THE SENSORY DETECTION OF WATER BORNE VIBRATIONAL STIMULI AND THEIR MOTOR EFFECTS IN THE NORWAY LOBSTER, *Nephrops norvegicus* (L.)

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Declaration:

I declare that this thesis represents, except where a note is made to the contrary, work carried out by myself. The text was composed by myself.

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10th OCTOBER 1988

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FOR MUM, DAD, SUZY AND MARK

"AD SUMMA NITOR"

CONTENTS

SUMMARY

The morphology and distribution of cuticular setae on the uropods and walking legs of the Norway lobster *Nephrops norvegicus* (L.) has been studied using both light microscopy and Scanning Electron Microscopy. Three types of setae are present on the uropods, **plumose setae, simple setae** and **guard hairs. Hair peg** and **hair fan organs** were also seen.

The propodus and dactyl of the 2nd and 3rd legs of *Nephrops* are similar in both their structure and in the form and distribution of their cuticular setae. Three main areas of setal distribution are found: **squamous setae** are distributed 1) in bunches on the flat surfaces of the propodus and dactyl and 2) along the lateral edges of the propodus and dactyl and 3) **hedgehog hairs** line the inner edges of the propodus and dactyl.

Most of the setae on the 4th and 5th legs are found around the propodus-dactyl (P-D) joint. Three rows of **simple setae** are found on the dactyl, and both **serrate setae with simple scales** and **squamous setae** are found overlapping the P-D joint. Also found near the joint are **CAP organs** and **hedgehog hairs.**

All of the setae on the uropods show responses to tactile and vibratory stimulation as do the hedgehog hairs, the serrate setae, the simple setae and the squamous setae on the legs.

The responses of afferents from the uropods and walking legs and of the abdominal interneurones have been tested in response to water borne vibrations of different frequencies produced both as surface waves and in an acoustic tube. The uropod afferents show range fractionation and have therefore been divided into three nested categories based on the upper limit of their frequency response. Low

frequency units respond from 2-20Hz, intermediate units from 2-50Hz and high frequency units from 2-100Hz. The leg afferents also show range fractionation and have also been divided into three nested catagories: low frequency units respond from 20-60Hz, intermediate units from 20-200Hz and high frequency units from 20-450Hz. Preliminary studies have indicated that the leg afferents show directional sensitivity. The abdominal interneurones have been categorised as either intermediate or high frequency; intermediate interneurones respond from 2-100Hz and high frequency interneurones from 2-200Hz. The receptive fields of mechanosensory interneurones have also been determined.

The postural responses of *Nephrops* to water borne vibrations have been studied using video analysis. An abdominal extension response is reliably elicited which varies with the frequency of stimulation in a distinct way. From 20-80Hz the animals respond immediately, and abdominal extension is accompanied by rapid leg movements, swimmeret beating and very occasionally, tail flipping. From 100-180Hz the response occurs with a delay, the duration of which seems unrelated to frequency within this range. No responses were seen above 180Hz.

The nervous control of the abdominal extension response has been studied by recording from abdominal motor roots (superficial root three and root two) which supply the two muscles involved (the superficial flexor and extensor muscles). It has been shown that abdominal extension is produced by both central and peripheral inhibition of flexor muscle activity in combination with excitation of the extensor muscle. The neuronal basis of the delay seen in the behavioural experiments has been investigated, and a number of

different patterns of nervous activity have been found which might produce this delay.

Behavioural studies have been conducted in the field to investigate the responses of freely moving animals to sound in their natural environment. Investigations have been conducted of changes in the emergence rhythm and changes in the transient behaviour of the animals. Tests to investigate changes in the burrow emergence rhythm with the underwater loudspeakers at 10m from the animal failed to produce any responses. However small changes occur in the transient behaviour of *Nephrops* when they are very close to the loudspeaker even though the sound pressure levels are similar to those used at 10m. These tests have been repeated in laboratory tanks where clear locomotory responses, predominantly backwards walking, are seen in response to stimuli from 20-80Hz in both blind and sighted animals.

Tests have been conducted in a free acoustic field to determine the behavioural response threshold of *Nephrops* to sound using the postural response as a monitor. The animals showed no responses with the loudspeaker at 1m but showed clear responses with the speaker at 0.09m even when the sound pressure levels were similar, yeilding a threshold in terms of particle displacement of the water of 0.874μm which is independent of frequency. This indicates that the *Nephrops* is sensitive to the particle motion component of sound rather than the pressure component.

Chapter 1

GENERAL INTRODUCTION

1. GENERAL INTRODUCTION

Sense organs of different modalities form an interface between an animal and its environment. These organs, through their connections with the nervous system, respond to some of the different forms of energy found in the animal's natural habitat. They thereby allow an animal to perceive its environment and the objects in it, as well as giving it information about its own spatial orientation and position. This information may then be used by the animal to modify its behaviour via a change in its motor output. Thus sense organs and sensory perception are the first steps in a chain of physiological events which may determine and pattern behaviour.

Forms of energy found in an animal's natural habitat include electromagnetic radiation such as light, mechanical energy and chemical energy, and animals use specially adapted sense organs to detect these stimuli. Vision, mechanoreception, chemoreception and other senses are used to recognise and localise predators, prey and con-specifics (Tautz, 1987). In some environments one or more of these forms of energy may be absent and consequently the corresponding receptors may be absent or vestigial in animals living there. For example, animals such as the mole and the thalassinid shrimp, *Calocaris* which previously lived above ground have adopted the burrowing habit and now live in complete darkness. Although the eyes are present in these animals, the visual sense is vestigial and the animals are blind. These animals perceive their environment using other senses such as chemo- and mechanoreception. In general, however, animals make use of all their sensory modalities, and indeed loss of function of one sensory modality may be very detrimental to an animal's survival.

In an aquatic environment it is especially important that animals

should be able to utilise several alternative sensory modalities to vision as water is an optically poor medium. Light levels are often low, especially in deep water, the transmission of light may be attenuated by scattering, and vision is often impaired by turbidity. Many animals possess adaptations which may partially compensate for the poor optical qualities of the aquatic environment (Shelton, Gaten and Chapman, 1985, Cronin, 1986) and exploit the fact that water is a very suitable medium for the propagation of other stimuli such as sound.

1.1 **Mechanoreception**

The mechanical sense is used to detect several forms of stimuli found in the aquatic environment and aquatic organisms reveal a vast diversity of receptors for this purpose. Organs such as statocysts detect changes in gravity (Cohen, 1960), the swimbladders of fish detect changes in hydrostatic pressure (Blaxter, 1981), the lateral lines of fish (Harris and van Berjick, 1962) and amphibia detect low frequency water displacements and the ears of mammals and fish detect sound (Stebbins, 1983). External receptors such as cuticular hairs are widely used among aquatic invertebrates for the detection of water currents, turbulence, touch and vibratory stimuli (Wiese, 1987).

The hair receptor is a common component of many of these mechanoreceptive systems and is found in the form of the hair cell of the vertebrate acoustico-lateralis system, as well as in the innervated cuticular hairs of crustaceans and insects. This reflects the fact that the ultimate stimulus in each case is the same, a mechanical displacement which causes movement of the hair structure thereby stimulating the underlying receptor cell. The differences between the various components of vertebrate acoustico-lateralis system, or between the various arthropod mechanosensory systems exist

in terms of the accessory structures which transduce or amplify the of original stimulus so that it can cause the required displacement of the hair receptors.

1.2 Sound as a biological stimulus

Sound is a very important biological stimulus in both aquatic and terrestrial environments. On land a distinction is usually made between sound and vibration in that **sound** is transmitted through the air medium whereas **vibration** is transmitted through solid substrates. In water there is no clear distinction between sound and water borne vibration and the terms may be considered synonymous. A distinction could be drawn between substrate- and water-borne vibrations, although that distinction may also be unclear in such media as a mud substrate.

There are many comprehensive reviews on the nature and physics of sound both in air and in water, (Hawkins and Myrberg, 1983, Rogers and Cox, 1987), and so it is appropriate here only to reiterate some points which demonstrate the importance of sound as a biological stimulus with special reference to the aquatic environment.

Sound is produced by the movement or vibration of an object in a material medium. It may be considered as a longitudinal mechanical wave producing an oscillatory rarefaction and compression of the medium. The speed at which sound travels depends on the density and elasticity of the medium. In water sound travels at about $1500ms^{-1}$ which is about 5 times faster than it travels in air but is 150,000 times slower than the speed of light in water. In a free sound field, ie. in water with no reflecting boundaries, sound may be transmitted over long distances because of its low attenuation and absorption which is several orders of magnitude lower than sound in air and many orders of magnitude lower than the attenuation of light and

electromagnetic radiation in water. Other advantages of sound over light and chemical stimuli are that it can travel through solid substances, especially at low frequency, and around corners pervading many areas that light cannot reach. Unlike vision sound reception is not impaired at night or by water turbidity.

Sound propagation however, may be strongly influenced by reflecting boundaries such as the sea bed and the sea surface. The acoustic impedance (pc, the product of the density and sound propagation velocity of the medium) of water (1.54×10^5 $gcm^{-2}s^{-1}$) is much higher than that of air (41.5 $gcm^{-2}s^{-1}$) This mismatch in the acoustic impedance between the two media means that sound does not propagate from one to the other easily and will be reflected by any air-water interface. This has important consequences for acoustic experiments carried out in small tanks surrounded by air (Parvelescu, 1964; Hawkins, 1981). The effect of the air water interface may only be completely avoided by conducting experiments in a free sound field.

An acoustic field may be defined either in terms of pressure, which is a scalar variable, or in terms of particle acceleration velocity or displacement, which are vectors. For any sound source the acoustic field may be divided into **near field** and **far field** (Harris and van Bergeijk, 1962) depending on the proximity of the detector to the sound source. In the far field, ie. beyond 0.2 wavelengths from the sound source, the advancing wavefront of sound is a plane wave. Under ideal conditions in the farfield the pressure (p) and particle velocity(v) are related by the simple plane wave equation ;-

$$\mathbf{P} = \boldsymbol{pc}\mathbf{v} \text{.....(1)}$$

where pc is the acoustic impedance.

Within the near field (less than 0.2 wavelengths distance from the source) the advancing wavefront of sound is spherical and the relationship between pressure and displacement is described by the

spherical wave equation.

$$d = \frac{P}{2\pi\rho cf}\sqrt{1+\left(\frac{\lambda}{2\pi r}\right)^2} \qquad \text{.....(2)}$$

where d = displacement (cm)

P = sound pressure (μbar)

f = frequency (Hz)

ρc= acoustic impedance of the medium = 1.54×10^5 $gcm^{-2}s^{-1}$

λ = wavelength=velocity/frequency = 1500x100/frequency (cm)

r = distance from the sound source (cm)

In the far field particle velocity decreases in proportion to the square of the distance from the sound source but in the near field it decreases as the cube of the distance from the sound source. It will be seen from equation 2 that close to the source (ie. in the near field) very high particle displacements may accompany a given sound pressure. In the near field therefore, where the displacement/velocity component of a sound is large, some animals or individual sense organs may be unable to differentiate between displacements produced as a result of DC hydrodynamic effects such as current and turbulence and those which are components of a sound stimulus (Hawkins and Myrberg, 1983). It is worth pointing out that all experiments conducted in tanks, unless the tank is very large, will be in the near field.

Sound is generally measured in terms of sound pressure using calibrated hydrophones, the **sound pressure level (SPL)** being expressed in **decibels** (db), which are logarithmic units, according to the following equation;

$$\mathbf{SPL = 20\log_{10}\ P/Pref} \qquad \text{.....(3)}$$

when the reference pressure (Pref) is 1 μbar then the SPL is expressed

as db re 1μbar.

NB. SPL is now expressed in db re 1μPascal(Pa)

eg. 0 db/1μbar = 1μbar = -20 db/1μPa

1.3 Vertebrate and Invertebrate hearing mechanisms

Sound can pervade all environments and all vertebrate animals and many invertebrates have mechanisms for the detection of sound. The receptors involved range widely from the vertebrate ear to the hair sensillae of insects. These structures provide animals with the sense of hearing. **Hearing** is defined as a behavioural response to sound (Stebbins, 1983), and it is a sense which is active at all times and allows animals to be constantly vigilant. Hearing mechanisms can provide an early warning system, helping animals to detect prey and to locate mates. Animal sound production systems have evolved closely with the sense of hearing and allow animals to communicate with and recognise each other over long distances even when they are out of sight of each other. As hearing is a behavioural response the characteristics of this response in individual animals are most reliably determined using behavioural tests but measurements of nerve activity in response to sound may provide much useful information about the coding and processing of sound stimuli within the nervous system. Hearing systems can detect only those sounds which are above the **auditory threshold**, the measurement of which determines the sensitivity and frequency range of the hearing mechanism.

Both terrestrial and aquatic mammals have a centralised hearing organ, the ear, which in addition to sound detection has a vestibular role. The outer and middle ear amplify and transmit sounds via a bone chain to the inner ear where hair cells in the cochlear duct are moved by the mechanical sound stimulus. Two types of hair cell are present in the cochlea, one of which is more sensitive and plays a role in

detecting acoustic stimuli near the auditory threshold and another which plays a key role in frequency analysis and coding. (for a review of vertebrate hearing mechanisms see Ades, Keidel and Neff (1974)). The frequency range and sensitivity of mammalian ears varies greatly between species. The human ear can detect sounds up to 20kHz.

Bony fish possess two types of hearing mechanism, the ear and the lateral line system (for reviews of fish hearing see Hawkins and Myrberg, 1983 and Hawkins, 1981). The lateral line system responds to local water movement and may also respond to near field low frequency sound (50-100Hz) (Sand, 1981). In most fish the lateral line consists of hair cells contained within canals which are open to the water. The hair cells are directionally sensitive to the rate of water movement along the canal (Flock and Russel, 1973).

The ear of fish has no cochlea and sound detection is performed by the otolithic components of the labyrinth. The otolith or ear stone is situated above and attached to the tectorial membrane which in turn contacts the underlying hairs cells. When stimulated by sound the sensory cells and the otolith are set in motion but the movement of the otolith lags behind that of the hair cell stereocilia, the bending of which excites the sensory cells. This alone does not provide a very sensitive hearing mechanism because of the weight of the otolith. The gas filled swimbladder situated close to the ear, and which in some fish is anatomically connected to it, helps to enhance the fishes hearing ability by converting the sound pressure wave to displacement, which is then transmitted to the sacculus (Blaxter, 1981). It is interesting to note that the swimbladder is also often used by fish in sound production.

Auditory thresholds and frequency ranges have been determined for many fish using classical conditioning methods (Hawkins, 1981). There

is a great deal of variation both between species and between individuals of the same species. Some fish like the plaice are sensitive to particle motion (Chapman and Sand, 1974) whereas others like the cod are pressure sensitive (Chapman and Hawkins, 1973). The audiogram of the cod is well established, showing a cut off at frequencies above 250Hz. Species such as the catfish are sensitive over a wider range of frequencies up to as much as 2KHz, with sensitivity thresholds as low as -40db re 1 μbar which are much lower than those of the cod (Poggendorf, 1952). Fish possess the ability to discriminate between sound from different directions (Schuijf et al., 1972) and the cod can discriminate sounds in the median, lateral and horizontal planes (Hawkins and Sand, 1977).

The word "hearing" is not generally used with reference to invertebrate acoustic systems. However, most invertebrates do have an acoustic sense which can detect some form of vibration or displacement of the surrounding medium. For example, a vast diversity of acoustic receptors is found among the insects, ranging in sophistication from the simple hair sensillae found on the anal cerci of the cockroach (Camhi, 1988) to the tympanal organs of the locust. The cockroach uses its anal cerci to detect both air currents and low frequency sound (below 1000Hz). Directional sensitivity is achieved through the structure and positioning of the hair and the directional responsiveness of the underlying receptor cell (Camhi, 1988). More sophisticated external cuticular structures such as Johnstone's organ function as acoustic receptors in the mosquito and respond to frequencies of between 200-400Hz, which is the wing beat frequency of the female mosquito. The acoustic sense in these animals is primarily used in mate location.

Locusts and other related insects have the most highly developed of the insect acoustic receptors, the tympanal organ. These are paired

organs which outwardly consist of a cuticular membrane located in the thorax or thoracic appendages. Chordotonal organs are attached to the inside of this membrane which generally covers a trachea or air space. Movement of the tympanum by sound stimuli stimulates the chordotonal organs directly. Thus the excitation of sensory cells is accomplished directly without the energy transformation steps found in the vertebrate ear and this is true for all invertebrate acoustic receptors. This may render the acoustic receptors of insects and other invertebrates less sensitive than those of vertebrates but detection of the stimulus is more rapid and the system is more compact which is particularly important in insects whose size is limited. Tympanal organs can detect sounds over a wide frequency range and are directionally sensitive (Schildberger, 1988). There is some evidence that the locust can discriminate between different frequencies of sound using the physical properties of the tympanum or Müller's organ (Michelsen, 1973) and this information is preserved in the nervous system.

Crustaceans have not been shown to possess a centralised acoustic receptor to date, although there has been some speculation about the possible role of the statocyst in acoustic detection (Horridge, 1971). The statocyst has a well documented and undisputed role in gravity detection (Cohen, 1960, Takahata and Hisada, 1979) and in addition has been shown to respond to substrate borne vibrations (Cohen, 1955). However, no conclusive information has been produced on the responses of these structures to water borne vibrations. Although their structure has many features in common with the otolith organs of fish, it seems unlikely that they perform the same function (Hawkins and Myrberg, 1983).

The Crustacea use some of the vast diversity of cuticular hairs

located all over their body surface to detect water borne vibrations. The morphology and diversity of some of these receptors has been studied in several decapod species eg. *Homarus* (Derby, 1982), *Nephrops* (Farmer, 1974) and *Austropotamobius* (Thomas, 1970) and their sensory responsiveness has been studied in detail (Taylor, 1975; Tazaki and Ohnishi, 1974; Tautz and Sandeman,1980; Wiese, 1976; Vedel and Clarac, 1976; Ebina and Wiese, 1984). There is a considerable body of data to suggest that these receptors are directionally sensitive and that they are sensitive to water borne vibrations of a particular frequency and amplitude (Wiese, 1976; Tautz and Sandeman, 1984). The responses of vibration sensitive interneurones of the Crustacea, in particular those of the crayfish, have also been the subject of much study (Wiese et al. 1976; Wiese and Wollnick, 1983; Tautz, 1987; Reichert et al. 1982). It seems that, at least in crayfish, the directional and frequency sensitivity of the afferent nerves is preserved in the central nervous system.

In contrast with the vast literature on the sensory responses of the Crustacea to water borne vibrations, there is a paucity of data on their behavioural responses. No studies exist of the motor control of a behavioural response to water borne vibrations. Behavioural responses have been studied in crayfish (Tautz, 1987) and the shrimp, *Crangon* (Heinisch and Wiese, 1988). However, both of these studies have been conducted in the laboratory where neither conditions for the animal nor the stimulus were optimal. Huber (1988) has stressed the need to study the behaviour of an animal in its natural environment if the results are to be meaningful. Removal of an animal from its natural environment may alter the response. Also, the acoustic conditions are very poor in experimental tanks due to the proximity of the air-water interface which acts as a very efficient reflector for the stimulus (Parvelescu, 1964). Under these conditions, unlike those

in the field, the sound pressure may be very small but the corresponding particle displacements very large.

Whether the receptors of crustaceans can be called true acoustic receptors is a matter of some doubt (Hawkins and Myrberg, 1983), because their responses have only been tested in the anomalous acoustic conditions of laboratory aquaria. In addition, their sensitivity appears to be much lower than that of vertebrate auditory systems. The sensory receptors of *Procambarus clarkii* have a sensory threshold of 0.1μm at 100Hz measured as displacement of the hair base, and it is likely that the behavioural thresholds of the response are even higher. In contrast the conditioned response of *Gadus* has a displacement threshold of $0.5 \times 10^{-4}\mu$m at 75Hz measured as displacement of the water particles(Chapman and Hawkins, 1973). Hawkins and Myrberg (1983) conclude that this relative insensitivity of crayfish would prevent them from detecting far field sounds since particle displacement is very low for a given sound pressure in the far field. However, the evidence presented above suggests that the sensory setae of the Crustacea may be very efficient at detecting low frequency near field sound and indeed this may be their primary function.

1.4 **Aims**

This study attempts to determine the response of a decapod crustacean species, the Norway lobster, *Nephrops norvegicus* (L.). to water borne vibrational stimuli. To date there is no information available on the response of *Nephrops* to water borne vibrations at either the sensory, motor or behavioural levels. As well as being of general biological interest this information is relevant to fisheries technologists, since *Nephrops* has been shown to respond to trawl gear

(Newland and Chapman, 1985). The process of trawling generates high sound levels in the water and information on the responsiveness of *Nephrops* to vibrational stimuli may help to improve gear design and fishing technique.

The nervous system of *Nephrops*, like that of other decapod crustaceans, consists of a chain of ganglia joined by paired connectives. Crustaceans have been used as model systems in neurobiology for some time because of the relative lack of complexity of their nervous systems compared with those of vertebrate and especially mammalian systems. Neurobiological research on the Crustacea has therefore advanced to a much more detailed level than similar research on vertebrates allowing for example individual nerve cells to be identified and their roles in neural circuitry to be characterised.

Until recently little was known about the neurobiology of *Nephrops*. Recent major neurobiological studies have focussed on the influence of sensory inputs on motor output systems (Priest, 1983, Neil and Miyan, 1986, Newland and Neil, 1987, Knox and Neil, 1987). The work of Miyan and Neil (1986) has emphasised the similarity between this species and the lobster, *Homarus*, whereas the work of Newland and Neil, (1987) has pointed out important differences between *Nephrops* and the crayfish. All of these studies have stressed the importance of the study of behaviour and the use of intact animals to the understanding of neurobiological mechanisms and this is also true of the present study.

This study has attempted to paint a more complete picture of the response of *Nephrops* to water borne vibrations by studying the response at sensory, interneurone and motor levels and by combining these neurophysiological studies with behavioural studies. A survey of the cuticular setae has been undertaken using the scanning electron

microscope and has allowed comparisons to be made between the receptors of *Nephrops* and those of other species (Chapter 2). The study of the sensory responses of the afferent nerves and interneurones under controlled acoustic conditions has allowed a determination of the frequency responsiveness and directionality of the system (Chapter 3). The study of morphology and sensory responses focussed on the walking legs and uropods but it should be borne in mind that other parts of the animal are also covered with sensory hairs and these may be involved in the responses reported here. Behavioural responses to water borne vibrations have been identified in two motor systems, those for locomotion and abdominal posture, and these have been studied both in the laboratory and the field (Chapters 4 and 5). The nervous control of the postural response was also studied. Behavioural studies are particularly relevant since hearing is a behavioural response. The behavioral studies were designed to allow direct comparisons between the animal's responses in the field and in the laboratory, and allowed the thresholds of the response to be determined in a free sound field.

1.5. ***Nephrops norvegicus*** (L.)

The Norway lobster, *Nephrops norvegicus* (L.), so called because of its kidney shaped eye, is a decapod crustacean belonging to the nephropid family of lobsters (Howard, 1982). The thorax bears one pair of large claws and four pairs of pereiopods or walking legs. The abdomen is divided into 6 segments, four of which bear a pair of pleopods or swimmerets which are used for assisting locomotion, for carrying the eggs in the female and for generating a current of water through the burrow. The tailfan is formed from the paired uropods and telson and is used during righting reactions and, together with the

powerful abdominal musculature, during escape swimming.

Nephrops norvegicus has a wide geographical distribution in the N.E. Atlantic, with populations being found from Iceland to Morocco in the Mediterranean and as far east as Egypt (Howard, 1982). The animals are found at depths of between 5-800m (Holt, pers.comm.; Howard, 1982) on sediments of fine cohesive mud in which they construct burrows. The burrows are generally 20-30cm in length, may have one or several entrances, and often include smaller adjoining burrows in which the juvenile animals live (Rice and Chapman, 1971). The primary function of the burrowing habit in *Nephrops* is to provide protection from predation as the natural habitat does not offer any other form of cover. This is especially true during and after moulting when the cuticle is soft and the animals are particularly vulnerable. The main predators around the coast of Scotland are fish such as cod, rays, dogfish and angler fish (Howard, 1982).

The animals spend the majority of their lives in the burrow, emerging for a short time in a 24 hour period to forage for food. Emergence occurs at an optimum light intensity which varies with depth, season and tidal state (Chapman and Howard, 1979). The time spent outside the burrow is thought to increase as the animal becomes larger, probably reflecting their increased ability to defend themselves against predation and their need for an increased food supply.

Mating occurs just after the female has moulted in the spring and she then carries the spermatophore until the eggs are laid on her pleopods in the autumn. The eggs are carried for around nine months until fully developed. During this time the female rarely emerges from the safety of the burrow. There are three free swimming planktonic larval stages in *Nephrops* (Santucci, 1926; Smith, 1987). Two subsequent metamorphic moults transform the larva into the juvenile

form which is bottom dwelling. The juvenile undergoes no further metamorphoses but progresses directly into the fully adult form through a series of moults. The larvae of *Nephrops* are carnivorous feeding on other larvae in the plankton as well as each other. The adults are omnivorous carnivores and forage actively for their food.

Nephrops is one of the most important shellfish resources in Scotland and landings in 1987 were valued at £32.6 million (Chapman, pers. comm.). Perhaps because of this much of the scientific research on this species has concentrated on its ecology and distribution around the coast of Scotland. Work on the behaviour of *Nephrops* includes studies of their emergence rhythm (Atkinson and Naylor, 1976; Chapman and Howard, 1979) and the modulating role of light. Not surprisingly therefore, considerable attention has recently been given to the structure and functioning of the *Nephrops* eye (Shelton, Gaten and Chapman, 1985; Gaten, 1988). These animals often live in low light conditions and they have a reflecting superposition eye which has increased light gathering ability. Recent research has shown that the retinal pigment in the *Nephrops* eye may be irreversibly bleached by exposure to surface daylight or artificial light. This could have important consequences for the fishery. The main method of fishing for *Nephrops* in Scotland is trawling and animals caught both by this method and by creeling must be of a minimum size before they can be landed. Smaller animals are therefore sorted from the catch and discarded. These animals are often exposed to light at capture and this may render them blind. Because of this, discarded animals may face an increased risk of predation which may reduce yields in the fishing industry. The laboratory studies reported here, unless stated otherwise, have been carried out on animals which have been blinded by continuous exposure to light, some experiments were also conducted

with visually intact animals. In some of the behavioural experiments the use of blind animals obviously removed any interactions with the visual system.

Chapter 2

THE MORPHOLOGY AND DISTRIBUTION OF CUTICULAR SETAE ON THE UROPODS AND WALKING LEGS OF ADULT AND LARVAL *Nephrops*

2.1 INTRODUCTION

Arthropods use mechanical senses for many different purposes, and mechanoreceptors are correspondingly numerous and diverse amongst this phylum. McIver (1975) stated that mechanical stimuli induce more behavioural activities than any other stimulus. Insects use mechanosensory setae to detect air currents both on the ground (Camhi 1985, 1988) and in flight (Guthrie, 1966) and as gravity, position and touch receptors (Martin and Lindhauer, 1966). Touch receptors are found on the proboscis of hematophageous insects which they use to determine when to feed (Rice et al., 1973). Many insects can also detect sound (Rheinlaender and Romer, 1986; Wolf, 1986; Boyan and Fullard, 1986).

The Crustacea possess a vast array of cuticular structures, many of which have been shown to have sensory functions covering a range of modalities (for review see Bush and Laverack, 1982; Debaisieux,1949). Crustaceans also make extensive use of mechanoreception to detect the presence of predators, prey, and con-specifics (Tautz, 1987), but their requirements differ slightly from those of terrestrial insects because of their generally aquatic lifestyles. Many of these cuticular structures in the Crustacea are bimodal and possess nerve endings which respond to both chemical and mechanical stimuli (Derby, 1982; Hatt, 1986). They have been shown to function as gravity receptors in the statocyst (Cohen, 1960), force receptors (Libersat et al. 1987), tactile receptors (Norris and Hartman, 1985), and pressure receptors (Laverack and Barrientos, 1985). Many aquatic crustaceans have receptors, comparable to the air current detectors of insects, which detect hydrodynamic stimuli (Wiese, 1976; Tautz and Sandeman, 1980; Ebina and Wiese,1984; Heinisch and Wiese, 1987; Vedel and Clarac, 1976).

Hydrodynamic stimuli include water currents, turbulence, sound and water borne vibrations the last two of which are the subject of this study. Hairs sensitive to vibrational stimuli may be responding to one of several components of the stimulus such as pressure, particle velocity, acceleration or displacement. Mammals, amphibians, fish and several invertebrate groups such as spiders and insects possess a hearing organ which is specialised to receive vibrational stimuli (Chapter 1.3). Fish also have lateral line organs (Harris and van Bergeijk, 1962) which respond to low frequency water movement. To date no conclusive evidence has been presented for the existence of such an organ in crustaceans, but these animals do use some of the large diversity of cuticular receptors distributed over the surface of the body, claws and walking legs to receive vibrational stimuli. If this is also true for *Nephrops* these animals may also use these receptors to detect not only natural stimuli but also man-made stimuli such as the water flow or sounds produced by trawl gear.

The entire body surface of *Nephrops* is covered in cuticular structures but those on the uropods and walking legs were chosen for study for several reasons. First, the uropods and legs are parts of the animal which are exposed to the environment, and are therefore likely to play a major part in the reception of external stimuli. Secondly, there is a dense concentration of setae on these areas of the body and a huge diversity of structures, especially on the tips of the walking legs. The fact that the uropods and walking legs are extremities means that electrophysiological recordings can be made without the need for complicated dissection, and recordings can be made from intact preparations as well as isolated ones. The large number of sense organs in these areas facilitates the isolation of sensory from motor activity in the recordings and ablations can be carried out with relative ease. The setae on the uropods and walking

legs can be relatively easily identified using the light microscope, which allows the responses of different individual setal types to the same stimulus to be determined. The uropods and walking legs have been studied in other species (Wiese, 1976; Derby, 1982) which allows important comparisons to be made between these species and *Nephrops*.

Hair types have already been classified by Factor (1978). A similar system of classification was used by Farmer (1974) to describe the setae from mouthparts and pereiopods of *Nephrops*. In the majority of cases the same system has been adopted here, but where the classifications of Factor and Farmer seemed inadequate some of the setae described here have been named after those described by Derby (1982). A note has been included in the text to indicate the origins of the classifications used.

The aims of this study were therefore: 1) to identify and describe some of the cuticular structures found on the uropods and walking legs of adult and larval *Nephrops*, to supplement previous work on this species (Farmer, 1974) and other decapod crustaceans (Derby, 1982; Thomas 1970, 1973) and 2) to allow the mechanical sensitivity and possible functions of the receptors to be tested physiologically as a prelude to studies of the mechano-sensory systems and motor behaviour of *Nephrops* in relation to vibrational stimuli (Chapters 3-6).

2.2 MATERIALS AND METHODS

2.2.1 Supply and Maintenance of animals

All experiments were carried out on adult male specimens of *Nephrops norvegicus* L. unless otherwise stated. These were obtained from the Universities Marine Biological Station at Millport, Isle of Cumbrae and maintained in a circulating seawater aquarium prior to use.

2.2.2 Methylene blue staining

The leg tips and uropods were removed from the animals and washed in filtered seawater before being stained in a 0.5% solution of methylene blue and filtered seawater for about 2 minutes. The methylene blue was then rinsed off leaving the cuticular setae stained blue. The setae were viewed using a Wild Type 126 269 microscope and photographed using the Wild NPS 51 camera attachment.

2.2.3 Scanning Electron Microscopy

The leg tips and uropods of adult and larval *Nephrops* were studied using scanning electron microscopy. Stage 1-3 larvae (L1-L3) were obtained from the Clyde Estuary at Millport using fine and medium mesh plankton nets and stage 1 post larvae (PL1) which had been fixed in formal saline were kindly donated by Dr R.J.A. Atkinson and Dr.R. Smith (U.M.B.S., Millport). The uropods and legs were removed from the adult *Nephrops* and washed in several changes of filtered seawater in an ultrasonic shaker (Polaron) to remove any debris. The leg tips were then separated from the rest of the body at the merus-carpus (M-C) joint. The larvae were washed by agitating them in filtered seawater as they were too fragile to be washed in the shaker. The specimens were then fixed as follows.

1.**Fixation**

Sodium cacodylate/sea water fixative

1% Glutaraldehyde for 1 hour at 4 ^{o} c

2.**Buffer Rinse**

0.1M Sodium cacodylate/sea water buffer

2-3 changes at 10 minutes each

3.**Post fixation**

2% osmium in sea water buffer

1 hour at 20 ^{o} c

4. **Buffer Rinse**

as 2.

5.**Dehydration**

Ethanol series

30%,50%,70%,90%,100%,100%

10 minutes in each

6.**Critical point drying**

1 hour 15 minutes

Flushing out every 15 minutes

7.**Mounting and Gold coating**

The specimens were taken from the critical point dryer and mounted on aluminium stubs using double sided tape. Quick drying silver paint was used to mount smaller specimens and was painted around the edges of all the stubs to help prevent charging while in the microscope. The mounted specimens were then coated with gold in a Polaron SEM coating unit (E5000) for 6 minutes. The specimens were viewed on a Phillips 500 SEM and photographed with the camera attachment using Ilford FP4 125 ASA film.

2.2.4. **Sensory Recordings**

En passent recordings were made from Root 2 and Root 3 which supply the uropod exopod and endopod respectively and the nerves supplying the walking legs. The preparations (described more fully in sections 3.2.2.a and b) were pinned out in a dish of saline.

Recordings were made using a suction electrode made from Portex tubing, the electrode was mounted on a Narashige micromanipulator. The signal was preamplified using an Isleworth A101 preamplifier and filtered by a Neurolog NL125 Filter before being viewed on a Tektronix 5115 Storage Oscilloscope and recorded on a Racal Store 4 tape recorder.

The preparations were stimulated with either tactile, hydrodynamic or vibrating water stimuli. Tactile stimuli were generated using either a fine paintbrush or a fine mounted insect pin which was bent at the end. Hydrodynamic stimuli were generated by water jets from a Pasteur pipette. Vibrating water stimuli were generated by a plastic ball attached by a metal rod to a Derritron vibrator. The ball was placed in the bath of saline close to but not touching the preparation.

2.3 RESULTS

2.3.1 Morphology of the uropods of *Nephrops* and distribution of cuticular setae on their surface

The uropods of *Nephrops* are biramous appendages comprising an endopod and an exopod which are similar in general outline. Together with the post-segmental telson these appendages form the tailfan.

The exopod (Fig. 2.1.A) is divided into two sections by an articulation which runs transversely across the blade, separating it into a large proximal portion and a smaller distal portion. The proximal portion bears internally two areas of muscle attachment, which have few setae. Three clearly defined fields of cuticular setae surround the muscle insertions. Along the outer edge of the proximal part of the uropod are several short cuticular spines which overlap the distal portion. Between these spines are bunches of setae. The dorsal surface of the distal uropod bears few setae which radiate out from the area of muscle attachment at its midline. The medial edge of the proximal portion and the entire edge of the distal portion have a dense fringe of several hair types, the largest of which has thick spines and side branches. Other setae are situated between these large setae, increasing the density of the fringe.

There are fewer setae on the ventral side of the exopod (Fig 2.1.B). Along the lateral edge of the proximal section is a fringe of setae which seems to be a medially pointing continuation of the laterally pointing fringe around the rest of the uropod. There are two sparse fields of setae on the ventral surface, one at the lateral edge and one in the midline, situated around the edge of the muscle articulation.

The endopod is undivided (Fig. 2.1.C), and has only one area of muscle attachment running vertically down its midline. This area bears

few setae but surrounding it are two fields of setae, a large lateral field and a smaller medial one. 5-6 very long setae project from the proximal lateral border of the endopod. There is a distal fringe of setae similar to that seen on the exopod. The ventral surface of the endopod is devoid of setae and bears only some small pore-like structures.

2.3.1.a **Scanning Electron Microscope studies of the uropods.**

The fields of setae which cover much of the dorsal surface of the exopod and endopod consist of many small **plumose setae** (Fig 2.2.A). Each seta arises from a base in a slight depression in the cuticle, the shaft tapers towards the tip and thin side branches project starting about 1/5 of the way up the shaft to the tip. Within each field all are generally orientated in terms of a line drawn from base to tip in a similar direction.

Hair fan organs are found in relatively large depressions in the cuticle on the lateral edge of the dorsal surface of the exopod and the ventral sides of both the exopod and the endopod. They have a thick shaft with many long projections originating from it close to the base which form a thick bunch of hairs.

Along the lateral border of the dorsal surface of the endopod are 5-6 long simple setae (Fig. 2.2.B) each of which is flanked by two smaller guard hairs of similar simple form (Fig. 2.2.C). The base of the seta has a hinge-like structure and its shaft bears no secondary structures or projections.

The distal fringes around the exopod and endopod, which are identical on both rami, comprise several setal types, but there are slight differences between the dorsal and ventral sides. On both sides the most predominant setae are long plumose setae which have thick

shafts with closely spaced side projections of a ribbon-like form along their length (Fig. 2.3.A). The setal shaft is smooth near the base but distally it becomes ribbed. Between these setae on the dorsal side are thin plumose setae (Fig 2.3.B). Both of the latter plumose types have sleeve-like structures around their bases. On the ventral side between each of the large plumose setae is a small bunch of 3-4 smaller plumose setae (Fig. 2.3.C).

2.3.2 The Morphology of the tips of the walking legs of *Nephrops* and distribution of cuticular structures on their surface

The 2nd and 3rd legs are similar in structure, but differ from the 4th and 5th legs. The former (Fig. 2.4.a) are sub-chelate, the propodus and dactyl forming a small claw or chela.

Cuticular setae were present on the 2nd and 3rd legs in three main areas (Fig 2.4.A). The setae along the lateral edges of the propodus and dactyl form a border which projects outwards from the leg and consists of one or two rows of simple setae. The setae are longer at the proximal end of the leg, tapering towards its tip. Small, tooth-like projections line the inner edges of the propodus and dactyl, a single row of which forms a continuous border extending as far as the distal tip of each segment. Hairs on the flat surfaces of the propodus and dactyl are located in discrete bunches or tufts at intervals along the two segments. Each bunch, consisting of about 20 setae, is contained within a slight depression in the cuticle. There are several (8-12) bunches of setae on the propodus and a similar number on the dactyl. Most of the bunches are located on the claw part of the propodus, but a few are also found more proximally.

The 4th and 5th legs are similar in structure but are not chelate. The dactly is long, and tetrahedral in shape (Fig. 2.4.B) and the propodus bears most of the cuticular setae of this leg on its

distal portion. Both the 4th and 5th legs bear the majority of cuticular setae on their posterior face. The pattern of setal distribution on these legs varies between the propodus and the dactyl. Consequently these two segments will be dealt with separately

The setae on the dactyl are arranged in three bands, 2-3 setae wide, which extend to the distal tip on each side of the dactyl. A small ridge of cuticle is present at the inner proximal end of the segment close to the propodus-dactyl articulation. On top of this ridge is a dense band of setae and below it a small field of setae.

Most of the setae on the propodus overlap the P-D joint. Bunches of setae are distributed further up the leg, mostly on the posterior side, but these do not generally extend into the proximal half of the segment. A dense bunch of **serrate setae** overlaps the P-D joint lying over the ridge of setae on the dactyl. Bunches of setae are also seen around the rest of the distal end of this segment.

2.3.2.a Scanning electron microscope studies of the setae on the 2nd and 3rd legs

The structure of the three previously described groups of setae was examined using the scanning electron microscope. Figure 2.5.A shows a low magnification view of the tip of the 2nd leg.

The setae on the flat surfaces of the propodus and dactyl are **squamous setae** (Fig. 2.5.B). The setal base is found within a depression in the cuticle and the seta is smooth from the base to about half way up the shaft, at which point there is an annulus. Above this annulus the hair shaft bears scales on one side which extend to the tip. (Fig. 2.6.B). These setae have a longitudinal ridge on the opposite side of the shaft which is otherwise smooth. A few setae located on the lateral edges of the bunches are orientated the other

way round.

The setae around the lateral border of the propodus and dactyl are also squamous setae (Fig. 2.5.A) like those on the flat surface of the propodus and dactyl.

The structures which line the medial edges of the propodus and dactyl appear under low power to be solid conical pegs (Fig. 2.5.B) However, under higher magnification it becomes evident that they have short blunt spines or filaments extending from one side (Fig. 2.6.A) These spines point towards the distal tip of the peg and lie flattened against its side. This type of hair is called a **hedgehog hair** (Derby, 1982).

2.3.2.b **Scanning electron microscope studies of the 4th and 5th legs**

Under the SEM a large variety of cuticular organs was visible on the 5th leg. Figure 2.7.A shows a low magnification view of the tip of the leg. The long setae on the dactyl were often broken and worn. They were present in three bands, although the bands were not always continuous. These were **simple setae**. The rest of this article was devoid of setae except for the ridge close to the P-D joint where a variety of small projections was found (Fig. 2.7.B) Lying to one side of the ridge was a row of blunt-ending peg-like structures. Below these on the slope of the ridge were a number of pointed projections which rested within small depressions in the cuticle in groups of 1-3 setae. These organs extended proximally under the overlapping setae projecting downwards from the propodus.

The largest bunch of setae projecting from the propodus and overlying the cuticular ridge of the dactyl were **serrate setae with simple scales**. The serrations were very large and generally projected in two rows from the medial side of the setae, that is, the side

facing the cuticle. The other side of the seta had triangular scales projecting from it (Fig. 2.7.C). The smaller setal bunches around the distal edge of the propodus and those found further up the propodus were squamous setae very like those found on the 2nd/3rd legs (Fig.2.7.D). Cuticular organs similar to the hedgehog hairs were found forming a border around part of the propodus and pointing down towards the dactyl (Fig. 2.7.B).

2.3.3. Morphology of the larval and post-larval (PL1) legs and uropods

The larval stages (L1-L3) and post larval (PL1) legs and uropods were examined using the scanning electron microscope so that the development of the appendages and their setae could be studied and compared with those already described in the adult (Sections 2.3.1 and 2.3.2). The L1-L3 larval legs are all biramous appendages having an endopod which develops into the walking leg or claw and an exopod which is lost in the partial metamorphosis from L3 to PL1. Stage L1 and L2 larvae do not have uropods but have a long forked caudal spine or telson which bears a fringe of setae around its distal end. The uropods develop at L3 on either side of this structure. The two stage metamorphosis which occurs from L3 across PL1 to PL2 changes the larva into a form which closely resembles the adult. The uropods and claws become fully differentiated and the uropods and the telson fully formed. At the subsequent moults the full adult characteristics are expressed.

2.3.3.a Stage L1

At L1 the larval claw is the largest of the legs (Fig. 2.8.A), with a large chela having long pointed tips on the propodus and

dactyl. These tips bear conical hairs on their surface which are orientated towards the distal tip of the leg. Long feather-like setae are present on the exopods of all the thoracic limbs, but the endopod or leg bears very few setae compared with the adult. The claw bears two types of structures: **Hedgehog-type hairs** on the inside of the chela at regular intervals and a few short setae on the flat surfaces of the chela (Fig. 2.8.B).

Leg 2 and leg 3 are very similar to each other. Their endopods are similar to that of the claw and have a chelate form (Fig. 2.8.C). These legs bear very few setae, most of which are concentrated on the propodus and dactyl. The relative length of the setae is greater than that of the adult setae and their structure is different. These setae are all **serrate setae** and have two rows of serrations which start about 1/5th of the way up the shaft and extend to the tip.

Leg 4 and leg 5 are similar in structure to each other, and both bear similar distributions of setae and setal types (Fig. 2.9.A). The dactyl of leg 4 has several setae projecting distally from its surface, which are identical in structure to those found on the other legs (Fig. 2.9.B). Leg 5 has fewer setae on the dactyl. Several long setae can be seen around the distal tip of the propodus on legs 4 and 5 which overlap the P-D joint.

The L2 larva is very similar in structure to L1 and so will not be described in detail here.

2.3.3.b **Stage L3**

The uropods of L3 are biramous appendages with an exopod and an endopod, and unlike the legs remain in this form throughout their adult life. The caudal spine is still present in much the same form seen in the previous two larval stages. The uropods have a distal fringe of setae which consists of long **plumose setae.** This border is

less dense than that found in post larval and adult stages. The dorsal surface of the uropod exopod has 5-6 setae along its lateral edge (Fig. 2.10.A) but is otherwise devoid of cuticular structures as are the dorsal surface of the exopod and the ventral surfaces of both the exopod and the endopod (Fig. 2.10.B).

The legs at L3 are very similar to those seen in the previous two stages, having a similar distribution of setae of the same types as previously described.

2.3.3.c **Stage post larval one (PL1)**

On a gross level PL1 looks very like the adult, certain larval tendancies such as the exopods of the legs and the caudal spine having been lost during metamorphosis. The structures on the walking legs at this stage are intermediate between those seen in the larval forms and those seen in the adult. On legs 2 and 3 (Fig 2.11.A) long setae form a border on the outside edges of the propodus and dactyl. These are relatively long compared with those of the adult but they retain their larval serrate structure. On the flat surfaces of the propodus and dactyl are small bunches of 1-5 setae. On the inner edges of the propodus and dactyl are small conical projections; these are widely spaced but presumably will develop into the hedgehog hairs seen in the adult.

Leg 5 of PL1 again appears to be intermediate in structure between the adult and larval forms. There are fewer setae than in the adult, but some order of distribution is seen at this stage. Most of the setae are situated on the propodus and overlap the PD joint. These setae appear to be distributed in groups with a dense group of long setae found on one side of the propodus and a couple of smaller bunches seen more proximally on the propodus. The dactyl has a few

setae projecting from it which appear to be situated in at least two rows running along this segment of the leg.

The telson is now fully developed and the exopod of the uropod is divided into two sections by a joint at its distal end (Fig. 2.11.B). The uropods have a dense fringe running around their distal ends and extending part of the way up their lateral and medial edges. This fringe consists of two setal types: 1) Plumose setae with thick shafts and many side projections which project caudally at an angle of approximately 45^{o} and 2) thin setae situated between and dorsally to the plumose setae, the fine structure of which was not clear. The dorsal surfaces of the uropods bear a few more cuticular structures than those of stage L3. The setae on the lateral edge of the endopod are still present.

2.3.4. Sensory responses of setae groups on the uropods and walking legs of *Nephrops*.

2.3.4.a. The uropods: general observations

The uropod setae were generally very sensitive to mechanical stimulation with all areas, incuding the distal border and the areas of muscle insertion which bore very few setae, producing responses. Figure 2.12 shows a typical example of a multi-unit record. Units can be seen which show clear responses to stimulation with a paintbrush of different areas of the uropod exopod. Units on the uropods were also very responsive to water jets directed onto the fields of plumose setae from the lateral, medial, rostral and caudal directions.

The uropod setae were also responsive to stimulation with a vibrating ball placed close to, but not touching them. One or more spikes per cycle phase locked with the stimulus wave were produced at low frequencies. Responses to stimuli at different frequencies were

subsequently analysed in greater detail (Chapter 3).

2.3.4.b. **Stimulation of identified setae types on the uropods**

Plumose setae (field setae type)

It was impossible to stimulate an identified seta within one of the major setal fields without stimulating some of the others around it. In order to demonstrate the response of single plumose field setae it was therefore necessary to choose ones which lay on the edge of the uropods outwith the major setal field. Figure 2.13. shows the position of one such seta on the right endopod which showed a low level of background activity at rest and was sensitive to movement in all 4 directions. The responses to stimulation in the lateral and medial directions were very similar: a short burst of 3-6 spikes was fired as the probe contacted the seta and throughout the movement. The spikes ceased when the seta reached its new position. Another short burst of spikes was fired as the seta was released and returned to its original position. Thus, in the lateral and medial directions, the setal units were only sensitive to the movement and were not able to monitor the position of the seta.

The setal units were generally much more sensitive to movement along the rostro-caudal axis of the uropod. An intense burst of spikes was fired at the onset of the movement and firing continued until the seta was released and returned to its original position. The units seemed especially sensitive when the seta was moved in the rostral direction, having a high rate of firing even when the movement was carried out very slowly. Increased sensitivity in the rostral/caudal directions would be in accord with the orientation of these setae on the tailfan.

These units also responded well to vibratory stimuli between 10-20Hz, the strongest response being at 12Hz where one spike per cycle

occurred.

Guard hairs

It was possible on occasion to stimulate guard hairs on the uropods individually without moving the neighbouring simple setae. These hairs showed a clear mechanosensory response (Fig 2.14). In this case there was quite a high level of background firing of the responsive units. The larger units responded only to movement, producing bursts of spikes as the hair was moved. The smaller unit responded to both position and movement preferentially in the rostral direction, with caudal movement producing some inhibition of firing.

2.3.4.c Sensory responses of identified hair types on the walking legs of *Nephrops*

Leg 2

A typical example of the response of a bunch of squamous setae on the dactyl of leg 2 to movement in 4 directions can be seen in Figure 2.15. The neurones responded well to stimulation in all 4 directions, producing a burst of spikes which continued to fire throughout the period of contact with the setae. The spikes showed a sharp cut off in firing when the stimulus stopped. Units of different size responded to stimulation in different directions. A small unit responded as described above when the setae were stimulated in all 4 directions. However, in addition, when the setae were pushed in the lateral and proximal directions larger spikes also appeared and fired throughout the stimulus period. It is possible that this may represent the same nerve cell which fired in response to both proximal and lateral movement or it may be that the firing was the response of 2 cells which had spikes of approximately the same size. This may also be true of the small unit. The larger spikes did not fire in the

medial and distal directions when only the smaller spikes were seen.

Figure 2.16 shows an example of the responses of units from both the propodus and the dactyl of Leg 2 to stimulation of the squamous setae on their outside edges. In both cases the units responded to only one direction of movement, from distal to proximal, remaining silent when the setae were moved in the opposite direction. Figure 2.17 shows an example where two different populations of units responded to opposite directions of movement: larger units to proximal movement and smaller units to distal movement. Thus, it would appear that there were two unidirectional populations of units responsive to proximal and distal movement respectively.

The hedgehog hairs on the inside of the chela were also mechanically sensitive although it was very difficult to stimulate these hairs in isolation as they were very stiff.

Leg 4

The setae on the dactyl were stimulated with a water jet directed distally, proximally and medio-laterally (Fig. 2.18). The setae responded to stimulation in all directions tested with high frequency bursts of large and small spikes.

Stimulation of the setal bunches on the propodus produced high frequency bursts of mixed populations of spikes to movement in all directions. The setae overlapping the PD joint were also responsive in a similar manner. It appeared therefore that all major groups of setae on legs 4 and 5 were mechanosensory and responded to directed water movement.

2.4 DISCUSSION

This study has highlighted the structure, position, variety and density of the cuticular organs on the uropods and walking legs of the adult and larval *Nephrops*. Although some authors have suggested that functions can be assigned to receptors through an in-depth study of their morphology (Thomas, 1970), it now seems generally accepted that the sensory functions and characteristics of receptors cannot be determined without studying the physiological responses of the sensory receptors. Laverack and Barrientos (1985) have stated that caution should be exercised when linking structure and function. Early studies of cuticular receptors concentrated on morphological aspects, from which sensory function was inferred (Farmer, 1974; Factor, 1978; Thomas, 1970). More recent studies (Wiese, 1976; Altner et al. 1986, Hatt, 1986) have attempted to link structure with function more directly and the present study adopts a similar approach in the case of *Nephrops*.

2.4.1 The uropods

The larger cuticular organs on the uropods of *Nephrops* fall into two categories. The majority are **plumose setae** (Factor, 1978) of which there were 4 types, three forming the dense border around the lateral edge (Fig. 2.3.B and C) and one covering most of the dorsal surface of each uropod (Fig. 2.1). Short plumose setae like those found on the dorsal surfaces of the uropods are likely to be the most suitable to function as low frequency mechanoreceptors (Tautz, 1979). The remaining setae are **simple setae**. Five to six setae of this type were seen along the lateral border of the endopod and each of these had two guard setae which were also simple setae. According to Ebina and Tautz (1984) simple setae are less likely to respond to low frequency water

movements as they do not couple to the water as well as plumose setae. Other smaller organs similar to those described as hair pegs and hair fans in the lobster (Laverack, 1962 a & b) were also seen but these were not studied in detail. The variety of setae encountered on the uropods of *Nephrops* has also been found on the uropods of the crayfish *Austropotomobius* (Thomas, 1970) and on the telson of *Procambarus clarkii* (Wiese, 1976) but they have not previously been studied in detail in other species or in *Nephrops*.

This study has shown that all of the uropod setal types in *Nephrops* are mechanosensory (in 2.3.4) responding both to tactile mechanical stimuli (Fig. 2.12) and to water borne stimuli of different frequencies. The plumose setae of the dorsal surface of the uropods and the guard hairs in addition show directional sensitivity along the long axis of the uropod blade (Figs. 2.13 and 14). Directional sensitivity has not been conclusively shown here for other individual setal types. All of the setae tested physiologically appeared to be innervated and this agrees with Laverack's statement (1988) that the majority of surface structures in the Crustacea are innervated and so must have some function in conveying information to the animal about its environment. Although no structures were found which were definitely not innervated, such structures have been found in other species (Bender et al. 1984) and it is possible that they may also exist in *Nephrops*.

It is unlikely that any of the setal types on the uropods of *Nephrops* will be chemosensory simply because of their position on the animal. The uropods are used primarily for steering during escape swimming and righting reactions and have never been shown to have a role in feeding or the detection of food. Derby and Atema (1982) saw no behavioural response indicative of food detection when food was touched by the telson or uropods of *Homarus*.

2.4.2 The lateral border of the uropod

The large plumose setae which form a border around the lateral edge of the uropod articulate in a sleeve-like structure which does not seem to offer a great deal of flexibility of movement at the base (Fig. 2.3.B). Mechanoreceptors generally have either a hinge or a ball and socket at their base and articulate in a flexible membrane in a depression in the cuticle (Laverack and Barrientos, 1985). These articulations allow the seta to be moved by the stimulus and cause excitation of the underlying nerve cells (Chapter 1.3). Although the long plumose setae were physiologically responsive to mechanical stimulation it seems unlikely that they will be sensitive to the small displacements produced by water borne vibrations because of the restraints which their articulation imposes on them.

It is possible that these setae are primarily mechanical in function, with any sensory functions being secondary to this. These setae may be used to increase the surface area of the *Nephrops* uropod to make the structure a more effective paddle. Maximisation of surface area by means of setae is common in other species such as *Austropotamobius* (Thomas, 1970) and in other body structures such as the pleopods and maxillipeds (Factor, 1978). It should be borne in mind however, that these setae have receptors which are sensitive to mechanical stimulation and must therefore have a sensory function. It is possible that they are sensitive to greater forces such as the water currents produced during escape swimming or the currents generated around the animal as it falls through the water column after a tailflip. This may give the animal some additional information to that generated by the statocysts about its position and orientation. Another possible function of such setae could be to tell the animal whether or not its tailfan is touching the substrate. *Nephrops* tend to

stand at rest with the abdomen flexed and with these large plumose setae just touching the substrate.

2.4.3.**The tailfan as a sensory array**

The other cuticular receptors on the uropods are sensitive to both touch and water borne vibrational stimuli. It is possible that these setae may collectively form a sensory array or raster (Schone, 1984) and thus the tailfan may act as an adjustable omnidirectional sensor in two planes. The tailfan may either be held vertically as it is when the animal is standing, or horizontally as it is when the animal is walking either forwards or backwards or turning. Superimposed on this range of movement the uropods are themselves independently mobile; the blades can open and close, either symetrically or asymetrically (Newland and Neil, 1987). When fully open the tailfan spans an angle of 160^{o} and when fully closed this angle is reduced to 40^{o}. Consider the orientations of the sensory setae and their axes of maximum sensitivity: the field setae are predominantly sensitive to stimulation along the longitudinal axis of the uropod with reduced sensitivity in the medio-lateral directions, the guard hairs are sensitive only to movement along the long axis. If the tailfan is held horizontal ly the guard hairs and plumose setae could be stimulated by water movement. The orientations of the setae and the broad span of the fully-open uropod blades would allow the animal the possibility of receiving stimuli in the horizontal plane from a 160^{o} arc both rostrally and caudally.

The shorter plumose setae of the lateral border are sensitive to movement in the dorso-ventral axis. If the abdomen is extended this gives a sensitivity in the vertical plane, and if the tailfan is flexed the resulting direction of sensitivity is in the horizontal

plane. The positioning and directional sensitivity of these setae may therefore provide a second sensory raster in the vertical plane perpendicular to the first.

Nephrops have been shown to orientate to water currents in the field and preferentially face downstream (Newland et al., 1988). This reduces drag over the body surface. Under these conditions all of the receptors in the operating plane of the raster could be constantly stimulated at a low level by water current flow. If we consider a stimulus from a particular direction acting on an extended tailfan, it will maximally stimulate those receptors which have a corresponding polarisation plane while other receptors with different orientations will be stimulated to a lesser degree or perhaps not at all. The question then arises, can the animal use this information to abstract the direction of the stimulus? A general principle of sensory systems, found in both vertebrates (Woolsey and van der Loos, 1970) and invertebrates (Jacobs and Miller, 1988), which might make this possible is the representation of certain properties of the sensory stimulus in a **computational map** (Jacobs and Miller, 1988). A **map** is defined as "an array of neurones through which there is a systematic variation in the tuning of neighbouring neurones for a particular parameter" (Jacobs and Miller, 1988). A **computational map** is one in which "the representation of sensory information is transformed into a place-coded probability distribution that represents the computed values of parameters by sites of maximum relative activity" (Knudsen et al., 1987). Examples of such maps are found in the vertebrate visual system (Hubel and Wiesel, 1963). If the array of mechanosensors on the *Nephrops* uropod represents such a sensory array biased stimulation in one set of sensory neurones may be passed on to a corresponding central map represented by line-labelled interneurones. Indeed it has been found that interneurones with precise receptive

fields do exist and receive inputs from particular sensory receptors (Chapter 3). Non-relevant information may be filtered out by central integration.

Using this system of sensory arrays the animals would also be able to process information from moving stimuli. When the stimulus moves a different population of receptors would be stimulated and the animal would be able to perceive the change in direction. Lateral inhibition would enhance this process and indeed Mittlestadt (1973) states that a process of contrast enhancement by lateral inhibition may be essential in such a map. Such a mechanism was proposed by Wiese (1984) for the crayfish water vibration detection system. However, the uropods themselves are mobile appendages and the net effect of movement of the uropods during a stimulus would be to make it seem as if the stimulus itself had moved. It is possible that the animal could use a system of proprioceptors at the uropod articulations to inform it that the uropod has changed its position, and this would help to resolve movement of the uropods from movement of the stimulus. A similar system is used by the rock lobster (Schone and Schone, 1967). These animals have very flexible antennules and when these move the statocyst at its base is stimulated. However, proprioceptive elastic strands at the base of each antennule provide the necessary additional information to differentiate between movement of the antennule and movement of the animal.

Whether or not *Nephrops* would use the potentially detailed source of information from the uropod array to influence motor output or behaviour is uncertain, and would depend on many other factors such as inputs to other mechanosensors on the body surface and to other sensory modalities such as vision. There are already examples in the Crustacea of complex sensory inputs which produce both correspondingly

complex graded responses (Schone, 1984) and very simple on/off responses (Newland and Neil, 1987). Even though the input system (the statocyst) is very complex much of the information seems to be lost during central integration. It is possible that the sense organs on the uropods of *Nephrops* may fall into either of these two categories and the motor output in response to vibrational stimuli has been studied in later chapters (Chapters 4 and 5).

2.4.4 **The walking legs**

This study has described the structure and diversity of the cuticular receptors on the propodus and dactyl of the 2nd-5th legs of *Nephrops* . There are 3 populations of setae on the 2nd and 3rd legs which are similarly distributed on the propodus and dactyl: the **hedgehog hairs** (Derby, 1982), the lateral **squamous setae** (Derby, 1982) and the bunches of **squamous setae** (Fig. 2.5.A). The setae on the 4th and 5th legs differ between the propodus and dactyl. **Simple setae** are present on the dactyl in three rows (Fig. 2.7.A) and close to the joint between the propodus and dactyl are **hedgehog hairs** and **CAP sensillae**-like structures (Laverack, 1978), (Fig. 2.7.B). The propodus has a dense bunch of **serrate setae with simple scales** (Factor, 1978) a few smaller bunches of **squamous setae** which overlap the propodus dactyl joint and some **hedgehog hairs** which are also found close to the joint.

There are several accounts of the setae on the walking legs and claws of other crustacean species (Bauer, 1981; Derby, 1982; Tautz and Sandeman, 1980; Norris and Hartman, 1985; Thomas, 1970,) and the setae on the walking legs of *Nephrops* were first described by Farmer (1974). This study has generally confirmed Farmer's findings but with one exception. He described the setae on the lateral edges of the propodus and dactyl of the 2nd and 3rd walking legs as **simple setae** but this

study has shown that these setae are **squamous setae** (Fig. 2.5.A) as described by Derby (1982) in the lobster *Homarus americanus*. The setae of *Nephrops* were generally very similar in their diversity and distribution to those of *Homarus* (Derby, 1982).

2.4.5. Setal Functions

1) Non-sensory

The setae on the tips of the walking legs are generally more diverse than those on the uropods but plumose setae are notably absent and there are very few simple setae. Possible non-sensory functions for some of the setae on the walking legs have been suggested by Farmer (1974) for *Nephrops* and by Derby (1982) for the lobster and Bauer (1981) for crayfish and other species.

Farmer (1974) suggested that some of the setae on the tips of the walking legs may serve a mechanical function in preventing the animal from sinking into the very soft mud substrate on which it lives. The simple setae on the 4th and 5th legs and the lateral squamous setae on the outside edges of the propodus and dactyl on legs 2 and 3 seem the most likely to fulfill this purpose.

Nephrops and other decapod crustacean species spend a large part of their time cleaning and grooming the body surface and several authors have suggested that some of the setae may be used to remove detritus particles from the body surface and in the case of the female to clean the eggs (Derby, 1982; Farmer, 1974; Bauer, 1981). Derby (1982) and Bauer (1981) suggest that the serrate setae are the most likely to fulfill this role.

2) Sensory

On the basis of their morphology Farmer (1974) suggested sensory

functions for several of the setae of *Nephrops*. However, the physiological recordings made in the present study represent the first attempt to assign sensory functions to the setae.

Chemoreceptors

One of the main functions of the walking legs of *Nephrops* is to locate and handle food. The animals are omnivorous and they and other crustacean species probe the substrate with their walking legs while searching for food (Bailey, pers. comm.; Derby and Atema, 1982). It is therefore likely that some of the cuticular organs on the walking legs of *Nephrops* will be chemosensory and used for food detection (Farmer, 1974). Although chemosensitivity has not been specifically tested in this study, *Nephrops* possesses organs which are morphologically similar to those which have been shown to be chemosensitive in other species eg. the bunches of squamous setae on the 2nd and 3rd legs (Hatt, 1986; Derby, 1982; Altner et al., 1983), hedgehog hairs (Shelton and Laverack, 1970) and the serrate setae on the 4th and 5th legs (Derby, 1982). Derby and Atema (1982) showed in the lobster that the change from searching to grasping food relied on stimulation of the sensors on the propodus and dactyl of the walking legs and it is likely that similar hairs on *Nephrops* will serve the same purpose.

Mechanoreceptors

All of the hair types on the walking legs of *Nephrops* were found to be sensitive to mechanical stimuli, (section 2.3.5). On the 2nd and 3rd legs, the squamous setal bunches responded to stimulation in 4 directions (Fig. 2.15) with populations of spikes of two different sizes suggesting that movement in different directions was coded by different nerve cells. The lateral squamous setae responded only in the distal and proximal directions with responses in these directions

again being coded by units of different spike amplitude. This is in accord with Wiese (1976) who found that 2 mechanosensory cells innervated each mechanoreceptive seta on the telson of the crayfish each of which coded for a different direction of movement. It is generally thought that most of the mechanosensory sensillae are innervated by two sensory cells (Laverack, 1987) but in the case of the setae on the walking legs, where many are likely to be chemosensory as well, the number of neurones is likely to be much greater. Chemosensory setae are generally innervated by a much greater number of neurones (Laverack, 1987).

According to Laverack, (1987) the hedgehog hairs may be innervated by up to 8 cells in the lobster, *H. gammarus*. In the present study these hairs were difficult to stimulate and it was not possible to record any response differences on stimulation in different directions. Derby (1982) stated that these hairs responded only to touch and bending. The hairs are stiff, and are probably stimulated both mechanically and chemically when the animal is feeding. Their position on the inside of the chela is highly suggestive of this. Of the setae on the 4th and 5th legs those on the dactyl showed the most vigorous responses to mechanical stimulation (Fig. 2.18) responding to stimulation in the 4 directions tested but with greater sensitivity in the proximal direction. The setae on the propodus of the 4th and 5th walking legs, although responsive to mechanical stimulation, seemed to be omnidirectional and may be less strongly involved in the process of mechanoreception than the other receptors on these legs. These setae, especially the serrate setae, are involved in grooming (Bauer, 1981) and a non-directional mechanical sensitivity might help the animals to locate particles of detritus on the body surface.

Studies on other crustacean species have shown that many of the setae on the walking legs are mechanosensory (Derby, 1982; Hatt, 1986; Libersat et al., 1987). Derby (1982) showed that both the hedgehog hairs and the squamous setal bunches on the 2nd and 3rd legs of the lobster responded to mechanical stimulation either by direct touch or by water borne stimulation, as did both the serrate setae and the smooth setae on the 4th and 5th legs. There is a wealth of literature which proposes that the neurones innervating some of these hair groups are unimodal and only respond to mechanical stimuli (see Hatt and Bauer, 1980). Other authors suggest that some only respond to chemical stimuli (Shelton and Laverack, 1968). Derby (1982) proposes that many of these hair types, with the exception of hair peg organs, are bimodal in function having separate chemosensitive and mechanosensitive neurones at their base. It is possible that this may also be true for *Nephrops*. Later studies by Hatt (1986) have shown that bunches of squamous setae on the walking legs of the crayfish may be innervated by neurones which are themselves bimodal and extremely sensitive to both modalities. It remains to be shown whether this will also be true for *Nephrops*.

Mechanosensitivity on the legs of *Nephrops* may be used in the same way as has been proposed for the tailfan, with setae on the walking legs acting as a complex array of sensory receptors capable of receiving water borne vibrational information from different directions. As the tips of the walking legs will often be in the substrate they may also receive substrate borne vibrations. However, they are not as conveniently located as the tailfan receptors for this purpose and do not form an organised array pattern. It is likely that they have additional mechanoreceptive functions. Tactile stimulation may be an important function of these receptors in both the location of food and grooming.

2.4.6. **The larvae (L1-L3) and post larvae (PL1)**

The setal structures on the uropods and walking legs of the stage 1-3 larvae and the stage 1 post-larva of *Nephrops* were described in this study. The gross morphology of the larvae of *Nephrops* was first described by Santucci (1926) and has since been extended by Farmer (1975) and Smith (1987). These studies provide useful descriptions of the larval life history and general morphology.

The stage 1-3 larvae are pelagic. Stages 1 and 2 have no uropods; these appear at stage 3 and this is the only significant change in appearance between the larval stages. The formation of a tailfan in L3 is a classic decapod feature (Williamson, 1982) and it is thought to have evolved to allow the animal some mechanism of rapid reversal of the direction of travel generally called the tailflip. Neil et al. (1976) noticed that in *Homarus gammarus* there was an improvement in mobility at L3 which coincided with the appearance of the uropods. The uropods increase the surface area of the tail and provide an effective paddle to steer the animal during tailflipping and righting reactions. The walking legs of the larvae are biramous and it is not until the moult between PL1 and PL2 that they become completely uniramous. In the larval forms the exopods are used for swimming and this is brought about by metachronous beating of the fringed exopods (Neil et al. 1976). The post larvae swim using the swimmerets and by tailflipping.

Perhaps the most striking feature about the setae on the walking legs and uropods of the larval forms of *Nephrops* shown in this study was that they were relatively few in number compared with the adult. The walking legs bore few long uniform setae which were not situated in distinct groups. Most of the setae seemed to be serrate setae (Factor, 1978) with the exception of small setal buds lining the inner edges of the large claw or chela. The adult *Nephrops* on the other

hand has an abundance of setae distributed in complex patterns and they have very diverse morphology. In *Austropotamobius pallipes* (Thomas, 1973) the walking legs of second stage larvae had the full complement of adult setal types. This was cerainly not true for *Nephrops* larvae in which the setae on the walking legs retain their larval form until PL1.

The development of the uropods at stage 3 introduced two other hair types, plumose setae and simple setae, which occur in their adult positions. These develop into the main hair type in the plumose hair border and into the simple lateral setae. Neither of which seem to be of major importance in mechanoreception in the adult, so it is possible that they have a sensory function in the larvae. Letourneau (1976) noticed that in the crayfish certain identified mechanoreceptive setae on the adult could be found as early as the first instar and traced throughout development. However, the adult uropods bear many hundreds of setae of which those present in the larval forms represent only a very small fraction.

At PL1 the animal bears a close resemblance to the adult, athough it does not take on the full adult characteristics until PL3 (Smith, 1987). The setae on the walking legs at this stage are still serrate although they are present in the positions which they will occupy in the adult. On the uropods the dorsal surface of the distal fringe consists of two setal types, as it does in the adult, and in addition to the lateral setae a few cuticular structures are present on the dorsal surface of the uropod blade. It is likely (Letourneau, 1976) that cuticular structures will be added each time the animal moults. However, at PL1 the hair types have not become fully differentiated and this may not occur until PL3.

Why differences occur between larval and postlarval setal

structure, position and number is not clear. It is likely that these differences reflect the major change in lifestyle, behaviour and size which occurs between stages. The larvae have an apposition eye, adapted to higher light levels than the superposition eye of the adult and they tend to orientate towards light rather than any other stimulus (Rice, 1964) as they have no functional statocyst (Neil et al., 1976). The change from positive to negative phototaxis which occurs at L3 may cause the larval *Nephrops* to sink to the bottom to test the substrate (Smith, 1987) in preparation for their change to a benthic lifestyle. The larvae are pelagic and swim in midwater eating other plankton as well as members of their own species. It is likely therefore that they would require chemosensors for this purpose. Mechanosensors would also be necessary for food handling as well as orientation to water currents and other mechanical stimuli. In order to be responsive over the same range and to the same kind of stimuli as the adult setae, the larval setae would need to be of the same size range. It is possible that there may be fewer setae on the larvae because the larvae are simply too small to support a vast array of diverse setae of the required size. The serrate setae which are present may be bi- or multi- modal and may therefore fulfill all of the functions required by the animal.

Figure 2.1 Diagram of the uropods of *Nephrops* showing the distribution of cuticular setae. Scale bar represents 1cm. Carapace length 6cm.

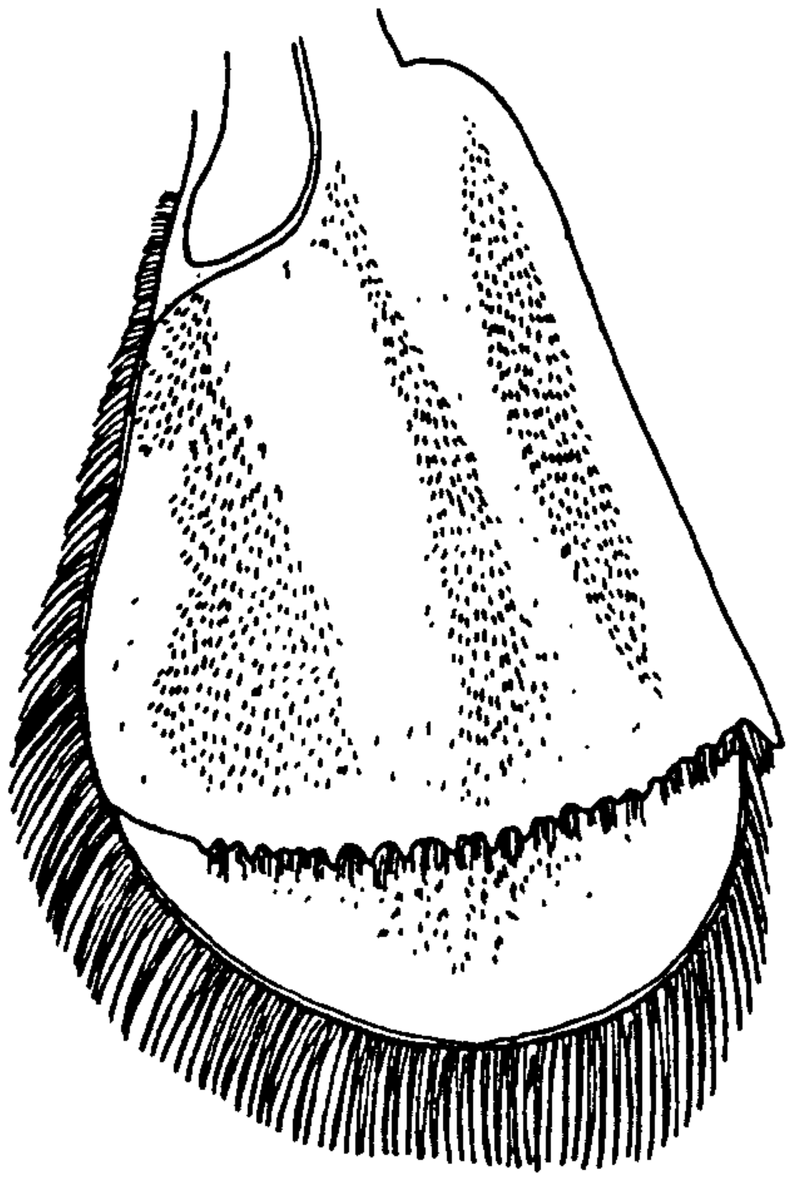
A. The dorsal surface of the right exopod

B. The ventral surface of the right exopod

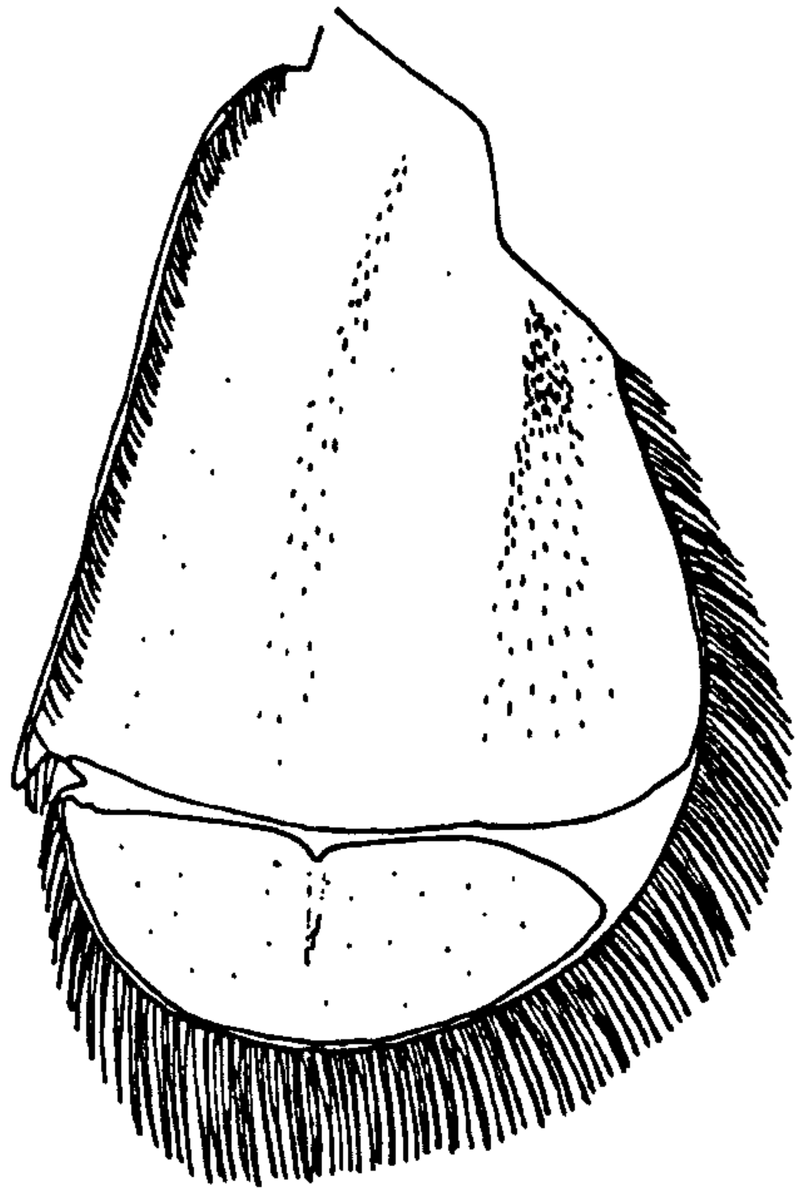
C. The dorsal surface of the right endopod.

PROXIMAL

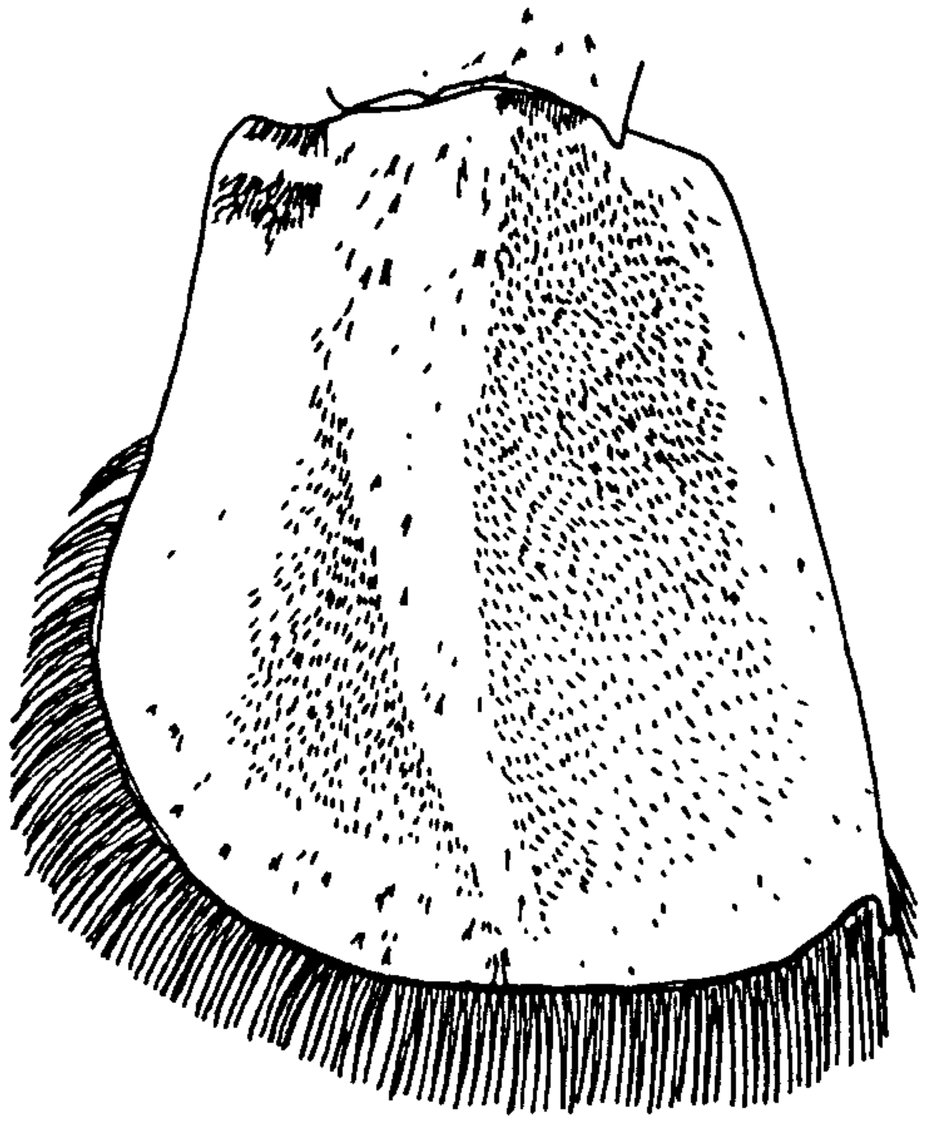
A



B



C



DISTAL

1 cm

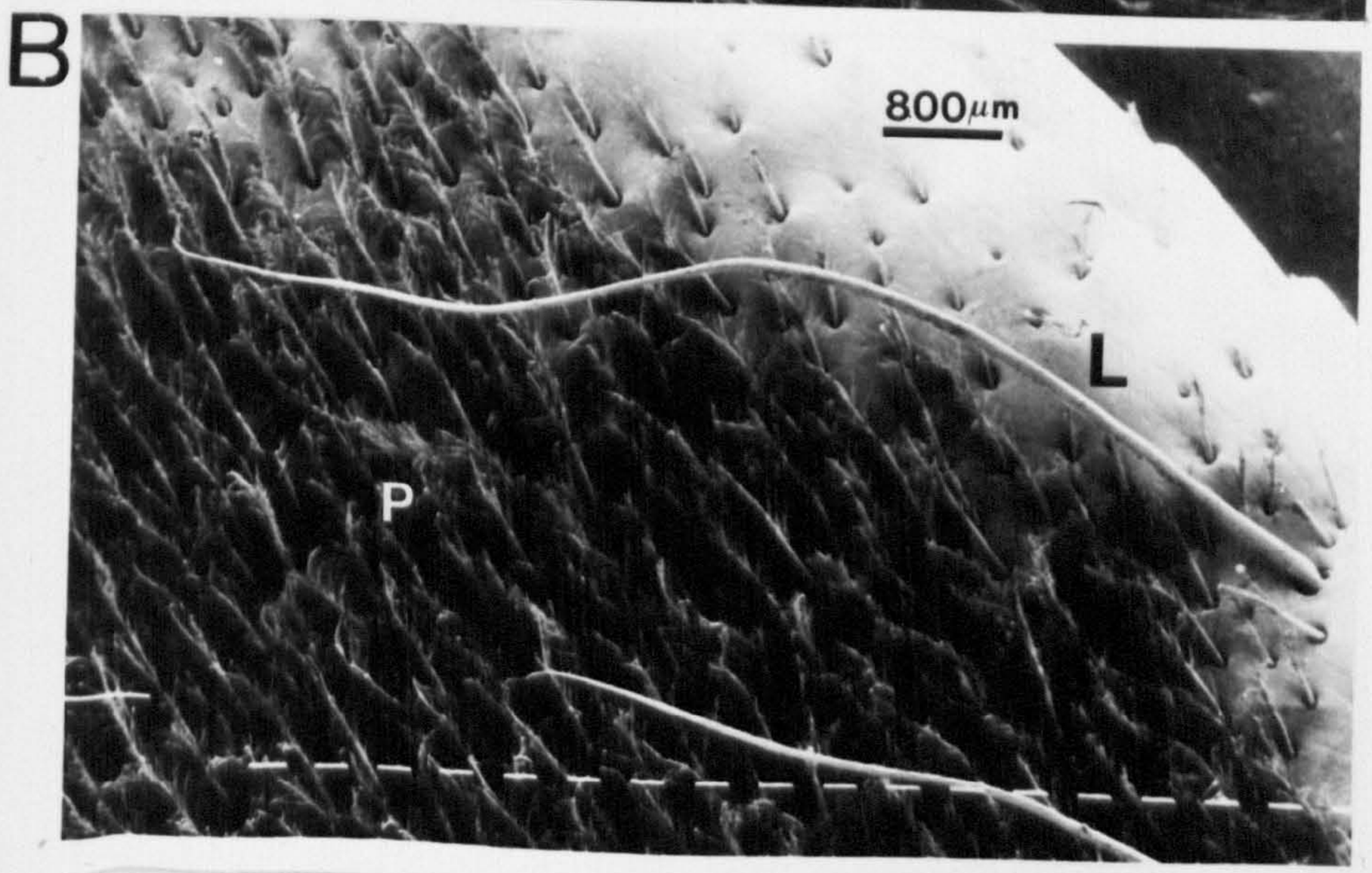
Figure 2.2 A. Scanning electron micrograph of part of one of the fields of plumose setae found on the dorsal surfaces of the endopod and exopod of *Nephrops* showing that the setae are all orientated in the same general direction ie. a line from base to tip points towards the distal tip of the uropod.

B. Scanning electron micrograph of the lateral border of the endopod of *Nephrops* showing the lateral field of plumose setae (P) and two of the long simple setae (L) found along the edge of this part of the uropod.

C. Scanning electron micrograph of the base of one of the simple setae (L) found along the lateral border of the endopod of *Nephrops* showing the hinge-like structure at the base of the seta (H) and a guard hair on either side (G).

A

B



C

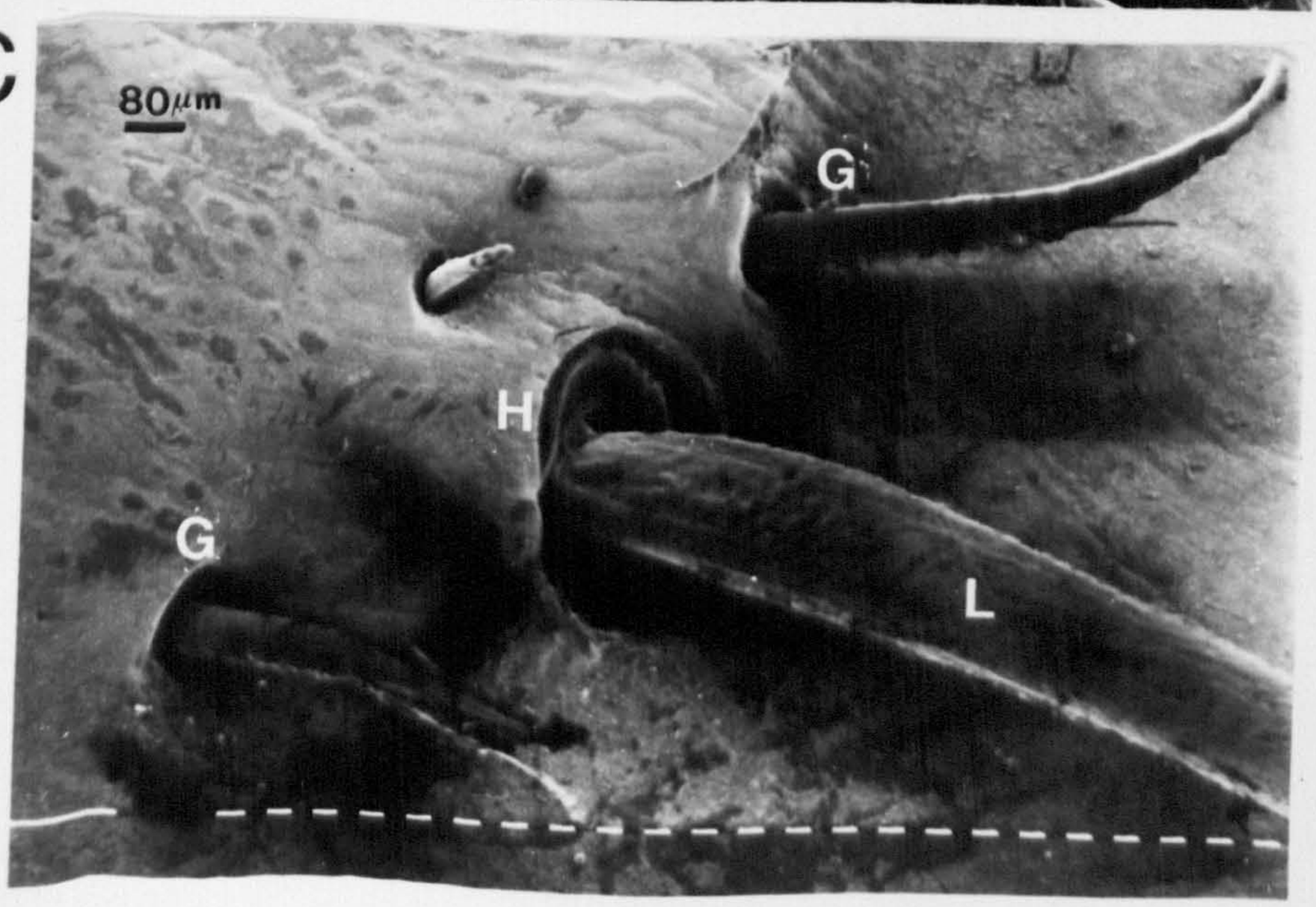
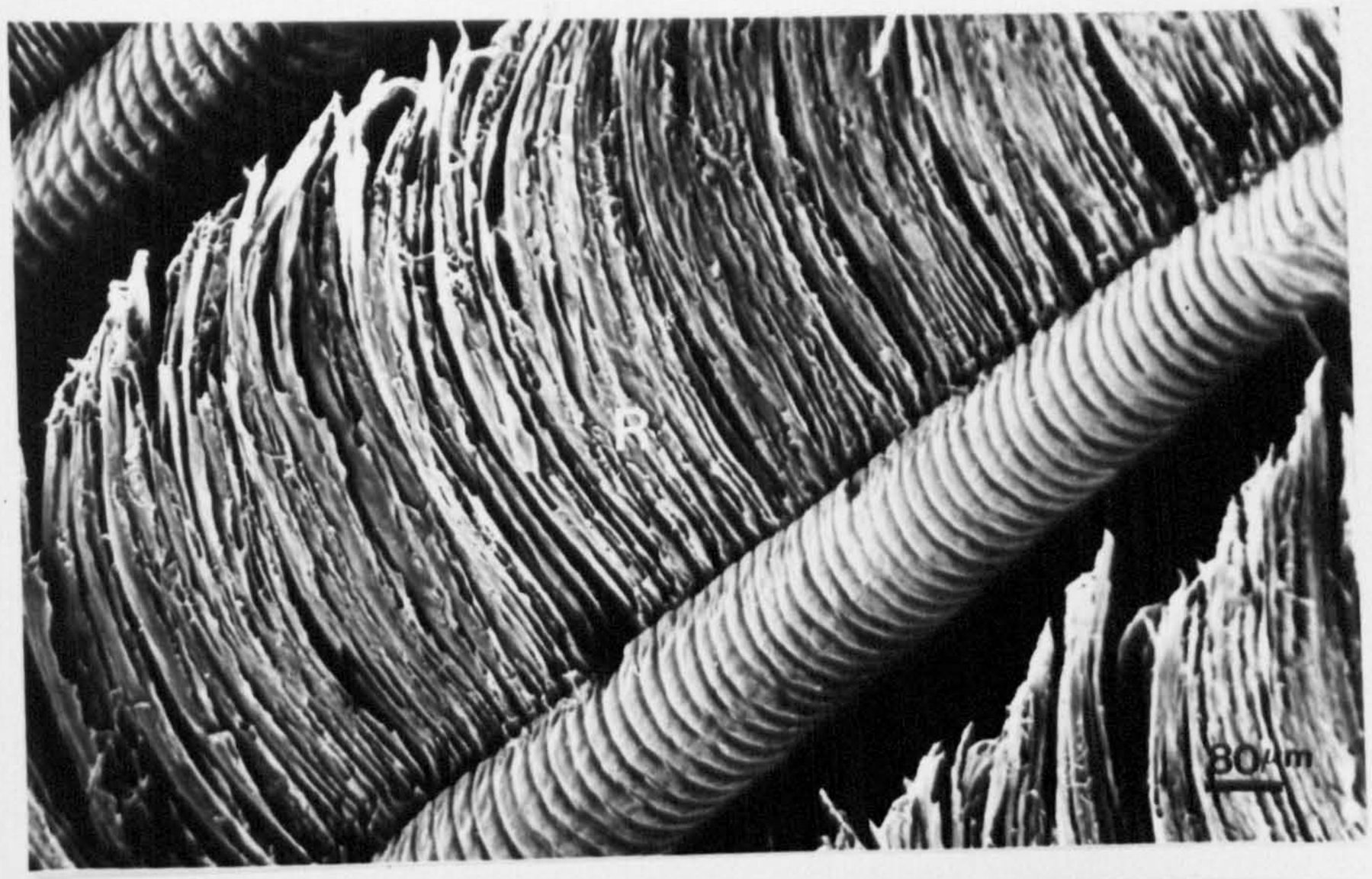


Figure 2.3 A. Scanning electron micrograph showing the fine structure of the long plumose setae, the most predominant hair type in the distal fringe around the uropods, showing the ribbed shaft and the ribbon-like side projections (R).

B. Scanning electron micrograph of the dorsal side of the distal uropod fringe showing the large plumose setae (P), the smaller plumose setae (PL) situated between them and the sleeve-like structure at the bases of both of these hair types (S).

C. Scanning electron micrograph of the ventral side of the distal uropod fringe showing the large plumose setae (P) and the bunches of smaller plumose setae (U) situated between them.

A



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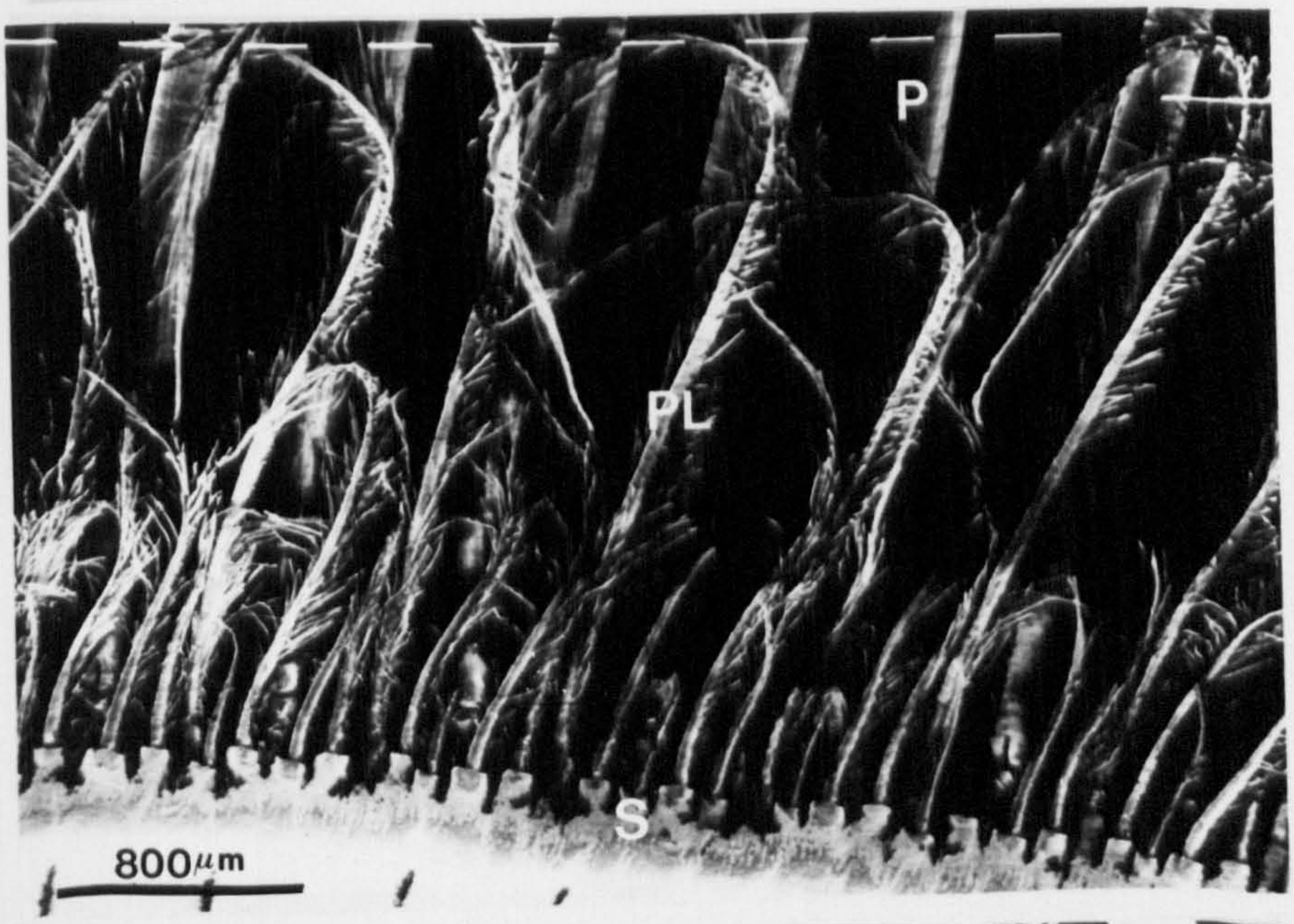

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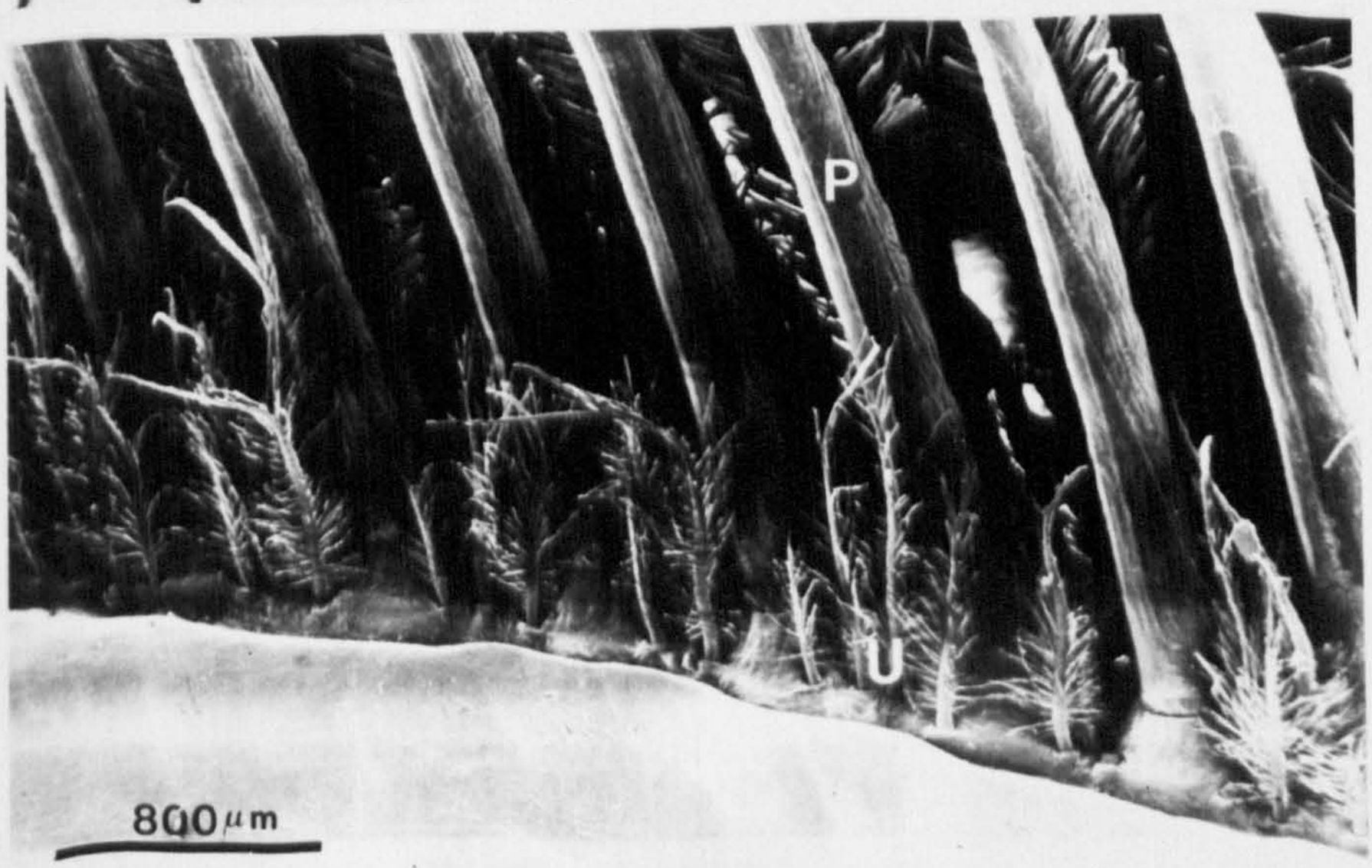
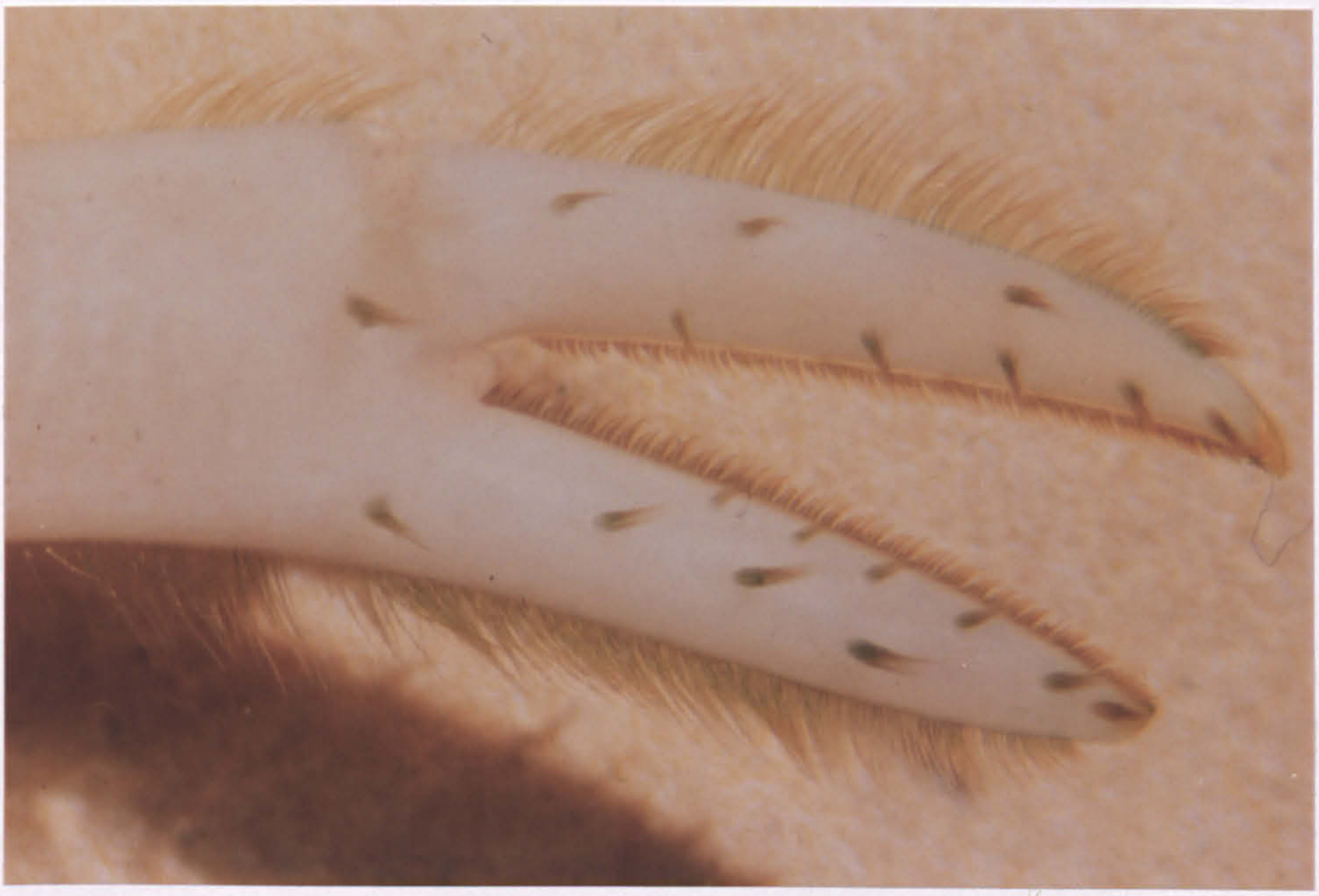


Figure 2.4 A. Photograph of the propodus and dactyl of leg two of *Nephrops* showing the distribution of cuticular setae which have been stained with methylene blue dye. B. Photograph of the propodus and dactyl of leg five of *Nephrops* showing the distribution of cuticular setae.



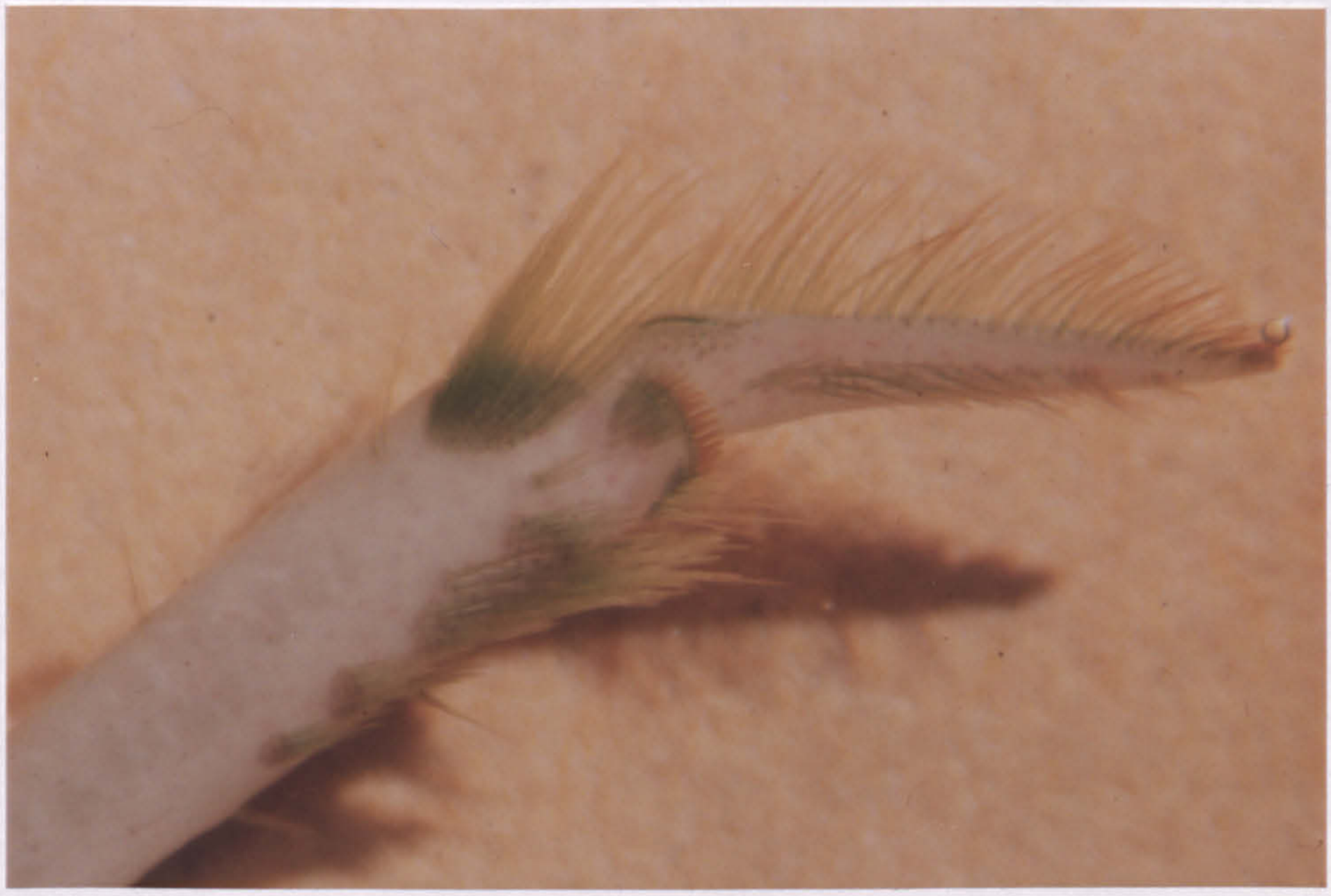
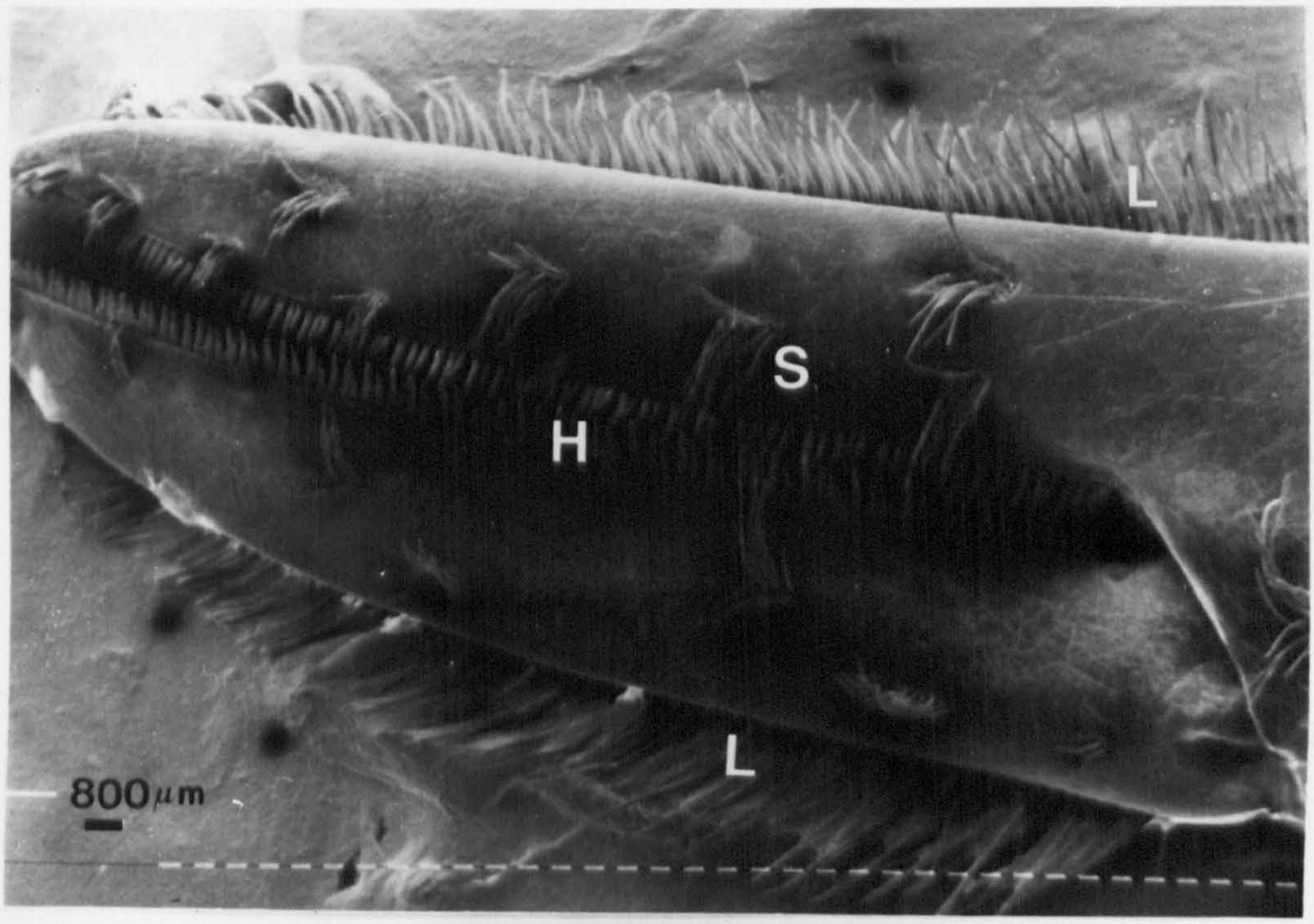


Figure 2.5 A. Scanning electron micrograph of the propodus and dactyl of leg two of *Nephrops* showing the simple setae around the lateral edges of the propodus and dactyl (L), the bunches of squamous setae on the flat surfaces of the propodus and dactyl (S) and the hedgehog hairs (H) lining the inner edges of both segments.

B. Scanning electron micrograph of the propodus and dactyl of leg two of *Nephrops* showing the bunches of squamous setae (S) found on the flat surfaces of the propodus and dactyl and the hedgehog hairs (H) lining the chela.

A



B

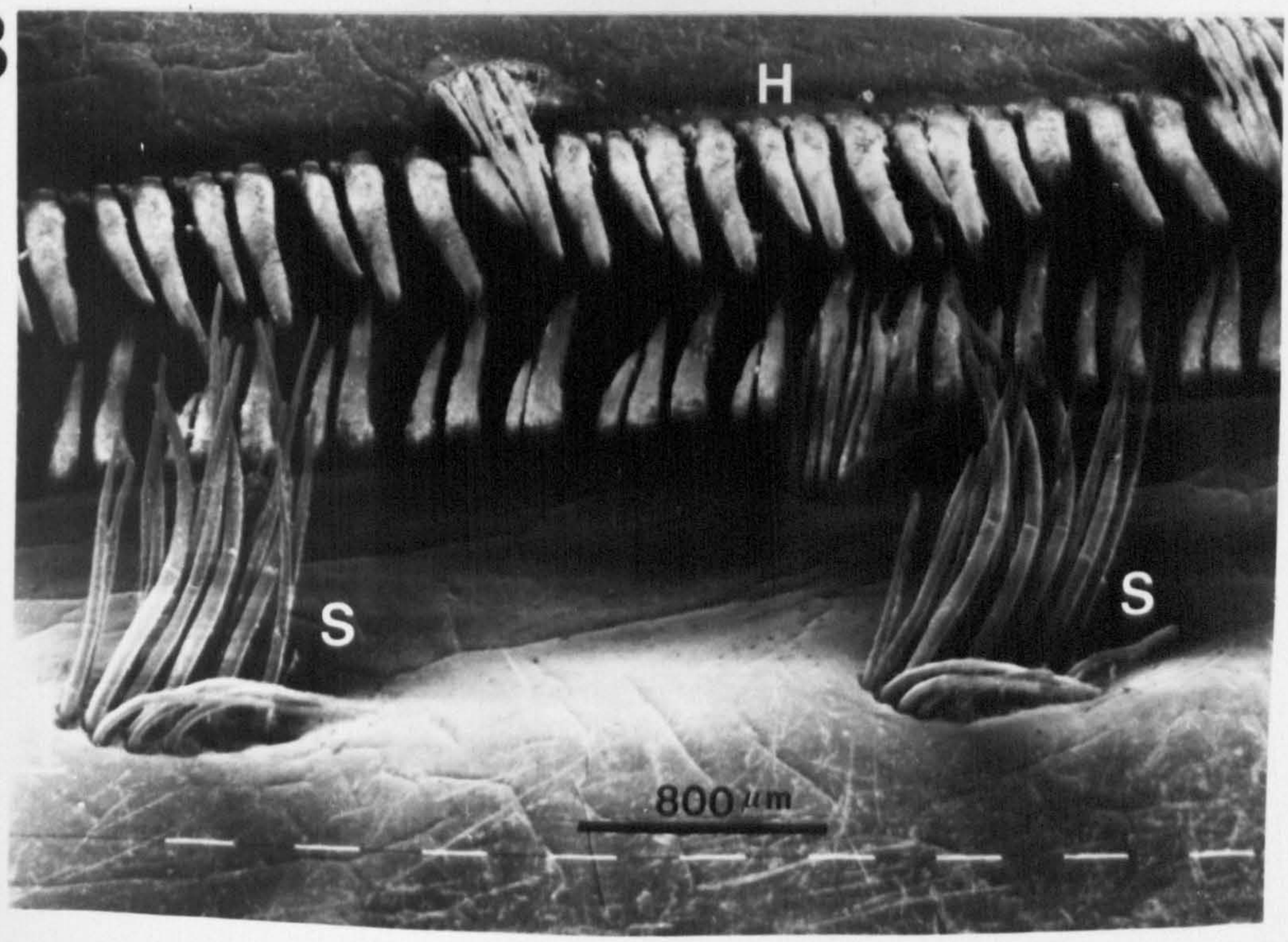
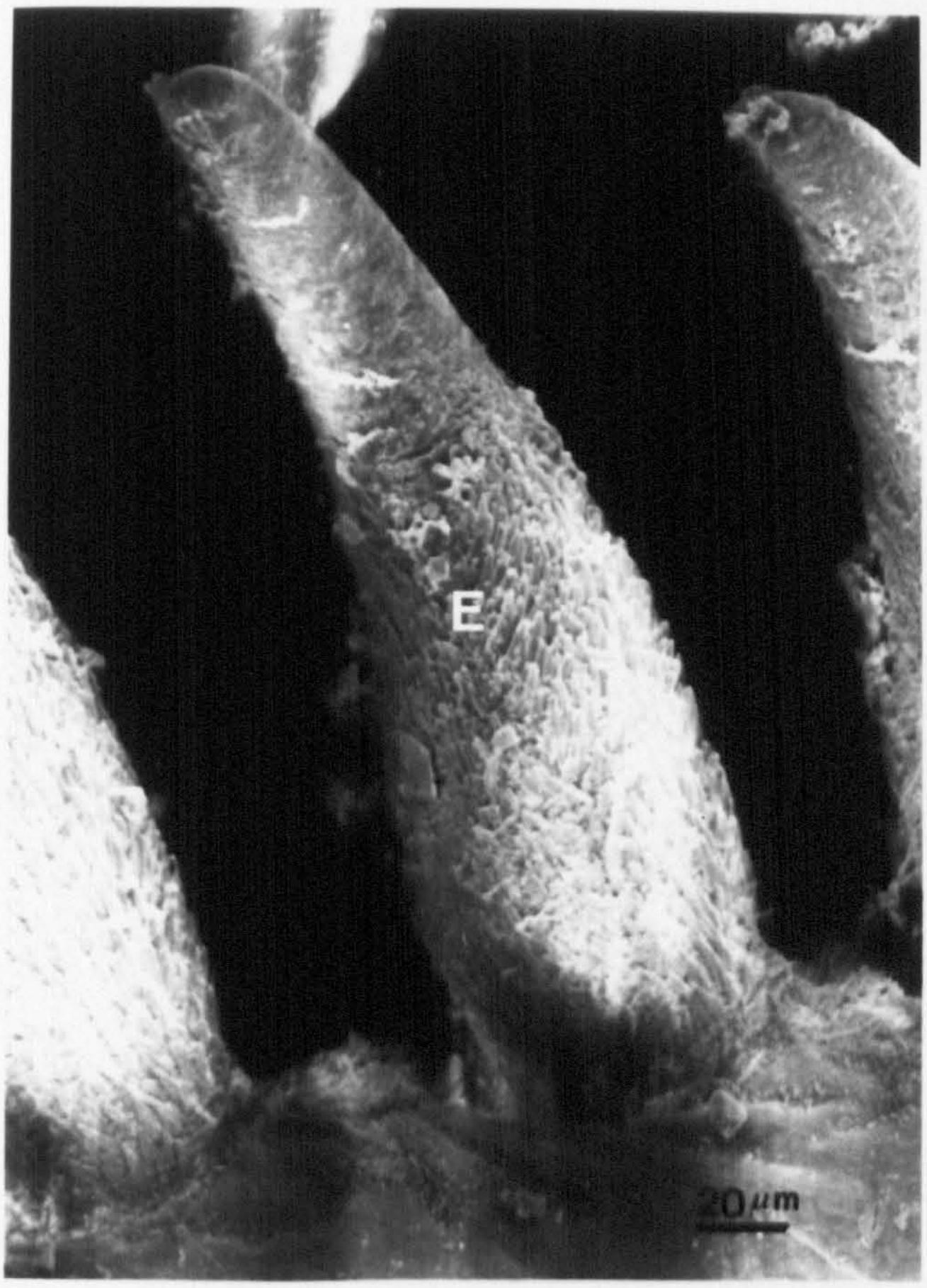


Figure 2.6 A. Scanning electron micrograph of the fine structure of a hedgehog seta from leg two of *Nephrops* showing the numerous blunt filaments extending from one side (E).

B. Scanning electron micrograph showing the detailed structure of the bunches of squamous setae from leg two of *Nephrops*. The micrograph shows the annulus (A) and the scales (S) which extend from the annulus to the tip of the seta on one side.

A



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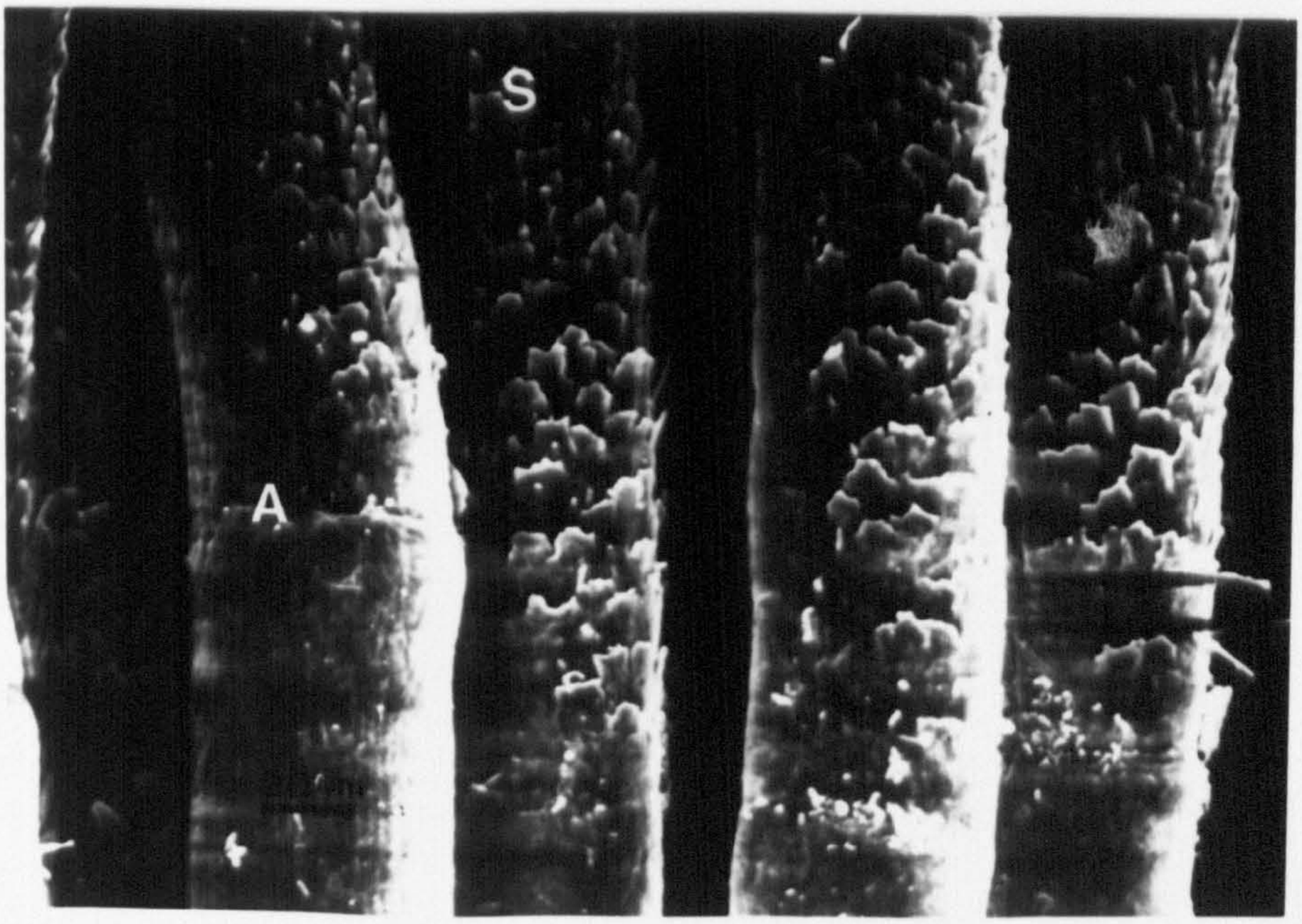


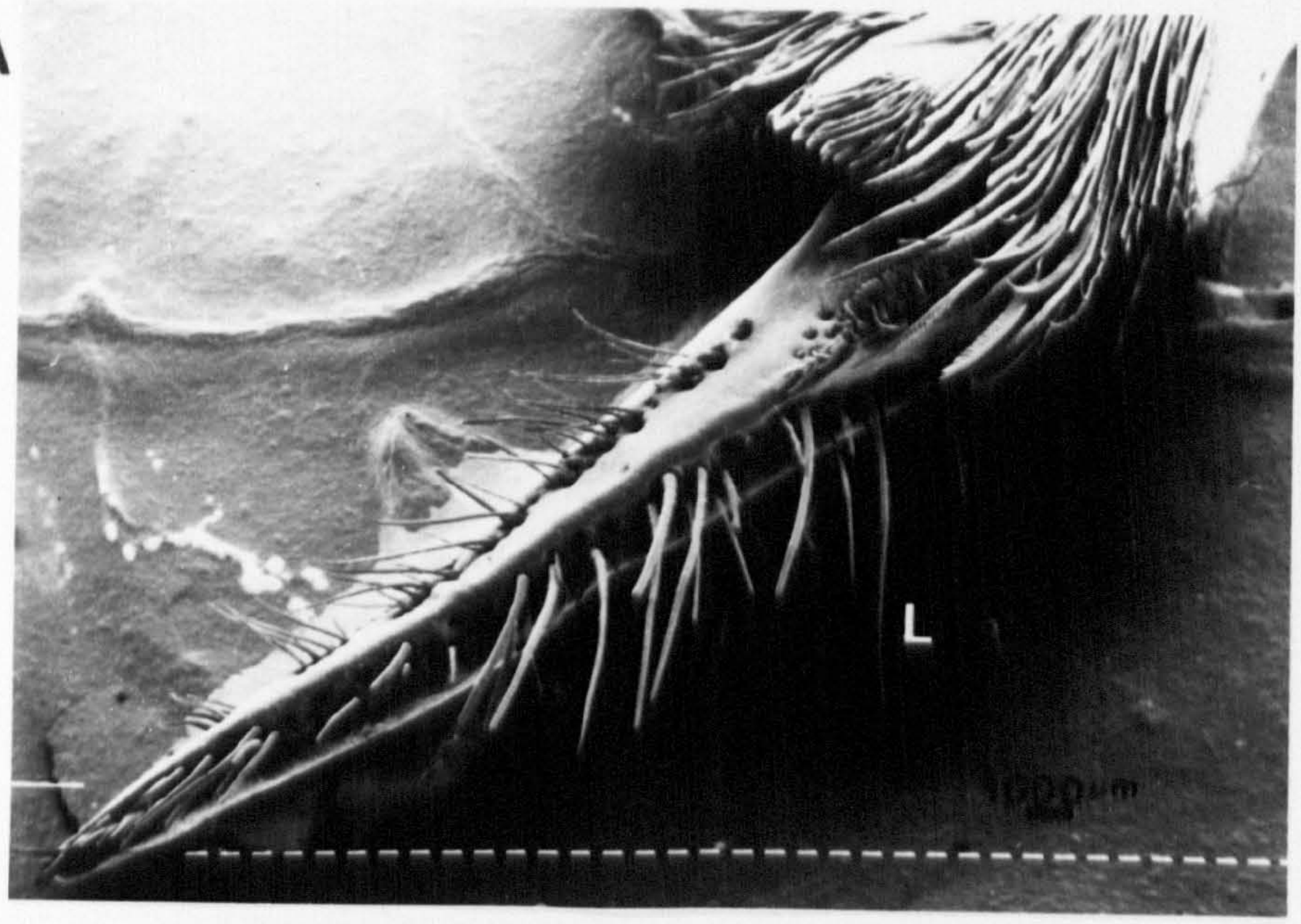
Figure 2.7 A. Scanning electron micrograph of the dactyl and part of the propodus of leg five of *Nephrops* showing the simple setae (L) which were present in three bands on the dactyl. Many of the setae are broken in this case.

