ASPECTS OF THE RESPIRATORY PHYSIOLOGY OF THE SWIMMING CRAB LIOCARCINUS DEPURATOR (LINNAEUS)

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DECLARATION

I hereby declare that this thesis represents, except where a note is made to the contrary, work carried out by myself. It has not been previously submitted for any degree.

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14th November 1986.

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Summary

The swimming crab, Liocarcinus depurator (L.), is one of the commonest portunid crabs in the inshore waters around the British Isles. Experimental animals were obtained from the Clyde Sea Area where the species is commonly trawled at a depth of 40 - 50 metres.

As background, the respiratory physiology of decapod crustaceans including blood chemistry is succinctly reviewed.

The branchial morphology of L. depurator is then described. It conforms to the typical portunid pattern.

Using the impedance pneumograph and pressure transducer techniques, the heart rate, scaphognathite rate and the branchial pressure were recorded and analysed. The heart and scaphognathite beat of L. depurator is extremely variable both between individuals and within an individual crab. Periods of cardiac arrest and ventilatory apnoea have been shown to occur. Cardiac arrest and apnoea occur synchronously. Reversals of the respiratory current occurred

regularly under normoxic conditions in crabs that were not buried. When the crab was buried in the substratum the scaphognathites maintained almost permanent reversal of the respiratory current. It was shown that after a minimum of twelve hours the incidence of reversals and the periodicity of cardiac arrests became relatively predictable and this was indicative of a relatively unstressed crab.

The oxygen consumption of L. depurator was determined using a closed system respirometer. It was found that a minimum of twelve hours was required in order to overcome the stress of handling prior to being placed in the respirometer and for an unstressed oxygen consumption rate to be determined. A Q_{10} value for L. depurator was determined and the difference in oxygen consumption between fed and unfed animals was also examined. Investigations of oxygen consumption during hypoxia produced a range of Pc values which were related to the wet weight of the crab.

CHAPTER 1 INTRODUCTION

1.1. Physiological Background

Investigations into the respiratory physiology of decapod Crustacea appear to begin with Milne-Edwards (1839). Milne-Edwards was the first to recognize the importance of the role of the scaphognathite in the production of respiratory currents in decapods and went on to state that the current was always in one direction in decapods, ie. anteriorly. Garstang (1896), however, showed that in the crab Corystes cassivelaunus the ventilation current was reversed when it was buried. This was the first time that reversed scaphognathite pumping was noted. Scaphognathite activity was investigated by subsequent workers, notably Segaar (1934), Fox & Johnson (1934), Johnson (1936), Lindroth (1938) and van Heerdt & Krijgsman (1939). Segaar (1934) recorded its activity by a system of levers attached directly to the scaphognathites. This method tended to overload the scaphognathites and Larimer (1964) developed a method whereby scaphognathite activity was recorded by pressure transducers

and catheters.

Arudpragasam and Naylor (1964a) investigated the role of current reversals in the shore crab Carcinus maenas, using cannula tubing implanted into the branchial chamber and connecting the tubing to a tambour beneath a recording arm. Branchial chamber pressure was recorded on a variable speed kymograph and current reversals were noted as spikes on the trace. Arudpragasam and Naylor (1964a) suggested that the main role of the reversals was to irrigate the upper surface of the gills and also to clean the gills. However, Hughes et al. (1969) argued against this hypothesis and proposed that the primary function of reversals is to clean the ventrally facing gill surfaces. Their function is still not fully resolved, see review by McMahon and Wilkens (1983).

The review of oxygen consumption in the Crustacea undertaken by Wolvekamp & Waterman (1960) shows that there are marked differences in oxygen consumption for the same species under apparently the same conditions. The authors recognised that the differences in techniques used by different workers and the different parameters to which the experimental animals were exposed, could account for this variation, but even after corrective factors were applied

this variability was still evident.

Butler et al. (1978), in an attempt to reduce this variability, used an experimental regime which resembled as closely as possible the conditions in the natural environment. Unrestrained lobsters were placed in a cylindrical, continuous-flow respirometer surrounded by sea water at 15^{0}C and partially covered by a sheet of black polyethylene which dimmed the light, allowed a natural photoperiod and prevented disturbance of the animals in the respirometer. As a result of their studies they were able to show that the lobster Homarus gammarus, took up to 48 hours to acclimatize to its experimental conditions. Halcrow and Boyd (1967) also found that the oxygen consumption of the amphipod Gammarus oceanicus decreased after several hours in the respirometer. Aldrich (1975a) working on the crabs Cancer pagurus and Maia squinado, described a large amount of variability in the rates of oxygen consumption between individual crabs. He went on to suggest, however, that there are two distinct levels of oxygen consumption, active or excited rates and standard or undisturbed rates.

Other workers, such as McMahon et al. (1974)

working on the crayfish Orconectes virilis, have confirmed that acclimatization to experimental conditions is important in reducing the variability in the oxygen consumption rates of Crustacea.

From the above work it is generally agreed that there is a marked amount of variability in rates of oxygen consumption between animals of the same size, sex and moult stage and even in the same individual depending on the length of time it has been allowed to acclimatize to the experimental conditions, it is therefore important that these factors are closely controlled.

Investigations into the heart and scaphognathite rates of Crustacea have shown that there is also considerable variability in these parameters both within and between species. Spaargaren (1973) demonstrated variability in the heart rate of the shrimps Palaemon serratus and Crangon crangon. Similarly, Florey and Kriebel (1974) confirmed variability in the heart rate of crabs. Cumberlidge and Uglow (1977), in summarizing knowledge of scaphognathite beat rates, indicated that most Brachyura studied showed periods of apnoea and all showed the presence of current reversals. From their own experiments on Carcinus maenas they suggested

that both heart and scaphognathite rates in this species can be divided into three levels, elevated, active and resting levels.

The techniques for recording heart and scaphognathite rates in decapods, have developed over a number of years. Some of the first heart rate recordings were carried out by Larimer (1962, 1964) who used an electrocardiogram (ECG) procedure. The impedance pneumograph technique of Hoggarth and Trueman, (1967), however, has proved more useful. Hoggarth and Trueman (1967) used the impedance pneumograph to record the valve movement of the bivalve mollusc Mya arenaria in the sand. The technique was also adapted to record the heart rate of molluscs, examples of which are, Donax vittatus and Cardium edule (Trueman, 1967); Mytilus edulis (Helm and Trueman, 1967; Bayne, 1971; Widdows, 1973); Chiton tuberculatus, Patella vulgata and Isognomon alatus (Trueman et al., 1973); Pecten maximus (Brand and Roberts, 1973); Arctica islandica (Taylor and Brand, 1975); Scrobicularia plana (Earll, 1975); Anodonta anatina (Brand, 1976) and Chlamys opercularis (A.C. Taylor pers. comm.). The technique (as applied to decapod crustaceans), involving implanted electrodes and recording by means of an impedance pneumograph, is fully described in

Chapter 3 and has subsequently been used by a number of workers in investigations of the respiratory physiology of decapod Crustacea.

Examples of some workers who have used the impedance pneumograph technique on Crustacea are: Ansell (1973) to record heart and scaphognathite rate in the crab Cancer pagurus when investigating the effects of starvation on the metabolic rate; Taylor (1976, 1977), on the respiratory responses due to declining oxygen tension and changing environmental salinity in Carcinus maenas; Cumberlidge (1977) and Depledge (1978), working on the cardiac and ventilatory activity of Carcinus maenas; McDonald et al. (1977) when investigating the heart and scaphognathite activity of Cancer magister; Hagerman and Uglow (1979) investigating the heart and scaphognathite activity of Palaemon adsperus; Burton et al. (1980) when investigating the effect of temperature increases on the cardiac activity of Callinectes sapidus; Costa (1980) on the effect of heavy metal pollutants on the heart rate of Gammarus pulex; Uglow (1980) on the effect of changes in salinity on the cardiac and ventilatory responses of Crangon crangon; Wheatly and Taylor (1981) when investigating the effect of increasing hypoxia on the cardiac and ventilatory responses of

Austropotamobius pallipes and Morris (1984) while investigating various physiological responses of Palaemon elegans. Dyer and Uglow (1978) modified the technique by using only one implanted electrode to measure heart and scaphognathite rates in the shrimp Crangon crangon. They found that using a single implanted electrode with a reference electrode in the water, reduced the weight that the animal had to bear thus giving it more freedom to move and reducing stress. Morris (1984) used a similar technique for another shrimp, Palaemon elegans. The preceeding list of workers is in no respect exhaustive and many other workers have used the impedance pneumograph technique to record physiological parameters in Crustacea.

Since the late 1960's and early 1970's with the advent of advances in microelectronics, primarily in the medical field, the technique for measuring oxygen consumption has been mainly by the use of an oxygen electrode to determine the oxygen tension in water. Prior to the 1970's the method used was the standard Winkler method, or a modified version of it (Fox & Wingfield, 1938), and even today micro-Winkler techniques are used, particularly for small animals or if the cost of oxygen micro-electrodes is prohibitive, (Agnew, 1985).

Until the early 1960's, measurement of oxygen consumption and ventilation were done in isolation. Larimer (1961) was one of the early workers who devised a system to measure the two simultaneously. This is generally regarded as the beginning of a holistic approach to crustacean respiratory physiology since, along with this work, Larimer and Gold (1961) investigated the role of haemocyanin in blood oxygen transport. In a continuation of this holistic approach, Arudpragasam and Naylor (1964a, 1964b) recorded gill ventilation volumes, oxygen consumption and respiratory rhythms simultaneously in Carcinus maenas. Johansen et al. (1970) conducted a study on the crab Cancer magister. This study involved an analysis of the respiratory properties of the haemocyanin, direct measurement of ventilation and oxygen extraction, an evaluation of the blood gas levels in unrestrained crabs and also the responses of these parameters to a provoked response. This study was one of the first detailed studies on the respiratory physiology of crabs involving several parameters simultaneously and in this respect it is important in the respiratory physiology of decapods. Prior to this, such complete studies had been carried out only on fish e.g. Stevens and Randall (1967).

Oxygen consumption has been measured in a number of marine invertebrates with molluscs (eg. Akerland, 1969; Bayne, 1973; Booth and Mangum, 1978; Bayne and Scullard, 1978 and Brand and Morris, 1984) and crustaceans being the groups most investigated. Most studies on decapod Crustacea have looked at the respiratory responses to change in environmental factors eg. Grainger (1956), who recorded changes in oxygen consumption in response to changes in temperature. In this study, Artemia salina was the major subject of investigation. With a change in temperature, an overshoot in the oxygen consumption was recorded and a return to a steady level characteristic of the new temperature followed after a short period of minor oscillations. In decapods, as in most invertebrates, respiration rate decreases with decreasing temperature and there is a displacement of the M-T Curve (metabolic rate versus temperature) to the left for animals from colder waters compared with animals from warmer waters (see Vernberg (1962) for a review of M-T Curves). Some species of crabs, however, show a relative insensitivity to temperature changes, as indicated by a low Q_{10} value. Leffler (1973) shows that there is very little change in the metabolic rate in juvenile Callinectes sapidus from a temperature range of 20 - 27 ^{o}C, whereas at temperatures greater than this range, there is a

dramatic increase in oxygen consumption rate. Vernberg (1969) showed that there is a range of temperatures (20 - 30°C) over which the metabolic rate of fiddler crabs, (Uca), do not change significantly. This can be explained in that this is the range of temperatures which Uca normally experiences in its intertidal habitat. Some other workers who have investigated the effect of temperature on the oxygen consumption of crabs are, Breteler and Klein (1975) working on juvenile Carcinus maenas and Burggren and McMahon (1981) on hermit crabs.

In studying the responses due to changes in environmental salinity , Hume and Berlind (1976) showed that in Carcinus maenas the heart rate increased when the animal was exposed to a lower salinity. Taylor (1977), also working on C. maenas, showed an increase in the oxygen consumption when the crab was exposed to reduced salinity. This increase in oxygen consumption was still evident after 3 - 4 days of exposure to water of reduced salinity. This increase in oxygen consumption in low salinities is also evident in Callinectes sapidus (Laird and Haefner, 1976). In contrast to this Birchard et al. (1982), found a decrease in oxygen consumption at lower salinities in the crab Ovalipes ocellatus also Davenport et al. (1980), found a decrease in

oxygen consumption in the hermit crab Pagurus bernhardus at lower salinities although he attributed this to a decrease in the ventilation rate of the crab.

Other external factors affecting the respiration rate in Crustacea have been studied. Examples of these are, the effect of starvation (Aldrich, 1975b and 1975c; Ansell, 1973; Armitage and Wall, 1982; Marsden et al., 1973 and Wallace, 1973), the effects of hypoxia and anoxia (Batterton and Cameron, 1978; Bradford and Taylor, 1981; Bridges and Brand, 1980a, 1980b; Burnett, 1979; Butler et al., 1978; Childress, 1971 Mangum and Van Winkle, 1973; McMahon and Wilkens, 1975; McMahon et al., 1974; Truchot, 1979; Wernick and Penteado, 1983 and Uglow, 1973) and the effect of activity on the oxygen consumption (Burke, 1979; ; Foulds and Roff, 1976; Halcrow and Boyd, 1967 and McMahon et al., 1979). These studies and others will be discussed in the relevant sections in the following chapters.

As previously stated, the work of Johansen et al. (1970) was one of the first in-depth studies of crab respiratory physiology. One of their major achievements was the integration of data on the heart beat and scaphognathite beat with the information on the role of the blood in oxygen

transport. Investigations into the respiratory properties of the blood have recently become a subject of growing interest.

1.2. Blood Oxygen Transport

Haemocyanin has been shown to be the only oxygen carrying pigment in decapods (Mangum 1983). It should be noted, however, that other groups of Crustacea such as the Branchiopoda and Ostracoda have been shown to have haemoglobin as the oxygen carrying pigment. In order to have a full understanding of the respiratory properties of the decapod blood, it is important to understand the factors which affect the haemocyanin's ability to take up, transport and release oxygen. One of the major factors is the affinity of haemocyanin for oxygen. This can be affected by a number of parameters such as environmental temperature, external salinity, external oxygen tensions and conditions within the blood ie. blood oxygen tensions, blood pH, concentrations and types of ions and other organic modulators.

The oxygen affinity of haemocyanin increases at low temperatures (Truchot, 1973, working on C. maenas). However, as an adaptation to colder environments, there appears to a trend to a general lowering of the haemocyanin oxygen affinity in colder water species as compared to warmer water ones (Mauro and Mangum 1982). This is regarded as a general

geographic trend.

With decreased salinity there is a lowering of the oxygen affinity of the haemocyanin (Truchot, 1973). However, with a decrease in the salinity of the external medium there is a compensating increase in the blood pH which in turn increases the oxygen affinity of the haemocyanin, thus balancing the decrease in affinity believed to be due to changes in the blood ion concentration associated with low salinity (Truchot, 1975). However, Mangum (1983), (citing unpublished work by R.P. Mason and C.P. Mangum) states that there is a reversible acclimation of the haemocyanin oxygen affinity involved. Taylor (1977) working on Carcinus maenas says that with decreased salinity there is an increase in oxygen consumption rate. This increase in oxygen consumption rate was highest in the 2 - 3 hour period after being exposed to the lowered salinity, after which the oxygen consumption rate dropped although it still remained higher than the rate recorded immediately prior to being exposedto the low salinity. The high oxygen consumption rate recorded in the 2 - 3 hour period after exposure is due mainly to the increased locomotory activity associated with a sudden decrease in salinity.

Aggregation state of the haemocyanin molecule appears to have an effect on its oxygen affinity, with polymers having a higher oxygen affinity than the monomers of haemocyanin (Pickett *et al.*, 1966 working on lobsters and Chantler *et al.*, 1973 working on *Carcinus mediterraneus*). Individual ions in the blood have been investigated in order to determine which are the most important. Generally, divalent cations such as Ca^{2+} and Mg^{2+} have been shown to increase the oxygen affinity of the haemocyanin, with Ca^{2+} having a greater affect (Miller and Van Holde, 1974). In portunids, Ca^{2+} is the critical ion in the blood (Truchot, 1975 and Mangum and Mason, unpublished data cited from Mangum, 1983). It is thought that the divalent cations either stabilize the haemocyanin protein in a high affinity conformation or promote polymerization to a higher affinity aggregate (Mangum, 1983). In contrast, however, it has been shown that divalent cations such as Ca^{2+} have no affect on the oxygen binding properties of isopod haemocyanin (Van Holde and Brenowitz, 1981).

Until recently, it was thought that in order to compare the haemocyanin oxygen affinity values of different species of crabs, the main consideration was the P_{50} value of the blood. However, it has been shown that there are factors

present in decapod blood which are known to have an effect on the oxygen affinity of the haemocyanin (Mangum, 1983). Therefore without knowing the concentrations of these factors, it is impossible to make direct comparisons of the haemocyanin oxygen affinity based solely on the P_{50} values.

Recently L-lactate has been identified as one of these factors affecting haemocyanin oxygen affinity. During anaerobic respiration, due to either increased locomotory activity or hypoxia, L-lactate is produced as an end product and has been shown to have an effect on the oxygen affinity (Truchot, 1980; Booth *et al.*, 1982; Graham *et al.*, 1983; Bridges *et al.*, 1984 and Taylor *et al.*, 1985) It appears that L-lactate affects the structure of the haemocyanin molecule which in turn increases the haemocyanin oxygen affinity. In addition to this effect, Taylor *et al.* (1985), have shown that an increase in L-lactate also decreases the cooperativity of the haemocyanin in *Atelecyclus rotundatus* and *L. depurator*, although only slightly. However, the overall result of an increase in L-lactate concentration, is an increase in oxygen affinity. At present the specific effect of L-lactate and other, as yet unidentified factors, on the oxygen affinity of the haemocyanin, is poorly understood and therefore most recent research is

concentrating in this area.

In addition to the haemocyanin transported oxygen there is also oxygen transported in the haemolymph as dissolved oxygen. The total amount of oxygen that can be transported is determined by the PO_2 value of the blood. In general, the mixed post-branchial blood PO_2's are sufficent to fully saturate the haemocyanin, eg. McMahon and Wilkens (1977) for Cancer productus, Taylor (1977) for Carcinus maenas, Taylor and Butler (1978) also for C. maenas, Burnett (1979) for Libinia emarginata and Mauro and Mangum (1982) for Cancer borealis.

It has long been established that the ventilatory rates in Crustacea are extremely variable even under normoxic conditions (see above). McMahon and Wilkens (1977) working on Cancer productus found that during ventilatory pausing, only a small amount of the stored oxygen (dissolved in the haemolymph and bound to the haemocyanin) was used. However, in contrast to this, Burnett and Bridges (1981) found that the oxygen store (dissolved and bound oxygen) was fully utilized during ventilatory pausing in Cancer pagurus. Recently, a number of investigations into the oxygen transport in the blood of decapods (Bridges et al., 1979;

Burnett, 1979; Taylor and Davies, 1981; Taylor and Davies, 1982; Bridges et al., 1984; Taylor, 1984; Morris et al., 1985; Morris and Bridges, 1985 and Taylor et al., 1985) has confirmed that in decapods, during ventilatory pausing, there is almost total utilization of the oxygen stores.

During moderate hypoxia, 150 - 30 Torr, there is a gradual decrease in the blood oxygen tension as the oxygen stores are used up. In an attempt to maintain oxygen saturation, there is an increase in ventilatory rate but as the external oxygen tension declines, it becomes increasingly difficult for sufficent oxygen to be absorbed in order to maintain aerobic respiration. There is always a small amount of anaerobic respiration, even when the blood haemolymph and haemocyanin are fully oxygen saturated. After the Critical Point or Pc has been past (the Pc is defined fully in Chapter 4), anaerobic respiration becomes the predominant metabolic pathway for ATP production with its associated production of L-lactate which increases the haemocyanin oxygen affinity. As anoxia is approached and past, there is a build up of L-lactate and a depletion of the glycogen stores which results the cessation of any respiration.

The preceeding short review of decapod crustacean respiratory physiology gives an indication of the diversity of previous work. Recent comprehensive reviews of crustacean respiratory physiology have been given by McMahon and Wilkens (1983), Mangum (1983), Vernberg (1983) and Cameron and Mangum (1983).

In the present study it was decided to combine some of the above techniques to investigate the respiratory physiology of a potentially active decapod crustacean species thought likely to show a wide range of variability in its activity. The species selected was the swimming crab, Liocarcinus depurator which is relatively common in the Clyde Sea Area and is readily trawled. In addition to the intrinsic value of studying what was thought to be an active crab, there is also the commercial implications for studying L. depurator. At present L. puber, the large or velvet swimming crab, has become a valuable fishery in the West coast of Scotland and interest is also being shown in L. depurator as a commercial fishery (C. Janick , Scottish Development Association, pers. comm.)

1.3. Liocarcinus depurator (Linnaeus)

The following review of generic and species names for Liocarcinus depurator has been given by Ingle (1983); Cancer depurator, Linnaeus, 1758; Portunus depurator, Leach, 1815; Macropipus depurator, Christiansen, 1969 and Liocarcinus depurator, Ingle, 1980.

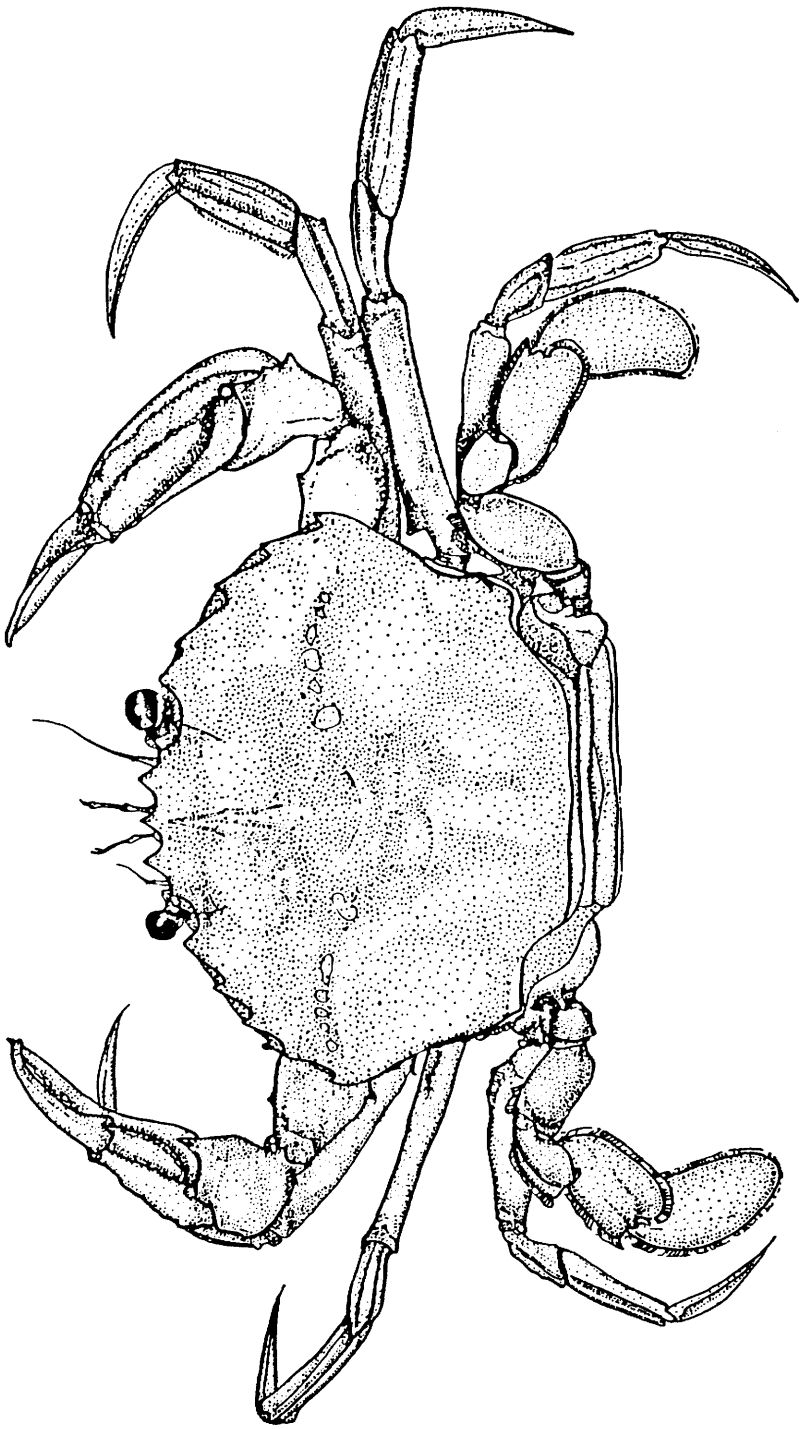
L. depurator has a relatively flat, oval carapace. The dorsal side is a reddish brown colour with the ventral sides of the pereiopods being pink and white. The carapace is much broader than it is long with relative lengths being 45mm and 36mm respectively in fully grown animals. For a fuller description of the animal see Ingle (1980, 1983). Plate 1.1. is a drawing of an adult male crab.

Ingle (1983) in citing Walker (1892), Grieg (1927) and Allen (1967) states that L. depurator had been reported from 5m - 146m on a range of substrata ie. sand, muddy-sand and gravel. Ingle (1983) states that it is common all round the British Isles but it also occurs around many coasts of N.W. Europe. Christiansen (1969) states that L. depurator was found from 1-2m - 450m on a similar range of substrata as Ingle (1983). She gives its distribution as being the coast

PLATE 1.1.

An adult male *Liocarcinus depurator*

Scale = *circa* 1.5 times normal size



of Norway (South of 68°N.) to the West coast of Sweden and the coasts of Denmark, Netherlands, Belgium and the East coast of Britain. She also states that they have been found as far south as the Spanish Sahara and at sites along both the North and South coasts of the Mediterranean. Allen (1967), in describing the Clyde population of L. depurator, has recorded them from depths of 6-125m on sand, muddy sand and gravel substrata and describes them as being common in this region.

Until now there has been very little experimental work performed on L. depurator. Margaria (1931) in studying osmotic changes in a number of marine invertebrates, states that L. depurator is isosmotic down to its lower limit of survival, which was 65% seawater. Uglow (1973) in a study on the effect of oxygen changes on the heart and scaphognathite rates of portunid crabs, compared L. depurator to L. holsatus and Carcinus maenas. His results are discussed in the relevant chapters. Recently, Taylor et al. (1985) have completed an investigation into the oxygen and carbon dioxide transport by the blood of three sublittoral crabs including L. depurator.

Glass (1985), studied the behaviour of L. depurator

from two different sites (Firth of Clyde and Loch Sween) in both the laboratory and in the field (using SCUBA diving) and showed that in the field L. depurator has a behavioural repertoire which includes walking across the surface of the sediment, remaining partially or wholly buried in the sediment, swimming in the water column and hiding in weed and behind boulders. By far the most common activity was simply sitting on the sediment surface (Plates 1.2.A., 1.2.B. and 1.2.C. illustrate different behavioural attitudes, swimming, sitting on the sediment and partially buried respectively). He noted that when threatened it was generally only the smaller crabs which would bury themselves in the sediment whereas the larger ones would swim away.

In his laboratory experiments, Glass (1985), noted that crabs from the shallow water of Loch Sween displayed rhythmic locomotor activity (correlated with tidal and diel variables) whereas the Clyde crabs were largely arrhythmic.

