

ORIGINAL PAPER

J. M. Burns · M. A. Castellini

Physiological and behavioral determinants of the aerobic dive limit in Weddell seal (*Leptonychotes weddellii*) Pups

Accepted: 3 July 1996

Abstract The aerobic dive limit, as defined by an increase in plasma lactate levels following dives, has to date only been determined in adult and juvenile Weddell seals (*Leptonychotes weddellii*). However, theoretical aerobic dive limits based on calculated total body oxygen stores, estimated metabolic rates, and dive duration frequencies have been published for several species. Using data collected over the past 3 years in McMurdo Sound, Antarctica, the aerobic dive limit of Weddell seal pups was determined by both the physiological and modeling methods. Time-depth diving recorders deployed on 36 pups between 2 and 14 weeks of age allowed the aerobic dive limit to be predicted from duration–frequency histograms. The aerobic dive limit was also calculated from estimates of total body oxygen stores and predicted diving metabolic rates. Finally, these two estimates were compared with aerobic dive limits determined from post-dive lactate levels in three pups between 5 and 7 weeks old. The aerobic dive limit of pups increased with age, but pup aerobic dive limits were still significantly shorter than those of yearlings and adults. In addition, the aerobic dive limits determined by the three methods were not equivalent for pups, yearlings, or adults, and indicate that care should be taken when modeling methods are used to estimate the aerobic dive limit in other species. Changes in hematocrit, plasma glucose, and plasma lactate levels during and between rest, diving, and recovery in pups were compared to known values for juveniles and adults. Plasma metabolite levels were more highly regulated in older pups, and together with the increasing aerobic dive limit, suggest that Weddell seal pups are not refined divers until after they are weaned, and that their diving ability continues to develop over several years.

Key words Aerobic dive limit · Weddell seal · Diving physiology · Development · Lactate

Abbreviations *ADL* aerobic dive limit · *BMR* basal metabolic rate · *DMR* diving metabolic rate · *Hct* hematocrit · *MR* metabolic rate · *RMR* resting metabolic rate · *TBO* total body oxygen · *TDR* time-depth recorder

Introduction

The concept of the ADL, the longest dive duration following which plasma lactate levels are not elevated above resting, has been used to place dive behavior within a physiological context. Dives shorter than the ADL are often referred to as more efficient, as they do not require extended recovery periods at the surface (Kooyman et al. 1980; Castellini and Kooyman 1989; Castellini et al. 1992). Early studies on the diving behavior of Weddell seals (*Leptonychotes weddellii*) demonstrated that the ADL determined from post-dive lactate levels could also be predicted from the dive behavior itself, as generally less than 5% of adult and juvenile dives exceeded the measured ADL (Kooyman et al. 1980; Kooyman et al. 1983). In addition, the ADL could also be accurately estimated from calculations of the TBO₂ stores and the DMR. As a result of the close agreement in adult and juvenile Weddell seals between the ADL determined from post-dive lactate levels, free ranging dive data, and that calculated from TBO₂ stores and MR, these three techniques have often been thought of as interchangeable. However, only the measurement of post-dive lactate levels directly determines whether dives were aerobic or anaerobic.

Because determining post-dive plasma lactate levels in freely diving seals is difficult, (it has only been accomplished for Weddell seals diving in McMurdo Sound, Antarctica), many researchers have estimated the theoretical ADL for other species using one of the

J.M. Burns (✉) · M.A. Castellini
Institute of Marine Science, School of Fisheries
and Ocean Sciences, University of Alaska Fairbanks, Fairbanks,
AK 99775–7220, USA Tel.: +1–907/474–5243,
Fax: +1–907/474–7204,
e-mail: ftjmm@aurora.alaska.edu

alternative techniques. DMRs have only rarely been measured, and are known to vary with dive type and duration (Kooyman et al. 1973, 1980, 1983; Castellini et al. 1992; Ponganis et al. 1993). Yet, frequently, either DMRs of twice the BMR (Kleiber 1961) or RMR are used with estimated TBO_2 stores to calculate the ADL (Feldkamp et al. 1989; LeBoeuf et al. 1989; Hindell et al. 1992; Lydersen et al. 1992; Lydersen and Hammill 1993b; Lydersen and Kovacs 1993; Lydersen et al. 1994; Slip et al. 1994). The proportion of monitored dives exceeding this calculated ADL is often reported as an indicator of inefficiency or, if the proportion is large as unusual behavior (LeBoeuf et al. 1989; Hindell et al. 1992; Lydersen et al. 1992; Slip et al. 1994). Alternatively, the fact that less than 5% of the dives exceed the calculated ADL is sometimes taken as proof that the modeled ADL is correct, as had been shown for adult Weddell seals (Kooyman et al. 1980; Kooyman et al. 1983). Despite the widespread use of these estimation techniques, the validity of applying methods tested only on a few mature Weddell seals to seals of different ages or species has not been evaluated, even when large numbers of dives exceed the modeled ADL. In this study, the relationship between the three methods used to determine the ADL were examined, and the applicability of these techniques to young developing Weddell seal pups evaluated.

Material and methods

There were two primary components to this study: the study of diving physiology and the determination of free-ranging dive behavior. The methodology used in the experimental study of diving physiology follows that first described in Kooyman (1968) and later used by Kooyman et al. (1980, 1983), Qvist et al. (1986), Castellini et al. (1988), Zapol et al. (1989), and Ponganis et al. (1993). An isolated experimental dive site was created by drilling two 1 m diameter holes in the 3 m thick sea ice of McMurdo Sound in the Ross Sea, Antarctica (approximate location 166.25° E, 77.73° S). This site was sufficiently remote from any other holes or cracks in the ice such that seals released in the holes had to return to them to breathe. A hut with a trap door in the floor was set up over one hole for use as a field laboratory. The exterior hole was closed during experiments so that the seal was obligated to surface inside the laboratory, but was opened at the end of the trial to provide the seal with an exit to the sea ice.

Animal handling

Four known-age Weddell seal pups (37–50 days old) were selected from the colony at Big Razorback Island in McMurdo Sound, Antarctica (166.48° E, 77.684° S) for use in the isolated hole dive experiment. Pups older than 4 weeks were chosen because by this age pups were routinely seen diving and resting alone. Pups were captured by manual restraint, weighed with a hanging electronic scale (accuracy ± 0.1 kg), and transported by sled to the experimental dive site. At the dive site, pups were anesthetized with a mixture of ketamine (0.1 mg kg^{-1}) and diazepam ($0.25 \text{ ml } 100 \text{ mg}^{-1}$) given intramuscularly (i.m.). After Lidocaine (2 ml) was injected subdermally around the insertion site, two catheters were implanted approximately 10 cm apart in the intravertebral extradural vein and advanced approximately 50 cm. Once the catheters were in place, antibiotics (Keflin, 5 g and Gentaved, 2 ml) were ad-

ministered intravenously through a catheter. Each external catheter was approximately 1 m long, and ended in a float and three-way valve. Catheters were held in place with neoprene pads glued to the fur with a cyano-acrylate adhesive (Loctite 422). This design allowed blood to be sampled while the seal was at the surface of the water. These blood sampling methods have been used extensively in Weddell seals (Kooyman et al. 1980, 1983; Castellini et al. 1988). While under anesthesia, a TDR (Wildlife Computers Mark 5) was attached around a rear flipper with an anklet (Burns and Testa, in press). Upon full recovery from an anesthesia, the trapdoor in the laboratory was opened, the seal was free to enter the water, and the experiment started.

