

The Effects of Environmental Oxygen Levels on the Respiratory Function of Hemocyanin in the Crabs, *Libinia emarginata* and *Ocypode quadrata*¹

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ABSTRACT The respiratory function of the hemocyanin (Hcy)-containing blood in the aquatic spider crab, *Libinia emarginata*, and the terrestrial ghost crab, *Ocypode quadrata*, was studied in animals exposed to normoxic and hypoxic conditions at 25°C. In *L. emarginata* low concentrations of Hcy, although 90% O₂ saturated at the gill, necessitate a high blood and water convection strategy. *O. quadrata* has blood with a higher concentration of hemocyanin which is 96% O₂ saturated at the gill, obviating a very high rate of blood circulation. During progressive hypoxia convection initially increases on both sides of the gill in *L. emarginata* while in *O. quadrata* cardiac output decreases. Blood pH increases with decreasing ambient P_{O₂}, below 60 torr in *L. emarginata*, inducing a greater hemocyanin O₂ affinity.

The problem of withstanding periods of hypoxic stress is particularly interesting in the decapod crustaceans, which include a large number of terrestrial species with adaptations such as changes in gill surface area and vascularization of the branchial cavity (Pearse, '29). Respiratory adaptations accompanying the transition of organisms from water to land have been widely studied in vertebrates but have received little attention in the invertebrates. The present study describes the in vitro and in vivo properties of oxygen binding to hemocyanin in two invertebrate species, representing an aquatic and an aerial mode of respiration, and seeks to explain compensatory changes in ventilation, oxygen extraction, cardiac output and blood gas transport at different levels of ambient oxygen availability. Attention is also given to the relation between cardiac output and the concentration of hemocyanin in the blood.

Oxygen uptake rates in many marine invertebrates show little evidence of oxygen independence in declining oxygen conditions (Mangum and Van Winkle, '73). As the respiratory role of the blood becomes more important, oxygen uptake is more strongly regulated. While oxygen independence in many groups may involve only passive, genetically fixed mechanisms such as the intrinsic properties of the blood, it has been known for many

years that active compensatory responses to hypoxia occur in decapod crustaceans. Examples of active compensations involve the circulatory (McMahon et al., '74; Hill and Koopowitz, '75; deFur and Mangum, '79), as well as the ventilatory system (Taylor, '76; McMahon et al., '78; Butler et al., '78; Batterton and Cameron, '78), but the importance of each, and their efficacy in opposing the effect of hypoxia on total aerobic metabolism have not been widely investigated.

Recent studies have elucidated various aspects of gas exchange in crabs, including the countercurrent nature of gill ventilation and perfusion (Hughes et al., '69), circulatory dynamics of blood (Blatchford, '71; Belman, '75) and respiratory properties of the hemocyanin-containing blood (Redmond, '55, '62, '68; Johansen et al., '70; McMahon and Wilkens, '75; Weiland and Mangum, '75; Taylor, '76; Mangum and Towle, '77). Hemocyanin, which is dissolved in the hemolymph, occurs in fairly low concentrations in both molluscs and arthropods. The low concentration is probably related to the contribution of hemocyanin to colloid osmotic pressure (Mangum and Johansen, '75). Oxygen carrying capacities in crab

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blood are therefore quite low and hemocyanin concentrations are quite variable both within and between species. Interspecific differences in hemocyanin concentration are related to cardiac output. Johansen et al. ('70) reported a relatively high concentration of the oxygen carrier in the hemolymph in *Cancer magister*. Accompanying this high hemocyanin concentration is a low cardiac output. While in vivo hemocyanin oxygenation states in *C. magister* and the blue crab *Callinectes sapidus* are similar, the oxygen carrying capacity of blue crab blood is smaller by half (Mangum and Weiland, '75). One result of decreased oxygen carrying capacity is an increase in the perfusion requirement, as in *C. sapidus*.

MATERIALS AND METHODS

Experimental animals

Spider crabs, *Libinia emarginata* (Leach), were obtained by trawling in North Inlet Estuary near Georgetown, South Carolina, or purchased from the Gault Fish Company in Beaufort, South Carolina. Ghost crabs, *Ocyropsis quadrata* (Fabricius), were collected at night on ocean beaches between Georgetown and Pawley's Island, South Carolina. Crabs were transported in insulated containers to Williamsburg, Virginia, where the in vitro oxygen binding characteristics of hemocyanin, heart rate and blood oxygen carrying capacity were determined, and to Columbia, South Carolina, where measurements of oxygen uptake, ventilation, percent oxygen extraction and in vivo P_{O_2} and pH were carried out. Spider crabs (mean wet weight 188 gm, range 60-235 gm) were kept in aerated sea water (30‰ salinity). Ghost crabs (mean wet weight 44 gm, range 10-87 gm) were kept individually in plastic boxes containing a small amount of sea water (30‰ salinity). Only male crabs of each species were used. Crabs were held under laboratory conditions at 25°C for at least 1 week prior to experimentation and fed a diet of fish and shrimp 2 to 3 times each week. Animals were starved at least 24 hours prior to each experiment.

