UiT The Arctic University of Norway

Faculty of Biosciences, Fisheries & Economics
Department of Arctic and Marine Biology

Nitrogen Excretion and Aspects of Water Balance in Fasting Hooded Seal Pups (*Cystophora cristata*)

Matthew Coyle

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Cover Page Image: Hooded Seal Pup (*Cystophora cristata*)

Credit: Anna Victoria Pyne Vinje, UiT, Norway

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Specialization in Arctic Animal Physiology

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Supervisor: Prof. Erling S. Nordøy, UiT – The Arctic University of Norway

Co-supervisor: Prof. Lars Folkow, UiT – The Arctic University of Norway

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Abstract

The post-weaning fast of hooded seal pups (*Cystophora cristata*) is not well understood. What energy sources are being used? How is water balance maintained? How are these mechanisms balanced with thermoregulation? This study aims to answer some questions on the physiological mechanisms in place to assist an Arctic seal pup in the early stages of their life with no source of food or freshwater. Hooded Seals have some remarkable adaptations to birth on the ice; they have the shortest lactation period of any mammal at 3-5 days and therefore the pup is highly precocious. After weaning, the pups will fast for an estimated 30 days, relying on their energy sources built up during lactation until they are capable of foraging for themselves.

Six pups were captured in the Greenland Sea at the end of, or shortly after, lactation. Measurements of metabolic rate and mass were made weekly alongside weekly sampling of blood and urine samples throughout their 30 days of post-weaning fast. Samples were then analysed for the concentrations of nitrogenous compounds and relevant ions (Cl^-, Na^+, and Mg^{2+}) to find how they may change over the month-long fasting period. From the analysis of nitrogenous products within the urine samples, the amount of protein catabolised over a 24-hour period was estimated, allowing for calculating the proportion of metabolic rate that is accounted for by the catabolism of proteins.

Results here for electrolyte concentrations in plasma and urine support previous literature demonstrating that hooded seals are capable of ingesting seawater in order to maintain homeostasis. Urine osmolality increased to values higher than seawater (1.3±0.13 osmol/kg, compared with 0.949 osmol/kg in seawater), while haematocrit values stabilised after entrance to seawater. Weight-specific metabolic rates were not detected to significantly decrease over the fasting period (at an average of 2.4±0.54 W/kg, 1.7 times estimated Kleiber value) and the proportion of this that is accounted for by protein catabolism remained low at an average of 3.4±3.1 %. The metabolism rate not being observed to decrease and the low rate of protein catabolism goes against some trends common among pinniped species. This may be due to the large amount of fat stores that hooded seals have, greater than other Arctic seal counterparts such as the harp seal. This may mean that sufficient energy is available that they can utilise their fat deposits for energy, without a requirement to reduce their metabolic rate or increase protein catabolism to supplement their use of fat as an energy source.

Table of Contents

1 Introduction 1

1.1 The Arctic Habitat -------- 1

1.2 Arctic Pinnipeds -------- 2

1.3 Hooded Seals -------- 3

1.4 Water Balance -------- 5

1.5 Metabolism -------- 6

1.6 Objectives and Hypotheses -------- 8

2 Methodology 10

2.1 Expedition -------- 10

2.1.1 Travel -------- 10

2.1.2 Seal Selection and Capture -------- 10

2.2 Animal Care -------- 12

2.2.1 On the Boat -------- 12

2.2.2 At the University -------- 12

2.3 Sampling -------- 13

2.3.1 Urine -------- 14

2.3.2 Blood -------- 15

2.3.3 Metabolic rate -------- 17

2.4 Sample Analysis -------- 22

2.4.1 Blood Sample Handling -------- 22

2.4.2 Urine Sample Handling -------- 23

2.5 Statistical Analysis -------- 26

3 Results 27

3.1 Mass loss -------- 27

3.2 Haematocrit -------- 27

3.3 Plasma ---------- 28

3.3.1 Plasma Nitrogenous Products ---------- 28

3.3.2 Plasma Electrolytes ---------- 30

3.4 Urine ---------- 33

3.4.1 Urine Nitrogenous Products ---------- 34

3.4.2 Urine Electrolytes ---------- 37

3.5 Metabolic Rate ---------- 40

4 Discussion 43

4.1 Protein Catabolism ---------- 43

4.1.1 Urine Excretory Products ---------- 45

4.2 Metabolic Rate ---------- 47

4.3 Changes to Blood Chemistry ---------- 49

4.4 Changes to Urine Production and Chemistry ---------- 50

5 Conclusion 53

Works cited 54

Appendix 65

Raw Data: ---------- 65

1 Introduction

1.1 The Arctic Habitat

The Arctic is most commonly identified by the Arctic Circle, which is the circle around the Northern Pole defined by the region above the latitude of 66.5°N. This, however, is not a perfect definition in order to understand the stresses faced by Arctic species. Sometimes a more helpful definition for understanding the adaptations to the thermal stressors of the Arctic environment is the 10-degree isotherm line. This is defined as the region of the Northern environment where the highest temperature reached during July does not exceed 10°C (figure 1).

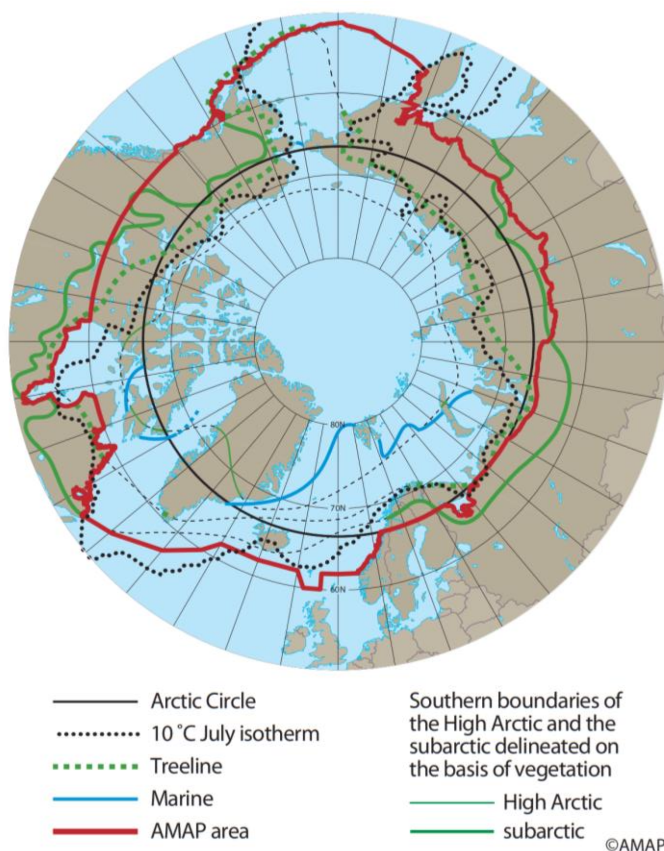

Figure 1: A map showing different definitions of what can be considered the Arctic. The Arctic circle is represented by a solid black circle while the 10-degree isotherm is represented by a black dotted line. Credit: Arctic Monitoring & Assessment Programme (AMAP, 1997)

Ecological diversity of an area is usually well correlated with the temperature of a given region, resulting in the Arctic generally having a lower species diversity than warmer regions; this 10-degree isotherm line can demonstrate this given that it is somewhat followed

by the tree line (figure 1). This is in part due to the lower solar energy that Arctic regions receive due to the angle of the sunlight reaching the ground.

These conditions of lower temperature and lower energy received to the Arctic regions results in some other stressors that resident animals in these regions must adapt to. Arctic regions have low air humidity due to the low temperatures reducing the saturated water vapour pressure of the air. Low water availability therefore becomes another stressor for animals in the Arctic.

Given that this study in particular focusses on an Arctic marine mammal species, the sea ice is of particular interest. The cover of sea ice varies significantly seasonally however there is a permanent centre of interlocking ice floes. The ice is formed north of Siberia before taking about 5 years to drift to the edge of Greenland (Mysak, 2001). Freshwater runoff from northern Russia contributes a large amount of low-salinity water (Aagaard & Carmack, 1989); the shallow seas then become less saline which allows for the pack ice to form. This pack ice within the Fram strait is an average of around 2.2m thick (Hansen et al., 2013) and is very extensive, causing it to have great ecological significance. Ice floes are particularly important for Arctic seal species given that is commonly where individuals will go to give birth and breed.

1.2 Arctic Pinnipeds

There are seven species of pinniped that are resident in the Arctic. Six of these are from the family Phocidae including the hooded seal (*Cystophora cristata*), harp seal (*Pagophilus groenlandicus*), ringed seal (*Pusa hispida*), bearded seal (*Erignathus barbatus*), ribbon seal (*Histriophoca fasciata*), and spotted seal (*Phoca largha*). Also present in the Arctic is the Walrus (*Odobenus rosmarus*) from the Odobenidae family. No species from the third extant family of pinniped Otariidae (eared seals) are truly present in the Arctic, however the northern fur seal (*Callorhinus ursinus*) and Steller Sea Lion (*Eumetopias jubatus*) can be found distributed in the Bering Sea. Also notably, there are a small population of harbour seals (*Phoca vitulina*) present around Svalbard, but they are an isolated population and therefore their definition as an Arctic seal is questionable.

For a seal to be considered an Arctic species they must give birth and breed in the Arctic. This notably causes some significant adaptations to be necessary for newborn seal pups in the Arctic. Arctic seal pups all develop lanugo fur, which is a fine long fur allowing for greater

thermal resistance before the blubber is thick enough to ensure thermal homeostasis. In the hooded seal and walrus, this fur is shed in the womb due to already significant blubber stores before birth, allowing pups to enter cold water sooner (Oftedal et al., 1991). Artic seals generally have a shorter lactation period compared with pinnipeds found at lower latitudes; from as low as 4 days in hooded seals (Bowen et al., 1985), to up to 39 days in ringed seals (Lydersen, 1995).

1.3 Hooded Seals

Figure 2: Map of Hooded Seal distribution. Credit: (Kovacs, 2009)

The hooded seal (*Cystophora cristata*) is a species of Arctic seal that can be found only in the North Atlantic Ocean (figure 2). The hooded seal is named for the inflatable bladder on the head of the male seals that is inflated with air, primarily used to demonstrate aggression to other males, but also to attract females (Ballard & Kovacs, 1995). The species can be found distributed around the North Atlantic, being found as south as Newfoundland, Canada. The hooded seal population is estimated to be around 340,000 individuals (Kovacs, 2016), however this estimate cannot be considered reliable with no recent surveys in Canada or Greenland. The Greenland Sea population of hooded seals is estimated to be around 77,000 individuals (ICES., 2019). Every year, around March, the seals return to their ancestral

breeding regions to give birth and breed for next year's births (Folkow et al., 1996). Due to the gestation period being approximately one year, delayed fertilization occurs, delaying the egg fertilization by 3-5 months (Atkinson, 1997).

They are deep divers compared to other Arctic seals found in the same regions. They have been observed to dive deeper than 1000m, but most dives are between 100-600 m and last for 5-25 minutes (Folkow & Blix, 1999). Their diet will often include species found deep such as the Boreo-atlantic Armhook Squid (*Gonatus fabricii*) (Potelev et al., 2000). The polar cod (*Boreogadus saida*), and the capelin (*Mallotus villosus*) are also common prey animals (Haug et al., 2004). These food sources are often inaccessible to other shallower diving species, meaning less competition for the same resources. Satellite telemetry data from hooded seal pups (Folkow et al., 2010) shows that although they are not as successful divers as adult hooded seals, they can dive to similar depths and durations as adult harp seals (Nordøy et al., 2008). The early feeding habits of hooded seal pups going on their first dives is not fully understood, however study of stomach contents has shown a high proportion of their diet to be composed of amphipod species (Haug et al., 2000). The difference in diet between pups and adults is therefore likely to be reflective of the diving behaviour.

The pups of hooded seals are colloquially referred to as "bluebacks" due to their blue-grey colouring on their backs with white bellies. The lanugo fur that is present on many Arctic seal pups is already shed in the womb (Oftedal et al., 1991), meaning that their blue-grey colouration is recognisable from birth. The pups are born with a thick layer of blubber already present which will protect them from cold ambient temperatures, meaning that the lack of lanugo fur poses them no significant threat of hypothermia. Kvadsheim and Aarseth (2002) found that, when in air, the contribution of their fur to the combined thermal resistance of fur, skin, and blubber, was 73%, compared with 90% in harp seal pups. This means that the hooded seal pups are less reliant on fur for insulation than harp seal pups. For submerged harp seal pups, the thermal contribution of fur was 65%, compared to 26% in hooded seal pups (Kvadsheim & Aarseth, 2002). Once submerged, the thermal resistance of fur drops by 84-94% which demonstrates how ineffective fur is for insulation when in water.

The early life of hooded seals, although not drastically different from other pinniped species, seems to be taken to an extreme. All Arctic seals have a relatively short lactation period, but the hooded seals have the shortest of all mammals, at a range of 3-5 days (Bowen et al.,

1985). This means that they must gain a significant amount of energy in this brief time period. It is for this purpose that the milk of the hooded seal is the most energy rich of all animals, at around 61% fat (Oftedal et al., 1988).

After this period of intense energy intake, the pups are left by the mother so she can then mate. The pup is then entirely self-dependent and will undergo a post-weaning fasting period until it is capable of catching prey. The pups remain on the ice floes for a while before leaving to the feeding grounds for their first meal. This period of post-weaning fast is estimated to last around 30 days (Folkow et al., 2010).

1.4 Water Balance

Arctic marine environments pose many problems to its inhabitants for the maintenance of water homeostasis. Seawater, having such a high osmolality compared to mammalian body fluids, is hard to properly utilise as a source of exogenous water. This is compounded by the problem of the cold air; cold air has a lower saturated water vapour pressure than air at body temperature. Cold air that enters the body becomes warmed, increasing its saturated water vapour pressure, causing water in the airways to vaporise and escape in the exhaled air. This is a process that will occur all over the world, and the effects are more significant at colder temperatures such as in the polar regions; but a caveat is that once temperature is dropping lower than 0°C, the saturated water vapour pressure is already very low, so it cannot drop much further. There are mechanisms in Arctic animals are in place to mitigate this, examples being the nasal heat exchange in the reindeer (*Rangifer tarandus*) (Blix & Johnsen, 1983; Langman, 1985) and phocid seals (Folkow et al., 1988).

For the first weeks of life in Arctic Seals, while still on the pack-ice, this maintenance of water balance has a simple solution since the animals are surrounded by freshwater in the form of snow and ice. After remaining on the icefloes for a time, the pups will enter seawater in search for feeding grounds, still fasting during this period (Folkow et al., 2010). During this period of fasting with no access to freshwater is where the water balance of these pups will be challenged the most; water cannot be sourced from food or snow, thus making seawater the only available exogenous water source.

Pinnipeds are known for being able to produce very concentrated urine, more concentrated than most terrestrial mammals (Ortiz, 2001) except for small desert rodents such as the kangaroo rat (*Dipodomys sp.*) (Schmidt-Nielsen, 1990). It has been suggested that seawater

drinking was used in pinnipeds as a way to assist in nitrogen excretion and thermoregulation (to reduce core body temperature), making it more likely to occur in tropical species rather than polar (Gentry, 1981). Despite this, hooded seals have already been demonstrated to drink seawater (Schots et al., 2017; Verlo, 2012); research on how this allows them to maintain water balance is still incomplete. Marine mammals do not have salt-secreting glands as many species of seabird and marine reptiles do which means that salt excretion must occur through the kidney. Despite a complex renal morphology which could allow for highly concentrated urine, the actual resulting urine produced by pinnipeds is not much more concentrated than seawater (Bester, 1975).

1.5 Metabolism

The metabolism of an animal is simply the total chemical processes that occur in the body. All reactions will release heat, so therefore, the metabolic rate of an animal can be measured as the total energy leaving an organism as heat. This measurement of heat loss is referred to as direct calorimetry which is considered an accurate measurement (provided that the organism is not doing work on its surroundings) but the problems with it lie in logistics. Direct calorimetry works on the simple principle that the heat production from an animal is directly proportional to the internal metabolic rate. Direct calorimetry, however, can be difficult to perform due to the difficulty of creating an appropriate setup. Large metabolic chambers are required with complex equipment to ensure that all heat loss is measured (DeLany, 2017). Indirect calorimetry instead utilises a proxy of either O_2 consumption or CO_2 production for calculating metabolic rate.

Indirect calorimetry works by having a specified flow of air through an airtight metabolic chamber. Both the input and output air can be measured for the percentage of air that is composed of both oxygen and carbon dioxide. From these values, and the total flow of air measured, you can calculate how much oxygen is being consumed within the metabolic chamber (VO_2, L/min), and how much carbon dioxide is being produced (VCO_2, L/min). The amount of energy produced per unit of gas consumed/produced varies based on the sources being oxidised (i.e., carbohydrates, lipids, or proteins). The respiratory quotient (RQ) is calculated by dividing the VCO_2 by the VO_2 and provides an indication of the types of energy sources that are catabolised to produce energy (table 1) (Kleiber, 1975; Richardson, 1929).

Table 1: The Respiratory quotient from different substrates

Energy Source	**Respiratory Quotient**
Carbohydrates	1.0
Lipids	0.71
Proteins	0.83

From this respiratory quotient you determine the food source to then use the correct conversion factor, converting oxygen consumed (or carbon dioxide produced) into joules of energy. Oxygen consumption is normally used due to oxygen consumption being less impacted by the food source than CO_2 production (Brown & Brengelmann, 1965).

Given that it is essentially a measurement of all chemical processes within the body, the metabolic rate is therefore affected by a broad range of factors. It can be affected by the age of the animal (Boily & Lavigne, 1997), sex (Ladds et al., 2017), season (McHuron et al., 2019; D. Rosen & Renouf, 1995) and more. It is a comprehensive measure of the biological functions within an animal. In fasting or starving animals, the food source that provides the energy for these functions is now gone, therefore it is reasonable to expect that the metabolic rate of these animals may therefore be impacted.

The study of the effects of fasting on metabolic rate is not new; a metabolic depression during periods of starvation has been demonstrated in rats (*Rattus norvegicus*) (Montemurro & F Stevenson, 1960), humans (*Homo sapiens*) (Grande et al., 1958), and adult pinnipeds such as the Steller sea lion (*Eumetopias jubatus*) (Rosen & Trites, 2002). During the post-weaning fast undergone by these seal pups, it may be expected that there will be a change to their metabolic rate. The existence of a post-weaning metabolic depression has been demonstrated in grey seal pups (*Halichoerus grypus*) (Nordøy et al., 1990) during their 50-day post-weaning fast. It therefore would be expected that hooded seal pups follow a similar trend, but this has not been demonstrated (Bue, 2015). The purpose of a post weaning fast metabolic depression is believed to be quite simple, to minimise loss of energy during this period of low energy intake.

Animals in polar regions experience strong seasonality, therefore it is common that food sources that will be regularly utilised one season will be unavailable in the other. Another challenge faced in these regions is the cold; stored fat (sometimes in the form of blubber) will always serve a dual purpose in polar species, as both insulation and energy source. Body protein is also important however, muscles contain large amount of protein and therefore a substantial proportion of protein stores is within muscles. These muscles cannot be allowed to deteriorate during starvation or when food becomes available, they will have difficulty obtaining it.

Moreover, it has been shown that a number of polar species exposed to starvation as a part of their life history will reduce catabolism of protein as an energy source during fasting. Emperor penguin (*Aptenodytes forsteri*) males will fast for 4 months during the Antarctic winter in order to brood the eggs of their mate; during this fasting period they show a decreased protein catabolism where it only accounts for 4% of their total energy requirements (Robin et al., 1988). Polars bears (*Ursus maritimus*) will reduce protein catabolism during denning at variable rates depending on available fat stores (Nelson et al., 1983; Whiteman et al., 2018). Arctic ground squirrels (*Urocitellus parryii*) have been observed to increase catabolism of proteins upon arousal of their hibernation (Whitten & Klain, 1968), after a relatively low rate during the hibernation (during which they are fasting).

Within the group of pinnipeds, grey seals undergo a post-weaning fast, where approximately 6% of their energy expended is derived from protein catabolism (Nordøy et al., 1990). In the harp seal (another Arctic seal species), pups in a post-weaning fast were initially measured to derive 9% of energy from protein catabolism for the first 3 days of the fast, before a drop down to less than 4% (Nordøy et al., 1993). Calculating the metabolic rate through the fasting period would, in combination with the nitrogenous products in the urine, allow for calculation of the proportion of energy that is accounted for by protein catabolism.

1.6 Objectives and Hypotheses

During the post-weaning fast, hooded seal pups will face challenges of dehydration, starvation, heat loss, and the balancing of these against one another. Therefore, the ultimate aim of this study is to gain further understanding into how these hooded seal pups undergo their post weaning fast in regard to energy expenditure and water balance.

Three primary objectives are of focus in order to gain this understanding:

1. To calculate the rate of excretion of nitrogenous compounds allowing to determine the rate of protein catabolism.
2. To measure the sleeping metabolic rate throughout the post-weaning fast to investigate the presence of a post-weaning fast metabolic depression.
3. Measure the amounts of important ions (Na^+, Cl^-, and Mg^{2+}) in the plasma and urine to further support the theory of seawater drinking after transition to a saltwater exogenous water source.