At the end of the experiment the laboratory trapdoor was closed and the outside hole was opened. The seal then emerged onto the ice surface where it was recaptured using manual restraint. The TDR and catheters were removed and the seal returned to the site of initial capture. Before release, a second injection of Gentaved (2 ml) was administered i.m. In all trials, the seal was in the water for no more than 14 h, and was returned to Big Razorback Island within 24 h of its initial capture. All pups handled prior to weaning were seen after the experiment with their mothers (identified by numbered flipper tags). One pup (22R) had been weaned prior to the experiment.

Blood collection and analysis

Blood was collected at the end of a dive by retrieving the floating catheter, clearing the line, and withdrawing a blood sample. The catheter was cleared of flush solution prior to blood collection by withdrawing more than 3 ml fluid (catheter volume was less than 2 ml). The blood sample was then collected directly into heparinized vacutainers (Becton-Dickenson), or into heparinized syringes and immediately transferred into heparinized vacutainers. This process took approximately 1 min, and all initial samples following dives were collected within 60 s of the seal surfacing. During long surface intervals blood was collected several times and the catheter flushed between each sample. When more than one sample was collected, the initial sample was considered the post-dive sample, and all subsequent samples considered recovery samples. The two main problems with repeatedly sampling blood through an external catheter in Antarctic waters were freezing and clotting. Both were prevented by flushing the catheter free of blood after each sample and filling the dead space prior to submergence with a 20% ETOH-saline solution to which heparin (1000 U l^{-1}) had been added. However, post-dive lactate levels were collected from only three of the four pups, as both catheters in the first pup (21R) froze soon after entry into the water. Samples from pups 75R, 24R, and 22R were collected at rest, after dives, and during dive recovery.

Blood samples were stored on ice prior to centrifugation and separation of the plasma. Hct was determined in duplicate using a battery-operated field micro-hematocrit centrifuge (Compur M1100, samples spun at 5400 g (11 500 rpm) for 3.5 min). The remaining blood was centrifuged and the plasma removed and frozen for later analysis. Samples were held in a liquid N_2 cooled CryoPac shipper (-196°C) while in the field, and then transferred and stored at -80°C in McMurdo prior to initial analysis for plasma lactate and glucose concentrations. Plasma glucose and lactate concentrations were determined within 1 week of collection using a YSI model 2300 STAT glucose/L-lactate autoanalyzer. The remaining samples were transported to University of Alaska Fairbanks and stored at -80°C .

Dive data

In the isolated hole dive experiments, once the seal was in the water and the experiment begun, the duration of all dives and surface events were recorded on a portable computer using a timing program written for QuattroPro. Time of sample collection was also recorded. Dive depth was determined once the TDR was recovered.

The TDR sampled depth at 1 s intervals with a resolution of 2 m, and dive depths were determined using analysis software (3M) provided by Wildlife Computers.

Free-ranging dive behavior

Data on free ranging dive behavior was collected in 1992 and 1993 from 36 pups between the ages of 2 and 14 weeks (19 males, 17 females) for a total of 119 records, and from 15 yearlings (10 males, 5 females). TDRs (Wildlife Computers Mark 5 or 6) were attached to a rear flipper with an anklet or glued dorsally to the fur (Devcon 5 min epoxy). TDRs were removed 3–10 days after deployment, and pups carried TDRs multiple times (Burns and Testa, in press). TDRs sampled data at 10 s intervals with a depth resolution of 2 or 3 m. Dives were decoded using programs provided by Wildlife Computers (ZOC, DA). Only dives which exceeded 30 s and 12 m were considered for further analysis in this report.

ADL determinations

The ADL was determined in three ways: from the post-dive lactate levels collected in the isolated hole experiment, from behavioral dive data gathered by TDRs, and from calculations based on published estimates of TBO₂ stores and DMR.

Physiological ADL

The physiological ADL was determined from post-dive plasma lactate samples collected during the isolated hole experiments. The ADL duration was estimated using two different methodologies. First, the duration of the longest dive following which plasma lactate levels did not exceed the highest resting value was set as the classical lactate ADL, as defined by published methods (Kooyman et al. 1980, 1983). Alternatively, the ADL was estimated using line-fitting statistical procedures designed to detect physiological thresholds. In this process, the initial post-dive lactate level was regressed against dive duration using an iterative process that determined the two best-fit linear regression lines which minimized the overall variance (Yeager and Ultsch 1989). Because the two

lines were not constrained to share a joint point, the threshold value was taken as the dive duration intermediate between the last point of the first line and the first point of the second line (Yeager and Ultsch 1989). This model was used to determine the threshold value for the raw data, as well as for log–log and semi-log transformed data (Beaver et al. 1985). In order to estimate the ADL of yearlings and adults statistically, post-dive plasma lactate concentrations were determined by digitizing published figures (Kooyman et al. 1980, 1983).

The appropriateness of the two-line threshold model versus a single line was assessed by comparing the significance of the regression equations and the resultant *r*² values and residual plots. In all cases, two lines were judged to fit the data better than a single regression line. The threshold value determined from these data sets is reported as the statistical estimate of the physiological ADL.

Behavioral ADL

Behavioral ADLs were determined from the TDR records of all free-ranging pups and yearlings, as well as from the dive records of the three pups in the isolated hole experiment. For each animal at each age, the dive duration that was exceeded by only 5% of the dives was determined. The mean value for all seals in each age group was then termed the behavioral ADL. The effect of mass, age, and sex on the behavioral ADL duration was assessed using stepwise linear regression procedures (STATSTIX), with significance assumed at *p* < 0.05.

Calculated ADL

The calculated ADLs were determined as ADL = TBO₂/DMR. TBO₂ stores were calculated based on mass specific values reported in the literature for blood volume, lung O₂ stores, and muscle mass (Lenfant et al. 1970; Kooyman et al. 1971; Kooyman 1975; Kooyman and Sinnett 1982; Fujise et al. 1985; Ponganis et al. 1993), and data collected in this project (Table 1). When possible, pup TBO₂ stores were estimated using values for subadult rather than adult Weddell seals (Fujise et al. 1985; Ponganis et al. 1993). As the DMR of pups has never been measured, it was approxi-

Table 1 Values used in the calculation of total body oxygen (TBO₂) stores for pup and yearling Weddell seals. Age categories are pup (P), yearling (Y), and adult (A)

Oxygen stores	Age	Content	Source
In Muscle			
% Body Mass	Y	30% muscle	Fujise et al. (1985)
Mb Content	Y	4.4 g per 100 g muscle	Ponganis et al. (1993)
O ₂ Content	A	1.34 ml O ₂ per g Mb	Kooyman and Sinnett (1982)
TOTAL		17.1 ml O ₂ per kg	
In Lung			
Diving Lung Volume	A	27.3 ml per kg	Kooyman et al. (1971)
O ₂ content	A	15%	Kooyman et al. (1971)
TOTAL		4.1 ml O ₂ per kg	
In Blood			
Plasma Volume	Y	7.8% body mass	Ponganis et al. (1993)
High HCT	P	58	This study
Blood Volume	P	18.5%	This study
Hb Content	Y	17.1 g Hb per 100 ml blood	Ponganis et al. (1993)
O ₂ Content	A	1.34 ml O ₂ per g Hb	Kooyman and Sinnet (1982)
Blood Distribution	A	33% Arterial, 66% Venous	Kooyman (1975)
Arterial Saturation	A	95 to 20%	Kooyman (1975); Lenfant et al. (1970)
Veinous Saturation	A	Arterial Content-5%	Kooyman (1975)
TOTAL		33.2 ml O ₂ per kg	
Total TBO₂ stores		55 ml O ₂ per kg	

