Oxygen stores and aerobic dive limit of ringed seals (*Phoca hispida*)

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In this paper we measured total lung capacity, myoglobin content of muscle tissue, and hemoglobin content of the blood of ringed seals (*Phoca hispida*). Based on this information and body composition analysis we estimated the total available oxygen stores of a diving average adult ringed seal (standard length 129 cm, body mass 73.7 kg) to be 4.5 L. The aerobic dive limit for a ringed seal of this size was estimated to be 8.9 min. Diving data from previous studies show that less than 4% of the dives of adult free-living ringed seals exceed this aerobic dive limit. Based on information from the literature on maximum breathhold capacity and observed maximum dive times of ringed and Weddell seals (*Leptonychotes weddellii*), the maximum breathhold capacity of adult ringed seals was suggested to be 26.1 min.

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Nous avons mesuré la capacité totale des poumons, le contenu en myoglobine des tissus musculaires, et le contenu en hémoglobine du sang chez des Phoques annelés (*Phoca hispida*). Ces mesures et l'analyse de la composition corporelle nous ont permis d'estimer à 4,5 L les réserves globales d'oxygène disponible chez un phoque plongeur adulte moyen (longueur standard 129 cm, masse corporelle 73,7 kg). La durée limite d'une plongée aérobique chez un phoque de cette taille a été évaluée à 8,9 min. Des données sur la plongée obtenues au cours de travaux antérieurs démontrent que moins de 4% des plongées de phoques adultes en nature excèdent cette limite de plongée aérobique. D'après les données de la littérature sur la capacité maximale de rétention de la respiration et d'après les durées maximales de plongée observées chez des Phoques annelés et des Phoques de Weddell (*Leptonychotes weddellii*), nous avons évalué à 26,1 min la capacité maximale de rétention de la respiration chez des Phoques annelés adultes.

[Traduit par la rédaction]

Introduction

The optimum diving regime for any marine mammal is one that allows it to spend maximum time underwater. Such an optimal regime is achieved by performing short aerobic dives rather than long and partly anaerobic ones (Kooyman et al. 1980). When anaerobic metabolism supplements energy production, the length of surface time for full recovery increases considerably, mainly due to an increased lactic acid concentration, which alters the body's acid–base balance. The aerobic dive limit (ADL) is defined by Kooyman (1989) as the maximum breathhold time that is possible without any increase in the blood lactic acid concentration during or after the dive. This limit is dependent on available oxygen stores, oxygen consumption rates, degree of peripheral vasoconstriction, and rate of lactic acid production and consumption, of which the first two are the major variables. Kooyman et al. (1983) demonstrated a close correspondence between the ADL calculated from known body oxygen stores and estimated metabolic rates, and the ADL determined from the intercept of constructed lactate versus endurance curves.

In this paper we calculate the ADL for adult ringed seals on the basis of oxygen stores in the lungs, blood, and muscles. We compare this limit with observed diving times of free-living ringed seals (Lydersen 1991) to estimate how often the estimated ADL is exceeded during voluntary diving.

Materials and methods

In connection with a larger study, 134 ringed seals were shot in Kongfjorden, Svalbard (78°55′N, 12°30′E), from April to June 1985–1987. Standard length was measured to the nearest 0.01 m. All animals were bled completely and then weighed to the nearest 0.1 kg. The sculp was dissected from the core and then weighed to the nearest 0.1 kg. The skin was then carefully dissected from the blubber, and the skin was weighed to the nearest 0.01 kg. The viscera were removed and weighed to the nearest 0.01 kg (N = 102). Most of the muscle tissue was removed from the bones, and the skeleton (N = 36) were packed in wire mesh and stored in the sea for 6–12 months. Carnivorous crustaceans then cleaned the bones of all remains. The skeletons were then dried and weighed to the nearest 0.05 kg. By applying information from St. Aubin et al. (1978), who reported that ringed seals have 226 mL of blood per kilogram of lean body mass (= core + skin), and solving the following equation: blood mass = 0.226 (bled core mass + skin mass + blood volume × 1.07), where 1.07 is the specific gravity of blood, we were able to calculate the blood mass for each seal and thus estimate their original body masses.

