

Water conservation in fasting northern elephant seals (*Mirounga angustirostris*)

Christopher W. Lester* and Daniel P. Costa

Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, CA 95064, USA

*Author for correspondence (e-mail: cwlester@comcast.net)

Accepted 21 August 2006

Summary

Prolonged terrestrial fasting is a key element in the life history of elephant seals. While on land seals typically fast without access to fresh water, and thus must maintain positive water balance by reductions in water loss such that they can subsist primarily on metabolic water production (MWP). The terrestrial apnea demonstrated by seals may reduce respiratory evaporative water loss (REWL) to levels that allow seals to make a net gain of water from MWP. We empirically measured REWL in 13 fasting northern elephant seal pups and determined the effects on water conservation of a breathing mode that incorporates a regular pattern of apneas, of ≥ 1 min in duration, followed by eupneic recovery, compared with a breathing mode with no apneas longer than 20 s and resembling typical breathing patterns in other mammals (normative breathing). Overall REWL fell 41% from 0.075 ± 0.013 g min⁻¹ (mean \pm s.d.) during normative breathing to 0.044 ± 0.006 g min⁻¹ during apneic breathing. The decline in REWL is attributed to a decrease in overall

ventilation rate, made possible by a decline in metabolic rate along with an increase in oxygen extraction that would occur during apneic breathing. Data on the range of ambient humidity conditions at the local breeding site were collected and used to bound the range of environmental conditions used in laboratory measurements. Our data showed that the observed variations in ambient humidity had no significant effect on REWL. A combination of apneic breathing and the complex nasal turbinates allows fasting elephant seals to reduce REWL well below the rate of MWP so that they can maintain water balance during the fast.

Supplementary material available online at
<http://jeb.biologists.org/cgi/content/full/209/21/4283/DC1>

Key words: fasting physiology, *Mirounga angustirostris*, Pinnipedia, respiratory evaporative water loss, water conservation.

Introduction

For those vertebrates that have secondarily returned from a terrestrial existence to the marine environment, one of the primary challenges has been that of water conservation. With the exception of polar species that have access to snow and ice as sources of fresh water (Kooyman and Drabek, 1968; Renouf et al., 1990), or those species that venture into estuarine and riverine environments, these animals must satisfy their needs for water with metabolic water production, the preformed water in their food, or seawater ingestion (Irving et al., 1935). Whereas seawater ingestion has been measured or observed in a variety of marine mammals (Costa, 1982; Costa, 2002; Costa and Gentry, 1986; Costa and Trillmich, 1988; Gentry, 1981; Hui, 1981; Lea et al., 2002), most earless or true seals (Phocidae) either do not or cannot drink seawater, and thus it is not considered a significant positive contributor to their overall water budget. This therefore leaves preformed water in prey and metabolic water production (MWP) as the primary sources of water for these species (Costa, 2002; Ortiz, 2001; Storeheier and Nordøy, 2001).

The challenge of maintaining water balance becomes even more severe in those species of marine mammals, such as the northern elephant seal, *Mirounga angustirostris* (Gill), that endure prolonged fasts in arid terrestrial environments (Ortiz et al., 1978). In most phocid species, the female does not leave the pup at all during nursing, but fasts during the entire period of lactation (Bonner, 1984). Likewise, males in the harem-forming phocid species must remain on land in order to defend their breeding territories (Le Boeuf, 1974). Both the males and females are thus prevented from leaving their territories or pups even to drink, eliminating seawater ingestion as a potential contributor to water balance in most cases.

Phocid pups undergo prolonged fasting as well (Bowen et al., 1987; Burns et al., 1999; Crocker and Costa, 2002; Lydersen et al., 1997; Oftedal et al., 1989). In northern elephant seals this post-weaning fast lasts for 9–12 weeks (Ortiz et al., 1978; Reiter et al., 1978), and it takes place in often arid or semi-arid terrestrial environments, ranging from central California to the southern Baja Peninsula (Stewart et al., 1994). Like the adults, elephant seal pups do not drink during the fast

(Ortiz et al., 1978), and must therefore rely on the catabolism of body stores as their sole source of water.

The northern elephant seal thus endures a severe challenge to water balance: prolonged terrestrial fasting, without access to fresh water and abstaining even from seawater consumption during the first few weeks after weaning. It is also a well-studied system, having long been the subject of active research covering aspects of water balance (Adams, 1991; Adams and Costa, 1993; Blackwell, 1996; Blackwell and Le Boeuf, 1993; Ortiz et al., 1978; Ortiz et al., 1996), cardiovascular and respiratory physiology (Hammond et al., 1968; Castellini et al., 1994a; Castellini et al., 1994b; Kohin et al., 1997; Milsom et al., 1996), diving and foraging behavior (Crocker et al., 1997; Le Boeuf et al., 2000; Le Boeuf et al., 1996), energetics (Crocker et al., 2001; Houser and Costa, 2001; Noren, 2002a; Ortiz et al., 1978; Ortiz et al., 1984), protein catabolism (Adams and Costa, 1993; Crocker et al., 1998; Houser and Costa, 2001), thermoregulation (Bartholomew, 1954; Noren, 2002b), etc. The elephant seal is thus ideally suited for study of the adaptive limits of mammals with regard to water conservation.

Ortiz et al. hypothesized that fasting weaned elephant seal pups could derive sufficient water from metabolic water production (MWP) and from the release of preformed water from catabolized tissue if water losses from excretion and respiratory evaporation were minimal (Ortiz et al., 1978). Huntley et al. (Huntley et al., 1984) documented the presence of complex nasal turbinates, convolutions in the air passages that act as a countercurrent heat exchanger and conserve both heat and moisture (Jackson and Schmidt-Nielsen, 1964; Schmidt-Nielsen et al., 1970). These turbinates would thus reduce respiratory evaporative water loss (REWL), which results from the evaporation of water across the epithelium of the respiratory tract and is a major route of water loss for all air-breathing endotherms. Adams and Costa (Adams and Costa, 1993) found that the urinary output of pups was high at first but decreased substantially over the course of the post-weaning fast, from $\sim 430 \text{ ml day}^{-1}$ in the early stages to $\sim 70 \text{ ml day}^{-1}$ by the end of the fast. Water loss through feces production is small, having been measured at 20 g day^{-1} in fasting pups (D.P.C., unpublished data), and loss through cutaneous evaporation is generally considered negligible in phocids due to a lack of eccrine sweat glands (King, 1983; Renouf et al., 1990). Together these studies confirm that fasting weaned elephant seals pups have a series of physiological and anatomical mechanisms that allow them to survive without an exogenous source of water by reductions in respiratory evaporative and urinary water loss.

Huntley et al. documented the presence of a nasal counter current heat exchanger (Huntley et al., 1984), but they did not directly measure REWL. REWL can be further reduced through apneustic breathing, a distinctive respiratory pattern seen in phocids in which periods of extended breath-hold are interspersed with periods of rapid, deep breathing (Bartholomew, 1954; Blackwell and Le Boeuf, 1993; Castellini et al., 1994a; Castellini et al., 1994b). Apneustic breathing is

thought to increase the efficiency of oxygen extraction, allowing the seal to take fewer breaths overall (Costa, 2002; Ortiz et al., 1978); this reduces the total volume of air humidified by the lungs in taking up a given quantity of oxygen, thereby reducing REWL. Blackwell (Blackwell, 1996) studied the breathing behavior of fasting pups and estimated total respiratory water loss of a hypothetical weanling with or without apneustic breathing. However, like Huntley et al. (Huntley et al., 1984), she was unable to measure water loss empirically, nor was it possible to characterize the impact of increasing apnea duration on water economy. Furthermore, while it is possible that ambient humidity will affect water conservation, it was unknown to what extent the differences in humidity actually experienced by fasting pups affected REWL.

In order to determine whether increasing apnea duration leads to a reduction in water loss, and thus whether the activity of apneustic breathing is itself a behavioral strategy for water conservation, we directly measured respiratory water loss in fasting elephant seal pups and examined the relationship between REWL and apnea duration. To determine whether humidity has a noticeable effect on water conservation, we performed these measurements under a range of humidity conditions consistent with what the animals experience at the rookery. If there were variations in local climate between sites at the rookery, and if humidity could be demonstrated to have an effect on REWL, then site selection could also be an important behavioral strategy for maintaining water balance. Finally, the collected data were used, in conjunction with the previous research on other aspects of water balance, to create a water budget for an early-stage weanling elephant seal.

Materials and methods

Experimental trials

Thirteen study animals were captured at the elephant seal rookery at Año Nuevo State Reserve in San Mateo County, CA, USA, and transported 30 km south to Long Marine Laboratory in Santa Cruz. Trials were performed between 26 April and 29 May 2003 and between 29 March and 22 May 2004; in both years these dates corresponded to the late stages of the post-weaning fast. Each subject was kept up to 10 days before being returned to the rookery. Based on molting status, the seals were all determined to be at least 2 weeks into the post-weaning fast (Reiter et al., 1978); the exact ages of pups were unknown, but regular censuses of nursing pups at the rookery revealed that all pups were weaned by 12 March 2003 and only three pups remained un-weaned by 9 March 2004 (P. Morris, personal communication). It can therefore be concluded that all pups were at least 6 weeks into the post-weaning fast when our trials began in 2003, and were almost certainly at least 20 days into the fast in 2004.

