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Standard metabolic rate at the surface and during trained submersions in adult California sea lions (*Zalophus californianus*)

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Summary

The metabolic rate (MR) of four adult California sea lions (*Zalophus californianus*), two males and two females, was quantified during trained submersion and stationing behavior in laboratory tanks. MR was measured, at rest and for single submersions of increasing duration (1–7 min), by measuring oxygen consumption using open-circuit, indirect calorimetry. Standard MR was measured under conditions defined for basal MR and was found to be 1.9 to 3 times that predicted for terrestrial animals of similar size. Submersion MRs were calculated from the post-submersion oxygen debt and declined to as little as 47 % of standard MR on the longest submersions. This hypometabolic response was proportional to the duration of submersion and was greatest for the maximum duration submersions. Short submersions produced MRs equivalent to measured standard MR. These data suggest that although California sea lions maintain an elevated metabolism under standard conditions, they are capable of reducing their metabolism in response to the needs of diving. Such metabolic flexibility enables sea lions to moderate their oxygen use during diving and to extend their aerobic diving capability.

Key words: hypometabolism, diving metabolic rate, standard metabolic rate, aerobic dive limit, California sea lion, *Zalophus californianus*

Introduction

Foraging marine mammals are constrained by the paradox of exercising while breath-holding (Castellini et al., 1985). These conflicting metabolic demands have inspired many investigations into the underlying physiological mechanisms that govern them. Unfortunately, a key variable in understanding diving physiology, metabolic rate (MR), has been difficult to measure. The limitations of measuring oxygen consumption in a breath-holding animal coupled with the aquatic lifestyle, large size and exotic nature of marine mammals have made precise MR measurements particularly difficult. Furthermore, MR is influenced by numerous confounding variables including age, size, digestive condition, thermal condition, activity level, reproductive state and experimental conditions. These constraints have resulted in data that are not always comparable and not collected using standardized criteria, which makes their analysis inherently difficult. Even an understanding of basal MR in these species continues to be controversial. Few studies (Lavigne et al., 1986) have provided comparable results based on variables established for the measurement of basal metabolism in terrestrial animals (Kleiber, 1975), and none of these studies was conducted on otariids (fur seals and sea lions).

These inherent obstacles to quantifying basal MRs in diving mammals are even more pronounced when determining the MR during diving, which is difficult to measure in the wild or to simulate in captivity. Nearly every study on dive metabolism has at least one and often a combination of confounding influences (e.g. age, reproductive status, digestive condition and others) that make comparisons problematic (Kooyman et al., 1973; Kooyman et al., 1980; Guppy et al., 1986; Castellini et al., 1992; Williams et al., 1991; Costa and Gentry, 1986; Costello and Whittlow, 1975; and others).

Early studies of diving metabolism consisted of ‘forced submersion’ experiments on restrained animals (Scholander, 1940; Andersen, 1959; Andersen, 1966; Pickwell, 1968) (note that we use the term ‘submersion’ instead of ‘dive’ to distinguish between shallow experimental submersions and field conditions where significant depths are obtained). These experiments provided the foundation for what became known as the ‘dive response’, consisting of bradycardia, widespread reduction in perfusion to the periphery, and the supplemental use of anaerobic energy production. The experiments also indicated a hypometabolic response to diving, demonstrated by a post-submersion rate of oxygen consumption that was insufficient to maintain a normal MR during submersion. It was suggested that this hypometabolic response extended available oxygen supplies, enabling an increased breath-hold duration (Scholander, 1940). However, these measurements

were carried out on physically restrained subjects and have not been reproduced using an unrestrained subject. Despite the reported reduction in MR upon submersion, this study was the first of many to report that basal MRs in marine mammals are higher than those of terrestrial animals of similar size. However, the fact that the experimental design involved the use of restraint leaves questions regarding the interpretation of these data unanswered.

Further evidence in support of hypometabolism during diving comes from reports of a reduced core body temperature in diving seals (Scholander et al., 1942; Kooyman et al., 1980; Hill et al., 1987; Qvist et al., 1986). Extremely cold water is a possible explanation for such a decline in body temperature, which is unusual for such a large mammalian homeotherm, but facultative hypometabolism is a more likely explanation, given the significant blubber layer of seals and the increase in insulation resulting from peripheral vasoconstriction during diving (Noren et al., 1999).

