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GAS EXCHANGE OF CAPTIVE FREELY DIVING GREY SEALS (*HALICHOERUS GRYPUS*)

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Summary

When at sea, phocids dive for long periods and spend a high percentage of their time submerged. This behaviour requires some combination of an increased oxygen storage capacity, rapid oxygen loading at the surface and reduced oxygen utilisation when submerged. To assess these adaptations, breath-by-breath ventilation was studied in four adult grey seals (two male, two female, 160–250 kg), freely diving in a large outdoor tank where surface access was restricted to one breathing hole. The dive patterns obtained were similar to those recorded from freely diving wild grey seals. Respiratory frequency during the surface periods was 40 % higher than that estimated from allometric relationships (19.4±0.7 breaths min^{-1}), and tidal volume (6.3±1.2 l) was approximately five times higher than that estimated from allometric relationships. These adaptations produce a high minute volume and enable gas exchange to occur at the surface.

Mean oxygen consumption rate ($\dot{V}_{O_2}$, measured for a dive+surface cycle) decreased with increasing dive duration. The aerobic dive limit was estimated as 9.6 min for a 150 kg grey seal (using the overall average $\dot{V}_{O_2}$ of 5.2 ml O_2 min^{-1} kg^{-1}), which is consistent with results from freely diving wild grey seals (only 6 % of dives exceeded 10 min). End-tidal oxygen values varied during a surface period, following a U-shaped curve, which suggests that there is limited oxygen uptake from the lung and/or blood oxygen stores during dives. This result was unexpected and indicates that these seals are utilising substantial physiological responses to conserve oxygen, even during shallow voluntary diving.

Introduction

Recent studies of some pinniped species (elephant seals, grey seals) have found that individuals may spend up to 90 % of the time at sea submerged, with some dives being longer than the predicted aerobic dive limit, but without extended recovery periods at the surface (Le Boeuf *et al.* 1988, 1989; Hindell *et al.* 1991, 1992; McConnell *et al.* 1992*a*; Thompson and Fedak, 1993). Thompson and Fedak (1993) recorded extremely low heart rates from freely diving grey seals during these extended dives (down to 4 beats min^{-1}),

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which are similar to those recorded from forcibly dived seals (Scholander, 1940). Similarly, low heart rates have been recorded occasionally during diving in northern elephant seals (Andrews *et al.* 1991).

It is not known how these very long dives are achieved. Our understanding of how marine mammals are able to remain submerged has changed over the last 50 years. Early laboratory studies suggested that seals performed long anaerobic dives with long recovery periods (based on data from forcibly submerged animals; Scholander, 1940; Scholander *et al.* 1942). Then, fieldwork studies of freely diving animals found that aquatic mammals and birds generally carry out relatively short aerobic dives with short recovery periods (e.g. Kooyman and Campbell, 1972; Butler and Woakes, 1979; Kooyman *et al.* 1980; Kanwisher *et al.* 1981; Fedak *et al.* 1988). This led to the concept of the aerobic dive limit (ADL; Kooyman *et al.* 1980, 1983), used to define the longest dive that an animal could perform without accumulating lactate in the blood.

Recent data indicate that some species can carry out very long dives with short recovery periods, during long bouts of continuous diving (Hindell *et al.* 1991, 1992; Thompson and Fedak, 1993). These different diving patterns suggest that seals use a number of physiological strategies during diving. There appear to be differences between species in the strategy used for diving. For example, both grey and Weddell seals exhibit very long dives but, whereas in Weddell seals surface periods increase in duration rapidly for dives over 20 min (which correlates to blood lactic acid levels; Kooyman *et al.* 1980), in grey seals surface periods are reasonably constant for dives over 7 min in duration (Thompson and Fedak, 1993). This may be due to different foraging patterns (i.e. sit-and-wait predators *versus* actively swimming predators; Fedak and Thompson, 1993; Thompson *et al.* 1993).

This study aimed to examine oxygen loading, storage and utilisation in order to understand how grey seals carry out extended dives without the need for long surface periods. Gas exchange variables were measured at the surface (i.e. respiratory frequency, tidal volume, respiratory flow rates). Rapid-response breath-by-breath end-tidal O_2 and CO_2 values were measured, and it was thus possible to compute respiratory minute volumes and gas exchange.

Materials and methods

Breath-by-breath oxygen and carbon dioxide concentrations were measured in the expired air of freely diving seals, using a custom-made mask. This enabled the rapid changes in breath-by-breath oxygen and carbon dioxide levels and respiratory airflow to be followed over a single breath and over a single surface breathing period.

Experimental arrangement

The work was carried out between June 1989 and July 1992 at the Institute for Forestry and Nature Management (IBN) in Texel, Netherlands. Four adult grey seals, two male and two female (mass ranges: Luke 235–250 kg; Snot 160–185 kg; Splodge 150–165 kg; Heinz 140–160 kg) were used. Seals were kept in unheated outdoor seawater tanks, 35 m by 6 m and 1.75 m deep. One tank was partitioned into four interconnecting sections, and

one section served as the respirometry chamber. This consisted of an area 8 m by 6 m, covered with netting, with one area roofed with plywood in which there was a hole. This breathing hole was covered by a mask designed to follow the shape of a seal's head and was freely available to the seal. Initially, the dead space in the mask when the seal was breathing was approximately 1–1.5 l; this variability was due to the size and height of the seal's head in the box. The dead space was reduced as much as possible by inner contouring of the corners of the mask. The minimum dead space was approximately 0.5 l (without accounting for the seal's nose); further reduction in dead space restricted the seals' access to the mask. The effect of dead space on the concentrations of end-expired O_2 and CO_2 was tested using the flushing mechanism of the mask (see system operation below). Different gas mixtures were fed into the mask to set the initial dead space gas composition (i.e. the mixture of gas which is present in the mask when the seal first begins the breathing bout). Two different flush gases were used: pure N_2 and a calibrated mixture of 12 % O_2 and 5 % CO_2.