Oxygen uptake

Oxygen uptake (\dot{V}_{O_2}) in *L. emarginata* was measured by monitoring the depletion of oxygen (Yellow Springs Instrument Co. Model 5720A oxygen electrode) in a closed vessel containing more than 5 l sea water. Animals were thoroughly scrubbed to remove epifauna, placed in the containers and the water aerated

for 1 hour prior to \dot{V}_{O_2} measurements. In *O. quadrata* aerial oxygen uptake rates were measured in large (300-500 ml) glass flasks attached to Warburg manometers. One milliliter sea water (30‰ salinity) was placed in each flask to provide a humid atmosphere and approximately one ml 20% KOH was present to absorb CO_2 . Crabs were allowed to remain in the flasks for 1 to 2 hours prior to measurement of oxygen uptake in commercially prepared gas mixtures containing 22, 37, 74 and 155 torr oxygen (Liquid Air, Inc.). The respiratory chambers were gassed for 10 minutes and sealed. Hypoxia was induced by gassing the flasks with progressively lower levels of oxygen. All measurements were made at approximately the same time of day. \dot{V}_{O_2} measurements on ghost crabs submerged in sea water were made using the YSI apparatus.

Oxygen equilibrium of hemocyanin

Blood was removed by hypodermic syringe from the base of one of the rear walking legs and allowed to clot. The clot was broken up in a tissue homogenizer and the blood sample centrifuged at $17,000 \times g$ for 30 minutes. The hemolymph was diluted with filtered sea water (30‰ salinity) and buffered with 0.05 M Bis-Tris-HCl Buffer (Sigma Chemical Company). Sea water was used to dilute the hemolymph since it closely approximates the major ionic constituents of crustacean blood (Prosser, '73). The Mg^{++} and Ca^{++} concentration differences between sea water and blood were ignored since their effective changes on hemocyanin O_2 affinity in this case are opposite (Truchot, '75a) and the dilutions were usually less than 3 parts sea water to 1 part blood. The samples were equilibrated in tonometers with various mixtures of oxygen and nitrogen, prepared with a Wösthoff Gas Mixing Pump (Type 1SA 27/2). High purity nitrogen (99.999%) was scrubbed with Fieser's oxysorbent and a 1% $CdSO_4$ solution before mixing with oxygen (Matheson Gas Products) and humidified. The tonometered blood samples were incubated in a thermostatted water bath ($\pm 0.1^\circ C$) and gently mixed with small magnetic stirring bars at each P_{O_2} for 7 minutes. The absorbance was measured at 335 nm.

Oxygen carrying capacity

The oxygen carrying capacity of the blood in *L. emarginata* was determined with a Lexington Instrument Corp. blood oxygen analyzer

(Lex O₂ CON-TL) after equilibrating a blood sample with air. Total oxygen content of air equilibrated blood samples taken from *O. quadrata* was measured with the Scholander microvolumetric oxygen analysis technique (Roughton and Scholander, '43; Scholander et al., '55) as modified by Hoffmann and Mangum ('70). Oxygen carrying capacity estimates for *L. emarginata* were verified by repetition with the Scholander microvolumetric oxygen analysis technique, and prediction from hemocyanin concentration based on the extinction coefficient for oxygenated hemocyanin at 335 nm in *Carcinus maenas* (13,719 l/g-atom Cu per cm; Nickerson and Van Holde, '71) and the assumption that two atoms of copper bind one molecule of O₂ (Redfield, '34).

In vivo blood P_{O₂} and pH

In vivo blood P_{O₂} and pH measurements were made at a variety of ambient oxygen levels ranging from 15-155 torr for *L. emarginata* and at 22, 37, 74 and 155 torr for *O. quadrata*. Water P_{O₂} was lowered progressively over a period of 30 minutes by bubbling nitrogen into the medium and then held constant at the desired level for at least 2 hours. Aerial hypoxic conditions were achieved in glass respirometers as described above. Sampling of postbranchial blood by needle puncture was facilitated in *L. emarginata* by drilling a hole partially through the carapace over the pericardium at least 24 hours prior to sampling. Prebranchial blood samples were obtained from the infrabranchial sinus at the base of the third or fourth walking leg. Each crab was pre-exposed to a given water P_{O₂} for at least 2 hours before sampling, which was completed within about 20 seconds. In all cases, post-branchial blood was sampled first. Blood P_{O₂} was measured with a Radiometer P_{O₂} electrode at 25°C. The remainder of the same sample was allowed to clot, the clot carefully disintegrated with a tissue homogenizer and centrifuged. The sample received minimal exposure to air and was kept on ice at all times until just prior to pH determination. pH values based on this procedure have been shown to vary little from values using completely anaerobic procedures (Truchot, '73). In addition, pH values determined in this study for *L. emarginata* are lower than values obtained in the same species at 23°C using a completely anaerobic technique (Mangum and Shick, '72), a result which indicates little if any CO₂ loss to the air. pH was measured at

25°C with a Fisher combination electrode (glass type) and a Model 130 Corning pH meter or with a Radiometer pH electrode (liquid junction) and an acid-base analyzer (Radiometer PHM71).

Ventilation

Water flow (\dot{V}_w) across the gills in *L. emarginata* was estimated from simultaneous measurements of oxygen uptake and percent oxygen extraction from the incurrent ventilation stream (Holeton and Randall, '67; Johansen et al., '70). Percent oxygen extraction from incurrent water (%Extr_w) was determined by continuously monitoring the oxygen partial pressure (Beckman O₂ electrode) of the excurrent ventilation stream leaving a wax mask (Utility Wax, Modern Materials Mfg., Inc.) placed over the mouthparts and excurrent opening of the gill chamber of the crab.