Work on the physiology of freely diving wild Weddell seals has significantly altered current perceptions of the 'normal' dive response and the associated mechanics of diving physiology. A broad range of physiological data can be collected on an unrestrained Weddell seal repetitively diving from the same ice hole (Kooyman and Campbell, 1972; Kooyman et al., 1973; Kooyman et al., 1980; Kooyman et al., 1983; Guppy et al., 1986; Hill, 1986). These studies provide a more moderate picture of diving energetics in Weddell seals, suggesting a variable use of the dive response. It appears that these animals are able to vary their MR and heart rate to appropriate levels for particular dives. In most cases, these animals dive aerobically and only rely on the maximum dive response when carrying out dives of extreme duration. These studies, while free of many restraint- or captivity-induced artifacts, are less able to control for the effects of activity, thermal condition or digestive condition. Nevertheless, they remain a central (perhaps, the primary) means of understanding the diving physiology of marine mammals. Equivalent research opportunities are not, however, available for other diving mammals. Such research is particularly needed for fur seals and sea lions as there are considerable differences between the diving abilities and behavior of phocids (true seals) and otariids (fur seals and sea lions) (Costa, 1992; Williams et al., 1991).

Given the difficulties of working with free-ranging animals in nature, trained animals can be used to provide the experimental rigour necessary to investigate discrete physiological variables without the stress artifact of forced submersion (Elsner, 1965). This study used trained animals to measure MR under standard conditions and as a function of submersion duration in an otariid, the California sea lion *Zalophus californianus*.

Materials and methods

Animals and facilities

Two adult male (M1 and M2) and two adult female (F1 and F2) California sea lions *Zalophus californianus* were trained to perform extended-surface and submerged stationing behavior in a 568,000 l three-tank complex at Long Marine Laboratory, University of California at Santa Cruz. The males were obtained as surplus animals from the Naval Ocean Systems Center in San Diego, CA, USA, 1 year prior to the onset of the study. The females were acquired as 2-year-olds following a previous rehabilitation period as stranded pups at Sea World of California in San Diego, after a short stay at the Scripps Institution of Oceanography. Experimental trials were conducted year-round in the deepest tank, which had a maximum depth of 3.1 m.

Training

Animals were trained using classical conditioning, operant conditioning and positive reinforcement techniques (Cover and Zeligs, 1991). For over a year prior to the onset of data collection, the animals were conditioned daily to station themselves in the metabolic chamber and at the bottom of the tank for increasing periods with increasing intervals between reinforcement. The extensive training promoted a cooperative relationship between the trainer and the animal and thus permitted the development of highly specific and controlled experimental protocols. The animals freely chose to cooperate with all data collection and were never restrained or confined during any experimental trials. To provide this freedom, the metabolic chamber contained a large (84 cm×69 cm) opening in the bottom, which was never blocked, allowing the animals to enter and exit the chamber at will or upon request.

Experimental apparatus

MRs were measured using open circuit indirect calorimetry following protocols similar to those of other marine mammal studies (Ashwell-Erickson and Elsner, 1981; Costa and Kooyman, 1982; Williams, 1987; Williams et al., 1991; Castellini et al., 1992). In all oxygen consumption trials, the subject was stationed in a metabolic chamber floating at the surface of the water. The chamber consisted of a Plexiglas dome (190 l air volume) resting on a submerged polyvinyl chloride (151 cm×84 cm×84 cm) cage. The cage provided an underwater platform and walls, which enabled the animal to maintain, with minimal effort, a stationary floating position by resting against the side of the cage. The dome was submerged approximately 5 cm under water to ensure an airtight seal. A wet/dry vacuum (model 17744, 2.0 peak HP, Sears Craftsman) continuously pulled ambient air through the dome, entering *via* a port and exiting the dome on the other side. Airflow was maintained at a constant rate of 60–70 l min^{-1} and was measured with a dry gas meter (model DTM-115, Singer, American Meter Division). A sub-sample of the main sample was continuously extracted using an internal system pump and was drawn through a series of columns. The first contained Drierite (Hammond Drierite Co., Xenia, OH, USA) to scrub the sample of water, the second contained Baralyme (Baralyme, St Louis, MO, USA) to remove CO_2 and a third contained further Drierite. The sample was then passed through

an oxygen analyzer (AMETEK S-3A, Pittsburgh, PA, USA) that measured the fractional oxygen content of the sample. The analyzer was previously calibrated with ambient air (20.94 % oxygen). The experimental values obtained (ranging at maximum approximately 1 % from ambient) were then logged by an MS-DOS-based laptop computer connected to the analyzer *via* an A/D converter (Remote Measurement Systems, model ADC1, Seattle, WA, USA). A Microsoft Basic program measured fractional oxygen content twice per second. Oxygen content was then averaged over a 10 s interval and logged to disk. The 10 s averages were converted to rates of oxygen consumption using equations 10 and 11 from Fedak et al. (Fedak et al., 1981). Prior to each experiment, the system was calibrated using the N_2 dilution technique described by Fedak et al. (Fedak et al., 1981). A respiratory quotient (RQ) of 0.71 was used for the calculations because the subjects had been fasted for 17–24 h at the onset of each experiment and were assumed therefore to be operating on a fat-based metabolism (Schmidt-Nielsen, 1975).

