

# Evidence of seawater drinking in fasting subadult hooded seals (*Cystophora cristata*)

Fernando Alvira-Iraizoz · Erling Sverre Nordøy

**F Alvira-Iraizoz** (Corresponding author) · **ES Nordøy**  
Arctic Chronobiology and Physiology research group,  
Department of Arctic and Marine Biology, UiT-the Arctic  
University of Norway, Postbox 6050, Langnes, Tromsø,  
Norway.

email: f.alvirairaizoz@gmail.com

Received: October 18, 2018 · Revised: November 22, 2018 · Accepted: November 22, 2018

**Abstract** The purpose of this study was to investigate the total water turnover rate of fasting subadult hooded seals in order to elucidate to what extent these animals rely in seawater drinking/mariposia at this life stage. Considering mariposia is important for later accurate estimations of food consumption using water turnover rate as a proxy. Five subadult hooded seals were kept fasting for 4 days in a seawater pool. Total body water ( $39.6 \pm 2.5$  % of total body mass) decreased by  $3.1 \pm 0.4$  % of initial body water over the experimental period. Turnover rates were  $16.7 \pm 3.9$  (influx) and  $24.6 \pm 4.6$  (efflux)  $\text{ml} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$  with a net water loss of  $710 \pm 51$   $\text{ml} \cdot \text{day}^{-1}$ . It was estimated that the seals drank approximately 947 ml of seawater per day, which corresponds to 61 % of total daily water influx. Initial body water was relatively low as a result of the high body fat ( $46.9 \pm 3.2$  % of initial body mass) shown in the animals. It is concluded that subadult fasting hooded seals drink significant amounts of seawater during fasting. Although mariposia stands out as the major source of free water in fasting hooded seals, the amount of seawater ingested is unlikely to provide a net gain of free water as it is provided by metabolic water. However, it may contribute to excrete the excess of urea produced during early phase I of fasting.

**Keywords:** marine mammals, mariposia, total body water, turnover rate, water homeostasis

## Introduction

Water balance in pinnipeds has been previously studied by use of the labelled-water method in order to understand the basic physiology underlying water homeostasis (Depocas et al 1971; How and Nordøy 2007; Lea et al 2002; Nordøy et al 1992; Ortiz et al 1978; Ortiz et al 2002; Skalstad and Nordøy 2000). The labelled-water method has also been used to estimate body composition and milk and food intake (Arnould et al 1996; Beauplet et al 2003; Carlini et al 2001; Crocker et al 2012; Donohue et al 2002; Lydersen and Hammill 1993; Lydersen and Kovacs 1996; Lydersen et al 1997; Noren et al

2003; Noren et al 2008; Reilly and Fedak 1990) in different stages of life of pinnipeds. Therefore, accurate estimates of water turnover rate ( $r\text{H}_2\text{O}$ ) are important to acquire a better insight about how pinnipeds maintain water homeostasis and to perform precise estimates of food/milk intake at different life stages.

Water homeostasis of a number of pinnipeds, such as the hooded seal (*Cystophora cristata*) for instance, is challenged throughout the entire life since such seal species spend most of their lifetime either swimming/diving in open waters or fasting. Folkow et al (2010), using satellite telemetry, determined the overall time spent in open seawater by young of the year hooded seals to be 76 %, covering a vast area of the north Atlantic. Subadult and adult (2-10 years old) hooded seals (Jan Mayen stock) also disperse after the moulting/breeding periods and their positions have been recorded by satellite-linked transmitters as far as southwest of Iceland, British Isles, Faeroe Islands, south of Bear Island, the Norwegian Sea and Svalbard. The trips performed by those animals lasted on average  $47 \pm 22$  days ( $n = 46$ ) (Folkow et al 1996). The northwest Atlantic subpopulation (Newfoundland and Labrador) have also been shown to travel large distances (Andersen et al 2009). During these migratory movements, seals have no access to fresh water. Moreover, hooded seals accomplish long post-weaning, moulting and breeding fasts (Folkow et al 1996; Lydersen et al 1997). In this case, they neither consume any food. The major effects of fasting have been described as profound mass loss, reduced metabolic rate, increased plasma osmolality, reduction in urine production and, ultimately, dehydration (Beauplet et al 2003; Nordøy et al 1993; Nordøy et al 1990; Nordøy et al 1992; Rosen and Trites 2002).

A number of studies have shown that pinnipeds effectively restrict water loss by a number of different adaptations. The efflux routes are respiratory evaporative water loss (REWL), urine, cutaneous evaporative water loss (CEWL) and water in faeces. Studies of the rate of REWL in

grey seals (*Halichoerus grypus*) have shown that water loss through respiration is relatively low due to a very well developed nasal heat and water exchange mechanism (Folkow and Blix 1987; Skog and Folkow 1994). In addition, Lester and Costa (2006) showed that apnoeic breathing, together with the complex nasal turbinates, allows northern elephant seals (*Mirounga angustirostris*) to reduce REWL by 41 %. Urine production, moreover, may also be effectively reduced when water is scarce due to a very high concentrating ability of the kidneys (Hong et al 1982; Storeheier and Nordøy 2001). Various studies of different species have shown that urine production is considerably reduced during fasting periods (Nordøy et al 1993; Nordøy et al 1990). CEWL has been generally considered very low in pinnipeds living in cold environments. In such conditions, seals increase or reduce body heat loss by adjusting perfusion to the flippers and heat transfer through the blubber (Kvadsheim and Folkow 1997). Nordøy et al (1992) estimated CEWL in fasting grey seal pups resting on land to be only about  $3 \text{ ml} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$  and active sweating has only been reported in California sea lions (*Zalophus californianus*) (Matsuura and Whittow 1974). Water in faeces in fasting seals is negligible since no food is ingested.

