

SHORT COMMUNICATION

VENTILATION FREQUENCIES AND STROKE  
VOLUMES IN ACUTE HYPOXIA IN *OCTOPUS*

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*Octopus* can regulate its oxygen uptake down to a  $P_{O_2}$  of 70 mmHg, and in some instances well beyond this (Borer & Lane, 1971; Maginnis & Wells, 1969; Wells & Wells, 1983). In part, this capacity depends upon a rise in the pH of the blood, which increases the oxygen affinity of the animal's haemocyanin as the external  $P_{O_2}$  falls (Houlihan, Innes, Wells & Wells, 1982). The fact that the blood can remain saturated down to quite low  $P_{O_2}$  values means that the animal does not need to circulate any more blood to keep the tissues supplied in an environment that is becoming depleted of oxygen. But it does have to pass more water through the gills. The percentage of the available oxygen that is extracted from the ventilatory stream sometimes increases a little as the inhaled  $P_{O_2}$  ( $P_{iO_2}$ ) falls; but the volume removed ( $\% \times \text{content}$ ) plainly does not (Wells & Wells, 1982). To maintain the same total uptake the animal must increase the ventilation frequency and/or the ventilation stroke volume. The purpose of the present work was to establish what happens.

Ten *Octopus vulgaris* (eight males and two females), from 666 to 1687 g, were used. The animals were kept at Banyuls during September 1984, at temperatures between 21 and 24°C. Temperature change during any one experiment was not greater than 1°C. The animals were undamaged and fed regularly on crabs. Each octopus was anaesthetized in 2.5% ethanol and had a 3-mm o.d. 'Portex' cannula sewn to the mantle adductor, just below the funnel in the midline of the animal. The cannula was led out through the muscle at the back of the mantle, as described in Wells & Wells (1982). The operation was very quick (<5 min) and could be carried out as soon as ventilatory movements ceased. Recovery, aided by passing a stream of sea water over the gills, was rapid. Tests were carried out not less than 1 h after breathing had begun again. Previous studies (Wells, O'Dor, Mangold & Wells, 1983; Wells & Wells, 1984) and work to be reported (D. F. Houlihan, G. Duthie, P. J. S. Smith, M. J. Wells & J. Wells, in preparation) have indicated that the maximum  $O_2$  debt that an octopus can sustain (about 20 ml kg<sup>-1</sup>, following exhausting exercise or prolonged anaesthesia) is paid off

within 2 h. The cannula sampling exhalant  $P_{O_2}$  ( $P_{eO_2}$ ) led to a Perspex chamber surrounding the electrode of an EIL 7130 dissolved oxygen meter. The water in the chamber was kept stirred by a paddle activated by a magnetic stirrer. The outflow was led to a beaker with a floating lid to minimize the free surface, and this water, collected at a rate of about  $30 \text{ ml min}^{-1}$ , was periodically returned to the respirometer. The respirometer itself has been described in Wells, O'Dor, Mangold & Wells (1983). The oxygen tension in the respirometer ( $P_{iO_2}$ ) was measured by circulating the water past an EIL 15A oxygen probe. The circulation pump, with an output of several  $l \text{ min}^{-1}$  also served to keep the tank water stirred.

The two oxygen electrodes were checked and matched to give the same output in saturated sea water before each experiment. Experimental runs normally lasted about 75 min, during which the animal would reduce the  $P_{iO_2}$  from about 140 to about 40 mmHg.

Ventilation frequency was observed directly, mantle movements being counted through the transparent ceiling of the respirometer while remaining, as far as possible, out of sight of the animal.

It was thus possible to record oxygen uptake, oxygen extraction from the ventilatory system, and ventilation frequency at the same time. Ventilation stroke volume ( $V_{s,R}$  in ml) was calculated as:

$$V_{s,R} = \frac{I_{O_2} - E_{O_2}}{\dot{V}_{O_2} \times f_R} \times 1000,$$

where  $I_{O_2}$  and  $E_{O_2}$  are the  $O_2$  contents ( $\text{ml l}^{-1}$ ) of the tank and exhalant streams respectively,  $\dot{V}_{O_2}$  is the animal's oxygen uptake in ml and  $f_R$  is the number of ventilation cycles.  $\dot{V}_{O_2}$  was measured over 10-min periods, with values for  $I_{O_2}$ ,  $E_{O_2}$  and  $f_R$  taken in the middle of each 10-min period.