The experimental setup is shown in Fig. 1. At the beginning of each trial the seal was secured on a fiberglass board. A collar made of PVC pipe, approximately 30 cm in diameter, was secured to the board in order to reduce head movement. The animal's nose typically protruded 1–5 cm beyond the collar;

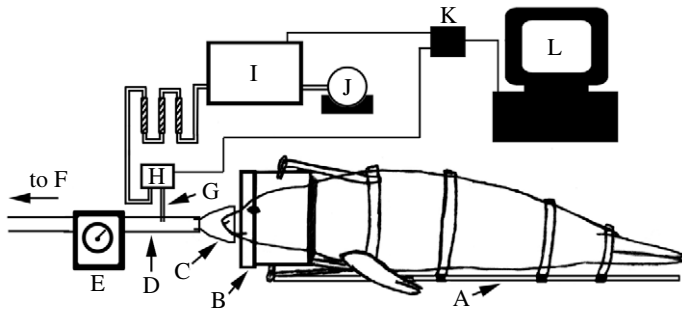


Fig. 1. Diagram of open-flow respirometry setup used in measuring respiratory evaporative water loss (REWL). Components are not drawn to scale. The fiberglass board (A) used in the experiment was fitted with sloping side-boards and carrying handles, which have been omitted from the diagram for the sake of simplicity. The other components were: B, PVC collar; C, Teflon funnel; D, 1 m-long Teflon tube; E, Singer dry gas meter; F, vacuum pump; G, a sample line which carried a fraction of the incurrent air to a relative humidity analyzer (H); I, oxygen sensor; J, air flow controller for sample line; K, analog-to-digital data converter; and L, computer used to record output. Solid lines (between H,I,K,L) represent data cables. For further details see Materials and methods.

this prevented accumulation of moisture on the collar and in the system. This was tested by using a variety of funnel-and-collar configurations such that there was no lag in the response time for relative humidity (RH) while the animal breathed or during entrance into apnea. Moisture accumulation in early iterations of the system was accompanied by a noticeable lag in the response of RH during apnea, and the design was adjusted until this lag no longer occurred.

REWL was determined by measuring relative humidity in an open-flow respirometry system (Withers, 1977). Exhalations were captured using a Teflon funnel (Welch Fluorocarbon, Dover, NH, USA) and drawn by vacuum into a 1 m-long Teflon tube (Harrington Industrial Plastics, Fremont, CA, USA), connected to a Singer dry gas meter (American Meter Company, Horsham, PA, USA). A sample line made of Bev-a-Line (Thermoplastic Processes, Inc., Warren, NJ, USA) carried a fraction of the incurrent air to a Sable Systems RH-100 relative humidity analyzer (Sable Systems, Las Vegas, NV, USA) 30 cm anterior to the funnel. Excurrent air from the RH-100 was carried to an oxygen sensor (I) (AEI Technologies, Sunnyvale, CA, USA). The use of Teflon and Bev-a-Line was necessary in order to minimize adhesion of water vapor and ensure maximum accuracy in humidity measurements. The accuracy of this technique for determining respiratory water loss has been previously verified, with a correlation coefficient of 0.999 (Hammarlund et al., 1986). The RH-100 was calibrated before data collection using dry compressed N_2 gas for the zero-point, and the saturation-point for a known air temperature was set by passing air through an aquarium aerator stone immersed in water. Air flow through the main line varied between 95 and 121 l min^{-1} . Air flow through the sample line in 2003 was 300 ml min^{-1} in 2003, 150 ml min^{-1} of which

passed through the O_2 sensor; and in 2004 was 400 ml min^{-1} , all of which passed through the O_2 sensor. These flow rates were necessary to ensure complete capture of exhalations and rapid clearance of water vapor from the system, and to allow coincident measurements of RH and O_2 . Relative humidity and O_2 depletion were recorded using Datacan data acquisition software (Sable Systems, Las Vegas, NV, USA).

Each seal was used in at least two trials, 1–5 days apart, typically lasting 3 h. Trial duration was dictated by the animal's behavior, which was monitored throughout the trial. Data were only used when the animal was calm and exhalations were being fully collected.

Defining breathing intervals

Two distinct modes of breathing were identified, and these were broken into discrete intervals for analysis. Each such interval is hereafter referred to as a 'measurement interval', and constitutes a single data point in analysis.

In the first mode, the animals alternated between apneas of ≥ 1 min and subsequent recovery periods of eupnea. During these recovery periods, rate and depth of breathing and the rate of oxygen consumption were increased with respect to periods of breathing that did not immediately follow a period of apnea. We designated this behavior as S-phase breathing, since this was principally observed during sleep (Castellini et al., 1994a; Castellini et al., 1994c; Huntley, 1984). Each interval of S-phase was measured from the beginning of an apnea to the end of the eupneic recovery period that followed it; the metabolic water production and respiratory water loss calculated for the recovery period were averaged over the entire cycle. We considered a recovery to be completed under either of two conditions: (1) if breathing ceased entirely and the animal returned to apnea, or (2) if deflections in the O_2 trace were reduced suddenly by more than 25% and then persisted at a steady intermediate level for at least 1 min before returning to apnea. This latter condition was interpreted to be a temporary shift to the second mode of breathing described below. However, if a period of breathing that followed an apnea was less than half the length, and the O_2 deflection was less than half as great as for the other eupneic periods following apneas during the same experimental trial, then the recovery was judged not to be complete and this apnea/eupnea period was combined with the next such period in the recording. The times for both apneas were then added together to determine the total apnea duration for the S-phase interval in question, and both eupneic periods were considered part of the recovery from apnea. Other cues to the end of a recovery period included a sudden reduction in the rate and depth of breathing, and/or resumed awareness and movement by the animal. Although the methods used to identify changes in breathing mode were largely qualitative, subsequent analysis showed a clear quantitative difference in breathing rate between the two modes that upheld the observations taken during the trials (see Results).

The second commonly observed mode of breathing resembled 'normal' breathing in other mammals. In this mode

there were no periods of breath-hold longer than 1 min, and depth and rate of breathing were variable but always less than was seen in a post-apnea recovery period during S-phase breathing. Because this behavior was commonly associated with wakefulness (Castellini et al., 1994a; Huntley, 1984), we have designated it as W-phase breathing. Sometimes an interval of W-phase breathing followed the recovery period of an S-phase interval, when the seal awoke briefly before returning to apnea and sleep; these instances were easily identified both by the change in breathing pattern and by the reduction in oxygen consumption mentioned above. At other times the animal would remain in W-phase for extended periods, sometimes in excess of 1 h, until the trial was concluded or the animal returned to S-phase once more.

Extended eupnea was often accompanied by activity and it was rare to record a long 'bout' of W-phase breathing without interruption. Therefore, data for W-phase breathing were only used when there were no vocalizations, no missed breaths (which could occur if the animal withdrew its nose from the funnel), and no post-apnea recovery (determined as described above), and when the interval of breathing was at least 1 min in duration (if following a recovery period and ending in a return to apnea) or at least 3 min in duration (if part of a longer period of wakefulness).

Humidity and temperature

RH data were transformed to measurements of absolute humidity ($\text{g H}_2\text{O min}^{-1}$), and polynomial baseline correction was used to subtract out ambient humidity, thus giving $\text{g H}_2\text{O enrichment min}^{-1}$ (i.e. REWL). The method was modeled after that used to calculate ml O_2 consumption from continuous $\% \text{O}_2$ measurements taken in respirometry (Withers, 1977), with the added complication that RH must be converted to absolute humidity by multiplying the measured RH by the saturation vapor density (SVD), which is temperature-dependent. Polynomial baseline correction is a partially automated feature included in the Datacan analysis software (Sable Systems, Las Vegas, NV, USA), and is described in detail in its operations manual. Briefly, the technique uses points throughout the data record that have been defined by the user as baseline (i.e. points at which ambient conditions are represented without added input from the test animal) to generate a polynomial curve that is then subtracted from the raw data curve, producing an output in which deviations from baseline are represented as fluctuations above and below zero. Integration of data over a baseline-corrected curve yields values of H_2O enrichment or O_2 depletion in which the baseline drift common in respirometry has already been taken into account. We used the animals' own periods of apnea as the primary points for baseline correction, since both O_2 concentration and RH returned to ambient levels while the animal was in breath-hold.

In 2004, absolute ambient humidity was modified to vary between 7.00 and 11.21 g m^{-3} using a dehumidifier (Sears, Roebuck and Co., Hoffman Estates, IL, USA). Temperature was monitored during the trial and used together with RH to

determine absolute humidity. The rate of oxygen consumption was determined according to Withers (Withers, 1977), assuming a respiratory quotient (RQ) of 0.711, calculated for the low protein catabolism of fasting elephant seals (Pernia et al., 1989; Houser and Costa, 2001).

