

THE EFFECTS OF REDUCING GILL AREA ON THE CAPACITY TO REGULATE OXYGEN UPTAKE AND ON METABOLIC SCOPE IN A CEPHALOPOD

BY M. J. WELLS AND J. WELLS

Department of Zoology, Cambridge and the Laboratoire Arago, Banyuls

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SUMMARY

Octopus vulgaris is able to maintain its oxygen uptake at a steady level down to a P_{O_2} of below 80 mmHg. This capacity is scarcely affected by surgical reduction of the gill area to between 30 and 50 %, a finding which confirms previous work suggesting that regulation is achieved by increasing the affinity of the haemocyanin rather than by circulatory adjustments. The same reductions in gill area seriously reduce the already very limited metabolic scope of *Octopus*. Experiments in an exercise wheel show that this is in the region of $115 \text{ ml kg}^{-1} \text{ h}^{-1}$ (against an SMR of 42 for a 1-kg animal at 21 C) in the intact *Octopus*; reduction of the gill area to 30–50 % approximately halves this figure. Animals with their gills reduced will run in the wheel until they have accumulated an oxygen debt in the region of 22 ml kg^{-1} , and then stop; recovery is slower than in intact animals.

INTRODUCTION

Cephalopods can regulate their oxygen uptake over a wide range of ambient oxygen tensions (Johansen, Brix & Lykkeboe, 1982; Maginniss & Wells, 1969; Wells & Wells, 1982). In *Octopus*, cardiac output does not increase as the inspired P_{O_2} falls (Wells & Wells, 1983). Regulation seems instead to be achieved by changing the pH of the blood, so that haemocyanin affinity is increased as the external oxygen tension declines (Houlihan, Innes, Wells & Wells, 1982).

Oxygen uptake in hypoxia might be enhanced by engorging the capillaries in the gills, so as to increase the blood residence time. If this were a contributory factor one would expect any reduction in the total gill area to reduce the capacity to regulate.

The scope for activity (Fry, 1957) of cephalopods appears to be very limited. *Loligo* and *Octopus*, in forced exercise, can increase their oxygen uptakes to only two or three times those found at rest (O'Dor, 1982; Wells, O'Dor, Mangold & Wells, 1983b). In *Octopus*, where the blood oxygen content has been measured directly *in vivo*, the venous blood is less than 15 % saturated even at rest (Houlihan *et al.* 1982); similar figures can be deduced from the venous P_{O_2} values found in *Loligo* and *Sepia* (Redfield & Goodkind, 1929; Johansen *et al.* 1982). There is thus little or no capacity for increasing oxygen delivery by expanding the difference in arterial and venous oxygen content (*Nautilus*, alone, could be an exception, with the venous blood 65 %

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saturated at rest; Johansen, Redmond & Bourne, 1978). Any increase in oxygen uptake must be met by an increase in cardiac output.

One might reasonably expect gill area to be matched to the maximum likely cardiac output, so as to preserve a minimal blood residence time in the gills. At rest there would then be considerable reserve capacity. In this case one would expect any reduction in gill area to reduce the scope for activity while not, necessarily, affecting the resting uptake. Alternatively, the exercising cephalopod might, as in hypoxia, increase the affinity of its blood pigment, so as to reduce the minimal residence time. In this case, any reduction in gill area should have similar effects upon the scope for activity in normoxia and the capacity to regulate in hypoxia. These matters are investigated in the experiments that follow.

METHODS

Five *Octopus vulgaris*, ranging from 792 to 1044 g, were used in these experiments. The animals were kept at the Laboratoire Arago, Banyuls, and fed daily on crabs. No animal was fed less than 12 h before a test. Temperatures ranged from 20 to 21 °C.

