UiT The Arctic University of Norway

Faculty of Biosciences, Fisheries and Economy
Department of Arctic and Marine Biology

Validating the Tritiated Water Method in Adult Harp Seals (*Pagophilus groenlandicus*).

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Bernhard Jakob Salen Sørli
Master's thesis in Biology BIO-3950, May 2022

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

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

Cover photo by Marie Aas Westvik.
Photo of Harp seal female with her pup on the ice.

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Abstract

As one of the top predators in the northeast Atlantic, and most numerous, the harp seal (*Pagophilus groenlandicus*), has a significant role in the higher trophic levels in the ecosystem. Their condition can be an indicator of changes in prey availability, competition with other species or even climate. It can also be a useful tool in predicting the pup production and breeding success. The tritiated water method is used to estimate an animal's total body water (TBW) and body composition. The method is based on the dilution volume of an injection of tritiated water into an animal's body water. But the method requires validation.

In order to validate the tritiated water method in a new species, the method is performed on the animal and compared with the results of another method that is as close to the correct value as possible. In this project that was a dissection, where the different values estimated by the tritiated water method could be obtained and determined through a thorough dissection.

In this study harp seals of the pack ice of the Greenland Sea population were used (n=5). They were captured in the end of March 2021. The tritiated water method was performed, blood samples collected both prior to and after injecton, and the seals were euthanized, frozen and brought back to Tromsø for further processing. In Tromsø they were dissected, and the relative percentage amount and water content of different tissues were determined. Plasma samples collected when performing the tritiated water method were analyzed to determine specific radioactivity and total body water.

In this study the tritiated water method underestimated the total body water of female breeding harp seals with on average 1.72±0.39% (SE). The average estimated TBW was 40.33±0.60%, while the determined value, calculated using the mass of tissues and water content was 42.04±0.42%.
By plotting the estimated TBW and percentage amount of tissue the regression equations were derived. Estimating the body composition using these equations did not differ significantly from the determined values obtained by the dissections (P-value<0.05).

Keywords:
Harp seal, *Pagophilus groenlandicus*, tritiated water method, tritiated water, tritium, body water, body composition, body condition

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TWC = Total water content

TBW = Total body water

CPM = Counts per minute

DPP = Deproteinized plasma

$HClO_4$ = Perchloric acid

SE = Standard error

TBB = Total body blubber

TBM = Total body muscle

2 Introduction:

Management of a species requires knowledge both about the species itself but also the ecosystem it lives in. Data on the population size, mortality and recruitment is important and may show if a population is stable, decreasing or increasing. There are many factors, however, affecting these parameters. Available prey, competition and changing habitats are important when managing species. The condition of an animal, in this study it´s percentage amount of fat, is linked to the factors mentioned. An animal in good condition has a high percentage amount of fat. The condition is also related to the breeding success, where animals in good condition have a higher probability of success. In a constantly changing climate and environment the ability to monitor different species` condition is a useful tool in the management of them.

2.1 The harp seal (*pagophilus groenlandicus*):

The harp seal (*Pagophilus groenlandicus*) is one of the true arctic seals. They are distributed from the Canadian archipelago south to Newfoundland and eastwards to the Laptev Sea. They are separated in three main populations, the northwest Atlantic, the Greenland Sea, and the White Sea populations. In this project, animals from the Greenland Sea population were used. The seals in this population migrates to the ice east of Greenland in March to breed (Folkow et al., 2004). They stay on and around the ice until July, when they migrate to the Barents sea to feed and gain weight (fig. 1) (Blix et al., 2013; Folkow et al., 2004). The harp seal is one of the top predators in the North Atlantic and due to their abundance, they constitute to an important component of the ecosystem (Haug et al., 2021).

Figure 1: The Greenland Sea population`s distribution. From satellite telemetry (Blix et al., 2013) .

Harp seals got their name from the characteristic harp shaped markings on their back which they obtain at an age of 3 – 5 years old (fig. 2). They Vary in body mass throughout the year. In March, just before the breeding season, they are at their fattest, adult males being average 140 kg and adult femalest 120 kg (Nilssen et al., 1997; Sivertsen, 1941).

The harp seals are affected by several different factors. Such as declining sea ice changing their habitats, change in prey abundance and competition with other species moving north. As the arctic is warming rapidly, the oceans get warmer and with that follows a decline in sea ice (Descamps et al., 2017). The warmer water creates new habitats for species usually found further south, such as Atlantic cod (*Gadus morhua*), which can be potential competitors for prey (Stenson et al., 2020).

Figure 2: Adult harp seals on the ice (March 2021). photo: Shona Wood

The harp seal has historically been an economically important animal and was hunted for blubber, meat and skins. Due to regulations, media pressure, a low demand for seal products and low fundings from the Norwegian government, seal hunting is at a minimum with only 10 284 animals caught in 2020 (Bjørge. et al., 2020), compared to the highest recorded number from 1925 with 347 920 seals caught (Statistisk_Sentralbyrå[SSB], 2000). The populations have then increased until recent years where numbers are going down again. Especially the Greenland Sea population shows this trend. And it is the number of pups born that are declining (Haug & Biuw, 2018). The current estimate for the total harp seal population in the Northeast Atlantic is between 1.5 and 2 million animals. Of this number, the Greenland sea population is estimated to 426 808 animals in 2019 (conf-int: 313 004 – 540 613) (Bjørge. et al., 2020).

The Northeast Atlantic Ocean is a large ecosystem containing a variety of different species. The seals foraging success when feeding in the Barents Sea can be linked to the prey abundance. The consumption of prey animals was estimated in 1998 to be 3.35 million tonnes for the two populations feeding in the Barents Sea with capelin (*Mallotus villotus*) being the most abundant prey (Nilssen et al., 2000). This is a significant amount of the total consumption and fisheries in the Barents Sea. The seals foraging success determines its condition, as the more it eats, the more energy it gains. Harp seals store their energy as blubber, so their condition is directly linked with the foraging success. Therefore, estimating their body composition can be useful as an indicator for the availability of prey species in the Barents Sea. A lowered condition can be an indicator for less available prey species. This can have several reasons, such as spatial distribution, competition with other species or low populations of prey.

A decline in pup production can be linked to among several reasons, a lowered condition. By estimating the body composition of the seals by non-invasive methods, their condition can be monitored over time. To avoid lethal sampling, the tritiated water method is one approach to do this. The method is used to estimate total body water and body composition, and from this the condition can be calculated.

2.2 The tritiated water method:

The tritiated water method is a widely used method to estimate water turnover rate, total body water and body composition. Tritiated water contains tritium, which is a radioactive isotope of hydrogen. Tritium has two neutrons and one proton in its nucleus, making it unstable. There are two other stable isotopes of hydrogen; protium and deuterium. Whereas protium has one neutron and deuterium has two. Protium is referred to as ordinary hydrogen, and deuterium is used in heavy water and can also be used to estimate body composition. Tritium has a half-life of 12,3 years and decays to ^{3}He by emitting β-electrons and an antineutrino (Tanabe, 2016). The hazard of being exposed to tritium is solely from the ionizing radiation. Radiation is well known for increasing the risk of cancer. Tritiated water is not differentiated from ordinary water and will dilute into all cells permeable to water (Bush, 1972). Tritiated water inside the body of an animal will after some time be lost through water flux. The specific activity of the injected dose, the radioactivity of the total dose and the water flux decide the total exposure to radiation.

The tritiated water method has the advantage that it is low invasive method, compared to other methods, eg dissection. It is efficient for estimating TBW and body composition. It is relatively easy to perform since it only requires an injection of tritiated water and subsequent blood samples after equilibrium, from the animal. This makes it possible to sample many animals in a relatively short time and avoid lethal sampling, compared to determining body composition through dissection in which all animals used will be killed. The animals are set free after sampling, and there are no visible effects of the experiment. The tritium will leave the body after some weeks trough body water efflux (Tanabe, 2016). A maximum noninjurious dose of tritiated water is suggested to be 100μCi*g body $mass^{-1}$ (Nagy & Costa, 1980), which is sufficient to be measured after being diluted in body water.

The method has been used on several species of pinnipeds (Bowen & Iverson, 1998), but it has not yet been validated in harp seals. In order to validate the tritiated water method in a new species, it must be compared with another method giving the exact results. In this study this was done by performing the method and later dissect the animals to determine body composition and water content and compare the results. Deriving equations from the estimated TBW and percentage amount of tissue is as done by Reilly and Fedak (1994), when validating the method in grey seals (*Halichoerus grypus*). Different species has a different body composition. Since the equations for estimating the body composition is made from the regression of the estimated total body water and the determined amount of tissue, the equations are species specific.

2.3 Aim of the study

The aim of this study is to validate the tritiated water method in adult harp seals, as described, in order to test if this is a useful method to estimate total body water and body composition in harps seals.
Validation was done by comparing the estimated TBW in five female harp seals using the tritiated water method, with the determined TBW obtained through total body dissection and measuring the water content of various tissues by desiccation, in the same animals.

3 Methodology

All animal experiments were approved by the Norwegian animal research authority (approved 07.02.2020, case id: 22693). Scientific capture was approved by the Norwegian directory of Fisheries and the Greenland Ministry of Fisheries, Hunting and Agriculture to capture the five adult female harp seals and associated pups.

Before departure syringes with tritiated water (n=5) for injection were made ready. The stock solution consisted of 4 ml tritiated water (PerkinElmer, 5mCi, 1mCi/g, 5ml) mixed with 50 ml physiological saline (B.Braun, 9mg/ml, i.m, i.u, s.c, B. Braun Melsungen AG, Melsungen, Tyskland). Syringes (n=5) were then filled with 9,5 ml of the solution. All syringes were weighed to the nearest 0.001g (vwr TA 314i, Radnor, Pennsylvania, USA) before and after filling to determine the exact mass of injected dose. The syringes were marked with numbers to separate them from each other. All syringes were sealed with a lid and stored in a cooler at 5°C for almost 3 months until being used. The prepared doses are below the maximum noninjurious dose, but estimated high enough to obtain radioactivity levels that can be used in further calculations.

3.1 Capturing the animals

A scientific cruise with R/V Helmer Hansen to the Pack ice of the Greenland Sea was done between the 16 of March and the 1 of April 2021. The purpose of the cruise was teaching of undergraduate students (BIO-2310) and scientific capture of harp and hooded seals for various research projects.
Adult female breeding harp seals (n=5) were caught on the pack ice of the Greenland Sea the 30th and the 31st of March 2021 using a hoop net. The net had a hoop with a diameter between one and one and a half meter and a long net attached. The only opening is through the hoop. The associated pups could be picked up and lifted on board. The seals caught were chosen at random. Female harp seals with pups tend to stay close to their pups and is therefore possible to catch by use of this net. By sneaking up behind seals and throwing the hoop net over their head they crawl into the net themselves as it is their believed escape route. As the pups does not escape it was also possible to lie and wait by the pup until the mother returned. All the pups were brought on board the ship, weighed, and then killed using a hakapik, as they were in the middle of lactation and would not survive on their own.
The adult seals were brought on board the ship and weighed, inside the hoop net and a lifting bag, using a mechanical scale (Salter scale, 250±1kg, Smethwick, UK).
The seals were left in individual cages with a size of 1.20m by 1.60m made of wood pallets, on board to avoid unnecessary stress, until the experiment could start (from immediately after capture to a couple of hours).

