

**THE EFFECT OF LIGHT-INDUCED EYE
DAMAGE ON THE BEHAVIOUR
OF *NEPHROPS NORVEGICUS***

A thesis submitted for the degree of
Doctor of Philosophy

by

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1996

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ABSTRACT

THE EFFECT OF LIGHT-INDUCED EYE DAMAGE ON THE BEHAVIOUR OF NEPHROPS NORVEGICUS

Submitted for the degree of Doctor of Philosophy by
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October 1996

Nephrops norvegicus is susceptible to irreversible eye damage following exposure to daylight when brought to the surface during fishing operations. This study investigated the significance of blinding on the behaviour of undersized eye-damaged *N. norvegicus* after being returned to the sea bed.

Field-based and laboratory-based experiments showed that sighted and blinded *N. norvegicus* possess similar 24 h nocturnal activity rhythms. In the laboratory, *N. norvegicus* performed a higher level of activity after they were blinded but in the field there was no significant difference in activity levels of the sighted and blinded individuals.

The effect of blinding on the predator avoidance behaviour of *N. norvegicus* was assessed using either the presence or odour of a predatory fish (cod, *Gadus morhua*). Avoidance behaviour of both sighted and blinded *N. norvegicus* was elicited by both types of cod stimuli, but blinded *N. norvegicus* were affected to a lesser extent than the sighted ones. It was also shown that avoidance behaviour of both sighted and blinded *N. norvegicus* is elicited equally by a predator species (*G. morhua*) and a non-predatory species (*Pollachius virens*).

Contests between individual *N. norvegicus* were studied in the laboratory and a full description is given of all types of the agonistic behaviour performed. The relative status of two opponents was unaffected if either the dominant or the subordinate opponent was blinded. The duration and content of contests were largely unchanged after blinding one opponent.

There was no evidence from laboratory-based experiments, that blinding has any effect on the ability of *N. norvegicus* to gain shelter and to locate food items. It was also shown that blinded *N. norvegicus* are able to find a baited creel in a laboratory situation.

These results provide evidence to suggest that, in respect of these important behaviours, there is no major difference between sighted and blinded *N. norvegicus*.

ACKNOWLEDGEMENTS

Thank you to Dr. Peter Shelton for his experience and advice on the experimental work and writing of this thesis. Many thanks to all those in the Zoology Department of the University of Leicester who have helped with this work, especially Maurice Andrews for his 'cheery optimism' (hands thrust deep in pockets, rub of the chin and sharp intake of breath, "Wrong time of year for....." / "You can't get the parts....."), Dr. Ted Gaten for his help in many matters from histology to computer image analysis and Dr. Frank Clark for his statistical expertise! Also many thanks to my other supervisor Colin Chapman and his team at SOAFD, Aberdeen, for the opportunity to work at Loch Torridon and in the Fish Behaviour Unit at SOAFD.

Finally, thanks to all my family and friends for their enduring support throughout this task; especially to my two cats, Dizzy and Tinker, for their helpful comments and incisive criticisms.

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Chapter 1 Introduction

It is now well established that the compound eyes of deep-water crustaceans are susceptible to permanent light-induced photoreceptor cell breakdown if exposed to daylight at the sea's surface; examples include the isopod *Glyptonotus antarcticus* (Meyer-Rochow, 1982), *Cirolana borealis* (Nilsson and Lindstrom, 1983) and the spiny lobster *Jasus edwardsii* (Meyer-Rochow and Tiang, 1984). In commercially important species such as *Nephrops norvegicus* (hereafter referred to as *N. norvegicus*) this causes concern about the fate of *N. norvegicus* smaller than the minimum legal size, that are returned to the sea bed. Undersized *N. norvegicus* may form up to 50% of the total catch (Shelton *et al.*, 1985), which suggests that many discarded *N. norvegicus* are returned blind or with extensive retinal damage.

In a previous study to compare the survival of sighted and blinded *N. norvegicus* in the field, individuals with 0%, partial (mean level = 75%) and 100% eye damage were tagged and released into the sea bed at Loch Torridon. From the individuals recaptured using baited creels over the period between 1985 - 92, it was found that recapture and growth rates were not affected by the degree of eye damage (Chapman *et al.*, 1989). However, such studies may not provide a true picture of the effect of blinding on the survival rate of a *N. norvegicus* population because it is possible that the trapping method may have acted differentially for the sighted and blinded *N. norvegicus*. For this reason it was necessary to extend this previous study and assess the effects of blinding on behaviour in *N. norvegicus*, using both laboratory and field based experiments. Aspects of behaviour that were examined in this thesis include: behaviour rhythms (including in-burrow and burrow

emergence activity) (chapters 3 & 4), agonistic behaviour during contests between conspecifics (chapter 5), predator avoidance behaviour (including predator detection and recognition) (chapter 6), and their ability to gain shelter and find food (chapter 7). For each aspect of behaviour, experiments were carried out to compare the responses of sighted and blinded individuals.

1.1 The *N. norvegicus* fishery.

Because of its status as a luxury food, the *N. norvegicus* fishery is of high commercial importance. No viable method of large scale artificial cultivation has been found, so it is important that the fishery is regulated in order to enable sustainable fishing of naturally occurring populations to continue.

Catches are taken at depths of 20 - 500 m using either trawl or creel techniques. *N. norvegicus* trawls have small mesh (30 - 70 mm) and are towed for 2 - 6 hours (Howard, 1989). Baited creels are used to catch *N. norvegicus* if the sea bed is unsuitable for trawling. Each creel is made from a wire frame covered with 25 - 30 mm mesh netting, with two openings for the *N. norvegicus* to enter. Baits commonly used are fresh or salted herring and mackerel. Creels are left on the sea bed for 1 - 2 days before hauling (Howard, 1989).

N. norvegicus are usually landed live and sorted on the deck of the fishing vessel. In 1988, an EEC regulation (no. 2024/88) was issued preventing the sale of *N. norvegicus* with carapace lengths of 20 mm or less in waters off the West of Scotland and in the Irish Sea, and 25 mm in all other areas (except Skagerrak and Kattegat where the legal landing limit is 40 mm). Although the mesh sizes on trawls and creels allow undersized *N. norvegicus* to escape, some small individuals can remain in the catch if they

cling to each other, to other species in the catch, or to the net itself. After sorting, the undersized *N. norvegicus* are returned to the sea bed as 'discards'.

The regulation of mesh sizes and sizes of individuals landed are the main technical measures for the conservation of *N. norvegicus* stocks. If discard survival is low, the minimum landing size becomes less effective as a conservation measure.

1.2 Survival of discards.

The survival of *N. norvegicus* discards has been of great interest to fisheries scientists. Previous studies have concentrated on the effects of physical damage to discards and physiological stress. Injury may be caused by fishing gear and contact with other individuals in the catch (Chapman, 1981). In one study, one third of those surviving and examined after three days, had lost one or both claws during trawl fishing. The damage was attributed to aggression from conspecifics, or damage by fishing gear (Gueguen and Charuau, 1975). The effects of rapidly changing temperatures and pressure as *N. norvegicus* ascends and then descends to the sea bed can also cause mortality. If exposure to air as the catch is landed and sorted is excessive, a large proportion can die from asphyxiation (Chapman, 1981). Prolonged exposure of discards to elevated temperatures (i.e. 14°C) on deck during sorting procedure can increase mortality; if kept on deck for 2.5 hours, survival was reduced by 30% (Chapman, 1981). Discards are also vulnerable to capture by sea birds as they are returned to the sea and other marine animals may take discards as they descend to the sea bed (Chapman, 1981). Finally, mortality of discards can be caused by returning them to an unsuitable substrate (Howard, 1989).

Various studies have been carried out to assess survival of discards using the technique of placing sorted undersized *N. norvegicus* in cages on the sea bed and calculating their rate of survival over a period of time. After 8 days, 97% of creel-caught *N. norvegicus* survived (Chapman, 1981). Studies of trawl-caught discards kept in a cage for 8 days found survival rates ranging from 19 - 79.8% (Charuau *et al.*, 1982; Morizur *et al.*, 1982). These studies of trawl-caught discard survival may overestimate mortality because there was a high incidence of fighting and cannibalism due to overcrowding in the cages used (Gueguen, 1975; Charuau *et al.*, 1982; Morizur *et al.*, 1982). It was also found that carnivorous amphipods entered the cages causing additional mortality (Charuau *et al.*, 1982; Morizur *et al.*, 1982). Cage studies may underestimate mortality because they do not include the effects of predation of discards as they fall to the sea bed.

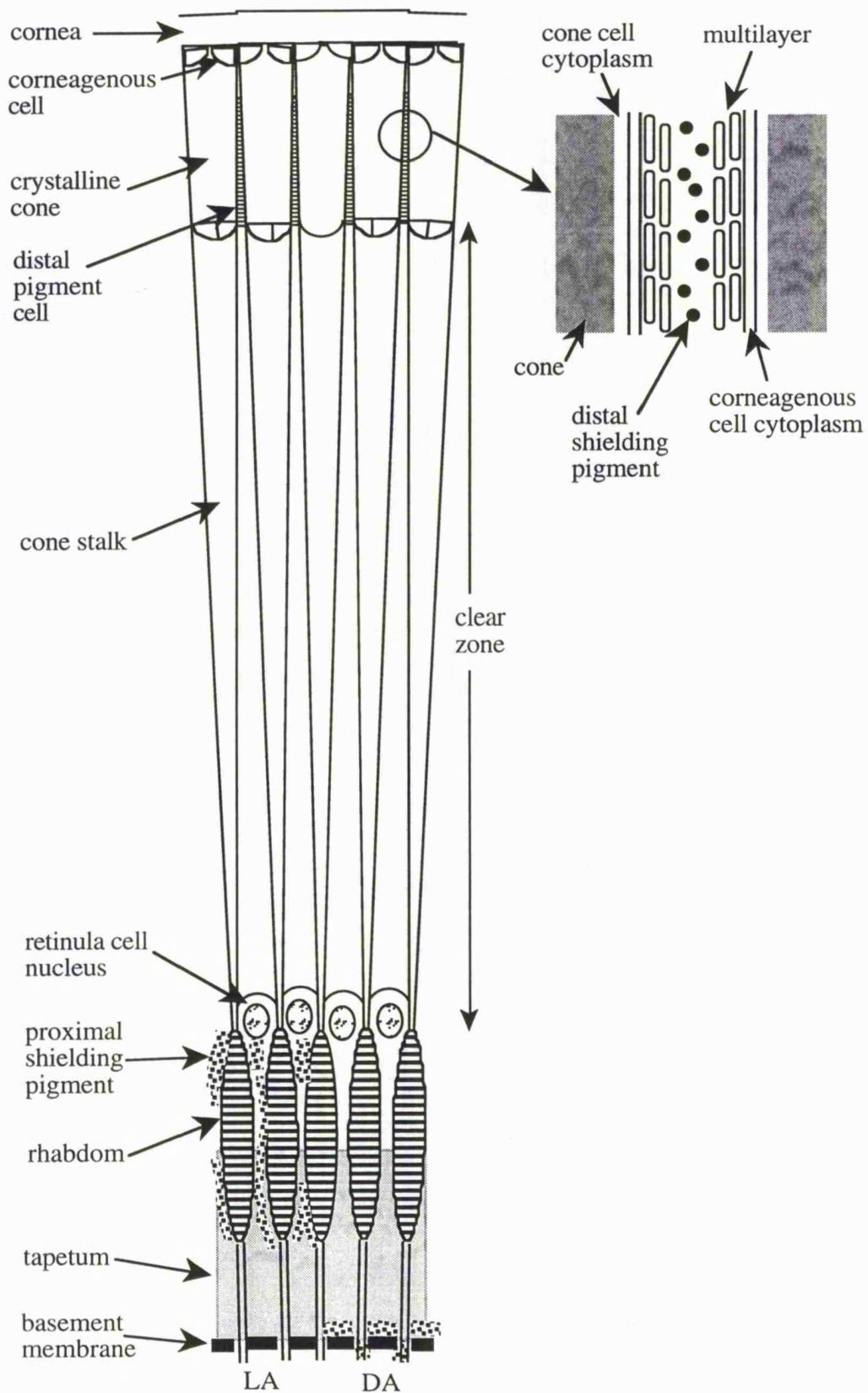
This thesis is part of the current effort to assess the fate of discards on the sea bed and concentrates on the impact of light-induced eye damage on the behaviour of *N. norvegicus*.

1.3 Vision in *N. norvegicus*.

1.3.1 Adaptations for low-light levels.

Because light from the sea surface decreases in intensity and becomes monochromatic with increasing depth, marine animals must have visual imaging systems designed to utilise the limited number of photons available and photoreceptors with spectral sensitivities appropriate to the wavelengths of light present at the depth at which the animals live (Lythgoe, 1987). The imaging system used by *N. norvegicus* is a reflecting superposition compound eye (Arechiga and Atkinson, 1975; Shelton *et al.*, 1985) (see Fig. 1.1 for a diagram of *N. norvegicus* eye structure). This

Figure 1.1. Structure of the reflecting superposition compound eye of *N. norvegicus*, showing several ommatidia in the light-adapted (LA) and dark-adapted (DA) states. The proximal pigment migrates from the basement membrane (DA) to a position around the rhabdoms (LA). The inset shows an enlarged distal pigment cell containing screening pigment and a multilayer of reflecting platelets (after Gaten, 1988).



type of eye is adapted to operate at low light levels and is typical of crustacea living in deeper water, such as lobsters and shrimps (Waterman, 1961; Nilsson, 1990). *N. norvegicus* has one of the largest pairs of eyes for a crustacean of its size. The eye has a distinctive kidney shape with lateral flattening (Gaten, 1992). The presence of these large well-developed eyes suggests that vision is likely to be important in behaviour and survival of *N. norvegicus*.

The reflecting superposition optics of a *N. norvegicus* eye increases its sensitivity at low light conditions, with up to 3,000 ommatidia co-operating to focus light onto a single blur circle on the reticular cell layer (Gaten, 1988). *N. norvegicus* has large fused rhabdoms made up of eight reticular cells (Gaten, 1988; 1992) and rhodopsin molecules are arranged within the rhabdom membranes of crustaceans so that they absorb the maximum amount of light (Shaw and Stowe, 1983). The tapetum is a layer of light-reflecting pigment cells lying behind the retinula cell layer and distal to the basement membrane. It improves sensitivity because it allows light rays which have passed through the photoreceptive cell layer without being absorbed to be reflected back into the eye.

1.3.2. Colour Vision.

N. norvegicus lives in an essentially monochromatic environment and it is unlikely to have colour vision. It possesses a tiered rhabdom of which the major part is derived from the microvilli of reticular cells R1 - R7. These are known to contain green sensitive visual pigment (maximum wavelength absorbed = 498 nm) (Loew, 1975). There is also a small distal rhabdom derived from reticular cell R8 and this is probably violet sensitive (Gaten, 1992). The role of the distal rhabdom remains obscure.

1.3.3. Spatial Resolution.

The spatial resolution of the eye in *N. norvegicus* does not appear to be very good. The retinula cell acceptance angle provides a useful estimate of an eye's resolving power (Snyder, 1979). In *N. norvegicus*, the acceptance angles for cells in the centre of the eye depend on the state of light or dark adaptation. In light-adapted eyes a value of 8.85° was obtained. This increased to 11.3° in the dark-adapted state (Shelton and Gaten, 1996). Compared with terrestrial arthropods that have superposition eyes these values are very large. For example, the crepuscular dung beetle *Onitis alexis* has superposition eyes with an acceptance angle of 4.0° (dark and light adapted) (Warrent and McIntyre, 1990).

The poor resolution of the eye in *N. norvegicus* may be partially due to spherical aberration. This leads to blurring of the image on the retina. From ray tracing work there is some evidence that image quality is better in the anterior and posterior parts of the eye (Gaten, 1992). Since the acceptance angle data was obtained from the centre of the eye it is possible that the resolving power of the anterior and posterior parts of the eye is better than that for the centre.

1.3.4. Shielding pigment migration.

Natural fluctuations in light levels require mechanisms for adjusting the sensitivity of the eye. Eyes may have to operate over considerable ranges. At the upper level, light intensity may be high enough to physically damage the eye, while at the lowest level the number of photons may be inadequate for image formation (Lythgoe, 1979). There are particularly large changes in light levels at dawn and dusk. *N. norvegicus* copes with such changes in light conditions by adjusting the sensitivity of its eye. Movements of shielding

pigment within the eye have a major role in this process (Shelton *et al.*, 1985). Pigment migration is controlled by an endogenous circadian rhythm entrained to a diurnal cycle by day-night light levels (Shelton *et al.*, 1985; Gaten *et al.* 1990). There are four accessory pigments: distal shielding pigment, distal reflecting pigment, proximal shielding pigment and proximal reflecting pigment. The proximal shielding pigment is the only one which migrates (Shelton *et al.*, 1985). In the light-adapted state, proximal shielding pigment is situated both distal to and around the rhabdoms. This reduces light reaching the rhabdoms and light passing between them. In the dark-adapted state the proximal pigment migrates to a position beneath the tapetal layer, allowing maximal tapetal reflection to occur (Shelton *et al.*, 1985).

The adaptational states of the eyes at particular times of day depend on the depths at which the *N. norvegicus* occur (Gaten *et al.*, 1990). Individuals from deep water are dark-adapted during daylight hours whereas those from shallow water are light-adapted. This means that the risks of light-induced eye damage in *N. norvegicus* raised to the surface during daylight hours become greater as the depth increases.

1.3.5. Light-induced eye damage.

The superposition compound eye is designed to maximise its sensitivity to light and so increases the risk to damage by elevated light levels. It has been widely shown that exposure to daytime light intensities causes major irreversible structural damage to the retinula cells of *N. norvegicus* (Loew, 1975; Shelton *et al.*, 1985,1990). Light also causes damage to the dioptric apparatus (Gaten, 1988). Thus, image quality can be affected by damage to the rhabdoms on which the image is focused, or by damage to the

dioptric systems of neighbouring ommatidia that help to form that image. Consequently, sensitivity and resolution of the eye will be reduced even if only part of it is damaged (Gaten, 1988).

In addition to retinula cell and dioptric system damage, the tapetal cells may also be destroyed by damaging light levels (Gaten, 1988). The changes to the eye structure appear within minutes after exposure. First rhabdoms break down, then the crystalline tracts detach from them and shorten. As a result of these changes proximal shielding pigment and debris from disorganised retinula and tapetal cells enter the damaged clear-zone (Shelton *et al.*, 1985; Gaten, 1988).

Previous work has assessed the amount of damage to the retinula cell layer of the *N. norvegicus* eye, caused by different periods of exposure to dim daylight ($180 \mu\text{mol m}^{-2}\text{s}^{-1}$) (Shelton *et al.*, 1985). If an individual is dark-adapted, only 15 s at this light level causes extensive retinula cell breakdown (76.75% retinal destruction). If it is light-adapted, 40 s is required to cause major breakdown (56.23% retinal destruction). After 5 minutes the retinula cell layer of eyes in both states of adaptation is almost totally destroyed (97.16% in light-adapted eyes; 98.97% in dark-adapted eyes). Different degrees of damage have been shown in eyes of *N. norvegicus* taken from different depths, with a greater susceptibility to light-induced damage in the deeper living animals (Gaten *et al.*, 1990). Thus, the degree of irreversible light-induced damage to an eye is dependent on light level, the adaptational state of the eye and the depth from which the individual is taken.

Based on such studies it is likely that the majority of *N. norvegicus* caught during daylight would suffer from extensive light-induced eye damage if they were kept on the deck of a fishing vessel for even a short period. This means that most discards are

returned to the sea bed with defective vision. Similarly at night, damage could be caused by a fishing vessel's deck lights.

1.4. The role of the arthropod compound eye in detecting objects in the environment.

The role of the compound eye in the behaviour of *N. norvegicus* is presently unknown. However, a great deal is known about the roles of compound eyes in the behaviour of other arthropods. Here there is extensive evidence that they are used for measuring the positions, sizes and velocities of objects in the visual field of many arthropod species (Wehner, 1981; Schwind, 1989).

Compound eyes have an important role in arthropods that have an active predatory behaviour. For example, stomatopod shrimps use their eyes for measuring distances and velocities of prey items. Stomatopods wait at the entrances of their burrows and using visual information can accurately strike, grasp or pierce swimming prey with their appendages (Waterman, 1961; Wehner, 1981). Distances can be measured in a number of ways. These include motion parallax, binocular cues and image size (Wehner, 1981; Schwind, 1989). The amount of binocular overlap may determine which mechanism is used. Those with minimal binocular overlap will have to depend on motion parallax or image size (Schwind, 1989). Observations on anterior eyeshine from *N. norvegicus* showed extensive binocular overlap (Richardson, unpublished) but whether or not binocular mechanisms are used by *N. norvegicus* is not known.

These mechanisms allow arthropods to receive visual information about objects in their environment. The following

section describes aspects of behaviour where there is evidence for visually mediated behaviour in arthropods.

1.5. The role of vision in arthropod behaviour.

1.5.1. Migration.

Visual cues are important in migratory behaviour in a number of species of arthropod. For instance, insects use their vision to collect information about landmarks, such as the positions of trees, to allow them to return to specific areas (Wehner, 1981). A number of amphipods live buried in the intertidal region and there is evidence that they use the moon's position and location of visual landmarks to return to their shelters after nocturnal foraging excursions (Enright, 1978). *N. norvegicus* is known to migrate from, its burrow over distances of up to 70 m in the case of males (Chapman et al., 1975). Visual information may be important during this activity.

1.5.2. Feeding.

Predators such as dragonflies and wasps use vision to capture fast moving prey while in flight (Wehner, 1981) and visual information about movement and size of an object can be used by arthropods to recognise prey. The dragonfly is a visually guided predator and it will capture all animals equal to, or less than a specific size. Movement of prey is also important for the feeding response of the American lobster (*Homarus americanus*), which will only respond to prey, such as the crab *Carcinus maenas* and sea urchins *Strongylocentrotus droebachiensis* if the prey is moving (Hirtle and Mann, 1978). Nothing is known about the role of vision in the feeding behaviour of *N. norvegicus*.

1.5.3. Mating behaviour.

Information about a potential mate must be accurate because it is important that individuals mate with members of their own species. There is evidence from crabs that vision is used during the initial approach to a potential mate and that olfaction is used for final identification (Wehner, 1981). The shore-living fiddler crabs (*Uca spp.*) use visual signals to attract mates. Male fiddler crabs have an enlarged and conspicuous claw which is used in a waving display when a female approaches. The female then follows the male, which leads the female to its burrow while performing a dance consisting of claw-waving and bows performed by bending its walking legs. The female will then enter the male's burrow (Waterman, 1961). Similar visual courtship signals have been observed in other crab species such as other ocypodids and grapsids (Wehner, 1981). The actual visual cues used by male fiddler crabs to recognise females have been studied using models. It was found that a dark object, with 2 small 'legs' pointing downwards and smooth movement, is required for a male to respond with characteristic claw-waving courtship behaviour (Wehner, 1981). Models were also used by Barlow *et al.* (1988) to investigate visual cues used by horseshoe crabs (*Limulus polyphemus*) to identify a suitable female. It was found that males were able to distinguish a cement cast of a female from a hemisphere or a cube of equal exposed surface area. The males also preferred casts from female crabs which had the same level of contrast to the background sand as that of a normal female crab. It was concluded that male horseshoe crabs use visual cues to recognise females. In the case of *N. norvegicus* detailed knowledge of mating behaviour is lacking and the importance of vision in courtship is unknown.

1.5.4. Fighting behaviour.

Many animals use visual signals before and during contests to indicate their body size and display their 'weapons' (Archer, 1988). This allows an individual to assess its chances of winning a contest and it enables it to 'decide' whether to attack or withdraw, before an interaction or during a contest (Krebs and Davies, 1987; Archer, 1988). Vision has been demonstrated to be important in crayfish fighting behaviour. Contests are resolved less efficiently when no visual information is available and different sensory cues are used (Bruski and Dunham, 1987). For several decapod crustacean species it has been shown that visual assessment of size differences between opponents greatly influences the outcome of a contest, with the larger opponent most likely to win (Archer, 1988; Smith *et al.*, 1994). Species found to use visual assessment of the relative size of an opponent range from the velvet swimming crab *Liocarcinus puber* (= *Necora puber*) (Smith *et al.*, 1994), to the prawn *Macrobrachium rosenbergii* (Barki *et al.*, 1991) and the mantis shrimp *Gonodactylus viridis* (Caldwell and Dingle, 1979).

Visual assessment is also important in threat displays performed by the many crustacean species that present their 'weapons' in order to increase their apparent size (Dingle, 1983). The swimming crab *Liocarcinus depurator* possesses a light coloured spot surrounded by a blue band on its swimming legs. The spots are held above the carapace during contests and used as an aggressive signal (Glass and Huntingford, 1980). Models made of wax or wood have been used to examine which visual characteristics elicit the greatest aggressive response in crustacea (Hazlett, 1972; Jachowski, 1974; Rubenstein and Hazlett, 1974). Models of the blue crab, *Callinectes sapidus*, demonstrated that opponents reacted in a similar way to models

and real opponents, suggesting that visual stimuli are very important in the resolution of contests between blue crabs (Jachowski, 1974). It was also shown that blue and white colouring on the blue crab's chelipeds serve as major aggressive signals during a contest (Jachowski, 1974). The most effective aggressive signals used in contests involving the hermit crab, *Calcinus tibicens*, are the white markings on the tips of the chelipeds when both chelipeds are raised in a meral spread (Hazlett, 1972). The use of such colour patterns has led to the use of the term 'contrast effect' to describe strongly contrasting display markings on an appendage (Scully, 1983).

Appendage position alone can also be an important visual indicator of an individual's intentions as it approaches a conspecific (Scully, 1983). Hermit crabs of the genus *Pagurus* will hold their chelipeds raised and pointing forwards if they intend to fight, but will carry them tucked under the carapace if an individual is in 'search mode' (Reese, 1962). Contests between *N. norvegicus* have been observed in the field (Chapman and Rice, 1971) and in the laboratory (Rice and Chapman, 1971; Farmer, 1974). However, fighting behaviour in *N. norvegicus* has not been described in detail and the role of visual signals in this behaviour is unknown.

1.5.5. Rhythms of behaviour.

Animals perform certain types of behaviour according to predictable cyclic patterns. These rhythms are dependent on two factors, an endogenous internal biological 'clock', and external cycles to which the 'clock' is coupled (Palmer, 1974; Arechiga and Naylor, 1976). These environmental variations, or 'entraining agents', act to correct the internal 'free-running' rhythms so that

an individual can adapt to its physical habitat cycles. One of the major entraining agents for the biological rhythms in crustacea is light. There is evidence that the day-night cycle is the entraining agent of locomotor rhythms; of the crayfish *Procambarus clarkii* and *Orconectes pellucidus* (Page and Larimer, 1972); the spiny lobsters *Panulirus argus* (Kanciruk and Herrnkind, 1973; Casterlin and Reynolds, 1979) and *Jasus edwardsii* (MacDairmid *et al.*, 1991); and the prawns *Penaeus semisulcatus* and *P. mondon* (Moller and Jones, 1975). Light is also able to entrain the vertical daily migrations of many zooplankton species (Forward and Hettler, 1992; De Coursey, 1983).

Activity rhythms of the spiny lobster, *Panulirus argus*, have been measured in laboratory conditions over a period of seven months (Kanciruk and Herrnkind, 1973). Lobsters were kept in a large tank, illuminated by a large window allowing them to experience natural ambient light cycles. The results showed a low level of activity during the day and a nocturnal peak of activity during which most individuals were emerged and walking around the tank. This 24 h locomotor activity rhythm was also seen to adjust to seasonal changes in periods of daylight (Kanciruk and Herrnkind, 1973). There is considerable evidence that *N. norvegicus* possesses a light-entrained endogenous activity rhythm (Arechiga and Atkinson, 1975; Chapman *et al.*, 1975; Atkinson and Naylor, 1976; Naylor and Atkinson, 1976; Hammond and Naylor, 1977a; Moller and Naylor, 1980).

1.5.6. Predator detection and avoidance by prey species.

Predation is a major cause of mortality within an animal population. To reduce the risk of being taken by predators, animals will adopt anti-predator behaviour, a vital part of which is the

accurate detection of predators. Some Crustacea, such as the mesopelagic amphipods and euphausiids, have double compound eyes, with the upper part designed for higher resolution of an image than the remainder of the eye (Land, 1990). It is thought that this is an adaptation to allow the eyes to accurately detect an image of a predator higher up in the water column against the downwelling light (Land, 1990). There is evidence that vision is used to detect fish predators by benthic Crustacea species, such as the American lobster *Homarus americanus* (Wahle, 1992) and juvenile signal crayfish *Pacifastacus leniusculus* (Blake and Hart, 1993). Predator response postures have been observed in *Procambarus gracilis*, *P. simulans simulans*, *P. acutus acutus* when using a predator model (Hayes, 1977). This showed that the visual stimuli from a predator are able to elicit an anti-predator response.

Many species of crustacea use burrows and shelters to protect themselves and avoid encounters with predators. Examples include the lobsters *Homarus gammarus*, *H. americanus*, *N. norvegicus* and the spiny lobsters *Panulirus argus* and *P. interruptus* (Cobb and Wang, 1982), the crayfish *Pacifastacus leniusculus* and *Orconectes propinquus*; the crabs *Uca pugilator* and *Carcinus maenas* (Schöne, 1961). Crustaceans may either modify pre-existing shelters, such as removing pebbles from a rock crevice, or will construct new burrows in mud or sand (Cobb and Wang, 1982; Schöne, 1961; Cobb, 1971). Previous studies have demonstrated that visual information is used by the American lobster *Homarus americanus* to select a suitable shelter (Cobb, 1971; Johns and Mann, 1987). The lobsters choose the shelter using negative phototaxis and prefer shelters giving them the darkest

conditions (Cobb, 1971; Johns and Mann, 1987). The role of vision in shelter recognition by *N. norvegicus* has not yet been investigated.

The preceding review shows that vision is very important to many arthropod species. Although little is known about visually mediated behaviour in *N. norvegicus*, it seems likely that it also uses vision in its behaviour. This study investigates the behaviour of sighted and blinded *N. norvegicus* in an attempt to assess the impact of light-induced blindness on subsequent behaviour. The information obtained from such studies can be used to assess the likely effects of light-induced eye damage on the behaviour of discards once they have been returned to the sea bed.

Chapter 2. General materials and methods.

2.1 Capture of *N. norvegicus*.

Nephrops norvegicus were captured from Loch Torridon (N.W. Scotland), using baited creels at depths of approximately 30 m. When light levels at the sea surface fell below $0.1 \mu\text{mol m}^{-2}\text{s}^{-1}$ the creels were raised. At this light level no damage should occur to their eyes (Shelton *et al.*, 1985). To prevent subsequent eye damage after capture and during experiments, all handling and examination of *N. norvegicus* was carried out in controlled light conditions using head mounted torches fitted with red gelatin cut off filters (transmission wavelengths $> 600 \text{ nm}$). It has been shown by Leow (1976) that the eyes of *N. norvegicus* are insensitive to and undamaged by red light. The captured *N. norvegicus* were sorted and those between 30 - 40 mm in carapace length (taken as the distance from the rear of the eye socket to the posterior edge of the carapace) were kept for use in experiments. 'Berried' females (i.e. females carrying eggs) were not used in this study.

Individuals were either used on site at the SOAFD field station at Torridon, or transported to the laboratories at Aberdeen and Leicester in light-tight boxes holding sealed opaque plastic bags containing oxygen and sea water. In the summer, bags of ice were used to maintain a low ambient temperature inside the containers.

2.2. Maintenance of *N. norvegicus*.

N. norvegicus were kept under similar conditions at the SOAFD field site at Torridon and the SOAFD Marine Laboratory in Aberdeen. They were maintained in circulating sea water (12°C) in

darkened tanks covered with black opaque sheeting. At Torridon, water was pumped from the loch into a header tank which was fed by gravity into a circular holding tank (diameter = 3 m). This holding tank was housed in a windowless shed and all handling of individuals took place under the sheeting covering the tank. *N. norvegicus* were kept in covered tanks with circulating sea water at the Marine Laboratory Aberdeen and similar precautions to prevent eye damage were taken.

At Leicester University, *N. norvegicus* were kept in light-tight holding tanks (base area = 0.6 m x 1.1 m) containing aerated sea water maintained at a depth of 0.25 m. The sea water was recirculated by a pump and filter system. The tanks were situated in a marine tank room fitted with a constant temperature refrigeration system set at 12°C. The lights in the tank room could be controlled to provide darkness for handling. A 12 h light/ 12 h dark cycle (dawn 07.00h, dusk 19.00h) was provided in these holding tanks using a modified photographic safelight, and a 25 W tungsten bulb, fitted into the lids of the tanks. A gelatin wide pass-band green filter (transmission maximum = 540 nm; bandwidth at 50% transmission = 53.64 nm) was fitted over this lamp and provided light conditions (light intensity = $0.24 \mu\text{mol m}^{-2}\text{s}^{-1}$) ($1 \mu\text{mol} = 6.023 \times 10^{17}$ photons $\text{m}^{-2}\text{s}^{-1}$) similar to those found in the field at 30 m depth in Loch Torridon at midday on a sunny September day (Shelton *et al.*, 1985).

In all three situations *N. norvegicus* in the holding tanks were provided with artificial shelters, made from 0.3 m lengths of 0.07 m diameter plastic pipe. All holding tanks were checked regularly so that any dead individuals or discarded limbs could be removed. To prevent individuals causing injury to each other in the holding tanks, their chelae were bound with rubber bands.

At Torridon, *N. norvegicus* were not fed, but in Aberdeen and Leicester individuals were fed on fish or squid every 4-5 days. Excess food remaining in the tank was removed after 3-4 hours.

2.3. Procedure for blinding *N. norvegicus*.

A standard system was used to cause 100% irreversible light-induced retinula cell breakdown to both eyes (hereafter referred to as blinding) of selected individuals.

Two quartz halogen car spot lamps were attached to opposite sides of a straight-sided glass tank (base area: 0.18 m x 0.24 m, height: 0.18 m) and a reflective material (aluminium foil) was wrapped around the sides and base of the tank (Fig. 2.1). A light-tight lid was lined with the same reflective material. The light intensity measured inside the tank when both spot lamps were switched on was $5,500 \mu\text{mol m}^{-2} \text{s}^{-1}$.

To prevent excessive shading no more than four individuals were blinded simultaneously in the apparatus. A previous study showed that *N. norvegicus* cannot completely dark-adapt out of phase with their endogenous rhythm (Shelton *et al.*, 1986). To ensure blinding occurred, the *N. norvegicus* had to be fully dark-adapted, therefore blinding was carried out at 21.00 h.

The tank was filled with fresh sea water and individuals were exposed to the light for a period of 10 minutes. The experiments in this thesis were designed to compare the behaviour of sighted and blinded individuals making it necessary to equalise the amount of stress during the handling and blinding procedure for sighted and blinded groups of individuals. The sighted 'control' individuals were treated similarly to the 'blinded' individuals, with the exception that the spot lamps were not switched on.

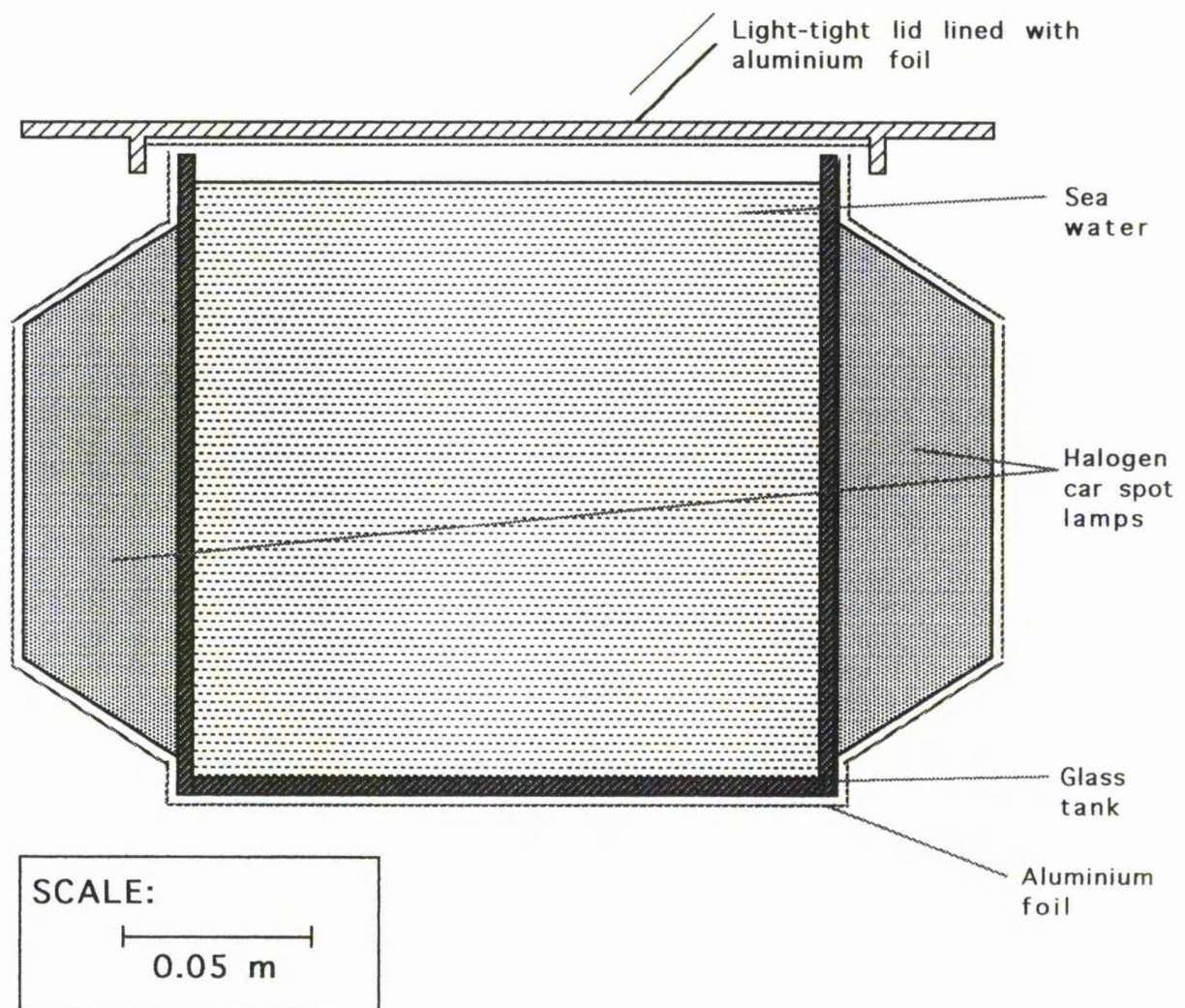


Figure 2.1. Apparatus used to blind *Nephrops norvegicus*.

After the blinding procedure, or 'handling control' procedure, individuals were returned to the holding tank for at least three days prior to being used in experiments.

If an experiment was designed to simultaneously record the behaviour of sighted and blinded individuals, the blinding and handling control procedures were both performed at the beginning of the experiment. Other experiments recorded the behaviour of individuals when sighted and then after these same individuals had been blinded. In these cases a handling control procedure using the sighted individuals was also performed at the start of the experiment.

2.4. Assessment of light-induced eye damage.

In order to check the degree of eye damage in each experiment, 5 sighted and 5 blinded individuals were randomly selected and their eyes examined histologically. Some experiments studied the behaviour of the same individual before and after it was blinded. In these cases, the eyes from sighted individuals were taken from individuals in the same population from which the experimental individuals had been selected. These eyes were examined at the end of the experiment with those of the blinded individuals.

The eyes were removed when in the dark-adapted state. Eyestalks were split to facilitate rapid penetration of the fixative and then all cuticle was removed. Isolated eyes were then washed with distilled water, dehydrated in an ethanol series, cleared in histoclear and embedded in paraffin wax. Sections (15 μm thick) were cut using a microtome (LKB Historange 2218) and mounted on slides. The sections were then stained with haematoxylin.

The eyes were fixed in Bakers Fomal Calcium up to 14 days after the individual had experienced light-induced damage. Damage to the eye was assessed by examining the retinula cell layer. The sample of eyes examined from sighted individuals used in the experiments in this thesis showed no evidence of damage to the rhabdoms (see Fig. 2.2 for an example of a section from an undamaged eye). However, the sample of eyes from the blinded individuals used in these experiments showed that no intact rhabdoms remained (see Fig. 2.3 for an example of a section from a totally damaged eye).

Preliminary behavioural observations demonstrated that after a *N. norvegicus* had been blinded it no longer responded to just the visual stimulus of an object. 10 sighted and 10 blinded *N. norvegicus* were tested using a 5 cm diameter black perspex disc fixed to a wire rod. A *N. norvegicus* was placed in a tank of sea water and the disc passed 1 cm above the water surface, or through the water just below the surface. In both cases the sighted *N. norvegicus* responded by moving backwards with their chelae raised, or with a rapid tail-flip. However, the blinded *N. norvegicus* only responded to the object when it was passed through the water, no response was evoked by the visual stimulus alone. When the blinded individuals did respond, they performed similar behaviour to the sighted ones.

2.5. Recording behaviour in a laboratory observation tank.

A tank (base area = 1.35 m x 0.8 m, height = 0.45 m), with glass windows in two sides, was used in order to study various aspects of *N. norvegicus* behaviour. The base of the tank was covered with a layer of fine gravel, approximately 10 mm thick. It

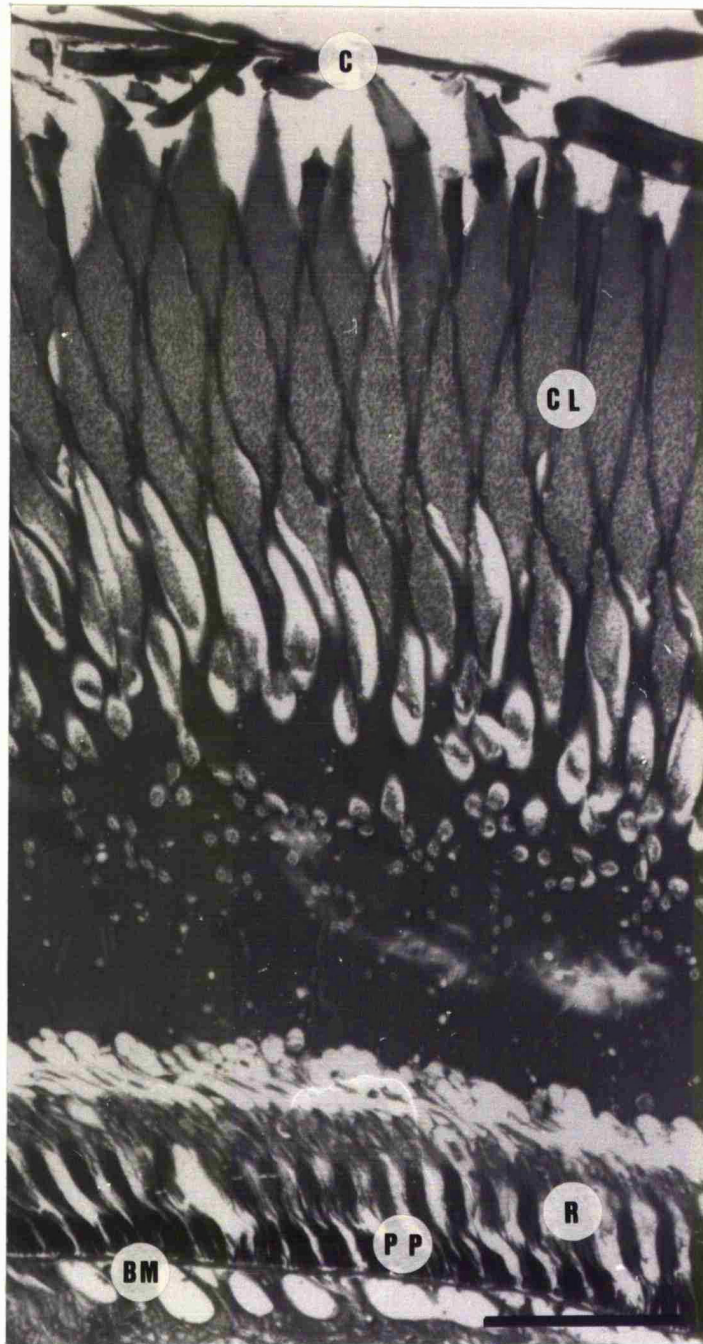


Figure 2.2. Haematoxylin-stained section of a typical undamaged eye from a sighted *N. norvegicus*. The rhabdoms are present between the cone cell layer and the basement membrane. This section shows proximal pigment in a dark-adapted state. Abbreviations: C - Cornea, CL - Cone Cell Layer, PP - Proximal Pigment, R - Rhabdoms, BM - Basement Membrane (Scale bar: 200 μ m).

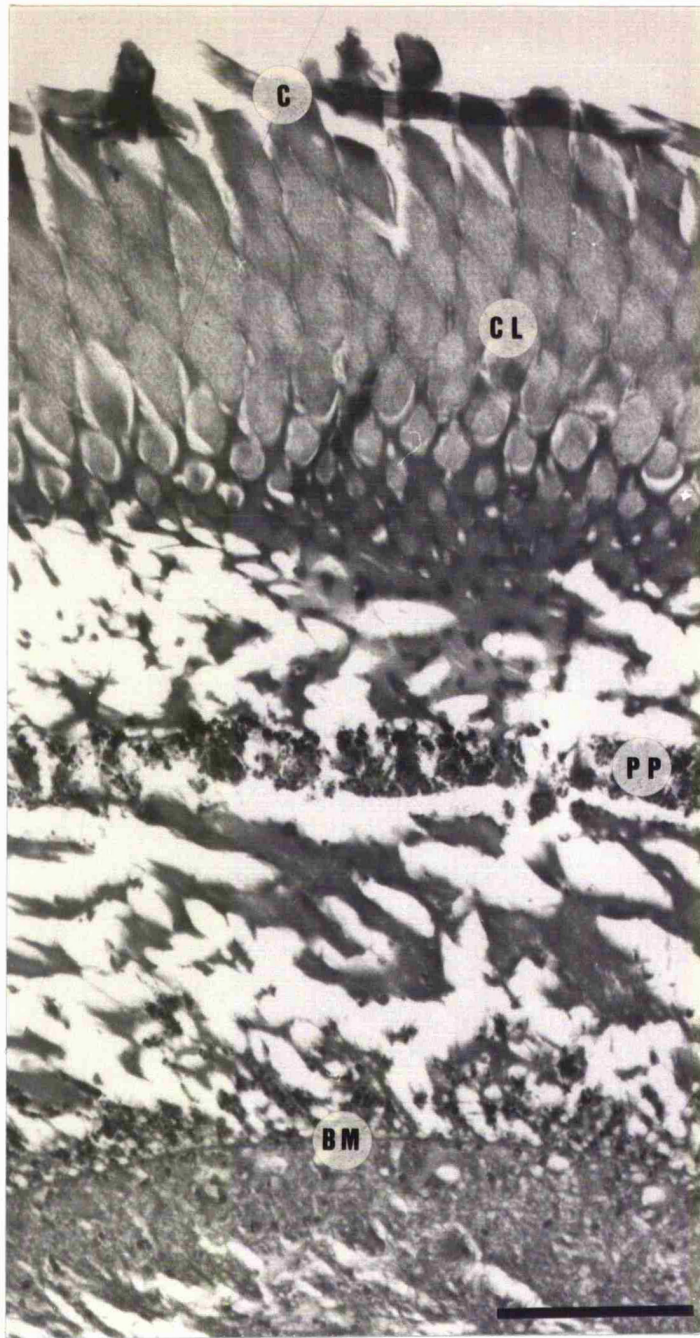


Figure 2.3. Haematoxylin-stained section of a typical damaged eye from a blinded *N. norvegicus*. The rhabdoms are completely absent and the proximal pigment is dispersed. Abbreviations: C - Cornea, CL - Cone Cell Layer, PP - Proximal Pigment, BM - Basement Membrane (Scale bar: 200 μ m).

was connected to the sea water circulation system situated in the marine tank room of the University of Leicester. The sea water in the tank was maintained at a depth of 0.4 m and a temperature of 12°C. The tank was isolated in a light-tight room so that lighting conditions could be controlled. A modified photographic safelight with a green wide pass-band gelatin filter (transmission maximum = 540 nm; bandwidth at 50% transmission = 53.64 nm) provided ambient light conditions in the tank at an intensity of 0.016 $\mu\text{mol m}^{-2} \text{s}^{-1}$. This light was arranged to correspond to that encountered by *N. norvegicus* on the sea bed during periods of emergence activity (Naylor and Atkinson, 1976; Chapman, 1980). The light level was controlled, to provide a 12 h light/12 h dark cycle (dawn 07.00h, dusk 19.00h), identical to that in the holding tanks in which they were previously housed.

Two additional photographic safelights were suspended above the observation tank and fitted with far-red cut off filters (Plexiglass PFR 700, with transmission wavelengths > 700 nm). The tank was continually illuminated with these far-red lights to which the *N. norvegicus* eye is insensitive (Loew, 1976). The far-red illumination was necessary for the operation of a far-red sensitive video camera (Hitachi HV620 fitted with an extended red tube allowing wavelengths up to 1800 nm to be detected by the camera). This enabled the behaviour of the *N. norvegicus* to be continuously recorded, even during the dark phase of the day/night cycle. Individuals could be viewed either laterally through a glass window in the side of the tank when the camera was mounted on a tripod, or from above when the camera was fixed to a 'Dexion'™ frame above the tank.

A time-lapse video recorder (Panasonic NVJ40) was used to allow continual remote recording for periods up to 24 h. The

running speed of the video tape could be set at 3 h mode (23.39 mm/s), 12 h mode (4.68 mm/s) or 24 h mode (2.60 mm/s). A monitor (Panasonic WV-5340) and the video cassette recorder were situated behind an opaque partition away from the tank.

2.6. Light measurement.

A Licor quantum light sensor (LI-185B) was used to measure irradiance in the 400-700 nm wavelength band. The light meter could be connected to sensors for use in air (LI-190SB) or water (LI-192SB).

2.7. Preparation of *N. norvegicus* for experiments.

Care was taken to ensure that only healthy, undamaged *N. norvegicus* were used in the experiments. Each individual was also checked to ensure that it was not approaching a moult (judged by the absence of paired white gastroliths under each side of the anterior carapace). The rubber bands binding an individual's chelae were removed before the start of an experiment.

Male and female *N. norvegicus* were used in field experiments carried out in Loch Torridon to measure behavioural rhythms (Chapter 3) but all other experiments were carried out using male *N. norvegicus* only. This dependence on males avoided the complications associated with the fact that females possess a seasonal rhythm of activity, with eight months spent inside their burrows while their eggs develop (Chapman, 1980). This results in a reduced level of out of burrow activity and could complicate interpretation of experiments using females at different times of the year.

2.8. The *N. norvegicus* burrow system and the artificial burrow systems used in the experiments.

In their natural habitat *N. norvegicus* construct a burrow system in the sea bed, which typically consists of two 'shafts' (predominantly vertical burrow components) interconnected by a single 'tunnel' (a predominantly horizontal burrow component) (Rice and Chapman, 1971). One shaft opens onto the sea bed in a wide shallow depression, this is generally described as the entrance to the burrow system (Rice and Chapman, 1971) and *N. norvegicus* are often observed at this entrance with their antennae and claws exposed (Chapman and Rice, 1971). The other shaft opens directly onto the sea bed with the mud lying flat around the opening (Rice and Chapman, 1971). However, *N. norvegicus* burrow systems have been found with only one shaft with a shallow entrance (Chapman and Rice, 1971).

For all experiments carried out in this thesis, *N. norvegicus* were provided with lengths of plastic tube or drain pipe to use as artificial burrow systems. The terms 'shelter' or 'burrow', when used in the context of the following experiments, refer to these artificial burrow systems. The limitations of using these artificial burrow systems are considered in each experiment and outlined in the 'Discussion' sections.

Chapter 3 Effects of blinding on burrow emergence rhythm - field experiments.

3.1 Introduction.

N. norvegicus has highly rhythmical behaviour and there is considerable evidence that the rhythms are controlled by light levels in their environment (Chapman, 1980). This part of the investigation was designed to assess the effects of loss of vision on burrow emergence rhythms in a mixed population of sighted and blinded *N. norvegicus*. *N. norvegicus* spend most of their time inside the burrow, only becoming fully emerged on the sea bed for a small fraction of each 24 hour period (Chapman *et al.*, 1975; Chapman, 1980). Investigations of burrow emergence rhythms have been carried out by counting the numbers of *N. norvegicus* within trawl and creel catches, by video or photographic recordings of activity on the sea bed, and using observations by SCUBA divers. These techniques all demonstrate clear diel variations of burrow emergence with peak levels of activity at night (i.e. nocturnal behaviour) in shallow water (< approximately 30 m), during the day (i.e. diurnal behaviour) in deep water (> approximately 120 m), and with dawn and dusk emergence peaks (i.e. crepuscular behaviour) at intermediate depths (Hoglund and Dybern, 1965; Chapman and Rice, 1971; Chapman *et al.*, 1972; Farmer, 1973; Arechiga and Atkinson, 1975; Chapman *et al.*, 1975; Chapman and Howard, 1979). The results from catch data, and direct observations show that as depth increases the times of the two crepuscular peaks of emergence behaviour change so that they occur earlier in the afternoon before sunset, and later in the morning after

sunrise (Chapman and Rice, 1971; Chapman *et al.*, 1972; Chapman *et al.*, 1975; Chapman and Howard, 1979). These observations led to the suggestion that *N. norvegicus* emerges at a specific range of sea bed light intensities (Chapman and Rice, 1971; Chapman *et al.*, 1972; Chapman *et al.*, 1975; Chapman and Howard, 1979).

Laboratory studies have been used for analyses of the behaviour rhythms of *N. norvegicus* in and around burrows using actographs and controlled environmental conditions (Arechiga and Atkinson, 1975; Atkinson and Naylor, 1976; Naylor and Atkinson, 1976; Hammond and Naylor, 1977; Moller and Naylor, 1980). Such actographs utilise an artificial burrow inside a tank, controlled lighting and a mechanism to automatically record the movements of an individual within the actograph. Light:dark regimes are used to simulate the luminance levels and cycles found in the natural habitat. Such studies reveal diel activity rhythms with nocturnal peaks (Arechiga and Atkinson, 1975; Atkinson and Naylor, 1976; Naylor and Atkinson, 1976; Hammond and Naylor, 1977 a, b; Moller and Naylor, 1980). Peaks of activity are found within the burrow ('in-burrow' behaviour), in the mouth of the burrow ('mouth-of-burrow' behaviour), as well as out of the burrow ('out-of-burrow' behaviour) (Atkinson and Naylor, 1973, 1976; Arechiga and Atkinson, 1975; Naylor and Atkinson, 1976). If the times of the actograph light:dark cycles are reversed, or the cycle is changed in some other way, behaviour is adjusted within 1 - 2 days so that the activity peaks occur during the new dark period (Arechiga and Atkinson, 1975; Hammond and Naylor, 1977a).

In actograph experiments emergence peaks are always nocturnal irrespective of the depth from which the animals are obtained and the daytime light levels in the actograph (Atkinson

and Naylor, 1976). The crepuscular and diurnal peaks of burrow emergence found in the field at depths greater than about 30 m never occur in actograph studies (Moller and Naylor, 1980). The causes of this difference between the rhythms of emergence found in the field and the laboratory are not fully understood, although it has been suggested that additional factors are responsible for influencing the rhythms seen in the field; these include lunar rhythms, tidal cycles, availability of prey, state of hunger and presence of predators (Naylor and Atkinson, 1976; Chapman, 1980; Moller and Naylor, 1980).

Nevertheless, all the evidence so far suggests that light is the most important factor controlling burrow emergence rhythms in *N. norvegicus*. The aim of the investigations, in this and the following chapter, was to discover whether light-induced blindness affects the levels and rhythms of *N. norvegicus* behaviour. Observations were made both in the field and in the laboratory. The field experiments concentrated on patterns of out of burrow activity and are dealt with in this chapter. The laboratory studies are described in Chapter 4 and were used to investigate both burrow emergence and in-burrow activity.

For the field investigation the *N. norvegicus* were contained in a large cage on the sea bed and were subjected to the natural rhythms of environmental factors, such as tides and light levels. Emergence patterns of sighted and blinded *N. norvegicus*, were recorded and any rhythms were identified. Comparisons were made between the patterns of behaviour displayed by the sighted and blinded individuals to see if loss of vision affects behaviour rhythms. Attempts were made to correlate any rhythms with environmental variables.

3.2. Materials and methods.

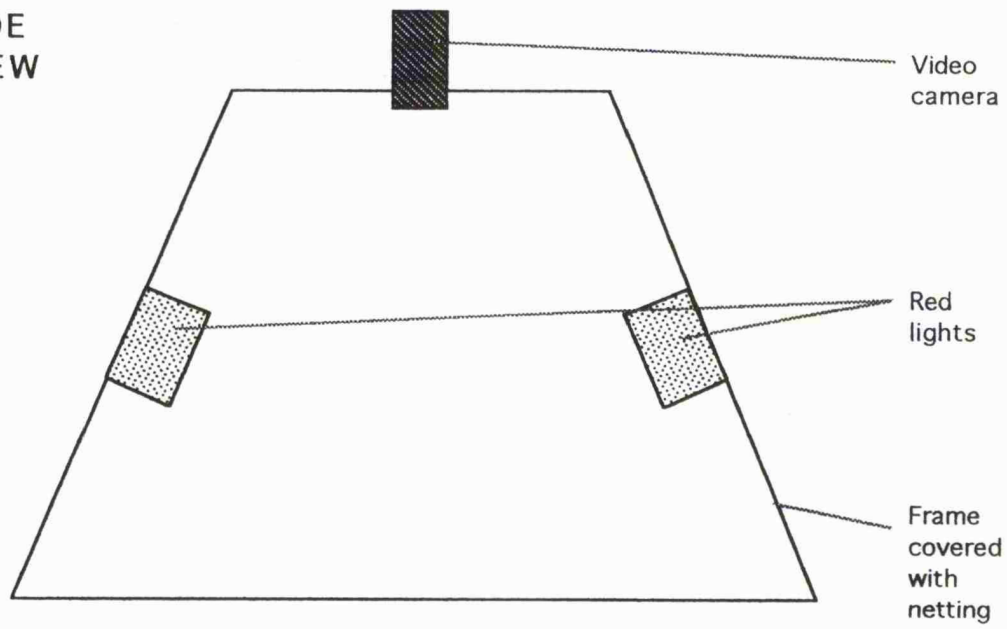
To observe the daily changes in the number of *N. norvegicus* fully emerged from their burrows under semi-natural conditions, a mixed group of sighted and blinded *N. norvegicus* was held within a net-covered cage on the sea floor. The netting was stretched over the top, sides and bottom of the cage. The cage was sited in Upper Loch Torridon, at a depth of approximately 30m (below chart datum), and close to the SOAFD field station. The first experiment using male *N. norvegicus* was carried out in September 1991; it was repeated using unberried females in September 1992. Thirteen artificial burrows were provided in the cage. They were made from lengths of plastic guttering (0.3 m long; 0.11 m diameter), each was blocked off at one end to leave a single entrance. They were inverted and attached to the netting on the base of the cage.

