

RESEARCH ARTICLE

Seasonal variation in energy expenditure is not related to activity level or water temperature in a large diving bird

Magella Guillemette^{1,*} and Patrick J. Butler²

¹Département de biologie, Université du Québec à Rimouski, Rimouski, Québec G5L 3A1, Canada and ²Centre for Ornithology, School of Biological Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

*Author for correspondence (magella_guillemette@uqar.qc.ca)

SUMMARY

There is considerable interest in understanding how the energy budget of an endotherm is modulated from a physiological and ecological point of view. In this paper, we used daily (24 h) heart rate (f_{H24}), as a proxy of daily energy expenditure (DEE) across seasons, to test the effect of locomotion activity and water temperature on the energy budget of a large diving bird. f_{H24} was monitored continuously in common eiders (*Somateria mollissima*) during 7 months together with measures of time spent flying and time spent feeding. f_{H24} varied substantially during the recording period, with numerous increases and decreases that occurred across seasons, although we did not find any relationship between f_{H24} and the time spent active (feeding and flying). However, inactive heart rate ($f_{H,inactive}$) decreased as locomotion activity increased, suggesting that common eiders were using some form of compensation when under a high work load. We were also unable to detect a negative relationship between water temperature and resting heart rate, a proxy of resting metabolic rate. This was unexpected, based on the assumption that high thermoregulation costs would be associated with cold waters. We showed instead that a high level of energy expenditure coincided with feather moult and warm waters, which suggests that the observed variable pattern of seasonal DEE was driven by these two factors. Nevertheless, our results indicate that compensation and possibly the timing of moult may be used as mechanisms to reduce seasonal variation in energy expenditure.

Key words: migratory bird, annual cycle, heart rate, daily energy expenditure, compensation.

Received 2 June 2011; Accepted 21 May 2012

INTRODUCTION

How animals deal with their intrinsic needs and environmental variability is a fundamental question in physiology and ecology. Energy expenditure is thought to vary considerably in birds during the annual cycle, as they need to reproduce, thermoregulate, grow feathers or even migrate. At the same time, food resources may vary seasonally in quantity and quality, while predation pressure may force animals to move and seek safer habitats. For these reasons, we expect the energy cost of life of birds to vary considerably in the course of the annual cycle. This reasoning has been labelled the increased demand (ID) hypothesis (Weathers and Sullivan, 1993). Recent studies on energetics of diving birds during the annual cycle support the ID hypothesis where peaks of energy expenditure are associated with productive costs, just like during pre-breeding and feather moult (Guillemette et al., 2007; Green et al., 2009; White et al., 2011).

However, metabolic ceilings may impose a limit to the level of energy expenditure (Kirkwood, 1983; Daan et al., 1990; Peterson et al., 1990; Weiner, 1992). In such a case, we might expect daily energy expenditure (DEE) to stay relatively constant despite variation in activity level, thermoregulation and productive costs (Guillemette et al., 2012). Despite large and sophisticated efforts devoted recently to the study of metabolic ceilings in endotherms (reviewed by Bacigalupe and Bozinovic, 2002; Speakman and Król, 2011), it appears that the identification of such limits to DEE are at best difficult. Such a difficulty is perhaps related to the possibility that an animal uses an array of behavioural or physiological

strategies in order to maintain itself below a metabolic ceiling or to minimise energy expenditure, thus making the identification of such a ceiling elusive (Guillemette et al., 2012). This is the energy budget limitation (EBL) hypothesis, which predicts that when facing such limits to DEE, the time–energy budget will be re-organised either by means of behavioural or physiological compensation (Pelletier et al., 2008). Pelletier et al. (Pelletier et al., 2008) found that a high level of flight, a costly activity for most wing-propelled diving birds such as the common eider, *Somateria mollissima* (Linnaeus 1758), was associated with a reduction of the rest of the energy budget. However, these authors could not discriminate whether this was the effect of EBL from the lack of any requirement to move rapidly by flight. In a recent paper, Guillemette et al. (Guillemette et al., 2012) selected a period of high energy turnover and compared DEE before and after moult migration and found that DEE was similar despite a large increase in foraging costs occurring before migration. Although this latter study supports the EBL hypothesis, we do not know whether such a hypothesis would be upheld during a longer time scale such as the annual cycle.