Each animal was subjected to a series of experiments over a period of about 1 week. It was first tested in an annular respirometer, an 'exercise wheel' (Wells *et al.* 1983b). The octopus was placed in the wheel and allowed to settle down. Oxygen uptake was monitored using an EIL 15A meter to compare the tensions at the inlet and outlet of the wheel; flow rates in the region of 1 l min^{-1} were used. When the animal had remained inactive for an hour or more, and the oxygen uptake appeared to be steady, an exercise run was begun by turning the wheel. *Octopus* has a well-developed optomotor response and normally paced to maintain its position relative to objects outside the transparent wheel. If it failed to do so and remained attached to the inside of the wheel, it was carried upwards to the top of the wheel where it was jostled by an air-filled ball. Normally the sight of the approaching ball was enough to induce the octopus to continue running. If the ball failed, it was possible to give small electric shocks through electrodes set into the outer rim of the wheel. Animals were exercised at 4 rev. min^{-1} (equivalent to 0.34 km h^{-1}) until reluctant to run despite occasional shocks and frequent contacts with the ball. By this time they were generally noticeably flaccid, stumbling and beginning to lose the dark brown colour typical of alert and active *Octopus vulgaris*.

After a recovery period of an hour or more, during which oxygen consumption fell to the pre-exercise value, the animal was removed from the wheel, and anaesthetized in 2.5% ethanol. Under anaesthetic the vertical muscular septum joining the mantle wall and the viscera was cut, and the mantle cavity folded inside out to reveal the gills. Each gill consists of 18 primary lamellae in two alternating rows linking the afferent and efferent branchial vessels. In octopods there is an accessory afferent vessel running inside the gill, and a minor lamella joining this and the efferent between each primary lamella (for a more complete description of the anatomy of the gills see Isgrove, 1909; Wells & Wells, 1982). Several of the primary lamellae (and their associated minor lamellae) were cut off from the circulation in each gill by ligating the afferent and efferent (and accessory afferent) vessels. The animal was revived by passing a stream of water over the gills, and returned to the exercise wheel. Next day

(or in one instance, later on the same day) the animal was run again in the wheel, again after measurement of its resting oxygen consumption. A further reduction in gill area was then carried out, and the experiment repeated as before.

After an overnight period of recovery, three of the octopuses were subjected to a third operation, in which the afferent branchial vessel was cannulated in one gill. They were then placed in a 20-l respirometer. Branchial heartbeat and the animal's oxygen consumption were monitored. When both had settled to steady values, the circulation was closed and the branchial pulse recorded while the octopus reduced the oxygen content of the respirometer (see Wells & Wells, 1983).

To date there has been no reported attempt to measure the gill area in any cephalopod. *Octopus vulgaris* has 18 primary lamellae, in two parallel rows. The first pair, next to the branchial heart, are a little smaller than the second, with the third pair the largest, after which the size tapers towards the tip of each ctenidium. For present purposes we have estimated the gill area remaining after each operation very crudely, treating each lamella as having the same area, 5.6% of the total. Operations were checked by examining the gills after the end of the experiments. In all instances the ligatures were intact, or had severed the blood vessels (which were well healed) and disappeared.

RESULTS

The capacity to maintain oxygen uptake after reductions in gill area

Fig. 1 shows how each of the three animals tested reduced the oxygen in a closed system, after reduction of their gill areas to 31–42% of the total available to an intact octopus. In two instances the animals were allowed to reduce the P_{O_2} to below 60 mmHg. The third experiment (animal 10B, which had the most extreme gill reduction, to 31%) was terminated at 80 mmHg, because the heartbeat was becoming erratic. It is clear that none of the three octopuses had difficulty in maintaining their uptake over the greater part of the range tested, despite a two-thirds reduction in gill area. Down to a P_{O_2} of 80 mmHg, oxygen uptakes ranged from 46.4 to 57.4 ml kg⁻¹ h⁻¹. This compares with the 51.6 to 55.4 ml kg⁻¹ h⁻¹ that would be predicted for intact animals of this size under normoxic conditions (Wells *et al.* 1983a; 341 records of routine oxygen consumption yielded the formula O_2 consumption = $0.501 \times \text{weight}^{0.67}$ at 21 °C).