3.2 Injection of dose

The seals were sedated inside the cage using an injection of Zoletil forte vet (VIRBAC,Carros Cedex, France) intramuscularly (0.5.1.5ml) (0.5-1.5 mg/kg i.m). After approximately fifteen minutes the seals were properly sedated for the project to start. The seal was placed on a seal board of and strapped to it using four leather straps, over hind flippers, back and neck, for physical restrainment.
A catheter (Secalon 16G/1.70x160mm, Argon Medical Devices, Argon Critical Care systems Singapore, Singapore) was inserted in the intervertebral extradural vein, by the fourth lumbar vertebrae, as this vein is relatively easy to locate and insert a catheter into. Other options are veins in the hind flippers, which is more difficult to locate. Prior to injection of tritiated water, ten ml blood samples (n=2) were collected for determining background radiation. The seal was then injected with its designated dose of tritiated water. Physiological saline was used to flush the catheter to ensure all tritiated water entered the seal. When injected the seal was left for one hour to let the dose equilibrate in the body water. When equilibrated, ten ml blood samples (n=10) were collected. Afterwards, the seal was euthanized using Euthazol (0.35 ml/kg) (Produlab Pharma B.V. Raamsdonksveer, Netherlands) injected through the venous catheter. The seal was then left outside to cool overnight in ambient temperature (-2℃ to -10℃). The next day the carcass was brought inside, thoroughly wrapped in plastic to avoid any evaporative water loss, and frozen at -20℃ in the ship's freezer. When back in Norway the carcasses were transported to the freezer at the Arctic marine biology facility and stored in a freezer at -20℃ until dissections were started.

3.3 Blood samples:

Ten ml Blood samples were collected into heparinized centrifuge vials (Heparin LEO, 5000IE/a.e./ml, LEO Pharma AS, Ballerup, Denmark). These were centrifuged immediately after sampling in a Heraus Labofuge 2000 (Thermo Fisher Scientific, Waltham, Massachusetts, USA) at 3000rpm for 10 minutes to separate plasma. Using a Pasteur pipette the plasma was transferred to 1,8ml cryotubes. These were marked with seal ID and frozen at -20℃ until further processing.

3.4 Dissection:

All seals were stored in a freezing room at -20℃ and each seal needed between 3-5 days to thaw, depending on size. The purpose of the dissections was to separate all parts of the seals and measure the amount in order to determine the body composition of each seal. Before dissections, the seals were weighed with a digital scale (Scaleit Dini Argeo MCWN T1, 300±0,1kg, Scaleit AS, Oslo, Norway) when still frozen to obtain total body mass (kg).

The seals were first skinned. The skinning was done so that the blubber stayed on the skin and could be removed later. The flippers were skinned separately. Then as much of the muscles outside the ribs were moved before the thorax was opened. To open the thorax a cut was made along the sternum through the cartilage. The intercostal muscles could then be removed. All ribs on one side were removed by cutting along the vertebrae to access the heart and lungs. The abdomen was opened in the same procedure and organ accessed. The organs were still frozen, even after several days thawing in all five seals. Therefore, they were taken out still frozen together and placed in a bucket to thaw. This allowed gathering of blood from the

abdomen and organs. When thawed, the organs and blood could be separated and weighed (Scaleit DHS 60, 60kg±0.15%). The internal organs also include the eyes and the brain. The eyes were carefully removed using scalpels without puncturing them and loosing mass. To access the brain the skull was cut in two parts, separating the front, and back using an ordinary hand saw.
All muscles were removed from the skeleton using knives and scalpels, by cutting and scraping it off. This was the most time-consuming part of the dissections and was done thoroughly. When done the different parts were weighed.
To determine the mass off the blood, the difference between the initial total weight (kg) and the total weight of tissues was calculated. But a large amount of blood was still left in veins and organs.

Sub samples for determination of water content were collected during the dissections. Approximately half a kilo of the different tissues was taken for sub samples. The brain was also stored for determining its water content. The brains total weight was only about 200 grams and therefore the whole brain was used for determining water content. All samples were put in plastic zip lock bags to avoid any water loss. Samples were frozen at -20°C until further processing. No samples of skeleton tissue were collected.

3.5 Radioactivity measurements

Plasma samples were taken out of the freezer at AAB and let to thaw in room temperature overnight. The method for background, stock and plasma samples are the same from point 3.5.2.
The pipettes used were all calibrated before the project started. Calibration was done by weighing a pipetted volume of distilled water and adjusting until the volume pipetted had a weight not ranging more than ±0.01g (vwr TA 314i) for a minimum of ten times.

3.5.1 Stock solution:

To determine the activity in the stock solution injected into the seals, a 100 times dilution of this was made for each seal and counted. 9.9 ml of physiological saline was pipetted (Biohit proline 1-5ml, Helsinki, Finland) into glass vials (n=7). To this 100 µl of stock solution was added (BioHit proline 20-200µl) in each and mixed on a vortex mixer (LabGenius MI0101001 Mini-Vortex Mixer). The stock solution must be diluted to obtain a radioactivity that is within the measuring range of the liquid scintillation counter. To avoid differences in radioactivity measurement because of reduced counting efficiency that can occur if the content in the vials is different, stock solution and uncontaminated plasma is mixed.
1ml uncontaminated plasma was transferred into two ml Eppendorph tubes (n=7) and to this 10µl of diluted stock solution was added (vwr, 2-20µl, Radnor, Pennsylvania, USA). Each vial of stock solution had its designated plasma sample, so that replication is possible. Thereafter the same procedure was followed as with the other plasma samples.

3.5.2 Perchloric acid

1ml of plasma was transferred from the cryotubes to centrifuge tubes using a pipette (Biohit proline 1-5ml). To this 200 µl of 70% perchloric acid ($HClO_4$) was added to precipitate plasma protein. Following the addition of perchloric acid, the solution was mixed thoroughly on a Vortex mixer (LabGenius MI0101001 Mini-Vortex Mixer, Daigger scientific, Illinois/ New Jersey, USA). Samples were then centrifuged using a Himac ct15re (Eppendorf Himac Technologies Co.,Ltd, Ibaraki, Japan) centrifuge at 3000 rpm for 10 minutes to separate the precipitated proteins from the plasma. The deproteinized plasma (DPP) were then transferred to microtubes. 1ml of DPP were transferred to 20ml scintillation vials and 10ml scintillation fluid was added (Ultima Gold, PerkinElmer Health Sciences B.V., Groningen, The Netherlands), using a dispenser (PerkinElmer 0-10ml). The vials were shaken to mix the content well and stored until the next day, when samples were put in the liquid scintillation counter (Tri-Carb 2900TR, PerkinElmer, Waltham, Massachusetts, USA) to measure radioactivity. The counter was set at counting samples 10 minutes each.

3.6 Determining water content in tissues:

The sub samples of different tissues from the dissections were frozen in plastic bags at -20 ℃ in a freezing room. Samples were frozen for approximately one month. Samples were taken out to thaw in a fridge overnight. Sub samples weighing approximately 100g of the samples were taken and cut into small pieces and grinded in an ordinary kitchen mixer (Wilfa xplode boost BLP1200B, 1200W, Oslo, Norway) until becoming a homogenous mass. Sub samples weighing between ten and thirty grams was put in Petri dishes, weighed, and covered with perforated aluminium foil to aid in water vaporisation and avoid contamination.
The samples were then put in a laboratory drying oven (Termaks TS4057, Termaks AS (Nordic Labtech AB), Kungsbacka, Sweden) at 60 ℃ for 48 hours. It was made three parallels of muscle, inner organs, brain, and heart. The blubber samples, when grinded, separated into two parts, one oil and one tissue part. These were decided to keep separated when drying. It was made five parallels of each of these. Several parallels are necessary as the mean weight loss is used to determine water content and it reduces possible errors with outliers.
Samples were weighed before drying and after 48, 72 and 96 hours. Before each weighing the samples were let to cool in a desiccator. The desiccator had a bowl of silica gel (unknown origin) in the bottom to absorb all water vapour and to avoid any condensation of water on the samples. The silica gel was dried in the drying oven several times throughout the project to ensure that it could still absorb water.

The tissue samples were weighed on a Mettler pc 180 digital scale (200±0,001g, Mettler Toledo, Columbus, Ohio, USA).
A selection (n=15) of Petri dishes were weighed before the project started, and the average weight was used for the rest. An average weight of 7,305 grams were determined.

The water content for skin is retrieved from (Gales et al., 1994). By drying 5cm^2 squares of harp seal pelt (skin with fur), they determined the average water content to be 58,1±2,4%. This value was used in further calculations. In lack of data on water content in the skeleton of seals, the data on water content in the human skeleton was used(Mitchell et al., 1945). In a study of the chemical composition of the human body, a 35 year old man who died of an acute heart attack, was used to determine the composition of tissues. The water content of the skeleton in humans were determined to be 31,81%

3.7 Equations for estimating total body water:

There were several equations used to estimate the total body water using the tritiated water method. The equations are based on those used by Schots et al (2017) but modified to fit the method in this study.
The water content in the samples must be corrected for and the specific activity in the dose injected and in seal plasma after equilibration must be calculated prior to estimating the TBW. Seal G1-21 is used as an example in the equations showed.

EQ 1: $$TWCps = 0.92PV + 0.30PaV = 980\mu l$$

To calculate the total water content in the plasma samples (TWC_{PS}) equation 1 is used. Where PV is the plasma volume used and PaV is the perchloric acid volume. The water content in plasma is 92% and in Perchloric acid, 30%. Added together this result in 980µl of water.

EQ 2: $$CF = \frac{980\mu}{920\mu} = 1.0562$$

The correction factor (CF) corrects for water content. It is obtained by dividing the total water content in the plasma samples by the water content in plasma.

EQ 3: $$S.A.plasma = 1.0562 * 2189 = 2331.7228$$

The specific activity in plasma (S. A. plasma) is determined by multiplying the correction factor with the average scintillation counting, corrected for background radiation. This determines the activity/ml.

EQ 4: $$S.A.\,body\ water = 2331.7228cpm * ml^{-1} * \left(\frac{1000}{920}\right) = 2533cpm * ml^{-1}BW$$

By using equation 4 the specific activity (S. A.) in the body water (BW) is determined.

EQ 5: $$TWC(stock) = 0.92PV + 0.30PaV + 10\mu l = 990\mu l$$

Again, the total water content must be calculated to be able to correct for water content. But since it here was added 10µl of stock solution this must be included. Abbreviations is the same as in EQ 1.

EQ 6: $$cpm(10\mu l) = S.A.(stock) * \left(\frac{TWC}{1000}\right) = 1270.071cpm$$

The activity in the stock was calculated by multiplying the specific activity in the stock, from the results of the scintillation counting and corrected for background radiation, with the TWC. To get the answer in cpm*ml^{-1}, the TWC is divided by 1000.