Fifty ringed seals were shot in Kongsfjorden in April and May 1990. Standard measurements were taken in the field. The lungs and trachea were carefully dissected out and placed in a plastic barrel filled with seawater. Total lung capacity was measured as the displacement volume when the lungs are inflated to maximum extension. Immediately after shooting, blood samples (N = 50) were drawn from the epidural vein for hemoglobin (Hb) concentration measure-

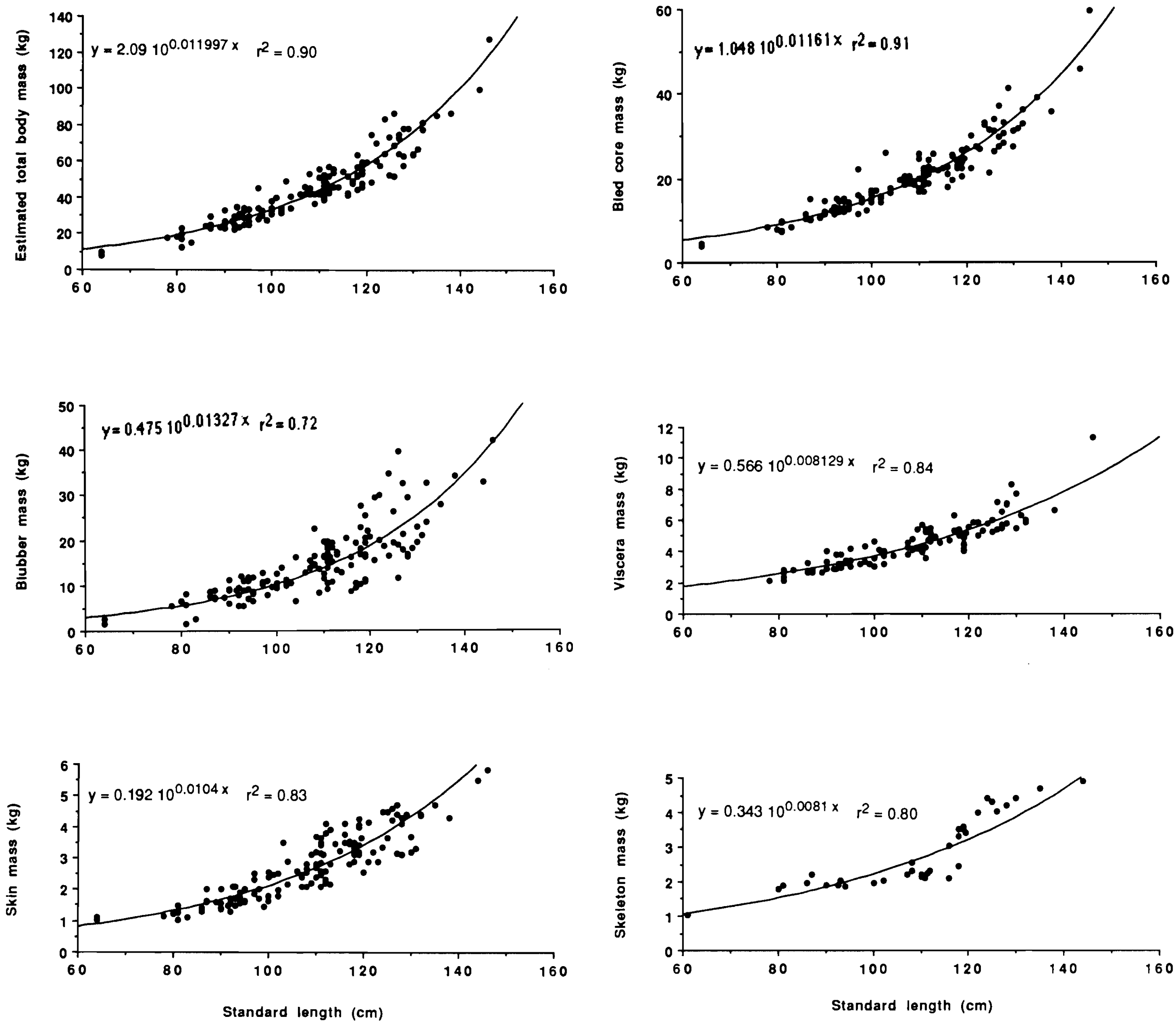

FIG. 1. Components measured or estimated during dissection of ringed seals from Svalbard.

