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# Drinking behaviour and water turnover rates of Antarctic fur seal pups: implications for the estimation of milk intake by isotopic dilution

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## Abstract

The estimation of milk consumption in free-ranging seals using tritium dilution techniques makes the key assumption that the animals drink no pre-formed water during the experimental period. However, frequent observations of unweaned Antarctic fur seal pups drinking water at Iles Kerguelen necessitated the testing of this assumption. We estimated water flux rates of 30 pups ( $10.7 \pm 0.3$  kg) in four experimental groups by isotopic dilution over 4 days. The groups were: (1) pups held in an open air enclosure without access to water to estimate fasting metabolic water production (MWP); (2) free-ranging pups not administered additional water; (3) pups held in an open air enclosure and given a total of 300 ml of fresh water to verify technique accuracy; and (4) free-ranging pups given 200 ml of fresh water. Pups without access to water exhibited water flux rates ( $20.5 \pm 0.8$  ml kg<sup>-1</sup> d<sup>-1</sup>), which were significantly lower than those observed for the free-ranging group ( $33.0 \pm 1.7$  ml kg<sup>-1</sup> d<sup>-1</sup>). Mean estimated pre-formed water intake for the free-ranging experimental groups was 12.6 ml kg<sup>-1</sup> d<sup>-1</sup>. Thus, MWP, measured as total water intake during fasting, may be significantly over-estimated in free-ranging Antarctic fur seal pups at Iles Kerguelen and at other sites and subsequently milk intake rates may be underestimated. © 2002 Elsevier Science Inc. All rights reserved.

**Keywords:** Otariid; Pinniped; Water drinking; Turnover; Milk intake; Metabolic water production

## 1. Introduction

All young otariids (fur seals and sea lions) undergo regular periods of fasting during the lactation period while their mothers are foraging at sea. Fasting periods may vary in duration from short overnight periods for some Antarctic fur seals, *Arctocephalus gazella* (Goldsworthy, 1999) up to a month for the Subantarctic fur seal, *A. tropicalis* (Georges and Guinet, 2000). During the fasting periods, pups must subsist on energy

reserves built up from a diet of lipid-rich milk supplied to them by their mothers during short attendance periods. This fasting period may be particularly physiologically demanding on pups. Fasting duration, pup age, size and body composition in addition to moulting stage all affect a pup's ability to sufficiently store blubber and thermoregulate effectively (Donohue et al., 2000).

Quantifying the milk consumed by pups is an essential component of estimating reproductive effort in mammals (Oftedal and Iverson, 1987), including fur seals. Accurate assessment of total water intake (TWI), comprised of water produced through metabolism (MWP) and milk water, is a

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critical component of mammalian milk intake estimates. MWP can be estimated independently by the doubly-labelled water method (Lifson and McClintock, 1966; Costa and Gentry, 1986; Donohue et al., in press), by indirect calorimetry (Higgins et al., 1988) or by using hydrogen isotope dilution for fasting and non-drinking animals, where MWP is essentially equal to TWI (Ortiz et al., 1984). In studies of fur seals a common method of determining MWP is to measure the in situ dilution of deuterated (HDO) or tritiated water (HTO) in fasting animals over a defined period, adequate to allow for metabolic turnover of the isotope to provide labelled vs. non-labelled ratios sufficient to provide accurate measurements. There are three potential sources of water that can contribute to pup TWI: preformed water in milk; water produced through metabolic processes (MWP); and exogenous water from drinking or prey consumed. Milk intake is estimated by measuring TWI under the assumption that TWI is derived only from MWP and milk (Ortiz et al., 1984). Thus, a common assumption of energetic studies examining milk intake by hydrogen isotope dilution (Costa, 1987) is that young pups consume nothing while fasting between suckling bouts.

Milk intake of many species of otariid seals has been estimated by the dilution of tritiated water (Costa and Gentry, 1986; Oftedal et al., 1987; Higgins et al., 1988; Arnould et al., 1996a) as it is relatively inexpensive when compared to other isotopes such as doubly-labelled water. If MWP is estimated by tritium dilution as the rate of water flux in fasting animals, water consumption during fasting periods will lead to over-estimates of MWP, and consequently underestimates of milk consumption. During the 1997/1998 austral summer at Cap Noir, Iles Kerguelen, water drinking was observed in Antarctic fur seals, *Arctocephalus gazella*, of all age classes, particularly pups (authors' personal observation). Fresh water drinking has not previously been reported in otariid seals, although seawater drinking (mariposia) has been observed in several species of otariid (Gentry, 1981; Costa and Trillmich, 1988), at least two species of phocid, the harp seal, *Phoca groenlandica* (Storehøier and Nordøy, 2001) and the hooded seal, *Cystophora cristata* (Skalstad and Nordøy, 2000) and the sea otter, *Enhydra lutris* (Costa, 1982).

In the subsequent austral summer (1998/1999), we undertook an experimental study to: (i) estimate the consumption of exogenous water ingested

by fasting pups; and (ii) quantify the frequency of water drinking in fasting fur seal pups. We aimed to estimate metabolic water production of fasting pups without free access to drinking water by isotopic dilution examine for comparison with free-ranging pups.

## 2. Materials and methods

This study was conducted at the Cap Noir Antarctic fur seal colony on the Courbet Peninsula, Iles Kerguelen (49°07'S, 70°45'E) from 2 to 28 February 1999. Pup production was estimated to be approximately 775 pups in that year (M.-A. Lea, unpublished data). Antarctic fur seals arrive and give birth to a single pup from late November to early January. Pups are suckled by their mothers during intermittent periods of 1–2 days ashore and may fast for periods of up to 17.9 days while their mothers are feeding at sea (Lea et al., 2001). Weaning occurs at approximately 4 months of age (Doidge et al., 1986).

