12. Mean numbers (± 1 S.E.) of crayfish surviving with each treatment in Experiment 2.6.

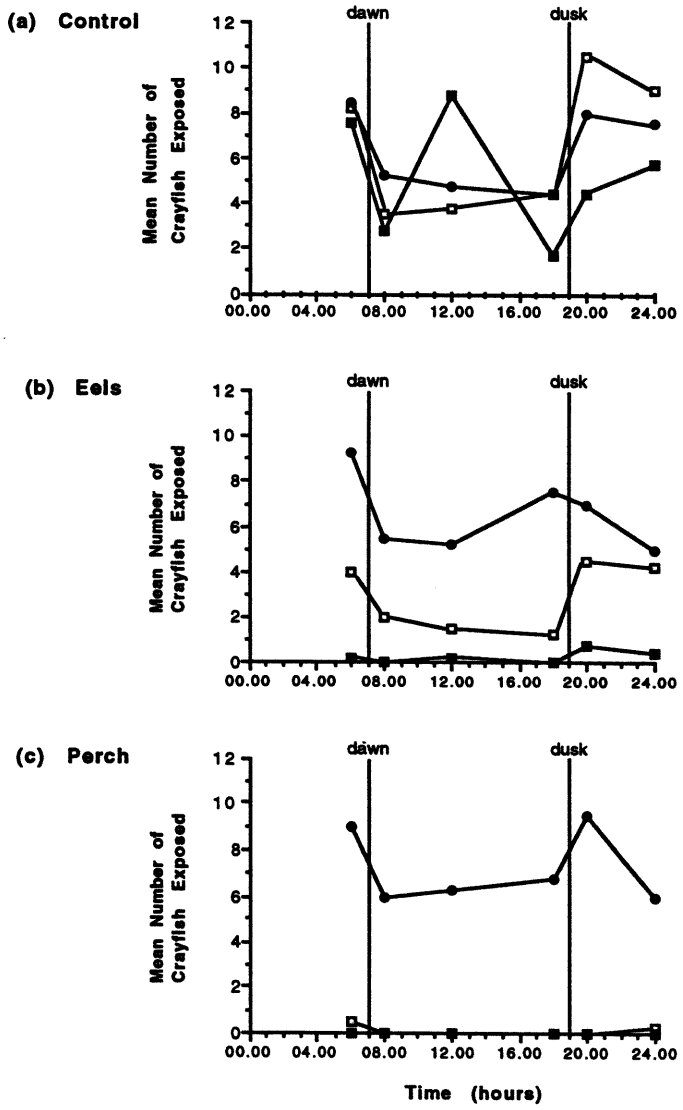


Figure 2.13. Mean numbers of crayfish exposed on the tank floor on day 0 ● , day 1 □ , and day 6 ■ , of Experiment 2.6 in a) control tanks, b) tanks with eels, and c) tanks with perch.

Crayfish Activity

Two representative time periods were chosen for the statistical analyses of the number of crayfish exposed (12.00 and 24.00 hours). Crayfish activity did not differ between treatments at 12.00 hours prior to the introduction of the fish (Fig. 2.13). After one day, the number of crayfish exposed fell in response to fish (Kruskal-Wallis test, $H=9.6$, $df=2$, $n=12$, $p<0.025$). In a pairwise comparison, the presence of perch reduced the number of crayfish exposed ($p<0.025$; Fig. 2.13c). A similar but smaller effect was produced by the eels, although this was not significant ($p>0.05$; Fig. 2.13b). These differences in crayfish exposure persisted after 6 days (Kruskal-Wallis test, $H=9.6$, $df=2$, $n=12$, $p<0.025$). Perch reduced the number of exposed crayfish (pairwise comparison with controls, $p<0.025$) and eels produced a similar result. These patterns were also similar at 24.00 hours.

Fewer crayfish were exposed in response to perch at 12.00 hours on day 1 and day 6 of the experiment (Kruskal-Wallis test, $H=10.5$, $df=2$, $n=12$, $p<0.01$; pairwise comparison between Day 0 and day 1 and day 0 and day 6, $p<0.025$). Also, fewer crayfish were exposed on day 6 in response to eels (Kruskal-Wallis test, $H=8.3$, $df=2$, $n=12$, $p<0.025$; pairwise comparison between day 0 and day 6, $p<0.025$). Similar trends were again evident at 24.00 hours (Fig. 2.13).

When expressing the number of crayfish exposed in each tank on the last day of the experiment (day 6) at 20.00 hours as a percentage of the total number of crayfish surviving at the end of the experiment, proportionally fewer crayfish were active in response to perch (Kruskal-Wallis test, $H=8.0$, $df=2$, $n=12$, $p<0.025$; pairwise comparison between perch and control tanks, $p<0.05$; Fig. 2.14). A similar trend was evident for crayfish exposed to eels but this was not significant ($p>0.05$).

Fish Activity

Both perch and eels were more active at night, with perch being more active than eels (Fig. 2.15). Most perch activity was outside the shelters whilst eels spent proportionally more time in the shelters with their heads exposed. Eels were not active at all on day 6, possibly as a result of a decline in foraging activity or exploratory behaviour. Perch activity was similar on day one and day six (Fig. 2.15).

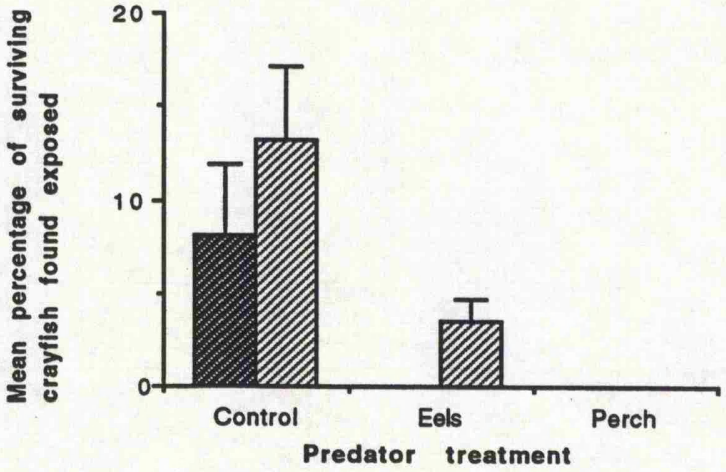


Figure 2.14. The percentage of the surviving crayfish which were exposed on the tank floor at a) 08.00 hours ▩, and b) 20.00 hours ▨, on the penultimate day of Experiment 2.16. Values are means (± 1 S.E.) for each treatment.

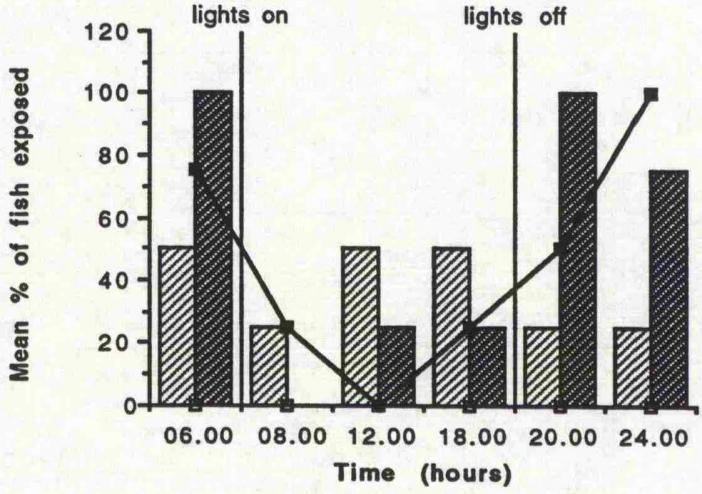


Figure 2.15. Mean percentage of fish exposed in the water column at each time period on day 1 and day 6 of Experiment 2.6. Columns refer to fish exposed on day 1: perch ▩, and eels ▨. Lines refer to fish exposed on day 6: perch —■—, and eels —⊟—.

2.7 GENERAL DISCUSSION

Eel and perch predation increased juvenile crayfish mortality over a 1 to 2 week period. In all three experiments, crayfish mortalities due to both predators were similar. This was true even when crayfish had access to abundant shelter (Experiment 2.6). Thus the hypothesis that eels would be more successful predators of crayfish was rejected. Either eels were not using scent to locate prey, or eels were using scent, but this did not allow them to capture crayfish more easily than perch. It is suggested that the second explanation is more likely. Eels are known to feed on benthic prey using chemoreception (Deedler, 1970; Tesch, 1977), and the foraging behaviour of eels feeding on juvenile crayfish in Chapter 4 (below) closely resembled the chemoreceptive foraging behaviour of American eels described by Helfman & Clark (1986).

Experiment 2.5, illustrated a difference in the foraging behaviours of eels and perch. Perch preyed rapidly on juvenile crayfish before they found shelter. Conversely, eels allowed crayfish exposure to pass without penalty of heavy predation. This may be partly a result of the relative inactivity of the eels as indicated from the observations on the dispersal of crayfish after their introduction into the tanks in Experiment 2.5. It may also be, that differences in foraging behaviour caused the differences in predation rates. The faecal analyses in Experiment 2.4 indicated that perch preyed more rapidly on juvenile crayfish than eels which fed well.

Diehl (1988) found that in an experimental situation, perch reduced numbers of chironomid larvae more rapidly than roach or bream. This was a result of the perch being able to feed by sight, whereas roach and bream fed by sifting chironomids from ingested silt, although roach fed by sight during the day. Over the course of the experiment, roach, bream and perch all took similar numbers of prey, suggesting that, although perch rapidly consumed large numbers of prey, capture rate quickly declined with declining prey density. Bream and roach fed at a lower intensity but maintained a more constant capture rate, even at low prey densities. The evidence from this example and from Experiment 2.5 suggests that when juveniles are first released, they may be more vulnerable to visual predators until they find shelter. This means that had prey densities been maintained at the original level in Experiment 2.5, then perch would have been more successful predators of crayfish than eels. Also, in natural populations, where newly released juvenile crayfish will be found in large numbers over a larger area, perch may consume more juvenile crayfish more quickly than eels.

Certain aspects of the experimental design may have facilitated perch predation in the tanks where it would not normally occur in the field. Firstly, the illumination from the algal culture during the simulation of night may have rendered crayfish visible to perch at a time when they are naturally active and when they would not normally be detected. In response to perch, juvenile *A. astacus* become increasingly nocturnal (Hamrin, 1987). Such anti-predator behaviour will have evolved if nocturnal

activity benefitted survival. Perch are primarily diurnal or crepuscular in their feeding behaviour (Disler & Smirnov, 1977), but in all three of the above experiments, perch were more active at night. A second factor likely to influence predation rates by perch is the relatively high activity levels shown by newly independent juvenile crayfish (Doroshenko, 1979). In experiments without predators, the motive activity of newly released juvenile *A. astacus* was high until they reached the end of stage III (prior to the third moult). During this period, juveniles spent a lot of time exploring the biotope.

Thirdly, in Experiment 2.4, the mean survival of crayfish in the control tanks was only 49%. This suggests that the feeding regime and shelter provided for the juveniles were not sufficient. Survival rates of 18 to 47% have been achieved after 80 days under stocking densities of 130/m^2 with varying feeding regimes and substrata (Mason, 1979). A lack of shelter stimulates crayfish activity (Westin & Gydemo, 1988). This would also be a likely result of poor food availability. Relatively poor substrata, poor feeding regimes, high stocking densities and the restricted area offered by the experimental tanks, will tend to increase juvenile crayfish activity and their vulnerability to visual detection and predation by perch.

Juvenile crayfish exhibited a preference for nocturnal activity even in control tanks, although it is conceivable that crayfish were reacting to chemical stimuli recirculated from tanks containing fish (Hazlett, 1985; 1990; Appelberg, pers. comm.). Hamrin (1987) found that juvenile *A. astacus* were mainly crepuscular, but became more nocturnal in the presence of perch. Such a preference has been shown in juvenile *P. leniusculus* by Appelberg & Odelström (1988) and has been shown to persist in adults (Abrahamsson, 1983). In Experiment 2.5, crayfish activity was reduced on the brick substatum but not on the pebble substratum in control tanks (with no predators). Mason (1979) and Westin & Gydemo (1988) found that locomotory activity declined in the presence of shelter compared with no shelter. This suggests that in Experiment 2.5, the pebble substratum offered no more shelter than was available in tanks which had bare floors, as crayfish activity was similar in these tanks.

In Experiment 2.6, crayfish responded to both perch and eels by increasing their use of shelter, particularly by day. This indicates avoidance of visual predators to be a strong selective pressure acting on juvenile crayfish behaviour. An increased use of shelter by juveniles under the threat of predation may have been driven by two mechanisms. Firstly, predation will be directed towards exposed crayfish, thus increasing the proportion of surviving crayfish in the shelters. Secondly, crayfish modify their distribution when faced with the threat of predation by increasing their use of shelter-providing substrata (Stein & Magnuson, 1976). It was not possible to isolate these causes, but evidence supports the suggestion that both were operating. In Experiment 2.4, this was demonstrated firstly, by the steady increase in the proportion of crayfish in the shelters throughout the experiment in response to perch, whilst the total number of surviving crayfish declined. Secondly, the number of crayfish

occupying the shelters increased two days after the introduction of perch but did not change in control tanks with no predators.

In Experiment 2.5, brick shelters appeared to enhance crayfish survival when the predators were perch. A similar pattern was evident when eels were present, but it was not possible to say whether the lack of effective predation in the eel/brick treatment was due to eels not feeding, or, due to eels being unable to catch crayfish. Good shelter can enhance juvenile crayfish survival when no predators are present (Mason, 1979) as locomotory activity and aggressive encounters fall with increasing shelter availability (Capelli & Hamilton, 1984), however, there was no indication of increased survival with increased shelter in control treatments (no predators) in Experiment 2.5.

Crayfish behaviour appeared to differ between situations when eels fed well and those when eel feeding was poor, although the data is limited. The presence of eels alone did not appear to stimulate avoidance behaviour. This suggests that stimuli characteristic of predation and not of predatory fish per se, induce defensive behaviour, although predator scent alone has been shown to stimulate avoidance behaviour in *A. astacus* (Appelberg, pers. comm.). In Experiment 2.6, crayfish exposure data suggests that either perch predation was again rapid, or that crayfish avoidance behaviour was more marked in response to perch than in response to eels. Perch were more active than eels and this might account for an increased avoidance response if fish activity were a measure of predatory threat. The magnitude of the behavioural response should match the predation risk (Endler, 1991). The stimuli that elicit defensive behaviour in crayfish are investigated are Chapters 3 and 4.

Locally dense juvenile crayfish populations occur when juvenile crayfish first become independent. At this time exploratory behaviour will be at a maximum (Doroshenko, 1979). Under such conditions in Experiments 2.5, perch were initially more detrimental to crayfish survival than eels. In crayfish populations, mortality rates are usually highest in juvenile crayfish and age-specific mortality regulates population sizes to within quite small limits which are often constrained by the habitat (Momot, 1984). Momot (1967) found that brook trout preyed upon newly hatched *O. virilis* but concluded that this was not an important population control mechanism. However, Appelberg (1990) suggests that high densities of perch may limit the abundance of newly hatched YOY *A. astacus* in some Swedish lakes.

Perch are largely restricted in the size of crayfish they can consume and rarely take crayfish greater than 70 cm long other than soft moults (Dehli, 1981). *P. leniusculus* reach maturity in an American river after 2 years (Shimizu & Goldman 1985). The size at maturity is 30.5 mm carapace length for males and 32 mm for females, corresponding to total lengths of about 70 cm. Thus perch are mainly restricted to preying on juvenile crayfish. Perch less than 20 cm seldom feed on 1+

crayfish and predation on young-of-the-year (YOY) crayfish is almost exclusively by small perch (Dehli, loc. cit.). Therefore, if perch regulate crayfish population size, then this is likely to occur when predation is directed at newly independent young, particularly in the first few days of independence. High relative and absolute densities would render juvenile crayfish vulnerable to both eel and perch predation at this time. Fish and crayfish tend to make up proportionally more of the diet of larger eels (Tesch, 1977). This suggests that eels may regulate crayfish populations as a result of predation on the brood stocks.

An important factor governing predation is predator selectivity, which is dependent on relative prey abundance and vulnerability as well as predator behaviour (Ware, 1973; Moore & Moore, 1976a & b). Hart et al. (unpublished) suggest that *Asellus* are more vulnerable to eel predation than juvenile crayfish due to the relatively poor avoidance response of *Asellus*. Flounder feeding on *Gammarus* and *Asellus* approached both prey from similar distances and at similar speeds (Moore and Moore 1976a). Flounder had a 100% capture success of *Asellus* but only 80% of *Gammarus* due to the latter having a more motile escape response. Environmental conditions were also important in controlling search success. *Gammarus* in the Severn Estuary were less vulnerable to predation when sheltering in weed (Moore and Moore, 1976b). Their relatively small size compared to other prey also rendered them less vulnerable to predation.

In Experiment 2.4, *Gammarus* were less often found in the shelters or on the tank floor than juvenile crayfish. Instead, they may have found shelter by remaining in contact with the tank walls. Also, very few *Gammarus* were found in eel faeces. It was not possible to determine with any degree of certainty whether *Gammarus* mortality was due to eel predation or to other factors. A comparison of the ratio of surviving *Gammarus* to crayfish in tanks with eels that fed (0:10, 3:6, 6:74) and in the tank where the eel did not feed (114:134), suggests eel predation was a factor governing *Gammarus* mortality, however, there was no evidence to suggest that eels preferred *Gammarus* to juvenile crayfish.

It is noted that certain problems exist with the work described above. First and foremost, was the low number of replicates per treatment and the resultant limitations in the power and confidence of the statistical analyses. A problem that was difficult to tackle was the variability of eel feeding behaviour. More replicates and a longer period of acclimatisation to the tanks may have countered this to some extent. It was decided that in future, more productive work could be done by limiting the use of eels in the laboratory to a more passive role. A detailed knowledge of eel prey selectivity with respect to species and size would provide a clearer insight into whether eels regulate crayfish populations, and if so, what mechanisms underly this. It was not possible to find suitable field sites where the diets of perch and eels could be compared. Therefore, the following work studied the possible differences that foraging eels and perch invoke

in crayfish avoidance and evasive behaviour, and the consequences of this for crayfish survival.

CHAPTER 3

3.0 THE BEHAVIOURAL RESPONSES OF JUVENILE SIGNAL CRAYFISH, *P. LENIUSCULUS* TO STIMULI FROM PERCH AND EELS.

3.1 SUMMARY

Experiments were designed to determine the relative importance of chemical and visual stimuli in eliciting predator avoidance behaviour in juvenile signal crayfish, *P. leniusculus*.

Crayfish placed in visual and/or chemical contact with one of two predators exhibited marked avoidance behaviour, spending less time walking and climbing and more time within shelters. The combined effects of both visual and chemical stimuli increased crayfish shelter use and reduced walking and climbing activity to a greater degree than either stimulus when presented alone.

Crayfish exhibited avoidance behaviour in response to chemical stimuli during periods of light and darkness. Visual detection of predators elicited avoidance behaviour during the day. It is suggested that the behavioural response of *P. leniusculus* to chemical stimuli reduces the likelihood of being detected by visual predators, and that chemical stimuli lower the response threshold for avoidance behaviour in crayfish reacting to visual stimuli. The adaptivity of using chemical cues to detect predators is emphasised

3.2 INTRODUCTION

Behaviour against predation can be categorised as either avoidance or evasive behaviour (Weihs & Webb, 1984; Sih, 1987). Evasive behaviour occurs after prey have encountered predators. Avoidance behaviour by prey occurs before an encounter with a predator, and reduces the rate of encounters between predators and prey.

Avoidance behaviours which bring about changes in microhabitat selection and temporal shifts in activity have been demonstrated in crayfish when in the presence of crepuscular predators (Stein & Magnuson, 1976; Stein, 1977; Hamrin, 1987; Appelberg & Odelström, 1988). In freshwater systems, chemical stimuli associated with predators have been shown to cause avoidance behaviour in prey (Petranka et al., 1987; Alexander & Covich, 1991). Visual, chemical and tactile stimuli elicit aspects of defensive behaviour in crayfish (Wine & Krasne, 1982; Hazlett, 1985).

Perch, *P. fluviatilis* and eels, *A. anguilla* are known to prey on crayfish (Svärdson, 1972; Dehli, 1981; Hogger, 1988 for review). Each species has a different foraging strategy. Anguillid eels are nocturnal and forage using chemoreception (Edel, 1975; Tesch, 1977). The mean stomach fullness of *Anguilla australis* (Richardson) was shown to increase throughout the night (Ryan, 1984). Perch are diurnal or crepuscular predators and forage visually (Disler & Smirnov, 1977). It is possible that crayfish respond to different predatory stimuli associated with the separate foraging techniques of perch and eels. Visual stimuli should be important for the detection of diurnal predators such as perch. A reliance on chemoreception or mechanoreception might be expected for the detection of nocturnal predators such as eels. This would be a likely product of the levels of illumination present when each predator forages. Crayfish of the Genus *Orconectes* respond to the loss of visual stimuli at night with compensatory increases in the use of mechanoreceptive organs such as the antennae and chelae (Bruski & Dunham, 1987; Smith & Dunham, 1990). No similar increase was found in the use of the antennules, a major site of chemoreception. The purpose of the following experiments was to determine the importance of visual and chemical stimuli, characteristic of eels and perch, in initiating defensive behaviour in the signal crayfish, *P. leniusculus*.

The predators were presented to the prey under conditions simulating nocturnal and diurnal light levels. Predators and prey were physically isolated so as to minimise the chances of crayfish detecting predators by means of mechanoreception. Under these experimental conditions it was hypothesised that if chemical stimuli were important in determining defensive behaviour in crayfish, such behaviour should occur under conditions of both light and darkness. If visual stimuli were important, two results would be expected. Firstly, defensive behaviour should only be observed in the light. Secondly, defensive behaviour should be more marked in response to an increase in the frequency of visual disturbances if the latter is a measure of potential

danger (Sih, 1987).

The following experiments describe the changes in behaviour observed in crayfish in response to perch and eels during four time periods in the diel cycle.

3.3 MATERIALS AND METHODS

Experimental animals

Juvenile crayfish were obtained from a crayfish farm near Gillingham, Dorset, England, and were stored in aquaria and fed part-boiled potato. Crayfish ranged between 16.3 to 23.5 mm carapace length. Eels and perch were obtained by electrofishing in the River Welland, Leicestershire and in a pond near Wallingford, Oxfordshire. All fish were stored in a 4 m^3 arena tank. The total lengths of eels ranged between 40 to 60 cm. The perch ranged between 15 to 20 cm total length.

Experimental Design

Crayfish were placed individually into 12 litre aquaria and were subjected to one of four treatments arranged in a 2 x 2 factorial design, based on the presence or absence of visual and chemical contact between predator and prey (Table 3.1).

Table 3.1. Description of the experimental design for Experiments 3.1 and 3.2 (X= no stimulus, √= stimulus).

				Presentation of stimuli			
				Predator:			
				EEL (Experiment 3.1)		PERCH (Experiment 3.2)	
Tank	Aquarium	Treatment	Description	Visual	chemical	visual	chemical
A and C	a	1	CONTROL. NO STIMULI	X	X	X	X
	b	2	VISUAL & CHEMICAL STIMULI	√	√	√	√
B and D	a	3	CHEMICAL STIMULI ONLY	X	√	X	√
	b	4	VISUAL STIMULI ONLY	√	X	√	X

Two 12-litre aquaria, filled to a depth of 18 cm, were placed adjacent to each other inside each of four 250 litre tanks. These tanks were filled to a depth of 15 cm. For Experiment 3.1, four eels were rotated between the test tanks. In Experiment 3.2, the eels were replaced by four perch. A single tank, containing two aquaria, was observed on each day of an experiment. Observations were made on each tank in rotation. In the tanks, the aquaria were subjected to pairs of treatments, as set out in Table 1. Eight replicates of each of the four treatments were performed. Initially, each crayfish was used twice, once in each of two different treatments. In some cases replicates had to be repeated. In Experiment 3.1, 22 crayfish were used in 32 replicates of the 4 treatments. In Experiment 3.2, 21 crayfish were used.

In treatments where the predator and the prey were to be in visual contact, the aquaria were transparent. The aquaria sides were covered with black plastic sheeting in treatments with no visual contact between predator and prey. Water was circulated between the aquaria and the tanks in the treatments where predator and prey were to be in chemical contact. Where no chemical contact was required, water was circulated within each aquarium. All tank and aquarium bottoms were covered with sand. Each aquarium was fitted with two pieces of plastic tubing (2 cm diameter by 4 cm long) which the crayfish could enter for shelter. To provide food for the crayfish, each aquarium was also supplied with part-boiled potato in excess. Water temperature in the tanks during the experiment was 7 to 9 °C. The tank room operated on an 8.5:15.5 light:dark regime. The lights came on at 09.30 hours and were turned off at 18.00 hours. The lights did not fade in or out.

Experimental Procedure

The following procedure was used in the preparation and observation of each tank. The water in each aquarium was replaced and aerated for 24 hours. The crayfish were then placed in the aquaria 48 hours before observations began. The water circulation pumps were started 24 hours before observations began. On the day of each experimental trial, crayfish were observed for 30 minutes over 4 time periods: pre-dawn (08.45 h), post-dawn (09.45 h), pre-dusk (17.15 h) and post-dusk(18.15 h). In Experiment 3.1, eels were placed in the tanks 15 minutes before the start of the pre-dawn and pre-dusk observation periods so as to ensure that they were active throughout all four periods of observation. In trials conducted prior to this experiment, eels became inactive when left in the tanks for long periods of time. In Experiment 3.2, perch were also placed into the tanks 15 minutes prior to the pre-dawn and pre-dusk observation periods.

Tank water was not changed between replicates. Water that had been occupied by a predator 48 hours previously, was pumped into aquaria that were to be treated with predator scent 24 hours prior to the new predators being placed in the tanks. As a result, the crayfish in these aquaria were in contact with old predator water for 24 hours prior to the onset of the experiment. The introduction of the predator into the

tanks thus constituted a fresh input of predator scent.

Crayfish Behaviour

Observations of crayfish activity were made using black and white video recording equipment sensitive to infrared light. Crayfish were filmed for four periods of half an hour as indicated above. These times were chosen for two reasons. Firstly, to ensure that the predators were active whilst filming. Secondly, crayfish change their activity in response to the changes in light intensity associated with dawn and dusk (Hamrin, 1987). Therefore, any behavioural changes associated with predator avoidance should have been discernible within these time periods. The videos were analysed and crayfish behaviour was noted every 30 seconds for each 30-minute filming period. The following categories of crayfish behaviour were observed in preliminary trials:

a) Activities inside shelter

Hidden - no part of crayfish visible.

Withdrawn - chelae visible but mostly inside the shelter.

Blocking - tip of rostrum visible inside the shelter. Chelae crossed in front of the carapace at the hide edge or held to the side of the carapace within the shelter.

Guarding - carapace visible. Chelae held to the side of the carapace outside the shelter or held across the front of the carapace outside the shelter.

Visible - carapace and tail outside the shelter. Crayfish remaining in contact with the shelter.

Investigating - crayfish moving in or out of shelter head first (i.e. chelae and carapace first).

Moving - crayfish advancing or retreating from a position inside the shelter.

b) Activities outside shelter.

Inactive - crayfish exposed but resting in the corner of the tank or in a corner between the hide and tank wall.

Walking - walking forward.

Walking backwards.

Climbing - climbing tank sides or water circulation tubes.

Excavation - crayfish moving sand substratum with chelae.

Feeding.

Maintenance - crayfish at rest but chelae or walking legs in motion.

Orientation - changing direction between walking and climbing.

Stillness - resting between walking or climbing.

Encounter - an encounter was recorded whenever a crayfish orientated towards a fish. When the crayfish were exposed, an encounter resulted in the crayfish adopting a confrontational posture. This is not described as aggressive or defensive because this posture was adopted regardless of whether it was followed by an advance or retreat.

In the experimental trials, only a small number of the above behaviours occurred with regularity during the periods of observation. As a result, some categories were combined for the purpose of the analyses. These categories are listed below:

Defensive shelter use - crayfish were withdrawn inside a shelter and were either not visible or the tip of their rostrum and chelae were visible within the shelter.

Active shelter use - crayfish were partly exposed outside a shelter with either their carapace or carapace and tail visible.

Total shelter use - includes both defensive and active shelter use.

Walking - forward walking in the open tank.

Climbing - climbing the tank sides or water circulation tubes.

Analysis of Behaviour

Counts were made of the number of 30-second observations in which each behaviour occurred. These counts were expressed as a percentage of the total number of 30-second observations made during the 30 minutes of filming. Within each time period, the influences of visual and chemical stimuli on crayfish behaviour were analysed using a 2 x 2 factorial, non-parametric analysis of variance by ranks (Meddis, 1984). The Wilcoxon Signed-Rank test was used to determine the effect of changes in light intensity on crayfish behaviour (Siegel & Castellan, 1988). The results for each crayfish were paired within treatments and across the following time periods: a) pre-dawn versus post-dawn and b) pre-dusk versus post-dusk. The null hypothesis employed for these analyses was that neither treatment nor light conditions affected crayfish behaviour. Nonspecific (two-tailed) alternative hypotheses were employed in all tests.

Table 3.2. The results of the 2 x 2 factorial analyses showing the effect of chemical and visual stimuli on crayfish behaviour for each period of observation in Experiment 3.1, (with eels as the predator). Values of H, and associated probability values, ($p<0.1$) are given in the body of the table. Figures in brackets denote the direction of the behavioural change.

Predator : EEL		Test employed			
Time period and Behaviour		Overall effect of treatments (df=3, n=8)	Effect of visual stimuli (df=1, n=16,16)	Effect of chemical stimuli (df=1, n=16,16)	Interaction effect of stimuli (df=1)
(a) PRE-DAWN					
In shelter	H value	8.07	ns	4.39 (+)	3.54
	p	<0.05		<0.05	<0.1
Walking	H value	11.36	ns	5.49 (-)	4.86
	p	<0.025		<0.025	<0.05
Climbing	H value	8.72	ns	7.34 (-)	ns
	p	<0.05		<0.01	
(b) POST-DAWN					
In shelter	H value	ns	ns	4.46 (+)	ns
	p			<0.05	
Walking	H value	ns	ns	ns	ns
	p				
Climbing	H value	ns	ns	3.19 (-)	ns
	p			=0.07	
(c) PRE-DUSK					
In shelter	H value	11.08	2.73 (+)	4.13 (+)	4.21
	p	<0.025	=0.09	<0.05	<0.05
Walking	H value	8.17	ns	3.02 (-)	4.95
	p	<0.05		=0.078	<0.05
Climbing	H value	ns	ns	2.92 (-)	ns
	p			=0.083	
(d) POST-DUSK					
In shelter	H value	ns	ns	ns	ns
	p				
Walking	H value	ns	ns	ns	ns
	p				
Climbing	H value	ns	5.09 (+)	ns	ns
	p		<0.025		

Table 3.3. The results of the 2 x 2 factorial analyses showing the effect of chemical and visual stimuli on crayfish behaviour for each period of observation in Experiment 3.2, (with perch as the predator). Values of H, and associated probability values, ($p<0.1$) are given in the body of the table. Figures in brackets denote the direction of the behavioural change.

Predator : PERCH		Test employed			
Time period and Behaviour		Overall effect of treatments (df=3, n=8)	Effect of visual stimuli (df=1, n=16,16)	Effect of chemical stimuli (df=1, n=16,16)	Interaction effect of stimuli (df=1)
(a) PRE-DAWN					
In shelter	H value	7.94	ns	6.31 (+)	ns
	p	<0.05		<0.025	
Walking	H value	ns	ns	5.32 (-)	ns
	p			<0.025	
Climbing	H value	ns	ns	5.56 (-)	ns
	p			<0.025	
(b) POST-DAWN					
In shelter	H value	7.07	ns	5.52 (+)	ns
	p	=0.68		<0.025	
Walking	H value	ns	ns	ns	ns
	p				
Climbing	H value	ns	ns	4.49 (-)	ns
	p			=0.05	
(c) PRE-DUSK					
In shelter	H value	9.35	4.78 (+)	3.74 (+)	ns
	p	<0.025	<0.05	<0.05	
Walking	H value	6.64	4.42 (-)	ns	ns
	p	=0.083	<0.05		
Climbing	H value	ns	ns	3.61 (-)	ns
	p			=0.054	
(d) POST-DUSK					
In shelter	H value	ns	ns	ns	ns
	p				
Walking	H value	ns	ns	ns	ns
	p				
Climbing	H value	ns	ns	ns	ns
	p				

3.4 RESULTS

Crayfish Behaviour In Response To Predatory Stimuli

During the pre-dawn, post-dawn and pre-dusk periods, crayfish spent more time in shelters in response to the scent of both eels and perch (Tables 3.2 and 3.3). Often the increase in shelter use was accompanied by a reduction in walking and climbing activity, although this was not always the case. Behavioural responses of crayfish to fish scent were most marked where the fish could also be seen (Figs. 3.1 and 3.2). Visual stimuli influenced crayfish behaviour most strongly during the pre-dusk period. Crayfish spent more time within shelters and walked less in response to seeing perch (H=4.8 and 4.4 repectively, df=1, m=16, n=16, $P<0.05$). There was a similar trend in response to eels but this was not significant ($p>0.05$).

Crayfish behaviour appeared to be influenced more by seeing eels than by seeing perch. Although it was not possible to test this , it may have been a consequence of the greater activity of the eels. This is indicated by the predator-prey encounter data (Table 3.4). Encounters were only detected in those treatments in which fish could be seen by the crayfish. Fifteen of 22 crayfish encountered eels a total of 77 times. Eight of 21 crayfish encountered perch a total of nine times. There was a clear difference in crayfish behaviour between treatments. Where the crayfish could see and smell the eels, the majority of the encounters occurred whilst the crayfish were in the shelters. Where the eels could only be seen, the crayfish were most often exposed. This was true in the light (Chi^2 =16.9, n=48, $p<0.001$) and the dark (Chi^2=10.1, n=29, $p<0.01$).

Table 3.4. The total number of visual encounters between juvenile crayfish and the fish predators in Experiment 3.1 and 3.2. Figures in brackets denote the number of individual crayfish responsible for the total number of encounters shown.

		Light Intensity:			
		Light		Dark	
		Crayfish position		Crayfish position	
Predator	Treatment	In shelter	Exposed	In shelter	Exposed
Eel	Visual and chemical stimuli	9 (5)	0 (0)	13 (5)	4 (2)
	Visual stimuli only	10 (5)	29 (7)	2 (2)	10 (4)
Perch	Visual and chemical stimuli	2 (2)	1 (1)	0 (0)	2 (2)
	Visual stimuli only	2 (2)	1 (1)	0 (0)	1 (1)

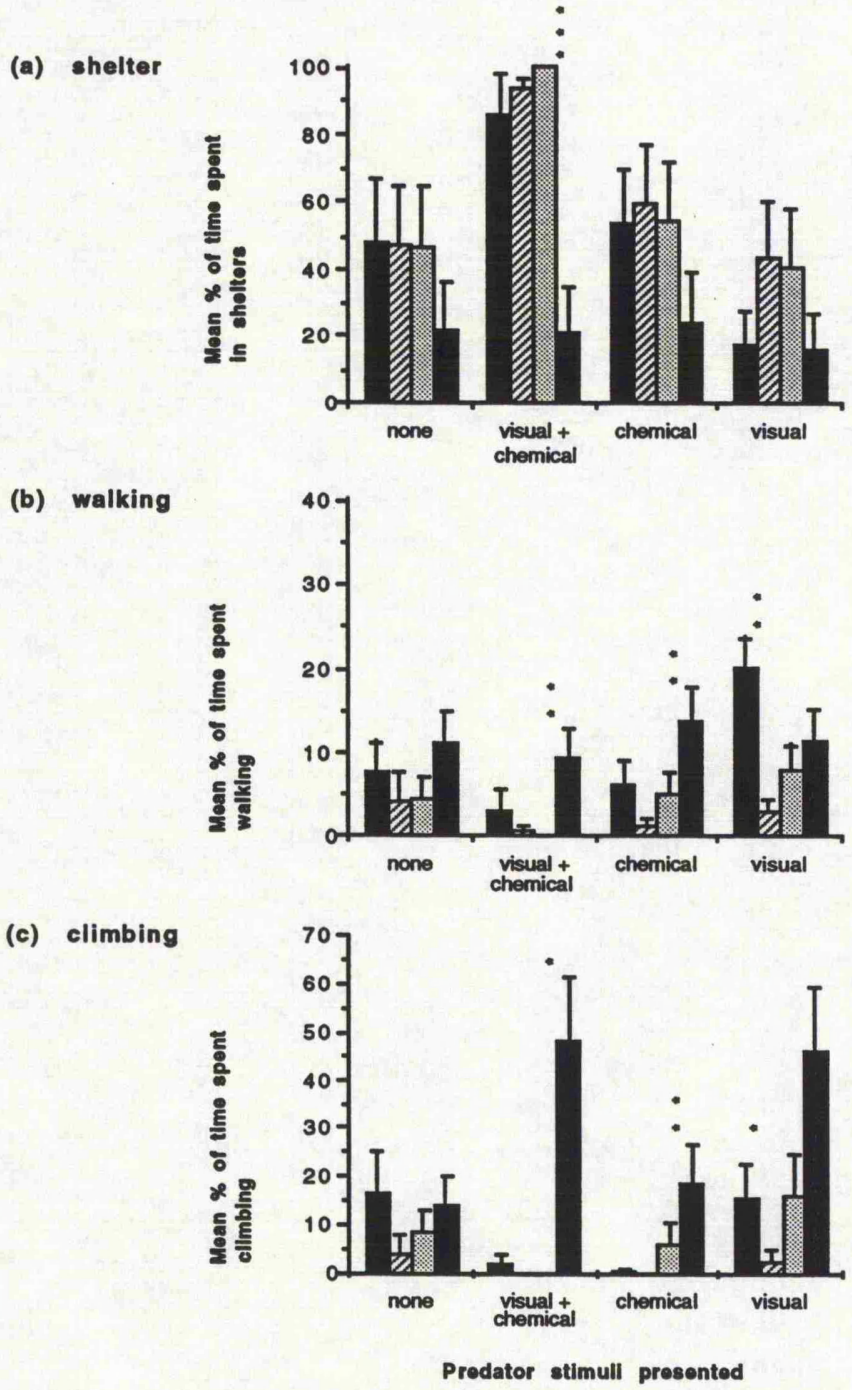


Figure 3.1. The percentage of time spent by crayfish a) in shelters, b) walking , and c) climbing in each 30-minute observation period in Experiment 3.1, (with eels as the predator). Values are means, (±1 S.E.) of the percentage of 30-second counts spent in each behaviour. Time periods are pre-dawn ■ , post-dawn ▨ , pre-dusk ▩ , and post-dusk ■ . Asterisks denote levels of significance for Wilcoxon pair-wise comparisons between adjacent time periods (*p<0.05; **p<0.025; ***p<0.01).

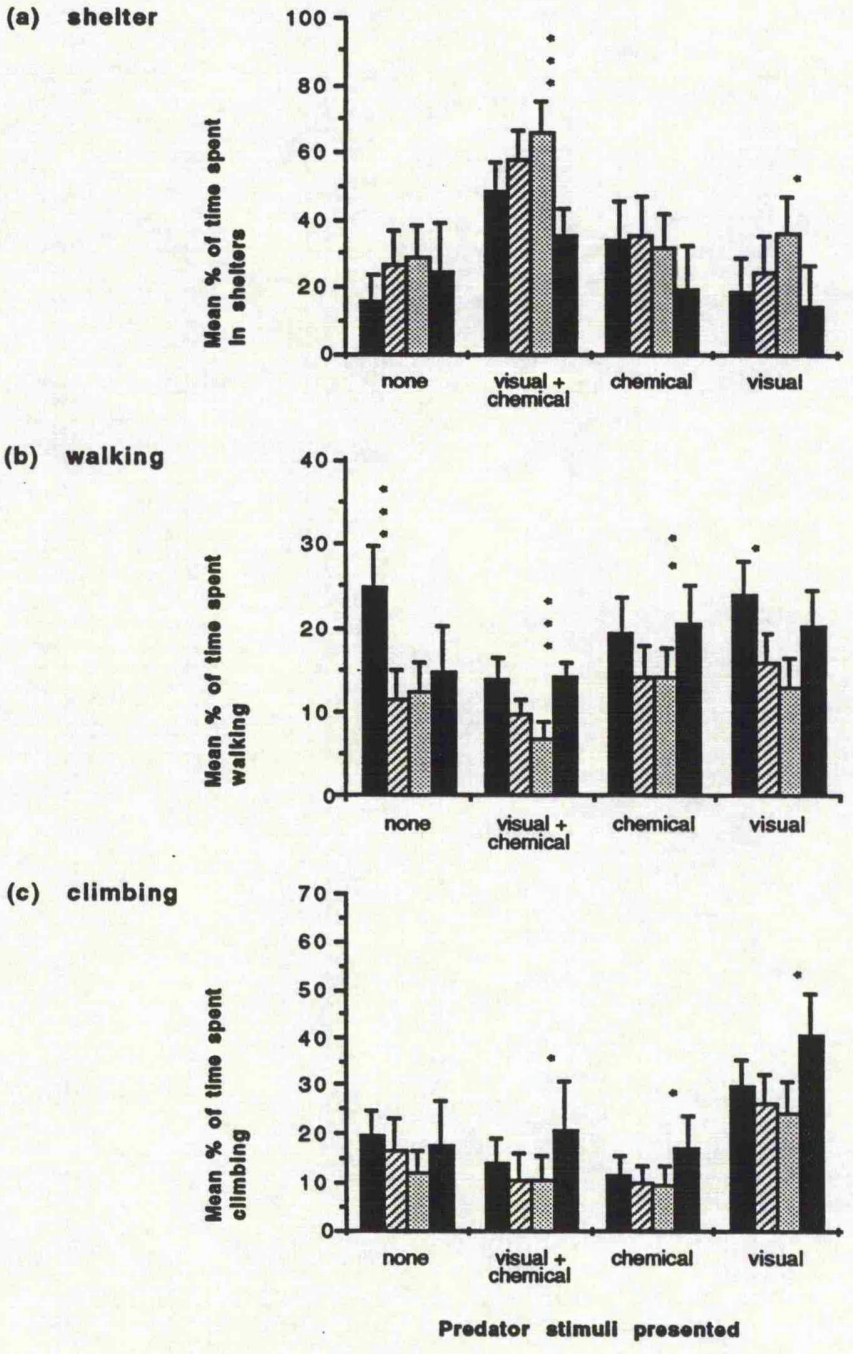


Figure 3.2. The percentage of time spent by crayfish a) in shelters, b) walking , and c) climbing in each 30-minute observation period in Experiment 3.2, (perch as the predator). Values are means, (± 1 S.E.) of the percentage of 30-second counts spent in each behaviour. Time periods are pre-dawn ■ , post-dawn ▨, pre-dusk ▩, and post-dusk ■ . Asterisks denote levels of significance for Wilcoxon pair-wise comparisons between adjacent time periods (*p<0.05; **p<0.025; ***p<0.01).

Crayfish did not orientate specifically to the head of the eels. They often reacted to the movement of the tail as it passed by. These data also indicate that crayfish were able to see predators during the dark, possibly because red light was emitted by the infrared lamps.

After dusk, crayfish behaviour did not alter in response to seeing and/or smelling the fish, with the exception of climbing behaviour in response to seeing eels ($H=5.1$, $df=1$, $m=16$, $n=16$, $p<0.025$). Climbing activity occurred more than twice as frequently in the two treatments where crayfish could see eels, and may have been influenced by the fact that these aquaria had clear sides (Fig. 3.1c). It may be that the crayfish could perceive the tank beyond the confines of the aquaria in these treatments and that this influenced their attempts to climb out of the aquaria. A similar trend in climbing behaviour was evident in response to perch, but this was not significant ($p>0.05$; Fig. 3.2c).

Crayfish Behaviour in the Shelters

Generally, crayfish did not show a preference for any class of behaviour when they were inside the shelters. Some crayfish that could see and smell eels took up 'defensive' positions more frequently during the two periods of light but these differences were not significant (Wilcoxon Signed-Rank test, $p>0.05$; Fig. 3.3). Crayfish which could see and smell perch took up more 'active' shelter positions during the two periods of darkness (Wilcoxon Signed-Rank test; pre-dawn, $T^+=28$, $n=7$, $p<0.05$; post dusk, $T^+=27$, $n=7$, $p<0.05$).

Crayfish Behaviour In Response To Changes In Illumination

In control treatments, crayfish behaviour was not influenced by changes in illumination except that crayfish spent less time walking after dawn in Experiment 3.2 (Table 3.5 and 3.6). Crayfish behaviour did not alter at dawn when crayfish could smell the fish. Most crayfish were already within shelters prior to dawn and remained so after dawn. Again, there was one exception. Crayfish that could smell but not see perch spent less time walking after dawn, although this was not significant ($p>0.05$). After dusk, crayfish that could smell fish became much more active, spending less time in shelters and more time walking and climbing (Table 3.5 and 3.6).

Crayfish that could only see eels spent less time walking and more time under shelter after dawn. Crayfish also spent less time walking in response to seeing perch (Fig. 3.1). After dusk, crayfish that could only see perch became more active (Fig. 3.2). No similar change in behaviour occurred in response to seeing eels.

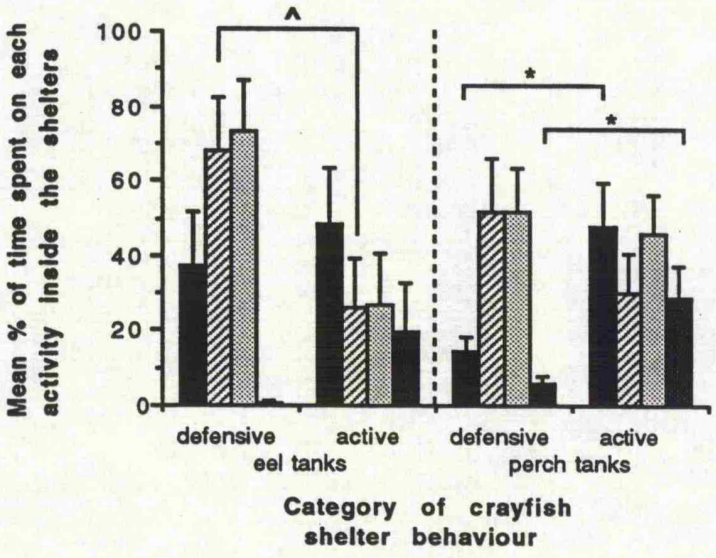


Figure 3.3. The behaviour of crayfish when using shelters in Experiments 3.1 and 3.2 in the treatment with chemical and visual stimuli present. Values are means, (± 1 S.E.) of the percentage of 30-second counts spent in each behaviour. Time periods are pre-dawn ■ , post-dawn ▨ , pre-dusk ▩ , and post-dusk ■ . Levels of significance for Wilcoxon pair-wise comparisons between each time period are: ^p<0.1, *p<0.05).

Table 3.5. The results of Wilcoxon Signed-rank analyses showing the effect of the change in light intensity at dawn on juvenile crayfish behaviour for each treatment in Experiment 3.1 and 3.2. Values of T+, sample sizes and associated probability values, ($p<0.1$) are given in the body of the table. Figures in brackets denote the direction of the behavioural change.

