UiT The Arctic University of Norway

Faculty of Biosciences, Fisheries and Economics, department of Arctic and Marine Biology

The metabolism of lean and fat hooded seal pups (*Cystophora cristata*)

How fat contributes to the total metabolic rate

Agnete Pedersen Evertsen

BIO-3950 Master's thesis in Biology, May 2021

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Supervisor: Lars Folkow, UiT – The Arctic University of Norway

Co-supervisor: Erling S. Nordøy, UiT – The Arctic University of Norway

Cover page picture:

Blueback - hooded seal pup

Photo by Michael Poltermann, Institute of Marine Research, Norway

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Agnete Pedersen Evertsen

Abstract

Hooded seal pups are highly adapted to their proximate environment from the moment they are born. With a substantial blubber layer being present already at birth, as well as the fact that they typically gain 20+ kg of body mass in the short span of time they are nursing (2-4 days), they are very well adapted to the rough, cold environment they are born into. With the body mass gained during nursing mainly being a result of fat deposition, it made them an excellent model to use when studying the fat metabolism and to what extent it contributes to the total metabolic rate.

I used indirect calorimetry through expired gas-analysis, with the O_2-consumption (VO_2) and the CO_2-production (VCO_2) and respiratory quotient (RQ) as proxies to calculate the resting metabolic rates of 12 hooded seal pups. We strived to include both lean, newborn pups and fat, weaned pups. We assumed that the differences in body mass was a result of mainly fat deposition. Some of the pups lacked data on fat percentage, and others lacked data on body length, so the fat percentage and condition index (CI = BM/BL) were predicted using linear regression models for all hooded seals captured from 2007-2019 with these data. There were three standard measurement conditions for all individuals: 1) They were within their thermoneutral zone, 2) They were post-absorptive and 3) They were sleeping, showing a characteristic apnoea-pattern in their breathing for the periods analysed. VO_2 was not used as the main proxy for calculations of the metabolic rates due to a drift in the system, with unstable baseline recordings for the O_2-measurements. VCO_2 was therefore instead chosen as the main proxy, using known RQ-values.

The results showed that there was a significant negative relationship between the weight-specific sleeping metabolic rate (SMR) and body fat percentage ($p = 0.0039$) using linear regression, indicating that the individuals with a higher body fat percentage has a relatively lower SMR compared to the individuals with a lower body fat percentage. A similar correlation was also found between the weight-specific SMR and condition index (CI = body mass/body length), with a significant negative relationship ($p = 0.0021$), showing that individuals with a higher condition index-value generally had a relatively lower SMR compared to those with a lower condition index-value. To compare, the individual with the lowest body fat percentage (22,4%) had a weight-specific SMR that was more than doubled

compared to the individual with the highest body fat percentage (53,9%): 3,75 Watts/kg vs. 1,51 Watts/kg. In conclusion, this study found that fat is relatively inert and contributes less than the lean body mass (LBM) to the total metabolism.

Keywords: *Hooded seal, Hooded seal pups, Cystophora cristata, Resting metabolism, Indirect calorimetry, Weight-specific resting metabolism, Expired gas analysis, Lactation period, Fat metabolism, Respiratory quotient, West Ice, Condition Index*

List of contents

List of figures

List of tables

List of abbreviations

BL – Body length

BM – Body mass

BMR – Basal metabolic rate

BP – Barometric pressure

CI – Condition index

CL – Curvilinear length

F_eCO_2 - CO_2-percentage of expired air during expired gas-analysis

F_eO_2 - O_2-percentage of expired air during expired gas-analysis

F_iCO_2 - CO_2-percentage of atmospheric air

F_iO_2 - O_2-percentage of atmospheric air

LBM – Lean body mass

LCT – Lower critical temperature

MR – Metabolic rate

RH – Relative humidity

RMR – Resting metabolic rate

RQ – Respiratory quotient

SDA – Specific Dynamic Action

SE – Standard error

SMR – Sleeping metabolic rate

S-W – Shapiro-Wilk test (test for normality)

T_{air} – Temperature of the air

TNZ – Thermoneutral zone

UCT – Upper critical temperature

VCO_2 – Volume of carbon dioxide produced by animal

VO_2 – Volume of oxygen consumed by animal

1. Introduction

The Arctic is a large area of the globe where the environment can be rather hostile for most living organisms. Due to low temperatures, unfavourable light-conditions in winter, lots of ice and snow during winter, scarce food-resources and generally rough conditions, there are few organisms that can survive under these conditions for longer periods of time. Animals that manage to withstand these conditions therefore typically have evolved numerous adaptations that make them more viable in the Arctic climate. These adaptions give them an increased fitness relative to those that do not have the same adaptations, such as thick, isolating fur or blubber, shorter limbs (typically causing less heat loss due to smaller surface area), hibernation (lowering metabolism during the roughest winter period, causing a substantially lowered energy requirement from food) and fat storage in general (e.g. large food-intake during late summer/fall, leading to a large weight-gain for when food resources are scarce and temperatures are lower during winter) (Blix, 2005).

Among the animals adapted to the Arctic climate are the Arctic seals, six seal species that spend most of their lives above the Arctic circle. These six species are spotted seals (*Phoca largha*), ringed seals (*Pusa hispida*), ribbon seals (*Histriophoca fasciata*), bearded seal (*Erignathus barbatus*), harp seals (*Pagophilus groenlandicus*) and hooded seals (*Cystophora cristata*). One could also include the harbour seal (*Phoca vitulina*), given that they have a population at Svalbard and are distributed far North (Wiig, 1989). The Arctic seals all, though not exclusively, have certain traits and behavior patterns in common:

1. They all give birth and breed on pack ice.
2. They all have a significant blubber layer that provides them with good isolation. Blubber contains very little water and lots of lipids, making it a great insulator for the seals and marine mammals in general (Castellini, 2018).
3. The pups of the Arctic seals all have lanugo furs. Some moult it in the uterus prior to birth (e.g. hooded seal pups), while most species moult it after birth. The hooded seal pup is instead born with a much greater blubber layer that gives good isolation.
4. They have a short lactation period, only lasting from a couple of days to a couple of weeks, depending on species and individual.

5. Their milk is very energy dense, which helps the pups gain lots of body fat, and henceforth isolation, rapidly. This makes them more viable when the lactation period is as short as it is, an adaptation that makes it possible for the mother to feed again and breed soon after birth.
6. They have subsequent mating and moulting, meaning that they moult not long after breeding. Typically, the birth/breeding and moulting occurs at different places, five to seven weeks apart.
7. All of the species have delayed implantation. Even though mating occurs shortly after birth, the fertilization is delayed so that the timing of the birth is more advantageous in terms of the surrounding environment. Originally the seals are only pregnant for about six months, but with the delayed fertilization the total gestation period is typically anywhere from nine to twelve months, depending on the nursing period, and therefore also timing of breeding for the different species (Atkinson, 1997).

1.1. Hooded seals

The hooded seal (*Cystophora* cristata) is one of the Arctic seal species, found mainly on thick pack ice when on land. Its name refers to the males' characteristic mating display, where they blow up their nasal sac and septum (fig. 1), looking like hoods when inflated. This inflation is used as a dominance display, both to attract females and deter competition towards other males, and it is otherwise used as a threat signal year-round (Blix, 2005; Kovacs, 2018). Also the pups are quite characteristic with their looks, mainly in two ways: 1. They are born without the lanugo fur that most of the other Arctic seals are born with. The reason for this, is that they moult in the uterus, and they are instead born with a considerable blubber layer that helps them survive the

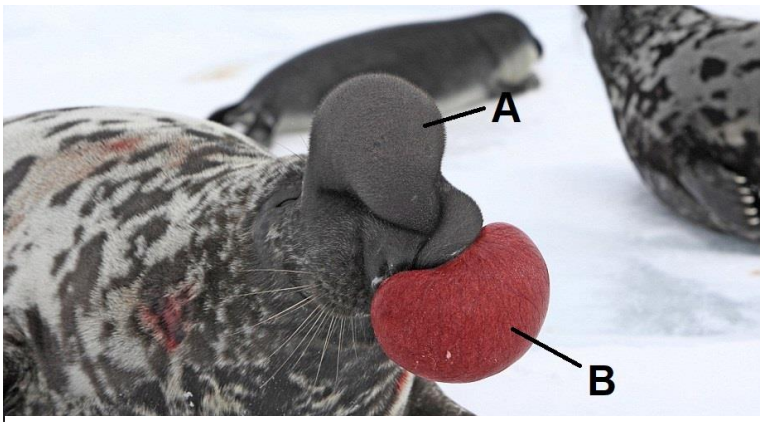

Figure 1: Hooded seal male that has blown up his nasal cavity (A) and septum (B) during mating display. Seen next to a hooded seal female and her pup. The male stays nearby until the nursing period is done to mate with the female. Photographer: Sylvain Cordier

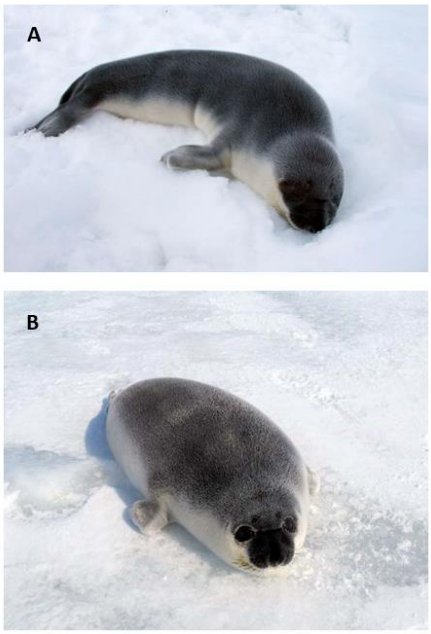

Figure 2: Shows the difference between a close to newborn hooded seal pup (A) and a weaned pup (B). This illustrates the difference in fat deposition and colour between the two.

Photographer, picture A: Raldi Somers

Photographer, picture B: Mike Hammill

typically rough, cold environment they are born into. The lanugo fur of the hooded seal is additionally brown, unlike the white lanugo fur seen on most other Arctic seals. 2. They have a dark, blueish back on an otherwise rather silver-colored body when weaned (fig. 2), which is the reason for why weaned hooded seal pups often are referred to as bluebacks (Blix, 2005).

The total hooded seal population is estimated to be approximately 670.000 individuals. The population consists of three stocks: The Greenland Sea-stock, with its breeding location near the coast of East Greenland and Jan Mayen, and with a stock size of approximately 77.000 individuals (ICES/NAFO/NAMMCO *Working group on harp and hooded seals*, 2019). Secondly, there is a stock that breeds in the Davis Strait every year. The last stock is divided into two sub-populations, and they breed in the Gulf of St. Lawrence and between Newfoundland and Labrador. ICES/NAFO/NAMMCO did not have any estimates on the two other stocks (Davis Strait and Gulf of St. Lawrence/Newfoundland) from 2019, but Hammill and Stenson (2006) estimated the population size to be approximately 593.500 individuals (SE = 67,200 and 95% Confidence Interval = 465,600 - 728,300) based on catches of hooded seal pups (pup production).

Getting access to hooded seals for population size estimations can be both challenging and expensive, mainly due to their way of living. They are considered a data-poor species, indicating that there is a lack of sufficient data for good population estimates. This because of limitations and uncertainties in data and estimations on pup production, reproduction and the structure of the stocks (Hammill & Stenson, 2007). One of the reasons for this is that they are out in the open ocean diving and foraging most of the year (Folkow et al. 1996). In addition to this, hooded seals are known to be deep divers. Results from a study conducted by Folkow and Blix (1999) using satellite-linked recorders glued on 10 adult hooded seal individuals, found that dives at 100 – 600m depth with a duration between 5 and 25 minutes were most frequent. As a result, it can be considerably challenging to estimate the population size of

hooded seal without a major cost due to expensive, specialized equipment needed in a large scale. Resultingly, there is not much data on the hooded seal population size, and the data we have gathered also come with substantial possibilities of error (Folkow et al. 1996).

Hooded seals gather on thick pack-ice each year to give birth and breed, and the pups are born between the end of March and early April. Their lactation-period is the shortest of not only the Arctic seals, but in fact for all mammals of similar size, only lasting 2-4 days before the mother mates again and further on leaves to forage in the ocean. Hence, the pups are left by themselves until they are ready to fend for themselves and start foraging in the ocean (Bowen et al. 1985; Oftedal et al. 1987; Bowen, 1991; Atkinson, 1997). The mother of the pups leaves to mate right after weaning, and there are often males waiting nearby during lactation, so that the mating occurs in the water immediately after weaning. (Kovacs, 2016). As the case is for seals in general, hooded seals delay their implantation so that the time of birth is more advantageous in terms of the environment. Because of this, they have a total gestation period of approximately 12 months, 1 year. The fertilization of the egg is delayed with 3-5 months (Atkinson, 1997).

As previously mentioned, the hooded seal pups are born without lanugo fur, given that they moult in the uterus of their mother before birth. One would think that this would be detrimental for their survival out on the ice in the Arctic, but the fact is that they instead are born with a considerable blubber layer, that is much thicker at birth compared to most of the other Arctic seal pups. This causes them to have good isolation from the rough surrounding environment even though the lanugo fur is not present. They do also have a thin fur, closer to that of the adults, though this does not contribute much in terms of isolation compared to the blubber, especially when wet (Kvadsheim & Aarseth, 2002). At birth the pups typically weigh between 20 and 25 kg, which is a lot compared to harp seal pups, that typically have a weight ranging from 10 to 12 kg at birth. In fact, the pups are normally in the range of 20-25kg at birth (Blix, 2005). The milk consumed by the pups during nursing is extremely energy rich, with a fat percentage can reach 61% (Oftedal et al. 1988)! In comparison, human breast milk has a fat percentage of approximately 4% (Lavigne et al., 1982), and heavy cream has a fat percentage of around 37% (TINE®, Norway). Given that their nursing period is as short as it is, this energy dense milk is an advantage for the pup for growing as much as possible before weaning, at which time they often have exceeded a body mass of 40kg, mostly due to fat deposition (Bowen, 1985).

Krogh's principle states the following: "*For such a large number of problems there will be some animal of choice, or a few such animals, on which it can be most conveniently studied*" (Krogh, 1929). Because of its unique life strategies, the hooded seal pup is such a model-animal when studying fat metabolism and its contribution to the total metabolism. First of all, they gain a lot of fat very quickly, and because all of the pups generally are born within a short span of time, it makes it possible to get access to several individuals at different stages of their nursing (from newborn to weaned). This helps us to compare the metabolic rate for a wide range of fat percentages and body sizes, so that the effect of fat percentage on the metabolism more easily can be studied and evaluated for a large span of fat percentages. In addition to this, the pups are generally quite easy to capture, to move back and forth between the metabolism chamber and their pens, both because they generally are calm and easy to carry, and they rapidly go to rest and fall asleep when left alone.

1.2. Metabolism and indirect calorimetry

To maintain life, all living organisms must be able to not only obtain energy, but also utilize it for essential energy demanding processes in their bodies, necessary for their survival. All animals are made up of atoms bound together to form their body and all its belonging tissues and organs. The body manages to keep this organization despite atoms continuously being switched out with atoms from the environment. The second law of thermodynamics states that if there is an internal change in an *isolated* system, the change will always increase the entropy, meaning that it always will go towards more disorder. Animals' bodies are, not isolated systems, and exchanges of atoms can be made between them and the environment. This means that energy can enter the system, which helps maintaining order in the body. Energy is defined as the capacity to do work, and in biological terms that means energy can be used to keep order in the internal organization. Without continuous inputs of energy, the body would not be able to function, given that the molecules making up the body then would go towards greater disorder. Blood circulation would eventually stop, the nervous system would not work given that the ion-flow necessary to produce an action potentials could not have been produced, and there would generally be a breakdown of numerous vital molecules, ultimately ending with the animal dying (Hill et al. 2018).

All organisms are categorized into two main groups based on how they obtain energy. Autotrophs, also called producers, produce the energy they require themselves by using an energy source (e.g. light) to produce organic energy from inorganic compounds. An example of this process is photosynthesis, where e.g. plants use solar light and CO_2 (inorganic compound) to produce glucose and water (H_2O). In the other category are all the organisms that cannot produce energy themselves using inorganic compounds, and therefore have to get organic compounds by consuming other organisms to obtain energy. These organisms are called heterotrophs, or consumers. The food they consume, may it be plants or other animals, have chemical energy stored in the bonds of the chemical compounds of their bodies. As the food is being digested, this energy is released and can be used to form other molecules necessary for bodily function and work. Not all of the energy in the food consumed can be used and stored. Some of the energy will be degraded to heat, which cannot be used for work and inevitably will be released to the environment. In addition to this, some of the energy will be released through urination and faecal disposal. (Hill et al. 2018).

Metabolism is often expressed as a metabolic rate, which is the energy used per unit of time. One of the most used units are Watts (Joules/second). There are often standardized conditions used when observing the metabolism of various animals, so that the data can be compared without adding potential errors due to conditions not being the same. Expressions that are much used in metabolism-related studies are the basal metabolic rate (BMR) and the resting metabolic rate (RMR). These are much used because they give an intel in how much energy is required for the basic body functions while the animal is resting. While the BMR tells us the minimum energy requirement to maintain the basic functions of an animal at rest, the RMR tells us the general energy expenditure while resting (Hill et al. 2018). Hence, measuring the BMR and/or the RMR does not include activity and extra work done, and can therefore often be used as a standard. Studying the metabolic rate of different organisms can be useful for a number of reasons. First and foremost, gathering data on the metabolism of several organisms makes it possible to generalize and compare them with each other. This could be helpful to compare different life strategies and evolutionary adaptations the organisms have to their respective environments. Knowing more about the differences in energy expenditure can also give information on differences within a species related to how energy is used and in what quantities it is required depending in age, sex and reproductive age. Metabolism-measurements and metabolism-related studies further on can tell us more about animals'

evolutionary adaptations to reduce energy expenditure, which in turn also tells us more about their general biology. Given that the metabolic rate is directly connected to how much food animals will need to maintain bodily functions and survive over time, studying their metabolism is important to know their energy needs.