During the present studies, L. depurator was observed in laboratory holding tanks over the period of the study. From these observations it was noted (in agreement with Glass, 1985) that it was generally the smaller crabs which buried themselves in the sediment with the larger ones

PLATE 1.2.

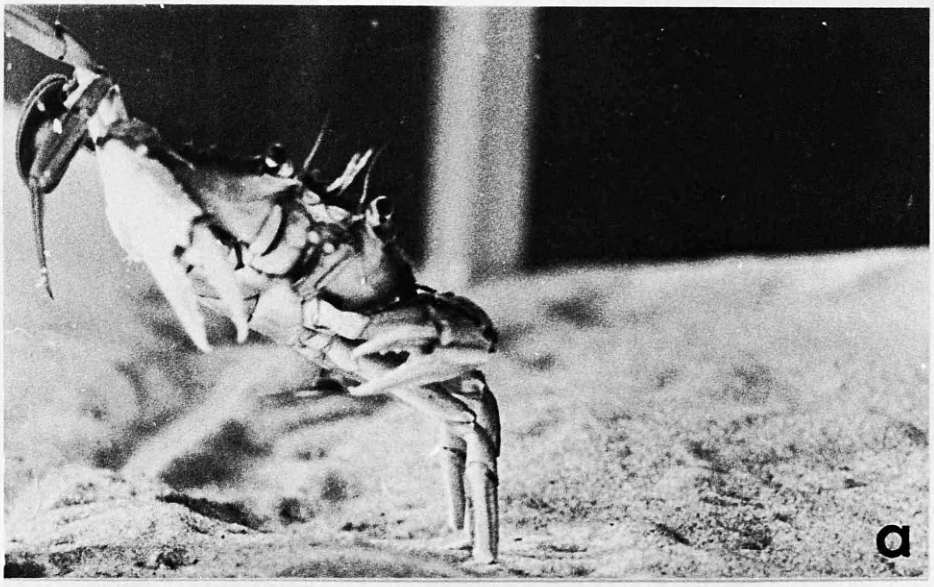
Some of the different behavioural attitudes of L. depurator.

1.2.A. Crab swimming.

1.2.B. Crab sitting on the sediment surface. This was found to be (by general observation) the most common attitude.

1.2.C. Crab partially buried in the sediment.

Scale = Actual size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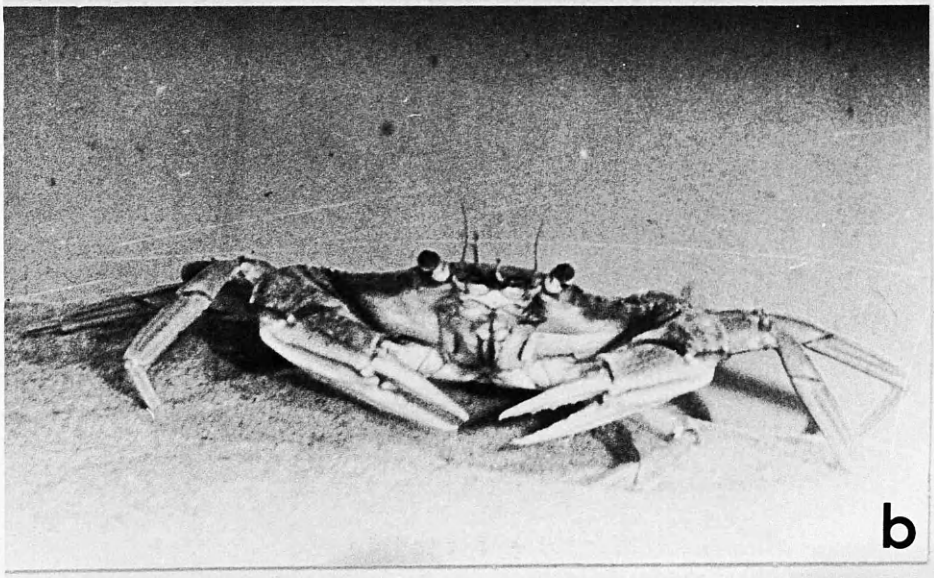
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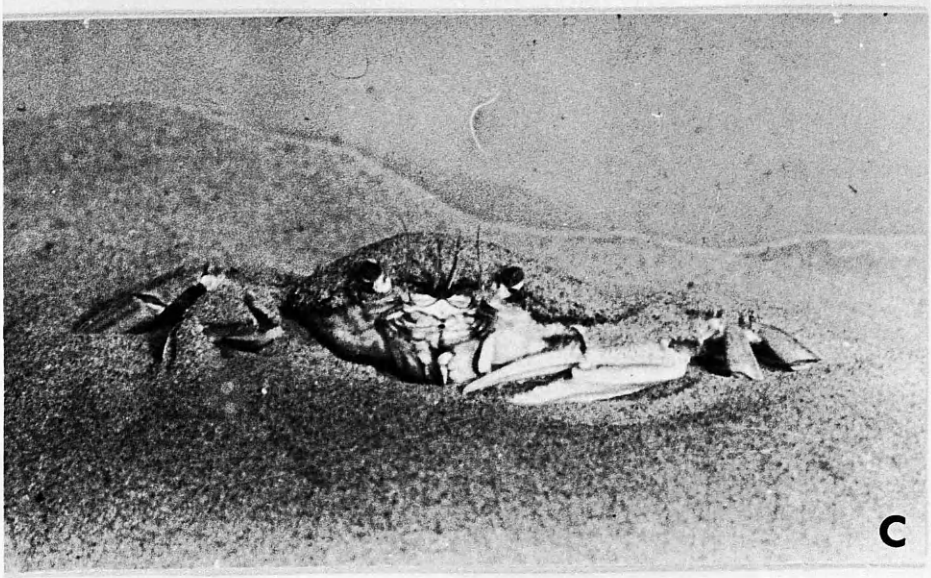
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CHAPTER 2 BRANCHIAL MORPHOLOGY

2.1. Introduction

The functional morphology of the Crustacea has been studied by a number of workers over the years. Pearson (1908) was one of the earliest workers to publish an in depth study of the morphology of a brachyuran crab. In this case it was a study of Cancer pagurus. His treatise has become a classic and forms the basis of present day knowledge of crab morphology. Another early work, devoted solely to the branchial morphology of a crab is that of Borradaile (1922) in which he examined the mouth-parts and the associated respiratory structures of Carcinus maenas. It was this study which introduced much of the basic terminology of the respiratory apparatus of crabs.

Since these early studies, numerous workers have studied the branchial morphology of different crabs usually in conjunction with aspects of the physiology of the animal. Notable among the recent work are the studies of Arudpragasam and Naylor (1964a) on Carcinus maenas, Hughes et al. (1969)

also on C. maenas, Schembri (1980) on Ebalia tuberosa, Hawkins et al. (1982) on Helice crassa and Macrophthalmus hirtipes, Greenaway and Farrelly (1984) on Ocypode cordimanus, Taylor (1984) on ventilatory behaviour of Atelecyclus rotundatus and Eshky (1985) on Ocypode saratan.

A comparative study was carried out on L. depurator to see to what extent the branchial morphology and gill structure differed from the basic portunid pattern and to see if there were any morphological adaptations for burrowing.

2.2. Materials and methods.

The branchial morphology of L. depurator was examined on a number of different levels. These were; (1) by dissection, by simply removing the carapace from around the branchial chamber and the gills, (2) preparing light microscope sections (both transverse and longitudinal) through the gill lamellae and (3) scanning electron microscopy of the gills.

The material for the SEM was prepared by fixing it in 25% glutaraldehyde for 30 minutes then transferring it to 0.2M sodium cacodylate buffer for a period of one hour, after which it was transferred to 2% osmium tetroxide. The material was then critical point dried and sputter coated with gold (20-50 nm). The samples were mounted on aluminium stubs using conductive silver paint. The material was examined and photographed using a Philips SEM 500 instrument at the Zoology Department of the University of Glasgow.

Material for light microscope examination was mounted in paraffin wax, sectioned to 8 - 10 μm. and cleared using the standard series of alcohols and xylol. It was then stained with standard haematoxylin - eosin stain.

2.3. Results and Discussion

As in other crabs, the branchial chambers of *L. depurator* are composed of two parts. 1. The pre-branchial chambers which contain the scaphognathites and 2. the branchial chambers which contain the gills. The chambers are roofed by a cuticular membrane and ventrally they are bounded by the body wall on the inside and exteriorly by the branchiostegite. The scaphognathite is responsible for drawing water through the branchial chamber and thus irrigating the gills. Movement of the scaphognathite was not analysed in this study since this has been studied in some detail by other workers, (Young, 1975; Mercier and Wilkins, 1985).

2.3.1. Pre-branchial Chamber.

There is a pre-branchial chamber situated on either side of the mouth. They are sub-circular chambers smaller than the branchial chambers to which they are connected. Anteriorly, each pre-branchial chamber connects to the exterior by means of the exhalant aperture. Plate 2.1.A. shows the pre-branchial chamber with the carapace removed.

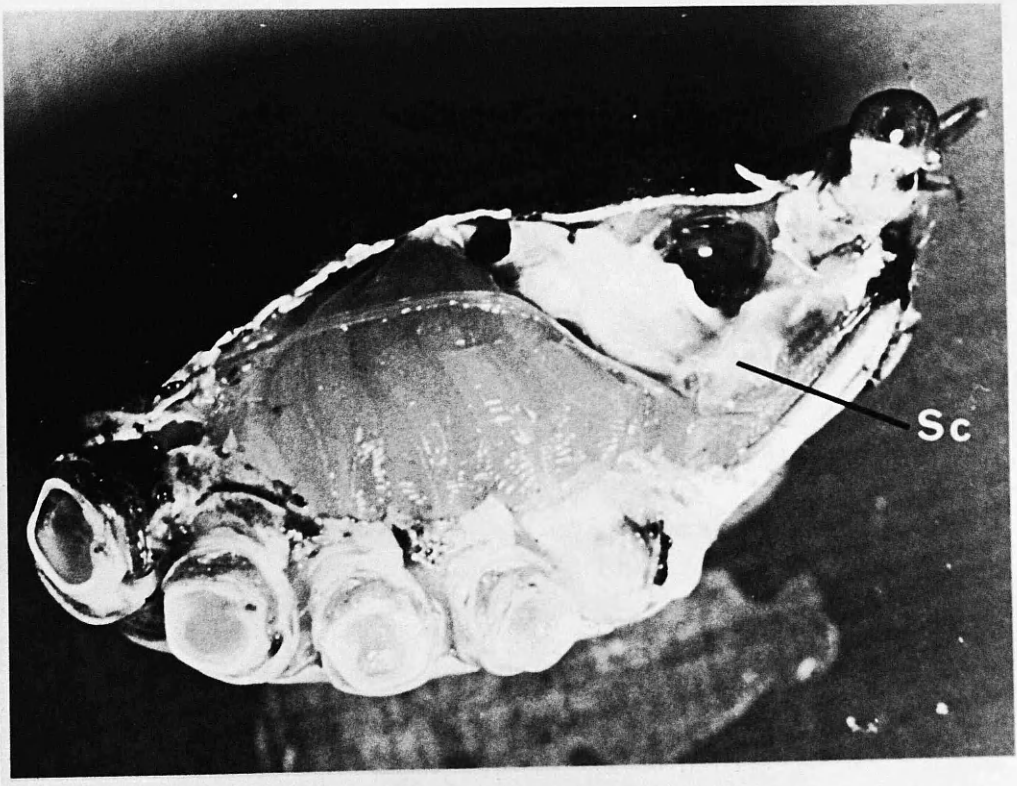
PLATE 2.1.

2.1.A. Pre-branchial chamber with the carapace removed. The scaphognathite is marked Sc and the exhalant aperture and the posterior aperture (connecting to the branchial chamber) are clearly visible

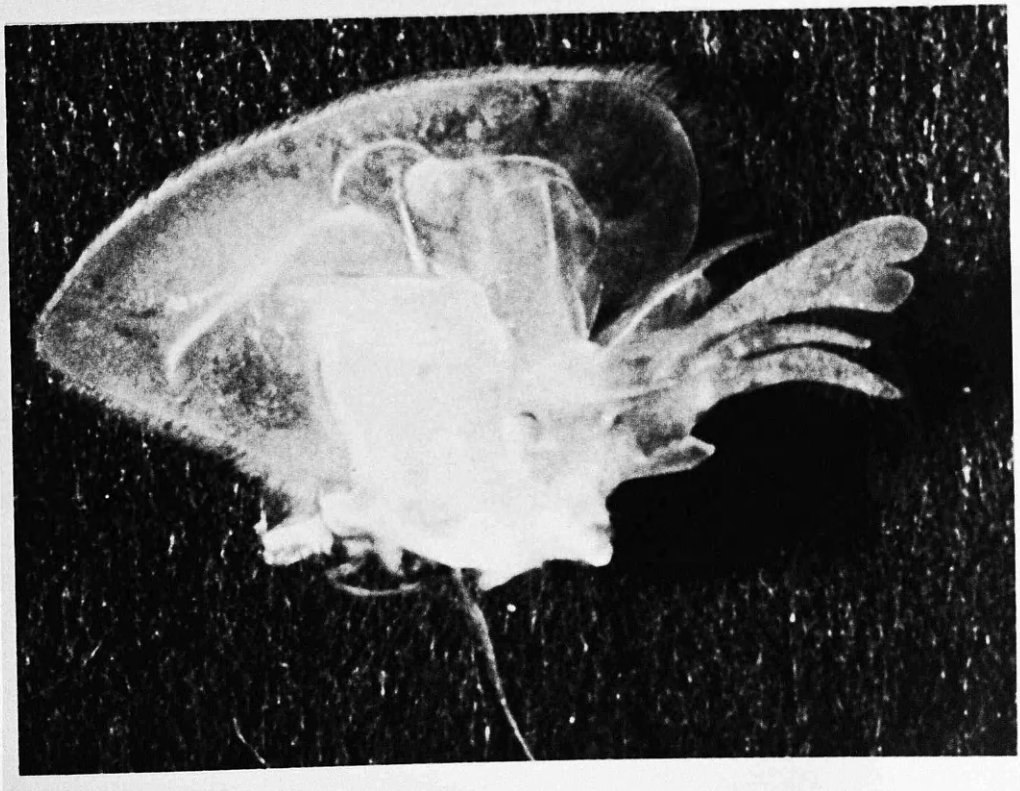
Scale = X 4

2.1.B. The scaphognathite dissected out of the pre-branchial chamber.

Scale = X 12



a



b

The exhalant aperture is clearly visible as well as the posterior aperture connecting to the branchial chamber. The scaphognathite is present in this illustration but cannot be readily distinguished. The walls of the chamber are produced by an ingrowth of the inner edge of the anterior part of the sub-branchial region of the carapace (Pearson, 1908).

Plate 2.1.B. shows the scaphognathite dissected out and Plate 2.2.A. shows a ventral view of the pre-branchial chamber with the walls removed to display the scaphognathite in situ. According to Borradaile (1922) the scaphognathite is derived from the 2nd maxilla. It is the exopodite and is borne on the outer edge of the protopodite of the 2nd maxilla. The scaphognathite has posterior and anterior hinges which allow the scaphognathite to undulate resulting in its pumping action. The alternative name "Gill Bailer" is an excellent description of the mechanism of the scaphognathite. During forward pumping the movement is in an anterior direction and reverse pumping is achieved by movement in the posterior direction. For support, there is a skeletal structure inherent within the scaphognathite. The actual movement is achieved by a system of levator and depressor muscles as described by Young (1975) for Carcinus maenas and also by Hughes et al. (1969) although they use a different

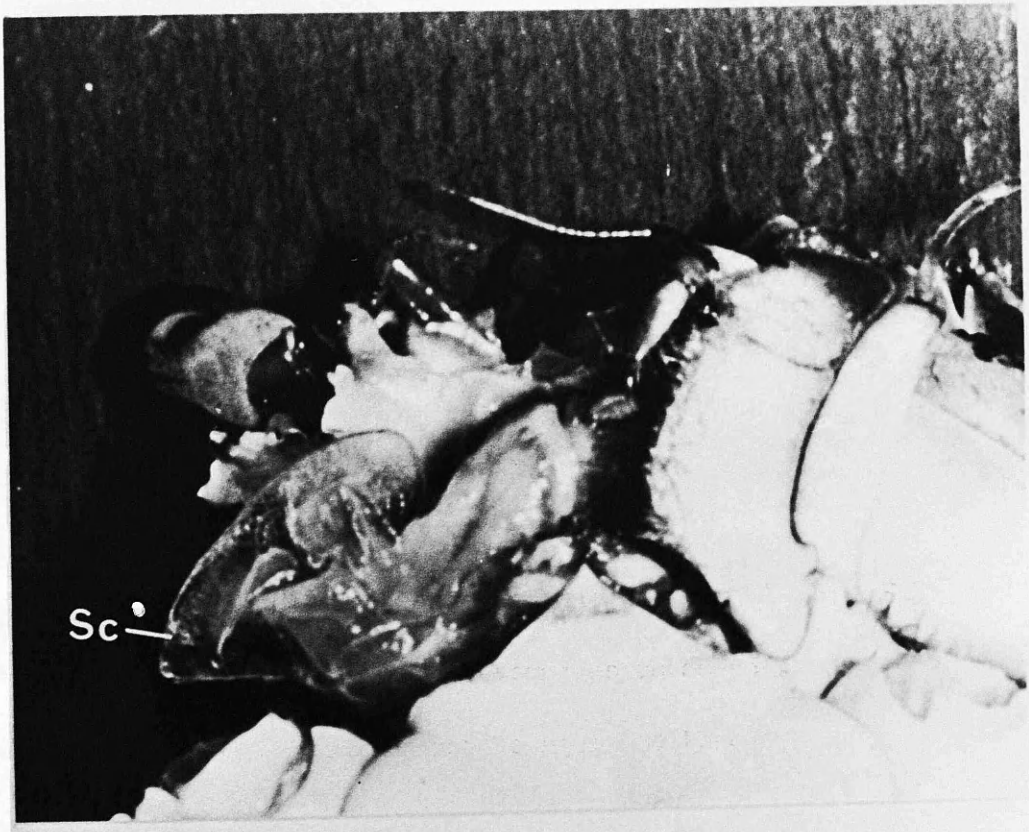
PLATE 2.2.

2.2.A. A ventral view of the scaphognathite *in situ* with the carapace removed. A fringe of setae is visible around the edge of the scaphognathite.

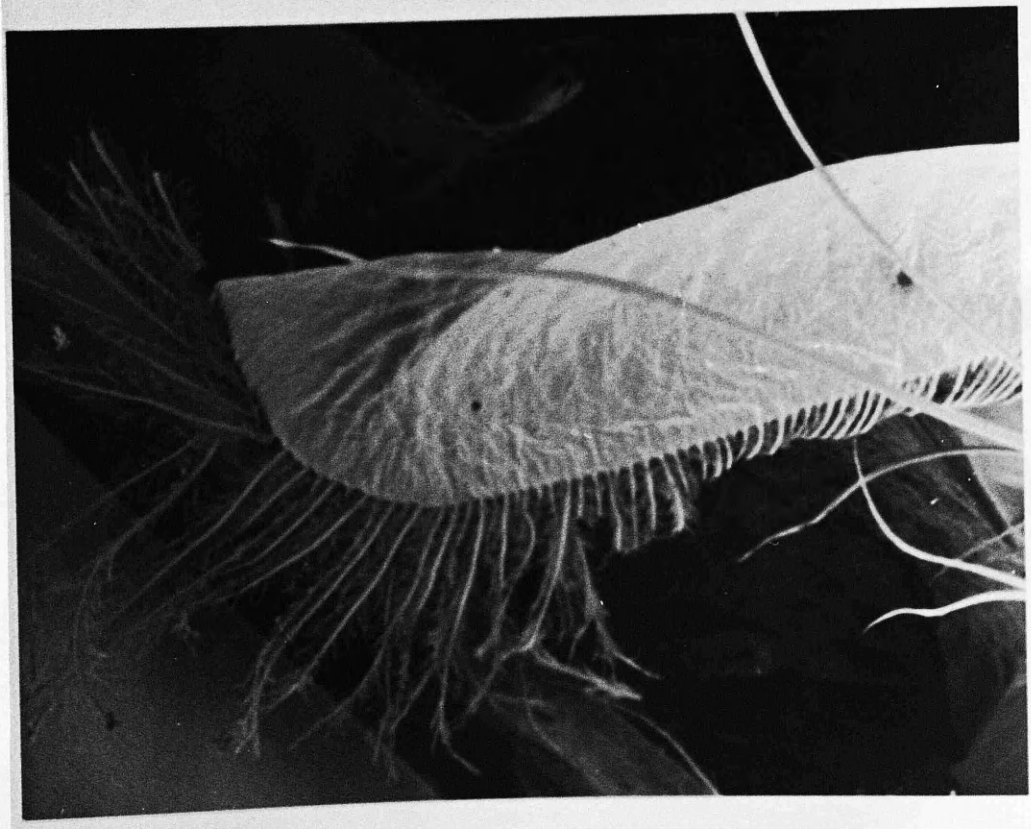
Scale = X 7.5

2.2.B. A scanning electron micrograph of the scaphognathite. Clearly visible is the fringe of setae.

Scale = X 2800



a



b

terminology to describe the muscles involved (extensor and flexor for levator and depressor).

Also visible in Plate 2.1.B. is the fringe of setae around the outer edge of the scaphognathite. The setae are shown in greater detail in Plate 2.2.B. which is an electron micrograph of the scaphognathite. The setae are used to create a seal between the scaphognathite and the walls of the pre-branchial chamber. The plumose nature of these setae enable them to form a tight seal which improves pumping efficiency.

2.3.2. Branchial Chamber

The branchial chamber is formed by a down-growth of the carapace (Pearson 1908). In L. depurator the chamber is more dorso-ventrally flattened than in Carcinus maenas. The walls of the chamber are smooth as illustrated in Plate 2.3.A. Also evident in the walls are what appear to be pores. Plate 2.3.B. is a high magnification electron photomicrograph of one the pores. It is not known what function these pores perform in L. depurator. In contrast, the pores present in the epibranchial "lungs" of some land crabs have a respiratory function (Diaz and Rodriguez 1977). No pores have been reported previously in wholly sublittoral brachyurans.

Plate 2.4.A. shows the branchial chamber with the carapace removed and the gills in situ. As stated in the previous section there is a connecting aperture to the pre-branchial chamber at the anterior end of the branchial chamber. At the posterior end of the branchial chamber and ventrally located, there are the inhalant openings, which open to the outside at the bases of the limbs. The most significant of these openings being the Milne-Edwards openings at the base of the chelipeds.

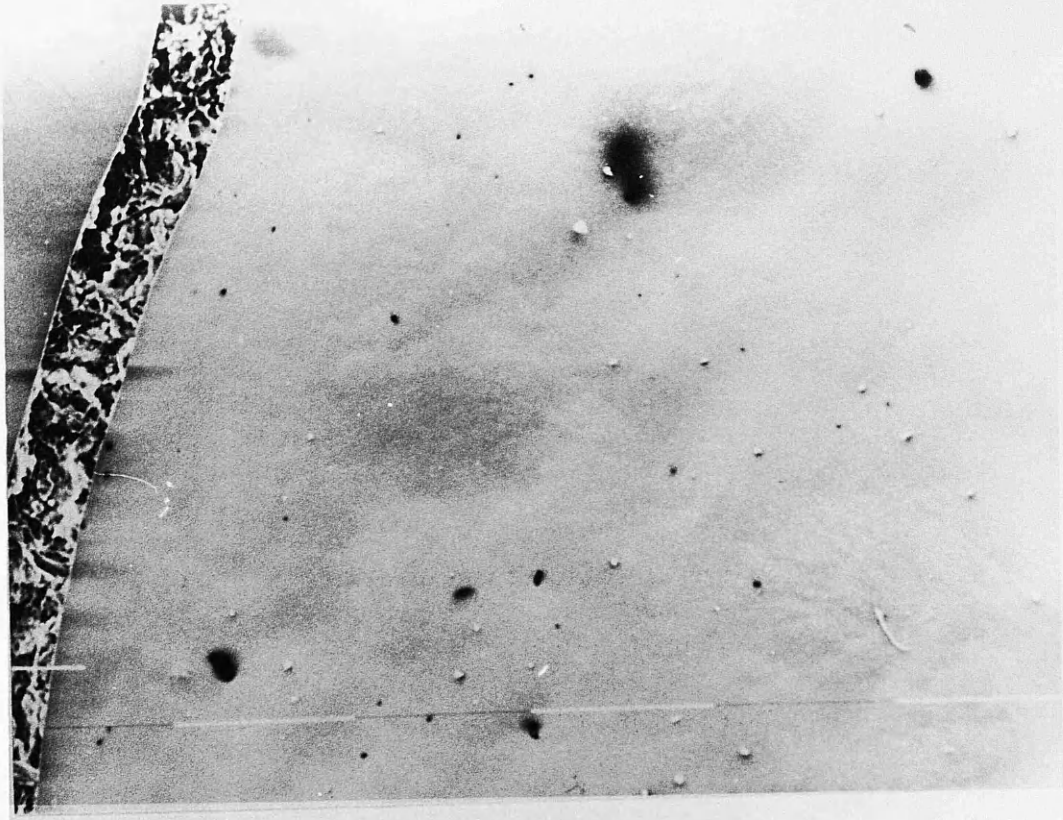
PLATE 2.3.

2.3.A. A low magnification scanning electron micrograph of the branchial chamber wall which shows the smooth surface of the wall and what appears to be pores.

Scale Bar = 100 μm

2.3.B. A high magnification scanning electron micrograph of one of the branchial chamber wall pores.

Scale Bar = 10 μm



a

b

PLATE 2.4.

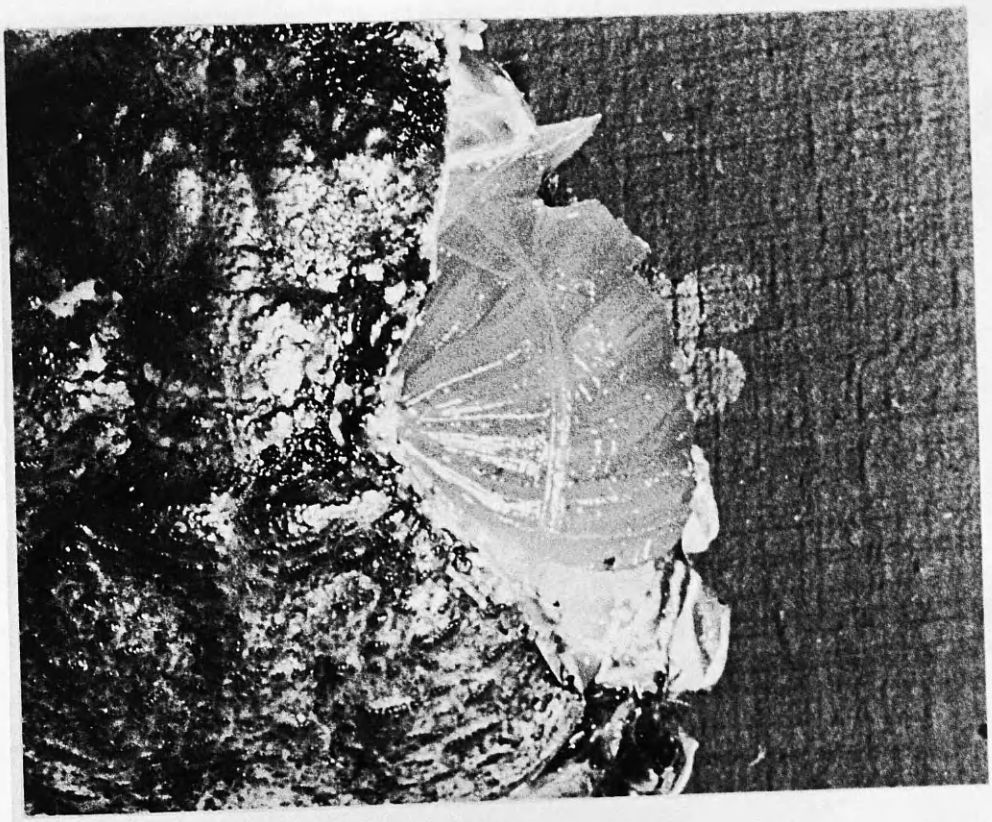
2.4.A. Dissection of the right branchial chamber providing a dorsal view of the gills <u>in-situ</u>.