Heart rate and volume

Heart rate in each of 2 spider crabs was recorded using an impedance pneumograph (E & M Instrument Co., Inc.) with 2 platinum electrodes implanted in the lateral regions of the pericardium and held in place with Periphery Wax (Lactona Surgident). Signals were amplified and recorded on a Physiograph Six.

Heart volume was determined by removing a portion of the carapace over the heart and injecting the ventricle with latex rubber until the heart was inflated and rubber appeared outside the heart. This preparation was allowed to set overnight in isopropyl alcohol and sea water. Ventricles were subsequently dissected and their volume determined by displacement.

Symbols and data analysis

The symbols and units used are those recommended by the International Union of Physiological Sciences in the Glossary on Respiration and Gas Exchange ('73).

Mean values were compared according to the Student's *t* test.

RESULTS

Oxygen uptake and branchial ventilation

When \dot{V}_{O_2} is plotted as a function of incurrent P_{O₂} (P_{1O₂}) (fig. 1) a moderate degree of regulation is apparent in both species. If these data are fit to the quadratic polynomial model of Mangum and Van Winkle ('73), the value for the coefficients describing the degree of regulation is -0.06×10^{-3} for *L. emarginata*

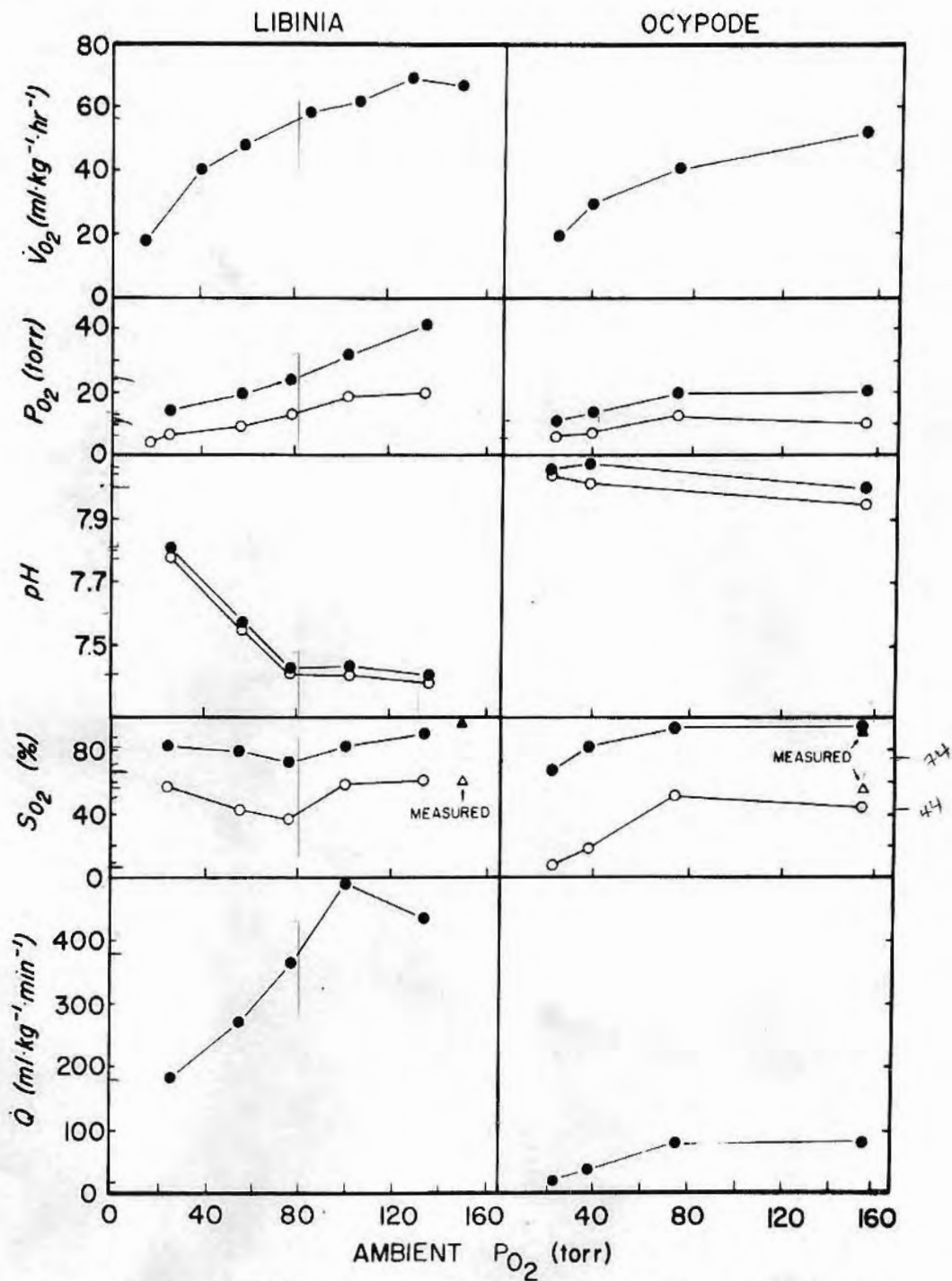


Fig. 1 Oxygen uptake ($\dot{V}O_2$), blood P_{O_2} , pH and percent oxygen saturation (S_{O_2}) (postbranchial, closed circles; prebranchial, open circles), and cardiac output (\dot{Q}) as a function of ambient P_{O_2} in *Libinia emarginata* and *Ocypode quadrata*. Direct determination of postbranchial S_{O_2} (▲) and prebranchial S_{O_2} (△) are also indicated.

