J. exp. Biol. **170**, 257–264 (1992)
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SHORT COMMUNICATION

LOW ARTERIAL P_{O_2} IN RESTING CRUSTACEANS IS INDEPENDENT OF BLOOD OXYGEN-AFFINITY

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Accepted 6 May 1992

In the literature about the respiratory physiology of water-breathers, the arterial partial pressure of oxygen, Pa_{O_2}, is reported to range from about 2 to 10–13 kPa (Shelton *et al.* 1986; McMahon and Wilkens, 1983). In fish, Shelton *et al.* (1986) reported that animals with low blood O_2-affinity exhibit high Pa_{O_2} and *vice versa*. But in laboratory conditions, the excitation state of the animals may explain much of this variability: high excitation is associated with high Pa_{O_2} values and low excitation with low Pa_{O_2} values (see McMahon, 1985, for relevant discussion on crustaceans). In conditions where animal excitability was reduced to a minimum, we reported that low Pa_{O_2} values correspond to an apparent set point in crayfish *Astacus leptodactylus* (Massabuau and Burtin, 1984), fish *Silurus glanis* (Forgue *et al.* 1989) and mussel *Anodonta cygnea* (Massabuau *et al.* 1991). Indeed, when the inspired P_{O_2} (PI_{O_2}) varies from 3 to 40 kPa, Pa_{O_2} remains in the range of 1–3 kPa. In resting crabs *Carcinus maenas* – contrary to all previously reported data (see McMahon and Wilkens, 1983) – and *Eriocheir sinensis*, we also reported that Pa_{O_2} in normoxic conditions is kept in a low range around 1–3 kPa. This is independent of the blood pigment concentration (Forgue *et al.* 1992).

Except for *A. cygnea*, which has no respiratory pigment, the above species exhibit mid values of blood O_2-affinity, with P_{50} (the values of P_{O_2} at which 50 % of the respiratory pigment is oxygenated) around 0.7–1 kPa at physiological pH (7.8–8.0) and 15 °C. The present study examined the effects of variation in O_2-affinity on the setting of Pa_{O_2}. We present data obtained, at rest and during normoxia, in crustaceans chosen for their distinctly different blood O_2-affinities with P_{50} varying from 0.2 to 2.0 kPa.

Seven species of freshwater (fw) and/or seawater (sw) crustaceans were examined from January to October 1991: *Procambarus clarkii* (fw), *Astacus leptodactylus* (fw), *Eriocheir sinensis* (fw and sw; studied in sw), *Carcinus maenas*

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Key words: oxygen affinity, blood gas transport, crab, fish, *Silurus glanis*, mollusc, *Anodonta cygnea*.

(sw), *Homarus vulgaris* (sw), *Cancer pagurus* (sw), *Maia squinado* (sw) and *Necora puber* (formerly *Macropipus puber*, sw). All were intermoult animals (see Table 1 for *N* values). They were either collected locally (*C.m., E.s., N.p.*) or obtained from commercial suppliers (*P.c., A.l., H.v., C.p., M.s.*) and adapted to laboratory conditions for at least 1 week prior to experimentation. During the maintenance period they were supplied with aerated sea water or fresh water at a temperature ranging from 8 to 22°C depending on the season. Experiments on *P. clarkii* and *A. leptodactylus* were performed in Strasbourg tap water (see Massabuau *et al.* 1991, for ionic composition). All experiments in sea water were performed in Arcachon sea water (salinity 30–32 ‰). The experimental conditions were as follow: temperature 15.0±0.5°C; $P_{I_{O_2}}$ 20–21 kPa; $P_{I_{CO_2}}$ 0.1 kPa and pH 7.8–7.9 or 8.3–8.4 depending on the titration alkalinity, which was 1.8–2.0 mequiv l^{-1} in sea water and 4.4 in fresh water. At least 5 days before experiments began animals were starved, adapted to the experimental conditions, and equipped for arterial blood sampling. The latter consisted of drilling a hole in the shell above the heart. A thin layer of cuticle was left in place and a piece of rubber was glued over it (Butler *et al.* 1978). Care was taken not to disturb the animals during this 5-day period. The experiments consisted (in less than 30 s) of removing an individual from water without disturbing the other animals (i.e. without inducing escape behavior), puncturing the rubber membrane with a capillary glass tube equipped with a needle and collecting arterial blood (180 μl). This sampling technique was critically assessed in Forgue *et al.* (1992) and considered to provide true *in vivo* Pa_{O_2} values comparable to those previously obtained with the use of extracorporeal techniques (Massabuau and Burtin, 1984; Forgue *et al.* 1989). All sampling was performed between 10:00 h and 18:00 h. Each individual was sampled only once. Values of Pa_{O_2} and arterial blood pH (pHa) were determined within 3 min of sampling using a Radiometer polarographic electrode and a pH Radiometer G299A capillary electrode thermostatted at 15°C. Immediately after arterial blood sampling, 500 μl of venous blood was collected from the base of a walking leg to determine total blood copper concentration, $[Cu]_b$ (Boehringer kit no. 124834). This was used as an index of the haemocyanin content. The O_2-binding curves of whole blood in *P. clarkii* and in *E. sinensis* were determined at 15°C using the diffusion chamber technique described by Sick and Gersonde (1969) and gas-mixing Wösthoff pumps.