Ultimately, gaining understanding in the form of these three objectives allowing for awareness of how a changing climate may affect the individuals, and therefore overall population, of this species. Current climate predictions would suggest that sea-ice will be greatly reduced in the Greenland Sea where hooded seals are known to give birth (Abdalati & Steffen, 2001; Sha et al., 2016). There has been a massive reduction in the pack ice distribution in this breeding site just observed by those who travel yearly to the Greenland Sea, travel time to reach the ice has increase over a few decades by an estimated 30% (Erling S. Nordøy, personal communication). This may result in pups being able to spend less time on the sea-ice and more quickly must transfer to seawater as their exogenous source of water. How this may affect the early stages of life in hooded seals is not yet known.

A primary hypothesis of this study is as follows:

Hooded seals are more dependent on insulation than pinnipeds at lower lattitudes due to low ambient air and water temperatures at weaning. Thus, during the post-weaning fast, they would use a higher proportion of protein and less fat (their primary method of insulation) as an energy source.

2 Methodology

To achieve the objectives as previously stated the methodology is as follows:

1. Capture six hooded seal pups at the end of lactation
2. Measure metabolic rate weekly through indirect calorimetry
3. Take blood and urine samples weekly
4. Analyse these samples for nitrogenous products and important ions to gain understanding of the post-weaning fast
5. Use urine nitrogenous products in tandem with metabolic measurements to determine the relative importance of protein catabolism

2.1 Expedition

2.1.1 Travel

The university vessel the R/V "Helmer Hanssen" was used for reaching the Greenland Sea pack ice. This is an expedition that goes yearly for the purpose of various research projects alongside teaching. After departing from Tromsø, Norway on the 21st of March 2021, arrival at the ice was on the 25th of March. Travel was to just east of the Greenland coast, around 71°N and 14°W. The intention was to arrive soon after the birth of hooded seals pups, so they would be at the end of their lactation period.

2.1.2 Seal Selection and Capture

A total of six seal pups were captured for this project from the 26th to the 31st of March. Permits were obtained to capture seals (permit no. 2021-80) from the Department of Fisheries, Hunting and Agriculture, Government of Greenland.

The aim was to capture blueback pups at the end of weaning. This was determined by the ice floe they were found on and the perceived weight of the animal. If a seal was found to be with the family group, but with a large amount of blubber, it was assumed to be close to the end of lactation. If a pup was found alone on its birth floe, then it was also considered to be found immediately after weaning. This is because the seals will often travel away from their birth floe soon after weaning. A birth floe could be easily identified by the presence of the placenta from its birth.

To capture the seal, a person would take the lift down to the ice floe and use a bag to capture the pup. Immediately after capture, the seals were brought onboard weighed to get an initial weight using a hanging scale (Heavy-duty hanging scale, PHS100-HD, Pesola Präzisionswaagen AG, 8834 Schindellegi, Switzerland) than can be used for later calculations (table 2). The seals were also weighed just before departure from the Greenland Sea since a significant amount of weight change can occur during the start of their fast (Nordøy et al., 1990). No measurements of body length/circumference were taken due to it being a less consistent measurement of body condition due to the variable dimensions a seal can have dependent on body position.

Table 2: Table of captured pups and initial measurements/observations

Seal Code	Seal Number	Capture Date	Initial Weight (kg)	Sex	Notes
K2-21	1	26/03/2021	44.15	M	Caught with mother on birth floe
K6-21	2	27/03/2021	42.27	M	Found alone on birth floe
K7-21	3	27/03/2021	33.69	M	Found alone on birth floe
K9b-21	4	29/03/2021	34.66	F	Caught with mother at end of weaning
K12-21	5	30/03/2021	36.22	M	Found alone on birth floe
K13-21	6	31/03/2021	48.97	F	Caught at end of weaning

2.2 Animal Care

2.2.1 On the Boat

Once the seal pups were brought up onto the boat, they were placed in pens that were constructed out of wooden pallets, measuring about 2.5 m by 3 m. They were provided freshwater in the form of snow collected on the ice floes (figure 3). After around three days of travel, we returned to Tromsø with the seals. Permit to import seals into Norway was issued by the NFSA (Norwegian Food Safety Authority, reference 2021/014701).

Figure 3: Seal lying on snow in the pens that were constructed for travelling.

2.2.2 At the University

The Department of Arctic and Marine Biology and the Arctic Chronobiology and Physiology Research Group maintains an animal research facility that is authorised by the NFSA (approval number 089) for the upkeep of seals for research purposes. The seals arrived at the facility and administered 0.02ml/kg ivermectin subcutaneously (Ivomec® vet., 10mg/ml, vnr. 525352, Boehringer Ingelheim Animal Health, 69007 Lyon, France) as an antiparasitic treatment. They were placed into the 40,000L pools (figure 4) which contain natural (untreated) seawater pumped up from the harbour southeast of the university. Within the pool rooms a light timing system was used to imitate the natural photoperiod that occurs in Tromsø. These seawater pools will also somewhat reflect the conditions in the wild as after a

period of remaining on the ice the seals will then spend time in open ocean travelling to the feeding ground for the first time (Folkow et al., 2010).

Figure 4: Pools used to keep the seal at the university.

In the pools, they were similarly not given food for the duration of the experiment and their only access to water was in the form of seawater. Hooded seal pups have previously been demonstrated to maintain water homeostasis when given access to only seawater (Schots et al., 2017).

Two of the seals (K9b-21 and K7-21) were taken out of the experiment at the beginning of week 4 so feeding could be done. This was due to animal welfare concerns about their weights falling to a critical mass (23.64 kg and 26.52 kg), dropping down to about 2/3rds of their original weight.

2.3 Sampling

The permit to conduct my experiments was issued by the NFSA (FOTS ID number 22751).

Both blood and urine samples were collected from each seal weekly, including on the day of their initial capture. Alongside these samples, weight and resting metabolic rate were measured each week. Generally, weighing occurred before blood sampling, before metabolic measurements were taken, which lasted from around 1.5 to 3 hours. Finally, the seals were placed in the urine collection box to collect sample over the next 24-hour period.

2.3.1 Urine

To obtain the urine samples the seals were placed in a box (length of 127 cm, width of 66 cm, height of 128 cm) for 24 hours (figure 5). The seals lie on a silicone lined steel mesh and the bottom 63 cm of the box is a funnel lined with silicone to direct the urine down to a collection flask. The flask contained one crystal of thymol in order to prevent bacterial growth and thus, the breakdown of nitrogenous products. After 24 hours, the seal was placed back into the pool and the total urine produced was measured. A subsample of 24 ml was taken and stored in polyethylene tubes at -20°C for analyses explained later.

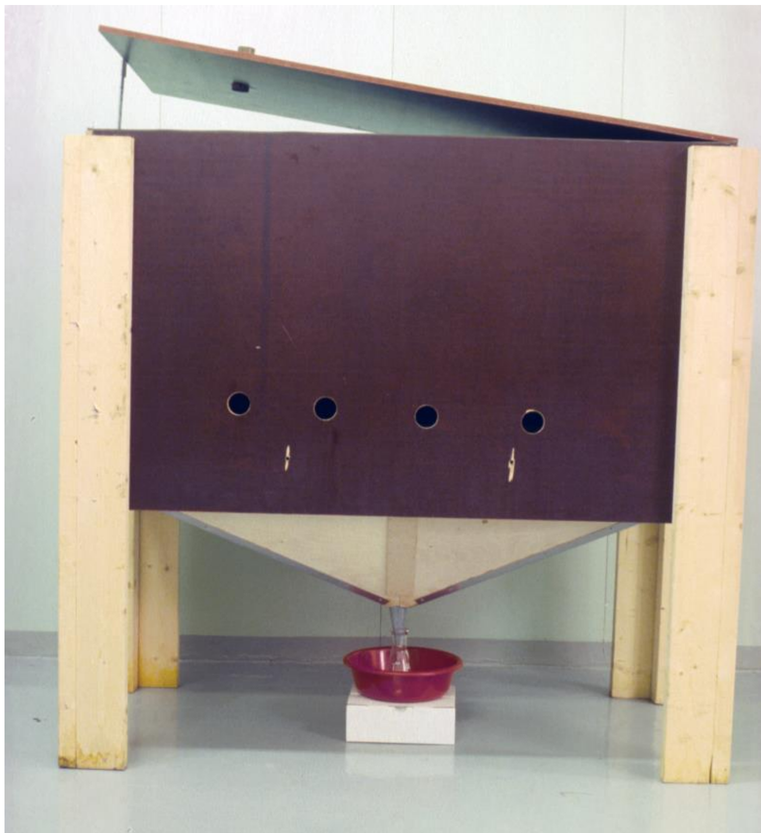

Figure 5: The box in which the seal was placed to collect urine. Photo Credit: Erling S. Nordøy

2.3.2 Blood

Obtaining blood samples from an Arctic seal comes with some challenges, in large part due to the thick stores of blubber surrounding the torso. This blubber layer makes major blood vessels in the seal torso difficult to locate; thus, in order to sample the blood two options were available. The first is by utilising a vacutainer™ container (BD, Franklin Lakes, NJ, USA) and needle to extract blood from a superficial vein in the hind flipper. Hind flippers are most suitable for this option as the hind flippers are well vascularised due to their role in thermoregulation (Tarasoff & Fisher, 1970), making the locating of a blood vessel easier. The other option is to extract blood using a catheter via the extradural intervertebral vein (figure 6). The catheter was chosen due to the increased amount of control that a catheter provides, and the speed of extraction. Given that this sampling provides a possible route of infection, it becomes important to ensure everything is properly disinfected with chlorhexidine (primarily the gloves and the point of insertion).

Firstly, the seal had to be kept stationary; sedatives were not used as they introduce their own inherent risk of mortality or injury (Arnemo et al., 2014; Chinnadurai et al., 2016). They were instead kept physically restrained during blood extraction as it is a short and safe procedure. This was achieved by strapping them to a wooden board to stabilise them during sample extraction. A head cover was used to assist in keeping the seals calm.

Figure 6: The set-up for the extraction of blood from a seal via the extradural intervertebral vein.

In order to extract the blood via the extradural intervertebral vein a few steps must be taken. Firstly, the location of the spine is identified by feel before an area around the lumbar vertebrae (3-4) is shaved. The reason for inserting the catheter around the lumbar vertebrae is that the gaps between the vertebrae are larger making for easier entrance (Erling S. Nordøy, personal communication). A thick needle is then used to puncture the skin. The catheter (Secalon-T™, 16G/1.70x160 mm, 128 ml/min, Argon Medical Devices, Singapore) needle is then inserted at a low angle, and it is inserted between the vertebrae. In Figure 7, the arrow points directly to the vein and just below that, is the spinal cord.

Figure 7: Longitudinal section of a hooded seal pup torso. From the top to bottom you can see the skin, blubber layer, muscle layer, vertebrae, extradural vein, and the spinal cord. The arrow shows the angle of entrance for a catheter between the lumbar vertebrae.

For each sampling of the seals, around 50 ml of blood were taken from the extradural vein into separate 15 ml centrifuge tubes (VWR® Centrifuge Tube, 15 ml, Conical-Bottom, Sterilized, VWR™ International, Radnor, PA, USA). This amount was taken to ensure there was sufficient amounts of sample for multiple types of analysis. In these tubes, two drops of sodium heparin (Heparin LEO, 5000IE/a.e./ml, 10htgl, á 5 ml, LEO Pharma AS, Industriparken, Ballerup, Denmark) were added to prevent clotting. A subsample of this was taken with a plastic disposable pipette to measure haematocrit while the rest was centrifuged at 2000 rpm for 15 minutes (Labofuge 200, Kendro Laboratory Products GmbH, Hanau,

Germany) to separate the cells from the plasma. The plasma was then extracted using a glass pipette and stored in cryotubes (Low Temperature Freezer Vials, 2 ml, VWR™ Intenational, Radnor, PA, USA) at -20°C for later analysis of nitrogenous products and important ions. Details on sample handling will follow.

2.3.3 Metabolic rate

The metabolic rate was measured each week alongside the samples being taken. The seals were placed into an airtight metabolic chamber (length of 114 cm, width of 57 cm, height of 61 cm) and air was pulled through holes in the back (diameter of 40 mm). A mass flow controller (FlowKit Mass Flow Generator, Field Version, FK-500-1, Sable Systems International, North Las Vegas, NV, USA) pulls air through the system at a relatively consistent 150 L/min while simultaneously sending the data on air flow to the computer. The reason for this 150 L/min is so that the flow is high enough to ensure the manifold quickly becomes filled with air, and the larger the air flow gets, the smaller the difference would be between the reference air and the exhaled air. A subsample of this air is drawn through to a hygrometer (RH-300, Sable Systems International, North Las Vegas, NV. USA) to measure the water vapour pressure (kPa) of the air. The air then flows either through a vacuum filter (CSL-849-150HG, Solberg® International, Ltd., Hellebæk, Denmark) to the manifold or through the excess output. A three-way stopcock allows for flow through to the analysing components from either the reference air or the air coming from the manifold (figure 8). The Foxbox® (Sable Systems International, North Las Vegas, NV, USA) itself pulls air at a consistent rate of 1.5 L/min so can be drawn consistently from the reference environmental air or the manifold (figure 8). The Foxbox® is what analyses the oxygen and carbon dioxide concentrations in the air, providing the concentration in volts and sending the data to a computer (figure 9).

Water vapour may cause damages to the cell of the oxygen analyser of the Foxbox® and must be removed, therefore a drying element is used as the final component before the analysis can occur. Calculations are made with “dry air concentrations” so this also assists in ease of calculating later. The drying agent is composed of two airtight cylinders joined in series containing granules of water-vapour absorbent silica gel (SiO_2, Sigma-Aldrich®, USA) and calcium chloride ($CaCl_2$, Merck KGaA, Germany). Both chemicals absorb the water vapour, but with calcium chloride, it is not clear when it has become saturated with water; the coloured silica gel, however, turns more transparent the more water it absorbs and thus is an

indicator of whether the granules require replacement. This usually resulted in replacement of granules in the first tube after two metabolic readings.

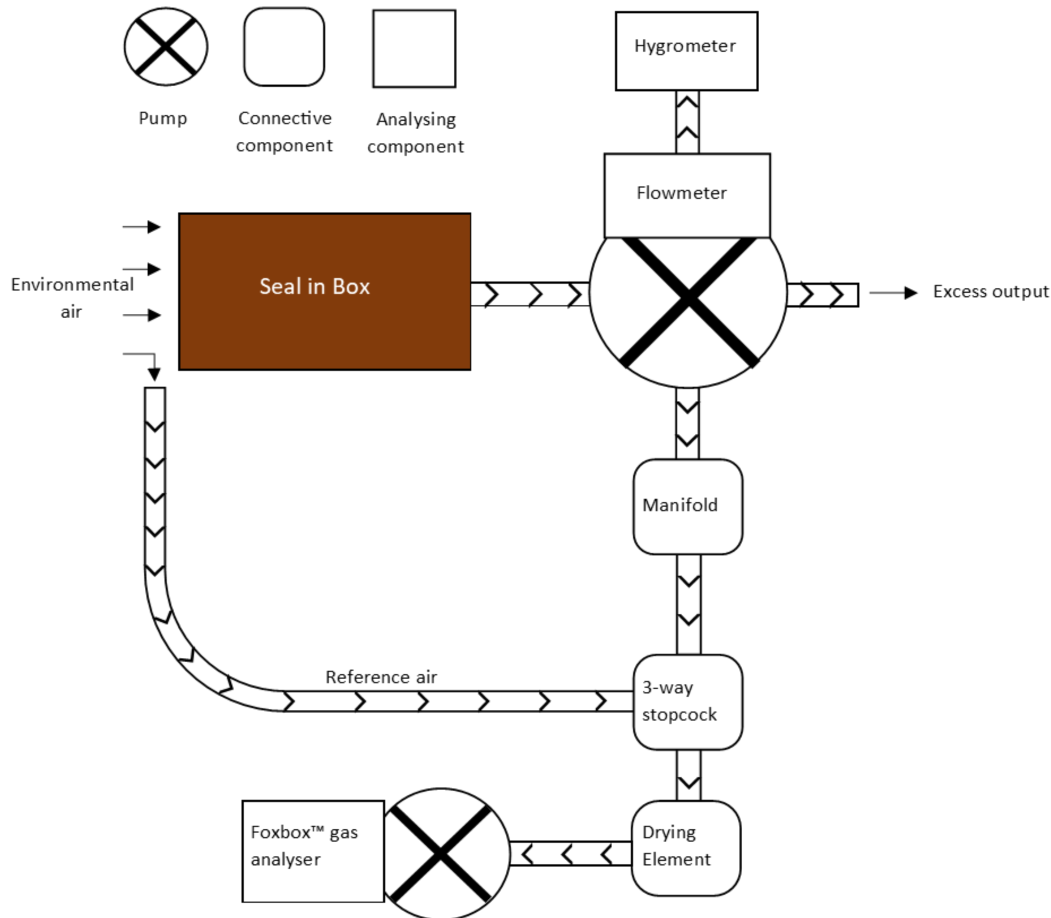

Figure 8: A diagram showing the setup used for metabolic analysis.

While the seal was contained within the chamber, I stood back with the computer and the equipment monitoring the values; this was to ensure that I did not stress the animal in the chamber, ensuring calm, resting readings. Readings were done in 20-minute periods before switching the stopcock to allow for reference air for 5 minutes. This was to ensure that reference air was consistently available for comparison of background oxygen levels. The aim was to get consistent behaviour from the seals to allow for comparison both between seals and between different stages of the fast. As such, the behaviour opted for was sleeping; this is relatively easy to recognise from the readings due to the presence of sleep apnoea that is typically displayed by pinnipeds (Castellini & Castellini, 1993). Sleep apnoea is a mechanism by which the seals will breathe periodically during sleep instead of constant

breathing, this can be detected by a pattern of rising and falling oxygen levels between breaths (figure 9). An average of the sleep apnoea cycle can be taken to represent the standardised sleeping metabolic rate of the seal.

The values of oxygen and carbon dioxide during the measurements could be observed on the Labchart programme (Labchart version 8, ADInstruments, Dunedin, New Zealand), given in values of voltage (figure 9).

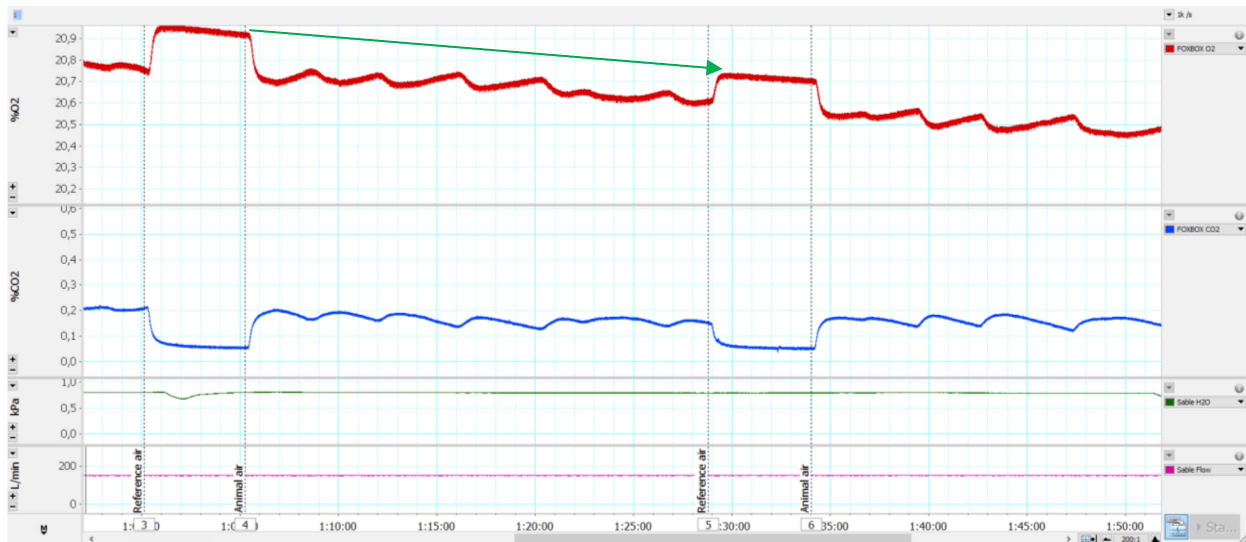

Figure 9: An example of the labchart readings showing the clear sleep apnoea cycles. From the readings, values are provided for (in order from top to bottom) oxygen content of air (V), carbon dioxide fraction of air (V), water vapour pressure (kPa), and total air flow (L/min). Green arrow starts in one reference point and goes to the next, showing the drift.

Due to inherent drift in readings for oxygen concentration (V) (figure 9), it was assumed there was a linear drift between the reference air values (green arrow of figure 9) for oxygen concentration. From these, an average can be found between the reference points to provide a new reference oxygen concentration (V).

2.3.3.1 Calibration and Calculations

Oxygen content of the environmental air (FiO_2*100) was assumed to be 20.95% (Cox & Pilachowski, 2002), and carbon dioxide content of the environmental air ($FiCO_2$*100) was assumed to be 0.04162% based on the NOAA data for April 2021 (National Oceanic and Atmospheric Administration, 2021).