Oxygen consumption and metabolic water production

Data were integrated over the measurement intervals to give REWL and ml O_2 consumed. Metabolic water production (MWP) was calculated from O_2 consumption using a conversion factor of $5.23 \times 10^{-4} \text{ g H}_2\text{O ml}^{-1} \text{ O}_2$, following the equation:

$$\text{MWP} = (\% \text{ lipid}) \times W_L \times \dot{V}_{\text{OL}}^{-1} + (\% \text{ protein}) \times W_P \times \dot{V}_{\text{OP}}^{-1}, \quad (1)$$

where W is the mass of metabolic water produced per gram of lipid or protein, \dot{V}_{O} is the volume of oxygen consumed per gram of lipid or protein, and $\% \text{ lipid}$ and $\% \text{ protein}$ are the relative contributions of each to metabolism. We used the values obtained by Costa and Williams (Costa and Williams, 2000) for these metabolic constants and assumed a contribution of protein catabolism of 6%, based on the mean mass losses from lean and adipose tissue reported by Rea and Costa (Rea and Costa, 1992) and Houser and Costa (Houser and Costa, 2001); see 'Estimating water budget' in the Discussion for a more detailed description of the calculations used.

Data analysis

Data were analyzed using SYSTAT 10.2 (Systat Software, Richmond, CA, USA). Unless otherwise noted, data are summarized as mean \pm s.d. REWL was divided by MWP to standardize for differences in metabolic rate, and the resulting water loss ratio was examined in an analysis of covariance (ANCOVA) against the following factors: individual seal (fixed effect variable), apnea duration and absolute humidity. All interaction terms were examined, and removed from the model if not significant. Additional analysis was performed on total water lost by respiratory evaporation for a measurement interval (EW_Total) in a multiple linear regression against apnea duration and the total oxygen consumed over the same interval ($\text{O}_2\text{-Con}$).

Measuring ambient conditions

Weather stations were placed at two sites at Año Nuevo State Reserve, 30 km north of Santa Cruz, CA. These sites reflected the extremes likely to be observed within the habitat used by the fasting pups. The station at Mid-Bight Beach (MBB) was located atop a small dune on a broad, sandy shelf approximately 3 m above sea level, directly exposed to wind coming in from the sea. The second station, at Año Point Dunes (APD), was more sheltered, sitting at the edge of a small tussock of arroyo willows (*Salix lasiolepis*) northeast of a broad sandy expanse that sloped gradually down toward the water. Monitor II weather stations (Davis Instruments, Hayward, CA, USA) recorded temperature and relative humidity from 23 March to 1 June 2002 (for APD) or 8 June 2002 (for MBB).

Data were collected continuously, averaged and stored every 15 min, and downloaded every 7 days. Data were grouped into equivalent 15 min blocks of the 24 h cycle and were averaged together to create an 'average day' for each site that was divided into 15 min increments.

Results

Effects of apnea duration and humidity on water loss

Mass of the 13 seals ranged from 56.0 to 135.0 kg, with a mean mass of 92.4 ± 20.9 kg (Table 1). Males and females were not significantly different with respect to mass (two-tailed *t*-test, $t=1.22$, $P=0.25$), nor was there any significant difference between study years ($t=0.29$, $P=0.77$). For details of individual seals, see Table S1 in supplementary material. There was no relationship between mass and the water loss ratio, REWL/MWP. Since the use of individual seals as a fixed effect variable resulted in a better overall fit to the data than did the use of mass as a continuous variable (ANCOVA multiple $R^2=0.400$ with individual effects *versus* multiple linear regression $R^2=0.212$ with mass effects), mass was not considered in further analysis.

When data for all breathing intervals were combined for each seal, mean values for the water loss ratio REWL/MWP ranged

from 0.309 to 0.538. Mean water loss ratios were significantly higher in 2004 than in 2003 ($t=4.656$, $P<0.01$), and males had significantly higher mean water loss ratios than females ($t=5.09$, $P\leq 0.001$).

An analysis of covariance indicated that water loss ratio was significantly related to apnea duration; the interaction term between individual seal and absolute humidity was also highly significant (Table 2). There were no other significant interaction terms. Absolute humidity for all lab trials ranged from 7.00 to 11.21 g m⁻³; in 2004, when humidity was experimentally manipulated, the absolute difference in humidity between trials for any individual seal ranged from 0.5 to 3 g m⁻³.

Water loss ratios showed high variance at short apnea durations, but became less variable and declined as apneas became longer (Fig. 2A). This pattern was similar to the trend in metabolic rate (Fig. 2B). A plot of total respiratory water loss (EW_Total) against total oxygen consumption (O₂_Con) revealed a strong linear trend (Fig. 2C). EW_Total increased linearly with both O₂_Con and apnea duration (Duration_{ap}), following the equation:

$$EW_Total = 0.0531 + 0.000199(O_2_Con) - 0.0155(Duration_{ap}), \quad (2)$$

where EW_Total is in g H₂O, O₂_Con is in ml O₂ and Duration_{ap} is in min (combined $R^2=0.777$, $F=693$, $P<0.01$).

The ratio of apnea duration to interval time during S-phase breathing was notably consistent across all seals; on average, 67.5%±5.5% of time during S-phase breathing was spent in apnea, with 32.5% of time being spent in recovery breathing. Post-apnea recovery was associated with a markedly higher breathing rate than breathing intervals not associated with apnea ($t=7.66$, $P\leq 0.001$): mean breathing rate during eupnea, BR_E, averaged 9.57±1.89 breaths min⁻¹ during the recovery periods of S-phase breathing but only 5.15±0.86 breaths min⁻¹ during W-phase breathing.

Whereas breathing during the recovery period itself was greatly increased, breathing rate as averaged over total time interval declined during S-phase breathing. We averaged the breaths taken in a given interval over the entire time period, including apnea, to produce mean overall breathing rate, BR_O. BR_O declined as apnea duration increased ($R^2=0.386$, $F=250$, $P<0.01$). During S-phase, mean BR_O across all seals was

Table 1. Summarized data for all seals

Variable	Mean ± s.d.*
Mass at weighing	92.4±20.9
Mass-specific metabolic rate (ml O ₂ kg ⁻¹ min ⁻¹)	
W-phase [†]	3.466±1.102
S-phase [‡]	2.751±1.002
Mean REWL (g H ₂ O min ⁻¹)	
W-phase [†]	0.075±0.013
S-phase [‡]	0.044±0.006
Mean MWP (g H ₂ O min ⁻¹)	
W-phase [†]	0.155±0.030
S-phase [‡]	0.121±0.016
Mean water loss ratio (REWL/MWP)	
W-phase [†]	0.492±0.093
S-phase [‡]	0.372±0.070
Mean water loss per breath (g)	
W-phase [†]	0.013±0.003
S-phase [‡]	0.015±0.004
Mean apnea duration (min)	5.14±1.10
Mean temperature (°C)	19.37±1.53
Mean absolute humidity (g H ₂ O m ⁻³)	9.040±0.801

*Data presented are for 13 seals (5 male, 8 female). Values listed are mean ± s.d. of the mean values for each of the individual seals.

REWL, respiratory evaporative water loss; MWP, metabolic water production.

[†]No apneas exceeding 1 min.

[‡]Breathing intervals contained apneas of 1 min or more.

Table 2. ANCOVA results for factors affecting water loss ratio, REWL/MWP

Source	SS	d.f.	MS	F-ratio	P
Seal	0.915	12	0.074	4.25	≤0.001
Absolute humidity	0.028	1	0.061	1.56	0.212
Apnea duration	1.156	1	1.243	64.39	≤0.001
Seal × absolute humidity	0.874	12	0.069	4.06	≤0.001
Error	6.642	370	0.018		

Total number of intervals (N)=397; multiple R=0.633; squared multiple R=0.400.

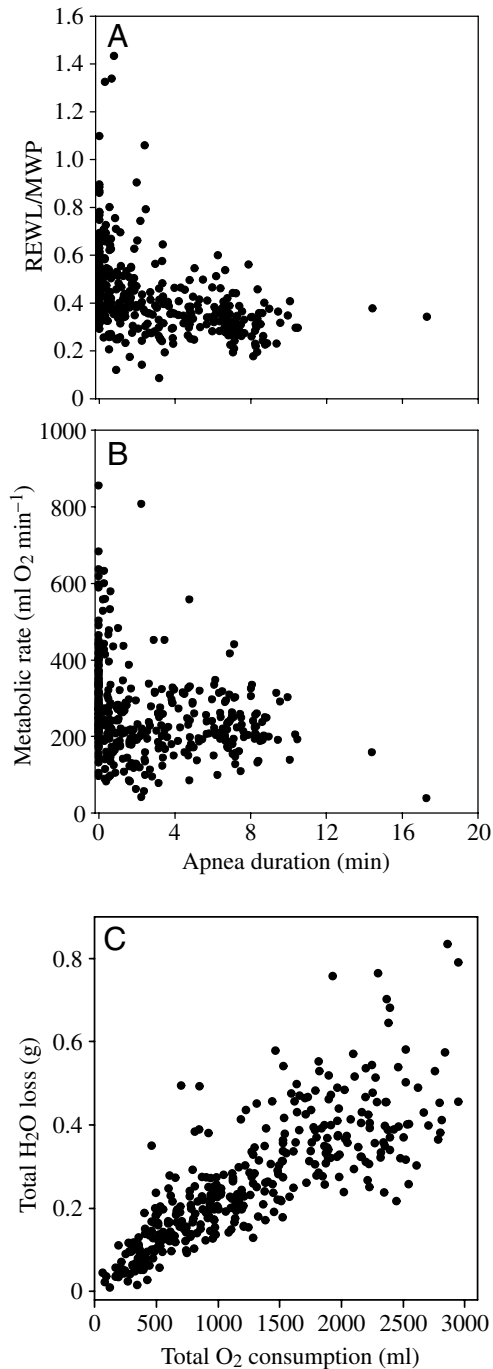


Fig. 2. (A) Water loss ratios plotted against apnea duration for all seals. Apnea duration is a highly significant contributor to the ANCOVA model for respiratory evaporative water loss/metabolic water production (REWL/MWP; $F=65$, $P \leq 0.001$). (B) Metabolic rate versus apnea duration. (C) Total respiratory evaporative water loss (EW_{Total}) plotted against total oxygen consumption (O₂Con). Each data point denotes a single breathing interval (apnea + recovery, or W-phase eupnea with no preceding apnea, as defined in Materials and methods), and the values for water loss and oxygen consumption are the amounts lost or consumed during that discrete interval. In the analysis, breathing intervals in which apnea duration was less than 1 min were considered W-phase breathing; an apnea duration of 0 min indicates a W-phase breathing interval in which no apnea took place.