The frequency distribution of all measured Pa_{O_2} values is presented in Fig. 1. In the resting conditions of the experiments, most of the values are low and the data are not normally distributed. The most frequently measured Pa_{O_2} values (i.e. the modes) are in the range of 1–3 kPa. They are similar in all species examined, irrespective (i) of the fact that some are so-called 'active' animals (*N. puber*, *C. maenas*) while other are 'sluggish' (*M. squinado*, *C. pagurus*) and (ii) of variations in sampling period (summer/autumn/winter, including the corresponding temperature changes between holding and experimental tanks). Note that higher Pa_{O_2} values of up to 12 kPa were also occasionally observed. Corresponding pHa values are presented in Table 1 with mean values for Pa_{O_2} and other

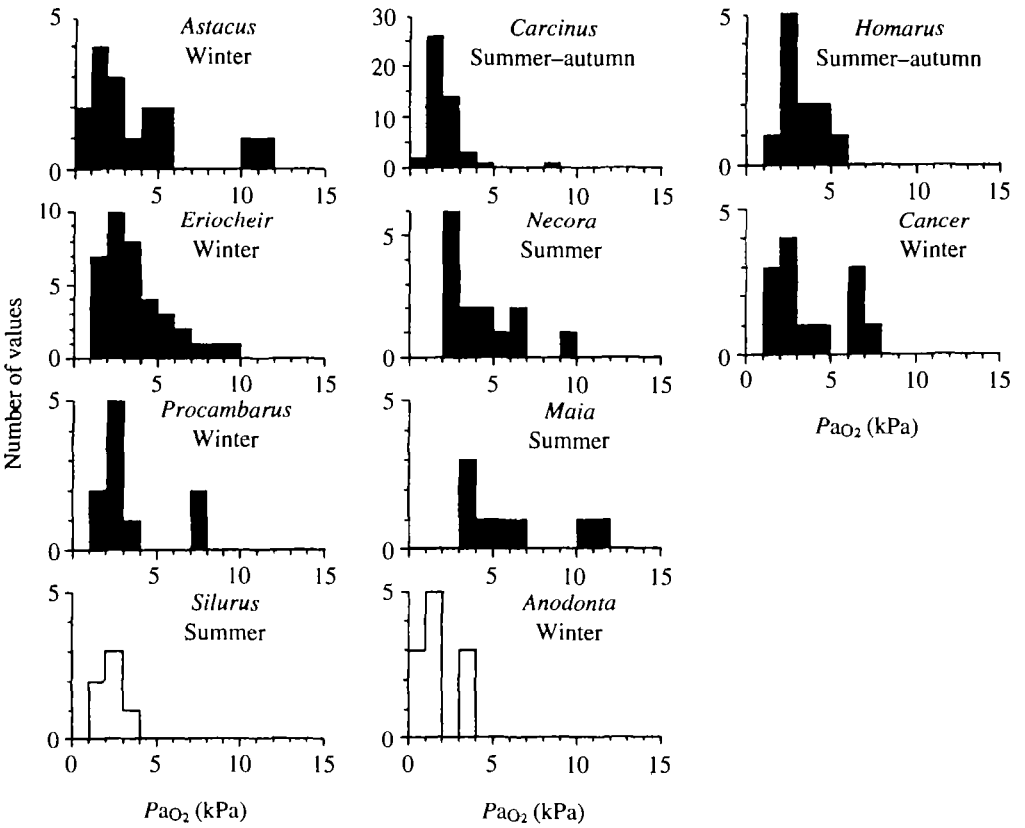

Fig. 1. Frequency distribution of arterial O_2 partial pressure (Pa_{O_2}) in various water-breathers at rest and in normoxia (P_{ICO_2} 0.1 kPa; T 13–15 °C; see Table 1 for N values). For all species, data were not normally distributed and the most frequently measured value of Pa_{O_2} was in the range 1–3 kPa. Filled histograms, crustaceans with P_{50} varying from 0.2 to 2.0 kPa; open histograms, data from the teleostean fish *Silurus glanis* (P_{50}=0.64 kPa, Forgue *et al.* 1989) and the freshwater mussel *Anodonta cygnea*, which lacks a respiratory pigment (Massabuau *et al.* 1991).

measured variables. Values of P_{50} from various authors are also reported for similar pH values. These results obtained in crustaceans are completed by data previously obtained in the mollusc *Anodonta* and the fish *Silurus* (open bars in Fig. 1; Massabuau *et al.* 1991; Forgue *et al.