For an experiment, an individual animal was introduced to the respirometry chamber and allowed to acclimate to the chamber for at least 1 h before recording began. The animals were fasted for at least 6 h before data recording, to eliminate any specific metabolic effects of feeding. Prior to each day's set of experiments, the flow and gas analysers were calibrated and checked (see section below). Recordings began once the equipment had been set up and the animal had been acclimated to the chamber. Behavioural observations were noted by hand on a chart recorder, although it was generally not possible to observe the animal in the chamber unless the water was very clear. Recordings were made at various times during the day (between 09:00 and 24:00 h) for periods of 1–8 h. At the end of an experimental run, the gates of the respiratory chamber were opened and the seal was released into the uncovered area of the tank.

Instrumentation

A schematic diagram of the breath-by-breath system used to sample the expired gases is shown in Fig. 1. The inspiratory and expiratory flows were measured using an ultrasonic flowmeter (BRDL Ltd, Birmingham University; 60 l s^{-1} version; see Woakes *et al.* 1987) mounted on the top of the mask so that the seal could breathe freely through the flowmeter. The gas analysers were positioned on a platform mounted on the mask to minimise the delay between sampling and analysis.

A continuous subsample of the seal's inspiratory and expiratory flows was drawn at a constant flow rate (600 ml min^{-1}) through a small-bore capillary tube (approximately 1.5 mm diameter and 20 cm length) from the centre of the seal's respiratory gas flow, passing through a Minisart drying filter (Sartorius; dead space approximately 0.1 ml), to two Servomex gas analysers (model 1505 Miniature Infrared CO_2 and 728 Zirconia O_2; Servomex UK Ltd, Crowborough, Sussex). The oxygen sensor required a reference gas, and the air in the vicinity of the respirometry mask was used after it had been passed through a separate drying filter. The maximum possible sample gas rates were used to minimise the delay of the system (400 ml min^{-1} for the O_2 sample, 250 ml min^{-1} for the O_2 reference gas, 200 ml min^{-1} for the CO_2 sample).

The data streams from the three analysers (flow, oxygen and carbon dioxide) were

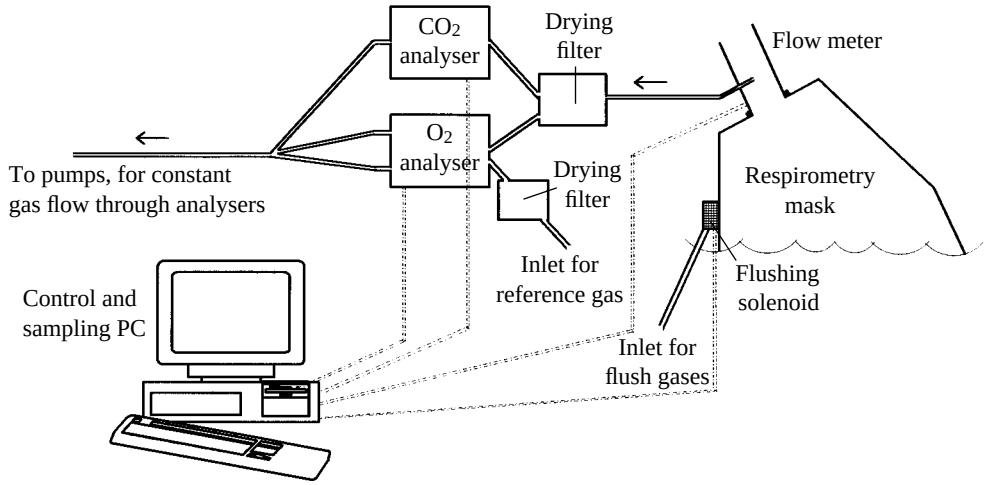

Fig. 1. Schematic diagram of the respirometry sampling equipment. The respirometry mask enables the seal to vent freely to the atmosphere, and the respiratory flows are sampled for analysis by the oxygen and carbon dioxide gas analysers. Data sampling and storage are controlled by the computer.

sampled at a rate of 100.37 Hz, using a PCL-812 12-bit analogue-to-digital converter (PC LabCard); the data sampling and digital storage were controlled by a 16 MHz 286 Dell PC with specifically designed and written software. The output from the analysers was also recorded onto chart paper using a two-channel Watanabe Linearcorder Mk VII chart recorder (Watanabe Instruments, Japan). Respirometry data were recorded simultaneously with any heart rate data (see below) onto magnetic audio-type tapes using a four-channel FM tape recorder (Data Acquisition Ltd), so that analysis of the heart rate recording (which was carried out after the experiment) could be matched up to surface and dive periods.

Calibration

The ultrasonic flowmeter calibration was checked against a Greer Electromanometer M12 with pneumotachograph flowhead (Mercury), which was only used for these calibration measurements and which had also been calibrated using commercial flowmeters (KDG flowmeters, Burgess Hill, UK). A custom-built gas syringe of 7 l was also used for volume calibration of the ultrasonic flowmeter. The overall respirometry system was calibrated in three stages. (1) The analogue-to-digital converter (ADC) input voltage gain was calibrated using constant voltage inputs and monitoring the digital output with a precision 4.5 digit electronic multimeter (Siemens B1021). (2) The gas sensors were calibrated using gas mixes (pure N_2; 12 % O_2 and 4 % CO_2 in N_2; 10 % CO_2 in N_2; supplied and certified to 0.01 % by Hoekloos Gases, Amsterdam, Holland), and the output voltages were monitored using the precision multimeter. (3) Overall system integrity was verified by monitoring the ADC output when calibration gases were passed through the gas analysers.

System operation

The computer continuously monitored the analyser outputs and data were buffered until a breath was detected. This triggered data storage until the PC detected the end of the breathing period. Each surface period was saved as a separate file, with time, seal identification, temperature, calibration data etc. in the file header. As soon as the seal submerged, the respirometry mask was automatically flushed with fresh air using a high-flow pump, by energising a solenoid valve attached to the base of the breathing box. This valve also enabled different gas mixtures to be introduced to the box, for the first inspiration or expiration of a seal upon surfacing, for various control experiments.

