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CANCER PAGURUS L.

BY X-RAY

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Some Aspects of the Respiratory Physiology of <u>Cancer</u> <u>pagurus</u> L.
(Crustacea: Decapoda) in Scottish West Coast Waters.

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ABSTRACT

For the largely immature population of crabs studied no
difference in length weight relationships could be detected
between males and females unless fresh weight was used for
comparison. Females and immature males showed isometric growth
throughout their life while mature males showed allometric growth.
The use of length, fresh weight, dry weight and ash-free dry
weight for the basis of comparative metabolic studies is
discussed.

Oxygen consumption rate was found to be related to the
0.799 power of body dry weight or ash-free dry weight for both
males and females and resting and active rates. Scope for
activity was constant at 3.7 across the entire body weight range.

A marked diurnal rhythm of oxygen consumption rate was found, the exact form of which depended on season being bi-modal in short day length and uni-modal in long day length.

The beat rates of heart and scaphognathite were found to show a high degree of correlation throughout all phases of the daily cycle. A typical short period rhythm of beat rates is shown during the resting phase.

Ventilation volume showed a linear relationship with scaphognathite beat rate up to active rates of pumping. Above this ventilation efficiency was impaired. The short period rhythm had a period of almost exactly twenty minutes and was divided into three phases, the high-rate, the low-rate and the changing phase. In order to conserve metabolic stores the duration of the low-rate phase is increased, at the expense of the level and duration of the high-rate phase, with increasing starvation. % utilisation of oxygen was at its highest during the high-rate phase (40 - 42%) and it appears that efficient oxygen exchange is limited to the rates of pumping of the active phase. Possible functions for the short period rhythm are discussed in the light of this observation.

Haemocyanin was more or less absent from the local population of Cancer but not Carcinus and Portunus. Reasons for this are discussed. A new method for measurement of

haemocyanin oxygen capacity was used. Effectiveness of oxygen uptake by the blood was low due to the lack of haemocyanin but effectiveness of oxygen removal from the ventilatory water was high due to a decrease in ventilation: perfusion ratio. Ventilation:perfusion ratio did not change greatly with increased starvation. The physiological significance of these measurements is discussed.

SECTION ONE

1.A. REVIEW AND GENERAL INTRODUCTION

There now exists a considerable body of knowledge on various aspects of respiration in the Crustacea, particularly the Decapoda. Before introducing the work carried out for this thesis it seems useful to review and discuss previous work.

Relatively recently fish physiologists have made considerable advances in the understanding of fish respiratory physiology by making use of new sensitive techniques for recording water and blood flow and oxygen and carbon dioxide levels (e.g., Albers, 1970; Randall, 1970a, 1970b; Riggs, 1970 & Shelton, 1970; all in Hoar & Randall, 1970; Hughes & Shelton, 1962; Hughes, 1964; Hughes & Morgan, 1973). Many of these techniques are specially designed to reduce interference with the normal behaviour of the fish to a minimum. The respiratory physiology of other aquatic groups is less well known and there have been, until recently, few attempts to apply the sensitive techniques of fish physiology to them.

The problems of gaseous exchange facing all aquatic animals are similar but are markedly different from those facing terrestrial animals. These differences are mostly due to the differences between water and air as respiratory media and are largely related to their physical and chemical properties, the

most important properties being oxygen content, density and viscosity. One litre of air at STP contains 209.5 ml of oxygen while the oxygen content of water varies considerably with temperature, barometric pressure and content of dissolved solutes. For sea-water (18‰ Cl) at $10^{\circ}C$ and 760 mm Hg atmospheric pressure one litre of sea-water contains 6.52 ml O_2. In spite of this large concentration difference between the two media the oxygen tension (pO_2) for an air/water system in equilibrium is the same (Henry's Law) at about 155 mm Hg. Water is some 1800 times more dense than air and about 100 times more viscous. Together these mean that the energy cost to an animal of ventilating the respiratory surface with water is far greater than ventilating with air. As the rate of diffusion of oxygen is also some 3×10^{6} times slower in water than air aquatic animals, particularly the most active forms e.g., fish and cephalopods, have been forced to develop exchange mechanisms which are highly efficient.

A further major difference between aerial and aquatic respiration concerns the fate of the waste carbon dioxide produced by cellular respiration. Carbon dioxide is 28 times more soluble in water than is oxygen and it is further taken up by a carbonate-bicarbonate buffer system especially in sea-water. The diffusion rate is also about 25 times that of

oxygen in water. Water may therefore absorb large quantities of carbon dioxide with little rise in pP^c_2. This results in the water at the exchange surface having a large capacity to absorb carbon dioxide and blood carbon dioxide tensions remain low. Blood carbon dioxide levels are thus of little use to aquatic animals as a means of controlling respiration.

The respiratory physiology of crustaceans may conveniently, as in other groups, be considered under two main headings,

i. the demand for oxygen in aerobic forms to carry out normal tissue metabolism and,

ii. the processes involved in gas transport which supply oxygen to and remove carbon dioxide from these tissues.

1.A.i. The Oxygen Demand

While all animals may release energy from metabolic substrates anaerobically to a greater or lesser extent, the advantages to the animal, in terms of energy gain, of aerobic metabolism are considerable. In aerobic forms the demand for oxygen will obviously depend on metabolic rate and in this context it is important to point out that several levels of metabolic rate have been defined usually in relation to locomotor activity. Basal metabolism is the level of metabolism at which only maintainance requirements are fulfilled, no

energy is put into locomotor activity. Standard metabolism or resting metabolism refers to that rate measured under conditions of minimum motor activity. Measurements of metabolic rate may be made at different rates of motor activity, the maximum sustainable rate being referred to as active metabolism (Prosser & Brown, 1961). While it is possible to measure oxygen consumption of fish at different swimming speeds and extrapolate back to zero motor activity and thus obtain basal metabolic rates the concepts are not so easily applied to the decapod crustaceans. For these the terms resting and active have been used to describe rates of oxygen consumption which refer to the minimum and maximum levels found throughout the natural daily cycle of activity (Ansell, 1973; Aldrich, 1975a, 1975b). Ansell (1973) has further defined an excited rate as that rate which is produced by handling and gross disturbance of the animal. The difference between the resting rate and active rate has been termed the scope for activity (Prosser, 1973) and is usually quoted as a ratio of active to resting rates. When intra- and inter specific comparisons of metabolic rates are being made then it is of obvious importance that values from comparable levels of metabolism are used.

In the Crustacea two factors have been mainly used as indicators of metabolic rate, the oxygen consumption rate and heart rate. Of the two the former is the more useful since the latter does not take into account possible changes in respiratory dynamics independent of heart rate. Maynard (1960) has, however, used a term the oxygen pulse (the amount of oxygen delivered to the body per heart beat) which may prove to be of more general use. Oxygen consumption rate gives a measure of aerobic metabolic rate and it is usually tacitly assumed that this represents total metabolism.

There are several factors which influence the demand for oxygen and these are conveniently divided into a) environmental or external factors such as temperature, salinity, pO_2 and pCO_2, and b) internal factors such as body size, metabolic substrate, activity level, etc.

1.A.i.a. Environmental factors

Temperature

As all metabolic processes are essentially chains of chemical reactions, clearly temperature changes will have an effect on their rate (law of Arrhenius) unless some temperature compensatory mechanisms are involved. This is particularly true in poikilothermous animals where body temperature is largely a function of environmental temperature, although some teleosts

and elasmobranchs are able to maintain body temperature up to $10^{\circ}C$ above environmental temperature by conserving heat produced by muscular activity (Carey & Teal, 1966, 1969a, 1969b, Carey et al., 1971).

The effects of temperature changes on the rates of metabolic processes are often quoted in terms of the Q_{10} value. This is given by:

$$Q_{10} = \frac{K_1}{K_2}^{\frac{10}{t_2 - t_1}}$$

where K_1 and K_2 are the rates measured at temperatures t_1 and $t_2 \, ^{\circ}C$.

The Q_{10} for respiration rate may be determined for the response to a large single sudden change in temperature, that is the acute response. Such determinations have been made for several species of crustaceans and values lie typically between 1 and 3.5 (a value of 2 represents a doubling in respiration rate for a $10^{\circ}C$ rise in temperature) (Waterman, 1960). The value of Q_{10} is not constant for any one species but varies across the temperature range (Brunow, 1911; Hoshi, 1951).

Apart from these direct responses to sudden changes in temperature many crustaceans are able to show a considerable

degree of adaptation to changes in temperature and are able to maintain metabolic rate at low temperatures or decrease it at high temperatures. For example Uca pugnax fully acclimates to temperature changes in about two weeks (Vernberg, 1959b) with a Q_{10} of 1.2 - 1.4 (Teal, 1959). Ahsanullah & Newell (1971) found that there was no acclimation of heart rate to temperature changes over a three week period. During this period, however, the animals were starved and several workers (Vernberg, 1959a; Roberts, 1957; McLeese & Watson, 1968; Ansell, 1973) have shown that there is a depression of heart and metabolic rate associated with starvation and this may have masked any acclimation.

Such temperature acclimation plays an important role in seasonal regulation of metabolism. The range of temperature to which a species can successfully adapt, however, may change considerably with geographical location. Members of a species living in colder waters often have a metabolic rate which is considerably higher than warmer water animals acclimated to the same temperature. This is a permanent response to living in cold water and is usually considered as a special adaptation which allows cold living animals to maintain activity levels comparable to those of warm living animals (Roberts, 1959).

Salinity and Ionic Concentrations

For many crustaceans living in fully marine habitats salinity is reasonably constant. Other species are either adapted to living in fresh-water or in regions where salinity fluctuates often from full sea-water to complete fresh-water. Some species e.g., _Artemia salina_ are specially adapted to living in waters where salinity is extremely high due to evaporation.

Animals which are hyperosmotic to the external medium i.e., fresh-water forms must perform metabolic work to ensure that the internal salt concentration remains high. Conversely animals which maintain the body hyposmotic to the medium, i.e., marine forms, must keep up body concentrations of water, again involving metabolic work. Even in animals which are isosmotic to their environment, selective accumulation and excretion of ions occurs. For the most part crustaceans maintain higher concentrations of Na^+, K^+ and Ca^{2+} and lower concentrations of SO_4^{2-} and Mg^{2+} than exist in sea-water, the Cl^- ion remaining more or less in equilibrium. The maintainance of these ionic gradients involves metabolic work.

On this basis it might be reasonable to expect that of comparable fresh-water and marine forms the fresh-water form should have the higher oxygen consumption. Thus the marine isopod _Idotea neglecta_ has an oxygen uptake about one third of

the fresh-water Asellus aquaticus. Marinogammarus marinus
and Gammarus locusta have lower oxygen uptake than Gammarus pulex
(Fox & Simmonds, 1933). When Carcinus (euryhaline species) is
transferred from 100% to 50% sea-water its oxygen consumption
increases by one third (Kinne, 1966) and for Artemia in sea-water
respiration was 25% higher than that in concentrated brine
(Gilchrist, 1956).

Care must be taken in interpreting these results however
as the minimum respiration rate does not occur at isotonicity.
Prawns, Palaemonetes varians, grown at a salinity close to
isotonicity of the body do have a minimum respiration rate
at this salinity. Prawns from habitats with a salinity of
1.3‰ show a respiration minimum at 6‰ (Lofts, 1956) even
though blood salt concentrations are likely to be little
different (Panikkar, 1941).

Many animals e.g., Palaemonetes, Pachygrapsus, and
Gammarus duebeni show an increase in locomotor behaviour when
placed in media of salinity different to that of their normal
medium resulting in elevated respiration rates (Gross, 1957).
Some species show no increase in respiration rate in different
salinities e.g., Eriocheir (Krogh, 1939) and it is possible
that energy to cope with salinity stress may be diverted from
other bodily processes such as growth and reproduction.

Oxygen and Carbon Dioxide Tensions in the Medium

As with salinity the majority of crustaceans live in environments which show little fluctuation in oxygen content. Those species which live in small enclosed waters or rock pools may be subjected to periods of low oxygen and high carbon dioxide tensions (especially in fresh-water).

Classically it is regarded that there are two types of response to a fall in oxygen tension, animals are classed either as conformers or regulators (Prosser, 1973). Those animals which show a respiration rate which declines linearly with declining oxygen tension are termed conformers. Regulators are able to maintain respiration rate over a wide range of oxygen tensions, by means of adapting the respiratory dynamics, until a critical oxygen tension below which they become conformers. There is clearly considerable adaptive value in the ability to regulate oxygen consumption in this way.

Examples of both regulators and conformers are found in the Crustacea. Sub-littoral forms rarely encounter water of low oxygen tension and might be expected to show no particular adaptation to it. This is perhaps borne out by the observations of Amberson et al. (1925) and Thomas (1954) who have shown that Homarus americanus, Callinectes sapidus and Homarus cammarus are conformers. McLeese & Watson (1968) have shown, however,

that _Homarus_ _americanus_ and _Chionectes_ _opilio_ (both sub-littoral)
can regulate oxygen consumption down to 1.7 and 2.8 mgO_2 l^{-1}
respectively. _Uca_ _pugilator_, _U._ _pugnax_, _Pacifastacus_ _leniusculus_,
Goniopsis _cruentata_, _Cardiosoma_ _guanhumi_, _Panulirus_ _interruptus_,
Cambaris _immunis_, _Pugettia_ _producta_, _Orconectes_ _virilis_, _O._ _nais_,
Cancer _pagurus_ and _Carcinus_ _maenas_ all show regulation of the
oxygen consumption (Helf, 1928; Weymouth, _et al._, 1944; Wiens &
Armitage, 1961; Teal & Carey, 1967; Winget, 1969; Moshiri, _et al._,
1970; Ansell, 1973; Young, 1973; Taylor, 1976).

Clearly the situation is not simple with animals being either
regulators or conformers. Bayne (1971a, 1971b) has described
the response of _Mytilus_ _edulis_ (Bivalvia) to declining oxygen
tension and has shown that those animals which had been freshly
collected from the shore were able to regulate oxygen consumption
whilst those which had been held in the laboratory for more than
three weeks were unable to regulate. He ascribed this difference
in response to declining oxygen to a starvation stress on
laboratory maintained populations. A similar response has also
been described by Taylor & Brand (1975) for the bivalve (_Arctica_
islandica. Bayne (1971a) has also shown that the degree of
respiratory independence depends on the size of the animal.

Walshe (1950) and Redmond (1955) have postulated that the critical oxygen tension corresponds to the pO_2 required to just saturate the haemocyanin. Young (1973) was not able to support this conclusion and suggested that critical tension was the point of onset of anaerobic metabolism. In fish it would appear that it is limitations in the systems for gaseous exchange that cause the onset of respiratory dependence (Fry, 1957; Hughes & Shelton, 1962; Hughes, 1964).

It has been suggested recently (Mangum & van Winkle, 1973; Taylor & Brand, 1975; Taylor, 1976) that the division of animals into conformers and regulators may be entirely false and results from attempting to force a continuously variable response into two discrete categories. In many studies, particularly those on invertebrates, the authors have neglected to define or control for the activity state of the animal and this is of considerable importance. Ansell (1973) has shown that resting Cancer pagurus are able to regulate oxygen consumption down to oxygen levels of 2.5 mgO_2l^{-1} whilst in the active state the maximum oxygen uptake is limited by oxygen tension and the animal therefore acts as a conformer. McMahon & Wilkens (1975) and Taylor (1976) have shown that respiration rate in Homarus americanus and Carcinus maenas is maintained by an increase in ventilation volume in resting animals. Active animals, which

are already ventilating at nearly maximal rates, have little
capacity to increase ventilation volume and thus behave as
conformers. These studies which show that the degree of
respiratory independence is affected by a variety of
ecological and physiological factors indicate that the
division of animals into either regulator or conformer classes
is unwarranted (e.g., Wolvekamp & Waterman, 1960; Lockwood,
1968) and that the regulation or conformation of oxygen
consumption are best regarded as the two extreme responses
of a variable capacity to maintain respiratory independence.

1.A.i.b. Internal Factors

Body Size.

For inter- and intraspecific comparisons of respiration
rate animals of the same size should be used. This is
obviously very limiting and often quite impractical. Weymouth
et al. (1944) have shown that 96% of the total variance in
observed respiration rates in the kelp crab, Pugettia producta
may be ascribed to the effects of size, the other 4% being due
to other factors. Before comparisons may be made then the
effects of different body sizes must be eliminated.

When large and small specimens of the same species are
compared it is found that the total respiration rate of the

larger animal is greater than the smaller one. When the weight specific metabolic rates (oxygen consumption per gram of tissue) are compared it is found that the smaller organism has the higher rate. The relationship between body size and respiration rate is usually described in terms of a power function:-

$$\dot{V}O_2 \; = \; a \, W^b$$

where $\dot{V}O_2$ = oxygen consumption rate, mgO_2hr^{-1}

   W = the weight in grams

and a & b are constants

This equation is more conveniently handled in its logarithmetic form:-

$$\log VO_2 \; = \; \log a + b \log W$$

This is the equation for a straight line of slope b and intercept log a. The value of a corresponds to the respiration rate of a "unit animal" of weight 1 g and is sometimes referred to as the weight independent rate. The slope of the line, b, gives the rate at which respiration rate increases with increasing size. If its value is equal to 1 metabolism increases in direct proportion to body weight. If its value is 0.67 the metabolism increases in proportion to body surface area.

Hemmingsen (1960) has reviewed and corrected to a standard temperature much of the published work and has found for all organisms that, except for a short transition zone of body weights from 10^{-7} to 10^{-4} grams, a value for b of 0.75 is universal to poikilotherms and homiotherms alike. The reasons for this universal value are still much discussed but lie beyond the scope of this review. Reviews of values of a and b are to be found in Altman & Dittmer (1971) and Prosser (1973). The data available for crustaceans has been reviewed by Wolvekamp & Waterman (1960) and most values lie between the two values of 0.67 and 1.0. Scholander et al. (1953) give an average value of 0.85 while Zeuthen (1953) gives a value of 0.8. More recently values ranging from 0.5 to 1.2 have been found in several brachyurans (Wallace, 1972; Leffler, 1973; Marsden et al., 1973; Aldrich, 1974; Breteler, 1975). The picture of common slope is complicated by the fact that the value for b may not be fixed for one species as Vernberg (1959a) has shown that, in Uca rapax from Jamaica and Florida, the value of b changes considerably with temperature.

Activity

The general levels of metabolism as related to activity have already been defined. To date no work has been done to directly relate oxygen consumption to activity rate. Several

authors have correlated periods of high motor activity and periods of high oxygen consumption e.g., Edwards (1950), Naylor (1958, 1962), Arudpragasm & Naylor (1964a). Many authors have shown the existence of tidal or circadian rhythms of activity and respiration rate and it seems reasonable to assume that high locomotor activity is supported by high oxygen consumption. The precise investigation of this relationship must await the development of a method of assessing respiration rate and activity together.

1.A.ii. Gas Transport

All aerobic metabolic processes in the Crustacea require that oxygen be supplied to the tissues and carbon dioxide carried away. In order that this should be achieved the metabolising tissues are coupled to the external environment by the haemolymph system as diffusion is inadequate in all except the smallest of organisms (Krogh, 1941). It is convenient to consider gas transport in several divisions although the functioning of each depends heavily on the others. The three main divisions of gas transport may be seen as:-

a) the exchange of oxygen and carbon dioxide across the exchange surface,

b) the transport of oxygen and carbon dioxide around the body

c) the exchange of oxygen and carbon dioxide with the tissues

1.A.ii.a. The Exchange of Respiratory Gases across the Exchange

Surfaces

Before the exchange of respiratory gases is considered the nature of the exchange surfaces will be dealt with. The smallest crustaceans e.g., the Copepoda and Ostracoda may have no particular areas of the body which are specifically specialised for gaseous exchange. These animals rely on diffusion of oxygen across the general body surface to supply metabolic demands. Spencer (1955) showed that, in Ligia, oxygen diffuses across the cuticle particularly at the base of the legs and the ventral surfaces. As crustaceans get larger dependence on general body surface area for exchange becomes less although Thomas (1954) has shown that, in Homarus, 3% of the exchange occurs at sites other than the gills.

Typically crustaceans have localised exchange surfaces which are well irrigated with the respiratory medium and well supplied with haemolymph. The typical crustacean has localised respiratory surfaces arising as one or more lobes or lamellae on the basal segment of each thoracic limb. The typical malacostracan gill is a podobranch arising from the coxa of a thoracic limb. Additional gills may arise from the arthrodial membrane at the base of the leg (arthrobranch) or from adjacent areas of the body wall (pleurobranch).

Many Crustacea have epipodites on thoracic limbs which are assumed to act as gills because of their large surface area, thin cuticle and well developed vascularisation. Among the Malacostraca, however, some forms lack such epipodites, but have areas which stain heavily with silver indicating high permeability e.g., the latero-ventral corners of the carapace in *Neomysis* *integer* and the ventral surface of the abdomen of *Asellus* *aquaticus* (H.·abe, 1949).

More often the malacostracan gill is completely[x] organised with a central axis containing efferent and afferent blood vessels and a profusion of branches which may be foliaceous (lophogastrid mysids), filamentous (Euphausiacea and many Macrura), dendritic (Penaeidae), or lammellar (Carida, the majority of Anomura and Brachyura). Four gill series on each segment are typical giving a maximum possible gill number of thirty-two to a side. The largest number reported is twenty-four in the penaeid *Benthesicymus*. Further reduction of gill number is general, leaving as few as three in *Pinnotheres*.

The gills in the Decapoda are covered by an outgrowth of the carapace, the branchiostegite, giving only limited access to the outside and allowing the development of more efficient ventilatory currents. The euphausids are, however, an exception to this carrying the gills external to the branchiostegite.

Drach (1930), Smyth (1942), Siewing (1956), Agrawal & Autar (1969), have studied the structure and morphology of decapod gills. The integument across the gill surface consists of a thin outer chitinous layer with an inner epithelial layer, the cells of which may contribute to a meshwork of cells within the gill. The chitin layer has been thought to impart a certain degree of physical strength to the gills and may also be useful in controlling salt balance in those species subject to osmotic stresses. The chitin layer in Eriocheir is, however, either extremely thin or completely absent (Webb, 1940).

Few other studies on the cytology of decapod gills have been made; Bernbecker (1909) made a general survey and Allen (1892), Bock (1925), Chen (1933) and Burggren et al. (1974) have studied the gills of Palaemonetes, Astacus, Grapsus and Procambarus clarkeii. More recently Viullemin (1967) has studied the gills of both terrestrial and aquatic forms. The gill structure of terrestrial crustaceans is specially adapted to withstand collapse in air. The gills of Cardiosoma and others have special ridges to hold the plates apart (von Raben, 1934) and the gills of Scylla serrata (Portunidae) have a series of teeth which serve the same function (Hill, pers. comm.). The respiratory adaptations to terrestrial life are reviewed by Edney (1960).

The gill area is of obvious importance in respiratory exchange. Table 1 summarises the measurements of gill area for several malacostracans and for several fish for comparison. On the whole the gill areas in these two groups are of the same order. Gill area is correlated with general activity level and degree of terrestrialness; the most active crabs having the highest gill areas and the most terrestrial crabs having the lowest. Terrestrial crabs also show a reduction of gill number (Pearse, 1929a, 1929b, 1950; Ayers, 1938). Gray (1957) has further shown that weight specific area and number of gill plates decreases as animals become larger within the species. This may be correlated with a decreased weight specific oxygen consumption with increased size.

Gaseous exchange across the gill surface is driven by the oxygen (or carbon dioxide) tension gradient between the water and blood. In order that the external tension of oxygen is kept high and carbon dioxide low water is continuously circulated over the gills of aquatic forms. This circulation is normally referred to as gill ventilation although Jones (1972) has used the term irrigation.

Movement of water over the gills is usually brought about by rhythmical beating of some post-oral appendages. Such pumping units may be either thoracical or abdominal. The Isopoda,

Table 1.

Showing the gill areas of decapods and those of several fish

for comparison. Fish species are marked with an asterisk.

Species	Body weight g.	Gill Area mm^2g^{-1}	Source
Carcinus maenas	48	660	Damant (1920)
" "	102	960	" "
" "	80	625	Webb (1940)
" "	40.6	777	Hughes et al. (1969)
Ocypode albicans	-	325	Gray (1957)
Uca minax	-	513	" "
Uca pugnax	-	770	" "
Uca pugilator	-	624	" "
Sesarma cinerea	-	638	" "
S. reticulata	-	579	" "
Panopeus herbstii	-	874	" "
Menippe mercenaria	-	887	" "
Libinia dubia	-	748	" "
L. emarginata	-	566	" "
Hepatus epheliticus	-	1099	" "
Portunus spinimanus	-	901	" "
P. gibesii	-	1003	" "
Ovalipes ocellatus	-	1288	" "
Areneus cribarus	-	1301	" "
Callinectes sapidus	-	1301	" "
*Scomber scombrus	182	1158	Gray (1954)
*Clupea harengus	85	640	Hughes (1966)
*Callionymus lyra	46	198	" "

Amphipoda and Stomatopoda use beating of the pleopods to ventilate the gills. Amongst those using the thoracic limbs, the branchiopods and mysids use all of the limbs whilst others may use only a specialised part such as maxillulary exopodite of the ostracods or the scaphognathite (epipodite of the second maxilla) in the decapods. Several forms, especially planktonic and pelagic forms, use the ventilatory current for feeding and locomotion as well as respiration.

Amongst the Malacostraca the ventilatory current is an anteriorly moving stream of water which is drawn into the branchial chamber around the edge of the branchiostegite. In the Mysidacea, Euphausiacea, Cumacea, Tanaidacea, and natantian decapods the edge of the branchiostegite is quite free and water may flow into the branchial chamber all around. In the reptantian decapods, especially brachyurous forms, the branchiostegite edge is closely applied to the body and the inflow of water is restricted to small areas above the base of each pereiopod. In many brachyurans there is a large opening at the base of each chela, the opening of Milne-Edwards, through which the majority of inhalent water flows (80% in _Carcinus maenas;_ Arudpragasm & Naylor, 1964b).

In the Decapoda the respiratory current is as follows:- the inhalent water is drawn into the hypobranchial chamber and

flows posteriorly and dorsally. The water passes through the spaces between the gill lamellae into the epibranchial space and then flows anteriorly over the scaphognathite and out through the exhalent opening on either side of the mouth.

The action of the scaphognathites in the decapods has received some attention. Paztor (1968, 1969) has studied the inervation of the muscles moving the scaphognathites and its sensory system in the crayfish Orconectes virilis. The muscles driving the scaphognathite act in two ways; one set moving the blade up and down while another set twist the blade about its axis. These observations have largely been confirmed for other species (Wilkens & McMahon, 1972; Pilkington & Simmers, 1973). In Homarus americanus the scaphognathite blade is not hinged and depends on being twisted to achieve proper angle of attack (Wilkens & McMahon, 1972). The blade of Cancer novae-zelandiae (Pilkington & Simmers, 1973) and Carcinus maenas (Young, 1975; Atkinson, pers. comm.) is hinged allowing the blade to flex during the beat.

Reversal of the scaphognathite beating occurs in several species of Reptantia and causes a reversal of the normal water flow through the animal. There have been several interpretations of the function of these reversals in decapods. Bohn (1901) suggested that they serve to rest the muscles of the scaphognathite.

Wilkens & Young (1975) and Young (1975) have shown that reversals are brought about by a different firing order of the muscles involved. Borradaile (1922) considered that reversals in Carcinus served to clean away particles which have become trapped on the gills and to ventilate the gills with air when the animal is trapped in shallow oxygen depleted water. Taylor & Butler (1973) and Taylor et al. (1973) have extended the observations of Borradaile (1922) and have shown that this behaviour raises the oxygen tension in the branchial chambers and the post-branchial blood. This is not a complete explanation however as Carcinus and other decapods show reversals when in fully oxygenated waters. Arudpragasm & Naylor (1964a, 1966) have studied reversals in several species of decapods and concluded that reversals serve to irrigate the more posterior gills in Carcinus and that addition of suspended matter to the medium did not alter reversal rate. They found that in Cancer pagurus, which has two major openings for inhalent water, one anteriorly and one posteriorly, reversal rate was low. If the posterior opening was blocked or if the crab buried reversal rate was increased. Hughes et al. (1969) measured hydrostatic pressure during normal and reversed pumping and found that there was a dead space around the posterior gill in Carcinus. They were unable, however, to demonstrate any increase in hydrostatic

pressure or oxygen tension on reversal.

Arudpragasm & Naylor (1964a, 1966) were able to obtain an increased reversal rate in response to suspended matter only in Homarus gammarus. Wilkens & McMahon (1972) have suggested that reversals serve to clean accumulated material from the hairs fringing the branchiostegite in Homarus americanus. Finally Blatchford (1971) has suggested that the rapid change from negative to positive to negative hydrostatic pressures in the branchial chamber may serve to flush blood through the gills.

Some crustaceans maintain a predominantly reversed water current through the gill chamber. This is particularly associated with the burrowing habit. The penaeids Solenocera and Metapenaeus, the anomuran, Emerita analoga, the cumacean Leucon siphonatus and the brachyurans Calappa, Parthenope, Heterocrypta, Carposporus, Lopholithodes and Corystes cassivelaunus all use the reversed mode of pumping to supply water to the gills (Garstang, 1896; Zimmer, 1941; Scäfer, 1954; Dall, 1958; Arudpragasm·& Naylor, 1966; Hartnoll, 1972).

There have been few measurements made on ventilation volume in crustaceans. Thomas (1954) gives a value of 0.5 ml g^{-1} min^{-1} for Homarus gammarus and Larimer (1961) gives a value of 0.6 ml g^{-1} min^{-1} for Procambarus simulans. Arudpragasm & Naylor (1964b, 1966) give values of 1 ml g^{-1} min^{-1} for small and

2 ml g^{-1} min^{-1} for large *Carcinus maenas*, *Cancer pagurus* and *Macropipus puber*. Hughes et al. (1969) give a similar value of 1 ml g^{-1} min^{-1} for *Carcinus* and Moshiri et al. (1970) have found a value of 0.3 ml g^{-1} min^{-1} for *Pacifastacus leniusculus*. All of these workers used a method which involved channeling the exhalent water. Johansen et al. (1970) have criticised this method and found values of 0.63 ml g^{-1} min^{-1} for large *Cancer magister*. These values were always higher than those determined by an overflow method.

There have been no studies of the mechanisms involved in the regulation of ventilation volume. Regulation may occur in three main ways. The rate of scaphognathite beating may be varied, the amplitude and angle of attack of the blade may be varied or the size of inhalent openings may be varied.

The rate of scaphognathite beating is relatively easy to determine and this has been done for several species mostly by direct observation of the tip of the blade. Wolvekamp & Waterman (1960) give a table of typical values. Lindroth (1938) suggests that ventilation volume varies directly with scaphognathite beat rate. Pilkington & Simmers (1973) suggest that beat amplitude does not vary a great deal with beat rate and so plays little part in the regulation of ventilation volume. There is no published work relating angle of attack to ventilation volume.

Obviously the size of the inhalent openings will affect ventilation volume but this aspect of regulation has not been investigated. Borradaile (1922) has shown that in _Carcinus_ the size of Milne-Edward's opening is regulated by the position of the epipodite of the third maxilliped. The branchiostegite may also be adducted onto the body to a varying degree by the action of the epimeral attractor muscle (Wilkens & McMahon, 1972).

The efficiency of exchange of oxygen has been examined in several crustaceans. Efficiency has mostly been quoted in terms of % utilisation (or % extraction) and values from the literature are shown in Table 2. It can be seen that in general the values found are usually low at about 20% although the values obtained by Hazelhoff (1938), Larimer (1961) and Taylor (1976) are higher than those obtained by other workers. Thomas (1954) found that it was possible to raise % utilisation by artificially slowing down the ventilation stream. The low values of % utilisation found are generally ascribed to the existence of a high barrier to diffusion caused by the layer of chitin at the gill surface.

More recently several workers have applied equations derived from heat exchangers by Hughes & Shelton (1962) to crustacean gas exchange. Values for effectiveness of oxygen removal from the water, Eb, lie at around 20% although this may increase

Table 2.

Showing the % extraction of oxygen from the ventilatory water by several species of crustaceans.

Species	% Sat.	% Ext.	Source
Isopoda			
<u>Anilocera</u> <u>physodes</u>	100	45	Hazelhoff (1938)
Macrura			
<u>Astacus</u> <u>astacus</u>	100	50-70	Hazelhoff (1938)
<u>Procambarus</u> <u>simulans</u>	100	45	Larimer (1961)
" "	100	50	Larimer & Gold (1961)
" "	33	65	" " "
<u>Orconectes</u> <u>virilis</u>	100	30-40	McMahon <u>et</u> <u>al</u>. (1974)
<u>Palinurus</u> <u>elephas</u>	100	36-52	Hazelhoff (1938)
<u>Scyllarides</u> <u>latus</u>	100	39-48	" "
<u>Homarus</u> <u>gammarus</u>	100	30.6	Thomas (1954)
" "	65	42.1	" "
" "	42	55.1	" "
<u>H</u>. <u>americanus</u>	100	36-40	McMahon & Wilkens (1972)
" "	67-100	22	McMahon & Wilkens (1975)
" "	13-20	73	" " "
" "	7-13	44	" " "

Species	% Sat.	% Ext.	Source
Brachyura			
Calappa granulosa	100	57-88	Hazelhoff (1938)
Dromia vulgaris	100	37-46	" "
Maia verrucosa	100	25-32	" "
Carcinus maenas	100	9-23	Arudpragasm & Naylor (1964b)
" "	100	7-23	Hughes et al. (1969)
" "	100	10-25	Taylor (1976)
Cancer magister	100	10-25	Johansen et al. (1970)

(McMahon & Wilkens, 1975) or decrease (Taylor, 1976) with increasing hypoxia. The effectiveness of oxygen uptake by the blood, Eb, is high being about 80% for <u>Carcinus</u> and 100% for <u>Cancer magister</u>. Values for the transfer factor, $To_2 ml O_2$ $kg^{-1} min^{-1} mmHg^{-1}$ lie between 5×10^{-3} and 9×10^{-3} (Johansen <u>et al</u>., 1970; McMahon & Wilkens, 1975; Taylor, 1976). Typical values for teleosts (rainbow trout) are: Ew, 11-30%; Eb, 95-100%; and To_2, 2×10^{-2} ml $kg^{-1} min^{-1} mmHg^{-1}$; and elasmobranchs (<u>Scyliorhinus</u>): Ew, 66%; Eb, 79%; and To_2 9×10^{-3} ml $kg^{-1} min^{-1} mmHg^{-1}$. (Randall <u>et al</u>., 1967; Piper & Baumgarton-Schumann, 1968).

<u>1.A.ii.b.</u> The Transport of Gases Around the Body

As diffusion processes only serve to transport gases adequately over very short distances, it is thus necessary for oxygen to be transported to and carbon dioxide from the respiring tissues by some other means. This is usually achieved by circulating the gases around the body either in simple solution in the blood or in combination with a respiratory pigment.

Respiratory pigments occur in only a few Crustacea, the majority relying on gases dissolved in the haemolymph. To date two types of pigments have been found in the Crustacea, these are haemoglobin (Hb) and haemocyanin (Hcy). The haemoglobins

found in the Crustacea seem to be higher polymers than the mammalian Hbs with molecular weights up to six times greater. They have been reported from the Branchiopoda, Ostracoda, Cirripeda, Copepoda and Branchiura.

