
Increased Metabolic Rate of Hauled-Out Harbor Seals (*Phoca vitulina*) during the Molt

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ABSTRACT

Harbor seals (*Phoca vitulina*) live in cold temperate or polar seas and molt annually, renewing their fur over a period of approximately 4 wk. Epidermal processes at this time require a warm skin; therefore, to avoid an excessive energy cost at sea during the molt, harbor seals and many other pinnipeds increase the proportion of time they are hauled out on land. We predicted that metabolic rate during haul-out 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-haul-out oxygen consumption (\dot{V}_{O_2}) in captive harbor seals during molt and postmolt periods. We recorded greater \dot{V}_{O_2} of seals while they were molting than when the molt was complete. Post-haul-out \dot{V}_{O_2} increased faster and reached a greater maximum during the first 40 min. Thereafter, \dot{V}_{O_2} decreased but still remained greater, suggesting that while metabolic rate was relatively high throughout haul-outs, it was most pronounced in the first 40 min. Air temperature, estimated heat increment of feeding, and mass also explained 15.5% of \dot{V}_{O_2} variation over 180 min after haul-out, suggesting that the environment, feeding state, and body size influenced the metabolic rate of individual animals. These results show that molting

seals have greater metabolic rates when hauled out, especially during the early stages of the haul-out period. As a consequence, human disturbance that changes the haul-out behavior of molting seals will increase their energy costs and potentially extend the duration of the molt.

Keywords: *Phoca vitulina*, harbor seal, marine mammals, pinnipeds, metabolic rate, haul-out, 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 seals spend more time on land during this time (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 that 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 be achieved only 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 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), gray 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*; Worthly et al. 1992). These opposing findings demonstrate the complexity of molt physiology, and therefore there is a clear need to better

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understand factors influencing the energetic cost of molt in phocid seals.

Among 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 of the molt, if not the entire period (Worthy et al. 1992; Boyd et al. 1993), although studies in southern elephant seals have identified occasional trips to sea while molting (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 do not fast 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 molt 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 harbor 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 harbor seals following hauling out to land. We predicted that metabolic rate during haul-out 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-haul-out oxygen consumption ($\dot{V}O_2$) in captive harbor seals during molt and postmolt periods. This allowed assessment of potential energy costs of human disturbance on seals during the molt.

Methods

Animals

Six male harbor seals, five adults and one subadult, 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 subadult was estimated to be less than 5 yr 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 postmolt 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 haul-out 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 haul-out 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 1 wk in the experimental setup and 1 wk 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 (± 0.1 kg) upon capture, opportunistically throughout the experimental period, and immediately before 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 nontransparent 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 1,700 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 FlowKit 500H, Sable Systems International, Las Vegas, NV). This resulted in a lag time to measurement of approximately 25 s and a time constant, as defined by Lighton and Halsey (2011), of 4 min and 52 s, giving a 95% equilibrium of 14.6 min. Air

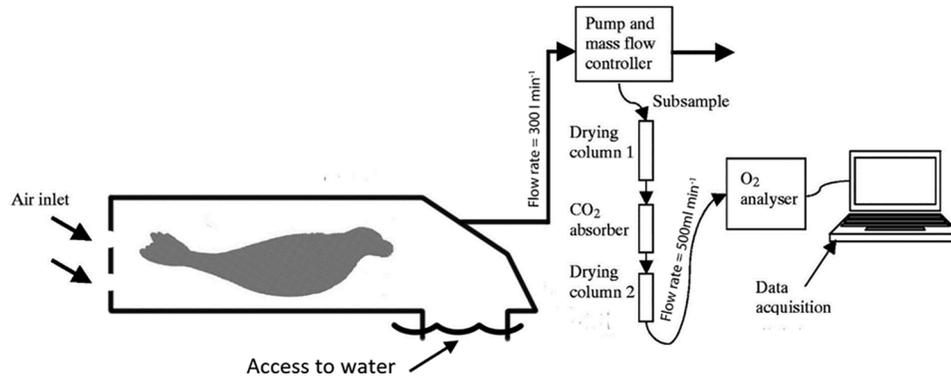


Figure 1. Schematic diagram of the open-flow respirometry system (figure adapted from Sparling and Fedak 2004). Arrows indicate the direction of air flow.

entered through holes in the rear of the chamber and exited through a 30-mm-diameter tube at the front, transferring air into the facility building, where a gas analysis system was located.