As mentioned before, during long migratory periods the seals have no access to fresh water in form of ice/snow. Snow and ice may be a source of water when hauling out on pack ice as described by Renouf et al (1990) in harp seals (*Phoca groenlandica*). Thus, most of water influx during these prolonged journeys in open waters must be obtained from free and metabolic water from food, inspired water and seawater drinking (mariposia) (Skalstad and Nordøy 2000). Depocas et al (1971) estimated inspired water rate to be  $93\text{--}108 \text{ ml} \cdot \text{day}^{-1}$  in harbor seals (*Phoca vitulina*) while Skalstad and Nordøy (2000) calculated respiratory influx to be about  $114 \text{ ml} \cdot \text{day}^{-1}$  or 5 % of total water influx in hooded seals fed capelin. Regarding seawater drinking, early studies concluded that marine mammals do not voluntarily drink seawater (Depocas et al 1971; Fetcher and Fetcher 1942; Irving et al 1935) and that mariposia is not an effective mechanism to obtain water (Tarasoff and Toews 1972). However, studies by Gentry (1981) and Skalstad and Nordøy (2000) showed evidences of spontaneous mariposia in a number of pinnipeds. Moreover, experimentally dehydrated harp seals were able to restore water balance with ad libitum access to seawater (How and Nordøy 2007). Early studies of ringed (*Pusa hispida*) and Baikal (*Pusa sibirica*) seals showed that these species are able to concentrate urine up to  $2000 \text{ mOsm} \cdot \text{kg}^{-1}$ , well above seawater concentration, which serves as a remarkable mechanism for water conservation (Hong et al 1982) and reinforces the potential of mariposia. Unfortunately, direct measurement of the amount of water entering the body by each of those routes in wild subadult hooded seals is nearly impossible. When food is deprived (fasting), catabolism of

subcutaneous fat has proven to be the main source of energy and water (Arnould et al 2001; Castellini et al 1987; Nordøy et al 1993; Ortiz et al 1978; Verrier et al 2009). Up to 1.07 grams of free water are produced per 1 gram of fat metabolized (Schmidt-Nielsen 1964).

The purpose of this study was, by use of a bolus injection of tritiated water ( $^3\text{H}_2\text{O}$ ), to estimate the total water influx (rH<sub>2</sub>O) in order to elucidate to what extent fasting subadult hooded seals depend on seawater drinking/mariposia at this life stage. An accurate estimation of the level of mariposia will improve our understanding of the water balance of young hooded seals, which may result in more accurate estimations of food and milk consumption when using the labelled water method.

## Materials and Methods

### Animals

Five subadult hooded seals, 3 females (no. 1, 3 and 5) and 2 males (no. 2 and 4), aged 1.5 years were used in this study. The animals were captured as weanlings in the Greenland Sea (70°N, 16°W) and transported on board RV “Helmer Hanssen” to the research facility of the Arctic Chronobiology and Physiology research group at UiT-the Arctic University of Norway. The animals were kept in the facilities in a 42000 l water tank with continuous exchange of seawater and exposed to simulated (70°N) seasonal variation in day length prior the experiment onset. This study was carried out in strict accordance with the recommendations of the Animal Welfare Act of Norway and the Norwegian Regulation on Animal Experimentation. All applicable institutional, national and/or international guidelines for the care and use of animals were followed. All procedures performed involving animals were in accordance with the ethical standards of the Norwegian Ethical Committee for Animal Research (Permit Number: FOTS ID: 5422). During an experimental period of four days, the animals were fasted in order to investigate this status. Initial body mass (BM<sub>i</sub>) ranged from 83.4 to 103.5 kg (Table 1) and seals appeared to be in excellent health before, during and after the experimental period.

**Table 1** Seal identification number (ID #), sex (F: female, M: male) and initial body mass (BM<sub>i</sub>) of the 5 seals used in the study.

ID #	Sex	BM <sub>i</sub> (kg)
Seal 1	F	103.5
Seal 2	M	87.6
Seal 3	F	96.4
Seal 4	M	83.4
Seal 5	F	87.5

### Experimental protocol

The seals were fasted for 24 h in the seawater tank prior to onset of the experiment. Then, a 1.0 ml intramuscular injection of a sedative (50 mg · ml<sup>-1</sup> tiletamine-50 mg · ml<sup>-1</sup> zolazepam, Zoletil Forte Vet, Reading, L 'Hay-Les-Roses, France) was administered and the animals were weighted (Dini Argeo MCWNT 1RF-1; 10-150 kg, accuracy ±0.5 kg; Spezzano di Fiorano, Italy). The animals were then restrained on an especially designed board and two catheters (Selacont™ 16G/1.70x160 mm, The Hague, the Netherlands) inserted into the extradural intravertebral vein (EDV) at the level of the 1<sup>st</sup> and 4<sup>th</sup> lumbar vertebra. After that, a bolus dose of <sup>3</sup>H<sub>2</sub>O (1 - 2.5 μCi · kg<sup>-1</sup>) was injected, as described by Reilly and Fedak (1990), through the catheter located at the 1<sup>st</sup> lumbar vertebra (EDV1). A volume of 10 ml of saline (9 mg · ml<sup>-1</sup> NaCl, Melsungen, Germany) was gently flushed through the catheter after the injection. Where after an additional 5 ml of saline were used to rinse the outer part of the catheter. Blood samples were collected from the catheter inserted at the level of the 4<sup>th</sup> lumbar vertebra (EDV2) 30, 60 and 90 minutes after <sup>3</sup>H<sub>2</sub>O administration. After every blood sampling the catheter was gently flushed with 10 ml of saline in order to avoid contamination of subsequent blood samples and blood clotting. After the initial <sup>3</sup>H<sub>2</sub>O experiment, the seals were reintroduced into the seawater tank. After 96 h of fasting with ad libitum access to seawater, seals were restrained on the same board and blood samples collected from the EDV in order to estimate rH<sub>2</sub>O. All blood samples were immediately centrifuged for 10 min at 2000 rpm (Kubota KS-8000, Tokyo, Japan) and plasma was transferred to cryovials (VWR International, Norway) and stored at -80°C for later analysis.

