FACULTY OF BIOSCINCES, FISHERIES AND ECONOMICS
DEPARTMENT OF ARCTIC AND MARINE BIOLOGY

Seawater consumption in dehydrated hooded seals (Cystophora cristata)

Are Verlo

BIO-3950 Master`s thesis in Biology

Spring 2012

Abstract

Studies based on satellite-tracking show that hooded seals (*Cystophora cristata*) spend many months out in the open sea each year. When the seals are out in the open sea they have no access to fresh water. Experimental studies have showed that hooded seals drink seawater and that they have the capacity to produce urine with Na^+ and cl^- concentrations equal to or higher than that of seawater. They may also produce urine with an osmolality twice that of seawater. This indicates that the hooded seal may use seawater to restore water balance and maintain homeostasis. The purpose of this study is to quantify the amount of seawater drunk as a result of a controlled experimental dehydration and to monitor the endocrine response to dehydration and seawater drinking. Following 24 hrs of fasting five subadult hooded seal males were dehydrated using the diuretic mannitol. They were then given *al lib* access to seawater for 48 hrs. For the duration of the experiment total body water and the turnover rates of water was estimated by tritiated water injections and subsequently isotope dilution. Plasma parameters such as Na^+, Cl^- were monitored and all seals maintained relatively stable plasma concentrations of the electrolytes for the duration of the entire experiment, while urinary excretion of Na^+ and cl^- increased after *ad lib* access to seawater. Plasma osmolality and hematocrit values indicated seawater intake. The amount of seawater drunk by the seals were calculated as the difference between total influx and water influx from respiration and endogenous reserves. The hooded seals drank on average 1900 ml of seawater each day. The role of the hormones ,ADH and aldoesterone ,in the osmoregualtion of hooded seals still remain somewhat unclear.

Table of contents

1.0 Introduction

1.1 About the Hooded Seal

The hooded (*Cystophora cristata*) seal was discovered in 1874 by the Norwegian sealer Edvard Johansen (Nansen 1924). The hooded seal has obtained it´s name from the inflatable nasal sac developed only by the male. The sac reaches its full size when the males sexually mature and are used for courtship displays. Adult males, weighing 200 to 360 kg are larger than adult females, weighing 150 to 250 kg.
The hooded seal has three breeding stocks; the Jan Mayen stock, the Davis Strait stock and the Newfoundland stock.

Studies based on satellite-tracking have showed that the Jan Mayen stock congregate west/north-west of Jan Mayen to give birth and then mate in late march (Folkow et al.1996). After moulting in July the stock disperse over vast areas of the North Atlantic (Folkow and Blix 1995, Folkow et al. 1996, Folkow et al. 2010). Satellite-tracking studies have showed that hooded seal pups spend 76.3% of the time in open water (Folkow et al. 2010). Birth takes place at the center of a large ice-floe. The pup weighs 25 -35 kg and is born with a subcutaneous layer of blubber. Sealers often refer to the pup as a blue back because of its white belly and blue-grey back. Lactation lasts 2- 4 days . During lactation the pup can grow at rates of up to 7 kg per day (Bowen et al. 1985). This impressive weight gain is due to the hooded seals´ milk being one of the most energy rich produced by any mammal (Oftedal 1984).

The hooded seal has a remarkable diving capacity, being able to dive to depths of more than 1000 meters and dive durations up to 1 hour (Folkow and Blix 1999). This diving capacity is due to a high capacity to store oxygen in the blood and the skeletal muscles, a low diving metabolic rate and a high tolerance to hypoxia (Burns et al. 2007, Folkow et al. 2008, Ramirez et al. 2007).

Prey items include Greenland halibut, red-fish, polar cod, capelin and squid (Sergeant 1976).

1.2 Maintaining homeostasis

During breeding and moulting the hooded seal has access to fresh water in the form of snow and ice. Several studies based on satellite-tracking have showed that the hooded seal pups may spend months out in the open water (Folkow et al. 2010). The seal is hypo-osmotic to its surroundings and during these weeks the seal can be said to live in a marine desert.

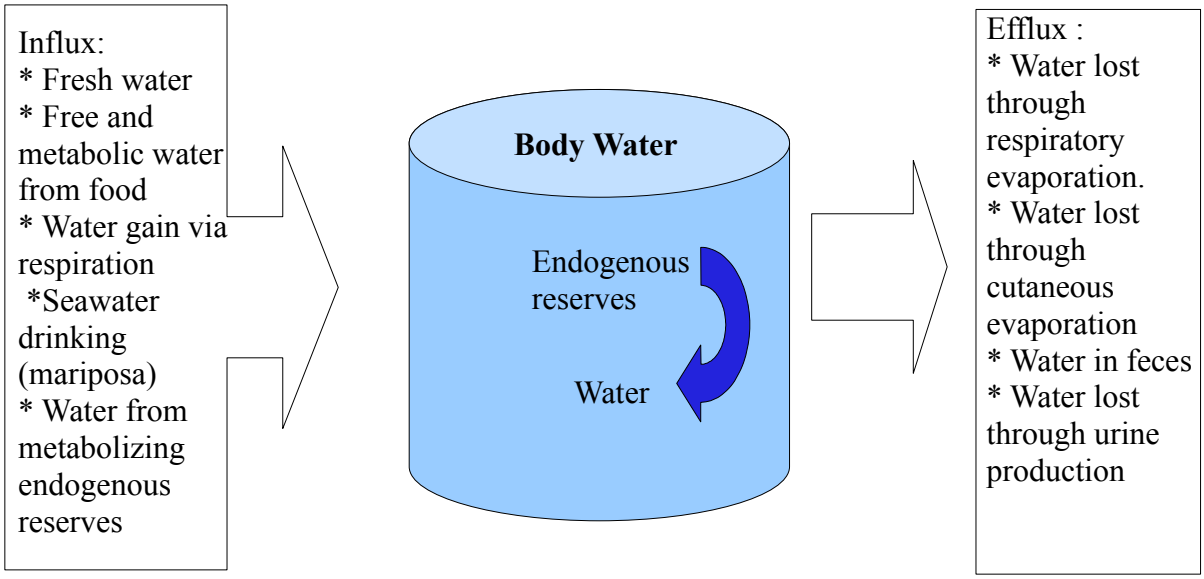

Fig. 1 shows the main routes of loss and gain of body water of a hooded seal.

1.2.1 Mammals have four main routes of water loss or efflux :

- Water lost through respiratory evaporation
- Water lost through cutaneous evaporation
- Water in feces
- Water lost through urine production

1.2.2 The hooded seal may have water influx from

- Fresh Water
- Water in food , both free and metabolic water
- Water influx/gain via respiration
- Water from metabolizing endogenous reserves
- potentially seawater drinking (mariposa)

1.2.3 Water lost through respiration

Inhaling and then exhaling will result in water lost. The air in the lungs have core body temperature and is 100% saturated with water. The capacity of air to hold water increases exponentialy with increasing temperature. When air with a temperature lower than core body temperature is inhaled both heat and water is transferred to the inhaled air, thus lost from the body. The water lost through respiratory evaporation is relative to the ambient temperature and humidity of the air. Seals have a nasal counter-current heat exchange (NHE) that reduces air and heat lost from the respiratory system (Skog and Folkow 1994, Folkow and Blix 1987). The NHE is primary a thermo- regulatory function, but it will reduce water lost through respiration. Studies have shown that respiratory evaporation of grey seals (*Halichoerus grypus*) is only 2 – 11% of total water loss (Folkow and Blix 1987). Without NHE the water lost through respiratory evaporation would be 10 – 33% of total water loss.

1.2.4 Water lost through cutaneous evaporation.

The seals have a thick coat of fur for isolation and a thick skin that keeps cutaneous evaporation to a minimum. The seals have few and inactive sweat-glands (Matsuura and Whitrow 1974). Evaporation from the skin´s surface would be an ineffective way to loose heat for a marine mammal. Overheating is not a threat for an animal that could cool down by submerging in the cool ocean.

1.2.5 Water lost with feces.

Water in the feces is iso-osmotic compared to body water. During fasting feces production is absent or nearly non-existent.

1.2.6 Water lost through urine production.

Studies have shown that harp seal (*Phoca groenlandica*) have maximum urinary of electrolytes (Na^+, Cl^-) higher than that of sea water (How and Nordøy 2007) and the maximum urine osmolarity was double that of seawater (Storeheier and Nordøy 2001, How and Nordøy 2007). During fasting urine production is kept to a minimum and urinary concentration of solutes increase (Nordøy et al. 1993). Urea is produced from metabolizing proteins. Urea has to be excreted at the expense of body water.

1.2.7 Fresh Water

Fresh water is available as snow and ice and would be readily available when the hooded seals congregate to breed and moult. When foraging near land fresh water from rivers would potentially be available. Seals out in the open seals would not have access to fresh water of any kind. Folkow et al. (2010) showed that bluebacks spent 76% of the time in the open waters during their first year.

1.2.8 Water from Feeding and metabolizing endogenous reserves

Irving (1935) found that harbor seal (*Phoca vitulina*) fed herring would have enough water available to completely replenish the water lost through urine production, respiratory evaporative water loss and feces under normal conditions. Depocas (1971) found that water from food is the most important influx route of water in the harbor seal. Water from feeding, both free and metabolic, plus inspired water accounts for 90% of total daily influx. When feeding on fish both free water (50 -70% of the fish body mass) and water from metabolizing fat and proteins is available. When metabolizing 1 g fat, 1,07 g water is made available, while metabolizing 1g proteins 0,39g water is made available. Urea accumulates from breaking down proteins and have to excreted at the expense of body water (obligatory urine production).

1.2.9 Water gained from respiration

As a marine mammal, the seal is breathing humid air. Still Skalstad and Nordøy (2000) found that the influx was so small that it was negligible. Depocas (1971) found that influx from inspired air corresponded to 93 – 108 ml per day.

1.2.10 Water gained from seawater drinking

Whether marine mammals drink seawater is a question many researchers have sought to answer. Early studies (Irving et al. 1935, Krogh 1939, Fetcher and Fetcher 1942) suggest that marine mammals do not drink seawater and the animals cover their daily water needs by feeding. Several studies have shown that marine mammals may drink seawater (Gentry 1981, Renoulf et al. 1990 , Skalstad and Nordøy 2000).

Studies have also shown that harbor seal is not able to drink seawater with a net gain of water (Tarasoff and Toews 1972). Hong et al. (1982) found that the ringed (*Pusa hispida*) and Baikal seals (*Pusa sibirica*) does not have the renal capacity to have a net water gain from drinking seawater. Depocas (1971) suggested that the seawater intake in harbor

seals is a result of feeding, since it increased linear with the amount of fish eaten. Newer studies (Skalstad and Nordøy 2000, Storeheier and Nordøy 2001, How and Nordøy 2007) suggest that hooded and harp seal does drink water, has the renal capacity to gain water through seawater drinking. Skalstad and Nordøy (2000) hand-fed the seals to prevent seawater intake related to feeding and still reported that the adult harp seals drank 900 ml seawater each day.

A fully hydrated seal strictly does not need to drink seawater. Studies have shown that under normal conditions feeding on fish and even clams provides enough water to maintain homeostasis. Some studies (Hong et al. 1982, Tarasoff and Toews 1972, Storeheier and Nordøy 2001) have applied a 2- 4 day fast to induce food and water deprivation. As shown by Nordøy (1992) grey seal pups can fast on land for 40 days without access to exogenous water without exhausting the animals defenses with regards to maintaining water balance and homeostasis.

It is therefor likely that the studies applying a short fast to achieve dehydration does not challenge the full capacity of the pinnipeds to utilize seawater as a source of exogenous water during dehydration.

1.3 The aim of this study

Studies based on satellite-tracking have shown that the hooded seal spend many moths each year out in the open seas. During these stays the seal only has access to water from feeding and metabolizing endogenous reserves. Several studies have suggested that hydrated marine mammals maintain water balance and electrolyte balance by feeding on fish and mollusks. Previous studies (Skalstad and Nordøy 2001) have showed that both the hooded seal and the harp seal have seawater intake that is not related to feeding. Studies such as How and Nordøy (2007) and Storeheier and Nordøy (2001) have showed that the harp seal can have urinary concentrations of Na^+ and Cl^- that equals or exceeds the concentration found in seawater. And that the maximum urinary osmolarity of harp seal urine can reach double the osmolarity of seawater. These are strong indications that seals, such as the harp seal, can drink seawater with a net water gain. How and Nordøy (2007) showed that experimentally dehydrated hooded seals given *ad lib* assess to seawater could restore electrolyte balance and homeostasis. However the design of the the study did not allow for quantification of the seawater drunk.

I aim to quantify the amount of seawater drunk by dehydrated hooded seals . Because previous studies have potentially failed to dehydrate the seal sufficiently, I will administer an intravenous dehydrating agent to fasting hooded seals. This will increase urine output and cause plasma concentrations of electrolytes to increase and lower the amount of total body water (TBW). For maximum effect of he dehydrating agent, the seals will be kept on land for 24 hours. The dehydrated seals will then be given ad-lib access to seawater for 48 hrs. The seawater will be the only source of exogenous water for the fasting seals.
TBW will be monitored during the study using isotope-dilution. The amount of seawater drunk will be calculated from change in TBW and water from metabolizing endogenous reserves of fat. I will also monitor the endocrine reactions as a consequence of seawater drinking.

2.0 Materials and Methods

2.1 Animals

Five hooded seal males aged 1 – 2 years were used for the experiments. The seals were caught as «blue-backs» in the West ice. They were transported to, and then kept at the Department of Arctic Biology at the University of Tromsø. All animals appeared to be healthy and in good general condition during the experiment.

Table 1 Average weight of the 5 hooded seals used in the experiment.

Animal	Weight (kg)
Seal 2	87 ± 3,5
Seal 3	77,5 ± 3,5
Seal 4	83,5 ± 2
Seal 5	96 ± 4,5
Seal 6	71 ± 2,5

Weighing was done using a Salter suspended weight (mod. 235, England 300 ± 1 kg)

2.1.1 Animal living conditions

Prior to the experiments the seals were kept in two separate indoor pools containing 42 m^3 of seawater. The seawater was kept fresh by filters and a steady supply of 50 liters of seawater per minute. The osmolarity of the pools was monitored during the experiments by taking water samples and analyzing them as described in 2.3.3. Osmolality ranged from 921 to 970 mosm/kg, with an average of 949 mosm/kg.

The temperature in the pool water was 9 - 10^{0}C and the temperature in the pool-room was kept at 9 -11^{0}C during the experiment. The artificial light regime was computer-controlled and simulated day/night rhythm at 70^{0}N. The temperature was measured with a hand held probe at 1.5 meters depth.

2.1.2 Feeding

Before the experimental period, the seals were fed 2.5 – 3.5 kg recently thawed herring per day. The herring was frozen in 20 kg blocks and the blocks were left to thaw in running water. The seal´s diet was also supplemented with antioxidant vitamin supplement formulated for marine animals. The animals were fed two tablets each day.

Table 2 The contents of each tablet fed the seals

Supplement	Dose
Vitamin A (retinol acetate)	10,000 I.U
Vitamin D_3 (cholecalciferol)	400 I.U
Vitamin C (Calcium ascorbate)	10 mg
Vitamin E (d-alpha tocopheryl succinate)	50 I.U
Vitamin B1 (thiamine mononitrate)	250 mg
Vitamin B2 (riboflavin)	2 mg
Vitamin B3 (niacinamide)	6 mg
Vitamin B6 (pyridoxine hydrochloride)	2 mg
Folic Acid	500 mg
Vitamin B12 (cyanocobalamin)	5 mg
D-Biotin	200 mcg
Kelp Plant Extract (*Laminaria digitata)*	20 mg
CLA (conjugated Linoleic Acid)	10 mg
Inositol	5 mg
Lycopene	300 mcg
Lutein	250 mcg

Note: 24 hrs prior to the experiments start the feeding was halted and the seals fasted for the duration of the entire experiment. See experimental protocol below for more details.

2.1.3 Animal Handling and the use of seditative

The seals were given an intramuscular injection (0.35 – 0.65ml) of zoletil (Zoletil forte vet. France. 50mg/ml Tiletamin, 50mg/ml Zolazepam) when needed to ease handling. For the remainder of the experiments zoletil was administered when the animals were to be restrained and blood samples collected.