Table 2 The age, sex, mass, sampling, and summary dive data for the four Weddell seal pups used in the isolated hole dive experiment in McMurdo Sound, Antarctica. Mean \pm standard deviation and (maximum) values are shown for dive depth and duration

Pup	Age (days)	Sex	Mass (kg)	No. of blood samples	No. of dives	Mean dive depth (m)	Mean dive duration (min)
21R	37	F	106	none	111	11 \pm 10 (60)	1.8 \pm 1.0 (4.3)
75R	41	F	137	25	95	14 \pm 15 (24)	2.2 \pm 1.3 (5.7)
24R	46	M	124	50	124	35 \pm 33 (123)	2.3 \pm 1.7 (7.4)
22R	50	F	107	55	126	14 \pm 15 (74)	2.5 \pm 2.3 (11.0)

mated in these calculations by the RMR of 4-week-old Weddell seal pups of similar mass measured by Elsner et al. (1977).

The calculated ADL of yearlings and adults was determined using published estimates of their TBO₂ stores and DMR (Kooyman et al. 1973; Castellini et al. 1992; Ponganis et al. 1993).

Diving metabolic rates

We used our estimates of the ADL (classical, statistical, and behavioral) to back-calculate the DMR of pups in this study according to the formula: $DMR = TBO_2 \text{ stores}/ADL$. These estimates were then compared to those from resting Weddell seal pups (Elsner et al. 1977) and pups of other species. All metabolic

rates are presented as $ml O_2 kg^{-0.75} min^{-1}$ due to the large range in body mass (Kleiber 1961).

Results

Isolated hole dive experiment

The age, sex, mass, and dive data for the four pups used in isolated hole experiments are reported in Table 2. All pups were successfully anesthetized and catheterized, and dove normally once in the water. Pups were unable to exit the water due to the distance between the surface of the water and the floor of the laboratory (1 m). Dive bouts lasted from 26 to 167 min, followed by periods of rest and sleep in the water ranging from a few minutes to more than an hour. This pattern of bout diving is similar to that seen in TDR records from free-ranging pup, juvenile, and adult Weddell seals (Castellini et al. 1992; Burns and Testa, in press). In comparison to free-ranging pups of the same age, pups in the isolated hole made dives of similar mean duration, but shallower mean depth (Burns and Testa, in press).

Blood samples were collected at rest and following dives made by three pups. From those samples Hct, plasma lactate, and plasma glucose concentrations were determined. Changes with dive duration in post-dive lactate levels are shown in Fig. 1, and post-dive Hct values in Fig. 2. Plasma glucose levels did not change significantly as dive duration increased, and averaged $7.5 \pm 0.4 \text{ mmol l}^{-1}$ for pup 75R, $8.2 \pm 1.1 \text{ mmol l}^{-1}$ for 24R, and $7.2 \pm 0.4 \text{ mmol l}^{-1}$ for 22R.

All three pups remained at the surface for extended periods after long dives which permitted multiple recovery blood samples to be drawn. During recovery from dives, Hct and plasma lactate levels declined steadily, while plasma glucose first increased and then decreased. Figure 3 illustrates an example of the pattern of changes in Hct, and plasma lactate and glucose concentrations seen during recovery after long dives. The long dives ($> 8 \text{ min}$) in Fig. 3 were made by pup 22R, and were characterized by an initial post-dive lactate level of greater than 3 mmol l^{-1} , and Hct of 54 or higher. Plasma lactate, glucose, and Hct declined to resting values (approximately 1 mmol l^{-1} , 7 mmol l^{-1} , and 45%) over a 20-min period. Recovery periods were shorter following shorter dives, or after dives with lower initial post-dive lactate and Hct levels. While neither pup 24R

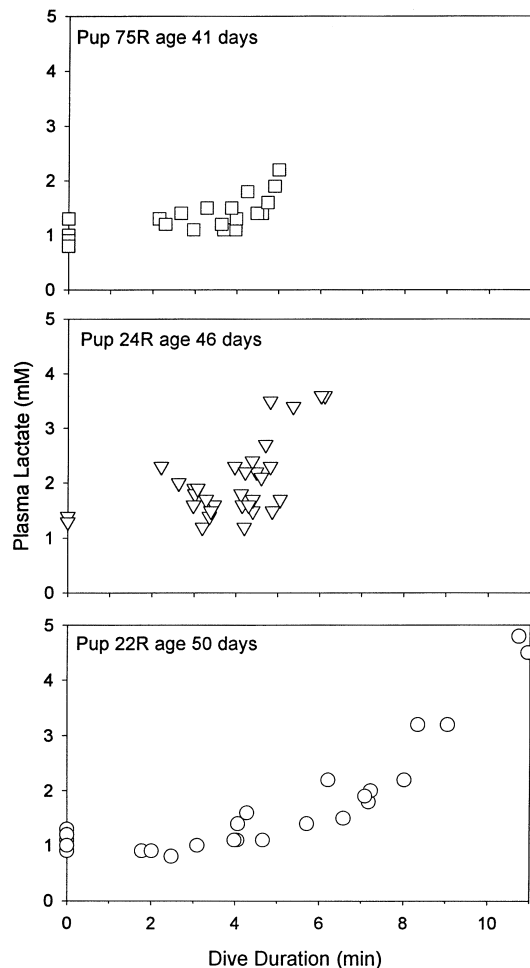


Fig. 1 Post-dive plasma lactate concentrations following dives made by the three Weddell seal pups in the isolated hole dive experiment in McMurdo Sound, Antarctica

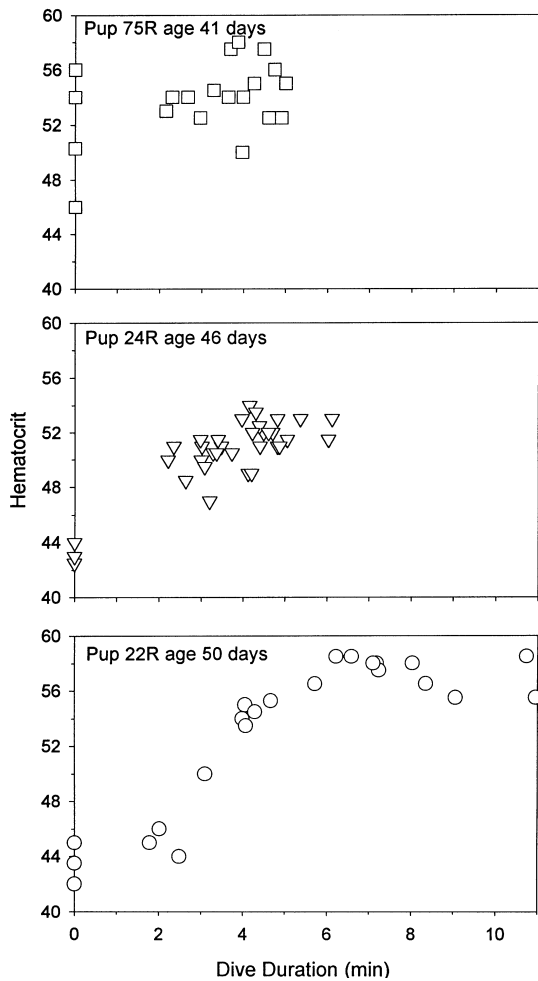


Fig. 2 Post-dive hematocrit levels following dives made by the three Weddell seal pups in the isolated hole dive experiment in McMurdo Sound, Antarctica

or 75R made dives as long as pup 22R, both showed similar recovery patterns following their longest dives.

