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RESPIRATORY AND CARDIAC PERFORMANCE IN *LOLLIGUNCULA BREVIS* (CEPHALOPODA, MYOPSIDA): THE EFFECTS OF ACTIVITY, TEMPERATURE AND HYPOXIA

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Summary

Lolliguncula brevis Blainville is a small euryhaline squid found at temperatures between 11 and 31 °C. Changes in $\dot{V}_{O_2}$, heartbeat and ventilation frequencies were observed throughout this temperature range and under a variety of conditions, including acute hypoxia and swimming by jet propulsion in a tunnel respirometer. Resting $\dot{V}_{O_2}$ showed a Q_{10} of 1·47, and heart rate and ventilation rate Q_{10} values of 1·92 and 1·73, respectively; oxygen uptake could exceed 1·01 $kg^{-1} h^{-1}$ at 30 °C even at rest. The squids regulated their oxygen uptake at all temperatures. Oxygen extraction rates were in the region of 5–10 % in saturated water, increasing to 15–20 % in hypoxic water or after exercise. One effect of this variability is that ventilation stroke volume can remain constant throughout the range of temperatures and oxygen concentrations that the animal is likely to encounter, a necessary condition since the ventilation stream is also the principal mode of locomotion by jet propulsion. Blood oxygen-carrying capacity (from the copper concentration) was 4·6 ± 1·8 vols%. Cardiac output and stroke volume were estimated from the observed $\dot{V}_{O_2}$ values and heartbeat frequencies. Resting at 25 °C, the output was close to 11·5 $l\,kg^{-1}$ body mass h^{-1}. The systemic heart of *Lolliguncula* weighed only 2·06 ± 0·62 $g\,kg^{-1}$. In exercise the cardiac output must exceed 14×10^3 $l\,kg^{-1}$ heart mass h^{-1}, pumping more than the heart's own mass of blood at each stroke.

Introduction

Ventilation and blood flow have been studied in some detail in *Octopus* (for reviews see Wells & Wells, 1983; Wells *et al.* 1987) but equivalent data for squids are scarce owing mainly to the difficulties of keeping these free-swimming predators in good health in small tanks and to the delicacy of their skin compared with that of other cephalopods (Hulet *et al.* 1979). Cardiac function and oxygen

Key words: respiration, cephalopods, cardiac performance, hypoxia.

transport in squids are interesting because their muscular activity appears to be fuelled by an obligatory carbohydrate metabolism (Hochachka *et al.* 1975) and their cost of transport at similar swimming speeds is estimated to be five times that of a similar-sized salmon (O'Dor, 1982). O'Dor (1982) was the first to provide data on adult squids (*Loligo opalescens*), although Hurley (1976) had provided preliminary data on hatchling *L. opalescens*. Substantial baseline data are now available on the oceanic squid *Illex illecebrosus* (DeMont & O'Dor, 1984; Webber & O'Dor, 1985, 1986; O'Dor *et al.* 1986). Both are cold-water species living at 8–18°C.

We report here data on the loliginid squid *Lolliguncula brevis*. This species is proving to be a suitable laboratory animal because of its relative hardiness (Hulet *et al.* 1980; Dubas *et al.* 1986). It is an estuarine species living along the south-eastern coasts of the United States which withstands a wide range of temperatures (11–31°C) and salinities (17–38‰). Furthermore it is not easily traumatized by trawl capture, is available year-round and can be maintained for months in aquaria (Hixon, 1980; Hendrix *et al.* 1981; Hanlon *et al.* 1983). In contrast to most other squids, the adult is small and translucent and thus heart and ventilation rates can be studied non-invasively with video recording equipment.

The objectives of this research were to determine (1) the resting oxygen uptake, (2) the efficiency of oxygen extraction, (3) the ventilation frequency and (4) the heartbeat frequency of *L. brevis* at different temperatures and oxygen concentrations and to make a preliminary study of what happens in exercise. The information was used to estimate the ventilation volume, cardiac output, and Q_{10} values for O_2 uptake, ventilation and heartbeat frequencies.

Materials and methods

Animals

Lolliguncula brevis Blainville were captured in March 1987 by bottom trawl in Galveston Bay or in the Gulf of Mexico immediately offshore from Galveston Island, Texas, and acclimated to laboratory conditions (19–21°C and 26–30‰) for at least 1 week prior to experiments. The squids ranged from 3 to 23 g wet mass and both sexes were used.

Water quality and culture systems

All water quality parameters were kept within the environmental tolerances of the squids during the experiments: pH 8·2, temperature 11–31°C, salinity 26–32‰, ammonia nitrogen $<0{\cdot}1\,mg\,l^{-1}$ (Hanlon *et al.* 1983). The only exception was for squids used to determine the oxygen extraction rate while swimming in the tunnel respirometer; ammonia nitrogen in this system ranged from 0·1 to $0{\cdot}7\,mg\,l^{-1}$, but exposure times were short, a matter of hours rather than days.

The squids were maintained in two 2-m diameter circular fibreglass holding tanks provided with biological, mechanical, chemical and adsorptive fibration (Hanlon *et al.* 1983). The water was circulated through ultraviolet sterilizers to limit bacterial populations (Spotte, 1979).

Water sampling and O_2 measurement

All oxygen measurements were made with either a Kent EIL model 7130 or a YSI model 58 oxygen meter. Both probes and meter were found to respond linearly when calibrated with a Winkler oxygen determination (Strickland & Parsons, 1972).

Oxygen extraction from well-aerated sea water

The cephalopod ventilatory system is through-flow and counter-current to the blood flow in the gills; the ventilation stream enters laterally above the gills and passes through the gills to a common postbranchial chamber before being expelled through the siphon (Wells & Wells, 1982). Oxygen extraction by *L. brevis* was measured continuously from samples taken through a cannula threaded through the tip of the abdomen, and along the postbranchial chamber, with the aperture in the base of the funnel. The first 10 cm or so of the cannula was of 1 mm o.d. 'Portex' tubing. It was not possible to get adequate flow rates without pumping along such narrow tubes, and the rest of the cannula was of wider bore, 3·25 mm o.d. 'Portex' tubing. Flow, at 5–10 ml min^{-1} past the electrode and magnetic stirrer, was achieved by gravity; it was important that the cannula opening, which was floating free in the funnel aperture, had insufficient suction to attach it to the inside of the funnel, and that flow rates represented only a small fraction of the total ventilation volume. The animals were free-swimming within the limits of the tether imposed by the cannula. *Lolliguncula brevis* spends most of the time hovering in its holding tank, keeping station with its fellows, and frequently moving only a few centimetres back and forth, over long periods. Under these conditions the cannula did not appear to impede swimming.

Oxygen extraction during acute hypoxia

O_2 extraction was also measured in animals that progressively reduced the O_2 concentrations of sea water in 2-l jars immersed in their holding tanks. The cannula arrangements are shown in Fig. 1A; sampling could be switched from animal to jar contents by clamping the appropriate cannula, with the water removed being replaced continuously through a third cannula in the lid of the jar. The total dead space of the cannula system was typically around 3 ml, so that there was a delay of 20–30 s between switching and the arrival of the new sample at the electrode. Typical experiments involved switching from animal to jar every 5 min, with results obtained within 2 min of the changeover being discarded.