2.2 Experimental protocol

The experiments were performed during September and the first week of October 2011. A pilot experiment was done first, thereafter the first experiment started 10th of September. Each experiment lasted for 5 days.

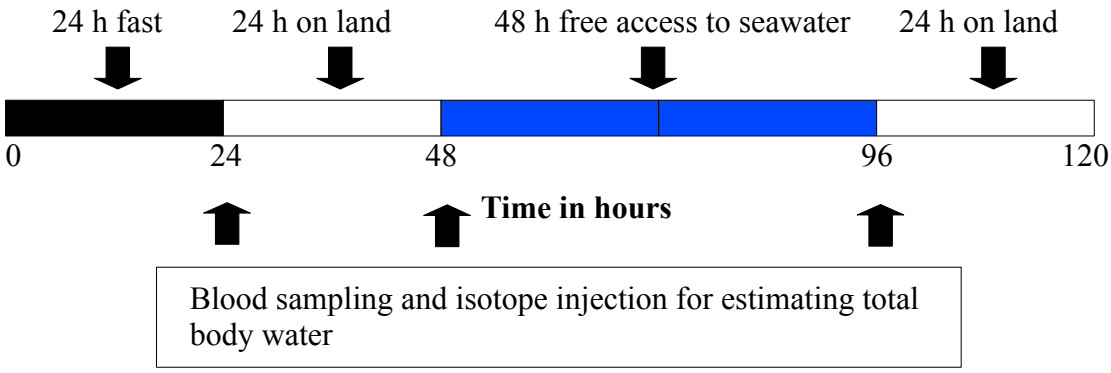

Fig. 2 Schematic presentation of the experimental protocol

At the start of each experiment:

- The pools were drained.
- The seals fasted for 24 hours.

After fasting the seals were caught with a hoop-net, weighted and restrained on a board. Blood was drawn by a catheter (16G/1.7 x 160mm Secalon T, Ohmeda, Swindon, UK) inserted in the extradural intravertebral vein at the level of the fourth vertebra.

The seals were then injected with a dose of tritiated water (2.2– 3.6 MBq/kg). After an equilibration period of 1.5h the isotope was distributed through the various body compartments (Skalstad and Nordøy 2000). Then blood was drawn using the inserted catheter.

The seals were then given a preheated dose (25 ml of Mannitol per kg body mass ,37^{0}C) of the diureticum Mannitol (150 mg/ml, 930 mosm/l, Fresenius Kabi,Uppsala,Sweden) over a period of 2.5 hrs, through the extradural vein, in order to induce dehydration.

Then the seals were then kept fasting on land for 24 hrs to achieve maximum dehydration. During this stay they were kept in special sealcage with a funnel under a metal grid to collect urine. The seals were then weighted, restrained and catheterized. Blood samples were collected and tritiated water was injected to estimate TBW once more.

The seals were then released into the pools and the pools were refilled with seawater at

the rate described previously. During their stay in the pool, the seals had ad-lib access to seawater.

After a 48 hrs stay in the pool, with ad-lib access to seawater, the seals were caught, weighted and restrained on a board. Blood was drawn and tritiated water was injected to estimate TBW. The seals were then transferred to a cage for 24 hrs to collect urine.

2.2.1 Fasting

The seals were fasted 24 hours prior to being caught and restrained on the first day. Such a short fasting period is not enough to cause dehydration. The seals were kept fasting for the duration of the experiment to ensure that food would not provide an exogenous source of water.

2.2.2 Blood sampling

The blood was drawn by inserting a catheter as previously described.

Immediately after blood was drawn it was transferred to a 10ml heparinized centrifuge tube, gently turned from side to side and briefly placed in ice-water. The centrifuge tubes were treated with two heparin (Nycomed Pharma, Oslo, Norway) droplets (ca 0,18g 5000 IU/ml) prior to the experiment, to prevent the blood from coagulating. The blood was then centrifuged (KS-8000, Kubota Corporation, Tokyo, Japan) for 10 minutes at 3000 rpm. The blood was then transferred to a 2,0 ml cryovial (Simport Plastic, Quebec, Canada) and frozen in a -80^{0}C freezer until analysis.

2.2.3 Urine sampling

Urine was collected when the seals were kept in the cages prior to and after their 48 hour stay in the pool. At the bottom of the cage there is a funnel and the urine was collected in bottles kept in ice-water under the funnel.
The urine was then transferred to 20 ml polyethylene vials and frozen at -20^{0}C. Prior to analysis the frozen urine was thawed and centrifuged for 10 minutes at 3000 rpm. The supernatant was transferred to a 2,0 ml cryovial.

2.3 Analysis of blood- and urine parameters.

Blood- and urine samples were analyzed for the concentration of Na^+, K^+, Cl^-, Mg^{2+} , urea and osmolarity.

Parameter	Source	Analytical procedure
Na^+	Plasma and urine	Indirect potentiometry
Cl^-	Plasma and urine	Indirect potentiometry
K^+	Plasma and urine	Indirect potentiometry
Mg^{2+}	Plasma	Spectrophotometry (Xilidyl blue)
Mg^{2+}	Urine	Spectrophotometry (o-cresolphtalein)
Urea	Plasma and urine	Enzymatic photometry
Osmolality	Plasma and urine	Cryoscopic osmometry
Aldosterone	Serum	Competitive radioimmunoassay (RIA)
Anti- diuretic hormone (ADH)	Plasma	RIA

2.3.1 Indirect potentiometry

In indirect potentiometry, a liquid ion exchanger membrane separates two aqueous solution, the internal reference whose concentration is fixed and the external test solution at varying concentration. The method is based on electrode potential of an Ion-selective electrode (ISE) that are measured relative to a reference electrode.
When two different concentration of similar electrolytes are separated by selective membrane , a membranes potential is developed across the membrane walls which is then measured.

ISEs do not measure the concentration of an analyte, they measure its *activity*. The activity of an ion is its concentration, times the activity coefficient. Dilute solutions exhibit nearly ideal behavior; activity coefficient ~1. In indirect potentiometry the solute is diluted, and any excluded volume (for instance lipids) will introduce an error, which is usually insignificant.

Based on Nernst´s equation it is possible to calculate the concentration of the electrolyte selected for by the ISE.

$E = E_0 + (RT/NF)\ln[fC]$
where;

E =the potential of the ISE at temperature T
E_0= the potential of the reference electrode
N= the number of electrons transferred
R= Ideal gas constant
f= Faraday´s constant

2.3.2 Spectrophotometry and enzymatic spectrophotometry

Spectrophotometry is based on the principle that absorbance of electromagnetic energy is quantitative.
For laboratory applications, it usually involves radiation in the ultraviolet and the visible regions of the spectrum.

Design of spectrophotometric methods:

- The analyte absorbs a specific wavelength
- The analyte reacts with a regent, to produce an abduct that absorbs a unique wavelength, called a chromophore
- The analyte is involved in a reaction that produces a chromophore.

The absorbed light at the given wavelength is the compared to the absorption of a control sample with a known concentration.

Urea in blood and urine is found by adding urease and an alkaline solution of phenol and hypochlorite, called Berthelot's reagent , to the sample. Urease catalyze the hydrolysis of urea into ammonia and CO_2. Ammonia binds to Berthelot's reagent and is then measured as a chromophore.

For Mg^{2+} in the urine, the test solution is acidified to release Mg^{2+} bound to proteins. But a o-cresolphtalein - Mg^{2+} complex forms at alkaline pH. The complex acts as a chromophore at wavelengths 570 -580nm. For Mg^{2+} in the blood, Xilidyl blue is used to complex magnesium. The chromophore absorbs light at wavelength of about 600 nm.

Analysis of plasma- and urine concentrations of Na^+, Cl^-, K^+, Mg^{2+} and urea was done using a Modular P (Roche, Penzberg, Germany)

2.3.3Cryoscopic Osmometry

Cryoscopic osmometry uses freezing point depression to determine the total osmolality of aqueous solutions. The freezing point of pure water (0^0C) is compared to that of the solution. A solution with a total osmolality of 1 Osmol/kg has a freezing point of - 1,858^{0}C.

The equation used calculate osmolality is;

$C_{osm} = \Delta T/K$	C_{osm} = osmolality [osmol/kg]
	T = freezing point depression [^{0}C]
	K = 1,858^{0}C kg/osmol freezing point constant

2.3.4 Hematocrit centrifuge

Capillary tubes are filled with blood and centrifuged for 10 minutes at 13000 rpm. The lid is removed and a hematocrit plate placed over the capillary tubes. The capillary plate is opaque and covered with %-increments. The plate is rotated to cover an individual capillary tube and the hematocrit is read manually by matching the %-increments to the blood in the tubes. A Hettich EBA 12 hematocritcentrifuge, Tuttlingen, Germany was used.

2.3.5 Competitive radioimmunoassay

Competitive radioimmunoassay is based on the principle of two or more bodies competing for associating with another substrate. The isotope labeled antigen (^{125}I-aldosterone) competes with unlabeled antigen (aldosterone) for binding with aldosterone specific antibodies. The unbound antigen is separated from the bound antigen and the radioactivity is measured. By comparing the measured radioactivity to a known standard, the concentration of aldosterone can be found.

A kit from Siemens Healthcare Diagnostics, Los Angeles, USA, was used to find plasma concentration of aldosterone.

A Kit from Bühlmann Laboratories AG, Schönenbuch, Switzerland was used to find plasma concentration of ADH. Prior to RIA, preparative cromatography was used to separate ADH from carrier proteines. Then RIA was used to find plasma concentration of ADH. The marked regent is ^{125}I-ADH.
Reference for all analytical procedures : Kaplan (2010)

2.3.6 Transport and analysis of blood- and urine samples.

The samples were kept frozen at -80^{0}C until the samples were transported to Rikshospitalet, Oslo. The samples were securely packed in a box with dry ice and during traveles by plane from Tromsø to Oslo. All the blood- and urine samples were analyzed for electrolytes and urea at Rikshospitalet, Oslo. Hormone analysis was done at Aker universitetssykehus, Oslo

2.4 Estimating Total Body Water (TBW) by hydrogen isotope dilution.

Both TBW and the rates of water influx and efflux can be measured in animals by injecting them with tritiated water, and then following the decline in the specific activity (SA) of the isotope (H*) over time.

The method is based on 6 assumptions provided by Lifson and McClintock (1966)

1. Body water volume remains constant during the measurement period
2. The rates of water influx and efflux remains constant
3. The isotope labels only water in the body
4. The isotope leaves the body only as water
5. The SA in the water lost is the same as in body water
6. labeled or unlabeled water from the environment does not enter the animal via the skin or through respiration.

If these assumptions are incorrect they may cause significant error in the calculated flux rates. Nagy and Costa (1980) provides a review of these assumptions and the potential errors they provide. They also provide alternate equations when conditions/assumptions are invalid.

2.4.1 Estimating TBW

TBW (ml) may be found by using the following equation:

$$TBW = \frac{\text{i. d. (cpm)}}{SA_E \text{ (cpm/ml)}}$$

Where; TBW= Total Body Water

i.d. = Injected dose (cpm)

SA_E = Isotope specific activity at equilibrium

cpm = counts per minute

To find SA_E use the following equation:

$$S.A._E = \frac{(S.A._{t=1} - S.A._B)}{\%\ H_2O \text{ plasma}}$$

Based on assumption 3 from Lifson and McClintock, the activity is distributed in the water of the plasma, not the solids. Thus we correct for the distribution by dividing the activity by the water content of plasma.

The plasma water content was found to be 90.26 %, by drying plasma at 60^0C over night and cooling the plasma in an exicator with silica gel crystals.

2.4.2 Scintillating Counting

To measure the activity in the blood samples 9 ml scintillation liquid (Ultima Gold XR, Waltham, USA) was added to 0,4 ml plasma in a 20 ml polyethylene vial. The vial was shook by hand for 1 minute. The sample were then analyzed in a scintillation counter (Packard Tri-Carb liquid scintillation spectrometer mod. 3375 Warrenville, Downers Grove, USA). When the isotope decays it emits energy and excites crystals in the scintillation liquid to emit a light burst. Each disintegration generates a light burst, but the counter will not count the bursts at 100% efficiency.

dmp = disintegration per minute

cpm = counts per minute

The counter will provide an estimate of the counting efficiency. This estimate is based on counting the activity of a control sample with a known activity.

2.4.3 Calculating water turnover

Turnover is found by using equation 4 and 6 from Nagy and Costa (1980)

$$\frac{\text{ml } H_2O \text{ efflux}}{\text{day*kg}} = \frac{2000\,(W_1 - W_2)\,LN\,(H_1 * W_1 / H_2 * W_2)}{(M_1 + M_2)\,LN\,(W_2 / W_1)\,t}$$

$$\frac{\text{ml } H_2O \text{ influx}}{\text{day*kg}} = \frac{\text{ml } H_2O \text{ efflux}}{\text{day*kg}} + \frac{2000\,(W_2 - W_1)}{t\,(M_1 + M_2)}$$

Where; M = body mass in g

W = body water in ml

H = specific activity of the isotope in body water in cpm/ml

t = time elapsed between initial and final body water samples in days

$_1$ and $_2$ is initial and final subscript

Then we apply equations 32 and 39 from Lifson & McClintock (1966) to correct for fractionation and exchange (assumption 3 and 5).

2.4.4 Fractionation

$$r_{H2O} = fi * r_G + r_L$$

where; r_{H2O} water turnover corrected for fractionation

r_G = the gaseous fraction of turnover

r_L = the liquid fraction of turnover

fi = the fractionation factor (tritium = 0.93, Sepall and Mason 1960)

Skalstad and Nordøy (2000) found that the fraction of turnover subject to fractionation was taken as the difference between total water turnover and urine production. Assuming 90% of total water turnover is subject to fractionation and multiplying by a fractionation factor of 0.93, the calculated turnover is an underestimate of about 6%.

2.4.5 Exchange

$$\frac{(r_{H20})calc}{r_{H2O}} = \frac{r^{R}_{H20} + r^{E}_{H20} - jr^{I}_{H2O}}{r^{R}_{H20} + r^{E}_{H20} - r^{I}_{H2O}}$$

where;

$(r_{H20})calc$ = uncorrected turnover

r_{H2O} = turnover corrected for exchange

r^{E}_{H20} = water lost via expiration

jr^{I}_{H2O} = water entry via inspiration

r^{R}_{H20} = water lost from all remaining routes

j = ratio of the specific activity of inspired air to that of the body water (= 0)

Skalstad and Nordøy (2000) found that by using the equations from Folkow and Blix (1987), Kleiber (1975) and Lifson and McClintock (1966), while knowing the ambient temperature and assuming 100% relative humidity in the air, that the calculated turnover was an overestimate of 4 – 5 %.

2.4.6 Respiratory minute volume

Water influx via respiration was be calculated by applying the following equation from Folkow and Blix (1987) and the Kleiber-equation (Kleiber 1975). The BMR found by the Kleiber equation will then be multiplied by two to find field metabolic rate since the seals are active and growing.

Folkow and Blix (1987): $V= 0,042\ MR + 0,119\ l\ min^{-1} * kg^{-0,75}$

where;

V = respiratory minute volume

MR = metabolic rate ($W *kg^{-0.75}$)

Kleiber (1975): $MR= 70*W^{0,75}$

where;

MR = Metabolic rate

W = body weight (kg)

Assuming 100% saturation of water vapor in the inspired air and using a known air temperature, we can find the partial pressure of water at this temperature.
Comparing the partial pressure of water to the standard pressure of one atm , 760mmHg, we can quantify the volume of the air inspired, by multiplying by the calculated respiratory volume per day.

The volume calculated by using the equation of Folkow and Blix (1987) is volume at standard temperature pressure dry (STPD) and must be corrected to include water vapor.

$V = V_{STPD} * P_{H2O} * (P_{tot} – P_{H2O})$

Where;

V = respiratory minute volume

V_{STPD} = volume at STPD

P_{tot} = total pressure (760 mmhg)

P_{H2O} = partial pressure of water vapor

2.5 Statistics

Blood and urine parameters were compared statistically by using a one tailed Student´s t test for paired data. A statistical significant difference between means was indicated by $P \leq 0.05$.