Animals had been previously trained to stand on a platform scale (model df2000, Duran 8000, Western Scale Co. Ltd., Vancouver, British Columbia, Canada) and mass was determined to within ±0.1 kg immediately prior to each experimental trial. Only one trial per day per subject was attempted.

Standard metabolic rate measurements

MR was measured following the conditions defined by Kleiber (Kleiber, 1975) for the measurement of basal metabolism. Since it is unclear whether these conditions result in an actual measurement of minimal (or basal) metabolic rate in this species, we chose to call the measurements standard metabolic rate (SMR) rather than basal metabolic rate (BMR). The standard conditions were as follows: (1) adult subjects were studied, (2) experimental studies were thermoneutral, (3) the animals were at a post-absorptive stage of digestion and (4) were quiescent (not sleeping) and at a steady state.

(1) Age. All subjects were adult animals (ranging in age from 5 to 14 years).

(2) Temperature. All experiments were conducted at water temperatures of at least 15 °C (maximum 20 °C), which is the reported lower critical temperature for California sea lions (Liao, 1990). Liao measured MR in three female sea lions (two adults) at 5 °C intervals from 5 °C to 35 °C. The temperatures were randomized to avoid acclimation. Each subject showed an increase in the rate of oxygen consumption below a lower critical temperature of 15 °C using multiple linear regression analysis. In our measurements of standard MR (conducted above 15 °C), we found no correlation between temperature and MR (r=–0.01, data from M1 and M2), further justifying this choice of thermoneutral range.

(3) Digestive state. Trials were conducted only after the animal had been fasted for a minimum of 17 h to ensure the post-absorptive condition. To maintain cooperation, the animals were occasionally fed small amounts of fish (approximately 0.2 kg capelin in total), usually towards the conclusion of the trial. MRs were closely monitored to ensure that there was no heat increment of feeding (HIF) effect during the trial. If this had occurred it would have manifested itself by an increase in MR. Although the HIF has never been measured in California sea lions, data on Steller sea lions *Eumatopias jubatus* suggest a prolonged increase in oxygen consumption that lasts between 6 and 10 h. In this species, the peak increase in oxygen consumption was 1.8–2.8 times control levels and occurred between 2.8 and 3.7 h after feeding 2 or 4 kg of fish (Rosen and Trites, 1997). This is consistent with measurements of HIF in sea otters and harbor seals (Ashwell-Erickson and Elsner, 1981; Costa and Kooyman, 1984). Given the small amount of food consumed by our sea lions and the fact that it was consumed towards the end of the metabolic measurements, we are confident that our MRs were unaffected by any measurable increase in MR due to the HIF.

(4) Activity level. During standard MR measurements, animals maintained stationary positions for up to 1 h under trained control. Although a longer trial duration may be preferable, the length of the trial duration was based on the cooperation of the subject so that errors associated with physical restraint could be avoided. Standard MR measurements consisted of a minimum of 15 min of stable oxygen consumption readings that exhibited a flat-line regression of oxygen consumption *versus* time (slope zero), indicating a steady state condition. There was no significant difference between trials lasting 15–25 min and those lasting 40–50 min (t=0.42, P=0.68, N=16), which indicates the steady state condition of the subject and validates the inclusion of the shorter duration measurements. Of the trials, 65 % lasted longer than 30 min. Standard MR determinations were used as baseline measurements for comparison with submersion MRs and as a control for the submersion experiments.

Submersion metabolic rate measurements

For these experiments, the subjects were maintained under standard conditions to avoid any metabolic increase that might alter the submersion MR. To establish that an animal was at standard MR prior to submersion, the subject was stationed in the metabolic chamber for 10–30 min until the baseline rate was within 15 % of the control standard MR. This was approximately equal to the maximum standard deviation from the individual standard MR measurements or the inherent variability of the individuals from day to day. Once a stable baseline had been achieved, the animal was commanded to dive directly to a sunken target at the bottom of the pool and remain there, stationary until recalled to the chamber. A year of training established distinct commands for each submergence duration and subjects were maintained at each duration for 1–2 weeks prior to the onset of data collection at that duration. At the completion of the submersion, the animal returned to the chamber and remained there for 10–40 min until the post-recovery MR had returned to within ±10 % of the baseline rate for that trial. (There was no significant