For these experiments glass tubing was used to link the respirometer jar with the O_2 electrode. This was necessary because at O_2 concentrations below about 5·0 mg l^{-1}, detectable quantities of O_2 leaked inwards during the 20–30 s residence time in Portex tubing exposed to the air outside the holding tank.

Oxygen extraction in a tunnel respirometer

Lolliguncula will swim against a current to keep station relative to objects in its field of view. We used a Brett-style respirometer (Brett, 1964) to study oxygen

extraction while swimming. The respirometer was constructed of polyvinyl chloride, with an inline titanium cooling coil and a 373-W pump that could provide flow down a 15 cm diameter swimming chamber at speeds of up to 45 cm s^{-1}. Preliminary tests showed that cannulated squids would swim for 30 min or more against a current of 9·5 cm s^{-1}, but tended to become exhausted after a few minutes at 16 cm s^{-1}. The cannula was normally slack and did not appear to impede jet propulsion despite the added drag on the squid.

Oxygen uptake in closed respirometers

Animals were placed in 2-l screw-top jars that were placed on the floor of the holding tanks, where the imprisoned squid could keep station with their free companions hovering alongside. The oxygen concentration of the water, originally that of the holding tank, was measured after a period estimated to be long enough to reduce the O_2 content to around 3·5 mg l^{-1}, this being above the concentration at which the squids had begun to show signs of stress in the acute hypoxia experiments.

In *Octopus*, heartbeat frequency and pulse amplitude are reduced at oxygen concentrations of about 3·0 mg l^{-1} and below, resulting from their own respiration in closed containers. Exactly the same effects are produced by transferring the animals to sea water in which the oxygen concentration has been reduced to similar levels by boiling. It is concluded from this that the circulatory response is driven by the lack of oxygen and not by the accumulation of metabolites such as CO_2 or ammonia (Wells & Wells, 1983). Thus metabolites accumulating in the jars were not monitored.

Video recording

Ventilation and heartbeat frequencies were determined from video recordings made with a Panasonic colour video camera (model WV-3250/8AF) with a standard lens (focal length 10·5–84 mm), zoom feature (8×) and built-in stop-watch (0·01 s recorded on-screen). Normal lighting (60 W fluorescent) was supplemented with a 650-W photography light directed from 1·5–2·0 m above the tank onto the squids. No information is available about light-adaptation in the eyes of *Lolliguncula*. Other cephalopods show a very quick (seconds) pupillary response followed by pigment migration, which is fastest in the lower part of the eye, that would be maximally affected by bright light from above; in *Octopus* the change is complete in about 4 min (Young, 1963). We allowed a minimum of several minutes for acclimation to the brighter light before any series of video recordings. Analyses of the 1·27 cm video tapes were aided by viewing the tapes on a Panasonic VCR (model NV-8950) player with multi-motion playback capability. Heartbeat frequency (easily visible) and ventilation frequency (counted from mantle contractions or the shadow of the mantle on the tank bottom) were counted at slow speed for 10- to 20-s periods. This was repeated 3–6 times and the rates averaged.

Blood oxygen-carrying capacity

The copper concentration of *L. brevis* haemolymph was determined on a Perkin–Elmer atomic absorption spectrophotometer (Perkin–Elmer, 1982). The haemolymph was withdrawn by a microcapillary inserted into the branchial hearts. The copper concentration was then used to predict the oxygen-carrying capacity of the haemolymph (vols%) based upon two atoms of copper carrying one molecule of oxygen (Ghiretti, 1966).

Statistical analyses

Differences between treatments (i.e. temperature, oxygen concentration, swimming rates) were tested using Student's *t*-test or analysis of variance (Sokal & Rohlf, 1969). Relationships between rates and temperature were analysed using linear regression analysis and the slopes of the lines determined for use with Q_{10} estimates.

Results

Oxygen extraction from well-aerated water

Measurements were made from seven squids swimming freely within the limits set by their trailing cannulae in their holding tank, in the tank containing the tunnel respirometer or in the tunnel respirometer. The animals ranged in mass from 6·10 to 14·15 g and the tests were run at 20–30°C. A further five animals were tested while hovering in 2-l jar respirometers at the start of experiments made to examine oxygen extraction during progressive hypoxia. These animals weighed 10·87–23·70 g and were tested at 15–27°C.

In all, more than 200 measurements were made. Table 1 lists typical values as well as the highest and lowest percentage extractions found. Additional results are shown in Figs 1 and 2. There would be little point in averaging the figures for extraction since this so plainly depends upon what the animal happens to be doing from one moment to the next, and on its immediate past history. Maximum extraction, up to 16% from well-aerated water (80% saturated and above), followed periods of vigorous jetting when the squid lay on the floor of its tank, ventilating but not jetting backwards or forwards. Minimal extraction, 2%, was seen during particularly vigorous jetting. Animals 'at rest' that had been hovering for several minutes could show extraction levels as low as 3%, but extraction values were typically in the 5–10% range.

Oxygen extraction during progressive hypoxia

Eight experiments were made with five squids (ranging in size from 10·87 to 23·7 g) at temperatures of 15–27°C. The results of four experiments are shown in full in Fig. 1, with samples from four further experiments included in Table 1B. As shown in Fig. 1, the animals regulate successfully, taking up oxygen at an almost steady rate down to as low as 3·0 mg l^{-1}. In five of the eight runs, extraction by

Table 1. *Oxygen extraction from the ventilatory stream*

Temperature (°C)	Animal	Mass (g)	Tankwater O_2 ($ml\,l^{-1}$)	Percentage extraction	Remarks
A In sea water greater than 80% saturated with oxygen					
20	L2	7·56	7·5	4	
22	L2	7·56	7·3	3	
20	L19	10·16	7·7	3	
			7·7	2	In tunnel at $9{\cdot}5\,cm\,s^{-1}$
			7·7	16	Recovery after exercise
21	L24	11·89	7·7	4	
			7·7	5	In tunnel at $9{\cdot}5\,cm\,s^{-1}$
			7·7	5	In tunnel at $16\,cm\,s^{-1}$
22	L16	11·38	6·6	8	
			6·5	11	
			6·4	13	
23	L15	14·15	6·0	4	
			6·1	8	
			6·1	10	
30	L21	6·10	6·7	8	
			6·7	6	Vigorous jetting
30	L22	14·00	6·7	10	
			6·7	9	In tunnel at $9{\cdot}5\,cm\,s^{-1}$
			6·7	9	In tunnel at $16\,cm\,s^{-1}$
			6·7	13	Recovery after exercise
B During progressive hypoxia (in 2-l jar respirometers)					
15	L27	23·7	7·4	7	
			6·3	7	
			5·4	7	
			4·4	10	
			3·7	11	Sometimes on bottom
			2·8	11	Resting on bottom
			2·3	11	Resting on bottom
18	L26	10·87	7·3	8	
			6·6	2	Vigorous jetting
			5·7	5	
			5·3	8	
			4·8	8	
			4·0	9	
			3·7	8	
24	L28	21·57	5·8	17	
			4·7	17	
27	L28	21·57	5·1	14	
			3·9	15	

Typical values; animals cruising gently in their holding tanks except where stated.
Further detailed examples are given in Figs 1 and 2.

hovering squids increased as the oxygen concentration fell (all cases in Fig. 1, and L27 in Table 1B). Further increases in extraction were seen at the lowest oxygen concentrations on occasions when the squids ceased swimming and lay on the bottom (Fig. 1A,C,D and L27 in Table 1B). Experiments were terminated soon after the animals had begun to show this sign of stress.