* 1989). Distributions and modes of Pa_{O_2} are similar in animals from the three phyla. As illustrated in Fig. 2A, the modes of Pa_{O_2} are independent of P_{50} at physiological pH. In Fig. 2B, the position of the Pa_{O_2} modes is plotted on O_2 saturation (Sa_{O_2}) or O_2 concentration (Ca_{O_2}) curves *vs* P_{O_2} for whole blood. Although all species exhibited rather low values of Pa_{O_2}, all Sa_{O_2} values are located in the upper half of the curves. The lowest saturation percentage, in the range 50–80 %, was observed in *Necora*, an animal considered to be very 'active' and 'O_2 sensitive'. The highest saturation, 98–99 %, was

Table 1. *Respiratory variables in various crustaceans, in the fish* Silurus glanis *and in the mollusc* Anodonta cygnea *kept in normoxia and with constant acid–base balance in the water* (P_{ICO_2}=*0.1 kPa,* T=*15°C)*

Species	P_{50} (kPa)	Pa_{O_2} Mode (kPa)	Pa_{O_2} Mean (kPa)	pHa	$[Cu]_b$ (μmol l^{-1})	Mass (g)	N	$\dot{M}_{O_2}$ (μmol min^{-1} kg^{-1})
Procambarus clarkii	0.26	2–3	3.4±0.7	8.06±0.02	654±78	35.2±2.6	10	14.4±0.7[1]
Astacus leptodactylus	0.83[2]	1–2	3.7±0.8	7.89±0.04	–	35.4±3.0	16	13.8±0.8[3]
Carcinus maenas	0.87[4]	1–2	2.0±0.2	7.81±0.01	972±58	65.6±2.0	47	19.3±1.1[5]
Homarus vulgaris	1.0[6]	2–3	3.0±0.5	7.88±0.01	1269±144	388±13	11	16.4±1.0[6]
Eriocheir sinensis	1.15	2–3	3.6±0.3	7.83±0.02	665±76	79.6±2.9	37	17.9±2.9[7]
Cancer pagurus	1.41[4]	2–3	3.8±0.6	7.87±0.02	779±92	336±39	13	7.5–15.0[8]
Maia squinado	1.91[4]	3–4	6.1±1.1	7.86±0.01	535±106	523±19	8	7.5–15.0[8]
Necora puber	2.05[4]	2–3	4.1±0.5	7.90±0.01	853±68	81.5±12.7	15	–
Silurus glanis	0.64[9]	2–3[9]	2.3±0.3[9]	7.96±0.02[9]	–	775±43[9]	6[9]	15.4±1.5[9]
Anodonta cygnea	No pigment	1–2[10]	1.8±0.3[10]	7.62±0.04[10]	No pigment	317±15[10]	11[10]	Approx. 2.3[10]