A television camera fitted with a silicon intensifier tube was mounted vertically above the cage base (Fig. 3.1). Four quartz-iodide lights with red perspex filters (see section 2.1 for details of filter) were fixed inside the cage. These lights remained on throughout the period that the cage was on the sea bed and provided illumination for the T.V. camera. The *N. norvegicus* eye is insensitive to this type of illumination (Loew, 1976). The images were relayed from the cage on the sea bed to a time-lapse video recorder (Panasonic NVJ40) and monitor (Sony) housed in a laboratory hut onshore.

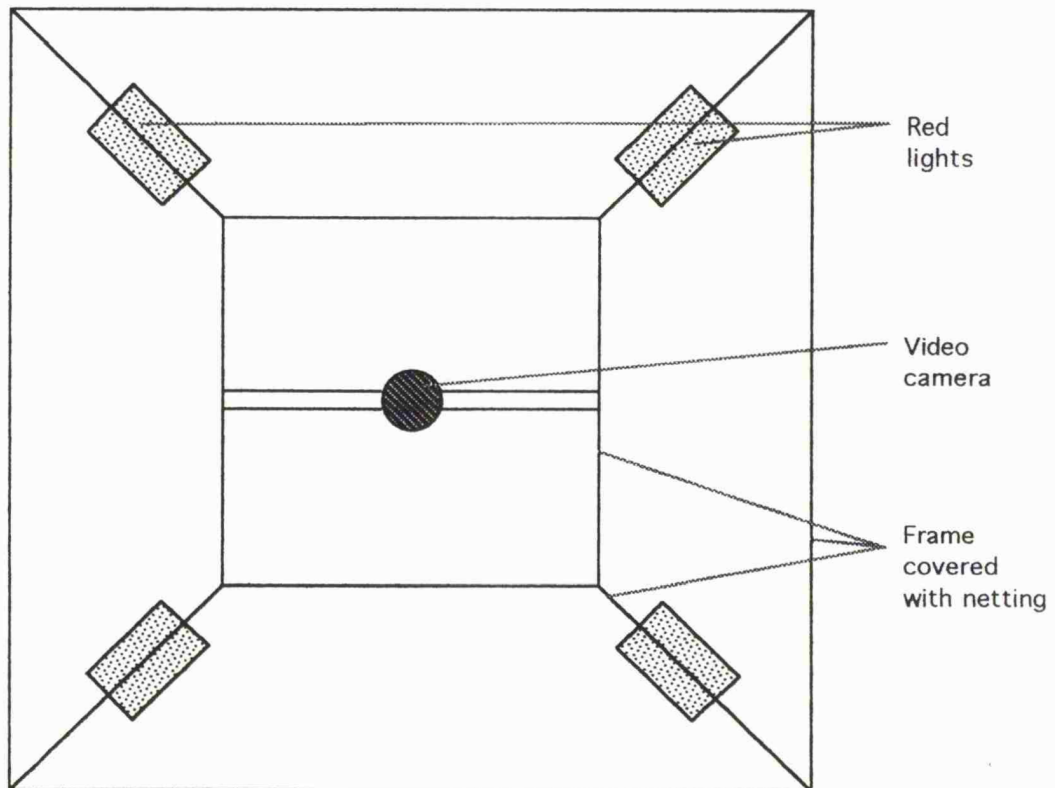
3.2.1. Preparation of *N. norvegicus*.

In September 1991, 10 male *N. norvegicus* (mean carapace length = 38.87 mm, s.d. = 0.86), and in September 1992, 8 unberried females (mean carapace length = 36.13 mm, s.d. = 0.86) were selected. These male and female *N. norvegicus* were both divided

SIDE
VIEW



TOP
VIEW



SCALE:

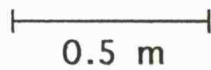


Figure 3.1 Schematic diagram of the cage (artificial shelters not shown).

into two equal groups. In each case, the first groups were blinded using the technique described in section 2.3; and individuals in the second groups were left sighted. A 'handling control' was carried out on the sighted individuals to equalise the amount of manipulation experienced by both groups. Each was labelled by fixing a white disc to the upper part of the merus of the cheliped using stainless steel wire (on left cheliped for sighted; right cheliped for blinded individuals). The discs were positioned so that they did not obstruct cheliped movements. Prior to their introduction into the cage, disc-marked *N. norvegicus* were kept in a holding tank for three days to recover from the immediate effects of the blinding process and handling.

3.2.2. Procedure.

After dusk, the *N. norvegicus* were transferred into the cage which was then lowered onto the sea bed at approximately 22.00 h and the video recording equipment and lights were switched on.

Ambient light conditions at the surface of the loch were sampled and recorded at the start of each hour within the period between sunrise and sunset using a Licor quantum light meter and air sensor. Light level readings were taken at 30 s intervals at the start of each hour, and the mean of six consecutive readings was calculated and noted. The surface light level data was used to estimate light levels on the sea bed in the area of the cage. The attenuation of light by the sea was measured by taking a series of readings at different depths within the range 1 - 34 m. The measurements were obtained from a site in the loch close to the position of the cage between 11.00 - 13.00 h, four days after the cage was placed on the sea bed. From these readings it was estimated that 0.07% of the surface light was reaching the sea bed

at the cage location (depth = 31m) (Fig. 3.2). From the hourly surface light readings and the attenuation data, a reasonable estimate of light levels at the sea bed could be calculated throughout the experimental period.

When the cage was raised at the end of the experiment, the *N. norvegicus* were removed and their carapace lengths noted. Both eyes from the *N. norvegicus* were removed and placed in formal calcium fixative for later histological examination of eye condition (see section 2.4). The cage remained on the sea bed for 8 days in the first experiment using male *N. norvegicus* (September 1991); and 5 days in the second experiment using females (September 1992). This difference in time of submergence was due to adverse weather conditions in September 1992.

3.2.3. Statistical analysis.

A 'mean square successive difference test' (Farnum, 1989) (see Appendix I for details of test) was applied to each series of observations to check that data was suitable for further time series analysis (i.e. data was autocorrelated). Autocorrelation analysis then was carried out (Chatfield, 1984) (see Appendix I for details of test). This type of analysis is commonly used to identify and describe the significant rhythms present in a series of observations.

Cross correlation analysis (Chatfield, 1989; Farnum, 1989; Bakus, 1990) (see Appendix I for details of the test) was also used to compare the characteristics of two different series. The results of the autocorrelation and cross correlation analyses were both plotted on correlograms (Chatfield, 1984; Bakus, 1990).

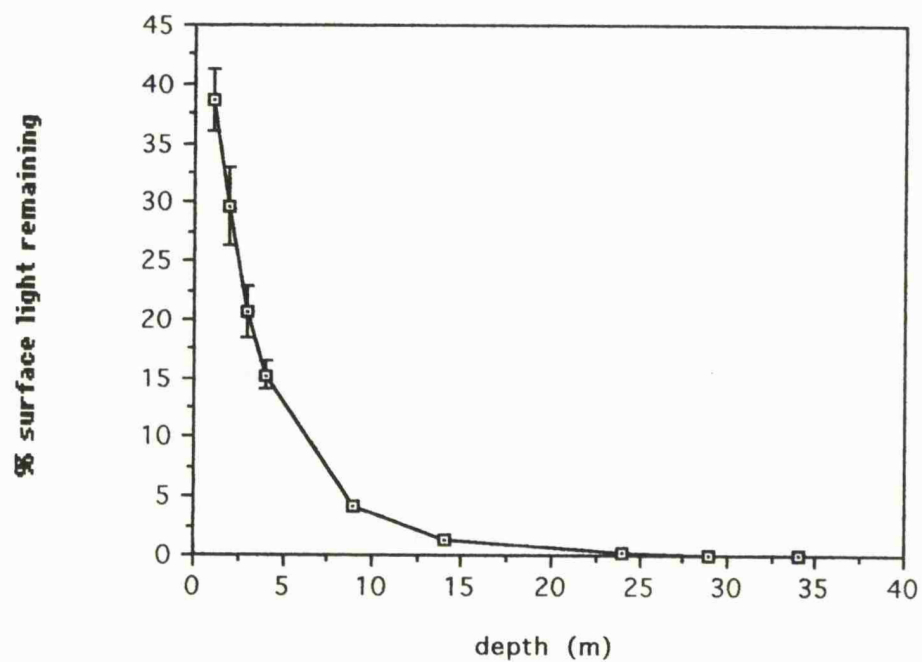


Figure 3.2. Percentage of surface light remaining at different depths at a site close to the shore and cage at Loch Torridon, September 1991 (each point is a mean of 5 readings, $\pm$ s.d.).

Once a significant rhythm was identified, a seasonal differencing technique (Chatfield, 1984; Farnum, 1989) (see Appendix I for details) was used to see how well this rhythm described the data in the original series.

To compare the amounts of emergence activity performed by a group of individuals it was necessary to form a subset of each series of observations to be used for a test (see Appendix I for details). A subset was selected by taking the seventh observation from a series, and then every subsequent seventh observation throughout the series. In the present study the activity levels were compared using a two-way ANOVA test. A probability level of 0.05 was used for all tests carried out.

3.2.4. Video analysis

Ten individuals (five sighted and five blinded) were allowed twelve hours to acclimatise to conditions in the cage and occupy shelters. Their behaviour was not analysed during this period. Video tape recordings, from the following eight days that the cage remained on the sea bed were analysed and the numbers of the blinded and sighted individuals seen fully emerged from their burrows were compared. This was done by reviewing a fifteen minute period at the beginning of each hour of the video recording. To sample the data the image was frozen at intervals of 30 seconds. The numbers of blinded and sighted individuals seen fully emerged on each frozen image were counted, and the totals seen in all images of each sample were noted. The total numbers of blinded or sighted individuals seen emerged were divided by the number in each group present in the cage to provide a mean index of

emergence activity for each hour during the experiment for the sighted and blinded groups.

For example, for the sighted group of *N. norvegicus*:

Mean emergence index for sighted individuals for 30 frozen images taken over the first 15 minutes of each hour of the experiment:

$$\frac{\sum (\text{number of sighted individuals emerged in frozen images})}{\text{number of sighted individuals in cage}}$$

3.3. Results.

3.3.1. Observations of the burrow emergence rhythms of sighted and blinded male *N. norvegicus*.

The results from the video analysis were plotted to reveal any possible rhythms in the numbers of sighted and blinded *N. norvegicus* fully emerged from their burrows (Fig. 3.3). Visual inspection of the graphs indicates the presence of a possible rhythm of behaviour in both series of observations. A preliminary test, the mean square successive difference test (Farnum, 1989), (see Appendix I for details) was applied to the series of observations and showed that both series were autocorrelated ($p < 0.05$). It was therefore possible to use autocorrelation analysis (Chatfield, 1984; Farnum, 1989) (see Appendix I for details) to identify the characteristics of any rhythms present in each series. Autocorrelation coefficients were calculated for each series and were plotted as correlograms (Chatfield, 1984) (see Appendix I for details).

3.3.1.1. Sighted *N. norvegicus*.

The autocorrelation coefficients, calculated from the series of observations of the number of sighted *N. norvegicus* seen fully emerged (Fig. 3.4), displayed a significant cycle of emergence behaviour which repeats itself approximately every 24 h. The autocorrelation coefficient peaks occur at 24 hourly intervals throughout the correlogram (Fig. 3.4 a) but as the time lags increase the size of these peaks show a gradual 'tailing-off'. A decrease in the degree of oscillation as time lags lengthen is expected in this type of analysis where the original time series is of a limited duration. At these larger lags, smaller proportions of the series are compared for the calculation of the coefficients,

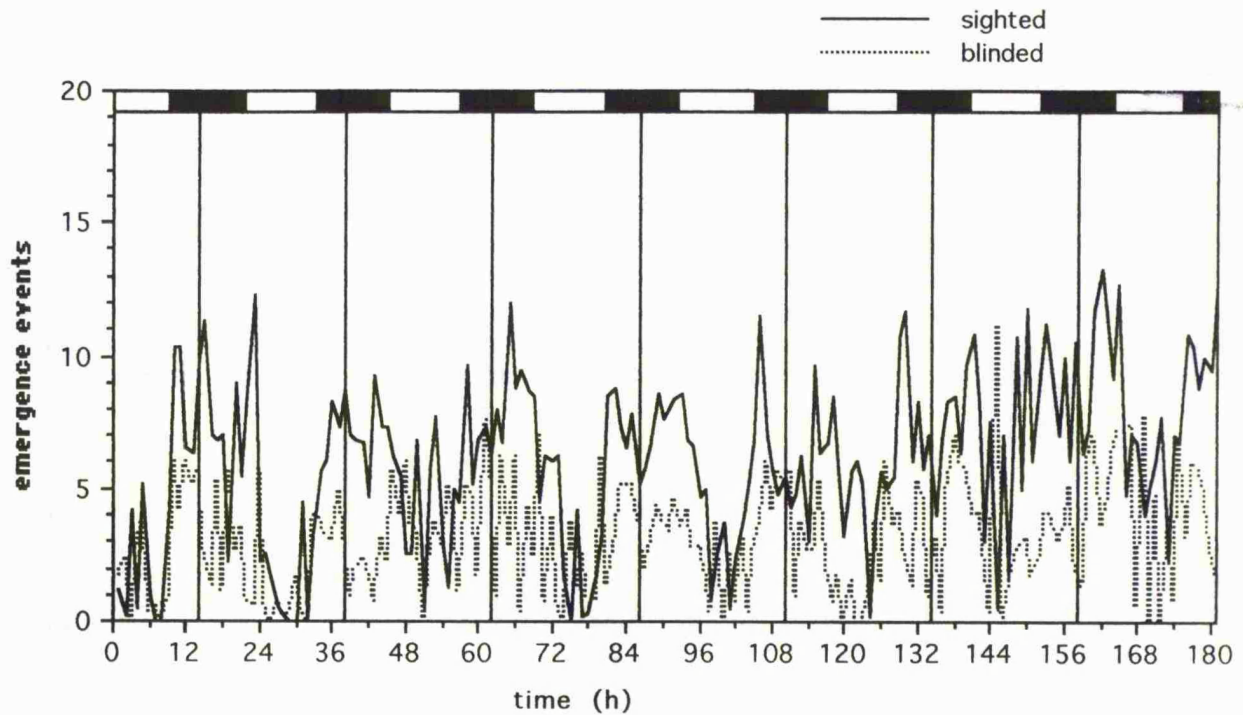


Figure 3.3. Series of the mean number of burrow emergence events performed by the male *N. norvegicus* while in the cage on the sea bed at a depth of approximately 30 m (first point denotes 10.00 h, day 1). Bar above graph indicates periods of daylight (empty bars) and night (shaded bars) and vertical line indicates midnight. A pattern of peaks of emergence behaviour is apparent, particularly for the sighted individuals.

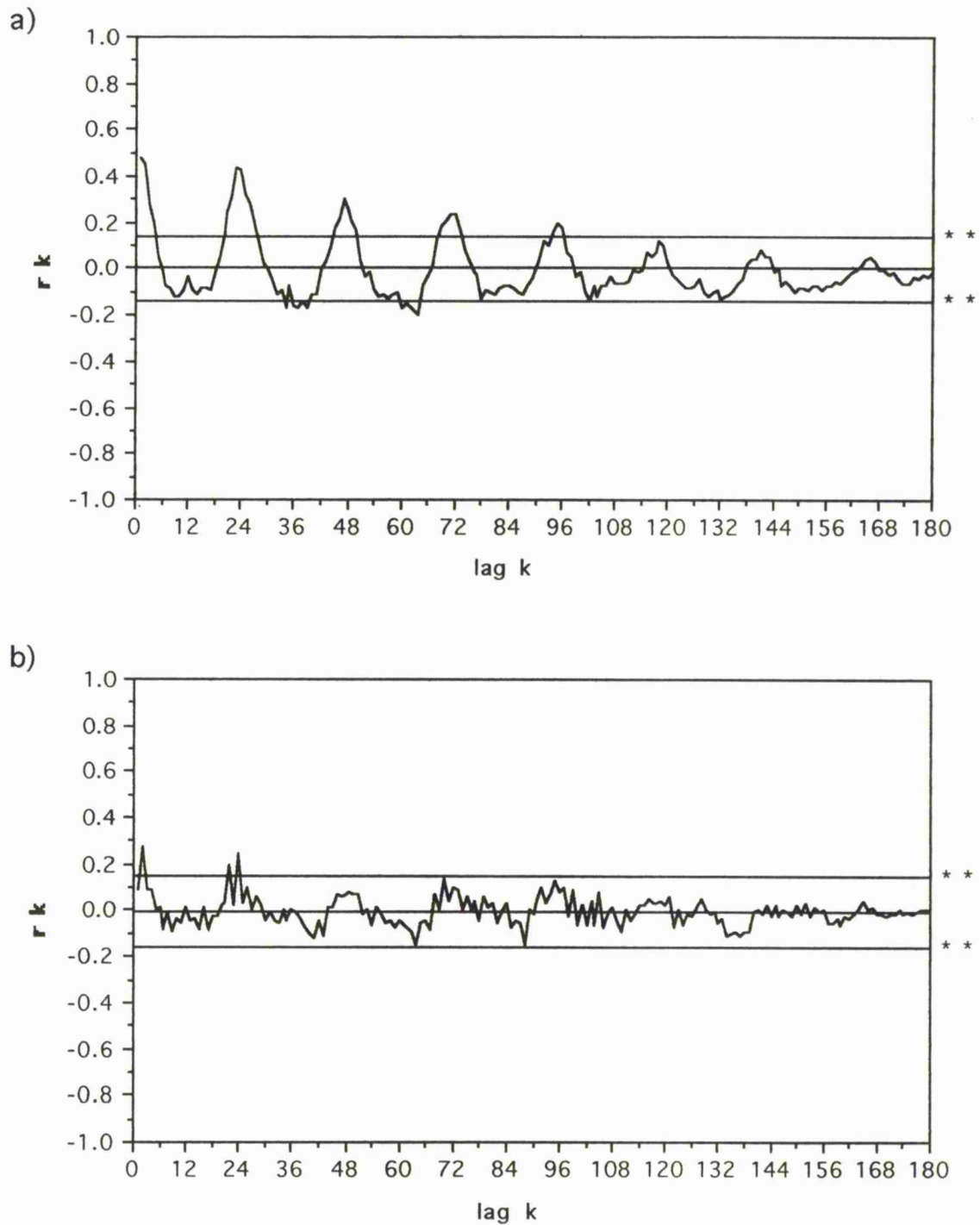


Figure 3.4. Autocorrelation coefficients (r_k) for the time lags (k) 1 to 180 h, calculated from the series of mean number of burrow emergence events by the a) sighted and b) blinded males. The significant peaks at 24 h and successive 24 h intervals indicate the presence of a 24 h rhythm in both groups. * * denotes 95% confidence limits.

meaning that random movements made by the individuals (i.e. movements that deviate from the underlying 24 h rhythm) have a greater influence in the calculation of the autocorrelation coefficients (Chatfield, 1984; Farnum, 1989). For a time series of 8 days this type of analysis is able to identify a 24 h rhythm in the observations.

Visual inspection of the series of the mean burrow emergence behaviour for the sighted *N. norvegicus* (Fig. 3.3) does not reveal any evidence of a decrease of rhythmic activity over the 8 days that they remained in the cage. A cross correlation analysis was used to compare the rhythms of burrow emergence behaviour during the first 4 days and second 4 days of this period. If a change in the emergence behaviour had occurred there would be a poor correlation between the two halves of the series. The result of this cross correlation analysis (Chatfield, 1984; Farnum, 1989) (see Appendix I for details) showed a strong correlation between the two halves of the series ($p < 0.05$), indicating no decrease in the amplitude of the rhythm. This would further suggest that the trend seen in the autocorrelation coefficients of the series is caused by the technique of calculation at the longer time lags. Any cycle detected in the initial part of the correlogram of autocorrelation coefficients can be said to persist throughout the 8 days that the behaviour was recorded.

To determine how well the 24 h rhythm explains the emergence behaviour observed in the sighted *N. norvegicus*, this 24h cycle was modelled and removed from the original series of observations using a seasonal differencing technique (Chatfield, 1984; Farnum, 1989) (see Appendix I for details). The resulting residuals show a great deal of oscillation but reveal no further significant rhythms (Fig. 3.5 a).

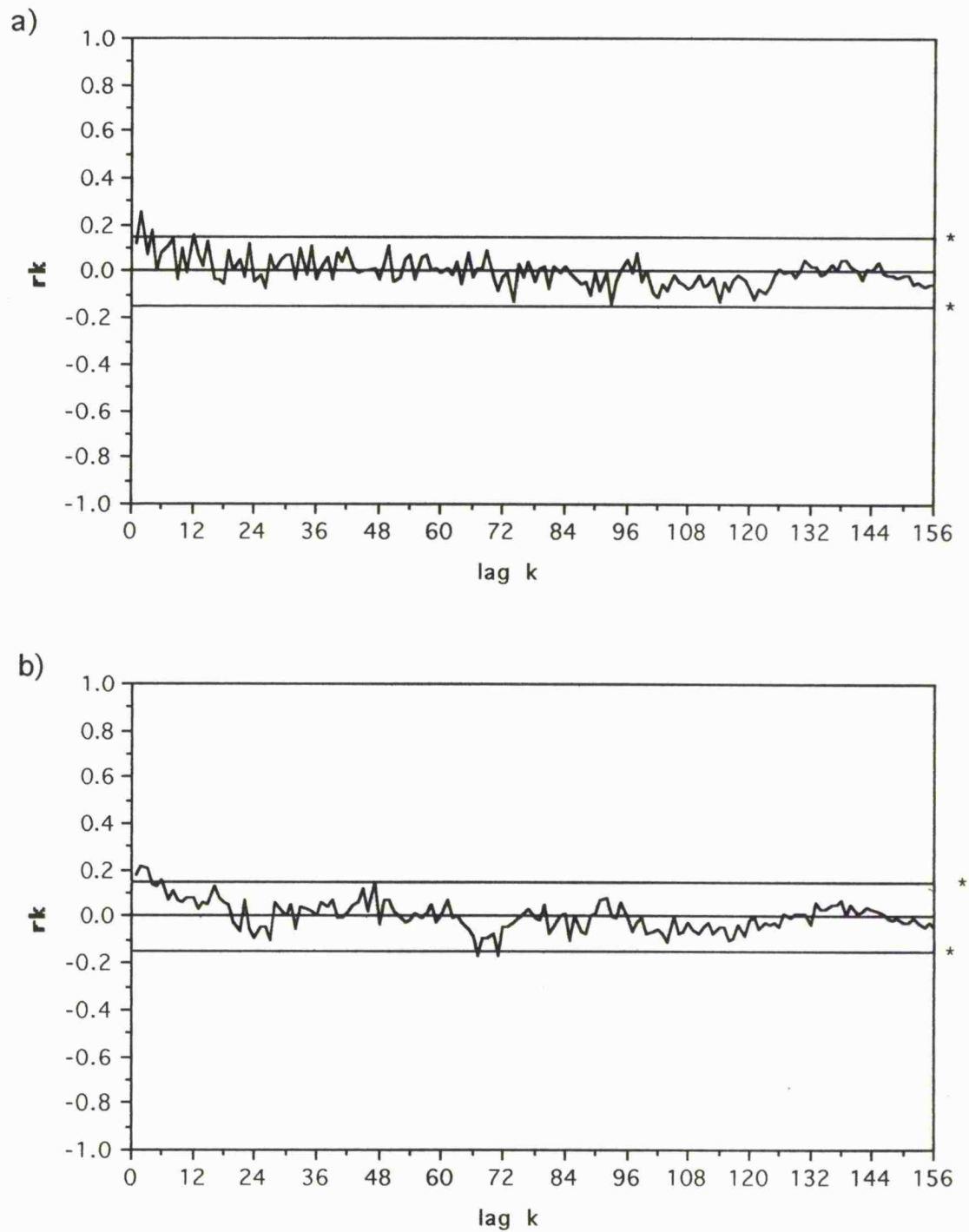


Figure 3.5. Autocorrelation coefficients (r_k) for time lags (k) 1 to 156 h, calculated from the residual series of mean number of burrow emergence events of males when a) sighted and b) blinded. No significant rhythms remain in each series.

* * denotes 95% confidence limits.

3.3.1.2. *Blinded N. norvegicus*.

The correlogram of the autocorrelation coefficients calculated from the time series for blinded *N. norvegicus* (Fig. 3.4 b), shows that at a time lag of 24 h the autocorrelation coefficient is significant ($p < 0.05$). When the series is lagged by greater hourly intervals there seems to be a pattern of peaks indicating a rhythm with a period of 24 h throughout the series, but these later peaks do not exceed the 95% confidence limits and are therefore not significant ($p > 0.05$). This suggests there is 24 h rhythm in the burrow emergence behaviour of blinded *N. norvegicus* that it is weaker than in sighted ones. Again, there seems to be a gradual decrease in the size of oscillations as the length of time lags increases, caused by the calculation of autocorrelation coefficients from a time series of limited duration. The original time series of emergence activity seems to show that this cyclical pattern of behaviour persists throughout the 8 days of the experiment (Fig. 3.3). The two halves of the series were also compared using cross correlation analysis which showed that there was no difference ($p > 0.05$) between patterns of behaviour in the initial four days and in the subsequent four days.

As before, a 24 h cycle was modelled and removed from the original emergence behaviour leaving a series of residuals. There were few fluctuations in this residuals series and no other rhythms were identified (using autocorrelation analysis and a significance level of 0.05) (Fig. 3.5 b).

3.3.1.3. *Comparison of the emergence activity rhythms of the sighted and blinded male N. norvegicus*.

A cross correlation test was carried out to measure the degree of synchronisation between the two series of observations

for sighted and blinded *N. norvegicus*. The results showed a significant direct correlation (i.e. when there was no time lag between the two series, $k = 0$) ($p < 0.05$) between the rhythms of emergence behaviour performed by the sighted and blinded individuals (Fig. 3.6). This suggests that the blinded individuals were carrying out burrow emergence behaviour with similar rhythmic characteristics to those observed in the sighted individuals.

There was also no significant difference between the numbers of sighted individuals seen fully emerged each hour on the cage base (mean events/h = 6.13, s.d. = 3.2) and the numbers of blinded individuals seen fully emerged (mean events/h = 3.19, s.d. = 2.16) (two-way ANOVA, two-tailed: $F = 1.68$, $p > 0.05$) (Table. 3.1, p.60).

3.3.1.4. The relationships between behavioural rhythms and environmental factors.

Having identified a 24 h cycle of burrow emergence in both sighted and blinded individuals, fluctuations in light levels and tidal cycles were examined in order to determine possible causal relationships.

The light levels in the cage during the experimental period were estimated from the surface light readings (Fig. 3.7). Light intensity follows a 24 h cycle and a cross correlation was used to see whether this rhythm and that of the *N. norvegicus* burrow emergence behaviour were simultaneous. The cross correlation function for sighted individuals and for light levels over the same period (Fig. 3.8 a) showed a clear negative relationship ($p < 0.05$) when the two series were directly compared (i.e. time lag, $k = 0$). This shows an inverse relationship between light level and burrow

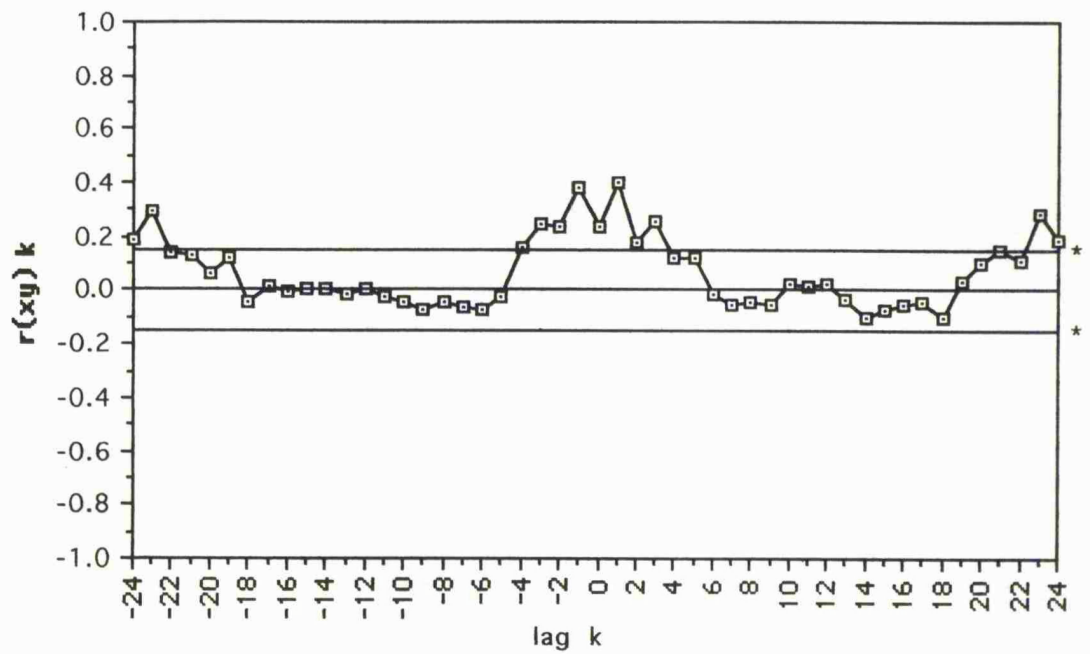


Figure 3.6. Cross correlation coefficients $(r(xy)k)$ for time lags (k) -24 to +24 h, coefficients calculated from the comparison of mean number of burrow emergence events of the sighted (x) and blinded (y) males. Analysis indicates that the rhythm of the sighted and blinded groups of individuals are simultaneous.

* * denotes 95% confidence limits.

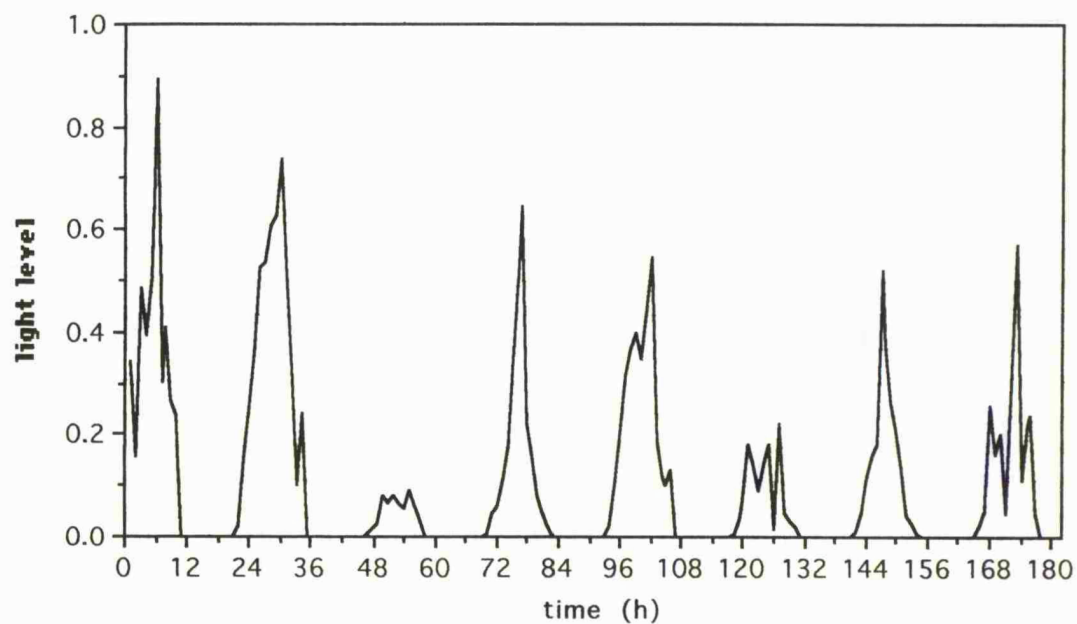


Figure 3.7. Light levels ($\mu\text{mol m}^{-2}\text{s}^{-1}$) on sea bed at 30 m depth, estimated from surface light readings taken during the same period that the behaviour of the male *N. norvegicus* was recorded.

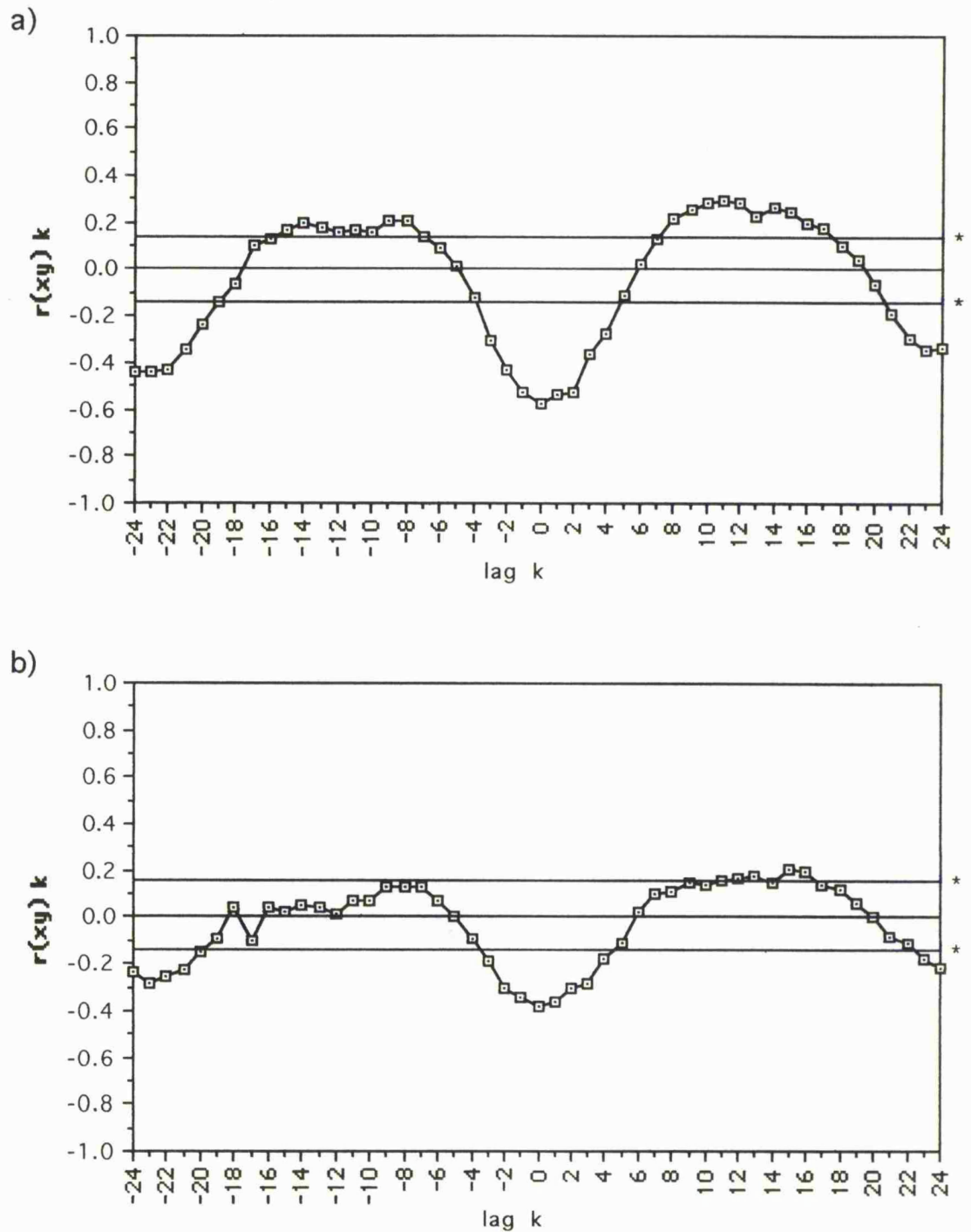


Figure 3.8. Cross correlation coefficients ($r(xy)_k$) for time lags (k) -24 to +24 h, coefficients calculated from the comparison of the light levels and mean number of burrow emergence events by a) sighted and b) blinded male *N. norvegicus*. Each series is significantly and negatively correlated when directly compared. This indicates an inverse relationship in each case.

* * denotes 95% confidence limits.

emergence, with peak emergence occurring at the lowest light levels, indicative of nocturnal behaviour.

There seem to be small dips in the cross correlation coefficients when the two series are lagged by periods of approximately ± 12 h (Fig. 3.8 a). This implies that there is a decrease in burrow emergence around midnight, which agrees with findings of other studies (Chapman and Rice, 1971). The cross correlation analysis between the burrow emergence series for the blinded *N. norvegicus* and the light level series for the same period also showed a significant negative relationship ($p < 0.05$) when the 2 series were directly compared (Fig. 3.8 b). Again, there is evidence for a slight decrease in nocturnal burrow emergence at midnight by the blinded *N. norvegicus*.

The tide height (Fig. 3.9) throughout the period of observations was also compared with the rhythm of burrow emergence behaviour for both sighted and blinded *N. norvegicus*. Cross correlation analysis (Fig. 3.10) revealed a weak correlation at lags of approximately 12 h, when the behaviour rhythms were lagged in the positive direction by 2 h. This indicates a weak positive relationship between *N. norvegicus* behaviour and daily tide cycle, with a delay of 2 h between them. However this correlation is not significant at the 0.05 probability level.

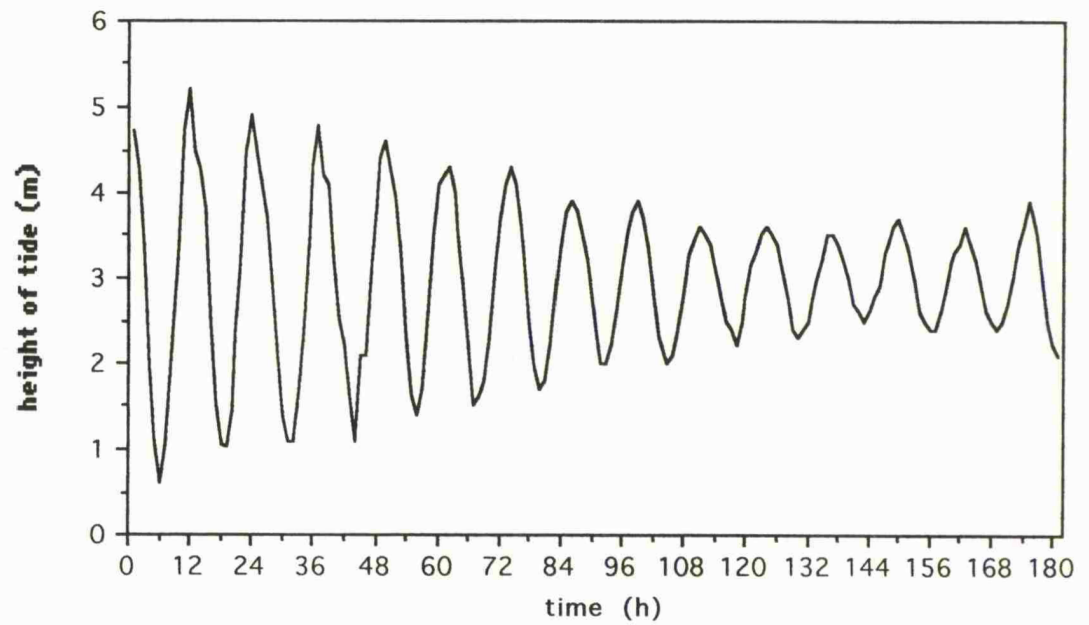


Figure 3.9. Tide height (m) during the period that the behaviour of the male *N. norvegicus* was recorded, September 1991.

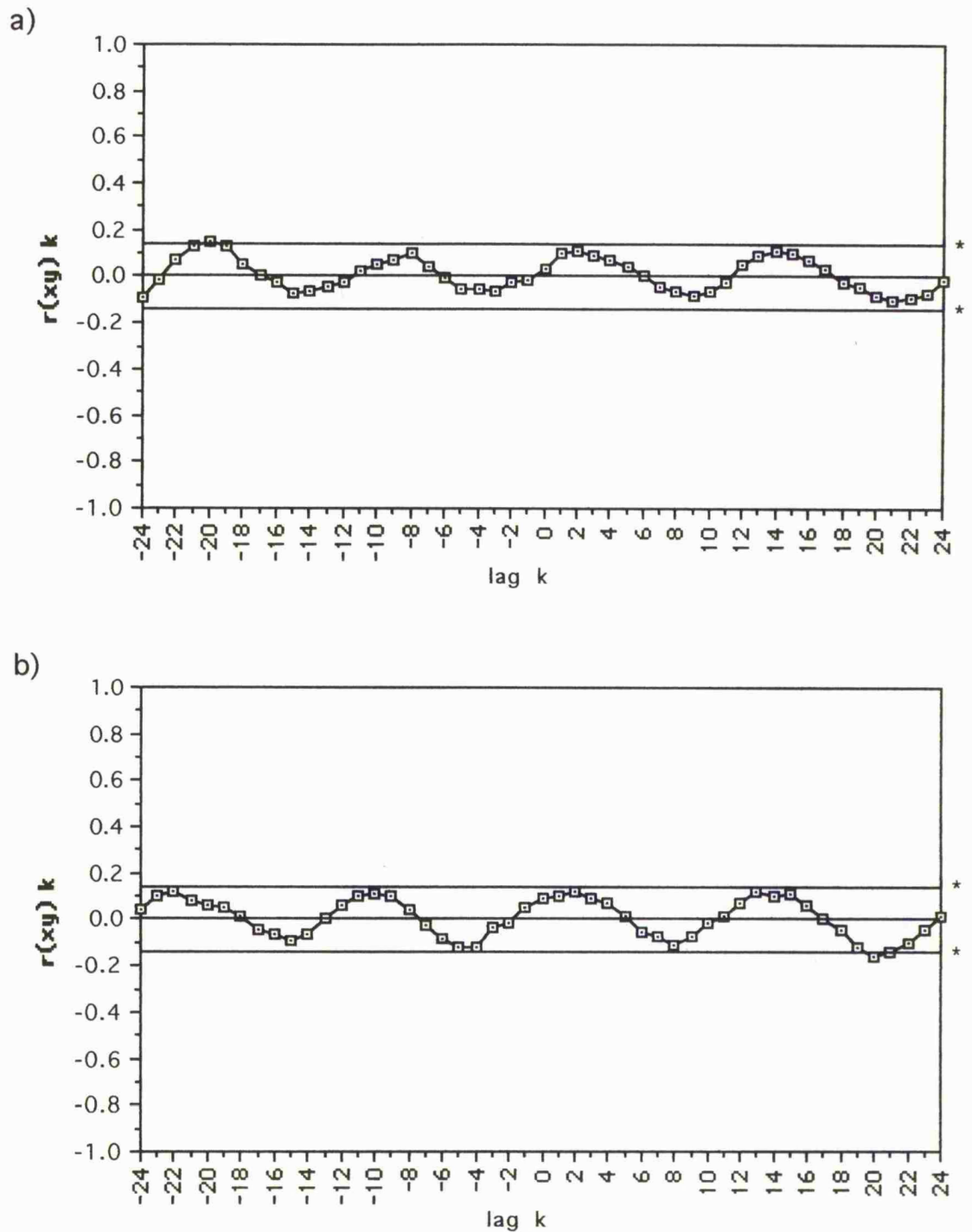


Figure 3.10. Cross correlation coefficients ($r(xy)k$) for time lags (k) -24 to +24 h, coefficients calculated from the comparison of tide height and burrow emergence events by a) sighted and b) blinded male *N. norvegicus*. Any relationships between tide and emergence are weak and not significant.

* * denotes 95% confidence limits.

3.3.2. Observations of the burrow emergence rhythms of sighted and blinded female *N. norvegicus*.

In September 1992, similar observations to those described for male *N. norvegicus* were carried out on sighted and blinded females using similar methods and statistical analysis. Because of severe weather conditions, the duration of the experiment (4 days observations) was shorter than that for males.

Visual inspection of the burrow emergence behaviour series (Fig 3.11) and a cross correlation analysis to compare behaviour in the first and second halves of the experiment both indicate that the patterns of burrow emergence behaviour of both sighted and blinded *N. norvegicus* did not change throughout the period of the experiment ($p > 0.05$).

The correlogram (Fig. 3.12 a) of the data series for emerged sighted individuals shows evidence of a 24 h rhythm, but only the coefficients calculated at time lags around 72 h were actually significant ($p < 0.05$). This suggests a weak activity rhythm with a periodicity of 24 h. When this 24 h cycle was removed from the data series for these sighted individuals the plot of the residuals showed no other significant rhythms present in the data (Fig. 3.13 a).

For the blinded individuals, there were two significant peaks at time lags of 24 h and 48 h ($p < 0.05$), and finally a peak at 72 h which was not significant ($p > 0.05$) (Fig. 3.12 b). These results indicate rhythmic behaviour with a periodicity of 24 h confirming the presence of a nocturnal rhythm for the blinded individuals over the four days of the experiment. After the removal of the 24 h cycle from the data series, further autocorrelation

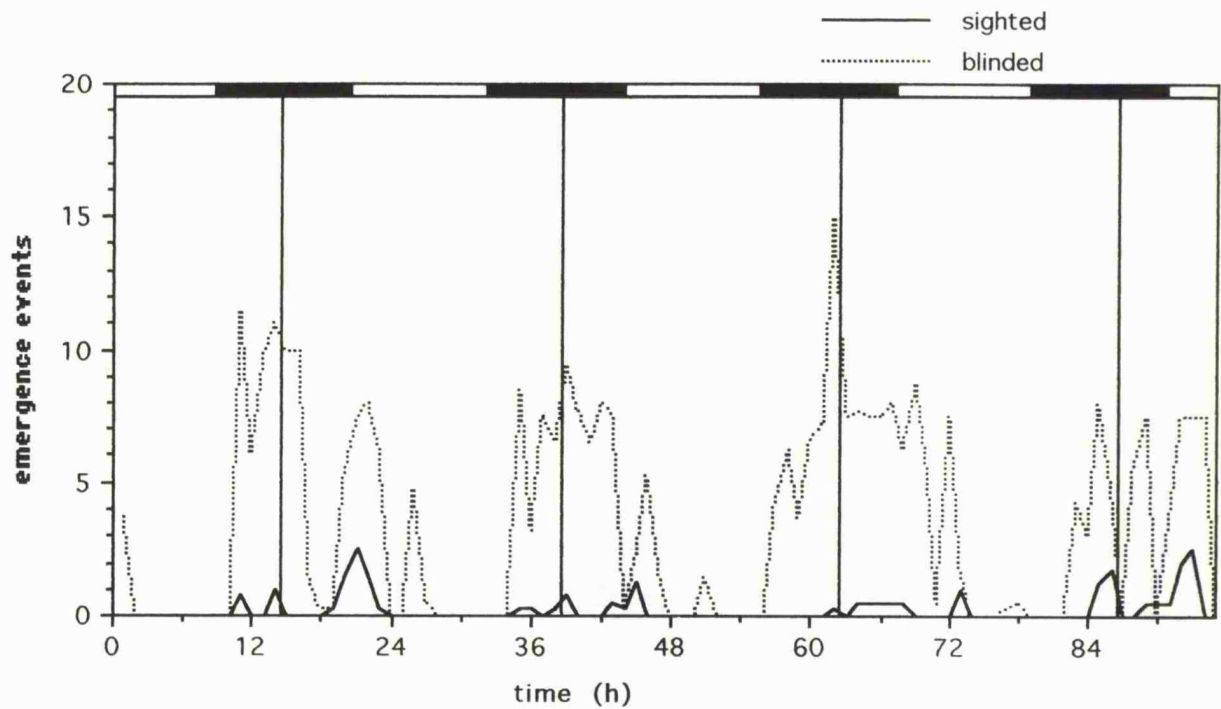


Figure 3.11. Series of mean number of burrow emergence events performed by female *N. norvegicus* while in a cage on the sea bed at a depth of approximately 30 m (first point denotes 10.00 h day 1). Bar above graph indicates periods of daylight (empty bars) and night (shaded bars) and vertical line indicates midnight. A clear pattern of peaks are apparent for sighted and blinded groups of individuals.

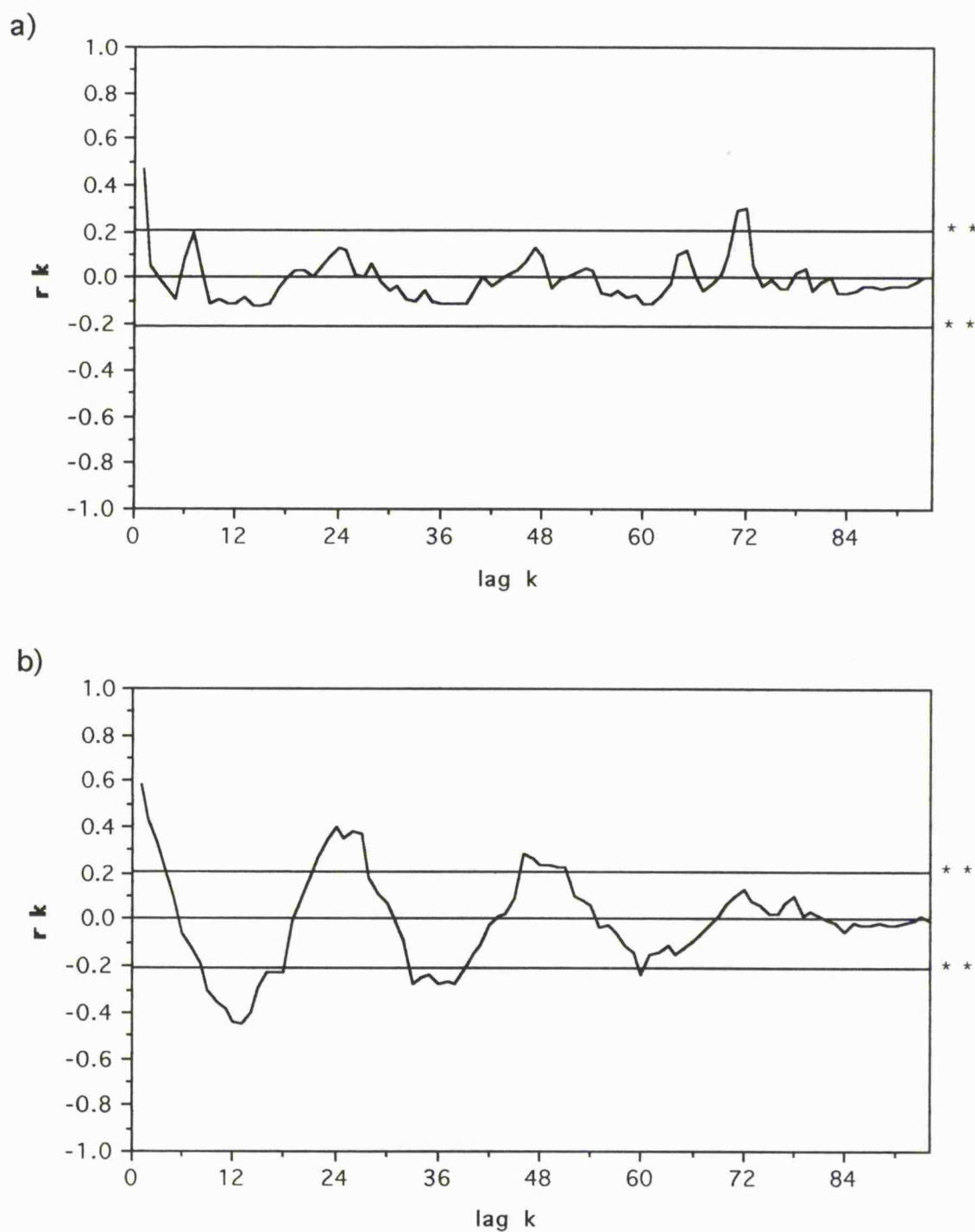


Figure 3.12. Autocorrelation coefficients (r_k) for time lags (k) 1 to 95 h, calculated from the series of burrow emergence events by female *N. norvegicus* when a) sighted and b) blinded. Peaks occur at 24h, 48h and 72h in both series, but not all are significant. Evidence for a 24 h rhythm in the sighted females is weaker than that for the blinded ones. * * denotes 95% confidence limits.

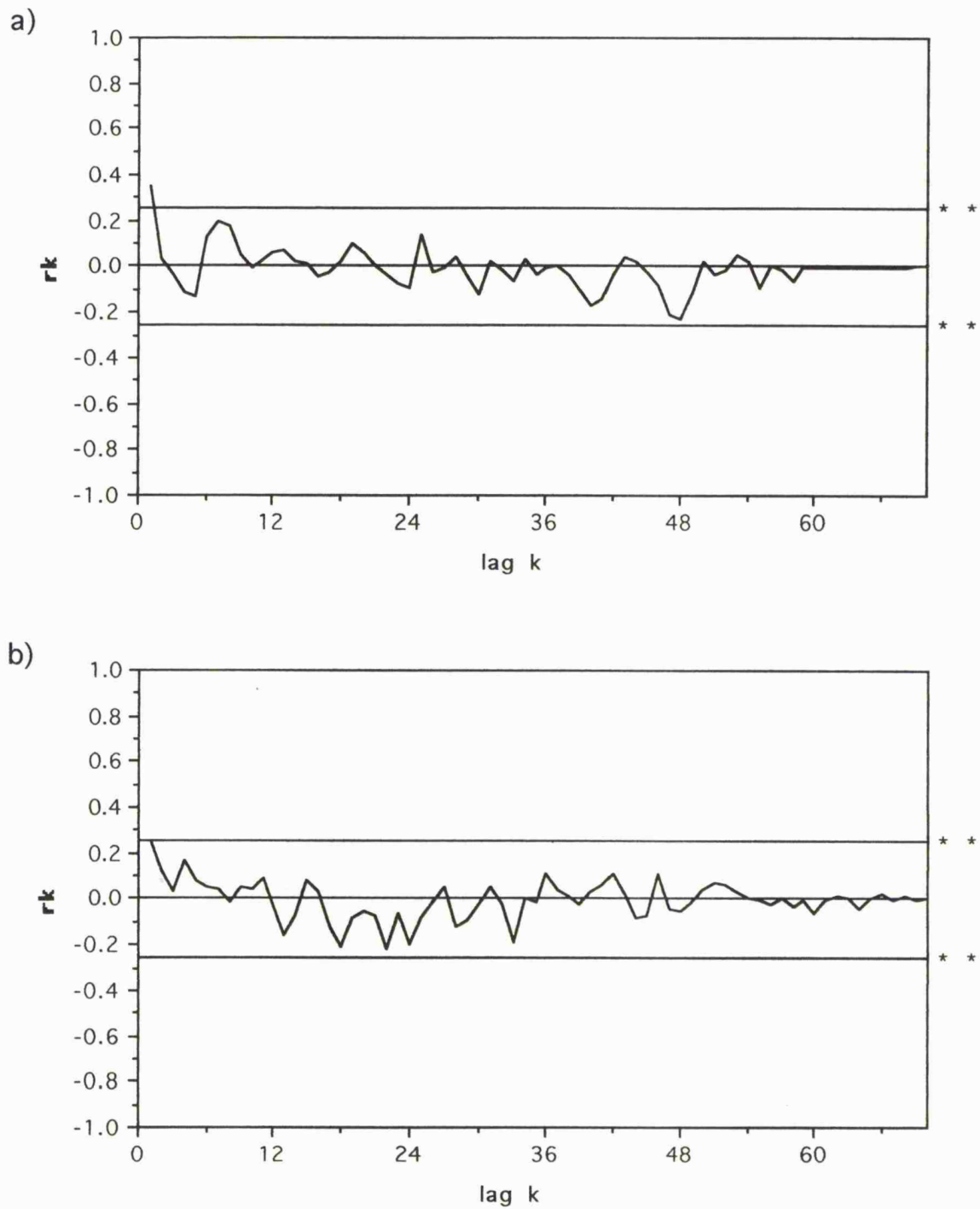


Figure 3.13. Autocorrelation coefficients (r_k) for time lags (k) 1 to 71 h, calculated from the residual series for female *N. norvegicus* when a) sighted and b) blinded. No further rhythms were detectable. * * denotes 95% confidence limits.

analysis did not detect any additional rhythms in the behaviour of the blinded individuals (Fig. 3.13 b).

*3.3.2.1. Comparison of the emergence rhythms of the sighted and blinded female *N. norvegicus*.*

A cross correlation analysis was used to examine for synchronisation between the 24 h rhythms of the sighted and blinded individuals. The results of this test (Fig. 3.14) showed a significant correlation ($p < 0.05$) when both series were directly compared (i.e. time lag, $k = 0$). This demonstrates that the 24 h rhythms of burrow emergence behaviour of the sighted and blinded individuals were simultaneous. The amplitude of the burrow emergence behaviour of blinded females (mean events/h = 3.62, s.d. = 3.83) was not significantly different from that of the sighted females (mean events/h = 0.25, s.d. = 0.54) (two-way ANOVA, two-tailed: $F = 1.68$, $p > 0.05$) (Table 3.1, p.60).

3.3.3. Comparison of the rhythms of activity of the sighted males and sighted females; and, the blinded males and females.