Terms used here are as follows:

VO_2 = Oxygen consumption of the animal (l/min)

VCO_2 = Carbon dioxide production of the animal (l/min)

Corrected flow = Flow rate of air through the metabolic chamber after correction by removing water vapour (l/min)

Total flow = Total flow rate of air through the mass controller before correction (l/min)

H_2O flow = Flow rate of water vapour that must be removed from the air before entering the analysis component (l/min)

FiO_2 = Fraction of dry environmental air that is composed of oxygen

$FiCO_2$ = Fraction of dry environmental air that is composed of carbon dioxide

FeO_2 = Fraction of dry expired air that is composed of oxygen

$FeCO_2$ = Fraction of dry expired air that is composed of carbon dioxide

Firstly, the air flow had to be calculated given that the water vapour was not able to enter the machine, and because all gas fractions used refer to dry air (Lighton, 2008):

$$H_2O\ flow = Total\ flow\ \times \frac{\frac{Relative\ Humidity}{100} \times 4.588 \times 10^{\frac{7.59 \times Temperature(°C)}{240.78 \times Temperature(°C)}}}{Barometric\ Pressure}$$

Since gas volumes are standardised to STPD conditions, and the mass flowmeter already provides values in standard temperature and pressure, the last condition to account for is the “D” (dry conditions). The corrected flow could then be calculated as:

$$Corrected\ flow = Total\ flow - H_2O\ flow$$

Calibration of the set-up occurred by performing a dilution of nitrogen gas at a rate of 2L/min through a smaller mass flow controller (FMA5518A Mass Flow Controller, Omega Engineering Inc., Norwalk, CT, USA). The principle is essentially that the nitrogen will not be detected by the analyser, and thus an injection of a specific amount of nitrogen will allow the new concentration of oxygen to be calculated precisely, allowing for a more accurate

calibration (McLean & Watts, 1976). From this the new percentage of oxygen can be calculated:

$$Nitrogen\ Dilution\ Oxygen\ content\ (\%) = \frac{20.95 \times (Corrected\ flow - 2)}{Corrected\ flow}$$

From this value, a ratio of Oxygen content (%) to voltage can be found:

$$Oxygen\ ratio\ (\%/V) = \frac{20.95 - Nitrogen\ Dilution\ Oxygen\ content\ (\%)}{reference\ voltage\ (V) - Dilution\ voltage\ (V)}$$

Therefore, the oxygen fraction in the output air (FeO_2) can be calculated as:

$$FeO_2 = \frac{20.95 - (Oxygen\ ratio \times (Input\ oxygen\ (V) - Output\ Oxygen\ (V)))}{100}$$

A ratio for carbon dioxide was used from a previous study, using the exact same gas analyser, for a carbon dioxide (%)/V ratio of 1.05±0.0478 due to a leak in the calibration gas that meant calibration for CO_2 could not be completed (Evertsen, 2021). From that, the carbon dioxide fraction in the output air (FeO_2) could be calculated as:

$$FeCO_2 = \frac{0.04162 + (Carbon\ dioxide\ ratio \times (Input\ CO_2\ (V) - Output\ CO_2\ (V)))}{100}$$

From these, the oxygen consumption (VO_2) and carbon dioxide production (VCO_2) (both in L/min) can be calculated as such (Lighton, 2008):

$$VO_2(L/min) = Corrected\ flow \times \frac{(FiO_2 - FeO_2) - FiO_2(FeCO_2 - FiCO_2)}{1 - FiO_2}$$

$$VCO_2(L/min) = Corrected\ flow \times \frac{(FeCO_2 - FiCO_2) + FiCO_2(FiO_2 - FeO_2)}{1 + FiCO_2}$$

From these values of oxygen consumption and carbon dioxide production, further calculations can be done to convert these into units of energy.

The respiratory quotient (RQ) is first calculated by dividing the VCO_2 by the VO_2:

$$Respiratory\ Quotient\ (RQ) = \frac{VCO_2}{VO_2}$$

The VO_2 and VCO_2 was then calculated by use of the Weir Equation (Weir, 1949) which calculated the metabolic rate independent of the RQ:

$$Metabolic\ rate\ (kcal/day) = 1440(3.94VO_2 + 1.11VCO_2)$$

This can then be converted into watts by simply dividing the value by 20.64 (kcal/day/W).

2.4 Sample Analysis

2.4.1 Blood Sample Handling

Firstly, the subsample of whole blood that was taken to measure haematocrit was analysed immediately after samples were taken. Proper mixing via inversion was ensured before the samples were placed into the capillary tubes to ensure no sedimentation of blood cells. Haematocrit was measured using a haematocrit centrifuge (EBA 12, Type 1000, Andreas Hettich GmbH, Tuttlingen, Germany), spinning at 12000 RPM for 15 minutes. An average of 6 capillary tubes was taken to get an average haematocrit (%) for the sample.

The plasma samples were sent to The Norwegian University of Life Sciences (NMBU) central laboratory for analysis of nitrogenous compounds (urea, uric acid, and creatinine) and relevant ions (Na^+, Cl^-, and Mg^{2+}). All samples were analysed by an Advia®1800 chemistry station (ADVIA 1800® Clinical Chemistry System, Siemens Healthineers AG, Erlangen, Germany) using solutions produced by Siemens Healthineers (Siemens Healthineers AG, Erlangen, Germany). Sodium (Na^+) and chloride (Cl^-) ions were analysed via the same method, an indirect potentiometric measurement. A buffer is added to the sample in order to maintain consistent pH and ionic strength, before the sample is passed through an ion selective electrode. The changes in electrical potential are detected and compared with a reference electrode to provide an accurate measurement. For magnesium ion analysis, the sample is mixed with xylidyl blue in an alkaline medium to form a chelate. This increases the absorbance of the sample at 505 nm in proportion to the concentration of magnesium in the initial sample.

The nitrogenous compounds in the plasma samples were similarly analysed by the Advia®1800 chemistry station using solutions/reactants produced by Siemens Healthineers (Siemens Healthineers AG, Erlangen, Germany). Urea is hydrolysed in the presence of water and urease to produce ammonia and CO_2. The ammonia then reacts with 2-oxoglutarate when catalysed by glutamate dehydrogenase and is reduced by NADH. The oxidation of NADH to

NAD is measured at 340 nm as an inverse rate reaction. Uric acid is converted by uricase to allantoin and hydrogen peroxide. The hydrogen peroxide then, catalysed by peroxidase, reacts with 4-aminophenazone and TOOS (3-(N-Ethyl-3-methylanilino)-2-hydroxypropanesulfonic acid sodium salt) to form a coloured complex. The absorbance of this complex can be measured at 545 nm to measure the amount of uric acid at the start of the reaction. Creatinine in a sample reacts with picric acid to form a colour complex. The rate of formation of this complex is measured at 505 nm proportionally to the concentration of creatinine in the sample.

Osmolality in both plasma and urine was measured using an Osmometer (Osmomat 030, Gonotec GmbH, Berlin, Germany) and calibrated using solutions produced by Gonotec (Osmoref® Reference solution, Gonotec GmbH, Berlin, Germany). It works as a cryoscopic osmometer that measures the freezing point in a sample to determine the total osmolality.

2.4.2 Urine Sample Handling

Urine samples were taken to UNN hospital to utilise the equipment available there for analysis. Samples were analysed by a Roche/Hitachi® cobas c system (Roche/ Hitachi® Cobas c 311, Roche Diagnostics GmbH, Mannheim, Germany) which is a modular system that allows for many types of analysis. Reagents used with similarly produced by Roche Diagnostics (Roche Diagnostics GmbH, Mannheim, Germany).

Sodium and Chloride were both measured using the Roche cobas 8000 ISE module (Roche/ Hitachi® Cobas c, Roche Diagnostics GmbH, Mannheim, Germany). An ion selective electrode (ISE) is used where the sample is passed through. The changes in electric potential can be detected and compared with a reference electrode to get the concentration of the analyte in the sample (in this case, sodium, and chloride ion concentration). For magnesium ion analysis, the Roche cobas c 702 (Roche/ Hitachi® Cobas c 702, Roche Diagnostics GmbH, Mannheim, Germany) module was utilised; it uses the colorimetric endpoint method. The sample is mixed with an alkaline buffer before xylidyl blue is added. The magnesium within the sample forms a coloured complex with the xylidyl blue, then the magnesium concentration can be measured photometrically from the reduction in absorbance of xylidyl blue.

Ammonia levels in the urine was analysed using an ammonia kit (Ammonia Assay Kit, MyBioSource, San Diego, CA, USA). The ammonia within the urine reacts with a

chromogen in an alkaline solution (reagents provided by MyBioSource) to produce a blue/green solution where the intensity is proportional to the concentration in the sample. The plate is then read between 630-670 nm and compared with known ammonium chloride standards to find the precise concentration of ammonia in the sample.

All other nitrogenous products (urea, uric acid, and creatinine) were analysed using the Roche cobas c 702 module (Roche Diagnostics GmbH, Mannheim, Germany) using catalysts and reagents provided by Roche Diagnostics (Roche Diagnostics GmbH, Mannheim, Germany). Urea in a sample reacts with water when catalysed by urease to produce ammonium and carbonate ions. Then the ammonium reacts with 2-oxoglutarate and coenzyme NADH (catalysed by GLDH) to L-glutamate, H_2O, and NAD^+. Two moles of NADH are oxidised for each mole of urea hydrolysed. The rate of reduction of NADH is directly proportional to the sample urea concentration and therefore the concentration can be measured photometrically. Uric acid in a sample will react with oxygen and water in the presence of uricase to form allantoin, carbon dioxide, and hydrogen peroxide. The hydrogen peroxide will then oxidise 4-aminophenazone (in the presence of peroxidase) to form quinone diamine dye. The intensity of colour in this dye is directly proportional to the uric acid concentration in the same so the increase in absorbance is similarly proportional. From this the concentration of the original sample can be calculated. Creatinine in a sample will hydrolyse in the presence of creatininase to form creatine. Creatine is then hydrolysed in the presence of creatinase to form sarcosine and urea. Sarcosin will then react with oxygen and water in the presence of SOD to form glycine, CH_2O and hydrogen peroxide. This hydrogen peroxide then reacts with 4-aminopehnazone and HTIBa to form a quinonimine chromogen, water, and hydrogen iodide. The colour intensity of the chromogen formed is directly proportional to the concentration of creatinine in the sample.

Once results were returned from UNN then further calculations could be performed. Analysis from UNN resulted in data with the units mmol/l or µmol/l. From these, the total amount of urinary nitrogen produced could be calculated.

Firstly, the molecular mass of nitrogen and measured nitrogenous products were found and the proportion of the compounds that are composed of nitrogen (table 3):

$$Nitrogen\ Fraction = \frac{14.0067 \times Number\ of\ Nitrogen\ atoms\ in\ Compound}{Molecular\ Mass\ of\ Compound}$$

Table 3: Table of molecular masses used in order to find the fraction of compounds that is accounted for by the element nitrogen (Cox & Pilachowski, 2002)

	Molecular weight	Number of nitrogen atoms	Nitrogen mass in compound	Nitrogen Fraction
Nitrogen	14.0067	1	14.0067	1
Ammonia	17.031	1	14.0067	0.822424
Urea	60.06	2	28.0134	0.466424
Creatinine	113.12	3	42.0201	0.371465
Uric Acid	168.1103	4	56.0268	0.333274

The values of compound concentration in the urine samples given in mmol/l or µmol/l are then converted to g/l using their known molecular mass (ammonia is being used as an example here):

$$Ammonia\ Concentration\ (g/l) = \frac{Ammonia\ Concentration\ (mmol/l) \times 17.031 (g/mol)}{1000 (mmol/mol)}$$

Then the nitrogen provided by an individual compound can be found as:

$$Nitrogen\ from\ ammonia\ (g/l) = Ammonia\ concentration\ (g/l) \times Ammonia\ nitrogen\ fraction$$

Once this has been done for all compounds, the total nitrogen concentration (g/l) can be found simply by summing the nitrogen provided by all compounds together:

$$Nitrogen\ Concentration\ in\ Urine\ (g/l) = Nitrogen\ from\ ammonia\ (g/l) + Nitrogen\ from\ urea\ (g/l) + Nitrogen\ from\ Uric\ acid\ (g/l) + Nitrogen\ from\ creatinine\ (g/l)$$

This can then be multiplied by the urine production over 24 hours (l/day) to get the nitrogen produced (g/day).

To convert this into grams of protein catabolised this value of nitrogen produced per day can be multiplied by 6.25 (Kleiber, 1975). Then the energy obtained by this catabolised protein can be found by multiplying the grams of protein by 4.8 to get the energy content of this protein (kcal) (Kleiber, 1975).

The energy provided by protein can then be divided by the total SMR to get the ratio of metabolic energy that is accounted for by protein catabolism.

2.5 Statistical Analysis

Initially data was organised in Microsoft Excel version 2203 (Microsoft Corporation, Redmond, WA, USA) Statistical analysis and graphs were made using R version 4.1.3 and RStudio version 2022.02.1.461 (RStudio, PBC, Boston, MA, USA). The ggplot2 library was installed and used to create graphs and RStudio was used to perform linear regression and two-sample t-tests.

Linear regression models were used to detect significant changes in values over time (in days of fasting). Models were run for the whole sample to detect significant changes in samples over the entire fasting period. They were also run with subsets of the data including just the data for the first two weeks in sampling (representing the shift between freshwater and saline exogenous water sources). Two-sample t-tests were also used in RStudio to detect the differences between the results before and after the first sampling, similarly representing the shift to a saltwater exogenous water source.

A p-value of less than 0.05 can be accepted as a rejection of the null hypothesis, showing a correlation and statistical significance. A p-value of greater than 0.05 means that the null hypothesis is accepted, however sometimes the results are still stated due to it possibly being the result of a lack of statistical power.

3 Results

3.1 Mass loss

As would be expected for a fasting animal, the pups lost weight over the fasting period (figure 10). A linear model applied to the data shows that on averages the pups lost 0.82±0.072% of their weight per day of fasting ($y = -0.82x+97.5$, $p=1.11e-12$, $R^2=0.809$, $n=33$). The seals ended their fast on an average of 74±3.5% of their initial weight. This average loss can also be given as 0.27±0.096 kg/day ($p=0.00921$).

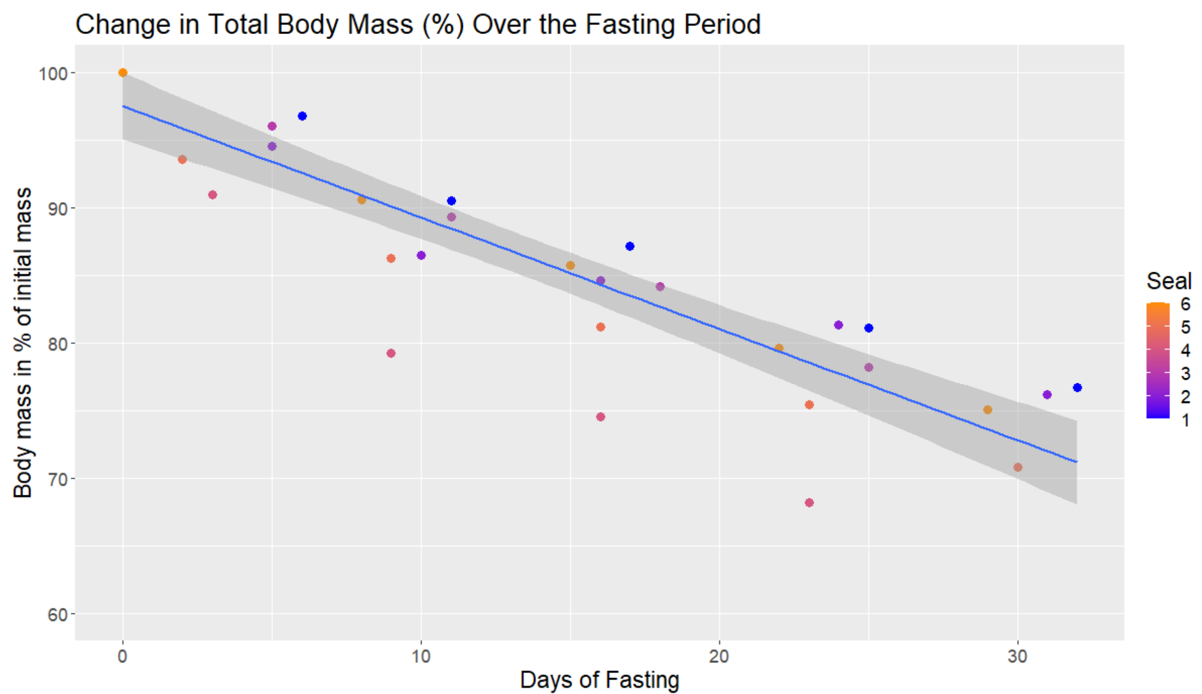

Figure 10: A scatter plot showing the change in total body mass (as % of initial mass) over the days of fasting.

3.2 Haematocrit

Haematocrit was observed to increase during the first week of fasting, at an increase of 0.58±0.18% per day ($y = 0.58x+55.3$, $p= 0.00807$, $R^2=0.521$, $n=12$), from an average of 55±1.8% to 61±5.3% (figure 11). After the initially shift from the first week, the change was insignificant ($p=0.318$).

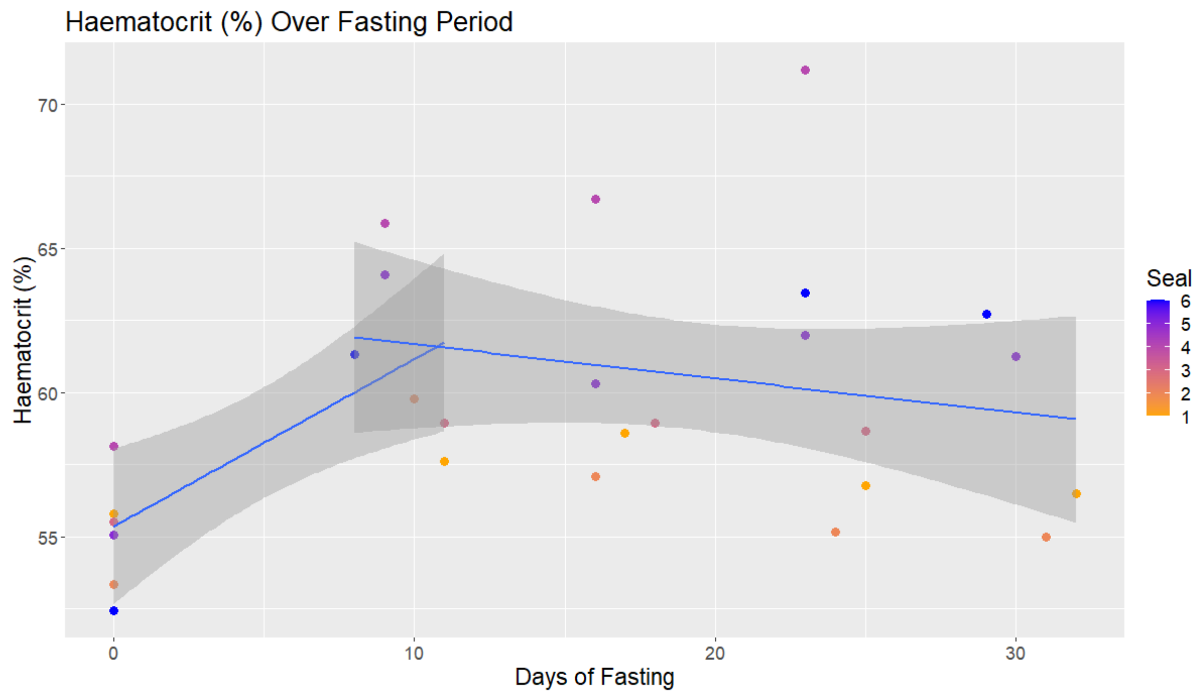

Figure 11: A scatterplot showing the change in haematocrit (%) over the days of fasting. Two regression lines are present to demonstrate the change in conditions before and after the first week of sampling.

3.3 Plasma

3.3.1 Plasma Nitrogenous Products

A linear increase in the concentration of urea in plasma was found. Averages going from 12±5.0 mmol/l on day 1 to 19±6.7 mmol/l at the end of the fasting period (figure 12). An increase of 0.24±0.10 mmol/l per day was found ($y = 0.24x+10.9$, $p= 0.0296$, $R^2=0.1694$, $n=28$).

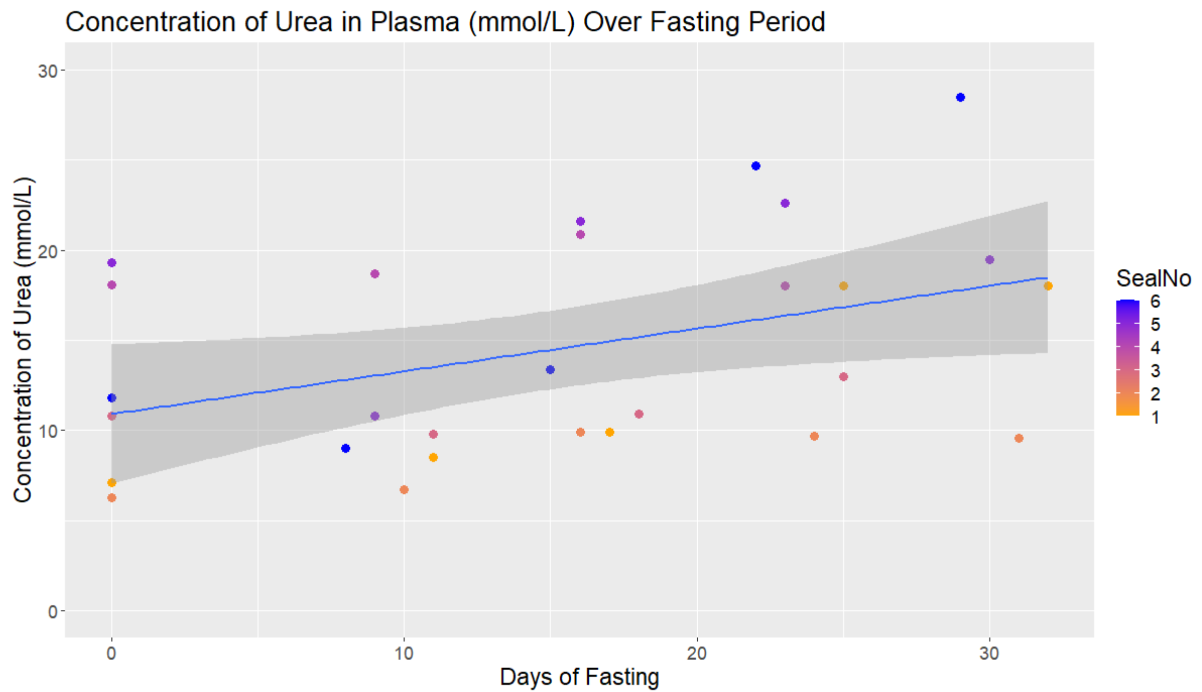

Figure 12: A scatterplot of the change in urea concentration (mmol/l) in the plasma over the days of fasting.