3.04 ± 1.36 breaths min^{-1} ; during W-phase, it was 5.96 ± 2.15 breaths min^{-1} . There was a positive linear relationship between BR_O and the rate of evaporative water loss, described by the equation:

$$\text{REWL} = 0.0204 + 0.007(\text{BR}_O), \quad (3)$$

where REWL is in $\text{g H}_2\text{O min}^{-1}$ and BR_O is in breaths min^{-1} ($R^2=0.482$, $F=370$, $P<0.01$).

Ambient weather conditions at breeding site

Temperature and humidity showed substantially different diurnal patterns at the two weather station sites. Temperature at APD ranged from a minimum of 4.7°C (06:15 h, 8 May) to a maximum of 26.5°C (13:15 h, 22 April); absolute humidity ranged from 5.19 g m^{-3} to 14.59 g m^{-3} . At MBB, temperature ranged from 2.7°C (05:00 h, 8 May) to 25.5°C (16:15 h, 24 April); absolute humidity ranged from 5.95 g m^{-3} to 15.49 g m^{-3} . Absolute humidity was approximately normally distributed for both sites; means were 7.84 g m^{-3} for APD and 9.53 g m^{-3} for MBB, a highly significant difference (Student t -test, $t=7.31$, $P<0.01$).

Discussion

Factors affecting respiratory water loss

Our results clearly demonstrate that S-phase breathing (i.e. long apneas followed by recovery periods) leads to a reduction in water loss, and that the efficiency of water conservation is a function of apnea duration (Fig. 2). Evaporative water loss varies widely during W-phase breathing (i.e. breathing that contains no long apneas), occasionally even exceeding the estimated amount of metabolic water produced over the same interval.

There appear to be two mechanisms by which S-phase breathing reduces evaporative water loss. First, S-phase breathing leads to, or at least is strongly associated with, a reduction in metabolic rate (Fig. 2B). Metabolic depression in conjunction with extended breath-hold has long been characterized as part of the dive response in marine mammals (Butler and Jones, 1982; Castellini et al., 1992; Hurley and Costa, 2001; Scholander, 1940), and the same correlation is evident here. Furthermore, the pattern observed here, in which variance is high during W-phase breathing and becomes constrained during extended apnea, is comparable to that observed in free-diving Weddell seals (Williams et al., 2004) and in California sea lions performing trained submersions (Hurley and Costa, 2001). In both diving animals and fasting elephant seals the hypometabolism induced by apnea results in fewer breaths being taken, which leads to decreased water loss as fewer lungfuls of air are humidified and expelled from the body.

The second way in which S-phase breathing reduces water loss is by facilitating increased oxygen extraction efficiency. Increasing apnea duration leads to an increase in oxygen extracted per breath (Irving et al., 1941; Kramer, 1988; Ridgway, 1972). This can be explained by both an increase in

the time allowed for oxygen exchange in the lungs and by the low partial pressure of oxygen in the blood after a long apnea, which results an increased partial pressure differential between the blood and the inspired air and a faster rate of oxygen diffusion. As oxygen is extracted more efficiently from the lungs, the seal can take fewer breaths, and again the amount of water lost by the animal is reduced.

We found no evidence that changes in ambient humidity affected respiratory water loss. The range of humidity conditions we were able to produce in the lab reflected the average range of absolute humidity measured at the Año Nuevo rookery, and over this range of values there was no relationship between humidity and the water loss ratio. The significant interaction term observed in the analysis of covariance between individual seals and absolute humidity (Table 2) resulted from the fact that different seals showed markedly different responses to the low- and high-humidity trials: some seals showed lower REWL/MWP during the high-humidity trials, but some showed higher values, and others showed little or no change. Therefore, we conclude that the interaction term is a statistical artifact, and that changes in REWL/MWP between trials were driven more by random variations in individual seal behavior than by differences in humidity. Given the known effect of ambient humidity on the performance of the nasal turbinates, this may appear counterintuitive (Huntley et al., 1984). However, the absolute humidity inside a mammal's lungs (assuming 37°C core body temperature and 100% saturation) is 44 g m⁻³. Thus, the humidity differential between the lungs and the ambient air over the range of conditions measured in the field is only about 9.5% greater at 7 g m⁻³ than it is at 11.2 g m⁻³; over that small a range, it is not surprising that there was no effect of changes in humidity on water loss. These results suggest that elephant seals are well-adapted to the near shore coastal environment in which they raise and wean their pups, and that even the driest conditions in this maritime climate will not adversely affect their overall water economy.

There are no clear explanations for why mean water loss ratios were higher in 2004 than in 2003, or why males showed higher water loss ratios than females. Given that the majority of our subjects were female in 2003, whereas in 2004 the majority were male, these two factors may well be intertwined. Given that there were no significant differences in mass either between years or between sexes, it seems unlikely that body size played a major role in the observed differences, nor can we identify any other single factor that seems likely to be responsible. Given the small sample sizes involved when comparing years or sexes ($N=5$ for 2003, $N=8$ for 2004; $N=5$ for female, $N=8$ for male), we would caution against assigning any great significance to the differences between these groups, as these differences could simply be the result of random variation in the study population. The differences observed within each of our individual seals, due to S-phase *versus* W-phase breathing, are far better attested and can be interpreted with much more confidence than the differences between sexes and study years; additional study of a much larger sample size

would be required to confidently ascertain whether there is a sex bias in water conservation during the post-weaning fast.

Role and origins of apnea

The central importance of S-phase breathing in improving water conservation in fasting seals leads to the question of when and how this behavior originated. Terrestrial apnea is common to all phocids (Knopper and Boily, 2000), including those that do not engage in prolonged fasting; since fasting is considered a derived trait in seals rather than the ancestral condition (Costa, 1993), it can be concluded that S-phase breathing did not originally evolve as an adaptation to this life history pattern.

Given the similarity in metabolic responses between terrestrial apnea and breath-hold while diving (Castellini, 1996), it seems evident that this behavior developed in response to the need for increased dive duration to improve foraging efficiency. A reduction in metabolic rate while diving reduces the rate at which oxygen stores are depleted, increasing foraging time while avoiding the long post-dive surface intervals associated with anaerobic diving (Costa et al., 2001; Hurley and Costa, 2001). Furthermore, the increase in oxygen extracted per breath caused by prolonged apnea allows for more rapid replenishment of oxygen at the surface and a reduction in the post-dive surface interval, so long dives are doubly effective in increasing foraging efficiency (Kramer, 1988). The reduction in water loss is a useful ancillary benefit that was probably exploited later to enable prolonged fasting behavior. This would make terrestrial S-phase breathing a classic example of exaptation (Gould and Vrba, 1982).

Although it is true that phocids dive on exhalation, whereas terrestrial apnea begins on inhalation, this need not mean that the two behaviors are unrelated. The ancestors of modern pinnipeds may well have begun by diving on inhalation, with their descendants only beginning to dive on exhalation when they expanded their range to forage at greater depths. This additional adaptation to the effects of pressure at depth need not have carried over to terrestrial apnea, where it is advantageous to hold breath on inhalation and thus extract as much oxygen from the lungs as possible. Terrestrial apnea might therefore represent a throwback to the ancestral pattern of diving, before the challenges of deep-water foraging necessitated a transition to diving on exhalation.

Nasal turbinates

We can estimate the effects of nasal turbinates by comparison with one of the seal's close phylogenetic relatives, the domestic dog (*Canis lupus familiaris*). Although there may be other differences in the respiratory systems of seals and dogs that could contribute to differences in water economy, the exceptional complexity of the phocid turbinates is the most striking difference between them. It must be cautioned that phocid lungs have a number of other important anatomical differences that set them aside from the typical mammalian structure, such as reinforced airways, a unilobular lung and unique alveoli (Drabek and Kooyman, 1984; Kooyman, 1973);

however, these differences are not likely to contribute to differences in water conservation, as they occur within the lungs themselves where, in both dogs and seals, the air is already warmed to body temperature and completely saturated with water (Schmidt-Nielsen et al., 1970).