The heart rate method using data loggers implanted in the body cavity of birds allows investigators to estimate the rate of energy expenditure continuously over a relatively long period of time, often in excess of a year (reviewed by Butler et al., 2004; Green, 2011). The heart rate method has been largely used for wild and farm animals, as well as humans (reviewed by Green, 2011; Brosh, 2007; Achten and Jeukendrup, 2003). With this method, heart rate (f_H) is calibrated against the rate of O₂ uptake (\dot{V}_{O_2}) in the laboratory and

f_H measured in the field. Studies using this method have shown that variation in f_H of wild birds is the major circulatory adjustment observed in relation to changes in oxygen demand and thus any sustainable response to that demand should be reflected by variation of f_H .

In the present paper, we test the two hypotheses stated above by quantifying seasonal variation of energy expenditure in a large sea duck, the common eider. Daily heart rate (f_{H24} ; beats min^{-1}), the total number of heartbeats occurring in 1 day divided by 1440 (the total number of minutes in 24h), was converted into energy expenditure (Hawkins et al., 2000) and used as an index of DEE. We determined whether average f_{H24} varies on a seasonal basis to test the ID hypothesis. Seasonal f_{H24} was characterised by various oscillations during the recording period (7 months) and was related to seasonal water temperature in an effort to interpret these variations. We also examined the influence of locomotor activity (A_{loc} ; time spent flying and feeding) to test the EBL hypothesis by partitioning f_{H24} into feeding heart rate (f_{Hfeed}), flight heart rate (f_{Hfly}) and 'inactive' heart rate ($f_{Hinactive}$). The analysis was performed first within (intra-) individuals and then conducted on the data from all the birds to examine how the pattern of seasonal variation in f_{H24} is correlated with A_{loc} .

MATERIALS AND METHODS

The study was performed on Christiansø Island (55°19'N, 15°12'E), an old Danish fortress located in the southern Baltic Sea, 18 km from the Danish island of Bornholm. The general approach of our work involved the monitoring and deployment of data loggers on breeding females, partitioning of f_H data and using f_H to estimate DEE.

Deployment of data loggers

We studied the breeding biology of common eiders by monitoring ~100 nests on the study plot every year (1999–2005). Nests of banded females were identified by numbered wooden sticks. In spring 2003, 20 females were surgically implanted with f_H and pressure data loggers (BioMetistics, Robin Hill, Atch Lench, UK). We obtained a licence from Dyreforsøgtilsynet (Royal Veterinarian Corporation) in Denmark and birds were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care. All surgical procedures were conducted indoors 100 m from the experimental plot. The 20 data loggers were 36 ± 0.5 mm long (mean \pm s.d.), 28 ± 0.2 mm wide and 11 ± 0.3 mm thick, and weighed 21 ± 0.3 g, i.e. 1.2% of body mass at implantation (Guillemette et al., 2002). Hydrostatic pressure and f_H were sampled every 2 s.

Eighteen (90%) of the experimental females returned to the study area 1 year later, which is similar to the previously reported survival rate in this species (Coulson, 1984). This is most likely related to the fact that implanted data loggers do not alter aerodynamic or hydrodynamic properties of the instrumented individuals (Guillemette et al., 2002). However, the number of days per bird for which we had available information in the present study was variable (ranging from 45 to 220 days), most likely because of battery failure of the data loggers. We analysed data from 13 individuals, as these birds had loggers that recorded continually for ~7 months ($N=186$ –220 days), which covered the summer and the beginning of winter (mid-December).

Time activity budget and partitioning of f_H data

The time budget data involved calculating the daily time spent: (1) flying, (2) feeding and (3) being inactive. The partitioning of f_H data

involved calculating the number of heartbeats associated with each of these categories of behaviour and subsequently counting the number of heartbeats occurring in one day (daily number of heartbeats). Flight schedules (number and duration of flights) were compiled for each bird following the method described by Pelletier et al. (Pelletier et al., 2007). This method is based on the dramatic increases and decreases of f_H upon take-offs and landings respectively, and a plateau phase during flight where f_H is typically three to four times the resting level. For every female, the daily time spent flying was obtained by summing the duration of all flights that occurred during one day.

In birds, dives are usually performed in a series, where time spent submerged alternates with time breathing at the surface, which constitute a dive cycle. A feeding bout (>1 dive) is defined as the succession of dive cycles, and the daily time spent feeding was obtained by summing all feeding bouts occurring in one day. Finally, the daily time spent 'inactive' was obtained for each day and each female sampled by subtracting the time spent active (feeding + flying) from 1440 min. From visual observations, we know that 'inactive' behaviour is composed of swimming, preening and resting (Guillemette, 2001).

