Heart rates and amplitudes were measured for both intact animals (in situ) and isolated hearts (in vitro) of the Dungeness crab, Cancer magister, exposed to low levels in oxygen. Recordings were also made of gill ventilation rate by measuring the frequency of scaphognathite (gill bailer) beats.

Both the in vitro heart and the in situ heart beat showed a marked decrease in amplitude and frequency under hypoxic stress. Upon the return of oxygen to the environment, the recovery of the hearts of the intact animals showed very short latent periods while the recovery of the isolated hearts was very slow.

The response of the scaphognathites to hypoxia was biphasic. Upon the initial decrease in oxygen concentration, they showed an increase in beating frequency; with continued lowering of the oxygen concentration, the rate fell off considerably.

In analysing the results it was concluded that such a decrease in
heart rate could be adaptive in that it could result in a lowered energy demand at low oxygen concentrations. The rapid recovery of the in situ hearts may indicate the presence of a receptor for oxygen.
Cardiac and Respiratory Responses to Hypoxia in the Crab, *Cancer magister* (Dana)

by

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Typed by Mary Jo Stratton for Daniel Francis Stiffler
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**Figure 14** Typical recording of scaphognathite beats using a pressure transducer.
CARDIAC AND RESPIRATORY RESPONSES TO HYPOXIA IN THE CRAB, CANCER MAGISTER (DANA)

INTRODUCTION

Responses of the crustacean heart to deficiencies in environmental oxygen concentration have not been extensively studied. Those investigations which have been made deal almost entirely with changes in heart rate, and have ignored concomitant changes in amplitude. The field has recently been reviewed by Maynard (1960a) and Lockwood (1967).

Burger and Smythe (1953) noted that the heart rate of the lobster, Homarus americanus, was slower when the animal was out of the water. In this situation the gill filaments would be collapsed and the effective exchange area would be reduced, thereby placing the lobster in an hypoxic condition. A similar observation was made on Carcinus maenas (Jones, 1964, personal communication to Lockwood, 1967). In his studies on the crayfish, Procambarus simulans, Larimer (1962, 1964b) demonstrated a marked bradycardia (decrease in heart rate) when oxygen was driven from the water. Thompson and Pritchard (1969) subjected the burrowing shrimp, Callianassa californiensis, to hypoxia and noted a reduction in heart rate only at very low oxygen tensions. The low threshold for bradycardia was interpreted as being part of the regulatory mechanism which allows these animals to exist in burrows where there is wide fluctuation in
oxygen concentration.

In contrast to the scarcity of information on cardiac responses to hypoxia among the crustacea, there exists considerable data regarding the respiratory adjustments. This has been extensively reviewed by Wolvekamp and Waterman (1960). An early notice of respiratory responses to hypoxia was reported in *Limulus polyphemus*, a xiphosuran, by Hyde (1906, cited by Waterman and Travis, 1953). It was noted that saturating the water with hydrogen (thereby driving off the oxygen) caused a decrease in the beating of the book gills. Fox and Johnson (1934) examined the responses of the respiratory appendages to oxygen deficiency in a variety of crustaceans. The crayfish, *Astacus fluviatilis*, was shown to accelerate scaphognathite beating during oxygen stress. The barnacle, *Balanus balanoides*, and the phyllopod, *Cheirocephalus diaphanus*, however, did not show an acceleration of respiratory movement under hypoxia. The isopods, *Asellus aquaticus* and *Ligia oceania*, were also examined: *Asellus* showed an increase in pleopod beating under hypoxic conditions; *Ligia* did not but did show a decrease in pleopod beating under hyperoxic conditions. For the amphipods examined, *Gammarus pulax* accelerated its pleopod beat while *G. locusta* showed only a transitory increase when oxygen was displaced from the environment. Continuing this investigation, Johnson (1936) reported the responses to hypoxia for several other crustaceans. The stomatopod, *Squilla mantis*,
accelerated beating of not only its pleopods but also of the epipodites of its maxillipeds. These are homologous to the scaphognathites of higher crustacea and have respiratory function in larval *Squilla* (Johnson, 1936). The prawn, *Pandalus borealis*, also showed an acceleration in rate of beating of its respiratory appendages, in this case scaphognathites, when subjected to hypoxia. The crab, *Carcinus maenas*, had an irregular scaphognathite beat rate and showed no changes which could be correlated with oxygen concentration. Arudpragasam and Naylor (1964), however, were able to demonstrate quite clearly the increase in ventilation rate in this crab. The isopods, *Idotea neglecta* and *Cirolana borealis*, both showed acceleration of pleopod beating while *Anilocra physodes* and *Nerocila bivittata* did not.

