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# Increased metabolic rate of hauled out harbor seals (*Phoca vitulina*) during the molt

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## Keywords

*Phoca vitulina*, harbor seal, marine mammals, pinnipeds, metabolic rate, haulout, molt

## What is already known?

Metabolic rate has been measured while molting in captive phocid seals in the water (Rosen and Renouf 1998, Sparling et al. 2006) and while on land (Ashwell-Erickson et al. 1986, Boily 1996). Previous studies have been conflicted in whether metabolic rate increases (Boyd et al. 1993; Boily 1996; Sparling et al. 2006) or decreases (Ashwell-Ericksen et al. 1986; Rosen and Renouf 1998) while phocid seals are molting. Theoretical predictions from thermal imaging by Paterson et al. (2012) suggested that metabolic rate increased in hauled out harbor seals during the first 30 minutes post-haulout to meet the cost of increasing skin surface temperature and the subsequent evaporative heat loss incurred while molting.

## What this study adds?

The results of the present study fill a knowledge gap in which very little is known about changes in metabolic rate of molting phocid species at the point immediately after transition from water onto land. This study presents conclusive, empirical measurements of increased metabolic rate in hauled out harbor seals during the molt while demonstrating how this effect varies within haulout periods and over the molting season. Metabolic rate during the molt was found to be relatively high over the first 40 minutes post-haulout compared to when the molt was complete, which highlights the importance of mitigation to protect phocid seals at haulout sites throughout the molting season.

# ABSTRACT

Harbor seals (*Phoca vitulina*) live in cold temperate or polar seas and molt annually, renewing their fur over a period of approximately four weeks. Epidermal processes at this time require a warm skin and therefore to avoid an excessive energy cost at sea during the molt, harbour seals and many other pinnipeds increase the proportion of time hauled out on land. We predicted that metabolic rate during haulout would be greater during the molt to sustain an elevated skin temperature in order to optimize skin and hair growth. To examine this, we measured post-haulout oxygen consumption ($\dot{V}O_2$) in captive harbor seals during molt and post-molt periods. We recorded greater $\dot{V}O_2$ of seals while molting than when the molt was complete. Post-haulout $\dot{V}O_2$ increased faster and reached a greater maximum at 40 minutes during the molt. Thereafter, $\dot{V}O_2$ decreased but still remained greater suggesting that while metabolic rate was relatively high throughout haulouts, it was most pronounced in the first 40 minutes. Air temperature, estimated heat increment of feeding (eHIF) and mass also explained 15.5% of $\dot{V}O_2$ variation over 180 minutes post-haulout, suggesting that the environment, feeding state and body size influenced the metabolic rate of individual animals. These results show that moulting seals have greater metabolic rates when hauled out, especially during the early stages of the haulout period. As a consequence, human disturbance that changes the haulout behaviour of molting seals will increase their energy costs and potentially extend the duration of the molt.

## Introduction

The molt period is an important phase in the annual life cycle of phocid seals. Each year shortly after the breeding season, hair over the entire body surface is shed and renewed and is a time when seals spend more time on land (Boily 1995). More time on land is necessary because the proliferation of phocid skin cells appears to be optimized at 37°C and ceases below 17°C (Feltz and Fay 1966). This is problematic in that thermal conductivity of water is 25 times greater than air, meaning maintenance of a warm skin for extended periods, for the shedding and renewal of hair, is energetically prohibitive in the cold temperate or polar seas where phocid seals are found (Nadel 1984). Elevation of skin temperature can therefore only be achieved by increasing the amount of time spent on land which reduces foraging time at sea (Watts 1996). This is also problematic in that phocid seals are capital breeders and must optimize foraging throughout the year to maximize success during the breeding season (Pistorius et al. 2004; Bowen et al. 2006). To counter this, phocid seals do show behavioral and physiological traits that allow a more rapid molt, the most obvious being to haul out on land and regulate blood flow through the blubber layer to increase skin temperature (Paterson et al. 2012).

Molting southern elephant seals (*Mirounga leonina*) (Boyd et al. 1993), grey seals (*Halichoerus grypus*) (Boily 1996; Sparling et al. 2006) and harbor seals (*Phoca vitulina*) (Paterson et al. 2012) have relatively high metabolic rates. In these studies, the cumulative effect of maintaining a warm skin in a cold environment coupled with active hair cell growth appears to be energetically demanding. Boyd et al. (1993) estimated that the energy required for molting in adult female southern elephant seals was approximately half that invested in pups during suckling. In contrast, resting metabolic rate has also been shown to be lower during the molt in harbor seals (Ashwell-Erickson et al. 1986, Rosen and Renouf 1998) and northern elephant seals (*Mirounga angustirostris*) (Worthy et al. 1992). These opposing findings demonstrate the complexity of molt physiology and therefore there is a clear need to better understand factors influencing the energetic cost of molt in phocid seals.

Amongst phocid seals there are two main molt types. In both northern and southern elephant seals, animals shed skin and hair as sheets of keratinized epidermis during a 'catastrophic' molt (Ling 1970). These species generally remain on land throughout the molt and therefore fast for the majority if not the entire period (Worthy et al. 1992; Boyd et al. 1993), although studies have identified occasional trips to sea while molting in southern elephant seals (Boyd et al. 1993; Chaise et al. 2017). In most but not all other phocids, hair is shed and renewed during a longer, more diffuse process that, while still requiring more time on land, is characterized by intermittent foraging trips. However, even in these species that are not fasting while molting, foraging may not be a priority. For example, voluntary reduction in food intake has been observed in harp seals (*Pagophilus groenlandicus*) (Lager et al. 1994) and harbor seals (Rosen and Renouf 1998), possibly indicating a response to predictable periods when some degree of fasting is required. The annual molt therefore represents a period when energetic demands are increased at a time when energy intake is reduced, as seals spend more time hauled out. Consequently, factors that prolong the moult on land could increase energy costs and delay foraging at sea.