3.0 Results

Body mass, plasma- and urinary parameters obtained at t = 1 day is considered baseline. Samples collected after t = 2 days are considered to be after dehydration and samples collected at t = 4 for plasma parameters and body mass and t=5 days for urinary parameters, are considered to be after rehydration. Averages are presented ± SD

3.1 Body mass

Body mass decreased with an average of 4,1 kg to an average of 82 ± 9 kg after dehydration ($P < 0.001$). After rehydration the average body mass decreased a further 1 kg ($P < 0.05$). Values range from 105 kg to 69 kg.

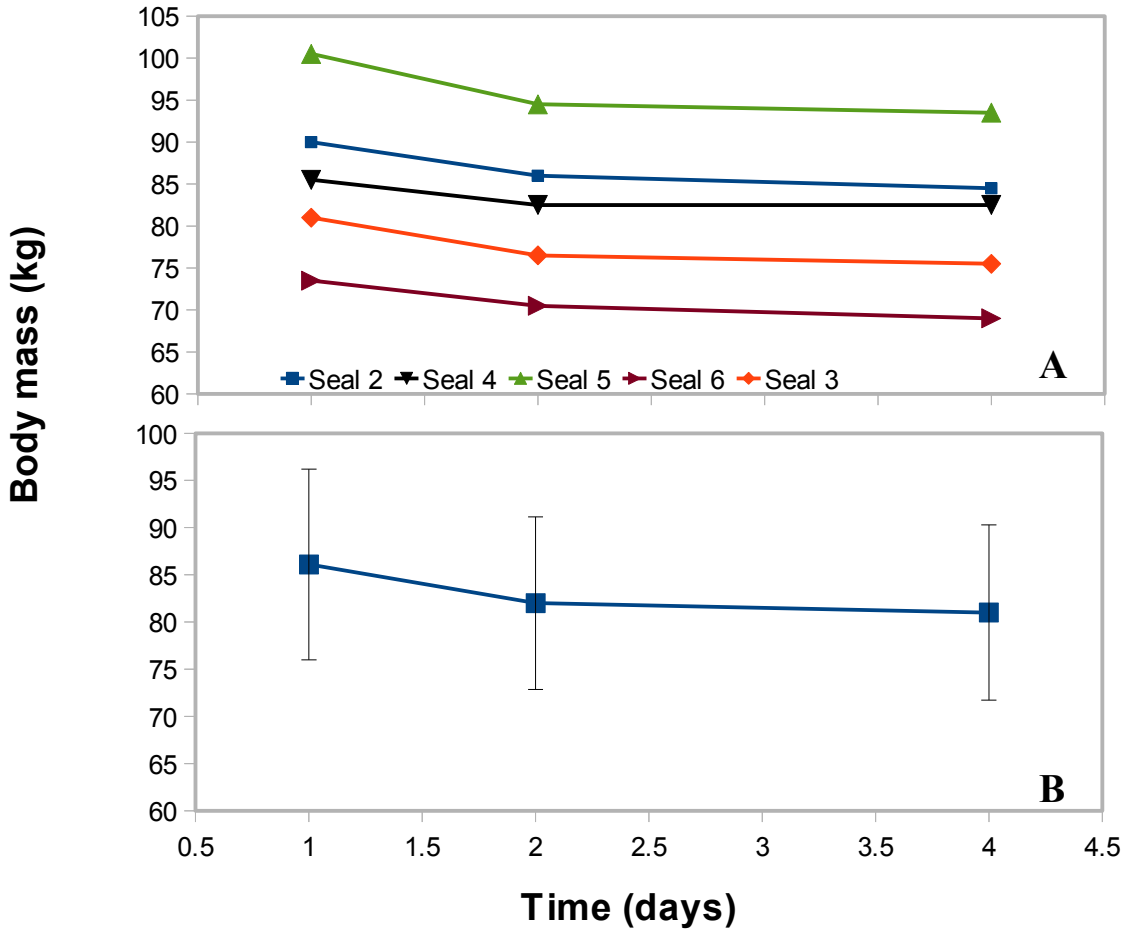

Fig 3 shows body mass (kg) as a function of time (days). (A) Individual seal´s weight as a function of time (days), (B) as average (n=5) ± SD

3.2 Plasma parameters

3.2.1 Plasma osmolality

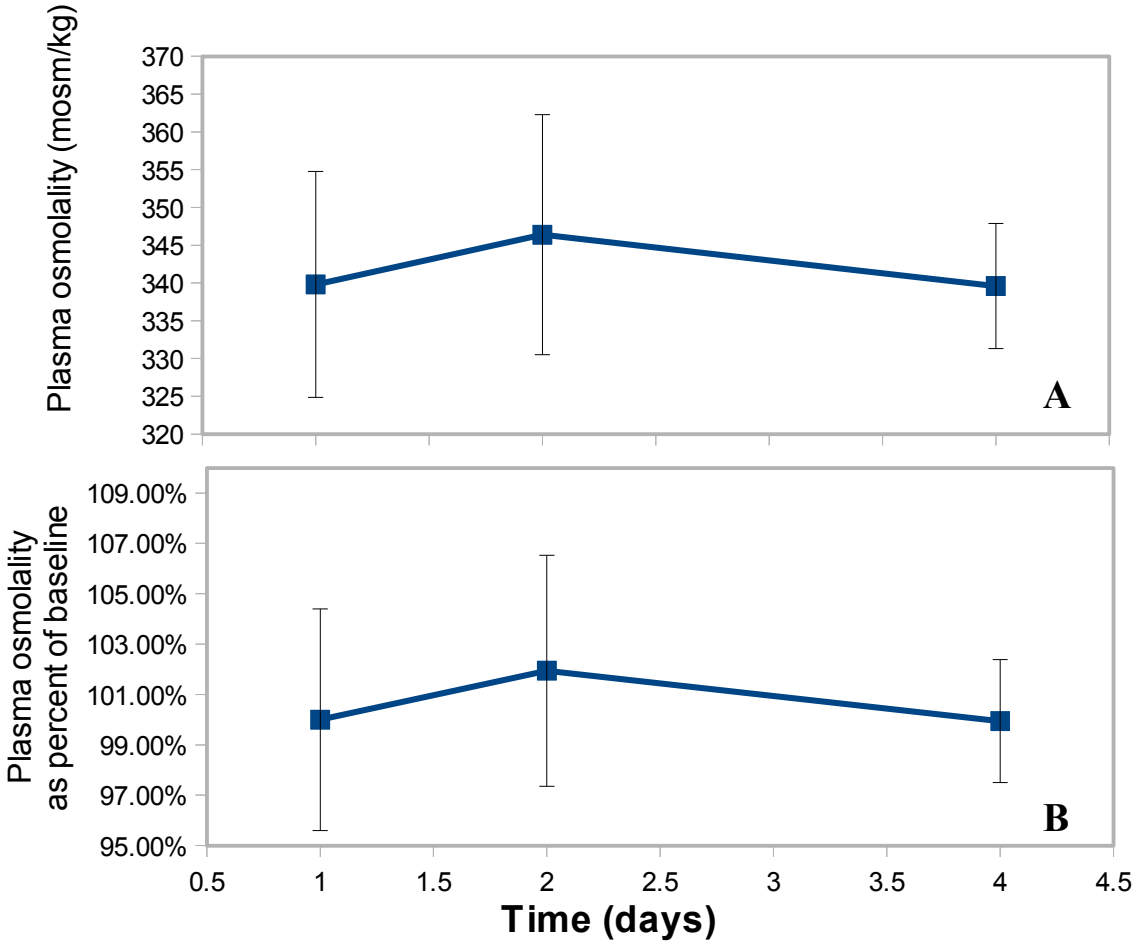

Fig. 4 Shows average (n=5) ± SD plasma osmolality as a function of time (days), (A) shows average as mmol/kg, and (B) shows plasma osmolality as percent of baseline osmolality (t = 1)

There was a tendency of rising plasma osmolality after dehydration, with values ranging from 330 – 366 mosm/kg at baseline and 333 – 373 mosm/kg after dehydration (P> 0.05). After rehydration there was a trend that plasma osmolality decreased, with values after rehydration ranging from 330 - 347 mosm/kg (P> 0.05).

3.2.2 Hematocrit

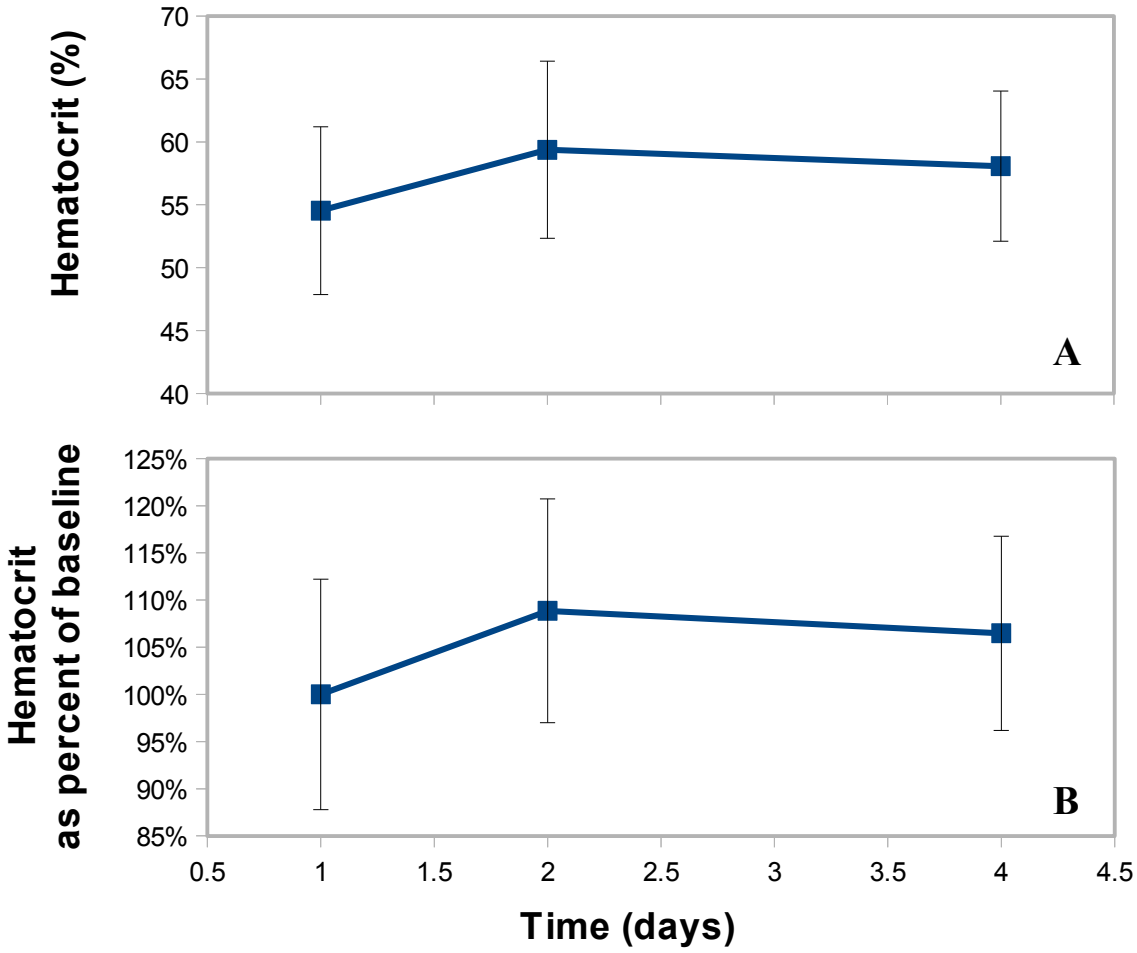

Fig. 5 shows hematocrit averages (n = 5) ± SD, (A) as hematocrit % and (B) as percent of baseline (t = 1).

Hematocrit had a significant increase after dehydration ($P < 0.05$), from an average of 54,5 ± SD % to 59,4 ± SD % after dehydration. After rehydration there was an decreasing trend with values ranging from 49 to 65% hematocrit , with an average decrease of 1 %($P > 0.05$). Values for the whole sample pool ranged from 45 to 65 % Hct.

3.2.3 Plasma electrolytes

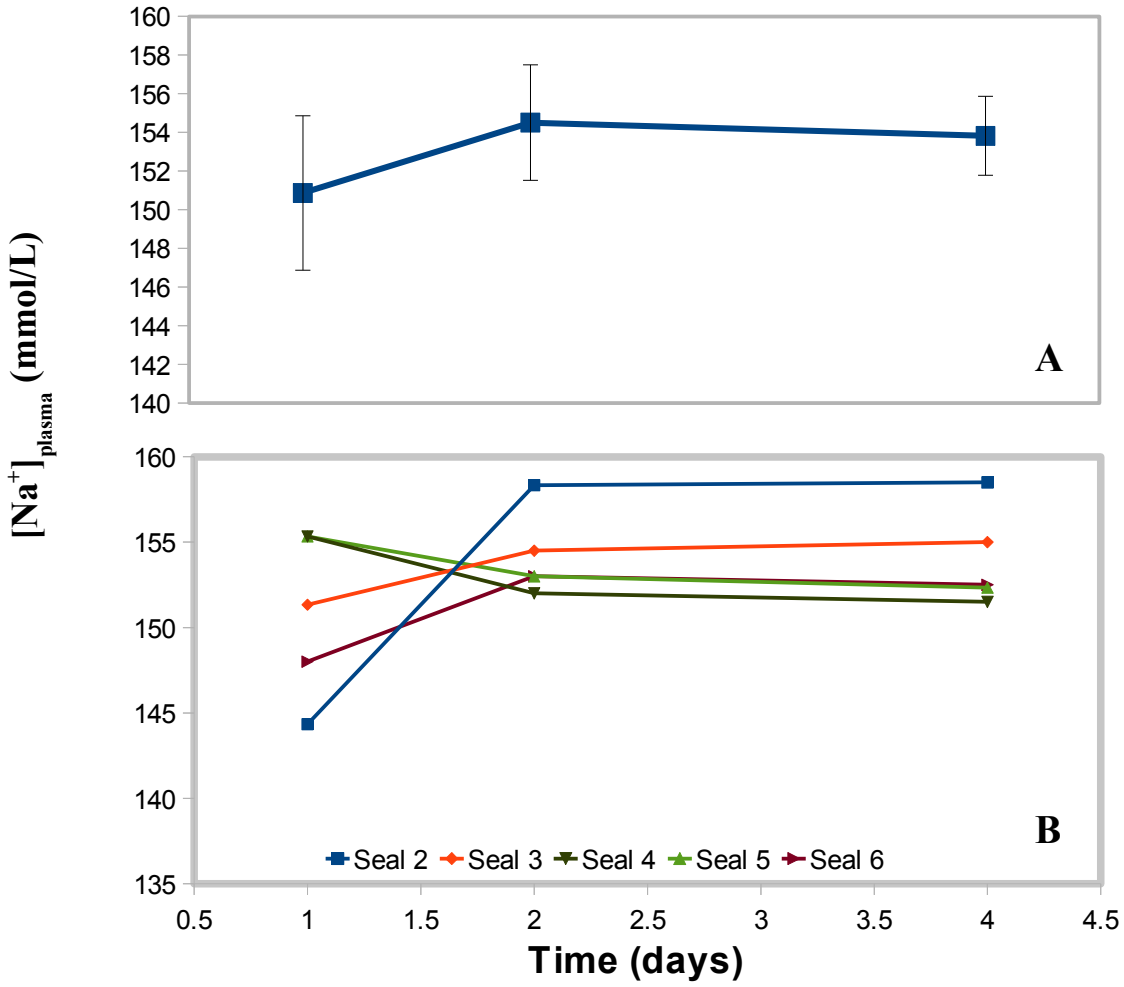

Fig. 6 shows plasma concentrations of Na^+ as a function of time (days), (A) shows average plasma concentration (n = 5) ± SD and (B) shows individual plasma concentrations.

There was no significant difference between averages at baseline, after dehydration and after rehydration ($P > 0.05$). Average values for plasma concentration of Na^+ range from 150 to 155 mmol/L, with an average of 153 ± 4 mmol/L The individual seals show varying trends of plasma Na^+ after dehydration as seen in panel B. While the average concentration of Na^+ is stable ranging from 151 to 154.5 mmol/L.