The f_H data were partitioned into useful quantities, i.e. feeding heart rate (f_{Hfeed}), flight heart rate (f_{Hfly}) and inactive heart rate ($f_{Hinactive}$). Thus, for every category of behaviour and for every female, we summed the total number of heartbeats associated with that behaviour and divided this by the number of minutes the bird spent engaged in that activity each day. Thus, we obtained averages of f_{Hfeed} , f_{Hfly} and $f_{Hinactive}$ for the 13 females. A similar procedure was followed for the total (daily) heart rate by dividing the total number of heart beats obtained in 1 day by 1440 min.

A customised computer program (written by J. M. Grandbois, Université de Québec à Rimouski, Canada) was run to calculate all these quantities from the raw data. Finally, we estimated the minimum f_H for each bird during each day of sampling and took this to be the resting heart rate (f_{Hrest}). To do so, we wrote a computer program in order to find the minimum average value within an interval of 5 min, which was then compared with similar 5 min intervals obtained through the day. This time interval was a compromise between smaller intervals, incompatible with the observed decrease in f_H during diving, and larger intervals that compose a larger portion of the day. Because each new 5 min interval was searched 20 s later than the one before, we obtained 4306 such intervals for each day of sampling. The end result of that procedure was the selection of the 5 min interval with the lowest mean f_H . We used that quantity as an estimate of the f_{Hrest} for that day and for a specific bird.

Conversion into rate of energy expenditure

We used the calibration study of Hawkins et al. (Hawkins et al., 2000) to convert f_H data into mass-specific metabolic rate. Hawkins et al. [see their table 4 (Hawkins et al., 2000)] related f_H (beats min^{-1}) and mass-specific rate of oxygen consumption, \dot{V}_{O_2} ($\text{ml O}_2 \text{ kg}^{-1} \text{ min}^{-1}$), for six common eiders that were monitored continuously for 2 days on a water flume, exercised at various speeds for up to 6 h per day and fed with waterfowl diet pellets. The functional (reduced major axis) relationship was: $\dot{V}_{O_2} = 0.146f_H + 9.677$ ($r^2 = 0.753$, $P = 0.023$). Each liter of oxygen consumed was multiplied by 20.083 kJ (Schmidt-Nielsen, 1997) to obtain the mass-specific metabolic rate of birds.

A different procedure was required to estimate flight costs, as it has been shown that there is a different relationship between f_H and \dot{V}_{O_2} when birds are swimming/running compared with when they are flying (Ward et al., 2002). Pelletier et al. (Pelletier et al., 2008)

used the two physiological models of Bishop (Bishop, 1997) based on heart mass and heart rate, and found that estimates of energy expenditure during flight were similar to estimates generated by allometric and theoretical models. Using the second model of Bishop (Bishop, 1997), Pelletier et al. (Pelletier et al., 2008) estimated mass-specific flight costs of flight to be 79.5 W kg^{-1} , and this estimate was used in the present study. Finally, mass-specific DEE was obtained by summing the metabolic rate of birds while flying, feeding and inactive.

One potential difficulty when converting f_H into estimates of DEE for migratory birds is that they show labile body and organ mass during the annual cycle (Biebach, 1998; Piersma, 1998; McWilliams and Karasov, 2001). This is because changes in organ mass and body composition may alter the relationship between f_H and \dot{V}_{O_2} and thus affect estimates of energy expenditure. This may well be the case in our study, as female common eiders recover from incubation fast and prepare themselves for migration, wing and body moult, and the onset of winter. Thus, an implicit assumption of the heart rate method is that heart mass is an isometric function of body mass. Variation in heart mass is a determinant of the cardiac stroke volume: for a given rate of oxygen uptake and oxygen extraction, f_H varies inversely with stroke volume (see Butler et al., 2004). Violation of that assumption would potentially confound our results regarding DEE. At the interspecific level, Bishop (Bishop, 1997) computed a reduced major-axis regression from 228 species of birds and found that heart mass scales with body mass with a slope of 0.93 ± 0.02 [mean \pm 95% confidence intervals (CI)], which is only slightly shallower than the theoretical (isometric) slope of 1. Guillemette et al. (Guillemette et al., 2007) collected female common eiders during winter and post-hatching phases and found that they varied greatly in terms of body mass (1191–2002 g) and heart mass (12.1–19.8 g). A reduced major-axis regression on these log-transformed data resulted in a slope of 1.03 (intercept = -2.12, $N=17$, $r^2=0.61$). These results indicate that variation in body mass is tracked by a similar variation in heart mass, suggesting that although there is some small variability in the relationship between heart mass and body mass, it is reasonable to assume that the relative stroke volume remains constant during the present study period. However, only measurements at that intra-individual level would properly test the assumption that heart mass variation tracks body mass variation.