In a study of breathing behaviors in dogs at varying levels of ambient temperature and exercise intensity, Goldberg et al. (Goldberg et al., 1981) found that the temperature of the air exhaled through the nose at rest (T_E) followed a positive linear relationship with ambient temperature (T_A) that was elevated above and shallower in slope than the line $T_E=T_A$. Mean temperature for our experimental trials was 19.4°C; at 20°C, the dogs in the aforementioned study had a mean expired air temperature of about 26°C (as judged by fig. 3 in the referenced paper). Given this, and assuming that expired air is effectively 100% saturated (Schmidt-Nielsen et al., 1970), percent recovery of respiratory water will follow the equation given by Collins et al. (Collins et al., 1971):

$$\% \text{ recovery} = 100\% \times (W_B - W_E)/(W_B - W_I), \quad (4)$$

where W_I is the water content of the inspired air, W_E is the water content of the exhaled air, and W_B is the water content of the air in the lungs (i.e. at body temperature). With $T_B=38^\circ\text{C}$,

$T_E=26^\circ\text{C}$ and an absolute humidity of 9.0 g m^{-3} (the mean value for our experimental trials), the percentage recovery by the canine nasal passages is 58.7%. Based on our findings – using empirical measurements of water loss, rather than the estimate given by Eqn 4 – the percentage recovery by the phocid nasal passages under the same conditions is 92.5% for W-phase breathing. Water loss per breath is thus only 7.5% of the water vapor added to the air in the lungs, whereas the dog's loss per breath (41.3%) is proportionately much higher. The use of S-phase breathing slightly increases water loss per breath (recovery is 91.2%), but the mean overall breathing rate (BR_O) is also reduced by 49%, and thus the efficiency of the entire breathing process is nearly doubled. If the dog's breathing rate while resting is comparable to that of the seal in W-phase, then a dog's REWL will be 9.6 times that of a seal in S-phase.

Comparisons to other homeotherms

Whereas REWL has rarely been measured directly, percentage water recovery has been estimated from expired air temperature for a variety of mammals and birds (Table 3). It is clear that the two phocid species examined, the elephant seal and the grey seal (*Halichoerus grypus*), both possess water conservation systems that are among the most efficient for any

Table 3. Estimates of respiratory water recovery for various species

Species (N)	T_a (°C)	Hum_a (g m ⁻³)	T_{ex} (°C)	% R_w	Reference
Terrestrial – arid					
Cactus wren (5)*	20.0	~3.5	23.6	64%	(Schmidt-Nielsen et al., 1970)
Giraffe (15)	21.1	13.3	28.0	56%	(Langman et al., 1979)
Kangaroo rat (12)	28.0	27.3	~29.5	88%	(Jackson and Schmidt-Nielsen, 1964)
	28.0	~0	~23.0	56%	(Jackson and Schmidt-Nielsen, 1964)
Waterbuck (3)	22.4	13.7	28.6	58%	(Langman et al., 1979)
Wildebeest (9)	23.9	13.9	35.4	24%	(Langman et al., 1979)
Mean				58%	
Terrestrial – temperate					
Cow (10)	21.1	12.1	23.6	47%	(Langman et al., 1979)
Dog (3)†	20	~4.1	~26	52%	(Goldberg et al., 1981)
Donkey (4)	23.6	13.6	32.3	34%	(Langman et al., 1979)
Goat (7)	23.4	13.2	29.5	55%	(Langman et al., 1979)
Human (4)	20.8	~0	29.9	32%	(Varène et al., 1986)
Rabbit (4)‡	20	NR	~34.5	26%	(Caputa, 1979)
Sheep (8)	24.1	13.8	35.3	24%	(Langman et al., 1979)
Mean				27%	
Marine					
Elephant seal (13)	19.3	9.0	NR	92%	This study
Grey seal (3)	-20.0	~0	~11	80%	(Folkow and Blix, 1987)
Herring gull (6)§	15.0	6.4	19.4	76%	(Geist, 2000)
Penguin (15)	5	<5	9	82%	(Murrish, 1973)
Mean				83%	

T_a , ambient temperature; Hum_a , ambient humidity; T_{ex} , expired air temperature; % R_w , % calculated water recovery. NR, not reported.

*Humidity reported as 10–30% RH, T_b as 40–42°C.

†Humidity reported as 25% RH, T_b as 38°C.

‡Estimated assuming $Hum_a=9.0 \text{ g m}^{-3}$ and $T_b=39.3^\circ\text{C}$.

§Humidity reported as 50% at 15.0°C; % R_w estimated from $T_b=39.4^\circ\text{C}$.

homeotherm, rivaling even the arid-adapted kangaroo rat (*Dipodomys* sp.). Their water conservation capabilities are far beyond those not only of dogs, but nearly every large mammal examined. Remarkably, estimated water recovery in grey seals reaches 80% even at -20°C , when the moisture content of the air is effectively zero (Folkow and Blix, 1987). This is particularly telling in light of the fact that the water recovery of the kangaroo rat under similarly dry conditions is only 56% (Jackson and Schmidt-Nielsen, 1964).

Another marine-adapted species, the herring gull (*Larus argentatus*), is also among the most water-efficient animals studied, with a water recovery of 76% under temperate, relatively dry conditions (Geist, 2000). Likewise, the Adelie and Gentoo penguins (*Pygoscelis adeliae* and *P. papua ellsworthi*) displayed water recovery rates of 81.9% at 5°C , when ambient humidity was less than 5 g m^{-3} (Murrish, 1973). It would appear that the pressure to conserve respiratory water in the marine environment is comparable to that seen in desert-adapted species.

Estimating water budget

Together with past findings on sources of water loss and metabolic water production, the data from this study can be used to model a complete water budget for a fasting elephant seal pup (Table 4). For simplicity's sake, we consider a pup in the early stages of the fast, when these animals enter the water rarely, or not at all (Thorson and Le Boeuf, 1994), and overall activity levels are minimal. Although our own study animals were older, the benefits of S-phase breathing should be similar for pups at any point in the fast, as Blackwell (Blackwell, 1996) found that the percentage of time spent in terrestrial apnea does not change over the course of the fast; only the length of mean apnea duration increases, from 4.0 min to 8.0 min (fig. 2.8 in referenced volume). Our results show that there is little change in REWL/MWP over this range, so we

may assume that the benefits of S-phase breathing do not change during the fast.

MWP is dependent upon the quantity and type of body stores used in metabolism. Houser and Costa (Houser and Costa, 2001) found that fasting pups lost 600 g day^{-1} of mass; Rea and Costa (Rea and Costa, 1992) reported a rate of 870 g day^{-1} in the first 4 weeks of the fast and 510 g day^{-1} in the second 4 weeks, whereas Noren et al. (Noren et al., 2003) reported a rate of 626 g day^{-1} for a seal with a weaning mass of 121 kg. To provide a conservative estimate of water available for our hypothetical seal we used a mass loss rate of 600 g day^{-1} . Rea and Costa (Rea and Costa, 1992) found that on average seals lost 7.2 kg of lean mass and 14.2 kg of adipose tissue between the sixth and twelfth week of life (i.e. weeks 2–8 of the postweaning fast), whereas Houser and Costa (Houser and Costa, 2001) reported mean losses of 6.5 kg of lean mass and 14.9 kg of adipose tissue. Taking the mean of these results, we find that the average mass loss across both studies was 32.0% lean tissue and 68.0% adipose tissue; we therefore assumed that 408 g of blubber and 192 g of lean tissue per day were contributed to metabolism. The values in Table 4 assume that blubber is approximately 10% preformed water (Crocker, 1995), lean tissue is 73% water (Pace and Rathbun, 1945), and lipid and protein catabolism produce 1.071 g and 0.396 g H_2O , respectively, per g of tissue (Costa and Williams, 2000).

REWL estimates are based on past observations of the activity budgets of fasting pups (Blackwell, 1996). From these observations, it can be calculated that a pup spends 38% of its time, or 9.12 h per day, in S-phase breathing. For an early-stage pup the remainder of the time, 14.88 h per day, is spent awake but relatively inactive on land, which equates well to the W-phase breathing measured in the lab. During S-phase breathing, the seals in this study lost water through respiration at an average rate of 0.044 g min^{-1} , or 2.64 g h^{-1} . During W-phase breathing, REWL was 0.075 g min^{-1} , or 4.5 g h^{-1} . With the time budget listed above, this leads to REWL of 24 g day^{-1} during S-phase breathing and 67 g day^{-1} during W-phase breathing, for a total REWL of 91 g day^{-1} .

All other factors in the water budget are drawn from previous studies (Table 4). Using these values, our model seal in the early stages of the fast shows a net water surplus of 51 g day^{-1} . Using higher proportions of adipose tissue consumption results in a greater surplus, up to 92 g day^{-1} when 90% adipose tissue use is assumed, as might be the case in a well-fed pup with a very large amount of adipose tissue. Even if we assume a minimum of 50% adipose tissue use, as might be the case in a very lean pup attempting to conserve blubber for insulation, the model still yields a surplus of 26 g day^{-1} . In reality, any excess water not lost to evaporation would result in an increased urine output and a lower urine osmotic concentration; however, since urinary water loss has been measured and is already incorporated into the model, it is likely that this additional water is instead being lost during the small amounts of activity displayed by pups in the early stages of the fast, since increased activity leads to an increase in REWL/MWP. It is worth noting that urinary water loss declines greatly over the course of the

Table 4. Estimated water budget for a fasting elephant seal pup

Water produced from 408 g blubber*:	+434 g day^{-1}
Water produced from 192 g lean tissue [†] :	+161 g day^{-1}
REWL from apneustic breathing (9.12 h day^{-1}):	-24 g day^{-1}
REWL from non-apneustic breathing (14.88 h day^{-1}):	-67 g day^{-1}
Cutaneous water loss [‡] :	-0 g day^{-1}
Fecal water loss [§] :	-20 g day^{-1}
Urinary water loss (week 1 of fast) [¶] :	-433 g day^{-1}
Net gain/loss:	+51 g day^{-1}

REWL, respiratory evaporative water loss.