EQ 7: $$ID = cpm(ml) * \frac{mass\ of\ injected\ dose}{1.0046} = 12378111966cpm$$

To determine the injected dose (Eq. 7) the specific activity in the stock is multiplied with the mass of the injected dose, divided by the specific gravity of saline to correct for that.

EQ 8: $$TBW = \frac{ID}{SA} = \frac{12378111966cpm}{2533cpm} = 48.84l$$

The total body water is then estimated (Eq. 8) by dividing the injected dose (ID) to the specific activity in the plasma (S.A.). The result is how much body water the injected dose was diluted into.

3.8 Estimating the body composition using the tritiated water method:

The method for making equations to estimate body composition is according to the method by Reilly and Fedak (1990) when estimating the composition in grey seals.
To estimate the body composition the estimated total body water in percent of body mass was plotted against the percentage amount of tissue. Making a regression line from the datapoints the equation for the line is used to estimate body composition.
When using the equations the y value equals the percentage amount of tissue and the x value, equals the estimated TBW (%).

3.9 Statistical analysis:

Statistics were done using Microsoft Excel 2016. Statistical analysis was done using the data analysis tool integrated in Excel. The regression analysis function was used to analyse the significance of the regressions. To test the difference between estimated body composition and determined composition, an Anova test was used. Results are presented as average and standard error of the mean (SEM)
Calculating the correlation coefficient of regressions was done using the function "Correlation" in Excel. The squared of the correlation coefficient is used to calculate the R^2.

3.10 Ethics:

In this study live seals were captured and used in the first part on the experiments before being euthanized. The seals were exposed to mild to moderate stress, but it was done a great effort to reduce both time exposed and stress level. The time between capture and experiment were kept to a minimum, with a maximum time between capture and sampling as low as a couple of hours. Only trained personnel handled the animals. The animals were sedated to assure they remained calm throughout the sampling time, when strapped to the board, and humanely euthanized by an injection of Euthazol.

3.11 Swab samples and radioactive waste:

Swab samples of surfaces of working areas eg. laboratory benches were taken after every workday, after cleaning. A small piece of paper with the swab sample was and put directly into scintillation vials after sampling and 10 ml scintillation fluid added. The swab samples should show radioactivity levels lower or equal to the background radiation, ensuring that no spill of radioactivity had occurred. Daily bench and equipment cleaning was done using cloth soaked with soap and water.

Radioactivity in the swab samples were measured after the laboratory work was done and functions as a safety check for later use of work areas. It is required by the health and safety administration to do swab samples when working with radioactive materials.

All waste were collected in designated waste buckets which could be sealed. The radiation from tritium cannot penetrate through plastic so ordinary waste buckets were sufficient. All waste was then weighed, labeled, and then sent to be destroyed.

4 Results:

4.1 Dissections:

In the dissections, the different parts of the seal were weighed, and their percentage amount of the total body mass was determined. As seen in table 1, blubber and muscles made up the largest parts of the seals. The brain was only weighed separately in three of the seals (G1, G2 and G4) as it was small and assumed to not have a significant effect on the total body water. The average percent brain mass of these three are used for the two that were not weighed. The blood mass used is equal to the total body mass minus the combined mass of tissues.

Table 1: Total body mass and mass of the different tissues (kg) in the harp seals. Values obtained by dissection.

Animal Id	Body mass (kg)	Blubber	Muscle	Internal organs	Skeleton	Skin	Brain	Blood
G1-21	115.6	41.56	34.78	12.74	10.40	8.66	0.22	7.24
G2-21	141.0	54.62	41.88	12.54	12.94	9.58	0.26	9.18
G3-21	149.7	55.74	46.92	15.56	12.46	11.62	0.23	7.40
G4-21	126.7	50.66	38.30	11.48	9.80	8.60	0.20	7.66
G5-21	139.7	55.38	43.46	13.18	9.66	7.52	0.23	10.50

From the weight of the different tissues the percentage amount of the total body mass for the tissues was calculated (table 2). There is low variation in percentage amount of the tissues between the seals, the highest standard deviation is in blubber amount which is the one that also varied the most in mass (kg).

Table 2: The percentage amount of tissue of the total body mass.

Animal Id	Blubber	Muscle	Internal organs	Skeleton	Skin	Brain	Blood
G1-21	35.95	30.09	11.02	9.00	7.49	0.19	6.26
G2-21	38.74	29.70	8.89	9.18	6.79	0.18	6.51
G3-21	37.23	31.34	10.22	8.32	7.76	0.18	4.94
G4-21	39.98	30.23	9.06	7.73	6.79	0.16	6.05
G5-21	39.64	31.11	9.26	6.91	5.38	0.18	7.52
Standard deviation	1.51	0.63	0.81	0.82	0.82	0.01	0.83

The blubber constituted to the most significant component of the carcass in all seals in this study. It contributed up to 40% of the total body mass.

4.2 Tissue water content:

The same method is followed for the different tissues. The water content after 48 hours of drying or when the water content stabilized is the values used in further calculations.

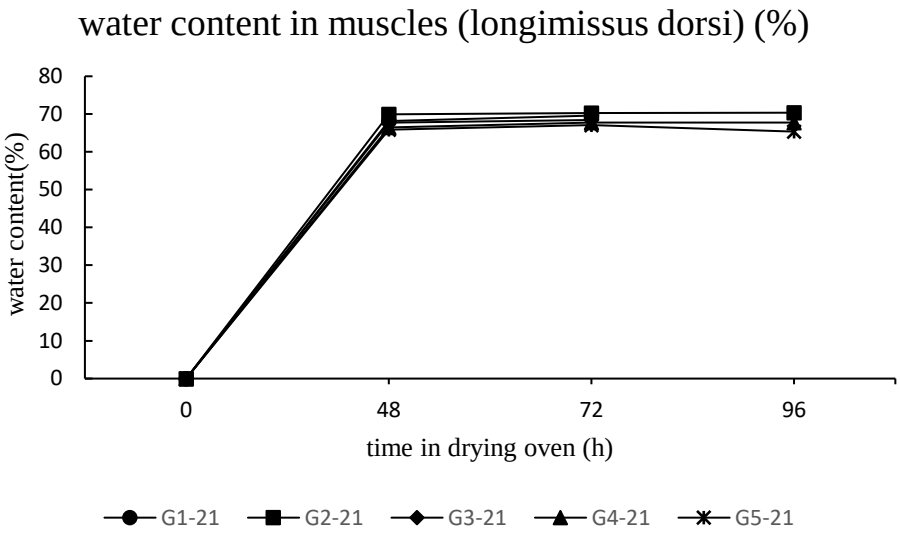

Figure 3: The percentage water content in muscle samples.

The water content in the muscles (fig. 3) is similar for all seals. After 48 hours of drying the determined water content was stable between 65 and 70%.

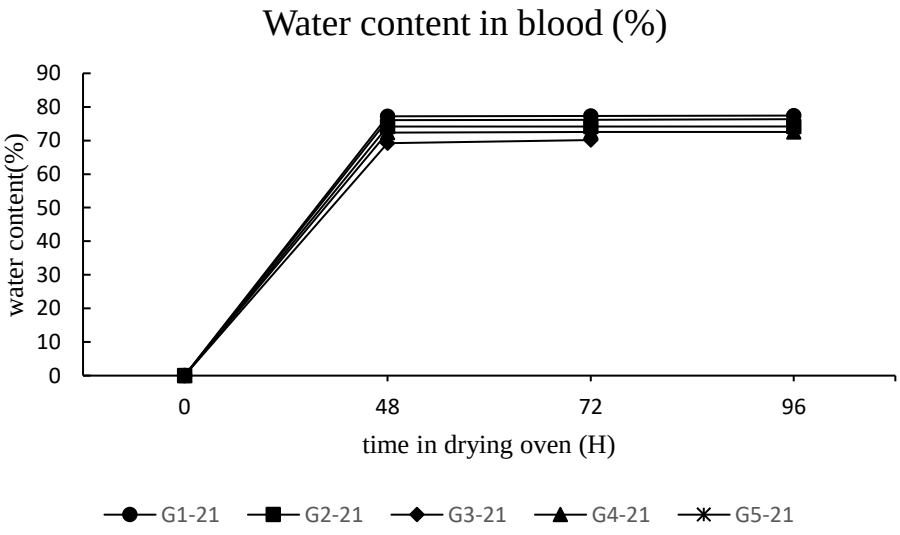

Figure 4: The percentage water content in blood samples.

The water content in the blood is (fig. 4) determined to be between 70 and 80%. Drying samples showed little variation.

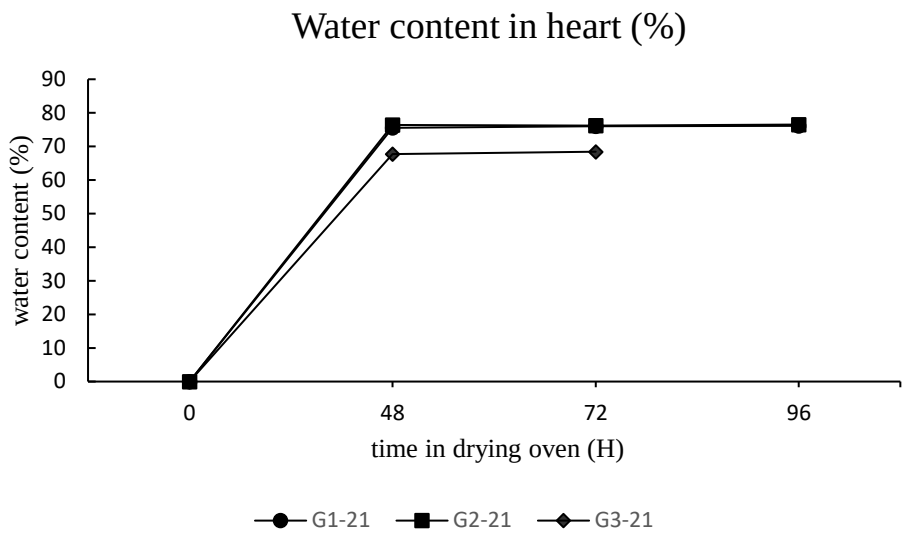

Figure 5: The percentage water content in heart samples

The water content in heart tissue (fig. 5) was only measured for three seals. It was assumed to have a similar water content as muscle tissue and therefore only three seals were sampled. For G1 and G2 the water content was 75 and 76%. For G3 it ended at below 70 %.