Branchial heartbeats in gill-reduced animals

Fig. 2 summarizes the records made during the experiments in a closed circulation, and subsequently after restoration of an open circulation. They may be compared with similar records made with animals having intact gills (Wells & Wells, 1983). The new measurements show that pulse pressures and frequencies in the afferent branchials are normal after gill area reduction, decline in a normal manner as the inspired P_{O_2} falls, and recover in a normal manner when the oxygen tension rises again.

These results, taken together with the records of oxygen consumption during acute hypoxia are important on two counts. (1) They show that successful regulation is not dependent upon gill area, or upon adaptive circulatory changes (thus confirming the

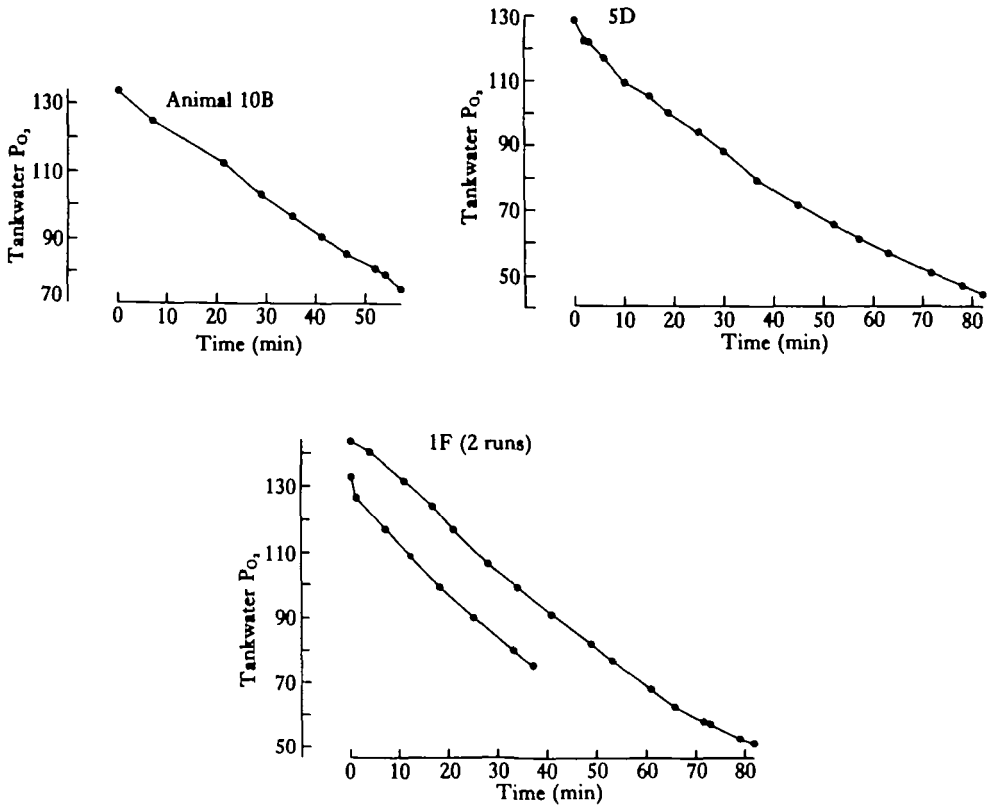


Fig. 1. Regulation after reduction in gill area. Oxygen uptake was followed in a closed respirometer of 20 l capacity. Blood pressures were measured during these experiments and are shown in Fig. 2.

finding with intact animals). (2) They show that the animals were still in good physiological condition at the end of the experiments. It should be remembered that the cannulation/regulation experiments were made at the end of the series, after the exercise experiments, following the third surgical operation carried out on each octopus. This is important because it will be shown below that gill reduction considerably reduces the scope for activity and the animals' willingness to run in the wheel, changes that might otherwise be attributed to operational trauma.

Scope for activity and gill reduction

Fig. 3 shows the performance of the five octopuses in the exercise wheel. All were run at a nominal 4 rev. min^{-1} , or 0.34 km h^{-1} , the speed being reduced a little towards the end of most runs, as the animals became reluctant to continue and had to be jostled with the ball to keep them moving.