A 24 h rhythm of emergence behaviour has been identified in sighted and blinded *N. norvegicus*, in both the males and females used in the present study. Cross correlation analysis was then used to compare the characteristics of the rhythms of the males and females. The results (Fig. 3.15) showed that when the rhythms of sighted females and sighted males were compared and those of blinded females and blinded males were compared, all emergence rhythms were significantly and positively correlated ($p < 0.05$). Therefore, there is no significant difference between the

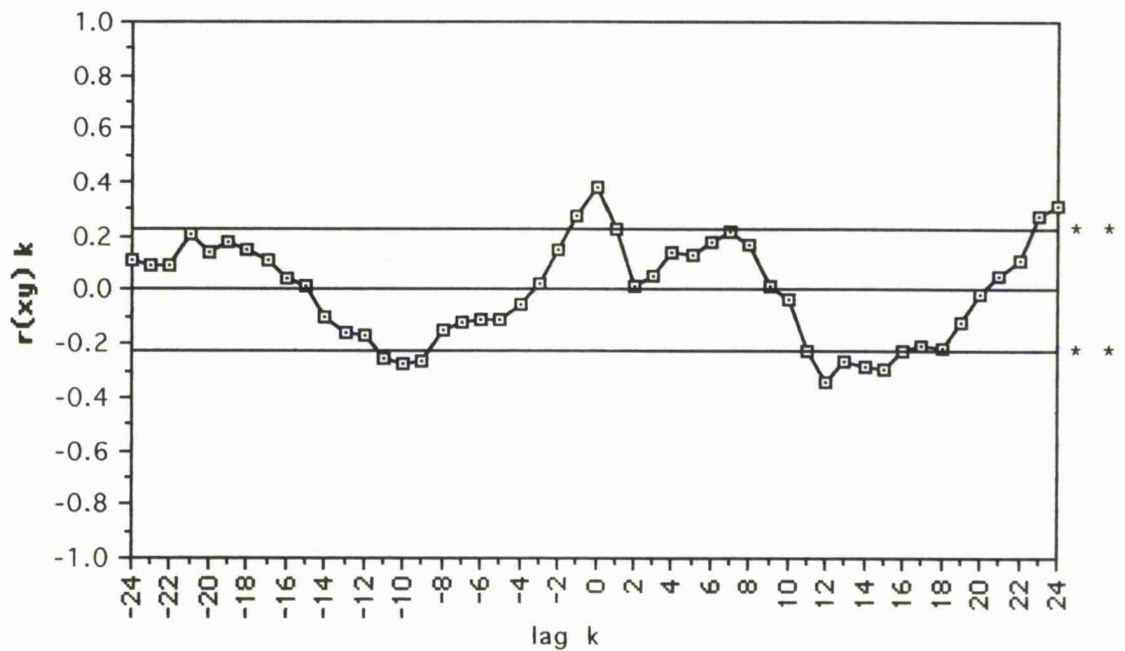


Figure 3.14. Cross correlation coefficients ($r(xy)k$) for time lags (k) -24 to +24 h, coefficients calculated from the comparison of burrow emergence events of sighted (x) and blinded (y) female *N. norvegicus*. Significant correlation at $k = 0$ indicates that the rhythms of burrow emergence of the sighted and blinded groups are simultaneous. * * denotes 95% confidence limits.

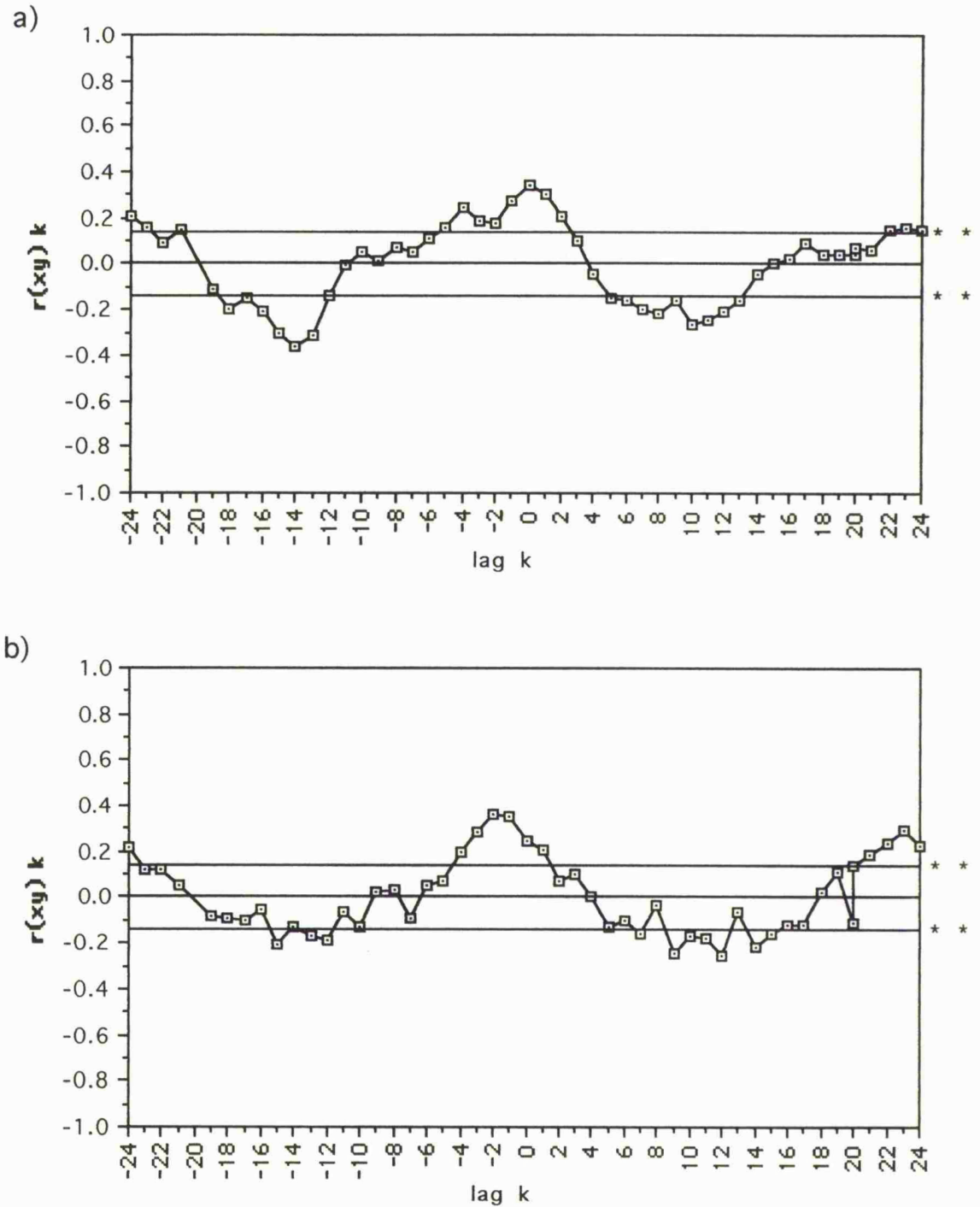


Figure 3.15. Cross correlation coefficients ($r(xy)_k$) for time lags (k) -24 to +24 h, coefficients calculated from the comparisons of burrow emergence events of a) sighted males (x) and females (y); and, b) blinded males (x) and females (Y).

* * denotes 95% confidence limits.

emergence rhythm of the females and that of the males recorded in the previous year.

3.3.4 Evidence for a nocturnal peak of activity.

The patterns of emergence behaviour of the male and female individuals over each 24 h period throughout the two experiments were examined. Looking at the series of observations of each sex separately, the mean of all observations at 10:00 h was calculated for each series in this experiment (i.e. sighted males, blinded males, sighted females, blinded females). This was repeated for each hour over a 24 h period (see Fig. 3.15). All four groups of individuals studied seem to show more emergence activity during the night.

Statistical analysis of the subset values of each series (see Appendix I and section 3.2. 'Materials and methods' for details of subset construction) showed that each set of individuals used in this experiment performed significantly more emergence events during the night (independent measures t-test, two-tailed $p < 0.05$). Therefore it is possible to say that sighted and blinded males and females showed a 24 h nocturnal rhythm of emergence behaviour.

3.3.5. Levels of activity of the male and female N. norvegicus.

A two-way ANOVA (for independent measures, two-tailed test) was carried out to find any effects of vision or the sex of an individual on the levels of activity performed (Table 3.1). The results of the test (using subsets of each series) demonstrated that there was a significant effect of the sex of an individual on its level of activity ($F = 4.56$, $p < 0.05$), but no effect of vision on activity ($F = 1.68$, $p > 0.05$).

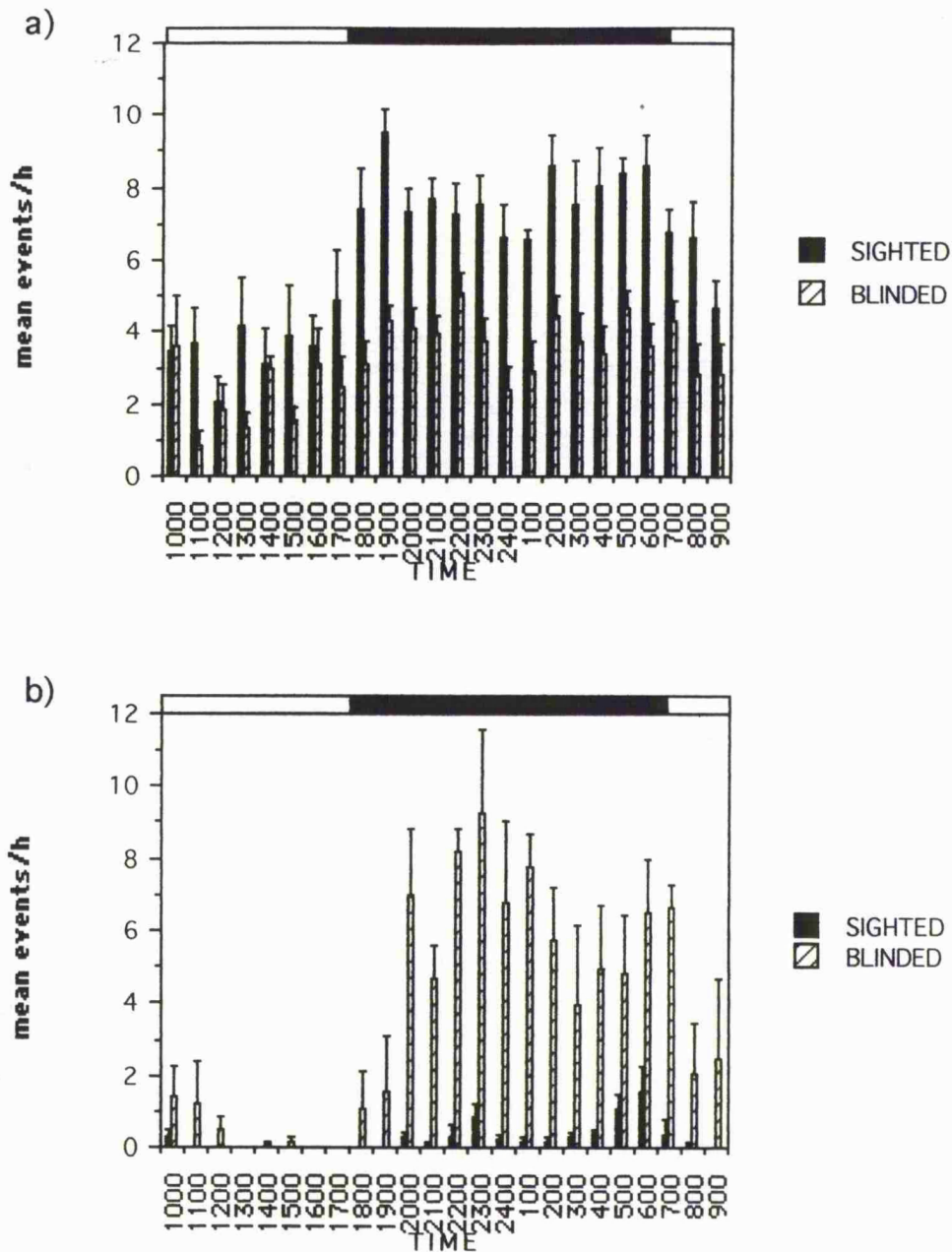


Figure 3.16. The mean 24 h rhythm of burrow emergence events for the (a) male and (b) female groups of *N. norvegicus* used in this experiment. In both cases greater activity occurs during the night (independent t-test, $p < 0.05$). Bar above graphs indicates day/night cycle.

	males	females
sighted individuals	6.13 (3.20)	0.25 (0.54)
blinded individuals	3.19 (2.16)	3.62 (3.83)

Table 3.1. Mean number of burrow emergence events per hour (s.d. in brackets) for sighted and blinded groups of individuals observed during two experiments studying the emergence activity of male individuals (September 1991) and female individuals (September 1992).

Levels of activity for sighted and blinded male individuals were not significantly different. This was also true for the females. However there was a significant difference ($p < 0.05$) in the levels of activity between the two cage experiments. For the blinded group, there was significantly more activity performed by the females than by males. For the two sighted groups of individuals the sighted females performed a lower level of activity than the males. This difference in activity was greater than the difference in activity between sighted and blinded males.

3.4. Discussion.

3.4.1. *The character of the emergence behaviour rhythms.*

The results of this study show that the blinded male and female *N. norvegicus* display a rhythm of emergence behaviour with similar periodicity to that of sighted ones. All showed a diel rhythm with a nocturnal peak of activity. For both male and female *N. norvegicus*, the results of cross correlation analyses failed to show any significant difference between the rhythms of the sighted and blinded individuals. In both cases there was also no change in the rhythm of the blinded individuals during the time that they were on the sea bed. No other rhythms were found in the emergence behaviour of all four groups of individuals studied. This rhythmic emergence behaviour is similar to that found in previous observational studies of *N. norvegicus* at 30 m in their natural habitat (Chapman *et al.*, 1972; Chapman and Howard, 1979).

In the case of the males, the autocorrelation analysis revealed a clearer rhythm (with significant autocorrelations at 24h intervals in the sighted individuals), than in the blinded ones which showed greater noise in the correlogram. On the other hand, the sighted females showed a weaker rhythm (with no pattern of significant autocorrelations), than the blinded ones. Where the rhythm was weaker (blinded males/sighted females) the number of emergence events was relatively low (blinded males) or very low (sighted females) compared with the other group of the same sex. These low numbers of observations make the underlying rhythms more difficult to detect.

Looking at the males and females separately, there was no significant effect of blinding on the levels of emergence activity. In the case of the females this could have been due to the large standard deviation in the data. However, there was a difference in

the levels of activity performed by the males and females in the two experiments. The sighted females performed significantly less activity than the sighted males, whereas the blinded females showed significantly more activity than the blinded males. The differences found between the behaviour of male and female individuals may be due to real sex differences. It is also possible that sea bed conditions were different between the two years that these two experiments were carried out. Further investigations would be required to clarify these differences seen in the emergence behaviour of male and female *N. norvegicus*.

3.4.2. Light as the entraining agent for emergence behaviour rhythms.

Results of previous studies (Atkinson and Naylor, 1976; Naylor and Atkinson, 1976; Chapman, 1980) strongly indicate that light is an important factor in the entrainment of the emergence rhythms of *N. norvegicus*. The results of the present study are consistent with this idea because the light level cycle was shown to be simultaneous and inversely related to the rhythms of emergence behaviour of both the sighted and blinded males during the experiment in the first year of this study (September, 1991). The tide cycle was also investigated as a possible entrainment agent for emergence behaviour. However, whereas there was a significant relationship between light levels and emergence behaviour, the relationship between tidal height and emergence behaviour was not significant at the same confidence level ($p > 0.05$).

3.4.3. *Limitations of studying captive N. norvegicus.*

The limitation of studies like this is that the cage is an artificial environment that could affect normal behaviour. Areas of behaviour possibly affected include migration between burrows, foraging activity, predator avoidance and burrow maintenance. The *N. norvegicus* were confined in the cage which would have restricted the area available for foraging, and the males would not have been able to make their long excursions (Chapman *et al.*, 1975). The densities in the cage (2.5 males/m² and 2 females/m²) were higher than has been reported for the naturally occurring population at 30 m in Loch Torridon (0.13 individuals/m²) (Chapman and Rice, 1971). This could have resulted in an increased frequency of conflicts between individuals. The individuals inside the cage may have been isolated from any predator stimulus and could have remained out of the burrows for longer periods because they were performing less predator avoidance behaviour. Finally, the use of artificial shelters may mean that it is only possible for *N. norvegicus* to perform a modified burrow maintenance behaviour. The fact that rhythms persist within the artificial environment of a cage suggest that they are robust.

3.4.4. *Limitations of making observations on a mixed population of sighted and blinded individuals.*

The present results clearly show that in a mixed population of sighted and blinded individuals, blinded *N. norvegicus* are likely to demonstrate the same rhythms as the sighted ones. This has clear relevance to the question of how blinding might affect discards. In such mixed populations it is possible that the activity rhythms of the sighted individuals could affect those of the blinded ones. In order to find out whether isolated blinded individuals

display the same rhythm as that found in the mixed population of blinded and sighted individuals as used in the present study, further laboratory based actograph experiments were carried out to investigate this problem (Chapter 4).

3.5. Conclusion.

The results of the present field study clearly demonstrate that sighted male and female *N. norvegicus* perform a 24 h rhythm, with a nocturnal peak, of emergence behaviour. This 24 h rhythm is also present in blinded males and females.

Blinding also did not appear to affect the level of emergence behaviour. However a difference was found between the level of emergence behaviour performed by male and female individuals, but it is unclear whether this is a real sex difference or a difference between the conditions in which the two experiments were carried out.

The ambient light level cycle was simultaneous with that of *N. norvegicus* emergence behaviour and it adds to the growing evidence that light is a major entrainment agent.

Chapter 4. Effect of blinding on burrow emergence rhythm - laboratory experiments.

4.1. Introduction.

In this laboratory study, movements within the burrow and burrow emergence were automatically recorded for each *N. norvegicus* while it remained in the controlled environment of an actograph. A 12 h light: 12 h dark regime was used, with the light level similar to that found in the natural habitat. The aim of this study was to identify rhythms in the behaviour of the *N. norvegicus* while in controlled conditions and to compare these rhythms with those found in the previous field experiments (see Chapter 3 for details). A major objective was to see whether the burrow orientated rhythms recorded in individual sighted *N. norvegicus* persisted after blinding. The field experiments involved mixed populations of sighted and blinded individuals. The actograph experiments included the study of isolated individuals and so excluded the possible complications of examining rhythms in mixed populations of sighted and blinded individuals.

4.2. Materials and Methods.

To study rhythms of burrow emergence and inside-burrow activity in the laboratory, an 'actograph' system was designed and constructed at the University of Leicester (Fig. 4.1). The actographs were made from opaque plastic tanks (height = 0.45 m, base = 0.33 m x 0.28 m) each with a 0.25 m length of plastic tube (0.065 m diameter) inserted into the side of the tank to provide an artificial burrow. A sub-gravel filter bed was fitted into the base of each actograph tank and the water was continually re-circulated through the filter. This circulatory system also provided aeration for the water. A thin layer of gravel was placed on the floor of the artificial burrow. The actograph contained sea water at a depth of 0.3 m, at a temperature of 12°C. The actograph tank was fitted with a light-tight lid, with a green gelatin filter inserted in it. A modified photographic safelight fitted with a 25 W tungsten bulb was suspended above an actograph tank and the light adjusted so that, when switched on, the maximum light intensity on the base of the actograph tank was close to that estimated at the sea bed at midday during a previous 8 day period in September 1991 (section 3.2 for details) (mean = $0.28 \mu\text{mol m}^{-2}\text{s}^{-1}$, s.d. = 0.07, n = 8). A timer switch was used to produce a 12 h light: 12 h dark cycle (light on at 07.00 h and off at 19.00 h) in the actograph identical to that in the holding tanks where individuals were kept prior to the experiment.

The movements of an individual inside the actograph were automatically monitored using reed-relay switches triggered by the individual passing underneath them. One reed switch was fitted on the roof of the artificial burrow 2 cm inside the entrance; and a second was fixed 2 cm outside the burrow entrance on a bar of perspex extending 3 cm from the burrow roof (Fig. 4.1). Resistors

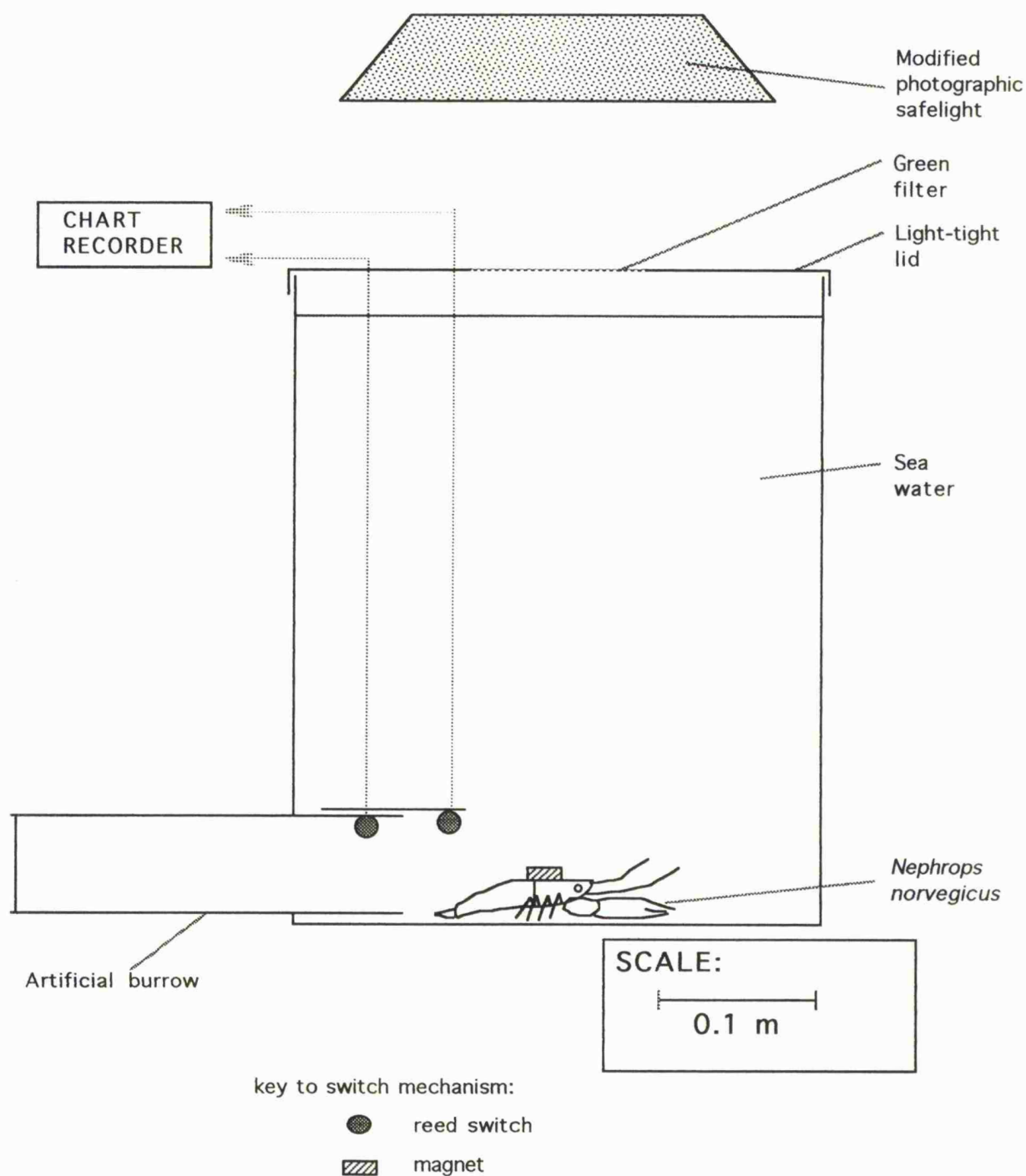


Figure 4.1. Actograph and switch mechanism.

were used in the switch circuits so that each switch produced a different output voltage when it closed in response to a magnet passing under the switch (Fig. 4.2). The output voltages from each switch were recorded on a Linseis flat bed chart recorder. By the use of a magnet situated on the dorsal part of the carapace of the *N. norvegicus* and using the chart recorder, it was possible to automatically monitor the movements of an individual as it passed under each switch. The number of times that an individual performed either an in-burrow movement (i.e. in-burrow behaviour event), or emerged from the burrow (i.e. an emergence behaviour event) was noted for each hour while it remained inside the actograph.

An initial study was carried out to assess the reliability of the switches in recording the movements of an individual. The results from simultaneous recordings from a television camera, and the switch mechanism, of the emergence behaviour of 4 individuals over 3 days were compared. A total of 112 emergence events was observed on the video tape and the switches recorded 97.3% of these events.

4.2.1. Preparation of *N. norvegicus*.

For each actograph run, male *N. norvegicus* were selected from the holding tank and a minimal amount of a resin-based filler ('Plastic Padding') was used to attach a small magnet (weight of magnet in water = 5.2 g) to the dorsal part of the carapace. In this position, the magnet reliably activated the reed switches as it passed underneath. After fitting the magnet, the *N. norvegicus* was returned to a holding tank for 3 days to recover from any immediate effects of handling. The presence of the magnet did not appear to interfere with the movement of the *N.*

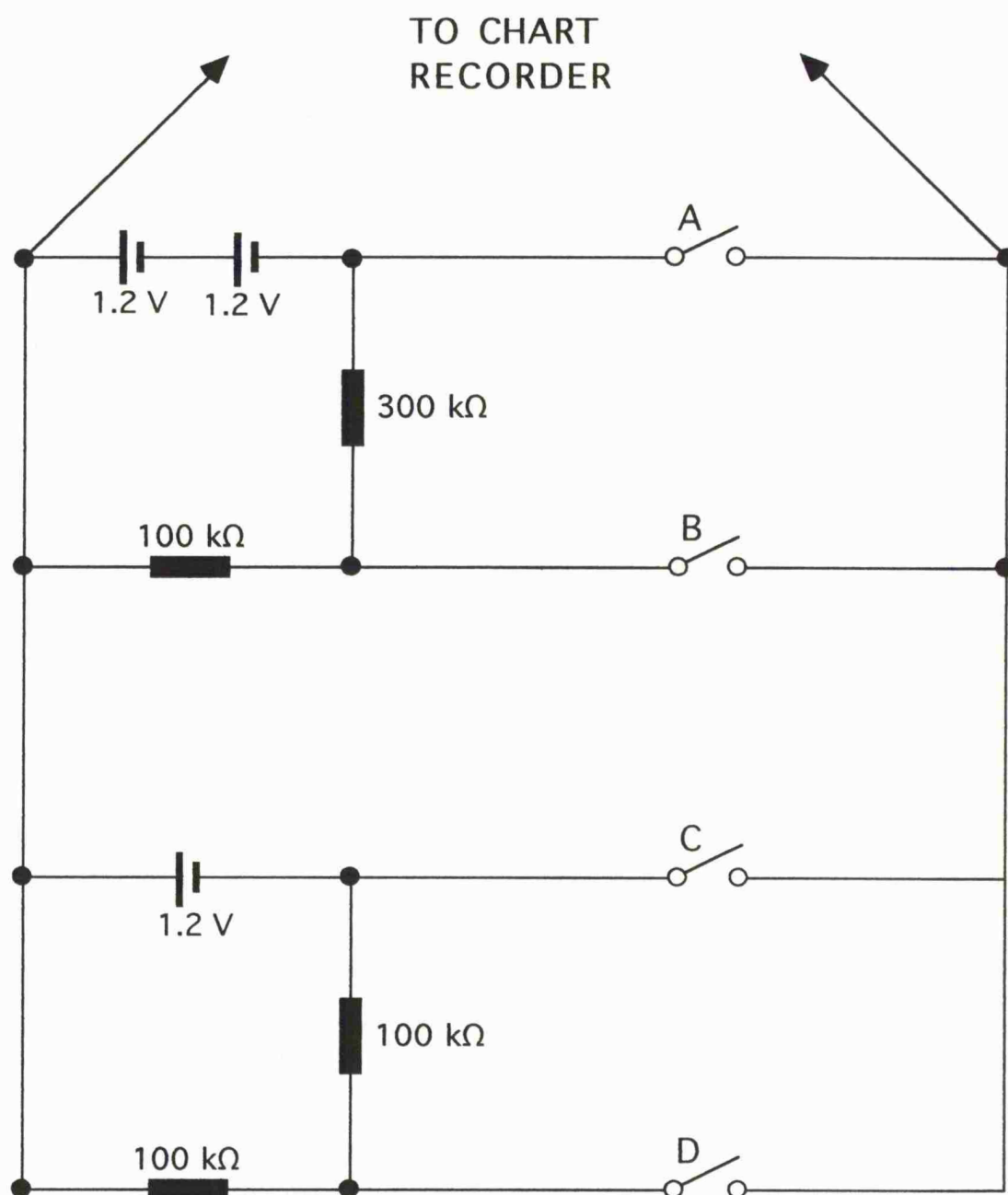


Figure 4.2. Diagram of a circuit indicating four different switches, A - D, each able to produce a different output to the chart recorder (of 2.4 V , 1.8 V , 1.2 V and 0.6 V respectively). Using this arrangement a single chart recorder was used to monitor the four switches in two actographs simultaneously.

norvegicus. Since all individuals observed in the actograph were treated equally, and all carried the same type of magnet, any effect would have been constant for all individuals. The mean carapace length of the 13 male *N. norvegicus* used was 35.2 mm (s.d. = 2.67 mm).

4.2.2. Procedure.

At least two hours before an individual was placed in an actograph, the sea water in the actograph was replaced. At 10.00 h on day 1 of each trial, a light-adapted *N. norvegicus* was transferred into the actograph and placed outside the burrow. The lid was then fixed onto the actograph and the light above it switched on. Burrow orientated behaviour was continuously recorded for seven days. The *N. norvegicus* were not fed while in the actograph and the lid was not removed.

At 10.00 h on day 8 of each trial, recording was stopped and the *N. norvegicus* removed at approximately 22.00 h on the same day. It was then blinded (see section 2.3 for blinding procedure) and returned to a holding tank for three days to recover from the immediate effects of the blinding procedure. The sea water inside the actograph was changed again. At 10.00 h on day 12 of each trial the blinded *N. norvegicus* was replaced into the same actograph and its activity behaviour recorded for a further period of seven days. At the end of the experiment, the *N. norvegicus* was removed and its carapace length was noted. It was then sacrificed and its eyes placed in formal calcium fixative.

This experiment was repeated 13 times using a different individual *N. norvegicus* to produce a total of 13 trials.

4.2.3. Statistical analysis.

Autocorrelation analysis (Chatfield, 1984; Farnum, 1989) (see Appendix I for more details of this test) was carried out on each set of seven day recordings of activity of an individual inside an actograph in order to identify and describe any significant rhythms present in a series of observations.

Once a rhythm was identified in a series of observations, cross correlation analysis (Farnum, 1989) (see Appendix I for more details of the test) was used to compare the rhythms of different series. Seasonal differencing technique (Chatfield, 1984; Farnum, 1989) (see Appendix I for details of technique) was used to see how accurately a rhythm described a series of observations.

Data which is autocorrelated, such as that in the series of observations described in this chapter, cannot be used for the statistics involved in comparing the amounts of activity. To compare levels of activity it was necessary to select a subset of each series of observations (see Appendix I for details). A subset was made from the seven day series of observations by taking every seventh observation. For comparison of activity levels of different series, a repeated measures t-test (two-tailed) and a two-way ANOVA test (for repeated measures, two-tailed) were used. A probability level of 0.05 was used for all tests carried out.

4.3. Results.

4.3.1. Identification of activity rhythms.

The combined data from all 13 individuals were used to calculate the mean number of events each hour throughout each 7 day experimental period for both in-burrow movements and out of burrow activity (Figs 4.3 and 4.4). Visual inspection of these behaviour series seems to show a diel rhythm with a nocturnal peak in both in-burrow and burrow emergence behaviour, both when the *N. norvegicus* were sighted and after they were blinded.

The results of a 'mean square successive difference' test (Farnum, 1989; see Appendix I for details) showed that the data in all four series were autocorrelated ($p < 0.05$) and that autocorrelation analysis (Chatfield, 1984) (see Appendix I for details) was justified for each series.

4.3.1.1. In-burrow activity.

The autocorrelation coefficients calculated from the two series of the in-burrow movements (i.e. before and after individuals were blinded) (Fig. 4.5), reveal a pattern of positive peaks at time lag 24 h ($p < 0.05$) and at subsequent 24 h intervals. This indicates the presence of a strong 24 h rhythm in sighted males, which then persisted after they were blinded.

The gradual decrease in the amplitude of the peaks and troughs of the correlogram coefficients as the size of the lags increases is a normal consequence of the application of this type of statistical test to a time series of limited duration. It does not represent a real decline in amplitude of the rhythm. The persistence of the rhythm is clear from visual inspection of the time series plot of in-burrow behaviour (Fig. 4.3). To further

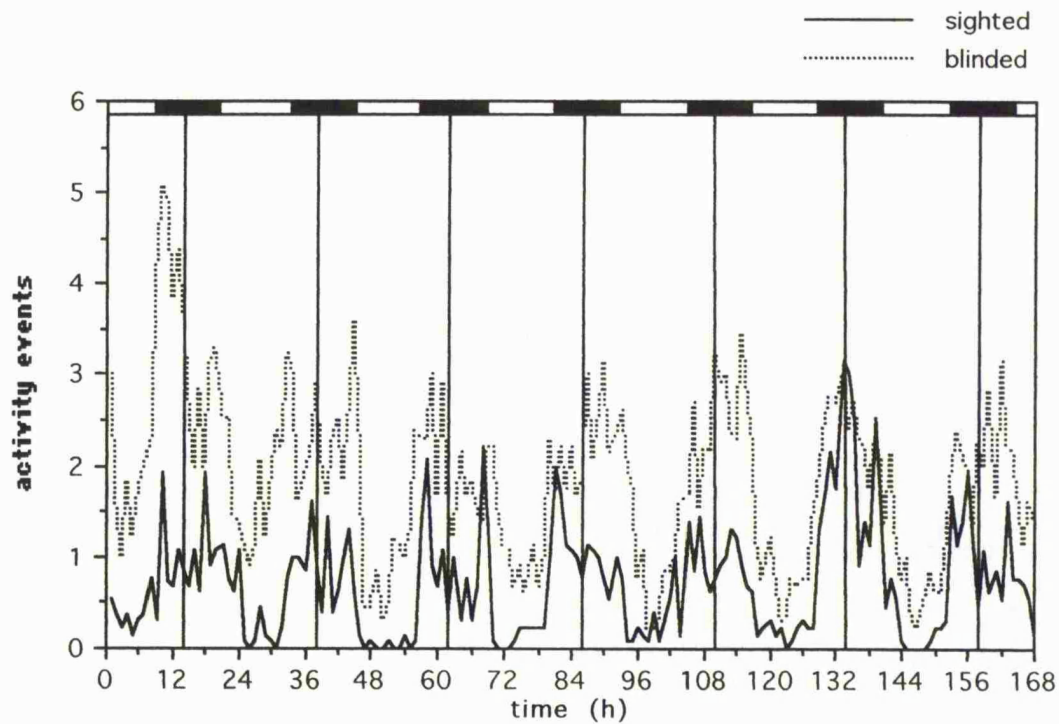


Figure 4.3. Series of mean numbers of in-burrow movements performed by 13 *N. norvegicus* while inside actographs for 7 successive days (starting at 10.00 h) when they were sighted and then after they had all been blinded. Bar above graph indicates light cycle in actographs (light period = empty bar, dark period = shaded bar) and vertical lines indicate midnight. Clear rhythms of in-burrow behaviour are apparent before and after blinding.

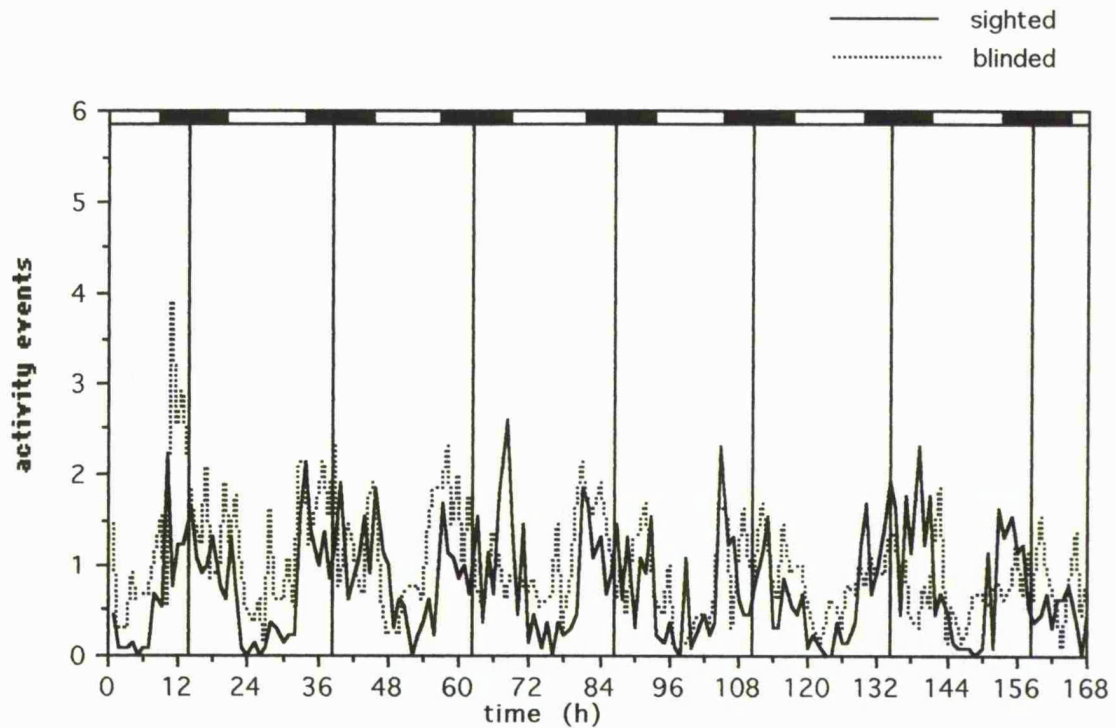


Figure 4.4. Series of mean numbers of burrow emergence events performed by 13 *N. norvegicus* while inside actographs for a period of 7 successive days (starting at 10.00 h) when they were sighted and then after they had all been blinded. Bar above graph indicates light cycle of actographs (light period = empty bars, dark periods = shaded bars) and vertical lines indicate midnight. Clear patterns of emergence activity are apparent in both sighted and blinded individuals.

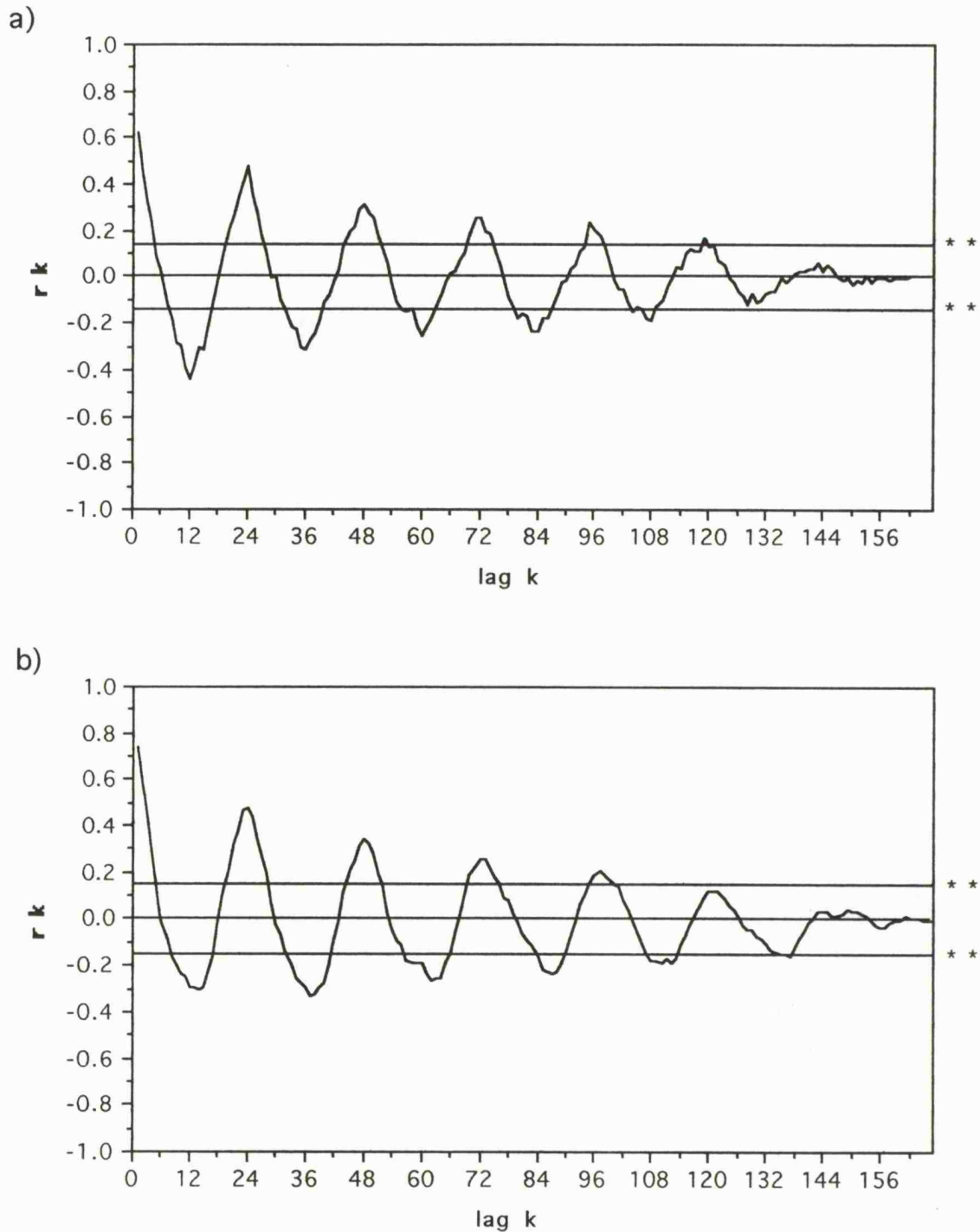


Figure 4.5. Autocorrelation coefficients (r_k) for time lags (k) 1 to 167 h, calculated from mean number of in-burrow events of the 13 *N. norvegicus* when a) sighted and b) blinded. Both indicate a strong rhythm. ** denotes 95% confidence limits.

examine the series for any temporal differences, the pattern of behaviour in each 7 day period was divided into half and the halves were compared using cross correlation analysis (Chatfield, 1984) (see Appendix I for details). The results of this analysis demonstrated no significant differences ($p>0.05$) in the rhythms recorded in the first and second halves of each of the two 7 day periods. Thus, the 24 h rhythm can be said to have persisted throughout the period that individuals remained inside the actographs.

To see how well a 24 h rhythm explains the cycle of mean numbers of in-burrow movements of 13 individuals before and after they were blinded, 24 h rhythms were modelled for each series (see Appendix I for details of the 'seasonal differencing' technique used to model a 24 h rhythm) and fitted to the data of both series. The difference between the estimated 24 h rhythms and those of the actual numbers of movements recorded were calculated and autocorrelation analyses were carried out to find any other rhythms in the residual series (Fig. 4.6). No further significant rhythms were found in the residual series for the *N. norvegicus* when they were sighted.

Cross correlation analysis confirmed that there was no significant change in the in-burrow rhythms of the 13 individuals after they were blinded ($p<0.05$) (Fig. 4.7).

The use of subsets (see Appendix I and section 4.2 'Materials and methods' for details) made it possible to show that the mean level of in-burrow activity was significantly higher (using two-way ANOVA, two-tailed: $F = 24.9$ $p<0.05$) after blinding (mean events/h = 1.83, s.d. = 0.88), than when sighted (mean events/h = 0.72, s.d. = 0.69) (see Table 4.1, p. 86). Therefore individuals were

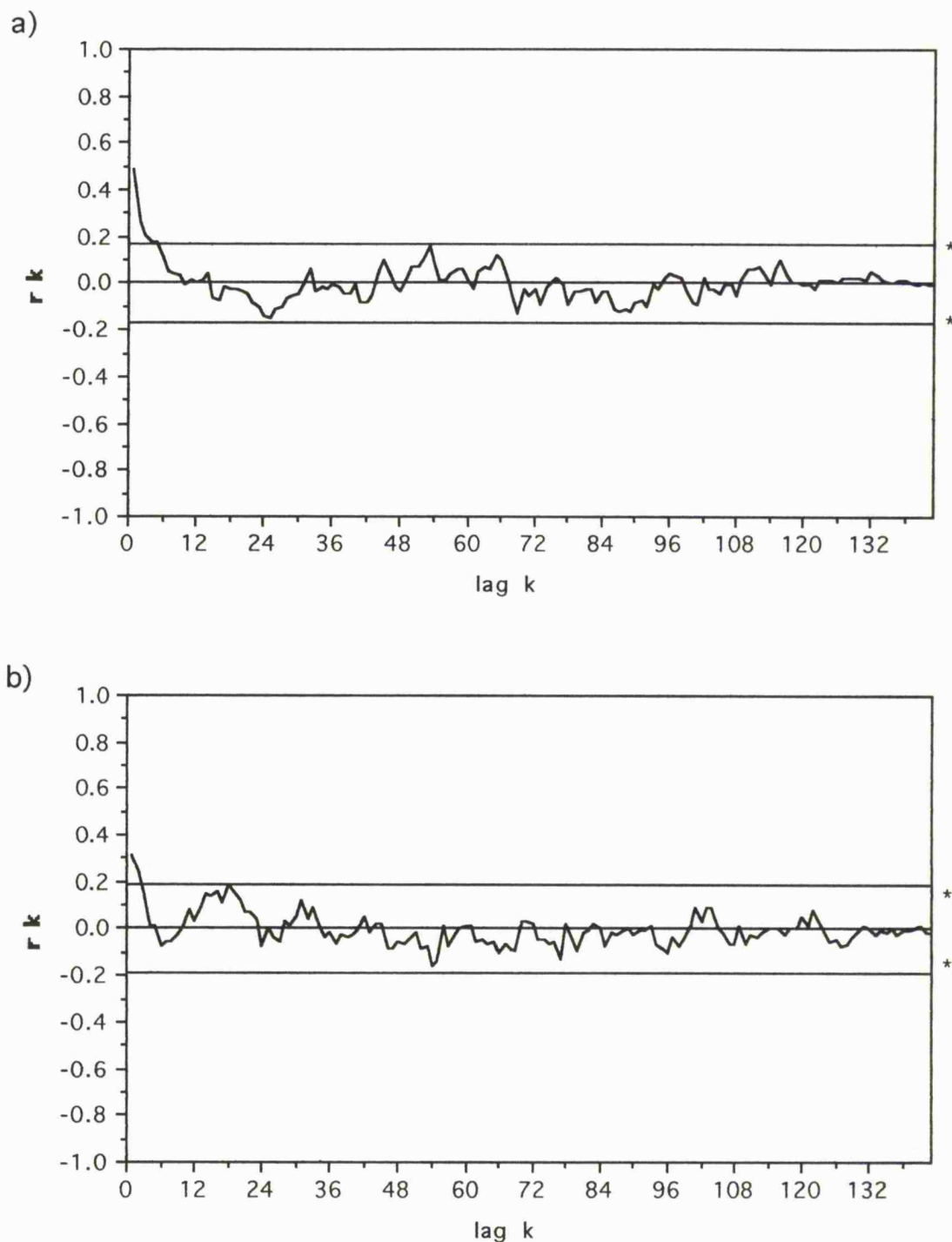


Figure 4.6. Autocorrelation coefficients (r_k) for time lags (k) 1 to 141 h, calculated from the residual series of in-burrow events of the 13 *N. norvegicus* when a) sighted and b) blinded. No further significant rhythms were present in these residual series. ** denotes 95% confidence limits.

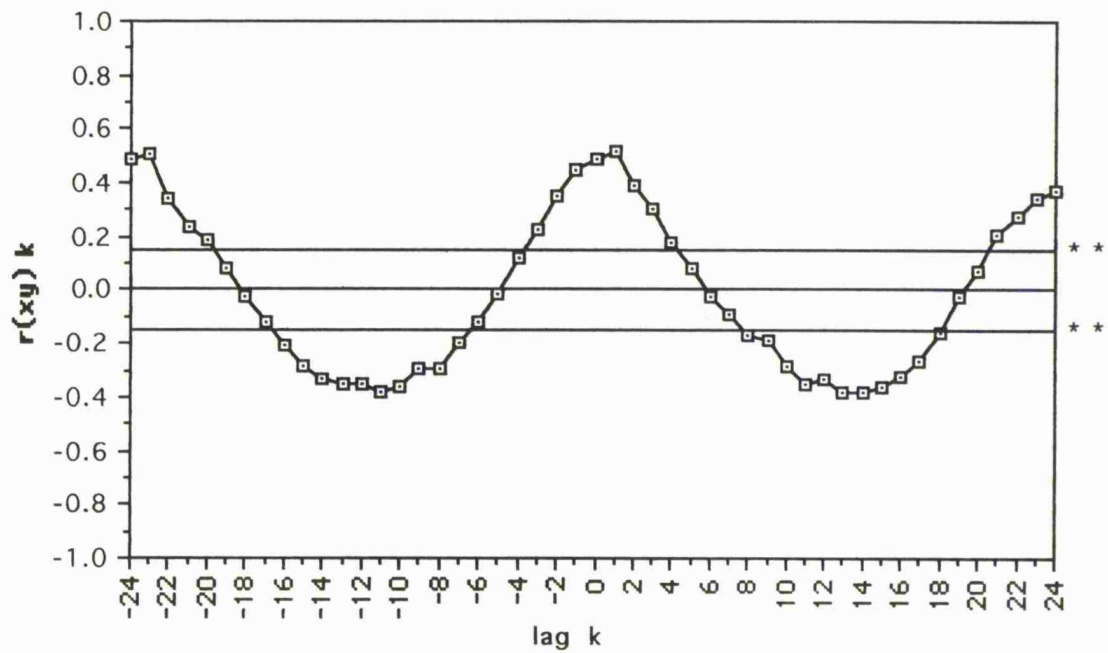


Figure 4.7. Cross correlation coefficients ($r(xy)k$) for time lags (k) -24 to +24 h, coefficients calculated from the comparison of in-burrow events of 13 *N. norvegicus* when sighted (x) and blinded (y). This shows that rhythms were not significantly different after blinding. ** denotes 95% confidence limits.

more active inside burrows after they had been blinded.

4.3.1.2. *Burrow emergence behaviour.*

The autocorrelation coefficients calculated from the series of burrow emergence events (Fig. 4.8) show evidence of a clear rhythm in the mean burrow emergence behaviour, both before and after blinding. The coefficients show peaks at time lags of 24 h and then at subsequent 24 h intervals, providing evidence of a daily cycle of burrow emergence behaviour. Visual inspection of both series of emergence behaviours (Figs 4.3 and 4.4) does not show any obvious change in the periodicity of the rhythm of behaviour with time and the results of cross correlation analyses, comparing the two halves of each series, did not show any significant difference ($p > 0.05$) in each case. This indicates that the 24 h rhythm persists throughout the 7 day period that the individuals remained in the actographs.

To show how well a 24 h rhythm fits the mean burrow emergence behaviour recorded from all 13 individuals when sighted and then after blinding, the rhythm was modelled in each case (using the seasonal differencing technique: see Appendix I for details of technique) and removed from the data (Fig. 4.9). No further rhythms were found in the residual series for individuals when sighted and after they had been blinded.

The rhythms of emergence behaviour of the individuals when sighted and then after having been blinded were essentially similar (using cross correlation analysis, $p > 0.05$) (Fig. 4.10).

The level of emergence activity was significantly greater after all 13 individuals had been blinded (mean events/h = 1.06,

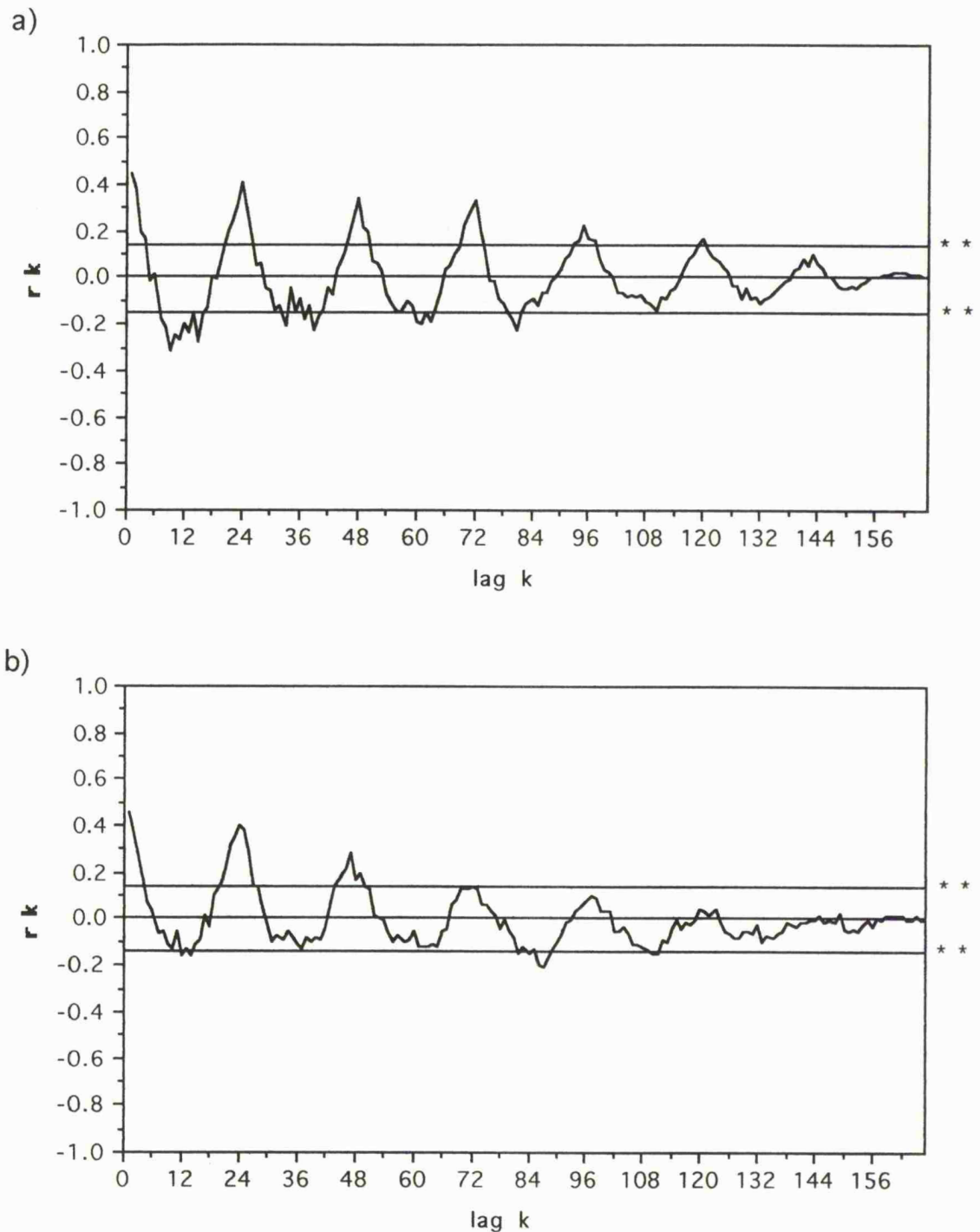


Figure 4.8. Autocorrelation coefficients (r_k) for time lags (k) 1 to 167 h, calculated from the series of mean number of burrow emergence events of the 13 *N. norvegicus* when a) sighted and b) blinded. In both cases there is evidence for a 24 h rhythm. ** denotes 95% confidence limits.

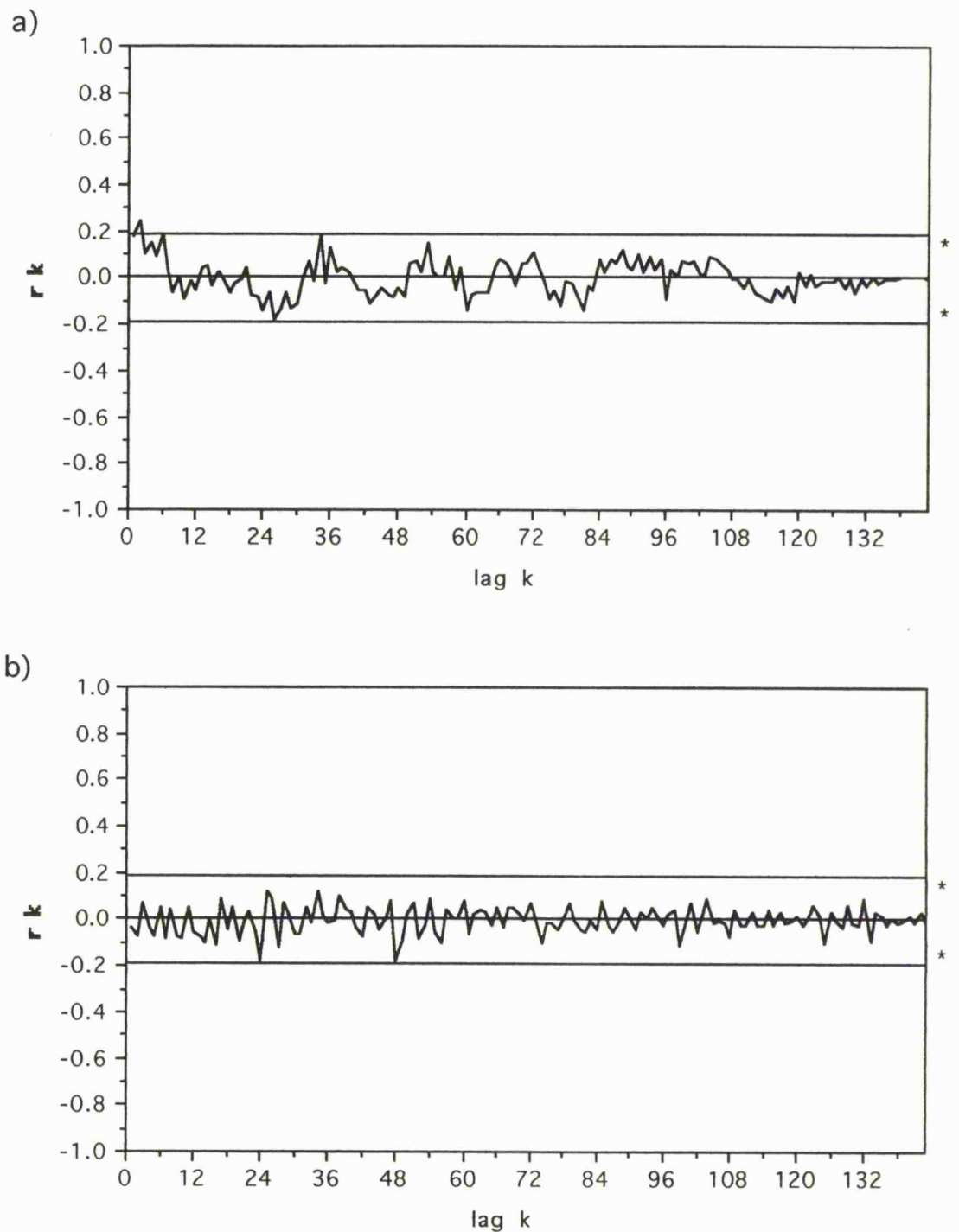


Figure 4.9. Autocorrelation coefficients (r_k) for time lags (k) 1 to 141 h, calculated from the residual series of burrow emergence events of 13 *N. norvegicus* when a) sighted and b) blinded. There are no other significant rhythms in either residual series. ** denotes 95% confidence limits.