The haemocyanins are copper based chromo-proteins with no prosthetic group. They have been recorded from two phyla; the Mollusca and the Arthropoda. In the Mollusca they are confined to the Gastropoda and Cephalopoda and in the Arthropoda have been reported from the Xiphosura, the Arachnida and the Crustacea. Haemocyanins are commonly found in the haemolymph of Stomatopoda and Decapoda and have also been found in Isopoda and Amphipoda (Berthet & Berthet, 1963; Manwell & Baker, 1963; Wieser, 1961, 1965a, 1965b).

Hcy always occurs in solution in the haemolymph and its high molecular weights, $6-8 \times 10^5$, have the advantage of restricting losses through the excretory system. In solution however the concentration is limited by the viscosity and colloid osmotic pressure. It is perhaps for these reasons that bloods containing Hcy have fairly low oxygen capacity. Table 3 shows typical values for the Crustacea. The oxygen capacity of most fish bloods lie between 4 and 10 vol % and the capacity of some mammalian bloods may be as high as 20 vol % (Prosser, 1973).

Table 3.

Showing the oxygen capacity of fully saturated blood of several species of crustaceans.

Species	Oxygen capacity vol %	Source
Macrura		
Astacus fluviatilis	2.4	Dhéré (1900)
Homarus gammarus	3.1	" "
" "	0.94	Spoek (1962)
H. americanus	1.95	Redfield et al. (1928)
" "	1.31	Redmond (1955)
Palinurus elephas	1.48	Winterstein (1909)
" "	1.25	Stedman & Stedman (1925)
Panulirus interruptus	1.99	Redmond (1955)
Brachyura		
Cardiosoma guanhumi	2.83	Redmond (1962)
Gecarcinus lateralis	2.17	" (1968a)
Loxorhyncus grandis	1.03	" (1955)
Maia squinado	0.84-1.13	Winterstein (1909)
" "	1.22	Stedman & Stedman (1925)
Xantho incisus	0.46	Truchot (1971)
Macropipus puber	1.41	" "

Species	Oxygen capacity vol %	Source
Cancer pagurus	1.85	Stedman & Stedman (1925)
" "	0.88	Truchot (1971)
" "	2.3	Dhéré (1903)
Cancer irroratus	1.23-1.69	Redfield et al. (1926)
Cancer borealis	1.4	Redfield et al. (1928)
Cancer magister	3.4	Johansen et al. (1970)
Carcinus maenas	1.14-1.16	Begemann (1924)
" "	1.04	Truchot (1971)

In most Crustacea haemocyanin occurs at fairly low concentrations of up to 50 mg 100 ml^{-1} (Ellerton et al. 1976). The oxygen capacity of *Jasus lalandei* Hcy is 25 ml O_2 per 100 g Hcy (Rawlinson, 1940) which is to be compared with a value of 134 ml O_2 per 100 g Hb. Reaction rates of oxygen with Hcy are comparable to those of Hb, the Hcy of *Maia squinado* reaching 50% saturation in 3×10^{-3} secs (Millikan, 1933) the reaction rate is pH dependent.

The Hcy of different animals reach saturation at different oxygen tensions. Those pigments which saturate rapidly and thus have a low p50 (pO_2 at which the pigment is 50% saturated) are termed high affinity pigments while those reaching saturation slowly, and thus having a high p50, are termed low affinity pigments. Thus the oxygen affinity of any pigment may be conveniently described in terms of its p50. The terms loading and unloading tensions are often used for p95 and p50. This use should be avoided since p95 and p50 do not necessarily correspond to actual loading and unloading tensions. Values for the p50 of some crustacean haemocyanin-containing bloods are given by Wolvekamp & Waterman (1960), Altman & Dittmer (1971) and Prosser (1973). In general the p50 values for crustaceans tend to be low indicating high oxygen affinity. Young (1972) has demonstrated a shift towards lower oxygen affinities with increased terrestrialness

in a series of Jamaican brachyurans.

When the percentage saturation of the pigment is plotted against pO_2 an equilibrium curve results. Generally these are sinusoidal in shape. From these curves it may be seen that the pigment tends to act as an oxygen buffer, releasing or combining with large amounts of oxygen for a small change in pO_2.

There are several factors which modify the shape of the equilibrium curve and thus the affinity for oxygen of the pigments. In most cases increased temperature causes the equilibrium curve to be displaced to the right so that oxygen affinity is reduced. This is a consequence of the exothermic nature of the oxygen binding process and Wolvekamp (1955) has pointed out that the increased oxygen requirements of poikilotherms may be in part met by this decrease in oxygen affinity and consequent easier unloading at higher temperatures. Excessive displacement of the equilibrium curve may, however, result in oxygen binding at the respiratory surface becoming difficult. Young (1972) has shown that p50 for Callinectes sapidus increases with temperature and Redmond (1955) and Johansen et al. (1970) found a similar situation for Loxorhyncus grandis, Panulirus interruptus and Cancer magister.

An important factor affecting the binding of oxygen with haemocyanin is the presence of carbon dioxide. The classical Bohr effect described a reduction of oxygen affinity of the pigment in conditions of increased pCO_2 but the term is now usually used to describe changes in affinity caused by either pCO_2 or pH changes although some fish haemoglobins show a change in oxygen affinity in response to pCO_2 changes at constant pH (Riggs, 1970).

Usually Bohr shift with increasing pCO_2 or pH is towards lower affinity and such shifts are referred to as normal or negative. Normal Bohr shifts have been reported for several crustacean species (Pantin & Hogben, 1925; Fox, 1945; Redmond, 1955, 1962, 1968; Johansen et al., 1970; Truchot, 1975). Some haemocyanins show an increased affinity to increased pCO_2 or acidity, this is a positive or reversed Bohr effect and has been shown in Busycon, Limulus and Helix.

The functional significance of the normal Bohr effect is that oxygen affinity is lowered at places of high internal pCO_2 thus liberating oxygen at the site of CO_2 production. The extent of the Bohr shift will depend on the buffering power of the blood as good buffering will require a large change in pCO_2 before pH changes significantly. Most of the studies of blood chemistry have been carried out in vitro with pH changes which are

extremely unlikely to occur in the animal. Johansen et al. (1970) found a marked Bohr effect in Cancer magister haemocyanin, in vivo measurements of arterial and venous pH revealed differences of less than 0.05 units implying that the Bohr shift has little significance in vivo.

The presence of divalent cations may have a considerable effect on the oxygenation properties of haemocyanins (Lontie, 1954; Redmond, 1955; Wolvekamp & Waterman, 1960; Manwell, 1964) but the problem has not been studied systematically. Larimer & Riggs (1964) have shown that dialysis of haemocyanin reduces oxygen affinity, the effect is largely reversed by the addition of 10 mM calcium or 60 mM magnesium. In view of the changes in serum calcium levels associated with the molt cycle this effect could be of physiological importance. The presence or absence of various ions may control the degree of aggregation of the sub-units of haemocyanin (Cohen & van Holde, 1962, 1963; DePhillips & van Holde, 1962; van Bruggen et al., 1962, 1963; Levin, 1963; van Holde & Harrison, 1963) although the effect of the aggregation state on oxygenation is largely unknown. Johnston et al. (1967) have shown that dissociation of Homarus haemocyanin into sub-units raises oxygen affinity but does not alter oxygen capacity.

The presence of ATP or other organic phosphates e.g., diphosphoglycerate have a considerable effect on the oxygen affinity of haemoglobin (Benesch et al., 1968; Lenfant et al., 1968). These effects are unstudied in the haemocyanins.

Once the oxygen has crossed the respiratory surface of the animal and is either in solution in the blood or combined with the pigments when present, it is carried around the body by physical circulation of the haemolymph. This is achieved in several ways. A dorsally situated heart is present in most crustaceans though a true heart is completely lacking in the copepods and ostracods. When the heart is absent its function may be served by somatic muscles modified to compress a vessel on contraction (Cannon, 1940) or may be brought about by body movements such as the raising and lowering of the body posterior in *Caligulus salva* (Gnanamuthu, 1948). These special adaptations may serve to augment heart function in crustaceans with better developed circulation (Burger & Smythe, 1953). Where a heart is present it may take several forms; ovoid in calanoid copepods, tubular in amphipods, isopods and stomatopods and rhomboidal in decapods.

Amongst the Decapoda the circulatory system has been studied for *Cancer pagurus* (Pearson, 1908), *Homarus americanus* (Burger & Smythe, 1953), *Caridina laevis* (Pillai, 1965) and

Panulirus interruptus (Belman, 1975). The general course of decapod circulation is as follows:- blood enters the heart through five pairs of ostia from the pericardium at diastole and is pumped from the heart through seven arteries, five of which are directed anteriorly and two posteriorly. The blood is conducted along these arteries to the various regions of the body. The blood then passes through a complex series of interconnecting sinuses which bathe all the organs. Burger & Smythe (1953) suggest that in the resting animal these sinuses form discrete pathways. Eventually the blood, by this time deoxygenated, collects in the hypobranchial sinus from where it flows up the dorsal gill face, across the gill lamellae and down the hypobranchial vein to the pericardium via the branchio-pericardial veins. Burger & Smythe (1953) showed that, in Homarus, all blood must pass through the gills before it can return to the heart.

Injection of indian ink into the system results in its rapid spread to all parts of the body indicating few, if any, barriers to the circulation and that direction of blood flow is maintained by pressure gradients.

Measurements of circulation pressure have been made in several decapods. Dubuisson (1928) found a systolic pressure of 5.5 cm H_2O and a pressure gradient of 0.6 cm H_2O across the

gills in _Maia squinado_. Picken (1936) has given measurements
of 0.5 to 2.6 cm H_2O and 0.75 to 2.2 cm H_2O from the sternal
sinus of _Carcinus maenas_ and _Potamobius fluviatilis_,
pericardial measurements being up to 4 cm H_2O. Prosser _et al._
(1947) give a cardiac pressure of 8.5 cm H_2O for _Astacus_. The
methods of Dubuisson (1928) and Prosser _et al._ (1947) resulted
in considerable damage and opening of the pericardium causing
considerable blood loss. The value of the results is thus
questionable.

Burger & Smythe (1953), Blatchford (1971), Belman (1975),
and Belman & Childress (1976) have made use of cannulae and
either strain gauges or pressure transducers to measure
haemolymph pressures. Burger & Smythe (1953) report a systolic
pressure of 17 cm H_2O and a mean arterial pressure of 14.3 cm H_2O
proximal to the heart. Blatchford (1971) has recorded
haemolymph pressures from several parts of the circulation
simultaneously in _Carcinus maenas_. Recorded systolic pressures
are similar to those of Burger & Smythe (1953) at 14 cm H_2O and
pressures elsewhere in the system reflect the systolic pulses.
The pressure in the leg sinuses remains positive with respect to
the infrabranchial sinus and haemolymph might be expected to
flow to the gills at all times. The lateral pericardium, which
receives blood from the gills always remains negative to the

gills and blood will thus flow through the gills continuously.
Haemolymph pressures in the dorsal pericardium are much higher
than the lateral pericardium and the mechanism by which this
is achieved without the use of valves or chambers remains
unclear.

Belman (1975) using the same techniques as Blatchford (1971)
has found much higher systolic pressures of 55 cm H_2O in
Panulirus interruptus. He also found diastolic pressures of
20 cm H_2O whereas diastolic pressure in Carcinus fell to zero
(Blatchford, 1971). In Panulirus arterial systolic pressure
equals cardiac systolic pressure, arterial diastolic pressure
remains higher than cardiac diastolic pressure. This is
explained by the presence of valves at the junction of the
artery with the heart, blood ejection from the heart only
occurring when systolic pressure exceeds arterial pressure.
There is a pressure drop of 17 cm H_2O across the body and a
drop of 20 cm H_2O across the gills indicating a similar
resistance to flow. Belman & Childress describe a similar
range of pressures for the mysid Gnathophausia ingens with
systolic pressures of up to 27 cm H_2O.

The values of systolic pressures found by Belman (1975)
for Panulirus are very much greater than any previously reported
for the Decapoda. Belman suggests that they are due to the high

resistance of the filamentous gill in the Palinuridae.

Fyhn et al. (1973) have found values of haemolymph pressure in the gooseneck barnacle, Pollicipes, of up to 250 cm H_2O.

Few determinations of cardiac output have been made in the Decapoda although there has been recent interest (Johansen et al., 1970; Blatchford, 1971; Ansell, 1973; Florey & Kriebel, 1974; Belman, 1975; Taylor, 1976; Mangum & Weiland, 1975; Spaargaren, 1976). Cardiac output is the product of stroke volume and beat rate. Burger & Smythe (1953) determined stroke volume crudely by plunging a glass tube into the heart and give values of 0.1 to 0.3 ml per beat. At a beat rate of 100 b.p.m. this gives a circulation time of 2-8 minutes.

Redmond (1955) has used data of Burger & Smythe (1953) and Weymouth et al. (1944) together with his own observations on blood gas levels to calculate the cardiac output of a 750 g Panulirus interruptus to be 80 ml kg^{-1} min^{-1} using the Fick principle. The value that Redmond (1955) used for oxygen uptake of Panulirus from Weymouth et al. (1944) was a mean value for several crustaceans and seems very low. Johansen et al. (1970) have recalculated cardiac output using a value of oxygen consumption from Winget (1969) and Redmond's (1955) blood gas levels and obtained values of about 400 ml kg^{-1} min^{-1}.

Johansen et al. (1970) consider that both of these values seem

to be very high for an invertebrate. Johansen et al. (1970)

used the Fick principle to calculate cardiac output in Cancer

magister and obtained values of 29.5 ml kg^{-1} min^{-1}. Assuming

a blood volume of 35%.body weight (Drach, 1939; Krogh, 1939;

Webb, 1940; Prosser & Weinstein, 1950; Flemister, 1958;

Zuckerkandl, 1960; Siebers & Lucu, 1973) this gives a

circulation time of twelve minutes. Ansell (1973) gives a value

of 39 ml kg^{-1} min^{-1} for Cancer pagurus. These values are of the

same order as those obtained for fish (Prosser, 1973).

Belman (1975) has calculated cardiac output for Panulirus

interruptus based on blood velocity measurements and obtained

values of 128 ml kg^{-1} min^{-1} and gives a circulation time of

2-3 minutes. Blatchford (1971) has calculated circulation time

to be 1 to 5 minutes in Carcinus. His figures for heart volume

and rate give a calculated cardiac output of 45 to 352 ml kg^{-1}

min^{-1}. Calculations of cardiac output based on rate alone do

not take into account any changes in stroke volume that may

occur with beat frequency. In fish it is thought that it is

stroke volume which plays the largest part in changes in cardiac

output (Randall & Stevens, 1967; Stevens & Randall, 1967a, 1967b;

Hanson, 1967 cited by Satchell, 1971). Beat rates and heart

volume taken together do allow, however, an estimate of the

maximum value likely.

The values of cardiac output of Belman (1975) seem to be very high when compared to the Fick method used by Johansen et al. (1970). Mangum & Weiland (1975) and Taylor (1976) have used the Fick method to calculate the cardiac output of Callinectes sapidus and Carcinus maenas and have obtained values of 207-235 ml kg^{-1} min^{-1} and 70 to 90 ml kg^{-1} min^{-1} respectively. The main reason for this discrepancy seems to be the large oxygen capacity of the blood of Cancer magister resulting in an arterial-venous turnover of 1.7 vol %. The a-v turnover in Carcinus is 0.46 vol % (Taylor, 1976) and 0.6 vol % in Callinectes (Mangum & Weiland, 1975).

Spaargaren (1976) has used a method of determining cardiac output in several crustaceans which is based on the rate at which the temperature of an animal equilibrates with its environment. The values found are, in general, higher than those obtained by the Fick method. Spaargaren gives an equation:

$$VF = 2.36 \ W^{0.69}$$

where V = stroke volume, F = beat frequency and W = weight calculated from data on several crustaceans. This gives a value of 277 ml kg^{-1} min^{-1} for a Cancer magister of weight 1 kg which Johansen et al. (1970) found to have a cardiac output of 34.1 ml kg^{-1} min^{-1} by the Fick method. The reasons for these

large differences in calculated values is not at all obvious but Spaargaren (1976) did not monitor ventilation rate and it is obvious that the rate of active temperature exchange across the gills will depend on this as well as the rate of blood flow through the gills. Against the Fick method it must be pointed out that the measured values of oxygen capacity of arterial and venous bloods are measured instantaneously while respiration rate is determined over a period and some error is bound to arise through this. Furthermore with the apparently long circulation time (based on low cardiac outputs) the venous tension taken may not correspond to that of the arterial tension at that moment but to that of some time earlier.

The regulation of cardiac output in the Crustacea has received very little attention. This is due to the great difficulty in measuring cardiac output. Ansell (1973), from data on the oxygen pulse and the analogue trace of heart beat, found that there was no need to postulate any change in stroke volume to account for the observed change in cardiac output. Florey & Kreibel (1974), relying heavily on data from other studies and several assumptions, postulate that stroke volume decreases with increasing beat frequency. Taylor (1976) found a decrease in cardiac output associated with progressive hypoxia in Carcinus and since heart rate remained more or less constant

over this period he argued that stroke volume declined. Spaargaren (1976) has determined cardiac output in several crustaceans over a range of heart beat rates and from his figures it would appear that in any one crab the stroke volume does not change with frequency.

Obviously the situation is complex and difficult to understand but since filling of the heart depends on elastic recoil in the suspensory ligaments it is difficult to imagine variable diastolic filling causing a change in stroke volume (Starling's Law). From this is might be argued that, in the Crustacea, heart rate gives a reasonable indication of cardiac output and the observations of Spaargaren (1976) support this hypothesis.

Blood oxygen levels determined recently (Johansen et al., 1970; McMahon & Wilkens, 1972; Taylor, et al., 1973; Taylor, 1976) are in marked contrast to those determined by Redmond (1955, 1962, 1968a, 1968b), Zuckerkandl (1957a) and Spoek (1962) who gave remarkably low values which were below the oxygen content of sea-water. Johansen et al. (1970) and Taylor et al. (1973) have discussed the reasons for this large difference and ascribe it to an inadequate sampling procedure. It is now fairly well established that for the most part crustacean pigments reach saturation at the gills, Johansen et al. (1970) report arterial

saturation of 99-100%. Taylor et al. (1973) emphasis the importance of monitoring heart beat and scaphognathite beat continuously so that the condition, such as sensory shocks, which cause the onset of acute bradycardia may be avoided (Larimer, 1964; Larimer & Tindel, 1966; Wilkens & McMahon, 1972; Taylor et al., 1973; Florey & Kreibel, 1974).

Of further importance to gaseous transport by the blood is the concentration of haemocyanin present. Zuckerkandl (1957b) has shown that haemocyanin is present in the blood of *Maia* *squinado* in significant amounts only immediately prior to moulting. On this basis the respiratory function of haemocyanin has been disputed as the animals seem to survive for long periods without it. Wieser (1965a) has reported that the haemocyanin levels of some isopods and amphipods decrease with starvation and this led him to postulate that haemocyanin acts as an organic store as well as having a respiratory function. Uglow (1969a, 1969b) using fluid film electrophoresis was able to show the presence of two haemocyanins (a "fast" and a "slow") and an apohaemocyanin. The quantitative role of each suggested a different physiological role, the "fast" Hcy, showing little monthly variation, was probably respiratory in function while the "slow" Hcy and the apohaemocyanin acted as organic stores.

More recently Spock (1974) has shown that _Homarus gammarus_ is able to maintain resting metabolism in the absence of Hcy but that the scope for activity is seriously limited by its absence. McMahon & Wilkens (1975) and Taylor (1976) have shown that, in _Homarus americanus_ and _Carcinus maenas_ in air saturated water, only about 25% of the oxygen delivered to the tissues is carried combined to the haemocyanin the rest being carried in solution. Although haemocyanin concentrations were not measured their values of oxygen capacity compare well with the figures of other authors.

1.A.ii.c. The Exchange of Oxygen and Carbon Dioxide with the
         Tissues.

To date there have been no studies of gaseous exchange between the blood and the tissues in the Crustacea. Presumably this exchange will rely on gradients of pO_2 and pCO_2 between the blood and tissues just as it does at the gills.

It is evident from this review that there has been considerable recent interest in the Crustacea, particularly the Decapoda. Much of the new information casts doubt on conclusions reached from the older work and has forced a re-appraisal of the respiratory physiology of decapods (e.g., Jones, 1972).

The present work was undertaken to investigate some aspects of the respiratory physiology of the common edible crab _Cancer pagurus_, collected from Scottish west coast inshore waters applying techniques which have given valuable data in other crustacean studies (e.g., Johansen _et al._, 1970; Taylor, 1976) partly as a preliminary to applying such techniques to comparative studies of other large decapods in the area. In particular attention has been directed towards investigating rhythmic changes in respiratory activity including those involved in a short period of heart and scaphognathite activity recently reported to occur during the resting phase of the daily cycle (Ansell, 1973).

SECTION TWO

MORPHOMETRIC RELATIONSHIPS

2.A. Introduction

In all studies of respiratory function it is necessary to relate the rates of oxygen consumption measured to some measure of body size of the individual organisms involved. In studies in the literature carapace length, total length, carapace width, fresh weight, dry weight and ash-free dry weight have all been used as such a basis. As a preliminary to the respiratory studies, therefore, the relationships between these measures of body size for the crab Cancer pagurus were investigated.

2.B. Methods

Specimens of the edible crab, Cancer pagurus L. (Decapoda: Brachyura) were caught sub-littoraly by aqua-lung diving within a three mile radius of the laboratory at Dunstaffnage, Oban. The majority came from shelly-gravel or sandy ground.

In the laboratory, sex, maximum carapace width, and length were recorded. The crabs were then suspended anterior end down until all free water had drained from the branchial chamber. They were then weighed to the nearest gram.

Crabs to be used for respiratory analysis were held in large polystyrene or fibre-glass tanks supplied with continuously renewed sea-water. Other crabs were killed, dried to constant weight at $80^\circ C$ (about six days) and then ground to a fine powder in a large mortar and a ball mill. A small sub-sample (c. 5g) was then taken and ashed overnight at $520^\circ C$.

In a few cases the amount of calcium carbonate in the dried crab was estimated by digesting a known quantity (c. 1g) of the crab powder in excess 1 N HCl for several hours on a hot-plate. The remaining acid was estimated by back titration with 0.1 N NaOH using phenolphthalein as indicator and calculating the carbonate as calcium carbonate (1 ml N HCl 0.050 g Ca CO_3).

The relationships between width, fresh weight, dry weight, ash-free dry weight and length were investigated by computing regression lines by the method of least squares (Snedecor & Cochran, 1968). The relationships between the various measurements of weight and length are best described by a power function:

$$W = a L^b$$

This was linearised by taking logarithms:

$$\log W = \log a + b \log L$$

where W = weight

    L = length

    a = intercept on Y axis

    b = slope of line.

In order that variability between crabs was reduced as much as possible only crabs in the hard C_4 moult stage (Drach, 1939) were used throughout this study. As the dry weight of a crab varies considerably with the moulting stage (Drach, 1939) a t test was used to compare all data points on the log dry weight vs. log length line to the calculated line (Snedecor & Cochran, 1968) and those points which showed a significant difference at the 95% level were eliminated from the analysis.

The slopes for males and females were compared against each other for each measurement of weight by a d test (Bailey, 1969) and also compared to a standard value of 3 by a t test (Snedecor & Cochran, 1968). The significance of each regression line was also calculated ($t = {}^b/sb$ Snedecor & Cochran, 1968). A covariance test could not be used in this case because a variance ratio test (F test) gave a significant result indicating heterogeneous variance between pairs of lines.

2.C. Results

The results of the regression analyses are shown in Figures 1 to 3 and Table 4. The results of the d test for significant difference between males and females showed that for log fresh weight against log length the difference in slope was significant (d = 6.36, P < 0.01) and for log ash-free dry weight against log length the difference was just significant (d = 1.96, P = 0.05) but there was no significant difference in dry weight lines (d = 1.28, P < 0.05). Inspection of the figures shows that the variance about the line was very small for fresh weight but greater for dry weight and ash-free dry weight.

In each case it can be seen that the slope of the lines was found to be approximately three. This reflects the common cubic relationship between length and body volume (and thus mass). The results of the t test (Table 5) comparing these slopes to the theoretical value of three indicated that the slope was significantly different only for log fresh weight and log ash-free dry weight for males (t = 9.0, P < 0.001 & t = 2.05, 0.02< P < 0.05 respectively).

All of the regression lines linking log weight and log length were highly significant (Table 4). Those linking % dry weight and % ash-free dry weight to length were not significantly

Figure 1.

Scatter diagram of fresh weight (g.) against
carapace length (mm.) with fitted regression
lines. Double log axes.

Δ = $\male$

+ = $\female$

log FW = 3.216 log L — 3.574

log FW = 2.938 log L — 3.113

FRESH WEIGHT
grams

♀ — ♂
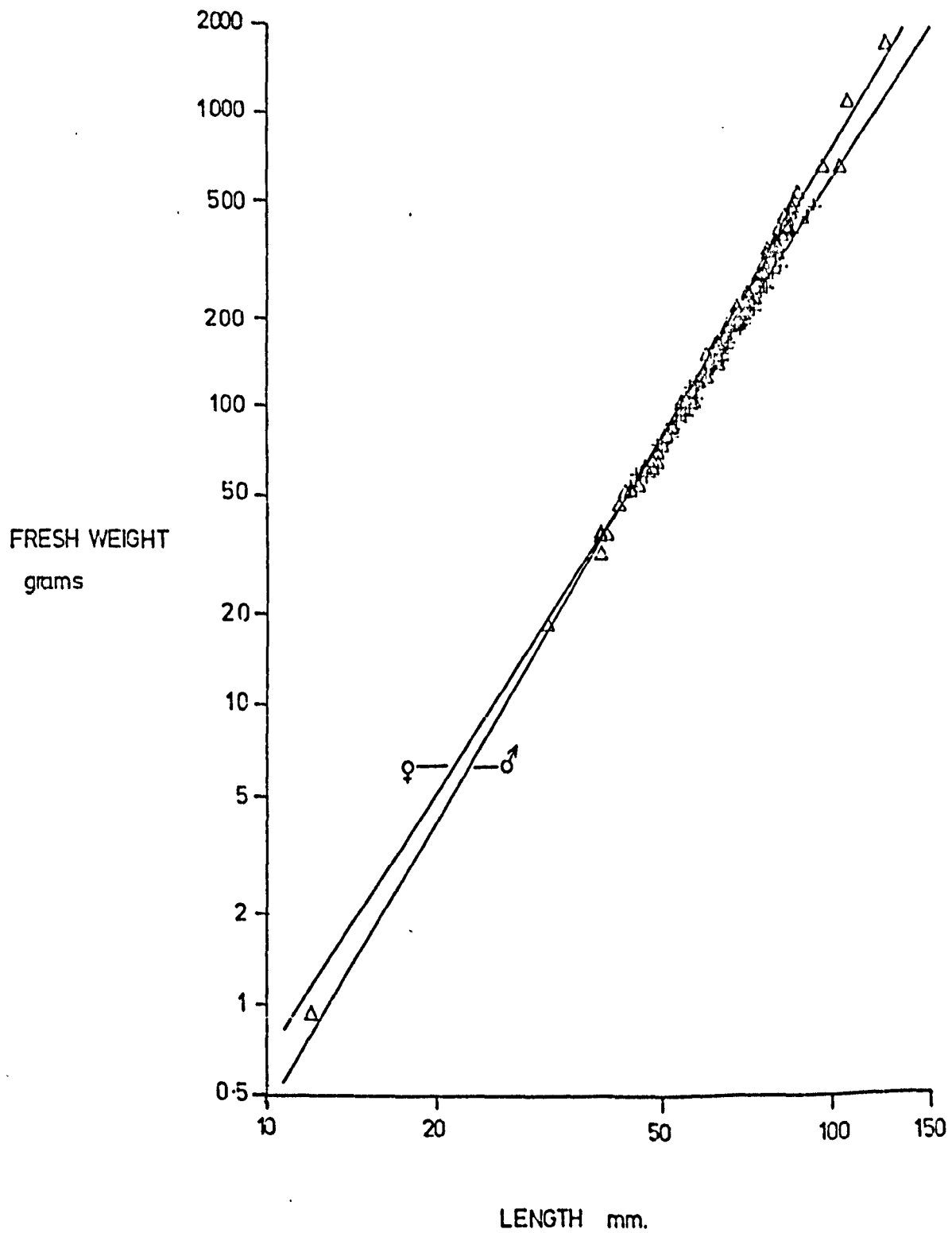
LENGTH mm.

Figure 2.

Scatter diagram of dry weight (g.) against carapace

length (mm.) with fitted regression lines.

Double log axes.

$\Delta = \male$

$+ = \female$

log DW = 3.179 log L − 3.973

log DW = 2.934 log L − 3.572

DRY WEIGHT grams.

LENGTH mm.

$\Delta = \delta$

$+ = \female$
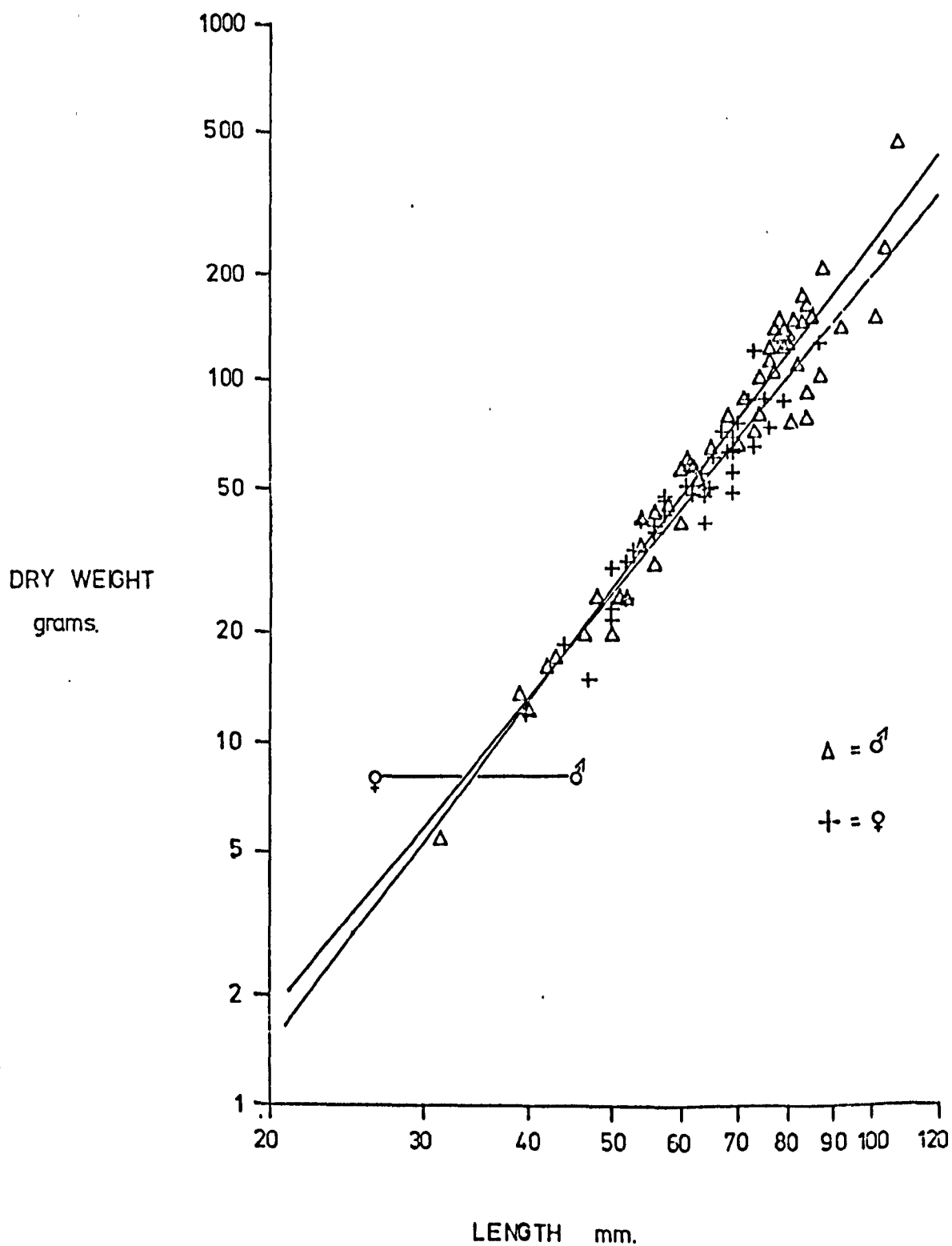
Figure 3.

Scatter diagram of ash-free dry weight (g.) against
carapace length (mm.) with fitted regression lines.
Double log axes.

Δ = ♂

+ = ♀

log AFDW = 3.375 log L − 4.687

log AFDW = 2.819 log L − 3.700
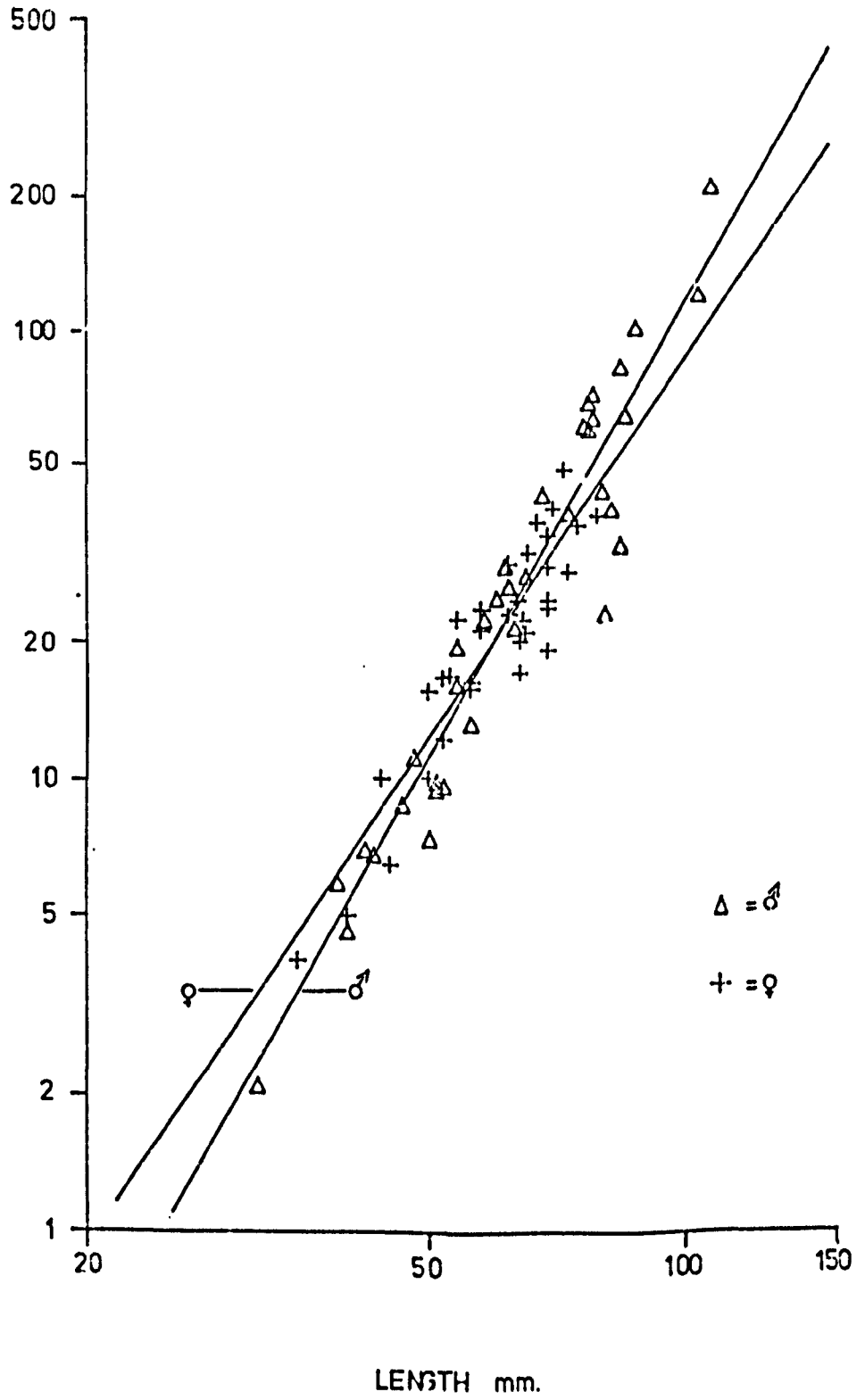
Table 4.