Two points stand out: gill reduction reduces maximum oxygen uptake, and gill reduction reduces the length of runs made. Both effects are progressive; octopuses with only a small area of gill left soon become reluctant to run and can take up only a fraction of the normal amount of oxygen while doing so. The scope for activity is approximately halved by a reduction in gill area to 50% (Table 1).

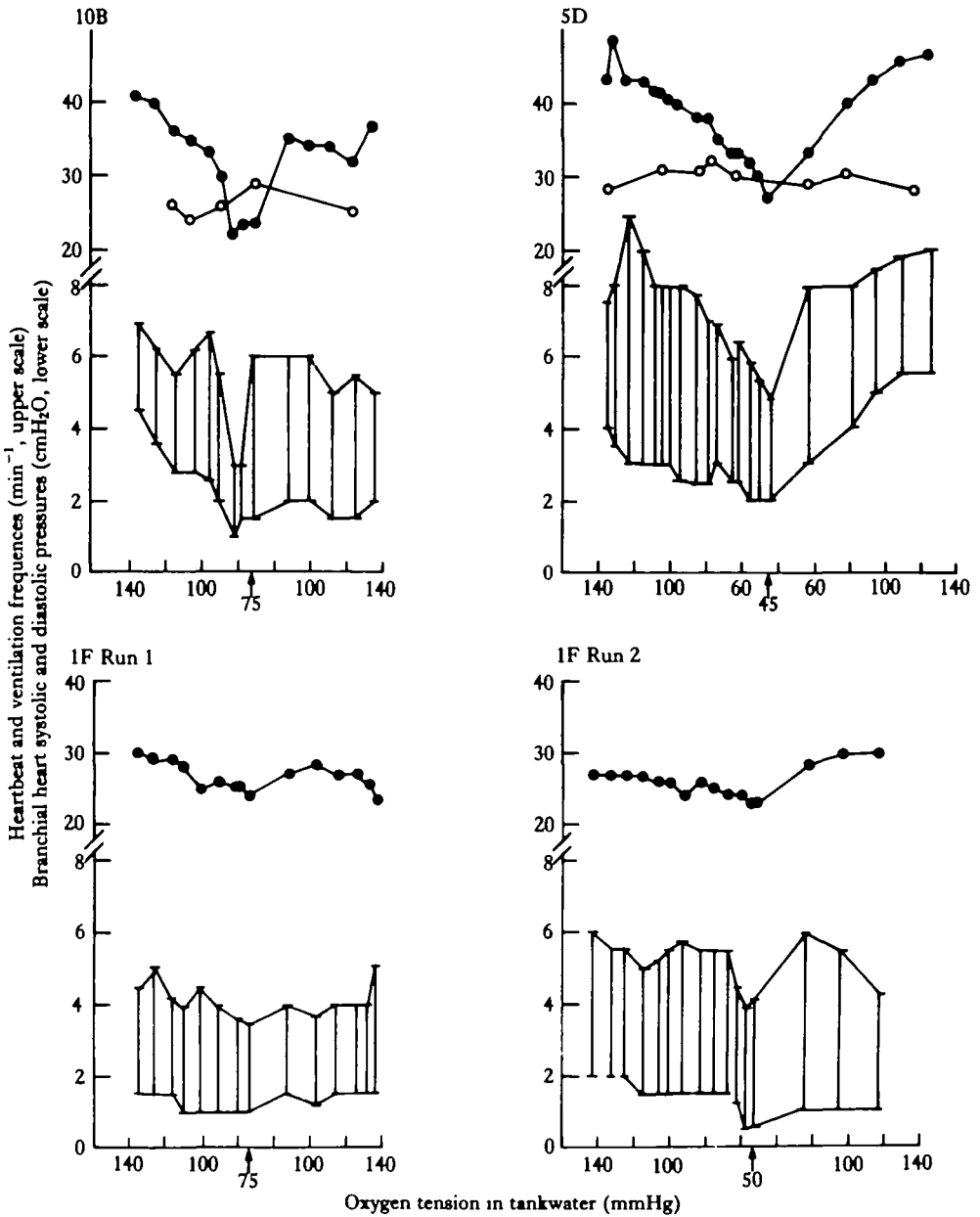


Fig. 2. Branchial heart pulse changes recorded from an afferent gill vessel during acute hypoxia in a closed respirometer; four experiments, with three individuals. At the point marked by an arrow in each experiment an open circulation was restored; heartbeat records continued during recovery. ●, Heartbeat frequency; vertical bars the systolic and diastolic pressures. ○, Shows the rate of mantle contractions.