Uric acid in the Plasma showed a linear decrease of -0.0051±0.0010 mmol/l per day (y = -0.0051x+0.30, p=3.52e-05, R^2=0.4885, n=28) (figure 13). Averages went from 0.33±0.080 mmol/l at the start to 0.16±0.0085 mmol/l at the end of fasting.

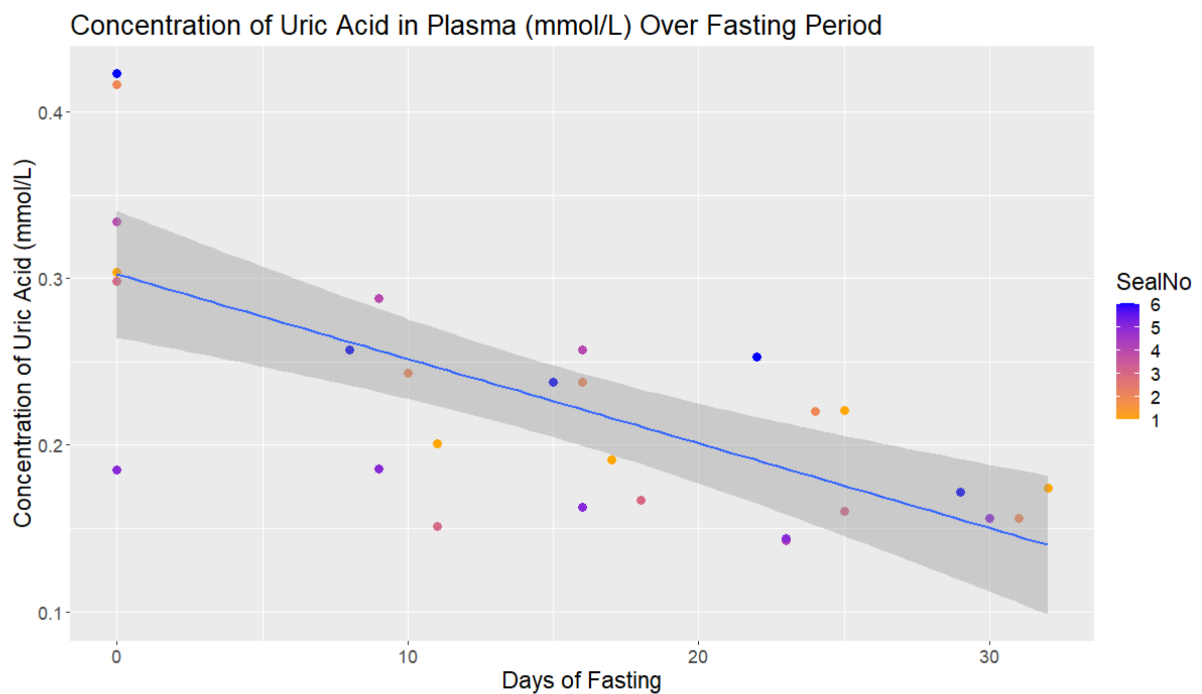

Figure 13: A scatterplot of the change in uric acid concentration (mmol/l) in the plasma over the days of fasting.

For plasma creatinine levels, no detectable change was detected by a linear model (figure 14) ($p= 0.778$). Urea:Creatinine (U:C, mmol/l:mmol/l) was calculated as an average of 181±80 with a slight increase in relation to days fasting ($y = 2.9x+138$, $p=0.0465$, $R^2=0.1439$, $n=28$).

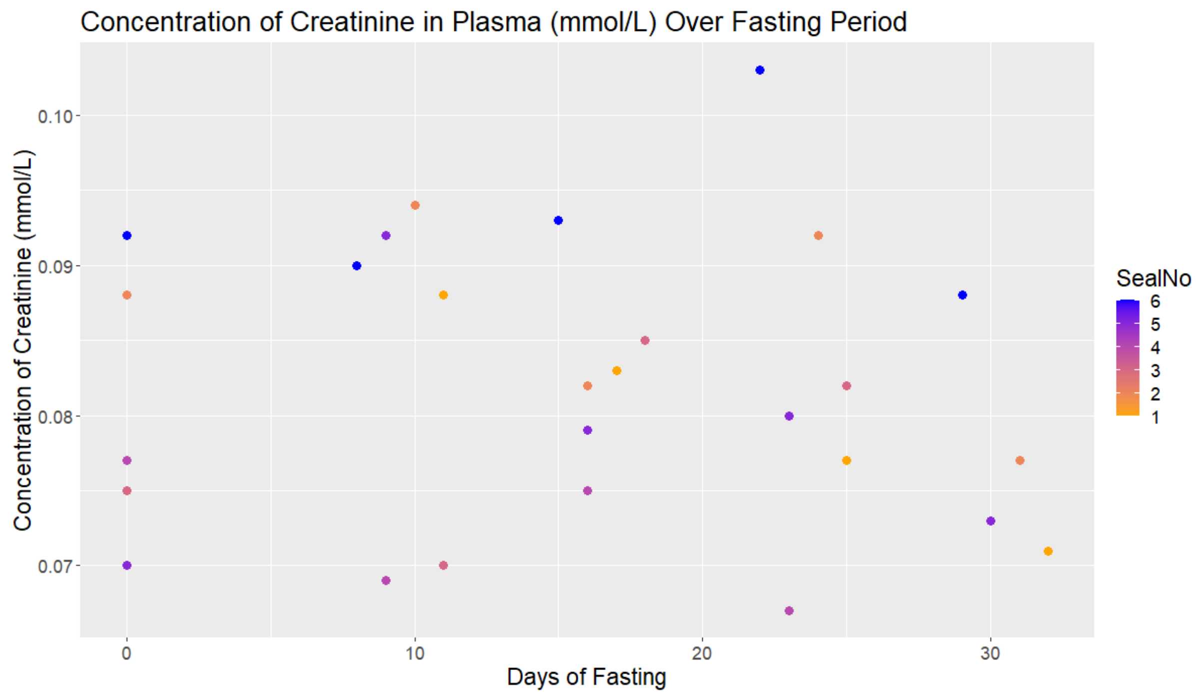

Figure 14: A scatterplot of the creatine concentration (mmol/l) in the plasma over the days of fasting.

3.3.2 Plasma Electrolytes

Chloride ion concentration in plasma showed a linear increase of 0.11±0.047 mmol/l per day ($y = 0.11x+103$, $p=0.0322$, $R^2=0.1646$, $n=28$) from an average of 103±1.8 mmol/l at the start to 108±2.6 mmol/l at the end of fasting (figure 15).

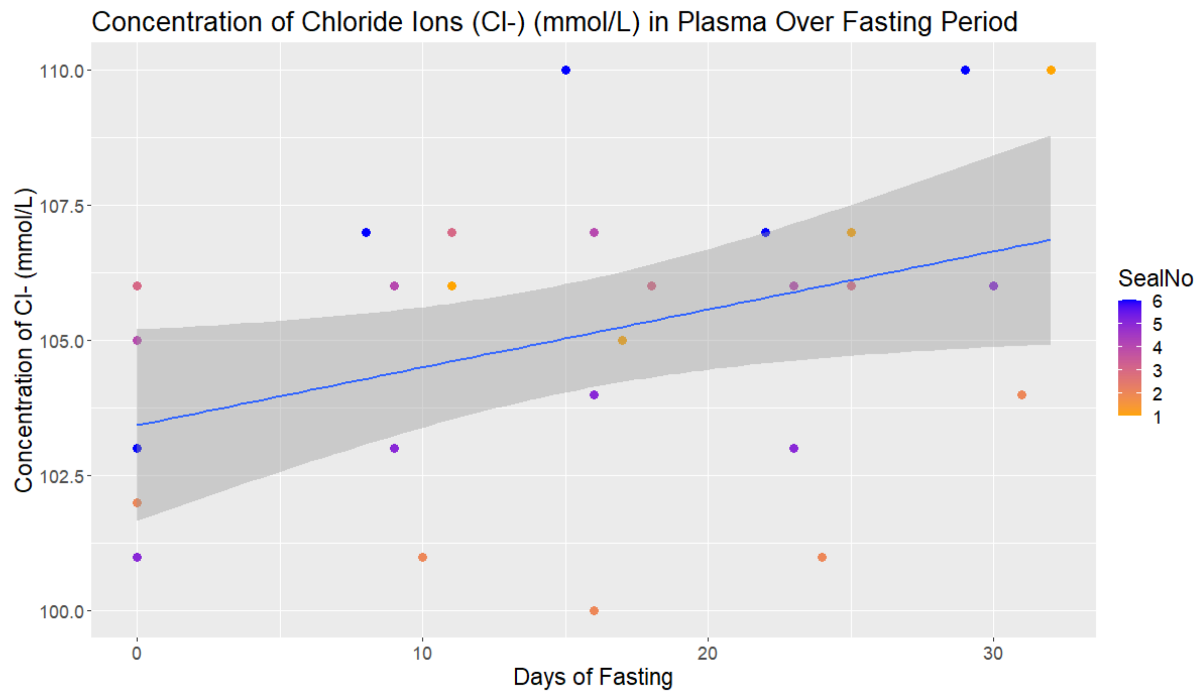

Figure 15: A scatterplot of the chloride ion concentration (mmol/l) in the plasma over the days of fasting.

Sodium ion concentration was not found to have a correlation with days passed (p=0.596) (figure 16). However, seal 1 (K2-21) was found to have a significant increase of 0.15±0.045 mmol/l per day (p=0.0457).

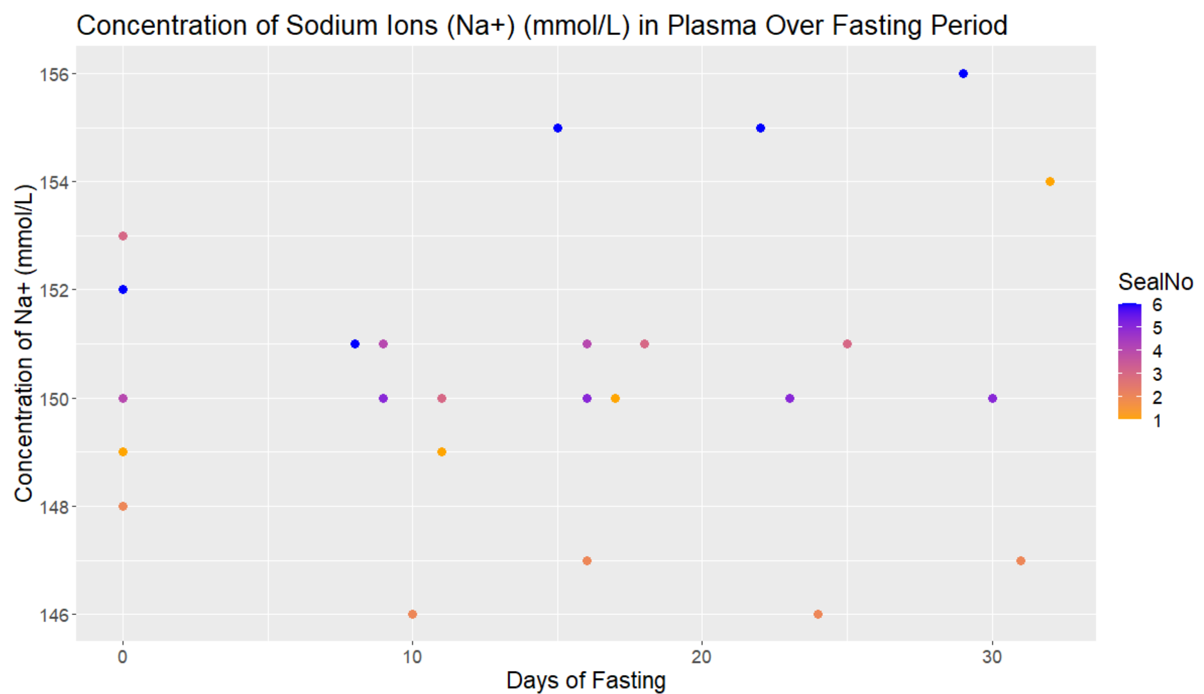

Figure 16: A scatterplot of the sodium ion concentration (mmol/l) in the plasma over the days of fasting.

Magnesium concentration in plasma is detected to rise significant at a rate of 0.016 mmol/l per day between the first and second sampling periods (y = 0.016x+0.94, p=0.0381, R^2=0.3633, n=12) before being an insignificant change (p=0.933) (figure 17). Average in the first sample was a concentration of 0.93±0.075 mmol/l compared with 1.2±0.19 mmol/l at the end of the fasting period.

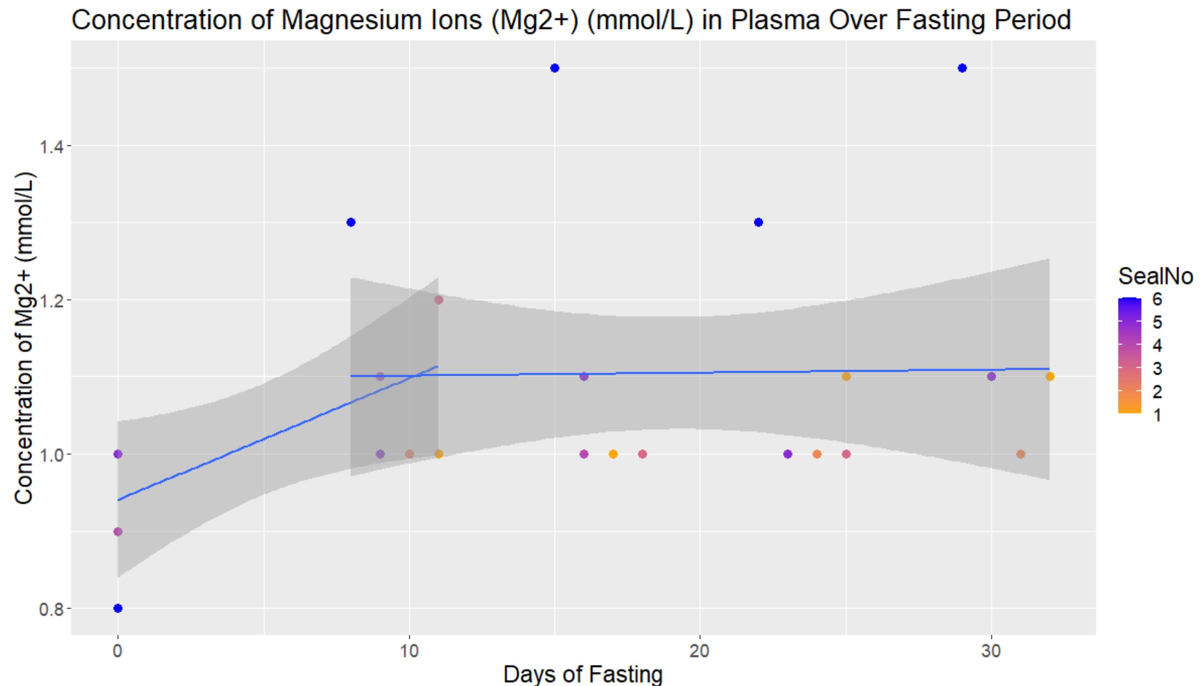

Figure 17: A scatterplot showing the change in plasma magnesium ion concentration (mmol/l) over the days of fasting. Two regression lines are present to demonstrate the change in conditions before and after the first week of sampling.

Plasma osmolality was measured to have a rise of 0.00091±0.00056 osmol/kg per day but this was not truly significant (y = 0.00099x+0.31, p= 0.0879, R^2=0.112, n=27). If the 3 major outliers in the data (figure 18) are excluded from the model then is becomes significant (y = 0.00068x+0.31, p=0.00918, R^2=0.2705, n=24) at a rise of 0.000681±0.00024 osmol/kg per day. Average osmolality at the start of the fasting period was 0.31±0.0056 osmol/kg and was 0.33±0.019 osmol/kg at the end of the fasting period.

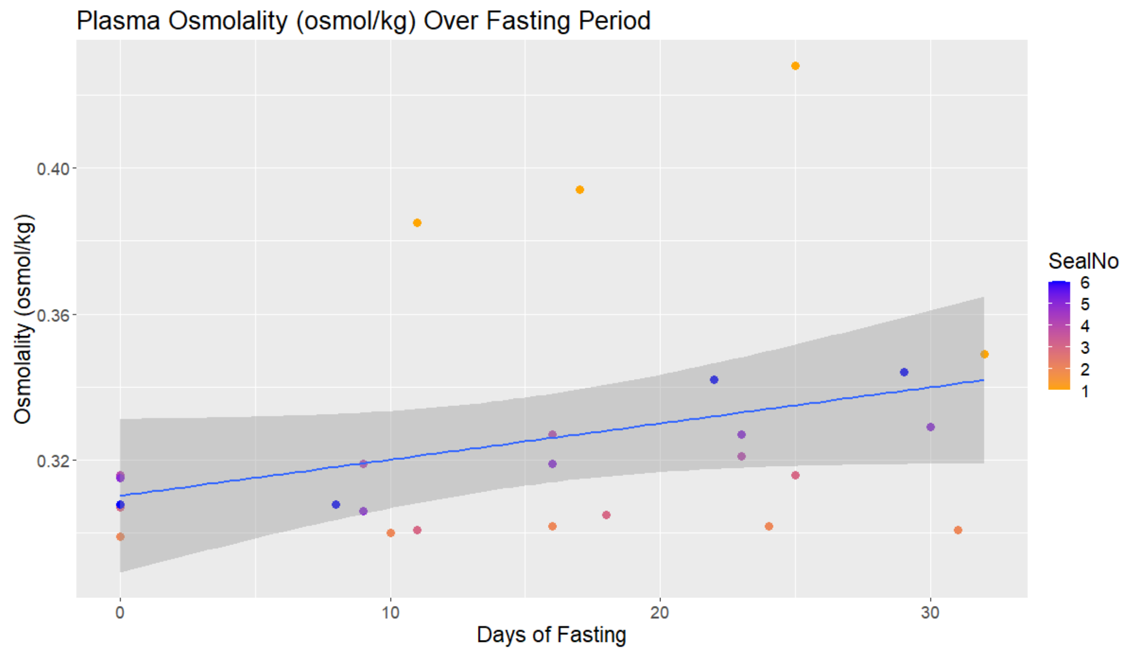

Figure 18: A scatterplot showing the change of osmolality in the plasma (osmol/kg) over the days of fasting.

3.4 Urine

Weight-specific urine Production, although insignificant, appeared to decrease (y = -0.13x+8.0, p=0.233, R^2=0.1385, n=12) over the fasting period at a rate of 0.13±0.099 ml decrease per day of fasting (figure 19). The average total production at the start of fasting was 324±220 ml/day, dropping to 143±45 ml/day at the end of the fasting period.

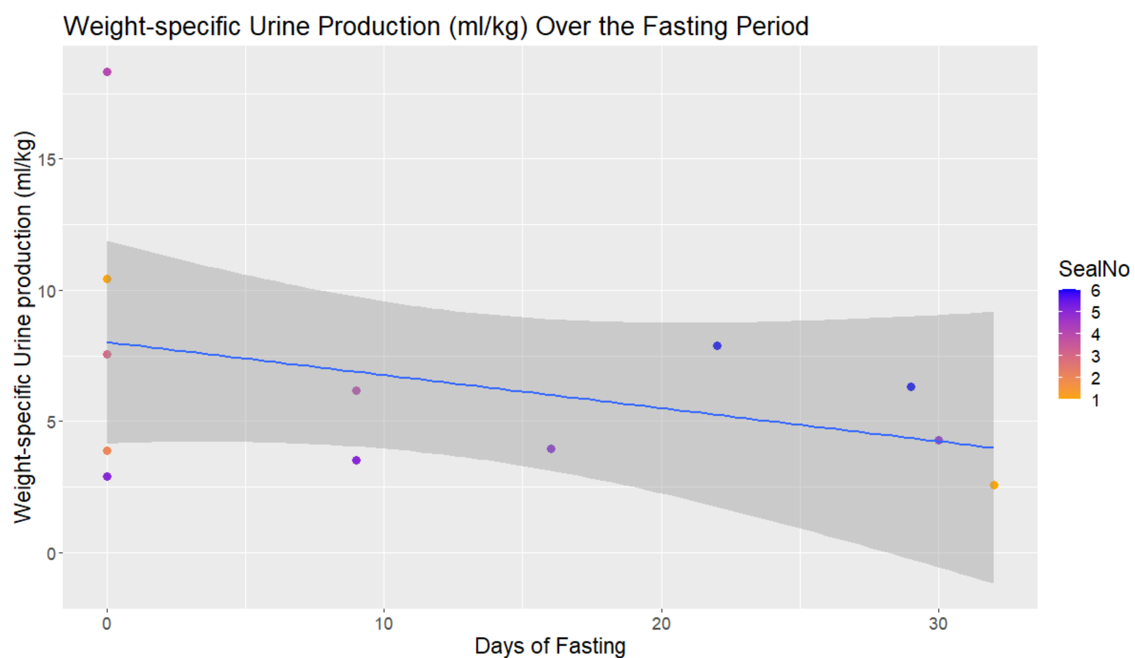

Figure 19: Weight specific urine production (ml/kg) over all seals over the days of fasting.