P_{50}, blood P_{O_2} for which 50 % of the respiratory pigment is oxygenated at normoxic pHa and about 15°C; Pa_{O_2}, O_2 partial pressure in the arterial blood expressed either as mode or mean value±S.E.; pHa, arterial pH; $[Cu]_b$, blood copper concentration, an index of haemocyanin blood concentration in crustaceans; N, number of animals in which Pa_{O_2}, pHa, $[Cu]_b$ and mass were measured; $\dot{M}_{O_2}$, resting oxygen consumption in normoxia at 13–15°C.

Data tagged refer to: 1, J. Forgue, unpublished data; 2, Angersbach and Decker (1978); 3, Massabuau and Burtin (1984); 4, Truchot (1971); 5, Taylor and Butler (1978); 6, Butler *et al.* (1978); 7, Forgue *et al.* (1992); 8, Aldrich (1975); 9, Forgue *et al.* (1989); 10, Massabuau *et al.* (1991).

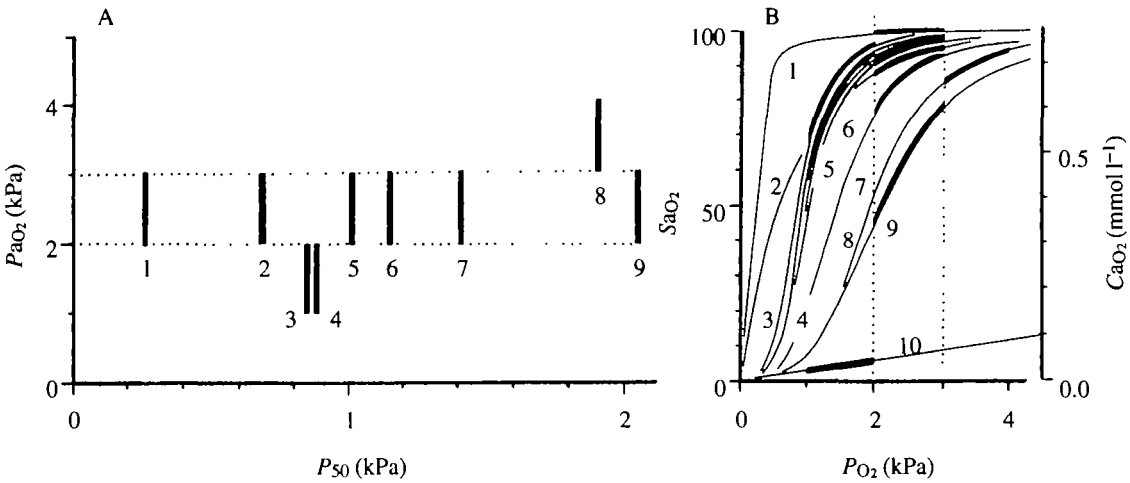

Fig. 2. (A) Arterial O_2 partial pressure (Pa_{O_2}, derived from the modes presented in Fig. 1) in resting water-breathers with different blood O_2-affinities: P_{50} ranged from 0.26 to 2.05 at pH 7.8–8.0 and 15°C. Normoxic conditions. The Pa_{O_2} value is independent of P_{50}. (B) The same Pa_{O_2} values (modes) presented on O_2 saturation Sa_{O_2} curves for species 1–9 (left abcissa) and on an O_2 concentration Ca_{O_2} curve for species 10 (right abcissa). Note that all Sa_{O_2} values are located in the upper half of the saturation curves. 1, *Procambarus clarkii*; 2, *Silurus glanis*; 3, *Astacus leptodactylus*; 4, *Carcinus maenas*; 5, *Homarus vulgaris*; 6, *Eriocheir sinensis*; 7, *Cancer pagurus*; 8, *Maia squinado*; 9, *Necora puber*; 10, *Anodonta cygnea*. Curves 1 and 6, present experiment; 2, Forgue *et al.* (1989); 3, Angersbach and Decker (1978); 5, Butler *et al.* (1978); 10, Massabuau *et al.* (1991); 4, 7, 8 and 9, Truchot (1971).

observed in *Procambarus*, an animal thought to be very O_2 resistant and known for its burrowing habits.