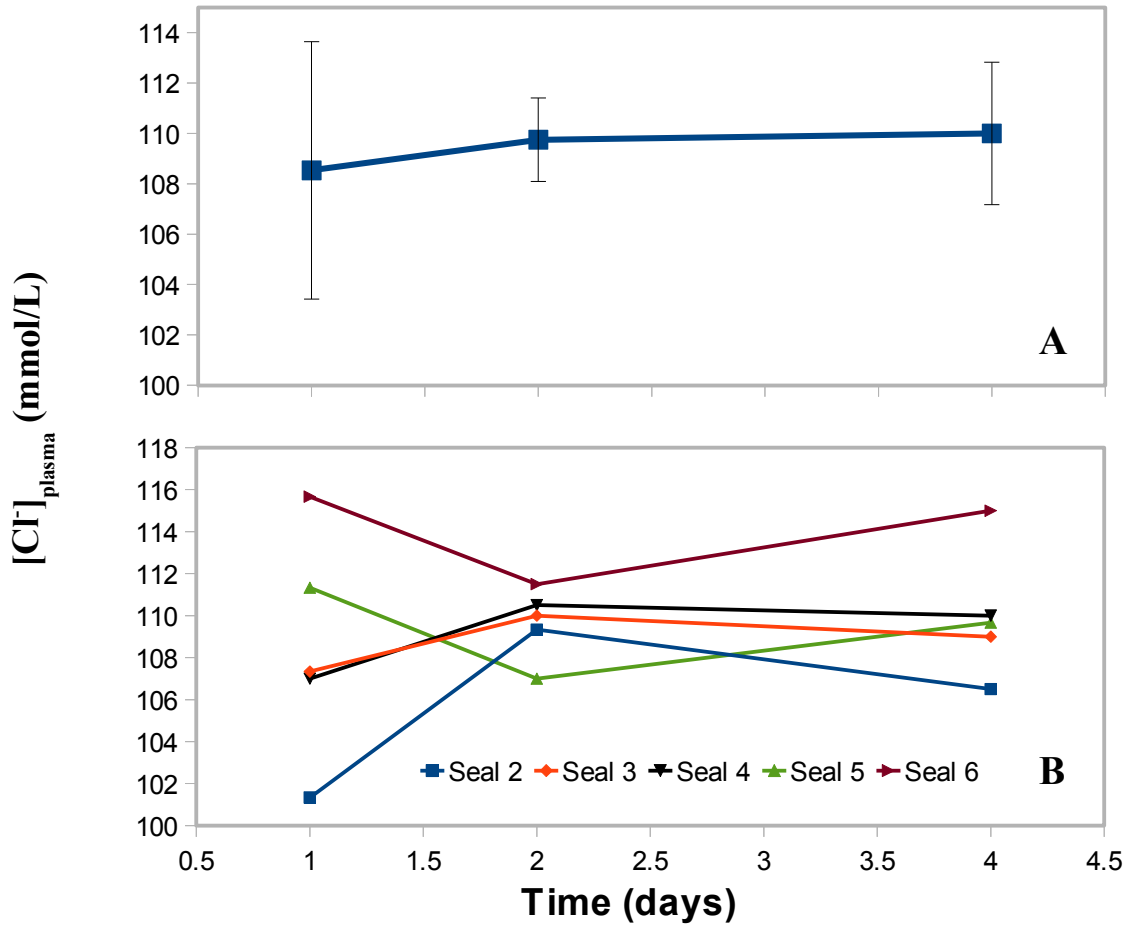

Fig7 shows plasma concentrations of Cl^- as a function of time (days), (A) shows average plasma concentration (n = 5) ± SD and (B) shows individual concentrations.

Plasma concentrations of Cl^- remained stable with values ranging from 101 to 115 mmol/L with an average of 109 ± 3.5. There was no significant difference between means at baseline, after dehydration and after rehydration ($P > 0.05$). There was a trend of individual variation decreasing with time.

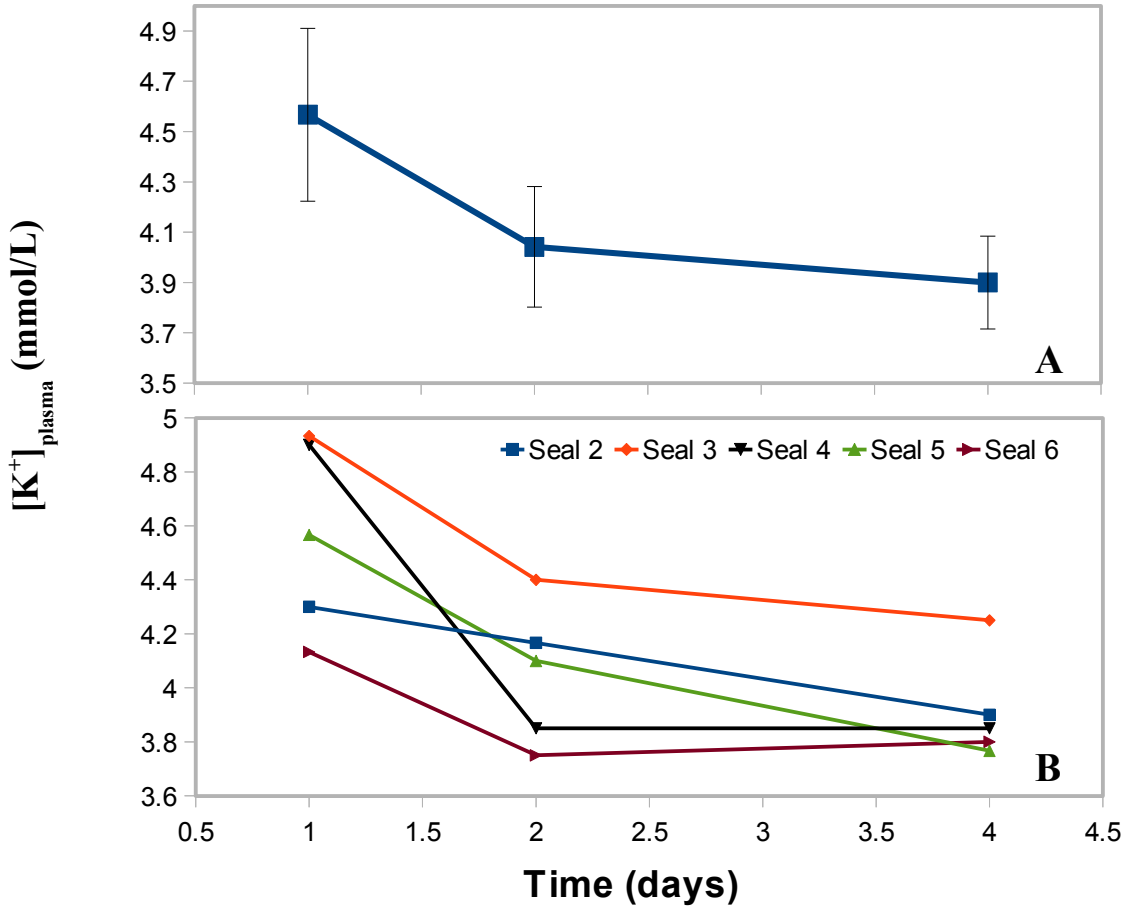

Fig. 8 shows plasma concentrations of K^+ as a function of time (days), (A) shows average (n = 5) ± SD and (B) shows individual concentrations.

Plasma concentration of K^+ had a significant decrease after dehydration, with average values decreasing from 4.5 ± 0.35 mmol/L to 4.0 ± 0.3 mmol/L. After rehydration there was a trend showing a weak decrease (P> 0.05).

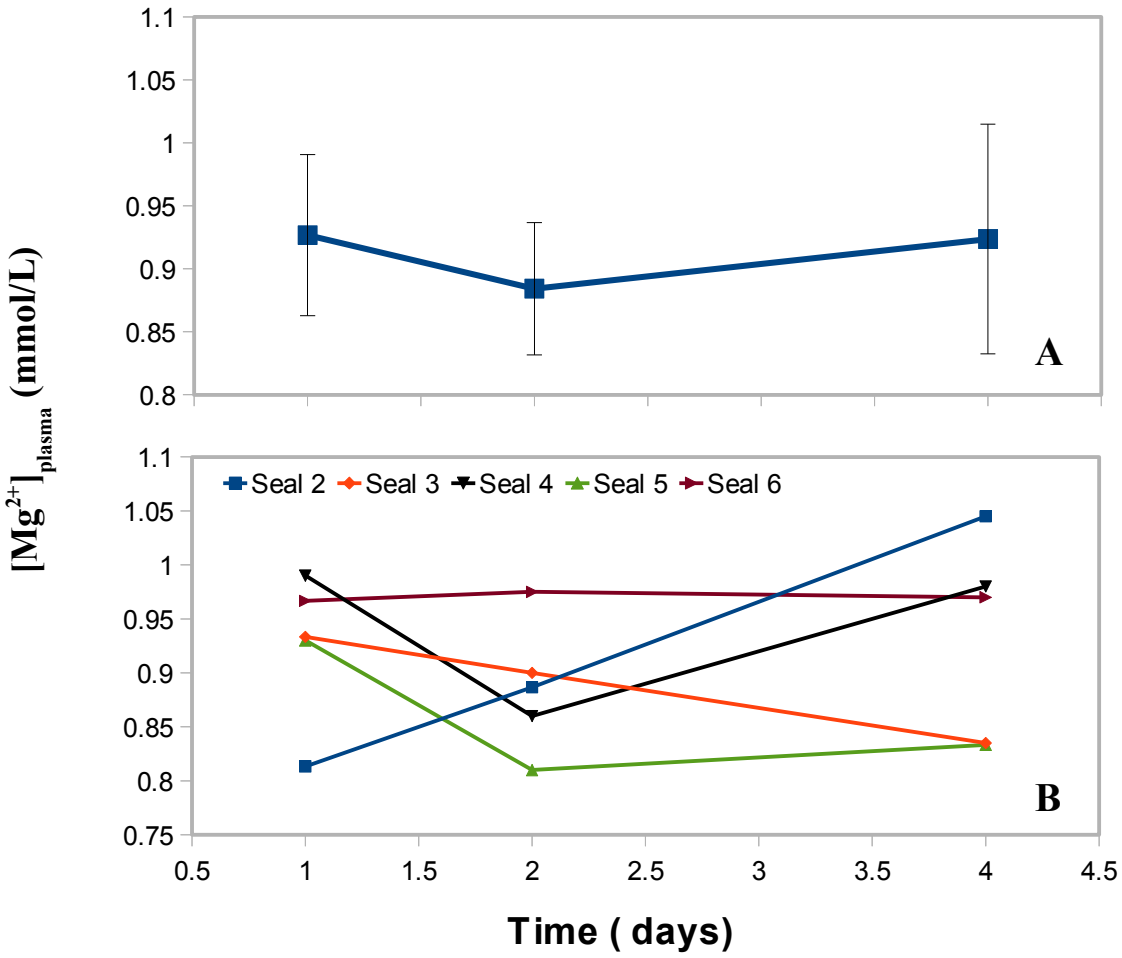

Fig. 9 shows plasma concentrations of Mg^{2+} as a function of time (days), (A) shows average concentration (n = 5) ± SD and (B) shows individual concentrations.

There was no significant difference between values obtained at baseline, after dehydration and after rehydration (P > 0.005). The average plasma concentration of Mg^{2+} at baseline was 0.93 ± 0.06 mmol/L, 0.88 ± 0.05 mmol/L after dehydration and 0.92 ± 0.9 mmol/L.

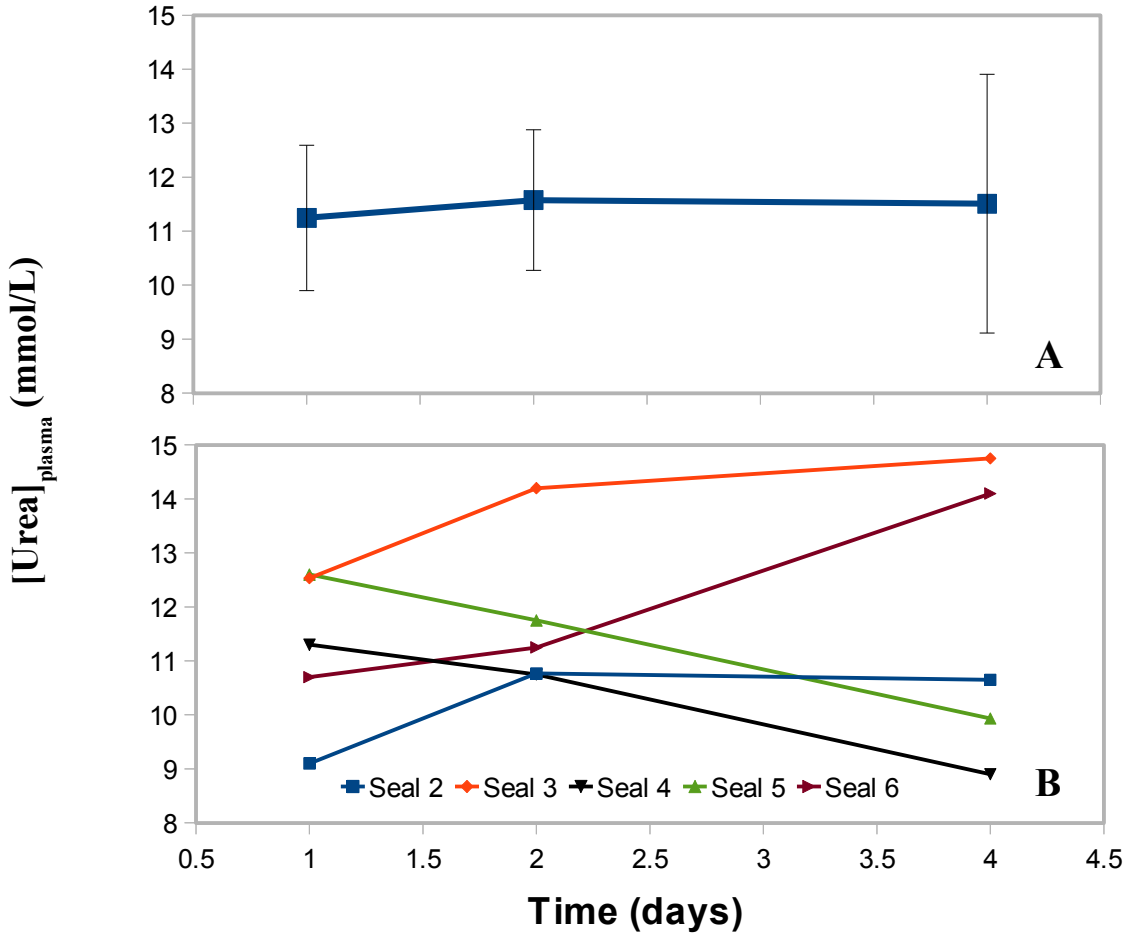

Fig. 10 shows plasma concentrations of urea, (A) shows average plasma concentration (n = 5) ± SD and (B) shows individual plasma concentrations.

Plasma urea ranged from 8.9 to 14.8 mmol/L, with averages at 11.4 ± 1.3, 11.5 ± 1.3 and 11.5 ± 2.4 mmol/L, for baseline, after dehydration and rehydration respectively. There is a trend suggesting a weak increase after dehydration and increasing variation between the seals after rehydration.

The highest plasma concentrations of Na^+, Cl^-, K^+, Mg^{2+} and urea observed in the study were 160, 115, 5.1, 1.07 and 14.8 mmol/L respectively.

3.3 Urine parameters

Seals 5 and 6 had no urinary output collected after baseline sampling (t = 1). Averages for urinary parameters are n = 3.