*1.071 g $\text{H}_2\text{O g}^{-1}$ lipid (Costa and Williams, 2000), 41 g preformed H_2O (Crocker, 1995).

[†]0.396 g $\text{H}_2\text{O g}^{-1}$ protein (Costa and Williams, 2000), 140 g preformed H_2O (Pace and Rathbun, 1945).

[‡](Renouf et al., 1991).

[§]D. P. Costa, unpublished data.

[¶](Adams, 1991).

fast (Adams and Costa, 1993), while at the same time activity is increasing (Blackwell, 1996). Because urine contains the surplus water beyond what is necessary to maintain normal hydration, it is difficult to tell what proportion of the high urine output early in the fast represents a constraint on activity (due to obligatory excretion) as opposed to being simply the result of a lack of activity. Urea concentration in the urine increases significantly over the course of the fast, even as total urinary nitrogen excretion declines (Adams and Costa, 1993). This suggests that early in the fast pups are excreting more water in the urine than is necessary for nitrogen excretion, and that at least during this initial period they have more water than is necessary to maintain homeostasis.

The complexity of the situation is further increased by the effects of the hormones used for regulation of urine output. Fasting elephant seal pups rely on the renin–angiotensin–aldosterone system (RAAS) in regulating conservation of both water and electrolytes; vasopressin (AVP) remains at relatively low and constant levels throughout the fast (Ortiz et al., 2000). Because the fasting pups are not drinking sea water, retention of electrolytes is important to prevent hyponatremia, and the sensitivity of the RAAS pathway is increased in these animals (Ortiz et al., 2000). However, angiotensin II and AVP are vasoconstrictors, whereas atrial natriuretic peptide (ANP) is a vasoconstrictor-inhibitor; during sleep apnea in elephant and Weddell seals, angiotensin II and AVP levels decline while ANP levels increase (Zenteno-Savin and Castellini, 1998). This is attributed to the decline in heart rate during apnea and the consequent increase in cardiac pressure, which stimulates ANP release (Ortiz, 2001; Stanton, 1991). Thus, the use of apnea in S-phase leads to a reduction in respiratory water loss, but also stimulates hormonal changes that could result in the loss of electrolytes. This indicates that there may be a tradeoff between respiratory evaporative water loss, which does not result in electrolyte loss, and urinary electrolyte and water conservation later in the fast. In the late stages of the fast, the need to conserve electrolytes may become more important than maintaining a low REWL/MWP.

The decline in urine production over the course of the fast can thus be seen partly as a result of declining urea production, partly as a means of electrolyte conservation, and partly as a result of more water being lost through respiration as the pup devotes more time to activity. Animals that are more active will have higher REWL/MWP, leading to a decline in the surplus water that would otherwise be lost in the urine; at the same time, hormonal regulation forces a decline in urine output in order to preserve electrolyte balance, and may set an upper limit on the amount of time that a fasting seal can afford to spend in S-phase breathing, since apnea is associated with increased production of hormones that promote electrolyte loss. The addition of diving behavior further complicates matters, as apnea during diving will result in metabolic suppression that will partly counteract the tendency of heightened activity to increase REWL/MWP. It is not possible to tease apart the relative importance of these factors to the overall water budget of a late-stage fasting seal from the available data.

Conclusions

The results of this study reveal that fasting elephant seals have at their disposal a range of behavioral and anatomical adaptations to restrict respiratory water loss. The physiological adaptation of the nasal turbinates provides an impressive benefit to water economy in its own right, whereas the use of apnea in S-phase breathing has the dual benefit of reducing metabolic rate and greatly increasing the oxygen extraction efficiency. The combination of these two factors leads to a drop in mean breathing rate of 49%, effectively cutting the animal's water costs in half as long as it remains in this mode of breathing.

If phocids had a respiratory system comparable to that of a terrestrial carnivore, such as the dog, they would lose 41.3% of the water their lungs added to every breath, instead of only 7.3%. Under these conditions, they could not produce adequate water through metabolism alone, and they would not be able to carry out the prolonged post-weaning fast without an exogenous source of water. The challenges of extended fasting in a terrestrial environment are severe, but our results show the importance of physiological mechanisms associated with the dive response – notably the capacity for extended breath-hold coupled with hypometabolism – in pre-adapting these animals to face extended bouts of water deprivation with the behavioral strategies necessary to survive them. At the same time, the capacity for water recovery displayed in their respiratory physiology shows them to be one of the most efficient homeotherms in minimizing REWL. As urine output declines over the course of the fast, more water becomes available for loss through respiration in conjunction with activity. The physiological and behavioral requirements for water conservation must be balanced against the requirements for development, but it is evident that elephant seal pups have the capacity to face them both.

The authors would like to thank the rangers at Año Nuevo State Reserve for their cooperation and the Earl and Ethel Myers Oceanographic Trust, the Friends of Long Marine Laboratory, and the Office of Naval Research (grant N00014-02-1-1012) for their generous financial contributions. Special thanks to P. Morris and G. Oliver for their helpful advice and tireless contributions in the field, and to D. Crocker for many valuable insights throughout the course of this study. Comments from two anonymous reviewers significantly improved this manuscript. This research constituted the MA thesis of C.W.L. This study was performed with the supervision and authorization of the UCSC Chancellor's Animal Research Committee and the National Marine Fisheries Service (Permit #836).

References

- Adams, S. H. (1991). *Changes in Protein Metabolism and Water Conservation in Northern Elephant Seal Pups During the Postweaning Fast*. MS thesis, Department of Marine Sciences, University of California, USA.
- Adams, S. H. and Costa, D. P. (1993). Water conservation and protein metabolism in northern elephant seal pups during the postweaning fast. *J. Comp. Physiol. B* **163**, 367-373.