In the analysis of the present results two points are of particular importance. First, data were obtained in a group of crustaceans and in a teleost fish which display a homogeneous level of resting O_2 consumption (Table 1). Second, in the resting (incidentally starved) and normoxic conditions we used, the constraints on the respiratory system were minimal and Pa_{O_2} was allowed to drift spontaneously to a resting value following handling (McMahon, 1985, and personal observation). Both points allowed us specifically to study the effect of the blood O_2-affinity on the setting of Pa_{O_2} at rest in water-breathers. We show that, in these conditions, Pa_{O_2} is mainly kept in the range 1–3 kPa, regardless of the blood O_2-affinity (for P_{50} varying from 0.2 to 2.0 kPa). This apparent set point of Pa_{O_2} is similar to that already reported in the mussel *A. cygnea*, which has no respiratory pigment but a seven- to 10-fold lower O_2 consumption (Table 1). Arguing from a largely theoretical basis, Malte and Weber (1987) discussed the effect of the shape and position of the oxygen equilibrium curve on extraction and ventilatory requirement in normoxic fishes. They compared the effects of low and high P_{50} (0.5 and 4 kPa) when O_2 saturation in the arterial and venous blood (Sa_{O_2} and $S\bar{v}_{O_2}$) were

fixed at 95 and 60%, respectively. By comparing theoretical curves with experimental P_{50} from carp and trout (P_{50} of 0.6–0.9 and 2.6–2.9 kPa, respectively), they concluded that a high affinity may allow an increase in O_2 extraction from water and a reduction in ventilation. In other words, a low Pa_{O_2} is sufficient to saturate a high-affinity pigment and this can be achieved with a lower ventilatory flow rate. Starting with the hypothesis that Sa_{O_2} and $S\bar{v}_{O_2}$ are fixed, these conclusions are not in question. But the main difference from our results is that we did not find a fixed Sa_{O_2} value. On the contrary, it is Pa_{O_2} that remained in a narrow range as an apparent controlled value (Fig. 2A). Any further detailed comparison appears rather speculative because (i) the maximum theoretical P_{50} of 4 kPa used by Malte and Weber (1987) is quite high in comparison to the presently studied range and (ii) trout have a much higher metabolic level than the animals we studied. However, it is clear from our results that reaching full saturation of the respiratory pigment is not a prerequisite, at least in resting and normoxic conditions. To summarize, based on both present findings and previous results cited at the beginning of this report, all the water-breathers we studied maintained Pa_{O_2} at rest in the range 1–3 kPa (at 13–15°C) irrespective of species and phyla, marine or freshwater origin, season (taking into account the 5-day acclimation period at 15°C), organization of the respiratory system (gill type, ventilatory pump, open *versus* closed circulatory system), presence or absence of respiratory pigment and, when present, concentration of respiratory pigment and blood O_2-affinity for P_{50} in the range of 0.2–2.0 kPa. Our present experiments were performed in normoxia but this was also observed at various values of PI_{O_2} in *A. leptodactylus, S. glanis* and *A. cygnea*.

In conclusion, the frequent occurrence of low Pa_{O_2} values in our experimental conditions, only slightly above the anaerobic Pa_{O_2} threshold (0.7–1.2 kPa) determined in deep hypoxia (Forgue *et al.* 1992), appears to be a strong intrinsic characteristic of gas-exchange regulation in resting water-breathers. The infrequent occurrence of higher Pa_{O_2} values must, however, also be taken into account in the analysis of the overall gas exchange.

The animals we studied represent a wide spectrum of physiologically different water-breathers, but are not representative of all species and all physiological conditions. Comparison with similar data obtained in animals with higher metabolic rates should be very interesting.

The crew of the *Côte d'Aquitaine* (INSU-CNRS) provided the crabs *Eriocheir sinensis*. Dr John Simmers corrected the English manuscript. J.F. was supported by a grant from Ministère de la Recherche et de la Technologie.

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