Scale = X 3

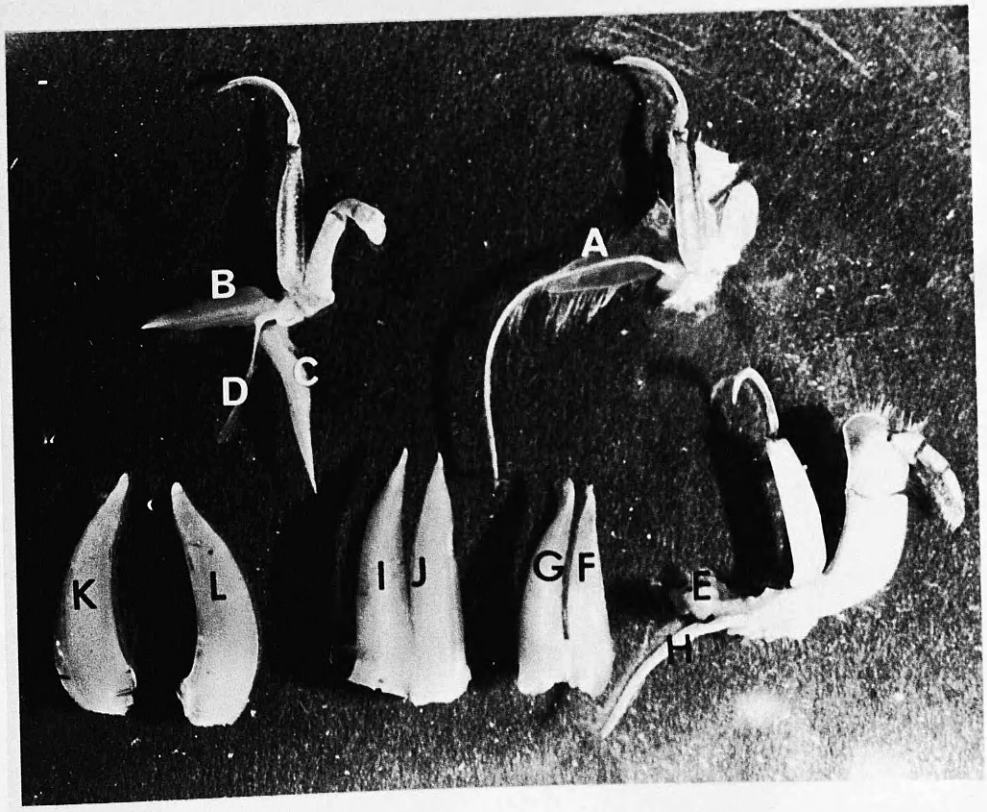
2.4.B. The individual gills dissected out.

Scale = X 3

See text for identification of A - L.



a



b

The epipodite (mastigobranch) of the 3rd maxilliped is clearly visible lying across the surface of the gills.

The gill formula for L. depurator was determined to be as follows:-

	Thoracic Somites							
	1	2	3	4	5	6	7	8
	1st maxpd.	2nd maxpd.	3rd maxpd.	cheliped	2 perei.	3 perei.	4 perei.	5 perei.
Podobranch		1	1					
Arthrobranch								
Ant.		1	1	1				
Post.			1	1				
Pleurobranch					1	1		
Epipodite	1	1	1					

As can be seen from the gill formula there are nine gills and three epipodites in each branchial chamber.

Plate 2.4.B. illustrates the nine gills and three epipodites dissected out individually.

They are :

A: Epipodite of the 1st maxilliped

B: Podobranch of the 2nd maxilliped

C: Anterior arthrobranch of the 2nd maxilliped

D: Epipodite of the 2nd maxilliped

E: Podobranch of the 3rd maxilliped

F: Anterior arthrobranch of 3rd maxilliped

G: Posterior arthrobranch of the 3rd maxilliped

H: Epipodite of the 3rd maxilliped

I: Posterior arthrobranch of the cheliped

J: Anterior arthrobranch of the cheliped

L: Pleurobranch of the 2nd pereiopod

K: Pleurobranch of the 3rd pereiopod

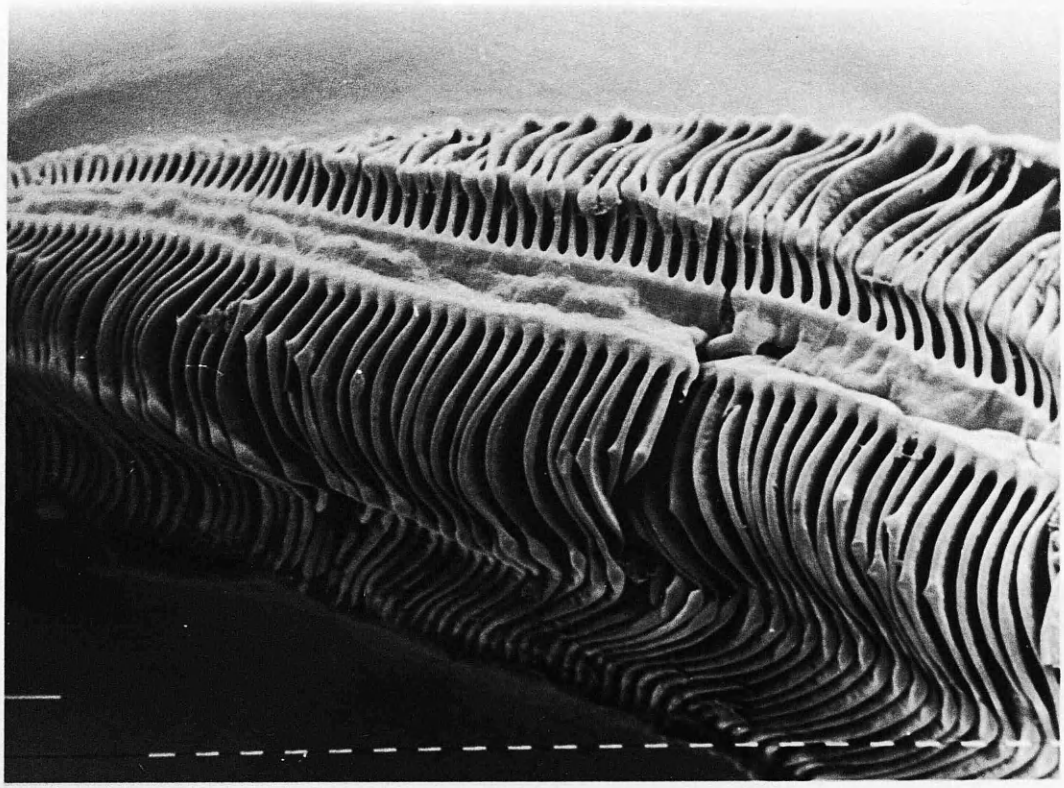
Plates 2.5.A. and 2.5.B. are scanning electron micrographs which show gill morphology and lamellae detail repectively. In both, nodules at the apex of the lamellae are easily discernible. These are thought to keep the lamellae separate and allow free flow of water between them. The nodules are not present on every lamella and there does not appear to be any obvious pattern to their distribution. Sclerotization is apparent on the edges of the lamellae and

PLATE 2.5.

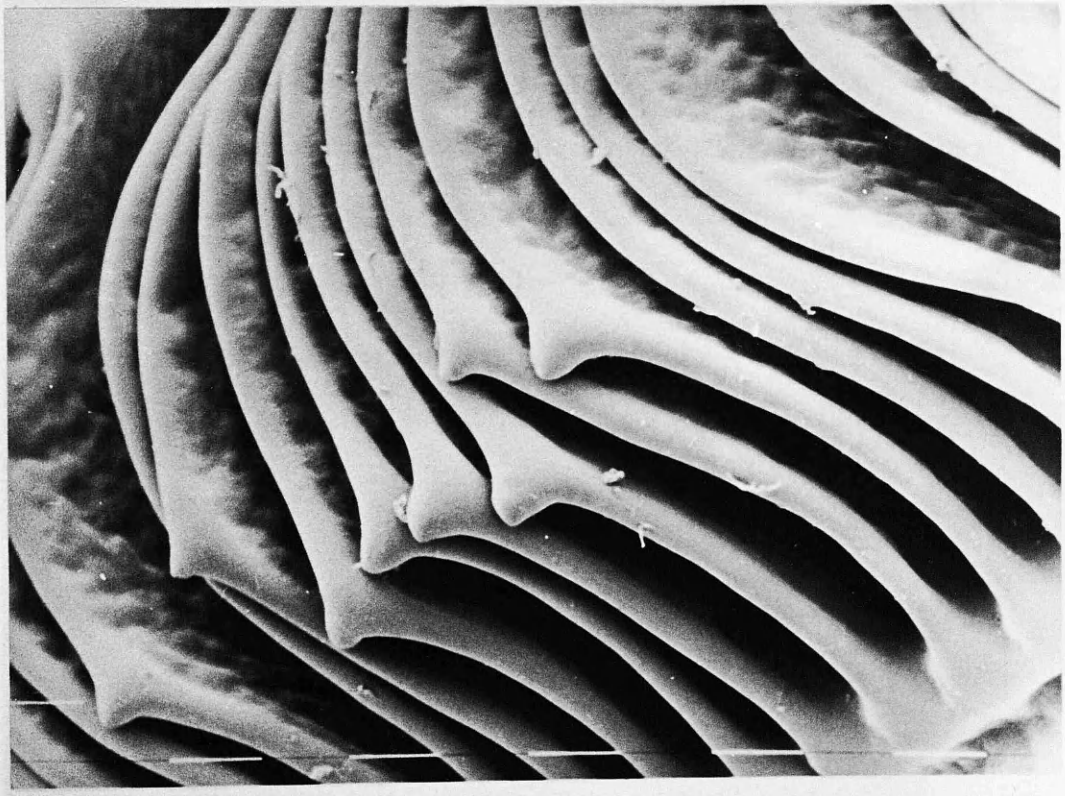
Scanning electron micrographs of the pleurobranch of the 2nd pereiopod to show detail of gill structure.

2.5.A. Scale Bar = 100 μm

2.5.B. Scale Bar = 100 μm



a



b

is thought to serve as a supportive mechanism for the lamellae.

Plate 2.6.A. is a longitudinal section through the gill. The outer lamellar sinus is contained within the nodular dilations along the lateral edge of the gill lamellae. The lamellar sinus can be seen more clearly in Plate 2.6.B. with bridges of epidermal cells across the lamellae. On the ventral side of Plate 2.6.A. the efferent branchial vessel is distinguishable. In Plate 2.6.B. blood cells are visible in the lamellar sinus.

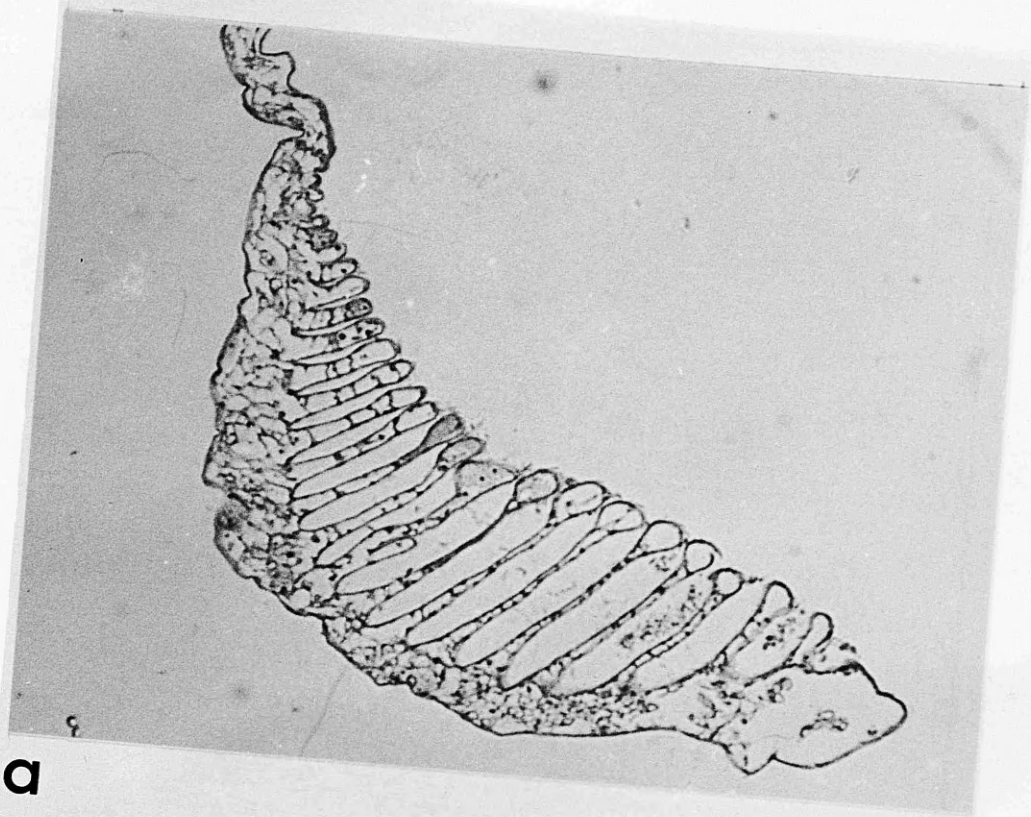
Plate 2.7. is another photomicrograph of the gill lamellae. On the surface of the lamellae there is apparently some type of microorganism. An attempt was made to identify it at the Department of Mycology of Glasgow University. Although no exact identification was made, it is thought that the growth was a species of Actinomycete. It is not known to what extent this affects the respiratory function of the gills nor the scale of the infection in the Clyde population of L. depurator.

PLATE 2.6.

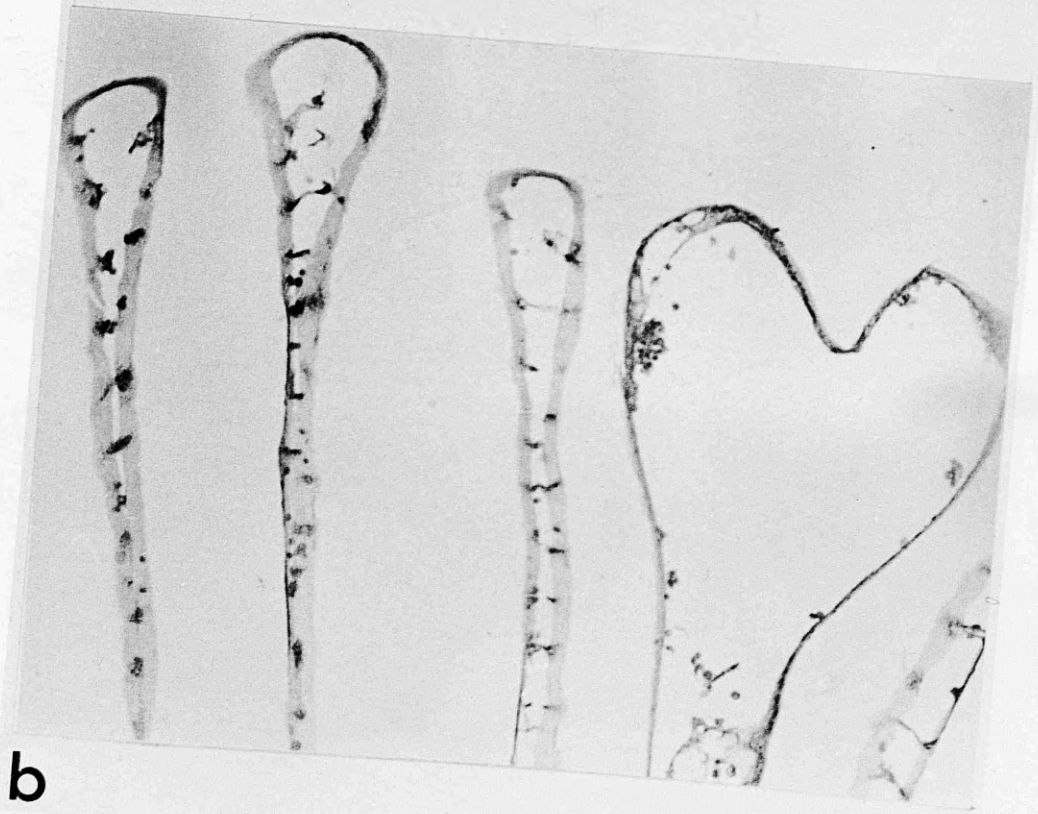
Light microscope preparations showing longitudinal sections through the pleurobranch of the 2nd pereiopod.

2.6.A. Scale = X 60

2.6.B. Scale = X 100



a

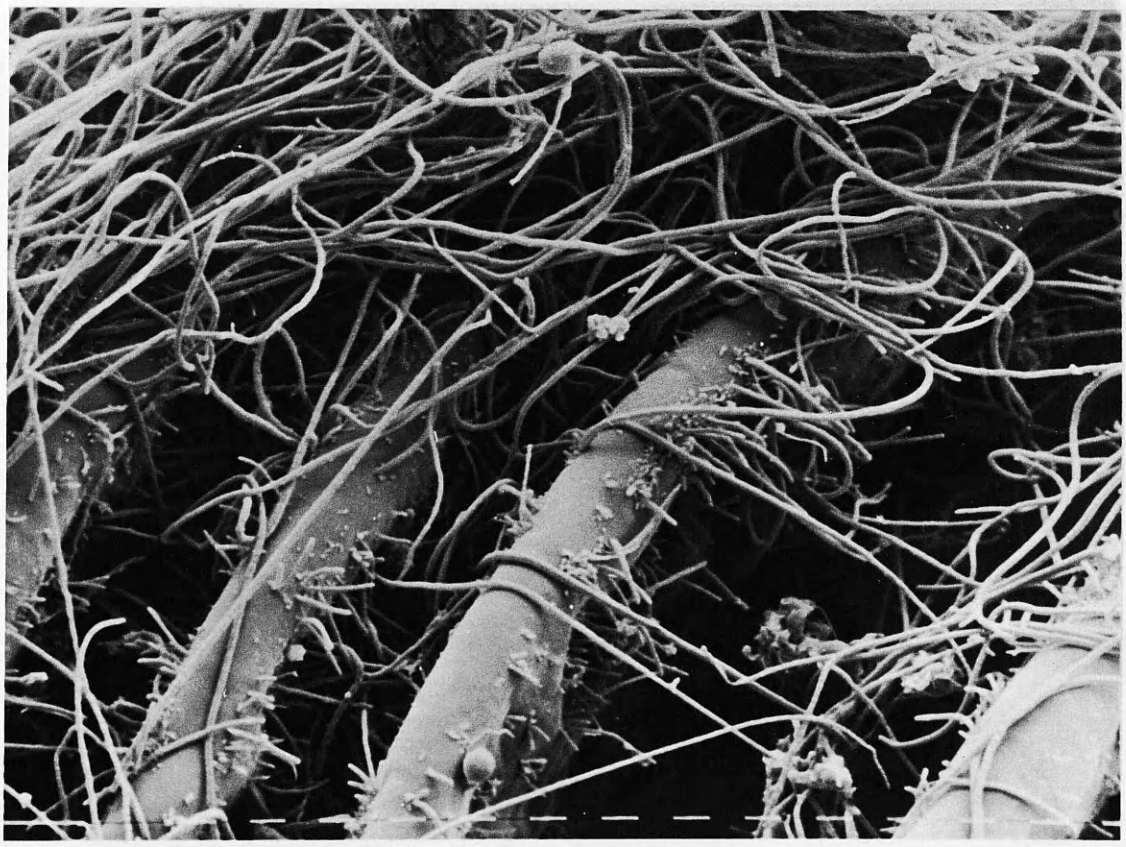


b

PLATE 2.7.

A scanning eletron micrograph of gill lamellae which has an unidentified growth on them.

Scale Bar = 10 μm



2.3.3. Water Flow

The direction of water flow over the gills was determined by injecting blue dye (pen recorder ink) into the water adjacent to the crab. It could readily be seen that the water was sucked in via the Milne-Edwards openings at the base of the chelipeds and to a lesser extent via the openings in between the other pereiopods, and exhaled via the exhalant openings adjacent to the mouthparts. Water was drawn in by the undulating movement of the scaphognathite which lies in the prebranchial chamber. This movement acts as a pump which creates negative pressure in the branchial chamber. Water is drawn into the hypobranchial space, passes between the gill lamellae into the epibranchial space and then passes anteriorly over the gill lamellae to the prebranchial chamber and from there to the exhalant openings. Gaseous exchange occurs as the water passes over the lamellae (Hughes et al., 1969). Details of ventilatory rates are given in Chapter 3.

An attempt was made to try to identify the major areas of respiratory gas exchange in the gills using the method of Bertolani (1933, 1934) for holothurians (as cited by Diaz and Rodriguez, (1977)) , Edney and Spencer (1955) for isopods and Diaz and Rodriguez (1977) for terrestrial crabs.

This involved the injection of a reduced solution of methylene blue (reduced by sodium sulphite) into the heart of the crab. With oxidation of the solution the tissues should stain blue at the site of oxygen uptake. Reduction and re-oxidation was achieved on the bench using this technique but unfortunately it was not successful in L. depurator. Similar problems in this technique were found by R.J.A. Atkinson (pers. comm.) and Eshky (1985).

In general the branchial morphology of L. depurator is similar to other Portunidae such as Carcinus maenas and Cancer pagurus. Although it is adapted for swimming and can also bury itself under the substratum, there are no obvious branchial morphological adaptations for these modes of behaviour.

CHAPTER 3 CARDIAC AND VENTILATORY ACTIVITY

3.1. Introduction

In the following experiments an attempt was made to determine the heart and scaphognathite rate of L. depurator and to see if the rates showed a predictable pattern. In order to do this an impedance technique was used. Impedance techniques permitted the recording of the heart rate and scaphognathite rate in L. depurator with minimum interference to crab movements and general activity during experimentation. Implantation of cannula tubing into the branchial chamber in order to detect branchial hydrostatic pressure changes also imposed only minimal interference on movement. The usefulness of these techniques is well established (Trueman, 1967; Ansell, 1973; Uglow, 1973; Cumberlidge & Uglow, 1977 and Taylor, 1976, 1977). See Chapter 1.

Experiments were also performed to determine the effect of stress on both heart and scaphognathite rates. Recordings of the pressure in the branchial chamber were

carried out simultaneously with recordings of the scaphognathite rate. The branchial pressure was recorded in order to confirm respiratory current reversals. The problems of stress effects and rate variability have already been introduced in Chapter 1.

3.2. Materials and methods

3.2.1. Collection and Maintenance of Animals

All the crabs were caught in the immediate vicinity of Millport by using a 2 metre beam trawl, trawled from the University Marine Biological Station's research vessels RV Aplysia and RV Aora. The trawl had a mesh size of 65mm and was trawled along the sea bed at 2 knots. The crabs were generally caught at a depth of around 40 to 50 metres, in the Largs - Fairlie Channel which has a heterogenous substratum consisting of varying proportions of mud and sand with gravel and shell debris (oversimplified in Deegan et al.,1973). The animals spent at most 2 - 3 hours in tanks on board the vessels before being brought ashore and put into laboratory holding tanks.

The holding tanks had sea water constantly flowing through them at ambient sea water temperature. This varied from 4°C in the winter to 14°C in the summer. Except in extremes of temperature i.e. very cold winter spells, outside tanks were used; therefore a natural photoperiod was maintained. The holding tanks were 110cm by 50cm by 30cm

and had a substratum of sand to a depth of 6cm.. The experimental animals were held in the holding tanks for at least two days and were not fed prior to experimentation. This was done to ensure that all the experimental animals were in the same nutritional state for the experiments since Ansell (1973) found a difference in the heart rate of fed and unfed Cancer pagurus and other workers have found similar differences in other species.

3.2.2. Recording Techniques.

Heart recordings were obtained by implanting two small silver wires through the carapace on either side of the heart. Similarly, the rate of beat of a scaphognathite was recorded by implanting two silver wires through the branchiostegite into the appropriate prebranchial chamber. Plate 3.1.A. and 3.1.B. show cardiac electrodes and scaphognathite electrodes implanted respectively. In order to facilitate this electrode implantation, holes were drilled in the carapace and the branchiostegite using either a dentist drill or a syringe needle. The electrodes were held in place with "Permabond C" (Permabond Adhesives Ltd.), a quick

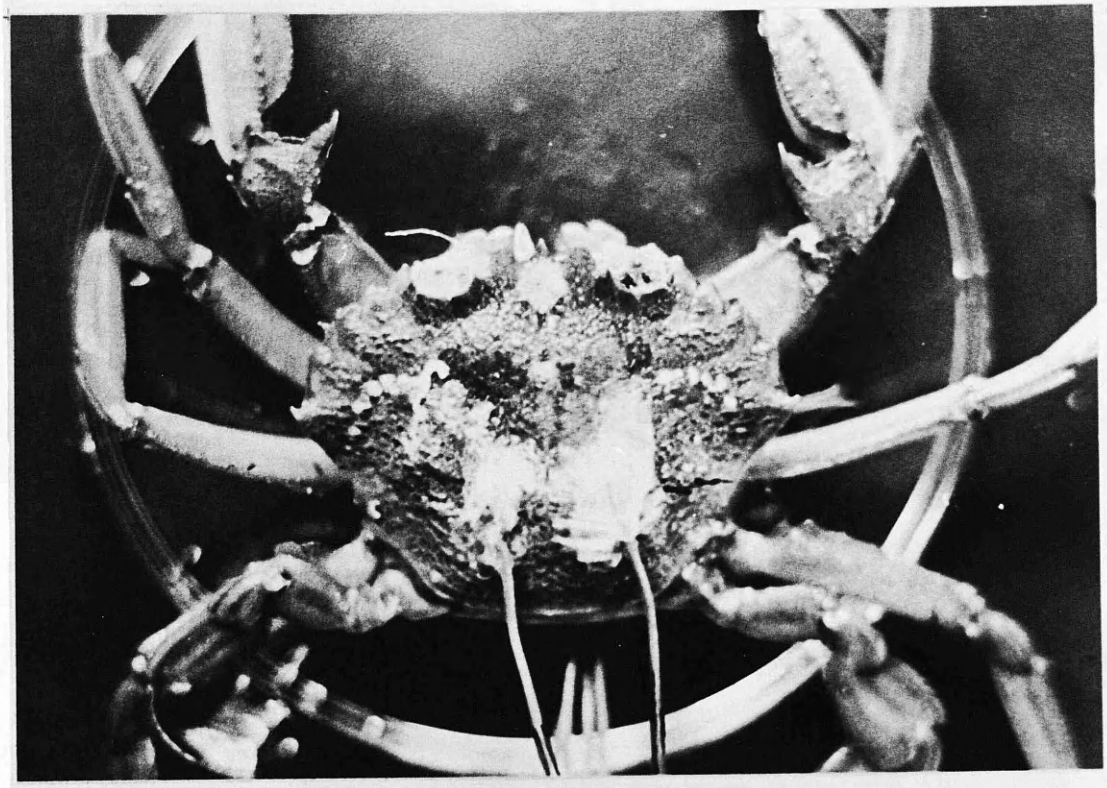
PLATE 3.1.

