Reference *Biol. Bull.*, **150**: 15-37 (February, 1976)

CIRCULATORY ADAPTATIONS TO THE OXYGEN MINIMUM LAYER IN THE BATHYPELAGIC MYSID *GNATHOPHAUSIA INGENS*

BRUCE W. BELMAN[1, 2] AND JAMES J. CHILDRESS

Department of Biological Sciences and Marine Science Institute, University of California, Santa Barbara, California 93106

Zones of minimum oxygen are found at intermediate depths in most of the world's oceans and although the dissolved oxygen in some of these "oxygen minimum layers" is considerably less than 0.5 ml/l, populations of metazoans exist there (Schmidt, 1925; Sewell and Fage, 1948; Banse, 1964). Previous studies have shown that crustaceans which live in such minimum layers are unusually effective at removing oxygen from water (Teal and Carey, 1967; Childress, 1968, 1969, 1971, 1975). The respiratory physiology of one of these organisms, the lophogastrid mysid, *Gnathophausia ingens* has been studied in some detail by Childress (1968, 1969, 1971). These reports show that *G. ingens* is able to live aerobically at oxygen partial pressures of 6 mm Hg at an oxygen consumption rate of 0.8 mgO_2/Kg wet wt/min. The ability of this species to maintain a very high flow rate of water over its gills (up to 8 ml/g wet wt/min), while removing 50 to 80 per cent of the oxygen is apparently an important factor in this animal's remarkable aerobic ability (Childress, 1971). The major remaining questions concern the removal of O_2 from the respiratory stream, its passage through the gills and its movement to the tissues by way of the circulatory system.

The relationship between respiratory characteristics and the internal circulation has remained largely unstudied in crustacea. Present understanding of crustacean haemodynamics is based primarily upon anatomical studies of several species and a small number of direct measurements, such as the blood pressure measurements made in crabs and lobsters (Dubuisson, 1928; Drach, 1939; Burger and Smythe, 1953; Blatchford, 1971). The use in intact crustaceans of newer, more sensitive techniques for the measurement of internal pressure and fluid velocities (techniques previously utilized primarily in fishes and other vertebrates) has now permitted more detailed analysis of crustacean circulatory dynamics (Belman, 1975; Cobbold, 1974). In this continued examination of the unique respiratory abilities of *G. ingens*, we have used such techniques to study the blood circulatory system of this species.

Materials and Methods

Most of the specimens of *Gnathophausia ingens* used were taken in the basins off southern California with a 3.1 meter square Tucker trawl from the R/V Oconostota. Immediately after they reached the surface, the animals were placed in four liter polyethylene jars containing sea water at 3° C. The animals were

[1] Current address: Department of Biology, University of California, Los Angeles, California 90024.

[2] Authors' names are in alphabetical order and do not imply a greater contribution by one or the other.

transported in refrigerated containers to the Marine Laboratory at the University of California, Santa Barbara, and maintained at either 4.5 or 5.5° C. Individual specimens of *G. ingens* have survived in this laboratory for more than two years on a diet of salmon and lobster. All of the experimental animals were sexually immature individuals of undetermined sex and had a wet weight of less than 18 g.

Blood pressure and velocity

Blood pressure determinations were made on animals restrained in a chamber of sea water held at 5° C. Blood pressures were monitored with Statham 23BB pressure transducers connected *via* polyethylene tubing to #27 gauge hypodermic needles inserted into the blood system. Needle placement was facilitated with micromanipulators and the needle positions checked by dissection following the experiment. Pressure output was recorded on either an inkwriting recorder or oscillograph. All values given below represent pressure in cm H_2O above ambient pressure.

Blood flow velocities were measured directly with a Paul Beckman Associates' blood fluid velocity probe. This device consists of a #25 gauge needle with a heated thermocouple at the tip and measures fluid velocity by means of heat dissipation from the heated thermocouple. Blood velocity was measured in the heart, dorsal abdominal artery and hepatopancreas. Using a micromanipulator, the probe was placed into the vessel or tissue of interest after being gently pushed through either tiny holes in the carapace or the articular membrane between somites. The animals were lightly restrained under the same conditions as for the pressure measurements.

The flow-probe was calibrated by direct volumetric readings when placed in a polyethylene tube of known diameter. The calibration fluid was sea water at 5° C. As the density of crustacean blood is only slightly greater than that of sea water at the same temperature (Maynard, 1960a), no correction factor was introduced. The response of the probe is logarithmic. The direct volumetric readings were used in preparing linear velocity calibration charts for each experiment. Determinations were made of blood velocity in cm/sec from the strip-chart recordings of output from the flow-probe using these calibration charts.

Since the time period of the acceleration of the blood during a systolic contraction of the heart is on the order of 300 milliseconds or more, it was necessary to determine as accurately as possible the response-time of the flow probe. This was done by comparing the response of the flow-probe to that of a Statham 23BB pressure transducer. The flow-probe and a #25 gauge hypodermic needle connected *via* polyurethane tubing to the transducer were placed in a 0.15 cm diameter polyethylene tube connected to a peristaltic pump (60 strokes/min). The outputs from each were recorded simultaneously on a Beckman Instruments RS Dynograph at a chart-speed of 125 mm/sec. While there was some variation in response-time of the flow-probe, the lag behind the pressure trace was never more than 15 milliseconds. This indicates that the maxium error due to response-time is about 5% or less and that the flow-probe can follow the flow changes occurring in the heart of this species.

Following each experiment in which blood velocity was measured, the animal was dissected, placement of the flow-probe checked, and the diameters of the heart or other vessel measured at the point where flow was measured.

In both the blood velocity and blood pressure experiments, measurements were made at both high and low oxygen concentrations. These were carried out by sealing the chamber in which the animal was restrained and then slowly introducing N_2-saturated water at the experimental temperature. The O_2 content of the water in the chamber was reduced to levels lower than 1.0 ml/l and held there for several hours. Oxygen partial pressure was continuously monitored with a Clark-type oxygen electrode.

Gill surface area

As previously noted (Childress, 1971), the gills of *G. ingens* are so irregular as to preclude any possibility of measuring their surface area by the usual methods. The following method was devised to deal with this difficulty. Gill surface area was determined by measuring the density of cast exoskeletal material (carapace), the average thickness of the gill exoskeleton from cast exoskeletons and the weight of the gills from cast exoskeletons. From the density (D), thickness (T), and total weight (W) of the cast gills, the gill area was computed by the relationship: area $= W/DT$.

The thickness of the gill exoskeleton was measured using a Filar eyepiece calibrated with a stage-micrometer. The weights were all taken as ash-free dry weight, ash forming about the same fraction of both gill and carapace exoskeleton.

Heart rate (EKG)

Heart rates (EKG's) were measured in response to low oxygen using paired silver electrodes placed on the heart through small holes in the carapace and glued in place with dental cement. The electrical signal was amplified with a Grass P-15 A-C pre-amplifier and recorded on an oscillograph. The animal was lightly restrained in the cooling-jacketed chamber described above and lowered oxygen levels were obtained by flowing N_2-saturated water through the chamber. Oxygen partial pressure was monitored with a Clark-type oxygen electrode.

Heart volume

Heart volume was calculated from the geometry of the heart. Measurements were made of heart diameter at four places along the longitudinal axis and of the heart length from the posterior valve to the anterior exit to the median cephalic artery in five animals. Three kinds of measurements were taken. First the diameters were determined at maximum filling by observing the heart in the intact animal. The dorsal carapace was then removed and while the heart continued to beat, a second set of measurements was made. Finally, the heart was dissected out of the animal, measured, and weighed. All three measurements agreed within 0.1 mm.

Results

Anatomy of the blood vascular system

Present knowledge of crustacean blood systems is not comprehensive. Only a few species are well known, and for most of these, only the arterial system has been examined in any detail (Maynard, 1960a; Pillai, 1965; Seaton, 1971). No detailed account of the anatomy of the blood system in the Mysidacea exists, although Delage (1883) and Alexandrowicz (1955) provide some information on the structure of the heart and arterial system in *Mysis* and *Praunus flexuosus,* respectively.

The anatomy of the arterial system and of the return pathways in *G. ingens* was examined in eight individuals. Injection of Evans Blue into the heart or pericardial chamber caused this dye to be circulated throughout the arterial system and rendered the transparent vessels visible. Similar injections of dye into the ventral sinus channels in living animals enabled the return pathways to be mapped. In three individuals, a silicone rubber injection material (*Microfil,* Canton Biomedical Products) was injected into the arterial system, allowed to set, and the resultant casts dissected free and examined.

The heart. The heart in *G. ingens* is a single chamber, generally tubular in shape, extending throughout the posterior half of the thorax. It is suspended within the pericardial sinus although tending to lie toward the lower surface of the chamber. Ligaments attach both dorsally and laterally along its entire length. Structurally the heart appears similar to that in other mysids (Gadzikiewicz, 1905). Observations of the beating heart *in vivo* suggest that the contraction begins near the ostial openings and proceeds as a peristaltic-like wave in both the anterior and posterior directions. The temporal sequence in the normal heartbeat therefore is similar to that in *Holopedium* (Storch, 1931). Systole and diastole are apparently not discrete events temporally and blood enters the heart through the ostia while systolic contraction proceeds distally.