During the course of a data collection period (1–8 h), the zero point of the flow and gas sensors tended to drift. The flow offset was corrected by assuming that the true average flow over a surface period (some 6000 samples) was zero and making the corresponding offset adjustment in software for processing. CO_2 and O_2 offsets were corrected by using the inspired air as a baseline (20.98 % O_2 and 0.03 % CO_2) and assuming that the sensors had returned, during the course of the 1.3 s (or so) of inspiration, to this value. The sensors and the capillary tubing introduced lags and delays into the system. The response time of the ultrasonic flowmeter was 12 ms (for 100 % response). The response time of the oxygen analyser (installed in the system) was approximately 500–600 ms, while that for CO_2 was approximately 300–400 ms. Both these values include the lag times for gas transport etc. The delays introduced by the sensors and the capillary pathways were estimated by calculating the delay between the flow transition from expiration to inspiration (where there is a very rapid change in the O_2 and CO_2 levels) and the peak end-tidal CO_2 and minimum end-tidal O_2.

Data processing

The data files saved by the sample program were analysed using a separate analysis program. Each breath cycle (expiration, inspiration, pause) was identified by the corresponding transitions of the flowmeter output. The diving pattern of the seals was analysed in cycles: a dive cycle was defined as a dive plus the following breathing bout. Since the animals' activity levels could only occasionally be observed, it was not possible to categorise the submersions into types of dives (e.g. active or asleep). Therefore, the term 'dive' is used throughout this paper for these voluntary submersions and does not indicate any level of activity.

Dive durations were measured from the chart paper recordings. The analysis program automatically calculated surface durations, durations of inspiration and expiration (in seconds), respiratory frequency at the surface (f_R; breaths min^{-1}), peak expiratory and inspiratory flow rates (PEFR and PIFR, in l min^{-1} at BTPS) and tidal volumes in litres (V_T, at BTPS). Minute volume ($\dot{V}_E$, l min^{-1}) was calculated as $V_T \times f_R$. Oxygen and carbon dioxide data were output as percentage end-tidal (minimum) O_2 and percentage end-tidal (maximum) CO_2 and converted to kPa (assuming relative humidity of air at 37 °C is 6.26 kPa). The breath-by-breath volumes of O_2 uptake and CO_2 production were calculated by integrating the product of the instantaneous fractional percentage of oxygen (or CO_2) and the expiratory flow rate at that instant. Owing to the response times of the gas analysers, the output signal was 'smeared' over a longer interval than that of the input

(known as convolution). Thus, the calculations for gas volumes are an underestimate (by less than 5 % for CO_2 and less than 8 % for O_2). The mass-specific metabolic rates for each animal (i.e. rate of oxygen consumption, $\dot{V}_{O_2}$, in ml O_2 min^{-1} kg^{-1}) were calculated by summing the volume of oxygen utilised in each of the breaths over one surface period and assuming this was utilised over the whole dive+surface period. Gas exchange volumes are tabulated at STPD.

Heart rate measurement and blood variables

Heart rates (f_H) were also recorded during some of the experiments, by real-time short-range telemetry using acoustic transmitters (Bard Holand, SINTEF, Trondheim, Norway). The transmitters were mounted laterally, just dorsal and posterior to the base of a foreflipper. Bioelectric potentials created by each heartbeat were detected *via* two electrodes mounted dorsally and ventrally, along the midline of the animal. The electrodes consisted of 15 mm copper discs and were pressed closely to similar-sized areas of shaven skin and held in place and insulated from the water by epoxy resin, which glued the electrode mounting to the surrounding fur (Fedak *et al.* 1988). The transmitters produced a signal (70–90 kHz) which was modulated by the heartbeat potential. Signals were received using an omnidirectional hydrophone and receiver (Vemco), and the output was recorded on magnetic tape. The electrocardiogram (ECG) signals were later manually decoded and logged during playback of the tape, using the Dell PC as an event recorder. The mean of 10 inter-beat intervals for each surface period was calculated and converted to surface breathing heart rates; the mean dive heart rate was calculated similarly (see Chabot *et al.* 1991 for a discussion on methodologies of heart rate measurements).

Blood was sampled on an opportunistic basis, whenever the seals were being restrained (on land) for some other purpose. Samples were taken either from the hind flipper plexus or from the epidural vein and were analysed for haematocrit (using a Whatman mini-centrifuge) and for haemoglobin content (using a standard Sigma kit for the spectrophotometric assay of haemoglobin concentration, [Hb]); each sample of blood was evaluated in triplicate.

Statistical analysis

The output from the analysis program was read into a Minitab spreadsheet for statistical analysis (Minitab: Pennsylvania State University 1981). Heart rates were also analysed using Minitab. The data are presented as the overall means (± standard error of the mean), calculated from the means of the four individual animals. For comparisons between animals, Student's *t*-tests were carried out; the level of statistical significance was taken at the 95 % ($P<0.05$) confidence level.

Results

Diving behaviour

Overall, the mean dive duration was 3.8±0.65 min, the mean surface duration was 0.8±0.14 min and the mean percentage of time spent submerged was 80.5±0.43 %. The

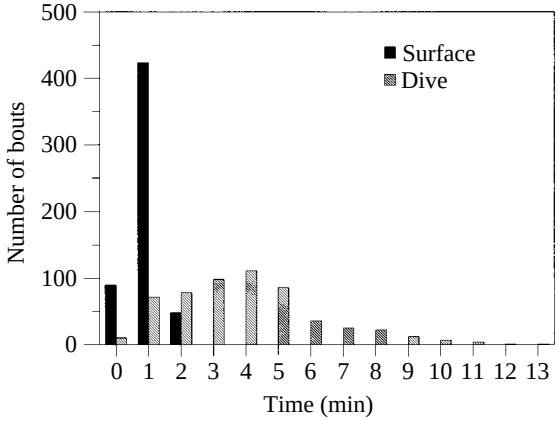

Fig. 2. Histogram showing the distribution of dive and surface times (in minutes), for all seals.

data are plotted as frequency histograms in Fig. 2. There was considerable individual variation in the dive patterns recorded. Mean dive times for the various individuals ranged between 2.9±0.3 and 5.7±0.2 min; maximum recorded dive times for each individual varied and ranged from 13.1 min (Heinz, ♀) to 9.2 min (Snot, ♂). The longer dives tended to occur in the late afternoon or evening when there were few outside events to disturb the animals.