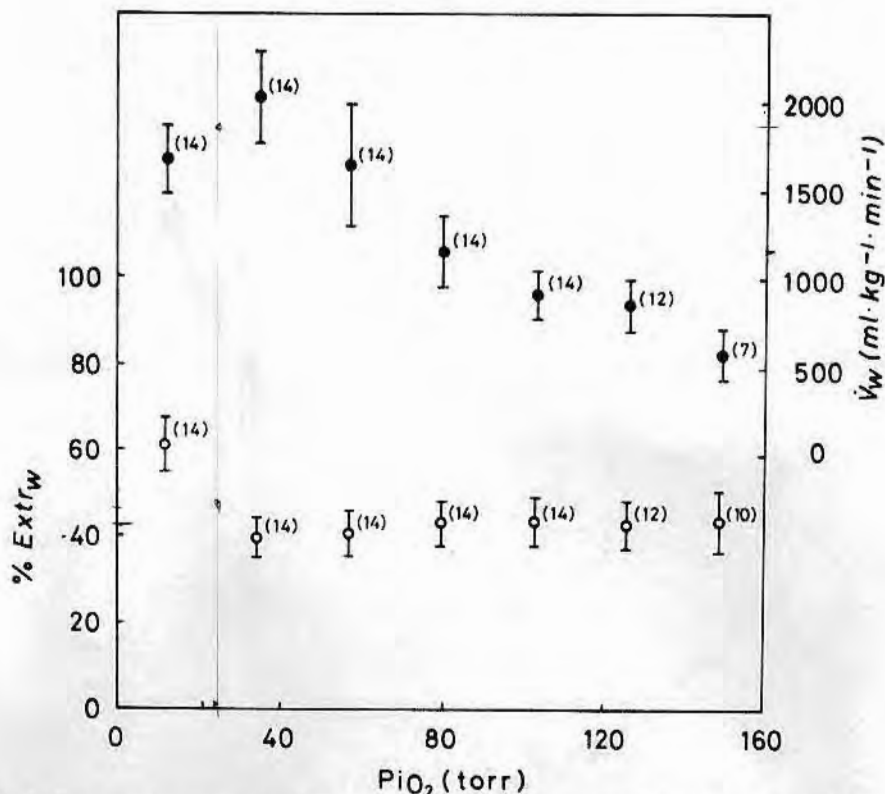


Fig. 2 Percent oxygen extraction (% $Extr_w$) from the incurrent ventilation stream (open circles) and estimates of branchial ventilation (\dot{V}_w ; closed circles) as a function of incurrent P_{O_2} (PiO_2) in *Libinia emarginata* (25°C; 30 ‰ salinity). Mean \pm S.E. (N).

and -0.03×10^{-3} for *O. quadrata*, resembling their curves F and D respectively. These coefficients indicate an appreciable regulation of oxygen consumption, more so in *L. emarginata* than in *O. quadrata*.

Rates of oxygen uptake in *L. emarginata* completely submerged in air saturated sea water are about twice as high as those obtained manometrically by King ('65) on crabs in only enough water to cover the opening of the gill chamber. It was found in the present study that vigorous aeration is necessary to maintain levels of ambient oxygen when animals are held in open containers. Oxygen levels drop very rapidly (less than 50% air saturation) when aeration ceases.

The \dot{V}_{O_2} values for *O. quadrata* in air (fig. 1) and water (100 ml/kg-hr; N = 4) are much lower than those found earlier (Ayers, '38; Flemister and Flemister, '51). Furthermore, the aerial rates closely fit a line (slope = 0.69) describing the logarithmic relationship between \dot{V}_{O_2} and body weight in *O. quadrata*

(Wolcott, '78). \dot{V}_{O_2} is much higher in submerged animals and shows complete oxygen dependence.

Oxygen extraction from the incurrent ventilation stream (% $Extr_w$) in *L. emarginata* averages 44.5%, remaining unchanged ($P > 0.10$) in the P_{O_2} range 33-155 torr, but increasing ($P < 0.005$) below 33 torr (fig. 2). Estimates of ventilation (\dot{V}_w) in *L. emarginata* average 575 ml/kg-min in normoxic water. The rates increase with decreasing PiO_2 to about 33 torr (fig. 2), below which there is a slight decrease. Several observations of flow direction using suspended clay particles failed to reveal ventilation reversals.

In addition to tolerating the imposed level of hypoxia, five specimens of *L. emarginata* survived anoxia for two hours. One crab tested for 13 hours also survived.