Illustration of electrode implantation for heart and scaphognathite recordings.

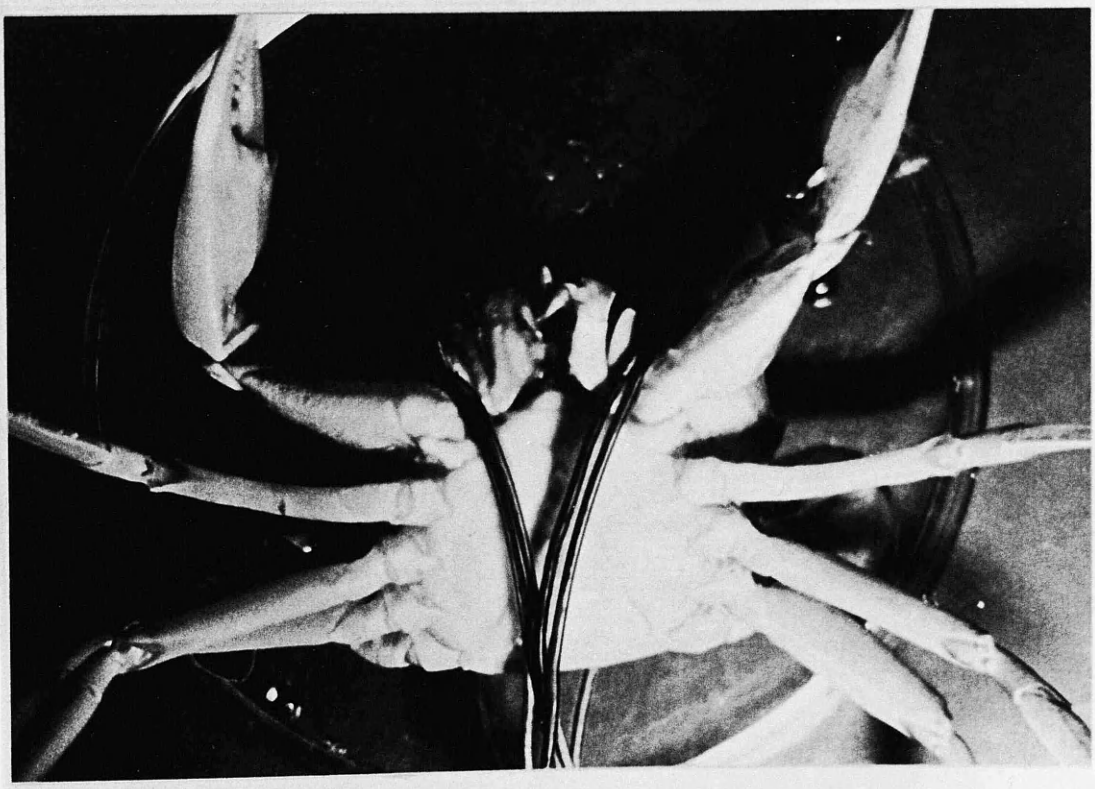
Scale = X 1.5

3.1.A. Dorsal view to show position of cardiac electrodes.

3.1.B. Ventral view to show position of bilateral scaphognathite electrodes.



a



b

setting cyanoacrylate glue. This was found to be non-toxic to the crab provided that activator, if applied, was not used in excess. Each pair of electrodes was connected to an impedance monitor (Strathkelvin Instruments) by means of single strand 10/0.1 wire (R.S. Components). The impedance monitor was connected to an Oscillograph 400 MD/2 (George Washington, Bio-Science) which enabled heart and scaphognathite activity to be amplified and displayed on a paper trace output.

In order to record branchial pressure a small hole was drilled dorsally into the branchial chamber. Narrow bore cannula tubing (diameter 1mm) was then inserted into the hole, taking care to ensure that the opening in the tubing remained unblocked by any tissue. The tubing was then connected to a PT 400 blood pressure transducer which was connected to a MD/2 oscillograph via a FC 137 strain gauge (all Washington Bioscience). This gave a trace of the hydrostatic pressure within the branchial chamber from which it was possible to deduce the direction of water flow through the chamber.

In order to determine the heart rate or scaphognathite rate in beats. min^{-1}, a thirty second period

of appropriate recording trace was taken and the beats counted and multiplied by two to give a rate in beats. min^{-1}

During recordings of heart and scaphognathite rates and of branchial pressure, the crabs were kept in tanks with a substratum of sand and supplied with flowing sea water. The tanks were approximately 67cm by 45cm by 17cm and the substratum of sand was between 2-6cm in depth. A 6cm depth of sand was found to be sufficent to allow the animals to bury themselves. A curtain was placed around the tank to prevent disturbance of the crabs. The temperature of the water in the experimental tanks was generally about 8-12°C with the exact temperature being dependent on the ambient sea water temperature. Only male crabs in the intermoult stage were used.

The levels of locomotory activity exhibited by L. depurator were observed using a closed circuit television system (National CCTV Video Camera, Model WV-1350 and National Video Monitor, Model WV 5310 9", monochrome) and recorded on reel to reel video tape (National Time Lapse Video Tape Recorder, Model NV-8030). The crabs were placed in an experimental tank (as previously described) and a red light (15 watts) was used for illumination. Recordings were

made over twenty-four hours and the tapes analysed to determine the time spent in actual movement. In some cases, video recordings and heart recordings were taken simultaneously.

3.2.3. Hydrostatic Pressure

Using facilities readily available at the University Marine Biological Station an experiment was run to see the effect of hydrostatic pressure on the heart rate thus simulating the depth at which the Clyde population of L. depurator is normally found. An aquarium tank, 35cm by 20cm by 20cm with a sand substratum of approximately 6cm. depth was placed in a therapeutic recompression chamber (Hunting 54" D.D.C.). A crab with heart electrodes implanted was placed in the aquarium tank and the leads connected via the recompression chamber's microphone link to a George Washington chart recorder outside the chamber. The pressure was increased to an equivalent depth of 50m (the depth at which L. depurator normally occurs in the Clyde) both by discrete steps and by continuous compression and brought up to surface pressure either in stages or continuously. The heart rate was monitored throughout these procedures.

3.3 Results

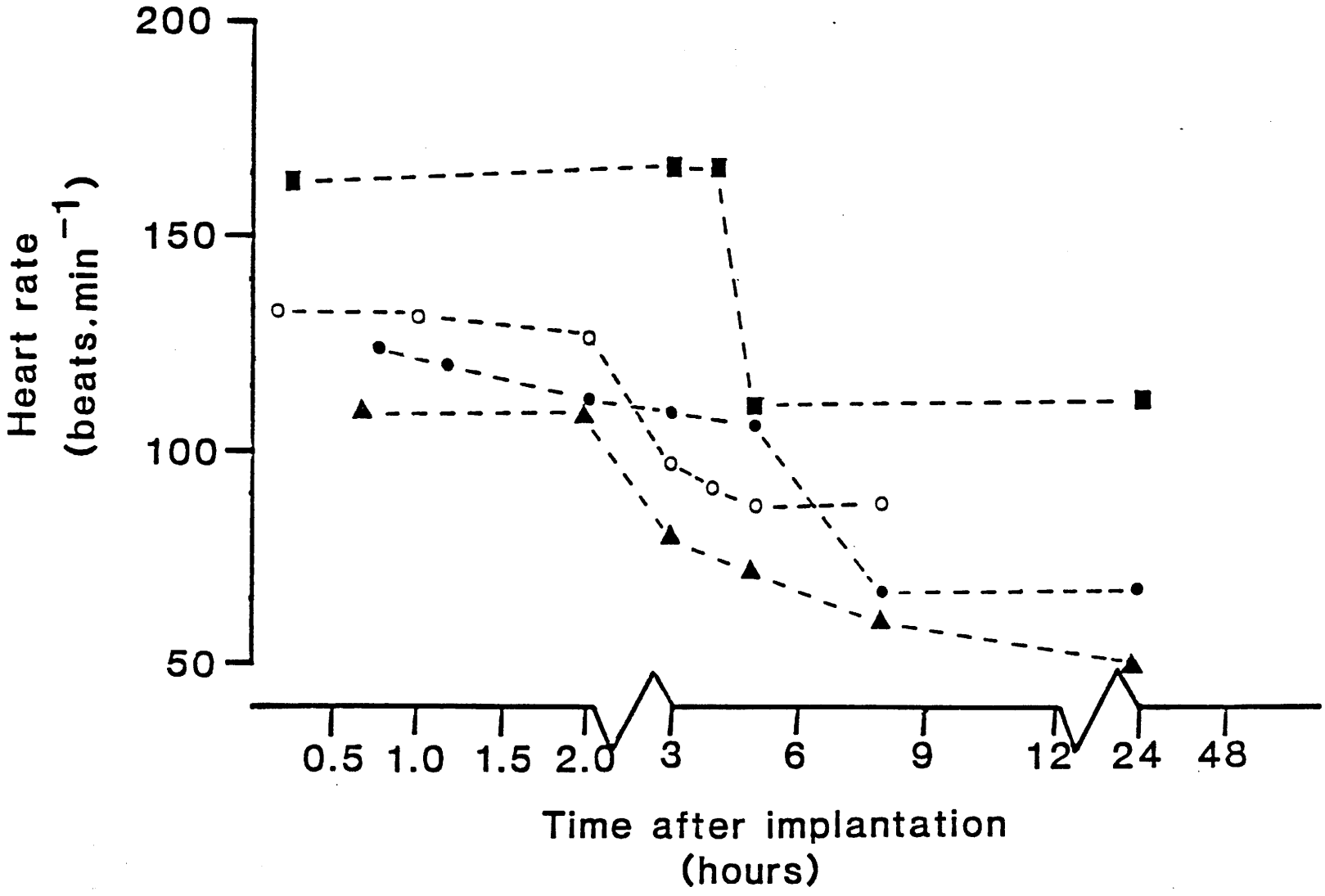
3.3.1. Heart Rate

3.3.1.1. Variability

Initial experiments showed that there was considerable variation in the heart rate of L. depurator both between individuals and in the same individual. A number of heart recordings were performed on a number of crabs at the same time under the same conditions (the crabs were held in laboratory aquarium tanks with flow through seawater at ambient seawater temperature). Figure 3.1. shows an example of the heart rate of four individual crabs, with heart rate related to the time after implantation of the electrodes. Heart rate was determined at 15 minute intervals for the first two hours of the experiment and thereafter at greater intervals for a period of 24 hours. The graph indicates a general decrease of heart rate with time and shows a range of rate from 166 beats. min^{-1} to 50 beats. min^{-1}

FIGURE 3.1.

Heart rates of four individual crabs against time after electrode implantation. The recordings were performed in a laboratory aquarium tank with flow through seawater at ambient seawater temperature. The weight range of the crabs was from 21.5 g to 27.8 g.

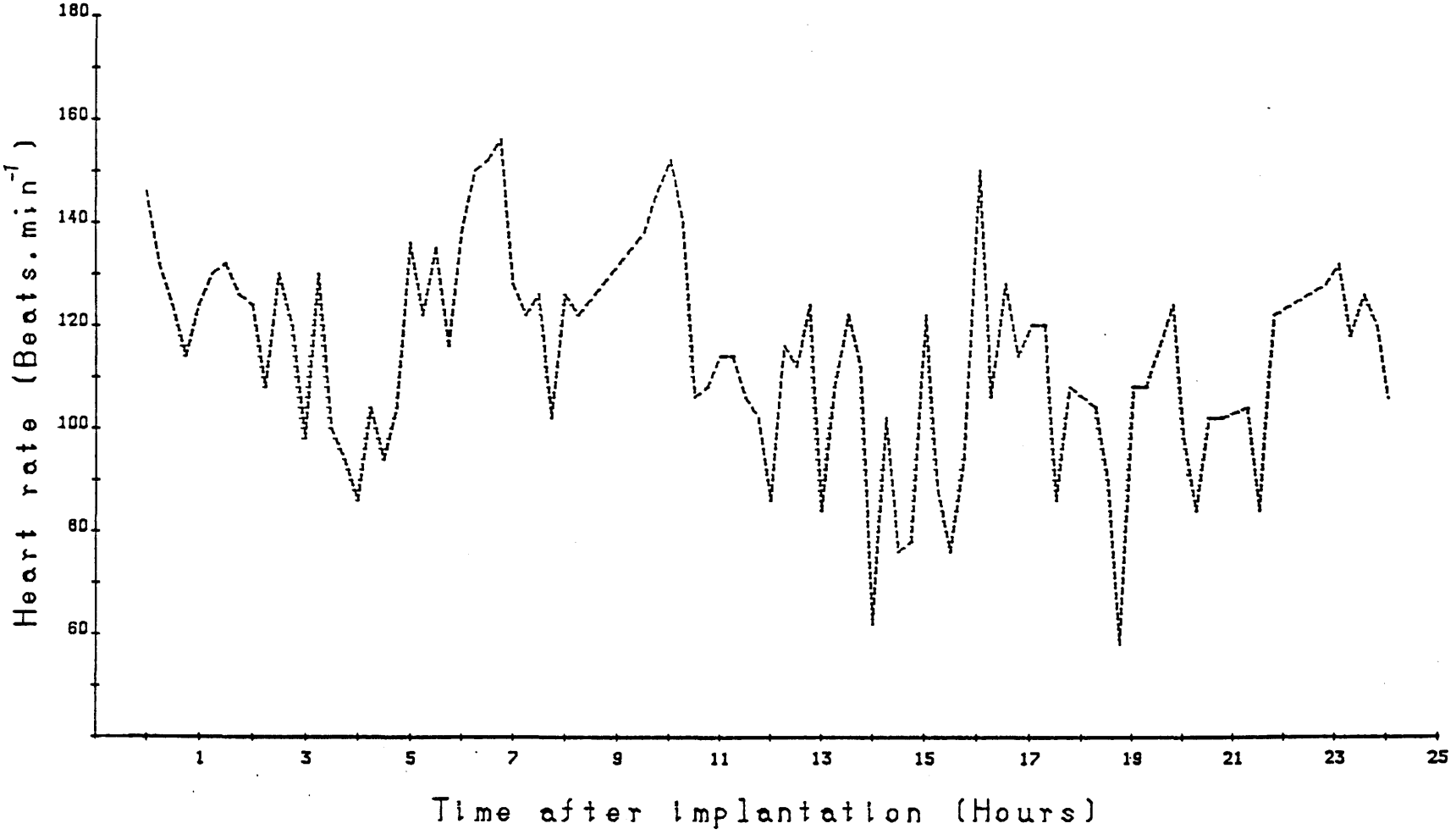

200
150
100
50
Heart rate
(beats.min^{-1})
0.5
1.0
1.5
2.0
3
6
9
12
24
48
Time after implantation
(hours)

During these recordings it was observed that the heart rate varied within short time periods. In order to determine accurately the degree of variability, a continuous 24 hour recording was made of the heart rates of some individual crabs.

Figure 3.2. illustrates a graph of a heart recording over 24h with rates being taken every 15 min. In this graph the heart rate in beats. min^{-1} is plotted against the time after electrode implantation. It can be seen that the rate is extremely variable and it would be inappropriate to determine an "average" heart rate. However, there appears to be a change in the range of heart rates after approximately 10-12 hours. Prior to this time the range was from 157-86 beats. min^{-1} and after 12 hours the heart beat range was 128-58 beats. min^{-1}.

FIGURE 3.2.

Heart rate of an individual male crab of 21.5 g in a flow through seawater tank at ambient seawater temperature. The heart rate was recorded at 15 min intervals over a period of twenty-four hours after electrode implantation.


Heart rate (Beats.min^{-1})
180
160
140
120
100
80
60
1
3
5
7
9
11
13
15
17
19
21
23
25
Time after implantation (Hours)

3.3.1.2. Cardiac Arrest

In addition to a variable heart rate, periods of cardiac arrest were also observed during which the heart stopped beating for differing periods of time. Figure 3.3. shows the frequency and duration of heart pauses over a period of 24 hours. From this it can be seen that a pause can vary from 5 seconds to 216 seconds. In the first two hours after electrode implantation there were no periods of cardiac arrest and it appears that the duration and frequency of the periods of cardiac arrest increased with time after electrode implantation. Since occasional beats may occur erratically during an obvious pause, a pause has been defined as a range of heart rates from 0 beats. min^{-1} over a minimum period of 5 seconds to a maximum of 20 erratic beats. min^{-1} over a maximum period of 15 seconds. This definition is illustrated in Figure 3.4. and has been developed from numerous observations on cardiac pausing behaviour in different individuals.

As Figure 3.4. illustrates, periods of cardiac arrest in L. depurator are not necessarily simple cessation of heart beat as there can be a few erratic beats during a period of arrest. Example 1 would be called a 'Simple' pause

FIGURE 3.3.

The relationship between length and frequency of cardiac pauses over a period of twenty-four hours after electrode implantation.

A regression analysis was performed on the data using the method of least squares. The regression line is shown.

The regresssion equation $Y = 32.0 + 0.0692\ X$ was determined; with Y being Length of pause and X being Time of start of pause after electrode implantation.

An r value of 0.584 was determined with $p<0.001$ ($n=102$).

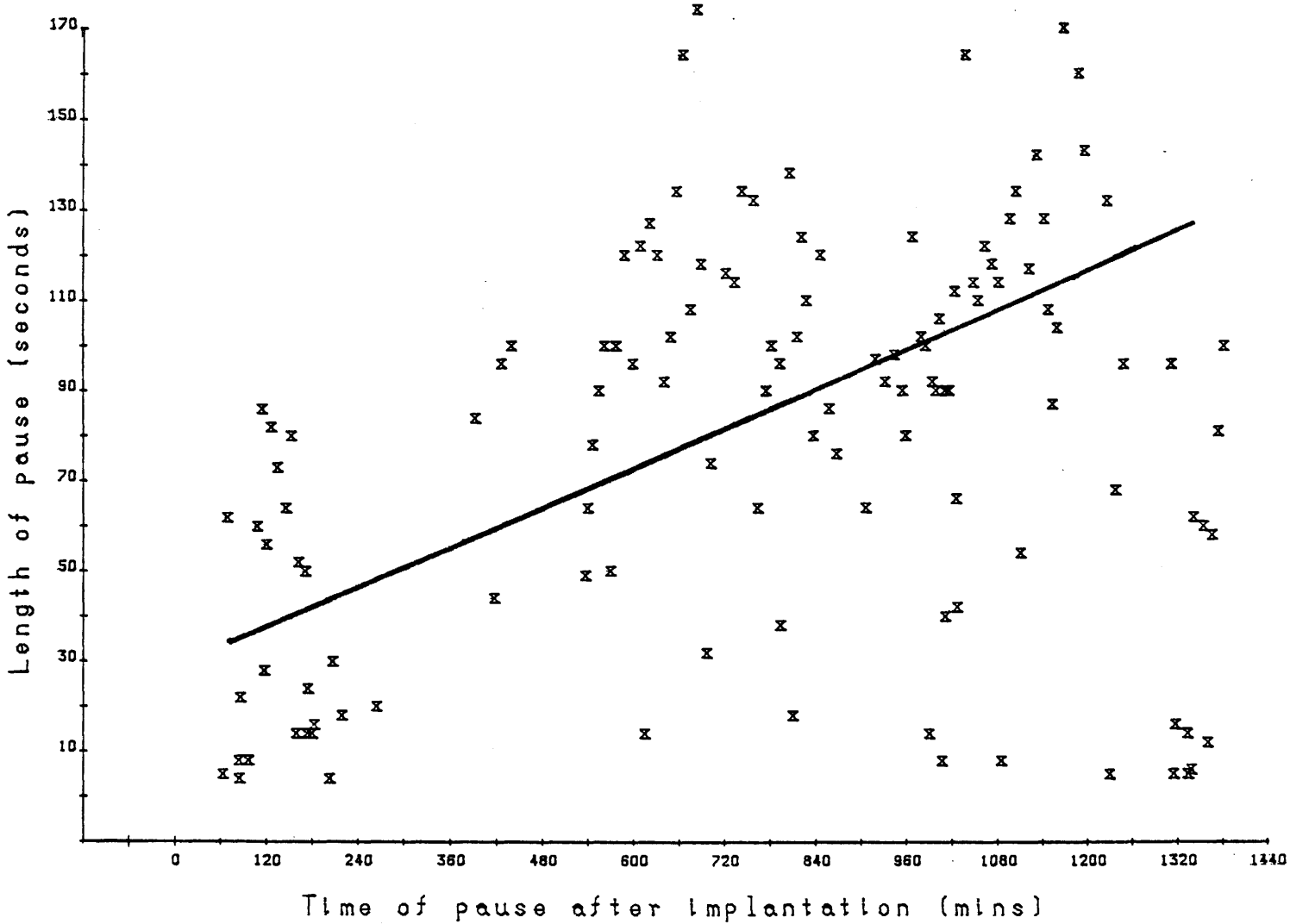
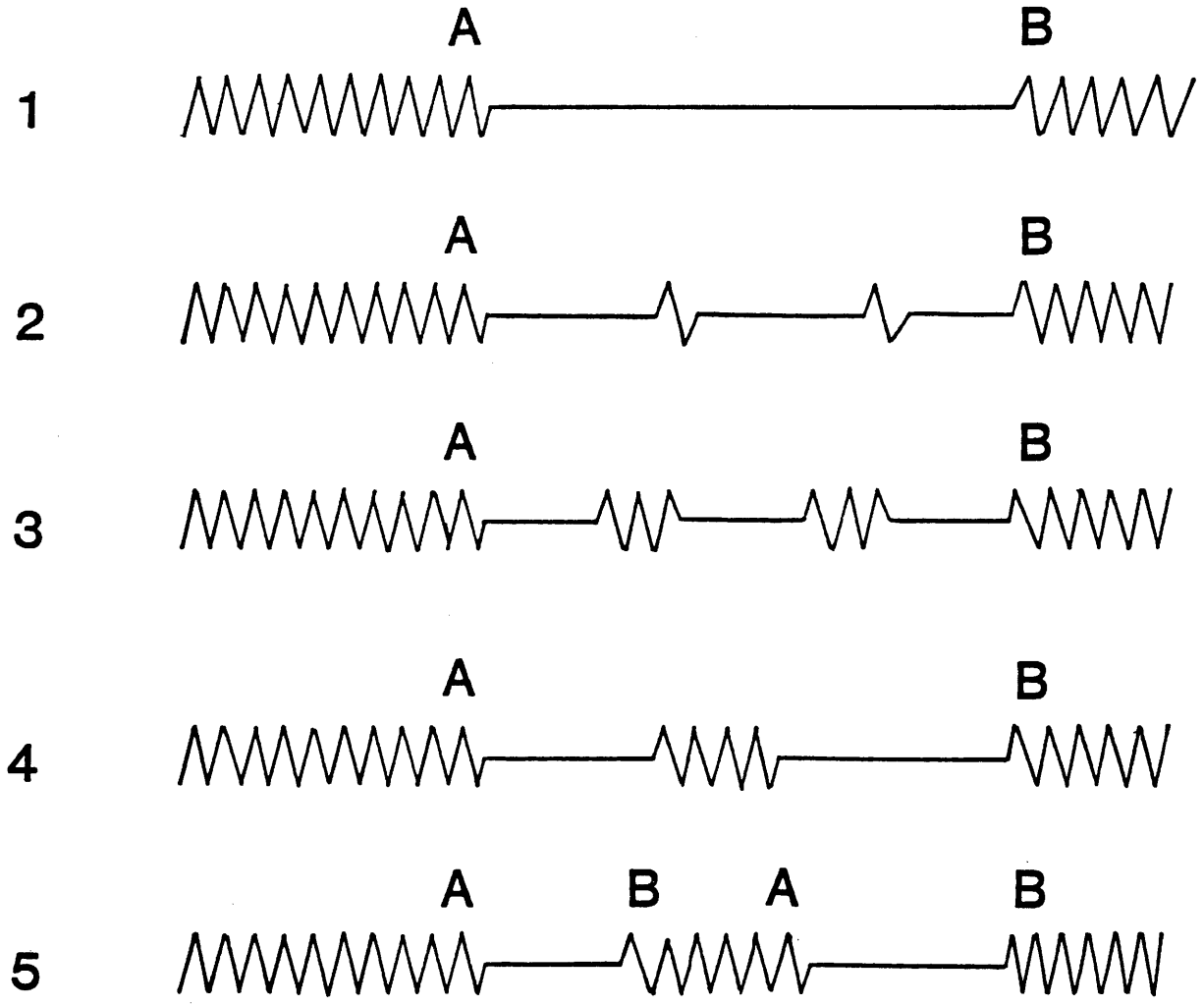

Length of pause (seconds)
170
150
130
110
90
70
50
30
10
0
120
240
360
480
600
720
840
960
1080
1200
1320
1440
Time of pause after implantation (mins)

FIGURE 3.4.

A diagramatic representation of a cardiac pause in L. depurator, with A - B being a cardiac pause period.


A
B
1
A
B
2
A
B
3
A
B
4
A
B
A
B
5

whereas examples 2-4 would be 'Compound' pauses with the period A-B being a 'Pause Episode' with example 5 illustrating two 'Simple' pauses. In the remainder of this thesis the term 'cardiac arrest' refers to both 'Simple' and 'Compound' pauses.

3.3.1.3. Normoxic Conditions

With closer examination of the heart trace between the pauses it can be seen that the heart rate gradually decreased from the point when one pause stopped to when the next commenced. This continual decline in rate explains the apparent variability of the heart beat when the trace is first examined (Fig. 3.5.A.). Figures 3.5.B. and 3.5.C. illustrate the decline in heart rate between pauses 20 hours and 96 hours respectively after electrode implantation. (Figure 3.5.A. is 10 hours after electrode implantation). The gradual reduction in the rate of the heart beat, increased with the length of time after electrode implantation and the length of time between the commencement of heart pauses became shorter. There appeared to be no difference between the rate of heart rate decline and time between pauses in

FIGURE 3.5.A.

Relationship of heart rate with time between cardiac pauses, 10 hours after electrode implantation.