Heart rates and blood variables

The overall mean diving heart rate was 28±2 beats min^{-1} and the mean surface heart rate was 99±2 beats min^{-1}. The heart rates for individual seals ranged from 93±0.4 to 102±0.4 beats min^{-1} while they were breathing at the surface and from 20±0.9 to 28±0.5 beats min^{-1} during diving. Mean haematocrit for the four seals was 50.8±2.17 %, and mean haemoglobin concentration was 18.3±0.85 g 100 ml^{-1} blood.

Respiratory variables

Table 1 presents mean values of the respiratory variables for the four seals. Fig. 3 presents the gas and flow traces recorded over a typical single breathing period; the pattern of breathing (expiration, inspiration, pause) can be clearly seen. The respiratory frequencies for the individual seals ranged from 18.0±0.3 to 20.6±0.3 breaths min^{-1}, and the tidal volumes ranged from 9.75±0.04 l (for the 250 kg male seal) to 4.86±0.02 l (for the 160 kg female seal). The total lung capacity (TLC) for each seal was estimated from the scaling relationship $TLC=0.10M_b^{0.96}$, where M_b is body mass (Kooyman, 1989), and the tidal volumes relative to TLC for each seal ranged from 49 % (for the 250 kg male seal) to 35 % (for the 185 kg male seal). For three of the four seals, the mean peak flows were significantly higher for expiration than for inspiration (for the 250 kg male seal: PEFR was 12.3±0.09 l s^{-1}, PIFR was 9.3±0.05 l s^{-1}). Peak flow rates increased with increasing tidal volume. There were no significant correlations between tidal volumes, peak flows or times for inspiration or expiration as a function of the time during the surface period (see Figs 4, 5).

Table 1. *Respiratory data recorded from the four grey seals*

Respiratory frequency (f_R; breaths min^{-1})	19.4±0.7
Expiration duration (s)	0.97±0.08
Inspiration duration (s)	1.14±0.11
Peak expiratory flow rate (PEFR; l s^{-1})	9.7±0.9
Peak inspiratory flow rate (PIFR; l s^{-1})	8.4±0.5
Tidal volume (V_T; l at BTPS)	6.3±1.2
Minute volume ($\dot{V}_E$; l min^{-1} at BTPS)	122.8±14.1
V_T/estimated TLC	0.4±0.03

Total lung capacity (TLC; in litres) is estimated from the scaling relationship TLC=0.10$M_b^{0.96}$ (Kooyman, 1989).

Values are means ± S.E.M.; N=4 for all variables.

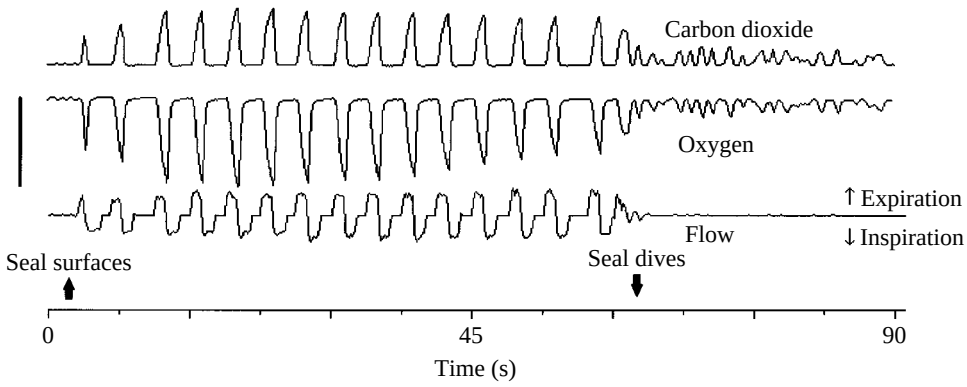

Fig. 3. Traces from a single surface period taken from the computer screen during data analysis. The upper trace is the carbon dioxide levels, the middle trace is oxygen and the lower trace is the flow. The scale bar (on the left) represents a 10 % change in the oxygen and carbon dioxide traces and 0 to +40 l s^{-1} (expiration) for the flow trace. The data displayed are only one-sixteenth of the data recorded (for more rapid display); hence, the traces shown are not smooth. The time axis relates to the flow trace, and the delays in the O_2 and CO_2 traces can be seen. Note the periodic breathing pattern (expiration, inspiration, pause) and the U-shaped curve followed by the end-tidal P_{O_2} values.

Gas exchange data

The overall mean end-tidal partial pressure of oxygen (P_{O_2}) was 11.5±0.23 kPa, and that of carbon dioxide (P_{CO_2}) was 4.6±0.38 kPa. The variation in end-tidal P_{O_2} during a surface breathing period is presented in Table 2. End-tidal gas values were not significantly correlated with tidal volumes or expiratory flow rates. The end-expiratory P_{O_2} varied throughout the surface interval, producing a wide approximately U-shaped curve (see Table 2; Figs 4, 6A), with the breaths with the lowest end-tidal P_{O_2} occurring towards the middle of the breathing bout (about 40 % of the way across the surface period; i.e. during the third to the fifth breath after surfacing; see Table 2). This phenomenon was also reflected by the (inverse) shape of the end-expiratory P_{CO_2} curve (see Figs 4, 6B).