The results obtained are summarized in Table 1. Oxygen uptake and percent extraction from the ventilatory stream both declined a little as the  $P_{iO_2}$  fell (the latter by about 20 % for a three-fold decline in  $P_{iO_2}$ ). Ventilation frequency and ventilation minute volume both increased. Ventilation stroke volume changed very greatly compared with ventilation frequency. The former increased by up to 400 %, the latter by a maximum of 50 % (average figures were 161 % and 32 %).

If the animals are ranked according to their capacity to maintain their  $O_2$  uptake as the  $P_{iO_2}$  fell (columns 3–5 in Table 1) and these rankings are compared with their capacity to increase ventilation frequency (columns 9, 10) and stroke volume (13, 14), it is immediately obvious that it is the stroke volume, and not the frequency, that matters so far as  $O_2$  uptake is concerned (Spearman Rank Correlation,  $r_s = 0.042$  for frequency, not significant and  $0.0756$ ,  $P = < 0.01$ , for stroke volume).

The lack of correlation between  $O_2$  uptake and ventilation frequency is in keeping with earlier results (Boyle, 1983; Wells & Wells, 1982) which have shown that frequency tends to increase with behaviour such as locomotion, defaecation, defence and arousal, activities that are not always correlated with immediate changes in  $O_2$  uptake; percent extraction normally falls on these occasions (Wells

Table 1. *Oxygen uptake, extraction, and ventilatory changes in acute hypoxia*

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Weight (g) and sex	Oxygen uptake (ml kg <sup>-1</sup> min <sup>-1</sup> ) at P <sub>102</sub> (mmHg)	140	95	45	140	95	45	min.	max.	min.	max.	min.	max.	Frequency	Stroke volume
					Percentage of available oxygen P <sub>102</sub> (mmHg)			ventilation frequency (min <sup>-1</sup> )		ventilation flow rate (ml min <sup>-1</sup> )		ventilation stroke volume ÷ body weight (×100)		Maximum increases in ventilation (%)	
Y4	826♂	1.2	1.3	1.0	46	45	47	24	34	420	1664	2.1	6.2	21	297
Y5	666♀	2.1	1.6	0.8	42	40	43	30	38	689	1050	2.9	4.9	27	71
Y12	1380♂	1.7	1.8	1.0	28	25	23	26	34	2342	4011	5.3	9.1	23	71
Y14	1687♂	1.4	1.4	0.9	36	33	37	32	34	1594	2961	2.7	5.2	-13*	79
Y16	1110♂	1.6	1.0	0.9	44	53	63	22	30	718	1914	2.7	6.6	36	143
Y17	1040♂	1.8	1.2	1.0	51	33	33	24	34	1043	1919	4.1	6.6	41	60
Y18	947♂	0.8	1.3	1.0	64	60	40	18	26	260	1717	1.5	7.5	44†	407†
Y19	628♀	1.4	1.4	1.0	62	50	40	20	30	272	1325	2.1	8.0	50	285
Y20	840♂	1.5	1.0	0.6	32	30	23	20	28	739	1409	3.6	6.4	40†	79†
Y29	1210♂	1.6	1.2	0.8	53	30	27	24	30	1003	2410	3.4	7.6	25	121
Means ± s.e.					46 ± 4	40 ± 4	38 ± 4					3.0 ± 3	6.8 ± 0.4	32 ± 4	161 ± 39

\*Decreased.  
†Ignoring periods of activity.

& Wells, 1982). Frequency generally increases as  $PiO_2$  falls. It rose by an average of 32% in the present series. Borer & Lane (1971), working with *O. briareus*, found that frequency rose by 4 cycles  $\text{min}^{-1}$  for each 12-mmHg drop in  $PiO_2$  below 85 mmHg. But the variability introduced by other factors swamps the relationship between frequency and the capacity to regulate.

There has been one previous attempt to measure ventilation volumes in *O. vulgaris*. Winterstein (1925) attached the exhalant funnel to the finger of a rubber glove and thence, *via* a condom, to a collecting vessel outside the respirometer. He found ventilation volumes ranging from 100 to 1500  $\text{ml min}^{-1}$  as the  $PiO_2$  fell in animals from the same size range as those considered in Table 1. Johansen (1965) used a similar technique to examine ventilation in the much larger *O. dofleini*. The difficulty about direct collection is that it is liable to impede flow, now known to be driven by very small pressure gradients, in the region of 0.5  $\text{cmH}_2\text{O}$  ( $\sim 0.05$  kPa) (Wells & Smith, 1985). The lack of obstruction achieved by using an indirect method presumably accounts for the considerably larger volumes (260 to 4000  $\text{ml min}^{-1}$ ) that we have now recorded.

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