3.3.1 Urine osmolality

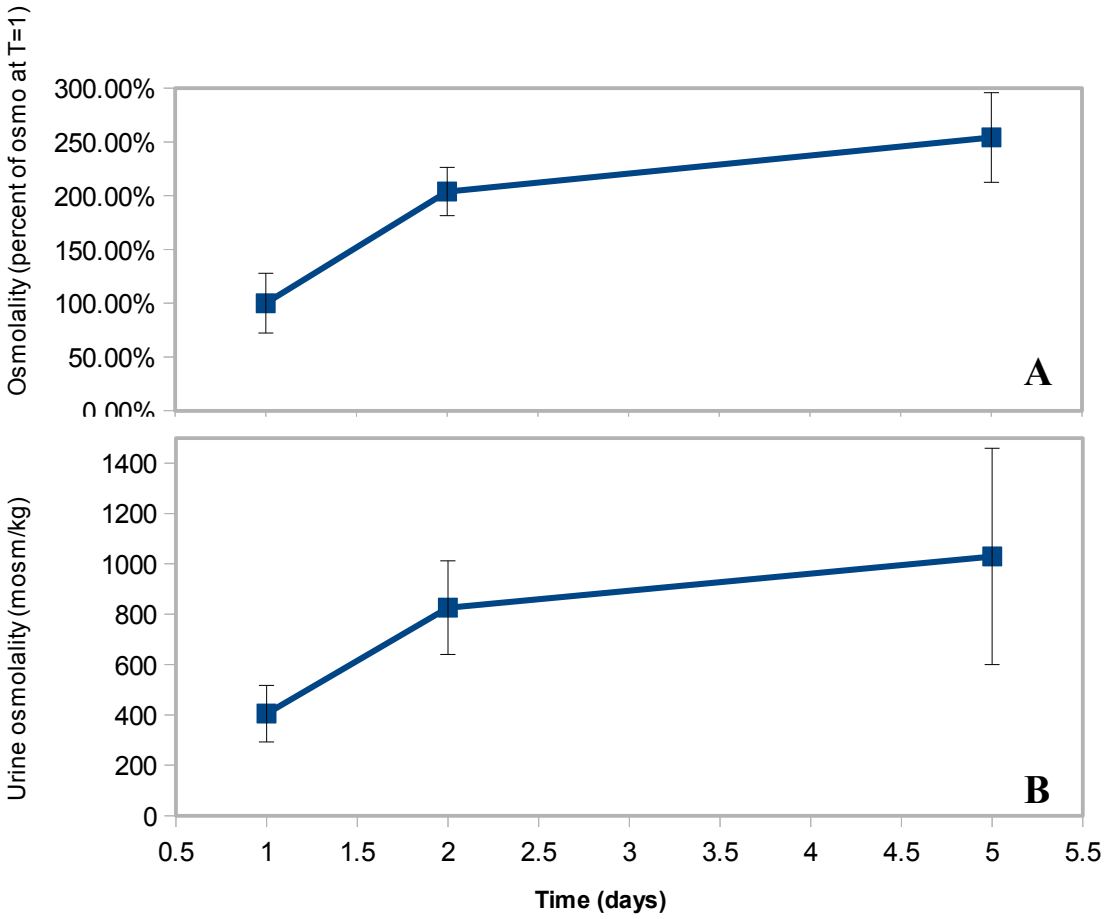

Fig 11 shows average (n = 3) urine osmolality (mosm/kg) as a function of time (T). **(A)** Shows urine osmolarity as a percent of baseline osmolality (t = 1) and (**B**) Shows average urine osmolarity (mosm/kg)

Urine osmolality ranged from 303 to 1502 mosm/kg, with average values of 405 ± 104, 825 ± 179 and 1029 ± 430 mosm/kg at baseline, after dehydration and after rehydration respectively. There is a significant increase in urine osmolality after dehydration ($P < 0.05$) and a trend of further increasing osmolality after rehydration ($P > 0.05$)

3.3.2 Urine electrolytes

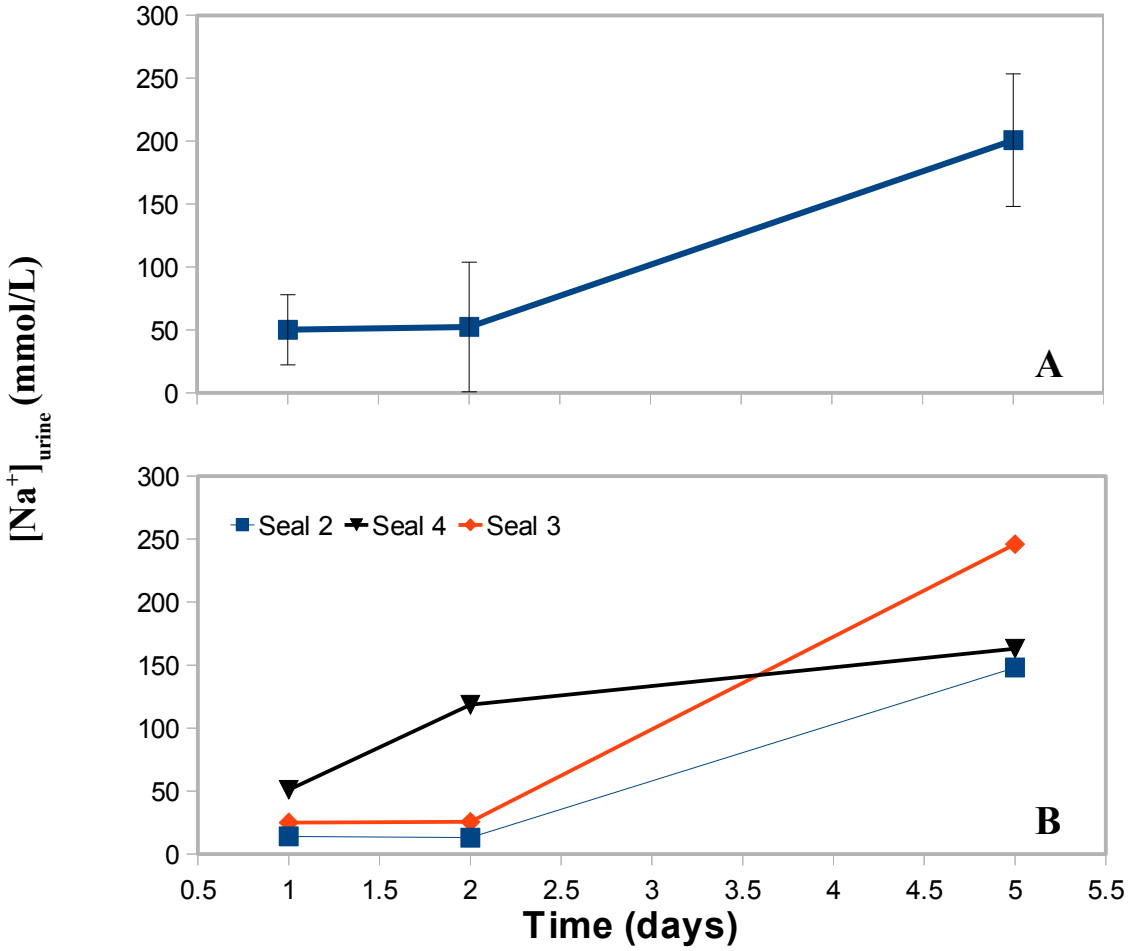

Fig. 12 shows urinary concentrations of Na^+ as a function of time (days), (A) shows average concentration (n = 3) ± SD, (B) shows individual concentrations.

There was no significant difference between means of urinary Na^+ obtained at baseline, after dehydration and after rehydration ($P > 0.05$). Values ranged from 12 to 248 mmol/L with averages for baseline, after dehydration and after rehydration at 50 ± 28, 52 ± 51 and 200 ± 52 mmol/L. There is a trend of rising Na+ concentration in the urine after rehydration.

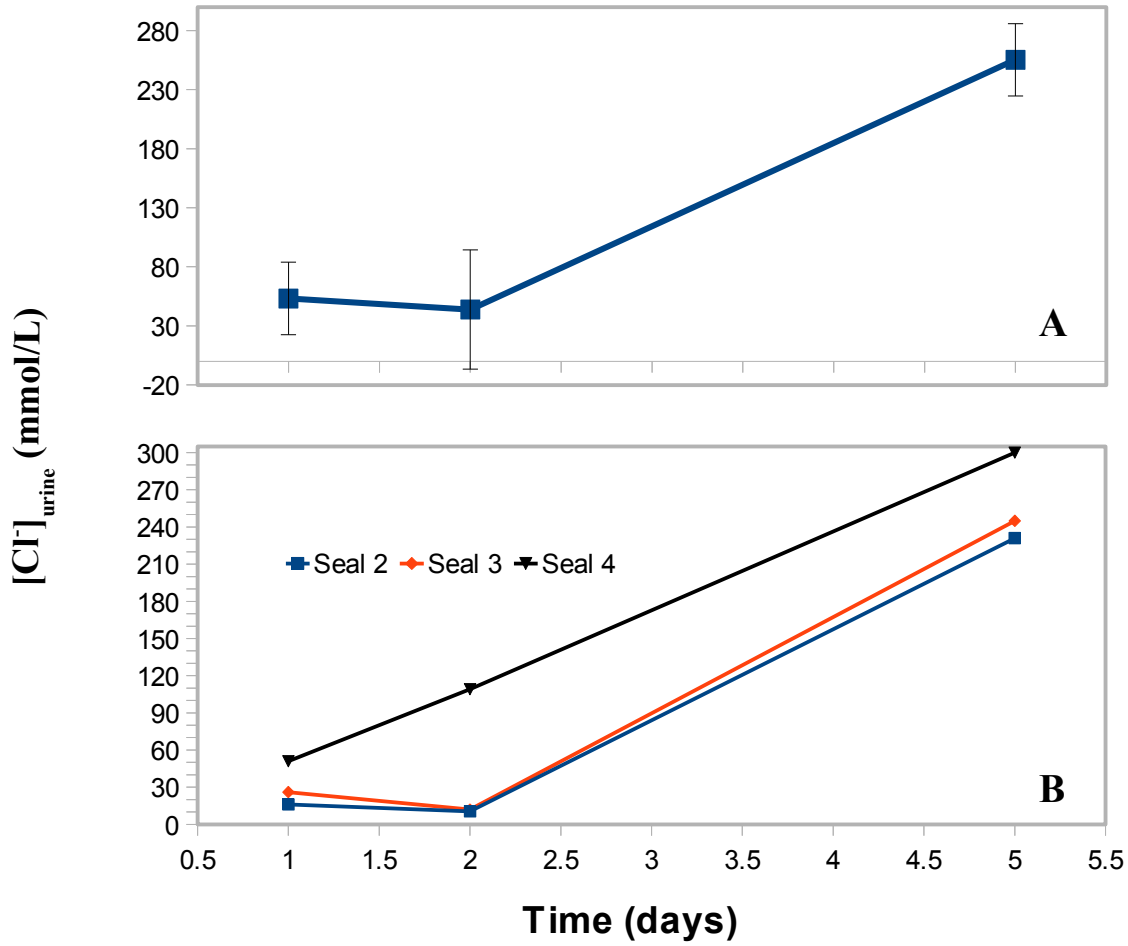

Fig. 13 shows urinary concentrations of Cl- as a function of time (days), (A) shows average concentration (n = 3) ± SD and (B) shows individual concentrations.

Urinary concentrations of Cl^- ranged from 10 to 300 mmol/L, with averages of 53 ± 30, 44 ± 50 and 255 ± 31 mmol/L at baseline, after dehydration and rehydration respectively. There was a significant ($P < 0.05$) increase in urine Cl^- concentration after rehydration.

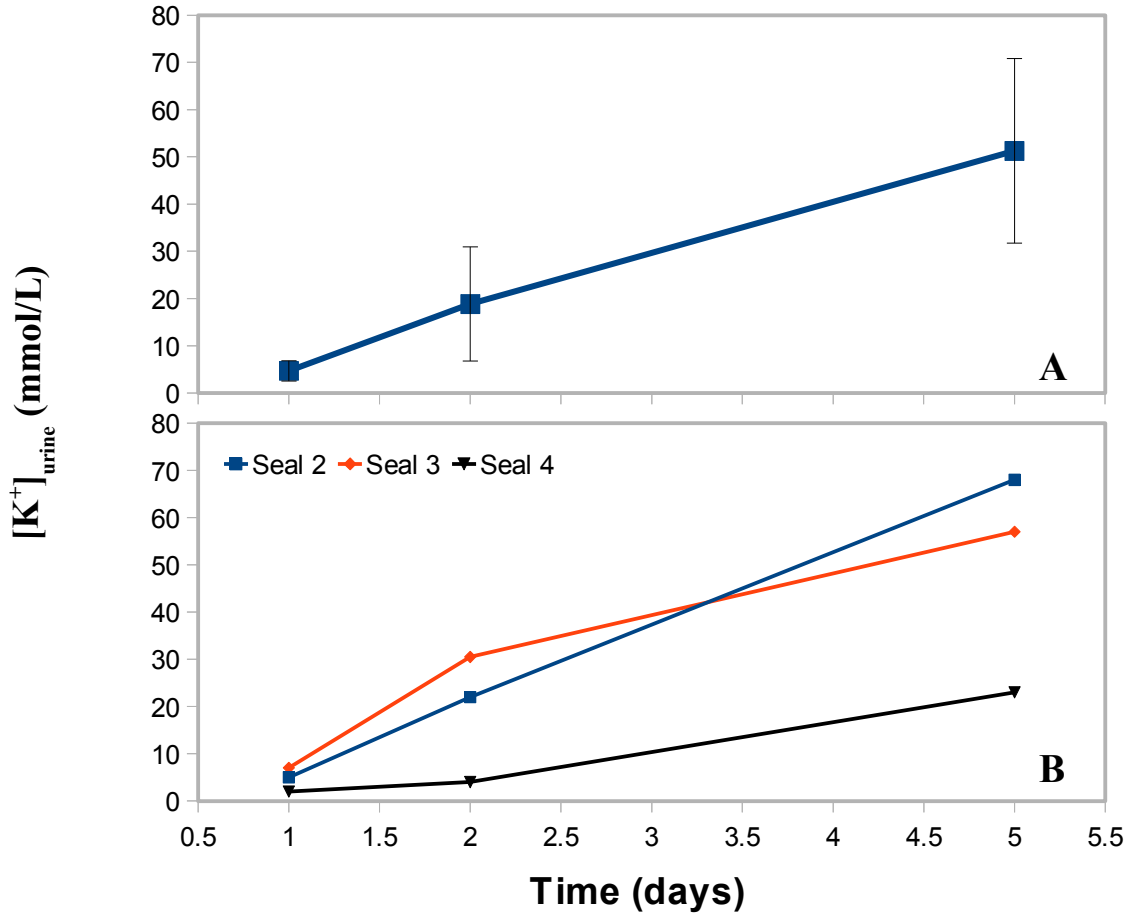

Fig. 14 shows urinary concentrations of K^+ as a function of time (days), (A) shows average concentration (n = 3) ± SD, (B) shows individual concentrations.

Urine K^+ concentration had a significant increase after rehydration ($P < 0.05$) increasing from 19 ± 12 to 51 ± 19.5 mmol/L. Average at baseline was 4.6 ± 2 mmol/L. Values ranged from 2 mmol/L at baseline to 67 mmol/L after rehydration. There was no significant difference between baseline and after dehydration ($P > 0.05$).

