

On the Relationship between P_{50} and the Mode of Gas Exchange in Tropical Crustaceans¹

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ABSTRACT: In general, the oxygen affinity of hemocyanin does not decrease when tropical decapod crustaceans carry on gas exchange in air instead of water. Other oxygenation properties such as cooperativity and the Bohr shift also change very little, if at all. The generalization of a higher oxygen affinity in tropical than in temperate zone species appears to be true but has exceptions of unclear origins, emphasizing the crudity of correlations between respiratory properties of the blood and gross features of the environment.

THE EXCHANGE OF RESPIRATORY GASES in air occurs in a medium far richer in oxygen than water. Among the vertebrates (e.g., amphibians), increasing dependence on air is accompanied by profound modifications of the sites and organs of gas exchange, and a clear decrease in the oxygen affinity (P_{50}) of the blood (Johansen and Lenfant 1972), presumably in response to higher levels of blood P_{O_2} .

However, the taxonomic scope of this generalization appears to be limited, and the relationship may even be reversed. Among the annelids, hemoglobin-oxygen (HbO_2) affinity is uniformly high in the earthworms, in which the gas exchange site is the general body wall epithelium, whereas it may become very low in the aquatic polychaetes, with their frequently elaborate gills (Mangum 1976). Despite morphological adaptations for air breathing, hemocyanin-oxygen (HcO_2) affinity under physiological conditions is about the same in pulmonates (Spoek, Bakker, and Wolvekamp 1964; Wells and Shumway 1980) and in several aquatic gas-

tropods (Jones 1972, Mangum and Lykkeboe 1979, Redmond 1968b, Wells and Wong 1978). One aquatic species actually has a lower HcO_2 affinity (Brix, Lykkeboe, and Johansen 1979) than pulmonates. No clear trend can be discerned among the chelicerates, where the comparison is limited by the scarcity of information as well as a surprising heterogeneity within the terrestrial arachnids (Mangum 1980). An enormous difference between HbO_2 affinities in larval, water-breathing chironomids and adult, bubble-breathing notonectids is related to the unique function of hemoglobin in the back swimmers, viz. buoyancy control, and not to the mode of gas exchange (Wells, Hudson, and Brittain 1981). In a study of water- and air-breathing fishes, Johansen, Mangum, and Lykkeboe (1978) found no clear relationship between P_{50} at pH 7.4 and the mode of gas exchange; only at the physiological pH characteristic of the particular species can significantly lower oxygen affinity be detected in air breathers. They concluded that the design of the gas exchange organs and the cardiovascular system is so different among the various groups of air breathers (see also Johansen 1970) that physiological determinants of oxygenation such as blood acid-base balance may counteract the influence of ambient oxygen availability. Thus, Fyhn et al. (1979; see also Table 3 in Riggs 1979), who controlled P_{CO_2} in the equilibration gas and allowed pH

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in the sample to vary with total CO₂ content, also found significant differences in several of the same species.

The relationship between HcO₂ affinity and the mode of gas exchange in crustaceans is uncertain. Redmond (1962, 1968*a*, 1968*b*) found a high oxygen affinity in one terrestrial species and a lower oxygen affinity in another, compared with several aquatic decapods. Young (1972) concluded that HcO₂ affinity in crabs decreases with emergence onto land. However, his comparison includes variables other than the intrinsic ligand affinity of the carrier, viz. blood pH and geographic distribution of the species. When the data are corrected to a common pH of 7.6, the values for the three most terrestrial species differ by less than 1 mm Hg, and the value for an intertidal species that breathes water as well as air suggests an oxygen affinity more than 10 mm Hg lower than in the three air breathers. While HcO₂ affinity in the subtidal water breather *Callinectes sapidus* was higher than any of the others, this comparison is confounded by a geographic variable; *C. sapidus* is the only species in the sample that extends throughout the temperate zone. Sevilla and Lagarrigue (1979) found that HcO₂ affinity decreases in isopods with increasing specialization of the organs of gas exchange for breathing air, but all the species in their sample are supratidal air breathers. The only data available for a water breather are for the giant, deep-sea (and therefore cold-water) species *Bathyonus giganticus* (K. E. Van Holde and M. Brenowitz, personal communication). With these additional differences in mind, and also with the reservation that none of the information for isopod hemocyanins has been obtained under strictly physiological conditions, the comparison also suggests no difference between *Bathyonus* and several of the air breathers, including one of the species with a tracheal system.