	Time period:	Pre-dawn v Post-dawn							
	Predator :	EEL				PERCH			
Treatment	Behaviour	T+	p	N	effect	T+	p	N	effect
Control. No stimuli	Shelter use		ns	3			ns	4	
	Walking		ns	4		36	<0.01	8	(-)
	Climbing		ns	3			ns	7	
Visual + Chemical Stimuli	Shelter use		ns	4			ns	8	
	Walking		ns	3			ns	7	
	Climbing		ns	2			ns	3	
Chemical stimuli only.	Shelter use		ns	3			ns	4	
	Walking		ns	4		15	<0.07	5	(-)
	Climbing		ns	1			ns	4	
Visual stimuli only.	Shelter use		ns	5			ns	5	
	Walking	28	<0.025	7	(-)	21	<0.05	6	(-)
	Climbing	21	<0.05	6	(-)		ns	7	

Table 3.6. The results of Wilcoxon Signed-rank analyses showing the effect of the change in light intensity at dusk on juvenile crayfish behaviour for each treatment in Experiment 3.1 and 3.2. Values of T^+, sample sizes and associated probability values, ($p<0.1$) are given in the body of the table. Figures in brackets denote the direction of the behavioural change.

	Time period:	Pre-dusk v Post-dusk							
	Predator:	EEL				PERCH			
Treatment	Behaviour	T+	p	N	effect	T+	p	N	effect
Control. No stimuli	Shelter use		ns	3			ns	6	
	Walking		ns	6			ns	7	
	Climbing		ns	5			ns	7	
Visual + Chemical Stimuli	Shelter use	36	<0.01	8	(-)	36	<0.01	8	(-)
	Walking	28	<0.025	7	(+)	36	<0.01	8	(+)
	Climbing	21	<0.05	6	(+)	21	<0.05	6	(+)
Chemical stimuli only.	Shelter use		ns	4			ns	4	
	Walking	28	<0.025	7	(+)	28	<0.025	7	(+)
	Climbing	28	<0.025	7	(+)	21	<0.05	6	(+)
Visual stimuli only.	Shelter use		ns	6		27	<0.05	7	(-)
	Walking		ns	7		25	<0.08	7	(+)
	Climbing		ns	8		27	<0.05	7	(+)

3.5 DISCUSSION

Predators behaved in a similar manner in the two experiments and crayfish responded similarly to both eels and perch. The time spent in the shelters appears to be the best indicator of the defensive state of the crayfish, as they reduced the chance of visual detection by a predator. The use of cover by crayfish to minimise detection has been shown by Stein & Magnuson (1976) and Appelberg & Odelström (1988). The use of shelter by *P. leniusculus* in these experiments is thus interpreted to be an avoidance response. Walking and climbing are most likely to occur in the absence of a predatory threat.

Crayfish (*A. astacus*) tend to be more active at night, particularly in the presence of a crepuscular predator (Hamrin, 1987). In this study, changes in light intensity exerted only a weak influence on crayfish behaviour in the control treatments. Over the first three time periods, crayfish behaviour changed in response to predator scent, confirming previous studies (Hazlett, 1985; Appelberg, pers. comm.). Crayfish showed their most marked changes in behaviour in response to both seeing and smelling the fish. These crayfish spent more time in shelters before dawn and during the day. After dusk there was a switch to locomotary activity. This behaviour corresponded to the loss of the dawn and the enhancement of the dusk peaks in locomotary activity found in *A. astacus* in response to the presence of perch (Hamrin, 1987).

It was hypothesised that visual stimuli would only cause crayfish to show defensive behaviour in the light. This proved to be the case in treatments where only visual stimuli were presented. Due to the experimental design, there was little difference in the behaviour of the two predator species in the tanks. Perch were expected to be more active than eels, thus providing a stronger visual stimulus to the crayfish. The encounter data shows the opposite was true, however, and we observed no conclusive evidence to support the hypothesis that an increased frequency of visual disturbance would produce a stronger avoidance response in crayfish.

If crayfish detect predators chemically, they should show behavioural responses in both the light and dark. This proved to be the case. Before dawn, crayfish increased their use of the shelters in response to predator scent. This behaviour persisted during the post-dawn and pre-dusk periods. As a result, crayfish that could smell the predators showed no change in shelter use in response to dawn. Where crayfish occur sympatricly with crepuscular predators, reducing exposure prior to dawn should be selected for, as this interrupts the chain of predator-prey interactions before a dangerous visual encounter can occur (Endler, 1991). It also enables crayfish to monitor the habitat from within a shelter thus reducing exposure to predators. Such an effect was demonstrated by the behavioural differences between the crayfish that could both see and smell eels and those that could only see eels. The

crayfish in chemical contact with eels were most often within their shelters when a visual encounter took place.

Remaining stationary in a confined space may not be a successful strategy for avoiding predation from predators such as eels that feed by chemoreception. Capture might even be made easier. Thus the increase in walking and climbing by crayfish in response to the reduction in light levels after dusk may reduce the chances of being eaten by nocturnal predators. A further result of the increased movement is that it may increase the chances of crayfish moving to habitats where there is a smaller risk of predation. Crayfish might be expected to commence feeding immediately during the dusk activity peak after a period of reduced diurnal activity. The crayfish, however, spent the majority of the post-dusk period either climbing or walking.

Crustaceans are sensitive to their chemical environment (Ache, 1982). The source of chemical information on the proximity of predators may be the predators themselves or their prey. Prey may release alarm chemicals as a result of physical damage inflicted by a predator, or disturbance chemicals as a result of being disturbed by a predator (Hazlett, 1985; Petranka et al., 1987; Alexander & Covich, 1991). Alarm chemicals are often low molecular weight hydrocarbons which differ between species (Carr, 1988 for review). Fish of the superorder Ostariophysi, posses an alarm chemical system and may learn to respond to other chemicals associated with the release of alarm chemicals. One such chemical stimulus is the scent of the predator (Smith, 1992 for review). Hazlett (1990) suggested that crayfish (*O. virilis*) respond to conspecific disturbance signals comprised of ammonia released from the gills, and pheromones released from the green gland in the excretory system. Whilst their behavioural responses to conspecific disturbance chemicals were greater in magnitude, crayfish also responded to disturbance chemicals produced by different taxa, including the leech *Macrobdella decora* and the darter *Etheostoma exile*. This suggests that some component of the disturbance chemicals, probably ammonia, was common to the different taxa. In the present study, crayfish responded to the predators scent, although it was not possible to determine the nature of the chemicals which produced the response.

Crayfish must obtain chemical information from water which is by nature a turbulant medium. Hence chemical concentrations will be patchy. By extension, information transfer will be discontinuous and additional sensory information is often required to improve search efficiency (Atema, 1988). This is likely to be the case for predator detection by crayfish. In the present study, an interaction of visual and chemical stimuli produced a greater behavioural reaction in crayfish than did either stimulus alone. Such an effect has also been shown in the cyprinid fish, *Leucaspius delineatus* (Heckel) (Rüppell & Gösswein, 1979) and in crayfish, whose latency of response to visual stimuli declined after being exposed to disturbance chemicals

(Hazlett, 1990). It is suggested that, in the present study, chemical perception of predators lowered the threshold of the crayfish avoidance behaviour in response to visual stimuli. This would make evolutionary sense. Behavioural responses should be related to the degree of threat (Stein, 1979). If it is assumed that chemical stimuli act over temporal and spatial ranges greater than those of visual stimuli, then chemical detection of a predator should predispose prey to adopt defensive behaviour before a visual encounter. The distance between predator and prey must be reduced for a visual encounter to occur. This will represent a more dangerous situation for many potential prey species and so the behavioural response should be greater.

CHAPTER 4.

4.0 EVASIVE BEHAVIOUR OF JUVENILE SIGNAL CRAYFISH, *P. LENIUSCULUS*.

4.1 SUMMARY

This study investigated the stimuli which elicit evasive behaviour in juvenile *P. leniusculus*. Juvenile crayfish were exposed to simulated attacks by model predators possessing different features. Evasive behaviour was found to be highly individual. Both mechanical and visual cues produced functional evasive responses although neither the size and shape of the model predators, nor the presence of conspicuous eye patterns affected this behaviour.

Visual and chemical stimuli warning crayfish of the presence of a predator, reduced the duration of subsequent escape responses. It is suggested that alerted crayfish 'assessed' risk more quickly than crayfish which were surprised by an attack, and that the model predators did not possess sufficient stimuli to maintain escape swimming in alert crayfish. This was supported by the fact that crayfish swam less far in escape when the model predators were visually conspicuous by comparison to less conspicuous models.

The adaptivity of crayfish evasive behaviour in response to visual and mechanical stimuli is discussed with respect to the probability of surviving predatory attacks by diurnal and nocturnal predators. This is related to observations on crayfish avoidance behaviour in response to eels and perch. It is suggested that perch are better able to catch crayfish than eels, as a result of their ability to chase fleeing prey. It is also suggested that the preference of crayfish for nocturnal activity is the most adaptive predator avoidance behaviour, as this exposes crayfish to less dangerous predators, and crayfish possess a functional evasive response to combat this risk.

4.2 GENERAL INTRODUCTION

The purpose of this study was to determine 1) the importance of visual, chemical and mechanical stimuli in eliciting evasive behaviour in juvenile *P. leniusculus* 2) to relate this to the likelihood of successful evasion of perch and eel predators by juvenile crayfish and 3) to discuss the implications this has for crayfish populations in the wild.

Predation can be an important factor controlling prey populations (Endler, 1986; Sih, 1987), including crayfish (Taub, 1972; Stein, 1977; Saiki & Tash, 1979). Once predators have encountered prey, the ensuing interactions are usually rapid and energetically costly (Endler, 1991). Prey must recognise a predator at a distance in order to allow time to execute a successful escape. The timing of the flight is critical. Fleeing too early or too late could prove fatal.

The crayfish evasive response consists of an initial tail-flip (startle response) mediated by giant axons, followed by truncated tail-flips (escape response) used in continuous swimming (Wine & Krasne, 1972; Webb, 1979). The initial flexion of the abdomen is stereotyped, but the following abdominal extension is subject to sensory modification, as are the subsequent tail-flips used in escape swimming (Krasne & Wine, 1984; Davey & Mcmillan, 1991). Davey & Macmillan (loc. cit.) found that individual crayfish (*Cherax destructor*) produced their own characteristic trajectories of flight.

The sensory channels used to detect predators should match closely the type of information most indicative of a predatory attack, and should be expected to be functional in response to the most dangerous predator (Endler, 1986). Within a habitat, crayfish must defend themselves against numerous predators with various predation strategies (Hogger, 1988), and therefore, might be expected to respond to more general predatory stimuli. Perch and eels are known to be important predators of crayfish, and both predators can be limiting to the development of crayfish populations (Svärdson, 1972; Dehli, 1981; Appelberg, 1987). Perch forage using mainly visual cues (Disler & Smirnov, 1977) and are crepuscular/diurnal in their feeding habits (Thorpe, 1977). Eels forage principally by using chemoreception (Tesch, 1977) and feed mainly at night (Ryan, 1984).

Crayfish use visual, chemical and mechanical stimuli in social interactions and at least visual and chemical stimuli in predator detection (Tierney & Dunham, 1984; Smith & Dunham, 1990; Hazlett, 1990). The sensitivity of crayfish (*Procambarus simulans*) to visual, chemical and mechanical disturbances was demonstrated by Larimer (1964). Crayfish scaphognathite beats arrested in response to these stimuli, and it was suggested that these responses were an integral part of predator-defence behaviour.

It was shown in (Chapter 3) that visual and chemical stimuli affected crayfish avoidance behaviour. Hazlett (1990), using *O. virilis*, showed that exposure to disturbance chemicals from conspecifics resulted in a reduction in the latency of reaction to threatening visual stimuli and to chemical stimuli associated with food. Smith & Dunham (1990), have shown that sensory deprivation of one effector organ results in compensatory changes in the use of other effector organs. The use of various systems of predator detection would be of great value when predator behaviours are varied.

Because crayfish predators are active by day and night, crayfish should possess systems of predator detection that function in both sets of conditions. The difference in light quality indicates that mechanoreception should compensate for the loss of the visual system at night. Both visual and mechanical stimuli induce a tail-flip startle response in crayfish (Krasne & Wine, 1972). Fast start escapes in teleost fish may be in response to single, often visual stimuli, or to several stimuli (Eaton & Hacket, 1984 for review). Sound, mechanical vibrations and electric fields may also be involved.

Although the structure of teleost fish and crayfish eyes are markedly different, the visual criteria governing escape responses to visual stimuli should be similar. Crayfish have been shown to react to the vertical edges of moving shapes by orientating towards them. They also responded more rapidly to larger objects (Gordan, 1971). Crayfish also exhibit a greater defensive response when confronted by predator movement (Garrison, 1976). Fish prey have been shown to initiate flight when the rate of change of the angle subtended by the predator at the prey's eye reaches a critical threshold value (Dill, 1974a). This visual stimulus 1) acts as a key stimulus that can be associated with numerous different predators 2) can be processed quickly, and 3) allows the reactive distance of the prey to be sensitive to the predator's size and velocity. The shape of the approaching predator also affects the escape response of fish prey (Webb, 1982). Fathead minnows (*Pimephales promelas*) exhibited a higher response threshold for flight when confronted by predators with a round as opposed to an elliptical cross-section. Round-bodied tiger musky (*Esox* spp.) caused fewer escape responses and were also more successful at capturing prey that exhibited an escape response.

Movements of fish produce mass movements of water and also cause vibrational disturbances within the water (Weise, 1988). Fish moving rapidly are preceded by fast pressure pulses of water which are detectable by other fish at distances of 2-3 body lengths (Gray & Denton, 1991). The mechanosensory system of crayfish is similar in design to the lateral line system of teleost fish, and should provide information on the direction of movement and the nature of a signal source (Weise, loc. cit.).

This study investigated the flexibility of the evasive reaction of juvenile crayfish in response to different predatory stimuli. Elements of a predatory attack

were simulated using model predators. Crayfish evaded an attacking predator by swimming backwards, propelled by a rapid series of tail-flips. Aspects of this evasive reaction were used to determine the importance of visual, mechanical and chemical stimuli in eliciting flight. The dynamics of the flight reaction in response to model predators (Sections 4.3 to 4.11) were related to the outcome of experimental interactions between juvenile crayfish and two predators with different foraging strategies, the European perch and the European eel (Section 4.12).

4.3 GENERAL MATERIALS AND METHODS

Experiments 4.3 to 4.11 were conducted in a plastic tank 1.5 m long by 1.0 m wide, divided by a partition into a holding area and a test arena (Figure 4.1). The holding area contained a transparent plastic holding chamber in which individual crayfish were contained prior to an experimental trial. Removal of a rubber bung at the bottom of the holding chamber allowed the crayfish to enter a second chamber with a sloping bottom. This chamber directed the crayfish through a partition door into a plastic walkway, which was situated on the floor of the test arena.

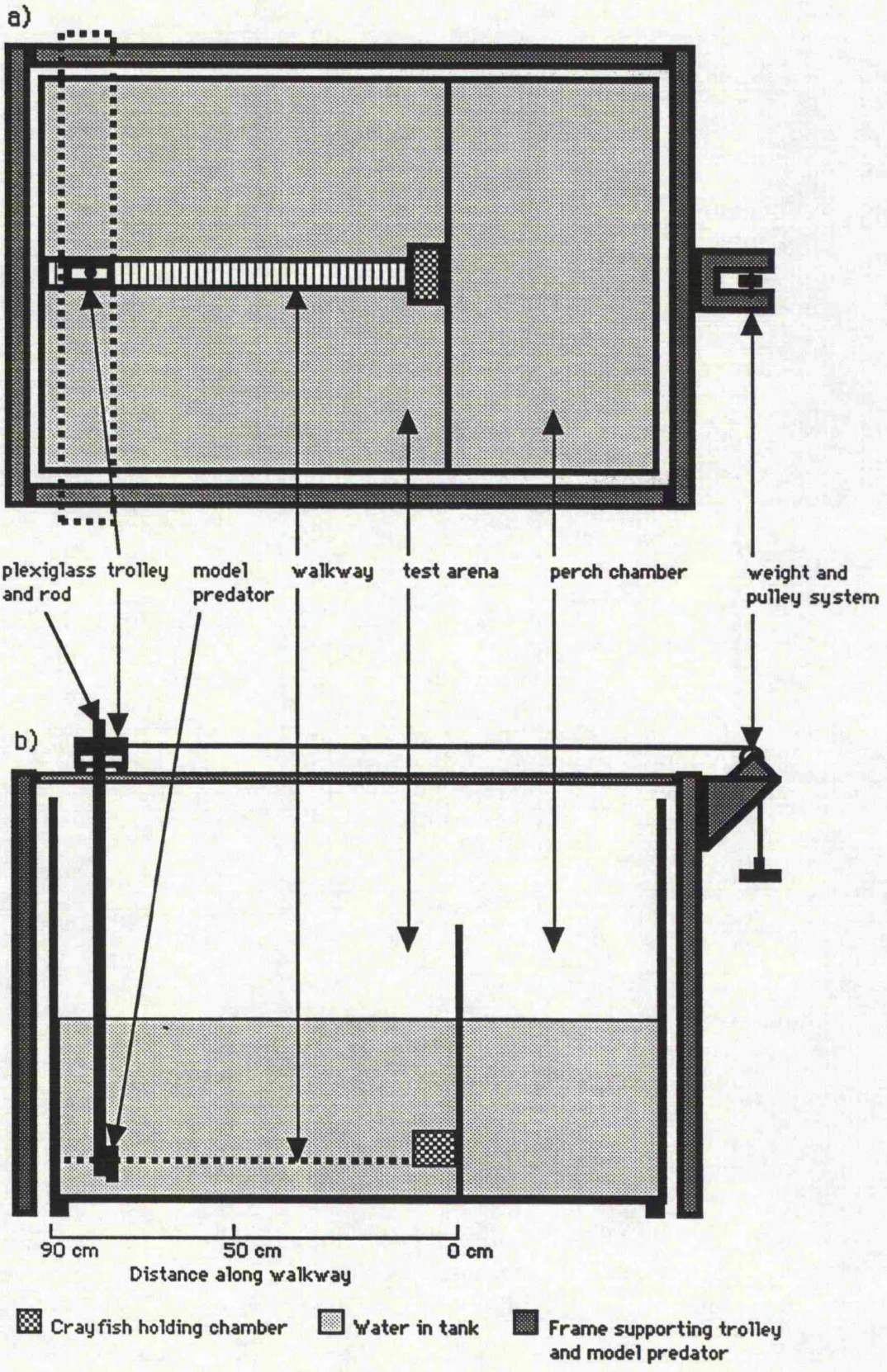
The first holding chamber was used in the initial two experiments (4.4 & 4.5), however, problems were encountered in getting crayfish to enter the walkway. A new system was subsequently employed within the test arena. A chamber with a sliding floor was placed over the walkway. Forty-eight hours before each experiment, crayfish were stored in aquaria adjacent to the experimental tanks. Forty-five minutes before each experimental trial, a crayfish was removed from an aquaria and was placed in the holding chamber in the experimental tank. Crayfish were released into the plastic walkway in the test arena by removing the sliding floor panel.

The plastic walkway was made from a 5 cm deep, 1 m length of house guttering, whose side walls sloped from a width of 10.5 cm at the top to 7 cm at its base. Crayfish entering the walkway were directed along its length by the walls. At the far end of the walkway a model predator was suspended on a 1 m long plexiglass rod, supported from above by a plexiglass trolley which was powered using a pulley and weight system. The use of plexiglass for these objects was designed to render them invisible to the crayfish eye. The trolley was housed on a supporting frame upon which it could move along the length of the test arena. The frame supporting the trolley and weights was isolated from the tank to minimise the transmission of vibrational disturbance from the trolley's movement to the test arena. In order to further minimise the transmission of vibrations, pads of paper were inserted between the floor and the trolley frame.

When crayfish had walked to a predetermined point along the walkway, the weight was released and the trolley was propelled forward. Initially, the movement of the trolley was arrested after 40 cm by two rubber clad sleepers. For Experiment 4.10 the sleepers were removed and the model predator was brought to rest by frictional forces.

The test arena was lit by two 60-watt lights which gave an illumination of 6 microeinsteins cm^{-2} sec^{-1}. The water temperature was 15 °C in both the test arena and the crayfish storage tanks. The crayfish were kept under a 10:14 light:dark light regime.

Figure 4.1. a) the top view, and b) the side view of the apparatus used in experiments testing the evasive response of juvenile crayfish. The figure shows the modified crayfish holding chamber used in Experiments 4.6 to 4.10.



In all experiments, the following behavioural variables were recorded for each trial: 1) Walking Speed. This was measured as the average walking speed of each crayfish along the walkway, towards the model predator. If walking and observation of the environment (i.e. vigilance) were mutually exclusive activities, then this measurement should give an indication of the state of alertness of crayfish. Slower average walking speeds could be an indication that crayfish were spending more time observing the environment for a potential threat. This could then affect crayfish behaviour in response to the experimental trials.

2) Model predator velocity. This was the average speed of the model predator over a predetermined distance from its starting point. This was measured for each simulated attack and varied to a small degree between experimental trials (See experiments described below). The distance the model predators travelled was also altered for different experiments. As a result, different model predator speeds were measured in each experiment, however, model predator speed was not used as a treatment variable either within or between experiments.

3) Stopping Distance. The distance from the advancing model predator at which crayfish stopped walking. (i.e. perceptive distance).

4) Reaction Distance. The distance from the advancing model predator at which crayfish initiated a tail-flip evasive response.

5) Swimming speed. The speed of the backward flight of crayfish, measured over the first 5 to15 cm of the swimming response. The distance varied depending on how far crayfish swam. If crayfish only swam a short distance (~10 cm), then only a small part of this distance involved powered swimming. The rest involved crayfish cruising to a stop with the tail extended. Therefore, to get a better estimate of escape speed, this was only measured whilst crayfish were actively swimming.

6) Swimming Distance. The total distance travelled by crayfish during the tail-flip evasive response.

Crayfish behaviour was recorded using a Panasonic video recorder and camera. The recording speed of this equipment was 50 frames per second. A built in stopwatch allowed behavioural events to be timed to 1/10 th of a second. Timings to 1/50th of a second were made by counting the individual frames passing within 1/10 th of a second on a video screen. Measurements of distance were made by reference to 0.5 cm wide, black and white striped markings which were drawn along the length of the crayfish walkway. Distances were measured at the anterior tip of the rostrum on the cephalo-thorax. Crayfish were obtained from farmed supplies at Riversdale Farm, near Gillingham, Dorset and Kingcombe Crayfish, near Beaminster, Dorset, England.

Experiment 4.4. THE EFFECT OF PREDATOR SHAPE ON THE EVASIVE BEAHAVIOUR OF JUVENILES.

4.4.1 INTRODUCTION

The shape of the approaching predator affected the escape response of fathead minnows, *P. promelas* (Webb, 1982). They exhibited a higher response threshold for flight when confronted by predators with a round as opposed to an elliptical cross-section. Round-bodied tiger musky (*Esox* spp.) caused fewer escape responses and were also more successful at capturing prey that exhibited an escape response.

This experiment was designed to determine whether predator shape influenced crayfish evasive behaviour. Four predator silhouettes were accelerated towards juvenile crayfish. These models provided a basis for determining whether vertical or horizontal predator dimensions affect the reaction threshold of juvenile crayfish. The four predator models were:

1) the transparent plexiglass rod, (this was used to mount the predator models and constituted a control),
2) a circular shape 3 cm in diameter,
3) an elliptical shape 3 cm wide and 7 cm tall,
4) an elliptical shape 7 cm wide and 3 cm tall.

4.4.2 MATERIALS AND METHODS

In this experiment, it was necessary to prevent crayfish from detecting the transmission of mechanical waves, produced by the movement of the model predator through the water. To this end, the model predator and plexiglass rod were contained within a square-sided glass aquarium, 30 cm wide and 45 cm long. To ensure that the predator models were visible to crayfish through the glass, the lights were situated above the aquarium which was also filled with water. Care was also taken to prevent the predator model from hitting the glass and thus giving rise to mechanical disturbances.

Crayfish between 32.8 and 44.6 mm in total length (mean 38.4 mm, S.D.=4.0) were used in the experiment. Crayfish were released into the test arena individually, and when they had walked along the walkway to a point 5 cm from the glass side of the aquarium, the weight attached to the plexiglass rod was released and the predator model accelerated towards the crayfish. The trial was repeated if a crayfish failed to walk along the walkway after 15 minutes or if it climbed out of the walkway. After three failures, crayfish were replaced in the storage tank and were not tested further that day.

Five trials were conducted on each day. Crayfish (*Orconectes* species) release chemical alarm signals in response to a predatory threat (Hazlett, 1990). As there was a possibility that *P. leniusculus* might also release alarm chemicals, the first trial per day was used as a control to ensure that these chemicals would be present in all the subsequent trials of each day. As a result of the need for five trials a day, five experimental treatments were used in Experiment 4.4 (Table 4.1).

Table 4.1. Visual stimuli used in experimental treatments in Experiment 4.4.

Treatment	Abbreviated name	Description of treatment
1	Scent control	A vertically extended elliptical model, 7 cm tall and 3 cm wide. This was used in the first trial of each day to control for the possible production of disturbance chemicals by crayfish.
2	Visual control	No predator model was attached to the end of the plexiglass rod used to carry the models in the other trials.
3	Circular model	A circular predator model 3 cm in diameter.
4	Vertical model	A vertically extended elliptical model, 7 cm tall and 3 cm wide.
5	Horizontal model	A horizontally extended elliptical model, 7 cm wide and 3 cm tall.

Five crayfish were tested on five consecutive days before being replaced by a second set of five crayfish. Crayfish and treatments were rotated with respect to the time of the day that they were used. Where possible, each crayfish was exposed once to each treatment. During the course of the experiment, 39 trials were recorded for the five treatments using a total of ten crayfish. The results were analysed to test for between-treatment differences in the frequency of evasive responses and the dynamics of crayfish evasive behaviour.

4.4.3 RESULTS AND DISCUSSION

Fifteen of 39 trials produced a crayfish tail-flip escape response. In all of the trials, crayfish stopped walking in response to either a predator model or the plexiglass rod. Table 4.2 shows the number of trials per treatment and the number of evasive reactions recorded per treatment. Trials were lost in some instances due to crayfish escaping from, or failing to walk along the walkway.

Table 4.2. Treatments in Experiment 4.4 in which the crayfish showed an evasive reaction. The percentage of trials for each treatment that elicited a reaction are given in brackets.

Treatment	Description	Stimulus presented	Number of trials	Evasive responses
1	control for scent	vertical model	8	6 (75)
2	visual control	plexiglass rod	8	0 (0)
3	visual	circular model	7	3 (43)
4	visual	vertical model	7	3 (43)
5	visual	horizontal model	9	3 (33)
Total			39	15 --

Table 4.3. Reaction variables measured for crayfish exhibiting evasive behaviour in response to each model predator treatment. Values are means with standard errors in brackets. Evasive reaction variables are pooled for the 3 model predator shapes.

Treatment	Reaction Variables				
	Walking speed (cm/s)	Predator speed (cm/s)	Stopping distance (cm)	Reaction distance (cm)	Swimming speed (cm/s)
Scent control (1st trial/day)	2.4 (0.4)	38.5 (1.8)	23.1 (2.8)	6.8 (0.8)	55.8 (6.0)
Visual control	2.8 (0.6)	37.3 (1.5)	27.2 (2.1)	---	---
Circular model	1.8 (0.3)	40.1 (1.6)	21.8 (3.7)		
Vertical model	2.9 (0.3)	39.5 (0.9)	25.1 (1.7)	12.6 (2.9)	60.1 (3.0)
Horizontal model	2.3 (0.5)	40.6 (1.8)	19.8 (4.4)		

Crayfish behaviour in the first trial of each day did not differ from crayfish behaviour in the subsequent trials of the day. This indicated that disturbance chemicals were either not released or that they were released but had no effect on crayfish behaviour. Also, the frequency of the evasive response did not differ between the first and subsequent trials of each day a) when only the data from the vertically extended predator model are included in the analysis, and b) when data from all the model predator shapes were included in the analysis (Fisher's Exact test; $p>0.05$, $n=15$,

and $p>0.05$, $n=39$ respectively).

No differences were found in walking speed, model predator velocity or stopping distance between treatments (Table 4.3). The frequency of tail-flip reactions was significantly greater in the three treatments with model predators than for the visual control treatment, with only the plexiglass rod (Fisher's Exact Test, $p<0.05$, $n=31$). This was also the case when readings from the initial trial of each day were combined with the other model predator treatments (Fisher's Exact test, $p<0.025$, $n=39$).

Walking speeds and stopping distances were similar for crayfish which showed an evasive response and for those which did not. The data for the visual control trials were excluded from this analysis.

Table 4.4. Days on which each crayfish exhibited an evasive response in Experiment 4.4. Treatments involved are given in the body of the table in brackets.

Crayfish	Trial number/crayfish 1	2	3	4	5	Reactions per crayfish	Trials per crayfish
1						0	4
2		√ (3)			√ (1)	2	4
3				√ (1)		1	2
4		√ (5)				1	4
5			√ (4)			1	3
6	√ (1)	√ (3)	√ (5)			3	5
7						0	5
8			√ (3)	√ (1)		2	4
9	√ (4)		√ (1)			2	4
10	√ (5)	√ (1)	√ (4)			3	4
Total reactions	3	4	5	2	1	15	
Total trials	5	8	9	10	7		39
% reactions per trial	60	50	56	20	14		

To investigate further the possible determinants of the crayfish evasive reaction, the data was inspected for an effect of either crayfish orientation at the time the model predator began to move, or habituation to the simulated predator attacks

with each trial (Table 4.4). Evasive reactions were spread across individual crayfish and no pattern was evident with respect to crayfish orientation, despite the possibility of the crayfish being to one side of the walkway and also of facing slightly away from the approaching predator model. The fact that only 3 of the 15 evasive reactions occurred on the last two days of the experiment indicates that crayfish may have habituated to the test apparatus.

The fact that crayfish did not exhibit evasive behaviour in response to all of the simulated predator attacks, suggests that either visual stimuli alone represent a weak threat to crayfish, or that predator models possessed too few visual 'predator' characteristics to elicit evasive behaviour consistently. This may have been a result of reflected light from the side of the aquarium preventing crayfish from seeing the advancing model predator clearly. This is unlikely, as 50% of the attacks did elicit an evasive response. As a result of limitations on the number of crayfish available for the following experiments and of the time available to conduct the experiments, it was important to be able to elicit evasive responses in crayfish consistently. Therefore, the following experiment attempted to determine whether important releasing stimuli were missing from the simulated predator attacks described above.

Experiment 4.5 THE ROLE OF MECHANICAL STIMULI IN ELICITING EVASIVE BEHAVIOUR.

4.5.1 INTRODUCTION

In the previous experiment (Experiment 4.4), crayfish exhibited evasive behaviour in only ~50% of the simulated predator attacks in response to visual stimuli. Simultaneous perception of several stimuli can produce a greater behavioural response than perception of a single stimulus in isolation. Rüppell & Gösswein (1979) found that pike isolated behind glass failed to produce the same degree of shoaling in the cyprinid *Leucaspius delineatus* Heckel as a free swimming pike. Chemical and/or mechanical cues were also important. It was the aim of this experiment to determine whether mechanical stimuli, produced by a model predator advancing through the water towards crayfish, were important in eliciting crayfish evasive behaviour when visual stimuli were also present.

4.5.2 MATERIALS AND METHODS

The experimental procedure for this experiment was as described for Experiment 4.4. Six treatments were used in Experiment 4.5. These are detailed in (Table 4.5). An elliptical model 7 cm tall and 3 cm wide was used as a visual representation of a predator. For the treatments without mechanical stimuli, the predator model or plexiglass rod were contained within a glass aquarium, as described above (Experiment 4.4). When mechanical stimuli were required, the aquarium was removed. Five trials were conducted per day. The first trial of each day was used to contol for the possible release of alarm chemicals into the test arena by startled crayfish. Two treatments were used to control for the effect of alarm signals (Table 4.5). These were used in the first trials of alternate days throughout the experiment.

Twelve crayfish, between 34.2 to 44.5 mm in total length (mean 39.5 mm, S.D.=2.7), were used in 49 trials of the six experimental treatments. Each individual crayfish was used in five trials except for five cases. Four of these were a result of two crayfish escaping from the storage aquaria. These crayfish were replaced by new individuals. The fifth case was a result of one crayfish persistently failing to walk along the walkway during it's final trial.

Table 4.5. Treatments used in Experiment 4.5. Model predators and crayfish were isolated by glass in the visual only treatments. Treatments 1 and 2 were always performed as the first trial per day (alternately) to control for the possible production of disturbance chemicals.

Treatment	Description	Predatory stimuli available		Modelpredator used
		mechanical	visual	
1	Visual control for the possible production of disturbance chemicals	no	yes	vertical ellipse
2	Mechanical+visual control for the possible production of disturbance chemicals	yes	yes	vertical ellipse
3	Only visual stimuli	no	yes	vertical ellipse
4	Visual only control	no	no	none (plexiglass rod only)
5	Mechanical + visual stimuli	yes	yes	vertical ellipse
6	mechanical + visual control	yes	no	none (plexiglass rod only)

4.5.3 RESULTS AND DISCUSSION

Of the 49 trials, 26 resulted in a crayfish tail-flip evasive response. Table 4.6 gives details of the number of trials and reactions per treatment. As in Experiment 4.4, crayfish stopped walking in response to all of the treatments.

Evasive behaviour did not differ between treatments but model predator velocity did (Kruscal-Wallis, $H=15.74$, $n=47$, $df=5$, $p<0.01$; Table 4.7). There was no difference in the frequency of evasive reactions or in crayfish evasive behaviour between the first and the subsequent trials of each day, indicating that either disturbance chemicals were not produced by startled crayfish, or they were produced but did not effect crayfish behaviour. As a result of this, data from the first trials of each day were combined with the corresponding data from the subsequent trials (treatments 1 and 3, and treatments 2 and 5 were combined). These pairs of treatments will be referred to as visual and mechanical + visual treatments below.

Table 4.6. Treatments in Experiment 4.5 in which crayfish showed an evasive reaction together with the percentage of trials per treatment eliciting a reaction. Figures in brackets denote trials for which data was pooled in subsequent analyses.

Treatment	Description	Stimulus presented	Number of trials	Evasive responses	(%)
1 (a)	visual control for scent	visual	5	2	40
2 (b)	mechanical + visual control for scent	mechanical +visual	4	3	75
3 (a)	Visual only	visual	10	6	60
4	Visual only control	none	10	2	20
5 (b)	Mechanical + visual	mechanical +visual	10	9	90
6	Mechanical +visual control	mechanical	10	4	40
Total			49	26	-

Model predator velocities were on average 3 cm/sec greater in the mechanical + visual treatments than in the visual treatments (Mann-Whitney U test, U=58, m= 14, n=15, $p<0.05$). Model predator velocities were also greater in treatments where an evasive reaction occurred than in treatments where crayfish failed to react (Mann-Whitney U Test, U=164.5, m=22, n=25, $p<0.05$). The frequency of evasive reactions tended to be greater when mechanical and visual stimuli were presented together than when visual stimuli were presented alone (Fisher's Exact test, $p=0.068$, n=29).

Because more evasive reactions occurred in response to mechanical + visual stimuli, and these treatments had faster model predator velocities, a test was conducted on the treatments with only visual stimuli to ascertain whether model predator velocity was a causal factor eliciting an evasive response. Model predator velocities were compared for those trials in which a response occurred and those in which it did not. No difference was found between these two categories (Meddis Rank Analysis, H=1.918, m=13, n=17, $p>0.1$). To further test the effect of model predator velocity on the evasive reaction, the reaction frequencies of the treatments using the vertical, elliptical models in Experiment 4.4 (treatments 1 and 4) were compared to those in Experiment 4.5 (treatments 1 and 3). The experimental procedures for these treatments were identical except that target speeds in the first experiment were 6 cm/sec greater (Mann-Whitney U Test, U=33.5, m=15, n=15, $p<0.01$). Despite this, the

frequency of evasive responses did not differ between the two experiments (Chi^2test, Chi^2=0, n=30, p>0.1).

Table 4.7. The reaction variables measured for crayfish evasive responses exhibited in each treatment. Values are means with standard errors in brackets. The two controls for the production of disturbance chemicals were presented as the first trial on alternate days.

Treatment	Reaction Variables					
	Walking speed (cm/sec)	Predator speed (cm/sec)	Stopping distance (cm)	Reaction distance (cm)	Swimming speed (cm/sec)	Swimming distance (cm)
Visual scent control	2.7 (0.5)	36.3 (1.6)	28.0 (4.0)	18.7 (9.7)	57.5 (7.5)	11.0 (2.0)
Visual + mechanical scent control	2.2 (1.2)	37.4 (1.4)	27.5 (2.0)	11.1 (1.9)	62.3 (2.9)	28.8 (10.7)
Visual only	1.6 (0.3)	34.6 (0.5)	22.2 (3.1)	8.4 (1.9)	56.7 (3.9)	30.8 (8.6)
Visual control	1.8 (0.4)	31.9 (1.0)	22.1 (3.2)	12.0 (1.5)	62.8 (2.1)	9.7 (0.7)
Mechanical + Visual	2.4 (0.5)	37.2 (0.9)	19.2 (2.3)	8.8 (1.8)	57.1 (3.8)	26.8 (6.0)
Mechanical + visual control	2.6 (0.3)	35.6 (1.0)	20.4 (2.4)	17.7 (4.8)	54.7 (1.5)	31.2 (10.9)

Although model predator velocities were variable, they are not considered to have affected crayfish reaction behaviour or the frequency of reaction in the above analysis. Instead, the greater reaction frequency in response to mechanical + visual stimuli in Experiment 4.5 is considered to be a response to mechanical stimuli. This would suggest that mechanical stimuli offered additional, pertinent information on the nature of the attacking model predator than that which was available from visual stimuli. In order to determine whether mechanical and visual stimuli interact in some way to elicit more evasive responses than either stimulus alone, a further experimental treatment should have been used that presented only mechanical stimuli to crayfish. This was not done in the above experiment, but an experiment is described below (Experiment 4.8) in which the behaviour of crayfish is recorded in response to either mechanical or mechanical + visual stimuli.

The differences in model predator velocity may be due to a systematic error in the reading of the distances and times for the different treatments. The presence of the glass aquarium in the visual treatments meant model predator velocity was measured over 20 cm and not 25 cm as in the mechanical treatments. This was due to

the glass slightly obscuring the model predator from the video camera in the former case. If the weight, and hence model predator were still accelerating from the 20 cm to the 25 cm mark, this may have caused the readings over the shorter distance to produce a smaller estimate of model predator velocity.

Significantly more evasive reactions occurred in response to mechanical treatments compared to the mechanical control (Fischer Exact test, $p=0.028$, $n=24$). Although there were more reactions in the visual treatments by comparison to the visual controls this was not significant (Fischer Exact test, $p>0.1$). It is clear that in some cases, crayfish reacted to both visual and mechanical stimuli produced by the plexiglass pole in the control treatments.

Table 4.8. Showing the days on which each crayfish exhibited an evasive response in Experiment 4.5. The treatments involved are given in the body of the table in brackets.

Crayfish	Trial number/crayfish 1	2	3	4	5	Reactions per crayfish	Trials per crayfish
1	√ (1)					1	1
2	√ (4)		√ (6)	√ (3)		3	5
3		√ (5)	√ (1)			2	3
4						0	4
5		√ (3)		√ (5)		2	5
6		√ (3)				1	4
7	√ (4)	√ (5)				2	2
8	√ (6)	√ (3)	√ (2)		√ (3)	4	5
9	√ (3)		√ (5)			2	5
10		√ (5)	√ (6)	√ (3)	√ (2)	4	5
11	√ (2)	√ (6)			√ (5)	3	5
12	√ (5)			√ (5)		2	5
Total reactions	7	7	5	4	3	26	
total trials	12	11	10	8	8		49
% reactions per trial	58	64	50	50	37		

The evasive reactions were spread between the crayfish although one crayfish showed no evasive reactions at all (Table 4.8). Inspection of the behavioural data suggested that there were individual differences in crayfish evasive behaviour, however, there were insufficient reactions from individual crayfish to test this.

Behavioural differences between individual crayfish may have obscured any differences in reaction to the various treatments. This was examined in Experiment 4.6.

Experiment 4.6. THE EFFECTS OF PREDATOR SIZE AND VISIBILITY ON THE EVASIVE RESPONSE .

4.6.1 INTRODUCTION

The two previous experiments attempted to determine the influence of predator shape and mechanical stimuli on the dynamics of the crayfish evasive reaction. This reaction was shown not to vary with predator model shape but was triggered more frequently in response to mechanical and visual stimuli combined than to visual stimuli alone. One factor involved in the failure to detect differences in crayfish behaviour in response to different treatments may be individual variation in evasive behaviour.

The following experiment was designed to test 1) the effects of predator size and visibility on the crayfish avoidance response and 2) the variability of the evasive response between individual crayfish. To maximise the frequency of the escape responses crayfish in the following experiments were placed in both mechanical and visual contact with the model predators.

4.6.2 MATERIALS AND METHODS

The experimental apparatus was essentially as described for Experiments 4.4 and 4.5. Certain modifications were made to the design. To prevent shadows being cast by the plexiglass trolley, the lights were relocated under the trolley. The method of introducing the crayfish into the test arena was also altered. The holding chamber was relocated on top of the walkway inside the test arena (Fig. 4.1). The new chamber consisted of a small plastic container separated from the walkway by a sliding trap door on its underside. In order to release the crayfish into the test walkway the trap door was opened and the crayfish dropped through into the walkway. The reason for changing this mechanism was to reduce the chances of crayfish escaping from the walkway by climbing up the angle formed by the partition and walkway walls. The new design prevented this.

Crayfish were subjected to four treatments. These consisted of combinations of a large or small target moved against a white or black background, as set out in Table 4.9. Both targets were vertically extended ellipses. The small target was 3 cm wide by 7 cm tall and the large target was 6 cm wide by 14 cm tall. The targets were black and their visibility to crayfish was altered by placing a black or white background behind them.

Ten crayfish were used ranging from 30.6 to 46.8 mm total length (mean 38.8

mm, S.D.=5.5). Each crayfish was initially subjected to each of the four treatments. One crayfish received one extra trial and five of the crayfish were each subjected to a further four trials to test for individual differences in evasive behaviour (Table 4.10).

Table 4.9. A description of the experimental treatments used to test the effect of predator visibility and size on crayfish evasive behaviour.

Treatment	Description	Model predator size	Background colour	Trials	Responses
1	Large conspicuous model predator	large	white	15	15
2	Small conspicuous model predator	small	white	15	15
3	Large inconspicuous model predator	large	black	16	16
4	Small inconspicuous model predator	small	black	15	15

Table 4.10. Showing the number of trials per treatment recieved by each crayfish. All trials produced an evasive response in the crayfish. Crayfish 1-5 were used to test for individual differences in evasive behaviour.

Crayfish	Predator model treatment				Total
	large conspicuous	small conspicuous	large inconspicuous	small inconspicuous	
1	3	3	1	1	8
2	3	2	1	2	8
3	1	2	3	2	8
4	1	1	3	3	8
5	2	2	2	2	8
6	1	1	1	1	4
7	1	1	1	1	4
8	1	1	1	1	4
9	2	1	1	1	5
10	1	1	1	1	4

Five trials were conducted per day due to the possibility that crayfish would produce disturbance chemicals and that this would cause crayfish behaviour between the first and the subsequent trials of each day to differ. The results were analysed to determine if this was the case.

4.6.3 RESULTS AND DISCUSSION

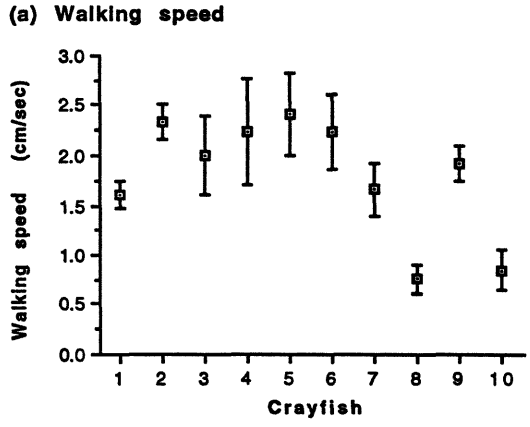
The ten crayfish showed an evasive reaction in all of the 61 trials to which they were subjected. Crayfish behaviour did not differ between trial one and the subsequent trials of each day. In the further analyses, the data for trial one of each day were included with the respective data from the other trials.

The evasive behaviour of each crayfish was compared to determine the extent of the individual variation. Initially this comparison was conducted on the first set of 4 trials for each crayfish and an overall difference was found in the walking speed, reaction distance and the swimming speed of the crayfish (Kruskal-Wallis test; walking speed, df=9, n=40, H=22.29, $p<0.01$; reaction distance df=9, n=41, H=21.85, $p<0.01$; and swimming speed, df=9, n=41, H=21.23, $p<0.02$; Fig. 4.2). As only four replicates per crayfish were available, five of the crayfish were tested for a further four trials. Using the data for these five crayfish, no differences were found in walking speed or swimming speed (Kruskal-Wallis test, df=4, n=40, H=5.478, $p>0.1$; and df=4, n=40, H=5.962, $p>0.1$ respectively). There were differences in reaction distance (Kruskal-Wallis test, df=4, n=40, H=16.54, $P<0.01$), and swimming distance (Kruskal-Wallis test, df=4, N=40, H=11.65, $p<0.05$).

The five crayfish used in the last analysis did not receive exactly the same number of attacks from the four experimental treatments (Table 4.10). It was assumed for the purpose of this analysis that treatment had no affect on crayfish evasive behaviour. This assumption proved invalid with respect to the distance crayfish swam in response to conspicuous and inconspicuous model predators (see below). Therefore, this analysis can only be used as an indication that individual crayfish may vary in their response to predator attacks.

In the light of the possible differences in the evasive responses of individual crayfish, between-treatment comparisons of the crayfish reaction dynamics were analysed using the Wilcoxon signed-rank test. Only the first evasive response of each crayfish to each treatment were used in these analyses. Results for each treatment were paired for each crayfish. There was no overall effect of the four model predator treatments on crayfish evasive behaviour (Friedman's Rank analysis). Data were grouped and the groups were tested individually for an effect of either model predator size or visibility.

As crayfish were exposed to each treatment, then when data were combined to test for an effect of either large versus small, or conspicuous versus inconspicuous predator models, the test used data from each crayfish more than once within each test category. Hence the data points were not strictly independent and measurements of the reactions of each crayfish were averaged within each category. No effects were detected except that crayfish tended to swim further in escape when the model predators were less visible, i.e. moved against the black background (Wilcoxon pairwise comparison, $T^{+}=47$, $n=10$, $p<0.05$). Figure 4.3 shows the mean swimming distances of crayfish recorded for each individual treatment.