Fasting pups ( $N=30$ ) were captured 2 days after the departure of their mothers to sea to allow for the prior absorption of milk obtained during the previous suckling bout and to ensure that all pups were at a similar stage of fasting (Oftedal and Iverson, 1987; Donohue, 1998). The four treatment groups ( $N=7-8$  per group) were comprised of pups of a similar range of body masses (e.g. 7.7–15.5 kg) and sex ratios (1:1.3 F/M). The initial body composition of pups in terms of total body water (see Section 2.1.1) was also compared between groups to identify any pre-existing differences in the composition of groups. Pups ranged in age from 40 to 70 days at this period and were weighed with a 25 kg  $\times$  20 g Salter Electro Samson spring balance (Salter™ Weigh-Tronix, West Bromwich, UK), sexed and a standard length measurement taken to the nearest 0.5 cm. Individual pups were identified by flipper tags located in the trailing edge of fore-flippers (Dalton Jumbo Tags®, Dalton Supplies Ltd., Henley-on-Thames, UK) or by a bleach mark (Clairol Born Blonde®, Bristol-Myers Squibb, Ryde, Australia).

### 2.1. Water intake experimental design

Water flux rates of pups in each experimental group were estimated over 4 days by the dilution of injected tritiated water. A standard procedure

was adopted as described in the following protocol and specific treatments are detailed in the relevant sections.

### 2.1.1. Protocol

A 2-ml blood sample was taken initially from the caudal gluteal vein in order to determine background levels of HTO in the blood. A 1-ml injection of 200  $\mu\text{Ci}$  HTO/ml tritiated water was then administered intramuscularly. The HTO dose was pre-weighed and sealed in 2-ml Wheaton vials (Milleville, NJ, USA) prior to the field study. The contents of the vial were injected and the vial rinsed twice with approximately 1 ml of sterile saline, also administered intramuscularly (Arnould et al., 1996a,b). Pups were subsequently held in the enclosure for 3 h (Arnould et al. 1996a) to allow for isotopic equilibration (Costa, 1987), after which time an equilibration blood sample (E1) was taken to determine initial total body water ( $\text{TBW}_i$ ). Body condition of the pups was also estimated on day 6 ( $\text{TBW}_f$ ) by repeating the procedure for  $\text{TBW}_i$ , using a lower concentration of HTO (1 ml 50  $\mu\text{Ci}$  HTO/ml).

### 2.1.2. MWP estimation

Water flux rates of eight pups held in an open air enclosure without access to water from day 2 to 6 of the fast (treatment 1 control), were compared with water flux rates of seven free-ranging pups calculated over the same period (treatment 2). Pups in treatment 2 were released after the 3-h HTO equilibration period on day 2, and remained free ranging until day 6, when a final TBW ( $E_f$ ) measurement was taken. In contrast, pups in the enclosed, control group were held in an open-air enclosure (3 $\times$ 2.5 m) within the colony, during the 4-day experimental period (referred to hereafter as day 2–6 of the fast).

### 2.1.3. Technique precision

A second enclosed group of seven pups was given a known quantity of fresh water (2 $\times$ 150 ml) on days 2 and 4 of the fasting period (treatment 3). We compared estimated and actual water flux rates of this experimental group to validate the precision of the tritiated water dilution technique.

The protocol was as for other groups and in addition pups were given 150 ml of fresh water

on days 2 and 4 following the collection of first and second equilibration blood samples, respectively. Water was administered by gastric intubation (15-mm diameter tube) and delivered using a 50-ml syringe. The intubation procedure took less than 1 min to complete in all cases. No more than five pups were held in the enclosure at any one time.

### 2.1.4. Estimation of water consumption.

As the consumption of water by experimental free-ranging pups (treatment 2) may or may not have occurred, a final experimental treatment estimating water flux rates were estimated for eight free ranging pups given a known quantity, 200 ml, of water ( $W$ ) on day 4 (treatment 4). The protocol was similar to that of free-ranging pups but in addition, the pups were given 200 ml of fresh water on day 4 after a second equilibration blood sample was collected. Unaccounted water flux rates during the 4-day period were estimated as the difference between TWI (d 2–6) and MWP (d 2–4) and the known quantity consumed [i.e.  $\text{TWI} - \text{MWP} - \text{consumed water (ml kg}^{-1} \text{d}^{-1})$ ].

## 2.2. Sample analysis

All blood samples were returned to the field hut and left to stand for 2–3 h. The plasma fraction was pipetted into Ependorf™ tubes and frozen at  $-20^\circ\text{C}$  until analysis in April 1999. Samples were thawed and three replicate 0.1-ml aliquots were distilled following the methods of Ortiz et al. (1978). The volume of distilled water was then measured and 3.5 ml of scintillation cocktail (EcoLite™ ICN, Costa Mesa, USA) was then added to each vial. Replicates were counted for 10 min in a Beckman LS6500 multipurpose scintillation counter. A set of standards created for each solution of HTO was run at the start of all sample runs to account for any discrepancies in counting.