Increased metabolic rate while molting may be partly attributable to having to synthesize new skin and/or hair (Ling 1970). There will also be an energetic cost from heat loss due to a high skin temperature on land (Paterson et al. 2012) and heat loss will be greater for species molting in colder, harsher environments. Animals that are fasting entirely on land or intermittently foraging may not have a sufficient energy intake to balance their energy needs. This is evident in longitudinal studies demonstrating mass loss while molting in species that fast throughout the molt (Worthy et al. 1992; Boyd et al. 1993; Chaise et al. 2018). Similarly, both longitudinal (Boily 1996) and cross-sectional (Chabot and Stenson 2002) studies show mass loss in species that periodically forage as the molt proceeds. This negative energy balance may be an important driver for conserving energy during the molt. For example, sustained lower food intake in harp seals is associated with a depressed metabolic rate (Ochoa-Acuna et al. 2009). Reduced metabolic rates have also been observed in harbor seals that voluntarily decreased food intake while molting (Rosen and Renouf 1998). This may partly explain why lower metabolic rates have been observed in molting harbour seals (Ashwell-Erickson *et al.* 1986, Rosen and Renouf 1998) and northern elephant seals (Worthy *et al.* 1992). However, this is complicated by the fact that for species that intermittently forage while molting, metabolic rate is likely to be temporarily elevated while prey is digested (Markussen et al. 1994).

The aim of this study was to examine the energy costs of molting harbour seals following haulout to land. We predicted that metabolic rate during haulout would be greater during the molt to sustain an elevated skin temperature in order to optimize skin and hair growth. To examine this, we measured post-haulout oxygen consumption ($\dot{V}O_2$) in captive harbor seals during molt and post-molt periods. This allowed assessment of potential energy costs of human distrurbance on seals during the molt.

## Methods

### *Animals*

Six male harbor seals, five adults and one sub-adult, were caught in the wild at either the Eden Estuary, Scotland (56°22’N, 02°48’W) or Ardersier, Scotland (57°35’N, 04°02’W). The sub-adult was estimated to be less than five years old which is the age of sexual maturity in male harbor seals determined by Bjorge (1992). Captured animals were immediately transferred to the captive facility at the Scottish Oceans Institute, University of St. Andrews. Two animals were brought into the facility in April/May in each of the years 2013, 2014 and 2015 and held until the post-molt period was complete around mid-October. When not in the experimental setup, animals were housed in separate outdoor holding pools in ambient temperature seawater surrounded by a haulout area exposed to ambient air temperature and solar radiation. Within the experimental setup and while respirometry measurements were being taken, animals also had access to seawater but were restricted to being kept within the respirometry chamber while breathing either in the water or when hauled out. When measurements were not being taken, animals remained within the experimental setup but had access to a platform (at ambient air temperature and solar radiation) surrounding the haulout respirometry chamber. Animals were always housed singularly and were trained to move voluntarily between the separate outdoor holding pools and the experimental setup, alternating between one week within the experimental setup and one week in holding pools.

Animals were fed a varied fish diet supplemented with multivitamins and ferrous gluconate (Aquavits, International Zoo Veterinary Group, Keighley, UK). Each individual was weighed (± 0.1kg) upon capture, opportunistically throughout the experimental period and immediately prior to release into the wild. All experiments with animals used in this study were conducted under Home Office License (60/4009 and 60/7806).

*Respirometry*

We measured metabolic rates of harbor seals hauled out in a respirometry chamber. This was constructed using non-transparent high-density polyethylene and incorporated into the structure of a large experimental pool within the facility (Fig. 1). The chamber itself was also covered in reflective insulating material to prevent excess heating under direct sunlight. Panels restricted access to the water surface while animals were in the pool so that all breaths were captured within the chamber. The approximate air space chamber volume was 1700 l allowing sufficient room for animals to haul out and to turn around if necessary. Animals entered and exited the chamber during experiments through a submerged internal hatch.

Mixing of air was achieved by way of multiple, equally spaced air inlets at the rear of the chamber. Air flow through the chamber was maintained at 350 l min$^{-1}$ by an air mass controller (Sable Systems Flow Kit 500H, Sable Systems International, Las Vegas, USA). This resulted in a lag time to measurement of approximately 25 seconds and a time-constant, as defined by Lighton and Halsey (2011) of 4 minutes 52 seconds giving a 95% equilibrium of 14.6 minutes. Air entered through holes in the rear of the chamber and exited through a 30mm diameter tube at the front, transferring air into the facility building where a gas analysis system was located.

### *Measurement of oxygen consumption during haulouts*

Oxygen consumption ($\dot{V}O_2$) over time was measured using open flow respirometry during voluntary haulouts. A subsample of air from the excurrent air flow drawn from the chamber was extracted at a rate of 500 ml min$^{-1}$. Water vapor and $CO_2$ were removed by passing the subsample through two desiccating tubes filled with calcium sulfate either side of a $CO_2$ absorbing tube filled with soda lime. Oxygen concentrations of the subsamples were measured continuously using a Sable Systems FC-10 Oxygen Analyzer (Sable Sytems International, Las Vegas, USA) and logged every three seconds. Baseline measurements of ambient air concentrations of $O_2$ were automatically recorded every hour to correct for drift in the system using LabAnalyst X software (M. Chappell, UC Riverside, Riverside, USA).