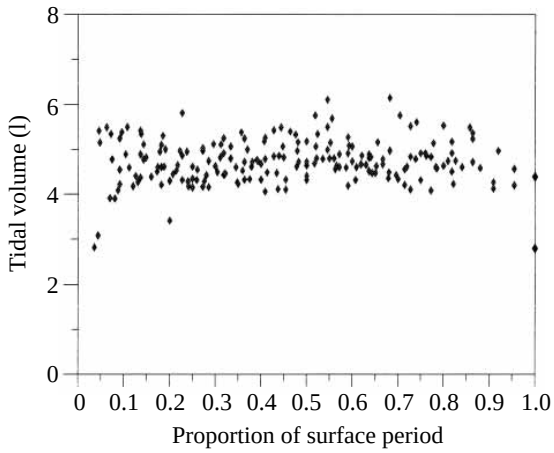

Fig. 4. Tidal volume (V_T in litres) for each breath over a surface period plotted against the time during a surface period (mean duration 52±2.3 s), expressed as a proportion; data from a set of 11 dives from a female seal (Heinz; 160 kg).

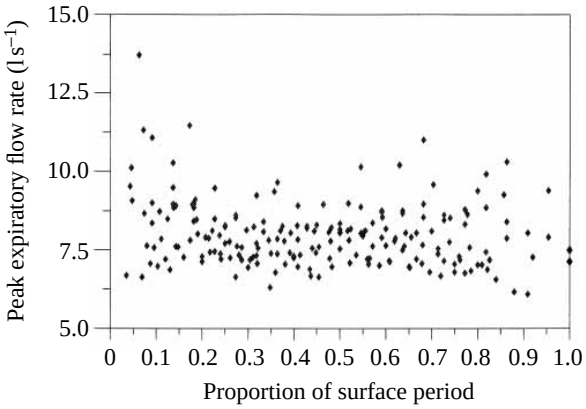

Fig. 5. Peak expiratory flows (PEFR, in l s^{-1}) for each breath over a surface period plotted against the time during a surface period (mean duration 52±2.3 s), expressed as a proportion; data from a set of 11 dives from a female seal (Heinz; 160 kg).

The U-shaped response of end-tidal P_{O_2} occurred after most of the dives, although to varying extents [for example, during bouts of very short dives (1–2 min) the surface period may only be for 2–3 breaths]. The U-shaped curve was also evident over a range of mask volumes (1.5–0.5 l of mask dead space). Fig. 6C shows the data from dives by Heinz from 1992 (mask volume 0.5 l; compare with Fig. 6A, when mask volume was approximately 1.0 l) and demonstrates that the position of the minimum end-tidal P_{O_2} value occurs approximately 35 % through the surface period regardless of dead space volume. It can be seen, with the smaller mask, that the minimum value of the end-tidal oxygen partial pressure is lower, although the position of the minimum occurs at the same time in both cases. Analysis of the end-tidal P_{O_2} during surface periods when either 12 %

Table 2. *Values for mean (± S.E.M.) end-expiratory* P_{O_2} *(in kPa) at the start and end of the surface period, the mean lowest end-tidal* O_2 *level and the percentage time through the surface period (the position in time across the surface period) at which this minimum oxygen occurs*

	Mean end-tidal P_{O_2} (kPa) at start of surface period	Mean lowest end-tidal P_{O_2}		Mean end-tidal P_{O_2} (kPa) at end of surface period
		P_{O_2} (kPa)	Position across surface period (%)	
Luke	11.6±0.12	8.6±0.04	39.4±1.28	12.5±0.07
Snot	12.6±0.23	10.1±0.08	41.5±2.97	13.0±0.18
Splodge	11.7±0.17	9.6±0.06	44.8±2.65	12.6±0.13
Heinz	12.1±0.10	9.2±0.05	35.7±1.09	13.5±0.06
Overall mean	12.0±0.23	9.4±0.33	40.4±1.91	12.9±0.21

O_2 or pure N_2 was used as the initial gas mixture in the mask demonstrated that the very first exhalation was significantly affected by the low oxygen content in only 9 out of the 25 trials. However, in all cases, subsequent breaths follow the previously observed U-shaped response, with both the position and value of the minimum P_{O_2} being consistent.

Oxygen consumption

The values for oxygen consumption rates are presented in Table 3, and it can be seen from this and from Fig. 7 that the rate of oxygen consumption decreases with increasing dive length, from $7.9\pm1.12\,ml\,O_2\,min^{-1}\,kg^{-1}$ for dives of less than 3 min to $3.5\pm0.26\,ml\,O_2\,min^{-1}\,kg^{-1}$ for dives of greater than 9 min.

Discussion

The dive durations recorded in this study on captive seals are similar to those recorded in the field from freely diving grey seals (Thompson *et al.* 1991; McConnell *et al.* 1992*b*; Thompson and Fedak, 1993). The very long dives occasionally performed by grey seals in the wild did not occur in this study, but the distribution of most dives around the 3–7 min range corresponds to the diving behaviour in the wild. Thus, it is possible to relate the respirometry data from this study to that in freely diving grey seals at sea.