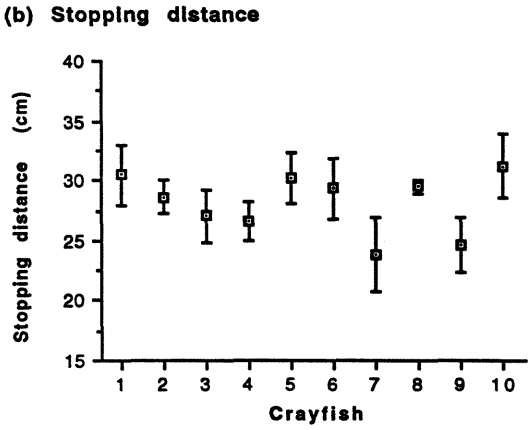
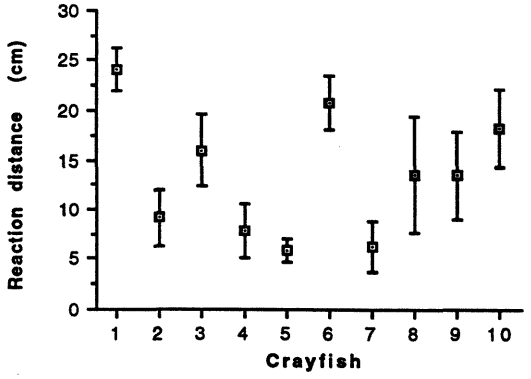
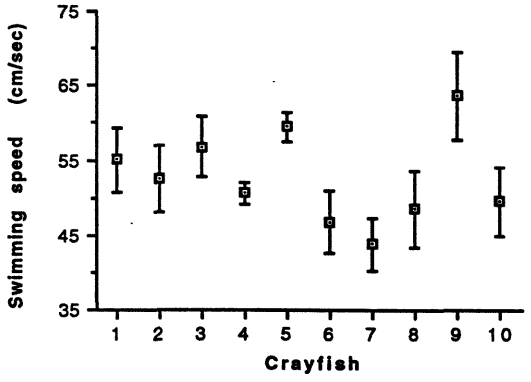


Figure 4.2. The mean values (± 1 S.E.) of reaction variables for the evasive responses of individual crayfish to simulated predator attacks. Reaction variables are a) walking speed, b) stopping distance, c) reaction distance, d) swimming speed, and e) swimming distance. (Number of trials (n) =8 for crayfish 1 to 5, n=4 for crayfish 6 to 8, & 10, and n=5 for crayfish 9).

(c) Reaction distance



(d) Swimming speed



(e) Swimming distance

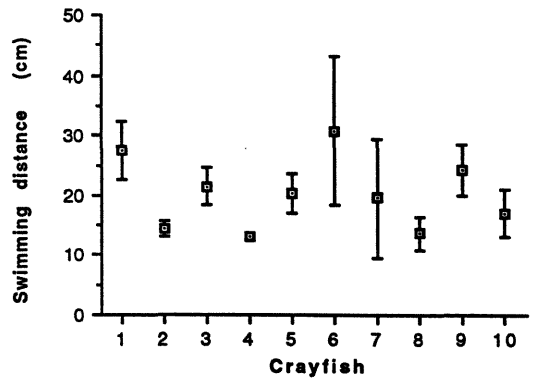


Figure 4.2. (continued)

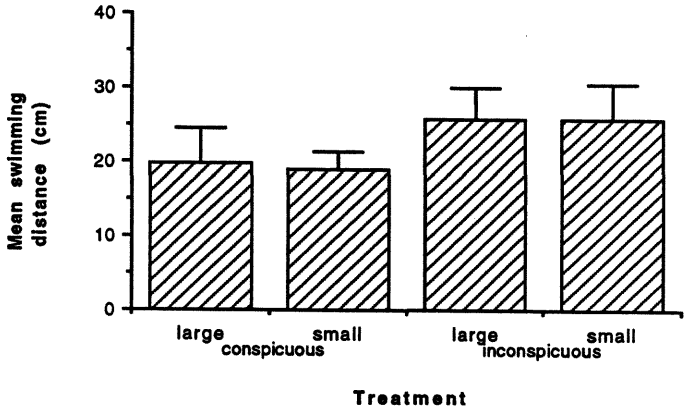


Figure 4.3. The mean swimming distances (± 1 S.E.) of crayfish responding to four simulated predator treatments.

Experiment 4.7. INDIVIDUAL VARIABILITY IN THE EVASIVE BEHAVIOUR OF JUVENILES.

4.7.1 INTRODUCTION

Experiment 4.6 illustrated individual variation in the crayfish evasive response. Stein (1977) showed that the escape behaviour in *O. propinquus* depended on individual body size, age and sex. In general, younger smaller crayfish swam shorter distances and more often terminated their flight with an attempt to hide. Also, males swam more slowly than females of comparable size. This was due to differences in the abdominal morphologies of the sexes. The aim of this experiment was to determine whether body or chelae size, sex, or morphological damage accounted for the individual variability in crayfish evasive behaviour demonstrated in Experiment 4.6.

4.7.2 MATERIALS AND METHODS

Using the apparatus described previously (Section 4.1.3), 57 crayfish, 35 females and 21 males, were exposed to a simulated predator attack once. The predator model was a vertical ellipse, 14 cm tall by 6 cm wide. This was moved against a white background. Crayfish were released from the holding chamber into the test arena after 45 minutes acclimatisation, by removing a sliding floor panel. Crayfish were allowed to walk to a point 55 cm along the walkway, which was 40 cm from the model predator, before the model was accelerated towards the crayfish. If crayfish had failed to move along the walkway after 15 minutes they were removed from the arena and replaced in the holding chamber for a further 30 minutes. Trials on individual crayfish were repeated until an escape response was recorded.

The following morphological information was recorded for each male and female crayfish: total body length (from the tip of the rostrum to the telson tip), maximum chelae length (from the tip of the propodus to the junction of the propodus and the carpus), and chelae to body length ratios (Table 4.11). The incidence of chelae loss or regeneration, and antennal loss or damage were also recorded.

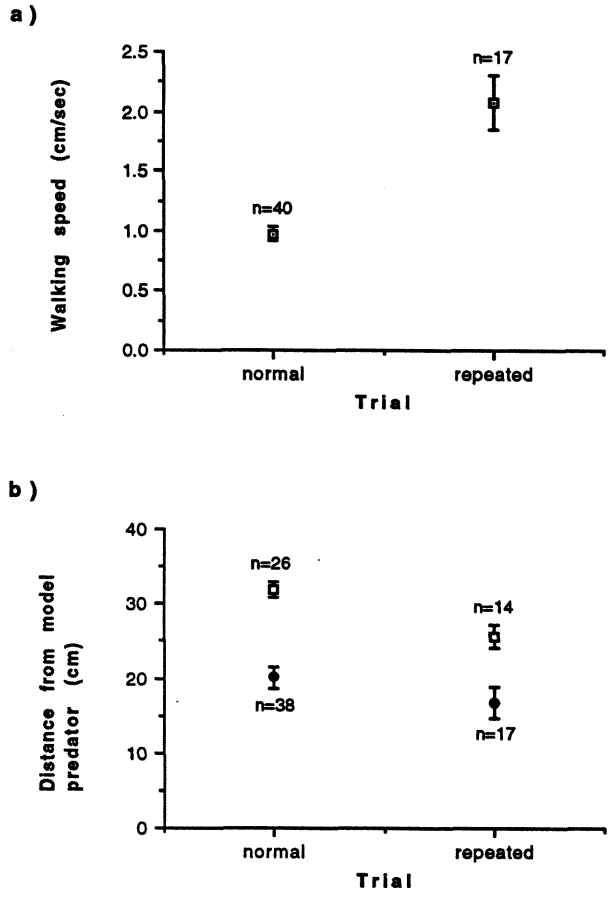


Figure 4.4. Differences in a) walking speed, and b) stopping distance □ , and reaction distance ● , between those crayfish showing an escape response in their first trial and those responding in repeated trials. Values are means (± 1 S.E.).

Table 4.11. Morphological data collected from crayfish used in Experiment 4.7.

Measurement	Sex	Mean	S.D.	N
Total Length	male	36.8	4.8	21
	female	34.6	5.4	35
Chelae length	male	10.8	1.7	21
	female	9.8	1.9	35
Chelae:total length ratio	male	0.29	0.01	21
	female	0.28	0.02	35

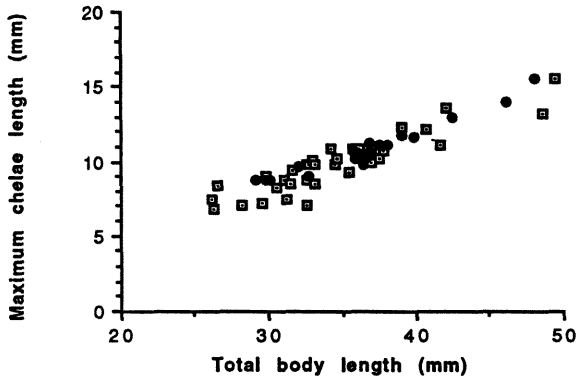


Figure 4.5. The relationship between total body length and maximum chelae length. Data is included for all female □ , and male ● , crayfish.

4.7.3 RESULTS AND DISCUSSION

Fifty-six of the 57 crayfish exhibited an evasive respone to the model predator. Seventeen crayfish required repeated trials. Crayfish in repeated trials walked faster and stopped walking closer to the advancing model predator than crayfish which did not require a repeat trial (Wilcoxon-Mann-Whitney test; walking speed, $U=81.5$, Chi^2 conversion=4.51, $m=17$, $n=40$, $p<0.01$; stopping distance, $U=71.5$, Chi^2 conversion=3.14, $m=14$, $n=26$, $p<0.01$; Fig. 4.4a, b). Repeated trials were required not because crayfish failed to react to the model predator, but because they failed to walk along the walkway in the previous trial. Repeating a trial altered crayish behaviour and for this reason behavioural data from the repeated trials were excluded from further analyses of crayfish evasive behaviour.

The effect of crayfish morphology on evasive behaviour

Morphological data were collected for 56 crayfish. The evasive behaviour of crayfish that had 1) missing or damaged chelae, or 2) damaged antennae, did not differ from the behaviour of crayfish that had no damage (Mann-Whitney test, $p>0.1$)

Maximum chelae length was positively correlated with total body length (Fig. 4.5). Using data from all of the crayfish, males tended to be larger than females with a difference in mean length of ~2 mm (Wilcoxon-Mann-Whitney test; $U=259$, Chi^2 conversion=1.84, $m=21$, $n=35$, $p<0.07$. Table 4.11). Males had longer chelae than females (Wilcoxon-Mann-Whitney test; $U=243.5$, Chi^2 conversion=2.10, $m=21$, $n=35$, $p<0.05$), but there was no difference in chelae:body length ratio. These trends were similar for those crayfish which reacted to the model predator in their first trial, but these were not significant ($p>0.05$). Evasive behaviour did not differ between the sexes.

There was an indication that larger crayfish swam faster and that crayfish with a greater chelae:body length ratio stopped walking closer to the advancing model predator (Spearman's Rank Correlation, both sexes combined, $R=0.37$, Chi^2 conversion=2.19, $n=37$, $p<0.05$; and $R=0.50$, Chi^2 conversion=2.44, $n=25$, $p<0.025$ respectively; Figs. 4.6a, b). In some cases, data on stopping distance and swimming speed were not available.

Crayfish evasive behaviour

The frequency distributions of the five measurements of crayfish evasive behaviour are presented in Figure 4.7. Walking speeds were skewed towards slower

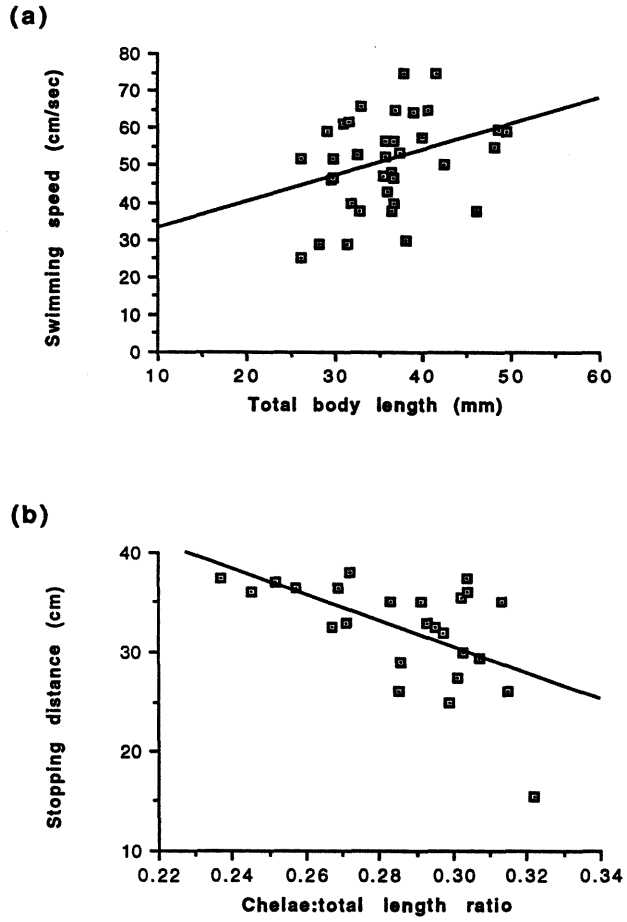


Figure 4.6. The relationship between a) total body length and swimming speed, and b) the chelae:total length ratio and stopping distance of crayfish reacting to the model predator in their first trial.

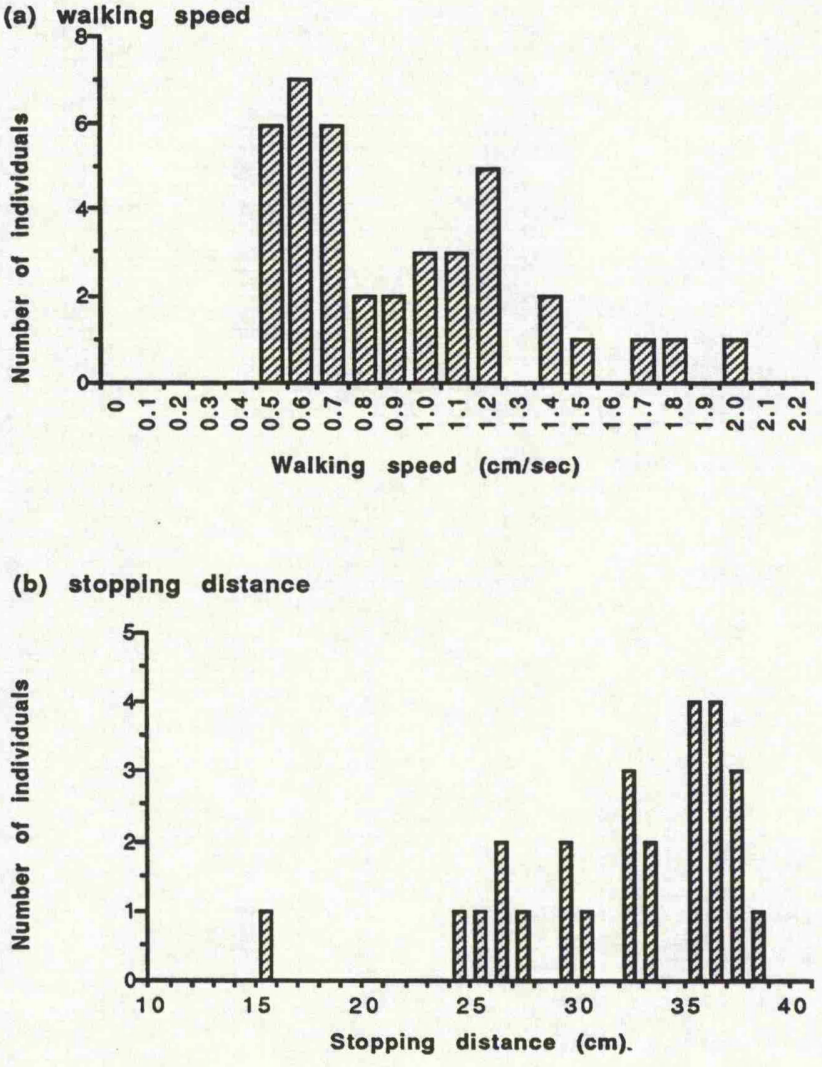
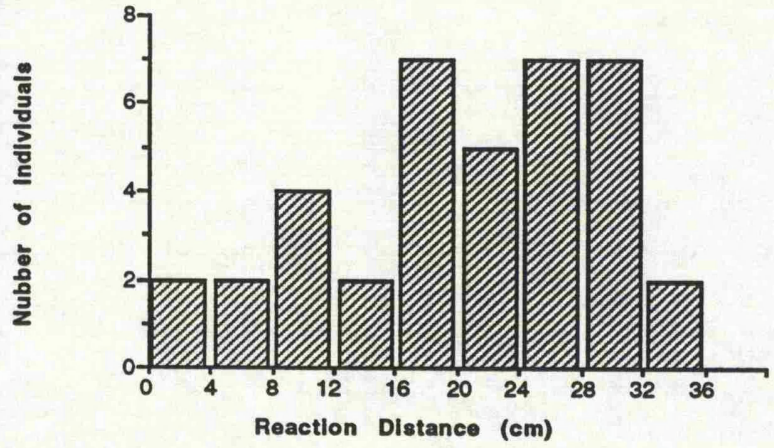
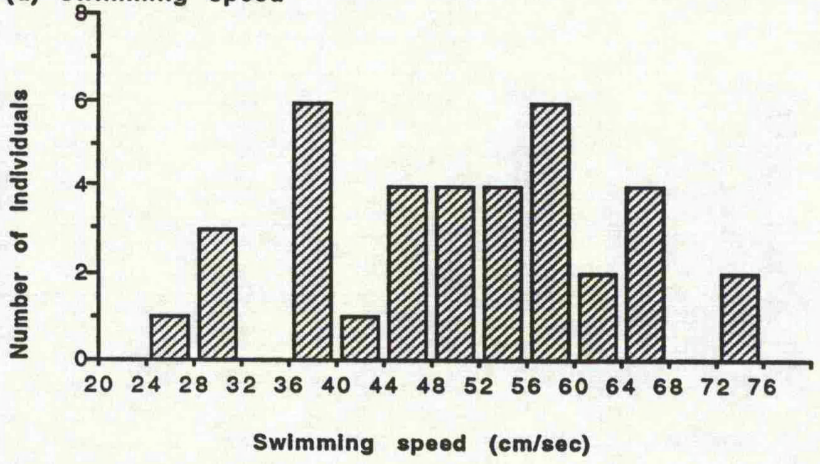


Figure 4.7. Frequency distribution patterns of crayfish escape response variables measured for crayfish in Experiment (4.7), only including data from crayfish which exhibited an escape response in their first trial. Frequency distributions are: a) average crayfish walking speed, b) the distance from the advancing model predator at which crayfish stopped walking, c) the distance from the advancing model predator at which crayfish initiated a tail-flip evasive response, d) the average backward swimming speed of crayfish during the evasive response, and e) the distance swam by crayfish during the escape response.

(c) reaction distance



(d) swimming speed



(e) swimming distance

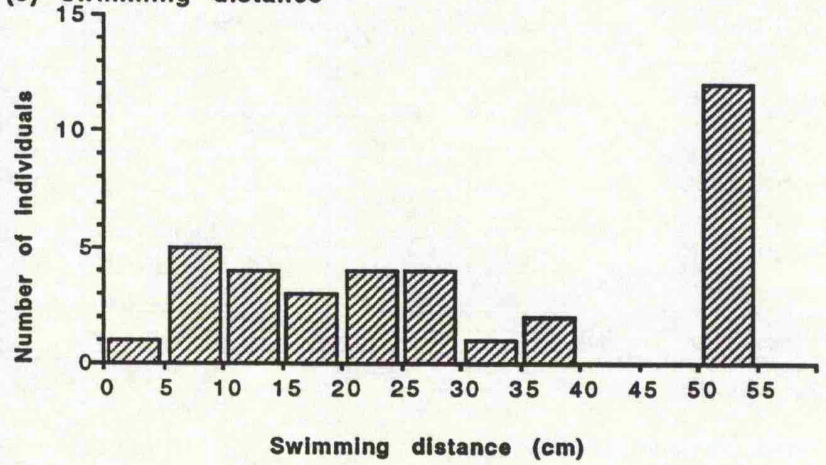


Figure 4.7 (continued)

velocities, whilst stopping and reaction distances were skewed towards larger distances. Swimming speeds appeared to be normally distributed whilst no clear pattern was evident for swimming distances. The peak at the longer distances was an artifact of the spatial constraints of the test arena. Crayfish reaching this point could swim no further.

Crayfish swimming distances were correlated with reaction distance and swimming speed (Spearman's Rank Correlation, R=0.49, Chi^2 conversion=2.85, n=36, $p<0.005$; and R=0.67, Chi^2 conversion=3.99, n=36, $p<0.001$ respectively; Fig. 4.8a, b). Measurements of swimming speed for crayfish which only swam a short distance (~ 10 cm) tended to be biased by the fact that crayfish were already decelerating during part of these measurements. Crayfish swimming longer distances (>10 cm) exhibited repeated tailflips and maintained a high speed throughout the measurement period.

The relationship between reaction distance and swimming distance may reflect individual variability in risk assessment, which manifests itself in both behaviours, i.e. crayfish that perceive more risk will tend to react earlier and also to prolong their escape. The relationship may also be a direct result of differential pursuit times. Crayfish that reacted early to the model predator were also 'chased' for longer, as the model predators travelled a constant distance. Increased pursuit time may have increased the perceived threat, which caused crayfish to swim further in escape.

Crayfish were between 36 to 40 cm from the model predator as it began to move. Crayfish that had already stopped walking when the model predator began to move were further from the model predator than crayfish that were still walking at this point in time (Wilcoxon-Mann-Whitney test; U=87, Chi^2 conversion=2.45, m=14, n=24, $p<0.025$). Also, crayfish which were already stationary when the model predator was accelerated towards them initiated a tail-flip response at a greater distance from the model predator (i.e. earlier) than crayfish that were walking when the model predator began to move (Wilcoxon-Mann-Whitney test; U=97.5, Chi^2 conversion=2.13, m=14, n=24, $p<0.05$; Fig. 4.9). A likely cause of this difference is the time required to stop walking and react to the advancing model. Although swimming distances were similar between crayfish which stopped before and after the movement of the model predator, crayfish that had stopped prior to the movement of the model predator might bias the positive relationship found between reaction distance and swimming distance, as these crayfish were furtherest from the model predator as it began to move. For this reason the relationship between reaction and swimming distances was re-examined including only those crayfish which were walking as the model predator began to move. The relationship persisted, but it was less strong (Spearman's Rank Correlation, R=0.46, Chi^2 conversion=2.19, n=23, $p<0.05$).

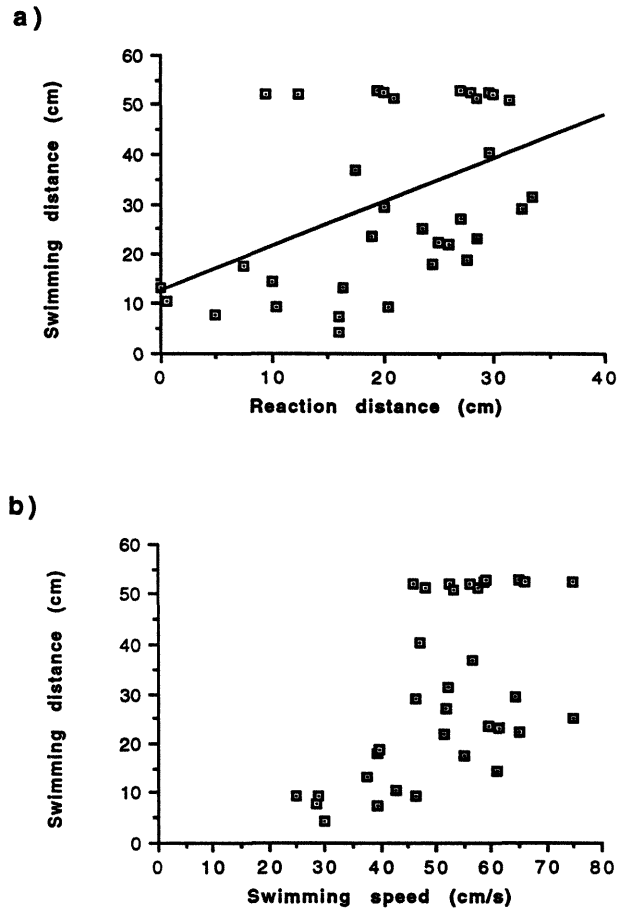


Figure 4.8. The relationship between a) reaction distance and swimming distance, and b) swimming speed and swimming distance, including data from crayfish that were already stopped when the model predator began to move. Data was only used from crayfish which reacted to the model predator on their first trial.

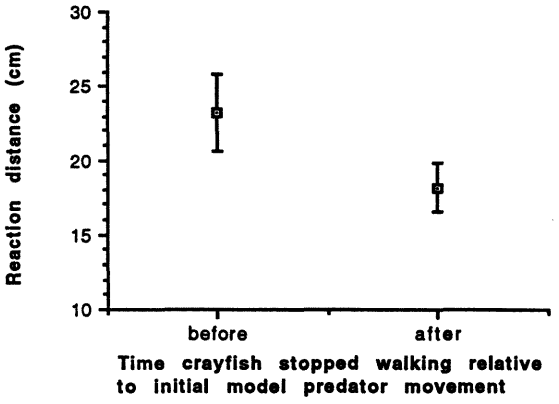


Figure 4.9. The reaction distances of crayfish which stopped walking 1) prior to, and 2) after the model predator began to move. Values are means (± 1 S.E.).

Experiment 4.8. THE IMPORTANCE OF MECHANICAL STIMULI IN ELICITING EVASIVE BEHAVIOUR.

4.8.1 INTRODUCTION

Crayfish of the Genus *Orconectes* increase their use of mechanosensory organs such as the chelae and antennae at night (Bruski & Dunham, 1987; Smith & Dunham, 1990). In the absence of light, mechanical stimuli should be an important determinant of crayfish evasive behaviour. It was the aim of this experiment to determine how crayfish evasive behaviour differed between day and night. An attempt was made to film crayfish evasive responses using infrared lights and infrared-sensitive video equipment. The infrared lamps also produced visible red light and it was decided that this might provide crayfish with too much visual information. Therefore, blind crayfish were tested against sighted crayfish to determine the importance of mechanical stimuli in eliciting evasive behaviour in crayfish.

It was hypothesised 1) that, as no visible information was available to blind crayfish, then blind crayfish would react later to an approaching predator than sighted crayfish, assuming that visual information acts over a greater distance than mechanical information, and 2) that if visual cues modify escape swimming, then blind crayfish should swim further in escape, as it is adaptive to overestimate rather than underestimate the danger of being captured (Bouskila & Blumstein, 1992).

4.8.2 MATERIALS AND METHODS

Thirty-nine crayfish were used in this experiment. Crayfish were divided into two groups and each crayfish was exposed to two simulated predator attacks, using an elliptical predator model 14 cm tall by 6 cm wide, advancing against a white background. When crayfish had reached a point 35 cm from the model predator , the model was accelerated towards them.

The first experimental trials took place between 9 to 20 December 1991. After the initial trial, the total body lengths and carapace lengths of each crayfish were recorded and crayfish were marked on the carapace with one of three coloured paints, so that individuals could be identified. Nineteen crayfish (the second group) were temporarily blinded by encasing their eye stalks and eyes in plastic cement. This allowed eye stalk movement but prevented light penetration. These crayfish later moulted, leaving the plastic cement eye caps with the exuviae. To control for the effects of handling, the first group of crayfish were treated in a corresponding manner to those that were blinded, but no plastic cement was administered. Crayfish were then exposed to a simulated predator attack for a second time between 7 to 16 January 1992. Group 1

crayfish (sighted) were between 31.1 to 46.6 mm total length (mean=35.1, S.D.=4.0), group 2 crayfish (blind) were between 27.9 to 39.1 mm total length (Mean=35.6, S.D.=2.9).

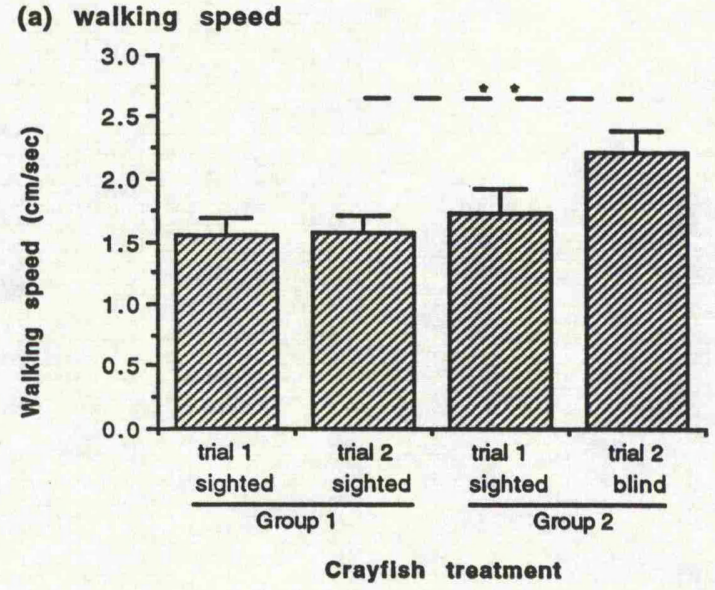
In between the two trial periods, some crayfish moulted and were excluded from further use. Thus only 13 sighted and 16 blind crayfish experienced two model predator attacks. Crayfish evasive behaviour was compared between groups and within trials and also within groups and between trials. A total of 65 evasive responses were recorded from 68 trials. Three crayfish from the first group did not exhibit an evasive respone in their first trial.

4.8.3 RESULTS AND DISCUSSION

In general, crayfish behaviour did not differ between the two crayfish groups prior to one group being blinded. There was an indication that the second group (which were to be blinded) swam further during the evasive response (Wilcoxon-Mann-Whitney Test, Chi^2 conversion=1.68, m=17, n=19, $p<0.1$; Fig. 4.10). Crayfish from the second group showed a marked difference in evasive behaviour after they had been blinded. In pairwise comparisons of individual crayfish behaviour before and after blinding, blind crayfish walked faster, stopped and initiated a tail-flip response closer to the model predator, and swam less far at a slower speed (Wilcoxon Signed Rank test; walking speed, Chi^2 conversion=1.78, n=16, $p<0.08$; stopping distance, $T^+=33$, n=8, $p<0.05$; reaction distance, Chi^2 conversion=3.41, n=16, $p<0.001$; swimming speed, $T^+=78$, n=13, $p<0.025$; swimming distance, $T^+=97.5$, n=15, $p<0.05$; Fig. 4.10).

Crayfish from group one, which were not blinded initiated evasive responses further from the model predator in their second trial by comparison to their first (Wilcoxon Signed Rank test; reaction distance, $T^+=58$, n=11, $p<0.025$; Fig. 4.10b). In a comparison of crayfish evasive behaviour between blind and sighted crayfish in their second trials, blind crayfish walked faster, stopped and reacted to the model predator later, and swam less far than sighted crayfish (Wilcoxon-Mann-Whitney test; walking speed, Chi^2 conversion=2.46, m=13, n=16, $p<0.025$; stopping distance, Chi^2 conversion=2.07, m=12, n=14, $p<0.05$; reaction distance, Chi^2 conversion=3.73, m=13, n=16, $p<0.001$; swimming distance, Chi^2 conversion=1.88, m=12, n=16, $p<0.07$; Fig. 4.10).

The distance after stopping at which blind crayfish initiated an escape response increased by comparison to sighted crayfish both within group 2 (Wilcoxon test; T^+36, n=8, $p<0.01$) and across groups (Wilcoxon-Mann-Whitney test; Chi^2 conversion=3.01,



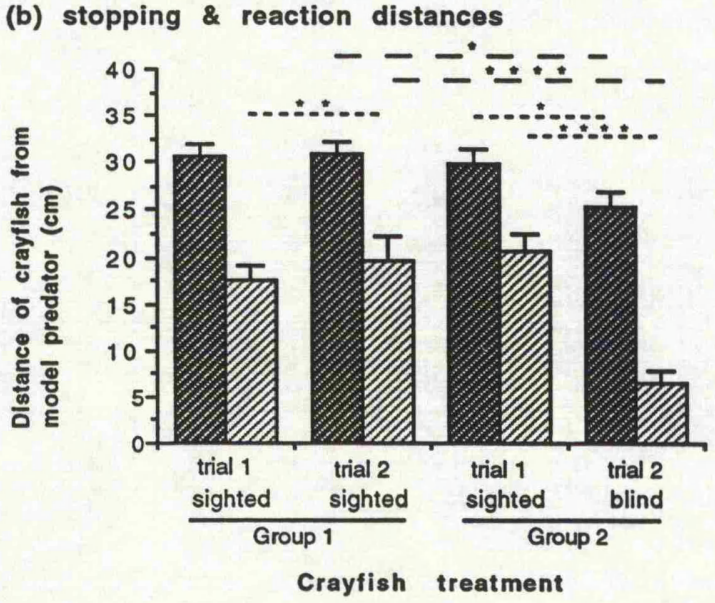
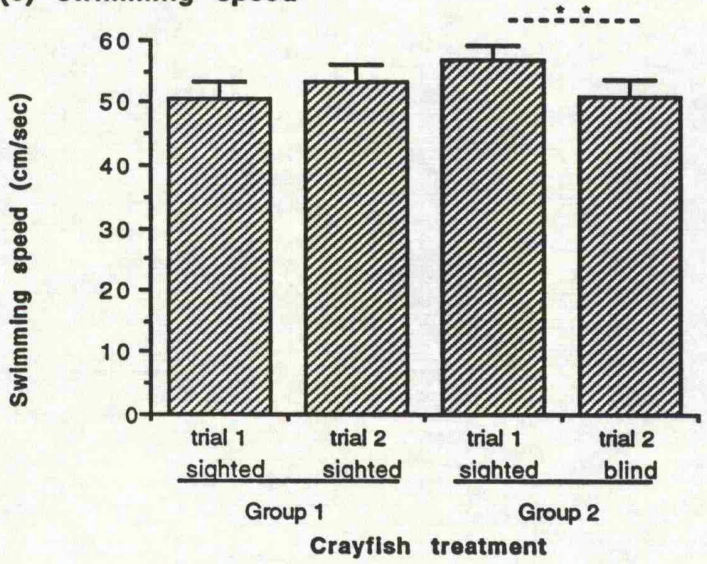


Figure 4.10. Crayfish escape reaction variables for two groups of crayfish, each exposed to two simulated predator attacks. Group 1 were sighted throughout. Group 2 were blinded after the first trial. The reaction variables are: a) walking speed, b) stopping distance ▩, and reaction distance ▨, c) swimming speed, and d) swimming distance. Values are means (± 1 S.E.). The following levels of significance are indicated: *p<0.05; **p<0.025; ***p<0.01; ****p<0.001. Bold lines — —, represent between group comparisons (Mann-Whitney test) light lines ------, represent within group comparisons (Wilcoxon test).

(c) swimming speed



(d) swimming distance

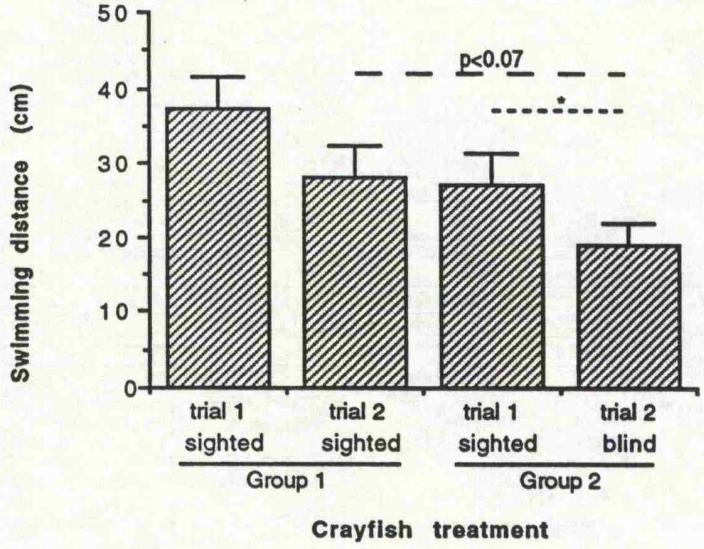


Figure 4.10 (continued)

m=12, n=14, p<0.001; Fig. 4.11). However, sighted crayfish from group 1 reacted more quickly after stopping in their second trial (Wilcoxon test; T^{+}26, , n=7, p<0.05) but group 1 crayfish reacted less quickly after stopping than group 2 crayfish, before the latter were blinded (Wilcoxon-Mann-Whitney test; Chi^2 conversion=1.97, m=11, n=12, p<0.05; Fig. 4.11).

These results illustrate the importance of visual stimuli in mediating an early evasive response to an approaching predator, and in maintaining bouts of escape swimming. The mean reaction distance of blind crayfish (6 cm) was, on average, 10 to 15 cm's shorter than that of sighted crayfish. The shorter stopping distances of blind crayfish in response to the model predator indicates that visual predator detection has an early warning function. The fact that, on average, blind crayfish only stopped walking 5 cm later than sighted crayfish suggests that mechanical stimuli, acting over distances of approximately 25 cm also serve as an early warning of an impending attack. These mechanical stimuli are likely to be of a vibrational nature similar to those produced by the motion of locomotary appendages of fish (Wiese, 1988). The reduction in the reaction:stopping distance ratio in blind crayfish suggests that directional mechanical stimuli characteristic of water movement induced an escape response in the crayfish, but that this stimulus acted over a shorter distance than visual stimuli.

The greater swimming distance of sighted crayfish may not simply be a result of the presence of visual stimuli per se. This may also be explained by the fact that sighted crayfish were reacting earlier to the model predator and were, therefore, effectively 'pursued' for a greater distance than blind crayfish which reacted later, because the model predator travelled a finite distance from a pre-set starting point.

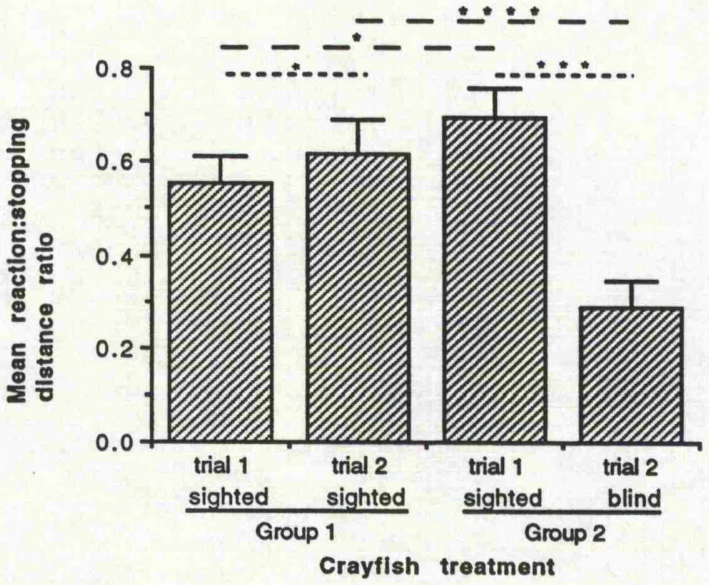


Figure 4.11. The reaction:stopping distance ratio for crayfish reacting to two simulated predator attacks. Group 1 were sighted throughout. Group 2 were blinded after the first trial. Values are means (± 1 S.E.). The following levels of significance are indicated: *p<0.05; **p<0.025; ***p<0.01; ****p<0.001. Bold lines — — , represent between group comparisons (Mann-Whitney test) light lines ------ , represent within group comparisons (Wilcoxon test).

Experiment 4.9. THE EFFECT OF PREDATOR SCENT AND VISUAL FEATURES ON EVASIVE BEHAVIOUR.

4.9.1 INTRODUCTION

This experiment was designed to determine how predator scent and distinguishing visual features affect crayfish evasive behaviour. Crayfish avoidance behaviour was shown to increase in response to seeing and smelling a predator (Chapter 3). Also, Garrison (1976) showed that *P. clarkii* increased defensive behaviour in response to simulated predator eyes. The response measured was tonic immobility, which was induced by pressure on the carapace. Although such recognition only occurred over relatively short distances, this experiment was designed to test whether *P. leniusculus* may also be able to detect specific threatening features of a predator.

In Experiment 4.6, crayfish swam shorter distances in response to model predators with a lower contrast to the background. This suggests that the highly visible model predator posed less threat than a less distinct one, and that to the crayfish, the model predators did not closely resemble real predators. Therefore, crayfish were exposed to simulated predator attacks by either a plain model predator, or one containing an exaggerated eye pattern. These model predators were presented to crayfish in the presence or absence of perch scent. It was hypothesised that if the eye stimulus and perch scent represented threatening stimuli, and hence a greater predatory threat, then crayfish evasive behaviour would be more marked in response to these stimuli. It was predicted that the greater response would manifest itself by crayfish swimming further from and possibly reacting earlier to the advancing model predator.

4.9.2 MATERIALS AND METHODS

Two model predators were used in this experiment. Both were vertically extended ellipses 7 cm tall by 3 cm wide. One was plain black, the other had two eyes painted on it 1.5 cm apart. The eyes were 17 mm in diameter and contained a pupil 5 mm in diameter surrounded by an orange iris. These two models were presented to crayfish either in the presence or absence of perch scent in a 2 x 2 factorial design (Table 4.12). Perch scent was presented using live perch constrained behind an opaque partition in the test arena. The tank water was common to the arena and the perch chamber, and was circulated between the two during experimental trials. When no scent was required, perch were removed from the holding chamber, and the tank was emptied and refilled with fresh tap water. This was aerated for 48 hours prior to recommencing experimental trials.

Twelve crayfish between 29.7 and 38.0 mm total length (mean 34.5 mm, S.D=3.0) were exposed to each of the four treatments, with the exception that one crayfish was exposed to only three treatments and two crayfish were only exposed to two treatments. These exceptions were due to crayfish failing to walk along the walkway in the test arena in these trials. In order to maximise the effect of the visual stimuli, crayfish were allowed to walk to within 20 cm of the model predator before it was accelerated towards the crayfish. Forty-five evasive responses were recorded for the twelve crayfish.

Table 4.12. The 2 x 2 factorial design of four treatments testing the effects of predator scent and visual stimuli on the crayfish escape response.

Visual stimulus		Chemical stimulus	
		Perch scent: Present	Absent
Plain black model predator:	Eye stimulus absent		
	Eye stimulus present		

4.9.3 RESULTS

Using Friedman's 2-way ANOVA (Siegel & Castellan, 1988) crayfish evasive behaviour was not found to differ between the four treatments (Table 4.13).

Table 4.13. Escape behaviour of crayfish in response to each model predator treatment. Values are means. Standard errors are given in brackets.

Reaction Variables	Treatment			
	No scent + Plain model	No scent + Eye stimulus	Scent + Plain model	Scent + Eye stimulus
Walking speed	1.36 (0.2)	1.2 (0.2)	1.2 (0.3)	0.9 (1.0)
Stopping distance	17.4 (1.4)	15.5 (1.4)	16.6 (1.1)	13.7 (1.7)
Reaction distance	11.5 (1.4)	8.9 (1.3)	12.5 (1.3)	9.9 (1.4)
Swimming speed	57.0 (2.1)	53.8 (3.3)	48.6 (3.2)	56.0 (2.0)
Swimming distance	41.1 (4.8)	43.1 (9.0)	24.5 (7.3)	34.3 (6.9)

Results were then grouped to test for an effect of either eye stimulus or scent on crayfish evasive behaviour. Individual crayfish were exposed to four treatments. Therefore, grouping the categories (i.e. scent versus no scent) would use data from individual crayfish twice within each test category. These sample points were not independent, and therefore, the two paired measurements of swimming distance for each crayfish per category were averaged. Crayfish swam further in response to a simulated predatory attack when they could not smell perch (Wicoxon signed rank test, $T^{+}=58$, $n=11$, $p<0.025$).

Experiment 4.10. THE EFFECTS OF PREDATOR DISTANCE AND ORIENTATION MOVEMENTS ON THE EVASIVE BEHAVIOUR OF JUVENILE CRAYFISH.

4.10.1 INTRODUCTION

In Experiment 4.7, crayfish which were stationary prior to the start of a simulated predator attack initiated an evasive response at a greater distance from the model predator than crayfish which were walking as the attack began. Stopping in response to a predator's orientation movement might prepare crayfish for an earlier evasive response. It was hypothesised that crayfish would react earlier to a simulated predator attack that was preceded by an orientation movement.

Prey being attacked from a shorter distance are likely to be more vulnerable to capture. Therefore, they might be expected to react more strongly. Crayfish reaction distances will tend to be shorter in response to a closer predatory attack. It was hypothesised that the subsequent escape behaviour would be more marked. Under the experimental conditions used below, this would mean that, given equal predator pursuit distances, crayfish should swim further in response to a closer attack.

In previous experiments, swimming distance was correlated with reaction distance. This may have been due to an increase in the distance over which crayfish were pursued if they reacted early to a simulated predator attack. To eliminate this possibility in the following experiment, an attempt was made to standardise the total distance travelled by the predator model from each of two starting distances, so that once crayfish had reacted to an advancing model predator, they were pursued over an equal distance.

4.10.2 MATERIALS AND METHODS

The original experiment was designed to test the effects of an attack by a close (6 cm) and more distant (18 cm) model predator, and the effect of predator model orientation movements on crayfish evasive behaviour. These effects were to be tested using a 2 x 2 factorial design (Table 4.14). Several problems were encountered during the trials. Firstly, some crayfish appeared to respond to mechanical stimuli rather than visual stimuli when attacked from a distance of 6 cm with no preceding predator orientation movement, and so, after ten trials, this treatment was discontinued. Secondly, crayfish failed to stop walking in response to the orientating model predator 18 cm away. Therefore, crayfish behaviour was not expected to differ between treatments with and without the orientation movement at this distance and the non -orientation trials from 18 cm were also discontinued. Thirdly, a number of crayfish moulted during the experiment. This limited the number of crayfish, and the time

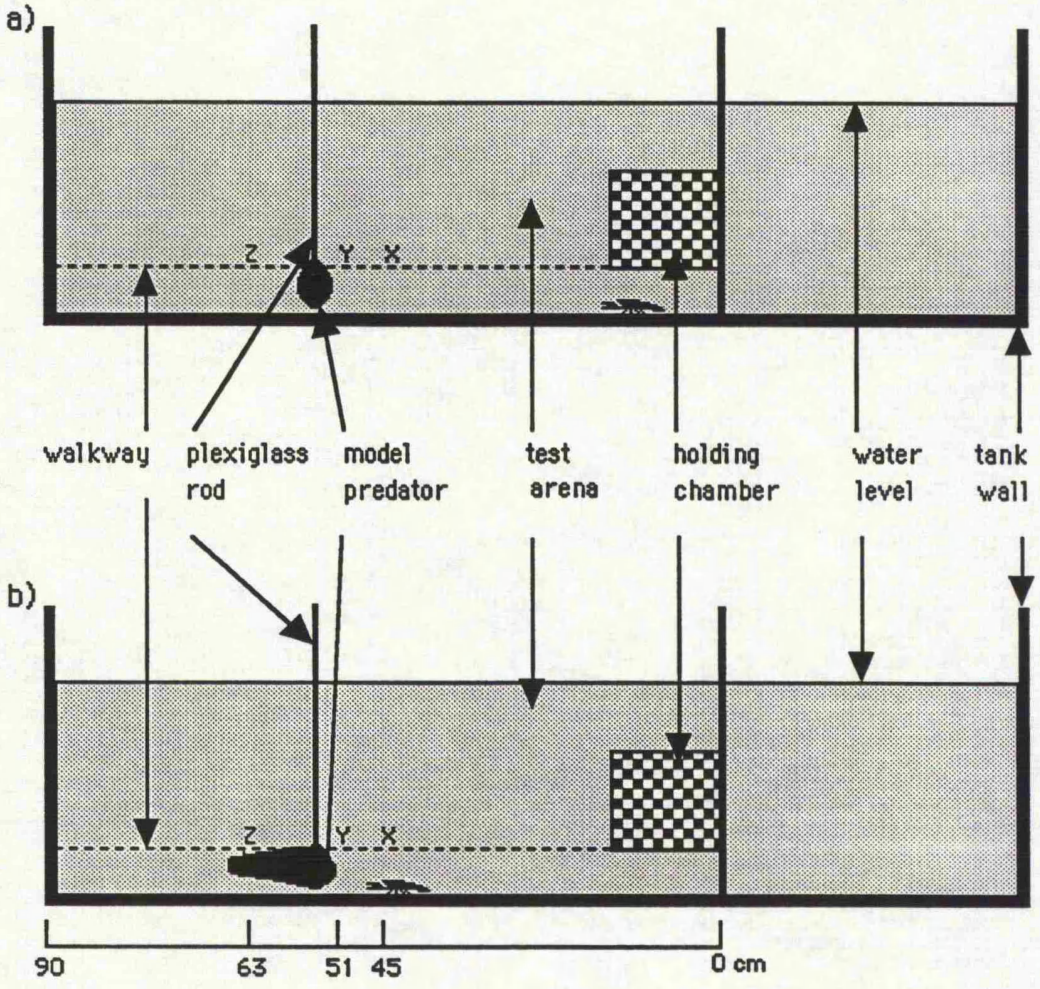


Figure 4.12. Schematic view of Experiment 4.10 showing the relative positions of crayfish and the model predator for a 'close attack'. a) The model predator faced 90° away from the crayfish before they reached point X. b) when crayfish reached point X, the model predator was orientated towards the crayfish and accelerated forwards in a simulated attack. The model predator was set at point Y for a close attack (from 6 cm) and at point Z for a distant attack (from 18 cm).

available to conduct this experiment. Therefore, crayfish behaviour was only tested in response to an orientating model predator attacking from either 6 or 18 cm.