## 2.3. Calculations

The body composition of pups was estimated by calculating total body water (TBW) using dilution space estimated by the dilution of a hydrogen isotope (HTO) during an equilibration period of 3 h. TBW was estimated using the equation (Arnould et al., 1996a):

$$\text{TBW (kg)} = 0.11 + 0.97 * \text{HTO space (kg)}$$

MWP was estimated in fasting animals as water influx during the fasting period using equation 6 in Nagy and Costa (1980):

$$\begin{aligned} \text{ml H}_2\text{O influx kg}^{-1} \text{ d}^{-1} \\ = \text{ml H}_2\text{O efflux kg}^{-1} \text{ d}^{-1} \\ + 2000 \frac{(W_2 - W_1)}{t(M_1 - M_2)} \end{aligned}$$

where  $W$  is body water volume (ml),  $M$  is body mass in grams and  $t$  the time period (days) between samples.  $H^*$  values represent counts per minute (CPM) of tritiated water present in blood samples. Water efflux rates were estimated using equation 5 in Nagy and Costa (1980):

$$\begin{aligned} \text{ml H}_2\text{O efflux kg}^{-1} \text{ d}^{-1} \\ = \frac{2000 \cdot W_1 \ln(W_2/W_1) \cdot \ln(H_1 * W_1 / H_2 * W_2)}{(M_1 + M_2) \cdot [1 - (W_1/W_2)]t} \end{aligned}$$

Mass-specific rates of mass loss (% d<sup>-1</sup>) of pups during the experiment were estimated using the equation (Guinet et al., 1999):

$$\text{MSRML} = \frac{(\log(M_f) - \log(M_i))}{\Delta t} \cdot 100$$

where  $M_i$  is initial mass (kg, day 2),  $M_f$  the final mass (day 6) and  $\Delta t$ , the time elapsed between the measurements of  $M_i$ .

#### 2.4. Drinking observations

Drinking was defined as the visible ingestion and swallowing of water (Gentry, 1981). All opportunistic observations of water drinking were timed to the nearest second and the type of water (salt or fresh) noted. Animals were categorised as a pup, juvenile, adult male, or adult female. All estimates of drinking bout duration are minimum estimates, as in many cases bouts had already commenced when the animals were first observed. Temperature (°C) was monitored during the study period at Cap Noir by a temperature probe mounted in the shade and data stored using the Weather Monitor II Station (Davis Instruments Corp. Hayward, CA 94545, USA).

#### 2.5. Statistical analysis

Rates of water flux were compared between treatment groups by analysis of variance (ANOVA). The Tukey post hoc test was used when differentiation between treatment groups was required. Differences in the initial body composition of pups in the four treatment groups were assessed by stepwise general linear model analyses, performed using SYSTAT statistical software (SYSTAT 9.0, SPSS, Inc. USA). Statistical significance was determined at  $P < 0.05$ . Data are reported as means  $\pm$  standard error of the mean (S.E.M.).

### 3. Results

#### 3.1. Body composition

The initial body condition of all pups in the four treatments was compared for any pre-existing differences in body condition prior to treatments (Table 1). A step-wise general linear model revealed that none of the interactions or the group had a significant effect on initial total body water (TBW<sub>i</sub>), but that TBW<sub>i</sub> was significantly related to both sex ( $F_{1,24} = 17.36$ ,  $P < 0.01$ , Fig. 1) and initial mass ( $F_{1,24} = 35.0$ ,  $P < 0.01$ ). The sex ratio of each treatment group was designed to be similar to account for these compositional differences (Section 2).

#### 3.2. Estimating metabolic water production (treatments 1 and 2)

The overall mean decrease in specific activity of the isotope was  $17.4 \pm 4.29\%$  ( $n = 30$ ) during the 4-day experiment and background levels of HTO in blood samples were low ( $48.0 \pm 8.05$  DPM,  $n = 30$ , range 0–180.7 DPM). Rates of water influx were significantly different between treatment groups (ANOVA,  $F_{3,26} = 20.79$ ,  $P < 0.01$ , Table 1) with fasting rates of water influx (MWP) for the enclosed group without access to water (mean =  $20.5 \pm 0.3$  ml kg<sup>-1</sup> d<sup>-1</sup>) being significantly lower than those for all other treatments (Table 1).

Mass-specific rates of mass loss (MSML) were not related to initial mass ( $r^2 = 0.059$ ,  $P > 0.05$ ) nor did they differ between treatment groups or as a function of pup sex or their interaction ( $F_{3,22} = 0.762$ ,  $P > 0.05$ ) when tested by a two-way ANOVA. Rates of water influx for female pups (Table

1) were significantly higher than those of male pups for treatment 3 ( $t_{5,[0.05]}=4.15$ ,  $P<0.05$ ) and treatment 4 ( $t_{6,[0.05]}=2.594$ ,  $P<0.05$ ). No inter-sexual differences in rates of water influx were apparent for pups without access to water ( $t_{6,[0.05]}=1.383$ ,  $P=0.216$ ) or for free-ranging pups ( $t_{5,[0.05]}=-0.776$ ,  $P=0.426$ ).

### 3.3. Accuracy of water influx estimates (treatment 3)

Rates of water influx for the enclosed pups given 300 ml water, once MWP (T1, 20.5 ml kg<sup>-1</sup> d<sup>-1</sup>, Table 1) had been taken into account (8.3 ml kg<sup>-1</sup> d<sup>-1</sup>), were not significantly different from the predicted rates of water influx for the quantity of water (300 ml) given to the pups (7.5 ml kg<sup>-1</sup> d<sup>-1</sup>, paired  $t_{6,[0.05]}=1.32$ ,  $P=0.117$ ). This confirmed the accuracy of the technique for the purposes of this study, i.e. to distinguish between water consumption and metabolic water production.