Measurement of Oxygen Consumption during Haul-Outs

\dot{V}_{O_2} over time was measured using open-flow respirometry during voluntary haul-outs. 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 on either side of a CO_2 -absorbing tube filled with soda lime. O_2 concentrations of the subsamples were measured continuously using a Sable Systems FC-10 oxygen analyzer and logged every 3 s. 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, University of California, Riverside).

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 haul-outs was then calculated using the following equation:

$$\dot{V}_{O_2} = \left(\frac{0.2094 V N_2}{0.8} \right) \left(\frac{\Delta C}{\Delta C^*} \right), \quad (1)$$

where ΔC denotes the change in O_2 concentrations ($\pm 0.1\%$; range = 0%–100%) during haul-outs, ΔC^* denotes the change in O_2 concentrations during calibration, and $V N_2$ denotes the volume of N_2 (L) used when calibrating the system. Errors associated with the respiratory quotient are accounted for in this equation by the inclusion of a correction factor (0.8) according to Fedak et al. (1981).

Measurements of O_2 concentration were recorded continuously throughout each haul-out period and then converted to \dot{V}_{O_2} ($\text{L O}_2 \text{ min}^{-1}$). Data were then averaged every 5 min 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 the breathing pattern while 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 taken when animals were in a resting state for at least 1 h up to a maximum of 3 h after haul-out were used for analysis.

Measurement of Haul-Out Activity

Haul-out activity was recorded using a closed-circuit video surveillance system with cameras (IR 37CSHR-IR 2M submersible, RF Concepts, Dundonald, UK) installed within the respirometry chamber. Video was recorded using a digital video recorder (Samsung SRD-470, Hanwha Techwin America, Teaneck, NJ) inside the facility building so that seals were unaware of any human presence during experiments. Experiments were carried out in the evening after 1700 hours, as this was a time when there was less activity and noise around the facility influencing haul-out behavior. This maximized the chances of taking measurements while seals were in a relaxed state on land. A seal haul-out began when approximately half of the animal's body had exited the water and similarly ended when approximately half of the animal's body entered the water. Only haul-outs lasting more than 1 h were used in this study. For haul-outs lasting more than 3 h, data were truncated at 3 h because of 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 ($\pm 0.1^\circ\text{C}$) was recorded inside the respirometry chamber using a temperature logger (Tinytag Plus 2 TGP-4500, Gemini Data Loggers, West Sussex, UK). Data were logged at a 5-min sampling interval.

Food Consumption

Markussen et al. (1994) showed that the effect of heat increment of feeding (HIF) in harbor seals was to increase \dot{V}_{O_2} within the first 30 min, after which it declined but was still evident for up to 15 h after consumption. In the present study, HIF could not be measured directly in terms of changes in \dot{V}_{O_2} because of the time constraints involved in running a separate suite of experiments.

Instead, on each experimental day, all boluses of food given to animals were weighed (± 0.01 kg), and the time of consumption was recorded. An estimate of HIF (eHIF) was then derived by assuming that the effect of all boluses of food decayed linearly to zero over a 15-h period after consumption. This allowed for the effect of eHIF to be quantified in terms of both the size of the bolus of food consumed and the 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 h were included in statistical models. In the hours leading up to experiments, animals were fed ad lib.

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. On the basis of 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 d either side of the peak molt date. \dot{V}_{O_2} measurements recorded during these periods were categorized, respectively, as molt and postmolt.

Statistical Analysis

We modeled how the response variable \dot{V}_{O_2} changed nonlinearly over time when seals were hauled out (minutes after haul-out) during both the molt and postmolt 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 postmolt) while simultaneously fitting separate smooths (thin-plate regression splines) of \dot{V}_{O_2} over minutes after haul-out 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 after haul-out. Separation of the two smooths for molt and postmolt 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 was included 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 be treated as linear predictors. A continuous-time autoregressive correlation structure was incorporated using the nlme library (Pinheiro et al. 2017) to account for autocorrelation of measurements taken within each haul-out. The autocorrelation structure penalizes smoothed lines for the response variable, assuming equally spaced time covariate measurements are 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 stepwise backward selection process using Akaike’s information criterion (AIC) with candidate models being chosen on the basis of having the lowest AIC score.