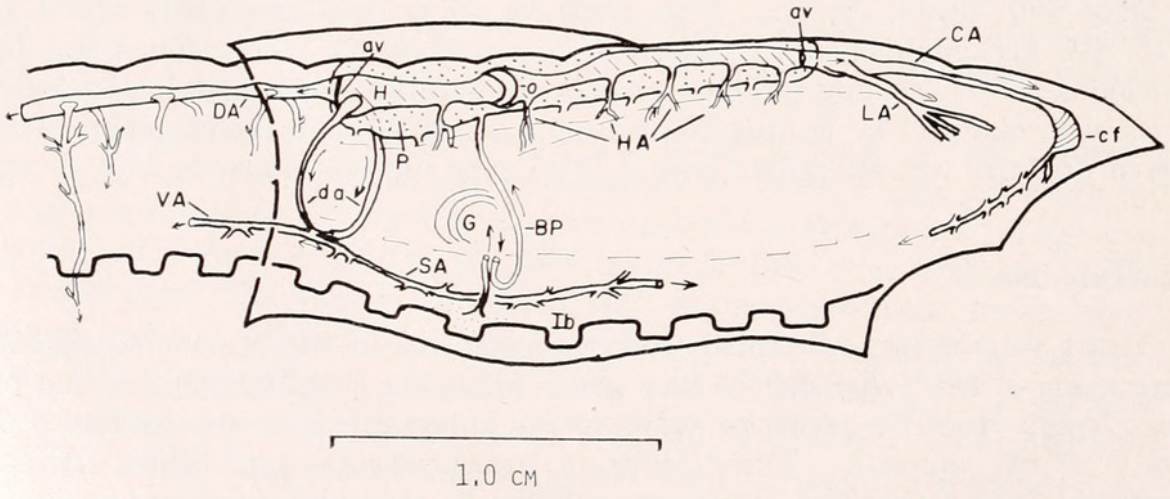

Figure 1. Arterial system of *Gnathophausia ingens.* Arrows denote direction of blood flow. H represents heart; av, arterial valves; o, ostial openings; DA, dorsal abdominal artery; da, descending arteries; SA, sternal artery; VA, ventral abdominal artery; HA, hepatic arteries; CA, median cephalic artery; cf, *cor frontale*; LA, lateral anterior arteries; Ib, infrabranchial sinus; BP, branchiopericardial vessels; and P, pericardium.

Arterial system. The arterial system of *G. ingens* is depicted in Figure 1. Blood leaves the heart through four main arteries and a number of smaller ones. Posteriorly, the dorsal abdominal artery (DA), of diameter nearly equal that of the heart itself, exists through a large valve. This artery passes along the dorsal wall of the abdomen with paired branches in each abdominal segment passing ventrally to divide further and supply the muscle of the abdominal wall and the pleopods. The descending arteries (da) exit the heart just anterior to its junction with the dorsal abdominal artery. The descending arteries pass laterally and then continue ventrally to rejoin, penetrate the center of the ventral ganglion, and terminate in the large horizontal, sternal artery (SA). The sternal artery extends anteriorly symmetrically giving rise to branches serving the periopods and more anteriorly, the maxillae. Posteriorly, the sternal artery continues along the ventral nerve cord becoming the ventral abdominal artery (VA).

Along the length of the heart, a series of small arteries arise which bifurcate and extend into the hepatopancreas. These are termed the hepatic arteries (HA). The supply of blood to the hepatopancreas is through multiple branches of these arteries and branches of the sternal artery, posterior abdominal artery and lateral anterior arteries.

The median cephalic artery (CA) leaves the heart anteriorly. After passing through the pericardial membrane, this vessel passes forward along the dorsal body wall providing a series of small branches to the intestine, cardiac stomach and various muscles. It then turns ventrally and enters the *cor frontale.* The *cor frontale* consists of four bundles of somatic musculature which surround an expanded section of the vessel some 5–7 mm in length. The muscles of the *cor* contract rhythmically, probably more or less in phase with the heart, although this could not be definitely determined. After the *cor* the aorta bifurcates and these lateral branches enter the nerve sheath of the circumoral connectives where the vessels again bifurcate, one branch (the larger) proceeding to the cerebral ganglion, the other proceeding along the connective.

The lateral anterior arteries (LA) leave the heart lateral to the median cephalic artery. Each then proceeds along the dorsal body wall toward the eye of that side. Several minor branches reach the hepatopancreas, the anterior portions of the stomach and various anterior musculature. The lateral anterior arteries continue forward with branches to the eyestalks and antennae, then terminate in the antennules.

In a 10.06 g animal, the diameters of all arteries leaving the heart were measured as accurately as possible: dorsal abdominal, 0.10 cm; descending (2), 0.04 cm; median cephalic, 0.05 cm; antennary (2), 0.02 cm; hepatic (estimate), 0.02 cm.

Return system. A series of complex sinuses collects blood from the tissue spaces in the anterior of the animal. These sinuses merge and form two main channels directed ventro-posteriorly which merge just posterior to the esophagus. Receiving a number of small channels from the hepatopancreas visceral tissues and the anterior appendages, this channel expands laterally to become the infrabranchial sinus supplying the gills. This sinus runs along the ventral midline and connects with the periopodal blood spaces and with the blood spaces of the pleopods *via* the ventral abdominal sinus. The infrabranchial sinus gives off branches to each of the gills.

TABLE I

The heart weight and heart volume as calculated from the geometry of the heart for G. ingens.

Whole animal wet weight (grams)	Heart weight (grams)	Heart volume (ml)
9.56	0.031	0.039
9.67	0.025	0.042
10.05	0.030	0.047
10.75	0.021	0.038
10.92	0.034	0.049

The branchio-pericardial vessels carry blood from the gills to the pericardial chamber. These vessels consist of discrete channels passing from the bases of the periopods dorso-laterally along the edge of each somite of the thorax. Each branchio-pericardial vessel enters the pericardial chamber directly.

Blood enters the heart from the pericardium through two large ostia located in the sixth thoracic somite. These ostia have large flaps of tissue which close the opening during systolic contraction.

Heart volume

The calculated heart volume and the heart weight of five *G. ingens* are presented in Table I. The heart volume for a 10 g animal is 0.047 ml. The heart weight in g is somewhat less than the volume in ml. Both heart weight and volume, when expressed as a percentage of the total body wet weight, are greater than that found in other crustacea of similar size (Maynard, 1960b).

Gill surface area

The thickness of gill exoskeleton showed a mean of about 1.001 microns with a range of from 0.873 to 1.178 microns from a series of over 400 measurements. All the different parts of the gill showed the same range of thickness and no parts were obviously specialized for support.

The gill surface areas were calculated for 15 individual *G. ingens*. The smaller individuals (<1 g) have gill surface areas of from 5 to 8 cm^2/g wet body wt. Animals between 1 and 3 g showed values between 9 and 13 cm^2/g, while larger ones are between 9 and 14 cm^2/g wet body wt. Gills freshly removed from living specimens of *G. ingens* have also been examined and the distance across which respiratory gases must diffuse ranges from 1.5 to 2.5 microns (exoskeleton plus living cells).

Blood pressure relationships

The blood pressures in the heart, pericardium, arterial system and infrabranchial sinus of *G. ingens* during normal cardiac cycling at high O_2 levels are shown in Figure 2. This figure is a summary of the pressures measured in fifteen individuals ranging in weight from 10.23 to 17.14 g wet wt. The range of pressures measured is not great. The maximum intracardiac systolic pressure was between 27 and

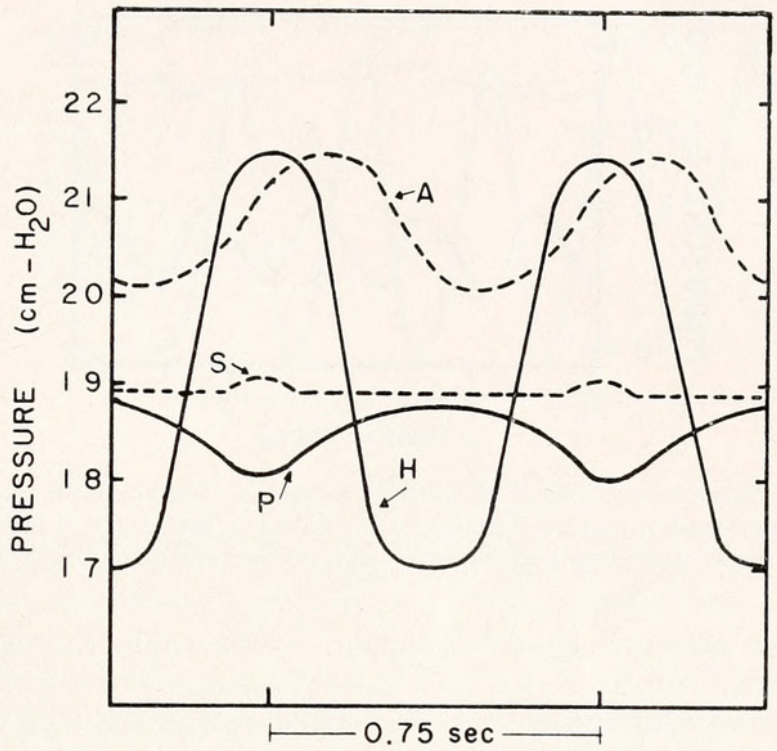

FIGURE 2. Graphical representation of normal circulatory pressures in *Gnathophausia ingens* from various regions during two systolic ventricular contractions; compiled from data obtained from 15 individuals. Heart is represented by (H); dorsal abdominal artery, (A); pericardium, (P); infrabranchial sinus, (S).

20.5 cm H_2O with a mean of 24 cm H_2O. Diastolic pressures ranged from 21.0 cm H_2O to 16.0 cm H_2O with a mean of 19 cm H_2O. The pulse pressure was between 3–6 cm H_2O with a mean of 4.5 cm H_2O. Arterial pressures (measured in the dorsal abdominal artery and in the median cephalic artery) are equal to that of the heart at systole but fall only 1–2 cm H_2O during diastolic filling. These arteries are apparently quite elastic as they expand in accepting the "pulse" of blood ejected from the heart at systole and then return slowly to the original size. The maintenance of a high arterial pressure at diastole is thus possible (Windkessel effect; Burton, 1972). There is no significant correlation between wet weight and either mean pulse pressure or maximum systolic pressure over the range of sizes of *G. ingens* examined ($P < 0.5$; *t*-test).