Showing the equations relating width (mm.) and body
weights (g.) to carapace length (mm.) derived from
regression analysis.

L   =   carapace length

FW   =   fresh weight

DW   =   dry weight

AFDW   =   ash-free weight

Sb   =   sample standard deviation of slope

R   =   correlation coefficient

N   =   number of points

Sex	Y	=	b.	X	±	a	Sb	R	N
♂	Width	=	1.675	length	−	4.150	0.010	0.998	97
♀	Width	=	1.587	length	−	0.669	0.027	0.990	74
♂	log FW	=	3.126	log L	−	3.574	0.024	0.997	97
♀	log FW	=	2.938	log L	−	3.113	0.036	0.994	74
♂L<70	log FW	=	3.107	log L	−	3.393	0.107	0.980	44
♂L>70	log FW	=	3.427	log L	−	3.971	0.029	0.998	53
♂	log DW	=	3.179	log L	−	3.973	0.112	0.968	97
♀	log DW	=	2.934	log L	−	3.572	0.155	0.951	74
♂	log AFDW	=	3.375	log L	−	4.687	0.183	0.952	97
♀	log AFDW	=	2.819	log L	−	3.700	0.217	0.917	74

Table 5.

Showing the results of the t test to compare the slope of the observed regression lines of body weight and length against a theoretical value of three.

line	slope	sb	t	dF	P	
♂ FW	3.216	0.024	9.00	95	> 99.9%	sig.
♀ FW	2.938	0.036	1.72	72	< 95%	n.s.
♂ DW	3.179	0.112	1.60	53	< 90%	n.s.
♀ DW	2.934	0.155	0.46	38	< 50%	n.s.
♂ AFDW	3.375	0.183	2.05	35	> 95%	sig.
♀ AFDW	2.819	0.217	0.834	32	< 60%	n.s.

$$t = \frac{b - \beta}{sb}$$

where b = slope of line

β = test slope (=3)

sb = standard error of line

different from zero at the 95% level. There was thus no
detectable dependence of % dry weight and % carbon on length.

The results of the analyses of calcium carbonate content
of dry powdered crab gave a mean value of 61.4% Ca CO_3
(s = 4.5, n = 20). There was no significant difference between
males and females (t = 0.64, P > 0.1). The mean value found for
% dry weight was 34.97% (s = 4.44, n = 85) and for % carbon 45.7%
(s = 5.73, n = 73). There was no significant difference between
males and females in either case (t = 0.66 for % DW and 0.65 for
% C).

2.D. Discussion

2.D.i. The relationship between weight and length

A value of three for the slope of log weight against log
length describes isometric growth. This is characterised by
an animal having an unchanging body form and unchanging specific
gravity throughout its life. Regression values of more than or
less than three characterise positive and negative allometric
growth respectively. That is if b is greater than three it
becomes "heavier for its length" as it gets longer and if b
is less than three it becomes "lighter for its length" as it
gets longer (Tesch, 1971).

Values of around three have been reported for the relationship between length and body weight for several species (Olmstead & Baumberger, 1923; Kubo et al., 1959; Heath & Barnes, 1970; Ahsanullah & Newell, 1971; Marsden et al., 1973; Bennett, 1974; Breteler, 1975) and for many fish species (e.g., Le Cren, 1951; Mann, 1976). Lasker et al. (1970) found that an equation of the form log w = bL-a best fitted their data on the growth of the harpacticoid Asellopsis intermedia. Mauchline (1967) found an average value of 2.59 for the slope of the line relating log body volume to log body length in several species of the Euphausiacea.

In this study only two lines are significantly different from three in slope; these are male log fresh weight against log length and males log ash-free dry weight against log length, the value of b being greater than three in both cases, i.e., positive allometry.

MacKay (1943b) has shown for Cancer pagurus and Hartnoll (1963, 1972, 1974) for several species of crabs that for male crabs the chelae show marked positive allometric growth after the puberty moult while the female chelae continue to grow more or less isometrically. This will result in male crabs having a larger weight for their length than female crabs after the puberty moult. If the lines for log fresh weight against

log length are inspected it can be seen that at length greater
than about 70 mm there is a tendency for the points for the
males to deviate from the fitted line and form a slightly
steeper slope. Crabs longer than 90 mm long are rare in the
Dunstaffnage area. As a result there are insufficient points
to show clearly whether this inflexion represents a transition
zone to a parallel line with greater elevation (i.e., growth
of the chelae returns to isometry) or whether the higher growth
rate is maintained. This change in fresh weight after the
puberty moult should also be reflected in the graphs of log
dry weight and log ash-free dry weight against length and,
although the log ash-free dry weight against log length is
greater than three it does not show a visible inflexion.
Any effect of the above nature is masked by the greater variance
about the line in both of these cases.

Regression lines for the males less than 70 mm long and
for those greater than 70 mm long have been calculated separately
(Table 4, Fig. 4). It can be seen that the smaller crabs have
a slope (3.107) which is much less than that for the larger
males (3.427). The variance of these lines is $s^2 = 6.25 \times 10^{-4}$
and 1.12×10^{-3} respectively. Both lines have been tested with
a d test as before against the line of log fresh weight against
log length for the females. For the larger crabs d = 4.33,

Figure 4.

Scatter diagram of fresh weight (g.) against carapace

length (mm.) for males only.

Double log axes.

Regression lines fitted for carapace length greater

than 70 mm. and less than 70 mm. separately.

$L > 70$ $\log FW = 3.427 \log L - 3.971$

$L < 70$ $\log FW = 3.107 \log L - 3.393$

These two lines cross at a length of 64.0 mm.

51b
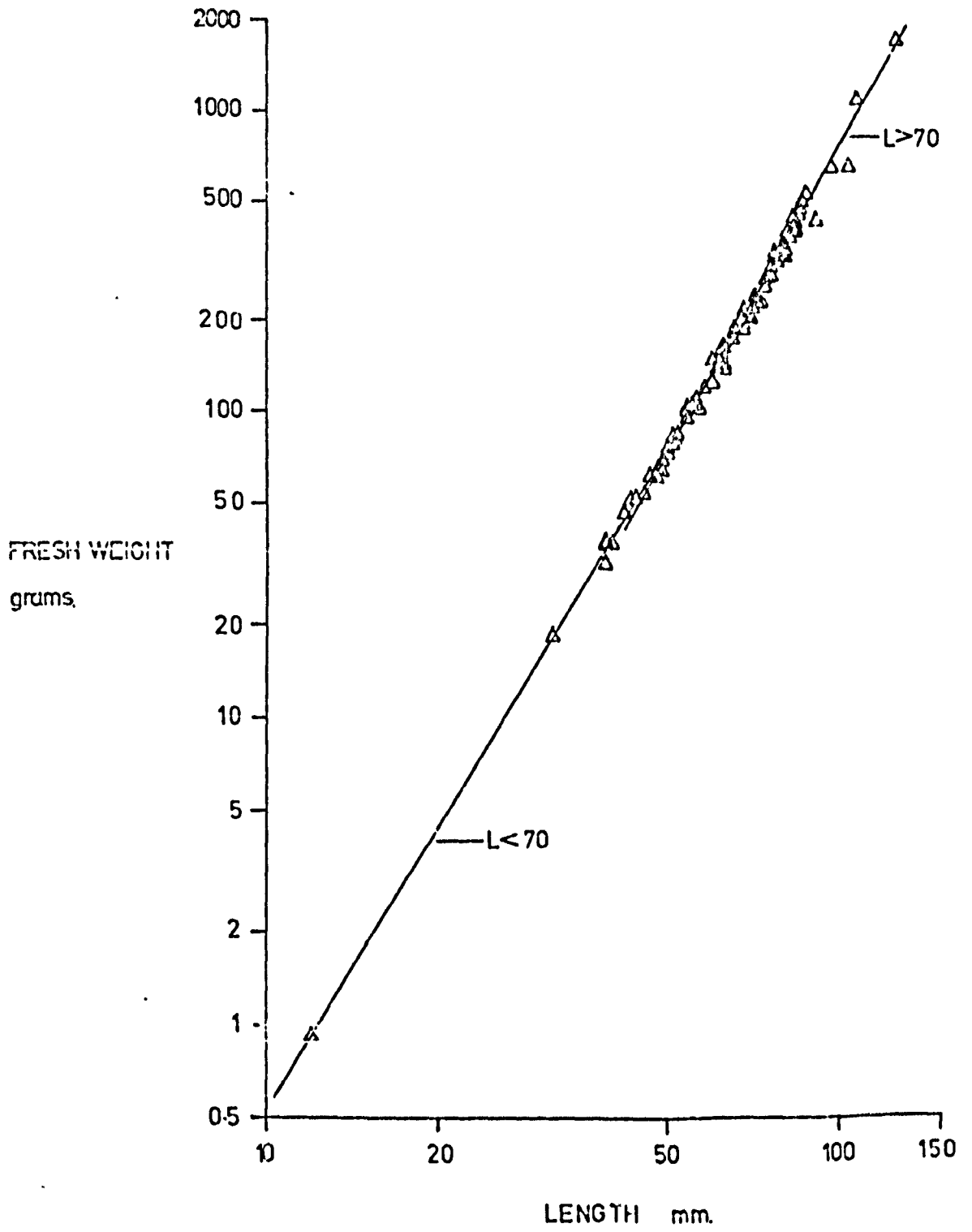
p < 0.001 and for the smaller crabs d = 3.63, P < 0.001, thus both lines are significantly different from the female line. These findings support the idea that there is an inflexion of the growth curve of the males and also there is a difference of growth of fresh weight with respect to length in males and females before puberty. A lack of large post-puberty crabs in the Dunstaffnage population has precluded a full analysis of the growth patterns. Orton (1936) found that Cancer pagurus attained maturity at a carapace length of 6.7 to 8.3 cm. MacKay (1943a, 1943b) found for the same species that maturity occurred at a length of between 5.9 and 7.4 cm. These figures overlap the point of 70 mm chosen by eye from the growth curve of Cancer in this study.

In general, then, it may be said that, for Cancer pagurus in this locality, weight increases in an isometric manner with respect to length until a size of about 70 mm in length is reached when growth becomes allometric for males but remains isometric for females.

53

2.D.ii. Fresh weight, dry weight and ash-free dry weight as
a basis for comparisons in metabolic studies.

A noticeable feature of the regression lines in Figures
1 - 3 is that the variances about the lines are different for
each measurement of weight. It can be seen that the variance
is the least for log fresh weight ($s^2 = 1.02 \times 10^{-3}$ ♂,
$s^2 = 4.84 \times 10^{-4}$ ♀) and the greatest for log ash-free dry
weight ($s^2 = 1.9 \times 10^{-2}$ ♂, and 1.02×10^{-2} ♀) with those for
log dry weight lying between ($s^2 = 9.22 \times 10^{-3}$ ♂ and
4.9×10^{-3} ♀). The fresh weight of an animal with a rigid
exoskeleton is dependent largely upon its volume and thus
the amount of tissue that it will hold. If any variation in
the amount of tissue does take place because of starvation or
changes of a seasonal nature, the spaces left by tissue loss
would be occupied by water or haemolymph with little resulting
change in density and thus overall weight. In order to test
this several crabs were starved in tanks for periods of up to
six months and weighed at intervals. In no case was any change
in fresh weight found.

Several workers have used dry weight as a better indicator
of respiring tissue in crabs (e.g., Newell et al., 1972;
Marsden et al., 1973). This measure obviously overcomes the
difficulty of a fluctuating water content but has its own

associated problems. Marsden et al. (1973) starved a group of Carcinus maenas for periods of up to three weeks and then compared their dry weights to those of a group which had been fed for the same period. The regression lines of log dry weight on log length showed no significant difference for starved and fed animals when tested by co-variance analysis.

Analysis of the data on body composition shows that of the fresh weight some 65% is water, of the remainder 45% is combustible organic material. Acid analysis of the dried material gave a mean value of 60% for $Ca\ CO_3$ so that agreement between the two methods of estimating the amount of respiring tissue is reasonable.

It can be seen then, that with the skeletal material forming some 55% of the dry weight a large change in the amount of organic tissue present must occur before differences between fed and starved populations of crabs can be detected statistically bearing in mind the large natural variance of the weights. This will be especially true in a crab such as Cancer pagurus which has a much thicker, more heavily calcified skeleton than Carcinus.

Although the skeletal material does form a large non-respiring part of the dry weight it still forms part of the metabolic load of the animal in that it has to be synthesised

and physically supported and carried around during locomotion. There is no evidence from present data that the skeleton grows allometrically with size and it will thus form a constant ratio to the amount of flesh. Wallace (1972) states that large crabs receive proportionately less support from the medium than do small crabs due to their relatively smaller surface area for their weight. This, however, is not the case. The support from the medium does not depend on surface area but on the relative densities of the animal and the medium: i.e.,

$$\text{Support} = V \left(\rho a - \rho m \right)$$

where V = volume of the animal

ρa = density of the animal

ρm = density of the medium.

Thus, provided that neither the density nor the volume of the animal changes with respect to weight as it grows all animals will receive the same proportional support. This effect, thus, has no differential effect on the active respiration rates of animals of different sizes as Wallace (1972) argues, even if it is assumed that all active rates are representative of muscular activity.

As it appears that the skeleton fraction does not grow allometrically with weight the most useful measure of weight to use for respiration studies is ash-free dry weight as it overcomes the problems of both fresh weight and dry weight. This measure has been used in crustacean work by Moshiri et al. (1970, 1971) and Breteler (1975).

SECTION THREE

RESPIRATION RATE AND ITS RELATIONSHIP TO BODY SIZE AND ACTIVITY

3.A. Introduction

Few authors have measured respiration rate in Cancer pagurus (Jolyet & Reynard, 1877; Ansell, 1973; Aldrich, 1975a, 1975b) and no data are available which allow an adequate prediction of the respiration rate of animals of different sizes. In order to fulfil this need and to act as the basis for further studies of respiratory dynamics the respiration rate of Cancer pagurus was determined in relation to sex, size and activity. As a large proportion of the animal consists of heavily calcified chitin and as the proportion of water changes considerably with the moult cycle (Drach, 1939) respiration rate was related to fresh weight, dry weight and ash-free dry weight.

3.B. Methods

Measurements of oxygen consumption were made in a manner essentially similar to that of Ansell, 1973. This method is a modification of the closed vessel system to allow long term monitoring of respiration rates. The apparatus is shown in Figure 5 . It consists of a large header tank holding sea-water which was vigorously aerated. Water in an equally large storage

Figure 5.

Diagramatic representation of the circulation
system supplying water to the respirometers.

A	=	aeration
CC	=	·cooling coil
CTT	=	constant temperature tank
HC	=	heating coil
HT	=	header tank
NRV	=	non-return valve
O_2E	=	oxygen electrode
P	=	pump
RC	=	respirometer chamber
SNRV	=	solenoid operated non-return valve
ST	=	storage tank

Lines with solid arrows represent direction of
circulation of water

A

HT

SNRV

NRV

O_2E

RC

P

HC   CC

ST

CTT
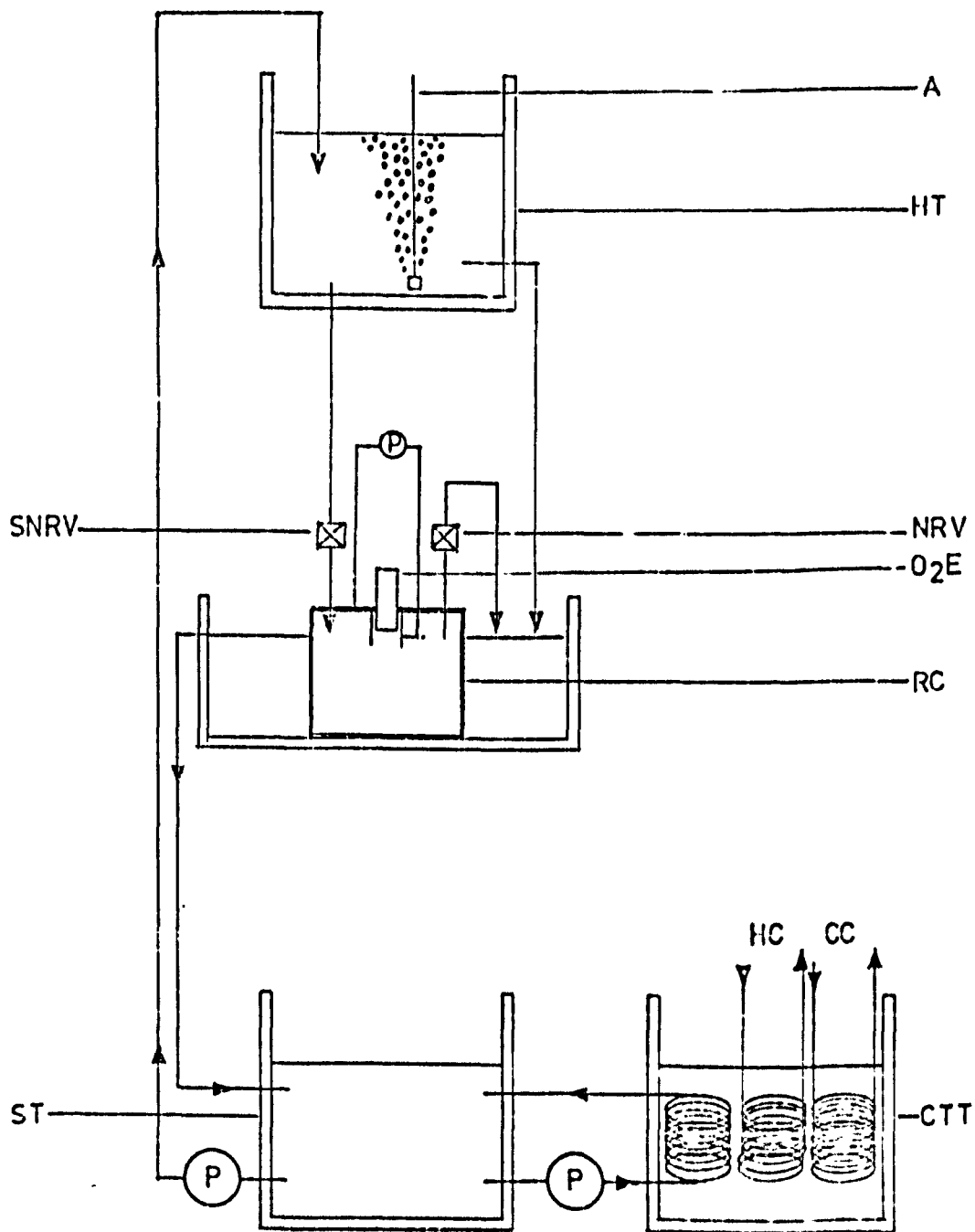
tank was pumped up to the header tank and allowed to return
by gravity through two intermediate tanks containing the
respirometers. Water from the storage tank was also pumped
through a long glass heat exchange coil immersed in a constant
temperature bath at $10^{\circ}C$ (Fig.5%). The whole experimental
set up was housed in an air conditioned room at $10^{\circ}C$ to
further stabilize the temperature. Water in the system was
renewed after each set of determinations were made. The room
was illuminated by natural light from a north facing window.

The respirometers consisted of circular perspex chambers
of several sizes from 500 ml to 20 l to cope adequately with
different sizes of animals. One of the respirometers was
made up of a series of tubes which could be bolted together in
different combinations to provide a larger range of volumes.
In general the size of respirometer was chosen so that at the
most active rates of respiration the oxygen tension of the
water was not reduced by more than c. 25% of saturation during
a 45 minute period (Stroganov, 1962). (Figure 6).

The lid of the chamber was fitted with ports to allow
inflow and outflow of water and to allow the connection of
electrodes implanted in the animal to recording equipment.
The lid also carried a housing for an oxygen electrode and
thermistor (Protech Advisory Services No 121C). Water in the

Figure 6.

Diagram showing the essential features of the
respirometer used for the determination of oxygen
consumption rate in <u>Cancer pagurus</u>.

FC   =   from circulation

NRV   =   non-return valve

O_2E =   oxygen electrode

P   =   pump

RC   =   respirometer chamber

SNRV   =   solenoid operated non-return valve

TC   =   to circulation

NRV

RC

TC

O₂E

P

FC

SNRV
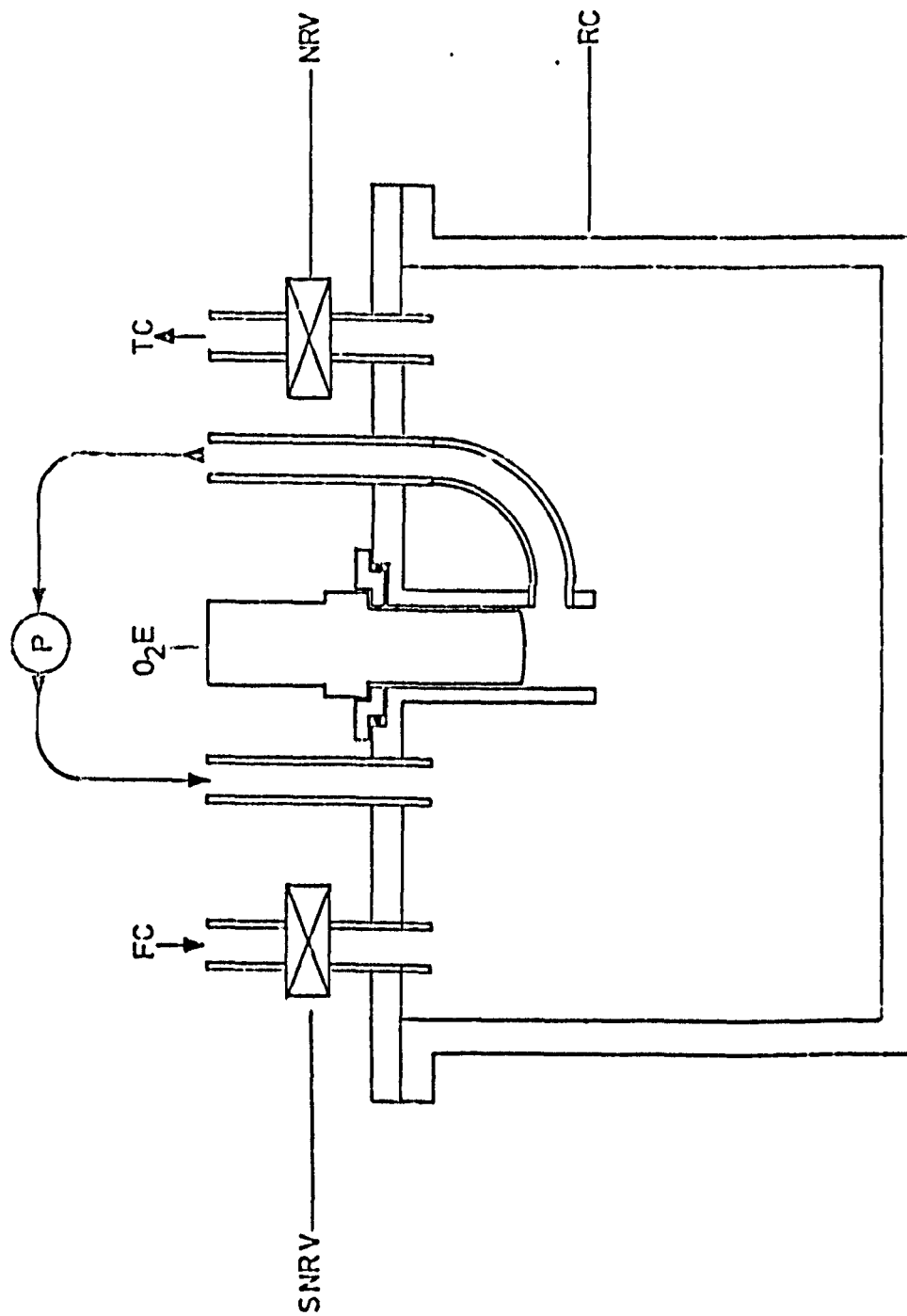
respirometer was continuously mixed and circulated past the electrode face by a small pump. The inflow to the respirometer was connected to the header tank and was controlled by a solenoid drain valve (Swiss Instruments type AV2E) controlled by a time switch. This switch held the solenoid valve open for a period of fifteen minutes allowing fully saturated water to flow through the respirometer thus restoring the oxygen tension, depleted by the previous 45 minutes of "closed system" operation, to full saturation. The output of the oxygen electrode was recorded, after suitable amplification, on a Leeds & Northrup flat bed potentiometric recorder. A typical trace and the method of extrapolation is shown in Figure 7.

In most cases the span and position controls of the recorder were adjusted so that the reading between 0 mm Hg O_2 and full saturation was 100 divisions on the chart. In this way the percentage change in PO_2 could be read directly from the chart line, extrapolated to one hour. The respiration rate was then calculated as:

$$\dot{V}O_2 = \frac{\Delta PO_2}{100} \cdot Vr \cdot a_{wO_2}$$

59a

Figure 7.

Showing a typical section of recording made during
the determination of oxygen consumption rate in
Cancer pagurus and the method of extrapolating the
record to obtain hourly consumption rates

Ps = % O_2 saturation at the start of
the period

Pe = % O_2 saturation at the end of
the period

ΔPO_2 = change in % oxygen saturation
over the hour period

TIME
hours
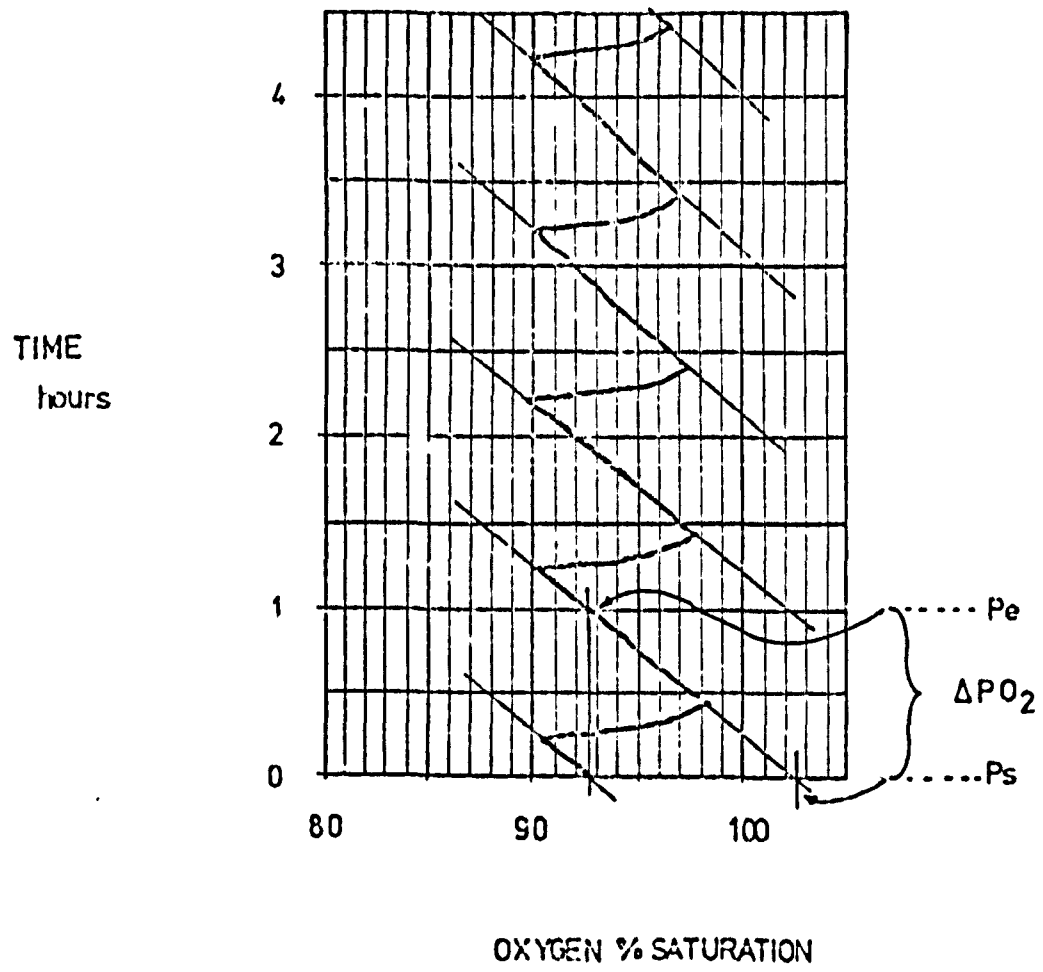
OXYGEN % SATURATION

where:

$$\dot{V}O_2 = \text{respiration rate. } mgO_2 \ hr^{-1}$$

$$\Delta PO_2 = \text{change in } \% \text{ saturation}$$

$$Vr = \text{respirometer volume in litres}$$

$$a_{wO_2} = \text{solubility of } O_2 \text{ in sea water. } mgO_2 \ l^{-1}$$

A check was made on the electrode calibrations and recorder span at frequent intervals. Several control experiments were made with no animals in the chamber. Before determinations were made crabs were kept in a tank at 10^oC to acclimate them to the working temperature. Time marks at hourly intervals were added to the trace by shorting the input to the recorder briefly with a time switch. These were synchronised to occur exactly on the hour. Sutcliffe, Carrick & Moore (1975) have used a similar respirometer, developed independently, for work on Austropotamobius pallipes.

As the volume of the crabs represented a significant proportion of the respirometer system in most cases, the volume of water used was determined for each crab separately, using a dye dilution technique. A five ml aliquot of $1 \ g \ l^{-1}$ aqueous solution of di-sulphine blue was injected into the respirometer after recording of respiration was finished. Fifteen minutes were allowed for complete mixing to occur and then three

replicate five ml samples were taken. The optical density

of the diluted dye solution was measured at 640 nm (peak

absorbance) on a Unicam SP200 spectrophotometer. The volume

of sea-water within the system was then calculated from a

previously prepared calibration curve and equation. Pilot

experiments had shown that no significant absorption of dye

took place by the crab over a period of six hours.

Estimates of the resting and active rates of oxygen

consumption (Beamish & Mookherji, 1964; Brett, 1964; Ansell,

1973) were made by examining the natural daily rhythm of

oxygen consumption found under the above conditions and taking

the lowest and highest values respectively. Single values

markedly different from the above values were ignored, as was

the period immediately after the introduction of the animal

into the respirometer. Measurements of size and weight, width,

length and fresh weight were determined before the animal was

placed in the respirometer, and dry weight and ash-free dry

weight after its removal by the methods described in the previous

section.

3.C. Results

3.C.i. The relationship between body size and respiration rate

Although measurements of oxygen consumption rate have been made on more than one hundred and twenty crabs at all times of the year the data are insufficient to allow an analysis of any seasonal changes which might occur in the relationship between body size and respiration rate. All data have therefore been pooled and analysed en bloc for each sex separately. As three measures of weight viz. fresh weight, dry weight and ash-free dry weight, were available for each crab, regression lines were calculated for each using the method of least squares. The relationships thus calculated are recorded in Table 6 and illustrated in Figures 8, 9 and 10. In these Figures, and in all others, the regression lines have been drawn to cover the range of sizes of animals used. No extrapolations from the data have been made.

Analysis of covariance (Snedecor & Cochran, 1968) was carried out to test for significant difference in slope and elevation between lines. In this case variance about the lines was homogenous so the test could be employed validly. Where no significant difference in slope was found at the 95% level a common slope was calculated. If no significant difference in slope and intercept was found at the 95% level a line of common

Table 6.

Showing the equations relating oxygen consumption rate $(mg.O_2 \ hr.^{-1})$ to body weight (g.) derived from regression analysis.

R   =   resting rates

A   =   active rates

FW   =   fresh weight

DW   =   dry weight

AFDW   =   ash-free dry weight

Sb   =   sample standard deviation of slope

R   =   correlation coefficient

N   =   number of points

Sex	Y	=	b.	X	±	a	Sb	R	N
♂	$\log \dot{V}O_2R$	=	0.869	log FW	−	1.201	0.052	0.91	62
♀	$\log \dot{V}O_2R$	=	0.692	log FW	−	0.797	0.054	0.850	65
♂	$\log \dot{V}O_2A$	=	0.921	log FW	−	0.769	0.069	0.869	62
♀	$\log \dot{V}O_2A$	=	0.654	log FW	−	0.139	0.066	0.779	65
♂	$\log \dot{V}O_2A$	=	0.850	log DW	−	0.726	0.050	0.907	62
♀	$\log \dot{V}O_2R$	=	0.745	log DW	−	0.548	0.058	0.849	65
♂	$\log \dot{V}O_2A$	=	0.896	log DW	−	0.288	0.067	0.866	62
♀	$\log \dot{V}O_2A$	=	0.707	log DW	+	0.091	0.072	0.779	65
♂	$\log \dot{V}O_2R$	=	0.801	log AFDW	−	0.380	0.047	0.909	62
♀	$\log \dot{V}O_2R$	=	0.779	log AFDW	−	0.345	0.061	0.851	65
♂	$\log \dot{V}O_2A$	=	0.844	log AFDW	+	0.109	0.063	0.866	62
♀	$\log \dot{V}O_2A$	=	0.735	log AFDW	+	0.288	0.075	0.779	65

Figure 8.

Scatter diagram of oxygen consumption rate $(mg.O_2 \ hr^{-1})$ against fresh weight (g.) with fitted regression lines. Double log axes.

Δ = $\male R$ = resting rates for male crabs.

∇ = $\male A$ = active rates for male crabs.

$+$ = $\female R$ = resting rates for female crabs.

X = $\female A$ = active rates for female crabs.