In addition, blood was sampled from the femoral vein to determine whether sampling site might have an effect when determining rH<sub>2</sub>O using labelled water. Nordgarden et al (2000) measured the directional flow in the extradural intravertebral vein (EDV) by use of Doppler flowmeter from non-diving to the diving situation in harp seals (*Phoca groenlandica*) and demonstrated changes in flow direction, although not correlated with diving. Pinnipeds have also proven to reduce their circulation to the flippers during exposure to low ambient temperatures (Hammel et al 1977). The major veins of the hind flippers are another important site for sampling blood. One may speculate that such circulatory changes may affect the rate of dilution of a radiolabelled substrate when using veins in these locations for sampling. However, no statistical differences between the specific activity (SA) of plasma samples from any of the sampling sites were found, therefore, this issue was not further investigated.

All plasma samples were deproteinised with 70 % perchloric acid (HClO<sub>4</sub>) (200 μl · ml<sup>-1</sup> plasma) for protein precipitation prior scintillation counting (Bray 1960). An aliquot of 0.5 ml deproteinized plasma was added to 9.5 ml of

scintillation fluid Ultima Gold™ (LSC cocktail, Sigma-Aldrich Química S.L., Tres Cantos, Madrid) and analysed in triplicates to determine radioactivity of <sup>3</sup>H<sub>2</sub>O in a liquid scintillation counter (Packard 1900 TR Beta-Teller Liquid Scintillation Analyser, Oslo, Norway) (How and Nordøy 2007; Reilly and Fedak 1990; Skalstad and Nordøy 2000). A set of standards was run prior every counting. The resulting data was adjusted to the real water content of deproteinized plasma samples. Plasma water content was calculated by desiccation of 1.0 ml plasma aliquots at 60 °C for 24 hours and HClO<sub>4</sub> water content was assumed 30%. Background level of tritium in plasma, 69 ± 54 cpm · ml<sup>-1</sup> (n = 5), was subtracted from SA at equilibrium (SA<sub>0</sub>).

### Body composition

Initial total body fat (TBF<sub>i</sub>) was calculated as described by Reilly and Fedak (1990) for grey seals: %TBF<sub>i</sub> = 105.1 - 1.47 (%TBW) where, %TBF<sub>i</sub> is the initial body fat as a percentage of body mass and %TBW is the total body water as percentage of body mass. Thus, initial lean body weight (LBW<sub>i</sub>) was calculated as %LBW<sub>i</sub> = 100 - %TBF<sub>i</sub>.

### Total body water (TBW) and rH<sub>2</sub>O

TBW was calculated as described by Lifson and McClintock (1966): TBW = i.d. / SA<sub>0</sub> where; i.d. is the injected dose in counts per minute (cpm) and SA<sub>0</sub> is the specific activity of <sup>3</sup>H<sub>2</sub>O in plasma at equilibrium in cpm · ml<sup>-1</sup>. rH<sub>2</sub>O, total water influx and efflux, was calculated using equations 4 and 6 provided by Nagy and Costa (1980) assuming a linear change in TBW. The amount of water entering and leaving through different routes from the main TBW pool was then estimated. According to Nordøy and Blix (1985), Nordøy et al (1990) and Reilly (1991), 94 % of the energy expended during the fasting period is derived from subcutaneous fat in grey seal pups. Moreover, Nordøy et al (1993) showed that only 2 - 4 % of the energy expended by harp seal pups was derived from protein catabolism after day 3 of fasting. To simplify calculations, it was assumed that fasting subadult hooded seals cover the energy expenditure by catabolizing fat exclusively. Thus, BM loss throughout the experimental period corresponded to subcutaneous fat loss. Metabolic water was calculated as a function of grams of fat metabolized, thus, 1 g of fat metabolized produced 1.07 g of water. Respiratory water influx was calculated as follows. Assuming 100 % relative humidity in air (Skalstad and Nordøy 2000), the partial pressure of water vapour (P<sub>H<sub>2</sub>O</sub>) in inspired air was calculated for the given room temperature (6.5 °C). Respiratory day volume was calculated from Folkow and Blix's equation (Folkow and Blix 1987): RMV = 0.042 · ADMR + 0.119; where RMV is respiratory minute volume in l · min<sup>-1</sup> and ADMR is the average daily metabolic rate

expressed in Watts. Then, daily water vapour influx ( $W_i$ ) was calculated using:  $W_i = RDV \cdot P_{H_2O} / 760$ ; where RDV is respiratory day volume and 760 is the standard pressure in air in mmHg.  $W_i$  was converted to  $ml \cdot day^{-1}$  of absorbed water using molar volume at the specific temperature and molar mass of water. Finally, the extent of mariposa was determined by subtracting metabolic water and respiratory influx from the total water influx determined by the  $^3H_2O$  method.

REWL was calculated using a similar approach as when calculating respiratory influx. Exhaled air temperature was estimated in grey seals, according to Folkow and Blix (1987), to be 29° C at the given room temperature.  $P_{H_2O}$  was adjusted to this temperature and introduced in the calculations previously described to determine REWL. CEWL was considered to be negligible in actively swimming hooded seals at a low ambient temperature since submersion and blood flow adjustments to the appendages are considered the ideal way to decrease body heat (Kvadsheim and Folkow 1997) rather than evaporation. Water loss in faeces was also assumed negligible in fasting seals (Nordøy et al 1992; Storeheier and Nordøy 2001). Urine production was determined by subtracting REWL from total water efflux.