Oxygen equilibrium of hemocyanin

Oxygen equilibrium curves for *L. emarginata* hemocyanin indicate a rather typical

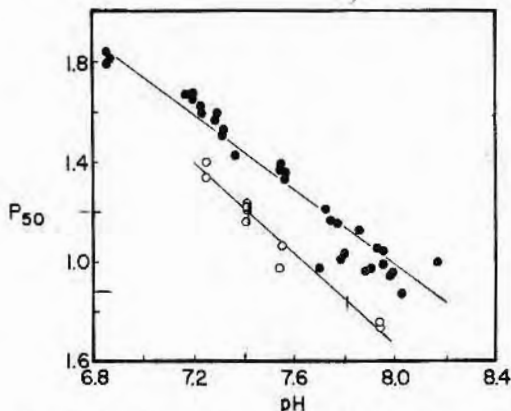


Fig. 3 The oxygen affinity (P_{50}) of *Libinia emarginata* hemocyanin (open circles) and *Ocypode quadrata* hemocyanin (closed circles) as a function of pH (25°C). The magnitude of the Bohr shift, indicated by the slopes of the fitted regression lines, is -0.92 in *L. emarginata* and -0.76 in *O. quadrata*.

oxygen affinity ($P_{50} = 16.7$ torr at 25°C and pH 7.41) and a normal Bohr shift ($\Delta \log P_{50}/\text{pH} = -0.92$ in the pH range 7.41 to 7.94) (fig. 3). Under the same conditions hemocyanin oxygen affinity in *O. quadrata* is lower ($P_{50} = 27.5$ torr) and the Bohr shift slightly smaller ($\Delta \log P_{50}/\text{pH} = -0.76$ at pH 7 to 8). The cooperativity of oxygen binding differs in the two species. The value of the Hill coefficient at about 50% oxygen saturation (n_{50}) and in the physiological pH range (7.4-7.8) is 2.3 - 2.7 in *L. emarginata* and 2.9 - 3.9 in *O. quadrata* (pH 8.0) (fig. 4).

Oxygen carrying capacity

The oxygen carrying capacity of blood in *L. emarginata* ($\text{HcyO}_2 + \text{Free O}_2$) averages 0.76 vol % oxygen (± 0.02 S.E.; $N=86$) with values ranging from 0.5 vol % to 1.5 vol %. The quantity of oxygen bound to hemocyanin is therefore 0.29 vol%. Similar values were obtained with a fundamentally different procedure using a Scholander microvolumetric oxygen analyzer (0.80 and 0.86 vol %). Spectrophotometric determination of hemocyanin concentration in four crabs predicted hemocyanin oxygen capacities of 0.29, 0.24 and 0.21 vol%. However, values obtained with a Lex O_2 CON-TL analyzer for five spider crabs freshly collected in Virginia during the summer yield an average value of 1.16 vol% (± 0.06 S.E.). Although the reason for this degree of variation is unknown it may not be due solely to geographic differences or to nutritional dete-

rioration in the laboratory. The same procedure used on the blood of two crabs freshly caught in Virginia the previous year gave values of 0.8 vol%.

In contrast, the blood oxygen carrying capacity in *O. quadrata* averages 2.55 vol% (0.08 S.E.; $N=57$) with values ranging from 1.26 to 4.38 vol%. The hemocyanin oxygen capacity is 2.06 vol%, much higher than in *L. emarginata*.

In vivo P_{O_2} and pH

Values for blood pH and P_{O_2} in *L. emarginata* (fig. 1) fall within the ranges reported for other decapod crustaceans in the same temperature range (Mangum and Shick, '72; Mangum and Weiland, '75). The oxygenation at the gill of *L. emarginata* is 90%, similar to values found in other species. In hypoxic water, blood P_{O_2} decreases (fig. 1), and blood pH remains fairly constant in the ambient P_{O_2} range 60-152 torr. Surprisingly, blood pH increases at water P_{O_2} 21-60 torr.

Data for *O. quadrata* indicate an oxygen transport function of the blood under normoxic conditions which is quite similar to that in *L. emarginata*. However, blood P_{O_2} of ghost crabs in normoxic conditions is much lower than in *L. emarginata*. Low postbranchial blood P_{O_2} in resting *O. quadrata* results in very high oxygen saturation since the blood has a high oxygen affinity. Moreover, a high cooperativity of Hcy- O_2 binding (n_{50}) even at low blood oxygen pressures, apparently permits the delivery of a large volume of oxygen despite a small blood P_{O_2} change between postbranchial and prebranchial blood.

Postbranchial blood pH in *O. quadrata* is significantly higher (Student's t test for paired observations; $P < 0.01$) than prebranchial blood pH only at ambient P_{O_2} 155 torr and 37 torr, but the small pH difference in normoxia induces an 8% increase in deoxygenation. Blood P_{O_2} does not decrease significantly at ambient $P_{\text{O}_2} = 74$ torr, although more pronounced hypoxia results in simultaneous decreases in blood P_{O_2} and cardiac output. Blood pH appears to rise during hypoxia, but the increase is significant only at ambient $P_{\text{O}_2} = 22$ torr and only in prebranchial blood.