Pericardial pressure decreases about 1 cm H_2O as the systolic heart pressure rises (Fig. 2). The pressures measured in the pericardium vary somewhat along the longitudinal axis of the chamber but average slightly greater than diastolic pressure. At the ostial apertures the pericardial pressure drops to its lowest value (equal diastolic) as the valves open when filling begins.

Infrabranchial sinus pressures are greater than diastolic heart pressures by about 1.5 cm H_2O. In Figure 3, pressures recorded in the infrabranchial sinus in the region of the fifth periopod are compared with pressures measured simultaneously in the heart. In this case, the pressure drop between artery (systolic pressure) and sinus is about 3 cm H_2O. This gives a reasonable measure of tissue resistance to blood flow. The resistance to blood flow across the gills is approximately the difference between infrabranchial sinus pressure (pre-branchial) and the

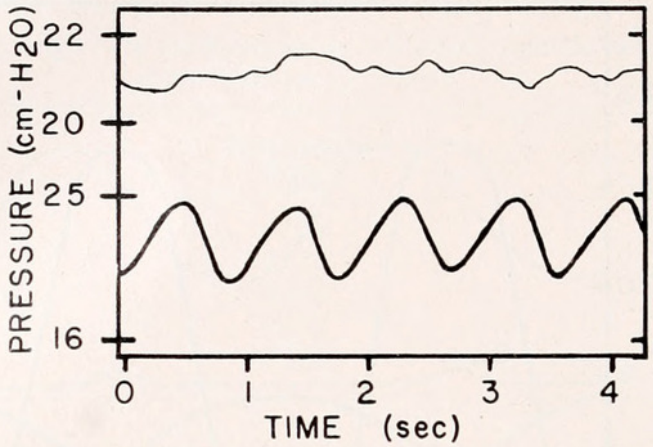

FIGURE 3. Synchronous recordings of blood pressure in *Gnathophausia ingens* in the infrabranchial sinus (upper trace) and the heart (lower trace). These are tracings of the original records which were made on an oscillograph and could not be reproduced properly for publication.

minimum diastolic pressure (post-branchial). This value averages about 2 cm H_2O pulse pressure.

Blood pressure measurements were also made on animals under sustained conditions of low oxygen such as occur in the oxygen minimum layer; *e. g.*, 0.5 ml O_2/l. Systolic and diastolic heart pressures, averaged for four animals (9.8, 10.5,

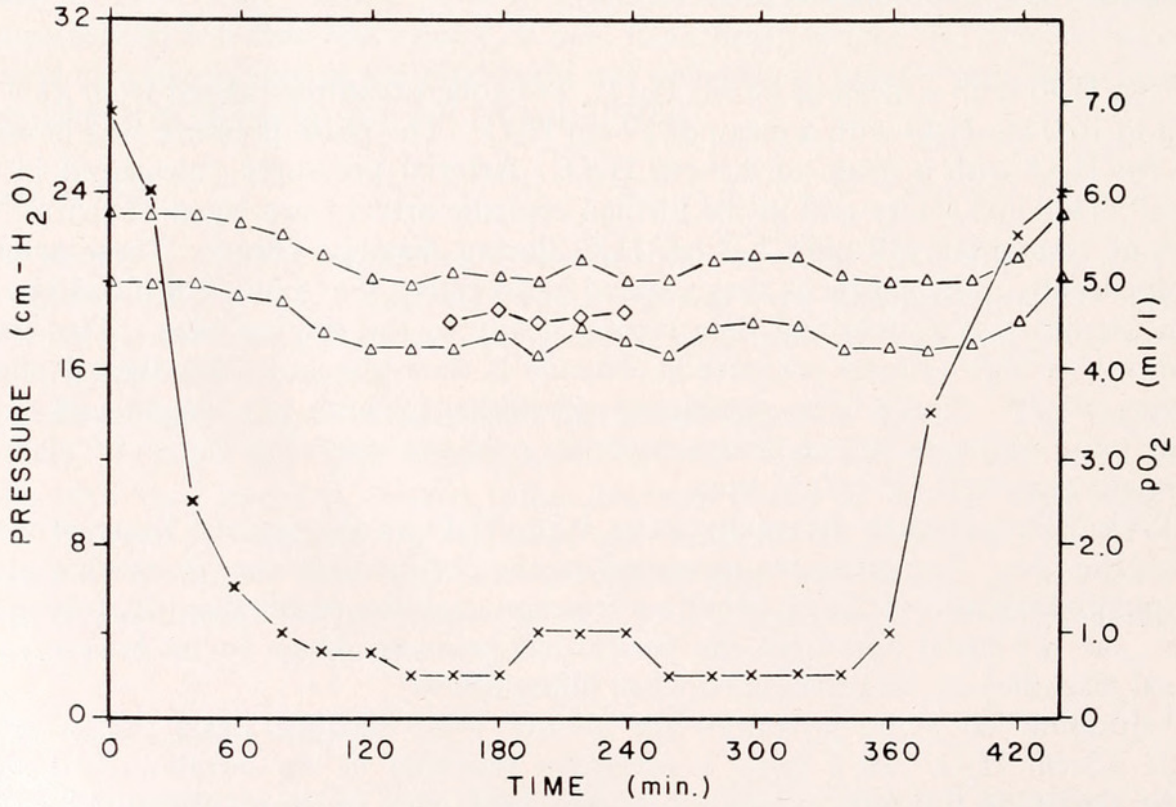

FIGURE 4. Summary of blood pressure changes occuring in four *Gnathophausia ingens* during exposure to low oxygen. Pressures measured in the heart are shown by triangles; upper line, systolic; lower line, diastolic. Diamonds show pressure measured in the infrabranchial sinus of two animals. The oxygen concentration during the experiment is shown by the symbol X.

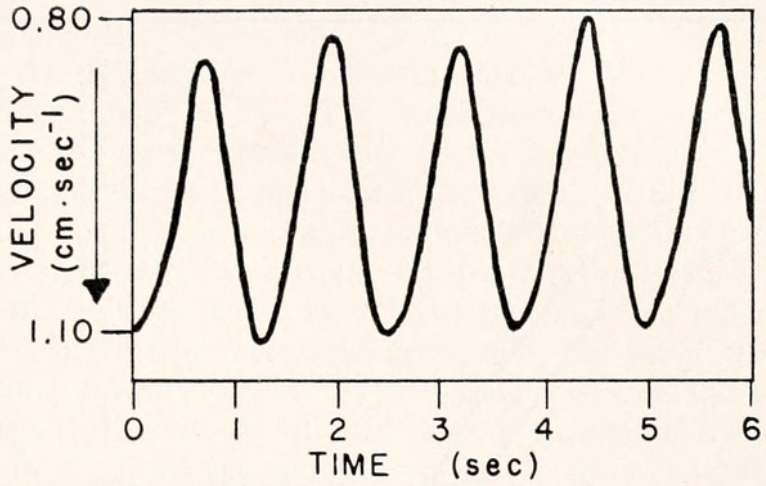

FIGURE 5. Record of blood velocity in the heart of a 10.56 g (wet weight) *Gnathophausia ingens* at low oxygen (0.50 ml O_2/l). This is a tracing of the original record which was made on an oscillograph and could not be reproduced properly for publication.

11.37, and 13.54 g wet wt), are plotted with the pO_2 against the time of the experiment in Figure 4. The range of values measured was not great; for example, the systolic range at high oxygen was 24.8 to 22.3 cm H_2O. As is shown in Figure 4, the pressures in the heart drop about 3 cm H_2O as the pO_2 drops below about 1.5 ml/l and remain within 1.0 cm H_2O of the lower value for the duration of the exposure to low oxygen. When the pO_2 is again elevated, the blood pressure returns to the high O_2 value. During the low oxygen portions of these experiments, the infrabranchial sinus pressures were measured in two animals. These pressures are plotted in Figure 4 also. The 13% drop in blood pressure at low oxygen is correlated with both a decrease in heart rate and a decrease in blood velocity in the heart (see below).

Blood velocity

The heart of *G. ingens* is a more or less tubular structure and each systolic contraction apparently moves a "pulse" of blood along its longitudinal axis (see anatomy section, above). For measurements of the blood velocity in the heart, the flow-probe was placed in the lumen of the heart midway between the posterior valve and the ostial openings (see Fig. 1). Heart-blood velocity measurements are, therefore, a measure of the velocity of the posteriorly-proceeding "pulse." A typical recording (10.56 g animal) made in this way is shown in Figure 5. At a heart rate of 48 beats/min, the maximum velocity reached is 1.10 cm/sec; the minimum velocity is 0.80 cm/sec.