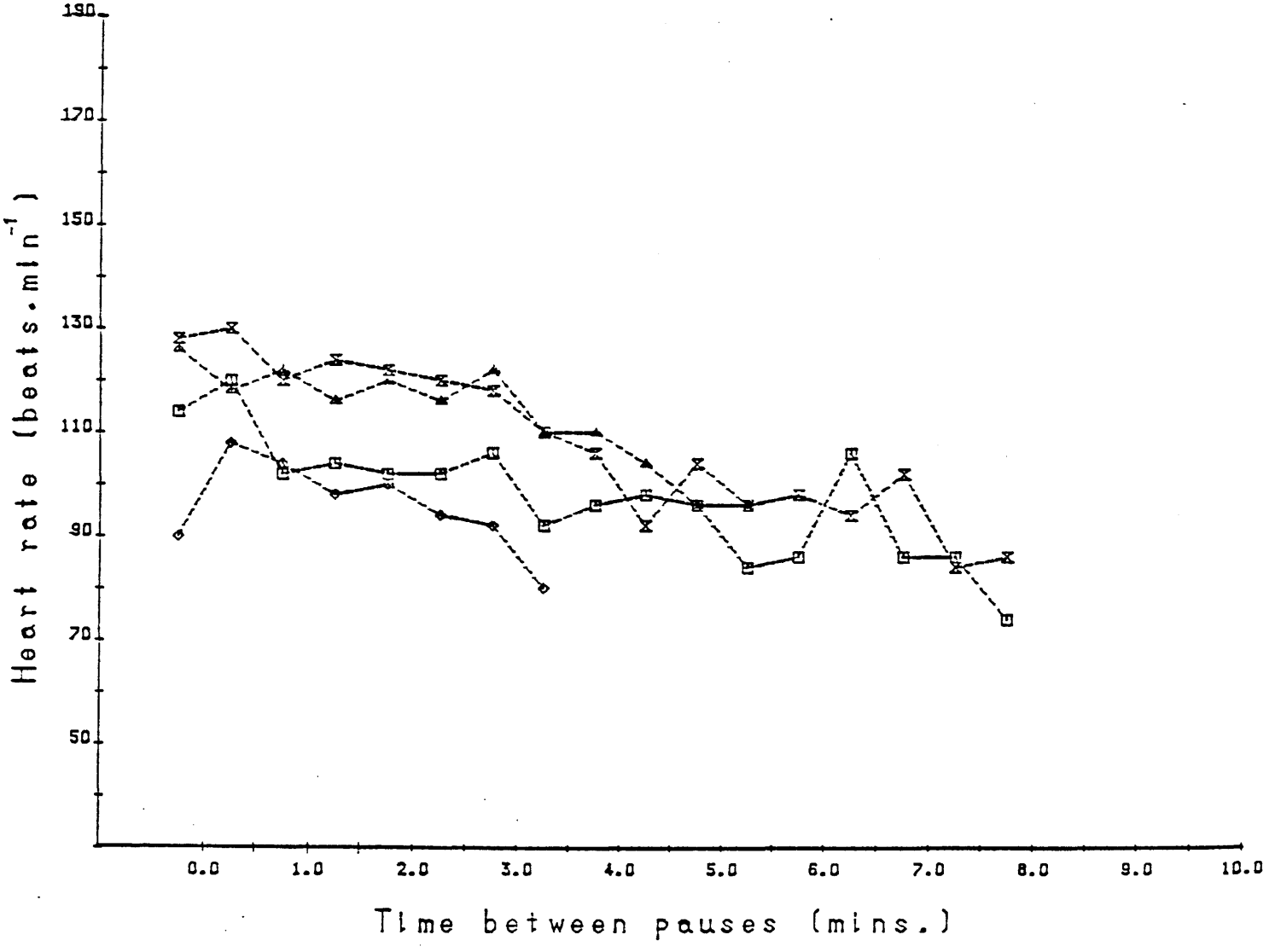

190
170
150
130
110
90
70
50
Heart rate (beats.min^{-1})
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
Time between pauses (mins.)

FIGURE 3.5.B.

Relationship of heart rate with time between cardiac pauses, 20 hours after electrode implantation.

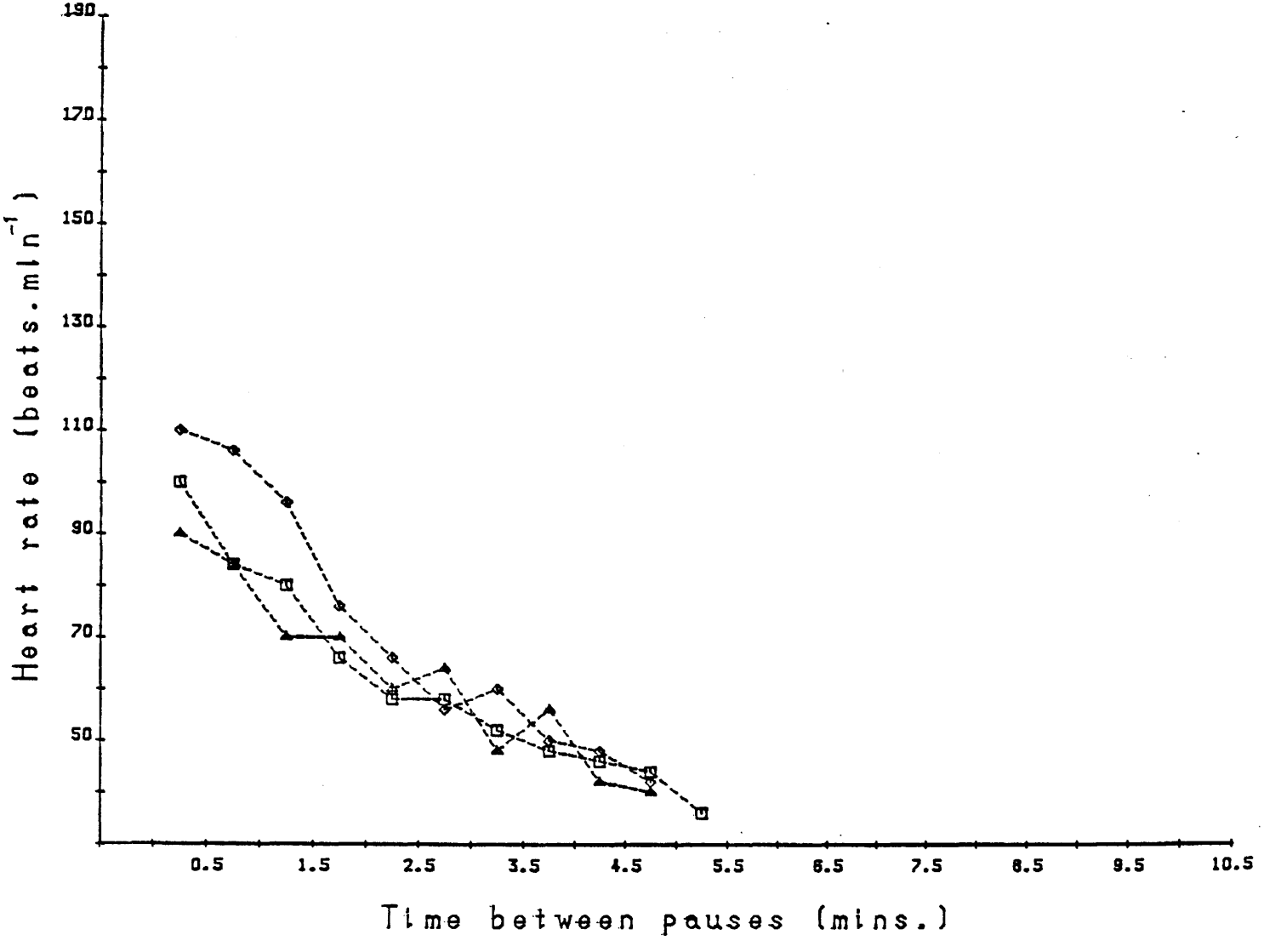
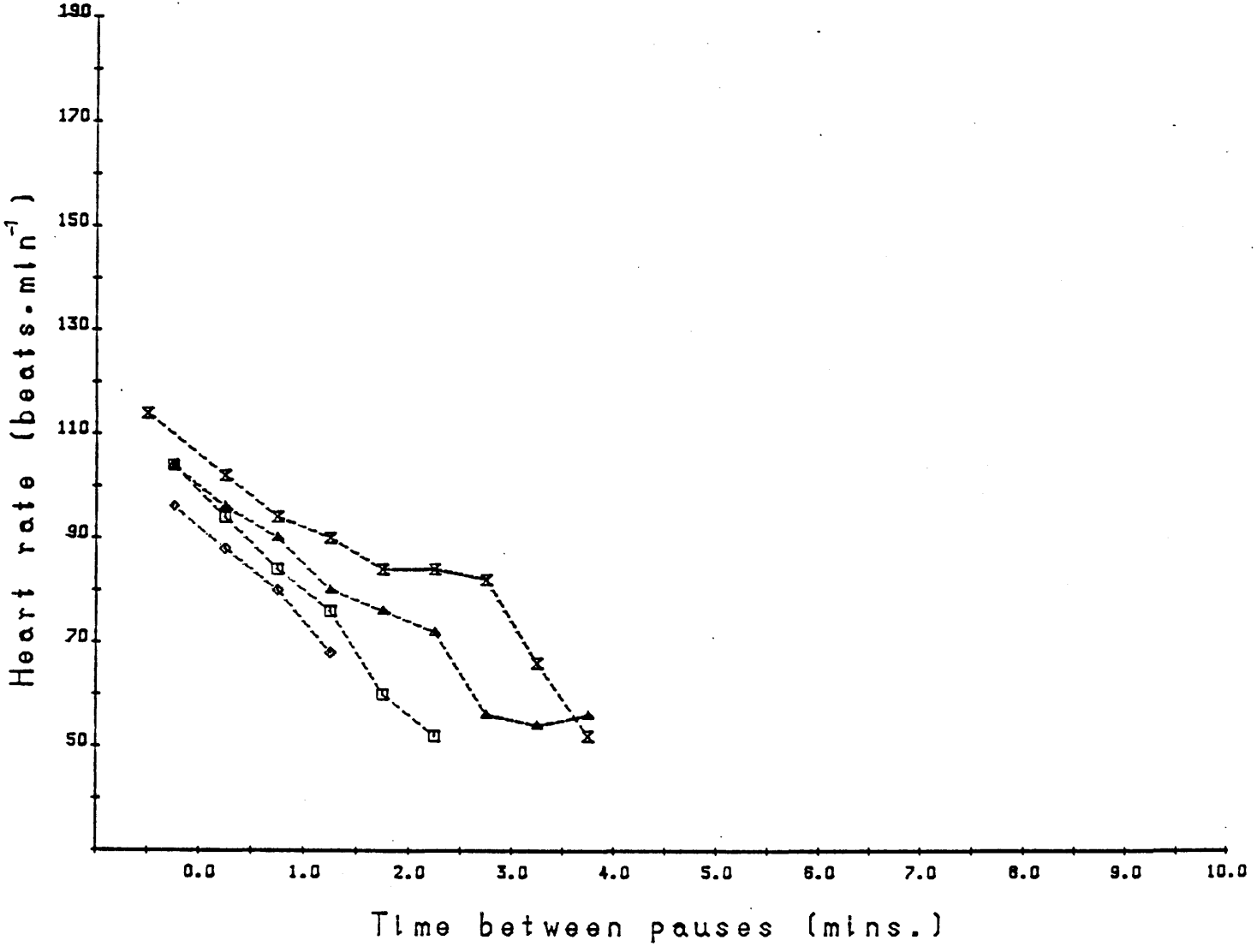

190
170
150
130
110
90
70
50
Heart rate (beats.min^{-1})
0.5
1.5
2.5
3.5
4.5
5.5
6.5
7.5
8.5
9.5
10.5
Time between pauses (mins.)

FIGURE 3.5.C.

Relationship of heart rate with time between cardiac pauses, 96 hours after electrode implantation.


190
170
150
130
110
90
70
50
Heart rate (beats.min^{-1})
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
Time between pauses (mins.)

heart recordings of 20 and 96 hours; (mean of 4-5 minutes). At 10 hours after electrode implantation the average time between commencement of pauses was 8-9 minutes.

The recording in Figure 3.6. illustrates the variation in heart rate immediately prior to and following a pause. The periods of cardiac arrest (pauses) are also illustrated. These periods of cardiac arrest are illustrated both vertically and horizontally, (the X-axis being the time after electrode implantation, 96-98 hours) It can be seen that the range of post-pause rates was from 80 - 100 beats. min^{-1}. and the pre-pause range was from 34 - 63 beats. min^{-1}. Therefore, there appears to be two distinct ranges of heart rate ie. 'pre-' and 'post-' pause. This, together with the decrease in heart rate between pauses makes it difficult to assign a standard heart rate for any individual under a particular circumstance.

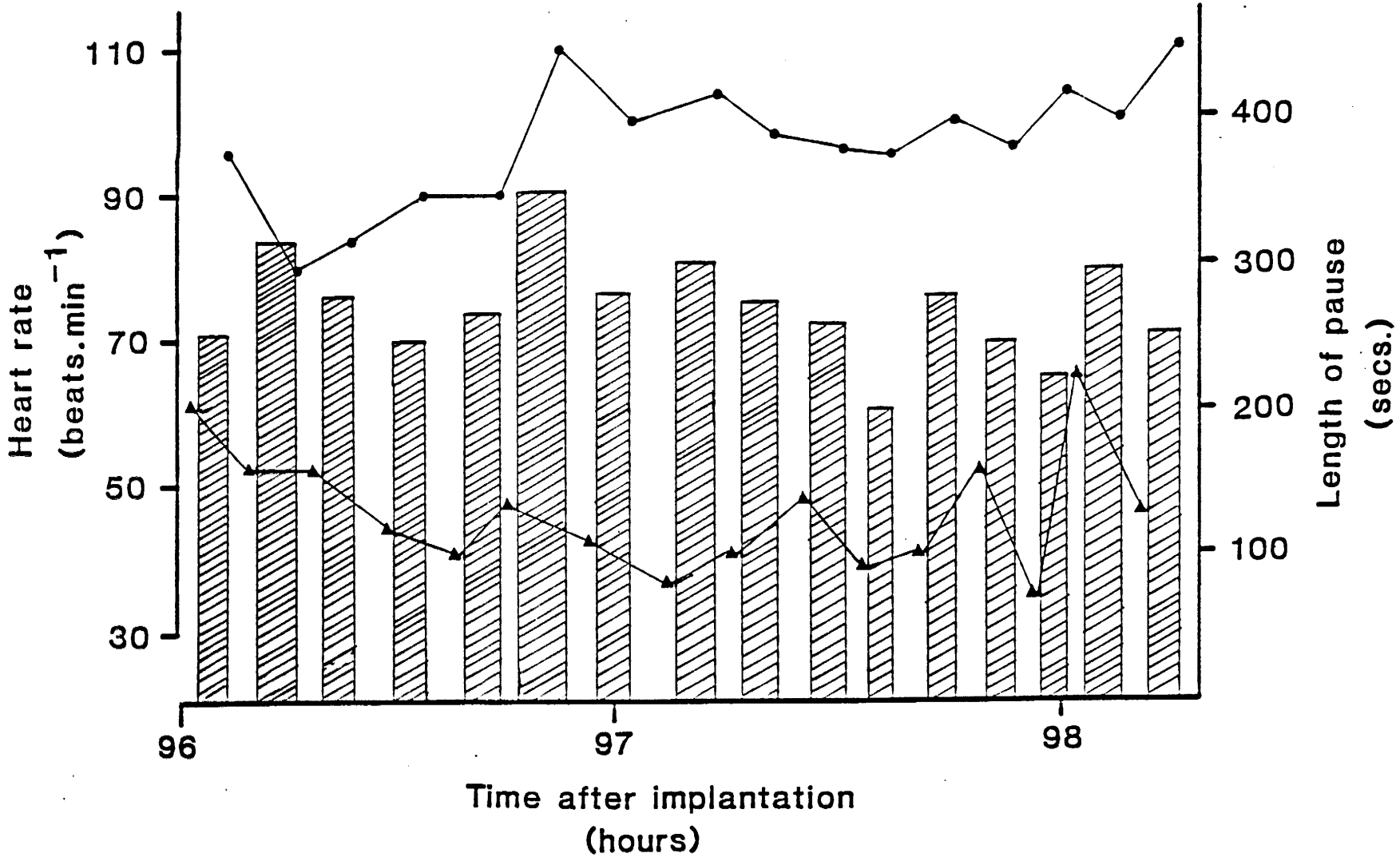
The cardiac arrests for this period had a mean duration of 4.5 $\pm$ 0.63 minutes and a frequency of one pausing episode per 8.75 minutes. The average time interval between the pauses was 3.9 $\pm$ 1.12 minutes. During the time of recording the heart activity, this animal was totally buried in the sand substratum. When the pausing frequency is

FIGURE 3.6.

Frequency of heart rate immediately before ▲——▲

and after ●——● a cardiac pause.

Cardiac pauses are illustrated by the hatched bars.


110
90
70
50
30
Heart rate
(beats.min^{-1})
400
300
200
100
Length of pause
(secs.)
96
97
98
Time after implantation
(hours)

compared to that in an animal which was continuously on top of the substratum (Fig. 3.3.) it can be seen that the pausing was more frequent and regular in the animal buried in the sand.

3.3.1.4 Effects of stress and activity under normoxic conditions

During concurrent recordings of the heart rate and video recordings of the locomotory activity, it could be seen that when the crab swam, walked or buried itself, the trace of the heart recording became indistinguishable due to electrical "noise" and analysis was impossible. This was probably due to interference to the electrode signal caused by the movement. Immediately after swimming etc. the heart rate was easily discernible and it could be seen that the rate was elevated compared with that recorded before activity. This elevation in the heart rate lasted for only one to two minutes before returning to the rate recorded immediately prior to moving. The time the animal spent in actual movement was usually very short and lasted for only a few seconds at a time.

Stress, in the form of electrode implantation, resulted in elevated heart rates and the lack of any periods of cardiac arrest. However, once the animal had overcome the stress induced by electrode implantation (minimum of 12 hours) and it was then disturbed by touching it with a probe, the heart would stop beating for a period of a few seconds and then exibit an elevated heart rate. This elevated heart rate would generally last for only a few minutes then return to the pre-disturbance rate.

3.3.1.5. Effects of Hydrostatic Pressure

In the initial experiments the pressure in the therapeutic recompression chamber was increased in stages to a pressure equivalent to a depth of 50m. (10 metres every 5 minutes). During this procedure the heart rate was monitored continuously. The heart rate pattern was similar to that recorded at normal atmospheric pressure for the entire range of depths. As the pressure in the chamber was increased there was a certain amount of audible noise (audible to the human ear) which evoked a response in the heart rate similar to that evoked by tactile stimulation. The same response was

evident when the chamber was brought back up to atmospheric pressure.

When the pressure was increased to a depth equivalent to 50m water in one continuous pressurization, the audible noise produced a response in the heart rate similar to the one when it was taken down in steps. When it reached a depth of 50m (after about 8 minutes) the heart rate took a few minutes to settle. After settling, the heart rate was similar to that recorded at atmospheric pressure. A similar result was noted when the pressure was brought back to atmospheric pressure in a continuous manner. It therefore appears that changes in hydrostatic pressure have no significant effect on the heart rate of L. depurator. The associated noise which elicits a response similar to tactile stimulation was a feature of experimental conditions and induced hydrostatically transmitted vibrations. It is thought that these vibrations induced the response in the heart rate similar to tactile stimuli.

3.3.1.6. Effect of Hypoxia

The effect of hypoxia on the heart rate is discussed fully in the following chapter.

3.3.2. Scaphognathite Rate

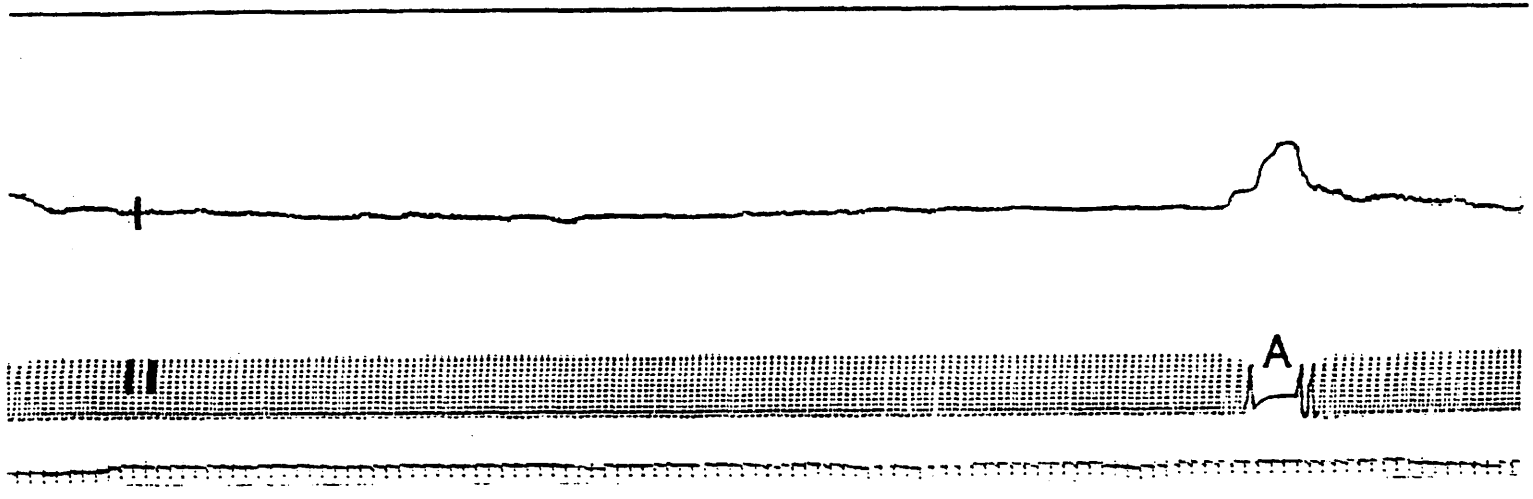
3.3.2.1. Patterns of Forward Pumping

Figure 3.7. illustrates a trace of scaphognathite forward pumping with a period of apnoea, together with the corresponding branchial pressure trace. When the scaphognathite is pumping in a forward direction a slight negative pressure is produced in the branchial chamber. The individual beats of the scaphognathite are also indicated by small pressure changes associated with each beat. As was observed for the heart rate, the scaphognathite rate is initially high immediately after electrode implantation into the prebranchial chamber. Similarly, there is a great deal of variability in the scaphognathite rate both within one individual, eg. 468-154 beats. min^{-1} and between individals (the highest value, 468 beats. min^{-1}, was recorded immediately after electrode implantation). Figures 3.8.A. and 3.8.B. illustrate the decline in both scaphognathite rates with time after electrode implantation.

FIGURE 3.7.

Recording trace of scaphognathite forward pumping (Trace I) with its associated branchial pressure trace (Trace II). A indicates a period of apnoea. These were recorded in normoxic conditions at ambient seawater temperature on a male crab of 18.5 g.

Time intervals (bottom trace) = 1 second.


A

As well as a variability in scaphognathite rate periods of apnoea were also recorded, where ventilatory activity ceased completely. These varied in duration from 5-520 seconds (a similar definition for a scaphognathite pause (apnoea) is used as for a period cardiac arrest). As can be seen in Figure 3.7., a period of apnoea results in a slight increase of pressure in the branchial chamber.

3.3.2.2. Unilateral and Bilateral Pumping

As illustrated in both Figures 3.8.A. and 3.8.B. both scaphognathites do not necessarily beat at the same rate (although in cases the rate was the same in both scaphognathites). In this particular example the difference in rates was between 8 - 86 beats. min^{-1}. This was particularly noticeable immediately after electrode implantation. Although the actual beat rate is not the same, the rate of decline in the rate of beating of both scaphognathites after electrode implantation was similar. However, although the beat rates of the two scaphognathites tended to differ, periods of apnoea generally occurred simultaneously and tended to have a similar duration (Figure 3.9.).

FIGURE 3.8.A.

Relationship of scaphognathite beat rate with time, 0 - 4 hours after electrode implantation.

LS..... Left Scaphognathite

RS..... Right Scaphognathite

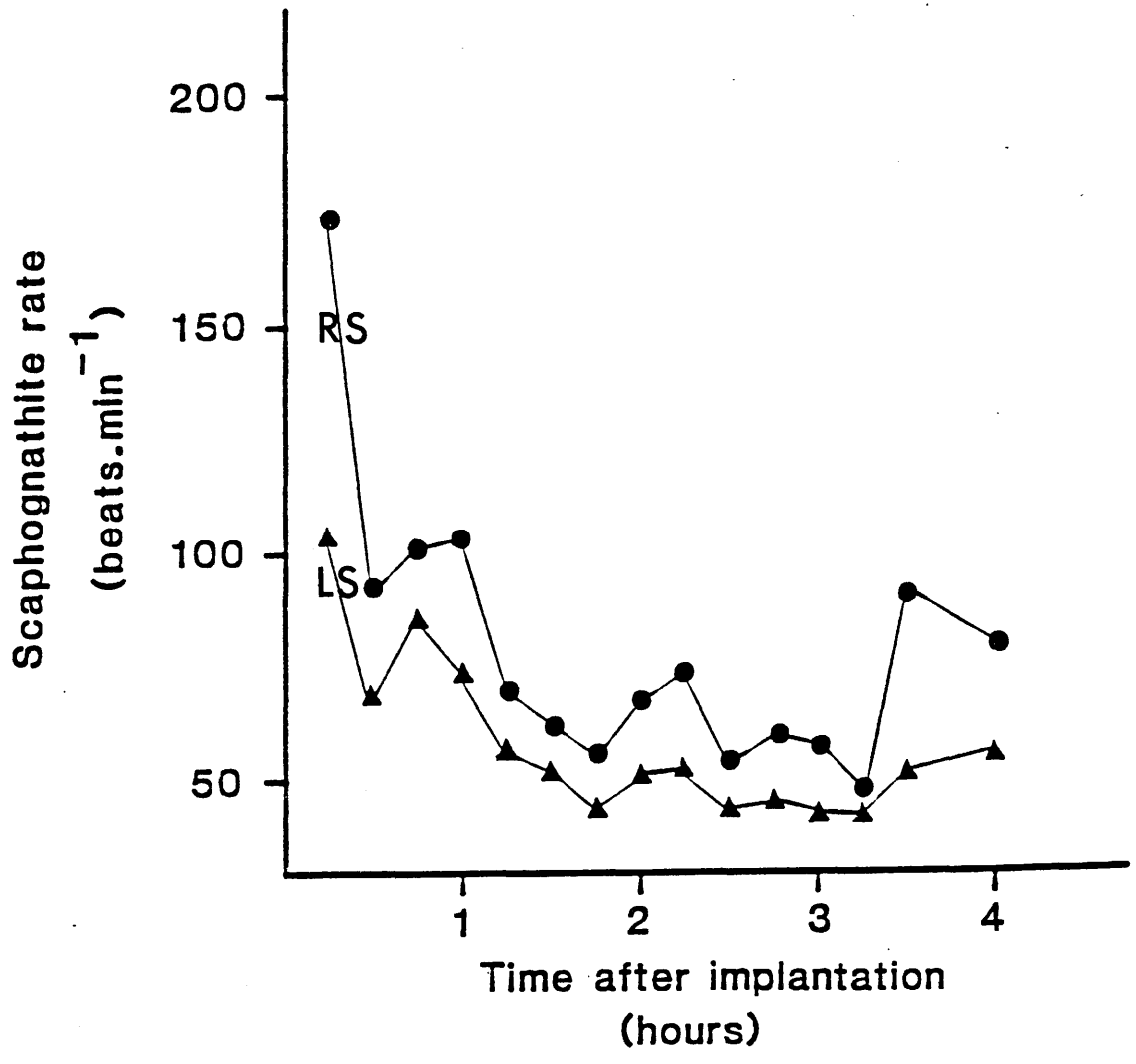

200
150
100
50
RS
LS
Scaphognathite rate
(beats.min^{-1})
1
2
3
4
Time after implantation
(hours)

FIGURE 3.8.B.

Relationship of scaphognathite beat rate with time, 17 - 23 hours after electrode implantation.

LS.... Left Scaphognathite

RS.... Right Scaphognathite

N/A = Not available

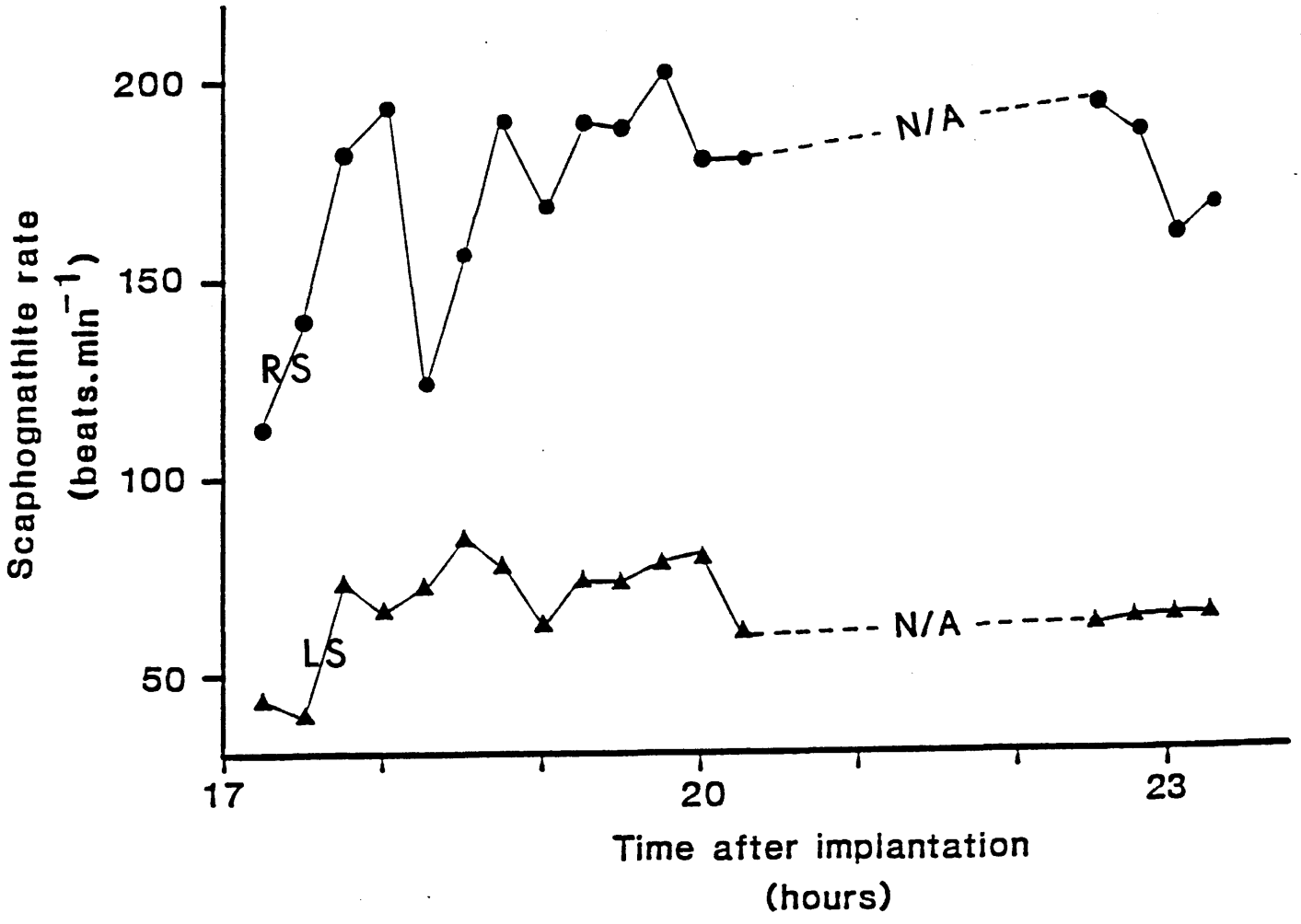

200
150
100
50
Scaphognathite rate
(beats.min^{-1})
R/S
L/S
N/A
N/A
17
20
23
Time after implantation
(hours)

FIGURE 3.9.

Recording of both scaphognathites and their branchial chamber pressure on a male crab of 22.5 g at ambient seawater temperature in normoxic conditions, showing simultaneous apnoea.

LS = Left scaphognathite

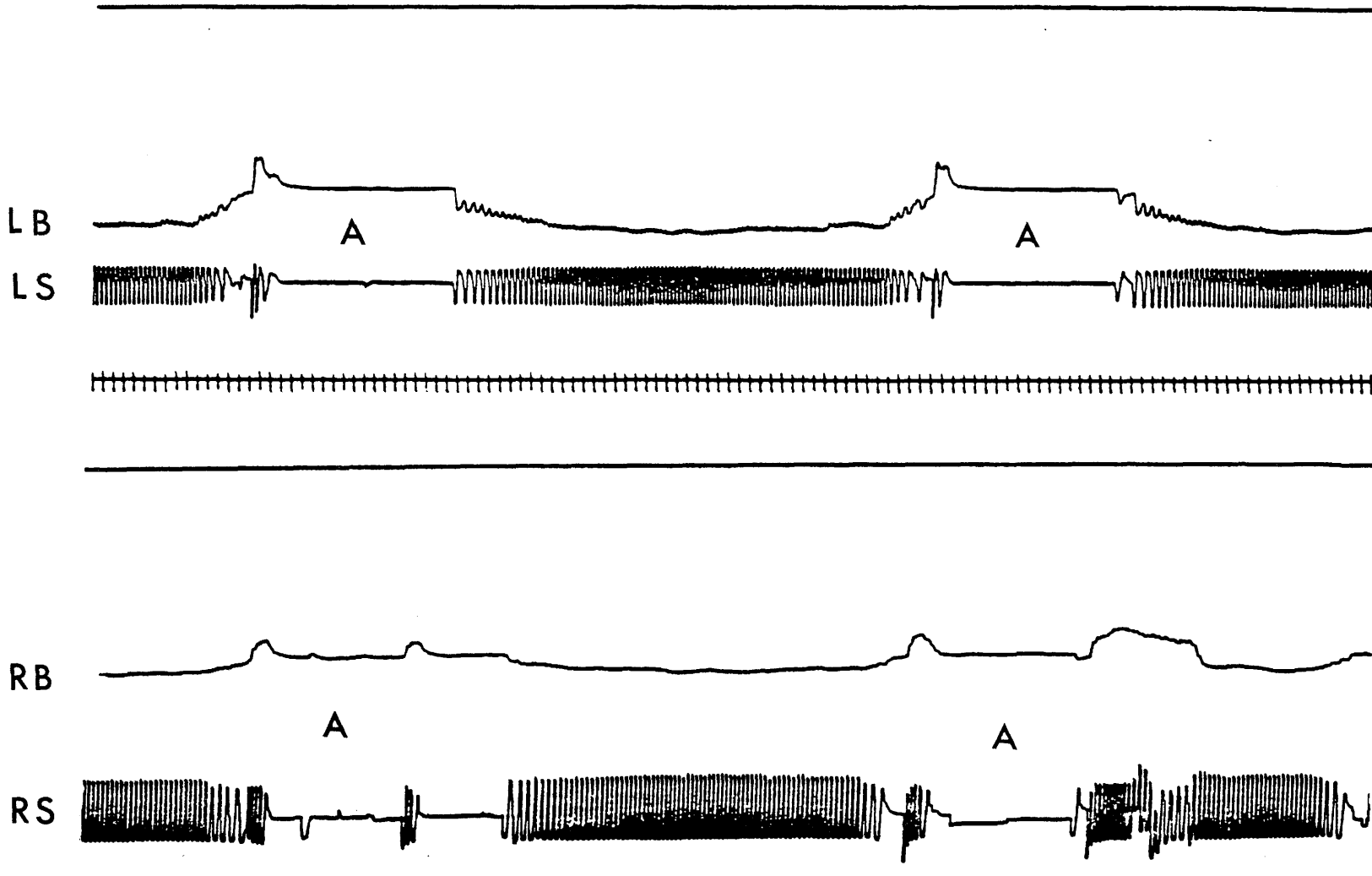
RS = Right scaphognathite

LB = Left branchial pressure

RB = Right branchial pressure

A = Apnoea

Time interval = 1 second.


LB
A
A
LS
RB
A
A
RS

3.3.2.3. Reverse Pumping

During reverse pumping, the scaphognathites change their pattern of beat and water enters the prebranchial chamber anteriorly. Reverse pumping is shown on the scaphognathite beat trace by a change in the pattern of the beats. Associated with reverse pumping is an increase in pressure within the branchial chamber which is indicated on the branchial pressure trace as a positive peak. Reversals occurred both when the crabs were on the surface of the sand and when partially or wholly buried in the sediment. When on top of the sediment the mean duration of reversals was 1-8 seconds and occurred with a frequency of 12-36 per hour (after a minimum of 12-14 hours after electrode and cannula implantation).

Reversals in both scaphognathites were found to be generally simultaneous (Figure 3.10.). Figure 3.10. shows an example of simultaneous reversals and it can be seen that the rate of scaphognathite beat increased during the reversal. In the left scaphognatyhite it was possible to calculate the beat rate during the reversal. The rate increased from 50 beats. min^{-1} immediately before the reversal to 120 beats. min^{-1} during the reversal, although the duration of this

reversal was only 5.0 seconds. In addition the increase in the branchial pressure can clearly be seen as indicated by the positive peak in the pressure trace. There was an approximate pressure increase of 65 mm of water during the period of the reversal.

When wholly buried, the scaphognathite would sometimes go into almost permanent reversal with only intermittent periods of forward pumping. This is similar to other burying crabs, eg. Atelecyclus rotundatus (Taylor, 1984).

FIGURE 3.10.

Recording of both scaphognathites and their branchial chamber pressure on a male crab of 23.8 g at ambient seawater temperatures under normoxic conditions, showing simultaneous reversals.

LS = Left scaphognathite

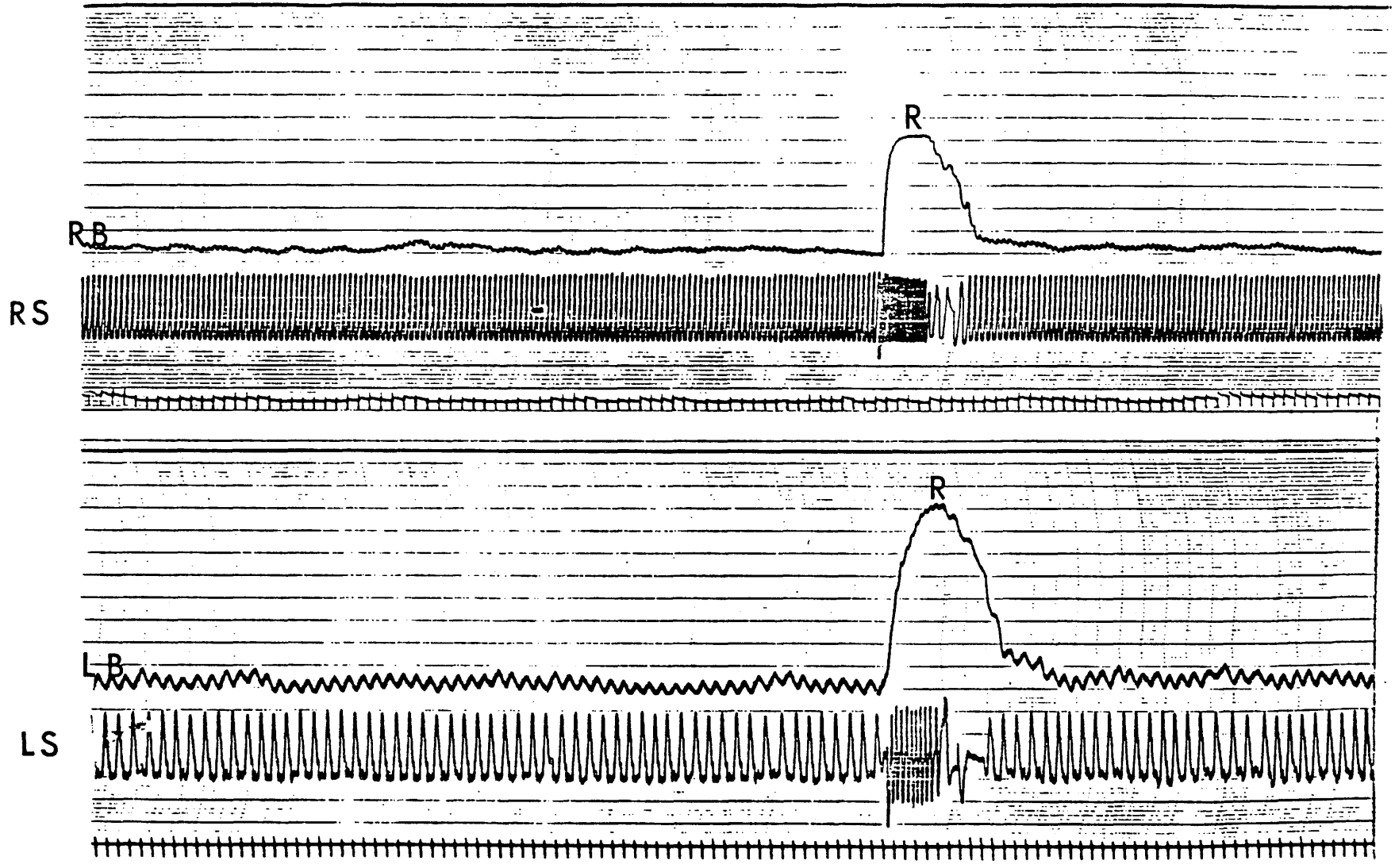
RS = Right scaphognathite

LB = Left branchial pressure

RB = Right branchial pressure

R = Reversal

Time interval = 1 second.


R
RB
RS
R
LB
LS

3.3.2.4. Effects of Stress and Activity

The stress of electrode and cannula implantation into the branchial chamber initially causes high scaphognathite rates (similar to the high initial heart rate caused by heart electrode implantation). Tactile stimulation also caused an increase in the rate. The response of the scaphognathites to tactile stimulation is much more dramatic and longer lasting than in the heart and results in an increased and erratic beat rate. When the crabs were active it was found that, as with the heart rate recordings, the trace became indistinguishable and comprehensive analysis was impossible. After movement had ceased the rate declined to the pre-stimulation rate after about five minutes.

3.3.2.5. Synchronous scaphognathite and cardiac activity

As the heart rate declined after electrode implantation so did the scaphognathite rate. In general, the heart and scaphognathite rates increased and decreased synchronously (during normoxic conditions), although the variation in scaphognathite rates was greater than the variation in heart rate. Scaphognathite pauses (apnoea) and cardiac arrests were also observed to occur synchronously. But as Figures 3.11.A. and 3.11.B. illustrate, scaphognathite pauses were more frequent than cardiac pauses (both 7-9 hours after electrode implantation, Fig. 3.11.A. and 21-23 hours after implantation Fig. 3.11.B.) Every heart pause was associated with a scaphognathite pause and in general the heart pause tends to commence either half way through the scaphognathite pause or towards the end of the scaphognathite pause.

FIGURE 3.11.A.

Relationship between periods of ventilatory apnoea and cardiac pauses, 7 - 9 hours after electrode implantation.

The lower graph (closed histograms) represent the periods of cardiac pauses and the upper graph (open histograms) represent the periods of apnoea.

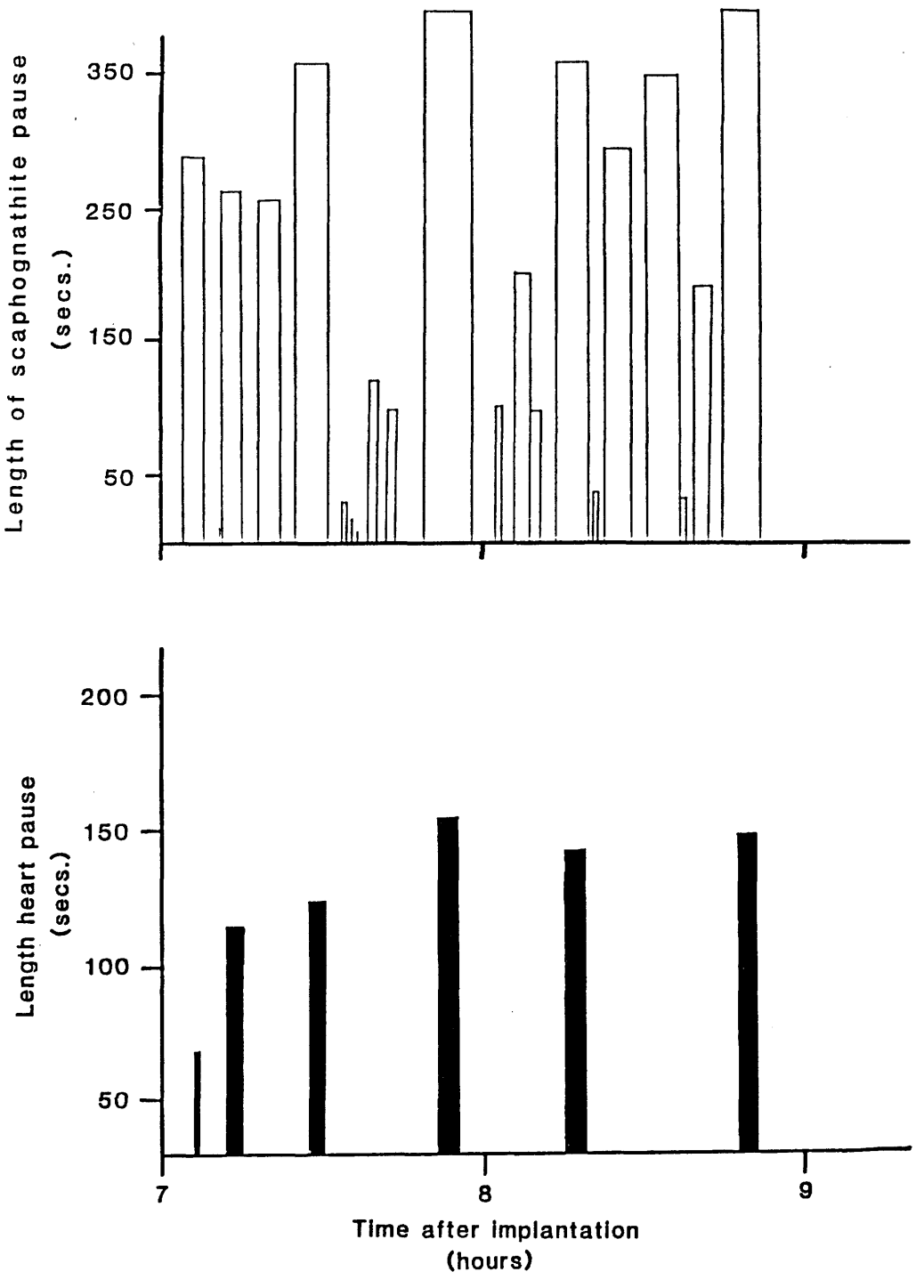
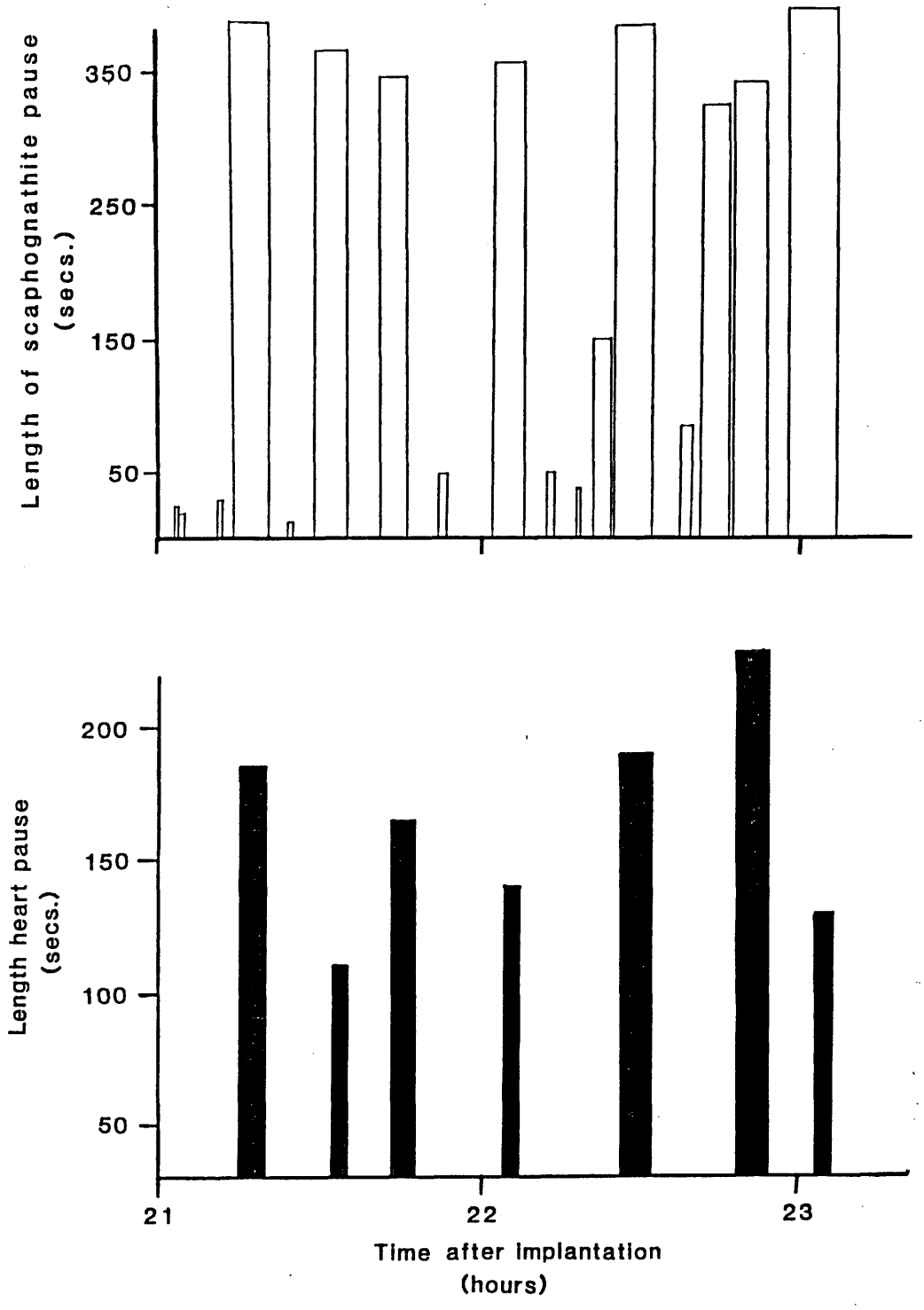

Length of scaphognathite pause (secs.)
350
250
150
50
Length heart pause (secs.)
200
150
100
50
7
8
9
Time after implantation (hours)

FIGURE 3.11.B.

Relationship between periods of ventilatory apnoea and cardiac pauses, 21 - 23 hours after electrode implantation.

The lower graph (closed histograms) represent the periods of cardiac pauses and the upper graph (open histograms) represent the periods of apnoea.


Length of scaphognathite pause (secs.)
350
250
150
50
Length heart pause (secs.)
200
150
100
50
21
22
23
Time after implantation (hours)

3.3.2.6. Effects During Hypoxia

The effect of hypoxia on the ventilatory activity will be discussed fully in the following chapter.

3.3.3. Behaviourial Observations

Whilst the crabs were being held in the experimental and in the holding tanks a number of behaviourial observations were made. It was noted that in the holding tanks the smaller crabs tended to bury themselves in the sand substratum whereas the larger ones would generally remain on the surface. Shadows or movements near the tank would elicit an antagonistic response from the crabs.

As confirmed by the video tapes, the crabs did very little actual swimming in the holding tanks and the main type of movement was walking over the surface of the sediment. When swimming did occur it was for very brief periods and over short distances. A number of different stimuli were observed to elicit the swimming response ie. ranging from close proximity to another crab,to external experimenter movement and vibration. It was observed, however, that the above stimuli did not cause swimming on every occasion and that spontaneous swimming occurred without any obvious stimulus.

3.4. Discussion

Variability in the heart rate of Crustacea has long been recognized (Larimer, 1964). Uglow (1973), comparing the heart rate of three species of portunid crabs, Carcinus maenas, Liocarcinus holsatus and L. depurator (Liocarcinus = Macropipus in Uglow 1973), found considerable variation within and between species. He divided the levels of heart rate into two distinct levels, Normal Resting Levels (NRL) and Elevated Levels (EL). He defined the EL as being the rate elicited by stimulation and the NRL as the rate resulting 30 minutes after handling once the animal had been in the experimental tank for "several hours". Uglow (1973) also recorded variability in the scaphognathite rate. Ansell (1973) working on Cancer pagurus, divided the heart rate into three levels; the Excited Rate which occurred immediately after gross handling and stress such as electrode implantation, the Active Level which occurred after the excited rate and was characterised by a more or less constant heart rate and the Resting Level which was characterised by a cyclical frequency of high and low rates.

Uglow (1973) described L. depurator as having an average heart rate of 140.2 $\pm$4.5 beats. min^{-1} and a

scaphognathite rate of 81.3 ±3.7 beats. min^{-1}. These results conflict with the rates obtained in this study in which the scaphognathite rate was extremely variable and was generally higher than the heart rate.

In a later study Butler, et al. (1978) reported that it took up to 48 hours for the heart rate of Homarus gammarus to settle from an elevated rate to a rate characteristic of quiescent animals. This agrees with the results achieved in this study since it was found that it took at least 12-24 hours for L. depurator to reach a quiescent heart rate. But as shown in the results, the quiescent rate is quite variable.

Cumberlidge and Uglow (1977) building on the work carried out by Ansell (1973) and by Uglow (1973) further defined the heart rate and scaphognathite rate in terms of different levels in Carcinus maenas. They defined three different levels, Elevated, Active and Resting. Each of these levels corresponded to a range of rates and length of time after electrode implantation. They also suggested that Resting heart rate levels are reached quicker when there is a sediment substratum present.

They go on to suggest that work designed to investigate the effects of environmental parameters on heart and scaphognathite rates should use only the changes in the frequency of the Active Levels as an indication of any physiological response in the crabs as reflected in heart or the scaphognathites beat rates.

In L. depurator, although there appeared to be a reduction in the range of heart rate (Fig. 3.2.), as an indication of quiescence, there was still variability in the heart rate. This variability was caused by the decrease in the heart rate prior to a period of cardiac arrest and the increase in beat immediately after the cardiac arrest (Figures 3.5.A. - 3.5.C.). It therefore appears that the most appropriate parameter which could indicate that a crab was unstressed, would be the duration and frequency of cardiac arrests since they appear to develop into a reasonably predictable pattern with time after electrode implantation. By using the periods of cardiac arrests to determine the stress level of a crab, the problem of the variability in heart rate is overcome. Because the scaphognathite rate is also extremely variable, it was thought to be unsuitable as a definitive indicator of the physiological status of a crab.

In L. depurator there is no period of cardiac arrest without an associated scaphognathite pause, however there are periods of scaphognathite pausing without a simultaneous heart pause. Periods of simultaneous bradycardia and apnoea have been observed in many other decapod crustaceans, eg. Homarus americanus (McMahon and Wilkens, 1972), Cancer pagurus (Ansell, 1973) and Cancer productus (McMahon and Wilkens, 1977). Recently Burnett and Bridges (1981) in studying the physiological properties of ventilatory pauses in Cancer pagurus, suggest that pausing behaviour optimizes the expenditure of energy during resting periods. They go on to suggest that oxygen stores which are built up during ventilation (both oxygen bound to the haemocyanin and dissolved in the haemolymph) are fully depleted during apnoea. As no specific study has yet been completed on the blood oxygen transport of L. depurator, it cannot positively be stated that the same is happening in L. depurator, but it is possible that during periods of bradycardia and apnoea, oxygen stores are fully utilized in L. depurator.

As the periods of cardiac arrest coincide with periods of scaphognathite apnoea there is a possibilty of a central controlling factor for the onset of pausing. As there

was no obvious external trigger causing the onset of the pausing behaviour (apart from when pausing in both the heart and scaphognathite were caused by experimental interference eg. prodding the crabs or by disturbance) it would appear that the pausing is induced by an internal mechanism. McMahon and Wilkins (1977) also observed simultaneous arrest in both the heart and the scaphognathites of Cancer productus. They suggested that there is a central neural control which coordinates the beating of both the heart and the scaphognathites. This suggestion has been confirmed by other workers, eg. Coyer, (1977); McDonald et al., (1977); Young, (1978) and Young and Coyer (1979). The work carried out by Burnett and Bridges (1981) (discussed above) suggests that it is the concentration of dissolved and bound oxygen in the blood which could trigger a central neural control. However, as previously stated, there are scaphognathite pauses without a simultaneous heart pause. This suggests that there could be another mechanism by which pausing behaviour in the scaphognathite is triggered, either independant of or in association with a central neural control.

Reversal of the ventilation current in decapod crustaceans is well documented and has been shown to occur in many species eg. Corystes cassivelaunus Garstang (1896);

Carcinus maenas Borradaile (1922), Arudpragasam and Naylor (1964b), Hughes et al (1969) and Taylor and Butler (1978) and Cancer magister McDonald et al (1977). In L. depurator reversals occur both when the crabs are on the surface of the sand and when buried. However, when they are buried in the substratum they appear on some occasions to maintain an almost continuous reversal of the ventilatory current. This behaviour has been observed in other burying crabs eg. Corystes cassivelaunus (Garstang 1896) and Atelecyclus rotundatus (Taylor, 1984). In A. rotundatus and C. cassivelaunus there are morphological adaptations which facilitate this, eg. an antennal tube is formed by two rows of setae on the second antennae and is connected posteriorly to a "chamber" (immediately in front of the mouth) which is formed dorsally by the rostrum and the anterior border of the carapace , laterally by the infra-orbital regions of the carapace and the floor of the "chamber" is formed by the anterior regions of the 3rd maxillipeds which are densely setose (to prevent entry of sediment into the chamber). In L. depurator there are no such obvious external adaptations nor are there any morphological adaptations in the branchial or pre-branchial chambers for burying. The morphological adaptations displayed by A. rotundatus suggest that being buried is a characteristic aspect of its life style whereas

in L. depurator being buried is helpful only in particular circumstances ie. when danger threatens it would prefer to swim away rather than bury itself. In general it is only the smaller (juvenile) crabs which tend to bury themselves in the substratum. The larger crabs generally tend to swim away from danger (Glass, 1985 and direct observation during this study). The increase in reversal rate and almost continuous reversal of the ventilatory flow when L. depurator is partially or wholly buried in the substratum is possibly due to an attempt to clear the inhalant openings and or to prevent sand from being drawn into the pre-branchial or branchial chambers.

As stated above in L. depurator, reversals also occur when the crab is sitting on the surface of the sediment. A number of reasons have been suggested why reversals occur in fully oxygenated water. One reason is the removal of particulate materials from the gills. However, in crabs it has been shown that both the epibranchial and hypobranchial surfaces are cleaned by the flabellae (Borradaile, 1922; Hughes et al., 1969), but particulate material from the interlamellar passages and the inhalant filter systems could be removed by the occurrence of reversals (McMahon and Wilkens, 1983).

The incidence of reversals has been shown to increase in L. depurator as it becomes apparently less stressed. This is similar to Cancer magister (McDonald et al., 1977). In addition, McDonald et al., 1977, has shown that oxygen uptake decreases during reversed current flow suggesting that reversals tend to be more frequent in situations of low oxygen demand. However Taylor, (1977) has shown that in Carcinus maenas, the incidence and frequency of reversals increase with changes in salinity. Assuming salinity changes induce stress on C. maenas, it would appear that low stress in C. maenas cannot be characterised by the reversal rate. At the present time there is still some debate on what the physiological reason for reversals is (see McMahon and Wilkens, 1983).

In conclusion it is suggested that a completely quiescent L. depurator exhibits an increase in periods of cardiac arrest and apnoea together with an increase in the frequency of ventilatory reversals.

Chapter 4 OXYGEN CONSUMPTION

4.1. Introduction

Oxygen consumption has been measured in Crustacea for a number of years. The review of oxygen uptake and consumption undertaken by Wolvekamp and Waterman (1960), shows that there are marked differences in oxygen consumption for the same species of animal in apparently the same conditions. The later review by Bliss (1983), confirms this assertion. Acclimitization to experimental conditions has also been shown to be important in affecting oxygen consumption rates.

In the following experiments an attempt was made to determine the basal rate of oxygen consumption of L. depurator and to investigate the effect of hypoxia on the rate of oxygen consumption and on the heart and scaphognathites.

4.2. Materials and methods

4.2.1. Collection and maintenance of animals

The experimental animals were caught and held in the laboratory holding tanks in the same manner as has been described for the animals used in the cardiac and ventilatory rate experiments.

4.2.2. Experimental apparatus

Only male crabs in the intermoult stage and in a fresh weight range of 12.0g. - 35.0g. were used for these experiments. Oxygen consumption values for L. depurator were determined by using a closed system, glass and perspex respirometer having a volume of 300ml. (Figure 4.1.). One of the major benefits of a closed system is that it minimises the disturbance on the experimental animal. Criticisms of a closed system are that there is a build up of CO_2 within the chamber. However, this can be overcome by flushing the chamber with water at the correct PO_2. An oxygen electrode (Radiometer E5046) was inserted into the lid of the

FIGURE 4.1.

A diagram of the respirometer apparatus.

AE.....Aerator

AL.....Air Line

SR.....Seawater Resevoir

P.....Pump

WI.....Water Inlet

WJ.....Water Jacket

WO.....Water Outlet

RC.....Respirometer Chamber

S.....Stirrer

OP.....Oxygen Probe

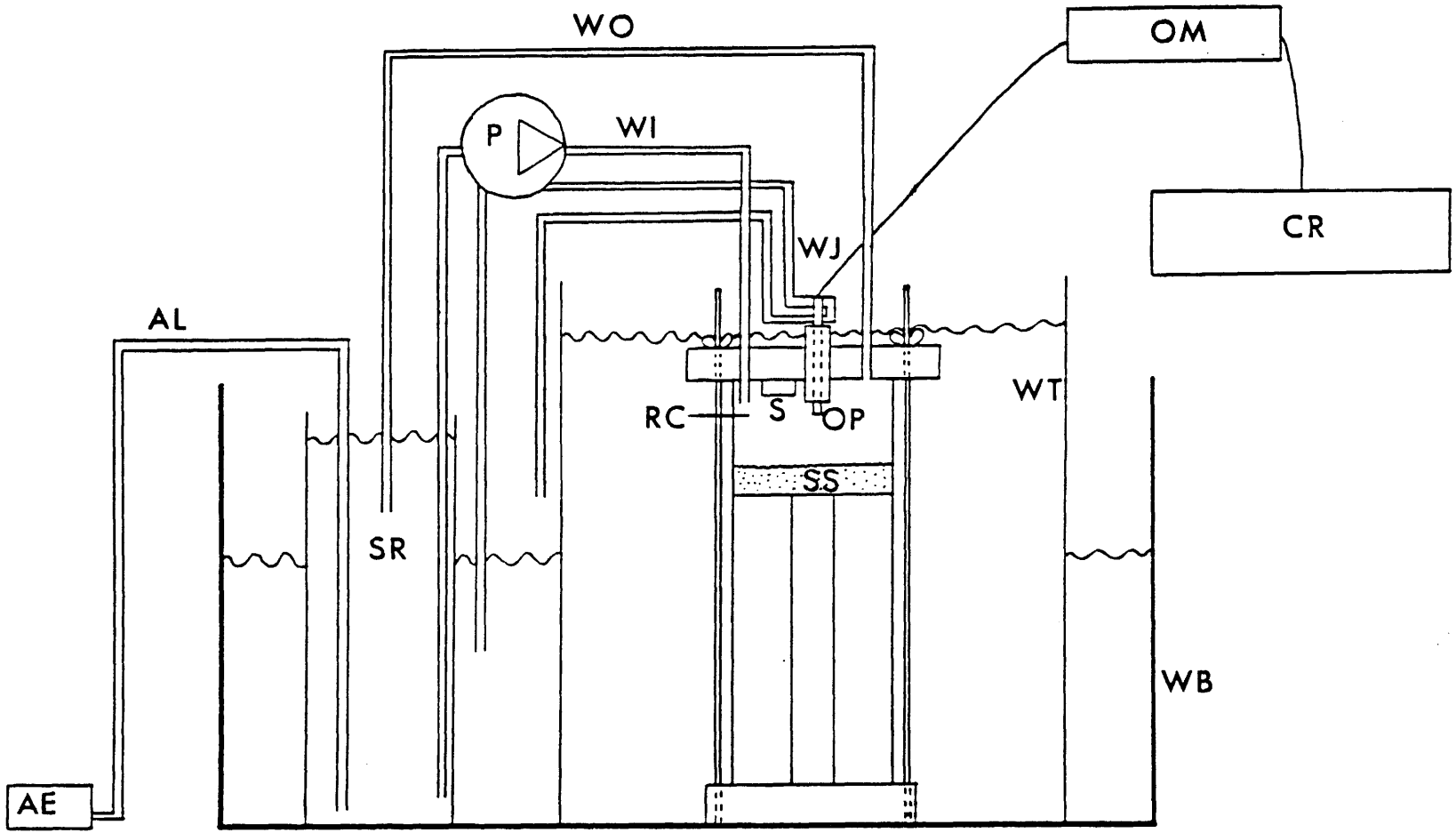
WT.....Water Tank

WB.....Water Bath

SS.....Sand Substrate

OM.....Oxygen Meter

CR.....Chart Recorder


WO
OM
P
WI
CR
WJ
AL
WT
RC
S
OP
SS
SR
WB
AE

respirometer chamber and held in place by means of a rubber bung. This ensured that the respirometer chamber was kept air and water tight. The electrode was connected to a digital oxygen meter (Strathkelvin Instruments) which was connected to a chart recorder (Tekman TE 200). This enabled a continuous trace of the rate of oxygen depletion inside the respirometer to be recorded. The respirometer was contained in a water bath and the temperature kept at a constant 10°C (except in those experiments in which the effect of temperature on oxygen consumption was investigated). The water in the respirometer chamber was constantly stirred, by a magnetic flea housed below the roof of the chamber, to prevent local depletion of oxygen and therefore maintain a uniform oxygen tension throughout the chamber.

A layer of autoclaved sand approximately 1cm deep was placed on the bottom of the respirometer chamber. This sand was provided in an attempt to reduce any stress which might have been caused by the lack of a natural substratum (von Oertzen, 1983). The water bath and the surface of the respirometer were curtained off using black-out curtains to reduce the effect of external disturbance which might cause stress to the crab and affect the rates oxygen consumption.

The Radiometer electrode was calibrated in a solution of 0.01M Borax and sodium sulphite which gave an oxygen saturation of 0% or a PO_2 value of 0 Torr and in air saturated sea water at 10°C to give an oxygen saturation of 100% or a PO_2 value of 150-160 Torr, depending on the barometric pressure of the day. The exact PO_2 was calculated using the following equations:

B.P. (mb.) = (B.P. mm.Hg. * 1.333224) - C.F.

PO_2 (Torr) = ((B.P. mb. / 1.333224) - W.V.) * 0.2093

B.P. mm.Hg. = Barometric Pressure of the day in mm.Hg.

C.F. = Correction Factor for height above sea level and temperature

B.P. mb. = Barometric Pressure of the day in millibars

W.V. = Water Vapour Factor (from tables)

A water jacket, at the same temperature as the water bath, was coiled around the top of the oxygen electrode in order to eliminate any effects due to temperature differences between the electrolyte in the probe (immersed in the respirometer chamber) and the electrolyte in the top of the electrode (which was exposed to the air). When the system was opened, air saturated sea water was pumped through the respirometer chamber by a peristaltic pump (Watson Marlow Ltd.) at a rate of 58ml per minute (initial experiments showed that this was the optimum flow rate to maintain 100% oxygen saturation when the system was open and a crab was in position). The sea water was filtered through a 0.3 micron in-line filter (Whatman) to remove any organic material which might affect the rate of oxygen depletion in the respirometer chamber and then held in a reservoir tank, which was constantly aerated to maintain saturation and was located in the water bath alongside the respirometer.

Before each recording, the wet weight of the crab was determined to enable the weight specific oxygen consumption to be calculated. In initial experiments which were performed in order to determine the length of time taken for the crabs to overcome the stress of handling, the animals were not allowed to deplete the oxygen in the respirometer

below approximately 110 Torr. This was to enable oxygen consumption under normoxic conditions to be calculated and to reduce any effect of hypoxia on $\dot{V}O_2$ (the weight specific rate of oxygen consumption).

In order to determine the responses of the cardiac and ventilatory activity to hypoxia, heart and scaphognathite electrodes were connected to crabs before placing them in the respirometer. The wires from the electrodes were led out through the roof of the respirometer along with cannula tubing implanted into the crab's branchial chamber (for determination of branchial chamber pressure and direction of pumping). (Methods of connecting the electrodes and cannula tubing to the crabs are described in the previous Chapter). A split rubber bung was inserted into the hole through which the wires were led and sealed with cyanoacrylate glue to ensure that the respirometer chamber remained air and water tight. The leads were connected to the recording equipment previously described and analysis of the recordings was performed in the same manner as described in Chapter 3.

4.3. Results

4.3.1. Acclimatization

Initial experiments showed that the quiescent rate of oxygen consumption was reached only after the animal had been in the respirometer for at least four to five hours and had been held in the laboratory holding tanks for at least two days prior to being placed in the respirometer.

In Figures 4.2.A. - 4.2.C. the rate of $\dot{V}O_2$ of an individual animal was recorded on three different occasions during a period of twenty four hours, at intervals of 5 mins, 3 hours and 24 hours respectively after being placed in the respirometer. When the three curves were compared, it could be seen that with time the crab's rate of oxygen consumption ($\dot{V}O_2$) decreased until a resting level was reached (Fig. 4.2.C.). Immediately after being placed in the respirometer, $\dot{V}O_2$ was at a maximum. In this example the maximum rate of oxygen consumption was 0.092 ml $O_2.g^{-1}.h^{-1}$. The maximum rate of oxygen consumption recorded during this study was 0.108ml $O_2.g^{-1}.h^{-1}$ which was recorded ten minutes after a freshly trawled crab had been placed in the respirometer chamber.

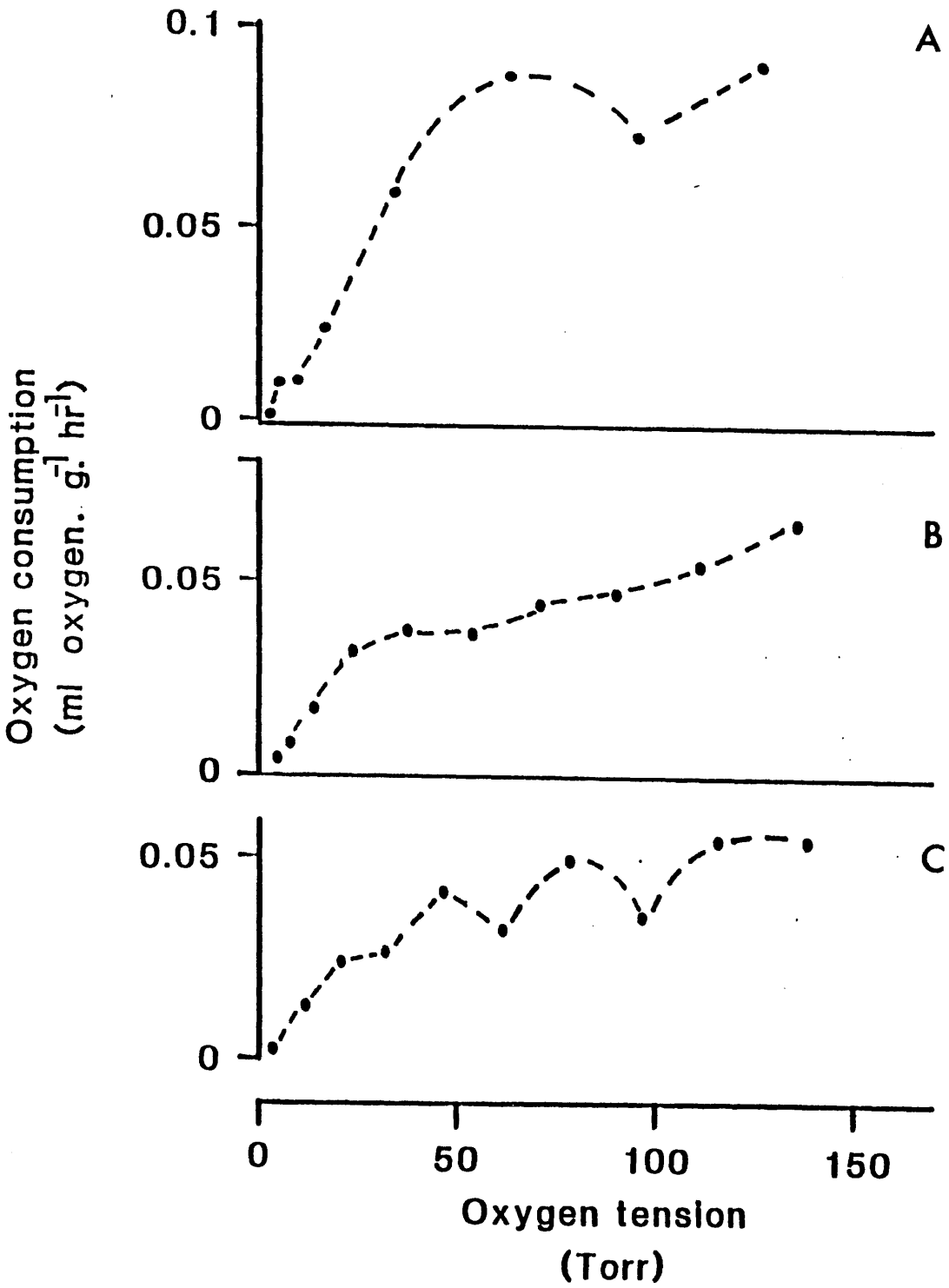
FIGURE 4.2.

Rate of oxygen consumption for an individual male crab of 24.6 g at a constant temperature of 10°C.

A... 5 mins in respirometer chamber

B... 3 hours in respirometer chamber

C... 24 hours in respirometer chamber


0.1
0.05
0
A
0.05
0
B
0.05
0
C
0
50
100
150
Oxygen tension
(Torr)
Oxygen consumption
(ml oxygen. g.-1 hr-1)

However, as previously stated in Chapter 3, it took from 12 - 24 hours for the heart rate to reach a relatively quiescent rate. It was therefore decided that the crab should be kept for a minimum of 12 hours in the respirometer under conditions of circulating aerated water before experimentation was commenced.

The term "quiescent" is somewhat misleading in the way that it suggests a steady rate. In this context it is taken to imply a lower, less variable rate compared with the extreme variability of oxygen consumption rate recorded when measured immediately after the animal was placed in the respirometer. This quiescent rate has been termed the "Basal Metabolic Rate", (B.M.R.), (Schmidt-Nielson, 1975). It was found that the crabs required a period of a least two days in a laboratory holding tank to allow recovery from the stress of trawling and capture, in addition to a twelve hour acclimatization period in the respirometer.

4.3.2. Weight Specific Oxygen Consumption

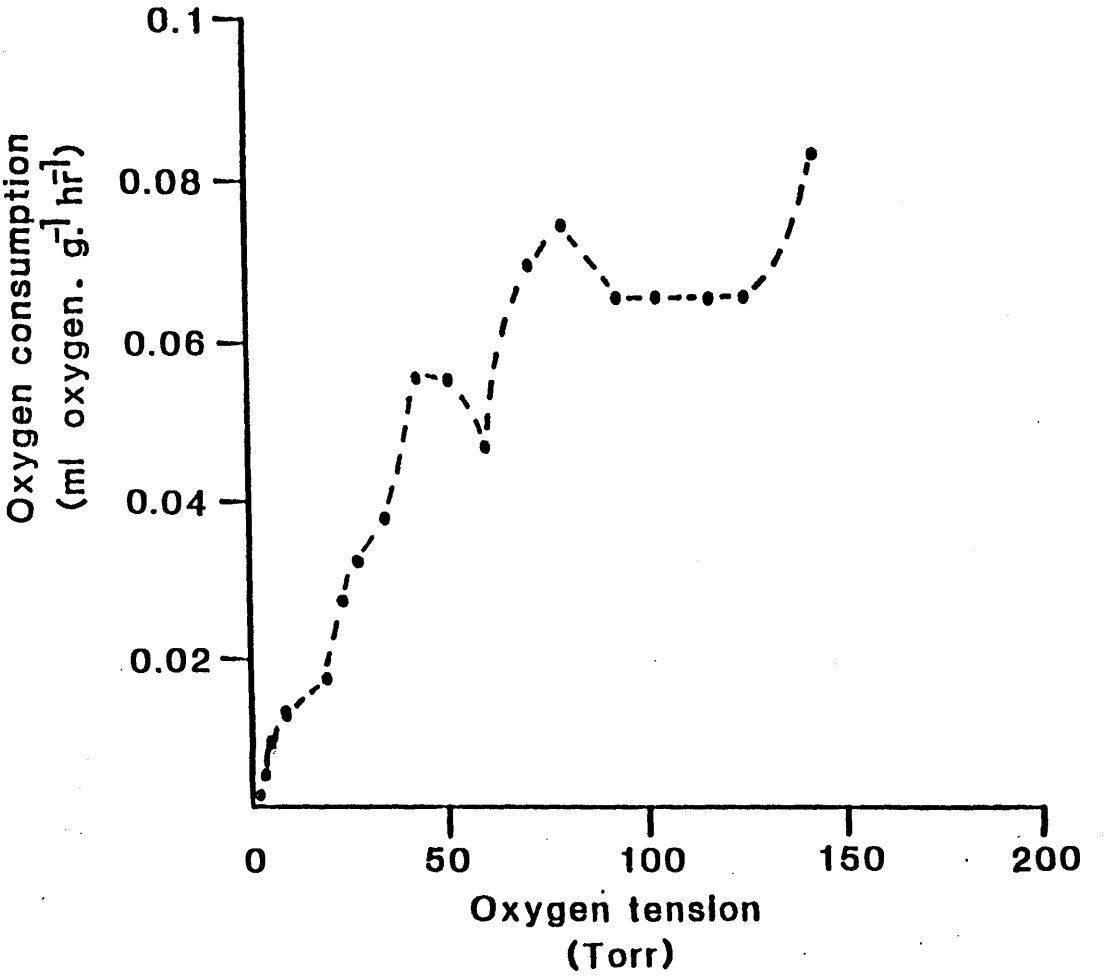
4.3.2.1. Quiescent Rates

After the initial acclimatization period, ie. minimum of 12 hours, the respirometer inlet valve was closed thus isolating the system from the circulating sea water. Following this isolation, the rate of oxygen consumption briefly increased and resulted in a rapid depletion of oxygen in the respirometer as demonstrated by a steep decline in the recorder trace. This steep decline continued until the oxygen tension in the respirometer reached a level of 120-130 Torr (equivalent to approximately 80%-90% oxygen saturation). The time scale of this depletion was variable , depending on the individual animal, and varied from one to two minutes to up to thirty minutes. From about 120 Torr to 50-60 Torr, the oxygen consumption rate was approximately constant. This lower, more constant rate is thought to represent the B.M.R. These factors are illustrated in Figure 4.3. which represents an oxygen consumption curves for an individual L. depurator.

An explanation of the initial rapid decline of the PO_2 in the respirometer after acclimatization, could be that stress is imposed on the animal when the inlet valve is

FIGURE 4.3.

Oxygen consumption curve for an individual male crab of 22.8 g at a constant temperature of 10°C after acclimatizing in the respirometer chamber for a minimum of 12 hours.


0.1
0.08
0.06
0.04
0.02
Oxygen consumption
(ml oxygen. g⁻¹ hr⁻¹)
0
50
100
150
200
Oxygen tension
(Torr)

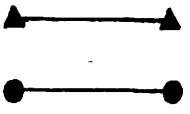
closed due to the attendant disturbance of the animal caused by experimenter movement and an abrupt cessation of the water current flow through the respirometer chamber even though the water in the chamber remains stirred.