The heart rates recorded from the four seals followed the bimodal pattern seen both in freely diving wild seals (Fedak *et al.* 1988) and in other laboratory studies (Ponganis *et al.* 1990; Williams *et al.* 1991): high while breathing at the surface and low while

Fig. 6. Variation of end-tidal P_{O_2} and P_{CO_2} (kPa) during the surface breathing period, for a series of dives. Note that the U-shaped curve is present in all graphs, although there is a reduction in deadspace (from approximately 1.0 l to 0.5 l) between 1991 and 1992 data. (A) P_{O_2} (kPa) during surface breathing periods for a series of 21 dives by a female seal (Heinz; 140 kg in 1991). (B) P_{CO_2} (kPa) during surface breathing period, for the same series of 21 dives as shown in A (Heinz; 140 kg in 1991). (C) P_{O_2} (kPa) during surface breathing periods, for a series of 20 dives by the same seal as in A and B (Heinz; 160 kg) in 1992, after considerable reduction in deadspace.

submerged. The heart rate values recorded in the present study are lower than the typical rates recorded for grey seals both in the wild and in the laboratory [i.e. heart rate while breathing: 119±1 beats min^{-1} (mean ± S.E.M.); heart rate while diving: 38±1 beats min^{-1}; Thompson and Fedak, 1993]. The low diving heart rate may be due to the restricted

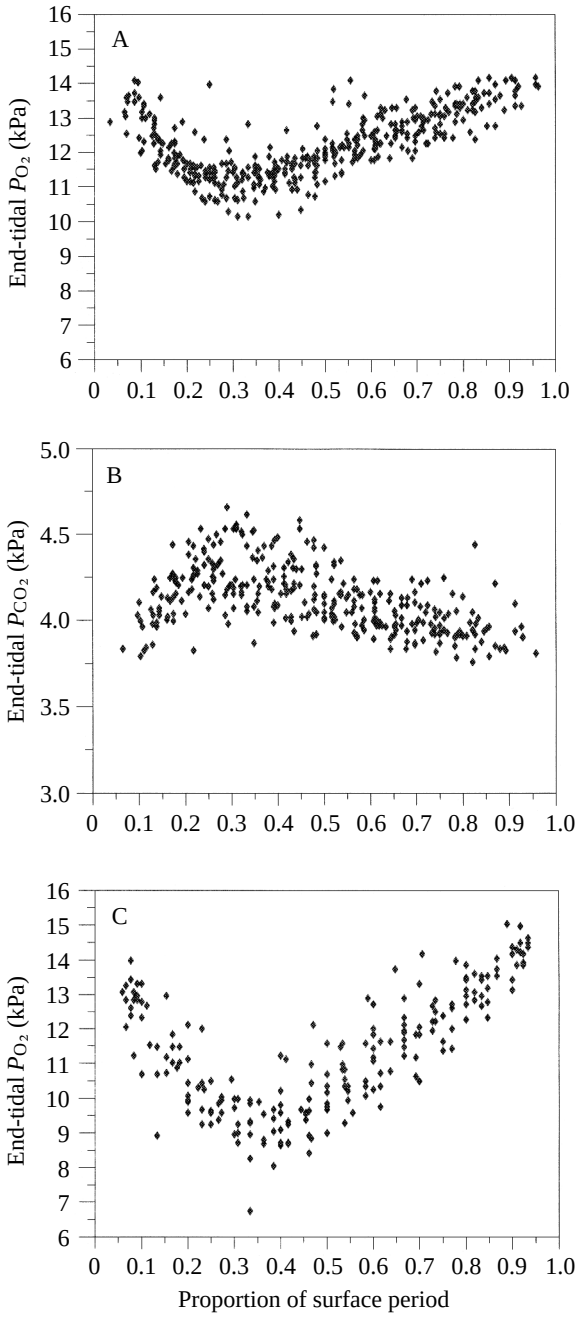

Fig. 6

Table 3. *Oxygen consumption for different dive durations*

Dive+surface time (min)	Oxygen consumption ($ml\,min^{-1}\,kg^{-1}$, STPD)	Number of dives
<3	7.9±1.12	12
3–6	5.4±0.26	31
6–9	4.0±0.22	17
<9	3.5±0.26	12
Overall mean	5.2±0.28	72

Data from all seals; values are means ± S.E.M.

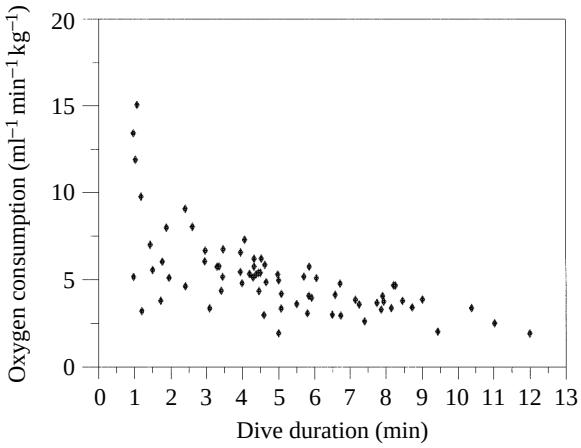

Fig. 7. Variation of the rate of oxygen consumption with dive duration ($\dot{V}_{O_2}$ is calculated over the whole dive+surface period). Oxygen consumption values are in $ml\,O_2\,min^{-1}\,kg^{-1}$. Data from four grey seals combined (72 dives).

situation. If the seals were slightly stressed, this may have produced a decreased heart rate while diving (i.e. a more extreme dive response than would ordinarily be the case). The lower heart rates while at the surface may be because these animals are not foraging and therefore do not need to maximise their heart rate at the surface; lower surface heart rates have been recorded from freely diving grey seals when they are around haul-out sites (Sea Mammal Research Unit, unpublished data).

The ventilatory data indicate the capacity for these seals to exchange large volumes of gases during each breathing bout. The tidal volumes are high since V_T is a large proportion of the total lung capacity (TLC). For the 250 kg male seal in this study, the estimated TLC is 20.8 l (from the scaling relationship $TLC=0.10M_b^{0.96}$; Kooyman, 1989), and the average tidal volume for this seal is 9.75 l (i.e. 47 % of TLC). In comparison, for a 450 kg horse, an athletic animal, the estimated TLC would be 32 l (from the relationship $TLC=0.063M_b^{1.02}$; Tenney and Remmers, 1963); the resting tidal volume is approximately 5 l, and tidal volume at a fast canter (approximately $12\,m\,s^{-1}$) is 14 l (Woakes *et al.* 1987; Butler *et al.* 1993); these values are 16 and 44 % respectively of TLC.