Cardiac output

Cardiac output (\dot{Q}) estimates for *L. emarginata* (fig. 1) calculated according to the Fick principle, are higher than those found in other species (Redmond, '55, '62; Johansen et al., '70; Belman, '75; Mangum and Weiland, '75;

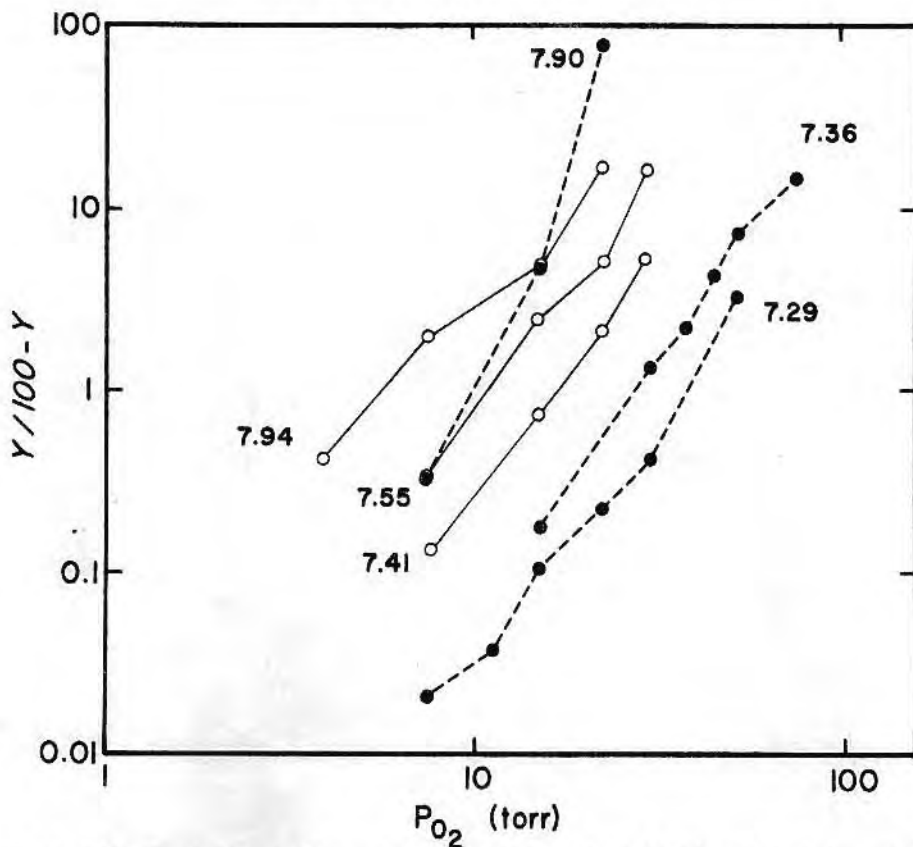


Fig. 4 A Hill plot showing the changes in slope with oxygenation in *Libinia emarginata* (open circles, solid line) and *Ocypode quadrata* (closed circles, dashed line).

Taylor, '76). Cardiac output estimated by the Fick principle involved using P_{O_2} and pH data and oxygen binding data to estimate the total oxygen content of postbranchial and prebranchial blood. Direct measurements of total blood oxygen content when animals were held in well-aerated conditions shows rather close agreement with estimated values of percent Hcy O_2 (fig. 1). In *L. emarginata* the total oxygen content of postbranchial blood is 0.78 vol% (± 0.06 S.E.; $N=6$) while prebranchial blood contains 0.46 vol% (± 0.04 S.E.; $N=7$). In *O. quadrata* the oxygen content of postbranchial blood is 1.89 vol% (± 0.11 S.E.; $N=14$) and prebranchial blood is 1.05 vol% (± 0.10 S.E.; $N=13$).

The heart volume of a spider crab of average weight (188 gm) is 0.75 ml. If the volume estimated from the injection of latex represents stroke volume, then the product of this quantity and heart rate (127 beats/min) gives an

independent estimate of cardiac output. This value is more than 67% of the Fick estimate for animals with a low blood oxygen carrying capacity (0.76 vol%) but 117% of the estimate for animals with a high blood oxygen carrying capacity (1.16 vol%). However, heart volume measured by latex injection is an estimate of total volume which undoubtedly overestimates stroke volume and thus overestimates cardiac output.

The present estimates of cardiac output in *L. emarginata* suggest an increase with declining P_{iO_2} down to 91 torr and a decrease below that level (fig. 1). In *O. quadrata* cardiac output, estimated according to the Fick principle, is an order of magnitude lower than in *L. emarginata* and there is no evidence of a compensatory response during hypoxia.

Convection requirements

The blood-convection requirement (\dot{Q}/\dot{V}_{O_2})

designates the volume of blood which must be passed over the tissues to supply a unit volume of oxygen (STPD). In normoxic water the blood-convection requirement in *L. emarginata* is 434 ml/ml O₂ (assuming a blood oxygen carrying capacity of 1.16 vol%), much higher than in *O. quadrata* (105 ml/ml O₂). This difference is probably due to the much higher O₂ carrying capacity of ghost crab blood.

The water or air convection requirement (\dot{V}_a or \dot{V}_w/\dot{V}_{O_2}) is the volume of ambient medium which must be passed over the gills to supply a unit volume of oxygen (STPD). In *L. emarginata* under normoxic conditions this quantity is 577 ml/ml O₂ and it increases in declining ambient oxygen.

Due to the very high estimate of cardiac output, the ventilation:perfusion ratio in *L. emarginata* lies between 0.77 and 1.38.

DISCUSSION

The very low concentration of hemocyanin in the blood of *L. emarginata* necessitates high rates of ventilation and circulation to support the oxygen requirement, especially when environmental oxygen availability decreases. Such high blood circulation rates, however, are unprecedented.