1.2.1. Factors affecting the metabolic rate

Several factors are known to affect the metabolic rate of animals, and it is indeed not just the body mass that determines their energy expenditure. Their environment plays a vital role in how the body has to utilize energy. One of the most important of these factors is the ambient temperature. All animals have a range of temperatures in which they do not have to increase their metabolic rate to maintain body homeostasis (a stable inner body environment), called their thermoneutral zone (TNZ). If the surrounding temperature exceeds the upper critical temperature (UCT – the upper temperature within the thermoneutral zone), extra energy must be used to get rid of excess heat (e.g. through panting and sweating) (Hill et al. 2018). This followingly causes the metabolic rate to increase. If the surrounding temperature falls below the lower critical temperature (LCT – the lowest temperature within the thermoneutral zone), the metabolic rate also increases. This because the body then has to produce extra heat to maintain a stable body temperature (Blix, 2005).

Food consumption and feeding rate are also factors that affect the metabolic rate of animals. First of all, food consumption increases the metabolic rate. This effect is named specific dynamic action (SDA), and is observed as a peak in the metabolic rate for some hours after food has been consumed. Though not all mechanisms causing the SDA are known, it is known that the process of digestion has a role in this increase of energy expenditure after feeding. It is also believed that deamination of amino acids in the liver is a major factor in the SDA-phenomenon (Buttery and Annison, 1973; Lavigne et al., 1982). The duration of the SDA depends on the composition and amount of food consumed, but after 10 hours the SDA should no longer be significant in terms of measuring the total metabolism (Gallivan & Ronald, 1981). Just as consumption of food increases the metabolic rate, not consuming food can also affect the metabolism. Going long periods of time without food can cause a fasting-induced metabolic depression so that energy can be preserved when food availability is scarce (Nordøy et al. 1990; Rosen & Trites, 2002).

1.2.2. Measuring metabolic rates: why and how we do it

For humans, one of the most important aspects of studying the metabolism and estimating the metabolic rates of various animals is related to sustainable management and optimalization of the economy of animal-related management by increasing the income and decreasing the expenses as much as possible. Estimations and models on metabolic rates are used to aid quota-calculations on various species each year. Some of the most important factors for calculating a quota for a species, is to know its reproductive rate (how quickly it reproduces and how stable the reproduction is) and to know its mortality rate (Hentati-Sunberg et al., 2014). Knowing the metabolic rate of the animal and its potential prey- and predator-species can help in assessing both factors. An example of how this is used in practice, could for instance be related to quota-calculations for Atlantic herring (*Clupea harengus*). If you know the energy requirements of the herring throughout the year, one can estimate the food that is required for it to survive, and this can then be put in correlation to population estimates and its reproductive rate to estimate a new population size based on catches and by-catches. The survival rate based on access to prey-density can then also be estimated, taking inter- (based on density of other species consuming similar prey-species) and intraspecific (based on population density) competition into account. Knowing the energy requirements and population size and density of the species predating on herring also help in assessing its mortality rate. By estimating how much herring the different predator species consume (e.g. seals and whales), one can followingly estimate the herring-mortality. Using all of this information to estimate the total reproduction and mortality can lastly be used to assess and calculate how much herring can be fished for it to be economically advantageous, while also maintaining the herring-population (Hentati-Sunberg et al., 2014).

Another example on how the metabolic rates of animals can be used in management, is seen in hooded seal-management. Many species have been affected by the climate change observed the more recent years, this including several Arctic animals that often require quite specific environments for their survival. It is expected that there will be declines in both harp and hooded seal populations (Learmonth et al., 2006; Kovacs & Lydersen, 2008), but due to the specific breeding pack-ice preferences for hooded seals (thick, solid pack-ice), they are considered to be one of the most sensitive species to climate change as a result of ice melting (Laidre et al. 2008). As a result, there are several management-procedures in play to maintain the population and getting data on their metabolic rate is an important factor to manage the

population properly. It is also important to have a good knowledge about the rest of the biology and life strategies of the seals when assessing their energy needs throughout the year, given that the energy requirements could be quite different depending on what time of the year it is. The metabolism can change substantially throughout the year, and if only one or very few measurements throughout the year are used to generalize for the entire year, the results could end up being far off from their realistic yearly energy utilization and requirements. This was demonstrated in a study conducted on adult harp seals by Lager, Nordøy and Blix (1994), where the food intake changed drastically throughout the year, reaching a top in the late summer/early autumn, while a bottom in food consumption was observed during winter. During the study the animals were fed ad libitum throughout the year, meaning that they could eat as much as they wanted. Reasons to

Several methods for measuring metabolism exist at this date, and these methods are divided into two main categories: direct and indirect calorimetry. While direct calorimetry measures the heat released from an individual directly, indirect calorimetry takes use of a proxy to measure the metabolic rate (e.g. O_2-consumption and/or CO_2-production). Though direct calorimetry tends to be more accurate in the metabolic rate-measurements, it can be not only expensive, but also quite complicated to set up, and indirect calorimetry is therefore used for many metabolism-studies on various organisms, especially larger ones (Hill et al. 2018). Methods for indirect calorimetry can either be in a closed circuit (e.g. in a respiration chamber), which means that no surrounding air enters the system, or it can be in an open circuit, with surrounding air entering the system. The open circuit, indirect methods of calorimetry include the expired gas-analysis.

There are several factors that should be considered when choosing a proxy for calculating the metabolic rate indirectly. First of all, the energy production is dependent on what foodstuffs that are being utilized. Looking at table 1, it is clear that using O_2 as a proxy should be more accurate if there is a mixture of foodstuffs compared to CO_2, given that the differences in energy production depending on substrate is smaller for O_2 than for CO_2. Because of this, oxygen is considered the ‘gold standard’ as a proxy for metabolic measurements (Hill et al. 2018). On the other hand, if O_2 cannot be used as a proxy, CO_2 is still a good alternative as proxy for indirect metabolic rate calculations.

Table 1: Overview of the ratio of the energy produced (Joules/ml) to O_2-consumtion and CO_2-production, depending on the substrate/foodstuff used for energy production (Brown & Brengelmann, 1965; Hill et al. 2019).

Substrate	J/ml O_2	J/ml CO_2
Fat	21,1	21,1
Proteins	19,8	27,9
Carbohydrates	18,7	23,3

1.2.3. Fat metabolism

Our bodies consist of many organs and tissues that all have varying energy requirements to function properly. This is because some of the tissues and organs have to do a lot of work (e.g. the heart that has to pump blood to the lungs and out to the body continuously, and the kidneys that have to produce a lot of urine to get rid of nitrogen-waste from the food we have consumed etc.), and they therefore require much energy to function properly (Wang et al. 2010). Other tissues, like adipose tissue, do not require as much energy, this mainly because it mainly serves as an energy storage for the rest of the body and only needs energy to produce triglycerides from fatty acids and glycerol, which then will be stored until the energy reserves are needed (Ahmadian et al. 2007). Studying fatty tissues and the mechanisms involved in fat metabolism is, though, still important. More knowledge on the topic can be used to develop new methods to help treat and prevent obesity and obesity-related problems and diseases, which has become a bigger problem among humans in the recent years (Ahmadian et al. 2007).

The topic of fat metabolism and how metabolically active fat is compared to the lean body mass (LBM) has been studied by several. It is a subject that is much discussed, with conflicting results on the metabolic contribution of fat. A couple of studies done on seals have found that fat is metabolically inert relative to the LBM. Among them is Rea and Costa (1992), who found that elephant seal pups had a significant decrease in their weight-specific metabolism, with a decrease of 47% when the body fat had an increase from 5 to 50%. This means that the heavier pups, with more body fat, had a relatively lower metabolic rate

compared to the smaller pups with less body fat. It has also been found that for adult harp seals, the weight-specific basal metabolic rate (BMR) decreased from 2,3 Watts/kg to 1,1 Watts/kg when their fat percentage went from 13% to 45% (Aarseth et al. 1999). This indicates that the weight-specific BMR was less than half with a fat percentage increase of 32%. Hence, both of these studies conducted conclude with fat being metabolically inert relative to the LBM.

On the other side, there is a study published by McNiven (1984), where she found that for sheep, there was no significant difference in the metabolic activity in fatty tissues and the LBM. The conclusion was that in this case the LBM and fat contributed almost equally to the total metabolic rate. On the other hand, it is important to note that the results could be different depending on species. Also, sheep and seals could have different types of fat (saturated vs. unsaturated), which in turn could affect the metabolic rate differently (e.g. van Marken Lichtenbelt & Westerterp, 1997). It is, though safe to say that there is more information to find on fat-contribution to the metabolism, and that it must be studied to a greater extent to get a better understanding on how fat acts metabolically. More knowledge on this topic is important to understand how the body composition and body mass can affect the energy needs of animals. This can in turn be used to more accurately calculate the animals' food requirements and aid the development of more sustainable and economically advantageous animal management.

1.3. Aim of study

Because there are some conflicting results with regard to how the fat metabolism contributes to the total metabolism, my aim is to look at the correlation between fat indices and the metabolic rate for hooded seal pups. The hooded seal pups gain a lot of fat during their short nursing-period, which makes them excellent model animals for studying how fat percentage affect the metabolic rate. Because they gain large amounts of fat through mainly fat deposition in a time span of only a few days, it creates a possibility to study the metabolic rate in two natural states of nutrition without it being affected by seasonal changes in the metabolism. The pups are otherwise more or less in the same state, making them excellent models for looking at how various fat percentages affect their metabolic rates. In this study, the metabolic rate of 12 hooded seal pups was measured indirectly by observing their oxygen

consumption and carbon dioxide production, as well as their weight and length were measured. Data from previous years (2007-2019) was also used with a goal of finding a correlation between fat percentages, body mass and body length for more individuals at various nutritional stages, given that specific fat percentages were not measured or estimated for most of the individuals with metabolism measurements.

2. Materials and methods

Because the cruise with R/V Helmer Hanssen to the West Ice was cancelled due to COVID-19 and the following restrictions, I did not get to perform the practical part of the masters. Instead, I used information on the methods used, which were explained in reports from earlier cruises, as well as additional, more detailed information on the execution from Lars. I also have some experience with practicing metabolism measurements using a set-up like that used for the data collection in 2019. Data organization and analysis is, on the other hand, done first-hand.

R/V Helmer Hanssen arrived at the western coast of Greenland the 20th of March 2019. Between then and the 25th of March there were 12 hooded seal pups captured, all at different stages in their postpartum development (between 71°49.705N - 72°52.740N and 15°56.010W - 17°26.199W). The metabolism measurements of the hooded seal pups were completed by Lars Folkow.

As a measure to get more data on length, weight and fat percentage on hooded seal pups, data gathered on cruises with R/V Helmer Hanssen from 2007 to 2019 was used to make models for the relationship between fat percentage and body mass, as well as between fat percentage and a chosen condition index (body mass/body length). This condition index is used because several studies have shown that there is a significant correlation between the fat percentage and this condition index for various seals (Reilly and Fedak, 1990; LeBoeuf et al. 1994; Arnould, 1995; Hall et al. 2001).

2.1. Animals from 2007 to 2019: length, weight and fat-measurements

2.1.1. Capturing and basic measurements

Basic measurements were done on all pups captured. For length, the curvilinear length was measured, meaning the length from the snout, over the body and to the root of the tail (fig. 1). Hence, the length-values used in this thesis is the *curvilinear length*, not the standard/linear length. This was measured using a measure tape. Additionally, the body mass was measured

using a DHS crane weight (Dini Argeo Scales – Weighing Systems, Italy) with a maximum weight capacity of 60kg and a resolution of ±0,15%. The pups were then put in a sack that enables breathing while confined, which then was hooked onto the crane weight. The sack weight was later subtracted from the total weight, leaving us with the body mass of the pup.

Figure 3: The black line illustrates the curvilinear length (CL) when measured on a hooded seal pup. Illustration of hooded seal pup from Jefferson et al. 2015. Paint used to make illustration of curved length.

2.1.2. Fat percentages

Fat percentages for hooded seal pups captured between 2007 and 2019 were found by weighing the fat mass of a few individuals that were dissected. Only a few individuals were dissected each year, as a part of a student-exercise in the course BIO-2310 (Arctic biology). Hence, to get more data on fat percentages, a model was made using the fat percentage-data for all years combined, a total of 24 individuals. The same was done with length-data related to fat percentage for 16 individuals captured between 2007 and 2019.

2.1.2.1. Dissection

Before dissection, the pups were euthanized with a hakapik and bled out. First, an incision was made from the top of the neck down to the anus using a sharp knife. Then the skin was flayed off with as much blubber as possible. The rest of the visible blubber on the remaining core body was removed, and the blubber was separated from the skin using sharp knives and scalpels. Once all of the blubber had been separated from the rest, it was all weighed. Prior to further dissection, the entire core body was weighed. All organs were then weighed: the heart, lungs, brain, liver, kidneys, the entire digestive system, spleen, reproductive organs, gallbladder, lymph nodes and eyes. Further on, the muscles were separated from the skin and

bones using knives and scalpels, also to be weighed separately. This left the skeleton, that was weighed in the end, when all other tissues had been removed. In the end, all of the separate weights were added together and compared to the total body mass of the pup prior to euthanasia, so that the total blood loss could be estimated.

For this thesis, the fat mass weighed during these dissections could be used to compare the total body mass prior to euthanasia. This could in turn be used to calculate the fat percentage using the following equation (eq. 1):

$$\boldsymbol{Eq.1}:\ Fat\ percentage = \frac{Fat\ mass}{Total\ body\ mass} * 100\%$$

Where fat mass and total body mass both are given in grams (g). The fat-percentage data was further used to make models that in turn were used to predict the fat percentage for the hooded seal pups captured in 2019 lacking this data.

2.1.3. Model-estimations

In 2019 there was a total of two hooded seal pups that did not have their length-measurement registered, as well as 10 hooded seal pups lacking data on fat mass. Hence, so that the data from 2019 could be used for further analysis on how fat contributes to the total metabolism of the pups, linear regression models were made on fat percentage and its correlation to total body mass and a chosen condition index (CI = BM/BL). This was done by using data on fat mass, body length and total body mass for the hooded seal pups captured between 2007 and 2019.

The equations from the linear regression models were further on used to estimate the fat percentages for the hooded seal pups lacking fat percentage-data, as well as to estimate the curvilinear length of the pups lacking length data. For the individuals lacking length data (table 3), the linear regression model for the correlation between fat percentage and body mass (fig. 11) was used for predictions. For the individuals lacking fat-percentage data (table 3), the linear regression model for the correlation between fat percentage and CI was used for predictions (fig. 12).

2.2. Animals from 2019: metabolism-measurements

As previously mentioned, hooded seals typically give birth and breed on thicker, less accessible ice floes compared to e.g. harp seals. Hence, R/V Helmer Hanssen had to press through a lot of sea ice before the hooded seals were reached. When the seals then were reached, they were brought on board the ship and put in pens (length = 114cm, width = 57cm, height = 61cm), that were filled with some snow so that they would have an enriched environment and have access to freshwater during their post-weaning fast.

2.2.1. Conditions for measuring metabolic rates

To compare the resting metabolism of all the pups, they should ideally be as similar as possible in their state. Firstly, they should be as calm as possible, not being stressed and/or moving around. Secondly, they should ideally be within their thermoneutral zone (TNZ). During metabolism-measurements the temperature in the metabolic chamber was kept at 2–5 °C, which was assumed to be within their TNZ based on thermophysiological data for newborn pups of other seal species (Nordøy & Blix, 1985; Hansen, 1995; Boily et al. 2011).

In addition to being in their thermoneutral zone, the hooded seal pups were also post-absorptive, meaning that their metabolism was not be affected by the Specific Dynamic Action (SDA) when measuring the sleeping metabolism. The hooded seal pups captured were kept in their pens until the following day before metabolic measurements were performed, and the SDA should therefore not have affected the metabolic rate. For optimal metabolism-measurements, the pups should not have been fasting for an extended period before the measurements either, given that it can cause a metabolic depression (Nordøy & Blix, 1985; Rosen & Trites, 2002). For the pups found while nursing, this was not an issue, but for the weaned pups it was more of a challenge to assess how long they had been fasting. Hooded seal pups are typically born late in March, early April. Given that the R/V Helmer Hanssen arrived around the 20th of March, it is unlikely that the weaned pups captured had been fasting for longer than a couple of days at most.

Lastly, the pups should ideally be as calm as possible, resting. Since I did not get to perform the metabolic measurements myself, and followingly did not get to observe the state of the

pups during the metabolic measurements, a standard for all individuals was used: when sleeping. Hooded seals, and seals in general, have a characteristic sleep-related apnoea breathing pattern (Castellini, 1996; Andrews et al. 1997; Falabella et al. 1999; Cummings et al. 2015). They then stop breathing for a short while, until they abruptly start breathing in lots of air again, only to stop breathing again, causing the following pattern of oxygen consumption (fig. 4). This was followingly used as a standard for the metabolic rates calculated in this thesis. It is also important to mention that the start and end of each period chosen was in the same cycle of the apnoea-pattern, so that the result would not be affected by a shift in the phases chosen.