Oxygen extraction during active swimming

Six experiments were made with five squids (ranging in mass from 9·40 to 14·00 g) at temperatures of 20–30°C. The results are summarized in Fig. 2 and Table 1A. Oxygen uptake rose when the animal swam against a current (P. G. Lee, in preparation) but extraction remained unchanged (at around 5–10 %), or even fell a little. At 16 cm s^{-1}, the squids rapidly became exhausted and were carried back against the downstream grid in the swimming chamber. When the flow was stopped, they lay on the floor of the tank, ventilating deeply and more slowly than usual (in Fig. 2C, last run, the ventilation frequency is recorded). On these occasions extraction could double to almost 20 % (Fig. 2; Table 1A).

Oxygen uptake from closed respirometers

Oxygen uptake from closed 2-l jars was noted in 46 experiments involving 23 animals. These ranged in mass from 3·00 to 23·70 g and temperatures ranged from 14 to 30°C. The results are shown in Fig. 3. $\dot{V}_{O_2}$ rose from an average of 410 ml kg^{-1} h^{-1} at 14–15°C to 764 ml kg^{-1} h^{-1} at 27–30°C with a Q_{10} of 1·47.

Heartbeat and ventilation frequencies

Video recordings were made in 69 experiments with 26 squids before confinement in 2-l jar respirometers. Size and temperature ranges were as in the $\dot{V}_{O_2}$ experiments summarized above.

The mean frequencies are plotted against temperature in Fig. 4; details of individual results are included in Fig. 5A,B. Heartbeat frequency increased as expected with temperature, with a Q_{10} of 1·92. Ventilation frequency rose to a plateau at about 25°C and the Q_{10} calculated for the 14–25°C range was 1·73.

The response to developing hypoxia is shown in Figs 6 and 7. Recordings were normally made from five squids (ranging in mass from 4·12 to 13·69 g) before and during confinement in 2-l jar respirometers at temperatures of 14, 20 and 27°C ($N = 6$ at 14°C). At the lower two temperatures, heartbeat frequency showed an initial response to confinement, rising by about 10 %, then it stabilized. Ventilation frequency (Fig. 7) was more variable than heartbeat frequency (Fig. 6) and showed no consistent trend as hypoxia developed. There was no response to confinement.

At 27°C, the animals were plainly stressed. Two of them died and one was dying as the oxygen fell to concentrations below 4·0 mg l^{-1} in the respirometer. Heartbeat frequency, already very high at 27°C, showed no increase due to confinement and fell progressively thereafter in four of the five animals.

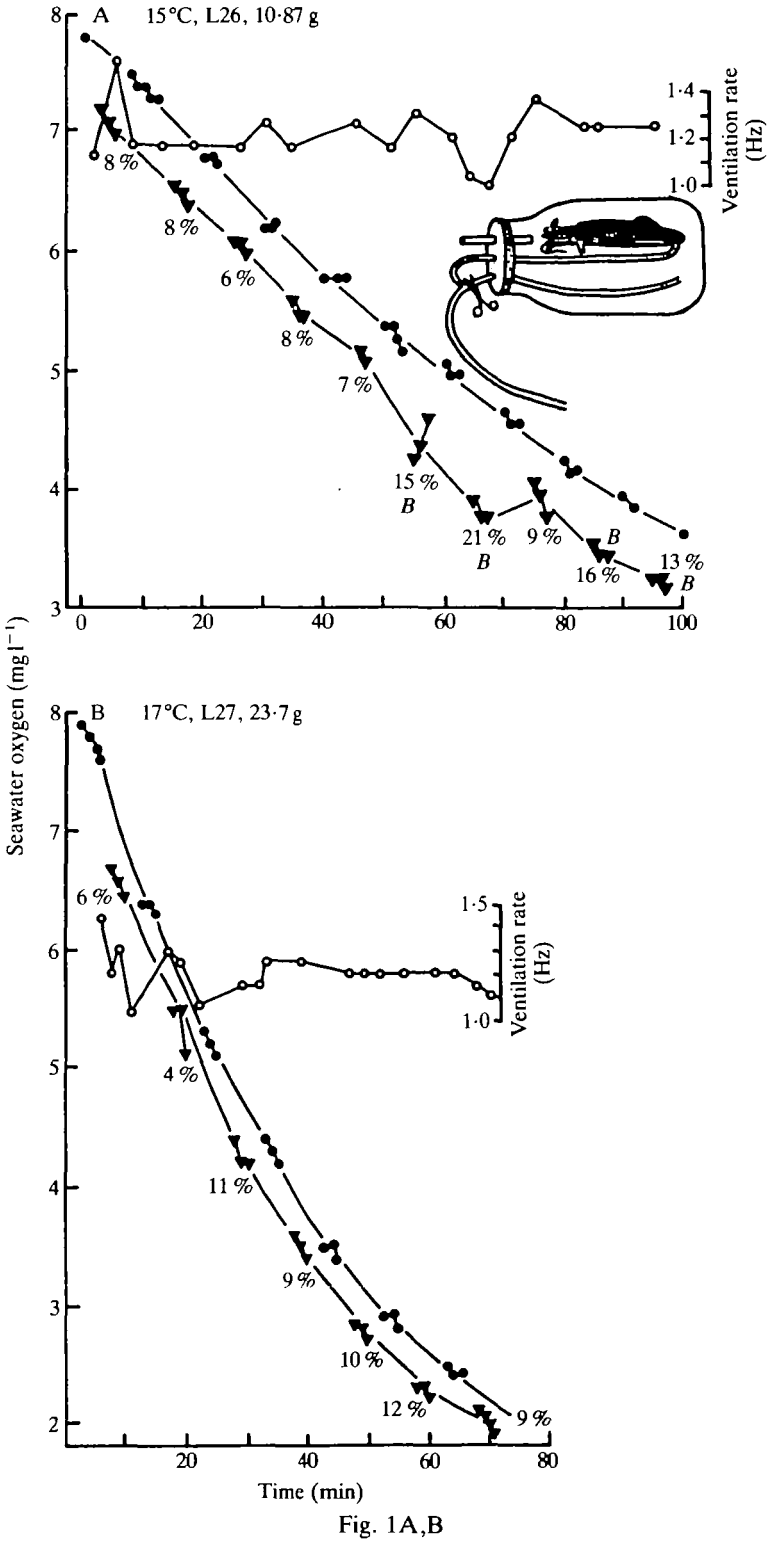
A 15°C, L26, 10·87 g
8 %
8 %
6 %
8 %
7 %
15 %
B
21 %
B
9 %
B
16 %
13 %
B
1·4
1·2
1·0
Ventilation rate (Hz)
B 17°C, L27, 23·7 g
6 %
4 %
11 %
9 %
10 %
12 %
9 %
1·5
1·0
Ventilation rate (Hz)
Seawater oxygen (mg l⁻¹)
Time (min)