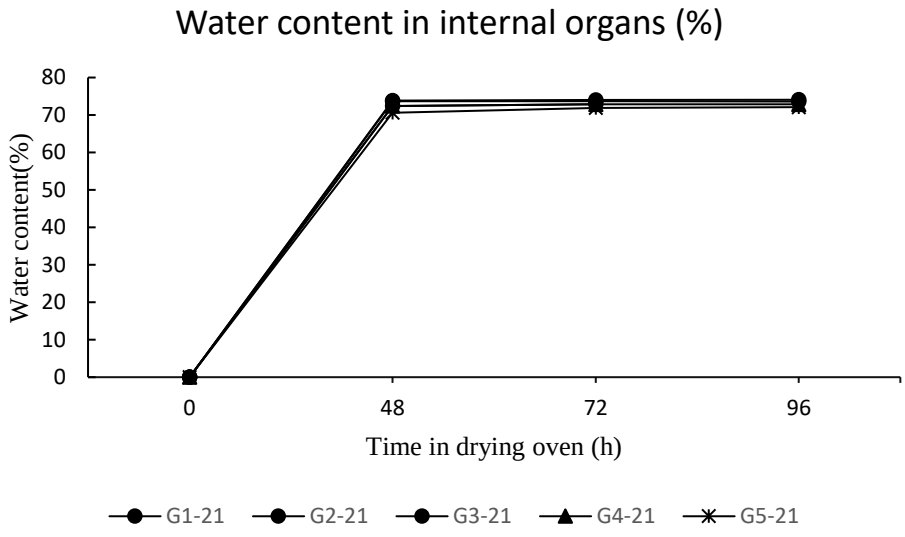

Figure 6: The percentage water content in internal organs

The water content in the internal organs (fig. 6) was determined to be approximately 70%. All samples had consistent results with very low variation. All internal organs including eyes and brain is included in this.

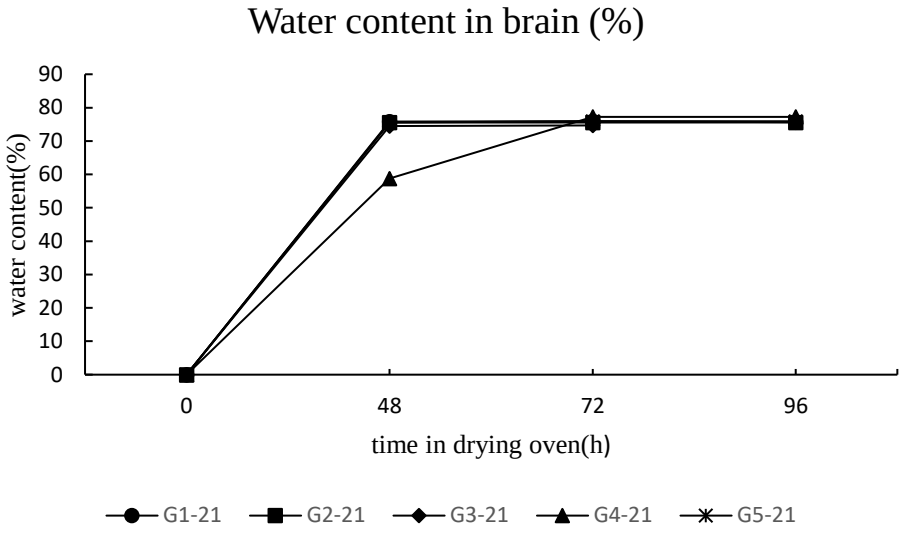

Figure 7: The percentage water content in brain samples

The water content in the brain (fig. 7) were determined to be between 74 and 80% for all seals except
G4-21 after 48 hours. But after 72 hours all seals had a similar water content the brain, and it stayed stable at that percentage. The value after 72 hours drying was therefore used in the further calculations.

Table 3: Determined water (%) content in tissues. The values are the average of the parallels (n=3), except blubber, skeleton and skin.

Animal Id	Blubber	Muscle	Internal organs	Skeleton	Skin	Brain	Blood
G1-21	7.5	68.32	73.58	31.81	58.1	75.64	77.41
G2-21	7.5	70.35	74.07	31.81	58.1	75.56	74.16
G3-21	7.5	68.42	72.86	31.81	58.1	76.15	70.13
G4-21	7.5	67.74	72.86	31.81	58.1	77.23	72.55
G5-21	7.5	65.37	72.10	31.81	58.1	76.15	76.34
Average	7.5	68.05	73.10	31.81	58.1	76.15	74.11
Standard deviation	0.00	1.60	0.68	0.00	0.00	0.75	2.65

The water content in the different tissues were determined by the average water content in parallel subsamples (Table 3). Water content in blubber, skeleton and skin were retrieved from other studies and therefore have no variation (Gales et al., 1994; Mitchell et al., 1945; Nordoy & Blix, 1985a).

4.3 Radioactivity measurements:

Table 4: Total body mass and body water of the five seals. Determined TBW calculated with the values in table 3.

Animal Id	TBW(L)	determined TBW (L)	difference(L)
G1-21	48.84	50.32	1.48
G2-21	55.44	59.49	4.05
G3-21	61.86	63.48	1.62
G4-21	50.98	51.89	0.91
G5-21	53.79	57.47	3.68

By using equation one to eight the TBW (L) could be estimated (Table 4). The difference between the estimated and the determined TBW (L) could then be calculated.

Table 4: Total Body Water as percent of total body mass.

Seal Id	Estimated TBW (%)	Determined TBW (%)	difference
G1-21	42.25	45.70	1.28
G2-21	39.32	44.52	2.82
G3-21	41.32	44.67	1.08
G4-21	40.24	43.38	0.72
G5-21	38.50	43.53	2.64

The TBW as percent of the total body mass (kg) was calculated (table. 5). Average underestimation was calculated to 1.72±0.39%.

4.4 Body composition

By plotting the relative amount of tissue (%) (Table 2) and the estimated TBW (%) (Table 5) and doing a regression, the equation for the regression line is obtained. The regression equation is used later to estimate the body composition.

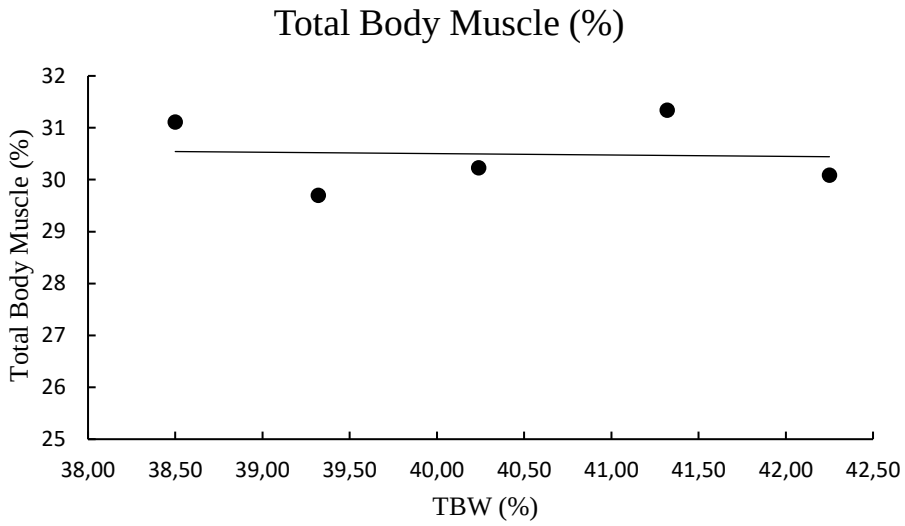

Figure 8: percentage muscle. Equation for the regression line: y = -0.0265x + 31.563. correlation coefficient: -0.05. P-value = 0.32.

The total body muscle (fig. 8) has a weak negative correlation with the TBW. It has a small decrease with an increasing TBW.

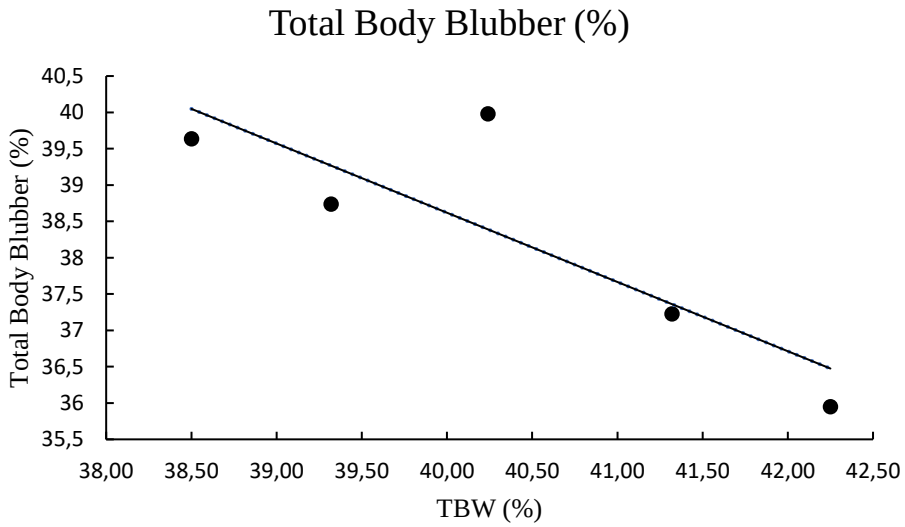

Figure 9: Total body blubber as a function of TBW. Equation for the regression line is y = -0.9531x + 76.741. Correlation coefficient: -0.84. (P-value = 0.07).

The percentage amount of blubber shows a clear decrease with increasing TBW (fig. 9). Four of the seals follow an almost linear decline in total body blubber except G4-21 which had the highest amount of blubber out of the five.

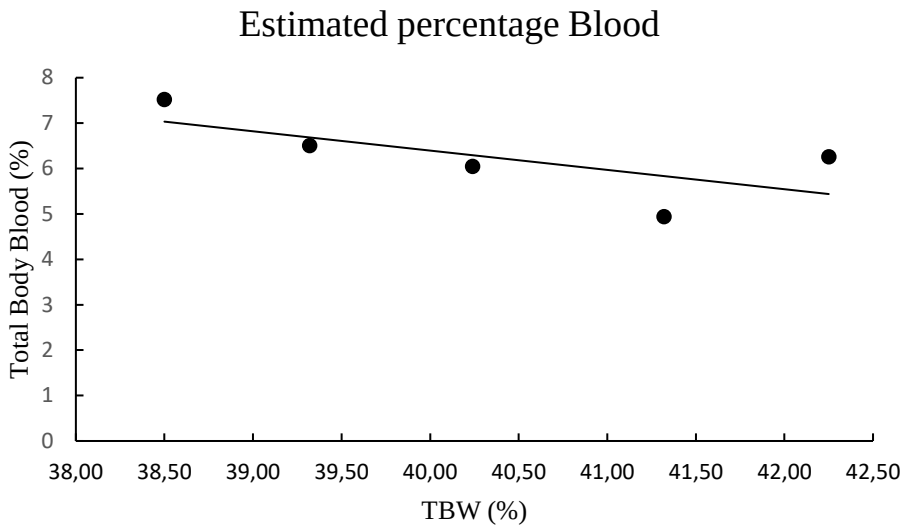

Figure 10: percentage blood, regression line equation: y = 0.4253x + 23.407, correlation coefficient: -0.69. p-value= 0.19

Percentage amount of blood is showing a decreasing trend with increasing TBW (fig. 10). The correlation factor indicates a moderate negative correlation between blood and TBW.