Perhaps the best calibration study performed so far between f_H and \dot{V}_{O_2} on a migratory bird is that by Portugal et al. (Portugal et al., 2009). These authors found that five out of the six relationships between f_H and \dot{V}_{O_2} , derived from various sampling periods throughout the year, did not differ significantly despite significant changes in body mass and body composition (Portugal et al., 2007). This indicates that the heart rate method is robust in relation to most of the seasonal variation of body condition. It suggests also that the single calibration study (Hawkins et al., 2000) used in the present paper to convert f_H into \dot{V}_{O_2} and then into energy expenditure of common eiders may be valid when comparing seasons within the whole study period (208 days on average). However, Portugal et al. (Portugal et al., 2009) found that the calibration curve derived from captive barnacle geese was different from that for wild geese, indicating that for a similar \dot{V}_{O_2} , f_H is lower for wild individuals. This suggests that our study may underestimate the actual value of energy expenditure. Therefore, our results are presented as a two-step process. In the first step, a minimum number of assumptions are made to test the effect of locomotion activity and water temperature on f_H . In the second step, estimates of DEE are made for the minima and maxima f_H observed during the period of

recording (7 months), giving an umbrella of values that can be compared with values from other studies.

Water temperature

The females nesting on Christiansø Island have been ringed for several years (Lyngs, 2000), and from this information we know that most females move from east to west during moult migration, mostly toward the eastern part of the Baltic and the southern part of the Kattegat, and some individuals into the Wadden Sea. Using this information together with the time spent flying and flight speed, we were able to position every female in this study into a spatial polygon. For each female in the Baltic, the surface water temperature was obtained from a monitoring programme (Finnish Institute of Marine Research) along the route of the ferry Finnpartner (Travemünde–Helsinki). In the Wadden Sea, similar information was obtained from the marine station of Oldenburg University (<http://las.physik.uni-oldenburg.de/landstation/>). Although this approach is coarse, it has the advantage of being robust when the increases and decreases in water temperature of these three regions are compared. Thus, for each day of recording, the water temperature was averaged across females.

Data analysis

We performed average and individual analyses of the data collected.

Individual level

One salient feature of the f_H data presented here is that they were recorded almost continuously, every 2 s, for a long period of time (208 days). We thus compute the Pearson correlation coefficient between two quantities for each individual separately (Table 1). We then calculated the average intra-individual correlation coefficients for which we calculate confidence intervals using the bootstrap method using 10,000 re-samplings (Lunneborg, 2000). Inspection of the sampling distribution resulting from that procedure demonstrated that the distribution was symmetric. Thus, we were able to use the Student's t version of the bootstrap (Lunneborg, 2000). When this interval excludes zero, we declared the average correlation coefficient to be significantly different from zero at the 95% level.

Average level

When averaged over the 13 experimental females, f_{H24} varies greatly on a seasonal basis with steady increases and decreases. Thus, the data points are obviously not independent from each other (Fig. 1A). This is an important point, as any positive autocorrelation will inflate the P -value of any statistical tests. By implication, positive autocorrelation reduces the effective sample size (Guillemette et al., 2004). We argue that this characteristic of the data does not interfere with the analysis proposed here. Clifford et al. (Clifford et al., 1989) and Dutilleul (Dutilleul, 1993) suggest independently a method to adjust the sample size in relation to the level of autocorrelation observed in serial data. When performing a statistical test (e.g. a correlation), we correct N_{sample} into N' by adjusting the observed P -value to its real (unbiased) value. Numerical simulations using artificial data with known autoregressive structures confirm that this method is valid (Dale and Fortin, 2002).