Results

Study Animals and Haul-Out Activity

Mean \pm SD mass of the five adult males used in this study was 85.62 ± 6.00 kg ($n = 31$) and 84.2 ± 4.92 kg ($n = 45$) during the molt and postmolt study periods, respectively. Mass of the one subadult male was 63.5 ± 1.33 kg ($n = 6$) and 61.0 ± 1.50 kg ($n = 8$) during the same periods. A total of 127 haul-outs were recorded over 113 d, with 52 during the molt and 75 after the molt over the course of the study. Mean \pm SD haul-out durations during the molt and postmolt periods were 156 ± 32.59 min ($n = 52$) and 156 ± 33.00 min ($n = 75$), respectively (for details on individual animals, see table 1).

Environmental Measurements

Mean \pm SD air temperature inside the respirometry chamber during haul-outs was greater during the molt period ($16.8^\circ \pm 2.05^\circ\text{C}$; $n = 52$) than during the postmolt period ($13.4^\circ \pm 2.41^\circ\text{C}$; $n = 75$).

Oxygen Consumption

GAMM predictions showed there to be a significant nonlinear relationship between \dot{V}_{O_2} and time since hauling out both during the molt ($P < 0.001$) and postmolt ($P < 0.001$) periods. Model predictions of \dot{V}_{O_2} during haul-outs are shown in figure 2. Predictions \pm SE \dot{V}_{O_2} showed that at 0 min after haul-out, \dot{V}_{O_2} was greater when animals were molting (0.70 ± 0.06 L O_2 min^{-1}) compared with when they were not molting (0.64 ± 0.05 L O_2 min^{-1}). During both molt and postmolt periods, \dot{V}_{O_2} increased to a maximum ca. 40 min after hauling out and then declined continuously until 180 min after hauling out (fig. 2). However, during the molt period, \dot{V}_{O_2} increased more rapidly and reached a greater maximum than during the postmolt period. Maximum \dot{V}_{O_2} measurements at 40 min 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 min after haul-out during the molt and postmolt 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 haul-outs, although air temperature appeared to reduce \dot{V}_{O_2} above $\sim 18^\circ\text{C}$ ($P < 0.001$). However, this may have been an artifact of having fewer 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 as a result of only one subadult male being included in the study. Decreasing eHIF was generally

Table 1: Number of haul-outs used for analyses as well as mean \pm SD mass (kg) for each study animal for both molt and postmolt periods in 2013, 2014, and 2015

	Adult (2013)	Adult (2013)	Adult (2014)	Adult (2014)	Adult (2015)	Subadult (2015)
No. haul-outs:						
Molt	5	7	14	6	11	9
Postmolt	15	5	13	20	11	11
Mass:						
Molt	83.5 \pm .55 (5)	76.2 \pm .46 (6)	86.0 \pm 1.54 (8)	88.6 \pm .09 (4)	92.1 \pm 2.53 (8)	63.5 \pm 1.33 (6)
Postmolt	79.9 \pm 1.05 (13)	80.3 \pm 1.09 (5)	92.0 \pm 1.42 (7)	85.8 \pm 1.75 (15)	83.6 \pm 1.78 (5)	61.0 \pm 1.50 (8)

Note. Numbers in parentheses indicate n .

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 that resulted in 35% of molt measurements effectively being measured under postprandial conditions, as 15 h had lapsed between feeding and experimental measurements taking place, whereas only 29% of postmolt measurements were considered postprandial because animals were feeding more frequently. Partial effects

predictions of molt stage as a factor showed there to be an overall reduction in $\dot{V}O_2$ during the postmolt period compared with 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 after haul-out was explained by the modeling approach used. Partial effects plots of each of the explanatory variables retained in the final model are summarized in figure 3, allowing