### 3.4. Estimating water consumption (treatment 4)

Rates of water influx (d2–d4) for free-ranging pups, ( $32.6\pm 2.0$  ml kg<sup>-1</sup> d<sup>-1</sup>), before a 200-ml drink was delivered, were not significantly different to the rates of water influx ( $33.0$  ml kg<sup>-1</sup>

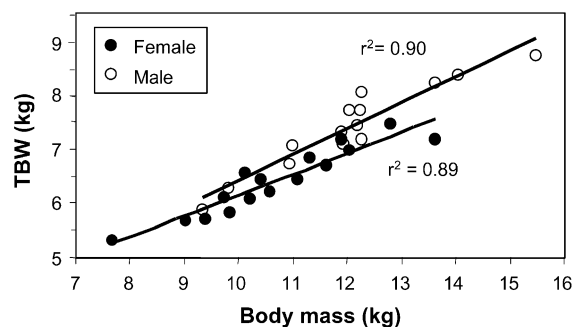


Fig. 1. The relationship between total body water (TBW) and body mass (kg) for male ( $n=14$ ) and female ( $n=16$ ) Antarctic fur seal pups.

d<sup>-1</sup>, Table 1) observed in the free-ranging control group ( $t_{13,[0.05]}=-0.178$ ,  $P=0.862$ ). Once actual water intake (200 ml) and estimated MWP from treatment 1 (20.5 ml kg<sup>-1</sup> d<sup>-1</sup>) were taken into account, the unaccounted rates of water influx for the two free-ranging groups,  $12.6\pm 1.4$  ml kg<sup>-1</sup> d<sup>-1</sup>, (range 2.7–23.2 ml kg<sup>-1</sup> d<sup>-1</sup>) were, on average, three times the rate for the known quantity of water given ( $4\pm 0.7$  ml kg<sup>-1</sup> d<sup>-1</sup>). The mean rates of unaccounted water influx for the two groups of free-ranging pups, 12.4 ml kg<sup>-1</sup> d<sup>-1</sup> (treatment 2) and 12.8 ml kg<sup>-1</sup> d<sup>-1</sup>, respectively, did not differ significantly ( $t_{13,[0.05]}=-0.120$ ,  $P=$

Table 1  
Rates of water flux for four experimental groups of *A. gazella* pups

	Treatment			
	1 Enclosed	2 Free-ranging	3 Enclosed	4 Free-ranging
Access to water	No	Yes	150 ml	Yes + 200 ml
<i>N</i>	8	7	7	8
Initial mass (kg)	$12.0\pm 0.4$	$10.4\pm 0.7$	$10.7\pm 0.4$	$12.2\pm 0.7$
Total body water <sup>d</sup> (TBW%)	$60.2\pm 0.7$	$62.6\pm 1.4$	$60.4\pm 0.8$	$60.1\pm 1.5$
MSML <sup>e</sup> (% d <sup>-1</sup> )	$-1.52\pm 0.1$	$-1.39\pm 0.1$	$-1.48\pm 0.1$	$-1.24\pm 0.1$
Water turnover rates (TWI, ml kg <sup>-1</sup> d <sup>-1</sup> )				
–Female pups	$21.6\pm 0.9$ (4)	$31.8\pm 3.0$ (4)	$30.8\pm 0.8^*$ (4)	$42.3\pm 2.8^*$ (4)
–Male pups	$19.4\pm 1.3$ (4)	$34.6\pm 0.6$ (3)	$26.4\pm 0.6^*$ (3)	$33.0\pm 2.3^*$ (4)
–Combined	$20.5\pm 0.8^*$	$33.0\pm 1.7$	$28.9\pm 1.0$	$37.7\pm 2.4$
TWI–MWP (ml kg <sup>-1</sup> d <sup>-1</sup> )	0	$12.4\pm 1.9$	$8.3\pm 0.7^a$	$17.2\pm 2.1$
Quantity of water given (ml kg <sup>-1</sup> d <sup>-1</sup> )	0	0	$7.5\pm 0.3^{a,b}$	$4.5\pm 0.2^c$
Unaccounted water flux (ml kg <sup>-1</sup> d <sup>-1</sup> )	0	$12.4\pm 1.9$	–	$12.8\pm 2.0$

Means expressed  $\pm$  S.E. Sample sizes are in parentheses.

\* Denotes significance at  $P<0.05$  *t* values in text.

<sup>a</sup> Not significant.

<sup>b</sup> 300 ml.

<sup>c</sup> 200 ml.

<sup>d</sup> Initial.

<sup>e</sup> Mass-specific rate of mass loss.

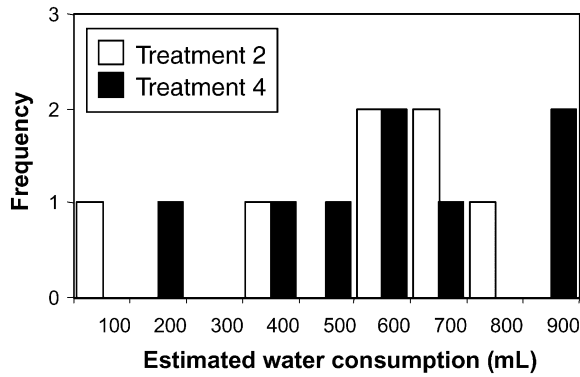


Fig. 2. Estimated water consumption rates over 4 days for free-ranging pups (treatment 2) and free-ranging pups given 200 ml of water (treatment 4).

0.906, Table 1). The estimated quantities of pre-formed water consumed during the 4-day experi-

Table 2  
Frequency of drinking observations by *A. gazella*, Iles Kerguelen

Age Class	n	Water type		Drinking bout duration (min)
		Fresh (n)	Salt (n)	
Adult males	8 <sup>a</sup>	5	3	0.93 ± 0.46
Juveniles	23	17	6	1.67 ± 0.35
Pups	27	19	8	2.46 ± 0.55 <sup>b</sup>
Total	58	41	17	1.9 ± 0.28

<sup>a</sup> Includes one sub-adult male.