A compilation of data collected under physiological conditions of inorganic ions and pH actually suggests that many terrestrial crabs have hemocyanins with higher oxygen affinities than those of their aquatic relatives (Mangum 1980). This comparison, however, retains the geographic variable. Most terrestrial crabs live in the tropics, and no data have

been obtained for strictly aquatic and also tropical species. The values for a single temperate zone air breather (Burnett 1979) do not differ from those for temperate zone water breathers (Mangum 1980).

The Philippines expedition of R/V *Alpha Helix* provided an opportunity to investigate oxygen affinity of the bloods of tropical animals. Although emphasis was placed on water-breathing decapods, the taxonomic scope was broadened to include other kinds of crustaceans, and several air breathers were studied to ensure against differences due to technique.

MATERIALS AND METHODS

Blood was taken from various sinuses into iced syringes, and expelled into a cell homogenizer where serum was expressed from the clot, if any. It was diluted 2 : 1 with an air-equilibrated suspension of yeast cells in Tris-maleate buffered seawater (final concentration 0.05 M). Oxygen equilibrium was then determined by the cell respiration method (described in detail in Johansen et al. 1978), using a Yellow Springs Instrument Co. Model 53 O₂ monitor and a high-sensitivity (0.0005 in.) Teflon membrane. The pH was also measured at the end of the experiment, following re-equilibration of the preparation with air.

RESULTS

Oxygen Affinity and the Mode of Gas Exchange

The values for air and water breathers are compared below in a physiological pH range that is often found at high temperature in both kinds of species (Mangum 1973, Burggren and McMahon 1981); more detailed effects of pH are shown in Figures 1 and 2.

When the O₂ affinities of the hemocyanins from all the species listed in Table 1 are compared according to the mode of gas exchange, the trend noted earlier (Mangum 1980) is highly significant ($P = 0.015$): HcO₂ affinity is not lower but rather higher in air and bimodal