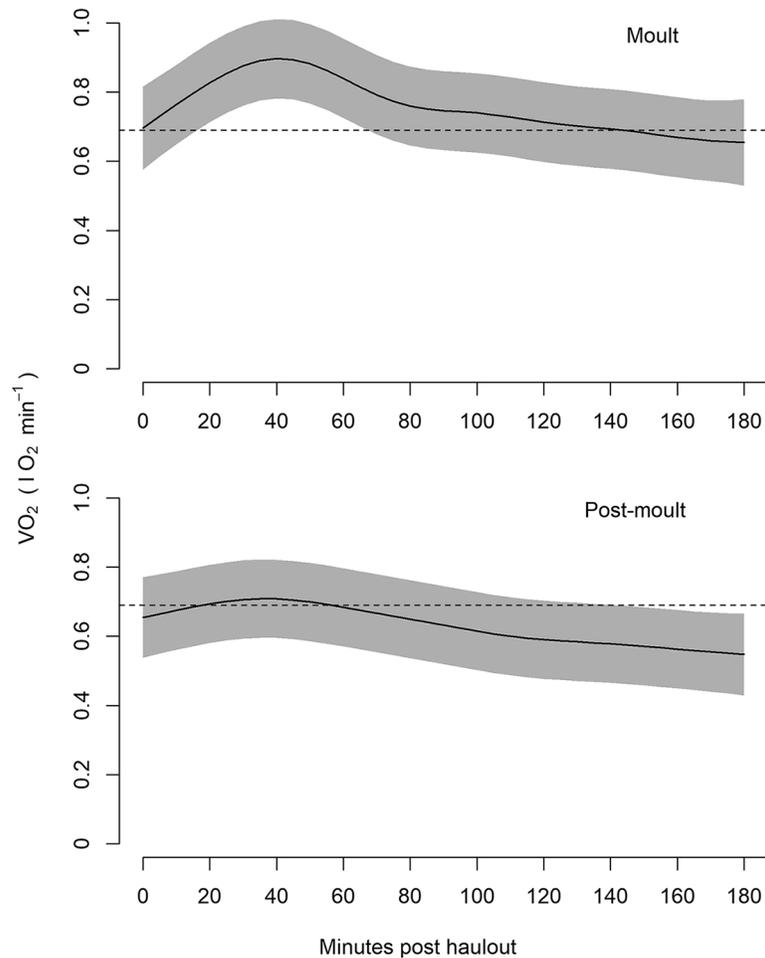


Figure 2. Smoothed model predictions of oxygen consumption ($\dot{V}O_2$; L O₂ min⁻¹) over minutes after haul-out during the molt and postmolt periods (solid lines). Shaded areas extend to 2 SEs on either side of the smooths. Dashed lines indicate the predicted mean $\dot{V}O_2$ for both study periods combined. Variations in air temperature, estimated heat increment of feeding, mass of animals, and molt stage are accounted for in model predictions.