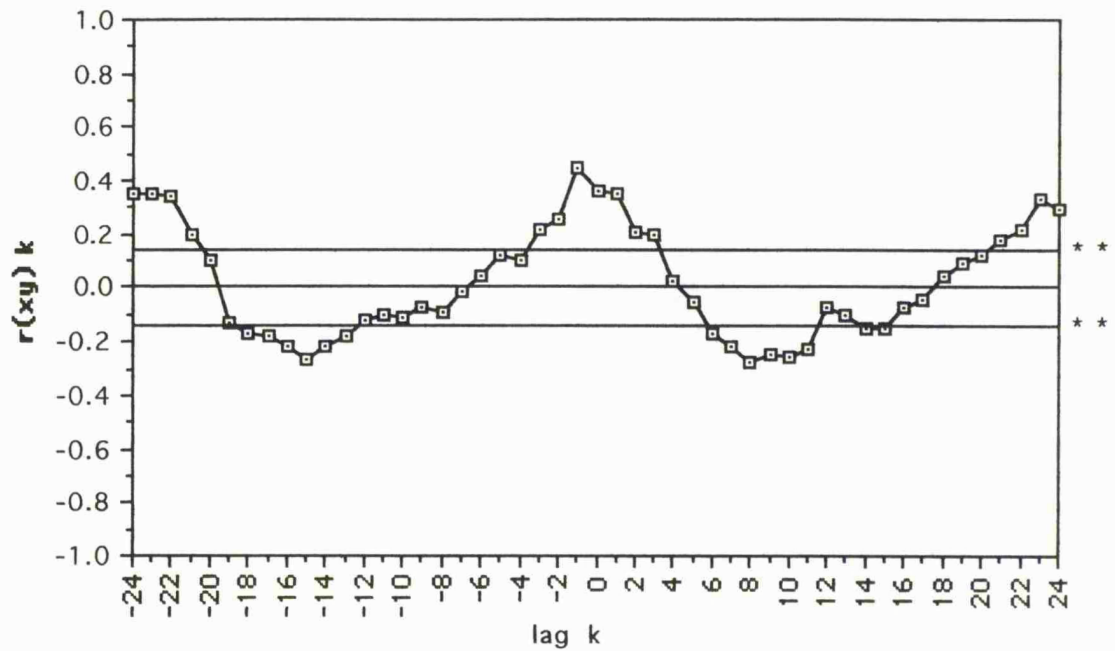


Figure 4.10. Cross correlation coefficients ($r(xy)k$) for time lags (k) -24 to +24 h, coefficients calculated from the comparison of burrow emergence events of 13 *N. norvegicus* when sighted (x) and blinded (y). This shows that the rhythms are similar before and after blinding. ** denotes 95% confidence limits.

s.d. = 0.55) than when sighted (mean events/h = 0.85, s.d. = 0.61) (two-way ANOVA, two-tailed: $F = 24.9$ $p < 0.05$) (see Table 4.1).

4.3.2. Comparison of in-burrow and burrow emergence rhythms of all individuals.

4.3.2.1. Rhythms of behaviour.

To see if the rhythm of in-burrow behaviour has the same characteristics as that of burrow emergence behaviour, a cross correlation analysis was carried out to compare them (Fig. 4.11 and 4.12). This analysis clearly demonstrates that both before and after individuals were blinded, the rhythms associated with the two types of behaviour are simultaneous when directly compared (i.e. at the time lag $k = 0$). Therefore, there was no difference between the mean rhythms of in-burrow and burrow emergence behaviours performed by the 13 individuals, either when they were sighted or when they were blinded.

4.3.2.2. Levels of activity.

The levels of in-burrow and burrow emergence behaviour for all 13 individuals were compared before and after the blinding procedure using a 2-way repeated measures ANOVA, 2-tailed test (see Appendix I for test and use of subsets) (see Table 4.1).

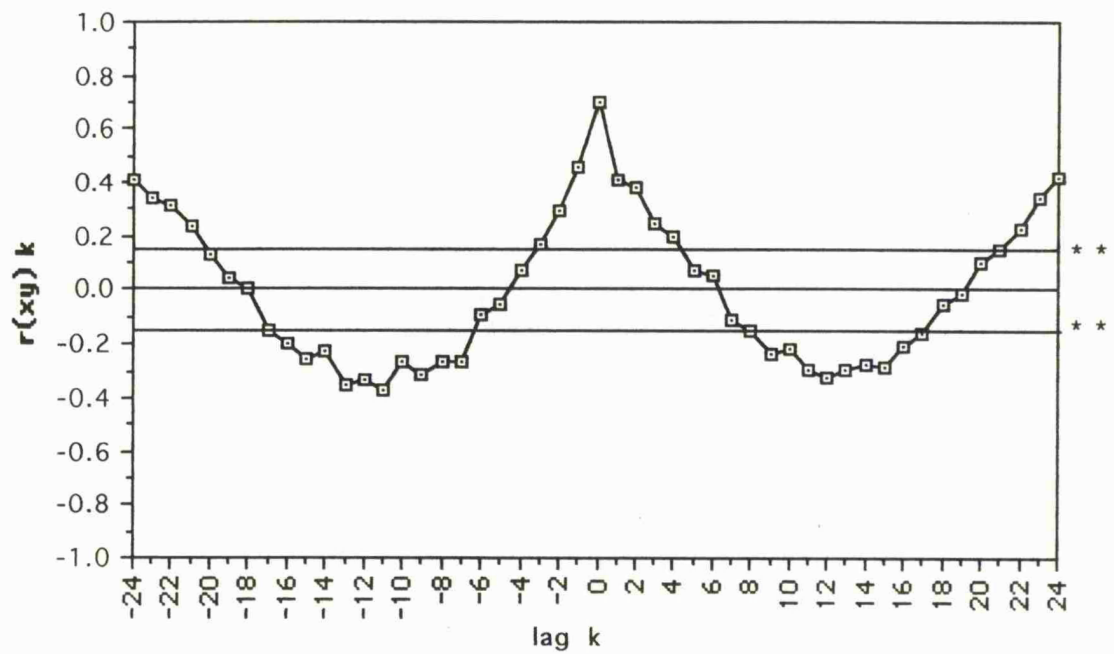


Figure 4.11. Cross correlation coefficients ($r(xy)k$) for time lags (k) -24 to +24 h, coefficients calculated from the comparison of in-burrow events and burrow emergence events of 13 *N. norvegicus* when sighted. This shows that for sighted individuals in-burrow and burrow emergence rhythms are similar.

** denotes 95% confidence limits.

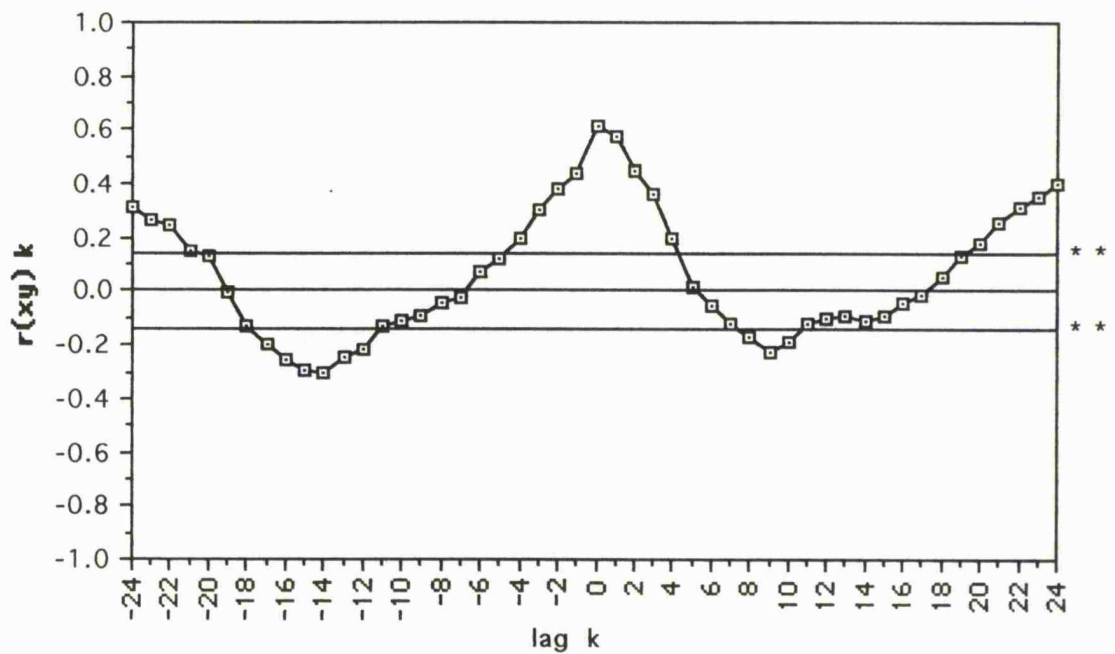


Figure 4.12. Cross correlation coefficients ($r(xy)k$) for time lags (k) -24 to +24 h, coefficients calculated from the comparison of in-burrow and burrow emergence events of 13 *N. norvegicus* when blinded. Therefore, in blinded individuals in-burrow and burrow emergence behaviour have similar rhythms.

** denotes 95% confidence limits.

	in-burrow events	emergence events
sighted individuals	0.72 (0.69)	0.85 (0.61)
blinded individuals	1.83 (0.88)	1.06 (0.55)

Table 4.1. Mean number of in-burrow and emergence events per hour (s.d. in brackets) for all 13 individuals when sighted and then after they had been blinded.

The results of this test showed a significant relationship between type of behaviour and the level of activity performed ($F = 10.7$, $p < 0.05$). While all 13 individuals were sighted they performed significantly more emergence events than in-burrow events. However, after all 13 individuals were blinded, they performed significantly less emergence events than in-burrow events. There was also a significant effect of blinding on both the number of in-burrow events and the number of emergence events performed by the individuals ($F = 24.9$, $p < 0.05$), with an increase in both after the individuals had been blinded.

This test also revealed an 'interaction effect' ($F = 45.31$, $p < 0.05$), where, after blinding, the increase in in-burrow events was greater than the increase in emergence events (Tukey test) (see Appendix I for details of this test).

4.3.3. Variation in rhythmic behaviour between individuals.

The in-burrow and burrow emergence behaviour of each individual used in this experiment was examined in order to find the number of individuals that clearly displayed the same 24 h rhythm of behaviour found in the combined results (section 4.3.1). Using autocorrelation analysis to identify any significant rhythm in the behaviour of individuals, it was found that the majority of individuals showed a 24 h rhythm of both in-burrow (12/13 sighted

individuals; 13/13 blinded individuals) and burrow emergence behaviour (10/13 sighted individuals; 12/13 blinded individuals). In each case, where no rhythm could be identified, there was very little activity over the seven days for which behaviour was recorded and so insufficient data was available for autocorrelation analysis to identify the presence or absence of a rhythm. Therefore, there was some variation between individuals in the expression of rhythms of in-burrow and burrow emergence behaviour, but no individuals were totally lacking in rhythmic behaviour. All individuals showed some rhythmic behaviour (in-burrow or burrow emergence behaviour) either when sighted, or blinded, or in both states.

4.3.4. The diel rhythm of in-burrow and burrow emergence behaviour.

The mean numbers of events per hour over 24 h for in-burrow and burrow emergence behaviour were examined for all individuals when sighted and then after they had been blinded. This gave four series of observations. For each series, the number of events between 10:00 - 10:59 h was counted for all 13 individuals and a mean number of events calculated. This was repeated for each subsequent hourly observation for a 24 h period (Fig. 4.13). The results show that individuals seem to perform most in-burrow and burrow emergence behaviour in the dark period, both when sighted and blinded.

Statistical analysis (i.e. independent measures t-test, two-tailed), using subsets (see Appendix I and section 4.2 'Materials and Methods' for details), confirmed that individuals performed significantly more in-burrow behaviour events in the dark period, both when sighted ($t = -3.14$, $p < 0.05$) and when blinded ($t = -2.97$,

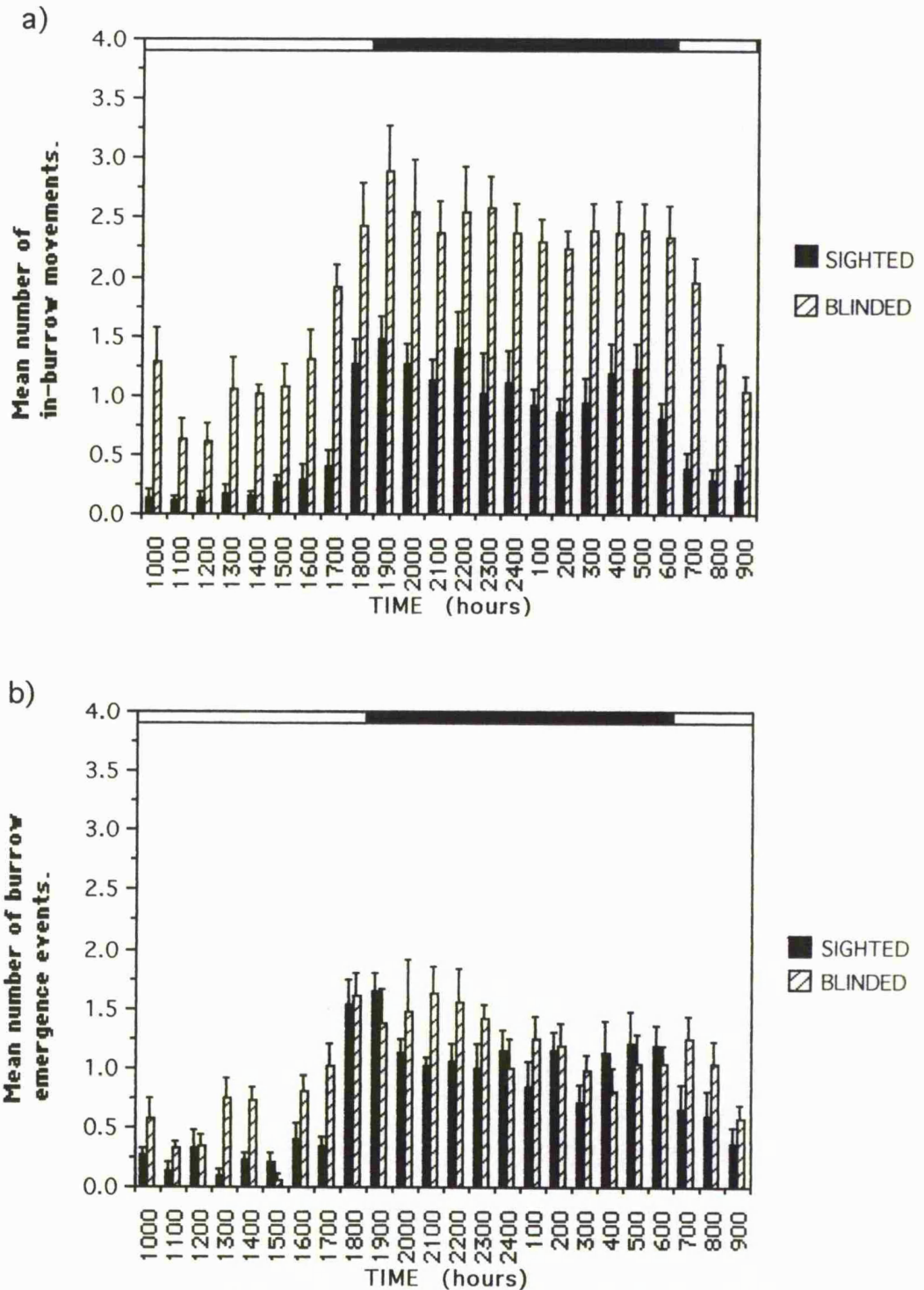


Figure 4.13. Mean 24 h cycle of a) in-burrow events and b) burrow emergence events. Individuals performed more in-burrow and emergence activity in the dark period when they were both sighted and blinded. Bar above graphs indicate 12 h light:12 h dark cycle in actographs (light periods = empty bars, dark periods = shaded bars).

$p < 0.05$). It was also revealed that individuals performed significantly more emergence behaviour events in the dark period, both when sighted ($t = -2.97$, $p < 0.05$) and when blinded ($t = -2.38$, $p < 0.05$).

Therefore it is possible to say that individuals performed a diel nocturnal rhythm of in-burrow and burrow emergence behaviour, when sighted and blinded.

4.4. Discussion.

4.4.1. Evidence for diel rhythms in individuals when sighted and then after blinding.

Clear diel rhythms of activity were found in the numbers of in-burrow and emergence behaviour events of sighted *N. norvegicus*. The results also demonstrated that the two rhythms were simultaneous. The sighted group of individuals exhibited a nocturnal peak of activity for in-burrow and burrow emergence behaviour. In a previous experiment (Chapter 3) a similar nocturnal rhythm was found in sighted *N. norvegicus* while observed in field conditions. These rhythms are also similar to those found in previous actograph studies using sighted *N. norvegicus* and comparable light:dark regimes (Arechiga and Atkinson, 1975; Hammond and Naylor, 1977; Naylor and Atkinson, 1976). The present study also showed that these rhythms persist and are unchanged after the same individuals were blinded. Cross correlation analyses failed to show any changes in the rhythms of in-burrow and burrow emergence behaviour after blinding. Cross correlation analyses also showed no changes in the rhythms of the blinded individuals during the period that they remained in the actographs. Similar rhythms also seemed to be unaffected by blinding in the previous field experiment (Chapter 3).

4.4.2. Levels of in-burrow and burrow emergence behaviour of individuals.

The results of the present study revealed that sighted individuals performed more emergence behaviour than in-burrow behaviour. This was reversed after individuals had been blinded. The reasons for this are unclear. In previous similar actograph studies measuring the same types of behaviour for sighted *N.*

norvegicus in 12 h light: 12 h dark regimes (Arechiga and Atkinson, 1975; Hammond and Naylor, 1976; Naylor and Atkinson, 1976) it was found that the levels of in-burrow and burrow emergence behaviour were similar. The field study results of the present actograph work are also at variance with those found in the field study described in this thesis (Chapter 3). This showed that there was no effect of blinding on the level of emergence behaviour of individuals. In the actographs there were significantly more emergence events after blinding. The reason for these differences is unclear.

The amount of in-burrow behaviour performed by individuals also increased significantly after blinding and did so significantly more than the increase in levels of emergence behaviour. Previous actograph studies also showed that in constant darkness *N. norvegicus* performs more in-burrow behaviour than emergence behaviour (Atkinson and Naylor, 1976).

4.4.3. Variation in behavioural rhythms between individuals.

Examination of the 24 h rhythms of in-burrow and emergence behaviour in all 13 individuals studied revealed that all showed rhythmic behaviour of one, or both, types of behaviour (in-burrow or emergence behaviour). Blinding had no effect on the presence or absence of rhythms. In the cases where no rhythm was found in a series of observations there was insufficient activity for the time series analysis to be carried out.

4.4.4. Limitations of the actograph apparatus.

The restricted and artificial conditions inside an actograph could have affected various aspects of behaviour. Normal in-burrow behaviour such as burrow maintenance (Naylor and Atkinson, 1976),

feeding on stored food items (Chapman, 1980) and migration between burrows (Chapman *et al.*, 1975) would not have been possible inside the actograph. Despite these artificial conditions, and the absence of other environmental factors (e.g. prey and predators), the individuals inside the actographs displayed a diel rhythm of either, or both, in-burrow and emergence behaviour with similar characteristics to that found in the previous field study (Chapter 3) and also observed in their natural environment (Chapman and Rice, 1971). Once again this suggests that the diel rhythms of *N. norvegicus* are extremely robust.

In the previous field experiment where emergence behaviour of *N. norvegicus* was observed in a mixed population of sighted and blinded individuals it was not known if there was any effect of the rhythmic behaviour of one group on the behaviour of the other. The results of the present actograph study suggest that there was no such interaction because isolated sighted and blinded individuals showed similar rhythms to those found in the field experiment.

4.5. Conclusion.

The results of the actograph study show that sighted *N. norvegicus* perform a 24 h rhythm with a nocturnal peak of activity for both in-burrow and burrow emergence behaviour. These rhythms of behaviour were unaffected by blinding.

Looking at the mean levels of behaviour performed, there was greater in-burrow and emergence behaviour after all individuals were blinded. It was also found that the sighted individuals performed more emergence behaviour than in-burrow behaviour, but after blinding this was reversed and they performed more in-burrow behaviour.

All individuals showed a 24 h rhythm for either in-burrow or emergence behaviour, or for both types of behaviour.

The results of this actograph study confirm those of the previous field study (Chapter 3) in demonstrating that blinded *N. norvegicus* display a 24 h rhythm, with a nocturnal peak, of emergence behaviour. However, in the actographs, blinding an individual resulted in a significant increase in its emergence activity. In a mixed population of blinded and sighted *N. norvegicus* in the field the levels of emergence behaviour were similar in both groups. The reasons for the disagreement of these two studies is unclear.

Chapter 5 The effect of predator stimuli on anti-predator behaviour.

5.1. Introduction.

Predation is a major source of mortality for most animals (Sih, 1987) and, in order to survive, an animal must be able to avoid detection and capture by predators. Anti-predator behaviour can be categorised into predator avoidance, escape and protective aggression (Edmunds, 1974). The following investigation was carried out to examine the possible effects of blinding on anti-predator behaviour of *N. norvegicus*. The main emphasis was on predator avoidance, but some observations were made on escape behaviour and aggression.

While sheltered inside its burrow *N. norvegicus* is protected from encounters with predators, but becomes vulnerable when it emerges onto the sea bed to perform tasks necessary for survival and future fitness (e.g. foraging) (Howard, 1989). Burrow emergence follows a rhythm of behaviour with an active period which has been shown to coincide with a specific range of low light intensities (Chapman, 1980). It has been suggested that emergence at these light intensities minimises predation risk from visual predators (Chapman and Rice, 1971; Chapman *et al.*, 1975). This is consistent with what has been found in other species. For example, pelagic species of *Artemia* (Forward and Hettler, 1992) and *Daphnia* (Meester *et al.*, 1995) descend at sunrise to avoid visual predators. Avoidance behaviour also occurs in benthic decapods such as *Homarus americanus* and *H. gammarus*. They emerge from shelter during specific low light intensities known to be associated with low predation levels (Cobb and Wang, 1982).

N. norvegicus is one of the main prey species of the cod (*Gadus morhua*) (Howard, 1989) and large numbers of *N. norvegicus* are found in their stomachs (Armstrong, 1982). *G. morhua* commonly forages using vision, but is also able to detect prey odours using chemoreceptors on its pelvic fins and ventral barbel (Brawn, 1969). Since it is potentially present at all times of the 24 h cycle (Dann, 1973; Hall *et al.*, 1990), *N. norvegicus* is vulnerable to predation whenever it is emerged on the sea bed.

Predator avoidance involving shelter use has been described in a number of species. *Homarus americanus* retreats from predators into rock crevices and may excavate its own burrows (Cobb, 1971). Spiny lobsters and rock lobsters (*Palinuridae*) aggregate together in coral and rock crevices during the day to avoid diurnally active predators (Eggleston and Lipcius, 1992). In the present study, burrow occupancy was used as a measure of predator avoidance behaviour in a laboratory population of mixed sighted and blinded *N. norvegicus*. The main question that was asked was: "Does shelter use by blinded *N. norvegicus* differ from that of sighted ones when there is an increase in predation risk?". Groups of *N. norvegicus* were held in a large tank and their emergence activities were monitored. The experiments were designed to see whether emergence activity would decline in the presence of a predator or its odour. Aquatic organisms can be extremely sensitive to low concentrations of chemicals. The blue crab (*Callinectes sapidus*) is able to detect amino acids at concentrations as low as 10^{-5} g/l (Derby and Atema, 1988).

The present investigation was conducted using two groups of *N. norvegicus*, one examined in April and the other in October 1993. For the first group, the effects of placing a cod in the same tank as sighted and blinded *N. norvegicus* were assessed. With the second

group the procedure was adjusted to measure the effects of both the physical presence of a cod and its odour alone. The second group was also used to test the response by *N. norvegicus* to a fish species that is not its natural predator. The aim of the experiment was to see if non-predatory species of fish have similar effects on emergence behaviour to those caused by a predatory one. The non-predatory species chosen was the saithe (*Pollachius virens*). This is a pelagic fish (Berstad, 1991; Sarno *et al.*, 1994) that feeds on pelagic decapod larvae, calanoid copepods and euphausiids (Robb, 1981; Hall *et al.*, 1990).

A final objective was to characterise the response of unsheltered *N. norvegicus* to the presence of a nearby predator. It is known that *N. norvegicus* possesses a 'tail flip' escape response (Newland *et al.*, 1988) and that it can adopt threatening postures by raising the chelae (Atema and Cobb, 1980). In the American lobster (*Homarus americanus*), rapid backwards locomotion using a 'tail flip' is also used to escape from predators, this both startles the predator and allows the lobster to distance itself from the immediate area of danger (Lang *et al.*, 1977). If escape is not immediately possible, the crayfish (*Procambarus clarkii*) may avoid capture by attacking the predator with its chelae (Hayes, 1977). This protective aggression (Edmunds, 1974) also startles the predator and increases the chance of the crayfish surviving the encounter (Sih, 1987). In the present study, observations were made on escape behaviour and aggression by *N. norvegicus* in response to approaches by cod and saithe.

5.2. Materials and methods.

Two investigations were carried out at the Fish Behaviour Unit at SOAFD Marine Laboratory, Aberdeen, using similar apparatus and procedures in each case.

The experimental tank was circular, 3 m in diameter and filled with sea water to a depth of 1.2 m. This tank was connected to the Fish Behaviour Unit sea water circulation system, providing the tank with a continuous flow of filtered and aerated sea water. A 20 mm layer of fine sand covered the tank base. The mean sea water temperature (monitored throughout both experiments) was 11.60°C (s.d. = 0.09) in April and 11.56°C (s.d. = 0.12) in October. A second smaller holding tank was also connected to the sea water circulation system to provide temporary housing for *N. norvegicus* before use in the larger experimental tank.

Both tanks were situated in a light-tight room allowing controlled lighting conditions. Two lamps fitted with green filters (peak transmission = 540 nm, band width at 50% transmission = 53.64 nm) were positioned above the circular tank so that the base of the tank was lit evenly. The resulting maximum light level on the tank floor ($0.19 \mu\text{mol m}^{-2}\text{s}^{-1}$) provided illumination similar to peak levels found at 30 m during September at Loch Torridon (Shelton *et al.*, 1985). Lights were computer controlled so that dawn and dusk could be simulated. After sunrise the lamps gradually came on over a period of 15 minutes and after sunset the light level fell over 15 minutes. The computer was programmed so that artificial sunset and sunrise occurred at local dusk and dawn (times set according to the Nautical Almanac, 1993).

Additional lighting for video recording was provided by four underwater lights ('Hydroproducts') fitted with red acrylic cut-off filters. These underwater lights were distributed inside the tank

and placed above the base so that they caused minimum shadow on the recorded image. The red lights remained switched on throughout the experiments so that activity in the tank could be monitored continually using a suitable video recording system.

The video camera (Panasonic, with a silicon intensifier tube), fitted with a 6.5 mm wide angle lens, was suspended vertically above the main circular tank and was fixed at a suitable height so that the whole of the tank base could be viewed. A Sony 9 inch monitor was used to display activity in the tank and a time lapse video cassette recorder was used for continuous recording throughout the experiment (see section 2.5 for details of video recording equipment).

Artificial burrows were constructed from 0.3 m lengths of plastic pipe (0.065 m diameter) sealed at one end so that they had a single entrance. Twenty five of these artificial burrows were randomly distributed on the floor of the experimental tank. Similar artificial burrows were placed in the temporary holding tank.

5.2.1. Preparation of N. norvegicus.

Twenty male *N. norvegicus* (mean carapace length = 36.14 mm, s.d. = 1.36) were temporarily housed in the smaller holding tank to acclimatise to the artificial day:night cycle of the tank room. After ten days, they were divided into two groups of ten individuals. Those in the first group were blinded and labelled using white discs of 10 mm diameter. These discs were wired on to the upper side of the merus of the left cheliped and positioned to allow unrestricted movement. The remaining ten *N. norvegicus* remained sighted and a 'handling control' was carried out with these individuals so that both groups experienced a similar amount of handling. Members of the sighted group were each labelled with

a white disc fixed to the right cheliped of each individual. Both groups were returned to the temporary holding tank for a further 3 days prior to the experiments.

5.2.2. Video tape analysis.

The video tape recordings were reviewed to assess the effects of different predator stimuli on the behaviour of *N. norvegicus* in the tank. Levels and rhythms of burrow emergence behaviour for both sighted and blinded individuals were noted during 15 minute sample periods starting at the beginning of each hour throughout the course of the experiments. The 15 minute periods were reviewed as follows: the image was frozen at 30 s intervals throughout each 15 minute period, and the number of sighted and blinded individuals seen fully emerged from their shelters were noted for each of the 30 resulting images. Emergence events for sighted and blinded individuals were treated separately. An emergence index was calculated by adding together the number of emergence events in all frozen images for that 15 minute sample. This was then divided by the total number of sighted or blinded individuals in the tank (maximum emergence index value = 30). Only individuals which were fully out of an artificial shelter were considered to be 'emerged' and no distinction was made between individuals which were stationary or moving around the tank.

5.2.3. Statistical analysis.

A two-way mixed design ANOVA (two-tailed) was used to compare the levels of emergence behaviour performed by sighted and blinded groups of individuals in the different stages of each experiment. It is not possible to use autocorrelated data with this

type of analysis. Therefore it was necessary to take a subset from each series to be compared. The seventh data point was taken from a series of emergence behaviour and each subsequent seventh data point in the series was added to form a complete subset (see Appendix I for further details).

An autocorrelation analysis (see Appendix I for details of test) was carried out on each five day series of emergence behaviour in order to identify rhythms present in the sighted and blinded groups of individuals used.

A probability level of 0.05 was used for all statistical analyses carried out.

5.3. Results.

The investigation involved two separate groups of male individuals examined in April (experiment 1) and October 1993 (experiments 2 and 3). The results for each experiment are presented separately below.

5.3.1. Experiment 1 - Effects of the physical presence of a cod on burrow emergence by sighted and blinded *N. norvegicus*.

The experiment took place over 15 days, divided into three periods of 5 days (see Fig. 5.1 below for details of procedure).

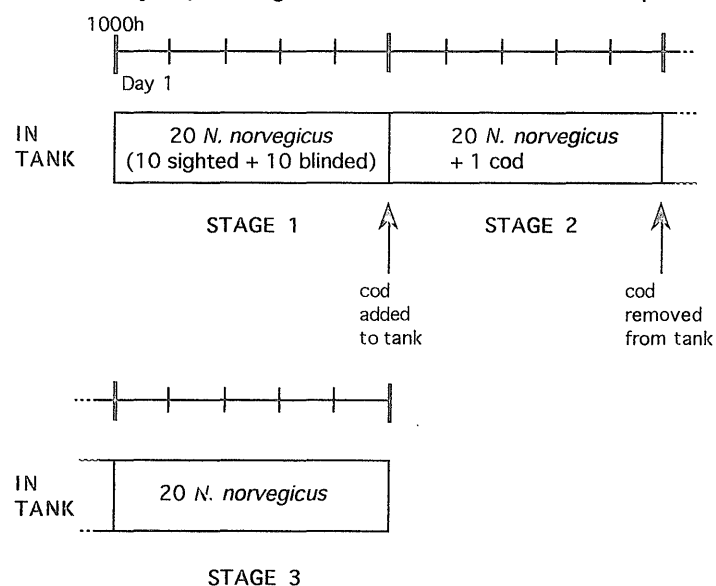


Figure 5.1. Procedure for experiment 1.

Ten blinded and ten sighted *N. norvegicus* were released into the main tank and continuous recording of their behaviour was begun at 10.00 h on day 1 of the experiment. At 10.00 h on day 6, a previously starved cod (total body length 0.51 m) was released into the main tank. It was removed at 10.00 h on day 11. At the end

of the experiment (i.e. 10.00 h, day 15) individuals were removed from the tank and their carapace lengths were noted. The eyes from each individual were removed and placed in formal calcium fixative for histological examination.

Throughout the experiment, levels of activity were monitored so that they could be compared for periods prior to, during and after introduction of the cod. Emergence levels for sighted and blinded individuals at each stage are recorded in Table 5.1 below and shown in Figure 5.2.

	stage 1 control period	stage 2 cod placed in tank	stage 3 control period
sighted group of individuals	3.98 (1.59)	0.081 (0.41)	9.89 (3.92)
blinded group of individuals	3.71 (2.29)	0.728 (1.36)	6.48 (4.60)

Table 5.1. Mean emergence index (with s.d.) for the sighted and blinded groups of individuals in experiment 1.

During the first control period (stage 1), both sighted and blinded individuals readily occupied the shelters and emerged to move around the tank base (Fig. 5.2. a). The ANOVA test (two-way mixed design, two-tailed) showed that the level of emergence behaviour performed by the sighted group of individuals in stage 1 was significantly higher than that of the blinded ones ($F = 6.22$, $p < 0.05$). After the cod was introduced into the tank (Fig. 5.2 b) both groups of individuals showed a reduced level of emergence activity, though the blinded group of individuals emerged significantly more than the sighted ones (ANOVA, two-way mixed

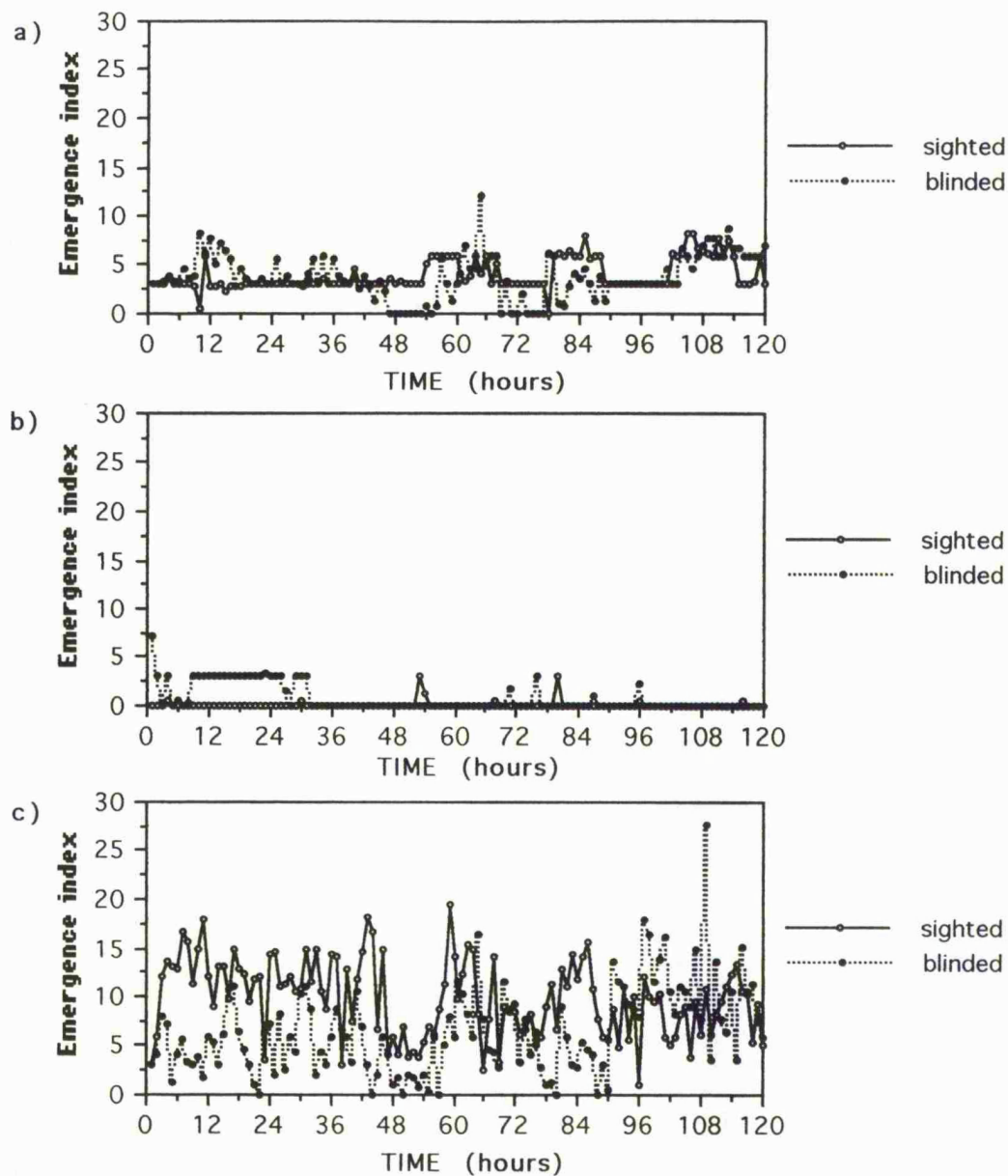


Figure 5.2. Experiment 1. The emergence behaviour of sighted and blinded groups of *N. norvegicus* a) before, b) during and c) after a cod was present in tank. All *N. norvegicus* showed reduced levels of emergence when cod was present.

design, two-tailed: $F = 6.22$, $p < 0.05$). When the cod was removed there was an immediate increase in emergence behaviour for both sighted and blinded individuals (Fig. 5.2. c), this was especially apparent for the sighted group (ANOVA, two-way mixed design, two-tailed: $F = 6.22$, $P < 0.05$).

An ANOVA (two-way mixed design, two-tailed) was performed to compare levels of emergence behaviour of sighted and blinded individuals in the three different stages of this experiment. This test showed that there was a significant effect of vision on levels of activity performed ($F = 6.22$, $p < 0.05$). It was also shown that the amount of emergence behaviour performed by each group of individuals in the tank (i.e. sighted group or blinded group) was significantly different in each of the three stages of the experiment ($F = 92.93$, $p < 0.05$; Tukey test). Activity of both groups of individuals was suppressed while the cod was present and increased after the cod was taken out of the tank. There was also significant interaction between visual state of an individual and stage of the experiment, with sighted individuals showing a markedly higher level of behaviour than blinded ones after removal of the cod from the tank ($F = 5.61$, $p < 0.05$; Tukey test).

These results indicate that the level of anti-predator behaviour was affected by visual status. During control periods blinded individuals spent more time inside their burrows than the sighted ones. However, when the cod was present in the tank, it was the sighted group of individuals that spent more time in their burrows.

An attempt was made to assess the effect of cod on the emergence rhythms of the *N. norvegicus* (see Fig. 5.3. for results of autocorrelation analyse). The only evidence for a significant 24 h rhythm of emergence behaviour was seen in the sighted group of

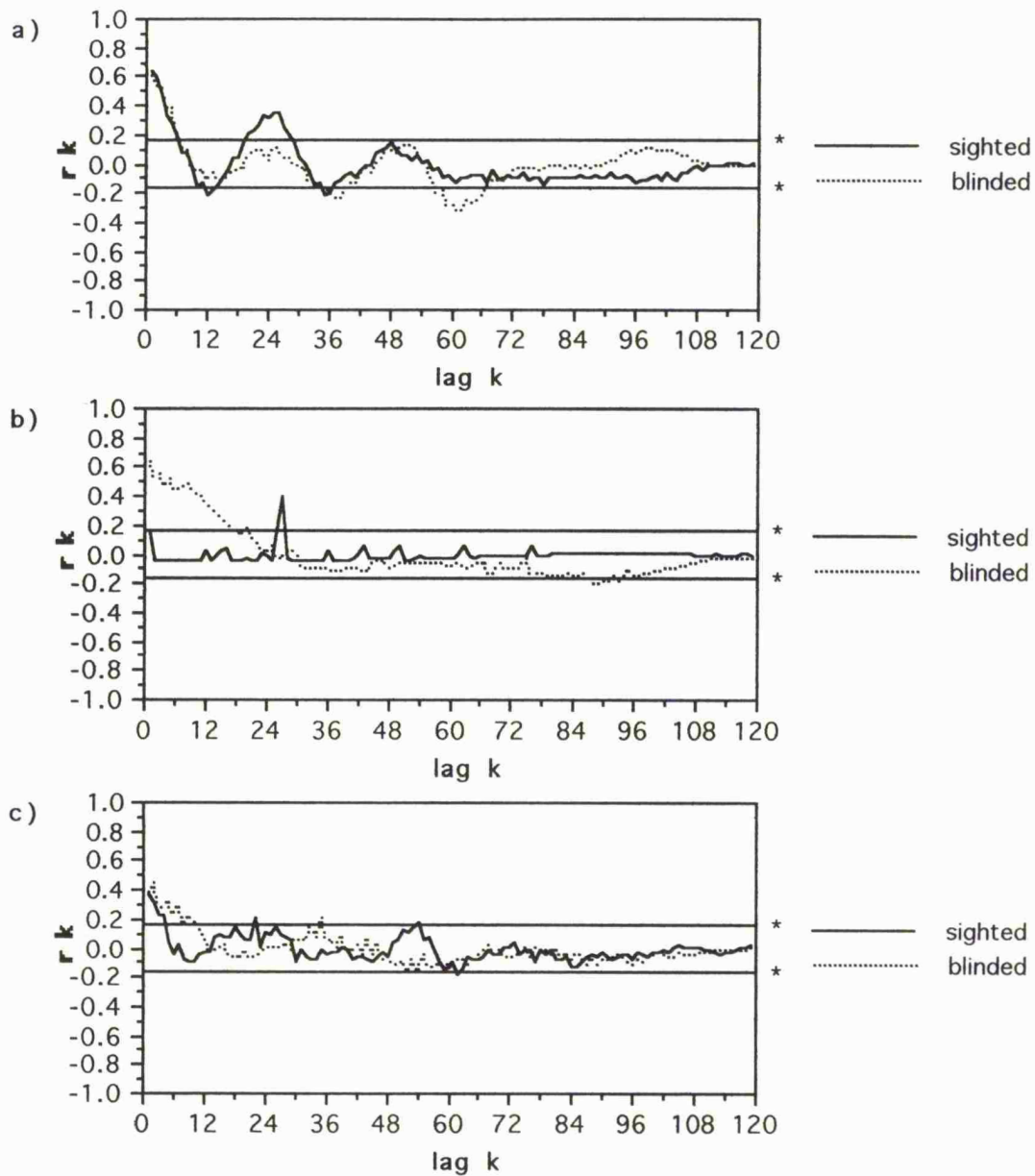


Figure 5.3. Experiment 1. Autocorrelation coefficients for series of emergence behaviour of sighted and blinded groups of *N. norvegicus* a) before, b) during and c) after a cod was present in the same tank. The only series showing clear evidence for a 24 h rhythm of behaviour was the sighted group, during the first control periods (a). * indicates 0.05 significance levels

individuals before and after the cod was in the tank. A similar 24h rhythm was detectable for blinded individuals, but it was not significant ($p>0.05$). In the presence of the cod, levels of activity were very low for sighted and blinded groups of individuals (mean emergence indices = 0.3% and 2.4% of maximum possible value for sighted and blinded individuals respectively). This low number of events meant that the correlogram cannot identify any rhythms in the series. The spike at 28 h (Fig. 5.3 b) was probably due to a correlation between the two peaks of emergence of sighted individuals which occurred at 53 - 54 hours and 80 hours after the cod was placed in the tank. After the cod was removed, only sighted individuals showed any evidence for a weak 24 h rhythm. Blinded individuals showed a complete absence of a 24 h rhythm (Fig. 5.3 c).

5.3.2. Experiment 2 - Burrow emergence of sighted and blinded *N. norvegicus* in response to either the physical presence of a cod or just the odour of a cod.

The experiment took place over 20 days and was divided into four periods of 5 days (see Fig. 5.4 below for details of procedure).

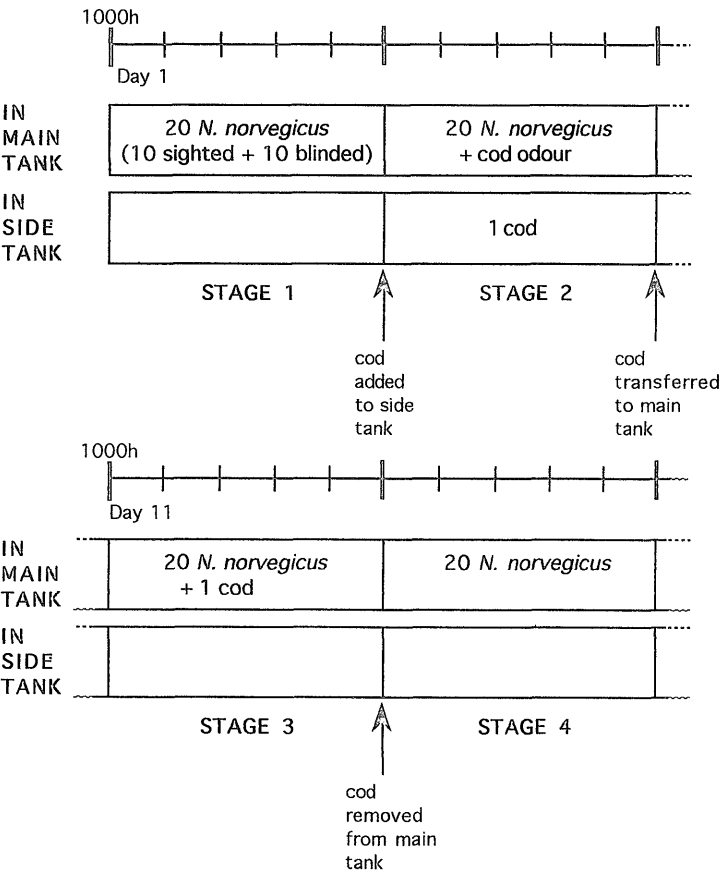


Figure 5.4. Procedure for experiment 2.

To deliver cod odour into the main tank another 2 m diameter circular tank (depth of sea water = 1.8 m) was positioned next to the one used in the previous experiment. Sea water from this tank

was then pumped (A.B.S. pump 240 V, flow volume = $3.7 \text{ m}^3\text{h}^{-1}$) into the main tank. When cod odour was required a single fish was placed in the side tank. From the main tank, the water flowed back into the Fish Behaviour Unit circulation system.

Light conditions and preparation of *N. norvegicus* were as described in experiment 1 (section 5.2.). Twenty labelled *N. norvegicus* (10 blinded and 10 sighted) (mean carapace length = 37.34, s.d. = 3.5) were released into the main tank on day 1 of the experiment and continuous recording was begun. The first 5 days of the experiment acted as a control period. A starved cod (total body length 0.5 m) was released into the side tank at 10.00h on day 6. This same cod was transferred into the main tank at 10.00h on day 11 and then removed on day 15. During the final 5 days of the experiment there was no cod stimulus. Emergence behaviour of both groups of individuals was recorded throughout the 20 days of this experiment (Fig. 5.5) and noted in Table 5.2.

	stage 1 control period	stage 2 with cod odour	stage 3 with cod in tank	stage 4 control period
sighted group of individuals	6.79 (3.70)	1.23 (1.72)	0.09 (0.49)	0.83 (1.64)
blinded group of individuals	10.19 (4.45)	6.94 (2.67)	1.18 (2.02)	3.43 (4.44)

Table 5.2. Mean emergence index (s.d. in brackets) for the sighted and blinded groups of individuals in stages 1 - 4 of experiment 2.

The first five days of the experiment (stage 1) acted as control period and the *N. norvegicus* readily occupied the shelters as before but both blinded and sighted individuals showed

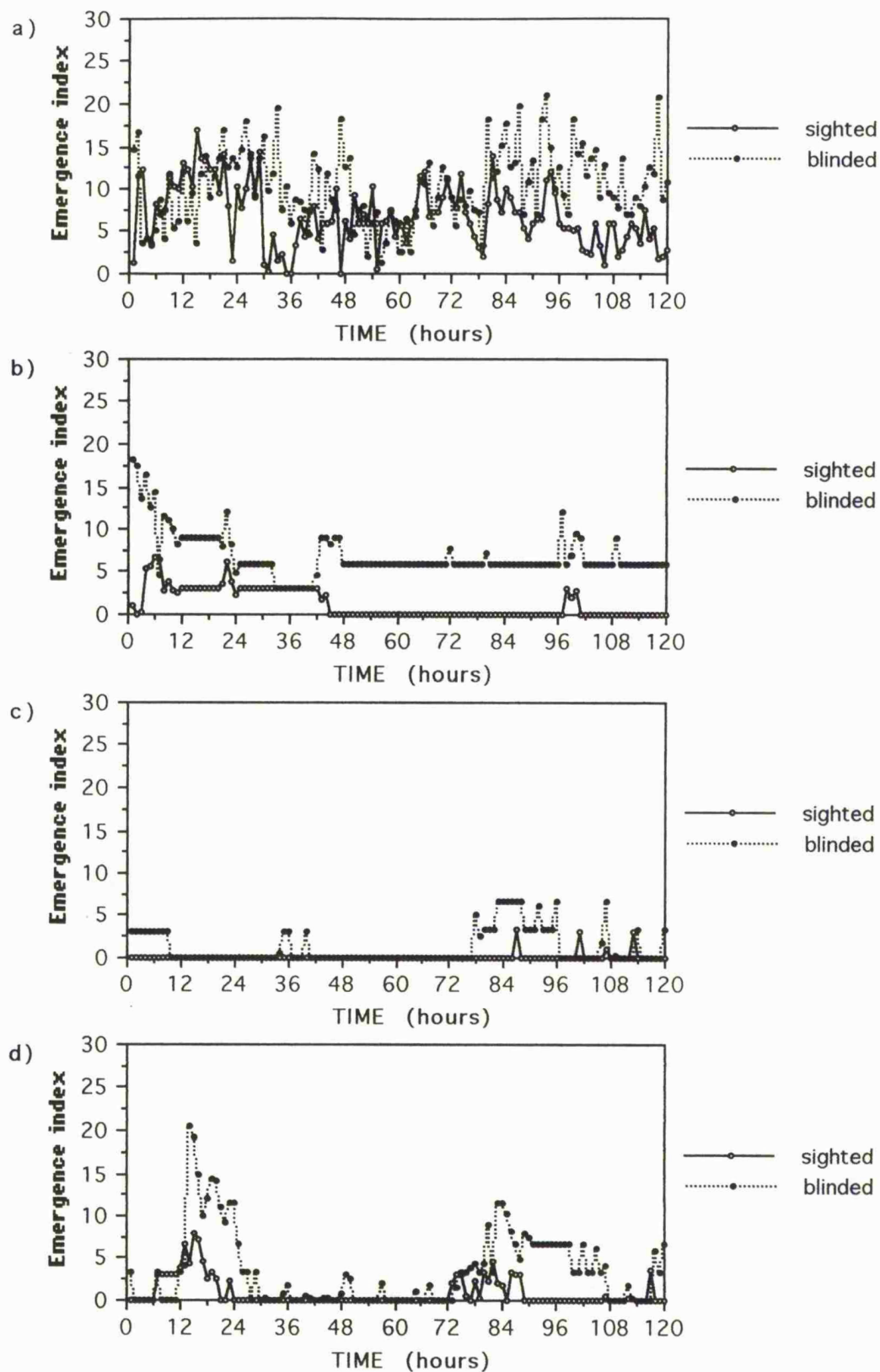


Figure 5.5. Experiment 2. The emergence behaviour of both groups is reduced after introduction of cod odour (b) and then further reduced when cod placed in same tank as *N. norvegicus* (c). Control periods before (a) and after (d) predator stimuli in tank.

considerable emergence activity (Fig. 5.5 a). After the cod was placed in the side tank (stage 2) the number of emergence events of both groups decreased (Fig. 5.5 b) and there were further decreases after the cod was transferred into the main tank (stage 3) (Fig. 5.5 c). The cod was then removed (stage 4) and the level of emergence behaviour of sighted and blinded individuals increased slightly (Fig. 5.5 d), although not to the level seen at the first stage of the experiment.

Statistical analysis (ANOVA, two-way mixed design two-tailed) was carried out to examine the effects of different predator stimuli (i.e. chemical or physical presence of a cod) on emergence behaviour of sighted and blinded groups of individuals. This test compared number of emergence events for sighted and blinded individuals in all four stages of the experiment (Table 5.2).

Blinding appeared to have had a significant effect on levels of emergence activity ($F = 23.13$, $p < 0.05$). In all four stages, blinded individuals displayed higher levels of emergence behaviour than sighted ones. It was also revealed that for each group of individuals, levels of emergence behaviour were significantly different in each of the four stages of the experiment ($F = 46.71$, $p < 0.05$).

For both groups of individuals, cod odour significantly reduced emergence behaviour which was further significantly reduced when the cod was physically present. After the cod was removed from the tank, emergence behaviour of sighted and blinded individuals significantly increased.

An interaction effect between stage and state of vision was also shown ($F = 3.01$ $p < 0.05$). The blinded individuals displayed particularly high levels of emergence activity in stages 1, 2 and 4 of the experiment.

Autocorrelation analysis was carried out for each of the four stages of the experiment (Fig. 5.6) and in no case was a significant 24 h rhythm detected. Since there was no evidence for a 24 h rhythm in the first control period no inferences can be drawn from the absence of 24 h rhythms in all subsequent stages.

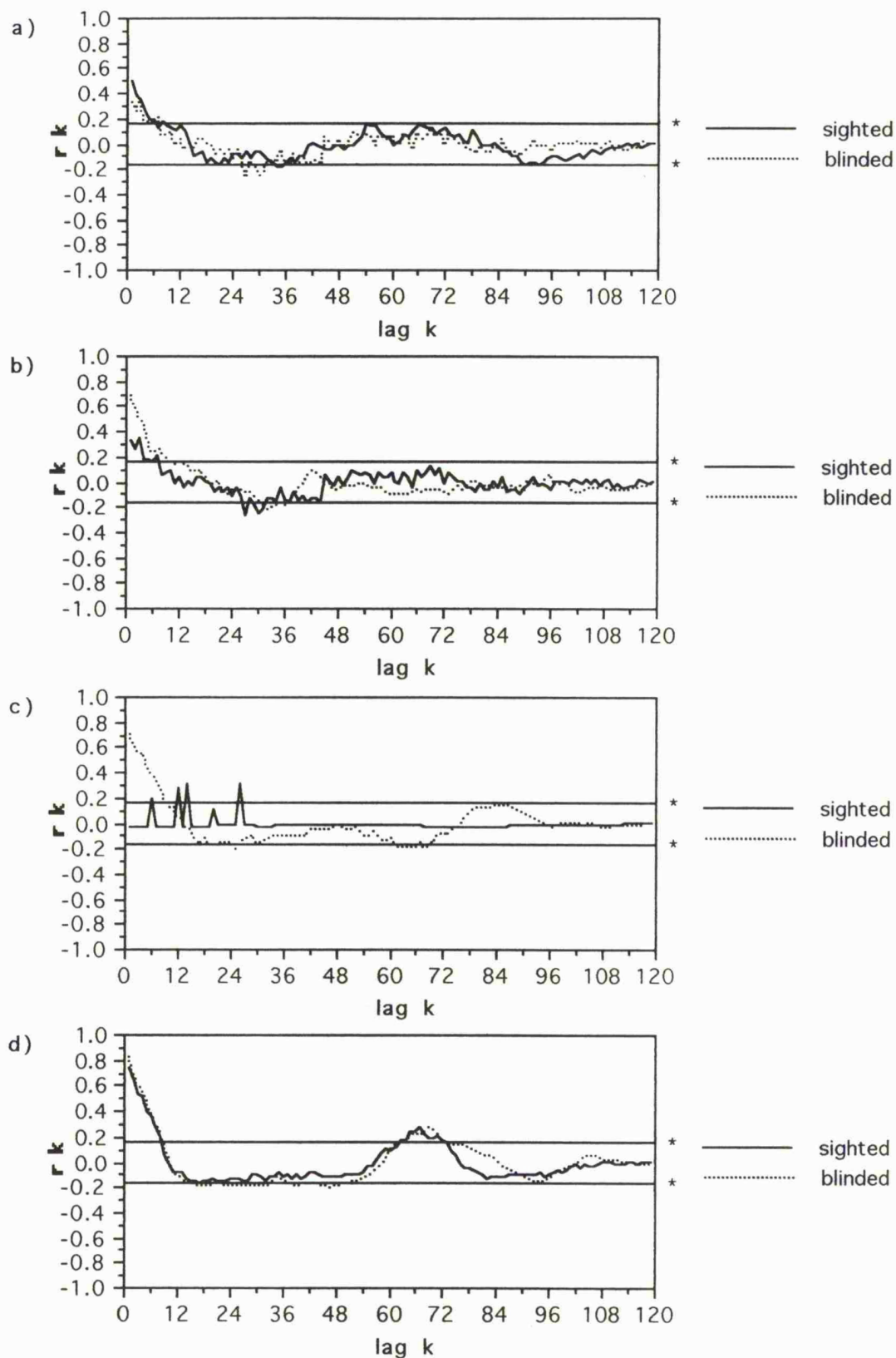


Figure 5.6. Experiment 2. Autocorrelation coefficients for emergence behaviour of individuals when cod odour (b) and cod (c) present in tank and control periods (a) and (d). No evidence for a 24 h rhythm in any stage. * indicates 0.05 significance levels.

5.3.3. Experiment 3 - Behavioural response of sighted and blinded *N. norvegicus* in the presence of a saithe.

In the previous experiments, both sighted and blinded *N. norvegicus* showed reduced emergence behaviour in response to the physical presence of a cod and its odour. Following experiment 2 (section 5.3.2.) the same individuals were used to assess their response to a pelagic fish species that does not normally predate on *N. norvegicus* (Sarno *et al.*, 1994). Thus, experiment 3 followed immediately after experiment 2 and the final control period of experiment 2 (i.e. stage 4) served as the initial control period of experiment 3 (see Fig. 5.7 overleaf for details of procedure).

At the end of the stage 4 control period, a previously starved saithe (total body length 0.67 m) was released into the main tank containing sighted and blinded individuals and left for 5 days (stage 5). At the end of stage 5, the saithe was removed, leaving the *N. norvegicus* in the tank for a final 5 day control period (stage 6). At the end of experiment 3 all individuals were removed and their eyes examined to check the degree of light-induced eye damage. Emergence behaviour of each group was monitored throughout the 15 days of this experiment (Fig. 5.8) and a summary of the results is given in Table 5.3 overleaf.

Introduction of the saithe led to a reduction in emergence behaviour of sighted and blinded groups of individuals (Fig. 5.8 b). Following the removal of the saithe there appeared to be only a slight increase in emergence events for sighted and blinded individuals (Fig. 5.8 c).