Nine crayfish were exposed to each treatment and 18 evasive responses were recorded. The crayfish measured between 32.6 and 43.1 mm total length (mean=37.3 mm, S.D.=3.3). The experimental apparatus is described above (Section 4.3), however in this experiment, the trolley carrying the model predator was allowed to come to rest as a result of frictional forces rather than using sleepers to arrest its motion.

The model predator faced 90° away from advancing crayfish and was orientated towards crayfish when they reached a predetermined point along the walkway. The model predator consisted of a 3-dimensional contoured head with an elliptical cross section, 7cm tall by 3 cm wide, with a 15 cm long black plastic strip shaped like a perch body extended behind the head. Immediately after the orientation movement the model predator was accelerated towards crayfish from each of the required distances (Fig. 4.12).

Table 4.14. The proposed 2 x 2 factorial design of four treatments testing the effects of attack distance (i.e. the distance between the model predator and the crayfish at the beginning of the attack) and predator orientation movements on crayfish evasive behaviour.

Attack distance	Predator orientation movement	
	Present	Absent
Near (6 cm)		
Far (18 cm)		

4.10.3 RESULTS AND DISCUSSION

Crayfish stopped walking in response to an orientation movement 6 cm away, but not to one 18 cm away. Crayfish initiated tail-flip evasive responses to simulated attacks from both distances. Crayfish reacted earlier (i.e. further from the model predator) to distant attacks and also swam further. (Wilcoxon Signed Rank test; $T^{+}=45$, $n=9$, $p<0.01$, for both; Fig. 4.13). The respective predator pursuit distances for the two attack distances are given in Table 4.15.

Model predators on average travelled 8.5 cm further during a distant attack.

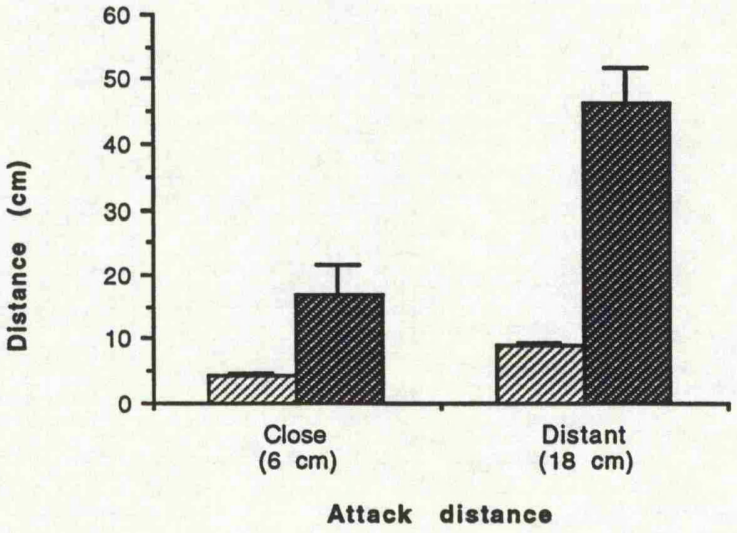


Figure 4.13. Crayfish evasive behaviour in response to simulated predator attacks from two distances. Reaction variables are reaction distance ▨ , and swimming distance ▩ . Values are means (± 1 S.E.).

This difference was only 4 cm if the distance at which crayfish initiated an evasive response was used as a measure. It is concluded that this small difference in pursuit distances is unlikely to have caused the large differences in crayfish swimming distances found in response to the different attack distances. Instead it is suggested that this was a result of the degree of threat percieved by crayfish.

Table 4.15. Distances travelled by the model predator during simulated attacks on crayfish, and the corresponding crayfish evasive behaviours. Values are all in cm's. Standard errors are in brackets.

Distance Measured	Treatment	
	Close attack (6 cm)	Distant attack (18 cm)
Average distance travelled by model predator throughout the simulated attack until coming to rest.	26.0 (0.3)	34.5 (0.3)
Mean model predator speed (cm/sec)	31.1 (1.8)	30.0 (0.8)
Average distance travelled by model predator after crayfish had stopped walking	26.0 (0.3)	32.0 (0.7)
Average distance travelled by model predator after crayfish had reacted	25.0 (0.8)	29.0 (0.7)
Mean crayfish stopping distance	5.9 (0.7)	12.2 (1.1)
Mean crayfish reaction distance	4.2 (0.5)	9.0 (0.6)
Mean crayfish swimming distance	16.7 (4.7)	47.7 (3.6)

Table 4.16 shows data from the experimental trials comparing crayfish evasive behaviour in response to a close predator attack either with or without a preceding orientation movement. Visual inspection of the data indicates a greater reaction distance but a reduced swimming distance in response to an orientation movement. The former result lends support the hypothesis that responding to an orientation movement allows crayfish to initiate an escape response earlier. Crayfish not exposed to an orientation movement probably reacted later as a result of having to stop walking prior to initiating tail-flip swimming.

The hypothesis that crayfish would swim greater distances in response to a close attack was not supported. Indeed the reverse was true. One complicating factor was that crayfish did not respond to a distant orientation movement. Therefore, they were not pre-warned of an impending attack as were crayfish only 6 cm from the model predator. A similarity exists between the crayfish responses to distant attacks and to close attacks which were not preceded by an orientation movement. In both cases, crayfish swam further than when an attack was preceded by an orientation movement. A common factor between these treatments was that crayfish were walking as the attack began. Therefore, there was an element of surprise in these attacks. It is possible that the absence of a warning signal prevented crayfish from assessing the nature of the predatory threat before being attacked. A sudden attack might be expected to produce a more violent escape response. It is suggested that the orientation movement allowed crayfish to assess the nature of the threat and that this modified their evasive behaviour.

Table 4.16. Individual crayfish behaviour in response to simulated predator attacks from a distance of 6 cm. Missing values are a result of: †the evasive response preceding the movement of the model predator, and * of the crayfish failing to walk along the walkway.

Crayfish	Reaction distance (cm)		Swimming distance (cm)	
	predator orientation		predator orientation	
	Yes	No	Yes	No
1†	3.0	NA	30.0	130.0
2	4.5	1.0	28.0	83.0
3 *	NA	0.5	NA	35.0
4	6.0	1.5	9.0	55.0

4.11 GENERAL DISCUSSION

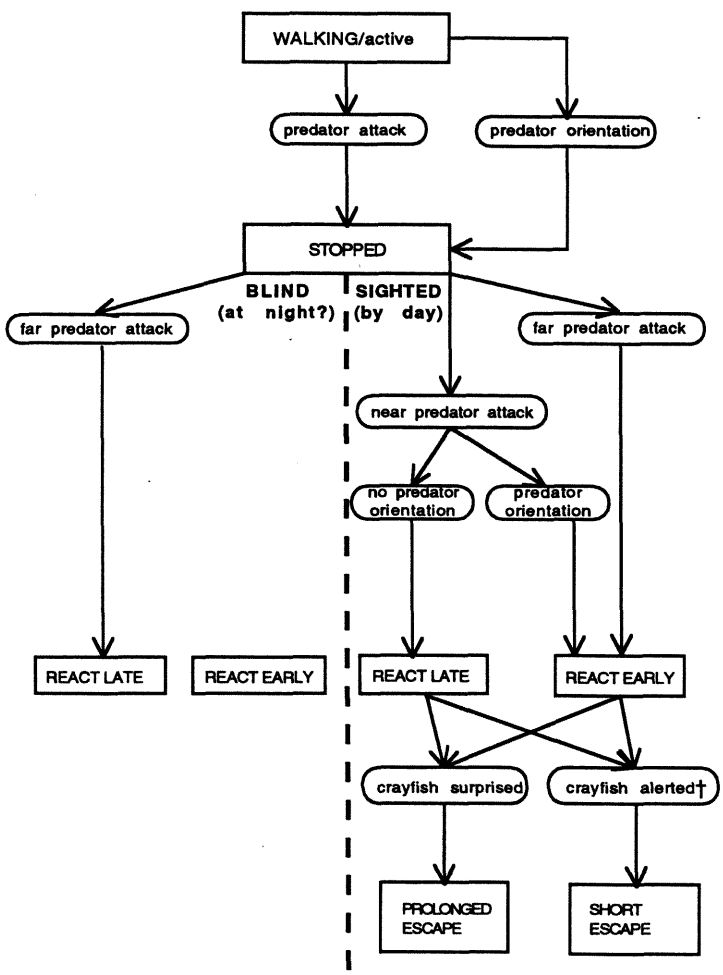
Although the mechanics of the crayfish startle response are extensively reported (Krasne & Wine, 1984), the variability of crayfish evasive behaviour in response to varying predatory threats is not well documented. The magnitude of a potential prey's evasive behaviour should vary with the degree of threat (Stein, 1979; Sih, 1987). Predator-prey interactions progress along a series of stages leading from an encounter to either consumption of the prey or to prey escape (Endler, 1991). The series of experiments described above dealt solely with the predator approach and attack. As predator and prey are in close proximity during this stage, the array of evasive responses available to prey are limited and escape often involves fleeing.

The fundamental questions posed in the above experiments were firstly, what stimuli elicit the evasive response in the crayfish? The sensory channels used to detect predators should match closely the type of information most indicative of a predator attack (Weise, 1988), and should be expected to be most functional in response to the most dangerous predators (Endler, 1986). Secondly, are these stimuli of a specific or general nature? The findings of the previous seven experiments concerning these questions are summarised in Figure 4.14.

Crayfish have many predators (Hogger, 1988 for review), and therefore might be expected to respond to stimuli common to many predators. One such stimulus is the rate of change of angle subtended at the prey's eye by an approaching predator (Dill, 1974a), or apparent looming threshold (Webb, 1982). This facilitates a quick response to many predators and allows the response to change according to predator size and speed. Ewert (1980) indicates that predator detection by prey usually depends on the size, motion and configuration of the threatening object. Fathead minnows (*P. promelas*), showed different escape response thresholds to different fish predators (Webb, loc. cit.). This was related to the configurational differences in the cross sectional shape of the approaching predators.

Whilst the above experiments highlighted differences in crayfish behaviour in response to the presence or absence of gross predatory stimuli, (i.e. visual or mechanical stimuli), limited information was obtained on crayfish behaviour in response to differences in the nature of the visual stimuli presented. One major factor may have been that the short distances over which these simulated attacks occurred did not allow sufficient variability in the behavioural responses for differences to be recorded. Alternatively, crayfish escape behaviour may not be determined by configurational features of predators so much as the characteristics of their movement.

Figure 4.14. Summary of the findings of Experiments 4.4 to 4.10. The figure shows the stages of crayfish behaviour (boxes) preceding and during a simulated predator attack. The oval boxes represent factors which influenced crayfish avoidance behaviour.



† refers to predators which could be smelt, orientated before attacking, or were highly visible (high contrast against background) but not threatening.

4.11.1 REACTION DISTANCE

The distance from the moving model predator at which crayfish initiated an escape response (Reaction Distance), was used as a mark of the degree of threat the model predator posed. Response thresholds were expected to decrease with a greater threat. This did not appear to be the case when crayfish could see different model predators approaching over similar distances, either because 1) crayfish detected no difference in the stimuli (i.e. stimuli were not more or less threatening) or 2) because the reaction threshold was determined by less variable stimuli (i.e. ALT) which did not change, or 3) that variations in response thresholds were limited by the constraints of the experimental system used. Support for the second explanation is taken from Dill (1974a). Zebra Danios (*Brachydanio rerio*) showed no difference in response thresholds to real or artificial predators. They did, however, swim away three times as fast in response to real predators, indicating that artificial predators lacked important stimuli which only influenced the escape response after the completion of the stereotyped startle response. It was possible that the 'missing' stimuli were a result of artifical predators failing to pursue fish for as long as real predators.

The effect of velocity on the reaction distance of fish was shown by Dill (1974a) and is consistent with a response determined by apparent looming threshold (Webb, 1982). No relationship between reaction distance and model predator velocity was found in Experiment 4.7 ($n = 54$), however, the range of target velocities was small (between 31 and 36 cm/s). But for the constraints of time, this relationship would have been examined further under conditions where differences in model velocity could have been controlled and exaggerated. Dill (loc. cit.) also showed that predator size can effect reaction distance, but a doubling of predator size in Experiment 4.6 failed to do so. It is possible that crayfish react to movement per se and not to rates of change of movement. Alternatively, the models may have been so close to the crayfish that their rates of movement exceeded a minimum response threshold value.

4.11.2 SWIMMING DISTANCE

The distance swam by crayfish during an escape reaction was expected to be more flexible and open to sensory modification (Davey & Macmillan, 1991). Crayfish evasive behaviour varied between individuals (Experiment 4.6), and reaction distance and swimming distance tended to be positively correlated. Crayfish that reacted earlier to an approaching model predator tended to swim further, although only 20% of the variation in swimming distance could be explained by the reaction distance (Experiment 4.7). This effect may have been influenced in several ways:

1) Crayfish which reacted earlier were "chased" by the predator model for longer and therefore would be likely to swim further (Experiments 4.7 & 4.8),

2) A more threatening stimulus might act to lower the response threshold for the startle response and would be likely to increase the threshold of inhibition for

escape swimming. Due to the more flexible nature of the escape response as opposed to the startle response, the latter should be a better indication of the degree of predatory threat 'recognised' by fleeing crayfish. This is indicated by the marked difference in escape swimming behaviour shown by crayfish in response to real and artificial predators (Experiments 4.4 to 4.10 compared to Experiment 4.12, below).

4.11.3 THE IMPORTANCE OF VISUAL, CHEMICAL AND MECHANICAL STIMULI

Both visual and mechanical stimuli elicited functional evasive responses. There was also evidence to suggest that the simultaneous perception of both mechanical and visual cues produced a more appropriate response than either stimulus presented alone. Such an effect was shown for avoidance behaviour in response to visual and chemical stimuli in Chapter 3. Avoidance behaviour was more marked when both stimuli were present. The absence of mechanical stimuli lowered the frequency of the evasive response to an approaching model predator, only eliciting a response in ~ 50% of the simulated attacks (Experiments 4.4 & 4.5). The presence of both mechanical and visual stimuli increased the response frequency to 89%, although response thresholds did not differ.

The use of mechanical and visual stimuli to determine evasive behaviour might be expected, as crayfish are exposed to predators under varying light intensities. Smith & Dunham (1990) found that crayfish used mechanosensory organs such as the antennae more in the dark by comparison to the light. In the present study, the loss of visual information reduced both the reaction and swimming distance of crayfish in response to an advancing predator model, but did not alter response frequency. It was hypothesised that mechanical stimuli presented alone would cause crayfish to swim further in response, as the nature of the predatory threat could not be accurately determined. This was not the case. Crayfish stopped walking in response to mechanical disturbances at a distance of ~25cm, but mechanical stimuli only elicited escape swimming over short distances (~6cm). Information provided by mechanical stimuli produced a functional escape response, but visual stimuli appear important for eliciting an early and prolonged flight.

Crayfish avoidance behaviour tends to reduce exposure to visual predators by day (Stein & Magnuson, 1976; Hamrin, 1987; Appelberg & Odelström, 1988; Blake & Hart, in press). Whilst escape behaviour may occur by day, as a result of avoidance behaviour, crayfish may most often encounter crepuscular or nocturnal predators. As a result, crayfish should be adapted to react not only to visual predator cues, but also to mechanical disturbances. This proved to be the case.

Responding to movement alone would lower the adaptivity of the escape response. Crayfish react to moving edges (Gordon, 1971), and to approaching objects.

Such stimuli may not only be characteristic of a predator attack, non-predatory fish may produce similar stimuli. Therefore, reaction to environmental motion alone might often be wasteful in terms of lost feeding activity and energy lost in unnecessary flight reactions.

Crayfish can gain information on the direction and nature of the source of mechanical disturbances in water using a system of receptors which function in a similar way to the lateral line system in fish (Wiese, 1988). In fish, information on the source and direction of hydrodynamic disturbances can be gained over a distance of a few body lengths (Kalmijn, 1988; Gray & Denton, 1991). Dijkgraaf (1963, cited by Dill, 1974a) found that mechanoreception allowed blind fish to adjust their escape reaction in response to varying sizes and speeds of a glass plate moving towards them. It seems likely that a similar system may operate in crayfish. Mechanical information coupled with visual information would make escape responses more specific.

In the present study, crayfish exhibited a functional evasive response when blind, however, the reaction was later and of shorter duration than in sighted crayfish. Visual cues were important in eliciting an early response. An earlier reaction may increase the chances of a successful escape (Endler, 1986). At night, visual cues will be limited. Nocturnal foragers, such as eels, are unlikely to pursue fleeing prey (Experiment 4.12, below), therefore, escape swimming would be an effective escape even over short distances, and it is suggested that mechanoreceptive predator detection is of great importance for *P.leniusculus* at night.

Crayfish exposed to perch scent were expected to be more vigilant. It was suggested that this would have been reflected in a slower walking speed. It was also hypothesised that the detection of perch scent might lower the evasive reaction threshold to an approaching predator. Despite being an important factor in modifying crayfish avoidance behaviour (Hazlett, 1990; Appelberg, pers.comm., Chapter 3), chemical stimuli appeared to have little effect on crayfish evasive behaviour. Whilst chemical information may serve to warn crayfish of the proximity of a predator, it may not convey any additional pertinent information during a predatory attack. In fact, perch scent appeared to reduce the distance crayfish swam during an escape response. It is possible that the presence of scent, whilst not altering the response threshold of the escape reaction, did alter the behavioural state of crayfish so that they were more vigilant. This may have allowed crayfish to assess more rapidly the nature of the approaching threat. Assuming that the model predators lacked important characteristics of real predators, crayfish that were vigilant might be expected to terminate an evasive response earlier. Walking speed was expected to provide an indication of the state of vigilance of crayfish but this did not appear to be the case, and it was not possible to ascertain whether crayfish that could smell perch were more vigilant.

4.11.4 THE NATURE OF THE STIMULI THAT ELICIT EVASIVE BEHAVIOUR

Evasive behaviour of prey may be induced by specific stimuli deriving from certain predators, or by more general stimuli characteristic of many predators. The startle response is stereotyped and is not open to sensory modification (Bennett, 1984; Davey & McMillan, 1991). Therefore, the startle response should be sensitive to general predatory stimuli. Such general stimuli may be sudden, novel, or of high intensity (see Dill, 1974a for review). Assuming prey are not ambushed, then immediately prior to and after a startle response is elicited, prey have an oppertunity to recognise predators and more accurately assess the degree of risk. The presence or absence of specific predatory stimuli may alter the response threshold of the startle response and the longevity of the escape response.

Predator detection by prey may be considered analogous to prey detection by predators. Cues used in prey detection and recognition are body size, movement, shape and contrast (Curio, 1976 for review). Omitting one of these stimuli may lessen the attack response, indicating stimuli summation. Roth (1986) suggests that properties of prey stimulate different tectal and retinal neurones which have broad response properties, and which together form a "recognition module" responsible for "universal prey detection". Summation and inhibition of different neurones determines more specific prey preferences. The critical nature of predator recognition may not require such detailed detection as prey recognition, suggesting the importance of general predator feature recognition such as apparent looming threshold (Dill, 1974a; Webb, 1982). Part of prey recognition is innate (Curio, loc. cit.). The same is true of predator detection (Lima & Dill, 1990), however, fish prey learn to distinguish between predatory and non-predatory fish (Csányi, 1985). The distiction can be based on visual cues associated with the configuration of the head, particularly mouth width and the distance between the eyes (Karplus & Algom, 1981). Prey that have evolved under heavy predation pressure tend to exhibit more pronounced avoidance behaviour when exposed to predators (Licht, 1989), and this behaviour will be flexible depending on the degree of risk (Coates, 1980).

Visual features of predators such as body shape (Experiment 4.4), size (Experiment 4.6), and eye patterns (Experiment 4.10), did not affect crayfish startle or escape behaviour. This indicates that juvenile *P. leniusculus* were using general visual stimuli associated with predator movement in their assessment of risk. This should be expected as these crayfish were young and their responses would tend to be innate. Older crayfish may respond to more specific stimuli as a result of experience. It is noted that the features presented may have been inappropriate. Also, if crayfish most often encounter predators at dusk, at night, or in turbid water, feature detection would be of little use and a general response to movement would be more adaptive.

Crayfish escape swimming differed in response to highly visible (high contrast with background), and less visible (poor contrast with background), predator models (Experiment 4.6). Crayfish tended to swim further in response to the low contrast model. This suggests that the less visible model predator was more threatening to the crayfish. A drawback of using model predators is that they inevitably lack important characteristics of live predators. Zebra danios escaped at three times the velocity in response to real rather than artificial predators (Dill, 1974a). The black model moving against a white background was clearly visible. The more vigorous response of crayfish to the less distinct model indicates that some image processing was taking place. The weaker response to the distinct model predator could mean that crayfish 'assessed' the model during the evasive response and, due to the absence of certain stimuli 'realised' that it was not a significant threat. The less contrasted model would give away less information. This would make a quick assessment of risk harder. As a result, indistinct objects should be treated as a threat as it pays prey to overestimate predatory hazards (Bouskila & Blumstein, 1992). The lack of a difference in reaction distance between the two treatments does not support this hypothesis, however, there is more scope for a differential response in swimming duration than reaction distance.

As the distance between predator and prey decreases, prey have less time to detect and react to a predator strike, and hence less time to effect an escape. Minimising the attack distance is important for successful prey capture and this may be achieved by the predator stalking the prey (Curio, 1976). After sighting potential prey, predators orientate towards the prey. This is a sign of predatory intent and may act as a warning stimulus to prey. This proved to be the case for crayfish 6 cm from a model predator, but not for crayfish 18 cm away (Experiment 4.10). This latter result is surprising considering crayfish responded to movements of live predators over similar distances in much poorer light conditions (Experiment 4.12).

The lack of response to an orientation by the more distant model predators is considered of key importance in the differences in escape behaviour displayed by crayfish reacting to a close and distant predator attack. Crayfish reacted further away from the distant predator attack, however, they also swam further in escape. It was hypothesised that a closer attack would stimulate a greater response. This was not the case and it is suggested that the orientation movement provided a warning to crayfish 5 cm from the model, which increased their levels of vigilance. This would have allowed crayfish to determine more accurately and more rapidly the nature of the threat. Conversely, a sudden attack with no pre-warning may produce a less controlled escape response. This hypothesis is supported by the limited data available for crayfish reacting to a simulated attack from a model predator 6 cm away, that was not preceded by an orientation movement. In these instances, crayfish again tended to swim greater distances, suggesting surprise was a key factor affecting the response. Prey responding to a stalking predator can gain advantage over a predator (i.e. in reaction time, reaction distance, and a planned escape route; Endler, 1986). Although stalking

behaviour was not simulated in the artificial attacks described above, both predator orientation movements and predator scent represented sources of early predator identification for the crayfish. In Experiments 4.9 & 4.10, both stimuli reduced the distance crayfish swam in escape.

Davey & Macmillan (1991) suggest that escape efficiency might be increased if crayfish adopt an efficient pre-escape response stance. Reacting to stalking movements and scent would allow crayfish to do this. Beall et al. (1990) found that backward walking and defence posturing inhibited tail-flip escape in crayfish (*P. clarkii*). These behaviours were incompatible because they used the same body parts. Similarly, forward walking and the escape response are mutually exclusive behaviours. Crayfish have to stop walking in order to tail-flip. Detecting stalking predators would allow crayfish to stop walking and to prepare for flight. It was determined in Experiment 4.7 that crayfish that were stationery when attacked reacted earlier. The time taken for walking crayfish to stop and react to an attack is at least one cause of this reaction latency. A possible explanation of the reduction in swimming distance after the detection of warning stimuli, is that such stimuli alert crayfish and make them more vigilant. An artificial predator may not possess sufficient stimuli to maintain bouts of escape swimming in vigilant crayfish. Again, levels of alertness or vigilance were not known for these crayfish.

4.11.5 INDIVIDUALITY OF CRAYFISH EVASIVE BEHAVIOUR

Aspects of the crayfish evasive response were individual (Experiments 4.6 & 4.7). Unpredictable behaviour is an effective defence against predators (Endler, 1991). This should hold true if the source of variation is within or between individual prey. Variable responses between prey may serve as a defence mechanism. Predator foraging efficiency improves with experience (Vinyard, 1982). If prey vary in their responses, predators may be less able to improve the timing of their attacks with successive interactions. Response latencies become critical in the attack phase of predator-prey interactions (Weihs & Webb, 1984) and prey capture is often dependent on the timing of the execution of a predatory strike and a prey's startle response (Nyberg, 1971; Weihs & Webb, 1984). As evasive and attack behaviour are initially highly stereotyped, and given that strike performances may differ between predator species, then crayfish populations exposed to different predators might be expected to vary in their response thresholds to attack. This may not hold true when an attack is followed by a pursuit. Fish may easily run down a fleeing crayfish, and hence response time may not affect the probability of escape (Webb, 1979; Experiment 4.12, below).

In Experiment 4.7, stopping distance was inversely correlated with the chelae length:body length ratio of crayfish (CL:BL ratio). Crayfish with longer chelae per unit body size stopped walking closer to the approaching predator, although CL:BL ratio

only accounted for 30% of the variation in stopping distance and also did not affect other aspects of escape behaviour. Chelae size affects dominance orders and reproductive success in *O. propinquus* (Stein, 1976) and *P. leniusculus* (Endsman & Jonsson, 1992). In the former study, crayfish with larger chelae were more likely to survive predation by smallmouth bass (*M. dolomieui*) although this was only a secondary use of the chelae in this species as non-mating males have a reduced chelae size. Greater chelae size may be more important for predator defence in *P. leniusculus* as this species does not alter chelae size seasonally.

Sih (1992) found that the tendency of certain salamander larvae (*Ambystoma barbouri*) to spend more time exposed was consistent over different situations. Individuals that were more active in the presence of predators, were at a greater risk of predation, but these individuals were also more active in the absence of predators, allowing a more rapid development, and were more active at at night, which increased their chances of moving to predator free habitats. A similar process may operate in *P. leniusculus* populations. More active crayfish may be more aggressive, may feed more and grow proportionally larger chelae, and may be less disposed to react to a predatory threat.

4.11.6 LIMITATIONS OF THE EXPERIMENTAL PROCEDURES

Experiments investigating predator-prey interactions are typically staged in artificial arenas that may alter natural behaviours (Webb, 1986). The conditions used in the above experiments placed artificial constraints on aspects of crayfish evasive behaviour. Some limitations of the apparatus and procedure are discussed below. Despite these drawbacks, it is considered that the major features of the evasive response were exhibited in these experiments and that the conditions did elucidate real differences in crayfish behaviour.

1) One major drawback of the system is the artificial nature of the simulated predator attacks. These inevitably lacked many subtle features associated with real predators. One important aspect of predatory behaviour, the approach phase, was ignored for the purposes of the experimental aims and also because of the difficulty of standardising such a variable. Crayfish escape behaviour was greater in response to real predators (see below) although the behaviour patterns were essentially similar in response to artificial predators.

2) The small area used as a test arena was necessitated by the limitations of the recording equipment. This may have restricted the possible variation in reaction distances in response to different treatments to indistinguishable levels. Fleeing crayfish occasionally collided with the tank wall before terminating their escape swimming. This was not considered to affect the results, as many crayfish stopped

swimming prior to reaching the tank wall. By design, the walkway limited the direction of escape swimming. Only in Experiment 4.10, when crayfish were attacked from 5 cm, did they escape at varying angles by swimming over the walkway walls.

3) Despite being left to acclimatise to the test apparatus for 30 to 45 minutes, crayfish were disturbed to some extent. Crayfish which were reused immediately after a failed trial appeared to be more disturbed (Experiment 4.7). For this reason crayfish were subsequently only used once per day irrespective of whether the trial was successful or not.

4) The artificial blinding of crayfish affected their behaviour. Walking speeds were greater in blinded crayfish. In Experiment 4.7, crayfish from repeated trials tended to walk faster than crayfish during their first trial. This may be analagous to the effect noted on walking speed by blinding crayfish. In Experiment 4.7, this also caused crayfish to stop walking later in response to a predator attack. A similar effect may be seen in Experiment 4.8 for blind crayfish. In the former experiment (Experiment 4.7), on average walking speed was 1 cm/s greater and stopping distance from the predator model 5 cm shorter in disturbed crayfish. Corresponding values for blind crayfish were 0.7 cm/s and 5 cm (Experiment 4.8).

5) Despite attempts to insulate the tank from the mechanisms driving the model predator, some sound was likely to have been transmitted. This in part may have alerted crayfish to the start of a simulated attack, causing them to cease walking. In the majority of cases crayfish appeared to respond solely to the advancing model predator. The effect on crayfish behaviour of the movement of the trolley used to carry the model predators should have been tested.

6) As illustrated in Figure 4.17, the recording equipment was of limited accuracy considering the speed of the interactions observed. Despite this, clear differences were found in crayfish evasive behaviour in response to different treatments.

7) A more detailed study of crayfish evasive behaviour under conditions of low light intensity would have proved informative. Avoidance behaviour will tend to limit crayfish accessibility to visual predators by day and encounters will be more frequent at dawn, dusk or at night. Problems with recording crayfish behaviour at low light intensities precluded this possibility.

8) The behaviour of crayfish used in the experiments can not be said to be strictly innate. Crayfish were taken from ponds containing trout. Therefore, during their time within these ponds (~ 6 months) experience of predatory attacks may have modified their escape behaviour.

Table 4.17. An example of the margins of error associated with measuring crayfish and model predator velocities in experiments testing the evasive response of crayfish (Chapter 4). The speed of the model predator in a typical simulated predator attack was measured over a distance of 25 cm to the nearest 0.2 seconds and 0.5 cm. Under these conditions an error of 0.2 s or 0.5 cm in readings leads to an error of 1 cm/sec.

Distance travelled by model predator (cm)	Time to travel distance (sec)	Average speed (cm/sec)
25.0	0.70	35.7 ~ (36)
25.0	0.72	34.7 ~ (35)
24.5	0.70	35.0 ~ (35)

Experiment 4.12. EVASIVE BEHAVIOUR OF JUVENILES IN RESPONSE TO PERCH AND EELS.

4.12.1 INTRODUCTION

The aim of this chapter was to relate the evasive behaviour of crayfish responding to mechanical, visual, and chemical stimuli to the likelihood of successful evasion of perch and eels. To better interpret the experimental data described in Experiments 4.4 to 4.10, crayfish evasive behaviour was observed in response to live predators. Interactions between perch or eels and crayfish were observed over a five month period between 10 October 1991 and 3 March 1992.

4.12.2 MATERIALS AND METHODS

Four perch (19 to 22 cm total length) and four eels (48 to 64 cm total length) were used over the 5 month period. Three 1.5-m^2 tanks filled to a depth of 30 cm were also used. Each tank was divided into two sections by a plastic partition containing a sliding door. One 1-m^2 section of each tank was used as a test arena and the floor was covered with a layer of sand. The second smaller section was covered with black plastic and was provided with plastic drainpipe shelters for the fish. At any time, only one fish was present in each tank. Fish were kept in the tanks for periods of 1 to 1.5 months. During this time the perch, and to a lesser extent eels, acclimatised to the tanks and began feeding. The water temperature was 15 °C and the tank room was kept on a 10:14, light:dark regime automatically.

Predator-prey interactions were filmed in one tank per night over a 6-hour period from 17.30 hours. The lights were turned off at 17.00 hours. A camera sensitive to infrared light and a video recorder with a film speed of 50 frames/second were used to record the interactions. The tanks were illuminated using two infrared lamps which also gave off some visible red light. This light source produced levels of illumination of 0.5 microeinsteins cm^{-2} sec^{-1} at the water surface.

One crayfish was placed in a tank at 17.30 hours on each filming occasion. At the end of each trial, live crayfish were removed from the tanks. Perch-crayfish interactions were filmed on 25 occasions and eel-crayfish interactions on 24. A crayfish escape response was recorded in 27 eel-crayfish encounters and 18 perch-crayfish encounters. These encounters were analysed to give information on the dynamics of the predator-prey interactions. In all, 22 crayfish (33.7 to 44.9 mm total

length; mean 40.2, S.D=3.2) were exposed to eels, and 22 crayfish were exposed to perch (32.5 to 42.1 mm total length; mean 34.5, S.D=2.3).

4.12.3 RESULTS

Crayfish reacted to fish movement by initiating tail-flip evasive responses. These reactions occurred in respone to incidental fish movement, directional approach swimming (stalking), and to predator attacks (strikes; Table 4.18). Only the later two categories are considered below. Details of the predator-prey reaction dynamics are given in Table 4.19.

Table 4.18. Predator-prey interactions between perch, eels and crayfish which produced an escape response in crayfish.

Predator behaviour	Predator involved	
	Eel	Perch
Predator strike	8	7
Predator stalk	11	7
Undetermined activity	1	2
Incidental predator movement	7	2
TOTAL	27	18

With one exception, all fish approaches and strikes were directed towards crayfish on the tank floor. Crayfish responded to stalking eels by swimming away from the predator over short distances along the tank floor. In response to an eel strike, escape swimming was of longer duration, and on every occasion crayfish swam to the water surface. In response to perch approaches and strikes, crayfish again swam to the waters surface, with the exception of two occasions when crayfish responded to an approaching perch by swimming 9 to 12 cm along the tank floor. Escape swimming was often curtailed by collisions with the tank walls. Also, perch often curtailed escape swimming by chasing and catching crayfish.

Eels struck at crayfish on 8 occasions, and one of these resulted in a successful capture. On this occasion the strike was directed laterally towards the carapace, as the crayfish had not orientated to face the eel at the time of the strike. Of the 14 perch-crayfish encounters, six resulted in crayfish capture, but crayfish ingestion followed capture only once. On the other five occasions crayfish escaped during handling by the

perch. One crayfish survived being held inside a perch's mouth for 6 minutes. Two of the captures, including the successful ingestion, resulted from perch strikes. Two captures resulted from perch chasing crayfish after an unsuccessful strike, and two captures resulted from perch chasing crayfish which initiated escape swimming in response to approaching perch. In all, 8 of the 14 crayfish escape attempts resulted in perch giving chase. By comparison, eels never pursued fleeing crayfish.

Table 4.19. Behavioural dynamics of interactions between eels, perch and crayfish in a 1-m^2 test arena. Values are means (± 1 S.E.) and sample sizes (n). Distances are in cm, speeds are in cm/sec. Significance levels are from between predator comparisons (Mann-Whitney Test, $p<0.1$).

Reaction Variable	Predator		Level of significance
	Eel	Perch	
Method of prey detection	chemical	visual	
Approach (stalk) speed	8.3 (1.7) n=18	7.2 (0.7) n=8	- -
Crayfish escape speed in response to a stalking predator	30.1 (3.4) n=9	54.5 (6.1) n=4	P<0.01
Crayfish swimming distance in response to a stalking predator	26.4 (3.4) n=10	62.4 (15.1) n=7	p<0.1
Crayfish reaction distance in response to a stalking predator	3.6 (1.1) n=7	7.7 (1.6) n=7	p<0.07
Crayfish escape speed in response to a striking predator	50.2 (9.6) n=4	66.7 (2.6) n=6	- -
Crayfish swimming distance in response to a striking predator	78.4 (22.0) n=6	45.7 (7.9) n=6	- -
Crayfish reaction distance in response to a striking predator	2.5 (0.5) n=4	3.2 (0.6) n=8	p<0.08

Perch that were stalking crayfish propelled themselves by movements of the pectoral fins. Perch typically moved in bouts of 5 to 10 cm interspersed with stops, during which pectoral fin beats were minimal. Eels stalked crayfish in a less direct fashion, sweeping their head from side to side. Often this lateral head movement caused crayfish to tail-flip away. Both eels and perch approached to within 6 cm of the crayfish before preparing for a strike.

4.12.4 DISCUSSION

Only limited data were available for crayfish behaviour in response to perch and eels. These data, however, do illustrate differences in the response of crayfish to real and artificial predators and between the two predator species. Crayfish swam greater distances in response to real predator attacks although crayfish swimming speeds were comparable. The trajectory of the escape swimming also differed in response to real and artifical predators. Crayfish tended to swim to the water surface and to continue swimming in the water column when attacked by perch and eels. Only the response of crayfish to an approaching eel resembled the response of crayfish to a simulated attack by a model predator, being directed along the tank floor and over relatively shorter distances. The difference in behaviour in response to real and artificial predators may be a function of the levels of illumination prevalent in each case. At low light intensities, fleeing into the water column might be adaptive if attacking fish remain on the floor and do not chase prey. With an increase in illumination, crayfish escape can be directed visually and should be directed towards shelter (Stein, 1977). This however was not tested. The flight response of crayfish in response to perch was often unsuccessful, as flight was often followed by a chase and capture within 50 cm. It should be noted that naturally crayfish will tend not to be exposed on open featureless habitats in the presence of predatory fish (Stein & Magnuson, 1976; Collins et al., 1983).

The only notable difference in foraging behaviour between perch and eels was that perch chased and more frequently caught prey. The approach and strike behaviour were similar although the approach of perch was more direct. Eels swept thier heads from side to side as they moved. This behaviour corresponds to the movement of eels across scent plumes which allows eels to gain directional information on the location of the source of the scent (Tesch, 1977).

Crayfish often reacted to the approach of perch and eels, but responded differently to the two approaching predators. Crayfish reacted earlier and swam further in response to an approaching perch, although the increase in swimming distance in response to perch may have been due to perch chasing the crayfish. The difference in reaction distance suggests that perch present a more threatening stimulus when approaching crayfish. This may be because perch are more visible to crayfish: 1) due to the height of perch relative to eels, 2) despite the shallow water depth, perch may have been more visible as they swam in the water column and were more likely to present a silhoutte against the tank walls, whereas eels remained on the tank floor and were likely to be less conspicuous, 3) perch may be inherently more visible due to the nature of their body surface and the light it reflects. Predator shape and size did not influence crayfish evasive behaviour in Experiments 4.4 and 4.6. This suggests that the relative height of perch and eels is not an important factor. Alternatively, the behaviour of perch and eels may have presented more or less threatening stimuli.

Perch approached crayfish in a direct line whereas eels tended to meander across a direct line to the crayfish.

Despite the greater response latency exhibited by crayfish in response to striking eels, eels were less successful at capturing crayfish than perch. The latency of response to eels may be a result of eels presenting less distinct visual cues than perch, but even if visual stimuli were absent, crayfish should still be able to escape in response to mechanical stimuli (Experiment 4.8). Crayfish evasive responses are highly adapted to evade a fast-start predator strike. From rest, crayfish accelerations are superior to those of fish (Webb, 1979), however, the performance of fish during sustained swimming is superior to that of crayfish. The ability to chase a fleeing crayfish therefore improves the chance of a successful capture.

This study attempted to determine the relative efficiency of eel and perch predation on crayfish. Perch were more successful at capturing crayfish as a result of their tendancy to chase fleeing prey. A similar effect was found between Tiger Musky (*Esox* spp) and three other predators feeding on Fathead Minnows (*P. promelas*) (Webb, 1982). Tiger musky struck at prey with greater success but failed to chase prey. The other predators had a low strike success rate (6 to 18%) but capture rate increased to 36 to 41% after chases were included. Although perch and eels only consumed one crayfish each, it is suggested that given smaller crayfish, perch ingestion rates would have been better as they caught more prey but failed to handle them successfully.

Conclusions about relative predatory efficiency must be treated with extreme caution. Predatory efficiency may change with habitat complexity and levels of illumination (Crowl, 1989; Mattila, 1992) and different predators may be more or less advantaged by such changes. It does appear that behaviourally, perch are better able than eels to capture crayfish during an attack. This conclusion is made in the knowledge that over diel and annual periods and across habitats, relative predatory success may change. One notable factor controlling success is the avoidance behaviour of crayfish. Limiting their exposure by day and their preference for more complex habitats will tend to reduce the success of perch predation (Stein & Magnuson, 1976; Hamrin, 1987; Appelberg & Odelström, 1988). Also, in the wild, bouts of escape swimming may be shorter and directed towards cover, thus reducing the risk of capture whilst in flight.

PART II

CHAPTER 5

5.0 HABITAT PREFERENCES AND SURVIVAL OF JUVENILE SIGNAL CRAYFISH (*P. LENIUSCULUS*) - THE INFLUENCE OF WATER DEPTH, SUBSTRATUM, PREDATORY FISH AND GRAVID FEMALE CRAYFISH.

5.1 SUMMARY

A critical stage in the life history of crayfish is that between hatching and finding a suitable 'safe' habitat. The activity and habitat use of gravid female crayfish, newly released juvenile crayfish themselves, and of crayfish predators will have a bearing on the survival of juvenile crayfish during this critical period. Field observations and experiments were conducted to determine the effects of perch (*P. fluviatilis*), gravid female signal crayfish (*P. leniusculus*), water depth and substratum on the distribution and survival of newly released juvenile signal crayfish.

In the laboratory, substratum governed the habitat choice of gravid female and juvenile crayfish, however, the habitat preferences of the two classes of crayfish differed. Perch reduced crayfish activity and reinforced the respective habitat preferences of both crayfish classes. The preferred habitats of juvenile crayfish provided the maximum protection from perch predation.

Observations in a pond in southern Sweden during May to July 1991, indicated that substratum was the major factor governing the distribution of *P. leniusculus*. As a result of their own preference for the stone substratum, gravid females determined the initial distribution of newly independent juvenile crayfish between silt and stone substrata. During the first four weeks of their independence, more juveniles were found in shallow water than deep water. It is suggested that this distribution was governed by differential mortality between habitats and not by juvenile habitat selection behaviour. It is further suggested that perch predation influenced this distribution. The influence of perch predation on juvenile abundance is discussed in relation to the effects of invertebrate and intraspecific predation.

5.2 GENERAL INTRODUCTION

The purpose of the following study was to investigate the importance of predation in relation to other factors influencing the survival and distribution of newly hatched juvenile signal crayfish, *P. leniusculus*. Three possible determinants of juvenile crayfish distribution are: 1) the behaviour and habitat preferences of gravid females (females bearing eggs), 2) the behaviour and habitat preferences of juvenile crayfish, 3) differential predation rates on different substrata.

Newly hatched juvenile crayfish disperse from brood females (females bearing newly hatched juveniles) and explore the surrounding habitat. During the early stages of juvenile development, brood females may produce a brood pheromone which attracts juveniles (Little, 1975). Munkhammar et al. (1989) found that over a nine day period after the first moult, the desire to remain with the female declined in juvenile *A. astacus*, and brood females became increasingly cannibalistic as contact with the young declined. Bovberg (1959) demonstrated that adult *Cambarus alleni* (Faxon) migrate away from high densities of conspecifics, and that the rate of migration is directly related to the initial crayfish density. Jonsson (1992) demonstrated that juvenile *A. astacus* leave females more quickly if a suitable shelter-providing substratum is available. Thus, the behaviour and habitat preferences of gravid females and of females bearing newly hatched young will have a direct bearing on the distribution of juvenile crayfish.

Predation is often heaviest on the smallest size classes of decapod Crustacea e.g. crayfish (Stein, 1977), spiny lobsters (Smith & Herrnkind, 1992), American lobsters (Wahle & Steneck, 1992), and is therefore likely to exert a strong influence over the distribution of juvenile age classes. Butler & Stein (1985) found that the distribution patterns of juvenile *Orconectes* species in experimental aquaria were an artifact of predation and not a response to it.

Juvenile American lobsters (*Homarus americanus*, Milne Edwards) quickly traverse substrata providing inadequate shelter, but tend to remain on substrata once suitable shelter is found (Wahle & Steneck, 1992). The distribution of crayfish may be influenced by substratum particle size (shelter size), macrophyte cover, water current speed and direction, and competition between and within crayfish species (Stein & Magnuson, 1976; Beingesser & Copp, 1985; Rabeni, 1985; Foster, 1992). Field observations have shown that smaller, more vulnerable crayfish are more closely associated with shelter-providing substrata in lakes containing high densities of predatory fish (Stein & Magnuson, 1976; Stein, 1977; Collins et al., 1983). Appelberg (1986) found that in Swedish lakes with large populations of predatory fish, crayfish distribution was related to substratum particle size and water depth. Juveniles were found in the shallow water, which also contained the smallest substratum particles.

Work on juvenile lobsters, *H. americanus* suggests that habitat selection is complex and related to stimuli characteristic of the habitat as well as lobster behaviour (Johns & Mann, 1987). Aspects of habitat choice changed between light and darkness, visual stimuli dominated in the former case and tactile stimuli in the latter. The positive relationship between *A. pallipes* and the size of shelter they occupy (Foster, 1992), suggests that tactile cues may also be of importance in crayfish shelter selection.

The following work was conducted between May and July 1991. The field work was designed to determine 1) how newly independent juvenile *P. leniusculus* were distributed within a pond in southern Sweden, 2) how quickly this distribution pattern became established, and 3) how perch, gravid females and juvenile crayfish influenced this distribution pattern. The field work was complemented by laboratory studies conducted at Simontorp Aquaculture A.B., Blentarp, Sweden to determine 1) the habitat preferences and behaviour of gravid female crayfish in response to perch, and 2) the effect of perch on juvenile crayfish habitat selection behaviour and survival.

5.3 GENERAL MATERIALS AND METHODS

Experimental animals

Gravid female crayfish that were carrying large numbers of healthy eggs were selected for use in Experiment 5.5. These were trapped in Rögle pond (described below) during May 1991, and were kept individually, indoors at Simontorp. Gravid females were left for at least two weeks to acclimatise to the light regime described below before being transferred to the experimental tanks The mean total length of a sample of the 72 crayfish used was 92.5 mm (range 83 to 106 mm, n=20).

Perch between 15 to 20 cm total length were obtained from fishermen, and were caught in nets at Vombsjön and Sövdesjön, two lakes in southern Sweden, between 8 May to 8 July. Both lakes contain small populations of crayfish. *P. leniusculus* are present in Vomsjön, and *A. astacus* in Sövdesjön. The perch, therefore, were likely to have experience of crayfish prey. It proved difficult to keep perch healthy for long periods of time in experimental and holding tanks. For this reason perch were obtained, as near as possible to the exact day on which they were required, and where possible, perch were placed into experimental tanks immediately on arrival, so as to minimise the stress due to handling. A stock of perch were also kept in holding tanks with a 1-m^2 bottom area, filled to a depth of 50 cm. Perch were placed into experimental tanks on the first day of each trial in Experiments 5.5 and 5.6, and on the third day of Experiment 5.7. Whenever experimental perch were found to be in poor condition, they were replaced by new fish from the holding tanks.