B. Scanning electron micrograph of the propodus-dactyl (P-D) joint of leg five of *Nephrops* showing the blunt ending peg-like structures (P) on the ridge of the dactyl, the CAP-like sensillae (A) above these, the long serrate setae with simple scales (SR) and the squamous setae (S) found on the propodus extending over the joint and the hedgehog hairs (H) which formed a border around the distal edge of the propodus.

C. Scanning electron micrograph of the fine detail of the serrate setae with simple scales which overlap the P-D joint showing the scales (S) found on one side of the seta and the serrations on the other.

D. Scanning electron micrograph of the fine detail of some of the squamous setae which overlap the P-D joint. Scale bar is the same as in C.

A



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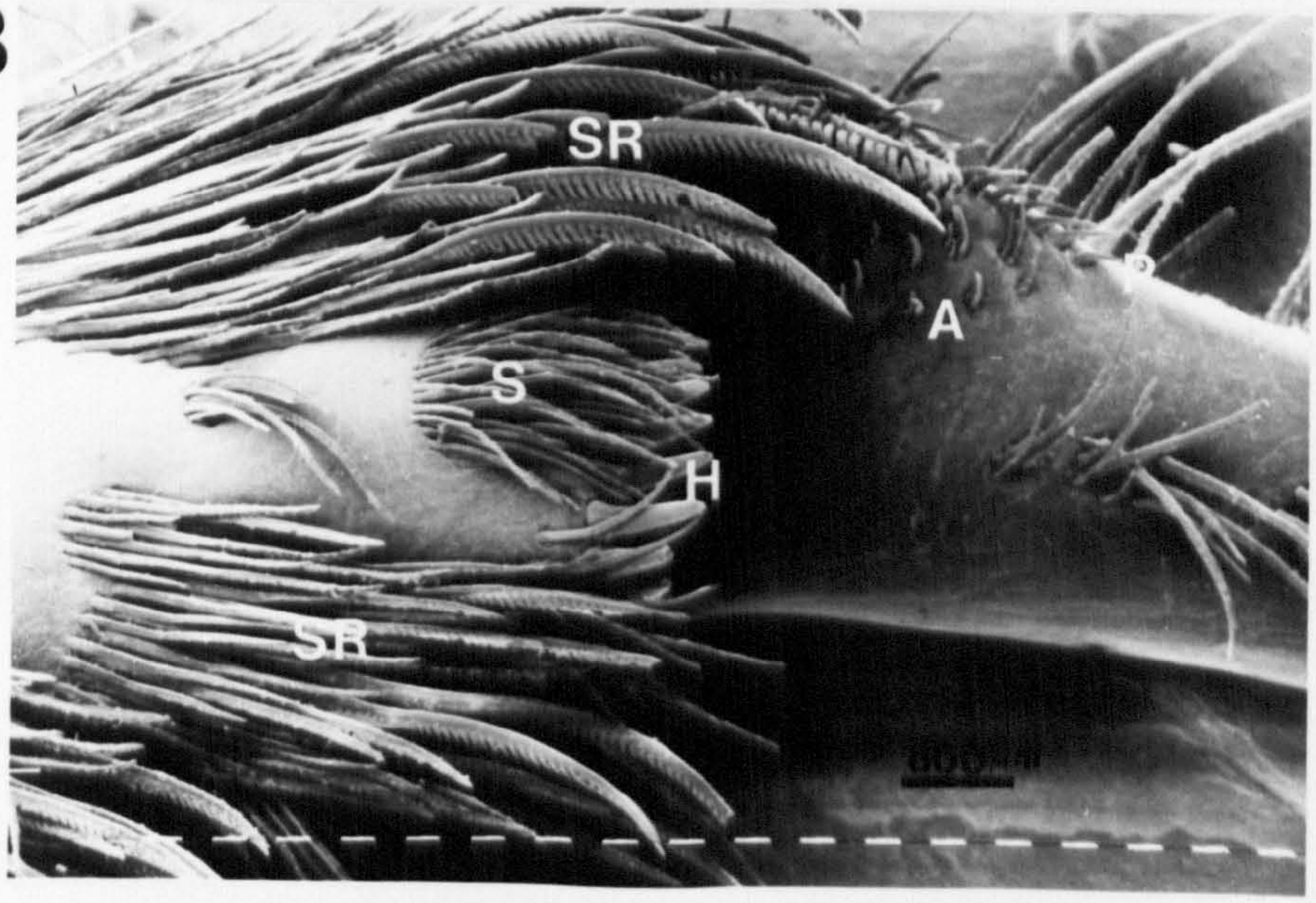
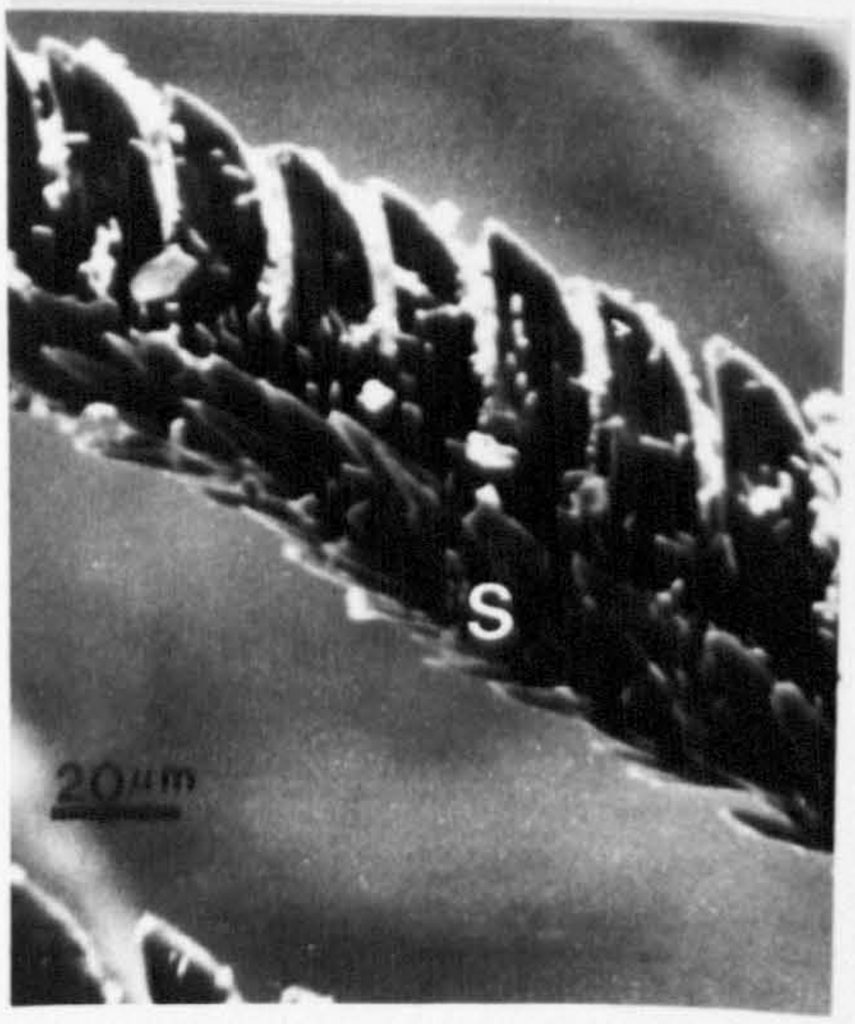


C



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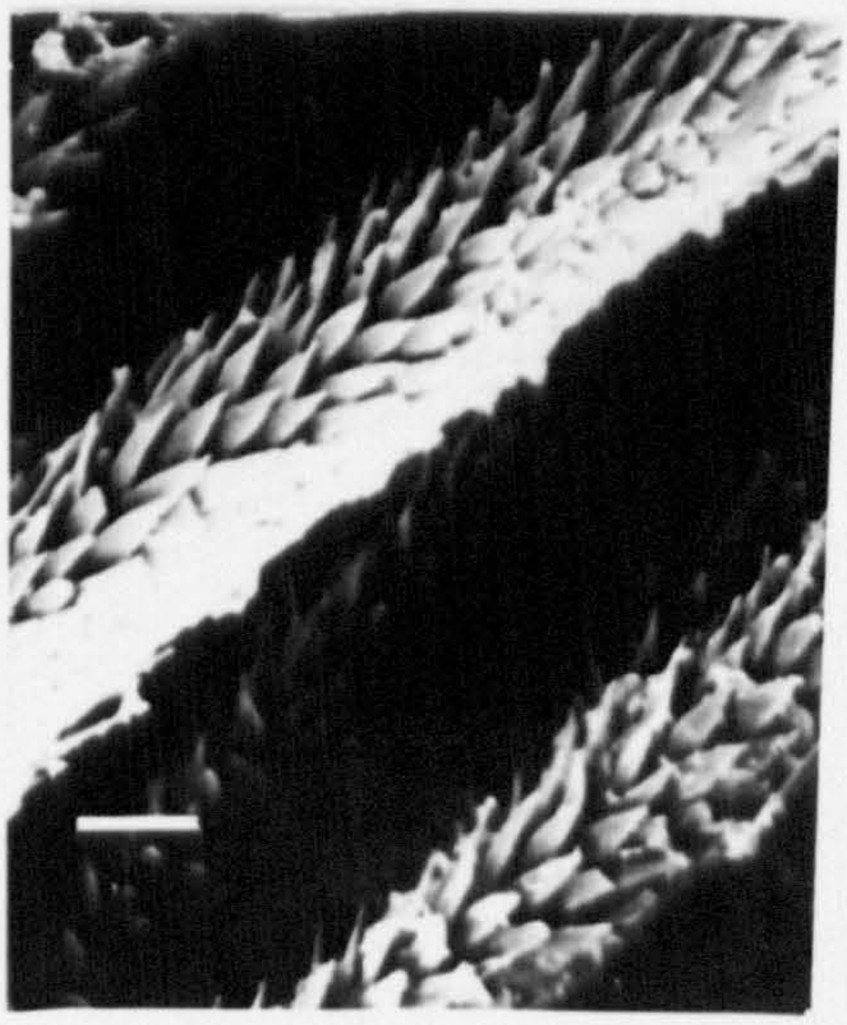
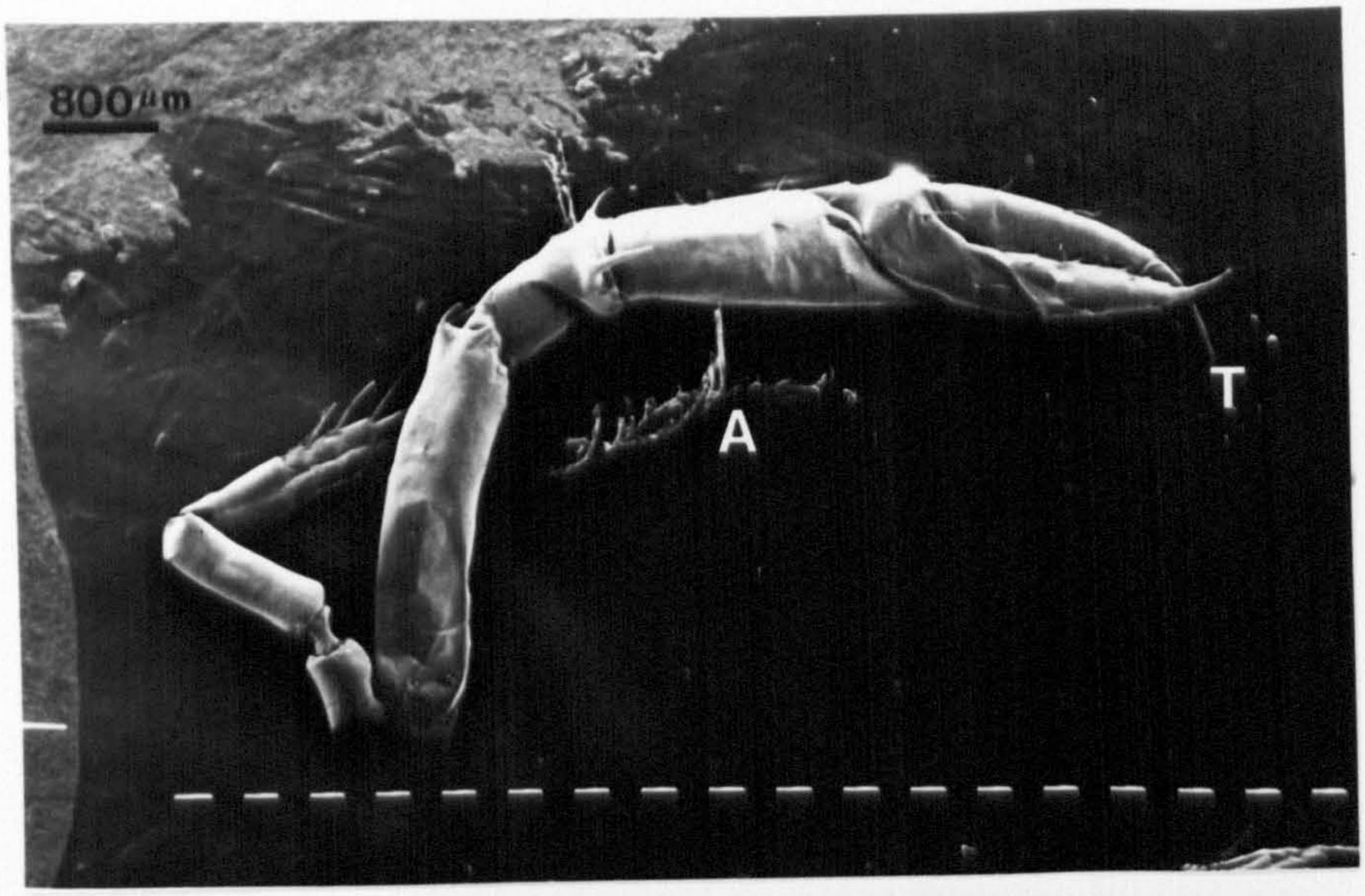


Figure 2.8 A. Scanning electron micrograph of the stage one (L1) larval claw of *Nephrops* showing the fringed exopod (A) and the long pointed tips on the propodus and dactyl (T).

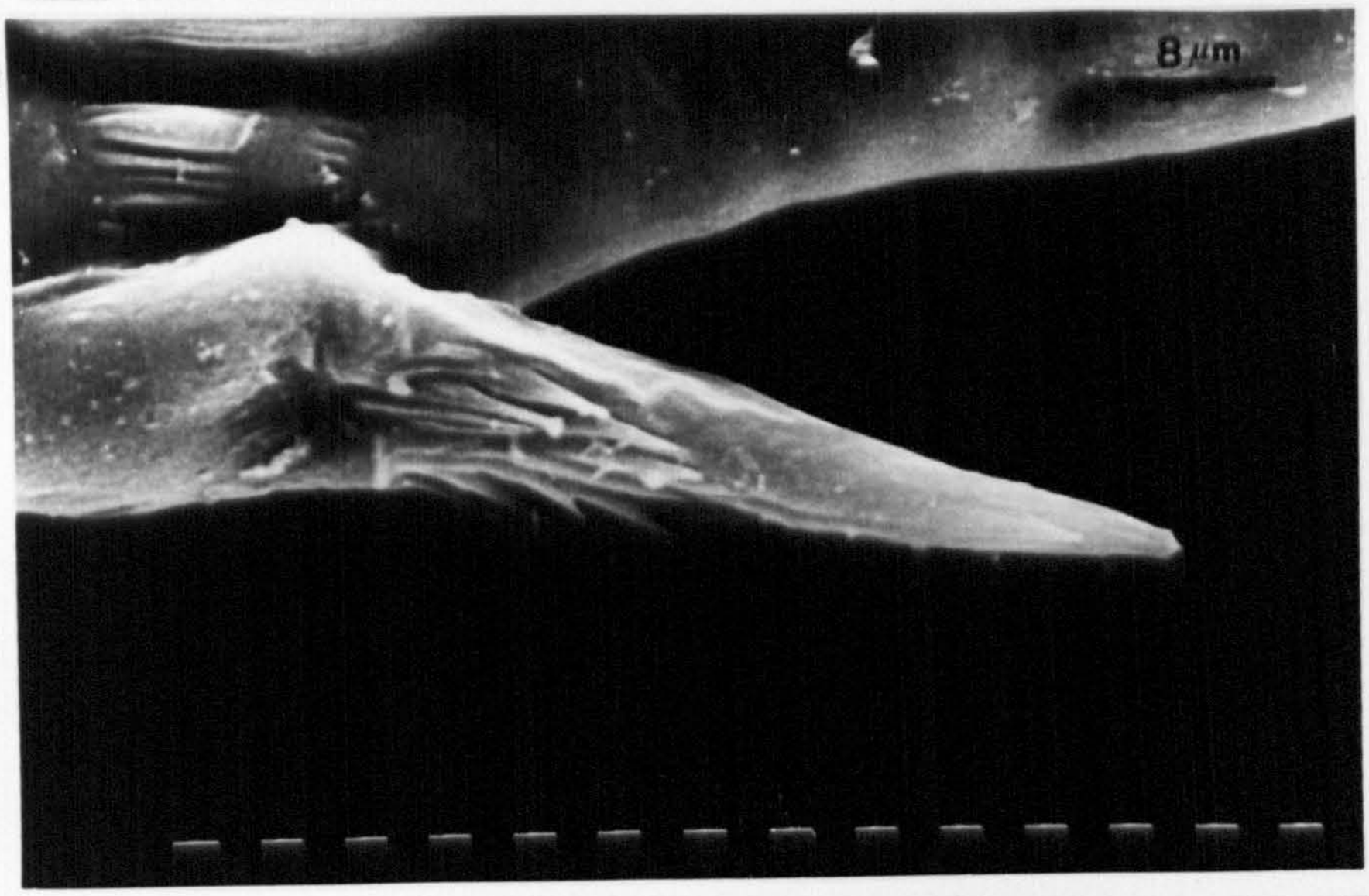
B. Scanning electron micrograph of one of the hedgehog-like setae which line the inner edges of the claw of *Nephrops* L1.

C. Scanning electron micrograph of leg two of *Nephrops* L1.

A



B



C

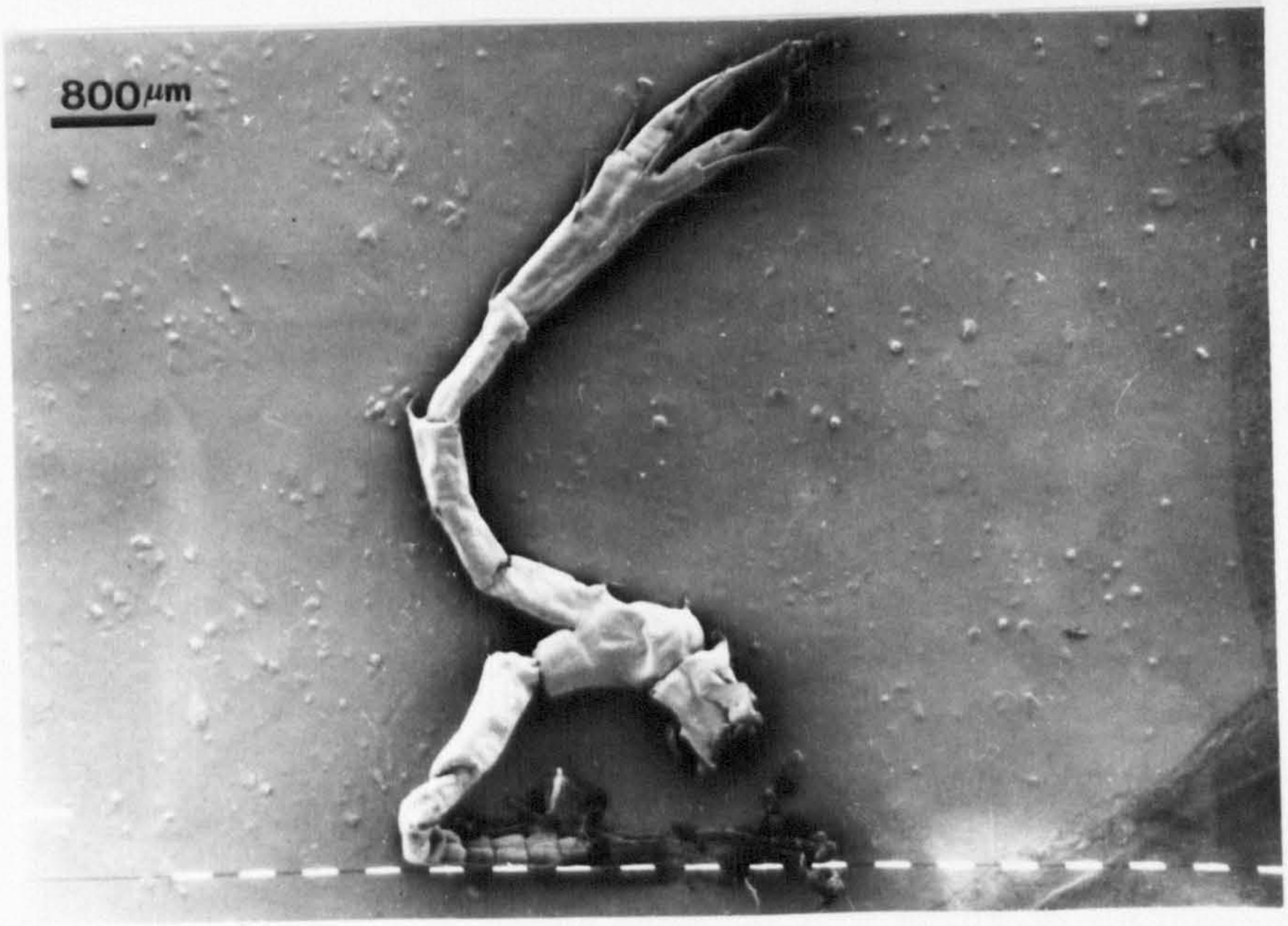
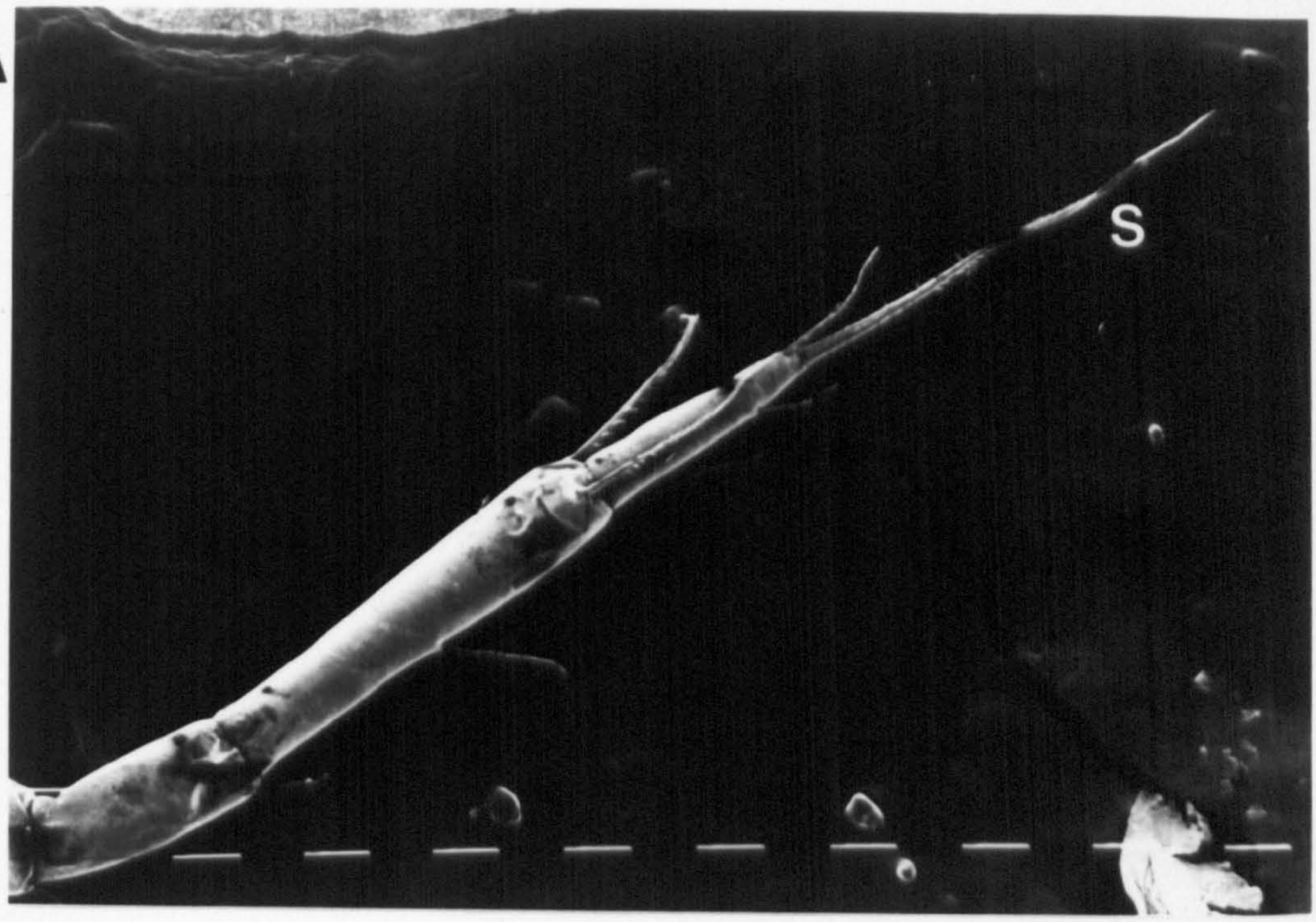


Figure 2.9 A. Scanning electron micrograph of the tip of leg four of *Nephrops* L1 showing the propodus and dactyl with its long pointed tip (S).

B. Scanning electron micrograph showing the fine structure of the larval serrate setae (S). This example is from leg 4 of PL1 but setae of similar form are also found in the other larval stages.

A



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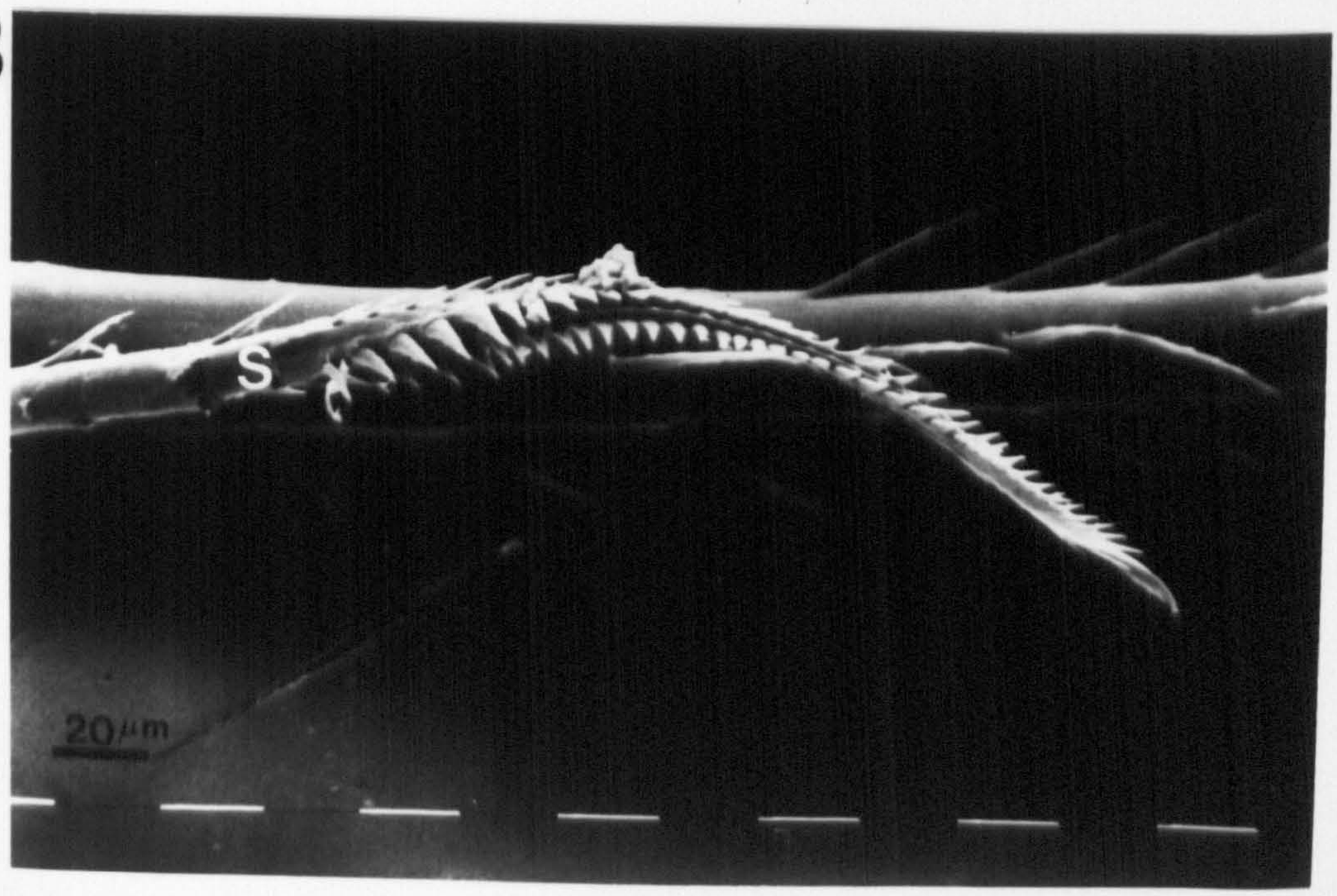
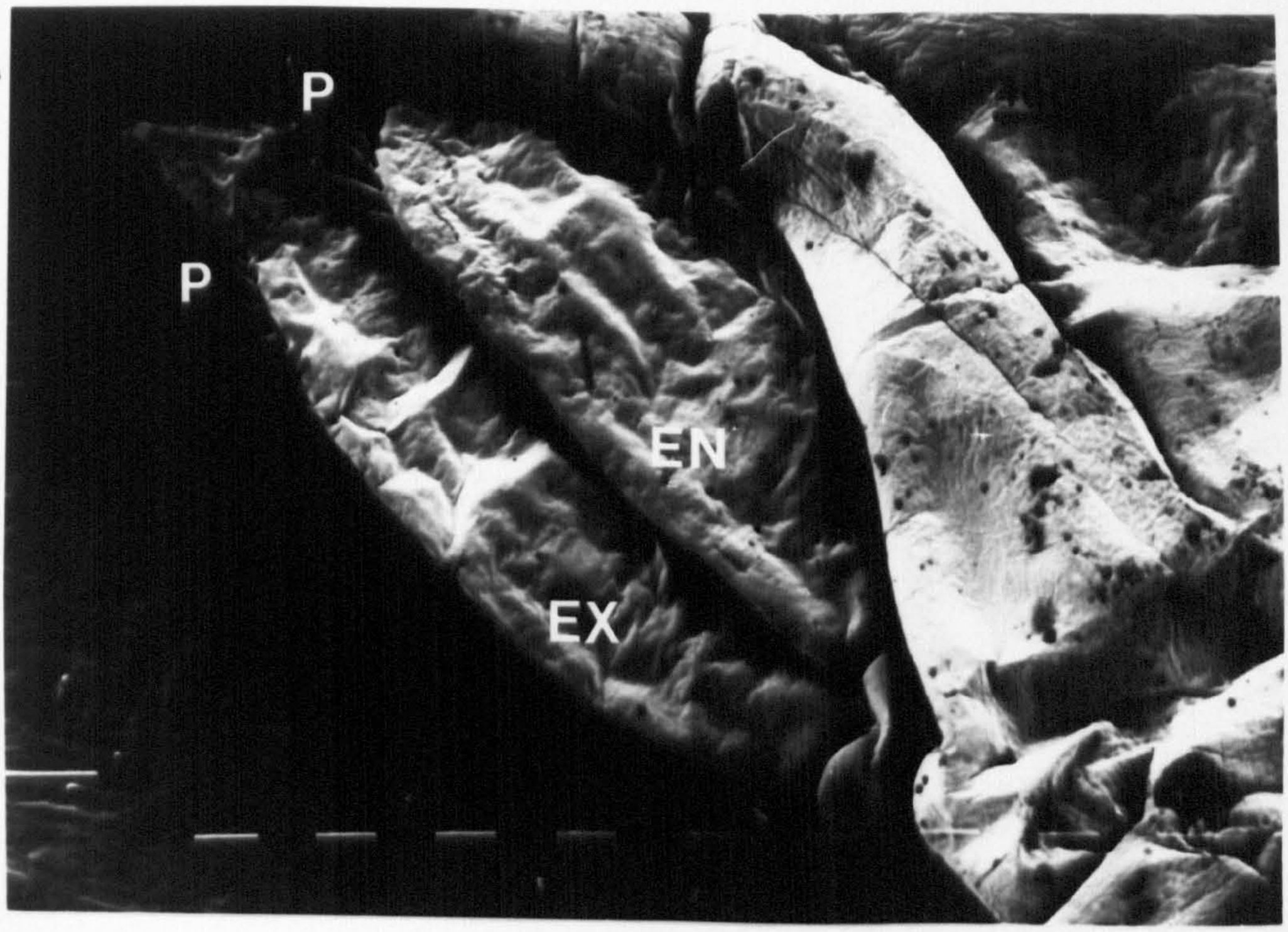


Figure 2.10 Scanning electron micrographs of the stage 3 (L3) larval uropods of *Nephrops*.

A. Dorsal view of the right uropods showing the exopod (EX), the endopod (EN) and the fringes of plumose setae (P) around the distal edges of both uropods.

B. Ventral view of the right uropods showing the telson with its fringe of setae (T). Other features as in A.

A

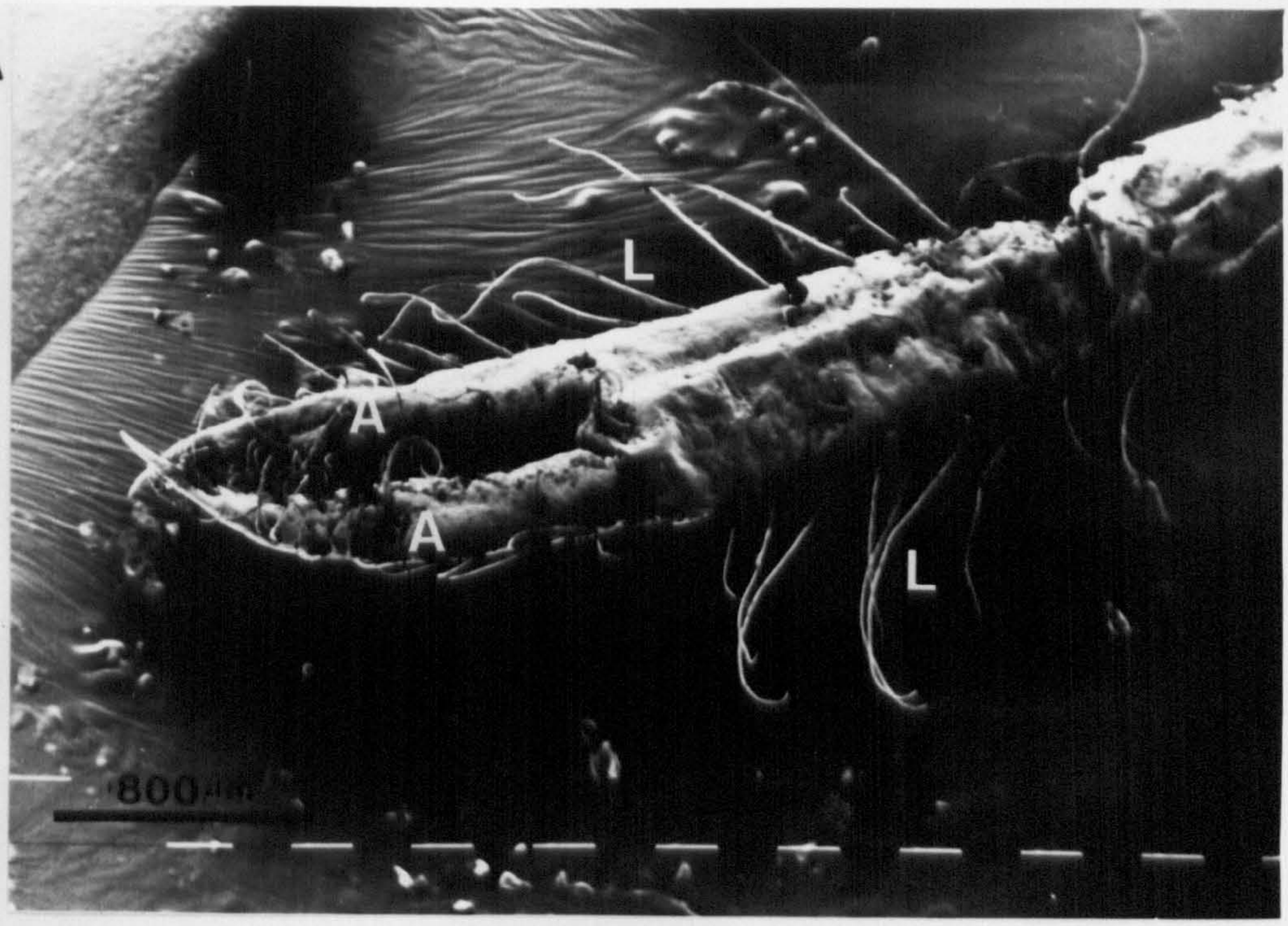


B

Figure 2.11 A. Scanning electron micrograph of the propodus and dactyl of leg two of the post larval one (PL1) *Nephrops* showing the long setae (L) which form a border around the lateral edges of the propodus and dactyl and the bunches of setae (A) on the flat surfaces of the propodus and dactyl.

B. The tailfan of the PL1 *Nephrops* showing the uropods and telson. The uropods and telson have a distal fringe (which in this case is missing from the telson) consisting of plumose setae (U). The joint of the exopod can also be seen (J).

A



B

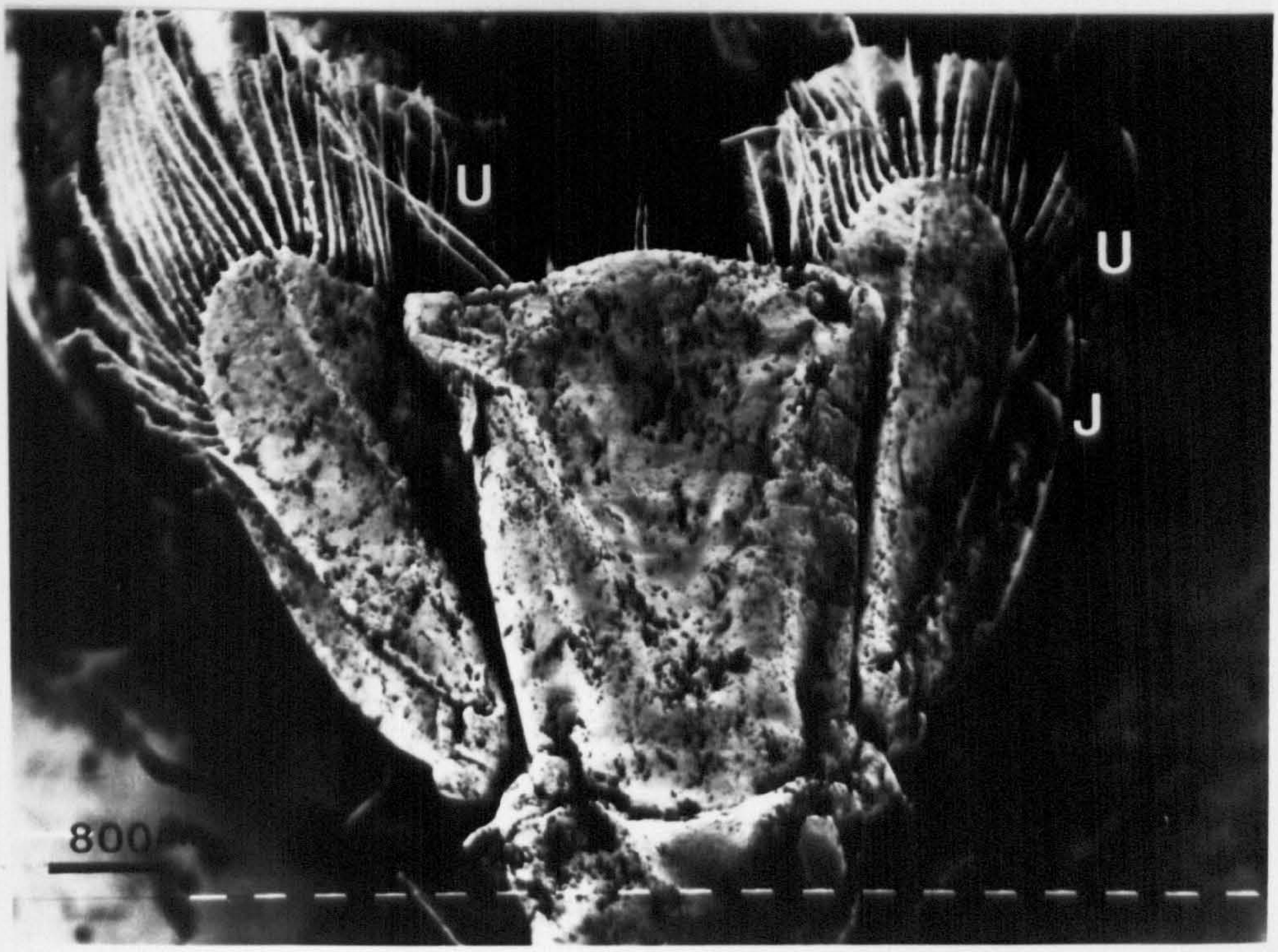


Figure 2.12 An example of multi unit recordings obtained from root 2 to G6 of *Nephrops*, carrying sensory information from the uropod exopod. The plot shows responses obtained when areas of the uropod were selectively stimulated with a paintbrush. Bars indicate stimulus. Scale bar shows time in seconds.

A. Responses to stimulation of the anterio-medial section of the exopod.

B. The anterio-lateral section of the exopod

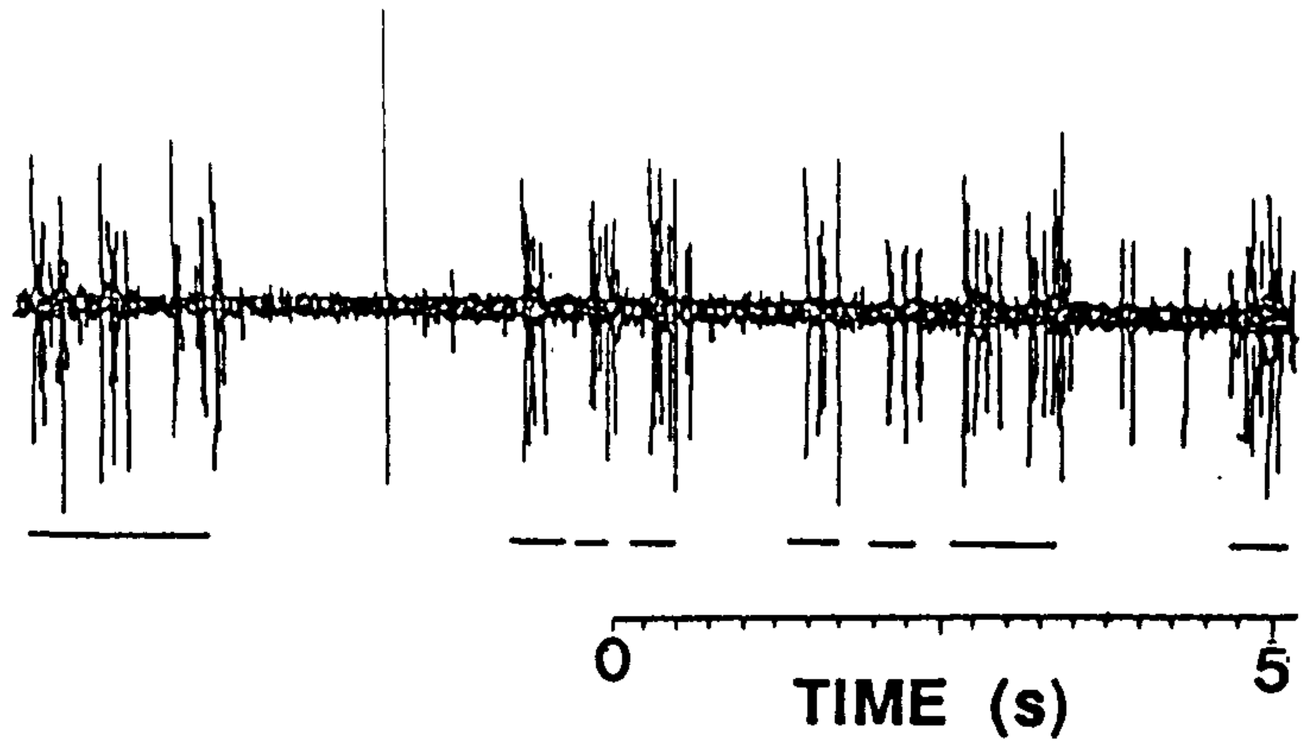
C. The posterio-lateral section of the exopod.

D. The posterio-medial section of the uropod.

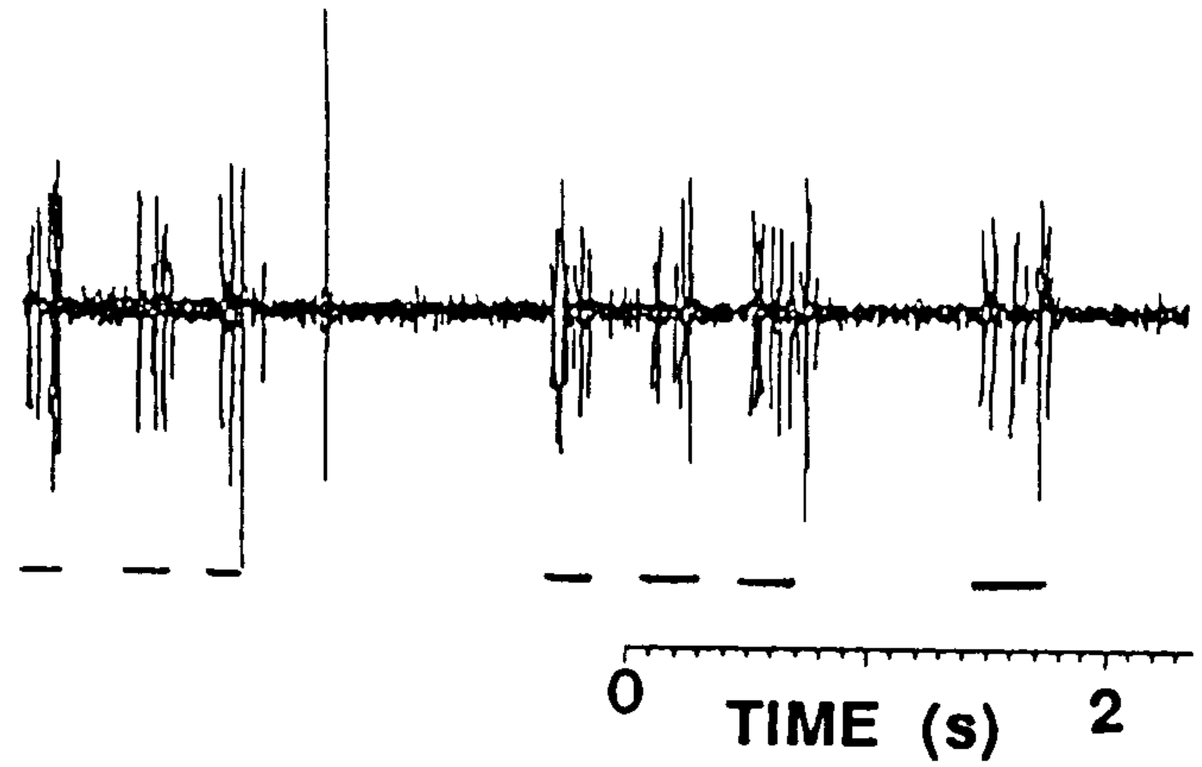
E. The area of muscle insertion.

F. The distal fringe of setae.

A



B



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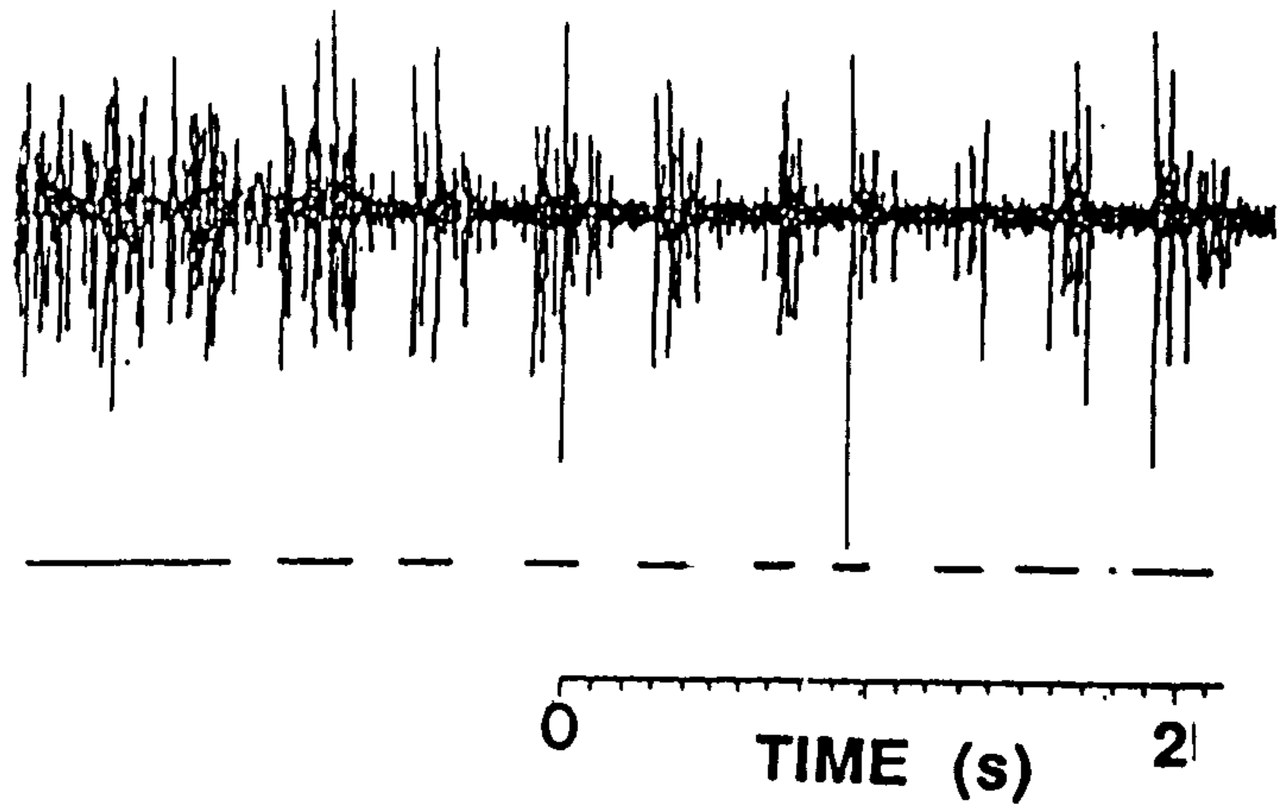

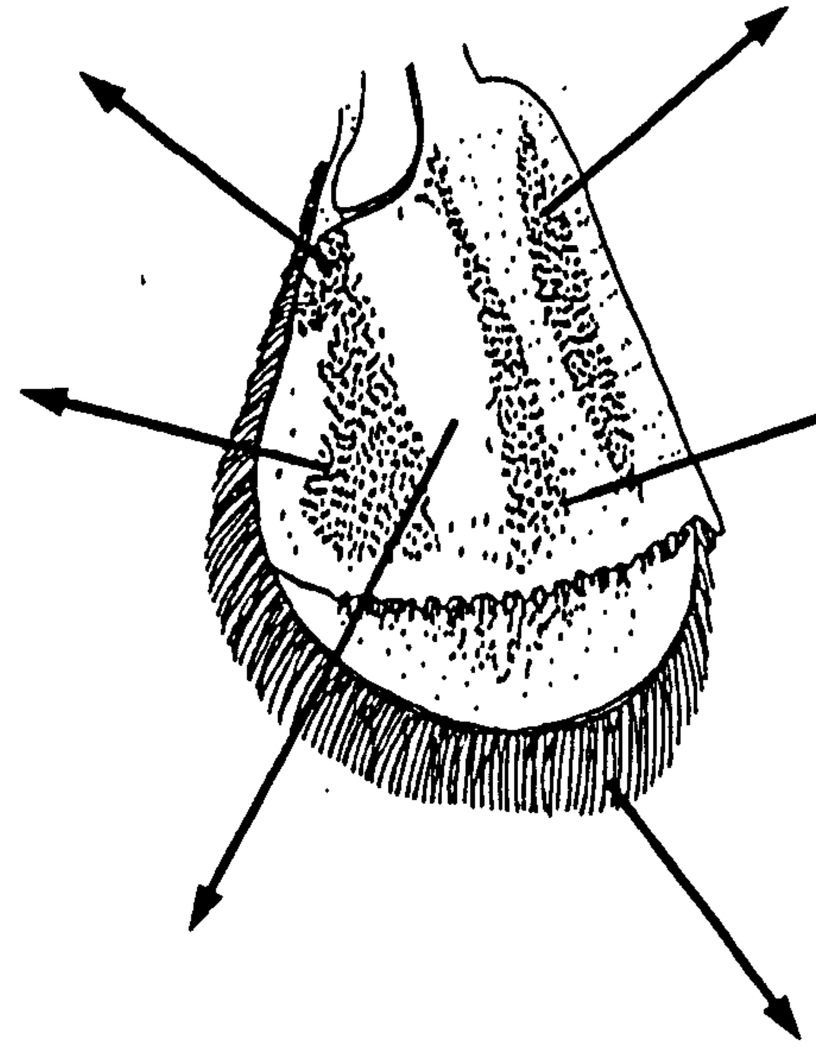

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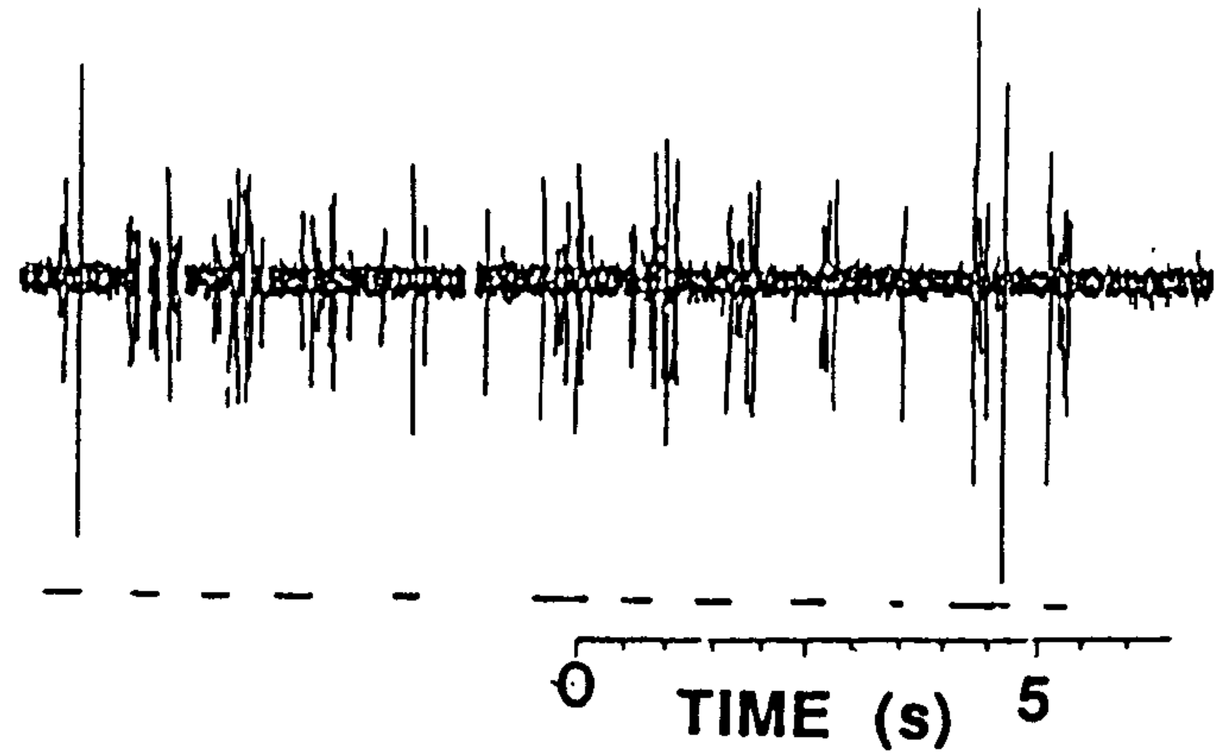
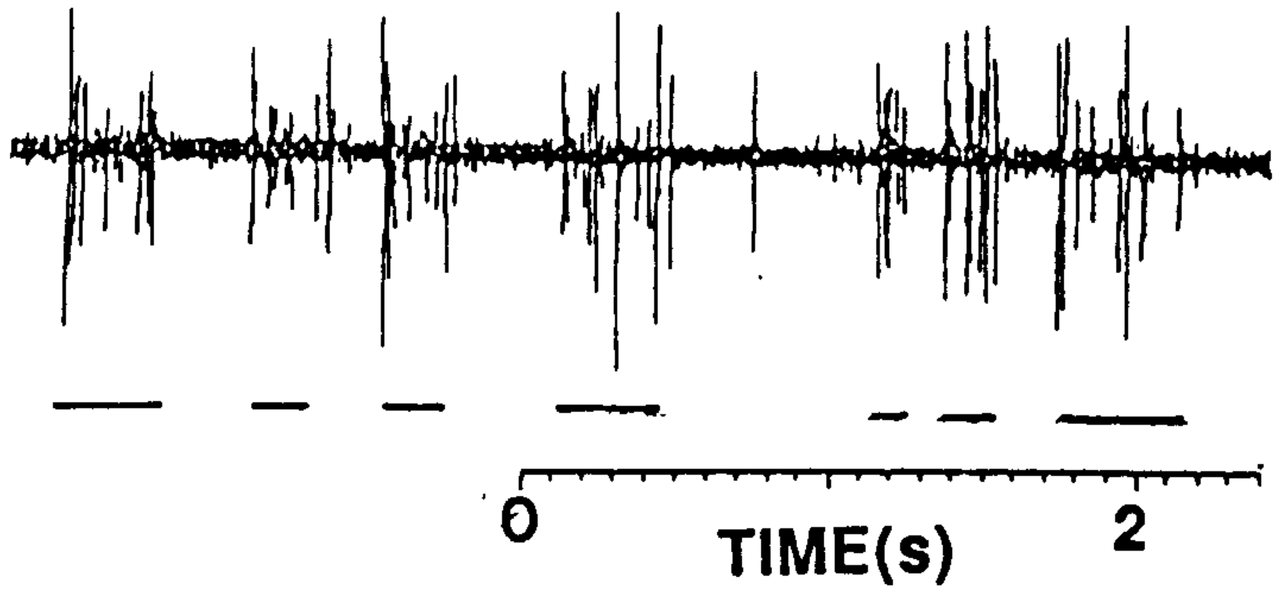


E



F

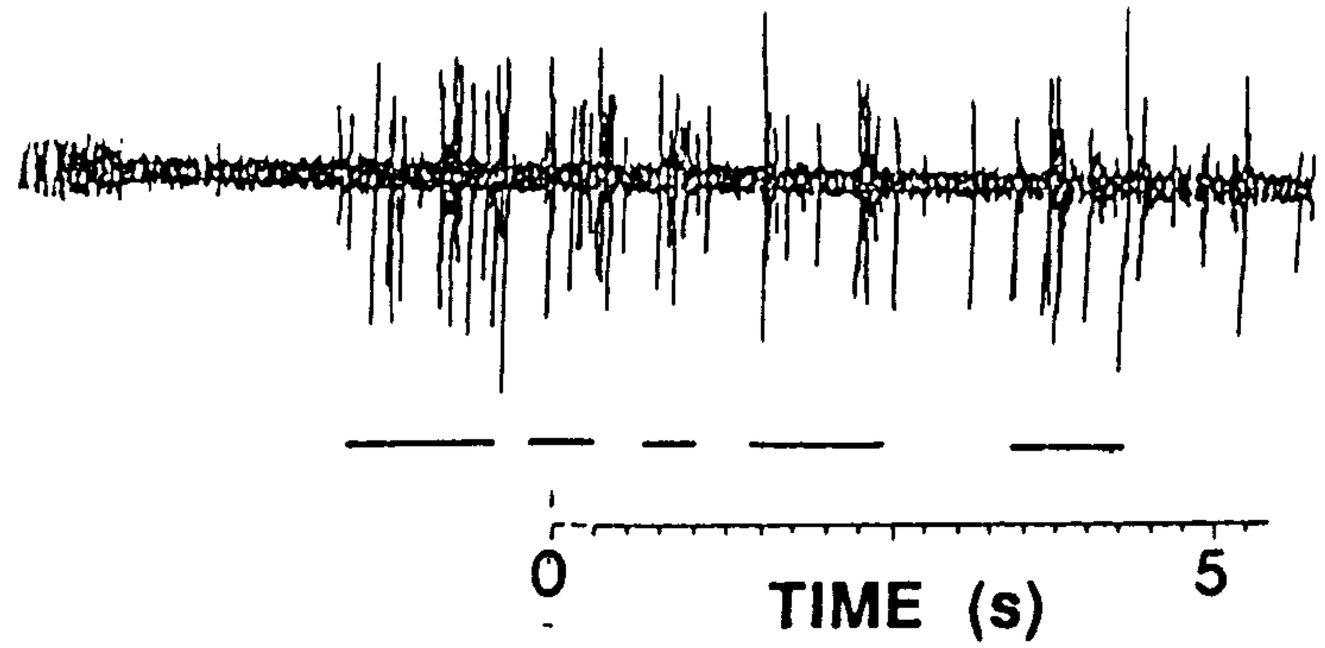


Figure 2.13 Responses recorded in root 3 of G6 carrying sensory information from the uropod endopod, to movement of an identified plumose seta from one of the dorsal fields using a mounted needle. The position of the seta is indicated by the star on the diagram of the endopod (centre). Nerve traces show the responses obtained when the hair was stimulated in the rostral, caudal, lateral and medial directions. Dotted areas indicate the fields of plumose setae. Scale bar shows time in seconds.

ROSTRAL

TIME (s)

0

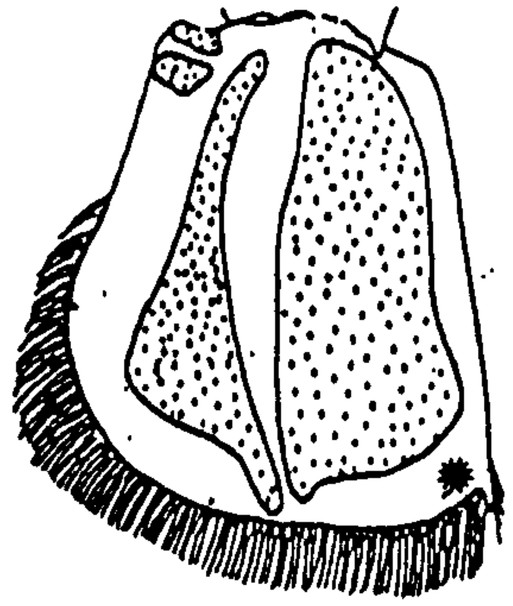
2·5

MEDIAL

TIME (s)

0

2·5



TIME (s)

0

2·5

LATERAL

CAUDAL

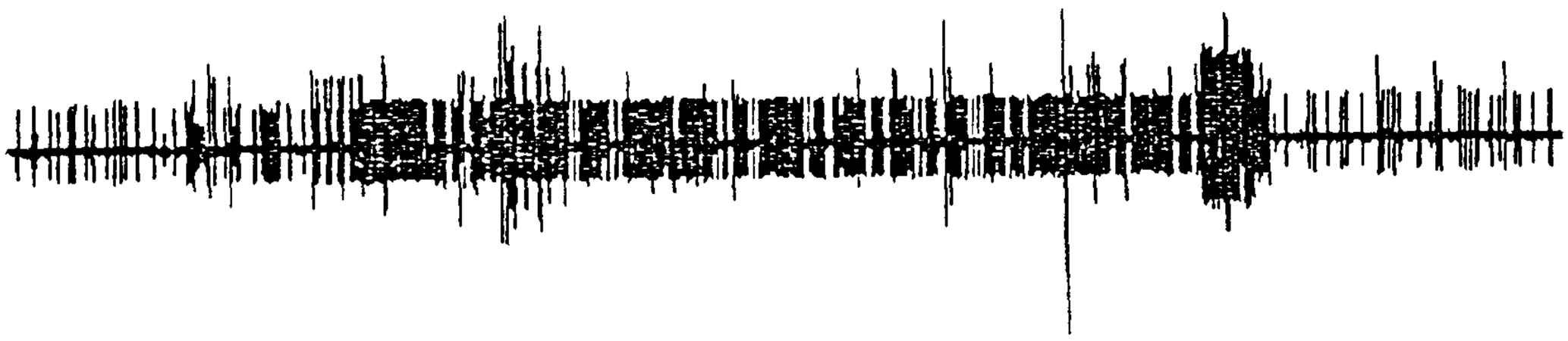
TIME (s)

0

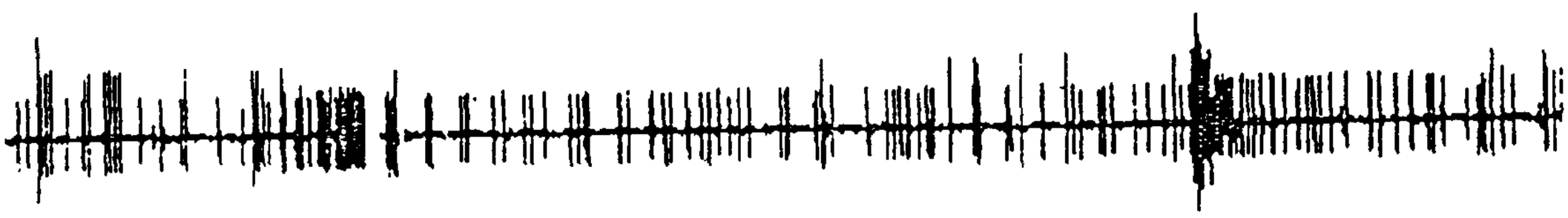
2·5

Figure 2.14 Responses recorded in root 3 of G6 to movement of a guard hair with a mounted needle. Traces show the responses to rostral (top) and caudal (bottom) movement of the seta. Bar indicates stimulus. Scale bar shows time in seconds.

A ROSTRAL



CAUDAL



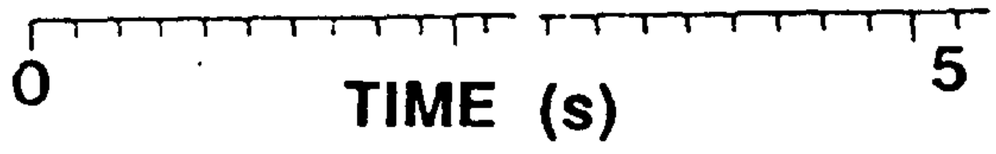


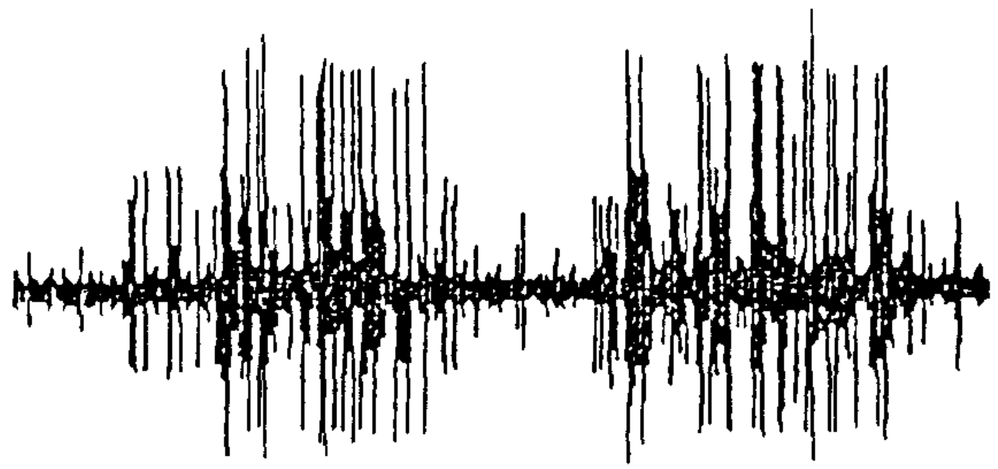
Figure 2.15 Responses recorded in the leg nerve from leg two of *Nephrops* to tactile stimulation of an identified bunch of squamous setae on the flat surface of the propodus. The traces show the responses to movement of the bunch of setae in the distal, proximal, lateral and medial directions. Bar indicates stimulus. Scale bar indicates time in seconds.

PROXIMAL

MEDIAL

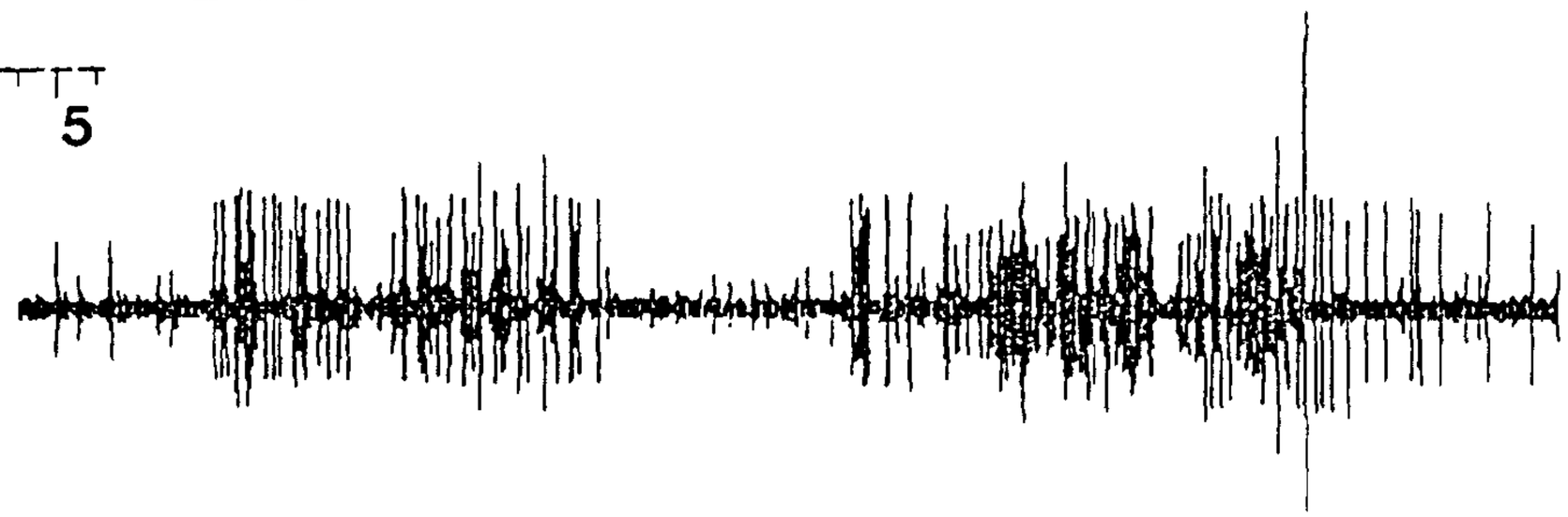
0 TIME (s) 5

LATERAL



DISTAL

0 TIME (s) 5



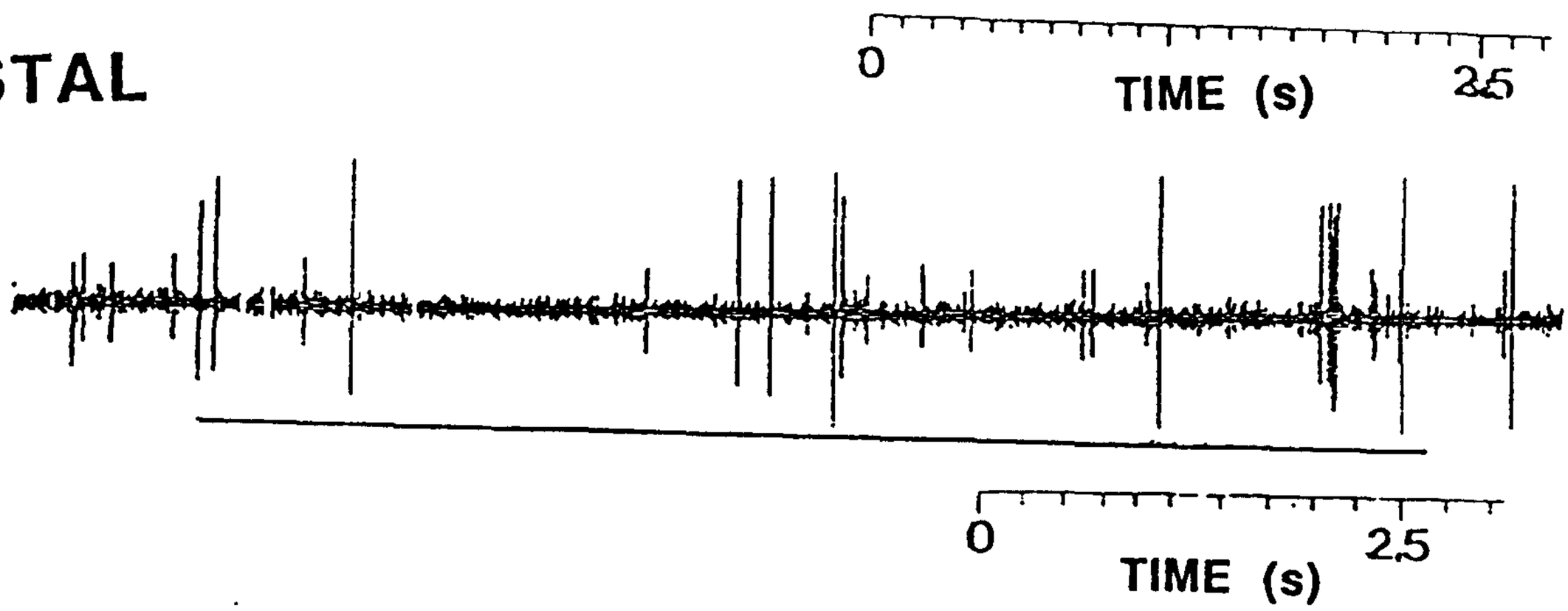
0 TIME (s) 5

0 TIME (s) 5

Figure 2.16 Responses recorded in the leg nerve from leg two of *Nephrops* to movement of the lateral squamous setae on both the propodus (upper) and the dactyl (lower) in the distal and proximal directions. Bar indicates stimulus. Scale bar indicates time in seconds.

PROXIMAL

DISTAL



PROXIMAL

DISTAL

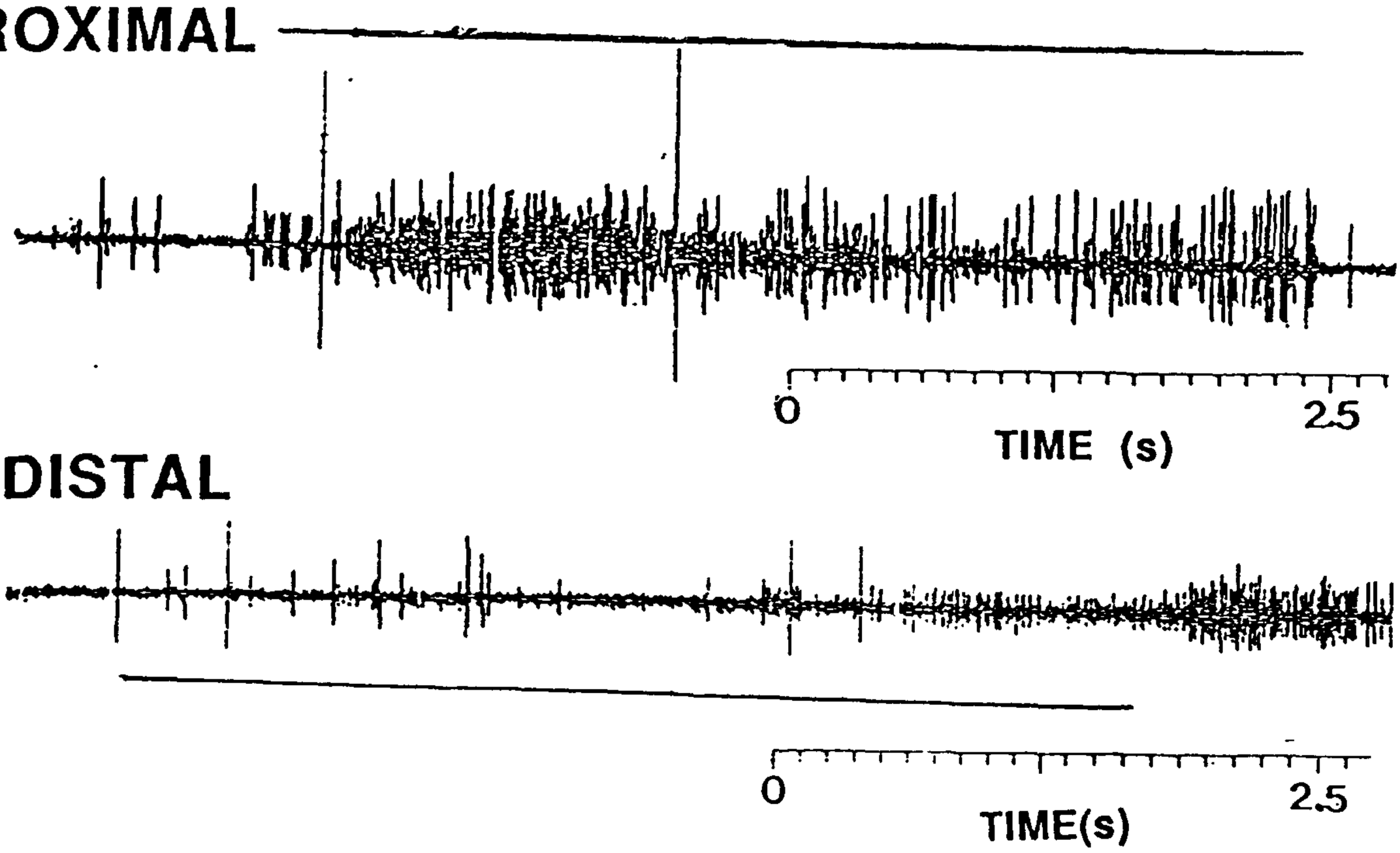
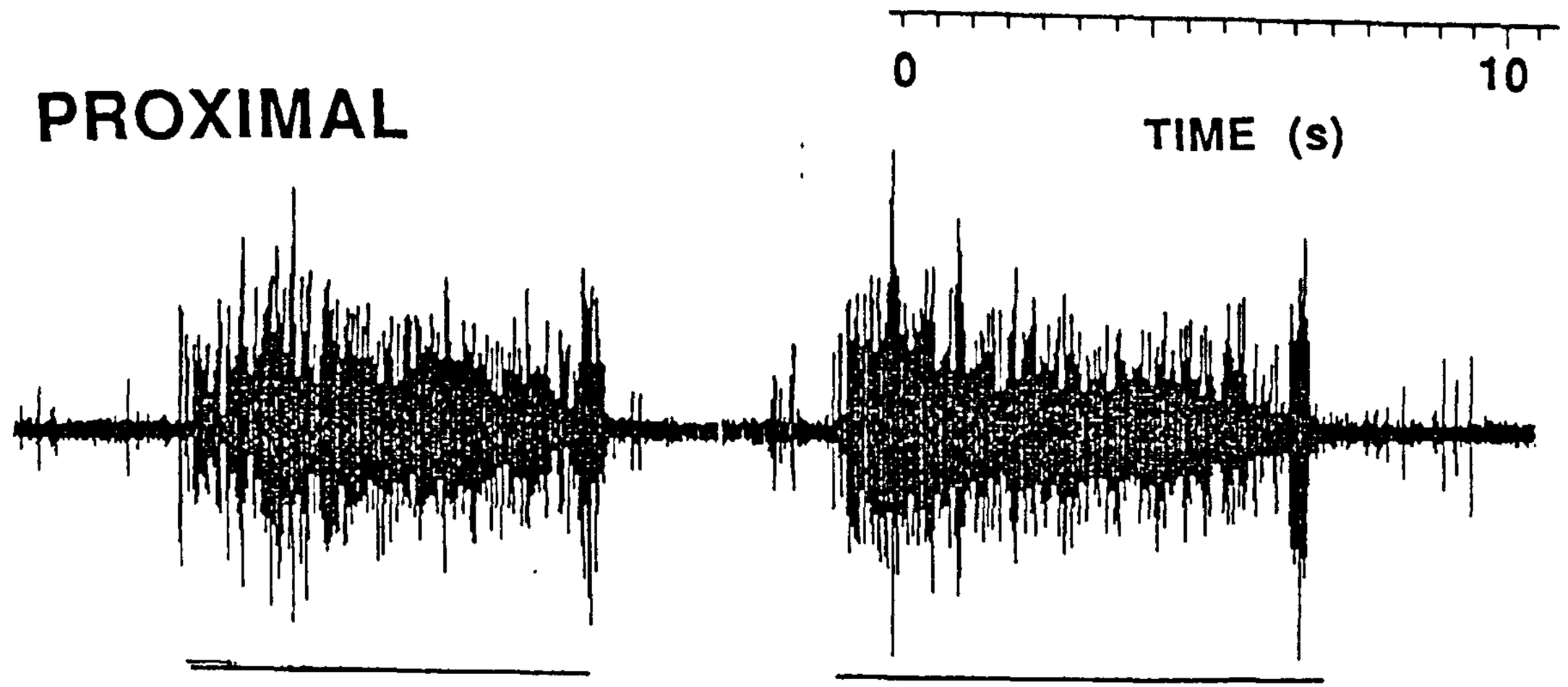


Figure 2.17 Responses recorded in the leg nerve from leg two to tactile stimulation of the lateral squamous setae in the distal and proximal directions. Bar indicates stimulus. Scale bar shows time in seconds.


PROXIMAL
0
10
TIME (s)

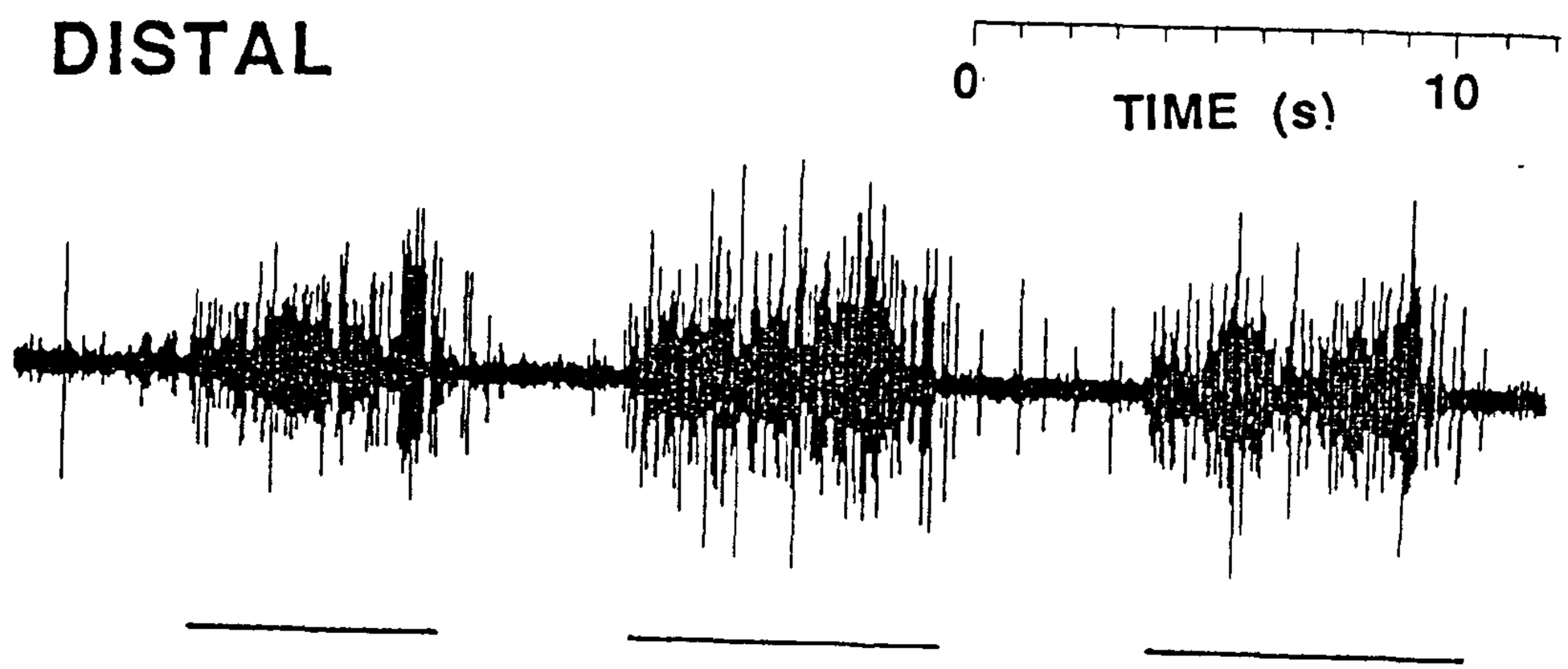

DISTAL
0
TIME (s)
10

Figure 2.18 Responses recorded in the leg nerve from leg four of *Nephrops* to stimulation of the hairs on the dactyl with a water jet in the proximal, distal and medio-lateral directions. Bar indicates stimulus. Scales bar shows time in seconds.

A PROXIMAL

TIME (s)

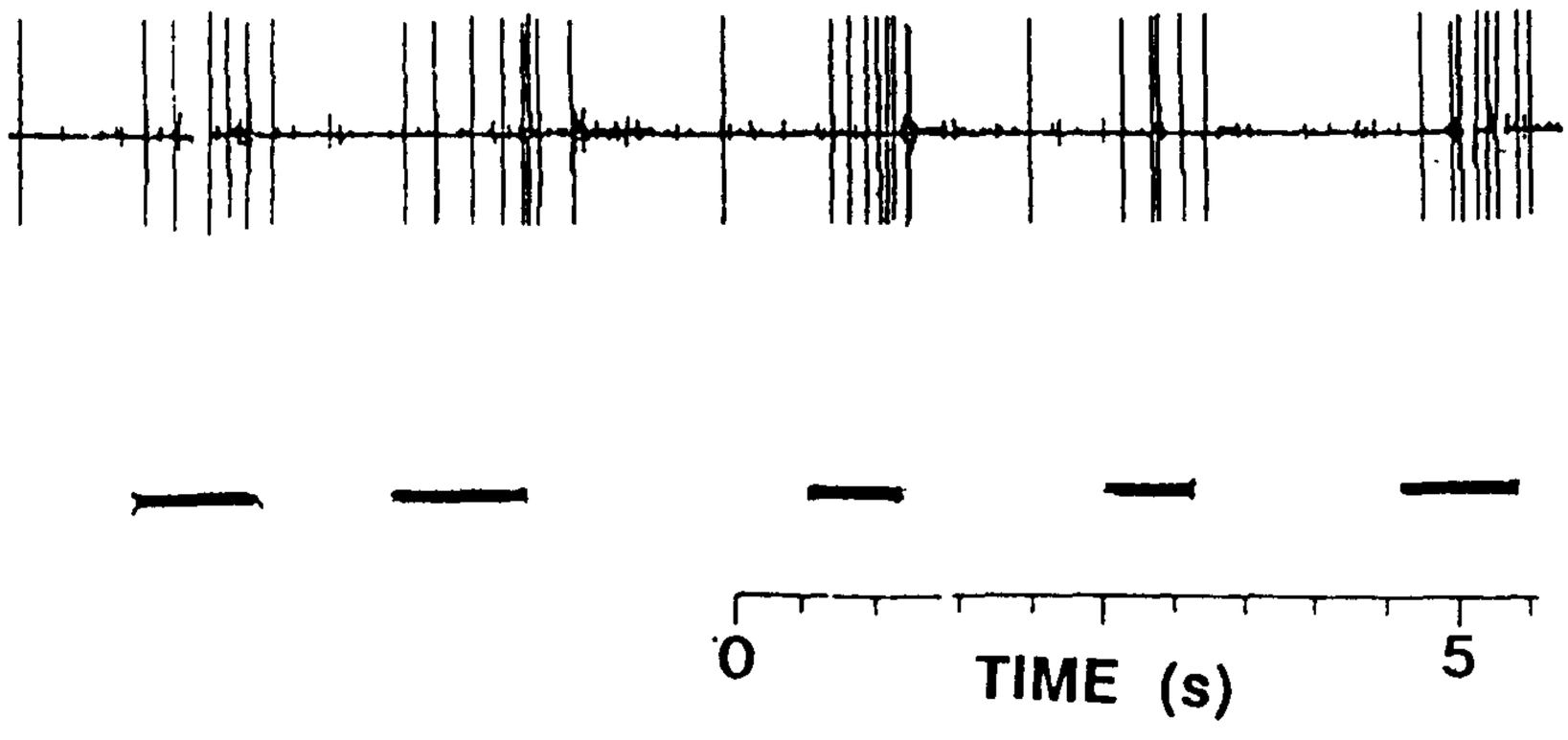
0 5

B MEDIAL LATERAL

TIME (s)

0 5

C DISTAL



TIME (s)

0 5

Chapter 3

RESPONSES SHOWN BY SENSORY AFFERENTS AND INTERNEURONES OF *Nephrops* TO WATER BORNE VIBRATIONAL STIMULI.

3.1 INTRODUCTION

There is a considerable body of data on the responses of crustacean mechanosensory systems to water borne vibrations, most of which has been carried out on the crayfish and the lobster. Some of these studies have concentrated on correlating the morphological characteristics of the setae with their sensory properties and have characterised the sensory responses of single neurones to water borne vibrations (Vedel and Clarac, 1976; Derby, 1982; Hatt, 1986; Shelton and Laverack, 1970) Intracellular studies have characterised the morphology and physiological response properties of abdominal and tritocerebral interneurones (Sigvardt et al., 1982; Reichert et al., 1982; Tautz, 1987).

Chapter 2 has shown that many of the hairs on the uropods and walking legs of *Nephrops* are mechanosensory, and preliminary experiments have demonstrated that the hair receptors respond to hydrodynamic stimuli such as directed water jets and water borne vibrations. Bending of the hair by the stimulus causes stimulation of the receptor cells at its base. It is evident from these recordings that populations of receptors exist at the hair bases which may respond to stimulation in opposite directions (Figs. 2.16 and 2.17). Wiese (1976) demonstrated, both morphologically and physiologically, that two sensory cells are present at the bases of the setae on the crayfish telson and that these cells responded to headward and tailward movements of the seta respectively. In addition, he found a hinge-like articulation at the base of the seta along with guiding structures which only allowed the shaft to move along one axis of movement which was the same for the entire population.

The majority of studies on the responses of the Crustacea to water borne vibrational stimuli fall into three main categories

according to which component of the stimulus has been varied: frequency, amplitude or direction. Knowledge of an animal's responses to these parameters yields useful information about the frequency and amplitude ranges over which it is sensitive. This can be related to the biologically relevant stimuli which might be found in the field and also to the animal's capability for stimulus localization.

3.1.1. **Stimulus parameters**

Studies of **frequency sensitivity** have reported that the afferent nerves from setae on the telson of the crayfish, *Procambarus* are most sensitive to water borne vibrations of 20Hz, although they show responses up to 100Hz (Wiese, 1976). The hydrodynamic receptors which are found all over the body surface of the rock lobster *Palinurus* showed maximal responses between 40-70Hz, and Vedel and Clarac (1976) have suggested that these receptors may be used as a kinetic sense organ by the animal during tailflipping when it has no contact with the ground. Hairs on the chelipeds of the crayfish *Cherax destructor* were maximally stimulated by frequencies of 150-300Hz (Tautz and Sandeman, 1980) and responses over a similar frequency range were found by Chichibu et al. (1978) while monitoring the activity of sensory receptors on the antenna of the crayfish *Procambarus*.

Plummer et al. (1986) characterised the responses of crayfish abdominal inteneurones to water borne vibrations of different frequencies and they grouped the units into three categories depending on the range of their frequency response. Low pass interneurones (LPI) were maximally stimulated at 30Hz and inhibited above 100Hz, broad band interneurones (BBI) were maximally stimulated between 30-60Hz but responded up to 80Hz and high pass interneurones (HPI) were inhibited at low frequency but responded up to as much as 400Hz. It is known that such inhibitory mechanisms operate in the vertebrate ear and are

used to highlight relevant frequencies. Some of these interneurones are known to be presynaptic to command elements controlling escape behaviour in the crayfish and others are command elements for abdominal posture. Therefore it is possible that when stimulated by the relevant frequencies these interneurones may be able to initiate motor behaviour.

Sensory thresholds can be measured in relation to one of several components of the stimulus, most commonly displacement, pressure or velocity. Threshold measurements are often combined with studies of frequency sensitivity as the lowest threshold is often reached at a particular frequency or range of frequencies (Wiese, 1976; Tautz and Sandeman,1980). Threshold sensitivity for *Nephrops* has not been calculated in this way but has been determined using behavioural measures, for reasons given in Chapter 5.

Studies of the **directional sensitivity** of the nervous systems of crustaceans have been mainly carried out on *Procambarus* by Wiese and his collaborators (for review see Wiese, 1987). Initial experiments by Wiese (1976) showed that the hairs on the telson of this species were directionally sensitive and that this sensitivity was preserved in the interneurones of the abdominal nervous system (Wiese et al. 1976). He concludes that stimuli parallel to the long axis of the body are most effective in inducing responses in afferents of root 4, which innervates the telson, while stimuli originating from the side are most effective in stimulating afferents in root 3, which innervates the endopodite. This is in accord with the theory of the tailfan as a sensory array (Chapter 2) as these stimuli are parallel to the axis of movement of the hairs on these two structures respectively. Wiese and Wollnick (1983) state that the extent of directional sensitivity of an interneurone is related to the extent of its receptive field, so that

an interneurone receiving input from only one segment will be highly directionally sensitive while one which receives input from many segments may lose its directional sensitivity. The presence of lateral inhibition in the displacement sensitive pathway of the crayfish *Procambarus* has also been investigated (Wiese and Wollnick, 1983; Wiese and Schultz, 1982).

3.1.2 Stimulus conditions

Few crustacean studies which have measured the sensitivity of the animal either to stimulus amplitude or direction have taken into account the acoustic conditions under which these measurements have been made. Most of the above studies have been carried out in a small tank using surface waves as the stimulus (Wiese, 1976; Wiese et al. 1976; Wiese and Wollnick, 1983; Ebina and Wiese, 1984). They will therefore have experienced distortion of the stimulus due to reflections from the air water interfaces surrounding the tank (Chapter 1.3.; Parvelescu, 1964). This makes it extremely difficult to make accurate measurements of the amplitude of the stimulus and to define its directional components. Results of experiments performed under these conditions cannot therefore be totally reliable. Parallel studies to measure the thresholds and directional sensitivity of fish to sound have attempted to avoid these problems (Hawkins, 1981). The ideal situation is to carry out the experiments in a free sound field such as the open sea and this approach has been followed in this project to determine behavioural thresholds (Chapter 5). However, this approach is not practical for the measurement of sensory thresholds using neurophysiological methods. To improve the acoustic conditions of a laboratory tank it can be lined with acoustically absorbant material, although the thickness of this lining must be a significant fraction of the wavelength of the stimulus. Hawkins (1981)

states that at 100Hz the stimulus wavelength is 15m so this solution is clearly not practical for use at low frequency. Alternatively, the stimulus may be generated in air around a thin walled tank, but this method will generate only sound pressure not particle motion. Hawkins and MacLennan (1975) describe an appropriate design of tank which may be used for such experiments constructed from tubular steel with sound projectors at either end. If the sound projectors move in phase with each other, producing alternating rarefaction and compression, then a predominantly pressure stimulus may be generated while if they are operated out of phase stimuli with higher particle velocity or displacement components may be generated. Modifications of this design which operate in the particle motion mode have been used in a few crustacean studies to measure both displacement thresholds and frequency sensitivity in the crayfish, *Procambarus* (Tautz and Sandeman, 1980; Plummer et al., 1986). The design by Tautz and Sandeman (1980) of a tube with a sound projector at one end and a sloping shelf at the other has been adapted for use in this study. Although not as acoustically ideal as the tubes described by Plummer et al.(1986) and Hawkins and MacLennan (1975) this design was thought to be more practical for use with the available equipment and allowed the stimulus to be measured with some degree of accuracy.

The present study attempts to determine the frequency responsiveness of two afferent sensory systems of *Nephrops*, the uropods and walking legs, and of the abdominal interneurones to water borne vibrational stimuli of different frequencies. Preliminary studies of directional sensitivity of the leg afferents and the abdominal interneurones were also undertaken and although incomplete these allow some comments to be made about the directional sensitivity of *Nephrops*. Some information about the directional sensitivity of the

afferent system was already known from experiments using surface waves (Chapter 2).

3.2 MATERIALS AND METHODS

Recordings were made from units in the uropods, walking legs, and interneurones of *Nephrops*. The preparations were stimulated using tactile stimuli and vibrating water stimuli both in a small dish and in an acoustic tube.

3.2.1 The acoustic tube

A cylindrical tube 45.5 cm in length and 12 cm in diameter was constructed from perspex by the workshop of the Zoology Department at Glasgow University (Fig. 3.1). At one end of the tube was a rubber diaphragm which had a Derritron vibrator fitted to it. The other end of the tube was open with a 45^{o} sloping shelf which helped to dissipate the water movement and so cut down reflections back into the tube. Halfway along the inside of the tube a small turntable was fitted on which the specimens to be recorded from could be fixed. This turntable allowed rotation of the specimens during the recordings. Above this turntable was a hole measuring 4 cm in diameter to allow access and movement of the electrode. This hole could be sealed during the experiments. The Derritron vibrator was driven by a 25 Watt power amplifier.

The design of the tube allowed measurements to be made of both the magnitude of the particle displacement in the tube and its phase lag relative to the voltage signal from the Derritron. The phase lag was measured using an accelerometer which was suspended in the tube directly above the specimen turntable. The magnitude of the displacement was measured using a calibrated electromechanical transducer (Schaevitz Linear Voltage Displacment Transducer-LVDT) (Sand, 1981) which was fixed outside the tube between the vibrator and the diaphragm. The assumption was made that the displacement of water

inside the tube would be the same as the displacements measured by the LVDT in air although it is likely that there would be some degradation in this signal at the specimen position. The voltage output of the Derritron and the output of the accelerometer at different frequencies were then recorded on an FM tape recorder (Racal Store 4) These recordings were then played back onto a pen recorder and the phase lags measured using a digitising tablet (Summagraphics MM1200) linked to a Tuscan S100 microcomputer.

The output of the LVDT was recorded on tape along with that of the Derritron. The recordings were played back through a CED interface to a Tandon microcomputer and the output was dumped to a printer. The displacements were measured directly from the paper traces produced. Both the phase lag and the magnitude of the particle displacement were plotted against frequency.

3.2.2 Specimen preparation and dissection

a)The uropod preparation

The posterior two segments were removed from the animal and pinned out in a small dish of saline (Miyan, 1984). The cuticular ribs and intersegmental membranes overlying the abdomen were removed to expose the 6th abdominal ganglion (G6) and its roots. The ventral surfaces of the 'protopodites' of the uropods on one side were removed to expose roots 2 and 3. All other roots were cut close to G6. The abdominal nerve cord anterior to G6 was held in forceps while the ganglion and the remaining nerve roots were freed from the underlying membranes and laid over the uropods which they supplied. The uropods were then dissected away from the rest of the now denervated preparation which was then discarded. This left a preparation consisting of the two uropod rami from one side together with their

nerve roots, G6 and a small section of the abdominal nerve cord from G5-G6. This preparation was then pinned out dorsal side upwards in the dish.

b) **The leg preparation**

The walking legs were removed from the animal by autotomy. The articular membrane was then broken at the C-P joint and the two sections of the leg carefully pulled apart. Generally this left the distal 2-3 segments of the leg with about 1.5cm of trailing nerve. This method of exposing the nerve did not seem to stretch or damage it if it was done slowly and carefully. The leg was pinned out in a dish of cooled saline.

c) **The interneurone preparations**

The whole animal preparation

This preparation was used both here and during later experiments carried out to determine the motor responses to vibrational stimuli (Chapter 4). Except where otherwise stated the whole animal was prepared in the following way. The claws were removed by autotomy and the animal was secured ventral side upwards to a wax-lined perspex platform using rubber bands, in a small tank of seawater. The sea water was cooled to 10^{o}C by passing the supply piping through a bath of coolant maintained at 7^{o}C by a Churchill thermo-circulator cooling unit. The water was circulated and filtered using an Eheim charcoal filter. A small window was made in the ventral intersegmental membrane between the cuticular ribs in each abdominal segment to expose the ventral nerve cord. The nerve cord was desheathed using insect pins. Recordings were either made *en passant* (section 3.2.3) or from the ends of cut nerve roots.

Isolated abdomen preparation

Because of the size limitations of the tube the whole animal preparation could not be used in it. Instead an isolated abdomen preparation was used under these circumstances. The posterior three or four segments of the abdomen together with the tailfan were removed from the rest of the animal and pinned out ventral side upwards in a Sylgard filled dish which formed the rotatable platform in the tube. The cuticular ribs and swimmerets overlying the abdomen were removed to expose the abdominal nerve cord. The cord was dissected free from its roots and the surrounding muscle tissue in the anterior two segments of the preparation so that it could be recorded from and rotated to different positions. The turntable was then placed in the tube and the tube was filled with seawater at 10^{o}C.

3.2.3. Recordings

En passant recordings were made using a suction electrode with a small diameter tip made from Portex polythene tubing. The electrode was mounted on a Narashige micromanipulator. The signal was preamplified using an Isleworth A101 amplifier and filtered by a Neurolog NL125 Filter before being viewed on a Tektronix 5115 Storage Oscilloscope and recorded on a Racal Store 4 tape recorder. In the case of the tube a modified electrode was used. This electrode had a tip made from Portex tubing and a 4cm glass tube (Clark Electromedical GC 100F-6) 100 μm in diameter was attached between the tip and the electrode to extend its length.

3.2.4 The stimulus

The leg and uropod preparations were stimulated with either tactile, hydrodynamic or vibrating water stimuli. Tactile stimuli were

generated using either a paintbrush or a fine mounted insect pin which was bent at the end. Hydrodynamic stimuli were generated by directed water jets from a Pasteur pipette. Vibrating water stimuli were generated either by the tube or as surface waves which were generated by a plastic ball attached by a metal rod to a Derritron vibrator. The ball was placed in the water close to, but not touching, the preparation. Frequencies from 2-600Hz were tested.

The receptive fields of the abdominal interneurones to mechanical stimuli were determined either using a paintbrush to produce tactile stimuli or using a directed water jet. The frequency responsiveness of interneurones was tested using vibrating water stimuli both in a small dish using a vibrating ball and in the acoustic tube. The directional sensitivity of the interneurones was also tested in the tube by rotating the turntable holding the abdomen to 8 possible positions. At each position the responses of the interneurones were tested to a range of frequencies from 2-300Hz.

3.2.5 **Analysis**

Two different computer systems were used in the analysis of the data. The first mode of analysis used a Tandon PCA20 microcomputer with a CED 1401 interface. Spike data were fed via window discriminators to the event inputs of the interface. Using the CED Spike 2 program, the times of events and analog to digital samples (taken down to 2 ms intervals) were written directly to the hard disc. From these stored data, views of the original records could be reconstructed and dumped to the printer, and various calculations could be performed to produce, for example, phase or interval histograms.

The second mode of analysis used a Tuscan S100 microcomputer and

interface. The recorded nerve data (Fig. 3.2.A) were fed via a window discriminator and interface to the computer. The driving voltage signal to the Derritron was fed via a zero point crossing device which generated a TTL pulse at a chosen point in the cycle. These timing signals in turn were fed to the computer to trigger the accumulation of phase histograms of spike data. Phase histograms were constructed over a selected number of cycles, (usually corresponding to 1 s of analysis at each frequency) (Fig. 3.2.B). Circular statistics were used to calculate the circular mean of the distribution, its standard deviation and the magnitude of the mean vector R_c which gives an indication of the 'peakiness' of the distribution (Batchelet, 1981).

3.2.6 **The statistical parameters**

The circular mean: The circular mean value gave an indication of the mean position of spike firing within the phase cycle. This gave an indication of the phase position of the majority of the spikes. The circular mean moved through the phase cycle as the frequency increased. Two examples of this for leg units are shown in Figure 3.3. The circular mean often showed a saw tooth pattern, initially increasing as the frequency increased, then jumping half a cycle and starting to move through the cycle again as the frequency increased until another transition point was reached. The correction for phase lag, also shown in Figure 3.3.A and B did not remove this jump but merely shifted it between different cycle positions. This implied that on either side of the transition point units were responding to opposite directions of water movement. The standard deviation (SD) around the circular mean was also calculated which showed whether the spread of spikes around the circular mean was broad or narrow. This gave some indication of the strength of phase locking, as a small SD indicated strong phase locking while a large or incalculable SD

indicated weak phase locking.

The Spike number: The spike number (SN) was calculated over 20 cycles in all cases. This value was often less than 1 spike per cycle so a line indicating where one spike per cycle occurred was marked on each graph. Figure 3.4 shows the spike numbers of three typical leg units, one from each category showing their frequency range. It was common in both the uropods and the legs that low frequency units had more spikes than the other two categories. The units often produced one or more spikes per cycle at the lower end of their range but the value for SN usually dropped as the frequency increased to less than one spike per cycle. This was due to the unit "missing" cycles and not to a progressive loss of responsiveness. Missing cycles did not necessarily mean that the response was poorly phase-locked. In order that the strength of phase locking may be checked the SD of the circular mean should be studied along with the spike number. If the spike number is low and the SD of the circular mean is small (Fig. 3.5.A-C, **a**) then the unit is firing at the same phase position each time even though it does not occur every cycle. However,, if the SD of the circular mean is large or unavailable, and the spike number is small (Fig. 3.5. A-C, **b** and Fig. 3.5.D-E, **a**) then the spikes could have been occurring over a wide range of phase positions in the cycle indicating a weak response.

The R_c value: The R_c value was strongly interconnected with the other two values and is derived from the circular mean (Priest, 1983). A high R_c value should indicate strong phase locking and a low R_c weak phase locking. This, however, can be very misleading unless the other factors discussed above are taken into account. If the R_c value and the spike number are high then the response is strongly phase locked

(Fig. 3.5.A-C, **c**). If the R_c is high and the spike number is low then the standard deviation of the circular mean must be taken into account. If this is also low then the response is phase locked but with "missing" cycles (Fig. 3.6.A-C, **a**). If the R_c value is low and the spike number is high then the response is strong but not phase locked. The standard deviation of the circular mean is likely to be high or unavailable in this case. If the R_c is low and the spike number is also low then it is likely that the unit is not responding to the stimulus.

3.3 RESULTS

3.3.1 Measurement of displacement and phase lag in the acoustic tube

Figure 3.7.A shows the phase lag of the particle displacement in the acoustic tube relative to the driving voltage signal of the Derritron amplifier at a range of frequencies. The relationship between the phase lag of particle displacement and the frequency does not become linear until above 20Hz and so reliable corrections for phase lag cannot be made below this level. Above 20Hz it shows a positive relationship with frequency.

Figure 3.7.B & C show the particle displacement (in cm) calculated at the speaker diaphragm for the two voltage levels used in the neurophysiological experiments. The magnitude of the displacement in each case falls off sharply with increasing frequency.

3.3.2 Responses of afferent hair units in the uropods and walking legs of *Nephrops* to water borne vibrational stimuli.

The results presented here were, in the case of the uropods, obtained from 26 animals, and in the case of the legs, obtained from 25 animals. The responsiveness of units was predetermined by general mechanical stimulation of the uropods and walking legs. Units were classed as responsive if the level of firing was above the resting level during the stimulus period. Afferent responses were obtained from the uropods in the range of 1.4-100Hz. In almost all cases no responses were seen above 100Hz. Leg units were responsive over a broader frequency range, from 2-450Hz in some cases, and these units were often not responsive to frequencies below 20Hz. Within these ranges the responses of units could be subdivided into three nested categories, low, intermediate and high depending on the upper

frequency limit of their response.

3.3.2.a **Responses of afferent hair units in the uropods**

Low frequency units: Low frequency units found in the uropods responded up to 20Hz and were by far the largest category of units. Phase histograms of the responses of typical low frequency units tested in a small dish to water borne vibrations can be seen in Figures 3.8 and 3.9 and the general response parameters may be determined from these data. Figure 3.8 shows phase histograms from a unit responding up to 20Hz. At lower frequencies of 1.4-2Hz, although there was a general excitation of the unit above the resting level, the response was not phase locked and spikes occurred throughout the cycle. From 3-6Hz the firing of the units showed two peaks of responsiveness which moved to different positions as the frequency increased, but maintained the same relative spacing. At 8Hz and 10Hz the response was very strongly phase locked, with all or most of the spikes occurring in a narrow phase band. At 12Hz and 16Hz, although the response was still phase locked, secondary peaks were again becoming evident and the spread of the distribution was quite broad. At 20Hz two peaks were visible both with a widespread distribution. No responses were seen above 20Hz. These general features of strong phase locking with large spike numbers between 4-10Hz and of shifting phase positions and decreasing spike number of response peaks with increasing frequency were found in all low frequency units (Fig. 3.9). The responses of low frequency units tested in the acoustic tube followed the same general pattern. Figure 3.10 shows the statistical values calculated for one such unit. The circular mean moved through the phase cycle twice in this case as the frequency increased, showing that the main body of the spikes occurred at different points in the cycle at different frequencies. The RC value showed two peaks, one at

4-8Hz and another at 14-20Hz.

Intermediate frequency units: Intermediate units found in the uropods responded from 2-50Hz. Examples of two such units can be seen in Figures 3.11 and 3.12. The first unit was active from 2-50Hz (Fig. 3.11) although at lower frequencies the response was not phase locked and the spike number was low. The response became phase locked above 8Hz, with the strongest response occurring from 20-40Hz at which frequencies the spike number increased slightly. The response started to decline at 50Hz and above this frequency no response was seen. The other unit typifies those which although they responded up to 50Hz were clearly tuned to lower frequencies of between 4-8Hz (Fig. 3.12).

High frequency units : Units in this category responded from 2-100Hz and very occasionally higher. Figure 3.13 shows the responses of a unit which was tested in a small dish. It became phase locked between 4Hz and 80Hz but was most narrowly tuned, with high spike numbers around 10Hz.

Statistical values from a high frequency unit tested in the acoustic tube are shown in Figure 3.14. In this case the unit had a broad distribution with the strongest responses between 30 and 50Hz.

3.3.3 The responses of afferent hair units in the walking legs of *Nephrops* to water borne vibrational stimuli of different frequency, direction and amplitude.

Low frequency units : Low frequency units in the walking legs were responsive up to 60Hz. Figure 3.15 shows the responses of a typical low frequency unit which in this case was from leg 4 and was also tested for directional sensitivity in three positions in the tube. The unit responded in all three positions tested and the overall frequency range of this response was from 1.4-30Hz although in

position 1 (Fig. 3.15) the unit only responded up to 20Hz. The statistical values for this unit are shown in Figures 3.16-18. On the whole there was no change in the phase position of the circular mean as the orientation of the preparation to the water movement was changed indicating an omnidirectional responsiveness of this unit.

Intermediate units: A large number of intermediate units were found. None of these units was tested for directional sensitivity. Although the upper end of the response range was 100Hz, the lower end of the range was more variable with some units responding to frequencies as low as 1.4Hz while in others the response did not become apparent until 20Hz.

High frequency units: Histograms showing the responses of a typical high frequency unit are shown in Figure 3.19. This unit was from leg 2 of the animal and responded from 20-300Hz. The response was phase locked throughout its response range with fewest spikes seen at low and high frequencies and more spikes seen in the mid range of the response, around 80-120Hz. Its responses were tested to water movement in two directions (also shown on Figure 3.19) 90^{o} apart. The histograms show that the responses shift by a half phase between position 1 and position 2. The spike numbers also showed marked differences between position 1 and position 2, indicating that position 1 was the preferred direction (Fig. 3.19). This would suggest that this unit was directionally sensitive. Changes in the amplitude of the stimulus did not appear to have any effect on the response of this unit.

3.3.4 The responses of mechanosensory interneurones in the abdominal nerve cord to water borne vibrational stimuli.

3.3.4.a Receptive fields of abdominal interneurones responsive to mechanical stimuli.

The receptive fields of 21 abdominal interneurones responsive to mechanical stimuli were determined (Fig. 3.20). The majority of interneurones had receptive fields falling into categories C and D in Figure 3.20 with the receptive fields restricted to the segment of recording (the 6th) and the tailfan or only the tailfan. Thus they received mostly ascending input. Class A interneurones were responsive to stimulation of all 6 segments and the tailfan and class B were responsive to stimulation of the posterior half of the abdomen and the tailfan. The latter two categories were therefore receiving both ascending and descending input from sensory cells. One interneurone was found which only responded to stimulation of the mouthparts and it is likely that there will be other abdominal interneurones with descending input from the thoracic part of the animal.

Most interneurones in classes B, C, and D were unilateral and had receptive fields which were ipsilateral to the site of recording. The intereurones in class A were most often bilateral and interneurones with bilateral receptive fields were seen in each of the other classes, many with predominant ipsilateral input, and a few with predominant or exclusively contralateral input. The presence of the bilateral and contralateral interneurones indicated that information must cross over in the abdominal nerve cord in both ascending and descending directions. Experiments were conducted to determine whether sensory information crossed over in the 6th ganglion by cutting roots 2 and 3 carrying sensory inputs from the uropods ipsilaterally to the site of recording. The contralateral uropods were then stimulated

causing bursts of activity in the interneurone. This showed that information did cross over in G6.

3.3.4.b. **Frequency response of abdominal interneurones to water borne vibrational stimuli**

The frequency responses of abdominal interneurones were tested in 13 animals. The overall frequency range of the sensitivity of the interneurones was from 2-200Hz. However, within this range the interneurones showed range fractionation and could be divided into two categories depending on the range of their response. Low frequency interneurones responded within the range 2-100Hz and high frequency interneurones responded up to 200Hz. Within these ranges the interneurones did not necessarily respond over the full range and wide and narrowly tuned interneurones were seen particularly in the low frequency band.

Low frequency interneurones : An example of histograms produced from a typical wide range low frequency interneurone can be seen in Figure 3.21. This unit showed wide range tuning and responded somewhat weakly to frequencies from 2-100Hz. The highest spike numbers were seen at low frequency, falling below the one spike per cycle line around 20Hz. The response was most strongly phase locked at low frequency, with the strongest phase locking occurring at 8Hz where the R_C value was at its highest and accompanied by a high spike number and low SD of the circular mean (Fig. 3.22).

The statistical data from a narrowly tuned low frequency interneurone can be seen in Figure 3.23. This unit responded from 3-8Hz; more typical narrow band units responded up to 20Hz. The spike numbers were high throughout the range, never falling below the one

spike per cycle line. Correspondingly, the R_c values were always above 0.5 and the SD of the circular mean was low showing strong phase locking throughout. The circular mean showed the typical pattern seen in the sensory units, moving through the phase cycle as the frequency increased and going through a half cycle phase shift between 4 and 6Hz.

Low frequency units which were tuned over an intermediate range were also seen generally responding up to 40 or 50Hz (Fig 3.24). The unit in Figure 3.24 was tuned to between 20 and 30Hz and had high spike numbers throughout its range with the spike number falling below the 1 spike per cycle line only above 35Hz.

High frequency interneurones: These responded from 2-200Hz and were often tuned around one particular frequency (Fig. 3.25) The illustrated unit responded up to 200Hz and was most strongly phase locked around 40 and 30Hz. The statistical parameters showed that around the optimum frequencies, the R_c values were high and the standard deviation of the circular means low (Fig. 3.26).

3.3.4.c. Directional sensitivity of abdominal interneurones tested in the acoustic tube.

The responses of mechanosensory interneurones from 13 animals were tested in response to water borne vibrations with different directions relative to the body. In general all of these units responded to water movements from each direction tested, but certain differences were seen in the numbers of spikes produced in a given time and at a given frequency when the direction of the stimulus was changed.

Figures 3.27 and 3.28 show three examples of the responses of mechanosensory interneurones tested in this way. The responses of the

interneurones shown in Figure 3.27 were tested in 8 directions to the stimulus. Histograms have been plotted for each direction which show the phase position of the spikes over 5 seconds. The phase changes at 45^{o} and 225^{o}. The circular plot shows the spike numbers plotted as percentages of the maximum spike number, which in this case occurred at 45^{o} (ie. a displacement to the ipsilateral side).

Figure 3.28 shows the responses of two other interneurones tested with stimuli of constant frequency over 6 directions. These units also showed maximum sensitivity at 45^{o} (ie. ipsilateral to the recording site). However, one of these (black squares) showed strong responses over half a cycle of rotation while the other (white squares) showed weak responses to directions other than 45^{o}.

It would appear from these results that the abdominal interneurones of *Nephrops* are directionally sensitive and may show wide or narrow directional tuning.

3.4 DISCUSSION

It is clear from the results presented in this chapter that the nervous system of *Nephrops*, like that of many other decapod crustaceans, is responsive to water borne vibrational stimuli. The responses of three systems, the uropod and walking leg afferents, and the abdominal interneurones, have been tested to water borne vibrations of different frequencies. The results of this study have shown that the afferent uropod nerves are responsive from 1.4-100Hz, the afferent leg nerves respond from 20-450Hz and the abdominal interneurones are responsive to frequencies of between 2-200Hz.

These frequency ranges are comparable with those which have been determined for other crustacean species. Wiese (1976) showed that hairs on the telson of *Procambarus* showed strongest responses to stimuli of 20Hz but responded over a wider overall range of 0.5-100Hz which is comparable with the uropod units of *Nephrops*. The hydrodynamic receptors of the rock lobster responded best to stimuli of 40-70Hz (Vedel and Clarac, 1976) which although higher than the optimal response frequencies of *Nephrops* still encompasses the same overall range. The abdominal interneurones of *Procambarus* (Plummer et al., 1986) responded from 4-400Hz, although some had narrower ranges comparable to those of *Nephrops*. The frequency responsiveness of the walking legs of a crustacean species has not been previously studied but the chelae of *Cherax* showed responses from 150-300Hz (Tautz and Sandeman, 1980), which is within the range of responsiveness shown by the *Nephrops* leg afferents.

3.4.1 Range fractionation.

Range fractionation of the response to frequency was seen in both

the afferent systems of *Nephrops* studied here and the abdominal interneurones. Low, intermediate and high frequency units have been identified on the basis of their frequency response. Plummer et al. (1986) point out that frequency selectivity is only one of several parameters which may be used to identify neurones. Therefore the categories in which these units have been placed in this study are purely arbitrary and identify the units solely on the basis of their frequency response. This does not imply that the units within each category are identical in the nature of their response.

The uropod units were divided into 3 categories, low (1.4-20Hz), intermediate (2-50Hz) and high (1.4-100Hz); low frequency units were the most commonly encountered category. It is interesting to note that these categories are nested and not completely separate, with the result that the system as a whole is highly sensitive to low frequencies. Most of the optimal response frequencies for these units were below 10Hz, the highest optimal frequency range being seen between 30-50Hz.

The abdominal interneurones of *Nephrops* were divided into two categories, low (2-100Hz) and high (2-200Hz) which were also nested. Wide, intermediate and narrow range tuning was seen within the low frequency category. The responses of the low frequency interneurones were of a similar overall range to the uropod units (2-100Hz) and the extent of tuning seen within this category was closely correlated with the classes of uropod units seen. It is likely therefore, that some of the inputs feeding onto these interneurones may be from uropod receptors. However, some of the abdominal interneurones responded up to 200Hz while the uropods never showed responses to such high frequencies. This implies that while it is possible that these high frequency interneurones may still receive inputs from the uropods, it is likely that they also receive inputs from other receptors, perhaps

on the abdominal segments or the telson which may be responsive to higher frequencies.