Fig. 1A,B

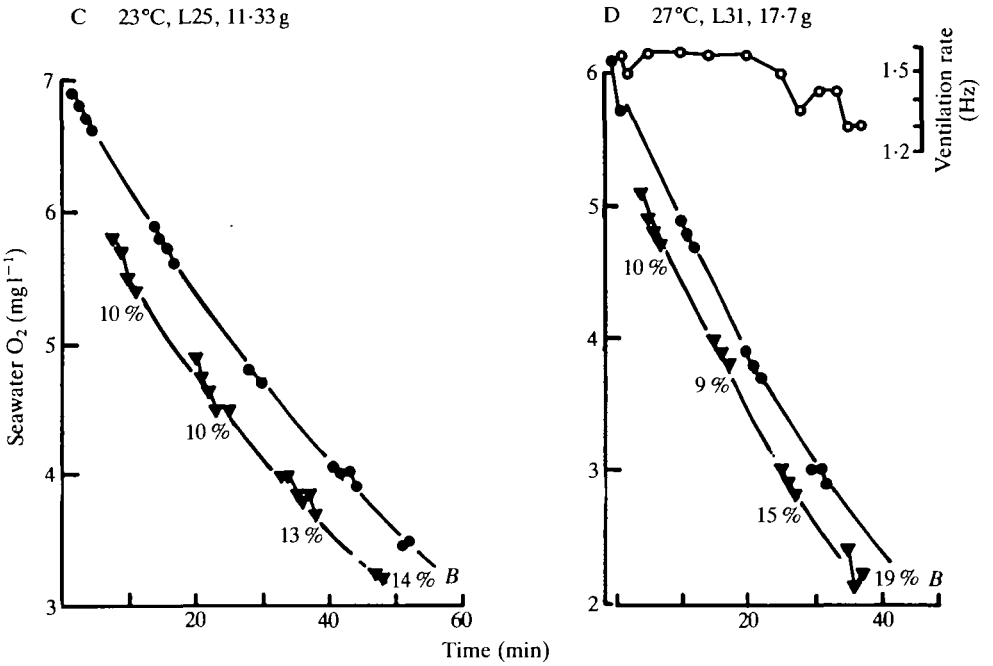

Fig. 1. Oxygen extraction during developing hypoxia. Animals had cannulae implanted as shown in A to sample the exhalant stream. Sampling was switched between this and the respirometer at approximately 5-min intervals. The ventilation rate (○) was observed and timed by stopwatch. (A–D) Performances at increasing temperatures, 15, 17, 23 and 27°C. $\dot{V}_{O_2}$ increased with temperature, but the animals regulated well throughout the temperature range, oxygen uptake being reduced only slightly as the concentration was reduced below 50 % saturation. The extraction rate rose as the external P_{O_2} fell. *B* indicates occasions when a squid was resting on the bottom of its respirometer. (●) O_2 (mg l^{-1}) in the respirometer jar; (▼) O_2 (mg l^{-1}) in the exhalant stream, showing percentage oxygen extraction.

Ventilation frequency showed little change in the two animals that remained in good condition until the end of the experiment. In nature this species tolerates temperatures up to 31 °C but these levels are reached after weeks of acclimation.

Derived values

Ventilation stroke volume

Where total oxygen uptake, oxygen extraction and ventilation rates are known it is possible to calculate the stroke volume of ventilatory movements (SVv) (for a 10-g squid) as follows:

$$SVv = \dot{V}_{O_2}(\text{ml kg}^{-1}\text{h}^{-1}) \times \frac{1}{100} \times \frac{1}{60} \times \frac{1000}{O_2 \text{ content (ml l}^{-1})}$$