Newly independent (stage II) crayfish were obtained from an indoor hatchery at Simontorp between 10 May to 7 July. These hatched from gravid females which were caught in Rögle pond between April and May 1991. During Experiment 5.6 & 5.7, juveniles were fed a standard quantity of a liquidised suspension of either egg, peas and earthworm, or fish. All experimental animals were stored and used under a 9:15 hours, light:dark regime. The lights were turned on at 07.00 hours and off at 16.00 hours, but did not fade in or out. This system was used to fit in with the normal working practices of Simontorp Aquaculture A.B.

Statistical methods

Unless otherwise stated, all statistical analyses are two-tailed and use a system of nonparametric analysis of variance by ranks to test the difference between two or more independent samples (Meddis, 1984). Time (weeks) was used as a blocking variable when comparing trappability (crayfish distribution) between experimental sites. Blocking variables are "qualities which cannot be controlled but must be taken into account even though they are not specifically relevant to the hypothesis under examination". In two sample tests, sample sizes (m and n) are given. For multiple sample tests, degrees of freedom (df) are given.

5.4 DISTRIBUTION AND ABUNDANCE OF SIGNAL CRAYFISH IN A SWEDISH POND.

5.4.1 MATERIALS AND METHODS

Field site

The trappability and distribution of gravid female crayfish and newly independent juvenile crayfish, and the diets of perch (*P. fluviatilis*) were studied in Rögle Pond 3, Skåne, southern Sweden. The pond has a surface area of about 20,000 m^2 and a maximum depth of 3 m. The upper littoral margins contain a substratum of stones, approximately 20 cm in diameter, which stretch from the bank for a distance of approximately 7 m into the water. The lower littoral area comprises a bed of silt. Dense growths of *Elodea* (spp) occur over the silted region. Emergent vegetation, mainly *Carex* (spp), reaches about 50 cm into the pond. The pond contains perch and pike, both of which prey on crayfish (Dehli, 1981; Hogger, 1988 for review). The water temperature during the study period (16 May to 28 June) rose from 12.0 to16.5 °C.

Distribution of adult crayfish

Data on the distribution of adult male, non-gravid female and gravid female crayfish (crayfish carrying eggs but not stage I or stage II young) was determined from test trapping conducted from 13 May to 4 July. Five double-ended funnel traps, baited with fish, were set in the pond at each of four sites (sites a,b,d,& e; Fig. 5.1), four times a week. The traps were set parallel to the west shore at midday and were collected after 24 hours. All the crayfish in the traps were sexed, measured for total length (from the tip of the rostrum to the telson tip) and examined to determine if they were bearing eggs or young, so that the stages of development of juvenile crayfish could be monitored. The crayfish were then replaced at the opposite side of the pond. Trappability was determined from catch per unit effort data (CPUE). CPUE, defined in this work as the number of individual crayfish caught per five traps per 24 hours, was used as a measure of relative abundance.

During the first two weeks of the sampling period, 778 gravid females were removed from the pond by Simontorp Aquaculture A.B. for use in their hatchery (Nyström, pers. comm.). Also, in 1990, 1616 gravid females were removed and 38 kg of female crayfish were returned to the pond after releasing their young.

Distribution of juvenile crayfish

The distribution and abundance of newly independent juvenile crayfish was estimated by counting the number of juveniles found in artificial substratum traps (bag traps) between the 10 June to 8 July. Initially, six bag traps were laid parallel to the west shore of the pond at each of the five sites described in Figure 5.1. After the first collection, six more traps were set each week at sites a,b,& c. Traps were set between

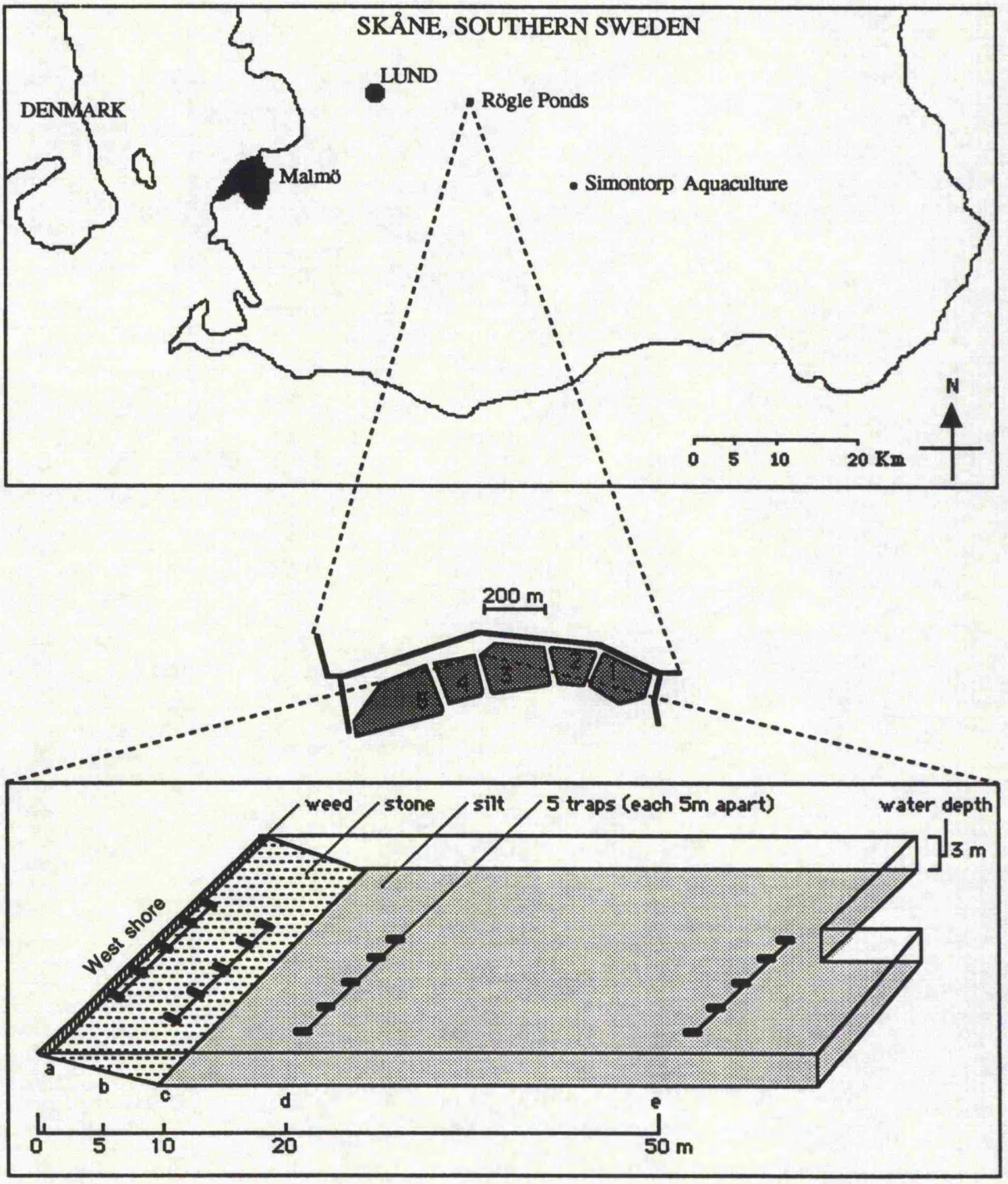


Figure 5.1. A map of Skåne, southern Sweden, showing the position of Rögle Ponds1 to 5, and the sites in pond 3 where adult and juvenile crayfish were trapped. Adults were trapped at sites a,b,d & e. Juveniles were trapped at sites a to e. The silt was covered with growths of *Elodea*. Traps were set at depths of 0.3 m at site a, 2.0 m at site b, and at 3.0 m at sites c,d & e.

09.00 and 12.00 hours and were left for 6 to 7 days before being collected, emptied and reset. The traps were constructed out of plastic net bags (50 x 25 cm, mesh size of 6 x 4 mm) a quarter filled by corrugated plastic cylinders 5 cm in diameter by 3 cm long. For the first week of sampling, the number of crayfish in the bags were pooled for each site. After this time, the number of crayfish found in each bag was recorded. On the 11 July, the total lengths (from the tip of the rostrum to the telson tip) of a sample of juveniles from the traps in the shallow and deep stone habitats (sites a & b) were recorded using vernier callipers.

In addition to sampling the juvenile crayfish from each site, counts were made of the other invertebrate fauna collected in the bag traps. For these counts, the number of individuals of each invertebrate category from the six bags per site were pooled.

Perch predation

On seven occasions between 30 May to 10 July, a standard benthic-survey gill net was set parallel to different areas of the shore, at a distance of 15 to 20 m, at midday for 24 hours. Perch were caught on three occasions prior to and on four occasions after juvenile crayfish had become independent. The net had eight 7.5-m sections, each with a different mesh size (7.6, 10.0, 12.5, 16.5, 22.0, 30.0, 40.0, 55.0 mm). Perch were removed, weighed and measured for total and fork lengths. Their stomachs were removed and frozen, and later the stomach contents of each fish were identified. Counts of the occurrence and quantity of each prey type were made for each stomach.

5.4.2 RESULTS

Distribution of adult crayfish

The CPUE data for male and female crayfish were analysed seperately. CPUE of adult male crayfish differed between habitats ($H=20.93$, $df=3$, $p<0.001$, $n=97$) and between weeks ($H=36.86$, $df=5$, $p<0.001$, $n=97$; Fig. 5.2a). CPUE of males was at a minimum between 28 May to the 1 June. This low CPUE persisted in all the habitats to the 7 June, after which time, CPUE rose markedly on the two stone habitats but remained low on the silt substratum.

Throughout the trapping period, fewer males were caught on the far silt habitat, 50 m from the shore, (Meddis 1984, multiple pairwise comparison between sites; far silt versus:- shallow stone, $p<0.025$; deep stone $p<0.001$; near silt, $p<0.001$). Trappability differed between the stone and nearby silt habitats (20 m from the shore) during the last two weeks of the trapping period (18 to 25 June). More males were found on the deep stone than the nearby silt habitat ($H=10.73$, $m=8$, $n=8$, $p<0.01$). This was also the case for the shallow stone and nearby silt habitats ($H=10.19$, $m=8$, $n=8$, $p<0.01$). Before this time, there was no difference in male abundance in the traps set on these habitats.

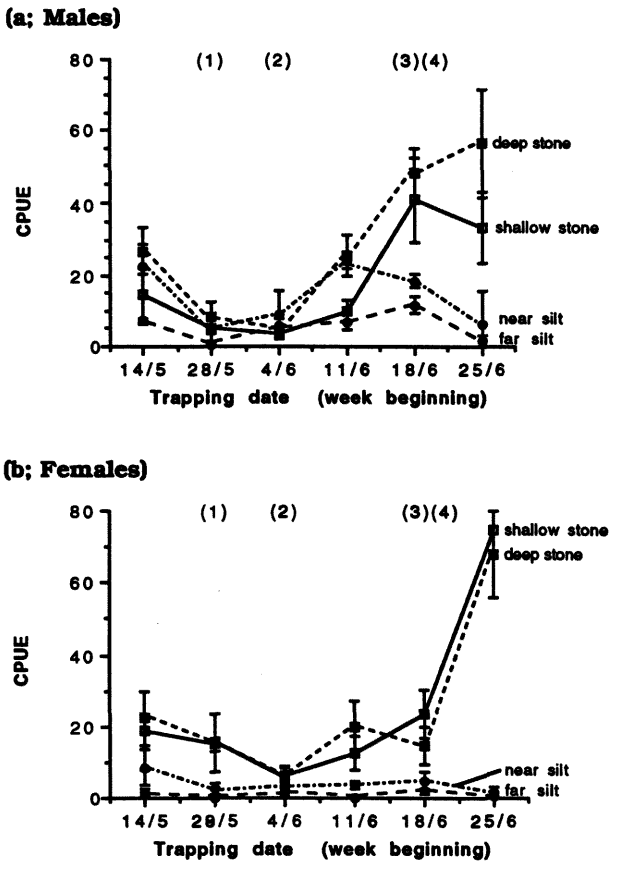


Figure 5.2. The CPUE of a) male crayfish and b) female crayfish in traps set in Rögle pond between 14/5/91 to 29/6/91. Values are the mean (± 1 S.D.) number of individuals caught per five traps per 24 hours, each week on the shallow stone ——, and deep stone - - - -, habitats and on the silt substratum 20 m ·····, and 50 m – – , from the shore. Figures in parentheses refer to first records of: (1) moulted crayfish, (2) females bearing stage I juveniles, (3) females bearing stage II juveniles, and (4) independent stage II juveniles.

Female trappability differed between habitats (H=69.95, df=3, p<0.001, n=97) and between weeks (H=15.10, df=5, p<0.01, n=97; Fig. 5.2b). Throughout the trapping period, more females were found in traps on the two stone habitats than on the two silt habitats (Meddis (1984), multiple pairwise comparison; shallow stone v near silt, p<0.01; shallow stone v far silt, p<0.01; deep stone v near silt, p<0.01; deep stone v far silt p<0.01). CPUE fell to a minimum between 4 to 7 June, but subsequently increased greatly on the stone substratum from 18 to 28 June (sites a and b). Very few females were found on the silt substratum at this time.

The increase in both male and female crayfish trappability from the 4 June coincided with an increase in the proportion of crayfish that were recent moults (Table 5.1). Proportionally fewer moulted females were found on the stone habitats than on the silt habitats (between 4 to 28 June, H=28.6, m=23, n=30, p<0.01), and a smaller proportion of the females on the stone habitats were recent moults compared to males (between 4 to 28 June, H=45.7, m=30, n=31, p<0.001).

Table 5.1. The percentage of male (m) and non-gravid female (f) crayfish in the traps in Rögle pond that were newly moulted at each site for each week. Values are means (±1 S.D.)(† Stage 1 young found; * independent Stage II young found)

Trapping Dates	HABITAT							
	Shallow stone (site a)		Deep stone (site b)		Near silt (20 m) (site d)		Far silt (50 m) (site e)	
	m	f	m	f	m	f	m	f
14-17/5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
28-31/5	0.0	0.0	5.0 (10.0)	0.0	3.1 (6.2)	0.0	0.0	0.0
4-7/6†	63.7 (43.8)	18.0 (23.7)	80.5 (14.1)	29.2 (34.5)	96.7 (5.8)	58.3 (38.2)	85.5 (17.1)	69.0 (27.1)
11-14/6	98.25 (3.5)	57.0 (15.6)	95.0 (2.6)	33.2 (19.4)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)	n/a
18-21/6*	100.0 (0.0)	27.0 (10.2)	100.0 (0.0)	37.7 (23.1)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)
25-28/6	100.0 (0.0)	21.7 (8.3)	100.0 (0.0)	17.2 (5.2)	100.0 (0.0)	66.5 (47.4)	100.0 (0.0)	100.0 (0.0)

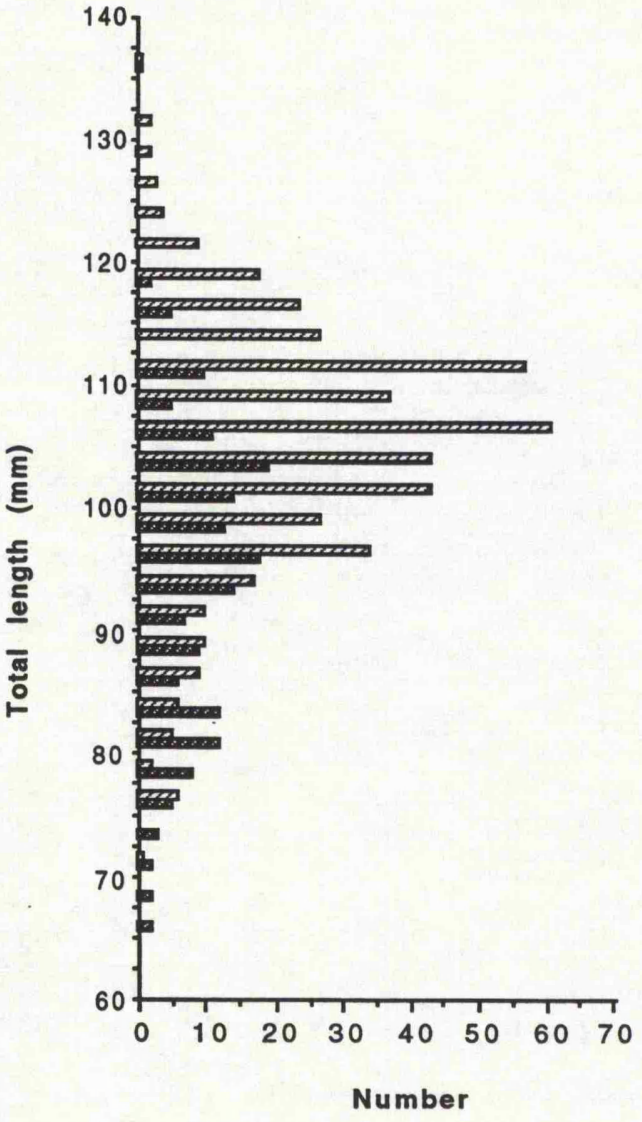


Figure 5.3. Size distribution of male ▨, and female ▩, crayfish caught in traps set at sites a,b,d & e between 18 to 21 June 1991 at Rögle Pond 3.

A sample of crayfish caught in the traps between 18 to 21 June showed that overall, the traps caught larger male crayfish than females (T-test, T=10.4, m=180, n=458, p<0.001; Fig. 5.3). This was true for each habitat. There was an indication that the mean size of both males and females differed between habitats (One factor ANOVA; males F=2.56, df=3, p=0.054; females, F=7.05, df=3, p<0.001; Fig. 5.4). Larger females were found on both the shallow and deep stone stone habitats than on the far silt habitat (Scheffe's F-Test; shallow stone v far silt, p<0.01; deep stone v far silt, p<0.001).

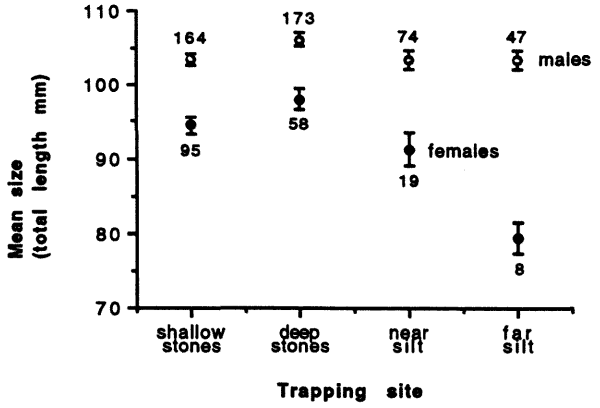


Figure 5.4. Mean (± 1 S.E.) body lengths of male ○ , and female ● , crayfish from traps set at each habitat in Rögle Pond 3 between 18 to 21 June 1991. Numbers denote sample sizes.

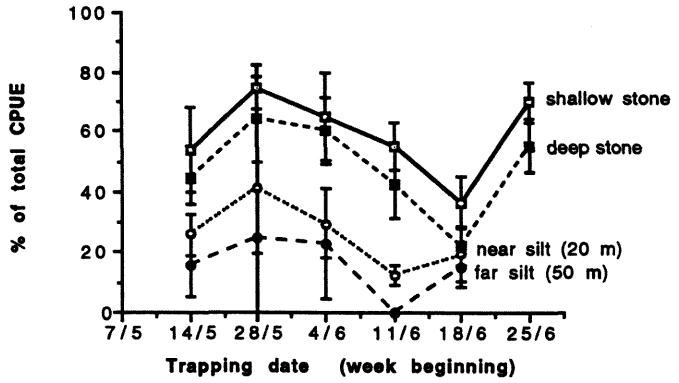


Figure 5.5. The proportion of females in the total CPUE of traps set at each habitat between 7/5/91 to 28/6/91 in Rögle Pond 3. Values are mean (± 1 S.D.) percentages of females in the total number of crayfish caught per 5 traps per 24 hours each week on the shallow stone —— , and deep stone --- , habitats and on the silt substratum 20 m ---- , and 50 m – – , from the shore.

Crayfish caught between the 14 May to 20 June were checked for chelae damage (Table 5.2). The proportion of the crayfish with damaged chelae did not differ between habitats or between males and females.

Table 5.2. The mean percentage (± 1 S.D.) of crayfish from traps set at sites a,b,d & e that had damaged chelae. Traps were set between 14/5/91 to 20/6/91.

Crayfish sex	Habitat:			
	shallow stone (site a)	deep stone (site b)	near silt (site d)	far silt (site e)
Males	14.2 (13.6)	13.6 (8.3)	12.6 (11.2)	14.0 (15.2)
Females	22.2 (8.6)	14.9 (10.8)	25.5 (31.9)	9.3 (18.8)

Throughout the trapping period, there was an overall difference in the proportion of females in the total number of crayfish caught in each habitat ($H=47.01$, $df=3$, $p<0.001$, $n=92$; weeks were used as a blocking variable, Meddis, 1984; Fig. 5.5). Individual comparisons between habitats (blocked for week) indicated that the proportion of females in the catches was inversely related to the distance from the shore. Proportionally more female crayfish were found in the traps on the shallow stone habitat than the deep stone habitat ($H=9.58$, $m=24$, $n=25$, $p<0.01$), on the deep stone habitat than on the silt habitat nearest to the shore ($H=12.46$, $m=23$, $n=25$, $p<0.001$) and on the silt nearest to the shore than on the silt habitat furthest from the shore ($H=3.89$, $m=20$, $n=23$, $p<0.05$).

Distribution of gravid females

The number of gravid females in the traps differed between weeks (14 May to 7 June, $H=10.01$, $df=2$, $p<0.01$) and between habitats ($H=22.74$, $df=3$, $p<0.001$; Fig. 5.6a). More gravid females were caught on the stone substratum than the silt substratum. There was no difference in the trappability with depth on the stones, except for the week of the 28 May to 1 June. In this, the final week prior to the detection of stage I juveniles, more gravid females were caught in the shallow water ($H=5.07$, $m=4$, $n=5$, $p<0.025$). Also, during this week, proportionally more of the females caught on the shallow habitat were gravid ($H=6.05$, $m=4$, $n=5$, $p<0.025$; Fig. 5.6b) and gravid females constituted proportionally more of the total catch of adult crayfish than in the deep stone habitat ($H=6.00$, $m=4$, $n=5$, $p<0.025$; Fig. 5.6c).

Distribution of juvenile crayfish

The first females bearing stage I juveniles were detected on the 4 June. Stage II young were first found on female crayfish on the 17 June, 13 days after stage I young were found. Newly independent juvenile (0+) crayfish were first found in the artificial substratum traps (bag traps) on the 20 June. These crayfish were mainly found on the

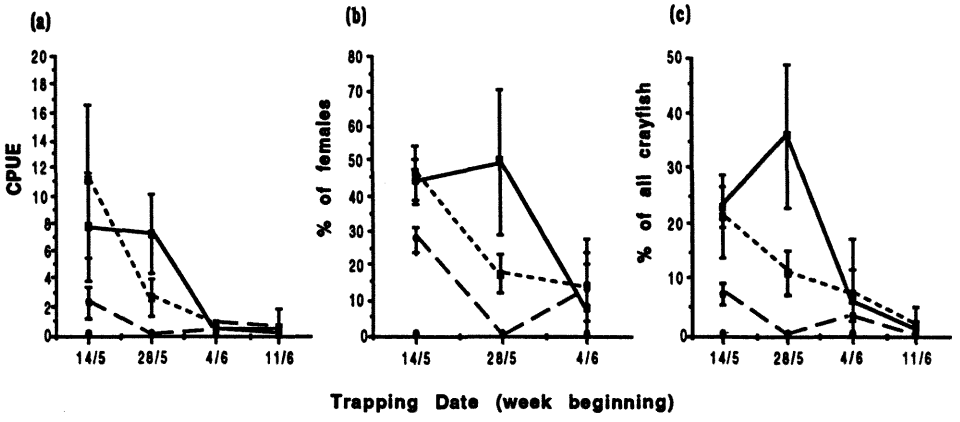


Figure 5.6. a) The CPUE of gravid females, b) the % of females that were gravid, and c) the % of the total catch that were gravid females, in the weeks prior to egg-hatching on the shallow stone ——, and deep stone- - - , habitats, and on the silt substratum 20 m — —, and 50 m (no line, values are all zero) from the shore. Values are means (± 1 S.D.).

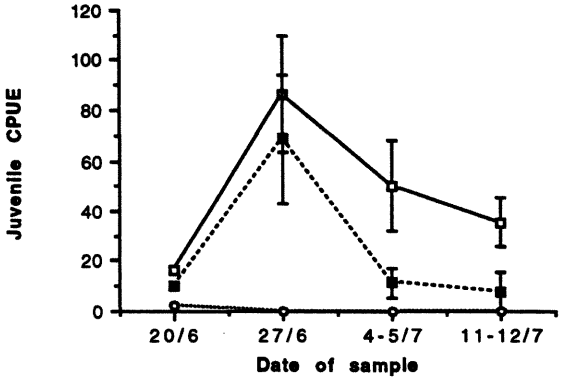


Figure 5.7. The abundance of independent (stage II) juvenile crayfish in traps set each week on the shallow stone □, and deep stone ■, habitats and on the silt substratum 10 m ○, from the shore. Values are the mean (± 1 S.D.) number of crayfish per trap.

stone habitats and were almost completely absent in traps set on the silt substratum (Fig. 5.7). The highest densities of 0+ crayfish were detected in the traps on the stone habitats on the first observation (7 days) after independent juveniles were first detected. On this day (27 June) densities of 0+ juveniles did not differ with depth. In the following two observations made on the 4 to 5 July and 11 to 12 July, there was a decrease in the number of 0+ crayfish caught in both the deep stone and shallow stone habitats. On these two occasions, greater densities of 0+ crayfish were found in the traps in the shallow stone habitat (4 to 5 July, $H=14.10$, $m=12$, $n=12$, $p<0.001$; 11 to 12 July, $H=15.92$, $m=12$, $n=12$, $p<0.001$). On the 11 July, juvenile crayfish from the shallow water traps were of greater mean length (14.1 mm, S.E.=0.3) than juveniles from the deep water traps (mean=12.7 mm, S.E.=0.3; $H=7.38$, $m=11$, $n=22$, $p<0.01$).

Distribution of Yearling (1+) Juvenile Crayfish

One year old (1+) juvenile crayfish were also found in the bag traps. During the trapping period, there was an overall difference in the abundance of the 1+ crayfish in traps set at different habitats ($H=20.33$, $df=4$, $p<0.001$, $n=36$; Fig. 5.8). More 1+ crayfish were found on the stone substratum than the silt substratum ($H=18.88$, $n=18,18$, $p<0.001$) but distribution did not alter with water depth on the stone substratum.

Distribution of Invertebrate Fauna

The distributions of the most common invertebrates found in the traps are shown in Figure 5.9. Table 5.3 gives a full list of invertebrates found.

Table 5.3. Common invertebrate fauna collected in the artificial substratum (bag traps at all sites in Rögle pond between 10/6/92 to 8/7/91.

Class		Order	Family	Further Identification
Crustacea		Isopoda		*Asellus*
		Amphipoda		*Gammarus*
		Decapoda		*P. leniusculus*
Insecta	(adult)	Hemiptera	Corixidae	
	(emergent nymphs)			
	(nymphs)	Plecoptera		
		Ephemeroptera		
		Odonata (Zygoptera)		
	(larvae)	Diptera	Chironomidae	
		Trichoptera (cased)		
		(uncased)		
Gastropoda		Pulmonata	Planorbidae	
			Lymnaeidae	

Also found: Platyhelminthes, Oligochaeta, Odonata

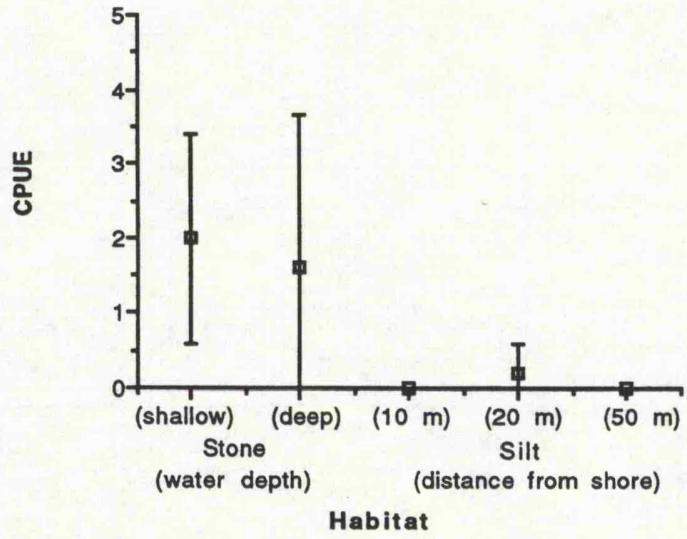


Figure 5.8. The abundance of yearling (1+) juvenile crayfish in traps set at each habitat between 3/6/91 to 11/7/91. Values are the mean (± 1 S.D.) numbers of crayfish caught per six traps per week.

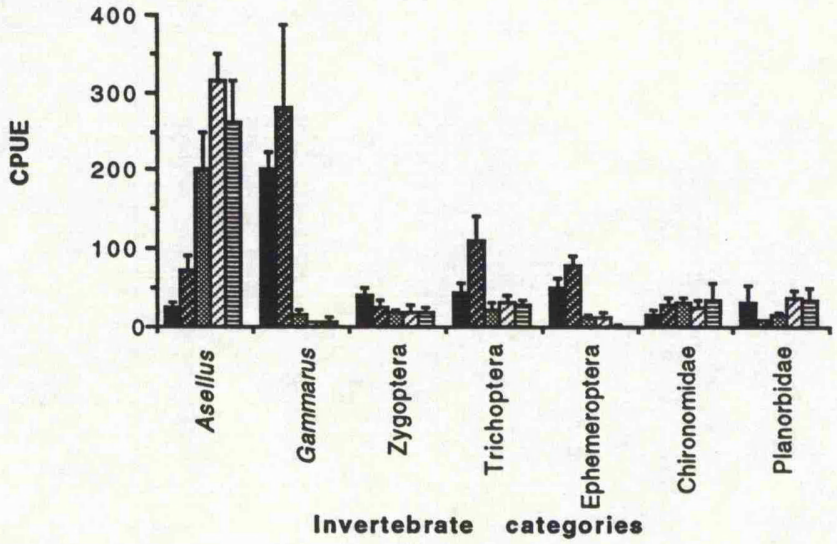


Figure 5.9. The abundance of the most common invertebrate groups in traps set at each habitat between 3/6/91 to 11/7/91. Values are means (± 1 S.E.) of the number of individuals of each category found per six traps per week on the shallow stone ■, and deep stone ▨, habitats and on the silt 10 m ■, 20 m ▨, and 50 m ▤, from the shore.

Asellus were the most abundant organism in the traps. They were found in greatest numbers in the silt habitats (Mann-Whitney U test, between the deep stone and silt habitat 10 m from the shore; U=11, m=9, n=9, p<0.01). The greatest densities of *Gammarus*, Trichoptera and the Ephemeroptera were found on the stone substratum (Mann-Whitney U test between the deep stone and silt habitat 10 m from the shore for *Gammarus;* U=0, m=9, n=9, p<0.01, for Trichoptera; U=4.5, m=9, n=9, p<0.01, and for Ephemeroptera; U=5.0, m=9, n=9, p<0.01). A similar trend was apparent for the distribution of Zygoptera. All of these except for Zygoptera were found in greatest numbers on the deep stone habitats. Chironomidae were associated with the deeper water and silt substratum and Planorbidae were found in greatest numbers in the shallow stone habitats and on the silt habitats furthest from the stones (Mann-Whitney U test between the shallow and deep stone habitat; U=16, m=9, n=9, p<0.05, and between the silt habitats 10 m and 20 to 50 m from the shore; U=17, m=9, n=9, p<0.05).

Perch predation

A total of 114 perch were collected between 10.7 and 38.4 cm total length (mean=19.2 cm, S.E.=0.3). The analysis of perch stomach contents was separated into the periods prior to (n=48) and after (n=66) the release of the 0+ (stage II) juvenile crayfish. Perch primarily fed on *Asellus* and insect nymphs and larvae. These were found in a high proportion of perch stomachs and constituted a large proportion of the total food items in the stomachs in which they occurred (Table 5.4).

Table 5.4. The diet of perch, showing a) the number (%) of stomachs containing each prey type, and b) the relative abundance of each prey in the stomachs in which they occurred. This is expressed as a percentage of the total number of prey items, excluding zooplankton, per stomach. Stomachs containing 1+ crayfish were excluded from the relative abundance data. Data are from perch sampled before (n=48) and after (n=66) the release of Stage II juveniles.

Prey Item	Occurrence: number (%) of stomachs		Relative abundance: Mean % of prey items/stomach (1 S.E.)	
	before	after	before	after
(0+) crayfish	- -	8 (12.1)	- -	21.9 (10.7)
(1+) crayfish	8 (18.7)	6 (9.1)	- -	- -
Asellus	7 (14.6)	44 (77.3)	59.7 (14.6)	69.2 (5.0)
Gammarus	3 (6.2)	2 (3.0)	37.0 (13.0)	57.0 (0.0)
Insect larvae and nymphs:				
Zygoptera	26 (54.2)	31 (47.0)	33.4 (1.1)	25.9 (6.0)
Trichoptera	6 (12.5)	29 (44.0)	20.4 (7.0)	26.0 (5.5)
Ephemeroptera	34 (70.8)	12 (18.0)	62.6 (6.4)	11.9 (4.3)
Plecoptera	6 (12.5)	0	36.7 (16.9)	0.0 (0.0)
Chironomidae	4 (8.3)	18 (27.3)	12.3 (3.9)	21.7 (6.0)
Insect pupae	13 (27.1)	8 (12.1)	30.2 (7.9)	6.9 (1.8)
Insect adults:				
Hemiptera	2 (4.2)	2 (3.0)	6.3 (3.2)	3.0 (0.0)
Zooplankton	0	46 (69.7)	- -	- -
Empty	0	6 (9.1)	- -	- -

Cladocera were abundant in perch stomachs after 0+ juvenile crayfish had hatched, but data on the relative abudance of cladocera in perch stomachs were not recorded.

Perch consumed both 0+ and 1+ crayfish (Table 5.5). Estimates of size classes of some of the crayfish present in the stomachs were only possible by extrapolating the total body lengths from the size of the chelae found, using Figure 5.10. This figure was derived from measurements of live crayfish caught in the pond. Both 0+ and 1+ classes of juvenile crayfish were found in 12% of the perch stomachs sampled during the time periods when they were respectively exposed to predation (0+, 8 out of 66 perch between 21 June to 10 July; 1+, 14 out of 114 perch between between 30 May to 10 July; Table 5.6). Newly independent (0+) juveniles on average comprised 22% of the prey items in the diets of the perch in which they were found. Yearling (1+) juveniles on average constituted 48% of the total number of prey items per stomach in which they were found, but, in terms of volume they constituted the majority of the diet. The relative abundance of juvenile crayfish in perch stomachs matched that of juveniles in the traps on the stone habitats, and particularly in the traps on the deep stone habitat (Spearman's Rank Correlation; perch and shallow water, rs=0.35; perch and deep water, rs=0.95, n=4; Fig. 5.11).

Table 5.5. The sizes of perch and the crayfish they consumed.

Predator size (cm total length)	Crayfish size (mm total length)			Number eaten
	Actual	Estimated male	Estimated female	
38.4	71			2
		85	104	
24.8*				1
24.1	58			1
22.8*				1
22.2		58	65	1
21.5*		38	40	2
21.4	70			1
21.0*				1
20.5*				1
20.4	51			1
20.2*	30			3
		38	40	
20.1		57	70	1
19.9	30			1
17.9*				1
21.1	0+ juveniles			1
19.9	"			10
19.6	"			6
19.3	"			1
19.2	"			7
18.7	"			1
17.7	"			1
17.5	"			5

* One crayfish too digested to measure.

Table 5.6. The number of two size classes of perch which fed on 0+ and 1+ juvenile crayfish in each week from the 30/5/91 to the 10/7/91. Numbers in brackets are percentages of the numbers caught.

PERCH CAUGHT	DATE 30/5	6/6	13/6	21/6	28/6	5/7	10/7	TOTAL
Numbers of perch caught:								
all sizes	22	9	17	10	21	11	24	114
>20 cm total length	0	1	8	2	9	8	10	38
<20 cm total length	22	8	9	8	12	3	14	76
Number of perch that fed on 1+ crayfish:								
>20 cm total length	0 (0)	1 (100)	7 (87)	0 (0)	1 (11)	2 (25)	1 (10)	12 (32)
<20 cm total length	0 (0)	0 (0)	1 (11)	0 (0)	0 (0)	0 (0)	1 (7)	2 (3)
Number of perch that fed on 0+ crayfish:								
both size classes	-	-	-	4 (40)	3 (14)	0 (0)	1 (4)	8 (12)

There was no size difference between perch that had and perch that had not fed on 0+ juvenile crayfish, however, perch which had 1+ juvenile crayfish in their stomachs were larger than those that did not (Mann-Whitney U test, U=174, Chi^2 conversion=4.41, m=14, n=93, $p<0.001$; Fig. 5.12). Only 3% of perch under 20 cm total length preyed on 1+ crayfish, whereas, 32% of perch over 20 cm total length contained 1+ crayfish (Table 5.6).

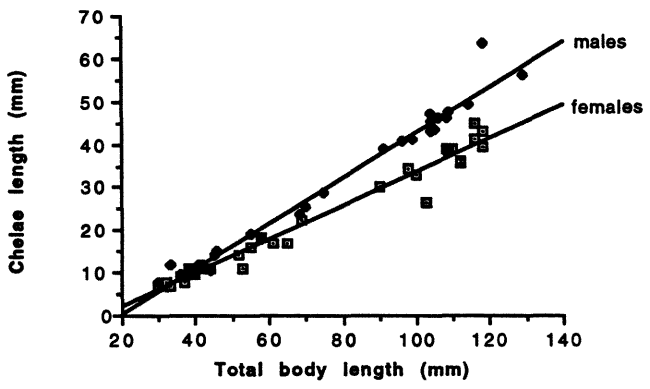


Figure 5.10. The relationship between chelae length and total body length for male and female crayfish trapped in Rögle Pond 3.

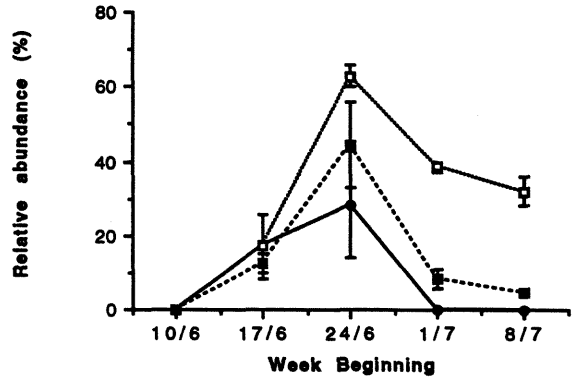


Figure 5.11. The relative abundance (mean percentage ± 1 S.E.) of 0+ *P. leniusculus* individuals in the total number of organisms found per perch stomach ● , and in the traps on the shallow stone □, and deep stone ■ , habitats.

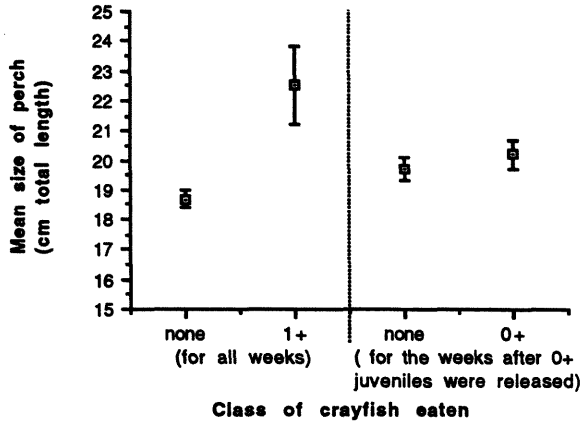


Figure 5.12. The mean (± 1 S.E.) total lengths of perch which consumed 0+ and 1+ juvenile *P. leniusculus*.

Experiment 5.5. HABITAT SELECTION BY GRAVID FEMALE CRAYFISH

5.5.1 MATERIALS AND METHODS

The first experiment tested the preference of gravid female crayfish for two types of substratum and two water depths. Crayfish were presented with two habitat treatments: a) with a deep water (30 cm) pebble substratum (particle size=12 to 29 mm), and a shallow water (10 cm) gravel substratum (particle size=8 to 6 mm), b) with a deep water gravel substratum and a shallow water pebble substratum. Crayfish were exposed to each habitat treatment with either perch present, or with no perch present (control). Thus, in total, there were four experimental treatments Table 5.7.

Table 5.7. Experimental design for testing the habitat preferences of gravid female and juvenile crayfish in Experiment 5.5 & 5.6.

Predator	Tank design	Water depth		Substratum		Treatment
Perch present	a	shallow	(10 cm)	gravel	(8-16 mm)	1
		deep	(30 cm)	pebble	(12-29 mm)	
	b	shallow	(10 cm)	pebble	(12-29 mm)	2
		deep	(30 cm)	gravel	(8-16 mm)	
Perch absent	a	shallow	(10 cm)	gravel	(8-16 mm)	3
		deep	(30 cm)	pebble	(12-29 mm)	
	b	shallow	(10 cm)	pebble	(12-29 mm)	4
		deep	(30 cm)	gravel	(8-16 mm)	

Over a three week period between 10 May to 31 May, twelve plastic tanks with a bottom area of 1 m^2, were used to run nine replicates of each treatment, giving a total of 36 experimental trials. Each trial ran for one week. For each trial, two gravid females, one of which was marked with liquid correction fluid for identification, were placed in each tank. Thus, 72 crayfish were used in the 36 trials.

All twelve tanks were fed by the same recirculating water system. Water temperature ranged between 10.0 and 15.5 ^{o}C for the experimental period. Circular trays 2 cm deep with a surface area of 572 cm^2 (diameter 27 cm) were used to contain the substrata for each habitat treatment. Two trays of each substratum were placed in each tank. Trays at a depth of 30 cm were placed on the tank floor. Trays at a depth of 10 cm were supported on upturned buckets within the water column. The sides of the buckets and trays were covered in plastic netting to enable the crayfish to climb them. Two shelters constructed from corrugated roofing tiles were placed in each tray and the appropriate substratum was then added. The substratum was used to cover the tiles so that each shelter had only one entrance. Plastic drain pipe shelters were placed in each

experimental tank. These were used as shelter by perch. Occasionally, crayfish were also found to be occupying these shelters.

Both crayfish and perch were placed in the respective tanks for two days before observations began. The crayfish were fed part-boiled potato in excess. A piece of potato, measuring approximately 1 cm^3, was placed on each habitat tray twice during each trial. Observations on the habitat use of each crayfish were made over a 24-hour period, on the third and fifth days after the animals were placed in the tanks. Six observation times were used: pre-dawn (06.30 h), post-dawn (08.00 h), day (11.00 h), pre-dusk (15.30 h), post-dusk (17.00 h), and night (20.00 h).

5.5.2 RESULTS

Not all the gravid females were found in shelters during the observation period. Crayfish were more active by night than by day in the controls, and when they were exposed to perch (Fig. 5.13). For all six time periods, over each of two days, more crayfish were exposed in the control tanks than in the tanks with perch. This was only significant during the post-dusk period on day 3 (Chi^2=5.06, df=1, $p<0.025$, n=72). The trend was also strong during the pre-dawn period on day 5 (Chi^2=3.38, df=1, $p<0.07$, n=72).

After three days, gravid females in the control tanks showed a preference for the substrata in the deep water during the day (post-dawn, Chi^2=4.2, $p<0.05$, n=24; pre-dusk, Chi^2=3.8, $p<0.08$, n=26; Fig. 5.14a). More individuals were found on the deep gravel habitats than the deep pebble habitats during these time periods. No habitat preferences were apparent during the night on day three, or at any time on day five of the experiment, due to the majority of the crayfish being exposed (Fig. 5.14b).

After three days, gravid female crayfish in the presence of perch, showed a preference for the gravel substratum during the night (post-dusk, Chi^2=4.6, $p<0.05$, n=14; night, Chi^2=5.4, $p<0.025$, n=15; Fig. 5.14c). There was a similar trend during the day but this was not significant for any time-period ($p>0.05$). Prior to dawn on day five, a preference was shown for the gravel substratum (Chi^2=3.86, $p<0.05$, n=21; Fig. 5.14d). No preference for depth was found. There was an indication that crayfish used the gravel substratum in the shallow water more when they were exposed to perch than in controls with no perch. No similar difference was found for any of the other three habitat combinations.

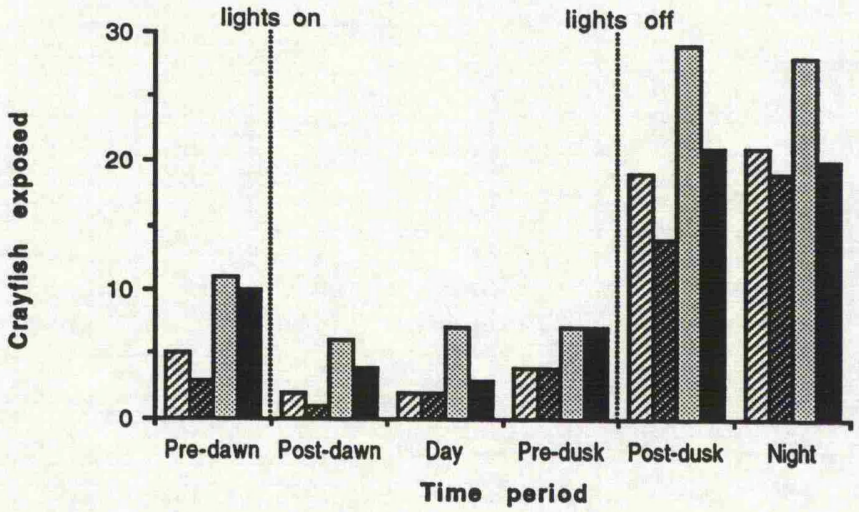


Figure 5.13. The activity of gravid female crayfish at six time periods on day three and day five of Experiment 5.5. Columns denote total numbers of crayfish exposed in tanks with perch on day 3 ▨ , and day 5 ▩ , and in control tanks (no perch) on day 3 ▩ , and day 5 ■ .