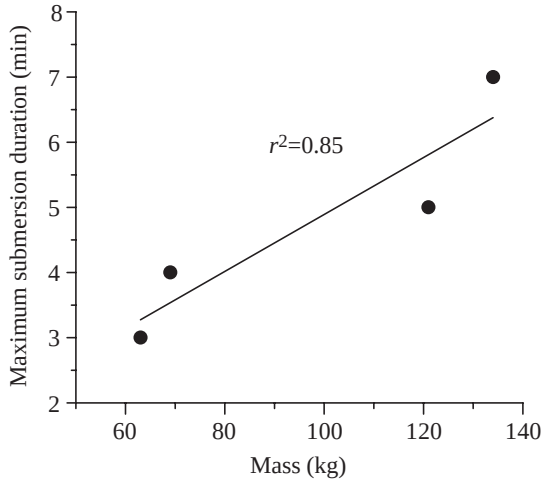

Fig. 1. Mean mass *versus* maximum submersion duration for all subjects (P=0.08). The solid line represents the least-squares linear relationship.

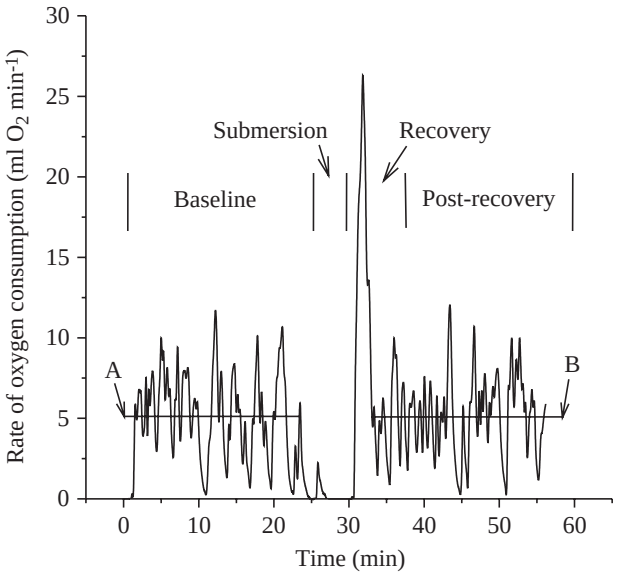

Fig. 2. Sample single-dive MR (rate of oxygen uptake) calculation using a 5 min dive trial with M1. Oxygen consumption was sampled every 10 s. Horizontal line A represents the baseline MR calculated from the area under the metabolic curve divided by the duration; A=5.12 ml O_2 min^{-1} kg^{-1}. Horizontal line B represents the post-recovery MR calculated from the area under the metabolic curve divided by the duration; B=5.09 ml O_2 min^{-1} kg^{-1}. The recovery interval contains all the post-dive O_2 debt, including 3 min of post-recovery MR, to ensure that all the O_2 debt has been included; recovery=46.18 ml O_2 kg^{-1} and covers a 4.67 min period. The average baseline rate is (A+B)/2=5.11 ml O_2 min^{-1} kg^{-1}. The portion of the recovery period MR which is accounted for by the baseline MR is 4.67 min×5.11 ml O_2 min^{-1} kg^{-1}=23.86 ml O_2 kg^{-1}. Therefore, the O_2 debt from the dive is 46.18 ml O_2 kg^{-1}–23.86 ml O_2 kg^{-1}= 22.32 ml O_2 kg^{-1}, and the dive MR is 22.32 ml O_2 kg^{-1}/5.0 min=4.46 ml O_2 $kg^{-1}$$min^{-1}$.

difference between submersion MRs calculated from trials with a maximum difference of 10 % and those where the difference was less than 2 % (t=1.01, P=0.31, N=81). We therefore chose to include data with a 10 % maximum differential between pre- and post-submergence metabolism to increase sample size.)

Single submersion MRs were measured in 1-minute increments until the maximum submersion duration (MSD) of the individual was achieved. Each subject had a unique MSD that correlated with size (or sex) (Fig. 1, r^2=0.85). The MSD is the point at which commands to go beyond that duration were met with a 100 % refusal rate.

Statistical analyses

Differences between means were tested for significance using a paired Student's *t*-test, while linear relationships were calculated by least-squares regression.

Standard MR was reported as a single average over the entire trial. Standard MR values were first regressed to ensure that the subject was at a stable, constant MR represented by a flat-line regression where the slope = 0.