Attempts to repeat the hypoxia-induced acceleration of scaphognathite beating in *Astacus* reported by Fox and Johnson (1934) have, in general, confirmed their findings. However, Segaar (1934) attempted to record this rate function mechanically with recording levers attached to the scaphognathites and showed an inhibition of ventilation rate. Jordan and Guittart (1938) duplicated the results of Fox and Johnson (1934) by not using levers; so it appears that hindrance of the scaphognathites changes their responses. Lindroth (1938) showed the hypoxia-induced acceleration of scaphognathite beat for *Astacus*. He also demonstrated an increased pumping volume under
these conditions. Van Heerdt and Krijgsman (1939) showed a response similar to that of Astacus in the wooly mitten crab, Eriocheir sinensis. Working with the lobster Homarus vulgaris, Thomas (1954) observed a decrease in scaphognathite rate when he subjected the animal to hypoxia. Larimer (1961) and Larimer and Gold (1961) reported that the ventilation volume increases in P. simulans subjected to hypoxia. The scaphognathite beat rate has also been recorded using a pressure transducer (Larimer, 1964a, b), and the characteristic acceleration was observed. Two burrowing shrimp, Callianassa californiensis and C. affinis, have been investigated by Farley and Case (1968). They report that hypoxia resulted in sustained hyper-ventilation in both species although the former beat its pleopods at the highest rate upon readmission of oxygenated water.

It appears that the response of most crustaceans to hypoxia is a cardiac slowing accompanied by an increased rate of pumping water across the gills. There are several exceptions to the response of the scaphognathite, however.

A few investigations have attempted to correlate the respiratory adjustments of crustaceans to their ecology. A study was made by Walshe-Maetz (1952) which attempted to demonstrate a relationship between these responses and the availability of oxygen in the normal environment. She compared respiratory responses to hypoxia in four species of amphipods and four species of isopods from four different
environments. The results showed that species from environments with a more constant oxygen concentration demonstrated little or no hyperventilation during oxygen stress. On the other hand, species from the more variable environments (such as "hot algae-choked lagoons") showed a very definite hyperventilation response. Of the 14 species examined by Fox and Johnson (1934) and Johnson (1936), the shore crab, Carcinus, the barnacle, Balanus, and the two isopods, Anilocra and Nerocila, did not show the acceleration of ventilation. The results of Arudpragasam and Naylor (1964), however, indicate that Carcinus does accelerate the beating of its scaphognathites under hypoxia. Balanus, which lives in the intertidal, is well aerated when covered, although it may experience oxygen stress at low tide. The two isopods are ectoparasites of fish and probably inhabit a relatively oxygen rich environment (Johnson, 1936). It appears then that there is at least some correlation between "constancy" of the environment and the ability to regulate ventilation rate. Not enough is known about the cardiac response to make a similar statement about the regulation of heart beat.

The present investigation is an attempt to establish the responses of the heart and the scaphognathites to hypoxia in the Dungeness crab, Cancer magister. Previous investigations of cardiac response to low oxygen have concentrated on heart rate. This study will also deal with the effect of hypoxia on amplitude.
It is not known for certain whether the slowing of the heart is due to the effect of lack of oxygen directly on the cardiac muscle or if there is a nervous or endocrine inhibition of the cardiac ganglion in response to reduced oxygen tension. Similarly, whether the recovery is due directly to the availability of oxygen or if it is signaled via nervous or endocrine elements is unknown. External control would, of course, imply the existence of an oxygen receptor.

Eyzaguirre and Koyano (1965) have presented direct evidence for a mammalian oxygen receptor located in the carotid artery. This was accomplished by recording nerve impulses, associated with changes in oxygen concentration, from a receptor neuron. There also exist several bits of indirect evidence for oxygen receptors in arthropods. Waterman and Travis (1953), working with the horseshoe crab, *Limulus polyphemus*, hypothesized an oxygen receptor to explain a very rapid recovery from an hypoxia-induced arrest of gill beats. When the oxygen concentration was lowered to 0.5 ml/l, the amplitude and frequency of the book gill beats decreased steadily. The final result of hypoxia was complete arrest of breathing. When oxygen was reintroduced into the water, the gill beat became normal within five seconds. Attempts to locate the site of the receptor were unsuccessful other than to establish that it was probably external in position.

Farley and Case (1968), in their study of the two burrowing shrimp, *Callianassa californiensis* and *C. affinis*, reported increased
pleopod beating a few seconds after reintroduction of aerated seawater into a container of hypoxia-stressed animals. This was interpreted as evidence for an external oxygen receptor. The animals were also observed to migrate towards the intake end of their container upon the reintroduction of aerated water.