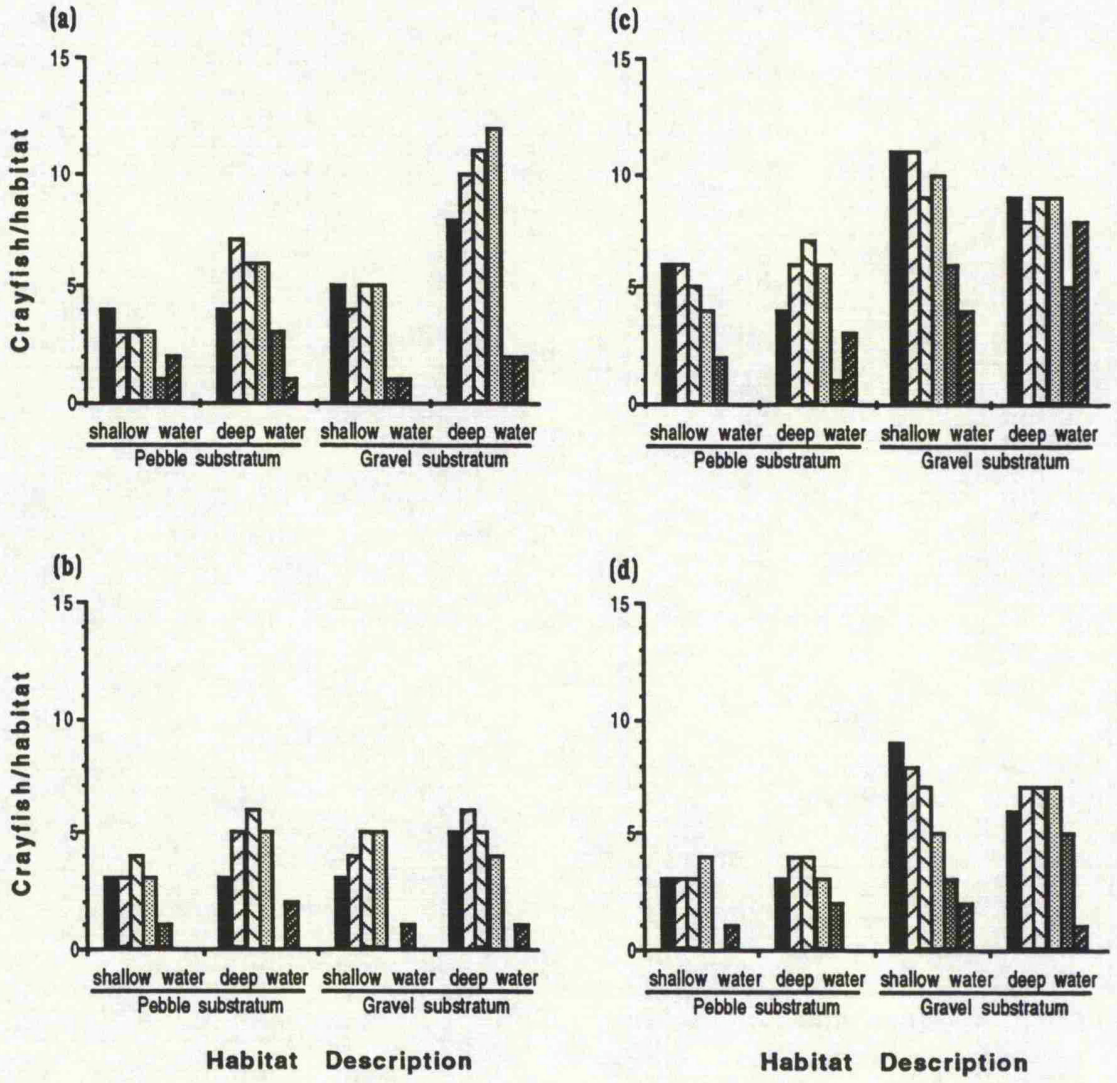


Figure 5.14. Habitat choice of gravid female crayfish, found in shelters in control tanks a) on day 3 and b) day 5, and in response to perch, c) on day 3 and d) day 5, at six time periods: Pre-dawn ■, Post-dawn ▨, Day ▧, Pre-dusk ▩, Post-dusk ■, and Night ▨.

Experiment 5.6. HABITAT SELECTION BY JUVENILE CRAYFISH

5.6.1 MATERIALS AND METHODS

Consideration was given to the relative importance of water depth and substratum in the habitat choice of newly hatched juvenile crayfish. The effect of perch on this habitat choice was also considered. The experimental tanks were set up to give two habitat treatments, as described in Experiment 5.5 (Table 5.7). The roofing tiles were removed from the substratum trays which were then filled to a depth of 2 cm with the respective substratum. One hundred newly independent (stage II) juvenile crayfish were placed in each tank. Fifty were placed on each substratum. Crayfish were exposed to each habitat treatment with either a) perch present, but restrained in cylindrical mesh cages approximately 25 cm diameter by 50 cm long, or b) with no perch, but with the mesh cages still present (control). Thus, as in Experiment 5.5, four treatments were used. Each treatment was replicated 9 times between 1 June to 16 June, giving 36 trials. Each trial lasted four days.

Juvenile crayfish and perch were placed in the tanks at the same time. On the third day after crayfish and perch were placed in the tanks, observations on crayfish activity were made. Levels of activity were determined from the number of crayfish exposed on each substratum/depth combination and on the bare tank floor. Counts of crayfish activity were made on six occasions over a 24 hour period, as described in Experiment 5.5. On the fourth day of the experiment, perch were removed and the numbers of crayfish present in each habitat and on the tank floor were counted. The water temperature in the tanks ranged between 10.0 to 12.0 °C.

5.6.2 RESULTS

In both the perch and control tanks there was an overall difference in the number of juvenile crayfish found on the four habitats (perch tanks, $H=24.86$, $df=3$, $p<0.001$; control tanks, $H=23.91$, $df=3$, $p<0.001$; Fig 5.15). In the control tanks, crayfish were more abundant in the deep water ($H=17.21$, $m=18$, $n=18$, $p<0.001$) and on the pebble substratum ($H=6.66$, $m=18$, $n=18$, $p<0.01$). Crayfish that were exposed to perch were also more abundant in the deep water ($H=11.70$, $m=18$, $n=18$, $p<0.001$), and on the pebble substratum ($H=13.15$, $m=18$, $n=18$, $p<0.001$). Significantly more crayfish were found on the shallow pebble habitats when perch were present than in the controls ($H=6.60$, $m=9$, $n=9$, $p<0.01$). No similar differences were found in the number of crayfish counted on each of the other three habitat combinations.

There was no difference in the survival of the juvenile crayfish between perch and control tanks or between habitat treatments within the perch and control treatments. The activity of juvenile crayfish was determined from counts of the

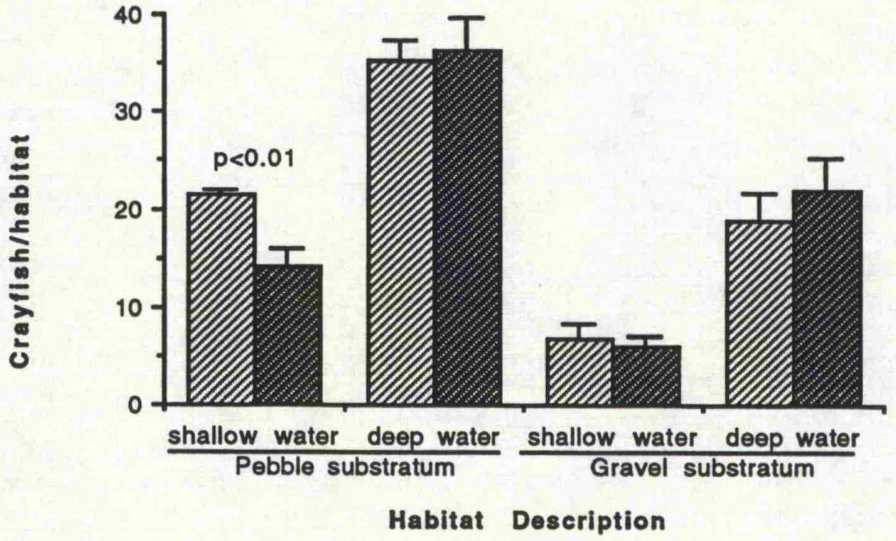


Figure 5.15. Habitat preferences of juvenile crayfish. Values are the mean number (± 1 S.E.) of crayfish counted per habitat at the end of Experiment 5.6, when perch were present but restrained ▨ , and in control tanks (no perch) ■ .

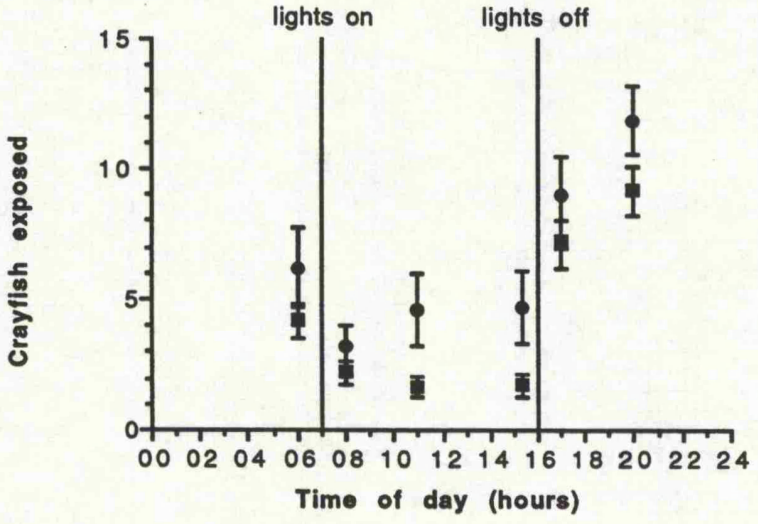


Figure 5.16. The mean (± 1 S.E.) number of crayfish found exposed during day 3 of Experiment 5.6 in tanks with restrained perch ■ , and in control tanks (no perch) ● .

number of juvenile crayfish exposed within the whole tank, at six time periods on the third day of the experiment. In three replicates of the two predator tank treatments, perch were dead on the morning of the activity observations, and were only replaced at midday. The absence of active perch may have altered juvenile crayfish behaviour in these tanks. For this reason, a second set of observations were made in these tanks and in the equivalent control tanks at 06.30, 08.00 and 11.00 hours on the following day. The average of the two results for these replicates is used in the analyses below.

Only a small number of juvenile crayfish were found exposed. Within each observation period, comparisons were made between the number exposed in tanks containing perch and the control tanks (Fig. 5.16). Prior to dusk, fewer crayfish were exposed in the perch tanks ($H=4.86$, $m=18$, $n=18$, $p<0.05$,). A similar trend was found in each of the other five time periods although these were not significant ($p>0.05$).

Experiment 5.7. THE EFFECT OF WATER DEPTH AND SUBSTRATUM ON JUVENILE CRAYFISH MORTALITY DUE TO PERCH PREDATION

5.7.1 MATERIALS AND METHODS

This experiment was designed to compare the effects of substratum and water depth on the predation of juvenile crayfish by perch. Twelve plastic tanks (1-m^2 bottom area) were organised to give four habitats each with a separate depth/substratum combination. Each tank contained four, 572-cm^2 trays filled to 2 cm with a single substratum (either pebbles or gravel; particle sizes as for Experiment 5.5) at water depths of either 25 cm (shallow) or 50 cm (deep) in a 2 x 2 factorial design. One hundred newly hatched (stage II) crayfish were placed on each habitat with either a) unrestrained perch, or b) no perch (Control), giving a total of eight treatments (Table 5.8). Six replicates of each tank design were run between 16 June to 14 July, giving a total of 48 trials. Each trial took seven days. The water temperature for the duration of the experiment ranged between 14.0 -18.0 °C.

On the third day after crayfish were placed in the tanks, perch were added to the relevant tanks, and were left for five days. The five day period was chosen because perch left with crayfish for this length of time in previous experiments reduced crayfish survival by up to 88% (Chapter 2).

Table 5.8. Treatments used to test the effect of water depth and substratum on predation of juvenile crayfish by perch. Numbers in the body of the table indicate different treatments.

Substratum	Water depth:			
	deep (50 cm)		shallow (25 cm)	
	Perch present	Perch absent (control)	Perch present	Perch absent (control)
pebble	1	3	5	7
gravel	2	4	6	8

5.7.2 RESULTS

Crayfish survival was compared between perch and control tanks for each habitat type (Fig. 5.17). Perch reduced crayfish survival on both the shallow and deep gravel habitats (Shallow gravel, $H=4.02$, $m=6$, $n=6$, $p<0.05$; deep gravel, $H=4.02$, $m=6$, $n=6$, $p<0.05$). There was an indication that perch reduced crayfish survival on the deep pebble habitat ($H=2.85$, $m=6$, $n=6$, $p=0.087$), but survival was not affected on the shallow

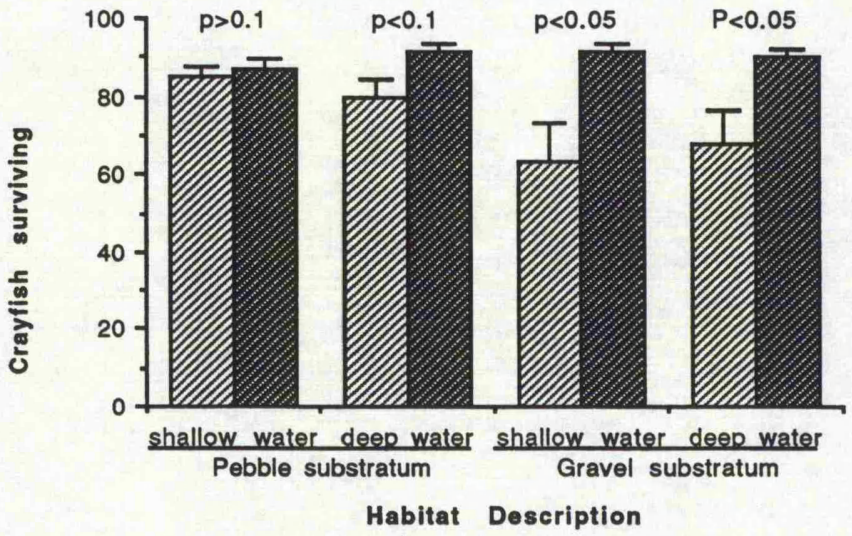


Figure 5.17. Numbers of juvenile crayfish surviving on each habitat after exposure to perch ▨ , and in control tanks (no perch) ■ . Values are means (± 1 S.E.).

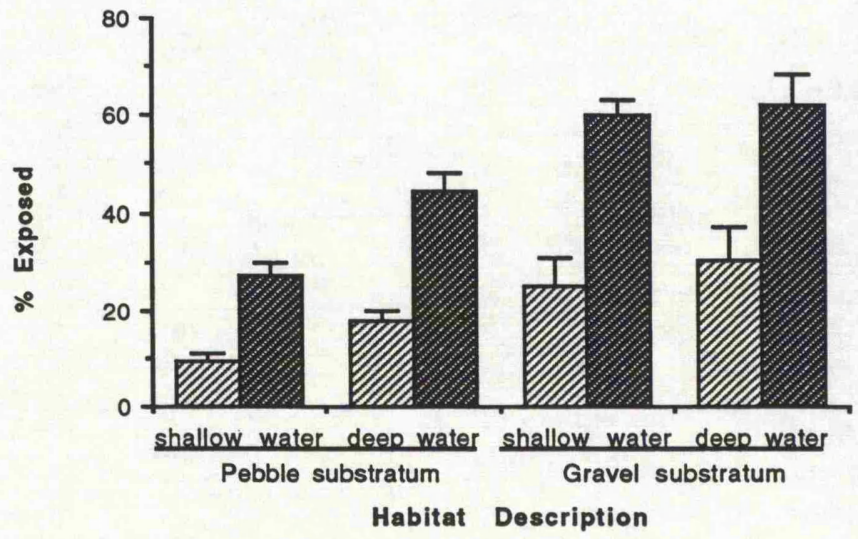


Figure 5.18. Activity of juvenile crayfish from different habitats when exposed to perch ▨ , and in control tanks (no perch) ■ . Values are the mean (± 1 S.E.) percentage of the surviving crayfish which were exposed on the tank floor.

pebble habitat ($p>0.1$).

Activity was determined from counts of the number of crayfish exposed on the bare tank floor upon the removal of the habitat trays at the end of the experiment. The activity of the crayfish is expressed as the percentage of the crayfish present in the whole tank which were exposed on the tank floor.

Fewer crayfish were exposed in tanks where pebbles formed the substratum, both when the perch were present ($H=6.95$, $m=12$, $n=12$, $p<0.01$) and in the control tanks ($H=12.84$, $m=12$, $n=12$, $p<0.001$; Fig. 18). Also, in tanks where pebbles formed the substratum, more crayfish were active in the deep water than in the shallow water (with perch present; $H=6.27$, $m=6$, $n=6$, $p<0.025$; control tanks, $H=7.03$, $m=6$, $n=6$, $p<0.01$). This trend was not significant in the tanks with a gravel substratum. In each habitat, activity was reduced by the perch (shallow pebble habitat, $H=8.31$, $m=6$, $n=6$, $p<0.001$; shallow gravel habitat; $H=7.44$, $m=6$, $n=6$, $p<0.01$; deep pebble habitat, $H=8.37$, $m=6$, $n=6$, $p<0.01$; deep gravel habitat; $H=5.03$, $m=6$, $n=6$, $p<0.025$).

5.8 GENERAL DISCUSSION

This study was designed to determine 1) how newly independent juvenile crayfish were distributed in Rögle pond, 2) how quickly this distribution was established, and 3) to what extent perch predation, and the behaviour of gravid female and juvenile crayfish influenced this distribution. Trapping data indicated that newly independent juvenile crayfish were restricted to the stone substratum, with very few found on the silt. Also, few gravid females were found on the silt, suggesting that gravid females may exert a strong influence on the initial distribution of the juveniles.

Juvenile crayfish reached their maximum density in the traps on the first sampling occasion (one week) after the first independent juveniles were detected. One week after this, there was a marked decline in the number of juveniles in deep water traps, and to a lesser extent in the shallow water traps. Thus, after two weeks, relatively more juveniles were found in traps on the shallow stone habitat than on the deep stone habitat. The fall in 0+ juvenile densities coincided with the second moult from stage II to stage III.

The number of juveniles sheltering in the traps will be influenced by population density and the availability of alternative shelter. Thus, fewer crayfish may have entered the deep water traps if natural shelter was more abundant there. In Rögle pond the stone substratum was uniform with depth, although weed, which is another potential source of shelter, was more abundant in the shallow water. It was not possible to determine whether juveniles migrated to the shallow water in Rögle pond, however, in Experiment 5.6, substratum influenced juvenile distribution but water depth did not. Thus, independent from other influences, where a substratum is uniform with water depth, as in Rögle, juvenile crayfish distribution should also be uniform.

Differential survival is another possible cause of the differential distribution of juveniles between deep and shallow water. The distribution of the crayfish in the traps is, therefore, likely to have been a function of: 1) protection offered by the weed in the shallow water, and 2) predation by perch (Dehli, 1981) and aeschnid nymphs (Dye & Jones, 1975; Gydemo et al., 1990; Jonsson, 1992), and/or competition and predation by adult and juvenile conspecifics. The greater abundance of juveniles in the shallow water implies that potential predators/cannibals or competitors were either less abundant or less successful in shallow water.

The relative abundance of 0+ juveniles in perch stomachs corresponded most closely to their relative abundance on the deep stone habitat, although only a small number of stomachs were analysed. The majority of the perch caught in Rögle pond were larger than 15 cm, and although no data on perch activity and habitat use were available, perch predation on juvenile crayfish was likely to be less successful in the shallow littoral margins, less than 30 cm deep. Both shallow water and weed limit fish

predation on crayfish (Saiki & Tash, 1979; Mather & Stein, 1990). Diehl (1988) and Matilla (1992) showed that perch predation on amphipods and chironomid larvae also declined in the presence of weed. Experiment 5.7 indicated that shallow water may reduce the success of perch feeding on crayfish on substrata providing good shelter (i.e. pebble substratum).

It has been estimated from predation rates by aeschnid nymphs in enclosed experiments that when aeschnid densities are high, these predators could account for up to 75-100% of juvenile crayfish (*O. virilis*) mortality in the first week of independence (Dye & Jones, 1975). Although large Odonata nymphs were occasionally found in the traps in Rögle, their density and distribution are not known.

Cannibalism is affected by the availability of alternative food, the density of the population and the behaviour and availability of the prey (Fox, 1975a). Adult male and female crayfish (either recently gravid or not) prey upon juvenile crayfish (Mason, 1977; Dye & Jones, 1975; Munkhammar et al., 1989; Gydemo et al., 1990; Jonsson, 1992). Cappeli (1980) found crayfish remains in 60% of the stomachs of adult and large juvenile *Orconectes propinquus* (Girard) collected from a lake at a depth of 1 m in June. The proportion of stomachs with crayfish remains declined with increasing water depth and over a period of several months. This suggested that cannibalism was mainly directed at newly independent young, which were most abundant in the shallow water. In a lake in Ontario, Momot (1992) found little evidence for cannibalism by adult *O. virilis* on juveniles. In laboratory studies, adults had difficulty in catching juveniles unless juveniles were immobile during moulting.

In Rögle pond, CPUE data indicated that feeding activity increased in recently moulted adult male and non-gravid female crayfish, and also in recent brood females between 11 to 28 June (Fig. 5.2, Table 5.1). This coincided with the increase in juvenile abundance as they became independent from the females. This situation fulfils criteria that favoured cannibalism by adult notonectids on juveniles (Fox, 1975b), namely, that there was an increase in the relative and absolute abundance of juveniles and that juveniles were most vulnerable to attack during ecdysis. This suggests that cannibalism by adult crayfish on juveniles was likely to occur in Rogle pond. Although juvenile crayfish survival was better in the shallow water, trap data did not indicate that adult crayfish were more abundant in the deep water. Even if densities were similar with water depth, the emergent vegetation in the shallow water might provide juveniles with greater protection from predation. Cannibalism between conspecifics of the same size is often density dependent (Polis, 1981). In laboratory studies, shelter has been shown to increase survival of juvenile *P. leniusculus*. (Mason, 1979). Thus the weed in the shallow water at Rögle may also reduce intraspecific interactions between juveniles.

Since 0+ crayfish survival appeared to improve in shallow, weedy water, this might explain the increase in the number of gravid females found in the shallow water in the week immediately prior to the hatch (i.e. prior to the first records of stage I juveniles). Gravid females (*P. leniusculus*) are very aggressive (Mason,1970), and Stein (1977) suggests that this enables gravid *O. propinquus* to secure shelter which renders them almost exempt from predation. However, the suggestion that females move to shallow water to release their young is tentative. The use of CPUE data for population studies has many associated problems (Brown & Brewis, 1979). The traps in the shallow water were likely to attract crayfish from a large area of deeper water and the increase in gravid females in traps in the shallow water may have been a result of competitive exclusion by adult male crayfish, either limiting female access to deep water traps or female abundance in the deeper water. Further work is required to clarify this discrepancy.

Given that the substratum was uniform with depth in Rögle, and assuming that the vulnerability of gravid females did not alter with depth, then selection might favour females which release their young in shallow water, if juveniles have a greater chance of surviving there. However, despite the indication that gravid females were present in greater numbers in the shallow water during the hatching period, the initial density of the juveniles at different depths did not reflect this. Although juveniles show a preference for shelter on suitable substrata over maternal protection, female sheltering behaviour and the use of brood pheromones indicates that selection has favoured females which choose habitats according to their own needs (Jonsson, 1992). In Experiment 5.5, the substratum preferences of gravid females differed from those of 0+ juvenile crayfish, and females did not show a preference for shallow water as indicated in Rögle pond, although the gravid females used in the laboratory were more than one week away from hatching their young.

It is difficult to separate the effects of predatory mortality and predator induced behaviour on the distribution of prey throughout a habitat. In Experiment 5.6, juvenile crayfish preferred the pebble substratum, and the presence of restrained perch reinforced this preference. Perch preyed more heavily on juveniles on the gravel substratum. Both mechanisms led to increased densities of crayfish on the pebble substratum. In pond experiments, sixty days after hatching, the abundance of young-of-the-year (YOY) *P. leniusculus* did not differ between ponds where perch were present and ponds with no perch, although perch reduced crayfish activity (Appelberg & Odelström, 1988). Conversely, Svensson (1992) found that perch and roach reduced YOY *A. astacus* survival in experimental ponds. Also, Appelberg (1987) suggested that perch limited *A. astacus* population recovery in Swedish lakes that had been limed to neutralise the effects of acidification. However, it was not known whether poor juvenile densities were a result of predatory mortality or of negative effects on juvenile crayfish activity and growth.

It is possible that juvenile abundance in Rögle was limited indirectly by predators. Momot (1992) found a negative relationship between growth and survival of juvenile *O. virilis* and suggested that adults increase juvenile mortality by inducing moult failure as a result of limiting juvenile feeding activity. Perch may also reduce crayfish activity and thereby growth (Appelberg & Odelström, 1988).

The survival of *P. leniusculus* from independence to sexual maturity (2 years) was reported to be 10% in a stocked population and 25% in a natural river population of *P. leniusculus* (Shimizu & Goldman, 1983; Fürst, 1977 cited by Fjälling & Fürst, 1988). The importance of predation during these two years is uncertain. Momot (1967) and Momot & Gowing (1977) suggest that fish and invertebrate predation are not important population control mechanisms. Goldman & Rundquist (1977) and Mitchell & Smock (1991) suggest that predation, intraspecific interactions and substratum availability interact to limit crayfish populations. Cappelli & Magnuson (1983) found that substratum was the single most important variable controlling crayfish abundance, although other variables also had significant effects. This is consistent with the present study, which suggests that adult *P. leniusculus* were mainly limited to the stone substratum. The distribution of gravid females influenced the distribution of juveniles between silt and stone substrata in Rögle pond, but probably had no influence on the stone habitat. It is suggested that differential mortality rather than juvenile behaviour resulted in greater numbers of juvenile crayfish being found on the stone substratum in the shallow water. Whilst experimental and field data indicate that perch predation influenced juvenile distribution, it was not possible to distinguish between the effects of perch, the effects of invertebrate and intraspecific predation and competition, or the influence of the emergent vegetation. This problem is addressed in the work at Rögle pond described in the following chapter.

CHAPTER 6.

6.0 THE EFFECT OF WEED, FISH AND ADULT CRAYFISH ON THE DISTRIBUTION, SURVIVAL AND GROWTH OF JUVENILE *P. LENIUSCULUS*.

6.1 SUMMARY

By manipulating areas of Rögle pond in southern Sweden, the effects of perch and emergent vegetation on newly independent juvenile crayfish distribution, survival and growth were investigated. Complementary laboratory experiments, using real weed, artificial weed or no weed habitats, tested whether the habitat preferences of newly independent juvenile crayfish were based on cover or food availability, and whether fish and adult crayfish altered the habitat preferences, activity and survival of juvenile crayfish. Small non-predatory fish were used to simulate the indirect effects of predatory fish, as it had previously been established that the behavioural responses of crayfish to these two types of fish were similar.

Although there was an indication that perch and weed exerted a weak influence over the distribution of newly independent juvenile crayfish in Rögle pond, it was concluded that other factors were exerting a stronger control over crayfish distribution and survival. Crayfish growth was greater in the shallow littoral margins (~ 30 cm deep) than in deeper water (~ 150 cm deep)

In laboratory studies, crayfish showed a weak preference for cover provided by real and artificial weed. Real weed benefitted crayfish growth. Juvenile crayfish became increasingly nocturnal in response to fish and increasingly diurnal in response to adult crayfish. Juvenile crayfish mortality increased in response to both non-predatory fish and adult crayfish but this effect was mitigated by real weed habitats. This suggested that mortalities were either a result of an increase in intraspecific interactions or of limited food availabiltiy associated with reduced activity. Cannibalism by adult crayfish may also have increased juvenile crayfish mortality.

The results of the laboratory studies suggest that adult crayfish may be important limiting factor to juvenile crayfish survival, distribution and growth in Rögle pond. The results showed that fish and adult crayfish produce conflicting avoidance responses in juvenile crayfish. The significance of this conflict in wild populations of crayfish is discussed and appropriate responses are suggested on the basis of minimising overall predation risk.

6.2 GENERAL INTRODUCTION

The work described below was designed to investigate the importance of perch, adult crayfish and vegetation on juvenile crayfish distribution, growth and survival. Chapter 5 described laboratory experiments in which substratum was shown to affect the distribution and survival of juvenile *P. leniusculus*. More crayfish were found on substrata with greater interstitial spacing, both as a result of juvenile crayfish habitat preferences, and also as a result of perch predation. Such effects have also been shown by Stein & Magnuson (1976), and Butler & Stein (1985). There was an indication that perch predation was further reduced in shallow water on substrata providing good protection from predation (Chapter 5, Section 5.7.2). A similar effect was shown for smallmouth bass (*M. dolomieui*) feeding on *Orconectes rusticus* and *O. sanborni* (Mather & Stein, 1990).

In laboratory experiments, crayfish did not select habitats with respect to water depth (Chapter 5, Section 5.6.2), yet juvenile crayfish were found in greater densities in traps set in the shallow margins in Rögle pond (Chapter 5, Section 5.4.2). A similar distribution pattern was found in populations of *A. astacus* (Appelberg, 1986). The pattern was most marked in lakes with large populations of predatory fish. The shallow water also contained the substratum which provided juvenile crayfish with the best protection from predation.

Juvenile lobsters (*H. americanus*) locate shelter using visual cues by day, choosing areas of shade, and using tactile cues at night, choosing shelters according to their complexity, (Johns & Mann, 1987). Juvenile lobsters also prefer real, as opposed to artificial weed cover, indicating a positive response to chemical characteristics of weed, or organisms associated with weed (Johns & Mann, loc.cit.). Spiny lobsters (*Panulirus interruptus* Randall) have a strong preference for specific den designs, but den preferences depend more on shade than on the presence of den walls (Spanier & Zimmer-Faust, 1988). Caribbean spiny lobsters (*P. argus* Latreille) choose shelters scaled to their own size when conspecific densities and predation risk are low. Lobsters become more gregarious at greater densities and use smaller shelters when predation risk rises (Eggleston & Lipcius, 1992). Wahle (1992a) found a linear relationship between body length of *H. americanus* and the minimum diameter of cobbles in which shelter was obtained, although lobsters also manipulated substrata to form shelters. Shelter selection was based on tactile cues. A correlation between body and shelter size has also been shown for freshwater crayfish (Rabeni, 1985; Appelberg, 1986; Foster, 1992).

The littoral margins of Rögle pond have a uniform stone substratum, and it was concluded in Chapter 5 that the observed pattern of young-of-the-year (YOY) crayfish distribution resulted from different mortality rates in the deep and shallow water habitats, rather than from juvenile crayfish migrating to shallow water.

It was suggested in Chapter 5 that perch and adult *P. leniusculus* were likely to be important predators of newly independent juvenile *P. leniusculus* in Rögle Pond. Dragonfly larvae were also present, and are known to prey upon juvenile crayfish (Dye & Jones, 1975; Witzig et al., 1986; Gydemo et al., 1990; Jonsson, 1992). Perch preyed upon YOY crayfish during their first few weeks of independence (Chapter 5, Section 5.4.2). A similar result was also found by Andersen & Helmgaard (1990), for perch feeding on *A. astacus*. Two weeks after their second moult, very few YOY crayfish were found in perch stomachs. Perch may , therefore, be of major importance in controlling the initial survival and distribution of YOY crayfish.

Perch predation was likely to be limited in the shallow water habitat in Rögle pond, either due to the physical restrictions of shallow water on perch access, or as a result of protection afforded to juvenile crayfish by emergent vegetation. Weed cover reduces largemouth bass (*M. salmoides*) predation on *O. causeyi* (Saiki & Tash, 1979), cunner (*Tautogolabarus adspersus* Walbaum) predation on juvenile lobsters (Johns & Mann, 1987), and perch and ruffe (*Gymnocephalus cernuus* L.) predation on *Asellus aquaticus* L. (Matilla, 1992). In the latter study, tall shading elements, such as reeds and aquatic plants, gave the best protection against fish that fed visually. Greater patches of vegetation also increased *Asellus* survival. Therefore, there may be survival benefits for YOY crayfish which select weedy habitats, in terms of growth, if food availability increases in association with weed cover, and in terms of shelter from predation. Also, vegetation may reduce intraspecific predation between juveniles, as shelter reduces crayfish activity and aggressive interactions and improves crayfish survival (Mason, 1979; Westin & Gydemo, 1988).

Momot (1992) suggests that adult crayfish control crayfish populations by inhibiting the growth of juvenile crayfish (*O. virilis*), thus promoting juvenile mortalities, although in enclosure experiments, adult crayfish reduced juvenile crayfish growth, but did not increase mortality (Maxwell, 1988 cited by Momot, 1992). Juvenile crayfish growth was enhanced by cover and low conspecific densities. Crayfish become less active in the presence of predators (Stein & Magnuson, 1976; Hamrin, 1987; Appelberg & Odelström, 1988). In the later study, this resulted in reduced growth of juvenile *P. leniusculus* in response to perch.

Four experimental investigations were proposed. The first (Section 6.3), involved a field manipulation to determine whether perch or emergent vegetation affect the distribution, survival and growth of newly independent YOY *P. leniusculus* in Rögle Pond, southern Sweden. The final three experiments were laboratory studies, designed to complement the field study. These experiments addressed the following questions:-

1) whether weed cover affects YOY *P. leniusculus* habitat use and, if so, whether habitat selection is based on shelter or food availability (Section 6.4)?

2) to determine whether juvenile crayfish activity, survival and growth are affected by a) shelter provided by aquatic weed, b) the presence of fish, and c) the presence of adult crayfish (Section 6.5)?

3) to determine the effect of aquatic weed on juvenile crayfish mortality due to perch predation? Time limits prevented the completion of the third experiment.

6.3 DISTRIBUTION, GROWTH AND SURVIVAL OF JUVENILES IN RESPONSE TO WEED COVER AND PERCH.

6.3.1 INTRODUCTION

The following field experiment was conducted in Rögle Pond 3 in southern Sweden between 20 May and 15 July 1992. The study examined the effect of emergent vegetation and perch predation on juvenile crayfish survival, growth and distribution by removing either perch and/or vegetation from areas of the pond. In the previous year's work at Rögle Pond (Chapter 5, Section 5.4), there was also an indication that gravid female crayfish were moving to shallow water before hatching their young. This was retested in the following study.

6.3.2 MATERIALS AND METHODS

Experimental Site

The work was conducted at Rögle Pond 3. This is described in Chapter 5, although several characteristics of the pond differed between 1991 and 1992. In 1991, the water temperature rose from 12 to 16.5 °C between the 16 May and the 28 June. In 1992, water temperature fluctuated between 19 and 23 °C between the 22 May and the 8 July. Growths of *Elodea* (spp) were absent from the pond in 1992, and the water was turbid and contained dense growths of planktonic algae. The stone substratum was also coated in a thick layer of sediment. This contrasted with the 3-m growths of *Elodea*, the relatively clear water, and the relatively sediment free stone substratum in 1991 (Chapter 5, Section 5.4.1).

The following work was concentrated in the littoral margins of the pond, which had a uniform stone substratum running from the water surface to an approximate depth of 2.5 m. Perch and weed were removed from areas of the littoral margin on the west shore of the pond in a 2 x 2 factorial design (Figure 6.1).

Emergent vegetation (*Carex* spp) was concentrated in a belt extending 0.5 m from the shore line. Two weeks before juvenile crayfish became independent, emergent vegetation was cut and removed from two adjacent 15-m stretches of the littoral margin; one inside and one outside a net enclosure. These adjacent lengths of shore were chosen to limit the possibility that benthic invertebrates might migrate to adjacent weedy areas of shore. It was assumed that a greater area of cleared shore line would limit these migrations, if they occurred.

Fish were excluded from a 30-m stretch of the littoral margin by suspending a small-meshed siene net (1 cm by 1 cm square mesh) in an arc, reaching 10 m into the

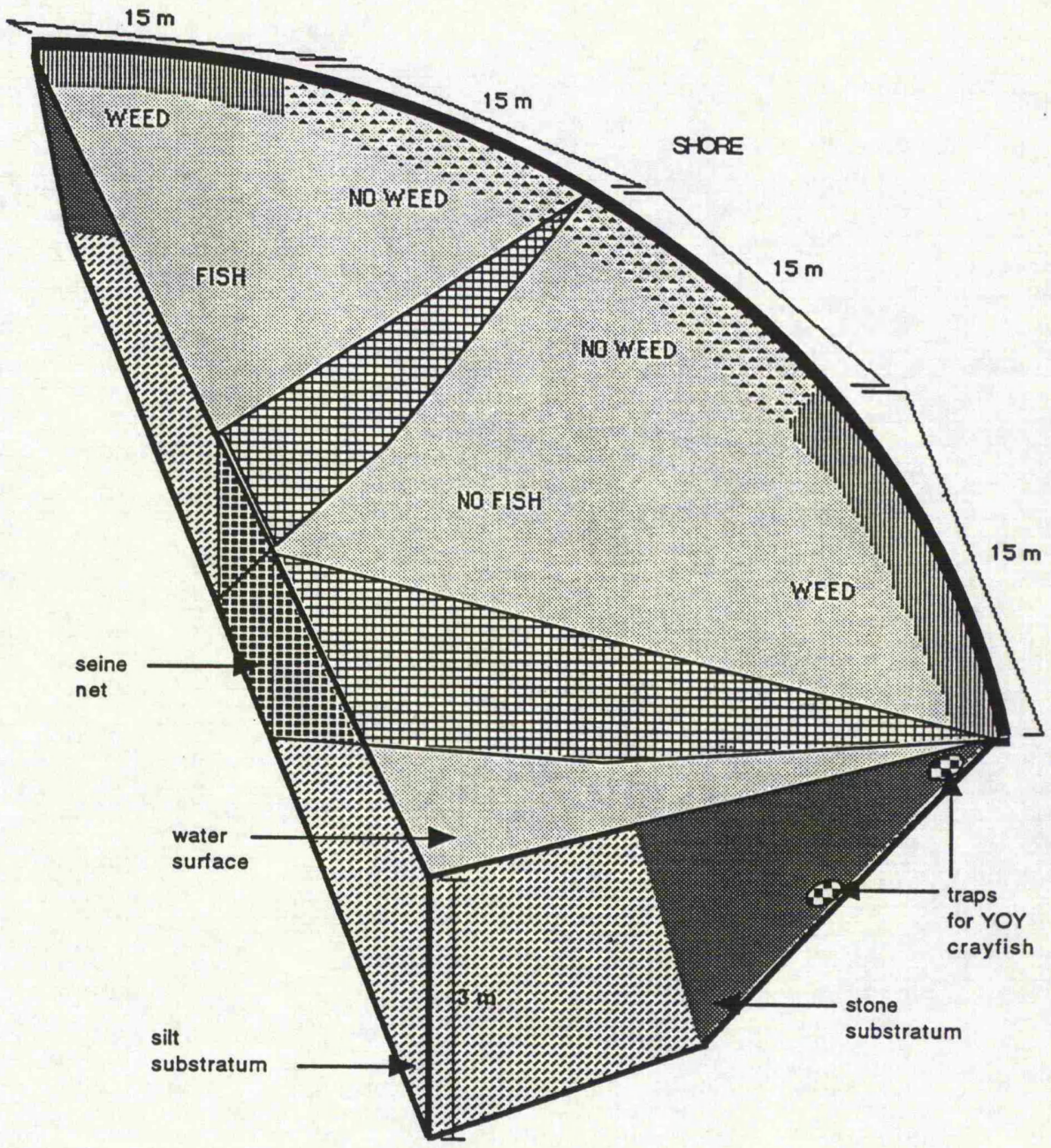


Figure 6.1. A schematic representation of the design of the field experiment carried out at Rögle pond.

pond from the shore. The net enclosed one 15-m stretch of shore with the weed removed, and another 15-m stretch with the weed intact. The bottom of the net was weighted and lay on the pond floor. The top of the net floated at the water surface. At the end of the experiment, the net was drawn towards the shore and a test electrofishing was conducted within the confines of the net to determine if any fish were present. No fish were caught, apart from juvenile perch (*P. fluviatilis*) and groplöja (*Leucaspius delineatus* Heckel) measuring less than 8 cm long. These fish were not of a size capable of feeding on YOY *P. leniusculus*. Adult perch and pike capable of preying on YOY *P. leniusculus* had access to the littoral margins outside the net.

During the course of the experiment, the net was pulled into the shore three times by members of the public. Each time it was reset as soon as it was detected. This did not allow predatory fish access to the shallow littoral area (<30 cm deep), as the net was not pulled in this far, however, predatory perch did have access to the deeper littoral areas for at least two periods of 2 days and one period of 4 days. This experiment ran for 7 weeks (30 May to 15 July 1992) after the net was first placed in the pond.

Juvenile Crayfish Distribution

Juvenile crayfish and other benthic invertebrates were trapped using plastic mesh bags (50 cm x 25 cm; mesh size of 4 x 6 mm) filled with corrugated plastic cylinders of 5 cm diameter and 3 cm long. Six bag traps were set at a depth of 30 cm at each of the four 15 m stretches of the littoral margin. Four traps were also set in the deep water (1.5 m), parallel to the shore at each of the four sites . All traps were set on the stone substratum.

Every week these traps were removed and counts were made of the number of juvenile crayfish and other invertebrate taxa in each bag, before replacing the bags in the pond for a further week. The catch per unit effort, (mean number of crayfish per bag-trap per site), was used as an indication of the distribution and survival of YOY crayfish. This technique was also employed to determine the distribution of other invertebrate taxa. Six weeks after the first YOY crayfish were found in the traps, a sample of YOY crayfish from each site was weighed to see if there were any differences in crayfish growth between the sites. Crayfish were blotted on absorbent paper to remove any surface moisture , and were then weighed to the nearest milligram.

Adult Crayfish Distribution

The distribution of adult male, non-gravid female and gravid female crayfish was determined between 20 May to 8 July, principally from CPUE data obtained from double ended funnel traps baited with fish. The CPUE for these crayfish refers to the mean number caught per trap per night. Traps were used:-

1) To test the relative abundance of adult crayfish at 30 cm and 1.5 m depths

on the stone substratum. Fifteen traps were set for 24 hours at each depth on six occasions between the 20 May to the 8 July. These traps were set in an area of the pond away from the experimental sites described above.

2) To test for the possible effects of weed removal and the net enclosure on adult crayfish abundance in both the deep and the shallow water habitats. On four occasions between the 28 May and 15 June, 5 traps were set at each experimental site in the shallow water and 5 traps were also set at each experimental site in the deep water. Again, all traps were set on the stone substratum.

In order to test more rigorously the distribution of gravid females in the weeks before and during hatching, 6 blocks of shelters were set at 30 cm and 1.5 m depths in the pond. These shelters were constructed of layers of corrugated plastic and provided 64 individual compartments in which crayfish could shelter. Traps were collected on three occasions between the 28 May and 11 June using SCUBA apparatus, and were initially wrapped in a mesh net before being brought to the surface. Crayfish were then removed from the shelters and were counted and sexed.

Unless otherwise stated, all statistical analyses are two-tailed and use a system of nonparametric analysis of variance by ranks to test the difference between two or more independent samples (Meddis, 1984). Time (weeks) was used as a blocking variable when comparing trappability (crayfish distribution) between experimental sites. Blocking variables are "qualities which cannot be controlled but must be taken into account even though they are not specifically relevant to the hypothesis under examination". In two sample tests, sample sizes (m and n) are given. For multiple sample tests, degrees of freedom (df) are given.

6.3.3 RESULTS

Adult Crayfish Distribution

In an area of the pond, away from the test sites, more adult crayfish were caught in the traps in the deep water than in the shallow water between 20 May to 8 July ($H=18.56$, $m=75$, $n=75$, $p<0.001$; Fig. 6.2). This was true of both adult male and female crayfish (males, $H=5.60$, $m=75$, $n=75$, $p<0.025$; females, $H=16.49$, $m=75$, $n=75$, $p<0.001$). Also, proportionally more of the crayfish from the deep water were female ($H=3.22$, $m=75$, $n=75$, $p<0.07$). This trend was similar for gravid females (Fig. 6.3). Between 20 to 29 May, more gravid females were caught in the deep water ($H=16.29$, $m=35$, $n=38$, $p<0.001$), and gravid females tended to make up proportionally more of the catch in the deep water in the two weeks leading up to the hatch (25 May, $H=5.59$, $m=10$, $n=13$, $p<0.025$; 29 May, $H=10.8$, $m=12$, $n=12$, $p<0.01$). The first stage I young were found on female crayfish on the 29 May.

Artificial hides proved difficult to manipulate. The numbers of adult crayfish,

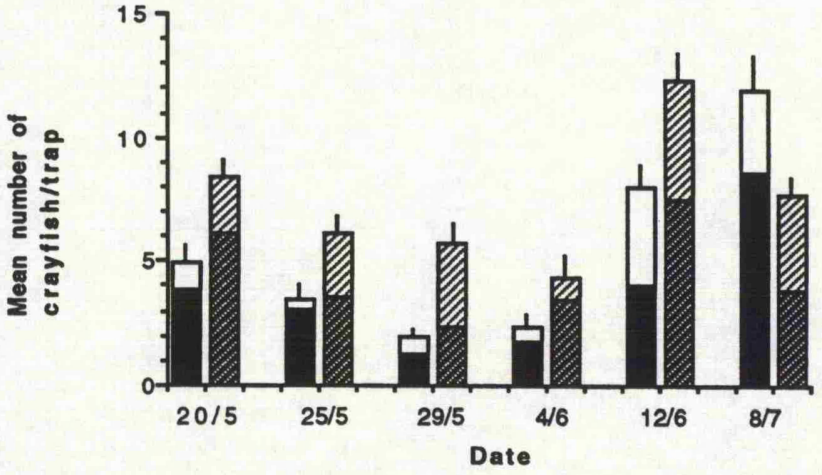


Figure 6.2. The mean number (± 1 S.E.) of adult male and female crayfish caught per trap night in deep and shallow water in Rögle pond between 25 May and 8 July 1992 (shallow water, males ■, females □; deep water, males ▩, females ▨).

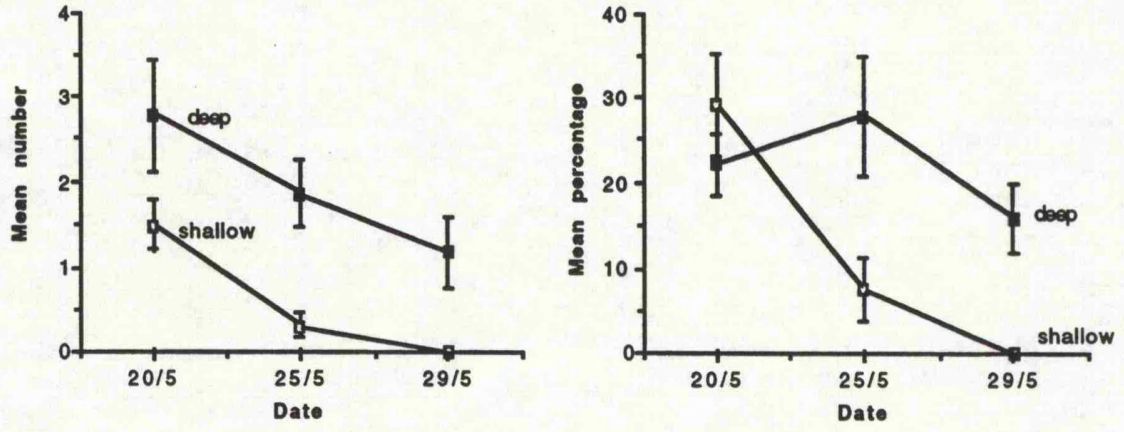


Figure 6.3. a) the mean number (± 1 S.E.) of gravid females caught per trap night, and b) the proportion (± 1 S.E.) of gravid females in the total catch per trap night, in the shallow water □, and deep water ■, in Rögle pond between 20 to 25 July 1992.

males or non-gravid females, did not differ between shelters in the deep and shallow water. No gravid females were were found in these shelters.

Within the experimental area, adult crayfish distribution did not differ between the manipulated experimental sites in either deep or shallow water, but overall, more crayfish were found in the deep water than the shallow water (all sites combined; $H=4.02$, $m=53$, $n=54$, $p<0.05$). This was also true of the male crayfish ($H=10.01$, $m=53$, $n=54$, $p<0.01$).

There was a difference in female crayfish distribution between the four sites within both shallow and deep water (shallow, $H=8.05$, $df=3$, $p<0.05$; deep, $H=7.33$, $df=3$, $p<0.07$). More females were caught outside the net enclosure (shallow water, $H=6.92$, $m=53$, $n=54$, $p<0.01$; deep water, $H=7.33$, $m=53$, $n=54$, $p<0.01$). Females also made up proportionally more of the catch in the habitats outside the net enclosure (shallow water, $H=8.19$, $m=26$, $n=27$, $p<0.01$; deep water, $H=8.17$, $m=26$, $n=28$, $p<0.01$). CPUE of females did not differ with water depth, however, proportionally more of the crayfish caught in the shallow water were females ($H=7.24$, $m=53$, $n=54$, $p<0.01$).