Emergence behaviour of both groups before, during and after a saithe was present in the same tank, were compared using an ANOVA technique (two-way mixed design, two-tailed). The results

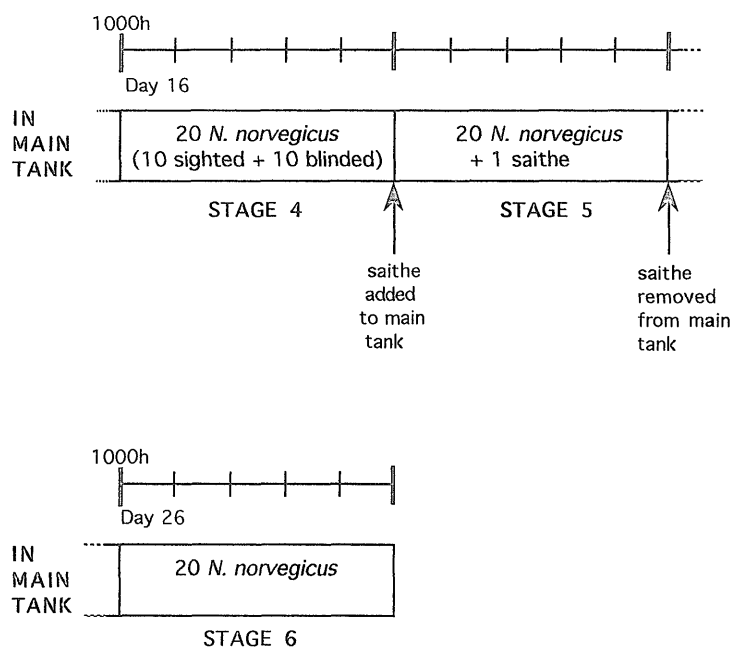


Figure 5.7. Procedure for experiment 3 (note that stage 4 was common to both experiments 2 and 3).

	stage 4 control period	stage 5 saithe placed in tank	stage 6 control period
sighted group of individuals	0.83 (1.64)	0.07 (0.34)	0.15 (0.42)
blinded group of individuals	3.43 (4.44)	1.21 (1.96)	1.71 (2.26)

Table 5.3. Mean emergence index (s.d. in brackets) for the sighted and blinded groups of individuals in experiment 3.

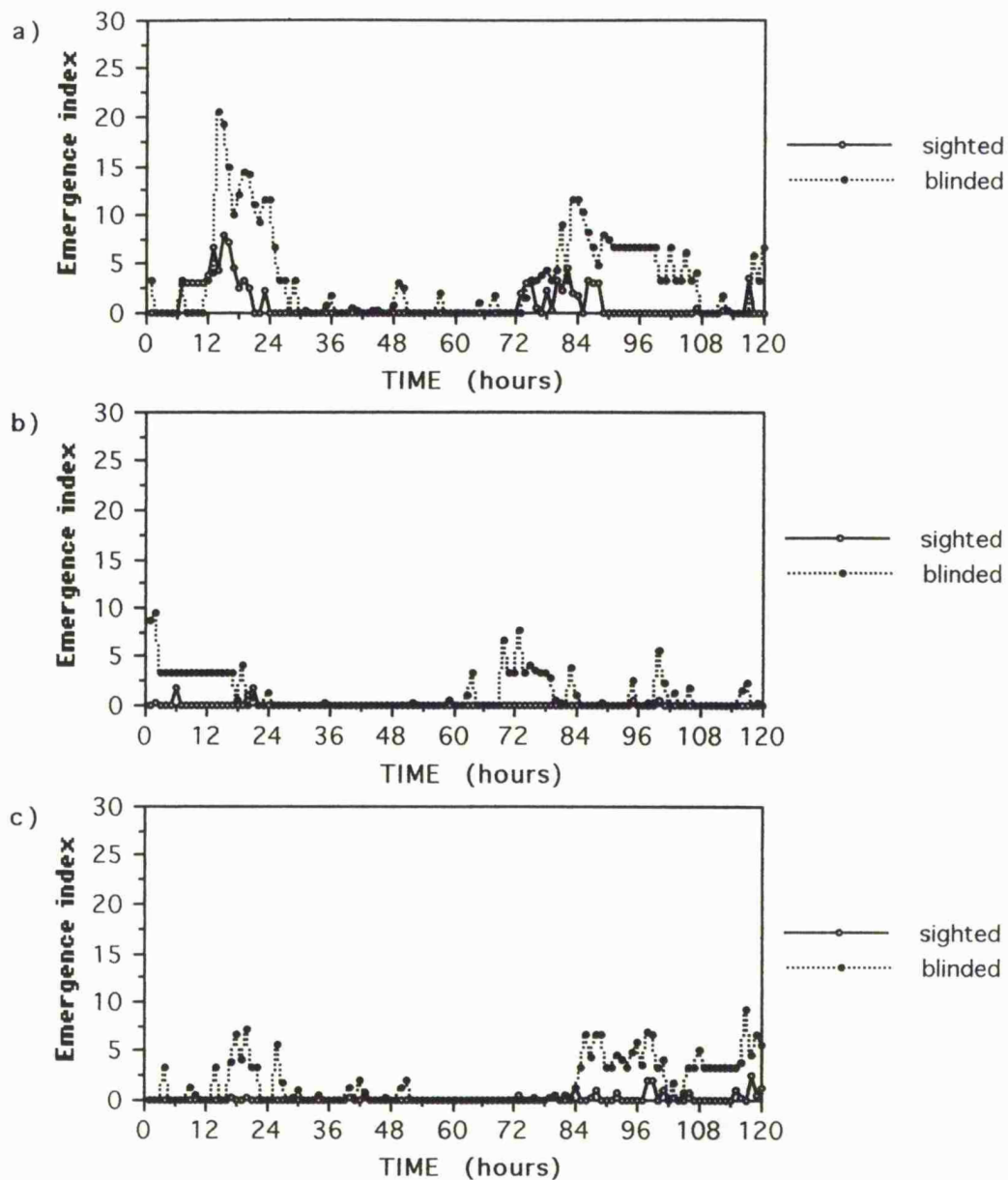


Figure 5.8. Experiment 3. The emergence behaviour of sighted and blinded groups of *N. norvegicus* prior to introduction of saithe (a), in presence of saithe (b) and after saithe removed (c). Both groups of individuals showed a reduction in emergence behaviour when the saithe was placed in the tank. (Note that stage (a) corresponds to stage (d) in Fig. 5.5).

showed that the blinded group of individuals performed a significantly higher level of emergence behaviour than the sighted ones in all three stages of the experiment ($F = 14.97$, $p < 0.05$). It was also shown that for both sighted and blinded groups, levels of emergence behaviour were significantly higher in the first control period (stage 4) than in the other two stages ($F = 5.77$, $p < 0.05$). In the latter two stages the emergence behaviour of the sighted individuals did not change significantly after the saithe was removed (Tukey test). The emergence behaviour of the blinded individuals also showed no significant change (Tukey test).

There was an interaction effect ($F = 3.54$, $p < 0.05$) where the blinded individuals showed an especially high level of activity in the initial control period.

Autocorrelation analysis revealed no 24 h rhythm of emergence behaviour at any stage of experiment 3 (Fig. 5.9).

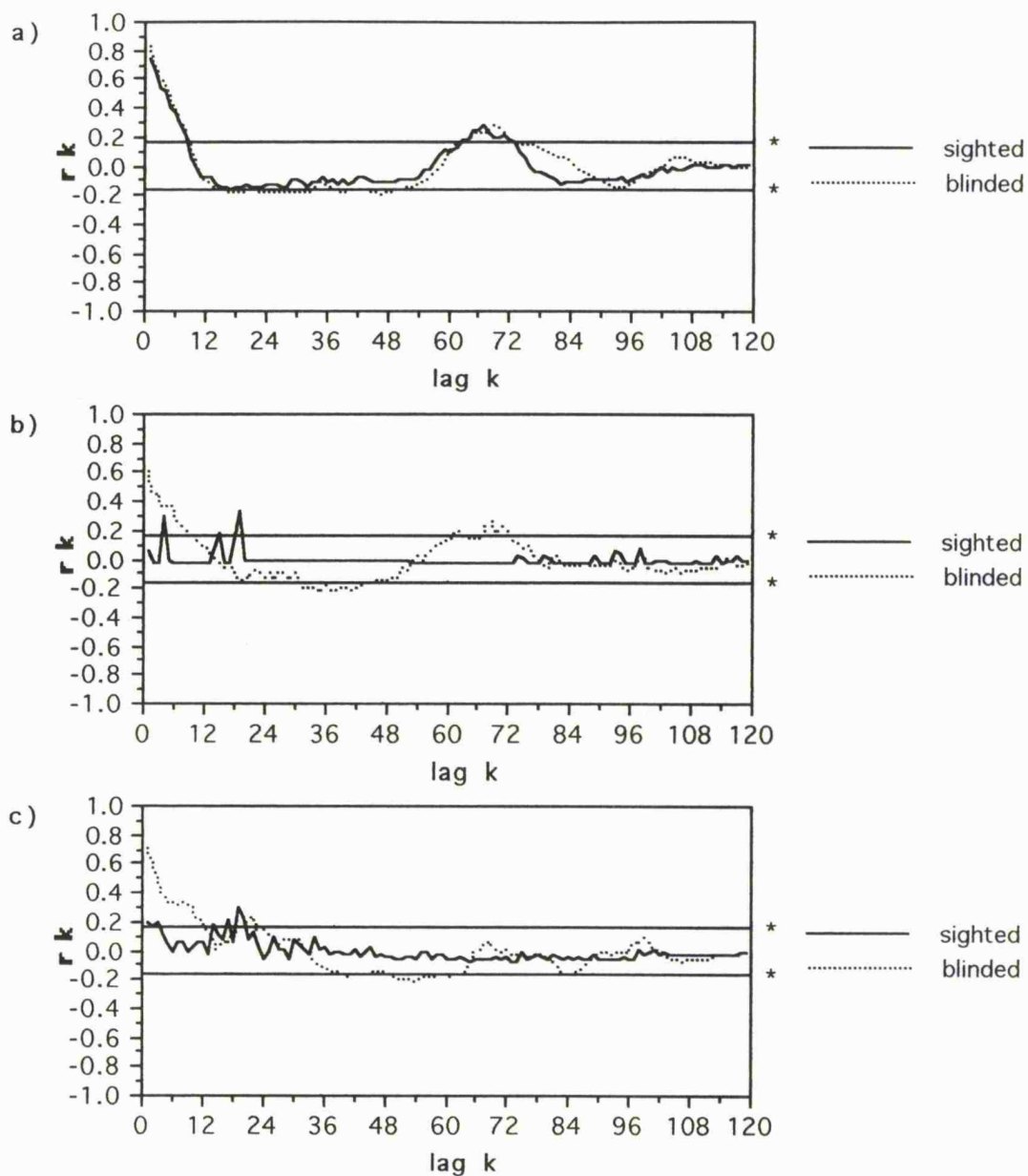


Figure 5.9. Experiment 3. The autocorrelation coefficients for emergence behaviour of sighted and blinded individuals prior to introduction of saithe (a), in presence of saithe (b) and after saithe removed from tank (c). No evidence for a 24 h rhythm in any stage. * indicates 0.05 significance levels.

5.3.4. Observations made on fish behaviour and responses of *N. norvegicus* to both cod and saithe.

Video recordings of experiments 1,2 and 3 were reviewed for sequences showing interactions between each species of fish and the *N. norvegicus*. In the case of the cod, the fish were seen swimming throughout the whole tank and did not seem to favour any particular area. The *N. norvegicus* inside the shelters often remained near to the entrances with parts of their chelae exposed. If the cod approached a shelter, its occupant was often seen to retreat rapidly further inside. The cod was occasionally seen moving shelters, sometimes causing the 'eviction' of a shelter's occupant. Occasionally the cod was seen to 'drag' an individual *N. norvegicus* out of its shelter. These individuals escaped and regained shelter. As the cod swam close to an emerged *N. norvegicus*, the *N. norvegicus* would either escape by walking rapidly backwards, or performing a tail flip. The *N. norvegicus* were also observed performing threat behaviour towards the cod, using cheliped displays (see Ethogram for descriptions of 'non-contact cheliped display' performed by *N. norvegicus* in aggressive interactions, section 6.3). On a few occasions, the cod was seen to flinch and rapidly move away from a *N. norvegicus* which was behaving aggressively towards the cod. When this happened it appeared that the cod had been physically contacted by the chelae of the *N. norvegicus*. Both sighted and blinded *N. norvegicus* were observed performing the types of behaviour described above.

In the third experiment, the saithe also swam throughout the whole area of the tank. Both sighted and blinded *N. norvegicus* performed the same types of escape and aggressive behaviours towards the saithe as they did with the cod. The saithe also investigated the artificial shelters and approached the *N.*

norvegicus but did not attack them or remove any from their shelters.

5.4. Discussion.

*5.4.1. Burrow emergence of sighted *N. norvegicus* in response to predatory and non-predatory fish stimuli.*

Sighted individuals performed less emergence behaviour when a cod or cod odour was present in the tank. However the effect of odour alone was less than that caused by the physical presence of the cod in the tank. Sighted individuals responded in a similar way to the presence of a species not normally predatory on *N. norvegicus*. However, although the saithe is not known to feed on *N. norvegicus*, it is possible that its behaviour in the tank may have been interpreted by the *N. norvegicus* as threatening. Close proximity to a *N. norvegicus* of any large species of fish may be adequate stimuli for eliciting anti-predator behaviour. Previous studies have found predator specific defences in a wide variety of invertebrate and vertebrate species (Edmunds, 1974). One example is the scorpion fish (*Scorpaena guttata*) which flees from an approaching octopus (*Octopus bimaculatus*), but just raises its spines if it detects approaching fish or sees an octopus at rest. The octopus is the only predator of a scorpion fish (Edmunds, 1974). In the current investigation no evidence was found to suggest that cod and saithe presented different levels of threat to a sighted *N. norvegicus* or that there was predator specific behaviour.

*5.4.2. Burrow emergence of blinded *N. norvegicus* in response to predatory and non-predatory fish stimuli.*

In the current investigation blinded individuals also showed a reduction in burrow emergence in the presence of all fish stimuli. However, in all cases such stimuli elicited less of an avoidance response in blinded individuals than in sighted ones. This suggests that blinded individuals may be less able to avoid predators.

*5.4.3. Sensory cues used by sighted and blinded *N. norvegicus* in anti-predator behaviour.*

The relative importance of different types of sensory cues in anti-predator behaviour has been studied in the crayfish *Pacifastacus leniusculus* (Blake and Hart, 1993). It was found that chemical and visual cues together had a greater effect on shelter use by crayfish than either stimulus alone. Wahle (1992) also found evidence that the American lobster remained exposed for shorter periods in the physical presence of a fish predator than if exposed to just the chemical presence of the fish alone. The present findings are in agreement with these previous studies because they show that in *N. norvegicus* sighted individuals respond more strongly to the presence of a fish than blinded ones do. However, the sighted individuals also responded more strongly to fish odour alone. The latter result is not easy to explain but it may be that damage to one sensory modality also affects behaviour elicited by other modalities. The mechanism for such an effect is not clear but could be due to a general lowering of sensory awareness in individuals that have been blinded. The results do suggest that all channels of sensory information are necessary in *N. norvegicus* for the accurate assessment of predation risk in their environment.

5.4.4. Other types of anti-predator behaviour performed by sighted and blinded individuals in response to the presence of a predatory and non-predatory fish.

In addition to seeking shelter, sighted and blinded individuals were observed carrying out other types of anti-predator behaviour (i.e. tail flip, retreat, attack). Detailed studies of the performance of these behaviours by sighted and blinded individuals was not

measured in the present study. However, in a previous study of evasive behaviour by crayfish (*Pacifastacus leniusculus*) it was found that the distance from an approaching model of a predatory fish at which the 'tail flip' response occurred was reduced in blinded crayfish. Also, blinded crayfish did not move away as far from the predator stimulus as when they were sighted (Blake, 1993). This suggests that blinding affects the chance of escape by a crayfish from an encounter with a predator. It would be interesting to measure any possible change in the distances at which sighted and blinded *N. norvegicus* react to a predator in order to see whether blinding impairs their responses.

5.4.5. Activity levels and behavioural rhythms of sighted and blinded N. norvegicus during experiments 1, 2 and 3.

The five day control periods at the beginning of experiments 1 and 2 each showed a significant difference between the activity levels of sighted and blinded individuals. However the differences were not consistent; in the first experiment sighted *N. norvegicus* performed significantly more emergence activity, whereas in the second experiment, it was the blinded group that showed greater activity. Results of previous chapters dealing with emergence activity of sighted and blinded *N. norvegicus* showed that the effect of blinding on levels of activity were similarly inconsistent (Chapters 3 and 4). In field conditions, there was no significant difference between sighted and blinded groups, but in the laboratory, blinded individuals performed more activity than sighted ones. In spite of the differences in the activity levels of sighted and blinded *N. norvegicus* at the start of experiments 1 and 2, the introduction of fish stimuli always had the same effect for

blinded and sighted groups. This was a reduction in emergence activity.

In the first experiment, the diel rhythm performed by both groups of *N. norvegicus* in the tank was not detected after the cod was placed into the tank (i.e. in stages 2 and 3). No persistent rhythms were found in any stage of the second or third experiment. The reasons for the absence of rhythmic behaviour are unclear, although in many cases there were low activity levels and there may have been insufficient data to identify any rhythms.

5.4.6. *The limitations of the techniques used.*

The artificial conditions of the tank could have affected various aspects of *N. norvegicus* behaviour, such as migration, foraging, and burrow maintenance. Movements of individuals in the tank would have been restricted and many moved around the base of the tank walls. There was no food available in the tank, so normal foraging would not have been possible. Normal burrow related behaviour would have also been limited in the artificial shelters provided (e.g. burrow maintenance) (Chapman, 1980). The individuals in the tank were kept at a density higher than found in their natural habitat (in tank = one *N. norvegicus*/0.35 m²; at Loch Torridon, 30 m depth = one *N. norvegicus*/8 m² (Chapman and Rice, 1971); at Loch Sween, 15 m depth = one *N. norvegicus*/10.87 m² (Tuck et al., 1994)).

The use of a mixed population of sighted and blinded individuals in the same tank may also have affected the results. One possibility is that behaviour of sighted individuals could have alerted blinded individuals to the presence of a threat. Alarm substances are produced by crayfish (*Orconectes virilis*) when they are disturbed by a predator, this substance can be detected by

other crayfish and can elicit predator avoidance behaviour (Hazlett, 1985). It is possible that in this present study some individuals in the tank could have alerted other individuals in a similar way. Further studies would be required to see if alarm substances are produced when *N. norvegicus* are disturbed by a predator. However, the rapid response of the majority of individuals in the tank to the fish stimuli suggest that they were responding directly to predator stimuli rather than to any alarm substance produced by any individual disturbed by the fish.

A further possible criticism of the experiment could be that, levels of fish odour were different when the fish was in the same tank (volume = 33.9 m³) as the *N. norvegicus* and when it was in the side tank. In the latter case the fish odour would have been further diluted by the additional volume of the side tank (3.6 m³). However, since the *N. norvegicus* clearly reacted to the stimulus coming from the side tank, the fact that chemical signal levels may have been lower does not alter the main finding that emergence activity is depressed by cod odour. When the cod was introduced to the tank containing the *N. norvegicus* there was a further reduction in emergence behaviour. This could have been due to the fact that they were also responding to the physical presence of the cod or to a higher concentration of fish odour. However since the volume of the side tank was approximately only one tenth of the volume of the main tank, any dilution effect would have been smaller and can possibly be neglected.

The results in this study do not actually illustrate what occurs in the natural habitat because a predator is unlikely to remain in such proximity to *N. norvegicus* for the period allowed in this experiment. Predator stimuli would be brief and it would be necessary for the *N. norvegicus* to react immediately to any

perceived threat. It would be interesting to extend this experiment to a field situation and examine the anti-predator behaviour of sighted and blinded *N. norvegicus* using cameras situated on the sea bed.

5.5. Conclusion.

This investigation was designed to assess the effect of blinding on the predator avoidance response of *N. norvegicus*. The amount of burrow emergence behaviour of sighted individuals was significantly reduced in the presence of predatory and non-predatory fish. Avoidance behaviour was also elicited by predator odour alone, but the effect was less marked than when the predator was physically present. Blinding did not change this basic avoidance response, but blinded individuals showed more emergence behaviour in the presence of predator and non-predator stimuli than sighted ones.

The results suggested that visual information about predator risk is necessary to elicit the appropriate level of avoidance behaviour by *N. norvegicus*. In the present study it was shown that the emergence behaviour of *N. norvegicus* is affected in a similar way by a predator species (*G. morhua*) and a non-predatory one (*P. virens*).

Chapter 6 Effects of blinding on the agonistic behaviour.

6.1. Introduction

Fighting behaviour has been observed in *N. norvegicus* both in the field, at burrow entrances between the occupants of burrows and intruders (Chapman and Rice, 1971), and in the laboratory (Rice and Chapman, 1971; Farmer, 1974). Damage is commonly seen on the claws of *N. norvegicus* caught in creels. Chapman and Rice (1971) found 62% males and 41% females possessing wounds in one sample at Loch Torridon. They suggested these injuries were caused by intraspecific fighting prior to capture. Such a large incidence of wounded *N. norvegicus* strongly suggests that there is a high level of fighting between individuals in the natural habitat (Chapman and Rice, 1971).

In the study of animal behaviour, 'aggressive' behaviour is commonly defined as any behaviour which involves the intent to cause harmful stimulation, and act destructively towards another individual (Manning, 1979). Aggressive behaviour can be used by an individual to compete actively for resources (e.g. space, mates, food). It can also be used by an animal to protect itself, other areas (e.g. territory) and its offspring, against a potentially damaging factor (e.g. a predator) (Archer, 1988). The term 'agonistic behaviour' is used to describe all possible behaviour performed during a fight, or an aggressive interaction, where at least one individual performs aggressively towards another (agonistic behaviour includes attack, conciliation and escape behaviour) (Manning, 1979; Huntingford and Turner, 1987).

Observations of *N. norvegicus* on the sea bed show that individuals frequently change their burrows (Chapman and Rice, 1971), some changing burrows daily (Chapman *et al.*, 1975). This suggests that there is probably a high level of agonistic behaviour occurring at burrow entrances because occupants are likely to have to repel many intruders. The female American lobster *Homarus americanus* selects a male and temporarily shares his burrow in order to mate with him and the male then has to maintain occupancy of the burrow so that he will have the opportunity to eventually fertilise her eggs (Atema and Cobb, 1980). The male lobster also guards the female in the burrow after copulation to prevent insemination by other males (Atema and Cobb, 1980). It is likely that sexual behaviour in *N. norvegicus* is similar, but this has not yet been confirmed. Using a video camera situated on the sea bed, *N. norvegicus* were seen engaging in contests for food (Nickell and Moore, 1992). It is concluded that agonistic behaviour is likely to be particularly important in the conflicts between individuals for occupancy of burrows and when competing for food. Observations on the sea bed and tagging studies suggest that *N. norvegicus* does not migrate great distances and that individuals remain in the same general area after release (Chapman *et al.*, 1975; Chapman, 1980). This situation could favour the establishment of dominance hierarchies.

During agonistic behaviour individuals are able to inflict costs on their opponents, but risk possible injury to themselves if an opponent retaliates and the interaction continues (Huntingford and Turner, 1987; Archer, 1988). In escalated stages of agonistic interactions, crustacea use their chelae as weapons to cause injury to their opponents (Dingle, 1983). In the

field, specimens of *H. americanus* have been seen with crushed claws and it is assumed that such injuries result from agonistic interactions. Observations in aquaria of contests between pairs of *H. americanus* have shown injury inflicted by one individual on another and interactions have been observed in which one individual even snapped off the dactyl of an opponent. Such damage to the claws may lead to cheliped autotomy (Atema and Cobb, 1980). In another study it was shown that injury and autotomy of chelipeds by the swimming crab *Liocarcinus puber* led to a loss of subsequent agonistic ability (Smith, 1990) and a lowered capacity of an individual to defend itself against predators (Thorp and Ammerman, 1978). In *N. norvegicus* cheliped damage can lead to increased mortality Chapman, 1981). Displays and fighting can also expose individuals to predators (Archer, 1988) and such exposure would occur in *N. norvegicus* when individuals are competing on the sea bed outside their burrows. Agonistic interactions also incur a high cost in time and energy (Huntingford and Turner, 1987; Smith and Taylor, 1993). This time is not available for other essential activities such as feeding and reproduction (Archer, 1988). Also, after engaging in agonistic behaviour an individual may have sufficiently reduced energy reserves that its ability to compete with conspecifics or to defend itself against predators is limited (Huntingford and Turner, 1987).

Since 1982, when game theory was applied to studies in animal behaviour by Maynard Smith, it has been used as the main theoretical basis for understanding the 'decisions' made by opponents during a fight. Game theory uses an economic approach where the different types of agonistic behaviour and fighting strategies are considered in terms of the costs and

benefits incurred to an individual's fitness (i.e. the ability to survive and to maximise the number of offspring produced during its lifetime). Game theory predicts that an individual will 'consider' the relative costs (e.g. risk of injury) and benefits (e.g. gaining shelter) of engaging in a conflict with another individual (Archer, 1988; Krebs and Davies, 1987). It also suggests that each individual will be constantly 'assessing' the relative ability of its opponent to inflict costs and use this information to 'decide' whether to continue or withdraw from the interaction (Maynard Smith, 1974; Huntingford and Turner, 1987; Archer, 1988).

The many models and mechanisms proposed to explain fighting behaviour all agree that for an individual to form an effective strategy during an interaction, communication between opponents is important (Huntingford and Turner, 1987; Archer, 1988). Although there are many possible modes of communication used during an interaction (e.g. tactile and chemosensory cues) (Huntingford and Turner, 1987), previous studies demonstrate that visual cues play an important role in the agonistic behaviour of other species of crustacean. In the spider crab *Microphrys bicornutus*, Hazlett and Estabrook (1974) used information analysis to show that visual cues are used for communication during agonistic interactions. The use of models showed that the blue crab, *Callinectes sapidus*, employs distinctive colouration patterns on its chelipeds to make threat displays to its opponents (Jachowski, 1974). The crayfish, *Orconectes rusticus*, changes its agonistic behaviour to compensate for loss of vision during conflicts in darkness by using more tactile behaviour (Bruski and Dunham, 1987; Smith and Dunham, 1990).

The present study was carried out to examine the possible effects of blinding an individual *N. norvegicus* on its agonistic behaviour. Initially, any change in the predicted outcome of a contest was assessed after one of a pair of opponents had been blinded. To do this, pairs of individuals were allowed to fight in order to establish which was the dominant individual of the pair. To assess the role of vision in the status and agonistic behaviour of each opponent, either the dominant or subordinate individual was blinded and the pair was allowed to fight again.

To record the outcome of each contest it is necessary to define the components of a complete agonistic interaction and to determine the criteria by which winners and losers can be recognised. Hyatt (1983) studied the agonistic behaviour of many crustaceans and produced a template of behaviour occurring during a 'typical' agonistic interaction. This template was used in the present study in order to describe a complete interaction, which is referred to as a 'bout' (Bruski and Dunham, 1988). A bout begins when one opponent approaches the other, which then responds in an agonistic manner. Both opponents then engage in agonistic behaviour until one opponent withdraws from the interaction and both opponents cease to display any agonistic behaviour (Hyatt, 1983). The loser of an agonistic interaction is commonly said to be the opponent that withdraws at the end of a bout and the winner is the opponent that forces the loser to retreat (Maynard Smith, 1974).

In the present study detailed analysis of bouts was carried out in order to discover any possible change in the agonistic behaviour and fighting strategies during a bout after one opponent had been blinded. In order to describe the range of behaviour during agonistic interactions, a complete description

of all possible recognisable agonistic acts performed by *N. norvegicus* was developed; such a description is known as an ethogram (Lehner, 1979). Ethograms have been used as valuable tools in previous studies of the agonistic behaviours of other crustacea including the crayfish *Orconectes virilis* (Heckenlively, 1970) and *O. rusticus* (Bruski and Dunham, 1988); the crabs *Callinectes sapidus* (Jachowski, 1974), *Clibanarius tricolour* and *C. antillensis* (Hazlett, 1975), *Potamon fluviatile* (Vannini and Sardini, 1971); and the prawns *Macrobrachium rosenbergii* (Barki *et al.*, 1990). Most of these agonistic ethograms fit into the basic template for crustacean interactions constructed for an 'average' decapod by Hyatt (1983). This was based on many observations of the agonistic behaviour of different species of crustacea and consisted of four main categories of agonistic behaviour: approach to an opponent, non-contact cheliped display, contact cheliped attack, retreat from an opponent. In this thesis the ethogram for *N. norvegicus* was described and found to conform to this model.

6.2. Materials and methods.

Studies on the agonistic behaviour of sighted and blinded *N. norvegicus* were carried out at the University of Leicester using the observation tank.

Two weeks prior to use in this series of experiments, individual male *N. norvegicus* were selected and placed in separate compartments (base dimensions = 0.18 m x 0.18 m, and height of walls = 0.3 m) situated within holding tanks in the marine tank room. This meant that each individual was isolated visually and physically from the others inside the holding tank, but individuals were not chemically isolated from each other because water was allowed to circulate between the compartments. The *N. norvegicus* were not fed while in these isolation compartments and they were kept on a 12 h light: 12 h dark day/night cycle using appropriate light levels. Each compartment contained an artificial burrow (0.2 m length of plastic drainpipe; 0.065 m internal diameter) and a fine gravel substrate.

To study the agonistic behaviour of the *N. norvegicus* inside the observation tank they were confined within a square 'arena' placed inside the tank. The arena had a base with an area of 0.6 m x 0.6 m and walls 0.4 m high, which was greater than the depth of the water in the tank so that the individuals inside the arena were unable to escape. The walls of the arena were made of transparent perspex and the *N. norvegicus* were observed through a window in the side of the observation tank. This permitted body and chela positions to be noted during agonistic bouts. A video camera fitted with a 16 mm (F:1.4) lens, was mounted on a tripod and adjusted so that the entire area within the arena could be monitored from the side. All behaviour was recorded onto video tape but it could also be observed simultaneously in a neighbouring room using a video

monitor (Sony, 9'') and a time-lapse video cassette recorder (Panasonic AG-6024).

6.2.1. Preparation of N. norvegicus.

48 *N. norvegicus* (mean carapace length 36.4 mm, s.d. = 0.4 mm) were kept in the isolation compartments and were matched into pairs of similar size (i.e. difference in carapace length ≤ 5 mm). Matched opponents remained paired throughout the study. The two individuals of a pair were kept apart until required for experimentation. Four of the pairs used had equal carapace lengths (i.e. difference < 0.1 mm) while the remaining 20 pairs had a mean carapace length difference of 1.9 mm (s.d. = 0.4 mm).

Each individual was labelled on the dorsal part of the carapace with black tape cut into a letter/number which was attached using cyanoacrylate glue. The labels were positioned so that each individual could be identified when pairing the opponents and when viewing the video tapes of agonistic interactions. These preparation procedures were carried out at least 48 h before a pair was placed in the arena.

6.2.2. Procedure.

A summary of the trials carried out is given in Figure 6.1. Agonistic interactions were recorded during the period between 09.00 h (2 hours after artificial dawn) and 17.00 h (2 hours before artificial dusk). A 'trial' was carried out as follows:

A central vertical opaque partition was placed into the arena to divide it in half. One opponent of the pair was placed in each half of the arena where the opponents were visually and physically isolated from each other by the opaque partition. They were left

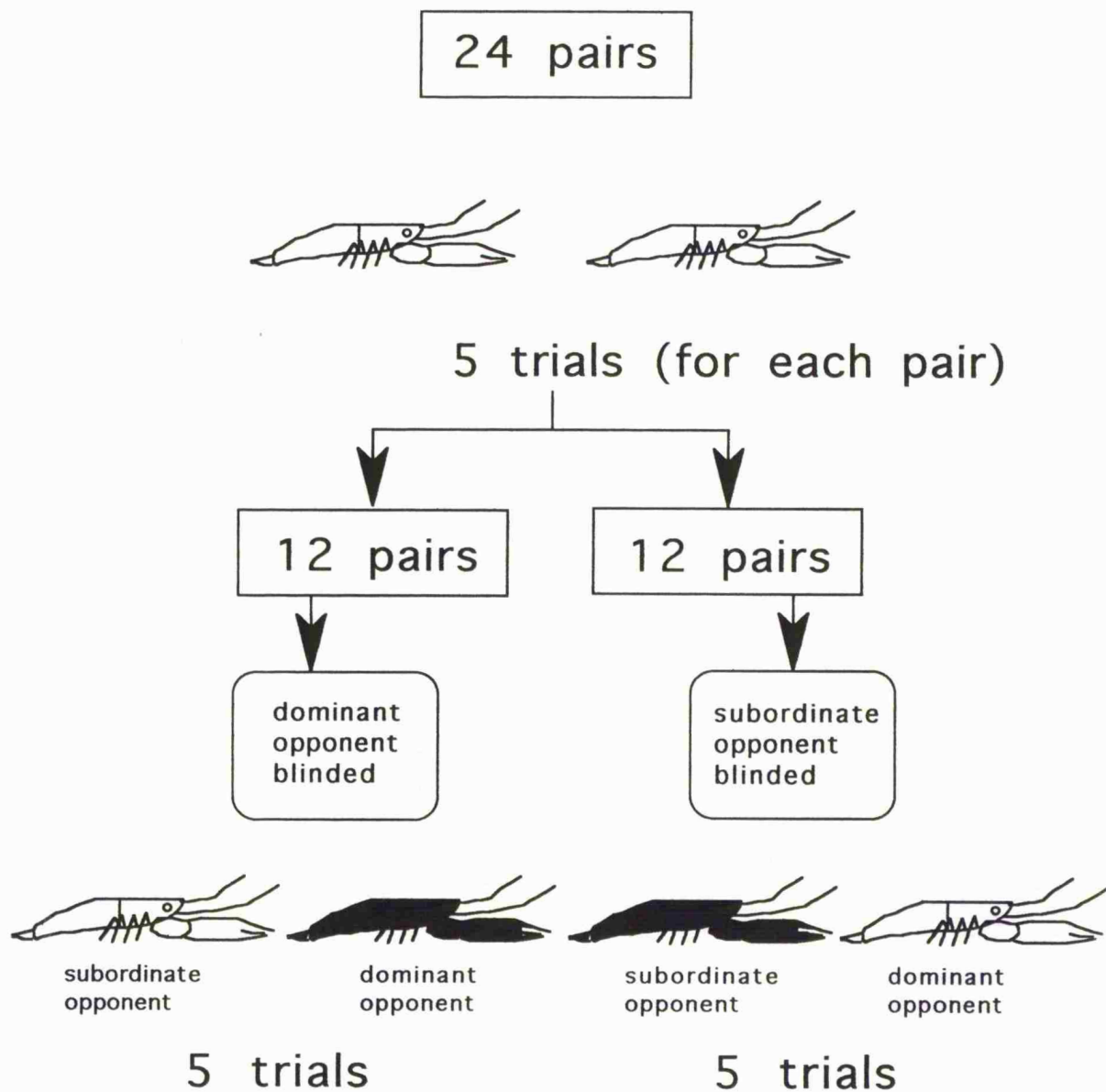
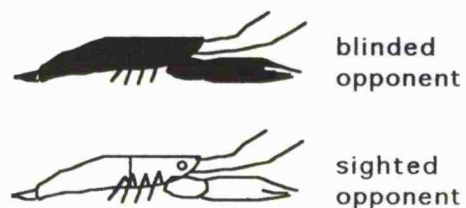


Figure 6.1. Diagram of procedure carried out with 24 pairs of *N. norvegicus* to discover if the outcome of trials changed after one opponent was blinded.

key:



for 0.5 h in order to allow them to recover from any immediate effects of handling and to acclimatise to the arena.

The central partition was removed by hand, causing as little disturbance as possible. At this point recording began and continued for 3 h. Afterwards, each *N. norvegicus* was returned to its separate isolation compartment. A total of five trials were carried out with each pair, with at least 24 h between trials. Within each trial there was a variable number of bouts, but there were never less than 12.

This procedure was carried out using all 24 pairs of *N. norvegicus*. An assessment was then made of the outcomes of interactions between each pair. An opponent was referred to as dominant in a trial if the number of bouts won by it was significantly greater from that expected on the basis of a random process (a chi-squared test was used, with a significance level of 0.05); the other opponent was referred to as subordinate. For the purposes of analysis, only the first 12 bouts of each trial were used. The 24 pairs were then divided into 2 groups of 12. For the first group of 12 pairs, the dominant opponent was blinded and for the second group of 12 pairs, the subordinate opponent was blinded. In each case a 'handling control' was carried out on the other sighted opponent. Three days were then allowed for the *N. norvegicus* to recover from the blinding/'handling control' procedures in their isolation compartments in the holding tanks. A set of five 3 hour trials was then carried out as before for all 24 pairs to see how blinding one of the opponents affected bout outcome.

At the end of the trials the eyes of both *N. norvegicus* were removed and placed in fixative for later analysis.

6.2.3. Statistical analysis.

i) Analysis of variance.

All data was tested for homogeneity of variance (using the $F_{\max}$ test (Fowler and Cohen, 1990) see Appendix I for details of test) and found to be suitable for analysis by the ANOVA test.

A two-way repeat measures analysis of variance (ANOVA) test (Meddis, 1973) was used to measure any significant effect of (i) blinding and (ii) the four different types of agonistic behaviour, on the various aspects of agonistic bouts (i.e. number of acts performed and time spent performing these acts during a bout). It was also possible to examine whether the number, or duration, of the four types of agonistic behaviour in a bout were affected differently by the blinding treatment (i.e. interaction between the blinding and different type of agonistic behaviour performed).

Once a significant effect was found in an ANOVA test, a Tukey HSD test (Fowler and Cohen, 1990) (see Appendix I for details of test) was used to indicate which means were significantly different.

ii) Sequence analysis.

A chi-squared test was used to test for an association between the behaviour of one opponent and that of the other, using data collected on transition matrices. The observed value in each cell of a matrix was compared with the corresponding expected value (Colgan, 1978; Fowler and Cohen, 1990).

For all tests a probability level of 0.05 was used.

6.2.4. Description of agonistic bouts.

The following section describes the behaviour observed when two individuals were placed in the arena. It includes a general description of an agonistic bout used by all subsequent analyses to

study the outcome, duration and content of bouts to determine any affects of blinding on an individual's agonistic behaviour.

The behaviour of two individuals was observed while together in an arena. While in the arena, the two individuals were seen to move throughout the area inside the arena and explore the walls. Individuals rested (i.e. remained motionless, body and chelipeds lowered) and groomed themselves using their walking legs. The two opponents also performed a sequence of agonistic bouts. One of the *N. norvegicus* would initiate a bout by approaching the other individual, or by using its chelipeds to 'threaten' the other opponent. This other individual would either not respond, or would react by performing agonistic behaviour. An agonistic bout was said to have begun only if the second opponent had responded. Both opponents would then engage in some/all of the types of agonistic behaviour described in the ethogram (see section 6.3). Attacking behaviour can be divided into 'approach' where individuals move so that they are nearer to an opponent, 'non-contact cheliped display' where an individual will move its chelipeds to increase its apparent size and threaten its opponent and 'contact cheliped attack' where an opponent will use its chelipeds to make physical contact with its opponent ranging from merely touching it, to intense wrestling sometimes resulting in one opponent being flipped over by the other. At any point in the bout one opponent would withdraw from the contest, using any behaviour described as 'retreat' in the ethogram. This withdrawal behaviour varied from a rapid tail-flip escape to slow backwards walking. When an opponent withdrew from a bout it was sometimes pursued by the other opponent which then re-initiated the bout by attacking the retreating one. This 'retreat-pursuit' sequence may occur many times throughout a bout. A retreating individual may

also not be pursued, with both opponents ceasing to display any further agonistic behaviour. It is then said that the agonistic bout has ended, the loser is the opponent that withdrew at this point and the winner is the other opponent that forced the loser to retreat. There were occasions when an opponent would initiate an agonistic bout and the second opponent responded by immediately retreating. If the first opponent did not then pursue and re-initiate the bout, this was the minimum number of acts which could occur in a bout.

6.2.5. Ethogram construction.

To analyse the agonistic behaviour occurring within bouts, it was necessary to classify the types of behaviour that may be seen in a contest between *N. norvegicus*. In order to build a comprehensive ethogram of the agonistic behaviour of *N. norvegicus* a total of approximately 720 hours of video recording tape was reviewed. During this review the agonistic behaviour of each opponent in a bout was noted. Aspects of behaviour recorded included the context of each act, positions and movements of chelipeds and the direction and approximate speed of an individual. The data was used to construct the ethogram. As subsequent bouts were analysed any agonistic behaviour not previously seen was described and added.

6.3. Ethogram of *N. norvegicus* agonistic behaviour.

The following ethogram is a set of descriptions of all possible agonistic behavioural acts performed during an intraspecific interaction. This allows the characterisation of the agonistic interactions of the *N. norvegicus* and facilitates the comparison of the content of interactions between two sighted opponents with those after one opponent has been blinded. Grabbed video images were enhanced using an Apple Macintosh™ computer, utilising NIH Image v1.54™, an image processing and analysis software package. For the purposes of describing the behaviour of each individual during an agonistic act, the opponent carrying out the act being described is referred to as opponent A, and the other is called opponent B.

Agonistic acts were divided into four categories of 'approach', 'non-contact cheliped display', 'contact cheliped attack' and 'retreat'. While performing 'approach' behaviour opponent A has chelipeds raised from the substrate, and either held parallel to each other and pointing forwards, or spread (but not as wide as the position described in the meral spread). Three types of 'approach' behaviour were recognised - direct approach (Plate 6.1), turn on axis (Plate 6.2) and chase (Plate 6.3). 'Non-cheliped display' an opponent's chelipeds were used for threat without actually touching the other opponent. 'Non-contact cheliped display' was sub-divided into meral spread (Plate 6.4), thrust (Plate 6.5) and raised chelipeds (Plate 6.6). 'Contact cheliped attack' involved one or both opponents performing overt contact fighting behaviour. There were seven types of 'contact cheliped attack' recognised - single cheliped contact (Plate 6.7), double cheliped contact (Plate 6.8), single interlock (Plate 6.9), double interlock (Plate 6.10), pushing (Plate 6.11), wrestling (Plate 6.12) and strike (Plate 6.13).

While performing 'retreat' behaviour an individual's chelipeds were held parallel to each other and pointed forward, or were spread (but not to the extent of meral spread). There were four types of 'retreat' behaviour recognised - move backwards (Plate 6.14), flee (Plate 6.15), tail flip (Plate 6.16) and turn away (Plate 6.17).

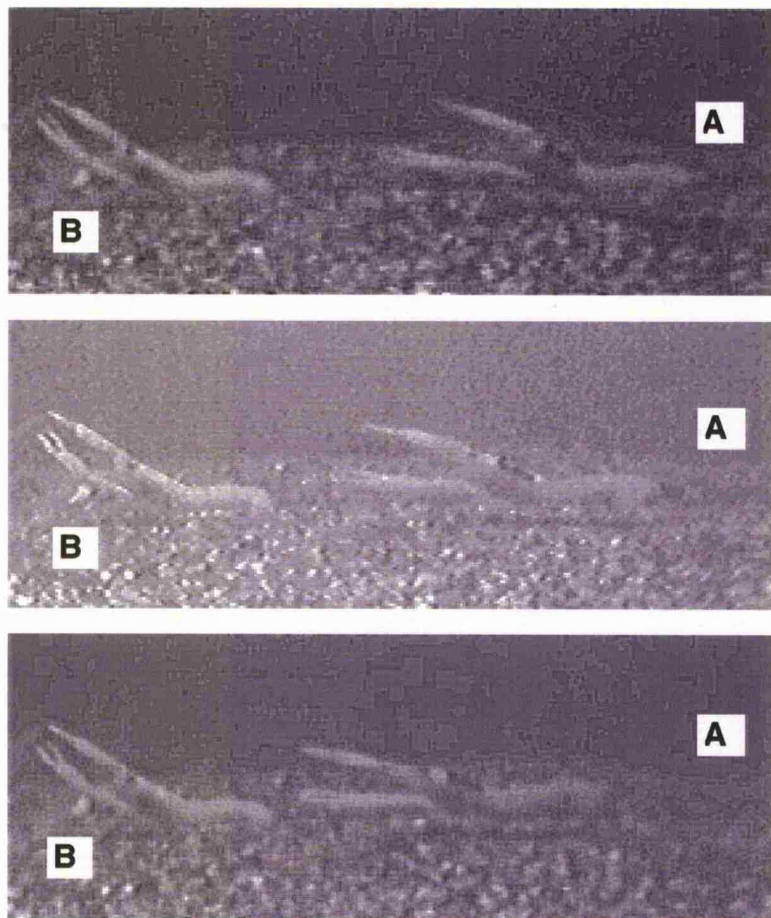


Plate 6.1. Direct approach - opponent A walks (≤ 0.05 m/s), towards opponent B, so that it reaches a position facing any point on the body of opponent B. This sequence shows three images where opponent A approaches opponent B.



Plate 6.2. Turn on axis - opponent A turns around so that it is facing any point on the body of opponent B. The sequence of four images shows opponent A turning to face opponent B.

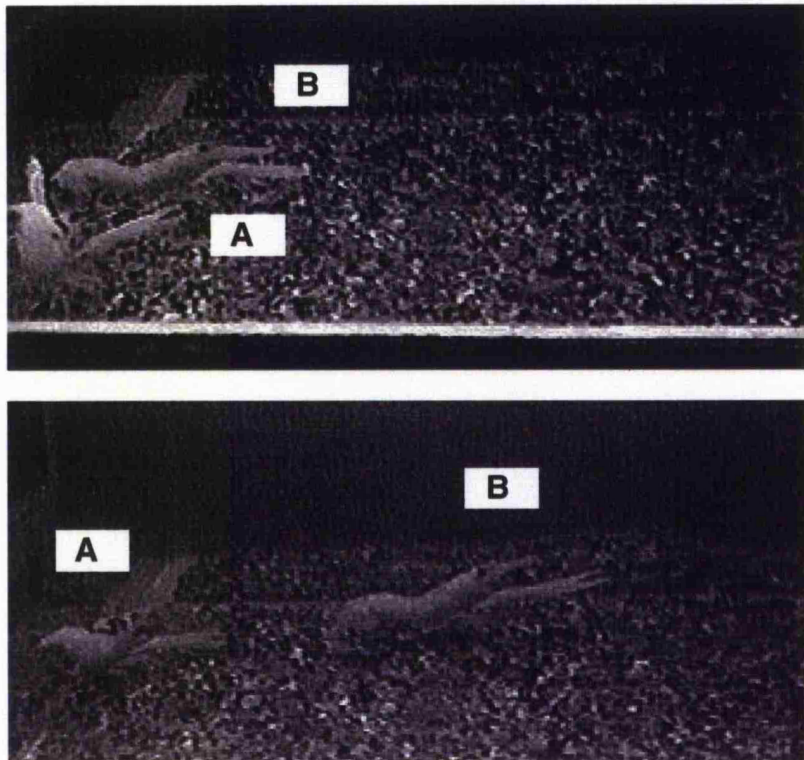


Plate 6.3. Chase - opponent A pursues opponent B, both opponents moving at the same time. In these two images opponent A is pursuing opponent B.

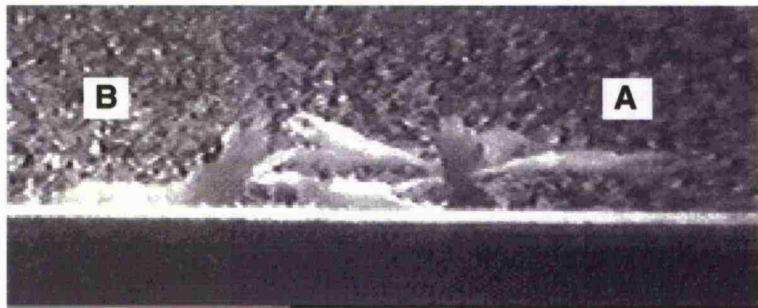


Plate 6.4. Meral spread - opponent A is stationary, both chelipeds are raised so that they are parallel to substrate and held fully spread apart and perpendicular to the side of its carapace. Opponent A will face any point on the body of opponent B. In this image opponent A is performing a meral spread.

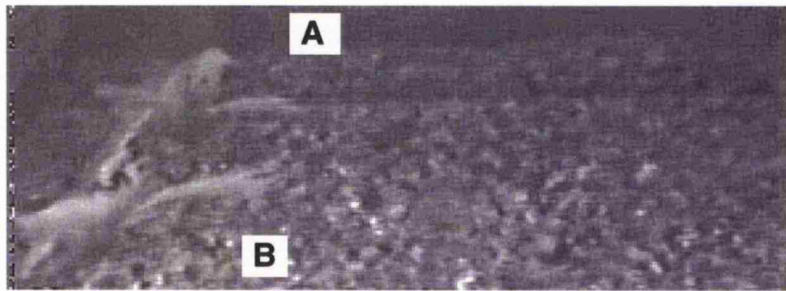


Plate 6.5. Thrust - opponent A, with chelipeds extended in the position of meral spread, moves forward rapidly ($\leq 0.05\text{m/s}$) and so that it is facing any point on the body of opponent B. In the above image opponent A is performing a thrust towards opponent B.

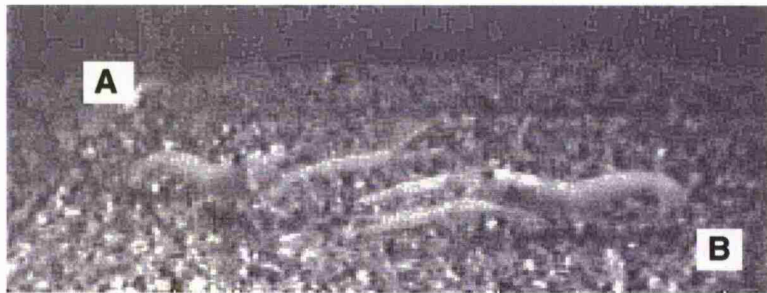


Plate 6.6. Raised chelipeds - chelipeds held by opponent A at a recognisable angle above the horizontal position. Chelipeds may be held parallel to each other, or spread (but not to the extent described in the meral spread). This can be carried out with opponent A facing any point on the body of opponent B. In this image opponent A has raised its chelipeds in a threat towards opponent B.

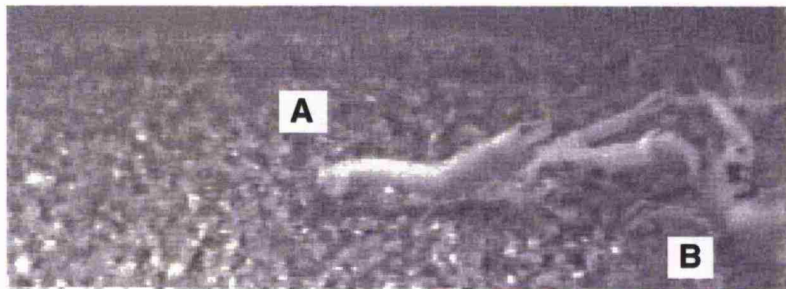


Plate 6.7. Single cheliped contact - one chela of opponent A holding one cheliped of opponent B. Chela of opponent A held shut. In this image the right chela of opponent A is holding the left cheliped of opponent B.



Plate 6.8. Double cheliped contact - closed chelae of opponent A holding both chelipeds of opponent B. In this image both opponents are held in double cheliped contact.



Plate 6.9. Single interlock - one closed chela of opponent A interlocked with closed chela of opponent B. In this image the left chela of opponent A is interlocked with the right chela of opponent B.

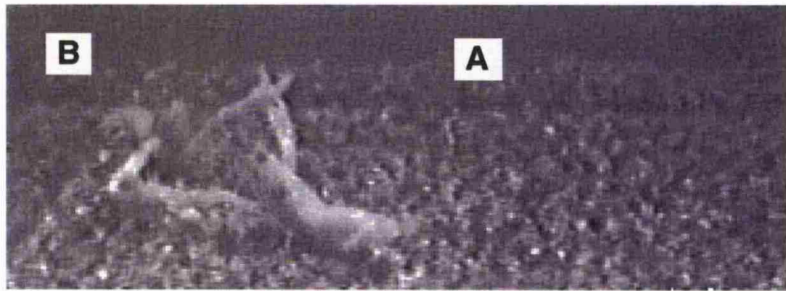


Plate 6.10. Double interlock - both chelae of opponent A interlocked with both closed chelae of opponent B. In this image both opponents A and B are held in the double interlocked position.

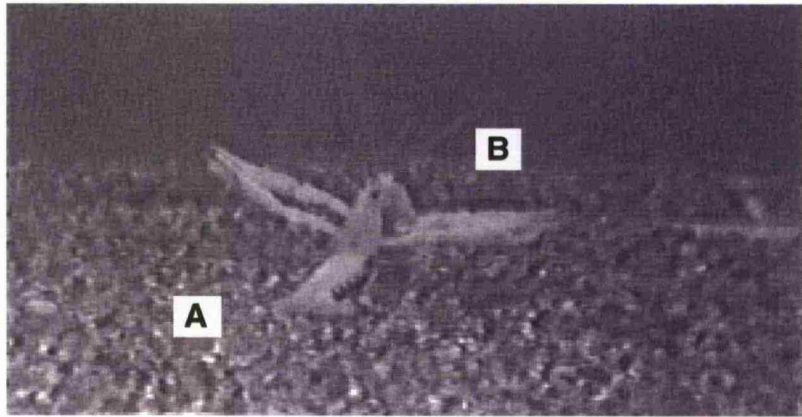


Plate 6.11. Pushing - chelipeds of opponent A held in meral spread while facing and pushing against the chelipeds of opponent B, also held in meral spread. Both opponents try to force the other backwards. In this image both opponents are engaged in pushing behaviour.

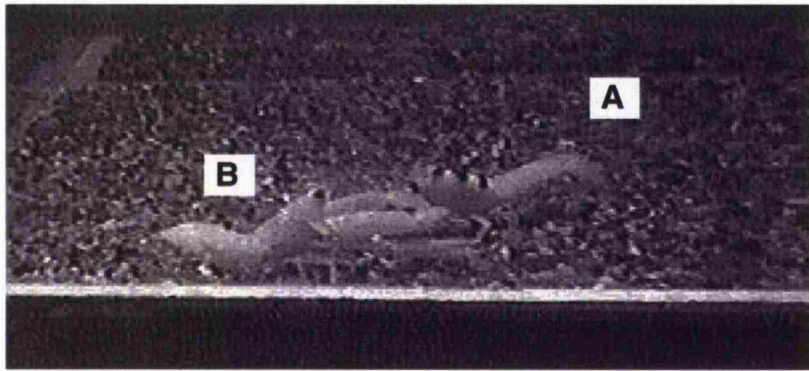


Plate 6.12. Wrestling - opponent A and opponent B facing each other and holding the other so that their chelipeds touch both sides of the others' carapace. Both opponents pushing and trying to force the other backwards. In this image both opponents are engaged in wrestling behaviour.

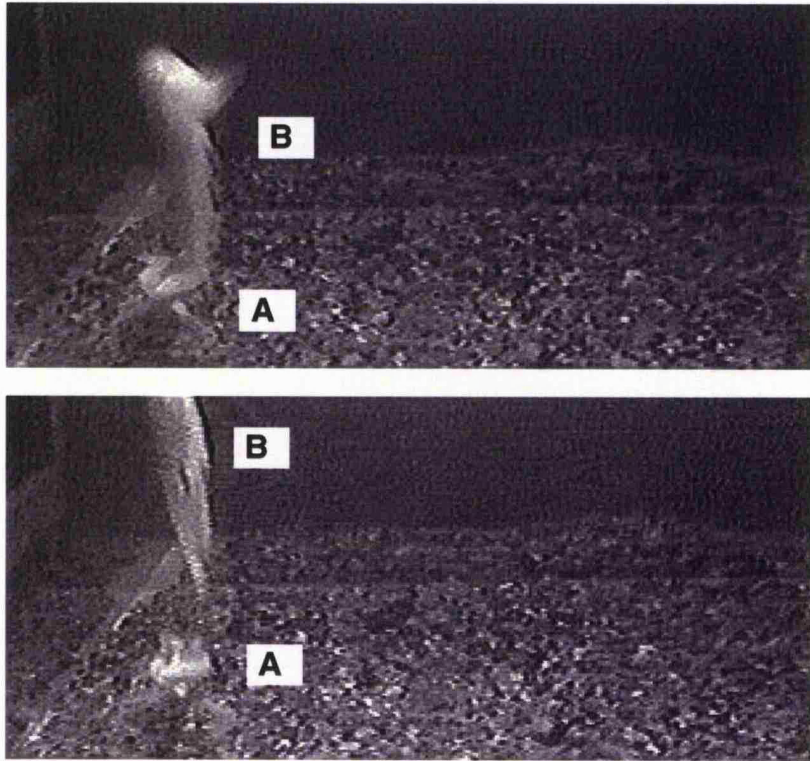


Plate 6.13. Strike - opponent A rapidly hits any part of the body of opponent B. In this image opponent A is using both chelipeds to strike both chelipeds of its opponent.



Plate 6.14. Move backwards - opponent A walks (≤ 0.05) backwards away from opponent B. Opponent B remains stationary. In this sequence opponent A is moving to the right of the frame, away from opponent B.

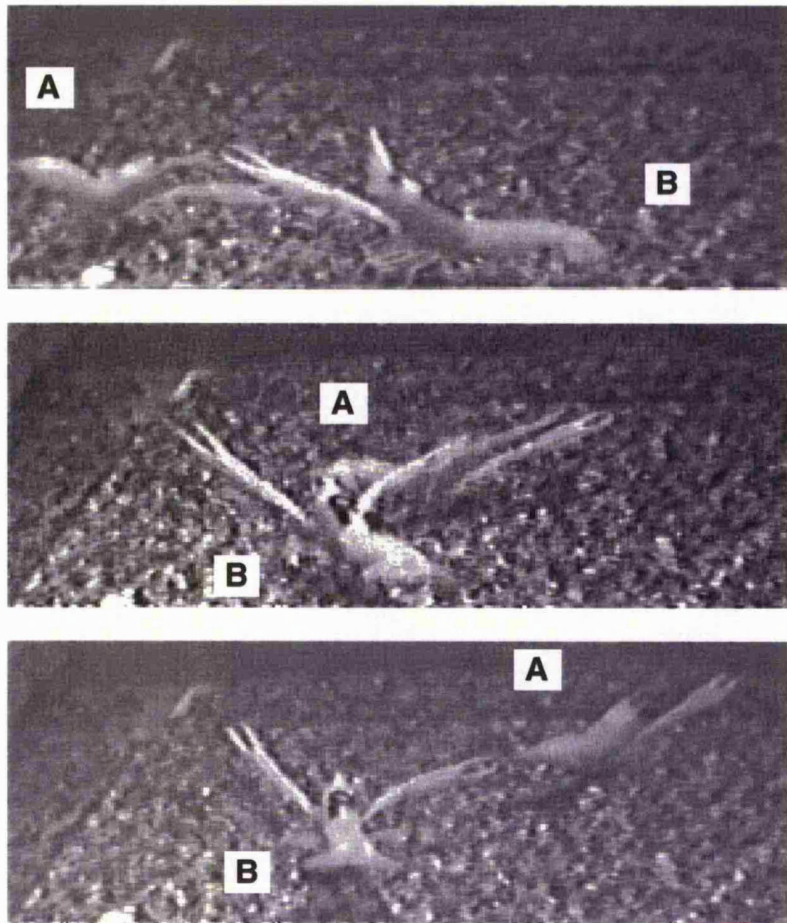


Plate 6.15. Flee - opponent A runs ($> 0.05\text{m/s}$) forward away from opponent B. Opponent B remains stationary. In this sequence opponent A flees away from opponent B. Opponent B moves but does not chase opponent A.