Additional evidence for an oxygen receptor comes from the study of Larimer (1964a) on the crayfish, *Procambarus simulans*. Upon recovery from gamma aminobutyric acid-induced cardiac arrest, there occurred a brief, reproducible scaphognathite beat inhibition. This was cited as evidence for an internal oxygen receptor located somewhere in the circulation. It was reasoned that during the cardiac arrest the blood lying stationary in the gills would become oxygen saturated. Upon return to circulation, this blood might encounter an oxygen receptor, thus triggering the observed inhibition of scaphognathite beat.

If the oxygen acts directly on the heart, one would expect no difference between the response of the isolated heart and the response of the heart of the intact crab to hypoxia. In an attempt to shed light on this problem, we have studied the *in vitro* heart in the same way as the *in situ* heart.
METHODS AND MATERIALS

This study was carried out during the summer of 1969 at the Oregon State University Marine Science Center at Newport, Oregon, and during the winter of 1969 to 1970 at the Oregon State University campus.

Adult Cancer magister having a carapace width ranging from approximately 10 to 18 centimeters were used in the study. All animals were collected in Yaquina Bay near the laboratory using a commercial crab trap belonging to the OSU Marine Science Center. In addition, a few crabs were taken by net from the Sally's Slough area further up the bay.

At the Marine Center, animals were maintained in either indoor or outdoor holding tanks supplied continuously with sea water.

The animals used in Corvallis were transported in buckets covered with wet towels. These crabs were maintained in polyethylene aquaria at 12°C in constant temperature rooms. Two animals were placed in each aquarium and the sea water was changed every two days.

All experiments were performed at 12°C ± 1°C, which approximates the temperature in Yaquina Bay. Temperature control of the experimental setups at Newport was achieved by circulating water maintained at 12°C using an Aminco constant temperature circulator. For the investigations at Corvallis, the temperature was maintained by combining a Precision Scientific Company portable cooling unit with
a Bronwill thermoregulator. The experimental containers in the constant-temperature baths were shallow polyethylene pans containing just enough water to cover animals used for scaphognathite rate determinations. For the in situ heart measurements the water line was kept below the patch of hypodermis which was exposed for the insertion of the myograph hooks.

The observed cardiac and respiratory responses were monitored with a desk model Physiograph, type DMP-4A, manufactured by the E and M Instrument Company of Houston, Texas. Heart rate and amplitude were recorded with an E and M type B Photoelectric Force Transducer Myograph. Scaphognathite beat rate was recorded with an E and M Linear Core Pressure Transducer, model P-1000. This transducer is sensitive to pressure changes of the magnitude produced by the beating of the scaphognathites. It was connected to the exhalant canal of one gill cavity via a distilled water column in 3/16" tygon tubing. Pressure changes corresponding to scaphognathite beats were transmitted via the column of water to the transducer.

Oxygen concentration in the experimental containers was manipulated by passing nitrogen or air directly into the water or perfusion solution. Nitrogen pressure from the cylinder was regulated with a Hoke-Phoenix Nitrogen Regulator. A Yellow Springs Instrument Company Model 54 Oxygen Meter and Model 5420 Oxygen Probe were used to monitor oxygen concentration in the testing bath.
The oxygen probe was re-calibrated at the beginning of each experiment against oxygen saturated sea water. Sea water was assumed saturated after one to two hours of aeration at low pressure. The low level of aeration was to prevent super saturation. Any instability in the meter indicated the necessity of replacing the membrane on the probe.

Preparation of animals for in situ recording of cardiac functions and scaphognathite beat rate was accomplished by cutting off the legs with bone cutters and crushing the stubs. This induced autotomy of the limbs. A few animals were used without removing the walking legs and there was no discernible difference in their responses. After the legs were removed, the animals were secured to a Lucite restraining board. After the removal of a piece of carapace overlying the heart, a bent pin was inserted into the hypodermis and connected by a thread to the myograph. A thermometer, the oxygen probe and air stones were placed in the container which was covered with a piece of Lucite to restrict the entry of air. The setup for scaphognathite beat recordings was similar except that the tube from the pressure transducer was inserted into an exhalant canal and tied in place.