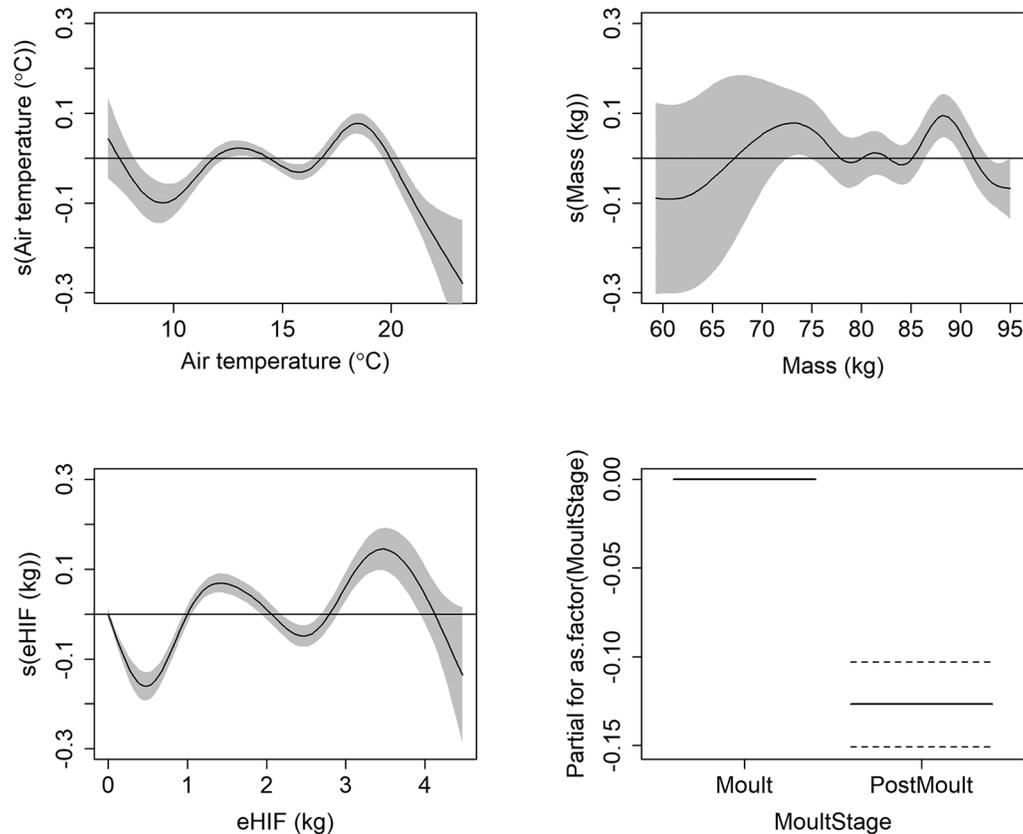


Figure 3. Partial effects for the relationship between oxygen consumption and each of the explanatory variables in the final model. Note that the effects for smoothed terms centered on zero, which is the mean partial effect of the variable. These are given for air temperature, mass, and estimated heat increment of feeding (eHIF). The predicted effect for the factor molt stage is also given, with molt being the reference value for postmolt. The shaded area for the smoothed terms extends to 2 SEs on either side of the smooth. Confidence intervals for the factor molt stage extend to 2 SEs on either side of the estimated effect.

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 was checked for meeting assumptions of homogeneity (fig. A1) and normal distribution (fig. A2) of residual errors. Candidate models used during model selection are summarized in table A1.

Discussion

We found that in harbor seals, post-haul-out $\dot{V}O_2$ was greater during the molt period compared with the postmolt period, indicating the importance of this life stage in their overall energy budget. Assuming that animals consumed an average $\dot{V}O_2$ of $0.76 \text{ L O}_2 \text{ min}^{-1}$ (during molt) and $0.62 \text{ L O}_2 \text{ min}^{-1}$ (after molt) over 3 h after haul-out (where $1 \text{ L of O}_2 = 19.7 \text{ kJ}$; 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 nonlinear over the duration of 3 h after haul-out during both the molt and postmolt periods. However, $\dot{V}O_2$ increased faster and reached a greater maximum at 40 min after

haul-out when seals were molting. Beyond 40 min, $\dot{V}O_2$ steadily declined, indicating that during the molt, the first 40 min after haul-out have a high energetic cost relative to the remainder of the haul-out.

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 after haul-out in molting harbor seals approximately doubled. The same study also showed that elevation of skin temperature while seals were molting reached an asymptote 30 min 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 haul-out to drive the physiological processes involved in achieving a high skin temperature and compensate 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 the metabolic rate declines. The results of both studies demonstrate that it is the initial stage of the haul-out that is relatively energetically demanding. Any increase in the frequency with which seals ended one haul-out 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 it 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 haul-out 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 haul-out sites, the frequency with which they initiate haul-outs will necessarily increase. Alternatively, if frequent displacement from haul-outs reduces the proportion of time hauled out, the duration of the molt process may be prolonged because of the inability to elevate skin temperatures when in the water.

The relatively long duration of the molt in harbor seals compared with 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 eHIF was used, which was assumed to decline linearly over time. While this approach simplifies the effect of 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 postabsorptive 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 ef-

fect of eHIF was lower during the molt because of reduced food intake means that the difference between $\dot{V}O_2$ in molting versus nonmolting 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 postabsorptive state for respirometry measurements, such as for harbor seals (see Ashwell-Erickson et al. 1986; Rosen and Renouf 1998), or 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 because of greater activity during foraging, suggesting that HIF would contribute more to the haul-out metabolic rate in wild seals.