The open flow respirometry system was calibrated before each experiment with known volumes of $N_2$ using a technique described by Fedak et al. (1981). $\dot{V}O_2$ during haulouts was then calculated using the following equation:

$$\dot{V}O_2 = (0.2094VN_2/0.8)(\Delta C/\Delta C^*), \quad (1)$$

where $\Delta C$ denotes the change in $O_2$ concentrations (± 0.1%, range = 0 – 100%) during haulouts, $\Delta C^*$ denotes the change in $O_2$ concentrations during calibration and $VN_2$ denotes the volume of $N_2$ (l) used when calibrating the system. Errors associated with the respiratory quotient (RQ) are accounted for in this equation by the inclusion of a correction factor (0.8) according to Fedak et al. (1981).

Measurements of oxygen concentration were recorded continuously throughout each haulout period and then converted to $\dot{V}O_2$ ($l\ O_2\ min^{-1}$). Data were then averaged every five minutes to account for the fact that while on land, harbor seals can exhibit a pattern of breath-holds (apnea) followed by rapid breathing (eupnea) similar to diving (Pasche and Krog 1980; Castellini 1996). By averaging data in this way, troughs and peaks in the data caused by apneic and eupneic breathing could be evened out. Only measurements during which animals were in a resting state for at least one hour up to a maximum of three hours post-haulout were used for analysis.

### *Measurement of haulout activity*

Haulout activity was recorded using a closed-circuit video surveillance system with cameras (IR 37CSHR-IR 2M Submersible, RF Concepts Ltd., Dundonald, UK) installed within the respirometry chamber. Video was recorded using a digital video recorder (Samsung SRD-470, Hanwah Techwin America, New Jersey, USA) inside the facility building so that seals were unaware of any human presence during experiments. Experiments were carried out in the evening after 17:00 as this was a time when there was less activity and noise around the facility influencing haulout behaviour. This maximized the chances of taking measurements while seals were in a relaxed state on land. A seal haulout began when approximately half of the animal’s body had exited the water and similarly ended when approximately half the animal’s body entered the water at the end of the haulout. Only haulouts lasting more than one hour were used in this study. For haulouts lasting more than three hours, data were truncated at three hours due to the uncertainty of the efficacy of calcium sulfate and $CO_2$ treatments of air samples beyond that point.

*Environmental measurements*

While experiments were in progress, air temperature (± 0.1°C) was recorded inside the respirometry chamber using a temperature logger (Tiny Tag Plus 2 TGP-4500, Gemini Data Loggers Ltd., West Sussex, UK). Data were logged at a five minute sampling interval.

*Food consumption*

Markussen et al. (1994) showed that the effect of HIF in harbor seals was to increase $\dot{V}O_2$ within the first 30 minutes, which then declined but was still evident for up to 15 hours post-consumption. In the present study, HIF could not be measured directly in terms of changes in $\dot{V}O_2$ due to time constraints involved in running a separate suite of experiments. Instead, on each experimental day, all boluses of food given to animals were weighed (± 0.01kg) and the time of consumption recorded. An estimate of HIF, here defined as eHIF, was then derived by assuming that the effect of all boluses of food decayed linearly to zero over a 15-hour period post-consumption. This allowed for the effect of eHIF to be quantified both in terms of the size of the bolus of food consumed and temporal changes while animals were hauled out. The effect of eHIF as a measure of the effect of the mass of fish (kg) consumed, and how that effect diminished over 15 hours, were included in statistical models. In the hours leading up to experiments animals were fed *ad libitum*.

### *Molt categorization*

Animals were observed daily to visually determine the date of peak molt and this was estimated to correspond to the day of maximum hair loss. Based on previous records of the maximum molt duration in harbor seals (Thompson and Rothery 1987), the start and end of molt in each animal was therefore estimated to be 16 days either side of the peak molt date. $\dot{V}O_2$ measurements recorded during these periods were categorized respectively as molt and post-molt.

### *Statistical analysis*

We modelled how the response variable $\dot{V}O_2$ changed non-linearly over time when seals were hauled out (minutes post-haulout) during both the molt and post-molt periods. A Generalized Additive Mixed Model (GAMM) was used with the gam function in the mgcv library (Wood 2004) using the statistics package R (R Development Core Team 2016). Comparisons between the two measurement periods were made by including molt stage as an explanatory factor with two levels (molt and post-molt) while simultaneously fitting separate smooths (thin plate regression splines) of $\dot{V}O_2$ over minutes post-haulout at each of those two levels. The use of thin plate regression splines allows for the automatic optimization of the degree of smoothness for the relationship of interest (Wood 2003) which in this case was changes in $\dot{V}O_2$ over minutes post-haulout.

Separation of the two smooths for molt and post-molt periods involved using the “by” option for smoothing parameters in the mgcv library where a separate smooth is derived at each level of the supplied factor variable. Air temperature within the chamber in the full model to assess changes in metabolic rate that may be associated with differing ambient conditions. The variable eHIF was included in the full model to account for the timing and quantity of food consumed. Air temperature, eHIF and mass were included as smooths in the full model. Smoothed terms were tested for significance to determine whether they should or should not be treated as linear predictors. A continuous time auto-regressive correlation structure was incorporated using the nlme library (Pinheiro et al. 2017) to account for autocorrelation of measurements taken within each haulout. The autocorrelation structure penalizes smoothed lines for the response variable assuming equally spaced time covariate measurements taken in succession and are therefore likely to be autocorrelated with one another (Pinheiro and Bates 2000). Additionally, individual was included as a random variable to account for the effect of particular individual animals that may bias the results either positively or negatively. Model selection was carried out in a step-wise backwards selection process using Akaike’s Information Criterion (AIC) with candidate models being chosen based on having the lowest AIC score.