$rH_2O$  was corrected for exchange using equation 39 provided by Lifson and McClintock (1966). Fractionation was corrected using a transformed version of equation 31 presented by Lifson and McClintock (1966):  $r_{H_2O}(calc) = r_{H_2O} (xf_1 + 1 - x)$ ; where  $r_{H_2O}(calc)$  is the corrected turnover rate,  $r_{H_2O}$  is the efflux rate,  $x$  is the fraction of turnover subjected to fractionation and  $f_1$  is the fractionation factor ( $f_1 = 0.93$ , according to Sepall and Mason (1960)). Exchange and

fractionation combined amounted in average ( $n = 5$ ) to an overall 6 % or  $104 ml \cdot day^{-1}$  overestimation of  $rH_2O$  which were subtracted from total water efflux.

*Statistical analysis*

Means for BM and radioactivity of each animal after isotope equilibration were compared to those at the end of the experimental period by T-test for paired samples. Data sets of each seal were previously analysed for normality using the Shapiro-Wilk test (for small sample sizes). In all cases, the data sets followed a normal distribution ( $p > 0.05$ ). Pearson correlation tests were run to investigate associations between body composition and  $rH_2O$ . Correlation was considered significant when  $p < 0.05$ . Pearson correlation coefficient was considered high when  $r < 0.5$  and assumed to be the most representative parameter due to the reduced sample size. All statistical analyses were carried out using SPSS-software.

**Results**

BM showed a significant ( $p < 0.05$ ) linear decrease down to an average ( $n = 5$ ) of  $96.9 \pm 0.4$  % of  $BM_i$  during the 96 h of experimental fasting. This amounted to  $710 \pm 58 g \cdot day^{-1}$ . Assuming all energy to be derived from subcutaneous fat reserves the amount of BM loss corresponded to an average ( $n = 5$ ) loss of  $2.8 \pm 0.2$  kg (Table 2) of fat/water over four days of fasting.  $TBF_i$  was in average ( $n = 5$ )  $43.1 \pm 5.4$  kg or  $46.9 \pm 3.2$  % of  $BM_i$  while  $LBW_i$  amounted in average ( $n = 5$ ) to  $48.5 \pm 3.9$  kg or  $53.1 \pm 3.2$  % of  $BM_i$  (Table 2).

**Table 2** Initial body mass in kg ( $BM_i$ ), final body mass in kg ( $BM_f$ ), total body mass loss in kg after 4 days of experimental fasting ( $\Delta BM$ ), initial total body fat in kg ( $TBF_i$ ), percentage of initial total body fat (% $TBF_i$ ), initial lean body weight in kg ( $LBW_i$ ), percentage of initial lean body weight (% $LBW_i$ ) for 5 subadult hooded seals. Average ( $n = 5$ ) values  $\pm$  SD are given in the bottom line.

	$BM_i$ (kg)	$BM_f$ (kg)	$\Delta BM$ (kg)	$TBF_i$ (kg)	% $TBF_i$	$LBW_i$ (kg)	% $LBW_i$
Seal 1	103.5	100.6	2.9	52.9	51.1	50.6	48.9
Seal 2	87.6	85.0	2.6	43.6	49.7	44.0	50.3
Seal 3	96.4	93.6	2.8	42.5	44.0	53.9	56.0
Seal 4	83.4	80.2	3.2	39.4	47.2	44.0	52.8
Seal 5	87.5	84.8	2.7	37.3	42.7	50.2	57.3
Av $\pm$ SD	91.68 $\pm$ 8.1	88.84 $\pm$ 8.2	2.8 $\pm$ 0.2	43.1 $\pm$ 5.4	46.9 $\pm$ 3.2	48.5 $\pm$ 3.9	53.1 $\pm$ 3.2

Av: Average; SD: Standard deviation

Tritium turnover rate was assumed to follow a linear decrease (Figure 1) and, thus,  $rH_2O$  was calculated assuming linear changes in TBW. Tritium activity in TBW decreased in average ( $n = 5$ )  $16 \pm 3$  % in 4 days of fasting.

TBW was on average ( $n = 5$ )  $39.6 \pm 2.5$  % of  $BM_i$ . Total water influx and efflux were calculated to be on average ( $n = 5$ )  $1491 \pm 294 ml \cdot day^{-1}$  or  $16.7 \pm 3.9 ml \cdot day^{-1} \cdot kg^{-1}$  and  $2200 \pm 333 ml \cdot day^{-1}$  or  $24.6 \pm 4.6 ml \cdot day^{-1} \cdot kg^{-1}$ , respectively. Overall daily water loss amounted to  $710 \pm 51$

$ml \cdot day^{-1}$  (Table 3). Net water loss matched BM loss as the result of assuming fat to be the only metabolic substrate during fasting. There was no correlation between  $BM_i$  and influx and/or efflux rates ( $0 < r < 0.5$ ). However, a high negative correlation ( $r < -0.5$ ) was shown between  $TBF_i$  and efflux or influx rates and all water pathways. In other words, a high  $TBF_i$  may contribute to reduce overall  $rH_2O$ . Lastly, no correlation was found ( $r \approx 0$ ) between  $LBW_i$  and efflux and/or influx rates.

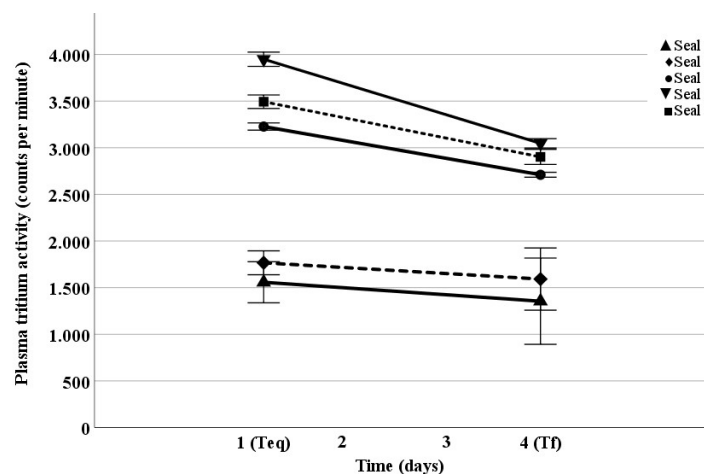
Respiratory influx, metabolic water and mariposia amounted to 6 % ( $1.0 \pm 0.0 \text{ ml} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$ ), 33 % ( $5.3 \pm 0.1 \text{ ml} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$ ) and 61 % ( $10.5 \pm 3.8 \text{ ml} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$ ) of total water influx, respectively. REWL and urine production amounted to 16 % ( $3.8 \pm 0.1 \text{ ml} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$ ) and 84 % ( $20.8 \pm 4.6 \text{ ml} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$ ) of total water efflux, respectively (Figure 2).