Johansen et al. ('70) concluded that a cardiac output of 80 ml/kg-min, calculated according to the Fick principle by Redmond ('55) for *Panulirus interruptus* is excessively high for an invertebrate. However, as pointed out by Mangum and Weiland ('75) heart rate in crustaceans is highly correlated with body size and with ambient temperature. Hence, the values of cardiac output for large animals such as *Cancer magister* (Johansen et al., '70) at low temperatures would be expected to be low. When cardiac output estimates in other organisms are converted to blood-convection requirements and plotted against the hemocyanin oxygen carrying capacity (fig. 5), a clear pattern matches low hemocyanin levels with very high blood-convection requirements. Species such as *O. quadrata*, *Cancer magister* and the octopus *Octopus dofleini* (Johansen, '65) have high hemocyanin concentrations and low blood-convection requirements, while the reverse is true of *L. emarginata*. Dejours et al. ('70), Dejours ('75) and Mangum ('77) cite several variations of this phenomenon.

Despite the low concentrations of hemocyanin in *L. emarginata*, blood P_{O₂} is about the same as in the blue crab, *Callinectes sapidus*,

at a comparable temperature. Hemocyanin oxygen affinity in both crabs is great enough to oxygenate the pigment nearly fully at post-branchial blood P_{O₂} and maintain a rather large venous reserve at prebranchial blood P_{O₂}.

The respiratory properties of blood at 25°C are somewhat different in the ghost crab. Despite the greater O₂ availability in air, a very large P_{O₂} difference exists across the gills, as in other terrestrial species (Redmond, '62, '68; Cameron and Mecklenburg, '73). This large P_{O₂} gradient probably arises in part from an increase in gill rigidity and thus greater diffusion distance of the gill epithelium, which accompanied the loss of buoyancy in the low density medium. With the transition to land, gill number and gill area were reduced as well (Gray, '57): gill number in *O. quadrata* is only 75% of that in *L. emarginata*, and gill area is only 57%. In addition, a reduction of the branchial system, which includes the volume of blood vessels (Pearse, '29; Ayers, '38) as well as the area of the gas exchange surface, results in a smaller residence time of the blood and thus a reduced equilibration time between a unit volume of blood and the external medium. Another terrestrial adaptation in the ghost crab is the vascularization of the branchial chamber (Pearse, '29). Although some gas exchange apparently takes place between the blood and air at these sites (Dias and Rodriguez, '78) their importance in overall oxygen uptake is unknown.

In spite of the low blood P_{O₂}, hemocyanin in *O. quadrata* is almost fully oxygenated at the gill due to its high oxygen affinity. The venous oxygen reserve (Sv_{O₂} = 44%) may be of some importance to these exceptionally active animals. During activity lower blood P_{O₂} and pH are likely to occur (Mangum and Weiland, '75). The net effect would be a decrease in the oxygen transported during a single circuit of the blood, and an insufficient O₂ supply to the tissues. When pursued on the open beach ghost crabs cannot run for longer than one minute, suggesting that the venous reserve, which is not unusually large, is quickly exhausted.

Thus, although blood pH and P_{O₂} are quite different in the two species, the properties of hemocyanin are adapted to the differences to perform a quite similar respiratory function. Blood P_{O₂} in both species shows a linear decrease with a decrease in ambient P_{O₂} (fig. 1). The decline in blood P_{O₂} necessitates compensation in either ventilation or circulation or