Submersion MRs were estimated from the oxygen consumption in excess of the baseline rate during the recovery. This is equivalent to the submersion MR calculations reported by Scholander (Scholander, 1940) (Fig. 2). These measurements were computed from the total area under the curve of oxygen consumption *versus* time. A program was written in Quick Basic to calculate and sum the area described by any two sequential data points. Post-recovery MR was required to be within ±10 % of the baseline rate, and the two rates were then averaged, multiplied by the duration of the recovery and subtracted from the total oxygen consumption during the recovery. The difference was assumed to represent the oxygen debt from the submersion and was divided by submersion duration to estimate submersion MR.

Results

Standard metabolic rate measurements

Standard MR measurements for all subjects were higher than the predicted mass-specific MR for terrestrial animals of an equal size (Table 1). The mean standard MR for the males was 6.43±1.0 ml O_2 min^{-1} kg^{-1}, which was 2.1 times the predicted basal MR calculated for a terrestrial animal of equal size (Kleiber, 1975). The mean standard MR for the females was 10.23±0.31 ml O_2 min^{-1} kg^{-1}, which was 2.9 times higher than the predicted basal MR.

Submersion metabolic rate measurements

A significant reduction in MR with submersion was found for all subjects (P<0.05) (Fig. 3). The relationship between submersion duration and submersion metabolic rate (SBMR) was linear in all subjects. Individual data for males were indistinguishable and thus were pooled and followed the relationship:

$$\text{SBMR} = -0.65 \times \text{submersion duration} + 7.6, \quad (1)$$

(for males 1 and 2, N=10, r^2= 0.83, P=0.0002), where MR is in ml O_2 min^{-1} kg^{-1} and submersion duration in min.

Table 1. *A comparison between the mean standard metabolic rate measured for four adult* Zalophus californianus *and the predicted basal metabolic rates*

Animal	SMR ($ml\,O_2\,min^{-1}\,kg^{-1}$)	Predicted BMR ($ml\,O_2\,min^{-1}\,kg^{-1}$)	Factor by which SMR exceeds predicted BMR	Mass (kg)	Total measurement duration (min)	*N*
M1	5.72±0.62	3	1.9	134	404	11
M2	7.14±1.12	3	2.4	121	341	10
F1	10.45±1.68	3.5	3	69	175	5
F2	10.01±0.35	3.6	2.8	63	81.5	5
Males	6.43±1	3	2.1	128	745	21
Females	10.23±0.31	3.5	2.9	66	256.5	10

Predicted basal metabolic rates (BMRs) were estimated according to Kleiber, 1975.
Values for standard metabolic rate (SMR) are means ± S.D., *N*=number of trials for each subject.

The regression equations for the two females were:

$$\text{SBMR} = -1.7 \times \text{submersion duration} + 14, \quad (2)$$

(for female 1, N=17, r^2= 0.5 P=0.001) and

$$\text{SBMR} = -1.6 \times \text{submersion duration} + 10.5, \quad (3)$$

(for female 2, N=12, r^2= 0.45 P=0.02). The data for individual females were distinct and were not combined. For each individual, oxygen consumption values for the shortest submersions were not significantly different from standard MR values (P>0.05); however, oxygen consumption for maximum submersion duration decreased to 61 % of standard MR on average and 47 % of standard MR at maximum (for male 1).

Although not significant, because of a sample size of 4, there was a positive linear relationship between maximum submersion duration (MSD) and mass (Fig. 1). The equation that best described this relationship was:

$$\text{MSD} = 0.04 \times \text{mass} + 0.52, \quad (4)$$

(r^2= 0.85, P=0.08), where submersion duration is in min and mass is in kg.

The recovery time was established as the period required for the MR to return to within ±10 % of the baseline rate, marking the onset of the post-recovery period. In all subjects maximum recovery time corresponded with maximum submersion duration. Three of the subjects showed a strong correlation between recovery time and submersion duration (M2: r=0.98, P=0.02; F1 r=0.98, P=0.02; F2: r=0.83, P=0.37). The largest male, M1, did not show a strong correlation between submersion duration and recovery time (r=0.26, P=0.62). The average recovery time was 3 min for the males and 2 min for the females; however, the males were submerged for greater durations than the females (Fig. 3).