The respiratory frequencies during the surface breathing periods were approximately 30–80 % higher than would be estimated from scaling relationships for resting mammals (f_R=0.84$M_b^{-0.26}$ where f_R is in Hz and M_b is in kg; Worthington *et al.* 1991). For example, for a 250 kg mammal, this relationship would predict a respiratory frequency of 12 breaths min^{-1}, which is 67 % of that of the larger male (250 kg) in this study. Thus, the minute volumes ($\dot{V}_E = V_T \times f_R$, in l min^{-1}) are much higher than would be estimated (from the allometric equation: lung ventilation rate=20$M_b^{0.75}$, in l h^{-1}; Schmidt-Nielsen, 1983). For a 250 kg mammal, the estimated minute volume would be 21 l min^{-1}, while the measured minute volume at the surface of the 250 kg male seal in this study was 159 l min^{-1}. However, if these data are recalculated as weighted values to include the time spent submerged (around 80 % of the dive+surface cycle), then the overall minute volumes approach those estimated from the allometric equations. Thus, both large tidal volumes and high respiratory frequencies enable these seals to carry out the gas exchange needed for the whole dive+surface cycle during the relatively short surface interval.

The oxygen consumption rates recorded in this study fall within the range of metabolic rates measured for seals diving in the laboratory and for freely diving Weddell seals. Fedak (1986) and Fedak *et al.* (1988) present data for grey seals swimming in a water channel; their oxygen consumption rates range between 3.5 and 15 ml O_2 min^{-1} kg^{-1}, increasing with increasing exercise load. Castellini *et al.* (1992) present data from freely diving Weddell seals, which show a decrease in rate of oxygen consumption with increasing dive duration (for short dives less than 14 min, $\dot{V}_{O_2}$ is 5.0±0.9 ml O_2 min^{-1} kg^{-1}; for long dives more than 14 min, $\dot{V}_{O_2}$ is 3.4±0.6 ml O_2 min^{-1} kg^{-1}). These are similar to the data from the present study (Fig. 7) and suggest that these seals can lower their metabolic rate during longer dives and thus conserve oxygen stores and reduce the need for anaerobic metabolism in longer dives.

The data on metabolic rates can be used to calculate the aerobic dive limits for these animals. The term ‘aerobic dive limit’ can be defined in three ways, and it should be made clear which usage is appropriate for different circumstances. The measured ADL is the longest dive duration possible, before the measured lactate levels in the blood increase appreciably after the end of the dive (Kooyman *et al.* 1980, 1983). The estimated ADL uses measured values of $\dot{V}_{O_2}$, and estimates of oxygen stores, to calculate the maximum dive time before whole-body anaerobic metabolism is required; and the predicted ADL uses allometric scaling relationships to obtain a value for $\dot{V}_{O_2}$ (and estimates of oxygen stores), and thus to calculate the ADL. In this paper, since the metabolic rate (i.e. $\dot{V}_{O_2}$) has been measured, the aerobic dive limit calculated (as total available oxygen stores/rate of oxygen consumption) is called the estimated ADL.

The muscle oxygen stores for a 150 kg grey seal can be estimated as 3.26 l of O_2, assuming that the muscle mass is 30 % of total body mass (Lenfant *et al.* 1970; Lydersen *et al.* 1992), the myoglobin concentration is 5.4 g 100 g^{-1} wet muscle mass (J. Z. Reed, P. J. Butler and M. A. Fedak, in preparation), and the myoglobin combining capacity is 1.34 ml O_2 g^{-1} myoglobin). The blood oxygen stores can be estimated as 3.31 l of O_2, assuming a blood volume of 120 ml blood kg^{-1} (Castellini *et al.* 1985), a haemoglobin concentration of 18.3 g 100 ml^{-1} blood (this study), a haemoglobin combining capacity of 1.34 ml O_2 g^{-1} Hb, and assuming that, since these seals hyperventilate at the surface, the

venous and arterial blood will have similar levels of oxygen at the end of the surface breathing period and that the oxygen that could be available during the dive is from 95 % to 20 % saturation (Lenfant *et al.* 1970; Kooyman *et al.* 1983). The lung oxygen stores can be estimated as 0.92 l of O_2, assuming a TLC of 12.3 l for a 150 kg grey seal (Kooyman, 1989), a diving lung volume of approximately 50 % of TLC (Kooyman *et al.* 1971; Kooyman and Sinnett, 1982) and that the utilisable oxygen in the lungs is 15 % (Kooyman, 1973). This gives a total available oxygen store of 7.49 l of O_2.

At the overall mean metabolic rate of 5.2 ml O_2 min^{-1} kg^{-1} (Table 3), this would give an ADL of 9.6 min. This value relates well to the diving behaviour recorded from freely diving grey seals (Thompson and Fedak, 1993), where only 6 % of the dives exceeded 10 min duration. However, since the rate of oxygen consumption, calculated for the whole dive+surface period, decreases with increased dive time, then using the mean metabolic rate possibly gives an underestimate of the ADL. If the lowest mean value for $\dot{V}_{O_2}$ is used (3.5±0.26 ml O_2 min^{-1} kg^{-1}), this gives an estimated ADL of 14.3 min for a 150 kg seal. The basal metabolic rate (BMR) can be estimated, according to the allometric relationship BMR=$0.0101M_b^{0.75}$ (in l O_2 min^{-1}; Kleiber, 1975); for a 150 kg grey seal this would give a value of 430 ml O_2 min^{-1}, corresponding to an ADL of 17.3 min. These calculations assume that the all blood, muscle and lung oxygen stores are available to the seal. However, the breath-by-breath end-tidal data from this study indicate that not all these stores may be universally available within the seal.