ADL determinations

Physiological ADL

The classical ADL for each pup was determined from the longest dive made in the isolated hole experiment following which plasma lactate levels were not elevated above the highest resting values (1.3 mmol l^{-1} , for pup 75R, 1.4 mmol l^{-1} for 24R, and 1.3 mmol l^{-1} for 22R). The ADL values were approximately 4 min for pups 75R and 24R (238 s and 252 s, respectively), while 22R, the oldest pup, had a physiological ADL of approximately 5 min (280 s). The classic ADL for yearlings (10 min) and adults (22 min) was taken from the literature (Kooyman et al. 1980, 1983).

The ADLs estimated using the statistical method of regressing plasma lactate levels versus dive time were

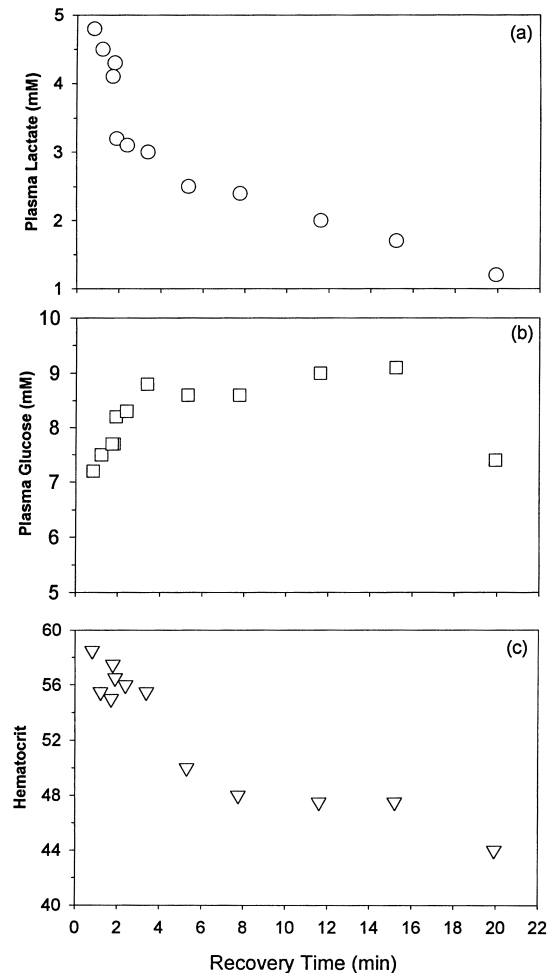


Fig. 3a–c Changes in (a) plasma lactate concentration, (b) plasma glucose concentration, and (c) hematocrit, during recovery at the surface following several dives longer than the measured aerobic dive limit. Dives were made by pup 22R

similar to those determined by the classical method (Table 3). While the different data transformations produced slightly different ADL estimates, no one method was clearly the best. This is evident in Fig. 4, which shows the physiological ADL determined by both classic and statistical methods for pup 22R. For all pups the mean value of the statistical estimates differed from the classical estimate by less than 1 min. For yearlings and adults the mean statistical ADL differed from the classical ADL by slightly more than a minute.

Behavioral ADL

The behavioral ADL, the dive duration which is only exceeded by 5% of dives (Kooyman et al. 1980), increased with age in both free-ranging and experimental pups (Fig. 5). The behavioral ADL determined for pups diving in the isolated hole was not significantly different from that of free-ranging pups of the same age. The

Table 3 The ADL (min) determined from post-dive lactate levels using the classical and statistical methods. The ADL estimated statistically is the midpoint between the two best-fit linear

regression lines used (Yeager and Ultsch 1989). Values for yearlings from Kooyman et al. (1983) and for adults from Kooyman et al. (1980)

Seal	Mass (kg)	Classic lactate ADL	Statistical ADL lactate vs. dive duration	Statistical ADL lactate vs. log(dive duration)	Statistical ADL log(lactate) vs. log(dive duration)
P75R	137	4.0	4.6	4.6	3.5
P24R	124	4.2	4.1	4.1	3.1
P22R	107	5.0	6.4	6.4	5.2
Yearlings	135	10	10.2	10.2	6.2
Adults	355 ± 59	22	25.7	25.7	19.8

behavioral ADL of yearlings (14.8 ± 3.4 min) was determined from the free-ranging dive behavior of yearlings in this study. The 26-min behavioral ADL of adults is an approximation from Kooyman et al. (1980).

To determine those factors responsible for the observed increase in the behavioral ADL of free-ranging pups, the ADL was regressed against animal age and mass (52.5–134 kg). As mass was not significantly correlated with pup age ($p = 0.47$), these two factors were treated as independent. Across the entire range (2–14 weeks), pup age accounted for approximately 57% of the variation in the behavioral ADL, while mass accounted for 4% ($p < 0.05$) (Fig. 5). However, as pups aged, the relative impact of mass and age shifted. Age accounted for 45% of the variation in behavioral ADL of pre-weaned pups (age to 7 weeks) while mass was not significant. In weaned pups (older than 7 weeks), age accounted for 18% and mass for 32% of the variation in the behavioral ADL. In yearlings, mass accounted for

82% of the observed variation in the behavioral ADL (Fig. 5). In all cases, larger and/or older seals had longer behavioral ADLs, and there was no difference by sex ($p > 0.05$).