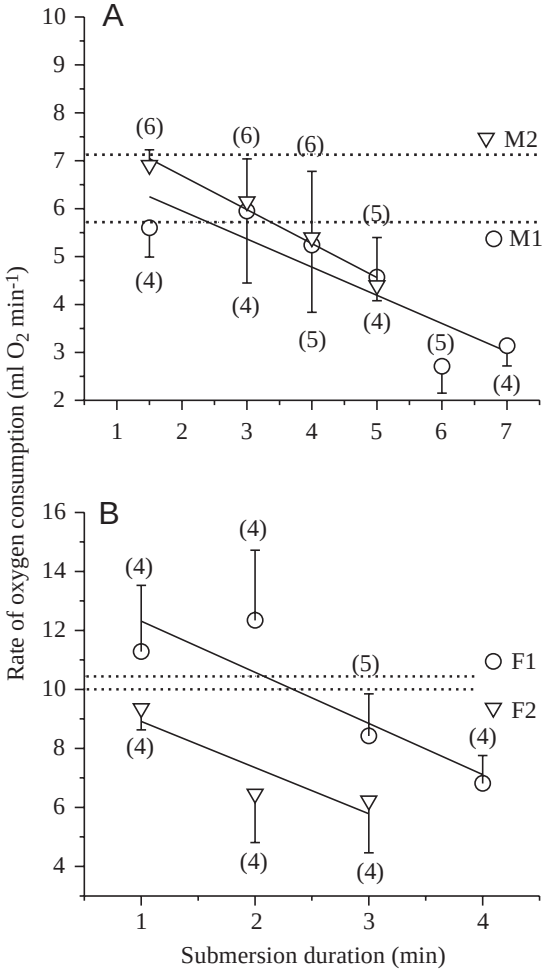

Fig. 3. Submersion MR *versus* dive duration for (A) males and (B) females. Solid lines represent least-squares linear relationships (M1: r^2=0.78, P=0.02; M2: r^2=0.96, P=0.01; F1: r^2=0.5, P=0.001; F2: r^2=0.45, P=0.02). Values are means ±1 S.D. The sample size is given in parentheses. Dotted horizontal lines represent standard MR for each animal for comparison.

Discussion

Relationship to free-ranging animals

An inherent weakness in any laboratory study is its applicability to wild animals. In this study, dive depth and

transport costs were well below those that would be experienced in nature. In addition, captive sea lions may lack the physical conditioning of free-ranging subjects, which could reduce their diving capability. In attempting to assess the potential implications for free-ranging animals, we therefore restrict the discussion to general processes and trends. However, a laboratory setting did allow for a precisely controlled examination of the metabolic responses to submersion that would otherwise have been impossible.

'Basal metabolic rate'

Our results indicate that California sea lions maintain elevated MRs under standard conditions ranging from 1.9 to 3 times the value predicted for terrestrial animals of equal size (Kleiber, 1975). These measurements conform to standardized criteria and are consistent with MRs reported for other otariids, which show a clear trend of MRs greater than predicted and greater than those measured in phocid seals (Matsuura and Whittow, 1973; Costello and Whittow, 1975; Liao, 1990; Costa and Gentry, 1986; Costa et al., 1989a; Costa et al., 1989b). Some studies reported increases of only 1.2–1.75 times the predicted values (Feldkamp, 1987; Ponganis et al., 1991). To our knowledge, ours are the first measurements of the MR of an otariid that rigorously adhered to conditions required to measure basal MR. Previous studies were conducted with juveniles (Matsuura and Whittow, 1973; Costello and Whittow, 1975; Feldkamp, 1987; Ponganis et al., 1991) or were confounded by reproductive status or activity (Costa and Gentry, 1986; Costa et al., 1989a; Costa et al., 1989b). Nevertheless, two of these studies reported resting MR values generally lower than those measured here. We suspect that these data may have been confounded by including measurements taken during apnea or sleep. Neither of the investigations attempted to record basal MR and instead reported values as resting MR.

Some of these previous studies 'selected' data, representing the lowest 10 min averages (Feldkamp, 1987). This 'selective' method of measuring resting MR does not ensure that the animal was in a steady state and may be confounded by apnea or other cyclic metabolic variation. Our measurements manifested a pronounced cyclic variation in rates of oxygen consumption (Fig. 4). We found 15 min to be the minimum period necessary to ensure that the animals were in a steady state, and we regressed the data to ensure we would not selectively choose a period of decreased respiratory rate that was frequently followed by a complementary rate increase.

The subjects of the present study were adult, thermoneutral, post-absorptive, awake, not moving and in a steady state. Taylor et al. (Taylor et al., 1987) found that standing MRs for several species of terrestrial animals were 1.5–2 times the predicted basal MR. They concluded, on the basis of associated heart rates, that these animals exhibited an elevated MR because of the anticipation and excitement inherent in the study conditions. Presumably, this elevated MR could also be related to the effect of maintaining a standing position. As our animals were either resting on the bottom of the cage or floating, there should be no metabolic increase due to posture maintenance. Furthermore, they had extensive desensitization to the experimental apparatus (over 1 year). Separate heart rate measurements, using the same experimental protocols as in this study, yielded heart rates for males (70±15 beats min^{-1}; mean ± S.D., N=134, range 34–128 beats min^{-1}) and females (86±9.6 beats min^{-1}; mean ± S.D., N=85, range 39–143 beats min^{-1}) (Hurley, 1996) that were within the range reported for this species measured during nocturnal haul-out periods on land (80±17.4 beats min^{-1}; mean ± S.D., N=22, range 55–130 beats min^{-1}) (Ponganis et al., 1997). We are therefore confident in our MR measurements.