The interpretation of velocity records such as that in Figure 5 is complicated by the fact that the flow-probe does not discriminate between turbulent and laminar flow and responds equally to flow in any direction. Using records of blood velocity pulses and blood pressure pulses obtained simultaneously in the heart of the same animal, we have ascertained that blood velocity records from the heart of *G. ingens* show the following. First, there is a marked increase in velocity during the systolic contraction with the peak velocity occurring just before the end of systole (about 20–30 milliseconds in the case shown in Fig. 5). This is followed by a decrease in

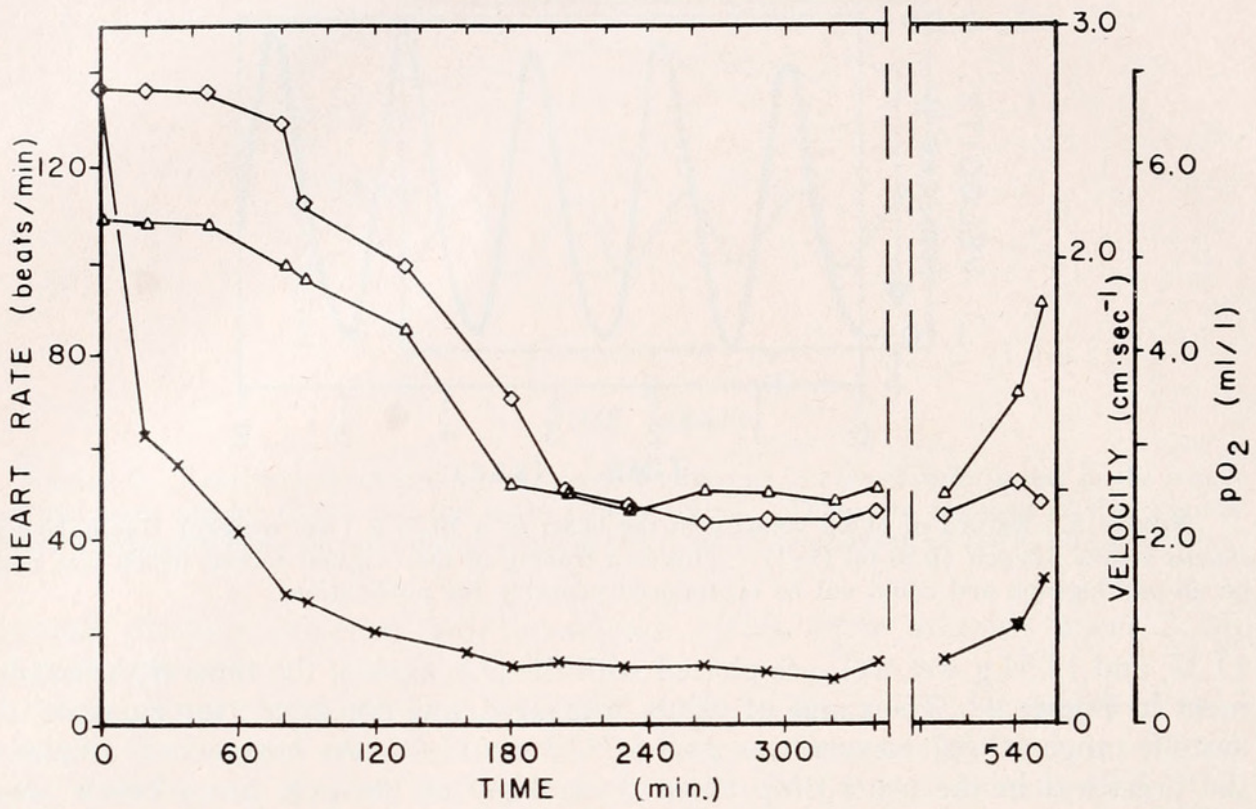

FIGURE 6. Effects of sustained low oxygen conditions on heart rate and blood velocity in a 9.38 g (wet weight) *Gnathophausia ingens*. The heart rate is shown by triangles, the blood velocity by diamonds and the oxygen concentrations by X.

velocity during the relaxation and filling phase of the cardiac cycle with the minimum velocity occurring well before the beginning of systole (about 70–100 milliseconds in Fig. 5). To derive a single value for the velocity of blood moving in the heart during systole, we have taken a typical one-minute series of contractions, calculated a representative value for the systolic portion of the cycle for each pulse and then a mean value for all the pulses occurring in that minute. To determine a representative velocity for a single systole, we have taken the mean velocity of the highest (most rapid) 75 per cent of the acceleratory portion of the velocity curve. A more sophisticated method, integration of that portion of each velocity curve from beginning to end of systole, yielded results that were within five per cent of this estimate. Since a large amount of data was involved, we chose to use the simpler method. In the case shown in Figure 5, the mean systolic velocity is 0.99 cm/sec.

The flow-probe was also introduced into the heart anterior to the ostial openings. The blood velocity measured in this region is similar in magnitude to that measured in the posterior of the heart. The recordings made here were generally inconsistent and the heart often ceased beating within a few minutes following introduction of the flow-probe. In what follows, heart-blood velocity refers only to records obtained from the posterior region of the heart.

Eight experiments were conducted in which the velocity of blood moving in the heart was measured under different oxygen tensions and under sustained low oxygen such as that occurring in the oxygen minimum layer (0.5 ml O_2/l). The results of one of these experiments (9.38 g animal), which we consider representative of the majority, are shown in Figure 6. Here the mean systolic velocity, heart rate and

oxygen tension are plotted against time. Above about 2.5 ml O_2/l the contraction frequency was apparently independent of external oxygen. As the pO_2 dropped further, the heart rate fell from over 100 beats/min to 50 beats/min or less as the oxygen level reached 0.5 ml O_2/l. The heart rate then remained within a few beats/min of the lower rate for the duration of the low-oxygen exposure.

In each of the experiments in this series, the mean systolic velocity varied with the heart rate as the pO_2 changed. In the experiment shown in Figure 6, the mean systolic velocity at high heart rates (over 100 beats/min) is 2.71 cm/sec. As the heart rate drops, the velocity drops as well, so that at low oxygen levels (0.5 ml O_2/l), where the heart rate is about 42 beats/min, the mean systolic velocity is 0.95 cm/sec. Like the heart rate, the velocity increases as the pO_2 is increased near the end of the experiment. In all of these experiments at higher oxygen levels (above 1.5 ml O_2/l), the heart rate varied from 120 to 86 beats/min, while the mean velocity varied from 4.71 to 2.35 cm/sec. At low oxygen levels (0.5 ml O_2/l), the heart rate varied from 48 to 36 beats/min, while the mean systolic velocity ranged from 1.65 to 0.54 cm/sec. The systolic contraction is of longer duration at lower heart rates. In several recordings the speed of recording the output from the flow-probe was increased so that the relative proportions of each beat due to systole and diastole could be determined. At heart rates of 118 beats/min, the duration of systolic contraction is about 0.1 sec or 20 per cent of the stroke time. At heart rates under low oxygen conditions (48 beats/min), the duration of systolic contraction is about 0.5 sec or 40 per cent of the stroke time (see Fig. 5).

The measurements of blood velocity in the heart of *G. ingens* are of interest primarily as a means of assessing possible changes in the cardiac pulse in response to changing external, and by implication, internal, oxygen levels. Of greater significance physiologically, and particularly useful for making interspecific comparisons of circulatory parameters, are measurements of arterial blood velocity. In two large specimens of *G. ingens* (13.4 and 14.7 g wet wt), blood velocities were measured in the dorsal abdominal artery, 0.5 cm posterior to the heart. The flow-probe was introduced into the vessel after inserting it through the articular membrane between the second and third abdominal somite. As the diameter of the artery at the point of measurement was about 0.1 cm, the flow-probe seemed to interrupt the flow to some extent by its presence and the velocity recordings proved difficult to interpret in most instances. Such data as were obtained suggested that arterial velocities of from 1 to 2 cm/sec occurred under low oxygen conditions and from 1.5 to 4 cm/sec under high oxygen conditions (above 1.5 ml O_2/l).

Attempts were also made to measure blood velocity in the infrabranchial sinus to compare with the arterial measurements. This sinus, at its largest dimension, consists of a wide, very thin chamber and the flow-probe surface could not be positioned free of tissue. While this resulted in erratic recordings, the results obtained suggest that flow in this sinus is fairly rapid, perhaps approaching to 3–4 cm/sec in an 11.45 g *G. ingens*.

The decreased heart rate, mean systolic velocity and arterial velocity under low oxygen conditions suggest that the output of blood from the heart (stroke volume) is significantly reduced. In order to determine if this is so, and hopefully to gain some idea of the magnitude of this reduction in flow, changes in the velocity of blood flowing through the hepatopancreas in response to lowering of the external oxygen

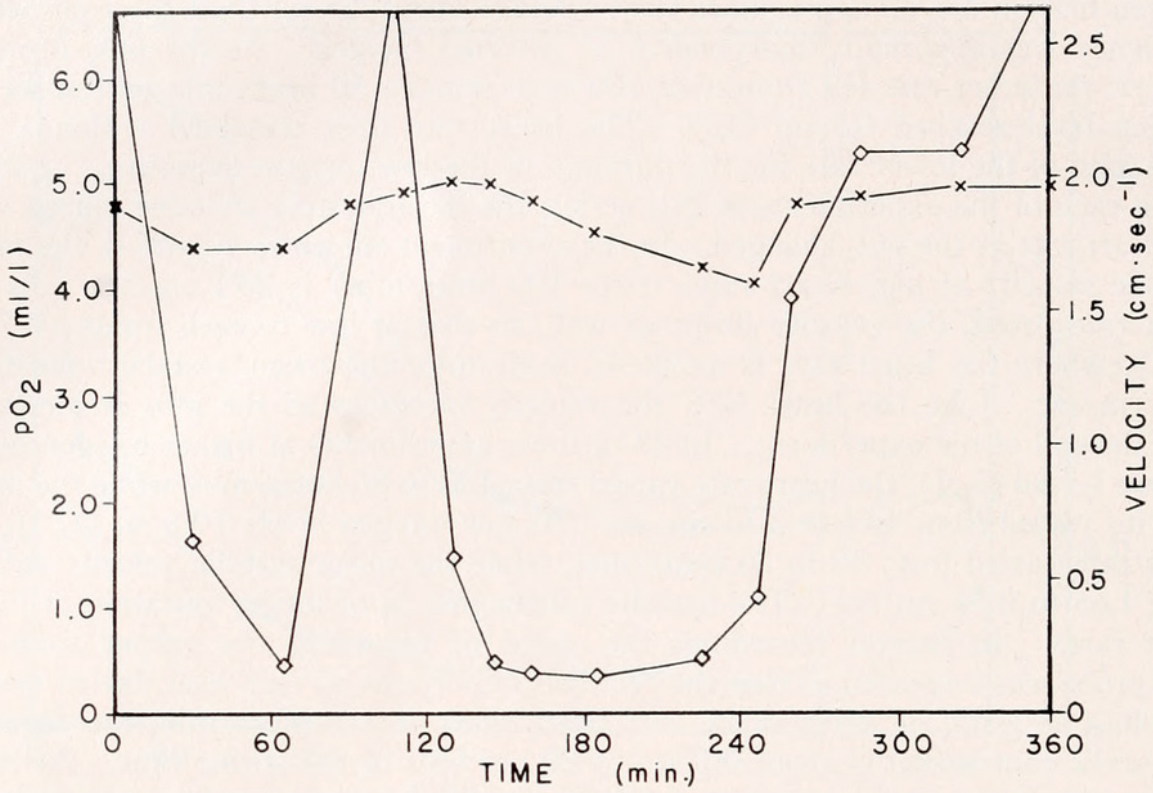

FIGURE 7. The effect of low oxygen on blood velocity in the hepatopancreas of a 10.93 g (wet weight) *Gnathophausia ingens*. Blood velocity is shown by the symbol X and oxygen concentration by diamonds.

tension were measured in three specimens of *G. ingens*. The hepatopancreas in this species is extremely large, occupying almost the entire (approximately 70%) interior of the animal with other tissues located next to the exoskeleton.