$\log \dot{V}O_2 \male R$ = 0.869 - 1.201

$\log \dot{V}O_2 \female R$ = 0.692 - 0.797

$\log \dot{V}O_2 \male A$ = 0.921 - 0.769

$\log \dot{V}O_2 \female A$ = 0.654 - 0.139

OXYGEN
CONSUMPTION
RATE

$mgO_2\ hr^{-1}$

Legend:
Δ = ♂R
$\triangledown$ = ♂A
+ = ♀R
X = ♀A

FRESH WEIGHT

grams.
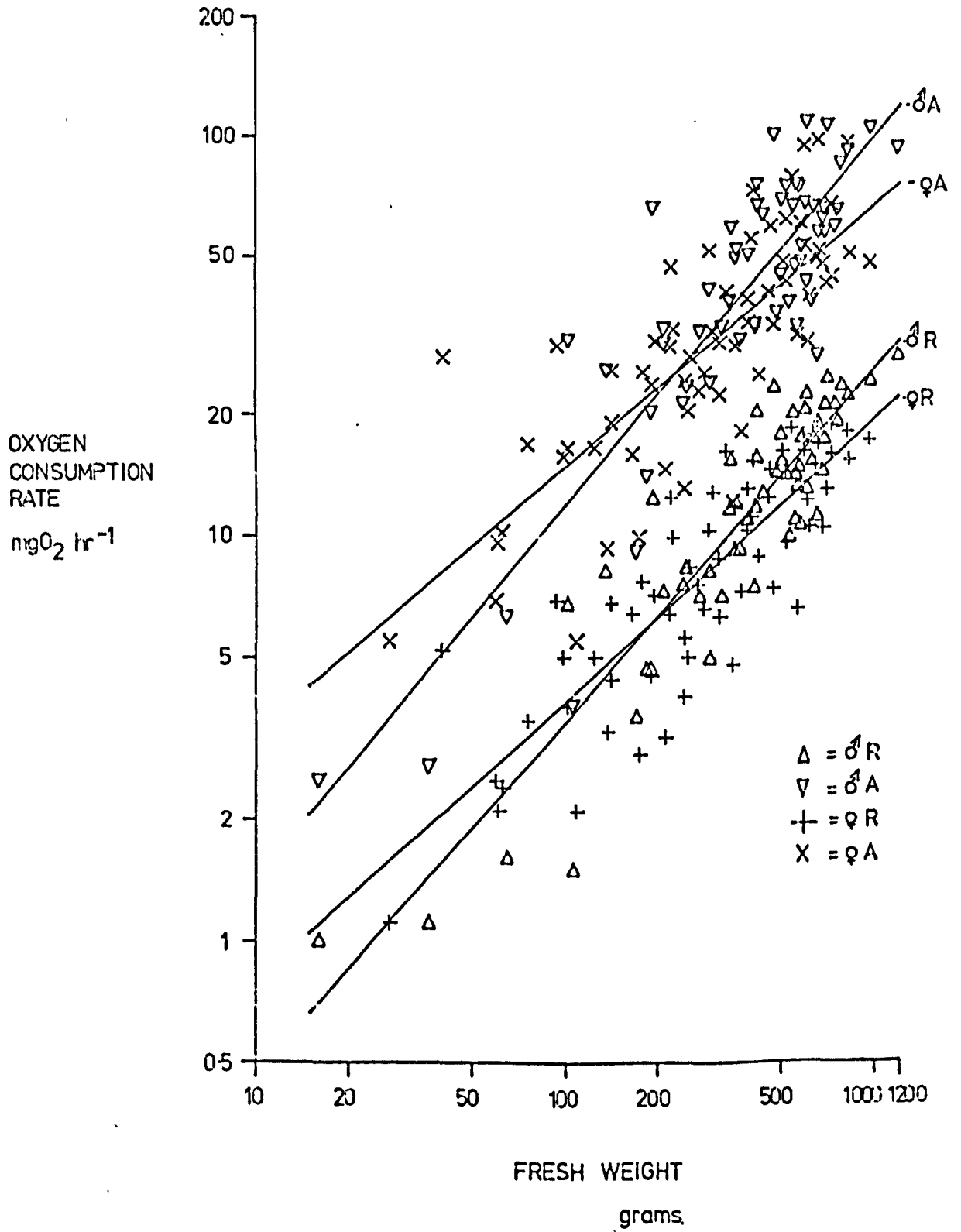
Figure 9.

Scatter diagram of oxygen consumption rate $(mg.O_2 \ hr^{-1})$
against dry weight (g.) with fitted regression lines.
Double log axes.

Δ = $\male R$ = resting rates for male crabs.

∇ = $\male A$ = active rates for male crabs.

$+$ = $\female R$ = resting rates for female crabs.

X = $\female A$ = active rates for female crabs.

$\log \dot{V}O_2 \ \male R$ = 0.850 log DW − 0.726

$\log \dot{V}O_2 \ \female R$ = 0.748 log DW − 0.548

$\log \dot{V}O_2 \ \male A$ = 0.896 log DW − 0.288

$\log \dot{V}O_2 \ \female A$ = 0.707 log DW + 0.091

Figure showing oxygen consumption rate versus dry weight. Axis labels: OXYGEN CONSUMPTION RATE, $mgO_2\,hr^{-1}$ (vertical); DRY WEIGHT, grams (horizontal).

Legend:
- Δ = ♂R
- V = ♂A
- + = ♀R
- X = ♀A

Curve labels: ♂A, ♀A, ♂R, ♀R
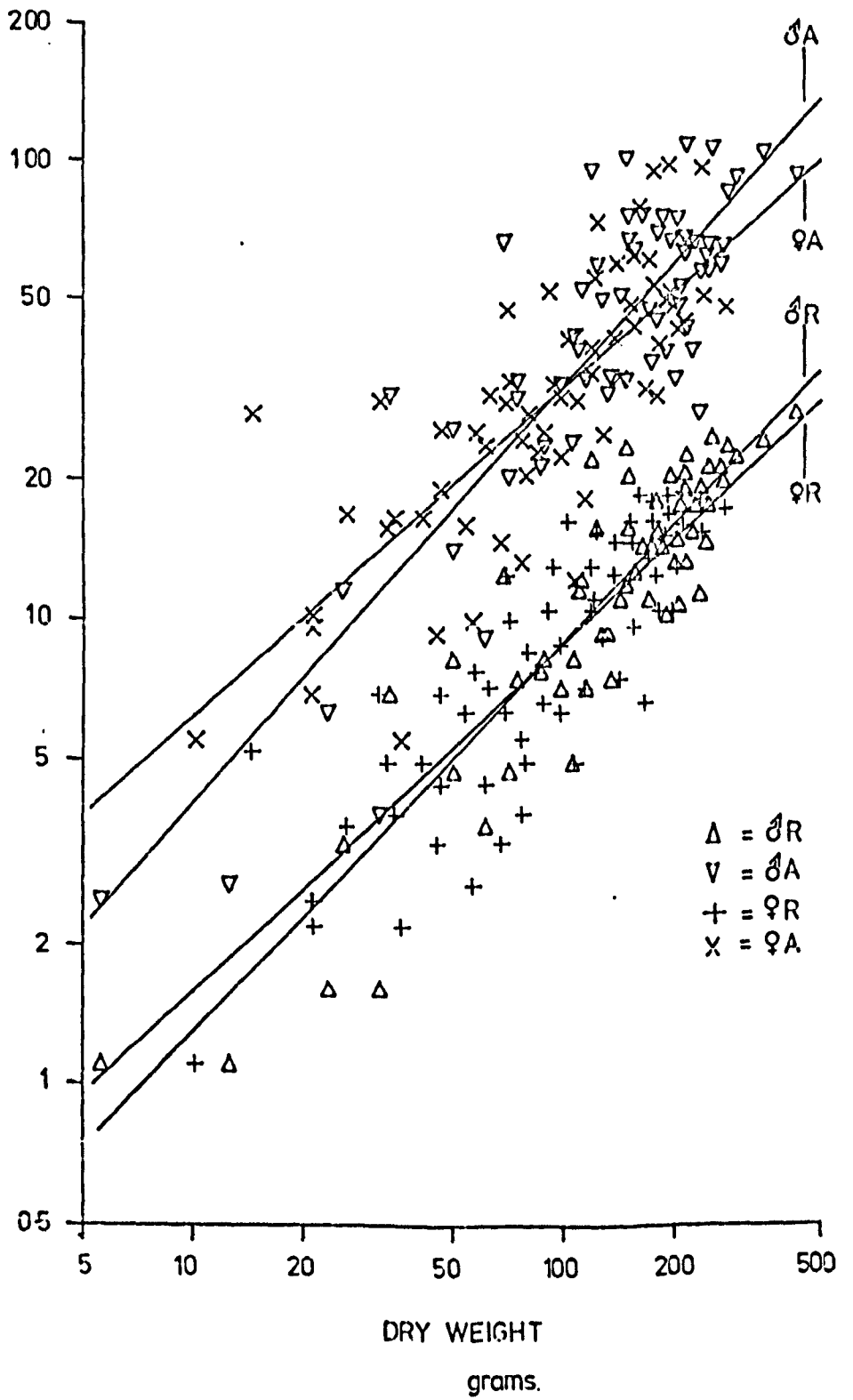
Figure 10.

Scatter diagram of oxygen consumption rate $(mg.O_2 \ hr.^{-1})$ against ash-free dry weight (g.) with fitted regression lines.

Double log axes.

Δ = $\male R$ = resting rates for male crabs.

∇ = $\male A$ = active rates for male crabs.

+ = $\female R$ = resting rates for female crabs.

$\times$ = $\female A$ = active rates for female crabs.

$\log \dot{V}O_2 \male R$ = 0.801 log AFDW - 0.380

$\log \dot{V}O_2 \female R$ = 0.779 log AFDW - 0.345

$\log \dot{V}O_2 \male A$ = 0.844 log AFDW + 0.109

$\log \dot{V}O_2 \female A$ = 0.735 log AFDW + 0.288

OXYGEN
CONSUMPTION
RATE

mgO_2hr^{-1}

ASH-FREE DRY WEIGHT

grams.
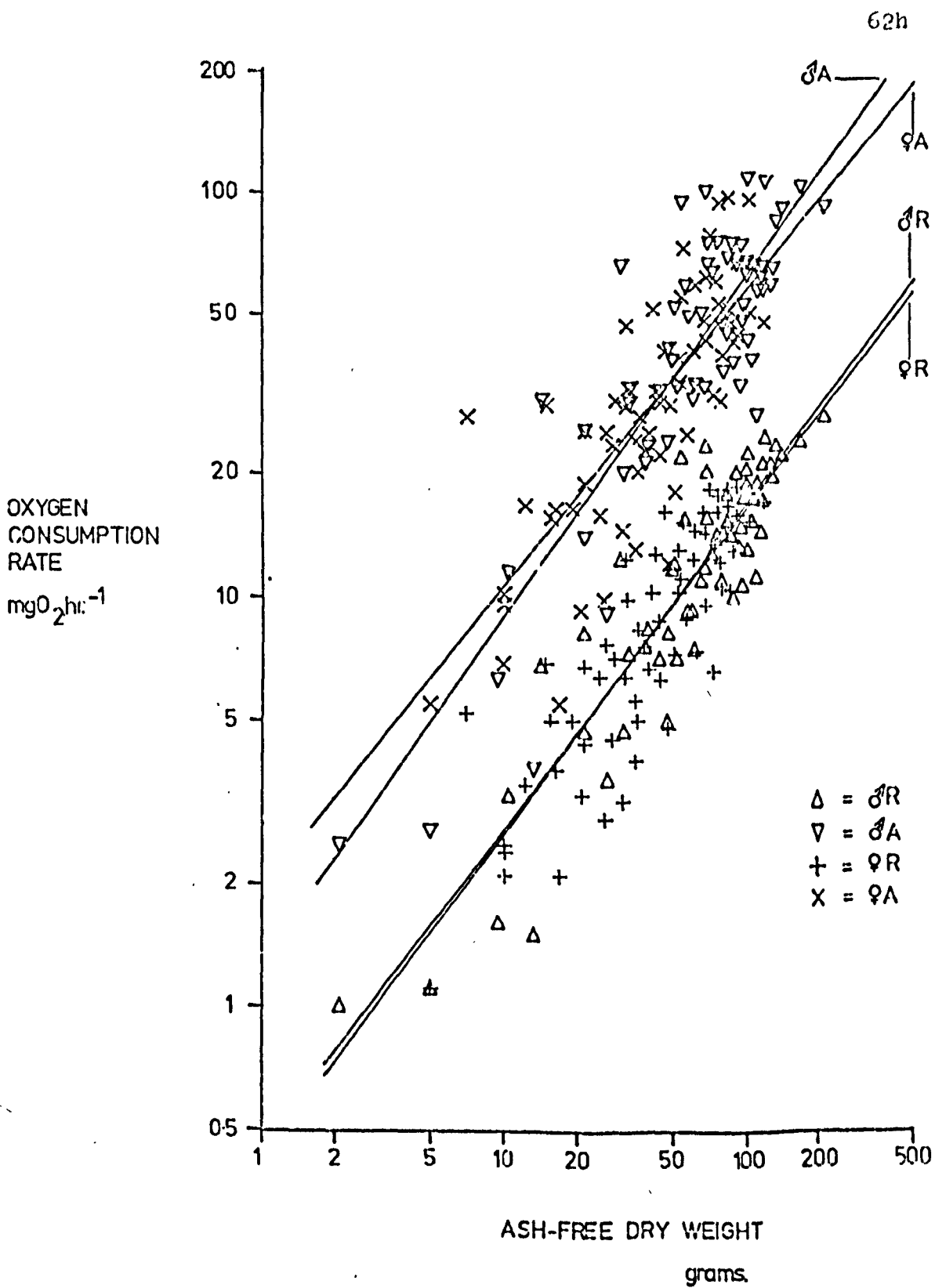
slope and intercept was calculated. Figures 11, 12 and 13 show the relationships thus derived for males and females for each of the measurements of body weight. The equations for these lines are given in Table 7 and the results of the covariance analysis are given in the appendix (Tables 14 to 16).

From these tables and figures several things are apparent. Firstly it can be seen that, in all cases, no significant difference in slope was found between resting and active lines within either sex within any weight measure at the 95% level. The lines for active and resting respiration rates are, thus, parallel throughout the range of weights examined. The ratio of active to resting metabolism is often called the scope for activity (e.g., Ansell, 1973; Prosser, 1973) and is defined, in this case, as the antilog of the difference in intercepts for active and resting rates, and gave a mean value of 3.7 for the scope for activity for males and females.

Secondly, although the lines were all parallel within males or females, there was a significant difference in slope between males and females for measurements based on fresh-weight. There was no significant difference in slopes for measurements based on either dry-weight or ash-free dry weight. It would thus appear that a difference may have existed between males and females if the comparisons were made on the basis of fresh weight.

Table 7.

Showing the equations relating oxygen consumption rate $(mg.O_2 \ hr.^{-1})$ to body weight $(g.)$ derived by analysis of covariance from the data in Figures 8, 9 and 10 and Table 6.

Sex	Y	=	b.	X	±	a
♂	$\log \dot{V}O_2 R$	=	0.895	log FW	−	1.267
♀	$\log \dot{V}O_2 R$	=	0.673	log FW	−	0.750
♂	$\log \dot{V}O_2 A$	=	0.895	log FW	−	0.702
♀	$\log \dot{V}O_2 A$	=	0.673	log FW	−	0.186
♂	$\log \dot{V}O_2 R$	=	0.804	log DW	−	0.661
♀	$\log \dot{V}O_2 R$	=	0.804	log DW	−	0.661
♂	$\log \dot{V}O_2 A$	=	0.804	log DW	+	0.201
♀	$\log \dot{V}O_2 A$	=	0.804	log DW	+	0.201
♂	$\log \dot{V}O_2 R$	=	0.795	log AFDW	−	0.371
♀	$\log \dot{V}O_2 R$	=	0.795	log AFDW	−	0.371
♂	$\log \dot{V}O_2 A$	=	0.795	log AFDW	+	0.197
♀	$\log \dot{V}O_2 A$	=	0.795	log AFDW	+	0.197

Figure 11.

Graph to illustrate the relationship between oxygen

consumption rate $(mg.O_2 \ hr.^{-1})$ and fresh weight $(g.)$

resulting from the analysis of covariance of the

data in figure 8.

Double log axes.

$\log \dot{V}O_2$ ♂R $=$ 0.895 log FW − 1.267

$\log \dot{V}O_2$ ♀R $=$ 0.673 log FW − 0.750

$\log \dot{V}O_2$ ♂A $=$ 0.895 log FW − 0.702

$\log \dot{V}O_2$ ♀A $=$ 0.673 log FW − 0.186
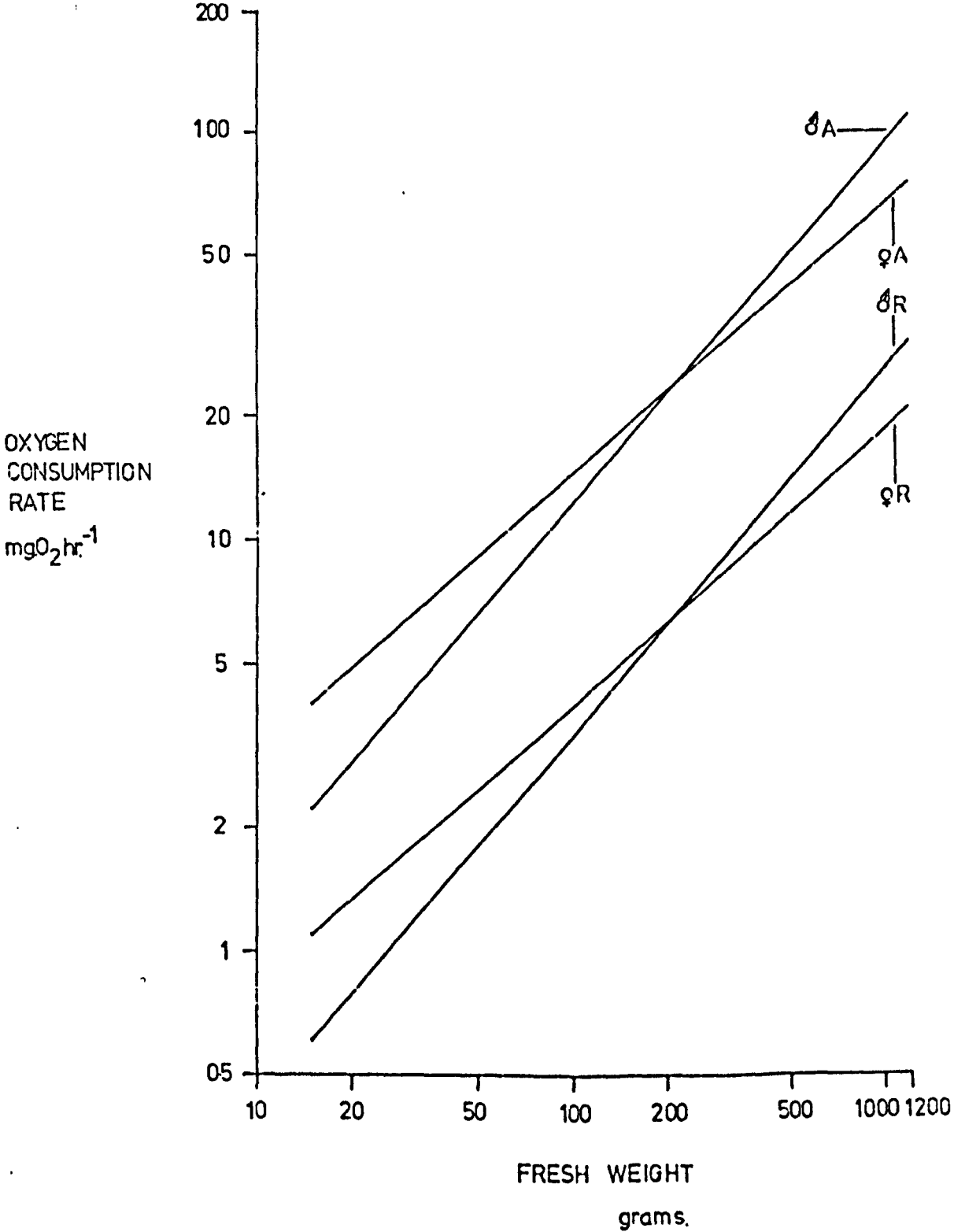
Figure 12.

Graph to illustrate the relationship between oxygen

consumption rate $(mg.O_2 \ hr.^{-1})$ and dry weight $(g.)$

resulting from the analysis of covariance of the

data in figure 9.

Double log axes.

$$\log \dot{V}O_2 \ \male R = 0.804 \log DW - 0.661$$

$$\log \dot{V}O_2 \ \female R = 0.804 \log DW - 0.661$$

$$\log \dot{V}O_2 \ \male A = 0.804 \log DW + 0.201$$

$$\log \dot{V}O_2 \ \female A = 0.804 \log DW + 0.201$$

200

100

50

20

OXYGEN
CONSUMPTION
RATE

$mgO_2.hr.^{-1}$

10

5

2

1

0·5

♂ and ♀ Λ

♂ and ♀ R

5   10   20   50   100   200   500

DRY WEIGHT

grams.
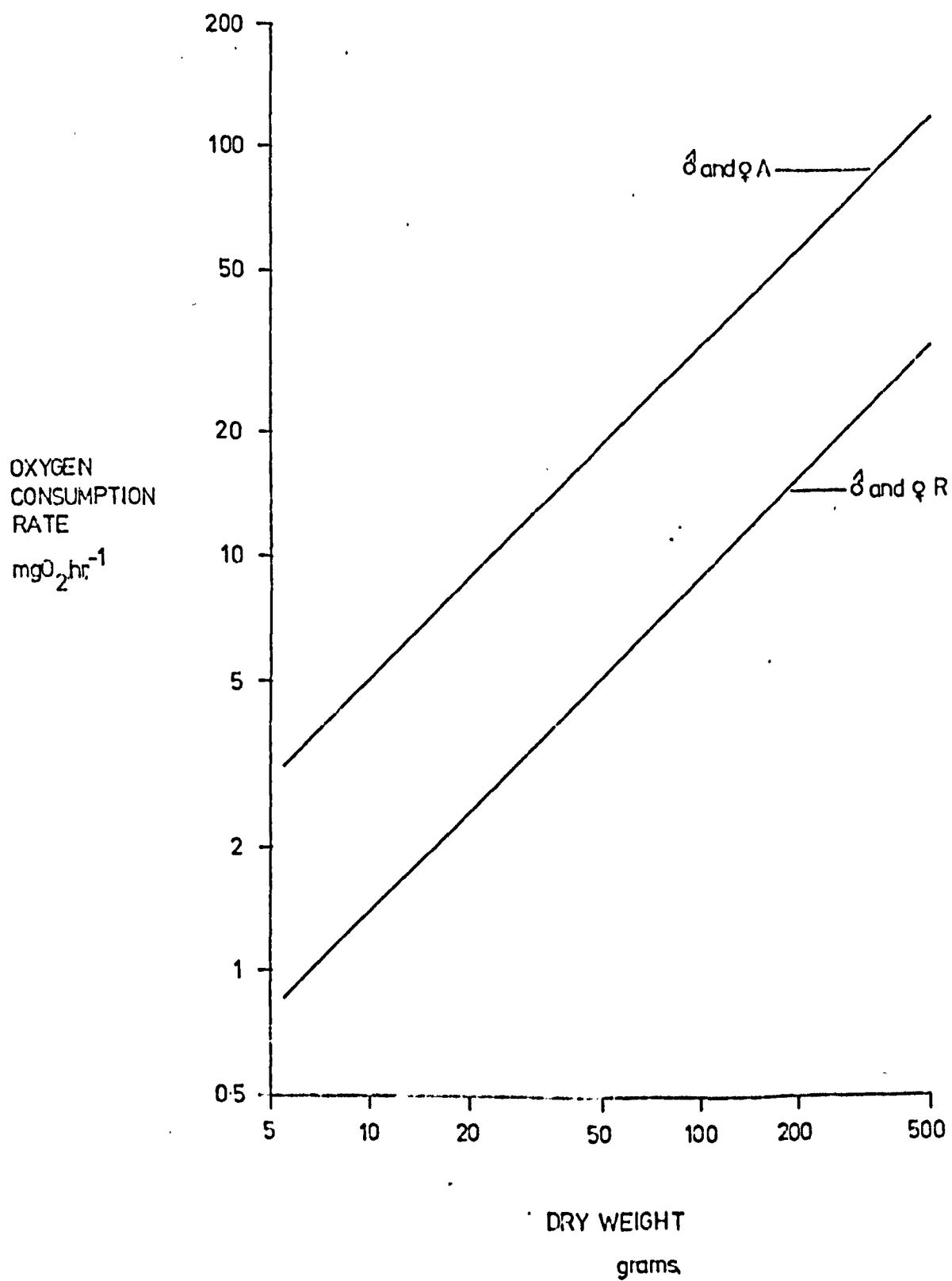
Figure 13.

Graph to illustrate the relationship between oxygen consumption rate ($mg.O_2$ $hr.^{-1}$) and ash-free dry weight (g.) resulting from the analysis of covariance of the data in figure 10.

Double log axes.

$$\log \overset{\bullet}{VO}_2 \, \male R = 0.795 \log AFDW - 0.371$$

$$\log \overset{\bullet}{VO}_2 \, \female R = 0.795 \log AFDW - 0.371$$

$$\log \overset{\bullet}{VO}_2 \, \male A = 0.795 \log AFDW + 0.197$$

$$\log \overset{\bullet}{VO}_2 \, \female A = 0.795 \log AFDW + 0.197$$

OXYGEN
CONSUMPTION
RATE

$mgO_2hr.^{-1}$

$\hat{\delta}$ and $\female$ A

$\hat{\delta}$ and $\female$ R

ASH-FREE DRY WEIGHT

grams.
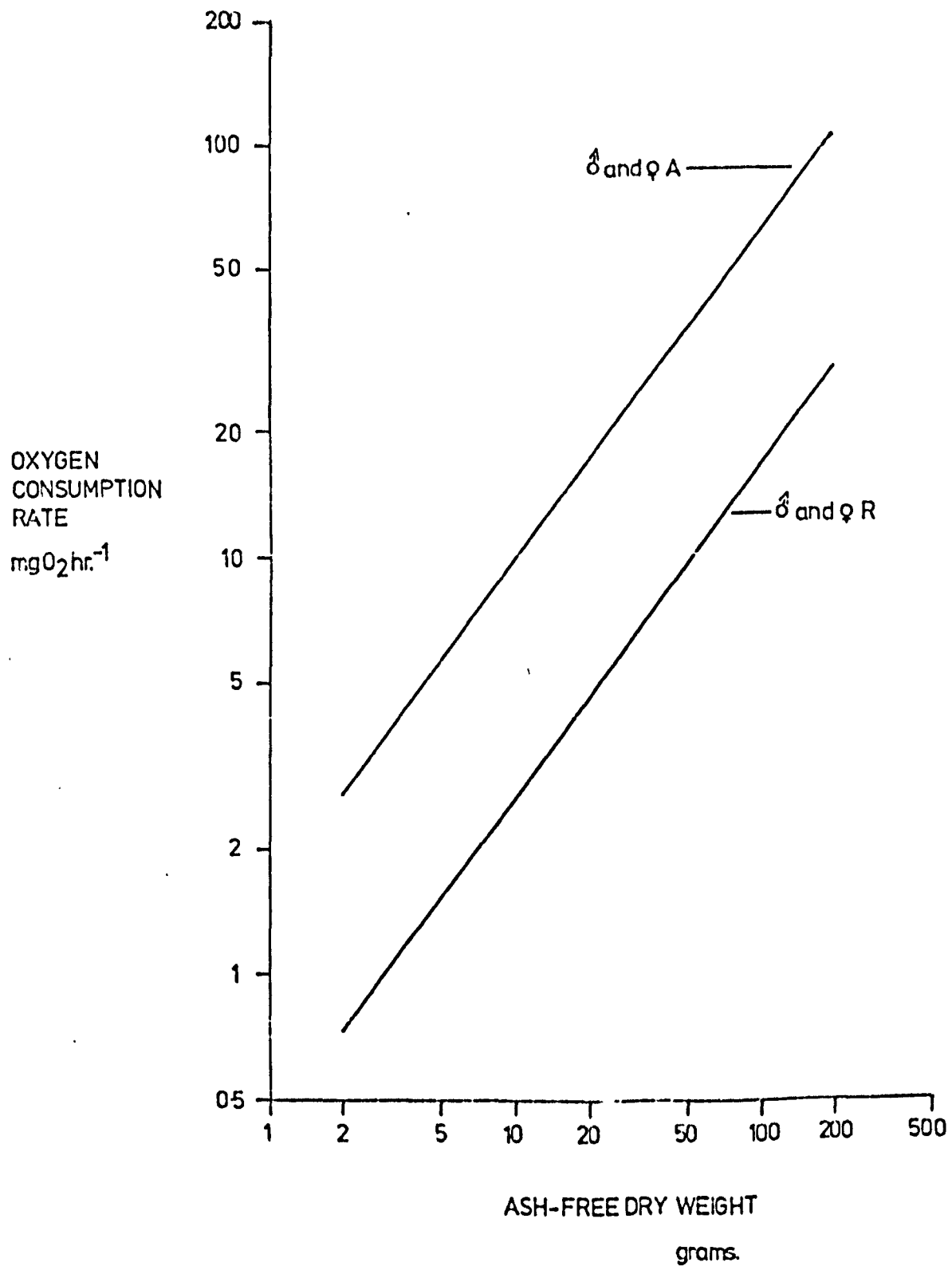
These differences were not apparent, however, if either dry weight or ash-free dry weight were used.

Thirdly, if these relationships are examined it can be seen that a choice of slopes is available for the relationship between oxygen consumption and weight depending, again, upon which measurement of body weight is used. The values of these slopes vary between 0.654 and 0.921. If Tables 14 to 16 showing the results of the covariance analysis are examined it can be seen that, within the males and within the females, there was no significant difference between slopes. When comparisons are made, however, between sexes it is found that, as well as the significant differences for fresh weight mentioned above, significant differences in slope between the lines for female-fresh weight-resting and male-dry weight-active. female-fresh weight-active and male-dry weight-resting, and female-fresh weight-active and male -dry weight-active were found. Because of these differences it would be misleading to calculate lines of common slope for each of the sexes separately as it would imply a difference between male and female rates for dry weight and ash-free dry weight which does not exist statistically. If the interfering factor of fresh weight is removed a common slope may be calculated for all lines except those for fresh weight and this is found to be 0.799 (s = 0.016,95% fiducal

limit = ± 0.031, t for goodness of fit = 49.55, P << 0.001)
(Table 8).

3.C.ii. The daily pattern of oxygen consumption

The continuous recordings, made in most cases over a period of four days, allow an examination of the daily rhythm under laboratory conditions. Under these conditions most environmental variables were constant (e.g., temperature, hydrostatic pressure). Light, however, followed the natural cycle and as respiratory measurements were made within a few days of capture the light regime was similar to that in wild conditions.

Figure 14 shows examples of the observed daily rhythm found in crabs in the laboratory at each month of the year 1975. Examples were chosen from determinations made at about the midpoint of each month and have been plotted together with the period of civil darkness (the period between the sun being 6° below the horizon in the west to 6° below the horizon in the east). All times are given in G.M.T. For each month the exact form of the rhythm was different for different animals, the greatest variation between animals being a difference in scope.

Table 8.

Showing the equations relating oxygen consumption rate $(mgO_2 \ hr^{-1})$ to body weight (g) derived by the analysis of covariance of the data in Figures 9 and 10 and Table 6 after the fresh weight data have been removed.

Sex	Y	=	b.	X	±	a
♂	$\log \dot{V}O_2R$	=	0.799	$\log$ DW	−	1.090
♀	$\log \dot{V}O_2R$	=	0.799	$\log$ DW	−	1.090
♂	$\log \dot{V}O_2A$	=	0.799	$\log$ DW	−	0.523
♀	$\log \dot{V}O_2A$	=	0.799	$\log$ DW	−	0.523
♂	$\log \dot{V}O_2R$	=	0.799	$\log$ AFDW	−	0.817
♀	$\log \dot{V}O_2R$	=	0.799	$\log$ AFDW	−	0.817
♂	$\log \dot{V}O_2A$	=	0.799	$\log$ AFDW	−	0.250
♀	$\log \dot{V}O_2A$	=	0.799	$\log$ AFDW	−	0.250

Figure 14.

Histograms of oxygen consumption rate $(mg.O_2 \ hr.^{-1})$

against time of day (hours G.M.T.) for each month

in the year.

The black bars on the baselines represent the

period of civil darkness (sun 6° below horizon in

west to 6° below horizon in east)

Data obtained from Nautical Almanac.

Oxygen uptake mg.O_2hr.$^{-1}$

TIME OF DAY.

Hours GMT.
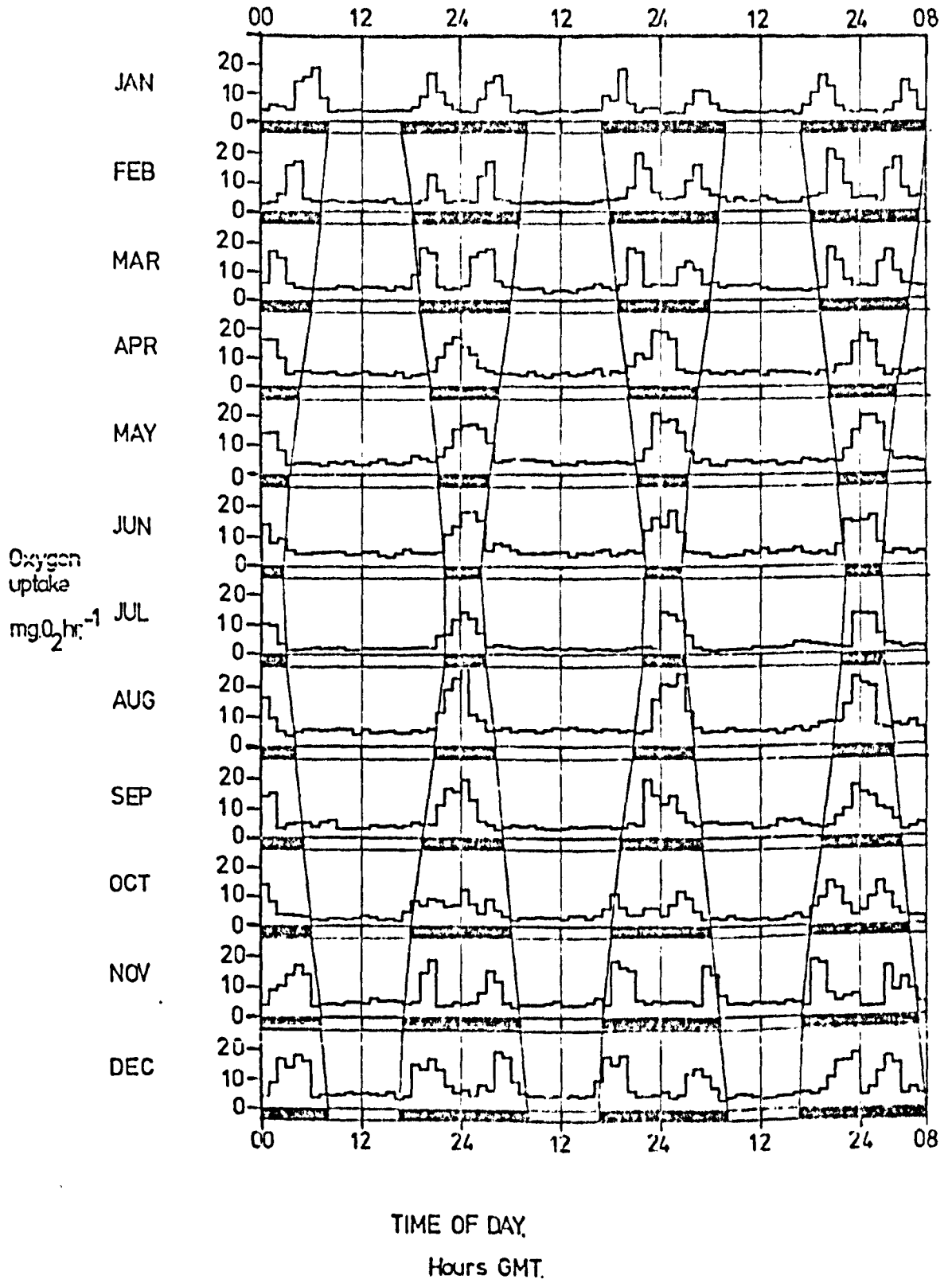
From the figure it can be seen that, during the late autumn, winter and early spring, there were two peaks of higher respiration rate occurring just after civil dusk and just before civil dawn. As the day length increased these two peaks tended to merge together until in mid summer, with very short periods of darkness at the latitude of Oban $(56°N)$, there was only one peak occurring at about midnight. As day length decreased towards winter the two peaks of activity became apparent again.