YOY crayfish distribution, abundance and growth.

Throughout the period 5 June to 15 July, more YOY crayfish were found in the deep water traps than in the shallows (all sites combined; $H=4.31$, $m=164$, $n=100$, $p<0.05$; Fig. 6.4a). This was mainly a result of the initial increase in the number of YOY crayfish in the deep water when crayfish were newly independent (between 5 to 12 June; $H= 51.35$, $m=32$, $n=48$, $p<0.001$). After this time, there was an overall tendency for more crayfish to be found in the shallow water ($H=6.49$, $m=68$, $n=116$, $p<0.025$), although this differed between weeks.

After 7 weeks, the densities of YOY crayfish in the shallow and deep water traps were similar, however, the percentage survival, extrapolated from CPUE data, was lower in the deep water (Fig. 6.4b). This was because YOY juveniles reached a greater maximum density in the traps in the deep water. Crayfish reached their maximum density in the shallow water traps one week later.

Throughout the experimental period, there was an overall difference in the number of YOY crayfish found at each experimental site in the shallow water ($H=28.97$, $df=3$, $p<0.001$; Fig. 6.5). Fewer crayfish were found in traps when both weed and fish were present by comparison to the other 3 sites (individual pairwise comparisons between weed/fish and a) weed/no fish, $p<0.01$; b) no weed/ fish, $p<0.01$; and c) no weed/no fish, $p<0.025$). This indicated that there was an interaction effect of the two variables on crayfish distribution.

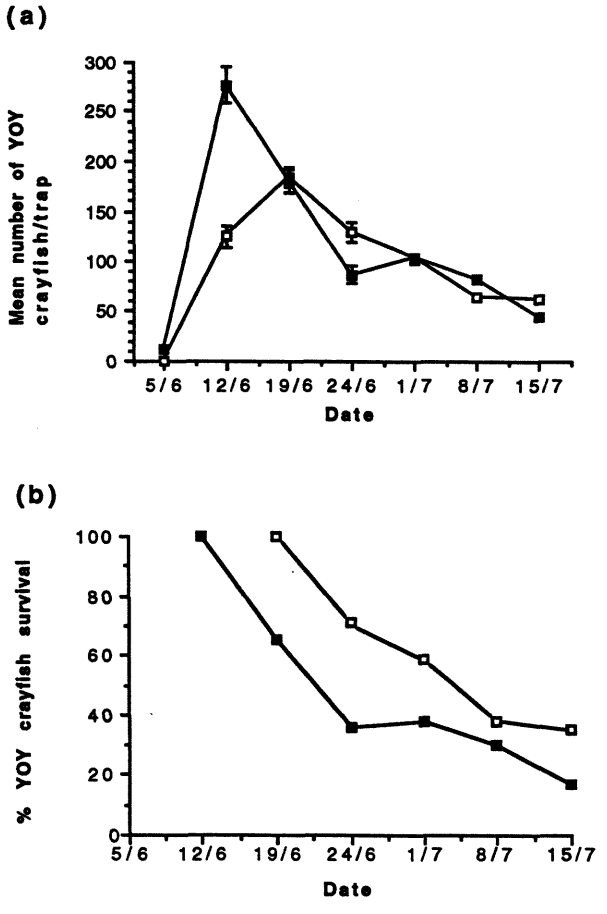


Figure 6.4. a) the mean number (± 1 S.E.) of YOY crayfish caught per trap night at each water depth in Rögle pond, and b) the average survival of YOY crayfish with water depth. The number of crayfish caught at each site is expressed as a percentage of the maximum number of crayfish caught at each site. Water depths are; shallow □ , and deep ■ .

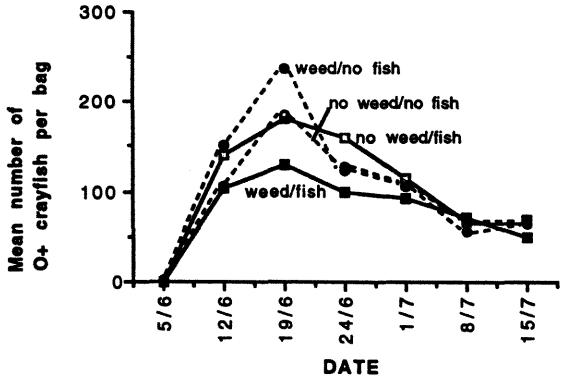


Figure 6.5. The mean number of newly independent crayfish caught per trap night in shallow water at each experimental site in Rögle pond, each week. Sites are weed/fish —■— , weed/no fish —●—, no weed/fish —□— , no weed/no fish —○—.

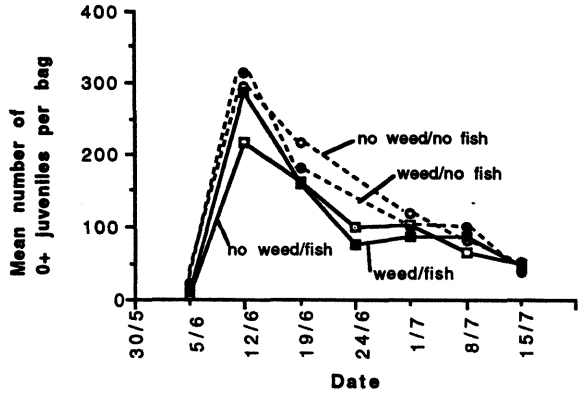


Figure 6.6. The mean number of newly independent crayfish caught per trap night in deep water at each experimental site in Rögle pond, each week. Sites are weed/fish —■— , weed/no fish —●—, no weed/fish —□— , no weed/no fish —○—.

There was an indication that crayfish distribution differed between the four experimental sites in the deep water between 5 June to 15 July (H=6.44, df=3, p<0.1, blocked for week; Fig. 6.6). More crayfish were found in the sites without fish (H=5.55, m=44, n=48, p<0.025). This pattern was not consistent when crayfish distribution was compared within each week.

Crayfish growth was determined from the weights of individual YOY crayfish collected from the traps at each experimental site on the 7 July. The data was $\log_{10}$ transformed for the following analyses. In the shallow water, crayfish growth was enhanced in habitats with fish (2-way parametric ANOVA; fish F=5.00, df=1, p<0.05; weed F=0.57, df=1, p>0.1; Fig. 6.7). In the deep water, crayfish growth was enhanced in the habitats with weed (2-way ANOVA; fish F=1.56, df=1, p>0.1; weed F=5.57, df=1, p<0.025). Crayfish growth was greater in the shallow water habitats by comparison to those in the deep water (T-test,T=6.33, m=204,n=239, p<0.001).

Yearling (1+) Juvenile Crayfish distribution.

Yearling crayfish distribution was not affected by fish or weed between the experimental sites, within either the shallow or the deep water. More 1+ crayfish were found in the deep water traps than in shallow water traps (All experimental sites combined; H=7.98, m=30, n=32, p<0.01).

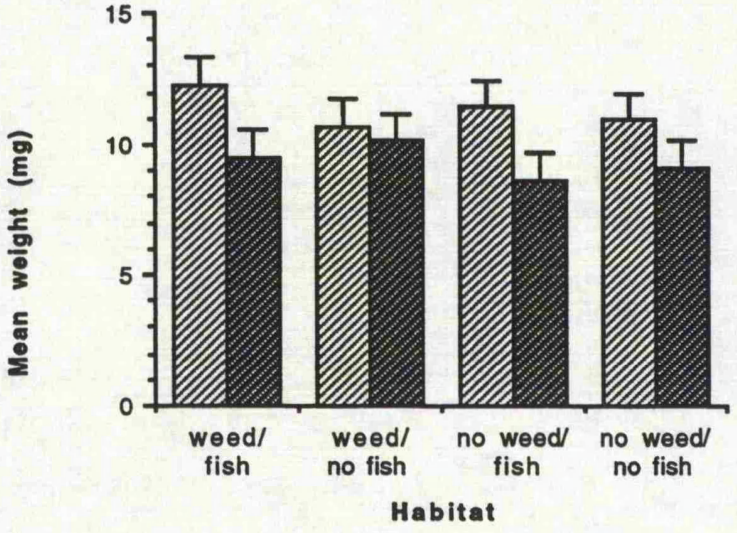


Figure 6.7. Mean weights (± 1 S.E.) of YOY crayfish from each of four experimental sites in shallow water ▨, and deep water ■ .

Distribution of Invertebrate Fauna

Only the distributions of the most common invertebrates found in the traps are considered below.

Gammarus - The number of *Gammarus* in the traps did not differ between the four experimental sites within either the shallow or the deep water. *Gammarus* were more abundant in the traps in the shallow water by comparison to the deep water (all sites combined; $H=117.10$, $m=90$, $n=143$, $p<0.001$; Fig. 6.8a).

Asellus - There was an overall difference in *Asellus* distribution between four sites within both the shallow and deep water (shallow, $H=25.72$, $df=3$, $p<0.001$; deep, $H=29.99$, $df=3$, $p<0.001$; Fig. 6.8b). In both cases, the weed/no fish site contained more *Asellus* than each of the other three sites. Overall, greater numbers of *Asellus* were found in the deep water than the shallow water (all sites combined; $H=20.74$, $m=40$, $n=144$, $p<0.001$).

Chironomidae - Greater numbers of chironomids were found in traps on sites containing weed in both the shallow water ($H=23.27$, $m=72$, $n=72$, $p<0.001$), and deep water ($H=5.51$, $m=41$, $n=48$, $p<0.025$; Fig 6.8c). There was also an indication that fewer chironomids were in the traps in sites with fish in the deep water ($H=3.29$, $m=43$, $n=46$, $p<0.07$). This was due to the large difference in chironomid numbers found between the weed/no fish and no weed/fish sites. Chironomid densities did not differ with water depth.

Ephemeroptera - Greater numbers of Ephemeroptera were found in traps in the shallow water than in the deep water (all sites combined; $H=5.09$, $m=24$, $n=120$, $p<0.025$; Fig. 6.8d). There was a difference in the number of Ephemeroptera found at each site within the deep water ($H=11.65$, $df=3$, $p<0.01$), as a result of the difference in number found in traps in the weed/no fish and the no weed/fish sites.

Trichoptera - More trichoptera were found in the deep water traps than in the shallow water (all sites combined $H=48.09$, $m=63$, $n=96$, $p<0.001$; Fig. 6.8e). There was also a difference in the distribution of Trichoptera between experimental sites within the deep water ($H=16.29$, $df=3$, $p<0.01$). This was a result of the different numbers found in the traps on the weed/no fish and no weed/fish sites.

Zygoptera - Zygoptera tended to be found in greater numbers in the shallow water traps than in the deep water (all sites combined; $H=3.42$ $m=47$, $n=72$, $p<0.07$; Fig. 6.8f). Zygoptera distribution also differed between sites within the shallow water ($H=12.32$, $df=3$, $p<0.01$). Again this was a result of the difference in numbers found in the weed/no fish and no weed/fish sites. In this instance more were found in the traps in the no weed/fish site.

Figure 6.8. Mean number (± 1 S.E.) of each invertebrate Taxa caught per trap night at each site in shallow ▨, and deep ■, water in Rögle pond. Data for all weeks (31/5-8/7/92) are combined. Taxa are a) *Gammarus*, b) *Asellus*, c) Chironomidae, d) Ephemeroptera, e) Trichoptera, and f) Zygoptera (see overleaf).

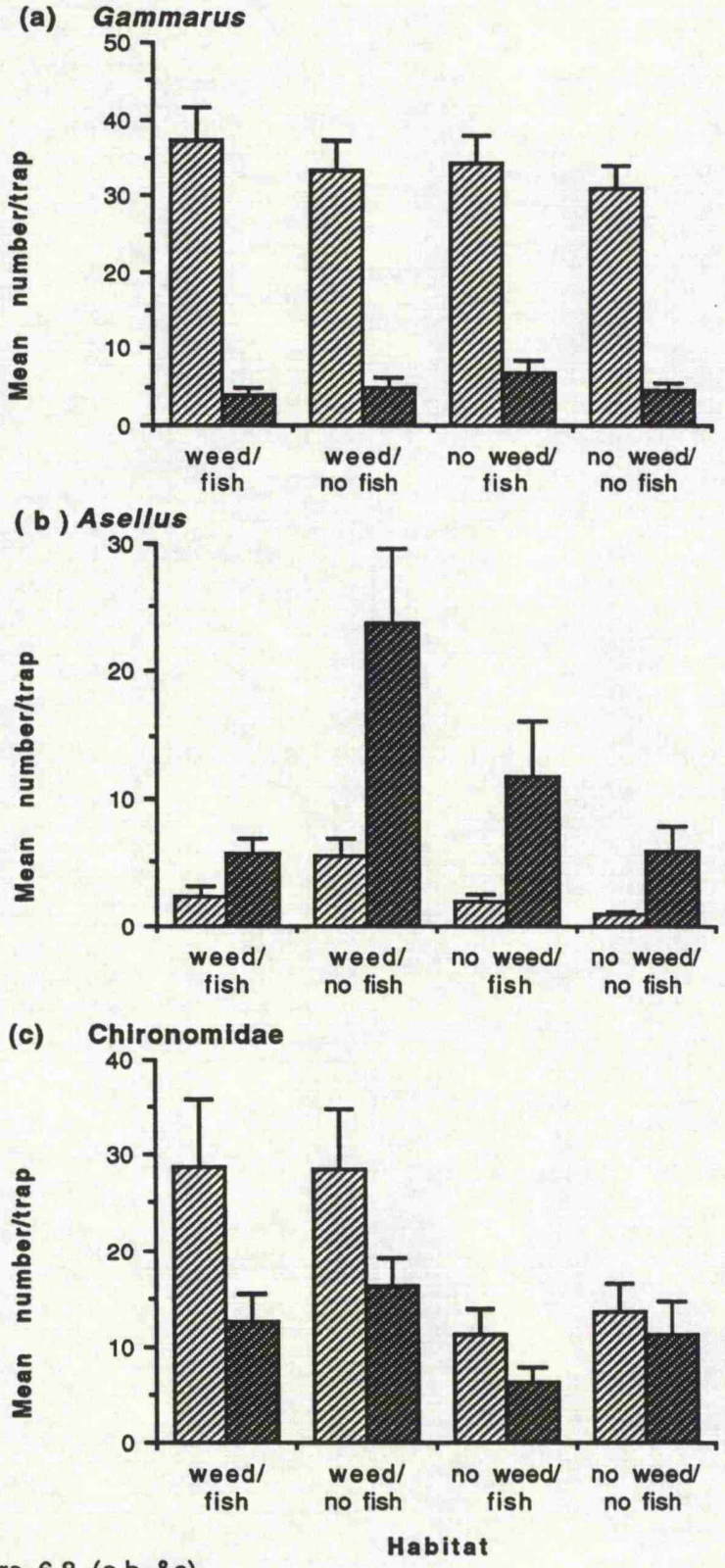


Figure 6.8 (a,b &c).

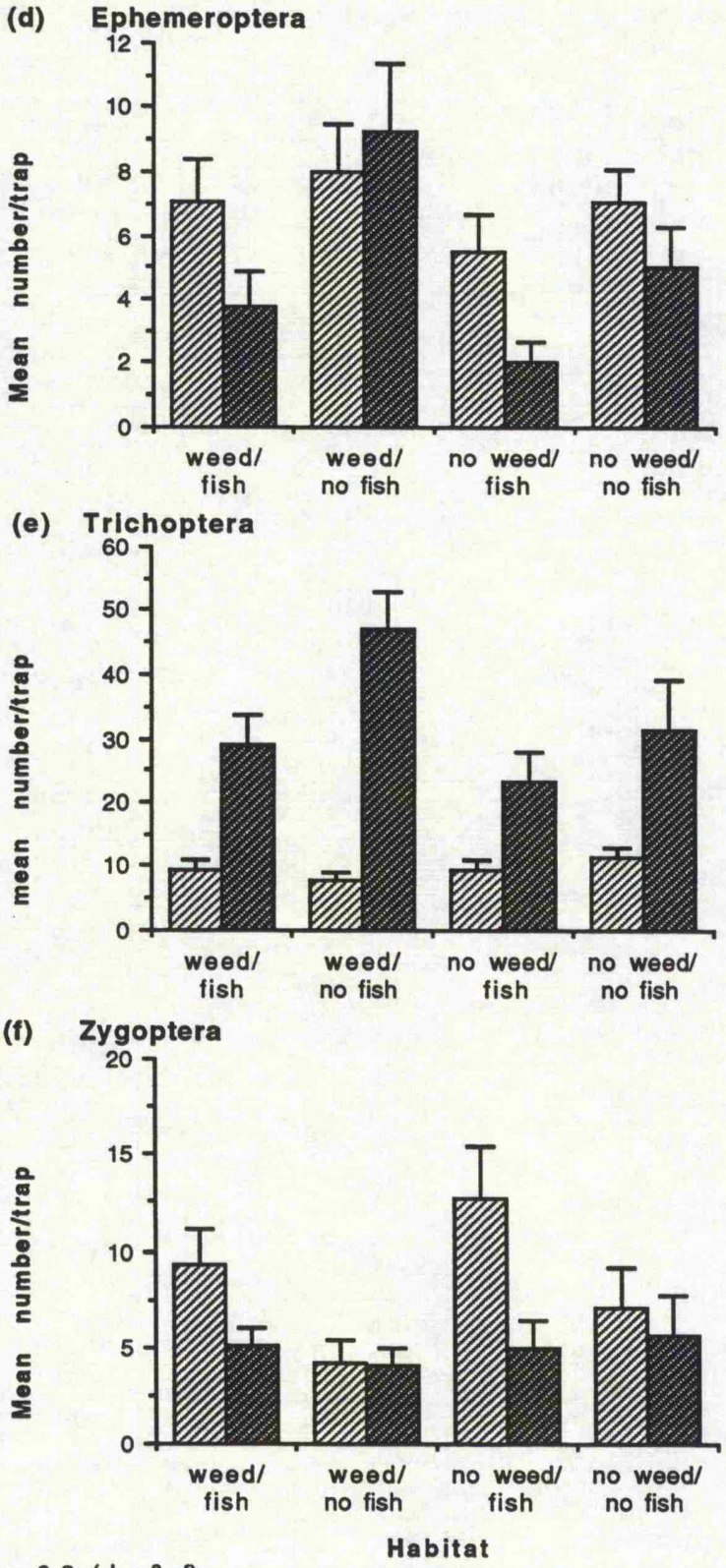


Figure 6.8 (d,e,& f).

Comparison between 1991 and 1992.

The trappability of adult crayfish was similar between the two years, but in 1992 more were found in the deep water than in the shallow water (Fig. 6.9). No statistical comparison of trappability between the two years was possible. The data from 1991 was collected as the number of crayfish per five traps, and sets of five traps were collected four times a week. Only one set of five traps was collected per week in 1992.

In most cases, the densities of invertebrates in the traps at each water depth were similar between years (Fig. 6.10) There were between-year differences in the number of *Gammarus* and Ephemeroptera in the deep water traps (Wilcoxon-Mann-Whitney test; *Gammarus*, W_K=23, m=6, n=9, $p<0.01$; Ephemeroptera, W_K=15, m=5, n=9, $p<0.01$) and the number of chironomids in the shallow water traps (Wilcoxon-Mann-Whitney test; W_K=68, m=6, n=9, $p<0.025$).

In 1992, YOY crayfish were found on female crayfish 3 weeks earlier, and in traps 15 days earlier than in 1991. Densities were greater in 1992 in both shallow and deep water habitats where both weed and fish were present (shallow water, H=20.7, m=18, n=36, $p<0.001$; deep water, H=22.9, m=11, n=33, $p<0.001$; Fig. 6.11). These differences were analysed for weeks 1 to 3 after YOY crayfish were first detected. Weeks were used as a blocking variable (Meddis, 1984). Greater densities of YOY crayfish were found in the other three experimental sites at both water depths in 1992. Unlike 1991, YOY crayfish distribution in 1992 did not differ with water depth, except for the first 3 weeks, when more YOY crayfish were found in the deep water.

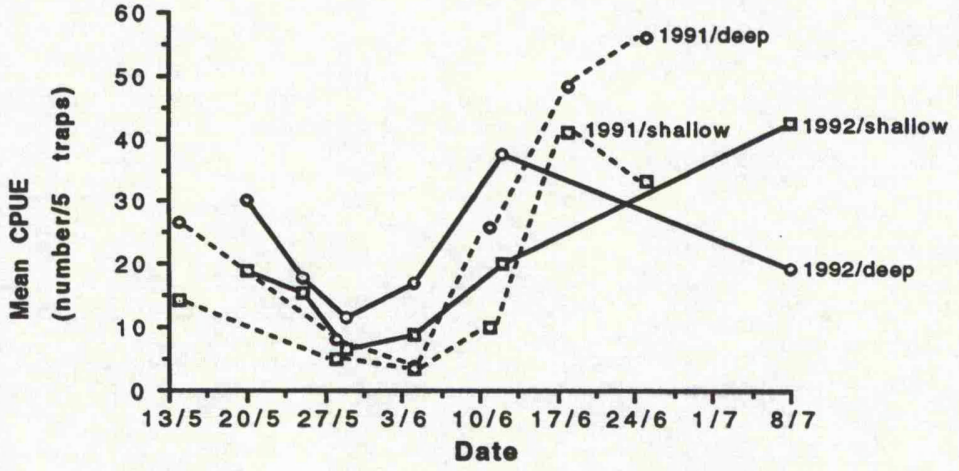


Figure 6.9. Mean numbers of adult crayfish caught per 5 traps per night during the spring/summer of 1991 -----, and 1992 ——, in shallow □ , and deep ○ , water sites.

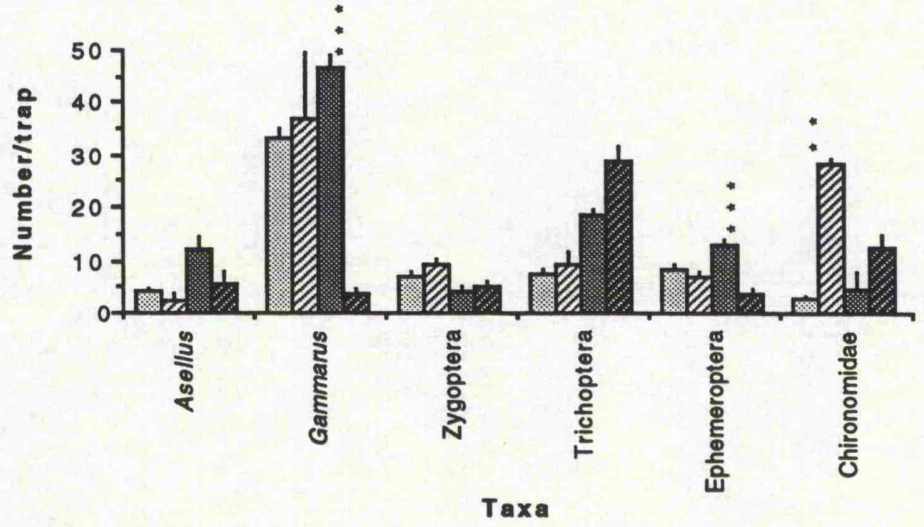


Figure 6.10. Mean numbers (± 1 S.E.) of invertebrate taxa found in 1991 in shallow water ▩ , and deep water ■ , and in 1992 in shallow water ▨ , and in deep water ▩ . Probability values are for Mann-Whitney tests between years within each water depth (**p<0.025, ***p<0.01).

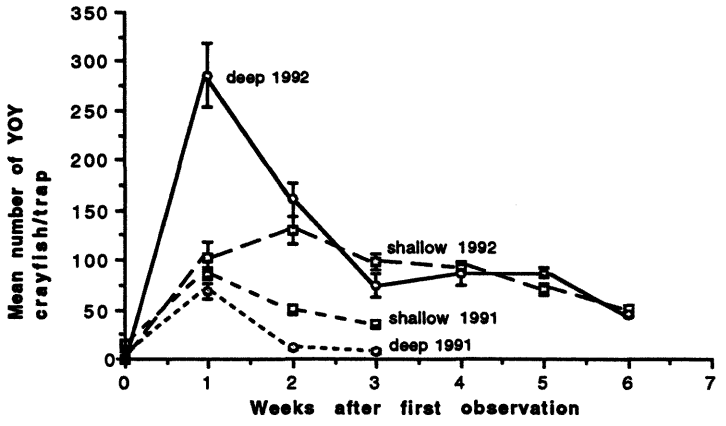


Figure 6.11. Mean numbers (± 1 S.E.) of YOY crayfish caught per 5 traps per night during the spring/summer of 1991, in shallow – – –, and deep -------, water sites, and in 1992, in shallow — —, and deep ———, water sites.

Experiment 6.4. THE IMPORTANCE OF WEED COVER IN HABITAT SELECTION BY JUVENILES EXPOSED TO FISH AND ADULT CRAYFISH.

6.4.1 INTRODUCTION

This experiment firstly tested whether newly independent (Stage II) juvenile crayfish positively selected habitats in response to weed cover, and whether this selection was based solely on the physical protection afforded by the weed, or whether it was a result of other factors associated with weed cover such as food availability. Crayfish were placed in indoor tanks and were offered a choice between a plain pebble substratum (no shelter), a pebble substratum with artificial plastic weed (shelter), and a pebble substratum with real weed (shelter and food). As crayfish prefer pebble substrata to gravel substrata (Chapter 5, Section 5.6.2), this experiment tested whether weed exerted any more influence on juvenile crayfish habitat choice than substratum alone. Secondly, the effects of fish and adult crayfish on juvenile crayfish habitat preferences were examined. Juvenile crayfish were expected to choose the habitats offering the most protection from predation in response to the predatory threats represented by groplöja (*L. delineatus*) and adult crayfish.

6.4.2 MATERIALS AND METHODS

Twelve 220 cm by 42 cm tanks (0.92 cm^2) filled to a depth of 15 cm were used in the following experiment. Eight replicates of 3 treatments were run in the tanks over a two week period between 30 May to 13 June 1992. Each replicate lasted one week. The tanks were arranged in two sets of six, but were all fed by the same recirculating water system. Each set of six tanks consisted of a line, 3 tanks long by 2 deep. The water temperature was between 15 and 16 °C, and the tanks were illuminated on a 9:15, light:dark light regime. The lights did not fade in or out.

Three 572-cm^2 circular trays, each containing 2 cm of pebbles measuring 12 to 29 mm diameter (n=25), were placed in each tank. Sixty 30-cm strands of *Elodea* were attached to the pebble substratum in one tray of each tank, so that the strands floated over the tray. Sixty plastic strips, 1 cm wide and 30 cm long, were attached to a second pebble substratum in each tank in a similar way. The third pebble filled tray in each tank was left with no cover. The three habitat trays were arranged randomly with respect to each other and tank inlet and outlets. The three experimental treatments consisted of tanks with no predator (control), with 3 groplöja (simulated fish predator), and with one adult crayfish (crayfish predator).

Adult crayfish were used to investigate the possibility that they were a cause of

juvenile crayfish mortality in Rögle Pond. Nyström (pers. comm.) found that YOY crayfish became less active in the presence of groplöja (*L. delineatus*), which are small cyprinid fish that normally grow to between 6 to 8 cm in length (Wheeler, 1978). Although these fish were incapable of preying upon YOY crayfish, the reported behaviour of juvenile crayfish in response to groplöja (Nyström, pers. comm.) is similar to that reported for juvenile crayfish in response to perch in other studies (Hamrin, 1987; Appelberg & Odelström, 1988; Chapters 2, 3 & 5).

In the previous two years, perch became diseased very easily in laboratory situations. Groplöja were more hardy (Nyström, pers. comm.) and so it was decided to use these fish as a substitute for perch in the following experiment, to determine the indirect effects of fish predators on crayfish habitat choice and survival.

Groplöja measuring 5 cm to 8 cm in total length were caught from a local pond at Simontorp Aquaculture A.B. Recently moulted adult male crayfish measuring 64 cm to 80 cm were trapped in Rögle Pond during the last week of May. Newly independent (Stage II) juvenile crayfish were obtained from the Simontorp indoor hatchery and 100 individuals were placed in each tank (0.92 individuals/m^2). After 24 hours, either 3 groplöja, 1 adult male crayfish or no predators were added to the tanks. After six days, the predators were removed and the number of crayfish on each habitat and on the bare tank floor were counted. Crayfish and groplöja were fed a standard quantity of either a liquidised suspension of egg, peas and earthworm or chironomid larvae; every second day.

Unless otherwise stated, the following statistical analyses used a one-way nonparametric analyses of variance by ranks (Meddis, 1984).

6.4.3 RESULTS

There was an overall difference in YOY crayfish survival between the three predator treatments ($H=7.86$, $df=2$, $p<0.025$; Fig 6.12). Adult crayfish reduced crayfish survival by comparison to controls (individual pairwise comparison, $p<0.025$).

At the end of the experiment, the proportion of the surviving crayfish that were exposed on the tank floor was used as an indication of activity. Activity differed between treatments ($H=5.98$, $df=2$, $p<0.05$). Proportionally fewer crayfish were exposed when together with adult crayfish by comparison to controls (individual pairwise comparison, $p<0.05$; Fig. 6.12).

To test the distribution of YOY crayfish between the three habitats, the number of juveniles found in each habitat per tank were expressed as a percentage of the total number of crayfish found in all three habitats per tank. This removed the effects of

differential crayfish activity between tanks. There was an overall difference in the proportion of crayfish that were found in the three habitats (Friedman 1-way ANOVA for all predator treatments combined, Chi^2 conversion=7.01, df=2, $p<0.05$; Fig. 6.13). Proportionally more crayfish were found in habitats with weed (*Elodea*) or plastic weed cover than with uncovered pebbles, although, individual pairwise comparisons between substrata were not significant ($p>0.05$). This pattern was evident when YOY habitat preferences were compared within each predator treatment, but again these were not significant ($p>0.05$).

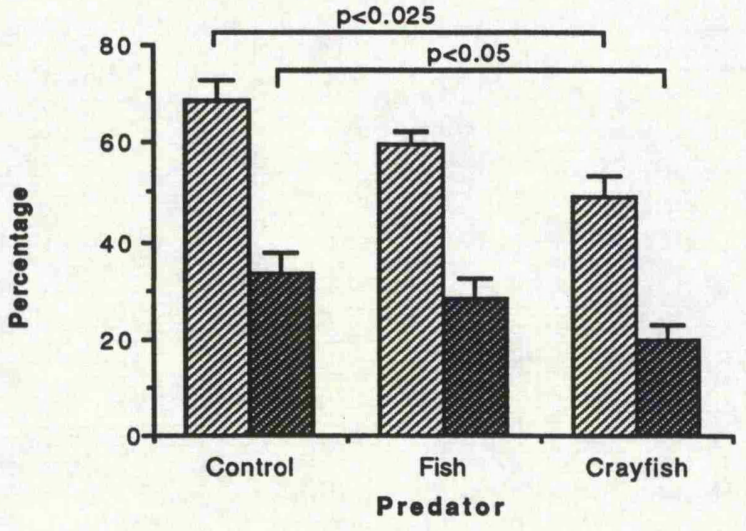


Figure 6.12. Mean percentages (± 1 S.E.) of crayfish surviving in each experimental treatment (light cross-hatching) and mean percentages (± 1 S.E.) of surviving crayfish that were exposed in 0.25 m^2 of the tank floor (dark cross-hatching) at the end of Experiment 6.4.

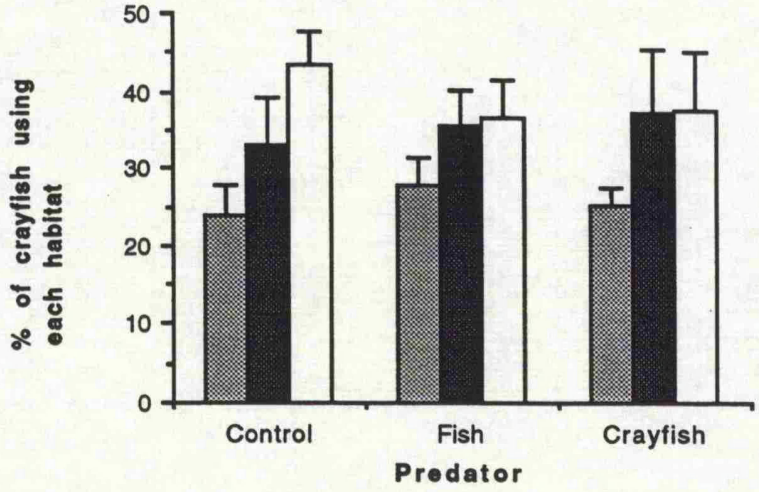


Figure 6.13. Mean percentage (± 1 S.E.) of the total number of YOY crayfish found using shelter that were found in each habitat type in response to three predator treatments. Habitats were; pebbles only ▨ , pebbles with plastic weed ■ , and pebbles with real weed □ .

Experiment 6.5. THE EFFECT OF WEED COVER, FISH AND ADULT CRAYFISH ON THE ACTIVITY, GROWTH AND SURVIVAL OF JUVENILES.

6.5.1 INTRODUCTION

In the following experiment, the possible benefits of weed cover in terms of food acquisition by crayfish and protection from predation were considered. Crayfish were placed in indoor tanks on one of three habitats; no weed (control), plastic weed (shelter only), and real weed (shelter and food), and were exposed to either adult crayfish, groplöja or no predators, as in Section 6.4.2. Thus the importance of weed as a source of food and of protection from predation were tested.

6.5.2 MATERIALS AND METHODS

Fifty-four 0.92-m^2 tanks were used, each filled to a depth of 15 cm. The tanks were arranged in 3 stacks of 18. Each stack of 18 tanks comprised 2 lines, each 3 tanks long and 3 tanks deep. As a result, light levels varied between lines ($H=11.81$, $df=5$, $p<0.05$) and heights ($H=45.13$, $df=2$, $p<0.001$). The light levels for each tank (Nyström, pers. comm.), their positions, and the experimental treatments used in each, are given in Figure 6.14. Each set of 18 tanks was supplied by a separate recirculation system, with water being pumped into the top tanks in each row and falling through the tanks below. Water temperatures ranged between 18 and 20 °C and the tanks were on a 9:15, light:dark light regime. The lights did not fade in or out.

For the purposes of the experiment, the tanks were divided into 2 groups of 15 and 2 groups of 12. Experimental trials were started in each group of tanks on consecutive days. All experimental trials ran for 26 days between the 15 June to 14 July 1992, and were started and terminated within a four day period.

The experiment consisted of nine treatments arranged in a 3 x 3 factorial design. Crayfish were given three habitat types and three predator situations as described in Figure 6.14. All tanks were supplied with 4 building bricks, each containing 24 holes to act as crayfish shelters. Bricks were either rested on a) 60 strands of Elodea (real weed cover), 30 cm long which floated around the brick, b) on 60 strands of black plastic (plastic weed cover), 1 cm wide and 30 cm long arranged in a similar fashion to the weed, or c) on the bare tank floor (no cover).

Groplöja measuring 5 cm to 8 cm in total length were caught from a local pond at Simontorp in early June. Recently moulted adult male crayfish measuring 65 cm to 80 cm were trapped in Rögle Pond between May and June. Newly independent (Stage II) juvenile crayfish were obtained from the Simontorp indoor hatchery and 100

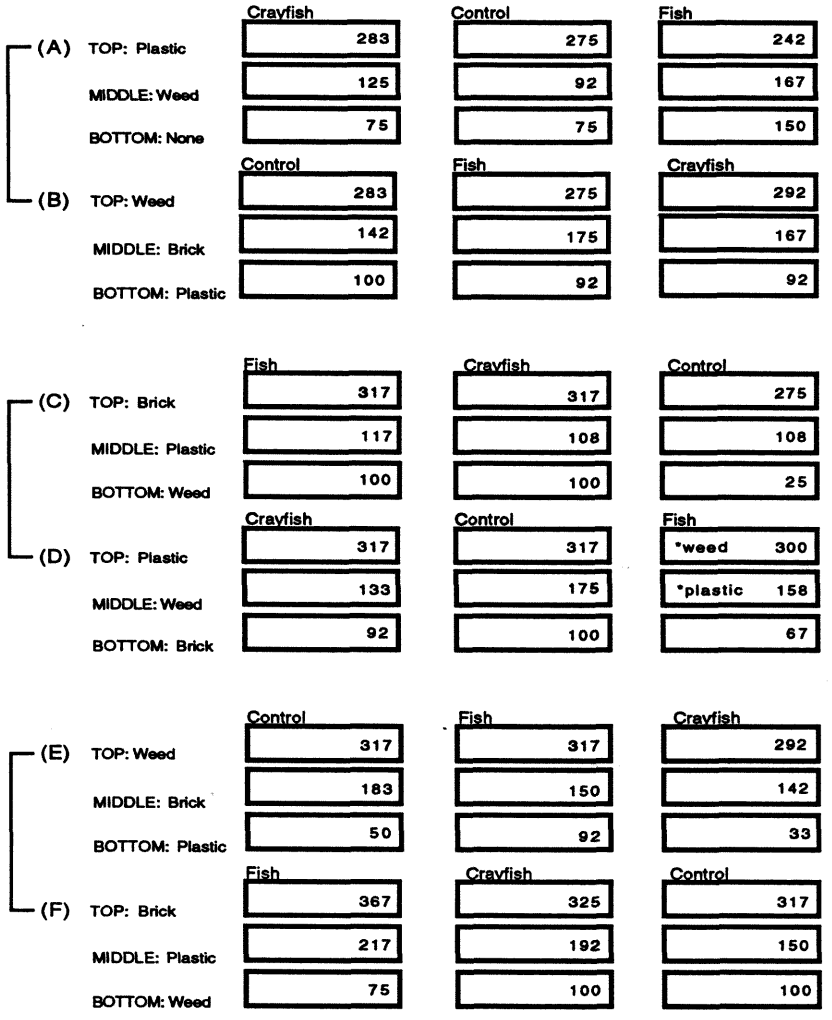


Figure 6.14. The tank designs used in Experiment 6.5. The diagram shows the predator treatments (tanks in vertical rows), the tank position and habitat treatments (tanks in horizontal lines), the illumination (lux) for each tank (numbers in boxes), and the 3 sets of 18 tanks, each supplied by a seperate water system (A+B, C+D & E+F).

individuals were placed in each tank. At the start of the experiment, the mean weight of 10 crayfish was 0.0207 g (S.E.=0.006, n=15). Thus the average weight of individual crayfish was ~2.0 mg. After 24 hours, four groplöja, one adult male crayfish, or no predators (control), were added to the respective tanks. Crayfish were fed a standard quantity of either a liquidised suspension of egg, peas and earthworm or of chironomid larvae, every second day, supplemented occasionally by a liquidised suspension of filamentous green algae.

After 26 days, groplöja and adult crayfish were removed from each tank and the surviving juvenile crayfish were counted. Individual crayfish were then weighed to the nearest mg to determine whether juvenile crayfish growth differed between treatments. Crayfish were weighed after excess moisture had been removed using absorbent paper. On two occasions during the experiment, days 4 to 7 and 14 to 17, and on the penultimate and last day of the experiment, days 25 to 26, juvenile crayfish activity was monitored by counting the crayfish exposed in a 0.25-m^2 area of each tank at 11.00 and 19.30 hours. The area chosen was free from any real or plastic weed cover.

Unless otherwise stated, all statistical analyses are two-tailed and use a system of nonparametric analysis of variance by ranks to test the difference between two or more independent samples (Meddis 1984). In two sample tests, sample sizes (m and n) are given. For multiple sample tests, degrees of freedom (df) are given.

6.5.3 RESULTS

The following analyses did not compensate for an effect of illumination, as the light levels in each tank were not correlated with either the mean weight of the surviving YOY crayfish in each tank or with crayfish survival (Spearmean's Rank Correlation $p>0.1$). The mean weight of crayfish from each tank was, however, correlated with crayfish survival (Spearman's Rank correlation; $R=0.41$, $n=53$, $p<0.01$).

YOY crayfish survival differed between treatments ($H=33.25$, $df=8$, $p<0.001$; Fig. 6.15). Survival was influenced by predators ($H=19.28$, $df=2$, $p<0.001$), and by habitat ($H=11.89$, $df=2$, $p<0.01$). Adult crayfish reduced YOY crayfish survival by comparison to controls (no predators) and groplöja (individual pairwise comparisons between adult crayfish and controls, $p<0.001$; groplöja, $p<0.025$). *Elodea* enhanced YOY survival by comparison to plastic weed and bare bricks (individual pairwise comparisons; both $p<0.01$). In a seperate analysis, groplöja were shown to increase YOY crayfish mortality by comparison to controls with no predators ($H=3.38$, $m=18$, $n=18$, $p<0.07$). This was not true when YOY crayfish had access to real weed cover, but was true when plastic weed or plain bricks were present ($H=6.73$, $m=12$, $n=12$, $p<0.01$).

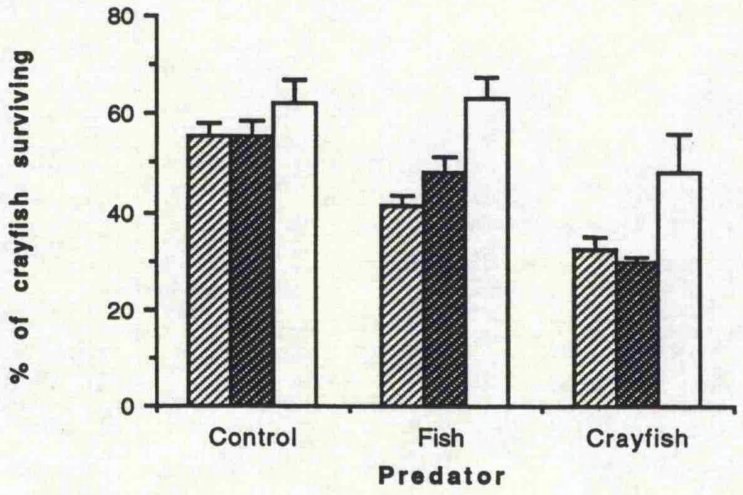


Figure 6.15. The mean percentage (± 1 S.E.) of YOY crayfish surviving in each predator treatment in Experiment 6.5 in plain brick habitats ▨, in brick habitats with plastic weed ■, and in brick habitats with real weed □.

The two following analyses express activity as the percentage of the surviving crayfish that were found exposed on 0.25 m^2 of the tank floor at 11.00 and 19.30 hours on the last day of the experiment. In both time periods, there was an overall difference in YOY crayfish activity between treatments (11.00 hours, $H=40.58$, $df=8$, $p<0.001$; 19.30 hours, $H=25.96$, $df=8$, $p<0.01$; Fig. 6.16). YOY activity differed in response to predators in both time periods (11.00 hours, $H=35.47$, $df=2$, $p<0.01$; 19.30 hours, $H=18.46$, $df=2$, $p<0.001$). By day, YOY crayfish were less active in response to groplõja (individual pairwise comparisons between groplõja and controls, $p<0.001$; groplõja and adult crayfish, $p<0.001$). At night, YOY crayfish were less active in response to adult crayfish (individual pairwise comparisons between adult crayfish and controls, $p<0.001$; adult crayfish and groplõja, $p<0.05$).

Wilcoxon pairwise comparisons were made between the number of YOY crayfish exposed within each tank at 11.00 and 19.30 hours on days 4 to 7, 14 to 17, and 25 to 26 of the experiment. In control tanks, YOY crayfish showed a slight preference for nocturnal activity (Fig 6.17a). YOY crayfish exposed to groplõja strongly favoured nocturnal activity (Fig. 6.17b), but this preference was reversed in response to adult crayfish (Fig. 6.17c). These preferences were also shown within each habitat treatment.

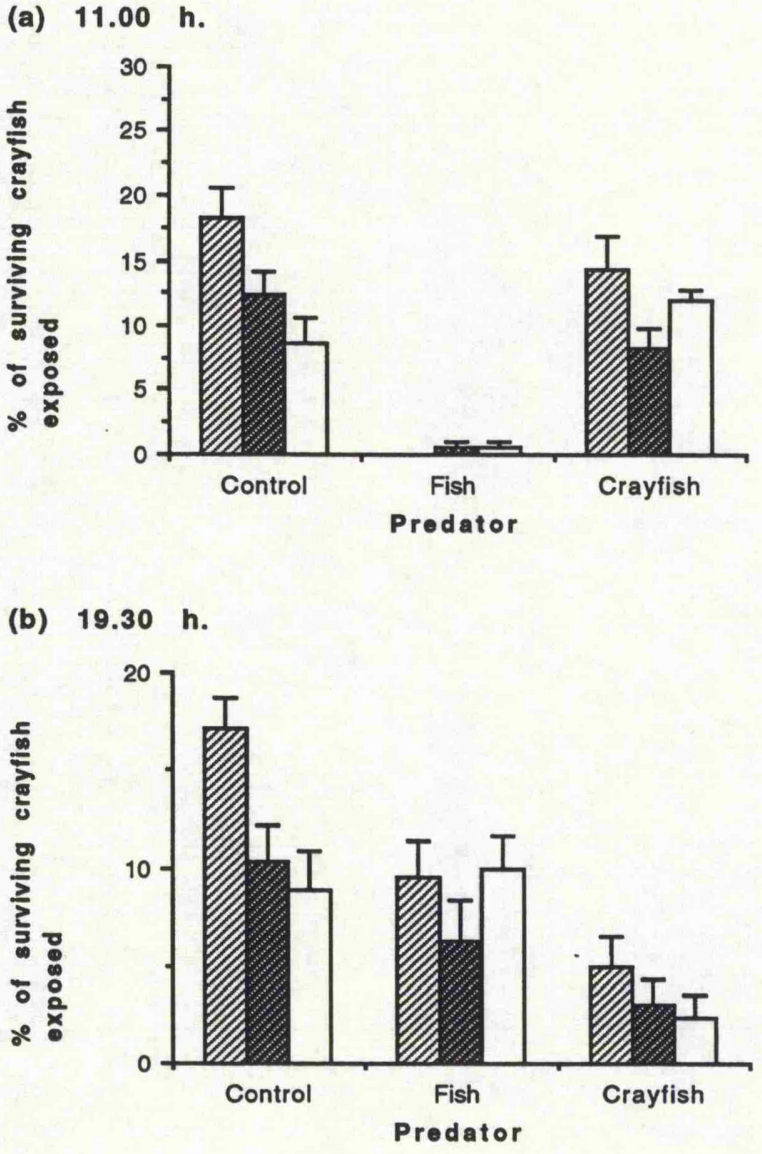


Figure 6.16. Crayfish activity in response to different predators at a) 11.00 and b) 19.30 hours on plain brick habitats ▨ , in brick habitats with plastic weed ▩ , and in brick habitats with real weed □ . Values are means (± 1 S.E.) of the percentage of surviving crayfish which were exposed in 0.25 m^2 of each tank.