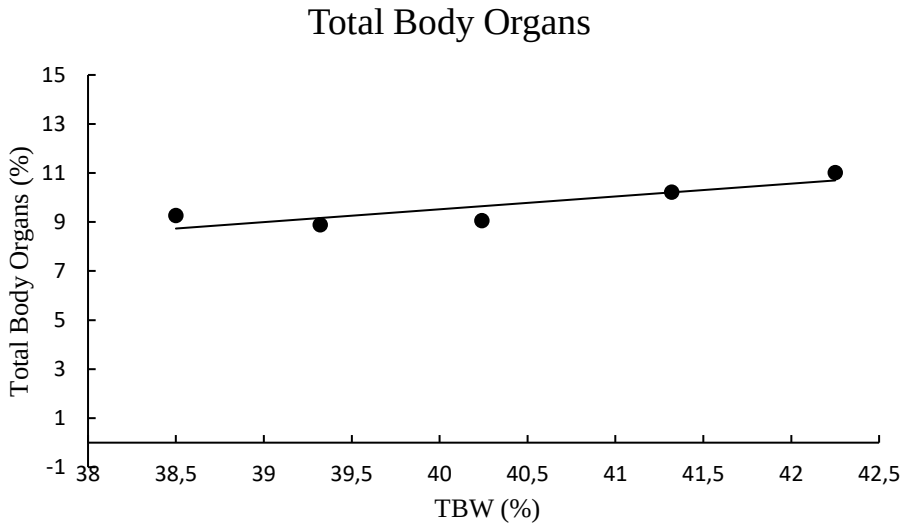

Figure 11: percentage internal organs. Regression line equation: y=0.523x – 11.401. correlation coefficient: 0.87. p-value=0.055

The total body organs (fig. 11) had an increasing trend with with an increasing total body water. the increase is low and statistically insignificant, with only just over 2% separating the highest and the lowest value for total body organs.

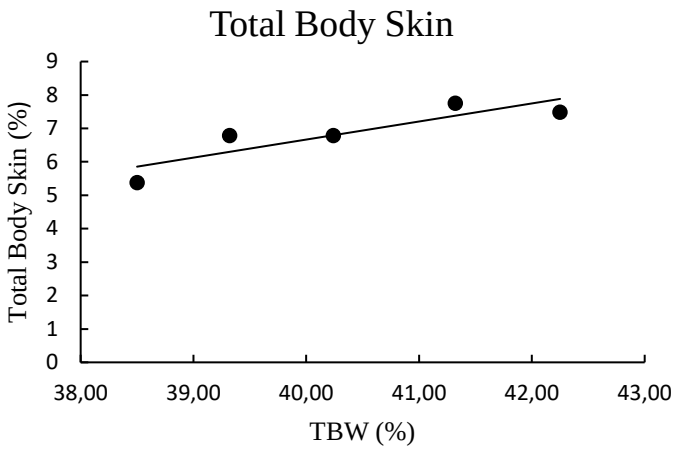

Figure 13: The skin with fur as a function of the TBW. Regression line equation: y=0.5402x – 14.944. correlation coefficient: 0.88. P-value=0.048

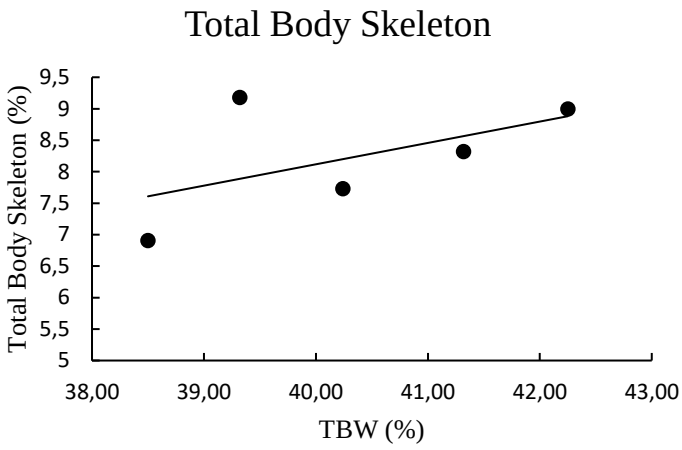

Figure 12: Total body skeleton as a function og the TBW. Regression line equation: y=0.3393x – 5.455. correlation coefficient: 0.55. P-value=0.34

Both the percentage amount of skin and skeleton increases with an increasing TBW (fig. 12; 13). The skin was assumed to increase as a larger animal has more volume and therefore a higher surface area. The skeleton had more variation, where one had a higher percentage amount of skeleton with a low TBW compared to the rest.

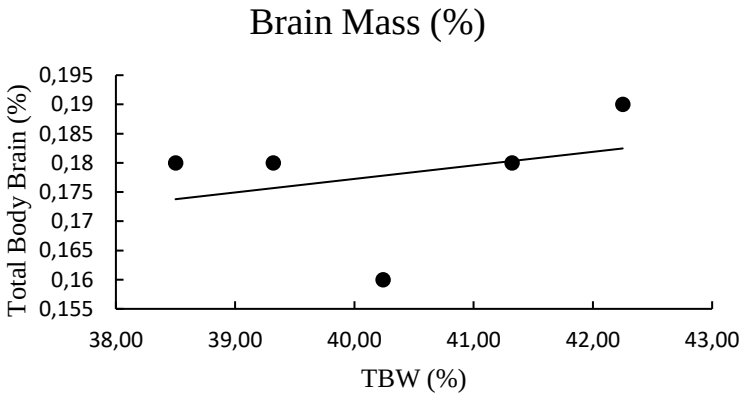

Figure 14: percentage brain as a function of the TBW. The regression line equation is y=0.0023x + 0.0845. correlation coefficient: 0.32. P-value: 0.60

The brain was also weighed to see if it was affected by the TBW. Except for one outlier it shows an increase with the increase of TBW (fig. 14). The brains low percentage amount of the total body mass does so that a difference in brain mass does not affect the TBW significantly, as it only contributes to under 0,2% of the body mass.

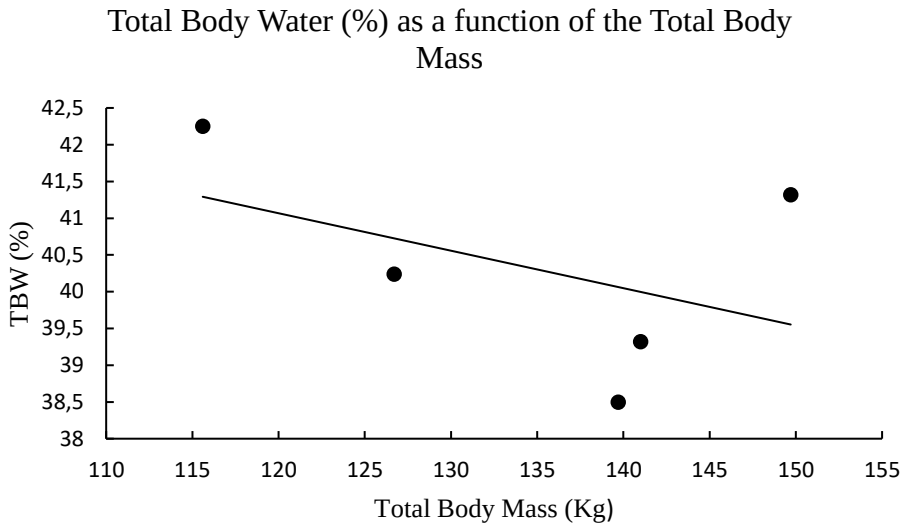

Figure 15: The total body water plotted against the total body mass of the five harp seals. Regression equation: y = -0,051x + 47.184. Correlation coefficient: -0.44. P-value = 0.44.

The total body water showed a decreasing trend when the body mass of the seal increased. The variation in the sample is high and no points are on the line. The correlation is not significant (P-value>0,05), but this could be affected by the low number of animals tested.

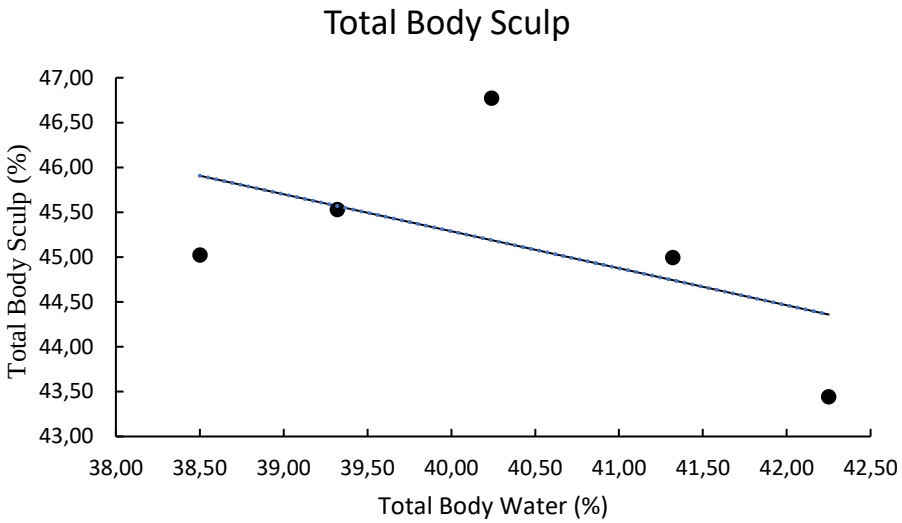

Figure 16:The Total Body Sculp as a function of the TBW. correlation coefficient: -0.52. Regression line equation: y = -0.4127x + 61.797. P-value = 0.37.

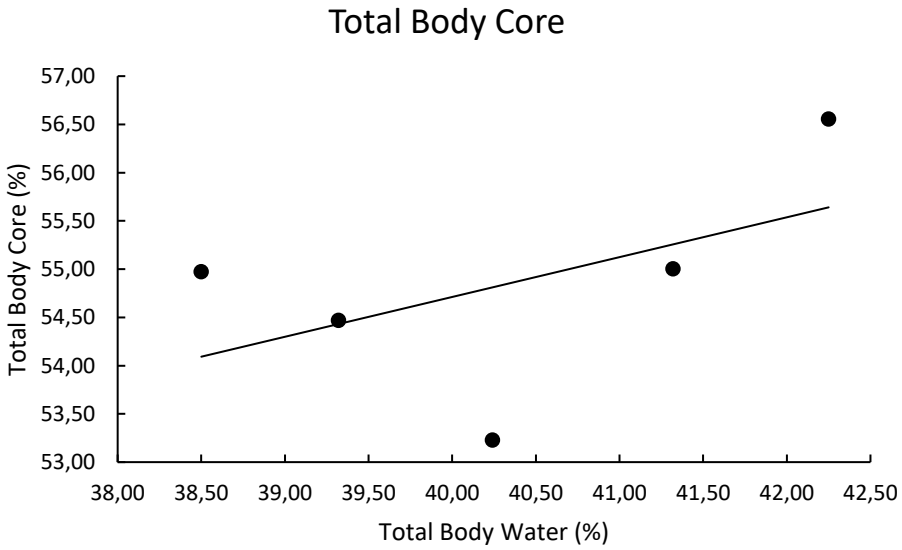

Figure 17: The Total Body Core as a function of the TBW. correlation coefficient: 0.52. regression line equation: y=0.4127x + 38.203. P-value = 0.37.

The total body sculp (fig. 17) and core (fig. 18) did not show a linear correlation when plotted against the total body water. The variation in the samples is high.