**Discussion**

On average, the hooded seals in this study lost  $710 \pm 58 \text{ g} \cdot \text{day}^{-1}$  or  $7.7 \text{ g} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$  while fasting. Grey seal pups during the prolonged post-weaning fast (over 7 weeks) with no access to water showed a BM loss of  $10.6 \text{ g} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$  (Nordøy et al 1992).  $\text{TBF}_i$  was on average ( $n = 5$ )  $46.9 \pm 3.2$  % of  $\text{BM}_i$ . Body composition compared well with recently weaned pups of other species.  $\text{TBF}_i$  has been reported to be  $48.5 \pm 0.9$  % in sub Antarctic fur seal pups (Verrier et al 2009), 50 % in elephant seal pups (Ortiz et al 1978),  $48.5 \pm 0.9$  % in subantarctic fur seal pups (Verrier et al 2009) and 40 % in grey seal pups (Sparling et al 2006), all just prior to weaning. These pups have all accumulated body fat for an extended period during lactation and, therefore, have a very high fat content and a very low TBW since subcutaneous adipose tissue contains only 10 % of free water (Ortiz et al 1978).

By using equations provided by Nagy and Costa (1980) total water influx and efflux were calculated to be  $16.7 \pm 3.9 \text{ ml} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$  and  $24.6 \pm 4.6 \text{ ml} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$ , respectively. No other studies have been done in subadult hooded seals, thus a direct comparison is difficult. On one hand, hooded seal pups during the post weaning fast have shown a daily water influx of  $15\text{-}16 \text{ ml} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$  (Lydersen et al 1997; Schots et al 2017) which is very similar to that measured in the present study. On the other hand, nursing hooded seal pups (Lydersen et al 1997) and actively feeding juvenile hooded seals (Skalstad and Nordøy 2000) have shown a total water influx

of approximately  $125$  and  $70 \text{ ml} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$ , respectively. This demonstrates that fasting subadult hooded seals with access to seawater show  $\text{rH}_2\text{O}$  clearly more similar to those of fasting (food and water deprivation) hooded seal pups than to those of nursing/feeding individuals. Other species, however, have shown different turnover rates under different conditions. Nordøy et al (1992) calculated water influx and efflux to be as low as 8 and  $12 \text{ ml} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$ , respectively, in fasting grey seal pups without access to any exogenous source of water. Elephant seal pups fasting under natural conditions showed, as well, an exceptionally low  $\text{rH}_2\text{O}$  of  $5.4 \pm 0.7$  (influx) and  $6.0 \pm 0.8$  (efflux)  $\text{ml} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$  (Ortiz et al 1978). Moreover, Verrier et al (2009) calculated influx and efflux rates in fasting subantarctic fur seal pups of  $7.9 \pm 0.9$  and  $8.8 \pm 1.0 \text{ ml} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$ , respectively. In contrast, enclosed (no access to seawater) and free-ranging (access to seawater) Antarctic fur seal pups showed a water influx of  $20.5 \pm 0.3$  and  $33.0 \pm 1.7 \text{ ml} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$ , respectively, during natural fasting (Lea et al 2002) having in both cases much higher  $\text{rH}_2\text{O}$  and percentage TBW than the hooded seals in the present study. This suggests ones more that different species of pinnipeds might be differently adapted with regard to water conservation when food and/or water are deprived as mentioned by Ortiz et al (1978). We could not find any correlation between  $\text{BM}_i$  and  $\text{rH}_2\text{O}$  suggesting that rates of influx and efflux are not dependant on the mass of the seal at the beginning of fasting. However, a negative correlation was shown between  $\text{TBF}_i$  and  $\text{rH}_2\text{O}$ . In this case, influx and efflux rates were lower in seals with higher  $\text{TBF}_i$ . This may suggest that seals with larger reserves of subcutaneous fat may better cope with situations of food/water deprivation not only by potentially producing more metabolic water but also by reducing overall  $\text{rH}_2\text{O}$ . It might be explained by the possibility of an even further decrease in metabolic rate and, consequently, the  $\text{rH}_2\text{O}$  (Reilly 1991; Richmond et al 1962), due to the larger isolative blubber layer.



**Figure 1** Tritium activity (cpm) plotted as a function of time (Teq, equilibration time; Tf, end of the experiment) in five subadult hooded seals. Seal #1 (▲), seal #2 (◆), seal #3 (●), seal #4 (▼) and seal #5 (■).