Range fractionation of the frequency response has also been observed in the interneurones of other decapod species. Plummer et al. (1986) defined three classes of interneurones in the abdominal nerve cord of *Procambarus* on the basis of their frequecy response. They found low pass (1-100Hz), high pass (6-400Hz) and broad band (1-80Hz) interneurones. Although the high pass interneurones found in *Procambarus* are responsive to higher frequencies than those of *Nephrops*, the low pass interneurones are responsive over a similar range. Plummer et al. (1986) also observed inhibition of the low pass interneurones at frequencies above 100Hz and of the high pass interneurones below 6Hz. There is no conclusive evidence to suggest the presence of inhibitory influences in *Nephrops*, but it is possible that an inhibition may operate at high frequencies as the abdominal interneurones in this case did not respond above 200Hz. If it exists the presence of inhibition may allow the nervous system to focus on behaviourally relevant frequencies of water borne vibrations using contrast enhancement (Wiese, 1987). Range fractionation has also been demonstrated by Ebina and Wiese (1984) with reference to the velocity thresholds of the abdominal interneurones of *Procambarus*.

It is likely that the range fractionation seen within the nervous system of *Nephrops*, in relation to different frequencies of water borne vibrational stimuli, reflects the fact that receptors with setae of different morphology may respond over different frequency ranges. There is a vast morphological diversity of setae on both the uropods and walking legs of *Nephrops* (Chapter 2) and the nerves in both of these appendage types show range fractionation. Structural differences in crustacean hair structure and their effects on the mechanics of

hair movement in a fluid medium have been considered by Tautz (1979). He states that hair shafts which are short or smooth will be best stimulated at comparatively high frequencies compared with hairs which have feather-like projections. These projections serve to improve the coupling of the hair with the water medium. Most of the setae on the uropod are feathered and this correlates with the fact that low frequencies are most effective at evoking responses from the uropods. The legs on the other hand are devoid of feather type hairs and have many longer hairs. Although these setae possess scales and setules they may be considered smooth for this purpose. The frequency ranges over which the legs were sensitive were correspondingly much higher.

3.4.2 **Directional sensitivity**

Preliminary experiments conducted during the course of this study have indicated that *Nephrops* may possess the capability for directional sensitivity in relation to water borne vibrational stimuli. Both directionally sensitive and omnidirectional units were found in the afferent leg nerves. Units recorded in leg 2 (Fig. 3.19.) showed circular mean values with different phase positions in response to the same frequencies of water borne vibrational stimuli when the leg was moved through 90^{o}. Correspondingly the spike numbers were also higher in the first position indicating possible directional sensitivity. Bunches of setae on leg 2 of *Nephrops* have already been shown to produce different sized spikes in response to movement of the setae in different directions (Fig. 2.15). Units recorded from leg 4 showed similar responses to stimulation in three different directions encompassing a 180^{o} arc (Fig. 3.15). Similar spike numbers, R_c values and circular means were obtained at each frequency tested in each position and it would seem from these data that this unit was omnidirectional. It was perhaps surprising in this case that the

circular means did not change as the leg was moved to different positions and the reason for this remains unclear. It is possible that the cuticular hair connecting with the latter units may have been one of the smooth hairs on the dactyl of leg 4, as experiments conducted in chapter 2 have indicated that such hair receptors may be omnidirectional (Fig. 2.18).

The results of initial tests of directional sensitivity in the abdominal interneurones were also suggestive of its existence within this system. This is in accord with the results presented in Chapter 2 which showed that some of the hair receptors on the uropods show directional responses to tactile stimulation in different directions. Directional plots (Figs. 3.27-28) showed the responses of two interneurones which responded over the full range of positions tested but showed maximal sensitivity (measured as highest spike number) in response to water borne vibrational stimuli from a certain direction. The stimuli causing maximal activity in two of these interneurones were broadly headward movements of the water which were asymmetrically distributed at 45^{o} to the rostro-caudal axis of the animal. Another interneurone (Fig. 3.28), although possessing the same direction of maximal sensitivity as the previous two interneurones, was not as narrowly tuned and had a high level of responsiveness to stimulation over almost half a cycle.

The directional plots shown here have been constructed in the same way as those of Wiese and Wollnick (1983). However, there were differences between that study and this one in the experimental method. Wiese and Wollnick (1983) moved the stimulus (in this case a source of surface waves) around the animals in a small tank. Not only does this lead to acoustic problems of reflection (Chapter 1) but it is possible that the stimulus may not have been uniform in amplitude

at all parts of the preparation due to attenuation. In the experiments presented in this chapter the preparation was moved relative to the stimulus source within an acoustic tube. Athough this made the exepriments technically much more difficult to perform it ensured that each area of the preparation received stimulation of uniform amplitude. Surface waves were not used here both because of the acoustic problems and also because this particular stimulus would have little relevance to *Nephrops* in the field as they live at a minimum depth of 5m under the sea surface.

The abdominal interneurones have been shown to receive inputs from distinct receptive fields within the abdomen, and there is evidence to suggest that these areas overlap (Fig.3.20). Some of these receptive fields consist of only one segment while others receive inputs from the entire abdomen. It has been suggested by Wiese (1987) that interneurones in the crayfish which have small receptive fields are more directionally sensitive than those with large receptive fields, which may have lost the capacity for directional sensitivity and therefore are omnidirectional. This may also be true for *Nephrops*. Directional sensitivity has been extensively investigated in the crayfish *Procambarus clarkii* (for review see Wiese, 1987). Initial studies demonstrated that the sensory hairs on the telson of the crayfish possessed two receptors at their base which responded to headward and tailward water movements respectively. It now seems generally accepted that the presence of two sensory cells is a common feature of crustacean mechanosensory systems. However, in functionally similar systems for detecting air currents and vibrations in terrestrial arthropods only one sensory cell is found at the hair base but within the population of hairs each has a different preferred direction of movement dictated by the orientation of the hinge at the base with the result that the population as a whole can respond to

stimulation from any direction. Wiese (1976) suggests that the structural dichotomy in these systems may stem from the fact that they are used in different media ie. water and air and that the two sensory cells found at the base of the hydrodynamic receptors may allow them to detect vibrations which are superimposed on a background of constant hydrodynamic flow.

Processing and transmission of the sensory information continues in the interneurones of the abdominal nerve cord and there is evidence to suggest that in the crayfish at least, the segregation of sensory information into headward and tailward categories is preserved in the abdominal interneurones (Wiese et. al. 1976). This may convey some additional information to the animal about the position of the stimulus source. Directionality plots have been established for many of these interneurones (Wiese and Wollnick, 1983; Wiese, 1987) both in the crayfish and the shrimp *Crangon crangon*. Wiese (1987) states that the interneurones are generally less directionally sensitive than the sensory afferents, which may be a result of convergence.

It is also evident from work on the crayfish system that mechanisms of lateral inhibition (as proposed in Chapter 2), do exist in the central nervous system of these crustaceans (Wiese and Schultz, 1982; Wiese, 1984) at the level of the second order interneurones. That inhibition is present centrally is in contrast with lateral line systems of fish and amphibians where inhibition of the response occurs peripherally, due to efferent innervation, and prevents stimulation of the receptors when the animal is itself moving. This mechanism helps to prevent transmitter depletion (Flock, 1971; Flock and Russell, 1973; 1976). Wiese (1984) proposes that contrast enhancement occurs in the crayfish pathway via common mode rejection which may serve to increase the directionality of interneurones with large receptive

fields and hence to accentuate detection of the direction of the stimulus.

Wiese (1987) states that in a directionally sensitive pathway the presence of both headward and tailward sensitive elements and contrast enhancement mechanisms are not a necessary requirement and proposes that these mechanisms operate in the nervous system of the crayfish in order that the animals might be able to locate sources of AC disturbance against a background of constant stimulation by gross DC movements of the medium. There is however, no strong conclusive evidence to date to back up this theory and it remains to be seen whether such an effect occurs in *Nephrops*.

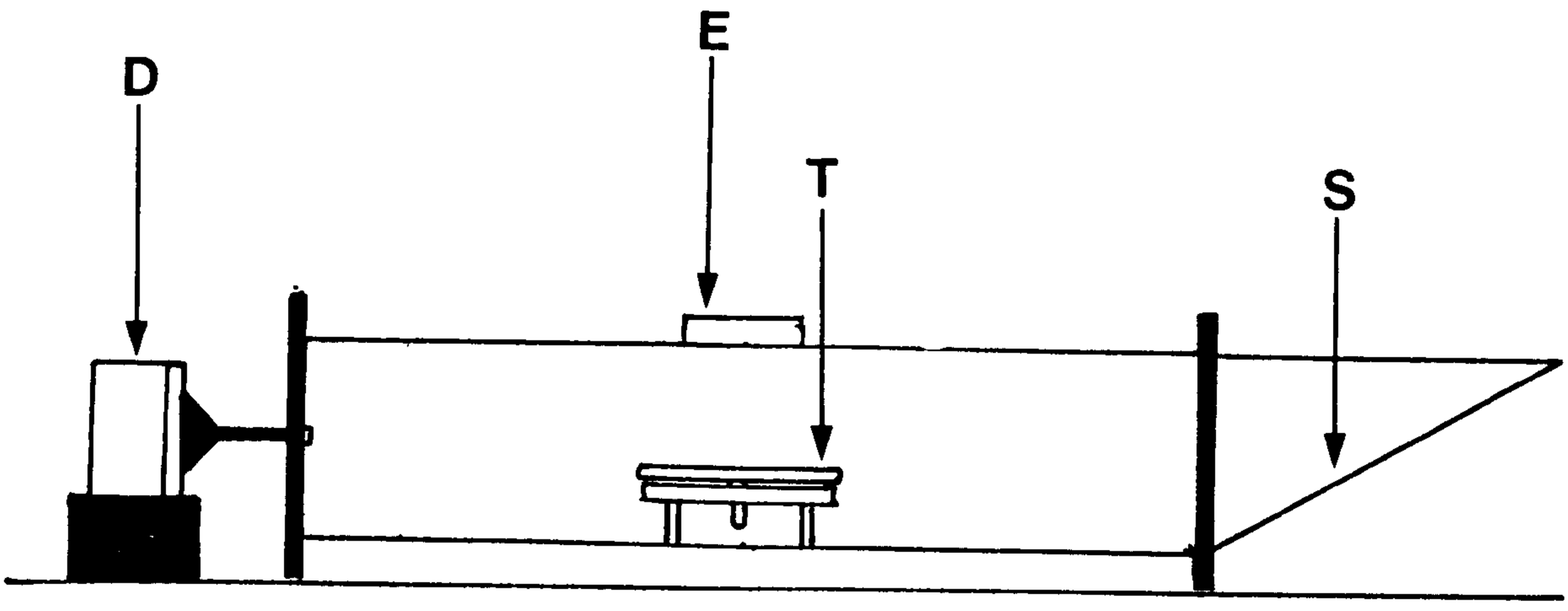
Figure 3.1 Diagram of the acoustic tube used in experiments to determine the sensitivity of the afferent nerves and interneurones of *Nephrops* to water borne vibrational stimuli.

T. Turntable

E. Hole to allow entry of electrode

S. Sloping shelf to allow dissipation of water movement.

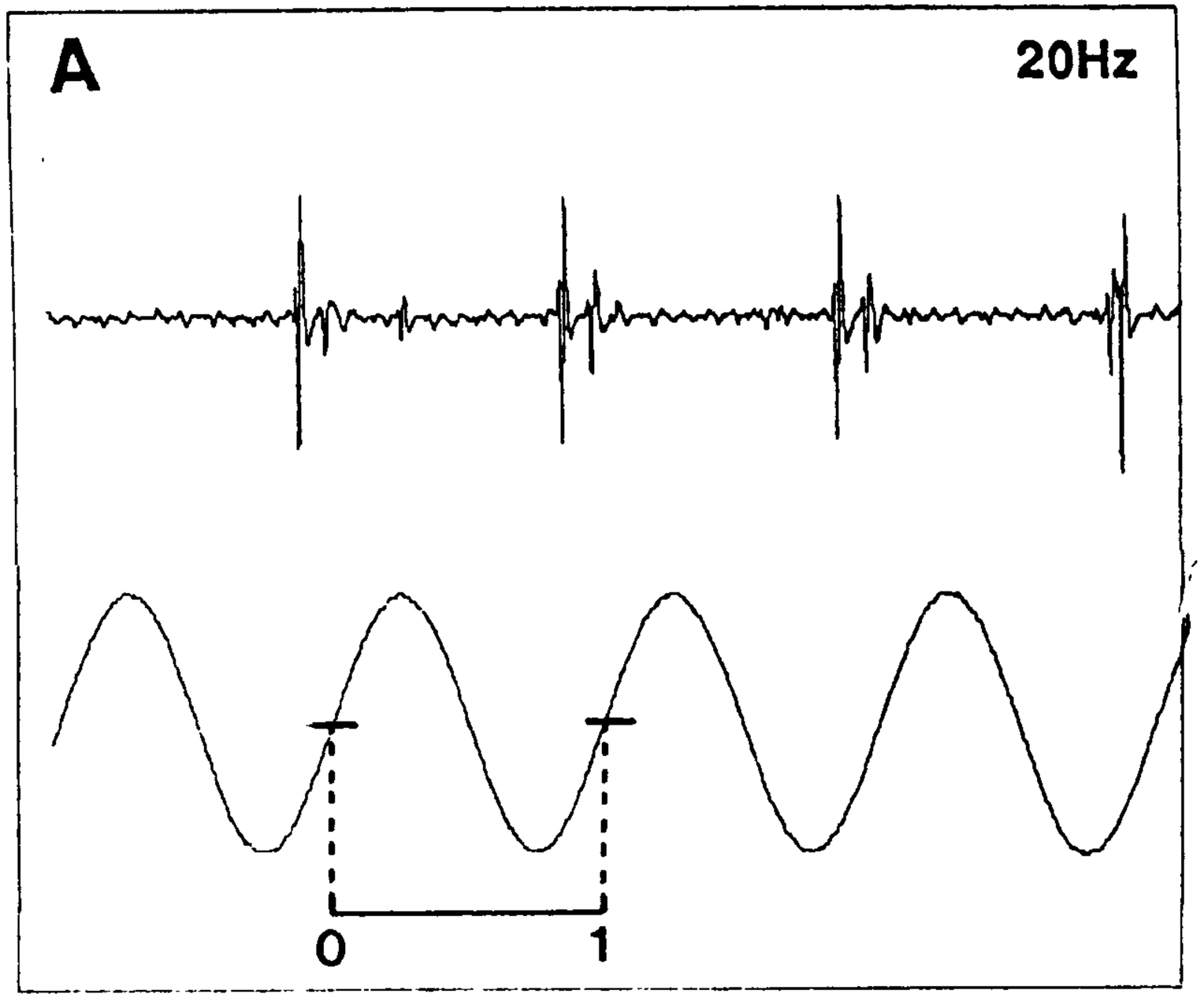
D. Rubber diaphragm and derritron vibrator

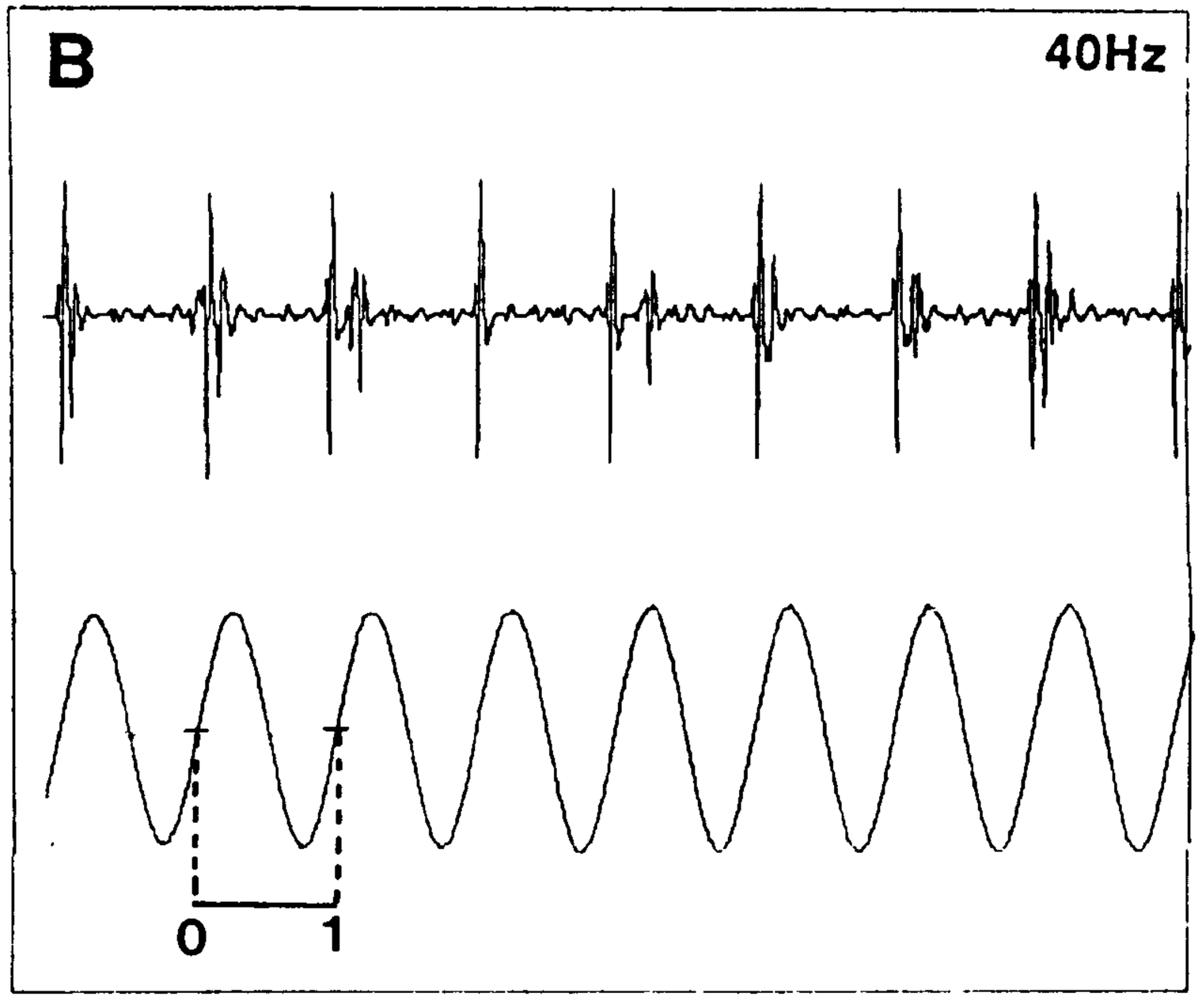


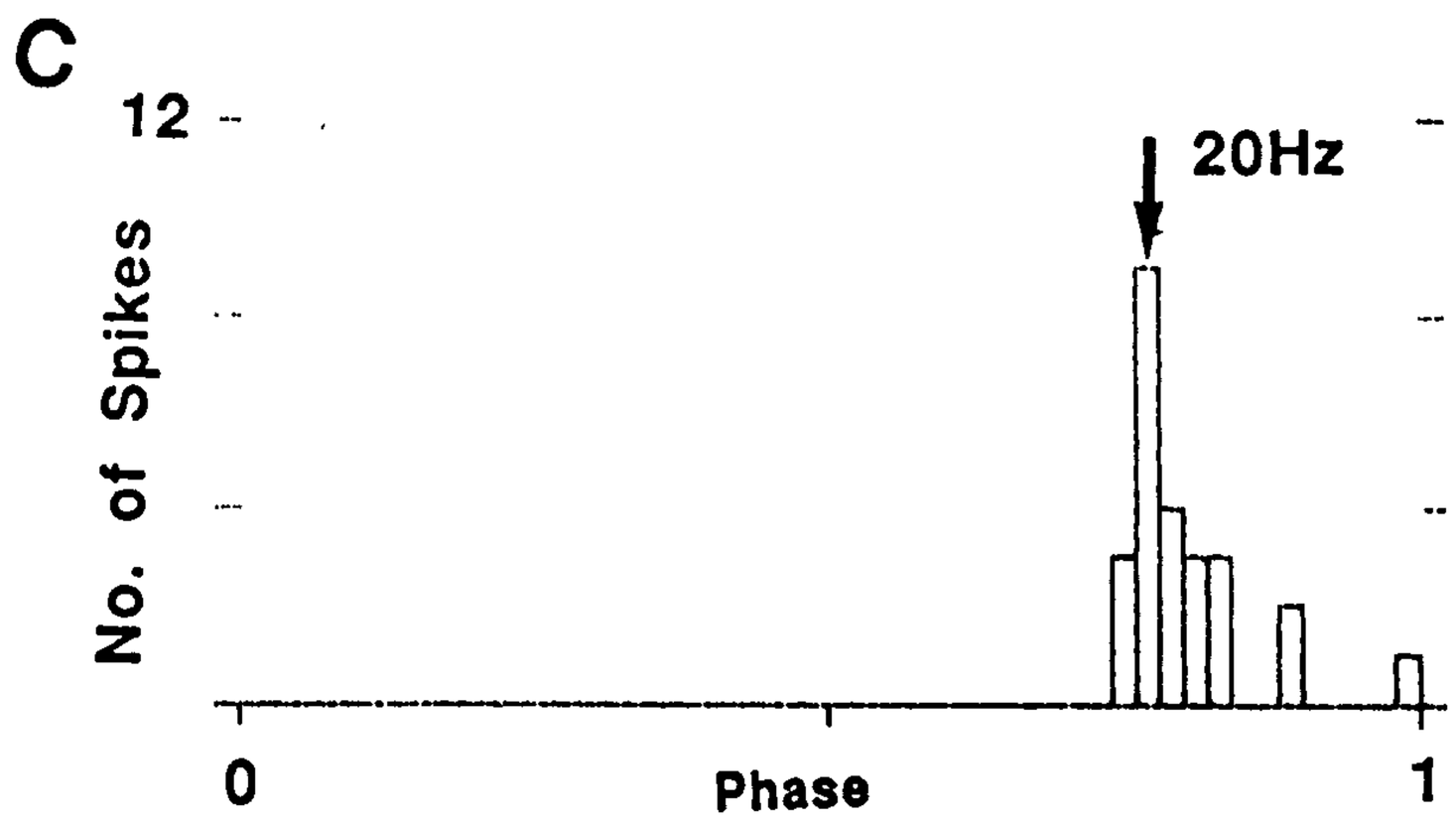
10cm

Figure 3.2 A and B. Raw nerve spike data recorded in the leg nerve from leg two of *Nephrops* in the acoustic tube showing the responses to water borne vibrations of 20 and 40Hz

C and D. Phase histograms produced from the above spike data showing the distribution of nerve spikes within the phase cycle gathered over 20 and 40 cycles respectively. The arrows on each histogram indicate the position of the circular mean.


A
20Hz
0
1


B
40Hz
0
1


C
12
20Hz
No. of Spikes
0
Phase
1

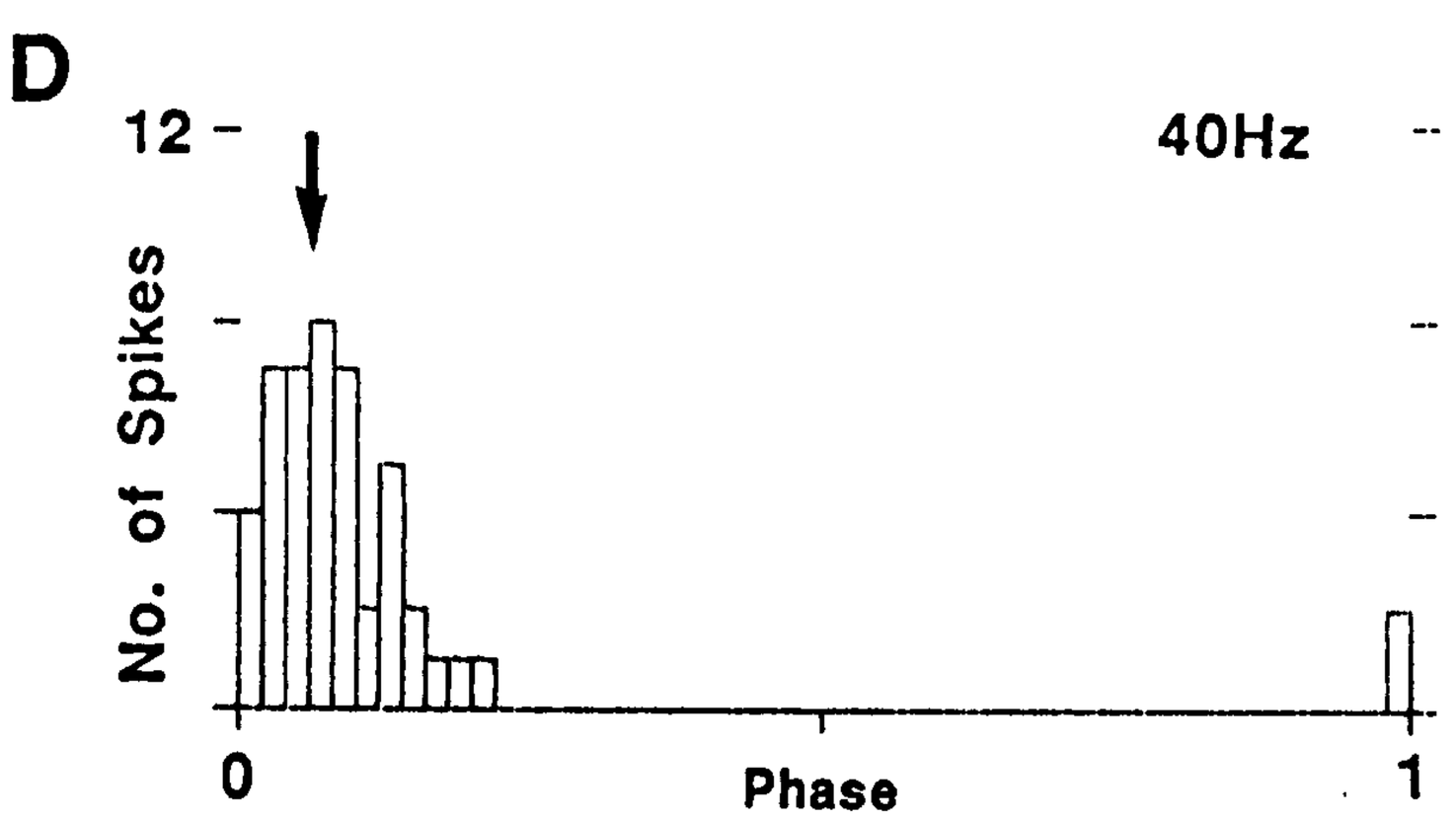
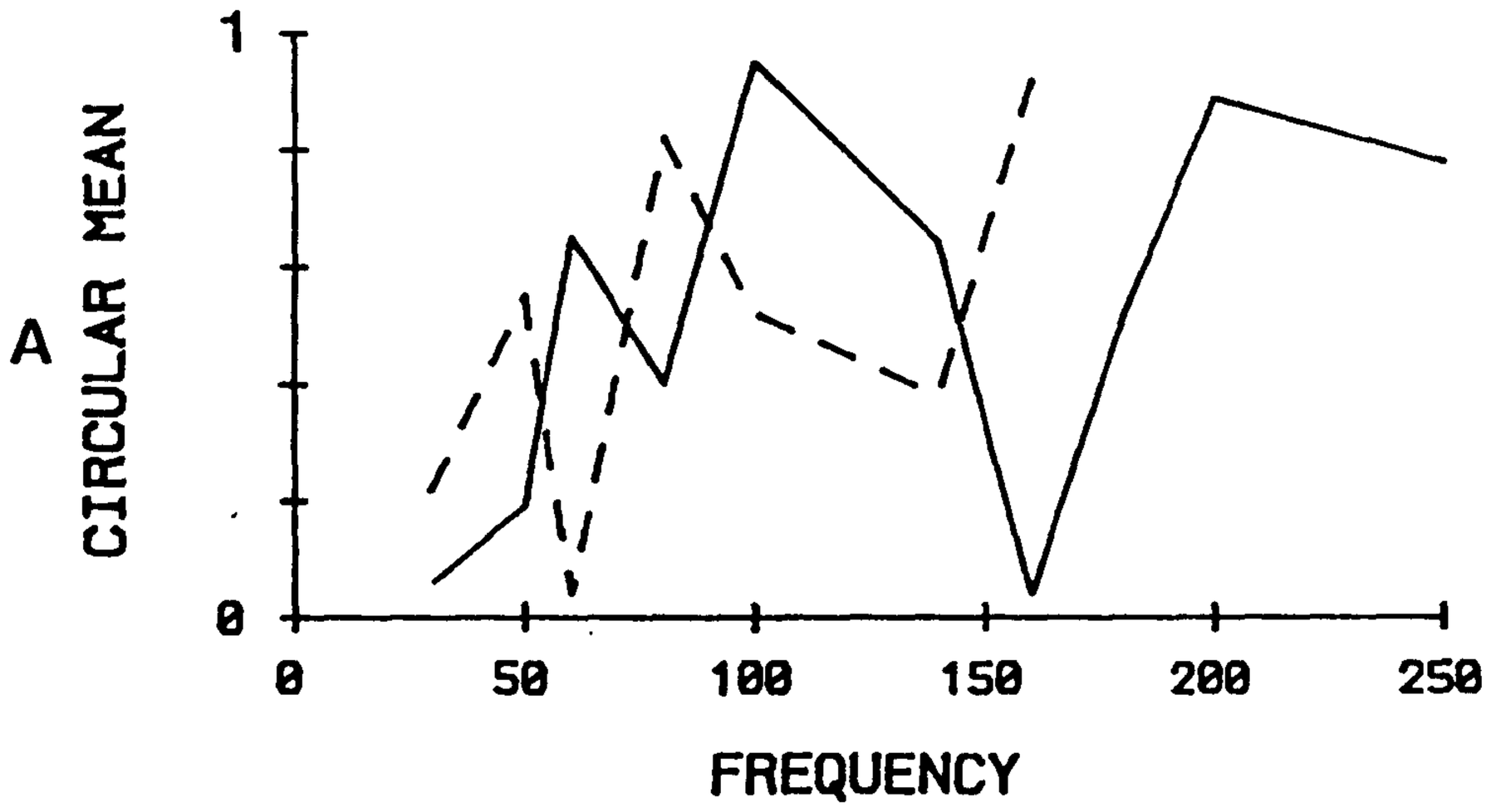

D
12
40Hz
No. of Spikes
0
Phase
1

Figure 3.3 Plots showing the phase shift of the circular mean with frequency in a high frequency (A) and an intermediate frequency (B) leg unit. The plots show the circular mean against frequency (Hz). The solid line shows the circular mean with reference to the driving signal of the Derritron vibrator, the dotted line shows the circular mean after a correction factor has been applied (Fig. 3.7.a) to account for the phase lag between the voltage signal and the actual water motion in the tube.

HIGH FREQUENCY UNIT



INTERMEDIATE UNIT

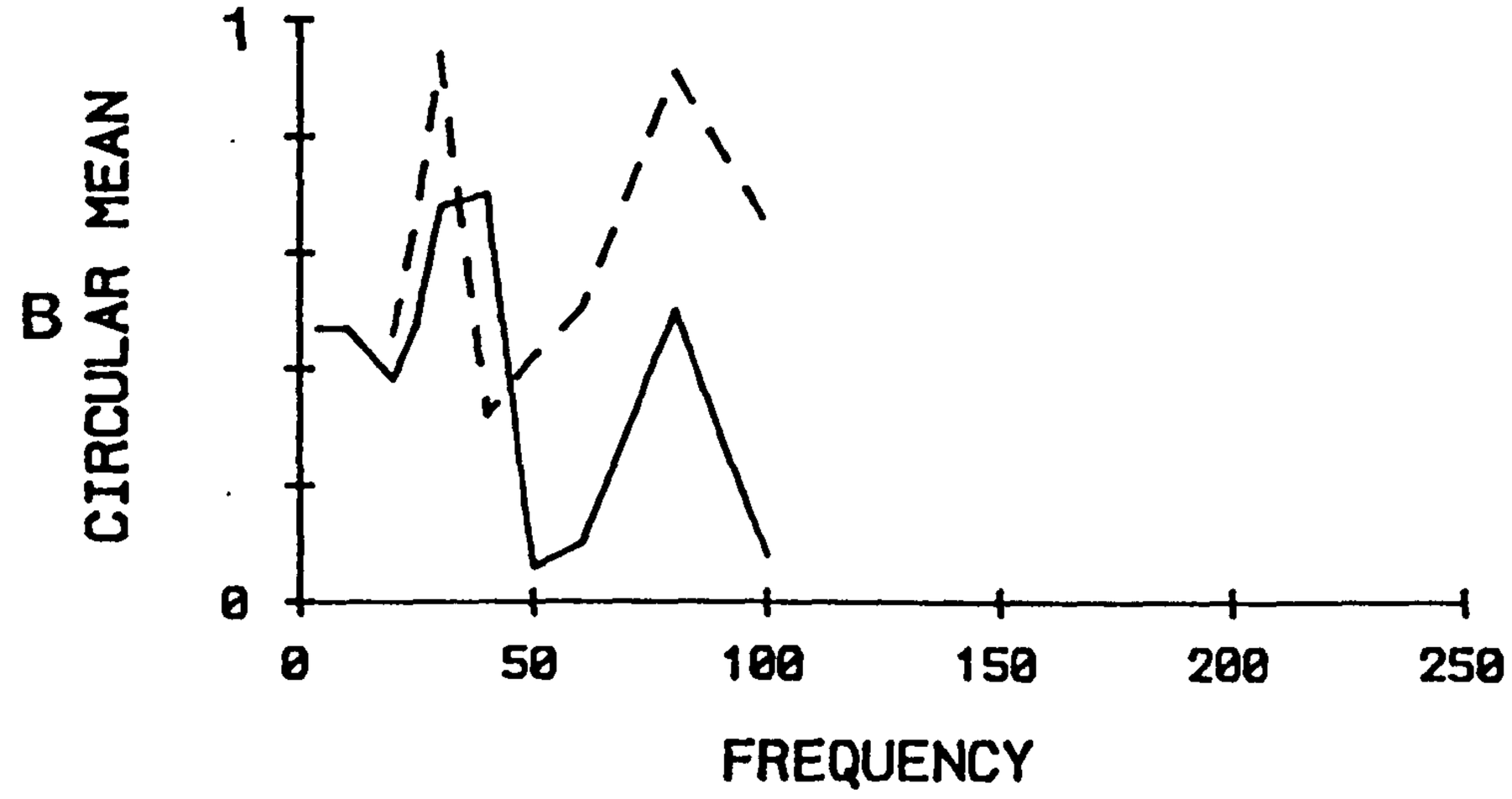


Figure 3.4 Plot of the spike number of low (a), intermediate (b) and high frequency leg units (C) showing the decline in spike number with frequency (Hz) in each case. The dotted line represents one spike per cycle.

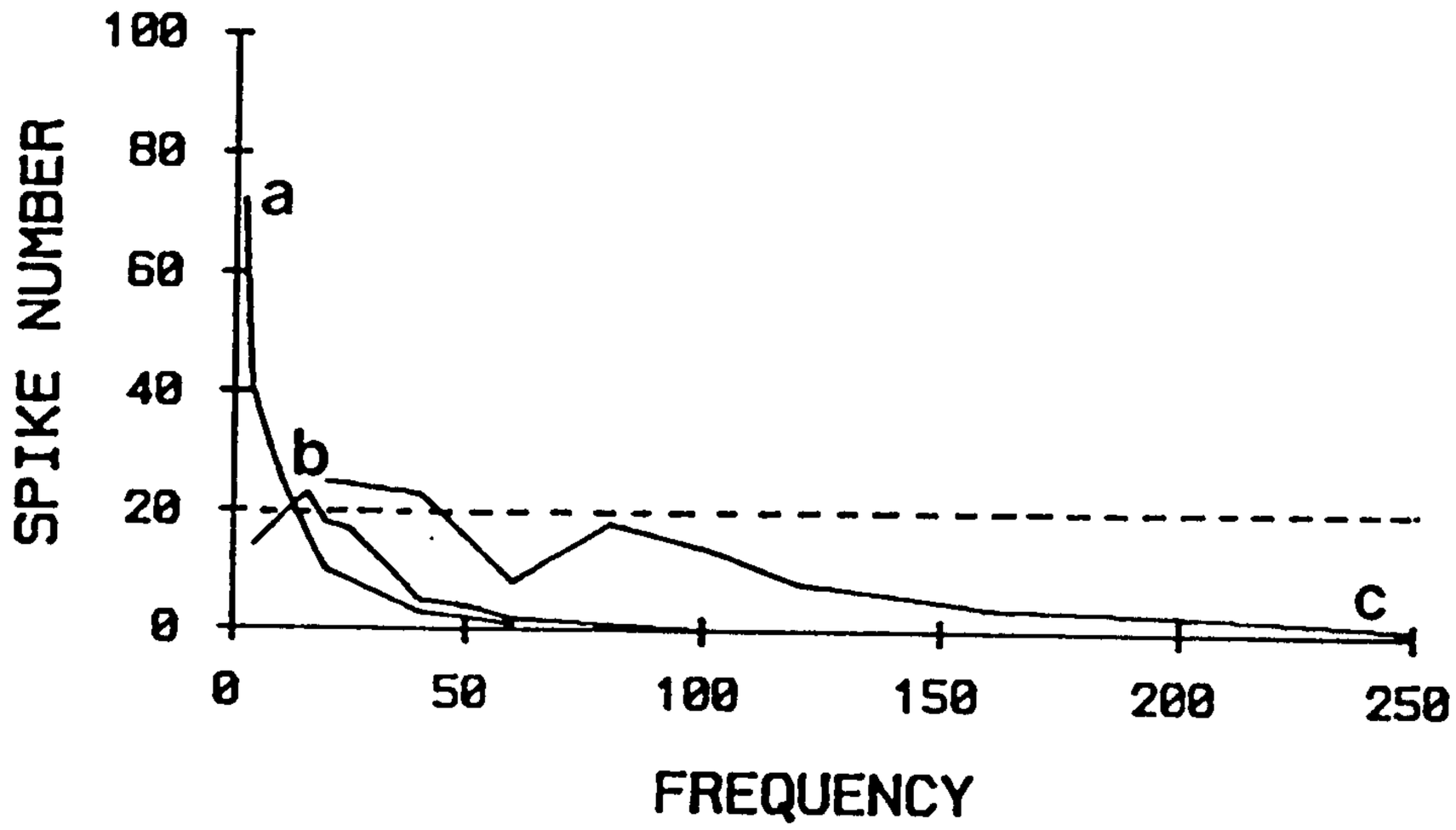
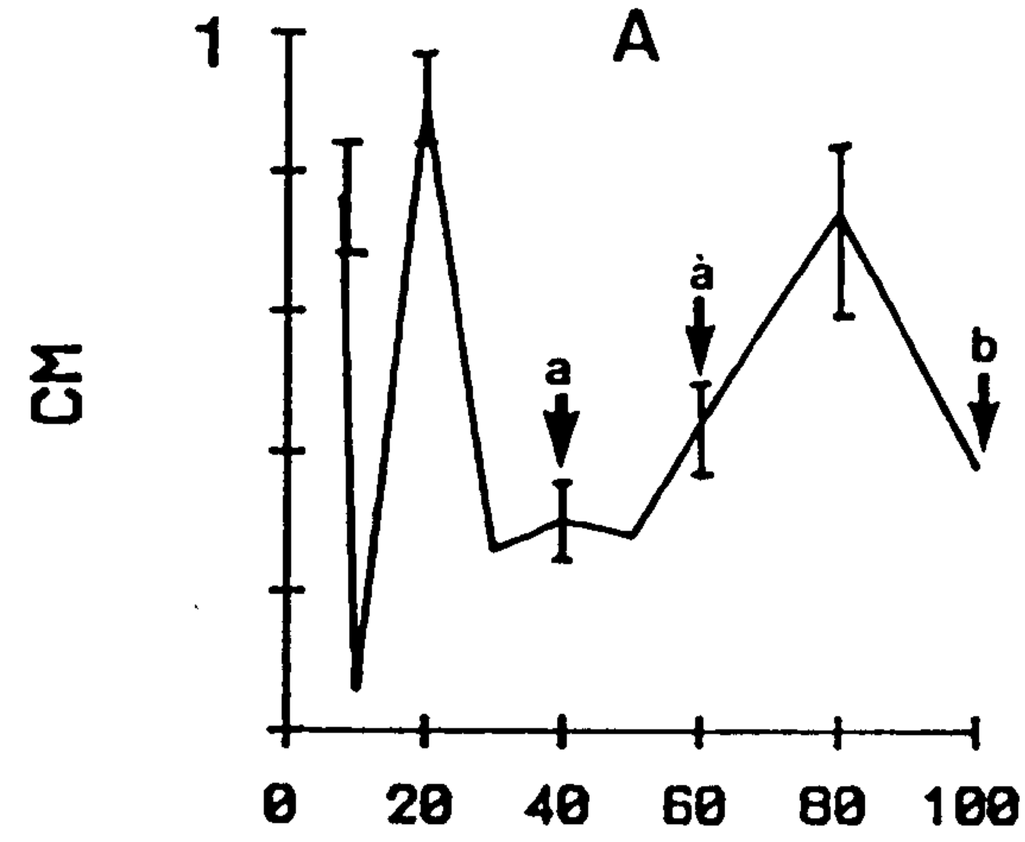
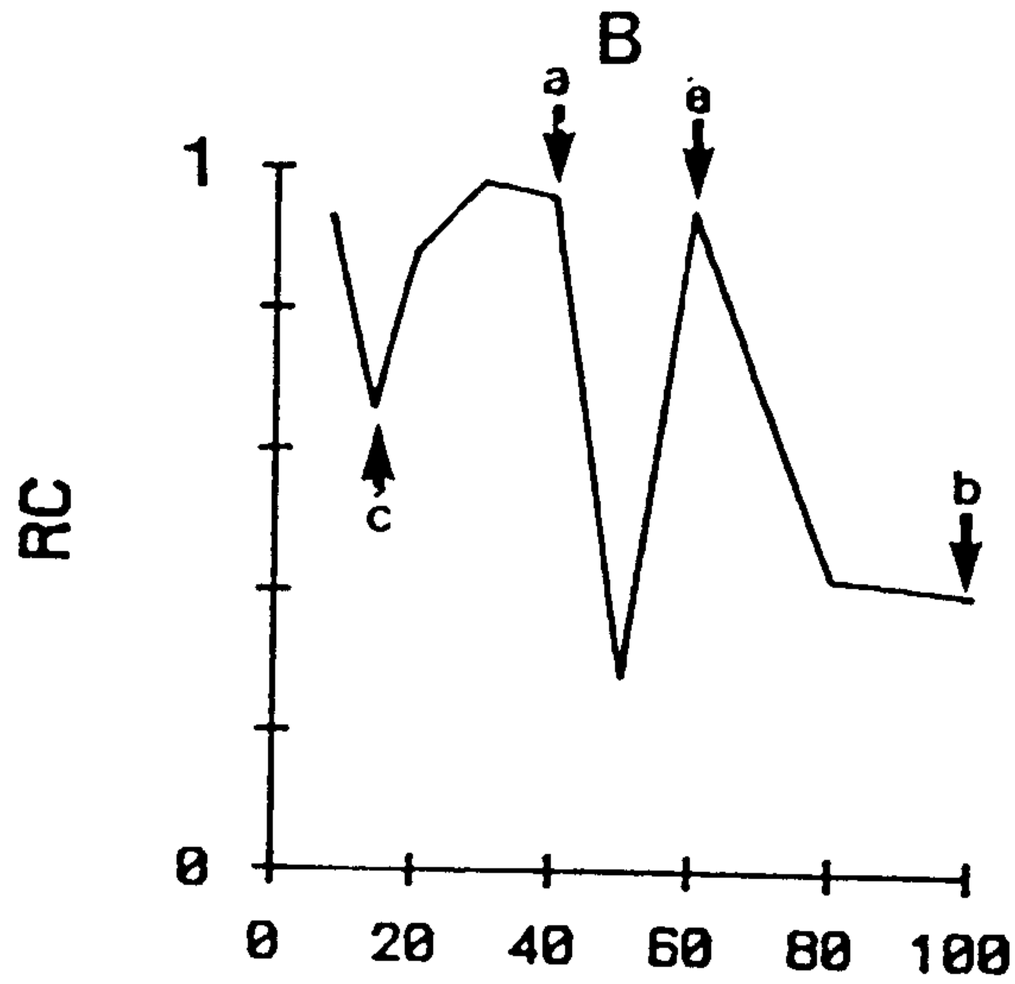
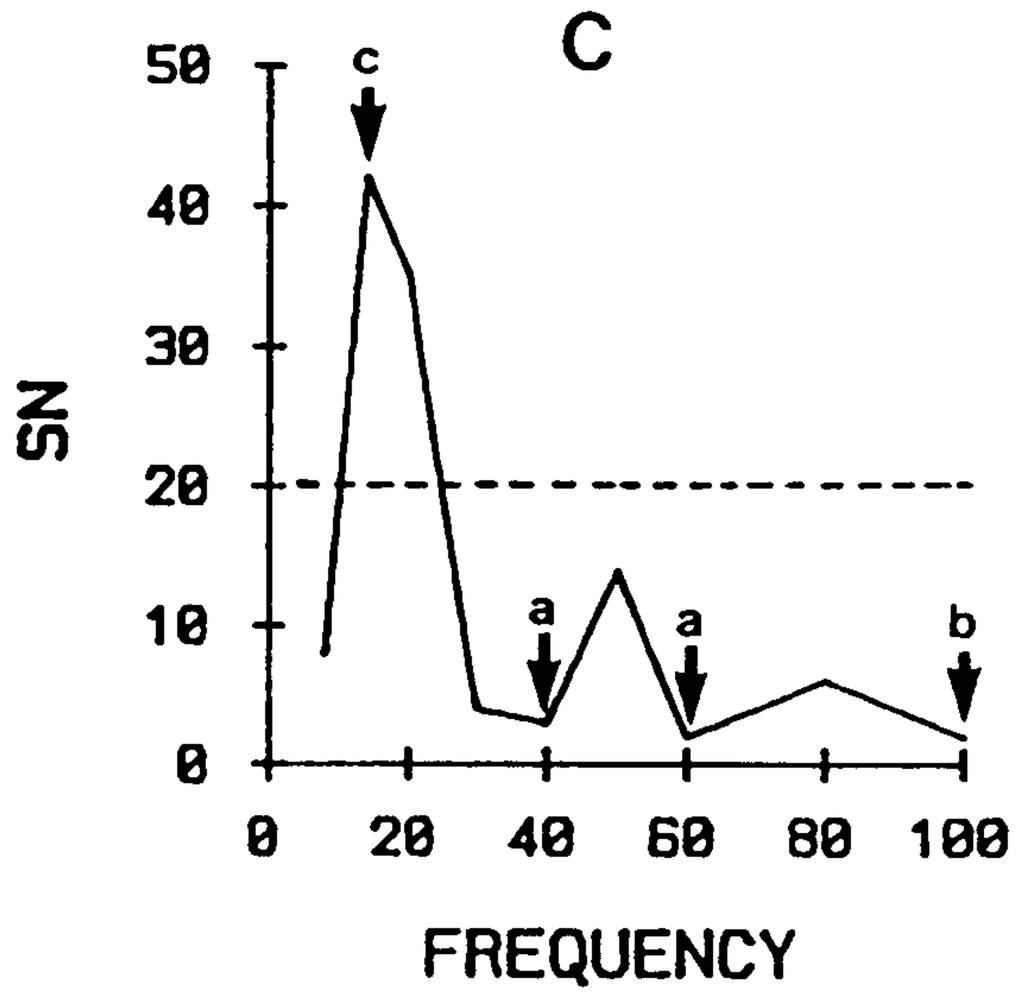
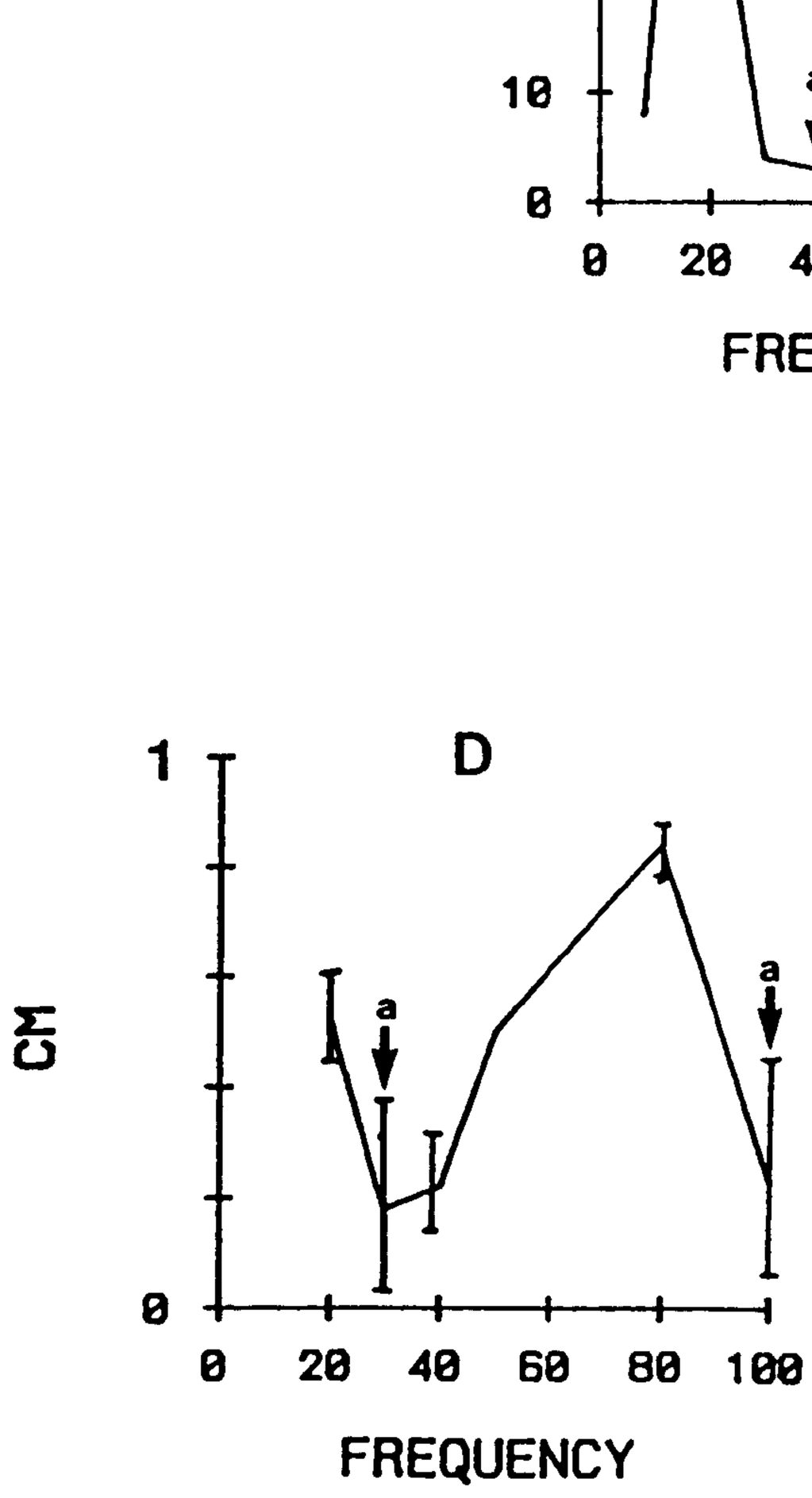

SPIKE NUMBER
100
80
60
40
20
0
a
b
c
0
50
100
150
200
250
FREQUENCY

Figure 3.5 Plots to illustrate various characteristics of the statistical parameters used in the analysis. Plots A and D show the variation in the circular mean with frequency (Hz). These have not been corrected for phase lag. Plot B shows the variation in R_c with frequency (Hz) and plots C and E show the variation in spike number with frequency. A-C show plots from the same unit, D and E show plots from another unit. All plots are from leg units. Small letters are referred to in the text p. 64-65.


A
1
CM
a
a
b
0
20
40
60
80
100
FREQUENCY


B
1
RC
a
a
c
b
0
20
40
60
80
100
FREQUENCY


C
50
40
30
20
10
0
SN
c
a
a
b
0
20
40
60
80
100
FREQUENCY


D
1
CM
a
a
0
20
40
60
80
100
FREQUENCY

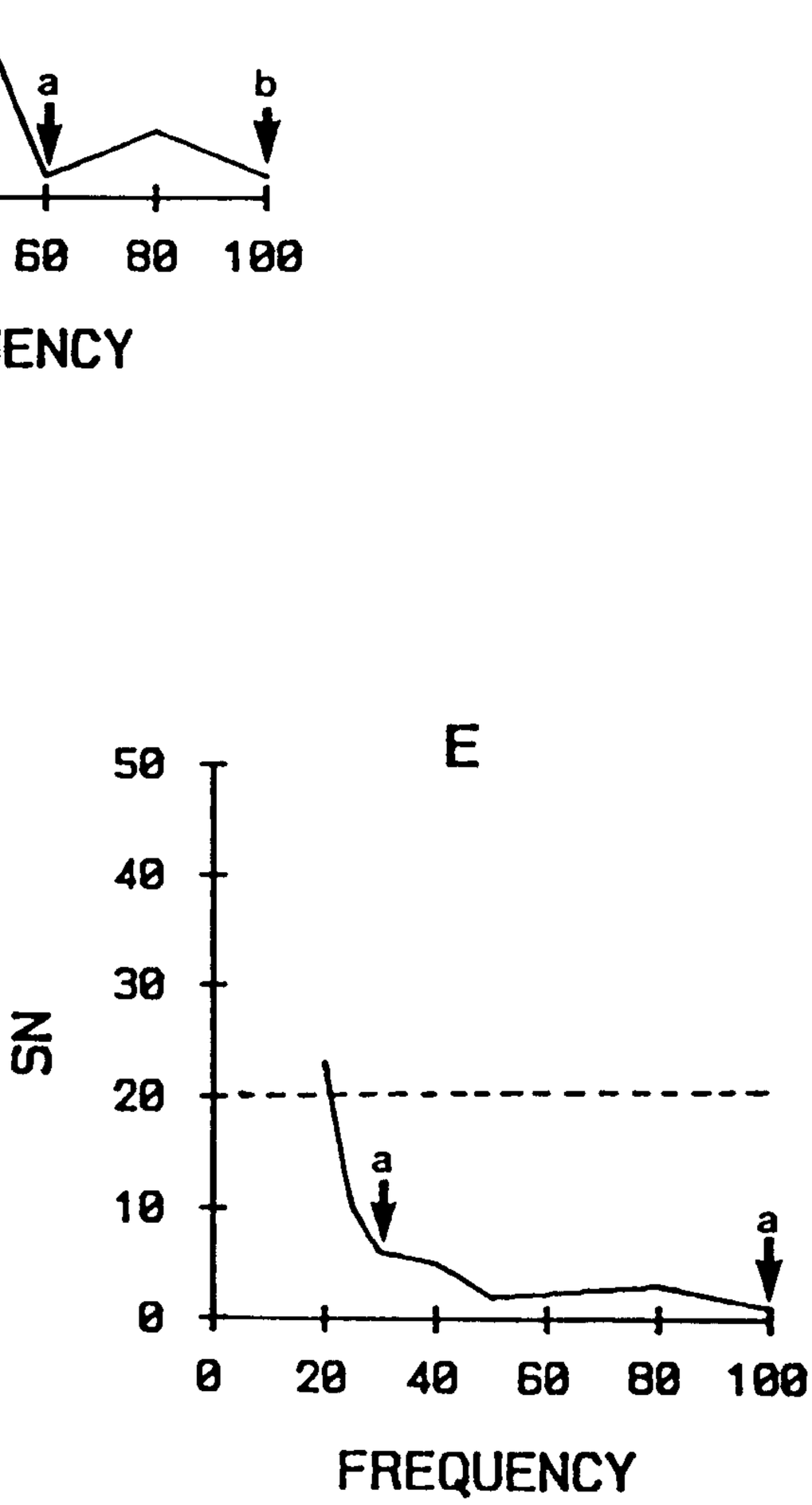

E
50
40
30
20
10
0
SN
a
a
0
20
40
60
80
100
FREQUENCY

Figure 3.6 Plots to illustrate various characteristics of the statistical parameters used in the analysis. Plots show the variation in the circular mean (A), the R_c value (B) and the spike number (C) of a leg unit with frequency. Small letters referred to in the text p. 64-65.

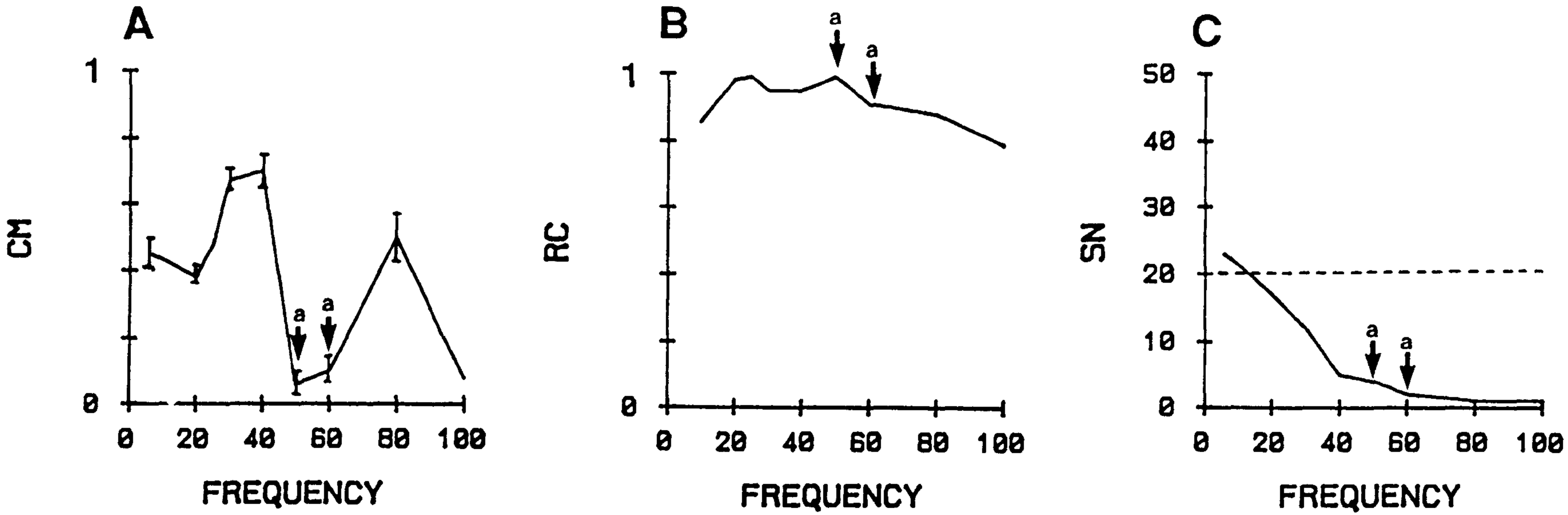
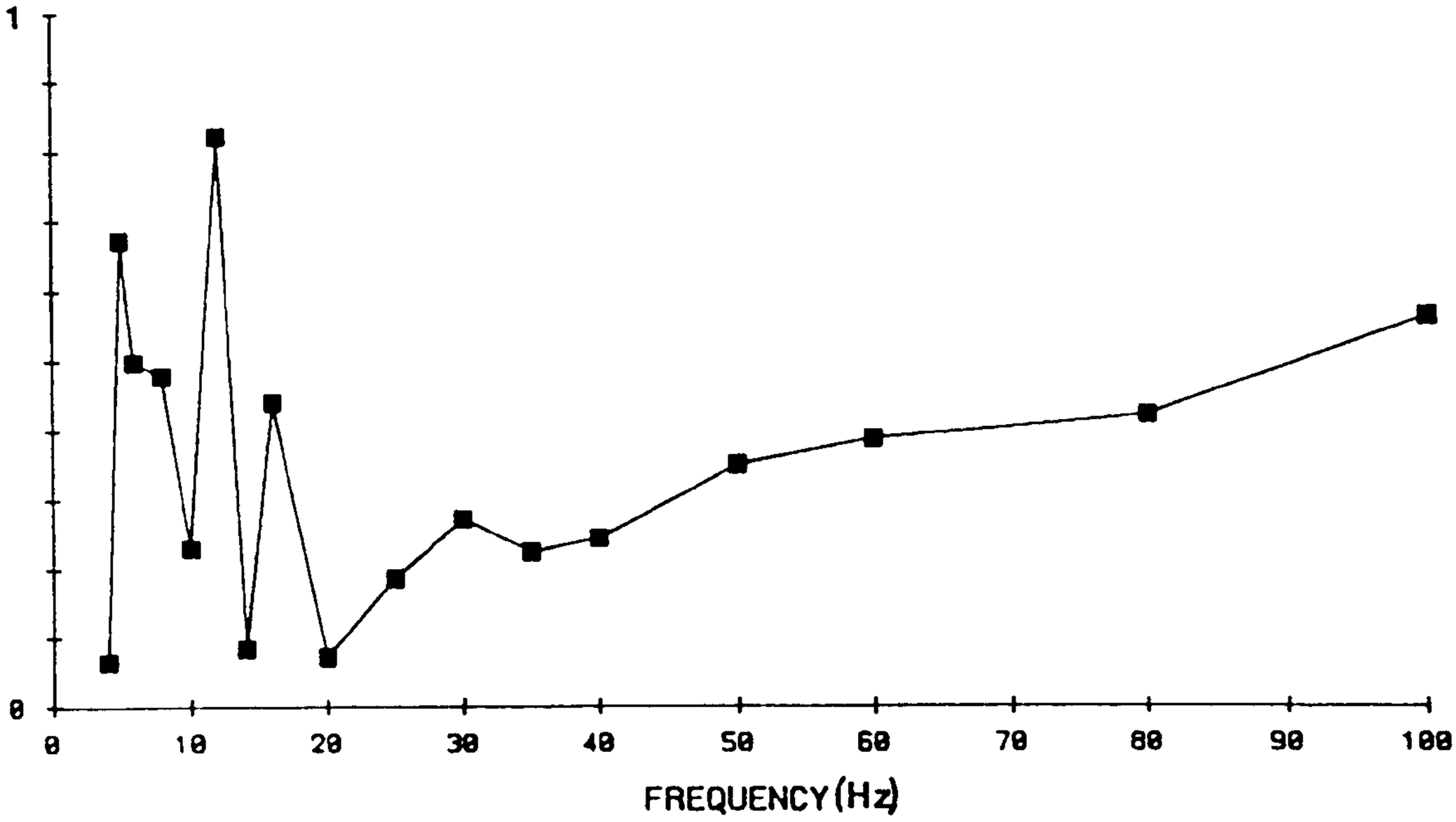

A
1
0
CM
a
a
0
20
40
60
80
100
FREQUENCY
B
a
a
1
0
RC
0
20
40
60
80
100
FREQUENCY
C
50
40
30
20
10
0
SN
a
a
0
20
40
60
80
100
FREQUENCY

Figure 3.7 A. The phase lag of the actual water movement in the acoustic tube relative to the voltage signal from the Derritron amplifier driving voltage. Plot shows the phase lag against frequency (Hz).

B. The displacement (cm $x10^{-3}$) produced in the acoustic tube at a range of frequencies (Hz) when the Derritron amplifier was used at an output of 1 (the most commonly used setting).

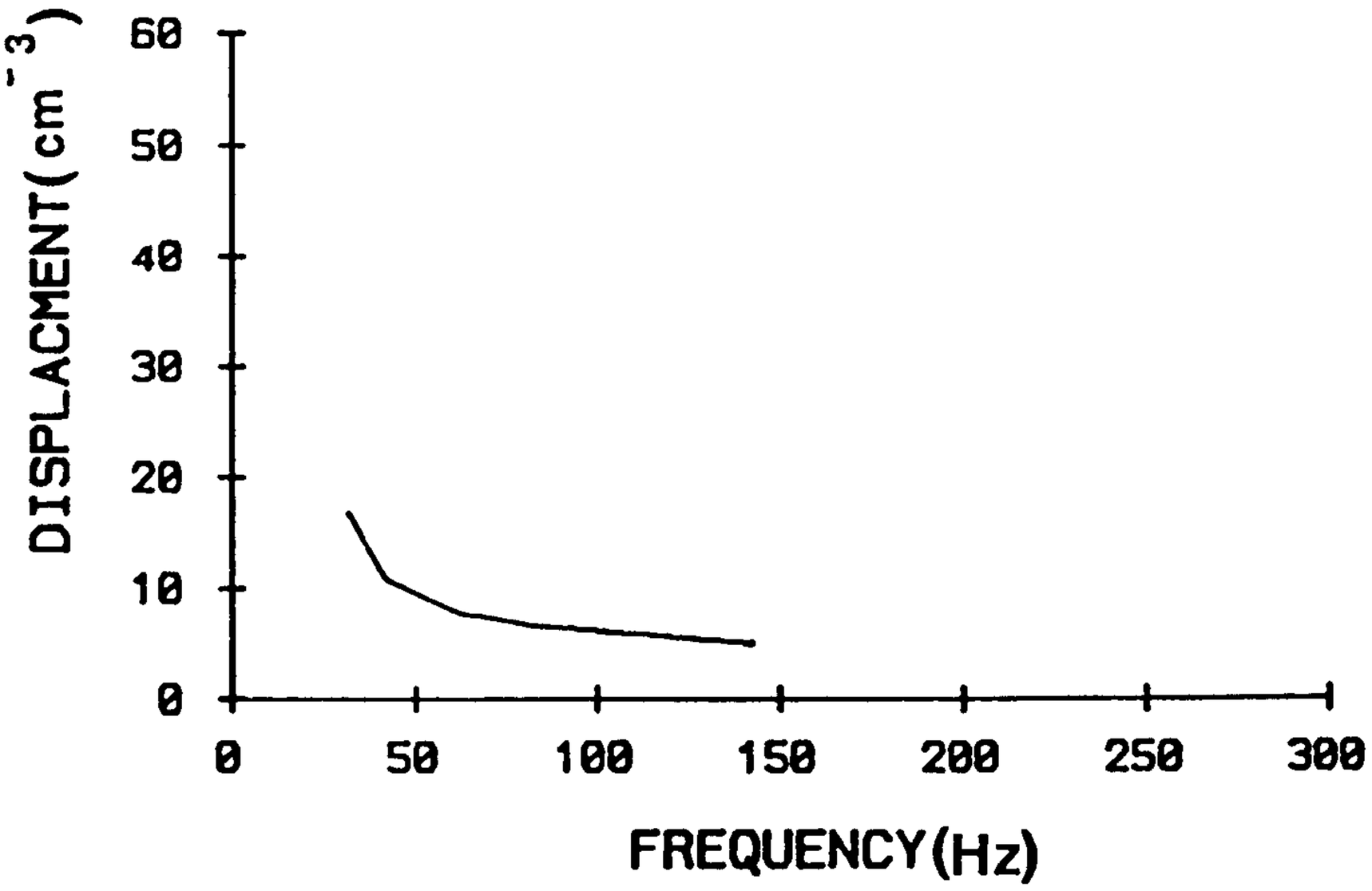
C. The displacement produced in the acoustic tube at a range of frequencies (Hz) when the Derritron amplifier was used at an output of 2.

A



OUTPUT 1

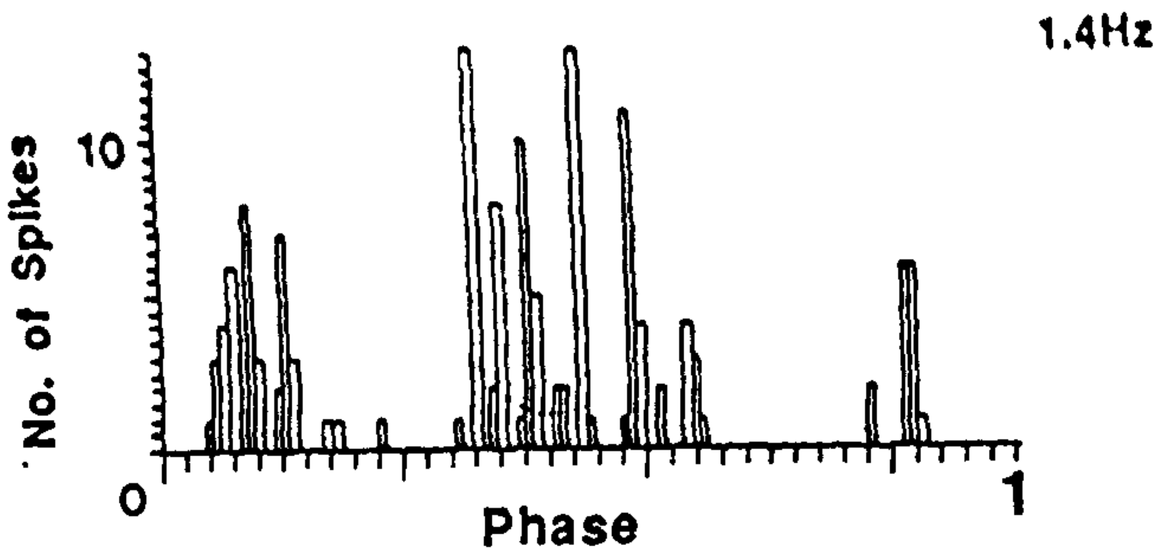
B

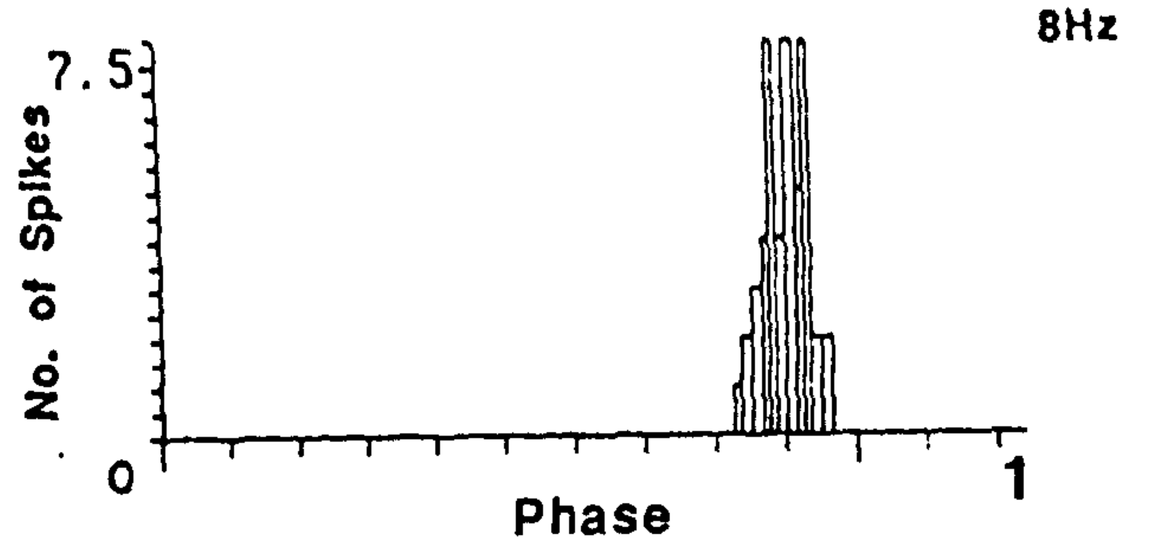


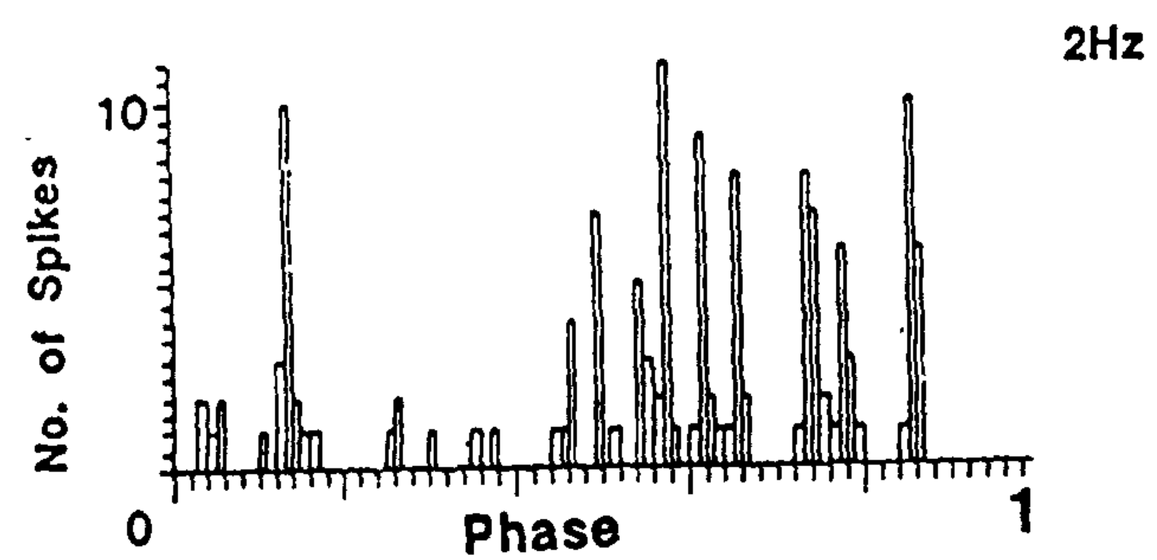
OUTPUT 2

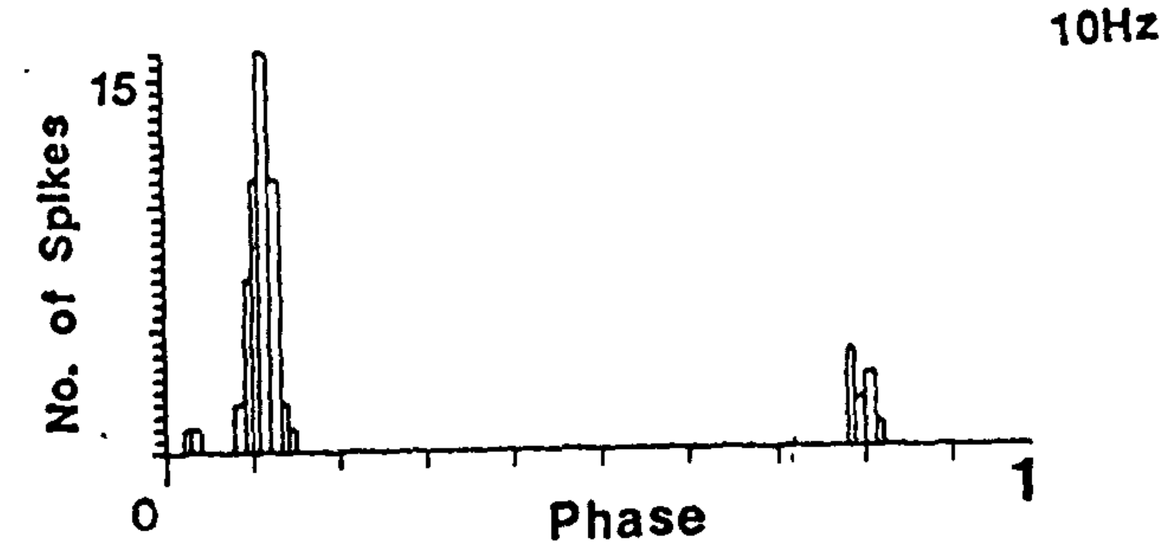
C

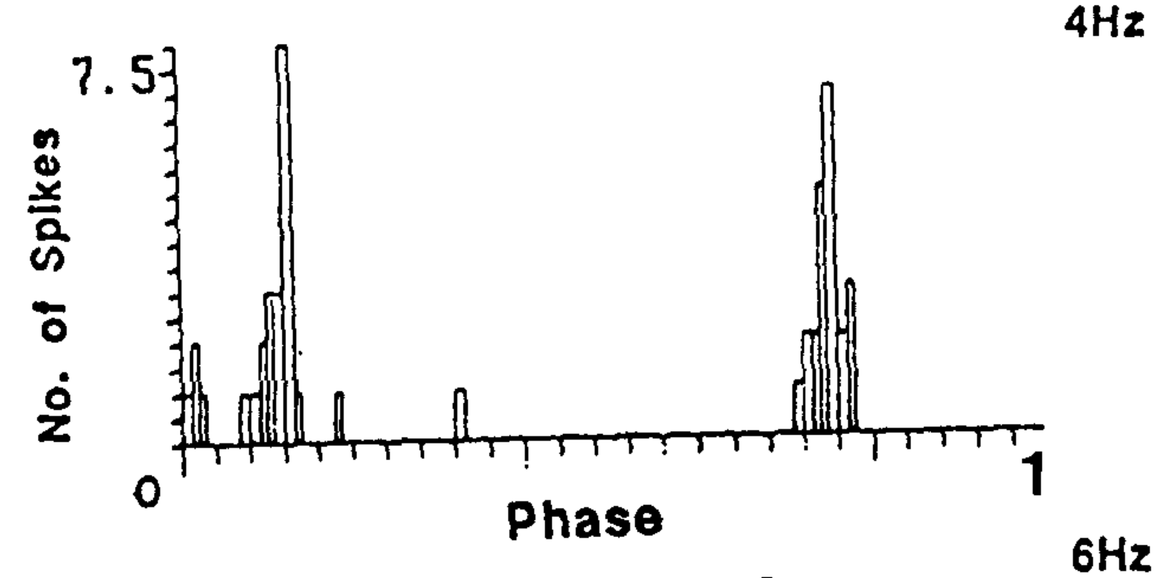
Figure 3.8 Phase histograms showing the responses of a typical low frequency uropod unit tested in a small dish. Plots show the spike number (vertical axis) against phase (horizontal axis) for a range of frequencies.

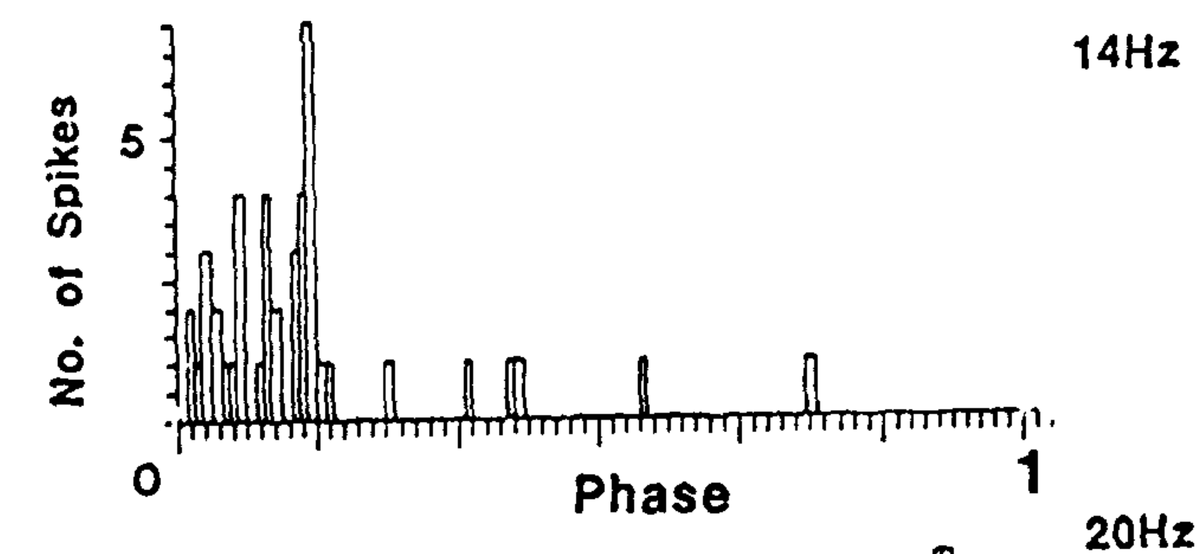

1.4Hz
No. of Spikes
10
0
Phase
1

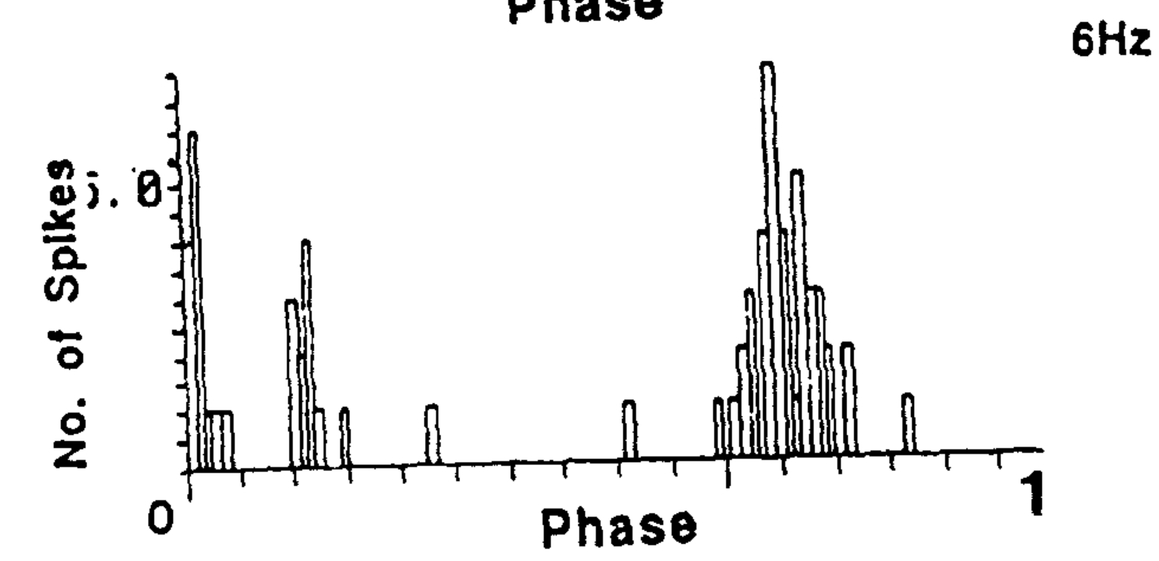

8Hz
No. of Spikes
7.5
0
Phase
1


2Hz
No. of Spikes
10
0
Phase
1


10Hz
No. of Spikes
15
0
Phase
1


4Hz
No. of Spikes
7.5
0
Phase
1


14Hz
No. of Spikes
5
0
Phase
1


6Hz
No. of Spikes
0
Phase
1

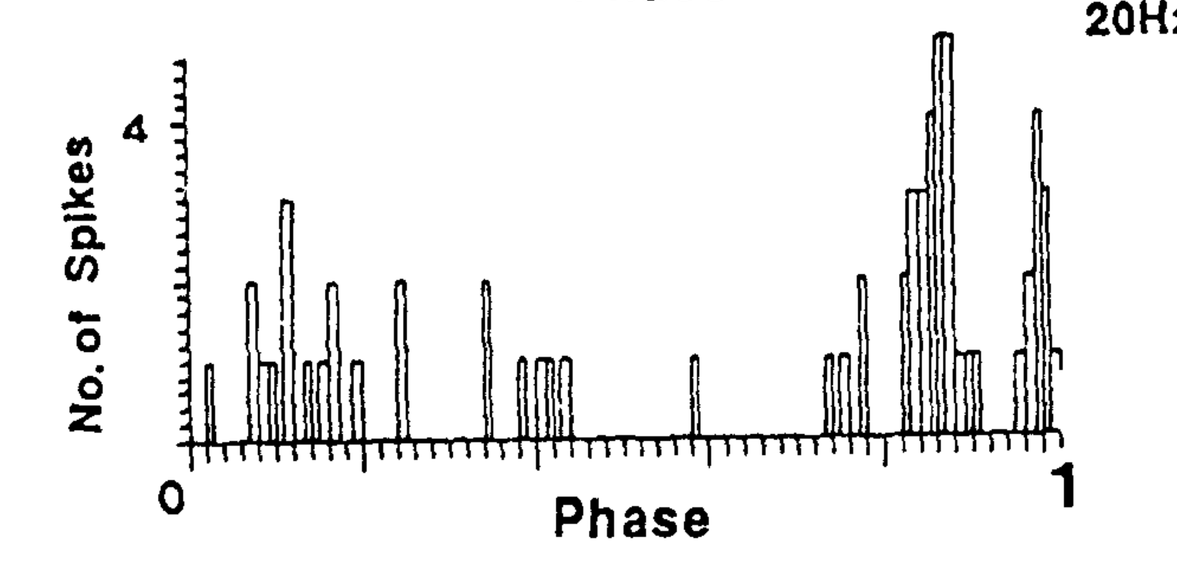
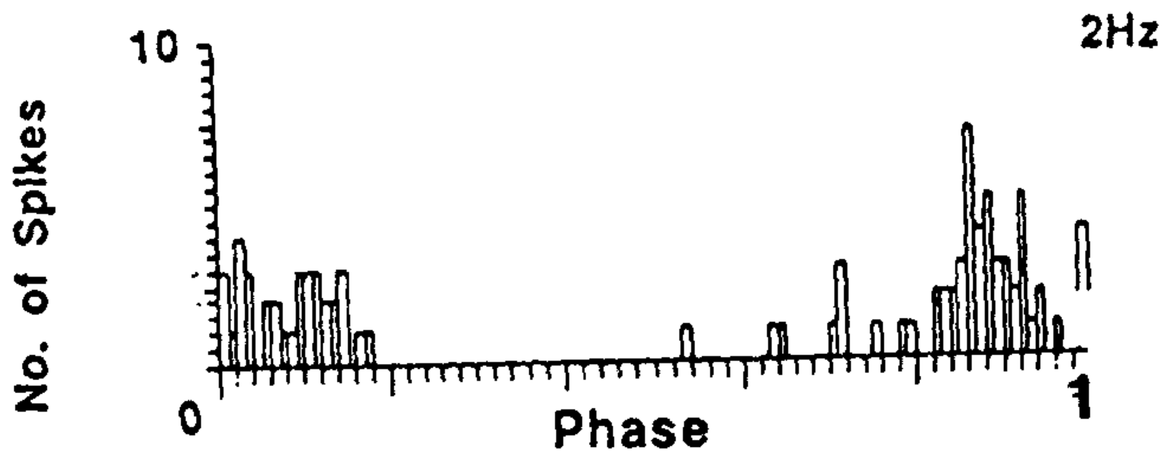
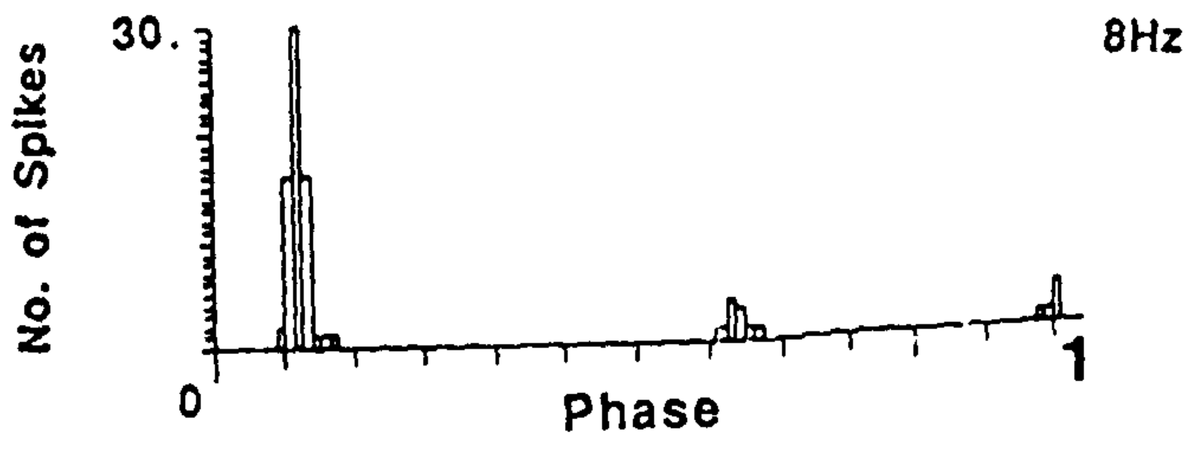
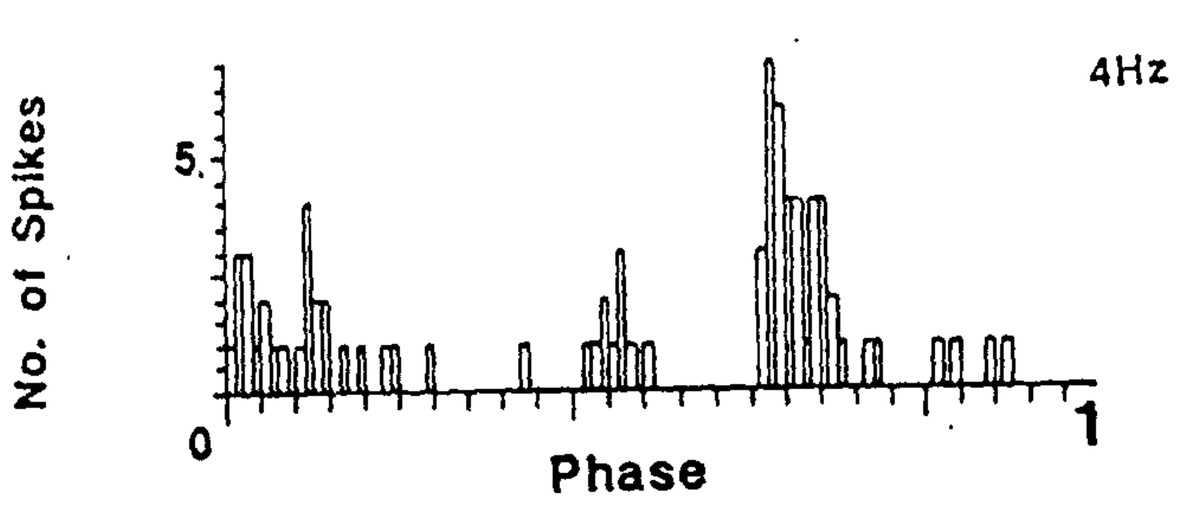
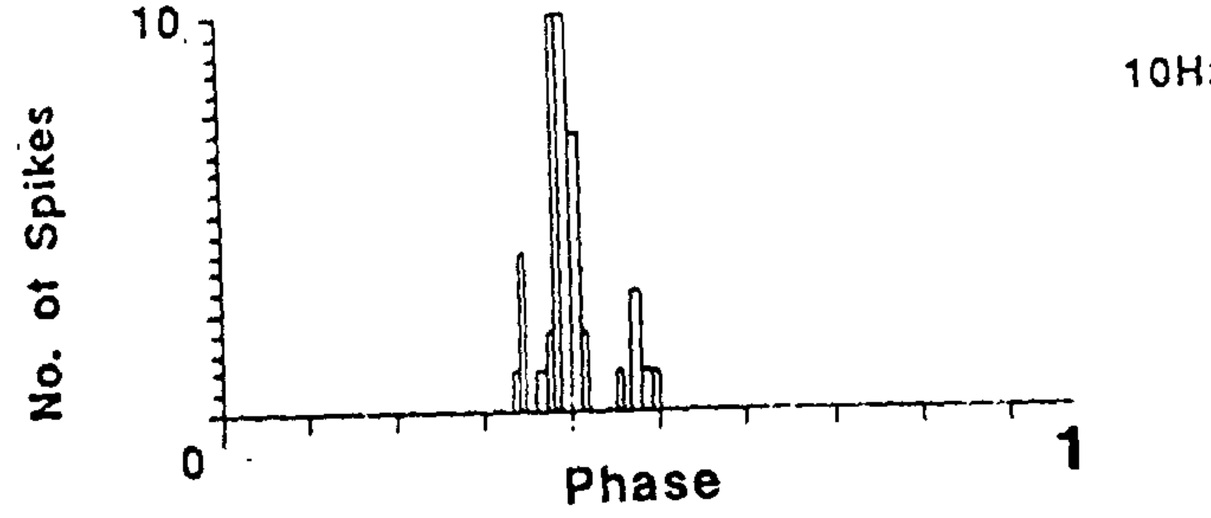

20Hz
No. of Spikes
4
0
Phase
1

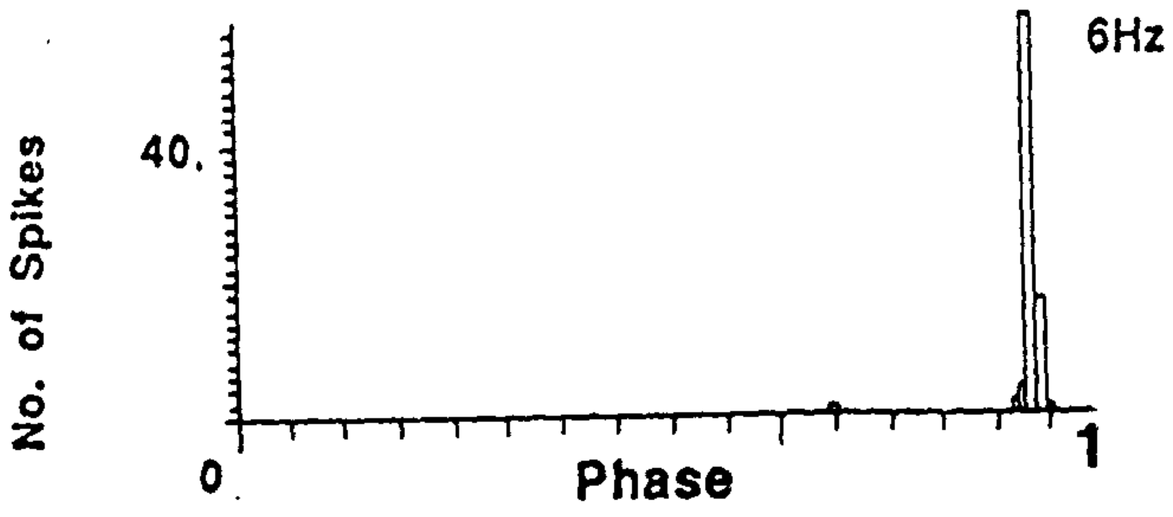
Figure 3.9 Phase histograms showing the responses of a typical low frequency uropod unit tested in a small dish. Plots show the spike number against phase.


No. of Spikes
10
0
Phase
1
2Hz


No. of Spikes
30.
0
Phase
1
8Hz


No. of Spikes
5.
0
Phase
1
4Hz


No. of Spikes
10.
0
Phase
1
10Hz


No. of Spikes
40.
0
Phase
1
6Hz

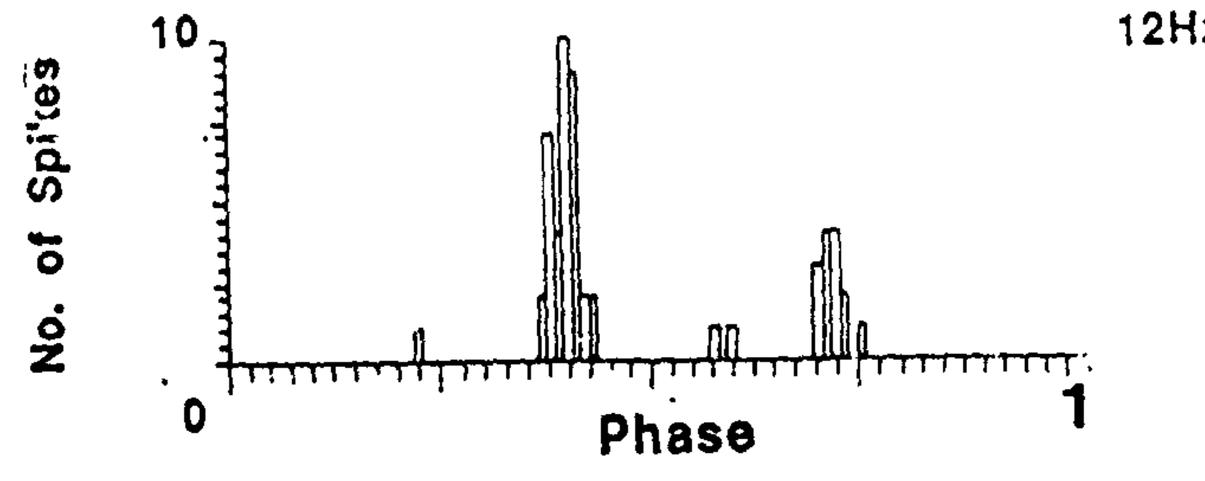
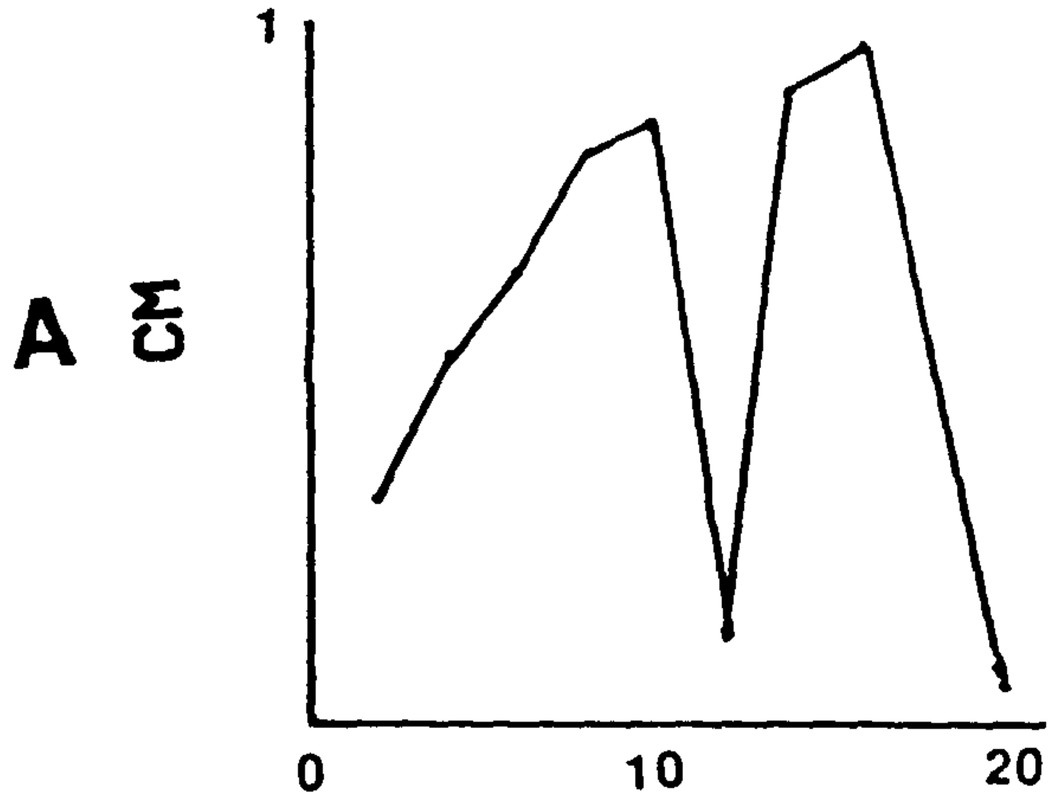
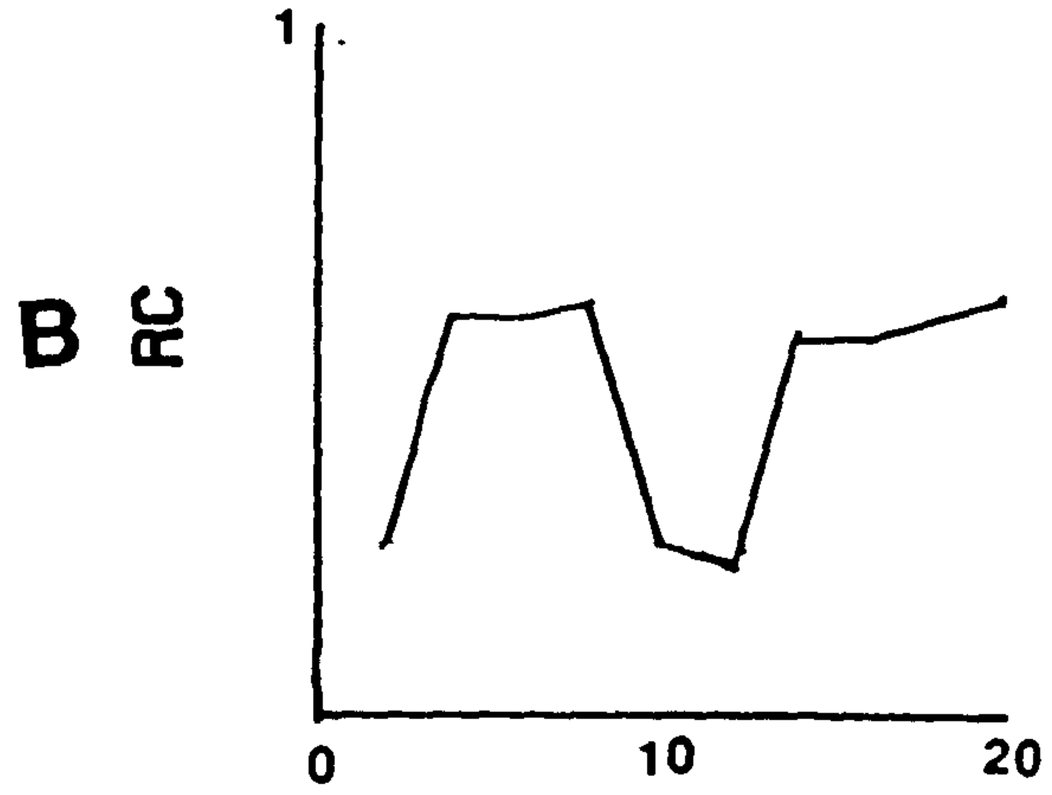

No. of Spikes
10
0
Phase
1
12Hz

Figure 3.10 Statitical values produced for a typical low frequency uropod unit which was tested in the acoustic tube. Plots show the variation in circular mean (A), R_c (B) and spike number (C) with frequency (Hz). The dotted line on plot C indicates one spike per cycle.


A CM
1
0
10
20
FREQUENCY Hz


B RC
1
0
10
20
FREQUENCY Hz

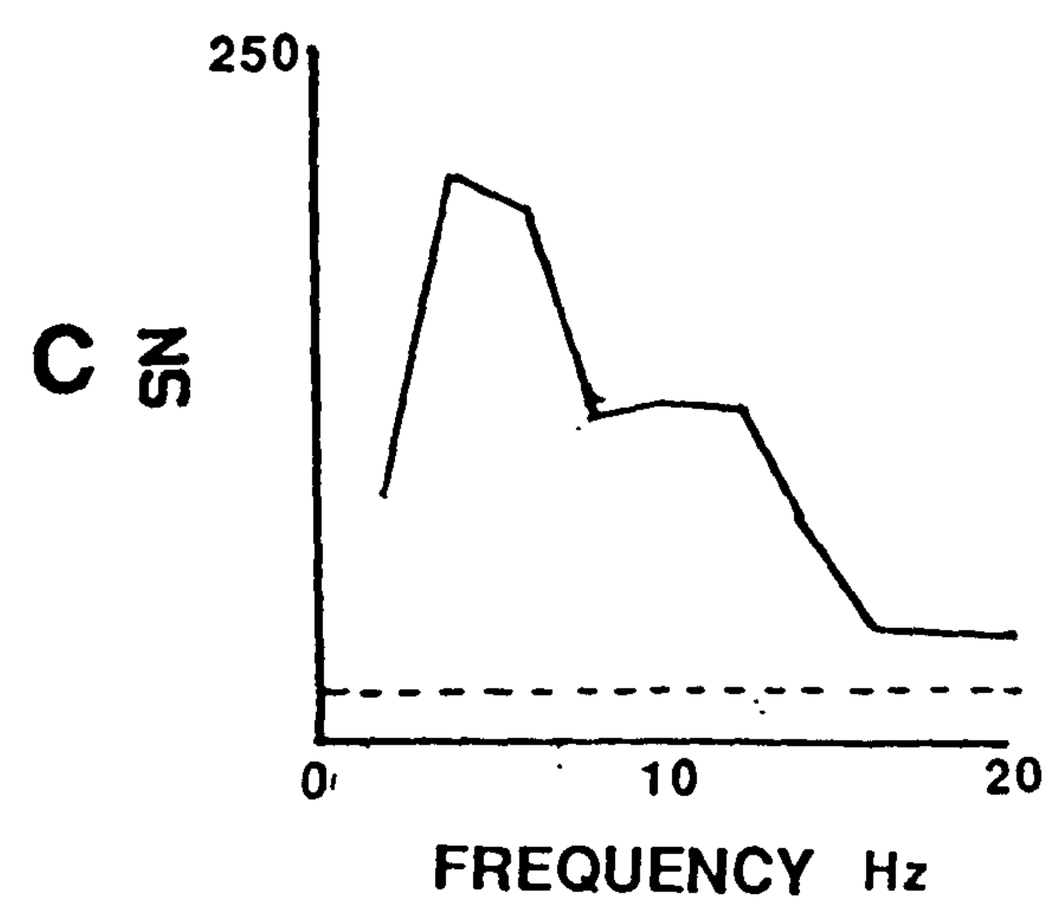
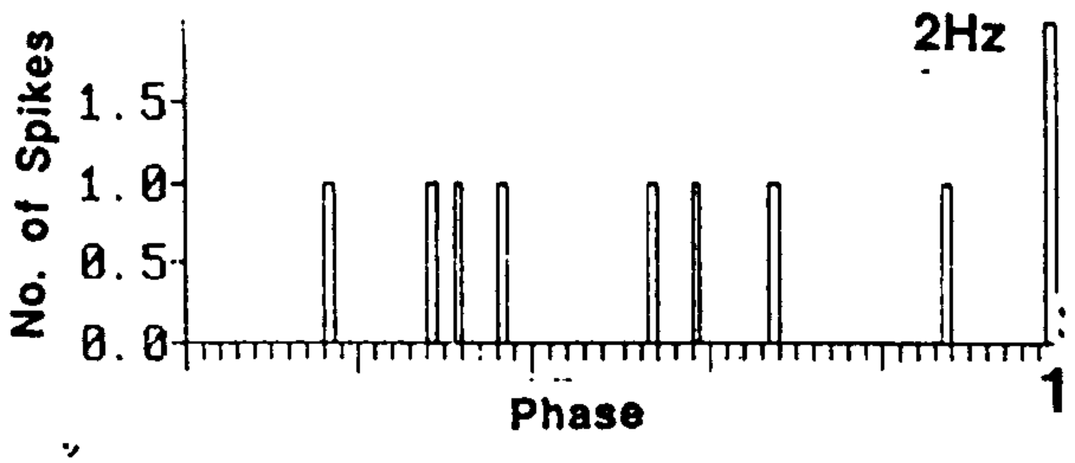
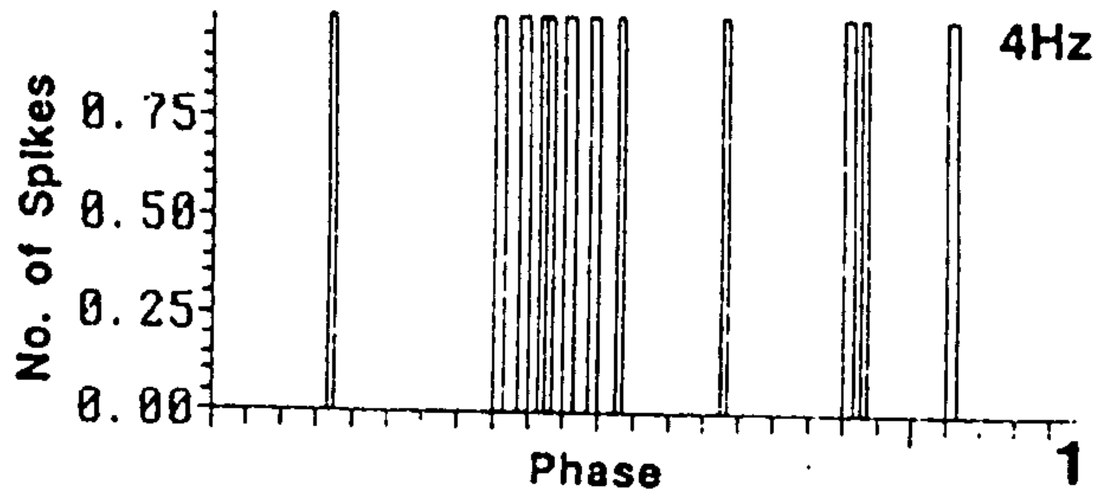
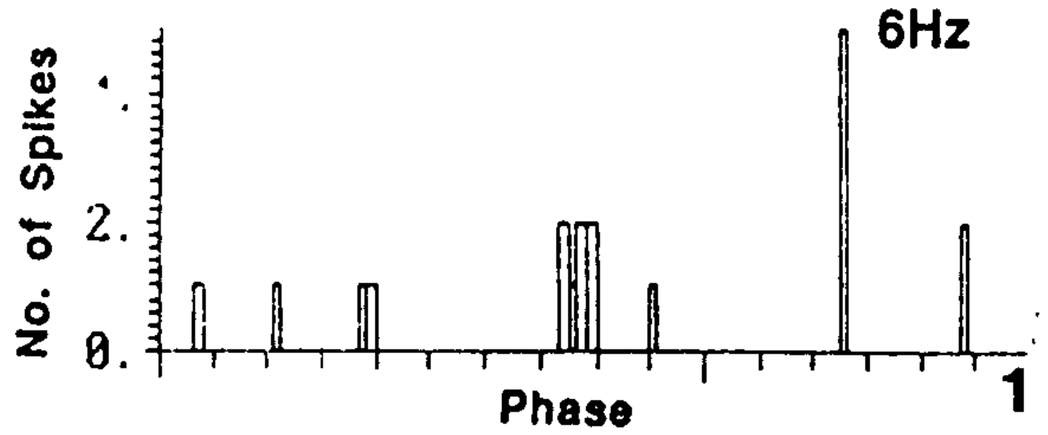
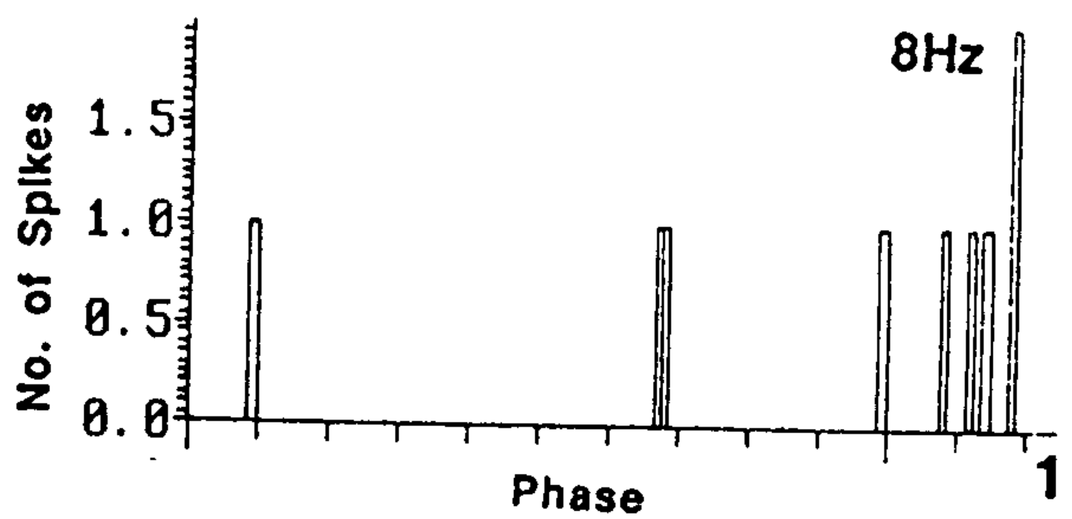

C SN
250
0
10
20
FREQUENCY Hz

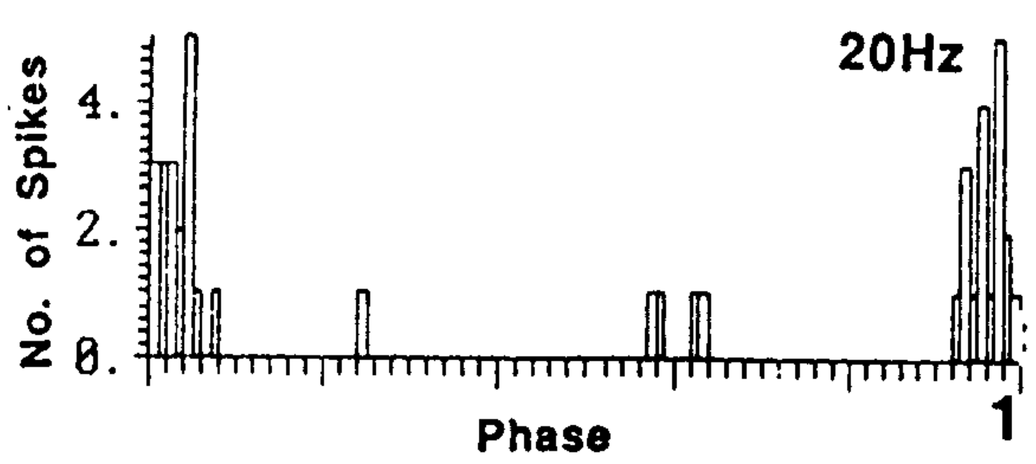
Figure 3.11 Phase histograms showing the responses of a typical intermediate frequency uropod unit to water borne vibrations produced in a small dish. Details as Figure 3.8.

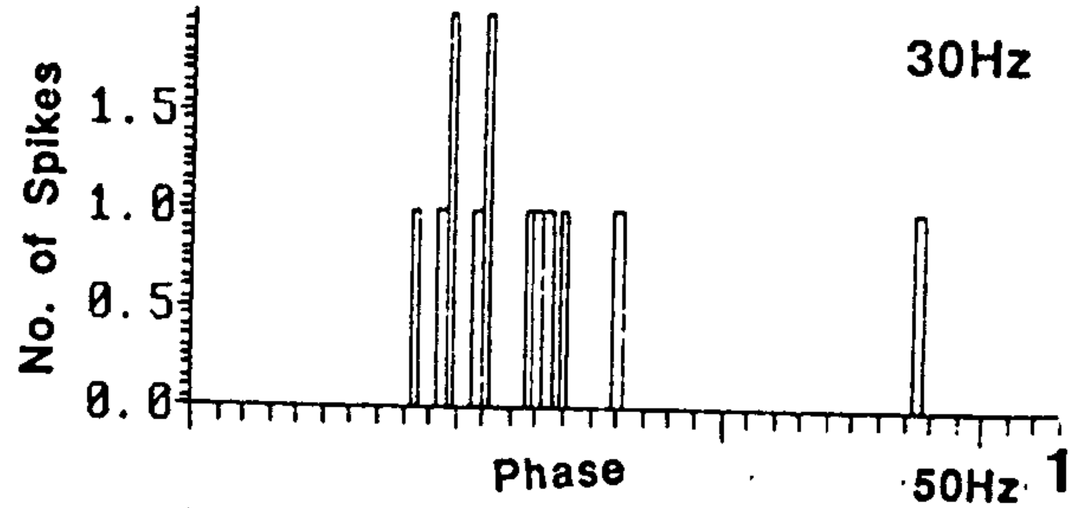

2Hz
No. of Spikes
1.5
1.0
0.5
0.0
Phase
1

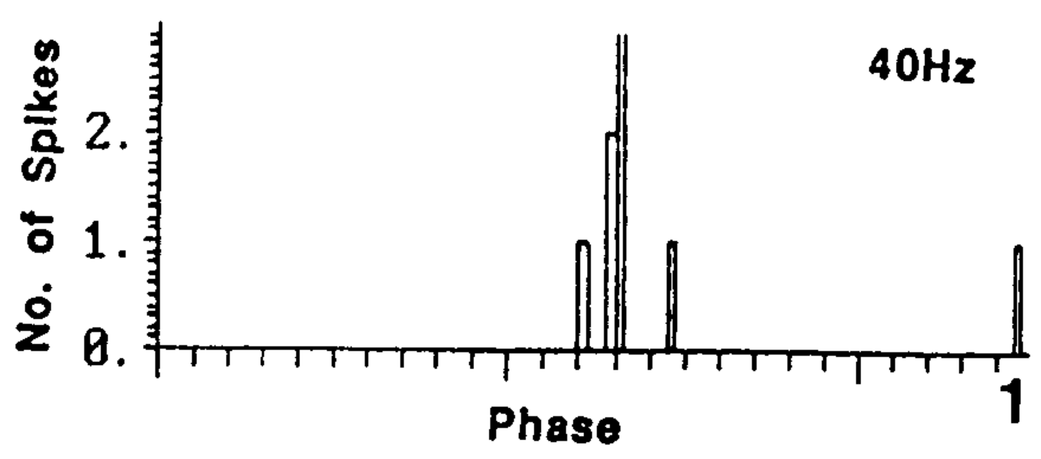

4Hz
No. of Spikes
0.75
0.50
0.25
0.00
Phase
1


6Hz
No. of Spikes
2.
0.
Phase
1


8Hz
No. of Spikes
1.5
1.0
0.5
0.0
Phase
1


20Hz
No. of Spikes
4.
2.
0.
Phase
1


30Hz
No. of Spikes
1.5
1.0
0.5
0.0
Phase
1


40Hz
No. of Spikes
2.
1.
0.
Phase
1

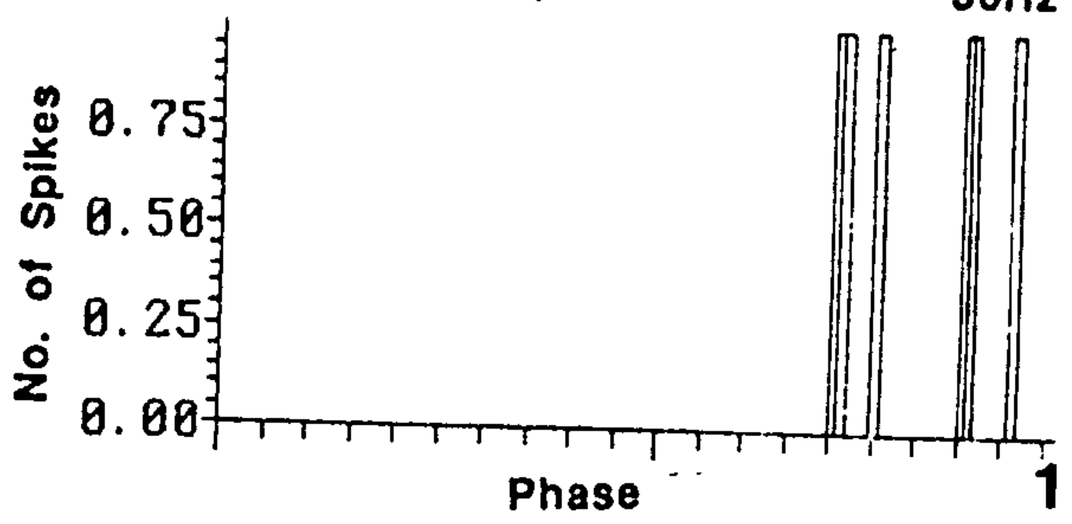

50Hz
No. of Spikes
0.75
0.50
0.25
0.00
Phase
1

Figure 3.12 Phase histograms showing the responses of a typical intermediate frequency uropod unit to water borne vibrations produced in a small dish. Details as Figure 3.8

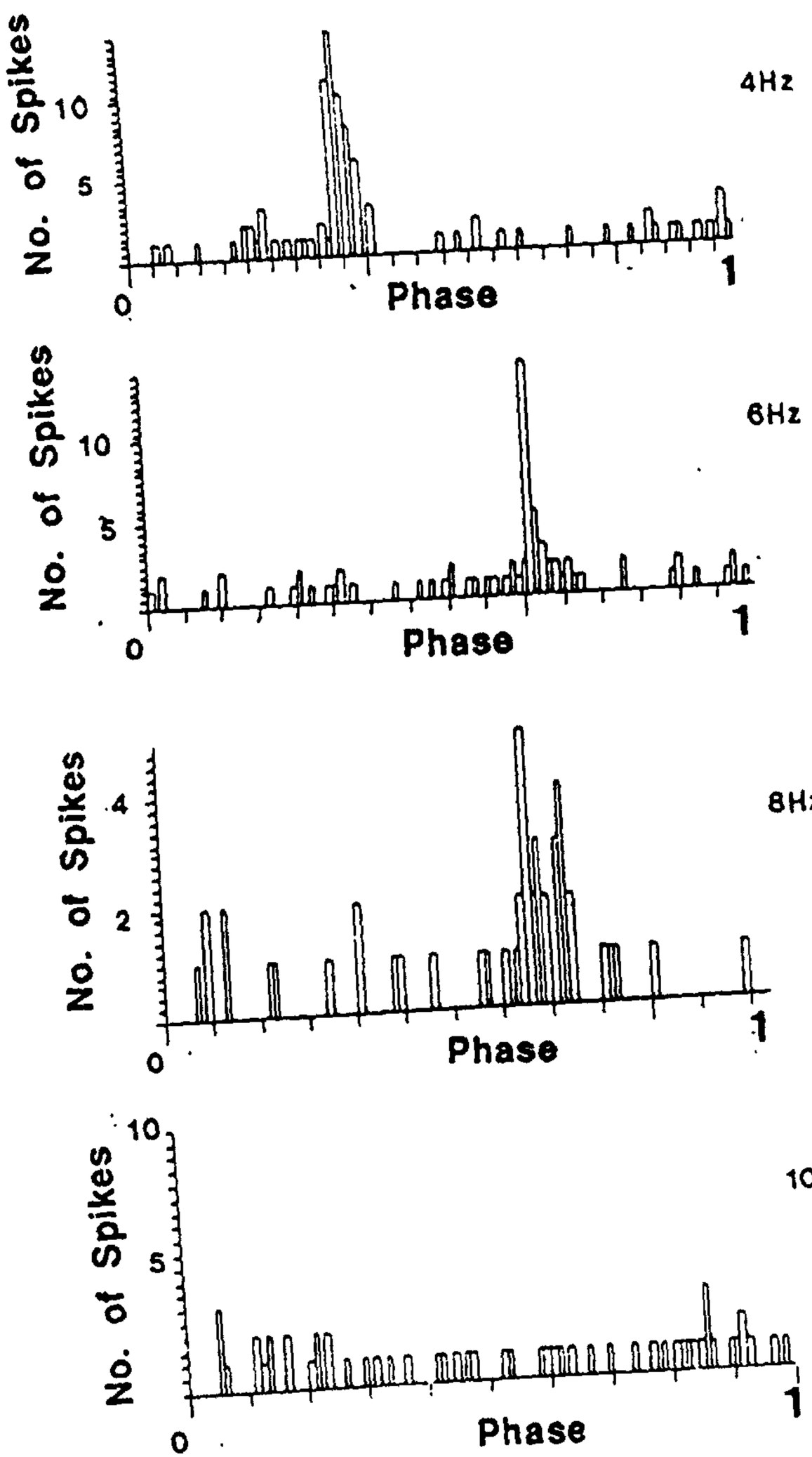

No. of Spikes
10
5
0
Phase
1
4Hz
6Hz
8Hz
10Hz
4
2
20Hz
30Hz
50Hz
40Hz
1.5

Figure 3.13 Phase histograms showing the responses of a typical high frequency uropod unit to water borne vibrations produced in a small dish. Details as Figure 3.8.