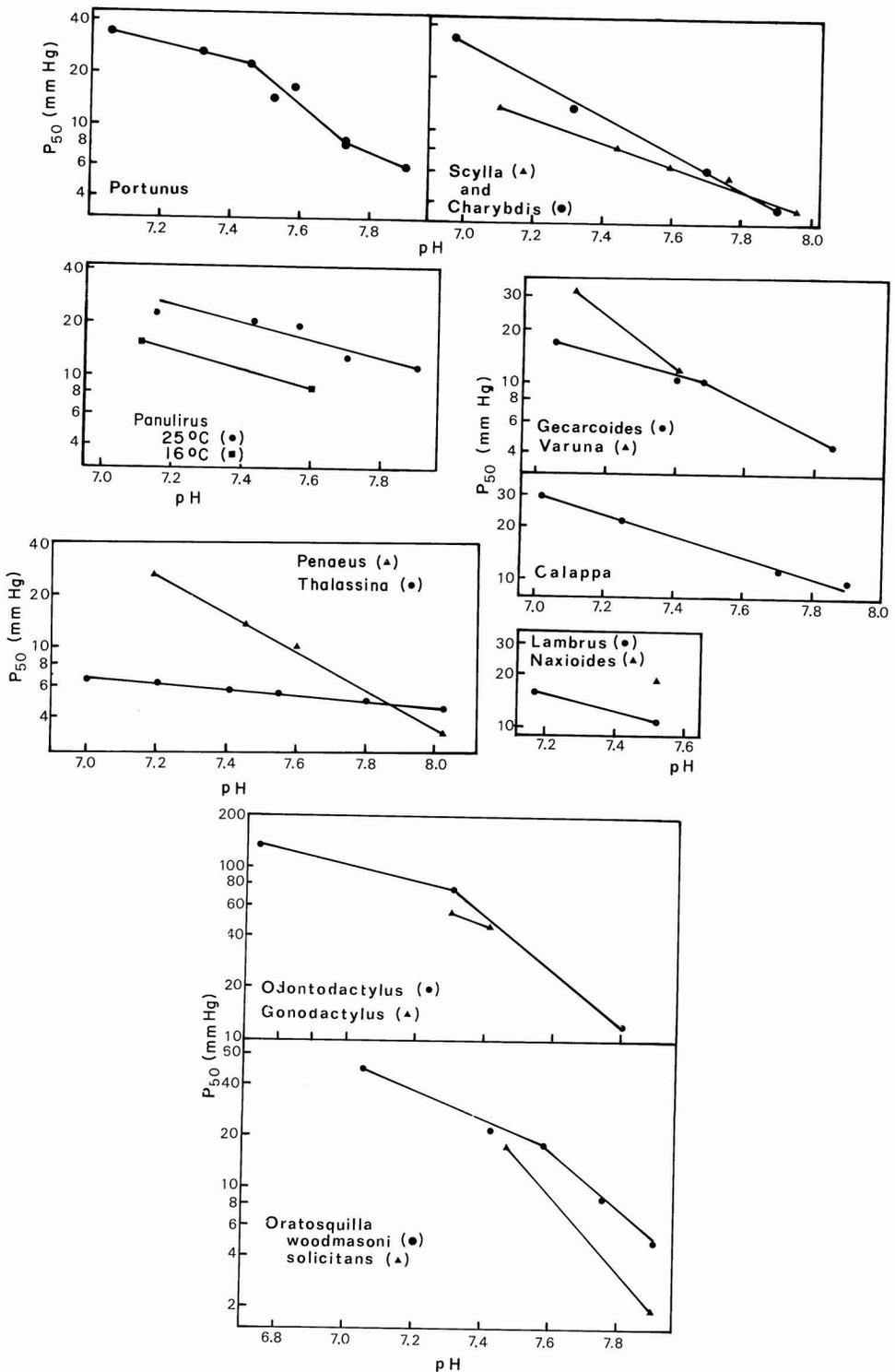


FIGURE 1. The relationship between hemocyanin-oxygen affinity (P_{50}) and pH in a variety of crustaceans from Bindoy, Philippine Islands, at 25°C; 0.05 M Tris-maleate buffered seawater (33–34‰).