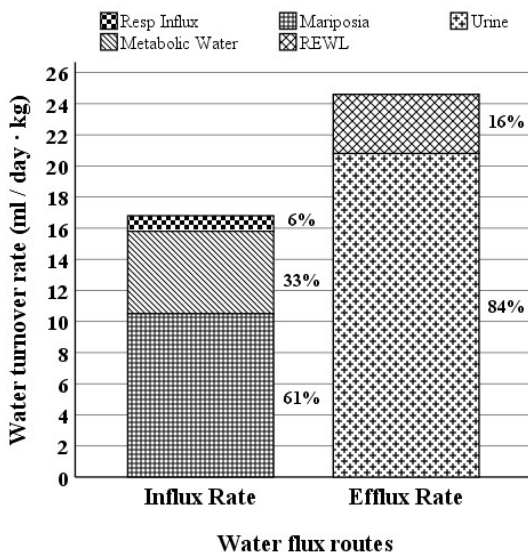
With regard to influx rates, metabolic water production is likely to be overestimated since fat was considered the only source of energy/water and catabolized to its end-products. However, it was considered to be more accurate to calculate the metabolic rate as a function of fat catabolized than using a multiplier of BMR predicted by the Kleiber equation (Kleiber 1961). Rapid changes in metabolic rate at the beginning of a fasting period have been shown in different species (Boily and Lavigne 1995; Cherel et al 1992; Hudson 1973; Keys et al 1950; Markussen 1995; Markussen et al 1992; Mrosovsky and Sherry 1980; Rosen and Trites 2002). Thus, assuming a constant metabolic rate during early stages of fasting may lead to errors. For instance, hooded seal pups during the first days

of the post-weaning fast have shown metabolic rates ranging 2.1 – 4.4 times the predicted BMR (Lydersen et al 1997). Besides, the calculated metabolic rate of the animals used in this study compared well with that of presumably less active fasting grey seal pups under similar conditions (Nordøy et al 1990). Respiratory influx and REWL were calculated as described by Folkow and Blix (1987) from empirical data obtaining similar results for REWL than in the present study. Moreover, this method has been validated in later studies of water balance (Skalstad and Nordøy 2000). Therefore, the rate of water influx through mariposia will be, if anything, underestimated.

**Table 3** Total water efflux (rH<sub>2</sub>O efflux) and influx (rH<sub>2</sub>O influx), efflux and influx rates per kg of BM and overall water loss per day of five subadult hooded seals. Average (n = 5) values ± SD are given in the bottom line.

	rH <sub>2</sub> O efflux (ml · day <sup>-1</sup> )	rH <sub>2</sub> O influx (ml · day <sup>-1</sup> )	rH <sub>2</sub> O efflux (ml · day <sup>-1</sup> · kg <sup>-1</sup> )	rH <sub>2</sub> O influx (ml · day <sup>-1</sup> · kg <sup>-1</sup> )	Δ H <sub>2</sub> O loss (ml · day <sup>-1</sup> )
Seal 1	2130	1406	20.9	13.8	724
Seal 2	1617	967	18.8	11.2	651
Seal 3	2344	1644	24.7	17.3	699
Seal 4	2627	1827	32.1	22.3	800
Seal 5	2286	1612	26.5	18.7	674
Av±SD	2200±333	1491±294	24.6±4.6	16.7±3.9	710±51

Av: Average; SD: Standard deviation



**Figure 2** Total water turnover (rH<sub>2</sub>O, ml · day<sup>-1</sup> · kg<sup>-1</sup>). Water influx rates via respiration, mariposia and metabolic water; and efflux rates via REWL and urine production of five subadult hooded seals. Percentage contribution of each different route of water influx and efflux to the total turnover rate is also given.

Mariposia contributed 10.5 ± 3.8 ml · day<sup>-1</sup> · kg<sup>-1</sup> or 61 % to total water influx of short-term fasting subadult hooded

seals. Despite of being the major contributor of free water during fasting, this amount of mariposia compares very well with that shown in actively feeding hooded seals (9 ml · day<sup>-1</sup> · kg<sup>-1</sup>) (Skalstad and Nordøy 2000). Although mariposia has been shown to restore water balance in dehydrated harp seals (How and Nordøy 2007) and potentially give a net gain of water (Storeheier and Nordøy 2001), ingested seawater shown in the present study was most probably used to create urinary osmotic space for urea, as described by Wolf et al (1959), rather than to provide a net gain of water. Storeheier and Nordøy (2001) showed that adult harp seals fasting for 2 days produced excess amount of urea during early phase I of fasting, which was more efficiently excreted when seawater was given as bolus administration. This voluntary seawater drinking may thus aid in eliminating the excess of urea produced in early stages of fasting due to protein catabolism. Therefore, this study supports the theory of “urea osmotic space” proposed by Wolf et al (1959) and further elucidated by Costa (1982) studying sea otters (*Enhydra lutris*).

It is concluded that also subadult fasting hooded seals drink significant amounts of seawater when available during early fasting. Although metabolic water remains as the most reliable source of free water for fasting subadult hooded seals, mariposia may play an important role in excreting the excess of urea produced during phase I of fasting.

## Acknowledgements

The authors wish to thank John Ness, Hans Lian and Hans-Arne Solvang, technical personnel at AAB, the Arctic University of Norway, who took care of the seals prior the experimental period and gave essential assistance during the experiments.