## Results

### *Study animals and haulout activity*

Mean ± S.D. mass of the five adult males used in this study was 85.62 ± 6.00kg (n = 31) and 84.2 ± 4.92kg (n = 45) during the molt and post-molt study periods respectively. Mass of one sub-adult male was 63.5 ± 1.33kg (n = 6) and 61.0 ± 1.50kg (n = 8) during the same periods. A total of 127 haulouts were recorded over 113 days with 52 during the molt and 75 post-molt over the course of the study. Mean ± S.D. haulout durations during the molt and post-molt periods were 156 ± 32.59 (n = 52) minutes and 157 156 ± 33.00 (n = 75) respectively (see Table 1 for details on individual animals).

### *Environmental measurements*

Mean ± S.D. air temperature inside the respirometry chamber during haulouts was greater during the molt (16.8 ± 2.05°C, n = 52) than during the post-molt (13.4 ± 2.41°C, n = 75) period.

### *Oxygen consumption*

GAMM model predictions showed there to be a significant non-linear relationship between $\dot{V}O_2$ and time since hauling out both during the molt ($p<0.001$) and post-molt ($p<0.001$) periods. Model predictions of $\dot{V}O_2$ during haulouts are shown in Figure 2. Predictions ± S.E. $\dot{V}O_2$ showed that at zero minutes post-haulout $\dot{V}O_2$ was greater when animals were molting (0.70 ± 0.06 l $O_2$ $min^{-1}$) compared to when not molting (0.64 ± 0.05 l $O_2$ $min^{-1}$). During both molt and post molt periods $\dot{V}O_2$ increased to a maximum ca. 40 minutes after hauling out and then declined continuously until 180 minutes post-haulout (Fig. 2). However, during the molt period, $\dot{V}O_2$ increased more rapidly and reached a greater maximum than during the post-molt period. Maximum $\dot{V}O_2$ measurements at 40 minutes were 0.90 ± 0.06 l $O_2$ $min^{-1}$ and 0.70 ± 0.05 l $O_2$ $min^{-1}$ decreasing to 0.65 ± 0.05 l $O_2$ $min^{-1}$ and 0.53±0.05 l $O_2$ $min^{-1}$ at 180 minutes post-haulout during the molt and post-molt periods, respectively.

The explanatory variables retained in the final model as smooths were air temperature, mass and eHIF. Molt stage, as defined above, was also retained as a factor. Air temperature showed no particular pattern over the range of values recorded during haulouts, though air temperature appeared to reduce $\dot{V}O_2$ above ~18°C ($p<0.001$). However, this may have been an artefact of having less data in that higher range of temperatures. Similarly, decreasing mass seemed to reduce $\dot{V}O_2$ ($p<0.001$), but data were limited at the lower range for mass due to only one sub-adult male being included in the study. Decreasing eHIF was generally shown to have a negative relationship with $\dot{V}O_2$ ($p<0.001$). This was associated with a reduction in feeding when not molting resulting in 35% of molt measurements effectively being measured under post-prandial conditions as 15 hours had lapsed between feeding and experimental measurements taking place, whereas only 29% of post-molt measurements were considered post-prandial due to animals feeding more frequently. Partial effect predictions of molt stage as a factor showed there to be an overall reduction in $\dot{V}O_2$ during the post-molt period compared to the molt period ($p<0.001$). The adjusted $R^2$ value for the final model was 0.155 meaning that 15.5% of the variation of $\dot{V}O_2$ over minutes post-haulout was explained by the modelling approach used. Partial effects plots of each of the explanatory variables retained in the final model are summarized in Figure 3, allowing visualization of the magnitude of the effect of each explanatory variable on $\dot{V}O_2$ at the different levels of each effect. The final GAMM model was checked for meeting assumptions of homogeneity (fig. S1, available online) and normal distribution (fig. S2, available online) of residual errors. Candidate models used during model selection are summarized in table S1, available online.

## Discussion

We found that in harbour seals, post-haulout $\dot{V}O_2$ was greater during the molt compared to a post-molt period, indicating the importance of this life-stage in their overall energy budget. Assuming that animals consumed an average $\dot{V}O_2$ of 0.76 (molting) and 0.62 l $O_2$ $min^{-1}$ (post-molt) over three hours post-haulout (where 1 l of $O_2$ = 19.7 kJ, (Schmidt-Nielsen, 1997)), the metabolic rate during molt was equivalent to an additional energetic requirement of 500 kJ (18.4% increase). This represents 2.5% of the daily energy requirement (20,000 kJ) of an adult harbor seal (Harkonen and Heidejorgensen 1991). Changes in $\dot{V}O_2$ were non-linear over the duration of three hours post-haulout both during the molt and post-molt periods. However, $\dot{V}O_2$ increased faster and reached a greater maximum at 40 minutes post-haulout when seals were molting. Beyond 40 minutes, $\dot{V}O_2$ steadily declined indicating that during the molt the first 40 minutes post-haulout have a high energetic cost relative to the remainder of the haulout.

In harbor seals, the process of molting is facilitated by hauling out and elevating skin temperature by shunting blood to the epidermis through anastomoses in the blubber layer (Ling 1970). Paterson et al. (2012) calculated that heat loss associated with elevating skin temperature post-haulout in molting harbor seals approximately doubled. The same study also showed that elevation of skin temperature while seals were molting reached an asymptote 30 minutes after hauling out. The results of the present study are lower but both studies indicate that molting seals increase metabolic rate in the initial part of a haulout to drive the physiological processes involved in achieving a high skin temperature and compensating for evaporative heat loss while drying out. Beyond the point at which skin temperature asymptotes and stasis is achieved, the need for a high metabolic rate is reduced and so metabolic rate declines. The results of both studies demonstrate that it is the initial stage of the haulout that is relatively energetically demanding. Any increase in the frequency with which seals ended one haulout and began another would therefore increase the amount of time in this elevated metabolic state. This effect may be exacerbated in inclement weather conditions that reduce skin temperature and/or increase the amount of time taken for seals to dry out, or may in fact be diminished if seals benefit from higher levels of solar radiation that speed drying of fur.