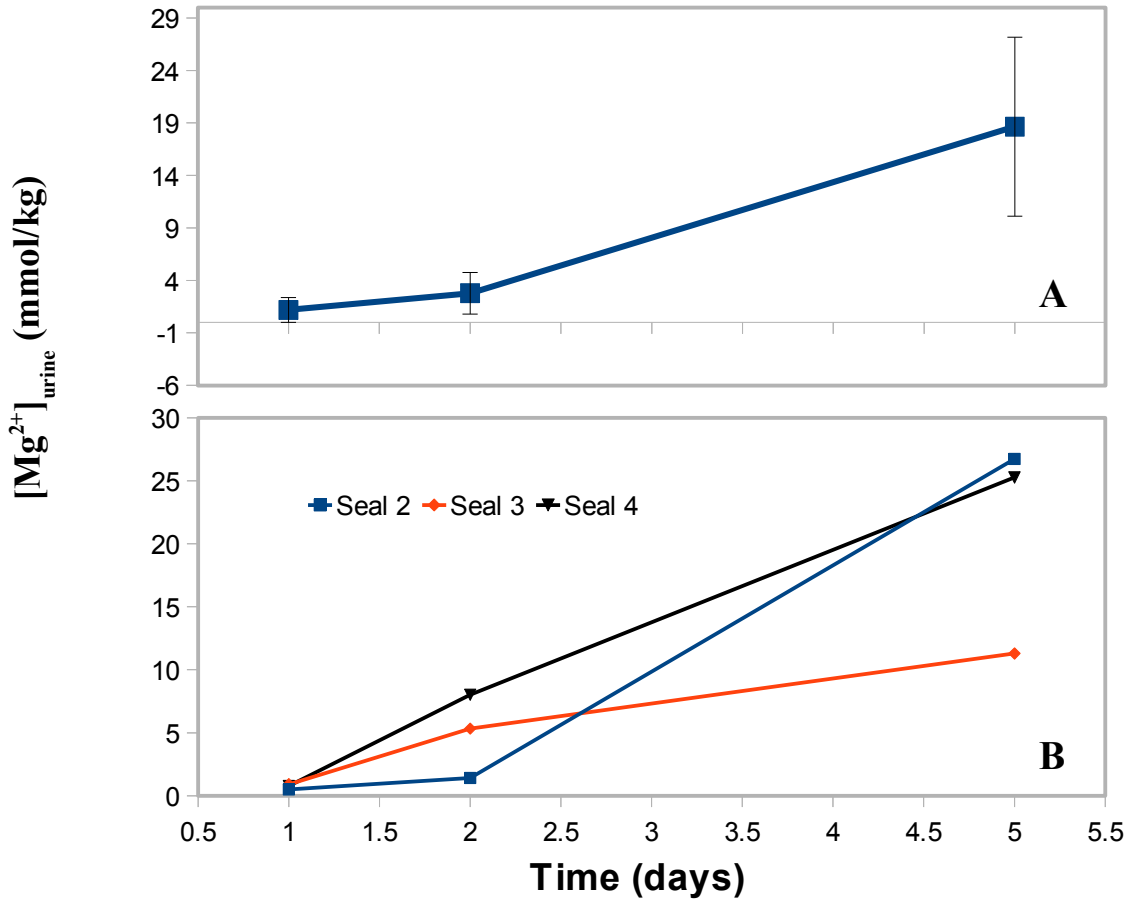

Fig. 15 shows urinary concentrations of Mg^{2+} as a function of time (days), (A) shows average concentration (n = 3) ± SD and (B) shows individual concentrations.

There is no statistical difference between values at baseline, after dehydration and after rehydration. There is a tendency of rising urine concentration of Mg^{2+} after rehydration. Baseline average is 1.18 ± 1.19 mmol/L, and averages obtained after dehydration and after rehydration are 2.77 ± 1.19 and 18.64 ± 8.52 mmol/L respectively.

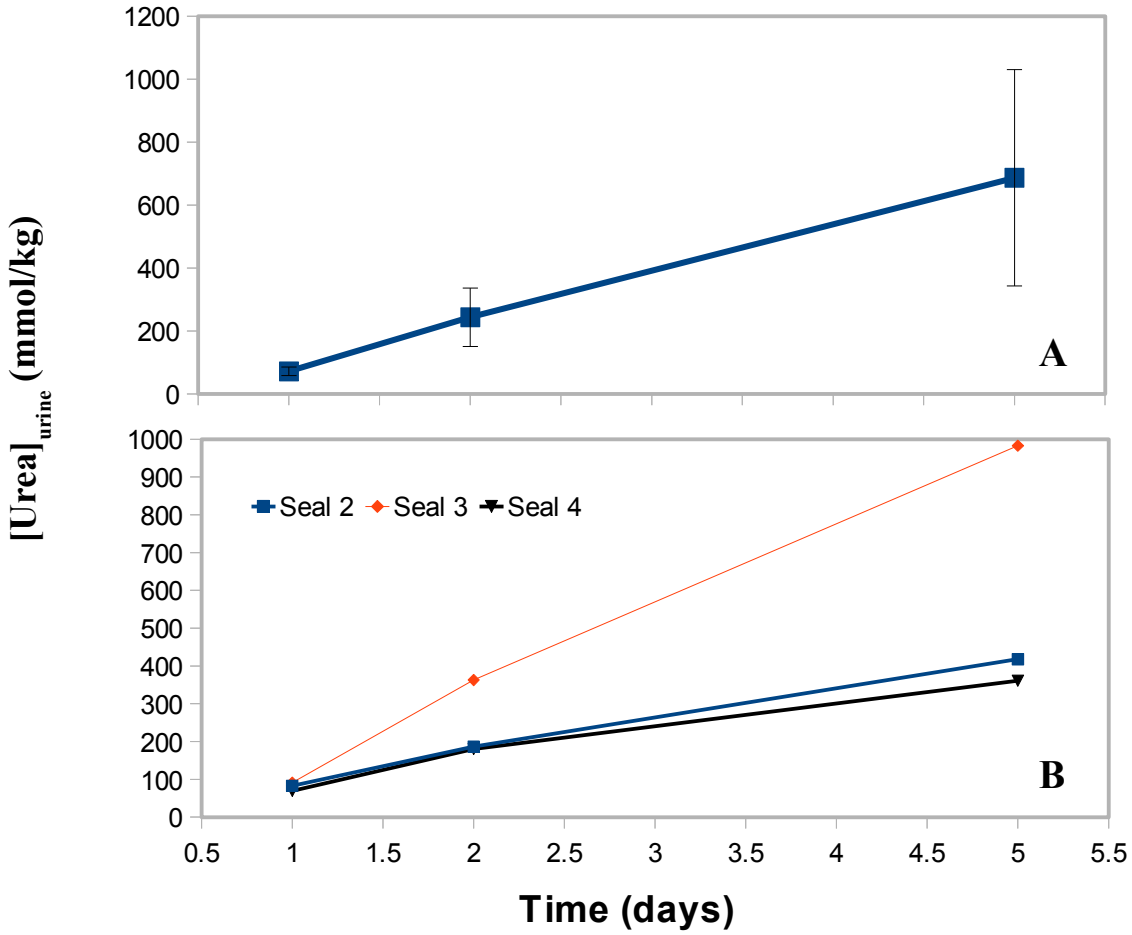

Fig. 16 shows urinary concentrations of urea as a function of time (days), (A) shows average urinary concentration (n = 3)± SD and (B) shows individual concentrations.

Urine concentrations of urea ranged from 54 to 991 mmol/L, with a maximum value observed after rehydration. The average values at baseline, after dehydration and after rehydration at 72 ± 14, 243 ± 93 and 682 ± 343 mmol/L. There was no significant difference between the averages at baseline, dehydration and rehydration ($P > 0.05$). However, there is a rising tendency after both dehydration and rehydration.

Maximum urine concentrations of Na^+, Cl^-, K^+, Mg^{2+} and urea was 248, 300, 68, 26.74 and 991 mmol/L respectively.

3.3.3 Hormone concentrations

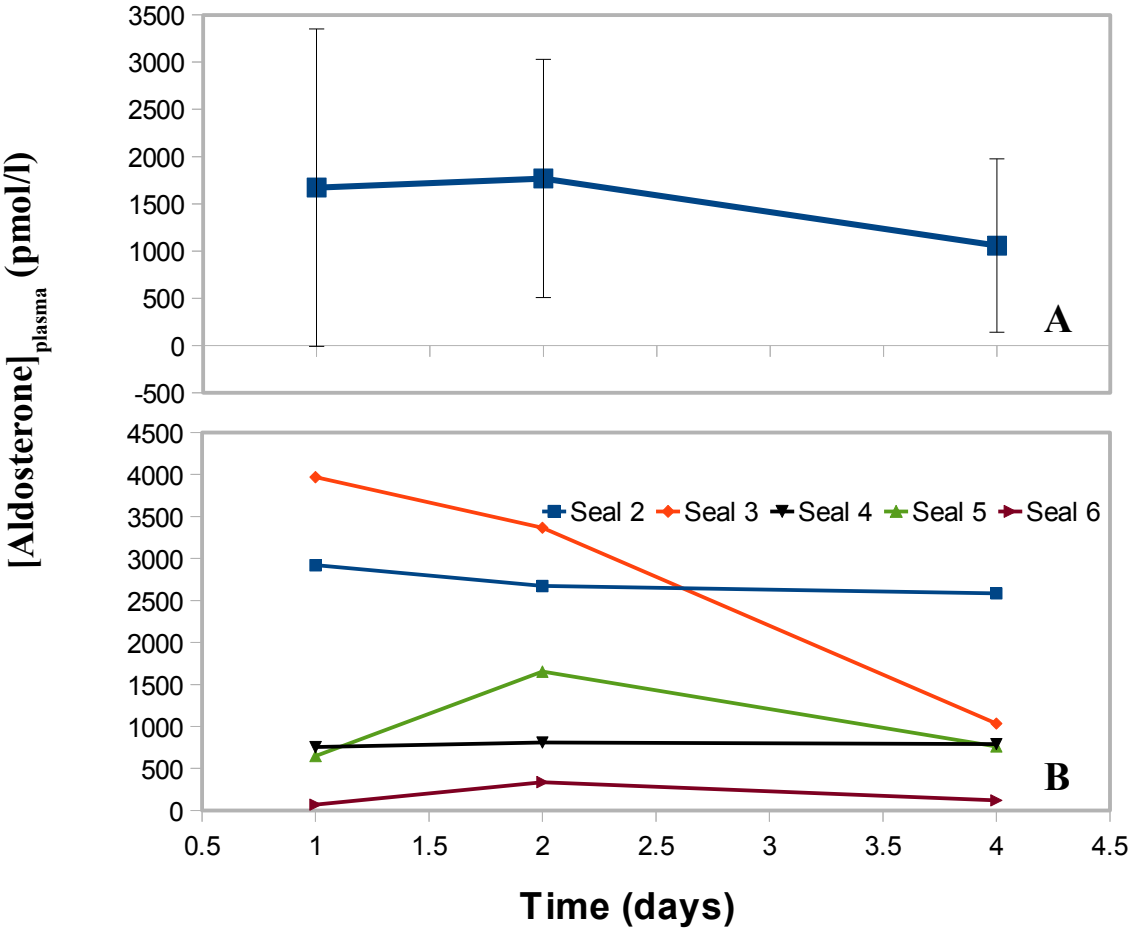

Fig. 17 shows plasma concentrations of aldosterone as a function of time (days), (A) shows average concentration (n = 5) SD and (B) shows individual concentrations.

There was no significant difference between values obtained at baseline, after dehydration and rehydration ($P > 0.05$). There was considerable individual variation within the seals. Values at baseline ranged from 69 to 3968 pmol/L, after dehydration observed values ranged from 337 to 2673 pmol/L and after rehydration the values ranged from 120 to 2585 pmol/L. The averages at baseline, after rehydration and after dehydrated was 1671, 1767 and 1058 pmol/L, respectively. The results show a trend of decreasing aldosterone concentration after rehydration.

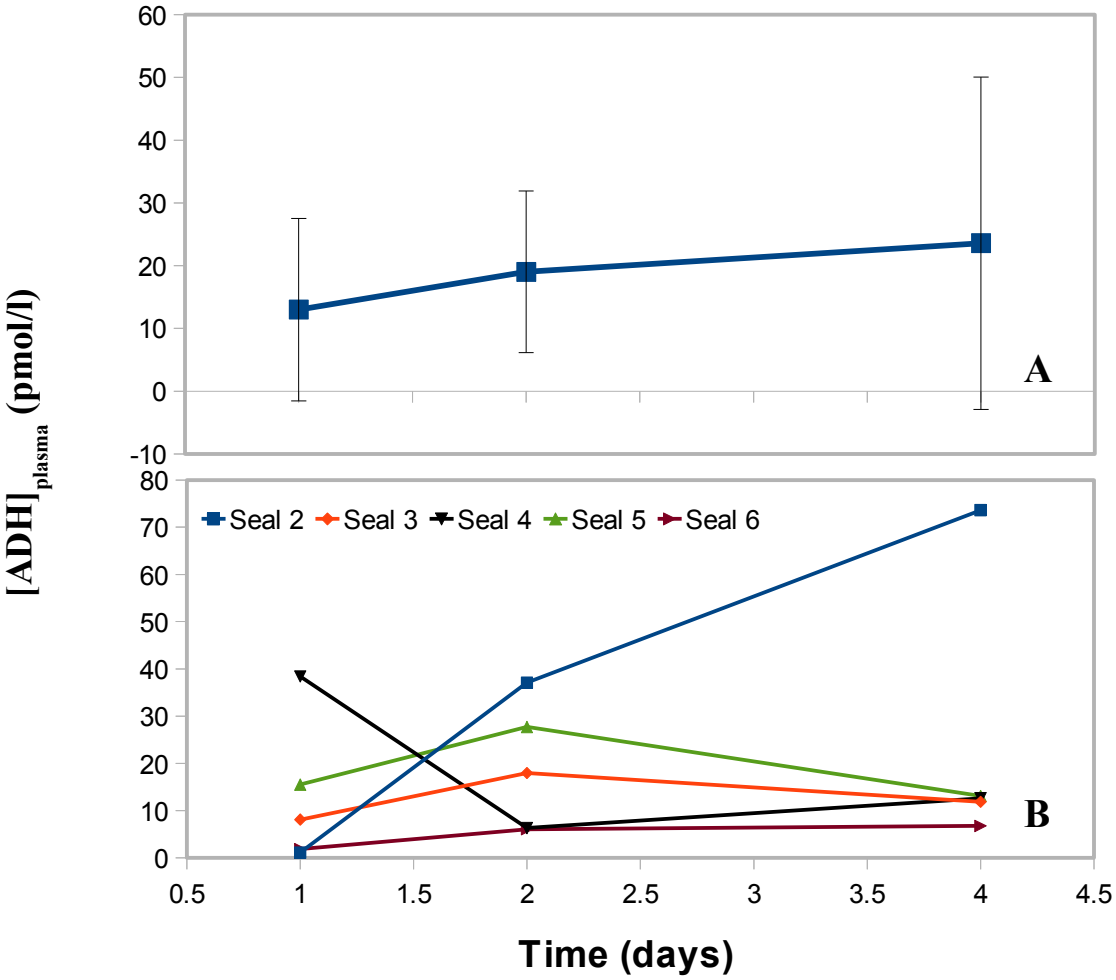

Fig. 18 shows plasma concentrations of ADH as a function of time (days), (A) shows average concentration (n = 5) +/- SD and (B) shows individual concentrations.

There is no significant difference between averages of plasma ADH at baseline, after dehydration and after rehydration. Values range from 1.0 to 73.6 pmol/L. 73.6 pmol/L was the maximum range of the kit used. Two samples had concentrations of ADH equal to or higher than 73.6 pmol/L. Both these were observed after rehydration for seal 2. Average values are 13, 19 and 24.5 pmol/L at baseline, after dehydration and after rehydration respectively.

3.4 Isotope dilution, total body water and turnover rates

Data based on isotope dilution is used for estimating both total body water (ml) and influx (both $ml*kg^{-1}*day^{-1}$ and $ml*day^{-1}$). Errors related to isotope dilution is included as maximum error expressed in percent of turnover rates (r_{H2O}).

3.4.1 Influx rates

Table3 Influx rates of water shown as influx ($ml*kg^{-1}*day^{-1}$) and ($ml*day^{-1}$), both for individuals and average (n = 6).

Parameter	Seal 2	Seal 3	Seal 4	Seal 5	Seal 6	Average (n =6) ± SD
Influx ($ml*kg^{-1}*day^{-1}$)	30.51	43.46	28.15	28.26	30.37	32,15 ± 6,4
Total influx ($ml*day^{-1}$)	2578	3281	2322	2642	2096	2584 ± 446
Metabolic influx ($ml*day^{-1}$)	527	485	518	596	453	510 ± 44
Respiratory influx ($ml*day^{-1}$)	151	139	148	163	130	146 ± 12,5
Influx seawater ($ml*day^{-1}$)	1900	2658	1656	1911	1513	1928 ± 442

Influx was calculated using isotope dilution as described in material and method.
Total influx was found by multiplying influx by body mass. Influx from seawater drinking was assumed to be the difference between total influx, metabolic influx and respiratory influx. The respiratory influx was calculated assuming 100% relative humidity in the air and a temperature of 10°C. The difference between volume at STPD and non-STPD was about 2 ml per day.