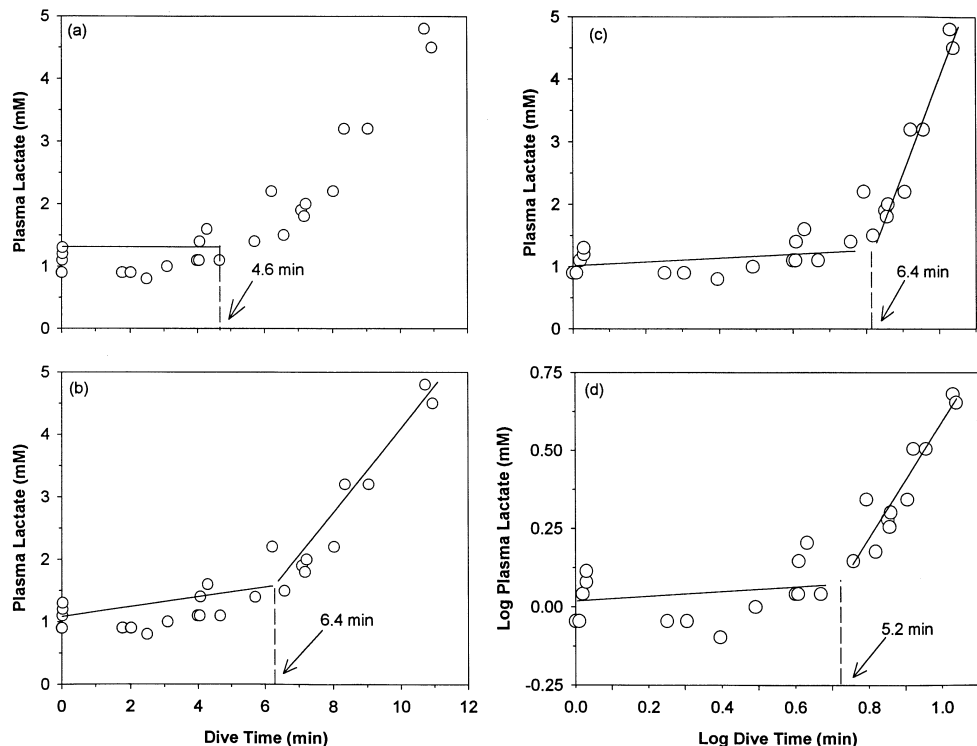
Calculated ADL

The calculated ADL for pups decreased with increasing age (and decreasing mass), from 7.5 min for the pup 75R, to 7.3 min for 24R, to 7 min for the oldest pup 22R. The calculated ADL of pups was much lower than that of yearlings (9.3 ± 0.4 min) or adults (18 ± 1.0 min).

ADL estimate comparison

Table 4 clearly shows that the modeling methods used to estimate the ADL produce values that are not equivalent

Fig. 4a–d The ADL determined from post dive plasma lactate levels in pup 22R using classical and statistical methods: **a** the ADL determined by the classical method. The *solid line* is the highest resting plasma lactate value; **b** statistical ADL, plasma lactate vs. dive duration; **c** statistical ADL, plasma lactate vs. log (dive duration); and **d** statistical ADL, log(plasma lactate) vs. log (dive duration). On all plots the *dashed line* indicates the ADL. The *solid lines* on **b**, **c**, and **d** are the best-fit linear regression lines. The statistical ADL was taken as the midpoint between the two lines



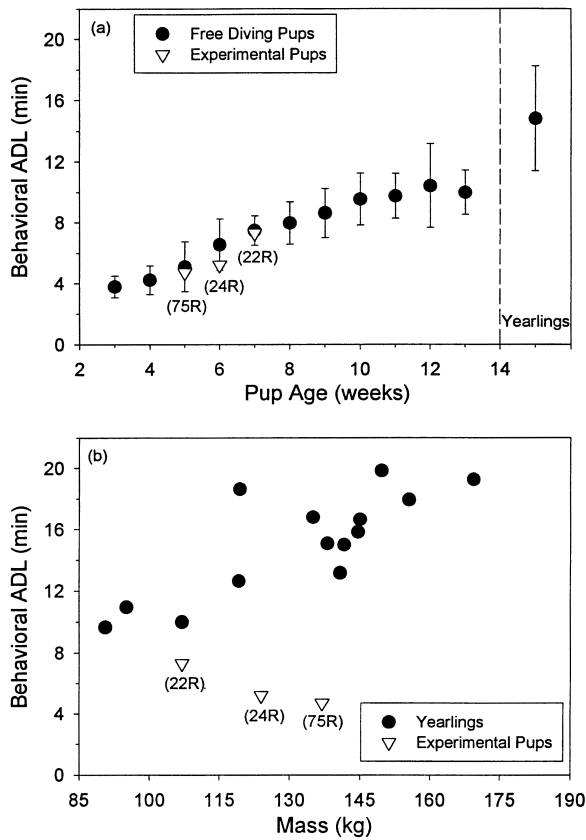


Fig. 5a The increase in the behavioral ADL with age determined from 38 free ranging pups, the 3 experimental pups, and 15 yearlings Weddell seals diving in McMurdo Sound. Mean and standard deviation shown for free ranging data. **b** The relationship between the behavioral ADL and mass in yearlings and the three experimental pups

to the ADL determined from post-dive lactate levels in pups, yearlings, or adults. In pups, the ADL determined from post-dive lactate levels using both classical and statistical methods was shorter than the ADL estimated from behavioral data or calculated from TBO₂ stores and MRs. However, while both the physiological and behavioral ADL increased as the pups aged, the calculated ADL was longer for the fatter, younger pups.

Table 4 A comparison of the ADL (min) determined for the Weddell seal pups in the isolated hole experiment from post dive lactate levels (classic method and statistical regression analysis of

While the magnitude of the differences between the ADL estimates will depend on the value accepted as ‘real’ it is important to note that for all animals (pups, yearlings, and adults) the behavioral ADL is markedly longer than that determined based on post-dive lactate levels.

Diving metabolic rate estimates

The DMR for pups estimated using their physiological and behavioral ADLs ranged from 25 to 59 ml O₂ kg^{-0.75} min⁻¹, and, in general, decreased with age (Table 5). When compared to MRs measured by respirometry for pups of other species, the values were 1–2 times higher than published rates, which ranged from a low of 10 to 20 ml O₂ kg^{-0.75} min⁻¹ for fasting northern elephant seal or harp seal pups swimming in a laboratory pool (Thorson & LeBoeuf 1994; Rea and Costa 1992; Worthy and Lavigne 1987) to a high of 25 ml O₂ kg^{-0.75} min⁻¹ fasting ringed seal pups and nursing Weddell seal pups on land (Smith et al. 1991; Elsner et al. 1977).

The DMRs calculated for yearlings and adults from their ADL and TBO₂ stores were similar to those measured by respirometry.

Discussion

Physiological development

Several studies have shown that as pups age there are shifts in metabolic and physiological processes associated with increasing dive capacity. As northern elephant seals (*Mirounga angustirostris*) age, the duration of spontaneous apneas increase, while apneic heart rates and MRs decrease (Castellini et al. 1986, 1994; Rea and Costa 1992; Thorson and LeBoeuf 1994). Older Weddell seal pups also appear to have greater cardiovascular control (personal observation). Along with an increased ability to regulate cardiorespiratory function, several studies have shown that older pups have lower resting

lactate vs. dive duration), behavioral dive data, and calculations using TBO₂ stores and MR. All values are mean ± standard deviation

Seal	Mass (kg)	Classic lactate ADL	Mean statistical lactate ADL	Behavioral ADL	Calculated ADL
P75R	137	4.0	4.3 ± 0.7	4.1	7.5
P24R	124	4.2	3.8 ± 0.6	5.2	7.3
P22R	107	5.0	6.0 ± 0.7	7.3	7.0
Yearlings	132 ± 23	10 ^b	8.9 ± 2.3	14.8 ± 3.4	9.3 ± 0.4
Adults	355 ± 59 ^a	22 ^a	23.7 ± 3.4	26 ^a	18.0 ± 10 ^c

^aKooyman et al. (1980)

^bKooyman et al. (1983)

^cPonganis et al. (1993)