On many occasions while the traces of oxygen consumption were being analysed it was noticed that the trace showed an undulating fall in oxygen tension within the respirometer, indicating a fluctuating rate of oxygen consumption by the crab (Figure 15). This phenomenon occurred only during the period when oxygen consumption was at its lowest throughout the day; it was never seen to occur during the active phase of the daily cycle.

3.D. Discussion

3.D.i. The relationship between body size and respiration rate

The data available on oxygen consumption rates in the Crustacea have been reviewed by Wolvekamp & Waterman (1960), Altman & Dittmer (1971) and Prosser (1973). Table 9 shows

Figure 15.

       Showing the undulations in the rate of fall in
oxygen tension within the respirometer typical of the
resting phase of the daily cycle of activity.

TIME
hours

OXYGEN % SATURATION
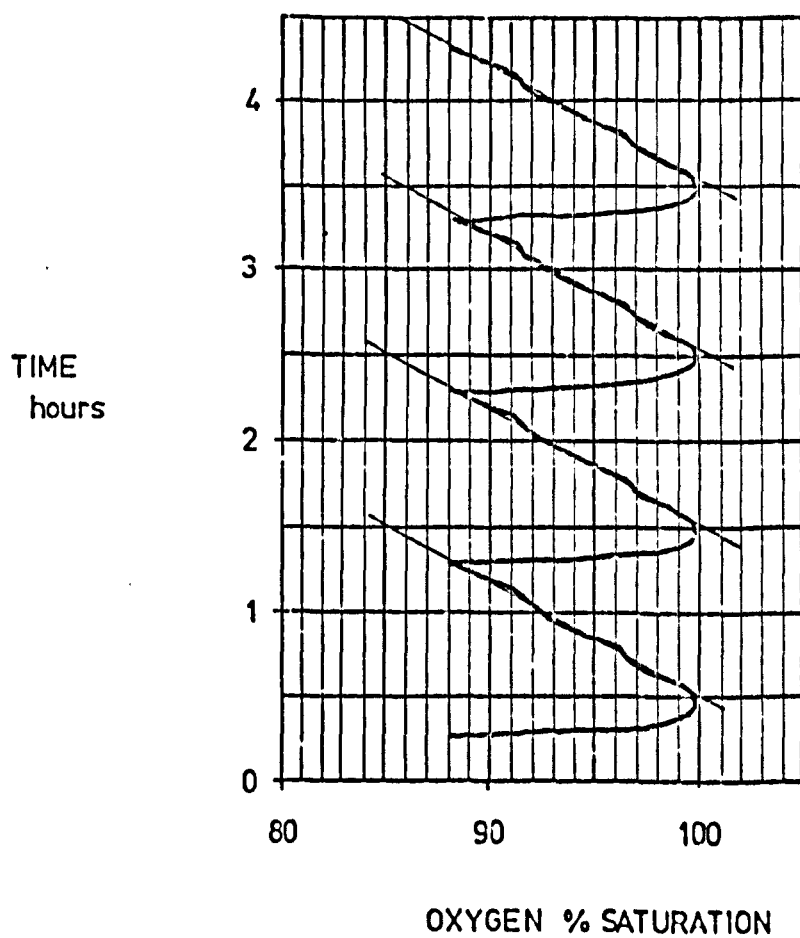
Table 9.

Showing values of oxygen consumption rate $(mgO_2 \ kg^{-1} \ hr^{-1})$ for the genus <u>Cancer</u>.

Species	Weight (g)	$\dot{V}O_2$ $mgO_2 kg^{-1} hr^{-1}$	Source
Cancer pagurus	–	153	Jolyet & Reynard (1877)
" "	200	14–113	Ansell (1973)
" "	200	6–15	Aldrich (1975a)
" " R	200	31	This study
" " A	200	114	" "
" " R	200	32	" "
" " A	200	115	" "
Cancer magister	1000	52.7	Johansen et al. (1970)

values published for <u>Cancer magister</u> and <u>Cancer pagurus</u> and it can be seen that the agreement between this and other studies is good.

There have been relatively few studies of the relationship between body size and respiration rate in the decapod crustaceans. Reviews of the data are available in Wolvekamp & Waterman (1960), Altman & Dittmer (1971), and Prosser (1973). Agreement between these values and those found in this study is good. From the analysis of covariance it would seem that if either dry weight or ash-free dry weight is used to compare males and females no significant difference is found. For the Dunstaffnage population this might be expected. The population contained very few post-puberty crabs and consequently little difference in overall shape exists between males and females (Weymouth & MacKay, 1936). As both % water and % carbon do not seem to change with increasing size (section 2) little difference between males and females is to be expected. Differences between males and females are more likely to be found in post-puberty crabs.

There has been much discussion about the significance of the value of the slope of the regression line linking log body size and log respiration rate (e.g., Weymouth <u>et al.</u>, 1944; Hemmingsen, 1950, 1960; Zeuthen, 1953; Wolvekamp & Waterman, 1960; Prosser,

1973). Hemmingsen (1950) has calculated that a common line of 0.75 is universal (section 1) and attempted to explain this value by arguing that there is an evolutionary tendency for metabolism to increase in proportion to mass and that this is in conflict with surface dependent limitations (e.g., heat loss in homiotherms). The value of 0.75 then is seen as a compromise between the tendency and the limitations. Others have attempted to explain the value on the basis of a changing body composition as animals become larger, the proportion of skeletal tissues increasing more rapidly than muscular tissue. Studies of the weight exponent in isolated tissue, however, give values of 0.75 (e.g., liver slices of rats and rabbits, Kleiber, 1941).

These arguments have all been based on measurements of basal metabolic rate and do not seem to take into account the fact that most animals are able to increase metabolic rate considerably when feeding or active. If surface area is limiting metabolism in any way then it will be the maximum rate that an animal can achieve that will be limited. Swan (1972) has suggested that the constant minimum energy expenditure necessary for life, a part of resting metabolism, is weight dependent and that the rest is surface area dependent. To investigate this he studied metabolism in a series of hibernating animals and concluded that resting metabolism could be divided into two

portions, the essential energesis (Mee) and the metabolism of obligate heat (MoH). Mee is the energy required to maintain body functions simply to stay alive and calculations from data in the literature show that this is insufficient in all except the largest homiotherms to maintain body temperature. MoH is the continuous expenditure necessary to maintain this temperature. The former is weight dependent, the latter surface area dependent and interaction between the two produces a value of 0.75.

Examination of the literature shows there to be considerable variation of the value of the weight exponent within any group of animals and the Crustacea are no exception. The value of 0.799 found in this study for Cancer pagurus lies close to the universal value of 0.75 and is comparable to values found in other crustacea.

Values for the slope of log active and log resting rates against log body weight were in all cases found to be parallel across the range of body weights used. There was thus no change in the scope for activity with body size. This is in contrast to the results of Wallace (1972) for Carcinus maenas who found the large crabs (10 g) had a scope of 2.57 and small ones (0.2 g) a scope of 1.2, his explanation of this difference being due to a change in the support that animals receive from the medium as they become larger but this seems to be based on a misconception

(section 2). Other data published on the scope for activity in crustaceans do not give any details of any effects of size. Newell & Roy (1973) have shown, however, that the scope for Littorina littorea remains constant at 6.4 over the range of sizes examined and remains independent of temperature.

Table 10 summarises the values for scope for decapods from the literature. It can be seen that most values lie below the value of 3.7 found here for Cancer pagurus. The fact that Aldrich (1975a) found scopes for excitement lower than those of this study is perhaps explained by his method of determination which involved placing a lid over an animal in an aquarium. This will result in disturbance of the animal and an elevated rate of oxygen consumption (Ansell, 1973) in his basal experiments and thus cause a reduction of the scope. Crabs used in this study were undisturbed during determinations and were not subject to the periodic stimuli of dropping floating covers onto the aquarium as in Aldrich (1975a, 1975b).

Spoek (1974) gives a value of 3 to 4 for the scope in Homarus gammarus under normal conditions. He has shown, however, that scope for activity is limited by the haemocyanin content of the blood. Animals which have the haemocyanin content of the blood reduced artificially are unable to maintain active rates of oxygen consumption for any period and the scope is reduced

Table 10.

Summarising the data available for measurements of the scope for activity $(VO_2A - VO_2R)$ in decapod crustaceans.

Species			Scope for Activity	Source
Homarus gammarus			3-4	Spock (1974)
Carcinus maenas	0.2 g		1.2	Wallace (1972)
"	"	10 g	2.57	" "
Cancer pagurus			5-6	Ansell (1973)
"	"		1.78	Aldrich (1975a)
"	"		3.7	This study

to two. Resting rates are unaffected. Decapods would therefore

seem to depend on the presence of haemocyanin in the blood to

maintain active rates of respiration. This presumably is due

to the much reduced oxygen capacity and thus oxygen carrying

capacity of the blood. Anthony (1961) has shown that goldfish

are able to survive for long periods in normoxic water even

with all of the haemoglobin of the blood blocked by carbon

monoxide. The anarctic ice-fish (Chaenichthyidae) are able

to maintain normal metabolism even though they completely

lack erythrocytes and haemoglobin (Rudd, 1954, 1965;

Hemmingsen & Douglas, 1970, 1972). Under these conditions

the animals depend for oxygen circulation on the oxygen

which is in simple solution in the blood. Obviously the

lower the environmental temperature the higher the volume of

oxygen dissolved in the blood and the lower the demand for

oxygen will be.

Observations on the whole blood of Cancer pagurus used

in this study have shown (section 6) that, in contrast with

Stedman & Stedman (1925), in no case was it possible to obtain

the blue colour associated with oxygenated haemocyanin

(e.g., Redfield, 1934; Rawlinson, 1940; Redmond, 1955;

Goodwin, 1960; Prosser, 1973). This was true even if pure

oxygen was bubbled through the blood. This would suggest

that, in the wild, the population of crabs studied have little
or no haemocyanin in the blood. The significance of this
observation is discussed later (section 6).

3.D.ii. The daily pattern of oxygen consumption

Williams & Naylor (1969) have shown that *Carcinus maenas*
exposed to intertidal conditions rapidly develop a tidal rhythm
of activity. Naylor (1958) showed that this rhythm may persist
overtly for about five days in constant laboratory conditions.
All of the *Cancer* used in this study were collected sub-littorally
and were thus not subject to the periodic exposure to air and
changes in temperature found in the inter-tidal environment.
It would seem that the exact synchrony of activity cycles with
the tidal cycle would be less important to sub-littoral crabs
such as *Cancer* than to intertidal crabs such as *Carcinus*. This,
together with the fact that the crabs were kept for four or
five days before experiments were carried out might explain
why no rhythm of tidal frequency was found.

It is interesting to note that the form of respiratory rate
of *Cancer* seems to change with the season. *Cancer* seems, in this
study, to be phasing its activity to dusk and dawn. The change
in rhythm form seems to be due to the changes in day length,
the shorter nights causing the two peaks to approach until they
merge. Personal observations indicate that, in mid-summer on a

clear night, the sky never goes completely dark. It would be of interest to study the daily rhythm of activity under wild conditions throughout the season. Observations made by diving indicated that during the day nearly all crabs were found either hidden in holes or buried up to the anterior edge of the carapace where the sediment structure permitted. Only very rarely were Cancer seen to be active or feeding during the day time. Atkinson & Parsons (1973) reported finding dusk and dawn peaks of activity in some specimens of Carcinus. It is impossible from the data available to examine any possible changes in total daily consumption with season as the recordings making up Figure 14 were all made from different animals. While these recordings are typical of each month there was a certain amount of variability between individual crabs.

Ansell (1973) has described the patterns of heart beat rate associated with changes in oxygen consumption rates throughout the daily cycle of oxygen consumption in Cancer pagurus. The active periods are characterised by a more or less constant high heart rate while the resting periods show a characteristic pattern of cyclical fluctuations between a high level approaching that of the active rate, and a low rate of a few beats a minute. This short period rhythm had a period of about twenty minutes.

The fluctuations found in the trace of oxygen tension had a
period which was very close to twenty minutes and it is
suggested that these fluctuations are probably manifestations
of the same short period rhythm as that described by Ansell (1973).
The fact that the fluctuations were only seen to occur during
the resting phase of the daily cycle is further evidence to
support this conclusion. Shields (pers. comm.) and Norfolk
(pers. comm.) have also found rhythms of similar frequency
in grapsid crabs in Australia and in Carcinus maenas. The
respirometer system was too insensitive to follow in detail
such short period rhythms in respiration rate so use was made
of another apparatus. This method and the results obtained
are described in section five.

SECTION FOUR

THE RELATIONSHIP BETWEEN HEART BEAT RATE AND SCAPHOGNATHITE
BEAT RATE

4.A. Introduction

In order that gaseous exchange at the gill surface should
be as efficient as possible the ratio of ventilation of the
gills by water and the perfusion of the gills be blood
(the ventilation/perfusion ratio) must be closely regulated.
This is particularly important in aquatic animals where the
cost, in terms of energy, to the animal is much higher than to
animals ventilating with air (Hughes & Shelton, 1962). In order
that this may be achieved a precise control of the outputs of
the ventilation and circulatory pumps must be maintained
(Rahn, 1966).

A close relationship between heart rate and buccal/opercular
ventilation pumps has been described for teleosts and elasmobranchs
(Schoenleim & Willem, 1894; Willem, 1924, 1941; Lyon, 1926;
Lutz, 1930a, 1930b; Satchell, 1960; Hughes, 1961; Shelton &
Randall, 1962; Randall & Smith, 1967). Several workers have
described the occurrence of an exact phase relationship between
heart beat and buccal/opercular pump rate. As the heart rate
is generally slower than the ventilation pump rate a whole number
ratio e.g., 1:2, 1:3, results.

Taylor & Butler (1971) and Hughes (1972) have re-examined this relationship using long term recording and have found that phase locking is the exception rather than the rule, occurring for less than 3% of the total time in the dogfish.

Although exact phase locking may not occur for a significant portion of the time it might be expected that the two pumps would show a good correlation of output as any difference between the capacity of the ventilation water to bring oxygen to the gills and the blood stream to carry it away would lead to a waste of energy.

The observations described in this section were made to investigate the relationship between heart rate and scaphognathite rates and as a preliminary to an investigation of respiratory performance during the short period rhythm which occurs during the resting phase of the daily cycle.

4.B. Methods

Both heart rate and scaphognathite rates were measured by using the impedance technique, developed by Hoff & Gedes (1967), and used extensively in many studies of invertebrate physiology (e.g., Hoggarth & Trueman, 1967; Ansell & Trueman, 1967; Trueman, 1967; Helm & Trueman, 1967; Jones, 1968; Blatchford, 1971; Brand, 1972; Ansell, 1973; Uglow, 1973; Brand & Taylor,

1974; Taylor, 1976).

In this technique the change in impedance to an A.C. current of very low voltage associated with movements of an organ between a pair of fine wire electrodes produces an analogous output voltage which is used to drive a pen recorder after suitable amplification (Impedance Pneumograph and Physiograph. Narco-Biosystems Inc.). The electrodes used consisted of short lengths of 32 s.w.g. (0.247 mm diam) silver wire. They were inserted through small holes drilled with the tip of a pointed scalpel through the carapace of the crab in an appropriate place. They were fixed in place on the carapace by applying a small drop of ethylcyanoacrylate (Permabond 240). The electrodes for heart rate were placed on the dorsal surface of the crab, one to each side of the heart as in previous studies (Blatchford, 1971; Ansell, 1973; Uglow, 1973). The electrodes for recording the scaphognathite beat placed one dorsal to and one ventral to the scaphognathite blade. It was found that this recording site gave a clear analogue trace consisting of a single peak rather than the often multimodal peaks which result from placement of the electrodes on the ventral surface. The unimodal peak was also much more effective in triggering the rate meters used.

The electrodes were connected to the impedance
pneumograph by means of a length of light screened cable.
The crabs with attached leads were allowed to move freely
around a large tank. In order to minimise drag and prevent
the leads becoming tangled a small floating cork was attached
to the cable and allowed to float at the water surface. Care
was taken at all times that the crab should be as little
disturbed as possible throughout the experimental cycle.
Natural light came from a north facing window over the tank,
and no artificial illumination was used.

The output from the impedance pneumograph was amplified
by a transducer-monitor-coupler (Narco-Biosystems Inc.) and
the output was used to drive the pens on a multi-channel
physiological recorder or to act as an input signal for
rate meters. Two types of rate meter were used to record the
beat rate of the heart and scaphognathite. The first was a
Neilsen type instantaneous rate meter (Devices Instruments Ltd.
type 2751) which gave a beat-by-beat reading of the rate. The
output from this rate meter was recorded at 10 second intervals
on a multi-point recorder (Kent Instruments Ltd.). An example
of the resulting chart is shown in Figure 17 where it can be
seen that a clear visual representation of the beat rate results.
These charts are, however, a little difficult to interpret

quantitatively. In order to overcome this difficulty a second type of rate meter, designed and built at the S.M.B.A. by Mr R. Bowers, was used. In operation it counts the number of beats occurring in a given period (one minute here) and then, during the next counting period, holds a steady output voltage analogous to the previous rate. This output was recorded, after being inverted, on a Leeds and Northrup flat bed recorder to result in a histogram of the beat rate.

Crabs which had three sets of electrode leads survived for long periods without the electrodes or leads becoming detached. The advantage of the cyanoacrylate adhesives over previously used black dissecting wax (e.g., Blatchford, 1971; Ansell, 1973; Uglow, 1973) is that the bond produced between the crab exoskeleton and the electrode wire is very much stronger and durable. The bond is so strong that the full weight of the crab may easily be supported on the leads. The adhesive could also be used whilst the exoskeleton was damp whereas it must be perfectly dry to produce a good bond with black wax. This, together with the extremely rapid setting time (c 30 secs) minimised the length of time for which the crabs were exposed to the air whilst having electrodes fitted, thus reducing operative stress.

4.C. Results

For the most part it was practical to record from the
heart and one scaphognathite only as, with the equipment
available, recording from rate meters could only be made on
two channels. McMahon & Wilkens (1972) and Wilkens & Young
(1975) have shown that, in the lobster Homarus americanus,
whilst the two scaphognathites may beat independently they
show a high degree of rate correlation for the majority of
the time. It is thus reasonable to use the rate of one
scaphognathite as an indicator of total pumping activity.
Several recordings of heart and both scaphognathites
movement were made on the "Physiograph" system to test this
hypothesis. The number of beats of heart and left and right
scaphognathite occurring in each thirty second period were
counted and the beat rate (beats per minute) for the left
scaphognathite has been plotted against the beat rate of the
right scaphognathite in Figure 16. It can be seen that there
was a high degree of correlation of beat rate between the two
scaphognathite rates although in this particular crab the left
scaphognathite beat at a rate which was consistently slightly
higher than the right scaphognathite. Other crabs showed the
reverse situation where the right scaphognathite beat faster
than the left. This situation is similar to that reported for

Figure 16.

Scatter diagram of left scaphognathite rate (b.p.m.) against right scaphognathite rate (b.p.m.) for a female crab of carapace length 46 mm. and fresh weight 60 g.
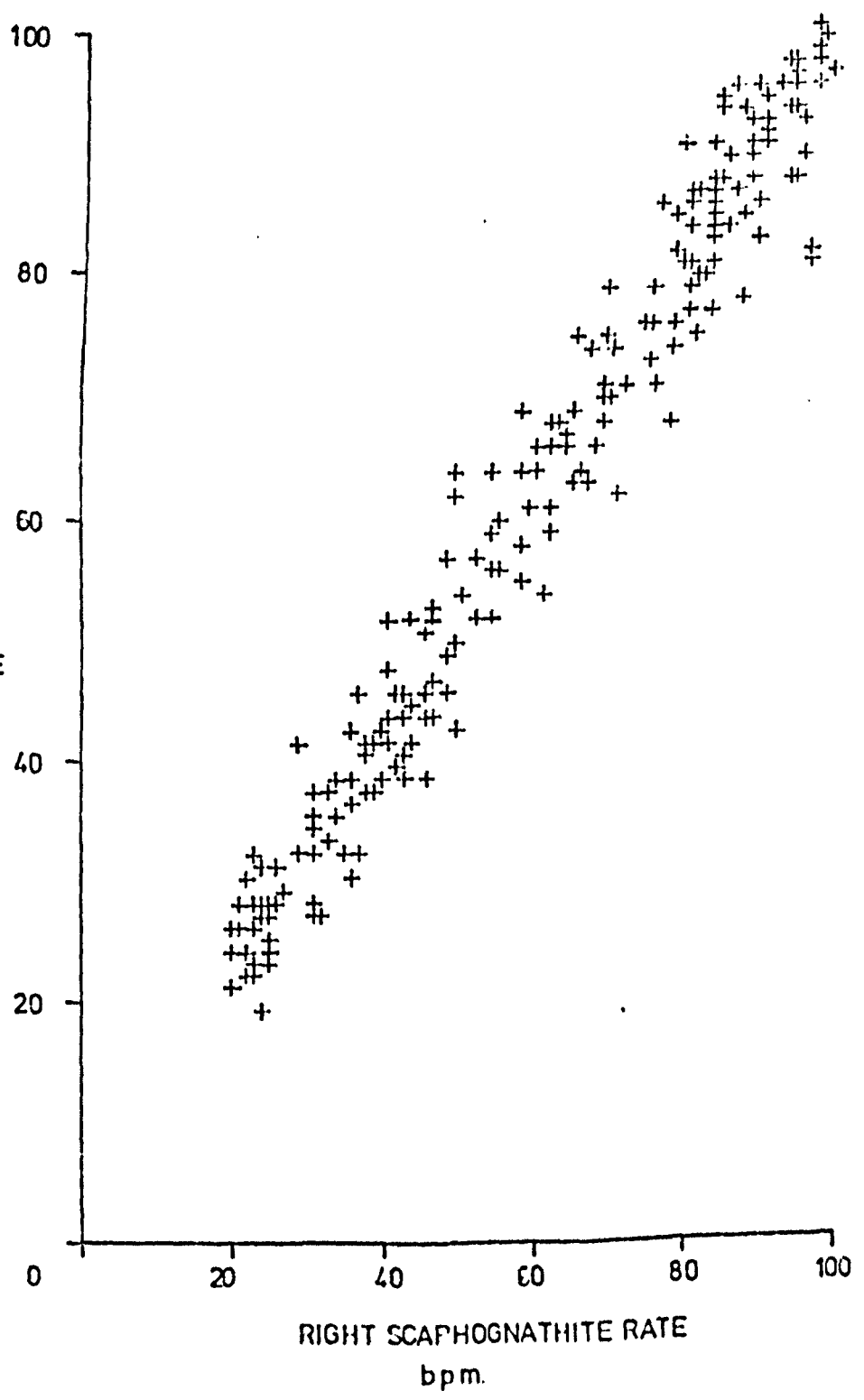
<u>Homarus</u> <u>americanus</u> by Wilkens & Young (1975).

Figure 17 shows a thirty six hour extract from a long term recording of heart and left scaphognathite made at the end of the month of March from an undisturbed crab which was free to move about a large tank. The extract was recorded on the third and fourth day after electrodes had been implanted so that the animal had recovered from the operation. It can be seen, quite clearly, that there were peaks of heart and scaphognathite activity just before sunrise and after sunset. During these peaks the heart and scaphognathite maintained a high steady beat rate (c 90 b.p.m.). These two peaks corresponded with the two peaks of respiration rate that are found at this time of the year (section 3). It can be seen that the timing of the peaks on successive days was slightly different. On the first day the dusk peak started at an earlier time (2020 hrs) than on the second day (2115 hrs), and the duration of the dusk peak was longer on the second day. The two dawn peaks are of approximately the same duration, but the second day peak occurred later than the first. The dusk peak of active pumping by heart and scaphognathite was of longer duration than the dawn peak of both days.

In contrast to these periods of active pumping by the heart and scaphognathite a different pattern was shown for the

Figure 17.

Recording of heart rate (b.p.m.) and left

scaphognathite rate (b.p.m.) over a one and a

half day period.

Recording made on multi-point recorder.

SCAPH. RATE BPM

HEART RATE BPM

SCAPH. RATE BPM

HEART RATE BPM

SCAPH. RATE BPM
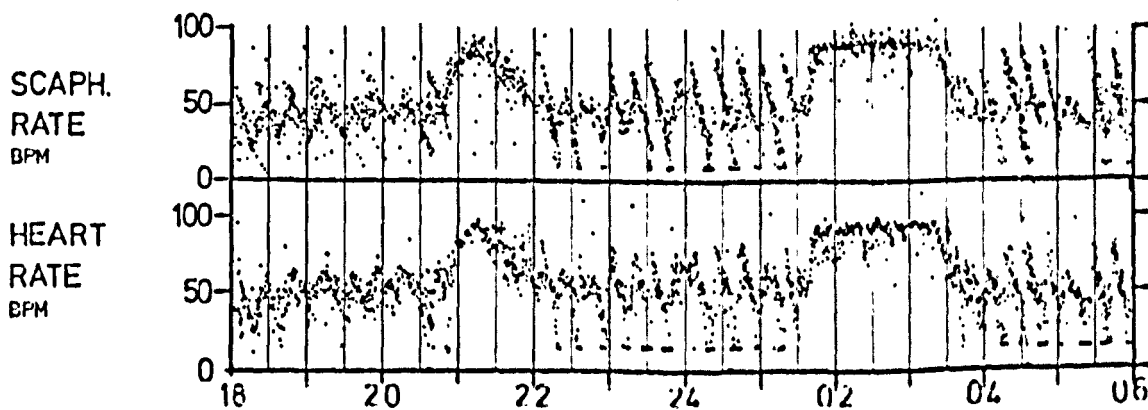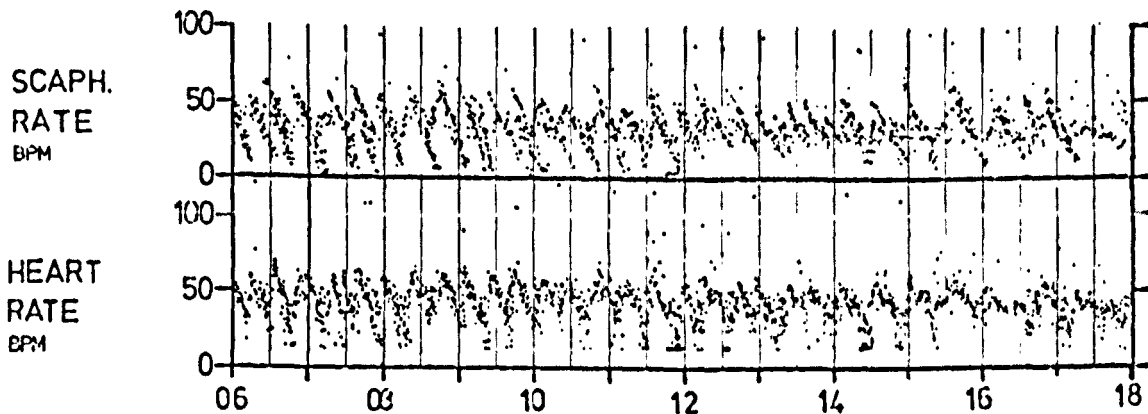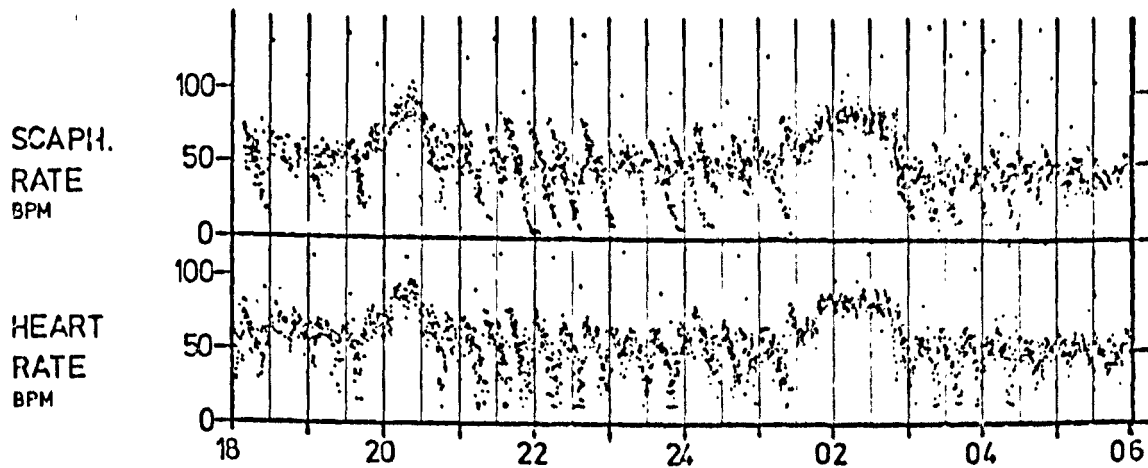
HEART RATE BPM

TIME OF DAY HOURS GMT

rest of the day when oxygen consumption rates were found to be low (section 3). During these resting periods the heart rate showed the characteristic short period cyclical fluctuations described by Ansell (1973). This rhythm is characterised by a change from high, near maximum active rates to very low rates of a few beats a minute. These cycles in the present data are very regular and have a period of almost exactly 20 minutes. The scaphognathite rate showed exactly the same rhythm as the heart rate. The two rhythms were exactly in phase throughout the period of the resting phase. Through limitations of rate meter triggering beat rates of less than about twenty b.p.m. did not record properly. Visual counting of the heart rate during these periods of low beat rate gave about three to five beats per minute whilst the scaphognathites were completely stopped.

This extract is typical of other recordings obtained from crabs under similar conditions. The exact form of the rhythm varied slightly between crabs but the period remained almost constant at twenty minutes in each case. Throughout these recordings it was noticed that any disturbance of the crab resulted in the loss of the short period rhythm. The rhythm was also lost if the crab was fed during the resting phase of the daily cycle.

4.D. Discussion

It has already been argued the heart rate and scaphognathite rate may be used, with caution, as indicators of cardiac output and ventilation volume as their stroke volumes do not seem to vary a great deal with frequency (section 1). This situation is in contrast to fish where differential pressure and flow resistance measurements have indicated, e.g., Callionymus and Scyliorhinus, that ventilation volume changes are associated with changes in depth of breathing as well as frequency (Hughes & Umezawa, 1968a, 1968b). Johansen (1962), Randall & Stevens (1967), Stevens & Randall (1967a, 1967b) and Hansen (1967, cited by Randall, 1970) have all shown that the cardiac output is regulated mostly by changes in the stroke volume. Stevens et al. (1972) and Priede (1976) have also shown that, in teleosts, the bulbus arteriosus plays an important role in maintaining aortic blood flow and may be responsible for up to 30% of the cardiac output.

It may be assumed then that the two peaks of activity shown by Cancer ~~at this time of the year~~ as peaks in respiration rate (section 3) are supported by an increased output from the cardiac and scaphognathite pumps. Both rates climb to approximately 90 b.p.m. and maintain this level steadily for the duration of the period of activity. Under

the conditions that the recording was made it might be expected that activity might be a little impaired and produce results which are not representative of the situation under wild conditions where the animal is free to feed. Under these recording conditions, however, it can be assumed that, for this particular crab, the high heart rate and scaphognathite rates represent the most efficient rates of movement of blood and water to ensure adequate oxygenation of the blood to support the oxygen demand.

During the resting phase of the daily cycle the crab shows the short period heart rhythm described by Ansell (1973). In exact phase with this heart rhythm is a rhythm of scaphognathite activity which must be correlated with increased pumping activity. At all times of the daily cycle the crab appears to exercise close control over both heart and scaphognathite rates in order that gaseous exchange should be closely controlled. In this context the function of the short period rhythm may be a little puzzling. Ansell (1973) suggests that there may be an optimum ventilation and/or circulatory flow rate and that a fall in oxygen demand during the resting phase below the level of oxygen consumption that this rate will support may cause the onset of intermittent ventilation and circulation. The crab may thus save energy by using the most efficient levels of ventilation

and perfusion for short periods. It may also be possible that the saw-tooth pattern represents a crude, inefficiently developed homiostatic mechanism when oxygen demand is low and the crab resting. Once the blood is fully oxygenated the respiratory pumps are shut off until the blood oxygen tension (or some other factor) falls to a low level when the pumps are re-activated.

The observations of the close coupling between heart rate and scaphognathite rates supports the observations of Wilkens et al. (1974) who showed that, for Cancer magister, there are certain command fibres in the circumoesophagael connectives which, when stimulated result in the modification of both heart and scaphognathite beating and presumably allow common control of heart and scaphognathite rates. The close synchrony between heart and scaphognathites may be taken as further evidence of common control.

SECTION FIVE

VENTILATORY PERFORMANCE DURING THE RESTING PHASE

5.A. Introduction

A complete analysis of respiratory performance demands a knowledge of respiration rate, ventilation rate, inhalent and exhalent oxygen tensions, blood gas levels and cardiac output. In practise, it is very difficult to measure all of these parameters simultaneously and calculations of some of the factors have to be used.

It is possible, by use of a continuous flow respirometer, to measure oxygen consumption rate continuously and to calculate, from inhalent and exhalent oxygen tensions, the ventilation volume. In this study though, the opposite approach was adopted, viz., the ventilation volume was measured directly and the oxygen consumption rate was calculated from inhalent and exhalent tensions.

Cardiac output may be measured by the Fick principle (Hoff & Scott, 1948) which involves a knowledge of blood gas levels. Due to technical difficulties it was practical to assess only ventilatory or circulatory performance at any one time. This section deals with ventilatory performance during the short period rhythm.

5.B. Methods

As stated previously (section 3) the respirometer system used was too insensitive to allow a close study of the short period rhythm. In order to understand better the form and possible function of the rhythm it is necessary to gain a knowledge of some of the factors involved. To this end an apparatus similar to that of Thomas (1954), Larimer (1961), Arudpragasm & Naylor (1964b, 1966) and Taylor (1976) was used. It is shown in Figure 18.