ments. Blood samples were transferred to heparinized tubes and stored at −20°C. Hb concentrations were measured using the hemiglobin cyanide method (Dade, 3186 Düdingen, Switzerland). In this method, Hb and its derivates are oxidized by potassium ferricyanide into hemiglobin, which, in turn, is converted by potassium cyanide into stable hemiglobincyanide. The compound is measured photometrically, and the photometer is calibrated with a hemiglobincyanide standard. Muscle tissue samples were taken from Musculus deltoideus, M. biceps femoris, and M. longissimus dorsi from each seal for myoglobin (Mb) concentration measurements. The muscle samples ($N = 150$) were packed in aluminum foil and stored frozen. For the Mb concentration analysis, the thawed muscle samples were homogenized in 25 volumes of 80 mmol · L^{-1} KCl to mimic intracellular ion concentrations and in 50 mmol · L^{-1} Tris-HCl at pH 8.0 on ice to prevent denaturation of Mb by acid produced during glycolysis (O'Brien 1986); for this procedure a Tissuemizer at setting 65 was used 3 times for 5 s with 30-s rest periods intervening. Mb was analyzed based on the procedure described by Theorell and De Duve (1947) at alkaline pH (Chu et al. 1978). The homogenate was 77% saturated with $(NH_4)_2SO_4$ and left overnight to extract the Mb and precipitate the Hb. Samples were centrifuged at approximately 5000 × g for 10 min to clarify the suspension, and the supernatants were ultrafiltered through a Millipore filter with 0.45-μm pore diameter. Filtrates were diluted twofold with deionized water and oxidized with 100 μmol · L^{-1} $K_3Fe(CN)_6$ for 20 min. The absorption was then determined spectrophotometrically at 580 nm to avoid interference by cytochromes. Oxidized equine Mb was used as a standard and had an extinction coefficient of 1.80 g$\%^{-1}$ · cm^{-1}, which is very close to that of acid metmyoglobin (Morton 1975).

Results and discussion

Adult ringed seals in Svalbard have an asymptotic standard length of 129 cm (Lydersen and Gjertz 1987). Figure 1 shows that a seal of this length has an estimated total body mass of 73.7 kg, a bled core mass of 33.0 kg, a blubber mass of 24.5 kg, a skin mass of 4.2 kg, a visceral mass of 6.3 kg, and a skeletal mass of 3.8 kg. Blood mass, calculated as difference, is 12.0 kg, corresponding to 11.2 L.

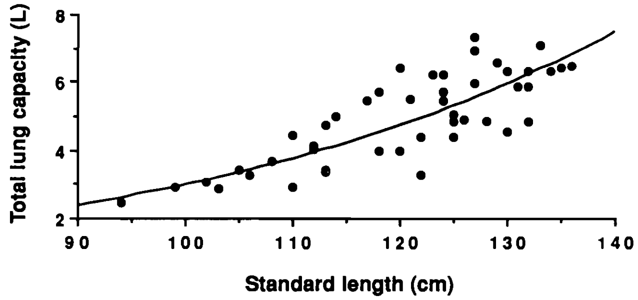

FIG. 2. Relationship between standard length and total lung capacity ($y = 0.3 \times 10^{0.01x}$, $r^2 = 0.70$) of ringed seals collected in Svalbard in spring 1990.

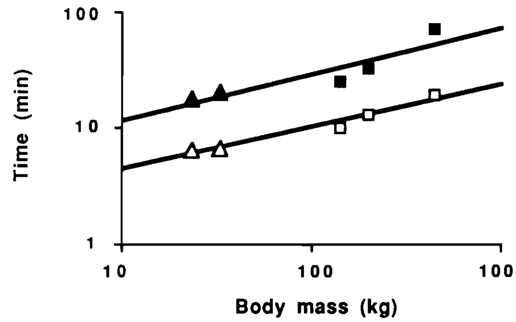

FIG. 3. Relationship between body mass and ADL (△ and □) ($y = 0.32x + 0.35$, $r^2 = 0.99$) and maximum registered breathhold (▲ and ■) ($y = 0.40x + 0.67$, $r^2 = 0.82$) for ringed (△ and ▲) and Weddell seals (□ and ■).

Oxygen stores in the muscles

The muscle mass in ringed seals was calculated as the difference between the bled core mass and the sum of the masses of all bled core components except muscle tissue (Fig. 1). The masses of claws, skin on flippers, eyes, brain, tendons, ligaments, and other tissues ("rest") were estimated to constitute 1.9% of the bled core mass. The muscle mass in an adult ringed seal, 129 cm, is thus estimated to be: 33.0 kg (bled core mass) − 3.8 kg (skeleton) − 6.3 kg (viscera) − 0.6 kg (rest) = 22.3 kg. This corresponds to 49.6% of the core mass and 30.3% of the total body mass. The mean Mb concentration of adult ringed seals was 4.1 ± 1.2 g (SD) per 100 g of muscle. There were no significant differences in Mb concentration between sexes. With an oxygen-combining capacity of 1.34 mL $O_2 \cdot g\ Mb^{-1}$, the adult ringed seal with 22.3 kg of muscle tissue will have a muscle O_2 store of 1.23 L.