Table 5 DMR (ml O₂ kg^{-0.75} min⁻¹) estimated from TBO₂ stores and the ADL determined from post-dive lactate levels, behavioral dive data, and balance equations. For comparison, the DMR

measured by respirometry by Elsner et al. 1977 (pups), Ponganis et al. 1993 (yearlings), and Castellini et al. 1992 (adults), are shown in the last column

Seal	Mass (kg)	TBO ₂ stores (ml O ₂)	Classic lactate DMR	Statistical lactate DMR	Behavioral DMR	DMR (D) or RMR (R) measured by respirometry
P75R	137	7535	47	45 ± 7	40	25 _(R)
P24R	124	6820	44	50 ± 8	35	25 _(R)
P22R	107	5885	35	30 ± 4	24	25 _(R)
Yearlings	132 ± 23	7260	19	22 ± 7	13	14 to 25(D)
Adults	355 ± 59	30609	17	16 ± 2	14	15 to 22(D)

and/or diving metabolic rates (Elsner et al. 1977; Rea and Costa 1992; Ponganis et al. 1993; Thorson and LeBoeuf 1994), and greater control over peripheral vasoconstriction (Cherepanova et al. 1993). In addition to declining rates of O₂ consumption, phocid post-weaning development is accompanied by the expansion of TBO₂ stores. Larger blood O₂ stores develop with age as a result of absolute increases in hemoglobin and Hct, as well as mass specific increases in blood volume (Ponganis et al. 1993; Thorson and LeBoeuf 1994; Rea 1995). Muscular stores of O₂ increase due to larger muscle mass and higher myoglobin content both in absolute and relative terms (Fujise et al. 1985; Cherepanova et al. 1993; Lydersen and Hammill 1993b; Thorson and LeBoeuf 1994). While changes in body composition and TBO₂ stores were not traced in this study, the measured post-dive lactate concentrations are an integration of both O₂ stores and utilization rates, and can be used as an indicator of physiological control.

The diving ability of Weddell seal pups, as measured by changes in average dive depth, duration, and frequency, has been shown to increase rapidly with age (Burns and Testa, in press). This study has indicated that the increase in dive ability with age is accompanied by an increase in the ability to regulate the metabolic processes associated with diving. In the two nursing pups (75R and 24R) almost all dives were followed by increased plasma lactate concentrations, and there was little evidence of Hct regulation. Dives of similar duration showed wide variation in measured Hct and plasma lactate values (see Figs. 1 and 2), which suggests poor control of diving physiology and/or large variation in diving O₂ stores and MR. However, the one weaned pup (22R) showed much greater regulation of both plasma lactate and Hct during dives and recovery. The changes in plasma metabolites and Hct seen following dives in this pup were similar in pattern to those seen following dives and during recovery in adult and subadult seals (Kooyman et al. 1980; Qvist et al. 1986; Castellini et al. 1988; Zapol et al. 1989). Long dives resulted in maximal Hct levels and high plasma lactate concentrations. During recovery from presumably anaerobic dives, pup 22R rested at the surface as Hct and plasma lactate levels decreased. Following the longest dives, complete physiological recovery to resting plasma lactate and Hct values took approximately 20 min (see Fig. 3), which is

comparable to reported recovery times for adults (Qvist et al. 1986; Zapol 1989). Similarly, for all pups and adults, shorter dives were followed by lower post-dive plasma lactate and Hct levels, and shorter recovery periods (Qvist et al. 1986; Zapol 1989; this study). These results suggest that the period around weaning may mark a threshold point in the development by pups of physiological control mechanisms similar to those employed by adults.

ADL development

The increase in dive capacity with age suggested by the blood metabolite data was also reflected by the changes in the ADL. While the scatter seen in post-dive lactate plots (Fig. 1) made it difficult to precisely determine the physiological ADL for these young animals, classical and statistical methods produced estimates that differed by less than a minute and indicate that there is little trouble identifying the inflection point in the lactate graphs. In addition, all methods suggested that the oldest pup had a longest ADL, and clearly identified the most important point: that all young pups have ADLs considerably shorter than juvenile or adult seals (Table 4). Moreover, the presence of statistically determinable threshold points for plasma lactate levels with respect to dive duration, supports the hypothesis that there is transition in the production or regulation of lactate during diving in pups similar to that in adults.

Changes in the behavioral ADL with pup age also indicated a rapid increase in dive ability. Prior to weaning, the behavioral ADL was most influenced by developmental state (age), and mass, which influenced the behavioral ADL of older animals, had little impact. It was only after pups were weaned and foraging on their own that mass accounted for more than a third of the variation in the behavioral ADL. In young bearded (*Erignathus barbatus*), ringed (*Phoca hispida*) and elephant (*Mirounga angustirostris*) seals age also had a stronger impact on diving behavior than did mass (Lydersen and Hammill 1993b; Lydersen et al. 1994; Thorson and LeBoeuf 1994). The decrease in the calculated ADL with increased pup age results from the reliance on mass in the equations used to predict TBO₂ stores and DMR, and is believed to be inaccurate.

Comparison of ADLs

While the three methods of determining the ADL of Weddell seals did differ in their values, the estimates were within a few minutes of each other (see Table 4). Absolute and proportionate differences between the ADL estimates are not given, because the 'true' ADL is not a fixed number and was not unequivocally determined. However, while both the physiological and behavioral estimates suggested that age played an important role in determining the ADL, the calculated ADL, estimated in the absence of information about age specific body composition, TBO₂ stores, or DMR, was determined exclusively by mass. Differences between ADLs in Table 4 indicate that both the duration of the estimated ADL, and the factors which influence it, can be affected by the method used, and suggests that an appropriate method for accurately estimating the ADL in the absence of post-dive lactate levels has yet to be determined. In the following discussion, the physiological ADL (without discriminating between the statistical or classical method) is accepted as the most accurate indicator of dive metabolism, and the modeling methods are evaluated by comparison.

For pups, the physiological ADL was the lowest of the three estimates. One possible explanation for the longer behavioral ADL is that pups diving in the isolated hole had elevated MRs due to stresses associated with handling, or with being at a non-familiar site over deep water. Higher MRs would have lowered the physiological ADL for pups in the experimental setting relative to those diving in the wild. However, as the behavioral ADL of pups diving in the isolated hole was not significantly different from pups of the same age diving in the wild, it is more likely that the use of 95% as a cut-off point for determining the behavioral ADL is not applicable to young, rapidly developing animals, perhaps for ecological reasons.

In McMurdo Sound, all age classes of Weddell seals are thought to forage on similar prey species which occur predominantly at depths greater than 200 m (Eastman 1985; Hubold 1985; Castellini et al. 1992; Burns and Testa; in press). Travel time on a foraging dive to 350 m is approximately 6 min, which is probably longer than the physiological ADL of newly weaned pups. In the absence of shallow-water prey, younger animals such as pups, or even yearlings, may be required to dive longer than their physiological ADL in order to maximize foraging efficiency (Ydenberg and Clark 1989; Houston and Carbone 1992; Fedak and Thompson 1993). As adult Weddell seals have a physiological ADL of approximately 20 min (Kooyman et al. 1980), most foraging dives probably occur within the ADL and 95% has been shown to be an appropriate delimiter for aerobic and anaerobic dives (Kooyman et al. 1980; 1983).