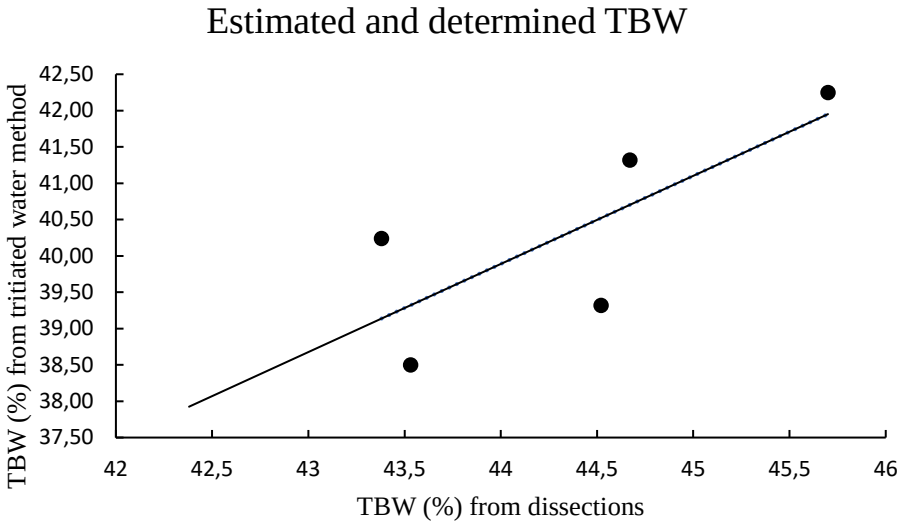

Figure 18: The estimated TBW for the five seals plotted against the determined values obtained by dissection. regression line equation: y=1.2127x – 13.469. P-value= 0.13

The estimated TBW from the tritiated water method was plotted against the determined TBW from det dissection (fig. 19). As seen the two TBW, is linearly correlated.

The equations 11 – 16 are obtained from the regressions of tissue and TBW. In the equations the percentage amount of tissue is the y – value. The estimated TBW from the tritiated water method is inserted as the x – value.

Eq. 11 $Muscles: y = -0{,}0265x + 31{,}563$

Eq. 12 $Blubber: y = -0{,}9531x + 76{,}741$

Eq. 13 $internal\ organs: y = 1{,}4457x + 26{,}371$

Eq. 14 $blood: y = -0{,}4253x + 23{,}407$

Eq. 15 $skin: y = 0{,}5402x - 14{,}944$

Eq. 16 $skeleton: y = 0{,}8778x + 33{,}104$

5 Discussion:

This study showed that the tritiated water method is a useful method to estimate the body composition of adult harp seals. This is due to the near linear correlation most tissues show when plotted. As the closer the points are to the regression line, the closer is the estimation to the determined value. The total body blubber shows a correlation with the TBW with one outlier (G4). The total body blubber tells the condition of the seal and is an important result of the tritiated water method. Considering this, the method can estimate the total body blubber well.

The different tissues and equations show different trends due to biological differences. Blubber has a negative trend as it has a low water content, and an increase in blubber will therefore lead to a decrease in TBW. All other tissues except muscles have an increasing trend with increasing TBW due to the high water content.

The range of TBW in this study is limited, ranging only from 38.5% - 42.5%.
Compared to when performed in grey seals by Reilly and Fedak (1990), which had a larger range from just above 40% to almost 70% (fig. 20). They also had a large sample size with eight seals.

The correlation between sculp and core to the TBW (fig. 17, 18) shows a large variation and not a linear trend. It is unclear why it was not linearly correlated. The sculp is the blubber and skin together and it was assumed that it would still follow a linear trend. But the skin had a positive trend and the blubber a negative one, and combined the variation became too large. As for the core, which is all that is inside the blubber in the seal, is the opposite of the sculp. It was assumed that the core would have a positive correlation with the TBW, as almost all tissues included had. The regression line shows a positive correlation, but the variation is too large to be able to estimate it accurately.

When plotted the estimated TBW and the determined TBW does not lie on the regression line as it would if they values were the same. Ideally the values would be the same and the line would have x equal to y. But the tritiated water method underestimates the TBW and the plots are therefore not on the line.

5.1 Dissection:

As the seals were all dissected by hand, to separate the different tissues, there are some possible errors related to this. Some of the muscle mass was weighed as skeleton and some skeleton tissue were included as muscle mass, as these tissues were sometimes hard to separate. The same also applies to some extent for blubber and skin.
The estimated mass of blood in this study was assumed to be the difference between initial total carcass mass (kg) and the sum of all the mass of all different tissues weighed after dissection. However, during further processing of samples, it was clear that many muscles and organs held a considerable amount of blood, that is not included in the blood mass. It is therefore probable that the blood mass is higher than the one measured.

The water content of skin and skeleton were not determined directly in this study due lack of a grinder that could effectively grind these tissues t a homogenous mass. The values for skeleton and skin water content were therefore obtained from other studies. For skeleton water content values from a study on human tissue composition were used (Mitchell et al., 1945). The value was determined to be 31.8%.
Regarding skin tissue, the value for water content in harp seal skin (68%) was taken from Gales et al. (1994).

The water content in the different tissues mostly stayed reasonable and were similar for the seals for the same tissues. The water content in the muscles, internal organs and the heart is similar. Probably due to the likeness of the tissues. It was assumed that the heart and the muscles would have a similar water content due to both being muscles.

When determining the water content of the brain (fig. 7), G4 had a lower water content at 48 hours than the rest. This is probably due to errors when weighing samples. Probably the weight was not reset properly before weighing the sample giving a higher weight, leading to an artificially low and unreliable water content at that point. But at the next weighing point it reached the same level as the rest. It kept stable at that weight and therefore that is used in further calculations. Unexpectedly the blood had a lower water content than protein in some of the samples. The blood of diving animals often has a high haematocrit (Castellini et al., 2010; Hedrick & Duffield, 1991), which causes a slightly lower water content.

Water content for blubber was determined by separating the two components of the blubber, the tissue and oil. This gave an inaccurate estimate of the total water content of blubber since the ratio between oil and tissue was not possible to determine. From a study on grey seal pups, the water content of blubber was determined to 7,4±1,1% (Nordoy & Blix, 1985b). This seems like a more reasonable result.
Using the water content in the blubber of grey seals instead of the one determined in this study, the estimated TBW is closer to the determined. As it should be according to (Bowen & Iverson, 1998). With the blubber water content at 7.4 %, the tritiated water method underestimates the TBW with on average 1.28%.

5.2 Quenching:

Quenching is when the radioactivity (cpm) in the samples measured by the scintillation counter is incorrect due to scintillations not being counted. It can occur because of a lowered number of scintillations or inability to count scintillations. It leads to a lowered or inconsistent measured radioactivity and therefore a higher dilution volume and an overestimation of TBW.

There were no immediate signs of quenching as the results were consistent and at a reasonable level. Calculations also gave a reasonable TBW and therefore the measurements were accepted. When measured immediately after processing the results showed signs of quenching. The main theory for this was that the air bubbles from the mixing affected the scintillation counting. After some time, these would float up to the surface in the vial and not affect the measurement.
To avoid errors in measurements the samples in the scintillation vials were made as similar as possible. The stock solution samples had uncontaminated plasma added to be similar to the plasma samples with tritiated water from the seals, as it was assumed that plasma could reduce the efficiency of the scintillation counter.

5.3 Body composition estimation:

Body composition of the seals were estimated using the Equations from the regressions (table. 6).

Table 6: Estimated body composition using the regression equations.

Estimated body composition (%)							
Seal Id	blubber	muscle	organs	blood	brain	skin	skeleton
G1-21	36.473	30.443	10.696	5.438	0.182	7.879	8.880
G2-21	39.265	30.521	9.163	6.684	0.175	6.297	7.886
G3-21	37.359	30.468	10.209	5.834	0.180	7.377	8.565
G4-21	38.388	30.497	9.645	6.293	0.177	6.794	8.198
G5-21	40.047	30.543	8.735	7.033	0.173	5.854	7.608

The estimates are close to the determined values with no consistent over or underestimation for any of the tissues.
Organs and blood have the largest differences between estimate and determined value. For internal organs, the estimates are 9% and 10% of total body mass, while the determined value varies from 8,7% to 13,4%. The same applies for the blood that is estimated to 6 and 7% of body mass while determined mass varies from 3,3% to 9,8%. the blood and organs were difficult to separate in the dissections because organs were filled with blood. This is probably one of the reasons for the variation. When the organs were left for some time, blood would leave the veins and then result in a lower weight of the organs. It is probable that values for blood should be higher, and lower values for organs. Using Anova tests, the difference between estimated values and determined values by dissection were statistically insignificant (P-value<0.05).

It was assumed that the TBW would increase with an increasing total body muscle (%), which it did not. The results showed a decrease. Unlike what other studies on other species have estimated. Due to the high water content in muscles it was assumed that a high total body muscle would make the TBW higher. But as seen in figure 20 the total body blubber shows an increasing trend with increasing total body muscle, although very low. This could be one of the factors explaining the decrease. As shown in the figure the total body muscle shows a downwards trend when the blubber amount increases. So it might be that with an increasing muscle mass the mass of blubber also increases which makes the TBW to be lowered because of the low water content in the blubber.

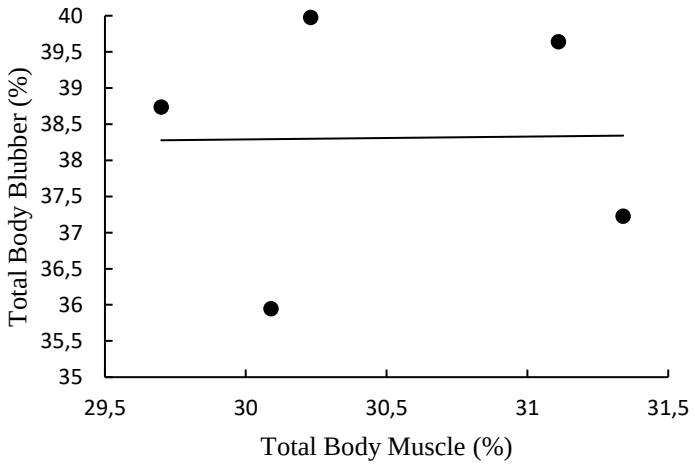

Figure 19: Total Body Blubber as a function of the Total Body Muscle. Regression line equation: y = 0,0392x + 37,113, Correlation coeffisient: -0.84 P-value:

The TBW decreases when the total body blubber increases. As it was expected because of the low water content in blubber. Therefore, the higher the percentage of the total body mass that is blubber the lower the TBW will be.