This study highlights that the molt is an energetically important stage in the annual life cycle of harbor seals and provides evidence that mitigation measures to protect seals from disturbance at haul-out sites, particularly during the molt, are important. Seals on haul-out 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 haul-outs 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 haul-out started while a seal is actively molting is likely to incur an energetic cost that would have a cumulative effect where disturbance of seals was prevalent. Potentially, human disturbance that changes the haul-out behavior of molting seals could therefore increase the overall energetic cost of the molt process. Mitigation measures to avoid disturbance that increases haul-out frequency in molting seals are therefore essential.

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APPENDIX

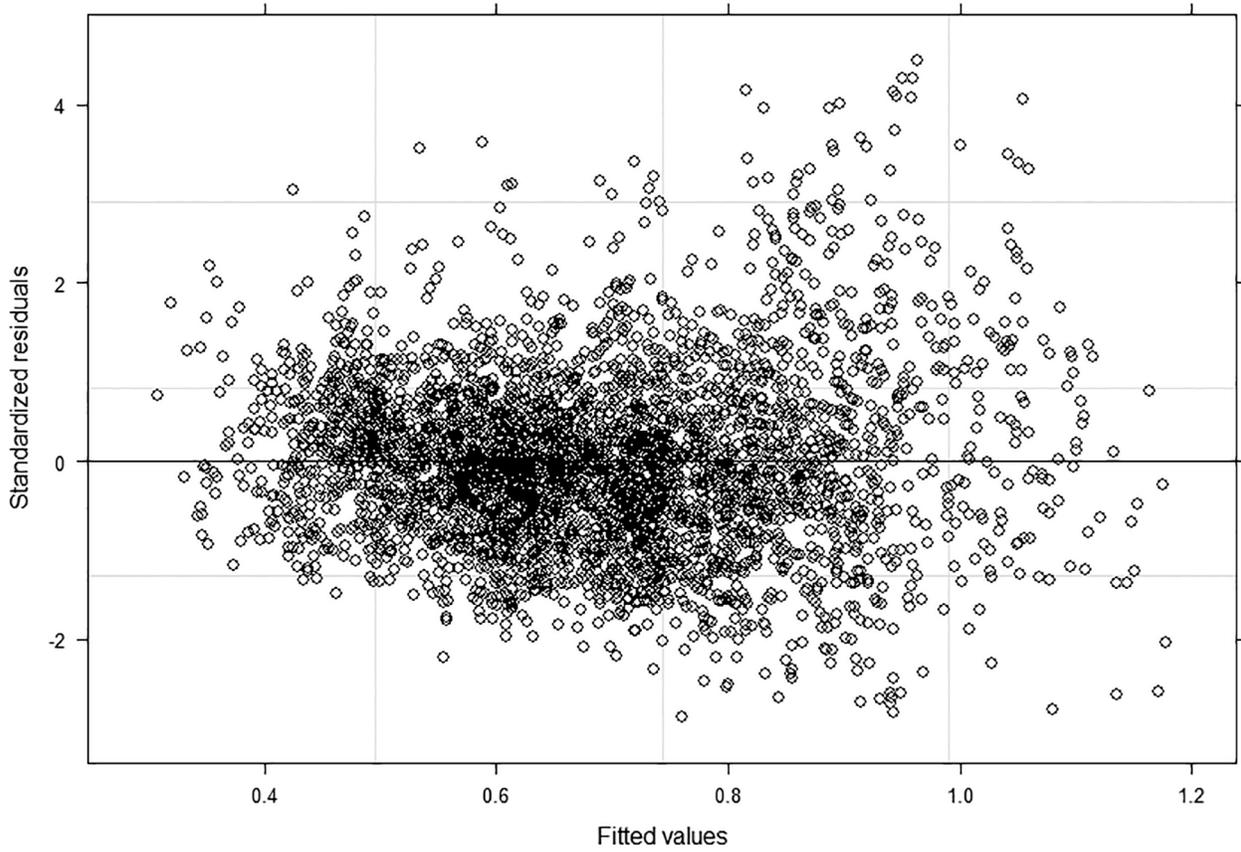


Figure A1. Relationship between standardized residuals and fitted values for the final generalized additive mixed model, demonstrating that the assumption of homogeneity of residual errors was met.