For pups, the ADL calculated from MR and TBO₂ stores was the highest of the three estimates (see Table 4). Inaccuracies in the calculated ADL probably result from difficulties in estimating both the TBO₂

stores and the DMR for young pups, two measures which vary with age and body composition. The modeled pup TBO₂ stores were derived from estimates of the separate component stores in Weddell seals of as close to the same age as the pups in this study as possible (see Table 1). However, mass-specific TBO₂ stores are known to be lower, relative to adult values, in northern elephant seal pups and yearling Weddell seals (Ponganis et al. 1993; Thorson and LeBoeuf 1994), and it is likely that our estimates of pup stores were high. It is also probable that using the RMR to approximate the DMR of pups resulted in an underestimation of DMR. Although DMRs are similar to RMRs in immature and adult Weddell seals (Castellini et al. 1992), pups do not appear to be as capable swimmers or divers (Kooyman 1968; Burns and Testa, in press; personal observation), and clearly do not have the physiological control of the older animals. In combination, the high TBO₂ stores and low DMRs produced calculated ADL estimates for pups that were too long and that decreased with age.

There were also differences between the physiological, behavioral, and calculated ADL estimates in yearling and adult Weddell seals. However, in contrast to the pups, the calculated ADL and physiological ADL were similar, and the behavioral ADL was the longest. The similarity between the calculated and physiological ADL is not surprising, as the DMR and many components of the TBO₂ stores were directly measured (Kooyman et al. 1983; Ponganis et al. 1993). However, the high behavioral ADLs relative to calculated values, especially in yearlings, suggests that even for older animals 95% may not always be an appropriate cut-off point. Long behavioral ADLs (relative to calculated estimates of the ADL) have previously been noted in pregnant northern and southern *Mirounga leonina* elephant seals, (LeBoeuf et al. 1988, 1989; Hindell et al. 1992; Slip et al. 1994) a period when adult physiology, MR, TBO₂ stores, and dive behavior are likely to be rapidly changing.

Overall, results suggest that differences between the ADL estimates are driven primarily by variations in activity patterns and/or TBO₂ stores and DMR that are themselves a result of age and/or physiological status. The long behavioral ADLs of both pups and yearlings suggest differences in dive patterns which may reflect foraging efficiency constraints, while the long calculated ADLs in rapidly developing pups reflect the difficulty of accurately estimating TBO₂ stores and DMR. As similar problems have been noted in other species (LeBoeuf et al. 1988, 1989; Feldkamp et al. 1989; Hindell et al. 1992; Lydersen et al. 1992; Slip et al. 1984), it is clear that care needs to be taken when the modeling methods are used to estimate the ADL or to interpret dive behavior.

Diving metabolic rate development

When the DMR of Weddell seal pups was calculated based on estimates of pup TBO₂ stores and the ADL determined from post-dive lactate levels and behavioral

data, the estimated values of 24 to 50 ml O₂ kg^{-0.75} min⁻¹ were only 2–4 times the BMR estimated using Kleiber's (1961) equation, and 1–2 times the resting value reported for young Weddell seal pups by Elsner et al. (1977). While these DMRs are higher relative to the RMR, than the DMRs measured for yearlings and adults (Castellini et al. 1992; Ponganis et al. 1993), they indicate that even very young Weddell seal pups have a high level of physiological control. The decline in the estimated DMR with age, from pre-weaned pup through yearlings to adults, probably reflects ongoing changes in physiological control, TBO₂ stores, and BMR associated with increases in age and body mass.

Unfortunately, it is impossible to compare the DMRs estimated for pups in this study to those of other species, for the DMR of pups in the wild has never been directly measured. The only approximation that exists is for ringed seal pups, but that estimate of 60 ml O₂ kg^{-0.75} min⁻¹ or 5.88 times BMR, was based on partitioning doubly-labeled water measurements of field MR according to the simultaneously measured time activity budget, and is highly speculative (Lydersen and Hammill 1993a). However, laboratory-based estimates of DMRs for fasting northern elephant seal pups are only slightly lower, and show a similar decline with increasing age (Thorson and LeBoeuf 1994). The bracketing of our DMR estimates by the only others available for comparison, suggests that using the ADL and TBO₂ stores to estimate DMR can produce reasonable results.

Conclusion

This study has demonstrated that it is possible to estimate the ADL in young, developing pups from post-dive lactate levels. The isolated dive hole protocol was used successfully with pups as young as 5 weeks, and in all cases pups dove naturally during the experiment and were soon returned to the colony. The blood samples and dive information collected make it clear that young pups are rapidly developing their diving skills and their physiological regulatory abilities, and this is reflected in the increase in the ADL with age. However, it is also evident that, regardless of the method used, the ADL of Weddell seal pups is still considerably shorter, and the DMR higher, than that of yearlings or adults. The comparison of the ADL determined from the post-dive lactate data to those estimated by modeling techniques reveals that, while the ADL estimates differed most in the youngest pups, they were not equivalent in yearling and adult Weddell seals either. Results suggest that the differences in the duration of the ADL and the factors which influence it, are due to age and/or condition related variation in dive physiology and ecology. The demonstration that the three methods currently used to estimate the ADL of marine mammals are not interchangeable, emphasizes the need to carefully consider the modeling technique used when producing or interpreting ADL estimates for other species.

Acknowledgements This work was funded by grant OPP-9119885 from the National Science Foundation (USA) to M. A. Castellini and J. W. Testa, and by a University of Alaska Fairbanks Chancellors Fellowship to J. M. Burns. Logistical Support was provided through the Office of Polar Programs at NSF, by Antarctic Support Associates, and the US Navy and Coast Guard. The following friends and colleagues gave help in the field and support in the lab: A. Ajmi, J. M. Castellini, B. Fadely, J. Fadely, E. Follmann, K. Hastings, P. Quang, L. Rea, B. Scotton, T. Zenteno-Savin, J. W. Testa, and K. Wynne. The research was carried out under permit # 801 of the Marine Mammal Protection Act. Experimental protocols for the studies were approved by the UAF Institutional Animal Care and Use Committee, and comply with the "Principles of animal care" publication No. 86–23 revised 1985 of the National Institute of Health, and the current laws of the United States of America.