Oxygen debt and the cost of transport

Since the animals were all run at the same speed, and the octopuses with their gills reduced were taking up less oxygen than the intact animals, they must have been

running partly anaerobically. The only available estimate of the oxygen debt that an octopus can sustain is that of Wells *et al.* (1983*b*). They examined oxygen uptake in octopuses recovering after exercise in the wheel, and found an average extra uptake of $17.6 \pm 2.3 \text{ ml kg}^{-1}$ from 11 runs with nine different animals, which included the

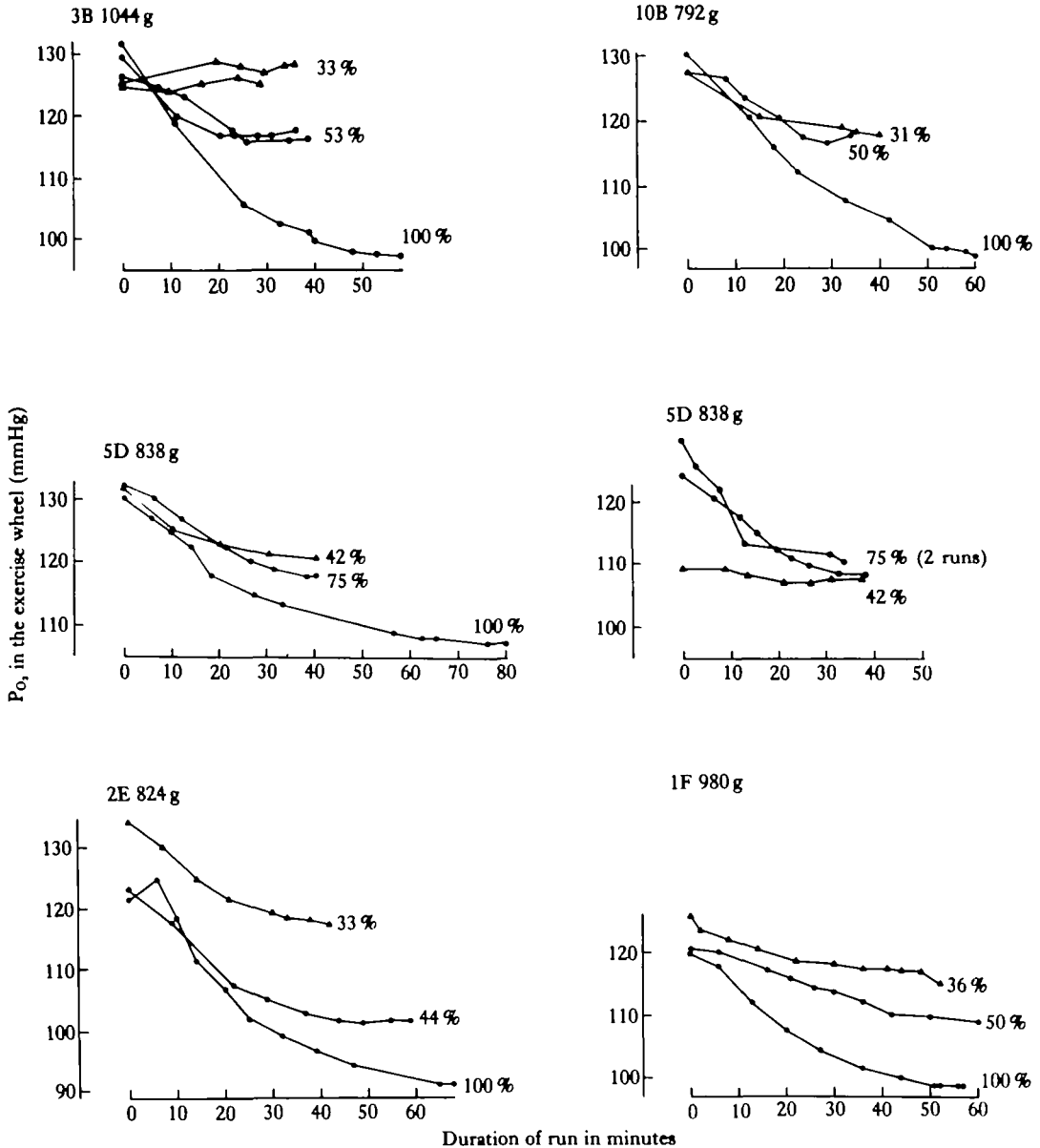


Fig. 3. Oxygen tensions in the outflow from an exercise wheel during runs at 0.34 km h^{-1} ; sea water through-flow rates varied slightly from one experiment to the next and averaged 1.31 min^{-1} . Each animal was run, first with its gills intact and then after each of two operations in which the gill area was progressively reduced; percentage figures show the proportion of the original gill area remaining. In some cases repeat runs were made after the first and/or second operation. The plots show how the capacity to remove oxygen and the length of runs made before exhaustion were reduced by progressive reductions in gill area. Actual oxygen consumptions are summarized in Tables 1 and 2.

Table 1. Oxygen uptake in the first 30 min of exercise runs ($\text{ml kg}^{-1} \text{h}^{-1}$), standard metabolic rate*, and scope for activity after successive reductions in gill area; percentage figures show the proportion of the total gill area remaining

Animal	Weight (g)	SMR† ($\text{ml kg}^{-1} \text{h}^{-1}$)	Intact	Metabolic scope ($\text{ml}^{-1} \text{kg}^{-1} \text{h}^{-1}$)	
				First reduction	Second reduction
3B	1044	40 (54.6)	117	45	32
				45	31
				53 %	33 %
10B	792	43 (68.5)	140	81	77
				50 %	31 %
5D	838	43 (70.6)	87	57	78
				64	
				75 %	42 %
2E	824	43 (75.8)	126	71	74
				44 %	33 %
1F	980	41 (79.5)	105	67	61
				50 %	36 %
Means	897	42 (69.8)	115	63	60
				51 %	36 %

SMR, standard metabolic rate.