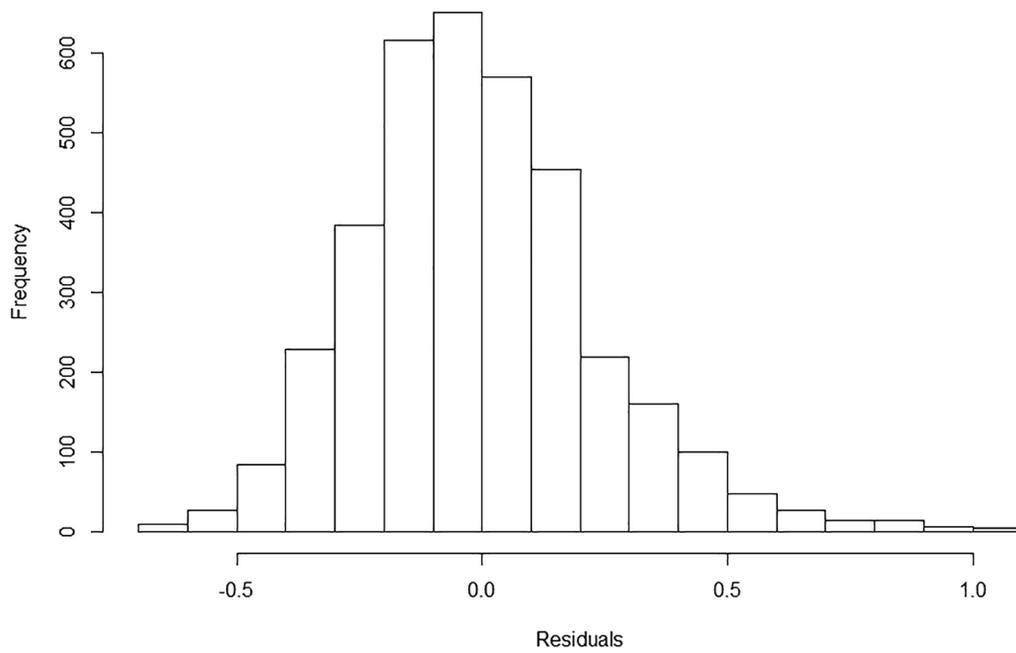


Figure A2. Distribution of residual errors for the final generalized additive mixed model, demonstrating that the assumption of errors being normally distributed was met.

Table A1: Generalized additive mixed models (GAMMs) for predicting $\dot{V}O_2$

GAMM formula	AIC	df	Dev (%)
$\dot{V}O_2 \sim s(\text{time by MS}) + s(\text{AT}) + s(\text{M}) + s(\text{eHIF}) + \text{factor}(\text{MS})^a$	-642.85	15	15.5
$\dot{V}O_2 \sim s(\text{time by MS}) + \text{AT} + s(\text{M}) + s(\text{eHIF}) + \text{factor}(\text{MS})$	-567.58	14	21.3
$\dot{V}O_2 \sim s(\text{time by MS}) + s(\text{M}) + s(\text{eHIF}) + \text{factor}(\text{MS})$	-565.48	13	20.5
$\dot{V}O_2 \sim s(\text{time by MS}) + s(\text{AT}) + \text{M} + s(\text{eHIF}) + \text{factor}(\text{MS})$	-578.72	14	13.6
$\dot{V}O_2 \sim s(\text{time by MS}) + s(\text{AT}) + s(\text{eHIF}) + \text{factor}(\text{MS})$	-578.79	13	12.8
$\dot{V}O_2 \sim s(\text{time by MS}) + s(\text{AT}) + s(\text{M}) + \text{eHIF} + \text{factor}(\text{MS})$	-495.38	14	14.9
$\dot{V}O_2 \sim s(\text{time by MS}) + s(\text{AT}) + s(\text{M}) + \text{factor}(\text{MS})$	-469.65	13	10.0
$\dot{V}O_2 \sim s(\text{time by MS}) + s(\text{AT}) + s(\text{M}) + s(\text{eHIF})$	-541.39	14	6.9

Note. Explanatory variables are molt stage (MS), air temperature (AT), mass of animals (M), and estimated heat increment of feeding (eHIF). Variables in parentheses following the letter *s* indicate that 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 (dev) explained are given for each model.

^aModel with the lowest AIC score and the final chosen model after backward selection.

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