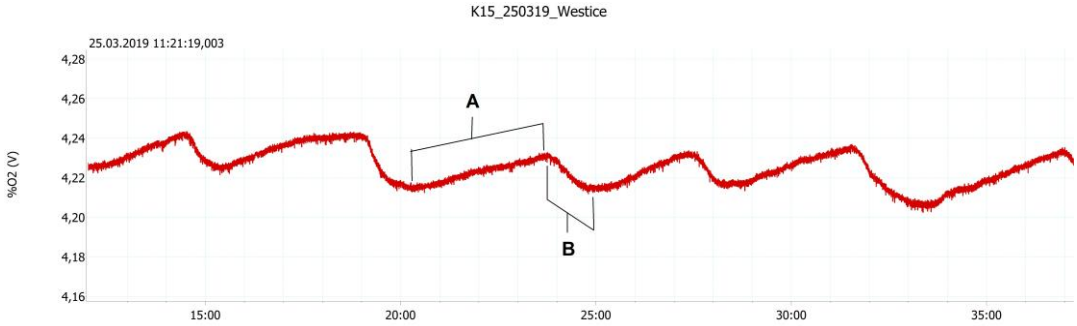

Figure 4: Typical sleeping pattern of hooded seals. Sleep apnoea, where the O2-levels increase as they hold their breath (A), and it decreases abruptly when they start breathing again (B). The x-axis shows the registered O_2-levels in the gas, given in Volts, and the y-axis shows the progression of times in minutes. This pattern typically repeats itself as the seals sleep. This is a part of the measurement done on pup K15.

2.3. Set-up

The metabolism-measurement was done using an open circuit, expired gas-analysis. This means that the system was open, and the surrounding air was pulled into the chamber in which the animal was placed. This caused there to be a mixture of ambient air and expired air from the animal, which was what entered the rest of the system for analysis (Fedak, 1981). The animal will take up O_2 from the surrounding air, and it will release CO_2 it has produced. This causes the air mixture from the reference air entering the system (atmospheric air) to change its composition due to changes in the O_2- and CO_2-proportions. These changes in O_2 and CO_2 caused by the presence of the animal is what is used as a basis for a calculation of its

metabolic rate. Hence, the volume of oxygen consumed (VO_2) and/or the volume of carbon dioxide produced (VCO_2) can be used as proxies when indirectly measuring the metabolic rate. There was a pump at the end of the system, which caused the pull and flow of air, with a continuous negative pressure throughout the system. This type of system is not very sensitive to leakages, given that the system either way was open to the surrounding air, and ambient air leaking *into* the system would not affect the results. The important factor was that the expired gas cannot leak *out* of the system, but because of the continuous negative pressure throughout the system, that was very unlikely to happen.

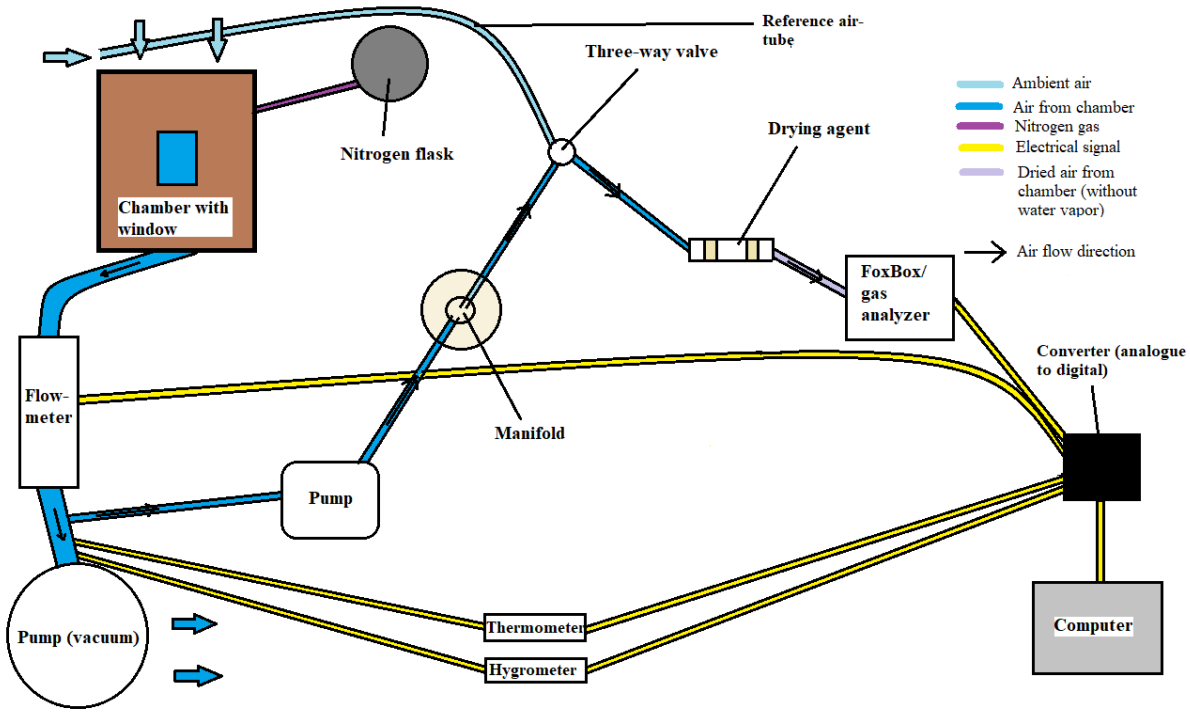

Figure 5: Sketch showing the schematic setup of the metabolism measurement-equipment and the flow through the system. The pup is places in the chamber up in the far left, where surrounding air enters the system through holes in the chamber. The vacuum/pump pulls the ait from the chamber through the tubes and flow meter, and a subsample is drawn by another pump after the flow meter. Further, the air is led to the manifold, which stabilizes the air pressure, and the air is lead from there through the three-way valve and to the drying agent (silica gel and calcium chloride). As the air then is dried, it can enter the gas analyser (Foxbox) for measurement of O_2 and CO_2 values in the air. The relative humidity and air temperature is measured using the hygrometer and thermometer respectively. All of the measurement tools (flow meter, hygrometer, thermometer and gas analyser) are connected to the A/D-converter, that converts the analogue signals to digital ones, making it possible for the computer to translate it to understandable units.The three-way valve also makes it possible to switch between chamber air and reference air, so that the comparison between the two easily can be made. Lastly, the flask with N_2-gas leads to the chamber with a tube, so that calibration through nitrogen dilution can be done after the animal is removed.

First off, the pups were placed in a wooden chamber (height = 61cm, width = 57cm, length=114cm) with a window on the top, so that the behavior of the pup could be observed, making sure that it stayed calm and that it was ok during the experiment. On the backside of the chamber there were four air-holes (diameter = 40mm) on the top of the wall, so that the surrounding air could be pulled into the system. There was also a small room in between the outer air holes and the chamber in which the pup was placed (length = 15cm), so that there was no risk of the pup breathing directly out of the holes. The separating wall also had four air holes (diameter = 40mm) at the bottom, so that the air could pass through to the pup without any outflow of the air it expires. On the other end of the box, there was a thick, enforced plastic tube with an inner diameter of 36mm and outer diameter of 47mm (Sable Systems International, USA) connected, which led the mixed air from the chamber to the flowmeter (In-Flow mass flow meter, Bronkhorst® High-Tech, Holland), that then measured the total air flow continuously. The flow was generated by a pump, a S6390 HEPA Silence vacuum (Miele, Germany), that was connected to the flowmeter using a thick tube like the one used from the chamber to the flowmeter. From this tube, there was a smaller marprene-tube (Watson Marlow Tubing, England) connected (Inner diameter = 1.6mm, Outer diameter = 3.2mm). Marprene-tubes are used because they are diffusion-proofed, hindering leakage of sample-air. A subsample of the mixed air from the chamber was pulled out by a pump, and then lead to a manifold. The gas-analyzer is sensitive to pressure fluctuations, and the manifold helps dampening these fluctuations. The air flow was high enough for the manifold to be completely filled with subsample-air, so that no ambient air can enter. From the manifold another marprene-tube leads the air from the manifold to the gas-analyzer. This flow is generated by the Foxbox

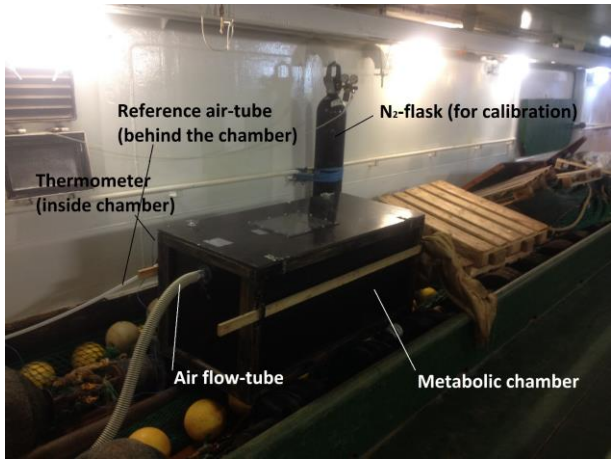

Figure 6: Picture of the setup of the metabolism measurement-equipment. Photo by Lars Folkow.

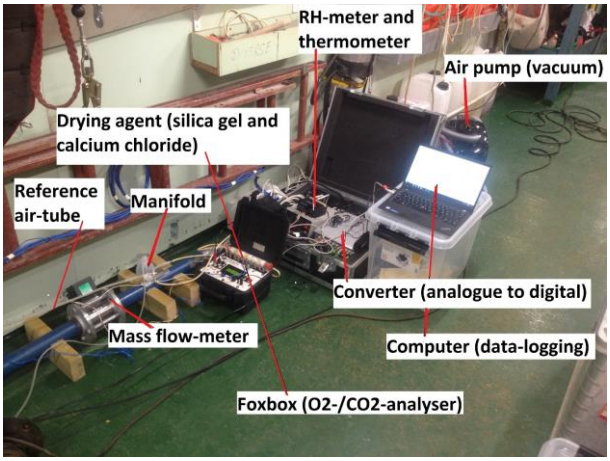

Figure 7: Picture of the setup of the metabolism measurement-equipment. Photo by Lars Folkow.

(Sable Systems International, USA), the gas-analyzer, that pulls the air from the manifold at a rate of between 1450-1550 ml/min, with a resolution of 1 ml/min.

Prior to the Foxbox, there is a three-way valve, which makes it possible to switch between the air from the chamber and the reference air (the surrounding air). Because the differences in O_2- and CO_2-levels between the expired air and the reference air is the basis of calculating the individuals' metabolic rate, this is very important. Next, there is a cylinder with drying agents connected to the tube prior to the Foxbox, filled with calcium chloride -$CaCl_2$ (Merck KGaA, Germany) and silica gel - SiO_2 (Sigma-Aldrich®, USA). This has to be done because the Foxbox is sensitive to water vapor, due to the O_2-analyzer consisting of burning cells that will produce electricity and heat if water reacts with them, which can cause overheating. The silica gel has a dual purpose, both serving as a drying agent and an indicator for when the drying chemicals are saturated with water and must be replaced. When efficiently drying, the silica gel is yellow in colour, and when it is saturated it is clear. After the air has passed through the drying agents, the dried air then goes through the Foxbox, where it first goes through the burning cells in the O_2-analyzer, then the infrared the CO_2-analyzer. Lastly, the air is pushed down to the bottom of the Foxbox to let the air out.

In addition to the flow and O_2- and CO_2-levels, the relative humidity (RH) and air temperature (T_{air}) was measured using a HMI32-hygrometer (Vaisala, Finland). The sensors were placed in the airstream at the outlet of the flowmeter, in order to obtain data on air humidity and temperature at the point at which the flow was determined. This is important because the temperature and relative humidity of the air is affected by the air pressure, and therefore also the flow. The temperature of the air was also measured using a separate AD595 CD-thermometer (Analog Devices, United States). T_{air} and RH of the air was then later used to decide the water vapor flow, which is needed to correct the total flow. The flow measured throughout the measurement includes the water vapor flow, and when looking at O_2- and CO_2-levels (where the air has been dried), the flow used for further calculations should exclude the flow of the water vapor. The exact calculations for this correction will be explained in the "calculations"-section.

All of the measuring equipment (flowmeter, Foxbox, hygrometer and thermometer) was connected to an ML796 PowerLab/16SP A/D-converter (ADInstruments, New Zealand), that

translated the analogue signals to digital signals that could be used by the computer and registered on the computer using Labchart® 8 (ADInstruments, New Zealand). The electrical signals received from the equipment is translated to voltage when registered by the computer. The voltage registered for each port (O_2-analyzer, CO_2-analyser, hygrometer, flowmeter, thermometer) is proportional to the O_2- and CO_2-percentages, relative humidity, air flow and temperature.

2.3.1. Calibration-procedures

A calibration method for this type of open circuit, expired gas analysis-system includes using pure nitrogen gas (N_2) that is leaked into the system at a known rate. This method, called one-step N_2-dilution technique (Fedak et al. 1981), is used to see how much a certain flow of N_2-gas corresponds to of the total flow in voltages. The calibration was done by first removing the animal from the chamber, and then leaked in N_2-gas at a known rate (2L/min) into the chamber using a FMA5518A mass flow controller (Omega Engineering Inc., United States) and let the rest of the system run as normal. Knowing that the atmosphere has approximately 20.95% O_2 (Hill et al. 2018), you know that if you have a total air flow of 150 L/min, you will have 148L of air with 20,95% O_2 and 2L with 0% O_2 (pure N_2-gas) passing each minute. This indicates that a reduction of 419ml (2 x 209,5ml) O_2-gas per minute from the reference air to the air diluted with 100% N_2-gas. Based on this knowledge, one can use the difference observed in voltage to calculate how much 1mV corresponds to in percentage of O_2-gas by using the following equation:

$$\boldsymbol{Eq.\,2}\text{: } \frac{\%O_2}{mV} = \frac{\Delta O_2}{\Delta voltage}$$

Where ΔO_2 is the difference in oxygen percentage between the reference air and the air during a nitrogen bleeding, and Δvoltage is the difference in voltage (measured in mV) between the reference air and during the nitrogen bleeding. This can be used further find the total oxygen consumption (which in turn indirectly indicates the metabolic rate). Exactly how this is done, will follow progressively in the methods-section.

To find the difference in % O_2 from the reference air to the nitrogen bleeding, the following equation is used:

$$\textbf{Eq. 3}:\ O_2(\%) = \frac{(Flow - 2) * F_iO_2}{Flow}$$

Where F_iO_2 is the inspired, or ambient, oxygen percentage (20,95%), and the flow is the average flow during N_2-bleeding corrected for water vapor given in L/min. It is corrected for water vapor, given that the gas analyzed by the gas analyzer is dried prior to analysis. The water vapor flow is calculated by using the following equation:

$$\textbf{Eq. 4}:\ Flow, H_2O = Total\ flow * \frac{\frac{RH}{100} * 4{,}588 * 10^{\frac{7{,}59 * T_{gas}}{240{,}78 + T_{gas}}}}{BP}$$

Where the total flow is the flow originally measured by the flowmeter and is given in L/min, RH is the relative humidity of the gas (measured with hygrometer) of the air, T_{gas} is the gas temperature in °C (measured with thermometer) and BP is the barometric pressure given in mmHg (Data measured each hour at R/V Helmer Hanssen, used the value closest to the measurement for each individual). With this value, one can find the correct flow for the O_2- and CO_2-voltages observed, by subtracting the value from the total flow:

$$\textbf{Eq. 5}:\ Corrected\ flow = Total\ flow - flow, H_2O$$

In addition to calibrating the O_2-measurement, the CO_2-measurement was also calibrated, to allow conversion of the analogue output of the Foxbox to understandable units, in terms of % CO_2. This is done after the nitrogen bleeding is finished, by again adding 100% N_2-gas purely into the system, so that the CO_2 (and O_2)-levels in the air analyzed is 0%. By doing this, the difference in voltage before and after the switch to 100% N_2-gas can be used in the same way that for the N_2-bleeding and O_2-calibration, using the following equation:

$$\textbf{Eq. 6}:\ \frac{\%CO_2}{mV} = \frac{\Delta CO_2}{\Delta voltage}$$

Where ΔCO_2 is the difference in carbon dioxide-percentage between the atmospheric air (0,0417775%, average of data from: https://scrippso2.ucsd.edu/osub2sub-data.html, https://www.esrl.noaa.gov/gmd/ccgg/trends/data.html) and during the calibration (0%), and Δvoltage is the difference in voltage from before and during calibration. Ideally the voltage should be 0 at 0% CO_2, and if the voltage is above that, this calibration corrects for that. All

in all, the entire setup of the metabolism measurement-equipment, as well as the flow of air throughout the system is illustrated in figure 5.

2.4. Choice of proxy for the metabolic rate

The conditions during this study makes the choice of proxy a little more complicated. Because there is a considerable drift in the system with regard to O_2, in addition to the drift not being constant, choosing O_2 as a proxy could potentially lead to a larger error rate than by using CO_2 as a proxy. Since it in this case is not known how the drift could have changed during the metabolism-measurements, the proxy used in this study is instead CO_2. The CO_2-measurements are not affected by a drift, and it is in general quite stable. Considering that the individuals also should be post-absorptive, using CO_2 as a proxy in place of O_2 seems like a logical choice to make.

2.5. Calculations

As previously mentioned, I have used data from periods with standardized activity only, consisting of sleeping, as identifiable from the very clear signs of sleep apnoea confirmed by visual inspection (fig. 4). The periods selected were the ones where the stability of the measurement was apparent (without too many abrupt changes from the apnoea-pattern). The time periods chosen lasted for a minimum of 15 minutes. <The period-length chosen for each individual is shown in table 1.

A typical O_2-data output, with an unstable run recording baseline during a metabolism measurement is shown in figure 8. You see the characteristic apnoea-pattern, observed in the O_2-measurement (red line), with a rising voltage/oxygen level when the individual holds its breath during sleep, and a quick decrease in the voltage/oxygen level when it abruptly breathes in again. It is also observed in the CO_2-measurement with the opposite pattern (fig. 9): a decrease in the voltage/carbon dioxide level when the individual holds its breath and an

abrupt increase in the voltage/carbon dioxide level when it abruptly breathes again. This type of period shows a template of a typical chosen period for each individual.