While harbor seals are molting, they spend a large proportion of time hauled out. For example, using telemetry data Lonergan et al. (2013) showed that the mean proportion of time hauled out during the molt was 0.72. By comparison, Cunningham et al. (2009) reported the proportion of time hauled out during a post-molt period as 0.34. A behavioral shift during the molt that results in seals spending more time on land makes them vulnerable to anthropogenic sources of disturbance that may cause them to enter the water at a higher frequency than normal. Previous studies have shown that harbor seals are highly site faithful (Yochem et al. 1987; Cordes and Thompson 2015), even when exposed to disturbance that causes them to temporarily leave preferred haulout sites (Andersen et al. 2014; Paterson et al. 2019). It is therefore likely that anthropogenic sources of disturbance that cause seals to enter the water will repeatedly affect the same animals around the point of disturbance (Paterson et al. 2019). In the context of the results of the present study, this is important as each time seals are forced into the water they are then faced with hauling out again with a corresponding increase in metabolic rate.

Molting harbor seals must balance the amount of time spent on land to complete the molt process and the amount of time at sea foraging. If seals continue to haul out for the same proportion of time even when frequently displaced from their haulout sites, the frequency with which they initiate haulouts will necessarily increase. Alternatively, if frequent displacement from haulouts reduces the proportion of time hauled out, the duration of the molt process may be prolonged due to the inability to elevate skin temperatures when in the water.

The relatively long duration of the molt in harbor seals compared to species that undergo a catastrophic molt requires that they forage intermittently while molting to meet their daily energetic requirements. In the present study, a derived estimate of the effect of HIF (eHIF) was used which was assumed to decline linearly over time. While this approach simplifies HIF on metabolic rate (Markussen et al. 1994), eHIF in this study was retained as a significant explanatory variable indicating that metabolic rate was higher when boluses of food were larger and had a greater effect when the time between food consumption and $\dot{V}O_2$ measurements was shorter.

Seals in this study did feed around the time $\dot{V}O_2$ measurements were taken, consuming less food during the molt which resulted in a lower eHIF effect. These results may reflect $\dot{V}O_2$ in wild animals more realistically than if they were kept in a post-absorptive state as harbor seals in the wild would be expected to continue to feed while molting, albeit at a lower rate. The fact that the effect of eHIF was lower during the molt due to reduced food intake means that the difference between $\dot{V}O_2$ in molting versus non-molting seals may have been greater if seals were consuming equal amounts of food in both periods. The lowering of metabolic rate in response to reduced feeding may also partly explain why metabolic rate has been found to be lower in molting seals in other studies. In these cases, animals were either kept in a post-absorptive state for respirometry measurements such as for harbor seals (see Ashwell-Erickson et al. (1986) and Rosen and Renouf (1998)), or were known to be fasting while on land such as in the study of northern elephant seals (Worthy et al. 1992). Seals in the wild are also likely to consume more food than in captivity due to greater activity during foraging suggesting that HIF would contribute more to haulout metabolic rate in wild seals.

This study highlights that the molt is an energetically important stage in the annual life cycle of harbour seals and provides evidence that mitigation measures to protect seals from disturbance at haulout sites, particularly during the molt, are important. Seals on haulout sites may be exposed to anthropogenic disturbances that cause them to enter the water at a greater frequency (Blundell and Pendleton 2015; Paterson et al. 2019), which should be avoided in molting seals that have a clear physiological need to be on land. Seals frequently forced from their haulouts lose heat on entering the water and must repeatedly elevate skin temperature when hauling out again (Erdsack et al. 2012). Our findings show that each new haulout started while actively molting is likely to incur an energetic cost which would have a cumulative effect where disturbance of seals was prevalent. Potentially, human disturbance that changes the haulout behaviour of molting seals could therefore increase the overall energetic cost of the molt process. Mitigation measures to avoid disturbance that increases haulout frequency in molting seals are therefore essential.

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## Tables

Table 1. Number of haulouts used for analyses as well as mean ± S.D. (n) mass (kg) are given for each study animal for both molt and post-molt periods in 2013, 2014 and 2015.

| | | **Animal** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Adult (2013)** | **Adult (2013)** | **Adult (2014)** | **Adult (2014)** | **Adult (2015)** | **Sub-adult (2015)** |
| **No. of haulouts** | **Molt** | 5 | 7 | 14 | 6 | 11 | 9 |
| | **Post-molt** | 15 | 5 | 13 | 20 | 11 | 11 |
| **Mass** | **Molt** | 83.5±0.55 (5) | 76.2±0.46 (6) | 86.0±1.54 (8) | 88.6±0.09 (4) | 92.1±2.53 (8) | 63.5±1.33 (6) |
| | **Post-molt** | 79.9±1.05 (13) | 80.3±1.09 (5) | 92.0±1.42 (7) | 85.8±1.75 (15) | 83.6±1.78 (5) | 61.0±1.50 (8) |