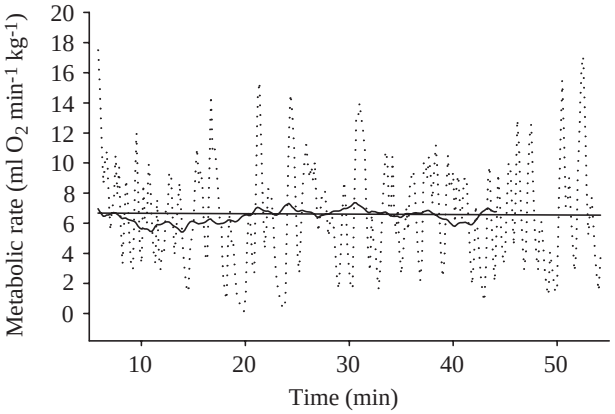

Fig. 4. An example of how the measurement period affects variation in oxygen consumption measurements in sea lions. The results of three analytical methods are shown. The dotted line shows the variability when a 10-second average is used, the bold line depicts a 10-minute running average and the flat line represents the regression of the entire data set (r^2=0.0001, P=0.84).

Submersion metabolic rate

Regardless of the argument regarding whether or not sea lions fall on the terrestrial mammalian curve for basal MR, our results indicate that aerobic MR, represented as the oxygen depletion rate, decreases with increasing breath-hold duration in sea lions. This is further reinforced by considering that our estimates are potentially greater than the actual submersion MRs because of the inclusion of post-submersion organ-specific hypermetabolism, which may account for some of the oxygen debt.

This picture of submersion or diving metabolism is consistent with data on the heart rate and renal blood flow measured with trained submergence and diving in sea lions (Elsner, 1965; Stone et al., 1973; Ponganis et al., 1997). Heart rate measurements using the same experimental protocols and subjects as those presented here (Hurley, 1996) revealed a pronounced bradycardia (35–19 % of surface resting values) that increased linearly with submersion duration. Heart rate recovery periods correlated with the time required for MRs to return to baseline levels (r=0.86, N=6, P=0.03). Ponganis et al. (Ponganis et al., 1997) reported a similar bradycardia during stationary submersion, submerged swimming and trained

diving (at sea) for juvenile California sea lions. Bradycardia and the associated widespread hypoperfusion are believed to be accompanied by reduced cardiac and renal rates of oxygen consumption (Sinnett et al., 1978; Davis et al., 1983; Zapol et al., 1979; Hochachka and Guppy, 1987). Whether the decrease in MR measured in this study was a result of a decrease in organ function as perfusion decreased or was an effect of hypoxia is uncertain. We conclude that the use of aerobic metabolism in these animals is highly flexible and apparently moderated to meet alternating surface and submerged conditions (or diving).

Several other studies have also reported a correlation between MR and submersion duration. Gallivan (Gallivan, 1981) noted that harp seals confined to a metabolic chamber showed decreasing MR with increasing submersion duration. Thorson (Thorson, 1993) reported the identical observation for elephant seals. Similarly, Webb et al. (Webb et al., 1998) found that the mean rate of oxygen consumption during submersion decreased by 26 % from resting values for elephant seals confined to a metabolic chamber. Castellini et al. (Castellini et al., 1992) measured diving MRs in wild, freely diving Weddell seals across a representative range of dive durations and found that the MR of diving declined with increasing dive duration.

Depending on the acceptance of our standard MRs as truly representative of basal rates, the submersion MRs can be seen as hypometabolic or merely appropriate conditions for the manifestation of basal MR. The results of this study are consistent with the only previous measurement of hypometabolism in marine mammals, which was reported by Scholander (Scholander, 1940). During 'forced dive' experiments with grey (*Halichoerus grypus*) and hooded (*Cystophora cristata*) seals, Scholander found a 60–70 % reduction in MR, which is comparable with the 47–65 % reduction found in the present study. Scholander concluded that the measured discrepancy in the rate of oxygen consumption could not be accounted for by supplemental anaerobic metabolism: both the measured lactic acid accumulated in the blood and the CO_2 levels (indicating respiratory quotient) were insufficient to compensate for the discrepancy.