<sup>b</sup> n = 20.

mental period for both groups of free ranging pups (combined n = 15) ranged from 74–876 ml (mean = 643 ± 247 ml, Fig. 2). A significant positive relationship was found between the estimated quantity of water consumed and total body water ( $r^2 = 0.586$ ,  $P < 0.05$ ,  $n = 7$ ) for pups in treatment

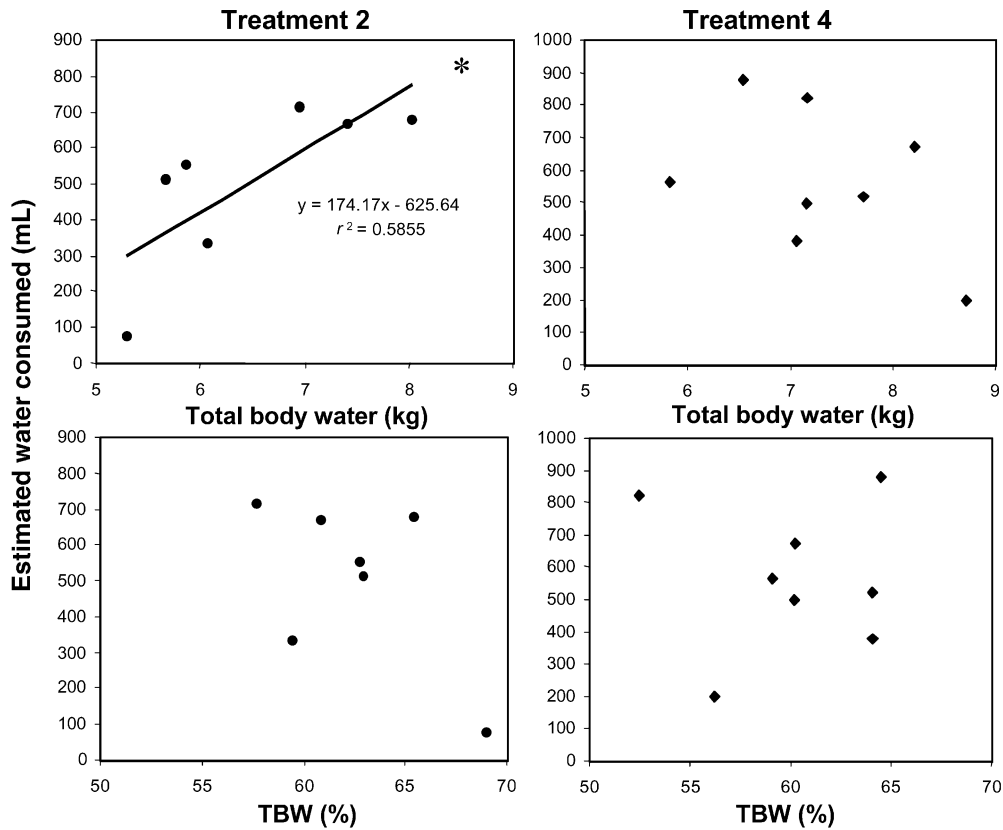


Fig. 3. The relationship between estimated water consumption (ml) and total body water (kg and %) for free ranging pups (treatment 2) and free ranging pups given additional water (treatment 4).

group 2 (Fig. 3), however, no relationship was found for pups in treatment 4 (Fig. 3,  $r^2=0.08$ ,  $P=0.259$ ). Total body water percentage was unrelated to the estimated water consumption of free-ranging seals ( $r^2=0.196$ ,  $P=0.177$  and  $r^2=0$ ,  $P=0.978$ , respectively).

### 3.5. Observations of water drinking

Drinking by Antarctic fur seals of varying age/sex classes was observed 58 times throughout the 1998/1999 austral summer (Table 2). There was no significant difference in the duration of drinking bouts between age/sex classes ( $F_{48,2}=1.97$ ,  $P=0.15$ ). Freshwater was consumed in the majority of cases for all age/sex classes, although seawater was consumed by 16 animals (29% of total observations). Pups were observed drinking more frequently than other age/sex classes, with freshwater consumption by pups accounting for 47% of all observations. Bouts of continuous drinking for 51 seals ranging from 5 s to 10 min were recorded (mean durations: saltwater  $1.1 \pm 0.3$  min,  $n=15$ ; freshwater  $2.2 \pm 0.4$  min,  $n=36$ ).

Pups exhibited a range of drinking behaviours, drinking frequently from freshwater seeps and streams, or drinking seawater from rock pools in transit to or from bouts of swimming ( $n=8$ ). Territorial males predominantly drank seawater from pools while maintaining their territory. In contrast, juvenile animals often hauled out and travelled directly to sources of fresh water such as seeps and small streams. Seals submerged their muzzles while drinking and the swallowing movement of the larynx was observed.

## 4. Discussion

The accuracy of the tritiated water method to estimate metabolic water production in the absence of water drinking was confirmed in this study by the similarity between actual and estimated water flux rates for pups given a known quantity of freshwater (treatment 3). A similar relationship between actual and estimated milk water intake was recorded by Arnould et al. (1996a) for Antarctic fur seal pups fed measured amounts of milk at Bird Island, South Georgia.

In this study, significantly lower rates of water turnover ( $20.5 \text{ ml kg}^{-1} \text{ d}^{-1}$ ) were recorded for pups without access to water, than for free ranging animals ( $33.0 \text{ ml kg}^{-1} \text{ d}^{-1}$ , Table 1). This varia-

tion may be due in part to: (i) water drinking by fasting, free-ranging pups; (ii) differences in activity levels between groups affecting the levels of metabolic water production; and/or (iii) milk stealing by free-ranging pups.