The apparatus consisted of a large perspex rectangular chamber (60 cm x 30 cm x 30 cm) fitted with an inlet and two stand pipes passing through the base. Around one of the stand pipes a cylindrical second chamber, 5 cm in diameter was fitted. The exhalent water from a crab could be channeled into this second chamber by means of a hood attached to the end of a short tube (12 mm i.d.) projecting from the base of the second chamber. The hood was made from a toy balloon with the blind end cut away and was stretched over the anterior end of the crab taking care not to occlude the inhalent openings above the chelae. The second chamber was made much smaller than that of Arudpragasm & Naylor (1964b, 1966) to improve temperature control and to prevent a build up of more or less stagnant water. Fully oxygenated water was supplied to the apparatus from the same system as

Figure 18.

Diagram of the apparatus used for the determination
of ventilation volume in <u>Cancer pagurus</u>.

C	=	cannula
FC	=	from circulation
Fl	=	float
Fu	=	funnel
M	=	magnet
MS	=	magnetic switch
O/P	=	output to counter and event recorder
P	=	pivot
R	=	relay
SDV	=	solenoid operated drain valve
TC	=	to circulation
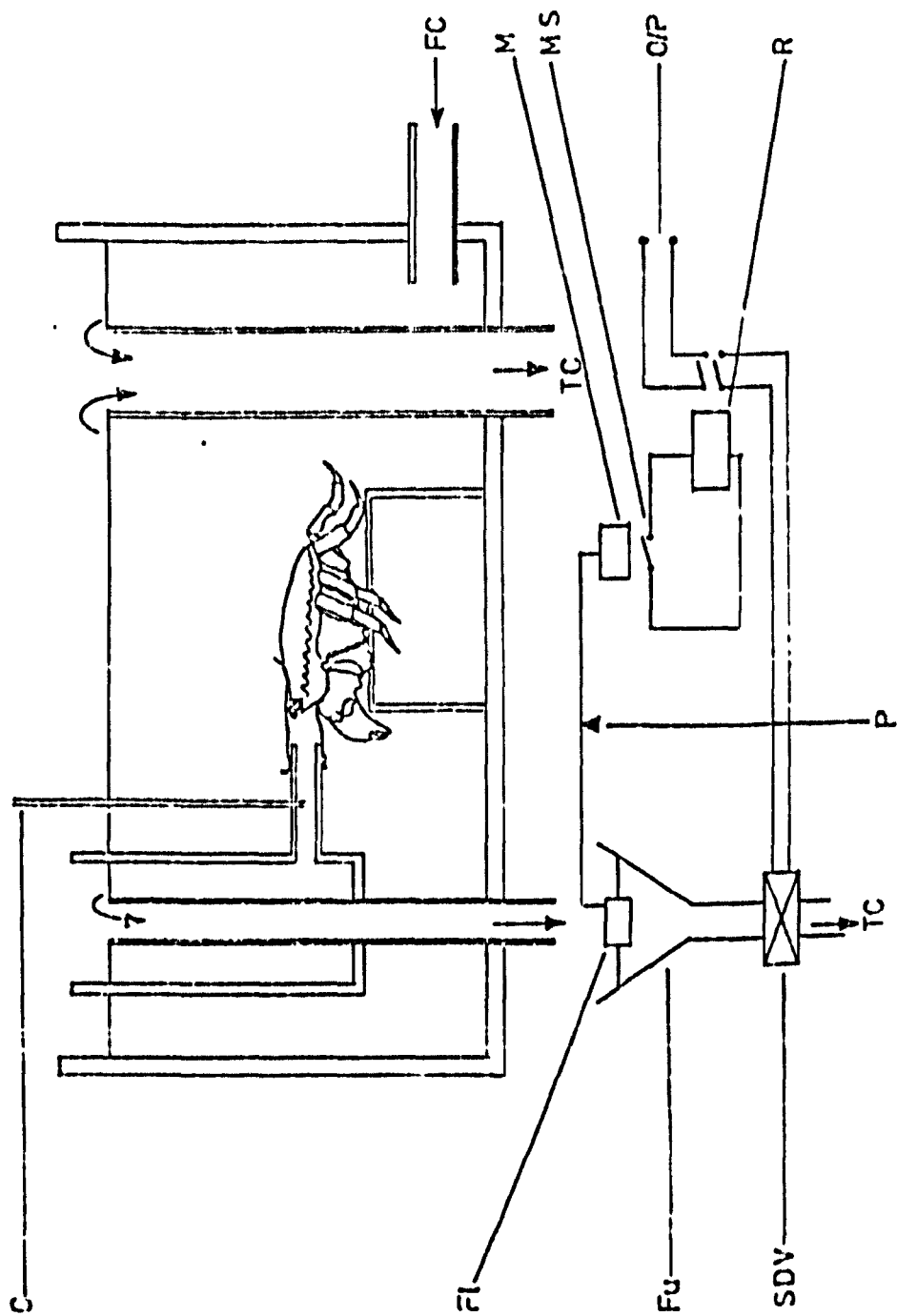
previously described.

The two stand pipes act to maintain a constant and identical water level inside and outside the second chamber so that no head of water existed to force water through the crab in either direction. Minor adjustments were made to this end by injecting a small amount of dye into the pipe connecting the two chambers and adjusting the level of one of the stand pipes until no movement of the dye could be seen. Once this had been done water flowing down the second stand pipe was equal to the ventilation volume of the crab.

The ventilation volume of the crab was determined either by collecting the water overflowing in a measuring cylinder for a known length of time or by using a switching system which allowed a fixed volume of water to escape for each operation. This was achieved by collecting the overflowing water in a funnel, the outlet of which was controlled by a solenoid drain valve. A float, at one end of a pivot arm, remained at the water surface in the funnel, and moved a magnet, at the other end, near to a magnetic switch. This completed the circuit and, via a relay, opened the drain valve until the circuit was broken by the falling water level raising the magnet. With this arrangement the water level in the funnel fluctuated between two levels allowing the same volume of water to escape

with each operation. The sensitivity of the measuring device could be changed by raising and lowering the funnel. In practise the maximum sensitivity that was achieved was c. 3 ml per operation. The ventilation volume could thus be obtained by counting the number of operations in a given time. For extended periods the number of operations was recorded on a counter or a Rustrak recorder.

A cannula was fitted to the connecting tube with its end at the centre of the lumen of the tube so that samples of exhalent water could be taken. These were analysed for oxygen content using a Radiometer acid-base analyser and oxygen electrode in a thermostatted cell. Inhalent oxygen tension was taken as that of the surrounding saturated water. Recordings of heart and scaphognathite rates were made as described in section 4.

From these data it is possible to calculate:-

a) % utilisation of oxygen from the ventilatory water:

$$U\% = \frac{P_iO_2 - P_eO_2}{P_iO_2} \times 100$$

where P_iO_2 and P_eO_2 are inhalent and exhalent oxygen tensions.

b) oxygen consumption rate:

$$\dot{V}O_2 = \frac{U\%}{100} \times \dot{V}_g \times a_{wO_2} \times 60$$

where: $\dot{V}O_2$ = oxygen consumption rate mgO_2 hr^{-1}

$\dot{V}_g$ = ventilation volume ml min^{-1}

a_{wO_2} = solubility of O_2 in sea-water. mlO_2 ml^{-1}

In this case: $\dot{V}O_2$ = $U\%$ x $\dot{V}_g$ x 5.592 x 10^{-3}

Experiments were carried out on crabs of carapace length up to 60 mm, larger ones being difficult to fit with adequate hoods. In preliminary experiments the crabs were not restrained in any way but they always moved and occluded the neck of the balloon preventing ventilation. To avoid this the crabs were clamped to the support by a retort clamp. It is appreciated that this position is somewhat unnatural but preliminary experiments showed that the crabs still showed the short period rhythm. As any disturbance of the crab under more natural conditions results in the rhythm disappearing (Ansell, 1973) it has been assumed that the crab was not grossly disturbed by clamping.

5.C. Results

5.C.i. The relationship between scaphognathite rate and ventilation volume

Simultaneous measurements of scaphognathite rate and ventilation volume have been made on several specimens of Cancer pagurus to provide information on the role of scaphognathite

beat rate in the regulation of ventilation volume. Typical results from two crabs are shown in Figures 19 and 20. In these Figures scaphognathite rate is presented as the mean rate of left and right sides. In no case was there a marked difference between the two rates although in both of these crabs the right scaphognathite beat at a rate which was consistently slower, by a few beats per minute, than the left scaphognathite. In other specimens this situation was reversed with the right scaphognathite beating faster.

Implantation of electrodes results in a very high excited rate of beating by heart and scaphognathite. This fact was utilised to extend the range of beat rates used. The normal active level of beat rate for the crabs in Figures 19 and 20 was 100 - 105 b.p.m.

It can be seen that ventilation volume decreased in a linear manner with decreasing mean scaphognathite rate. The fitted regression line passes close to the origin in each case. This implies that, over the majority of the beat rate range measured, there was little change in the stroke volume of the scaphognathite. The exception to this, however, is the portion of the data which comes from crabs in the post-operative excited state. In both crabs the ventilation volume falls below the regression line for these beat rates implying that stroke volume

Figure 19.

Showing the relationship between ventilation volume

$(ml\ min^{-1})$ and mean scaphognathite beat rate (b.p.m.).

$Y = 0.193 X + 0.45$

VENTILATION
VOLUME
ml min.$^{-1}$

MEAN SCAPHOGNATHITE RATE

b.p.m.
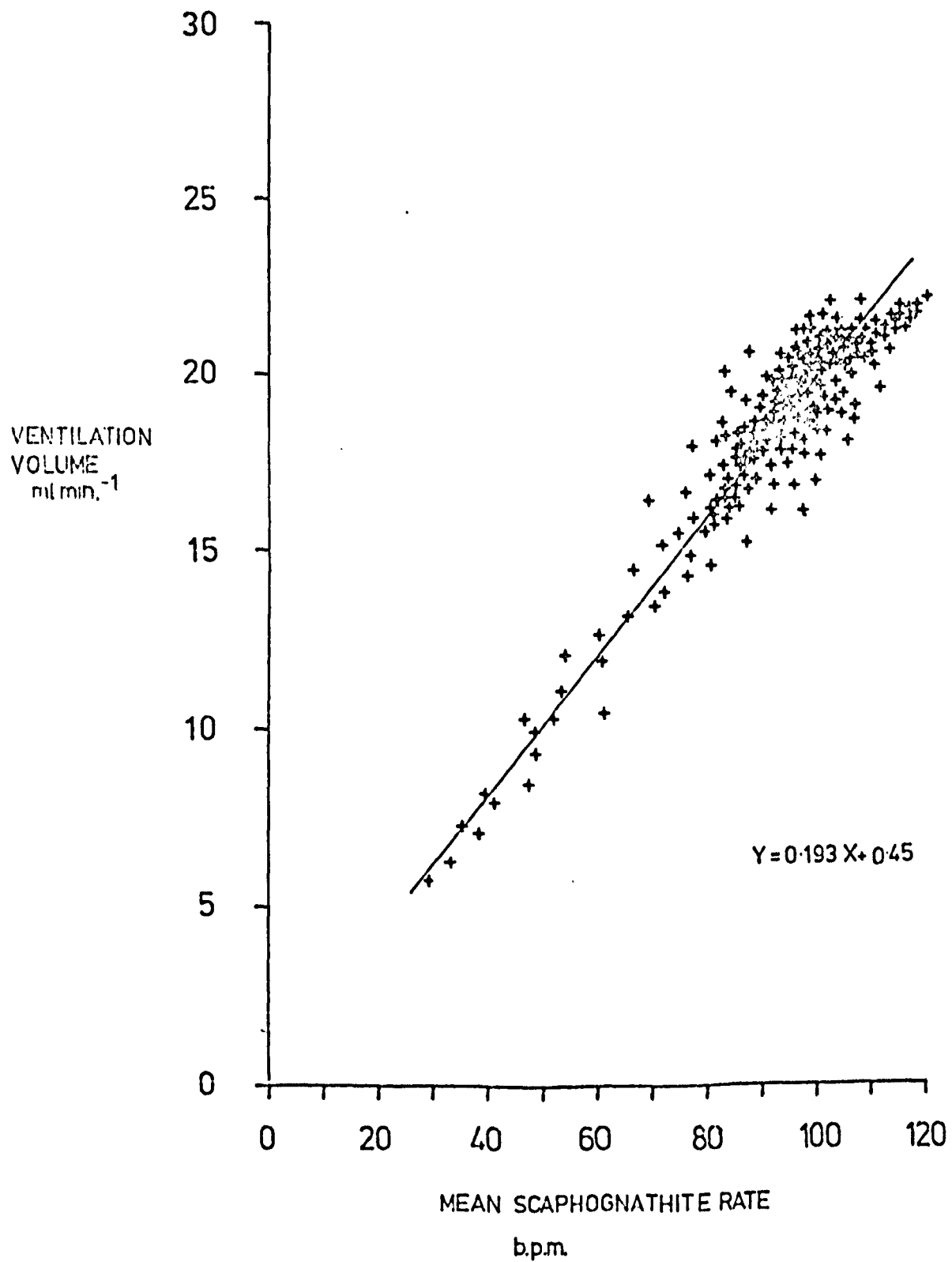
Figure 20.

Showing the relationship between ventilation volume ($ml\ min^{-1}$) and mean scaphognathite beat rate (b.p.m.).

$$Y = 0.251 \, X + 0.08$$

VENTILATION
VOLUME
$ml.min.^{-1}$

MEAN SCAPHOGNATHITE RATE
b.p.m.
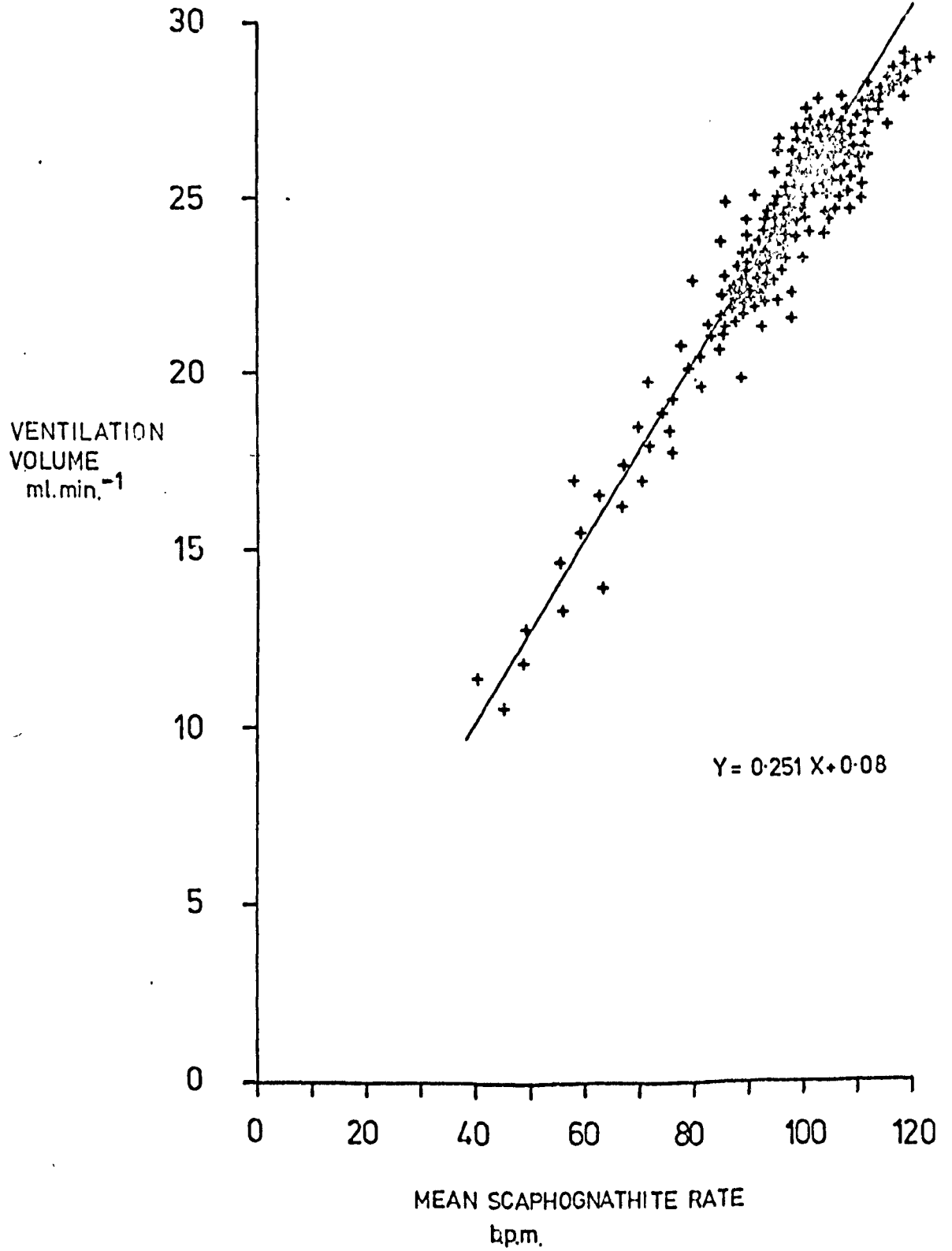
decreased. It can also be seen that, during these high excited rates, the variability in the data was much less than that at the normal active rates.

It can also be seen that for the same beat rate the first crab pumped less water than the second one, the slopes of the regression lines being 0.193 and 0.251 respectively.

5.C.ii. The form and effects of starvation on the short

period rhythm

Ansell (1973) has examined the changes that occur with starvation in the proportion of the daily cycle spent in the active and resting phase in Cancer pagurus. The form of the short period rhythm and the effects of starvation on that form will be considered here.

Figure 21 shows an extract of a recording of the heart rate made during the resting phase of the daily cycle from a crab in the apparatus described above. It illustrates the typical form of the rhythm. It can be seen that the heart rate was maintained at a steady high rate (c. 100 b.p.m.) for a short period (c. 12 mins) and then fell off, over a minute or two, to a steady low level (c. 20 b.p.m.) for about 5 - 6 minutes. The heart rate then returned to the high rate of c. 100 b.p.m. and the cycle was repeated. The period of each cycle was almost exactly twenty minutes. As pointed out in section 4 the rate meters

Figure 21.

Graph of heart rate (b.p.m.) against time (mins.)
to illustrate a section of the resting phase of
the daily cycle of activity when the crab was
showing the short period rhythm.

HEART RATE
b.p.m

TIME   mins.
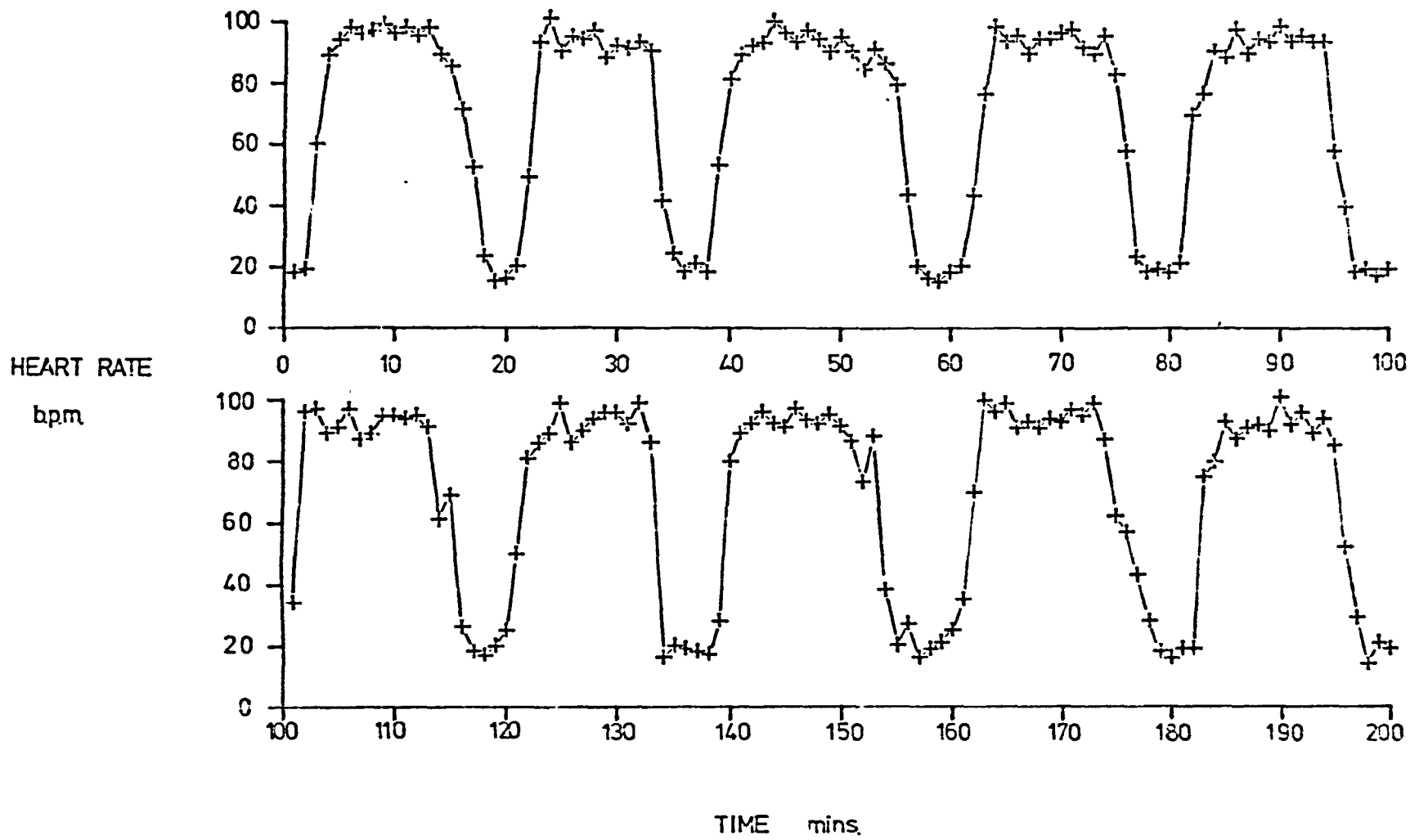
used did not trigger properly below beat rates of c. 20 b.p.m.
Visual counting of beat rate during these periods gave rates
of 4 - 5 b.p.m. Later this problem was overcome by reducing
gain at the impedance pneumograph and appropriate filtration
at the rate meter input.

Figure 22 shows the changes in form of the rhythm which
are associated with progressive starvation, three cycles from
each day are presented. Data on the ventilation volume were
also available, but these have been omitted for the sake of
clarity. Recording commenced on the first day after capture
from the wild. Whilst the period of the rhythm seemed to be
unaffected by starvation, remaining at about twenty minutes,
the high-rate phase of the rhythm was considerably affected.
The rate of heart beating decreased from about 100 b.p.m. on
day 1 to about 70 b.p.m. on day 18. The duration of the high-
rate phase also decreased with starvation, from about 13 minutes
on day 1 to 6 - 7 minutes on day 18.

The amount of time spent in each phase (high, changing,
and low) has been calculated as a percentage of each cycle and
the results are shown in Figure 23. The amount of time changing
between high and low rates did not alter with increasing
starvation. The percentage of the time spent in the high-rate
phase decreased almost linearly with time, this decrease

Figure 22.

Graph of heart rate (b.p.m.) against time (mins.)
to illustrate the changes in shape of the short
period rhythm with progressive starvation (days).

HEART RATE b.p.m

TIME mins.

+ = DAY 1
△ = DAY 7
□ = DAY 13
× = DAY13
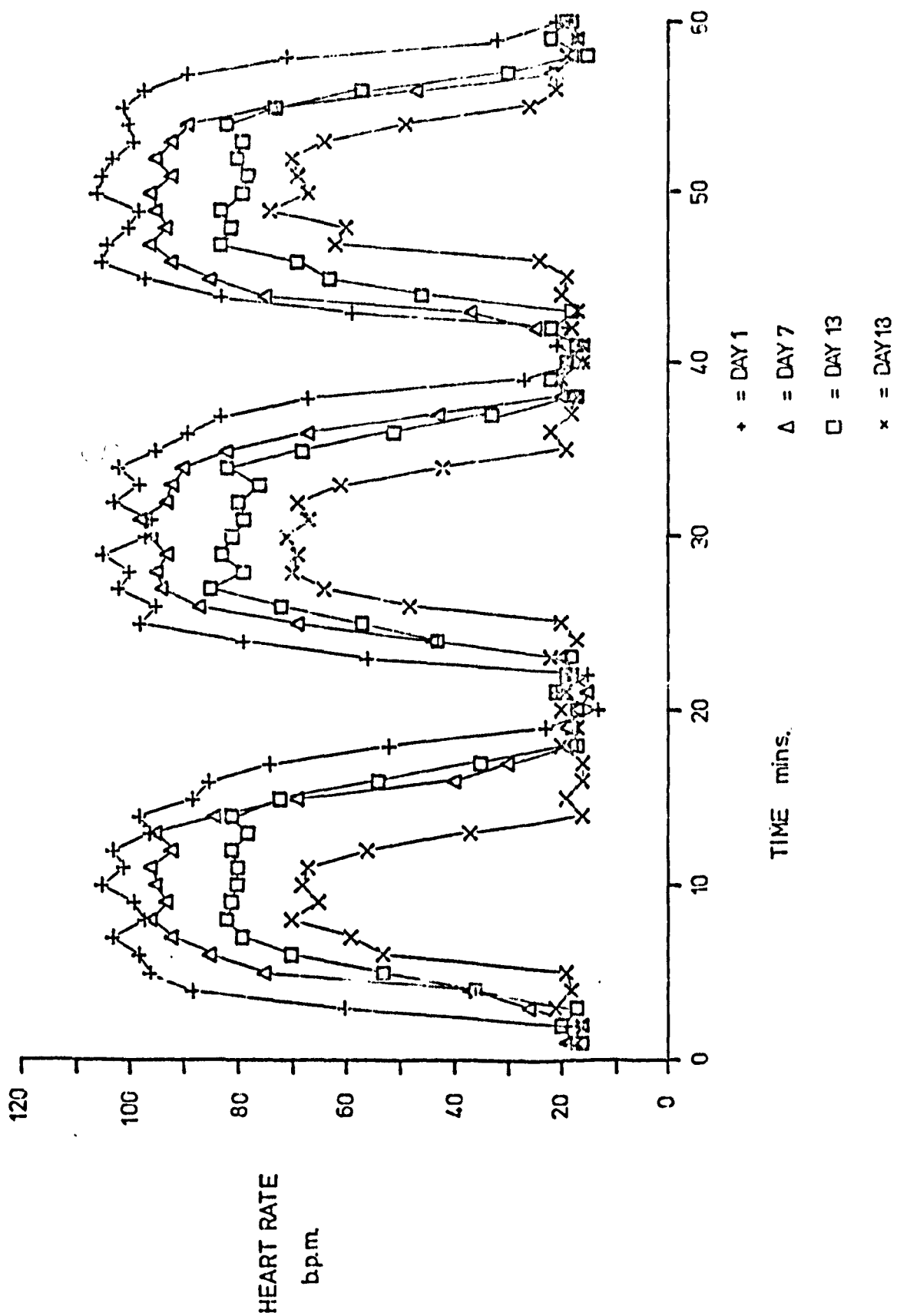
Figure 23.

Showing the changes in the proportion of time spent in each phase of the short period rhythm that are associated with increasing starvation.

PROPORTION OF
EACH CYCLE
SPENT IN EACH
PHASE

%

HIGH-RATE

CHANGING

LOW-RATE

DAYS STARVATION
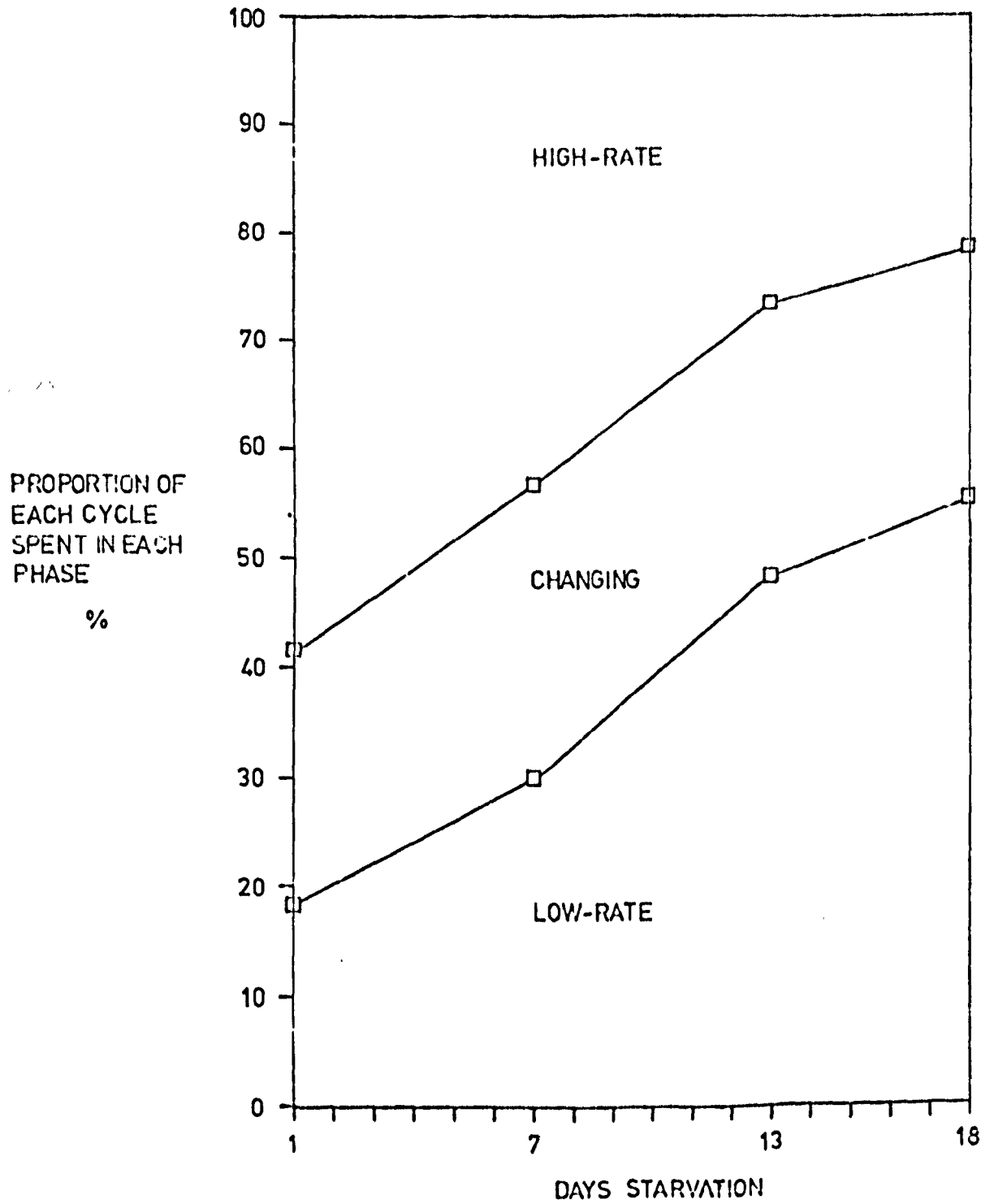
appearing almost completely, as an increase in the low-rate phase. Similar changes in the pattern of ventilation volume have been recorded. Mean curves for heart rate and ventilation volume have been calculated from about five hours continuous recording for each day and the data are presented in Figure 24. The curves obtained for ventilation volume on each day followed very closely the curve for heart rate as might be expected from the observations in section 4.

The changes in ventilation volume with progressive starvation were very similar to those of heart rate with both the level of pumping and the period of pumping during the high rate phase decreasing with starvation. The changes in ventilation volume did not seem, however, to be quite so marked as those of heart rate, ventilation volume falling to about 80% of its initial rate while heart rate fell to 70% of its initial rate.

Observations of movements of dye through the connecting tube at periods of low rate phase indicated that no flow occurred. This is borne out by recordings of heart and scaphognathite activity during a low-rate phase (Fig. 25). It can be seen that, although the heart did continue to beat at a very low rate, activity in the scaphognathites was almost completely absent.

Figure 24.

Graphs showing the changes of the shape of the mean
curves for heart rate (b.p.m.) and ventilation
volume (ml. min.$^{-1}$) that occur with progressive
starvation (days).
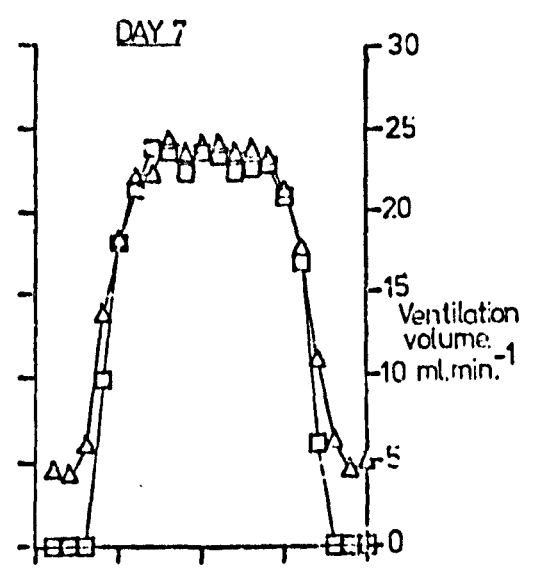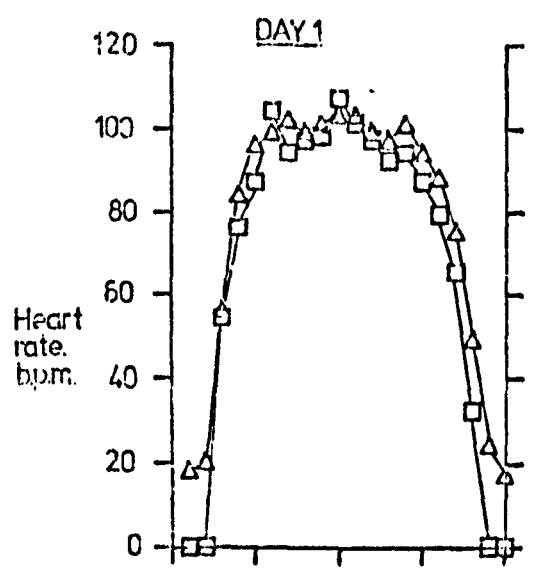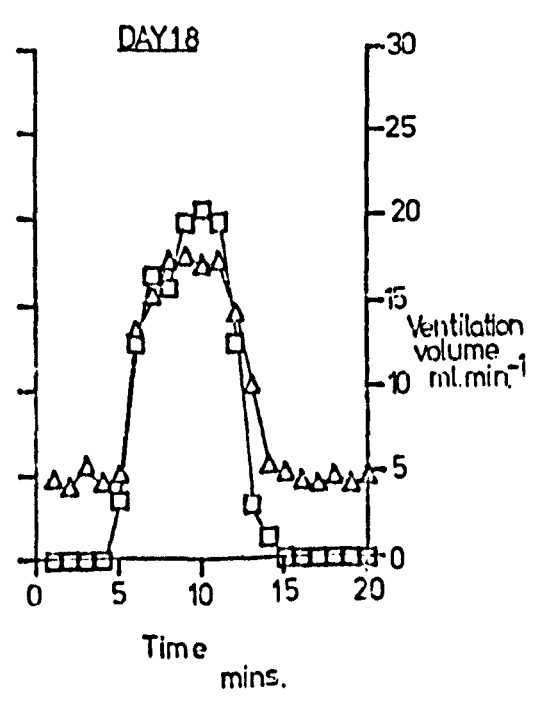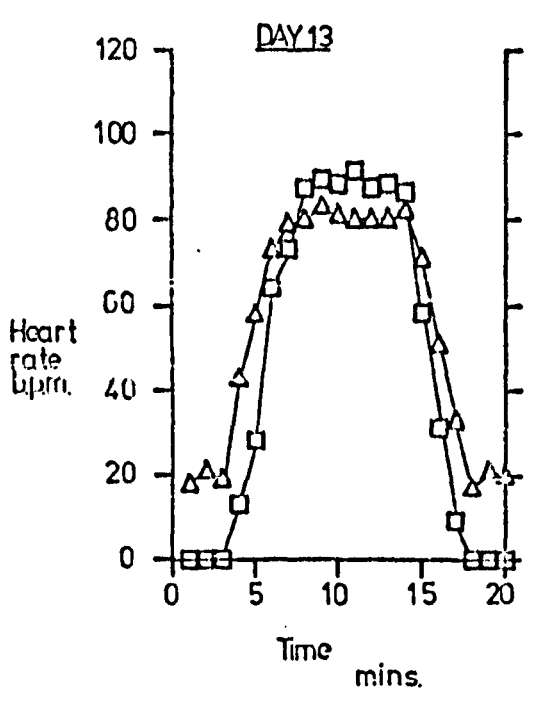
Δ = Heart rate   ☐ = Ventilation volume

Figure 25.