## References

- Andersen JM, Wiersma YF, Stenson G, Hammill MO (2009) Movement patterns of hooded seals (*Cystophora cristata*) in the northwest atlantic ocean during the post-moult and pre-breed seasons. *Journal of Northwest Atlantic Fishery Science* 42:1-11.
- Arnould JP, Green JA, Rawlins DR (2001) Fasting metabolism in antarctic fur seal (*Arctocephalus gazella*) pups. *Comparative Biochemistry and Physiology: Part A Molecular and Integrative Physiology* 129:829-841.
- Arnould JPY, Boyd IL, Speakman JR (1996) Measuring the body composition of antarctic fur seals (*Arctocephalus gazella*): validation of hydrogen isotope dilution. *Physiological Zoology* 69:93-116.
- Beauplet G, Guinet C, Arnould JP (2003) Body composition changes, metabolic fuel use, and energy expenditure during extended fasting in subantarctic fur seal (*Arctocephalus tropicalis*) pups at Amsterdam island. *Physiological and Biochemical Zoology*. doi:10.1086/367951
- Boily P, Lavigne DM (1995) Resting metabolic rates and respiratory quotients of gray seals (*Halichoerus grypus*) in relation to time of day and duration of food deprivation. *Physiological Zoology* 68:1181-1193.
- Bray GA (1960) A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Analytical Biochemistry*. doi:http://dx.doi.org/10.1016/0003-2697(60)90025-7
- Carlini AR, Marquez ME, Ramdohr S, Bornemann H, Panarello HO, Daneri GA (2001) Postweaning duration and body composition changes in southern elephant seal (*Mirounga leonina*) pups at King George Island. *Physiological and Biochemical Zoology*. doi:10.1086/322168
- Castellini MA, Costa DP, Huntley AC (1987) Fatty acid metabolism in fasting elephant seal pups. *Journal of Comparative Physiology B*. doi:10.1007/BF00691828
- Costa DP (1982) Energy, nitrogen, and electrolyte flux and sea water drinking in the sea otter *Enhydra lutris*. *Physiological Zoology*. doi:10.2307/30158441
- Crocker DE, Houser DS, Webb PM (2012) Impact of body reserves on energy expenditure, water flux, and mating success in breeding male northern elephant seals. *Physiological and Biochemical Zoology*. doi:10.1086/663634
- Cherel Y, Robin J-P, Heitz A, Calgari C, Maho Y (1992) Relationships between lipid availability and protein utilization during prolonged fasting. *Journal of Comparative Physiology B*. doi:10.1007/bf00260757
- Depocas F, Fisher HD, Hart JS (1971) Sea water drinking and water flux in starved and in fed harbor seals, *Phoca vitulina*. *Canadian Journal of Physiology and Pharmacology* 49:53-62.
- Donohue MJ, Costa DP, Goebel E, Antonelis GA, Baker JD (2002) Milk intake and energy expenditure of free-ranging northern fur seal, *Callorhinus ursinus*, pups. *Physiological and Biochemical Zoology*. doi:10.1086/338284
- Fetcher ES, Fetcher GW (1942) Experiments on the osmotic regulation of dolphins. *Journal of Cellular and Comparative Physiology*. doi:10.1002/jcp.1030190112
- Folkow L, Mårtensson P-E, Blix A (1996) Annual distribution of hooded seals (*Cystophora cristata*) in the Greenland and norwegian seas. *Polar Biology*. doi:10.1007/BF02329206
- Folkow LP, Blix AS (1987) Nasal heat and water exchange in gray seals. *American Journal of Physiology* 253:R883-R889.
- Folkow LP, Nordøy ES, Blix AS (2010) Remarkable development of diving performance and migrations of hooded seals (*Cystophora cristata*) during their first year of life. *Polar Biology*. doi:10.1007/s00300-009-0718-y
- Gentry RL (1981) Seawater drinking in eared seals. *Comparative Biochemistry and Physiology* 68:81-86.
- Hammel HT, Elsner RW, Heller HC, Maggett JA, Bainton CR (1977) Thermoregulatory responses to altering hypothalamic temperature in the harbor seal. *American Journal of Physiology-Reg I* 232:R18-R26.
- Hong SK, Elsner R, Claybaugh JR, Ronald K (1982) Renal functions of the Baikal seal (*Pusa sibirica*) and ringed seal (*Pusa hispida*). *Physiological Zoology*. doi:10.2307/30157892
- How OJ, Nordøy ES (2007) Seawater drinking restores water balance in dehydrated harp seals. *Journal of Comparative Physiology B*. doi:10.1007/s00360-007-0152-9
- Hudson JW (1973) Torpidity in mammals. *Special Aspects of Thermoregulation* 1973:97-165.
- Irving L, Fisher KC, McIntosh FC (1935) The water balance of a marine mammal. The seal. *Journal of Cellular and Comparative Physiology* 6:387-391.
- Keys A, Brožek J, Henschel A, Mickelsen O, Taylor HL (1950) The biology of human starvation. Oxford, England.
- Kleiber M (1961) The fire of life. An introduction to animal energetics. New York, United States. doi:10.1126/science.134.3495.2033
- Kvadsheim PH, Folkow LP (1997) Blubber and flipper heat transfer in harp seals. *Acta Physiologica Scandinavica*. doi:10.1046/j.1365-201X.1997.00235.x
- Lea MA, Bonadonna F, Hindell MA, Guinet C, Goldsworthy SD (2002) Drinking behaviour and water turnover rates of antarctic fur seal pups: implications for the estimation of milk intake by isotopic dilution. *Comparative Biochemistry and Physiology: Part A Molecular and Integrative Physiology* 132:321-331.
- Lester CW, Costa DP (2006) Water conservation in fasting northern elephant seals (*Mirounga angustirostris*). *Journal of Experimental Biology*. doi:10.1242/jeb.02503
- Lifson N, McClintock R (1966) Theory of use of the turnover rates of body water for measuring energy and material balance. *Journal of Theoretical Biology* 12:46-74.
- Lydersen C, Hammill MO (1993) Activity, milk intake and energy consumption in free-living ringed seal (*Phoca hispida*) pups. *Journal of Comparative Physiology B* 163:433-438.
- Lydersen C, Kovacs KM (1996) Energetics of lactation in harp seals (*Phoca groenlandica*) from the Gulf of St. Lawrence, Canada. *Journal of Comparative Physiology B* 166:295-304.
- Lydersen C, Kovacs KM, Hammill MO (1997) Energetics during nursing and early postweaning fasting in hooded seal (*Cystophora cristata*) pups from the Gulf of St Lawrence, Canada. *Journal of Comparative Physiology B* 167:81-88.