Breath-by-breath measurements of end-tidal oxygen and carbon dioxide concentrations can be used to indicate the arterial O_2 and CO_2 levels in steady-state conditions, and to date there have been no data published on the variation of breath-by-breath end-tidal variables over a breathing bout from any pinniped. Kooyman *et al.* (1973) obtained end-expiratory O_2 and CO_2 measurements, using a syringe to sample the expiration of Weddell seals, and they found end-tidal P_{O_2} values ranging between 16.5 kPa (pre-dive) and 5.3 kPa (post-dives for dives lasting longer than 20 min). The end-tidal data from this study fall within this range. However, the data from the present study suggest that the partial pressures of arterial gases vary over the breathing bout, with the minimum end-tidal P_{O_2} occurring approximately 40 % into the surface period (see Fig. 6, Table 4). This U-shaped curve was unexpected. Intuitively, it would be expected that, after a breath-hold dive, the first breath would be lowest in oxygen, and then end-tidal P_{O_2} would increase monotonically throughout the surface period.

Experiments were carried out to assess whether this U shape could be due to dead space in the mask. The main objective was to determine whether the occurrence of relatively high initial values, preceding the minimum oxygen value, was a result of dead space. Hence, qualitatively, it is only required to investigate the presence of high initial values and lower subsequent values. These experiments demonstrated that, although there was some dead space mixing, the U shape is a 'real' response. The evidence that the U-shaped response is a physiological response, and is not an artefact of the sampling system, can be summarised as follows. (i) During the course of this study (1989–1992), dead space in the breathing box was reduced approximately fourfold, yet the same shape of curve persisted. (ii) The same shape of curve is seen over the range of tidal volumes in the four seals. (iii) The introduction of 12 % oxygen (or nitrogen or air) into the box for the first breath

had no effect on the following breaths, which still followed the same U shape as seen normally.

These experiments demonstrate that, although there was some dead space mixing in the respirometry mask (and therefore the end-tidal O_2 values quoted must be considered maximum values), the U-shaped response is a genuine animal response, as demonstrated by its invariant response over different mask volumes and different initial gas concentrations. These data can thus be interpreted as an indication of low levels of gas exchange between the lung air and the active tissues during submersion. This could be due either to a lack of gas exchange at the lung–capillary boundary or to low levels of perfusion of the active tissues.

Gas exchange at the lung–capillary interface could be reduced by shunting of the blood away from the diffusion surfaces. No anatomical lung shunts have been demonstrated, although there have been several detailed anatomical dissections of both phocids and otariids (e.g. Howell, 1930). Evidence of physiological lung shunts (caused by alveolar collapse) have only previously been related to deep diving (>50 m; Kooyman and Sinnett, 1982), and it is unlikely that this could be the cause in these shallow-diving laboratory seals. Maybe these seals are able to cause partial alveolar collapse by contracting the abdominal muscles and/or diaphragm and expelling air voluntarily from the alveoli into the lower airways (which are non-exchange surfaces; Denison and Kooyman, 1973).

The other, more likely, hypothesis is that the actively respiring tissues (such as the muscles) are being under-perfused during the dive, and that the blood oxygen stores are not being utilised by the tissues. Thus, there is little transfer of oxygen between blood and muscle during the dive; the muscles use oxygen stored in myoglobin, and these stores are depleted during the dive. On surfacing, therefore, the lung air oxygen levels are high. The normal muscle blood flow is restored, and the muscle oxygen stores are replenished, depleting the blood oxygen stores. Oxygen is taken up from lungs, and the end-tidal P_{O_2} decreases. Once tissues are reoxygenated, the end-tidal oxygen increases. The relatively low diving heart rates recorded in these seals suggest that there is a reduction in perfusion during diving. Heart rate recordings from freely diving wild seals show that there is usually a pre-surface tachycardia, as the seal is swimming to the surface, and this may allow mixing of the depleted muscle oxygen stores with the blood oxygen stores, to minimise the surface time; in this study, no pre-surface tachycardia was recorded.

Resting muscle metabolic rates have been estimated at approximately 1.5–2.0 ml O_2 min^{-1} kg^{-1} (McGilvery, 1975); if the muscle oxygen store is approximately 70 ml O_2 kg^{-1} (from 5.4 g myoglobin 100 g^{-1} muscle and 1.34 ml O_2 g^{-1} myoglobin), then this would last for 35 min, at resting muscle metabolic rate. Thus, the myoglobin stores would be adequate for muscle metabolism during long low-activity dives. However, if the lowest mean $\dot{V}_{O_2}$ of 3.5 ml O_2 min^{-1} kg^{-1} recorded in this study is used, the ADL of a 150 kg seal (using only the muscle stores) would be 6.2 min, but this includes the metabolism of of tissues other than the muscles, which would be reliant on the blood oxygen stores. Studies of wild seals have recorded periodic tachycardia during diving (Thompson and Fedak, 1993), and it is possible that these bursts of higher heart rates are to pulse blood through the muscles, to ‘top up’ the muscle oxygen stores during a dive.

To test these hypotheses, it would be necessary to take blood samples from captive grey seals diving freely in similar situations to those used in the present study, to measure the blood gas levels and to compare the level of blood oxygen with the lung oxygen levels seen on surfacing. Whatever the mechanism may be, that such extreme physiological responses occur in a grey seal during shallow voluntary dives poses questions about the 'normal' dive response in these animals and suggests that they may utilise profound oxygen-conserving adaptations as a routine response to diving in some circumstances. That this is the case is also demonstrated by the extremely low heart rates recorded from freely diving grey seals, during normal foraging (Thompson and Fedak, 1993). Thus, these seals have a suite of physiological responses and can adapt flexibly to various diving situations.

In conclusion, this study has demonstrated that grey seals have high rates of gas exchange (i.e. rapid respiratory frequency and large tidal volumes) at the surface. Also, these seals have low rates of oxygen consumption during a dive bout, which decreased with increasing dive duration. These combined adaptations would enable rapid oxygen loading at the surface and low rates of oxygen utilisation while diving, thus allowing a high proportion of the time at sea to be spent submerged. Most importantly, when these grey seals are breathing at the surface, the end-tidal P_{O_2} values follow a U-shaped response, which indicates that they are utilising relatively extreme cardiorespiratory responses, even during voluntary dives in shallow water.

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