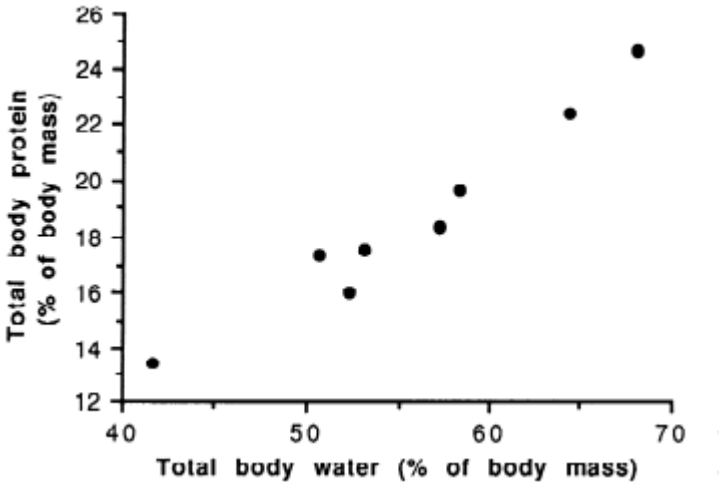

Figure 20: total body protein as a function of total body water. Collected from (Reilly & Fedak, 1990)

The muscle percentage of total body mass decreased with an increasing TBW water in the experiment. This was not as assumed, as the muscle had an average water content of 68,047±0,194%. When validated in grey seals the muscle showed a clear increase with increasing TBW (Reilly & Fedak, 1990). The grey seals in the study did not have a TBW as low as the harp seals in this study. Most of them had a TBW in percent of body mass, over 50%. While the harp seals were between 38,50% and 42,25%. The grey seals total body blubber and muscle were also lower than in harp seals, and the amount varied much more. As seen in the figure the grey seals have a total body protein as low as below 14% up to around 25%. While the harp seals in this study have a total body muscle between 29% and 32%.

5.4 Other studies on the method:

The method for validation was according to when done in grey seals by Reilly and Fedak, (1990). Although the grey seal is not considered an arctic seal, they have several similarities with the Arctic seals, such as a thick blubber layer, milk with a high fat percentage (Baker, 1990), and even a post weaning fasting period (Noren et al., 2008). The size range of females are also similar (Sparling et al., 2006).

Mostly the regressions between TBW and tissues was similar between the two species, but in the harp seals the muscles had a negative trend when plotted. While the grey seals have the opposite trend. Due to the muscles high water content (water content) it was expected that there would be a positive trend.

In this study the tritiated water method underestimated TBW with on average 1.72±0.39%. In grey seals the TBW was overestimated with on average 2.8±0.9% using the tritiated water method (Reilly & Fedak, 1990). Also when validated on Antarctic fur seals (*Arctocephalus gazella*) the tritiated water method overestimated the TBW with 2,80±0,68% (Arnould et al., 1996). When used in ringed seals (*Phoca hispida*) to estimate water flux it again overestimated the TBW with 1.6%(Lydersen et al., 1992). When the tritiated water method was reviewed for the use in pinnipeds, the method consistently overestimates the TBW (Bowen & Iverson, 1998).
Considering these results, it was expected that the TBW would be overestimated in this study as well. Which it did not.

Reilly and Fedak (1990) listed their equations to estimate the Total body fat and total body protein (Eq. 17; Eq. 18). They based their equations on the regression between the total body tissue and the determined TBW by desiccation. While it in this study the equations are based on total body tissue and estimated TBW from the tritiated water method.

Eq. 17 $$Total\ Body\ Fat\ (\%) = 105.1 - 1.47 * TBW(\%)$$

Eq. 18 $$Total\ Body\ Protein\ (\%) = 0.42 * TBW(\%) - 4.75$$

The equation for estimation of blubber (%) is similar to the one for harp seals in this study. Slope and intercepts are comparable, also considering the biological factors. The equation for estimating total body protein has lower intercept and a positive slope, while the equation from this study has a negative slope. The standard deviation of the residuals in the plots by Reilly and Fedak (1990) is for Total body fat: 1.1 and total body protein: 0.8. In this study the standard deviation of the residuals was for TBB: 0.81 and TBM: 0.62. Indicating that this study had a lower difference between the observed values and the estimated values. This however does not explain the different results. The R^2 For the regression for Eq. 17 is 0.994, and for Eq. 18 it is 0.955. In this study the R^2 of TBB was calculated to 0.71., and the R^2 of TBM was calculated to be 0.003. The R^2 . A R^2 close to 1 indicates that the model fits the prediction. The TBM has a low R^2, indicating no correlation between the TBW and the TBM.

5.5 Statistical analysis:

In this project only five adult female harp seals were used. Due to it being a Master project with limited time and resources available this number of animals were chosen. A larger number of animals would most probably give results with a higher statistical correlation. When compared to the study by Reilly and Fedak (1990), equations for estimating TBB and TBM, calculations indicate that the equations in this study has weaker statistics than those for grey seals. This is probably due to the limited range in total body water, body composition and number of animals in this study.

The correlation between pups and condition in mothers are limited because of a low sample size. A larger sample size and from different times of the lactation period, including before and after as well. The seals are at their fattest before breeding and lose mass during it. Therefore estimating condition of both adults and pups through the breeding period, could provide measurements with a strong correlation.

6 Potential errors

There are several potential errors when validating the tritiated water method. Both for the validation and the performing of the method. For the tritiated water method Nagy and Costa (1980) and lifson and Mcclintock (1966) have studied the method and analyzed possible errors that must be accounted for when performing the method. Since a validation study is a two-part study, both have potential errors. Errors when performing the tritiated water method, will in most cases lead to over and underestimations, but errors in the dissection will result the determined values being wrong. This would make it seem like the tritiated water method estimates wrong but in reality it might not.

6.1.1 Determined water content

As there was no possible way to grind and homogenise a total skeleton of the seals this was not done. This required large machines with the capacity to grind hard components. As not many has grinded only the skeleton from seals, a reliable source was hard to find. However, the water content in the human skeleton has been determined and was used in this project(Mitchell et al., 1945). As there are differences between humans and seals, there is reason to assume that there is a difference in water content. However, the determined value 31.8%, was used anyway, in lack of other sources.
The skin was also not possible to grind, even as it could be cut into smaller pieces. The water content in the skin was retrieved from a study on harp seals (Gales et al., 1994) and was seen as a reliable source for this project.

6.1.2 Evaporative water loss

Any loss of water from the seal after euthanasia would make the determined value for TBW lower. This will cause an overestimation of TBW by the tritiated water method, as the determined volume it is compared to is lowered. Water evaporating from the seal when frozen was considered the most important contribution to water loss. The seals were therefore tightly wrapped in plastic to avoid this. But some water could have evaporated still and condensate on the plastic. Especially from the eyes, mouth, and nose. This water loss would make the determined TBW lower than the “true” value and create a larger difference between the estimated and determined values.

It is assumed that some water was lost during the dissections due to evaporation. the dissection lasted for approximately one whole day and tissues were exposed during that time. Since the different tissues were separated and exposed, their surface area was also increased allowing for more evaporative water loss. This water loss was included in the blood mass, as it was the difference between initial body mass (%) and after dissections.
It can also create errors when determining the water content of different tissues. How large an error it causes depends on the time tissued were allowed to dry.

6.1.3 Accuracy in measurements:

The validation of the tritiated water method requires a high level of accuracy in all measurements. The main possibility where this can occur is when pipetting the different substances. All pipettes were calibrated before being used, but there is still possible that the volume pipetted was too small or too large. This would lead to either over or underestimation of the TBW. As no errors directly related to this were detected it was assumed that volumes were correct. If there was a wrong volume pipetted it is likely it was the same in all samples. Therefore, it would not be detected if the error is not significantly large.

6.2 Equilibration time:

The time needed for the tritiated water to equilibrate in the body water varies between different sources. The range is from over a day to just half an hour, depending on the study. In this project the equilibration time was set to one hour. This was assumed to be a sufficient time to let the tritiated water equilibrate into the body water. Equilibration time in seals has been researched and was determined to be between 0,5 and 3,0 hours (Bowen & Iverson, 1998). When performed in grey seals equilibration time was set to between 75 and 90 minutes (Bowen et al., 1999) even though stating that it should be equilibrated in well under 75 minutes. The tritiated water method has been used in sub adult harp seals and used an equilibration time of one hour (Lager et al., 1994), But stated that pilot studies showed a complete equilibration after only 30 minutes. Considering this the 60 minutes equilibration time was used in this study.

Some tritiated water will be lost between the time of injection and collection of blood samples. Water loss through the skin, urine and through respiration is a possible error creating a higher dilution volume and an overestimation of the total body water.
Harp seals has an advanced turbinate system to avoid respiratory water loss (Folkow et al., 1988), but some water is still lost to the surroundings.
The skin of seals is thick and water permeability is limited (Montagna & Harrison, 1957). Being a marine mammal spending most of its time in water, avoiding water exchange through the skin is important to keep a stable water balance.
Tritiated water lost through urine is also one possibility. The urine increases the dilution volume and was not measured as a part of the total body water. No urine was observed lost during the equilibration time, but it is assumed that the tritiated water injected also equilibrated with the urine lost after euthanasia. The error in determined TBE is therefore equal to the volume of the lost urine.

Equilibration time is linked to the listed ways of losing tritiated water, causing errors, as a too long equilibration time increases the tritium lost. The total body water volume is assumed constant, so the water influx is equal to efflux. But tritium is lost with the water efflux and not returned to the body. The lowered amount of tritium results in a higher dilution volume and an overestimation of TBW.

7 Further studies using the tritiated water method:

7.1.1 Body condition related to body mass (kg)

The most important result of the tritiated water method is the amount of blubber the seals have . The percentage amount of blubber can be seen as the seals body condition, where a high percentage means good condition, and a low means a bad condition.
It is not necessarily the largest seal that is in the best condition (fig. 21). For the seals used in

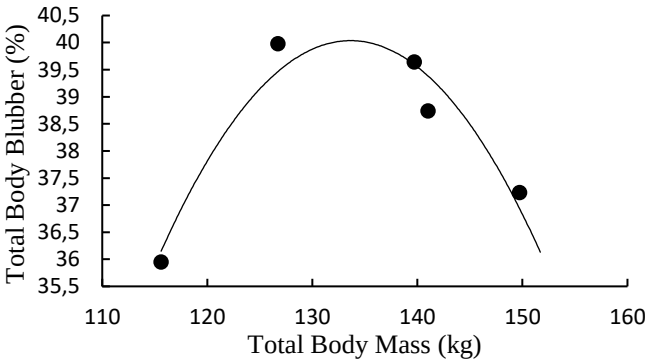

Figure 21: Total Body Blubber as a function of the seal Total body mass (kg)

this project, the medium sized seals were those with the largest percentage amount of blubber. The assumption that a large animal is the one in best condition is not always correct. The largest seal used in this study was over 30 kg heavier than the smallest, but it only had 1,28% higher percentage amount of blubber. But the seals used were in the middle of lactation and it is a low sample size. Those with the lowest total body blubber also had the largest pups. Meaning they transferred more energy and lost more blubber to the pups than the rest. During the breeding period the seals lose a considerable amount of mass (Nilssen et al., 1997). To conclude if there is a correlation like this, more seals without the energy loss to pups should be used.

7.1.2 Effect of mass of pups:

The seals were caught in different stages of the lactation period, and during that, energy is transferred from the mother to the pup. The harp seals store energy mostly in blubber, and therefore it was assumed that the females with the largest pups had the lowest relative amount of blubber. As seen in the figure 23 there is a negative trend in total body blubber and pup mass (r=-0.72).