$$\times \frac{1}{\quad} \times \frac{1}{\quad}$$

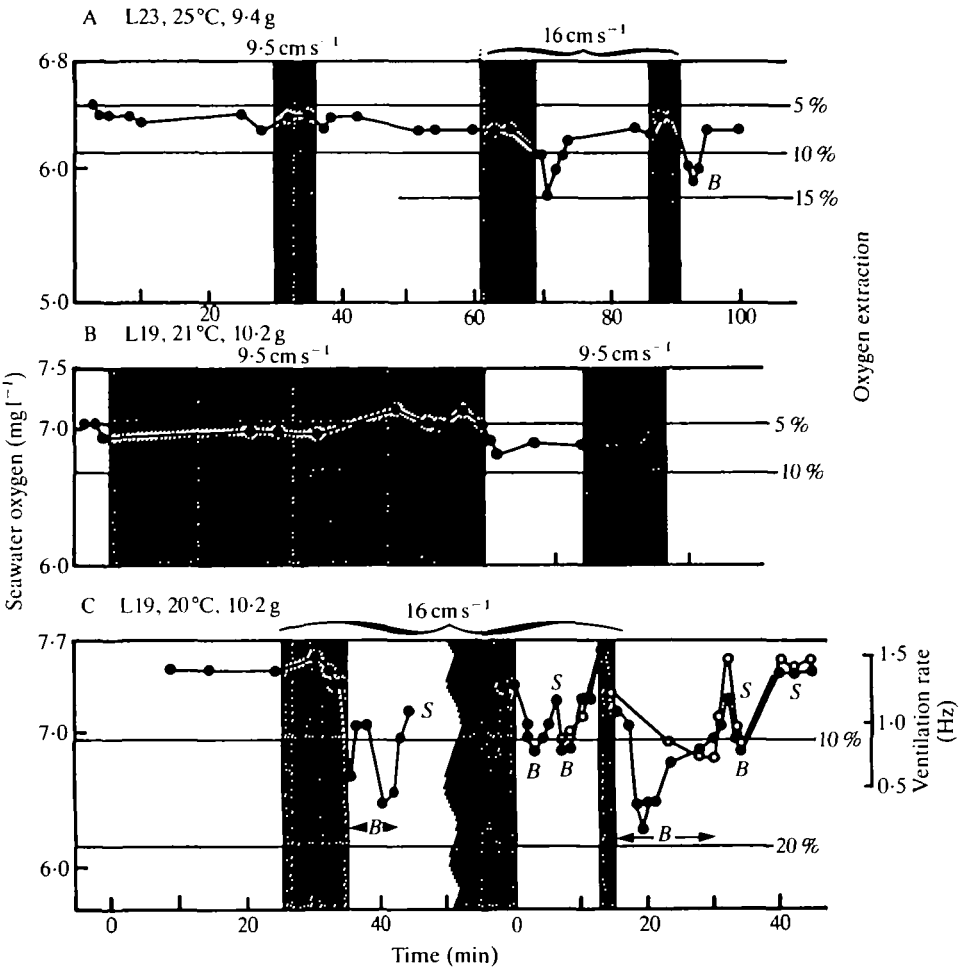

Fig. 2. Oxygen extraction in a tunnel respirometer. Animals, cannulated as shown in Fig. 1A, were allowed to swim freely in a tank. From time to time they were introduced into a tunnel respirometer (shaded areas) within the tank with water flowing at 9·5 or 16 cm s^{-1}. In the tunnel the animals swam against the current, maintaining their position. At 9·5 cm s^{-1}, swimming (using both fins and jet) could be maintained for long periods (B). At 16 cm s^{-1} the animals depended mainly upon powerful jets and rapidly became exhausted (A,C). If the flow was then stopped they either lay down in the tunnel or swam out and lay on the floor of the tank (B). On these occasions oxygen extraction (●) rose and ventilation rate (○) fell (C). *S* shows that the animals were again swimming actively after a resting period. In the second run in C the squid entered the flume by itself and had been swimming for an unknown time before measurements of O_2 extraction were started.

where fv is ventilation frequency. This, however, makes no allowance for possible cutaneous O_2 uptake. In *Octopus* of around 1000 g, this is in the region of 10 % of the total O_2 uptake and does not change as the external P_{O_2} falls (Wells & Wells,

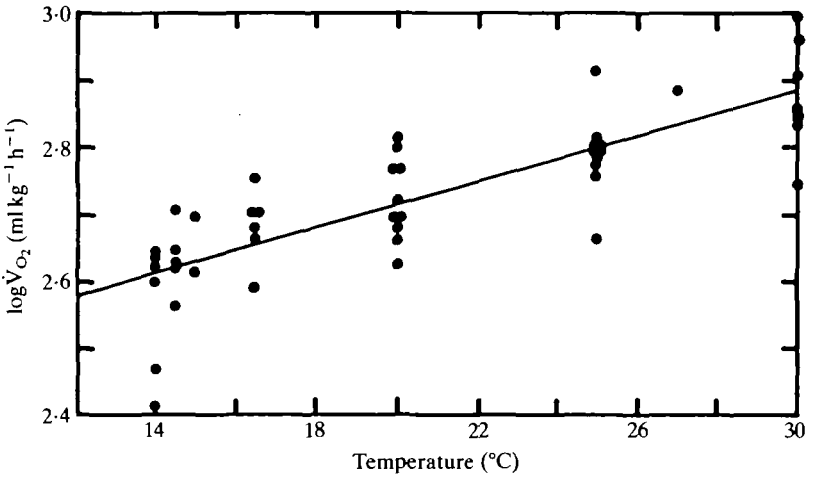

Fig. 3. The relationship between $\dot{V}_{O_2}$ and temperature. $\log\dot{V}_{O_2}$ (in ml kg^{-1} h^{-1}) = 0·0168T(°C) + 2·38, with a correlation coefficient, r, of 0·824.

$$Q_{10} = \left(\frac{R_2}{R_1} \times \frac{10}{T_2 - T_1}\right), \quad \log Q_{10} = 10\left(\frac{\log R_2 - \log R_1}{T_2 - T_1}\right) = 0{\cdot}168, \quad Q_{10} = 1{\cdot}47,$$

where R is $\dot{V}_{O_2}$ and T is temperature.

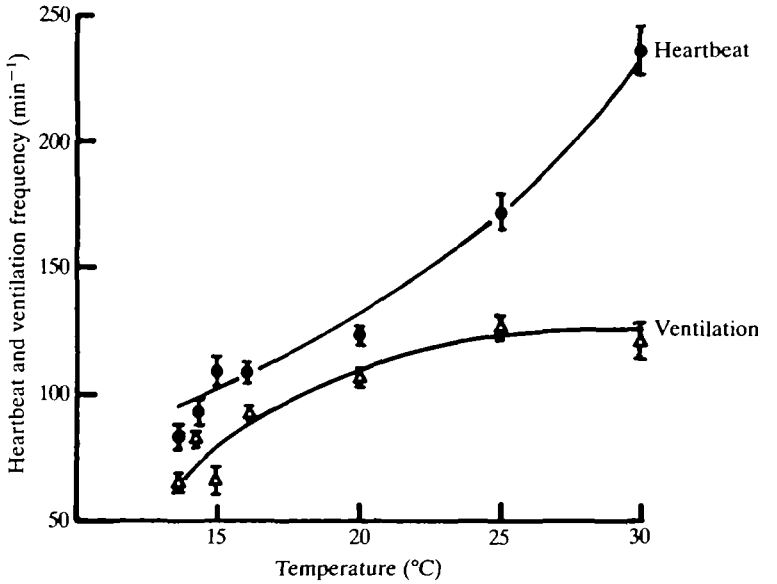

Fig. 4. Heartbeat and ventilation frequency as functions of temperature. Heartbeat frequency rose exponentially with temperature, but ventilation frequency reached a plateau at about 25°C. Mean and S.E.M. in each instance. 69 experiments with 26 squids.

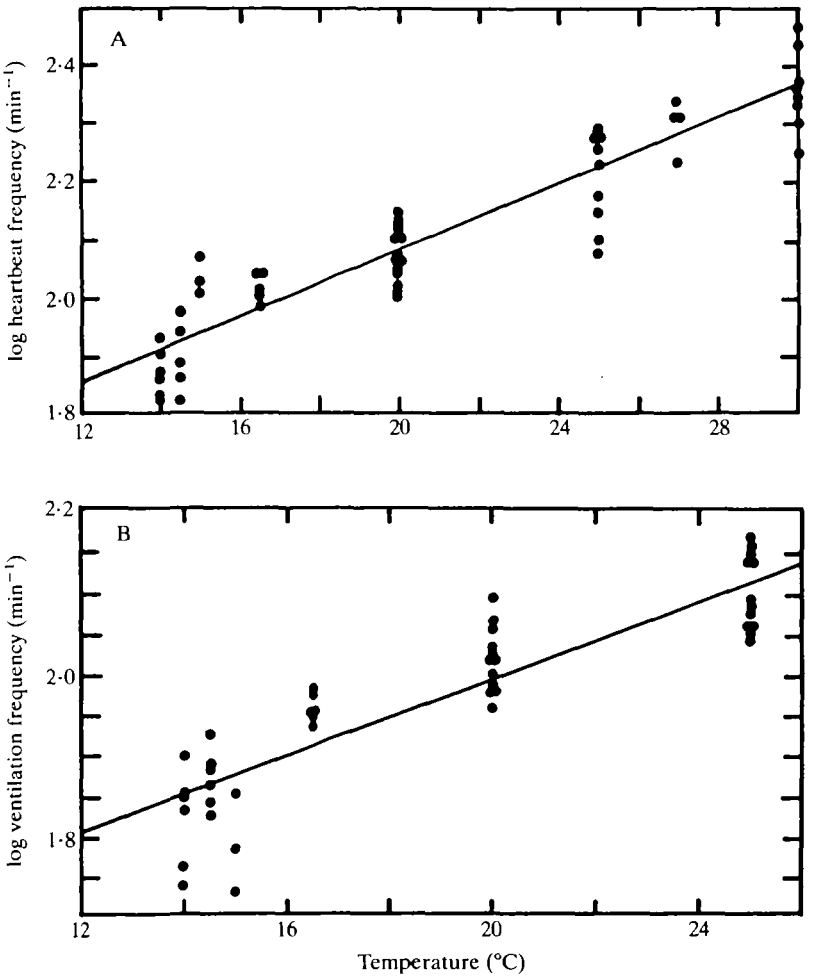

Fig. 5. Measurements used to establish Q_{10} values for heartbeat and ventilation frequencies. (A) Heartbeat frequency rose exponentially with temperature throughout the range tested, 14–30°C. Q_{10}, computed as in Fig. 3, was 1·92. (B) Ventilation frequency did not rise in a straightforward way with temperature. Q_{10} fell as temperature increased; see Fig. 4. The value computed for the range 14–25°C, shown here, was 1·73.