Showing a recording of heart and scaphognathite activity during a low-rate phase of the short period rhythm.

HEART

L. SCAPHOGNATHITE

R. SCAPHOGNATHITE

30sec.
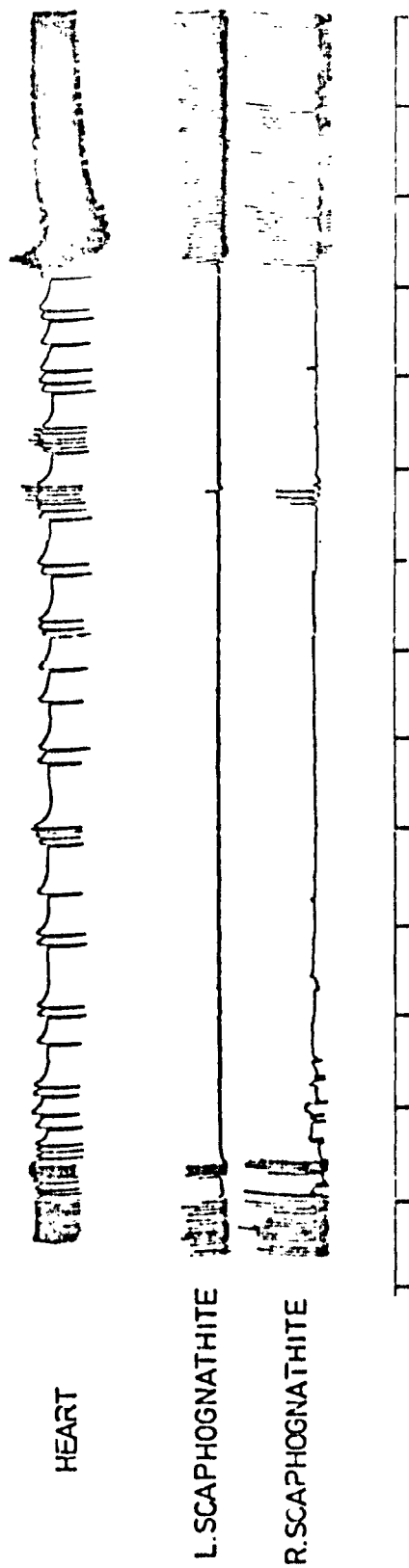
5.C.iii. % Utilisation during the short period rhythm

Measurements of ventilation volume and exhalent and inhalent

oxygen tensions have been made throughout eight cycles of the

short period rhythm and the results of these and calculated

values for respiration rate and % utilisation are presented for

three different crabs in Figures 26, 27 and 28. In each case

only four cycles are presented as typical of the whole period.

Due to technical limitations, such as the response time of the

oxygen electrode, it was not possible to measure exhalent pO_2

or ventilation volume continuously. The measurements of heart

rate were made as before and are subject to the same triggering

limitations. The results in Figure 28 were obtained after these

limitations had been overcome and represents the true low-rate

phase rate. The data were all obtained from crabs freshly

caught from the wild to avoid the effects of starvation.

In all three examples the heart rate shows the typical rhythm

found previously, the high-rate varied between crabs because of

the different sizes of the animals. In each case ventilation

volume reflected the pattern of heart rate as previously

described (section 5.C.ii.). Although not marked in these

figures, ventilation volume fell away to zero during the low-rate

phase.

Figure 26.

Showing the relationships between heart rate (histogram, b.p.m.), ventilation volume (Vg ml min^{-1}) percentage utilisation (% U) and oxygen consumption rate (VO_2, mg hr^{-1} x 10) during the short period rhythm.

HR.
+ = Vg
× = U%
△ = VO₂

+ HR.
× Vg
△ VO₂

TIME mins

Figure 27.

Showing the relationships between heart rate (histogram,
b.p.m.), ventilation volume $(Vg, ml\ min^{-1})$ percentage utilisation
(% U) and oxygen consumption rate $(VO_2,\ mg\ hr^{-1} \times 10)$ during the
short period rhythm.

HR
V̇_G
U%
V̇O₂

+ =
× =
△ =
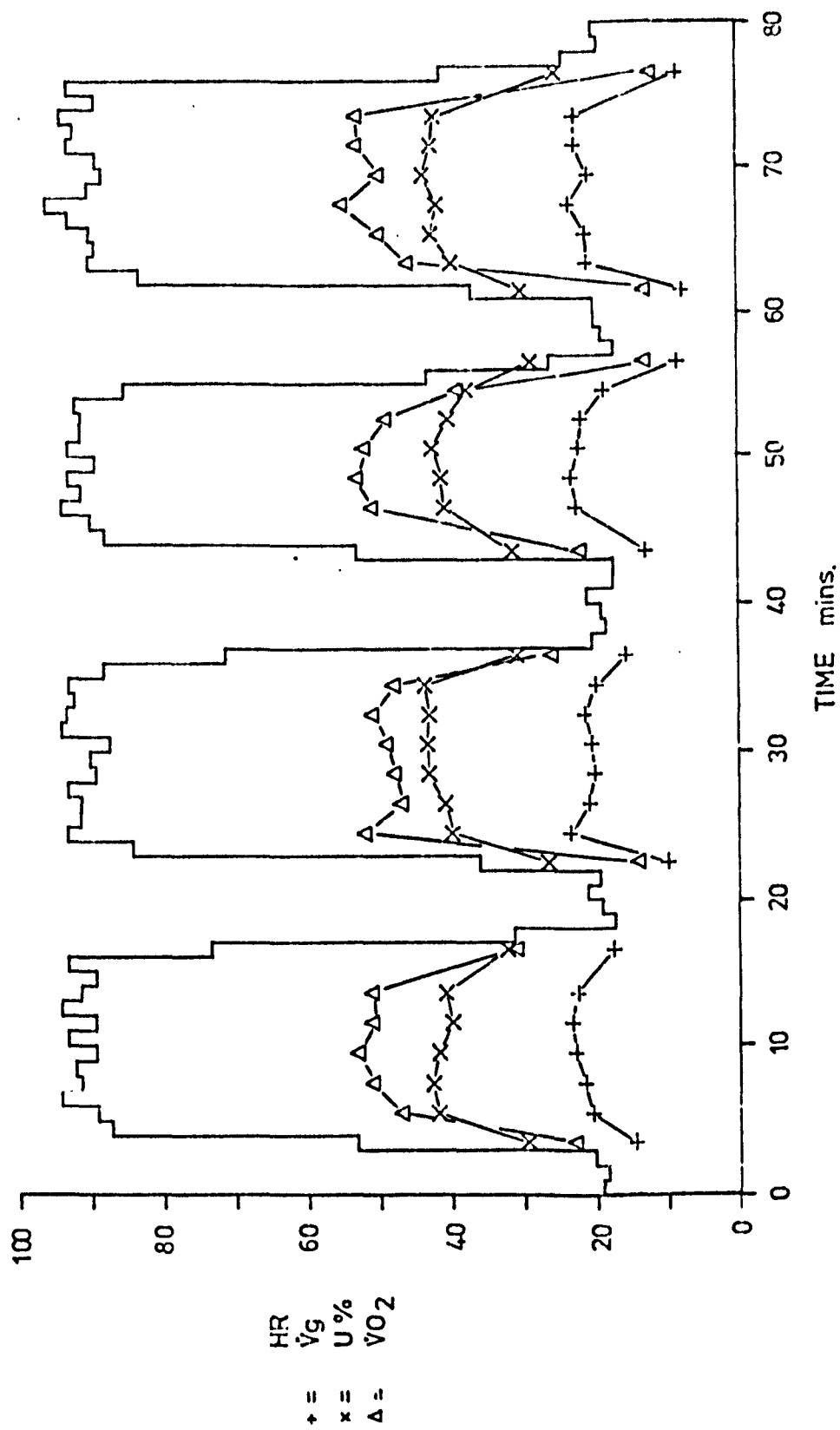
Figure 28.

Showing the relationships between heart rate (histogram, b.p.m.), ventilation volume (Vg, ml min^{-1}) percentage utilisation (% U) and oxygen consumption rate (VO_2, mg hr^{-1} x 10) during the short period rhythm.

TIME mins
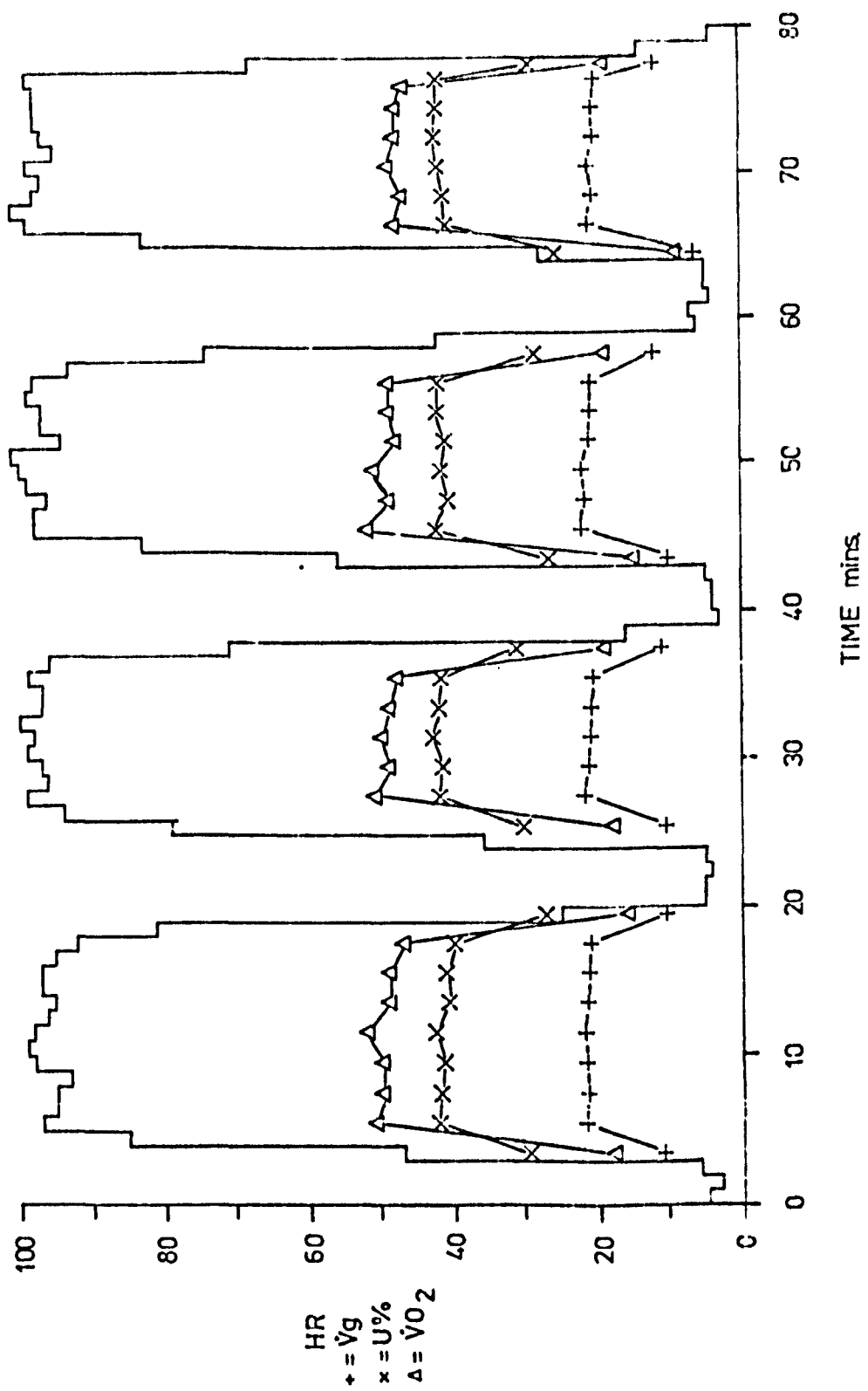
Measurements of exhalent oxygen tension showed that it was
at its lowest level (c. 87 - 91 mm Hg) when both the heart and
scaphognathites were pumping actively. As the beating of these
pumps slowed down the exhalent tension rose to a higher level
of about 110 mm Hg. Throughout the experiment inhalent tension
remained constant at air saturation. The changes in exhalent
tension have been expressed in Figures 26, 27 and 28 in terms
of the % utilisation of oxygen and it can be seen that the
periods of greatest utilisation (40 - 42%) coincided with the
periods of the high-rate phase. As the heart and scaphognathites
slowed down the efficiency of oxygen exchange decreased (25 - 30%).

These changes in ventilation volume and % utilisation are
reflected by the calculated rate of oxygen consumption, the rate
was at its highest level when ventilation, utilisation and heart
rate were greatest. Minor fluctuations in the oxygen consumption
during the high-rate phase seem also to be roughly correlated
with fluctuations in the heart rate.

As experimental technique improved more measurements were
made during each cycle (Figures 27 and 28). The pattern of the
changes found remained essentially similar in all cases.

5.D. Discussion

5.D.i. The relationship between scaphognathite rate and ventilation volume.

Pilkington & Simmers (1973) suggested that scaphognathite beat frequency is the main factor controlling ventilation volume in Cancer novae-zelandiae as their observations showed little change in beat amplitude with increasing frequency. This suggestion is supported by the present observations of ventilation volume and scaphognathite frequency in Cancer pagurus. For the normal range of beat rates the ventilation volume appears to be directly proportional to scaphognathite frequency and thus implies a constant stroke volume. There does remain, however, the possibility that both the amplitude of the beat and the angle of attack change with beat frequency but that these two change in such a way as to maintain stroke volume constant.

At the high beat rates which are shown in the post-operative recovery period, the relationship between scaphognathite frequency and ventilation volume changes and results in a decreased stroke volume. This may be due to changes in the scaphognathite beat amplitude and/or angle of attack of the blade. The gain to the animal of these very high ventilation rates, in terms of oxygen supplied to the animal, decreases as the muscles driving the scaphognathites consume more and more of the oxygen available,

viz., the efficiency of the ventilation system decreases. It is thus to the crab's benefit to use those rates of ventilation and circulation which supply the maximum amount of oxygen for the minimum energy consumption by the pumps, i.e., those rates which correspond to normal active periods of respiration.

When a crab is recovering from stress, however, it may sacrifice efficiency of operation for a rapid re-oxygenation of its tissues. As operations were carried out in air it is possible that an oxygen debt was incurred due to inefficient gaseous exchange with air and that the very high rates of heart and scaphognathite beating were associated with a repayment of such an oxygen debt. Further evidence which possibly supports this idea it that very high rate of scaphognathite and heart rates and oxygen consumption rate were found when a crab was recovering from six hours of total anoxia due to a power failure to the respirometer pumps.

From these observations then it would seem that there is a limit to efficient scaphognathite beating which lies at about normal active rates. The significance of this is discussed later (section 5.D.iv.).

It is also characteristic of the recovery period that there is little fluctuation of the scaphognathite (and heart) beat rate

(Figures 19 and 20). The greater variability in the data in
the normal range may be due to natural fluctuations in the
relationship or, more likely, to the occurrence of reversals
of the scaphognathite beat and thus a reduction of the
ventilation volume for that period of measurements. Reversals
were never observed during the period of recovery from an
operation.

5.D.ii. The form and the effects of starvation on the short
period rhythm.

Several workers have studied the effects of starvation
on the respiration rate of crustaceans (Ansell, 1973; Wallace,
1973; Aldrich, 1975a, 1975b, 1975c). Generally starvation
results in a depression of the active rate of oxygen consumption
and a lengthening of the resting phase of the daily cycle. To
these effects may be added the observation that starvation
depresses both the rate of heart and scaphognathite beating
and the duration of the high-rate phase of the short period
rhythm. Over the period of time studied both of these parameters
seem to decrease approximately linearly with time.

Ansell (1973) found that oxygen consumption rate in
Cancer pagurus declined to a steady level after 3 days for resting
rates and 8 days for active rates. This is in contrast to the
continual decline over the 18 day period found in this study.

The difference is possibly due to the fact that Ansell (1973) used crabs which had been maintained and fed in the laboratory whilst animals used in this study were collected fresh from the field just prior to experiments.

As both scaphognathite rates and heart rates decline with starvation it would be reasonable to assume that the overall oxygen consumption rate of the animal will decline with starvation thus conserving the metabolic stores of the animal. It seems likely that the function of the short period rhythm is to reduce the energy cost of ventilation and circulation (section 5.D.iv.) and that the reduction of the high-rate phase of the rhythm increases this saving.

5.D.iii. % utilisation during the short period rhythm.

As has already been pointed out (section 5.D.i.) it would seem that there is an upper limit to the rate of efficient scaphognathite beating. The data obtained on % utilisation during the different phases of the short period rhythm indicates that, as ventilation volume and heart rate decrease, efficiency of the respiratory exchange decreases. This results in the crab pumping more water to obtain the same amount of oxygen and there is thus an increase in energy cost per unit of oxygen supplied to the body.

The cause of this change in % utilisation may be directly related to ventilation rates. At low ventilation rates the velocity of water over the gill platelets will be low and flow will tend to be laminar. This will result in incomplete mixing of the water over the gills and a build up of a stagnant layer at the gill surface. The barrier to diffusion will thus increase because of this boundary layer. At higher ventilation rates the water velocity between the platelets will be higher and may result in the break down of laminar flow and result in turbulent flow. This turbulence will result in the maintainance of higher pO_2's at the gill surface and more efficient diffusion and exchange.

The values of % utilisation found in this study are higher than the majority of studies in other decapod species (Table 2). Thomas (1954) using a similar method found that utilisation in Homarus was increased if the ventilation was artificially decreased and Johansen et al. (1970) have suggested that this method increases resistance to flow and results in a lower ventilation volume. The high values of utilisation found in this study may have been due to increased ventilation resistance but this does not seem to be likely as values calculated for oxygen consumption from this data agree well with values found for crabs of this size in respirometry (section 3).

5.D.iv. Possible function for the short period rhythm.

Ansell (1973) suggests that there may be optimum rates of ventilation and circulation and once the oxygen demand of the animal falls below the level supported by these rates intermittent ventilation and gill perfusion may occur. The above observations would seem to support this idea.

Efficient ventilation rates seem to be limited to the normal active rates by changes in beat efficiency above this rate (i.e., the excited levels) and changes in exchange efficiency below this level. The energy cost to the animal of obtaining a given quantity of oxygen will be minimum if it is able to use these most efficient levels of ventilation and perfusion. These rates are adequate to supply the oxygen demands of active metabolic rates. During the resting phase, however, the oxygen consumption is, on average, 3.7 times lower (section 3) and it is more efficient for the animal to use the high efficient rates of ventilation and perfusion intermittently than to use less efficient rates, adequate to supply oxygen to the reduced oxygen demand, continuously.

Intermittent ventilation may also serve to increase respiratory efficiency by another means. During the low-rate phase of the short period rhythm oxygen within the body will be depleted by tissue respiration. On the resumption of active

pumping by the heart this will result in a low venous oxygen tension and hence an increase in the diffusion gradient across the gills making oxygen uptake more efficient. This idea is not supported however, by the observations of % utilisation made throughout the cycle.

It is possible to predict from the above that, under certain circumstances, the short-period rhythm might be lost during the resting phase. This will occur if oxygen uptake during the high-rate phase is inadequate to maintain a period of non-pumping by the scaphognathites and heart. This will occur if exchange efficiency during the high-rate phase is somehow impaired or if resting oxygen demand is increased.

Exchange efficiency would be reduced by a decrease in external oxygen tension and Shields (pers. comm.) has shown that, in Australian grapsid crabs, the frequency of the rhythm is dependent on external pO_2 and that below a certain pO_2 the rhythm disappears. In several experiments the temperature of the experimental tank was raised above $12^{o}C$. This would cause an increase in metabolic rate and therefore oxygen demand in the crab and would also reduce the oxygen capacity of the water and the affinity of the haemocyanin. Under these conditions the animal is subject to some respiratory stress compared to lower temperatures and it is significant that the short period rhythm was rarely evident at higher temperatures.

SECTION SIX

CIRCULATORY PERFORMANCE DURING THE RESTING PHASE

6.A. Introduction

Some of the changes in oxygen consumption rates and ventilatory performance during the short period rhythm have been examined in section 5. It is the aim of this section to examine the changes in circulatory performance which accompany the ventilatory changes and then to synthesize the data from both sections to assess the overall respiratory performance during the rhythm.

6.B. Methods

Experiments on the circulation were carried out in the apparatus described in section 5.B. Samples of arterial blood were drawn through a polythene cannula (i.d. 0.95 mm, o.d. 1.32 mm) implanted into the pericardium through the branchiostegite. Venous blood samples were obtained by the same means from the infra-branchial sinus. Cannulae were attached to the exoskeleton with cyanoacrylate adhesive. Cannula placement was checked by pushing a small piece of coloured plastic down the cannula and later dissection. In all cases cannulae were kept as short as possible to minimise dead space.

Samples of blood were analysed for oxygen bound to the haemocyanin (oxygen capacity) by a modification of the method of Laver et al. (1965) for haemoglobin and subsequently used for chlorocruorin and haemerythrin (Wells & Dales, 1974, 1975; Economides & Dales, 1975). This method is based on the fact that when blood is mixed with an excess of potassium ferricyanide solution in a closed vessel, the oxygen in combination with the pigment is released and a rise in oxygen tension in the chamber occurs. The oxygen capacity of the pigment is calculated from this pO_2 rise.

Ferricyanide ions, however, do not displace oxygen from the binding sites of haemocyanin (Cook, 1927; Holde quoted by Miller et al. (1976). It has been shown, though, that thiocyanate ions will combine with the oxygen binding sites of crustacean and gastropod haemocyanins releasing oxygen (Rombauts & Lontie, 1960a, 1960b; DeLey & Lontie, 1968; Rombauts, 1968), one thiocyanate ion combining with one oxygen binding site. The ferricyanide solution was thus replaced by thiocyanate.

The oxygen carrying capacity of the haemocyanin was determined in the following manner:- a small volume of blood, about 150 μl, sufficient to clear the dead space of the cannula was withdrawn and discarded. A 200 μl sample was immediately

taken and injected into air-equilibrated c. 2 mM thiocyanate solution in a cuvette and the resulting rise in oxygen tension was measured by means of a Radiometer oxygen electrode.

The oxygen capacity of the haemocyanin was calculated from:-

$$O_2 \text{ concentration} = \frac{V_T \cdot a_{wO_2} \cdot (pO_2\,_{BT} - pO_2\,_T)}{760} \text{ ml. } O_2$$

and

$$cO_2 = \frac{100}{V_B} \cdot \frac{V_T \cdot a_{wO_2} \cdot (pO_2\,_{BT} - pO_2\,_T)}{760} \text{ vol \%}$$

where:- V_T = vol. of thiocyanate used, ml.

V_B = vol. of blood sample used, ml.

a_{wO_2} = solubility of oxygen in distilled water ml ml^{-1}.

$pO_2\,_{BT}$ = pO_2 of blood/thiocyanate mixture

$pO_2\,_T$ = pO_2 of air eqilibrated thiocyanate solution.

For the volumes and conditions used in this study the oxygen capacity of the haemocyanin (cO_2 vol %) is given by:-

$$cO_2 = 7.18 \times 10^{-2} (pO_2\,_{BT} - pO_2\,_T).$$

This calculation makes the assumption that the solubility of oxygen in the thiocyanate solution is not markedly different

from that in distilled water.

The cuvette used in these experiments was specially designed to accommodate the Radiometer electrode. It was made from "Perspex" and had a central circular chamber, c. 2.5 cm in diameter and c. 2.5 cm deep, containing a small magnetic flea. A lid, with double 'O' ring seals, was fitted with a Luer lock socket to allow the injection of blood samples. The lid was also fitted with a long length (20 cm) of capillary tubing which served to maintain pressure within the vessel at atmospheric pressure while its long length acted as a large diffusion barrier to re-equilibration of oxygen tension with the atmosphere. The exact volume of the chamber was 13.62 ml and when filled with c. 2 mM potassium thiocyanate solution there was approximately 150 times excess thiocyanate assuming a blood oxygen capacity of 10 vol %. Blood oxygen capacities for crustacean haemocyanin-containing bloods are all well below 5 vol % (section 1.A.ii.b. Table 3). A diagram of the apparatus is given in Figure 29.

Samples of blood from Cancer pagurus, Carcinus maenas, Portunus puber and Palinurus elephas (= vulgaris) were equilibrated with air and the fully saturated oxygen capacity determined as described above. Except for Cancer pagurus a 200 μl sample of blood proved to be adequate for a determination

Figure 29.

The cuvette used for the determination of oxygen capacity

of the blood of crustaceans.

LS   =   Luer-lock socket

OT   =   Overflow tube

OS   =   O-ring seals

CC   =   Central chamber

MF   =   Magnetic flea

O_2E =   Oxygen electrode.

107b

LS   OT
                    20cm

OS   CC   MF   O₂E
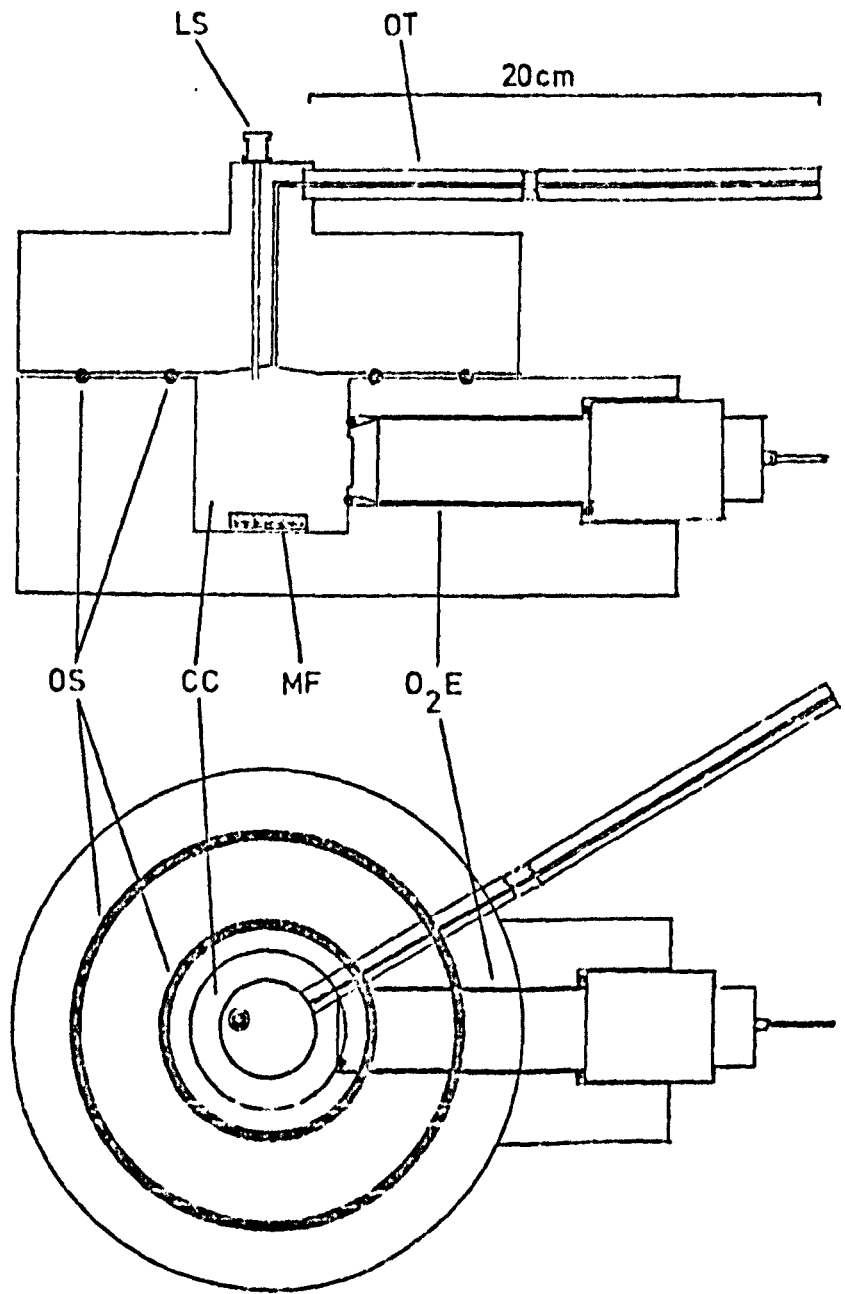
Scale: ⊢——⊣
          5 cm

of oxygen capacity. The measurements made on <u>Cancer</u> gave a

very low oxygen capacity and a much larger sample (500 - 1000 μl)

was necessary to produce a measurable rise in oxygen tension.

Blood copper content was determined by atomic absorption

spectrophotometry, at Stirling University, on diluted (5 or 10

times) blood.

Blood oxygen tensions were measured using the same

sampling technique and a Radiometer electrode housed in a

thermostatted cuvette. A sample of 100 ul was used.

6.C. Results

6.C.i. Blood Oxygen Capacity and Copper Content

Figure 30 shows the results obtained for the oxygen

capacity and copper content of the blood of the species of

decapods used. For <u>Carcinus</u> and <u>Portunus</u> the oxygen capacity

of the blood was found to be between 0.7 - 1.5 vol % and

copper levels were about 5-9 mg Cu per 100 ml. The data

showed a certain degree of correlation between oxygen capacity

and copper level but a more extended study would be needed to

confirm this. The values obtained for <u>Portunus</u> were on the

whole higher than the values for Carcinus, the former lying

between 1 and 1.5 vol % and the latter between 0.7 and 1.2 vol %.

The single value of 0.5 vol % obtained for the blood of <u>Palinurus</u>

Figure 30.

Showing the relationship between haemocyanin oxygen

capacity (vol %) and blood copper concentration (mgCu 100 ml^{-1})

for several decapod crustaceans.

Λ = <u>Portunus</u> <u>puber</u>

□ = <u>Carcinus</u> <u>maenas</u>

◐ = <u>Palinurus</u> <u>elephas</u>

+ = <u>Cancer</u> <u>pagurus</u>

HCY
OXYGEN
CAPACITY

vol %

BLOOD COPPER
mg 100ml^{-1}
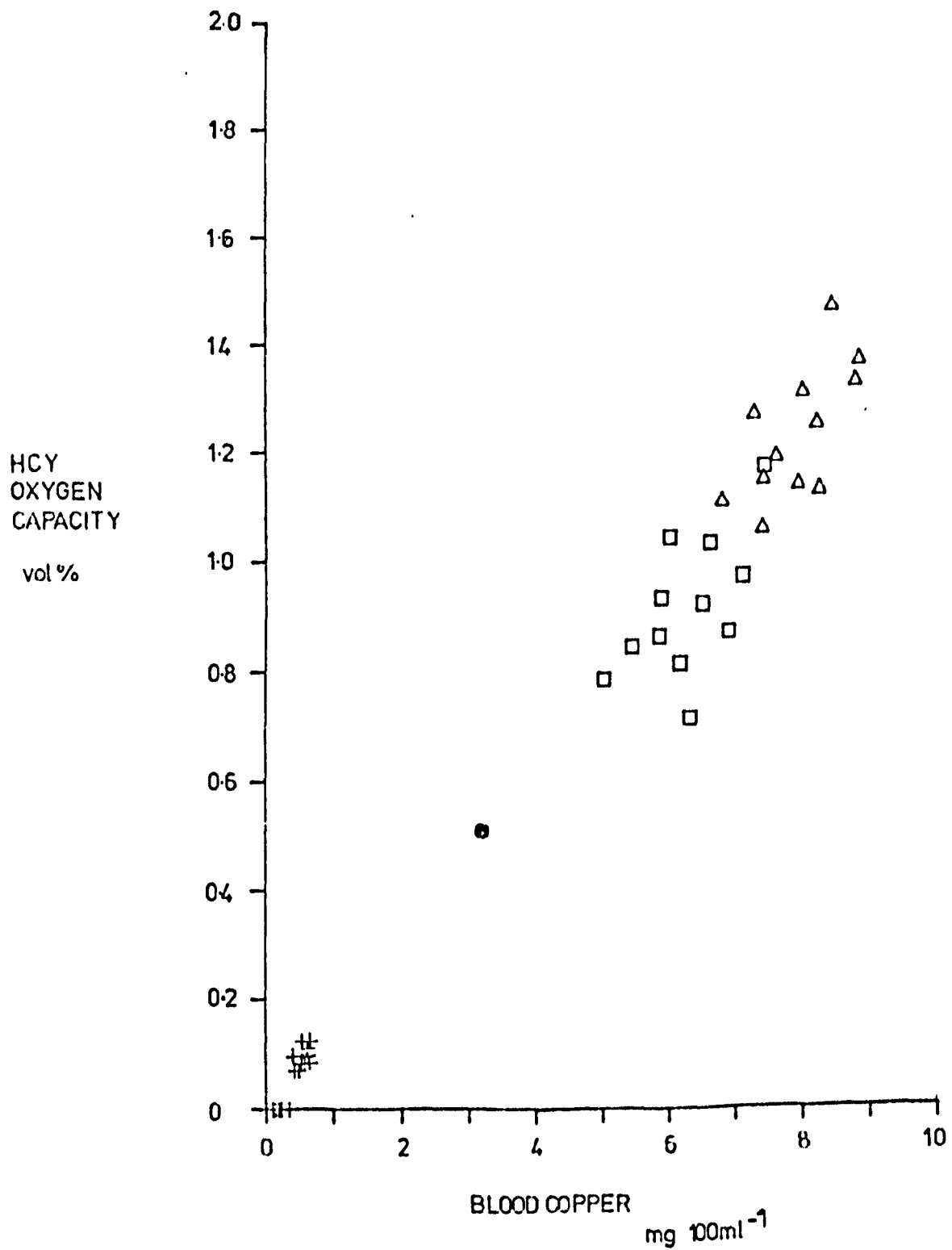
was lower than either of these two. It was, however, obtained from an animal which had been held in captivity for some time and had, perhaps, been subjected to a starvation stress. The oxygenated blood of all of these species was blue coloured to the eye.