References

- Beaver WL, Wasserman K, Whipp BJ (1985) Improved detection of lactate threshold during exercise using a log-log transformation. *J Appl Physiol* 59: 1936–1940
- Castellini MA, Costa DP, Huntley AC (1986) Hematocrit variation during sleep apnea in elephant seal pups. *Am J Physiol* 251: R429–R431
- Castellini MA, Kooyman GL (1989) Behavior of freely diving animals. *Undersea Biomed Res* 16: 355–362
- Castellini MA, Davis RW, Kooyman (1988) Blood chemistry regulation during repetitive diving in Weddell seals. *Physiol Zool* 61: 379–386
- Castellini MA, Kooyman GL, Ponganis PJ (1992) Metabolic rates of freely diving Weddell seals: correlations with oxygen stores, swim velocity and diving duration. *J Exp Biol* 165: 181–194
- Castellini MA, Rea LD, Sanders JL, Castellini JM, Zenteno-Savin T (1994) Developmental changes in cardiorespiratory patterns of sleep-associated apnea in northern elephant seals. *Am J Physiol* 267: R1294–R1301
- Cherepanova V, Neshumova T, Elsner R (1993) Muscle blood flow in diving mammals. *Comp Biochem Physiol* 106A: 1–6
- Eastman JT (1985) *Pleuragramma antarcticum* (Pisces, Nototheniidae) as food for other fishes in McMurdo Sound, Antarctica. *Polar Bio* 4: 155–160
- Elsner R, Hammond DM, Denison DM, Wybum R (1977) Temperature regulation in the newborn Weddell seal *Leptonychotes weddellii*. In: Llano GA (ed) Adaptations within Antarctic ecosystems. Smithsonian Institution, Washington, D. C. pp 531–540
- Fedak MA, Thompson D (1993) Behavioural and physiological options in diving seals. *Symp Zool Soc London* 66: 333–348
- Feldkamp SD, DeLong RL, Antonelis GA (1989) Foraging behavior of California sea lions, *Zalophus californianus*. *Can J Zool* 67: 872–883
- Fujise Y, Hidaka H, Tatsukawa R, Miyazaki N (1985) External measurements and organ weights of five Weddell seals (*Leptonychotes weddellii*) caught near Syowa Station. *Antarct Rec* 85: 96–101
- Hindell MA, Slip DJ, Burton HR, Bryden MM (1992) Physiological implications of continuous, prolonged, and deep dives of the southern elephant seal (*Mirounga leonina*). *Can J Zool* 70: 370–379
- Houston AL, Carbone C (1992) The optimal allocation of time over the dive cycle. *Behav Ecol* 3: 255–265
- Hubold G (1985) The early life history of the high Antarctic silverfish, *Pleuragramma antarcticum*. In: Siegfried WR et al. (eds) Antarctic nutrient cycles and food webs. Springer-Verlag, Berlin, pp 445–451
- Kleiber M (1961) *The fire of life*. Wiley, New York
- Kooyman GL (1968) An analysis of some behavioral and physiological characteristics related to diving in the Weddell seal. *Antarct Biol Ser* 11: 227–261

- Kooyman GL (1975) Physiology of freely diving Weddell seals. In: Ronald KW, Mansfield AW (eds) Rapp P. v. Reun Cons Int Explor Mer "Biology of the Seal", pp 441-444
- Kooyman GL, Sinnett EE (1982) Pulmonary shunts in harbor seals and sea lions during simulated dives to depth. *Physiol Zool* 55: 105-111
- Kooyman GL, Kerem DH, Campbell WB, Wright JJ (1971) Pulmonary function in freely diving Weddell seals, *Leptonychotes weddellii*. *Respir Physiol* 12: 271-282
- Kooyman GL, Kerem DH, Campbell WB, Wright JJ (1973) Pulmonary gas exchange in freely diving Weddell seals, *Leptonychotes weddellii*. *Respir Physiol* 17: 283-290
- Kooyman GL, Wahrenbrock EA, Castellini MA, Davis RW, Sinnett EE (1980) Aerobic and anaerobic metabolism during voluntary diving in Weddell seals: evidence of preferred pathways from blood chemistry and behavior. *J Comp Physiol* 138: 335-346
- Kooyman GL, Castellini MA, Davis RW, Maue RA (1983) Aerobic diving limits of immature Weddell seals. *J Comp Physiol* 151: 171-174
- LeBoeuf BJ, Costa DP, Huntley AC, Feldkamp SD (1988) Continuous, deep diving in female northern elephant seals, *Mirounga angustirostris*. *Can J Zool* 66: 446-458
- LeBoeuf BJ, Naito Y, Huntley AC, Asaga T (1989) Prolonged, continuous, deep diving by northern elephant seals. *Can J Zool* 67: 2514-2519
- Lenfant C, Johansen K, Torrance JD (1970) Gas transport and oxygen storage capacity in some pinnipeds and the sea otter. *Respir Physiol* 9: 277-286
- Lydersen C, Hammill MO (1993a) Activity, milk intake and energy consumption in free-living ringed seal (*Phoca hispida*) pups. *J Comp Physiol B* 163: 433-438
- Lydersen C, Hammill MO (1993b) Diving in ringed seal (*Phoca hispida*) pups during the nursing period. *Can J Zool* 71: 991-996
- Lydersen C, Kovacs KM (1993) Diving behavior of lactating harp seal, *Phoca groenlandica*, females from the Gulf of St. Lawrence, Canada. *Anim Behav* 46: 1213-1221
- Lydersen C, Ryg MA, Hammill MO, O'Brien J (1992) Oxygen stores and aerobic dive limit of ringed seals (*Phoca hispida*). *Can J Zool* 70: 458-461
- Lydersen C, Hammill MO, Kovacs KM (1994) Diving activity in nursing bearded seal (*Erignathus barbatus*) pups. *Can J Zool* 72: 96-103
- Burns JM, Testa JW (In press) Developmental changes and diurnal and seasonal influences on the diving behavior of Weddell seal (*Leptonychotes weddellii*) pups. In: Battaglia B et al. (eds) Antarctic communities. Cambridge University Press, Cambridge
- Ponganis PJ, Kooyman GL, Castellini MA (1993) Determinants of the aerobic dive limit of Weddell seals: analysis of diving metabolic rates, postdive end tidal PO₂s, and blood and muscle oxygen stores. *Physiol Zool* 66: 732-749
- Qvist J, Hill RD, Schneider RC, Falke KJ, Liggins GC, Guppy M, Elliot RL, Hochachka PW, Zapol WM (1986) Hemoglobin concentrations and blood gas tensions of free-diving Weddell seals. *J Appl Physiol* 61: 1560-1569
- Rea LD (1995) Prolonged fasting in pinnipeds. Thesis, University of Alaska Fairbanks
- Rea LD, Costa DP (1992) Changes in standard metabolism during long-term fasting in northern elephant seal pups (*Mirounga angustirostris*). *Physiol Zool* 65: 97-111
- Slip DJ, Hindell MA, Burton HR (1994) Diving behavior of southern elephant seals from Macquarie Island: an overview. In: Le Boeuf BJ, Laws RM (eds) Elephant seals: population ecology, behavior, and physiology. University of California Press, Berkeley, pp 253-270
- Smith TG, Hammill MO, Taugbol G (1991) A review of the developmental, behavioural and physiological adaptations of the ringed seal, *Phoca hispida*, to life in the Arctic winter. *Arctic* 44: 124-131
- Thorson PH, LeBoeuf BJ (1994) Developmental aspects of diving in northern elephant seal pups. In: LeBoeuf BJ, Laws RM (eds) Elephant seals: population ecology, behavior, and physiology. University of California Press, Berkeley, pp 271-289
- Worthy GAJ, Lavigne DM (1987) Mass loss, metabolic rate, and energy utilization by harp and gray seal pups during the post weaning fast. *Physiol Zool* 60: 352-364
- Ydenberg RC, Clark CW (1989) Aerobiosis and anaerobiosis during diving by western grebes: an optimal foraging approach. *J Theor Biol* 139: 437-449
- Yeager DP, Ultsch GR (1989) Physiological regulation and conformation: a BASIC program for the determination of critical points. *Physiol Zool* 62: 888-907
- Zapol WM, Hill RD, Qvist J, Falke K, Schneider RC, Liggins GC, Hochachka PW (1989) Arterial gas tensions and hemoglobin concentrations of the freely diving Weddell seal. *Undersea Biomed Res* 16: 363-373

Communicated by F. Heldmaier