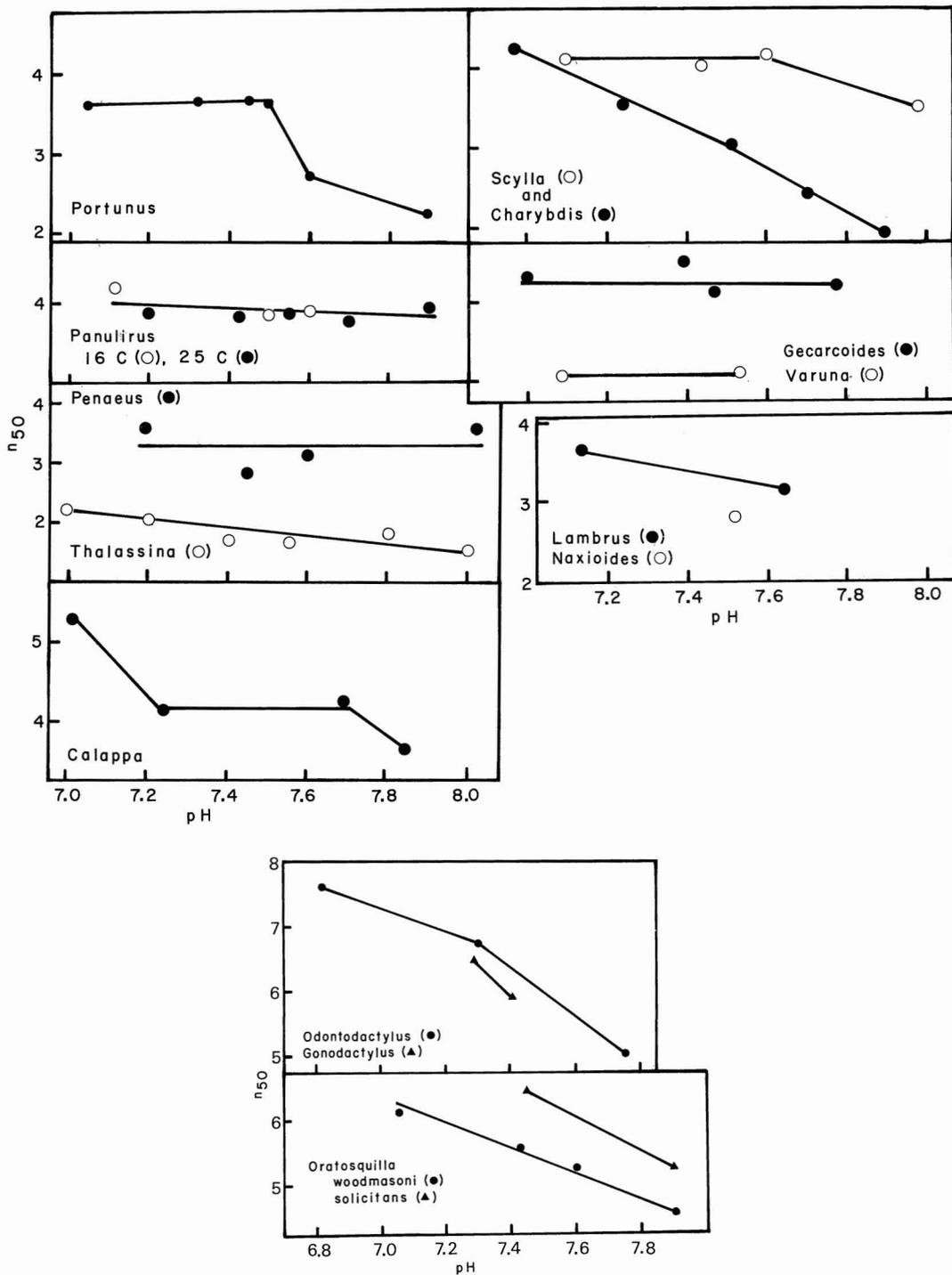


FIGURE 2. The relationship between the cooperativity of hemocyanin-oxygen binding and pH in crustaceans from Bindoy, Philippine Islands, at 25°C; 0.05 M Tris-maleate buffered seawater (33-34‰).

TABLE 1
 OXYGENATION PROPERTIES OF AIR- AND WATER-BREATHING CRUSTACEANS FOUND IN THE TROPICS

	MODE OF GAS EXCHANGE	P_{50} at pH 7.5 (mm Hg)	n_{50}	$\Delta \log P_{50}/\Delta \text{pH}$	SOURCE
Hoplocarida					
Squillidae					
<i>Oratosquilla sollicitans</i> Manning	Aquatic	17.5	6.1	- 0.5	Present data
<i>O. woodmasoni</i> (Kemp)	Aquatic	22.5	5.3	- 2.0	Present data
Gonodactylidae					
<i>Gonodactylus smithi</i> Pocock	Aquatic	~ 40	~ 5.6	- 0.5	Present data
<i>Odontodactylus cultrifer</i> (White)	Aquatic	39.5	6.1	- 1.7	Present data
Eumalacostraca					
Decapoda					
Dendrobranchiata, <i>Penaeus monodon</i> Fabricius	Aquatic	12.5	3.1	- 1.3	Present data
Pleocyemata, Austroastacidae					
<i>Panulirus argus</i>	Aquatic	5.5			Redmond (1968b)
<i>P. versicolor</i> (Latreille)	Aquatic	19.0	3.9	- 0.4	Present data
Anomura					
<i>Birgus latro</i>	Aerial	22.1	3.9	- 0.9	Burggren and McMahon (1981)
<i>Coenobita brevimanus</i>	Aerial	15.1	3.8	- 0.4	Burggren and McMahon (1981)
<i>C. clypeatus</i>	Aerial	~ 5		- 0.8	Burggren and McMahon (1979)
<i>Thalassinia anomala</i> (Herbst)	?Aerial	6.0	2.0	- 0.2	Present data
Brachyura					
<i>Aratus pisoni</i>	Aerial	7.8		- 0.5	Young (1972)
<i>Calappa philargius</i> (Linnaeus)	Aquatic	17.0	4.2	- 0.4	Present data
<i>Cardisoma carnifex</i>	Aerial	12.1	3.6	- 0.7	Burggren and McMahon (1981 and pers. comm.)
<i>C. guanhumii</i>	Aerial	3.0	2.6		Redmond (1962)
<i>Charybdis riversandersoni</i> Alcock	Aquatic	10.0	3.0	- 1.0	Present data
<i>Gecarcinus lateralis</i>	Aerial	13.3		- 0.3	Redmond (1968b)
<i>G. ruricola</i>	Aerial	8.5		- 0.3	Young (1972)
<i>Gecarcoides lalandi</i> H. Milne Edw.	Aerial	10.5	4.1	- 0.8	Present data
<i>Goniopsis cruentata</i>	Bimodal	19.7		- 0.6	Young (1972)
<i>Lambrus validus</i> de Haan	Aquatic	12.5	2.8	- 0.5	Present data
<i>Menippe mercenaria</i>	Aquatic	21.4	4.5	- 1.3	Mauro and Mangum (1982)
<i>Naxioides manillata</i> (Ortmann)	Aquatic	19.0	3.3		Present data
<i>Portunus pelagicus</i> (Linnaeus)	Aquatic	18.0	3.6	- 1.3	Present data
<i>Scylla serrata</i> (Forskål)	Aquatic	7.9	4.0	- 0.7	Present data
<i>Varuna litterata</i> (Fabricius)	Aerial	7.8	3.1	- 1.4	Present data

NOTE: Present values of n calculated around P_{50} and Bohr factor ($\Delta \log P_{50}/\Delta \text{pH}$) in pH range 7.4-7.6, 25°C.

breathers ($P_{50} = 10.7 \pm 1.6$ SE mm Hg, $N = 13$) than in water breathers (18.7 ± 2.7 SE mm Hg, $N = 14$).