Plate 6.16. Tail-flip - opponent A escapes from opponent B by rapidly flexing its abdomen so that opponent A is propelled backwards and upwards, away from opponent B. In this image opponent A escapes to the left of the frame, away from opponent B.

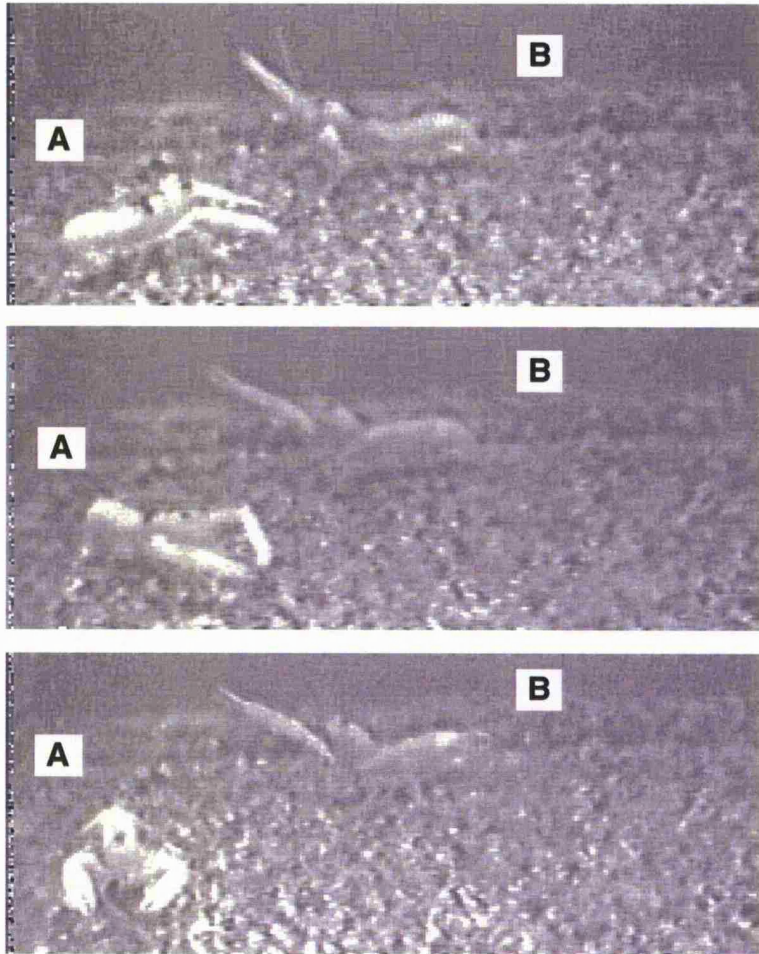


Plate 6.17. Turn away - opponent A turns around so that it faces away from opponent B. In this sequence opponent A turns to face away from opponent B.

6.4. Results.

6.4.1. *Effect of blinding on the status of an opponent.*

The dominant opponent of each trial was the opponent winning a significant number (using chi square test and 0.05 probability level) of the first 12 bouts of a trial, the other opponent was referred to as subordinate. From the study of all 24 pairs of *N. norvegicus* it was found that one individual in each pair was dominant in all 5 trials carried out when both opponents were sighted. Dominant opponents maintain their dominant status in the majority of the trials following the blinding of one of the opponents (Tables 6.1 and 6.2). Only 3 of the 24 pairs showed any change in the identity of the dominant opponent and only for a single trial (out of five). Of the 21 pairs showing a difference in carapace length between opponents, there were 16 cases where the larger one was dominant (Tables 6.1 and 6.2). It is possible that size difference influences the outcome of agonistic bouts.

Having found that the competitive status of an opponent appeared unchanged after either the dominant or subordinate opponents were blinded, further analysis of the interactions was carried out to examine for possible changes in agonistic behaviour during bouts. Bout duration was examined for all 24 pairs to see if blinding has any effect on the lengths of the bouts (see section 6.4.2.). A number of pairs were then selected for a more detailed investigation of agonistic behaviour performed during bouts before and after blinding one opponent; parameters investigated included (i) numbers of the different types of act and times spent carrying out different acts (sections 6.4.3. and 6.4.4.) and (ii) sequences of different types of agonistic acts in a bout (section 6.4.5.).

Individuals	Carapace length (mm)	Mean number of bout wins per trial (n=5) (s.d. in brackets)	
		Trials 1 - 5; both opponents sighted.	Trials 6 - 10; after dominant opponent blinded.
1	35.4	2.4 (3.1)	4.2 (3.4)
2	39.0	9.6 (3.1)*	7.8 (3.4)*
3	38.4	12 (0)*	10.5 (0.9)*
4	38.5	0 (0)	1.5 (0.9)
5	38.4	0.8 (0.4)	1.8 (2.2)
6	38.7	11.2 (0.4)*	10.2 (2.2)*
7	39.0	12 (0)*	11.8 (0.4)*
8	37.3	0 (0)	0.2 (0.4)
9	38.1	2.6 (1.6)	4.2 (1.6)
10	35.2	8.4 (1.6)*	7.8 (1.6)*
11	38.8	11.8 (0.4)*	11.6 (0.4)*
12	38.7	0.2 (0.4)	0.4 (0.4)
13	38.2	2.4 (1.6)	1.2 (1.6)
14	41.4	9.6 (1.6)*	10.8 (1.6)*
15	36.4	2.6 (2.7)	3.4 (1.6)
16	37.2	9.4 (2.7)*	8.6 (1.6)*
17	32.9	11 (0.7)*	10.7 (1.3)*
18	33.0	1 (0.7)	1.3 (1.3)
19	39.8	0.6 (0.9)	0 (0)
20	39.8	11.4 (0.9)*	12 (0)*
21	35.7	0 (0)	0.4 (0.4)
22	38.7	12 (0)*	11.6 (0.4)*
23	34.1	8.4 (1.6)*	8.3 (1.3)*
24	31.9	3.6 (1.6)	3.7 (1.3)

Table 6.1. Results for cases where the dominant opponent was blinded in trials 6 - 10, number of wins of all opponents.* denotes the dominant opponent for all five trials before and after the blinding procedure. Results indicate that the dominant opponent remains dominant after the dominant opponent was blinded (pairs outlined are those where the status of the opponents was reversed for one trial).

Individuals	Carapace length (mm)	Mean number of bout wins per trial (n=5) (s.d. in brackets)	
		Trials 1 - 5; both opponents sighted.	Trials 6 - 10; after subordinate opponent blinded.
A	35.1	0 (0)	0.2 (0.4)
B	35.0	12 (0)*	11.8 (0.4)*
C	37.2	0.8 (1.3)	0 (0)
D	37.3	11.2 (1.3)*	12 (0)*
E	36.3	9.7 (0.5)*	11.4 (0.5)*
F	35.2	0.3 (0.5)	0.6 (0.5)
G	34.9	10.8 (0.8)*	11.4 (0.9)*
H	29.7	1.2 (0.8)	0.6 (0.9)
I	34.4	11.6 (0.5)*	11.2 (1.3)*
J	34.8	0.4 (0.5)	0.8 (1.3)
K	31.5	1.4 (1.1)	3.8 (1.1)
L	33.5	10.6 (1.1)*	8.2 (1.1)*
M	42.8	1.0 (1.7)	1.5 (1.3)
N	42.8	11.0 (1.7)*	10.5 (1.3)*
O	35.6	11.8 (0.4)*	10.4 (0.5)*
P	32.1	0.2 (0.4)	1.6 (0.5)
Q	41.5	11.2 (0.8)*	10.4 (0.9)*
R	37.7	0.8 (0.8)	1.6 (0.9)
S	33.8	11.6 (0.5)*	10.8 (0.4)*
T	32.1	0.4 (0.5)	1.2 (0.4)
U	34.3	11.2 (1.8)*	10.4 (1.7)*
V	34.0	0.8 (1.8)	1.6 (1.7)
W	35.3	12 (0)*	8.4 (2.1)*
X	35.3	0 (0)	3.6 (2.1)

Table 6.2. Results for cases where the subordinate opponent was blinded in trials 6 - 10, number of wins of all opponents.* denotes the subordinate opponent for all five trials before and after the blinding procedure. Results indicate that the subordinate opponent remains dominant after the subordinate opponent was blinded (pairs outlined are those where the status of the opponents was reversed for one trial).

6.4.2. Effect of blinding on the duration of agonistic bouts.

A comparison was made of the length of time taken to resolve a bout in the period when both opponents were sighted and then after one opponent had been blinded. All 24 pairs of *N. norvegicus* were used for the analysis and the duration of each of the first 12 bouts was measured for: (i) trials 1-5 (both opponents sighted), and (ii) trials 6-10 (one opponent blinded). For each pair, mean bout durations were calculated separately for trials before and after blinding. A two-tailed repeated measures t-test was used to compare the mean bout duration before and after blinding. A 0.05 probability level was used throughout these tests.

Where the dominant opponent was blinded, mean bout length was shorter in the trials following blinding for 9 of the 12 pairs, (Fig. 6.2). However, the difference was only significantly shorter in four cases. In the second group of 12 pairs, where the subordinate opponent was blinded, there was no similar pattern of differences in bout duration before and after blinding; one pair showed significantly longer bouts and one pair had shorter bouts, after blinding (Fig. 6.3).

6.4.3. Trends in the duration of successive bouts during trials.

Data from the first 5 trials (when both opponents were sighted) were used to detect any possible trend in the duration of successive bouts within a trial that might be attributable to recognition and memory of the outcome of previous bouts with the same opponent, or fatigue from repeated interactions.

The mean duration of the first bout from each of the first five trials (i.e. when both opponents were sighted), of all 24 pairs used, was calculated (i.e. $\sum[\text{1st bout durations}] / [n=120]$

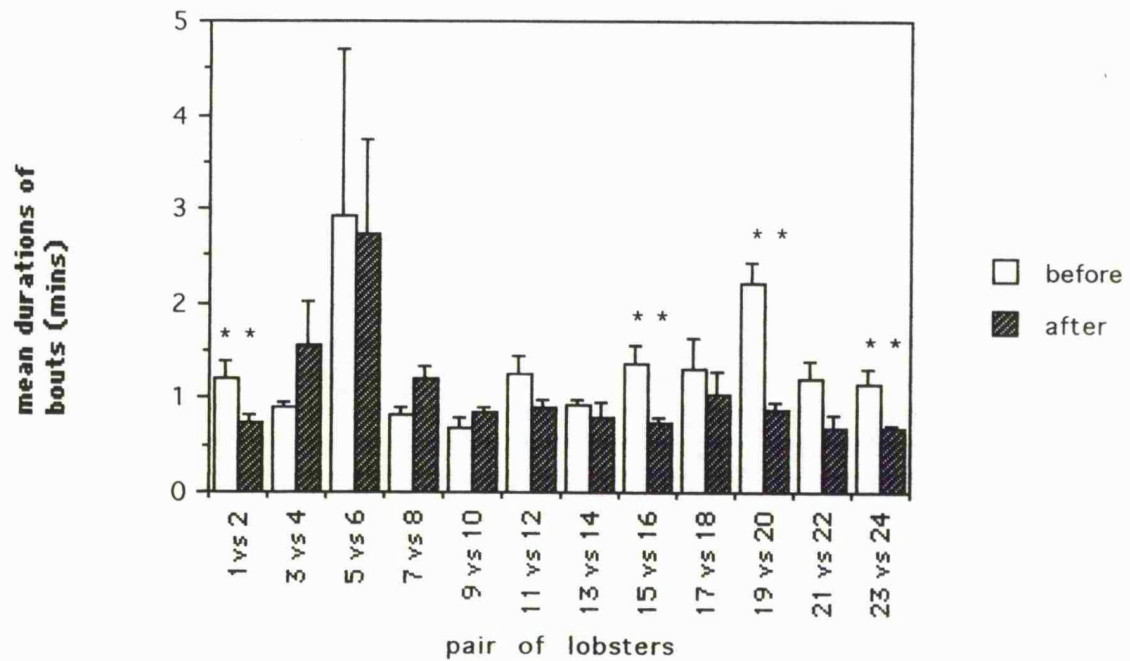


Figure 6.2. The mean duration of agonistic bouts (s.e.) before and after the dominant opponent was blinded. ** denotes a significant difference (using repeated measures t-test, $p = 0.05$). Mean bout duration was shorter in 9 out of the 12 cases, but only 4 were significantly different.

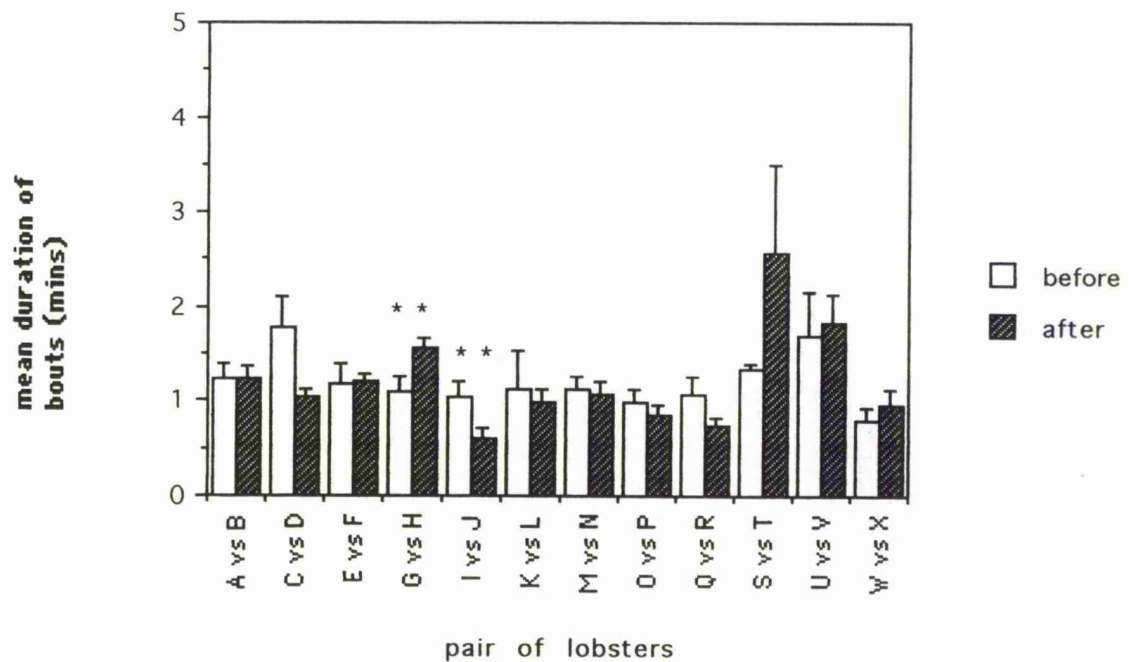


Figure 6.3. The mean duration of agonistic bouts (s.e.) before and after the subordinate opponent was blinded. ** denotes a significant difference (using repeated measures t-test, $p = 0.05$). In this case there was no clear trend in bout lengths after blinding. Where there were significant differences bout length was longer in 1 case and shorter in the other.

bouts (24 pairs x 5 trials)]. This was repeated for each of the 12 successive bouts within the trials and the means were plotted (Fig. 6.4). Visual inspection of the means reveals no obvious pattern.

To test whether there was any evidence for learning after the first bout between two opponents, the durations of the first two bouts were compared from all five trials when both opponents were sighted for all 24 pairs used in this experiment. A repeated measures t-test (two-tailed) showed that there was no significant difference ($p > 0.05$) between the duration of the first and second bouts. This suggests there was no evidence for learning affecting bout duration in the second of two bouts in a trial.

To check further for any possible difference between mean bout duration throughout a trial, mean duration of the bouts during the first six bouts was compared with mean bout duration during the last six bouts. An independent t-test was used and this showed that there was no significant difference in the mean bout duration (two-tailed, $t = 0.8$, $p > 0.05$). There was no evidence for any significant change in the duration of successive bouts in a trial of sighted opponents.

6.4.4. Agonistic bout structure.

To study the possible effects of blinding an individual on the agonistic behaviour of both opponents, agonistic interactions were analysed before and after blinding to look at (i) frequency of different types of acts; (ii) times spent performing these acts; and, (iii) sequences in which acts occurred.

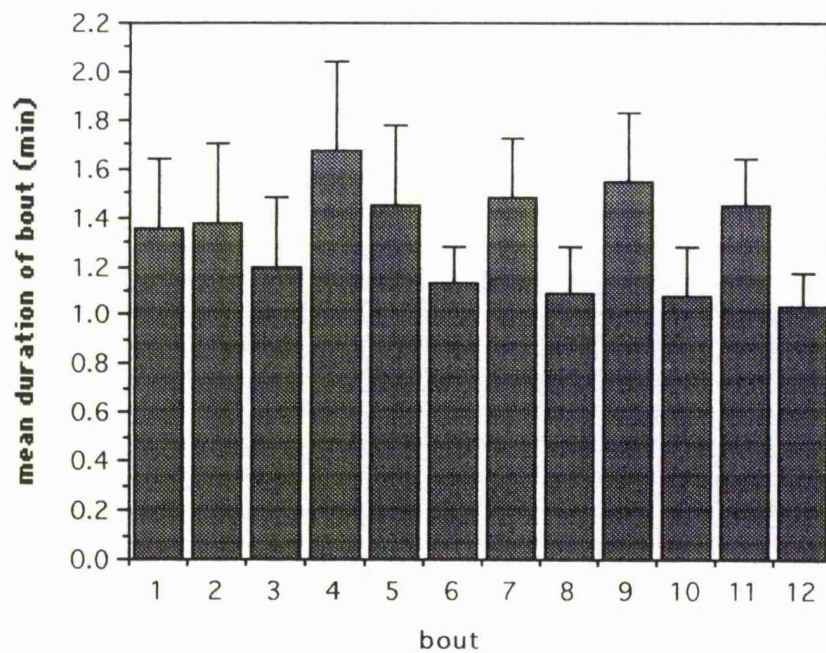


Figure 6.4. Mean duration (error bars = s.d.) of successive bouts (from first to twelfth) of trials when both opponents were sighted, for all 24 pairs of *N. norvegicus*. Over the 12 bouts there appeared to be no obvious trend in bout length.

Of the 24 pairs referred to in figure 6.5, 11 pairs were selected to represent the results from blinding the dominant or subordinate opponent and used for more detailed analysis. Pairs were chosen in which bout lengths were unchanged (groups A and C) or different (Groups B and D) after blinding (see Fig. 6.5 and 6.6).

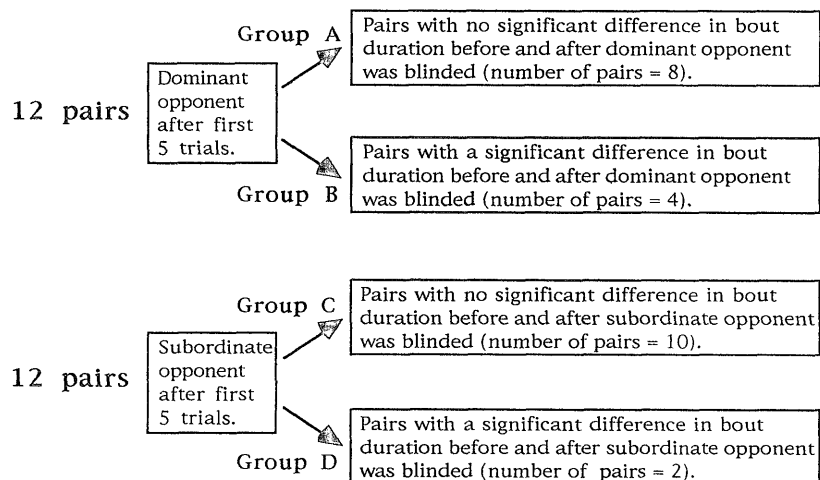


Figure 6.5. Pairs were assigned to different groups (A - D) according to whether or not the dominant or subordinate opponent was blinded and the effect that this had on bout duration.

Pairs selected for detailed analysis:

	Pairs in which dominant opponent was blinded after trial 5.	Pairs in which subordinate opponent was blinded after trial 5.
Pairs with no significant difference in bout duration before and after one opponent was blinded.	Group A: 5 vs 6 11 vs 12 13 vs 14	Group C: 19 vs 20 21 vs 22 23 vs 24
Pairs with a significant difference in bout duration before and after one opponent was blinded.	Group B: E vs F Q vs R W vs X	Group D: G vs H I vs J

Figure 6.6. Only some of the pairs were selected for analysis of bout structure. For each of groups A, B and C, three pairs were randomly selected (using paper balls drawn from a bag). For group D, both pairs were used for analysis.

Analysis of the effects of blinding one opponent on agonistic behaviour of a pair was carried out for each of the four groups separately and in the following way.

The video record of a bout was reviewed and sequences of agonistic behaviour were classified according to the ethogram (see section 6.3). Only the four main categories ('approach', 'non-contact cheliped display', 'contact cheliped attack', 'retreat') were used for the analysis. The total number of times that each category of agonistic acts occurred and the total time spent in displaying that act during a bout were recorded.

For the purposes of figures 6.7-6.16 the following abbreviations were used for the four main categories of agonistic acts being described in the graphs :-

- A - 'Approach' behaviour
- N/C - 'Non-contact cheliped display' behaviour
- C - 'Contact cheliped attack' behaviour
- R - 'Retreat' behaviour

6.4.4.(i) The effect of blinding on the occurrence of the four main classes of act performed during agonistic bouts.

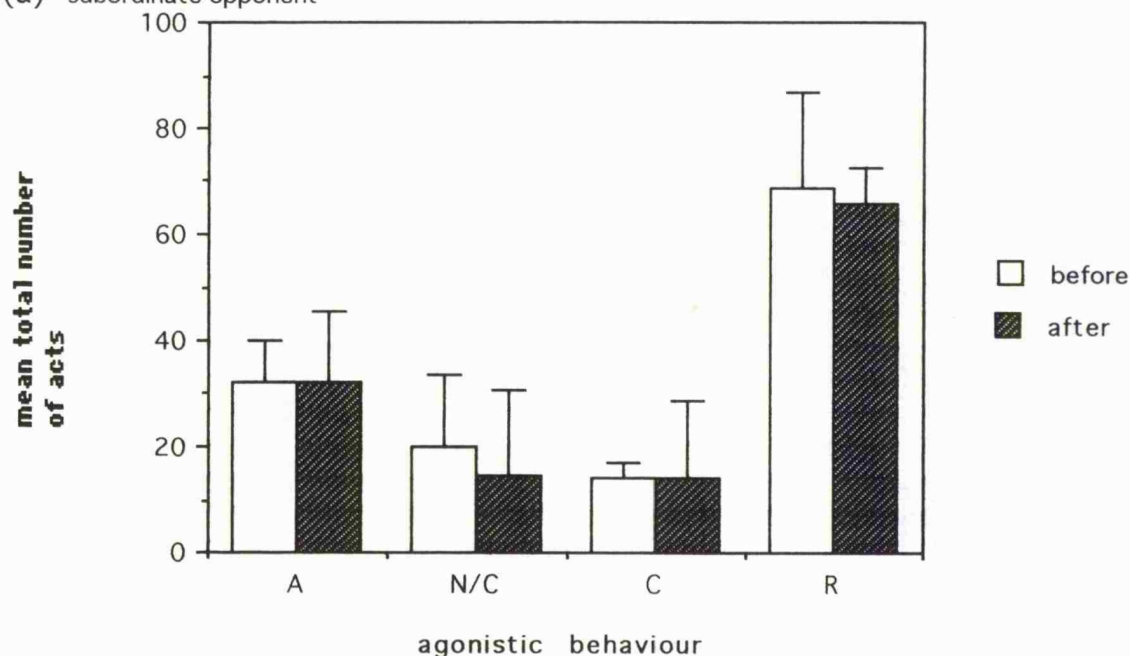
An analysis was carried out to compare the number of times each of the four main types of agonistic act occurred in the five trials before, and the five trials after either the dominant or subordinate opponent was blinded. In each case the total numbers of occurrences of the four categories of agonistic act for each individual in all five trials were noted for each pair (data for trials before and after blinding were calculated separately). For each of groups A, B and C there were three pairs per group. Here the mean total number of acts performed by each pair of each type was calculated for each of the groups and plotted (Figs 6.7 to 6.9). For group D each of the two pairs of individuals was analysed separately and total numbers of acts for each individual were plotted (Figs 6.10 and 6.11). The results were analysed using a two-way repeated measures ANOVA (two-tailed).

Pairs in which the dominant opponent was blinded before the second set of trials.

a) Pairs with no change in bout duration after blinding the dominant opponent (group A).

The results (Fig. 6.7) in these cases reveal that when sighted, the dominant opponent shows more 'approach' acts than all the other three types of agonistic act ($F = 10.46$, $p < 0.05$; Tukey test). Prior to blinding its opponent, the subordinate individual shows that more 'retreat' acts are performed than the other three types ($F = 56.7$, $p < 0.05$; Tukey test). In both opponents the other three types of agonistic behaviour are performed in similar numbers (Tukey

(a) subordinate opponent



(b) dominant opponent

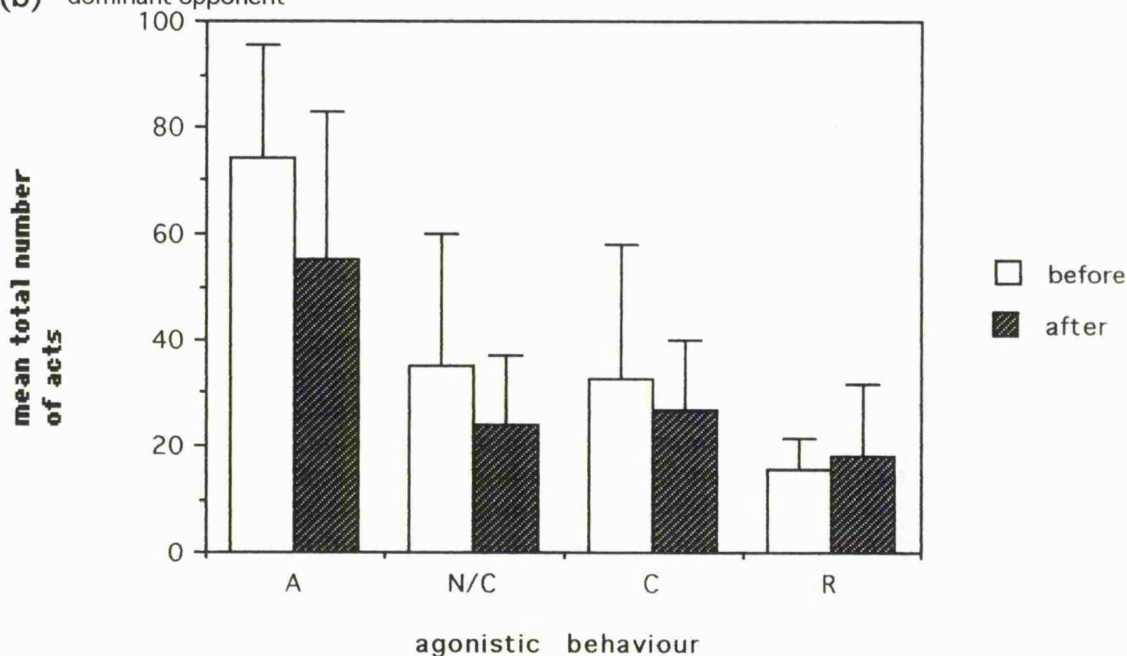


Figure 6.7. Mean total number of acts (s.d.) both opponents, the subordinate opponent performed more retreat acts and the dominant opponent performed more approach acts than any other types of act. Blinding of the dominant opponent had no effect on this pattern of behaviour (two-way ANOVA, $p = 0.05$). Results from the three pairs with similar mean bout durations before and after blinding the dominant opponent (i.e. group A).

test). After the dominant opponent had been blinded, bout structure is similar with no significant change in the trends of the number of acts performed by the dominant opponent ($F = 0.15$, $p > 0.05$) or by the subordinate opponent ($F = 0.18$, $p > 0.05$) (Fig. 6.7).

b) Pairs with significantly shorter bouts after blinding the dominant opponent (group B).

The results (Fig. 6.8) for these pairs show that prior to blinding, the sighted dominant opponent performs more 'approach' acts than the other three types of behaviour ($F = 20.11$, $p < 0.05$; Tukey test), all of which were performed in similar numbers (Tukey test). The numbers of acts of each of the four different types were unaffected by blinding the dominant opponent ($F = 1.3$, $p > 0.05$). The subordinate opponent performed more 'retreat' acts ($F = 18.7$, $p < 0.05$; Tukey test) than any other type of behaviour (which were all performed in similar numbers, Tukey test) and this result was also unaffected by the blinding of the dominant opponent ($F = 2.67$, $p > 0.05$).

Pairs in which the subordinate opponent was blinded during the second set of trials.

a) Pairs with similar bout durations before and after the subordinate opponent (group C).

Before the subordinate opponent was blinded the results (Fig. 6.9) revealed similar patterns to the previous sets of pairs. More acts of the 'approach' type were performed by the dominant opponent ($F = 37.52$, $p < 0.05$; Tukey test), than the other three types which were all performed similarly (Tukey test). As before, the subordinate opponent performed more 'retreat' acts ($F = 10.00$,

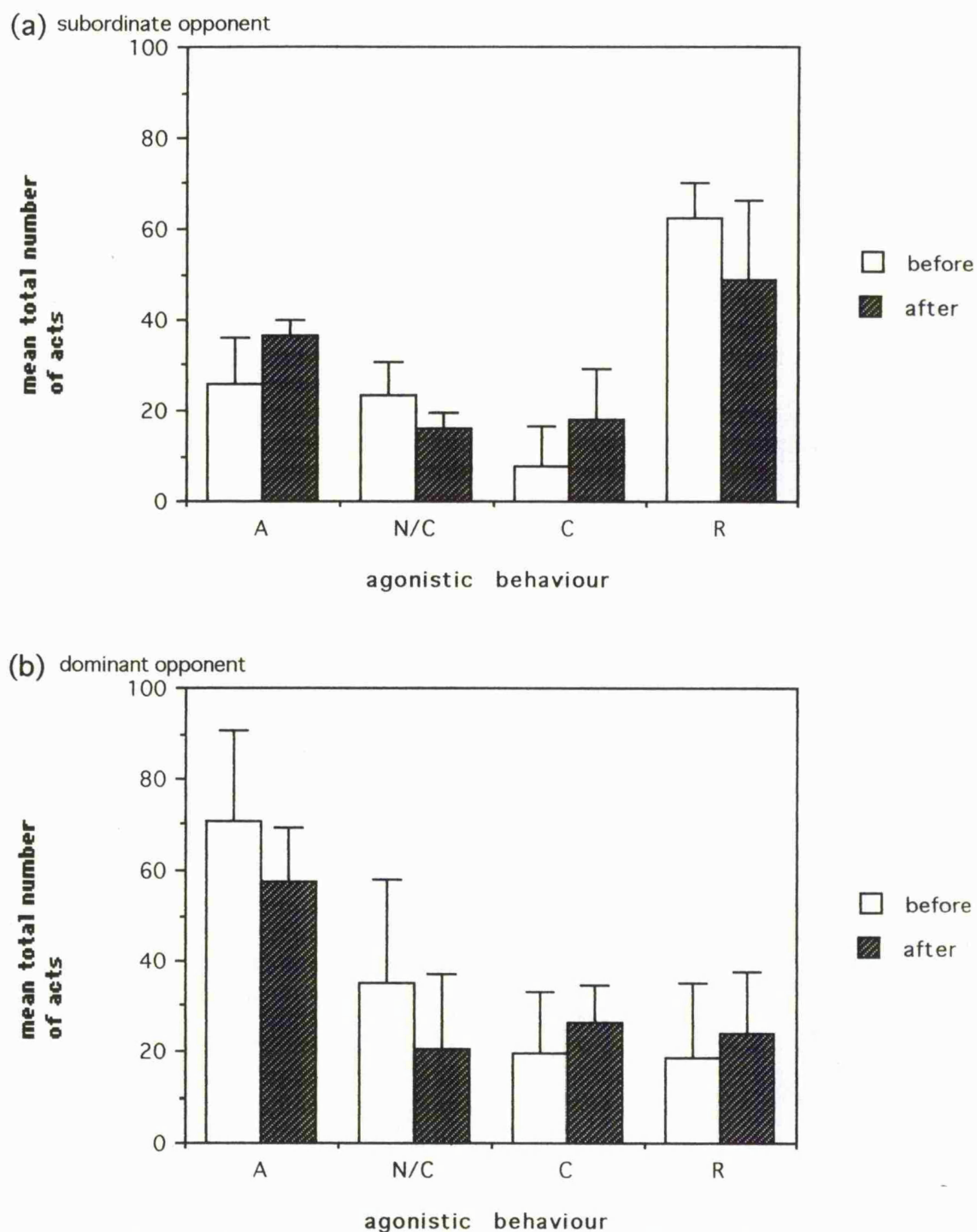
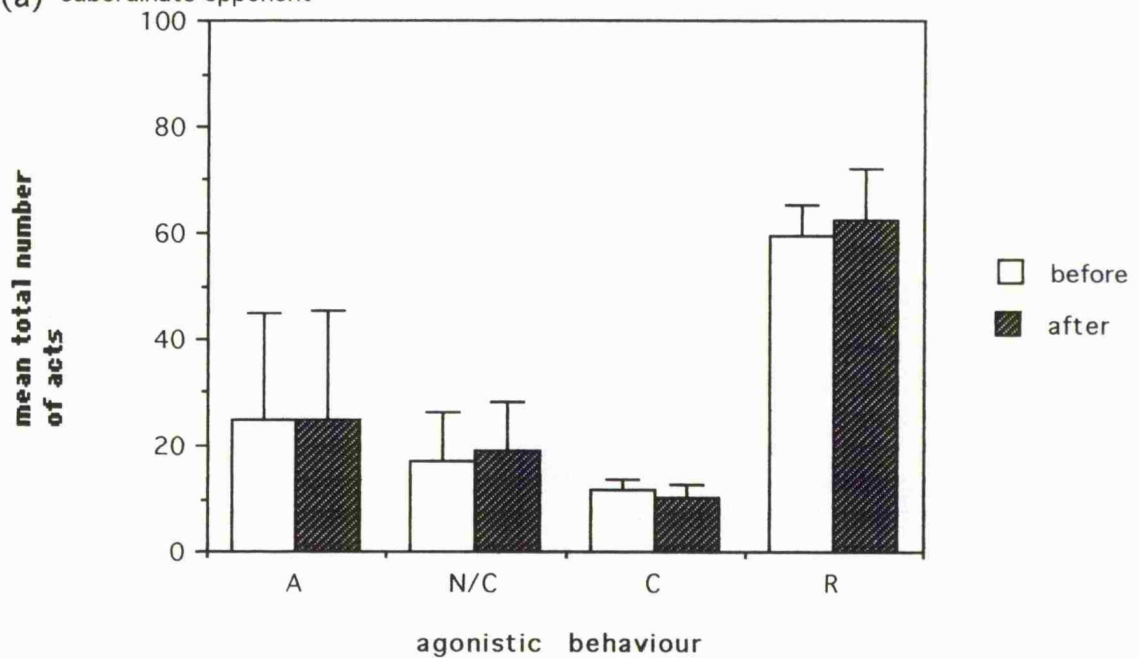


Figure 6.8. Mean total number of acts (s.d.) both opponents, the subordinate opponent performed more retreat acts and the dominant opponent performed more approach acts than any other types of act. Blinding of the dominant opponent had no effect on this pattern of behaviour (two-way ANOVA, $p = 0.05$). Results from the three pairs with shorter mean bout durations after blinding the dominant opponent (i.e. group B).

(a) subordinate opponent



(b) dominant opponent

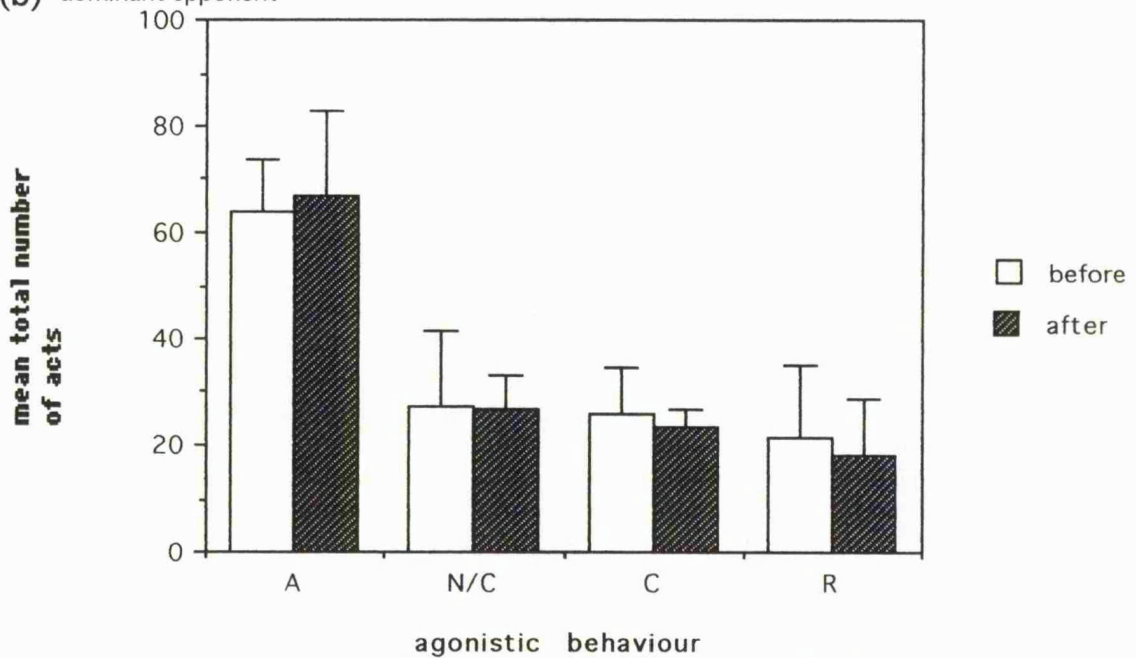


Figure 6.9. Mean total number of acts (s.d.) both opponents, the subordinate opponent performed more retreat acts and the dominant opponent performed more approach acts than any other types of act. Blinding of the subordinate opponent had no effect on this pattern of behaviour (two-way ANOVA, $p = 0.05$). Results from the three pairs with similar mean bout durations before and after blinding the subordinate opponent (i.e. group C).

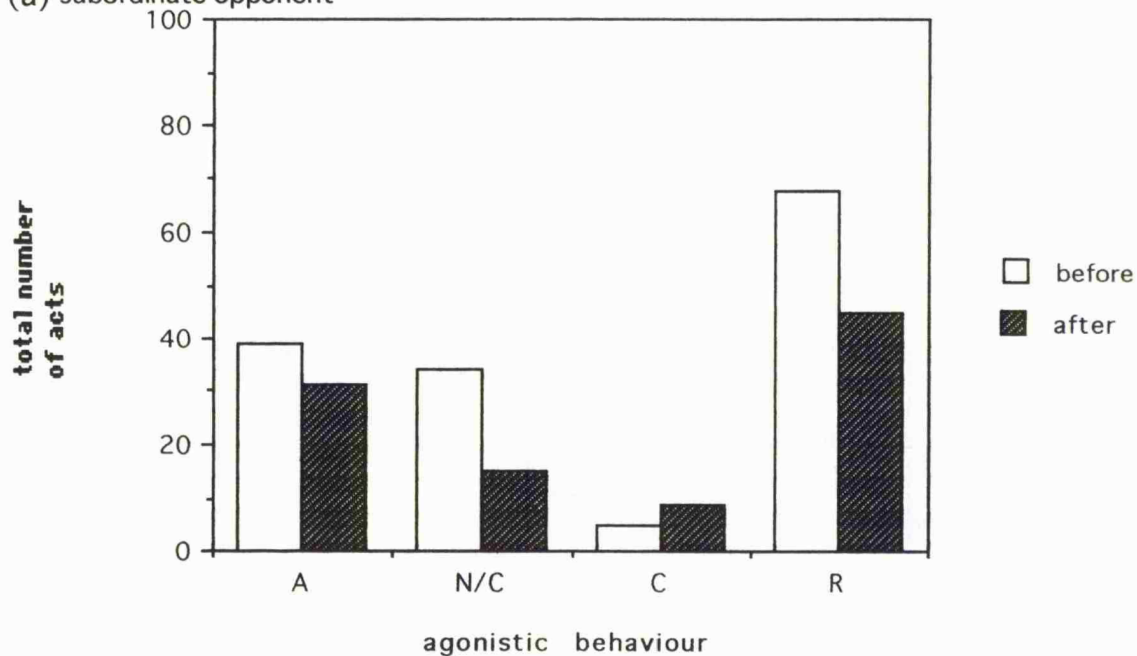
$p < 0.05$; Tukey test) than the other three types which were again performed with similar numbers (Tukey test). The blinding of the subordinate opponent had no effect on either opponent's behaviour (dominant opponent: $F = 0.41$, $p > 0.05$; subordinate opponent: $F = 0.32$, $p > 0.05$).

b) Pairs with significantly different bout durations after blinding the subordinate opponent (group D).

The results (Fig. 6.10 and 6.11) for these two pairs are presented separately because one pair had longer bouts following the blinding of the subordinate opponent and the other had shorter bouts. Because there was only one pair of each class, the data for statistical analysis of bout structure in group D had to be treated in a different way from the data sets in group A - C. In the latter case mean frequencies of the different types of acts for each of the three pairs in a group could be used in the ANOVA test. With only one pair available for analysis in each case it was necessary to compare the data for each of the ten different trials (five trials before and five trials after blinding) because there must be more than one sample when using the ANOVA test.

Pair with shorter bouts after blinding the subordinate opponent - when both opponents were sighted the dominant opponent performed more 'approach' acts ($F = 24.25$, $p < 0.05$; Tukey test) than the other three types which were all performed similarly (Tukey test). The subordinate opponent performed more 'retreat' acts than the other three types of agonistic behaviour ($F = 145.41$, $p < 0.05$; Tukey test). Again, blinding the subordinate opponent had no effect on the trends of behaviour for either

(a) subordinate opponent



(b) dominant opponent

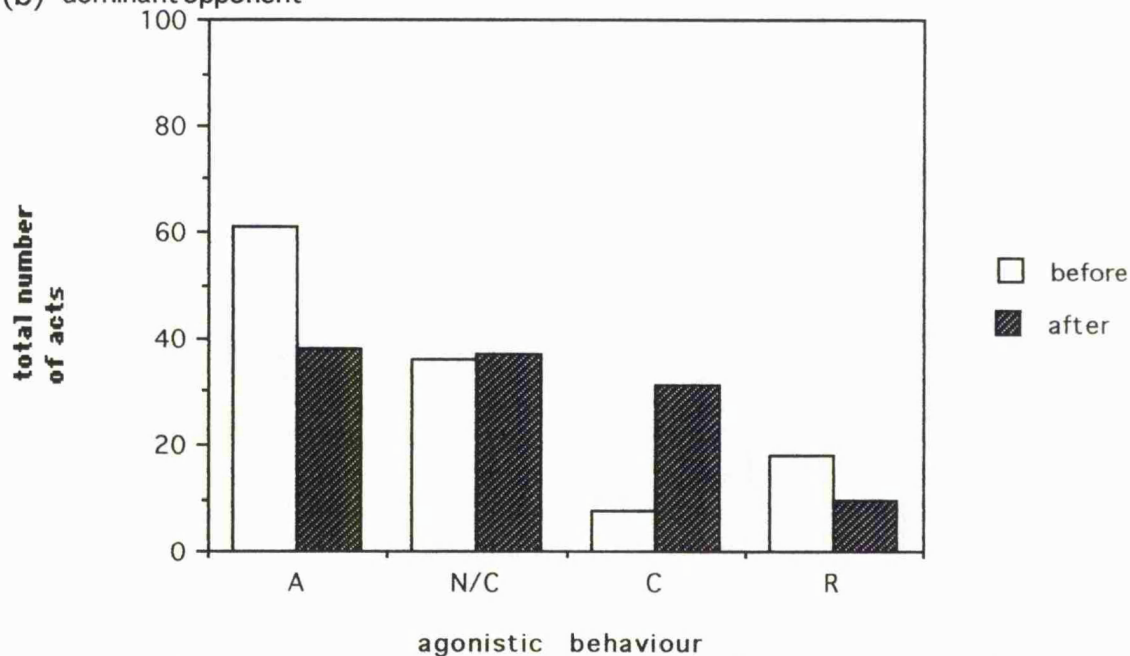
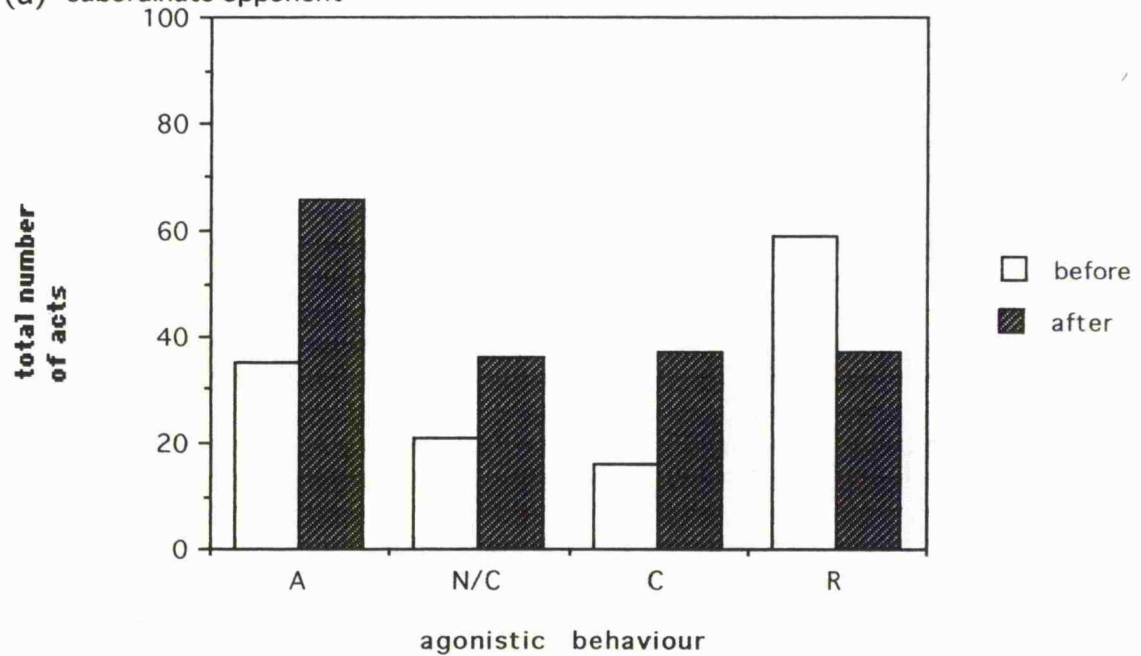


Figure 6.10. Total number of acts displayed by both opponents. The subordinate opponent performed more retreat acts and the dominant opponent performed more approach acts than any other types of act. Blinding of the subordinate opponent had no effect on this pattern of behaviour (two-way ANOVA, $p = 0.05$). Results from the pair with shorter mean bouts after the subordinate opponent was blinded (i.e. group D).

(a) subordinate opponent



(b) dominant opponent

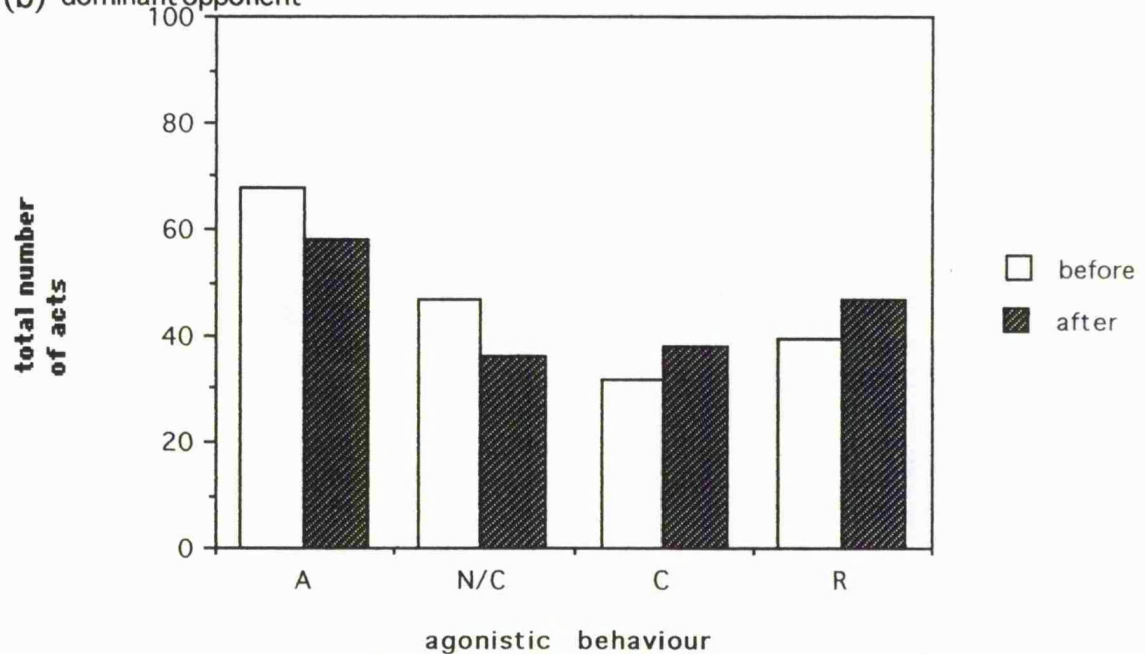


Figure 6.11. Total number of acts displayed by subordinate opponent (a) and dominant opponent (b). In both cases individuals performed each type of act in similar numbers and this was not affected by blinding (two-way ANOVA, $p > 0.05$). Results from the pair with longer bouts after blinding the subordinate opponent (i.e. group D).

opponent (dominant opponent: $F = 1.48$, $p > 0.05$; subordinate opponent: $F = 3.21$, $p > 0.05$).

Pair with longer bouts after blinding the subordinate opponent - in the case where mean bout length was longer all four types of acts were performed in similar numbers when both opponents were sighted, for the dominant opponent ($F = 2.44$, $p > 0.05$) and the subordinate opponent ($F = 1.03$, $p > 0.05$). Blinding had no effect on either opponent's behaviour (dominant opponent: $F = 0.98$, $p > 0.05$; subordinate opponent: $F = 1.13$, $p > 0.05$).

In summary, the groups of pairs examined (groups A - D) show that the dominant opponent's behaviour is characterised by more 'approach' acts and the subordinate opponent performs more 'retreat' acts. This difference in behaviour is unchanged by the blinding of either opponent.

The only exception to this was the single pair of individuals with longer bouts after the subordinate opponent was blinded. For this pair there was no significant difference in the number of each of the four types of acts performed by both opponents when they were sighted. There was no change after the subordinate opponent had been blinded.

6.4.4.(ii) The effect of blinding on the time opponents spent performing the four main classes of act during agonistic bouts.

The mean total time per bout spent performing each type of act by each opponent was calculated for the two periods before and after the blinding of one opponent (either dominant or subordinate). Treatment of data for graph plotting (Figs 6.12 to 6.16) and statistical analysis were similar to those used for data relating to the number of acts per bout (section 6.4.4. (i)).

Pairs in which the dominant opponent was blinded during the second set of trials.

a) Pairs with similar bout duration before and after the dominant opponent was blinded (group A).

When both opponents were sighted the dominant opponent spent more time performing the 'approach' type of act ($F = 15.54$, $p < 0.05$; Tukey test) than all other three types. The subordinate opponent spent significantly longer performing the 'retreat' type of agonistic behaviour ($F = 6.96$, $p < 0.05$; Tukey test). In both cases all other three types of agonistic behaviour were performed for similar amounts of time (Tukey test). The blinding of the dominant opponent had no effect on these trends (dominant opponent: $F = 0.09$, $p > 0.05$; subordinate opponent: 0.91 , $p > 0.05$) (Fig. 6.12).

b) Pairs with significantly shorter bouts after the blinding the dominant opponent (group B).

Prior to blinding the dominant opponent spent more time performing 'approach' agonistic behaviour ($F = 17.03$, $p < 0.05$; Tukey test) (Fig. 6.13) than any other types of act and the

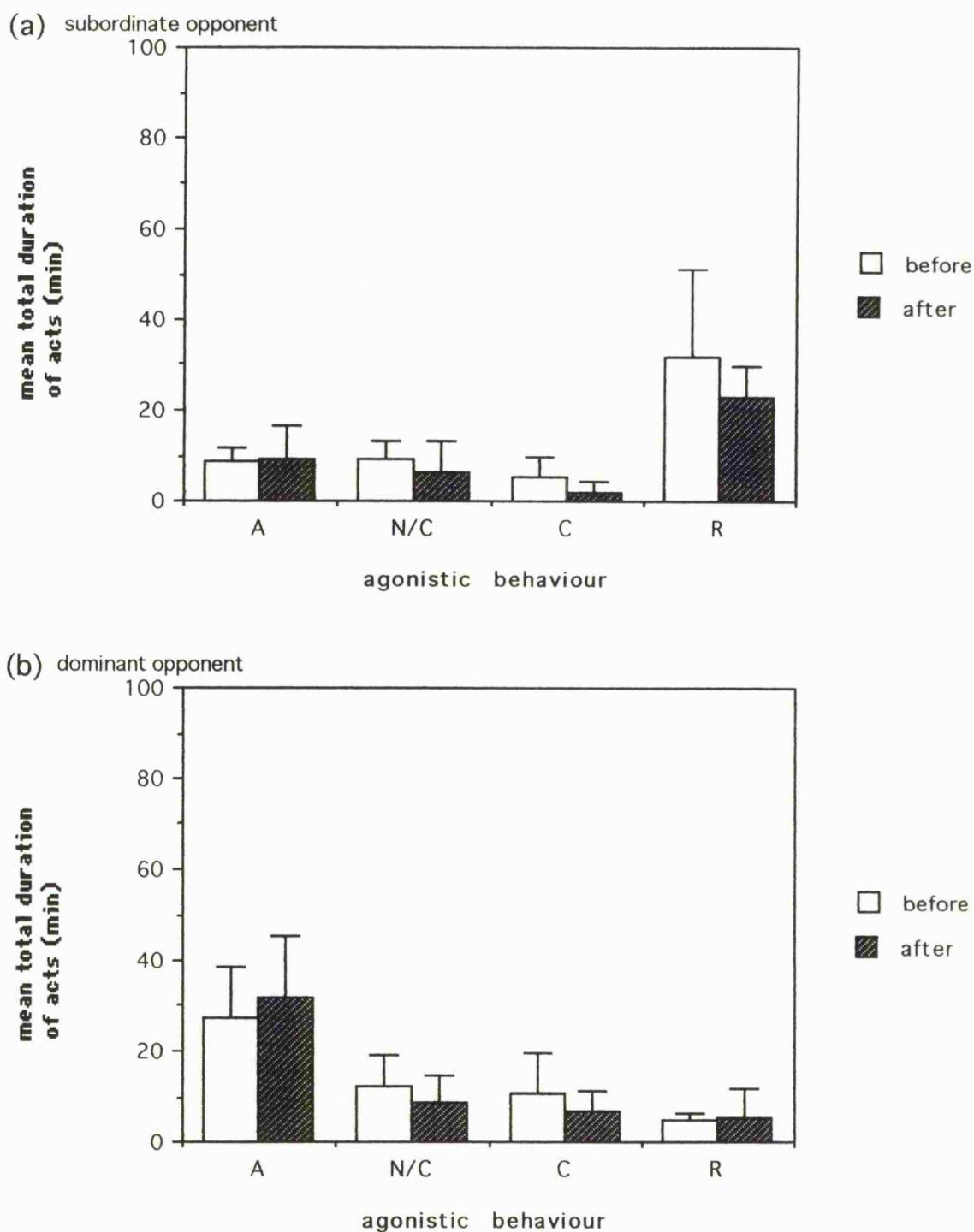


Figure 6.12. Mean total time spent performing acts (s.d.) by both opponents. The subordinate opponent spent significantly more time performing retreat acts and the dominant opponent spent more time performing approach acts. Blinding of the dominant opponent had no effect (two-way ANOVA, $p = 0.05$). Results from the three pairs with similar mean bout durations before and after blinding the dominant opponent (i.e. group A).