3.4.1 Urine Nitrogenous Products

Ammonia concentration in Urine showed no significant change over the sampling period ($p=0.885$). Average concentration was 85±33 mmol/l over the fasting period (figure 20). Ammonia on average accounted for 15±5.6% of the total nitrogen excreted.

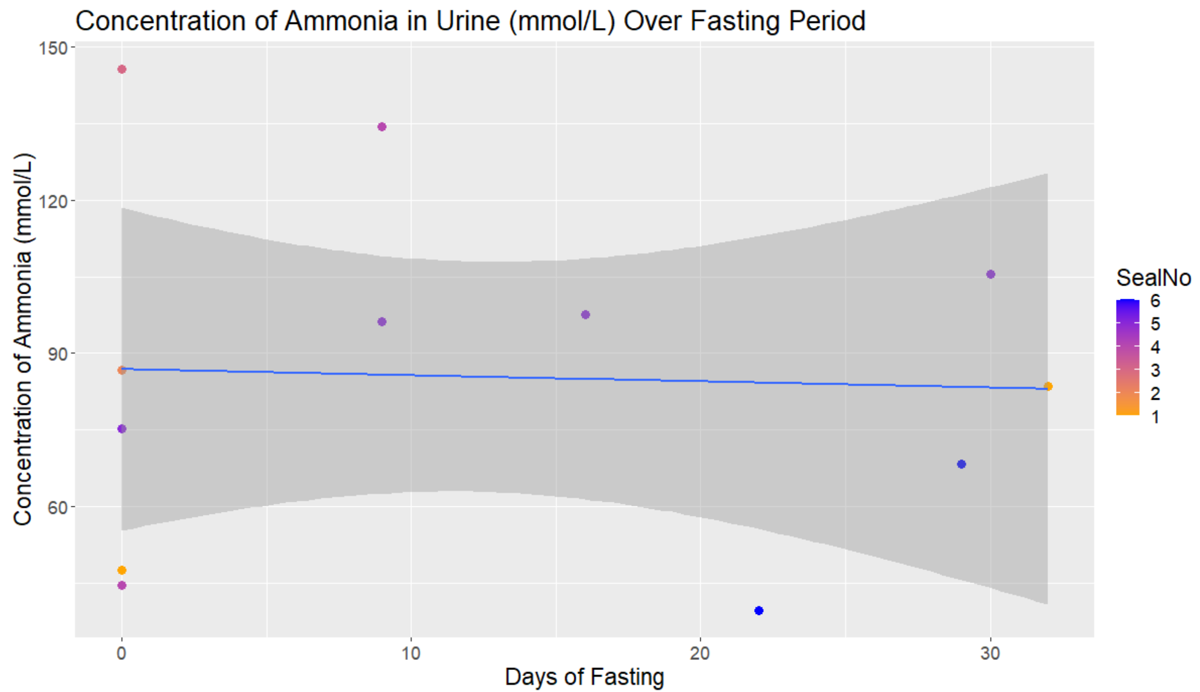

Figure 20: A scatterplot showing the insignificant change to urine ammonia concentration (mmol/l) over the fasting period.

Urea concentrations in the urine are observed to decrease at a rate of -2.2±1.9 mmol/l per day but this was insignificant ($y = -2.2x+263$, $p=0.256$, $R^2=0.1268$, $n=12$). Average concentration at the start of fasting was 269±78 mmol/l and 221±57 mmol/l at the end of fasting (figure 21).

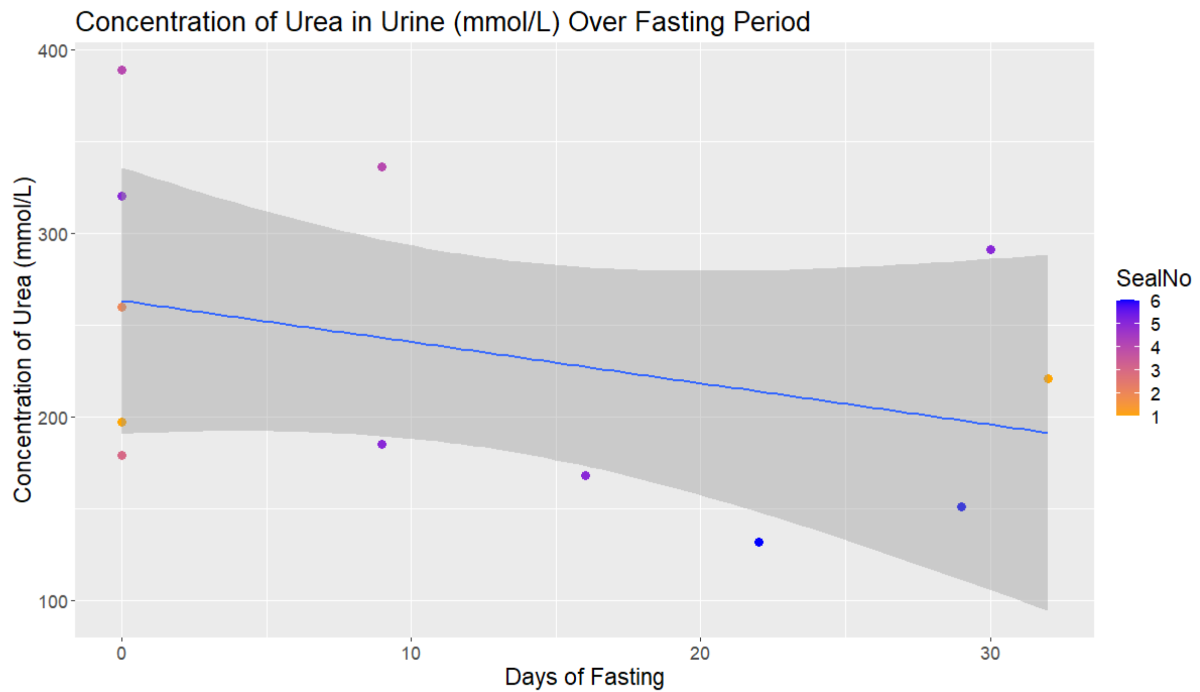

Figure 21: A scatterplot showing the concentration of urea (mmol/l) in urine over the days of fasting.

Uric acid concentrations in the urine were similarly observed to decrease (figure 22) at a rate of -0.0093±0.0067 mmol/l per day however it was also statistically insignificant ($y = -0.0093x + 0.76$, $p=0.194$, $R^2=0.1622$, $n=12$). Initial concentrations at the start of fasting averaged at 0.80±0.32 mmol/l and decreased to 0.52±0.14 mmol/l at the end of fasting.

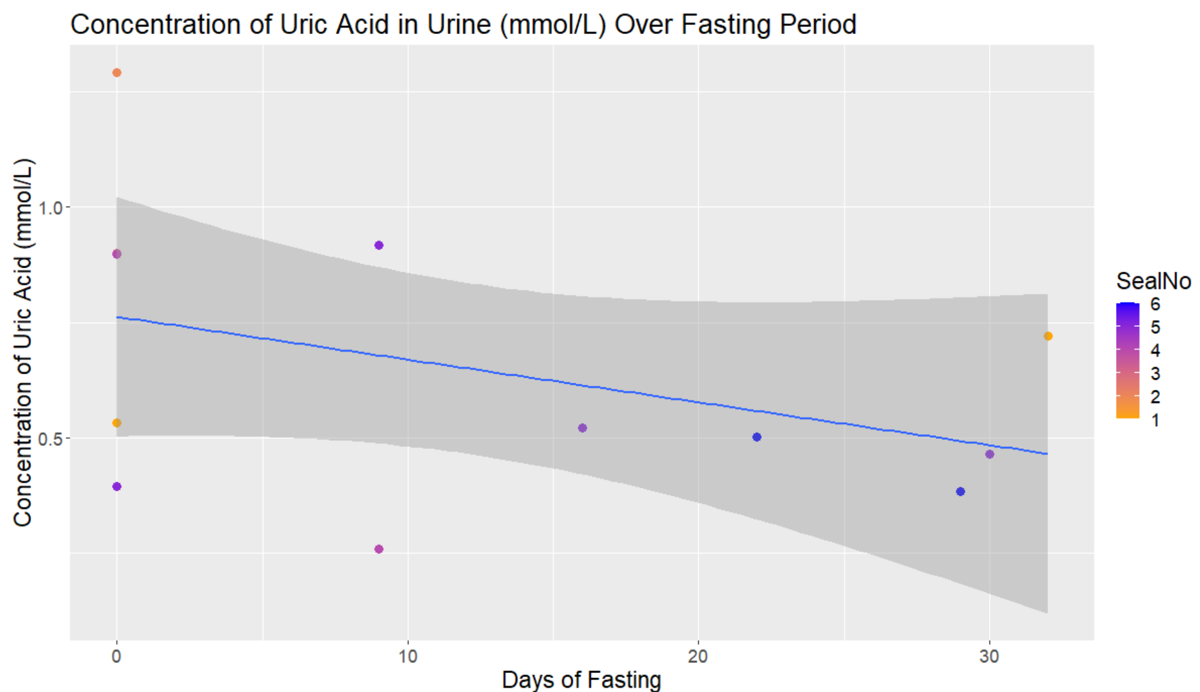

Figure 22: A scatterplot showing the concentration of uric acid (mmol/l) in urine over the days of fasting.

Creatinine concentration in the urine was the only nitrogenous product observed to rise (at a rate of 0.19±0.13 mmol/l per day, figure 23) but was insignificant (y = 0.19x+9.4, p= 0.173, R^2=0.1769, n=12). Starting average concentration was 7.5±4.2 mmol/l before rising to an average of 13±0.61 mmol/l at the end of fasting.

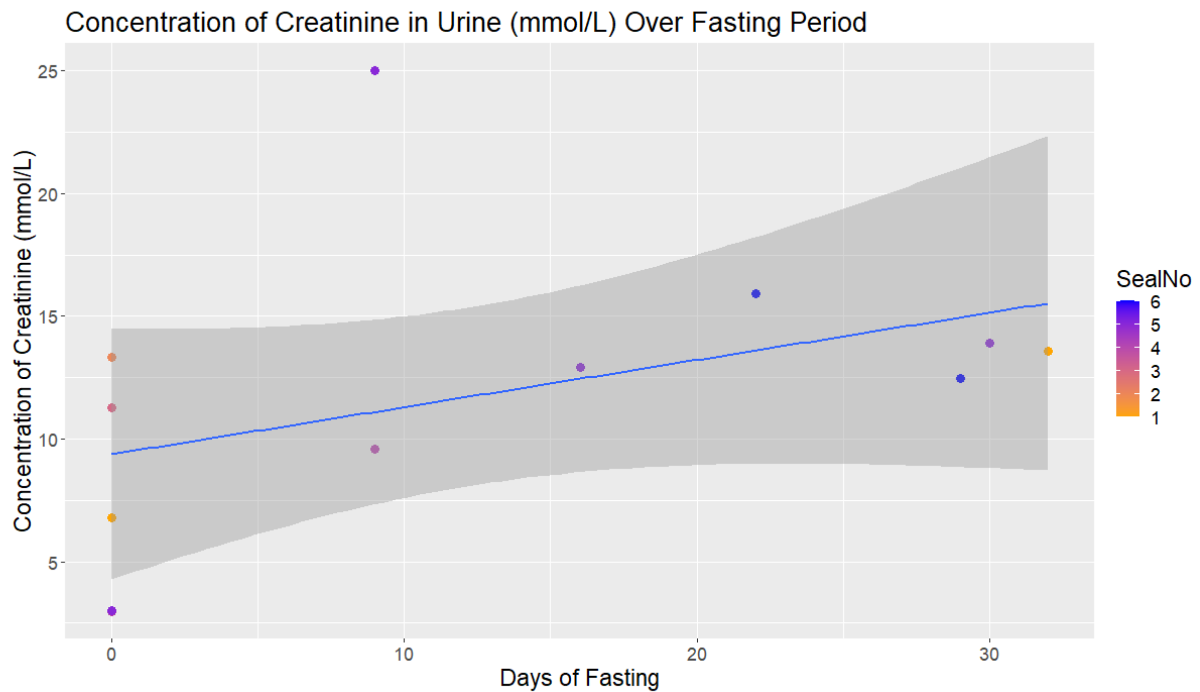

Figure 23: A scatterplot showing the concentration of creatinine (mmol/l) in urine over the days of fasting.

The protein catabolised per unit weight (g/kg) was observed to have a downward trend (-0.0101±0.0073g per day, figure 24) despite being insignificant (y = -0.0101x+0.47, p=0.199, R^2=0.1594, n=12). The average value at the start of fasting was 0.51±0.43g/kg/day and dropped to an average of 0.21±0.061g/kg/day at the end of the fasting period.

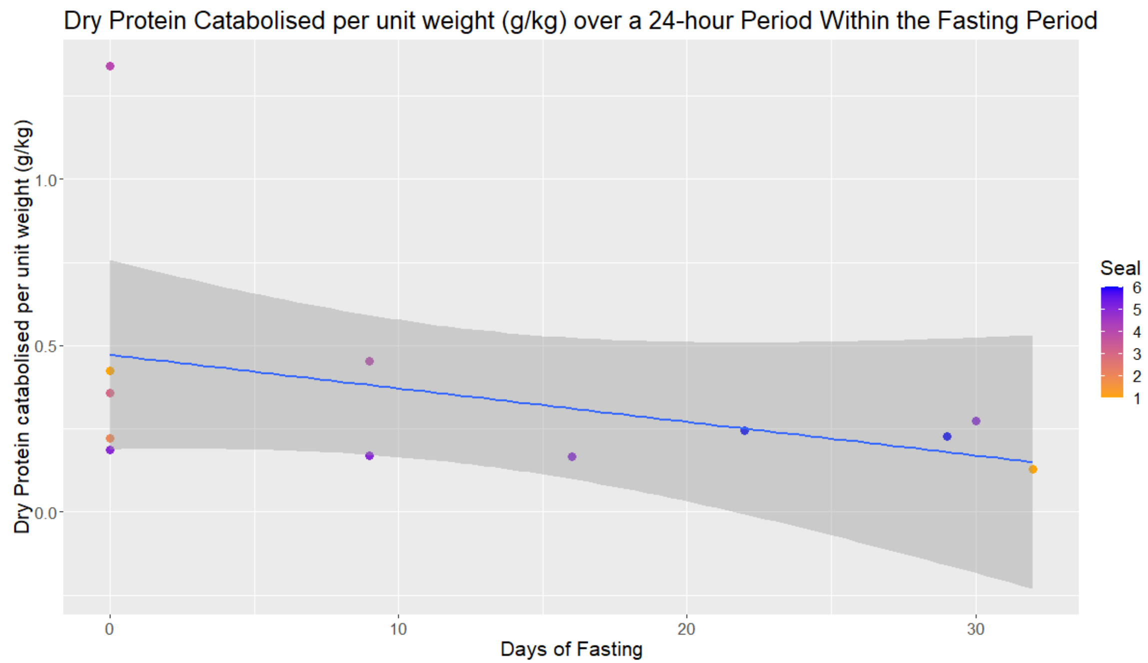

Figure 244: A scatterplot showing the amount of protein catabolised per unit weight over a 24 hour period within the days of fasting.

3.4.2 Urine Electrolytes

The concentration of chloride in the Urine over the fasting period showed a linear increase over time of 4.2±1.6 mmol/l per day (y = 4.2x+54, p=0.0265, R^2=0.4033, n=12). Average concentration at the start of fasting was 51±15 mmol/l and rose to an average of 140±18 mmol/l by the end of fasting (figure 25). The total excreted Cl^- started at a rate of 18±14 mmol/day before increasing to 29±36 mmol/day.

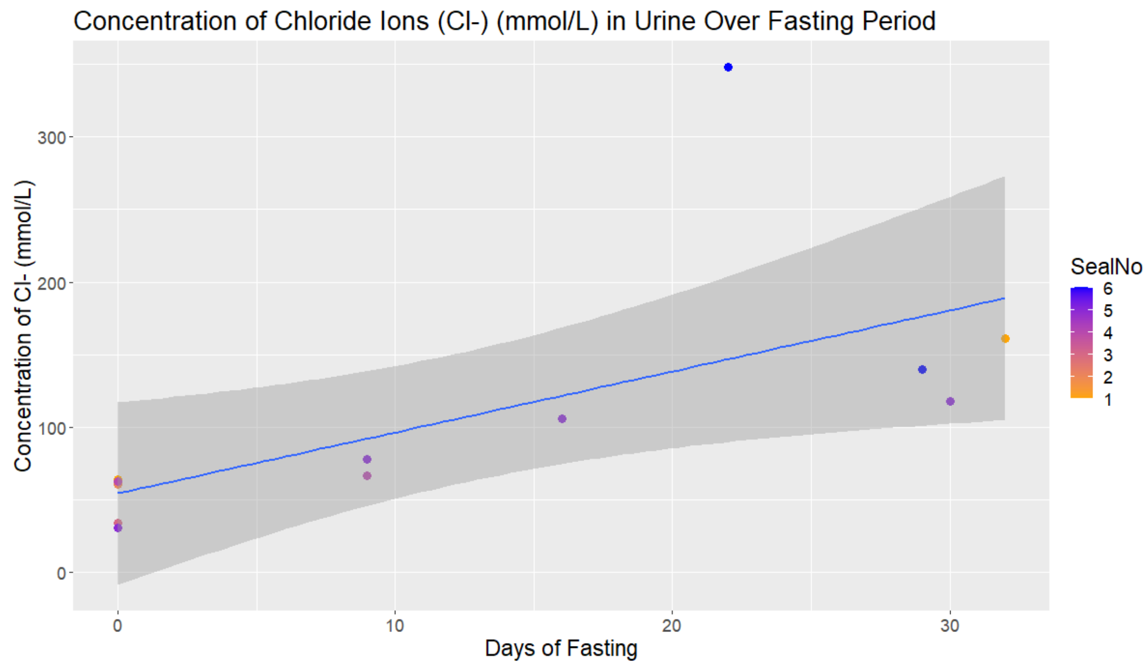

Figure 255: A scatterplot showing the concentration of Chloride ions (mmol/l) in urine over the days of fasting.

Sodium concentration in the urine similarly rose over time at a rate of 2.5±1.2 mmol/l per day but was not significant (y = 2.5x+90, p=0.0714, R^2=0.2891, n=12). Starting average concentration of 87±24 mmol/l at the start of fasting before rising to an average of 136±25 mmol/l at the end (figure 26). Total Na^+ excreted started at a rate of 29±19 mmol/day before dropping slightly to 27±28 mmol/day.

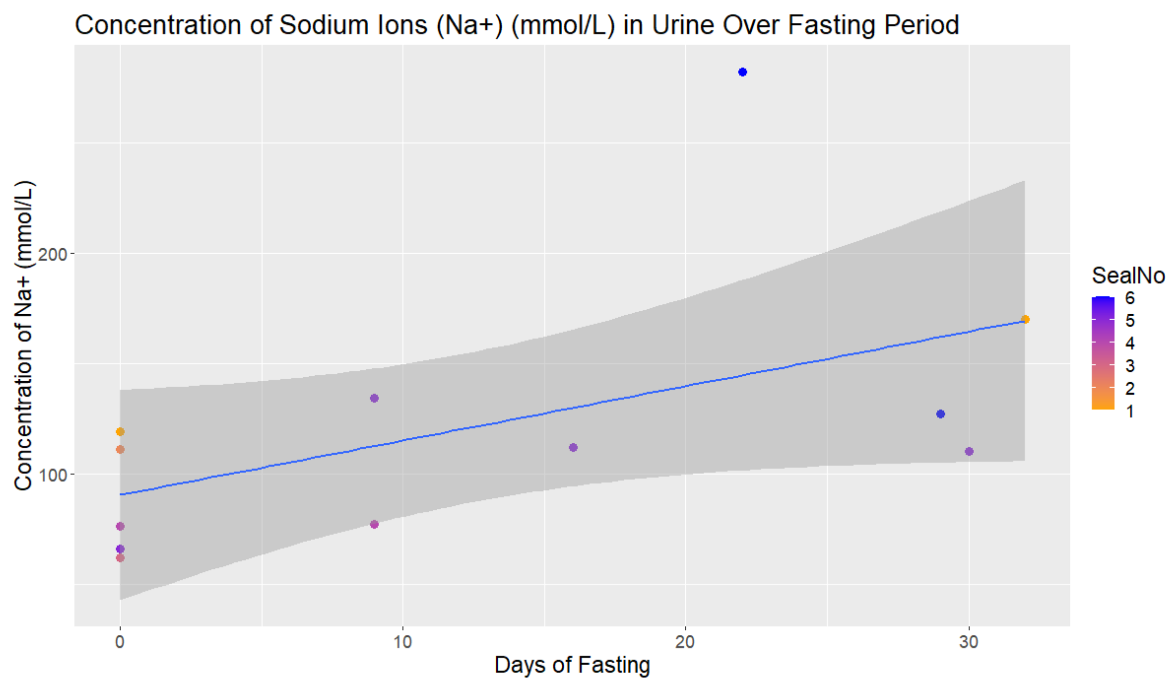

Figure 266: A scatterplot showing the concentration of sodium ions (mmol/l) in urine over the days of fasting.