The blood velocity and pO_2 are plotted for the duration of an experiment with a 10.93 g animal in Figure 7. Since the flow-probe tip was in contact with tissue, the absolute velocity recorded in this experiment is probably incorrect. However, the relative change in the velocity of blood flowing through this organ as the pO_2 decreases is of particular interest. In this case, the velocity decreases by no more than 16 per cent after 110 minutes of oxygen less than 1.0 ml O_2/l. In the other two such experiments, the decrease in blood velocity in the hepatopancreas was similar: 11.45 g animal, 21%; 12.02 g animal, 14%. Assuming that the physical dimensions of the circulatory channels within the hepatopancreas do not change, it appears that the decline in cardiac contraction frequency and mean systolic blood velocity are paralleled by a decrease in blood flow through this organ. The absolute decrease in volumetric flow is, due to the complexity of the arterial supply to the hepatopancreas, impossible to calculate. However, again assuming that the dimensions of the blood channels do not change, a reduction in blood velocity of from 14–21% would mean a reduction in total flow of the same relative magnitude.

Heart rate and EKG

Data on heart rate at different oxygen levels can, of course, be derived from either the pressure-pulses or the blood velocity pulses recorded in those particular experiments. To determine whether or not the physical presence of these monitor-

ing devices affected the rate of contraction, EKG recordings were made both with and without the pressure-needle or flow-probe in place. The results of these experiments showed that the shape of the EKG and the expected frequency of contraction were apparently unaffected. Due to rather high noise levels in the EKG records, no further use was made of these data, all heart rate information being derived from either the pressure pulses or the blood-velocity pulses.

All of the heart rate information obtained in this study was combined and plotted against oxygen tension. At oxygen concentrations above 1.5 ml/l, the rate is variable but averages 110 beats/min (range: 128–78). At low oxygen concentrations (less than 1.0 ml/l), the average is 42 beats/min (range: 86–30). The decline in heart rate to a steady, low rate does not follow the drop in pO_2 (below about 2.0 ml O_2/l) directly but lags by several minutes. This lag in cardiac response to diminished external oxygen suggests that the oxygen-sensitive mechanism is internal and that an excess of oxygen remains in the blood (or tissues) for some time beyond the external decrease.

Discussion

In oxygen minimum layer conditions such as occur off the coast of Southern California, populations of *Gnathophausia ingens* live aerobically at oxygen concentrations as low as 0.20 ml/l (Childress, 1968, 1969, 1971). The youngest and oldest individuals may live at somewhat higher oxygen concentrations (Childress, 1969); however, the individuals studied here and previously, all fall in the intermediate size range which is characteristically found in oxygen minimum layer depths (500–900 meters). It is important to remember therefore that in evaluating our results, the "normal" oxygen environment of this species is between 0.20 and 1.0 ml O_2/l. This is in contrast to previously studied species which either live continuously near oxygen saturation or live in intertidal zones where periodic low oxygen stress is dealt with by air breathing, anaerobiosis or behavioral modifications. For these reasons, in the following discussion, we will generally compare the performance of *G. ingens* at 0.50 ml O_2/l with that of other species near air saturation.

Gnathophausia ingens is remarkably effective at extracting sufficient oxygen for routine metabolism from water at low partial pressures. At the organismal level this effectiveness is the result of the combination of three factors. First, *G. ingens* maintains an exceptionally high ventilation volume (up to 8 body volumes/min; Childress, 1971). This is seen most readily when this species is compared to other water breathers. The ratio of ventilation volume to oxygen consumption ($\dot{V}_{O_2}$) in *G. ingens* is about 7000 ml H_2O/ml O_2 STPD. The same ratio is about 800 for octopus, 350 for crayfish (Dejours, Garey and Rahn, 1970) and 1200 for *Cancer magister* (Johansen, Lenfant and Mecklenburg, 1970). Secondly, this species has a relatively low rate of oxygen consumption (0.034 ml/g/hr; Childress, 1971). Thirdly, *G. ingens* shows a very high percentage utilization of oxygen (50–80%; Childress, 1971).

As we have not examined the mechanisms involved in maintaining a high ventilation volume, we will not deal with the first factor in this discussion. The second factor, low $\dot{V}_{O_2}$, has been discussed by Childress (1975). We are concerned here with the mechanism(s) underlying the maintenance of a high per cent utilization at high ventilation volumes and environmental partial pressures as low as

6 mm Hg O_2. The major problem is how this animal can transport relatively large amounts of oxygen (0.034 ml/g/hr) from the exterior to the mitochondria with such a low oxygen partial pressure gradient (maximum of 6 mm Hg at external partial pressure of 6 mm Hg). The critical points in this transport are: a) the first diffusion step, inhaled water to blood; b) the circulation of blood from the gills to the tissues; and c) the second diffusion step, blood to mitochondrion. Adaptation to deal with a low gradient might occur at any of these levels. We would not expect extensive adaptations at the mitochondrial level since mitochondria apparently function at very low oxygen partial pressures generally. We have sought answers to the central question in the first diffusion step (water to blood) and in the circulation.

At the first diffusion step the critical factors are: 1) the rate of delivery of water to the gill surface, 2) the partial pressure of oxygen at the gill surface, 3) the gill surface area, 4) the diffusion distance across the gills, 5) the permeation coefficient across the gills, 6) the partial pressure of the blood in the gills, and 7) the flow rate of blood through the gills.

The high per cent utilization indicates that delivery of water to the gill surface is quite effective with little dead space. The complex, foliaceous nature of the gills is undoubtedly a factor in this (Childress, 1971). Although we have made no measurements of the partial pressure in contact with the gills, a high ventilation volume and the observation that virtually all of the respiratory water passes through the gills (Childress, 1971) suggests that the partial pressures here probably range from equal to those in the inhaled water to slightly less than those in the exhaled water.

The gill surface areas measured in *G. ingens* are larger than the areas measured for most previously studied crustaceans and fishes, and are about equal to the greatest areas measured in those other species (Gray, 1954, 1957). These other crustaceans and fishes, however, are larger and have comparable or higher $\dot{V}_{O_2}$'s than *G. ingens*. It seems apparent that one of the adaptations of *G. ingens* to increase oxygen diffusion across its gills in the presence of a low oxygen gradient is a very large gill surface area relative to its $\dot{V}_{O_2}$. In Table II we have compiled data from the literature on the oxygen consumption rates and gill surface areas for a variety of species of fishes and crustaceans and computed the ratio. Table II shows that the O_2 uptake per unit area of gill ($\dot{V}_{O_2}/A$) in *G. ingens* is much less than in all fishes examined and lower than all crabs except the largest and most sluggish.

Another aspect of the gill surface data is the suggestion that small specimens of *G. ingens* have appreciably less gill surface area per unit wet weight. This is different from the usual pattern in which larger animals have less gill area/unit wt (Hughes, 1927b, Gray, 1957). This may indicate that some elements of the total transport system become disproportionately less effective as the animals grow in size. However, the thickness of the gill exoskeleton remains roughly constant over the entire range of sizes studied. Another possible explanation might be that the smaller individuals rely to a greater extent on extrabranchial oxygen exchange than do the larger individuals. Childress (1971) demonstrated that the larger individuals do not use extrabranchial oxygen exchange to any measurable degree.

The diffusion distance across the gills (exoskeleton plus living cells) is about 1.5 to 2.5 microns in *G. ingens* and this is comparable to that found in fishes with the shortest distances and less than in many fishes (Hughes, 1972b). Unfortunately,

TABLE II

Ratio of oxygen consumption rate ($\dot{V}_{O_2}$) to gill surface (A) in a variety of species. Letters following $\dot{V}_{O_2}$ indicate standard (S) routine (R) or active (A) rate.