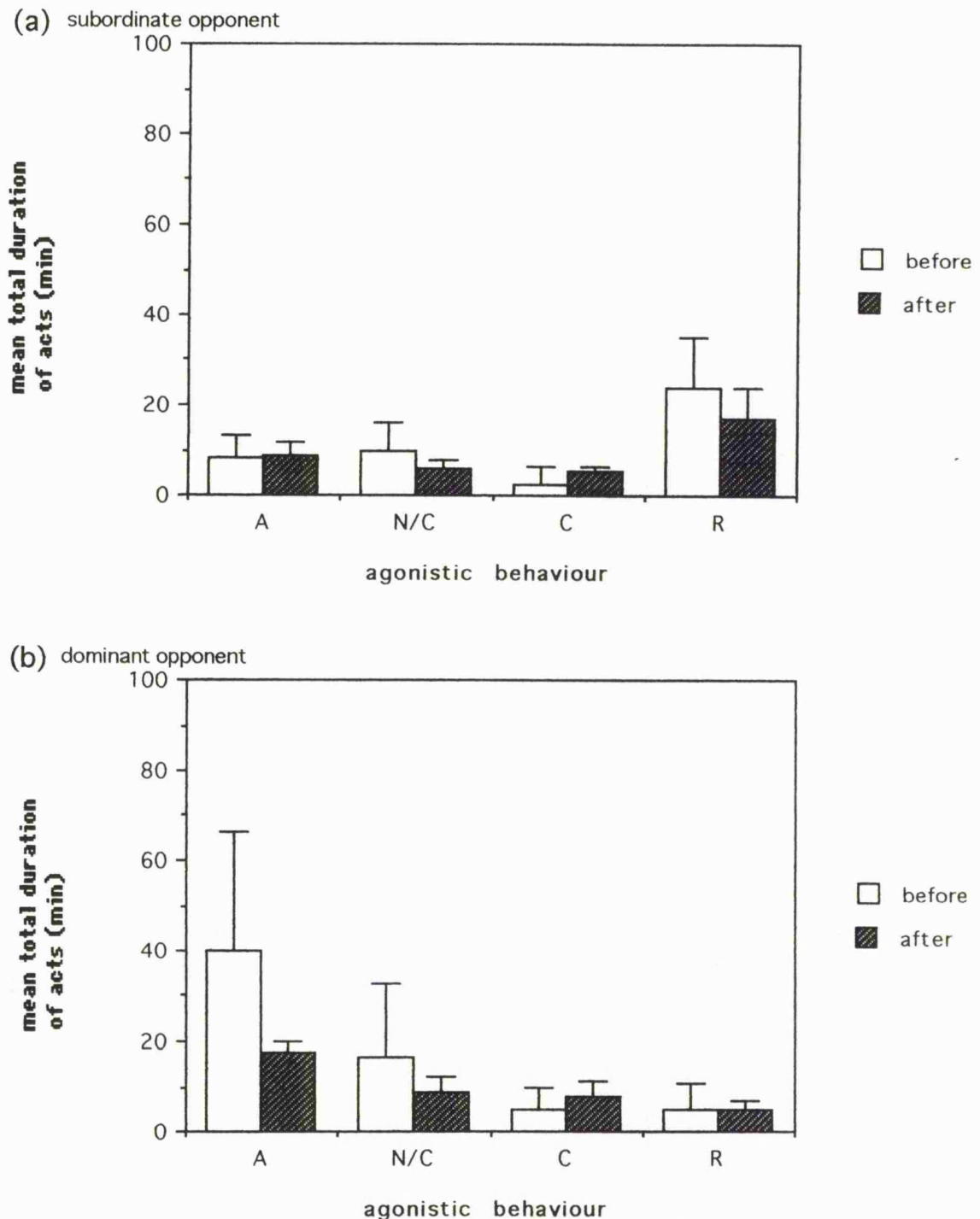


Figure 6.13. Mean total time spent performing acts (s.d.) by both opponents. The subordinate opponent spent significantly more time performing retreat acts and the dominant opponent spent more time performing approach acts. Blinding of the dominant opponent had no effect (two-way ANOVA, $p = 0.05$). Results from the three pairs with shorter mean bout durations after blinding the dominant opponent (i.e. group B).

subordinate opponent spent most time performing 'retreat' behaviour ($F = 50.79$, $p < 0.05$; Tukey test). Both opponents spent similar amounts of time performing the other three types of agonistic act. Again, the blinding of the dominant opponent had no statistically significant effect on the behaviour of either opponent (dominant opponent: $F = 1.6$, $p > 0.05$; subordinate opponent: $F = 1.18$, $p > 0.05$).

Pairs in which the subordinate opponent was blinded in the second half of the experiment.

a) Pairs with similar bout duration before and after blinding the subordinate opponent (group C).

During the period before the subordinate opponent was blinded the dominant opponent spent more time performing 'approach' acts ($F = 12.23$, $p < 0.05$; Tukey test) than the three other types of behaviour. The subordinate opponent spent longer performing 'retreat' acts ($F = 51.26$, $p < 0.05$; Tukey test) than any other type of behaviour. In both cases all other types of act were performed for similar amounts of time (Tukey test). The blinding of the subordinate opponent had no significant effect on the behaviour of either opponent (dominant opponent: $F = 0.98$, $p > 0.05$; subordinate opponent: $F = 1.02$, $p > 0.05$) (Fig. 6.14).

b) Pairs with different bout durations after blinding the subordinate opponent (group D).

Pair with shorter bouts - although the differences between the times spent performing the different types of agonistic act were less pronounced than in the previous groups studied, the analysis revealed that the dominant opponent spent a significantly longer time performing 'approach' behaviour ($F =$

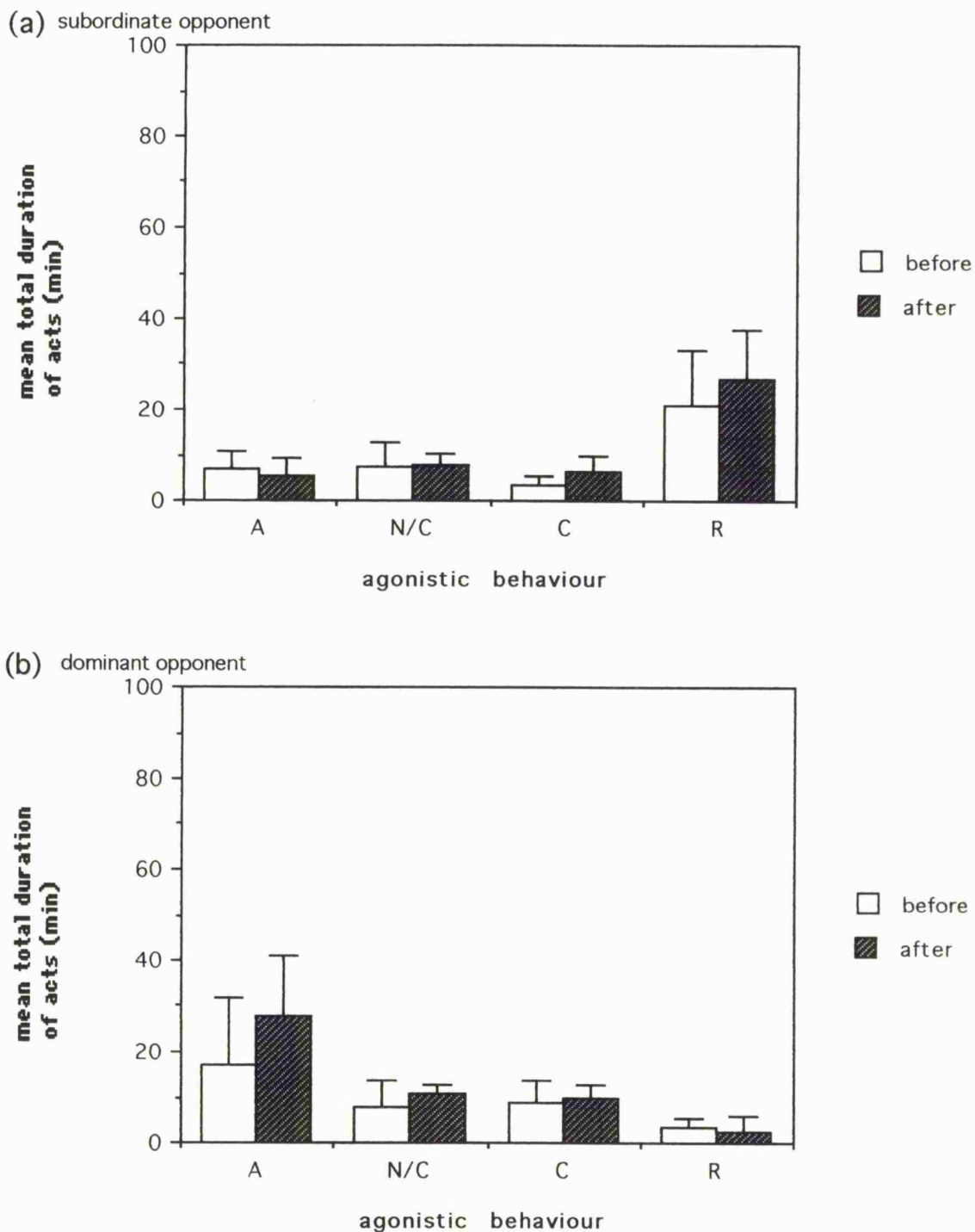


Figure 6.14. Mean total time spent performing acts (s.d.) by both opponents. The subordinate opponent spent significantly more time performing retreat acts and the dominant opponent spent more time performing approach acts. Blinding of the subordinate opponent had no effect (two-way ANOVA, $p = 0.05$). Results from the three pairs with similar mean bout durations before and after blinding the subordinate opponent (i.e. group C).

14.53, $p < 0.05$; Tukey test). Also the subordinate opponent spent longer performing 'retreat' behaviour ($F = 43.02$, $p < 0.05$; Tukey test) than the other three types of behaviour studied. For both opponents these other types of behaviour were performed for similar amounts of time (Tukey test). Blinding of the subordinate opponent had no significant effect on behaviour of either opponent (dominant opponent: $F = 0.31$, $p > 0.05$; subordinate opponent: $F = 1.0$, $p > 0.05$) (Fig. 6.15).

Pair with longer bouts - before the subordinate opponent was blinded there was no significant difference in the time spent engaged in four different types of agonistic act for both opponents (dominant opponent: $F = 0.83$, $p > 0.05$; subordinate opponent: $F = 1.12$, $p > 0.05$). The blinding of the subordinate opponent had no effect on the behaviour of either opponent (dominant opponent: $F = 0.53$, $p > 0.05$; subordinate opponent: $F = 1.11$, $p > 0.05$) (Fig. 6.16).

In summary, most groups of pairs studied (groups A - D) showed that the dominant opponent spends significantly more time performing 'approach' acts and the subordinate opponent spends most time performing 'retreat' acts. The only exception was the pair where bouts were longer after the subordinate opponent was blinded. In this case both opponents showed similar amounts of time spent performing all four types of act studied. In all cases blinding one opponent does not affect these trends in behaviour. These results are consistent with the results for the total numbers of each of the four types of act.

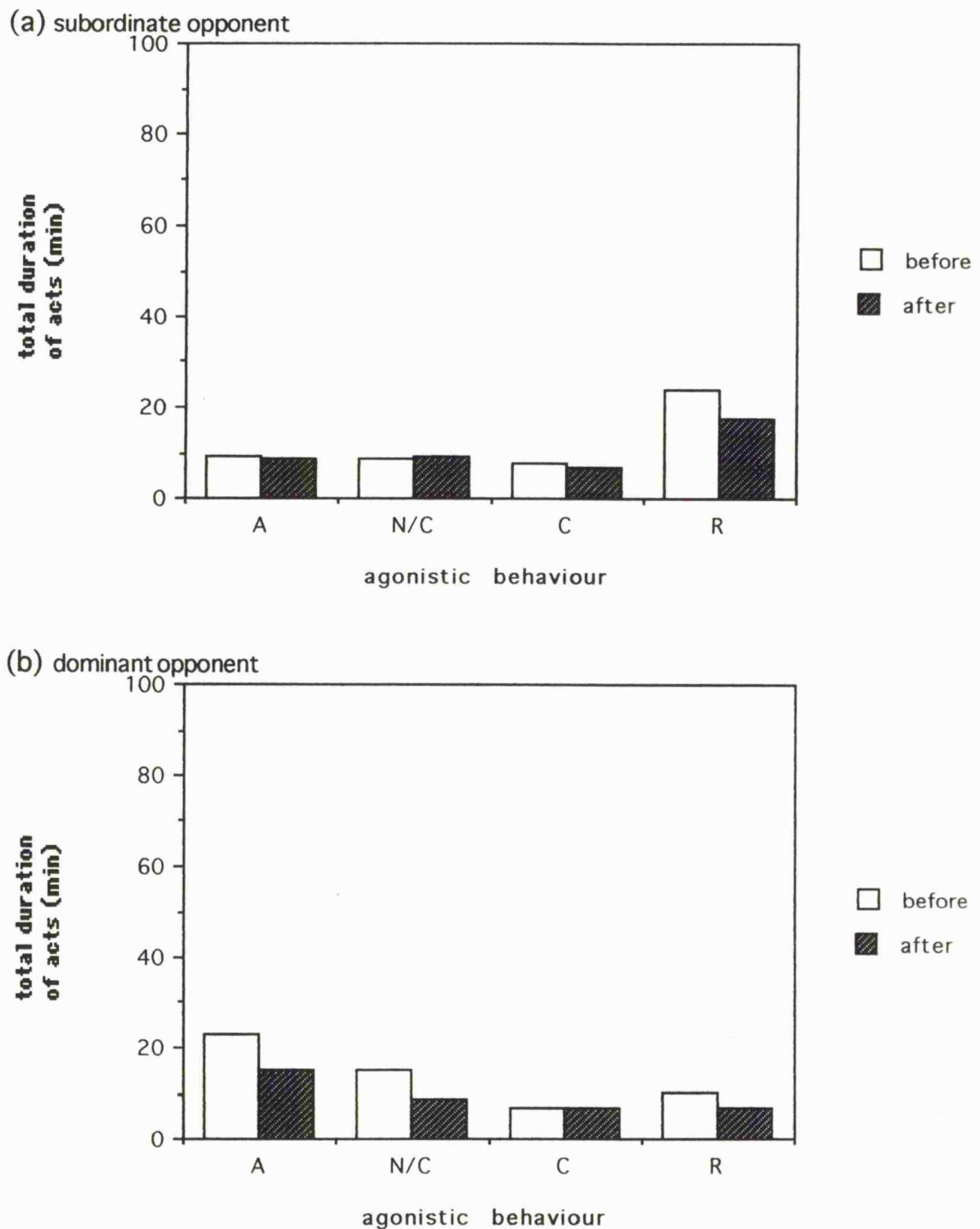


Figure 6.15. Mean total time spent performing acts (s.d.) by both opponents. The subordinate opponent spent significantly more time performing retreat acts and the dominant opponent spent more time performing approach acts. Blinding of the subordinate opponent had no effect (two-way ANOVA, $p = 0.05$). Results from the three pairs with shorter mean bout durations before and after blinding the subordinate opponent (i.e. group D).

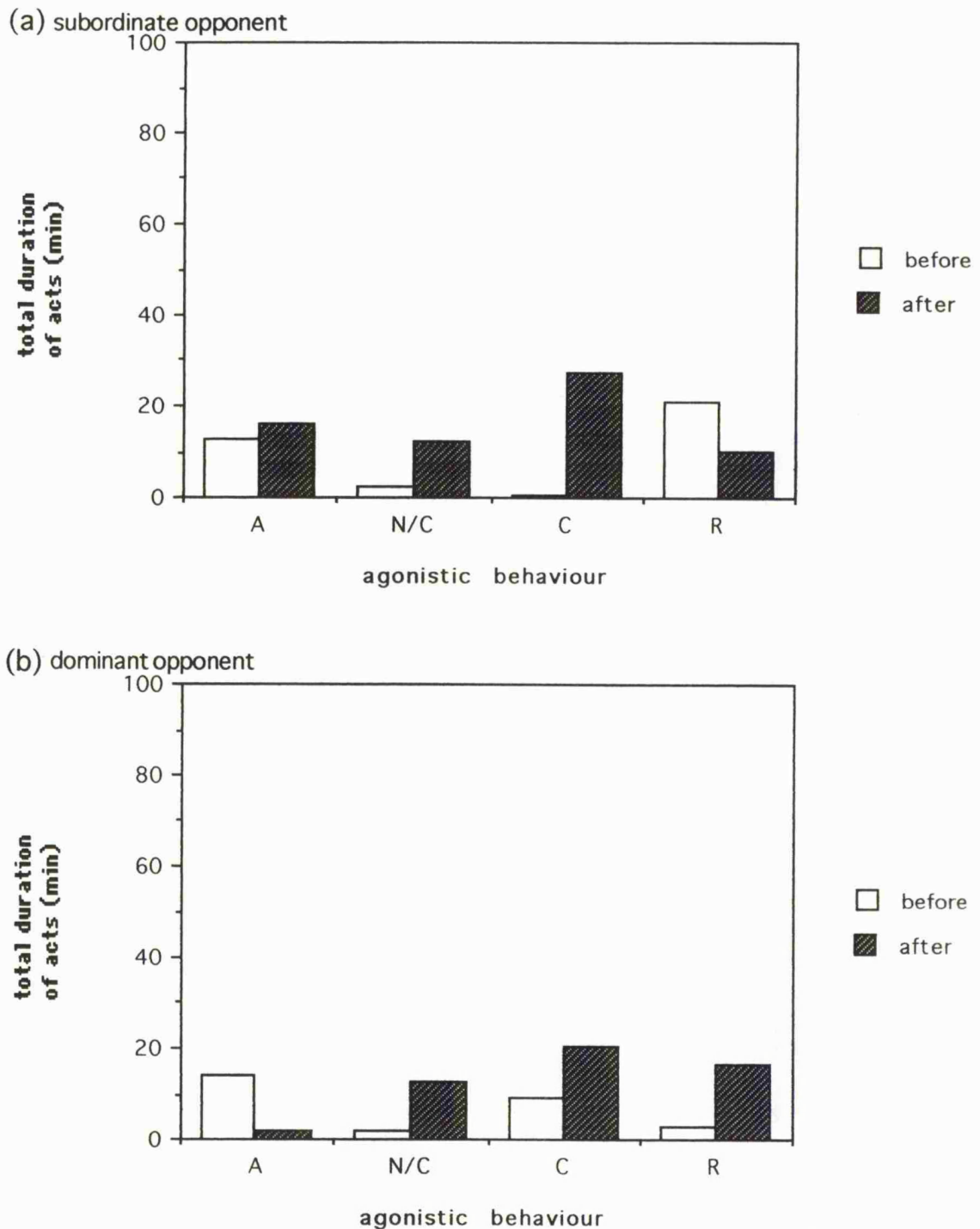


Figure 6.16. Bar charts to show results from the pair with longer bouts after the blinding the subordinate opponent (i.e. group D). Total duration of acts displayed by subordinate opponent (a) and dominant opponent (b). In this case each opponent spent similar amounts of time performing each type of act, before and after the subordinate opponent was blinded (two-way ANOVA, $P > 0.05$).

6.4.4.(iii) *Sequence of acts in agonistic bouts.*

Bout structure was also analysed in order to describe sequences of agonistic behaviour when both opponents were sighted, and after one opponent had been blinded. 'Sequence analysis' is a set of techniques used to analyse the succession of behavioural acts between two opponents and can demonstrate whether the behaviour of one individual is affected by that of the other (i.e. inter-individual communication has occurred) (Colgan, 1978). The application of these techniques to sequences of agonistic behaviour was used to assess whether the actions of one opponent affect those of the other opponent, when both opponents are sighted, and then after one opponent has been blinded.

For this analysis a first-order Markov chain (Colgan, 1978) was used to model the sequences within the bouts. This assumes that the probability of a given act occurring is dependent on the identity of the preceding act only (Slater, 1973), and allows each bout to be represented as a series of discrete pairs of acts. These can then be treated as independent for statistical tests to show whether each pair occurs more often than would be expected by chance.

For the analysis, video tapes of the same 11 pairs previously selected for detailed analyses of bout structure were used to study sequences of acts in the first 12 agonistic bouts of all 5 trials before, and in the 5 trials after, one opponent had been blinded.

The video tape recordings for these pairs were reviewed and the behaviour of a pair of opponents within each bout was described as a sequence of acts using the four main categories of agonistic behaviour previously described in the ethogram (i.e. 'approach', 'non-contact cheliped display', 'contact cheliped attack',

and 'retreat'). Occurrences of the four types of act were identified and noted in the orders in which they were observed (see Fig. 6.17. for an example).

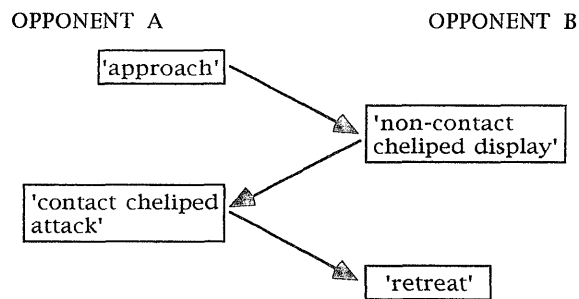


Figure 6.17. An example of a bout of agonistic behaviour between opponents A and B as a sequence of acts.

The sequence of agonistic acts within a bout was then divided into pairs of acts between the opponents (to be referred to as 'dyads' (Lehner, 1979)), starting with the first act by the opponent initiating the bout (see Fig. 6.18. for an example).

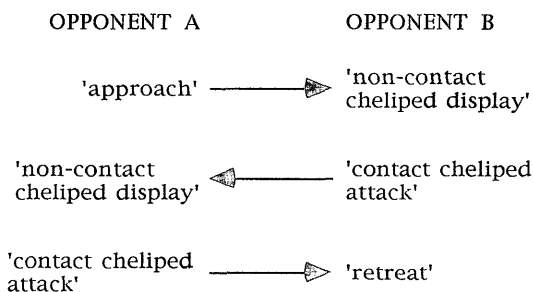
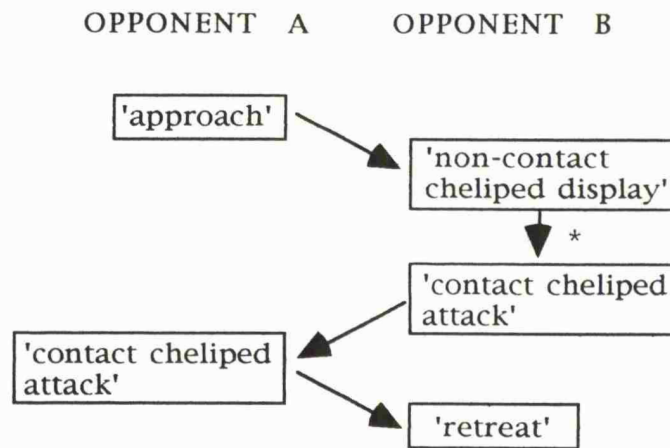


Fig. 6.18. the previous sequence of acts divided into dyads.

These pairs of acts were collected onto transition matrices to show the total numbers of each type of dyad occurring between

a pair of individuals. The transition matrices were arranged so that the horizontal rows represented the first act of a dyad performed by the first opponent (to be called the 'transmitter' (Lehner, 1979)) and the vertical columns represented the second act of the dyad performed by the other opponent (to be called the 'receiver' (Lehner, 1979)). Four sets of matrices were required to illustrate the agonistic behaviour between a pair of opponents; two matrices showing the dyads before one opponent was blinded, one of which had the 'transmitter' as the subordinate opponent and the other with the 'transmitter' as the dominant opponent; and, two matrices showing the dyads occurring after the blinding process (see Fig. 6.20 for transition matrices).

During the bouts there were occasions when one opponent (the 'transmitter') performed an agonistic act and the other opponent (the 'receiver') continued to perform the type of agonistic behaviour it was displaying earlier. It was not possible to know whether this 'receiver' was not responding to the behaviour of the 'transmitter' (either by choice, or was unable to detect the behaviour of the 'transmitter'), or that the 'receiver' was actually responding by performing its previous behaviour. For the purposes of this analysis it was decided to assume that the 'receiver' was not responding to the behaviour of the 'transmitter' and when recording the sequence of acts in a bout this behaviour was described as 'no response' for the 'receiver' (see Fig. 6.19. for an example).



(*Opponent A continued to display 'approach' behaviour and did not respond to 'non-contact cheliped display' of opponent B.)

This bout may then be divided into the following dyads:

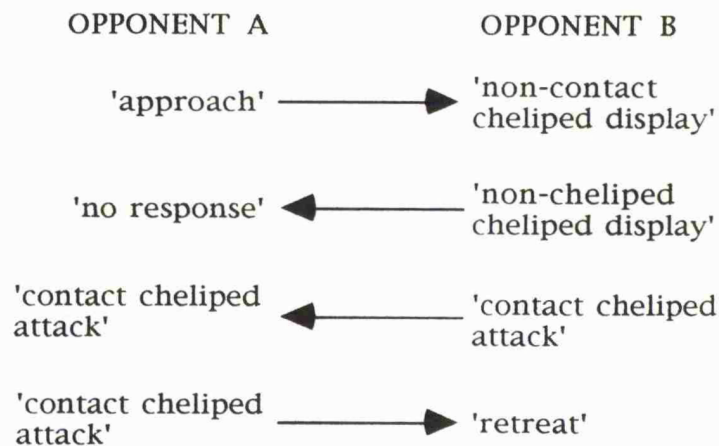


Fig. 6.19. An example of the description of the sequence of a behaviour of a bout between opponent A and B with the occurrence of one opponent not responding to the behaviour of the other.

The 'no response' column commonly contained high counts, indicating that there was often no change in the behaviour of an opponent in response to that of the other opponent.

The data in most transition matrices satisfied the conditions of a chi-squared test. The only exceptions were matrices representing the transitions between pairs where the subordinate opponent was blinded and there was a significant difference in bout length after the blinding procedure (Fig. 6.20.). In these cases there was insufficient data to proceed with a chi-squared test because each matrix contained the results from one pair of individuals only. Therefore no further sequence analysis could be carried out on these pairs of individuals. For all other transition matrices tested, it was shown that the agonistic behaviour of each opponent was related to the behaviour of the other (i.e. a number of transitions occurred more frequently than expected by chance) (see Fig. 6.20 for outcomes of chi-squared tests), irrespective of whether both opponents were sighted or one was blinded.

Significant dyads occurring in bouts.

Examining each transition matrix separately, those dyads which occurred more or less often than expected by chance (i.e. significant dyads) were identified in the following way. Taking a single matrix the pair of acts contributing the highest deviation from expected values was assigned a value of zero (i.e. a logical zero (Slater, 1973)) and a chi-squared test on the altered matrix was carried out. This procedure was repeated, removing each successive pair of acts showing the highest deviation from the expected values, until the chi-squared test failed to find a significant association between the behaviour of the individuals in the matrix. Arrow diagrams were drawn for each matrix to illustrate the significant dyads identified with this procedure ($p < 0.05$) (Fig 6.20).

Initially, patterns were looked for in the significant dyads identified from the agonistic bouts of all groups (i.e. groups A - C) in the first five trials, when both opponents were sighted. There were many differences in the significant pairs of acts found in the agonistic bouts of these individuals, however, the following significant pairs of acts were found in all groups of pairs examined when both opponents were sighted:

i) 'Retreat' inhibits the response of 'retreat' by the other opponent, if either the dominant or subordinate opponent withdraws from an agonistic bout.

ii) 'Contact cheliped attack' by a dominant opponent stimulates the subordinate opponent to respond with 'contact cheliped attack' and both opponents engage in overt fighting.

iii) If the dominant opponent performs a 'retreat' act, the subordinate opponent will re-initiate the bout with an 'approach' act.

iv) An 'approach' act by the dominant opponent will cause the subordinate opponent to 'retreat' and withdraw from the contest.

In summary, these common pairs of acts and those others in the sequence diagrams show that the subordinate opponent performs aggressive behaviour. The only indication of how the subordinate opponent possibly loses a bout is the immediate withdrawal response performed by the subordinate opponents if the dominant opponent displays 'approach' behaviour.

After one opponent had been blinded there was also a variation in the significant dyads between groups of opponents. However, when looking at the significant pairs of acts common to the agonistic behaviour of all pairs when one opponent had been blinded they showed little change from those when both opponents were sighted after the dominant opponent had been blinded dyad (i)

(see above description) was significant for all groups of pairs studied and dyads (ii), (iii) and (iv) were significant in most but not all of the groups studied. After the subordinate opponent had been blinded dyads (i), (ii) and (iv) (see above description) were significant for all groups of pairs studied. The only significant dyad not seen in all pairs after blinding the subordinate opponent was dyad (iii) (see above description).

No other significant dyads were common to all groups of pairs studied after one opponent had been blinded. On balance it seems that the significant responses of the subordinate and dominant opponents were largely unchanged after they had been blinded.

Figure 6.20. Transition matrices of agonistic behaviour between two *N. norvegicus*. The UNDERLINED dyads indicate the pairs of acts which occur more often than expected by chance (using chi-squared test and a probability level of 0.05). The OUTLINED dyads indicate those which occur less often than expected by chance (using chi-squared test and a probability level of 0.05).

Data in each matrix is the sum of the number of each dyad observed in all bouts examined. In this figure the results are written as a percentage of the total number of all dyads of all types recorded in the matrix.

Key to types of agonistic behaviour:

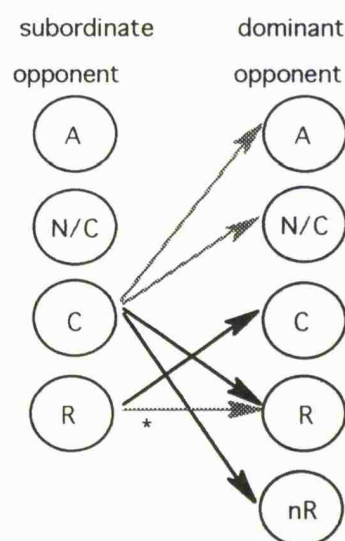
A - 'approach'
 N/C - 'non-contact cheliped display'
 C - 'contact cheliped attack'
 R - 'retreat'
 nR - 'no response'

Key to figures:

—————> transitions of behaviour occurring more often than expected by chance.
> transitions of behaviour occurring less often than expected by chance.

		receivers' behaviour (dominant opponent)				
		A	N/C	C	R	nR
transmitters'	A	12.2	7.3	3.1	5.9	3.8
behaviour	N/C	3.5	5.6	3.8	2.4	4.2
(subordinate	C	<u>1.0</u>	<u>0.7</u>	1.0	<u>3.1</u>	<u>5.9</u>
opponent)	R	10.5	10.8	<u>9.8</u>	<u>1.0</u>	4.2

(chi square = 65.74, $p < 0.05$)



		receivers' behaviour (subordinate opponent)				
		A	N/C	C	R	nR
transmitters'	A	3.3	4.5	<u>0.2</u>	<u>26.3</u>	14.6
behaviour	N/C	2.1	2.6	1.2	8.8	7.2
(dominant	C	0	0.1	<u>5.5</u>	8.1	7.4
opponent)	R	<u>2.4</u>	0.1	0.2	<u>0.7</u>	2.9

(chi square = 105.60, $p < 0.05$)

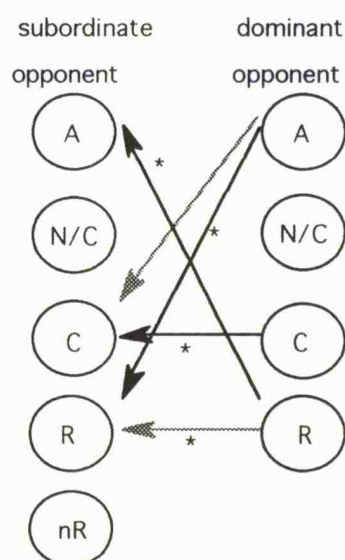
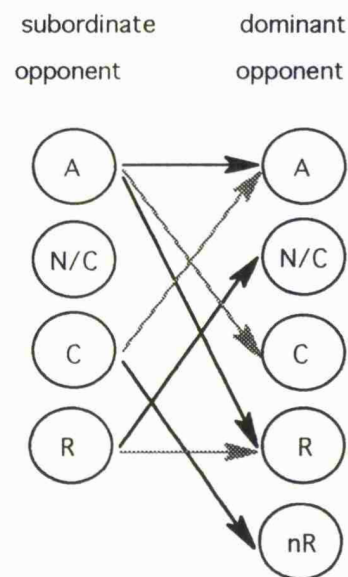


Figure 6.20 (i). Transition matrices of the sequence analysis for pairs in which the bout duration was similar before and after the dominant opponent was blinded. Results before dominant opponent was blinded. * indicates significant pairs of acts found in all groups of sighted pairs analysed (chi-squared, $p < 0.05$).

		receivers' behaviour (dominant opponent)				
		A	N/C	C	R	nR
transmitters'	A	<u>10.1</u>	6.7	<u>2.6</u>	<u>8.9</u>	7.1
behaviour	N/C	1.9	1.9	2.2	2.2	5.6
(subordinate	C	<u>1.1</u>	1.5	4.1	0.7	<u>7.5</u>
opponent)	R	5.6	<u>12.3</u>	8.2	<u>2.2</u>	7.5

(chi square = 56.96, $p < 0.05$)



		receivers' behaviour (subordinate opponent)				
		A	N/C	C	R	nR
transmitters'	A	3.5	3.2	<u>0</u>	<u>36.2</u>	9.6
behaviour	N/C	2.3	<u>4.1</u>	0.9	3.8	5.8
(dominant	C	0.3	<u>0.3</u>	<u>5.2</u>	9.9	4.7
opponent)	R	1.7	<u>2.3</u>	1.5	<u>0.3</u>	<u>4.4</u>

(chi square = 129.62, $p < 0.05$)

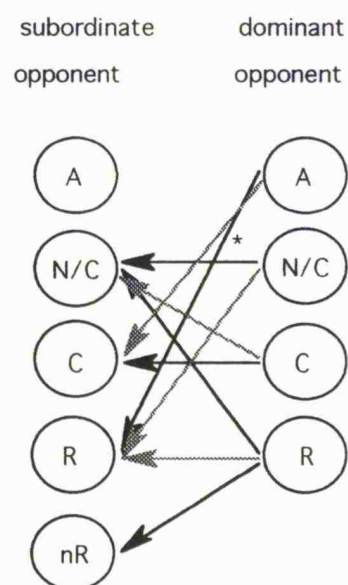
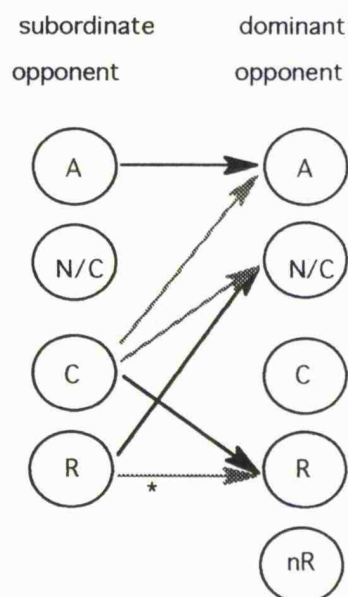


Figure 6.20 (ii). Transition matrices of the sequence analysis for pairs in which the bout duration was similar before and after the dominant opponent was blinded. Results after the dominant opponent was blinded. * indicates significant pairs of acts found in all groups of blinded pairs analysed (chi-squared, $p < 0.05$).

		receivers' behaviour (dominant opponent)				
		A	N/C	C	R	nR
transmitters'	A	<u>8.5</u>	5.3	2.8	4.9	8.9
behaviour	N/C	2.8	2.8	2.8	3.7	5.3
(subordinate	C	<u>0.4</u>	<u>0.8</u>	4.9	<u>8.5</u>	4.5
opponent)	R	5.7	<u>13.8</u>	9.8	0	3.7

(chi square = 84.64, $p < 0.05$)



		receivers' behaviour (subordinate opponent)				
		A	N/C	C	R	nR
transmitters'	A	3.2	4.8	<u>1.6</u>	<u>34.9</u>	11.1
behaviour	N/C	1.9	2.9	2.5	<u>4.1</u>	5.4
(dominant	C	0	1.6	<u>4.1</u>	10.2	5.4
opponent)	R	<u>2.9</u>	<u>2.2</u>	0.3	0	1.0

(chi square = 100.26, $p < 0.05$)

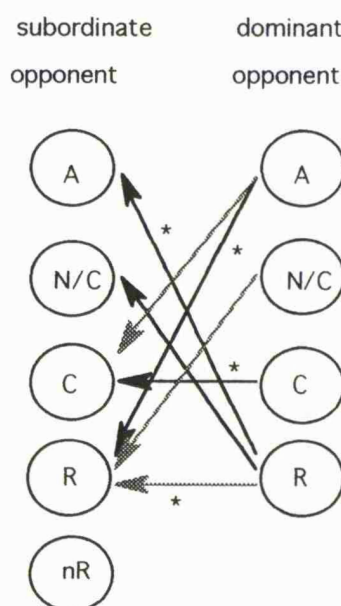
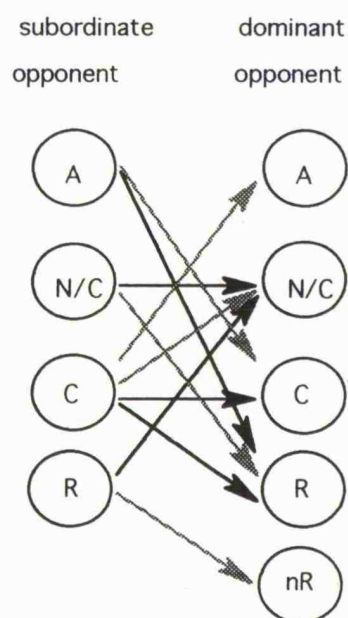


Figure 6.20 (iii). Transition matrices of the sequence analysis for pairs in which the bout duration was similar before and after the subordinate opponent was blinded. Results before the subordinate opponent was blinded. * indicates significant pairs of acts found in all groups of sighted pairs analysed (chi-squared, $p < 0.05$).

		receivers' behaviour (dominant opponent)				
		A	N/C	C	R	nR
transmitters'	A	10.2	6.0	<u>1.5</u>	<u>13.9</u>	8.3
behaviour	N/C	2.6	<u>8.3</u>	1.9	<u>1.1</u>	5.6
(subordinate	C	0	<u>0.4</u>	<u>4.5</u>	<u>8.6</u>	4.9
opponent)	R	6.4	<u>8.6</u>	4.9	0	2.3

(chi square = 101.89, $p < 0.05$)



		receivers' behaviour (subordinate opponent)				
		A	N/C	C	R	nR
transmitters'	A	2.7	7.7	<u>2.0</u>	<u>23.5</u>	11.7
behaviour	N/C	3.0	2.7	2.0	<u>3.0</u>	<u>12.4</u>
(dominant	C	0	2.0	<u>4.7</u>	6.7	3.7
opponent)	R	2.0	<u>5.0</u>	2.0	<u>0.3</u>	2.7

(chi square = 91.45, $p < 0.05$)

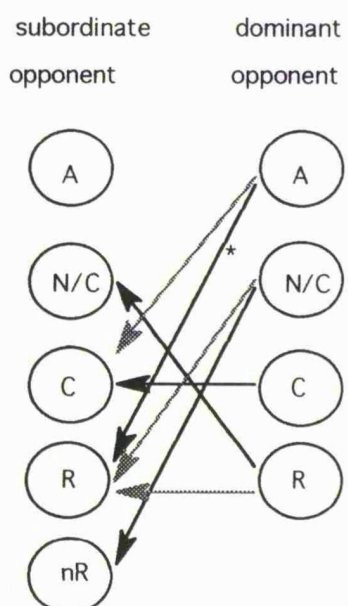
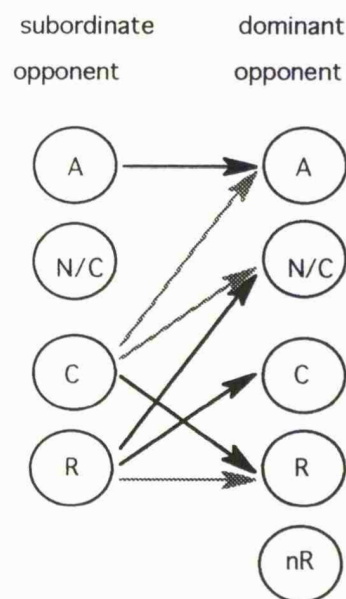


Figure 6.20 (iv). Transition matrices of the sequence analysis for pairs in which the bout duration was similar before and after the subordinate opponent was blinded. Results after the subordinate opponent was blinded. * indicates significant pairs of acts found in all groups of blinded pairs analysed (chi-squared, $p < 0.05$).

		receivers' behaviour (dominant opponent)				
		A	N/C	C	R	nR
transmitters'	A	<u>10.7</u>	3.9	2.9	10.1	10.4
behaviour	N/C	1.3	3.6	1.3	2.6	7.8
(subordinate	C	<u>0.3</u>	<u>0.3</u>	3.3	<u>7.5</u>	5.9
opponent)	R	6.2	<u>8.1</u>	<u>6.2</u>	0	7.5

(chi square = 86.16, $p < 0.05$)



		receivers' behaviour (subordinate opponent)				
		A	N/C	C	R	nR
transmitters'	A	7.8	3.6	2.7	<u>26.3</u>	12.2
behaviour	N/C	1.5	0.9	0.9	3.6	5.9
(dominant	C	0	2.4	<u>5.4</u>	9.6	6.9
opponent)	R	1.8	1.5	0.9	<u>0.9</u>	<u>5.4</u>

(chi square = 57.94, $p < 0.05$)

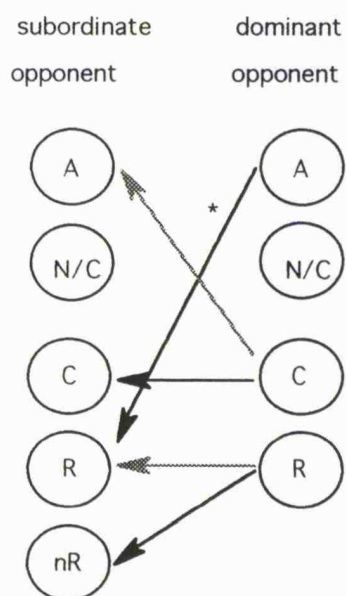


Figure 6.20 (vi). Transition matrices of the sequence analysis for pairs in which the bout duration was significantly shorter after the dominant opponent was blinded. Results after the dominant opponent was blinded. * indicates significant pairs of acts found in all groups of blinded pairs analysed (chi-squared, $p < 0.05$).

		receivers' behaviour (dominant opponent)				
		A	N/C	C	R	nR
transmitters'	A	8.3	7.3	0.9	6.4	11.0
behaviour	N/C	5.5	8.3	1.8	4.6	10.1
(subordinate	C	0	0	0	3.7	0.9
opponent)	R	3.7	13.8	0	0	13.8

		receivers' behaviour (subordinate opponent)				
		A	N/C	C	R	nR
transmitters'	A	2.9	9.7	0	38.8	4.9
behaviour	N/C	1.9	7.8	0.9	8.7	7.8
(dominant	C	0	0	1.9	6.8	0
opponent)	R	1.9	1.9	1.9	0	1.9

Figure 6.20 (vii). Transition matrices of the sequence analysis for pairs in which the bout duration was significantly shorter after the subordinate opponent was blinded. Results before the subordinate opponent was blinded. There are no sequence diagrams because there was only one pair in this group and therefore insufficient data.

		receivers' behaviour (dominant opponent)				
		A	N/C	C	R	nR
transmitters'	A	7.1	17.6	1.2	5.9	15.3
behaviour	N/C	7.1	3.5	4.7	1.8	5.9
(subordinate	C	1.8	0	0	9.4	3.5
opponent)	R	4.7	11.8	0	0	1.8

		receivers' behaviour (subordinate opponent)				
		A	N/C	C	R	nR
transmitters'	A	2.9	4.8	0.9	17.1	19.0
behaviour	N/C	0.9	2.9	0	10.5	8.6
(dominant	C	0	0	5.7	15.2	0.9
opponent)	R	0.9	7.6	0	0.9	0.9

Figure 6.20 (viii). Transition matrices of the sequence analysis for pairs in which the bout duration was significantly shorter after the subordinate opponent was blinded. Results after the subordinate opponent was blinded. There are no sequence diagrams because there was only one pair in this group and therefore insufficient data.

		receivers' behaviour (dominant opponent)				
		A	N/C	C	R	nR
transmitters'	A	20.6	8.2	3.1	3.1	3.1
behaviour	N/C	0	5.2	3.1	5.2	5.2
(subordinate	C	0	3.1	5.2	0	8.2
opponent)	R	10.3	8.2	3.1	0	5.2

		receivers' behaviour (subordinate opponent)				
		A	N/C	C	R	nR
transmitters'	A	9.1	9.1	0	28	9.1
behaviour	N/C	0	7.3	0	7.3	4.5
(dominant	C	0	0	7.3	11.8	2.7
opponent)	R	0	0.9	2.7	0	2.7

Figure 6.20 (ix). Transition matrices of the sequence analysis for pairs in which the bout duration was significantly longer after the subordinate opponent was blinded. Results before the subordinate opponent was blinded. There are no sequence diagrams because there was only one pair in this group and therefore insufficient data.

		receivers' behaviour (dominant opponent)				
		A	N/C	C	R	nR
transmitters'	A	9.4	14.5	3.6	9.4	9.4
behaviour	N/C	2.2	3.6	0	2.2	7.2
(subordinate	C	0	2.2	5.8	10.9	5.8
opponent)	R	5.8	3.6	2.2	0	2.2

		receivers' behaviour (subordinate opponent)				
		A	N/C	C	R	nR
transmitters'	A	10.1	7.3	0	14.5	10.1
behaviour	N/C	0	2.1	1.1	3.6	8.8
(dominant	C	0.6	1.7	8.1	4.5	8.4
opponent)	R	4.5	7.3	0	0	7.3

Figure 6.20 (x). Transition matrices of the sequence analysis for pairs in which the bout duration was significantly longer after the subordinate opponent was blinded. Results after the subordinate opponent was blinded. There are no sequence diagrams because there was only one pair in this group and therefore insufficient data.

6.5. Discussion.

From the outcomes of agonistic bouts it was demonstrated that the opponent found to be dominant when both individuals were sighted remained dominant after either it, or the subordinate opponent, was blinded.

Although the dominant opponent won significantly more of the first 12 bouts examined in each trial, the subordinate opponent was also occasionally able to win a bout. Looking at all 24 pairs used in this experiment, there was variation between pairs in relative fighting abilities of opponents.

The results also showed that there was a large number of cases where the larger opponent of a pair was the dominant one. Size is known to influence the outcome of contests for other crustacea species such as the velvet swimming crab *Necora puber* (= *Liocarcinus depurator*) (Glass and Huntingford, 1988; Smith *et al.*, 1994) and the crayfish *Orconectes virilis* (Rubenstein and Hazlett, 1974). In these previous studies the larger opponent was often found to be the winner of a contest and they also suggested that visual cues are used to assess relative size of an opponent. The results of the present study indicate that vision is not necessary for opponent assessment in *N. norvegicus* contests.

In the five trials after one opponent was blinded there were three pairs (out of twelve) where for one trial (out of five) the individual that was subordinate in the other nine trials became dominant. This suggests that reversals in dominance status of two individuals are possible but rare.

Having found no effect of blinding on the status of a pair of opponents, the possible effects of blinding on time taken to resolve a bout were investigated. In cases where the dominant opponent was blinded, mean bout length was either unaffected after blinding

(8/12 cases) or shorter (4/12 cases) but in no case was mean bout length longer. When the subordinate opponent was blinded, mean bout length was usually unchanged (10/12 cases). In the remainder, one was shorter and the other longer. Bruski and Dunham (1987) showed that bouts of agonistic behaviour by the crayfish *Orconectes rusticus* were longer when both opponents were in darkness. A logical development of the present study would be to blind both opponents and see whether this has any effect on the length of agonistic bouts.

In the present study bout lengths measured for the same pair of opponents varied greatly. Variation in bout duration is consistent with the theoretical predictions that an individual will adopt a strategy of changing its persistence between contests (Maynard Smith, 1974).

Evidence that learning affects subsequent contests has been found in studies of *Homarus americanus* agonistic behaviour (Karavanich and Atema, 1993a). In this species after a single contest, the loser immediately adopts retreat behaviour if approached by the other and actively avoids any further interactions. If these same individuals are isolated for up to a week they retain the memory of this previous interaction and perform retreat behaviour or avoid any further interactions with the previous winner (Karavanich and Atema, 1993b). A similar recognition and avoidance of a dominant individual by a previously defeated individual has been demonstrated in the stomatopod *Gonodactylus festai* (Caldwell, 1979). No evidence was found in the present study to indicate that experience of previous bouts influences the outcome of subsequent bouts. Both opponents engaged in aggressive behaviour during all twelve bouts of a trial and there was no indication that a subordinate opponent avoided

the other opponent after the first bout. There was also no statistical evidence for any learning between the first and second bouts, or during the course of the twelve bouts of a trial.

Based on the fact that dominance status is not affected by blinding one of a pair of individuals and that mean bout duration is unchanged after blinding, it seems that blinding has little effect on the resolution of an agonistic bout between two *N. norvegicus*. To identify any effects of blinding on agonistic behaviour the content and structure of bouts was examined in more detail. An ethogram was constructed to catalogue all types of agonistic behaviour recognised and to be used to describe bouts during this study. The agonistic behaviour of *N. norvegicus* fitted the four basic categories proposed by Hyatt (1983) to describe an 'average' decapod agonistic interaction (i.e. approach, display, attack, retreat). The different categories of agonistic behaviour within the four main headings in *N. norvegicus* were very similar to those described for the prawn *Macrobrachium rosenbergii* (Barki *et al.*, 1991) and the crayfish *Orconectes virilis* (Heckenlively, 1970; Bruski and Dunham, 1987), with the exception that crayfish use their antennae and antennules during a bout. The definition of a bout described in those studies was similar to that used here for *N. norvegicus*.

The four main ethogram categories for agonistic behaviour in *N. norvegicus* (i.e. 'approach', 'non- contact cheliped display', 'contact cheliped attack' and 'retreat') were used to describe the content of bouts. It was shown that blinding one individual has no significant effect on total number, or duration, of each of the four types of act performed by either opponent. This was true for interactions between all pairs examined in detail, including those with different bout lengths after one opponent had been blinded.

Previous studies of agonistic behaviour of the crayfish *Orconectes rusticus* have found that individuals perform more aggressive behaviour in darkness and show more use of the meral spread display (Capelli and Hamilton, 1984; Bruski and Dunham, 1987). These studies also found that more acts were performed per bout in the dark (Capelli and Hamilton, 1984; Bruski and Dunham, 1987). This suggests that in the crayfish, visual cues are important for agonistic interactions. In the current study the effects of blinding only one opponent were examined, so a direct comparison cannot be made with these previous studies. However, the fact that dominance status and bout structure remain largely unchanged after one opponent has been blinded, suggests that visual cues are not as important in the agonistic contests of *N. norvegicus*.

Looking at the four main classes of agonistic behaviour displayed by a pair of opponents it was found that a dominant opponent carries out significantly more 'approach' acts and spends a significantly longer period of time performing them, than the other three types of agonistic behaviour. It was also found that a subordinate opponent performs significantly more 'retreat' acts and spends significantly more time performing them. These acts, characteristic of the status of opponents, were largely unchanged by blinding either a subordinate or dominant opponent. This was true for ten of the eleven pairs studied in groups A - D. The only exception was one pair in group D where bouts were significantly longer after the subordinate opponent was blinded. The agonistic behaviour of this pair revealed no significant difference in the number of, or time spent performing, all four types of acts before and after blinding. Therefore in the majority of cases where there was a significant change in bout length after one opponent was

blinded, four out of five pairs showed the same trends of behaviour as those with similar bout lengths throughout all trials.

These results are consistent with the study of Bruski and Dunham (1987) on agonistic behaviour in the crayfish *Orconectes rusticus*. It was shown that winners of agonistic interactions performed more approach types of behaviour and losers performed more retreat types of behaviour (all other agonistic acts were performed in similar numbers). A similar study on the prawn *Macrobrachium rosenbergii* showed that the number of submissive acts (i.e. retreat behaviour) determined which opponent was the loser (Barki *et al.* 1990).

Sequence analysis techniques applied to the contents of bouts revealed that there was a significant behavioural interaction between opponents. This association was found when both opponents were sighted and after one opponent was blinded. This type of analysis can indicate possible communication between two individuals. Communication is said to occur when the probability of one individual performing one type of behaviour is altered by the actions of the other (Colgan, 1978). Results of the present work suggest that *N. norvegicus* are able to communicate during an agonistic interaction irrespective of whether one or both opponents are sighted. However, caution must be exercised before assuming that communication has occurred since the analysis has merely shown that certain pairs of acts occur more frequently than expected by chance alone.

Although the use of a first-order Markov model and a correlation technique such as the chi-squared test are the most appropriate techniques for sequence analysis (Slater, 1973), two limitations need to be considered. The first is that it is possible that the act performed is also dependent on factors other than just

the previous act. It is possible that their occurrence may also be caused by external or internal factors stimulating each opponent independently (Slater, 1973). The second is that the analysis assumes 'stationarity', where the probability of observing each different transition of agonistic behaviour between opponents is constant throughout all stages in a bout (Colgan, 1978). This is unlikely to be true because 'approach' is more likely to occur than any other type of behaviour at the start of a bout, and 'retreat' is most likely at the end. However, there was insufficient data to treat each stage in the bouts separately, and it is suggested that stationarity rarely exists in behavioural data because so many unknown factors may have an influence (e.g. biological clocks, hormone levels) (Slater, 1973).

Significant pairs of acts were identified by sequence analysis, but they were highly variable between groups. However, there were a few significant transitions between acts which were seen in all groups studied and some were unaffected by the blinding of either opponent. The significant pairs of acts found in all groups before and after one opponent had been blinded clearly show that both the subordinate and dominant opponent can be stimulated to respond aggressively and submissively during the bouts.

Previous studies investigating the role of vision in agonistic interactions of crustacea found that an opponent lacking visual information about its opponent would compensate with more use of other sensory channels. The Bruski and Dunham (1987) study, on contests between the crayfish *Orconectes rusticus* in light and dark conditions, found that in darkness they perform more tactile actions. These include the use of 'antennae taps', where the antennae of an opponent are swept down and onto the anterior region of the other opponent; chelae attack behaviour and pushing.

In addition the bouts are longer in darkness. 'Blindfolded' American lobsters (*Homarus americanus*) display more contact agonistic behaviour than sighted ones (Kaplan *et al.*, 1993), showing that behaviour can be modified to compensate for loss of visual cues. In the results presented here there was no evidence that loss of vision in *N. norvegicus* leads to any form of compensatory behaviour.

In the present study only male *N. norvegicus* were used. Differences in agonistic behaviour performed during interspecific contests by males and females is found in many animal species (Archer, 1988) including crustacea such as the green shore crab (*Carcinus maenus*), where male crabs show more aggressive behaviour during a contest than females (Berrill and Arsenault, 1982). Therefore similar studies of female *N. norvegicus* agonistic behaviour would be of value.

6.6. Conclusion.

The opponent which was dominant when both individuals were sighted, remained dominant after either it, or the subordinate opponent was blinded.

Looking at the mean duration of bouts, in the majority of pairs (18 out of 24 pairs) bout duration was unchanged after either the dominant or subordinate opponent was blinded.

An ethogram was constructed to describe each type of agonistic behaviour seen during contest between *N. norvegicus*. This ethogram was used to investigate bout contest and the sequences of different types of acts occurring in the bouts. Looking at bout content in the contests between sighted individuals it was found that the dominant performed significantly more of the 'approach' types of acts and the subordinate opponent performed

significantly more of the 'retreat' types of acts. In both cases individuals performed the other types of acts in similar numbers. It was also shown that the dominant and subordinate opponents spent significantly more time performing the 'approach' and 'retreat' type of acts respectively, than they did carrying out the other types of act. There was no effect of blinding on the content of bouts after either the dominant or subordinate opponent had been blinded.

Sequence analysis showed that both the dominant and subordinate opponent were able to respond submissively or aggressively during a contest. Several significant pairs of acts were identified in all pairs both before and after either opponent had been blinded, but only one indicated a possible set of acts responsible for the subordinate opponent losing and the dominant opponent winning a contest. This was the significant occurrence of a 'retreat' type of act by the subordinate opponent following an 'approach' type of act by the dominant opponent. Finally, sequence analysis shows that there is a significant association between the behaviours of two opponents during a contest. This suggests that communication occurs throughout the contest and that blinding one opponent does not change this exchange of information.

The findings of this investigation provide evidence that loss of vision does not seriously affect the agonistic behaviour of *M. norvegicus* and their ability to compete with a sighted conspecific.

Chapter 7 Effect of blinding on the ability to gain shelter, to find food and the rate of capture in a baited creel.

General introduction.

For a blinded *N. norvegicus* to survive on the sea bed it must be able to obtain shelter and food. With respect to the likelihood of discard recapture it is necessary to know whether creel finding ability and rate of entry into baited creels are affected by blinding. The following experiments were designed to assess any effects of blinding on shelter gain, location of food items and capture in a baited creel.

Part 1 - Effect of blinding on the ability to gain shelter.

7.1. Introduction.

Like other burrow dwelling animals, *N. norvegicus* uses burrows for avoidance of predators. Each burrow consists of a tunnel with one or more wide sloping main entrances and a smaller rear one, all opening onto the mud surface (Rice and Chapman, 1971). *N. norvegicus* remains inside the burrow for most of the time, but emerges onto the sea bed for short periods mainly to forage for food. Emergence is governed by a 24 h rhythm and this behaviour is thought to coincide with periods of minimum predation risk (Chapman, 1980). At the end of an activity period, it is important that an individual is able to gain shelter quickly in order to minimise the predation risk (Lima and Dill, 1989).

In *N. norvegicus*, the females normally return to the same burrows, but males may travel short distances and in these cases they may need to find a new burrow (Chapman and Rice, 1971).

The purpose of the present study was to investigate the effects of blinding on the time taken for a group of individuals to gain shelter. The experiments were carried out in a laboratory observation tank using sighted *N. norvegicus*. The same individuals were then blinded and the time taken to gain shelter was recorded as before. In addition, the behaviour of sighted and blinded individuals when investigating burrows and entering them, was observed.

7.2. Materials and methods.

Observations on burrow finding ability of sighted and blinded male *N. norvegicus* were carried out at the University of Leicester using a large observation tank and video recording equipment.

Artificial burrows were constructed from 0.22 m lengths of 0.11 m diameter plastic guttering. Each length of guttering was blocked at one end to leave a single entrance. Eight artificial burrows were placed onto the gravel in the tank and arranged in a radiating pattern so that the midpoint of each entrance was at an equal distance (0.38 m) from the central point of the observation tank base. The shelters were roughly equidistant from each other.

A 12h light/12h dark, green light cycle was used for this experiment. The light cycle was simultaneous with that in the holding tanks where the experimental animals were temporarily housed. A video camera (fitted with a 6.5 mm (F:1.5) lens) was fixed vertically above the observation tank so that an image of the entire tank could be recorded.

7.2.1. Trial procedure.

Each trial was begun at 16:30. A square opaque tube (cross section = 0.2 m x 0.2 m, height = 0.35 m) was placed vertically on the floor of the observation tank with the lower end positioned over the central point of the tank base. Eight individuals were transferred into the tube and video recording was begun. At the start of a trial the tube was then raised vertically out of the water releasing all individuals together. Each trial continued until 16:00 h of the following day, when all individuals were temporarily placed in another tank to allow the pattern of shelters to be rotated about the central point so that their positions were changed from trial to trial. 30 minutes were allowed for

rearranging the burrows. At 16:30 h the individuals were returned to the observation tank for the next trial. Eight trials were carried out using the same group of eight sighted individuals. The burrow entrances were always kept at the same distance (0.38 m) from the centre of the tank. At the end of the eighth trial using sighted individuals they were all blinded and returned to the holding tank for three days. A further eight 24 h trials were carried out following blinding.

Once all sixteen trials had been completed (eight when the individuals were sighted and eight when they were blinded), the carapace lengths of all individuals were noted (mean carapace length = 37.84 mm, s.d. = 5.07 mm; $n = 8$). All individuals were sacrificed and their eyes were removed and stored in formal calcium fixative.

7.2.2. Video tape analysis.

The video tape recordings of the eight trials with sighted individuals and the eight after they were blinded, were analysed separately, to compare the times taken to gain shelter by individuals when sighted and blind. The video tape of each trial was reviewed and each individual followed until it was seen to completely enter one of the artificial burrows. The time taken for an individual to gain shelter was noted and all subsequent movements of this same individual were then ignored for the remainder of that 24 h trial. An individual would often enter an already occupied burrow, but the artificial burrows could only be occupied by a single individual at any one time. An individual was said to have gained shelter if it was able to evict the original occupant and remain inside the burrow.