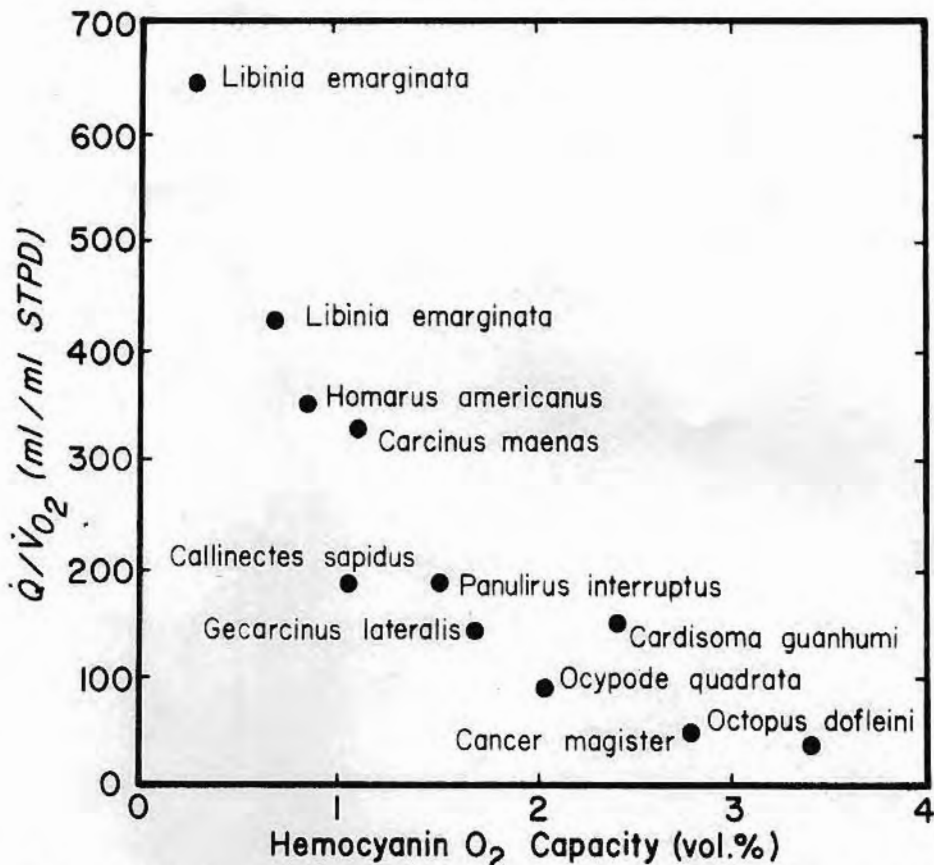


Fig. 5 The blood convection requirement (Q/V_{O_2}) of a variety of animals as a function of oxygen binding capacity of the hemocyanin in blood. Based on data presented by Redmond ('55, '62, '68), Johansen ('65), Winget ('69), Johansen et al. ('70), Truchot ('71), Cameron and Mecklenburg ('73), Taylor and Butler ('73), Cameron ('75), Mangum and Weiland ('75), McMahon and Wilkens ('75), and Taylor ('76).

both if the uptake of oxygen is to be regulated. However, this type of compensation involving convection at the gill surface may not be necessary if the respiratory pigment is able to transport sufficient quantities of O₂ during hypoxic stress. This may be the case during mild hypoxia. Another mechanism may involve a change in the properties of the blood during the hypoxic situation to render it better able to transport oxygen to the tissues, i.e., an increase in oxygen affinity.

Each of these types of adaptation is apparent to some degree in *L. emarginata* and *O. quadrata*. The most fundamental difference, however, lies in the reduced oxygen carrying capacity and diffusion rate in sea water in the case of the spider crab. Water is the poorer medium for making oxygen available and thus the convection requirements, even in normox-

ia, are much higher than for animals breathing air (Dejours, '75). *L. emarginata* compensates for a reduced O₂ supply by passing larger quantities of water over the gills. There is no evidence for such compensations in the terrestrial crabs (Cameron and Mecklenburg, '73).

The decline in blood P_{O₂} in *L. emarginata* during moderate hypoxia results in slightly less O₂ transported by hemocyanin per circuit through the organism. To compensate for this change, cardiac output increases resulting in the regulation of oxygen uptake. Although an increase in branchial ventilation during hypoxia is common among aquatic crustaceans (von Schlieper and Peters, '37; Lindroth, '38; Larimer, '61; Larimer and Gold, '61; Arudpragasam and Naylor, '64; Taylor, '76; Taylor et al., '77; Batterton and Cameron, '78),

the compensatory increase in cardiac output in *L. emarginata* is less common (deFur and Mangum, '79).

Ocyropsis quadrata shows no such circulatory compensation. Cardiac output remains nearly the same when ambient P_{O_2} is reduced to 74 torr. Regulation of oxygen uptake appears to be related to the ability of the blood to transport nearly the same quantity of oxygen to the tissues.

At lower ambient P_{O_2} 's the postbranchial-prebranchial blood oxygenation differences are as large or larger than control values in both species; however, cardiac output shows a marked decline. Ultimately this results in smaller quantities of oxygen transported to the tissues and the regulation of V_{O_2} fails.

In addition to cardiac and ventilatory adjustments there is a somewhat surprising increase in blood pH at low ambient oxygen levels (fig. 1). Truchot ('75b) and McMahon et al. ('78) also noted an increase in blood pH in crabs exposed to hypoxia. A similar result was obtained by the present author for the mud crab, *Panopeus herbstii*, in which average prebranchial blood pH at 28°C, 27‰ salinity and $P_{O_2} = 155$ torr is 7.51 (± 0.02 S.E.; N=8) and 7.75 (± 0.02 S.E.; N = 8) after 90 min when $P_{O_2} = 23$ torr. The pH shift raises oxygen affinity and thus permits appreciable oxygenation at low postbranchial blood P_{O_2} . Thus, even at very low ambient oxygen levels (21-24 torr) postbranchial blood is 82% O_2 saturated while prebranchial blood is 56% O_2 saturated.

Oxygen uptake in *L. emarginata* is clearly regulated during environmental hypoxia. The concentration of hemocyanin in the blood is very low. Therefore, compensation is strongly dependent on the regulation of fluid flow on both sides of the gill epithelium. The most obvious limitation of such a system is the energy requirement of operating the pumps to circulate blood and water, which is poorly understood. Direct measurements of the metabolic expenditure of resting and working hearts and scaphognathites have not been made. However, a limitation is apparent in other phyla at very low levels of oxygen where at least enough oxygen must be extracted from the ventilation stream to operate these pumps (Mangum and Burnett, '75).

The spider crab and ghost crab employ very different strategies to achieve similar levels of oxygen uptake at 25°C. Sufficiently high quantities of hemocyanin are present in the blood of the ghost crab to make it very effi-

cient in transporting oxygen at low blood flow. The blood of the spider crab, on the other hand, contains extremely low concentrations of hemocyanin, forcing the alternative strategy of a much faster blood flow rate to deliver oxygen to the tissues. While one strategy demands more physical work, the other requires the synthesis of more blood protein. The energetic demands of the two alternatives cannot be evaluated at present. The convection strategy, however, is clearly more sensitive to short term environmental perturbations that characterize aquatic habitats, and thus its exploitation by the aquatic species is hardly surprising.

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