Species	Weight g	$\dot{V}_{O_2}$ ml/g/hr	° C	A cm²/g	Weight g	$\dot{V}_{O_2}$/A µl/cm²/hr
Fishes:						
Anguilla rostrata	40	(1) 0.088 (S)	17	(2) 3.02	428	29.2
Ameiurus nebulosus	~160	(3) 0.046 (S)	20	(3) 2.43	163	18.9
Chaenocephalus aceratus	500–1800	(4) 0.016 (S)	0	(5) 1.20	100–1000	13.3
Cotostomus commersoni	62– 200	(3) 0.060 (S)	20	(3) 2.85	206	21
Cyprinus carpio	~190	(3) 0.066 (S)	20	(3) 2.66	184	24.8
Mugil cephalus		(6) 0.2 (R)	24	(2) 9.54	166	21
Salmo gairdnerii	300– 600	(7) 0.027 (S)	15	(8) 2.40	100	8.9
Scyliorchinus stellatus		(9) 0.038 (S)		(10) 2.60	100	14.6
Tinca tinca	30	(1) 0.067 (S)	18	(9) 3.83	140	17.5
Crustaceans:						
Callinectes sapidus		(11) 0.68 (R)	27	(11) 13.7	143	5
Carcinus maenas		(12) 0.094 (R)		(12 7.8		12.2
Gnathophausia ingens	5–10	(13) 0.017 (S)	5.5	(14) 10.0	5–10	1.7
	5–10	(13) 0.034 (R)	5.5	(14) 10.0	5–10	3.4
	5–10	(13) 0.151 (A)	5.5	(14) 10.0	5–10	15.1
Libinia dubia		(11) 0.025 (R)	27	(11) 13.7	143	5
Menippe mercenaria		(11) 0.031 (R)	27	(11) 8.9	163	3.5
Panopeus herbstii		(11) 0.056 (R)	27	(11) 8.7	19.2	6.4
Uca pugilator		(1) 0.10 (S)		(11) 6.2	2.3	9.6
Uca pugnax		(1) 0.06 (S)		(11) 7.7	2.1	1.3

(1) Prosser (1973), (2) Gray (1954), (3) Saunders (1962), (4) Holeton (1970), (5) Hughes (1972a), (6) Nicol (1967), (7) Holeton (1971), (8) Hughes (1972b), (9) Hughes (1970), (10) Baumgarten-Schumann and Piiper (1968), (11) Gray (1957), (12) Hughes, Knights and Scammell (1969), (13) Childress (1971), (14) this study.

there are as yet no measurements of this diffusion distance for other crustaceans. However, one can make a rough comparison of the functioning of the gills of specimens of *G. ingens* and those of other crustaceans and fishes by using the concept of diffusing capacity (Hughes, 1972b). This parameter (D, ml O_2/min/kg wet wt/mm Hg) describes the amount of oxygen transferred across the gills of an animal for each 1 mm Hg O_2 of gradient. It may be estimated from measurements of gill area, blood-water diffusion distance and the permeation coefficient in which case it is referred to as $D_\dagger$. It may also be estimated from the $\dot{V}_{O_2}$ and the diffusion gradient across the gills (ΔP_G) in which case it is known as D_g. Hughes (1972b) says that in general, D_g is about one tenth of $D_\dagger$ due to factors not accounted for in these calculations. Since information is available for $\dot{V}_{O_2}$ and ΔP_G in two crustacean species, it is possible to calculate D_g for them and compare it with the $D_\dagger$ for *G. ingens*. These comparisons are shown in Table III where we have listed diffusing capacities for a number of species of fishes and crustaceans. Calculations in this table are based on the equation $\dot{V}_{O_2} = (K/A/P_{O_2})/t$ from Hughes (1972b). K is the Krogh permeation coefficient expressed as ml/min/cm²/µ/mm Hg and taken as $1.71/10^{-4}$ for crustaceans and $1.45/10^{-4}$ (Krogh, 1941) for fishes and corrected for temperature (2%/° C, Randall, 1970). A is the gill surface in cm²/kg; t is the water-blood distance in µ; and $\dot{V}_{O_2}$ is expressed in ml O_2/kg/min. $D_\dagger$ for

TABLE III

Diffusing capacities (D_g = transfer factor) for a number of species of fishes and crustaceans and calculated diffusing capacities (D_t) of their water-blood barriers. Either D_g or D_t figures in parentheses are estimated values assuming $D_t = 10\ D_g$. The lower ΔP_g values in parentheses have been estimated from the relationship $\Delta P_g = \dot{V}_{O_2}/D_t$ while the higher values have been estimated from $\Delta P_g = \dot{V}_{O_2}/10/D_t$. [Table adapted from Hughes (1972b)].

Species	Gill area cm^2/g	Wet weight g	Water-blood barrier (t) μ	Diffusing capacity (ml/min/kg/mm Hg)		ΔP_g mm H_g
				Calculated D_t	Measured D_g	
Fishes:						
Salmo gairdnerii (1)	2.40	100	6	0.050	(2) 0.0080	56
Scyliorhuinus stellatus (3)	2.60	100	11	0.0272	(4) 0.0087	73
Tinca tinca (3)	2.50	100	3	0.0525	(1) 0.007	77
Chaenocephalus aceratus (5)	1.20	100	6	0.030	(6) 0.0043	63
Ameiurus nebulosus (7)	1.50	100	10	0.023	(8) 0.0015 =	(9) (33–520)
Scomber scombrus (10)	11.58	182	(1) 1.2	1.3	(0.13)	
Trachurus trachurus (10)	10.74	125	(1) 2.22	0.70	(0.071)	
Pleuronectes platessa (10)	4.33	86	(1) 3.85	0.17	(0.017)	
Crustaceans:						
Cancer magister (11)		1000		(0.09)	0.0086	60.5
Panulirus interruptus (12, 13)		600		(0.08)	0.0077	140
Gnathophausia ingens (14)	10.0	10	2.0	0.60	(0.06)	(0.373–3.73)

(1) Hughes (1972b), (2) Randall, Holeton and Stevens (1967), (3) Hughes (1970), (4) Baumgarten-Schumann and Piiper (1968), (5) Hughes (1972a), (6) Holeton (1970), (7) Steen and Berg (1966), (8) Fisher, Coburn and Forster (1969), (9) calculated using $\dot{V}_{O_2}$ from Saunders (1962), (10) Gray (1954), (11) Johansen, Lenfant and Mecklenburg (1970), (12) Redmond (1955), (13) Winget (1969), (14) Childress (1971) and this study.

G. ingens is about 100 times greater than D_g for *Cancer magister* and *Panulirus interruptus*, suggesting that the diffusing capacity of the gills of *G. ingens* is considerably greater than that of either of these animals. If one makes the further approximations shown in Table III, the ΔP_g of *G. ingens* can be estimated roughly at about 4 mm Hg. The interesting thing about this estimate is that it falls within the possible range for this animal at 6 mm Hg external O_2 and is much smaller than the measured ΔP_g of other species of crustaceans and fishes. It appears then that *G. ingens* achieves a high diffusion capacity by having a large gill surface relative to its $\dot{V}_{O_2}$ (Table II) as compared to fishes. Compared to other crustaceans, it seems that the higher diffusion capacity is either a function of a much thinner blood-water diffusion barrier or a much lower physiological dead space in the gills of *G. ingens*. During the evolution of this species to survive under low oxygen conditions, it appears to have developed the structures and functions so far considered to levels near the limits of effectiveness in oxygen transport found in aquatic animals.

Our observations concerning the circulatory system of *G. ingens* indicate that this system has been evolved for relatively high rates of blood flow. Among the modifications which favor high flow rates in this species are quantitative increases in size and capacity of circulatory system components. For example, the blood system of *G. ingens* follows the typical mysid and decapod crustacean pattern.

TABLE IV

Arterial diameters in three crustaceans.

Species	Wet weight (g)	Diameter of median cephalic artery (cm)	Diameter of dorsal- or posterior-abdominal artery (cm)	Reference
Gnathophausia ingens	12	0.050	0.100	This study
Pachygrapsus crassipes	20	0.042	0.065	Seaton (1971)
Panulirus interruptus	622	0.110	0.250	Belman (1975)

However, the arterial system in *G. ingens* appears proportionately quite large when the diameters of the main arteries exiting the heart are compared to those of corresponding arteries in lobsters and crabs as we have done in Table IV.

The single chamber tubular heart of *G. ingens* appears to be structurally similar to that of other mysids (Gadzikiewicz, 1905). However, the heart in this species is apparently relatively large for a crustacean of its size. This conclusion is based on a comparison of the data in Table I (10 g *G. ingens* heart weight = 2.8×10^{-2} g) with the predicted heart weight in a 10 g crustacean (1×10^{-2} g, from regression of heart weight on body weight for tropical decapods, Maynard, 1960b).

Since in the evolution of crustaceans there is a tendency for each vessel to supply a particular organ or organ system and to evolve to a size suitable to the needs of that region of the organism (Maynard, 1960a), we can also draw certain conclusions about blood flow from the relative diameters of vessels leaving the heart. Both the median cephalic artery and the posterior abdominal artery are disproportionately large in *G. ingens* compared to other crustaceans on a weight basis (Table IV) and compared to other vessels exiting the heart in this species (results section, this paper). The median cephalic artery also has a *cor frontale* located in such a position that it can assist in overcoming the resistance to flow imposed by the fine arterial divisions feeding into the cerebral and associated ganglia. These apparent priorities in the circulatory system agree with what we know from other points of view. That is, *G. ingens* has a well developed nervous system which explains the need for the median cephalic artery development. The development of the posterior abdominal artery is almost certainly related to the fact that this species has a considerable scope for activity which is largely accounted for by movement of the abdominal pleopods which account for about 50% of the O_2 consumption at "normal" levels of activity and much larger percentages at higher levels (Childress, 1968, 1971). This information concerning the anatomy of the circulatory system of *G. ingens* supports the suggestion that this species may have high blood flow rates.

The blood pressure data for this mysid also indicate circulatory modifications of adaptive significance. While absolute blood pressures over 20 cm H_2O in *G. ingens* may appear to be in conflict with the general characterization of crustacean blood systems as operating at low pressures (Prosser, 1973), other work in our laboratory indicates that the pressures observed in *G. ingens* are comparable to those found in a variety of littoral crustaceans (Belman, unpublished). The recordings of blood pressure (summarized in Fig. 2) suggest that the circulation in *G. ingens* is primarily heart driven and that body movements play only a limited role. The pattern of pressure in *G. ingens* differs from that in other crustaceans in that about 40 per cent of the peripheral resistance occurs across the gills in this

species, while in other crustacean species investigated (Burger and Smythe, 1953; Blatchford, 1971; Belman, 1975) less than 20 per cent of the peripheral resistance occurs across the gills. This difference may be the result of either longer or finer blood channels in the gills of *G. ingens* or in a relative reduction in the resistance of other parts of the circulatory system. In either case, *G. ingens* puts a greater fraction of its cardiac work into movement of blood through its gills than do crustaceans from higher oxygen habitats.