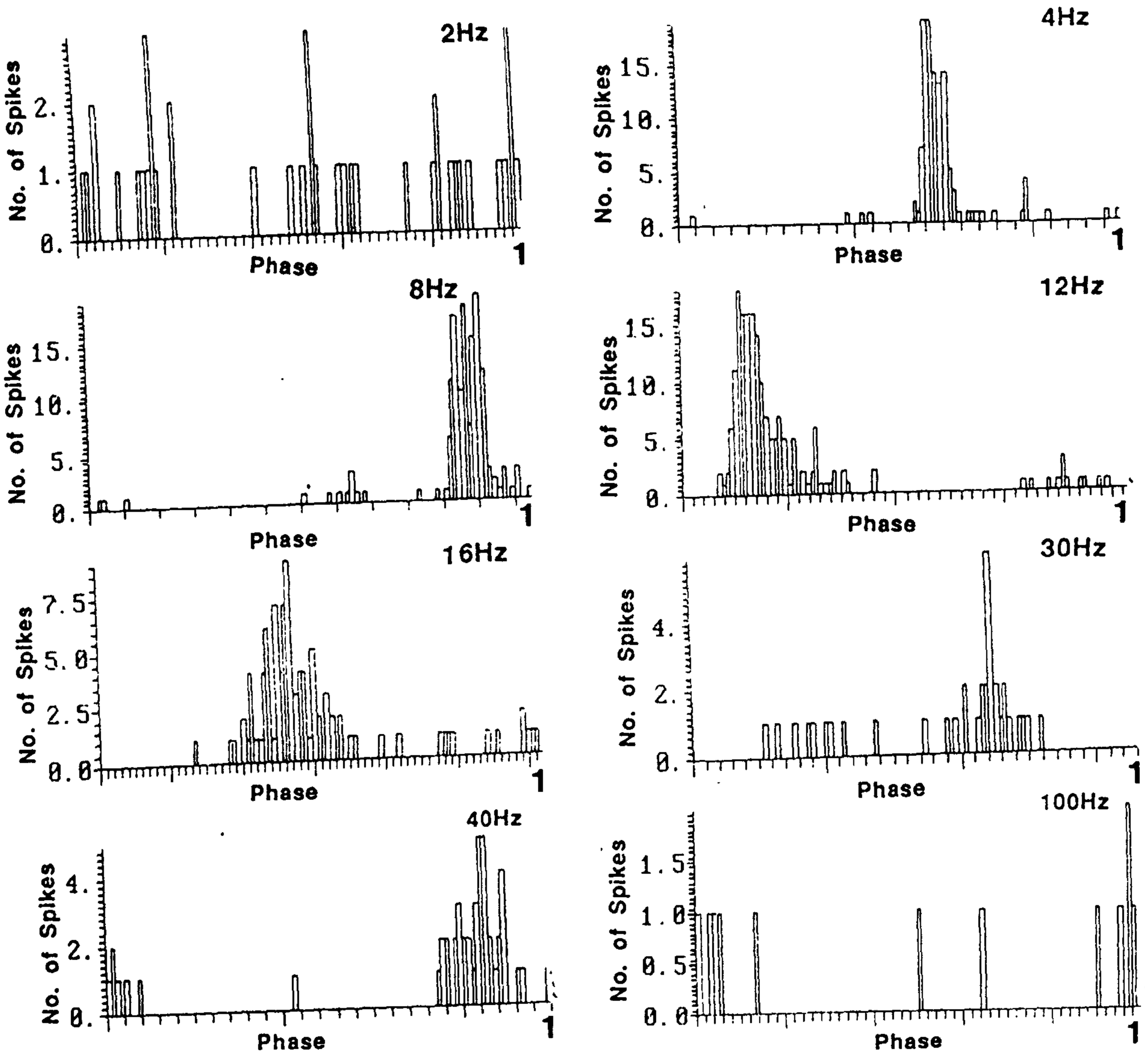
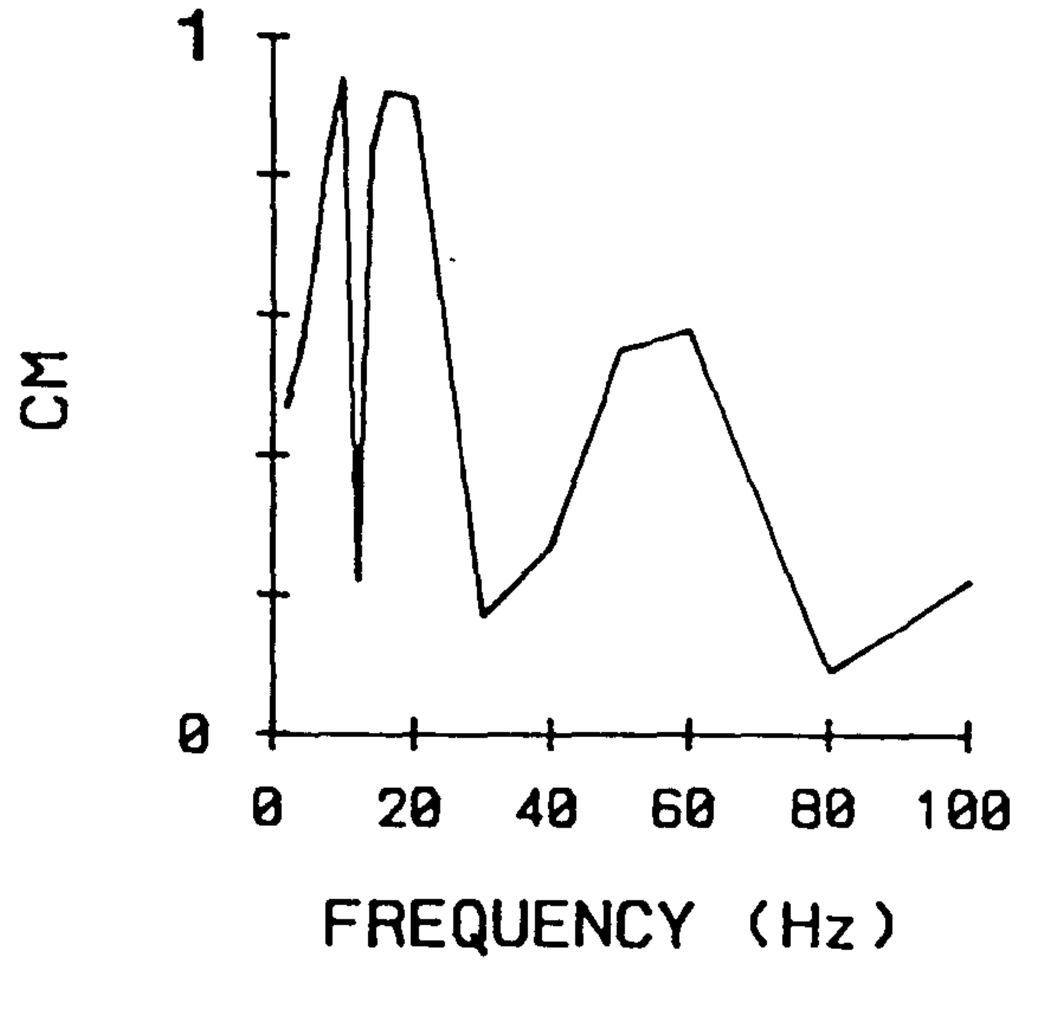
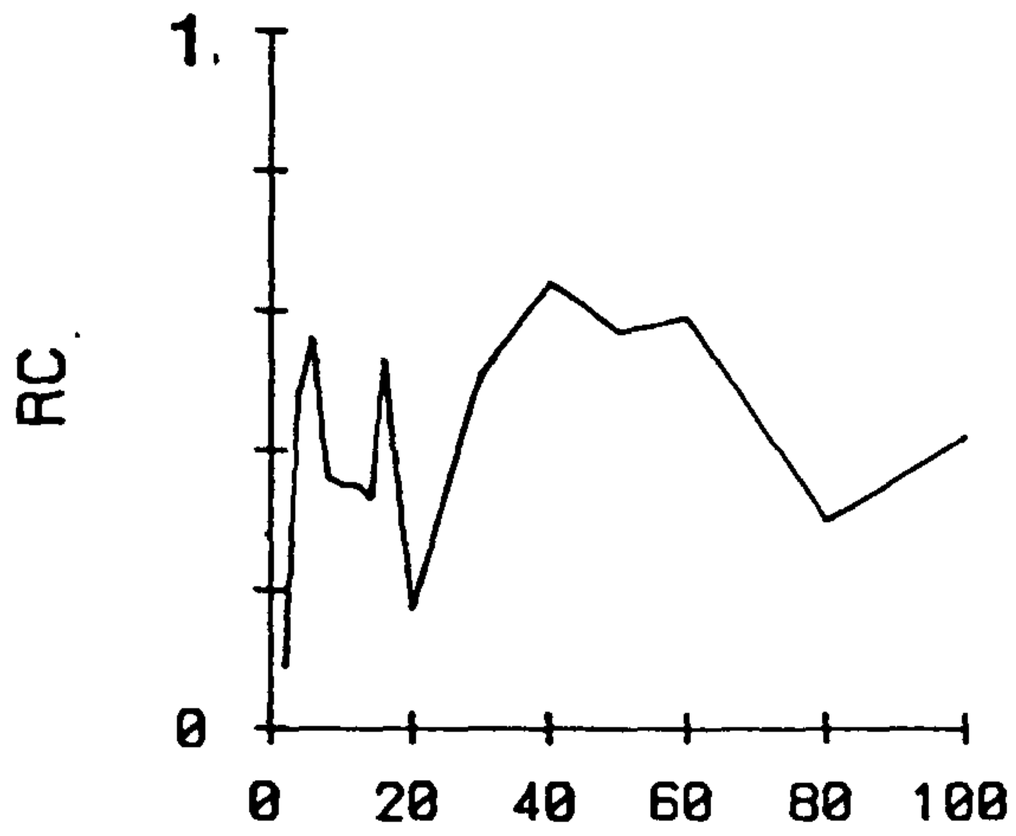

2Hz
No. of Spikes
2.
1.
0.
Phase
1
4Hz
No. of Spikes
15.
10.
5.
0.
Phase
1
8Hz
No. of Spikes
15.
10.
5.
0.
Phase
1
12Hz
No. of Spikes
15.
10.
5.
0.
Phase
1
16Hz
No. of Spikes
7.5
5.0
2.5
0.0
Phase
1
30Hz
No. of Spikes
4.
2.
0.
Phase
1
40Hz
No. of Spikes
4.
2.
0.
Phase
1
100Hz
No. of Spikes
1.5
1.0
0.5
0.0
Phase
1

Figure 3.14 Plots of statistical values produced from a typical high frequency uropod unit tested in response to water borne vibrations in the acoustic tube. Plots show the variation in the circular mean (A), the R_c value (B) and the spike number (C) with frequency (Hz). The dotted line on plot C represents one spike per cycle.


1
CM
0
0 20 40 60 80 100
FREQUENCY (Hz)


1.
RC
0
0 20 40 60 80 100
FREQUENCY (Hz)

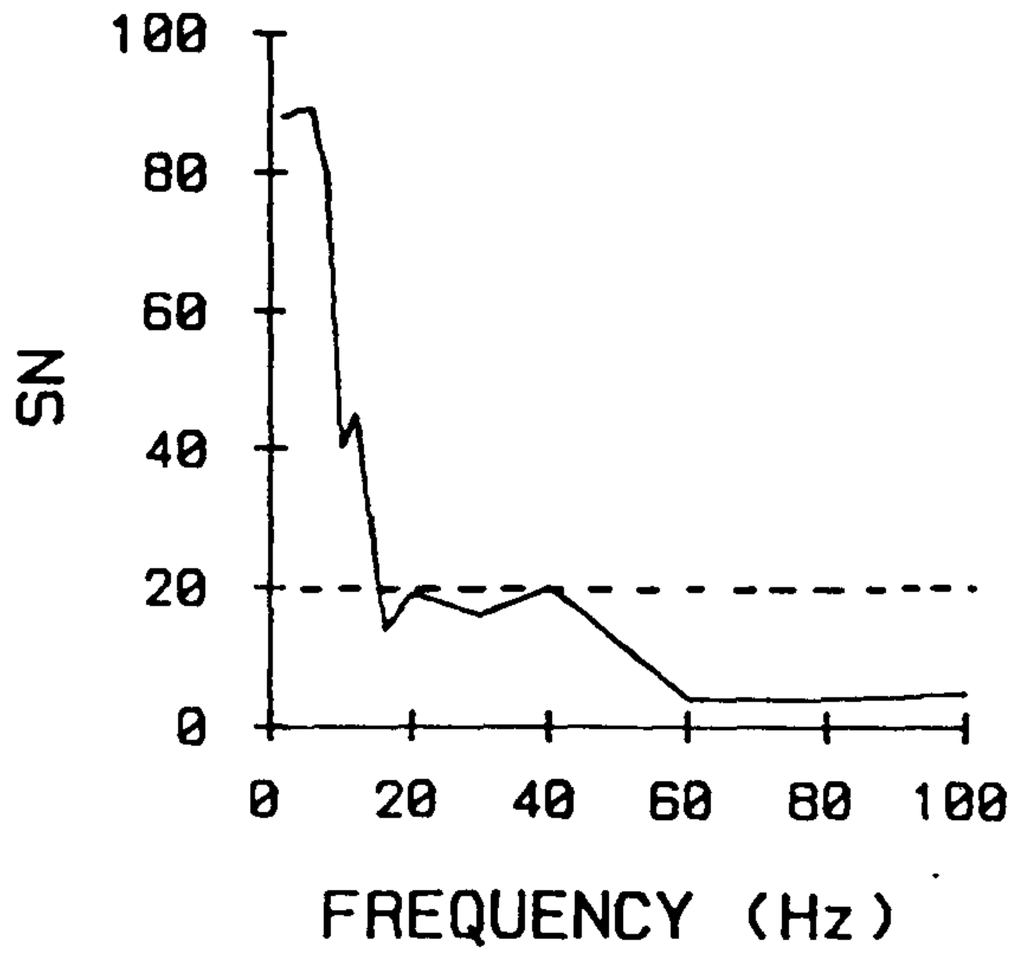

100
80
60
40
20
0
SN
0 20 40 60 80 100
FREQUENCY (Hz)

Figure 3.15 The responses of a low frequency leg unit (leg 4) tested with water borne vibrations from 3 directions in the acoustic tube. The plot shows phase histograms of the responses in each direction at 3 frequencies. The arrows within the circle indicate the position of the dactyl in each direction, the arrow outside the circle indicates the position of the loudspeaker.

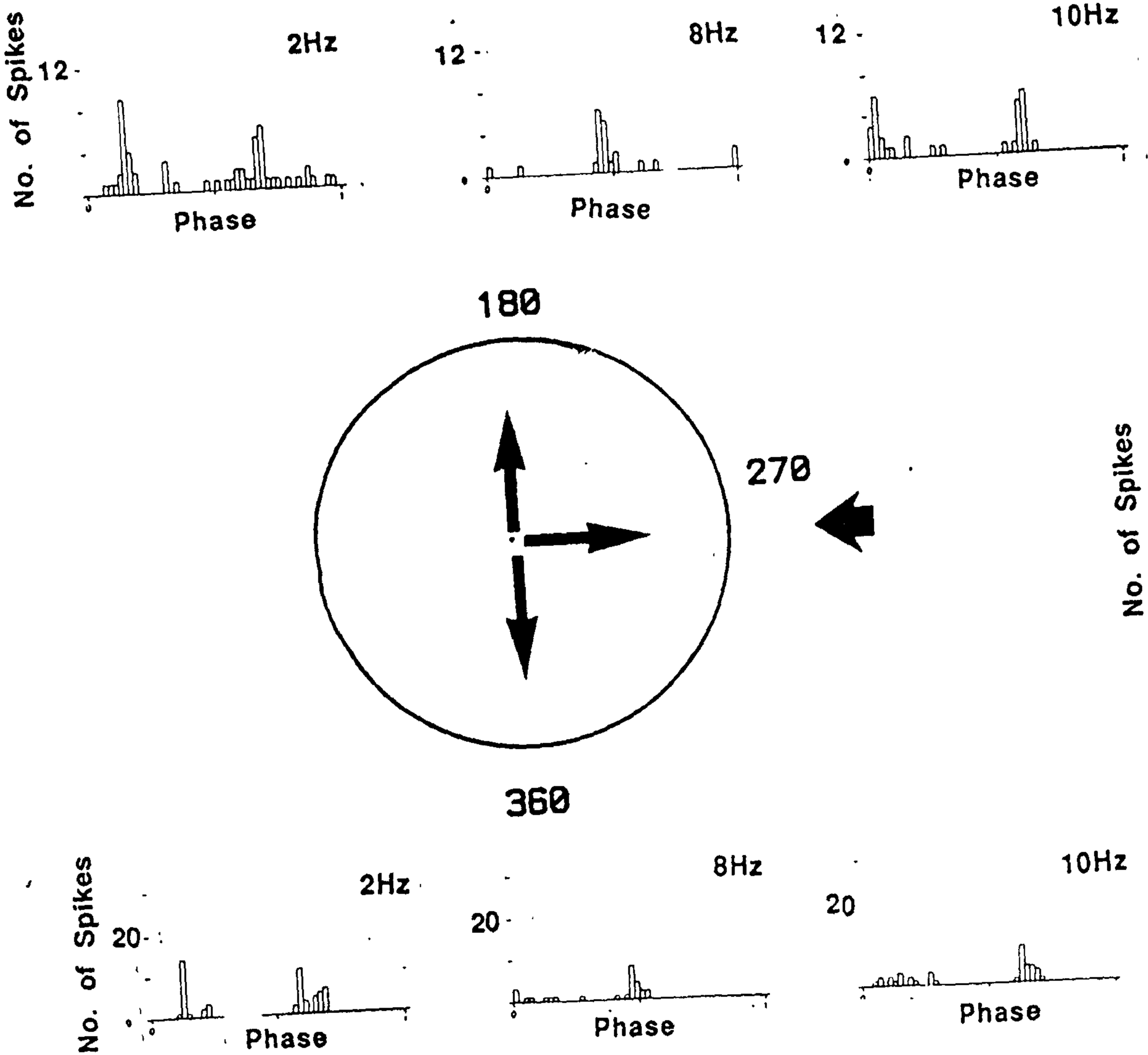

No. of Spikes
12
2Hz
Phase
12
8Hz
Phase
12
10Hz
Phase
180
270
360
No. of Spikes
20
2Hz
Phase
20
8Hz
Phase
20
10Hz
Phase
No. of Spikes
16
2Hz
Phase
12
8Hz
Phase
12
10Hz
Phase

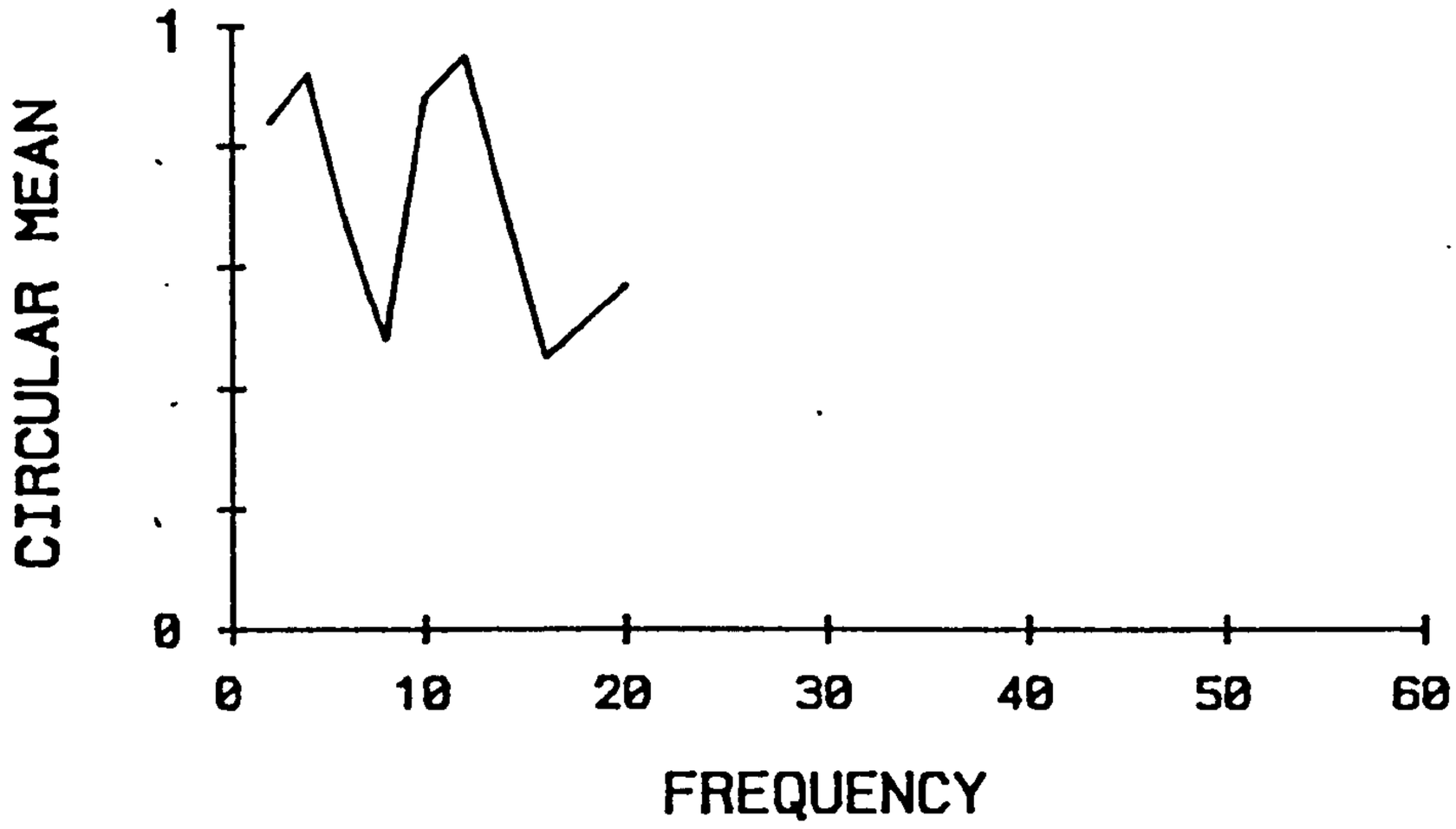
Figure 3.16 Plots of the variation in the circular mean with frequency (Hz) of the low frequency leg unit from Figure 3.15.

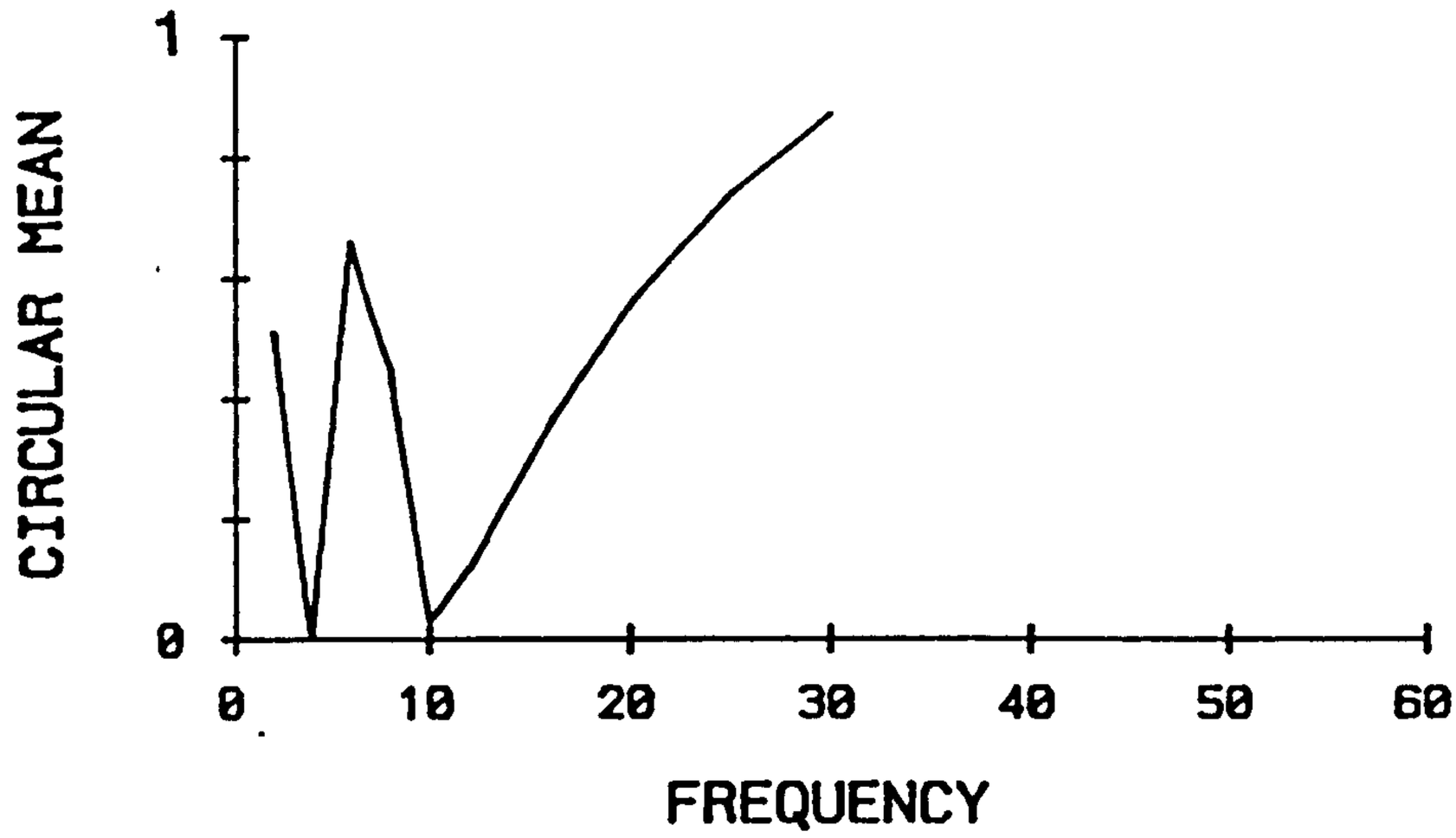
A. 180^{o}

B. 270^{o}

C. 360^{o}

180


1
CIRCULAR MEAN
0
0
10
20
30
40
50
60
FREQUENCY


1
CIRCULAR MEAN
0
0
10
20
30
40
50
60
FREQUENCY

360

1
CIRCULAR MEAN
0
0
10
20
30
40
50
60
FREQUENCY

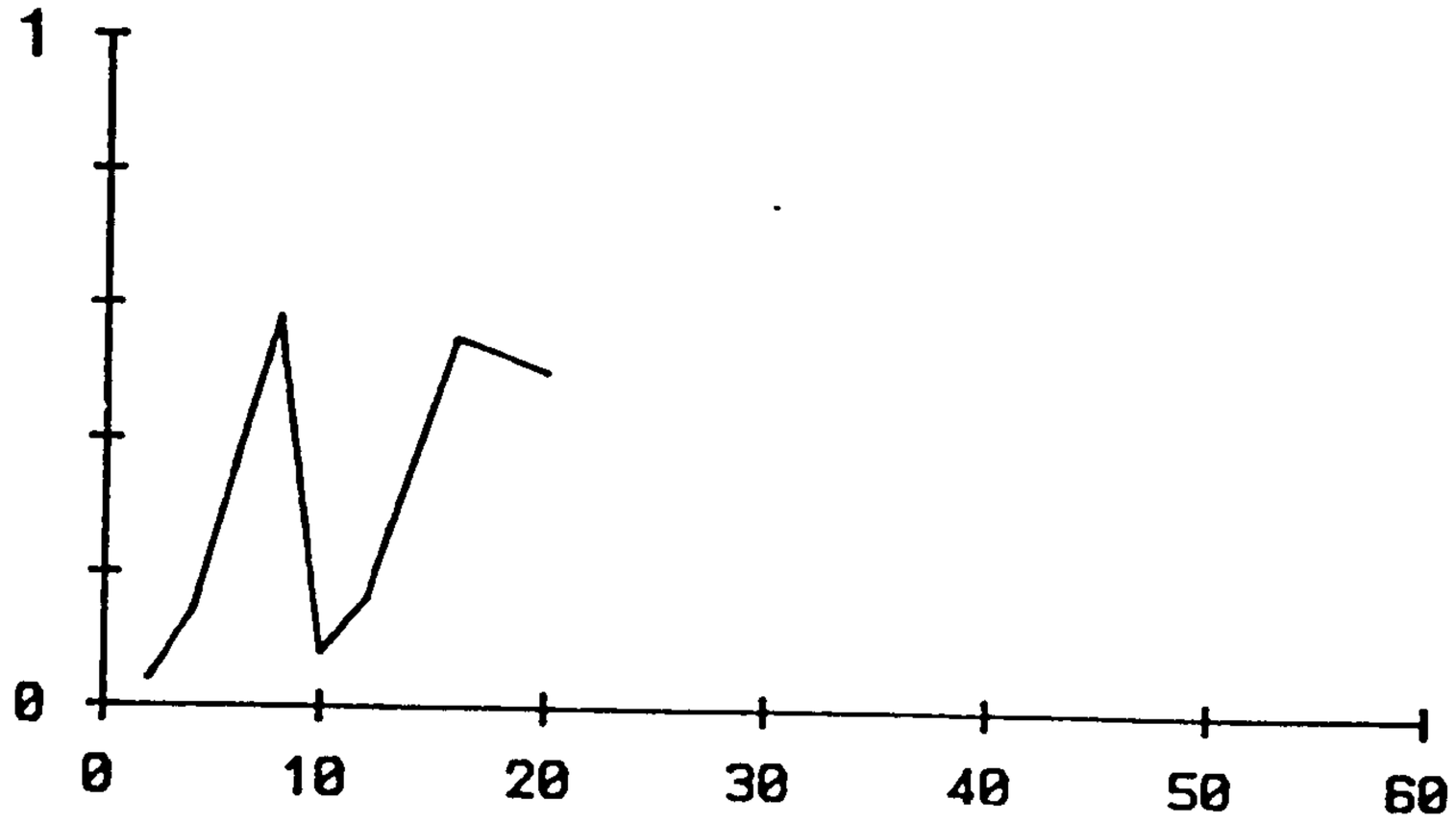
Figure 3.17 Plots of the variation in R_c with frequency of the low frequency leg unit from Figure 3.15.

A. 180°

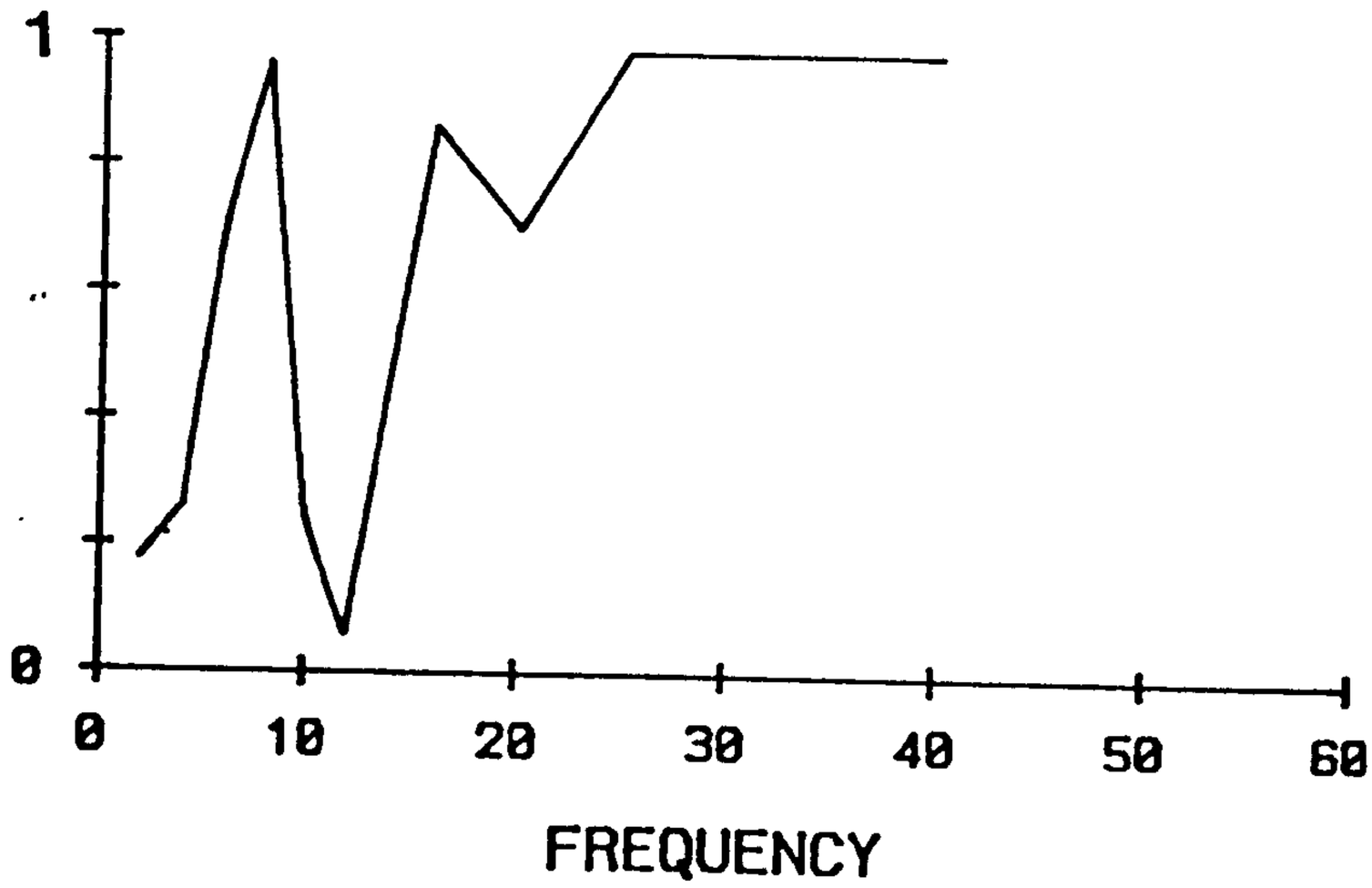
B. 270°

C. 360°

180


1
RC VALUE
0
0
10
20
30
40
50
60
FREQUENCY

270


1
RC VALUE
0
0
10
20
30
40
50
60
FREQUENCY

360

1
RC VALUE
0
0
10
20
30
40
50
60
FREQUENCY

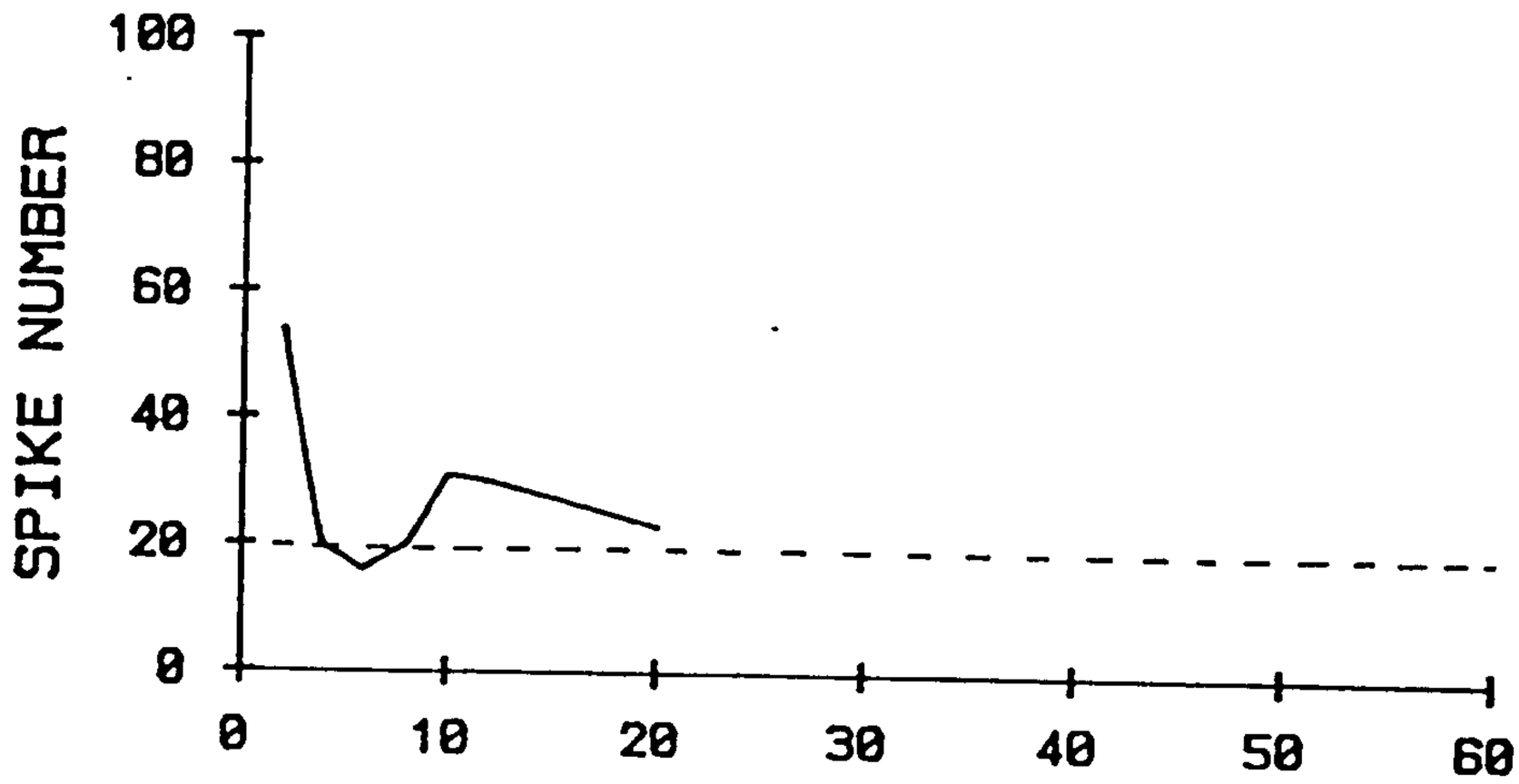
Figure 3.18 PLots of the variation in spike number with frequency of the low frequency leg unit from Figure 3.15.

A. 180°

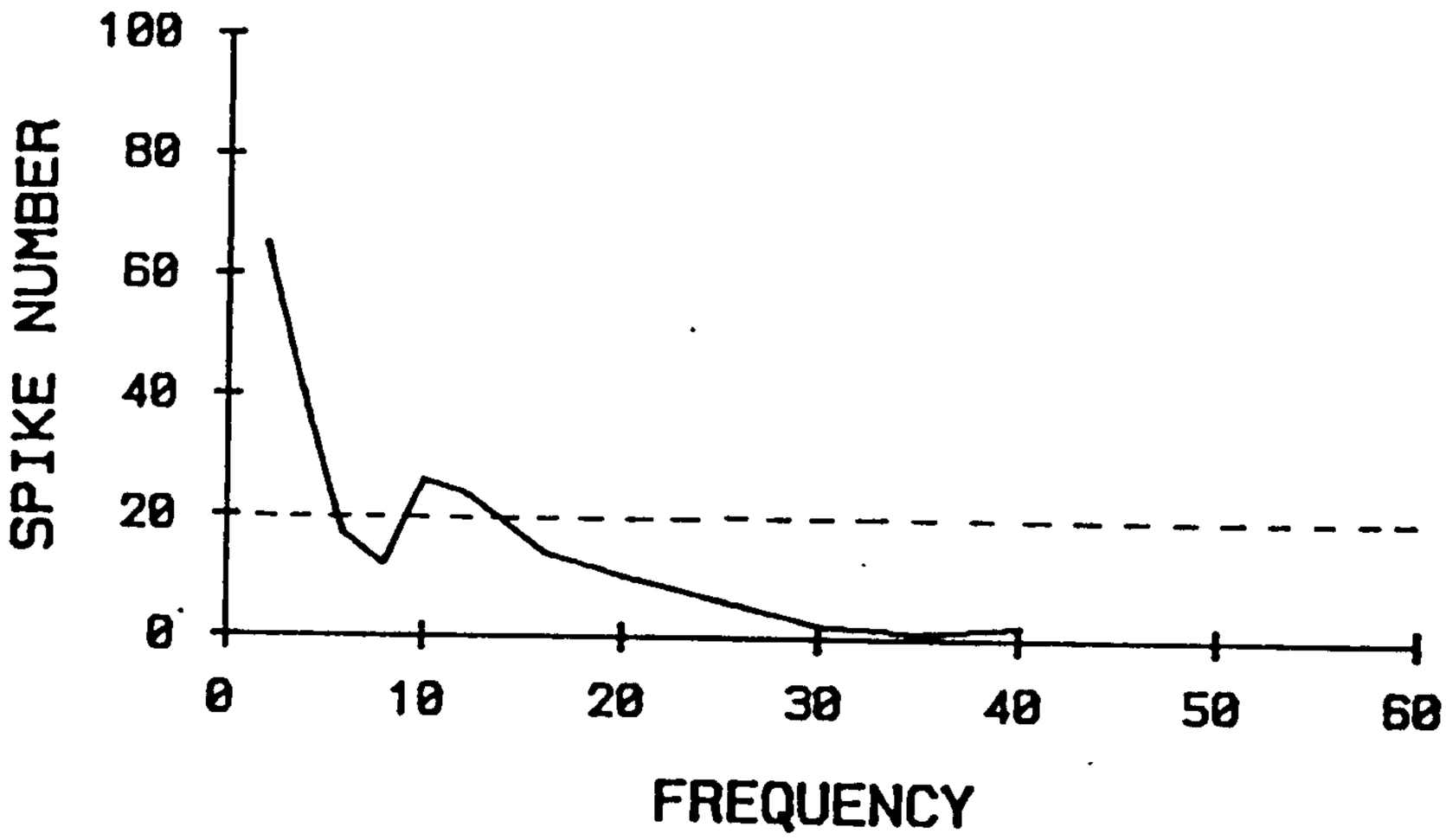
B. 270°

C. 360°

180


SPIKE NUMBER
100
80
60
40
20
0
0
10
20
30
40
50
60
FREQUENCY

270


SPIKE NUMBER
100
80
60
40
20
0
0
10
20
30
40
50
60
FREQUENCY

360

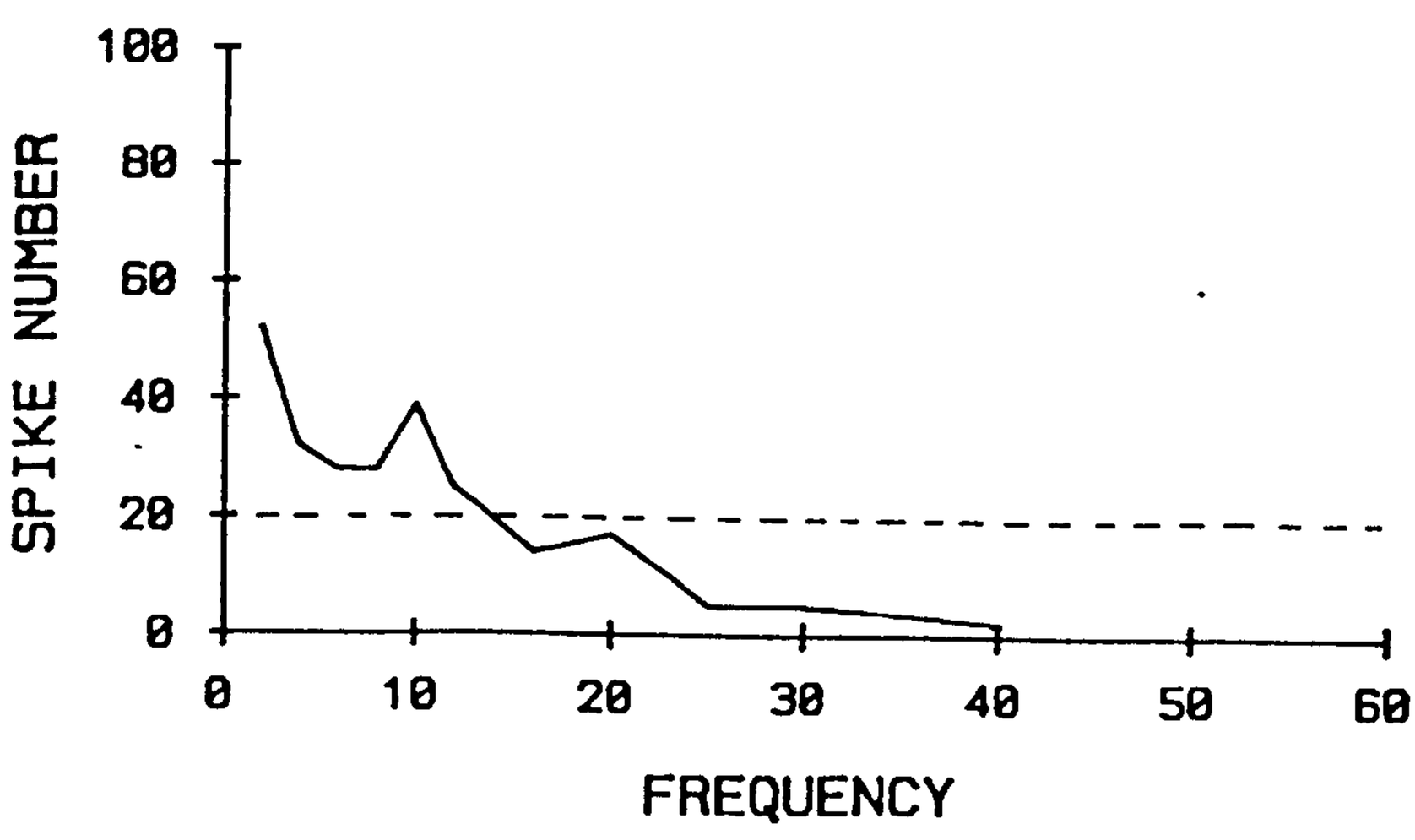
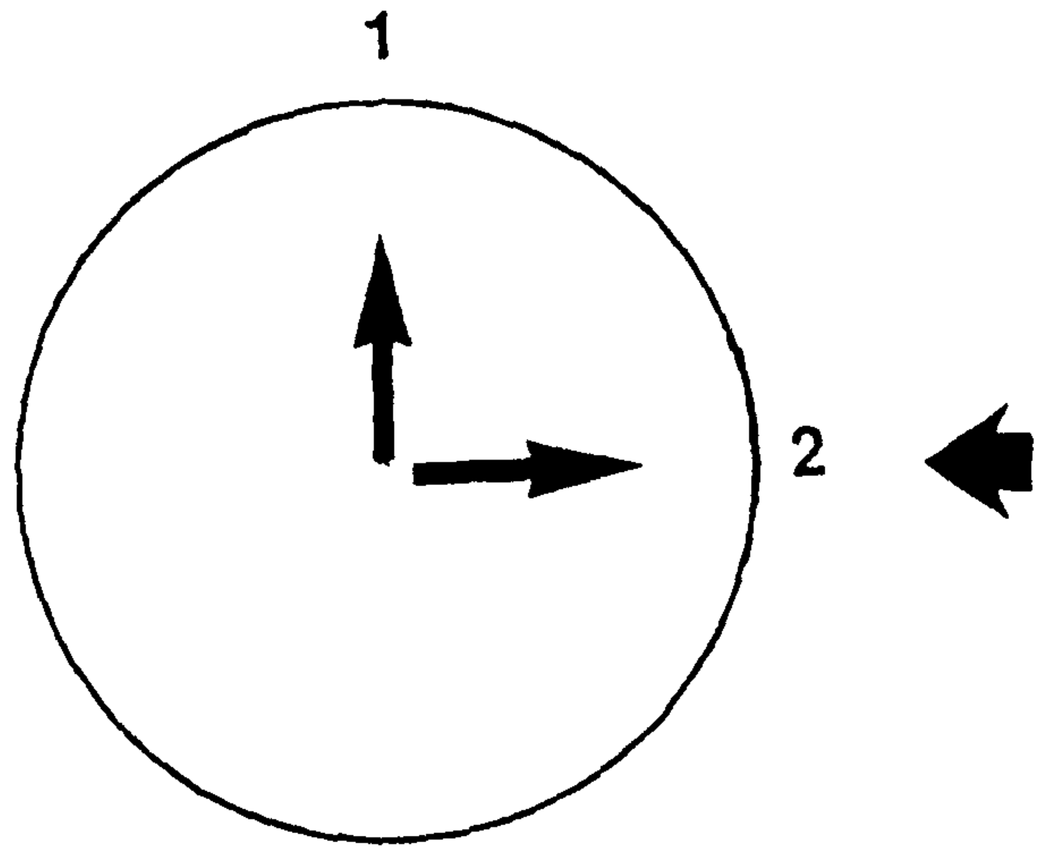

SPIKE NUMBER
100
80
60
40
20
0
0
10
20
30
40
50
60
FREQUENCY

Figure 3.19 Phase histograms showing the responses of a high frequency leg unit to water borne vibrations of 2 different directions produced in the acoustic tube. The plots show the number of spikes against phase at 20 and 80Hz in each direction (1 and 2). Arrows within the circle indicate the position of the distal part of the leg during the tests, the arrow outside the circle indicates the position of the loudspeaker.


1
20Hz
No. of Spikes
12
0
Phase
80Hz
No. of Spikes
12
0
Phase


1
2

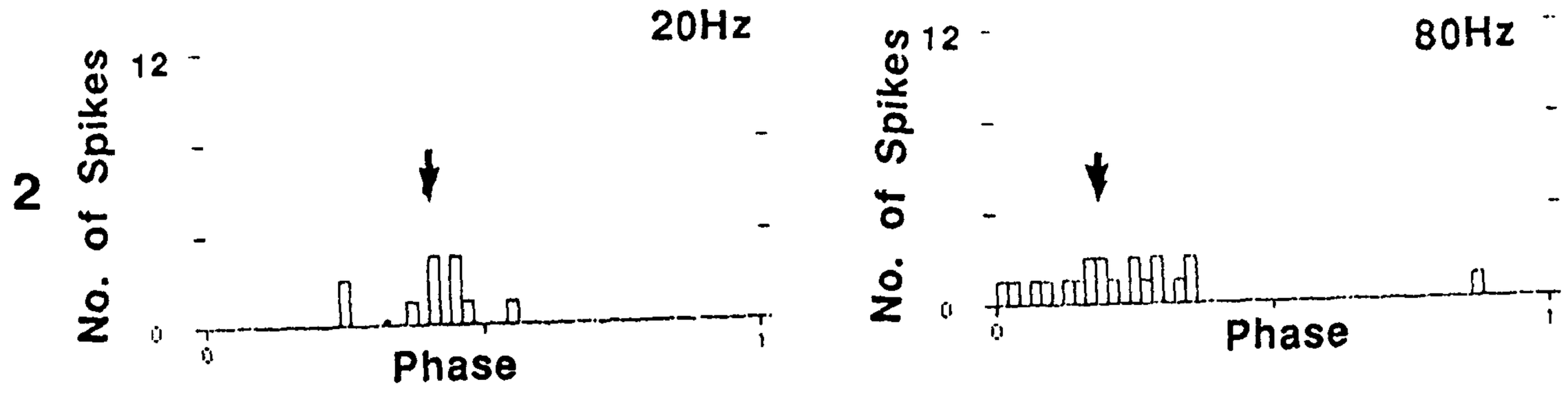
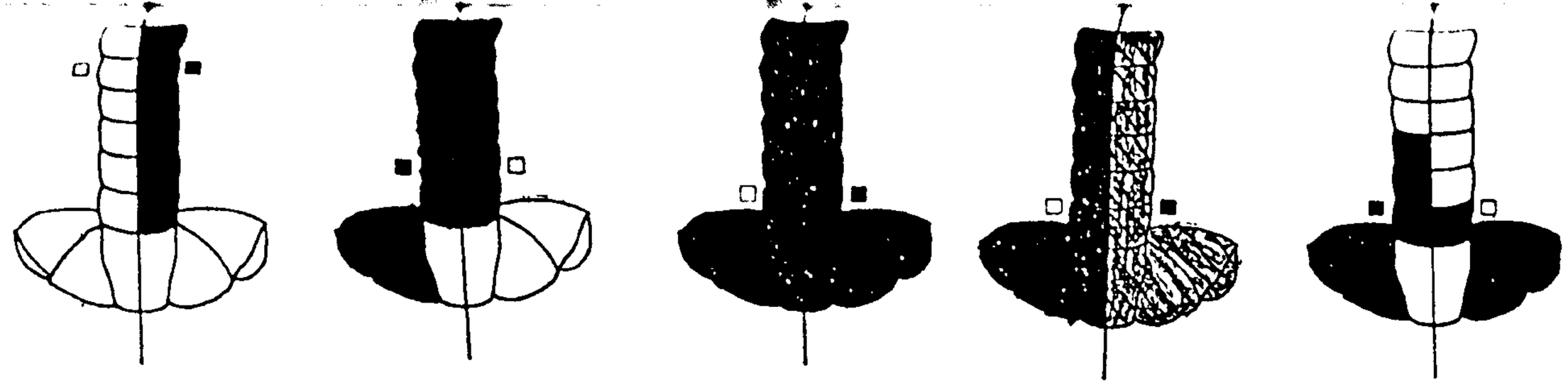
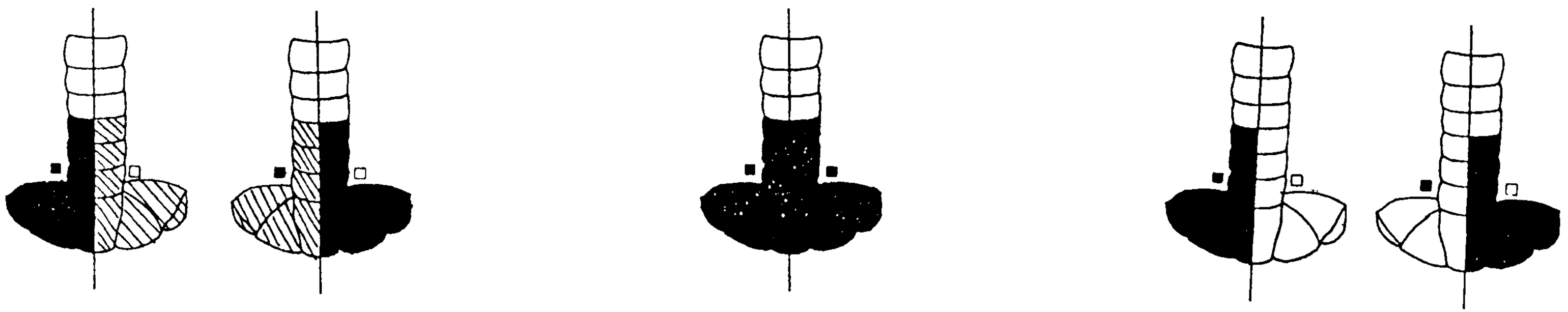

2
20Hz
No. of Spikes
12
0
Phase
80Hz
No. of Spikes
12
0
Phase

Figure 3.20 Diagrams of the abdomen of *Nephrops* showing the receptive fields of 21 mechanosensory interneurones. Dark shading indicates a strong response, hatched areas indicate weak responses. Black squares indicate the side and segment of recording, open squares indicate the conralateral partner of each interneurone.

A



B



C

D

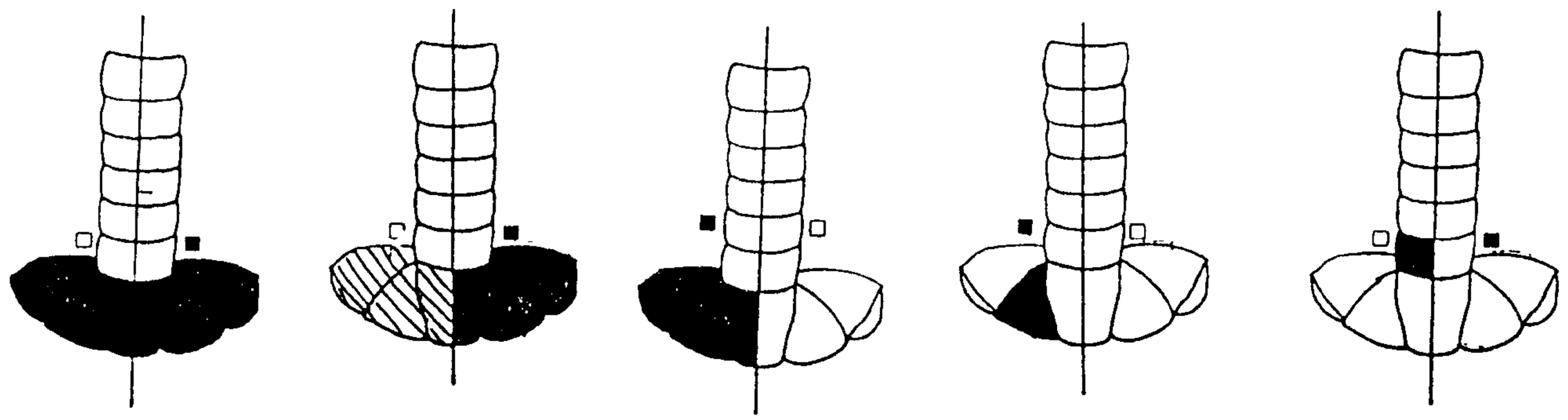
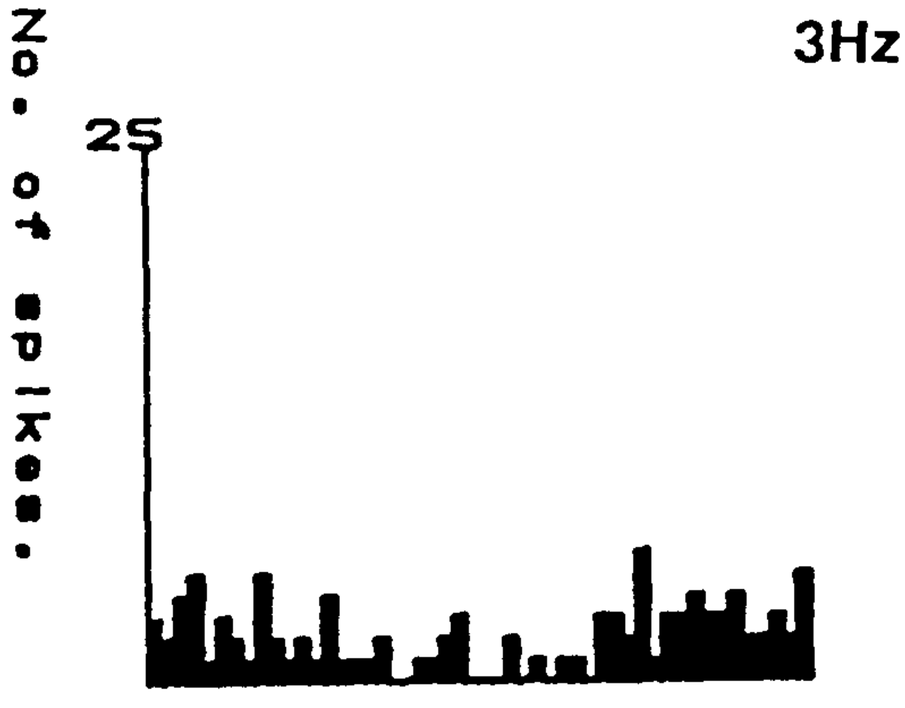
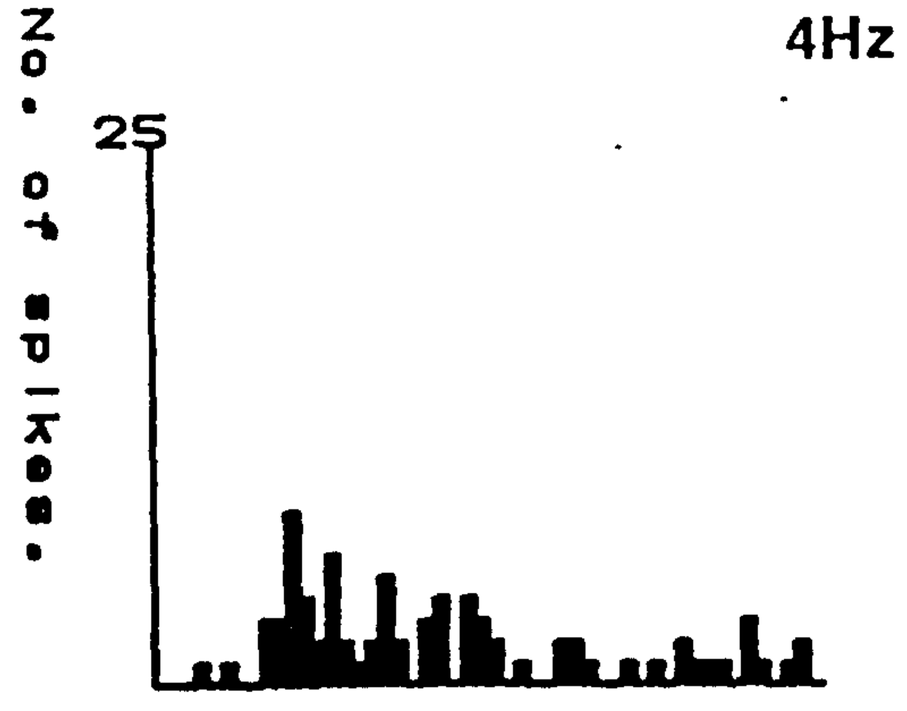
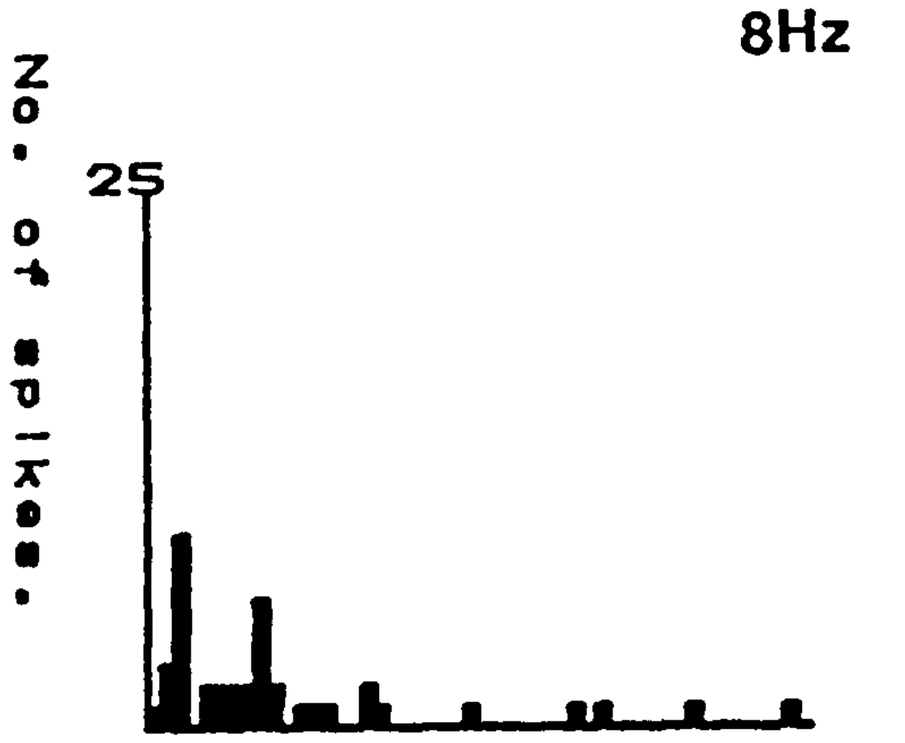
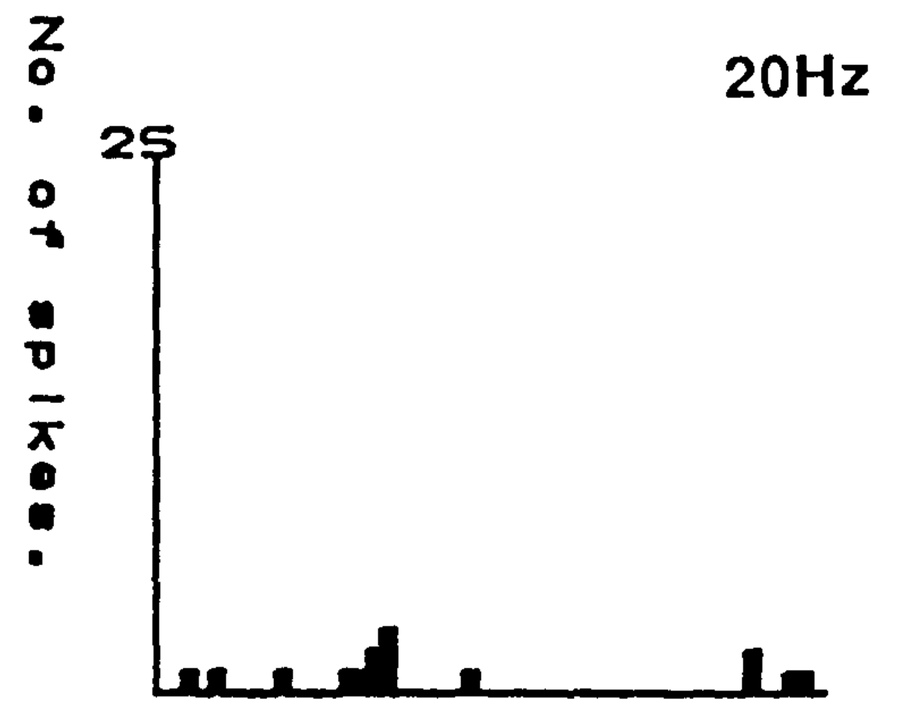


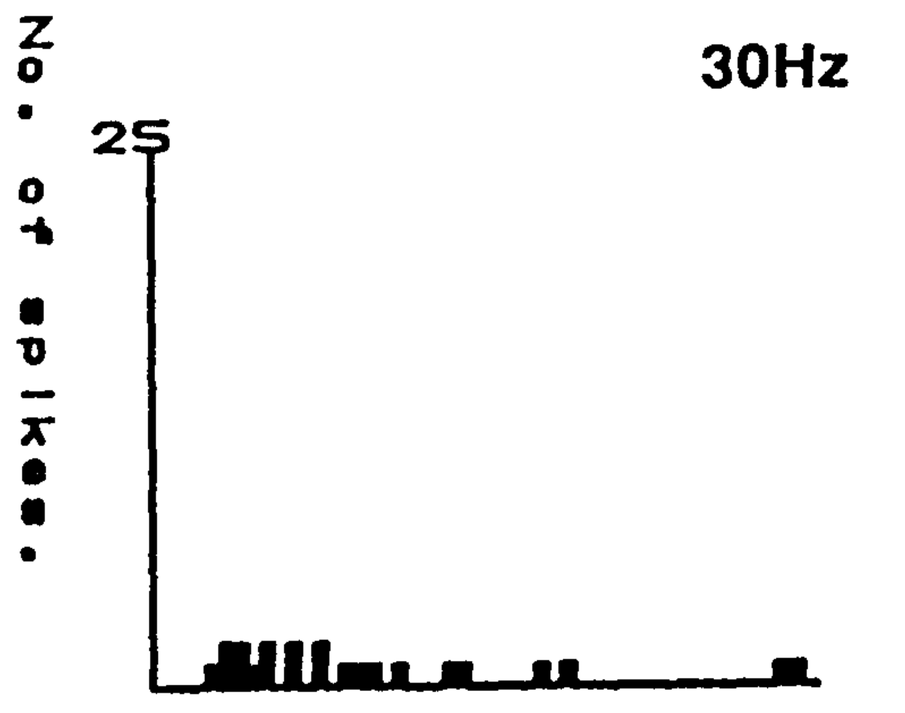
Figure 3.21 Phase histograms showing the responses of a typical wide range low frequency interneurone. Plots show the number of spikes against phase for a range of frequencies.

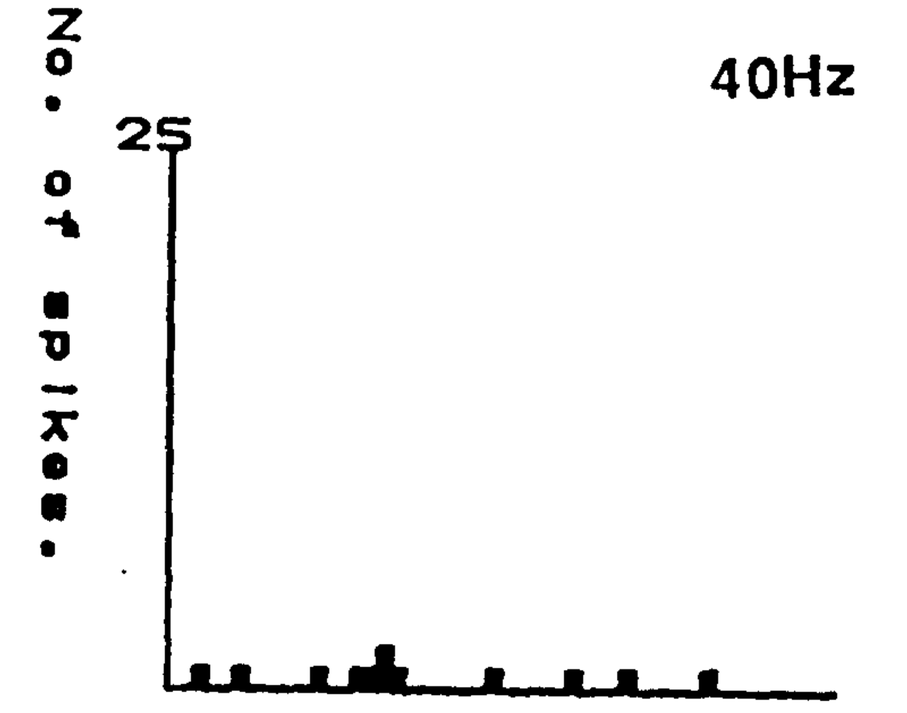

3Hz
25
No. of spikes

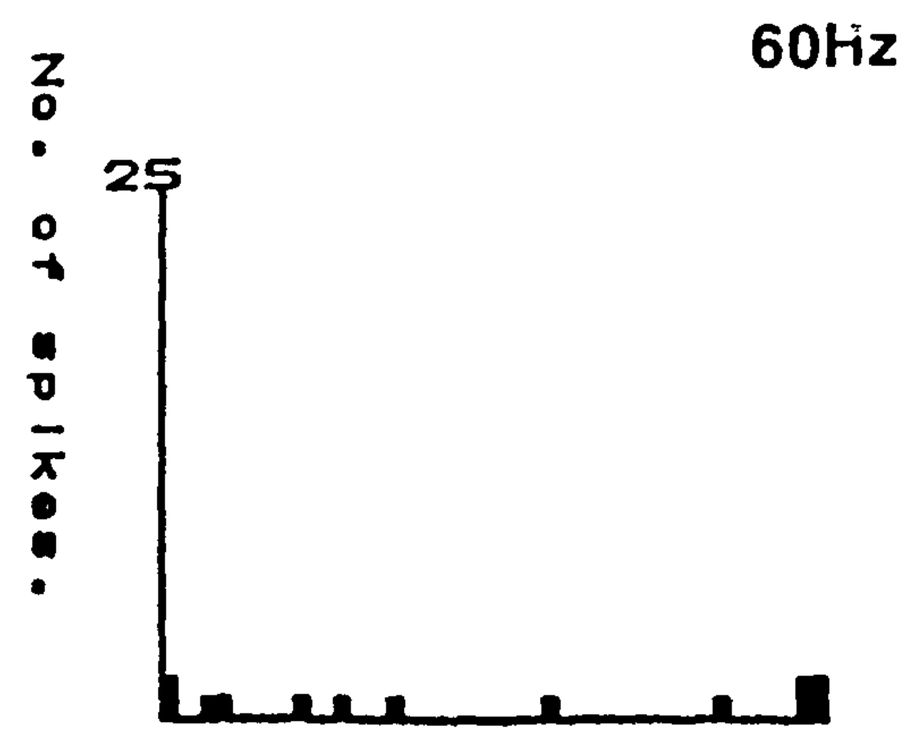

4Hz
25
No. of spikes


8Hz
25
No. of spikes


20Hz
25
No. of spikes


30Hz
25
No. of spikes


40Hz
25
No. of spikes


60Hz
25
No. of spikes

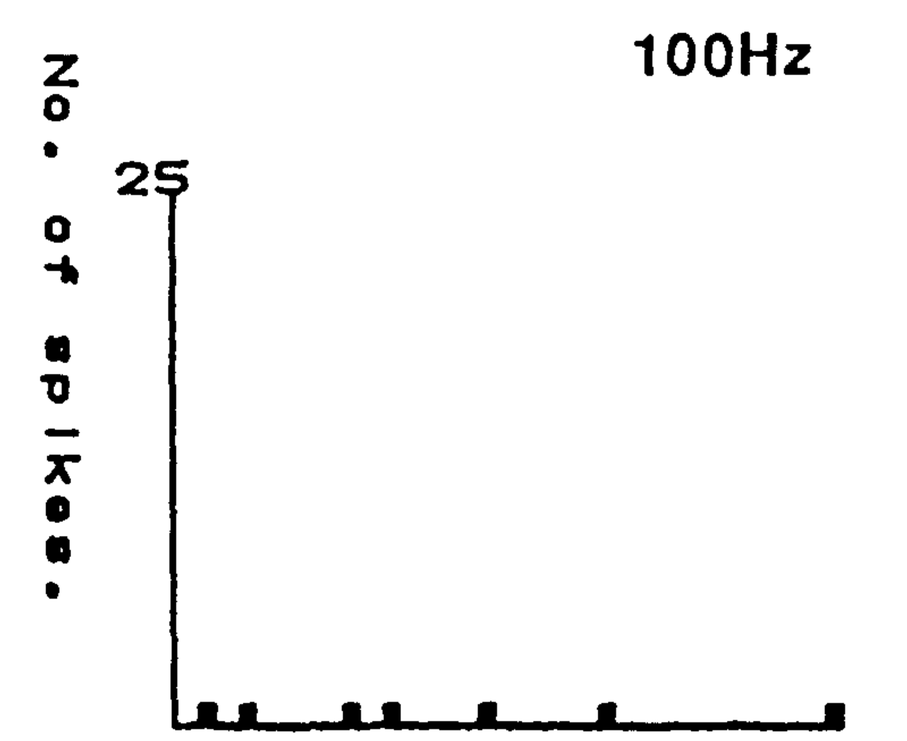

100Hz
25
No. of spikes

Figure 3.22 The statistical data for the interneurone shown in the previous figure. Plots show variation in the circular mean (A), The R_C value (B) and the spike number (C) with frequency (Hz).

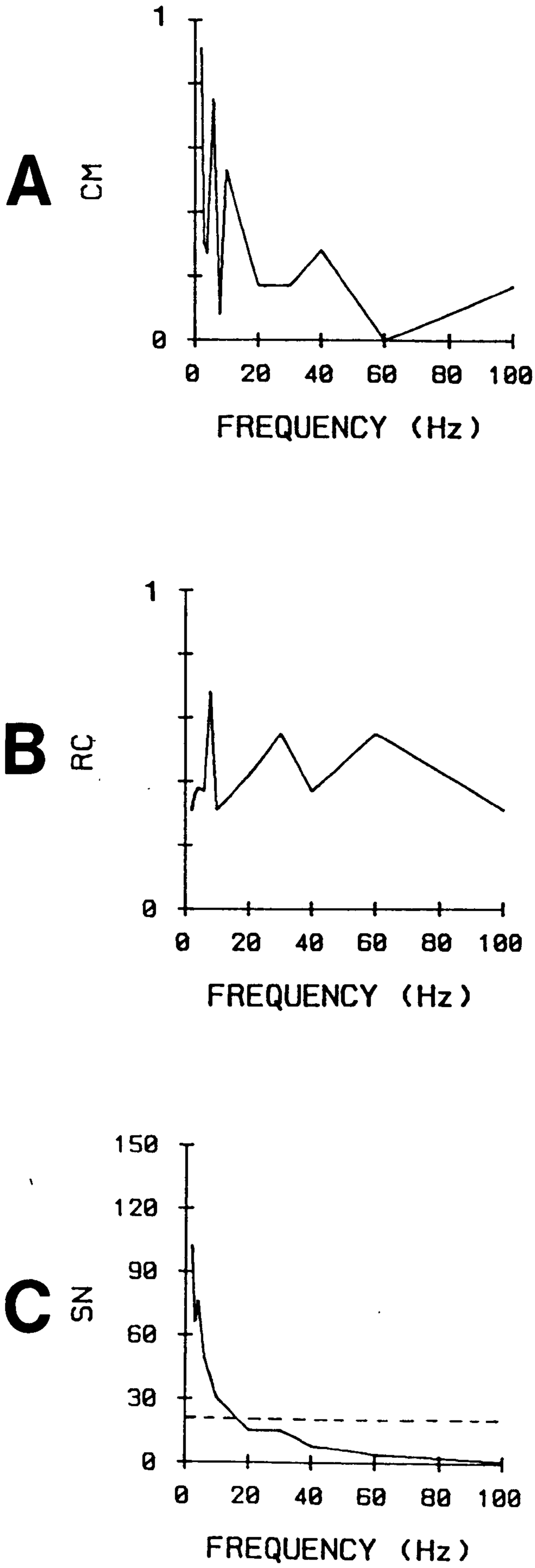
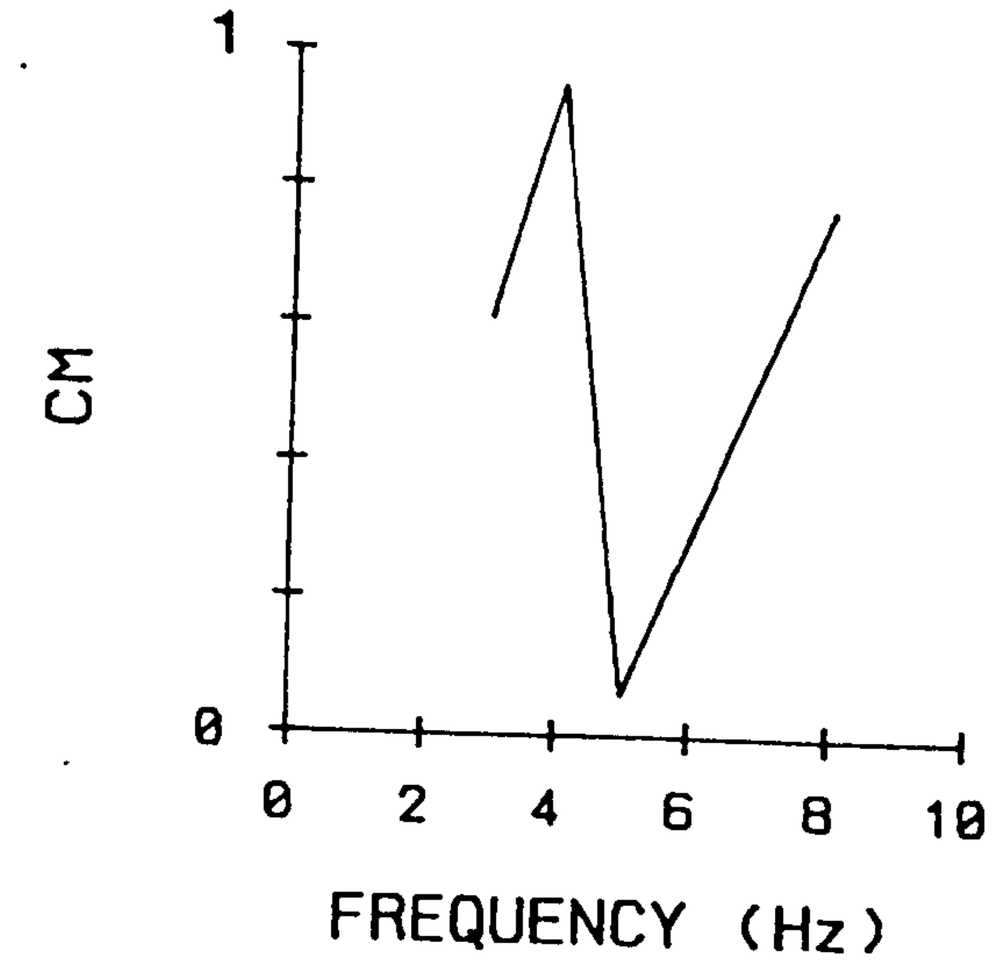
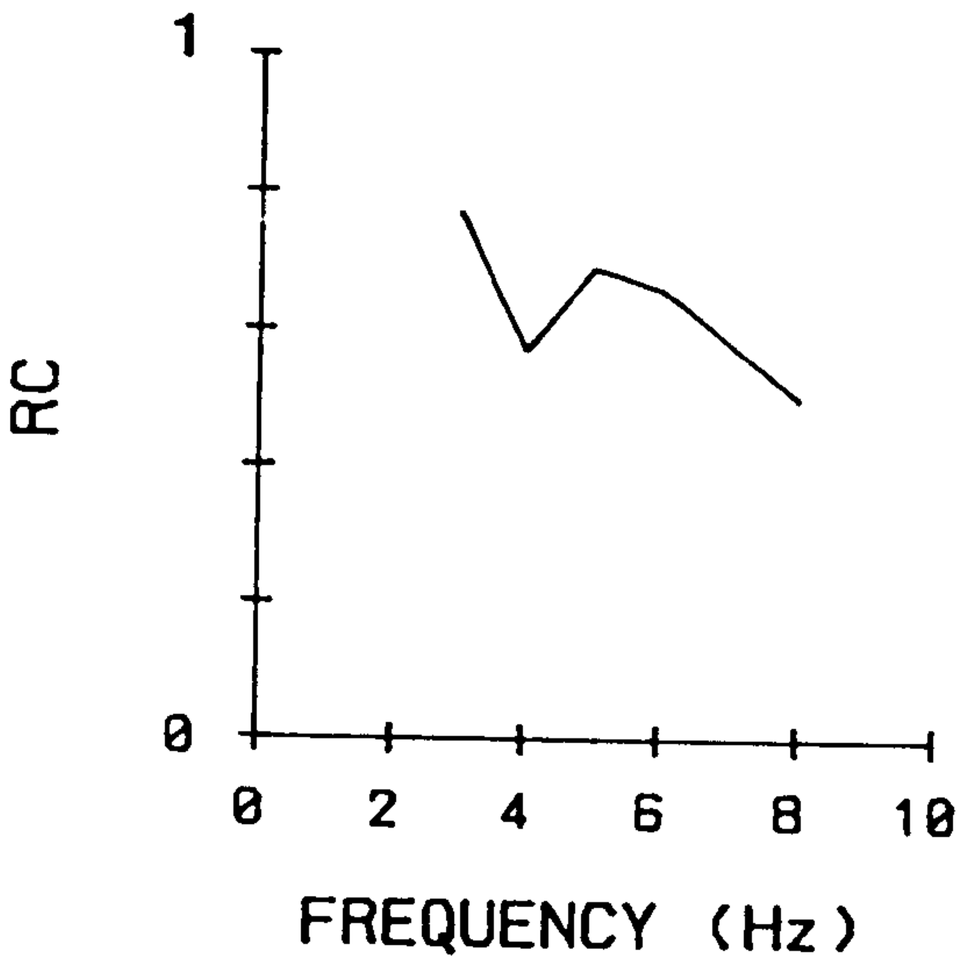

A
CM
1
0
0 20 40 60 80 100
FREQUENCY (Hz)
B
RC
1
0
0 20 40 60 80 100
FREQUENCY (Hz)
C
SN
150
120
90
60
30
0
0 20 40 60 80 100
FREQUENCY (Hz)

Figure 3.23 Statistical parameters from a narrow range low frequency interneurone responding from 3-8Hz. Details as Figure 3.22.


1
CM
0
0
2
4
6
8
10
FREQUENCY (Hz)


1
RC
0
0
2
4
6
8
10
FREQUENCY (Hz)

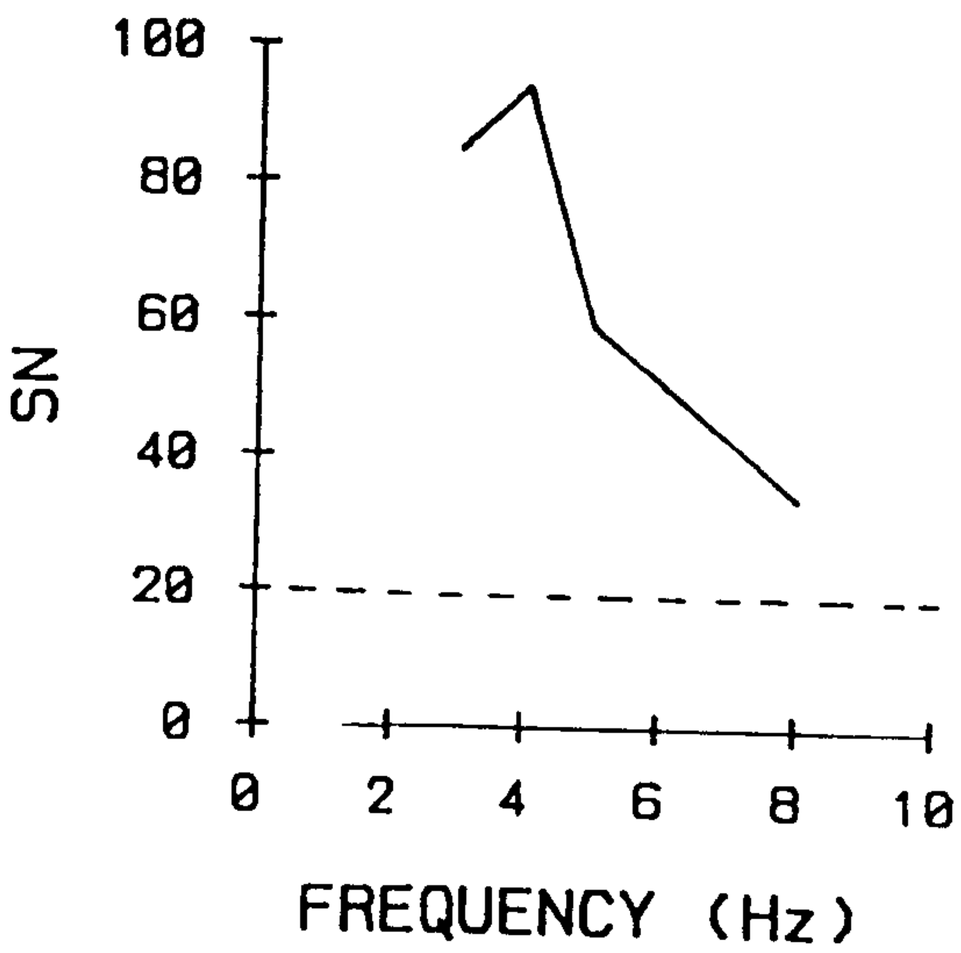
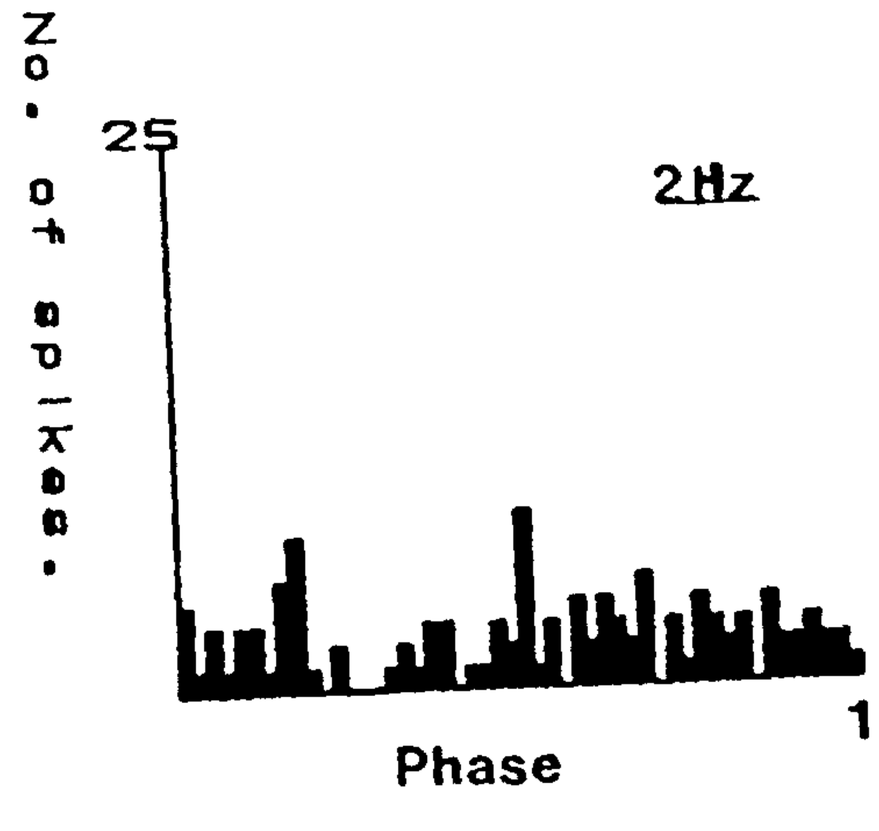
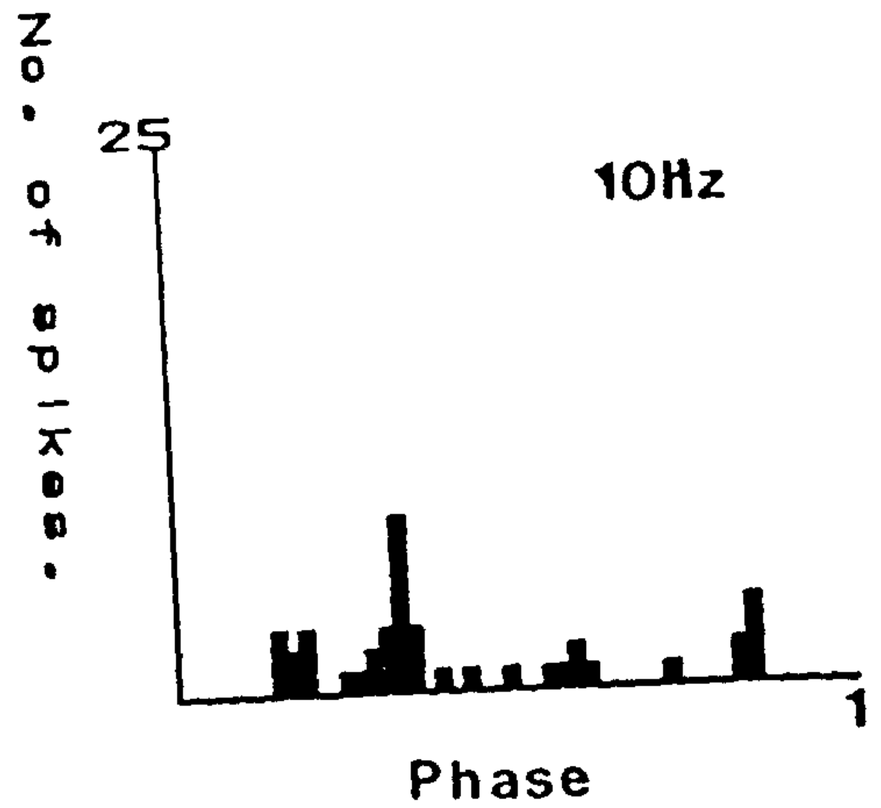
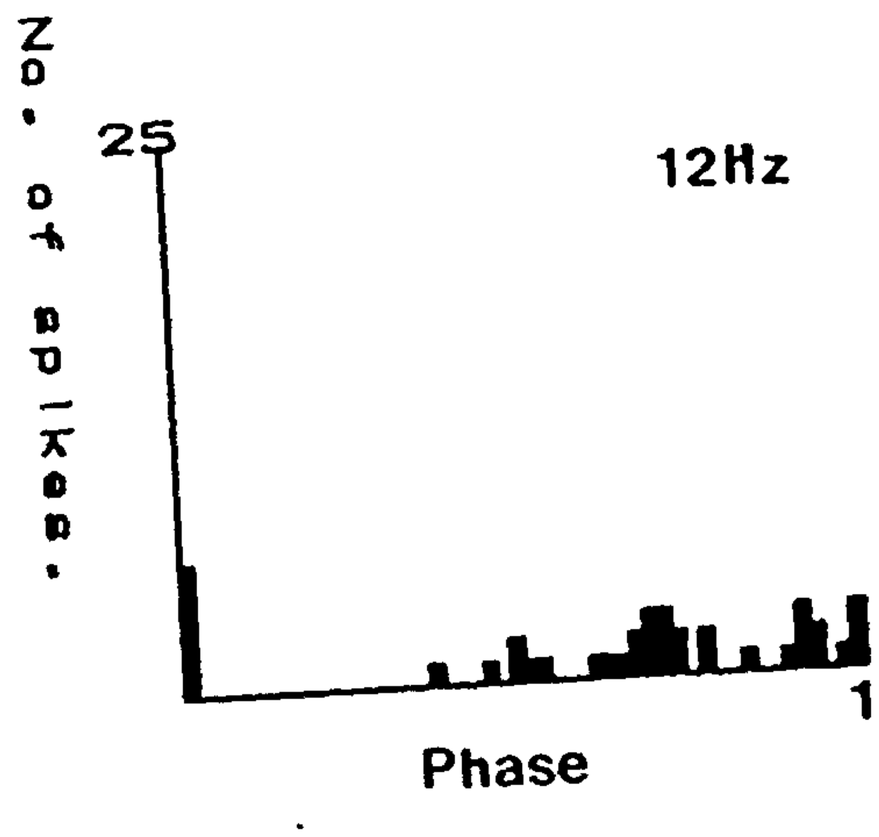
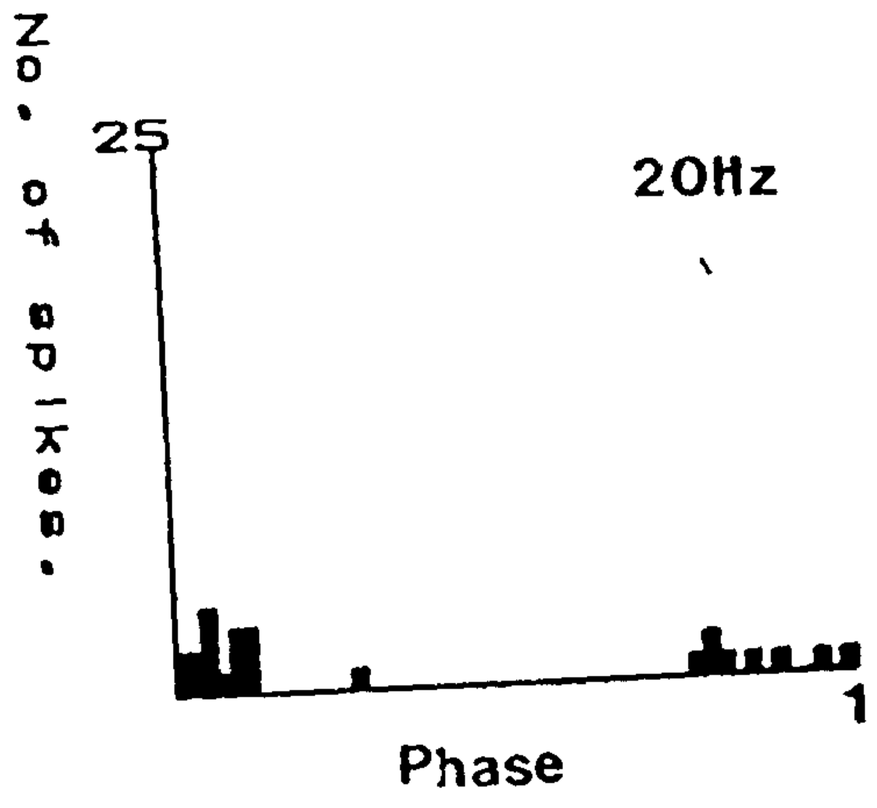

100
80
60
40
20
0
SN
0
2
4
6
8
10
FREQUENCY (Hz)

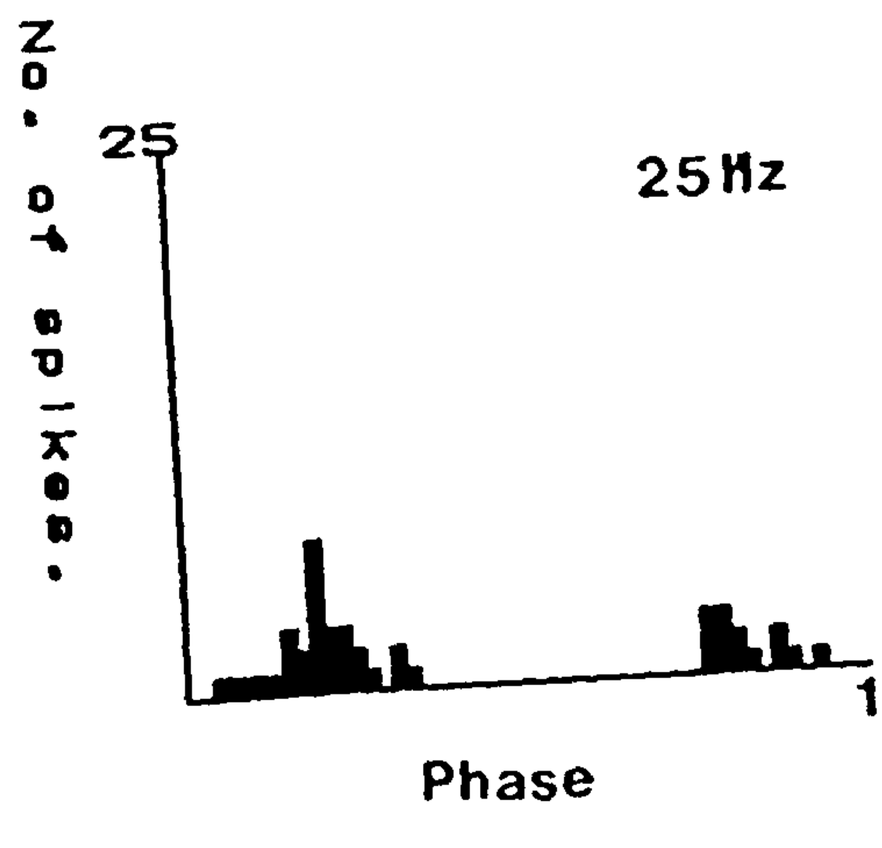
Figure 3.24 Phase histograms showing the responses of a typical intermediate frequency interneurone to water borne vibrations. Details as Figure 3.21.

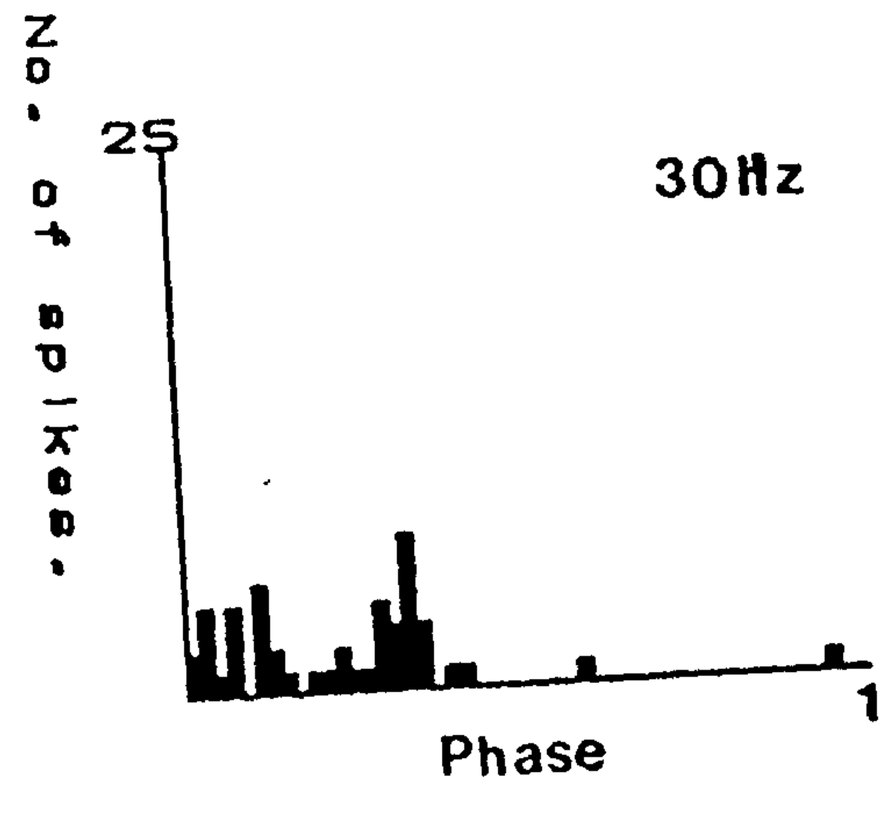

No. of spikes.
25
2Hz
1
Phase

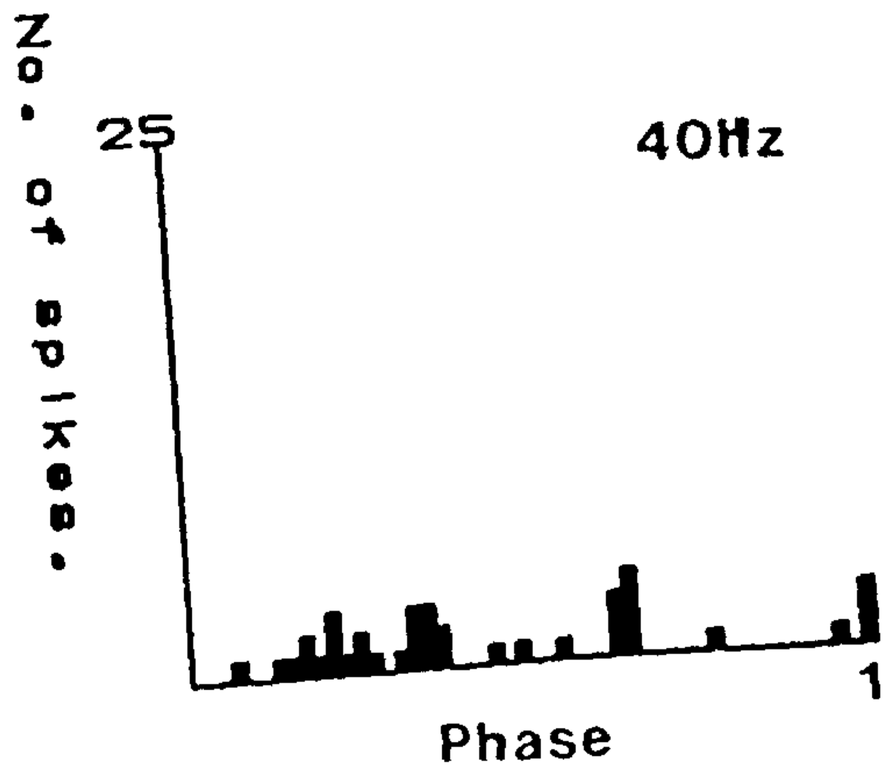

No. of spikes.
25
10Hz
1
Phase


No. of spikes.
25
12Hz
1
Phase


No. of spikes.
25
20Hz
1
Phase


No. of spikes.
25
25Hz
1
Phase


No. of spikes.
25
30Hz
1
Phase


No. of spikes.
25
40Hz
1
Phase

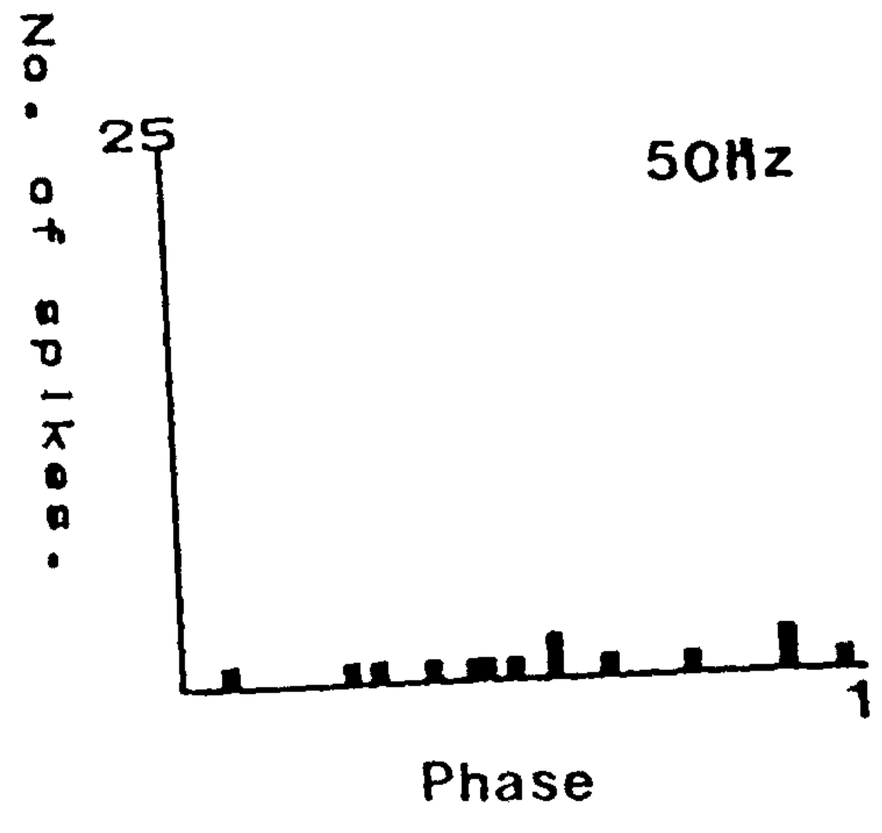
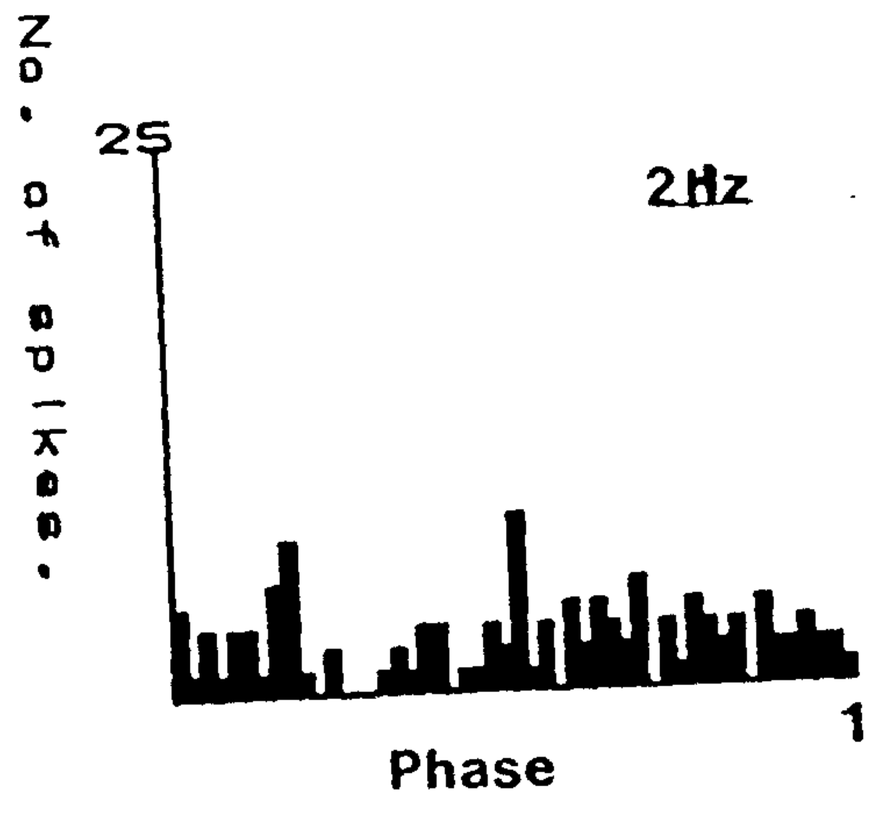
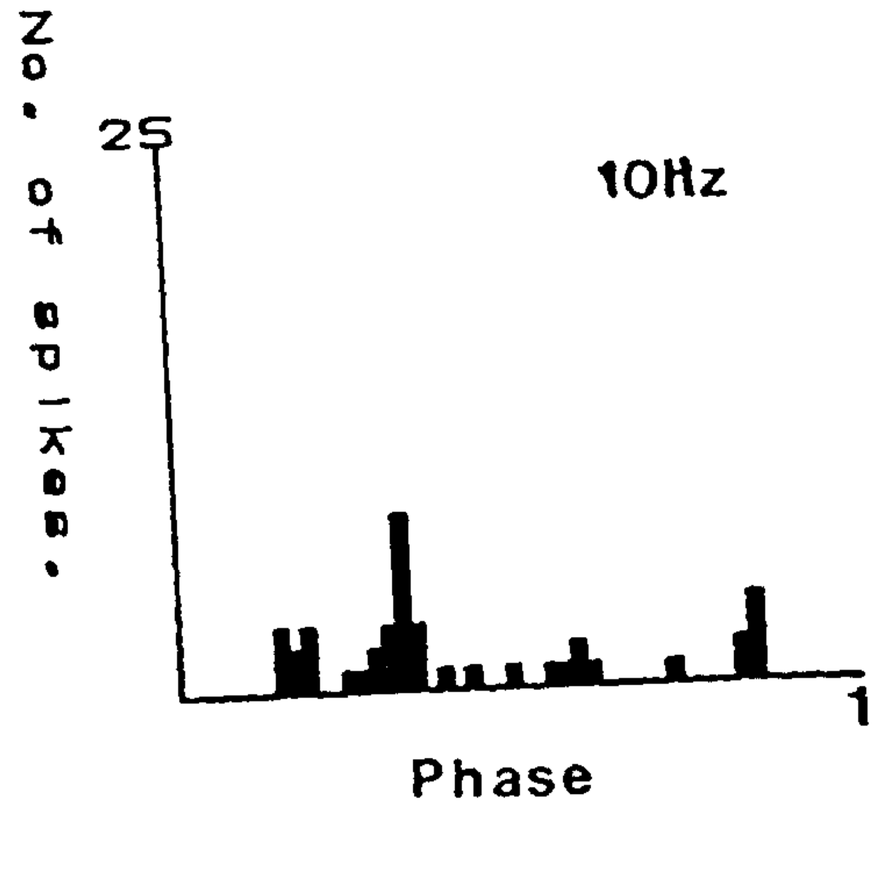
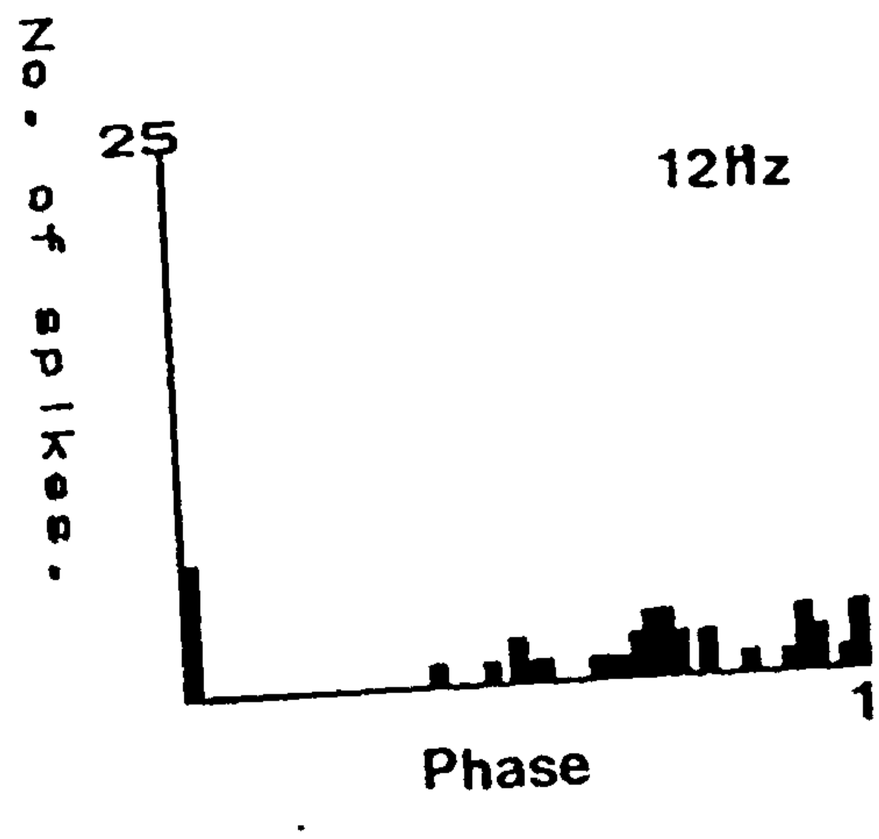
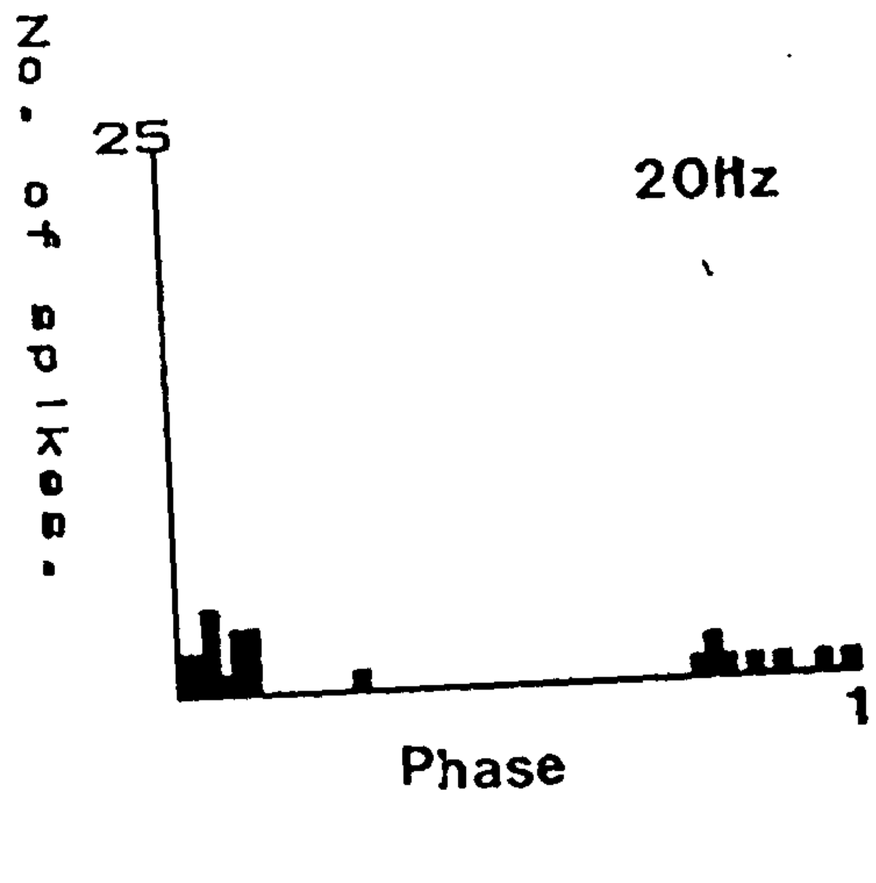

No. of spikes.
25
50Hz
1
Phase

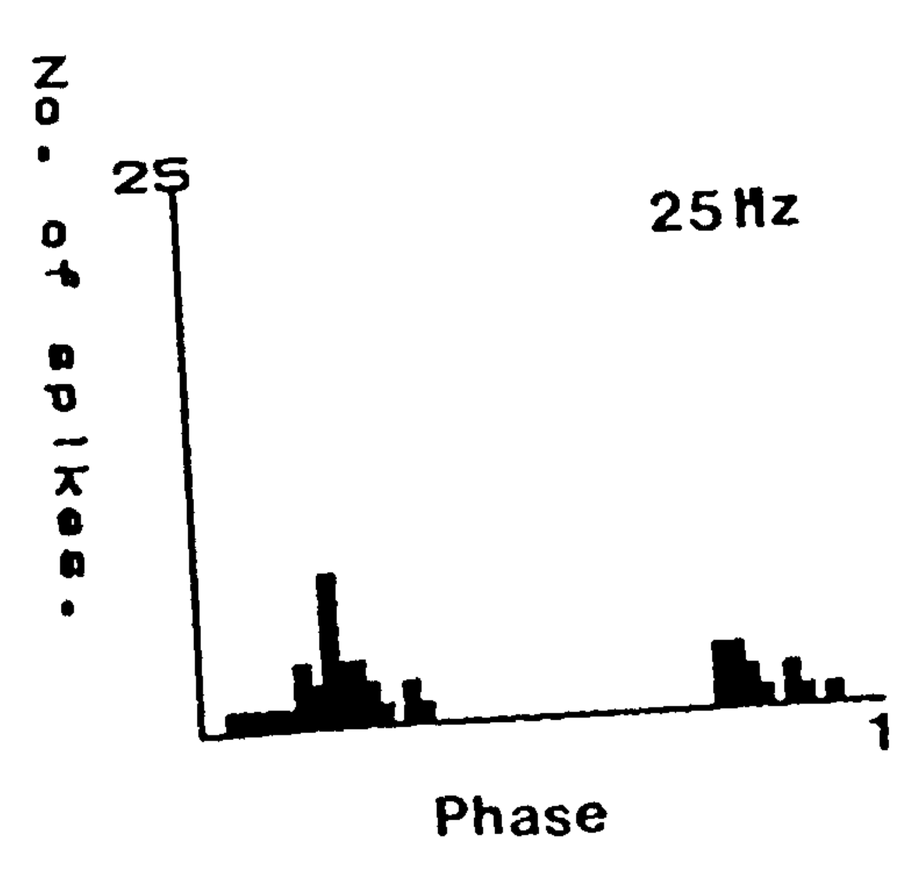
Figure 3.24 Phase histograms showing the responses of a typical intermediate frequency interneurone to water borne vibrations. Details as Figure 3.21.