Magnesium Concentration in the urine over the fasting period rose at a rate of 0.31±0.13 mmol/l per day (y = 0.31x+7.1, p=0.04, R^2=0.3577, n=12). Starting average concentration was 5±2.7 mmol/l before rising to an average of 12±0.21 mmol/l (figure 27). Total excreted Mg^{2+} rose from 1.5±0.84 mmol/day to 2.7±2.3 mmol/day.

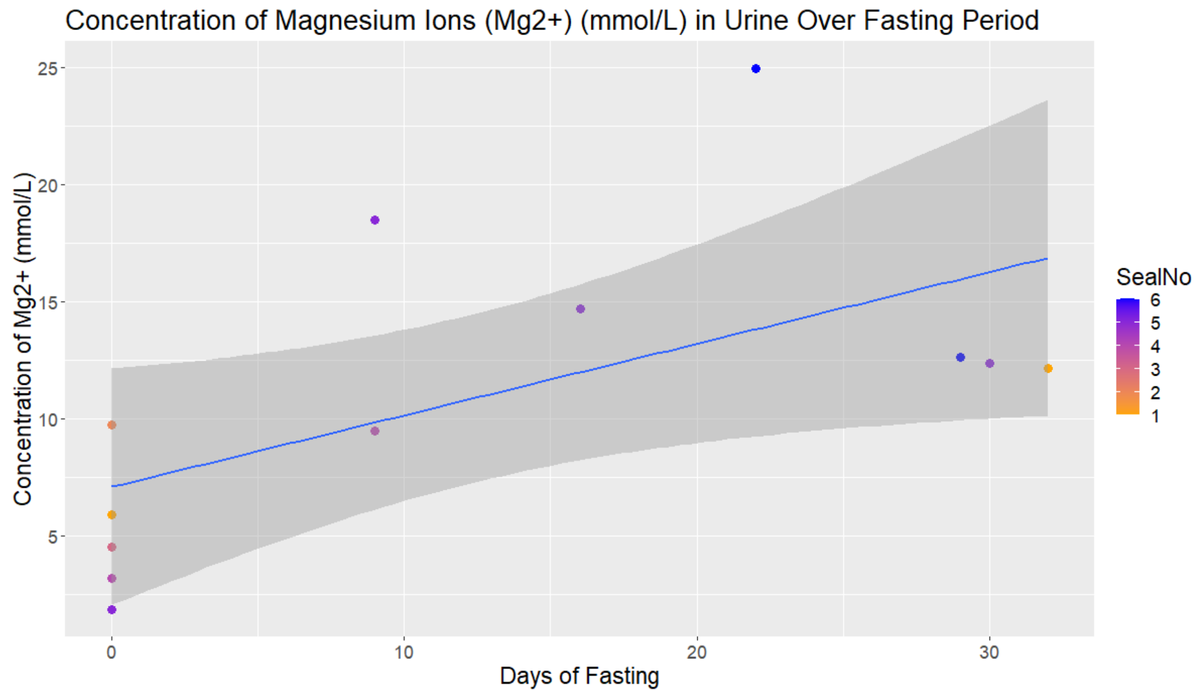

Figure 277: A scatterplot showing the concentration of magnesium ions (mmol/l) in urine over the days of fasting.

The Osmolality of urine over the fasting period showed a linear increase of 0.014±0.0055osmol/kg per day (y = 0.014x+0.83, p=0.0261, R^2=0.405, n=12), from an average of 0.81±0.21osmol/kg up to an average of 1.3±0.13osmol/kg (figure 28).

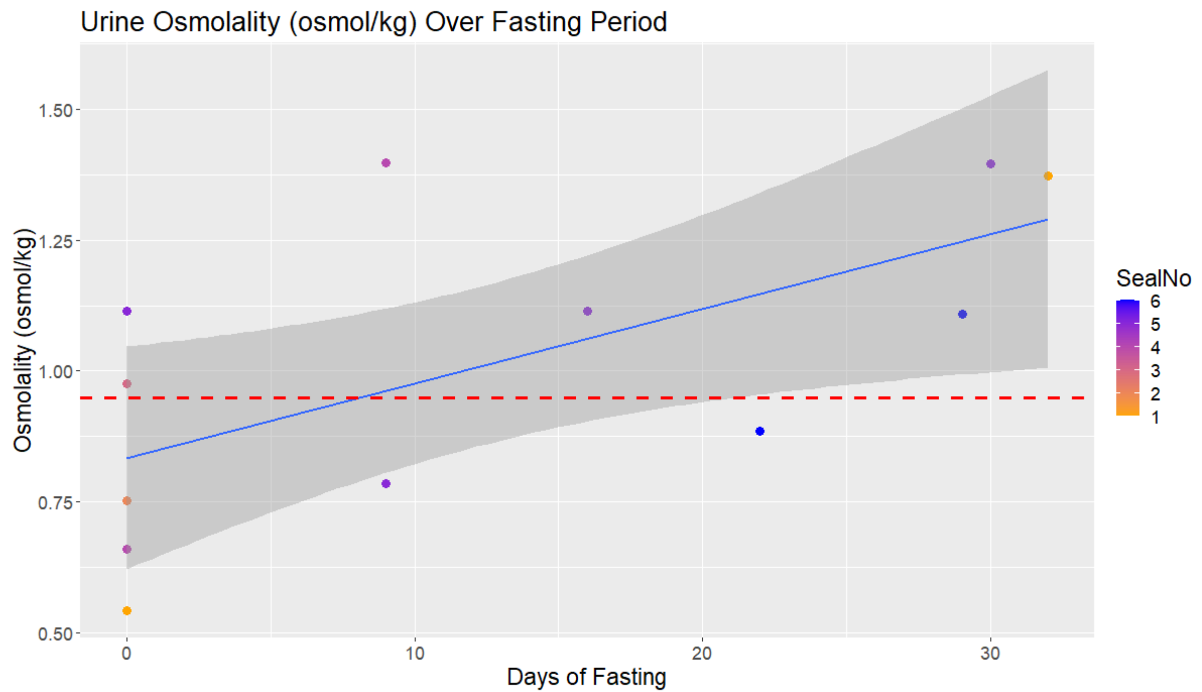

Figure 288: A scatterplot showing the osmolality of urine (osmol/kg) over the days of fasting. The dashed red line represents the average osmolality value for seawater in the tanks (Verlo, 2012).

3.5 Metabolic Rate

The weight-specific sleeping metabolic rate (SMR) was measured to be insignificant in its change over the fasting period ($p=0.43$) (figure 29), averaging at 2.4±0.54W/kg. A 2-sample t-test showed an insignificant difference between the first sampling period and those that come after ($p=0.701$).

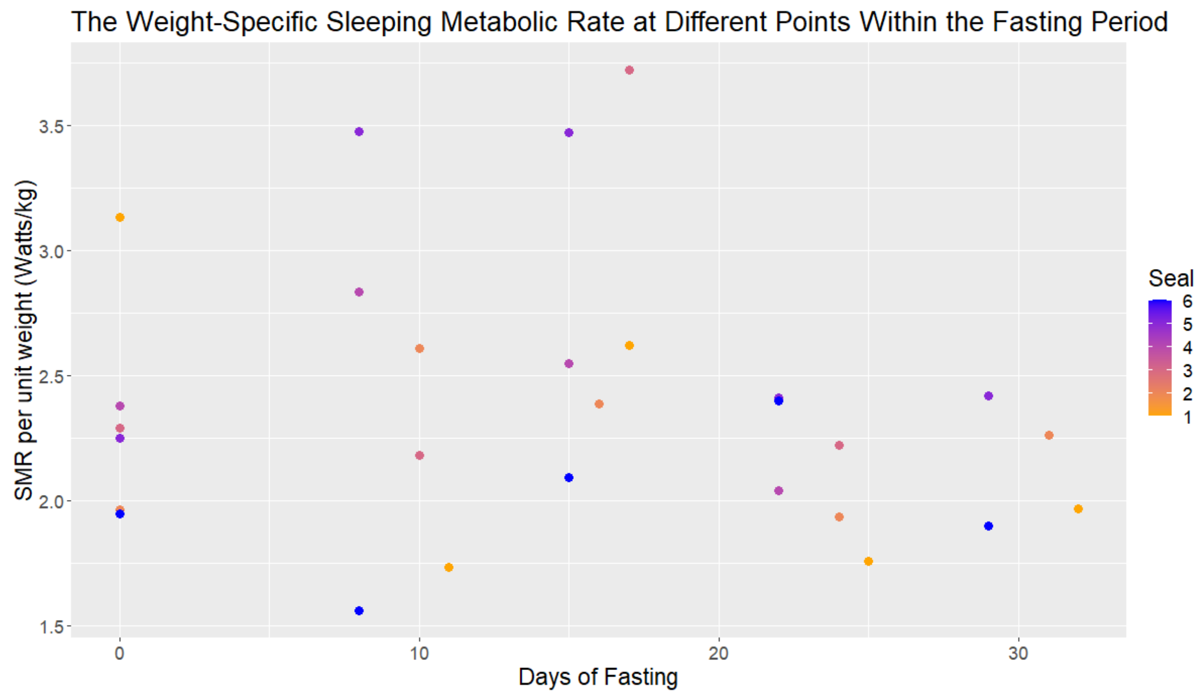

Figure 29: The weight-specific SMR (Watts/kg) of the fasting seal pups over the fasting period.

No change was measured in the respiratory quotient (RQ) over the fasting period ($p=0.69$) (figure 30).

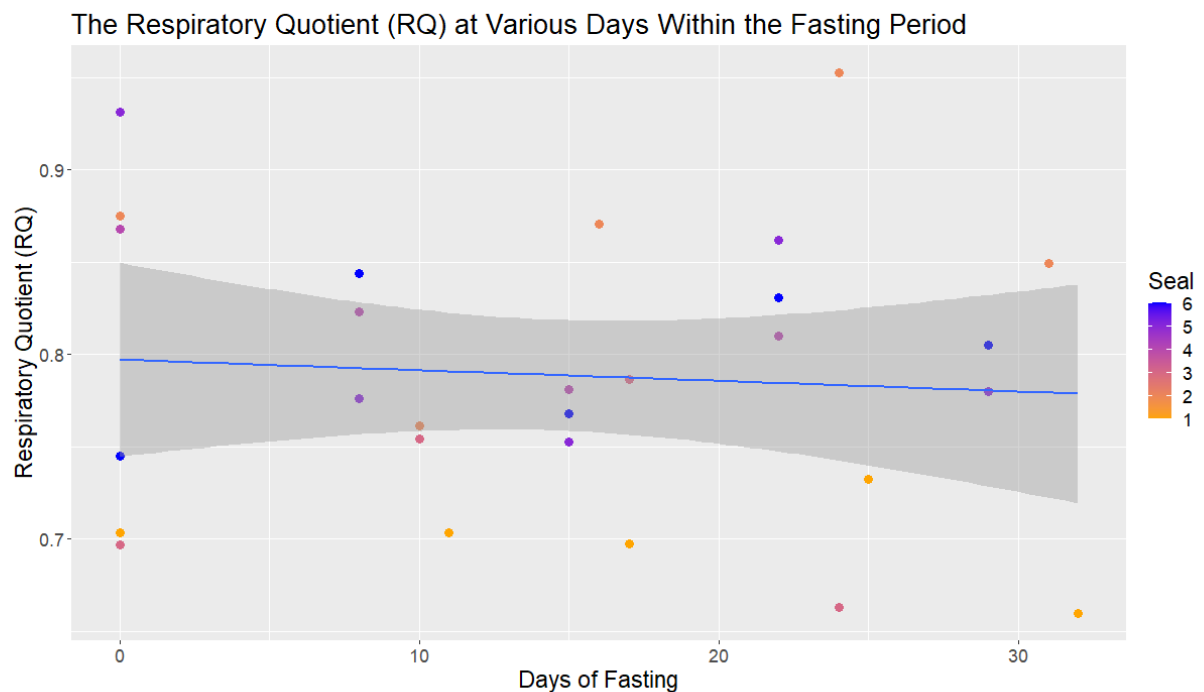

Figure 290: The respiratory quotient (RQ) of the fasting seal pups over the fasting period..

The average percentage of SMR accounted for by protein metabolism was calculated as 3.4±3.1% with no significant change over the fasting period (figure 31) ($y = -0.87x+4.4$,

p=0.259, R^2=0.1255, n=12) through an observed decrease of -0.087±0.072 %/day was observed (dropping from an average of 4.9±4.1% to 2.7±0.56%).

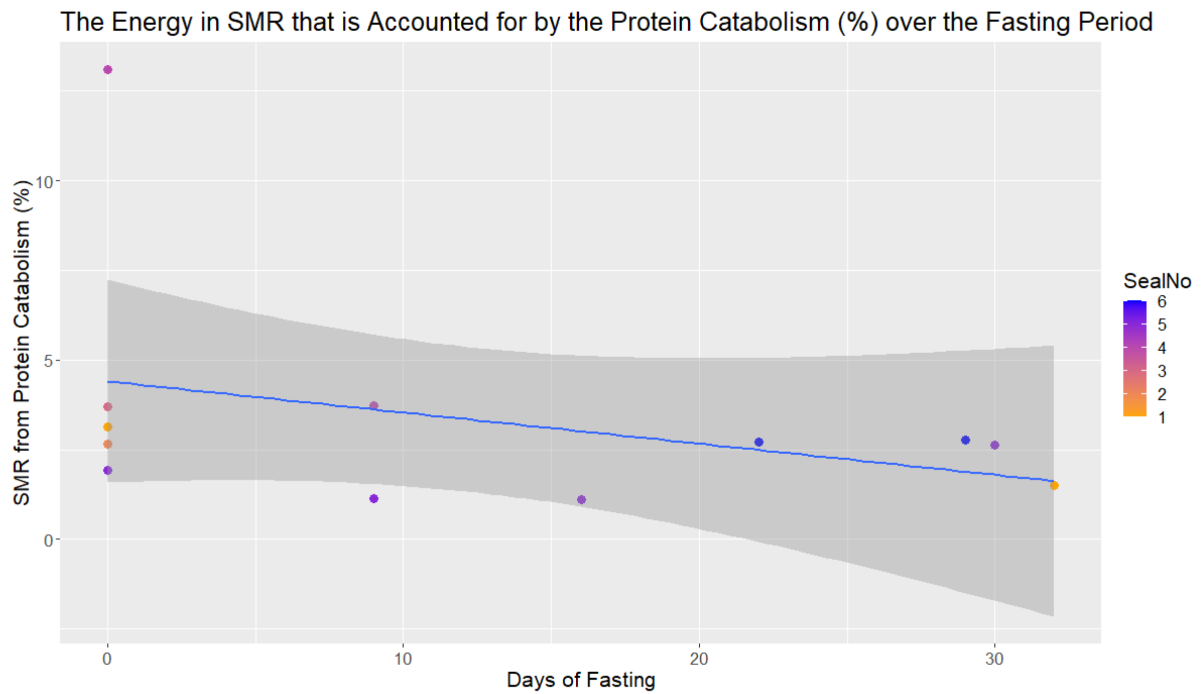

Figure 301: The proportion of SMR accounted for by protein catabolism (%) of the fasting seal pups over the fasting period.

4 Discussion

4.1 Protein Catabolism

Although protein metabolism was observed to drop, it was statistically insignificant, suggesting that there was no change in the percentage of SMR that was accounted for by protein metabolism ($p=0.259$, figure 31). This means I must reject my primary hypothesis that hooded seals would use a higher proportion of protein during as an energy source, when compared with seals found at lower latitudes. the average proportion of SMR that is accounted for by protein catabolism is lower than expected at 3.4±3.1%. This is vastly different to that which was estimated by Schots et al. (2017), where it was measured to be 15±8.1% at the start of the fast before dropping to just 10±10.3% at the end. But these results with the large standard deviations suggest that this was not completely consistent. Alongside this, Schots et al. (2017) estimated the protein catabolism from the total body protein loss via a tritiated water method, contrary with this study, where protein catabolism was estimated based on nitrogen excretion. Although this method based on nitrogen excretion has been documented to have its drawbacks (Mariotti et al., 2008), the value is most commonly expected to overestimate protein concentration, which would suggest that the error in my method is not causing an underestimation.

There is a postweaning fast present in many pinniped species, thus the energy sources during a pinniped post-weaning fast have been investigated previously. Northern Elephant seals (*Mirounga angustirostris*), despite being often distributed in subarctic conditions, breed in warmer conditions further south in California and Mexico (Lowry et al., 2014). The protein catabolism in northern elephant seal pups have been found to contribute less than 4% of total energy expenditure both during and after the lactation period (Houser & Costa, 2001). Grey seals breed in subarctic or temperate regions, so therefore are exposed to less thermal stress than hooded seals. This may suggest that grey seals would be expected to burn a higher proportion of fat for energy, given that it is less required for thermal homeostasis. They have been found to have about 6% of their metabolic expenditure accounted for by protein catabolism (Nordøy et al., 1990), compared to 94% of energy derived from fat oxidation (Nordøy & Blix, 1985). Harp seals are an Arctic species that breed in the Arctic (frequently in the same places as hooded seals). They have been demonstrated to initially (at the start of fasting) have 9% of energy expenditure provided by protein catabolism, before dropping down to less than 4% after the third day of fasting (Nordøy et al., 1993). From these species

that are born at different latitudes, a trend can be seen, where the further north a seal pup is born, the greater its protein catabolism contribution to energy expenditure. From this trend, it was expected that hooded seal pups would follow a similar trend with a higher protein catabolism proportion of energy expenditure, as was previously estimated by Schots et al. (2017), but this was not the case. Notably, the highest value observed was in the first week with seal 4 (K9b-21) with a value of 13.1% of SMR accounted for by protein metabolism. This, however, is an outlier even among the other samples obtained that week, with the average for the other four samples being just 2.9±0.66%.

The explanation for this discrepancy that hooded seals represent in this trend is not clear however some suggestions can be made. Harp seal pups are born with almost no subcutaneous blubber (Blix et al., 1979), while also initially have a significantly higher proportion (9%) of their energy expenditure supplied by protein catabolism (Nordøy et al., 1993). Hooded seals however are already born with blubber stores greater than most other species of seals, meaning they do not even require lanugo fur after birth. These significant stores already present mean that energy stores are already large, and they are ensured to gain much more during lactation. Previous experimentation on rats has shown that obese rats (therefore with greater fat stores) showed diminished protein catabolism during fasting when compared with normal control rats (Goodman et al., 1980), demonstrating the ability of animals with larger fat stores being able to conserve protein. Other Arctic seals such as harp seals, born with little to no blubber, must begin depositing energy stores only after birth, missing out on the “head start” that hooded seals have.

Hypotheses regarding the order that tissues would be utilised by seals in a fasting/starvation period has been put forward by Brodie & Pasche (1980). The first source would be the utilisation of fat nodules that can be found around internal organs. Secondly would be the use of the insulative blubber that would be utilised until it reaches a thickness of which any lower would be detrimental to its thermal balance. Thirdly would be use of the protein sources in muscles, similarly down to a critical mass below which it would not be able to successfully remain a predator. The final source would be to utilise the last of the blubber layer, prioritising the functionality of the muscles over the thermal importance of the blubber (Brodie & Pasche, 1980). If this sequence is followed by the hooded seal pups in their post-weaning fast, it would suggest that they never went beyond the second source of energy, due to the low protein catabolism observed over the entire fasting period.

Shortly after lactation the Arctic seals pups will begin swimming between ice floes before they begin leaving for the feeding grounds. For this purpose, the lanugo fur present on harp seal pups will lose much of its insulating properties, reducing its effectiveness by 84-92% upon entering the water (Kvadsheim & Aarseth, 2002). Hooded seals on the other hand, are not relying on fur with a blubber layer already present. This may go some way to explain the lower proportion of protein catabolism in hooded seal pups when compared with harp seal pups. However, the thermoregulatory challenges faced by Arctic seal pups is a big aspect for the reasoning for why they would be expected to catabolise a higher proportion of protein. It has previously been theorised that the higher catabolism of proteins observed in Arctic seal pups is to minimise the loss of blubber (Nordøy et al., 1993), which serves a vital role in maintain thermal homeostasis.

I would also like to acknowledge that although the metabolic readings were all made during resting/sleeping, thus giving the SMR, the nitrogenous products produced in the urine will include the proteins that were broken down during regular behaviour in the pools. This means that the proportion of SMR that is accounted for by protein will be calculated as higher than if related to the presumably higher true metabolic rate.

Despite being insignificant, the amount of protein metabolised per unit weight (g/kg) was observed to slightl drop over time from 0.51 g/kg/day to 0.21 g/kg/day. This is similar to the proportion of SMR accounted for by proteiyn catabolism which dropped from an average of 4.9% to 2.7% by the end of the fasting period (figure 31). Even with this drop however, the protein catabolism rates are much lower than expected.

The plasma ratio of Urea:Creatinine (mmol/l:mmol/l) was calculated to average at 180, with a slight increase. This is far higher than observed in other fasting species such as black bears (Nelson et al., 1984), polar bears (Derocher et al., 1990), and even other fasting seal pups (Lester & Costa, 2006). The slightly rising ratio would suggest an increase in protein catabolism however that is not what is observed here; instead, this seems to be a consequence of increasing plasma urea concentration, while the creatinine remains stable.