Table S1. GAMM models for predicting $\dot{V}O_2$. Explanatory variables are abbreviated as molt stage (MS), air temperature (AT), mass of animals (M) and estimated heat increment of feeding (eHIF). Variables in s() indicate a smooth function has been used. Otherwise the variable was treated as linear. The use of separate smooths for molt stages is indicated with “by”. Akaike’s Information Criterion (AIC), degrees of freedom (df) and deviance explained (%) are given for each model. The model in bold with the lowest AIC score is the final chosen model after backwards selection.

| GAMM formula | AIC | df | dev. (%) |
|---|---|---|---|
| **$\dot{V}O_2$~s(Time by MS)+s(AT)+s(M)+s(eHIF)+factor (MS)** | **-642.85** | **15** | **15.5** |
| $\dot{V}O_2$~s(Time by MS)+AT+s(M)+s(eHIF)+factor(MS) | -567.58 | 14 | 21.3 |
| $\dot{V}O_2$~s(Time by MS)+s(M)+s(eHIF)+factor(MS) | -565.48 | 13 | 20.5 |
| $\dot{V}O_2$~s(Time by MS)+s(AT)+M+s(eHIF)+factor(MS) | -578.72 | 14 | 13.6 |
| $\dot{V}O_2$~s(Time by MS)+s(AT)+s(eHIF)+factor(MS) | -578.79 | 13 | 12.8 |
| $\dot{V}O_2$~s(Time by MS)+s(AT)+s(M)+eHIF+factor(MS) | -495.38 | 14 | 14.9 |
| $\dot{V}O_2$~s(Time by MS)+s(AT)+s(M)+factor(MS) | -469.65 | 13 | 10.0 |
| $\dot{V}O_2$~s(Time by MS)+s(AT)+s(M)+s(eHIF) | -541.39 | 14 | 6.9 |

## Figure Legends

Figure 1. Schematic diagram of the open flow respirometry system (figure adapted from Sparling et al. (2004). Arrows indicate the direction of air flow.

Figure 2. Smoothed model predictions of $\dot{V}O_2$ (l $O_2$ $min^{-1}$) over minutes post-haulout during the molt and post-molt periods (black solid lines). Shaded areas extend to two standard errors either side of the smooths. Black dashed lines indicate predicted mean $\dot{V}O_2$ for both study periods combined. Variations in air temperature, eHIF, mass of animals and moult stage are accounted for in model predictions.

Figure 3. Partial effects for the relationship between $\dot{V}O_2$ and each of the explanatory variables in the final model. Note that the effects for smoothed terms centred on zero which is the mean partial effect of the variable. These are given for air temperature (a), mass (b) and estimated heat increment of feeding (eHIF) (c). The predicted effect for the factor molt stage is also given with molt being the reference value with post-molt (e). The shaded area for the smoothed terms extends to two standard errors either side of the smooth. Confidence intervals for the factor molt stage extend to two standard errors either side of the estimated effect.

Figure S1. Relationship between standardized residuals and fitted values for the final GAMM model, demonstrating that the assumption of homogeneity of residual errors was met.

Figure S2. Distribution of residual errors for the final GAMM model, demonstrating that the assumption of errors being normally distributed was met.

# Figures

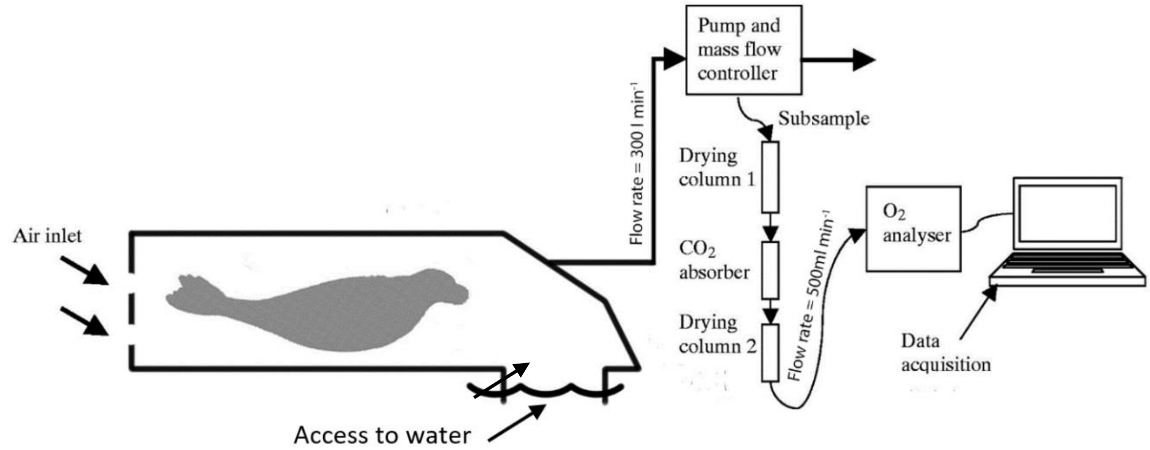

Figure 1.