Metabolic water influx was calculated assuming a field metabolic rate of twice the size of BMR found using Kleibers equation and assuming the entire energy expenditure was covered by metabolizing fat.
Influx from seawater was estimated to an average of 1928 ± 442 ml/day. The influx from seawater drinking was estimated to be 74.6% of daily water influx.

3.4.2 Fractionation

The fraction of turnover subject to fractionation was assumed to be the difference between total turnover and urine production. Based on Skalstad and Nordøy (2000) we assume that 90% of the water was subject to fractionation. Using eq. 32 from Lifson and McClintock (1966)an underestimate of 6% is indicated. If we assume no urine output, then the error introduced would be a 7% underestimate.

3.4.3 Exchange

Using the calculated respiratory influx and eq. 39 from Lifson and McClintock an overestimate of water turnover is found to be from 2 – 5%, with an average of 3%.

Combined exchange and fractionation yields an overestimate of water turnover of a magnitude of 1 – 4 %.

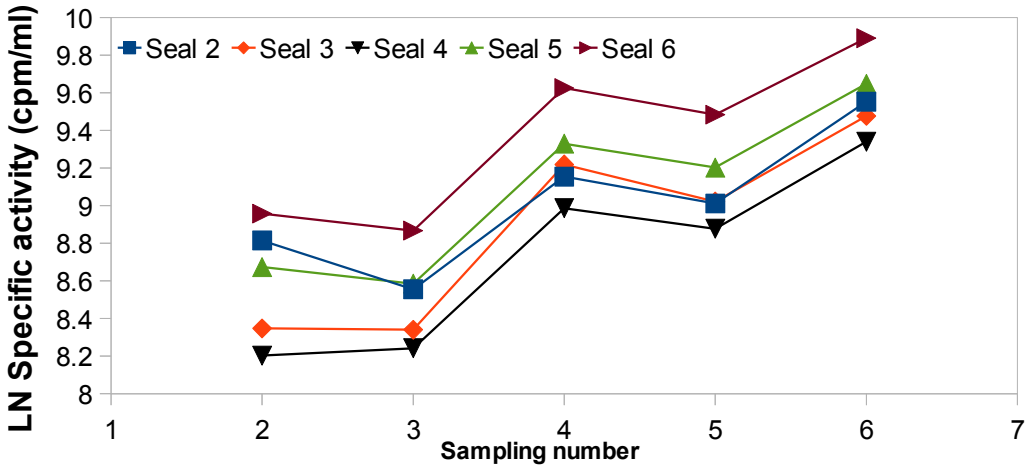

Fig. 16 shows the variation of the SA with time. Sampling numbers 2 and 3 are a pair representing initial and final SA of the body water during dehydration. Sampling numbers 4 and 5 represent initial and final SA of the body water during rehydration.

The figure shows that the SA of the blood decrease with time. Sampling number 2 represent the SA 1.5 h after injection of the isotope at baseline. Sampling 3 is the "background " activity of the blood at the end of the 24 h dehydration on land. These values are representing initial and final SA when calculating turnover during dehydration. Sampling 4 and 5 are representing initial and final SA of the body water during rehydration. The values obtained at sampling 1 and 6 are used for calculating body water and are not used for calculating isotope-dilution.

4.0 Discussion

4.1 Dehydration

24 hours after mannitol administration there was a significant decrease in body mass. And a significant increase in hematocrit values. Both are good indicators on the hydration state of the seals. Nordøy (1990) found that fasting grey seal pups lost on average $20g*kg^{-1}*day^{-1}$ during the first 10 days of fasting. The hooded seals used for this study lost body mass at more than twice that rate: $50g*kg^{-1}*day^{-1}$ during the 24 hours following the mannitol infusion. Increased Hct% and rising plasma osmolality is used in human medicine as initial indicators of dehydration. Hct measurements in pinnipeds are quite variable. Ortiz (2000) found that Hct increased by 35% during the postweaning fast of northern elephant seal pups, while Worthy and Lavigne (1982) reported no increased Hct in fasting harp seal pups. Kohin (1998) explained the increased Hct in northern elephant seal pups as an increase in red blood cell volume associated with the pups developing their diving capacity. Hooded seals are born with almost the same Hct as adults (Burns 2007) and I would attribute the increasing Hct % during dehydration to decreased total body water volume. Dehydration was an essential part of the experimental protocol and based on the elevated Hct% and the loss of body mass I conclude that the seals were dehydrated.

4.2 Isotope dilution and turnover rates

For an animal to be in water balance the daily water influx has to match the daily efflux. For animals with changing body water there must be a difference between daily influx and daily efflux. Nagy and Costa (1980) provide equations for calculating turnover when total body water, TBW, remains constant, when TBW changes linearly with time or changes exponentially with time. Calculating water turnover with the wrong equation introduces an error dependent on what equation is chosen and the percentile change in body water. The use of the “steady state” equation produces a large error if TBW is changing. The other equations yields small errors (<5%) unless TBW changes more than 40% from initial to final sampling. Assuming the seals were dehydrated, as strong evidence suggest, TBW decreased linearly with time. This assumption is based on the reasoning that the rate of water loss is largest during mannitol infusion. Mannitol has a biological half-life of 100 min in

humans and thus 6 hours after administration, only 8% of the diureticum remains in the seal. For the remaining hours of the stay in the cage, I assume a linear loss of TBW. The error for using the wrong equation would produce an <5% error for the turnover during the brief period where the efflux would qualify as non-linear.

The isotopic hydrogen atoms of water can disassociate and freely exchange with hydrogen atoms from organic molecules and with water vapor entering the lungs. This will cause a lower SA in body water and overestimate turnover rates. The error introduced is called exchange. By assuming a field metabolic rate twice the BMR calculated by the Kleiber equation, and by using an equation from Folkow and Blix (1987) to calculate daily daily respiratory volume, exchange was calculated to cause an overestimate of turnover in the order of + 2 -5 %

Labeled water, such as tritiated water, is heavier than water and evaporates slower than water. This phenomenon causes an underestimate of total turnover. Due to the seals having a 48 stay in a pool with free access to seawater, we have no measurement of how large fraction of total efflux is due to urine output. Assuming that the seals produced no urine these 48 hrs, the entire efflux would be subject to fractionation. F is the fractionation factor of tritium and is reported to be 0.92 at 30°C and 0.94 at 50°C (Sepall and Manson 1960). The air in the lungs have deep body temperature and 0.93 was used as fractionation factor. With no urine produced, fractionation produces an underestimate of 7% for water turnover. Skalstad and Nordøy (2000) found that 90% of total turnover was subject to fractionation. That gives (0.9 *0.93 +0.1) an underestimate of 6%. Combined fractionation and exchange largely cancel each other out.

Influx from metabolizing endogenous reserves was calculated using a field metabolic rate equal to BMR (from the Kleiber equation) multiplied by two. Oxidation of fat produces proportionately more water than the oxidation of other substrates, allowing fasting seals to rely primarily on water from metabolizing fat (Ortiz et al 1978) I assume that the entire energy requirement is covered by metabolizing fat. Metabolic water influx is then 20% of daily water influx.

If I had assumed the daily energy need was met by metabolizing a combination of fat and proteins, then the fraction of daily influx from metabolic influx would be lower.

Influx from inspired air was calculated using FMR (2x BMR from the Kleiber equation). Relative humidity was set to 100% and temperature to 10°C. Daily influx from inspired air was found to be 5.5 %of total influx. This compares well to the calculated value from Skalstad and Nordøy (2000), who found that respiratory influx was 5.3% of daily influx. The difference between total efflux and influx (respiratory water and metabolic water) suggest that the hooded seals drink seawater. On average the seals drank 1900 ml per day, or 23ml*kg^{-1}*day^{-1}. For the hooded seals this amounts to ca 75% of influx. Skalstad and Nordøy (2000) reported that seawater drinking amounted to 14% of total daily influx.

The equation from Folkow and Blix (1987) was derived from respiratory data collected from grey seal. The grey seal has no special respiratory adaptions compared to the hooded seal. The equation gives a good estimate of daily respiratory volume.

To support the conclusion drawn from seawater drinking calculated by the difference between influx and efflux of water, both plasma and urine parameters was arranged in a scoring plot, with a binary scoring system: + if the trend for that particular parameter supports seawater drinking and – if the parameter does not support seawater drinking.

4.3 Urine parameters

4.3.1 Urine production

The design of the experiment prevented the collection and quantification of total urinary production (Fig 2). This is because urine is only collected while the seals are on land. Tarasoff and Toews(1972) found that urine production for fasting harbor seal weighing 45 kg was 14 ml*h^{-1}. Fasting grey seal pups weighing 44 – 53 kg have a urine production 12 ml*h^{-1} (Skog and Folkow 1994), while Nordøy (1990) found that fasting grey seal pups with weights from 38.5 – 44.4 kg have urine production of ca 150 ml*day^{-1} after 2 days of fasting. Adult Baikal seal at 50 kg (n = 2) and ringed seal at 70 kg (n = 2) had an average urine production of respectively 5 and 12 ml*h^{-1} 24 hours after feeding. Skalstad and Nordøy (2000)

reported that hooded seals (n=3) kept in seawater had average urine production of 197 ± 18 $ml*day^{-1}$.

4.3.2 Urine osmolality

Urine osmolality for the hooded seals increased by 400 mosm/kg after dehydration , this is an 100% increase and during the same period urea concentration in urine increased by 180 mmol/L (240%). Other studies have showed that grey seal can produce urine with an osmolality of 2161 $mosm*kg^{-1}$(Skog and Folkow 1994), while Baikal seal have produced urine with osmolality of 2374 $mosm*kg^{-1}$ (Hong 1982) and hooded seals can produce urine with osmolality of 1592 $mosm*kg^{-1}$ (Skalstad and Nordøy). The seal urine with the highest osmolality was produced by grey seal pups 2741 $mosm*kg^{-1}$ (Reilly 1991). The seals maximum urine osmolality and urine:plasma ratio his higher than in humans, but much lower than those for rodents living in the desert. The kangaroo rat and the hopping mouse have maximum urine osmolality of 5500 and 9400 $mosm*kg^{-1}$ (Schmidt – Nielsen 1990). The osmolality itself is a measure of the total concentration of solutes. Hong (1982) showed that urea represented 83% of the urine osmolality and Na^+, Cl^- and K^+ constitute less than 10%. After seawater administration urea was reduced to constitute 60% of total osmolality, while Na^+, Cl^- and K^+ had increased to represent 23% of total osmolality. Intake of seawater will cause an increased intake of electrolytes, such as Na^+ Cl^- . In order to maintain homeostasis the extra electrolytes is excreted with the urine. This will cause an increase in urine osmolality after seawater drinking.

A core assumption is that in order to have water gain from drinking seawater, the electrolytes, primarily Na^+, Cl^- and Mg^{2+} have to be excreted in concentrations equal to or higher than that of seawater.

4.3.3 Urine electrolytes

Seals drinking seawater have to handle the excess salts related to seawater intake. Seawater has high concentrations of Na^+, Cl^- and seawater drinking causes an increased concentration of these electrolytes in the extracellular fluid (ECF). To maintain homeostasis these excess electrolytes have to be excreted in the urine. Unless urinary salt concentration can exceed that of the ingested fluid, it should not be feasible for that organism to sustain itself on seawater (Wolf 1959). But by applying the theory of osmotic (Wolf 1958) space animals could sustain themselves on seawater if they qualify the following conditions:

- There has to be at least one exogenous source of water in addition to the endogenous water, such as seawater and water made available by oxidizing fat and proteins.
- The osmotic “ceiling” of the urine must be higher than the osmolality of the exogenous source of water. In effect this means that the maximum urine osmolality must be higher than that of seawater. As shown in results, the maximum urinary osmolality was ca 1500 mosm*kg^{-1} and the water in the pool had an average of 949 mosm*kg^{-1}. (2.1.1 animal living conditions)
- The endogenous and the exogenous water source must contain different osmolytes. The endogenous water contains primarily urea, while the seawater contains Na^+ and Cl^-

The hooded seal can produce urine with higher osmolality than seawater. Even if the seal can not produce urine with the same concentrations of Na^+ and Cl- as found in seawater, it could use the seawater volume to excrete urea since it´s urine has a higher osmotic “ceiling” than seawater. The water volume from the endogenous source, fat, could then be used to excrete Na^+ and Cl^-.

In this study the urine concentrations of the electrolytes Na^+, Cl^- and Mg^{2+} was lower than that of seawater (Krogh 1939). The maximum urine concentration of Na^+, Cl^-, K^+ and Mg^{2+} observed was 248, 300, 68, 26 mmol/L respectively. K+ was found in higher concentration in urine than in seawater. This excretion of K^+ is most likely coupled to the reabsorption of Na^+.

4.4 Plasma parameters

4.4.1 Plasma osmolality

There was a weak trend indicating a slight increase after dehydration. But for all purpose and intent I consider the plasma osmolality to be stable during the entire experiment. Average values for plasma osmolality was 340 at both baseline and after rehydration. These values compares nicely to data from Skalstad and Nordøy (2000). They reported average plasma osmolality during seawater exposure to be 332 ± 4. Other studies show similar high plasma osmolality for other seal species (Hong 1982, Reilly 1991, Skog and Folkow 1994) while Nordøy (1992) reports similar high plasma osmolality in fasting grey seal pups. The high plasma osmolality is mainly due to high plasma concentrations of urea, Na^+, Cl^- and K^+ (Hong 1982). Fetcher (1939) calculated that the high plasma osmolality ease the osmotic workload by up to 10%. This is due to the energy required to move osmolytes against the concentration gradient.

4.4.2 Plasma electrolytes

Plasma concentration of K^+ had a significant decrease after dehydration. Plasma concentrations of the other electrolytes and urea remained relatively stable during the entire experiment. Skalstad and Nordøy (2000) reported that plasma parameters remained stable during their experiment and therefore the hooded seals maintained homeostasis, despite the significant amounts of seawater drinking. The decrease of plasma K^+ concentration observed in this study might be related to Na^+ reabsorption in the distal tubuli. When plasma volume decreases increased Na^+ reabsorption is mediated via the renin-angiotensin-aldosterone-system (RAAS for short). Increased Na^+ reabsorption leads to increased H_2O reabsorption and increased K^+ excretion. The discussion of what role RAAS plays in maintaining homeostasis is presented in later (4.5 hormones)

4.5 Hormones

In mammals, the primary hormones responsible for maintaining homeostasis are angiotensin (I, II), ANP, aldosterone and ADH (vasopressin). Renin converts angiotensinogen to angiotensin I. Angiotensin I is converted to angiotensin II by angiotensin-converting enzyme

(ACE). Angiotensin II stimulates the release of aldosterone from the adrenal gland, which in turn increases the Na^+ reabsorption in the distale tubule. ADH is the most potent anti-diuretic hormone. ADH stimulates the synthesis of aquaporins in the collecting duct. The actions of ANP oppose those of angiotensin II and aldosterone by inhibiting the synthesis and release of renin.

Zenteno- Savin and Castellini (1998) used RIA to prove presence of and the concentrations of the hormones: AVP, ANP and angiotensin II in harbor seals, Weddell seals (*Leptonychotes weddellii*), northern elephant seals (*Mirounga angustirostris*), ringed seals, California sea lions (*Zalophus californianus*) and Steller sea lions (*Eumetopias jubatus*) . A negative correlation between circulating levels of aldosterone and Na^+ reabsorption was not observed by Hong (1982) prompting Hong et al. to question the role of the RAAS in seals with regards to maintaining homeostasis.. However Malvin et al. (1978) and Ortiz et al. (2000) have obtained evidence of a functional RAAS in California sea lions and fasting northern elephant seal pups. This suggests that under normal conditions electrolyte balance is regulated by the RAAS. A negative correlation ($r = 0.492$) between Δ aldosterone and water efflux in fasting adult male northern elephant seal males (Ortiz et al. 2006) indicates that water loss is abated by increasing aldosterone.

Several studies suggest that the reabsorption of solute- free water from the collecting duct is mediated by ADH (Bradley et al. 1954, Page et al. 1954, Hong 1982, Skog and Folkow 1994) Under normal fasting conditions, the renal conservation of water and electrolytes in northern elephant seal pups appears to be an increased response in the RAAS without an increase in AVP concentration (Ortiz et al. 2000). The contribution of ADH to the osmoregulation in marine mammals continues to be ambiguous. For mammals collectively it appears that water and electrolyte reabsorption are mediated by ADH and RAAS, respectively.