4.1.1 Urine Excretory Products

Given that urine solutes are observed to change over the fasting period, but are consistently statistically insignificant, it seems there may be insignificance due to a lack of statistical power. A smaller number of urine samples obtained than intended resulted in a lack of

statistical power. With only six seals present it was important that urine samples were obtained each week however this was not the case due to lack of urination. Less than half of sampling dates resulted in a urine sample being obtained, especially after the shift to a saltwater exogenous water source. It was believed, from previous research with harp seal pups (Nordøy et al., 1993), that the seals would not be able to go 24 hours without urinating and would thus always provide a sample. A solution to this issue may be through use of a catheter to ensure that any urine in the bladder will be passed through such as those that have been used in adult harp seals (Storeheier & Nordøy, 2001). But this is not an ideal solution due to difficulties in placing a catheter in a seal, being very difficult in females and almost impossible in males (Erling S. Nordøy, personal communication). A urine catheter would also be expected to cause discomfort and stress in the animal, impacting animal welfare. Significantly more time in the box may also not be an ideal solution given that this study investigated the water balance of these pups and evidence suggests that access to seawater may be important for maintaining water homeostasis (Schots et al., 2017). Still, a short increase of time (such as just one extra day) may better ensure sample collection.

Ammonia within the urine samples showed no clear change over the fasting period, appearing to be unaffected by time as a variable; this is very clearly reflected by its p-value of 0.885 (figure 19) which is somewhat expected given that it is not the main nitrogenous product used for excretion by mammals as a whole. The ammonia concentration in this study averaged at 85±33 mmol/l where it accounted for an average of 15±5.6% of the total nitrogen excretion. Ammonia being used as an end product for fasting animals is well documented, initially in humans where it was documented to account for up to 42% of the nitrogenous products (Owen et al., 1969). This is believed to be an adaptation to titrate the loss of ketone bodies in the urine (Owen et al., 1969). Ketone bodies are molecules produced from the oxidation of fatty acids, which can serve as a fuel for the body during a state of starvation (Kolb et al., 2021; Robinson & Williamson, 1980), alongside serving as a signal to regulate the breakdown of proteins (Finn & Dice, 2006). Ketone bodies have been detected to massively increase in concentration during the grey seal pup fast (Nordøy & Blix, 1991), with evidence suggesting that ketone bodies may partly replace glucose as a vital energy source.

High urine ammonia concentration has similarly been documented in other fasting seal pups such as elephant seals where the ammonia contributed to 6.4-8.5% of total nitrogen excreted

(Adams & Costa, 1993), and the grey seal, where ammonia contributed to 20% of total nitrogen excreted (Nordøy et al., 1990). Due to the values in fasting seal pups being lower than fasting humans, it may be expected that, given these pups are adapted for this fast, that they have better physiological mechanisms in place to ensure that ammonia urine concentrations remain low.

Urea is the primary product of excretion for pinnipeds (Smith, 1936) which makes it a more important product to analyse; from my analysis, the urea contributed an average of 78.1±8% of the total nitrogen excreted (ranging from 66% to 93%). This is very comparable to results found in similar species such as fasting grey seals pups, with the urea contributing to between 70-80% of the nitrogen excreted over the fasting period (Nordøy et al., 1990). The urine urea concentration appeared to show a downward trend after starting at an average of 269 mmol/l before dropping down to an average of 221 mmol/l (figure 21) over the fasting period, likely due to the observed slight reduction in protein catabolised.

4.2 Metabolic Rate

Despite there being a significant drop of metabolic rate over the fasting period (-0.875 Watts/day, figure 29) this significance was lost after conversion to weight-specific metabolic rate. This would imply that although the metabolic rate does drop over the fasting period, it is not out of line with what would be expected with its weight loss. This would initially suggest that there is no post weaning metabolic depression. Grey seals have been demonstrated to have a drop in weight-specific basal metabolic rate during fasting, from an initial value of 2.95 W/kg down to 1.62 W/kg after 10 days of fasting (Nordøy et al., 1990). In harp seals, there is evidence suggesting the presence of a post-weaning metabolic depression, but it has not been measured (Nordøy et al., 1993). Here in this study the average weight-specific SMR was calculated as 2.4±0.54 W/kg and the weight specific RMR found by Bue (2015) averaged at 2.5±0.4 W/kg. These are very similar values where the small difference could be accounted for by the seals not sleeping in Bue's study. Repeated studies on this matter gives greater credit to the hypothesis that a post-weaning fast metabolic depression is not occuring in hooded seal pups.

It is possible that the greatest shift that occurred in the weight-specific metabolic rate was in the short time period after the end of lactation, as suggested by Nordøy et al. (1990). This could be caused by the initial metabolic rates after weaning still reflecting the metabolic rates

during lactation before their stomach is emptied. This, however, is further unsupported by the t-test that demonstrated there was no significant shift in values from the first sampling period to the next (p=0.806). Still, to further improve clarity on this point, it may be worth to complete daily measurements of indirect calorimetry during the first few days of the fast. Worthy & Lavigne (1987) had previously come to the conclusion that there was no post-weaning metabolic depression in grey seal pups, after beginning their measurements some days after capture. Three years later, Nordøy et al. (1990) instead found there was a significant decrease in BMR during the first few days of the fast; this demonstrates the importance in ensuring that measurements begin right after weaning, during the first crucial days of fasting.

Problems were faced in the analysis of the indirect calorimetry. Using the equipment, oxygen was expected to remain constant when measuring the reference (environmental) air but an inherent drift (due to instability of the oxygen analyser unit of the Foxbox) was observed in the readings. To solve this, the drift was assumed to be linear between the reference points during readings (figure 9). This allows for a new calculation of the oxygen content (in voltage) of the reference air; however, this may introduce error if the drift observed was not truly linear. The results for the respiratory quotient (RQ) from the metabolic measurements were different from previous metabolic measurements of hooded seal pups (Evertsen, 2021). The Weir equation was instead used to estimate energy expenditure while not using the RQ (Weir, 1949). This equation has been previously validated in humans (Mehta et al., 2015), but not seals, possibly meaning it is not the ideal equation for the study.

My data collected here does not support the hypothesis that there is a post-weaning metabolic depression in hooded seals, leading me to keep the null hypothesis that there is no post-weaning metabolic depression. This may be refuted by metabolic measurements taken more frequently during the first few days after capture, however previous study has similarly suggested that hooded seals do not undergo a post-weaning metabolic depression (Bue, 2015). If it is the case that a metabolic depression is not present, it may be because it is unnecessary. The larger energy stores present in hooded seal pups (Guerrero, 2018) compared to grey seals (Nordøy & Blix, 1985) or harp seals (Worthy & Lavigne, 1983) may mean that it is not necessary to undergo a metabolic depression.

4.3 Changes to Blood Chemistry

The only haematocrit change detected from the collected blood samples was found between the first (after capture) and second (after introduction to the pool) samplings with a significant increase of over 5% (from an average of 55% to 60.9%). This seems to reflect the change in exogenous water source from freshwater (as snow) to seawater. The rise in haematocrit observed in the present study may also reflect an adaptation to their subsequent diving behaviour that will occur soon after weaning. Study in northern elephant seal pups has suggested that the haematocrit rises from weaning up until their first foraging trip at sea (Thorson & le Boeuf, 1994). After the haematocrit has risen, it then shows no significant changes but can be observed to slightly decrease. This may support the previously demonstrated ability of hooded seal pups to drink seawater; if the pups were to become dehydrated, then it would be expected that their haematocrit would be observed to rise (Costill & Sparks, 1973). The haematocrit has been demonstrated in Northern Elephant seal pups to not be associated strongly with thyroid hormones or erythropoietin (EPO) (Somo et al., 2015) and thus is likely to be controlled by other regulatory factors. Whether the increase in haematocrit observed here is a result of a stimulated synthesis of red blood cells or changes in water concentration of the blood is unclear. Existing data on hooded seal haematocrit (Burns et al., 2007) has shown that hooded seal pups, despite having significantly lower total body oxygen stores (TBO_2), have haematocrit and haemoglobin concentrations similar to that of adults, likely reflecting their precocial nature.

Haematocrit is a measurement that is affected by many factors, so consistency was important in these measurements. The spleen is an organ that serves as a storage of red blood cells which can be released during dives made by hooded seal to temporarily increase oxygen stores (Cabanac et al., 1999). It is well understood that stress from capture or handling can result in the contraction of the spleen in many animals (Schalm, 1965); this coupled with the large size of the spleen in pinnipeds (Castellini & Castellini, 1993; Ponganis et al., 1992) can result in a significant change to the haematocrit measurements due to handling stress. The effects of this stress during handling are expected to be somewhat mitigated, given that the handling/sampling methodology remained consistent over the experiment.

The osmolality of the plasma was observed to increase over the fasting period, from an initial average of 0.31 osmol/kg to 0.33 osmol/kg. This is not a large increase but was significant ($p=0.00918$). Causing this increase in osmolality is in part due to the increases of the

concentrations of chloride and magnesium ions (increases of +5 mmol/l and +0.27 mmol/l respectively). Increased osmolality of plasma would be expected to be caused by an influx of seawater, in these seals that have previously been demonstrated to drink seawater (Schots et al., 2017).

Magnesium concentration increasing from 0.93±0.075 mmol/l to 1.2±0.19 mmol/l shows an increase of 29% after ingesting seawater. This value is similar to observed in harp seals by How & Nordøy (2007), where a 26% increase in plasma Mg^{2+} concentration was detected after 1 litre of orally administrated seawater was given to the seals. However in this study, this rise in Mg^{2+} could also be a consequence of the cessation of fresh water consumption, resulting in less total body water and thus higher concentration of magnesium. To distinguish these two possibilities, a future experiment where both seawater and freshwater are deprived for a number of days, to investigate what effect a lack of water source would have on the plasma and urine Mg^{2+} concentration. Regardless, this early increase in concentration followed by stability (figure 17) would suggest that water homeostasis is maintained after an inital acclimation to the saline conditions, possibly due to the ability of these seals to undergo mariposa (voluntary seawater drinking).

4.4 Changes to Urine Production and Chemistry

Firstly, as previously stated, it seems that there is a lack of statistical power when running linear models based on the urine data, due to a small urine sample size. Despite the insignificance, there is an observed reduction in weight specific urine production as has been observed in almost all prior studies (Adams & Costa, 1993; Nordøy et al., 1990, 1993). It has also been shown in hooded seals that the production of urine decreases upon switching from a freshwater to saltwater exogenous water source (Skalstad & Nordøy, 2000). Skalstad & Nordøy (2000) found that in subadult hooded seals, upon switching from freshwater to saltwater, urine production dropped from 365 ± 62 ml/day to 197 ± 18 ml/day; this is similar to that observed in my study, with a drop from 324 ± 98 ml/day to 140 ± 30 ml/day just days after entering seawater. This lower urine production observed in my seals compared to Skalstad & Nordøy (2000) is easily explained by the fact that the seals in their experiment were older and thus larger. Therefore, the results seen here are as expected, however, it is just lacking significance due to the limited sample number.

This reduction of urine production is a critical adaptation for many marine mammals that allows for maintenance of water homeostasis. This mechanism will reduce the unnecessary loss of water in a cold saline environment which works in tandem with other water homeostasis mechanisms such as nasal heat exchange (Folkow & Blix, 1987; Skog & Folkow, 1994) and resting apnoeic breathing patterns (Lester & Costa, 2006).

Both the chloride and sodium ion concentration in the urine were observed to increase over time at a rate of 4.20 mmol/l per day (from 51 to 140 mmol/l) and 2.47 mmol/l per day (from 87 to 136 mmol/l) respectively however the latter was just insignificant ($p=0.0714$). These are quite low values of ion concentration when compared with values recorded in the harp seal, provided with ad lib access to seawater (How & Nordøy, 2007). The insignificance in the sodium concentration increase may be a result of the lack of statistical power previously mentioned so further understanding may benefit from a study that has a greater number of urine sample available over the sampling period. The magnesium concentration in the urine was also detected to rise but at a lower rate than the chloride and sodium, from 5 mmol/l to 12 mmol/l. Drinking seawater will increase the influx of magnesium ions and result in increased excretion of Mg^{2+} (Skalstad & Nordøy, 2000). The rise in Mg^{2+} concentration in the urine may be what allows the Mg^{2+} concentration in the plasma to stabilise after its initial rise.

The total excretion of ions in the urine may better represent the changes in electrolyte influx and clearance (in terms of mmol/day) but this was not clear. No significance was found in these changes due to large variance but the Cl^- and Mg^{2+} appeared to be excreted at a higher rate upon entrance of seawater. The Na^+ excretion rates remained quite stable, only changing from 28 to 27 mmol/day, possibly by the way of increased reabsorption. Previous study on harp seals has suggested that aldosterone in fasting Arctic seals is increased significantly (10-fold) (Nordøy et al., 1993), which stimulates Na^+ reabsorption. This reabsorption of Na^+ would work to increase the reabsorption of water, possibly being the main way that hooded seals are able to reduce their urinary loss. This could be reflected by a low Na^+ urinary excretion rate as is observed in this study.

The urine osmolality, starting off at 0.81±0.21 osmol/kg, was initially lower than in seawater. Producing urine osmolality higher than seawater at this point would have been unnecessary given that the main source of exogenous water was in the form of freshwater snow. After the

shift to a seawater source, the osmolality of the urine rose to 1.3±0.13 osmol/kg by the end of fasting, higher than the osmolality of the seawater itself. This suggests that the pups in this study were also drinking seawater as has been previously demonstrated in hooded seal pups.

5 Conclusion

In conclusion the protein catabolism accounted for only a small proportion of sleeping metabolic rate, at 3.4%. This is lower than previously estimated in hooded seals pups and harp seal pups but closer to estimated values in more southernly breeding species. In this study no metabolic depression was detected during the hooded seal post-weaning fast, with measurements remaining stable at an average of 2.4 W/kg, which is equivalent to 1.7 times the predicted Kleiber value (Kleiber, 1975). Changes in osmolality and electrolyte concentrations observed in both urine and plasma are indicative of mariposa when hooded seal pups enter seawater. The low protein catabolism and stable metabolic rates may be the consequence of the larger stores of fat that hooded seals deposit during lactation (and before birth), which is a greater source of energy than is available to the harp seal pups. This greater source of blubber may provide hooded seals with the ability to undergo the post-weaning fast, with no mechanisms in place to reduce their metabolic rate or increase protein catabolism, while keeping their thermoregulatory homeostasis intact.

Works cited

Aagaard, K., & Carmack, E. C. (1989). The Role of Sea Ice and Other Fresh Water in the Arctic Circulation. *Journal of Geophysical Research Atmospheres*, *94*(C10), 485–499. https://doi.org/10.1029/JC094iC10p14485

Abdalati, W., & Steffen, K. (2001). Greenland Ice Sheet melt extent: 1979-1999. *Journal of Geophysical Research*, *106*(D24), 983–1016. https://doi.org/10.1029/2001JD900181

Adams, S. H., & Costa, D. P. (1993). Water conservation and protein metabolism in northern elephant seal pups during the postweaning fast. *Journal of Comparative Physiology B*, *163*(5). https://doi.org/10.1007/BF00265640

AMAP. (1997). *Arctic Pollution Issues: A State of the Arctic Environment Report*.

Arnemo, J. M., Evans, A. L., Fahlman, Å., & Caulkett, N. (2014). Field Emergencies and Complications. In G. West, D. Heard, & N. Caulkett (Eds.), *Zoo Animal and Wildlife Immobilization and Anesthesia* (2nd ed., pp. 139–147). John Wiley & Sons, Inc.

Atkinson, S. (1997). Reproductive biology of seals. In *Reviews of Reproduction* (Vol. 2, Issue 3). https://doi.org/10.1530/ror.0.0020175

Ballard, K. A., & Kovacs, K. M. (1995). The acoustic repertoire of hooded seals (*Cystophora cristata*) . *Canadian Journal of Zoology*, *73*(7). https://doi.org/10.1139/z95-159

Bester, M. N. (1975). The functional morphology of the kidney of the Cape fur seal, *Arctocephalus pusillis* (Schreber). *Madoqua*, *2*(4).

Blix, A. S., Grav, H. J., & Ronald, K. (1979). Some aspects of temperature regulation in newborn harp seal pups. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *5*(2). https://doi.org/10.1152/ajpregu.1979.236.3.r188

Blix, A. S., & Johnsen, H. K. (1983). Aspects of nasal heat exchange in resting reindeer. *The Journal of Physiology*, *340*(1). https://doi.org/10.1113/jphysiol.1983.sp014772

Boily, P., & Lavigne, D. M. (1997). Developmental and seasonal changes in resting metabolic rates of captive female grey seals. *Canadian Journal of Zoology*, *75*(11). https://doi.org/10.1139/z97-807

Bowen, W. D., Oftedal, O. T., & Boness, D. J. (1985). Birth to weaning in 4 days: remarkable growth in the hooded seal, *Cystophora cristata*. *Canadian Journal of Zoology*, *63*(12), 2841–2846. https://doi.org/10.1139/z85-424

Brodie, P. F., & Pasche, A. J. (1980). *Density-Dependent Condition and Energetics of Marine Mammal Populations in Multispecies Fisheries Management*.

Brown, A. C., & Brengelmann, G. (1965). Energy Metabolism. In Ruch T. C. & H. D. Patton (Eds.), *Physiology and Biophysics* (19th ed., pp. 1030–1049). Saunders.

Bue, M. E. S. (2015). *Metabolic adaptations and leptin expression in hooded seal (Cystophora cristata) pups during intense lactation and post-weaning fasting* [Masters Thesis]. UiT – The Arctic University of Norway.

Burns, J. M., Lestyk, K. C., Folkow, L. P., Hammill, M. O., & Blix, A. S. (2007). Size and distribution of oxygen stores in harp and hooded seals from birth to maturity. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, *177*(6). https://doi.org/10.1007/s00360-007-0167-2

Cabanac, A. J., Messelt, E. B., Folkow, L. P., & Blix, A. S. (1999). The structure and blood-storing function of the spleen of the hooded seal (*Cystophora cristata*). *Journal of Zoology*, *248*(1). https://doi.org/10.1017/S0952836999005087

Castellini, J. M., & Castellini, M. A. (1993). Estimation of Splenic Volume and Its Relationship to Long-Duration Apnea in Seals. *Physiological Zoology*, *66*(4). https://doi.org/10.1086/physzool.66.4.30163811

Chinnadurai, S. K., Danielle, S.-H., Fiorello, C. v, Harms, & Craig A. (2016). Best-Practice Guidelines for Field-based Surgery and Anesthesia of Free-ranging

Wildlife. I. Anesthesia and Analgesia. *Journal of Wildlife Diseases*, *52*(2), S14–S27. https://doi.org/10.7589/52.2S.S14

Costill, D. L., & Sparks, K. E. (1973). Rapid fluid replacement following thermal dehydration. *Journal of Applied Physiology*, *34*(3). https://doi.org/10.1152/jappl.1973.34.3.299

Cox, A. N., & Pilachowski, C. A. (2002). Allen's Astrophysical Quantities. In *Allen's Astrophysical Quantities* (4th ed.). https://doi.org/10.1007/978-1-4612-1186-0

DeLany, J. P. (2017). Energy requirement methodology. In A. M. Coulston, C. J. Boushey, M. G. Ferruzzi, & L. M. Delahanty (Eds.), *Nutrition in the Prevention and Treatment of Disease* (4th ed., pp. 85–102). Academic Press. https://doi.org/10.1016/B978-0-12-802928-2.00004-7

Derocher, A. E., Nelson, R. A., Stirling, I., & Ramsay, M. A. (1990). Effects of Fasting and Feeding on Serum Urea and Serum Creatinine Levels in Polar Bears. *Marine Mammal Science*, *6*(3). https://doi.org/10.1111/j.1748-7692.1990.tb00243.x

Evertsen, A. P. (2021). *The metabolism of lean and fat hooded seal pups (Cystophora cristata): how fat contributes to the total metabolic rate* [Masters Thesis]. UiT The Arctic University of Norway.

Finn, P. F., & Dice, J. F. (2006). Proteolytic and lipolytic responses to starvation. In *Nutrition* (Vol. 22, Issues 7–8). https://doi.org/10.1016/j.nut.2006.04.008

Folkow, L. P., & Blix, A. S. (1987). Nasal heat and water exchange in gray seals. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *253*(6), 883–889. https://doi.org/10.1152/ajpregu.1987.253.6.r883

Folkow, L. P., & Blix, A. S. (1999). Diving behaviour of hooded seals (*Cystophora cristata*) in the Greenland and Norwegian Seas. *Polar Biology*, *22*(1). https://doi.org/10.1007/s003000050391

Folkow, L. P., Blix, A. S., & Eide, T. J. (1988). Anatomical and functional aspects of the nasal mucosal and ophthalmic retia of phocid seals. *Journal of Zoology*, *216*(3). https://doi.org/10.1111/j.1469-7998.1988.tb02439.x

Folkow, L. P., Mårtensson, P. E., & Blix, A. S. (1996). Annual distribution of hooded seals (*Cystophora cristata*) in the Greenland and Norwegian seas. *Polar Biology*, *16*(3). https://doi.org/10.1007/BF02329206

Folkow, L. P., Nordøy, E. S., & Blix, A. S. (2010). Remarkable development of diving performance and migrations of hooded seals (*Cystophora cristata*) during their first year of life. *Polar Biology*, *33*(4), 433–441. https://doi.org/10.1007/s00300-009-0718-y

Gentry, R. L. (1981). Seawater drinking in eared seals. *Comparative Biochemistry and Physiology -- Part A: Physiology*, *68*(1). https://doi.org/10.1016/0300-9629(81)90321-2

Goodman, M. N., Larsen, P. R., & Kaplan, M. M. (1980). Starvation in the rat. II. Effect of age and obesity on protein sparing and fuel metabolism. *American Journal of Physiology - Endocrinology and Metabolism*, *2*(4). https://doi.org/10.1152/ajpendo.1980.239.4.e277

Grande, F., Anderson, J. T., & Keys, A. (1958). Changes of basal metabolic rate in man in semistarvation and refeeding. *Journal of Applied Physiology*, *12*(2). https://doi.org/10.1152/jappl.1958.12.2.230

Guerrero, M. A. (2018). *Body composition changes of hooded seal (Cystophora cristata) pups during extreme lactation* [Masters Thesis]. UiT - The Arctic University of Norway.

Hansen, E., Gerland, S., Granskog, M. A., Pavlova, O., Renner, A. H. H., Haapala, J., Lïyning, T. B., & Tschudi, M. (2013). Thinning of Arctic sea ice observed in Fram Strait. *J. Geophys. Res. Oceans*, *118*, 5202–5221. https://doi.org/10.1002/jgrc.20393

Haug, T., Nilssen, K. T., & Lindblom, L. (2000). First independent feeding of harp seal (*Phoca groenlandica*) and hooded seal (*Cystophora cristata*) pups in the Greenland Sea. *NAMMCO Sci Publ*, *2*, 29–39.

Haug, T., Nilssen, K. T., & Lindblom, L. (2004). Feeding habits of harp and hooded seals in drift ice waters along the east coast of Greenland in summer and winter. *Polar Research*, *23*(1). https://doi.org/10.1111/j.1751-8369.2004.tb00127.x

Houser, D., & Costa, D. (2001). Protein catabolism in suckling and fasting northern elephant seal pups (*Mirounga angustirostris*). *Journal of Comparative Physiology - B Biochemical, Systemic, and Environmental Physiology*, *171*(8). https://doi.org/10.1007/s003600100214

How, O. J., & Nordøy, E. S. (2007). Seawater drinking restores water balance in dehydrated harp seals. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, *177*(5). https://doi.org/10.1007/s00360-007-0152-9

ICES. (2019). ICES/NAFO/NAMMCO Working Group on Harp and Hooded Seals (WGHARP). *ICES Scientific Reports*, *1*(72), 193. https://doi.org/10.17895/ices.pub.5617

Kleiber, M. (1975). *The Fire of Life: An Introduction to Animal Energetics* (2nd ed.). Robert E. Kreiger Publishing Company.

Kolb, H., Kempf, K., Röhling, M., Lenzen-Schulte, M., Schloot, N. C., & Martin, S. (2021). Ketone bodies: from enemy to friend and guardian angel. In *BMC Medicine* (Vol. 19, Issue 1). https://doi.org/10.1186/s12916-021-02185-0

Kovacs, K. M. (2009). Hooded Seal: *Cystophora cristata*. In W. F. Perrin, B. Würsig, & J. G. M. Thewissen (Eds.), *Encyclopedia of Marine Mammals* (2nd ed., pp. 569–573). Academic Press. https://doi.org/10.1016/B978-0-12-373553-9.00132-2

Kovacs, K. M. (2016). *Cystophora cristata*. *The IUCN Red List of Threatened Species 2016*.

Kvadsheim, P. H., & Aarseth, J. J. (2002). Thermal function of phocid seal fur. *Marine Mammal Science*, *18*(4). https://doi.org/10.1111/j.1748-7692.2002.tb01084.x

Ladds, M. A., Slip, D. J., & Harcourt, R. G. (2017). Swimming metabolic rates vary by sex and development stage, but not by species, in three species of Australian otariid

seals. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, *187*(3). https://doi.org/10.1007/s00360-016-1046-5

Langman, V. A. (1985). Nasal heat exchange in a northern ungulate, the reindeer (*Rangifer tarandus*). *Respiration Physiology*, *59*(3). https://doi.org/10.1016/0034-5687(85)90133-1

Lester, C. W., & Costa, D. P. (2006). Water conservation in fasting northern elephant seals (*Mirounga angustirostris*). *Journal of Experimental Biology*, *209*(21). https://doi.org/10.1242/jeb.02503

Lighton, J. R. B. (2008). Measuring Metabolic Rates: A Manual for Scientists. In *Measuring Metabolic Rates: A Manual for Scientists*. Oxford University Press. https://doi.org/10.1093/acprof:oso/9780195310610.001.0001

Lowry, M. S., Condit, R., Hatfield, B., Allen, S. G., Berger, R., Morris, P. A., le Boeuf, B. J., & Reiter, J. (2014). Abundance, distribution, and population growth of the northern elephant seal (*Mirounga angustirostris*) in the united states from 1991 to 2010. *Aquatic Mammals*, *40*(1). https://doi.org/10.1578/AM.40.1.2014.20

Lydersen, C. (1995). Energetics of pregnancy, lactation and neonatal development in ringed seals (*Phoca hispida*). *Developments in Marine Biology*, *4*(C). https://doi.org/10.1016/S0163-6995(06)80033-8

Mariotti, F., Tomé, D., & Mirand, P. P. (2008). Converting nitrogen into protein - Beyond 6.25 and Jones' factors. *Critical Reviews in Food Science and Nutrition*, *48*(2). https://doi.org/10.1080/10408390701279749

McHuron, E. A., Sterling, J. T., Costa, D. P., & Goebel, M. E. (2019). Factors affecting energy expenditure in a declining fur seal population. *Conservation Physiology*, *7*(1). https://doi.org/10.1093/conphys/coz103

McLean, J. A., & Watts, P. R. (1976). Analytical refinements in animal calorimetry. *Journal of Applied Physiology*, *40*(5). https://doi.org/10.1152/jappl.1976.40.5.827

Mehta, N. M., Smallwood, C. D., Joosten, K. F. M., Hulst, J. M., Tasker, R. C., & Duggan, C. P. (2015). Accuracy of a simplified equation for energy expenditure

based on bedside volumetric carbon dioxide elimination measurement - A two-center study. *Clinical Nutrition*, *34*(1). https://doi.org/10.1016/j.clnu.2014.02.008

Montemurro, D. G., & F Stevenson, J. A. (1960). Survival and body composition of normal and hypothalamic obese rats in acute starvation'. *American Journal of Physiology*, *198*(4), 757–761.

Mysak, L. A. (2001). Patterns of arctic circulation. In *Science* (Vol. 293, Issue 5533). https://doi.org/10.1126/science.1064217

National Oceanic and Atmospheric Administration. (2021, April 17). *Monthly Carbon Dioxide Measurements*.

Nelson, R. A., Beck, T. D. I., & Steiger, D. L. (1984). Ratio of serum urea to serum creatinine in wild black bears. *Science*, *226*(4676). https://doi.org/10.1126/science.6494914

Nelson, R. A., Folk, G. E., Pfeiffer, E. W., Craighead, J. J., Jonkel, C. J., & Steiger, D. L. (1983). Behavior, Biochemistry, and Hibernation in Black, Grizzly, and Polar Bears. In *Bears: Their Biology and Management* (Vol. 5).

Nordøy, E. S., Aakvaag, A., & Larsen, T. S. (1993). Metabolic adaptations to fasting in harp seal pups. *Physiological Zoology*, *66*(6). https://doi.org/10.1086/physzool.66.6.30163747

Nordøy, E. S., & Blix, A. S. (1985). Energy sources in fasting grey seal pups evaluated with computed tomography. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *18*(4). https://doi.org/10.1152/ajpregu.1985.249.4.r471

Nordøy, E. S., & Blix, A. S. (1991). Glucose and ketone body turnover in fasting grey seal pups. *Acta Physiologica Scandinavica*, *141*(4). https://doi.org/10.1111/j.1748-1716.1991.tb09118.x

Nordøy, E. S., Folkow, L. P., Potelov, V., Prischemikhin, V., & Blix, A. S. (2008). Seasonal distribution and dive behaviour of harp seals (*Pagophilus groenlandicus*)

of the White Sea-Barents Sea stock. *Polar Biology*, *31*(9), 1119–1135. https://doi.org/10.1007/s00300-008-0453-9

Nordøy, E. S., Ingebretsen, O. C., & Blix, A. S. (1990). Depressed metabolism and low protein catabolism in fasting grey seal pups. *Acta Physiologica Scandinavica*, *139*(2), 361–369. https://doi.org/10.1111/j.1748-1716.1990.tb08935.x

Oftedal, O. T., Bowen, W. D., & Boness, D. J. (1988). The composition of hooded seal (*Cystophora cristata*) milk: an adaptation for postnatal fattening. *Canadian Journal of Zoology*, *66*, 318–322.

Oftedal, O. T., Bowen, W. D., Widdowson, E. M., & Boness, D. J. (1991). The prenatal molt and its ecological significance in hooded and harbor seals. *Canadian Journal of Zoology*, *69*(9), 2489–2493. https://doi.org/10.1139/z91-351

Ortiz, R. M. (2001). Osmoregulation in marine mammals. In *Journal of Experimental Biology* (Vol. 204, Issue 11). https://doi.org/10.1242/jeb.204.11.1831

Owen, O. E., Felig, P., Morgan, A. P., Wahren, J., & Cahill, G. F. (1969). Liver and kidney metabolism during prolonged starvation. *The Journal of Clinical Investigation*, *48*(3). https://doi.org/10.1172/JCI106016

Ponganis, P. J., Kooyman, G. L., Sartoris, D., & Jobsis, P. (1992). Pinniped splenic volumes. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *262*(2 31-2). https://doi.org/10.1152/ajpregu.1992.262.2.r322

Potelev, V., Nilssen, K. T., Svetochev, V., & Haug, T. (2000). Feeding habits of harp (*Phoca groenlandica*) and hooded seals (*Cystophora cristata*) during late winter, spring and early summer in the Greenland Sea. *NAMMCO Scientific Publications*, *2*. https://doi.org/10.7557/3.2970

Richardson, H. B. (1929). The respiratory quotient. *Physiological Reviews*, *9*(1), 61–125.

Robin, J. P., Frain, M., Sardet, C., Groscolas, R., & Maho, Y. L. (1988). Protein and lipid utilization during long-term fasting in emperor penguins. *American Journal of*

Physiology - Regulatory Integrative and Comparative Physiology, *254*(1). https://doi.org/10.1152/ajpregu.1988.254.1.r61

Robinson, A. M., & Williamson, D. H. (1980). Physiological roles of ketone bodies as substrates and signals in mammalian tissues. In *Physiological Reviews* (Vol. 60, Issue 1). https://doi.org/10.1152/physrev.1980.60.1.143

Rosen, D. A. S., & Trites, A. W. (2002). Changes in metabolism in response to fasting and food restriction in the Steller sea lion (*Eumetopias jubatus*). *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology*, *132*(2). https://doi.org/10.1016/S1096-4959(02)00048-9

Schalm, O. W. (1965). *Veterinary Hematology* (2nd ed.). Bailliere.

Schmidt-Nielsen, K. (1990). *Animal Physiology: Adaptation and Environment* (4th ed.). Cambridge University Press.

Schots, P. C., Bue, M. E., & Nordøy, E. S. (2017). Hooded seal (*Cystophora cristata*) pups ingest snow and seawater during their post-weaning fast. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, *187*(3), 493–502. https://doi.org/10.1007/s00360-016-1048-3

Sha, L., Jiang, H., Seidenkrantz, M. S., Muscheler, R., Zhang, X., Knudsen, M. F., Olsen, J., Knudsen, K. L., & Zhang, W. (2016). Solar forcing as an important trigger for West Greenland sea-ice variability over the last millennium. *Quaternary Science Reviews*, *131*. https://doi.org/10.1016/j.quascirev.2015.11.002

Skalstad, I., & Nordøy, E. S. (2000). Experimental evidence of seawater drinking in juvenile hooded (*Cystophora cristata*) and harp seals (*Phoca groenlandica*). *Journal of Comparative Physiology - B Biochemical, Systemic, and Environmental Physiology*, *170*(5–6). https://doi.org/10.1007/s003600000116

Skog, E. B., & Folkow, L. P. (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, H. W. (1936). The composition of urine in the seal. *Journal of Cellular and Comparative Physiology*, *7*(3). https://doi.org/10.1002/jcp.1030070314

Somo, D. A., Ensminger, D. C., Sharick, J. T., Kanatous, S. B., & Crocker, D. E. (2015). Development of dive capacity in northern elephant seals (*Mirounga angustirostris*): Reduced body reserves at weaning are associated with elevated body oxygen stores during the postweaning fast. *Physiological and Biochemical Zoology*, *88*(5). https://doi.org/10.1086/682386

Storeheier, P. v., & Nordøy, E. S. (2001). Physiological effects of seawater intake in adult harp seals during phase I of fasting. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, *128*(2). https://doi.org/10.1016/S1095-6433(00)00311-1

Tarasoff, F. J., & Fisher, H. D. (1970). Anatomy of the hind flippers of two species of seals with reference to thermoregulation. *Canadian Journal of Zoology*, *48*(4). https://doi.org/10.1139/z70-144

Thorson, P. H., & le Boeuf, B. J. (1994). Developmental Aspects of Diving in Northern Elephant Seal Pups. In B. J. le Boeuf & R. M. Laws (Eds.), *Elephant Seals: Population ecology, behaviour, and physiology* (pp. 271–289). University of California Press. https://doi.org/10.1525/9780520328150-017

Verlo, A. (2012). *Seawater consumption in dehydrated hooded seals (Cystophora cristata)* [Masters Thesis]. UiT – The Arctic University of Norway.

Weir, J. B. (1949). New methods for calculating metabolic rate with special reference to protein metabolism. *The Journal of Physiology*, *109*(1–2). https://doi.org/10.1113/jphysiol.1949.sp004363

Whiteman, J. P., Harlow, H. J., Durner, G. M., Regehr, E. v., Amstrup, S. C., & Ben-David, M. (2018). Phenotypic plasticity and climate change: can polar bears respond to longer Arctic summers with an adaptive fast? *Oecologia*, *186*(2), 369–381. https://doi.org/10.1007/S00442-017-4023-0

Whitten, B. K., & Klain, G. J. (1968). Protein metabolism in hepatic tissue of hibernating and arousing ground squirrels'. *American Journal of Physiology*, *214*(6).

Worthy, G. A. J., & Lavigne, D. M. (1983). Changes in energy stores during postnatal development of the harp seal, *Phoca groenlandica*. *Journal of Mammalogy*, *64*(1). https://doi.org/10.2307/1380753

Appendix

Raw Data:

Table 4: Mass measurements over the fasting period

Seal	Day of fasting	Mass (kg)
1	0	44
1	6	43
1	11	40
1	17	38
1	25	36
1	32	34
2	0	42.27
2	5	39.98
2	10	36.56
2	16	35.76
2	24	34.38
2	31	32.2
3	0	33.92
3	5	32.58
3	11	30.3
3	18	28.56
3	25	26.52
4	0	34.66
4	3	31.53
4	9	27.48
4	16	25.84
4	23	23.64
5	0	36.22
5	2	33.9
5	9	31.26
5	16	29.42
5	23	27.32
5	30	25.66
6	0	48.97
6	8	44.38
6	15	42
6	22	39
6	29	36.78

Appendix

Table 5: Results of Blood Analysis

Seal Number	**Day**	Chloride (mmol/l)	Creatinine (µmol/L)	Magnesium (mmol/l)	Sodium (mmol/l)	Urea (mmol/l)	Uric Acid (µmol/L)	Osmolality (osmol/kg)	Haematocrit (%)
1	0	102	77	0.9	149	7.1	304	0.308	55.78563
1	11	106	88	1	149	8.5	201	0.385	57.61092
1	17	105	83	1	150	9.9	191	0.394	58.57451
1	25	107	77	1.1	151	18	221	0.428	56.75323
1	32	110	71	1.1	154	18	174	0.349	56.49962
2	0	102	88	1	148	6.3	416	0.299	53.3497
2	10	101	94	1	146	6.7	243	0.3	59.77285
2	16	100	82	1	147	9.9	238	0.302	57.10001
2	24	101	92	1	146	9.7	220	0.302	55.16988
2	31	104	77	1	147	9.6	156	0.301	54.98409
3	0	106	75	1	153	10.8	298	0.307	55.4994
3	11	107	70	1.2	150	9.8	151	0.301	58.9539
3	18	106	85	1	151	10.9	167	0.305	58.93041
3	25	106	82	1	151	13	160	0.316	58.64331
4	0	105	77	0.9	150	18.1	334	0.316	58.11988
4	9	106	69	1.1	151	18.7	288	0.319	65.87775
4	16	107	75	1	151	20.9	257	0.327	66.6973
4	23	106	67	1	150	18	143	0.321	71.18605
5	0	101	70	1	152	19.3	185	0.315	55.06377
5	9	103	92	1	150	10.8	186	0.306	64.08638
5	16	104	79	1.1	150	21.6	163	0.319	60.30877
5	23	103	80	1	150	22.6	144	0.327	61.98912
5	30	106	73	1.1	150	19.5	156	0.329	61.23218
6	0	103	92	0.8	152	11.8	423	0.308	52.42438
6	8	107	90	1.3	151	9	257	0.308	61.30614
6	15	110	93	1.5	155	13.4	238	NA	NA
6	22	107	103	1.3	155	24.7	253	0.342	63.45237
6	29	110	88	1.5	156	28.5	172	0.344	62.71952

Appendix

Table 6: Results of Urine Analysis

Seal	Day	Ammonia (mmol/l)	Sodium (mmol/l)	Chloride (mmol/l)	Magnesium (mmol/l)	Urea (mmol/l)	Uric Acid (µmol/L)	Creatinine (mmol/l)	Osmolality (osmol/kg)	Produced (ml/day)
1	0	47.47107	119	64	5.9	197	532	6.8	0.542	460
2	0	86.70484	111	61	9.72	260	1292	13.31	0.753	164
3	0	145.7384	62	34	4.52	179	901	11.26	0.975	256
4	0	44.45309	76	63	3.18	389	899	2.96	0.659	635
5	0	75.27309	66	31	1.84	320	395	2.99	1.115	106
4	9	134.3981	77	67	9.48	336	259	9.59	1.398	170
5	9	96.30751	134	78	18.5	185	918	25.01	0.785	110
5	16	97.63359	112	106	14.71	168	522	12.93	1.115	117
6	22	39.5603	282	348	24.96	132	501	15.91	0.886	332
1	32	83.5954	170	161	12.13	221	721	13.58	1.373	87
5	30	105.5901	110	118	12.38	291	464	13.92	1.395	110
6	29	68.23113	127	140	12.64	151	384	12.48	1.108	232

Table 7:Results of Metabolic Measurements

Seal	Mass (kg)	Date	FiO2 Fraction	FeO2 Fraction	FiCO2 Fraction	FeCO2 Fraction	VO2 (L/min)	VCO2 (L/min)
1	44.15	26/03/2021	0.2095	0.206852	0.000416	0.002402	0.419995	0.295509
2	41.93	27/03/2021	0.2095	0.207936	0.000416	0.001821	0.240242	0.210181
3	33.22	28/03/2021	0.2095	0.20805	0.000416	0.001495	0.231261	0.161091
4	34.66	30/03/2021	0.2095	0.207931	0.000416	0.001817	0.240907	0.209141
5	36.22	31/03/2021	0.2095	0.207949	0.000416	0.001882	0.234769	0.218668
6	48.97	31/03/2021	0.2095	0.207685	0.000416	0.001844	0.286799	0.213632
1	39.98	06/04/2021	0.2095	0.208177	0.000416	0.001408	0.210385	0.147935
2	36.56	06/04/2021	0.2095	0.207678	0.000416	0.001876	0.285678	0.217481
3	30.3	07/04/2021	0.2095	0.208238	0.000416	0.001419	0.198323	0.149575
4	27.48	07/04/2021	0.2095	0.208013	0.000416	0.001687	0.229998	0.189355
5	31.26	08/04/2021	0.2095	0.20743	0.000416	0.002102	0.324181	0.25165
6	44.38	08/04/2021	0.2095	0.20818	0.000416	0.001568	0.203458	0.171736
1	38.48	12/04/2021	0.2095	0.207575	0.000416	0.00185	0.306771	0.213984
2	35.76	12/04/2021	0.2095	0.207871	0.000416	0.001874	0.249518	0.217275
3	28.56	14/04/2021	0.2095	0.207474	0.000416	0.002084	0.316468	0.248904
4	25.84	14/04/2021	0.2095	0.208243	0.000416	0.001445	0.196357	0.153409
5	29.42	15/04/2021	0.2095	0.207553	0.000416	0.001962	0.30635	0.230553
6	42	15/04/2021	0.2095	0.20782	0.000416	0.001772	0.263039	0.201998
1	35.82	20/04/2021	0.2095	0.208297	0.000416	0.001349	0.190031	0.13917
2	34.38	20/04/2021	0.2095	0.208231	0.000416	0.001637	0.191017	0.181992
3	26.52	21/04/2021	0.2095	0.208374	0.000416	0.001219	0.180702	0.11983
4	23.64	21/04/2021	0.2095	0.208579	0.000416	0.001193	0.142964	0.115764

5	27.32	22/04/2021	0.2095	0.208245	0.000416	0.00153	0.192869	0.166271
6	39	22/04/2021	0.2095	0.207715	0.000416	0.001953	0.275962	0.229212
1	33.86	27/04/2021	0.2095	0.208229	0.000416	0.001319	0.204246	0.134713
2	32.2	27/04/2021	0.2095	0.208113	0.000416	0.001633	0.213656	0.181467
5	25.66	29/04/2021	0.2095	0.208316	0.000416	0.001385	0.185247	0.14452
6	36.78	29/04/2021	0.2095	0.208168	0.000416	0.001534	0.206976	0.166651