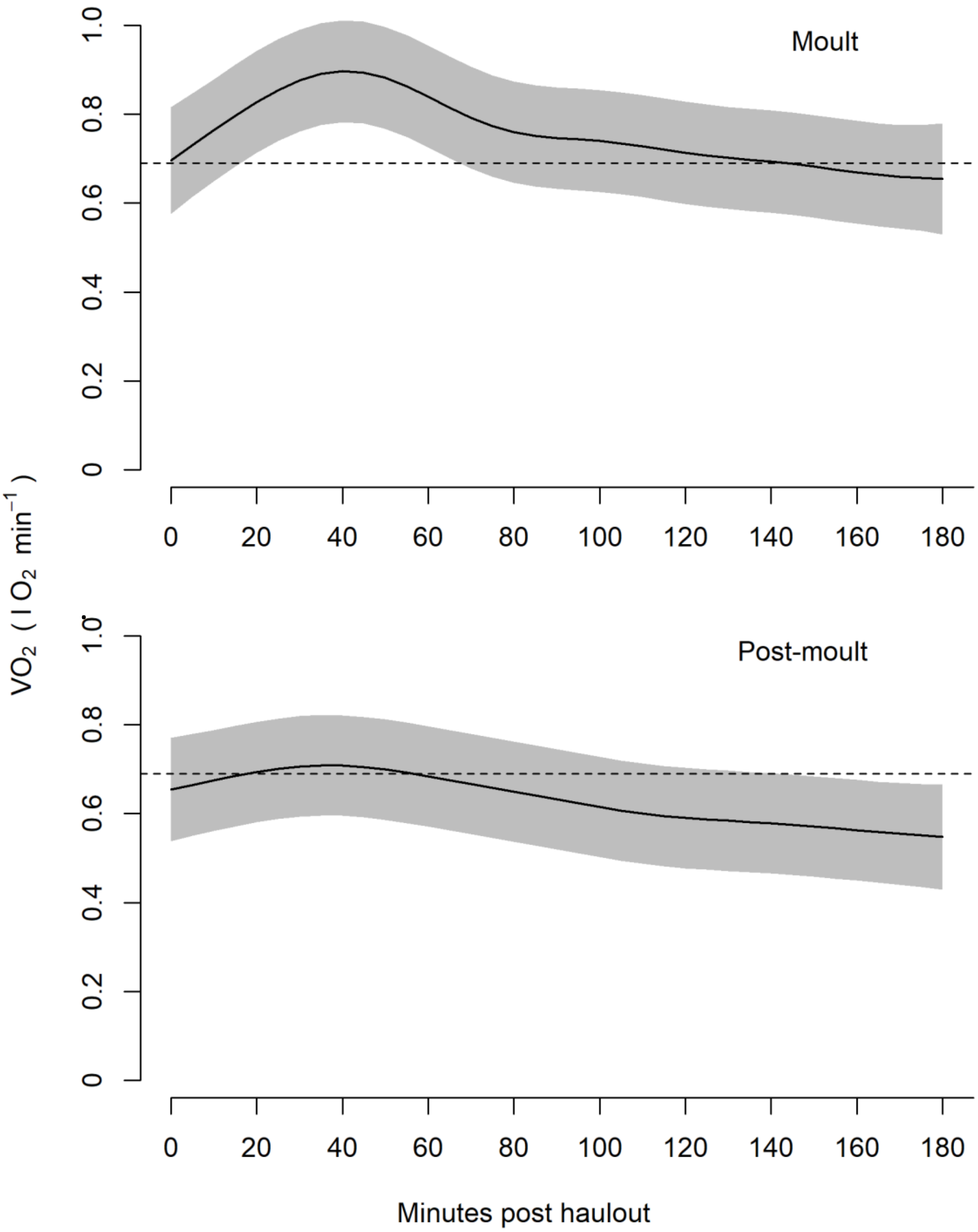
Moult
Post-moult
VO$_2$ ( l O$_2$ min$^{-1}$ )
0
0.2
0.4
0.6
0.8
1.0
0
20
40
60
80
100
120
140
160
180
Minutes post haulout

Figure 2.

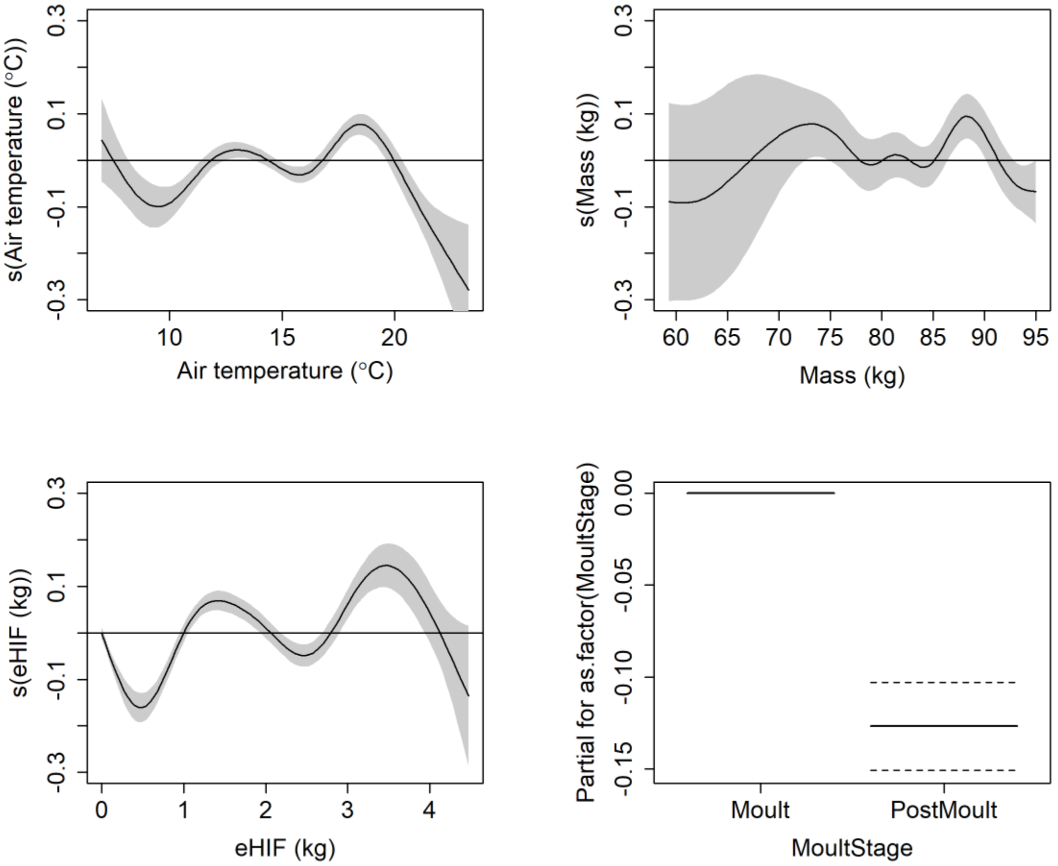
s(Air temperature (°C))
0.3
0.1
-0.1
-0.3
10
15
20
Air temperature (°C)
s(Mass (kg))
0.3
0.1
-0.1
-0.3
60
65
70
75
80
85
90
95
Mass (kg)
s(eHIF (kg))
0.3
0.1
-0.1
-0.3
0
1
2
3
4
eHIF (kg)
Partial for as.factor(MoultStage)
0.00
-0.05
-0.10
-0.15
Moult
PostMoult
MoultStage