- Markussen NH (1995) Changes in metabolic rate and body composition during starvation and semistarvation in harbour seals. In: Blix AS, Walløe L, Øyvind U (eds) *Developments in Marine Biology*. Elsevier Science, Tromsø, pp 383-391. doi:[http://dx.doi.org/10.1016/S0163-6995\(06\)80040-5](http://dx.doi.org/10.1016/S0163-6995(06)80040-5)
- Markussen NH, Ryg M, Øritsland NA (1992) Metabolic rate and body composition of harbour seals, *Phoca vitulina*, during starvation and refeeding. *Canadian Journal of Zoology*. doi:10.1139/z92-034
- Matsuura DT, Whittow GC (1974) Evaporative heat loss in the California sea lion and harbor seal. *Comparative Biochemistry and Physiology*. doi:[http://dx.doi.org/10.1016/0300-9629\(74\)90847-0](http://dx.doi.org/10.1016/0300-9629(74)90847-0)
- Mrosovsky N, Sherry D (1980) Animal anorexias. *Science*. doi:10.1126/science.6928327
- Nagy KA, Costa DP (1980) Water flux in animals: analysis of potential errors in the tritiated water method. *American Journal of Physiology* 238:R454-465.
- Nordgarden U, Folkow L, Walløe L, Blix A (2000) On the direction and velocity of blood flow in the extradural intravertebral vein of harp seals (*Phoca groenlandica*) during simulated diving. *Acta Physiologica Scandinavica* 168:271-276.
- Nordøy ES, Aakvaag A, Larsen TS (1993) Metabolic adaptations to fasting in harp seal pups. *Physiological Zoology*. doi:10.2307/30163747
- Nordøy ES, Blix AS (1985) Energy sources in fasting grey seal pups evaluated with computed tomography. *American Journal of Physiology* 249:R471-476.
- Nordøy ES, Ingebretsen OC, Blix AS (1990) Depressed metabolism and low protein catabolism in fasting grey seal pups. *Acta Physiologica Scandinavica* 139:361-369.
- Nordøy ES, Stijfhoorn DE, Raheim A, Blix AS (1992) Water flux and early signs of entrance into phase III of fasting in grey seal pups. *Acta Physiologica Scandinavica*. doi:10.1111/j.1748-1716.1992.tb09324.x
- Noren DP, Crocker DE, Williams TM, Costa DP (2003) Energy reserve utilization in northern elephant seal (*Mirounga angustirostris*) pups during the postweaning fast: size does matter. *Journal of Comparative Physiology B*. doi:10.1007/s00360-003-0353-9
- Noren SR, Boness DJ, Iverson SJ, McMillan J, Bowen WD (2008) Body condition at weaning affects the duration of the postweaning fast in gray seal pups (*Halichoerus grypus*). *Physiological and Biochemical Zoology*. doi:10.1086/528777
- Ortiz CL, Costa D, Le Boeuf BJ (1978) Water and energy flux in elephant seal pups fasting under natural conditions. *Physiological Zoology* 51:166-178.
- Ortiz R, Wade C, Costa D, Ortiz C (2002) Renal effects of fresh water-induced hypo-osmolality in a marine adapted seal. *Journal of Comparative Physiology B*. doi:10.1007/s00360-002-0254-3
- Reilly JJ (1991) Adaptations to prolonged fasting in free-living weaned gray seal pups. *American Journal of Physiology* 260:R267-272.
- Reilly JJ, Fedak MA (1990) Measurement of the body composition of living gray seals by hydrogen isotope dilution. *Journal of Applied Physiology* 69:885-891.
- Renouf D, Noseworthy E, Scott MC (1990) Daily fresh water consumption by captive harp seals (*Phoca groenlandica*). *Marine Mammal Science*. doi:10.1111/j.1748-7692.1990.tb00251.x
- Richmond CR, Langham WH, Trujillo TT (1962) Comparative metabolism of tritiated water by mammals. *Journal of Cellular and Comparative Physiology*. doi:10.1002/jcp.1030590106
- Rosen DAS, Trites AW (2002) Changes in metabolism in response to fasting and food restriction in the steller sea lion (*Eumetopias jubatus*). *Comparative Biochemistry and Physiology: Part B Biochemistry and Molecular Biology*. doi:10.1016/S1096-4959(02)00048-9
- Schmidt-Nielsen K (1964) Desert animals: physiological problems of heat and water. *Physiological Zoology* 37:338-339.
- Schots PC, Bue ME, Nordøy ES (2017) Hooded seal (*Cystophora cristata*) pups ingest snow and seawater during their post-weaning fast. *Journal of Comparative Physiology B*. doi:10.1007/s00360-016-1048-3
- Sepall O, Mason S (1960) Vapor/liquid partition of tritium in tritiated water. *Canadian Journal of Chemistry* 38:2024-2025.
- Skalstad I, Nordøy ES (2000) Experimental evidence of seawater drinking in juvenile hooded (*Cystophora cristata*) and harp seals (*Phoca groenlandica*). *Journal of Comparative Physiology B* 170:395-401.
- Skog EB, Folkow LP (1994) Nasal heat and water exchange is not an effector mechanism for water balance regulation in grey seals. *Acta Physiologica Scandinavica*. doi:10.1111/j.1748-1716.1994.tb09742.x
- Sparling CE, Speakman JR, Fedak MA (2006) Seasonal variation in the metabolic rate and body composition of female grey seals: fat conservation prior to high-cost reproduction in a capital breeder? *Journal of Comparative Physiology B*. doi:10.1007/s00360-006-0072-0
- Storeheier PV, Nordøy ES (2001) Physiological effects of seawater intake in adult harp seals during phase I of fasting. *Comparative Biochemistry and Physiology: Part A Molecular and Integrative Physiology* 128:307-315.
- Tarasoff FJ, Toews D (1972) The osmotic and ionic regulatory capacities of the kidney of the harbor seal, *Phoca vitulina*. *Journal of Comparative Physiology A*. doi:10.1007/BF00696628
- Verrier D, Groscolas R, Guinet C, Arnould JP (2009) Physiological response to extreme fasting in subantarctic fur seal (*Arctocephalus tropicalis*) pups: metabolic rates, energy reserve utilization, and water fluxes. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology*. doi:10.1152/ajpregu.90857.2008
- Wolf AV, Prentiss PG, Douglas LG, Swett RJ (1959) Potability of sea water with special reference to the cat. *American Journal of Physiology* 196:633-641.