The preparation for in vitro heart beat recordings involved a modification of a method described by Welsh and Smith (1960). After removal of all legs, a large section of carapace overlying the viscera was carefully removed, taking care not to damage the hypodermis. The
pericardial cavity was then opened and ligatures were tied around the posterior lateral connectives of the heart. These later served as a convenient means of handling the heart while removing it from the pericardial cavity. The next step was to make a small incision in the abdominal artery and insert a polyethylene canula approximately 1/16" in diameter, with a flared end. The canula was tied off and tested for leaks with perfusion solution. The final step before removal of the heart was to tie off the anterior arteries leaving sufficient thread to tie to the myograph. The heart was then cut free from the pericardial cavity and placed in the recording chamber (Figure 1). During the isolation of the heart care must be taken so as not to damage the hepatopancreas; enzymes from this organ might otherwise destroy the heart. The canulated heart was finally mounted on the perfusion tube and perfusion was initiated. The rate of perfusion was regulated with a Teflon needle valve manufactured by the Manostat Corporation.

Shortly after initiating perfusion the heart resumed its rhythmic beat with a fairly steady rate and amplitude. Before a preparation was judged usable it had to meet the following criteria: display of reasonably stable rate and amplitude; recovery to normal rate and amplitude after oxygen stress; and response to changes in perfusion rate with corresponding changes in rate and amplitude. An increase in perfusion rate has been shown to result in an increase in heart rate and amplitude for Maia squinado (Izquierdo, 1931).
Perfusion solution

Thermometer

O₂ probe

To the myograph

Air or N₂

Drain

Canula

Rubber stopper

Figure 1. Isolated heart chamber.
All hearts used were perfused with a solution first used by Pantin (1934) and later modified by Davenport (1941). The solution was as follows:

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<th>Concentration</th>
<th>Volume</th>
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<tr>
<td>NaCl</td>
<td>0.53 m</td>
<td>1 liter</td>
</tr>
<tr>
<td>KCl</td>
<td>0.53 m</td>
<td>25 ml</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.53 m</td>
<td>30 ml</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>0.53 m</td>
<td>25 ml</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>1.00 m</td>
<td>2 ml</td>
</tr>
<tr>
<td>Dextrose</td>
<td>-</td>
<td>1 gm</td>
</tr>
</tbody>
</table>

The same protocol was used in the experiments with intact animals (in situ preparation) and isolated hearts (in vitro preparation) in determining cardiac and respiratory responses to hypoxia. After the recording instruments were attached, the animal was allowed to stabilize (about ten minutes) and the oxygen concentration of the water was recorded. Nitrogen was then passed through the water for a short period. After the preparation and the oxygen concentration had again stabilized and the oxygen concentration had been recorded, the procedure was repeated. This was done repeatedly until the oxygen concentration had fallen below 1 mg O₂ per liter of sea water. Air was then re-introduced into the water and the rate and amplitude functions were allowed to return to normal. During the recovery period the oxygen concentration was periodically recorded in order to evaluate the latency of the response to oxygen.
RESULTS

Response of the Heart to Hypoxia

The heart rates of the crabs examined were found to vary somewhat from animal to animal, but were fairly constant from one experiment to the next for a given animal. The mean frequency for in situ hearts when beating at maximum rate was 79 beats per minute with a range of from 72 to 92. The mean for in vitro hearts at maximum rate was 54 beats per minute with a range of from 37 to 81.

Since this investigation is concerned only with relative values, the data are best examined in terms of the percent of maximum frequency. Maximum frequency is defined as the highest heart rate achieved during the course of an experiment whether or not that rate came before or after the stress period. To prevent the possibility of random accelerations of short duration from biasing the data, these maximum rates had to be sustained for a period of at least three minutes. The resultant values are best termed relative frequency.

The amplitude recorded by the myograph was directly related to the absolute amplitude of contraction of the heart but varied with the tension on the thread connecting the heart to the myograph and the adjustment of the amplifier. It was impossible to precisely control the tension on the thread so that it would be constant from one animal to the next. Similarly, the need to adjust the amplifier separately for
each animal precluded any constancy in settings. As a result, interpretation of the data in terms of absolute amplitude is impossible. It is possible to convert the data to relative amplitude if the tension on the thread and the setting on the amplifier are kept constant for each experiment. One other problem still had to be resolved: in some of the recordings amplitude varied slightly from contraction to contraction but was maintained at an overall general level. To determine relative amplitude in these cases, the heights of ten consecutive peaks, chosen at random, were averaged.

The response of the in situ heart to reduced oxygen concentration in the surrounding environment was shown in numerous tests to be a marked bradycardia accompanied by a decrease in amplitude. The response was quite reproducible. Figures 2 and 3 display results of eight runs on six different crabs. Both amplitude and frequency dropped to less than 50 percent when the oxygen concentration went below one mg $O_2$/l.