1983). *Lolliguncula brevis* is two orders of magnitude smaller than *Octopus*, which will give it a considerably greater surface area/volume ratio. If the two were isomorphic, the ratio would be 4·6 times as great in the squid, suggesting a greater capacity for cutaneous respiration. *Lolliguncula* has a metabolic rate some 10 times that of *Octopus*. In the absence of any estimates of surface area and of the likely partial pressure gradient across the skin, we can only make a guess at cutaneous uptake in squids. In Table 2 we have used 20 % as the proportion of the total O_2 uptake entering through the skin at all temperatures, with a consequent reduction in ventilation stroke volume. It seems likely that the proportion of O_2

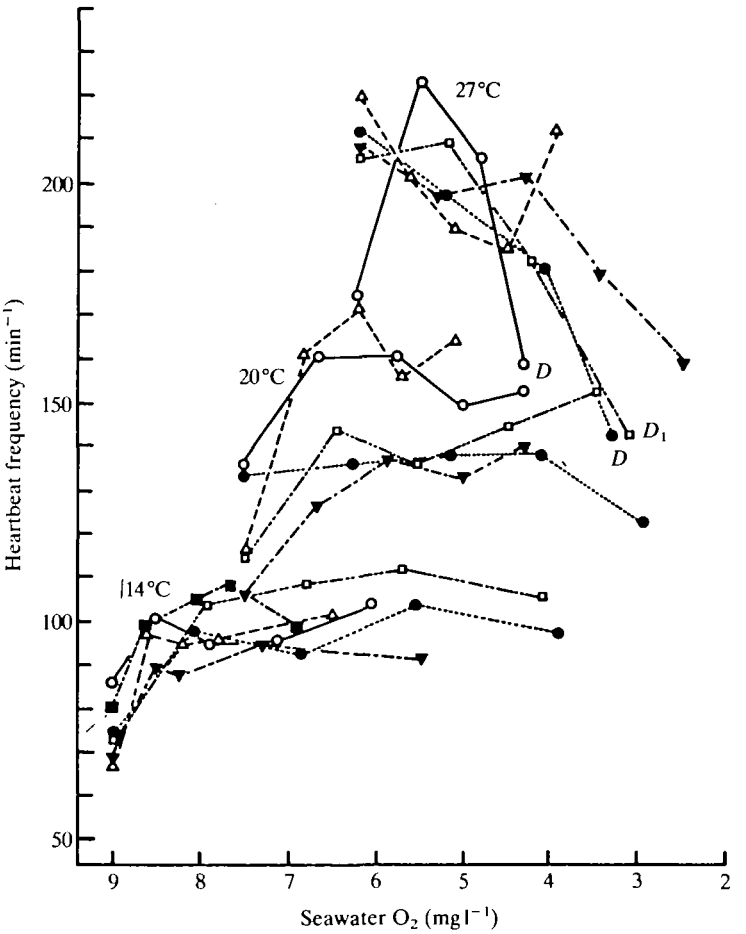

Fig. 6. Heartbeat frequency changes during developing hypoxia. Each animal was first observed swimming freely in the tank at the temperature concerned, before being caught and placed in a 2-l jar that was placed on the floor of the tank. The oxygen concentration of the tank water and the oxygen concentration of the jar at the end of the experiment were measured. Video recordings of the beating hearts were made before, and at intervals during, the period of confinement in the jar, without disturbing the animals. The oxygen concentration of the jar at the time of each recording was estimated on the assumption that oxygen uptake was maintained at a steady rate throughout the period of confinement. Catching animals and placing them in the jars caused an immediate increase of about 10 % in the heartbeat frequency at 14 and 20°C. There was no further increase as the oxygen concentration was reduced at 14 or 20°C. At 27°C, the animals failed to show the 'confinement' increase, and heartbeat frequency fell as hypoxia developed; two of the animals died (D) and a third was dying by the end of the experiment (D_1).

entering through the skin will not change greatly with temperature. Cephalopod skin is a very active tissue, with many small nerves and muscles controlling the chromatophores and skin texture (Packard, 1988), and probably uses much of the