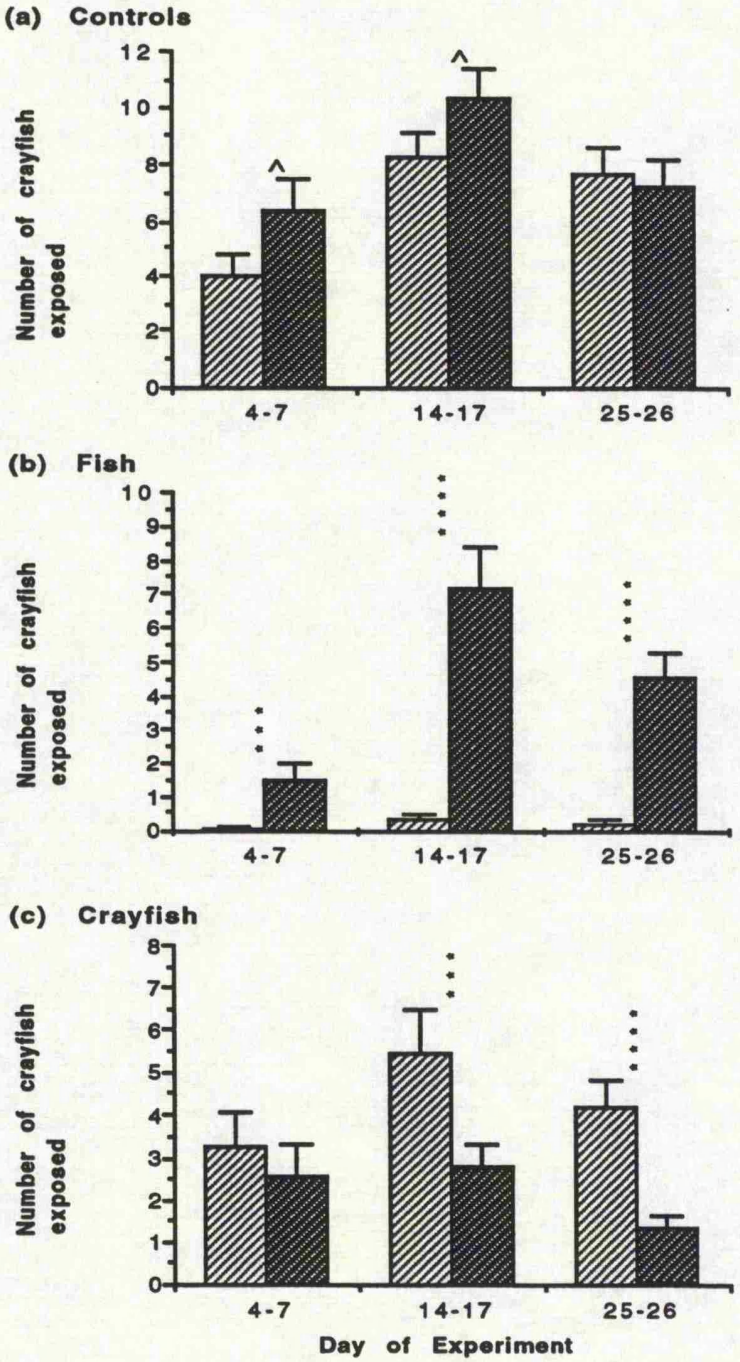


Figure 6.17. YOY crayfish activity in response to changes in illumination. Values are means (± 1 S.E.) of the number of crayfish exposed in 0.25 m^2 of each tank, on three occasions during Experiment 6.5 at 11.00 (light) ▨, and 19.30 (dark) ■, hours in a) control tanks, b) tanks with fish, and c) tanks with adult crayfish. Levels of significance are given for Wilcoxon pairwise comparisons between light and dark (^p<0.1, ***p<0.01, ****p<0.001).

The minimum number of YOY crayfish surviving in any one tank was 17. The weights (mg) of these crayfish and of 17 crayfish from each of the other 53 tanks were log $_{10}$ transformed and the effect of habitat and predators on crayfish growth were tested. In a 2-way parametric ANOVA, growth was shown to be affected by habitat type ($F=19.02$, $df=2$, $p<0.001$), but not by predator ($F=2.09$, $df=2$, $p>0.1$), although there was an interaction effect ($F=4.35$, $df=4$, $P<0.01$; Fig. 6.18). A one-way parametric ANOVA was conducted to test for differences in crayfish growth between habitats. YOY crayfish growth differed between habitats ($F=18.70$, $df=2$, $p<0.001$). Crayfish grew more quickly in habitats with real weed (*Elodea*) by comparison to plastic weed and bare brick habitats with no cover (individual pairwise comparisons, both $p<0.001$).

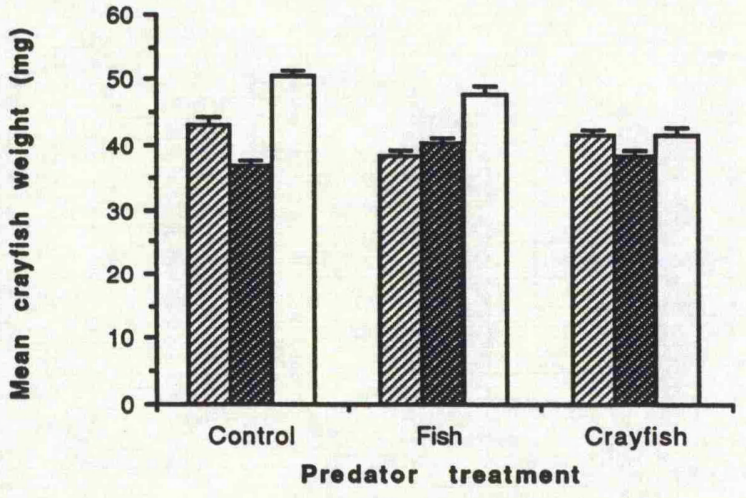


Figure 6.18. Mean (± 1 S.E.) weights (mg) of crayfish from each predator treatment in Experiment 6.5, in plain brick habitats ▨, in brick habitats with plastic weed ■, and in brick habitats with real weed □.

6.6 GENERAL DISCUSSION

6.6.1 THE EFFECT OF HABITAT MANIPULATIONS

The field experiment was designed to test the effects of perch predation and vegetation cover on YOY crayfish distribution in Rögle pond. It also tested the effect of water depth.

Removing emergent weed was likely to reduce the fauna of the upper littoral margin. Subsequent analysis of manipulated areas will, therefore, have been influenced by the experimental process rather than the experimental treatments. Manipulations of weed and predatory fish did affect the distribution of invertebrate fauna and of adult female crayfish (Section 6.3.3).

The situation is different with respect to studying YOY crayfish distribution. YOY crayfish were absent when the disturbances occurred, and were, therefore, affected by the experimental treatments. Adult crayfish, particularly gravid females, can influence YOY crayfish distribution (Capelli & Hamilton, 1984; Beingesser & Copp, 1985; Gore & Bryant, 1990; Chapter 5). Therefore, YOY crayfish distribution may have been indirectly affected by the experimental process as a result of changes in the distribution of adult female crayfish and of other invertebrate fauna. Too few gravid females were caught to assess distribution patterns between experimental sites.

6.6.2 YOY CRAYFISH DISTRIBUTION AND SURVIVAL

Initially, more YOY crayfish were caught in the deep water (Fig. 6.4). Before juveniles hatched, more gravid females were also caught in the deep water (Fig. 6.3). It is possible that this influenced the initial distribution of YOY crayfish. However, baited traps do not give an indication of absolute abundance and catches may be subject to competition effects between different ages, sexes and sizes of crayfish (Brown & Brewis, 1979). Also, in 1991, the initial distribution of YOY crayfish in traps did not differ with water depth, although more gravid females were found in the shallow water prior to the hatch (Chapter 5, Section 5.4.2).

In 1992, YOY abundance, extrapolated from YOY trappability data (Fig. 6.4), was greater in the deep water habitats, although, after five weeks, densities were similar at both depths. The one week lag in maximum YOY densities found in the traps in the shallow water by comparison to deep water in 1992 may reflect migration by YOY crayfish away from high conspecific densities in the deep water.

The rapid decline in abundance of YOY crayfish in the deep water traps in 1992 after the first week of independence, resembled the pattern in 1991 (Chapter 5,

Section 5.4.2). In many other respects YOY crayfish distribution differed between 1991 and 1992. Initial densities were 2 to 3 times greater in 1992, and densities were initially greater in the deep water. This may be explained, in part, by the warmer water and levels of sediment present in 1992. The size of the brood stock will also influence initial YOY crayfish densities, but no data was available on this.

YOY *A. astacus* (up to 10 mm carapace length), feed principally on detritus, benthic microcrustacea, and macrophytes (Appelberg, 1990). This suggests that food availabilty for YOY *P. leniusculus* was extremely good in Rögle pond in 1992, as detrital deposits were widespread and abundant in the littoral margins. Good hatching conditions were indicated by the fact that young became independent three weeks earlier in 1992. Climatic conditions were found to be important in YOY crayfish production in two lakes in Canada (Momot, 1992). The length of the growing season and water temperature were two factors controlling growth rates. Cold wet springs were detrimental to production but hot dry springs and summers increased production. Higher water temperatures also improved the growth of juvenile *P. leniusculus* in laboratory studies (Mason, 1979).

Two weeks after becoming independent, low densities of YOY crayfish were found in the traps in the shallow water habitat containing weed and fish by comparison to the other three sites (Section 6.3.3). Either 1) fish predation lowered YOY crayfish survival in this site, or 2) fewer crayfish were using the bag traps, as a result of the alternative shelter offered by weed. It is likely that these factors interacted to produce the poor adundance of YOY crayfish at this site. It is also possible that this area of the pond initially contained lower densities of YOY crayfish as a result of chance distribution. An interaction of fish and weed was found to affect the abundance of other invertebrate fauna in the traps, largely as a result of differences in the number of invertebrates found in weed/no fish and no weed/fish habitats.

Weed was effectively absent from the substratum in the deep water and was not found to affect YOY crayfish distribution in the deep water habitats (Section 6.3.3). There was an indication that perch reduced juvenile distribution in deep water. This result is treated with caution. Perch appeared to reduce YOY survival in the three week period after YOY crayfish reached their maximum density in the traps (Fig. 6.6), but subsequently, similar numbers of crayfish were found in sites with and without fish.

If perch were preying on crayfish and were prevented from foraging in the shallow water, then YOY survival should be better in the shallow water. This was indicated when YOY abundance data from the four weeks after YOY crayfish had reached their maximum density in the shallow water traps were analysed together. However, this effect was not consistent within individual weeks (Fig. 6.4).

6.6.3 THE EFFECT OF PERCH

Perch tend to prey heavily on YOY crayfish for two weeks after they first become independent (Chapter 5, Section 5.4.2; Anderson & Helmgaard, 1990). Therefore, perch predation may have influenced the rapid decline in YOY abundance in the deep water sites two to three weeks after crayfish became independent. Newly independent stage II young are very active, investigating their environment, but YOY crayfish activity decreases with growth, declining markedly over a 9 day period approaching the moult to stage III (Doroshenko, 1979). Predation by fish and invertebrate predators will be limited if YOY crayfish grow quickly (Momot et al., 1978) or if they change their temporal and spatial distribution in response to predators (Stein 1977). Wahle & Steneck (1992) found that attacks by fish on tethered lobsters fell drastically with small increases in body size. Attacks fell from 60/hour for size classes between 4 to 5 mm carapace length, to less than 10/hour for size classes over 8 mm. In years when water temperatures are high and food is abundant, YOY crayfish growth should be rapid. In such years, fish predation might be expected to have less effect on newly independent crayfish survival, although in 1991 perch between 17.5 to 21.1 cm long fed on YOY crayfish (Chapter 5, Section 5.4.2). These perch are able to prey on larger crayfish (Dehli, 1981), although size selection should favour predation on smaller crayfish (Stein, 1977).

There is conflicting evidence concerning the impact of fish predators on invertebrate community structure. Many lentic studies have found an effect of fish predation, whilst lotic studies have produced less clear results (Flecker, 1984 for review). Of the lotic studies, field manipulations of fish densities often failed to affect invertebrate communities. The study conducted by Flecker (loc. cit.) found that, although sculpins (*Cottus* spp) did not effect prey abundance, the combined effects of several vertebrate predators did. Even so, invertebrate abundance was more closely associated with plant detritus. A similar result was found by Flecker & Allen (1984), although in this study, fish did not affect macroinvertebrate communities. Conversely, the dietary value and feeding preferences of the marine amphipod *Ampthoe longimana* for host plants do not match field distributions. Instead, host plants providing better protection from predation contained the greatest amphipod densities (Duffy & Hay, 1991). Wahle & Steneck (1992) found greater densities of juvenile lobsters (*H. americanus*) in shelter-providing habitats during the early benthic stages of their life. They concluded that the proximate cause of this distribution was habitat selection behaviour and that predation was the evolutionary process reinforcing this behaviour. In 1991, predation rather than juvenile habitat selection appeared to control the initial distribution of YOY crayfish in Rögle pond (Chapter 5, Section 5.8). The substratum in Rögle is uniform with water depth and therefore substratum selection was not thought to influence YOY crayfish distribution. The results from 1992 do not support these conclusions.

6.6.4 THE EFFECT OF VEGETATION

Dense patches of vegetation are usually associated not only with an abundance of macroinvertebrates, but also with an abundance of fish predators. Bigger patches of vegetation and plants with tall shading elements provide *Asellus* with protection from predation by two percid species (Matilla, 1992). Vegetation does not always provide protection however. Prey must be capable of utilising these structures (Savino & Stein, 1989) and predators may change their foraging behaviour in more complex habitats, to maintain search efficiency (Savino & Stein, 1982). Deihl (1988) found that perch search efficiency remained relatively high in complex habitats by comparison to other fish species. The limited effect of vegetation on YOY crayfish distribution may have been because the weed did not form a habitat of sufficient complexity to reduce predation. Either the weed was not dense enough and/or the area covered by the weed was small by comparison to the area colonised by the YOY crayfish.

In Experiment 6.4, more YOY crayfish were found in both real and artificial weed habitats compared to habitats with no cover. Real weed reduced YOY crayfish mortality both in the presence and absence of predators (Experiment 6.5). The increase in juvenile survival in association with weed may have resulted from reduced intraspecific competition due to increased food and shelter availability in control and groplöja tanks, and/or due to increased protection from predation by adult crayfish. Real weed also enhanced crayfish growth.

YOY crayfish growth is a product of food availability and of crayfish activity, which are regulated by water temperature, photoperiod, densities of adult and juvenile conspecifics, availability of shelter, and predatory fish (Mason, 1979; Appelberg & Odelström, 1988; Figiel et al., 1991; Maxwell, 1988 cited by Momot, 1992. In Rögle pond, YOY crayfish in the shallow water were larger in 1991 (Chapter 5, Section 5.4.2), and heavier in 1992 (Section 6.2.7) by comparison to crayfish from the deep water. Perch did not reduce YOY crayfish growth in the deep water, as this effect was seen both within and outside the net enclosure. Also, in shallow water, growth was better in YOY crayfish exposed to perch.

It was not possible to say whether growth in the deep water was limited as a result of: 1) greater initial YOY densities and hence increased competition (Fig. 6.4). Maxwell (loc. cit.) found that high YOY densities retarded growth. 2) Greater competion from adult crayfish (trappability data indicated that adults were more abundant in the deep water; Fig. 6.2), or 3) better food resources in the shallow water associated with emergent vegetation. There was an indication that weed enhanced YOY crayfish growth in the deep water in Rögle Pond (Section 6.2.3). This is surprising because weed only reached 0.5 m in to the pond and the deep traps were set

5 m into the pond, and because no similar effect was found in the shallow water. Both this, and the positive effect of perch on YOY growth in shallow water were counter intuitive. It is suggested that these results should be verified with more closely controlled experiments using field enclosures.

From the above data, it is concluded that perch and weed exerted only a weak influence on YOY crayfish distribution and survival in Rögle Pond in 1992, and that other factors were exerting a stronger influence.

6.6.5 THE INFLUENCE OF ADULT CRAYFISH AND FISH

The laboratory studies suggest that adult crayfish may affect YOY crayfish distribution in Rögle pond. Momot (1992) found that YOY crayfish growth and mortality were inversely related in unexploited crayfish populations. Adult crayfish were found to suppress YOY crayfish growth as a result of suppressing YOY crayfish activity. This study provides evidence that adult crayfish do suppress YOY crayfish activity, but, YOY growth was not affected over the three weeks of the experiment. Over a longer time period, reduced activity could suppress feeding and growth, so leading to increased moult failure and to an increase in the length of time during which YOY crayfish are vulnerable to predation from fish and invertebrate predators (Momot, 1992). As discussed above, a major defence against predation by juvenile crayfish is a rapid growth rate (Momot, 1984). After one year, crayfish have outgrown most predators (Momot et al., 1978). Therefore, growth is at a premium for newly independent crayfish. As a result, YOY crayfish should spend the maximum amount of time possible feeding and any factor that reduces YOY crayfish activity is also likely to increase mortality.

Both adult crayfish and groplöja reduced juvenile crayfish activity and survival. Mortality in response to groplöja may have resulted from successful predation, or from chronic injuries sustained in unsuccesful predation attempts, although neither was observed. Such attacks were found when bullheads attacked crayfish too large to consume whole (Foster, pers. comm.). Dead crayfish were found with their legs missing. Alternatively, the increased mortality of juvenile crayfish exposed to groplöja may have resulted from reduced food intake associated with the reduced activity exhibited by these crayfish in response to the fish. Thirdly, the fact that groplöja only increased crayfish mortality in tanks without real weed suggests that intraspecific competition for food and/or shelter may have increased juvenile mortalities.

Adult crayfish increased YOY crayfish mortality to a greater degree than groplöja. YOY mortality increased in all three habitats, although survival was better in real weed habitats. Again, increased mortalities may have resulted from poor food

consumption associated with reduced activity, and/or from an increase in intraspecific interactions. As YOY mortality was so high (67 to 70% with no weed and 52% with real weed over 3 weeks) it is considered that cannibalism was also occurring. Momot (1992) found no evidence for cannibalism in analyses of adult crayfish stomachs from two Canadian lakes, and also observed that adult crayfish were unable to catch YOY crayfish. Smith & Herrnkind (1992) and Wahle & Steneck (1992) report that decapod predators were inefficient at catching and handling lobster prey, however, adult *O. virilis* were observed to prey on immobile, moulting YOY crayfish in laboratory conditions (Momot, loc. cot.) and cannibalism has been reported in field populations of *O. propinquus*, where large numbers of inter-moult young were consumed (Capelli, 1980). The experimental tanks in this study are likely to have increased encounter rates between adult and YOY crayfish and would therefore tend to enhance cannibalism.

6.6.6 PREDATOR AVOIDANCE BY YOY CRAYFISH

YOY crayfish predator-avoidance behaviour was shown to be flexible in response to groplöja and adult crayfish. If groplöja were not capable of preying on YOY crayfish, then the response to groplöja appeared maladaptive because of the reduction in crayfish activity and the increase in crayfish mortality. YOY crayfish were presumably reacting to stimuli characteristic of predatory fish. This raises the question of whether crayfish would have modified their behaviour in response to groplöja over a longer time period.

There is much evidence suggesting that animals can assess and behaviourally influence their risk of predation, within their life time, and across periods of days or hours (Lima & Dill, 1990 for review). Both aquatic vertebrates and invertebrates discern between predators and non-predators (Peckarsky, 1980; Peckarsky & Dodson, 1980; Heads, 1985), active and inactive predators (Alexander & Covich, 1991), hungry and satiated predators (Licht, 1989), and also the type of predator (DeWitt, 1992). The ability to detect dangerous predators has been shown to be related to the previous ecological history of predation in sticklebacks (Giles & Huntingford, 1984), minnows (Magurran, 1986), guppies (Licht, 1989), and crayfish (Shave et al., in press), although in the first two studies it was not known whether this was a result of within life-time experience or of natural selection acting on heritable differences.

Vertebrate prey may quickly learn to respond to specific predators. Csányi (1985) found that naive paradise fish (*Macropodus opercularis* L.) habituate to the presence of satiated predators and non-predators. If attacked by hungry predators, however, these fish quickly learned to avoid these predators on the basis of species characteristics regardless of whether they were hungry or satiated. Crayfish (Stein & Magnuson, 1976), and lobsters (Wahle, 1992b), undergo ontogenetic shifts from close

associations with shelter to a more free ranging existance. In lobsters both perceived levels of predation risk and shelter-based food availability affect this transition. Juvenile *A. astacus* also exhibit a variable behavioural reponse to the scent of different fish species. The scent of the most dangerous predators caused a total cessation of diurnal activity (Appelberg, pers. comm.).

Newly hatched crayfish in this study had no previous experience of predation. At this stage, crayfish have an acute conflict of interests. They are small and extremely vulnerable to predation, but rapid growth is an important means of lowering predation risk. Therefore, newly independent YOY crayfish should initially possess suitable predator avoidance/escape behaviour but this behaviour should adapt quickly to the prevailing predation situation.

The persisting avoidance response of YOY crayfish to groplöja is, therefore, of great interest. Lima & Dill (1990) and Bouskila & Blumstein (1992), suggest that assessment of predation risk is based on simple, conservative rules-of-thumb, as a mistake is likely to be fatal. One such rule could be "assume attack is likely until experience allows a more detailed assessment of risk" (Lima & Dill, loc. cit.). Other fish species of similar size to groplöja prey on YOY crayfish. Svensson (1992), has shown that roach between 10 to 17 cm long and perch 12 to 15 cm long both reduce YOY *A. astacus* survival to between 10 to 47%. The survival in non-predator controls was between 91 to 92%. Crayfish may not be able to distinguish between the characteristics of individual species in the wild. Fish movement does stimulate defensive behaviour (Chapter 2 and 3) but may not differ sufficiently between species. It is suggested that this response is strongly based on the evolutionary history of predation from fish.

Most animals live in environments containing many predators. Often prey have one particularly dangerous predator, however, Lima (1992) suggests that the presence of less dangerous predators may significantly affect anti-predator behaviour. The change from nocturnal to diurnal activity in response to different predators in isolation, shows that crayfish avoidance behaviour is flexible. Previously, only nocturnal activity shifts in response to diurnal predators have been reported in crayfish (Hamrin, 1987; Chapter 2 and 3). This raises the question as to how YOY crayfish should respond in sympatric populations of crayfish and fish? Mixed populations exist in the majority of crayfish habitats. To avoid both would severely restrict activity and growth. Wahle & Steneck (1992), and Smith & Herrnkind (1992) found that fish, which accounted for 88% of all observed attacks, were more common predators of juvenile lobsters than decapod crustaceans and other invertebrate predators. Fish were also more successful predators. If foraging activity becomes too limited in response to both fish and adult crayfish, then YOY crayfish should reduce their diurnal activty to avoid fish predators and should continue to be nocturnal. Whilst this study indicates that this would place

YOY crayfish at risk from competition and predation by adult crayfish, it is considered that the associated mortality risk of this behaviour would be less than that of exposure to diurnal fish predators.

6.6.7. CONCLUSION

It is concluded that both perch and adult crayfish affected YOY crayfish distribution in Rögle pond in 1991. The observed patterns of YOY crayfish growth in 1991 and 1992 suggest that feeding activity and/or food availability were limited in the deep water. Both perch and adult crayfish and also vegetation may have affected this, but in 1992 it is concluded that intraspecific interactions between YOY crayfish and competion from adult crayfish were likely to be most important in controlling YOY crayfish distribution, survival and growth. Although weed and fish interacted to effect the distribution of other invertebrate fauna, no strong effect was found on YOY crayfish distribution. This may have been a result of the high YOY crayfish densities. Caddy (1986), suggested that many shelter dependent Crustacea may experience a recruitment bottle-neck after settlement if shelter-providing habitat is limited. When crayfish populations are dense, habitat availability and intrapecific competition between juveniles and adult crayfish are major factors controlling YOY crayfish distribution, survival and growth (Hogger, 1988; Momot, 1992). In less dense populations, fish predation may be a more important controlling factor.

DISCUSSION

CHAPTER 7.

7.0 DISCUSSION

This study addressed three main questions: 1) how do juvenile crayfish respond to different predators? 2) how do predators influence juvenile crayfish habitat use ? 3) how important is predation as a source of juvenile crayfish mortality in nature? These questions are discussed below.

7.1 THE RESPONSE OF JUVENILE CRAYFISH TO DIFFERENT PREDATORS

Defensive behaviour in *O. propinquus* changes with ontogeny (Stein, 1977). Defensive behaviour may also differ depending on other factors such as the need to feed and reproduce (Lima & Dill, 1990 for review). At different stages of development, the defensive behaviour of *P. leniusculus* may be altered to a greater or lesser extent depending on the function of the behaviour and on the experience of individual crayfish. Part I of this study was concerned with predation by perch and eels on juvenile crayfish, and investigated avoidance and evasive behaviour in response to both predators. In addition, Part II of this study investigated the avoidance behaviour of YOY crayfish in response to perch, adult crayfish and non-predatory groplöja.

YOY crayfish exhibited flexible avoidance behaviour in response to different predators, but exhibited inflexible behaviour in response to groplöja, which appeared maladaptive. 'Inflexible' is used in the sense that crayfish hatched and immediately behaved in such a way as to avoid predation. 'Flexible' is used to describe the way these initial responses are modified through experience. This discussion does not attempt to draw a formal distinction between these categories of behaviour, but treats them as a continuum. These categorisations resemble 'fixed' and 'reactive' behavioural patterns as described by Stein (1979). Fixed behaviours do not require the presence of predators to elicit them, and are assumed to be a result of long-term predation pressure acting over evolutionary time. Reactive behaviours only occur in response to the presence of a predator. Examples of these categories of behaviour were shown by Heads (1985). *Ischnura elegans* larvae are more active at night in the absence of predators, reflecting fixed behaviour. Larvae exhibited a reactive response in the light, moving less when predators were present.

The tail-flip evasive response of juvenile crayfish tended to be inflexible in response to both fish and adult crayfish (pers obs). This should be expected, as the behavioural options leading to a successful escape are limited (Endler, 1986). By contrast, there is more scope for flexible avoidance behaviour because of the greater range of possible predator-prey interactions. For example, YOY crayfish avoided

perch by reducing diurnal activity and avoided adult crayfish by decreasing nocturnal activity (Chapters 5 and 6).

The preference of YOY crayfish for nocturnal activity in response to perch, demonstrated in Chapters 1, 2 and 4, confirms previous studies on *P. leniusculus* (Appelberg & Odelström, 1988), and on *A. astacus* (Hamrin, 1987). In the present study, eels induced similar avoidance behaviour in juvenile *P. leniusculus* as did perch, which had not been previously reported. Hamrin (loc. cit.) found that YOY *A. astacus* were crepuscular in the absence of perch, but when perch were present, crayfish became increasingly nocturnal. In the present study (Chapters 2, 5, and 6), YOY *P. leniusculus* tended to be nocturnal in the absence of predators. This preference was less strong in the older, yearling (1+) crayfish used in the studies described in Chapter 4. The presence of fish predators reinforced the preference of both YOY and 1+ crayfish for nocturnal activity. This behaviour varied depending on the predatory stimuli that were available, and was most pronounced when predators could be seen and smelt.

At first sight, the response of 0+ crayfish to perch and eels in Chapter 2 appeared to be inflexible and inappropriate, as both perch and eels were also more active at night. In these experiments (Experiment 2.5 and 2.6), light from an algal culture may have facilitated perch foraging at night. Perch are crepuscular predators (Hamrin, 1987) and were able to feed on crayfish successfully, even at very low light intensities (Chapter 4, Experiment 4.12). Perch may, therefore, have been responding to the increase in crayfish activity at night. However, the response of crayfish may still be considered appropriate in this situation, as visual detection of prey will be more difficult in the poor lighting conditions at night. Diehl (1988) found that the predatory success of perch feeding on chironomid larvae declined markedly at night. The adaptivity of the response to eels is less clear, although the ability of crayfish to escape using only mechanical cues, and the failure of eels to chase fleeing crayfish (Chapter 4) suggest that active, exposed crayfish will be better able to evade eel attacks than crayfish which are constrained within a shelter.

This study provided no evidence that eels are more detrimental to crayfish populations than perch and failed to support predictions 4 to 6 (Section 1.3). The evasive behaviour (Chapter 4, Experiment 4.12), and possibly the avoidance behaviour (Chapter 2, Experiment 2.6) of YOY crayfish was more marked in response to perch than to eels. This suggests that eels are not as conspicuous predators as perch for reasons discussed in Chapter 4. Despite this, eels were less successful than perch at capturing crayfish due to the abilitiy of perch to chase fleeing crayfish.

There was an indication that perch were able to feed more rapidly on newly independent YOY crayfish then were eels (Chapter 2, Experiment 2.5). A similar result was found by Diehl (1988), for perch and bream feeding on chironomid larvae. Perch quickly consumed large numbers of chironomid larvae. Capture rate declined as prey

density declined although perch were not satiated and continued searching for prey. By contrast, bream foraged with equal intensity throughout the experiment and eventually consumed similar numbers of prey. The perch fed visually but bream fed by sifting sediment for prey. In the present study, perch consumed the majority of juvenile crayfish in the first 48 hours of Experiment 2.5. Eels fed more slowly, but over a longer period of time eels consumed similar numbers of crayfish to perch.

If eels do have a damaging effect on crayfish populations, then it is suggested that this is a result of the differential abilities of perch and eels to prey on larger size classes of crayfish, and therefore effect the brood stock and juvenile recruitment. If crayfish populations are more vulnerable to eel predation, then either the size selectivity of eels, their population densities, or competition between eels and crayfish for food or habitat might cause this. These aspects of the predator-prey interactions of crayfish and perch or eels could not be tested.

Adult crayfish invoked a total switch in YOY crayfish avoidance behaviour from principally nocturnal to principally diurnal diel activity, demonstrating flexibility in the avoidance behaviour of *P. leniusculus* (Chapter 6). Conversely, the response of YOY crayfish to non-predatory groplöja was inflexible and appeared maladaptive when considered in isolation. Crayfish were less active by day in response to groplöja. This resulted in greater crayfish mortality, possibly due to an increase in the incidence of aggressive intraspecific interactions for food or shelter.

The inflexible avoidance behaviour in response to groplöja suggests that crayfish have 'predator images' which determine the nature of the defensive response. Predator images may be similar to prey (search) images used by predators to detect prey (Curio, 1976; Roth, 1986). Predator images used by crayfish may be based on visual, chemical and mechanical cues. Evidence from Chapter 3, suggests that predator movement is a key visual cue forming a predator image. Evidence from Chapter 6 suggests that this cue is independent of predator size or shape. This is further suggested by the similar responses of crayfish to groplöja, perch and eels. If movement alone stimulates avoidance behaviour, this would explain the inflexible response of YOY crayfish to groplöja. It may also explain why, in Chapter 2, eels that fed inconsistently failed to produce avoidance behaviour in YOY crayfish.

Further evidence for the general nature of visual predator stimuli is described by Shave et al. (in press). New Zealand crayfish (*Paranephrops zelandicus* White) responded to the movement of both native long-finned eels and introduced brown trout, but only responded to the scent of eels and not trout. The responses of prey to predators are influenced by the co-evolutionary history of predators and prey (Lima & Dill, 1990). Shave et al. (loc. cit.) suggested that the differential response to eel and trout was a reflection of the different evolutionary experience that these crayfish had had of the two predators.

The inflexible response of juvenile *P. leniusculus* to groplöja suggests that crayfish use a conservative rule-of-thumb to avoid fish predation. The environmental movement perceived by newly independent crayfish may more often derive from predatory than from non-predatory fish. The response of YOY crayfish to groplöja may have changed over a longer period of time than the three weeks of the experiment. Responses of prey to predators alter during ontogeny in crayfish (Stein, 1977), and in lobsters (Wahle, 1992a), although the mechanisms underlying the change in the perception of risk are not clear.

O. propinquus from lakes in Ontario are more nocturnal and more shelter bound if the lakes contain abundant predators. This behaviour persisted for at least three weeks in aquaria without predators (Collins et al., 1983). Sih (1987) identified this response latency as an inherent asymmetry in predator avoidance behaviour. An increase in predation pressure should produce a rapid response whilst a decrease in predation pressure might have little effect. For prey to distinguish the degree of predatory threat they must sample the environment. This will increase the risk of predation and, therefore, this should only occur when predation risk is low, when the time required to gather this information is low, when the cost of using shelter is high, and when the benefits of exposure are high (Stein, 1979; Sih, 1987). In newly independent crayfish, the cost of using shelter may be high in terms of lost feeding opportunity, however, the fatal result of exposure to predators must be an overriding factor shaping the response to fish movement.

Adult *Orconectes* spp. quickly distinguish between restrained and free predators (Butler & Stein, 1985). DeWitt (1992) showed that freshwater pulmonate snails (*Physa*) responded differently depending on whether they could smell fish and crayfish predators, and whether these predators are consuming snails or not. The response varied with the degree of predation risk. Appelberg (pers. comm.) found that the scent of predatory and non-predatory fish produced different responses in YOY crayfish and Hazlett (1990) showed that crayfish respond to disturbance chemicals from conspecifics which have been attacked but are unharmed. The present study demonstrated that the avoidance behaviour of YOY *P. leniusculus* in response to visual cues was less marked than in response to chemical or a combination of chemical and visual cues (Chapters 3 and 4). This evidence suggests that chemical cues may be more sensitive to the differentiation of predators than visual cues.

Chemical cues facilitate predator avoidance before a visual encounter occurs (Chapter 3). Once a visual encounter has occurred, then visual stimuli are important in eliciting an early and prolonged evasive response. However, evidence from Chapter 4 suggests that the evasive response becomes more specific if both visual and mechanical cues are present. Crayfish are nocturnal as a result of predator avoidance, therefore, crayfish should be expected to use sensory pathways other than vision to detect predators. This is confirmed by the demonstrated ability of crayfish to evade an

attacking predator on the basis of mechanical stimuli alone (Chapter 4).

7.2 THE EFFECT OF PREDATION ON JUVENILE CRAYFISH HABITAT USE AND GROWTH

Part II of this study investigated the influence of predation on crayfish distribution, survival and growth. The distribution of YOY crayfish is often related to shallow water, vegetation cover and 'safe' substrata i.e. substrata providing good protection from predation (Rabeni, 1985; Appelberg, 1986). Crayfish body size is correlated with the size of the particles forming the substratum in which they are concealed (Abrahamsson & Goldman, 1970; Rabeni, 1985; Foster, 1992). Safe substrata, a good food supply and high water temperatures allow crayfish populations to achieve high densities (Abrahamsson & Goldman, 1970; Shimizu & Goldman, 1983; Rabeni, 1985), and affect crayfish activity (Mason, 1979; Abrahamsson, 1983; Westin & Gydemo, 1988).

Rabeni (1985) and Mitchel & Smock (1991) suggest that crayfish distribution is determined by an interaction of substratum/habitat quality, competition and predation. Crayfish activity is stimulated in dense populations which promotes the dispersal of crayfish (Bovberg, 1959; Westin & Gydemo, 1988; Ackefors et al., 1989). Juvenile crayfish are also competitively excluded from habitats by larger conspecifics (Rabeni, 1985). Predatory fish also modify the distribution and activity of crayfish (Stein & Magnuson, 1976; Stein, 1977; Collins et al., 1983; Hamrin, 1987; Appelberg & Odelström, 1988).

If the environmental stimuli that control YOY crayfish habitat selection are known, then the relative importance of habitat, competition and predation on YOY crayfish distribution can be better predicted. Juvenile crayfish exhibited a preference for nocturnal activity which was reinforced by predators (except adult crayfish). Crayfish activity was also reduced on complex habitats (i.e. habitats with more shelter) indicating that shelter acquisition inhibits searching activity (Chapter 2, Experiment 2.5). This suggests that newly independent *P. leniusculus* are stimulated by predators to increase their search for shelter, but that this occurs through a promotion of nocturnal and not diurnal activity. A similar effect has been shown for American lobsters (Wahle & Steneck, 1992). Lobsters were active until a safe habitat was found. Lobsters which settled on poor substrata quickly traversed this habitat, but lobsters that settled on a good habitat rarely left it.

In laboratory experiments (Chapter 5), crayfish preferred substrata affording the best protection from predation, or put another way, crayfish sought shelter and more complex substrata supported larger densities of crayfish. This preference for shelter existed without the influence of predation, but was enhanced by the presence of

restrained predators, confirming Stein & Magnuson's study. Juvenile *P. leniusculus* did not choose habitats with respect to water depth, although there was an indication that crayfish mortality due to perch predation was reduced in shallow water (~ 30 cm; Chapter 5, Experiment 5.7). A similar effect was shown by Mather & Stein (1990). Crayfish also exhibited a weak preference for habitats with weed cover (Chapter 6, Experiment 6.4).

Both predation and the behavioural response to predation produced similar patterns of YOY crayfish distribution with respect to shelter provided by good substrata and weed. Similar distribution patterns, observed in tanks with no predators, may have been a result of intraspecific interactions (Mason, 1979; Chapter 6, Experiment 6.4 & 6.5). Juvenile crayfish may not be able to detect safe habitats from a distance unless chemical cues are used. When YOY crayfish are released from the females, they have a period when they must actively search for a safe habitat. Thus, whilst crayfish will tend to congregate on safe habitats as a result of their behaviour, the distribution of newly independent juvenile crayfish may be influenced more rapidly by predation if crayfish have to travel long distances to find shelter.

Work in Rögle pond produced conflicting evidence as to the relative influences of habitat selection behaviour, competition and predation on YOY *P. leniusculus* distribution. It is suggested that the uniform substratum at Rögle essentially limited the effect of crayfish habitat choice. Weed was not found to influence crayfish distribution in the shallow water, although it is possible that weed had a negative effect on the efficiency of the bag traps used to sample this distribution, and this may have obscured any such effect (Chapter 6). Weed was shown to enhance juvenile crayfish growth in laboratory experiments (Chapter 6), and weed may have had a similar effect on crayfish growth in the shallow (~ 30cm) littoral margins in Rögle pond. However, there was no evidence that this enhanced growth benifitted crayfish survival.

The initial distribution of YOY crayfish in Rögle pond was determined by the distribution of gravid females. Crayfish were released on the stone and not the silt substrata. As discussed in Chapter 5 and 6, gravid females probably did not exert a strong influence on the distribution of YOY *P. leniusculus* on the stone substratum in Rögle pond. In 1991, it was suggested that perch predation affected the distribution of YOY between the shallow and deep water stone habitats. In 1992, YOY crayfish abundance did not differ between shallow and deep water stone habitats, and neither perch predation nor vegetation cover were found to influence YOY crayfish distribution. In the laboratory, adult crayfish suppressed juvenile crayfish activity and caused greater YOY crayfish mortalities (Chapter 6, Experiments 6.4 and 6.5). It was suggested that intraspecific density-dependent population regulation was likely to be an important factor controlling juvenile *P. leniusculus* distribution in Rögle pond as found in populations of *O. virilis* (Momot, 1992).

Capelli & Magnuson (1983) found that the availability of suitable shelter-providing habitat was the major limiting factor affecting the abundance of crayfish in lakes in North America. If predators are present, either actual predators (perch, adult crayfish and eels) or 'ghost' predators (groplöja), then juvenile crayfish are stimulated to find shelter and to reduce activity. This may promote density-dependent competition for shelter and food which may regulate juvenile crayfish recruitment. The significance of predation as a source of crayfish mortality, and hence as a factor affecting crayfish abundance, is discussed below.

7.3 THE SIGNIFICANCE OF PREDATION AS A SOURCE OF JUVENILE CRAYFISH MORTALITY

Juvenile crayfish mortality is often a function of growth rate which controls the stock-recruitment relationship (Momot, 1984 for review). Density-dependent growth and mortality have their greatest effect on juvenile crayfish. Usually low food availability and poor nursery areas regulate population densities to narrow limits, despite the initial size of each year's cohort (Capelli & Magnuson, 1983; Hogger, 1988). Momot & Gowing (1983) showed that recruitment of young *O. virilis* in two lakes in Michigan, U.S.A, was limited by the carrying capacity of the nursery areas. Capelli & Hamilton (1984) found that limited shelter increased aggression in crayfish to a greater degree than limited food. Habitat availability has an important controlling influence on the distribution of other decapod crustacea (Wahle and Steneck, 1992 for review). Caddy (1986) suggests that recruitment in many shelter-seeking Crustacea is limited by the availability of shelter-providing habitats.

Predation and food/shelter availability can be classified as top-down and bottom-up factors respectively. This classification system has been used to describe the effects of trophic interactions and resource availability on community structure (Menge, 1992 for review). Top-down and bottom-up factors were considered in isolation but are increasingly considered as a continuum, with the interactions of these factors becoming an important issue. The importance of predation as a source of crayfish mortality should be considered in relation to the physical and chemical constraints of an environment.

Crayfish may be found on exposed substrata in habitats with poor or non-existant predator populations (Stein, 1977; Collins et al., 1983). Predation has been shown to influence the abundance and distribution of short-lived, highly fecund species of crayfish (Saiki & Tash, 1979). Populations of such species tend to be unstable (Momot, 1984). Predation appears to be less important as a population control mechanism in more stable populations of long-lived, less fecund crayfish species such as *P. leniusculus*, where recruitment is often density-dependent. This is particularly

true of salmonid predators (Momot, 1967; Mason, 1975). It has been suggested that rainbow trout predation caused differences in crayfish densities between two Californian lakes where suitable safe habitats were limited (Goldman & Runquist, 1977). Momot (1984) suggests that this may have resulted from high nutrient loads in one lake producing uninhabitable microhabitats for juvenile crayfish, which limited recruitment.

Percid populations may exert a greater control on crayfish populations than salmonid populations (Taub, 1972; Lorman & Magnuson, 1978; Appelberg, 1987; 1990). In the latter two studies, perch were suggested as a major factor limiting *A. astacus* populations, however, it was not known whether the recovery of *A. astacus* populations was prevented as a direct result of predation, or as a result of indirect predator effects such as reduced crayfish activity or competition for food. Appelberg & Odelström (1988) showed that perch reduced YOY *P. leniusculus* activity but not abundance. Similarly, Collins et al. (1983) found that dense percid populations produced behavioural changes in crayfish, without affecting crayfish densities on good habitats. Conversely, Svensson (1992) found that perch and roach 10 to 17 cm long reduced YOY *A. astacus* survival in pond experiments.

Direct field evidence of predation as a process structuring the distribution and abundance of Crustacea is difficult to obtain (Wahle & Steneck, 1992). Momot (1967) and Momot et al. (1978) suggest that fish and invertebrate predators have little effect on YOY crayfish survival. In Rögle ponds, there was a suggestion that perch limited juvenile crayfish distribution in deep water in the first four weeks of their independence in 1991. This was not verified in a direct test the following year. It is likely that, if fish predation does exert an influence on crayfish abundance, this will be a result of juvenile crayfish being limited to nursery grounds (i.e. areas which offer shelter against predation). In Chapter 2, Experiment 2.6, perch reduced YOY crayfish survival on a substratum providing plentiful shelter, although this may have been influenced by poor food availabiltiy and high crayfish densities. However, in Experiments 2.5 and 5.7, survival improved with increased shelter availability. When predator populations are large and nursery grounds and food availabiltiy are limited, predation may directly and indirectly affect crayfish populations. Predators may indirectly limit recruitment through density-dependent intraspecific competition, to the levels set by the carrying capacity of the nursery grounds.

Rögle ponds support dense crayfish populations. Abrahamsson (1966) found slow growing populations of *A. astacus* with a high incidence of chelae damage, indicating a large amount of intraspecific aggression. Crayfish plague removed *A. astacus* from the ponds but *P. leniusculus* were introduced in 1963 (Abrahamsson, 1971) and have also developed dense populations (Nyström, pers. comm.) indicated by the high levels of chelae damage; 12 to 14% of males and 9 to 25% of females from 4 sites (Chapter 5).

In dense populations, adult male *O. virilis* regulate YOY crayfish recruitment (Momot, 1992). It was suggested that this was through the suppression of YOY crayfish growth, which led to increased mortality, rather than as a result of cannibalism. Adult *P. leniusculus* may limit YOY crayfish recruitment in Rögle pond, as evidenced by the increase in mortality and reduced activity of YOY crayfish in the presence of adult crayfish (Chapter 6, Experiment 6.5). Perch may have enhanced this effect through the suppression of crayfish activity (Chapter 5, Excperiment 5.6 and 5.7). A further possibility exists. Groplöja reduced YOY crayfish activity in the laboratory (Chapter 6, Experiment 6.5). Groplöja were present in Rögle pond and could conceivably cause a similar effect on YOY crayfish growth and survival as adult crayfish and perch. Groplöja and YOY crayfish are likely to have been in artificially close proximity in experimental tanks, however, both tend to be found in the shallow littoral margins of lakes and so, non-predatory fish activity might be an additional factor influencing YOY crayfish activity and hence, YOY growth and survival.

Lastly, Momot et al. (1978) suggested that predation on larger crayfish may cause recruitment over-exploitation and so limit crayfish populations directly. Recruitment may also be reduced if heavy predatory mortalities occur in yearling (1+) crayfish cohorts. Yearling crayfish are small enough to be consumed by perch (Dehli, 1987) and occurred in 33% of perch greater than 20 cm long in Rögle pond (Chapter 5). As YOY crayfish mortalities reached between 65-83% after only 7 weeks in Rögle pond (estimated from trapping data), sustained predation pressure by perch feeding on 1+ crayfish may be a possible limitation to recruitment. Momot (1984) identified three important life stages in crayfish populations; 1) shelter seeking by newly hatched juveniles, 2) growing juveniles leaving littoral areas for deeper water, and 3) adults directing energy towards reproduction rather than growth. Wahle & Steneck (1992) suggest that if the size range of available shelters is insufficient, then the transition of animals at stage 2 to a new habitat may have a high associated risk of predation. In Rögle pond, 1+ crayfish were found in the deeper water (Chapter 6). Movement away from the littoral fringes to deeper water may therefore not only increase the chances of predation by perch, but may increase competition with adult crayfish.

From this work, a system of interactions affecting juvenile crayfish distribution growth and survival has been summarised in a model, involving the effects of crayfish behaviour, predation and environmental constraints (Fig. 7.1).

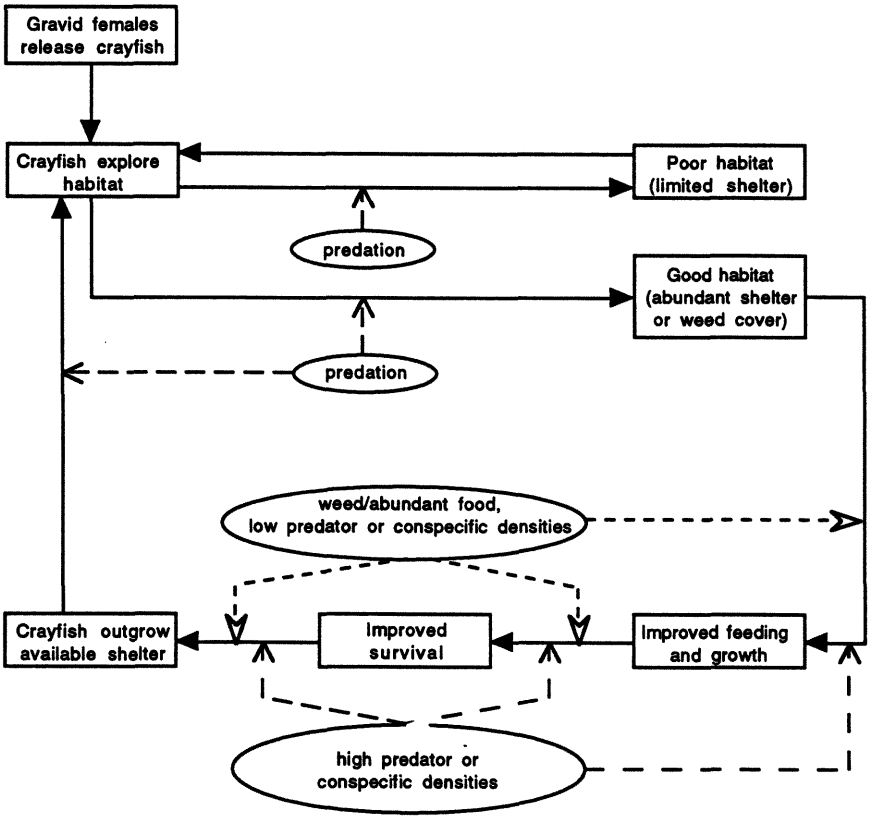


Figure 7.1. Schematic representation of factors influencing the defensive behaviour of crayfish against predation. Solid lines and boxes represent crayfish behaviour, ovals and dotted lines denote factors having a positive - - ➤, and negative — ➤, effect on crayfish behaviour and survival.

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