- Bartholomew, G. A.** (1954). Body temperature and respiratory and heart rates in the northern elephant seal. *J. Mammal.* **35**, 211-218.
- Blackwell, S. B.** (1996). Terrestrial apnea in northern elephant seals, *Mirounga angustirostris*: development and role in water economy. PhD thesis, Department of Biology, University of California, USA.
- Blackwell, S. B. and Le Boeuf, B. J.** (1993). Developmental aspects of sleep apnoea in northern elephant seals, *Mirounga angustirostris*. *J. Zool. Lond.* **231**, 437-447.
- Bonner, W. N.** (1984). Lactation strategies in pinnipeds: problems for a marine mammalian group. In *Physiological Strategies in Lactation*. Vol. 51 (ed. C. H. Knight), pp. 253-272. London: Academic Press.
- Bowen, W. D., Boness, D. J. and Oftedal, O. T.** (1987). Mass transfer from mother to pup and subsequent mass loss by the weaned pup in the hooded seal, *Cystophora cristata*. *Can. J. Zool.* **65**, 1-8.
- Burns, J. M., Castellini, M. A. and Testa, J. W.** (1999). Movements and diving behavior of weaned Weddell seal (*Leptonychotes weddellii*) pups. *Polar Biol.* **21**, 23-26.
- Butler, P. J. and Jones, D. R.** (1982). The comparative physiology of diving in vertebrates. In *Advances in Comparative Physiology and Biochemistry*. Vol. 8 (ed. O. Lowenstein), pp. 179-364. New York: Academic Press.
- Caputa, M.** (1979). Temperature gradients in the nasal cavity of the rabbit. *J. Therm. Biol.* **4**, 283-286.
- Castellini, M. A.** (1996). Dreaming about diving: sleep apnea in seals. *News Physiol. Sci.* **11**, 208-214.
- Castellini, M. A., Kooyman, G. L. and Ponganis, P. J.** (1992). Metabolic rates of freely diving Weddell seals – correlations with oxygen stores, swim velocity and diving duration. *J. Exp. Biol.* **165**, 181-194.
- Castellini, M. A., Milsom, W., Berger, W., Costa, D. P., Jones, D., Castellini, J., Rea, L., Bahra, S. and Harris, M.** (1994a). Long duration sleep apnea in elephant seal pups. *Am. J. Physiol.* **266**, R836-R869.
- Castellini, M. A., Milsom, W. K., Berger, R. J., Costa, D. P., Jones, D. R., Castellini, J. M., Rea, L. D., Bharma, S. and Harris, M.** (1994b). Patterns of respiration and heart rate during wakefulness and sleep in elephant seal pups. *Am. J. Physiol.* **266**, R863-R869.
- Castellini, M. A., Rea, L. D., Sanders, J. L., Castellini, J. M. and Zentenosavin, T.** (1994c). Developmental changes in cardiorespiratory patterns of sleep-associated apnea in northern elephant seals. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **36**, R1294-R1301.
- Collins, J. C., Pilkington, T. C. and Schmidt-Nielsen, K.** (1971). A model of respiratory heat transfer in a small mammal. *Biophys. J.* **11**, 886-914.
- Costa, D. P.** (1982). Energy nitrogen and electrolyte flux and sea water drinking in the sea otter, *Enhydra lutris*. *Physiol. Zool.* **55**, 35-44.
- Costa, D. P.** (1993). The relationship between reproductive and foraging energetics and the evolution of the Pinnipedia. *Symp. Zool. Soc. Lond.* **66**, 293-314.
- Costa, D. P.** (2002). Osmoregulation. In *Encyclopedia of Marine Mammals* (ed. W. F. Perrin, B. Würsig and J. G. M. Thewissen), pp. 837-842. San Diego: Academic Press.
- Costa, D. P. and Gentry, R. L.** (1986). Free-ranging energetics of northern fur seals. In *Fur Seals: Maternal Strategies on Land and at Sea*. Vol. 18 (ed. R. L. Gentry and G. L. Kooyman), pp. 79-101. Princeton, NJ: Princeton University Press.
- Costa, D. P. and Trillmich, F.** (1988). Mass changes and metabolism during the perinatal fast: a comparison between Antarctic (*Arctocephalus gazella*) and Galapagos fur seals (*Arctocephalus galapagoensis*). *Physiol. Zool.* **61**, 160-169.
- Costa, D. P. and Williams, T. M.** (2000). Marine mammal energetics. In *The Biology of Marine Mammals* (ed. J. Reynolds and J. Twiss), pp. 176-217. Washington: Smithsonian Institution Press.
- Costa, D. P., Gales, N. J. and Goebel, M. E.** (2001). Aerobic dive limit: how often does it occur in nature? *Comp. Biochem. Physiol.* **129A**, 771-783.
- Crocker, D. E.** (1995). Reproductive effort and fasting physiology of female northern elephant seals, *Mirounga angustirostris*. PhD thesis, Department of Biology, University of California, USA.
- Crocker, D. E. and Costa, D. P.** (2002). Pinniped physiology. In *The Encyclopedia of Marine Mammals* (ed. W. F. Perrin, B. Würsig and J. G. M. Thewissen), pp. 926-931. San Diego: Academic Press.
- Crocker, D. E., Le Boeuf, B. J. and Costa, D. P.** (1997). Drift diving in female northern elephant seals: implications for food processing. *Can. J. Zool.* **75**, 27-39.
- Crocker, D. E., Webb, P. M., Costa, D. P. and Le Boeuf, B. J.** (1998). Protein catabolism and renal function in lactating northern elephant seals. *Physiol. Zool.* **71**, 485-491.
- Crocker, D. E., Williams, J. D., Costa, D. R. and Le Boeuf, B. J.** (2001). Maternal traits and reproductive effort in northern elephant seals. *Ecology* **82**, 3541-3555.
- Drabek, C. M. and Kooyman, G. L.** (1984). Histological development of the terminal airways in pinniped and sea otter *Enhydra lutris* lungs. *Can. J. Zool.* **62**, 92-96.
- Folkow, L. P. and Blix, A. S.** (1987). Nasal heat and water exchange in gray seals. *Am. J. Physiol.* **253**, R883-R889.
- Geist, N. R.** (2000). Nasal respiratory turbinate function in birds. *Physiol. Biochem. Zool.* **73**, 581-589.
- Gentry, R. L.** (1981). Sea water drinking in eared seals. *Comp. Biochem. Physiol.* **68A**, 81-86.
- Goldberg, M. B., Langman, V. A. and Taylor, C. R.** (1981). Panting in dogs: paths of air flow in response to heat and exercise. *Respir. Physiol.* **43**, 327-338.
- Gould, S. J. and Vrba, E. S.** (1982). Exaptation – a missing term in the science of form. *Paleobiology* **8**, 415.
- Hammarlund, K., Riesenfeld, T. and Sedin, G.** (1986). Measurement of respiratory water loss in newborn lambs. *Acta Physiol. Scand.* **127**, 61-65.
- Hammond, D. D., Elsner, R., Simison, G. and Hubbard, R.** (1968). Submersion bradycardia in the new born elephant seal, *Mirounga angustirostris*. *Am. J. Physiol.* **216**, 220-222.
- Houser, D. S. and Costa, D. P.** (2001). Protein catabolism in suckling and fasting northern elephant seal pups (*Mirounga angustirostris*). *J. Comp. Physiol. B* **171**, 635-642.
- Hui, C. A.** (1981). Seawater consumption and water flux in the common dolphin *Delphinus delphis*. *Physiol. Zool.* **54**, 430-440.
- Huntley, A. C.** (1984). *Relationships Between Metabolism, Respiration, Heart Rate and Arousal States in the Northern Elephant Seal*. MA thesis, Department of Biology, University of California, USA.
- Huntley, A. C., Costa, D. P. and Rubin, R. D.** (1984). The contribution of nasal countercurrent heat exchange to water balance in the northern elephant seal, *Mirounga angustirostris*. *J. Exp. Biol.* **113**, 447-454.
- Hurley, J. A. and Costa, D. P.** (2001). Standard metabolic rate at the surface and during trained submersions in adult California sea lions (*Zalophus californianus*). *J. Exp. Biol.* **204**, 3273-3281.
- Irving, L., Fisher, K. C. and McIntosh, F. C.** (1935). The water balance of a marine mammal, the seal. *J. Cell. Comp. Physiol.* **6**, 387-391.
- Irving, L., Scholander, P. F. and Grinnell, S. W.** (1941). The respiration of the porpoise, *Tursiops truncatus*. *J. Cell. Comp. Physiol.* **17**, 145-168.
- Jackson, D. C. and Schmidt-Nielsen, K.** (1964). Countercurrent heat exchange in the respiratory passages. *Proc. Natl. Acad. Sci. USA* **51**, 1192-1197.
- King, J. E.** (1983). *Seals of the World*. Ithaca, NY: Cornell University Press.
- Knopper, L. D. and Boily, P.** (2000). Relationship between apnea duration and rate of oxygen consumption in captive female gray seals while sleeping or resting on land. *Mar. Mamm. Sci.* **16**, 676-681.
- Kohin, S., Williams, T. M. and Ortiz, C. L.** (1997). Metabolic effects of hypoxia and hypercapnia in Northern elephant seal pups. In *Annual Meeting of the Professional Research Scientists on Experimental Biology 97, New Orleans, Louisiana, USA, April 6-9, 1997*.
- Kooyman, G. L.** (1973). Respiratory adaptations in marine mammals. *Am. Zool.* **13**, 457-468.
- Kooyman, G. L. and Drabek, C. M.** (1968). Observations on milk blood and urine constituents of the Weddell seal, *Leptonychotes weddelli*. *Physiol. Zool.* **41**, 187-194.
- Kramer, D. L.** (1988). The behavioral ecology of air breathing by aquatic animals. *Can. J. Zool.* **66**, 89-94.
- Langman, V. A., Maloio, G. M. O., Schmidt-Nielsen, K. and Schroter, R. C.** (1979). Nasal heat exchange in the giraffe (*Giraffa camelopardalis*) and other large mammals. *Respir. Physiol.* **37**, 325-334.
- Lea, M. A., Bonadonna, F., Hindell, M. A., Guinet, C. and Goldsworthy, S. D.** (2002). Drinking behaviour and water turnover rates of Antarctic fur seal pups: implications for the estimation of milk intake by isotopic dilution. *Comp. Biochem. Physiol.* **132A**, 321-331.
- Le Boeuf, B. J.** (1974). Male-male competition and reproductive success in elephant seals. *Am. Zool.* **14**, 163-176.
- Le Boeuf, B. J., Morris, P. A., Blackwell, S. B., Crocker, D. E. and Costa, D. P.** (1996). Diving behavior of juvenile northern elephant seals. *Can. J. Zool.* **74**, 1632-1644.
- Le Boeuf, B. J., Crocker, D. E., Costa, D. P., Blackwell, S. B., Webb, P. M. and Houser, D. S.** (2000). Foraging ecology of northern elephant seals. *Ecol. Monogr.* **70**, 353-382.
- Lydersen, C., Kovacs, K. M. and Hammill, M. O.** (1997). Energetics during nursing and early postweaning fasting in hooded seal (*Cystophora cristata*)