It may be more meaningful to restrict the comparison taxonomically and morphologically to the pleocyemate decapods in which gas exchange is essentially unimodal. The inclusion of *Thalassina anomala* may also complicate the result for two reasons: (1) it is uncertain whether this animal carries on exclusively aerial gas exchange, or whether it can adopt aquatic gas exchange in its burrow at peak high tide; and (2) the ionic conditions used in the measurements proved to be unphysiological. The ionic composition of the blood (determined by ion-selective electrodes, as described by Mangum and Towle, this issue) taken from animals held overnight in seawater, which they did not leave, was 239 mM Na^+ (seawater, 445 mM); 9.80 mM Ca^{2+} (seawater, 8.75 mM); 9.0 mM Mg^{2+} (seawater, 45.6 mM); and 281 mM Cl^- (seawater, 503 mM). Thus, the dilution of the sample with buffered seawater considerably enriched the levels of NaCl and Mg^{2+} , which could raise O_2 affinity.

When the comparison is restricted, the trend remains the same; HcO_2 affinity is higher in air breathers ($P_{50} = 11.3 \pm 1.6$ mm Hg, $N = 11$) than in water breathers (14.5 ± 1.9 mm Hg, $N = 9$), but it diminishes and it no longer meets a rigorous criterion of significance ($P = 0.10$).

Other Oxygen-Binding Properties and the Mode of Gas Exchange

From the data in Table 1, cooperativity appears to be somewhat smaller in the air breathers ($n_{50} = 3.3 \pm 0.3$, $N = 7$) than in the water breathers (4.0 ± 0.4 , $N = 13$), but the difference is not significant ($P = 0.075$) and it is absent from the more restricted comparison ($P = 0.65$, $N = 14$). The Bohr shift appears to be smaller in air breathers ($\Delta \log P_{50}/\Delta \text{pH} = -0.6 \pm 0.1$, $N = 12$) than in water breathers (-1.0 ± 0.2 , $N = 12$). But here, too, the difference is not significant ($P = 0.075$), and it disappears from the restricted comparison ($P = 0.45$, $N = 10$).

DISCUSSION

The Relationship between HcO_2 Affinity and Latitude

Redmond (1968b) pointed out the correlation between latitude, or thermal regime, and HcO_2 affinity in two species of panulirid lobsters. At the same experimental pH and temperature, blood O_2 affinity is higher in a tropical than in a temperate zone species. The same trend is found in less closely related species of crabs belonging to different families and separated by even greater geographic distances; however, it is actually reversed at the respective levels of blood pH (Mauro and Mangum 1982). In hoplocarids, HcO_2 affinity in the temperate zone *Squilla empusa* is lower ($P_{50} = 30$ mm Hg at pH 7.5 and 22°C) than in the two tropical species of *Oratosquilla* (Table 1), which are also members of the family Squillidae. The diversity found in different families of tropical mantis shrimp is unexpected, however, and it will be interesting to learn whether it reflects comparable diversity in blood gases. Finally, it is somewhat unexpected to learn that *Palinurus versicolor* Hc differs so much from that of *P. argus* (Table 1) if the geographic relationship is common.

The Relationship between HcO_2 Binding and Mode of Gas Exchange

Earlier inferences of a decrease in hemocyanin-oxygen affinity with the emergence of species of crustaceans from water into air are based on comparisons that lack the critical control, viz. data for subtidal species from the same latitude at the same pH. The present findings indicate that HcO_2 affinity is generally higher in air-breathing decapods than in a variety of aquatic crustaceans, although the distinction between unimodal air- and water-breathing decapods is either small or nonexistent.

Branchial epithelia are highly permeable to water, a problem that is solved only in part by the reduction of gas exchange surface in terrestrial species (Bliss 1968). Although this comparison lacks the control of data for

aquatic and also tropical species, it is clear that postbranchial blood P_{O_2} in air breathers is often low (Burggren and McMahon 1981, Redmond 1968a), suggesting that the respiratory consequences of water conservation outweigh those of enhanced O_2 availability in air.

A correlation between other O_2 binding properties and the mode of gas exchange is even less obvious. The importance of these properties must be more closely related to the physiological variables that actually determine blood oxygenation and that cannot always be deduced from gross features of the environment. Perhaps more important, these variables appear to be the most adaptable components of the HcO_2 transport system.

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