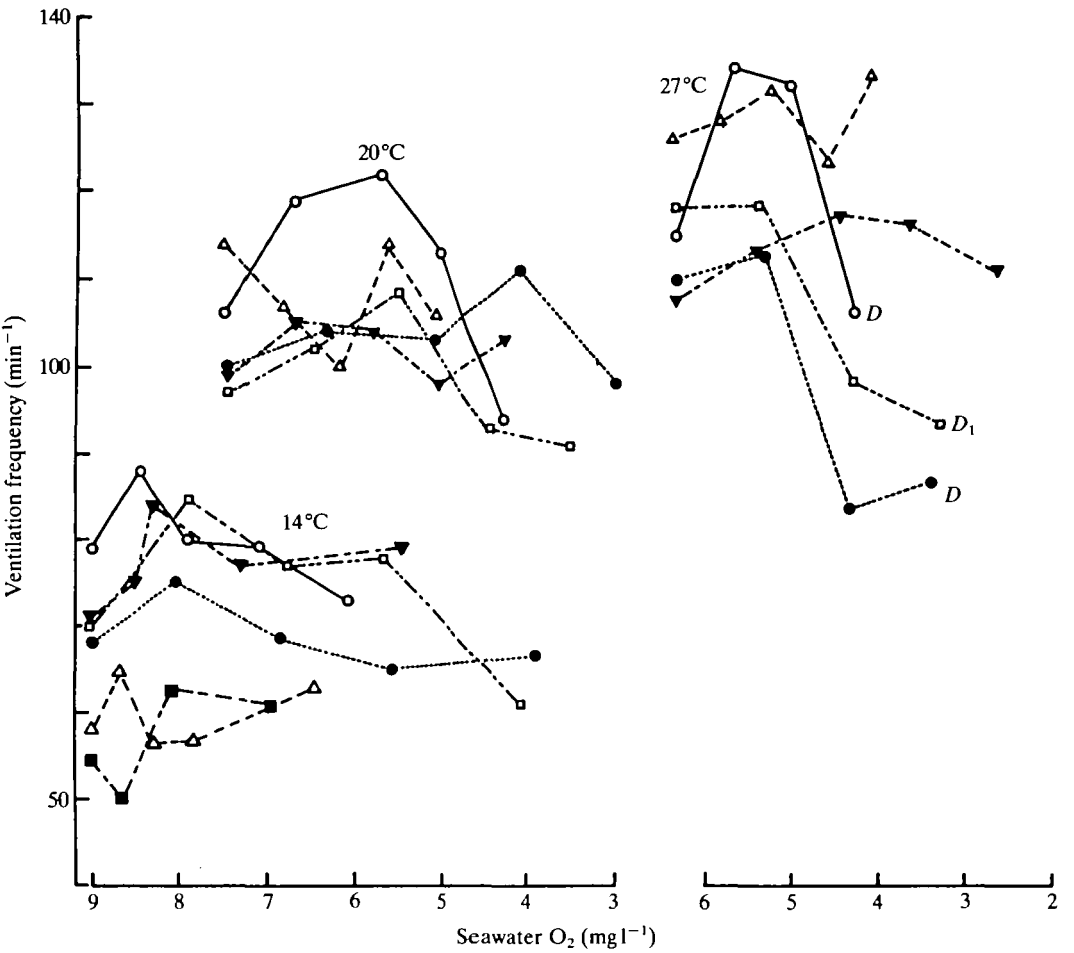

Fig. 7. Ventilation frequency changes during developing hypoxia. Data from the same experiments as in Fig. 6. The plots from results at 27°C are displayed to the right to avoid overlap with the 20°C data. Ventilation frequency did not change on capture and confinement, and there was little increase with temperature above 20°C. Once again, there was no change in frequency as the oxygen concentration fell at 14 and 20°C. At 27°C the animals were badly stressed (D and D_1, as in Fig. 6).

cutaneous oxygen uptake itself. One might expect the skin to use oxygen at a rate proportional to the oxygen uptake of the whole animal.

Table 2A shows values derived for ventilation stroke volume on these assumptions, taking values for O_2 uptake from the jar respirometer experiments (Fig. 3) and ventilation rates from video recordings of the same individuals immediately before each was placed in a respirometer (for mean ventilation rates, see Fig. 4). Information about extraction rates was not available for these individuals, so ventilation volumes were estimated on the basis of 5 % and 10 % O_2 extraction from saturated sea water by squids keeping station in their tanks, since nearly all

Table 2. *Ventilation: the relationship between stroke volume and temperature*

A Animals where $\dot{V}_{O_2}$ and fv were measured sequentially

	Temperature (°C)			
	14–15	20	25	27–30
Number of squids	15	12	12	12
Mean mass of animals tested (g)	8·02	10·78	8·90	9·21
Mean $\dot{V}_{O_2}$ ($ml\,kg^{-1}\,h^{-1}$)	410 ± 18	539 ± 22	619 ± 23	764 ± 43
O_2 content of sea water at 29‰ saturation ($ml\,l^{-1}$)	6·02	5·38	4·91	4·63
Ventilation frequency (fv; beats min^{-1})	69 ± 2	104 ± 3	126 ± 4	118 ± 4
Stroke volume (ml) for a 10-g animal, assuming 20% cutaneous respiration at:				
5% extraction	2·63	2·56	2·68	3·72
10% extraction	1·32	1·28	1·34	1·86

B Animals where $\dot{V}_{O_2}$ and fv were measured simultaneously

	Temperature (°C)		
	14	20	27
Number of squids	6	5	2
Stroke volume at 5% extraction (ml)	2·40	2·28	2·79

Values are means ± S.E.M. for $\dot{V}_{O_2}$ and ventilation frequency.

the measured values (Table 1; Figs 1, 2) fell within this range. Table 2B also shows the very similar values derived from the experiments summarized in Fig. 7 where $\dot{V}_{O_2}$ and ventilation frequency (fv) were derived simultaneously rather than sequentially.

The general conclusion from these results is inescapable, despite the assumptions that have to be made: in *L. brevis*, ventilation stroke volume remains constant despite the considerable increases in $\dot{V}_{O_2}$ as temperature rises. At the very highest temperatures tested, 27–30°C, stroke volume appeared to rise somewhat. We do not know if this effect is real (the assumptions made about cutaneous uptake invalidate statistical comparisons of such small differences) but it appears that it arises because some of the animals under these conditions stop swimming from time to time, lie on the bottom of the tanks and extract oxygen at a level in excess of 10% (as in the experiments illustrated in Fig. 1). Whatever the mechanism, the animals at 27–30°C were approaching their physiological limits, as Figs 6 and 7 show, and perhaps were not fully comparable with those tested at lower temperatures.

Cardiac output and heart stroke volume

By using the mean $\dot{V}_{O_2}$ for each temperature (Table 2), heartbeat frequency from the same animals (Fig. 5A), estimates of the total oxygen-carrying capacity of the blood derived from its copper concentration (4·6 ± 1·8 vols%, $N = 6$) and Redfield & Goodkind's (1929) measurement of 93 % for the proportion of the available oxygen removed at each circuit in *Loligo*, it is possible to compute the volume of blood pumped by the heart as follows:

$$\text{cardiac minute volume (ml min}^{-1}) = [\dot{V}_{O_2}(\text{ml min}^{-1}) - 20\,\%] \times \frac{100}{0{\cdot}93 \times 4{\cdot}6}.$$

Cardiac stroke volume (SVH) is this value divided by the observed heartbeat frequency (Table 3).

As in Table 2, the final values in Table 3 are corrected for a standard 10-g animal. These values of SVH decline as the temperature rises. The extra oxygen demand is met by increasing heartbeat frequency.

Discussion

In *Octopus* and *Sepia*, a fall in the oxygen concentration of the ventilatory stream is associated with a rise in the pH of the blood, increasing the oxygen affinity of the blood pigment such that the arterial blood continues to remain saturated as the external P_{O_2} falls (Houlihan *et al.* 1982; Johansen *et al.* 1982). The *in vitro* behaviour of squid and cuttlefish bloods are similar (Brix *et al.* 1981) and there is no reason to suppose that the blood of *L. brevis* will behave differently *in vivo* from that of the cephalopods already studied.

On this assumption, *L. brevis* should have no trouble maintaining blood O_2 saturation as the environmental P_{O_2} falls. The heart will not have to pump more

Table 3. *The volume of blood pumped by the hearts*

	Temperature (°C)			
	14–15	20	25	27–30
Number of squids	14	13	9	12
$\dot{V}_{O_2}$ from values in Table 2 (ml min^{-1})	0·0548	0·0968	0·0918	0·1173
Minute volume (ml min^{-1}) with blood O_2 delivery at 4·3 vols%, assuming 20 % cutaneous respiration	1·02	1·80	1·71	2·18
Heartbeat frequency (beats min^{-1})	85 ± 4	121 ± 3	169 ± 7	225 ± 8
Stroke volume (ml)	0·0119	0·0149	0·0101	0·0097
Stroke volume per 10-g of animal	0·0150	0·0138	0·0113	0·0105

Values for heartbeat frequency are means ± S.E.M.

blood until the P_{O_2} falls to very low concentrations. This prediction is shown to be correct by the lack of change in heartbeat frequency as the oxygen is reduced in closed respirometer chambers (Fig. 6); the slight initial increase, which has already been noted as occurring when the animals are caught and placed in jars, is evidently a response to confinement rather than a response to oxygen concentration.