† Figures in brackets show the resting metabolic rates for these animals, measured with their gills intact. These were all well above the expected SMRs because the animals had been fed before the start of the experiments; regular feeding can double the SMR (Wells, O'Dor, Mangold & Wells, 1983c).

* Calculated as $0.214 \times \text{weight}^{0.76}$; (Wells, O'Dor, Mangold & Wells, 1983a).

five used in the present series of experiments, run with their gills intact.

Debts of the same order were found after gill reduction (Table 2). There is, inevitably, considerable scatter in the values found. Oxygen debt was assessed from the extra oxygen consumed during a recovery phase, which was judged to end when an individual's oxygen uptake had returned to the level observed in that animal at rest before the run. Since this condition is approached exponentially, the end point is difficult to judge, particularly in the case of the gill-damaged animals which increased their oxygen consumption only a little and took a long time to return to normal.

The cost of transport for *Octopus* can be measured from the extra oxygen uptake in locomotion. Wells *et al.* (1983b) derived a figure of $252 \pm 8 \text{ ml kg}^{-1} \text{ km}^{-1}$ (mean and s.e.m.) from 22 animals in the size range 420 to 1044 g running at 0.34 km h^{-1} . That figure did *not* include oxygen debt, which could not be measured in 11 instances, when the animals became spontaneously active during the recovery period. Including the oxygen debt adds about 20 % to the cost of transport in animals with fully intact gills, bringing it up to about $300 \text{ ml kg}^{-1} \text{ km}^{-1}$. The proportion of the total oxygen consumed *after* the end of the run is much greater in gill-damaged animals; 47 % for the 10 instances listed in Table 2. The total oxygen cost of transport nevertheless works out about the same as in intact octopuses, $241 \text{ ml kg}^{-1} \text{ km}^{-1}$ as against $295 \text{ ml kg}^{-1} \text{ km}^{-1}$ for the five animals concerned in the present set of experiments. One must conclude that gill-damaged animals will run until they have accumulated the maximum sustainable oxygen debt, and are then obliged to stop. The result, which