Aerobic dive limit and anaerobic metabolism

The possible contribution of anaerobic metabolism to the overall submersion MR cannot be addressed by this study since our attempts to measure blood lactate levels failed. However, we do not believe that anaerobic metabolism contributed significantly to the energetic balance during submersion.

Ponganis et al. (Ponganis et al., 1997) reported the only measurement to date of the aerobic dive limit (ADL) for an otariid. They used training protocols comparable with the ones in the present study, and found that the point of increase in blood lactate over resting levels (ADL) was the same point at which the animals refused to make further increases in their dive duration. We have no reason to believe that our subjects would be any more willing to engage in significant anaerobic metabolism. In Weddell seals, the only other species for which aerobic dive limits have been measured, the evidence suggests that the animals are generally unwilling to exceed their aerobic limit except under extreme circumstances and do so in the wild less than 5 % of the time (Kooyman et al., 1980).

Table 2. *A comparison of the estimated maximum aerobic limits of* Zalophus californianus *with the maximum submersion duration*

Animal	MSD (min)	Estimated MAL (min)
Male 1	7	7.0
Male 2	5.5	5.6
Female 1	4	3.8
Female 2	3.5	3.8

MSD, maximum submersion duration; MAL, maximum aerobic limits.

Values for MAL were estimated using measured standard metabolic rates and average oxygen storage capacity ($40\,ml\,O_2\,kg^{-1}$) (Ponganis et al., 1997).

For the sake of argument, we calculated the maximum aerobic limit (MAL) of the subjects in our study on the basis of our measured standard MRs and the oxygen storage measurements from Ponganis et al. (Ponganis et al., 1997) and these are shown in Table 2. We are calling these estimates of the maximum aerobic limit since our animals were stationary during breath hold, whereas ADL calculations are based on a more active metabolic condition (i.e. swimming and diving). These MALs must be considered only rough estimates since the oxygen storage values were measured on smaller, juvenile animals. Nevertheless, these estimates of MAL are consistent with the maximum submersion duration measured in our sea lions. This further supports our hypothesis that anaerobic metabolism was minimal during these submergences.

Finally, lactate processing would presumably have manifested itself somewhat in the post-submersion MR, elevating the post-submersion rate of oxygen consumption beyond levels that would indicate hypometabolism. It is likely, however, that if lactate were produced during the dive, it may itself have been used to some extent as a substrate for metabolism (Murphy et al., 1980). This would not produce a resulting oxygen debt, which would result in an error in the estimate of the respiratory quotient (RQ).

Error in the estimate of the respiratory quotient

When the difference in fractional content of oxygen in the air entering *versus* the air exiting represents a deflection of not more than 1 %, there will be an error of 0.1 % for each 0.1 RQ unit difference between estimated and actual measurements (Fedak et al., 1981). As our animals were post-absorptive, we assumed a fat-based metabolism with an RQ of 0.71. In the unlikely event that the animals completely utilized anaerobic metabolism during a dive the RQ would be 1.0 (carbohydrate) and this would result in a 29 % overestimate of MR. The use of anaerobic metabolic products would presumably not occur

during the resting periods (when oxygen is readily available), but would contribute during the post-submersion recovery. Therefore the standard MRs should be unaffected by this potential error, but the submersion MR estimates could be as much as 29 % lower than estimated. Given the already low submersion MRs, we conclude that a substantial error of this kind is unlikely to have occurred. We believe that this further suggests that the animals were unlikely to have been utilizing any substantial amount of anaerobic metabolism.

An alternative explanation for our results would be a protracted, slight increase in MR during the post-submersion surface interval, which would ultimately repay the remaining oxygen debt. This postulated increase would be within the error in the system and, therefore, undetectable. The fact that the baseline MR was greater than the post-recovery rate in 58 % of the trials, tends to refute this possibility. In addition, there was no significant difference between submersion MRs calculated from trials where baseline MR exceeded post-recovery MR and those where post-recovery MR exceeded baseline MR (t=1.16, P=0.45, N=81). Therefore, we believe such an error is unlikely.

From this study we conclude that California sea lions (and possibly other otariids) exhibit elevated MRs when measurements are carried out using standardized criteria. In addition, they exhibit an ability to control their MRs, thus allowing adjustments in metabolism that are appropriate for extending their maximum aerobic dive capability. Such a hypometabolic response during submergence is consistent with the classic study by Scholander (Scholander, 1940) and, to our knowledge, is the first demonstration of such a response in an unrestrained subject.

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