Isolated hearts showed the same general response pattern to hypoxia as hearts of intact crabs. Again, the decrease in frequency and amplitude was marked and reproducible. Figures 4 and 5 represent six runs involving four different isolated hearts. Here again it is seen that both rate and amplitude fell below 50 percent when the oxygen concentration fell below one mg $O_2$/l.

The time course for the recovery of the hearts to near the
Figure 2. Heart rate (± S. E.), expressed as a percent of the maximum, versus oxygen concentration (mg O₂/l sea water) for in situ hearts. The curve represents mean results from eight experiments on six animals.

Figure 3. Heart amplitude (± S. E.), expressed as a percent of the maximum, versus oxygen concentration (mg O₂/l sea water) for in situ hearts. The curve represents mean results from the same eight experiments shown in Figure 2.
Figure 4. Heart rate (± S.E.), expressed as a percent of the maximum, versus oxygen concentration (mg O₂/l sea water) for isolated hearts. The curve represents mean results from six experiments on four hearts.

Figure 5. Heart amplitude (± S.E.), expressed as a percent of the maximum, versus oxygen concentration (mg O₂/l sea water) for isolated hearts. The curve represents mean results from the same six experiments shown in Figure 4.
maximum rate upon readmission of oxygen to the environment differed greatly between the \textit{in situ} and \textit{in vitro} preparations.

The \textit{in situ} hearts demonstrated a very rapid and reproducible recovery upon reoxygenation of the water. Within one minute after the initiation of aeration, the frequency rose from below 35 percent to above 85 percent of maximum (Figures 6 and 8). The amplitude rose from below 25 percent to above 50 percent during the same period (Figures 7 and 8).

In the case of the \textit{in vitro} heart preparation, the recovery was relatively slow. As noted in Figures 9, 10 and 11, about six minutes elapsed between the beginning of aeration, when rate and amplitude were 45 percent and 59 percent of maximum, and a recovery of 75 percent frequency and 85 percent amplitude.

As a precaution against the possibility that the response may be a mechanoreceptive one, originating with the turbulence caused by the bubbling, several crabs were subjected to sudden vigorous nitrogen bubbling while under oxygen stress (Figure 12). In no case was there a duplication of the aeration response. In fact, it was noted that on several occasions, a cardiac arrest occurred upon nitrogen bubbling when the heart was beating at maximum or near maximum rate (Figure 8 top trace).
Figure 6. Time course for the recovery from hypoxia of the in situ heart. Closed circles are the heart rate ($\pm$ S.E.) expressed as a percent of the maximum. Open circles are oxygen concentration ($\pm$ S.E.) in mg O$_2$/l sea water. These curves represent mean results from 19 experiments on nine animals. The arrow indicates the initiation of aeration.
Figure 7. Time course for the recovery of in situ hearts from hypoxia. Closed circles are amplitude (± S.E.) expressed as a percent of the maximum. Open circles are oxygen concentration (mg O₂/1 sea water). The curves represent the mean results from the same 19 experiments shown in Figure 8. The arrow indicates the initiation of aeration,
Figure 8. Typical recording of the heart beat of an in situ heart. The top trace shows the pre stress rate. The O\textsubscript{2} stress period corresponds to the period during which nitrogen was passed through the water. The bottom five traces show the effects of, and recovery from, hypoxia. Oxygen concentrations are indicated in mg O\textsubscript{2}/l sea water.
8.3 mg/l  start \text{O}_2  7.8 mg/l  \text{O}_2\text{stress}

\text{start air}

0.6 mg/l

2.8 mg/l  4.0 mg/l

5.2 mg/l  6.2 mg/l
Figure 9. Time course for the recovery of isolated hearts from hypoxia. Closed circles represent heart rate (± S.E.) expressed as a percent of the maximum. Open circles represent oxygen concentration (mg O₂/1 sea water). The curves represent mean results from 19 experiments on seven hearts. The arrow indicates the initiation of aeration.
Heart Rate (% maximum)
Oxygen Concentration (mg O₂/l)

Time (minutes)
Figure 10. Time course for the recovery of isolated hearts. Closed circles represent heart amplitude (± S.E.) expressed as a percent of the maximum. The open circles represent oxygen concentration (mg O₂/l sea water). The curves represent mean results from 19 experiments on the same seven hearts shown in Figure 11. The arrow indicates the initiation of aeration.
Figure 11. Typical recording of the heart beat of an isolated heart. The top trace is the pre stress rate. The $O_2$ stress period corresponds to the period during which nitrogen was passed through the water. The bottom five traces show the effects of, and recovery from, hypoxia. Oxygen concentrations are indicated in mg $O_2$/l sea water.
6.8 mg/l

start N<sub>2</sub>

0.7 mg/l

0.6 mg/l

start air

1.0 mg/l

2.5 mg/l

3.7 mg/l

5.4 mg/l

6.1 mg/l

6.6 mg/l

7.2 mg/l

0.2 stress
Figure 12. Recordings showing the failure of the in situ heart to respond to turbulence caused by vigorous nitrogen bubbling. Oxygen concentration is indicated as mg O₂/l sea water.
0.7 mg/l $N_2$ air 1.6 mg/l $N_2$