As all of the <u>Cancer</u> used for this study were caught fresh, conditions in captivity cannot explain the extremely low values found for blood oxygen capacity and copper levels. The maximum value of oxygen capacity found was 0.13 vol % corresponding to a copper level of 0.65 mg Cu per 100 ml blood. In many cases the oxygen capacity was too low to measure even taking larger samples of blood (500 or 1000 µl). In no case was the haemolymph taken from freshly caught <u>Cancer</u> blue even when equilibrated with pure oxygen.

6.C.ii. Blood gas levels during the short-period rhythm and the effects of starvation.

As haemocyanin appears to be more or less absent from the blood in this population of crabs it may be assumed that the majority of oxygen transport in the body is mediated by the physical solution of oxygen in the blood. Because of this, and because the large samples of blood necessary to determine blood oxygen capacity would have soon caused a severe depletion of the blood volume, arterial and venous (post- and pre-branchial)

oxygen tensions were measured. The oxygen capacity of the
blood was then calculated using a solubility of 6.52×10^{-3}
mlO_2 ml^{-1} blood and a straight line dissociation curve.

No more than one or two determinations of arterial and
venous oxygen tension could be made during the high-rate phase
of any one cycle. In order to obtain measures throughout the
cycle samples were taken at known intervals after the start
of the change from low to high rates. For convenience these
data have been pooled and plotted under an idealised cycle
with the high-rate drawn at the mean level for all the cycles
measured. The results obtained in this way are shown in
Figure 31. It can be seen that, throughout the high-rate phase
of the cycle the arterial pO_2 (p_aO_2) was around 118 mm Hg and
the venous pO_2 (p_vO_2) about 18 mm Hg. During the low-rate phase
these values are lower with a p_aO_2 of 105 mm Hg and p_vO_2 of
8 mm Hg. It can be seen that the rise in p_aO_2 which accompanied
the onset of high-rate phase pumping by the heart preceeded by
about four minutes the rise in p_vO_2.

At the end of the experiment samples of arterial and venous
blood were analysed for oxygen capacity $(c_aO_2$ and $c_vO_2)$. The
samples were taken during the high-rate phase of consecutive
cycles and the values found were $c_aO_2 = 0.113$ vol % and
$c_vO_2 = 0.032$ vol %. In order to obtain these values samples of

Figure 31.

Showing the changes in arterial $\left(p_a O_2\right)$ and venous $\left(p_v O_2\right)$ oxygen tensions throughout a cycle of the short period rhythm.

$$\Delta = p_a O_2$$
$$\nabla = p_v O_2$$
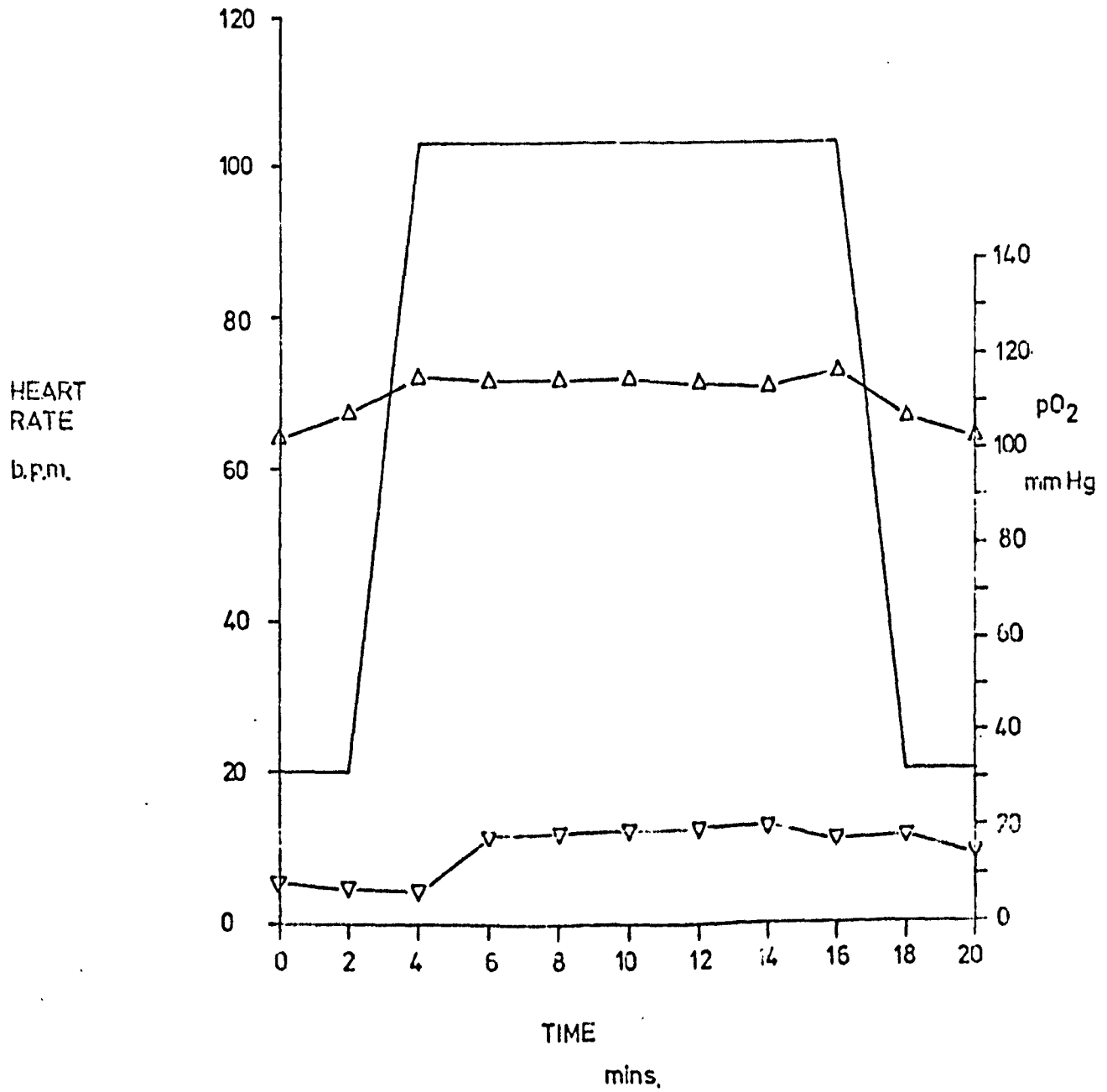
750 µl were taken and the total volume of blood removed throughout the experiment was c. 6 ml.

Experiments were also carried out on starved crabs. Blood samples taken at the beginning of each experiment showed that the fully oxygenated oxygen capacity was always less than 0.14 vol %. Preliminary experiments had shown that the oxygen capacity of the haemocyanin of Cancer was so low as to be immeasurable after one week's starvation. For these reasons, and to avoid reducing the blood volume of the crab excessively measurements were made at the same intervals as in section 5, viz., on day 1, 7, 13 and 18. The results obtained for $p_a O_2$, $p_v O_2$ (mean values of three measurements made on each day) $c_a O_2$, $c_v O_2$ (measured once on each day) and respiration rate are presented in Table 11. Cardiac output was calculated from the Fick equation:-

$$\dot{Q} = \frac{\dot{V}O_2}{\Delta c O_2}$$

For convenience the oxygen consumption rates are presented in $mlO_2 \ min^{-1}$ rather than $mgO_2 \ hr^{-1}$ as before. The former may be converted to the latter by multiplying by 42.

Table 11.

Showing the results obtained for the analysis of blood gas levels throughout the cycle of the short-period rhythm (crab weight = 147 g.).

$c_a O_2$ = oxygen capacity of arterial blood, vol %.

$c_v O_2$ = oxygen capacity of venous blood, vol %.

$\Delta c O_2$ = oxygen delivered to tisses by haemocyanin, $mlO_2\ ml^{-1}$ blood.

$P_a O_2$ = arterial oxygen tension, mm Hg.

$P_v O_2$ = venous oxygen tension, mm Hg.

$\Delta s O_2$ = oxygen delivered to tissue in solution, $mlO_2\ ml^{-1}$ blood.

$\dot{V}O_2^{HR}$ = oxygen consumption rate during the high-rate phase, $mlO_2\ min^{-1}$.

$\dot{V}O_2\ R$ = oxygen consumption rate during the resting phase, $mlO_2\ min^{-1}$.

$\dot{Q}HR$ = cardiac output during high-rate phase.

Days Starv.	c_aO_2 vol%	c_vO_2 vol%	ΔcO_2 ml ml^{-1} x10^2	P_aO_2 mmHg	P_vO_2 mmHg	ΔsO_2 ml ml^{-1} x10^2	$\dot{V}O_2HR$ ml min^{-1} x10^2	$\dot{V}O_2R$ ml min^{-1} x10^2	$\dot{Q}HR$ ml min^{-1}
1	0.126	0.045	0.081	115.0	17.3	0.411	7.35	4.29	14.94
7	–	–	–	114.1	18.1	0.404	5.95	2.58	14.73
13	–	–	–	118.6	17.8	0.424	5.48	1.46	12.92
18	–	–	–	117.7	16.9	0.424	5.25	1.14	12.38

No measurable amount of oxygen bound to the haemocyanin was found after one week's starvation although in each case some copper was present (0.1 mg Cu 100 ml^{-1}). Even on the first day after capture from the wild the amount of oxygen transport mediated by haemocyanin was only 19.7% of that carried in solution in the blood. The levels of oxygen tension in the arterial and venous blood, however, changed little with starvation, giving an oxygen supply to the tissues (ΔsO_2) of about 4.16 x 10^{-3} mlO_2 ml^{-1} blood. Oxygen consumption during the high-rate phase declined with starvation as was found in section 5. Resting oxygen consumption declined more rapidly than high-rate phase oxygen consumption due to the shortening of the high-rate phase (section 5). The values calculated for cardiac output also declined with progressive starvation. This fall was due almost entirely to the fall in oxygen consumption rate as ΔsO_2 changed little with starvation.

6.D. Discussion

6.D.i. Blood Oxygen Capacity and Copper Content.

The values of oxygen capacity found for Carcinus maenas, Portunus puber and Palinurus elephas in this study by the new method agree well with other published values for crustacean haemocyanins (section 1.A.ii.b., Table 3). The values, except

those of Redmond (1962) and Johansen et al. (1970), lying below 2 vol %. Some difference may arise through the use of different techniques as the method used in this study determines the oxygen bound to the haemocyanin whilst previous studies have used either van Slyke apparatus or vacuum extraction which determines total oxygen content.

The values of oxygen capacity found for blood taken from freshly caught Cancer pagurus in the Dunstaffnage area are very much lower than values for other species in the same genus (0.88 - 3.4 vol %, Table 3). This implies a very low haemocyanin concentration in the blood of Cancer pagurus in this locality. Values of oxygen capacity calculated from the measured copper content of the blood, assuming a 1:1 atomic combining ratio of copper to oxygen (Goodwin, 1960) (i.e., 5.67 mgCu 100 ml^{-1}: 1 vol %) gives values which agree closely with the directly measured values. Values for copper concentration in other crustaceans are:- Cancer magister, 4.2 - 8.4 mgCu 100 ml^{-1} (Ellerton et al. 1970); Maia squinado, 0.24 - 8.2 mgCu 100 ml^{-1} (Zuckerkandl, 1957b) and Callinectes sapidus, 16.2 mgCu 100 ml^{-1} (Mangum & Weiland, 1975), and Spoek (1974) gives a value of 11 mgCu 100 ml^{-1} for Homarus gammarus.

The very low blood copper levels are a general feature of the local population and at no season was a crab found with

significant copper levels. Blood protein levels were not measured but observations on the clotting of _Cancer_ blood showed that it never clotted under normal circumstances. Blood samples taken from _Carcinus_, _Portunus_ and _Palinurus_ clotted readily and were a blue colour when oxygenated. Stewart _et al._ (1967) and Stewart & Li (1969) found that measurement of serum protein provided a good index of the condition and nutritional status of the lobster _Homarus americanus_. Stewart _et al._ (1966) have shown a linear relationship between clotting time and fibrinogen concentrations in _Homarus americanus_, clotting time will therefore increase with increasing starvation.

Dall (1974), working on the western rock lobster _Panulirus longipes_, found that, although blood protein concentration did decline with increased starvation, this decline was due to an increase in blood volume. Total blood protein remained constant throughout starvation and moult cycle. He suggests that blood protein level should be used as an index of nutritional status only if blood volume is measured and taken into account.

Zuckerkandl (1957b) has shown that copper concentration in _Maia squinado_ varies throughout the moult cycle, being present in significant quantities only before moulting. Copper levels

in the whole blood volume, unlike the protein levels of Dall (1974), followed the same pattern as the copper concentration in the blood. There was thus little diluting effect of a change in blood volume associated with moult cycle (Drach, 1939). Zuckerkandl (1957b) does not give any details of sampling procedures or animal maintainance but, as the duration of some of the moult stages is so short, it might be assumed that they were held in captivity and were therefore possibly subjected to a starvation stress. All of the decapods used in this study were in the hard C_4 intermoult stage and from the observations of Zuckerkandl (1957b) would be expected to have low haemocyanin concentrations. The results obtained for Carcinus and Portunus seem to contradict the observations of Zuckerkandl (1957b) as high levels of oxygen capacity and copper content were found. All of the Carcinus and Portunus measurements, however, were made on animals which were fully grown and therefore in terminal anecdysis (Carlisle, 1957), and thus, presumably, had an internal chemistry which did not fluctuate in the cyclical manner associated with the moult cycle. Maia squinado also shows the ceasing of the moult cycle and growth at the moult when it becomes mature (the puberty moult) (Teissier, 1935) and may cease to show cyclical fluctuations of blood copper content. To date no work has been done, however, on copper

metabolism in crabs in terminal anecdysis. Zuckerkandl (1957b) must, however, have worked with immature moulting crabs. The situation in Cancer pagurus, however, is different. In Cancer the moult cycle never ceases and growth continues indefinitely (Carlisle, 1957). It might be expected, then, that blood copper levels would show cyclical changes in copper concentration and thus blood oxygen capacity associated with the moult cycle. However, as at no time throughout the year was any significant amount of copper found in the blood and as the blood contained little or no protein, the lack of copper and haemocyanin in the blood of Cancer pagurus would appear to be due to a starvation stress under wild conditions rather than changes associated with the moult cycle.

In this locality Carcinus and Portunus are able to synthesise haemocyanin in apparently normal quantities and it is not obvious why Cancer can not. During collecting dives Cancer has been observed feeding, mostly on Ensis. Deshimaru & Shigeno (1971) have shown, however, that in the prawn Penaeus japonicus growth is markedly affected by the amino-acid balance of the food given as well as by the gross bulk of protein given. If these results are applicable to crustaceans in general it would appear that food quality is important in crustacean nutrition and that some lack in the diet naturally available to Cancer may have caused the apparent starvation in the population studied.

Of previous studies on respiration rate in <u>Cancer</u> only

Johansen <u>et</u> <u>al</u>. (1970) (Table 9) give any indication of the

physiological condition of the animals by quoting a high

value of 3.4 vol % for blood oxygen capacity, corresponding

to a calculated copper concentration of 19.3 mgCu 100 ml^{-1}

assuming all of the oxygen to be bound to haemocyanin.

Respiration rates of <u>Cancer</u> <u>magister</u> (Johansen <u>et</u> <u>al</u>., 1970) and

<u>Cancer</u> <u>pagurus</u> in this study agree quite closely. In spite

of its lack of haemocyanin, then, <u>Cancer</u> <u>pagurus</u> is able to

maintain its respiration rate at levels comparable to those

of animals in presumed good physiological condition. This

implies considerable adaptation of the respiratory dynamics

and, in particular, the circulatory system.

<u>6.D.ii</u>. Blood gas levels during the short period rhythm.

The levels of p_aO_2 and p_vO_2 found in <u>Cancer</u> <u>pagurus</u>

during the high-rate phase do not differ markedly from levels

found in other crustacean studies (Table 12). The largest

difference found is the somewhat higher level of arterial

oxygen tension. This higher level is possibly due to the

lack of haemocyanin in the blood of <u>Cancer</u> <u>pagurus</u> reducing

the oxygen buffering power of the blood and resulting in a

greater rise of p_aO_2 for the same quantity of oxygen diffusing

across the gill surface. Such an increase in p_aO_2 will reduce

the average oxygen tension gradient across the gill surface and

Table 12.

Comparing the values of arterial (p_aO_2) and venous (p_vO_2) oxygen tensions found for Cancer pagurus in this study with those of other crustaceans from the literature.

Species	p_aO_2	p_vO_2	Source
Cancer magister	91	21	Johansen et al. (1970)
Cancer antennarius	98	19	" " " "
Paralithodes camchatica	95	35	" " " "
Carcinus maenas	82	–	Taylor et al. (1973)
" "	97	18	Taylor (1976)
Callinectes sapidus	35	14	Mangum & Weiland (1975)
Homarus americanus	69	18	McMahon & Wilkens (1975)
Cancer pagurus ·	118	18	This study

might result in less efficient exchange. Venous oxygen levels found agree well with values found in other studies.

The lower values of p_aO_2 and p_vO_2 found during the low-rate phase are due to oxygen utilisation from the blood during the period of bradycardia and apnoea. The level of p_aO_2 rises rapidly to the normal high-rate phase levels upon resumption of active pumping as this is a measure of the oxygen level of blood entering the heart directly from the gills.

The lag in the rise of p_vO_2 of about three minutes is a crude measure of the circulation time in Cancer pagurus as it represents the continued arrival of oxygen depleted blood from the low-rate phase, the increased p_vO_2 corresponding to the increased p_aO_2 of three minutes previously at the onset of the high-rate phase.

The data presented in Table 11 allows an estimate to be made of circulation time in Cancer pagurus using the value for cardiac output found and assuming a blood volume of 35% as before (section 1.A.ii.b.). This gives, for a cardiac output of 14.94 ml min^{-1} and a blood volume of 51.5 ml, a circulation time of 3.4 minutes.

This is in good agreement with the value found above but is considerably shorter than the value calculated from the cardiac output data of Johansen et al. (1970) (11.9 minutes).

The value of 3.4 minutes does, however, agree well with other
studies of circulation time in crustaceans (reviewed in
section 1.A.ii.b.). The main reason for this difference in
circulation time between this study and that of Johansen et al.
(1970) lies in the large difference in cardiac output.
Johansen et al. (1970) give a value of 29.5 ml kg^{-1} min^{-1} for
a 1000 g Cancer magister whilst the cardiac output for the
Cancer pagurus above (147 g) is 101.6 ml kg^{-1} min^{-1}. The
latter value is in good agreement with other studies (see
section 1.A.ii.b.). A value of 73.9 ml kg^{-1} min^{-1} may be
calculated for a crab of this weight from the equation of
Spaargaren (1976).

From the data available values for the effectiveness of
removal of oxygen from the ventilatory water, $E_w\%$ =
$(P_iO_2 - P_eO_2 / P_iO_2 - P_vO_2)$ x 100, and the effectiveness of
oxygen uptake from the blood, $E_b\%$ =
$(P_aO_2 - P_vO_2 / P_{sat}O_2 - P_vO_2)$ x 100 may be calculated.
Both of these factors are the ratio of the actual uptake to
the maximum possible uptake. For freshly collected Cancer
pagurus E_w = 49.6% and E_b = 74.0%. Johansen et al. (1970)
found in Cancer magister mean values of 17.2% and 95.6% for
E_w and E_b respectively while Taylor (1976) found for Carcinus
maenas E_w = 21.6% and E_b = 77.6%. McMahon & Wilkens (1975)

give a value of 27% for E_w for Homarus americanus.

In spite of the lack of haemocyanin in its blood Cancer pagurus is able to maintain a high efficiency in the use of oxygen supplied to it in the ventilatory water, the value of E_w being considerably higher than previous values found for crustaceans. This is further supported by the observations of the high extraction efficiencies found in section 5 (40 - 42%) whereas Johansen et al. (1970), McMahon & Wilkens (1975) and Taylor (1976) report values between 16 and 22% at normoxia.

Taylor (1976) reports a value for E_b for Carcinus which is very close to that found here for Cancer pagurus. His values for oxygen capacity are, however, derived from a dissociation curve published by Truchot (1971) and not from actual measurements on his animal population. As there appears to be the possibility of considerable differences in condition between different populations the dissociation curves from different animals must be used with caution. The higher value of E_b reported for Cancer magister by Johansen et al. (1970) is explained by the presence of haemocyanin in the blood. Where haemocyanin is present the blood will reach full saturation at a pO_2 which is equal to the p 100 for the blood, usually much lower than the pO_2 of the environment. Where

animals are relying on oxygen in solution in the blood the
arterial oxygen tension must reach very high levels $(= p_i O_2)$
before it becomes fully saturated giving high values of E_b.
With a counter current exchange system it is theoretically
possible to reach complete exchange in this way in the
absence of a respiratory pigment but only in a system of
infinite area or permeability.

In order to maintain its oxygen uptake at high levels
then, Cancer pagurus with no haemocyanin in its blood, is
able to increase the effectiveness of oxygen removal from
the ventilatory water. As it has no haemocyanin this cannot
be achieved by an alteration of blood chemistry (e.g., pH)
to obtain a more efficient oxygen uptake at the gills or by
changes in the a-v turnover as, without a pigment, very large
changes would have to be made to achieve a significant change
in carrying capacity. The only responses left to the animal
then are changes of the exchange efficiency which rely on
the mechanics of the exchange processes.

It is difficult to imagine crabs being able to change
the physical characteristics of the gill structure as there
are no muscles associated with the gills as in fish where
the gill sieve pore size may be regulated (Hughes, 1966).
The relative flow rates of the ventilatory water and blood

perfusing the gill system is of obvious importance in controlling the rate of oxygen transfer across the gill surface and the ventilation:perfusion ratio gives some measure of this. Johansen et al. (1970) give a mean value of 22 for Cancer magister and Taylor (1976) gives a value of 3.8 for Carcinus maenas although this figure is based on data from Truchot (1971) and, as before, must be treated with care. Values calculated for Cancer pagurus in this study are about 1.7. This is lower than the values quoted above and indicates that the cardiac output is raised compared to the ventilation rate. This will act to maintain the diffusion gradient at a high level across the gill membrane and will thus facilitate diffusion of oxygen into the blood. The maintainance of a steep diffusion gradient will be important when there is no haemocyanin in the blood to act as an oxygen buffer and absorb oxygen without a corresponding increase in pO_2.

In order to maintain its rate of oxygen consumption the main response of Cancer pagurus, in the face of a lack of haemocyanin in its blood, seems to be to increase the cardiac output in relation to the ventilatory rate, to achieve thus an increase in efficiency of the exchange of oxygen across the gills. This is in contrast to the response of Carcinus maenas when faced with decreasing external oxygen concentration which

also causes a decrease in diffusion gradient across the gills.
In _Carcinus_ the ventilation:perfusion ratio increases from
3.8 to 82.5 when external pO_2 decreases from 151 to 30 mm Hg.
This increase is due to both an increase in ventilation
volume and a decrease in cardiac output (Taylor, 1976).

Starvation has already been shown to affect the
respiratory rate and pattern in _Cancer pagurus_ (Ansell, 1973
and section 5). The changes in oxygen consumption rate noted
in section 5 are accompanied by circulatory changes, the
cardiac output falling from 15 ml min^{-1} to 12.4 ml min^{-1} over
the 18 day period of the experiment. This represents a fall
to 83% of the initial value. This is similar to the fall to
70% for heart rate and to 80% for ventilation volume found
in section 5. The changes in circulatory parameters during
the period of the experiment are set out in Table 13. Even
after the loss of the small amount of haemocyanin present on
day 1, there was little change in either ventilation:perfusion
ratio or effectiveness of oxygen uptake by the blood (E_b)
indicating the small role haemocyanin plays in gas exchange
and transport even though at an arterial oxygen tension of
about 118 mm Hg any haemocyanin present must be fully saturated
(assuming a dissociation curve similar to that of _Cancer magister_,
as determined by Johansen et al., 1970). There appears, thus,

Table 13.

Showing the values of some circulatory parameters over a period of starvation of <u>Cancer pagurus</u>. The data is taken from Table 12 and Figure 24.

$\dot{V}_g$ = ventilation volume

$\dot{Q}$ = cardiac output

$\dot{V}_g / \dot{Q}$ = ventilation:perfusion ratio

E_b = effectiveness of oxygen uptake by the blood

Days Starv.	$\dot{V}_g$ ml min^{-1}	$\dot{Q}$ ml min^{-1}	$\dot{V}_g / \dot{Q}$	E_b %
1	25	14.94	1.67	74
7	23	14.73	1.56	70.1
13	22	12.92	1.70	73.0
18	19	12.38	1.53	73.0

to be little adaptation of exchange efficiency to increasing starvation, the main response is simply to reduce oxygen demand by decreasing the length of time spent in the active phase (Ansell, 1973)· and the duration of and level of the high-rate phase of the short-period rhythm in the resting phase (section 5). It is possible that the crab is already operating at near maximal exchange efficiency when brought into the laboratory from the wild due to the apparent starvation existing in wild conditions and has no further capacity to increase the exchange efficiency of the system.

SECTION SEVEN

<u>SUMMARY</u> <u>AND</u> <u>CONCLUSIONS</u>

<u>SECTION</u> <u>ONE</u>

1. The literature of crustacean respiratory physiology was
reviewed and discussed in detail. The review was, for
the most part, confined to the Decapoda.

<u>SECTION</u> <u>TWO</u>

2. The morphometric relationships between carapace width,
fresh weight, dry weight, ash-free dry weight and carapace
length were investigated and regression equations were
calculated. Of the equations linking the various measures
of weight and length only those for male fresh weight and
male ash-free dry weight were found to have a slope
significantly greater than the isometric growth value of
three. It was concluded that males which are longer than
c 70 mm show allometric growth whilst females show isometric
growth throughout their size range.

3. The relationship between log fresh weight and log length
for males was significantly different from the relationship
for females at all size ranges studied.

4. There was no significant difference between the relationships
for log dry weight against log length and log ash-free dry
weight for males and females.

5. The calcium carbonate content of the crabs was analysed and no significant difference between males and females was found.

6. The values for % dry weight and % carbon were calculated and no significant difference between males and females was found. Further, no dependence of either % dry weight or % carbon on length was found.

7. The use of length, fresh weight, dry weight and ash-free dry weight as the basis for comparisons in metabolic studies is discussed. It is concluded that length, fresh weight and dry weight are unreliable and ash-free dry weight should be used.

SECTION THREE

8. The relationship between resting and active oxygen consumption rates and fresh weight, dry weight, and ash-free dry weight were examined by respirometry and regression and covariance analysis. Equations relating oxygen consumption to body weight were calculated for each sex, activity state and measure of body weight separately.

9. By the use of covariance analysis it was shown that in all cases the lines relating oxygen consumption rate to body size for each activity state were parallel across the range of sizes studied indicating a constant scope for activity (3.7).

10. Only when fresh weight was used as the basis of a comparison between males and females was there found to be any difference in slope of the lines.

11. There was no significant difference in slope between any of the lines relating oxygen consumption rate to either dry weight or ash-free dry weight. A line of common slope was thus calculated and its value was found to be 0.799. This value and its relationship to the "universal" value of 0.75 was discussed.

12. The daily pattern of oxygen consumption was examined and it was found that the crabs show a marked diurnal rhythm which varied with the season. The peaks of oxygen consumption rate appeared to phase to dusk and dawn. During the late autumn, winter and early spring this resulted in a bi-modal pattern. During the summer dusk and dawn were so close together that only a single peak was detected.

13. The possible existence of a rhythm of short period of oxygen consumption rate was noted.

SECTION FOUR

14. The relationships between heart beat rate and scaphognathite beat rate, and the pattern of beating of these throughout the day were examined. At all times of the daily cycle

the heart and scaphognathite rates showed a high degree
of correlation with the rhythms of beat rate being exactly
in phase. During the active phase the beat rates were at
a high steady rate while during the resting phase the
beat rates showed the short period rhythm previously
reported. Possible functions for this short period rhythm
were discussed.

SECTION FIVE

15. The ventilatory performance of the crab during the resting
    phase was investigated by means of an apparatus which
    allowed ventilation volume to be measured directly.

16. Over the normal range of scaphognathite beat rates a
    direct relationship between ventilation volume and beat
    rate was found. At high, excited rates of beating,
    however, ventilation was found to be less efficient,
    resulting in higher energy cost per unit volume pumped.

17. The form of the short period rhythm of heart and
    scaphognathite beating was examined. It had a rhythm of
    almost exactly twenty minutes. Each cycle was divided
    into a low-rate phase and a high-rate phase and a period
    of changing rate between each phase.

18. It was found that both the level and the duration of the high-rate phase decreased almost linearly with increasing starvation. It was concluded that this reduction served to further conserve the animal's metabolic stores.

19. Measurements of % utilisation of oxygen from the ventilatory water were made throughout several cycles of the short period rhythm. % utilisation was at its highest (40 - 42%) during the high-rate phase, decreasing to 20 - 25% during the changing phase.

20. It was concluded the most efficient oxygen exchange was limited to the rates of beating of heart and scaphognathite normally found during the active phase, and that the short period rhythm minimised the energy cost of oxygen exchange during the resting phase.

SECTION SIX

21. The circulatory performance during the resting phase was investigated. Measurements of blood oxygen capacity were made by a new technique. The concentration of copper in the blood was also measured.

22. The values of haemocyanin oxygen capacity found for Carcinus maenas and Portunus puber agreed well with previously published values. Values found for Cancer pagurus however were exceptionally low as was blood copper. It was

concluded that haemocyanin was more or less absent from the local _Cancer_ population due to starvation in the wild. Reasons for this apparent starvation were discussed.

23. Arterial and venous oxygen capacities and tensions were measured at different points in the cycle of the short period rhythm and, except for oxygen capacity, the values found did not differ greatly from those previously published for decapods.

24. It was found that oxygen bound to haemocyanin was responsible for only c 20% of the oxygen transport in the body in freshly captured animals.

25. From these measurements a figure of about 3 minutes is suggested for circulation time.

26. Values were calculated for cardiac output and compared to other decapod studies. Calculations from the cardiac output gave a value of 3.4 minutes for circulation time.

27. Values for the effectiveness of oxygen removal from the ventilatory water and the effectiveness of oxygen uptake by the blood were calculated. Effectiveness of oxygen uptake by the blood was found to be lower than earlier studies due to the lack of haemocyanin. The effectiveness of oxygen removal, however, was much higher than earlier studies and it was concluded that this was a response to the lack of haemocyanin.

28. The increased effectiveness of oxygen removal was shown to be associated with a decreased ventilation:perfusion ratio when compared to other studies.

29. It was concluded that the main response of Cancer pagurus to maintain its respiration rate in the face of the lack of haemocyanin was to increase cardiac output relative to ventilation.

30. The cardiac output was found to decrease with increasing starvation. This was accompanied by a decrease in ventilation and thus resulted in little change in ventilation:perfusion ratio and effectiveness of oxygen uptake by the blood.

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APPENDIX

Table 14.

Showing the values of F obtained and the corresponding
significance level from the analysis of covariance for
significant difference between slopes of the data in
figures 8, 9 and 10.

F values:	upper segment
Significance level:	lower segment
degrees of freedom =	1:123

F at 95%	=	5.15
F at 99%	=	8.18
NS	=	non-significant difference

		FW				DW				AFDW			
		♂R	♀R	♂A	♀A	♂R	♀R	♂A	♀A	♂R	♀R	♂A	♀A
FW	♂R		5.76	0.33	6.50	0.09	2.54	0.08	3.47	1.03	1.36	0.11	2.22
	♀R	>95%		6.95	0.20	4.58	0.50	5.69	0.03	2.31	1.14	3.36	0.25
	♂A	N.S.	>95%		7.72	0.70	3.68	0.07	4.60	2.12	2.40	0.67	3.27
	♀A	>95%	N.S.	>95%		5.48	1.12	6.60	0.29	3.27	1.88	4.32	0.69
DW	♂R	N.S.	>95%	>95%	>95%		1.17	0.30	2.70	0.51	0.83	0.01	1.62
	♀R	N.S.	N.S.	N.S.	N.S.	N.S.		2.77	0.20	0.50	0.13	1.23	0.01
	♂A	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.		3.72	1.37	1.67	0.32	0.52
	♀A	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.		1.23	0.58	2.07	0.09
AFDW	♂R	N.S.	>95%	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.		0.09	0.30	0.55
	♀R	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.		0.55	0.19
	♂A	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.		1.21
	♀A	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	

Table 15.

Showing the values of F obtained and the corresponding

significance level from the analysis of covariance for

significant difference between intercepts of the data in

figures 8, 9 and 10.

        F values: upper segment

        Significance level: lower segment

        degrees of freedom =   1:124

        F at 95%   =   5.15

        F at 99%   =   8.18

        NS   =   non-significant difference

		FW				DW				AFDW			
		♂R	♀R	♂A	♀A	♂R	♀R	♂A	♀A	♂R	♀R	♂A	♀A
	♂R		2.36	379	225	194	111	774	517	326	202	813	526
F	♀R	N.S.		380	364	130	121	827	614	329	206	873	535
W	♂A	>99%	>99%		1.39	18.9	17.8	114	67.1	7.88	5.81	200	127
	♀A	>99%	>99%	N.S.		26.7	32.0	115	71.3	2.96	0.64	200	122
	♂R	>99%	>99%	>99%	>99%		0.03	380	342	105	74.5	700	515
D	♀R	>99%	>99%	>99%	>99%	N.S.		338	364	106	76.2	751	524
W	♂A	>99%	>99%	>99%	>99%	>99%	>99%		0.01	74.5	52.8	66.1	45.3
	♀A	>99%	>99%	>99%	>99%	>99%	>99%	N.S.		96.5	84.1	64.9	45.1
	♂R	>99%	>99%	>95%	N.S.	>99%	>99%	>99%	>99%		0.01	380	351
A	♀R	>99%	>99%	>95%	N.S.	>99%	>99%	>99%	>99%	N.S.		341	365
F D	♂A	>99%	>99%	>99%	>99%	>99%	>99%	>99%	>99%	>99%	>99%		0.00
W	♀A	>99%	>99%	>99%	>99%	>99%	>99%	>99%	>99%	>99%	>99%	N.S.	

Table 16.

Summarising tables 14 and 15 , showing the results of the covariance analysis for significant differences between slopes and intercepts for the data in figures 8, 9 and 10.

☆ = significant difference at the 95% level

☆ = significant difference at the 99% level

upper segment = slopes

lower segment = intercepts

	FW				DW				AFDW			
	♂R	♀R	♂A	♀A	♂R	♀R	♂A	♀A	♂R	♀R	♂A	♀A
F ♂R		☆		☆								
W ♀R			☆				☆					
♂A	☆	☆		☆								
♀A	☆	☆			☆		☆					
D ♂R	☆	☆	☆	☆								
♀R	☆	☆	☆	☆								
W ♂A	☆	☆	☆	☆	☆	☆						
♀A	☆	☆	☆	☆	☆	☆						
A ♂R	☆	☆	☆		☆	☆	☆	☆				
F ♀R	☆	☆	☆		☆	☆	☆	☆				
D ♂A	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆		
W ♀A	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆		