

**"Can energy expenditure of free-ranging kittiwakes be estimated by body acceleration?"**



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*”Kan hende jeg seiler min skute på grunn,  
Men så er det dog deilig å fare”*

- Falk, i Kjærlighetens komedie av Henrik Ibsen -

## Preface

Denne masteroppgaven ble skrevet ved Avdeling for arktisk biologi, Norges arktiske universitet. Tromsø i samarbeid med Norsk Polarinstitutt. Feltarbeidet ble utført i Kongsfjorden, Svalbard, i juli-august 2012. Feltarbeidet ble utført med støtte fra Svalbard Science Forum.

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## Abstract

The way energy is used and acquired are fundamental questions in animal biology and figure greatly into conservation of a species. Accurate estimates of energy expenditure are critical in understanding how successful animals are throughout their lifespan and in quantifying population energy budgets and their role and impact on an ecosystem. Two methods have been commonly used to estimate daily energy expenditure (DEE) of free ranging animals: the doubly labeled water (DLW) method and the heart rate method. A third, less invasive method uses activity data captured by accelerometers. The recent development of miniature accelerometer data loggers have made it possible to use this method on small free-ranging animals; however, the method needs to be validated on the species of interest. Believed to be the most numerous gull species, the black-legged kittiwake (*Rissa tridactyla*) is well studied in the Arctic and therefore a valuable candidate for elucidating alternative methods of energy expenditure.

We deployed miniature accelerometer data loggers on eight breeding kittiwakes in Kongsfjord, Svalbard, and recorded body acceleration continuously over a three day period. From recorded acceleration in three axes, overall dynamic body acceleration (ODBA) was calculated for each bird. To validate accelerometry, we estimated the birds' energy expenditure using the DLW method. In addition, a control group of twelve birds was only treated with the DLW method to determine if the deployment of the loggers had an effect on the energy expenditure of the birds.

Using the DLW method, we found the mean DEE for birds with and without loggers was 1147 kJ/day ( $\pm 217$  SD) and 974 kJ/day ( $\pm 219$  SD), respectively. The loggers were not found to have an effect on DEE ( $t_{18} = 1.733$ ,  $p = 0.1$ ), nor was there any correlation between calculated ODBA and estimated DEE ( $r = -0.174$ ,  $t_6 = -0.432$ ,  $p = 0.681$ ) for the kittiwakes studied. With the removal of an apparent outlier, the correlation between the ODBA and estimated DEE was strengthened and evidence of a significant effect of loggers on DEE was revealed.

The question that remains unanswered is if kittiwakes have a correlation between ODBA and DEE. It is possible that locomotion does perhaps not constitute a large enough part of EE in kittiwakes to be reflected accurately by ODBA. However methodical errors should not stand in the way of the promise of accelerometry as an accurate method of measuring energy expenditure in free-ranging seabirds.

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## List of Abbreviations

EE	energy expenditure
DEE	daily energy expenditure
DLW	doubly labeled water
EQ	caloric equivalent
H	Hydrogen
O	Oxygen
H <sub>2</sub> O	Water
CO <sub>2</sub>	Carbon dioxide
g	acceleration force
ATP	Adenosine triphosphate
ODBA	overall dynamic body acceleration
BM	body mass
N	Number of animals in sample
APE	atom per cent excess
<sup>2</sup> H	deuterium
Hz	Hertz
LiAlH <sub>4</sub>	lithium aluminum hydride
IRMS	isotope ratio mass spectrometry
ppm h <sup>-1</sup>	parts per million (ppm) isotopes over time
I <sub>X</sub>	initial levels for isotope X
F <sub>X</sub>	final levels for isotope X
B <sub>X</sub>	initial levels for isotope X
h	hours
N	pool size
Mol <sub>inj</sub>	moles of injectate,
E <sub>mix</sub>	equilibrium enrichment
E <sub>wat</sub>	background level and
E <sub>inj</sub>	estimate of the injectate enrichment
N <sub>H</sub>	pool size for <sup>2</sup> H
N <sub>O</sub>	pool size for <sup>18</sup> O
N <sub>H</sub> /N <sub>O</sub>	pool size ratio
TBW	total body water
r <sub>CO2</sub>	rate of CO <sub>2</sub> production
k	fractional turnover rates of <sup>18</sup> O and <sup>2</sup> H
kJ	kilojoules
mL	milliliter
$\bar{A}$	static component
A	total acceleration
ODBA <sub>sum</sub>	sum of the dynamic acceleration for the three axes
ODBA <sub>vec</sub>	vectorial product of ODBA
SD	standard deviation
RQ	respiratory quotient

# 1 Introduction

## 1.1 Estimating energy expenditure

The way energy is used and acquired are fundamental questions in animal biology.

Understanding the rate of energy expenditure (EE) is important in questions of how successful animals are throughout their lifespan, e.g. reproduction and survival, and to quantifying population energy budgets and their role and impact on the ecosystem (Brown et al. 2004). Different methods have been developed to estimate the EE of animals (Halsey 2011). Direct and indirect calorimetry are the most precise techniques, but have the disadvantage that the individual studied must be enclosed in the instrument itself (Speakman 1997). By estimating EE for specific behaviors in the laboratory and combining this with time budgets obtained from observing wild animals, it is possible to obtain time energy budgets and estimate the daily energy expenditure (DEE) of a species (Buttemer et al. 1986). This method has its disadvantages in difficulties in deriving accurate estimates of EE for all behaviors and obtaining complete behavioral data (Goldstein 1988). The two most widely used methods for estimating DEE in free ranging animals over the last decade have been the heart rate method (Butler et al. 1992) and the doubly labeled water (DLW) method (Lifson and McClintock 1966). The heart rate method is an invasive procedure that requires surgery to implant data loggers and, due to the size of the devices, it is not preferable on animals under 1 kg (Butler et al. 2004). In addition, cardiovascular adjustments that do not affect EE can have a significant impact on the estimates (Green 2011). It is also a more expensive method to use on small animals compared to the DLW method.

### 1.1.1 DLW method

The DLW method is an isotope based technique of estimating a single time-average value for EE. Isotopes of oxygen ( $^{18}\text{O}$ ) and hydrogen ( $^2\text{H}$ ) are injected into an animal and, based on the different turnover rates of the isotopes ( $k_X$ ),  $\text{CO}_2$  production can be estimated, and hence  $\text{O}_2$  consumption can be quantified. A caloric equivalent (EQ) is estimated from an assumed or known diet composition and total EE for the duration of the experiment can be estimated and thereafter DEE calculated ( $\text{kJ d}^{-1}$ ) (Speakman 1997).



The DLW method is based on five main assumptions, the core elements of which are listed below. Lifson and McClintock (1966) outlined the assumptions that need to be met for the DLW method to produce valid results. Nagy (1980) and later Speakman (1997) have explored these assumptions in more detail. Some of the assumptions have shown to represent greater issues than others and, by improving calculation methods, it has been possible to minimize the potential effects of violated assumptions.

1. Rates of carbon dioxide production and water losses/gains and pool size are constant.
2. Isotopic species leaving the body of the animal do so at the same abundance as in the body at that time.
3. Isotopes turn over in the same pool, which is equal to the body water pool, meaning they only react with H<sub>2</sub>O and CO<sub>2</sub>.
4. All substances entering the animal are labeled at the background level and there is no entry of unlabelled CO<sub>2</sub> and H<sub>2</sub>O via the skin.
5. Background levels of isotopes are constant.

For a free-ranging bird, the first assumption will never be satisfied. One example is that when an animal is active, it will have an elevated CO<sub>2</sub> production compared to when it is resting. Intake of exogenous water will vary, and the production of metabolic water will as well. The change in body water volume is, in most cases, linear. When using the two sample method, which has one initial and one final sampling point, changes in body mass would have to be higher than 50 % for those changes to have any significant effect on body water volume (Nagy 1983). The second assumption is also impossible to satisfy, but the violation of this assumption can be adjusted for in the calculations of the estimates. The heavier <sup>18</sup>O and <sup>2</sup>H isotopes have almost the same physical characteristics as the normal O and H atoms. But when molecules change phase, e.g. when water evaporates, the isotope concentration is lower in the gas phase than in the liquid water phase. The implications of this fractionation effect have been well studied in the history of the DLW method and different equations have been used to correct for this (Lifson & McClintock 1966)(Nagy 1983). Today, the equation that has shown to account best for fractionation effects in validation studies is equation 7-17 from

Speakman (1997). This equation assumes both that 25% of total water loss comes from evaporation, and a different fractionation factor for evaporation. The third assumption addresses the problem that the isotopes can exchange with other molecules than H<sub>2</sub>O and CO<sub>2</sub>. If isotopes combine with substrates leaving the body the estimated elimination rates will be too high. If isotopes combine with substrates within the body, the volume the isotopes of H or O are diluted within the body (i.e. pool sizes), will be overestimated. Hydrogen isotopes exchange with other molecules at a greater rate than oxygen isotopes, but, for animals under 4 kg, validation studies have shown that the consequences are negligible when using the single pool model, where the body water volume is estimated only on O pool size (Speakman 1997). The two last assumptions are not accounted for in the equations and may lead to error in the estimates. Unlabelled CO<sub>2</sub> entering the body would have a much lower isotope concentration than the body water in the injected animal. This would be detected as a CO<sub>2</sub> loss, but under normal conditions accessible CO<sub>2</sub> does not reach levels that would be sufficient to make a significant difference. The fifth assumption could be a problem in animals where the isotope levels in the body water are low and/or the experiment lasts for long periods. For small animals injected with highly enriched DLW for experiments over short time periods in stable environments, the possible changes in background levels have shown to be negligible.

### **1.1.2 Estimating DEE in kittiwakes by the DLW method**

The DLW method has been used on a range of species (Speakman 1998) and one species that has been well studied is the black-legged kittiwake (*Rissa tridactyla*, Linnaeus; hereafter referred to as ‘kittiwake’) (Bech et al. 2002). With a circumpolar distribution, the kittiwake is a medium sized, long-lived bird and believed to be the most numerous gull species in the world (Coulson 2011). Considerable reductions in population size for unknown reasons over the last decade in the Barents Sea have put this species on the Redlist (Strøm 2006)(miljøstatus.no 2014).

The first study using DLW in kittiwakes was conducted in the 80’s (Gabrielsen et al. 1988) and this has since been followed up by numerous other studies. The study area of the present study in Kongsfjord, Svalbard, has previously been used several times for a variety of studies on EE in kittiwakes resulting in a long term data set (e.g.(Gabrielsen et al. 1987)(Bech et al. 2002)(Fyhn et al. 2001)(Welcker et al. 2010)(Schultner et al. 2010). These studies have

shown high consistency of the estimates of DEE between years (Welcker et al. 2010). On an individual level, kittiwakes show variable DEE that seems to reflect the birds' behavior and EE at the time of the experiment (Lassen 2012). This makes the kittiwake a very suitable species to test and validate new methods of estimating DEE in free ranging birds.

### **1.1.3 Accelerometry**

The first studies to use body acceleration to estimate EE on humans were performed in the 1960's (Cavagana et al. 1963). The recent development of miniature accelerometer data loggers have made it possible to deploy loggers on smaller animals and use recorded acceleration as a proxy for EE (Wilson et al. 2006). Energy expenditure in adult individuals is composed of the following four main bodily functions: basal metabolic rate, temperature-dependent energetic expenditure, specific dynamic action, and movement (Wilson et al. 2006). Movement normally represent the most variable and major factor in energy budgets for free ranging birds (Weibel and Hoppeler 2005). The quantification of body movements can be made using accelerometers to measure acceleration force (g). Studies on humans and animals have shown that acceleration data correlates with the rate of EE assessed by indirect calorimetry (Wilson et al. 2006). Today's technology has made it possible to manufacture miniature accelerometer data loggers that are small enough and light enough to even be deployed on birds with body mass less than 1 kg without surgery (Elliott et al. 2012).

Energy is the potential to do work, and, for animals, energy is stored in the form of chemical bonds. Animals use energy to contract muscles. This leads to the acceleration of limbs and movement of the animal's body. This body acceleration can be recorded by an accelerometer. Adenosine triphosphate (ATP) induces the shortening of the muscles and is the link between chemical energy and mechanical work. This link between acceleration produced by muscular contraction, mechanical power, and ATP should, in theory, make it possible to use accelerometry as a proxy for EE (Gleiss et al. 2011). Loggers can record acceleration (g) on one axis (X), two axes (X, Y) or three axes (X, Y, Z) with tri-axial loggers giving the most accurate data. The recorded total acceleration composes of two parts, static acceleration, which is the gravity of the earth, and the dynamic acceleration, which is induced by movement from the animal. The overall dynamic body acceleration (ODBA) is a variable derived from the dynamic part of the acceleration from the three axes and combines them into

a single variable (Halsey et al. 2009). The ODBA can either be the sum of the absolute dynamic acceleration from the three axes or it can be calculated as the vector of the three axes. While the first has been used most commonly, the latter may be more promising on small animals as the exact positioning of loggers on the body is challenging (Gleiss et al. 2011). Energy expenditure related to basal metabolic rate, digestion, and thermoregulation has to be estimated by other methods, but these are easier to model than mechanical work (Gleiss et al. 2011). Like the heart rate technique, accelerometry cannot be used as a direct measurement of EE, but it can offer a good proxy of oxygen consumption.

By calculating ODBA and calibrating it against direct or indirect calorimetry, accelerometry can obtain good estimates of EE of free-ranging animals (Halsey et al. 2009). The most frequently used method is to simultaneously measure oxygen consumption or CO<sub>2</sub> production while recording body acceleration, but this demands advanced equipment and the need to estimate the EE and body acceleration over a range of behaviors in the laboratory. Using ODBA as a proxy of EE in seabirds does have a great potential as activity, i.e. movement, normally constitutes the largest part of EE. Recent research has shown that ODBA derived from miniature accelerometers can be a valid method in predicting DEE in seabirds (Elliott et al. 2012). By calibrating ODBA against DLW data estimated simultaneously on the same individuals, ODBA may prove to be a good predictor of DEE in free-ranging kittiwakes.

## **1.2 Effect of loggers on kittiwakes**

Kittiwakes, as most seabirds, fly long distances from the breeding colonies on their foraging trips making visual observation difficult. As kittiwakes always return back to their colony, capturing and recapturing of individual birds is possible. This makes kittiwakes well suited for logging studies. Nevertheless, the deployment of loggers has the potential to have negative physiological and behavioral effects (Vandenabeele et al. 2011). Birds with low wing loading, such as kittiwakes, experience less constraint in mass specific mechanical power output relative to payload mass compared to species with higher wing loads (Vandenabeele et al. 2012). Seabirds that feed on the sea surface and do not dive for their food will not be affected by the increased hydrodynamic drag caused by loggers (Vandenabeele et al. 2012). As kittiwakes have low wing loading and are surface feeders, loggers less than 3% of body mass (BM) are not expected to have a significant effect on stress levels, foraging success, breeding

performance, or any other behavioral or physiological parameters related to EE (Heggøy 2013)(Vandenabeele et al. 2011).

### **1.3 Aims of study**

In this study, we estimated DEE for 41 breeding kittiwakes (*Rissa tridactyla*) using the DLW-method over a 72-hour period while, simultaneously, recording body acceleration with accelerometers for 18 of the birds using accelerometers. We aim to: (1) evaluate if ODBA could be used as a proxy for EE in free ranging kittiwakes and the possibility to replace the DLW method in future studying by examining the correlation between DEE and ODBA; (2) investigate what effect attaching miniature loggers has on the birds' DEE.

## 2 Material and Methods

### 2.1 Study area and study species

The fieldwork of the present study was conducted in a kittiwake colony located on the island Blomstrandhalvøya (78° 59' N, 12° 07' E)(Figure 2.1) in Kongsfjord, Svalbard, in the period 15.07.2012 -15.08.2012.



**Figure 2.1** Map showing Kongsfjorden, Svalbard. The kittiwake colony's (78° 59' N, 12°07' E) location is marked by the black ring.

The kittiwake is a circumpolar distributed seabird that forages offshore. During the summer, birds migrate in large numbers to coastal cliffs to breed in dense colonies (Cullen 1956). In Svalbard, birds arrive at their colonies around March – April and, after the breeding period, return to the open ocean in September. Kittiwakes catch food by plunge diving or swimming on the surface (Strøm 2006). The diet consists primarily of small fish (polar cod *Boreogadus saida* and capelin *Mallotus villosus*) and invertebrates. The clutch size is normally two eggs, but one or three egg clutches occur; the clutch is incubated for 25-32 days. Both parents take equal share in building nests, as well as in feeding and protecting the chicks. For the first two weeks, the chicks are completely dependent on the parents and are brooded regularly. The

chicks leave the nests after 35 - 40 days. During the intense summer season kittiwakes at the study sight do not perform a behavioral diurnal rhythm (Gabrielsen et al. 1988). Kittiwakes have a high activity level and utilize both flapping and gliding flight (Gabrielsen et al. 1987). They are calm and easy to handle during experiments. This make the species well suited for experiments that demand the recapture of individuals.

## **2.2 Field procedure**

Two groups of birds were used in the study. Group 1 (N=23) received both a doubly labeled water injection and had a micro accelerometer data logger deployed dorsally to investigate the correlation between DEE and activity data recorded from accelerometers. Group 2 (N=18) was only subjected to the DLW method to measure DEE. The DEE from birds in Group 2 was used as a control to determine potential effects of the logger deployed on birds in Group 1. The experimental birds were chosen based on nest accessibility at the lower section of the colony where it was possible to catch them using a long pole with a nylon noose at the end. The catching and untangling took only about 15 seconds per bird. Lying in a pre-weighted cotton bag, the birds' body mass was recorded using a Pesola spring balance (0-500 gram,  $\pm 1$  gram). Standard biometric measurements were taken. For head-bill length and tarsus length a caliper was used (Starret,  $\pm 0.1$  mm), and a ruler was used to measure the maximum wing length. For birds with unknown sex a small blood sample was collected for later molecular sexing (Griffiths et al. 1998). To make recapture and observation easier, the heads and breasts of sampled birds were color coded by sex, red for females or blue for males. Observations to record whether sampled birds were present on the nest were done every 30 min when working in the colony. Return time after the birds were released was also noted if observed.

### **2.2.1 Doubly labeled water and blood sampling**

For estimating DEE in Group 1, DLW was used following the two-sample protocol (Lifson & McClintock 1966)(Speakman 1997). After weighing, the bird was injected intraperitoneally with 1.25 ml of DLW using a gastight syringe (Hamilton Microliter Syringe). All birds were breeding birds possessing a distinct brood patch of exposed naked skin that made it easy to observe possible leakages or if the solution did not enter the body cavity. Three DLW mixtures with different concentrations of heavy isotopes were used due to availability. Each

individual was given an injection from only one the mixtures. The concentrations were 36.53, 34.60 and 36.22 atom per cent excess (APE) deuterium ( $^2\text{H}$ ) and 65.15, 62.34 and 64.00 APE oxygen-18 ( $^{18}\text{O}$ ). To allow full equilibration of the heavy isotopes with the body water, the birds were kept in a perforated plastic tube hanging in a cotton bag for 1 hour. After equilibration, an initial blood sample was obtained for DLW- analyses by collecting about 200  $\mu\text{L}$  blood from one of the brachial veins using a heparinized syringe. Then the blood was rapidly transferred into four 50  $\mu\text{L}$  heparinized microcapillary tubes and the ends of the tubes were immediately flame sealed using a butane torch. After initial sampling, the birds were colored and released. If a bird was observed when returning back to the nest, the return time was registered. We intended to recapture the birds after three days, preferable 72 hours after initial sampling. At recapture, a final blood sample was obtained in the same manner as the initial blood sample. To determine the natural isotopic background levels for the birds in the colony, blood samples from six additional birds that were not part of the study were obtained. After returning to the laboratory at the end of the day, the tube ends were controlled under microscope to confirm that the ends were completely sealed. As it was confirmed during the first days that the sealing method was successful this step was not continued throughout the whole experimental period.

### **2.2.2 Accelerometers**

The birds in Group 2 (N=18) were given accelerometer (Axy-1, TechnoSmArt, Rome, Italy) in addition to a DLW-injection. The DLW procedure was the same for both groups. To protect the loggers from the harsh environment, they were sealed in heat shrink tubes. The devices were deployed to the birds' dorsal feathers using water resistant Tesa<sup>TM</sup> tape. The weight of the whole device including tape was 7 grams. Great care was taken to fasten the loggers tight to the birds' body to secure that the loggers could not move independently and that the orientation of the loggers was the same on all birds. The upper part of the logger was placed close to the birds' neck pits. The loggers recorded acceleration in three axes (heave, surge, and sway) with a sampling resolution of 8 bit,  $\pm 4\text{g}$  and sampling rate at 25 Hz.



## 2.3 Analyses

### 2.3.1 Doubly labeled water analyses

The analysis of the blood samples was performed at the Department of Zoology, University of Aberdeen, Scotland. The blood samples were first vacuum distilled into glass Pasteur pipettes (Nagy 1983). To determine deuterium ( $^2\text{H}$ ) enrichment, first hydrogen gas ( $\text{H}_2$ ) was derived from the water in the distilled blood samples by reaction with lithium aluminum hydride ( $\text{LiAlH}_4$ ) (Ward et al. 2000). Then isotope ratio mass spectrometry (IRMS) described in Speakman & Król (2005) was performed. For analysis of  $^{18}\text{O}$  enrichment, distilled water was equilibrated with  $\text{CO}_2$  following the small sample equilibration technique and IMRS (Speakman et al. 1990).

### 2.3.2 Calculations and estimates

The average natural background level of the labeled isotopes in the colony was analyzed from the blood samples collected from the six uninjected birds, and then used to correct the initial and final samples from the injected birds. The fractional turnover rates for the labeled isotopes are expressed as change in parts per million (ppm) isotopes over time ( $\text{ppm h}^{-1}$ ) and are calculated by:

$$k_X = \frac{[\log_e(I_X - B_X) - \log_e(F_X - B_X)]}{\text{time}}$$

$I_X$ ,  $F_X$  and  $B_X$  are the initial, final and background levels for isotope X, respectively. Time is the interval between initial and final blood sample ( $\text{h}^{-1}$ ). This model assumes that full equilibration of isotopes are completed exactly at initial sampling time and is referred to as the plateau model (Speakman 1997). This is the preferred model when using the two-sample method with initial and final samples because EE estimates are not affected by unnatural behavior during the hour the bird is kept in captivity for isotope equilibration (Speakman & Król 2005). The dilution space (N) of the isotopes, also referred to as pool size, is the volume of the body water in which the isotopes are diluted. The initial pool size for  $^{18}\text{O}$  ( $N_{\text{O-int}}$ ) is estimated by the extent of dilution of the isotope from injection to equilibration after 1 h.

$$N_{O-int} = \frac{Mol_{inj}(E_{mix} - E_{inj})}{E_{wat} - E_{mix}}$$

$Mol_{inj}$  is the moles of injectate,  $E_{mix}$  is the equilibrium enrichment,  $E_{wat}$  is the background level and  $E_{inj}$  is the estimate of the injectate enrichment. We assume that the initial blood sample was taken exactly when full equilibration of the isotopes is reached (1 h). Assuming that the percentage of body water is constant during the experiment period, final pool size for  $^{18}O$  ( $N_{O-fin}$ ) can be estimated by expressing  $N_{O-int}$  as percentage of the initial body mass and multiplying this with the final body mass. Average pool size for  $^{18}O$  ( $N_O$ ) can then be calculated from  $N_{O-int}$  and  $N_{O-fin}$ . Performing the same calculations for  $^2H$ , the average pool size for  $^2H$  ( $N_H$ ) was determined. This is used to calculate the pool size ratio ( $N_H/N_O$ ). In general, the ratio should be in the range of 0.97 – 1.10 (Speakman 1997), for kittiwakes the long temporal trends have shown that the ratio should be in a narrower range from 1.04 – 1.06 (Welcker, pers.com.). Speakman (1997) recommends following the one pool model (Visser & Schekkerman 2000) to estimate total body water (TBW) in small animals, in which  $N_O$  equals TBW. In a DLW study, Jodice and colleagues (2002) TBW in kittiwakes to be around 60% of BM.

In order to estimate the rate of  $CO_2$  production ( $r_{CO_2}$ , mol  $CO_2$   $h^{-1}$ ) the equation 7 – 17 in (Speakman 1997) was used:

$$r_{CO_2} = \frac{N}{2.078} \cdot (k_O - k_H) - 0.0062 k_H \cdot N$$

The constant 2.078 accounts for the larger amount of O in  $CO_2$  compared with  $H_2O$ ,  $k$  is the fractional turnover rates of  $^{18}O$  and  $^2H$ . As the heavy isotopes are subjected to fractionation, this is taken into account by the constant 0.0062 that has proved to be the best correction in *in vivo* studies (Visser & Schekkerman 2000). Metabolic rate was then estimated assuming a caloric equivalent of 27.639 J/mL  $CO_2$  (Welcker et al. 2010). The caloric equivalent is the mean value with negligible annual variation obtained from regurgitation samples taken over 5 years at the study site and assuming adult having the same diet as they feed the chicks (Thorvaldsen 2013).

### 2.3.3 Accelerometer data analysis/calculations

The recorded raw data (ard-format) was downloaded from the loggers using the software Axy Manager (TechnoSmArt). The same software was used to convert the ard-files to csv-files. A scatter plot was made for each data set to identify acceleration pattern at release and recapture of the birds. On and off nest patterns and times were identified. The recorded data set was then trimmed by deleting data from before release and after recapture. Comparing the release and recapture times based on the logger data with actual times noted in the field log revealed a time drift in the loggers. The time drift was assumed to be linear and was corrected for by dividing overall dynamic body acceleration (ODBA) by the deployment time based on the logger and multiplied by the actual deployment time as recorded in the field:

$$ODBA_{corrected} = \frac{ODBA_{logged}}{logger\ time} \cdot actual\ time$$

The recorded total triaxial acceleration data is the sum of the gravitational pull of the earth (static acceleration) and the movement of the bird (dynamic acceleration)(Halsey et al. 2011). To estimate ODBA, the static acceleration had to be removed from the total acceleration. First, the static acceleration was approximated by applying a smoothing function to the total acceleration recorded for each of the three axes (Shepard et al. 2008). A running mean of 2 seconds was used to estimate the static component ( $\bar{A}$ ) (Gleiss et al. 2011). Then the dynamic acceleration was determined by subtracting the static component ( $\bar{A}$ ) from the total acceleration (A) for each of the three axes and converting them to absolute positive units (Shepard et al. 2008). The ODBA of each bird can either be calculated as the sum of the dynamic acceleration for the three axes (ODBA<sub>sum</sub>) or be calculated as the vectorial product (ODBA<sub>vec</sub>)(McGregor et al. 2009).

$$ODBA_{sum} = |A_x - \bar{A}_x| + |A_y - \bar{A}_y| + |A_z - \bar{A}_z|$$

$$ODBA_{vec} = \sqrt{(A_x - \bar{A}_x)^2 + (A_y - \bar{A}_y)^2 + (A_z - \bar{A}_z)^2}$$

Correlations for the two estimates were high for all the loggers ( $R^2 > 0.97$ ). The vectorial product is less sensitive to individual variation in the position of the logger on the body of the

birds (Elliott et al. 2012). As it is difficult to deploy the loggers in precisely the same way on all the birds,  $ODBA_{vec}$  was preferred over  $ODBA_{sum}$  to minimize the effect of these discrepancies.

## 2.4 Datasets and statistics

Blood sample analyses and accelerometer data with deficiencies were not used in the statistical analysis (appendix table 6.1). In Group 2 ( $N = 18$ ), final blood samples could not be collected from two birds. In addition, two samples were not sealed properly and the final enrichment of another sample was too close to background levels. These samples were excluded from further analyses. In Group 1 ( $n=23$ ), five final samples could not be obtained, three samples were not sealed properly, and four samples had final enrichments too close to background level. Two birds were able to remove their loggers and one bird evaded recapture. Estimated DEE for the sampled birds ranged from 381 – 1388 kJ/day. As BMR for kittiwakes has been estimated to average 314 kJ/day (Gabrielsen et al. 1987), a DEE of 381 kJ/day was regarded as too low for a breeding bird, and hence this sample was also removed from the data set. For the final statistical analyses, data was comprised from twenty birds (Group 1:  $N=8$  and Group 2:  $N=12$ , Appendix Table 6.1).

To determine the correlation between ODBA and DEE, a Pearson correlation test was run. The potential effect of the loggers on DEE was tested by running an ANCOVA including body mass and sex as covariates to account for variability caused by these factors. The DEE of one bird (#529) in Group 1 was substantially lower than for the other birds that had a logger deployed. However, the DEE of this bird was not outside of the range compared to the birds in Group 2 that did not have loggers. Examination of the bird's DLW analysis and accelerometer data did not show any apparent error and could therefore not be determined as an obvious outlier. Hence, all statistical tests were run on the data sets both including and excluding this individual. Statistical procedures were conducted and plots were made with R 3.0.2 (R Core team 2013). Significance level was set at  $p \leq 0.05$ .

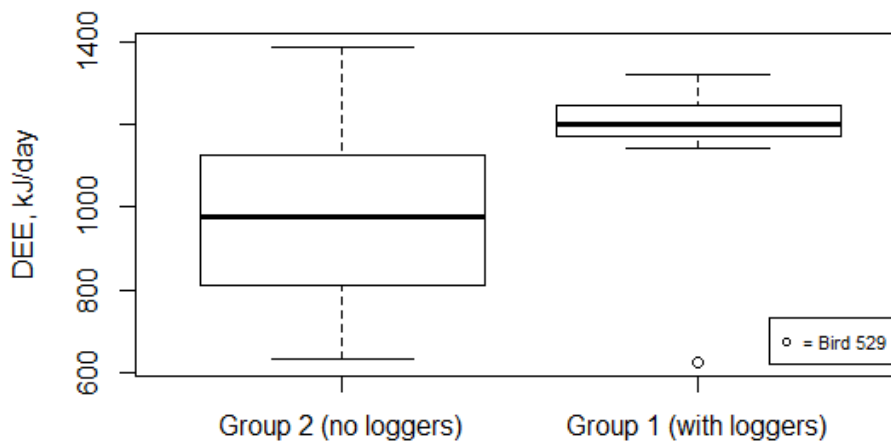
## **2.5 Permissions**

Permission to carry out the fieldwork was given by the Governor of Svalbard (Sysselmannen, RIS 3828, 2011/00488-90). Permission to perform DLW and deploy logger on the birds was provided by the Norwegian National Animal Research Authority (Forsøksdyrsutvalget; ref. FOTSID 4232 &4226)

### 3. Results

#### 3.1 Doubly Labeled Water

Data from 20 kittiwakes was used in the analyses. To estimate daily energy expenditure (DEE) (kJ/day) the doubly labeled water method was used on all birds (N=20). In addition, birds in Group 1 (n=8) also had a miniature accelerometer data logger deployed dorsally to record body acceleration. The birds in Group 1 consisted of four females and four males, while Group 2 included seven females and five males (Table 3.1). The data for each individual bird are listed in table 6.2 in the Appendix. The birds in Group 1 had a mean ODBA of 727 224.9 g/day ( $\pm 242\ 343.7$  SD) and a mean DEE of 1147 kJ/day ( $\pm 217$  SD). In Group 2, the mean DEE was 974 kJ/day ( $\pm 219$  SD). Birds in Group 1 had DEE well distributed in the range of the group, while the DEE for birds in Group 2 were aggregated in the higher end (1051 – 1319 kJ/day) except bird #529 (623 kJ/day) (Figure 3.2). The estimated DEE of the birds was in the expected range according to data from previous studies. Testing for the effect of the logger on the DEE excluding bird 529 from the analysis showed that the loggers had a significant effect on DEE. Due to small data set and DEE of bird 529 was within the range of expected DEE a test was also run with bird 529. This showed the loggers to not have a significant effect on DEE.



**Figure 3.1.** The DEE (kJ/day) derived from the DLW method is displayed for 20 birds divided into two groups, one with miniature accelerometer data loggers (box on right, N=8) and the other without (box on left, N=12). The middle of the box represents the median value DEE for the group, the top and bottom are the 1<sup>st</sup> and 3<sup>rd</sup> quartiles while the whiskers represent the minimum and maximum values. Values far outside a normal distribution are displayed as open circles such as Bird 529 in the bottom right of the figure.

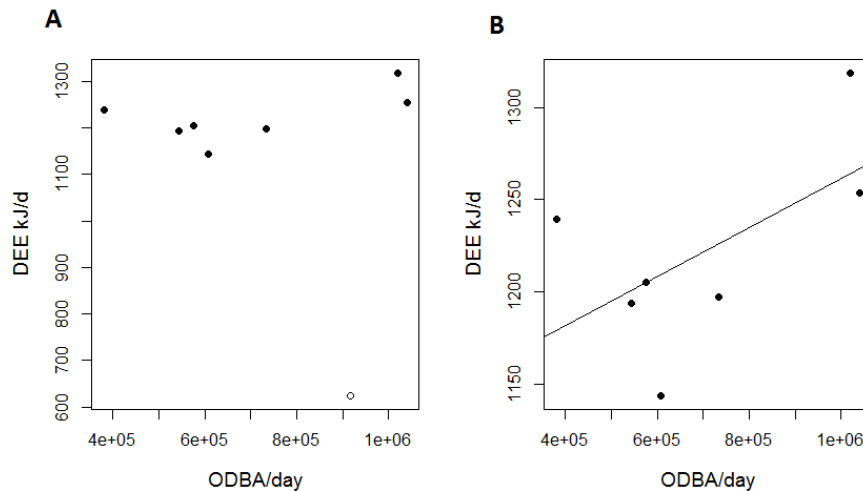
The mean ratio of the pool sizes ( $N_D/N_O$ ) was in Group 2 1.01 ( $\pm 0.02$  SD) and in Group 1 1.00 ( $\pm 0.02$  SD). The different sexes had the same mean within the groups, ratios for all birds ranged from 0.98 to 1.05. The body water volume for the birds were very different with the highest being 73% of body mass, and the lowest was 59% of body mass (Table 3.1). Mean body mass 364 grams ( $\pm 28$  SD).

**Table 3.1:** Twenty birds were used in the analyses. Birds in Group 1 had both logger deployed and were treated with DLW, Group 2 only DLW. The table shows mean, maximum and minimum rate for the estimated values from the DLW analyses. Values for each individual bird can be found in the appendix, table 6.1.

	number	Mean BM	Max BM	Min BM	Mean $N_D/N_O$	% BW	Max % BW	Min % BW	Mean DEE (kJ/day)	Max DEE (kJ/day)	Min DEE (kJ/day)
Only DLW Female	7	355	411	324	1,00	63	66	59	895	1224	738
Only DLW Male	5	391	411	381	1,00	65	68	59	1085	1388	821
Logger and DLW Femal	4	339	362	324	1,01	67	71	62	1085	1319	623
Logger and DLW Male	4	383	399	361	1,01	68	73	66	1209	1240	1194

### 3.2 Correlation between ODBA and DEE

The Pearson correlation test showed no correlation between DEE and ODBA in Group 2 ( $r = -0.174$ ,  $t_6 = -0.432$ ,  $p = 0.681$ )(Figure 3.2A). Removing the data of bird #529 from the data set resulted in a stronger yet still non-significant correlation ( $r = 0.593019$ ,  $t_5 = -1.6469$ ,  $p = 0.161$ )(figure 3.2B). The deployment of the loggers did not have a significant effect on the birds' DEE ( $t_{18} = 1.733$ ,  $p = 0.100$ ). However, excluding bird #529 from the data set resulted in a significant effect of the loggers ( $t_{17} = 2.904$ ,  $p = 0.009$ ) with a mean DEE of 1221 kJ/day ( $\pm 55$  SD) in Group 1 compared to 974 kJ/day ( $\pm 219$  SD) in Group 1.

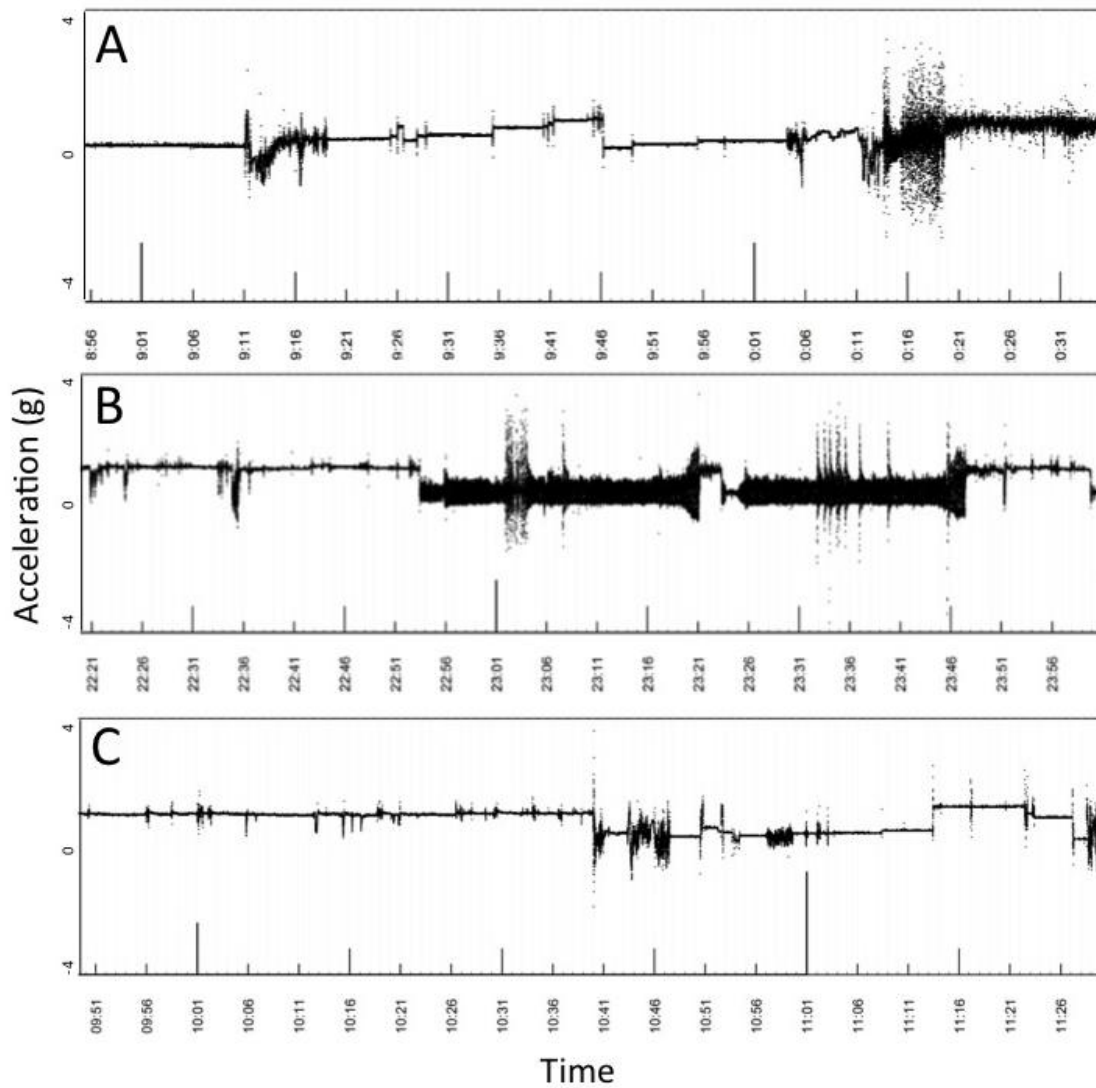


**Figure 3.2:** Estimated daily energy expenditure (kJ/day) using DLW on the Y-axis and calculated overall dynamic body acceleration in g (ODBA/day) on the X-axis was used to investigate if accelerometry can be used to estimate energy expenditure in kittiwakes. Including all birds in the analyses (A) showed no correlation. Removing bird 529 (open circle) that had a much lower DEE than the rest of the group (closed circles) from the data set showed a trend that higher body acceleration increased DEE (B), but this was not found to be significant.

### 3.3 Accelerometry

The plots made from the accelerometry data reveals distinctive patterns for different behaviors that the birds executed during the experimental period (Figure 3.3). A flat and narrow line showed the birds staying on the nests or resting away from the colony. A wider curve with large oscillations showed flying patterns indicating commuting flights or searching for food. Higher and narrower peaks on the graph probably showed plunge diving, but this was not verified by observation (Figure 3.3B). Release time and recapture time were recognized by acceleration patterns that differed from the rest of the graphs by its more boxy appearance followed by or after an abrupt transition to either natural behavior (Figure 3.3A) or removal of the logger (Figure 3.3C).





**Figure 3.3:** The figure show behavior patterns obtained from accelerometers in kittiwakes. On the x-axis is the time of day and on the y-axis is the acceleration in g. The top figure (A) shows the pattern during the one hour equilibration time before the initial blood sample performing the DLW method and subsequent release of the bird; (B) shows a bird staying on the nest (flat and narrow curve), flying (flat and wide curve) and the high and narrow peaks most likely shows a plunge diving pattern; (C) shows recapture pattern with abrupt transition followed by a boxier pattern indicative of the removal of the logger.

## 4 Discussion

The aim of the present study was to investigate the potential accelerometry may have to estimate DEE in kittiwakes. By comparing ODBA<sub>vec</sub> with DEE estimates from the DLW method, we wanted to determine the correlation between the body acceleration recorded by accelerometers and the birds' DEE. If a correlation was found we aimed to investigate the possibility of using recorded body acceleration to estimate DEE in the future. Elliot and colleagues (2012) have demonstrated that ODBA can be an accurate measure for EE in a seabird species using the thick-billed murre (*Uria lomvia*).

The accelerometry method has particular advantages over the DLW method concerning the flexibility, effort, and expense of the procedure. Birds injected with DLW need to be recaptured for a final blood sample before the isotope levels reach background level. If the blood is sampled too late no data can be obtained. Accelerometers save the recorded data, and even if the battery runs flat, the data can be recovered. Accelerometers do not require expensive lab analyses and can be reused, which lower the cost of operation. Limitations in how large a volume of DLW can be injected in a bird puts limitations to how long an experimental period can be to obtain valid EE estimates. For data loggers weight is the main limitation with regards to length of data collection. Ongoing improvements of the technology insure that smaller loggers and better batteries will be available in the near future. This will make it possible to obtain EE estimates over much longer periods. Where DLW method provides an average EE over a period of time, accelerometers offers higher scale resolution on behavior and time budgets. This allows for more detailed estimates of EE during specific behaviors.

During the breeding season kittiwakes are highly active due to the need to regularly commute between their foraging areas at sea and their nest site, and hence locomotion should represent a major part of their EE. It is therefore plausible to assume a close association between body acceleration and EE in breeding kittiwakes. However, in the present study a correlation between ODBA<sub>vec</sub> derived from miniature accelerometers externally attached to the body of the birds and DEE estimated using the DLW method was not found. The ODBA values varied substantially across the different individuals sampled indicating that the activity patterns differed among individuals which should be reflected in the DEE of the birds. However, the birds' DEE estimates, except that of one individual, were grouped in the upper range of the

values obtained for the control group, resulting in the apparent independence of ODBA and DEE values. Yet, as no significant correlation was found irrespective of whether or not the apparent outlier was included in the analysis, this unexpected result was likely caused by other factors.

## **4.1 Evaluation of the methods and their validity on kittiwakes**

### **4.1.1 Accelerometry**

If we assume that EE related to other factors than locomotion,( e.g. BMR, digestion and thermoregulation) do not vary significantly between the individuals, the work performed by skeletal muscles should represent most of the variable part of the DEE of a bird. The clearest limitation of the technique is that it can only directly estimate EE associated with the mechanical locomotion performed by an animal (Gleiss et al. 2011). Validations of estimating EE by the use of miniature accelerometer has been performed using oxygen consumption (Wilson et al. 2006), heart rate (Halsey et al. 2008), and DLW method (Elliott et al. 2012). Even though ODBA has proved to be a good proxy for EE in free ranging animals the rationale behind the method is by far not as well explored or understood as it is for the DLW and heart rate techniques.

Body mounted accelerometers record movement induced by ATP in the skeletal muscles. But not all force generated by ATP can be recorded. During isometric muscle contraction, chemical energy is utilized without generating movement and thus will not be recorded by an accelerometer. Many birds utilize gliding flight to a varying extent. In contrast to flapping flight, gliding costs relatively little energy and is dominated by isometric contraction (Weimerskirch et al. 2000). When a kittiwake performs gliding flight, the muscles would have to stabilize the position of the wings by isometric contraction. This muscle activity increases EE but would not increase body acceleration and therefore be poorly determined by ODBA. Also, if BM is altered, e.g. by carrying prey, this would affect EE more than changed acceleration indicates (Gleiss et al. 2011). This means that the amount of ODBA for one behavior not necessary correspond to the same EE as the same ODBA in another behavior. Different speed and locomotion modes mobilize different muscles, and maybe even muscle fibers, which may call for multiple regression to explain correlation between ODBA and EE

(Elliott et al. 2012). The accelerometer should ideally measure the acceleration of the center of mass, but in reality the accelerometers are positioned externally as close to center of mass as possible. Most often, as in this study, the devices are attached on the dorsal side of the trunk of the body. In addition, the acceleration of center of mass does not necessarily represent the work executed by the entire muscular system. The effects of these factors can result in ODBA values that do not reflect the total work performed by the skeletal muscles and therefore may not correctly reflect individual differences between the kittiwakes. Furthermore, it is difficult to identify and quantify possible differences between individuals. But still, acceleration of center of mass should usually correspond well with the overall mechanical work executed (Fednak, Heglund, and Taylor 1981). So it is reasonable to assume that the ODBA values calculated in the study would reflect differences in activity levels and hence EE.

The positioning of the loggers on the body of the animal is important in order to minimize different effects among individuals. Minor differences in the orientation of the loggers on the body will not be a problem when using tri-axial devices as a decrease in acceleration in one axis is mostly canceled out by a corresponding increase in one or two of the other axes. Nevertheless, variation in the position of the devices should be kept to a minimum to reduce the added error to the estimates (Halsey et al. 2011). To minimize this error tri-axial loggers were used, and to guarantee a standardized positioning of the devices, deploying the loggers was practiced in the field before the onset of the experiment.

The ODBA estimates varied considerably among the different individuals suggesting that ODBA gives a good impression of the birds' activity level and hence the level of EE. The accelerometers only record that a bird's body moves or accelerates in a certain direction. It cannot tell how much energy the different movements or locomotion modes cost. Jodice and colleagues (2002) present that kittiwakes loafing close to the colony spent about 60% less energy than what they spent loafing during a foraging trip. These are activities that would be recorded with similar ODBA values. The loafing during foraging trips tended to happen after capturing prey, which can lead to increased EE up to 80% due to heat increment of feeding (Baudinette et al. 1986). Loafing on foraging trips has been estimated to represent 21% of the time activity budget for kittiwakes, costing 4.5 times BMR (Jodice et al. 2002). If one bird is loafing away from the colony and one bird is loafing around the colony they will show similar acceleration of center of mass but have vastly different EE associated with the activity pattern.

#### 4.1.2 DLW method

In contrast to the ODBA data, the DEE estimates from the DLW method grouped with one exception in the higher range compared to the data of the control group. The analyses and calculations of the DLW-samples gave results that diverge from what was expected. The ratio of the two pool sizes was generally low, and the calculated percentage of body water of the birds were unnaturally high. In addition, data from some birds had to be removed from the final analyses because isotope enrichment values indicated that sampling and/or analyses had not been conducted correctly. Therefore, irregularities connected to the DLW method could be the reason that no correlation was found. To evaluate these possible discrepancies it is important to look at the limitations of the DLW-method and the stages of the analysis that are especially prone to error when conducting this method.

As mentioned in the introduction, the DLW method comprises many assumptions. How big the effects of the potential violations of these assumptions are differs among species with different body size and activity level. For birds less than 1 kg the assumptions have similar effects, and, in kittiwakes, many studies have been conducted using the DLW method and the method's validity for kittiwakes is well established (e.g. Fyhn 1999). Existing long-term data shows high inter-annual consistency of the DEE of kittiwakes estimated by the DLW method in Kongsfjord where the present study took place (Schultner et al. 2010)(Lassen 2012)(Gabrielsen et al. 1987).

The DLW method assumes that BM is constant for the birds during the experiment. In this study, the highest change in BM between capture and recapture was <6%. This is much lower than the 50% change that is necessary to alter the estimates significantly (Nagy 1983). Therefore, the observed change in BM should not have affected the estimates. Total body water was estimated from the initial <sup>18</sup>O enrichment using the single pool model recommended by Speakman (1993) for animals <4kg. As hydrogen can exchange with other substances in the body to a greater extent than oxygen, the hydrogen pool size (N<sub>2</sub>H) is normally estimated to be 3-4% greater than for oxygen (N<sub>O</sub>) (Speakman 1997). For kittiwakes, long-term data from Kongsfjord suggests that N<sub>2</sub>H exceeds N<sub>O</sub> by about 4-6% with relatively little inter-individual variation (Welcker, pers.com). The average ratio in this study was low (1.01 ±0.02 SD for birds with logger, and 1.00 (±0.02 SD) for birds without logger, Table 5.2). The ratios ranged from 0.98 to 1.05. The abnormal ratios indicate that there have been problems with the sampling and/or analyses, or the isotope enriched water

from the producer may have had an isotope concentration that differed from what was claimed.

The fractionation effect is adjusted for in the calculation (equation 7-17 by Speakman 1997) which assumes an average evaporative water loss of 25%. Birds, and specifically seabirds, minimize water loss by producing uric acid, in addition to urea, which is secreted in the feces. A uric acid molecule consists of 4 H, 3 O and 4 nitrogen atoms. This is equal to the excretion of two water molecules plus one oxygen atom. If hydrogen and oxygen are excreted in a 2:1 ratio this will not affect the estimated CO<sub>2</sub> production. However, for uric acid the ratio is 4:3. This will result in an overestimation of CO<sub>2</sub> production. For kittiwakes that mainly feed on polar cod and capelin this overestimation will be about 5% (Fyhn 1999). If this were to have an effect on the correlation between ODBA and DEE there would have to be great individual differences in the production of uric acid. It is unlikely that birds with diets of similar diet composition could secrete vastly different amount uric acid such that it could affect the DEE estimations.

Kittiwakes breed in dense colonies with nests that can be situated right next to each other. In theory, two injected birds that nest next to each other can inhale labeled CO<sub>2</sub> that was exhaled from the neighbor bird. However, this pathway for contamination is unrealistic due to the amount of CO<sub>2</sub> that would need to be exchanged between birds and thus can be ignored (Butler et al. 2004). If the isotopic background levels vary to a great extent within or across individuals this could lead to misleading results and incorrect DEE estimates (Speakman 1997). For experiments that last a short time period with small animals that feed in the same area the individual variations in background levels will normally be small (Nagy 1987). Previous studies on kittiwakes have shown stable background levels (Gabrielsen, pers.comm.) The samples to determine background levels were collected throughout the experimental period and did not show high fluctuations over time or between individuals. Assuming that the background levels for the sampled birds is within the same range as experimental birds is quite likely, but deviations cannot be completely ruled out.

If the injected volume of DLW differed from the intended volume this could lead to skewed estimates of pool sizes. This can happen by filling the syringe with inaccurate volumes, or that not all of the DLW enters the body cavity. The likelihood of this was minimized by paying extra attention when filling the syringe with the correct volume and when observing the injection spot after injection to confirm that no DLW leaked out. In fact, no such leakage was

observed. Also, the person performing the injections was trained before the experiment to fill the syringes with the correct amount of DLW. After the experiment, a test was run to check if the amount of DLW filled in the syringe was constant. This did not show any sufficient discrepancies that could invalidate the experiment.

Another factor that can affect the DLW results is if there is any contamination of the samples. It was paid great attention to avoid this and no direct contamination was observed. Using an open flame to heat-seal the ends of the capillary tubes can cause water to condense inside the tube walls and contaminate the samples with unlabeled water. CO<sub>2</sub> production is estimated from the difference in the isotope turnover rate. Because the enrichment of the isotopes are equally affected this would not lead to a bias in the estimates, but it can affect water turn over estimates. The tubes that were used had very narrow openings. Working with capillary tubes of this type is not likely to have significant impact on the samples (Nagy 1983) and therefore contamination of samples is unlikely to have caused the observed irregularities in the DLW data.

The respiratory quotient (RQ) is the ratio of the moles of CO<sub>2</sub> produced to the moles of O<sub>2</sub> consumed (CO<sub>2</sub>/ O<sub>2</sub>). For a diet solely containing carbohydrates, the moles of CO<sub>2</sub> produced will correspond closely to the moles of oxygen consumed. Such a diet would give a ratio close to 1.0. A diet of lipids will give a lower production of CO<sub>2</sub> than O<sub>2</sub> consumed resulting in a RQ of approximately 0.7. A protein diet will yield an RQ of around 0.8. In a laboratory, the diet can be fully controlled, but for a free-ranging seabird this is not possible. The diets of the birds have to be determined without direct observation of the birds. For breeding kittiwakes it is possible to sample the diet by collecting regurgitated food which the adults bring back to the chicks. By assuming that the adults have the same diet as their chicks, and do not metabolize stored energy, it is possible to estimate the RQ by analyzing the components of the collected food samples. This has been done previously in the same colony as the present study and show a high consistency of RQ over a 5 year period (Welcker et al. 2010). The mean conversion factor (27.639 J/mL CO<sub>2</sub>) obtained from 5 years of data was used to convert estimated CO<sub>2</sub> production to energy equivalents in this study. Food samples were also collected in the present study, and preliminary analyses imply that they follow the same diet composition.

If the regurgitated food samples did not represent the adults diets or the estimated conversion factor did not represent the diet during the year of the present study, this could lead to

estimates of DEE that are either too high or too low. However, as this would be a systematic error affecting all estimates of DEE in the same manner, it would not compromise the correlation between DEE and ODBA. By using the same conversion factor for all individuals, we assume they have the same diet or the differences are ignorable. If some individuals fed on prey of a significant different energy composition this could give misleading DEE estimates that could affect the correlation with ODBA. A study by Gessaman and Nagy (1988) using a fixed RQ of 0.8 showed that this only lead to an error of maximal 5% for birds metabolizing food. The prey items for kittiwakes may vary to some extent, but the composition would anyway mainly be of proteins and lipids, not carbohydrates, which would have little effect on RQ. A slightly erroneous RQ on the individual level should therefore not repeal a potential correlation completely. On the extreme end, if a bird is fasting instead of metabolizing prey this could induce error. A bird metabolizing stored lipids would have a low RQ of about 0.7, and using a RQ estimated on a mixed diet would give a higher CO<sub>2</sub> production than what would be the real case. In this study we did not observe a large BM loss in any of the sampled birds, so it is not likely that they were metabolizing stored lipids to a great extent.

The weather during the experimental period was not extreme, and there was no observable change in birds foraging and breeding behavior from the norm. The DEE of kittiwakes differs throughout the breeding season (Fyhn et al. 2001). To account for this, the adults were sampled when they had chicks of similar age (18-22 days). This is also the same age as for previous studies. This made it possible to compare the analyses from 2012 with studies from previous years. In 2013, the breeding season after the present study, DLW samples were collected from the same colony following the same sampling protocol as in 2012 by the same person. The estimated values from the DLW analyses from 2013 follow the long-term average from previous years. In 2012, the pool size ratio (N<sub>2</sub>H/NO) was lower than the values found in the other studies, averaging 1.00 when values ranging from 1.04 – 1.06 have been found in earlier studies. The biggest source of error when estimating DEE using the DLW method is related to the analysis of the isotope enrichments in the laboratory (Nagy 1983). The analyses of the DLW samples were performed at the same laboratory as in 2013, Schultner (2010) and Lassen (2012). Speakman (1995) reported that the gas preparation error contributed 80% to the total precision error of the lab analysis, and mass spectrometry 20%. The laboratory was asked to examine whether there had been any irregularities during the analyses of the 2012 samples. Results from analyses of samples performed at the laboratory before and after this study's samples did not show any abnormalities. Furthermore, other studies using DLW from



the same batches as in the present study were controlled for unexpected results, but no discrepancies in those studies were found. Explanations for the low N<sub>2</sub>H/NO ratio could not be found by the laboratory, nor why samples gave results that indicate that the tubes had not been properly sealed. As the sealed ends were controlled under a microscope and deemed satisfactory, how leakages could have occurred is unknown.

Investigations of the analysis process and sampling in field did not lead to any answer as to why there were so many samples that had indications of bad sealing or gave biologically impossible results regarding percentage of body water, nor why the samples that were possible to analyze showed abnormal values for N<sub>2</sub>H/NO ratio. The analyses protocol performed by the laboratory has been validated to have high precision and the DLW-method have been validated to have high accuracy for breeding kittiwakes. However, the birds that had estimated DEE within the range that have been found in other studies and acceptable N<sub>2</sub>H/NO were used for further investigation to compare with activity levels recorded by the accelerometers.

## **4.2 Potential effects of the methods on the studied animals**

The two methods used are believed to have no or minimal effects on the birds' behavior and EE. However, if they did cause a change in behavior or EE, the estimated DEE and ODBA would not represent natural levels. Both methods involve handling of the birds. The two sample DLW method requires that the birds are kept for one hour to allow for full equilibration of the two isotopes with the body water, but when released birds do not carry with them any external device that could potentially alter their natural behavior post capture. In contrast, deploying miniature accelerometers demand less handling time but the externally attached logger may affect the birds in several ways potentially leading to changes in their behavior. As the present study implies, behavior is directly linked to EE and therefore altered behavior due to the methods will give values that do not represent natural levels. However altered behavior should be reflected in changed EE, and therefore not have an impact on a potential correlation between the two methods used.

#### **4.2.1 DLW**

The two sample DLW method has been conducted in many studies in kittiwakes, from the first one in the 1980's (Gabrielsen et al. 1987) to more recent studies (e.g. Lassen 2012). It is a method that is recognized to generally have low impact on the studied individuals (Speakman 1997), including kittiwakes (Fyhn et al. 2001)(Jodice et al. 2002). However, a comparative study of the two sample DLW method and the less invasive one sample DLW method in kittiwakes did show that the post-treatment behavior following the two sample DLW method was altered to a larger degree than that of birds treated with the one sample DLW method (Schultner et al. 2010). The effect of the two sample DLW method can be reduced by prolonging the time between the two samples because the greatest change in behavior is observed shortly after release (Schultner et al. 2010). In the present study, a 72 hour interval between samples was maintained. Due to limited observations of the birds behaviors and nest attendances the DLW data from the present study was not examined against natural behavior in a control group or earlier studies. But the two sample DLW method should provide accurate enough data to be compared against ODBA (Elliott et al. 2012).

#### **4.2.2 Accelerometry**

Deploying data loggers has the disadvantage that the birds studied have to some degree be taken out of their natural pattern of life and have a device attached to their body that is alien for the bird. Even though the bird is released to its natural environment, the loggers may have negative effects on the bird's physic and/or behavior. In spite of the extensive use of loggers on free ranging birds, the effect of the loggers are often poorly considered (Vandenabeele et al. 2011). In studies on kittiwakes, loggers weighing up to 9% of the BM of the birds have been used without any apparent effect on BM or behavior of the birds (Gabrielsen and Melhum 1988). Loggers in this study were on average less than 2% of BM. In Gabrielsen and Melhum (1988) and other studies determining possible device effects on kittiwakes have typically constituted a minor part of the study, but ever less no significant effect of the loggers has been identified (Bogdanova et al. 2011)(Chivers et al. 2012)(Daunt et al. 2002) (Gabrielsen and Melhum 1988)(Kotzerka, Garthe, and Hatch 2010)(Paredes et al. 2012). A recent study focusing on the effect of instrumentation of kittiwakes showed higher levels of

the stress hormone corticosterone and extended feeding trips for breeding kittiwakes (Heggøy 2013). Devices that constitute 2.5% of the body mass should increase the cost of mechanical work by about 2% per day (Caccamise and Hedin 1985)(Fyhn 1999). Considering the precision of the DLW method and individual variation this is an increase that would not be possible to detect by the DLW method. The deployment of VHF transmitters weighing less than 2.5% of the body mass has not shown any effects on DEE estimated by the DLW method on kittiwakes (Fyhn 1999).

The accelerometers used in the present study had a total mass of 7 gram which was less than 2.5% of the average body mass. Because of the low DEE for bird 529 the results are ambiguous whether the logger had an effect on the birds DEE or not. As the data set is very small it is difficult to conclude if the birds with loggers had a naturally high DEE or if it was an effect of the loggers. By removing the bird that stands out with a much lower DEE than the other logger birds from the data set, the logger shows a strong effect. This is different from what have been found in other studies using low-weight loggers. One explanation for this can be that it was not the mass of the loggers that gave the apparently higher DEE in group 1 but maybe the way the loggers were attached to the feathers on the birds' back. To enlighten this further studies are necessary. If the higher DEE is caused by higher activity level this should not affect the correlation between the two methods. To determine the effect of placement and size of loggers experimentally, new study using different attachment methods and logger sizes would need to be conducted.

### **4.3 ODBA correlation with DLW**

To our knowledge, to date only one study has been published on the relationship between ODBA and DEE estimated using the DLW method. Elliot and colleagues (2012) showed that ODBA can be a good predictor for DEE in thick-billed murre (Uria lomvia). Using different equations for the different locomotory modes they were able to provide even more accurate estimates for DEE. Contrary from what we expected we could not find such relationship in kittiwakes. A thorough study of the methods and analyses used did not give a clear answer if the discrepancies in the methods and analyses had a too low precision to elucidate a possible correlation. Systematical errors in the methods and analyses could give inaccurate ODBA and DEE estimates that do not represent the real values. However, systematical errors should

not affect the correlation between the two methods because it would apply the same error to all individuals.

The question that remains unanswered is if kittiwakes have a correlation between ODBA and DEE. Locomotion does perhaps not constitute a large enough part of EE in kittiwakes to be reflected accurately by ODBA. It has been suggested that kittiwakes can regulate their resting metabolic rate in times of high energy use such as during the breeding season thus compensate DEE independently (Welcker et al. submitted 2014). Together with other biological factors (e.g. high thermoregulation in the Arctic) the variations across individuals perhaps masks possible relationships between ODBA and EE. In the limited data set, we did find a weak trend for increasing ODBA correlated with higher EE. In the present study, it is reasonable to believe that there was some error with the DLW procedure that gave the negative results. What exactly was the cause of this has not been possible to point out. As systematical errors should not affect a possible correlation, it is not likely to be calculations or the enrichment values used. It is more likely that either the analyses in the lab were for some unknown reason not reliable, or that the sampling in field was not consistent. A new study with a larger sampling size in the future could give a clearer answer if there is a correlation of ODBA and DEE. The acceleration data revealed promising behavioral data that has the potential to give EE estimates with high resolution of kittiwake natural activities. Keeping in mind the expected improvement of the technology, the use of accelerometry to estimate more fine scale EE over longer periods is very likely.

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## 6 Appendix

**Table 6.1** Table gives an overview of all the birds that were sampled during the study. It shows how many birds were included in the analyses and which were excluded.

	Only DLW (N = 18)	DLW and logger (N = 23)
<b>Sampled birds</b>	18	23
<b>Enrichment to close to background</b>	1	4
<b>No final sample</b>	2	5
<b>Cap leaks</b>	2	3
<b>Bird with DEE to close to BMR</b>	1	0
<b>Total birds not used in analyses</b>	6	12
<b>Birds used in DLW analyses</b>	12	11
<b>Loggers not retrieved</b>		3
<b>Logger stopped recording before recapture</b>		6
<b>Loggers with good ODBA-data</b>		14
<b>Birds with both good DLW and ODBA</b>		8

**Table 6.2** Table shows the different values for each individual bird that were used in the analyses.

Bird ID	Year	Logger	ODBA/day	Sex	BM	Nd/No	% BW (No)	DEE (kJ/day)
501	2012	Nologger		F	395	1,00	61	799
502	2012	Nologger		F	332	0,99	64	1032
503	2012	Nologger		F	345	1,03	61	851
505	2012	Nologger		F	347	1,00	59	989
510	2012	Nologger		F	324	1,04	66	1224
516	2012	Nologger		F	351	0,98	66	633
517	2012	Nologger		F	335	0,99	63	738
506	2012	Nologger		M	393	1,01	59	1175
507	2012	Nologger		M	411	1,01	63	1074
508	2012	Nologger		M	381	0,99	68	821
509	2012	Nologger		M	381	0,99	64	966
515	2012	Nologger		M	391	1,00	75	1388
521	2012	Yeslogger	607366	F	362	1,01	68	1144
527	2012	Yeslogger	1020222	F	324	1,00	67	1319
529	2012	Yeslogger	916433	F	336	1,03	71	623
539	2012	Yeslogger	1039893	F	333	1,02	62	1254
519	2012	Yeslogger	380108	M	390	0,99	64	1240
522	2012	Yeslogger	542851	M	380	0,99	73	1194
532	2012	Yeslogger	576230	M	399	1,02	66	1205
536	2012	Yeslogger	734696	M	361	1,05	71	1198