The difference in gas-percentage between the baseline (F_iO_2 and F_iCO_2: gas-percentage of the reference air) and the average of the gas-percentage during the period chosen is what is used as the basis for the calculation of the individuals' metabolic rate (F_eO_2 and F_eCO_2: gas-percentage of the air mixed with expired air from the animals). Because of the unstable baseline for O_2-measurements, VCO_2 was instead used as the main proxy. Regardless, VO_2 was also calculated with means to compare the values obtained using VCO_2 to when VO_2 was used. To correct for the drift in the O_2-measurements, a line was drawn from the first reference air-point to the next, then a parallel line was placed under the first line at the point where the area over and under the graph was the same (figure 8). An area-tool was used in a program called SketchAndCalc (https://www.sketchandcalc.com/, Elliott M. Dobbs, 2011) to make sure that the area over and under the line was the same. The distance was then measured as accurately as possible using a ruler. Next, the difference in voltage between two specific areas on the O_2-line, as well as the distance in mm between these two points (e.g. point A and B in figure 8) was measured. This data was then used to calculate what 1mm translated to in voltage (eq. 7).

$$\boldsymbol{Eq.7}: Volts\ per\ mm = \frac{\Delta V}{\Delta mm}$$

Where ΔV is the difference in voltage (mV) and Δmm is the difference in mm between the two points. With this information, one can use the ratio to calculate the difference in voltage between the top line and the average line (eq. 8).

$$\boldsymbol{Eq.8}: \ \Delta O_2 = volts\ per\ mm * distance\ measured\ with\ ruler$$

Where volts per mm is the value given by eq. 7, and distance measured with ruler is the distance between the two parallel lines given in mm (line between the two reference air-measurements and the line for the average O_2-percentages during chamber air-measurement).

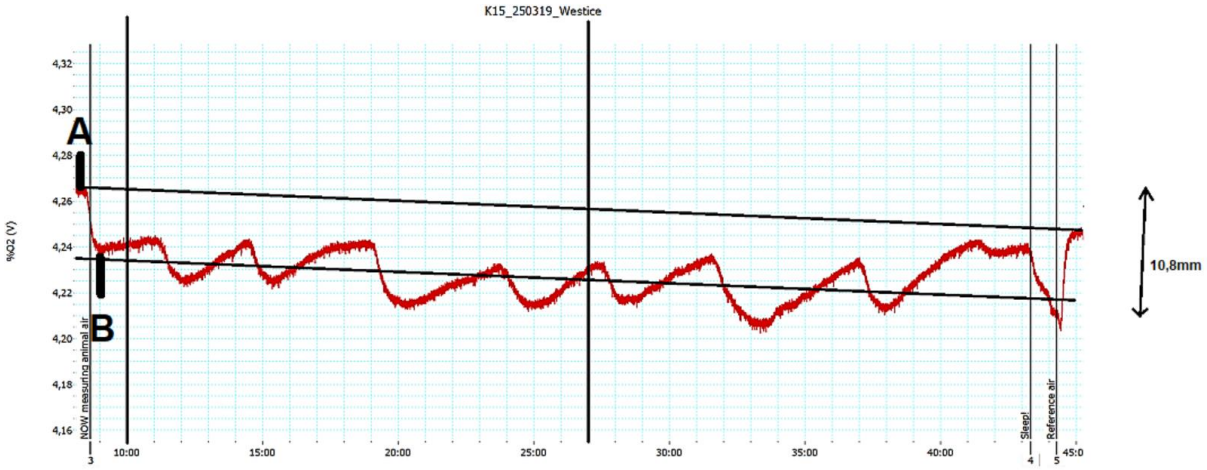

Figure 5: An example showing how the average voltage-difference between the reference air and the expired air was calculated for pup K15. The y-axis is the O_2-percentage expressed in volts, the x-axis is the progression of time (minutes). Point A and B are the two point chosen to calculate how much 1mm is in voltage for this specific graph. The two-sided arrow and the belonging value represents the distance between the two parallel lines.

With the data on how much 1 mV corresponds to in % O_2 and % CO_2 (eq. 2-6) for each individual's measurement, one can than translate the voltage to % O_2 and %CO_2. With these percentages, one can calculate the total O_2-consumption using the following equations published by Lighton (2018):

$$\boldsymbol{Eq.9}\text{: } VO_2 = Flow\ rate * \frac{(F_iO_2 - F_eO_2) + (F_iO_2 * (F_eCO_2 - F_iCO_2))}{1 - F_iO_2}$$

Where:

VO_2 = O_2-consumption by the animal given in L*O_2/min

VCO_2 = CO_2-production of the animal given in L*CO_2/min

Flow rate = flow rate corrected for water vapor (eq. 5) given in L/min.

F_iO_2 = O_2-percentage of inspired air (reference air)

F_eO_2 = O_2-percentage of expired air (percentage calculated based on voltage)

F_iCO_2 = CO_2-percentage of inspired air (reference air)

F_eCO_2 = CO_2-percentage of expired air (percentage calculated based on voltage)

In contrast to the baseline for the O_2-output, the baseline for the CO_2-output is stable. Because of this, it is enough to know the difference in CO_2 voltage output and use eq. 6 to translate to CO_2-percentage for each individual, and then calculate the total CO_2-consumption using the following equations published by Lighton (2018):

$$\boldsymbol{Eq.\,10}:\ VCO_2 = Flow\ rate * \frac{(F_eCO_2 - F_iCO_2) + (F_iCO_2 * (F_iO_2 - F_eO_2))}{1 + F_iCO_2}$$

Where:

VO_2 = O_2-consumption by the animal given in L*O_2/min

VCO_2 = CO_2-production of the animal given in L*CO_2/min

Flow rate = flow rate corrected for water vapor (eq. 5) given in L/min.

F_iO_2 = O_2-percentage of inspired air (reference air)

F_eO_2 = O_2-percentage of expired air (percentage calculated based on voltage)

F_iCO_2 = CO_2-percentage of inspired air (reference air)

F_eCO_2 = CO_2-percentage of expired air (percentage calculated based on voltage)

Further, the VO_2 was also calculated using VCO_2 and the respiratory quotient (RQ) of the animals. This was used as the main data for the metabolic calculations in this thesis. The reason for this, is the fact that there was a considerable drift in the system for the O_2-measurements (see fig. 8), while the CO_2-measurements did not (see fig. 9). Since the study animals included in the study were in a post-absorptive state (The SDA should not affect the metabolic rate), the RQ should theoretically be approximately 0.7, given that they in theory mostly should metabolize their stored fat (Kleiber, 1961; Blix, 2005; Hill et al. 2018). The RQ was calculated for each individual using the following equation:

$$\boldsymbol{Eq.\,11}:\ RQ = \frac{VCO_2}{VO_2}$$

Where VCO_2 is the production of CO_2 and VO_2 is the consumption of O_2. To get a more accurate RQ-value for each individual, the RQ was calculated for each time there was a switch from reference air to the expired air from the animal, and the average of these values was used for further calculations. This was done because there could have been small variations in RQ due to a slight mismatch in rate of CO_2-production and O_2-consumption, seen that the air analysed passed through the O_2-analyzer before passing through the CO_2-analyzer. This was done by converting voltages to O_2/CO_2-percentages, respectively, and then inserting the values into eq. 9 and 10 and lastly into eq. 11 to obtain the RQ-values. To reduce errors in the values due to the unstable baseline in the O_2-measurement, the O_2- and CO_2-values chosen were the ones right before and after the switch from reference air to chamber-air, as well as right before and after the switch from chamber-air to reference air (fig. 9). By doing this, the time between A and B, as well as between C and D shown in figure 9 is so short that it is expected that no major drift could have occurred within this time slot. Lastly, the values for O_2-consumption and CO_2-production are used to calculate the RQ for that individual at those points (eq. 9)

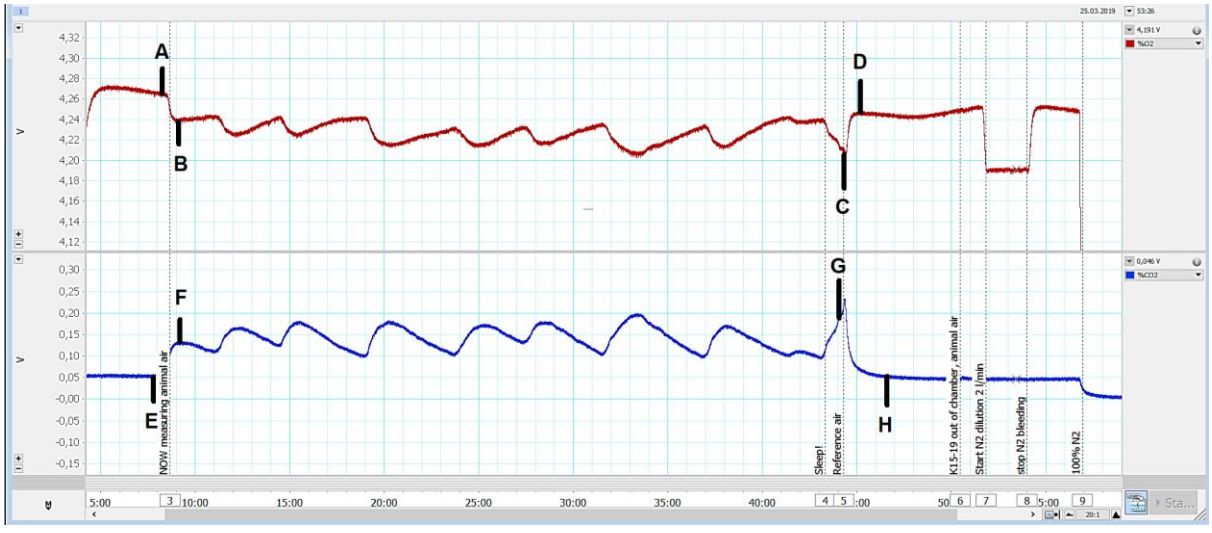

Figure 6: Illustration of how RQ was calculated using Labchart. B is subtracted from A, and the value is then multiplied with the mV to O_2-percentage ratio for that individual. Then E is subtracted from F, where the value is multiplied with the mV to CO_2-percentage ratio for that individual. The value for VCO_2 is then divided by the value for VO_2, whick gives the RQ. The same is then done by subtracting C from D for O_2 and by subtracting H from G for CO_2.

The metabolic rate can further on be calculated using the VO_2 calculated. First, the energy used per litre of O_2 is calculated using the following equation published by Lighton (2018):

$$\boldsymbol{Eq.\,12}: Energy/\,LO_2 = 16 + (5{,}164 * RQ)$$

Where the energy/LO_2 is the energy produced per liter oxygen consumed (given in Joules (J)), and RQ is the respiratory quotient calculated separately for each individual. When the energy produced per liter consumed is known, this can further be used to calculate the metabolic rate using the following equation:

$$\boldsymbol{Eq.\,13}:\ MR = \frac{VO_2 * \frac{energy}{LO_2}}{60}$$

Where MR is the metabolic rate given in Joules per second (J/s), VO_2 is the O_2-consumption, either calculated directly from VO_2 (eq. 7) or indirectly using VCO_2 and RQ (eq. 8 and 9), and it is given in L/min. Energy/LO_2 is, again, the energy produced per litre of O_2 consumed, and it is all divided by 60 s/min so that the unit is J/s (Watts - unit of metabolic rate) instead of J (unit of energy).

Lastly, the presentation of the metabolic rates in this study is given as the weight-specific MR (Watts/kg), so that it is easier to see the relative differences between the metabolic rates of the different hooded seal pups studied. This is simply done by dividing the metabolic rate with the body mass (eq. 14).

$$\boldsymbol{Eq.\,14}:\ Weight - specific\ MR = \frac{MR}{BM}$$

Where MR is the metabolic rate (J/s) and BM is the body mass (kg).

2.6. Statistical analysis

All of the statistical analyses were performed using RStudio (R-4.0.4) (RStudio, PBC, Boston, Massachusetts). Regression models were made using the ggplot2-library and the ggplot-function in RStudio.

Linear regression was used to assess the correlation for all variables, given that the variables studied in this case were continuous, making a regression model appropriate for the data. The normality of the data was tested using the Shapiro-Wilk-test. Percentages were log-

transformed, because the data otherwise would approach 100% asymptotically, which would indicate that the correlation could not be linear when the other variable theoretically could be any value.

Normality was for all data tested using the Shapiro-Wilk (S-W) test in R. P-values over 0,05 for the S-W-test were assessed as the null hypothesis (H_0) not being discarded. H_0 was in this case normal distribution of the data. For correlation-significance, a p-value under 0,05 is to discard the null-hypothesis that there is no correlation.

3. Results

All the results using an average is presented as mean ± standard error (SE). For several values there is not a mean used, and that value alone is given.

The main proxy for RMR used for further analysis is CO_2, but the data and regression models using O_2 as a model is also included to compare the two proxies to one another.

3.1. Animals from 2019: Body mass, length and fat percentage

Table 2: Overview of the basic information about the hooded seal pups captured in 2019 and included in this study (n=12). For weight and length, the values in bold, underlined text are predicted values. The rest of the values are the observed values.

Individual	Capture date	Sex	Weight(kg)	Curvilinear length(cm)	Date of metabolism measurement
K1A	20.03.2019	M	43,71	119	21.03.2019
K2A	21.03.2019	F	28,4	**125**	22.03.2019
K3	21.03.2019	F	21,25	**110**	22.03.2019
K4A	22.03.2019	F	32,72	104	23.03.2019
K6A	23.03.2019	F	31,36	106	24.03.2019
K8	23.03.2019	M	33,96	106	24.03.2019
K12	24.03.2019	M	31,08	105	25.03.2019
K13	24.03.2019	M	36,83	111	25.03.2019
K14	24.03.2019	F	44,18	114	25.03.2019
K15	25.03.2019	M	26	95	26.03.2019
K16	25.03.2019	M	40	96	26.03.2019
K18	25.03.2019	F	42,48	109	26.03.2019

There were 12 individuals captured during the 2019 cruise to the West Ice with R/V Helmer Hanssen, with a wide range of body mass and length. The body mass-data of the pups was considered normally distributed (Shapiro-Wilk(S-W) test: $p=0.73$), as well as for the length-data (S-W: $p=0.82$).

3.2. Labchart-run

An example of run in Labchart during a metabolic measurement is shown in figure 10. The example shows the run for individual K15, a male hooded seal pup weighing 26 kg. An in-depth description on how this is used for further calculations is found under the methods and materials-section.

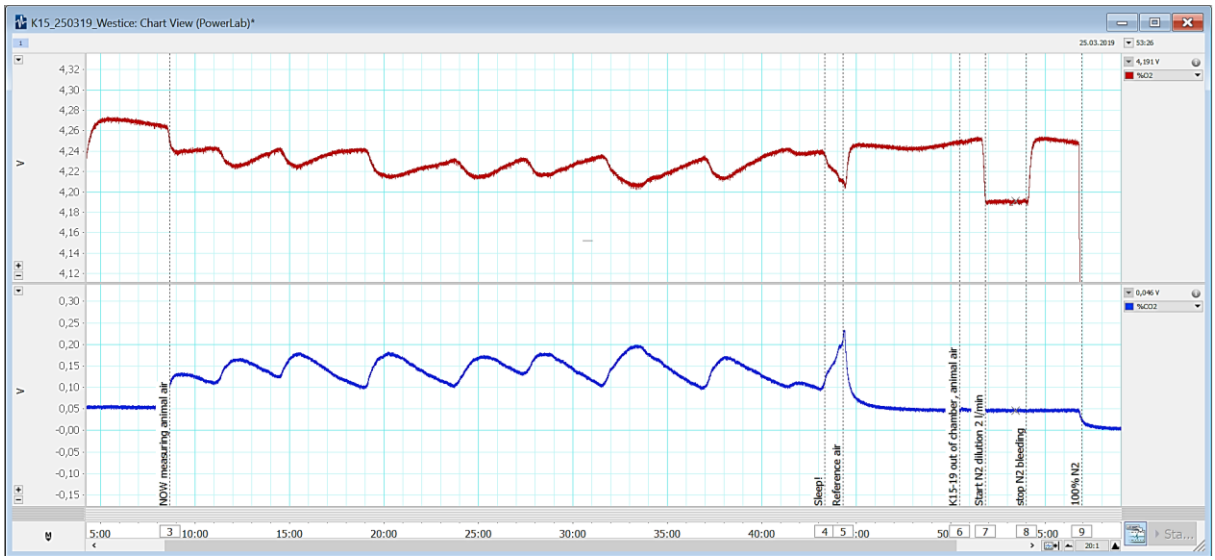

Figure 7: Run of metabolism measurement in Labchart for individual K15. It shows the O_2-levels in voltage on the top (red line), then the CO_2-levels on the bottom (blue line).

3.3. Animals from 2007 to 2019: Correlations - body mass, condition index and fat percentage

Table 3: Overview of the basic information about the hooded seal pups captured and included in this study (n=12). For weight and length, the values in bold, underlined text are predicted values. The rest of the values are the observed values.

Individual	Body mass (kg)	Curvilinear length(cm)	Fat mass (g)	Fat percentage	CI (BM/BL)
K6-07	45	119	22648	50,32888889	0,378151261
K14-07	23	98	8694	37,8	0,234693878
K3-12	24	105	5956	24,81666667	0,228571429
K5-12	26	105	7048	27,10769231	0,247619048
K16-12	39	**NA**	14582	37,38974359	**NA**
K1A-13	49,8	121	23804	47,79919679	0,411570248
K2A-13	47,5	120	23896	50,30736842	0,395833333
K4-13	28,85	**NA**	6008	20,82495667	**NA**

K7-13	26,7	109	5568	20,85393258	0,244954128
K2A-15	28,1	101	10362	36,87544484	0,278217822
K1A-15	25	105	6632	26,528	0,238095238
K4A-16	20,5	104	5052	24,64390244	0,197115385
K6A-16	19	**NA**	4414	23,23157895	**NA**
K9-16	37	**NA**	18900	51,08108108	**NA**
K10-16	31,7	**NA**	14100	44,47949527	**NA**
J6865	29,5	**NA**	8180	27,72881356	**NA**
J6869	44	**NA**	22898	52,04090909	**NA**
J6873	48	**NA**	27280	56,83333333	**NA**
J6876	30,5	89	14580	47,80327869	0,306692913
K2-18	38,95	127	16899	43,38639281	0,387758621
K4-18	44,98	116	22956	51,03601601	0,288333333
K8A-18	31,14	108	9182	29,48619139	0,320377358
K8-19	33,96	106	14374	42,3262662	0,296
K12-19	31,08	105	11676	37,56756757	0,342696629

The body mass-data for the hooded seal pups captured between 2007 and 2019 (n=24) was also normally distributed (S-W, p = 0.17). The linear regression model showed a significant correlation between the total body mass and fat percentage (p = 0.0000024). The correlation between the body fat percentage and body mass is explained by the following equation:

$$\boldsymbol{Eq.16}:\ y = 0.0123x + 1.15 \qquad (R^2 = 0.64)$$

Where y is the logarithm of the body fat percentage (log10(fat percentage)) and x is the body mass in kg (figure 11).

The length-data for the pups captured between 2007 and 2019 also had a normal distribution (S-W, p = 0.44). The linear regression model for the correlation between the condition index (body mass/length) and fat percentage also showed a significant correlation (p = 0.000019), with the following equation to explain the correlation:

$$\boldsymbol{Eq.16}:\ y = 1.69x + 1.05 \qquad (R^2 = 0.76)$$

Y is the logarithm with a base of 10 of the fat percentage, and x is the condition index (CI=BM/BL).

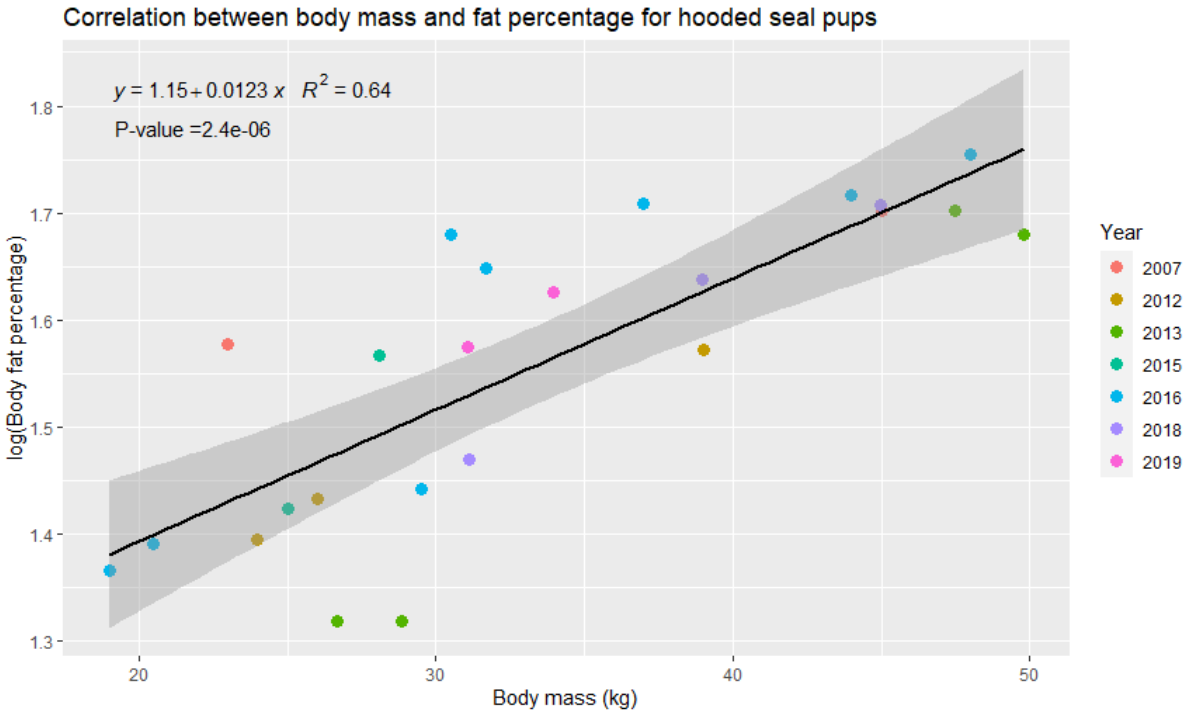

Figure 8: Linear regression model with 95% confidence interval, showing the correlation between the body mass and fat percentage for hooded seal pups that have been captured during the cruise with R/V Helmer Hanssen between the years 2007 and 2019 (n=24). There is a significant positive correlation, with $p<0.05$, and 64% of the observed variation can be explained by this regression model, with $R^2=0.64$. Regression done using ggplot in R.

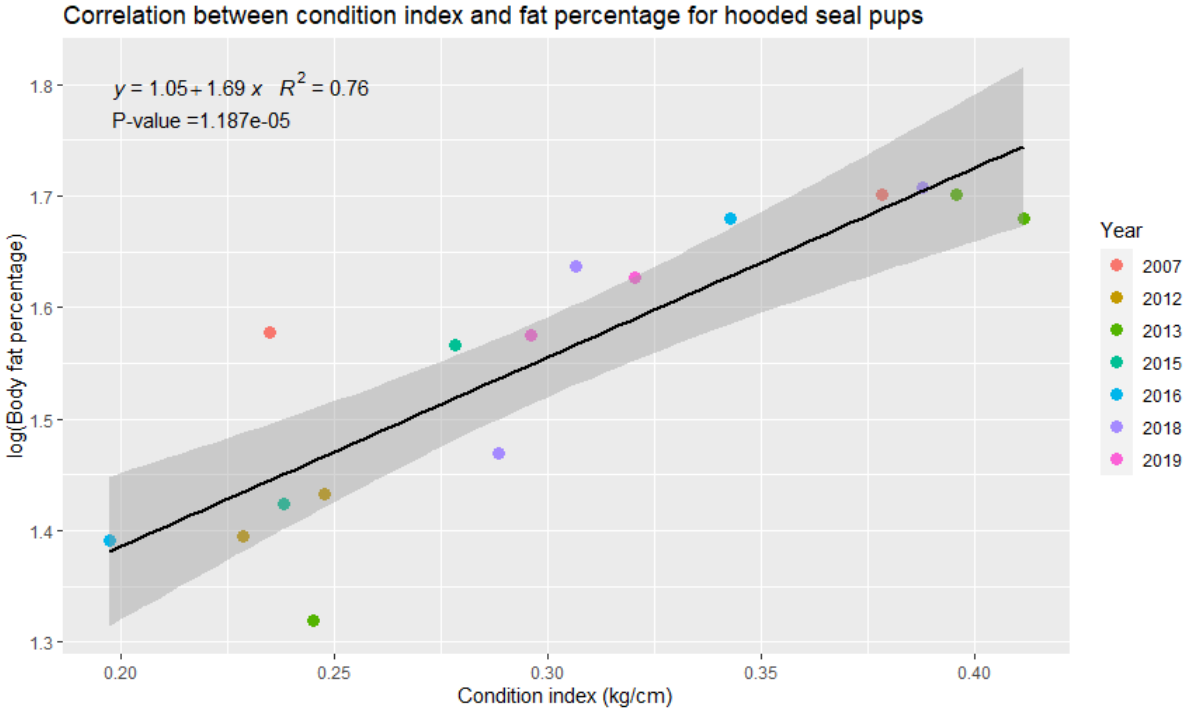

Figure 9: Linear regression model with 95% confidence interval, showing the correlation between the condition index (body mass/body length) and fat percentage for hooded seal pups that have been captured during the cruise with R/V Helmer

Hanssen between the years 2007 and 2019 (n=16). There is a significant positive correlation, with p<0.05, and 76% of the observed variation can be explained by this regression model, with R^2=0.76. Regression done using ggplot in R.

3.4. Animals from 2019: Metabolism-measurements

Table 4: Overview of the metabolism-data gathered for each individual hooded seal pup. The values with bold, underlined text are predicted values using equations from figure 11 and 12, while the rest are observed and calculated values. RQ is given with the mean value ± SE (standard error). The length of period refers to the length of the metabolism-measurement chosen for analysis. RMR is the weight-specific MRs measured for the individuals (Watts/kg).

Individual	Fat %	CI (BM/L)	RMR, O2	RMR, CO2	Ratio (O2/CO2)	RQ	Period-length
K1A	**46,96**	0,367	2,818	2,652	0,94	0,694 ± 0,019	15 min
K2A	27,15	**0,227**	2,276	3,09	1,36	0,708 ± 0,015	18 min
K3	22,35	**0,193**	3,563	3,751	1,05	0,710 ± 0,011	15 min
K4A	**39,51**	0,315	2,636	3,031	1,15	0,690 ± 0,014	1 hour and 22 min
K6A	**36,86**	0,296	2,857	3,239	1,13	0,759 ± 0,008	22 min
K8	40,33	0,32	2,984	2,689	0,9	0,717 ± 0,012	15 min
K12	36,88	0,296	3,304	3,194	0,97	0,720 ± 0,008	21 min
K13	**41,94**	0,332	1,981	2,648	1,34	0,727 ± 0,006	41 min
K14	**49,82**	0,388	1,796	1,82	1,01	0,759 ± 0,032	16 min
K15	**33,73**	0,274	3,069	2,482	0,81	0,702 ± 0,001	17 min
K16	**53,94**	0,417	1,609	1,509	0,94	0,697 ± 0,013	24 min
K18	**50,13**	0,39	1,852	2,776	1,5	0,705 ± 0,007	20 min

The body mass- and length data for the hooded seal pups used for metabolism measurements in 2019 had a normal distribution (S-W, body mass: p=0.73 and S-W, length: p=0.82).

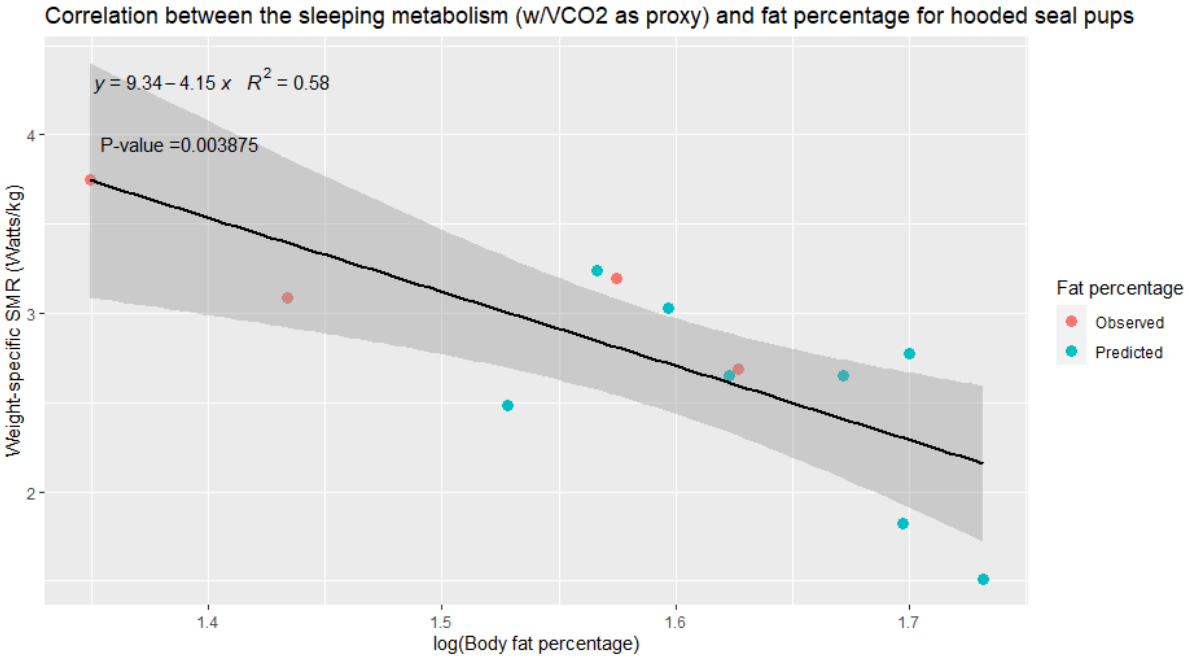

Figure 13: Linear regression model with 95% confidence interval, showing the correlation between the fat percentage and weight-specific SMR (SMR) for the pups, using the rate of carbon dioxide production (VCO_2) as a proxy for the metabolism. Both observed (orange) and predicted (blue) values of fat percentage are included. There is a significant negative correlation, with $p<0.05$, and 58% of the observed variation can be explained by this regression model, with $R^2=0.58$. Regression done using ggplot in R.

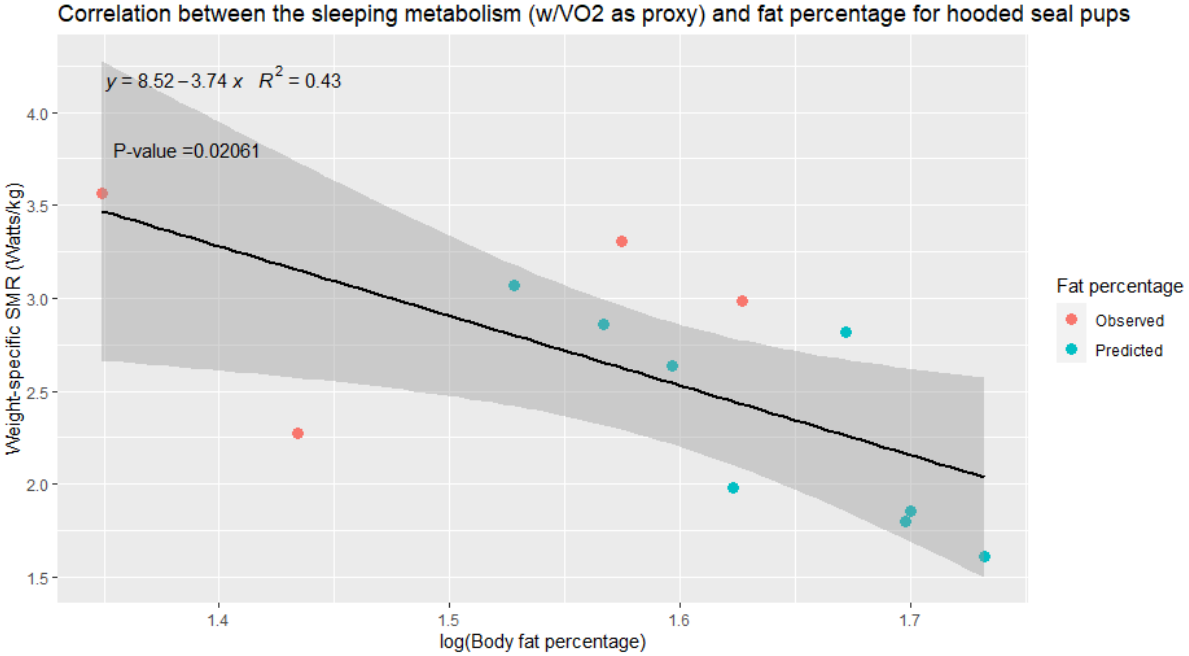

Figure 14: Linear regression model with 95% confidence interval, showing the correlation between the fat percentage and the weight-specific SMR (SMR/body mass) for the pups, using the rate of oxygen consumption (VO_2) as a proxy for the metabolism. Both observed (orange) and predicted (blue) values of fat percentage are included. There is a slightly significant

negative correlation, with p<0.05, and 43% of the observed variation can be explained by this regression model, with R^2=0.43. Regression done using ggplot in R.

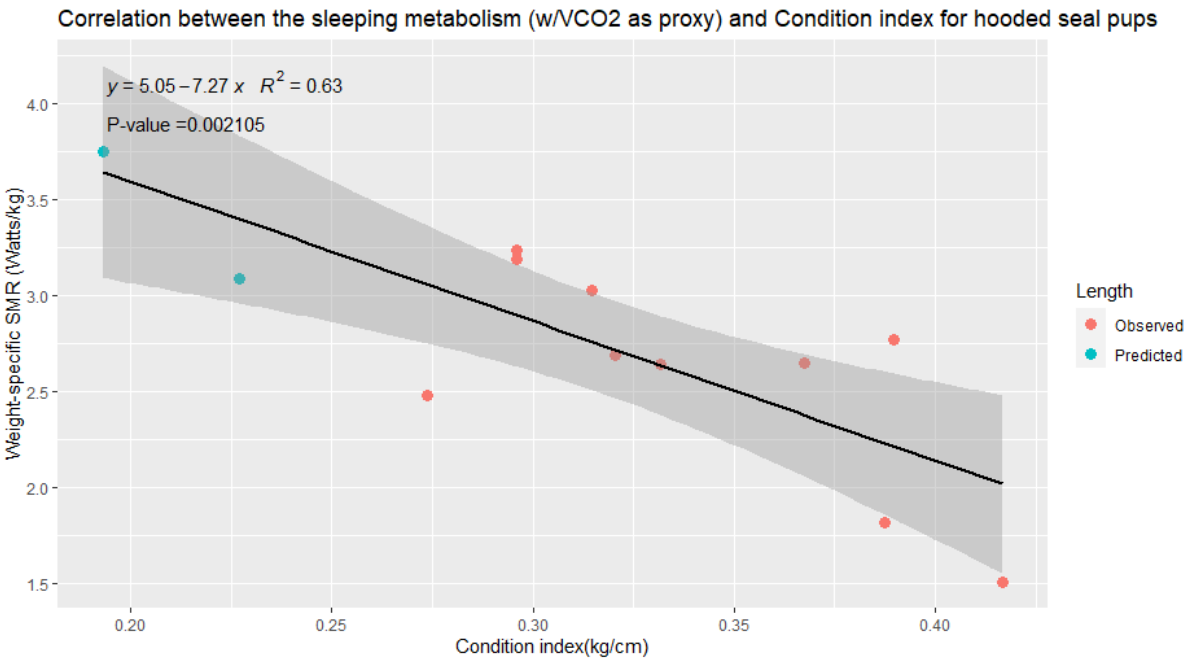

Figure 15: Linear regression model with 95% confidence interval, showing the correlation between the condition index (body mass/body length) and the weight-specific SMR (SMR/body mass) for the pups, using the rate of carbon dioxide production (VCO_2) as a proxy for the metabolism. Both observed (orange) and predicted (blue) values of body length are included. There is a significant negative correlation, with p<0.05, and 63% of the observed variation can be explained by this regression model, with R^2=0.63. Regression done using ggplot in R.