4.3.2.2. Effect of Feeding

Experiments were carried out to compare the oxygen consumption curves for fed and unfed crabs. The crabs were allowed to acclimatize to the experimental conditions in the respirometer for 24 hours (prior to this they were kept in a laboratory holding tank for 3 days without food) and then the rates of oxygen consumption were recorded over a period of 5 hours from which the weight specific oxygen consumption rates were calculated for unfed crabs. The crabs were then fed bits of Queen Scallops (Chlamys operculatis) and left another 20 hours before further recordings were taken to calculate weight specific rates for fed crabs. Figure 4.4. illustrates an individual example and shows the oxygen consumption curve for an unfed crab and the curve after it has been fed. It can be seen that once the animal has been fed, the rate of oxygen consumption increased by a factor of approximately two. This is similar to the results obtained by

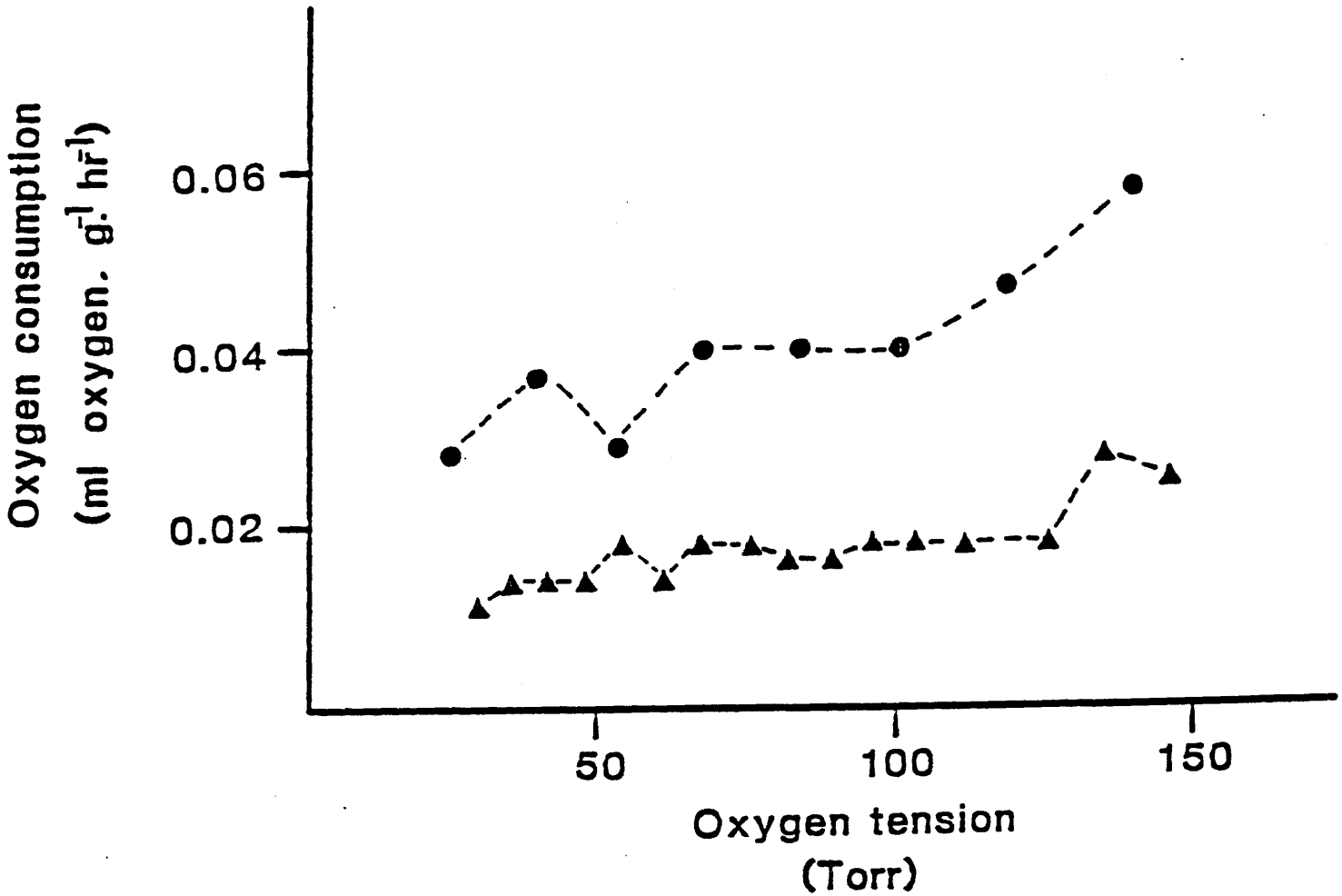
FIGURE 4.4.

Oxygen consumption curves of an individual male crab of 17.8 g at a constant temperature of 10°C.



Curve for unfed

Curve for fed


Oxygen consumption
(ml oxygen. g-1 hr-1)
0.06
0.04
0.02
50
100
150
Oxygen tension
(Torr)

McLeese and Watson (1968) who showed that feeding increased the routine oxygen consumption by a factor of 1.5 in the spider crab Chionoecetes opilio. A similar result was also obtained by Ansell (1973) working on Cancer pagurus.

4.3.2.3. Effect of Temperature

The weight specific oxygen consumption rates for different temperatures were calculated. Rates were recorded over a temperature range from 5°C to 20°C for similar sized crabs. It could be readily seen that the oxygen consumption increased as temperature increased. In order to quantify the increase in oxygen consumption with increased temperatures, the Q_{10} value for L. depurator was determined.

In order to determine Q_{10} values for Liocarcinus depurator, oxygen consumption values were calculated at temperatures of 5°C and 15°C for 10 crabs. Values were determined at 124 Torr (80% oxygen saturation) and at 140 Torr (90% oxygen saturation). Both levels of oxygen tension were used as there is no definitive level of oxygen tensions at which to calculate Q_{10} values been described in previous literature. The values recorded were 2.22 for 80% oxygen saturation and 2.38 for 90% oxygen saturation. When these values were averaged, a Q_{10} of 2.30 was determined. The Q_{10} value for L. depurator is given as 2.30.

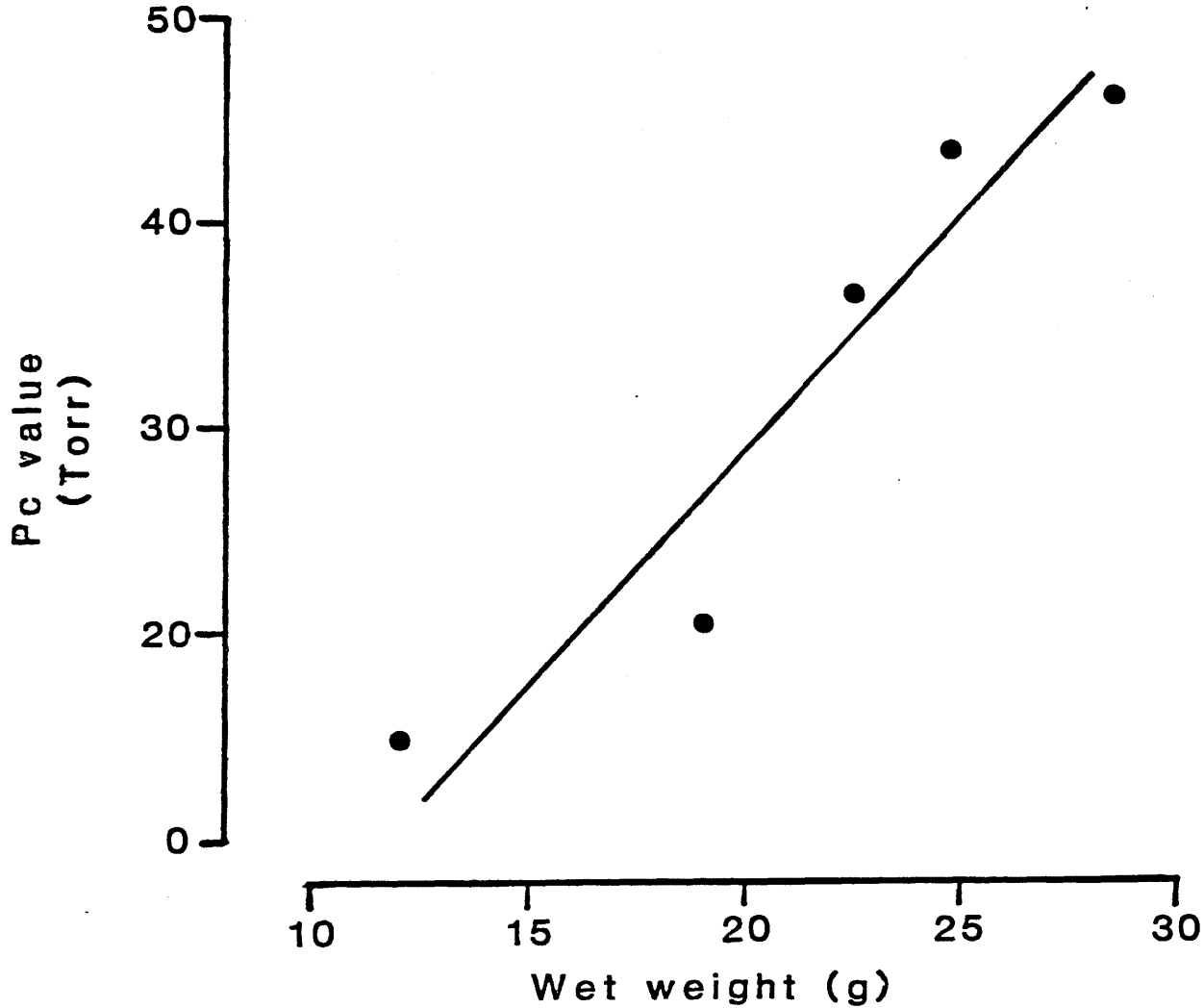
4.3.3. Oxygen Consumption During Hypoxia

Oxygen consumption remains relatively constant under hypoxic conditions when the PO_2 of the water is reduced from 150-160 Torr to approximately 30 Torr (apart from the initial high rate recorded at the start of the experiment). However, a feature noted in the majority of the oxygen consumption curves obtained during this study, is that at a PO_2 of between 15-45 Torr there is a noticeable but slight increase in the rate of oxygen consumption . The peak of this increase, immediately before the oxygen consumption starts to decline, has been interpreted as the "Critical PO_2" or Pc , and when plotted against the wet weight of the crab in grams, gives a positive straight line relationship (Figure 4.5.). A regression equation was fitted to the data using the method of least squares and the correlation coefficent calculated. The regression equation of the line fitted to the data was, Y =-13.6 + 2.15X (Y being Pc and X being weight) with r=0.954. The regression coefficent was found to be significant at the 5% level.

The Pc generally delimits the end of a period of constant $\dot{V}O_2$, and has been seen to be in a range of 15-45 Torr. At very low oxygen tensions ie. below the Pc value,

FIGURE 4.5.

The relationship of Wet Weight to Pc. The regression line fitted to these data is also shown.


50
40
30
20
0
Pc value
(Torr)
10
15
20
25
30
Wet weight (g)

the oxygen consumption rapidily decreased. This reduction in the rate of oxygen consumption, continued until the conditions were completely anoxic.

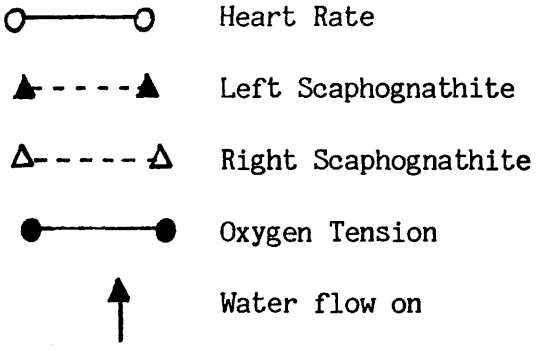
4.3.4. Cardiac and ventilatory responses to hypoxia

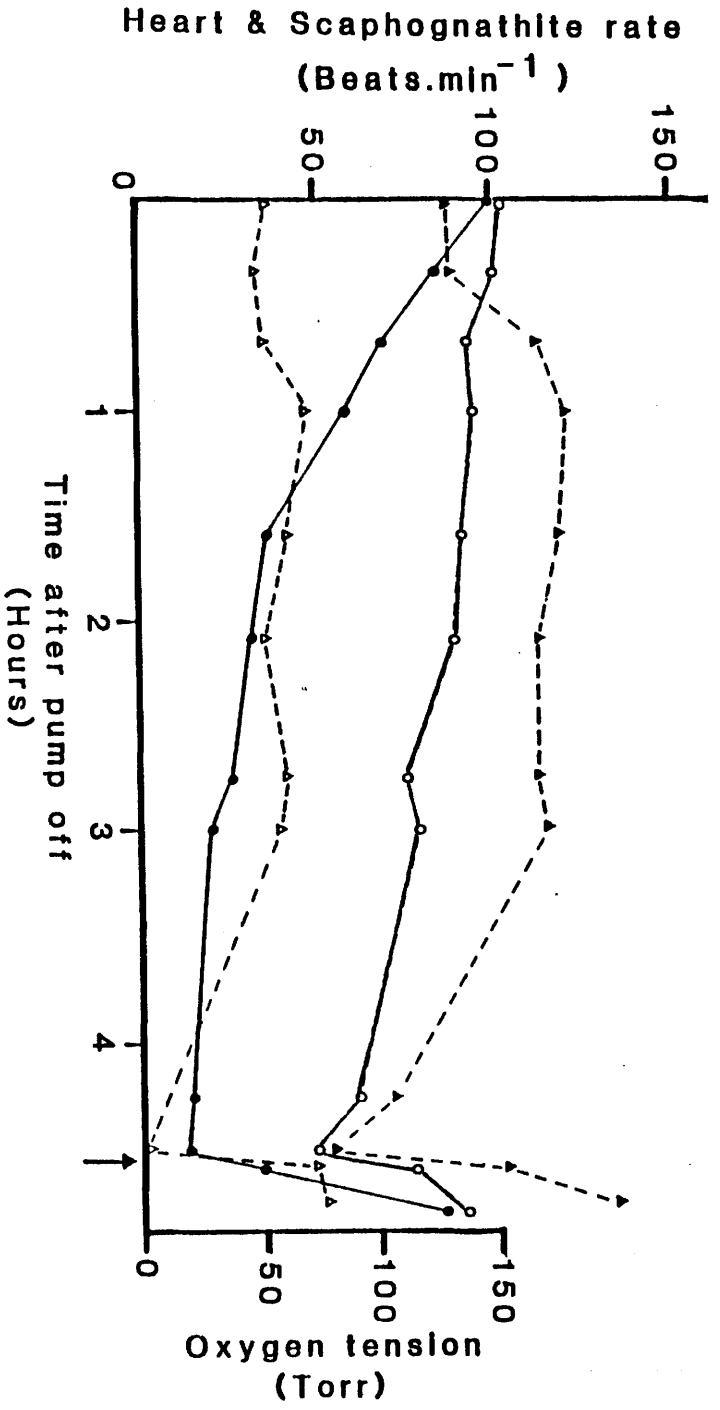
Heart, scaphognathite and respiratory current reversals under normoxic conditions have been described in the previous chapter.

Figure 4.6. illustrates the rates of beat of the heart and of both scaphognathites of one individual under conditions of declining oxygen tension. The heart rate generally followed the decline in oxygen tension ie. as the oxygen tension declined the heart rate declined and with an increase in oxygen tension there was a corresponding increase in the heart rate. With the scaphognathites the opposite was shown ie. with declining oxygen tension there was an increase in the scaphognathite rate until the PO_2 was reduced to a level close to the Pc. Immediately prior to the Pc the scaphognathite rate rapidly increased. As the PO_2 of the water was reduced to below the Pc, the scaphognathite rate rapidily decreased. It was observed that when the water was anoxic there was only the odd scaphognathite beat. When the respirometer inlet valve was opened and fully oxygenated sea water was pumped into the chamber, thus increasing the oxygen tension within the chamber, the scaphognathite rate rapidly increased, when normoxia was reached the scaphognathite rate

FIGURE 4.6.

The relationship of heart rate and scaphognathite rate with declining oxygen tension and time after water flow was stopped.



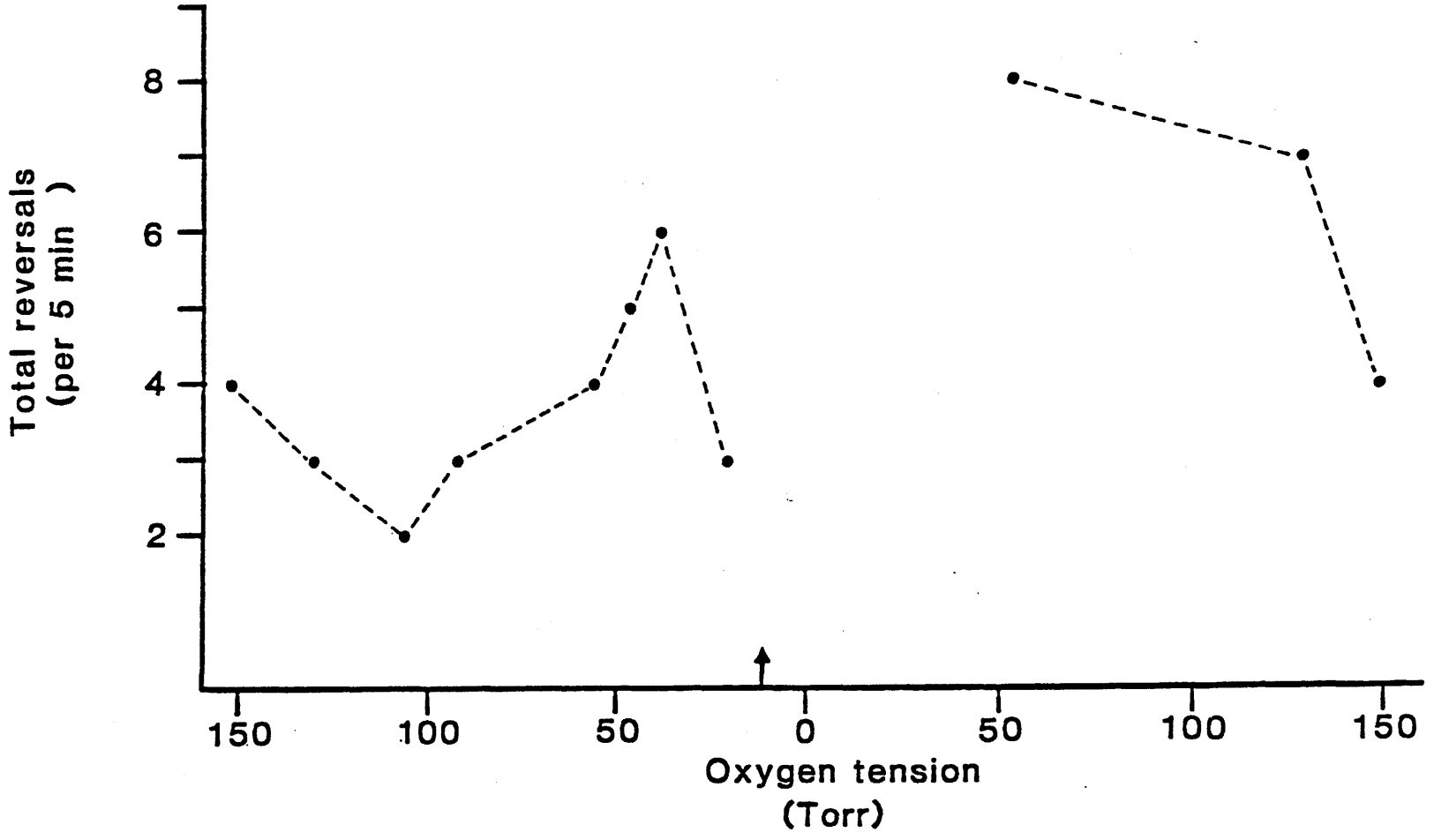

Heart & Scaphognathite rate
(Beats.min^{-1})
0
50
100
150
Time after pump off
(Hours)
1
2
3
4
0
50
100
150
Oxygen tension
(Torr)

had decreased to the level recorded under normoxic conditions.

The frequency of ventilatory reversals were also determined under declining oxygen tensions. Figure 4.7. illustrates the frequency of reversals per five minute intervals with declining and then with increasing oxygen tensions. The frequency of reversals of both scaphognathites were summed to give total reversal frequency. During declining oxygen tensions, the reversal rate dropped initially as the PO_2 was reduced from 156-100 Torr then increased dramatically as the PO_2 was reduced further. As anoxia was approached the reversal rate once again dropped. During the rapid increase in oxygen tension when the respirometer was opened, the reversal rate rapidily increased then decreased as normoxia was reached. Both the reversal rate and the scaphognathite beat rate declined to a minimum as anoxia was approached ie. below the Pc point.

FIGURE 4.7.

The relationship of total scaphognathite reversals per 5 min intervals with changing oxygen tension.


Total reversals
(per 5 min)
8
6
4
2
150
100
50
0
50
100
150
Oxygen tension
(Torr)

4.4. Discussion

Measurement of oxygen consumption has been used as an indication of aerobic metabolic rate in decapod crustaceans for a number of years and is the most common parameter measured. The extreme variability in the observed oxygen consumption rates is a major difficulty in the comparison of oxygen consumption rates between animals of the same species and even within individual animals recorded at different times under apparently the same environmental conditions. Bennet (1978) has defined a range of oxygen consumption values for any particular species as the "aerobic metabolic scope". This ranges from the maximum value of oxygen consumption at the maximum sustained activity level to the minimum possible value, this range is known as the "scope for activity".

Some variability is inherent but some will be due to the differing experimental regimes and techniques. As far as practicable, it would be of benefit to have an experimental regime resembling, as close as possible, the animals natural environment. Butler *et al.* (1978) approached this with lobsters (experimental regime described in

described in Chapter 1). The time taken for animals to acclimatize to the experimental conditions has also been shown to be important in the variability of oxygen consumption rates. Many workers have reported this in different species (McMahon et al., 1974; McMahon and Wilkens, 1975, 1977; Butler et al., 1978; Taylor and Butler, 1978). In L. depurator a minimum period of at least four to five hours in the respirometer is needed for the crabs' rates of oxygen consumption to reach relatively quiescent levels. It was interesting to note, however, that the minimum heart and scaphognathite rates were recorded only after the animals had been left undisturbed for 12 - 24 hours.

The variability in the oxygen consumption rate might be partially explained by the variabilities in the both the heart and scaphognathite rates. Burnett and Bridges (1981), have shown that during periods of apnoea in Cancer pagurus there is a decline in aerobic metabolism probably due in part to the decrease in energy expended due to the cessation of the scaphognathites and the decline in heart rate. After periods of apnoea, during which oxygen stores in the blood are fully utilized, there is an increase in scaphognathite beat rate and an increase in the rate of oxygen uptake compared to immediately prior to the

ventilatory pause. This increase in oxygen uptake is required to repay the oxygen debt incurred during the apnoea and to compensate for the increase in the ventilatory rate.

The nutritional state of decapod Crustacea has been shown to influence the level of aerobic metabolism. In general it has been shown that in starved animals there is a depression of their oxygen consumption rates (Ansell, 1973; Marsden et al., 1973; Wallace, 1973 and Aldrich, 1975a,b). This depression has also been confirmed in L. depurator in agreement with Ansell (1973). The nutritional state of the animal has also been shown to affect the haemocyanin concentration in the blood of Carcinus maenas (Uglow, 1969). Depledge (1985) has shown that in well fed Carcinus maenas the heart beat was regular and uniform and in starved crabs the heart beat was irregular with periods of cardiac arrest. Although it is not specifically stated, it is assumed, from their data that these observations were made on crabs that had been starved for 28 days.

Many investigations into the changes in oxygen consumption in relation to declining environmental oxygen tension (hypoxia) have been carried out in a number of decapod crustaceans, some of which are, Uca pugnax, U.

pugilator and Sesarma cinereum, (Teal and Carey, 1967); Carcinus maenas, (Taylor, 1976); Cancer pagurus, (Bradford and Taylor, 1981) and Clibanarius vittatus, (Wernick and Penteado, 1983). L. depurator appears to have generally the same response to declining oxygen tension as other brachyuran species. During moderate hypoxia (150 - 30 Torr), L. depurator compensates for the reduction in oxygen tension by increasing ventilation volume by increasing the scaphognathite rate. This response has been shown in other crabs, eg. Callinectes sapidus (Batterton and Cameron, 1978) and Libinia emarginata (Burnett, 1979). As hypoxia progresses, oxygen dissolved in the haemolymph is utilized and haemocyanin bound oxygen is begins to be depleted (refer to Chapter 1). With further hypoxia, the declining oxygen tension is such that the increased scaphognathite rate cannot supply a sufficent volume of water with the lowered oxygen tension, sufficient to meet the oxygen requirements of the crab. Concurrent with this, there is a gradual decrease in aerobic respiration and an increase in anaerobic respiration with its associated build up of L-lactate, which is a end product of anaerobic respiration. L-lactate has been shown to increase the haemocyanin oxygen affinity (see Chapter 1) which facilitates the haemocyanin oxygen transport and oxygen uptake at the gills. This occurs until the oxygen stored in

the haemocyanin is totally depleted and appears to correspond with the "Critical Point" Pc (Taylor and Butler, 1973; Taylor, 1976).

The Pc in L. depurator, occurs in a range of 15 - 40 Torr (dependent on the size of the crab). Below the Pc the remaining oxygen in the surrounding water is rapidly depleted and anaerobic respiration becomes the predominant metabolic pathway resulting in increasing amounts of L-lactate. It has been observed that crabs can fully recover after being exposed to environmental oxygen tensions of zero for up to 2 hours and presumably anaerobic respiration continues during this period. It should be noted, however, that this was not observed in all individual crabs exposed to anoxia. In some cases crabs did not recover even after being exposed to a few minutes of anoxia. It is also possible that some individuals could survive even longer periods of anoxia (greater than 2 hours) but this was not attempted.

When Pc was plotted against the wet weight of the crab (Fig. 4.5.) there was a positive straight line relationship. This is unusual since previous work (Dejours, 1981 citing Job, 1955) has given a negative relationship. Portner et al. (1985) have shown that in the sipunculid

Sipunculus nudus that the Pc shifted to a higher partial pressure with increasing animal size. The reason they give for this is the increased diffusion distance in the coelomic fluid. This explanation appears to be reasonable for Sipunculus but does not seem relevant to crabs. At the moment there does not seem to be any obvious explanation for this Pc to weight relationship in L. depurator and although only five different weights were plotted, they did span the entire weight range of the Clyde population of L. depurator.

CHAPTER 5

General Conclusions

Originally L. depurator was thought to be an active crab, but as it has been shown by both this work and by Glass (1985), L. depurator is not particularly active unless provoked. However the physiological parameters; heart rate, scaphognathite rate and oxygen consumption rates have been shown to be extremely variable both between individuals and within a single individual.

For L. depurator an indication of unstressed animal appears to be a heart rate with regular periods of cardiac arrest. Towards the end of this work a small study was conducted on Liocarcinus puber the Velvet or Large Swimming crab. The results obtained for the heart rate were similar to L. depurator in that it was extremely variable and that an unstressed crab was characterised by the appearance of periods of cardiac arrest. However in L. puber the time taken after electrode implantation to reach this stage was approximately 20 - 24 hours. Experiments on oxygen

consumption suggest that there is two Pc points. However as these experiments were only performed on a few individuals it is difficult to draw any significant conclusions. It was also noted that L. puber could be exposed to anoxia for 2 - 3 hours and still fully recover.

Work done by Kershaw (1986), has shown that Velvet Crabs which are held in flowing seawater tanks at ambient seawater temperature for two to three days prior to packing for transportation, have a higher survival rate than crabs which are packed immediately on landing. This suggests that time is needed to overcome the stress of catching.

In general it can be said that most crustacean respiratory physiology has been done on only five or six species, (McMahon and Wilkens, 1983), with Carcinus maenas being the most popular due to its ready availability. Also analysis of heart and scaphognathite rate traces have mainly been performed by taking blocks of data at regular time intervals. As the graphs in Chapter 3 illustrate, this has the potential for leading to misinterpretation.

Although great variability in heart and scaphognathite activity is evident in both stressed and

unstressed crabs, a pattern can be detected which can be used to determine and interpret stress. It is important to be able to identify physiological stress if experimental work is to be meaningful. In addition, from the commercial aspect, knowing the stress level of a crab can have far reaching economic affects.

It should also be noted that experimental physiological investigations performed on decapods generally necessitate the removal of the animal from its environment, with its associated trauma and the placing the animal in laboratory conditions (although effort is generally made to make the conditions as similar to the environment as is possible) before experimentation takes place. In order to perform physiological monitoring experiments on decapods, it is necessary to penetrate the carapace for electrode implantation. This results in immunological defence mechanisms being activated. The effect of this on the physiology, particularly at the cellular level, is as yet to be investigated.

In conclusion it should be stated that every crab is an individual with its own individualistic responses. Although experiments can be repeated on a number of similar

specimens, responses can be extremely varied and care should be made before assigning generalized species responses to differing circumstances.

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