The hooded seals in this study showed a wide range of values for both ADH and aldosterone. The expected trend after seawater drinking is a decrease in plasma concentration after rehydration. Large amounts of Na^+ need to be excreted and low aldosterone concentrations results in low reabsorption of Na^+. During dehydration the expected response was in increase

of plasma ADH. This would stimulate the synthesis of aquaporins in the collecting duct and cause an increased reabsorption of water. The data provided by this study makes no meaningful contribution to the roles of ADH or RAAS with regards to maintaining electrolyte- and water balance.

4.6 Scoring plots

When the dehydrated hooded seals drink seawater the expected physiological response is to have an increased urine output. Due to the experimental design any increase in urine volume was not recorded while the seals had *ad lib* access to seawater. The ingestion of seawater causes a rise in Na^+ and Cl^- in the ECF. To maintain homeostasis these electrolytes need to be excreted. The urine concentrations of Na^+ and Cl^- are expected to rise after seawater drinking (Tarasoff and Toews 1972, Hong et al. 1982, Skalstad and Nordøy 2000, Storeheier and Nordøy 2001, How and Nordøy 2007). Plasma and urine concentrations of Mg^{2+} are kept in a very narrow range during normal conditions. Divalent ions such as Mg2+ are absorbed at a lower rate than monovalent ions (Fine et al. 1991). Albrecht (1950) administered 1000 ml seawater to two harbor seal. The incomplete absorption of Mg^{2+} caused diarrhea. As a response to seawater drinking I expect a slight increased of plasma Mg^{2+} and an increased urine concentration of Mg^{2+}. I expect an increase in urine concentration of urea when dehydrated seals are given an exogenous source of water.
K^+ is expected have decreasing urine K^+ concentration, this is related to the expected increased excretion of Na^+.

Plasma parameters are expected to remain stable (Skalstad and Nordøy 2000). Mg^{2+} might be an exception and might show a slight increase. Hct% of rehydrating seals is expected to decrease. Body mass is expected to decrease at a slightly lower rate because the seals are still fasting and metabolizing endogenous reserves, but they will drink to restore water lost.

Table 4 Scoring plot for each parameter per seal

Parameter	Seal 2	Seal 3	Seal 4	Seal 5	Seal 6
Plasma Na^+	+	+	+	+	+
Plasma Cl^-	+	+	+	+	+
Plasma K^+	+	+	+	+	+
Plasma Mg^{2+}	+	-	+	+	+
Plasma Osmo	+	+	+	+	+
Plasma Urea	+	-	+	+	-
Hct %	-	+	+	+	+
Urine Na^+	+	+	+		
Urine Cl^-	+	+	+		
Urine K^+	+	+	+		
Urine Mg^{2+}	+	+	+		
Urine Osmo	+	+	+		
Urine Urea	+	+	+		
Body Mass	+	+	+	+	+

Seal 5 and 6 had no urine production during the last stay on land (Fig 2 96 - 120 hrs) Scoring was subjectively based on the following criteria: any seal with a – for urine Na^+ or urine Cl^- was evaluated to not have extra support for the conclusion drawn from the calculated volume of drank seawater. The other parameters were judged on a case by case basis.

Seal 2 had a negative scoring for Hct%. All other parameters support seawater drinking. The increase in Hct was a 1% increase. The scoring plot supports seawater drinking for seal 2.

Seal 3 had a negative scoring for decreasing plasma Mg^{2+} and it has the lowest increase in urine Mg^{2+}. It also has negative scoring for slightly increasing plasma urea. Since Na^+, Cl^- and

Mg^{2+} have trends supporting seawater drinking I consider the overall scoring in favor of seawater drinking.

Seal 4 has uniform support for seawater drinking.

Seal 5 had no urine output after rehydration. The other parameters support seawater drinking. I conclude that there is weak support in favor of seawater drinking in seal 5

Seal 6 with no data on urine concentrations after rehydration it is hard to score in favor of seawater drinking. With negative scoring for rising plasma urea I would suggest that there is support for seawater drinking based on Hct and body mass changes

The scoring plot is a supplement to the calculated seawater drinking. It is impossible to assay seawater drinking based only on the scoring. High urine concentrations of Na^+ and Cl^- are still good indicators of seawater drinking and seals 2 - 4 increased excretion of Na^+ and Cl^- after rehydration.

4.7 Potential errors

All *in-vivo* experiments may be affected by the stress levels of the experimental animals. Stressed mammals release ACTH (adrenocorticotropic hormone) that stimulates release of cortisol and aldosterone. The seals used for this study were somewhat used to being handled, but they showed signs of stress and resisted being restrained. During the experiment one of the seals broke free of the restraints and had to be recaptured and restrained. The possibility of stress affecting blood and urine parameters is very real for this study. To ease the stress handling we administered the sedative zoletil.

Samples analyzed at Rikshospitalet and Aker Sykehus were marked with tags before transport but there is a slight risk that samples were mis-tagged before departure for Oslo. And instructions on what kind of samples were marked with what kind of tags could have been clearer. This would have reduced the risk of analyzed samples being reported as a different plasma or urine parameter.

Some of the data from the isotope-dilution is flawed. Several parallel samples for the same parameter had clear trends of decreasing values. Other parallel samples of the same parameter had had huge variation. This caused me to refute all isotope results from seal 1 and 2 for day 1.
For seal 4 the background activity had increased instead of declining as seen on fig 16, the SA increased 24 hrs after injection. Possible explanations are contamination, mixing samples or possibly adding an increased plasma or decreased plasma volume to the counting vials prior to scintillation counting.

4.8 Conclusion

1. This study has showed that dehydrated seals drink seawater. The average amount drank was 1928 ± 442 ml *day-1 or 23ml*kg^{-1}*day^{-1} which amounts to 75% of daily water influx.
2. Hooded seals could take advantage of the osmotic space while drinking seawater to excrete urea and electrolytes.
3. The roles of aldosterone and ADH in osmoregulation for hooded seals remain unclear.

Based on the calculated volumes of seawater drinking and on the trends for the individual parameters I concluded that dehydrated hooded seals drank sea water when given access. The urinary concentrations of Na^+ and Cl^- were lower than that of seawater. Based on the theory of osmotic space they would still have a net water gain by drinking seawater. The maximum urine osmolality was higher than of seawater and thus the seals satisfy the 3 conditions stipulated by the theory of osmotic space. Sadly the data obtained for aldosterone and ADH were inconclusive and the role of ADH and aldosterone with regard to osmoregulation in hooded seals remains unclear.

Acknowledgments

This master thesis was done at the Department of Arctic Biology, University of Tromsø.
My supervisors were professor, dr. philos Erling Sverre Nordøy and professor, dr. philos Karl-Arne Stokkan.

I want to thank:
Erling S. Nordøy for invaluable help, extraordinary patience and for not giving up on me when my progress was slow.
Karl-Arne Stokkan for always keeping his office door open and providing good advice.

Ina Jakobsen for invaluable help during the experimental procedures.

The staff at the Department of Arctic Biology for technical assistance.

My fellow students at the Department of Arctic Biology for making me smile on a cloudy day.

My wife, Lise Verlo for being the glue that kept the family together while I spent late nights at the university.
And my son, Trym Verlo for patiently waiting for daddy to come home.

6.0 References

Albrecht CB. 1950. Toxicity of sea water in mammals. Am. J. Physiol.163: 370 – 385

Bradley SE & RJ Bing. 1942. Renal function in the harbor seal (*Phoca vitulina L*.) during asphyxial ischemia and pyrogenic hyperemia. J.Cell. Comp. Physiol. 19: 229 – 237

Bowen WD, OT Oftedal & DJ Boness. 1985. Birth to weaning in 4 days: Remarkable growth in the hooded seal, *Cystophora cristata*. Can. J. Zool. 63: 2841-2846.

Burns JM, KC Lestyk, LP Folkow & AS Blix. 2007. Size and distribution of oxygen stores in harp and hooded seals from birth to maturity. J. Comp. Physio.l B. 177: 687–700

Depocas F, J Hart, & HD Fisher. 1971. Seawater drinking and water flux in starved and fed harbor seals, *Phoca vitulina*. Can. J Physiol. Pharmac. 49:53 – 62.

Fetcher ES Jr. 1939. The water balance in marine mammals. Q. Rev. Biol.14: 451 – 459.

Fetcher ES & GW Fetcher. 1942. Experiments on the osmotic regulation of dolphins. J. Cell. Comp. Physiol. 19:123 – 130

Folkow, LP & AS Blix.1987. Nasal heat and water exchange in gray seals. Am. J. Physiol. 253: R883 – R889

Folkow LP & AS Blix. 1999. Diving behaviour of hooded seals (*Cystophora cristata*) in the Greenland and Norwegian Seas. Polar. Bio.l 22:61 – 74

Folkow LP & AS Blix. 1995. Distribution and diving behaviour of hooded seals. In: Blix AS, Walløe L, Ulltang Ø (eds) Whales, seals, fishsh and man. Elsevier,.Amsterdam. pp 193 - 202

Folkow LP, ES Nordøy & AS Blix. 2010. Remarkable development of diving performance and migrations of hooded seals (*Cystophora cristata*) during their first year of life. Polar Biol. 33(4): 433 – 441.

Folkow LP, JM Ramirez ,S Ludvigsen ,N Ramirez & AS Blix . 2008. Remarkable neuronal hypoxia tolerance in the deep-diving adult hooded seal (*Cystophora cristata*). Neurosci. Lett. 446: 147–150.

Folkow LP, PE Mårtensson & AS Blix. 1996. Annual distribution of hooded seals (*Cystophora cristata*) in the Greenland and Norwegian Seas. Polar. Biol. 16:179 – 189

Gentry RL.1981. Seawater drinking in eared seals. Comp Biochem Physiol. A 68: 81–86.

Hong SK, R Elsner, JR Claybaugh & K Ronald. 1982. Renal functions of the Baikal seal *Pusa sibirica* and ringed seal *Pusa hispida*. Physiol. Zool. 55: 289 – 299.

How OJ & ES Nordøy. 2007. Seawater drinking restores water balance in dehydrated harp seals. J. Comp. Physio.l B. 177:535 - 542

Irving L, KC Fisher & FC McIntosh. 1935. The water balance of a marine mammal, the seal. J. Cell. Comp. Physiol. 6: 387 – 391.

Kaplan LA & A Lawrence. 2010. Clinical Chemistry: theory, analysis, correlation / edited by Lawrence A. Kaplan, Amadeo J. Pesce. -5th edition. Elsevier. St Louis

Kleiber M. 1975. The fire of life. Krieger, New York

Kohin S. 1998. Respiratory physiology of northern elephant seal pups: Adaptations for hypoxia, hypercapnia and hypometabolism. PhD dissertation, University of California, Santa Cruz. 145Pp

Krogh A. 1939. Osmotic regulation in aquatic animals. Cambridge Univ. Press. Cambridge. Dover.

Lifson N & R McClintock. 1966. Theory of use of the turnover rates of body water for measuring energy and material balance. J theore Biol.12:46 - 74.

Malvin RL, S Ridgway & L Cornell. 1978. Renin and aldosterone levels in dolphins and sea lions. Proc. Soc. Exp. Biol. Med. 157: 665–668.

Matsuura DT & GC Whittow. 1974. Evaporative heat loss in the California sea lion and harbor seal. Comp. Biochem. Physiol. 48A:9 - 20.

Nagy KA & DP Costa. 1980. Water flux in animals: analysis of potential errors in the tritiated water method. Am. J. Physiol. 238: R454 - R465.

Nansen F. 1924. Blant sel og bjørn. Jacob Dybwad. Christiania. pp. 173.

Nordøy ES, A Aakvaag & TS Larsen. 1993. Metabolic adaptations to fasting in harp seal pups. Physiol. Zool. 66: 926 - 945

Nordøy ES, DE Stifjoorn, A Raheim & AS Blix. 1992. Water flux and early signs of entrance into phase III of fasting in grey seal pups. Acta. Physiol. Scand. 144:477 - 482.

Nordøy ES, OC Ingebretsen & AS Blix. 1990. Depressed metabolism and low protein catabolism in fasting grey seal pups. Acta. Physiol. Scand. 139: 361 – 369

Oftedal OT. 1984. Milk composition, milk yield and energy output at peak lactation: a comparative review. Symp. Zoo. Soc. Lond. 51:33 - 85

Ortiz RM. 2001. Osmoregulation in Marine marine mammals. J. Experimenta.l Biol.. 204:1831–1844

Ortiz RM, CE Wade & CL Ortiz. 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.

Ortiz CL, D Costa, & BJ Le Boeuf. 1978. Water and energy flux in elephant seal pups fasting under natural conditions. Physiol. Zool. 51:166 - 178

Ortiz RM, DE Crocker, DS Houser,& PM Webb. 2006. Angiotensin II and aldosterone increase with fasting in breeding adult male northern elephant seals (*Mirounga angustirostris*). Physiol. Biochem. Zool. 79: 1106-1112.

Ortiz RM, SH Adams, DP Costa & CL Ortiz. 1996. Plasma vasopressin levels and water conservation in fasting, postweaned northern elephant seal pups (*Mirounga angustirostris*). Mar. Mamm. Sci. 12: 99 – 106.

Page LB, JC Scott-Baker,GA Zak, EL Becker & CF Baxter. 1954. The effect of variation in filtration rate on the urinary concentrating mechanism in the seal, *Phoca vitulina L*. J. Cell. Comp. Physiol. 43: 257 – 269

Ramirez JM, LP Folkow & AS Blix.2007. Hypoxia tolerance in mammals and birds: from the wilderness to the clinic. Ann. Rev. Physiol. 69:18.1–18.31

Reilly JJ. 1991. Adaptations to prolonged fasting in free-living weaned gray seal pups. Am. J. Physio. 260:R267 – R272

Reilly JJ & MA Fedak. 1990. Measurement of body composition of living grey seals by hydrogen isotope dilution. J. Appl. Physio.l69:885 – 891

Renouf D, E Noseworthy & MC Scott. 1990. Daily fresh water consumption by captive harp seals (*Phoca groenlandica*). Mar. Mammal Sci. 6: 253 – 257.

Schmidt-Nielsen K. 1990. Animal Physiology: Adaptation and Environment. Fourth edition. New York: Cambridge University Press

Sepall O & SG Mason. 1960. Vapor/liquid partition of tritium in tritiated water. Can. J. Chem. 38: 2024 - 2025

Sergeant DE. 1976. History and present status of populations of harp and hooded seals. Biol. Conserv. 10:95 – 118

Skalstad I & ES Nordøy. 2000. Experimental evidence of seawater drinking in juvenile hooded (*Cystophora cristata)* and harp seal (*Pagophilus groenlandicus*). J. Comp. Physiol. B. 170: 395 - 401

Skog EB & LP Folkow.1994. Nasal heat and water exchange is not an effector mechanism for water balance regulation in grey seals. Acta. Physiol. Scand. 151: 233 – 240.

Smith HW.1936. The composition of urine in the seal. J Cell Comp Physiol. 7: 465 – 474.

Storeheier PV & ES Nordøy. 2001. Physiological effects of seawater intake in adult harp seals during phase I of fasting. Comp. Biochem. Physiol. A 128: 307 - 315

Tarasoff F & D Toews.1972. The osmotic and ionic regulatory capacities of the kidney of the harbor seal, *Phoca vitulina*. J Comp Physiol. 81: 121 – 132.

Wolf AV. 1958. Thirst: The Physiology of the Urge to Drink and Problems of Water lack. Springerfield: Thomas. 536Pp

Wolf AV, PG Pentiss, LG Douglas & RJ Sweet. 1959. The Potability of sea water with special reference to the cat. Am. j. Physiol. 196: 633 – 641

Worthy GAJ & DM Lavigne. 1982. Changes in blood properties of fasting and feeding harp seal pups, *Phoca groenlandica*, after weaning. Can. J. Zool. 60: 586 – 592

Zenteno-Savin T & MA Castellini. 1998. Plasma angiotensin II, arginine vasopressin and atrial natriuretic peptide in free ranging and captive seals and sea lions. Comp. Biochem. Physiol. 119C: 1 – 6.