- pups from the Gulf of St. Lawrence, Canada. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **167**, 81-88.
- Milson, W., Castellini, M., Harris, M., Castellini, J., Jones, D., Berger, R., Bahra, S., Rea, L. and Costa, D.** (1996). Effects of hypoxia and hypercapnia on patterns of sleep-associated apnea in elephant seal pups. *Am. J. Physiol.* **271**, R1017-R1024.
- Murrish, D. E.** (1973). Respiratory heat and water exchange in penguins. *Respir. Physiol.* **19**, 262-270.
- Noren, D. P.** (2002a). Body energy reserve utilization during the postweaning fast of northern elephant seals (*Mirounga angustirostris*): implications for survival. PhD thesis, Department of Ecology and Evolutionary Biology, University of California, USA.
- Noren, D. P.** (2002b). Thermoregulation of weaned northern elephant seal (*Mirounga angustirostris*) pups in air and water. *Physiol. Biochem. Zool.* **75**, 513-523.
- Noren, D. P., Crocker, D., Williams, T. M. and Costa, D. P.** (2003). Energy reserve utilization in northern elephant seal (*Mirounga angustirostris*) pups during the postweaning fast: size does matter. *J. Comp. Physiol. B* **173**, 443-454.
- Oftedal, O. T., Bowen, W. D., Widdowson, E. M. and Boness, D. J.** (1989). Effects of suckling and the postsuckling fast on weights of the body and internal organs of harp and hooded seal pups. *Biol. Neonate* **56**, 283-300.
- Ortiz, C. L., Costa, D. and Le Boeuf, B. J.** (1978). Water and energy flux in elephant seal pups fasting under natural conditions. *Physiol. Zool.* **51**, 166-178.
- Ortiz, C. L., Le Boeuf, B. J. and Costa, D. P.** (1984). Milk intake of elephant seal (*Mirounga angustirostris*) pups: an index of parental investment. *Am. Nat.* **124**, 416-422.
- Ortiz, R. M.** (2001). Osmoregulation in marine mammals. *J. Exp. Biol.* **204**, 1831-1844.
- Ortiz, R. M., Adams, S. H., Costa, D. P. and Ortiz, C. L.** (1996). Plasma vasopressin levels and water conservation in fasting, postweaned northern elephant seal pups (*Mirounga angustirostris*). *Mar. Mamm. Sci.* **12**, 99-106.
- Ortiz, R. M., Wade, C. E. and Ortiz, C. L.** (2000). Prolonged fasting increases the response of the renin-angiotensin-aldosterone system, but not vasopressin levels, in postweaned northern elephant seal pups. *Gen. Comp. Endocrinol.* **119**, 217-223.
- Pace, N. and Rathbun, E. N.** (1945). Studies on body composition. II. The body water and chemically combined nitrogen content in relation to fat content. *J. Biol. Chem.* **158**, 685-691.
- Pernia, S. D., Costa, D. P. and Ortiz, C. L.** (1989). Glomerular filtration rate in weaned elephant seal pups during natural long term fasts. *Can. J. Zool.* **67**, 1752-1756.
- Rea, L. D. and Costa, D. P.** (1992). Changes in standard metabolism during long-term fasting in northern elephant seal pups (*Mirounga angustirostris*). *Physiol. Zool.* **65**, 97-111.
- Reiter, J., Stinson, N. L. and Le Boeuf, B. J.** (1978). Northern elephant seal development: the transition from weaning to nutritional independence. *Behav. Ecol. Sociobiol.* **3**, 337-367.
- Renouf, D., Noseworthy, E. and Scott, M. C.** (1990). Daily fresh water consumption by captive harp seals (*Phoco groenlandica*). *Mar. Mamm. Sci.* **6**, 253-257.
- Ridgway, S. H. (ed.)** (1972). Homeostasis in the aquatic environment. In *Mammals of the Sea: Biology and Medicine*, pp. 590-747. Springfield, IL: Charles C. Thomas.
- Schmidt-Nielsen, K., Hainsworth, F. R. and Murrish, D. E.** (1970). Counter-current heat exchange in the respiratory passages: effect on water and heat balance. *Respir. Physiol.* **9**, 263-276.
- Scholander, P. F.** (1940). Experimental investigations on the respiratory function in diving mammals and birds. *Hvalradets Skrifter* **22**, 1-131.
- Stanton, B. A.** (1991). Molecular mechanisms of ANP inhibition of renal sodium transport. *Can. J. Physiol. Pharmacol.* **69**, 1546-1552.
- Stewart, B. S., Yochem, P. K., Huber, H. R., Delong, R. L., Jameson, R. J., Sydeman, W. J., Allen, S. G. and Le Boeuf, B. J.** (1994). History and present status of the northern elephant seal population. In *Elephant Seals: Population Ecology, Behavior, and Physiology* (ed. B. J. Le Boeuf and R. M. Laws), pp. 29-48. Berkeley, CA: University of California Press.
- Storeheier, P. V. and Nordøy, E. S.** (2001). Physiological effects of seawater intake in adult harp seals during phase I of fasting. *Comp. Biochem. Physiol.* **128A**, 307-315.
- Thorson, P. H. and Le Boeuf, B. J.** (1994). Developmental aspects of diving in northern elephant seal pups. In *Elephant Seals: Population Ecology, Behavior, and Physiology* (ed. B. J. Le Boeuf and R. M. Laws), pp. 271-289. Berkeley, CA: University of California Press.
- Varène, P., Ferrus, L., Manier, G. and Gire, J.** (1986). Heat and water respiratory exchanges: comparison between mouth and nose breathing in humans. *Clin. Physiol.* **6**, 405-414.
- Williams, T. M., Fuiman, L. A., Horning, M. and Davis, R. W.** (2004). The cost of foraging by a marine predator, the Weddell seal *Leptonychotes weddellii*: pricing by the stroke. *J. Exp. Biol.* **207**, 973-982.
- Withers, P. C.** (1977). Measurement of oxygen consumption carbon dioxide production and evaporative water loss with a flow through mask. *J. Appl. Physiol.* **42**, 120-123.
- Zenteno-Savin, T. and Castellini, M. A.** (1998). Changes in the plasma levels of vasoactive hormones during apnea in seals. *Comp. Biochem. Physiol.* **119C**, 7-12.

Table S1. Summarized data for individual seals

Seal	Sex	Total time of data intervals used (min)	Mass at weighing (kg)	Mean MR (ml O ₂ kg min ⁻¹)		Mean REWL (g min ⁻¹)		Mean MWP (g min ⁻¹)		Mean water loss ratio (REWL/MWP)		Mean water loss per breath (g)	
				W-phase*	S-phase [†]	W-phase*	S-phase [†]	W-phase*	S-phase [†]	W-phase*	S-phase [†]	W-phase*	S-phase [†]
2003													
A	F	207.4	94.0	2.965	2.723	0.057	0.036	0.144	0.128	0.396	0.281	0.012	0.017
B	F	178.1	87.2	3.335	3.050	0.062	0.039	0.153	0.140	0.405	0.279	0.011	0.015
C	M	156.3	135.0	3.345	1.713	0.073	0.042	0.181	0.120	0.403	0.350	0.013	0.017
D	F	244.0	56.0	5.617	4.522	0.068	0.046	0.162	0.130	0.420	0.354	0.013	0.015
E	M	142.4	110.2	4.551	2.402	0.071	0.039	0.181	0.136	0.392	0.287	0.022	0.027
2004													
F	M	72.4	83.2	5.627	5.118	0.087	0.053	0.156	0.141	0.558	0.376	0.010	0.011
G	F	192.6	96.0	3.529	2.489	0.088	0.048	0.177	0.129	0.497	0.372	0.012	0.014
H	M	129.5	112.2	2.637	2.136	0.098	0.056	0.172	0.125	0.570	0.448	0.015	0.015
I	F	153.9	85.0	2.762	2.543	0.080	0.039	0.130	0.108	0.615	0.361	0.012	0.012
J	M	218.6	67.4	2.817	2.600	0.061	0.048	0.108	0.093	0.565	0.516	0.009	0.013
K	M	179.2	88.6	2.866	2.509	0.064	0.045	0.134	0.120	0.478	0.375	0.009	0.012
L	M	149.5	110.4	2.417	1.624	0.092	0.040	0.210	0.104	0.438	0.385	0.014	0.011
M	M	149.5	76.6	2.596	2.333	0.073	0.044	0.110	0.096	0.664	0.458	0.012	0.012
Mean values		167.2	92.4	3.5	2.8	0.075	0.044	0.155	0.121	0.492	0.372	0.013	0.015

*No apneas exceeding 1 min.

[†]Intervals included apneas of 1 min or more.

Table S1. *Continued*

Seal	Sex	Mean apnea duration [†] (min)	Ratio of apnea duration/interval duration [†]	Mean breaths min ⁻¹ during eupnea		Mean temp (°C)	Mean absolute humidity (g H ₂ O g m ⁻³)
				W-phase*	S-phase [†]		
2003							
A	F	6.56	0.72	4.81	7.37	16.4	8.112
B	F	4.73	0.68	5.72	9.03	17.4	8.719
C	M	7.23	0.74	5.61	10.24	17.8	8.009
D	F	4.87	0.61	5.07	15.33	18.2	9.006
E	M	5.87	0.72	3.17	8.92	19.1	9.979
2004							
F	M	4.21	0.81	4.05	8.64	19.9	8.570
G	F	6.49	0.70	5.90	10.19	20.0	8.185
H	M	3.63	0.58	6.00	9.16	19.8	8.242
I	F	4.25	0.64	5.96	8.63	20.2	9.893
J	M	5.17	0.65	4.94	8.44	21.7	9.664
K	M	3.86	0.63	6.05	9.67	21.3	10.373
L	M	4.96	0.68	4.73	9.20	20.0	9.206
M	M	5.05	0.68	5.01	9.56	20.0	9.564
Mean values		5.14	0.68	5.15	9.57	19.4	9.040

*No apneas exceeding 1 min.

[†]Intervals included apneas of 1 min or more.