Considering only the range (up to 25°C) over which the animals appear to be unstressed as the P_{O_2} falls, one might expect an increase in ventilation stroke volume to result from the diminished O_2 concentration of the ventilation stream. In fact this does not happen, because oxygen extraction rises as the external P_{O_2} falls. An increase in the percentage extraction within the limits found could balance the fall in oxygen concentration so that ventilation stroke volume remains constant (Table 4).

We have no figures yet for the oxygen cost of locomotion in *L. brevis*. By analogy with other cephalopods (Wells *et al.* 1983, *Octopus*; Webber & O'Dor, 1985, *Illex*) one would expect the maximum $\dot{V}_{O_2}$ to be 2–3 times the 'resting' $\dot{V}_{O_2}$. Experiments such as those summarized in Fig. 2 show that animals swimming against a current in a tunnel respirometer do not increase their extraction rate. Video tapes of squid L19 swimming at speeds of 9·5 and 16 cm s^{-1} at 20°C showed ventilation frequencies of 80 and 90 min^{-1}, actually slower than the 106 min^{-1} averaged by squids simply keeping station at this temperature. A single run with squid L22, at 30°C swimming at 9·5 cm s^{-1}, showed a ventilation rate of 115 min^{-1}, the same as in quiet cruising in the holding tank. It is difficult to believe that the animals can manage sustained swimming even at 9·5 cm s^{-1} without some increase in $\dot{V}_{O_2}$. This must certainly be the case at 16 cm s^{-1}, since the animals quickly become exhausted, indicating that they have exceeded the factorial scope available to them. Since the two records made during swimming (above and in

Table 4. *Ventilation stroke volumes during progressive hypoxia for a 10-g* Lolliguncula

Oxygen content		Oxygen extraction	Ventilation stroke volume (ml)		
($mg\,l^{-1}$)	($ml\,l^{-1}$)	(%)	14–15°C	20°C	25°C
7·0	4·9	7	2·31	2·01	1·91
6·0	4·2	9	2·10	1·82	1·74
5·0	3·5	11	2·06	1·79	1·71
4·0	2·8	13	2·18	1·89	1·81
3·0	2·1	15	2·52	2·18	2·09
$\dot{V}_{O_2}$ ($ml\,min^{-1}$)			0·0683	0·0898	0·1032

$\dot{V}_{O_2}$ and ventilation frequency from Table 2.

Figures given here for SVv assume 20 % cutaneous respiration, perfect regulation of oxygen uptake and a progressive increase in extraction from 7 % to 15 %, as indicated in Fig. 1 and Table 1.

Fig. 2C) indicate that they do not increase their ventilation frequency and they certainly do not increase their oxygen extraction, ventilation stroke volume must rise in exercise. One would expect it to do this because of the need to produce a more powerful jet.

In recovery after exhausting exercise, animals typically lie on the bottom. Oxygen extraction can double and ventilation frequency can halve (Fig. 2C). These would cancel each other, with no resultant increase in $\dot{V}_{O_2}$ to pay off the oxygen debt if ventilation stroke volume did not rise. It certainly appears to do so when the animals are viewed from above. However, the effects may in part be an illusion since the mantle is flattened by the weight of the animal resting on the floor of its tank.

If, as one must suppose, oxygen uptake increases in exercise, cardiac minute volume must rise. At present we have only two records of heartbeat frequency made during swimming (at $9{\cdot}5\,cm\,s^{-1}$) in the tunnel respirometer. One of the animals, L19, was at 21°C and the other, L22, was at 30°C; the values obtained were 151 and 261 beats min^{-1}. The latter is about 10 % higher than the average for 'resting' animals at the same temperature, the former no higher than normal. If we assume that these results are typical, increases in oxygen delivery must be met by increasing cardiac stroke volume in *Lolliguncula*, as they are in exercising *Octopus* (Wells *et al.* 1987).

The volume of blood that must be pumped by the heart in these circumstances is impressive. With an O_2 delivery capacity of 4·3 vols%, and a maximum $\dot{V}_{O_2}$ of $1500\,ml\,kg^{-1}\,h^{-1}$ at 25°C (2·5 times the resting value), the systemic heart of a 10-g squid would have to deliver $28\,l\,kg^{-1}$ body mass h^{-1}. The systemic hearts of four *Lolliguncula* of approximately this size averaged $2{\cdot}06 \pm 0{\cdot}62\,g\,kg^{-1}$ (similar to the value found for *Illex* by Martin & Aldrich, 1970). So the 0·02-g heart of a 10-g *L. brevis* at 25°C would have to pump 0·027 ml at each stroke, more than its own mass of blood at each rapid contraction.

Finally, it should be pointed out that the extraction rates (at 5–10 %) found in *Lolliguncula* are very low by the standards of the other coleoids. *Octopus* and *Sepia* typically remove 40–50 % of the oxygen from the ventilatory stream (Wells & Wells, 1982). There are also differences in the response to progressive hypoxia. *Octopus* varies the stroke volume of its ventilatory movements by 150 % or more, with an increase of up to 30 % in ventilation frequency, but no change in oxygen extraction (Wells & Wells, 1985*b*). *Lolliguncula brevis*, in contrast, keeps SVv and fv constant, but doubles percentage oxygen extraction. SVv and fv only change if the squid stops swimming and lies on the bottom. This is, of course, the key to the difference in response. Squids use the ventilatory flow for locomotion and the volume of water pumped through the mantle while the animal is swimming is determined by this. Normally the jet requirement is well in excess of the volume needed to support $\dot{V}_{O_2}$. The gills need only extract a small proportion of the oxygen passing through. *Nautilus* is in a similar situation, dependent upon jet propulsion, and it too shows low percentage extraction rates, comparable with those reported here for *Lolliguncula* (Wells & Wells, 1985*a*). It is possible that

large changes in stroke volume are in any case impossible for squids because of the constraints imposed by the massive collagenous tissues included in the wall of the mantle and associated with efficient jet propulsion (Gosline & Shadwick, 1983). Neither *Octopus* nor *Sepia* (a relatively inactive decapod) uses jet propulsion as its routine locomotory mechanism. *Octopus* crawls with its arms and normally uses the jet only for escape or to pounce on prey and *Sepia* hovers using buoyancy control in the cuttlebone and the lateral fins that run the whole length of the abdomen, calling on the jet only for sudden manoeuvres. Both have evidently been selected for efficiency in oxygen extraction (it is relevant that one spends most of its time in holes, the other buries itself in sand) rather than for jet propulsion. The cephalopod mantle and gill complex have evolved along two divergent pathways, depending upon the life styles of the animals concerned.

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