The data on heart rate in *G. ingens* at different oxygen partial pressures are similar to those of crustacean species whose normoxic environment is near air saturation, showing comparable rates and lowered heart rates at lower environmental oxygen partial pressures (Larimer, 1962, 1964; Larimer and Gold, 1961; Stiffler and Pritchard, 1972). The suggestion for these other species has been that the lower heart rates are not an adaptive response but a result of oxygen stress on the heart near or below the lower critical oxygen partial pressure (P_c). The data on *G. ingens* are difficult to understand in this light, since this animal regulates its oxygen consumption down to very low partial pressures and its normoxic condition is one of low oxygen. Therefore, the heart rate of *G. ingens* is at least not interfering with this regulation. There are at least two possible explanations for this phenomenon in *G. ingens*. First, the high heart rates at high oxygen partial pressures may be a result of excitement of the animal and therefore do not indicate what the "normal" rates would be. Secondly, the heart rate may not actually be a good indication of volume flow of blood which is the physiologically important parameter. The first of these may well be an important factor in these observations since the experiments lasted only a few hours and the animals generally displayed a markedly high level of pleopod activity. This could result in $\dot{V}_{O_2}$ as much as 4.5 times greater than the routine rate and since, as Childress (1968, 1971) has shown, P_c increases as a function of increasing $\dot{V}_{O_2}$, the P_c could go as high as 25 mm Hg O_2 (about 1.25 ml O_2/l). It seems apparent that our data (Figs. 4, 6, and 7) describe the maximum heart rate, blood pressure, and blood flow which the animal maintains at the maximum $\dot{V}_{O_2}$ which it can sustain at a given P_{O_2}. This is supported by the fact that the major drop in heart rate occurs at about the P_c for a maximally active animal.

The second possible explanation for the decline in rate with declining P_{O_2} is supported by two parts of our findings which suggest that blood flow may decline more slowly than does heart rate. The first is the observation that the decrease in heartbeat frequence is coincident with an almost fivefold increase in the duration of the systolic contraction. The lower rate of spread of the contractile event from its origin to its terminus may allow more complete emptying of the heart, increasing the effective stroke volume of the heart. Due to this, minute volume might decrease proportionately less than heart rate. The second piece of data supporting the suggestion that volumetric flow declines less than heart rate is the data on blood flow in the hepatopancreas (Fig. 7). In the animals examined, apparent blood velocity decreased about 17% over the range where heart rate would be expected to decline about 60%.

The recordings of blood velocity in the intact circulation system of *G. ingens* are, to our knowledge, the first direct measurements made in such a small crustacean. The mean velocity of blood in the heart of a 10 g *G. ingens* would be about 2.7

cm/sec at high oxygen and 1.0 cm/sec at low oxygen (Fig. 6). The velocity in the posterior abdominal artery is on the order of 1.5 to 4 cm/sec under higher O_2 and about 1 to 2 cm/sec at low O_2 levels. The flow in the infrabranchial sinus may approach 3 to 4 cm/sec. These velocities range from about the same as to about one-half those found in 700 g lobsters (*Panulirus interruptus,* Belman, 1975). The velocities in *G. ingens* seem remarkably high when considered relative to the small size of this species and to the relatively large size of the blood vessels leaving its heart. For the reasons discussed above, these measurements probably represent maximum values at particular oxygen partial pressures.

The most important parameters of circulation concern the volume pumped in relation to time (minute volume) and in relation to total blood volume (turnover time). These can be estimated in a variety of ways as described below. The simplest method of estimating cardiac output is to assume that heart volume equals stroke volume and to multiply this by the heart rate in beats per minute. The minute volume calculated by this method for a 10 g *G. ingens* ranges from 197 ml/kg/min (0·5 ml O_2/l, 42 beats/min, heart volume = 0.047 ml) to 517 ml/kg/min (greater than 1.5 ml O_2/l, 110 beats/min). These values represent the absolute maximum rates which this system could produce. Since stroke volume is certainly somewhat less than the heart volume, higher estimates must have to be in error and actual values would be expected to be lower.

The data presented here on blood velocities provide another method of estimating cardiac output. By the Poiseuille-Hagen equation, $F = P/\pi/8/1/n/R^4/L$, total blood flow (F) in a given length of blood vessel (L) is proportional to the pressure drop (P), inversely proportional to the absolute viscosity (n), and proportional to the fourth power of the radius (R) (Ganong, 1969). Knowing the flow (F) in one of the vessels (assuming an equal pressure drop in all) using this relation, since the viscosity, pressure and length factors will all be small relative to the size factor (R) and can be presumed constant for the argument. It follows (equation 1) that $F = K/R^4$, where F is flow in cm^3/sec, K is a constant and R the radius in cm. A value for K can be derived from total flow (F) in the dorsal abdominal artery. In a 12.2 g *G. ingens* the velocity of blood in the dorsal abdominal artery was observed to be 1.5 cm/sec at low P_{O_2} and the arterial radius at the point of measurement was 0.05 cm (area = 0.008 cm^2). The flow in this artery is the product of the area times the velocity: 0.12 cm^3/sec or 0.72 ml/min. It follows (equation 2) that $K = F/R^4 = (0.012)/(0.05)^4 = 1.92/10^3$. Using the values given in the results for the diameter of the vessels leaving the heart of a 10.06 g individual and K from equation (2), the flow in each artery was calculated by equation (1). The sum of these flows is an approximation of the minute volume (0.805 ml/min). The corresponding cardiac output is 80 ml/kg/min. The full range of flows from the highest measured at high P_{O_2} to the lowest measured at low P_{O_2} is from about 225 ml/kg/min to 55 ml/kg/min. These values are possible since they are lower than the maximum values estimated above and when they are compared with the maximum values suggest that stroke volume is equal to about 25 to 50% of heart volume.

One can also estimate minute volume from the Fick principle if one knows the pre- and postbranchial blood O_2 concentrations and the $\dot{V}_{O_2}$. While we lack measurements of blood (O_2), we can make an estimate based on the $\dot{V}_{O_2}$ of this

species at 6 mm Hg environmental oxygen concentration (0.56 ml O_2/kg/min, Childress, 1971), assuming that the solubility of oxygen in the blood is the same as in sea water (no respiratory pigment). Using such assumptions one arrives at an estimate of a minute volume of 0.02 l/min for a 10 g *G. ingens*. This is impossibly high, exceeding the maximum possible value by fourfold. It suggests that this animal must have a respiratory pigment and that the capacity of blood for O_2 at 6 mm H_g O_2 must be at least 9 to 36 times greater than sea water (that is, between 1.9 and 7.8 ml O_2/l), depending on the actual blood flow rates.

An accurate Fick principle estimate will have to wait until the internal oxygen concentrations of this animal can be measured. Of the other two estimates, those based on heart volume suggest that this species has the potential for high blood flow rates, while the values based on measured flow rates indicate that this animal does indeed realize rather high minute volumes. For comparison with the cardiac output values of 55 to 225 ml/kg/l in *G. ingens* we can examine the lobster *Panulirus interruptus* which has cardiac output estimates between 80 and 150 ml/kg/min for a 700 g animal (Belman, 1975; *P. interruptus* is especially good for comparison since the $\dot{V}_{O_2}$ of a 700 g individual at 16° C is nearly the same as that of a 10 g *G. ingens* at 5.0° C; Winget, 1969). The cardiac output of a 1.0 kg *Cancer magister* (Johansen *et al.*, 1970) is 29 ml/kg/min and for a 0.45 kg *Homarus americanus* (Burger and Smythe, 1953) it is 22 to 67 ml/kg/min. Apparently *G. ingens* is capable of maintaining blood flow greater than most crustaceans examined and about as great as the highest values estimated previously. This clearly is of value for an animal living as *G. ingens* does, at very low P_{O_2}.

A value useful in comparing circulatory dynamics is the turnover time; that is, the time required for the complete circulation of a unit volume of blood. Assuming a total blood volume of from 20 to 30% of the wet weight (a reasonable estimate for crustaceans; Maynard, 1960a), and using a cardiac output between 55 ml/kg/min and 225 ml/kg/min, the turnover time will be from 0.9 to 5 minutes in a 10 g *G. ingens*. Calculated turnover times in decapods are 2.1 min in a 0.65 kg *P. interruptus* and 10 min in a 1.0 kg *C. magister* (Belman, 1975); 1–8 min in a 0.45 kg *H. americanus* (Burger and Smythe, 1953); 1–5 min in a 0.22 kg *C. maenas* (Blatchford, 1971). These comparisons show that circulation in *G. ingens* is about as rapid as in those crustaceans which exhibit the most rapid blood flows.

From values for pulse pressure and minute volume one can estimate the metabolic cost of cardiac output. Assuming that heart tissue is about 15 % efficient (Burton, 1972), the cardiac work for a 10 g *G. ingens* is about $3.5/10^{-4}$ cal/ml while for a 0.6 kg *P. interruptus* it is about $2.4/10^{-3}$ cal/min. This difference in efficiency probably is the result of the proportionately larger diameter and shorter blood vessels in *G. ingens*. The higher efficiency may be of significance as an energy conserving mechanism for *G. ingens* which lives at relatively food-poor greater depths (Childress, 1975).

In conclusion, with respect to those aspects of oxygen uptake considered in this manuscript and in Childress (1971), *G. ingens* appears to possess systems which are developed to as high a degree of effectiveness as found in any aquatic animals. What makes *G. ingens* unique among those species already studied and makes possible its unusual effectiveness at removing oxygen from water is not an unusual degree of development of one system, or some sort of special mechanism, but rather

the high degree of development and effectiveness of all of the mechanisms so far studied.

This research was supported by NSF GA 33232 and NSF GB 18929 to J. J. Childress.