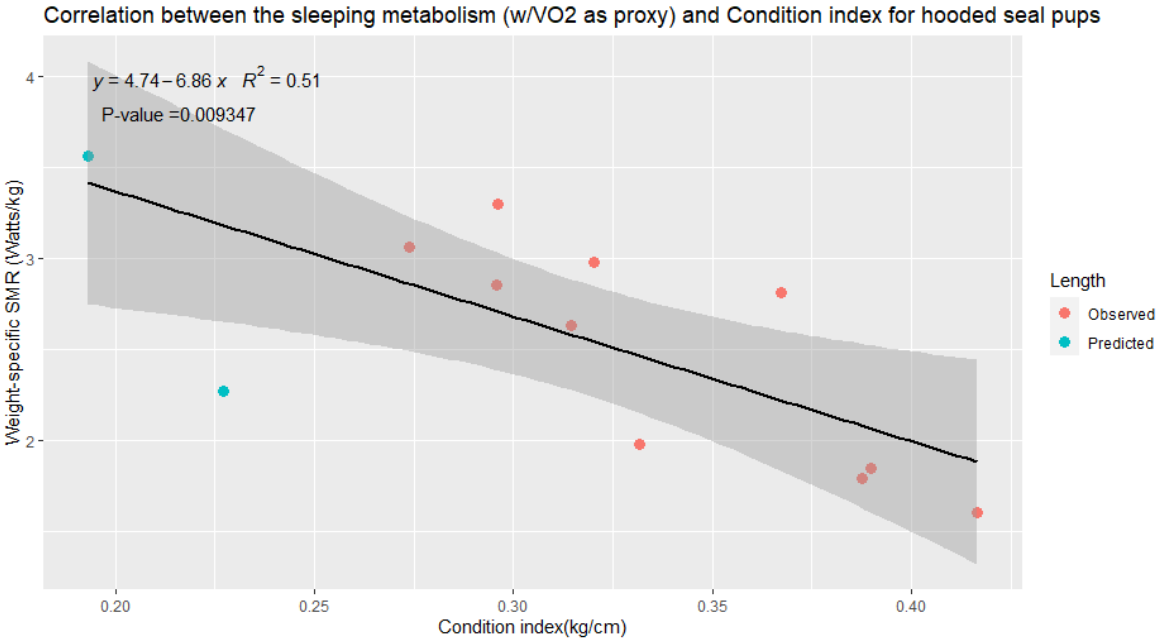

Figure 16: Linear regression model with 95% confidence interval, showing the correlation between the condition index (body mass/body length) and the weight-specific SMR (SMR/body mass) for the pups, using the rate of oxygen consumption (O_2) as a proxy for the metabolism. Both observed (orange) and predicted (blue) values of body length are included. There is a significant negative correlation, with $p<0.05$, and 51% of the observed variation can be explained by this regression model, with $R^2=0.51$. Regression done using ggplot in R.

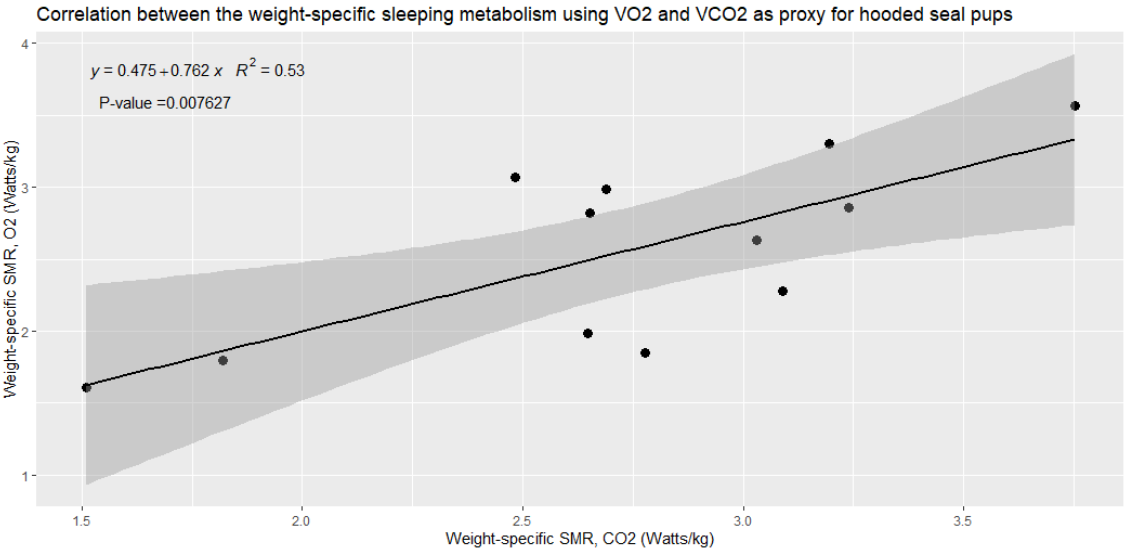

Figure 10: Linear regression model with 95% confidence interval, showing the correlation between the weight-specific SMRs (SMR/body mass) with VCO_2 as a proxy for metabolism and the weight-specific SMR with VO_2 as a proxy for metabolism. There is a significant positive correlation, with $p<0.05$, and 53% of the observed variation can be explained by this regression model, with $R^2=0.53$. Regression done using ggplot in R.

4. Discussion

4.1. Metabolic rates and fat metabolism

The results obtained this study are in line with what was observed by Rea and Costa (1992) in elephant seal pups, and by Aarseth et al. (1999) on adult harp seals: the weight-specific MR decreases as the fat percentage increases. In this study, there was a significantly higher weight-specific SMR for the leaner pups ($R^2 = 0.58$, $p<0.05$) than for the pups with a higher body fat percentage. Rea and Costa (1992) used elephant seal pups as a model for studying fat metabolism, where the pups were studied over a 4 month-period, including a 4-week nursing-period and a subsequent 10 week fasting-period. Their results showed an initial significant decrease in the weight-specific MR as the pups increased their body fat percentage from approximately 4% at the beginning of the nursing-stage to approximately 48% at weaning. They also found that the changes in LBM was more strongly correlated with the metabolic rate ($R^2 = 0.51$, $p<0.01$) compared to the body fat. An important difference between this study and the study conducted by Rea and Costa, is that the elephant seal pups have a nursing period lasting about 7 times longer than that of hooded seal pups. This means that there is a much longer period in which various factors, such as hormonal changes and environmental changes, could affect the MR of the pups. Rea and Costa specifically took most of these factors into account for the elephant seal pups in their study, but it makes a point that using the hooded seal pups, with only 2-4 days of nursing, as a model could be more advantageous.

Aarseth et al. (1999) also obtained similar results when studying three female, adult harp seals. They found that the weight-specific MR for the same adult individuals significantly followed the pattern of increasing when their body fat percentages were low, while it decreased when their body fat percentages were high. In fact, they observed more than a doubling in the weight-specific RMR, from 1.1 W/kg to 2.3 W/kg, when the body fat percentage went from 13% to 45%. Their study was conducted over several months, and the body composition of the seals was manipulated through controlled feeding so that the seals would obtain different levels of fatness throughout the study-period. The fact that the different body condition states were separated several weeks in time could be a source of error due to potential seasonal changes in the metabolic rate. In this study the metabolic measurements were only separated by a couple of days, indicating that seasonal changes should not have a

substantial effect on the results. In addition to this, Aarseth et al. (1999) manipulated the body composition of the seals through feeding, which could have been a source of stress for the animals and furthermore could have affected the results. Another important discussion-point is that they fasted the seals for 5 days prior to each measurement, and that this could have affected the individuals differently depending on their body composition each time. If they already had insufficient energy intake to reach their energy requirements over time, metabolic depression could have taken place. Fasting for an extra 5 days before metabolic measurements could have increased the probability of metabolic depression taking place. On the other hand, Aarseth et al. (1999) studied the changes in the weight-specific RMR on the same individuals over time, which removed potential errors surfacing when different individuals are used for different levels on fatness, like in this study.

McNiven (1984) found that there was no significant difference in the metabolic activity in the body fat and LBM, indicating that they contribute equally to the total metabolic rate. This is not in line with the results from this study. The reason for this could be related to the difference in the body composition of sheep compared to seals, where there could be differences in the type of fat stored. Also, in the study conducted by McNiven, the sheep were fed throughout the study. The sheep were divided randomly into three feeding categories, so that they would achieve different fatness levels to study. Hence, the conditions were not natural, but were instead simulated. Lastly, the specific body composition was not determined, which made it difficult to conclude how much fat specifically contributed to the total metabolic rate. In another study conducted on mice, they also found that fat was comparable to the LBM in metabolic activity (Kaiyala et al. 2010), and that fat contributes more to the energy expenditure than predicted from the energetic cost of fatty tissues. In this study they were also fed to different types of food (high fat diet and chow - low fat, but they accounted for the fact that the differences in the diets of the mice would influence the relationship between the metabolic rate and LBM.

In this study, the results also showed a stronger significant correlation between the weight-specific SMR and the used condition index (BM/BL) ($p<0.05$) compared to the correlation observed between the weight-specific SMR and fat percentage for the pups. Figure 15 shows that more of the variation in the data is explained by the linear regression model using the condition index instead of the fat percentage, as well as the p-value being lower. First of all,

this could be related to the fact that the fat percentage-values used in this study mostly consist of predicted values using the linear regression equation seen in figure 11. Because there is variation and potential errors in this model, using it to predict values for the pups in this study could have introduced a higher probability of error. There are also predicted values on length for two individuals in figure 16 using the equation in figure 12, but for most of the individuals the length data is empirically observed. Calculations of the fat percentage was done by dissecting some of the pups and weighing the blubber. This makes it possible to make an error if not all fat is successfully removed and weighed, or if there is additional fat in/on other organs that was not included. However, regardless of whether fat percentage or the condition index is used to assess the correlation to the weight-specific SMR, both show a significant negative correlation.

There is some variation in the data, with a couple of outliers seen in most models. There could be several reasons for these results. First of all, there has been shown to be a negative correlation between the time spent in sleep apnoea and the metabolic rate observed (Kohin et al. 1999). This means that if the pups had been in a sleep apnoea-phase for a longer period than others, this could potentially have affected their metabolic rate measurement, and have caused some of the variation. Otherwise, the variation could also be explained by the fact that some of the values used for calculations were solved graphically and judged by the eye with a ruler. Small changes in the measured distance in eq. 8 could lead to quite large changes in the energy output calculated. The same was observed for different values of RQ (eq. 11), where small changes in the RQ-value caused somewhat large changes in the energy output. Given that the average RQ was used instead of the RQ at the point of the measurement, this should probably have stabilized the differences.

Another important aspect to discuss regarding potential errors in the measurements of the metabolic rates of the hooded seal pups, is whether they were in a fasting state or not. As previously mentioned, the pups were ideally supposed to be post-absorptive, but not yet in a fasting state, given that fasting over a longer period of time can cause a metabolic depression to preserve energy when food is not consumed (Nordøy et al. 1990; Rosen & Trites, 2002). The fact is that there was no way of knowing how long the weaned individuals had been without their mothers, it could have been everything from hours to days. As a result, it is possible that some of the individuals indeed had reached a stage of metabolic depression due

to a longer fasting period. In fact, Nordøy et al. (1990) found that for grey seal pups, the metabolic rate decreased with as much as 45% from day 2 to day 10 of fasting. It then follows that if any of the weaned hooded seal pups in this study had been fasting for a longer period of time before being captured, the resting metabolic rates measured could have been underestimated compared to the other pups. On the other side, the arrival of the cruise was right in the beginning stage of the breeding-period of the hooded seals, meaning that it is unlikely that the weaned pups had been fasting for long, if at all, before being captured.'

Lastly, whether the measurements were done in a temperature within the thermoneutral zone of the pups or not is important to take into account. It was presumed that the temperature-range of 2-5 °C was within their thermoneutral zone. First of all, Kvadsheim et al. (2005) There is a lot of data supporting this, such as a study by Hansen (1995), who found that for grey seal pups (*Halichoerus grypus*) the TNZ ranged from -7°C to 23°C during their postweaning fast (Boily et al. 2011). It was also found that the average TNZ for 3-year-old harbor seals (*Phoca vitulina*) was at a temperature range from -12,9° to 28,6°C (Hansen and Lavigne, 1997). Nordøy and Blix (1985) performed a study looking at the fasting physiology and energy source utilization during fasting for grey seal pups, and they defined the thermoneutral zone to be between -4,5°C and +4,5°C. Though hooded seal physiology is not completely the same as that of grey and harbor seals, one could assume that their TNZ is somewhat similar.

4.1.1. Correlations: body mass, length and fat percentage

Body fat percentage and body mass for the pups captured from 2007-2019 showed a significantly positive correlation (fig.11), indicating that the of heavier hooded seal pups had the higher body fat percentage. A significant positive correlation is also found between the fat percentage and condition index (BM/BL) for the same individuals, and this model has a higher R^2-value (0.76 vs. 0.64), meaning that the model explains more of the variation seen in the data. This could be explained by the fact that the condition index helps differentiate between the pups to a greater extent. If a pup is short and heavy, the condition index indicates a good condition, and if another pup weighs the same, but is longer and skinnier, the condition index is lower. Arnould (1995) conducted a study to find appropriate condition

indices for female Antarctic seals (*Arctocephalus gazella*), and the results were very similar to those seen in this study, with a significant positive correlation between body fat percentage and BM/BL ($p<0.0001$), and 77% of the variation in the data explained by the model ($R^2 = 0.77$).

Linear regression models made for the correlation between body fat percentage and CI for all the individuals included from 2007-2019 (fig. 12) were used to predict the length of two of the individuals (K2A and K3). Even though the model showed a significant correlation between fat percentage and the condition index (BM/BL), only 76% of the variation in the data can be explained by the model, meaning that 24% of the data-variation is not. Hence, there is a possibility that the two length-predictions could be quite wrong. If the predicted length of K2A and K3 is compared to the other length data of the study-animals from 2019, the values are close to that of the other individuals. The average length-measurement for all pups is 106.5 ± 2.33cm, and given that K2A and K3 are the two individuals with the lowest body mass, it is unlikely that both are longer than most of the other individuals. K2A was 28.4kg and was predicted to be the longest of all individuals (125cm), while K3 was 21.25kg and predicted to be fourth longest of all individuals (110cm). Though these predictions are possible in theory, they seem unlikely, and it is likely that there is a slight over-estimation when using the equation generated from the linear regression model for the hooded seal pups captured from 2007 to 2019.

4.1.2. Choice of proxy for metabolic rates

First and foremost, what is observed from the data, is the fact that the data fit better with the models made using the SMR-data with VCO_2 as proxy instead of VO_2. This is the case with both the regression models looking at the correlation between SMR and body fat percentage (fig. 13 and 14), as well as the regression models looking at the correlation between SMR and condition index (BM/BL) (fig. 15 and 16). When VO_2 was used as a proxy for SMR and the correlation with body fat percentage was plotted and regressed, the linear model had both a higher p-value ($p = 0.0206$) and a lower R^2 (0.43) compared to when VCO_2 was used as a proxy ($p = 0.0039$, $R^2 = 0{,}58$). When VO_2 was used as a proxy for SMR, the correlation with the used condition index (BM/BL) showed similar results, with a higher p-value ($p = 0.0093$) and lower R^2 (0.51) compared to when VCO_2 is used as a proxy ($p = 0.0021$, $R^2 = 0.63$). All

in all, this indicates that the models fit better when VCO_2 is used as a proxy. This alone is not a good enough rationale for choosing VCO_2 over VO_2 as a proxy for the metabolic rate, especially given that VO_2 is considered the 'gold standard' for metabolic measurements. The unstable baseline observed in the O_2-measurements compared to a very stable baseline observed in the CO_2-measurements for all measurements indicate that the VO_2-data can be an unreliable when using the O_2-measurements directly, which strongly argues for using VCO_2 and RQ to indirectly calculate VO_2.

The SMR measured using VO_2 as a proxy for the metabolic rate should ideally be the same as when VCO_2 is used as a proxy, but that was not observed in this study. As figure 17 shows, the linear correlation between the weight-specific SMRs using VO_2 and VCO_2 as proxies has a slope of 0.762x. If the SMR-values were the same for each individual regardless of the proxy used, the slope would have been 1x. There is, on the other hand, a significant correlation between the two ($p<0.05$), and the linear regression model could explain 53%. The reason for the variance could be related to the unstable baseline observed for the O_2-measurements. Since the baseline was stable for the CO_2-measuments, this could have caused a mismatch in the calculated SMR for some of the individuals.