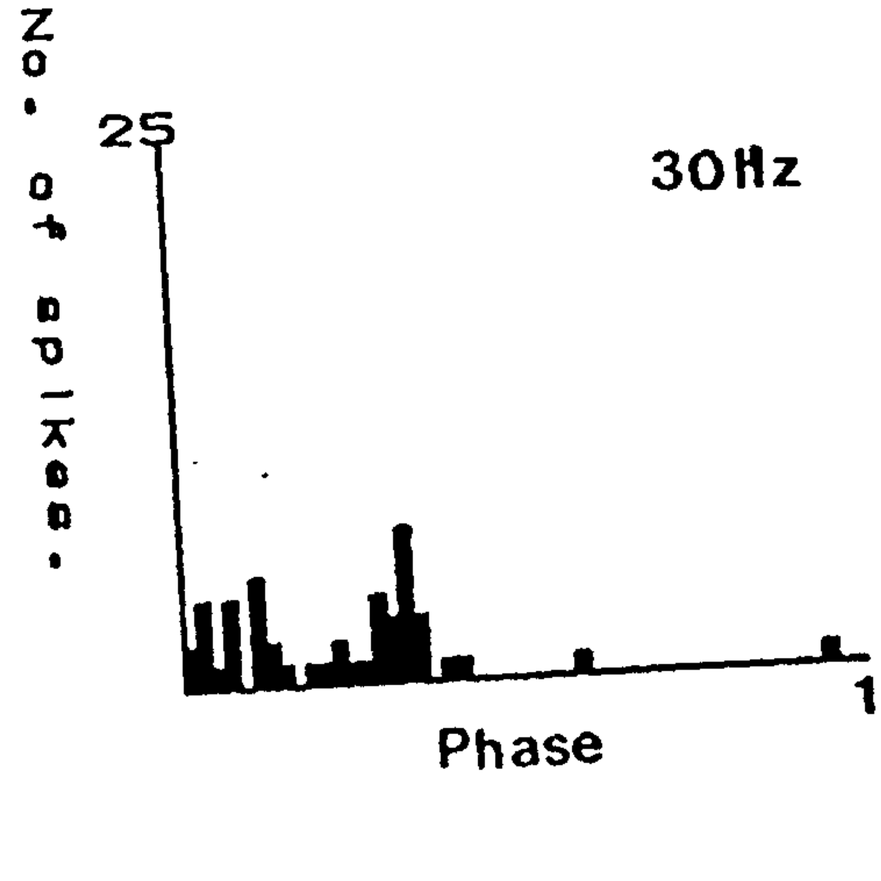

No. of spikes.
25
2Hz
1
Phase


No. of spikes.
25
10Hz
1
Phase


No. of spikes.
25
12Hz
1
Phase


No. of spikes.
25
20Hz
1
Phase

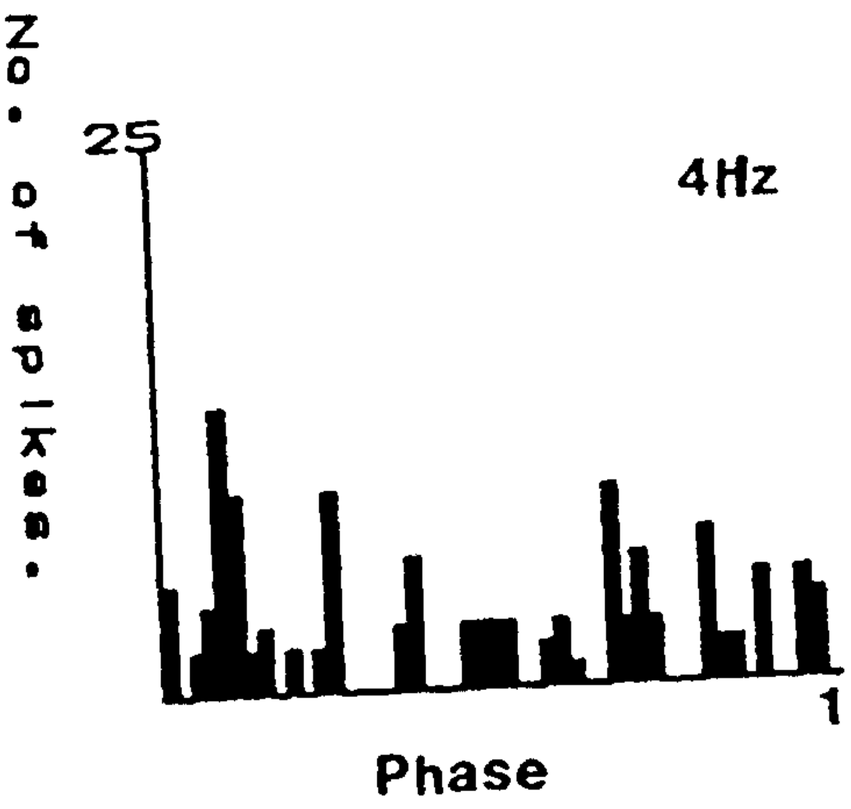

No. of spikes.
25
25Hz
1
Phase

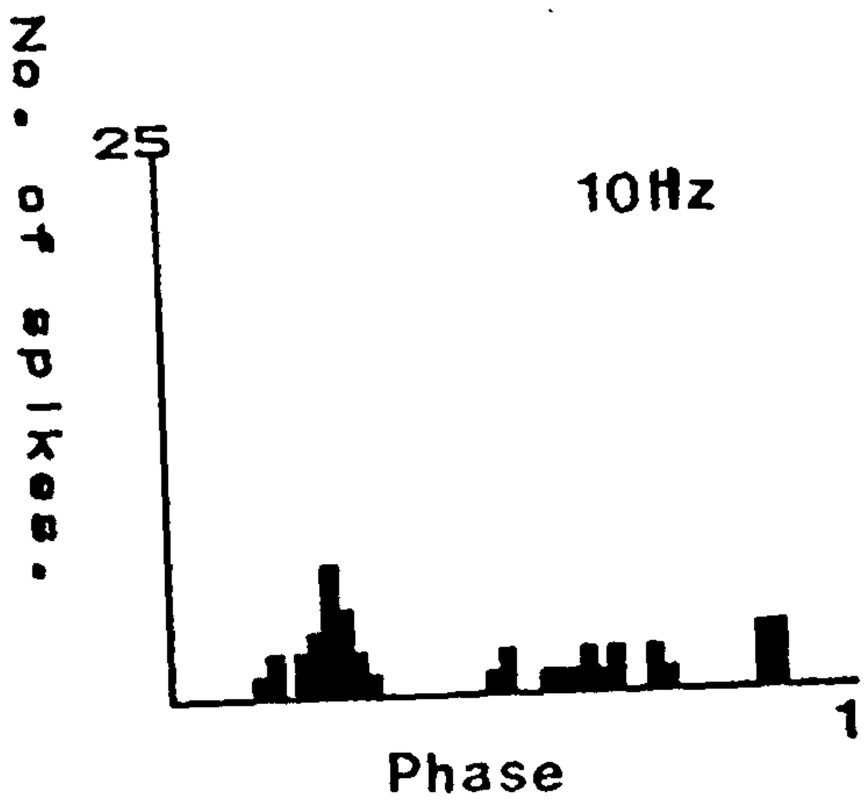

No. of spikes.
25
30Hz
1
Phase

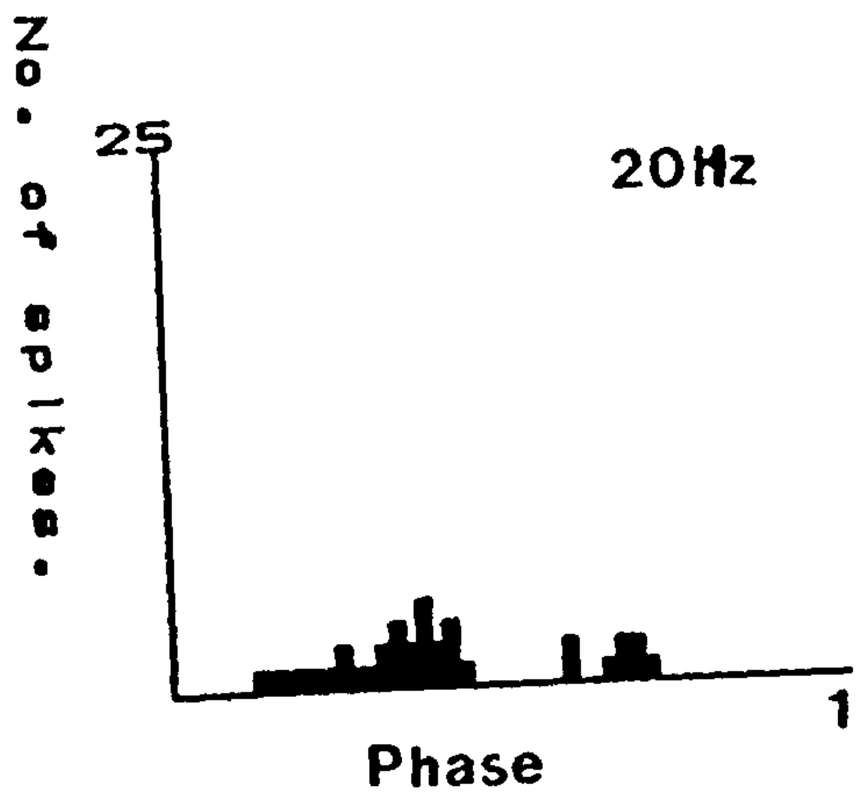
No. of spikes.
25
40Hz
1
Phase

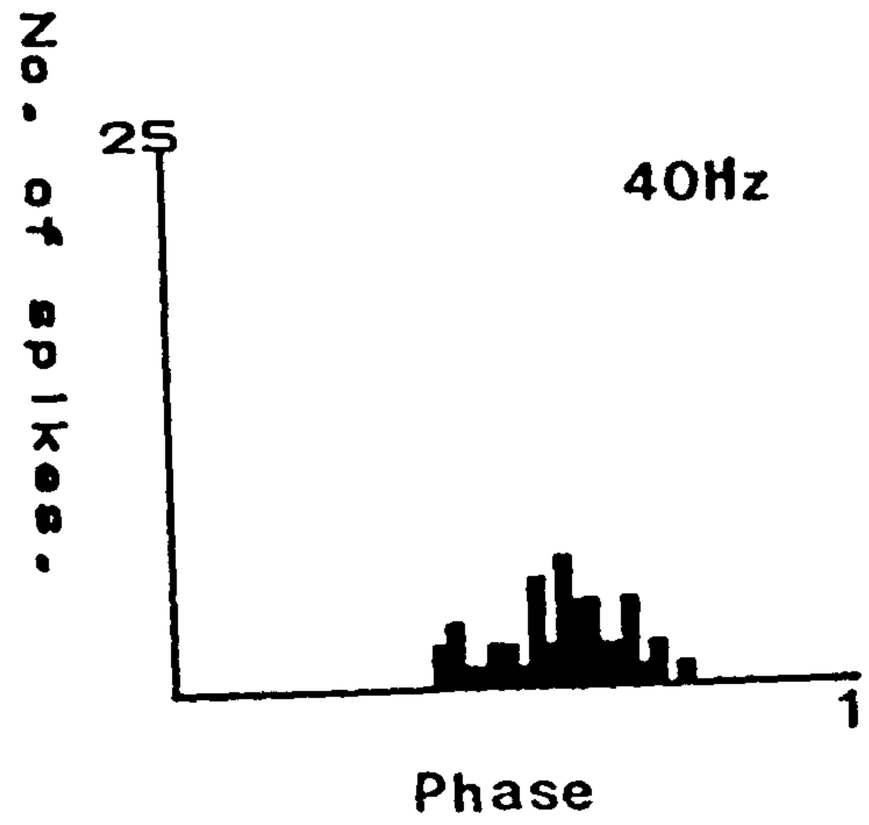
No. of spikes.
25
50Hz
1
Phase

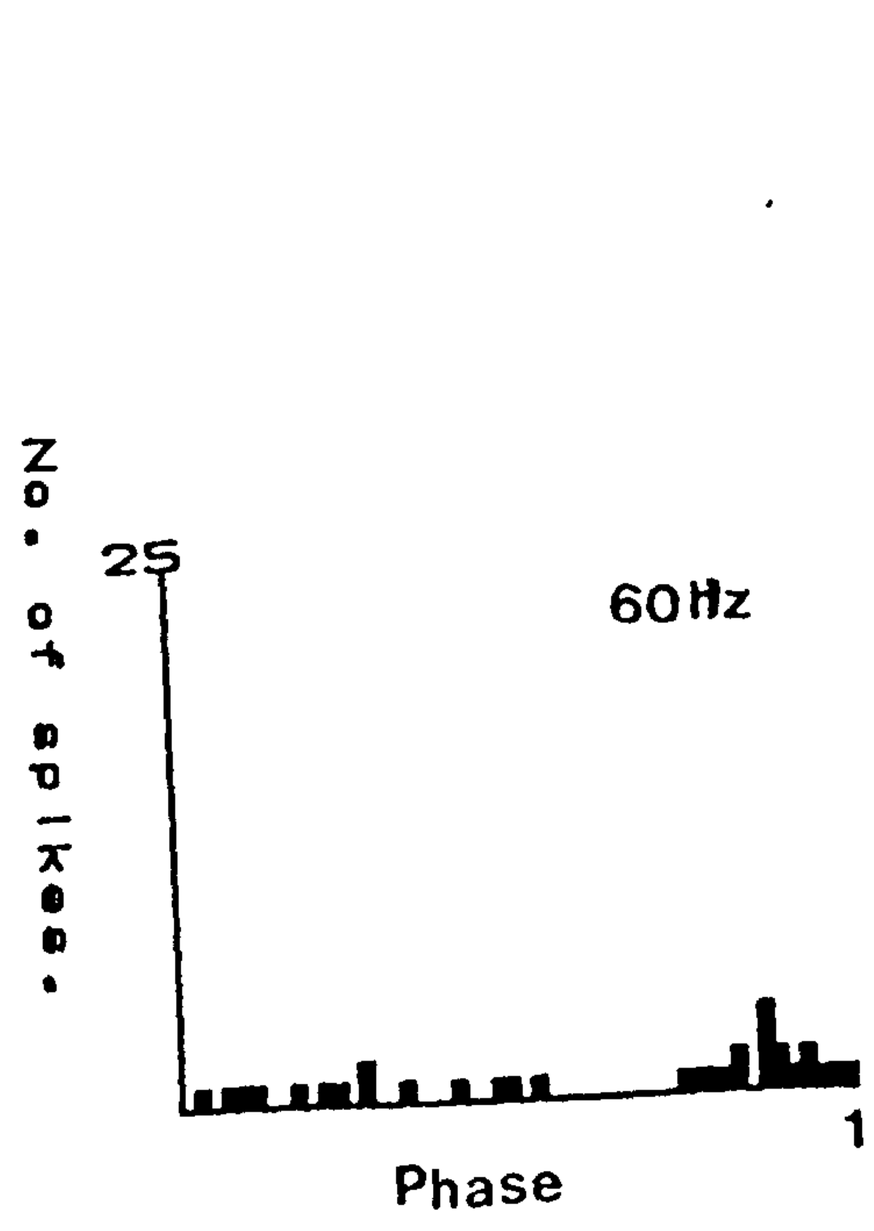
Figure 3.25 Phase histograms showing the responses of a typical high frequency interneurone to water bonre vibrations. Details as Figure 3.21.


4Hz
No. of spikes.
25
1
Phase


10Hz
No. of spikes.
25
1
Phase


20Hz
No. of spikes.
25
1
Phase


40Hz
No. of spikes.
25
1
Phase


60Hz
No. of spikes.
25
1
Phase

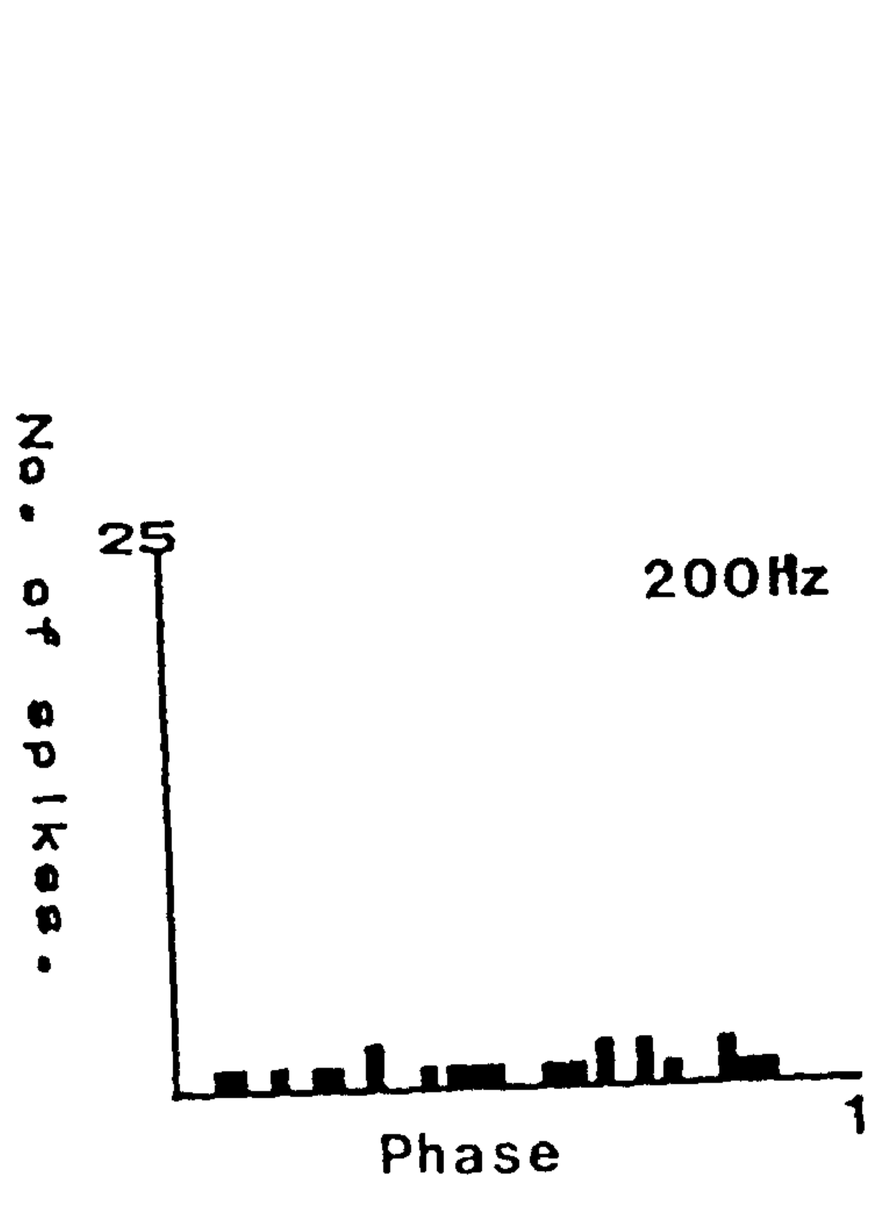
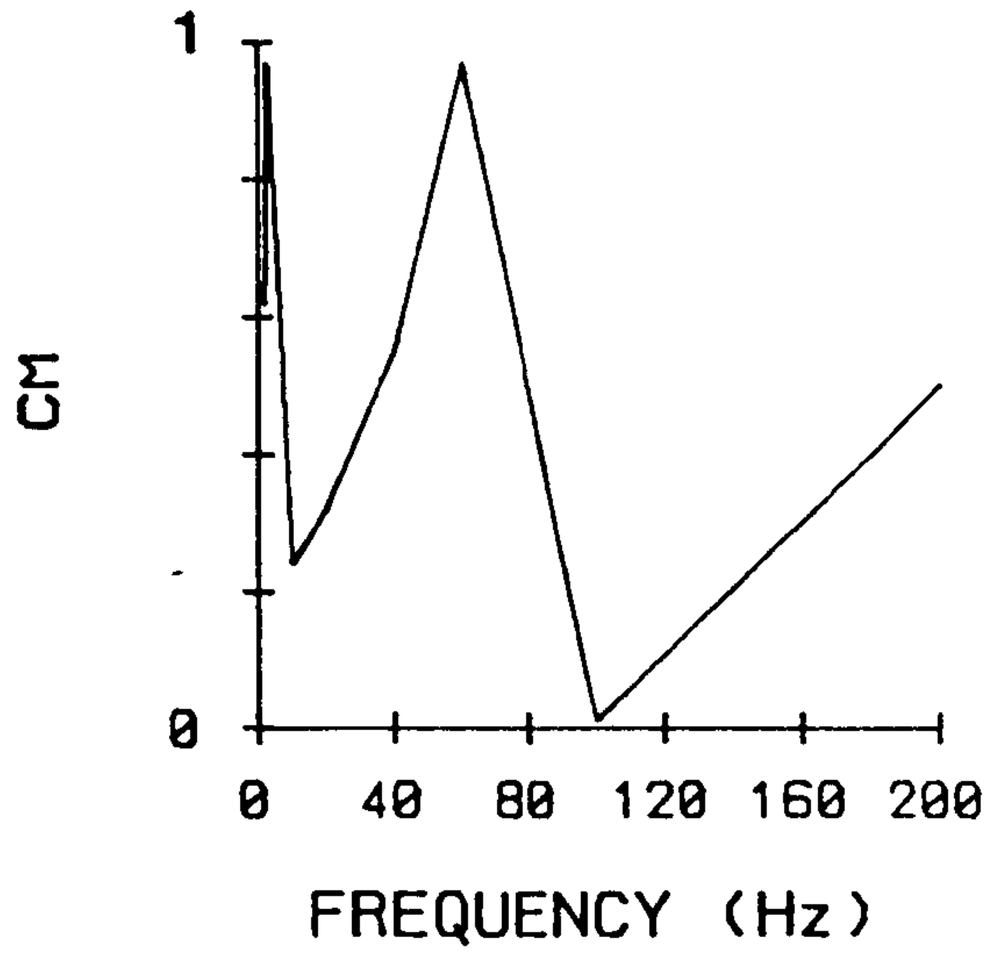
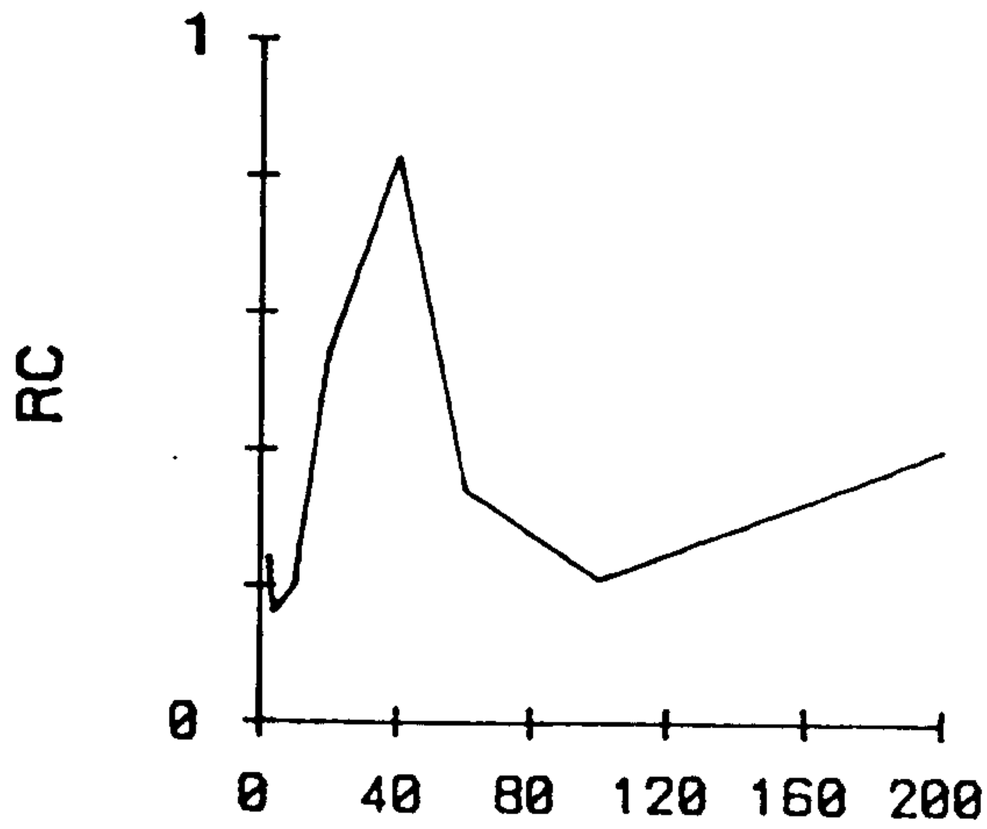

200Hz
No. of spikes.
25
1
Phase

Figure 3.26 Statistical parameters for the unit shown in Figure 3.25. Details as Figure 3.22.


CM
1
0
0
40
80
120
160
200
FREQUENCY (Hz)


RC
1
0
0
40
80
120
160
200
FREQUENCY (Hz)

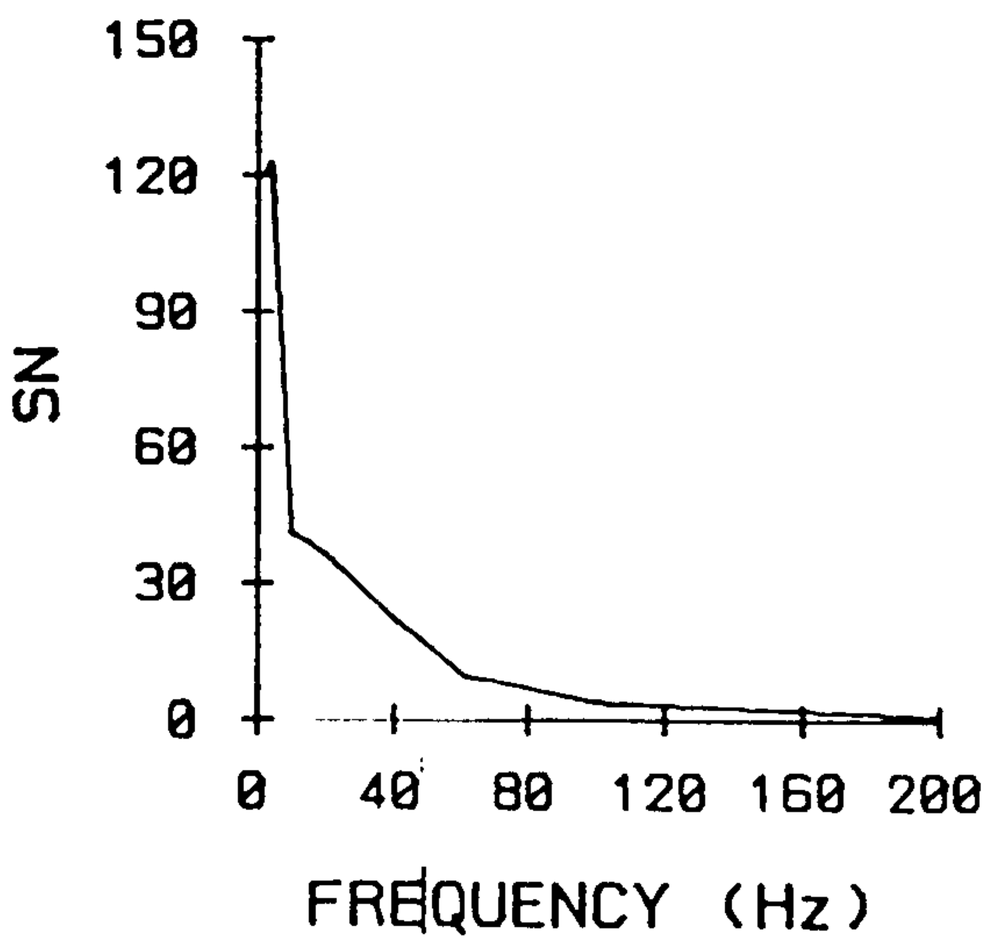

SN
150
120
90
60
30
0
0
40
80
120
160
200
FREQUENCY (Hz)

Figure 3.27 Circular plots showing the strength of the response of an abdominal interneurone of *Nephrops* to water borne vibrations from 8 directions. The strength of the response is plotted as a percentage of the maximum spike number obtained over 5 seconds. The plot shows the responses of the interneurone at 30Hz. Also shown are phase histograms produced at each direction showing the spike number against phase. 0/360 indicates the position of the loudspeaker.

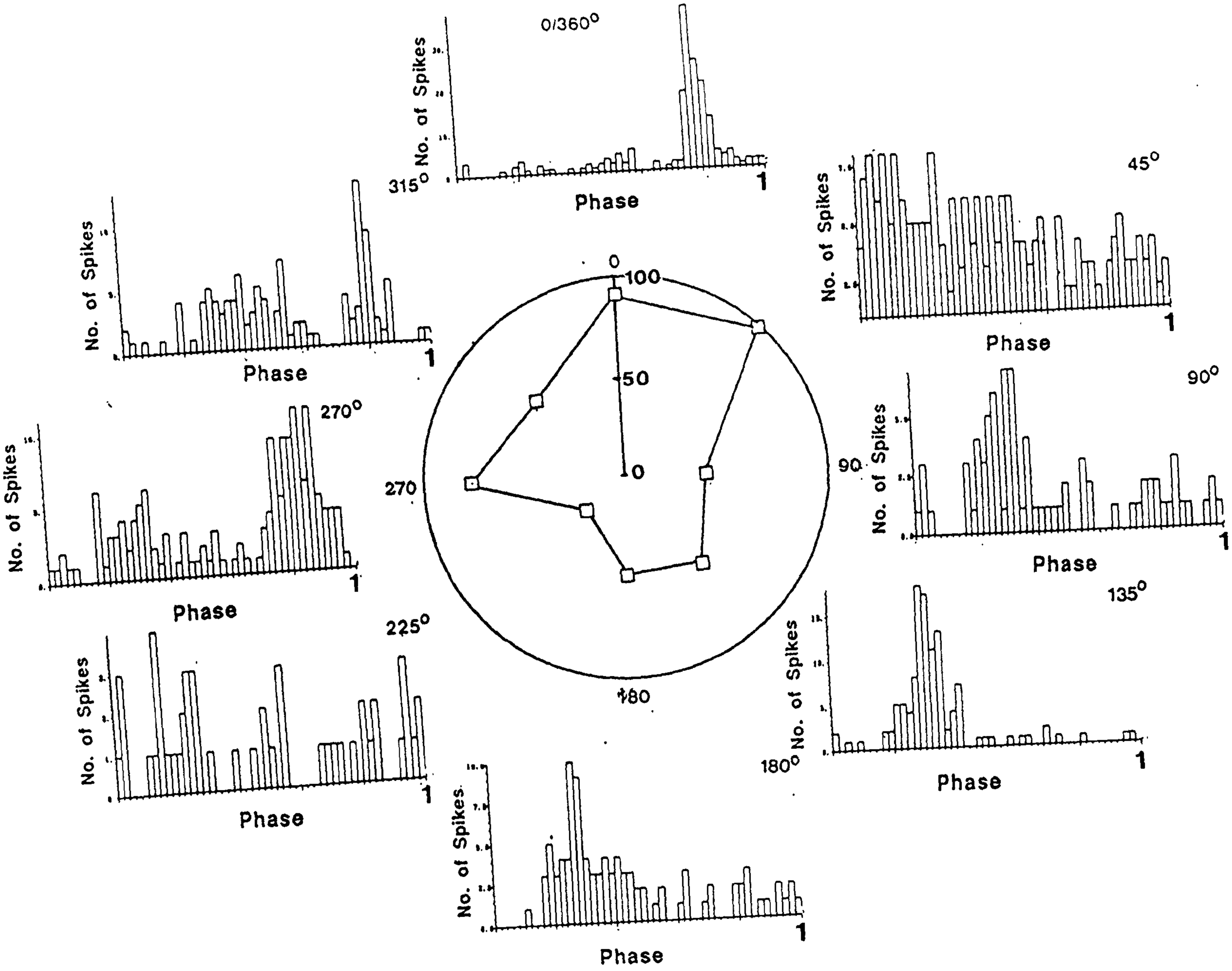
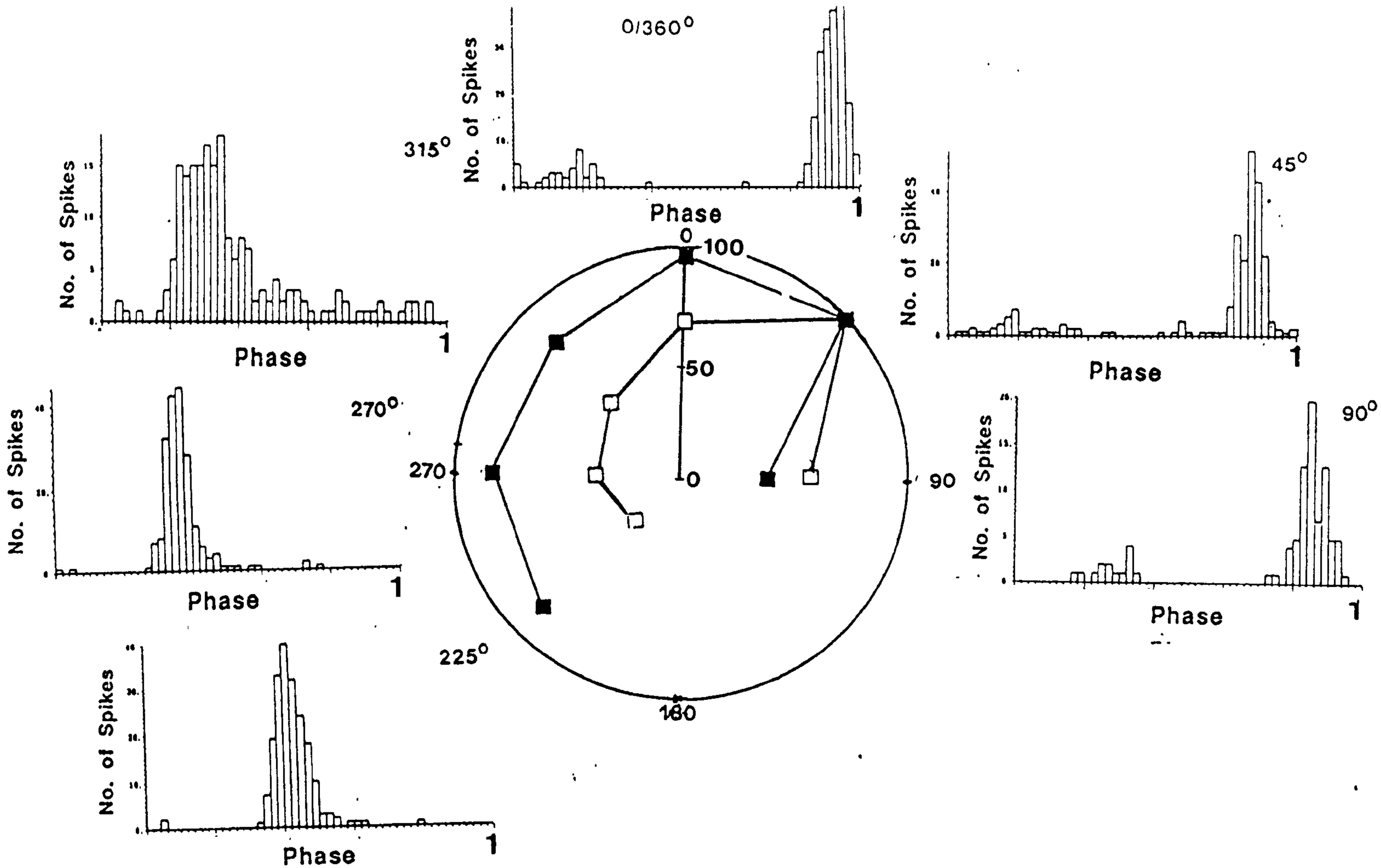

0/360°
45°
90°
135°
180°
225°
270°
315°
No. of Spikes
Phase
1
0
100
50
0
90
180
270

Figure 3.28 Circular plot showing the responses of two mechanosensory interneurones to water borne vibrations from 6 directions. The histograms correspond to the interneurone represented on the plot by the black squares. Details as Figure 3.27.


0/360°
315°
45°
270°
90°
225°
180
0
100
50
No. of Spikes
Phase
1

Chapter 4

POSTURAL RESPONSES SHOWN BY *Nephrops* TO WATER BORNE VIBRATIONAL STIMULI AND THEIR MOTOR CONTROL.

4.1 INTRODUCTION

The abdomen of *Nephrops*, like that of other decapod crustaceans is divided into 6 segments and is involved in many complex behaviour patterns such as reproduction, burrow ventilation, escape swimming, startle reactions, and other forms of locomotion and posture. Four sets of muscles are used to perform all of the abdominal movements required for these behaviour patterns. For example, the escape reaction, a series of tailflip swimming movements, is produced by phasic contractions of the deep extensor and flexor muscles which occupy most of the abdominal cavity (Wine and Krasne, 1982). Postural and locomotory movements of the abdomen, however, are produced by the tonic superficial extensor and flexor muscles which form thin dorsal and ventral sheets respectively (Parnas and Atwood, 1966). Posture is controlled by the balance between the activity in these two muscle groups which can also act reciprocally to produce the cyclic pattern of extension and flexion of the abdomen seen during backwards walking (Kovac, 1974 a & b; Moore and Larimer, 1988).

Each group of postural muscles is innervated by 6 motor neurones in each segment, of which 5 are excitatory, the flexor excitors (FE), and extensor excitors (EE), and 1 in each case is inhibitory, the flexor inhibitor (FI), and extensor inhibitor (EI). The flexor neurones alone comprise superficial root 3 (SR3) while the extensor neurones run in root 2 (R2) of ganglia 1-5 along with other motor and sensory axons and are consequently rather difficult to identify separately. The 6 axons supplying each muscle group have been extensively studied in the crayfish, first by Kennedy and Takeda (1965) who reported that each axon can be identified on the basis of both morphological and physiological characteristics. In the present study the amplitude of the extracellularly recorded potential was used

to identify the units from 1 (the smallest) to 6 (the largest) (Page, 1982). Simultaneous intracellular recordings from the fibres of the superficial flexor muscle and SR3 in *Nephrops* show that units 1,2,3,4, and 6 are excitatory, producing EPSP's (FE 1-4, 6) and unit 5 is inhibitory, producing IPSP's (FI 5) (Neil, unpublished obs.). A similar situation exists in the extensor motor neurones, E1-E4 & E6 are excitatory and E5 is inhibitory. The smaller axons generally have more tonic characteristics and the larger are more phasic, although all have been classed as tonic neurones (Kennedy and Takeda, 1965).

As a consequence of this clearly defined system of organisation at the motor level, and its responsiveness to sensory stimuli eg. mechanosensors (Kotac and Page, 1987) control of posture by the antagonistic flexor and extensor muscles has been the subject of much study, mostly in the crayfish (Kennedy and Takeda, 1965; Kovac, 1974; Larimer and Eggelston, 1971) and more recently in the lobster (Thompson and Page, 1982). Some studies have also been carried out on *Nephrops* to determine the response of the abdominal motor system to tilt (Knox and Neil, 1987).

4.1.1 **The control of abdominal posture**

Abdominal position is monitored by abdominal proprioceptors in each segment. The Muscle Receptor Organs (MRO's) respond to stretch produced by flexion of the abdomen and are involved in the regulation of abdominal posture via two reflex pathways (for review see Page, 1982). Information about the position of the abdomen is also provided by nerve cord stretch receptors and ventral mechanoreceptors (Page, 1982).

Many of the existing studies on abdominal posture have focussed on the control exerted by interneurones known as "command elements" (Larimer et al. 1986). There are more than a hundred of these cells

which can be divided into groups which produce either flexion, extension, or central inhibition of all tonic motor activity (as occurs in the tailflip) (Evoy and Kennedy, 1967). Electrical stimulation of these cells has been shown to cause the abdomen to assume particular positions (Kennedy et al, 1967) and some of these command elements control the reciprocity between the two sets of muscles. Using dye filling and intracellular recording techniques Moore and Larimer (1987, 1988) and Larimer et al. (1986) have found that these command neurones project onto separate sets of premotor interneurones in the abdominal nerve cord, which in turn initiate postural movements or cyclical activity. However, to date little is known about the mode of action of these interneurones.

The activity of the neurones which control abdominal posture may be altered by sensory stimuli such as touch and body tilt, and this has been studied at both the motor and premotor levels. Jellies and Larimer (1986) showed that tactile stimulation of the body surface caused activation of the motor neurones producing abdominal movements. Kotak and Page (1987) reported that tactile stimulation of the swimmeret surface caused activation of EE and FI units and inhibition of FE and EI units. Knox and Neil (1987) showed that abdominal posture could be modified by sensory inputs from the statocyst system, producing extension on pitch head up and flexion on pitch head down.

No studies to date have shown the effect of non-tactile vibratory stimuli on abdominal posture. This study attempts to show the effect of water-borne vibratory stimuli on abdominal posture in *Nephrops* using both behavioural measures and neurophysiological recordings from both the flexor and extensor motor neurones.

4.2 MATERIALS AND METHODS

4.2.1 Analysis of abdominal posture in relation to water borne vibrational stimuli

These experiments were carried out in a glass walled tank (Tank A) measuring 100x38x32 cm (Fig. 4.1). The floor of the tank was covered with coarse sand to allow the animal to stand normally. The tank was filled with sea water at 12^{o}C from the departmental circulating supply which was oxygenated between experiments.

A stainless steel rod was glued to the animal's carapace using dental cement. This allowed the animal to be held in a fixed position in the tank so that it was unable to move forwards or backwards but could move its thoracic appendages and abdomen freely. The rod was attached to a clamp stand using a X block, allowing the animal to be raised or lowered in the water. A J9 loudspeaker was suspended at the other end of the tank in midwater using a stainless steel frame such that neither the J9 nor the frame touched any part of the tank. The speaker diaphragm faced the animal. The J9 was driven by a 25W output Derritron power amplifier. Initially the animal was positioned in the tank in as normal a standing posture as possible with the thorax tilted upwards at an angle of approximately 10^{o} to the horizontal (Knox, 1987) and the walking legs in contact with the substrate. The animal was filmed from the side using a Panasonic video camera and the responses were recorded on video tape for later analysis.

The stimulus frequency ranged from 20-300Hz which included those frequencies to which the sensory system was most responsive (Chapter 3). It was not possible to use frequencies below 20Hz due to the technical limitations of the loudspeaker. The stimuli were presented at 5 amplitudes giving a total of 75 tests. The tests were carried out in a random order and repeated so that each test was carried out on

more than one animal and some were repeated on the same animal. A total of 8 animals were used. The experiments were carried out with the animal standing and were then repeated with the animal hanging in midwater so that no part of it was in contact with the substrate.

Initially two habituation tests were carried out to determine the duration of the animals' responsiveness. During the first test the animal was presented with a 60Hz stimulus at maximum output. The stimulus was presented for 10 seconds in every 60 seconds over a period of 10 minutes. During the second test the animal was presented with the same stimulus but played continuously for 5 minutes. The stimulus regime finally selected was based on the results of these tests.

Data analysis and presentation

The video tapes were analysed by a BBC microcomputer via a video interface. The computer program used to analyse the responses in this case required the input of three pairs of points on the animal (each represented by a co-ordinate pair) per video frame. The following pairs of points were digitised: the rostrum and the posterior end of the thorax; the end of the thorax and the posterior end of the 3rd segment; the posterior end of the 3rd segment and the tip of the tail. A line joining the first of these points was taken as the baseline for expressing the orientation of the lines joining the other pairs. These values were then calculated for each analysed frame, typically one per second, and then plotted.

The data were plotted as a change in the angle of the anterior and posterior parts of the abdomen with reference to the stationary thorax. Two lines were plotted on each graph, the top line representing the movement of the 3 posterior segments of the abdomen which for convenience of presentation in each case, was offset by 90^{o},

and the bottom line representing the movement of the 3 anterior segments which was offset by 50^{o} in each case.

4.2.2 The nervous control of posture

a)Recordings of flexor activity

Extracellular recordings were made *en passant* from SR3 in a whole animal preparation as described in section 3.2.3. using a suction electrode with a narrow tip. The signal was amplified and recorded as described in section 3.2.3.

Vibrating water stimuli were generated by a plastic ball as described in section 3.2.4. The preparation was stimulated at a range of frequencies from 20-200Hz by placing the vibrating ball at either the anterior or posterior ends of the animal. Various mechanical stimuli were also used, including forced flexion or extension of the tailfan or posterior part of the abdomen, tactile stimulation of the legs and pinching of the uropods.

Single recordings were made from SR3 in one segment and combined recordings were made from either the left and right SR3 of one segment, or the SR3 in two adjacent segments on one side. Double recordings allowed the activity of the same cell in different segments to be compared using cross correlation analysis which was carried out using a Tandon computer via a CED 1401 interface. Ablation experiments were also carried out in which the abdominal nerve cord was cut to remove inputs from certain areas of the body. Transections were most commonly made 1) at the circumoesophageal connectives to remove inputs from the head regions 2) at a point anterior to SR3 in the first segment to remove inputs from the head and thorax and the thoracic appendages, 3) at a point posterior to the recording electrodes to remove inputs from the tailfan and posterior parts of the abdomen. On

some occasions cuts were made between the recording electrodes. The stimulus regime was repeated before and after these cuts were made.

b)Recordings of extensor activity

Recordings were made from the second abdominal nerve roots (R2) normally in segment 4. Recordings were made either from R2 alone or from both R2 and SR3 in the same segment to show the relationship between the firing of the motor units to the extensor and flexor muscles respectively in response to vibratory stimuli.

The dissection was carried out as described in section 3.2.2.c with the following additional modifications. The cuticular rib was removed along with the surrounding membranes to expose the underlying ganglion and its roots. Root 2 was cut to remove sensory activity from the cuticular hairs and the MRO. The cut end was then taken up into a large diameter suction electrode. Recordings were made from SR3 using an *en passant* arrangement.

c) Data analysis and presentation

The responses shown by SR3 and R2 are not suitable for analysis using post stimulus time histograms or phase histograms as the responses were not phase related to the stimulus in any way. Data were therefore analysed on the Tandon computer via the CED 1401 interface using a mean frequency calculation. The mean frequency of spike firing was calculated by the computer every 0.5s, and was plotted along with the raw spike data against time.

4.3 RESULTS

4.3.1 Postural responses of *Nephrops* to water borne vibrations in tank A.

The following results were obtained from 8 animals which were tested in tank A, the smaller of the two laboratory tanks, in response to 15 frequencies of vibration from 8-300Hz at 5 voltage settings on the amplifier. The response to frequency has been analysed in detail. The response was also tested in tank B, described in chapter 5 to check that it was not solely a result of the acoustic conditions in tank A. The same results were obtained under both conditions. Tethered *Nephrops* showed a distinct and reproducible response to certain frequencies of water-borne vibrational stimuli in tank A. This response took the form of extension of the abdomen and is represented diagramatically in Figure 4.2. This occurred throughout the stimulus period whether the animal was facing the loudspeaker or facing away from it, and was accompanied by several other behavioural responses which are described later. Abdominal extension was therefore used as a monitor of the animal's responsiveness to water borne vibrational stimuli of different frequencies.

4.3.1.a Habituation.

The level of habituation was determined in two different tests, described in the methods, and the results from one example of each are shown in Figures 4.3 and 4.4. Figure 4.3. shows the responses of an animal to a 60Hz stimulus presented for 10 seconds every 60 seconds.

The graph showed a continuous pattern of extension and flexion closely linked with the stimulus. Typically extension occurred immediately when the stimulus was switched on and continued until the stimulus was switched off. The posterior segments underwent the

largest movement. During the inter-stimulus interval the abdomen was flexed into the normal resting position and the animal generally remained stationary. Occasionally extension was seen in the inter-stimulus period (1). The magnitude of extension varied from full extension, occuring most of the time (2) to slight extension (3). However, this variation did not seem to be related to habituation as the animal was still capable of producing full extensions at the end of the stimulus period. These tests therefore showed that *Nephrops* do not habituate to closely spaced stimuli and would still respond over a long time period.

Figure 4.4 shows an example of results from the second type of habituation test where a 60Hz stimulus was played continuously for 5 minutes. The animal extended its abdomen fully and very rapidly with an initial angular velocity of $10.66^{o}\ s^{-1}$. The animal continued to maintain this fully extended posture for 70 seconds. After this period the abdomen started to flex very slowly at an average angular velocity of $0.94^{o}\ s^{-1}$ and had almost reached its resting position after 89 seconds when the animal started to flex its abdomen again. This pattern of extension followed by flexion was repeated once more before the stimulus was switched off, when the tail, which had previously been held above the substrate, dropped onto the substrate. The animal remained stationary thereafter. This pattern was repeated in other animals. Thus, it seemed that animals remained responsive to continuously presented stimuli as well as repetitive ones and although in this case the animals remained in a state of reduced responsiveness they did not habituate and continued to show an "off" response when the stimulus was turned off.

A stimulus regime was selected to allow the animals to rest between presentations of stimuli and to minimise effects due to muscle fatigue. Experiments were therefore conducted every 10 minutes with a

stimulus duration of 30s.

4.3.1.b **Abdominal extension in *Nephrops* produced in response to water borne vibrational stimuli of different frequencies.**

The animals were tested in 2 postural states: 1) normal standing with legs and tail in contact with the substrate and the thorax at an angle of 10^{o} rostrum up to the substrate (Knox 1987) and 2) with the animal suspended in mid-water so that the legs and tail had no contact with any part of the tank. The thorax was held at the same angle as before. Stimulus presentation and data recording were as described in section 4.2.2.

Animal in contact with the substrate .

Graphs showing the response of the abdomen to different frequencies of vibration while the animal was in the standing position are shown in Figures 4.5-4.8. Abdominal extension at low frequencies was generally complete and vigorous. Up to 80Hz at all amplitude settings the animal always responded and no negative results were obtained. Up to 60Hz the response always occurred simultaneously with the onset of the stimulus and generally continued throughout the stimulus period. The range of the initial velocity of tail extension was from $8.7\text{-}31^{o}\ s^{-1}$ between 20 and 60 Hz and the average velocity was $17.4^{o}\ s^{-1}$ at these frequencies. Below 80Hz there was no direct relationship between the angular velocity of the movement and either the frequency or the amplitude.

From 80 Hz up to 180Hz there was usually a delay between the onset of the stimulus and the onset of full abdominal extension. Often in such cases a slight initial movement or twitch of the abdomen was seen at the onset of the stimulus. Above 80Hz negative responses also occurred. The delay in the response varied widely but not

systematically with frequency (compare Figs. 4.6.C, 4.7.A & 4.7.C). After this delay the rate of abdominal extension was as rapid as it was to low frequency stimuli.

Animal suspended in midwater

Figures 4.9 and 4.10 shows examples of the responses of animals to vibrational stimuli while they were suspended in midwater. Animals stimulated in this condition showed the same patterns of response as animals which were in contact with the substrate. The only major difference was in the period of time for which the abdominal extension was maintained. When the animal was in contact with the substrate extension was generally maintained throughout the stimulus period and frequently for some time thereafter. However,, when the legs were not in contact with the substrate the period of extension was much shorter, with flexion generally starting within 10 seconds. The flexion seen during this period was generally very slow and in occasional cases extension occurred again after the abdomen had returned to its original position (Fig 4.9.D).

4.3.1.c Other behaviour patterns

Leg movements: These responses were considered an important accompaniment to abdominal extension so have been studied in greater detail than the other behaviour patterns listed below. The leg movements either took the form of defined "walking" movements or were more random movements which could not be classified as attempts at walking. 22 of the tests were chosen at random to study the timing of the leg movements (Table 4.1). 12 of these used stimuli of 80 Hz and below and 10 used stimuli above 80 Hz. Out of these tests 19 were positive for abdominal extension and of these 17 also showed leg

movements. In 14 of these cases the legs responded simultaneously with the abdomen but in 3 the abdominal response preceeded the leg response. In no cases did the leg movements preceed the abdominal extension.

Stimulus frequencies which produced a delay between abdominal extension and leg movements corresponded to those at which a delay had also occurred between the onset of the stimulus and abdominal extension (ie. above 80Hz). At these higher frequencies the leg movements were generally concurrent with this later phase of extension, not the initial twitch.

Swimmeret beating: In all cases swimmeret beating only occurred if the abdomen was extended, although it did not always occur under these conditions, particularly in response to high frequency stimuli. The rate of beating seemed to increase with the strength of the abdominal response and was most effective in response to low frequency stimuli.

Antennal movements and claw waving: These were two other behaviour patterns seen in response to water borne vibrational stimuli. Although seen quite frequently they were more difficult to measure and so have not been studied in detail.

Tail flipping: Occasionally low frequency stimuli caused all of the above behavioural reactions to occur very vigorously and to culminate in one or more tail flips.

4.3.1.d **Ablation of the abdominal nervous system.**

Preliminary experiments were carried out to determine the effect of nerve ablation on the abdominal extension reflexes. The effect of ablation on the nervous control of posture is dealt with in more detail in a subsequent section (4.3.2).

A cut was made in the abdominal nerve cord at a point between the

first and second ganglia, posterior to SR3 in that segment. The animal was then tested as above in the standing position. Under these conditions the animal was able to extend only the first segment of its abdomen but not the other five segments. The other behavioural responses were still seen under these circumstances, with the exception of swimmeret beating. Three conclusions can be drawn from the results of this experiment:-1) Both the abdominal region (abdomen and tailfan) and the thoracic region (thorax, head and legs) must be involved in the sensory detection of water borne vibrations as the two systems were capable of operating separately from each other. The leg cycling was still seen when input from the tail region had been removed.

2) The other responses such as leg cycling but with the exception of swimmeret beating, were not dependent upon abdominal extension for their occurrence.

3) Full abdominal extension could not be generated from within the abdomen under these conditions as the only segment which extended was the one still receiving thoracic input ie. segment 1. Nervous input from the thorax must therefore be necessary for the full abdominal extension to occur.

4.3.1.e **The effect of stimulus amplitude on the response in tank A.**

The amplitude of neither the sound pressure nor the particle displacement could be measured in this tank with any degree of accuracy. However, the animals were tested using five different driving voltages to the vibrator at each frequency to determine whether this had any effect. Figure 4.11 shows the results of these tests at 4 frequencies. In most cases there was no systematic relationship between stimulus amplitude and response strength.

However, the characteristic changes of the response with frequency, including the appearance of a delay at high frequency, did occur.

4.3.2 **The nervous control of abdominal posture in relation to water borne vibrational stimuli**

Most of the following study of the nervous control of abdominal extension was carried out by monitoring activity of the peripheral inhibitor (F5) to the slow flexor muscle since this unit could readily be identified as the only one active in SR3 during extension movements. Later studies used recordings from both the flexor and extensor roots to monitor reciprocal activity in the two muscles. The results of this study were obtained from 29 animals.

4.3.2.a **SR3 activity.**

General observations and response to mechanical stimuli.

Figure 4.12 shows a section of resting activity in SR3 with the units numbered following the convention described in section 4.1. All units could be spontaneously active at rest, with the smaller units (F1 and F2) and F5 showing the highest tonic activity. In this study identification of FE and FI units was routinely confirmed by inducing resistance reflexes. Forced extension of the abdomen is known to induce reflex flexion and was found to induce FE units to fire in SR3 (Fig. 4.13.a) while the FI was silent. Forced flexion of the abdomen is known to induce reflex extension and was found to induce activation of the FI and central inhibition of FE units (Fig. 4.13.B.). Stimulating the legs and the thorax generally caused the activation of FI but this effect was not as reliable as an identification test as the forced flexion of the abdomen (Fig. 4.13.C&D.)

The response of SR3 to water borne vibrations of different frequencies; a typical response pattern.

Water borne vibrations caused activation of FI. This response was shown to frequencies between 20 and 100Hz and occasionally up to 140Hz. FI did not fire in response to frequencies below 20Hz.

Figure 4.14 shows typical responses recorded in SR3 to a frequency series of water borne vibrations. In this case F5 was already firing at rest but when the stimulus was switched on the firing of FE ceased while that of FI increased markedly especially at low frequency, reaching as much as 30 ips. at 30Hz (Fig. 4.14.A). The frequency of firing reached a peak rapidly and then started to decline, reaching its resting level after the stimulus was switched off. The strength of this response decreased as the frequency increased. At 80 and 100Hz (Fig. 4.14. D,E) the maximum mean frequency of 5 ips. was accompanied by activity in some of the smaller FE units, indicating a degree of activation of the flexor muscle at these frequencies.

An atypical response to water borne vibrations.

The response pattern described in the last section occurred in most preparations. Occasionaly the opposite response pattern was seen in which FE units were activated during the stimulus period. Figure 4.15 shows an example of this pattern. Although the FI responded to forced flexion of the abdomen (Fig. 4.15.B) it was not active at rest and did not respond to the stimulus (Fig. 4.15.C,D). Instead one of the small FE units responded to water borne vibrations and to leg stimulation.

Differential stimulation of the posterior and anterior regions of the animal.

Animals were stimulated with the vibrating ball in two positions 1) at the posterior end and 2) at the anterior end. Figure 4.16 shows examples of the responses of SR3 in segment 2 when the animal was stimulated in this way. Stimulation at the anterior end (Fig. 4.16) the typical pattern of response, with FI firing while the stimulus was on and FE units firing when the stimulus was switched off. FE units were rarely seen when the stimulus was on. The maximum response frequency during stimulation of the anterior end was 8ips.

Stimulation of the posterior end produced a different pattern of response (Fig. 4.17). FE units fired throughout the stimulus period and although FI clearly increased its firing level at the onset of the stimulus, the frequency of firing was much lower than when the animal was stimulated anteriorly

Ablation of the tail regions.

Ablation of the abdominal nerve cord posterior to the recording site removed ascending input from the posterior abdomen and tailfan regions. Figure 4.18 shows a recording made in segment 2 after the cord had been cut in segment 4. FI had responded in the typical manner to both touch and water borne vibrations before the cut was made. After the cut the general level of activity was slightly lower, and the response to forced flexion and extension of the posterior abdomen was absent. However, responses to water borne vibrations and to stimulation of the legs still occurred suggesting that the former responses were generated within the abdomen.

Ablation of thoracic regions.

Cutting the nerve cord anterior to the recording site removed any

descending inputs from the thorax and other anterior regions. This was done both within the abdomen and at the level of the circumoesophageal connectives. Figure 4.19 shows an example of recordings made in the 4th segment before and after the cord was cut in segment 1. Before the cord was cut FI responded in the typical way to frequency and to other mechanical stimuli (Fig. 4.19. 1.A,B). However, after the cord was cut in segment 1 the unit did not respond to either leg stimulation or to water borne vibrations (Fig. 4.19.2.A,B) although quite a vigorous response remained to forced flexion of the abdomen (Fig. 4.19.2.C). Cutting the cord at the circumoesophageal connective in a number of preparations generally had a similar effect to that shown above, removing much of the activity but not all of it.

Dual recordings from SR3

Recordings made from 2 SR3's either bilaterally or ipsilaterally in different segments were used to check whether the firing of the 2 FI's was correlated either at rest or during the stimulus period. Fig. 4.20 shows a typical example of recordings made from bilateral SR3's in segment 2. Both FI's fired regularly at rest along with some of the smaller FE units. Although superficially this firing seemed to be related in the two roots, a cross correlation test showed that there was no strict relationship. During the tone stimulus the two FI units seemed to respond in a similar way, increasing their firing rates to similar extents. However, their activity was not correlated and the cells appeared not to be coupled.

Figure 4.21 shows examples of the typical activity of two ipsilateral SR3's in segments 2 and 3. Like the bilateral recordings, although the two FI's appeared to be firing together in response to both touch and vibrational stimuli, there was no positive correlation between the firing of the two cells. This was also true for

correlations between other segments.

These results therefore demonstrate that different SR3's respond in a very similar way to vibrational stimuli, suggesting a common interneuronal drive onto them, but that this occurs without any direct coupling between them.

4.3.3 Reciprocal activity in Root 2 and SR3 during water borne vibrations and other mechanical stimuli.

Although monitoring the activity in SR3 alone gave a good indication of the nervous control of the animals response to water borne vibrations, a more complete picture was obtained by also monitoring the activity in R2 which supplies the slow extensor muscles.

Figure 4.22 shows a series of typical responses of R2 and SR3 to forced flexion of the abdomen and to water borne vibrational stimuli of different frequencies. During abdominal flexion (Fig. 4.22.A) the EE units in R2 and in FI SR3 fired at a very high rate , that of the former being generally higher. At lower frequencies of vibration the response was very strong in both roots but declined with frequency until at 120Hz no response was seen. The responses to higher frequency took several forms (Fig. 4.23). At 60Hz the reciprocal units in the two roots started firing immediately and increased their firing rate with time. At 120Hz however, there was a clear delay from the onset of the stimulus to the firing of the two units, with FI firing before EE. Figure 4.24.A shows the response to 180Hz, where the FI was active throughout the stimulus period but the EE were only active at a very low level and for a very short time at the beginning of the stimulus period. This pattern of motor unit activity corresponds well with the behavioural observations.

4.4 DISCUSSION

The results presented in this chapter have shown that vibratory stimuli between 20 and 80 Hz cause immediate extension of the abdomen accompanied by other behavioural responses such as leg movements, swimmeret beating and antennal and claw movements. Between 80-180Hz these responses are either absent or occur after a delay period. No extension responses could be evoked in *Nephrops* above 180Hz. Neurophysiological recordings from SR3 have shown that vibratory stimuli between 20-80Hz, and occasionally up to 140Hz, typically cause suppression of the flexor excitatory units (FE) and activation of the inhibitor (FI). This will prevent flexion of the abdomen. Activation of the extensor excitatory units occurs concurrently with this, and continues up to 180Hz in some cases. The behavioural result of this combination of nervous events would almost certainly be immediate extension of the abdomen, at least in the 20-80Hz frequency range, and in this respect the nervous and behavioural responses are strongly related.

4.4.1 Delayed responses and atypical responses: possible causes and implications for methodology

This study attempted to identify the possible neural basis for the delayed and negative responses. It is clear that several possible patterns of nervous activity in the 4 axon groups innervating the flexor and extensor muscles, as well as possible central influences such as central inhibition, could have caused these particular behavioural reactions to occur. It is important to bear in mind that the responses of the animal could in part be controlled by "motivation" and although it is known that the animals can sense certain frequencies and amplitudes of water borne vibrations (Chapter

3 and 5) they may not respond to them because of a change in arousal.

During this study several possible patterns of nervous activity were seen which could account for either a negative response or delay in abdominal extension. Negative behavioural responses could have been produced by a continuing low level of activity in some of the FE units and no activity in the EE (Fig. 4.22.E); or by inhibition of the flexor muscle but only a brief activation of the extensor (Fig. 4.24.A). Figure 4.23. shows an example of the nervous responses which might lead to a delayed response. At 120Hz neither excitation of the extensor muscle nor inhibition of the flexor muscle occurred until approximately 5 seconds after the onset of the stimulus. Synergistic activation of the excitatory units in both the flexor and extensor nerves which was observed in some cases, may also have produced a delay if thereby the forces produced by the two muscle groups remained in balance.It was clear therefore from the results of this study that although the stereotyped behavioural responses occurring below 80Hz were generally caused by fairly stereotyped patterns of nervous activity, those in the higher range could be caused by a variety of different patterns of nerve activation. One of the variants of motor behaviour observed (Fig. 4.15) involved predominant flexor activity, although abdominal flexion was never observed under the same conditions in the postural behavioural experiments.

It is important to bear in mind that the postural conditions during these experiments differed from the conditions which the animal experienced when it was tethered in the tank, the latter conditions being a closer approximation of normality than the former. In experiments in the dish the animals were held with most of the abdomen experiencing forced extension and it is possible that the patterns of nervous activation under these conditions may differ from those seen under more normal conditions. It would be necessary to repeat the

experiments using implanted electrodes to establish that the same nervous responses occurred while the animals were actually performing the behaviour. This observation calls into question some of the previous experiments on posture in both crustaceans and insects, many of which have been carried out on animals which have been restrained in some way. Only that of Jellies and Larimer (1986) has attempted to control for the effect of restraint by using both an "unrestrained" preparation, which was similar to the restrained one used here with the tailfan and the rostral segments free to move while the abdomen was restrained, and a "restrained" preparation in which tailfan was also immobilised. They reported no differences between the recordings obtained from these two preparations, but perhaps this is not surprising since a considerable degree of restraint still existed.

4.4.2 Possible central influences on the response of the abdominal motor system to vibratory stimuli.

Neither the central control pathways nor a central pattern generator for the abdominal posture system have been clearly defined in either crayfish (Moore and Larimer, 1988) or in other crustaceans. This study did not attempt to precisely determine the central pathways of this system; abdominal extension was primarily used as an indication that the animal could both receive and respond to a certain stimulus. However, the effect of differential stimulation and ablation of certain body areas were studied and the results of these experiments allow some statements to be made about possible central influences on this response.

Differential stimulation of the anterior and posterior ends of the animal produced different intensities of response in SR3. Stimulation of the anterior end always induced a higher firing rate in

FI (Fig. 4.16) while the response to posterior stimulation was weaker (Fig. 4.17). Although we cannot account for transmission of the stimulus to the other end of the tank, it is certain that the stimulus would have been stronger at the point of origin, thus having a greater influence on the adjacent part of the animal. These results would seem to suggest that thoracic inputs to the abdominal motor system have more influence than those produced from within the abdomen itself. During anterior stimulation the animal often became very active and would wave its legs which were often unrestrained. This "waving" often seemed to induce patterns of activation in FI like those seen in Figure 4.24.B where rhythmic activation of FI was seen. In view of the fact that a cyclic pattern of extension and flexion is seen during backwards walking (Kovac, 1974) and I observed both backwards walking (Chapter 5) and abdominal extension in response to this stimulus it is likely that inputs from the legs will have an important influence on this response.

Cutting the nerve cord in the first abdominal segment to remove all thoracic inputs removed all modulation of activity by vibratory stimuli. This seems to confirm that this response is controlled or modulated by thoracic inputs. This again might have implications for methodology as most recent studies have used isolated preparations consisting of only the abdominal nerve cord (Moore and Larimer, 1988; Kotak and Page, 1987). If thoracic inputs are important for the responses seen here they may also be important for others and it seems unlikely that the control pathways for abdominal extension will ever be determined if an intact preparation is not used. The response to forced flexion of the abdomen remained after the cord was cut in segment 1 and it is likely that this response was a local reflex produced internally within the abdomen by proprioceptors such as the ventral mechanoreceptors (Page, 1982) which cause inhibition of the

flexor muscle and do not require inputs from thoracic regions or integration at a higher level in the CNS. Cuts made posterior to the recording site had no effect on the response to vibratory stimuli (Fig. 4.18) but they did remove the response produced by forced flexion suggesting that the ventral mechanoreceptors may not operate unisegmentally but may need inputs from adjacent segments.

Cuts made at the COC again did not remove tonic activity and there was still a weak response to stimulation present, albeit greatly reduced. Thus it seems likely that central integration or inputs from central regions are required for modulation of the activity of the abdominal motor system by vibratory stimuli to occur, with sensory inputs from the abdominal mechanoreceptors and leg mechanoreceptors projecting to these regions rather than initiating the responses via local pathways. Inputs from the thoracic inputs must undoubtedly be important but these too may require central integration before the response can be produced.

Table 4.1 The time interval between the stimulus onset (time zero) and the onset of abdominal extension (s) and between abdominal extension and the onset of leg movements (s) for stimuli of different frequencies. F or B after the number in column three indicates whether the leg movements were indicative of forwards or backwards walking.

Table 4.1

Frequency (Hz)	Interval between stimulus onset and abdominal extension (S)	Interval between extension and leg movements (s)
10	0	0 (F)
20	0	0 (TF)
25	0	0 (F)
30	0	0 (B)
50	0	0 (F)
50	0	0 (F)
60	.36	0 (F)
60	.96	0 (F)
60	0	0 (F)
80	0	0 (?)
80	6.54	0 (?)
100	---	---
120	1.30	19.77 (B)
120	1.78	2.36 (B)
120	6.00	0 (F)
120	---	---
140	---	---
140	---	---
140	.76	---
160	2.00	---
180	.38	21.80 (F)

Figure 4.1 Photograph of tank A (4.2.1), showing the J9 loudspeaker (left) supported on a metal frame and the tethered animal (right) standing on a substrate of coral sand.

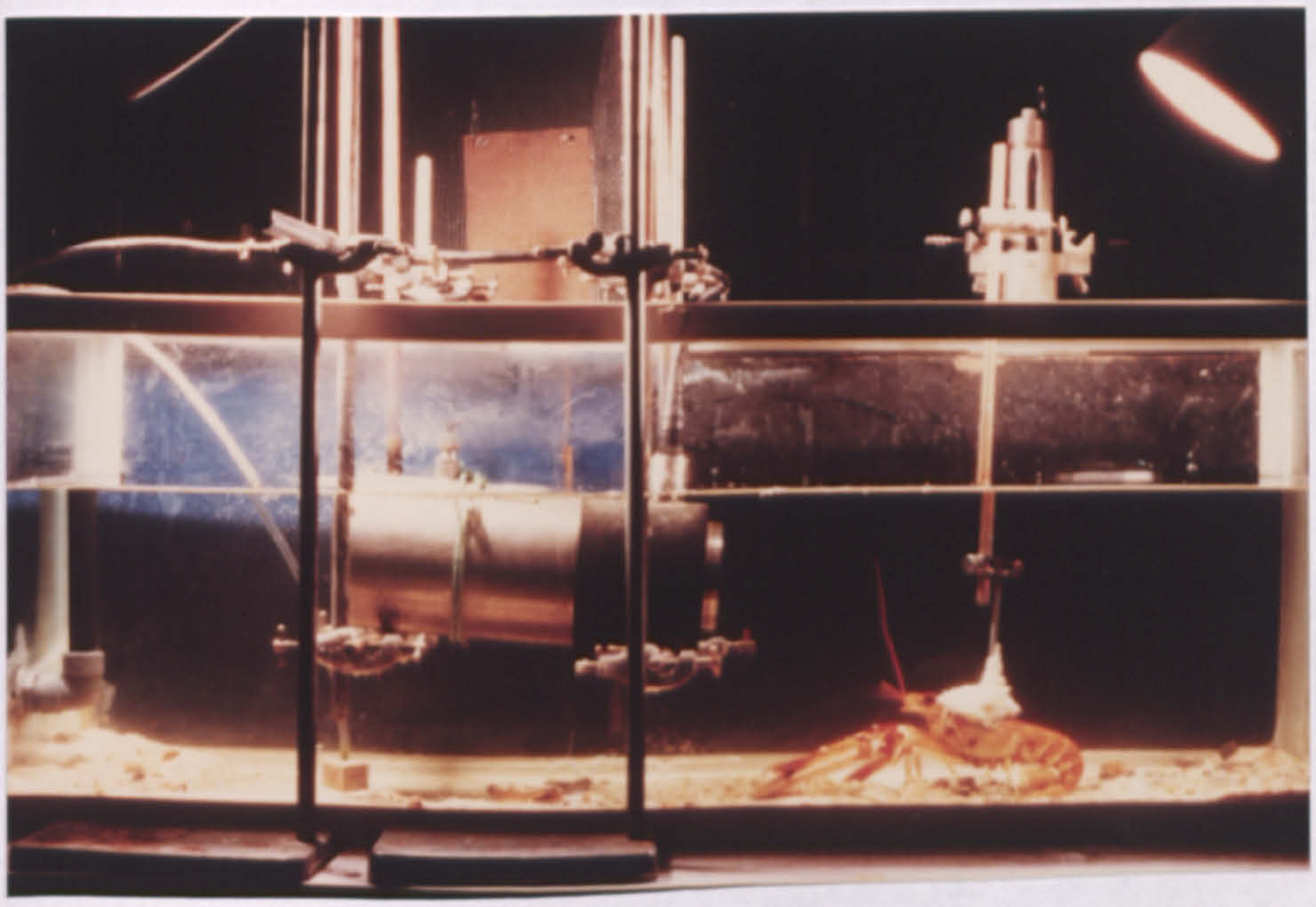
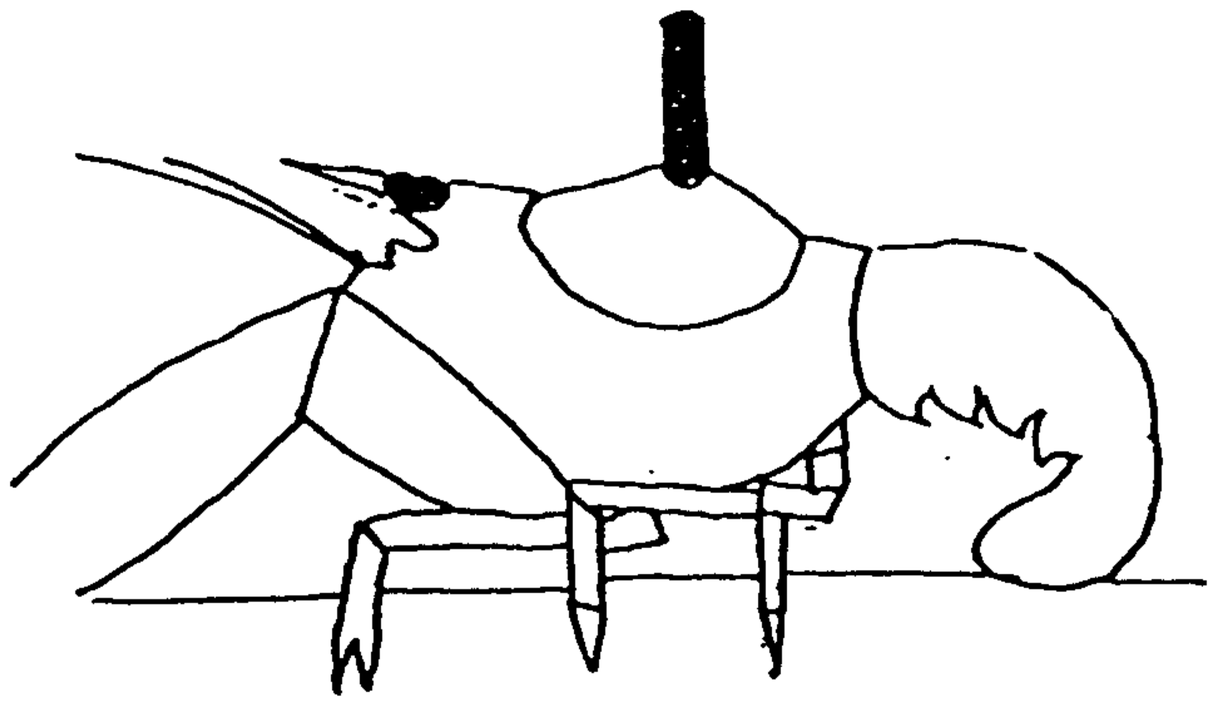
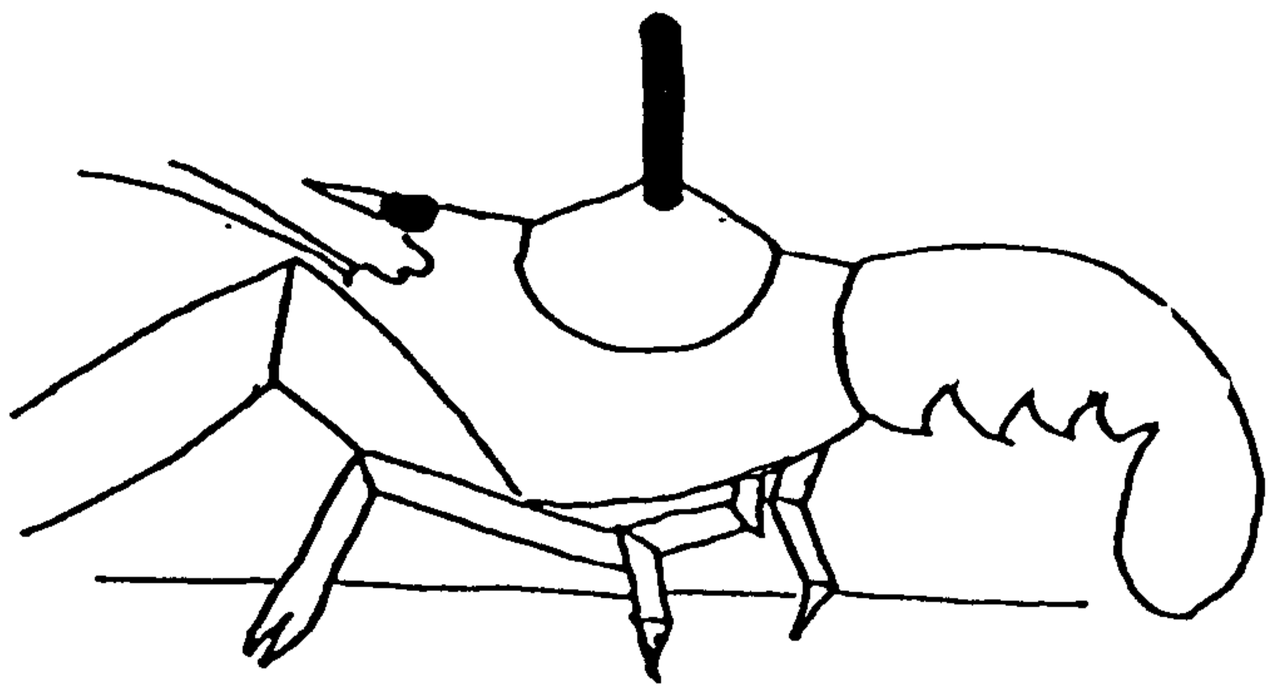


Figure 4.2 Diagramatic representation of the time course (seconds) of abdominal extension in a tethered *Nephrops* in response to a water borne vibrational stimulus of 30Hz. These diagrams are tracings of digitised TV images.

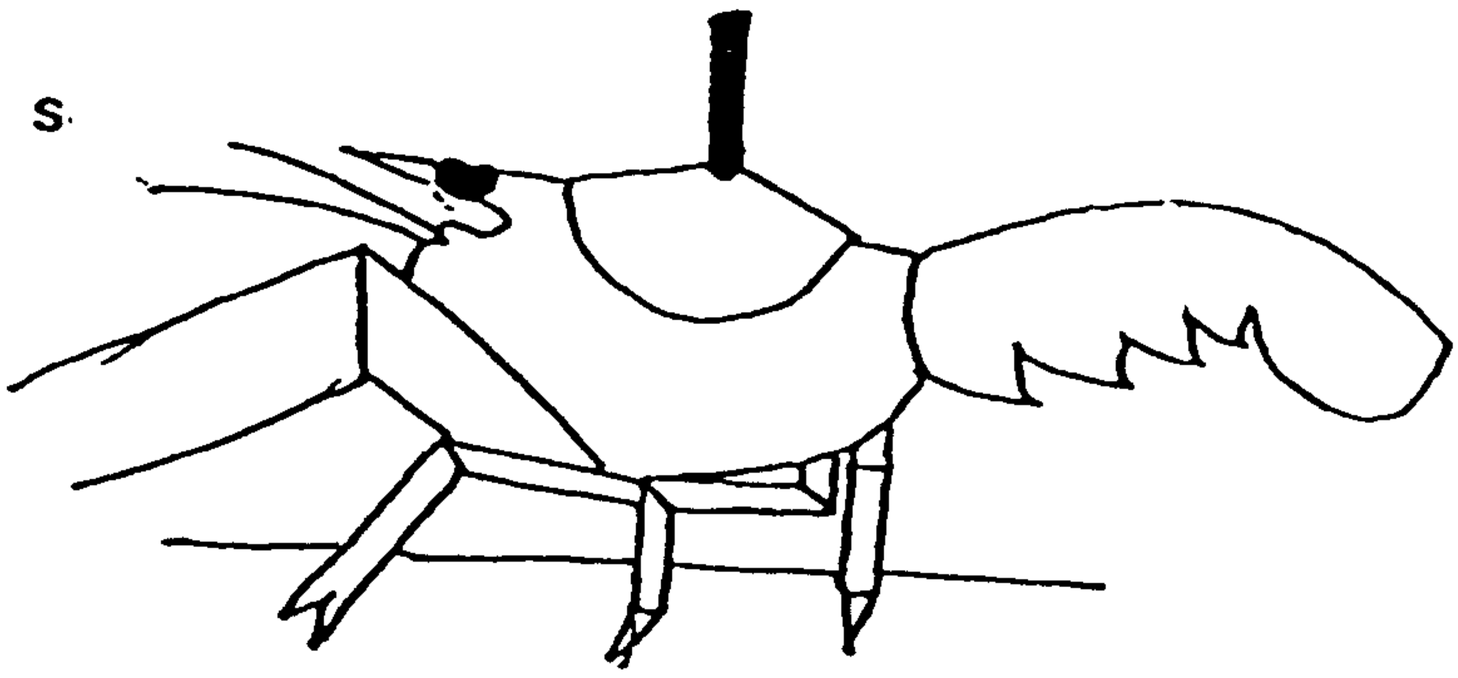
0 s



0.05 s



0.11 s



0.72 s

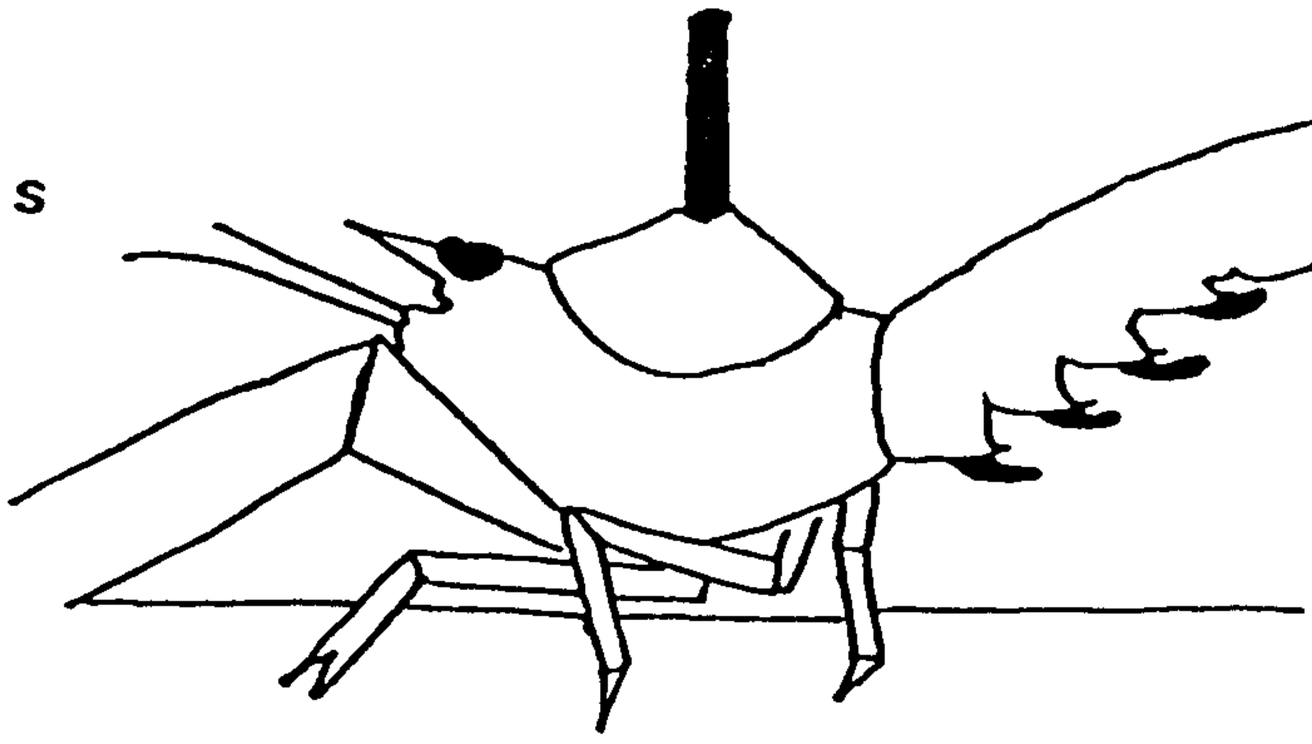
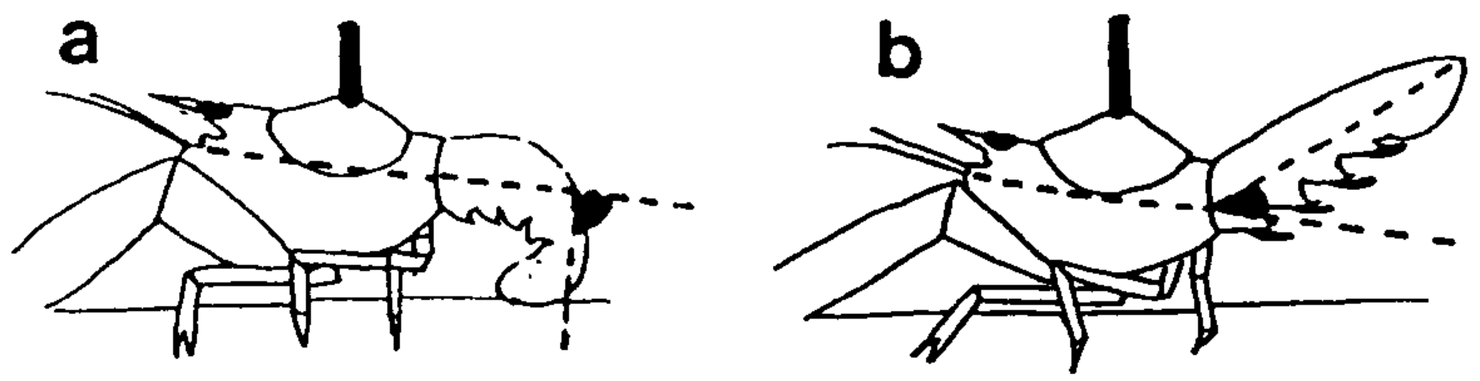


Figure 4.3 Plot of change in angle (degrees) of the posterior three abdominal segments of *Nephrops* (solid line) with reference to the stationary thorax over time (seconds) during habituation test 1. Arrows indicate the onset of a 60Hz stimulus of 10 seconds duration which was presented at 60 second intervals. The numbers are referred to in the text. Decrease in angle represents extension and increase represents flexion. Horizontal dashed line indicates angle of thorax. Insets a and b show the angle of the posterior three abdominal segments and the thorax (solid segment) at extreme flexed and extended positions respectively.


a
b

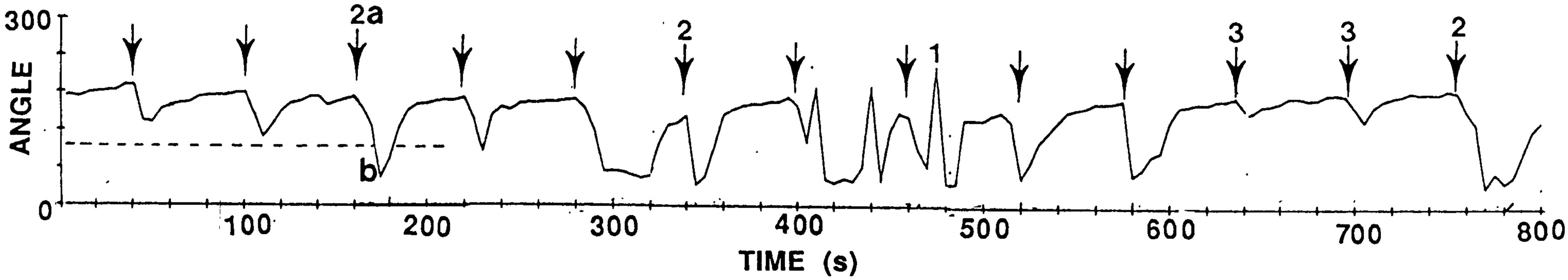

300
ANGLE
0
2a
2
1
3
3
2
b
100
200
300
400
500
600
700
800
TIME (s)

Figure 4.4 Plot of change in angle (degrees) of the posterior three abdominal segments (upper curve) and the anterior three abdominal segments (lower curve) with reference to the stationary thorax over time (seconds) during habituation test 2: continuous presentation of a 60Hz stimulus.

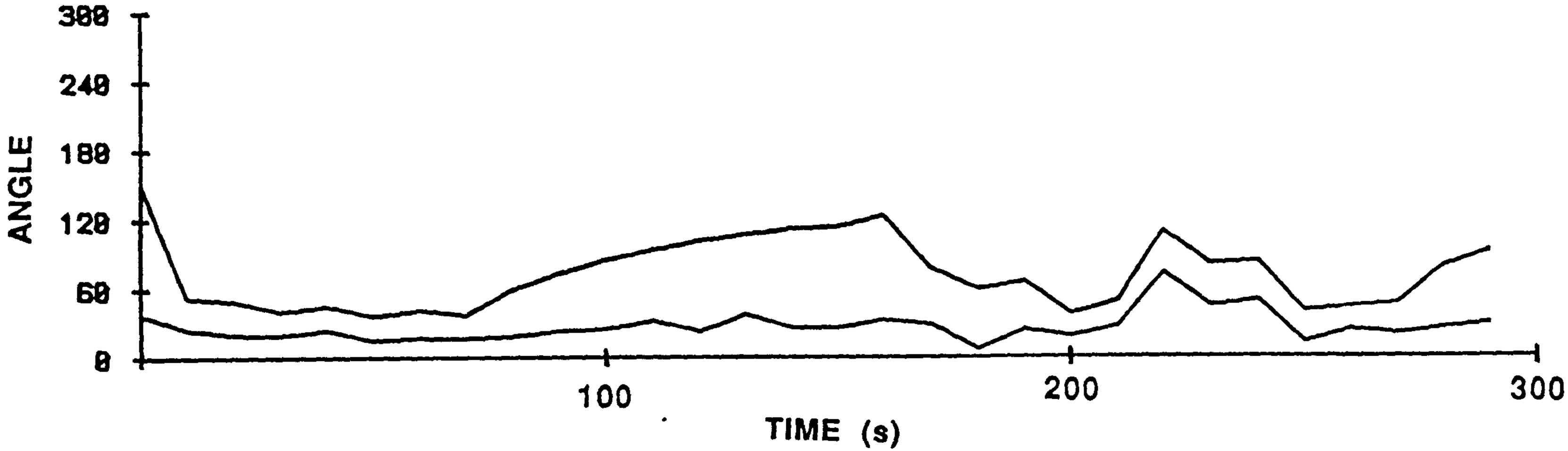

300
240
180
120
60
0
ANGLE
100
200
300
TIME (s)

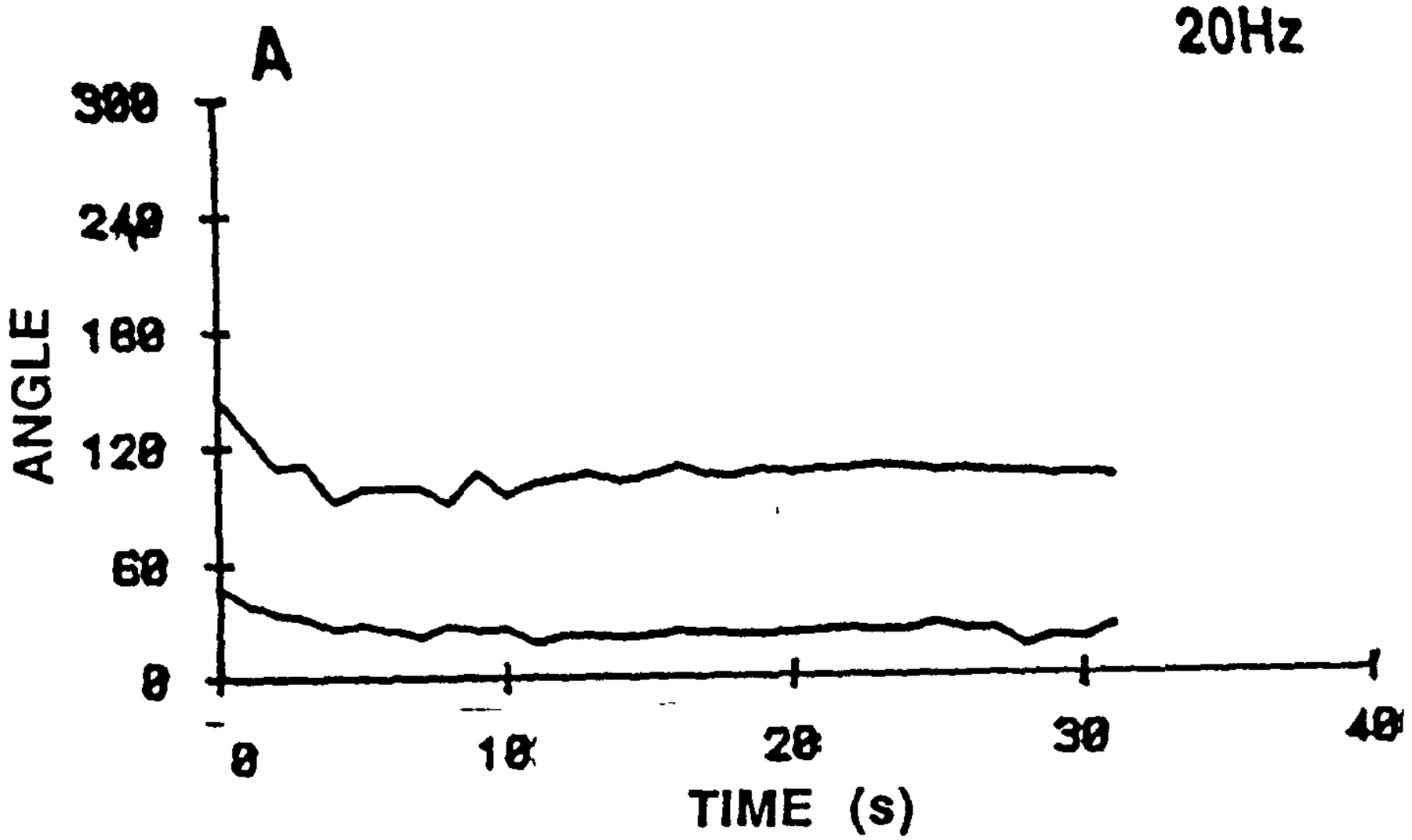
Figure 4.5 Plots of change in angle (degrees) of the posterior three abdominal segments (top line) and the anterior three abdominal segments (bottom line) with reference to the stationary thorax over time (seconds) during water borne vibratory stimuli of different frequencies presented for 30 seconds to standing animals. A,C and D are from the same animal, B is from a different animal.

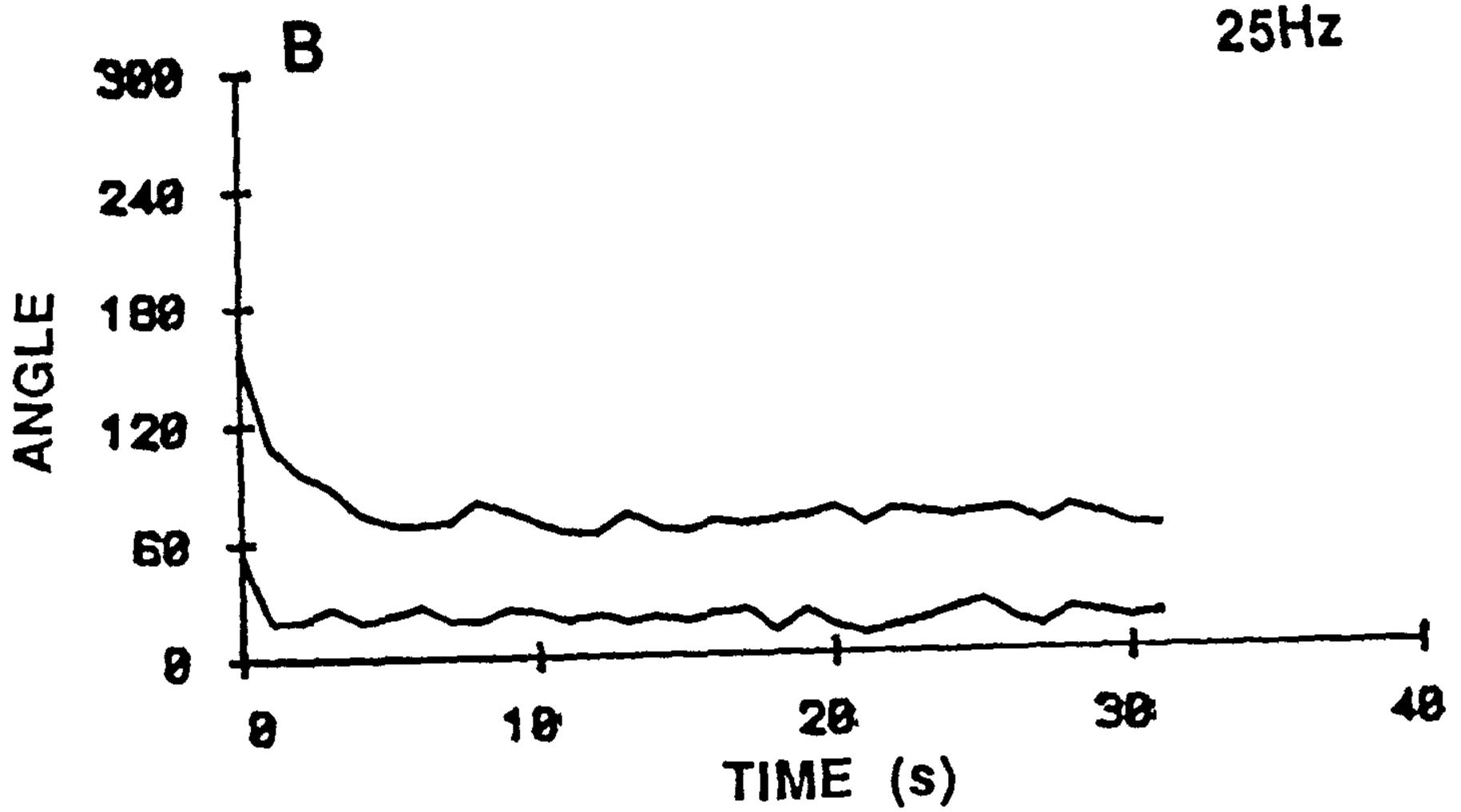
A. 20Hz

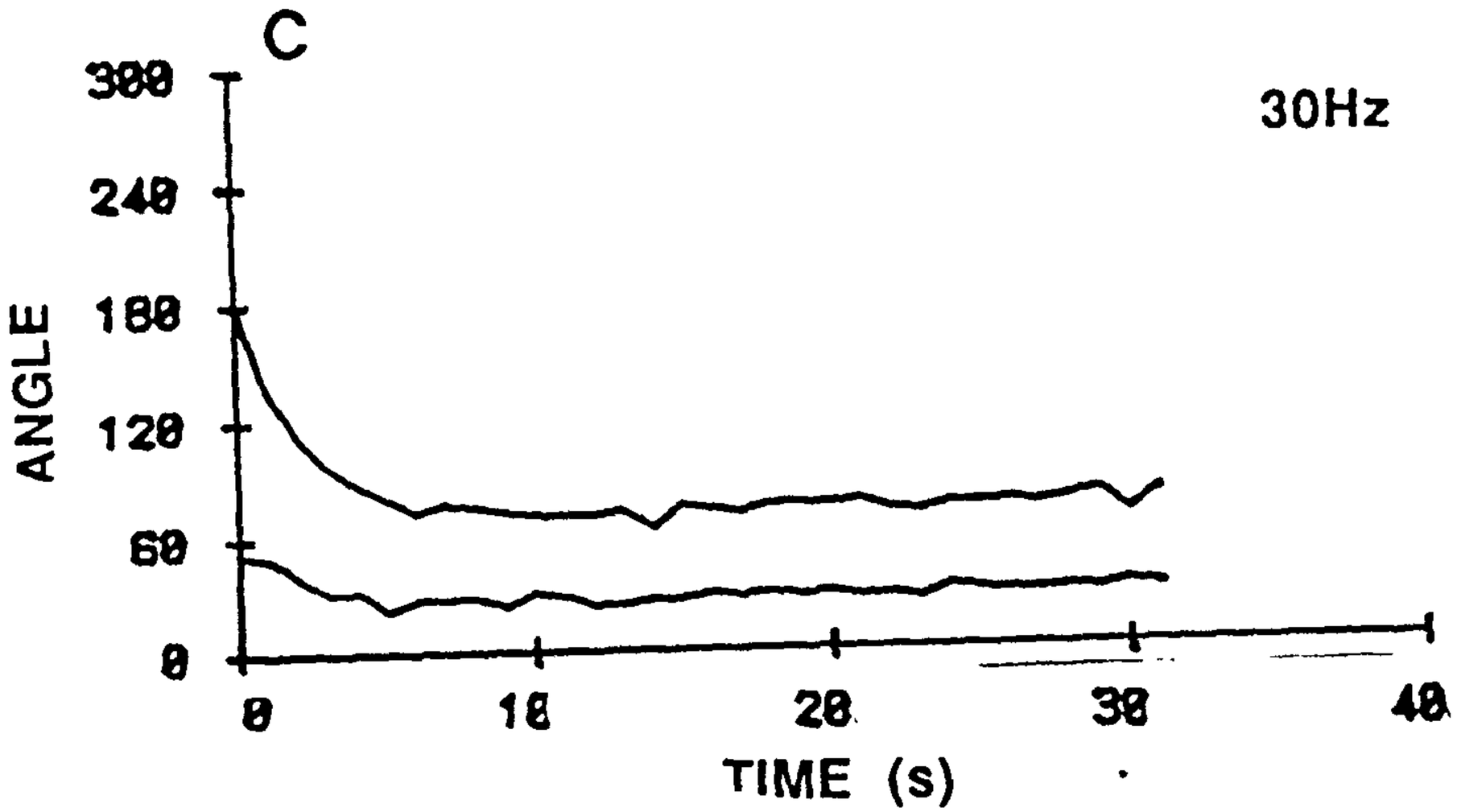
B. 25Hz

C. 30Hz

D. 40Hz


A
20Hz
300
240
180
120
60
0
ANGLE
0
10
20
30
40
TIME (s)


B
25Hz
300
240
180
120
60
0
ANGLE
0
10
20
30
40
TIME (s)


C
30Hz
300
240
180
120
60
0
ANGLE
0
10
20
30
40
TIME (s)

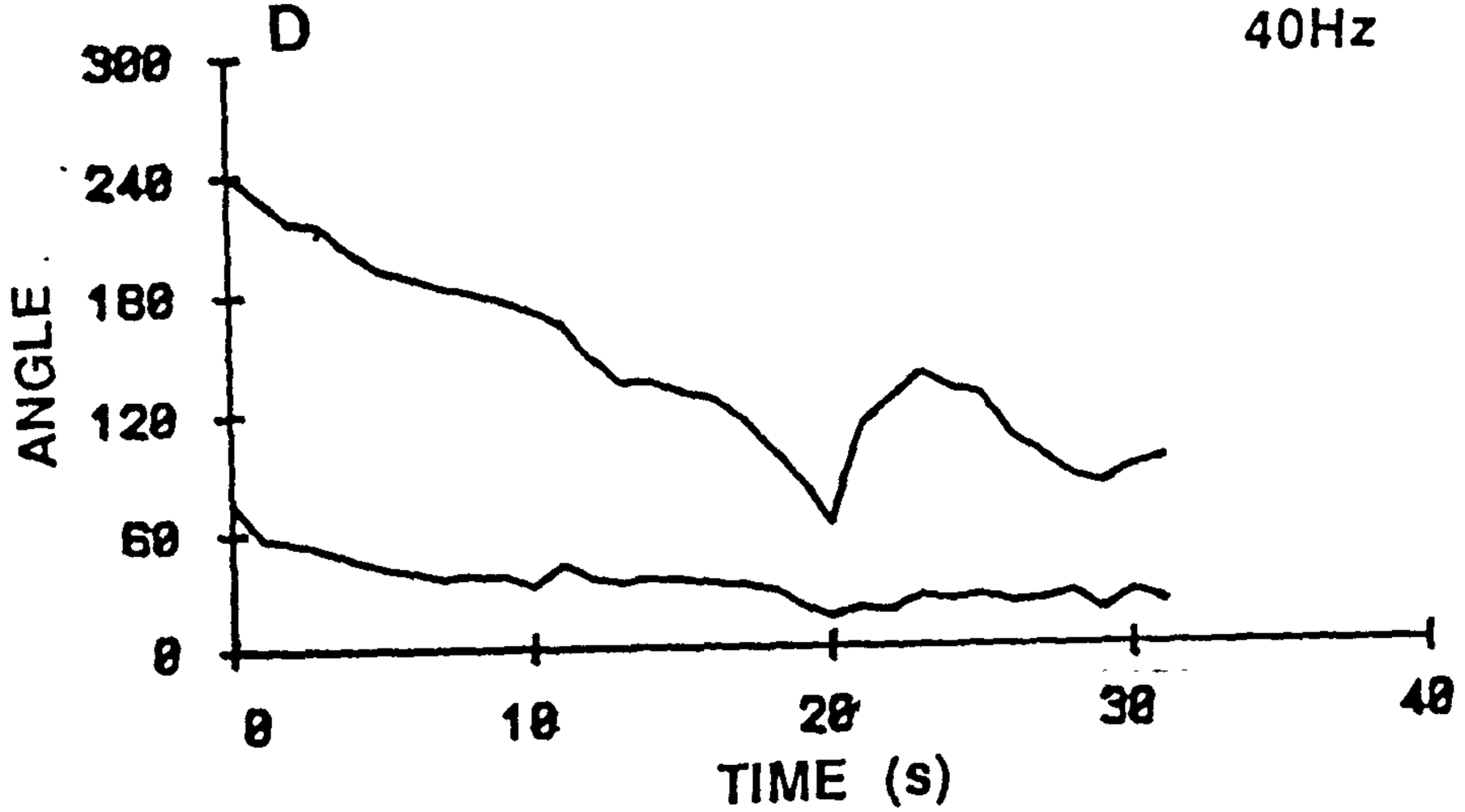

D
40Hz
300
240
180
120
60
0
ANGLE
0
10
20
30
40
TIME (s)

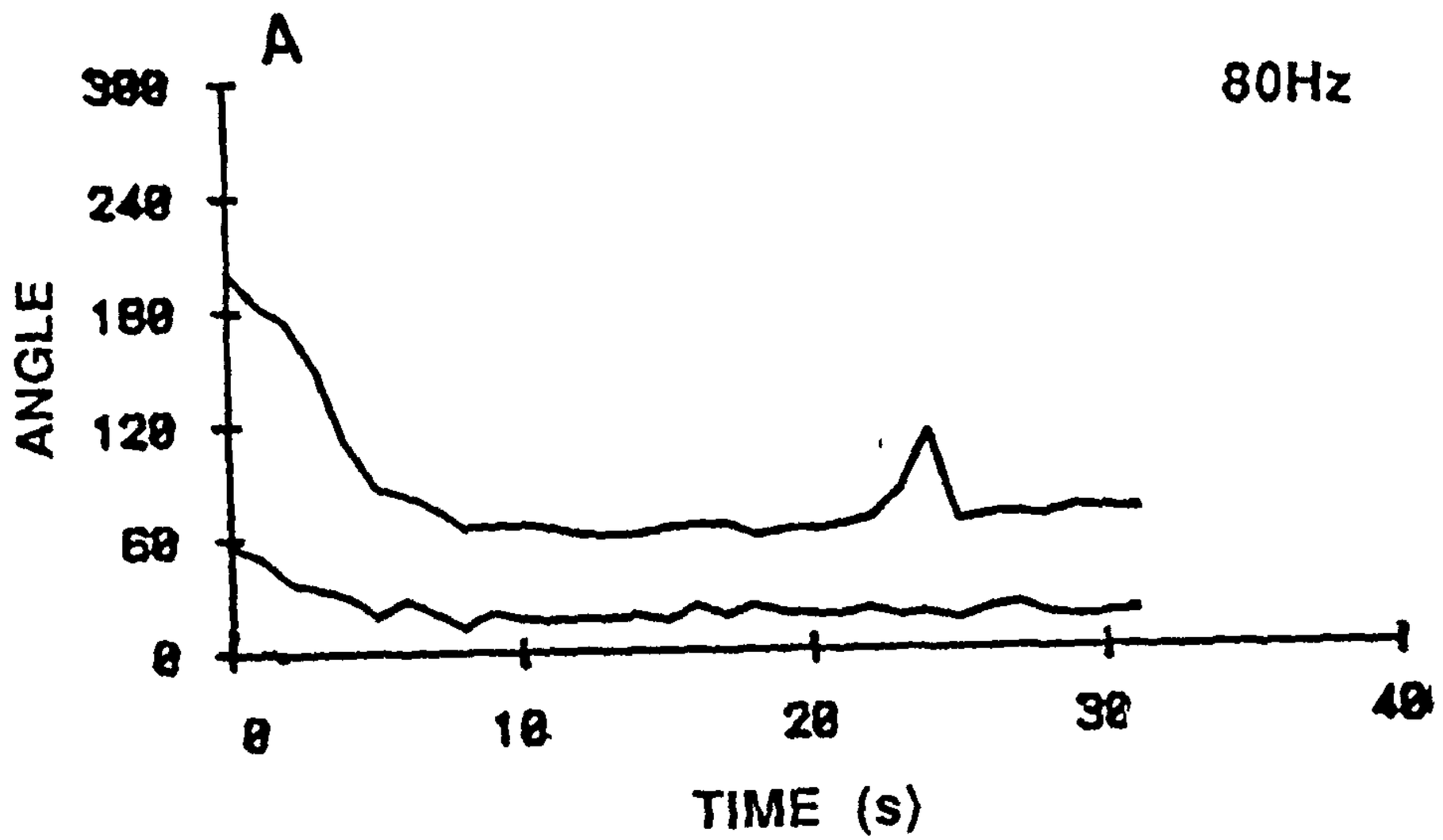
Figure 4.6 Plots of change in angle (degrees) of the posterior three abdominal segments (top line) and the anterior three abdominal segments (bottom line) with reference to the stationary thorax over time (seconds) during water borne vibratory stimuli of different frequencies presented for 30 seconds to standing animals. B,C and D are from the same animal, A is from a different animal.

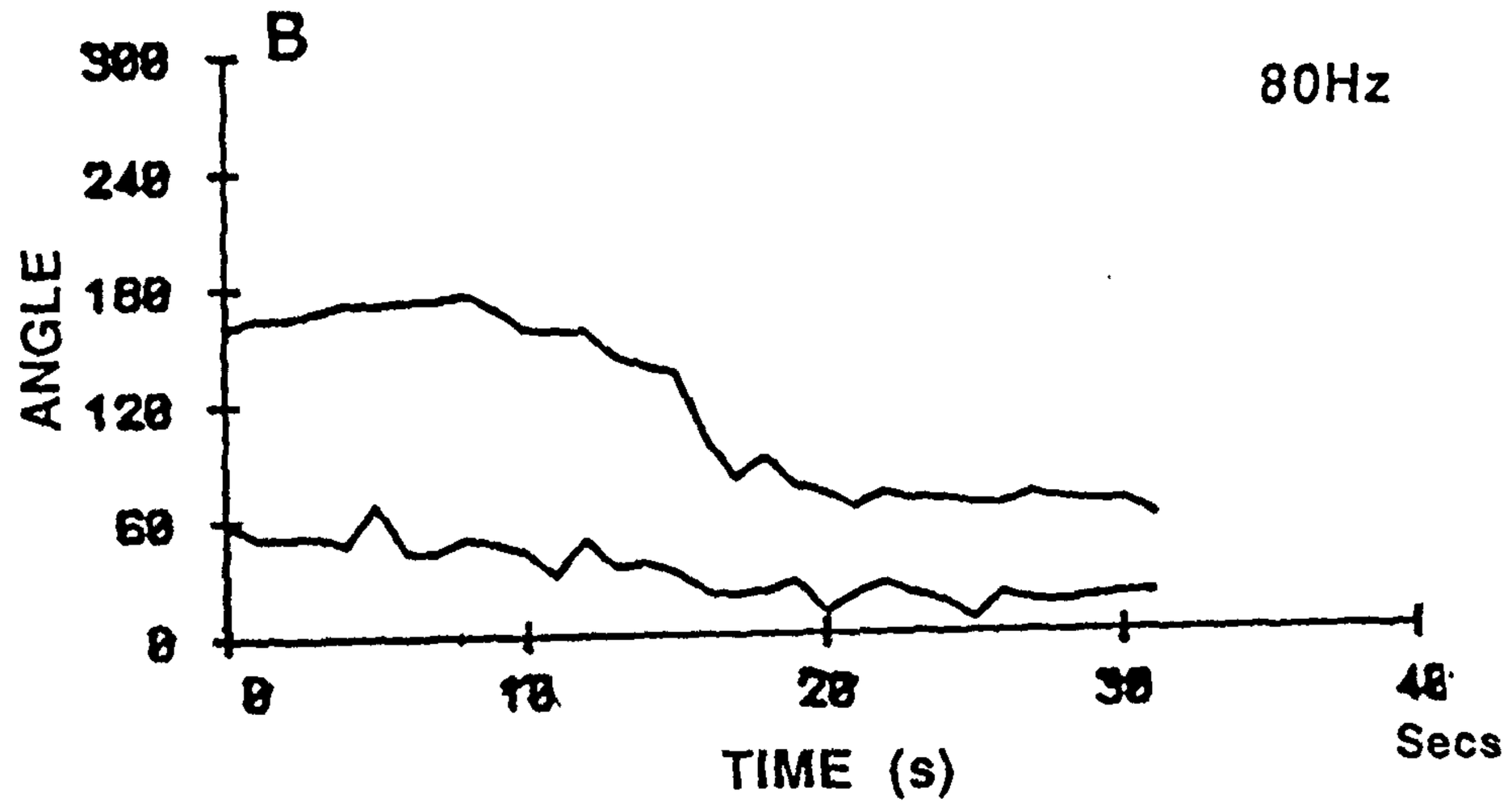
A. 80Hz showing immediate response

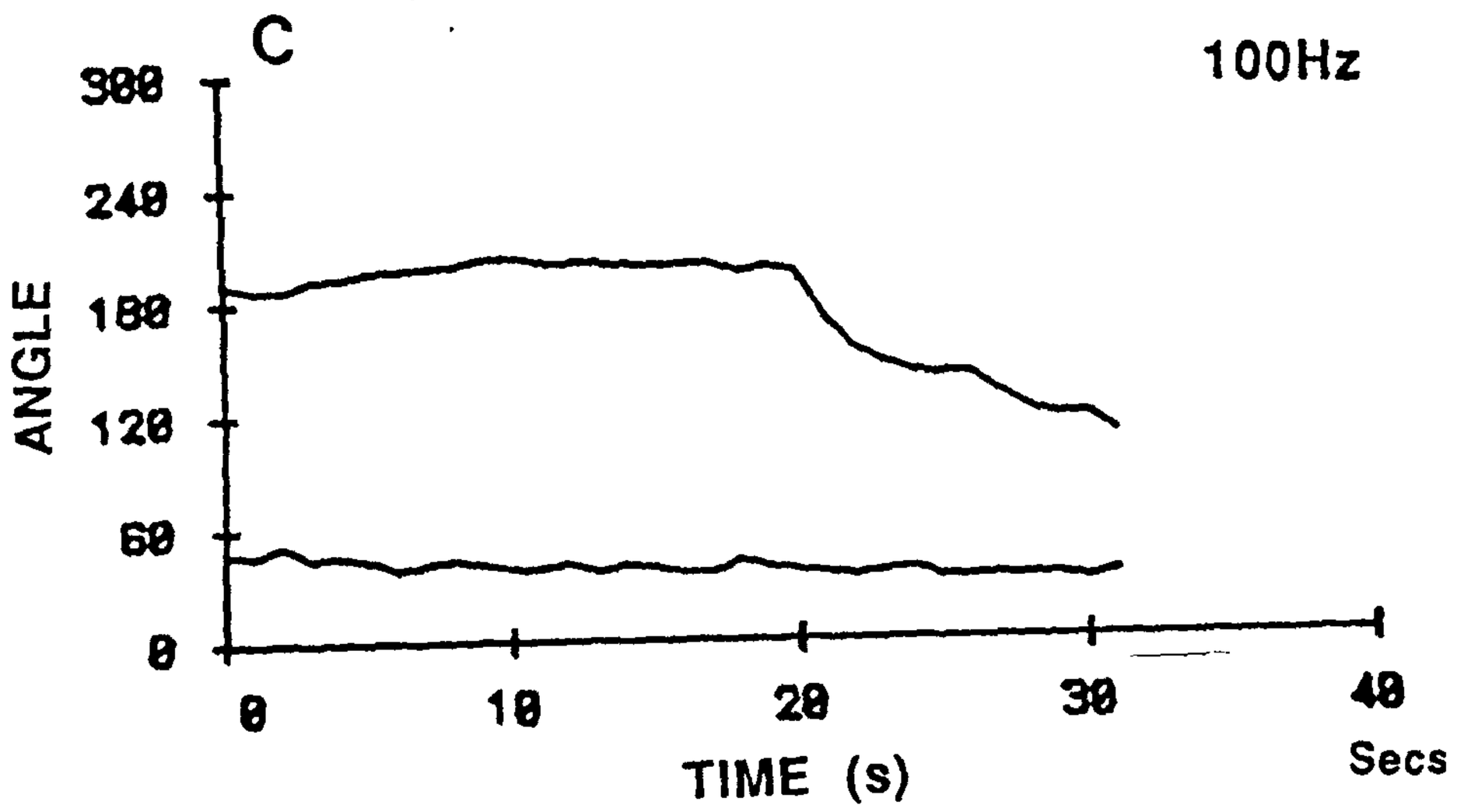
B. 80Hz showing delayed response

C. 100Hz showing delayed response

D. 100Hz showing negative response


A
80Hz
300
240
180
120
60
0
ANGLE
0
10
20
30
40
TIME (s)


B
80Hz
300
240
180
120
60
0
ANGLE
0
10
20
30
40
Secs
TIME (s)


C
100Hz
300
240
180
120
60
0
ANGLE
0
10
20
30
40
Secs
TIME (s)

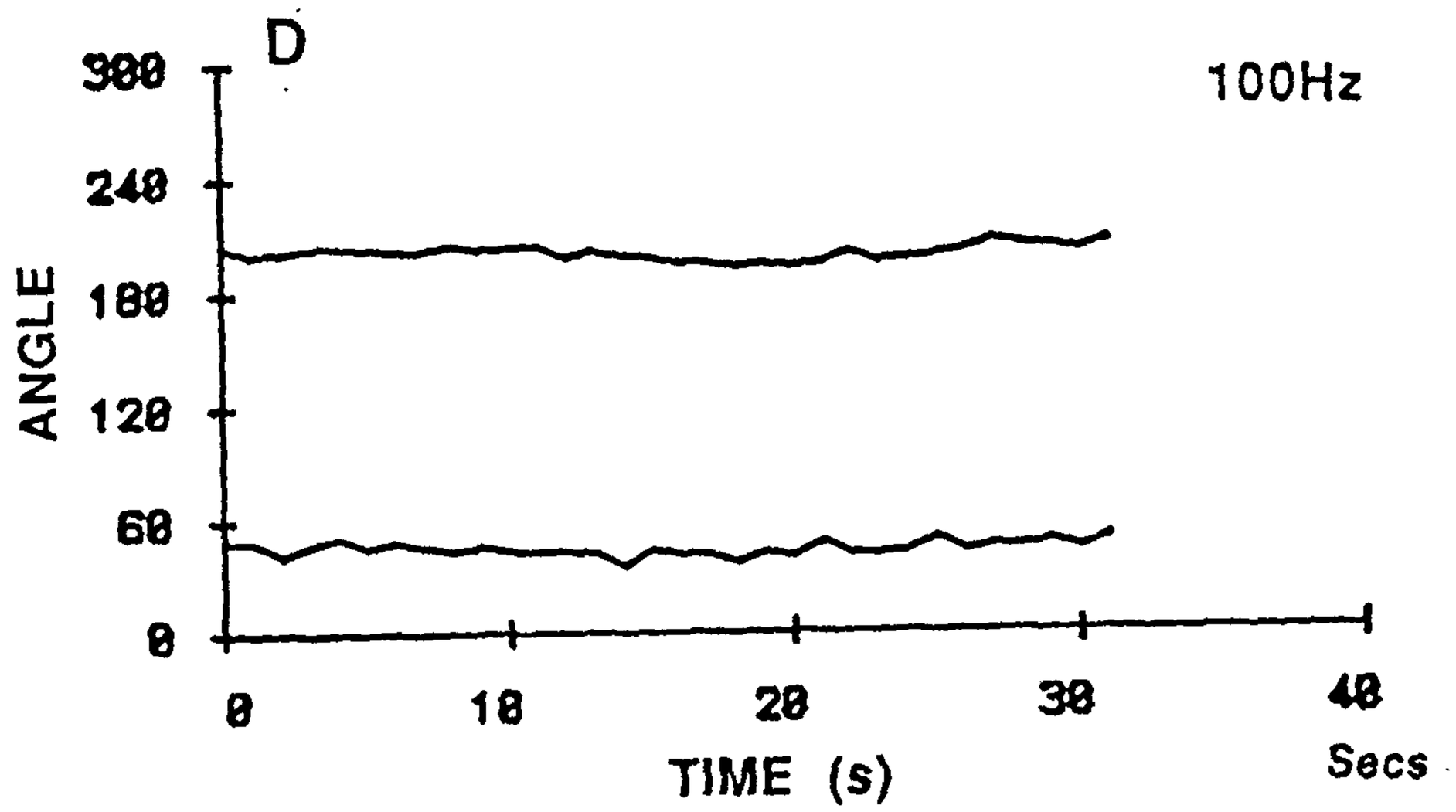

D
100Hz
300
240
180
120
60
0
ANGLE
0
10
20
30
40
Secs
TIME (s)

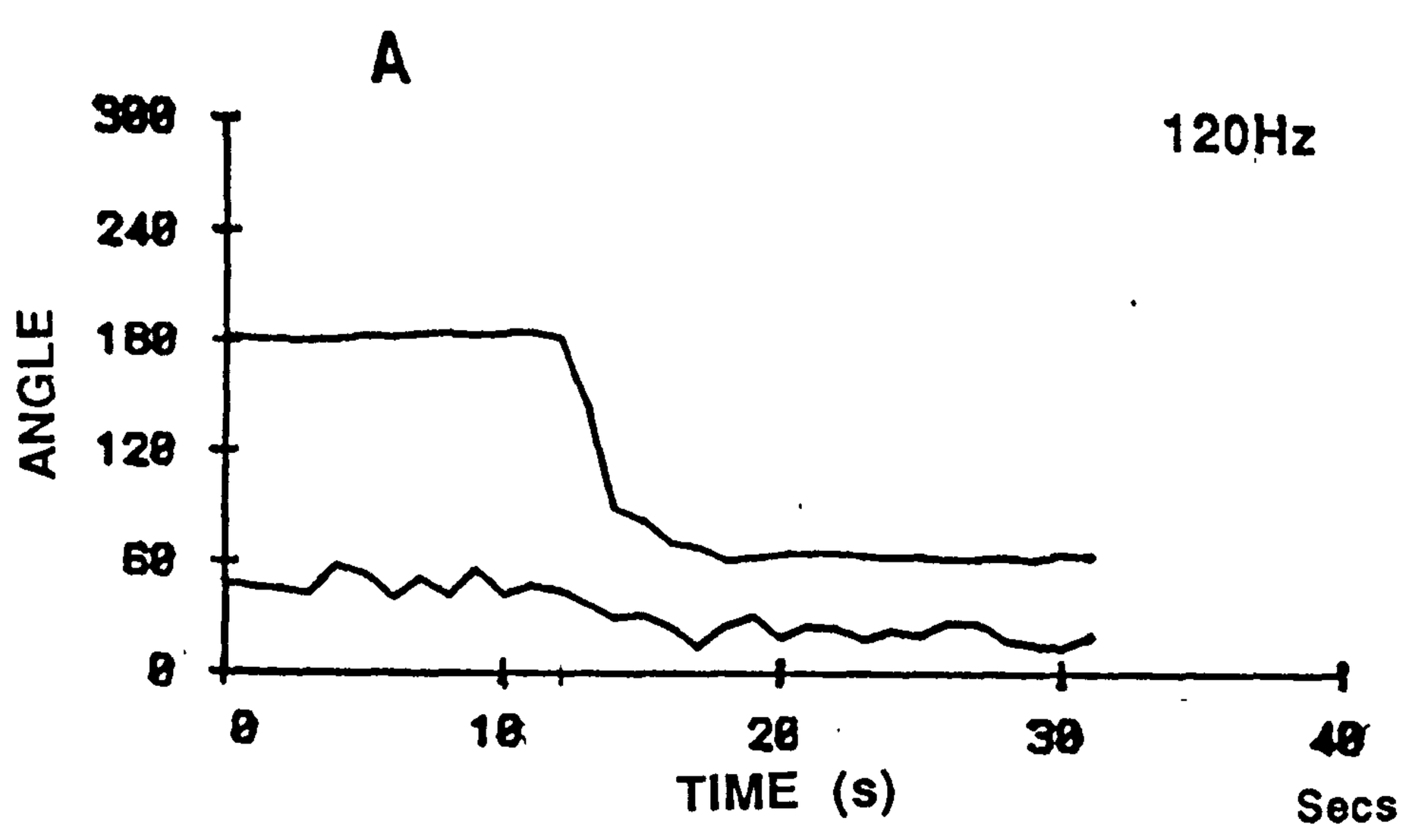
Figure 4.7 Plots of change in angle (degrees) of the posterior three abdominal segments (top line) and the anterior three abdominal segments (bottom line) with reference to the stationary thorax over time (seconds) during water borne vibratory stimuli of different frequencies presented for 30 seconds to standing animals. A,B and C are from the same animal, D is from a different animal.