It is likely that activity levels of pups held in the enclosure were reduced in comparison to those of their free-ranging counterparts and that lower rates of fasting MWP were consequently recorded. Free-ranging pups conceivably expend more energy travelling and thermoregulating while swimming (Donohue et al., 2000) than captive pups resulting in higher rates of MWP. Milk-stealing behaviour has also been observed in Antarctic fur seal pups at South Georgia (Lunn, 1992), and both successful ( $n=7$ , 0.5–18-min duration) and unsuccessful attempts, where the pup is stopped by the filial pup or mother ( $n=14$ , 66.6%), were observed during the 1998/1999 season at Cap Noir (authors' personal observations). Thus, both factors could conceivably contribute to the higher water turnover rates recorded for free-ranging pups. However, the similarity in mass-specific rates of mass loss for all treatment groups suggests that differences in activity levels were not marked or were perhaps masked by the consumption of exogenous water. The relatively low rate of non-drinking TWI (i.e. MWP) calculated for non-drinking pups falls within the range of MWP estimated for fasting free-ranging northern fur seal pups ( $16.1\text{--}22.1 \text{ ml kg}^{-1} \text{ d}^{-1}$ ; Table 3) estimated by both fasting isotope dilution (Costa and Gentry, 1986) and the doubly-labelled water method (Donohue et al., in press). Rates of water influx for free-ranging, conspecific pups at South Georgia were related to the age of the pup, with female pups averaging  $28.4 \text{ ml kg}^{-1} \text{ d}^{-1}$  ( $n=87$ ) and males  $26.5 \text{ ml kg}^{-1} \text{ d}^{-1}$  ( $n=80$ ) (Arnould et al., 1996a). These rates are intermediate between non-drinking and free-ranging influx rates recorded at Iles Kerguelen, and higher than those reported by Costa and Gentry (1986) for Antarctic fur seal pups at the same site (Table 3). These higher rates may represent the ingestion of pre-formed water at a lower rate than recorded in this study, however, this appears unlikely as drinking behaviour was rarely observed at this site (J. Arnould, personal communication). Alternatively, the difference could result from a genuinely higher metabolic rate due to environmental differences (temperature and absolute humidity) and/or differences in thermoregulatory capabilities.

Table 3  
Fasting rates of metabolic water production for Antarctic and Northern fur seal pups

Species	Sex of pup	Fasting water influx rates (ml kg <sup>-1</sup> d <sup>-1</sup> )	Source
<i>Antarctic fur seals</i>			
Iles Kerguelen	Combined sexes	33.0 ± 1.7 (7) Free ranging 20.5 ± 0.8 (8) Enclosed	This study
South Georgia			
–Free-ranging	Males	26.5 ± 0.9 (80) <sup>a</sup>	(Arnould et al., 1996a)
	Females	28.4 ± 0.4 (87)	
–Enclosed	Males	18.6 ± 0.7 (8) <sup>a</sup>	(Arnould et al., 2001)
	Females	22.3 ± 1.3 (9)	
	Combined	20.6 ± 0.9 (17)	
<i>Northern fur seals</i>			
St Paul Island	Combined	16.1 ± 0.3–22.1 ± 0.7 (102) <sup>b</sup>	(Donohue et al., in press)
	Combined	20.8 ± 0.4 (5)	(Costa and Gentry, 1986)

<sup>a</sup> Male and female flux rates significantly different.

<sup>b</sup> Data derived using the doubly-labelled water method, sample sizes in parentheses.

Climatic conditions may explain to some extent the occurrence of freshwater drinking by pups at Cap Noir (Iles Kerguelen) but not at Bird Island (South Georgia). Mean daily temperatures were relatively high at Cap Noir (8.2 ± 3.3 °C) during February 1999 ranging from 3.6–22.8 °C. No weather data were available for the same study period as Arnould et al. (1996a), however, mean daily temperatures at Bird Island in February 2001 were considerably lower at 3.7 ± 1.2 °C (range 0.0–9.0 °C, BAS, Cambridge, UK). It is possible, therefore, that increased thermal stress and the availability of water at Iles Kerguelen influenced the frequency of water drinking behaviour in pups.

The majority of pups studied were moulting (16.7% pre-moult, 80.0% moulting and 3.3% post-moult), as were those studied by Arnould et al. (1996a) at South Georgia. Donohue et al. (2000) studied the thermoregulatory capabilities of pre-moult and post-moult Northern fur seals, finding that post-moult pups sustain significantly lower metabolic costs in water than pre-moult pups, whilst mass-specific field metabolic rate (FMR) peaks during moulting (Donohue et al., in press). Thus the differences in moult stage for 20% of the pups studied may have contributed to variability in MWP and ideally all pups studied should be at a similar moult stage to minimise such effects.

If the unaccounted rates of water influx for both the groups of free ranging pups in this study are thought to represent only the ingestion of water (they may also be related to differences in pup activity levels), then water consumption during the

4-day experimental period ranged from minimal quantities of water to up to one litre. Such large quantities represent a considerable source of error, possibly equalling actual MWP in fasting pups. However, given the similarity between enclosed rates of MWP for Antarctic fur seals in this study (Table 3) and that at South Georgia (Arnould et al., 1996a), if we were to assume that free-ranging (as opposed to captive) rates of MWP were also similar to those at South Georgia (26.5–28.4 ml kg<sup>-1</sup> d<sup>-1</sup>), then water consumption may only be half of estimated quantities.