Nicholson et al. (1996) published a study on how reliable indirect calorimetry with expired gas analysis was for VO_2, VCO_2 and RQ, using the Europa GEM calorimeter. They found that there was a higher error percentage for VCO_2 ($1.3 \pm 1\%$) than for VO_2 ($0.3 \pm 2\%$). On the other hand, as the results indicate, the standard error (SE) for VO_2 is also doubled that of VCO_2. Based on their result, they also found an error rate for RQ of $1.4 \pm 1.5\%$. Hence, if VCO_2 is used as a proxy and VO_2 has to be calculated indirectly by also using RQ, this adds an extra error rate to the indirect calculation. This shows that it can be challenging to decide what proxy to use when indirectly measuring metabolic rates. It depends not only on what equipment is used, but also the exact conditions in which the measurements are done. Additionally, the instruments in use are important to take into account when deciding what proxy to use. In this set-up, it was clear that the O_2-channel was inherently unreliable due to an observed drift in the O_2-trace in Labchart, which made the choice of proxy challenging.

Another important aspect to consider in the choice of proxy for metabolic rate-measurements, is that the potential for error using VO_2 as a proxy is smaller than for VCO_2 with regard to the

substrates being metabolized. As shown in table 1, the difference in energy output depending on whether carbohydrates, fat or proteins are being utilized for producing energy is smaller when looking at O_2-consumption instead of CO_2-production. It is in this study assumed that the main substrate being used by the pups are lipids from the fat stores they had built up to different degrees, depending on their stage in nursing at capture. However, this need not be the case, given that other stores (e.g. carbohydrate and muscle stores) also could be utilized to some extent. Hence, using VO_2 as proxy ideally would give more accurate data on metabolic rates. In spite of this, the inconsistent and considerable drift in the O_2-measurements seen during measurements in this study makes it difficult to use O_2 as a proxy alone. As a result, VCO_2 was instead used as the main proxy for the metabolic rates of the pups.

4.1.3. Respiratory quotient

The respiratory quotient (eq. 11) depends on the substrate being catabolized for energy production. If lipids are utilized, RQ will have a value of approximately 0.71, if proteins are utilized, RQ will be approximately 0.83, and if carbohydrates are utilized, RQ will be approximately 1.0. (Kleiber, 1975; Hill et al. 2018). The substrates used for catabolism are on the other hand typically a mixture of all three, and RQ will then have a value in between 0.7 and 1.0, meaning that an RQ of about 0.83 need not indicate pure protein substrate utilization, but potentially a mix of substrates.

The RQ-values obtained in this study were generally quite low, with the average value being close to 0.7 for every individual (table 3), and the mean for all individuals combined was 0.72 ± 0.007 (table 3). This was expected since the pups were post-absorptive and were thought to generally take use of fat stores built up during nursing for required energy production. Some of the variation in the RQ-values could be explained by varying CO_2-washout as a result of the pups, and seals in general, tolerating high arterial pressure of CO_2 (Elsner et al., 1970; Bue, 2015 (Msc-thesis)). This could for instance happen because they held their breath periodically during sleep (sleep apnoea). It was tried to start and end each chosen period in the same phase, but it might be that some of the chosen period could have been slightly out of phase. There were some values below 0.7, with the lowest values being close to 0.6. Though this could be a result of the varying CO_2-washout, or simply just variation due to the

equipment used, such low values could potentially also be explained by how the fat is metabolized. When the fatty acid-chains are desaturated, O_2 is being utilized. In the process heat is released, but not CO_2, meaning that the volume of O_2 consumed gets higher, while the volume of CO_2 produced gets lower, resulting in a lower RQ-value (Owen et al. 1998; Bue, 2015 (Msc-thesis).

Another potential explanation to the lowest RQ-values observed, could be related to metabolization of ketone bodies. When fatty acids are broken down excessively, ketone bodies are produced, and they can later be used to produce energy through metabolization. Owen et al. (1967) found that when ketone bodies are metabolized, RQ-values could reach below 0.55. On the other side of the scale, there were also a couple of high RQ-values relative to the mean, reaching values close to 0.8. This could, again, possibly be explained by varying CO_2-washouts or variation in the equipment used, but it could also be a result of the pups using other substrate stores, such as carbohydrate-stores or proteins from muscle-tissue for energy production (Kleiber, 1961; Hill et al. 2018). The average RQ-values were, though, all close to 0.7, indicating that the variation in RQ-values instead could be a result of a slight mismatch in the registered O_2-consumption and CO_2-production. Because the analysed air passes by the O_2-analyzer before the CO_2-analyzer, there is a slight delay in the registration of the electrical signals sent to the converter. Using the average RQ instead of the point-values should have helped reducing this problem.

4.1.4. Metabolic rate

Studying the correlation between MR (metabolic rate) and relative sizes of different organisms and trying to find a universal equation to show this correlation, is something that many has tried to do. Kleiber's law, or Kleiber's equation, (eq. 17) is such an equation, and it seems to fit well with a variety of animals (Kleiber, 1975).

$$\boldsymbol{Eq.17}\text{: } BMR = 70BM^{0.75}$$

Where BMR is the basal metabolic rate given in kcal/day and BM body mass given in kg. It was found that the metabolic rate of adult harp seals fit this equation quite well (Gallivan, 1977; Lavigne et al., 1982). If the data on the weight-specific SMR using VCO_2 as a proxy from this study is compared to the expected values using Kleiber's equation, the SMR-values

from this study are approximately doubled for all individuals, with an average ratio of 1,93 ± 0,11 times the expected value using Kleiber's equation. This is expected, due to the extra energy required and utilized by the pups as they are growing rapidly, indicating that they need extra energy to rearrange and build up the body properly, and this is characteristic for immature animals in general (Denckla, 1970; Nordan et al., 1970; Lavigne et al., 1982; Kovacs & Lavigne, 1986). Based on this knowledge, the data from this study seems to be in line with what was expected. It has to be considered that the metabolic rate-results in this study are SMR-data. The Kleiber-equation bases itself on RMR, the metabolic rate of animals while at rest. Although the difference might not be large, this could still have an effect on the ratio. In fact, Zhang et al. (2002) found that for humans, the average SMR- and RMR-values were quite similar, but that the average SMR tends to be lower than the average RMR for those who are obese (BMI> 30), while it tended to be lower than the average RMR for non-obese people (BMI<30). Hence, though the difference might not be large, it could still cause small divergences from the expected value using Kleiber's equation.

4.2. Body mass, length and fat percentage

The hooded seal pups captured from 2007-2019 had a wide range of body mass, which was to be expected because of the fact that they were captured at different stages of their nursing-period, and some individuals followingly had gained more fat than others as a result. Hooded seal pups, typically only being nursed 2-4 days, can increase their body mass with up to 7kg per day in this short span of time, mainly as a result of fat deposition (Bowen, 1985). The body mass range was from a minimum of 19kg, all the way up to 49,8kg for all individuals included in the linear regression for figure 11 and 12, that were used for fat percentage determination, while the range was from 21,25kg to 44,18kg for the pups that had their metabolic rate measured (pups caught in 2019).

As a result of me not doing all the measurements myself, there is uncertainty on whether the measurements were performed accurately all individuals. The length- and mass-measurements were quite standard, and all students were instructed how to measure correctly. Hence, there should not be much error in this data, unless there was a systematic error with the digital crane weight. Dissections of the hooded seal pups and the methods used for finding the fat

mass could, on the other hand, be a potential source of error. First of all, it is challenging to get all of the blubber off the skin and core body, meaning that it is not unlikely that some blubber-residue could have been overlooked. Also, there could be additional fat on and in the organs (e.g. liver) that did not get included in the total fat mass, which would have led to an under-estimation of the body fat percentage. In addition to this, the total blood loss was estimated by subtracting the mass of all body compartments weighed from the total body mass measured before the animals were euthanized. In some of the student reports the blood loss estimations were very high, and in some cases it did not add up to the total body mass. This indicates that there might have been an error in the weighings and/or calculations.

5. Conclusion and future aspects

The environment into which the hooded seal pups are born can be very rough. To be able to survive these sometimes hostile conditions, the hooded seal pups have adapted to more easily stay warm and insulated by not only being born with a substantial subcutaneous layer of blubber, but also by depositing a lot of fat during the very short and intense nursing-period, gaining up to 7kg per day predominantly in fat (Bowen, 1985). This made them a great model for studying fat metabolisms role in the total metabolism. In this study it was found that there is a significant negative correlation between the body fat percentage and weight-specific SMR for the pups, meaning that individuals with a higher body fat percentage have a lower weight-specific SMR, a relatively lower SMR compared to individuals with a lower body fat percentage. Though, because the body fat percentages are not observed values, but are predicted using a regression model created by data from hooded seal pups captured previous years, as well as the fact that the models had quite a lot of variance, more elaborate studies should be done, preferably with more individuals with observed fat-percentages included, as well as a set-up with more reliable O_2-measurement for more accuracy in the data.

Future studies on fat metabolism and how it contributes to the total metabolism can be a very helpful and important tool for management and making bioenergetic models that are as accurate as possible. This in turn could improve the prediction models used to assess seal prey consumption, which is important not only for management of quotas and ecosystems, but also in understanding more about their ecological footprint, general physiology and biology of seals.

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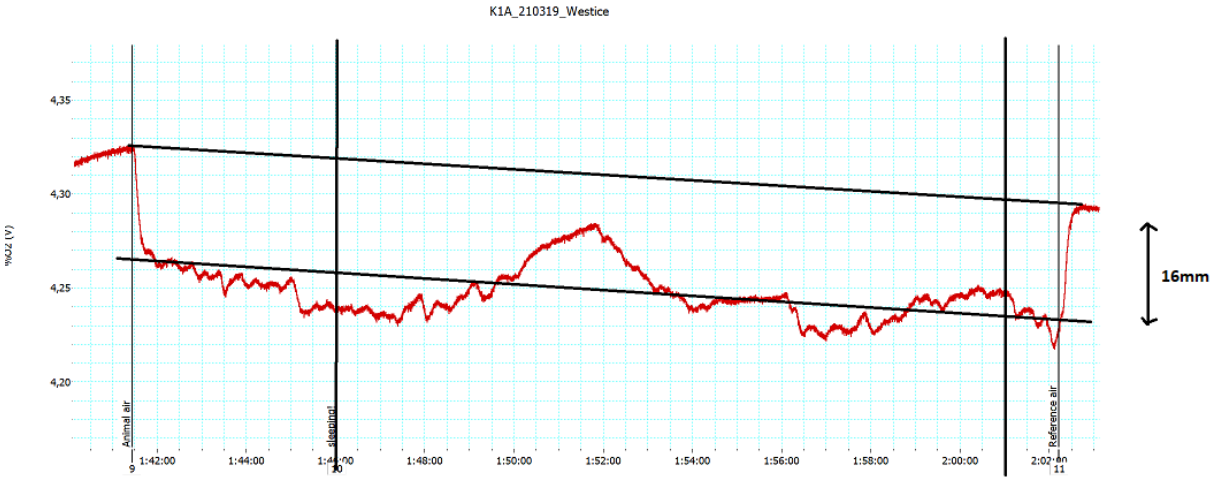
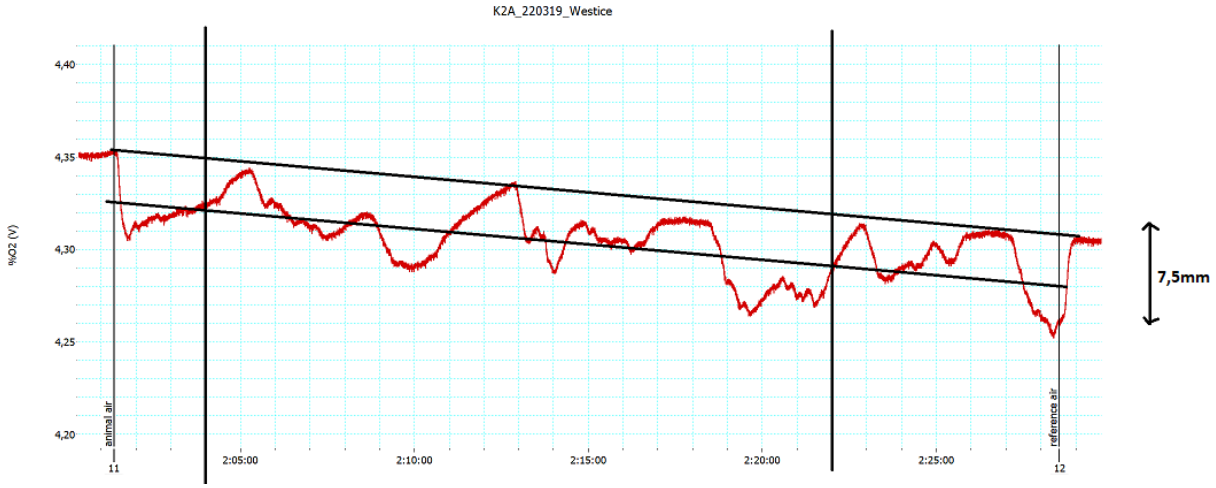
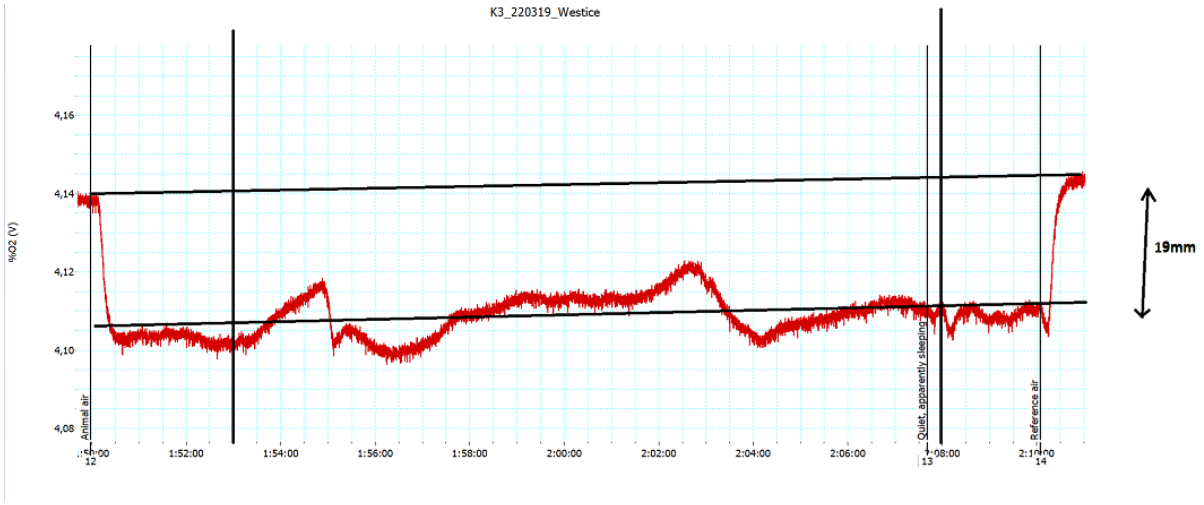
7. Appendices

7.1. Appendix A: Measurements and graphical solutions for all individuals

Figures showing the periods chosen for analysis with the graphic solution and measurements made in Paint for each individual used in this study.