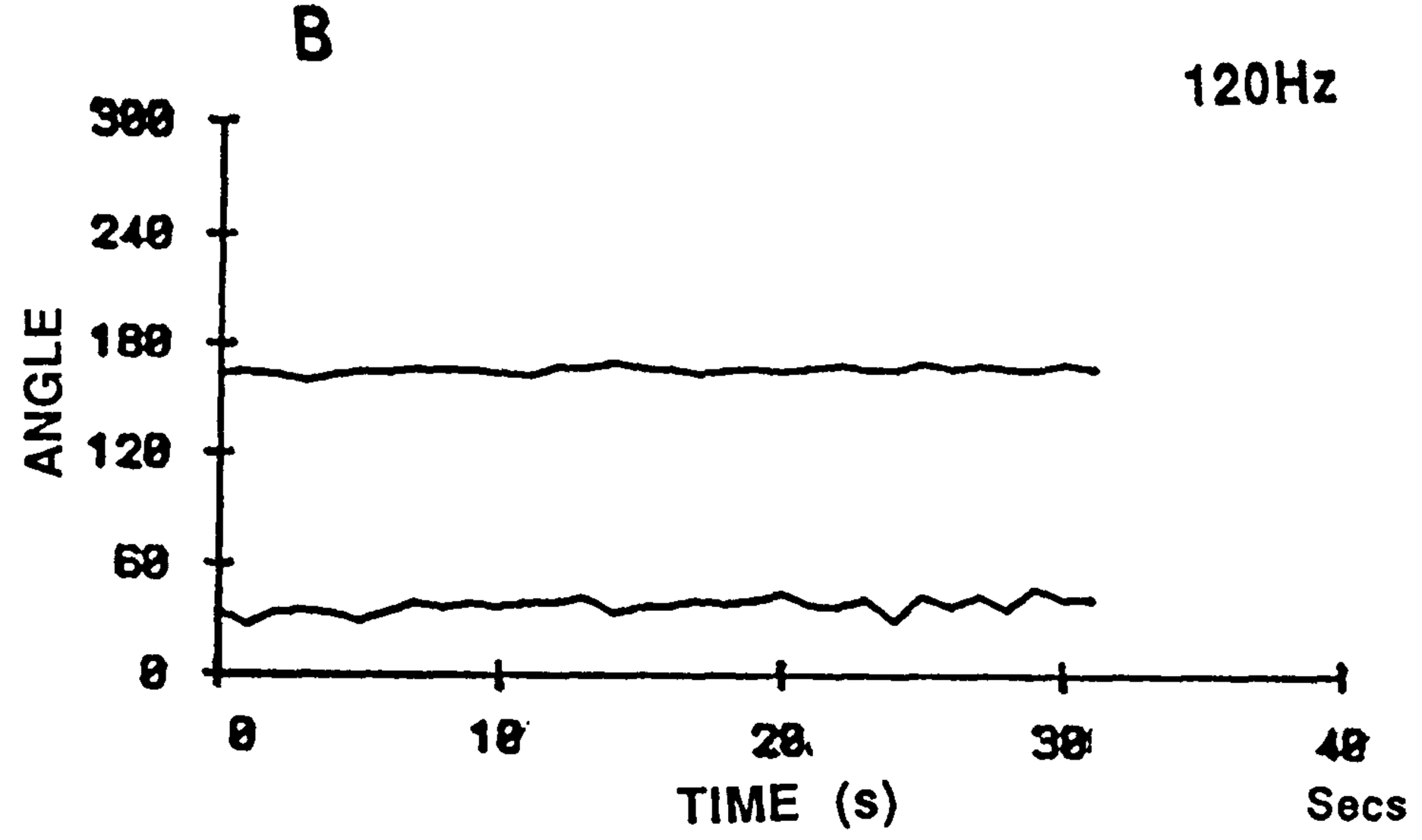
A. 120Hz showing delayed response

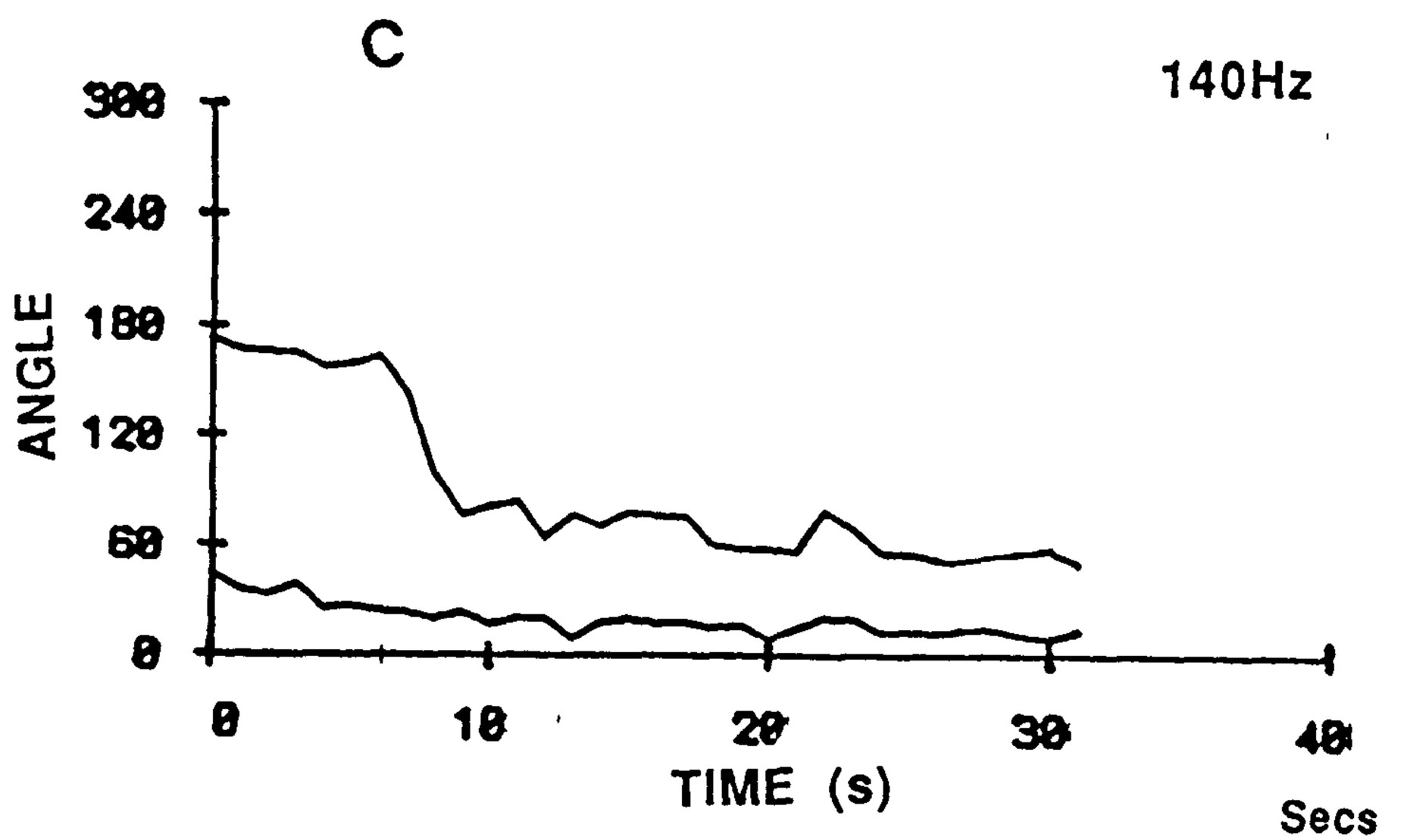
B. 120Hz showing negative response

C. 140Hz showing delayed response

D. 140Hz showing negative response


A
120Hz
300
240
180
120
60
0
ANGLE
0
10
20
30
40
TIME (s)
Secs


B
120Hz
300
240
180
120
60
0
ANGLE
0
10
20
30
40
TIME (s)
Secs


C
140Hz
300
240
180
120
60
0
ANGLE
0
10
20
30
40
TIME (s)
Secs

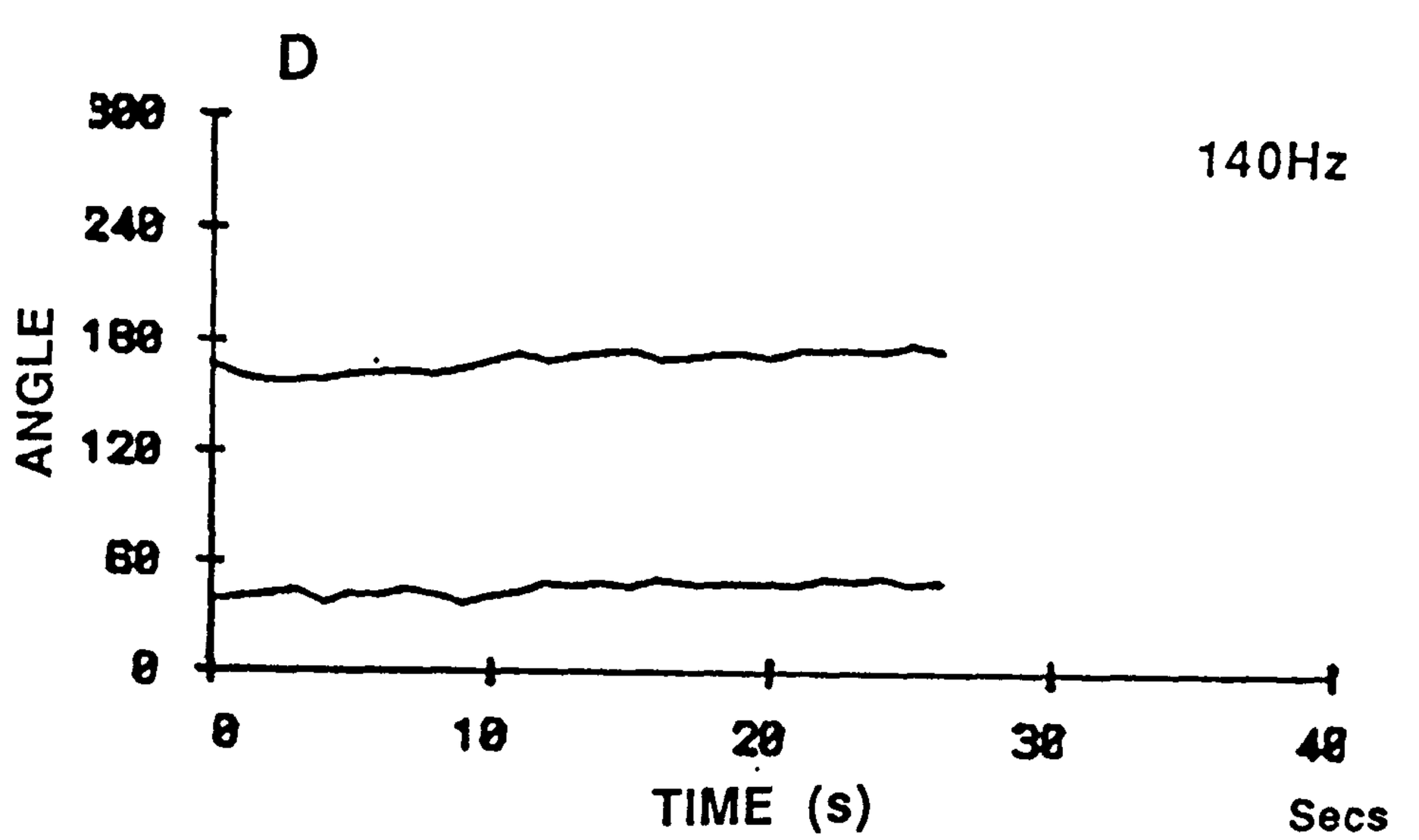

D
140Hz
300
240
180
120
60
0
ANGLE
0
10
20
30
40
TIME (s)
Secs

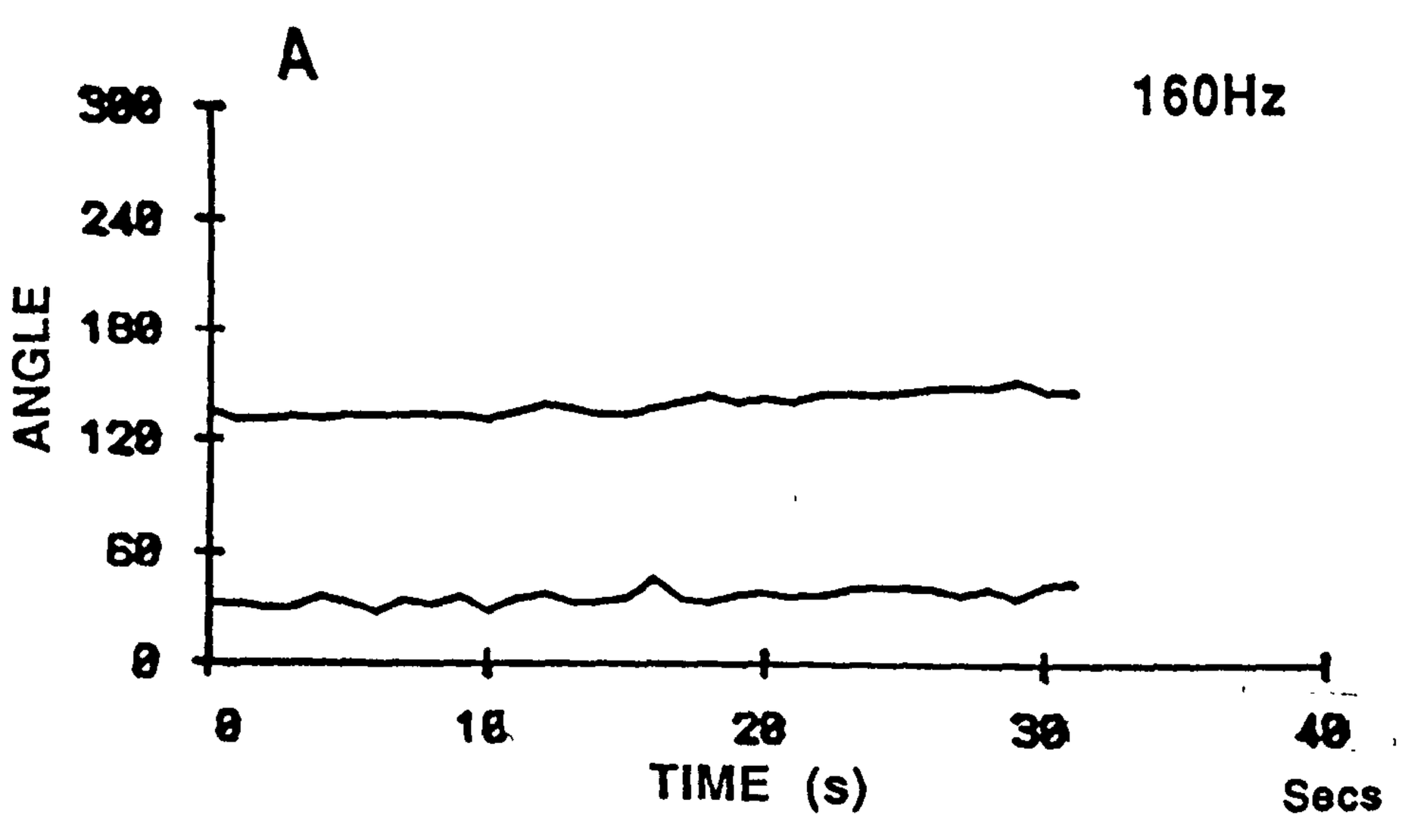
Figure 4.8 Plots of change in angle (degrees) of the posterior three abdominal segments (top line) and the anterior three abdominal segments (bottom line) with reference to the stationary thorax (zero) over time (seconds) during water borne vibratory stimuli of different frequencies presented for 30 seconds to standing animals. All are from the same animal.

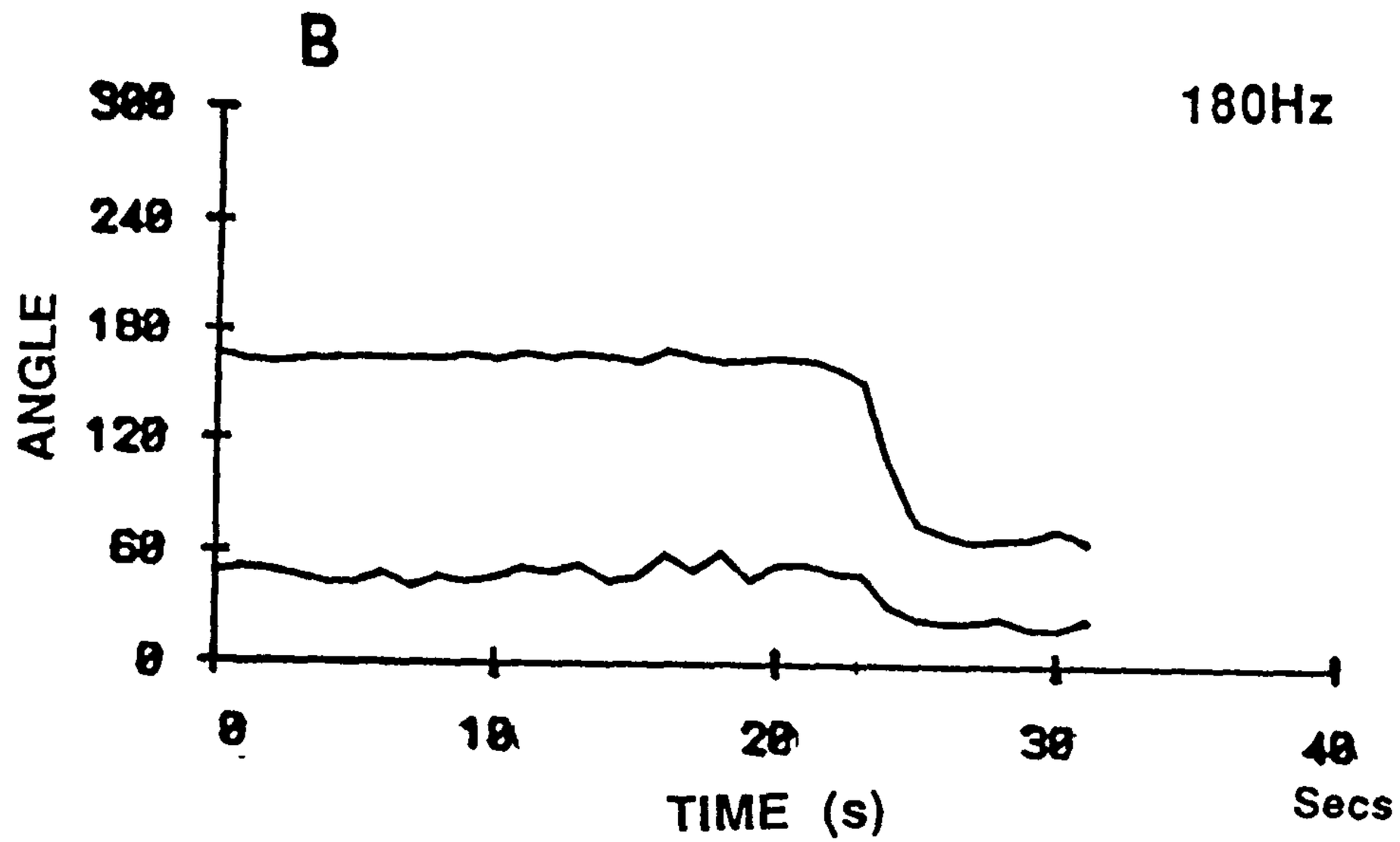
A. 160Hz

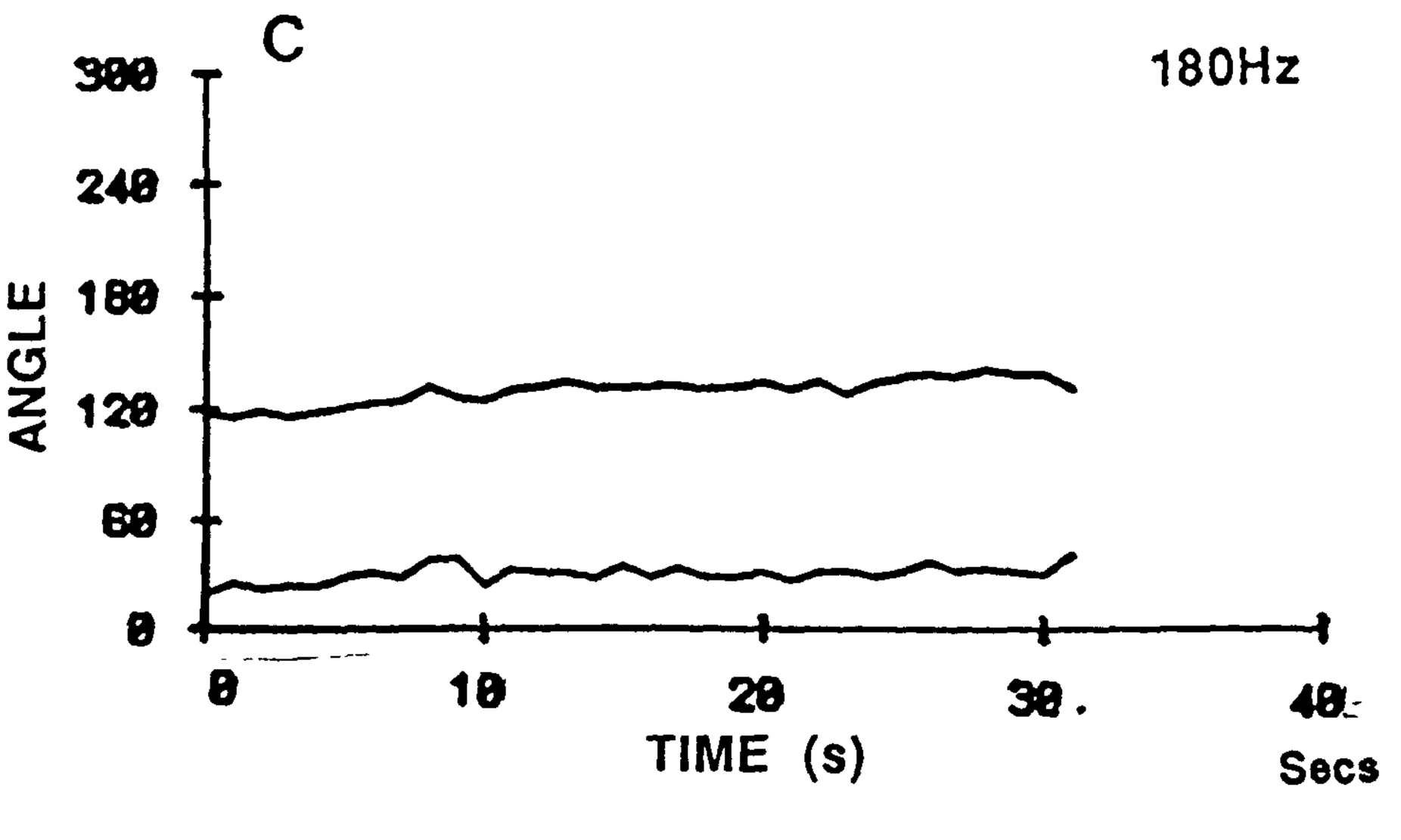
B. 180Hz showing delayed response

C. 180Hz showing negative response

D. 300Hz


A
160Hz
300
240
180
120
60
0
ANGLE
0
10
20
30
40
TIME (s)
Secs


B
180Hz
300
240
180
120
60
0
ANGLE
0
10
20
30
40
TIME (s)
Secs


C
180Hz
300
240
180
120
60
0
ANGLE
0
10
20
30
40
TIME (s)
Secs

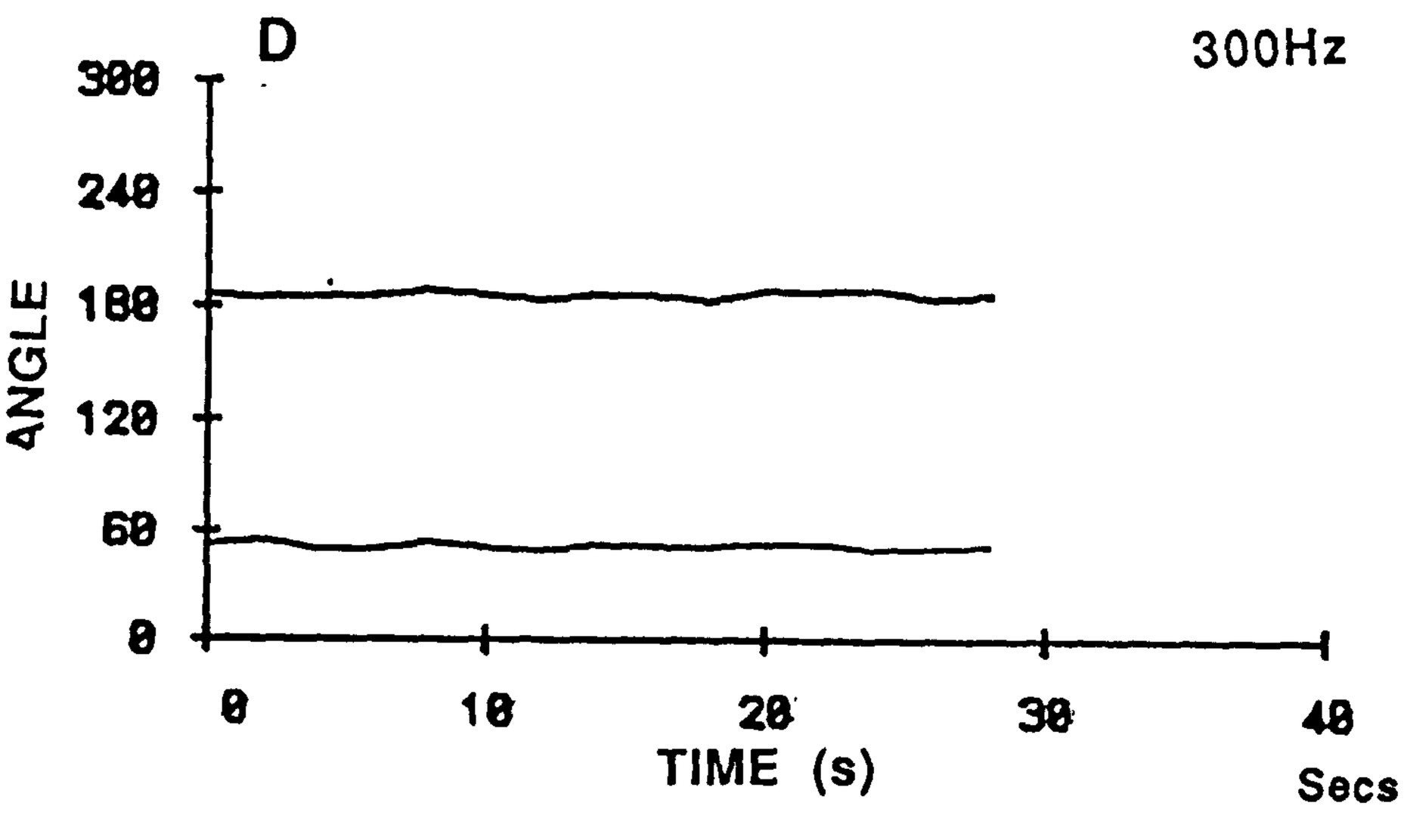

D
300Hz
300
240
180
120
60
0
ANGLE
0
10
20
30
40
TIME (s)
Secs

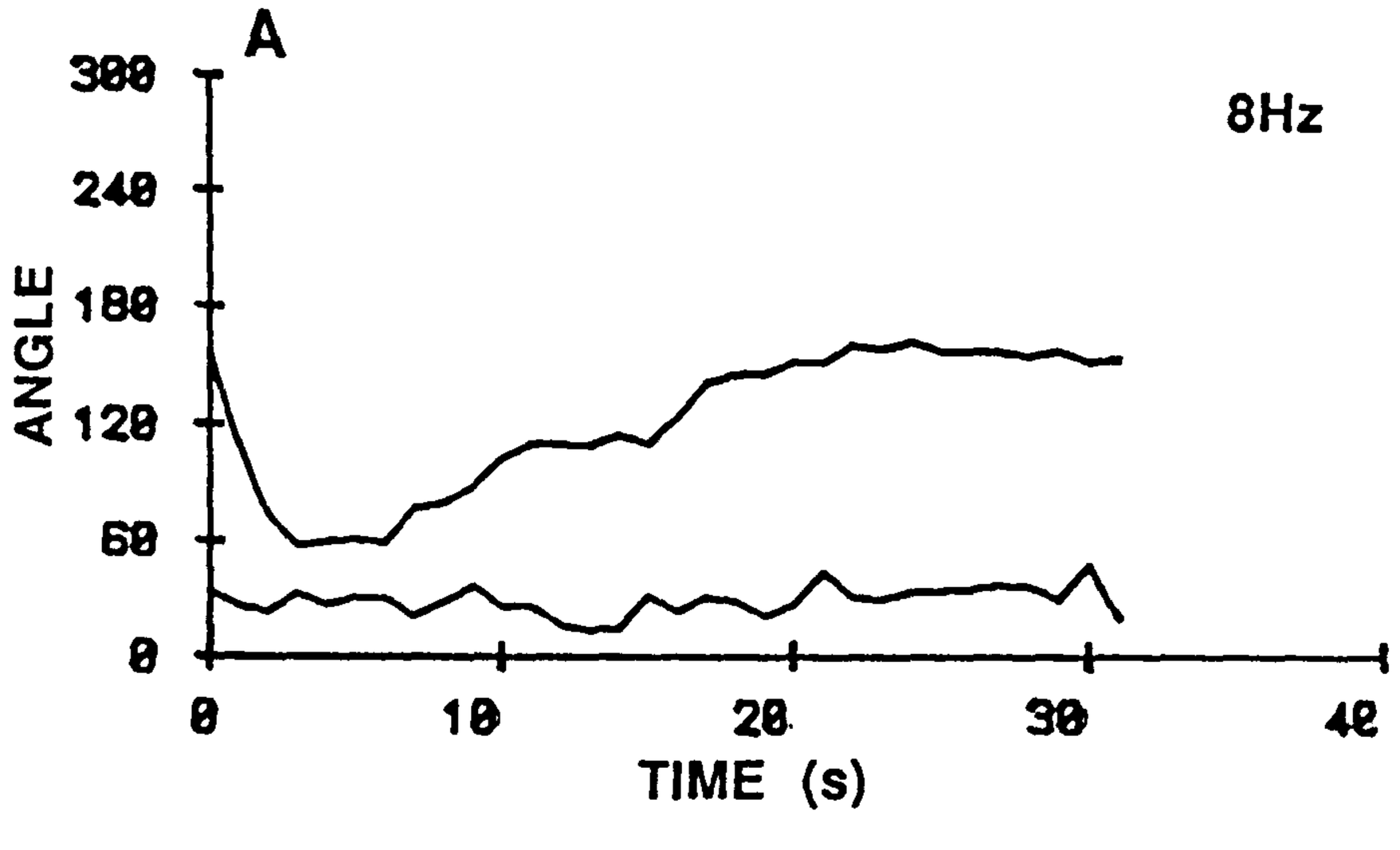
Figure 4.9 Plots of change in angle (degrees) of the posterior three abdominal segments (top line) and the anterior three abdominal segments (bottom line) with reference to the stationary thorax over time (seconds) during water borne vibratory stimuli of different frequencies presented for 30 seconds to animals suspended in mid water. B,C and D are from the same animal, A is from a different animal.

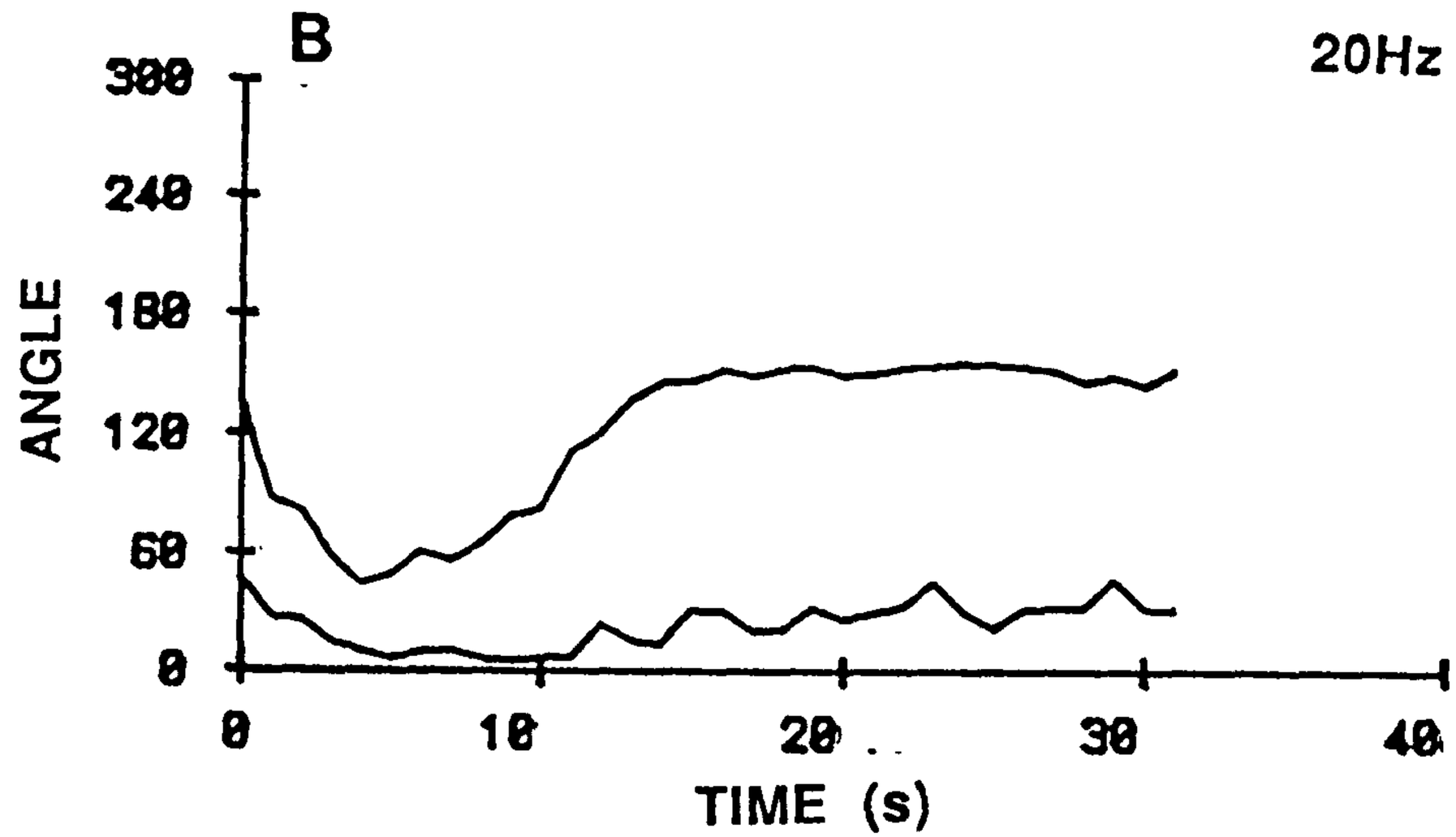
A. 8Hz

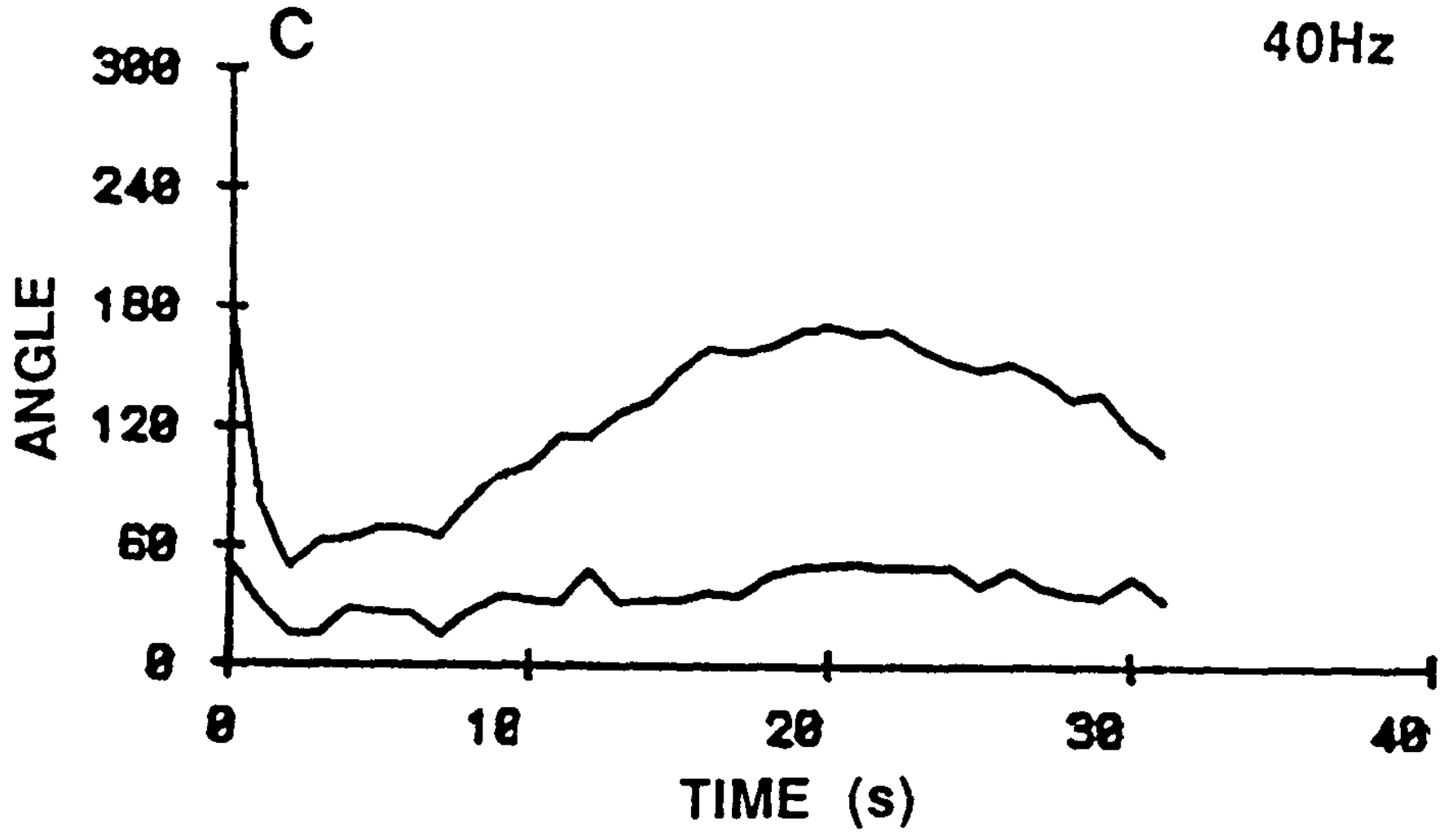
B. 20Hz

C. 40Hz

D. 50Hz


A
8Hz
300
240
180
120
60
0
ANGLE
0
10
20
30
40
TIME (s)


B
20Hz
300
240
180
120
60
0
ANGLE
0
10
20
30
40
TIME (s)


C
40Hz
300
240
180
120
60
0
ANGLE
0
10
20
30
40
TIME (s)

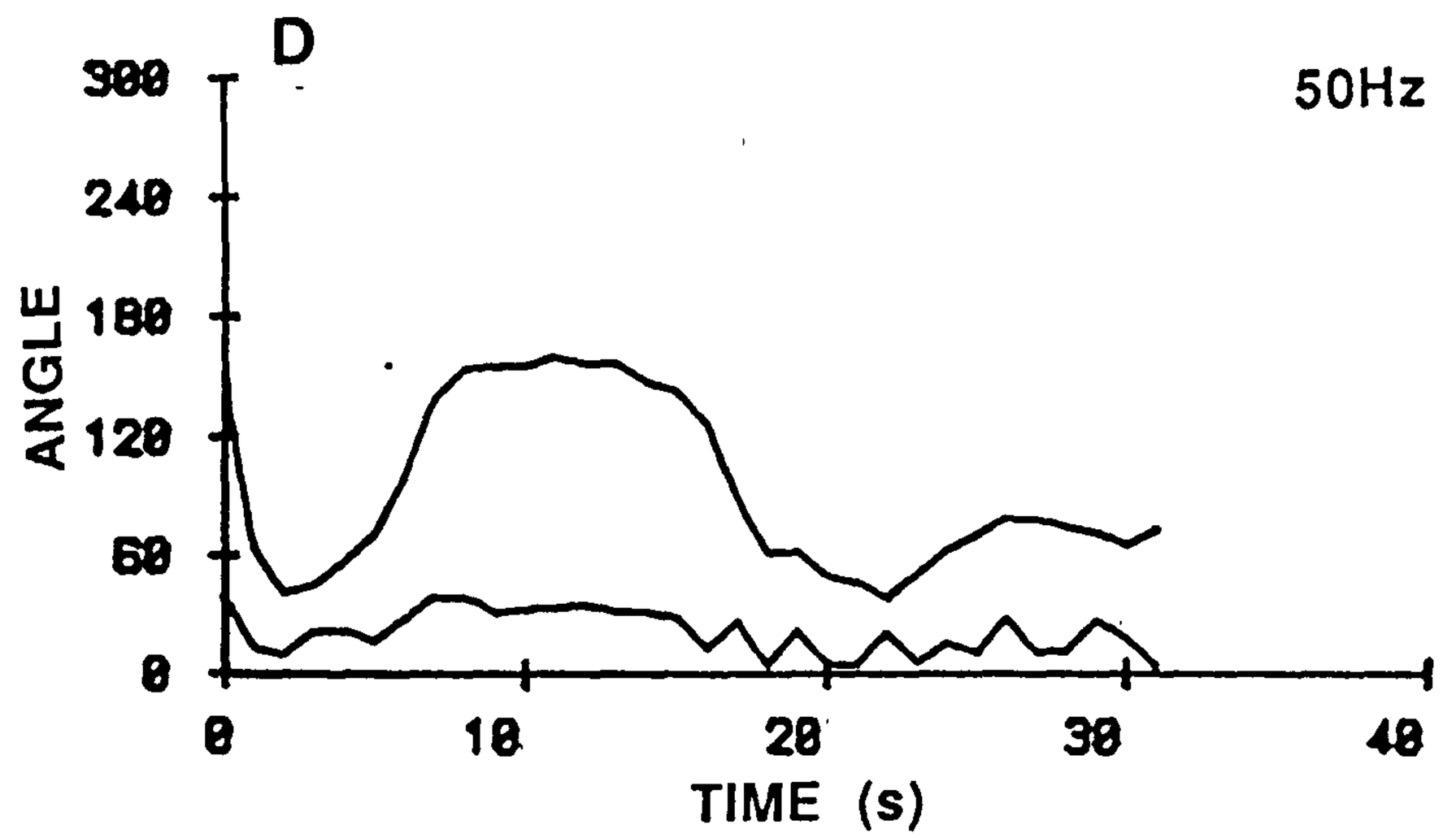
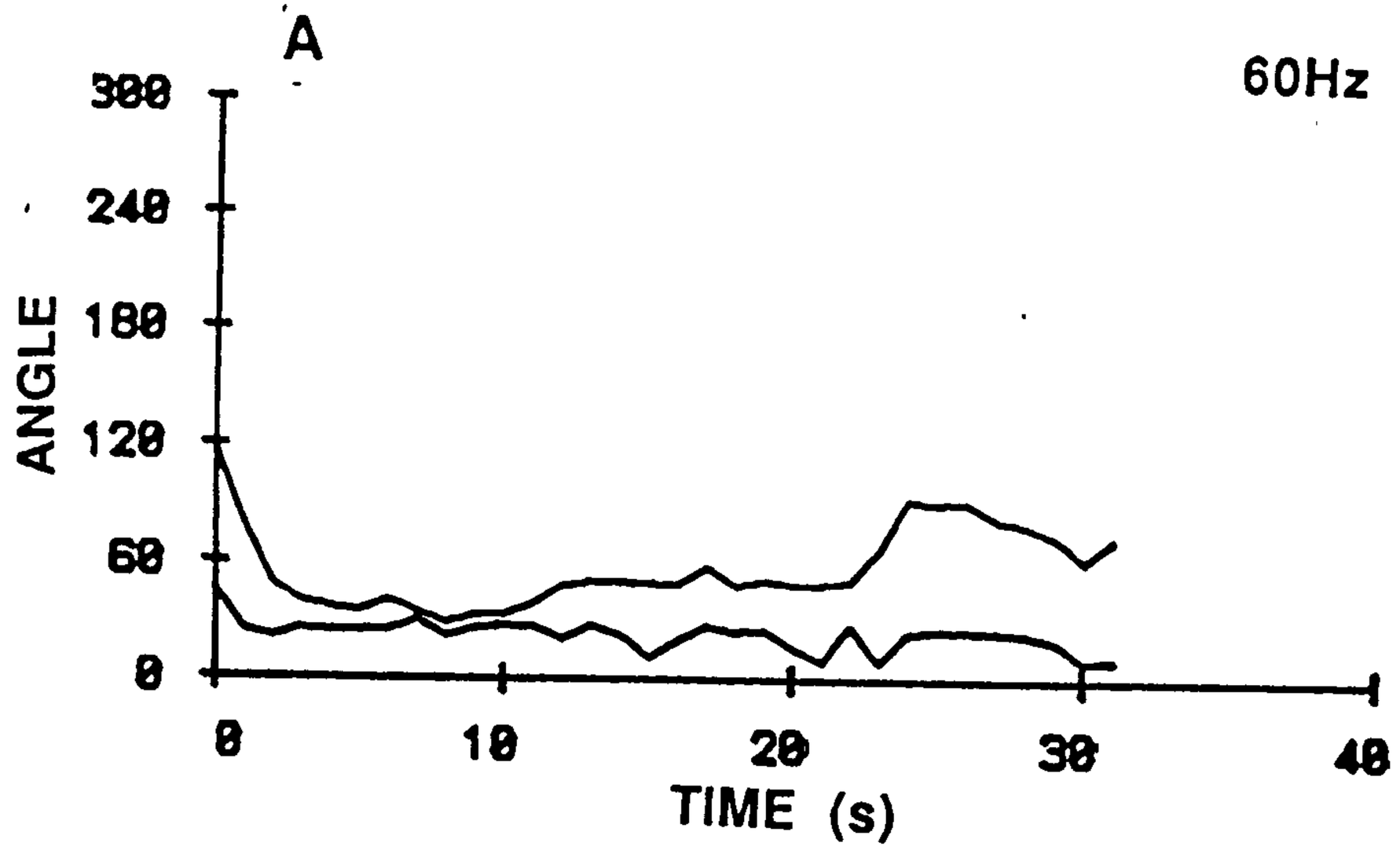

D
50Hz
300
240
180
120
60
0
ANGLE
0
10
20
30
40
TIME (s)

Figure 4.10 Plots of change in angle (degrees) of the posterior three abdominal segments (top line) and the anterior three abdominal segments (bottom line) with reference to the stationary thorax over time (seconds) during water borne vibratory stimuli of different frequencies presented for 30 seconds to animals suspended in mid water. Data from one animal.

A. 60Hz

B. 120Hz


A
60Hz
300
240
180
120
60
0
ANGLE
0
10
20
30
40
TIME (s)

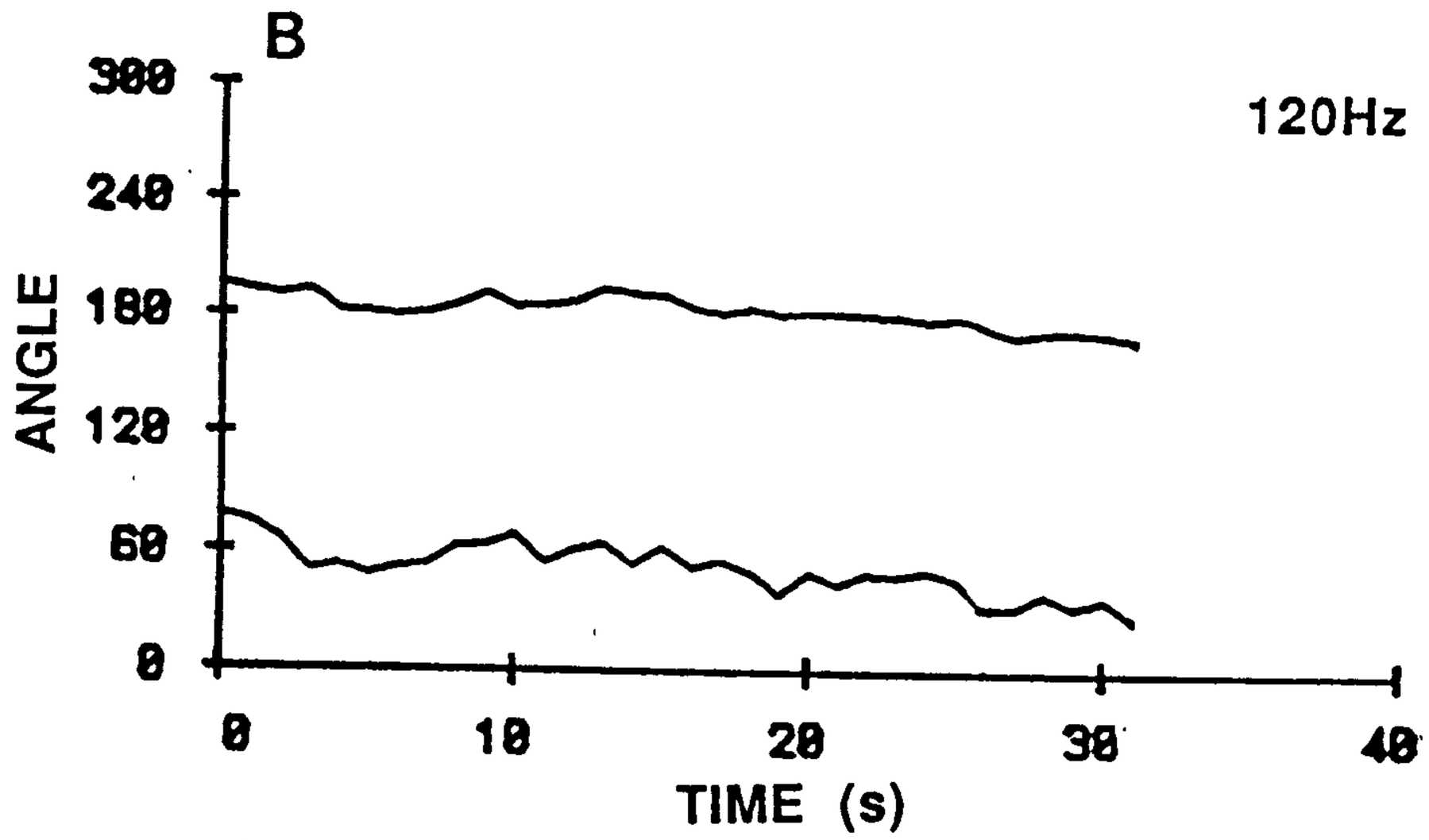

B
120Hz
300
240
180
120
60
0
ANGLE
0
10
20
30
40
TIME (s)

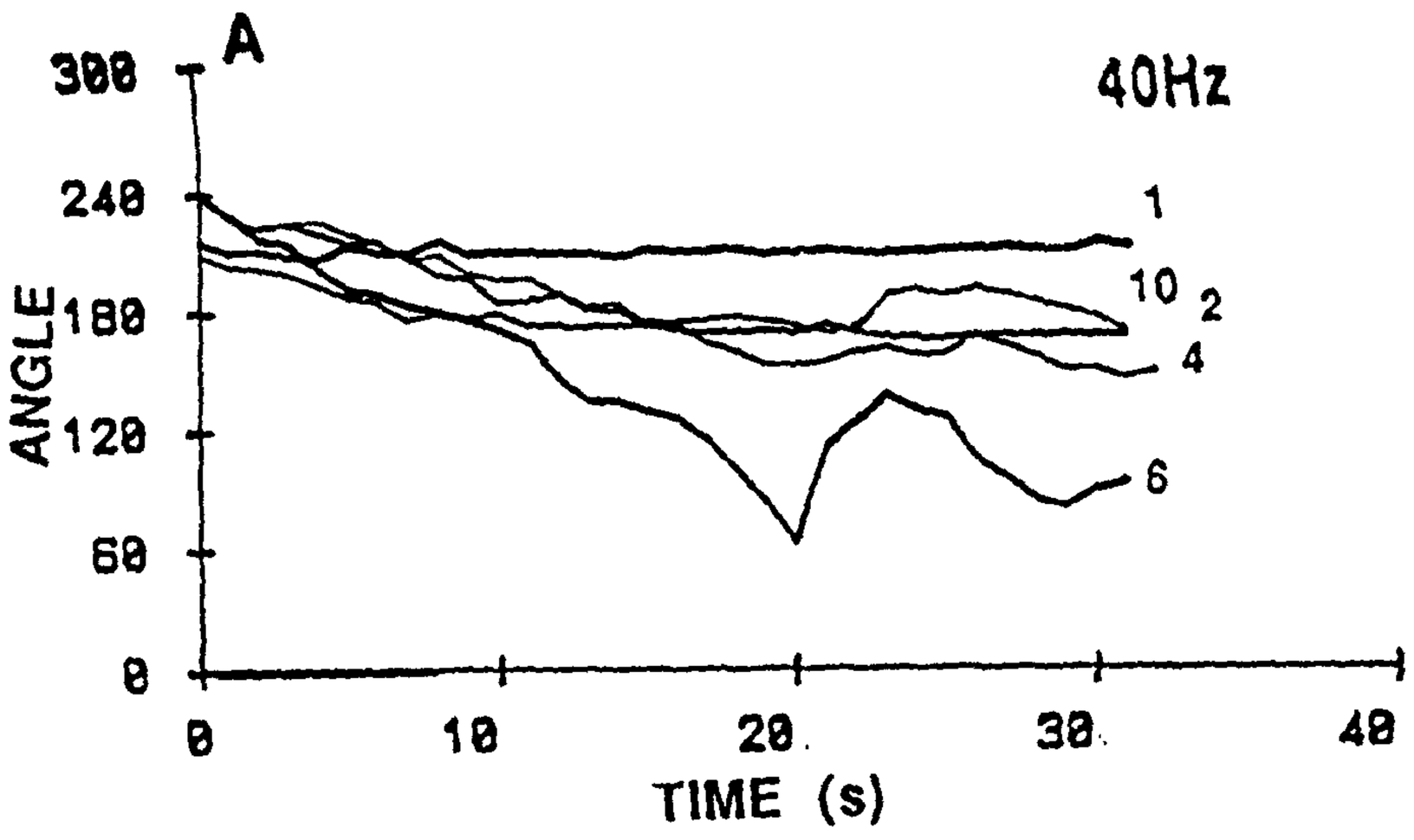
Figure 4.11 Plots of change in angle (degrees) of the posterior three abdominal segments with reference to the stationary thorax over time (seconds) during water borne vibratory stimuli of different frequencies and amplitude presented for 30 seconds to animals standing with legs in contact with the substrate. Numbers 1,2,4,6 and 10 refer to increasing voltage output signals supplied by the Derritron amplifier. A and C are from the same animal, B and D are from a different animal.

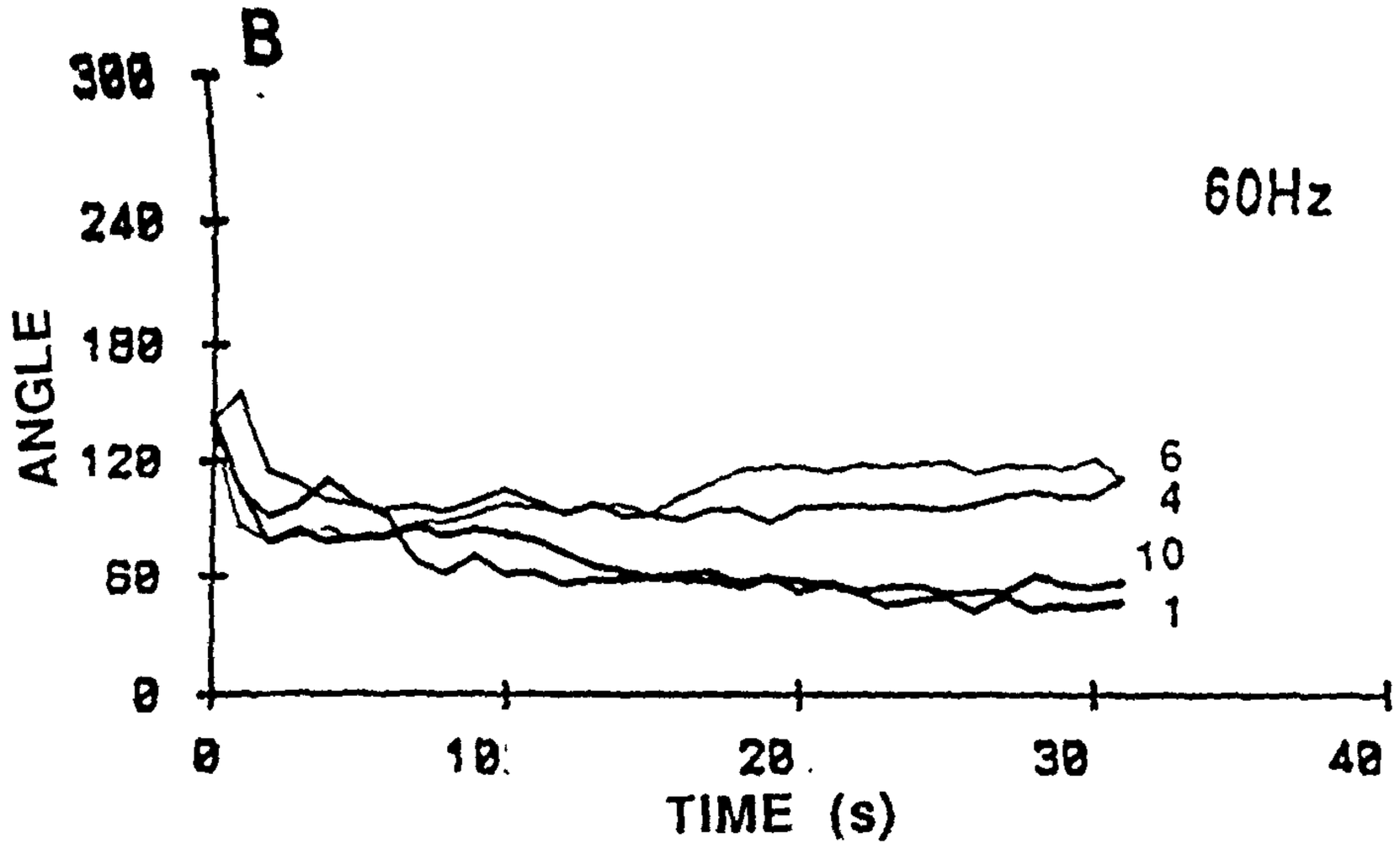
A. 40Hz

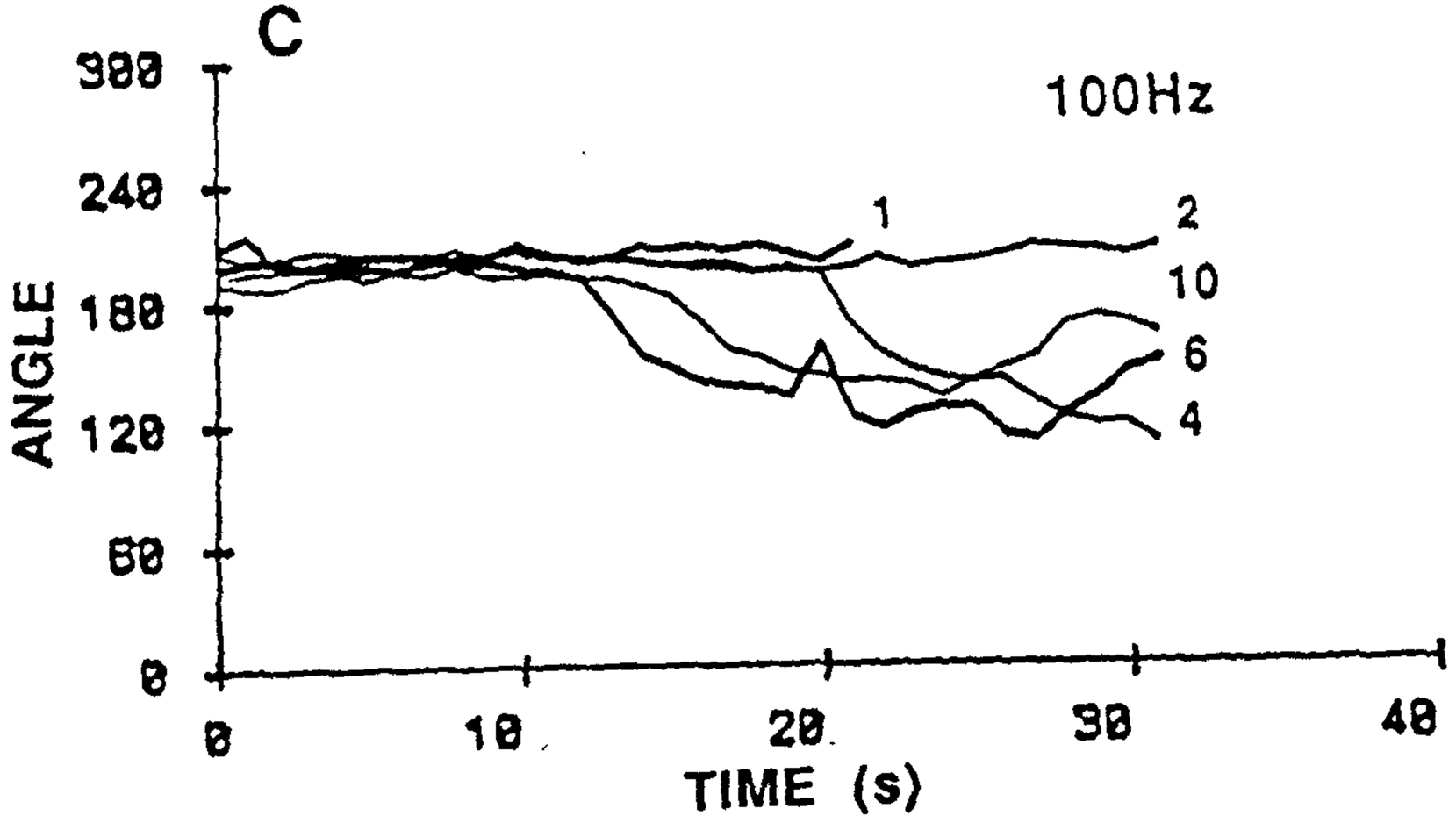
B. 60Hz

C. 100Hz

D. 120Hz


A
40Hz
300
240
180
120
60
0
ANGLE
1
10
2
4
6
0
10
20
30
40
TIME (s)


B
60Hz
300
240
180
120
60
0
ANGLE
6
4
10
1
0
10
20
30
40
TIME (s)


C
100Hz
300
240
180
120
60
0
ANGLE
1
2
10
6
4
0
10
20
30
40
TIME (s)

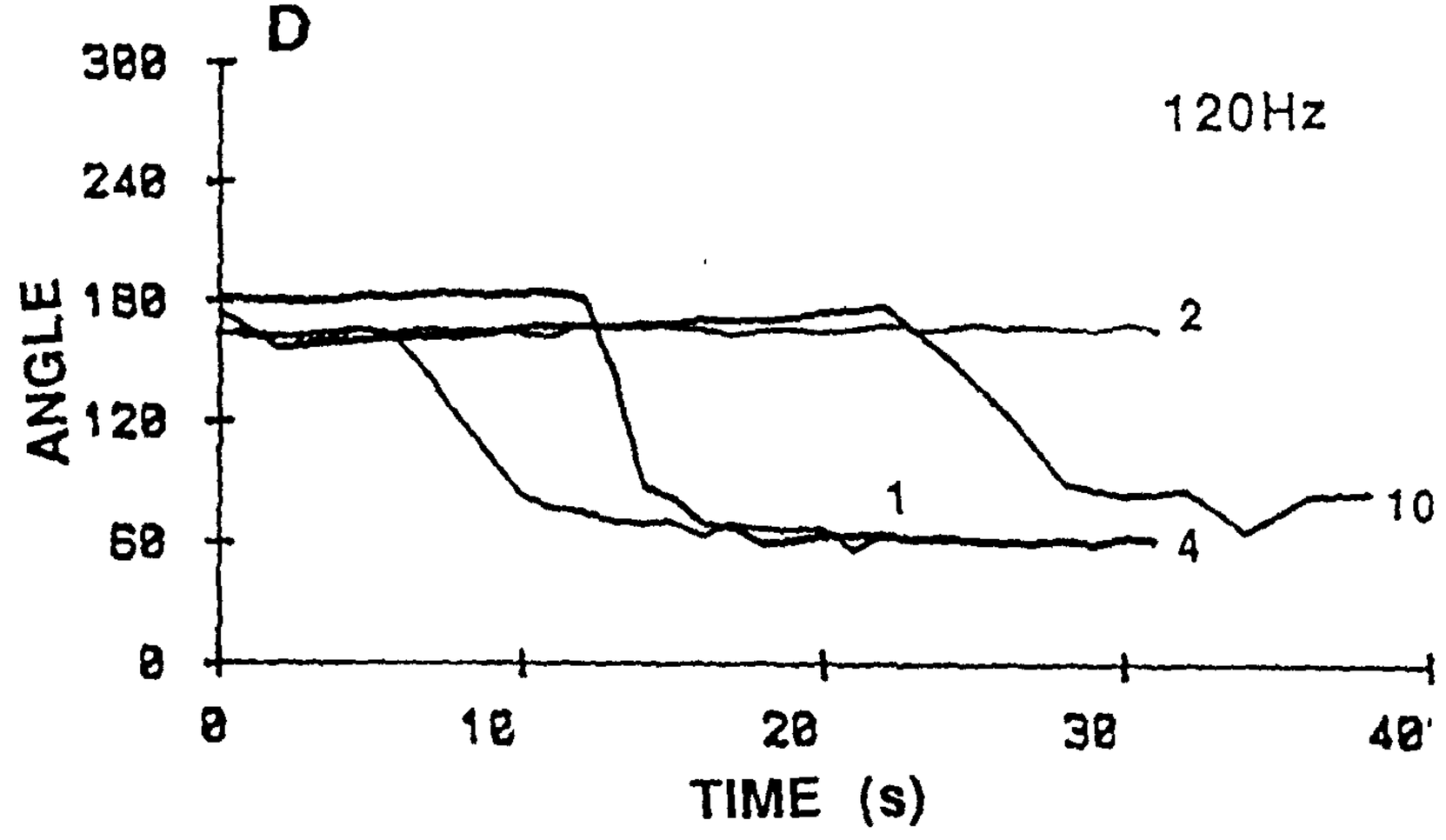

D
120Hz
300
240
180
120
60
0
ANGLE
2
1
4
10
0
10
20
30
40
TIME (s)

Figure 4.12 Spontaneous activity in Superficial Root Three (SR3) showing numbering of Flexor Excitatory (FE) units 1,2,3,4 and 6 and the Flexor Inhibitor (FI) unit 5. Horizontal axis shows time (seconds) vertical axis shows voltage (volts). This trace and subsequent traces in this chapter are modified screen dumps of data processed by the CED Spike 2 analysis program.

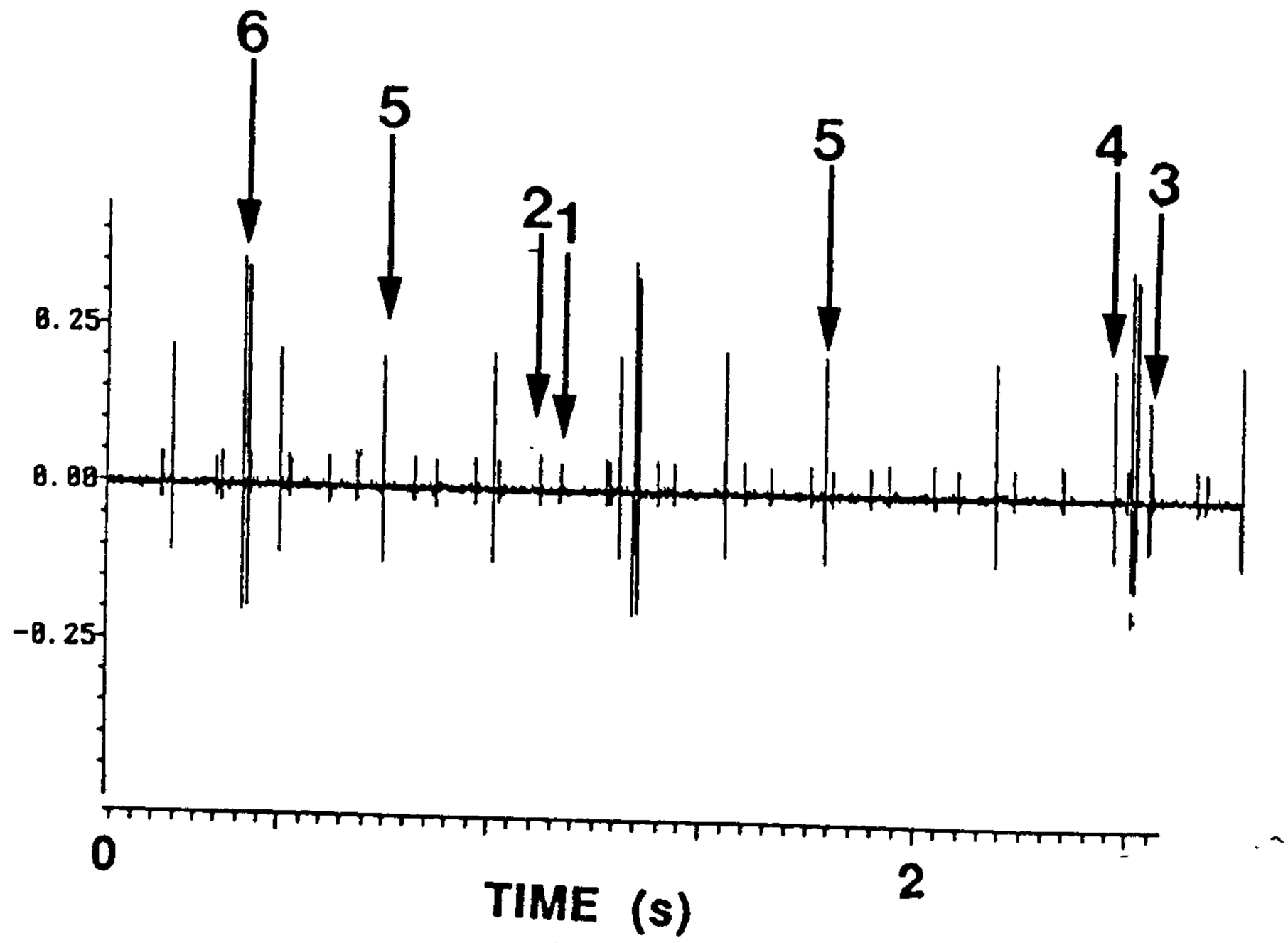

6
5
2
1
5
4
3
0.25
0.00
-0.25
0
2
TIME (s)

Figure 4.13 Responses of units in SR3 to tactile stimulation. Plots show the raw nerve spike data (top trace), and the mean frequency of firing in FI (i) (bottom trace). Scale bar shows time in seconds.

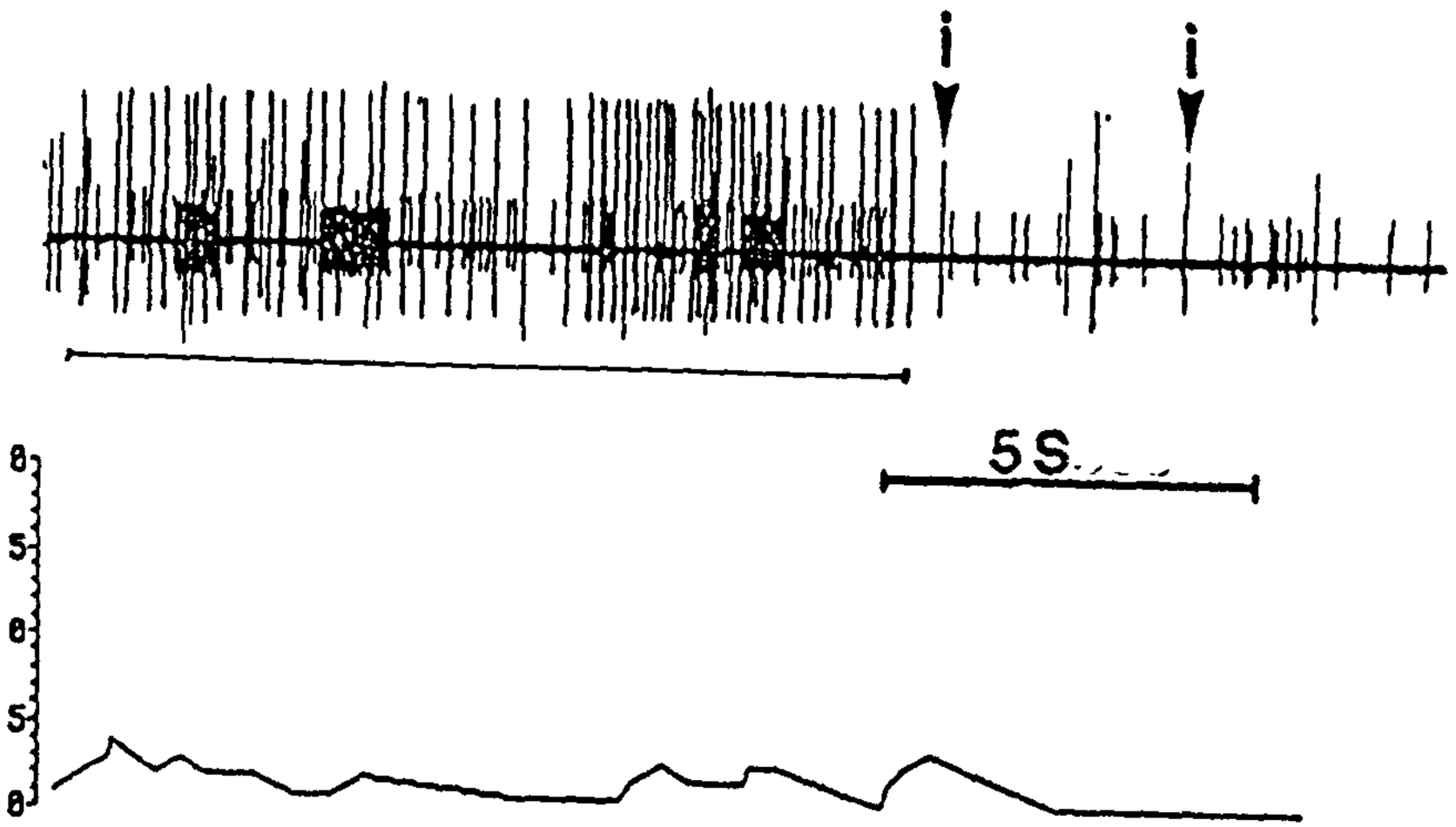
A. The response of SR3 to forced extension of the abdomen. The responsive units in this case are FE.

B. The response of units in SR3 to forced flexion of the abdomen. The responsive unit is FI.

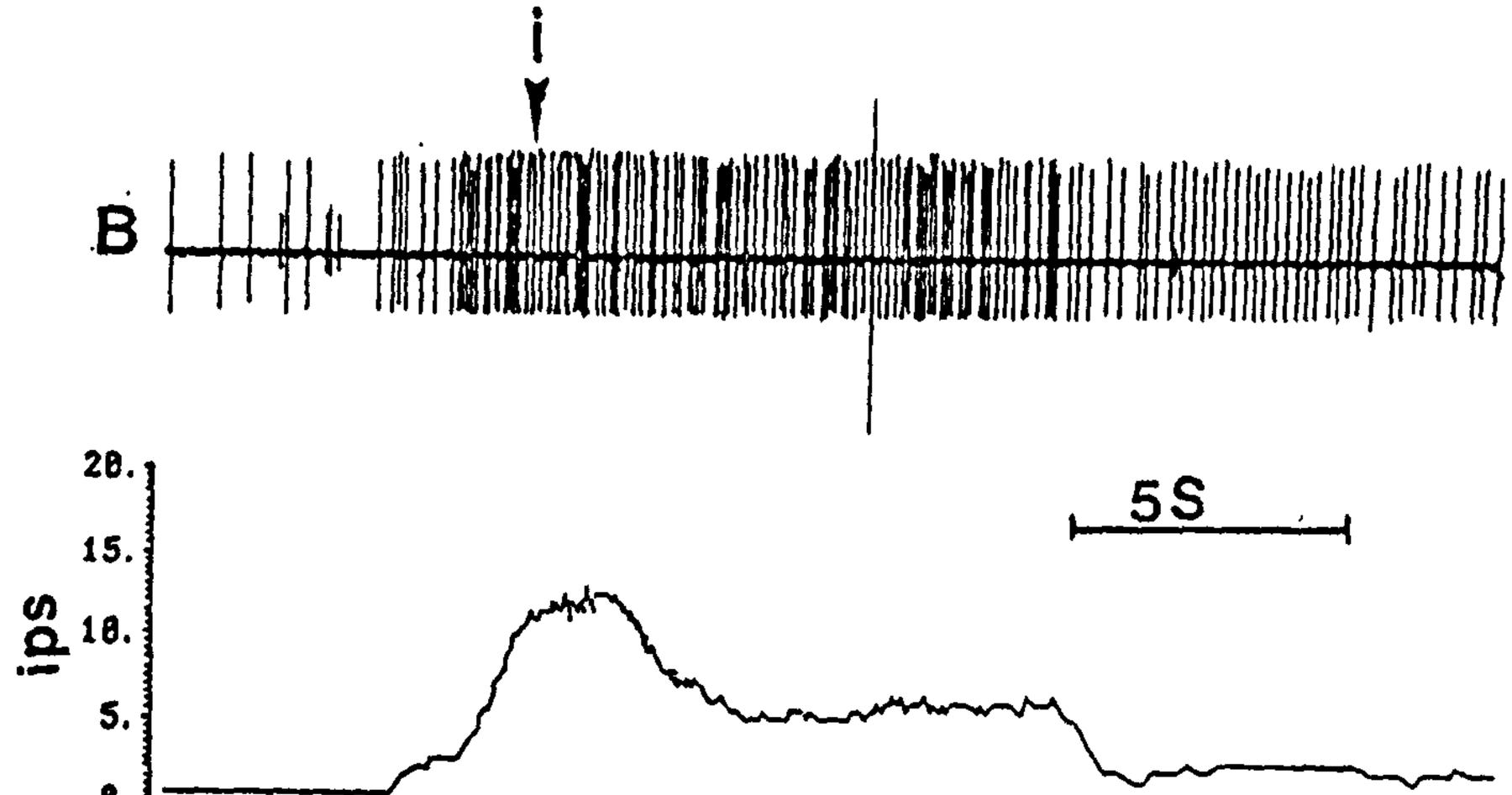
C. The response of units in SR3 to tactile stimulation of the walking legs.

D. The response of units in SR3 to tactile stimulation of the thorax.

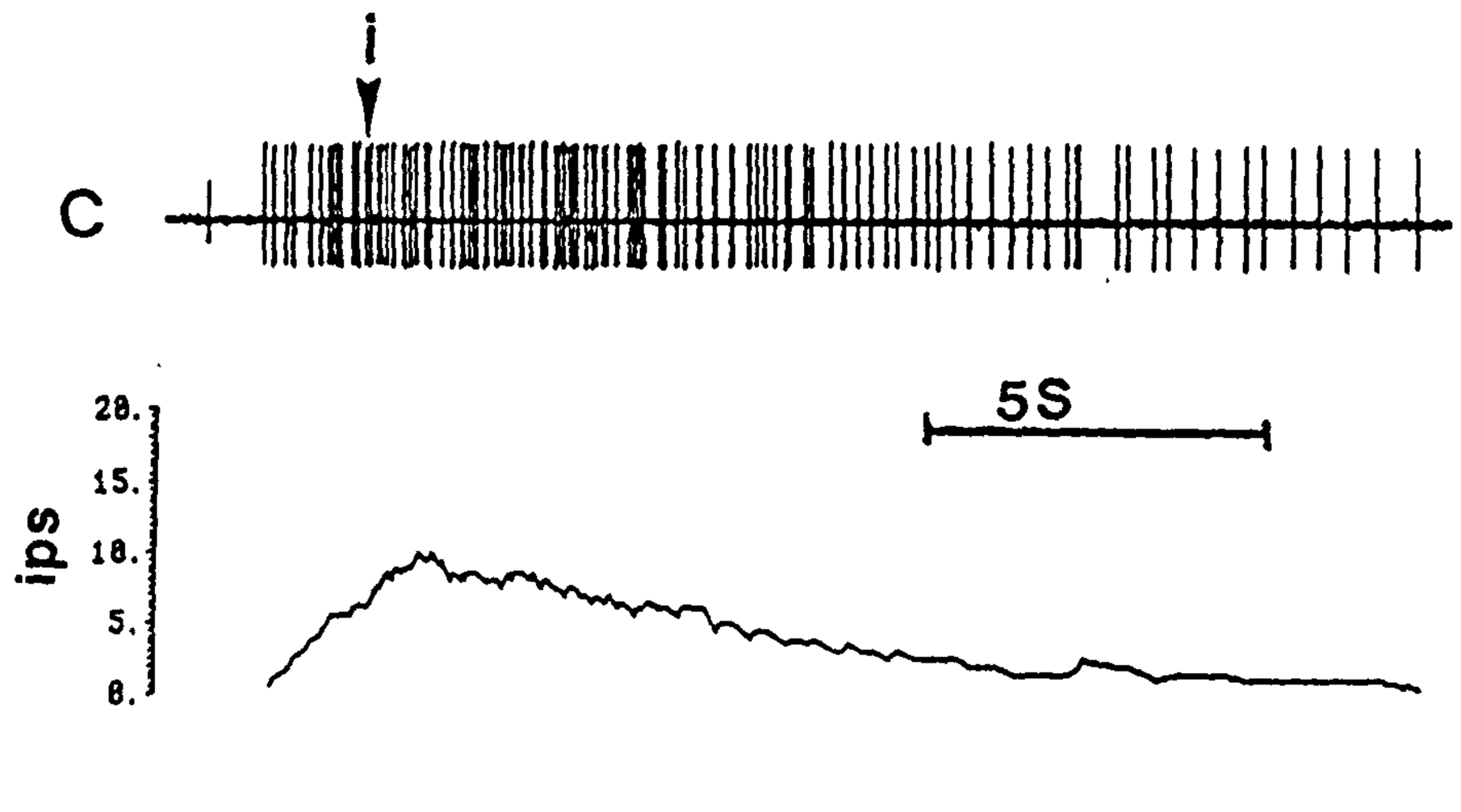
A


i
i
5S
ips
10.0
7.5
5.0
2.5
0.0

B


i
5S
ips
20.
15.
10.
5.
0.

C


i
5S
ips
20.
15.
10.
5.
0.

D

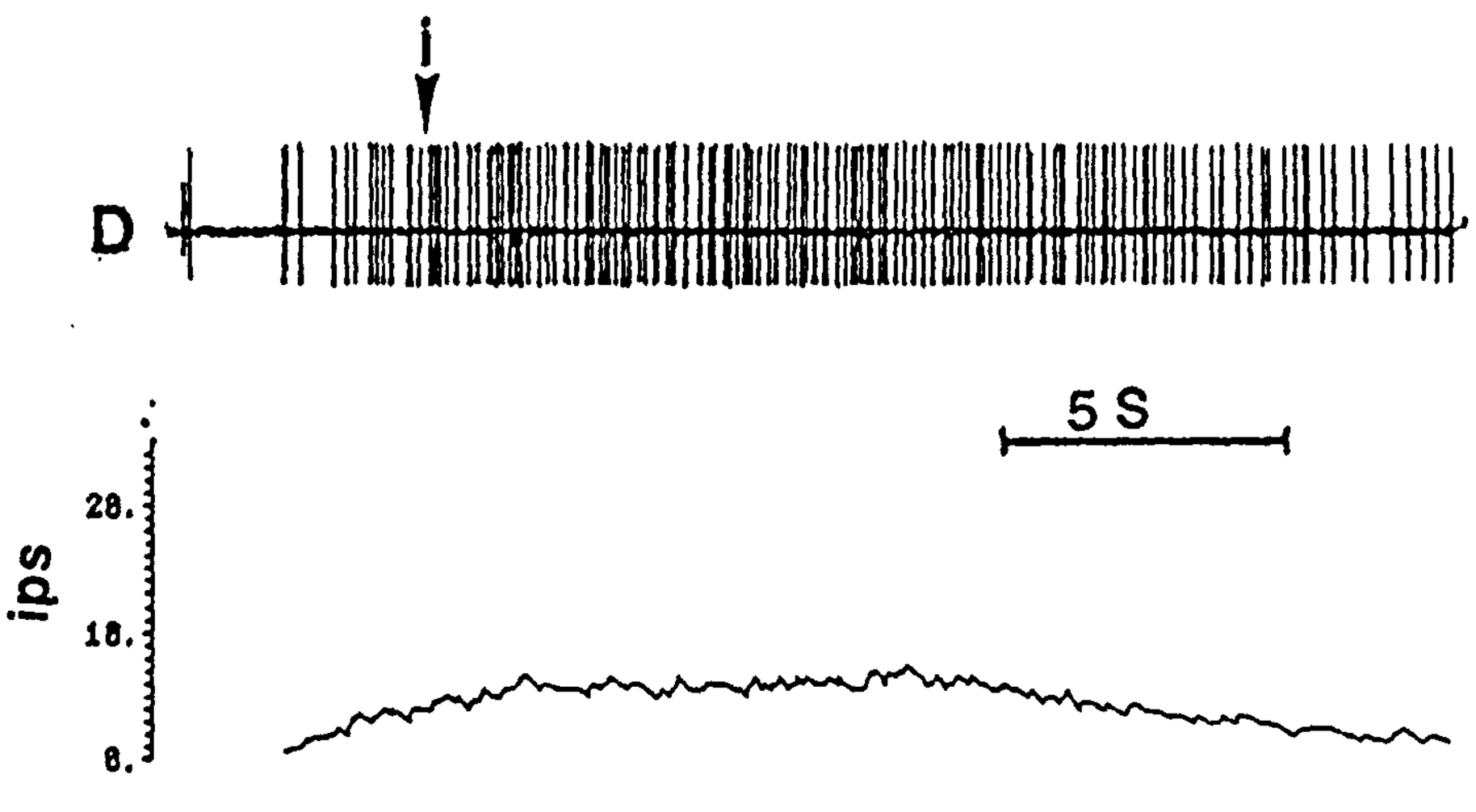

i
5 S
ips
20.
10.
0.

Figure 4.14 The typical pattern of responses shown by SR3 to water borne vibrations of different frequencies. The plots show the raw nerve spike data (top trace), the mean frequency of firing of the FI unit (i) (middle trace) and the stimulus trace (bottom trace). The scale bar indicates time in seconds.

A. 30Hz

B. 40Hz

C. 60Hz

D. 80Hz

E. 100Hz

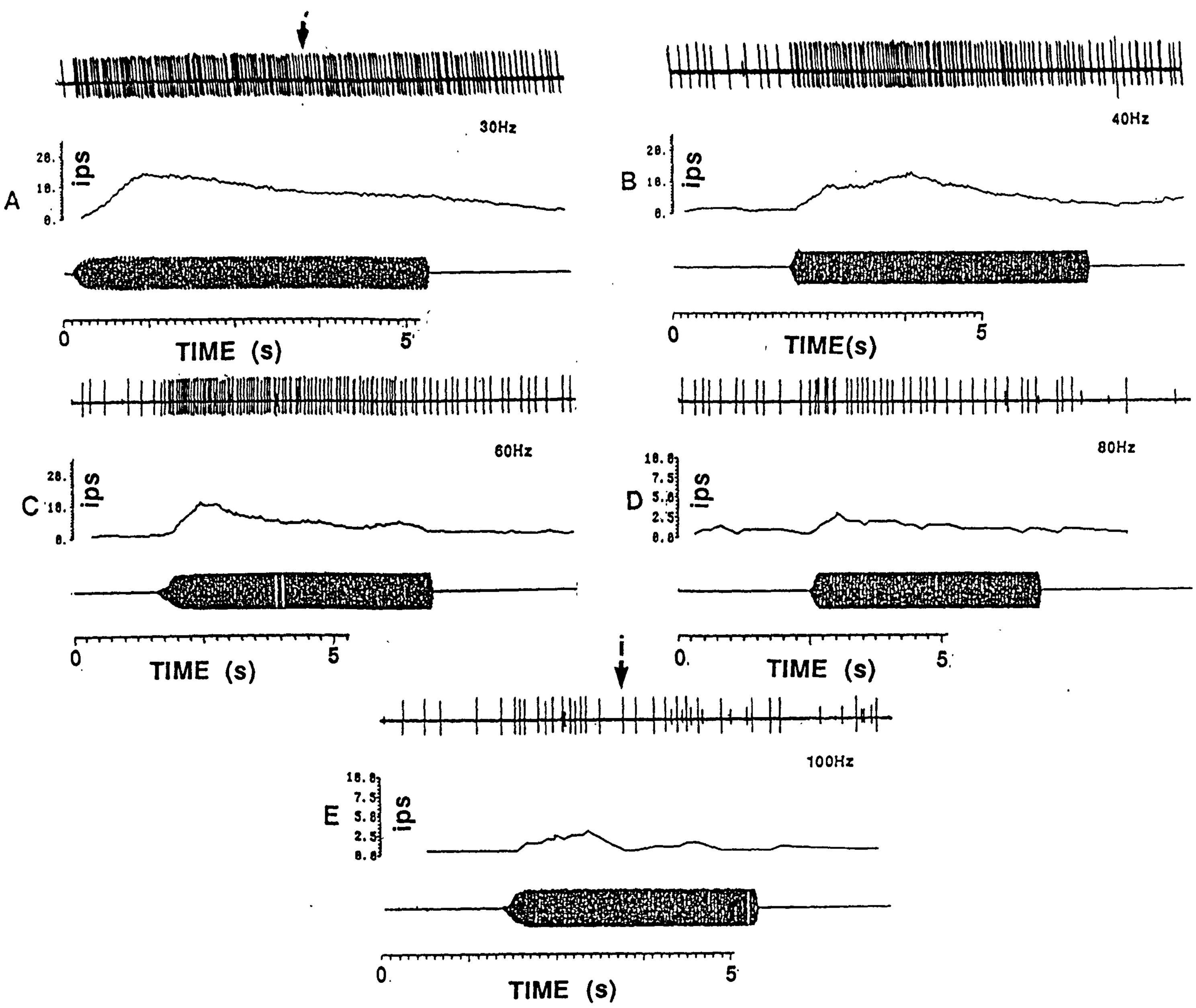

30Hz
A
20.
10.
0.
ips
0
TIME (s)
5
40Hz
B
20.
10.
0.
ips
0
TIME(s)
5
60Hz
C
20.
10.
0.
ips
80Hz
D
10.0
7.5
5.0
2.5
0.0
ips
i
0.
TIME (s)
5
100Hz
E
10.0
7.5
5.0
2.5
0.0
ips
0.
TIME (s)
5

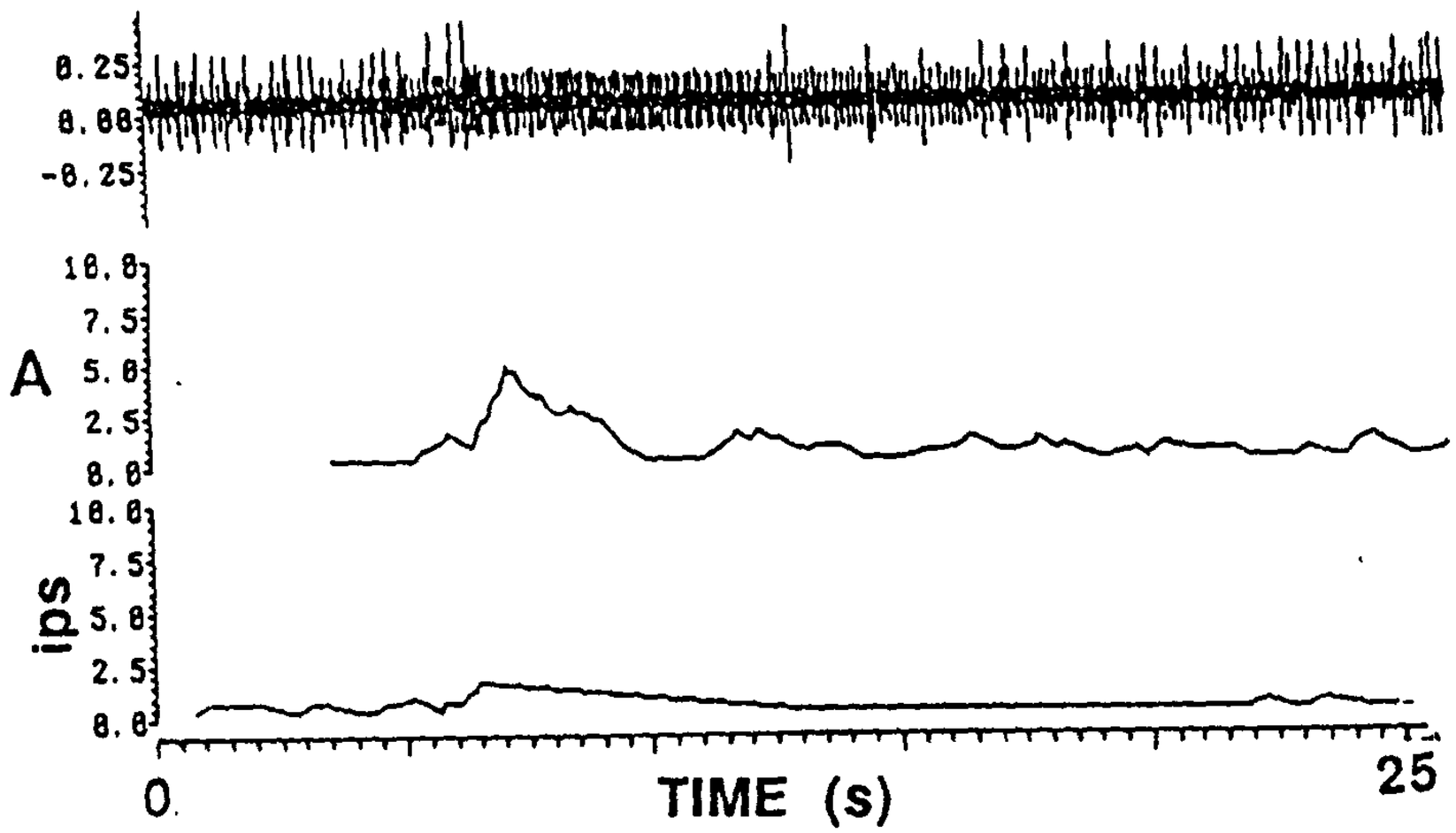
Figure 4.15 An example of the atypical responses occasionally shown by SR3 to both tactile stimulation of the walking legs and water borne vibrations of different frequencies. The scale bar indicates time in seconds.

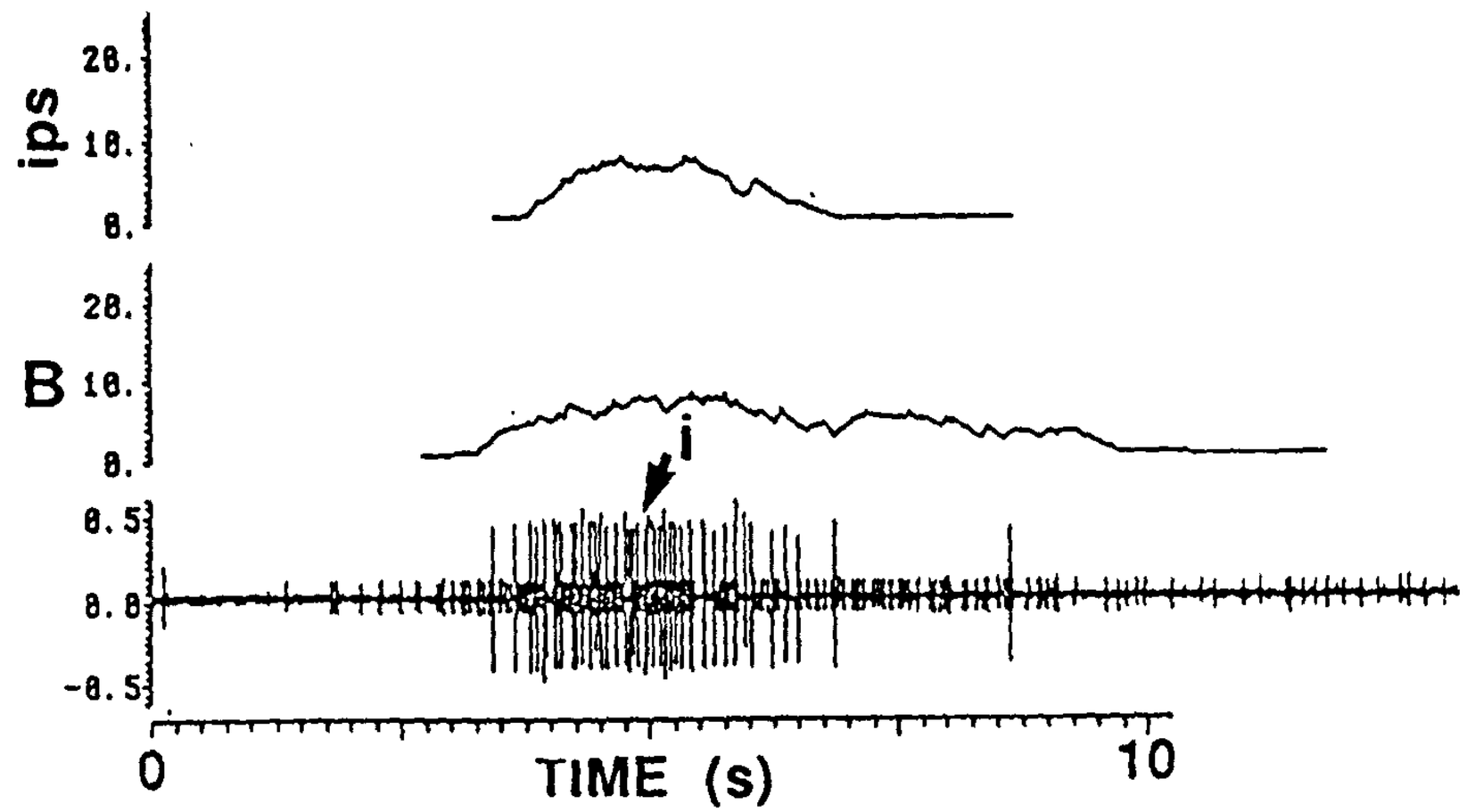
A. Responses shown by SR3 to tactile stimulation of the legs. The plot shows the raw nerve spike data (top), the mean frequency of firing in the small FE unit (middle) and the mean frequency of firing of a larger FE unit (bottom).

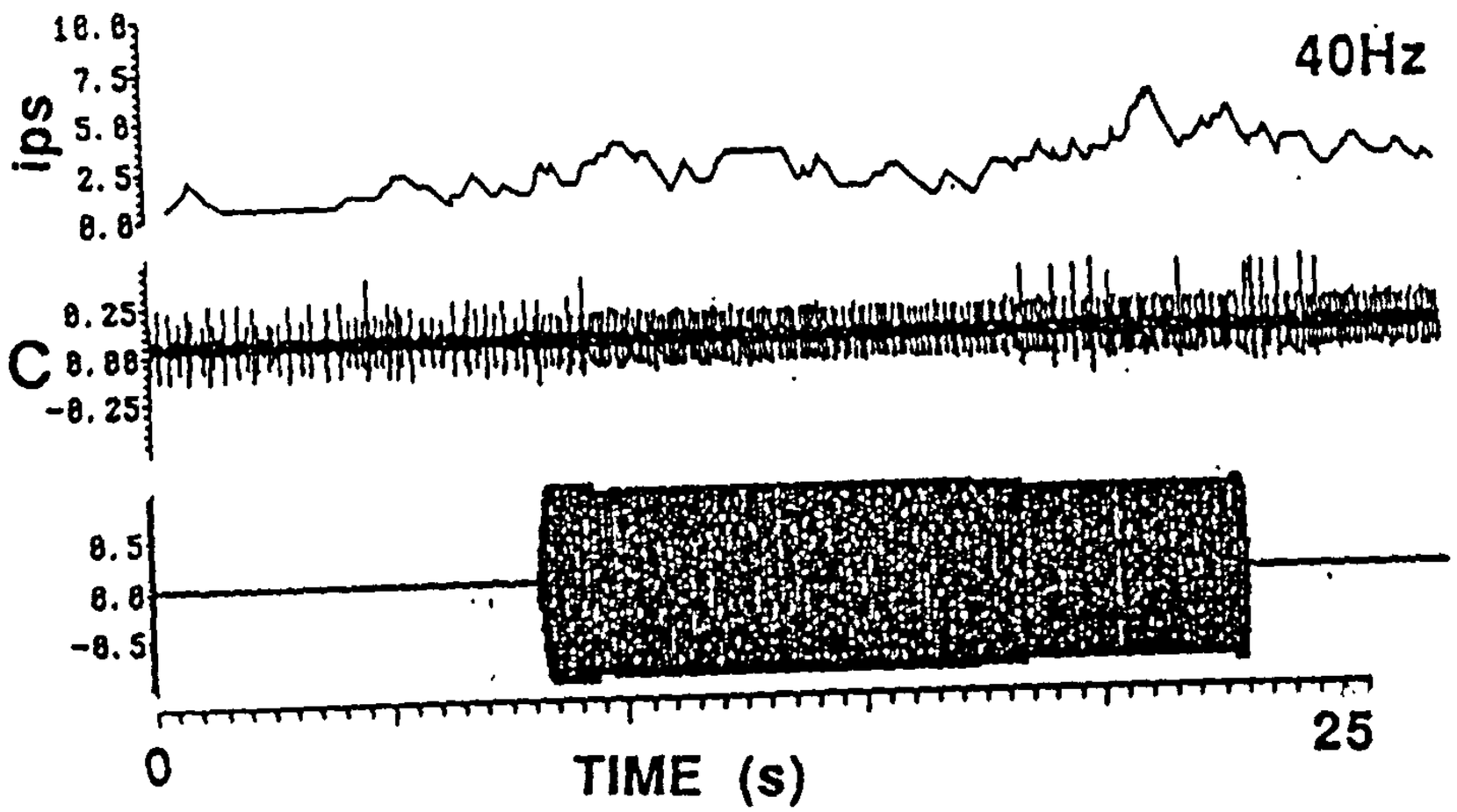
B. Responses shown by SR3 to forced flexion of the abdomen. The plot shows the mean frequency of firing in the small FE unit (top), the mean frequency of firing in the FI unit (i) (middle) and the raw nerve spike data (bottom).

C. Responses shown by SR3 to a water borne vibrational stimulus of 40Hz. The plot shows the mean frequency of firing in the small FE unit (top), the raw nerve spike data (middle) and the stimulus trace (bottom).

D. Responses shown by SR3 to water borne vibrations of 60Hz. The plot shows the mean frequency of firing in the small FE unit (top), the raw nerve spike data (middle) and the stimulus trace (bottom).


A
0.25
0.00
-0.25
10.0
7.5
5.0
2.5
0.0
ips
0
TIME (s)
25


ips
20.
10.
0.
B
i
0.5
0.0
-0.5
0
TIME (s)
10


40Hz
ips
10.0
7.5
5.0
2.5
0.0
C
0.25
0.00
-0.25
0.5
0.0
-0.5
0
TIME (s)
25

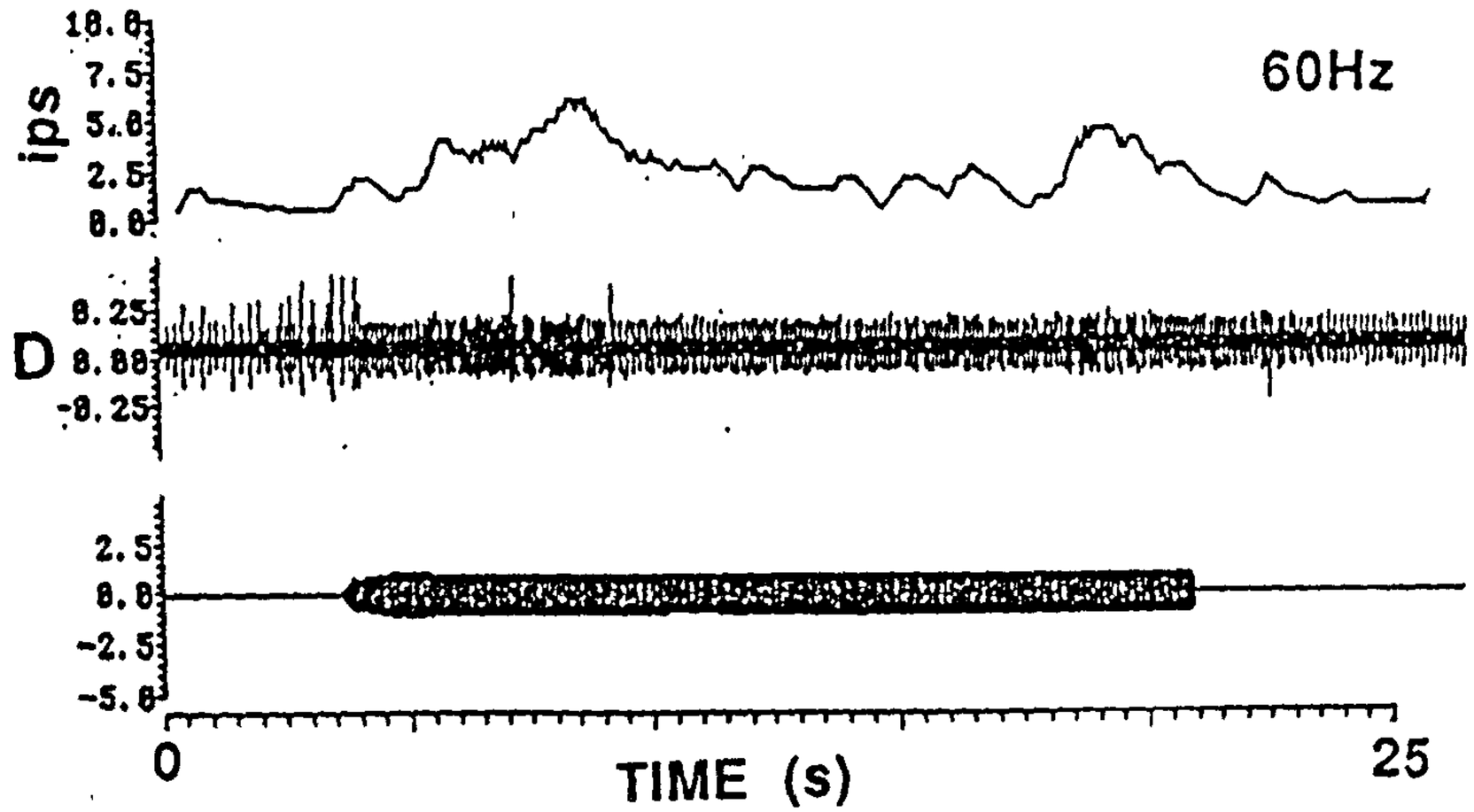

60Hz
ips
10.0
7.5
5.0
2.5
0.0
D
0.25
0.00
-0.25
2.5
0.0
-2.5
-5.0
0
TIME (s)
25

Figure 4.16 Responses shown by SR3 to differential stimulation of the anterior end of the animal with water borne vibrations of different frequencies. Recordings were made in the 2nd abdominal segment. The plots show the mean frequency of firing of the FI unit (i) (top), the raw nerve spike data (middle) and the stimulus trace (bottom). The scale bar indicates time in seconds.

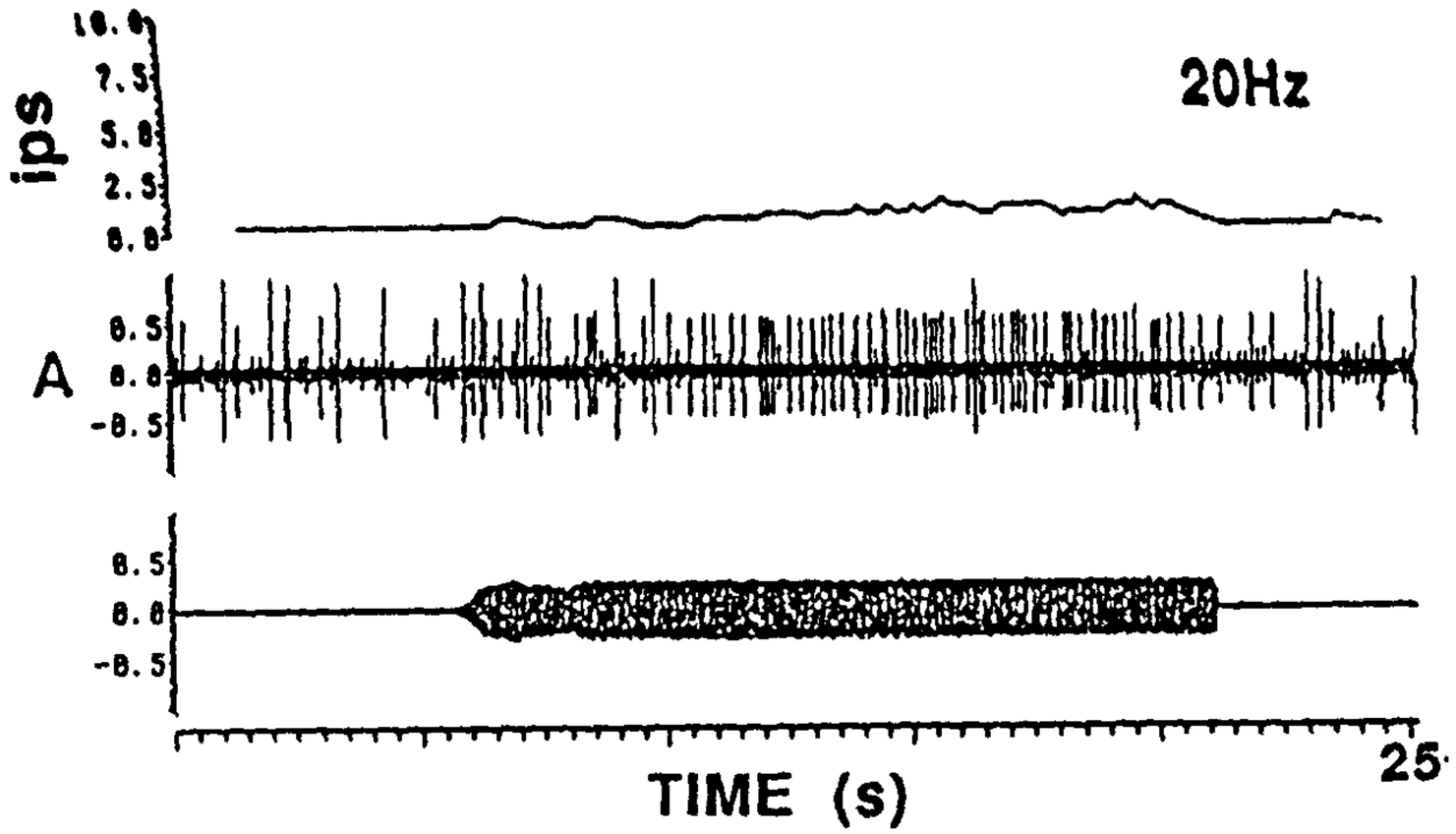
A. 20Hz

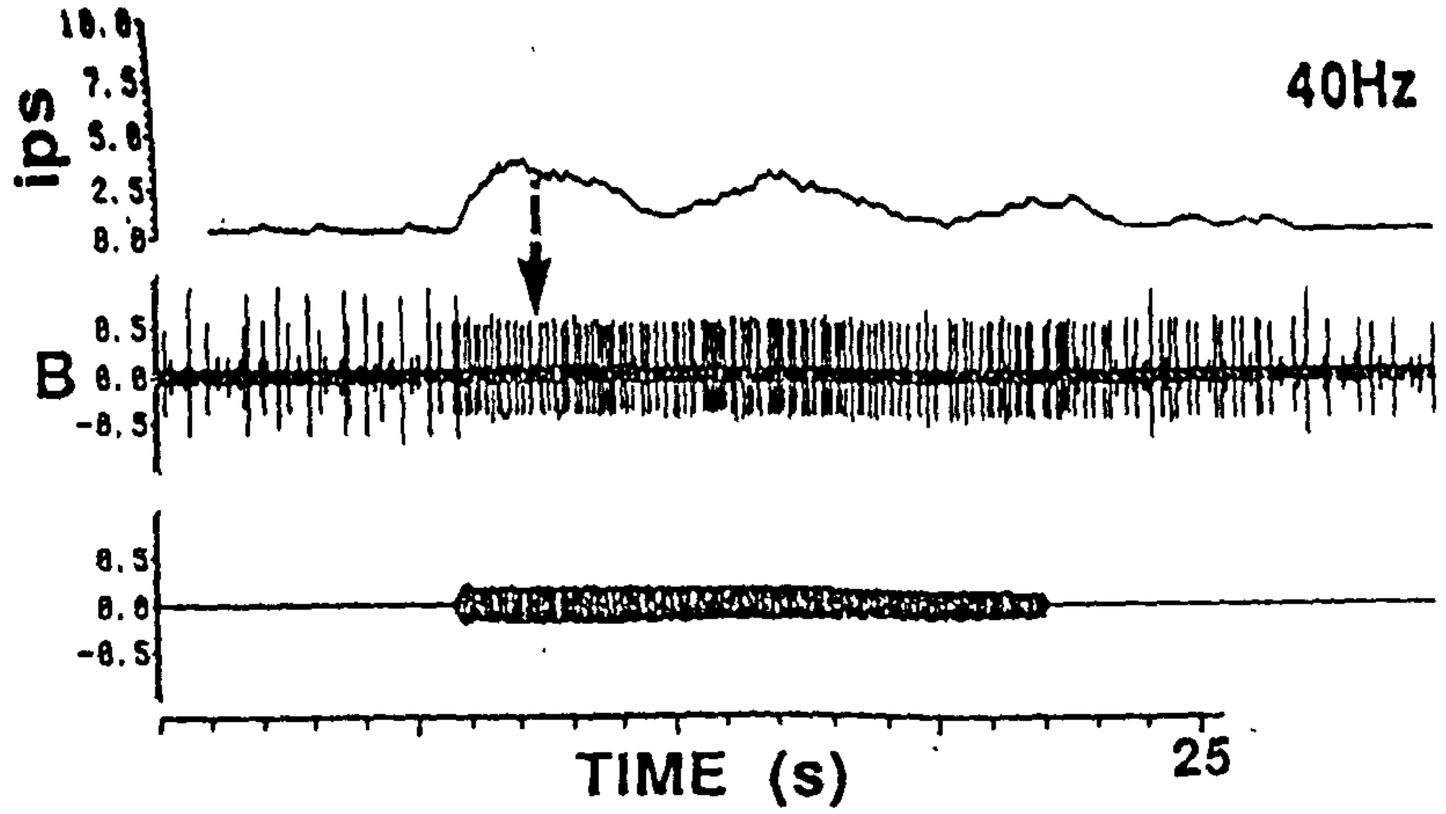
B. 40Hz

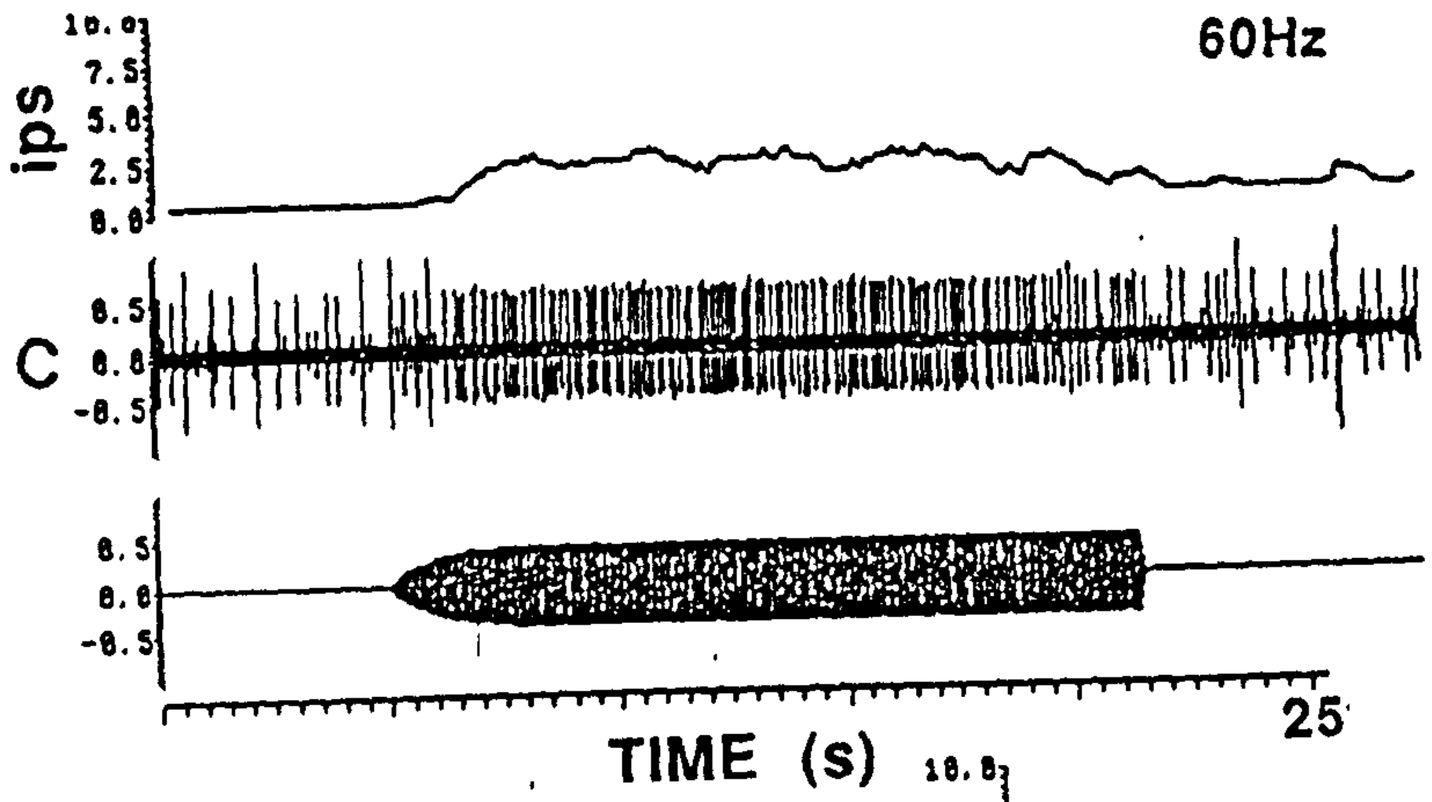
C. 60Hz

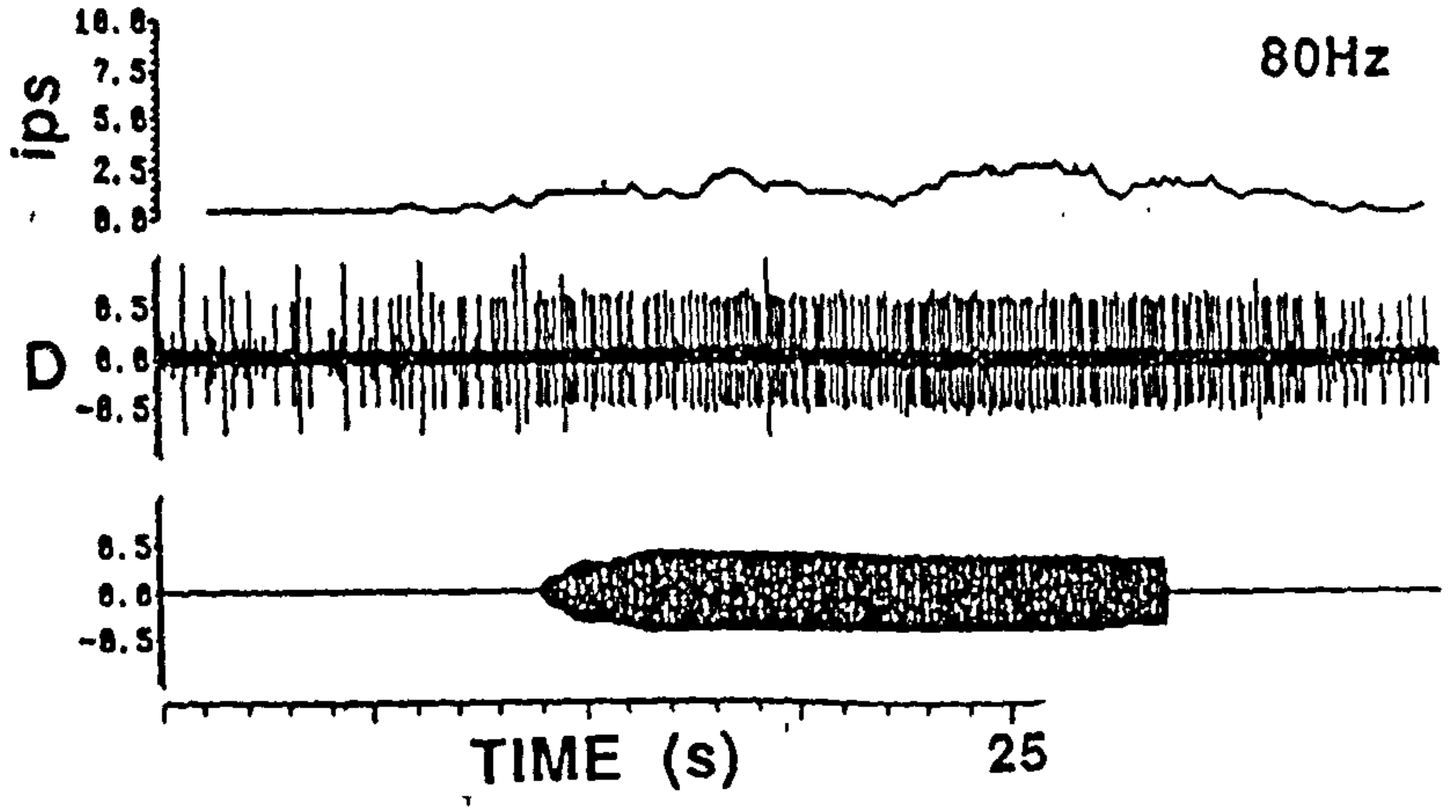
D. 80Hz

E. 100Hz


20Hz
ips
10.0
7.5
5.0
2.5
0.0
A
0.5
0.0
-0.5
TIME (s)
25


40Hz
ips
B
TIME (s)
25


60Hz
ips
C
TIME (s)
25


80Hz
ips
D
TIME (s)
25

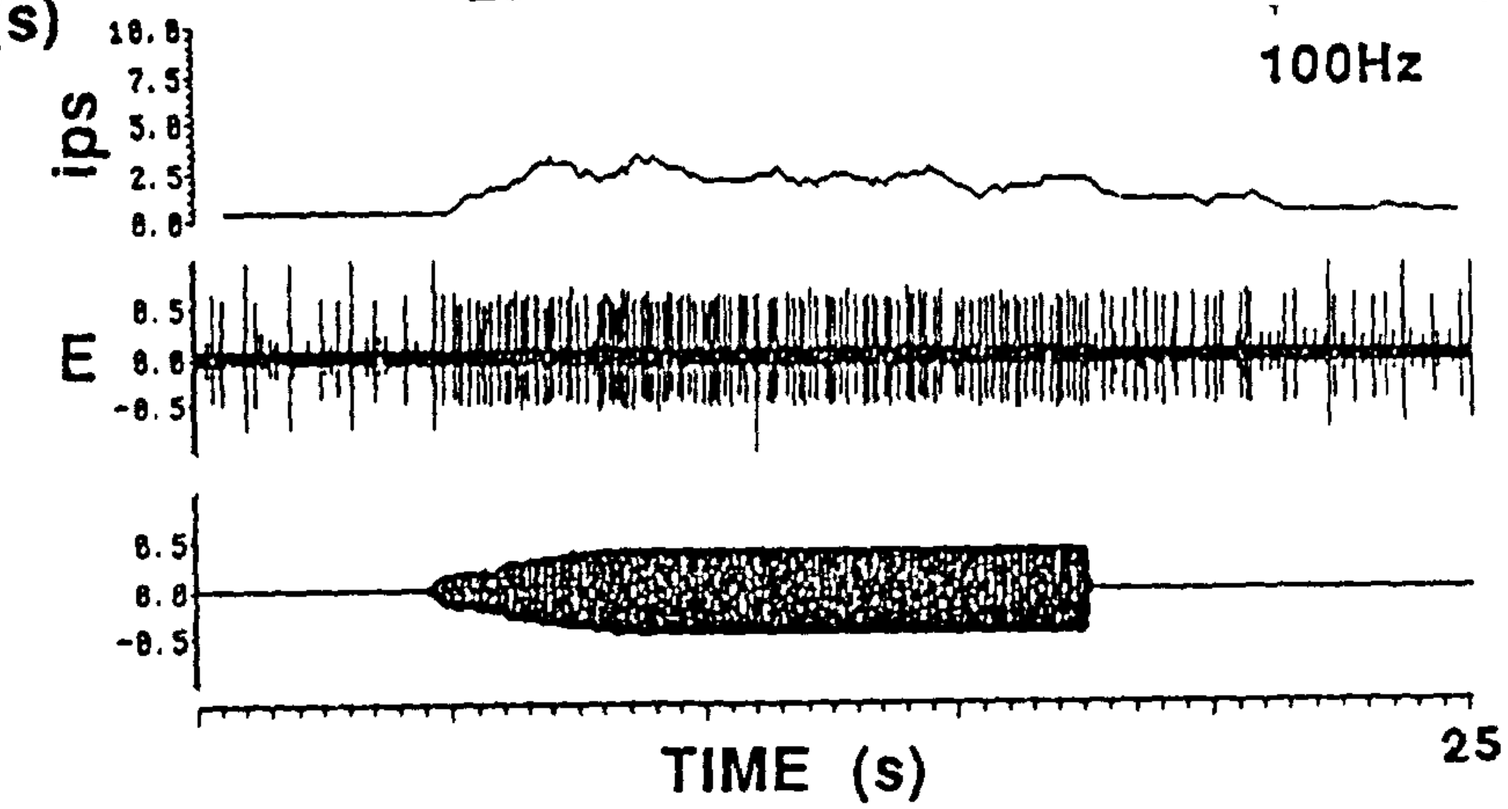

100Hz
ips
E
TIME (s)
25

Figure 4.17 The response shown by units in SR3 to differential stimulation of the posterior end of the animal with water borne vibrations of different frequencies. Recordings were made in the 2nd abdominal segment. Plots show the mean frequency of firing of the FI unit (i) (top), the raw nerve spike data (middle) and the stimulus trace (bottom). The scale bar indicates time in seconds.

A. 40Hz

B. 60Hz

C. 80Hz

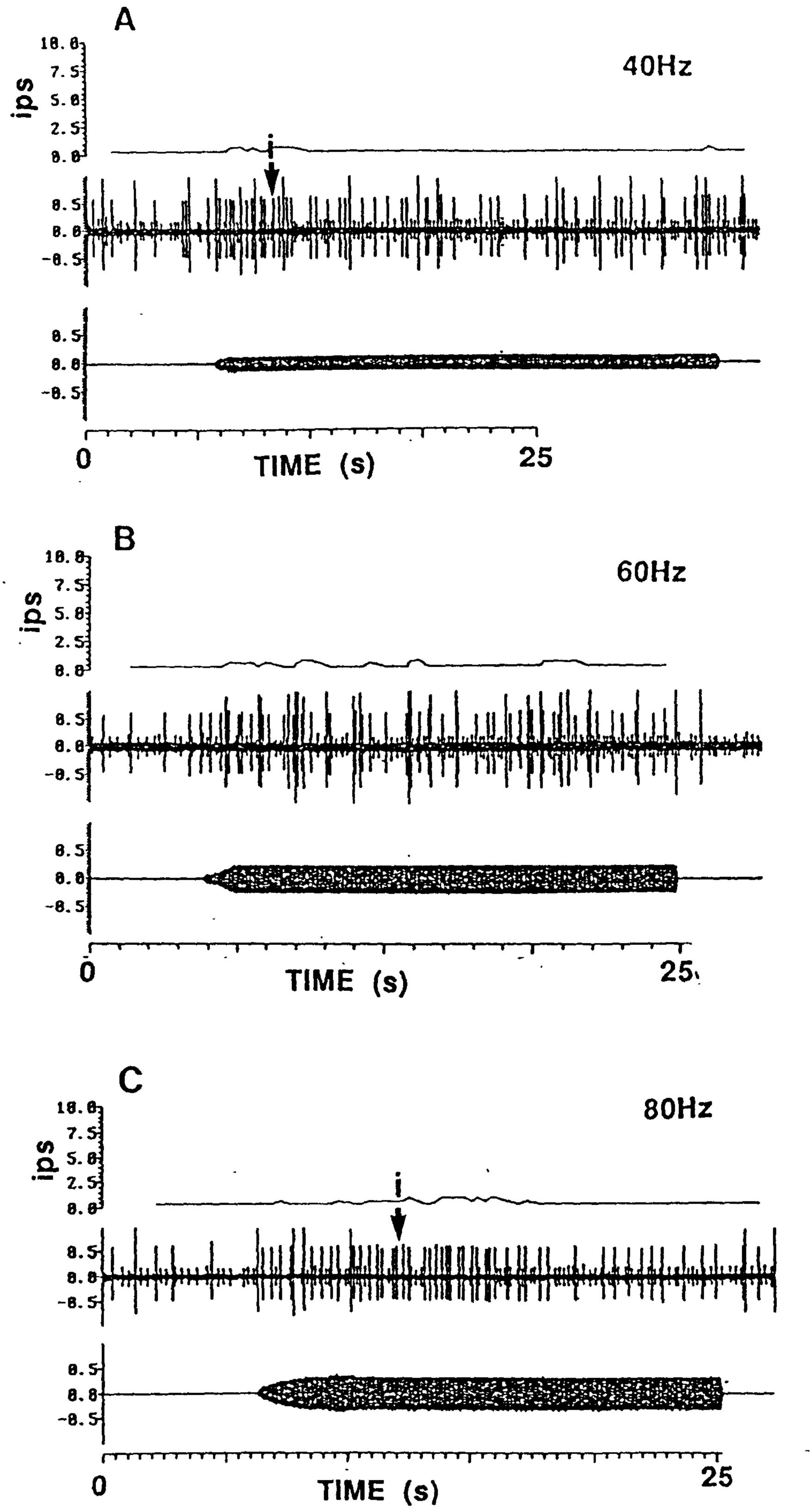

A
40Hz
ips
10.0
7.5
5.0
2.5
0.0
i
0.5
0.0
-0.5
0.5
0.0
-0.5
0
TIME (s)
25
B
60Hz
ips
10.0
7.5
5.0
2.5
0.0
0.5
0.0
-0.5
0.5
0.0
-0.5
0
TIME (s)
25
C
80Hz
ips
10.0
7.5
5.0
2.5
0.0
i
0.5
0.0
-0.5
0.5
0.0
-0.5
0
TIME (s)
25

Figure 4.18 Responses shown by units in SR3 recorded in segment 2 to tactile stimulation and water vibrations after a bilateral transection of the abdominal nerve cord in segment 4. The plots show the raw nerve spike data (top), the mean frequency of firing in the FI unit (i) (middle) in ips. and in B the stimulus trace (bottom). The scale bar shows time in seconds.