It is, therefore, possible to estimate the potential error in total water intake caused by the inclusion of incorrect MWP values in milk intake calculations. If we were to assume that unaccounted flux rates (12.6 ml kg<sup>-1</sup> d<sup>-1</sup>) equalled water consumption, then TWI for a 10-kg pup would be over-estimated by 40% for a milk intake of 1 l and 20% for a milk intake of 4 l. These errors would be halved if we assumed that MWP due to differences in activity levels between captive and free-ranging pups represented half of unaccounted water influx (i.e. MWP similar to that recorded by Arnould et al., 1996a).

The behaviour of water drinking, whether salt or fresh, appears to be more prevalent in a range of seal species than once thought. Observations of drinking behaviour were common for pups, juveniles and adult males, suggesting that drinking may be important in the water balance of Antarctic fur seals. Drinking has not previously been reported for Antarctic fur seals, although anecdotal



observations of conspecifics drinking seawater have been noted at Macquarie (S. Robinson, personal communication) and Heard Islands (A. Wellington, pers. comm.) and both salt (J. Arnould, pers. comm.) and freshwater (D. Costa, pers. comm.) at South Georgia. Our study at Iles Kerguelen, demonstrates that Antarctic fur seals pups consume quantities of primarily fresh water, and some seawater, while fasting during maternal foraging trips. These trips may last up to 18 days (Lea et al., 2001), with up to 71% of the lactation period spent fasting (Arnould et al., 2001), during which time pups do not usually receive any nourishment. Although the factors influencing the consumption of water by Antarctic fur seal pups at Iles Kerguelen were not the focus of this study, levels of absolute humidity, the proportion of fat in milk, pup body composition and the consequent level of protein-sparing metabolism while fasting, could all influence the water balance of fasting pups and the degree to which they drink. The ease of accessibility to freshwater in the form of pools, seeps and streams to seals of all ages at Cap Noir (Iles Kerguelen) is undoubtedly an important factor in determining the extent of drinking behaviour at some sites.

Juvenile seals were also observed on several occasions to come ashore and move directly to a source of fresh water to drink for several minutes. This behaviour may be unrelated to that observed in pups, and may result rather from a high protein diet. Adult females were never observed to drink water while ashore, which could imply a difference in diet between adult female and juvenile Antarctic fur seals. The metabolism of protein requires large amounts of water for nitrogen excretion (Ortiz, 1987) and may contribute to the differences observed in water drinking rates between these two age classes. Further research addressing such possibilities is required in order to gain a better understanding of factors influencing water-drinking behaviour in Antarctic fur seals and pinnipeds in general.

No examples of fur seals drinking freshwater in captivity have been reported in the literature, however, captive harp seals have been observed to regularly take water in the form of ice cubes (Renouf et al., 1990). In a recent study of the fasting metabolism of Antarctic fur seal pups at South Georgia, (Arnould et al., 2001) have shown that pups adopt a protein-sparing strategy by day 3 of their fasting period (phase I). This implies

that added water would not be necessary for protein catabolism indicative of phase II and III fasting (Cherel et al., 1988). The estimated amount of water consumed by the treatment 2 pups was significantly positively correlated to total body water. Further experiments of a larger sample of water-drinking pups would be necessary in order to properly identify the relationship between size, body condition, milk composition and rates of water consumption.

Recently, studies of harp (Storeheier and Nordøy, 2001) and hooded seals (Skalstad and Nordøy, 2000) have demonstrated that both species may drink considerable quantities of salt water. Skalstad and Nordøy (2000) concluded that seawater drinking could not be excluded as a source of error when using labelled water techniques to estimate food consumption of free-ranging harp and hooded seals. In addition, Storeheier and Nordøy (2001) were able to demonstrate that the kidney of adult harp seals prevented net loss of body water after the ingestion of saltwater and that the drinking of seawater may increase urinary osmotic space, serving as a mechanism to excrete additional urea produced during phase I of fasting. Studies of mariposia in other marine mammals including sea otters, *Enhydra lutris* (Costa, 1982), and the common dolphin, *Delphinus delphis* (Hui, 1981), have also concluded that seawater may aid in urea elimination by increasing urinary osmotic space. Perhaps those pups observed drinking seawater at Iles Kerguelen had reached a more advanced phase of fasting.

In conclusion, it is probable that metabolic water production estimated by tritium dilution, in fasting Antarctic fur seals at Iles Kerguelen was overestimated by the consumption of exogenous water. In some instances, the consumption of water may account for up to 50% of the estimated rate of metabolic water consumption, representing the consumption of up to 1 l of water over 4 days for a 10-kg pup and consequently considerable underestimation of milk water flux. Future studies examining the metabolism of fasting otariids using the tritium dilution technique should not exclude the possibility of water consumption as a source of error without careful prior study of fasting animals. In instances where exogenous water consumption is expected, the doubly-labelled water method or indirect calorimetry may provide more accurate measurements of MWP of free-ranging and captive animals, respectively.