Table 2. *Oxygen uptake, oxygen debt and cost of transport*

Animal	Weight (g)	Run (min)	Gill area remaining (%)	A Extra oxygen uptake in run (ml)	Recovery period (min)	B Extra oxygen uptake in recovery (ml)	A + B Total oxygen cost of run (ml)	Cost of transport ($\text{ml kg}^{-1} \text{km}^{-1}$)
3B	1044	58	100	81.5	90	20.8	102.3	298.1
		29	53	20.2	165	51.4*	71.6	417.3†
10B	792	60	100	83.2	80	14.1	97.3	361.3
		34	50	17.4	67	11.7	29.1	190.7
		41	31	20.4	110	9.1	29.5	160.0
5D	838	80	100	84.4	115	22.6	107.0	281.7
		36†	75	31.9	47	12.3	44.2	264.2
		40	42	25.0	60	6.1	31.1	163.7
2E	824	68	100	96.4	90	17.2	113.6	357.7
		59	44	52.4	140	39.9	92.3	335.1
		42	33	34.2	120	28.5	62.7	319.7
1F	980	57	100	47.4	62	8.3	55.7	176.0
		60	50	25.3	100	13.7	39.0	117.0
		52	36	24.1	75	27.2	51.3	177.6

† Average of two runs with the gills reduced to 75%.

* Record shows almost no decline in oxygen uptake for a long period after the run; the animal sat quietly throughout, so this is not attributable to activity.

100% figures from Wells, O'Dor, Mangold & Wells (1983b). The number of runs considered here is four less than in Table 1; if the additional uptake during the run is very small, or the animals are spontaneously active during the recovery period meaningful figures cannot be obtained.

indicates an oxygen debt averaging 27 ml kg^{-1} (22 ml kg^{-1} if one excludes the very high and possibly unreliable figure for 3B), confirms existing results which indicate that the sustainable oxygen debt in cephalopods is very small by vertebrate standards (see O'Dor, 1982; Wells *et al.* 1983b).

DISCUSSION

The results summarized above confirm the finding of Wells & Wells (1983) that regulation is achieved by changes in the properties of the blood rather than by alterations in cardiac output. They extend the previous work by showing that reduction in the available gill area is without effect upon the capacity to regulate. This indicates that the change in blood pigment affinity associated with regulation under hypoxic conditions is by itself sufficient to ensure a constant oxygen uptake without any increase in residence time. There is no need to postulate changes to the capillary circulation in the gills, an interesting contrast to the situation in fish (see, for examples, Booth, 1979; Hughes, Peyraud, Peyraud-Waitzenegger & Soulier, 1982).

We do not yet know how a cephalopod produces adaptive changes to the pH of its blood during acute hypoxia. So we do not at present know whether such changes are incompatible with the rise in metabolic rate associated with exercise. If the two were not incompatible, a cephalopod might be able to pump more blood through the same

capillaries in less time and compensate for this by increasing the affinity of its blood pigment. Evidence presented above, however, suggests that the animal does *not* behave in this manner. Cutting down the gill area reduces the maximum oxygen uptake in exercise (as in fish, see Duthie & Hughes, 1982) without altering the oxygen uptake at rest. This implies *either* that in the resting animal the residence time is well in excess of the minimum necessary *or* that the resting animal has a high proportion of its gill capillaries shut down. It should be noted that the osmotic pressure of cephalopod blood is only very slightly less than that of sea water (Schipf & Hevert, 1981), so that cephalopods are scarcely affected by the need to balance the desirability of having a large gill area against the metabolic cost of salt transport (Muir & Hughes, 1969).

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