A. Responses to tactile stimulation of the walking legs.

B. 60Hz

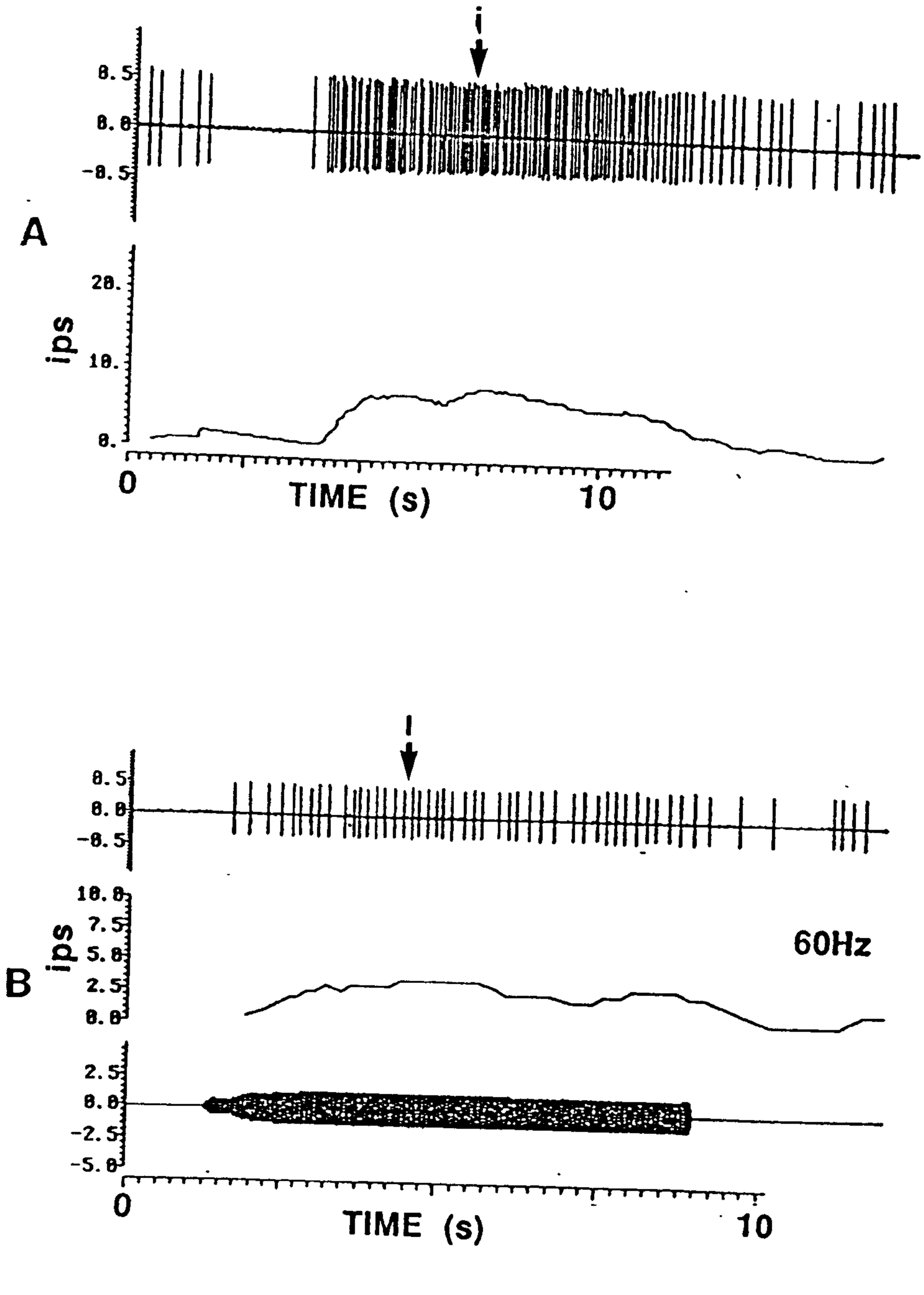

i
8.5
0.0
-0.5
A
ips
20.
10.
0.
0
TIME (s)
10
i
8.5
0.0
-0.5
B
ips
10.0
7.5
5.0
2.5
0.0
60Hz
2.5
0.0
-2.5
-5.0
0
TIME (s)
10

Figure 4.19 Responses of units in SR3 recorded in segment 4 to tactile stimulation and water borne vibrations before and after a bilateral transection of the abdominal nerve cord in segment 1. The plots show the mean frequency of firing (ips.) of the FI (i) unit (top), the raw nerve spike data (middle) and in 1.b and 2.b, the stimulus trace (bottom). The scale bar indicates time in seconds.

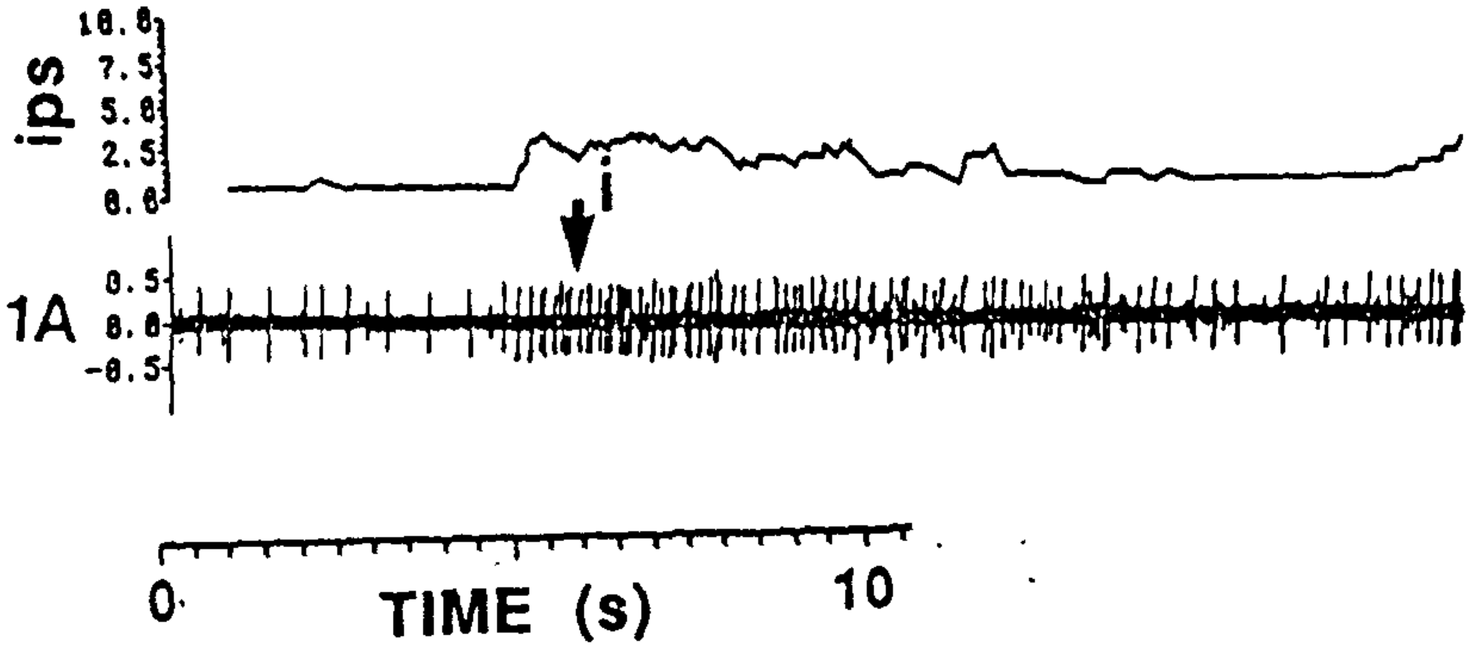
1.A Response to tactile stimuation of the legs before the transection.

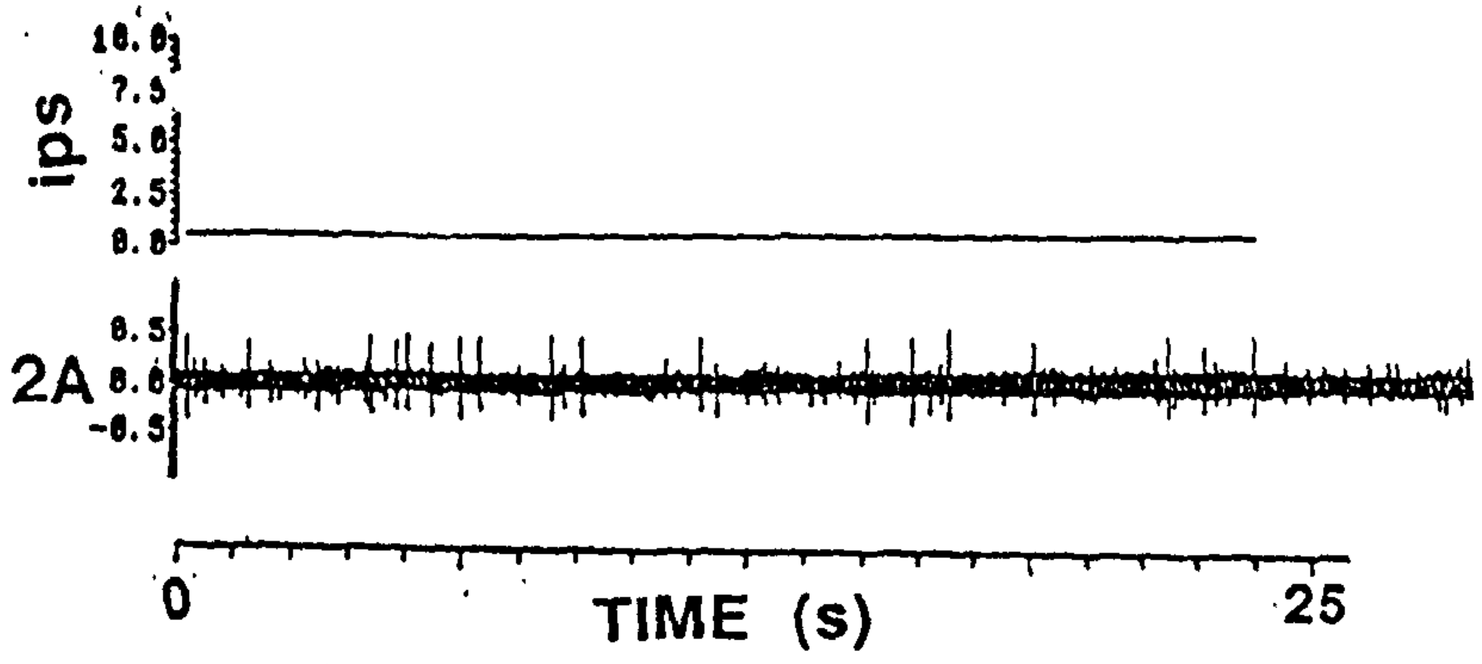
1.B Response to 60Hz stimulus before the transection

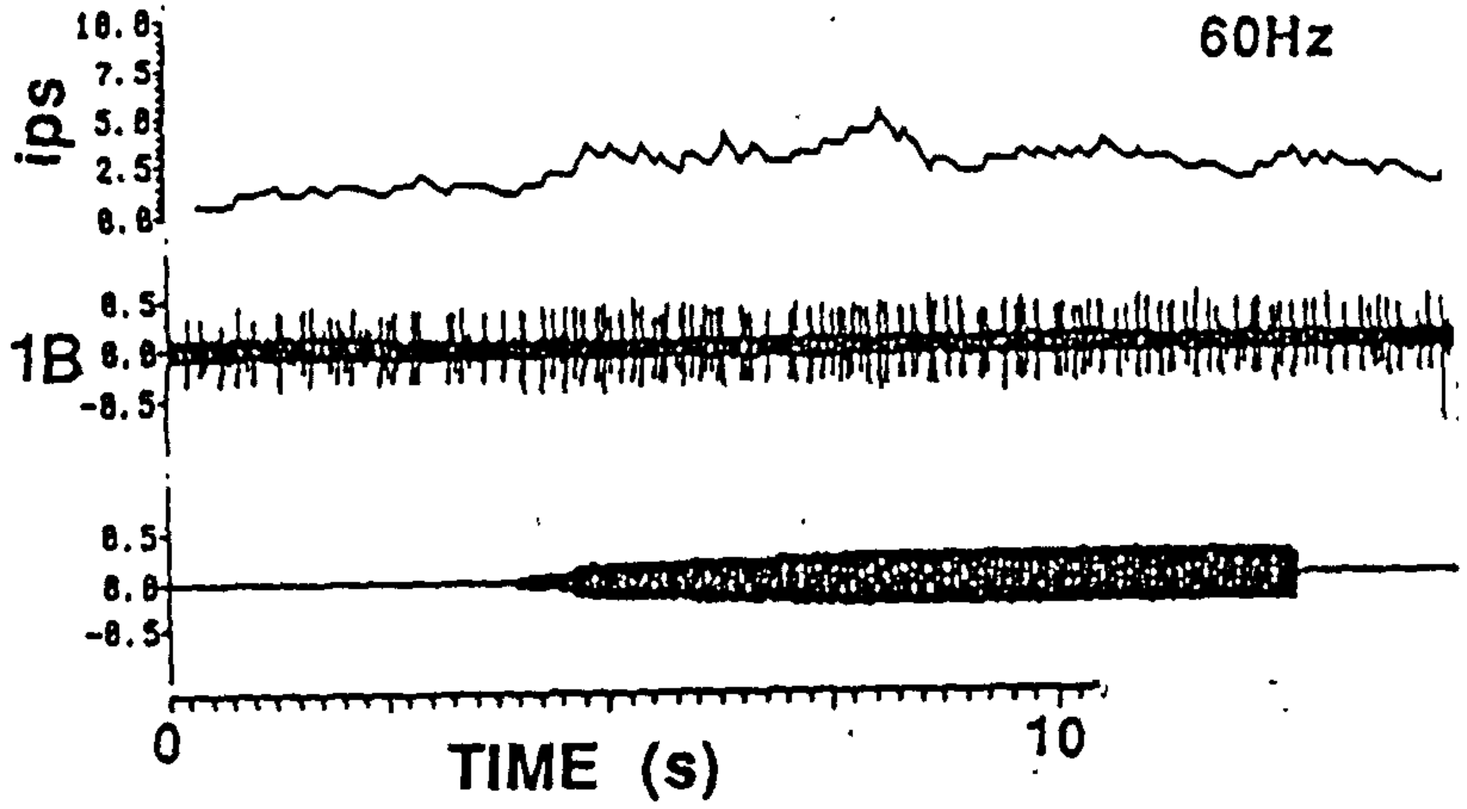
2.A Response to tactile stimulation of the walking legs after the transection.

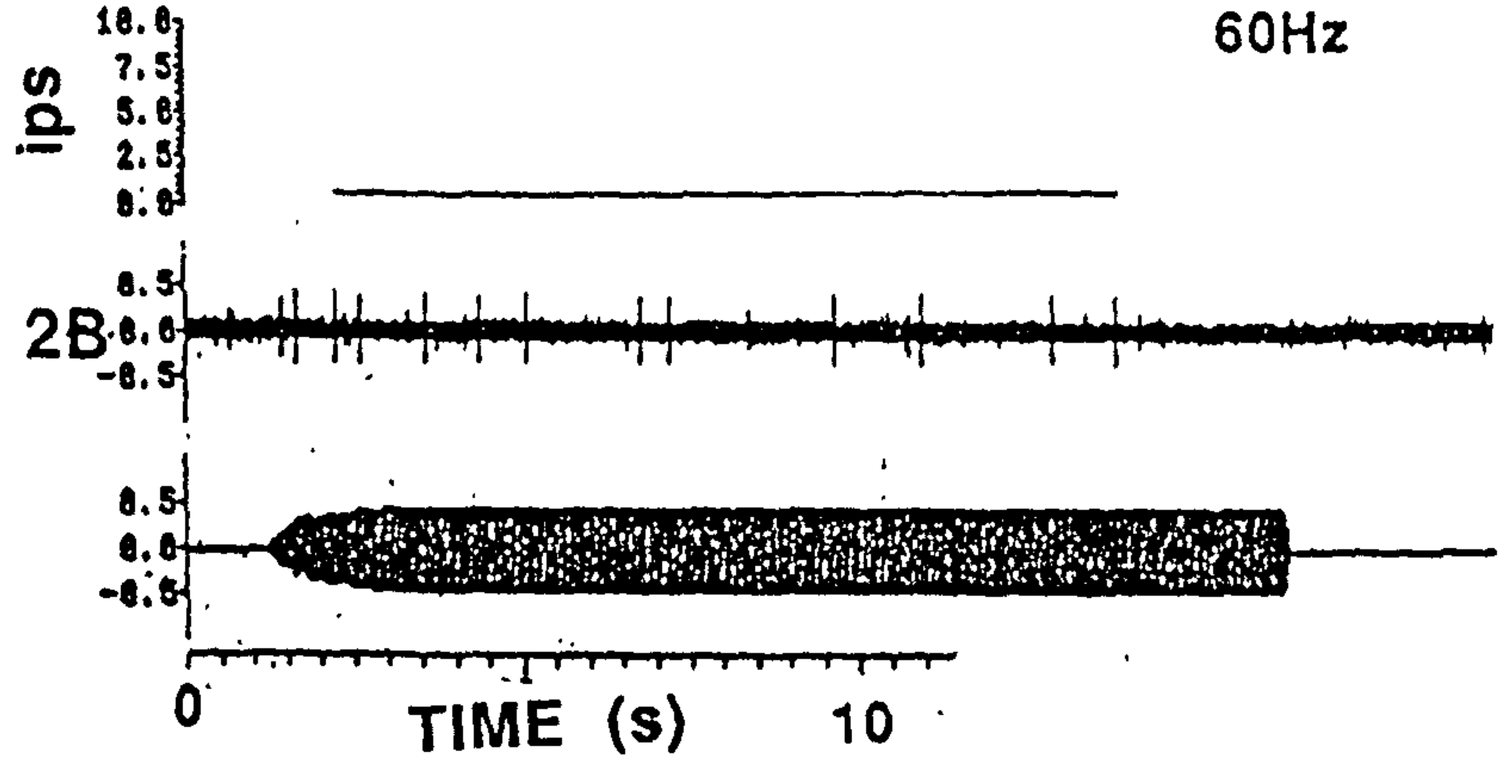
2.B Response to 60Hz stimulus after the transection.

2.C Response to forced flexion of the abdomen after the transection.


ips
18.0
7.5
5.0
2.5
0.0
1A
0.5
0.0
-0.5
0
TIME (s)
10


ips
18.0
7.5
5.0
2.5
0.0
2A
0.5
0.0
-0.5
0
TIME (s)
25


60Hz
ips
18.0
7.5
5.0
2.5
0.0
1B
0.5
0.0
-0.5
0.5
0.0
-0.5
0
TIME (s)
10


60Hz
ips
18.0
7.5
5.0
2.5
0.0
2B
0.5
0.0
-0.5
0.5
0.0
-0.5
0
TIME (s)
10

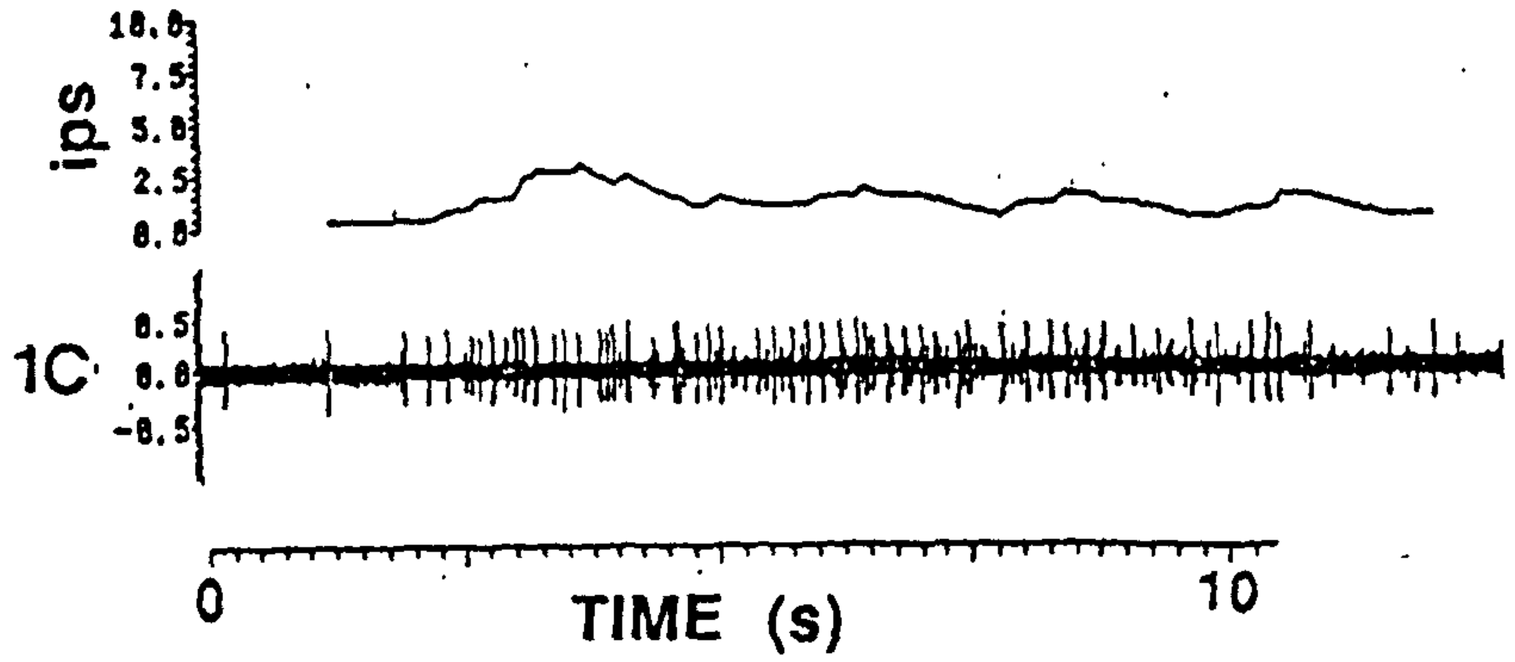
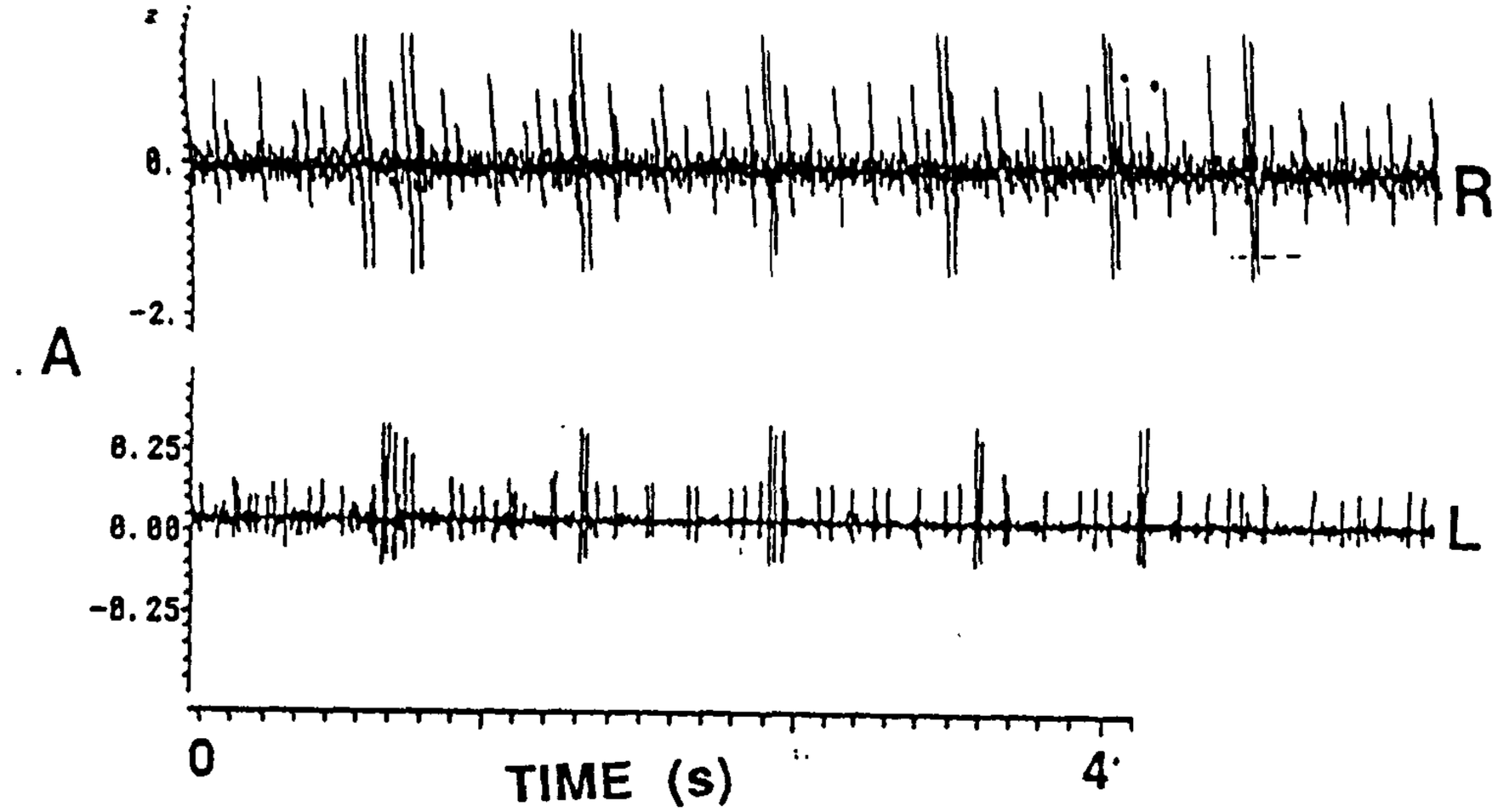

ips
18.0
7.5
5.0
2.5
0.0
1C
0.5
0.0
-0.5
0
TIME (s)
10

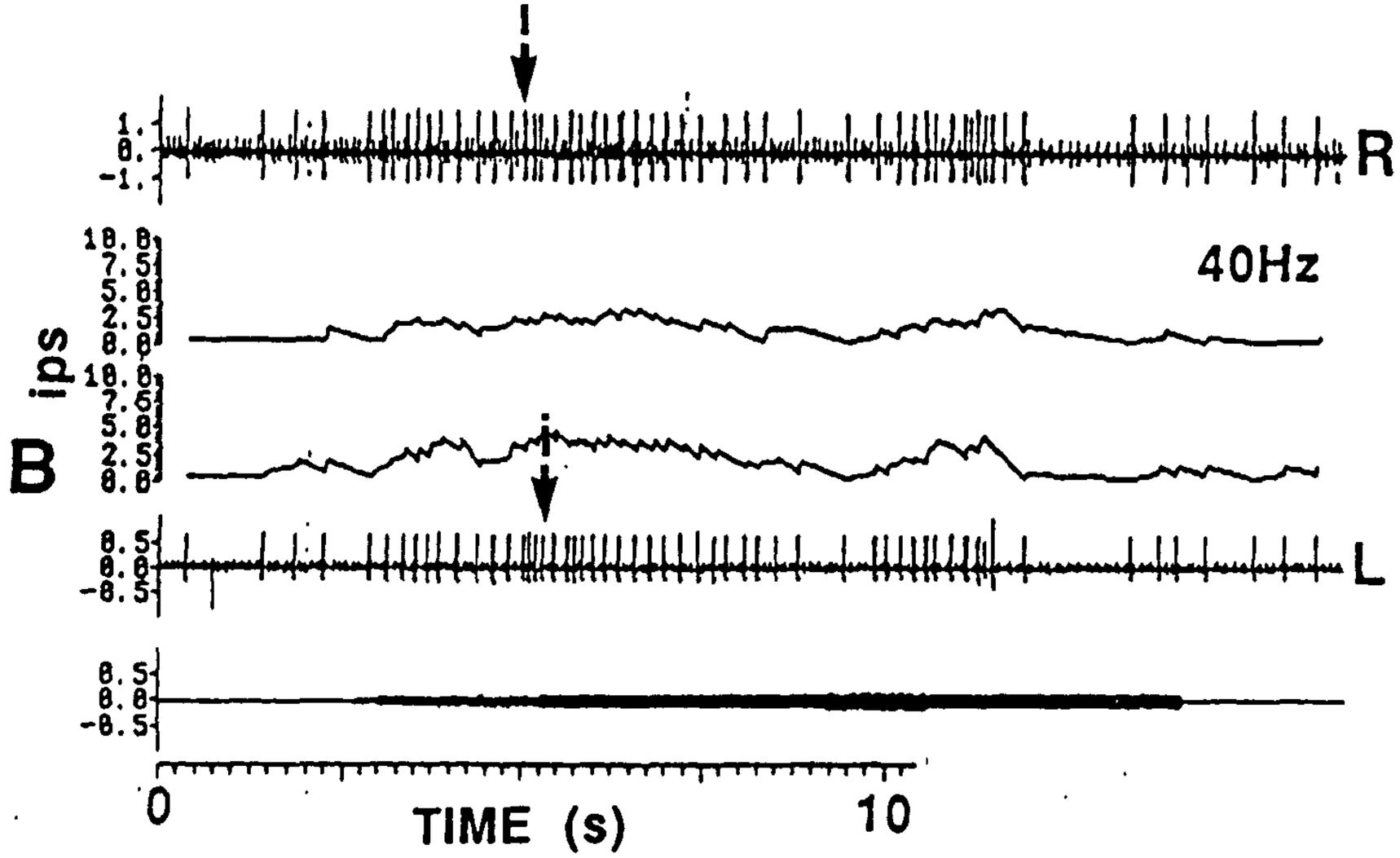
Figure 4.20 Activity of units in left and right SR3 recorded in segment 2 and responses of these nerve roots to water borne vibrations. Scale bar shows time in seconds.

A. Resting activity in the two nerve roots, left (top) and right (bottom). Scale bar shows time in seconds.

B. Response of units in bilateral SR3 to water borne vibrations of 40Hz. Plots show the raw nerve spike data of right SR3 (top), mean frequency of firing (ips.) of the right FI (i) unit (below), mean frequency of firing (ips.) of the left FI (i) unit (below), raw nerve spike data of left SR3 (below) and the stimulus trace (bottom). Scale bar indicates time in seconds.

C. As B 60Hz.


A
R
L
0
TIME (s)
4


B
ips
40Hz
R
L
0
TIME (s)
10

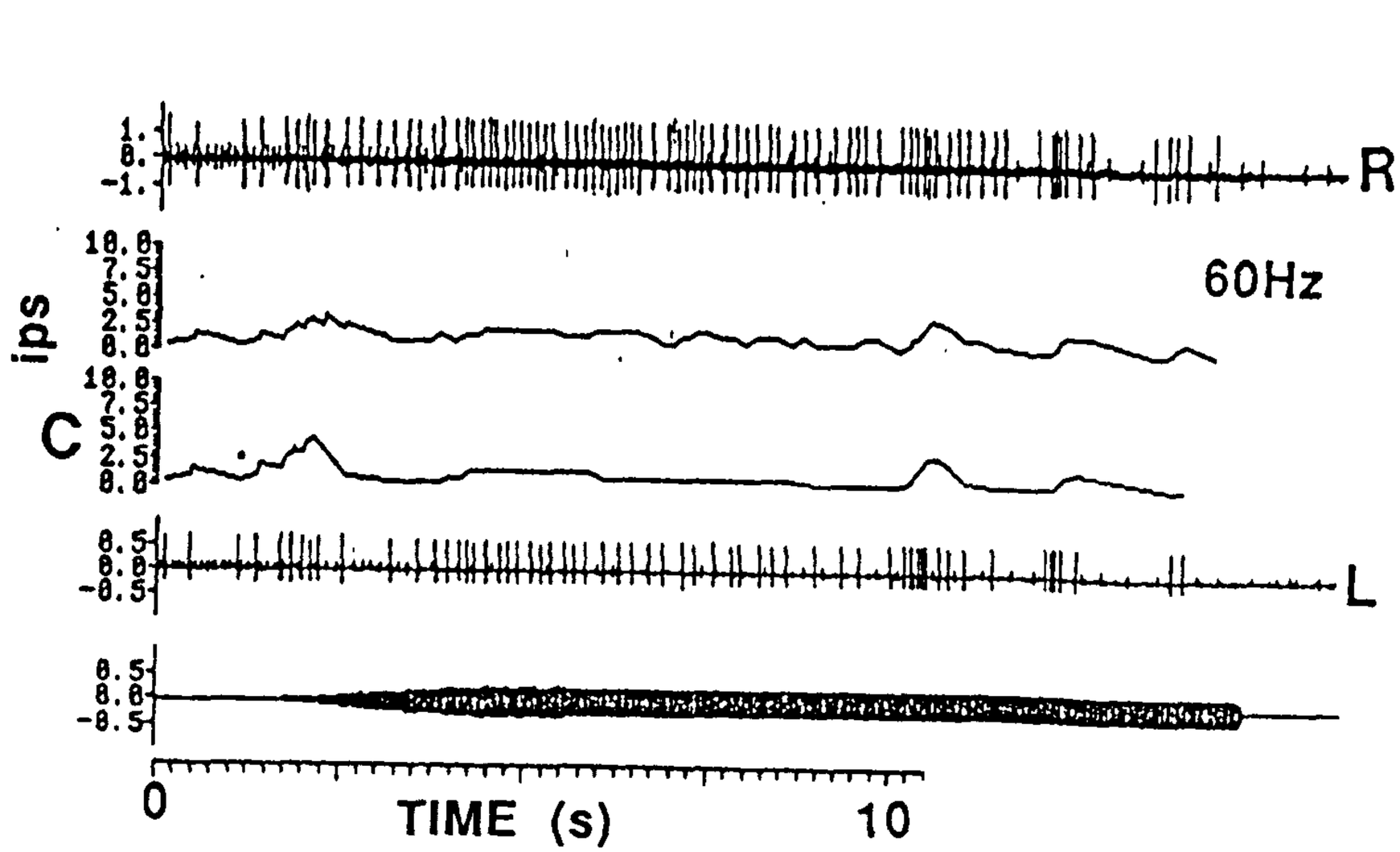
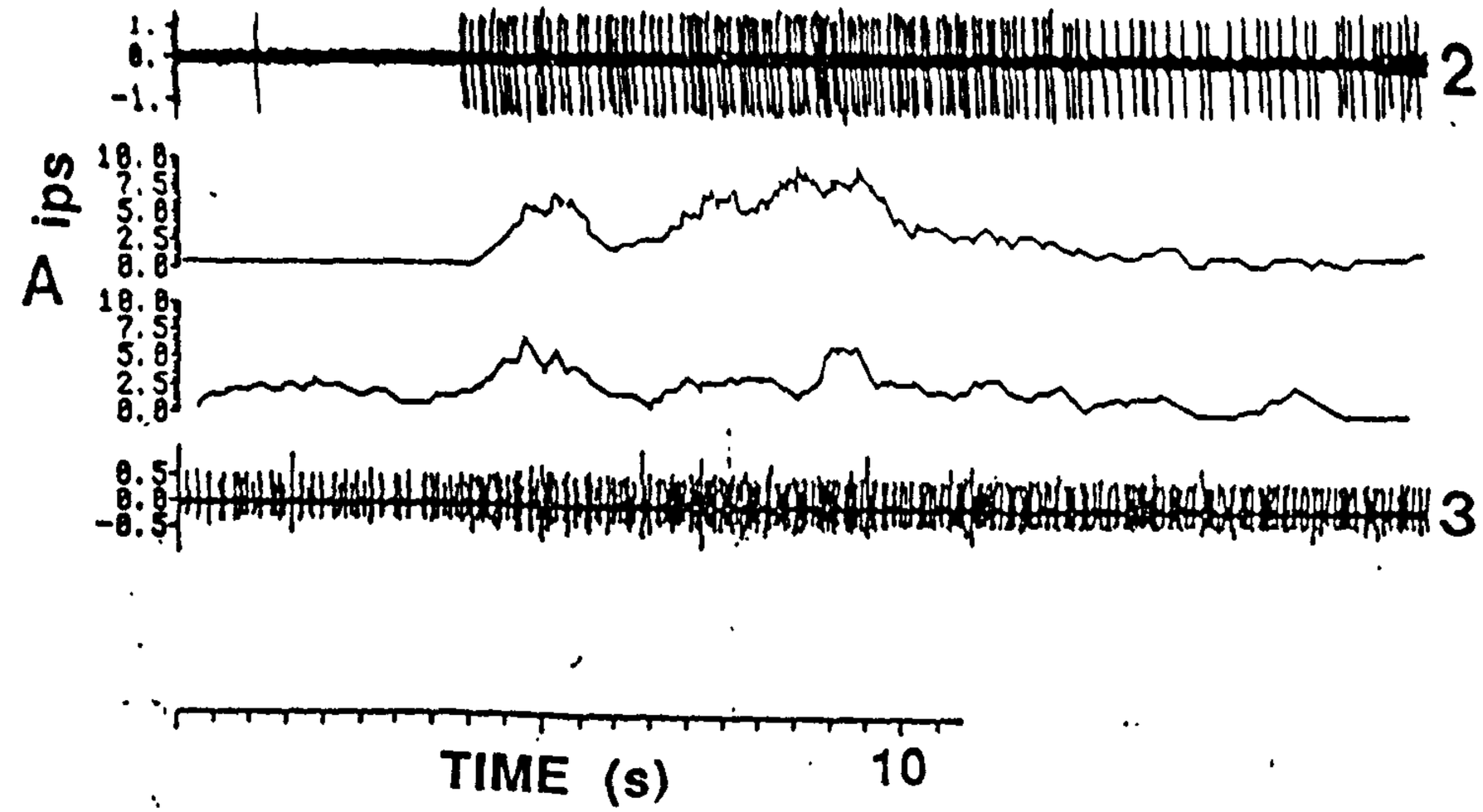

C
ips
60Hz
R
L
0
TIME (s)
10

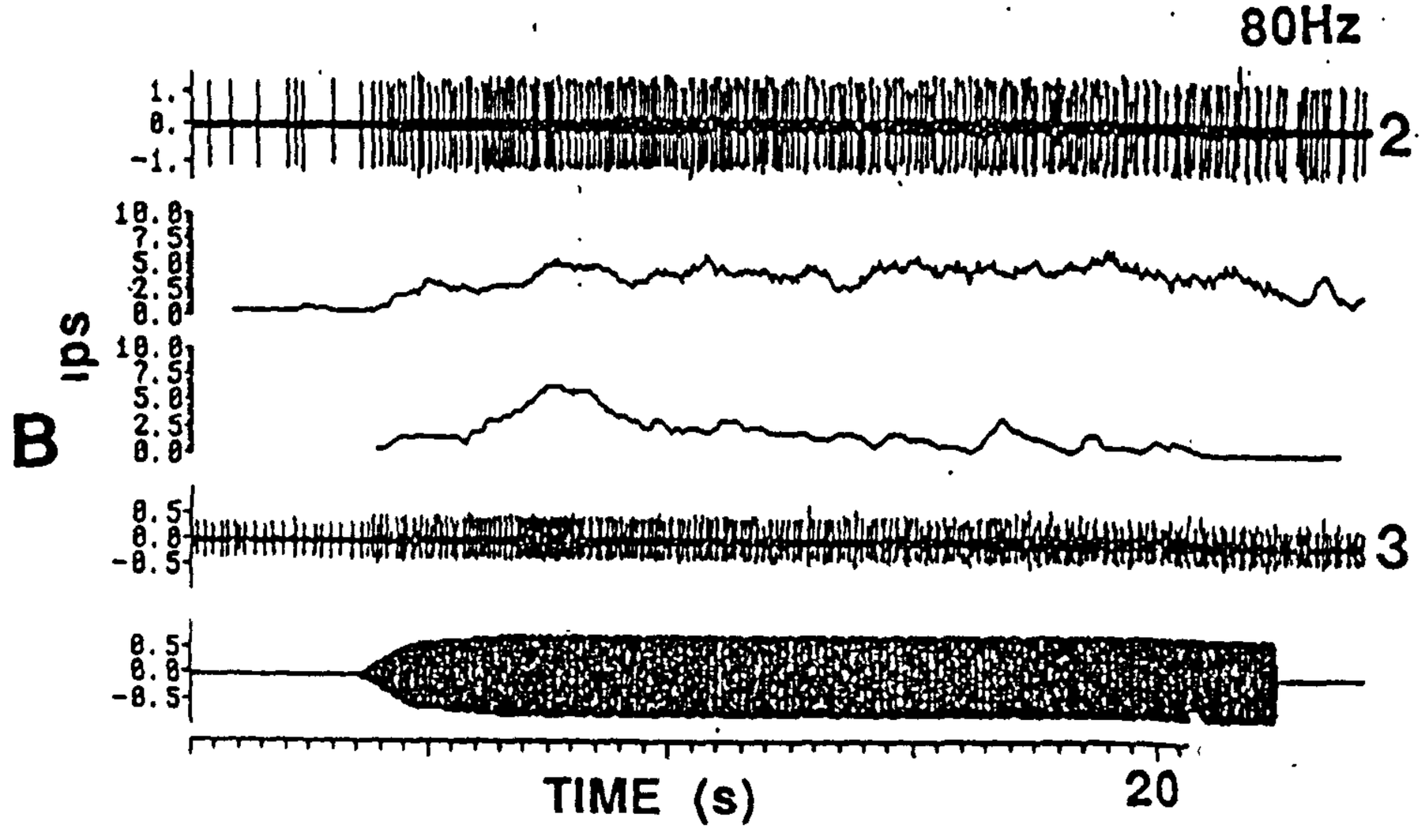
Figure 4.21 Responses of SR3 recorded in segments 2 and 3 to tactile stimulation and water borne vibrations of different frequencies. Plots show the raw nerve spike data in segment 2 (top), the mean frequency of firing (ips) of the FI (i) unit in segment 2 (below), the mean frequency of firing (ips) of the FI (i) unit in segment 3 (below), the raw nerve spike data in segment 3 (below) and in the case of B and C, the stimulus trace (bottom). The scale bar indicates time in seconds.

A. Tactile stimulation of the walking legs.

B. 80Hz

C. 60Hz


A
ips
2
3
TIME (s)
10


80Hz
B
ips
2
3
TIME (s)
20

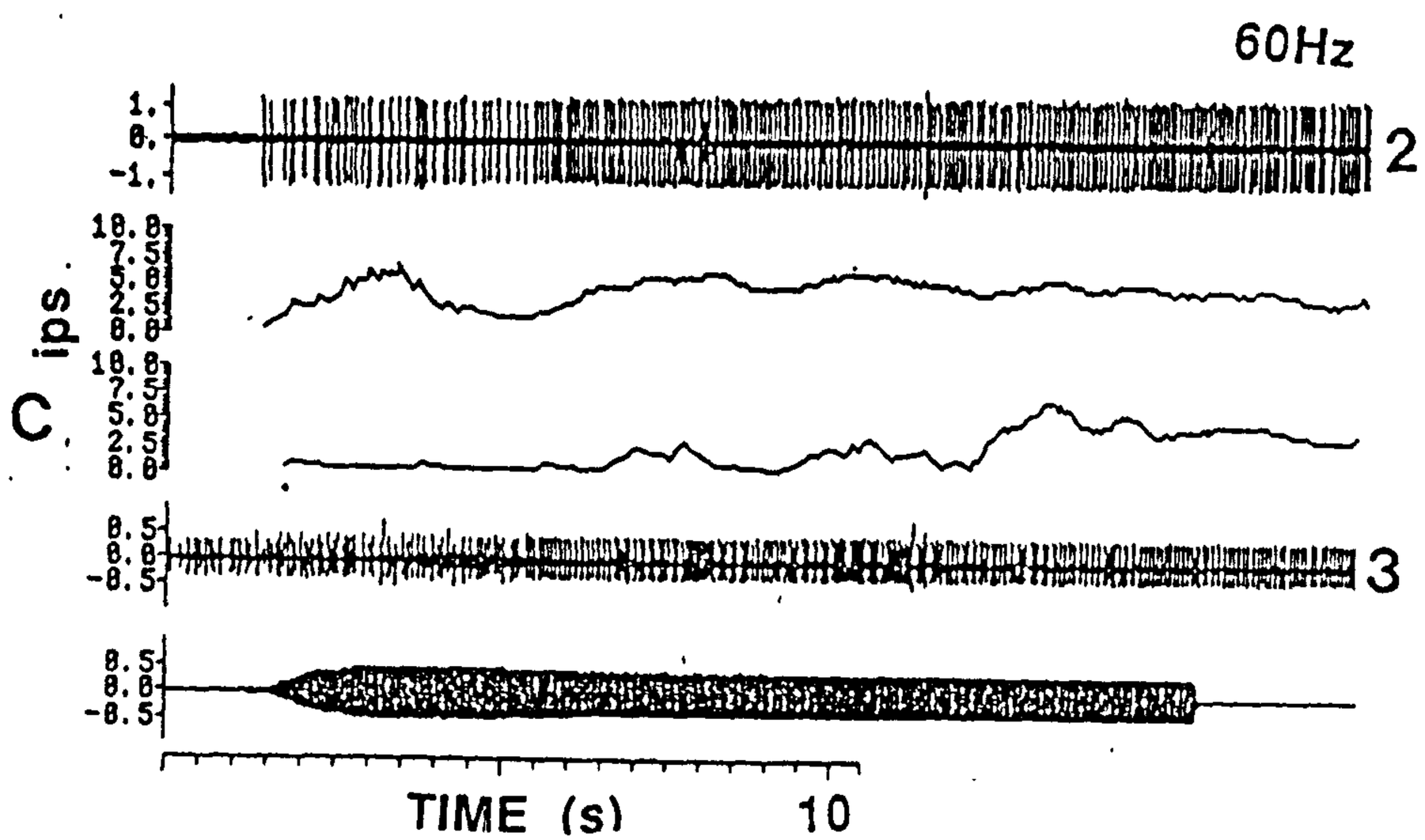

60Hz
C
ips
2
3
TIME (s)
10

Figure 4.22 Responses of units in SR3 and R2 to tactile stimulation and water borne vibrations of different frequencies. Plots show the raw nerve spike data from R2 (E) (top), the mean frequency of firing (ips) of EE units (e) (below), the mean frequency of firing (ips) of the FI unit (f) (below), the raw nerve spike data from SR3 (below) FI is indicated by an arrow and in the case of B,C,D and E the stimulus trace (bottom). The scale bar indicates time in seconds.

A. Forced flexion of the abdomen

B. 60Hz

C. 80Hz

D. 100Hz

E. 120Hz

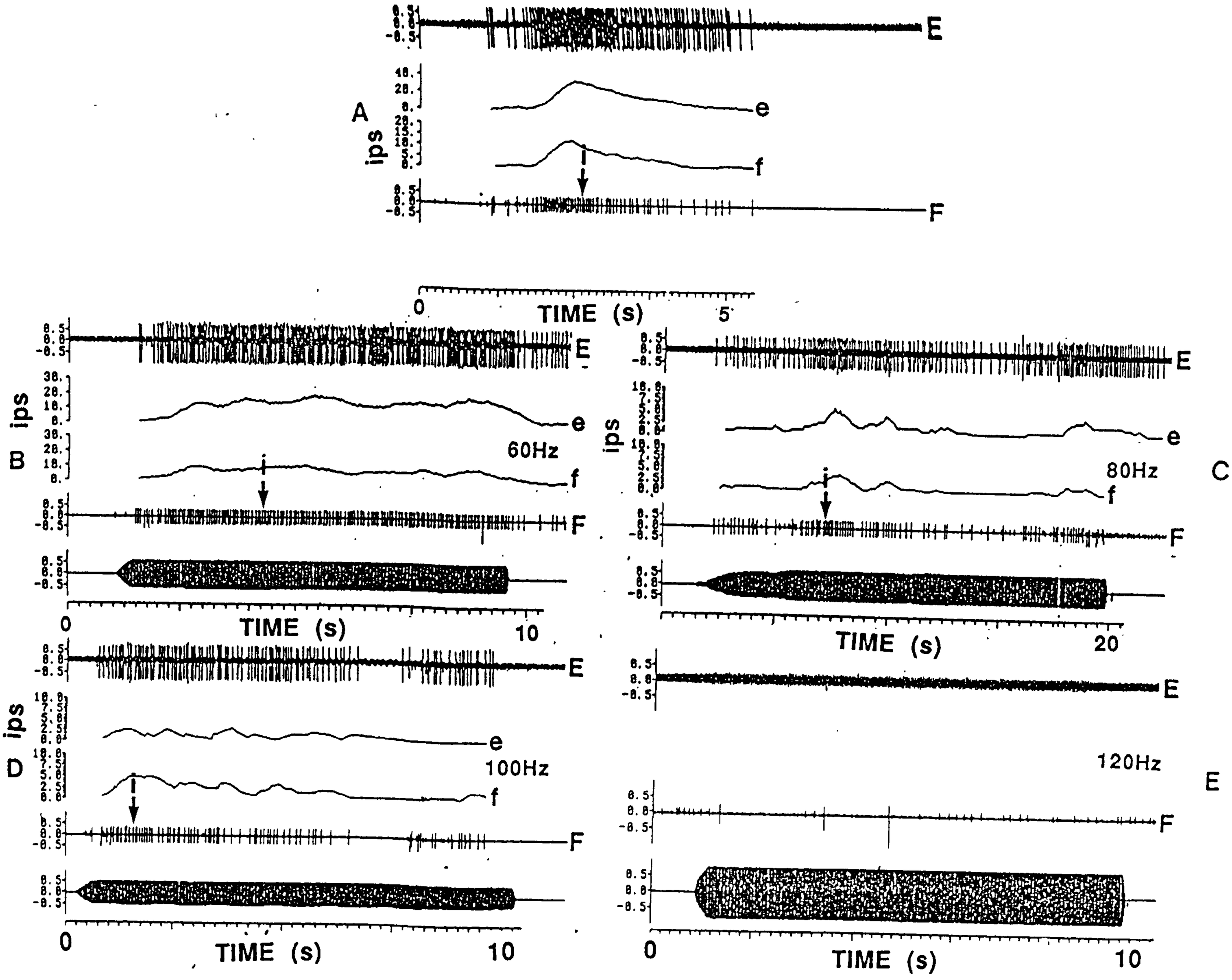

A
ips
E
e
f
F
0
TIME (s)
5
B
ips
60Hz
10
C
80Hz
20
D
ips
100Hz
E
120Hz

Figure 4.23 Responses shown by units in SR3 and R2 to water borne vibrations showing possible nervous basis for delayed responses. Plots show traces as detailed in Figure 4.22. Scale bar indicates time in seconds, i indicates the FI unit.

A. 60Hz

B. 120Hz

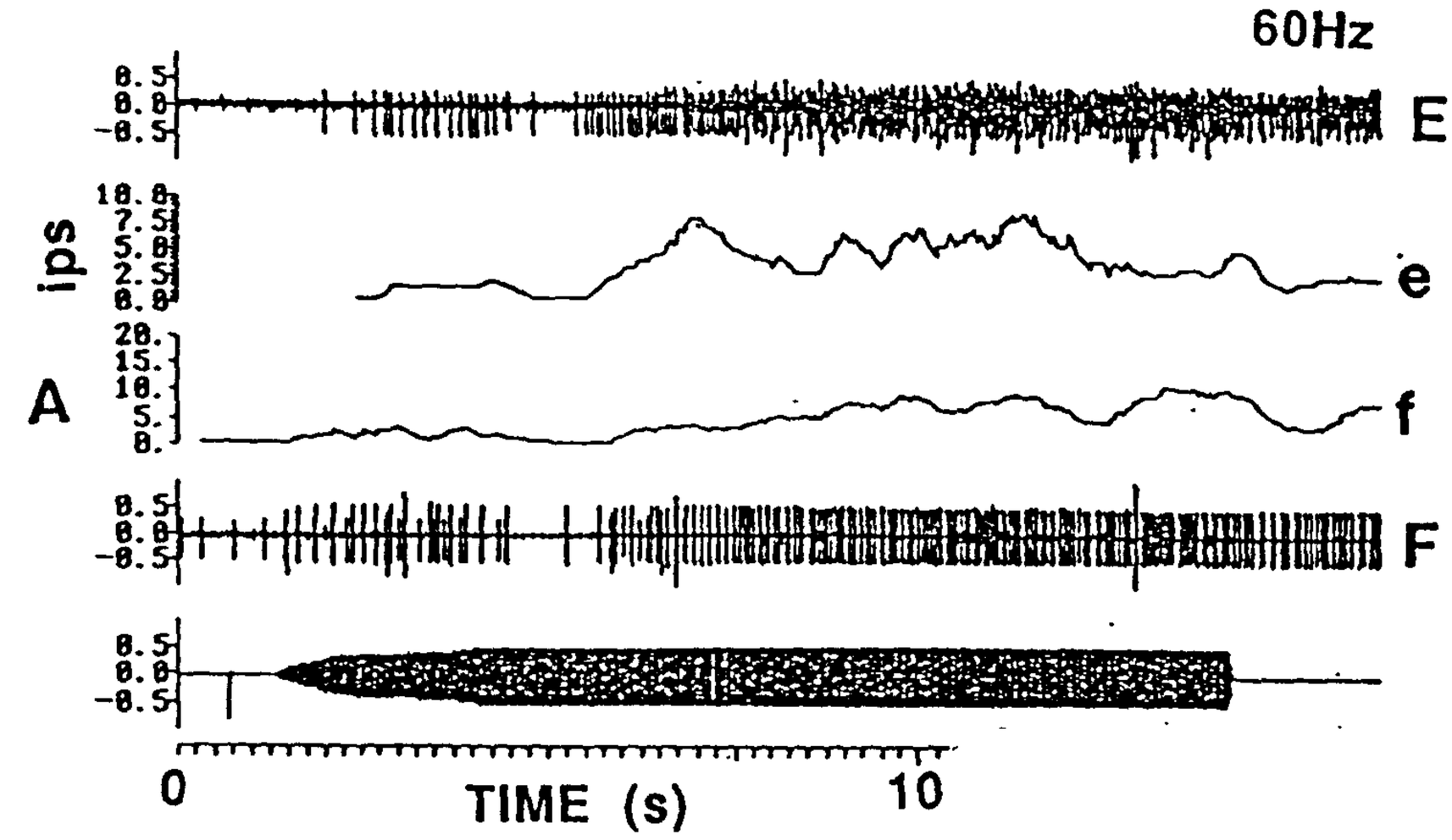
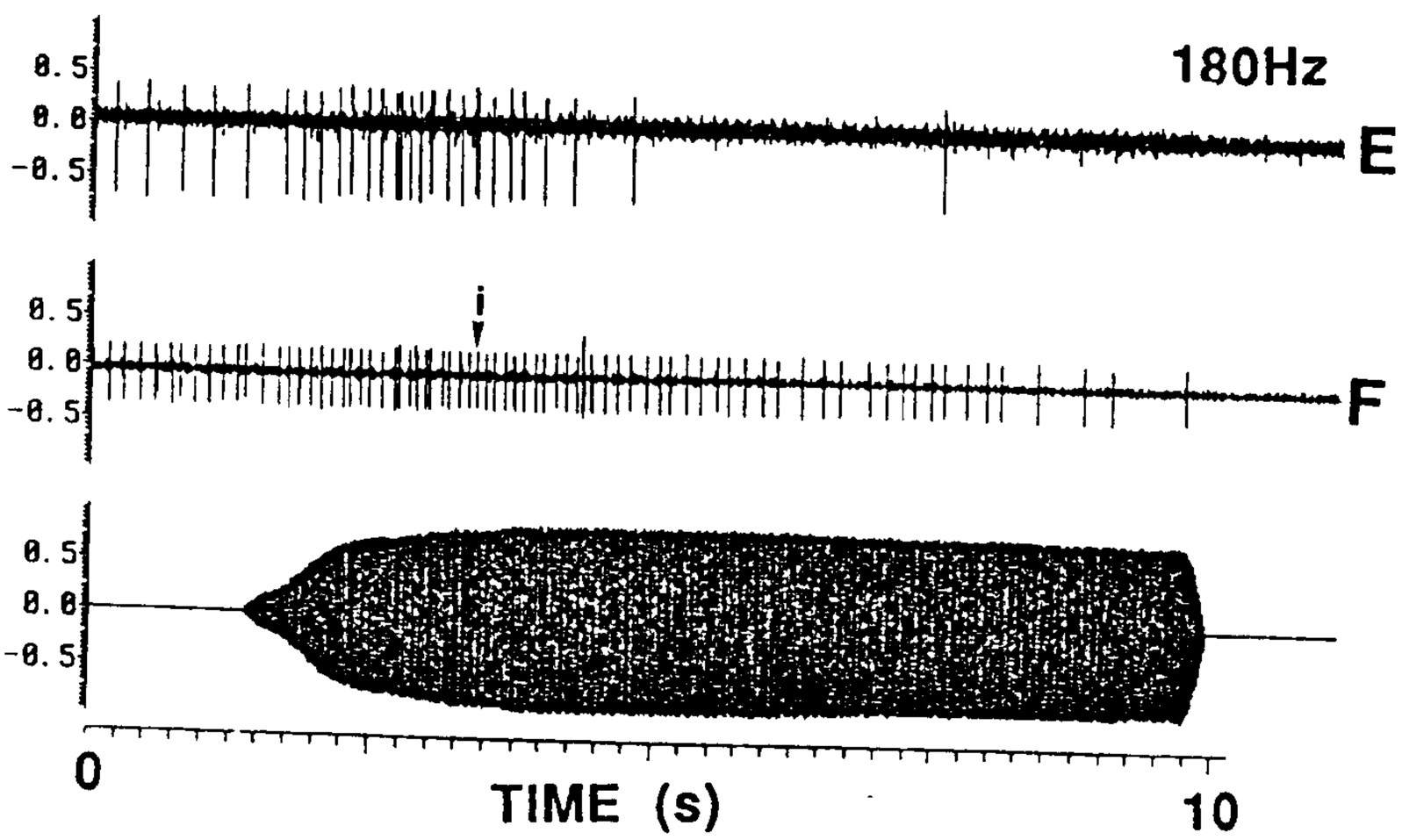
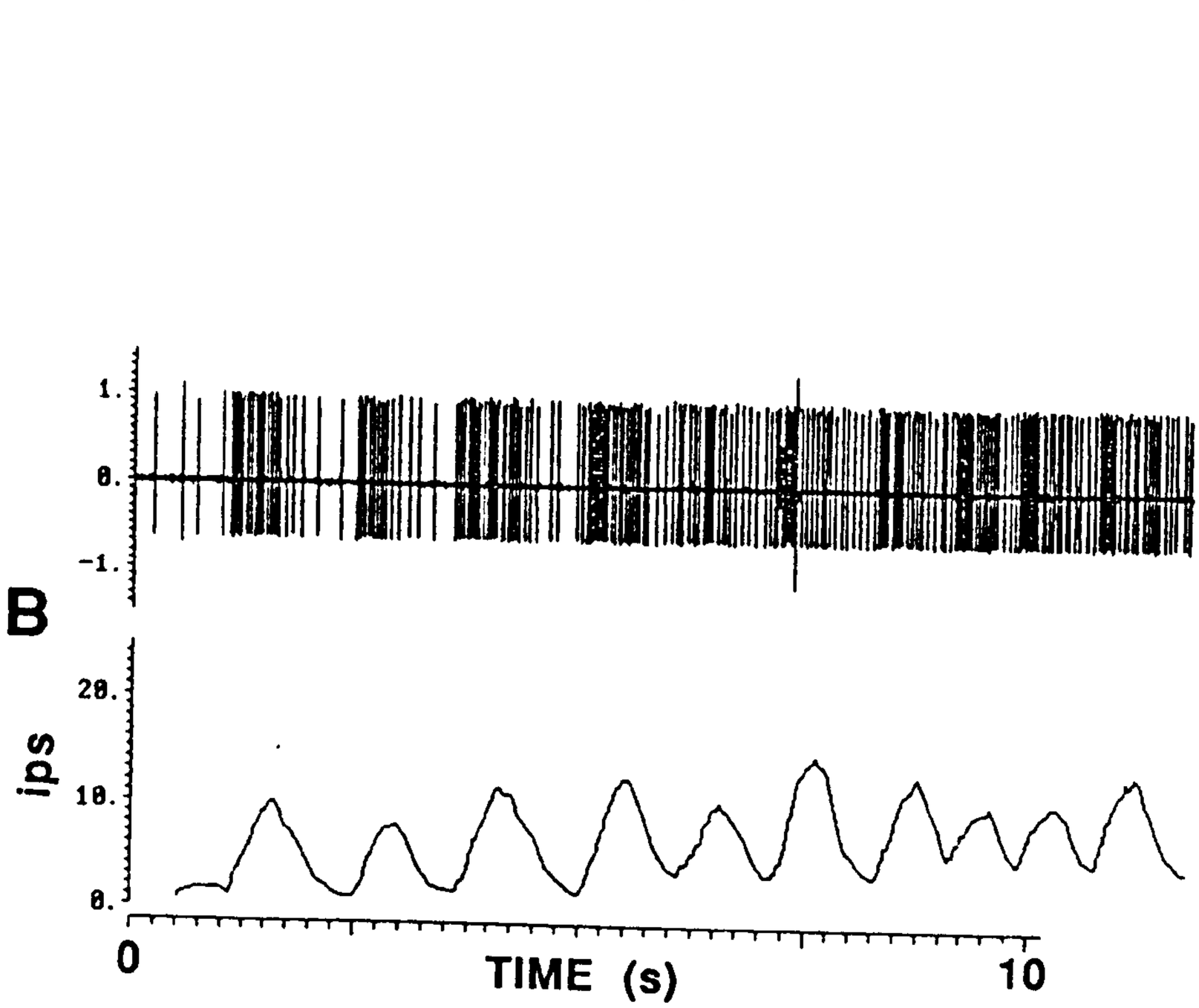

60Hz
E
e
f
A
ips
F
TIME (s)
0
10
120Hz
B

Figure 4.24 A. Responses shown by units in SR3 and R2 to water borne vibrations of 180Hz showing possible nervous basis for a delayed response. Plot shows the raw nerve spike data from R2 (top), the raw nerve spke data from SR3 (middle) (i indicates the FI unit), and the stimulus trace (bottom). Scale bar indicates time in seconds.

B. Spontaneous activity in SR3 during leg waving. Top trace shows the raw nerve spike data, bottom trace shows the mean frequency of firing in the FI unit (i).


0.5
0.0
-0.5
180Hz
E
A
0.5
0.0
-0.5
i
F
0.5
0.0
-0.5
0
TIME (s)
10


1.
0.
-1.
B
20.
10.
0.
ips
0
TIME (s)
10

Chapter 5

BEHAVIOURAL RESPONSES OF *Nephrops* TO SOUND AND CALCULATION OF RESPONSE THRESHOLDS IN A FREE SOUND FIELD.

5.1 INTRODUCTION

While a great deal of data is available on the sensory responses of members of the Crustacea to vibrational stimuli and general mechanical disturbances, there is a paucity of published data on their behavioural responses either in the field or in the laboratory. Chapter 4 has shown that *Nephrops* exhibit postural responses to water borne vibrations in the laboratory in the form of abdominal extension. The present study attempted 1) to determine the responses of freely moving animals both in the laboratory and in their natural habitat and 2) to repeat the postural experiments of Chapter 4 in a free sound field in order to calculate thresholds for these behavioural responses. This study represents the first attempt to record the behavioural responses of a crustacean species to sound in a free acoustic field.

Several recent studies have reported the existence of orientation mechanisms in decapod crustaceans. Many of these mechanisms are, at least in part, related to hydrodynamic cues. The spiny lobster *Panulirus argus* performs annual mass migrations to areas with greater food supply and which provide them with a thermally stable environment for larval hatching (Herrnkind, 1985). The animals form long queues which help to reduce drag by as much as 50% (Herrnkind and Kanciruk, 1978). There is evidence that this behaviour, which has been observed both in the field and in the laboratory, is influenced by hydrodynamic stimuli, as it occurs spontaneously in the field after a period of stormy weather (Herrnkind, 1985). Walton and Herrnkind (1971) demonstrated that blind lobsters cannot orientate without hydrodynamic cues and show straight and directional movements when currents or wave surges are present. This would suggest that these stimuli have an important role in lobster behaviour and the authors suggest that

cuticular hairs and joint proprioceptors may be the receptors involved. The blue crab has also been shown to orientate to hydrodynamic cues and exhibits escape behaviour towards deeper water in response to wave surges when it is feeding near shore (Nishimoto and Herrnkind, 1978).

Recent reports on the responses of *Nephrops* to hydrodynamic stimuli and in particular water currents have shown that these animals preferentially orientate downstream (Newland et al., 1988a). Field observations of *Nephrops* (Newland and Chapman, 1985) have shown that most animals seen out of their burrows were facing downstream to the direction of current flow. There is evidence that this posture helps the animals to reduce drag over their body surface (Newland et al., 1988a). Newland and Chapman (1985) also tested the responses of *Nephrops* to a trawl groundrope dragged over the substrate. They reported that half of the animals responded before they were contacted by the groundrope, when it was at a mean distance of 0.24m away from them. This response could involve visual stimuli since Newland (1985) showed that visually intact animals responded more frequently than blind animals before contact was made. This does not, however, exclude the possibility that this response may be partly due to inputs from mechanosensors responding to either the currents or vibrations produced by the rope as it is dragged along the sea bed.

There are a few isolated studies on the behavioural responses of crustacean species to vibrational stimuli and, although no attempt has been made to conduct these studies in the field, where acoustic conditions are better, they still provide some interesting information on the nature of the responses. Salmon and Astaides (1969) found that the fiddler crab, *Uca pugilator* showed behavioural reactions to substrate borne vibrations and were most sensitive to vibrations of 20Hz. More recent studies (Tautz, 1987; Heinisch and Wiese, 1988) have

shown that the crayfish *Orconectes* and the shrimp *Crangon* respond to vibrational stimuli with antennal movements. The results of Heinisch and Wiese (1988) should perhaps be viewed with caution as the conditions under which they obtained these results are unlikely to occur in the field. Tautz (1987) showed that freely walking crayfish exhibited behavioural reactions to frequencies of 10-90Hz, and to touch stimuli. The antennal movements he observed were occasionally accompanied by locomotory movements. Crayfish antennae have been shown to possess water vibration receptors (Masters et al. 1982) and the antennae are used by the crayfish to localise objects by touch (Zeil et al. 1985; Sandeman, 1985)

It is known that many marine crustaceans can produce sounds. The ghost and fiddler crabs for example produce sounds by the use of stridulatory structures or by thumping their legs or body against the substrate. Other sound producing crustacean species include the spiny lobsters, the snapping shrimps and the mantis shrimp (Hawkins and Myrberg, 1983). It therefore seems reasonable to expect that such animals should have mechanisms which enable them both to receive and respond to these stimuli and perhaps to recognise the sounds which their predators make. There is no evidence to suggest that *Nephrops* can produce sounds except during fights when they clash their claws together (Chapman and Rice, 1971) and most interactions with other members of their species must use other sensory cues. One of the main predators of *Nephrops* around the coast of Scotland is the cod, *Gadus morhua*, which is known to produce a low frequency grunting sound (Brawn 1961; Hawkins and Rasmussen, 1978). It is important therefore to determine if *Nephrops* may be able to respond behaviourally to such low frequency stimuli, especially since they display a sensory responsiveness to such stimuli (Chapter 3). It would also be

interesting to know whether *Nephrops* can respond to the sounds produced by trawlers and trawl gears. Studies on fish, which are known to possess a good sense of hearing, have shown that they respond to vibrating objects and sound in the field, in the absence of vision, and show strong avoidance reactions (Blaxter and Batty, 1985, 1987).

There is a wealth of information on the responses of fish to sound (Chapman and Sand, 1974; Chapman and Hawkins, 1969, 1973; Hawkins and Myrberg, 1983). Quantification of the parameters of the response is an important part of any study and has formed a major part of these studies of fish hearing. The resulting audiograms yield information about the range of frequencies and amplitudes over which an animal is sensitive. In fish these parameters can be determined using the cardiac conditioning technique (Hawkins 1981). Variation of the distance between the sound source and the animal has been used extensively in fish work to determine whether the animals are pressure or displacement sensitive (Chapman and Sand, 1974). Fish such as the plaice are displacement sensitive (Chapman and Sand, 1974) whereas the cod is also pressure sensitive (Chapman and Hawkins ,1973).

There are some published data on the determination of sensory thresholds to water borne vibrations in crustacean species (Wiese, 1976; Ebina and Wiese, 1984). However, the determination of sensitivity thresholds to different frequencies using cardiac conditioning has only been attempted on one crustacean species, *Homarus americanus* (Offut, 1970). It is generally accepted that cardiac conditioning is a very difficult technique to perform on crustaceans as their resting heartbeat is very erratic. Offut (1970) gave a response threshold in terms of particle displacement for *Homarus americanus* of 8.1 x 10^{-4} μm at 75 Hz, but attempts to repeat this work on the European lobster , *H. gammarus* have been unsuccessful

(A.D. Hawkins, unpublished observations). No other measurements of behavioural response thresholds for crustacean species have been made either in the field or in the laboratory. Furthermore, no conclusive data exist to confirm whether the Crustacea may be sensitive to the pressure component of the sound as well as to the particle displacement component which has been shown in the many studies of the nervous system. As hearing is a behavioural response (Stebbins, 1983) it is important that attempts should be made to calculate behavioural thresholds as well as neurophysiological ones.

Two main sets of experiments were therefore undertaken during the course of this study using freely moving animals. The first was conducted to determine whether vibrational stimuli could alter the behaviour of *Nephrops* in the field, either transiently or on a more long term basis by causing a change in the burrow emergence rhythm. The second was conducted in a laboratory tank under similar acoustic conditions to those which have been employed by other investigators. It should be remembered that all experiments in small tanks encounter all of the problems described in Chapter 1 due to the presence of reflecting surfaces. It is very difficult to reproduce ideal acoustic conditions in the laboratory and such tanks that are available are generally not suitable for behavioural experiments as the animal is not accessible to the experimenter (Hawkins and MacLennan, 1975).

A third set of experiments was conducted in a free sound field to determine in unconditioned animals their response thresholds to water borne vibrational stimuli and to determine whether the animals were sensitive to pressure as well as displacement.

5.2 MATERIALS AND METHODS

5.2.1 Field investigation of the behaviour of *Nephrops* in response to sound with particular reference to the burrow emergence rhythm

All field experiments were conducted at a field station belonging to D.A.F.S. by Upper Loch Torridon, Wester Ross, (Fig. 5.1). The natural population of *Nephrops* in the loch was viewed using a red-light sensitive underwater TV camera (Osprey OE1231A) with pan and tilt (Dennard Type 330) mounted on a Dexion frame (Fig. 5.2.). It was lowered onto the sea bed at a site approximately 250m from the shore with cables leading back to the laboratory hut. The water depth was 30m. A 500 watt light with a red perspex filter transmitting only wavelengths greater than 600nm was mounted alongside the camera to illuminate the field of view. Light of this wavelength is outside the absorption band of the *Nephrops* eye (Loew, 1976; Chapman, 1985). Furthermore Chapman and Howard (1979) showed that the intensity of light from this source had no effect on the movement of *Nephrops* in its vicinity.

Instead of presenting the animals with pure tones, noises of varying frequency and bandwidth were used (Table 5.1). All of these stimuli, including the low frequency components of the white noise stimulus, fell within the range of sensitivity of the mechanosensory systems studied (Chapter 3). The sounds were generated using a sine random generator (Bruel and Kjaer Type 1024) and fed via a 25W power amplifier (Derritron) to the sound projectors.

Two sound projectors (Dyna Empire Types J9 and J11) were positioned on the sea bed. The J9 was buried in the mud to simulate substrate borne vibrations. Both sound projectors were positioned within 10m of the camera.

The sound stimuli and ambient sea noise were monitored by a calibrated hydrophone (Plessey) mounted beneath the camera. The output signal was amplified and filtered using a Brookdeal amplifier and measured using a narrow band frequency analyser and level recorder (Bruel and Kjaer Types 2107 and 2305). The sea noise, the noise of the pan and tilt mechanism and the sounds played via the loudspeakers were analysed using this system. The analyser scans through the frequencies within a pre-set range and gives the sound pressure level at each frequency. The sound pressure level was then converted to the spectrum level value using the formula;-

$$\textbf{spectrum level} = \textbf{sound pressure level} - 10 \log \Delta F \quad \ldots\ldots (1)$$

where Δ F is the measuring filter bandwidth.

This study was undertaken at night following the observations of Chapman and Rice (1971) who stated that *Nephrops* in shallow water in Loch Torridon were nocturnal in June and emerged at dawn and dusk in September. This study was conducted between 29th August and 5th September and the emergence rhythms for *Nephrops* in Loch Torridon at this time were unknown. Observations were made on 4 nights in three periods of 10-12 hours and one shorter period of 3 hours. Sounds were presented every alternate hour for an hour, except on night 4 when they were alternated every half hour. All experiments began with a quiet, ie. sound off, period. Table 5.1 shows the experimental regime for each night. All activity was recorded on video tape for later analysis. The video signal was mixed with a timing signal allowing accurate timing of events to 0.01s.

5.2.2 Investigation of changes in the transient behaviour of *Nephrops* in the field

A second set of field experiments was conducted at the same site in Loch Torridon, to determine whether any transient change in the

behaviour of *Nephrops* could be induced by sound in the field.

A frame constructed by D.A.F.S. (Fig. 5.2) was lowered onto the sea bed at the same site and depth as previously described. An underwater TV camera, 4 red lights, two J9 loudspeakers and a calibrated hydrophone were mounted on the frame. The camera was mounted 0.9m above the sea bed so that the entire base of the frame was within the field of view. This meant that no animal was ever more than 2m from the loudspeaker during presentation of the sound stimulus.

The stimuli were pulsed using a Synthi sound synthesiser to give a total pulse time of 0.6s with a rise time of 0.1s. to avoid transients in the sound signal. The frequency range of the stimuli varied from 40-600Hz, and noise bandwidths of 100Hz and 30Hz as well as pure tones were used. Sounds were amplified as previously described (section 5.2.1.) and presented in a random order. Animals were also presented with taped recordings of trawl noise supplied by J. Main and G. Sangster (D.A.F.S.). One stimulus was presented each time an animal entered the field of view and was played until the animal left the field. The sounds were monitored and measured as previously described. All experiments were conducted at night and included the dawn and dusk periods.

5.2.3 Aquarium study of changes in the locomotory behaviour of *Nephrops* in response to vibrational stimuli.

These experiments were conducted in a square fibreglass tank 1.2m x 1.2m x 0.6m laid on top of a high density rubber mat which helped to isolate it from extraneous external vibrations. The floor of the tank was covered with a layer of coral sand to give the animals a suitable substrate on which to walk. A rod marked with black tape at 10cm

intervals was placed in the bottom of the tank to calibrate the video recordings and allow accurate measurements to be made. A hole was cut in the side of the tank to allow the diaphragm of a Dyna Empire J11 loudspeaker to be fitted into the wall. The edge of the hole was sealed with "Aquaseal" to prevent leakage and the tank was filled with circulating sea water at 12.5^{o}C. The J11 was powered by a 25W amplifier (Derritron).

Sound levels in the tank were monitored by a hydrophone suspended in one corner of the tank. The output of the hydrophone was amplified by a Brookdeal amplifier and fed into a Bruel and Kjaer frequency analyser and pen recorder.

A Panasonic video camera with wide angle lens was mounted above the centre of the tank so that the entire tank was within the field of view. Experiments were recorded on a Panasonic time-lapse video recorder, the video signal having been previously mixed with a timing signal. The output from the hydrophone was also recorded on the audio channel of the video recorder.

Two groups of animals were used; the first group was obtained from Upper Loch Torridon by creel fishing at night and had been transported back to the laboratory in light-tight plastic bags so that their sight was not damaged. The second group was obtained from U.M.B.S., Millport and had been blinded by continuous exposure to light. The sighted animals were tested in groups of two or four and the blind animals either singly or in pairs. Testing of the sighted animals required certain special conditions which did not apply to the blind animals. The animals were held in tanks under a reverse light rhythm so that experiments could be conducted during the day. Red light of an intensity low enough not to damage the eyes was switched on between 6am and 6pm to simulate the night period and red and green

lights were switched on between 6pm and 6am to simulate the day period. These conditions were maintained during the experiments. The animals were also supplied with pieces of drainpipe to serve as artificial burrows. The blind animals were not supplied with artificial burrows. All of the animals were marked with black paint to allow easy identification while in the tank.

The sighted animals were tested in response to frequencies of between 20 and 600Hz. These stimuli included both pure tones and broad and narrow band noise. Based on the results of these experiments the blind animals were tested in response to pure tones of between 20-200Hz. The amplitude of the stimuli was adjusted to avoid saturating the hydrophone system, the output of which was monitored on an oscilloscope. The stimuli were presented in a random order and only if the animals were stationary so that there was a common starting point to all recorded responses.

Data analysis and presentation

The video tapes were analysed using a video interface which overlaid cross hairs onto the video image. After alignment, co-ordinate points could be entered directly to a microcomputer (Tuscan S100) by depressing a switch. Two programs were used to analyse the data. One required the input of a pair of co-ordinates per frame at regular intervals (typically 1 per second) throughout the sequence. The reference points used in this case were the rostrum and posterior tip of the tailfan. The program then calculated the following parameters; 1) on each frame: the angle of orientation of the body relative to the sound source 2) between successive frames: displacement, velocity and acceleration of the anterior reference point. The displacement and orientation of the animals were then plotted for typical responses. The second program required the input

of an initial and final co-ordinate pair for each test. These were the co-ordinate pair showing the orientation of the animals at the onset of the sound and the orientation of the animal 10 seconds later. By using the Rayleigh statistical test (Baschelet, 1981) on the pooled data from several tests it was possible to acertain whether the animals orientation relative to the loudspeaker changed significantly during the tests.

5.2.4 Behaviour of animals in response to vibrational stimuli in an acoustic tube

Experiments were conducted to observe the behaviour of *Nephrops* in the acoustic tube (See Chapter 3) and to compare this with the previous behavioural studies in the field and the laboratory (4.3.1 and 5.3.1-5.3.3). Other decapod crustaceans were also studied in the tube so that their behaviour to vibrational stimuli could be compared with that of *Nephrops*. The characteristics of the tube allowed the animals to be presented with a stimulus which was composed mainly of displacement rather than sound pressure. For each test the tube was filled with cooled oxygenated sea water and an animal was placed inside and left for about 30 minutes to acclimatise. Its responses were then tested to a selection of frequencies presented in the previous experiments. The responses were noted in detail. Two other marine species, *Homarus gammarus* (L.) and *Munida rugosa* (Fabricius) were tested in the same way. One freshwater species, *Pasifastacus leniusculus* (Dana) was also tested.

5.2.5 The postural responses of *Nephrops* to water borne vibrations in the field: determination of the response thresholds.

These experiments were conducted during one week's field study in

Loch Thurnaig (Fig.5.1) which is part of Loch Ewe in Wester Ross, Scotland. The laboratory, which is part of the Loch Ewe field station complex belonging to D.A.F.S., was situated on top of a floating pontoon moored in Loch Thurnaig. The pontoon was supplied with electricity from a generator located on the shore. The depth of water in this part of the Loch was about 30m.

A frame was constructed by D.A.F.S. from durapipe tubing on which was mounted a low light under water TV camera and hydrophone (Fig. 5.3). The camera viewed horizontally a perforated perspex box containing the test animal attached to a rod (as described in section 4.2.1) secured to the lid of the box. The animal was able to stand in a normal posture inside this box, side on to the camera. The camera position was adjusted so that the animal filled the video screen. The frame and the box were made of material which had the same acoustic impedance as water and so would not lead to any problems of acoustic mismatch (Chapter 1).

The frame and a J9 loudspeaker were both suspended from a horizontal metal bar and lowered from the pontoon to a depth of 12m. Tests were conducted with the J9 at distances of 1m and 0.09m from the animal to vary the ratio of particle displacement and pressure components of the sound.

Tests were carried out at random time intervals using pure tones from the frequency range which had already been tested in the laboratory (25-200Hz). The responses of the animals were recorded on video tape as described in section 5.2.1 and sounds were generated and monitored using the systems also described in that section.

In the field, as in the laboratory both leg movements and the initial tail movement (4.3.1) preceded the full abdominal extension response and occurred in its absence at lower stimulus amplitides. By including these other responses as indicators of a behavioural

response it was possible to extend the frequency range over which the threshold could be measured without overdriving the loudspeaker.

Thresholds were determined using the "staircase" method in which the tone stimulus was attenuated by 5db steps until a point was reached where the response no longer occurred. The stimulus amplitude was then sequentially increased by 5db and attenuated by 5db until the threshold was confirmed (see Fig. 5.12). This process was repeated for each frequency. Blank tests were carried out randomly throughout the experiments by attenuating the signal by 30db to ensure that the animals were not responding to cues other than the stimulus.

Tone stimulus amplitudes and background sea noise levels were measured as described in section 5.2.1. In some experiments the behaviour of *Nephrops* was monitored in response to the sound generated by an outboard motor.

Data analysis and presentation

The threshold at each frequency was determined by calculating the percentage of positive responses at each SPL tested and plotting these values against the sound pressure level (Fig. 5.13). The SPL at which 50% of positive responses occurred (read from the graph) for each frequency was taken to be the threshold for that frequency. The sound pressure threshold values were then plotted against frequency for each animal and a linear regression line was fitted to these values.

5.3 RESULTS

5.3.1 Changes in the diurnal emergence rhythm.

a) Sound analysis

The ambient sea noise, noise of the pan and tilt mechanism, and the sounds played via the sound projectors are shown in Figure 5.4. The sea noise measurement showed that the components of the noise with the highest spectrum level were at low frequencies. The sea noise measurement also showed some variation with the sea state and weather conditions. High winds and rain generated higher sound levels at low frequencies. The spectrum levels of the sea noise varied from -17db re 1μbar at 30Hz to -55db re 1μbar at 7250Hz.

The noise produced by the pan and tilt mechanism (Fig. 5.4.B) varied from -10db to -45db re 1 bar with a broad peak between 100 - 300Hz. As the pan and tilt sound components were greatest at low frequency where the animals might be responsive it was decided not to operate this apparatus during the experiments.

Analysis of some of the sounds played via the J9 sound projector are shown in Figure 5.4.C. These were made at the end of each experiment. The 20-50Hz stimulus had the highest spectrum level measurement of +11db re 1μbar while the other two stimuli 40-340Hz and 20-20,000Hz reached maximum values of -20db and -15db re 1μbar respectively.

b) The emergence rhythm

The number of *Nephrops* observed in the field of view of the TV camera per hour was counted from the video tapes for the control and the sound transmission periods. The first night was used as a control period and no sounds were transmitted. This allowed the emergence rhythm of the animals to be determined. On subsequent nights (study periods 1-3) alternate hours were experimental and control periods

which to avoid confusion with the main control period have been called "quiet" and "experimental" periods. In each case the animals were divided into resident and non resident groups. The resident population comprised those animals entering or leaving burrows within the field of view. Some of these animals emerged and retreated many times into the same burrow for a whole study period. The non-resident group were all those animals crossing the field of view without entering a burrow. Each time an animal entered the field of view it was counted as a new arrival but it is possible that after leaving some animals turned round and re-entered the field so that the same animal could have been counted more than once.

The results for the control period for the two groups are shown in Figure 5.5. The non-residents showed a peak in numbers four hours after sunset and a smaller peak after sunrise. The resident group showed an increase in activity towards the middle of the night. Some of this activity was due to animals emerging and leaving their burrows, but a substantial component was due to the regular appearance of animals at the burrow entrance. As the latter animals did not fully vacate their burrows this probably represents in-burrow activity which is generally considered to be nocturnal (Atkinson and Naylor 1976).

In study period 2 (test stimulus noise band 40-340Hz) the non resident group maintained their emergence times of sunrise and sunset although the relative size of the peaks was reversed (Fig. 5.6). The resident group in this case emerged only at sunrise and sunset, with no animals seen outside these periods. There was a slight decline in the total numbers of *Nephrops* with reference to the control period. During study period 3, (noise band 20-50Hz), the numbers of animals declined markedly with reference to the control with both resident and non resident groups showing a small peak in numbers towards sunrise

(Fig. 5.7). The 4th study period again showed fewer *Nephrops* with a peak near sunset (Fig. 5.8).

Despite the decline in numbers of *Nephrops* in successive study periods the most obvious feature of these results was the presence of the diurnal emergence rhythm. It is generally difficult to detect changes present on an already very strong rhythm like the emergence rhythm, which should persist even in the event of disturbance. There seemed to be no clear relationship between the presence or absence of the noise stimulus and the numbers of *Nephrops* seen. A Mann and Whitney U test showed that there was no significant difference in the numbers of *Nephrops* observed in the sound and control periods within each study period.

5.3.2 Field investigation of transient changes in the behaviour of *Nephrops* .

5.3.2.a Sound analysis

Figure 5.9.A shows sea noise spectra averaged over all the study periods. Like the previous sea noise measurements the spectrum level was high at low frequencies and declined with increasing frequency. The range of the sea noise spectrum level was from -41 to -64.5 db re 1μbar.

Figure 5.9.B shows the spectrum level measurments during the transmission of taped trawl noise. Tapes C and E had the highest spectrum levels and tape D the lowest. The overall range was from +2 to -59dB re 1μbar.

5.3.2.b The behaviour of *Nephrops* in response to the sound stimuli

81 sightings of animals were made during the course of the experiments. Some animals did not completely enter the field of view and were not tested. Many of the stimuli presented to the animals fell

within the physiological range of frequencies to which the sensory system of *Nephrops* is responsive (Chapter 3), and the sound pressure levels of the sounds played fell between +4 to +26 db re 1 μbar. No clear changes in the behaviour of the animals were seen in response to any of the stimuli. During the presentation of the sound stimuli the animals generally continued walking across the field of view without any change in direction. Very occasionally when an animal was close to one of the speakers the onset of the stimulus gave rise to a tentative response in the form of a few steps of backwards walking or probing the mud area around the speaker with the claws.

5.3.3 Aquarium study of changes in the locomotory behaviour of *Nephrops* in response to vibrational stimuli.

Observations were made of the responses of blind and sighted *Nephrops* to water borne vibrational stimuli. The conditions under which the sighted group were tested represented an attempt to provide the animals with as close an approximation of their natural conditions as possible. However, quantitative measurment of any behavioural parameters from the sighted group proved difficult for several reasons;- 1) due to the unusual light conditions required for these animals the contrast in the tank was very poor, making the animals very difficult to see. 2) the animals were placed in the tank in groups of 4, so interactions were taking place between them which may have affected their behaviour in response to the stimulus. 3) the animals were supplied with "burrows" and they generally stayed inside these in preference to walking around the tank, which meant that the period in which they could be tested was short. 4) the animals were fed during the course of each day which again may have affected their behaviour in response to the stimulus. The tests of the blind group

were made under rather unnatural conditions and this should be borne in mind.

5.3.3.a Observations of the behaviour of the sighted group.

These animals showed a variety of behaviour patterns in response to the stimulus. The strongest and most frequently observed was backwards walking. This response was shown to low frequency tones (below 80Hz), and generally took the form of a retreat back into the "burrow" entrance. Animals engaged in other activites frequently stopped and retreated back into the burrow, often remaining at the burrow entrance for a while after the sound had been switched off. On occasions when the stimulus amplitude was very high, tailflipping back into the burrow was observed. This response was also seen in animals which had been starved for several days if the stimulus was switched on while they were feeding. Activities such as feeding, walking towards food and fighting with other animals in the tank could be interrupted by the onset of the stimulus.

Emergence from the "burrow" was seen in response to frequencies from 80Hz -200Hz , the animals emerged from their burrows waving their claws and sometimes probed around the edge of the tank with their claws. This behaviour only persisted for a short time after which the animals generally retreated back into their burrows. This probing behaviour was also induced in animals which were already out of their burrows when the stimulus was switched on. No responses were seen to frequencies above 200Hz.

5.3.3.b Observations of the behaviour of the blind group.

This group of animals responded to frequencies between 20 and 80Hz. No responses were observed to frequencies above 80Hz. The most

frequently seen response was backwards walking but forwards walking also occurred. The animals sometimes probed the area around the loudspeaker with their claws, if they were close to it, when the stimulus was switched on. Between 20 and 80Hz the frequency of the stimulus did not seem to affect the response, so these data have been pooled for the purposes of analysis.

Figure 5.10 shows two plots based on 34 tests in each case of the initial orientation of the animals at the start of the stimulus period (top) and their orientation after 10 seconds (bottom). These data were tested using a Rayleigh test to see if either the initial or the final orientations of the animals were significantly different from random, and whether they were related to the position of the loudspeaker. The r value (mean vector) was low in each case. The statistics obtained for the initial orientations showed that the animals were significantly orientated ($p<0.05$) but the final orientations did not differ significantly from random. The variances around the mean overlapped in each case showing that the initial mean orientation was not significantly different from the final mean orientation.

Figure 5.11.A-G show plots of the actual movement of several animals for 10 seconds following the onset of the stimulus. The position of the animal was plotted every second to give some indications of both the orientation of the animal relative to the stimulus source, its speed and direction of movement. Five patterns of movement were seen:

1) At the onset of the stimulus the animal moved slowly backwards with a straight trajectory. (Fig. 5.11.B). This response was seen most often.

2) The animal made a very small initial movement forwards lasting about 1 second followed by a longer period of rapid backwards walking. (Fig 5.11.A and E).

3) The animal initially moved backwards and then moved forwards starting to turn as it did so until it was facing in the opposite direction and it then started to walk backwards (Fig. 5.11.F)

4) The animal initially moved forwards and then started to walk backwards with a curved trajectory. (Fig. 5.11.C and D). This response was also seen without the initial forwards movement. The direction of the turn was not related to the position of the loudspeaker and could be performed in any direction.

5) The animal initially moved backwards and then started to walk forwards very quickly (Fig.5.11.G).

In all of these cases the direction of the movement seemed to be unrelated to the position of the loudspeaker.

5.3.4 The behavioural responses of *Nephrops* and other crustacean species to water borne vibrations in the acoustic tube

The behavioural responses of *Nephrops* and three other crustacean species were tested with water borne vibrations in the acoustic tube. All animals were able to walk freely during these experiments.

5.3.4.a *Nephrops norvegicus* (L.)

The responses of the animals were tested to pure tones in the frequency range 12-200Hz at amplitudes of either 1 or 2 on the voltage scale of the Derritron. The actual displacements at these amplitudes are shown in Figure 3.7.b and c. Very clear responses were seen in this species throughout the frequency range. The responses took several different forms depending on the frequency. At frequencies up to 20Hz the animal walked backwards very rapidly at the onset of the stimulus. This component of the behaviour was brief and was followed by a period of forwards walking which lasted until the animal had

reached the end of the tube. Above 20Hz up to 80Hz the backwards component of the response was just as rapid, but lasted longer and occurred whether the animal was facing towards or away from the loudspeaker. At these frequencies the next component of the behaviour was more variable and three different sequences were seen. The animal either:

1) continued to walk backwards although more slowly than the initial movement

2) The animal started to walk forwards with the abdomen fully extended, the tailfan open and the claws raised

3) the animals remained stationary after the initial movement.

Above 80Hz, although the behaviour sequences occurring between 20 and 80Hz were sometimes seen, more typical responses were subtle movements of the abdomen and tail. The animal often remained stationary and the abdomen was positioned in one of the following ways:

1) The abdomen was raised slightly at the onset of the sound, the tailfan was opened and the abdomen held in a straight line at an acute angle to the ground. The animal often rocked back and forth.

2) The abdomen was raised into an S shape and the tailfan held vertically and open. In cases 1 and 2 the animal raised itself off the ground using its legs.

3) The animal raised its claws and flexed its abdomen curling its tailfan under its body.

5.3.4.b *Homarus gammarus*(L.)

The responses of these animals were tested with tone frequencies from 20-1000Hz as their responsive frequency range was not known. In this species responses were only obtained when the animal was facing away from the loudspeaker. The high frequency stimuli had little

effect but the animals showed some responses to frequencies below 100Hz. These responses took 2 forms:

1) The animal raised its body off the ground using its legs; the swimmerets were generally beating during this movement.

2) The animal raised itself off the ground at the onset of the sound and started to walk backwards towards the speaker. The animal always tried to turn at some stage in this behaviour pattern but usually failed due to the size limitations of the tube.

In all cases the responses of *Homarus* were not as strong as those seen in *Nephrops* and where backwards walking was seen it was much slower than in *Nehrops*

5.3.4.c *Pasifastacus leniusculus* (Dana)

Like *Homarus* the crayfish only showed responses to the stimuli when it was facing away from the speaker. The crayfish was tested from 6-100Hz. The clearest responses were to frequencies of 10Hz and below. The animal generally backed slowly towards the speaker and then turned round so that it was facing it. If close enough to the speaker it generally started to probe it with its claws. Above 10Hz and up to 40Hz the only response was a slight waving of the antennae even at high stimulus levels.

5.3.4.d *Munida rugosa* (Fabricius)

This species was tested from 8-1000Hz. The animals showed no clear responses to any of the frequencies tested, except for a slight movement of the antennae which was seen up to 100Hz.

5.3.5 Postural responses shown by *Nephrops*; response thresholds calculated in a free sound field

Sounds were first presented with the J9 sound projector at a distance of 1m from the animal. The sounds were presented over the range of frequencies known to cause abdominal extension in the laboratory (25-200Hz) and at high stimulus amplitudes (+50-+60db re 1 μbar). These very high sound pressure levels were at the upper limit of the technical capability of the J9, and at such high sound pressures there was a danger of overdriving it. No responses were shown by the animals in any of these tests. The behavioural responses of the animals were also observed in response to the outboard motor noise produced by 45HP and 25HP engines. The boats were driven at high speed close to the pontoon. The sound pressure levels generated in the case of the larger 45HP engine were in excess of +40db re 1/μbar. Again the animals showed no reactions to any of these stimuli.

Very clear responses were shown when the speaker was moved to a distance of 0.09m from the animal. The pattern of the response was essentially similar to that seen in the laboratory. Low frequency vibrations (20-80Hz) caused abdominal extension to occur and this was accompanied by the previously described accessory behaviour patterns. Higher frequency vibrations (80-200Hz) caused the response to occur with a delay. The fact that responses were produced at 0.09m and not at 1m , even though the sound pressure levels were the same in both cases, strongly suggests that *Nephrops* are sensitive to the particle motion component of the stimulus rather than the sound pressure.

Examples of the threshold determination by the staircase method from each animal can be seen in Figure 5.12. These indicate the sound pressure levels at which positive and nil responses occurred. Figure 5.13 shows examples of the method of estimating the thresholds at

different frequencies. These thresholds, calculated for each frequency, are shown in Figure 5.14.A for the pooled data for two *Nephrops*. The graphs show that the threshold expressed in terms of sound pressure was positively correlated with frequency ($P<0.05$.). From Figure 5.14.A it can be seen that at 40Hz the sound pressure level required to produce a response was +32db re 1μbar while at 200Hz the required sound pressure was +60db re 1μbar. The thresholds were initially calculated with reference to sound pressure as the hydrophone used to measure the responses was pressure sensitive. Sound pressure thresholds were converted to particle displacement using the following equation (Chapman and Sand, 1973).

$$d = \frac{P}{2\pi\rho c f}\sqrt{1+\left(\frac{\lambda}{2\pi r}\right)^2} \qquad \ldots\ldots\ldots\ldots(2)$$

where d= displacement (cm)

P= sound pressure (ubar)

f= frequency (Hz)

ρc= acoustic impedance of the medium= 1.54×10^5 $gcm^{-2}s^{-1}$

λ = wavelength = velocity/frequency = 1500x100/frequency(cm)

r= distance from the sound source = 9cm

These displacement values were then plotted against frequency for the pooled data in Figure 5.14.B. In this case the slope of the regression equation was not significantly different from zero. Under these circumstances a horizontal line drawn through the mean value of the data gives an estimate of the average threshold level at .874μm. These data suggest that *Nephrops* are sensitive to particle motion (here expressed in terms of displacement) but that this was independent of frequency. There may be some inaccuracy in the

measurement of the displacement levels as it was impossible to determine the exact acoustic centre of the loudspeaker. Measurements were made from the animal to the loudspeaker diaphragm which may have resulted in an overestimation of the displacement.

The conclusion drawn from these experiments is that *Nephrops* are sensitive to the particle motion rather than the pressure component of the stimulus.

5.4 DISCUSSION

5.4.1 The emergence rhythm

The field results obtained in section 5.3.1 allowed measurement of the emergence rhythms for late August/early September which had not previously been determined. Chapman and Howard (1979) and Chapman and Rice, (1971) have measured the emergence rhythms for *Nephrops* at this location in Loch Torridon at different times of the year. They find that in March and April (Chapman and Howard, 1979) the animals are broadly nocturnal in their emergence and this is also true in June (Chapman and Rice, 1971). The animals emerge at dusk, forage in darkness and return to their burrows around dawn. In mid September *Nephrops* showed a crepuscular rhythm, foraging at dusk and dawn and returning to their burrows during darkness (Chapman and Rice, 1971). The results presented here (Figs. 5.5-5.8) for non-resident groups show a similar rhythm to that calculated by Chapman and Rice (1971) for September. The resident group showed noctural activity.

The apparent decrease in the numbers of animals over the experimental periods may have been due to deterioration of the weather conditions, which increased the turbidity of the water. This significantly reduced the field of view making it much more difficult to see the animals.

5.4.2 Behavioural responses of freely moving *Nephrops* to sound

Chapters 2 and 3 have attempted to describe the responses of *Nephrops* to water borne vibrational stimuli in terms of the responses which this stimulus evokes in the sensory neurones and interneurones of the nervous system. This is a perfectly valid approach which has been pursued in many studies. However, in order to gain a complete

picture of the response of *Nephrops* to water borne vibrations and the implications which this might have for the animal it is necessary to look at the motor outputs and how these are expressed in the behaviour. This neuroethological approach is the only way in which some understanding can be obtained of how different sensory and motor systems interact to produce a co-ordinated behaviour which is meaningful to the animal. Huber (1988) states also that it is important to study the behaviour of an animal in its natural habitat. All of an animal's sensory and motor systems have evolved and been adapted to cope with situations which they will encounter in the natural habitat. If the animal is removed from this environment or is restrained in any way, as is often necessary during lab based behavioural experiments or neurophysiological experiments, then the chances of seeing completely natural responses may be diminished. Conducting behavioural experiments in the laboratory may change the animals level of motivation as well as the nature of the stimulus itself.

It was partly for these reasons that the behaviour of *Nephrops* in relation to acoustic stimuli was studied in the field as well as the laboratory. The animals used in the field study were the natural population, they were visually intact and were disturbed as little as possible. Another very important reason for choosing to study response to sound in the field was that the stimulus itself requires optimal conditions which are not found in the laboratory. As has already been stated (Chapter 1) only the sea environment can provide a free sound field. The natural benthic environment of *Nephrops* (sections 5.3.1 and 5.3.2) cannot provide perfect acoustic conditions because of the proximity of the sea bed. However, the top layer of the soft mud substrate on which *Nephrops* lives is acoustically very fluid so that reflections from this surface may be small in comparison to

the strongly reflecting sea surface.

The first set of field experiments (Section 5.3.1) was conducted in the far field, where particle displacements for a given sound pressure are very small. The maximum stimulus SPL at 20-50Hz was +11db re 1μbar at 10m (equivalent to 31 db re 1μbar at 1m). The second set of experiments was conducted in the near field where the particle displacements for a given sound pressure are much larger. The source level in this case was +26db re 1μbar at 1m. Under free field conditions it is possible to define the sound field accurately. This is not so in the laboratory where the sound is reflected both from the walls of the tank and from the air water interface (Chapter 1). Under these conditions large particle displacements may be associated with a very small sound pressure (Parvelescu, 1964). Therefore, although it is often necessary to repeat field experiments in the laboratory as was the case here, the field results are important as they demonstrate the true responses of the animal to the measured stimulus in its natural environment.

The results presented in this chapter have shown that acoustic stimuli from a wide range of frequencies (20-600Hz), many of which were known to evoke behavioural and sensory responses in the laboratory (Chapters 3 and 4), did not significantly alter the behaviour of *Nephrops* in the field. Sounds presented in the laboratory however, caused very strong behavioural reactions in the form of rapid backwards locomotion. This response to water borne vibrations was also seen in the laboratory study made by Tautz (1987). The apparent discrepancy between the field and laboratory results demonstrates only too clearly the need to conduct such studies in the field. There are several reasons which may explain why the experiments gave positive responses in the laboratory while giving negative results in the

field. Firstly, it should be remembered that acoustic experiments in the field are conducted against a background of high ambient noise and this may mask the lower frequency sounds (Hawkins and Myrberg, 1983). In the laboratory, background noise levels can be reduced by careful insulation of aquaria. Secondly, in the field the ratio of pressure to particle displacement is relatively high compared with the situation in the laboratory where the dominant stimulus component is almost certainly particle displacement. It is possible that the animals may respond only to the particle motion and not to sound pressure. While the particle motion in the laboratory was above the animals threshold, those in the field experiments were not so unless the *Nephrops* were very close to the sound source (ie. well within the near field). This is discussed further in the next section

5.4.3 Calculation of response thresholds

By moving the J9 from 1m to 0.09m it was possible to study the effect of increasing the displacement component relative to the sound pressure level. It was apparent from the results of the experiment conducted with the J9 at 1m and those with the outboard motor noise that *Nephrops* did not respond even to high sound pressure levels. By moving the loudspeaker to 0.09m it was possible to evoke responses in the form of both abdominal extension and leg movements. This relatively small change in sound source distance makes a considerable difference to the amplitude of particle displacement, eg. for a 30Hz stimulus at a sound pressure of 1 μbar at 0.1m the displacement is 0.274×10^{-5}cm; the same sound pressure level at 1m produces a particle displacement of 0.276×10^{-8}cm. The fact that responses were obtained at a sound source distance of 0.09m but not at 1m even though higher sound pressures were tested strongly suggests that *Nephrops* are sensitive to the particle displacement component of the sound.

In terms of sound pressure, the results showed that the thresholds of *Nephrops* were positively correlated with frequency. In terms of displacement the threshold was 0.874μm and this was independent of frequency.

The fact that *Nephrops* appear to be particle displacement sensitive is perhaps not unexpected as previous sensory thresholds to sound in crustaceans have been presented in terms of displacement. Offut (1970) who determined response thresholds of the lobster *Homarus americanus* in a small tank claimed that these animals had displacement and pressure thresholds which varied with frequency. He states that the lowest pressure threshold of -13db was obtained at 37.5Hz and the lowest displacement threshold of 8.1 x$10^{-4}\mu$m was obtained at 75Hz. Although these frequencies are within the range to which *Nephrops* is sensitive, the threshold values are many orders of magnitude smaller. Sensory thresholds calculated for the crayfish *Procambarus clarkii* (Wiese, 1976, Tautz and Sandeman, 1980) were between 0.1 and 0.6μm at 100Hz, these were also determined in the laboratory but are close to the displacement thresholds of *Nephrops* calculated here. It should be borne in mind however, that these are sensory thresholds and it is likely that the behavioural thresholds for this species will be even higher. This would suggest, as did the experiments in section 5.4.4 that the crayfish may be less sensitive than *Nephrops*.

Hearing thesholds for some fish species have been calculated under free sound field conditions. Displacement sensitive fish such as flatfish have displacement thresholds of 4 x$10^{-5}\mu$m at 110-160Hz and thresholds of 5 x$10^{-4}\mu$m at frequencies of 40 and 250Hz (Chapman and Sand, 1974). Pressure sensitive fish such as the cod, *Gadus morhua*, in which the swimbladder seems to act as a pressure-displacement transducer, have displacement thresholds of 0.5 x$10^{-4}\mu$m at 75Hz and

the salmon, *Salmo* has a threshold of 3.0 x10^{-4}μm at 75Hz. These are many orders of magnitude lower than the response thresholds of *Nephrops* demonstrating the difference in sensitivity between fish and crustaceans such as *Nephrops* and the crayfish. The threshold of *Homarus* (Offut, 1970) is close to that of fish species but it is strange that there should be such a large discrepancy between the lobster and *Nephrops* as the animals are closely related. Tests conducted in the acoustic tube (section 5.4.4), although not quantitative suggested that the lobster was in fact less responsive than *Nephrops*.

Hawkins and Myrberg (1983) conclude that it is the relative insensitivity of crustacean auditory systems which would prevent them from detecting the low amplitude water movements associated with sound in the far field and question whether these systems may be called "auditory" systems. This study would tend to support this comment since it is clear that *Nephrops* are unlikely to detect any naturally occurring far field sound and even in the near field the displacement threshold is extremely high. It is possible, however, that the dipslacement sensitive system of *Nephrops* is analogous to the lateral line system of fish rather than to the hearing system.

The lateral line responds primarily to water movement and it has been claimed that it also responds to low frequency near field sound (Sand, 1981). Studies have shown that the lateral line systems of fish are most sensitive to frequencies of 50-100Hz and have displacement thresholds of between 0.1.-0.5μm. These are within the frequency range and close to the sensitivity of the *Nephrops* system.

5.4.4 Biological significance of the behavioural responses of *Nephrops* to the particle displacement component of sound

The behavioural responses shown by *Nephrops* to particle displacement stimuli occurred in two forms: untethered animals showed locomotory responses while tethered animals (section 4.3.1 and 5.3.5) showed postural changes in the form of abdominal extension and leg movements. In the laboratory the locomotory response took the form of backwards locomotion both in the tank and in the acoustic tube. These responses were not related to the position of the loudspeaker (Fig. 5.11). *Nephrops* spend a large proportion of their time sitting at the entrances of their burrows. The purpose of the response to sound may be to convey the animal backwards down its burrow It is known that during backwards walking a cyclic pattern of abdominal extension occurs (Kovac, 1974) and it may be that if the animal cannot walk backwards the extension response may still be expressed. In tethered animals leg movements always accompanied abdominal extension (section 4.3.1), and tailflipping was sometimes seen to low frequency tones at high amplitudes (section 4.3.1). It is possible that these three behaviour patterns, backwards walking, abdominal extension and tailflipping are successive steps in a behavioural hierarchy, ie. during backwards locomotion abdominal extension occurs; if locomotion cannot take place abdominal extension will occur more vigorously; if the animal remains stressed by the stimulus and cannot move away from it using either of these behaviours it may then resort to tailflipping.

The inevitable question arising from this study is, would *Nephrops* ever encounter above threshold particle displacements from sound sources in their natural environment? This study has shown that it is very unlikely that *Nephrops* will be able to respond to the

grunting sounds produced by their natural predator, the cod, unless it is very close to them. It also seems unlikely that *Nephrops* will be able to detect man-made sounds such as trawl gear except at very close range. The lateral lines of fish are used to detect the presence of other fish during schooling (Blaxter, 1981) and it is possible that *Nephrops* may be able to detect the displacements produced by cod as they swim through the water.

It should be remembered that in the near field it may be difficult for animals to differentiate between vibratory sound stimuli and hydrodynamic effects such as water turbulence and currents (Hawkins 1985). It is conceivable that the present sound experiments stimulated sensory receptors of *Nephrops* which normally function in response to other types of stimulus.

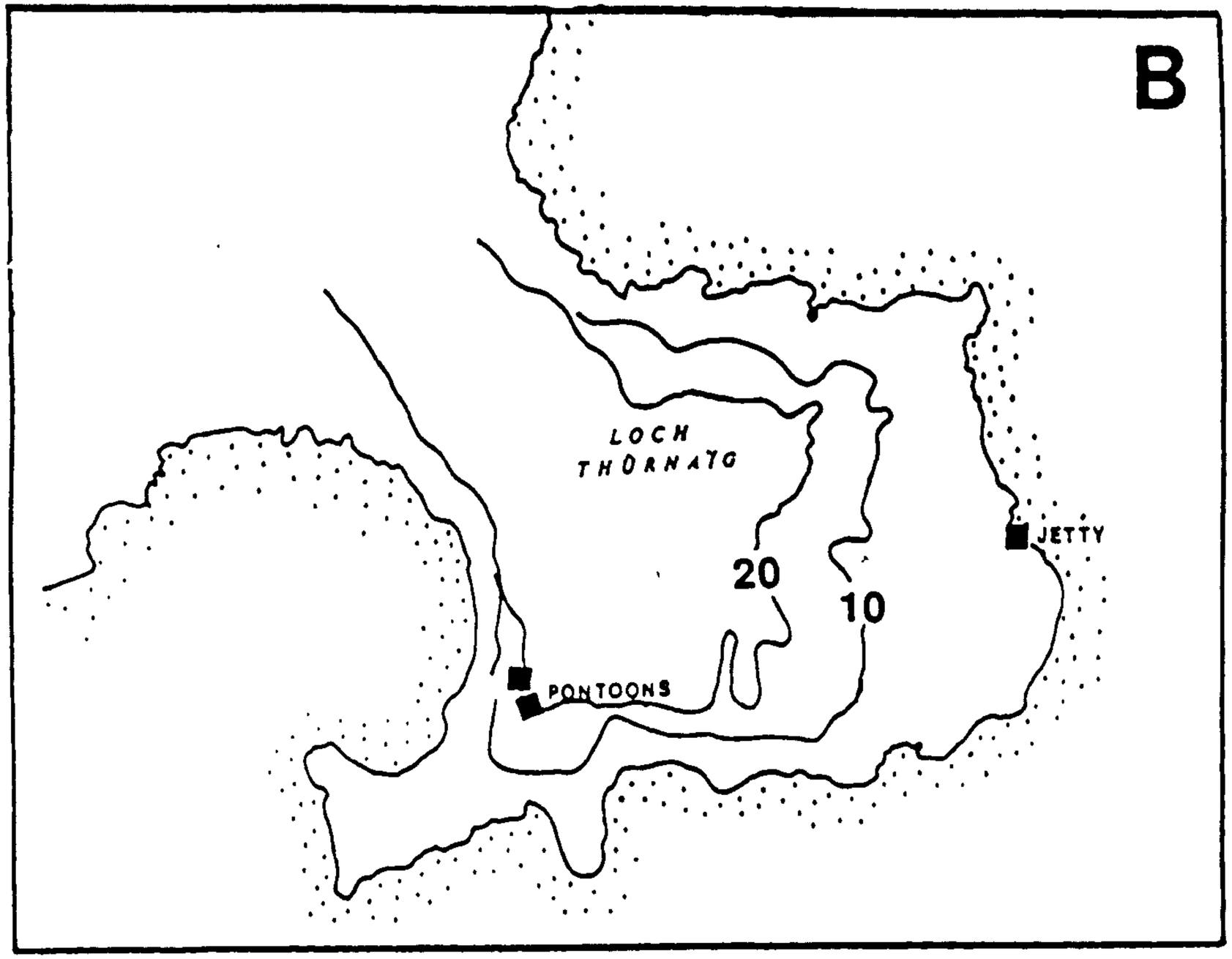
TABLE 5.1

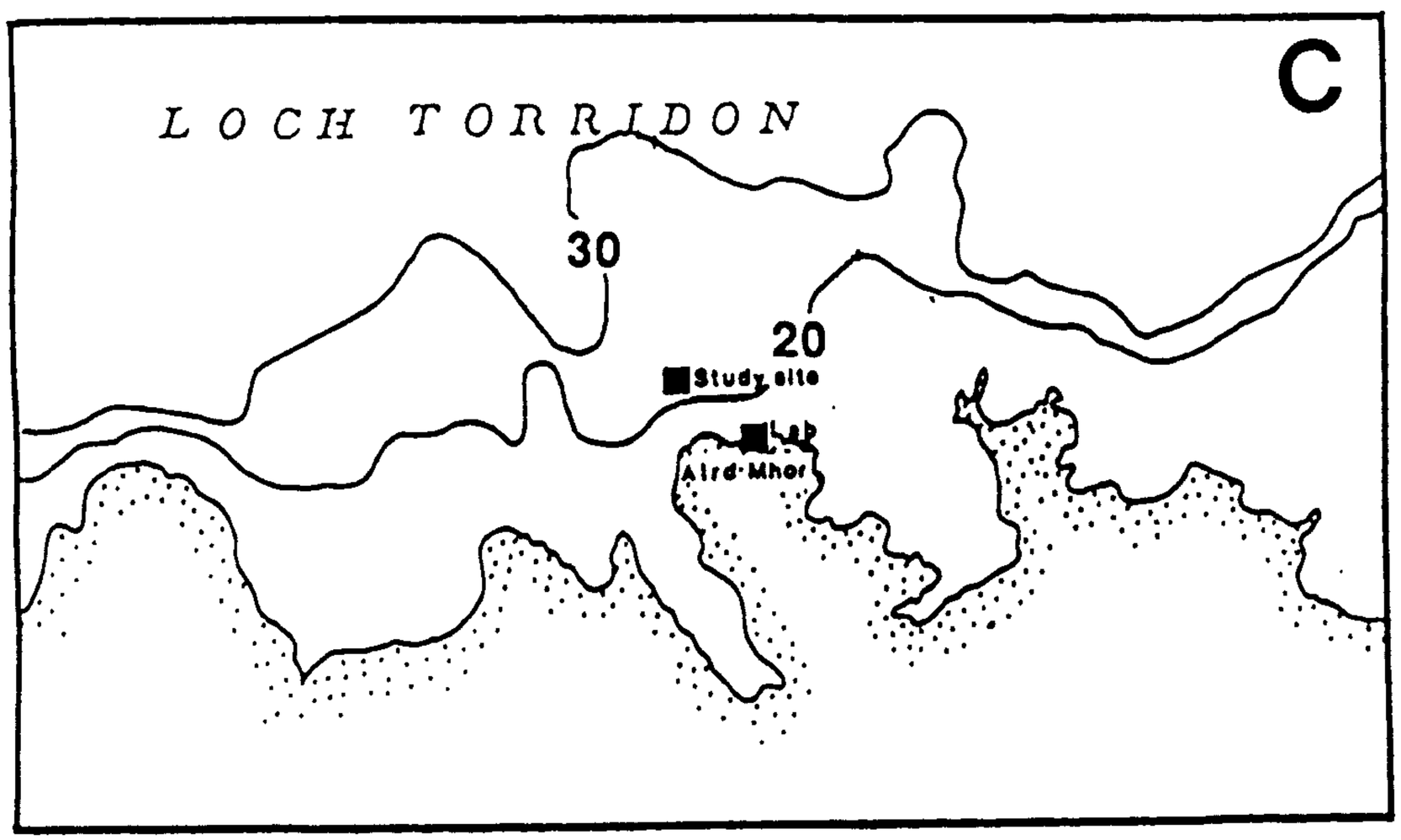
Date	Sound Frequency	Start of test period	End of test period	Rate of presentation
29/9/86	Control- no sound	9pm	8am	
31/8/86	40-340Hz	8pm	6am	Every alternate hour
2/9/86	20-50Hz	8pm	5am	Every alternate hour
4/9/86	20-20,000Hz	7.30pm	10.30pm	Every alternate half hour

Figure 5.1 A. Map of Scotland showing the distribution of *Nephrops* grounds (shaded areas) around the coast. Also marked (open squares) are the locations of the two study sites, Loch Ewe and Loch Torridon.

B. Chart of Loch Ewe, Wester Ross, showing the position of the lab pontoons and jetty (shaded squares). The depth of water (metres) is indicated on the contour lines around the study area.

C. Chart of part of Upper Loch Torridon , Wester Ross, showing Aird Mohr point and the position of the shore lab and study site. The depth of water (metres) is indicated on the contour lines around the study site.


B
LOCH
THORNATG
JETTY
20
10
PONTOONS


C
LOCH TORRIDON
30
20
Study site
Aird Mhor

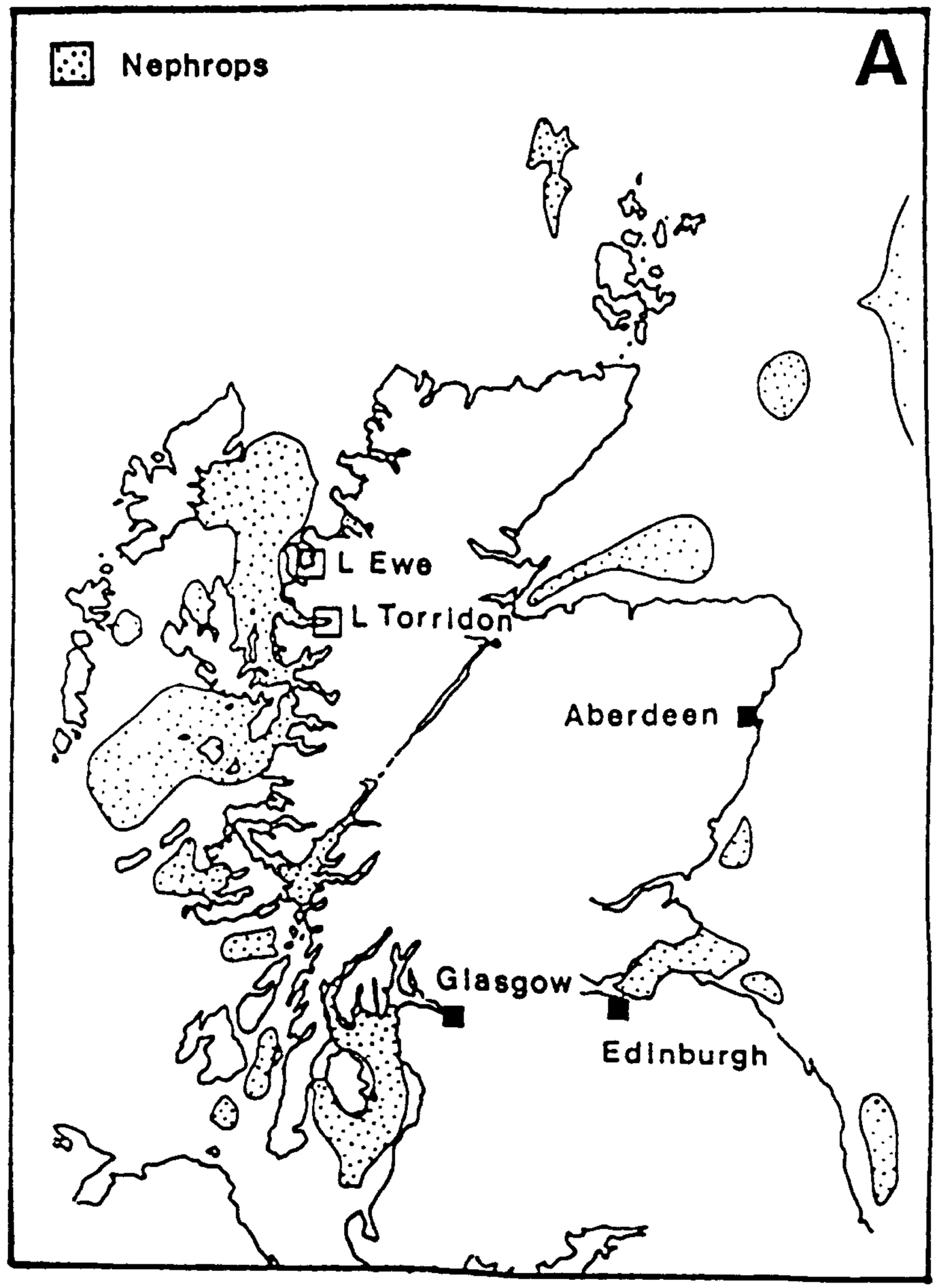

Nephrops
A
L Ewe
L Torridon
Aberdeen
Glasgow
Edinburgh

Figure 5.2 A. Diagram of the camera frame used during experiments in Loch Torridon to study changes in the burrow emergence rhythm of *Nephrops* in response to sound (5.2.1). The base of the frame was 1.2m square and the frame was 0.6m high.

C underwater TV camera

P pan and tilt

L red light source

H hydrophone

B. Diagram of the experimental frame used during experiments in Loch Torridon to study transient changes in the behaviour of *Nephrops* in response to sound (5.2.2). The base of the frame was 1.8m square, the top of the frame was 1.2m square and the frame was 0.9m high. The hydrophone was positioned so that it was exactly 1m from each J9 loudspeaker. The camera lens was 0.9m above the base of the frame.