Oxygen stores in the blood

The HB concentration was 249 ± 27 $g \cdot L^{-1}$, which is similar to the values reported earlier in ringed seals (Geraci and Smith 1975; Ferren and Elsner 1978; Engelhardt 1979). There were no significant differences in Hb concentration between sexes or age groups. For the calculations of available oxygen stores in the blood, we assumed that one-third of the total blood volume of 11.20 L (see above) is arterial and the rest venous, giving the adult ringed seal an arterial blood store of 3.73 L and a venous blood store of 7.47 L. With a Hb combining capacity of 1.34 mL $O_2 \cdot g\ Hb^{-1}$, this corresponds to 1245 mL O_2 in the arteries and 2492 mL O_2 in the veins. Assuming that the available O_2 stores in the arteries and veins are from 95 to 20% and from 90 to 15% saturation, respectively (Lenfant et al. 1970; Kooyman et al. 1983), available O_2 stores in the blood are 1245 (0.95−0.20) + 2492 (0.90−0.15) = 2803 mL O_2.

Oxygen stores in lungs

The mean total lung capacity (TLC) of the ringed seals was 5.04 ± 1.24 L (range 2.44−7.35 L). There was no significant difference in TLC between sexes. An adult ringed seal of 129 cm standard length had a TLC of 5.85 L (Fig. 2). This is 10.5% lower than the value predicted from the general equation $TLC = 0.10 \cdot M^{0.96}$, where M is body mass in kilograms (Kooyman 1989). The only available information on lung volumes of freely diving phocid seals comes from Weddell seals (*Leptonychotes weddellii*) (Kooyman et al. 1971); lung volume was shown to vary considerably, with an apparent relationship between length of dive and diving lung volume. An average diving volume of 56.9% of TLC was found, and transferring this value to the ringed seal gives a diving volume of 3.33 L. Only 15% of the lung volume is utilizable oxygen (Kooyman 1973), resulting in a total available O_2 store in the lungs of the adult ringed seal of 0.50 L.

Aerobic dive limit

The adult ringed seal with a body mass of 73.7 kg will have a total available O_2 store of 4.5 L. The ADL will depend on the diving metabolic rate of the seal. In the literature the average daily energy requirement is often set at 2 times the basal metabolic rate (BMR) (Lavigne et al. 1982; Stewart and Lavigne 1984; Feldkamp et al. 1989), where $BMR = 0.0101\ M^{0.75}$ L $O_2 \cdot min^{-1}$ and M is the body mass in kg (Kleiber 1975). This gives an ADL of 8.9 min for the ringed seal.

The only available information from the literature on the behaviour of freely diving ringed seals under natural conditions comes from Lydersen (1991). The adult seal had a standard length of 130 cm and should thus have had an ADL of 8.9 min. Of the 1321 monitored dives of this seal, only 4% exceeded 8 min. Elsner et al. (1989) registered 251 voluntary dives of a young ringed seal (30 kg) diving in a man-made pond. The ADL of a ringed seal of this size would, according to information from the present study, be 6.9 min, and 92% of the dives recorded by Elsner et al. (1989) lasted less than 6 min. These results show that most of the voluntary dives of ringed seals are shorter than the predicted ADL. Similar observations have been made on Weddell seals (Kooyman et al. 1980) and California sea lions (*Zalophus californianus*; Feldkamp et al. 1989), in which the ADL was exceeded in only 5 and 4% of the dives, respectively. The present results further document the fact that dives of free-living pinnipeds are primarily aerobic. Diving regimes of relatively short, aerobic dives result in a much longer overall time spent underwater compared with long anaerobic dives that may require long recovery at the surface (Kooyman et al. 1980).

For phocid seals with similar Mb and Hb concentrations and similar relative blood and muscle masses, it is reasonable to assume that ADL varies mainly as a function of body mass and diving metabolic rate. Data from ringed seals (this study) and Weddell seals (Kooyman et al. 1983) show that oxygen stores and body mass are related as O_2 (L) = 0.059 · M (kg) + 0.07 ($r^2 = 1$). Both ADL and maximum breathhold capacity (MBC) are also significantly correlated with body mass (Fig. 3). The slopes of the regressions of log ADL and log MBC to log body mass were not significantly different (ANCOVA, $F = 0.17$, df = 1,6). The MBC of the smallest ringed seal (23 kg, 17.85 min) was found by forced submersion to the point of an abrupt increase in heart rate or erratic cardiac activity (Ferren and Elsner 1978), and the MBC for the other ringed seal (32 kg, 20.7 min) was the longest of the 6854 registered dives of a captive seal (Parsons 1977). We calculated ADL for these 2 ringed seals on the basis of their body masses and information from this study. The ADLs for Weddell seals of 140, 200, and 450 kg are the values from the lactate endurance curves in the studies of Kooyman et al. (1980, 1983), and the MBC values are the longest observed dives for the same seals. Based on Fig. 3, an adult ringed seal should have an MBC of 26.1 min.

This study provides new information on ringed seal body composition and oxygen stores, enabling the ADL to be calculated for ringed seals of different sizes. It also demonstrates that the great majority of the dives performed by free-living ringed seals monitored so far are within this aerobic limit.

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