$N_2$ 0.9 mg/l $N_2$ $N_2$ 0.8 mg/l

air 2.0 mg/l start air
Response of the Scaphognathites to Hypoxia

The recordings of scaphognathite beat rate during hypoxia showed considerable variability. The mean value for maximum ventilation rate was 128 beats per minute with a range of from 76 to 182. As was done in the case of heart rate, relative frequency was computed as a percent of the maximum frequency. No attempt was made to determine amplitude changes but the general trend seemed to be an increase in amplitude concomitant with the increase in frequency.

The response of the scaphognathite beat rate to hypoxia was quite different from the cardiac response (Figures 13 and 14). The gill bailer frequency gradually increased as oxygen concentration declined to a certain level, at which the trend reversed and the frequency decreased. Figure 13 represents 12 runs on ten animals. Figure 14 is a portion of a typical recording of the scaphognathite beat. This recording shows the apparent direct relationship between frequency and amplitude. Upon return of oxygen to the water the response of the scaphognathite was reversed, gradually accelerating to its maximum, then slowing at high oxygen concentration.
Figure 13. Scaphognathite beat rate (± S. E.), expressed as a percent of the maximum, versus oxygen concentration (mg O₂/1 sea water). The curve represents the mean results from 12 experiments on ten different animals.
Figure 14. Typical recording of scaphognathite beats using a pressure transducer. Each line is a portion of the recording at the indicated oxygen concentration. Periods of nitrogen bubbling are indicated by the symbol N$_2$. 
28.7 mg/l

30 sec.

5.3 mg/l

3.8 mg/l

2.2 mg/l

stop N₂

0.4 mg/l
DISCUSSION

The variation observed in the maximum heart rate probably results from several factors. Since the animals were held for different lengths of time without feeding, their nutritional state might have varied. There is evidence that body size effects heart rate (Maynard, 1960b) although the effects are over a wider range in sizes than was employed here. No effort was made to determine the age of the individuals; therefore, it could have had an effect on the variability. In addition to these possibilities there is some evidence that there may be a relationship between intensity of illumination and heart rate. Maynard (1960a) points out that there are differences in heart rate between light and dark adapted Daphnia. Finally, it must be stated that differing levels of "trauma" following preparation of the animals undoubtedly had some effect on the level of heart rate.

It is felt, however, that since relative frequencies and amplitudes were employed and since the rates and amplitudes were quite stable within a given preparation, the variability did not enter into the interpretations of the data that follow.

A persistent feature of all in situ recordings was a periodic cardiac arrest occurring to varying degrees and at various times among different animals. This arrest was observed to occur both in systole and in diastole and was often seen either accompanying or just preceding limb or limb stub movement. Needham (1954) observed
similar inhibition in the isopod, *Asellus aquaticus*, when the heart beat briefly ceased prior to limb movement. Maynard (1960b) reports several such cardiac arrests in the lobster, *Panulirus argus*, chiefly in the second stage larvae. One such cessation of heart beat was accompanied by an abdominal flexure. Cardiac arrest has also been associated with feeding behavior in the crayfish, *Procambarus clarkii* (Larimer and Tindel, 1966).

The arrests reported here were in intact animals, never in isolated hearts so it appears that they were dependent on a nervous input. In light of this, and considering the correlation with muscular activity in other crustaceans, it seems probable that the inhibition of heart beat was of proprioceptive origin.

The observed decrease in heart rate resulting from hypoxia appears to be a general response in crustacea. Larimer (1962), studying the crayfish, *P. simulans*, observed a decrease in heart rate from approximately 115 beats per minute to less than 60 beats per minute, i.e., there was nearly a 50 percent decrease. Thompson and Pritchard (1969) reported a greater than 50 percent decrease in heart rate for *Callianassa californiensis* under oxygen stress. One insect (the larval form of the mosquito *Anopheles quadrimaculatus*) also has been shown to reduce its heart rate under conditions of almost complete anoxia (Jones, 1956).