C underwater TV camera

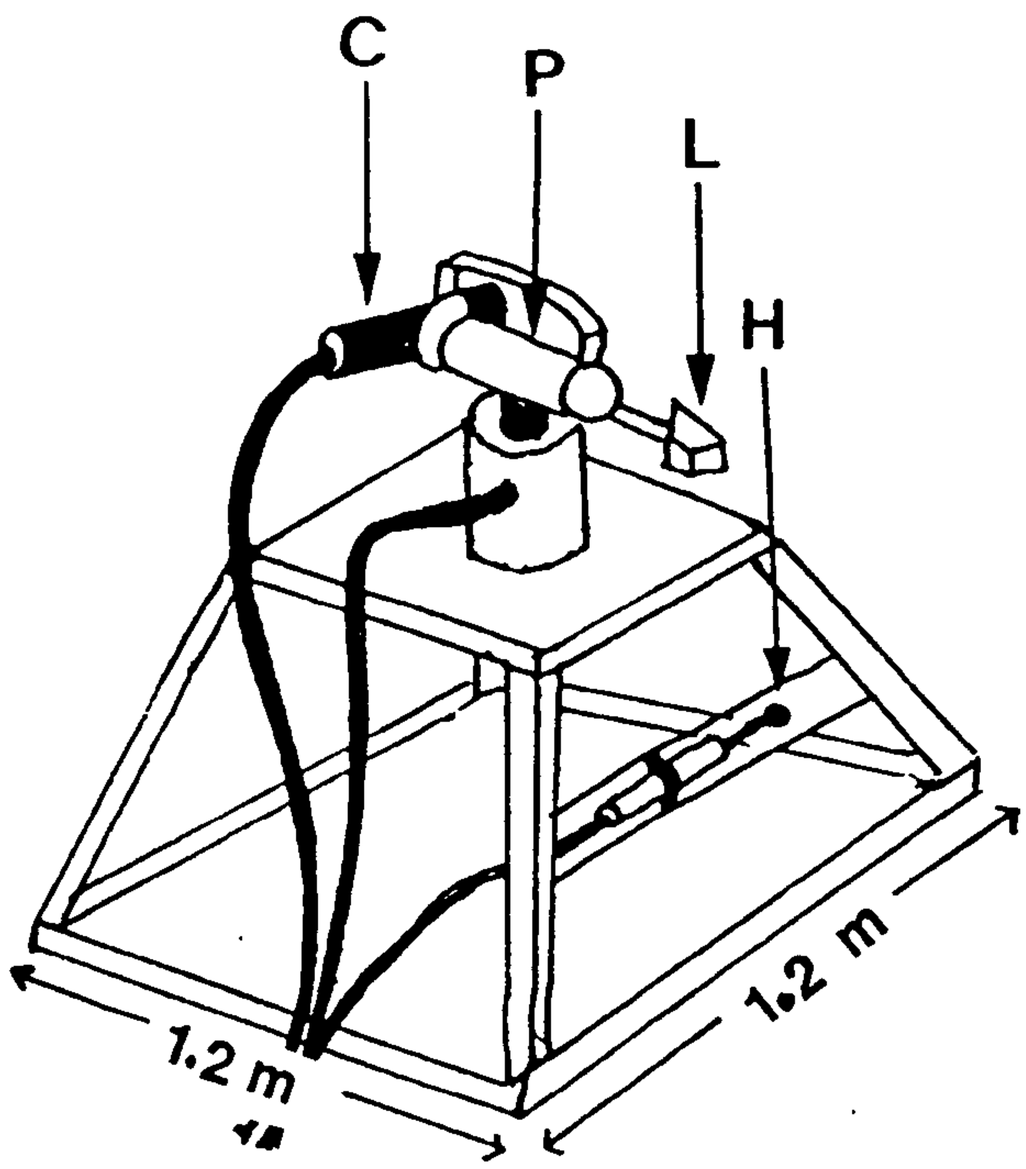
P pan and tilt

J J9 loudspeaker

L red light source

H hyrophone

A



B

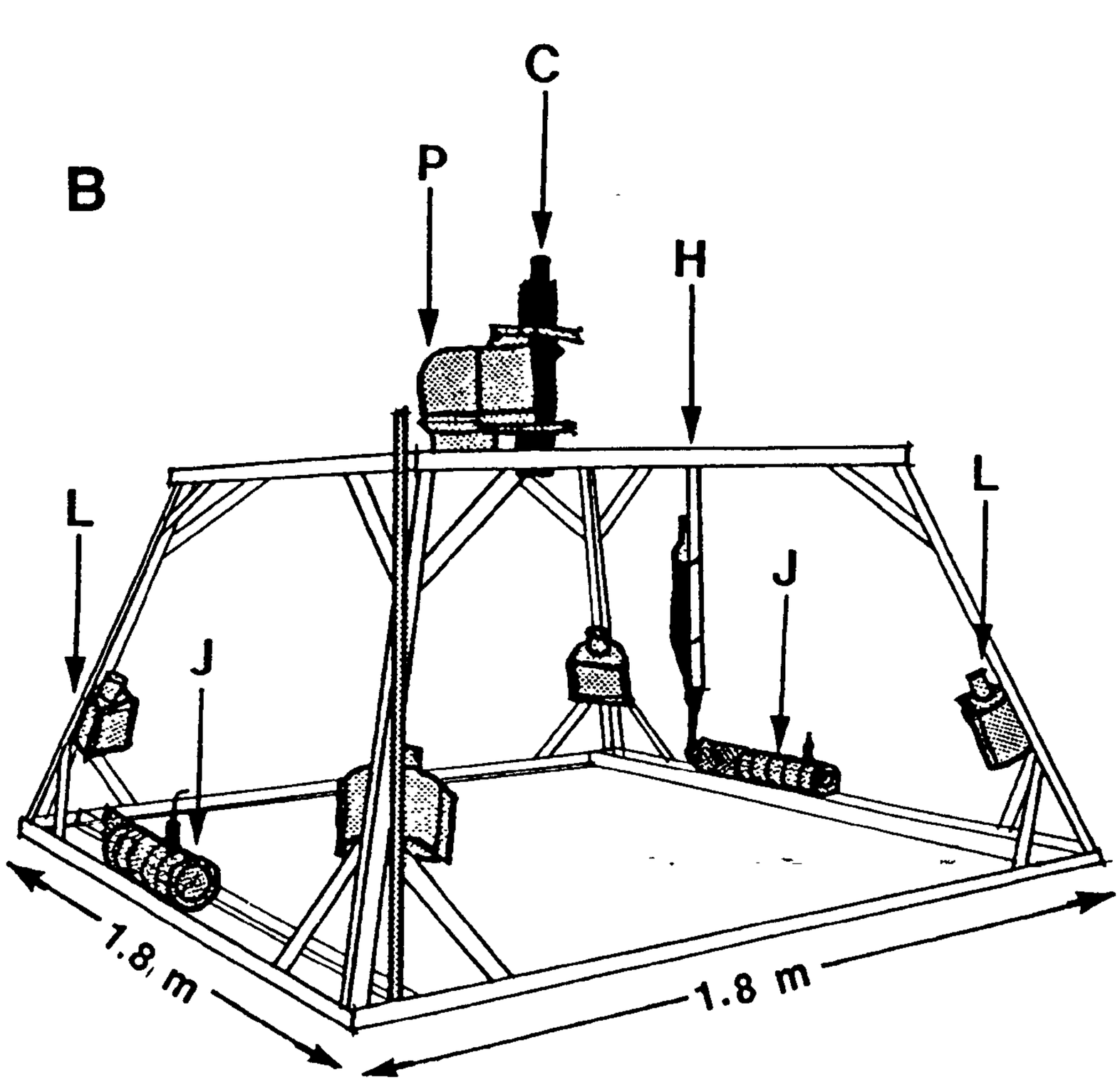


Figure 5.3 Photograph of the experimental frame used during experiments in Loch Ewe, Wester Ross, to calculate the response thresholds of *Nephrops* to sound (5.2.5). The animal was contained within a perforated perspex box (B), the hydrophone (H) was mounted directly above the animal, the underwater TV camera (C) faced the side of the box, the J9 (J) was positioned exactly 0.09m from the animal in the same plane.


H
C
B
J

Figure 5.4 A. Frequency analysis of the ambient sea noise measured during each of the 4 study periods at a depth of 30m in Loch Torridon (5.3.1). The plot shows the spectrum levels (db re 1μbar) against frequency (Hz).

□ study period 1 (control)

▲ study period 2 (40-3430Hz)

■ study period 3 (20-50Hz)

–· study period 4 (20-20,000Hz)

B. Frequency analysis of the noise produced by components of the pan and tilt mechanism. Plot shows spectrum levels (db re 1μbar) against frequency (Hz).

□ tilt up

— tilt down

■ pan right

▲ pan left

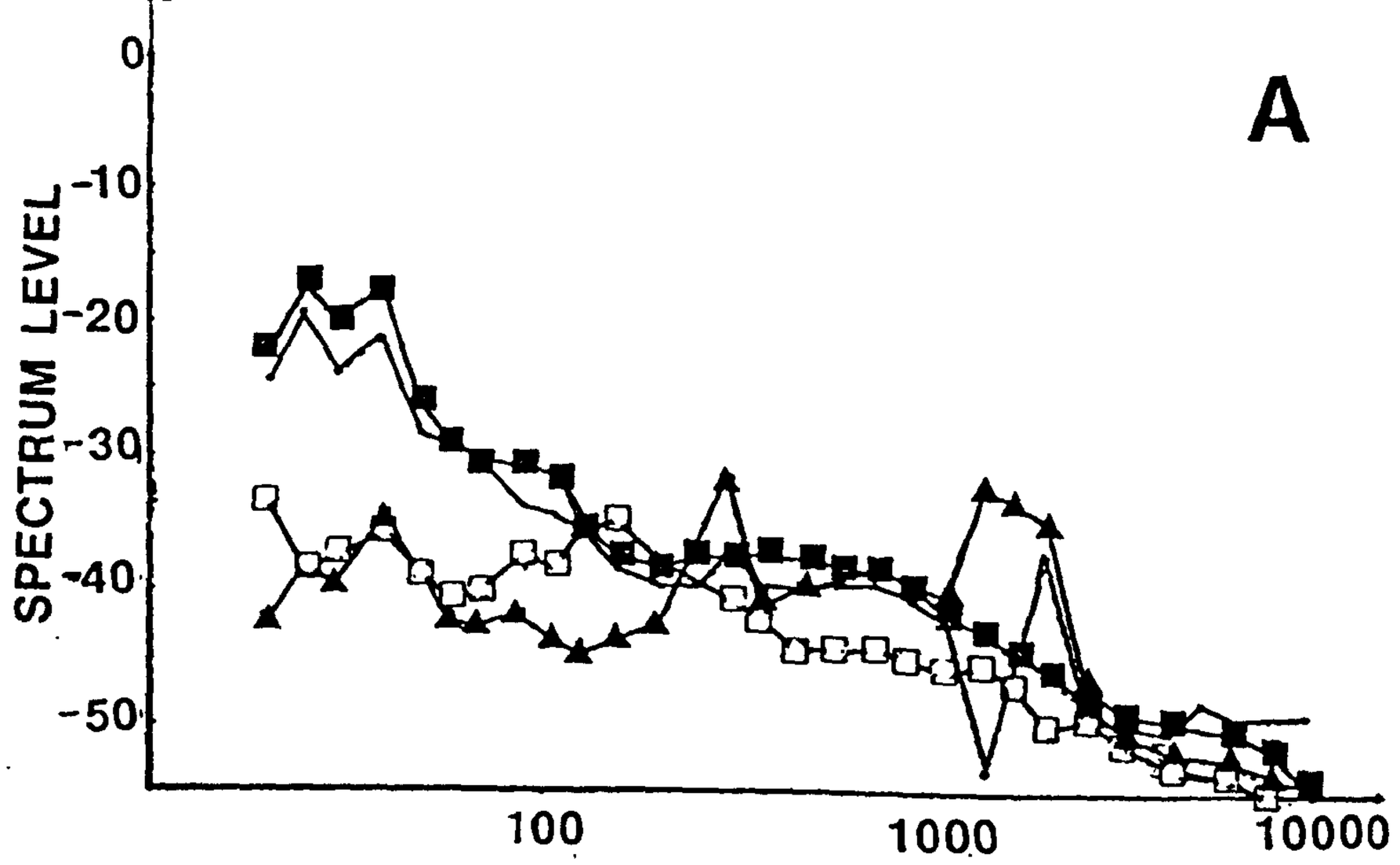
C. Frequency analysis of the sounds played via the J9 sound projectors. The plot shows the sound pressure levels (SPL in db re 1μbar) against frequency (Hz).

□ 20-50Hz

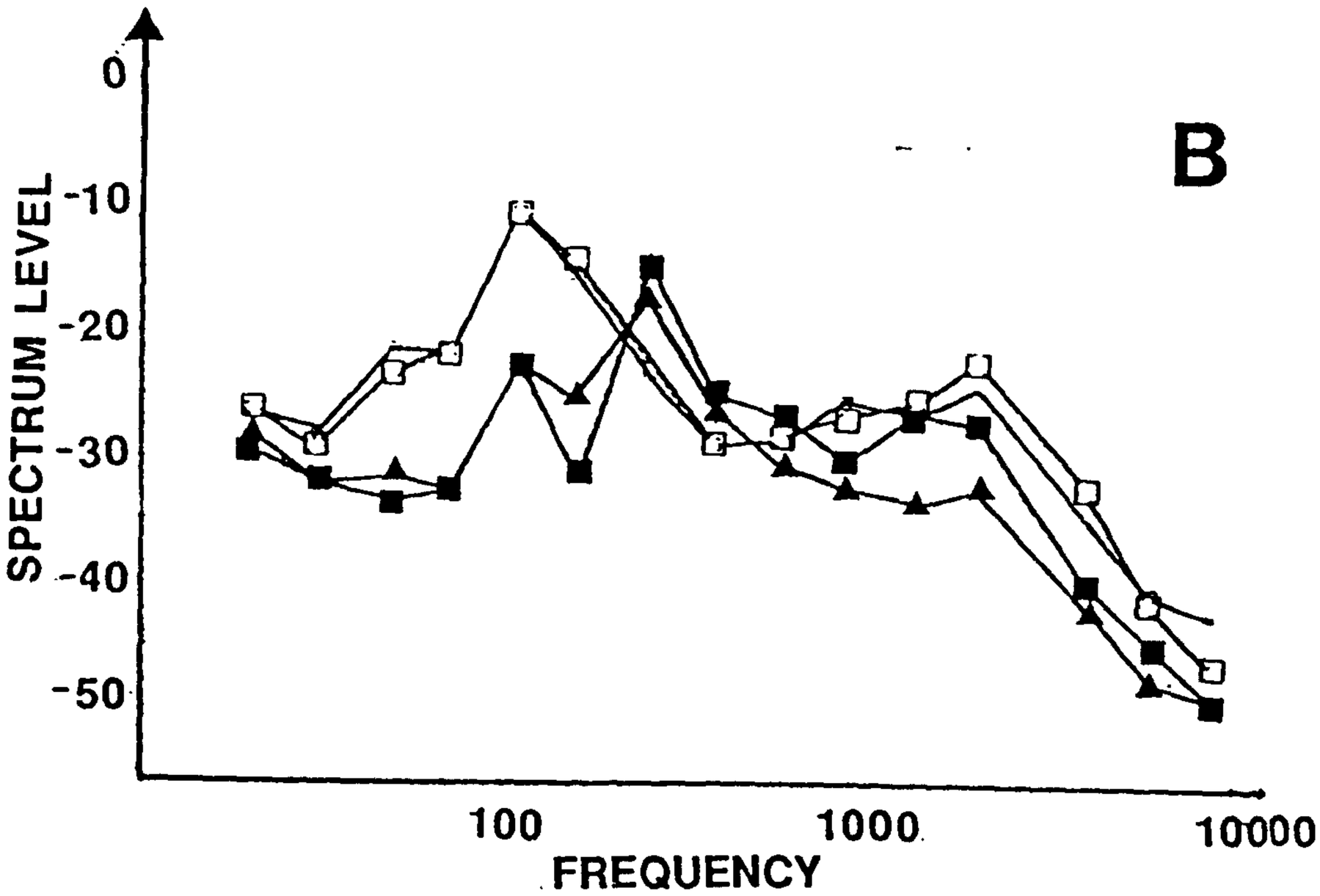
■ 40-340Hz

▲ 20-20,000Hz

A


SPECTRUM LEVEL
0
-10
-20
-30
-40
-50
100
1000
10000
FREQUENCY

B


SPECTRUM LEVEL
0
-10
-20
-30
-40
-50
100
1000
10000
FREQUENCY

C

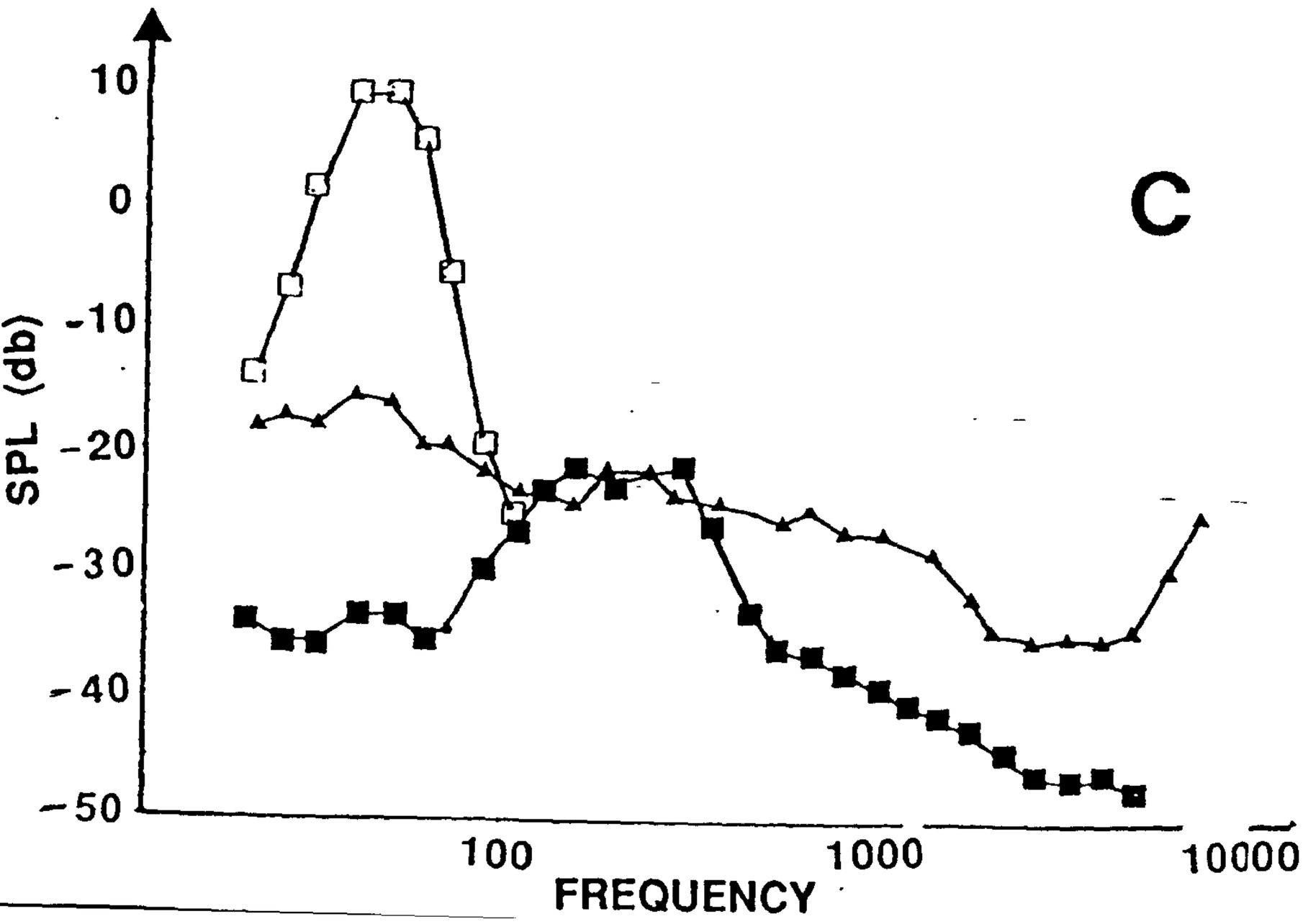
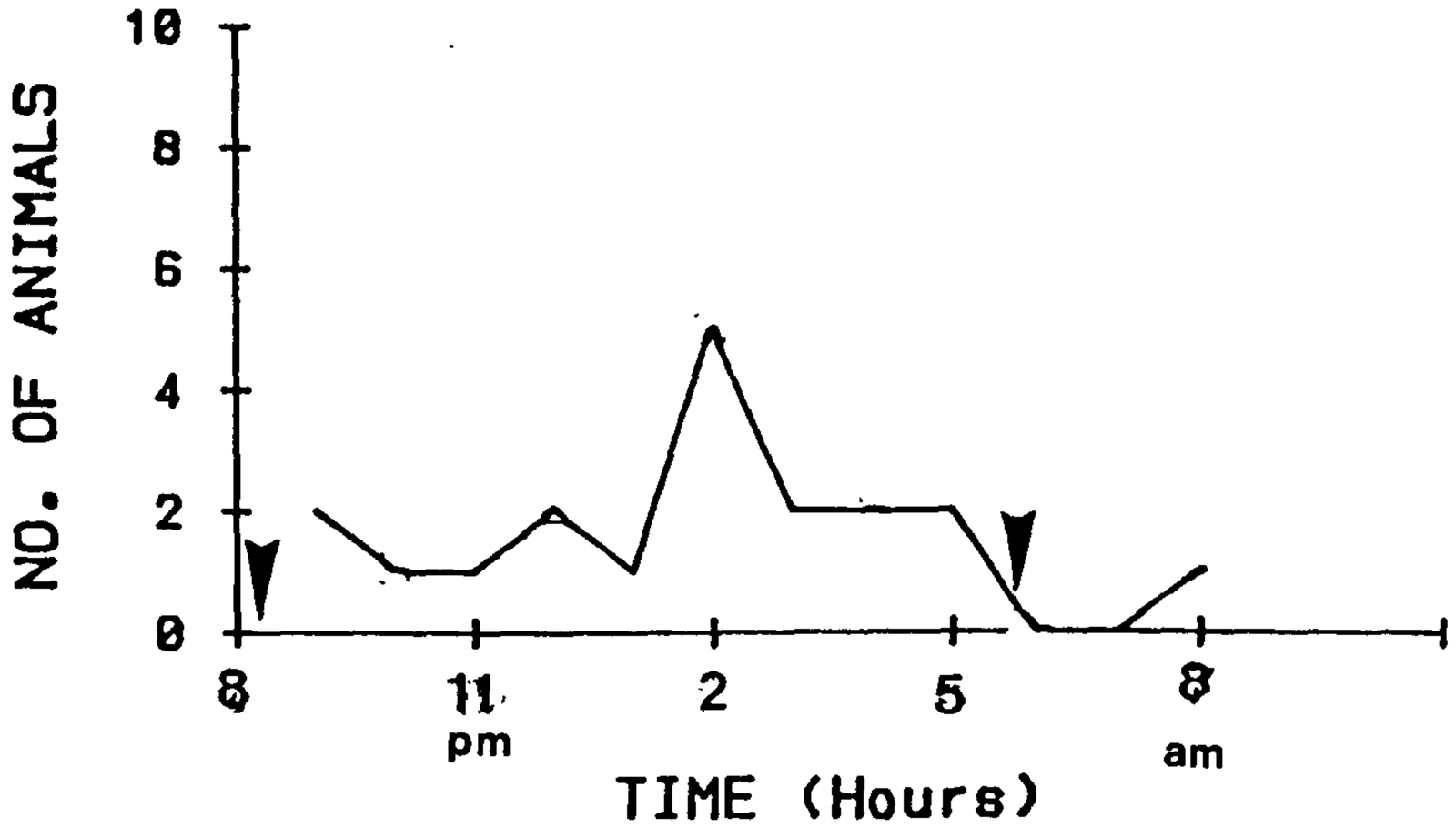

SPL (db)
10
0
-10
-20
-30
-40
-50
100
1000
10000
FREQUENCY

Figure 5.5 Numbers of resident (A) and non-resident (B) *Nephrops* seen within the field of view each hour during study period 1 (control). Plot shows animal numbers aginst chronological time (hours, B.S.T.). Arrows indicate times of sunrise and sunset.

A


RESIDENT CONTROL
NO. OF ANIMALS
10
8
6
4
2
0
8
11
pm
2
5
8
am
TIME (Hours)

B

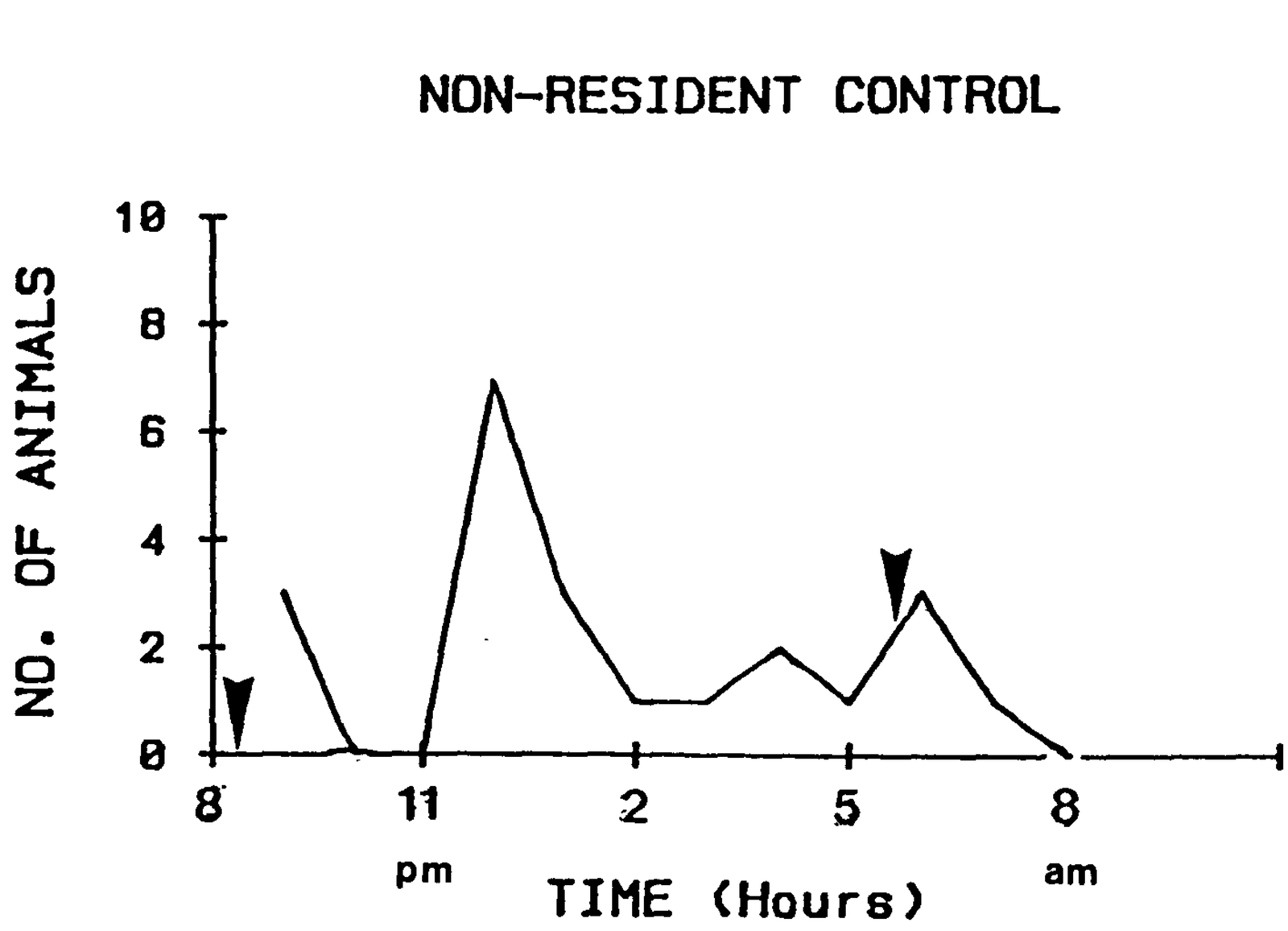
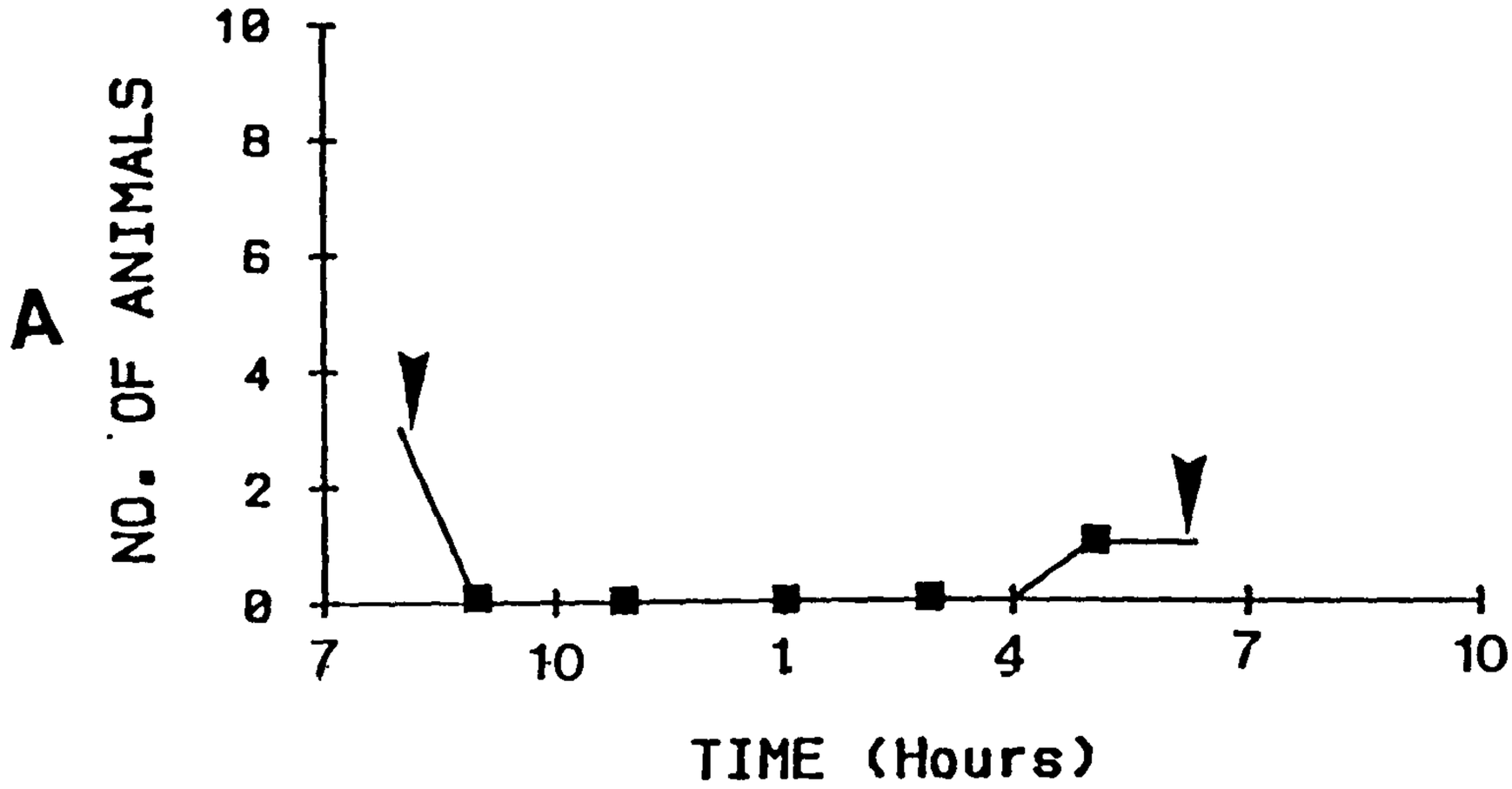

NON-RESIDENT CONTROL
NO. OF ANIMALS
10
8
6
4
2
0
8
11
pm
2
5
8
am
TIME (Hours)

Figure 5.6 Numbers of resident (A) and non-resident (B) *Nephrops* seen within the field of view during study period 2 (40-340Hz). Plots show numbers of animals against chronological time (hours, B.S.T.). Arrows indicate the times of sunrise and sunset. Black squares indicate the start of an experimental period (sound on) of one hours duration.

40-340HZ RESIDENT



40-340HZ NON-RESIDENT

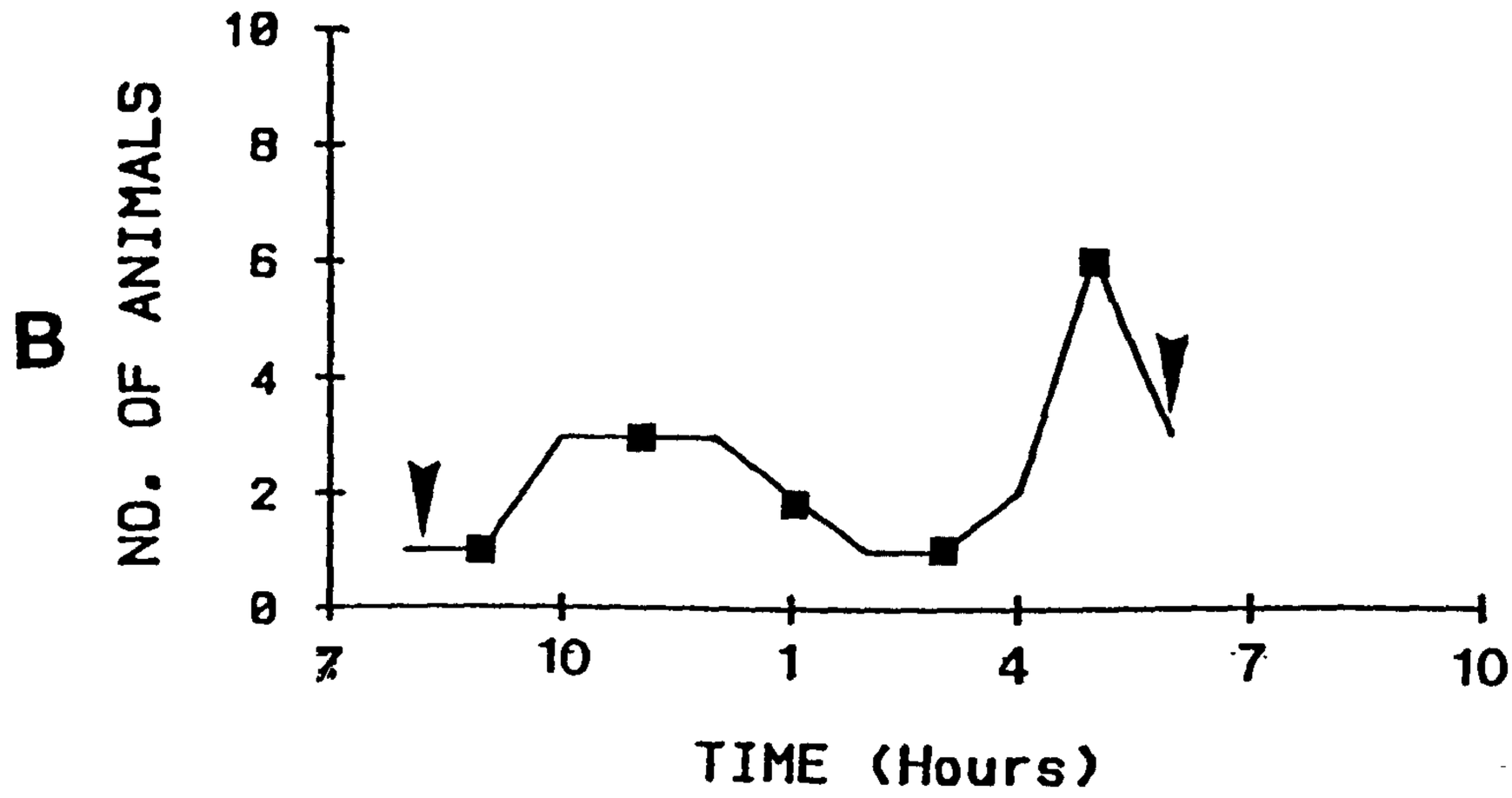
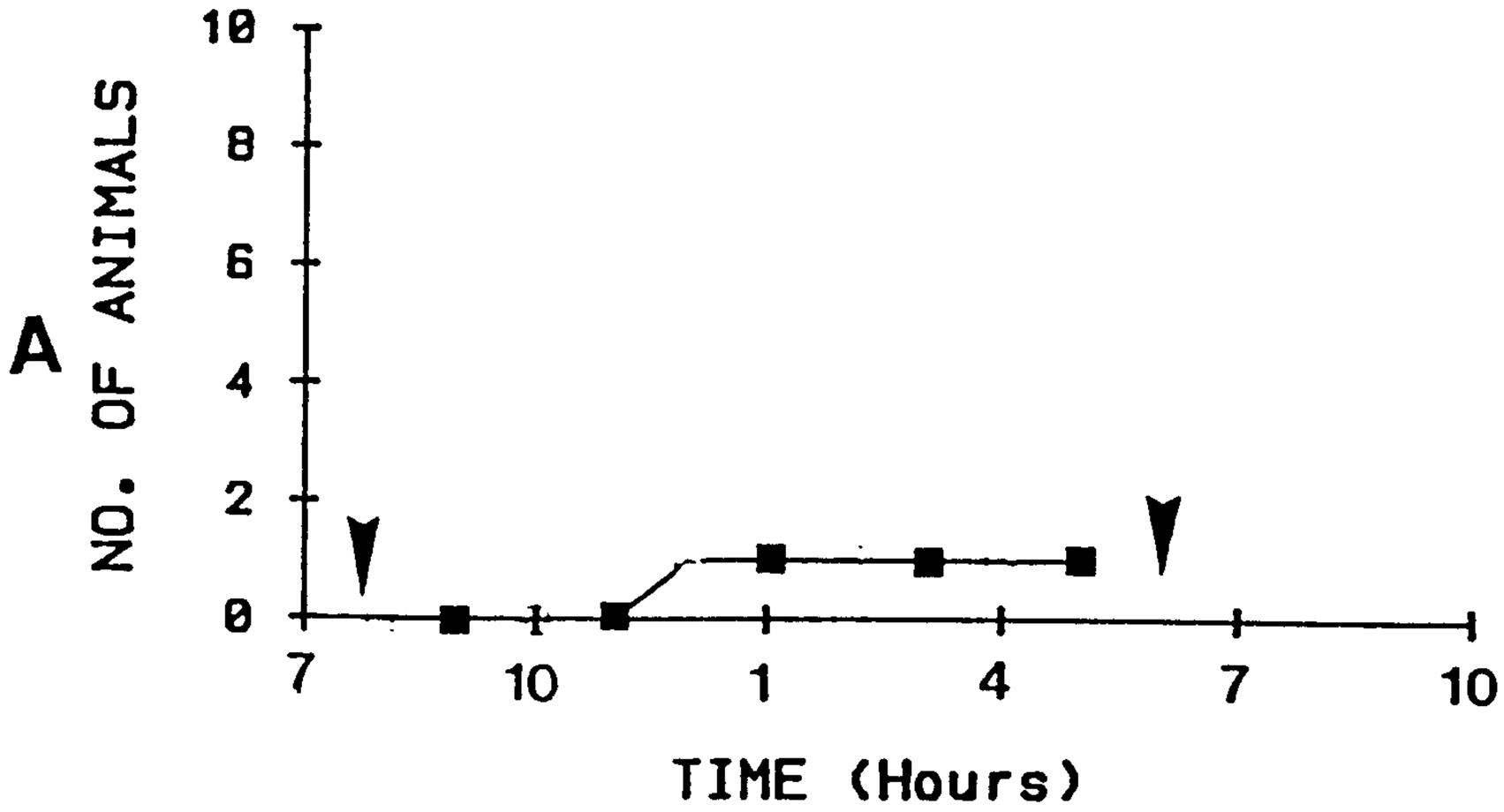


Figure 5.7 Numbers of resident (A) and non-resident (B) *Nephrops* seen within the field of view during study period 3 (20-50Hz). Details as Figure 5.6.

RESIDENT 20-50HZ



NON-RESIDENT 20-50HZ

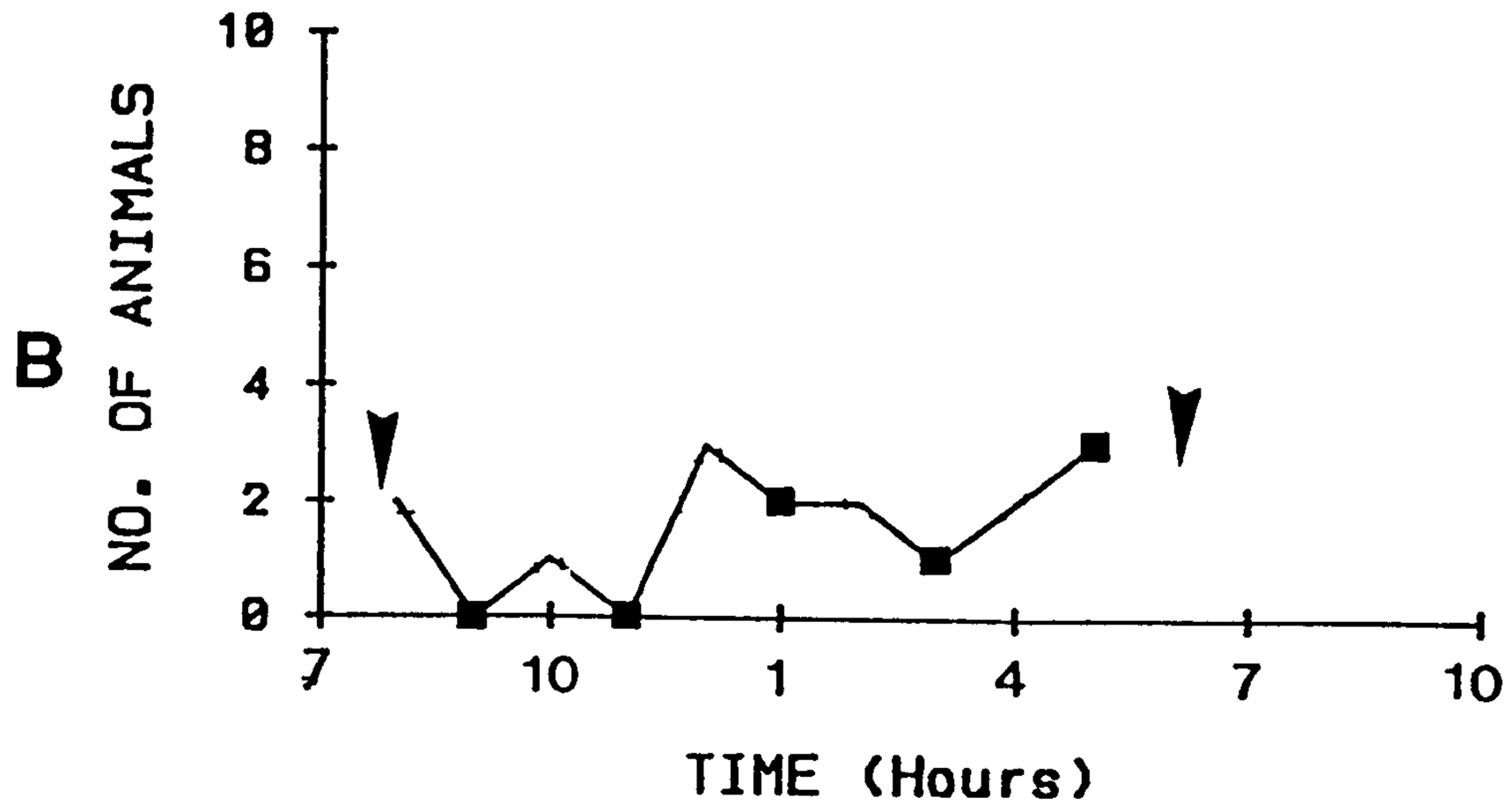
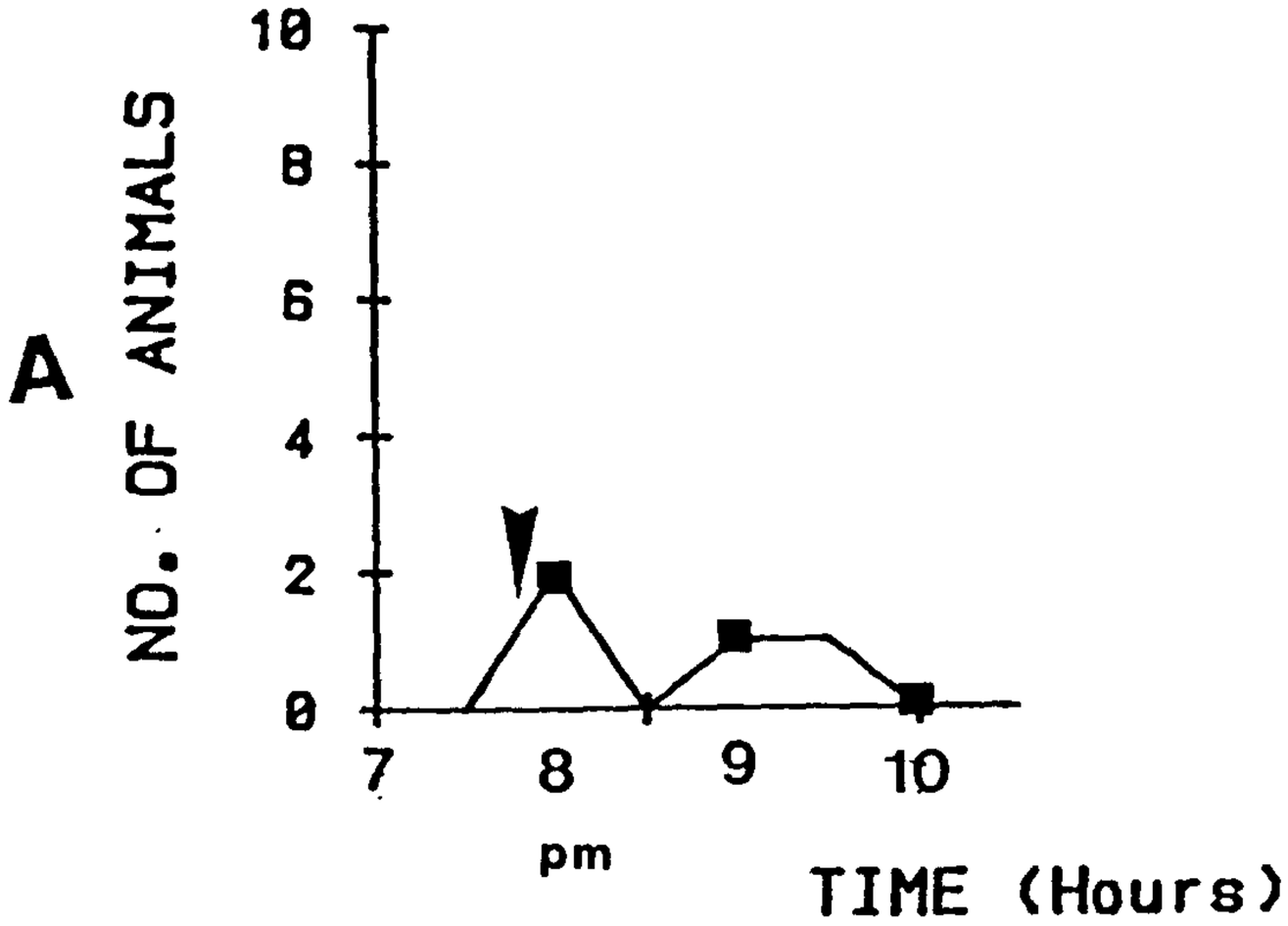


Figure 5.8 Numbers of resident (A) and non-resident (B) *Nephrops* seen within the field of view during study period 4 (20-20,000Hz). Black squares indicate the start of an experimental period of 30 minutes duration. Other details as Figure 5.6.

A


RESIDENT WHITE NOISE
NO. OF ANIMALS
10
8
6
4
2
0
7
8
9
10
pm
TIME (Hours)

B

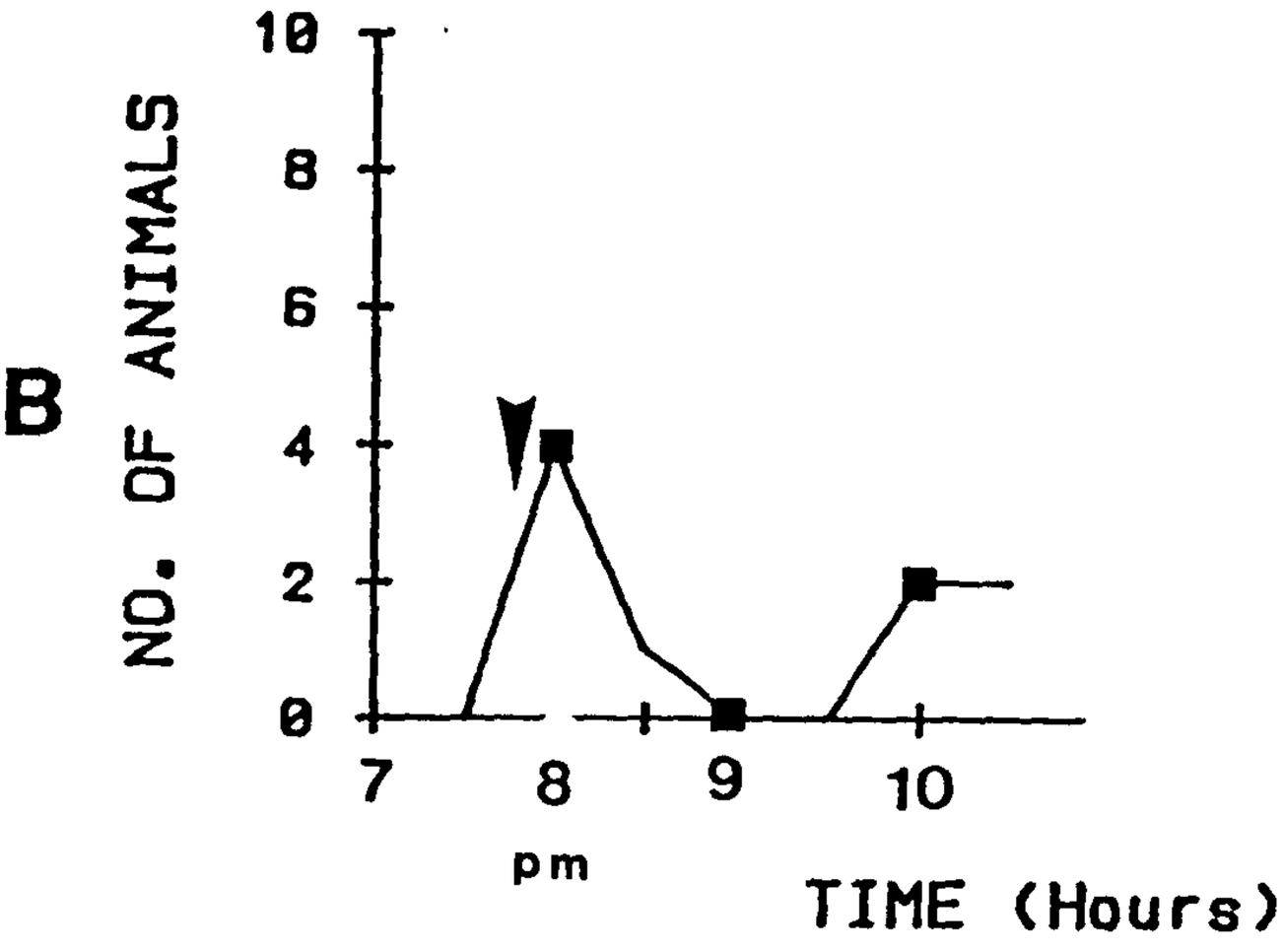

NON-RESIDENT WHITE NOISE
NO. OF ANIMALS
10
8
6
4
2
0
7
8
9
10
pm
TIME (Hours)

Figure 5.9 A. Frequency analysis of the ambient sea noise (5.3.2) showing the average sea noise levels measured over the entire study period. Plot shows the spectrum level (db re 1μbar) against frequency (Hz).

B. Frequency analysis of the taped trawl noise (5.3.2). Plots show the spectrum level (db re 1μbar) against frequency (Hz).

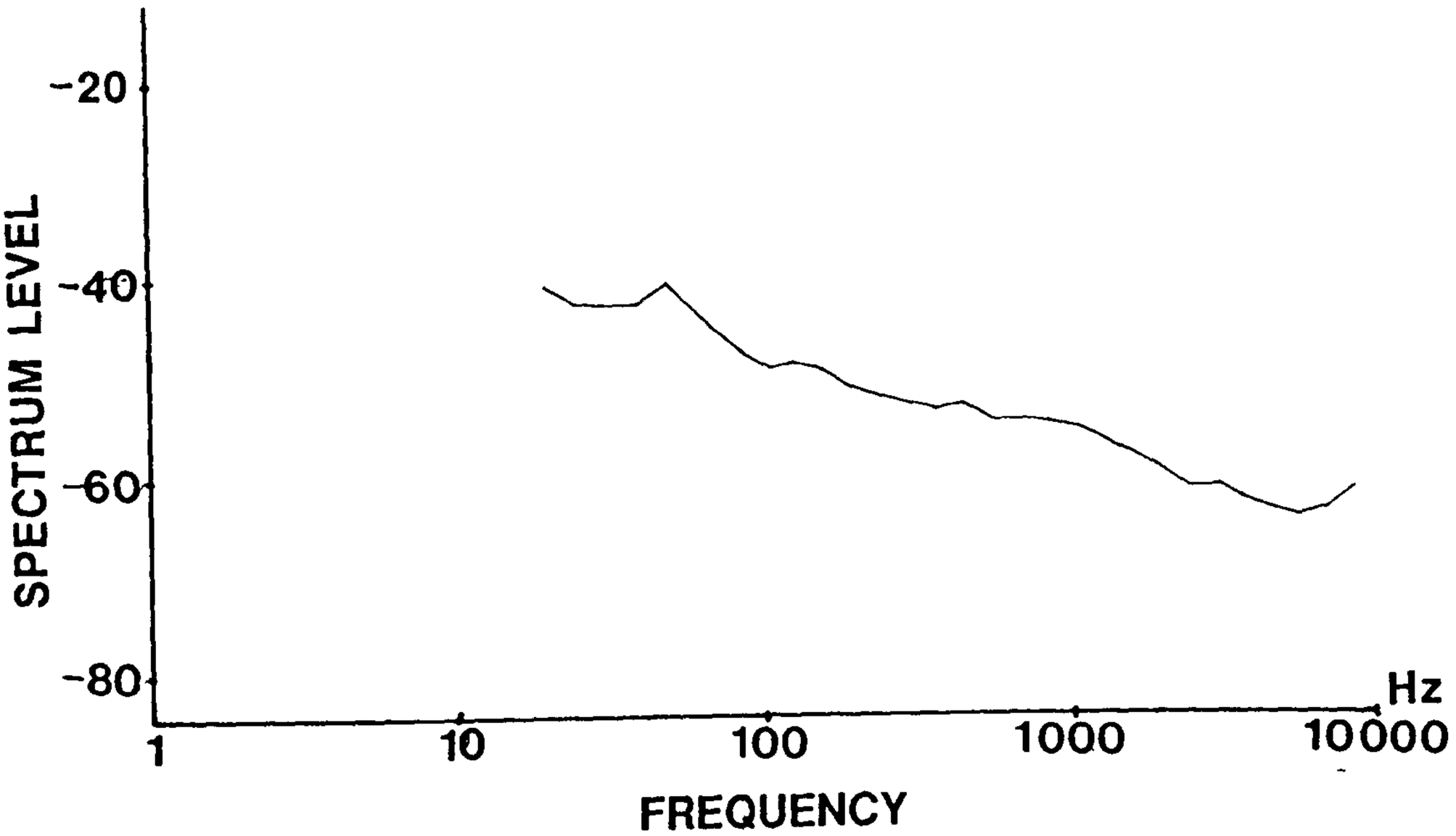
C Bobbin chain gear on hard ground

D Above starboard door

E Above footrope

F Above bobbins

A


SPECTRUM LEVEL
-20
-40
-60
-80
Hz
1
10
100
1000
10000
FREQUENCY

B

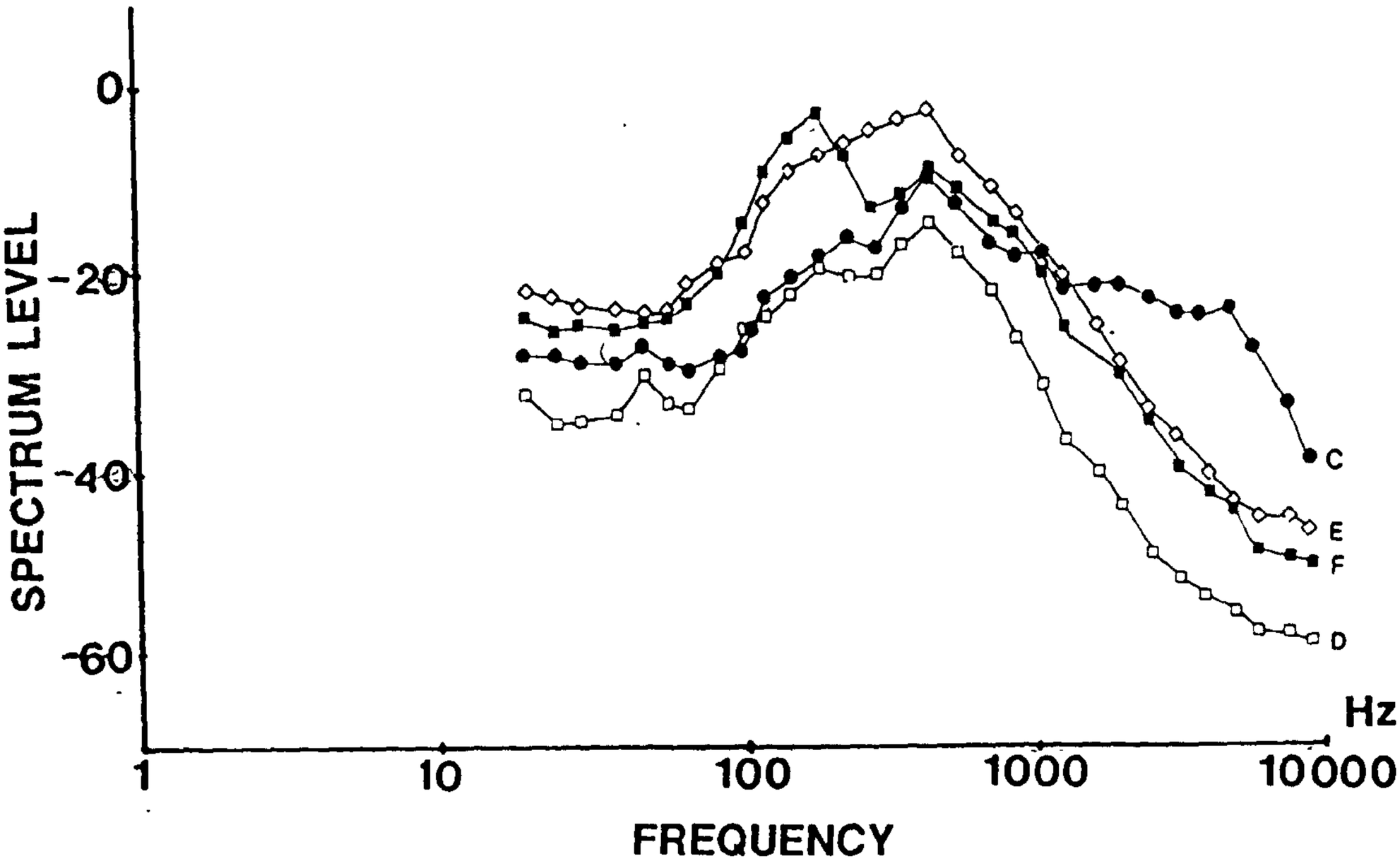
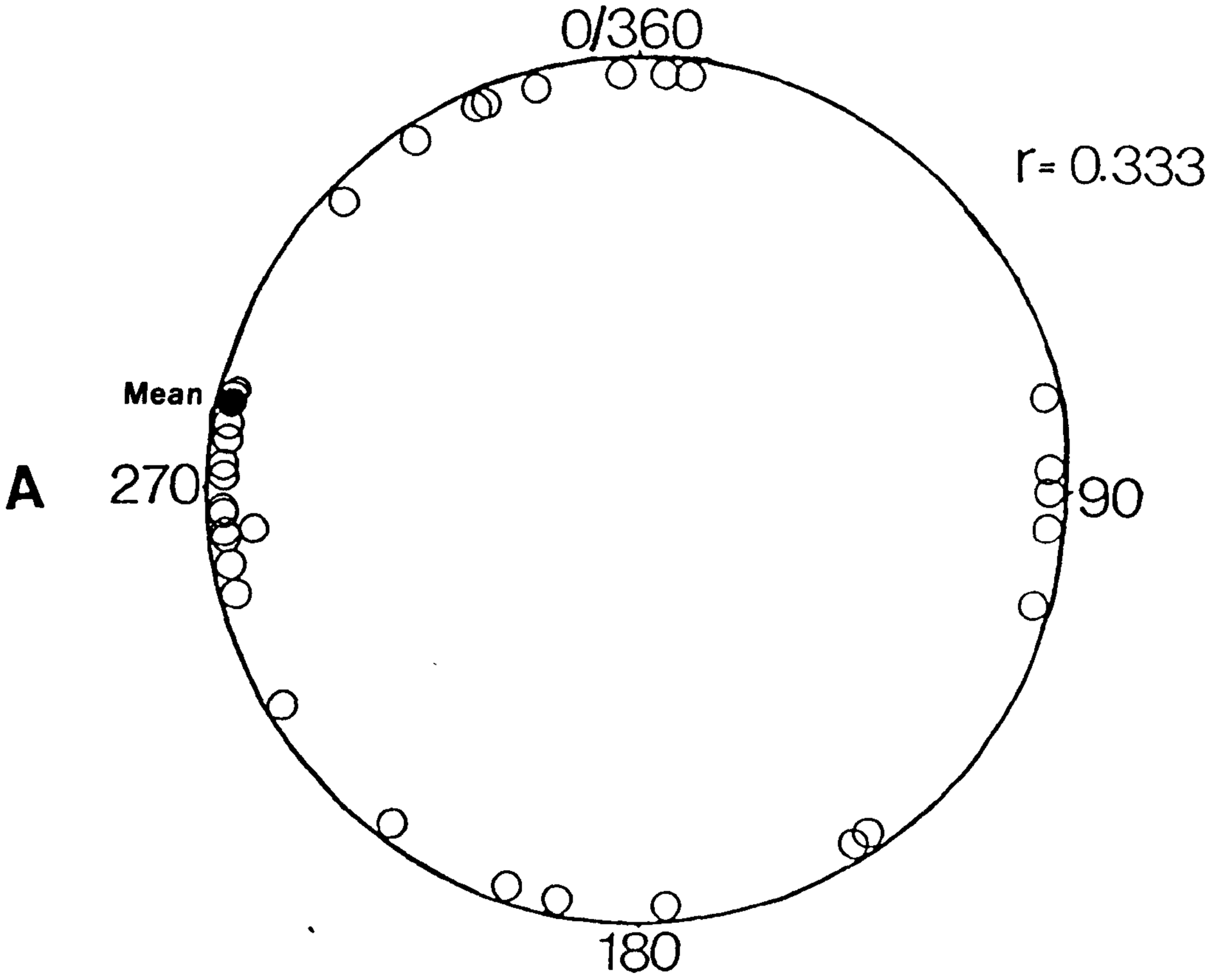

SPECTRUM LEVEL
0
-20
-40
-60
C
E
F
D
Hz
1
10
100
1000
10000
FREQUENCY

Figure 5.10 Circular plots showing the orientation (degrees) of *Nephrops* before (A) and after (B) water borne vibrational stimuli between 20-80Hz of 10 seconds duration. Plots show data from 34 tests which were tested for significance using a Rayleigh test. In plot A all animals were stationary.


A
0/360
90
180
270
Mean
r= 0.333

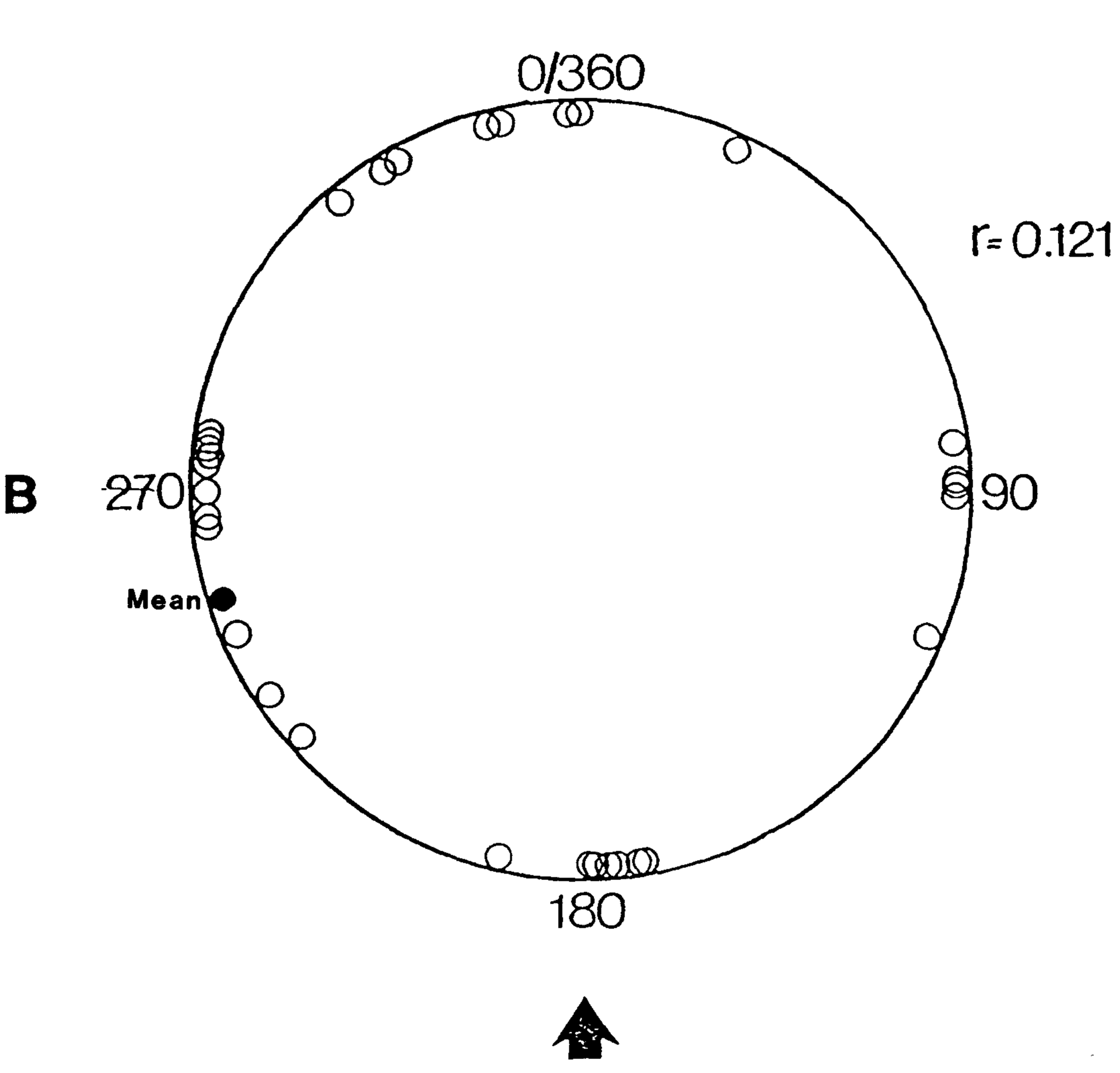
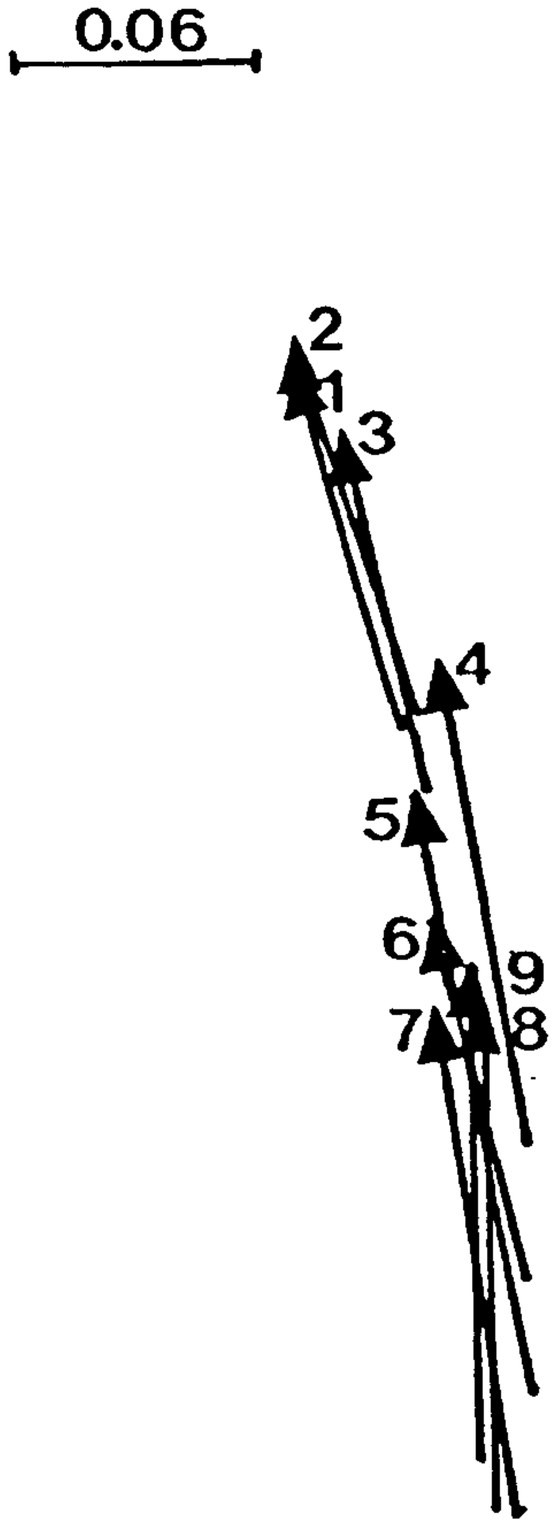
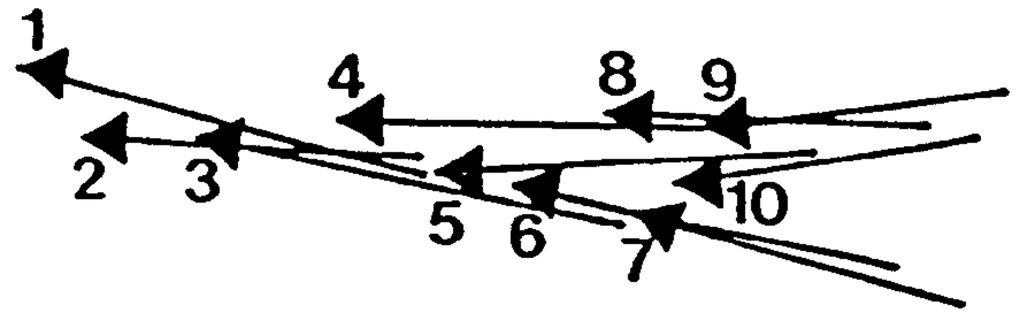

B
0/360
90
180
270
Mean
r= 0.121

Figure 5.11 A-G. Locomotory response patterns shown by *Nephrops* in response to water borne vibrations of frequencies from 20-80Hz. Plots show the position and orientation of the animals plotted every second for 10 seconds after the stimulus onset. Arrows indicate the animal's anterior end, numbers indicate the time in seconds. The scale bar indicates distance (metres). The large arrow indicates the position of the loudspeaker with reference to the animal but not its exact distance from the animal. Letters A-G are further explained in the text.

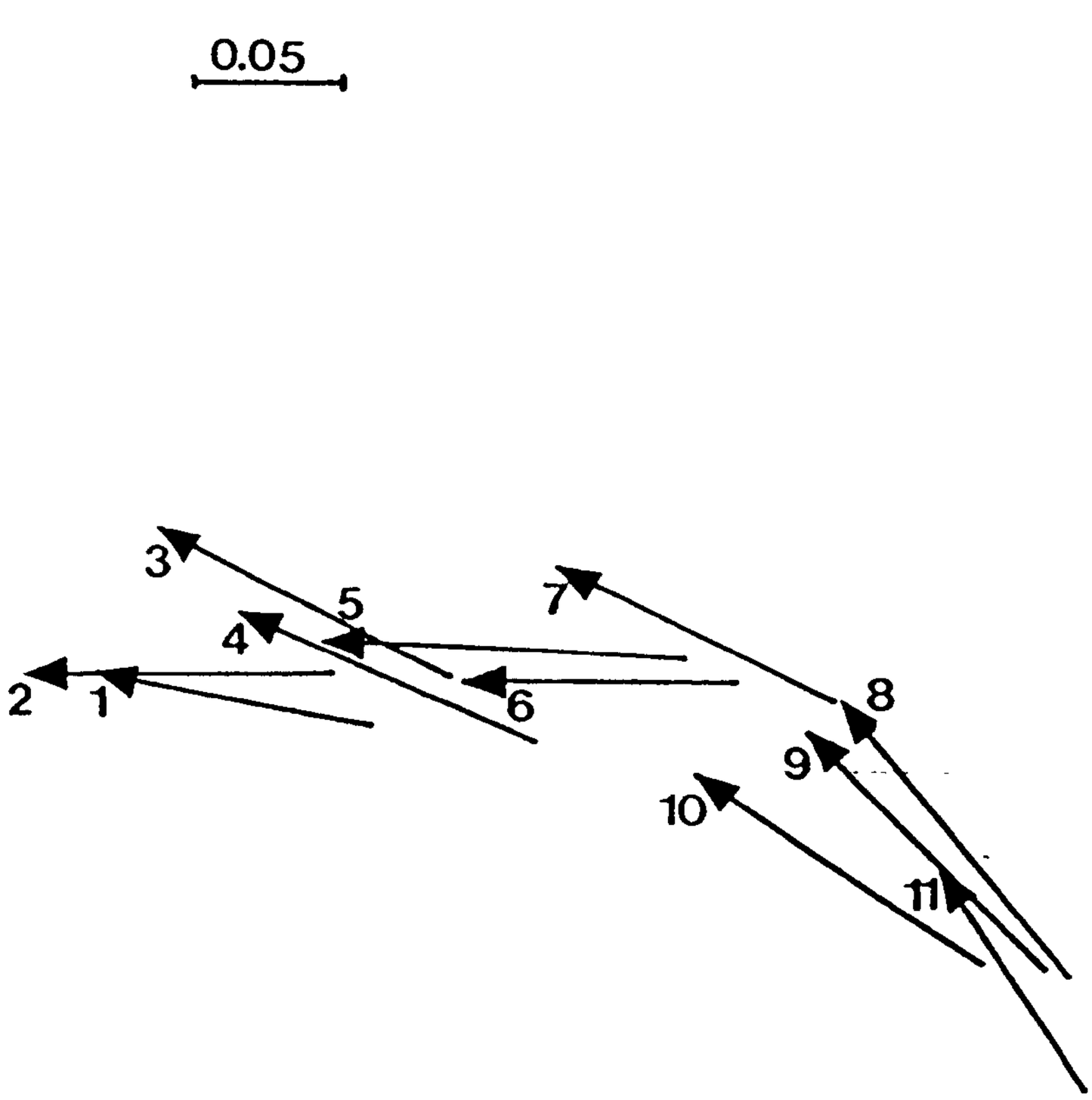
A



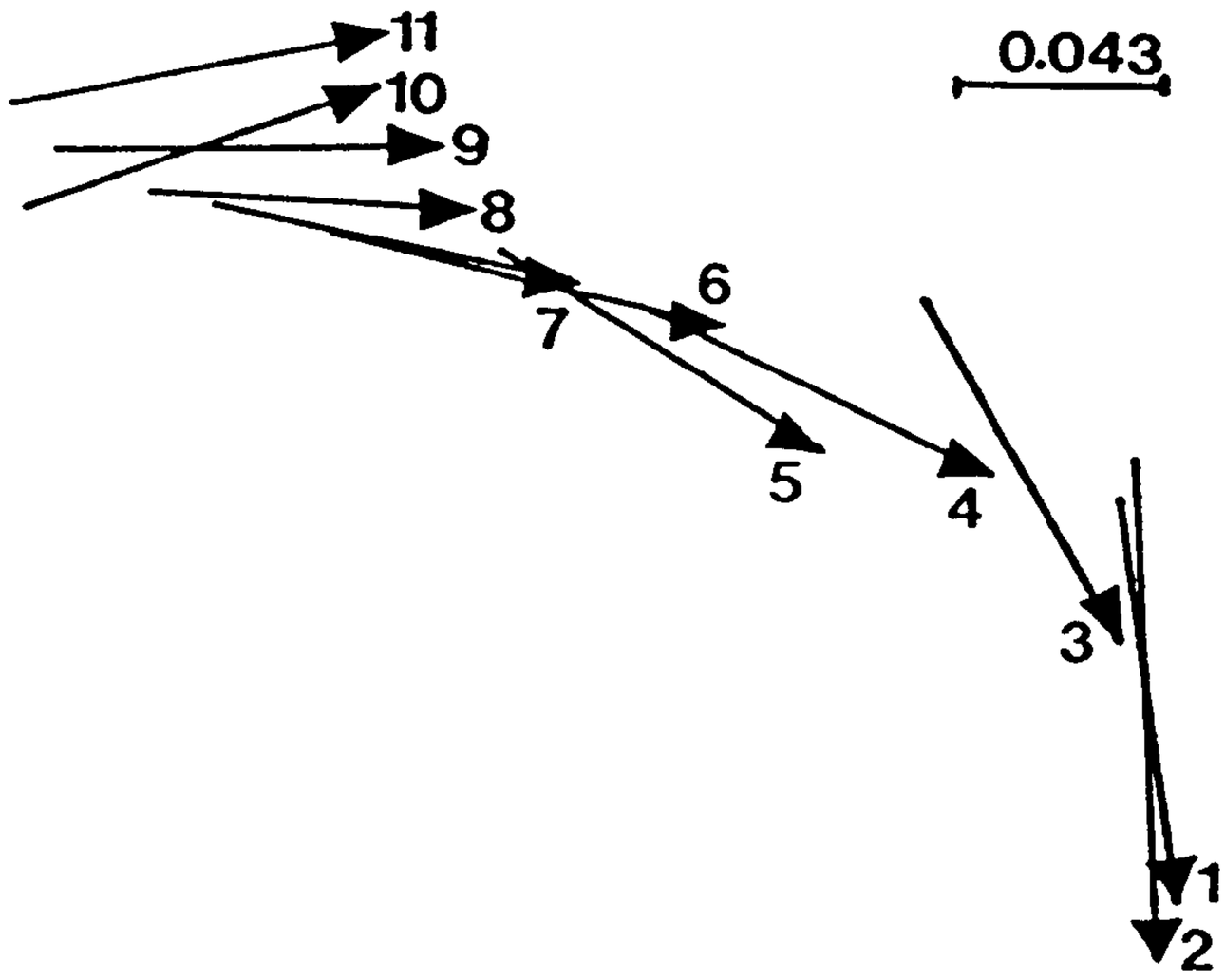
B



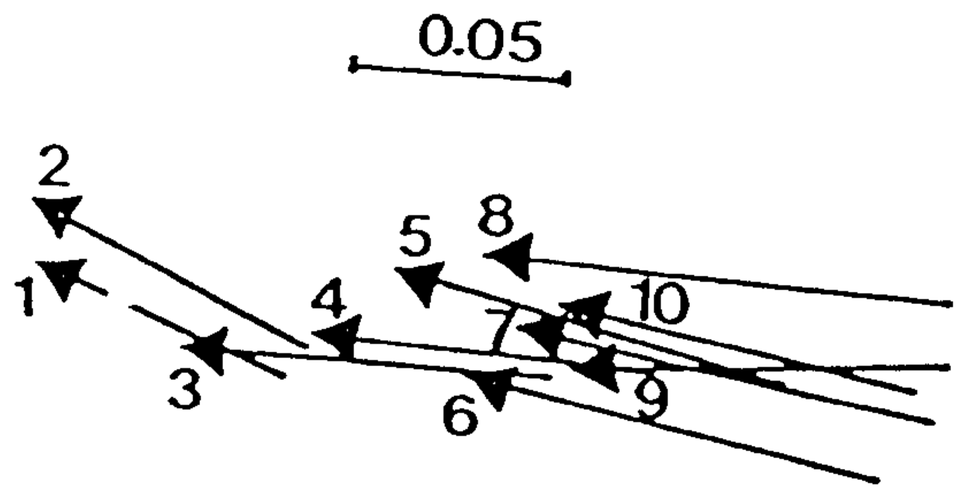
C



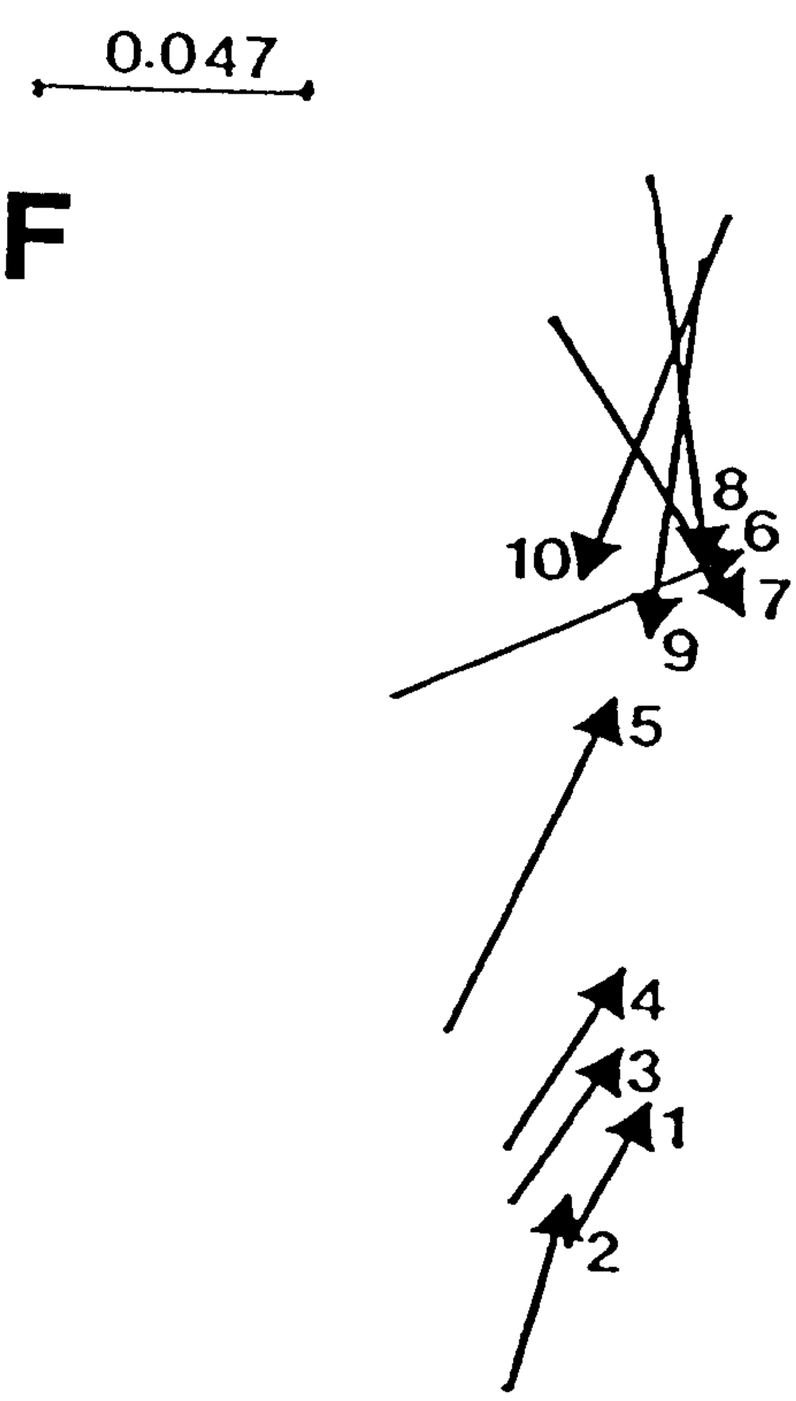
D



E



F



G

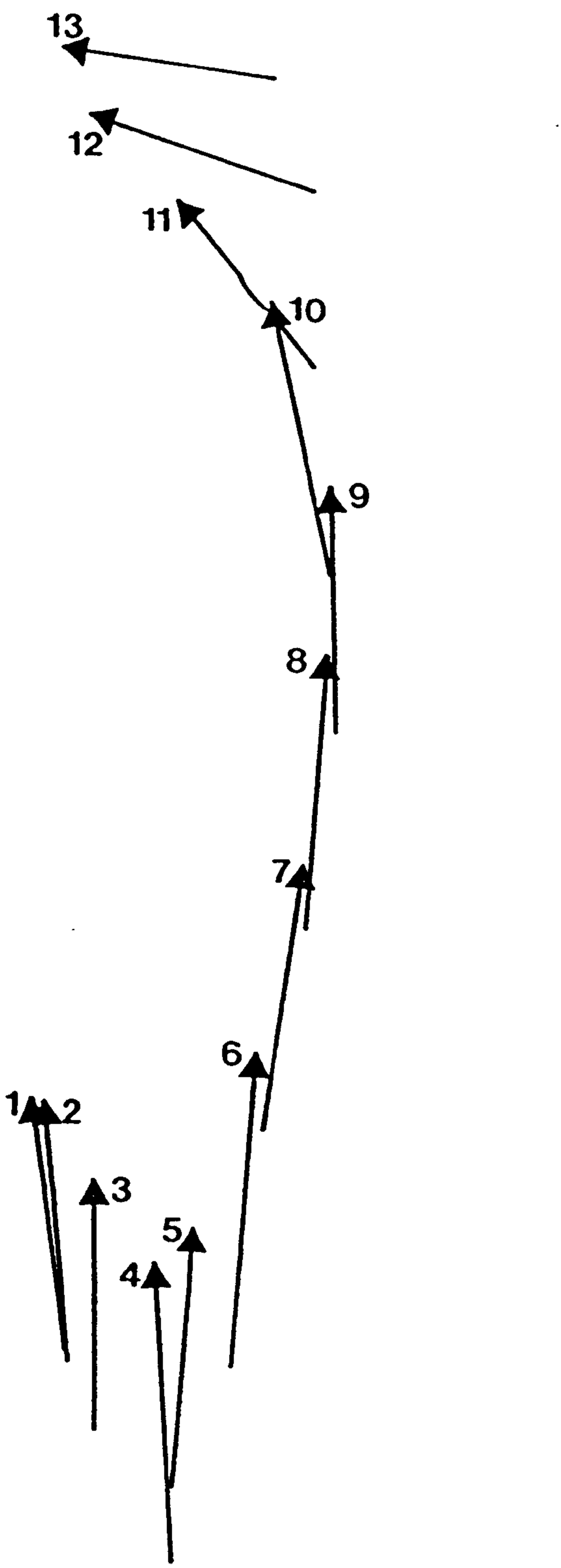


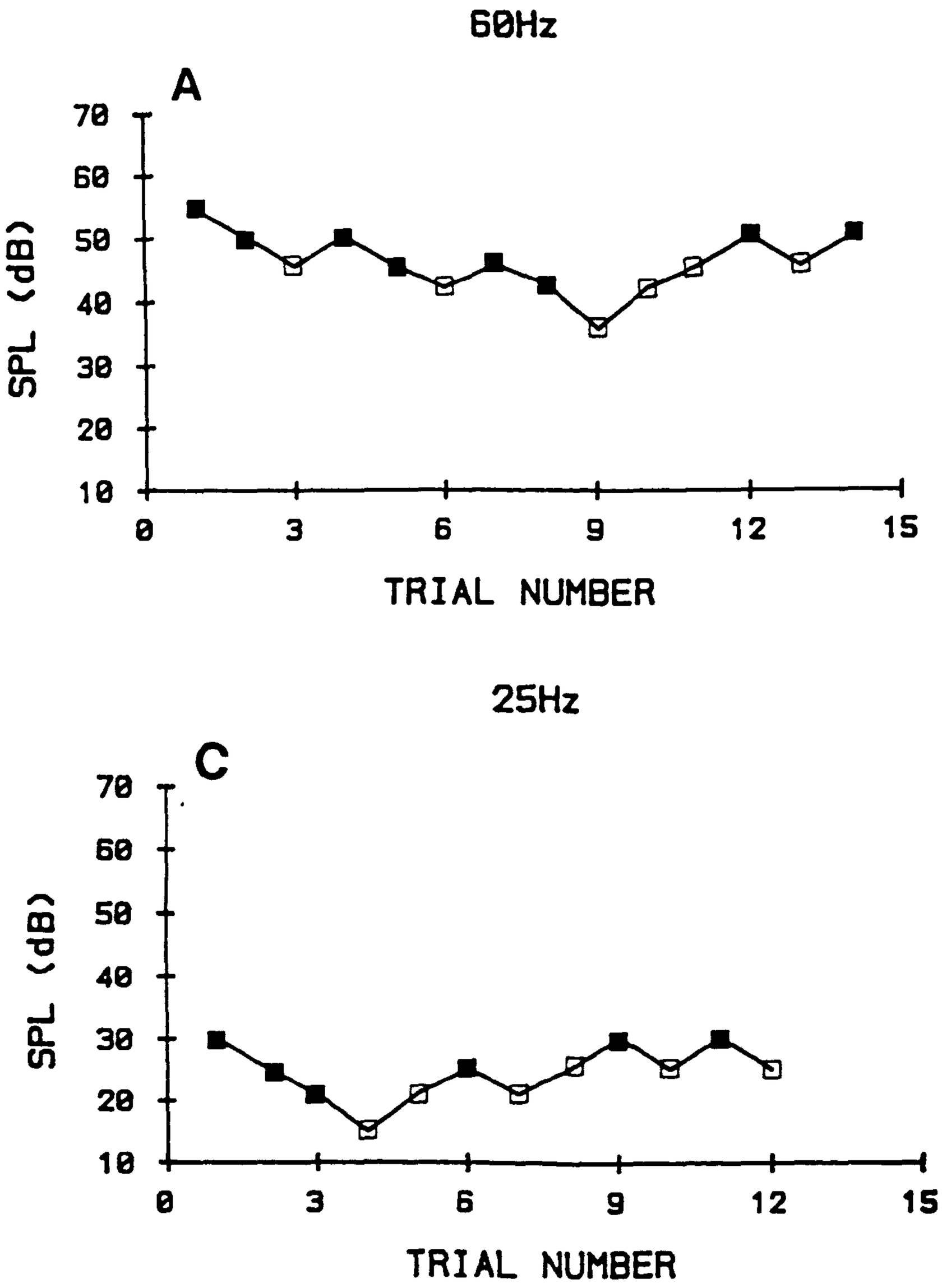
Figure 5.12 Examples of the staircase method used to determine the response thresholds (in terms of sound pressure) of *Nephrops* to tones of different frequencies. Plots show sound pressure level (db re 1 μbar) at the position of the animal against trial number (horizontal axis). Black squares indicate a positive response, open squares indicate no response. Plots A and B are from the same animal and plots C and D are from another animal.

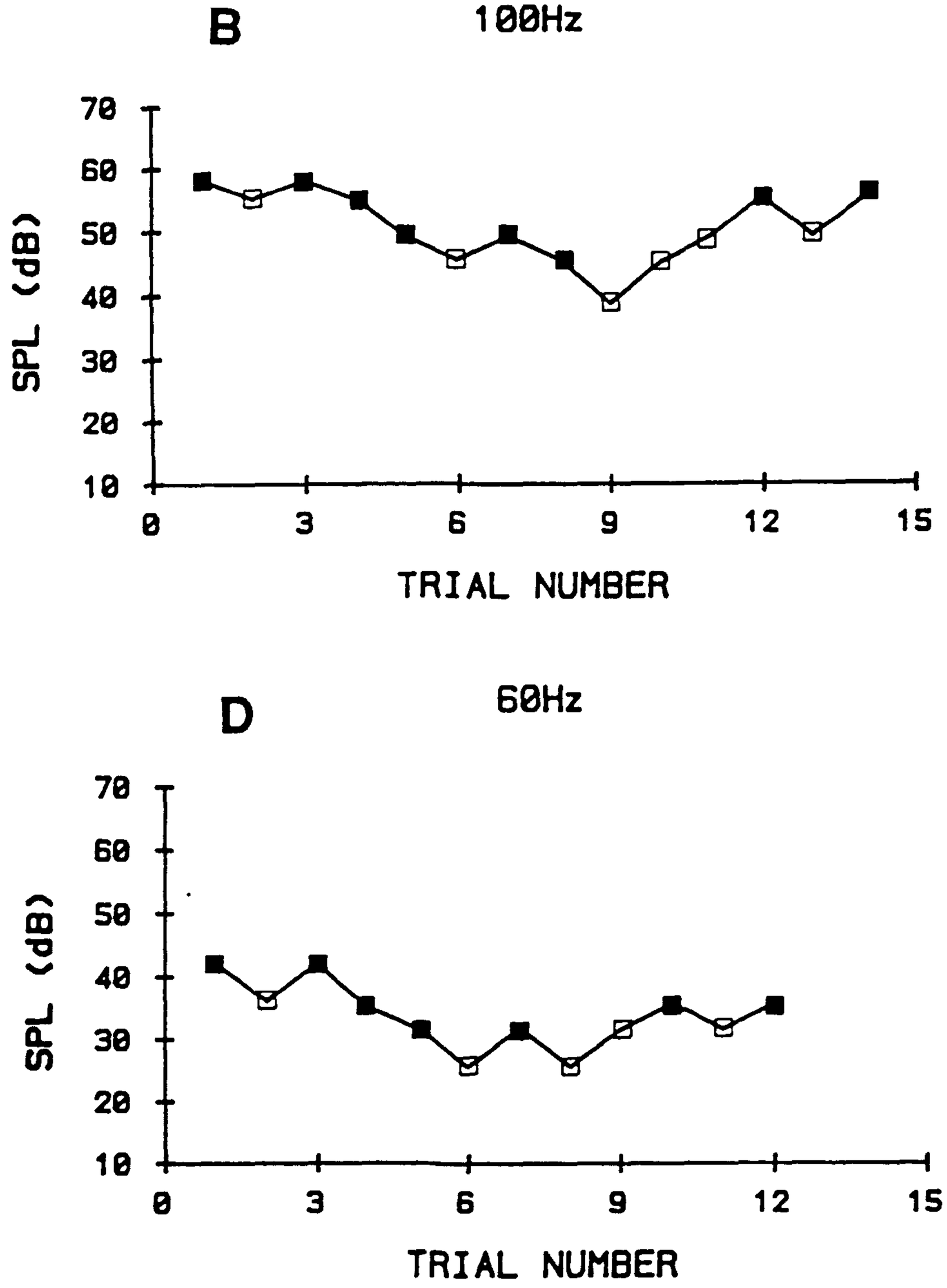
A. 60Hz

B. 100Hz

C. 25Hz

D. 60Hz

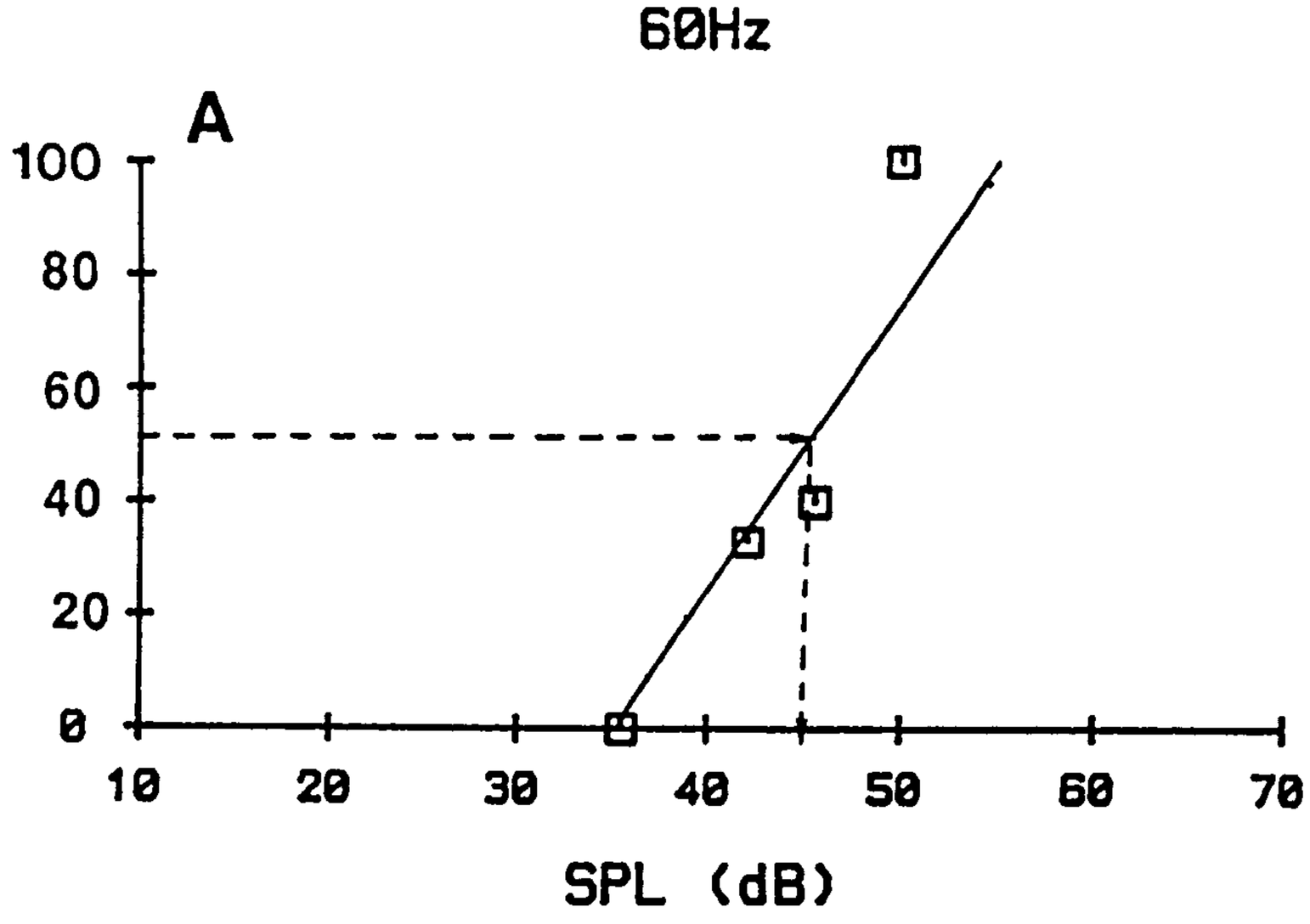

60Hz
A
70
60
50
40
30
20
10
SPL (dB)
0
3
6
9
12
15
TRIAL NUMBER

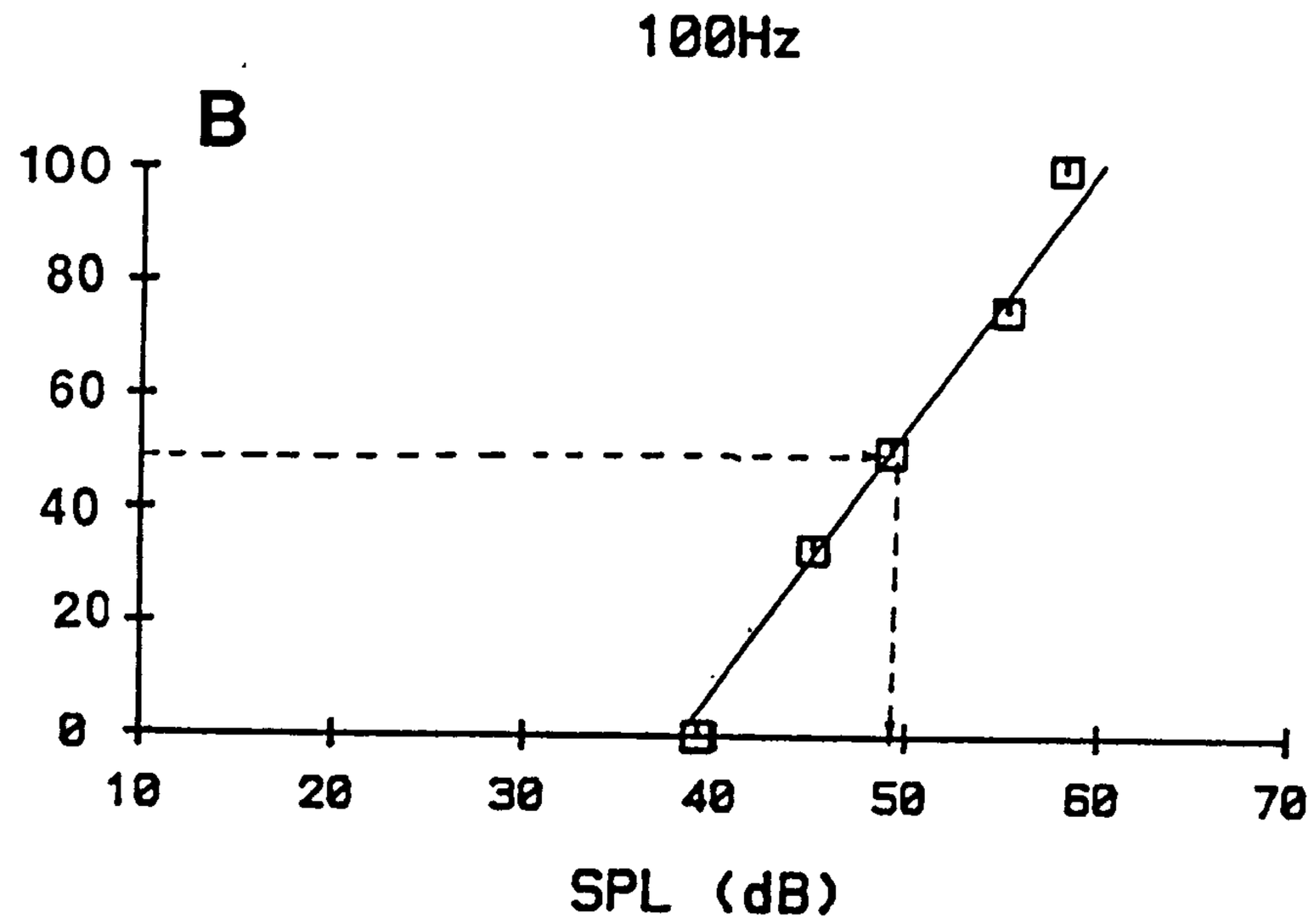

B
100Hz
70
60
50
40
30
20
10
SPL (dB)
0
3
6
9
12
15
TRIAL NUMBER

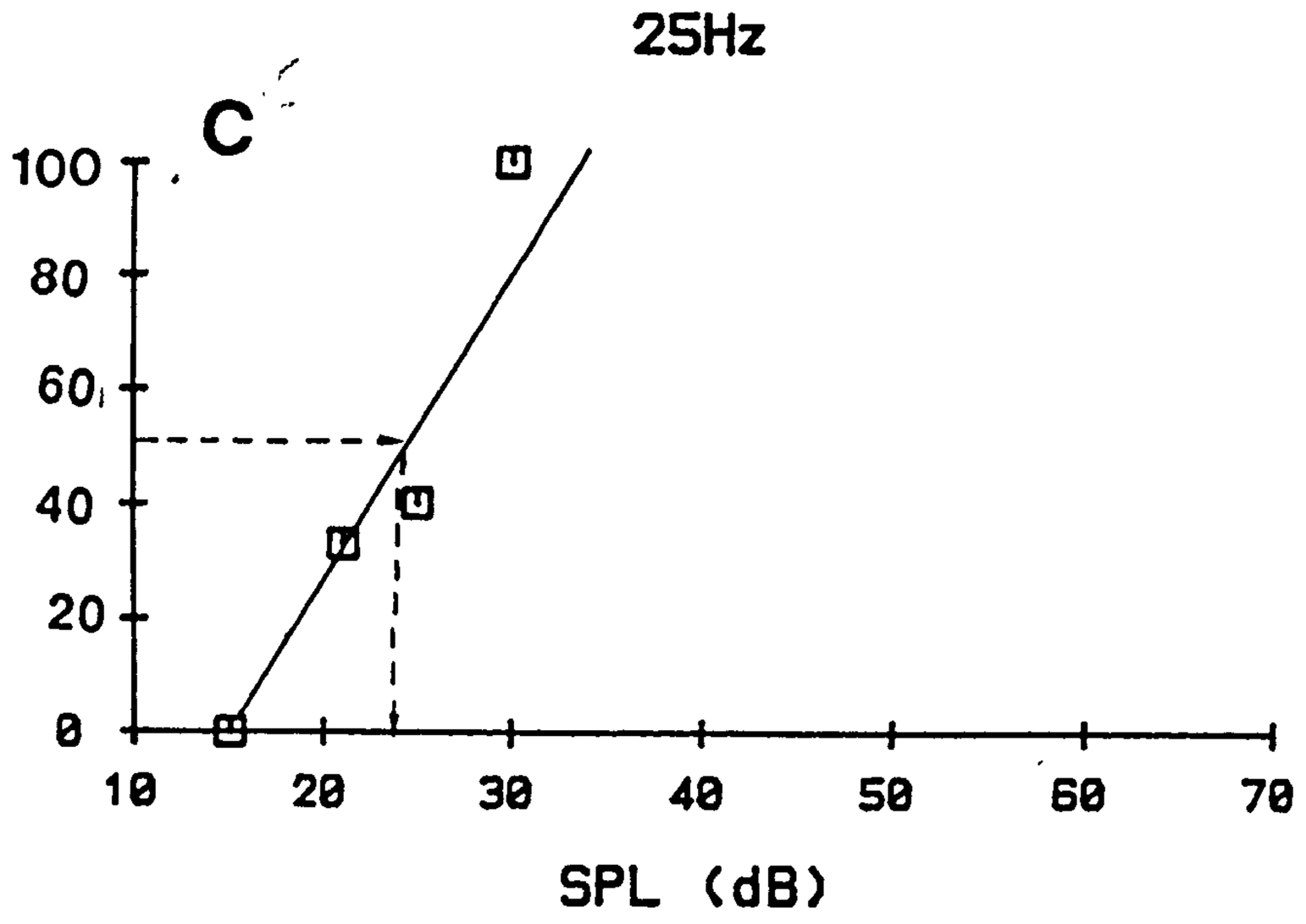
25Hz
C
70
60
50
40
30
20
10
SPL (dB)
0
3
6
9
12
15
TRIAL NUMBER

D
60Hz
70
60
50
40
30
20
10
SPL (dB)
0
3
6
9
12
15
TRIAL NUMBER

Figure 5.13 Plots of threshold determination using the information from Figure 5.12 the animals and frequencies are the same as in that figure. Plots show the percentage of positive responses at each sound pressure level against the sound pressure level (db re 1 μbar). The best fit line was then drawn between these points (solid line). The threshold value for each frequency was the sound pressure level at which 50% of positive responses occurred (dotted line)


60Hz
A
100
80
60
40
20
0
10
20
30
40
50
60
70
SPL (dB)


100Hz
B
100
80
60
40
20
0
10
20
30
40
50
60
70
SPL (dB)


25Hz
C
100
80
60
40
20
0
10
20
30
40
50
60
70
SPL (dB)

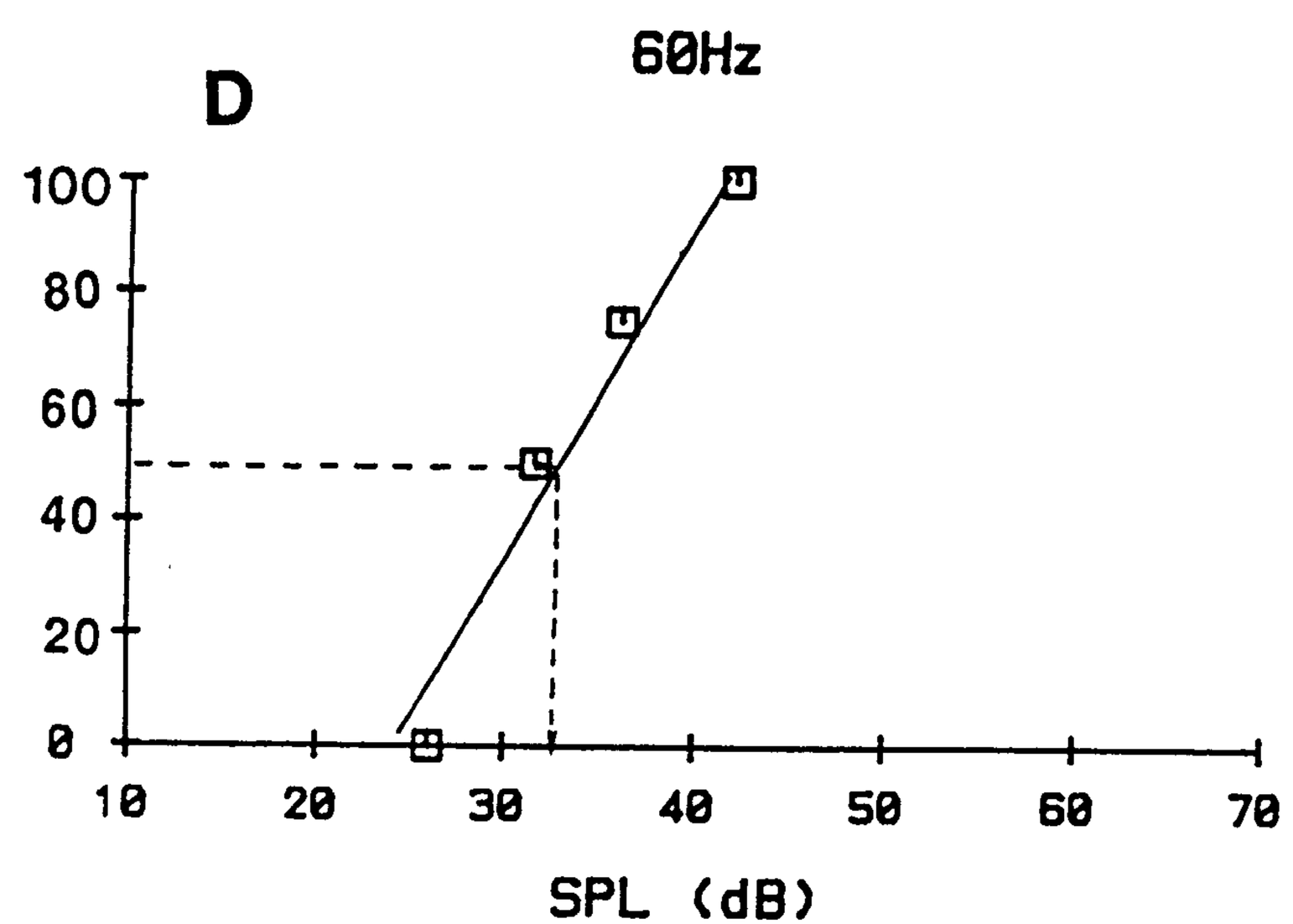
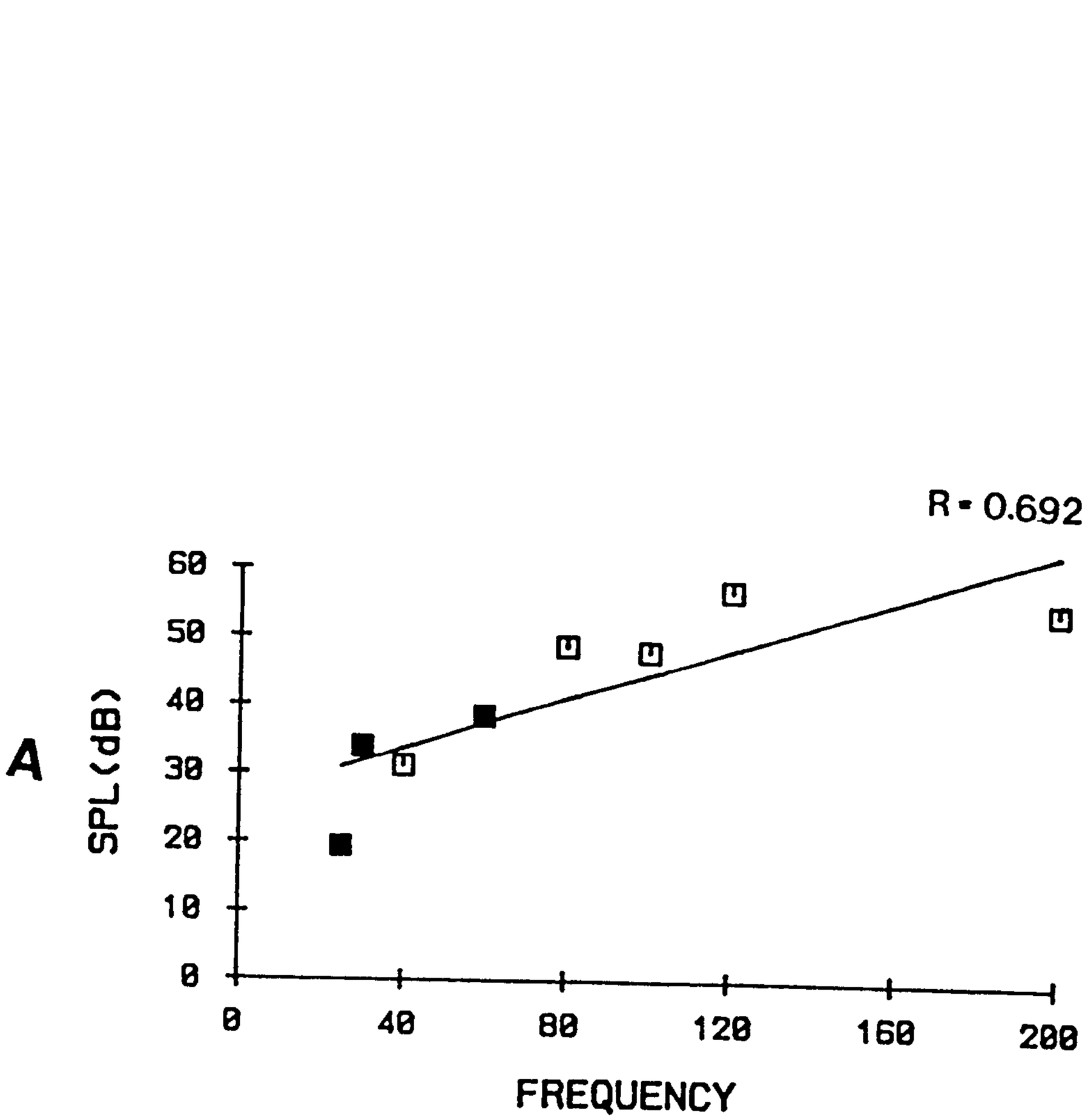
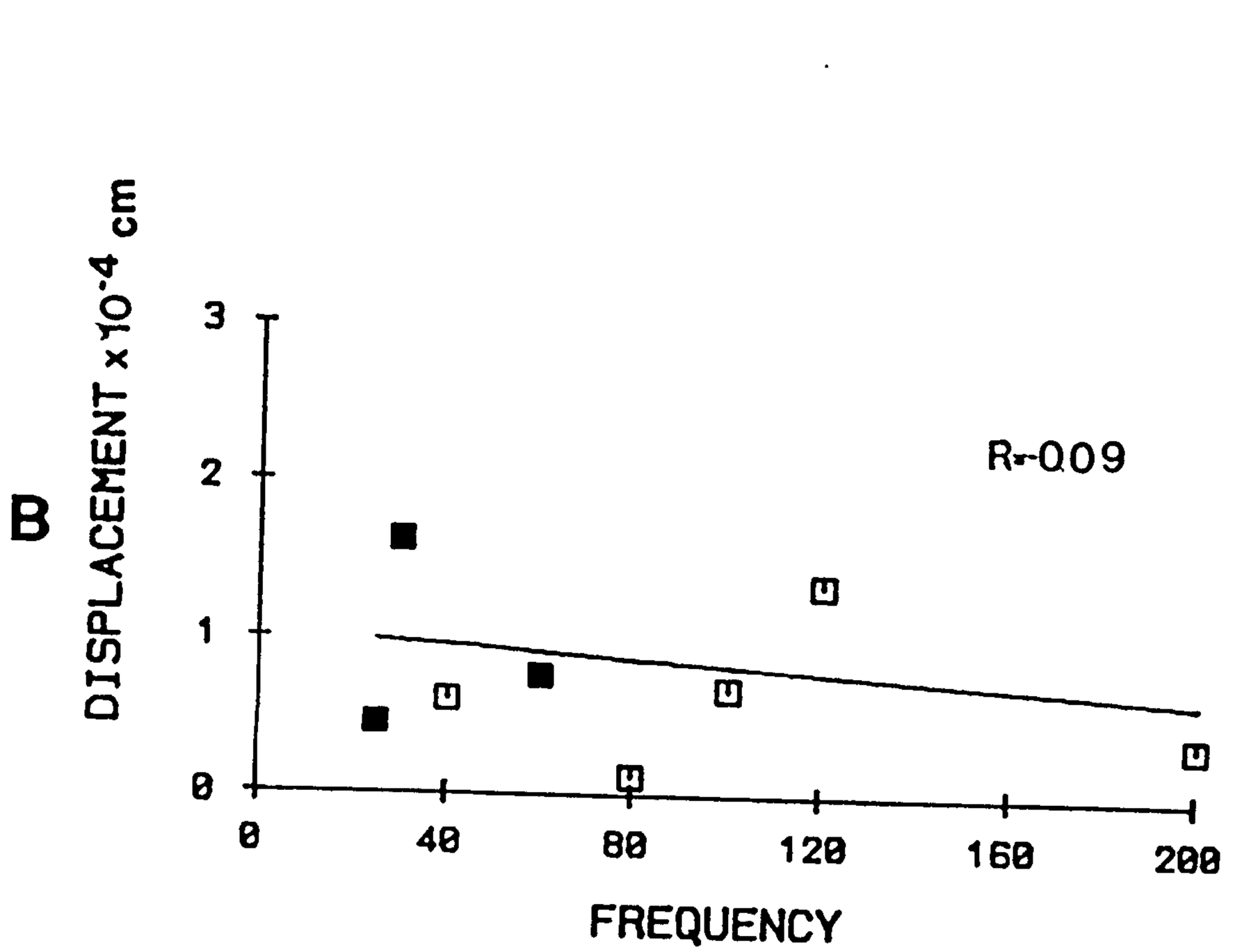

60Hz
D
100
80
60
40
20
0
10
20
30
40
50
60
70
SPL (dB)

Figure 5.14 A. The response threshold of *Nephrops* to sound expressed in terms of sound pressure. The plot shows sound pressure (db re 1 μbar) against frequency (Hz) and was plotted using data from 2 animals. Black squares indicate that the data is the mean value from two animals, open squares indicate that the data is from one animal. A linear regression line (solid line) has been fitted to the data ($R=0.692$, $P<0.05$)

B. The response thresholds of *Nephrops* to sound expressed in terms of displacement. The plots show displacement (cm) against frequency (Hz) and were plotted using data from two animals as above. ($R=0.09$, N.S.).


R = 0.692
A
SPL(dB)
60
50
40
30
20
10
0
0
40
80
120
160
200
FREQUENCY


B
DISPLACEMENT x10-4 cm
3
2
1
0
R=-009
0
40
80
120
160
200
FREQUENCY

Chapter 6

GENERAL DISCUSSION

6. GENERAL DISCUSSION

Nephrops possess cuticular mechanoreceptors similar in form to those which are known to receive water borne vibrational stimuli in other decapod Crustacea, and these have also been shown to be mechanosensory in this species (Chapter 2). The nervous system of *Nephrops* is clearly capable of both the detection of water borne vibrational stimuli and the processing of information on its frequency, amplitude and direction (Chapter 3). Much of this very specific information however, does not appear to be expressed to a great extent, in motor behaviour. For example, studies here have demonstrated that the sensory interneurones are capable of encoding the stimulus direction (Chapter 3) but this information would appear to have little influence on the locomotory responses of freely moving animals which generally walk rapidly backwards in response to the stimulus irrespective of its point of origin (Chapter 5).

The sensory system seems capable of discriminating between different frequencies at both the afferent and interneuronal levels and this information seems to be partially used to dictate the pattern of motor output. Both of the most prominent behavioural responses, backward walking and abdominal extension, occurred over a particular frequency range and the latter was modulated by frequency in a distinct way. Both of the behavioural responses and the sensory responses of the abdominal nervous system were strongest below 100Hz, suggesting that stimuli up to this frequency are biologically most significant for *Nephrops*. It would appear that this response is based on evasion rather than attraction as tailflipping was seen as the final event in the behavioural hierarchy and it may be primarily used by *Nephrops* to avoid predators.

It seems appropriate in conclusion to pose two questions, the

answers to which may not have been fully revealed by this study but which may provide the basis for further study in this field. Both of these questions are based upon the above assumption that the response shown by *Nephrops* is evasive.

6.1 Is the stimulus sufficient to be detected?

Studies here (Chapter 5) have shown that *Nephrops* produce behavioural responses to the particle motion component of sound. The response threshold for leg and initial abdominal movements calculated in a free sound field in terms of displacement was 0.874μm and *Nephrops* are therefore relatively insensitive compared with fish. Behavioural responses in the form of backwards walking (5.3.3) and abdominal extension (4.3.1) were reliably elicited in laboratory aquaria because the acoustic conditions therein ensured that the displacement component of the stimulus was above the response threshold. In the field leg and initial abdominal movements were only produced when the J9 sound projector was very close to the animal and responses could not be elicited in free moving animals with the J9 at either 10m (5.3.1), 0-2m (5.3.2) or 1m (5.3.5). The most likely explanation for this, which has been borne out by this study, is that the displacements produced were not above the response threshold. In the light of these findings it seems unlikely that *Nephrops* will be capable of responding to naturally occurring far field sound or indeed to many near field stimuli unless the stimulus source is very close to the animal or the displacements produced are very large.

Nephrops are not known to communicate with each other using sound and the most likely purpose of an acoustic sense in these animals is predator detection. Cod, a major predator of *Nephrops* are known to make low frequency grunting sounds (Hawkins and Rasmussen, 1978; Brawn, 1961) and are also known to make sudden swimming sounds

(Myreberg, 1981) both of which could potentially be detected by *Nephrops*, as they fall within the appropriate frequency range. However, it seems unlikely that these sounds will be above the response threshold of *Nephrops* unless the fish is very close to it in which case the danger of attack will be great. Sensory mechanisms do not operate completely independently of one another, however,and it is possible that interactions with the visual system may be very important in this case. The visual sense in *Nephrops* is well adapted to function in the low light, deep water conditions in which they are often found (Shelton et al., 1985). It is possible that the primary detection and identification of a predator by *Nephrops* is visual. Sensory detection of the water borne vibrations produced by the approaching predator may follow this (it is highly likely that the sensory threshold for the detection of water borne vibrations will be lower than the behavioural threshold). The behavioural threshold response may not be produced if evasion is not considered appropriate and then only if the predator is very close to *Nephrops*. At close proximity also it is known that threatening inputs to the crayfish visual system can produce escape reactions (Wine and Krasen, 1972) so it is likely that in *Nephrops* interactions between the acoustic and visual systems result in the appropriate behaviour being produced in response to the appropriate stimulus if thresholds in both systems are surpassed. This may partially explain the results of experiments conducted in section 5.3.2 during which the animals only showed very slight responses of a very short duration when very close to the stimulus source. It may be that more vigorous responses were not produced in this situation because the stimulus source was not visually identified as threatening.

It would seem appropriate therefore that further studies might

try to identify the effect of interactions between the visual and acoustic systems of *Nephrops* on their behavioural responses to acoustic stimuli, particularly those which occur in the animal's natural habitat.

6.2 Is the response sufficient to produce evasion?

The behavioural responses of *Nephrops* observed in the laboratory studies here (sections 4.3.1, 5.3.3 and 5.3.4) formed a behavioural hierarchy in which successively more intense responses were elicited if the normal expression of locomotor behaviour was prevented. Thus, in freely moving animals, rapid backwards walking was seen in response to water borne vibrations between 20-80Hz. This response may be regarded as the lowest step in the hierarchy, which normally provides an effective evasion from the source of the stimulus. If the animals were restrained their locomotory movements became ineffective, and it was only under these conditions that more vigorous reactions: abdominal extension, rapid leg movements and swimmeret beating were recruited. Although abdominal extension does occur during backwards walking (Kovac, 1974), the response seen here was much more vigorous. The abdominal extension facilitates the propulsive forces produced by the swimmerets, but also represents a preparation for the ultimate evasive act, the rapid flexion of the tail flip. This reaction occurred only infrequently and was always preceded by the vigorous leg and swimmeret movements.

The neuronal mechanisms controlling this hierarchy could provide a very interesting subject for future study as the responses are very reliably elicited by this stimulus. Behavioural and neuronal heirarchies have been studied in other animals (Wine, 1984; Davies, 1985). It is likely that in *Nephrops* the preferred response of the

animals to the stimulus is locomotion to produce evasion with the least energy cost. The failure of this behaviour when the animal is restrained would be detected by joint proprioceptors and water current detectors all over the body surface. The next step in the heirarchy, abdominal extension, may only be recruited if inputs from these proprioceptors to the CNS inform the animal that it is not moving. If subsequent sensory feedback to the CNS then reports that evasion has still not been successfully accomplished, and the stimulus is sufficiently intense then tailflipping may occur. This may also involve central gating. This behaviour is energetically very expensive and it also generally causes the inhibition of all other motor activity which may be antagonistic to its occurrence (Camhi, 1984), this is contrary to the previous step in the hierarchy, abdominal extension, during which leg movements still occurred.

The hierarchy of motor behaviours described here in response to water borne vibrational stimuli represents a set of predictable responses which varies systematically with stimulus parameters. This reliability could be exploited to advantage to study the neuronal basis of postural control, and would more clòsely answer Huber's call (Huber, 1988) for neurobiological invesitgations to be carried out as far as possible under natural conditions.

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