Summary

1. The anatomy of the blood circulatory system in *G. ingens* is similar to that in other crustacea, although proportionately both the heart and arterial channels are quite large.

2. *G. ingens* gill surface area ranges from 5–15 cm^2/g wet body weight. These gill surface areas are as large as the greatest areas measured in other, much larger crustaceans. A large gill surface area relative to a low oxygen consumption rate permits an increased oxygen diffusion across the gills despite a low oxygen gradient.

3. The generalized intra-ventricular blood pressure in *G. ingens* is 24/19 cm H_2O. Mean arterial pressure matches the systolic pressure. These pressures are comparable to those in the large brachyuran *Carcinus maenas*.

4. Blood velocities of from 0.54 to 4.71 cm/sec occur in the heart and arterial system of *G. ingens*. Under normoxic conditions ($pO_2 = 6$ mm Hg) heart rates average 42 b/m and arterial velocities average 1–2 cm/sec.

5. The circulation of blood in *G. ingens*, when compared to other crustaceans on the basis of minute volume, cardiac output and turnover time, appears to be remarkably effective. It is suggested that this effectiveness explains, in large part, the unique ability of *G. ingens* to remove large percentages of the available oxygen while moving large volumes of water over the gills.

LITERATURE CITED

Alexandrowicz, J. S., 1955. Innervation of the heart of *Parunus flexuosus* (Mysidacea). *J. Mar. Biol. Ass. U.K.*, **34**: 47–53.

Banse, K., 1964. On the vertical distribution of zooplankton in the sea. Pages 53–125 in M. Sears, Ed., *Progress in oceanography, Volume II*. Pergamon Press, Oxford.

Baumgarten-Schumann, D., and J. Piiper, 1968. Gas exchange in the gills of resting unanesthetized dogfish (*Scyliorhinus stellaris*). *Resp. Physiol.*, **5**: 317–325.

Belman, B. W., 1975. Aspects of the circulatory physiology of the spiny lobster *Panulirus interruptus* (Randall). *Mar. Biol.*, **29**: 295–305.

Blatchford, J. G., 1971. Haemodynamics of *Carcinus maenas* (L.). *Comp. Biochem. Physiol.*, **39**: 193–202.

Burger, J. W., and C. M. Symthe, 1953. The general form of circulation in the lobster, *Homarus*. *J. Cell. Comp. Physiol.*, **42**: 369–383.

Burton, A. C., 1972. *Physiology and biophysics of the circulation*. 2nd Ed. Yearbook Medical Publishers, Inc., Chicago, 226 pp.

Childress, J. J., 1968. Oxygen minimum layer: Vertical distribution and respiration of the mysid *Gnathophausia ingens*. *Science*, **160**: 1242–1243.

Childress, J. J., 1969. The respiratory physiology of the oxygen minimum layer mysid *Gnathophausia ingens*. *Ph. D. thesis, Stanford University*, Stanford, 142 pp.

Childress, J. J., 1971. Respiratory adaptations to the oxygen minimum layer in the bathypelagic mysid *Gnathophausia ingens*. *Biol. Bull.*, **141**: 109–121.

Childress, J. J., 1975. The respiratory rates of midwater crustaceans as a function of depth of occurrence and relation to the oxygen minimum layer off Southern California. *Comp. Biochem. Physiol.*, **50**: 787–799.

COBBOLD, R. S. C., 1974. Transducers for biomedical measurements: Principles and applications. Wiley, New York, 486 pp.

DEJOURS, P., F. GAREY, AND H. RAHN, 1970. Comparison of ventilatory and circulatory flow rates between animals in various physiological conditions. *Resp. Physiol.*, **9**: 108–117.

DELAGE, Y., 1883. Circulation et respiration chez les Crustacés Schizopodes (*Mysis* Latr.). *Archs. Zool. Exp. Gén.*, **1**: 107–130.

DRACH, P., 1939. Mue et cycle d'interne chez les Crustacés Décapodes. *Ann. Inst. Oceanogr., Monaco*, **19**: 103–391.

DUBUISSON, M., 1928. Recherches sur la circulation du sang chez les Crustacés. 2 note: Pressions sanguines chez les Décapodes Brachyures. *Arch. Biol., Paris*, **38**: 9–21.

FISHER, T. R., C. F. COBURN, AND R. E. FORSTER, 1969. Carbon monoxide diffusing capacity in the bullhead catfish. *J. Appl. Physiol.*, **26**: 161–189.

GADZIKIEWICZ, W., 1905. Über den feineren Bau des Herzens bei Malakostraken. *Jena. Z. Naturw.*, **39**: 203–234.

GANONG, W. F., 1969. *Review of medical physiology*. Lange, Los Altos, California, 628 pp.

GRAY, I. E., 1954. Comparative study of the gill area of marine fishes. *Biol. Bull.*, **107**: 219–225.

GRAY, I. E., 1957. A comparative study of the gill area of crabs. *Biol. Bull.*, **122**: 34–42.

HOLETON, G. F., 1970. Oxygen uptake and circulation by a hemoglobinless Antarctic fish (*Chaenocephalus aceratus* Lonnberg) compared with three red-blooded Antarctic fish. *Comp. Biochem. Physiol.*, **34**: 457–471.

HOLETON, G. F., 1971. Oxygen uptake and transport by rainbow trout during exposure to carbon monoxide. *J. Exp. Biol.*, **54**: 239–254.

HUGHES, G. M., 1970. Morphological measurements on fish gills in relation to their respiratory function. *Folia Morphol.*, **18**: 78–95.

HUGHES, G. M., 1972a. Distribution of oxygen tension in the blood and water along the secondary lamella of the icefish gill. *J. Exp. Biol.*, **56**: 481–492.

HUGHES, G. M., 1972b. Morphometrics of fish gills. *Resp. Physiol.*, **14**: 1–25.

HUGHES, G. M., B. KNIGHTS, AND C. A. SCAMMELL, 1969. The distribution of P_{O_2} and hydrostatic pressure changes within the branchial chambers in relation to gill ventilation of the shore crab *Carcinus maenas* L. *J. Exp. Biol.*, **51**: 203–220.

JOHANSEN, K., C. LENFANT, AND T. A. MECKLENBURG, 1970. Respiration in the crab, *Cancer magister*. *Z. Vergl. Physiol.*, **70**: 1–19.

KROGH, A., 1941. *The comparative physiology of respiratory mechanisms*. Univ. of Pennsylvania Press, Philadelphia. 172 pp.

LARIMER, J. L., 1962. Responses of the crayfish heart during respiratory stress. *Physiol. Zool.*, **35**: 179–186.

LARIMER, J. L., 1964. The patterns of diffusion of oxygen across the crustacean gill membranes. *J. Cell. Comp. Physiol.*, **64**: 139–148.

LARIMER, J. L., AND A. H. GOLD, 1961. Responses of the crayfish *Procambarus simulans* to respiratory stress. *Physiol. Zool.*, **34**: 167–176.

MAYNARD, D. M., 1960a. Circulation and heart function. Pages 161–226 in T. H. Waterman, Ed., *The physiology of crustacea. Vol. I.* Academic Press, N.Y.

MAYNARD, D. M., 1960b. Heart rate and body size in the spiny lobster. *Physiol. Zool.*, **33**: 241–251.

NICOL, J. A. C., 1967. *The biology of marine animals*. 2nd Edition. Pitman, London, 699 pp.

PILLAI, R. S., 1965. The circulatory system of *Cardina laevis* Heller. *Crustaceana*, **8**: 66–74.

PROSSER, C. L., 1973. *Comparative animal physiology*. 3rd Edition. Saunders, Philadelphia, 965 pp.

RANDALL, D. J., 1970. The circulatory system. Pages 133–172 in W. S. Hoar and D. J. Randall, Eds., *Fish physiology, Volume IV*. Academic Press, New York.

RANDALL, D. J., G. F. HOLETON, AND E. D. STEVENS, 1967. The exchange of oxygen and carbon dioxide across the gills of rainbow trout. *J. Exp. Biol.*, **46**: 339–348.

REDMOND, J. R., 1955. The respiratory function of hemocyanin in crustacea. *J. Cell. Comp. Physiol.*, **46**: 209–247.

SAUNDERS, R. L., 1962. The irrigation of the gills in fishes. II. Efficiency of oxygen uptake in relation to respiratory flow, activity and concentration of oxygen and carbon dioxide *Can. J. Zool.*, **40**: 817–862.

SCHMIDT, J., 1925. On the contents of oxygen in the ocean on both sides of Panama. *Science*, **61** : 592–593.

SEATON, P. K., 1971. The circulatory system of *Pachygrapsus crassipes*. *M.A. thesis, San Diego State University*, San Diego, 90 pp.

SEWELL, R. B. S., AND L. FAGE, 1948. Minmum oxygen layer in the ocean. *Nature*, **162** : 949–951.

STEEN, J. B., AND T. BERG, 1966. The gills of two species of hemoglobin-free fishes compared to those of other teleosts—with a note on severe anemia in an eel. *Comp. Biochem. Physiol.*, **18** : 517–526.

STIFFLER, D. F., AND A. W. PRICHARD, 1972. A comparison of *in situ* and *in vitro* responses of crustacean hearts to hypoxia. *Biol. Bull.*, **143** : 247–255.

STORCH, O., 1931. Über die Mechanik des Herzschlages bei Cladoceren. Eine Analyse mit Hilfe der Mikrozeitlupe. *Z. Vergl. Physiol.*, **14** : 709–736.

TEAL, J. M., AND F. G. CAREY, 1967. Respiration of euphausiid from the oxygen minimum layer. *Limnol. Oceanogr.*, **12** : 548–550.

WINGET, R. R., 1969. Oxygen consumption and respiratory energetics in the spiny lobster *Panulirus interruptus* (Randall). *Biol. Bull.*, **136** : 301–312.