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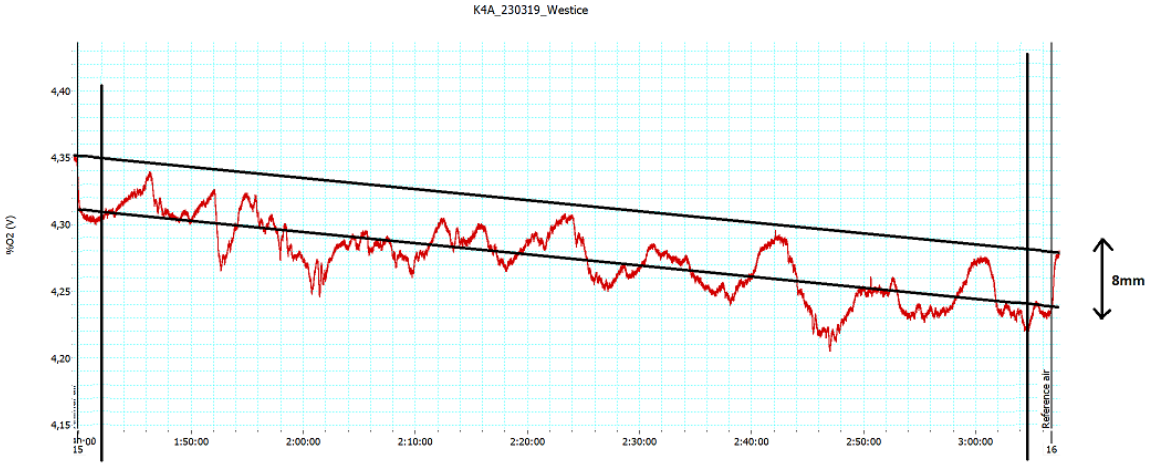
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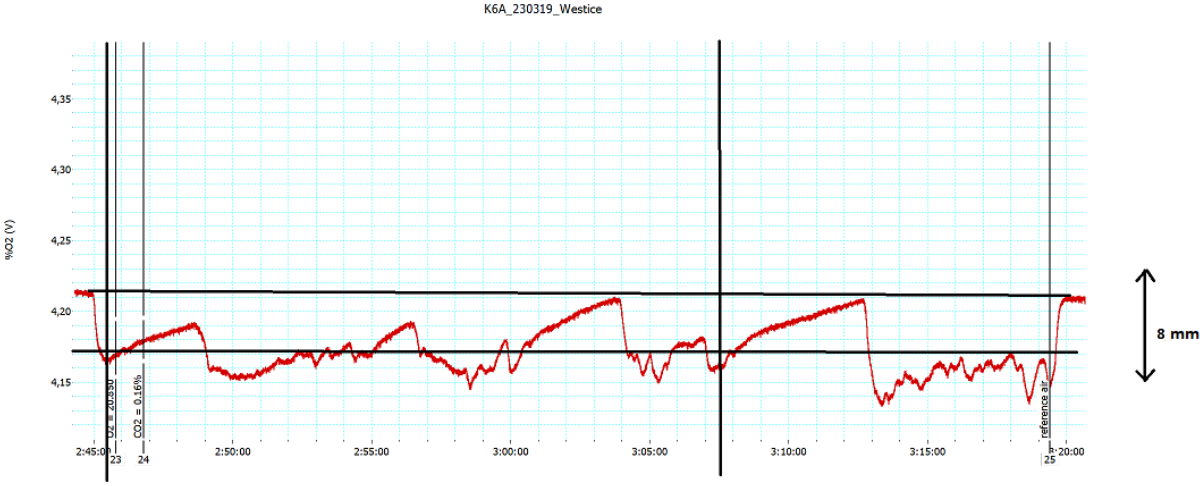
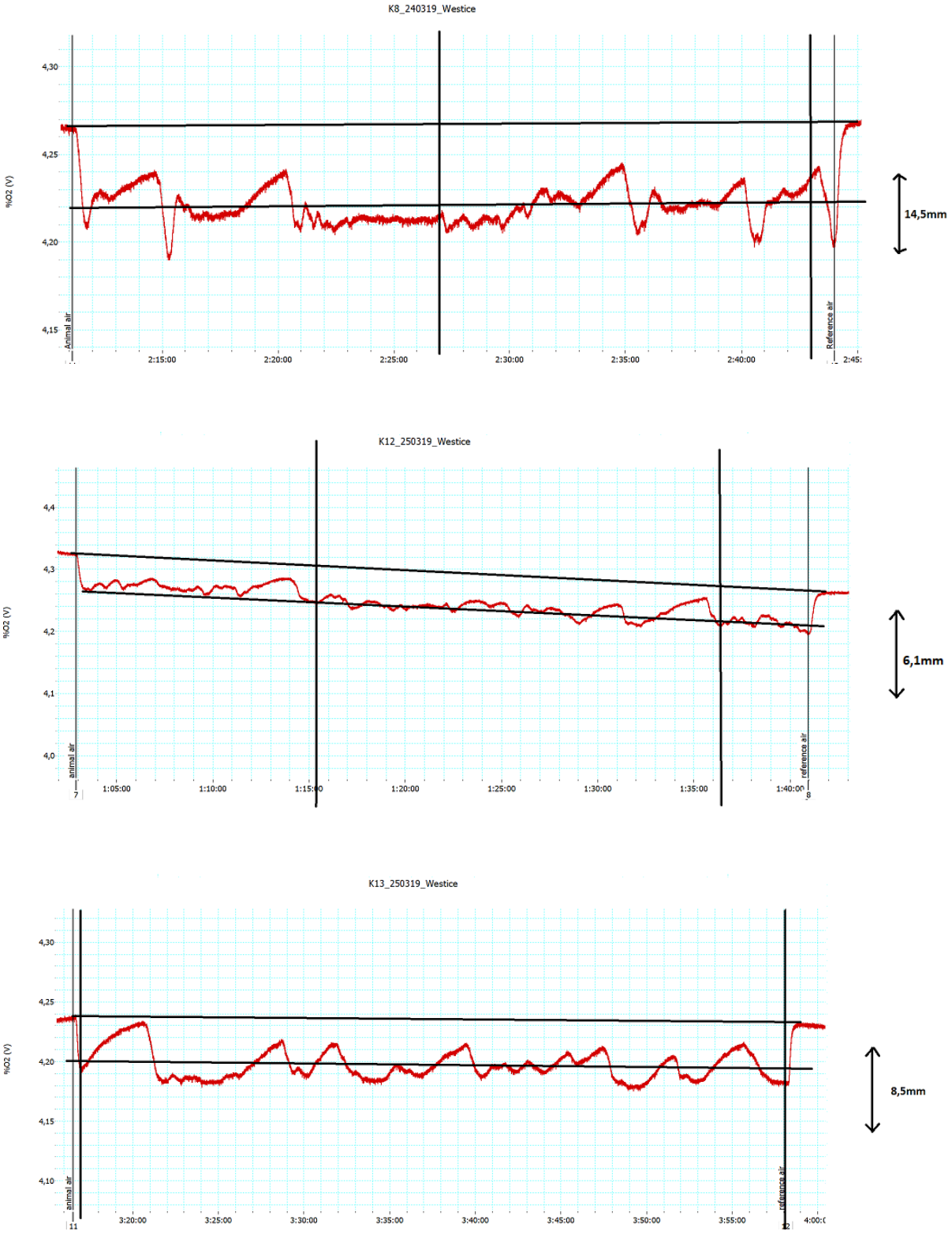
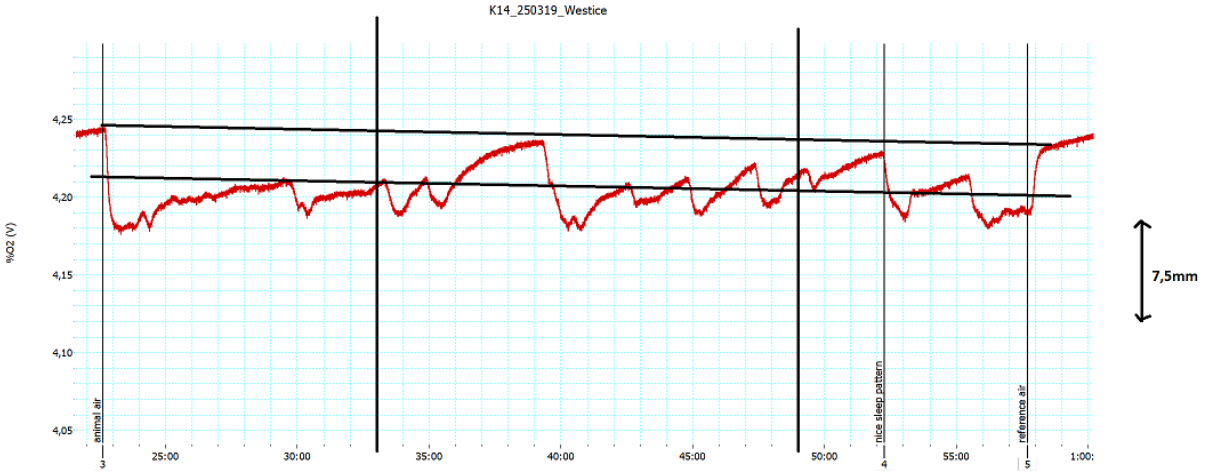
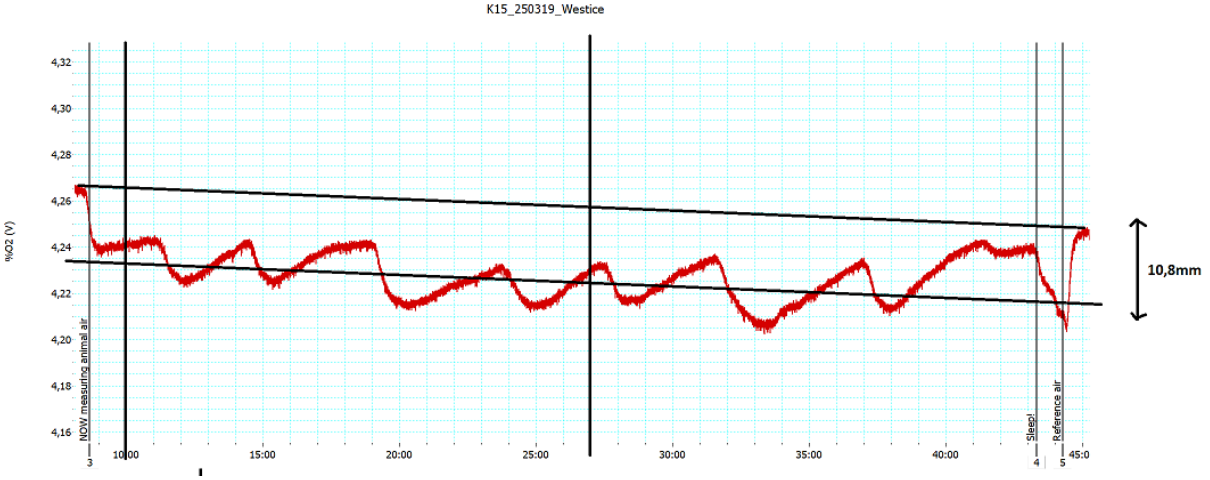
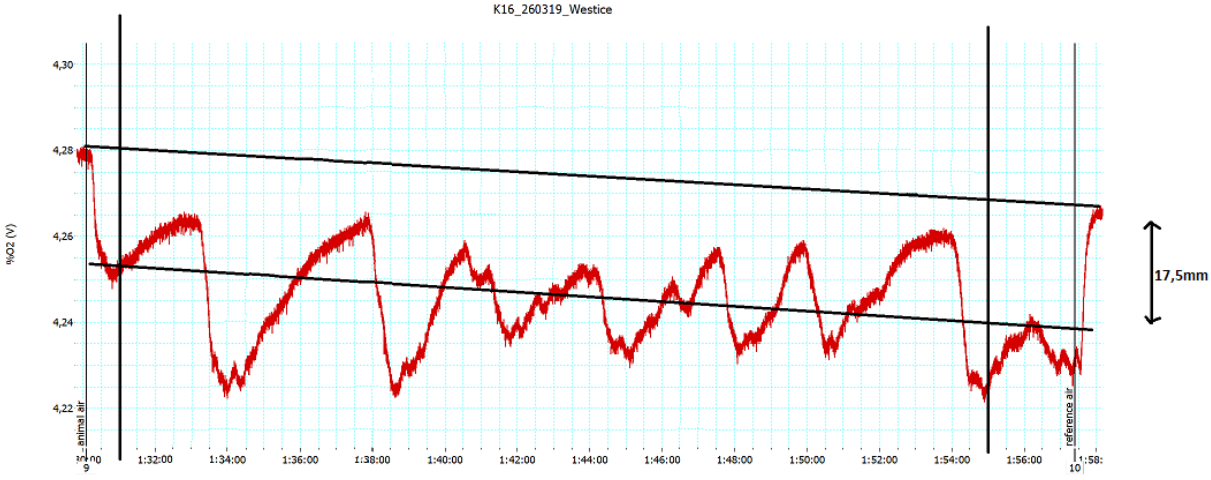
4,20
4,15
1:50:00
2:00:00
2:10:00
2:20:00
2:30:00
2:40:00
2:50:00
3:00:00
Reference air
15
16
8mm

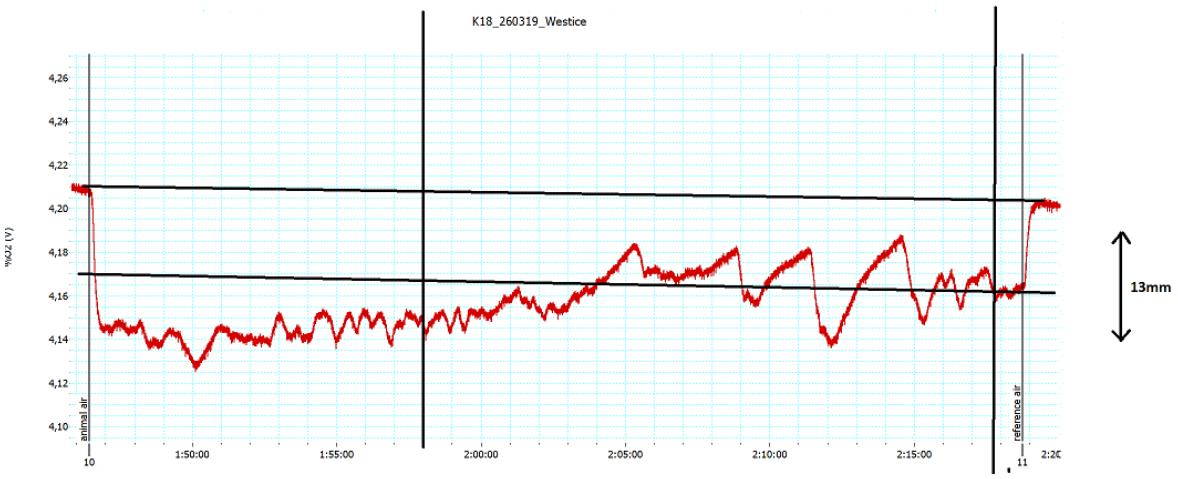

K6A_230319_Westice
%O2 (V)
4,35
4,30
4,25
4,20
4,15
2:45:00
2:50:00
2:55:00
3:00:00
3:05:00
3:10:00
3:15:00
3:20:00
O2 = 20,93%
CO2 = 0,16%
reference air
23
24
25
8 mm


K8_240319_Westice
%O2 (V)
4,30
4,25
4,20
4,15
Animal air
Reference air
2:15:00
2:20:00
2:25:00
2:30:00
2:35:00
2:40:00
14,5mm
K12_250319_Westice
%O2 (V)
4,4
4,3
4,2
4,1
4,0
animal air
reference air
1:05:00
1:10:00
1:15:00
1:20:00
1:25:00
1:30:00
1:35:00
1:40:00
6,1mm
K13_250319_Westice
%O2 (V)
4,30
4,25
4,20
4,15
4,10
animal air
reference air
3:20:00
3:25:00
3:30:00
3:35:00
3:40:00
3:45:00
3:50:00
3:55:00
8,5mm


K14_250319_Westice
%O2 (V)
4,25
4,20
4,15
4,10
4,05
animal air
nice sleep pattern
reference air
25:00
30:00
35:00
40:00
45:00
50:00
55:00
7,5mm


K15_250319_Westice
%O2 (V)
4,32
4,30
4,28
4,26
4,24
4,22
4,20
4,18
4,16
NOW measuring animal air
Sleep!
Reference air
10:00
15:00
20:00
25:00
30:00
35:00
40:00
10,8mm


K16_260319_Westice
%O2 (V)
4,30
4,28
4,26
4,24
4,22
animal air
reference air
1:32:00
1:34:00
1:36:00
1:38:00
1:40:00
1:42:00
1:44:00
1:46:00
1:48:00
1:50:00
1:52:00
1:54:00
1:56:00
17,5mm



7.2. Appendix B: All RQ-values

RQ-values calculated for each shift from reference air to chamber-air and from each shift from chamber-air to reference air. O-values (O1, O2, O3 etc.) and C-values (C1, C2, C3 etc.) are the differences in mV between reference air and chamber-air for O_2 and CO_2 respectively. mVperO and mVperC is how many percentages of O_2 and CO_2 one mV translates to. RQ is the value calculated based on the percentages.

Individual	O1	C1	mVperO	mVperC	RQ1	O2	C2	RQ2	O3	C3	RQ3	O4	C4	RQ4	O5	C5	RQ5
K1A	130	471	0,00479	0,00101	0,76255	130	402	0,65084	87	268	0,64835	121	387	0,67316	60	197	0,69105
K2A	84	291	0,005	0,00101	0,69951	83	289	0,70307	76	237	0,62968	48	144	0,60576	43	160	0,75133
K3	47	163	0,00476	0,00101	0,73453	48	156	0,68834	42	141	0,71103	48	147	0,64863	18	63	0,74129
K4A	88	299	0,00473	0,00101	0,7248	92	282	0,65387	53	177	0,71241	65	195	0,63996	42	145	0,73646
K6A	99	335	0,00457	0,00101	0,74646	84	276	0,72481	77	258	0,73914	78	265	0,74946	39	131	0,74097
K8	52	184	0,00474	0,00101	0,75402	39	144	0,78681	51	176	0,73538	40	131	0,69788	32	107	0,71253
K12	63	210	0,00462	0,00101	0,72815	45	152	0,73786	36	118	0,71602	37	123	0,72618	55	169	0,67122
K13	75	262	0,00469	0,00101	0,75219	33	106	0,69164	42	143	0,73312	36	123	0,73568	33	113	0,73732
K14	50	180	0,00478	0,00101	0,76039	63	221	0,74095	64	256	0,84488	44	144	0,69127			
K15	20	69	0,00497	0,00101	0,70084	35	121	0,70229									
K16	54	183	0,00478	0,00101	0,7158	57	201	0,74483	56	176	0,66384	30	97	0,68295	30	103	0,72519
K18	60	217	0,0049	0,00101	0,74413	41	145	0,72765	46	158	0,70671	47	160	0,70043	56	184	0,67604

O6	C6	RQ6	O7	C7	RQ7	O8	C8	RQ8	O9	C9	RQ9	O10	C10	RQ10	O11	C11	RQ11	AvgRQ
75	264	0,74086																0,69447
31	115	0,74906	21	77	0,74038	47	169	0,72606	46	170	0,74623	32	113	0,71303	71	253	0,71952	0,7076
37	120	0,68691	50	172	0,72858	37	124	0,70981	24	75	0,66187	35	126	0,76247	36	126	0,74129	0,71043
36	116	0,68736	40	127	0,67729													0,69031
45	160	0,78434	66	227	0,75871	50	173	0,76326	72	259	0,79353	50	180	0,79414				0,75948
26	82	0,67207	55	180	0,6974	57	184	0,68788	68	235	0,73643							0,71717
61	204	0,73054	62	203	0,71523	58	195	0,73443										0,71996
30	98	0,70339	45	153	0,7321	45	150	0,71774	51	176	0,74307							0,72736
																		0,75937
																		0,70156
34	107	0,66472	24	73	0,64246	36	125	0,7334										0,69665
43	142	0,67945	61	200	0,67459	44	157	0,73415	33	114	0,71077	46	156	0,69776	57	196	0,70749	0,70538