7.2.3. *Statistical analysis.*

In order to detect any effect of blinding on the pattern of time taken to gain shelter by each subsequent individual over the course of a trial, a two-way ANOVA test (for independent measures, unequal n) was used (Meddis, 1973; see Appendix I for details) to compare the times that individuals took to gain shelter before and after blinding.

To make data suitable for analysis by an ANOVA test, a logarithmic transformation was used (Fowler and Cohen, 1990; see Appendix I for details of transformation). The transformed data showed homogeneous variance (using $F_{\max}$ test (Meddis, 1973; Fowler and Cohen, 1990; see Appendix I for details) allowing the application of ANOVA analysis to this data.

A probability level of 0.05 was used throughout.

7.3. Results.

There was a total of nine trials (out of a total of 16 trials), where some individuals (up to four) did not gain shelter at any point throughout an entire trial. The total number of individuals not gaining shelters was greater over the first eight trials when they were all sighted (total number of individuals not gaining shelter over eight trials, when sighted = 10; when blinded = 5).

Analysis was then carried out to examine for trends in the mean times, over the eight trials, taken for individuals 1 to 8 to gain shelter in each trial (Fig. 7.1). The shape of the histogram showed no obvious change in the ability of individuals to find shelter after they had been blinded. An ANOVA test (two-way for independent measures and unequal n, two-tailed) confirmed that blinding had no effect on the mean time taken for all eight individuals to gain shelter ($F = 0.92$, $p > 0.05$).

7.3.1. Observations of shelter investigation and competition.

More detailed observations were made to assess any differences in the manner in which sighted and blinded individuals entered the shelters. Video recordings over a 3 h period were made of a single sighted individual with an empty artificial shelter (shelter of the type used in the tank during the previous experiment) in the observation tank. All recordings were carried out between 10:00 h and 16:00 h when individuals would be expected to seek shelter. This was carried out six times using a different sighted individual each time. The same procedure was carried out to observe the behaviour of six blinded individuals investigating and entering a shelter. Special attention was paid to

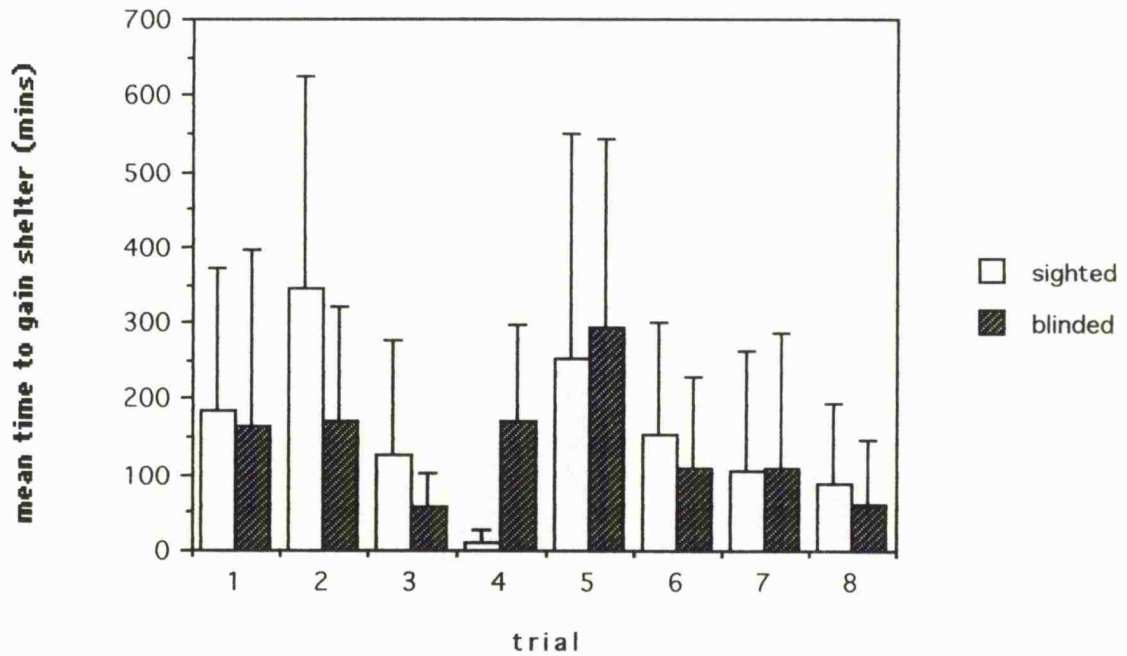


Figure 7.1. Mean time (error bars = s.d.) for a group of eight *N. norvegicus* to find shelter before and after they were blinded. Results showed no significant effect of blinding (two-way ANOVA, two-tailed, $F = 0.92$, $p > 0.05$).

the use of chelipeds, walking legs and antennae, as an individual searched and entered a shelter.

Shelter investigation by sighted and blinded individuals was carried out using either one or both chelipeds. A cheliped was placed inside the shelter entrance and moved so that it contacted one or both of a shelter's sides. Individuals were also seen to move either backwards, or forwards, into the unoccupied shelter, with no preliminary investigation. When entering forwards into a burrow the chelae were held together and slightly raised. There was no significant difference between the percentage of times that sighted and blinded individuals entered shelters without preliminary investigation (sighted 71%, s.d. = 17%; blinded 62%, s.d. = 19%) (independent t-test: $t = 0.84$, $p > 0.05$). When an individual moved forwards into a shelter, the antennae were held back over the carapace, whereas if it moved backwards into a shelter the antennae were held forwards. This occurred for both sighted and blinded individuals.

7.4. Discussion.

7.4.1. Effect of blinding on the ability to gain shelter.

The results revealed no significant effect of blinding on the time taken to gain shelter.

Individuals gaining shelter in a trial did not always remain inside for the entire trial. Many were observed emerging from the first shelter and entering another or remaining emerged on the tank base for the rest of a trial. Despite this movement of individuals between shelters, as a trial proceeded, the number of occupied shelters increased and individuals wanting shelter had to search for an empty one, or compete and try to evict a shelter's occupant. However, the results indicate that blinding does not affect this behaviour.

In cases where individuals did not gain shelter throughout a trial they spent prolonged periods at the edge of the tank base, or beside a shelter. The same individuals did not remain out of the shelters in every trial. More individuals remained out of burrows during the trials when they were sighted, but the reasons for this are unclear.

7.4.2. Limitations of using artificial shelters.

The results clearly demonstrate that *N. norvegicus* is highly motivated to take shelter and will readily use artificial shelters. All individuals that gained shelter in a trial, obtained shelter within the first 14 h and on average, the first four individuals to gain shelter had found shelter after 2 h. The artificial shelters were readily used despite their lack of resemblance to the natural burrows of *N. norvegicus*. In previous studies, the American lobster (*Homarus americanus*) utilised artificial concrete square shelters (Sheehy, 1976), and plastic tubes similar to those used in the

present study have been used successfully in many previous experiments with *N. norvegicus* (Atkinson and Naylor, 1976; Naylor and Atkinson, 1976; Hammond and Naylor, 1977 a, b).

In the present study only the ability to gain shelter in existing burrows was investigated. In the natural environment *N. norvegicus* constructs new burrows (Chapman, 1980) and in previous laboratory situations it has been seen to make new ones when no other shelter was available (Rice and Chapman, 1971; Farmer, 1974). This suggests that ability to find and compete for empty and occupied burrows is only one option for obtaining shelter because they can construct new ones. However excavation of burrows demands time and energy, so using an existing burrow could be more cost-effective.

In areas inhabited by *N. norvegicus*, the density of burrows on the sea bed varies widely, from 0.1/m² at Lough Ine to 3 - 5 /m² in the Sound of Jura (Chapman, 1980). Therefore the density of artificial burrows used in this present experiment (i.e. 1.08 burrows/m²) fell within the range found in the natural environment. The number of empty burrows is also highly variable, but is generally between 25 - 30% (Chapman, 1980). In the present study there was no occasion when all shelters were occupied and at least one or two were empty at most points in a trial.

The shelters were rearranged after each trial to prevent individuals learning entrance positions. *Uca pugilator* is able to return to its hole after foraging excursions of up to 12 m using learned cues (Bambridge, 1961). A previous study (Chapman et al., 1975) tracked *N. norvegicus* in the natural environment, and found that a female individual made excursions of up to 5 m and returned to the same burrow. This suggests that *N. norvegicus* has the ability to locate and return to particular burrows.

7.4.3. *Timing of the start of trials and the day/night cycle.*

In the present study, trials began at 2.5 h before simulated dusk. This meant that on average the first four individuals to gain shelter did so in the light part of the regime. Individuals 5, 6 and 7 found shelter in the dark and the last individual gained shelter after dawn. Since the time taken for individuals to gain shelter was similar for sighted and blinded *N. norvegicus*, it appears that shelter finding ability would not be affected by the presence or absence of light. Further experiments with continuous illumination and continuous darkness could be conducted to clarify this point. The important outcome of the present experiment is that there is no evidence that blinding alters the time taken for individuals to gain shelter.

7.4.4. *Observations of behaviour when entering and competing for shelter.*

In the present study, contests were frequently observed at a burrow entrance between an intruder and the shelter occupant. Intruders were also seen to enter shelters and compete with occupants inside the burrows. Contests between *N. norvegicus* have also been observed in their natural environment, where individuals were seen fighting at burrow entrances (Chapman and Rice, 1971). In a previous laboratory study (Chapter 6), *N. norvegicus* were observed defending their burrows against intruders using cheliped displays and overt fighting (Farmer, 1974).

Observations of shelter entrance behaviour showed that sighted and blinded individuals investigate the shelter with one or both chelipeds. *Homarus americanus* has been observed using one or both chelipeds to probe crevices to investigate potential shelter

(Pottle and Elnor, 1982). A previous study (Rice and Chapman, 1971) showed that *N. norvegicus* uses its antennae for orientation in the burrow system by holding the flagella against the tunnel ceiling pointing in the direction in which the individual is moving. In the present study the antennae were not seen to be used to investigate the shelters but were held in a position to aid entrance into the shelter (over abdomen if entering forwards and held forwards if entering backwards).

N. norvegicus was also observed entering either backwards or forwards into shelters with no preliminary investigation. Similar behaviour has been observed in *Homarus americanus* (O'Neill and Cobb, 1980). There was no difference in the number of times that a sighted or blinded individual entered a shelter without any preliminary investigation. Therefore, it seems that the blinded individuals do not compensate for their loss of vision by changing their behaviour when entering a shelter.

7.5. Conclusion.

In the present study there was no detectable effect of blinding on the time taken for the *N. norvegicus* to gain shelter. Also blinding did not seem to have any obvious effects on behaviour when investigating and entering a shelter.

Part 2 - Effect of blinding on feeding behaviour.

7.6. Introduction.

N. norvegicus feeds on a wide variety of organisms occurring both on and beneath the surface of the sediment (Chapman, 1980; Cobb and Wang, 1982). Both live prey and dead animals are consumed (Thomas and Davidson, 1962) and items found in *N. norvegicus* stomachs include crustaceans, polychaetes, echinoderms, molluscs and fish (Sarda and Valladares, 1990). *N. norvegicus* also browses on organisms such as algae, hydroids and polyzoans found on stones and shells (Thomas and Davidson, 1962; Howard, 1989). In the present investigation the importance of visual cues in the location of food items by *N. norvegicus* was assessed.

Previous studies have demonstrated that visual stimuli are not necessary for feeding behaviour in the crab *Hemigrapsus oregonensis* (Symonds, 1964) and the banana prawn *Penaeus merguensis* (Hindley, 1975). Both studies showed that the major sensory system used for the location and recognition of food items was chemosensory (Symonds, 1964; Hindley, 1975). Many other studies have demonstrated the importance of chemoreception in the feeding behaviour of lobsters and crabs such as *Homarus gammarus* (Mackie, 1973), *Homarus americanus* (Hirtle and Mann, 1978), *Panulirus interruptus*, *Cancer antennarius* and *Loxorhynchus grandis* (Zimmer-Faust and Case, 1982).

To study the effect of blinding on the ability to find food items a group of *N. norvegicus* was presented with food items placed on the surface or buried within, a fine gravel substrate. The time taken to find these items was recorded when all individuals

were sighted and could use all sensory channels. All individuals were then blinded and the procedure was repeated to see if food searching was impaired without the use of visual cues.

7.7. Materials and methods.

All observations were carried out at the University of Leicester, using the observation tank and video recording equipment. A video camera fitted with a 6.5 mm (F:1.5) wide angle lens was fixed above the observation tank so that the whole tank could be viewed. Nine artificial burrows (constructed from 0.22 m lengths of 0.11 m diameter plastic guttering) were placed in the observation tank in a row along the left hand side of the tank.

7.7.1. *Preparation of N. norvegicus.*

Seven male individuals were taken from the holding tanks (mean carapace length = 37.96 mm, s.d. = 2.2). For identification each individual was labelled with a different combination of white discs fixed onto the upper part of the merus of the cheliped (using stainless steel wire) and a disc of reflective tape attached to the dorsal part of the cephalothorax (using cyanoacrylate glue). The discs attached to the chelipeds did not appear to obstruct movement. Individuals were allowed 48 h to recover from the handling procedure. 24 h before a trial began (10.00 h), seven labelled *N. norvegicus* were transferred into the observation tank. Video recording was started and continued throughout the experiment using a time-lapse facility on the video cassette recorder. Individuals were allowed 24 h to acclimatise to the observation tank conditions and to occupy shelters.

7.7.2. *Preparation of food items.*

Pieces of squid tissue (mass = 1g) were used as food items in this experiment.

For each trial four food items and one control item were prepared. Each of the four food items was prepared by tying a 1g

square of frozen squid onto a clean microscope slide using fishing line. This was then allowed to defrost. The control item consisted of a piece of fishing line attached to a microscope slide. Care was taken that line and slide were clean and that no squid odour was transferred onto the control.

The four food items and single control item were placed randomly on the base of the observation tank in five different positions. A random number table was used to generate 5 sets of co-ordinates.

In each case the glass slide was buried under the gravel. For the covered food items (2 and 5), the squid was also buried in the gravel (approximately 5 mm deep). The squid on the other two items (1 and 4) remained exposed. To indicate the position of each item on the video tape recording, a white disc was temporarily placed at the position of each of the 5 items for 30 seconds at the start of each 24 h trial.

7.7.3. Trial procedure.

24 hours after introducing the *N. norvegicus* into the tank the sea water circulation to the observation tank was turned off at 10:00 h. The five food items were placed in the observation tank and left for a period of 23 h, until 09.00 h on day 3 when all five items were removed. The top 5 - 10 mm of gravel was then removed from the tank and replaced with clean gravel. The circulation was turned on and left for 1 h to clear any remaining squid odours (inflow = approximately $0.58 \text{ m}^3 \text{ h}^{-1}$, volume of observation tank = 0.43 m^3). Then at 10.00 h tank circulation was stopped and another trial was started with five new food items in new positions placed on the tank base as before.

A total of six of these 3 day trials was carried out. All seven *N. norvegicus* were then blinded (see section 2.3. for blinding procedure). They were allowed three days to recover in the holding tanks. Using these blinded individuals a further six trials were then carried out exactly as above. Finally, all seven individuals were removed from the tank, their carapace lengths recorded and their eyes removed and placed in formal calcium fixative.

7.7.4. Tape analysis.

The video tapes of the six trials when the individuals were sighted individuals and the six when they were blind were reviewed. In each trial the interval between the start of the trial and the first discovery of each of the 5 items by one of the individuals was noted. Any subsequent individuals finding an item previously discovered were disregarded. An item was said to be found if an individual was seen to perform feeding behaviour (e.g. manipulating the item with walking legs (pereiopods) (Thomas and Davidson, 1962)).

7.7.5. Statistical analysis.

To assess the effect of blinding and type of food item on the ability of a *N. norvegicus* to locate the item, a two-way repeated measures ANOVA (two-tailed) was used (Meddis, 1973). For this type of analysis it was necessary to use the time that an item remained undiscovered by the group of individuals in each trial so that there were equal numbers of data in each category. If an item was not found throughout the entire period of a trial, a maximum value of 24 h was given. A logarithmic transformation was applied to the data so that variance was homogeneous (using $F_{\max}$ test (Fowler and Cohen, 1990)) for purposes of the ANOVA analysis (the

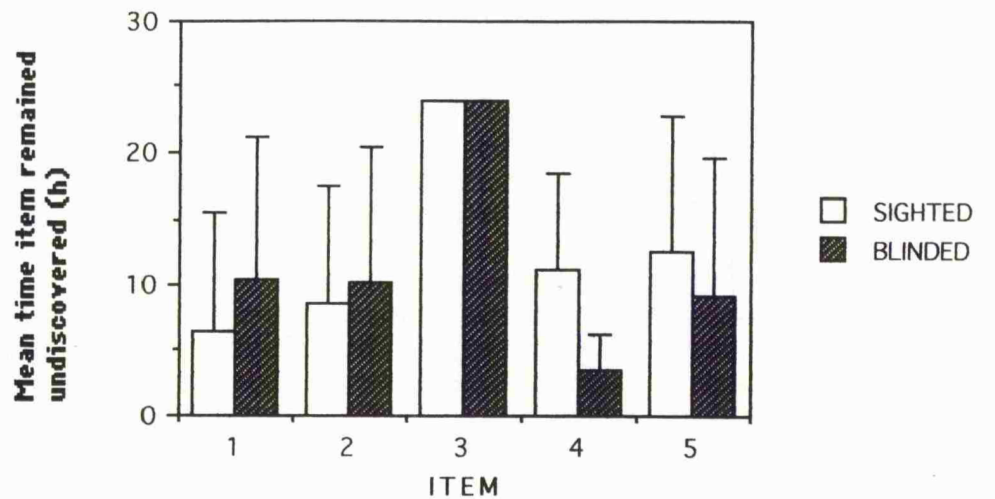
logarithmic transformation was used because the variance of the original data was greater than the mean (Fowler and Cohen, 1990)). A probability level of 0.05 was used throughout all statistic analyses.

7.8. Results.

The group of individuals moved around the whole tank base and clearly could be seen feeding on the food items provided. They were observed pulling pieces of food from the cubes of squid attached to the glass slides using their walking legs and then passing these pieces of food to their mouth parts.

There was a large variation between trials in the times taken to find the covered and uncovered food items. There were several cases when some of the food items remained undiscovered throughout a trial. There were three such trials when one uncovered item was not discovered. Similarly, for covered food items, there were also three trials where one of the items remained undiscovered. There was also one trial when both covered food items were not discovered.

The mean times that covered and uncovered items remained undiscovered in a trial appeared to be similar and the times for blinded individuals were similar to those for sighted ones (Fig. 7.2). Control items were never found in any trials. A two-way ANOVA (for repeated measures, two-tailed) confirmed there was no significant difference in the mean time that covered and uncovered food items remained undiscovered by sighted and blinded individuals ($F = 5.62$, $p < 0.05$; Tukey test). However, it is clear from the histogram (Fig. 7.2) and the ANOVA test that the food items were discovered significantly faster than the control items which remained undiscovered in all trials ($F = 5.62$, $p < 0.05$; Tukey test). The ANOVA test also revealed that there was no change in the ability of the group of individuals to locate covered and uncovered food items after they had been blinded (no effect of blinding: $F = 0.1$, $p > 0.05$ and no interaction effect: $F = 2.15$, $p > 0.05$).



key of items: 1 - uncovered food
 2 - covered food
 3 - control
 4 - uncovered food
 5 - covered food

Figure 7.2. Mean time (error bars represent s.d.) that each item remained undiscovered in the six trials before and the six trials after the seven individuals were blinded. The bar chart shows that the control item was always undiscovered and there was no difference in the times taken for uncovered and covered food items to be discovered when the individuals were sighted and blinded (two-way ANOVA, $p < 0.05$).

7.9. Discussion.

The results of the present study provided no evidence that blindness reduces the ability of *N. norvegicus* to locate static food items. In the first part of the experiment there was no significant difference in the time taken to find covered and uncovered food items by a group of sighted individuals. This suggests that visual cues are not used to find such food items or that *N. norvegicus* can compensate for loss of vision by using other senses. The control item was not found in any trial and it was never investigated although individuals moved near to it.

The maximum amount of food ingested by a *N. norvegicus* with a mean body weight of 20 g is 0.5 g per day (Sarda, 1990). Therefore, if an individual paid several visits to food items and ingested the maximum amount it could ingest in a day (i.e. 0.5 g) there would have been a maximum of 24 h before it could have consumed more. Only a fraction of the seven individuals in the tank was active in each trial and only a proportion of the group fed throughout the trials. Symonds (1964) compared the reactions of crabs *Hemigrapsus oregonensis* to food stimuli and found that although starvation increased their responsiveness to food, satiated crabs also located food items when presented with food stimuli. This suggests that those individuals that ingested food items in previous trials would still respond to the presence of food items in subsequent trials.

In this experiment the food was located in static water, any dispersal of food odour in the tank would have occurred by molecular diffusion and water currents created by the movements of individuals (Nickell and Moore, 1992). In the natural environment of *N. norvegicus*, sea water currents would aid the dispersal of food odours.

In the natural environment the diet of *N. norvegicus* also includes live items (Thomas and Davidson, 1962). In that case visual stimuli may be more important and there could be differences between sighted and blinded individuals.

7.10. Conclusion.

Within the context of this experiment the ability of *N. norvegicus* to find carrion was not affected by blinding. It would be of value to investigate the response of blinded *N. norvegicus* to live food.

Part 3 - Effect of blinding on the response to a baited creel.

7.11. Introduction.

A previous field experiment looked at the effectiveness of live intact prey, injured prey and excised tissue when trapping different species of crustacea, such as the spiny lobster *Panulirus interruptus* and the crab *Cancer antennarius* (Zimmer-Faust and Case, 1982). They found that excised tissue attracted the largest number of individuals and demonstrated that the chemical odour of the bait directed the foraging behaviour of the species tested (Zimmer-Faust and Case, 1982; Zimmer-Faust *et al.*, 1984). This suggests that chemoreception is primarily responsible for the capture of these species by baited traps. The following experiment was designed to examine the capture of *N. norvegicus* in baited creels when they were unable to use visual cues. The recapture rates of sighted and blinded *N. norvegicus* were measured using a baited creel of the type commonly used in the Scottish fishery. A difference in the recapture rates of blinded and sighted *N. norvegicus* would be of considerable relevance to the fishery. For this experiment the behaviour of a group of sighted and blinded *N. norvegicus* was monitored while in a tank containing a baited creel.

7.12. Materials and methods.

The experiments were carried out at the Fish Behaviour Unit at the SOAFD Marine Laboratory, Aberdeen. A circular tank (3 m diameter) was filled with circulating sea water to a depth of 1.5 m (mean temperature of water throughout experiment = 10.9°C, s.d. = 0.4). The base of the tank was covered with fine sand and 13 artificial shelters, made from inverted 0.3 m lengths of 0.11 m diameter plastic guttering, were placed onto the tank base in a random manner. A green light (see 5.2 for details) was used to provide daytime illumination. The maximum light level measured on the tank base was $0.16 \mu\text{mol m}^{-2}\text{s}^{-1}$, which is within the range found on the sea bed at a depth of 30 m (Shelton *et al.*, 1985). The light was controlled by computer which was programmed to simulate dawn and dusk by raising and fading the light levels over 15 minute periods. The computer program was set to the times of sunrise and sunset at the latitude of Aberdeen for the time of the experiment. Two red lights fitted with red perspex filters (section 2.1 for properties of filters) were fixed above the tank to provide constant illumination for the TV camera (Panasonic video camera, fitted with a Silicon intensifying tube).

A time lapse video cassette recorder (Panasonic time-lapse NVJ40) and monitor (Sony) were positioned in a separate room. Activity within the tank was recorded throughout each three day trial.

7.12.1. Preparation of *N. norvegicus*.

A smaller temporary holding tank (1 m x 1.5 m x 1 m) was set up in the same tank room with artificial shelters, identical to those used in the main tank. 30 male individuals (mean carapace length = 30.07, s.d. = 1.4) were placed in the holding tank and

allowed to acclimatise to the tank room light cycle. After ten days, individuals were divided into two groups of fifteen. Those in the first group were blinded and then labelled with a 25 mm diameter circle of silver reflecting tape ('Duct Tape'™) attached to the dorsal part of the cephalothorax using cyanoacrylate glue. The second group remained sighted and was treated similarly to the others except that they were not blinded (i.e. handling control). The sighted individuals were labelled with a 25 mm diameter circle of transparent polythene attached to the dorsal part of their carapace. All individuals were returned to the temporary holding tank for at least three days before use in the experiment.

7.12.2. Procedure.

24 h before the beginning of each trial, 5 sighted and 5 blinded *N. norvegicus* were taken from the temporary holding tank. These individuals were released into the main circular tank at 10.00 h and recording equipment was switched on. During this first 24 h period individuals were allowed to acclimatise to the tank conditions and occupy the artificial burrows. At 10.00 h on the next day when a trial began, the circulation to the tank was turned off and a creel baited with 50 g of salted mackerel was placed on the base of the tank, as near to the centre as possible. The creel remained in the tank for 48 h (a similar period to that normally used in commercial *N. norvegicus* fisheries (Howard, 1989)) after which the trial was concluded. At this point individuals were removed from the tank, their carapace lengths were recorded, and their eyes were removed and placed in formal calcium fixative. The creel was removed from the tank, any remaining bait was discarded and it was washed to remove any traces of the bait. The tank was left for a further 24 h and the circulation was turned on so that

any trace of the mackerel bait was expelled from the tank (inflow rate = $3.5 \text{ m}^3\text{h}^{-1}$, volume of water in tank = 10.6 m^3). The trial procedure was repeated twice more, using 10 different individuals (5 sighted and 5 blinded) and a freshly baited creel for each trial.

7.12.3. Video tape analysis.

At the end of each trial, the creel was removed and number of individuals captured was counted. The video tapes were then reviewed to note what time they were captured and to check that no individuals escaped after entering a creel.

In addition, the video tapes were reviewed to measure the times spent by the sighted and blinded groups of individuals in different areas of the tank base in order to assess their reaction to the baited creel. For this, the image of the tank base was divided in the following manner. A transparent acetate sheet was fitted over the monitor screen and the position of the creel in each trial was marked on this sheet. On this acetate sheet bands of equal width of 10 mm (10mm on screen was equivalent to a mean of 710 mm (s.d. = 96.5) in tank) were drawn around the creel until the whole screen image was filled (Fig. 7.3). The bands were labelled A to L, with band A directly around creel and bands B to L moving away from the creel. It was necessary to re-draw the creel and bands for each of the three trials reviewed because the position of the creel was slightly different in each trial.

To gain a measure of activity in different parts of the tank the first 15 minutes of each hour of video tape recording were analysed. Any individual seen to emerge from an artificial shelter was followed until it regained shelter or the sampling period ended. The time spent in each band in this 15 minute

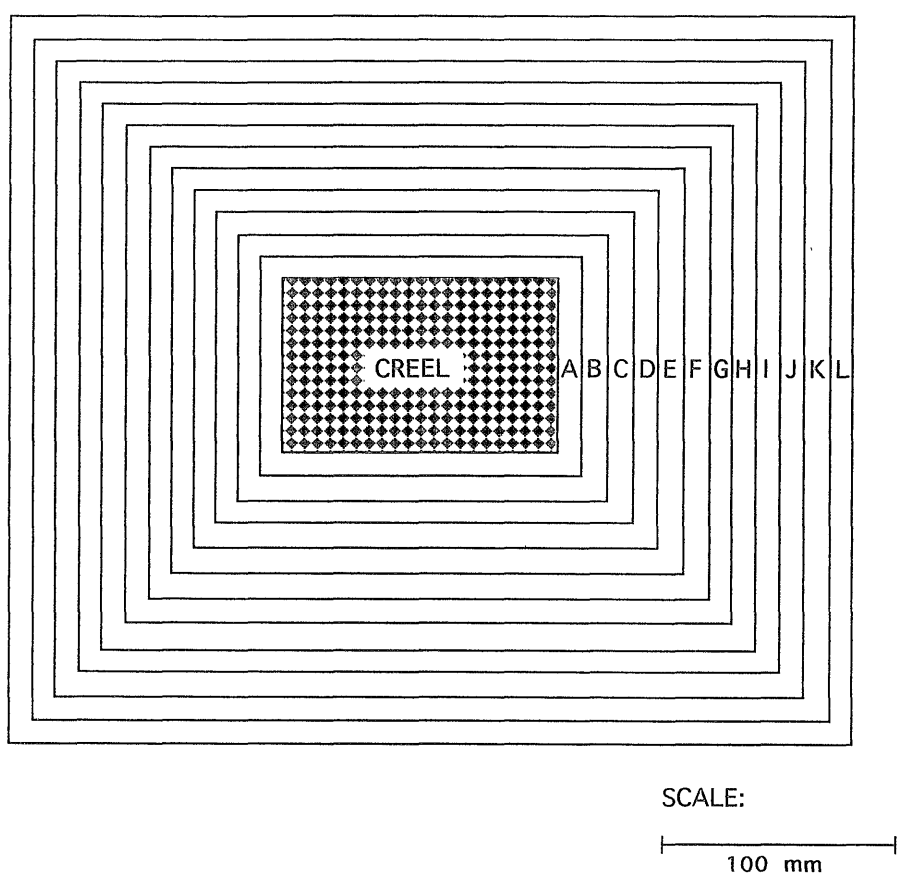


Figure 7.3. Creel position and bands as drawn on acetate sheet for video tape analysis. Scale indicates measurements of markings made on acetate sheet (10 mm on acetate is equivalent to a mean of 710 mm (s.d. = 96.5) on tank base).

period was noted, using the centre of the cephalothorax to indicate an individual's position. The visual status of each emerging individual (blinded or sighted) was noted.

The total time per unit area (s/m²) that the sighted and blinded groups of individuals spent in each band was calculated. A scale of known dimension was used to correct the minor distortions of the screen image.

7.12.4. Statistical analysis.

All data was tested for homogeneity of variance using the $F_{\max}$ test (Fowler and Cohen, 1990) and found to be suitable for analysis by a two-way ANOVA technique for mixed design (two-tailed) (Meddis, 1973). This was used to compare the total times (s/m²) spent by the sighted and blinded groups of individuals in the 12 bands A - L. The test was used to assess the effects of vision and band position on the time a group of individuals spent in each band.

All tests were carried out using a probability level of 0.05.

7.13. Results and Discussion.

Only two, out of a total of thirty sighted and blinded individuals, actually entered the creel. In both cases they entered the baited creel before dawn, between 04.00 h and 05.00 h. Because so few individuals entered the creel over the period of the trials, little can be drawn from the fact that both individuals were blind.

In order to get some idea of the response of *N. norvegicus* to a baited creel, the total times per m² spent by the sighted and blinded groups of individuals in each band was assessed (Fig. 7.4). The ANOVA test revealed that blinded individuals spent significantly more time out of their burrows than the sighted ones ($F = 9.12$, $p < 0.05$) and showed a significant preference for bands A and B, the two bands closest to the creel ($F = 4.87$, $p < 0.05$, Tukey test). There was no significant difference in the use of all other bands for the sighted and blinded groups of individuals. The sighted individuals showed no such preference for any bands on the tank base.

In a previous laboratory experiment (Chapter 4) using isolated male *N. norvegicus*, it was demonstrated that individuals performed significantly more emergence behaviour after they had been blinded. However, this change in behaviour was not found in a mixed population of sighted and blinded individuals in the field where, sighted and blinded *N. norvegicus* performed similar levels of emergence behaviour (Chapter 3). Because of this discrepancy between the two experiments it was unclear whether blinding does affect the level of emergence behaviour. The results from the present experiment seem to support the previous laboratory findings using isolated individuals. However, it is also possible that in the present experiment the presence of the baited creel may

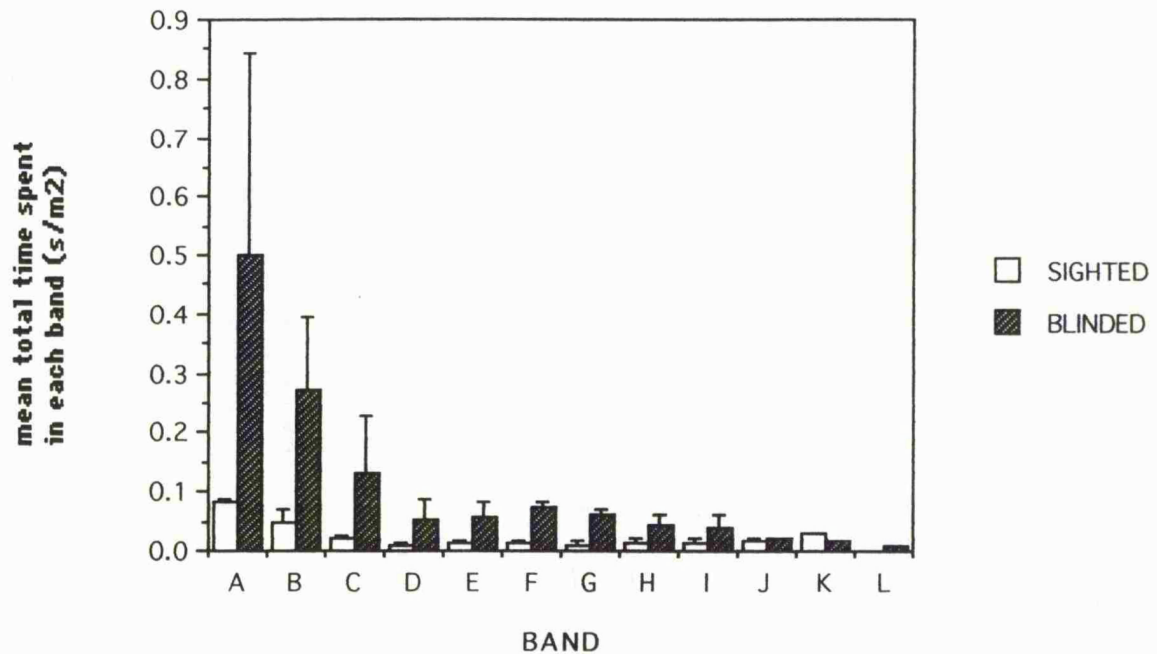


Figure 7.4. Mean total time spent by groups of sighted and blinded individuals in each of the 12 bands A - L surrounding a baited creel (band A was directly adjacent to the creel) (mean taken from 3 trials and error bars represent s.d.). Results show blinded individuals spent significantly more time in bands A and B than other bands. Sighted individuals spent similar amounts of time in all bands (using a 2-way ANOVA and probability level of 0.05).

have had a differential effect on the blinded individuals causing their increased level of emergence. Further experiments are required to distinguish between the influences of the blinding procedures and the presence of a baited creel on the levels of emergence behaviour of blinded individuals.

In a previous field study the recapture rates of different decapod crustaceans was measured using creels (Zimmer-Faust and Case, 1982). The results showed recapture rates of 5.4% for the spiny lobster *Panulirus interruptus* and 10.7% for the crab *Cancer antennarius*. A similar tagging experiment was carried out for *N. norvegicus* over the period between 1977 - 81 and demonstrated a recapture rate of 11% (Chapman and Bailey, 1987). In both studies creels remained on the sea bed for times similar to the two day periods used in the present recapture experiment. Such field studies can underestimate actual recapture rates because they ignore such factors as mortality (natural and discard-related) and migration from the release site after individuals are released on the sea bed. Thus, the apparently low recapture rate for *N. norvegicus* in the present experiment is entirely consistent with known recapture rates in the field. Previous experiments using creels in similar conditions and techniques to those used in the present experiment, were also unsuccessful in capturing velvet swimming crabs *Necora puber* (Kinnear and Bova, pers. comm.).

7.14. Conclusion.

Only two individuals entered the creel during the three trials and these were both blind. Although the present experiment was of limited scope, it provided some evidence that blinded individuals were more likely to be attracted to a baited creel than sighted ones.

Chapter 8. General discussion.

Given the complex anatomy and prominence of the *N. norvegicus* eye, together with the importance of vision in other arthropods with compound eyes, blinding seems to have surprisingly little effect on the behaviour of *N. norvegicus*. The results of the present study show that, after blinding, behavioural rhythms persist, agonistic interactions appear to be unaffected, food and shelter can be found and predators can be detected. Such findings raise questions concerning the role of the *N. norvegicus* visual system in normal behaviour. The failure of any of the experiments described in the present thesis to demonstrate large scale differences in behaviour between sighted and blinded individuals is consistent with observations on survival and growth of visually impaired *N. norvegicus* in a previous field tagging experiment (Chapman *et al.*, 1989). Sighted, partially blinded and totally blinded *N. norvegicus* were tagged and released onto the sea bed (at Loch Torridon, Scotland) and similar numbers of each category were recaptured over a period of five years (Chapman *et al.*, 1989).

8.1. The significance of vision in the entrainment of behavioural rhythms of *N. norvegicus*.

Previous observations of *N. norvegicus* activity rhythms show diel rhythms of in-burrow and burrow emergence behaviour with a nocturnal peak of activity at similar depths or light conditions to those used in the current experiments (Chapman *et al.*, 1975; Atkinson and Naylor, 1976; Naylor and Atkinson, 1976; Hammond and Naylor, 1977 a and b; Chapman and Howard, 1979). In the current field and laboratory investigations (chapters 3 and 4) there

was evidence for such rhythms in both sighted and blinded *N. norvegicus*.

Earlier work on the nature of the behavioural rhythms in *N. norvegicus* has demonstrated a diel entrained circadian rhythm of emergence and in-burrow behaviour (Atkinson and Naylor, 1973; Arechiga and Atkinson, 1975; Atkinson and Naylor, 1976; Naylor and Atkinson, 1976; Hammond and Naylor, 1977 a; Moller and Naylor, 1980). There is also abundant evidence that light is a major factor in the entrainment of these rhythms in *N. norvegicus* (Arechiga and Atkinson, 1975; Chapman *et al.*, 1975; Atkinson and Naylor, 1976; Naylor and Atkinson, 1976; Hammond and Naylor, 1977 a; Moller and Naylor, 1980). In the current field experiments, the diel rhythm found in sighted and blinded male *N. norvegicus* in the cage on the sea bed was simultaneous with the cycle of light changes recorded over the same period. Similar rhythms were also found inside the controlled conditions of the actographs. The results of both studies add to the body of evidence that light is a major entraining factor for the behavioural rhythms of *N. norvegicus*.

There are two types of behavioural rhythm found in animals, endogenous and exogenous. Both can be entrained by environmental factors, but only endogenous rhythms persist in constant conditions (Cloudsley-Thompson, 1961; Palmer, 1974). In previous actograph studies, *N. norvegicus* has been subjected to constant darkness and was shown to perform according to an endogenous circadian rhythm (i.e. with a cycle length slightly greater than 24 h) of burrow related activities ('in-burrow', 'out-of-burrow' and 'mouth-of-burrow' behaviour) (Atkinson and Naylor, 1973, 1976; Naylor and Atkinson, 1976). In one of these previous studies, as the *N. norvegicus* remained in the actographs, the time between

activity peaks gradually increased indicating the presence of an endogenous circadian rhythm, until after 11 days the activity peak occurred in the period when these *N. norvegicus* previously experienced light (Atkinson and Naylor, 1976; Naylor and Atkinson, 1976). In the current field and laboratory studies *N. norvegicus* behaviour was monitored for up to 11 days after they were blinded and there was no evidence for any effect of blinding on the periodicity of their behavioural rhythms. It is concluded that the entrained diel rhythm persists in the blinded *N. norvegicus*.

If light is entraining the rhythm of sighted and blinded *N. norvegicus* this suggests that an extraretinal mechanism is used to detect light level changes. In a previous study of *N. norvegicus* that had been subjected to 100% retinal ablation, activity rhythms were abolished and could not be entrained by light (Arechiga *et al.*, 1980). This might suggest that extraretinal photoreception is not involved in entraining the activity rhythms of *N. norvegicus*. However, blinding by surgical ablation of the retina involves extensive damage of the eyestalk. The eyestalk contains a group of neurosecretory cells called the x-organ, which is believed to be the location of the 'clock' controlling rhythms in many crustacea (Arechiga and Naylor, 1976; Naylor and Atkinson, 1976). Eyestalk extracts contain hormones able to change the locomotor rhythms of many crustacean species (Brown, 1961) and eyestalk extract has been demonstrated to inhibit neural activity in *Carcinus maenus* (Arechiga *et al.*, 1976) and in *N. norvegicus* (Arechiga *et al.*, 1980). Therefore it is not possible to know whether rhythms are abolished by the loss of vision or as a result of eyestalk damage. Extraretinal control of activity rhythms in *N. norvegicus* cannot be excluded. No such site has been characterised in *N. norvegicus*, but non-visual photoreception has been found in other crustacean species, such as

in the 6th abdominal ganglion of the crayfish *Procambarus clarkii* (Edwards, 1984; Simian and Edwards, 1990); and, in the terminal abdominal ganglion of the squat lobster *Galathea strigosa* (Maitland *et al.*, 1991).

Although blinding had no apparent effect on the periodicity of the behavioural rhythms there was evidence that blinding caused an increase in the levels of in-burrow and burrow emergence behaviour in the actograph study. However in the field, the levels of emergence activity were similar for sighted and blinded individuals. Further study is needed to clarify the differences between the two sets of results.

8.2. The effect of loss of vision on the agonistic behaviour of *N. norvegicus*.

When two sighted individuals were allowed to compete, one opponent was consistently dominant and its status remained unchanged after either the dominant or subordinate opponent was blinded. In the majority of pairs, mean duration of agonistic contests was also not affected by blinding and there was no apparent change in behavioural content of contests.

To explain different strategies in contests between animals, a number of models have been developed. These models concentrate on the abilities of an individual to gain information on the relative fighting abilities of opponents (Parker and Rubenstein, 1981; Archer, 1988). This information is used to estimate the chances of winning a contest and the costs that may be incurred (i.e. time, energy, risk of injury) (Maynard Smith, 1974; Parker, 1974; Parker and Rubenstein, 1981; Enquist *et al.*, 1990). If a blind *N. norvegicus* is to compete successfully it still has to be able to obtain accurate information about the relative fighting ability of an opponent

before and during a contest. This requires communication between the individuals when one opponent is blind.

Communication between two opponents can be demonstrated if the acts of one are shown to affect those of the other. In the present analysis of contests between *N. norvegicus* an association between the behaviour of one opponent and the other was demonstrated because an agonistic act by one led to an agonistic act by the other. This was true when both were sighted and after one opponent had been blinded. In addition, the fact that the dominance status was unchanged after blinding one opponent shows that status can be communicated irrespective of visual status. This suggests that the blinded opponent is able to receive information about its opponent using non-visual sensory channels. One possible source of information was the water disturbance caused by an opponent's movements. Meral spread is used by the crayfish *Orconectes rusticus* during contests in darkness and it is thought that this species uses the mechanical signal associated with the meral spread to provide information about cheliped size. It is argued that the amount of water displacement is directly related to the size of chelipeds (Bruski and Dunham, 1987). A blinded *N. norvegicus* would also be able to gain information about the strength of its opponent when performing different elements of contact cheliped behaviour, such as when opponents push against each other. Both opponents would hold their chelipeds in the meral spread, while facing each other. In this position each will push against its opponent and attempt to move the other one backwards. The use of pushing behaviour to assess strength asymmetries has been demonstrated in the swimming crab *Necora puber* (Smith *et al.*, 1994) and the crayfish *Orconectes rusticus* (Bruski and Dunham, 1987). In this same position, they will also 'clash' their chelipeds

against those of their opponents. This has been observed in the field and was detected as a 'snapping' sound by a hydrophone (Chapman *et al.*, 1968). This cheliped 'clashing' behaviour may be a further source of sensory information about the relative size and strength of an opponent.

Chemical communication can also be important during the fighting behaviour of crustacea. This may take the form of long range signalling using pheromones or short range surface contact between individuals (Laverack, 1988). Crayfish perform more aggressive acts in water previously containing fighting crayfish (Thorpe and Ammerman, 1978). There is also evidence that the American lobster *Homarus americanus* releases a chemical signal in its urine during contests. This chemical is able to inhibit aggressive behaviour in a defeated opponent after a single contest (Breithaupt and Atema, 1993; Kaplan *et al.*, 1993). This signal is used for individual recognition of a previous opponent (Karavanich and Atema, 1993). Chemical recognition of previous opponents has also been demonstrated in the banded shrimp *Stenopus hispidus* (Johnson, 1977) and the stomatopod *Gonodactylus festai* (Caldwell, 1979). In the current experiment there was no evidence for recognition behaviour in *N. norvegicus*. When two opponents remained together, both persisted in attacking the other in subsequent bouts and there was no pattern of changes in bout durations that would indicate learning. More prolonged pairing of two opponents and examination of any chemicals present in the water during a contest would be required to examine the possible role of chemosense in the agonistic behaviour of *N. norvegicus*.

In the current study there seemed to be no escalation of fighting as a contest proceeded, suggesting therefore, that the results do not appear to fit predictions of the sequential

assessment model where this would be expected (Enquist *et al.*, 1990). The structure of contests in the present study seems to be more consistent with some of the basic predictions of game theory. Game theory predicts that during a fight an animal must not transmit information about its intentions to continue or to withdraw until the end of a contest (Maynard Smith, 1982). The fact that both *N. norvegicus* perform aggressive and submissive behaviour throughout a bout suggests that neither opponent betrays its status during contests until the end when the one with the lower status withdraws. Studies of contests of other crustacea such as the swimming crab *Liocarcinus depurator* (Glass and Huntingford, 1988) and the prawn *Macrobrachium rosenbergii* (Barki *et al.*, 1991) also found no difference in agonistic behaviour of the eventual winner or loser until the end of a contest. However, in the present study it was also found that subordinate *N. norvegicus* perform more 'retreat' acts and the dominant opponents perform more 'approach' acts during a contest. Further information would be required to see whether these differences occurred throughout, or at the final stage of a contest.

It was clear that agonistic behaviour in *N. norvegicus* did not entirely rely on visual cues and that when an opponent was blinded its dominance status remained unchanged.

8.3. The importance of vision in the predator avoidance response of *N. norvegicus*.

In its natural habitat, it would be important for *N. norvegicus* to be able to assess levels of predation risk before performing anti-predator behaviour (e.g. remaining in burrow or escaping into a burrow) or carrying out other behaviour (e.g. foraging) (Lima and Dill, 1989). An individual must also be able to assess the potential

threat of a predator so that it can perform the type of behaviour most likely to aid its escape (e.g. enter/remain inside a burrow, 'tail flip', or attack) (Sih, 1987; Lima and Dill, 1989). If assessment of predation risk by *N. norvegicus* is less accurate when it is blind, this could mean a reduction in its chances of survival and foraging efficiency.

During the current study the responses of sighted and blinded *N. norvegicus* to the presence of two species of fish (i.e. cod, a common predator of *N. norvegicus* and a saithe, known not to feed on *N. norvegicus*) (Sarno *et al.*, 1994) and the odour of a predator (i.e. cod) were assessed. Blinded individuals performed predator avoidance behaviour (i.e. increased use of shelters) and other forms of protection from predation (i.e. the tail-flip escape response and aggressive behaviour towards a fish) in the same way as the sighted *N. norvegicus*. This suggests that the blinded individuals can use other sensory channels to detect a predator.

The responses of sighted and blinded individuals to fish odour clearly demonstrate that chemical detection of predators occurs in *N. norvegicus*. In an aquatic environment chemical signals from either a predator or from stressed and damaged conspecifics, can serve as a warning to others of the presence of a predator (Carr, 1988). Previous studies on decapod crustacea have shown predator avoidance in response to chemical stimuli in species such as the American lobster *Homarus americanus* (Wahle, 1992) and crayfish *Pacifastacus leniusculus* (Blake and Hart, 1993). In both cases, responses to chemical stimuli were lower than those elicited by the physical presence of the predator. It has been suggested that chemical stimuli can serve to warn prey about the presence of a predator at greater distances than other stimuli such as visual stimuli, which operate over shorter distances (Blake and Hart,

1993; Martel and Dill, 1993). Another possible explanation for the fact that *N. norvegicus* responds to the presence of fish by seeking shelter is that it releases alarm signals in response to stresses caused by the introduction of a predator. Alarm chemicals have been identified in the crayfish *Orconectes virilis* (Hazlett, 1985). In a previous experiment the remains of *N. norvegicus* after removal of the abdomen (i.e. 'tailing') were placed in creels and as a result the catch was reduced by 58% (Chapman, 1981). This could be explained by alarm substances released by damaged tissues of the 'tailed' *N. norvegicus* deterring others from entering the creel. In the current experiment it is possible that retreat into shelters was due not only to the direct effect of the presence of the fish but also involved the secondary one of alarm signal release.

The presence of a cod predator elicited a greater response than odour alone in sighted and blinded individuals. This implies that both sets of individuals also received additional sensory information from the physical presence of the cod, over and above the odour stimulus. It is possible that blinded individuals were using mechanoreception to detect water disturbance caused by the swimming cod. Water displacement is known to cause a tail-flip escape response in the crayfish *Procambarus clarkii* (Wiese, 1988). *N. norvegicus* also responds to water borne vibrations using one or more tail-flips to propel itself backwards from a stimulus (Newland and Chapman, 1985; Newland *et al.*, 1988 a, b). Using a length of rope to simulate part of the trawl fishing gear it was shown that 20% of the sighted individuals that reacted to the gear, responded before contact with the rope by tail-flipping in the opposite direction. The same experiment was repeated using blinded individuals and it was found that only 4% reacted before tactile contact with the rope. Mean reaction distance was reduced

from 0.23 m for visually intact individuals, to 0.17 m for blinded ones (Newland and Chapman, 1985). This suggests that blinded *N. norvegicus* may be less able to escape from the direct approach of a predator.

With respect to predator avoidance behaviour, sighted and blinded *N. norvegicus* could not distinguish between a predator and a non-predator species of fish. This suggests that the cues stimulating *N. norvegicus* to perform avoidance behaviour are common to cod and saithe. Although the sighted individuals may have been using visual cues, the blinded ones must have used other sensory modalities because they also responded to both species of fish.

According to the current study, blinded *N. norvegicus* can use sensory systems other than vision to detect a predator. However, blinded individuals showed less use of shelters in the presence of a predator stimulus. If blinded individuals in their natural habitat are less likely to retreat into, or remain inside, burrows in the presence of predators this could reduce their chances of survival.

8.4. The ability of sighted and blinded *N. norvegicus* to gain shelter.

Shelter gain is very important for survival of animals such as *N. norvegicus*, which uses shelters as protection against predators (Edwards, 1984). The current study showed that blinding did not effect the ability of *N. norvegicus* to gain shelter and that there was no effect of blinding on time taken for an individual to gain shelter. Individuals were seen using their chelipeds to examine shelter entrances. It is possible that they were using tactile and/or chemical information to investigate potential shelters. Other crustacea, such as the American lobster *Homarus americanus*

(Pottle and Elner, 1982) and hermit crabs *Pagurus bernhardus* (Schone; 1961), also use their chelipeds to assess the suitability of a potential shelter.

Cobb (1971) found that lobsters use positive thigmotaxis (i.e. a preference for vertical contact with solid objects (Vines and Rees, 1972)) when choosing a shelter. This is also used by stomatopods, which will occupy both clear and opaque glass tubes (Hazlett, 1962). It is possible that thigmotaxis is used by *N. norvegicus* when seeking and selecting shelter.

Further investigations are needed to confirm that blinded *N. norvegicus* is able to identify burrow entrances in its natural environment. However, there are already some previous unpublished preliminary observations on tethered *N. norvegicus* which showed that blinded individuals are able to find burrows on the sea bed (C.J. Chapman and J.A.M. Kinnear, pers. comm.).

8.5. The importance of visual cues in the recognition, and location of food and a baited creel, by *N. norvegicus*.

There is a great deal of evidence that crustacea primarily use their chemosense to locate and recognise food items (Carr, 1988). There is evidence that low molecular weight substances such as amino acids stimulate feeding behaviour in the European lobster, *Homarus gammarus* (Mackie, 1973), California spiny lobster, *Panulirus interruptus* (Zimmer-Faust *et al.*, 1984), American lobster, *Homarus americanus* (Derby and Atema, 1978), and crabs, *Cancer antennarius* and *Loxorhynchus grandis* (Zimmer-Faust and Case, 1982). These amino acids are abundant in the tissue of prey and are released into the environment when these tissues are damaged or decay (Zimmer-Faust and Case, 1982). The banana prawn, *Penaeus merguensis*, is unable to detect the presence of a

food item if presented with visual stimuli alone, but can do so in the presence of chemical stimuli (Hindley, 1975). The crab, *Hemigrapsus oregonensis*, shows no significant change in ability to locate food items before and after blinding (Symons, 1974). Both studies clearly demonstrate that visual cues are not used to elicit feeding behaviour and that the chemosense is important for detection and location of food items. The results for *N. norvegicus* are consistent with these findings. During the current investigations, it was demonstrated that *N. norvegicus* is able to find both uncovered and hidden food items using non-visual sensory information. In addition both sighted and blinded *N. norvegicus* were attracted to a baited creel.

One major aspect of *N. norvegicus* behaviour not included in the present study was sexual behaviour. Vision has been demonstrated to be important in the courtship behaviour of fiddler crabs (Waterman, 1961) and identification of suitable mates in the horseshoe crab, *Limulus polyphemus* (Barlow *et al.*, 1988). Very little is known about the mating behaviour in *N. norvegicus* and visual signals used for sexual behaviour have not been studied in species similar to *N. norvegicus*. Chemical communication has also been found to be important in the sexual behaviour of many species of crustacea, including the crab, *Carcinus maenus* (Eales, 1974), the crayfish, *Procambarus clarkii* (Ameyaw-Akumfi and Hazlett, 1975) and the American lobster, *Homarus americanus* (Atema and Engstrom, 1971). In the American lobster a pheromone is released by females at pre-moult when they are in a receptive state for mating (Atema and Cobb, 1980). A study of pheromone production in the blue crab, *Callinectes sapidus*, showed that such chemical communication is used for initial recognition and attraction of

mates and that other sensory cues (e.g. tactile and visual) are used during copulation (Gleeson, 1984). Observing *N. norvegicus* within a burrow would be technically difficult and females in pre-moult state are only available for a limited period during the spring. These difficulties do not make such a study impossible, but did make it beyond the scope of this present investigation.

8.6. GENERAL CONCLUSION.

The results of the present study provide no evidence that *N. norvegicus*, returned to the sea bed after receiving extensive light-induced eye damage, would suffer any decrease in their chances of survival. Ever since it was discovered by Leow (1975) that *N. norvegicus* is susceptible to light-induced blindness, there has been concern about the likely effects this would have on the behaviour and survival of discarded individuals. Previous to the present study very little was known about the behaviour of blinded *N. norvegicus*. Newland and Chapman (1985) showed that blinded individuals had greatly reduced mean reaction distances to simulated trawl gear, but apart from this work little else was known. The present study shows that in many aspects of their behaviour blinded individuals are indistinguishable from sighted ones. This finding provides an explanation for the fact that in a tagging and recapture experiment, the growth and recapture rates for blind individuals were not significantly different from those for sighted *N. norvegicus* (Chapman *et al.* 1989).

APPENDIX I. Statistical analysis.

Mean square successive difference test.

This is a preliminary test used for time series data to see whether a series is random, or if the data is autocorrelated (Farnum, 1989). This test statistic is calculated from the series of data by taking the ratio of the sum of the squared first differences, to the sum of the squared deviations from the mean:

$$M = SS_{\Delta y} / SS_{yy}$$

A table of critical values is then used to decide what this statistic M demonstrates about the character of the series (i.e. random/autocorrelated) (Farnum, 1989).

In order to test for the significance of the difference between levels of activity of two different autocorrelated series it was necessary to select a subset from a series, taking observations at regular intervals so that the new subset was not autocorrelated (using a mean square successive difference test).

Autocorrelation analysis.

This is a technique of time series analysis and is used to identify rhythms within a series (Chatfield, 1984; Farnum, 1989; Bakus, 1990). A time series of data is compared with itself, with different intervals (called time lags, or k) between each observation in the series. All tests carried out using lags, k = 1 h, 2 h, 3 h n (where n = the number of hourly observations) - 1. Autocorrelation coefficients (or, rk's) were calculated (using MINITAB® Statistical Software) for each time lag k (Chatfield, 1984; Farnum, 1989; Bakus, 1990).

Correlogram.

A correlogram is a graphical presentation of autocorrelation coefficients calculated from a series of data (Chatfield, 1984; Bakus, 1990). The time lags (k) used are plotted on the x-axis, against the autocorrelation coefficients on the y-axis. 95% confidence limits are plotted on each correlogram. These are calculated using the 'rule of thumb' (Farnum, 1989), where the autocorrelation coefficients will be approximately normally distributed (mean = 0, s.d. = $1/\sqrt{n}$). 95% of a normally distributed population falls within two standard deviations of the mean (Farnum, 1989), so any coefficient exceeding these limits indicates a significant point at the 0.05 significance level.

Upper limit: $+2/\sqrt{n}$

Lower limit: $-2/\sqrt{n}$

A significant coefficient shows the presence of a rhythm in the original series of data, with a period equal to the corresponding time lag. There are commonly significant coefficients at multiples of this time lag, showing the presence of a persistent rhythm throughout the series of observations (Chatfield, 1984; Farnum, 1989; Bakus, 1990).

Cross correlation analysis.

This technique is similar to autocorrelation analysis and involves the calculation of correlation coefficients between two series at different lags (k) (Chatfield, 1984; Farnum, 1989; Bakus, 1990), but in this type of analysis the features of two individual series of observations (labelled 'series x' and 'series y') are compared. Both series are directly compared (i.e. time lag, $k = 0$) to test whether they are simultaneous, and with the series lagged

slightly in the positive and negative directions to investigate whether there is a similar pattern in one of the series either k time lags ago, or k time lags hence, of the other. These cross correlation coefficients ($r_{(xy)k}$) are plotted onto a correlogram against the time lags (k). Once again the 'rule of thumb' technique was used to calculate the 95% confidence limits for a correlogram using two standard deviations of the coefficients in the correlogram (Farnum, 1989).

Seasonal differencing.

The technique used to model and remove the 24 h cycle is 'seasonal differencing' (Chatfield, 1984; Farnum, 1989). The original series is lagged by the period of the cycle to be removed (e.g. 24 h), and the difference between each point of the original series and the corresponding point of the lagged series is calculated:

$$\Delta y_t = (y_t) - y_{(t-c)}$$

where, Δy_t = seasonal difference
 y_t = point of series at time t
 c = length of seasonal cycle
 (e.g. 24 h)

Data Transformation.

A transformation is used to make a series of observations into a normal distribution (i.e. 'normalised') and suitable for use in statistical tests (Fowler and Cohen, 1990). The logarithmic transformation is used when the data is highly positively skewed. If the original values are zero, or near to zero, it is necessary to add a constant value of one to each value before transformation of the data (Fowler and Cohen, 1990).

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