Before one can make a statement concerning an effect on rate of
circulation he must determine two factors; the frequency of pumping (heart rate) and the stroke volume. A heart beating at a reduced rate but with an increased stroke volume may move blood through the circulatory pathways as rapidly as a faster beating heart with a smaller stroke volume. Indeed, such a slower heart is more efficient as it does the same amount of work with a lesser expenditure of energy. Changes in stroke volume may be crudely estimated by changes in amplitude. Amplitude as well as frequency decreases when the animal is subjected to hypoxia (Figures 2 and 3). In fact, amplitude decreases to a greater extent in the in situ heart than does frequency, although the reason for this is not clear. The fact that both amplitude and frequency decrease may indicate an overall decrease in circulation rate.

The response of ventilation rate, as estimated by measured changes in scaphognathite beat frequency and amplitude (Figures 13 and 14), agrees with that found for the majority of crustaceans discussed in the Introduction. The diphasic nature of the curve relating scaphognathite beat rate to oxygen concentration (Figure 13) probably reflects a steady increase in ventilation rate down to a critical oxygen level where the mechanism seems to break down. This critical point cannot be determined from the data as the oxygen concentration at which the maximum rate occurred varied widely among the various animals. This is why the average curve does not reach 100 percent at
any point.

The ventilation rate is only part of the picture. The efficiency of oxygen uptake is also of prime importance. As this investigation was mainly concerned with cardiac responses this parameter was not measured; however, it deserves some discussion.

The efficiency of oxygen uptake, which may be altered by ventilation rate, has been determined for only a few crustaceans. Percent utilization (or percent extraction), the function usually measured, refers to the percentage of available oxygen extracted from the water flowing over the gills. Arudpragasam and Naylor (1964) found that Carcinus maenas, when subjected to hypoxia, increased both its ventilation rate and its percent utilization of oxygen. It therefore appears that this crab is able to maintain a constant level of oxygen uptake over a considerable range. The picture for other crustaceans is not quite so clear. Thomas (1954) in investigating Homarus found that the percent extraction of oxygen increased with decreasing environmental oxygen concentration. The scaphognathite beat rate was found to be independent of the oxygen concentration, however. The crayfish, Procamburus, on the other hand, showed a decrease in percent extraction during hypoxia. Here, however, the scaphognathites showed an increase in rate of beating (Larimer, 1961, 1964a, b; Larimer and Gold, 1961). It was pointed out that a low percent extraction of oxygen from a large volume of water per unit
time might result in the same absolute amount of oxygen uptake as a high percent extraction from a small volume of water per unit time.

It might be argued that the steepened concentration gradient across the gill surfaces arising from a lower percent extraction might enhance the absolute amount of oxygen taken up. There is, as Larimer (1964a) has pointed out, one serious drawback to this argument. The in vivo oxygen concentration of crustacean blood may be very low. Redmond (1955) found this to be true for Homarus, Panulirus and Loxorynchus. A similar situation exists in Procambarus (Larimer, 1964a). If Cancer follows this pattern then the normal condition would be a steep diffusion gradient for oxygen across the gills. In such a case a slight increase in the gradient by hyperventilation would probably be insignificant.

There are other ways of improving the efficiency of oxygen uptake in the face of hypoxia. Altering the rate of circulation through the gills could accomplish this.

Larimer (1964a) reports that the slowing of the crayfish heart with gamma aminobutyric acid, without an effect on scaphognathite beat rate, actually increases the percent extraction of oxygen. Thomas (1954) attributed the increase in extraction efficiency of the lobster to circulatory affects but made no attempt to explain them. Larimer postulated an increased stroke volume along with a decreased resistance to flow leading to an increased circulation rate as the
The results reported here indicate indirectly that the stroke volume is decreased for Cancer under hypoxic conditions. It seems unlikely that a decrease in peripheral resistance as proposed by Larimer (1964a) could speed circulation when the stroke volume is decreased. The indication is, therefore, that there is a decrease in circulatory rate during hypoxia.

This reduction in circulation rate does not at first seem adaptive. With a low concentration of oxygen in the environment the obvious adaptation would be to move the blood through the gills faster to get more oxygen to the tissues at a higher rate. An increased gill perfusion rate, however, may not result in much of an increase in oxygen uptake if crustacean gills have a low permeability to oxygen. This possibility has been suggested by Redmond (1955) to explain the fact that crustacean blood never becomes fully saturated, even in oxygen rich water.