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## References

- Arnould, J.P.Y., Boyd, I.L., Socha, D.G., 1996a. Milk consumption and growth efficiency in Antarctic fur seal (*Arctocephalus gazella*) pups. *Can. J. Zool.* 74, 254–266.
- Arnould, J.P.Y., Boyd, I.L., Speakman, J.R., 1996b. Measuring the body composition of Antarctic fur seals (*Arctocephalus gazella*): validation of hydrogen isotope dilution. *Physiol. Zool.* 69, 93–116.
- Arnould, J.P.Y., Green, J.A., Rawlins, D.R., 2001. Fasting metabolism in Antarctic fur seal (*Arctocephalus gazella*) pups. *Comp. Biochem. Physiol. A* 129, 829–841.
- Cherel, Y., Robin, J.P., Le Maho, Y., 1988. Physiology and biochemistry of long-term fasting in birds. *Can. J. Zool.* 66, 159–166.
- 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., 1987. Isotopic methods for quantifying material and energy intake of free-ranging marine mammals. In: Huntley, A.C., Costa, D.P., Worthy, A.J., Castellini, M.A. (Eds.), *Approaches to Marine Mammal Energetics*. Society for Marine Mammalogy, pp. 43–66.
- Costa, D.P., Gentry, R.L., 1986. Free-ranging energetics of Northern fur seals. In: Gentry, R.L., Kooyman, G.L. (Eds.), *Fur Seals: Maternal Strategies on Land and at Sea*. Princeton University Press, New Jersey, pp. 79–101.
- Costa, D.P., 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.
- Doidge, D.W., McCann, T.S.C.,roxall J.P., 1986. Attendance behaviour of Antarctic fur seals. In: Gentry, R.L., Kooyman, G.L. (Eds.), *Fur Seals: Maternal Strategies on Land and at Sea*. Princeton University Press, New Jersey, pp. 102–114.
- Donohue, M.J., 1998. Energetics and development of northern fur seal, *Callorhinus ursinus*, pups. Ph.D thesis, University of California, Santa Cruz, 176 pp.
- Donohue, M.J., Costa, D.P., Goebel, M.E., Baker, J.D., 2000. The ontogeny of metabolic rate and thermoregulatory capabilities of Northern fur seal, *Callorhinus ursinus*, pups in air and water. *J. Exp. Biol.* 203, 1003–1016.
- Donohue, M.J., Costa, D.P., Goebel, M.E., Antonelis, G.A., Baker, J.D., in press. Milk intake and energy expenditure of free-ranging Northern fur seal, *Callorhinus ursinus*, pups. *Phys. Biochem. Zool.*
- Gentry, R.L., 1981. Seawater drinking in eared seals. *Comp. Biochem. Physiol.* 68, 81–86.
- Georges, J.-Y., Guinet, C., 2000. Maternal care in the subantarctic fur seals on Amsterdam Island. *Ecology* 81, 295–308.
- Goldsworthy, S.D., 1999. Maternal attendance behaviour of sympatrically breeding Antarctic and subantarctic fur seals, *Arctocephalus* spp., at Macquarie Island. *Polar Biol.* 21, 316–325.
- Guinet, C., Goldsworthy, S.D., Robinson, S., 1999. Sex differences in mass loss rate and growth efficiency in Antarctic fur seal (*Arctocephalus gazella*) pups at Macquarie Island. *Behav. Ecol. Sociobiol.* 46, 157–163.
- Higgins, L.V., Costa, D.P., Huntley, A.C., Le Boeuf, B.J., 1988. Behavioural and physiological measurements of maternal investment in the Steller sea lion, *Eumetopias jubatus*. *Mar. Mamm. Sci.* 4, 44–58.
- Hui, C.A., 1981. Seawater consumption and water flux in the common dolphin, *Delphinus delphis*. *Physiol. Zool.* 54, 430–440.
- Lea, M.-A., Hindell, M., Guinet, C., Goldsworthy, S.D., 2001. Variability in the diving activity of Antarctic fur seals, *Arctocephalus gazella*, at Iles Kerguelen. *Polar Biol.* DOI 10.1007/s00300-001-0339-6.
- Lifson, N., McClintock, R., 1966. Theory of use of the turnover rates of body water for measuring energy and material balance. *J. Theor. Biol.* 12, 46–74.
- Lunn, N.J., 1992. Fostering behaviour and milk stealing in Antarctic fur seals. *Can. J. Zool.* 70, 837–839.
- Nagy, K.A., Costa, D.P., 1980. Water flux in animals: analysis of potential errors in the tritiated water method. *Am. J. Physiol.* 238, 454–465.
- Oftedal, O.T., Iverson, S.J., 1987. Hydrogen isotope methodology for measurement of milk intake and energetics of growth in suckling young. In: Huntley, A.C., Costa, D.P., Worthy, A.J., Castellini, M.A. (Eds.), *Approaches to Marine Mammal Energetics*. Society for Marine Mammalogy, Lawrence, pp. 67–96.
- Oftedal, O.T., Iverson, S.J., Boness, D.J., 1987. Milk and energy intakes of suckling California sea lion, *Zalophus californianus*, pups in relation to sex, growth and predicted maintenance requirements. *Physiol. Zool.* 60, 560–575.
- Ortiz, C.L., 1987. Measurement of protein metabolism in naturally fasting phocids. In: Huntley, A.C., Costa, D.P., Worthy, A.J., Castellini, M.A. (Eds.), *Approaches to Marine Mammal Energetics*. The Society for Marine Mammalogy, Lawrence, pp. 29–42.

- Ortiz, C.L., Costa, D.P., 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., Costa, D.P., 1984. Milk intake of elephant seal pups: an index of parental investment. *Am. Nat.* 124, 416–422.
- Renouf, D., Noseworthy, E., Scott, M.C., 1990. Daily fresh water consumption by captive Harp seals (*Phoca groenlandica*). *Mar. Mamm. Sci.* 6, 253–257.
- Skalstad, I., Nordøy, E.S., 2000. Experimental evidence of seawater drinking in juvenile hooded (*Cystophora cristata*) and harp seals (*Phoca groenlandica*). *J. Comp. Physiol. B.* 170, 395–401.
- Storeheier, P.V., Nordøy, E.S., 2001. Physiological effects of seawater intake in adult Harp seals during phase I of fasting. *Comp. Biochem. Physiol.* 128A, 307–315.