Figure 3.

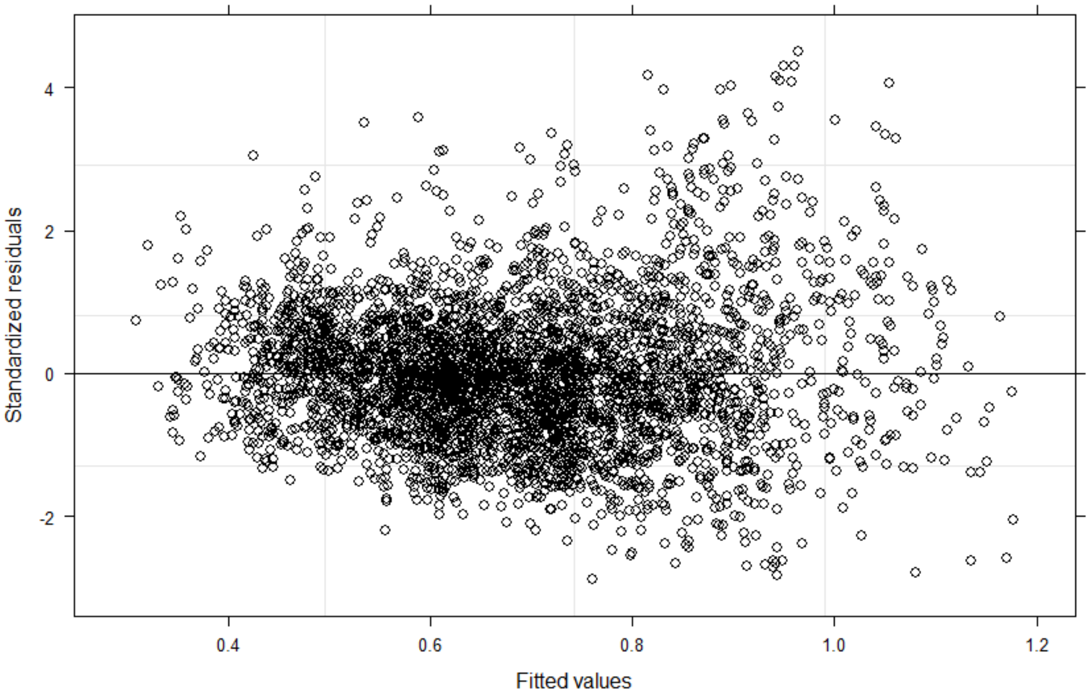

Figure S1.

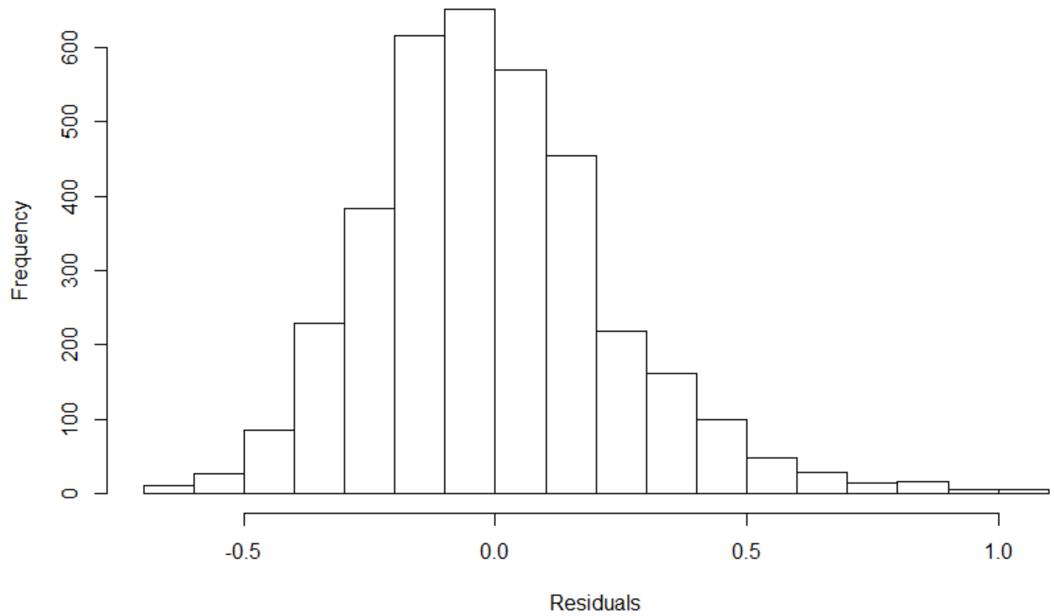

Figure S2.