Such an increase in circulation may result in an increase in energy expenditure. Conversely, a decrease in circulation rate may result in a decrease in energy expenditure and therefore a decrease in oxygen demand. If the in vivo hemocyanin of Cancer holds as little oxygen as does the hemocyanin of the crustaceans investigated by Redmond (1955) there may be a capacity for loading additional oxygen, even at low external oxygen concentrations. If this is the case, one
might speculate that a small volume of slowly moving blood, because it remains in contact with the gaseous exchange site in the gills longer, can take up as much oxygen as a larger volume of faster moving blood. Thus even though the blood would be reaching the tissues at a reduced rate, it might be delivering as much oxygen and the animal would be operating more efficiently. Direct measurement of blood oxygen as it leaves the gills in both normal and oxygen-stressed crabs might yield interesting results. Such a decrease in circulation rate would not, however, account for the increase in percent extraction of oxygen in certain crustaceans noted by Thomas (1954) and Arudpragasam and Naylor (1964).

The isolated hearts showed the same decrease in rate and amplitude under hypoxic conditions as observed in the in situ hearts. This would suggest that the inhibition of heart beat is by the direct action of hypoxia and not via inhibitory nerves. Although no attempt was made to see how long the heart would beat under oxygen stress it may be quite resistant to anoxia. There is evidence for sustained anaerobiosis in the octopus heart (Pritchard, Huston and Martin, 1963) and this capacity might extend to other invertebrates.

Perhaps the most striking feature of the results of this investigation is the very short latency in the recovery of near maximal heart rate and amplitude upon reaeration of the water. This recovery was noted only in the intact crabs and was far too short to be merely the
result of a direct action of oxygen on the heart. In fact much of the
time elapsed between initiation of aeration and the response is probably
required for the oxygen to diffuse through the water to the animal.
The time required for a comparable recovery in the isolated heart is
over ten times as long (Figures 9 and 10).

A possible explanation for this short latency is the existence of
a chemoreceptor for oxygen. The response cannot be attributed to
mechanical stimuli from the turbulence associated with aeration as
the initiation of vigorous bubbling of nitrogen will not reproduce it. In
fact, on several occasions, transient cardiac arrest in a normally
beating heart was observed at the onset of nitrogen bubbling. In one
animal, pure oxygen was substituted for air and the response was
seen to occur even sooner.

Removal of the antennae was unsuccessful in eliminating the
circulatory response. Outside of this, nothing in the way of localiza-
tion of the receptor or receptors was attempted. In speculating on
the possible routes of control of heart rate by an oxygen receptor, two
possibilities emerge. The first is via the nervous system and the
second possible route is via neurosecretory elements.

In the nervous system proper there are two pairs of nerves,
leading to the cardiac ganglion, which have been demonstrated to be
acceleratory in several decapods, including *Cambarus* (Wiersma and
Novitsky, 1942), *Panulirus* (Maynard, 1953), and *Pacifastacus*,
Orconectes and Homarus (Florey, 1960). These nerves originate in
the subesophageal ganglion and join with an inhibitory fiber on each
side at the lateral pericardial plexus to form a single nerve which leads
to the cardiac ganglion (Alexandrowicz, 1932; Krijgsman, 1952). It is
possible that an oxygen receptor could send a sensory input to the
central nervous system which would then relay the information to the
cardiac ganglion via the accelerator neurons resulting in the increase
in rate and amplitude.

The neurosecretory elements are termed pericardial organs
(Alexandrowicz, 1953) and are located in the pericardial cavity.
These are arranged in such a manner so as to release neurohormone
into the circulation as it returns to the heart. Alexandrowicz and
Carlisle (1953) have shown that extracts of pericardial organs cause
acceleration of the heart. Electrical stimulation of the pericardial
organs releases an active agent (Cooke, 1964). It appears that the
active agent is composed of two peptides (Belamarich, 1963;
Belamarich and Terwilliger, 1966) and that it acts directly on the
cardiac ganglion (Cooke, 1966).

There is also an anterior ramification of the pericardial organ
which extends forward to the area of musculature of the scaphognathites (Maynard, 1961). It has been suggested by Maynard that this
system might regulate a "loose coordination" between ventilation rate
and heart rate thus facilitating gaseous exchange. An oxygen receptor
located either externally or internally would be a logical part of such a system.

As could have been anticipated, this investigation has raised more questions than it has answered. The role of the changes in ventilation in adapting this animal to hypoxia remains obscure. In order to fully understand the dynamics of gaseous exchange, the ventilation volume, percent extraction of oxygen and the role of hemocyanin will have to be worked out. A better estimate of the changes in the stroke volume associated with environmental oxygen fluctuations would be useful in assessing the role of the circulatory system in these adaptations.

One of the more interesting questions to come out of this investigation is where the proposed oxygen receptor is located. The relatively short latency of response would tend to indicate an external location as is hypothesized by Farley and Case (1968). Further research into the possible location of such a receptor might prove fruitful.
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