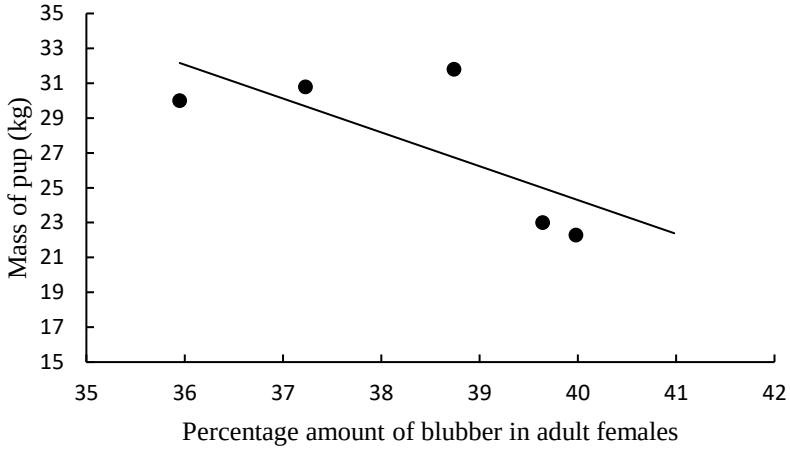

Figure 22: Pup mass (kg) as a function of the percentage amount of blubber in the mother. Correlation factor: -0.72. P-value: 0.50.

but the correlation is not significant (p-value>0,05). But since the females and pups were caught at different stages of lactation, is a low sample size, it cannot be used to say that the lactation does not affect the female's percentage blubber amount. The sample size for this is relatively low, and it might be that with a higher number of animals used at different times of lactation, will result in a significant change. The harp seal is at its fattest just before breeding, and loses mass through the breeding and following moult, being at its leanest in June and early summer (Nilssen et al., 1997). Capture of seals at the end of the breeding and moulting should increase the range of total body blubber and TBW.

The seal with the lowest condition also has the largest pups. Suggesting that the lowered condition comes from the pup drinking milk and energy is transferred from the mother to the pup. When the mass of the pup was plotted against the lean body mass of the mother, there is a positive trend (r=0.40). But the variation in the samples is high and there is no clear linear correlation. The pup with the smallest mother is also as large as the one with the largest mother. Indicating the lean body mass of the mother has a low effect on the mass of the pups.

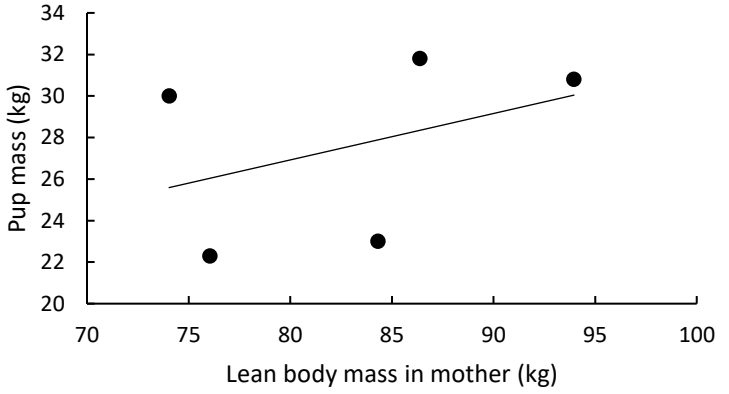

Figure 244: The mass of the pups (kg) as a function of the mother's lean body mass (kg)

The weight gain of the seal pups when lactating comes from the mother only and is almost a transfer of blubber from one to the other.
As seen with many species, a lowered condition often results in in a lower breeding success. Either less offspring is born or fewer survives. This is shown in harp seals as well (Øigård et al., 2013), where blubber thickness in the 1+ age group had a clear effect on the pup production in the white sea area. The data from these five seals indicates that the relative amount of blubber in the mothers has an effect on the growth of pups. It does not show if there is a difference if the mother started with a relative small or large amount of blubber. But one could assume that with a low initial blubber percentage the total possible energy transfer to the pup is lower than with a high initial percentage. This could lead to the mother using her reserves and might leave the pup before it has gained enough weight. This will again leads to a lowered breeding success. So to estimate the condition of harp seals could be useful as an indicator for breeding success and pup production.

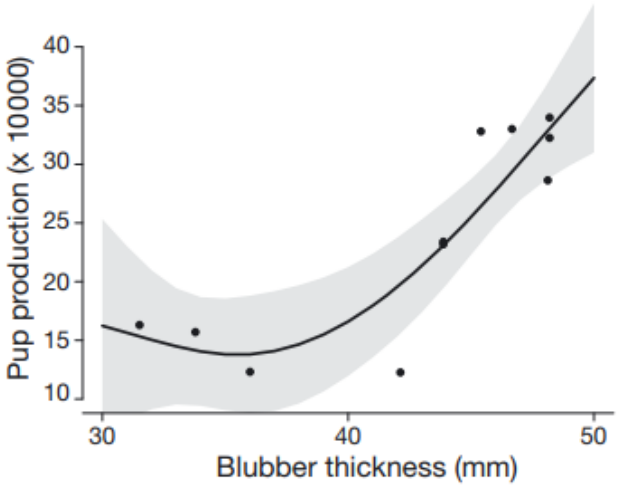

Figure 25: The effect of blubber thickness on the pup production.(Øigård et al., 2013)

7.1.3 Condition in relation to competition and prey abundance

Competition with other species for prey could affect the seal's condition. As less prey for the seals to eat results in a lower condition. The Barents Sea inhabits a variety of different predators that feed on the same species as the harp seals. Collapses in the populations of prey species is as important and has happened several times with different fish species due to overfishing. Therefore, good management including multiple species is necessary (Lindstrøm et al., 1998).

Harp seals have a varied diet consisting of both fish and crustaceans. Diets depend on where the seal is located in the Barents Sea as the different prey species are located in different areas(Lindstrøm et al., 1998). In seals captured in Olgastredet near Svalbard, the amphipod *Themisto libellula* was most frequently found in stomach content (Nilssen et al., 1991). While in the south east Barents Sea, Herring (*Clupea harengus*) and polar cod (*Boreogadus saida)* dominated the diet (Lindstrøm et al., 1998).

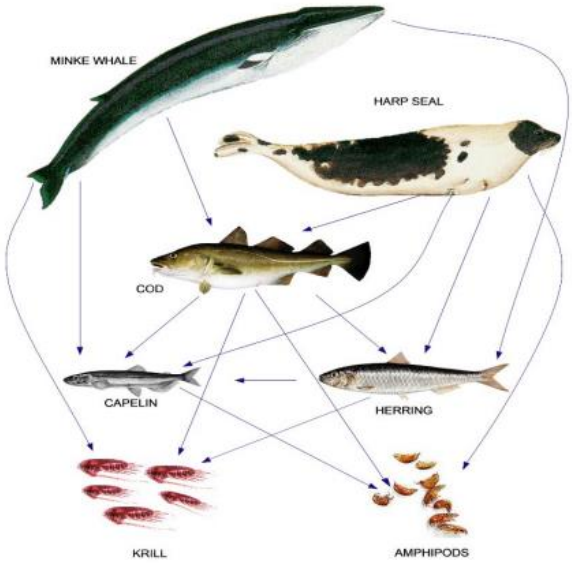

Figure 25: Foodweb including selected species in the Barents Sea. Arrows indicating relationship between predator and prey, pointing from predator to prey. (ICES, 2019)

Atlantic cod (*Gadus morhua*) and minke whale (*Balaenoptera acutorostrata*) are two other top predators in the Barents Sea which is possible competitors to the harp seals for prey (fig. 25) (Bogstad et al., 2015; ICES, 2019). The cod diet consists of a large variety of species with capelin being a significant contributor (Holt et al., 2019). The population of cod in the Barents Sea is high have been increasing and climate change and warmer water is one of the reasons for this (Kjesbu et al., 2014). The minke whales diet consists of mainly herring, capelin and krill (*Thysanoessa sp*), but it too can change its diet depending on what is available (Bogstad et al., 2015).

The different diets of the predators mentioned overlaps and indicates competition. So increasing populations or competitors leaves less prey for the harp seal. The same happens with decreasing populations of prey, where there is less available prey for all predators. example is the capelin, which is predated on by most predators in the Barents Sea. To ensure enough capelin as food for animals in the Barents Sea there is a set spawning population of 200 000 tonnes with a 95% confidents interval at the 1. of April each year (ICES, 2021).

The condition of the seals can be an indicator of several changes happening in the ecosystem. A lowered condition can indicate competition, low prey availability, changes in the composition of prey species. Monitoring the body composition and condition over time can be used as an indicator of an unbalance in the ecosystem. All commercially exploited species are monitored and regulated. Using the already available information and in the future the condition of the harp seal, a functioning multispecies management can be done to ensure a healthy harp seal population.

8 Conclusion:

The tritiated water method seems to be a useful method to estimate the body composition of harp seals. The tissues had a linear trend when plotted against the estimated total body water, even though there is some variation. The regression equations can therefore be used to estimate the body composition.
The total body blubber which can be seen also as the seals condition, clearly follows a linear trend except for one point. Statistics indicate that the equations for estimating the TBB has sufficient statistical strength to estimate TBB. When using the equations to estimate body composition, the estimated values did not differ significantly from determined values (P-values<0.05). The tritiated water method could therefore a useful method to estimate the seal's condition.

Due to study design and technical aspects the skeleton and skin were not dried. The water content in blubber determined in this study, were not used as the results showed to much variation. Instead, these values were collected from other studies. Further studies on the water content of these should be done to obtain an accurate result.

A repetition of the study using the method and equations made in this study to estimate the body composition of a higher number of seals with a wider range of TBW, would be useful. This means capturing the seals at different times of the year when they are both lean and fat. That provides more variation in percentage amount of tissue, and significant correlation to the TBW can be obtained.

The condition has a direct correlation with available prey and breeding success. It is also related to a changing climate and with that a change in sea ice extent and competition with other species. The tritiated water method could be a useful method to further study the correlation between the condition and listed factors affecting it.

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Appendix:

Appendix Table 1: Data on capture time, location and weight(kg). Data collected on board R/V Helmer Hansen immediately after capture.

ID	Time	Date	Coordinates	Sex	Weight (kg) with bag (pups,+ sack)	Weight (kg)	Length (cm)	Age
G1-21	18:00	30.03.2021	70.46N 015.14W	Female	131		162	Adult
G1B-21	18:00	30.03.2021	70.46N 015.14W	Female		30	104	Pup
G2-21		31.03.2021	70.43N 014.56W	Female		158	176	Adult
G26-21		31.03.2021	70.43N 014.56W			31,8		Pup
G3-21	10:00	31.03.2021	74.30N 015.55W	Female	168	163	181	Adult
G3B-21	10:00	31.03.2021	74.30N 015.55W	Female	35,5			Pup
G4-21	10:45	31.03.2021	70.44N 014.53W	Female	145		178	Adult
G4B-21	10:45	31.03.2021	70.44N 014.53W	Female	27*			Pup
G5-21	11:40	31.03.2021	70.45N 014.51W	Female	155	155	178	Adult
G5b-21	11:40	31.03.2021	70.45N 014.51W	Female		23		Pup