One important point in the application of this method, however, is that all autocorrelation lags must be included in the calculation of the effective sample sizes, even if the individual values do not seem to be significant. If this is not done, an error in the correction factor may arise because negative autocorrelations require deflation rather than inflation (Dutilleul, 1993; Dale and Fortin, 2002). Given that the

Table 1. Pearson correlation coefficients (r) and coefficients of determination (R^2) between various combinations of daily heart rate (f_{H24}), resting heart rate (f_{Hrest}), inactive heart rate ($f_{Hinactive}$) and locomotion activity (A_{loc}) at the intra-individual level for 13 female common eiders

Individual ID	$f_{H24}-f_{Hrest}$		$f_{H24}-f_{Hinactive}$		$f_{H24}-A_{loc}$		$f_{Hinactive}-A_{loc}$	
	r	R^2	r	R^2	r	R^2	r	R^2
WB	0.856	0.733	0.917	0.841	-0.293	0.086	-0.614	0.377
BR	0.799	0.638	0.985	0.970	0.096	0.009	0.282	0.080
OO	0.694	0.482	0.990	0.979	0.229	0.052	-0.050	0.003
OR	0.808	0.653	0.942	0.888	-0.068	0.005	-0.278	0.077
OY	0.500	0.249	0.951	0.905	0.092	0.008	-0.177	0.031
YR	0.649	0.421	0.831	0.691	0.253	0.064	-0.206	0.042
YW	0.875	0.765	0.960	0.921	-0.014	0.000	-0.391	0.153
YY	0.574	0.329	0.770	0.592	0.591	0.349	-0.007	0.000
RW	0.868	0.754	0.936	0.875	0.156	0.024	-0.156	0.024
WO	0.403	0.162	0.935	0.874	0.462	0.213	0.166	0.028
YB	0.342	0.117	0.960	0.921	-0.004	0.000	-0.230	0.053
RR	0.733	0.537	0.943	0.889	0.047	0.002	-0.155	0.024
RB	0.466	0.217	0.937	0.879	0.175	0.031	-0.128	0.016
Mean \pm s.d.	0.659 \pm 0.186	0.466	0.927 \pm 0.061	0.863	0.132 \pm 0.227	0.065	-0.150 \pm 0.227	0.070
CI	0.108		0.036		0.132		0.133	
CI+	0.767		0.963		0.264		-0.017	
CI-	0.551		0.891		0.000		-0.282	

Intra-individual correlation coefficients were averaged, for which we calculated confidence intervals (CI) using the bootstrap method. When this interval excludes zero, we declared the average correlation coefficient to be significantly different than zero at the 95% level.

Individual ID is based on colour code.

level of autocorrelation of the data presented here is alternatively positive and negative (Fig. 1) with a preponderance of negative values (data not shown), this suggests that our sample size should be increased, not decreased. Nevertheless, we used the original number of days recorded in our analysis ($N=208$) as a conservative estimate of the P -value derived from our correlation analysis.

The EBL hypothesis predicts that f_{H24} and A_{loc} are not related and is similar to the statistical null hypothesis of no relationship. However, when we remove all the heartbeats associated with A_{loc} from f_{H24} ($\equiv f_{Hinactive}$), the EBL hypothesis predicts that $f_{Hinactive}$ and A_{loc} should be inversely related. We first computed the correlation coefficient for the original data and then chose permutation re-samples from the data without replacement in a way that is consistent with the null hypothesis of no relationship. We then constructed the permutation distribution of the statistic from its values in 10,000 re-samples (the permutation approach is distribution free). Finally, we found the P -value by locating the original statistic on the permutation (Lunneborg, 2000).

When averaged across individuals, f_{H24} varies greatly on a seasonal basis with various oscillations (Fig. 1A). We used that characteristic of the data to perform our analysis and test whether the various decreases or increases of f_{H24} occurring during the annual cycle were different from zero. For each individual, we averaged f_{H24} for a 5 day period (lows and highs indicated in Fig. 1A) that we subtracted from the average obtained during the preceding 5 day period to obtain a difference (Δ) that estimates the rate of change of f_{H24} . By repeating this over all 13 individuals sampled, we obtained the average rate of change for which we calculated 95% CIs using the bootstrap method and 10,000 re-samplings, as described above. The statistical significance was set at $P=0.05$. Resampling Stats v.2 (www.resample.com) was used for statistical analyses. Values are means \pm s.d. unless otherwise stated.

RESULTS

f_H and DEE

One obvious feature of the curve depicted in Fig. 1A is the numerous oscillations in f_H that occur during the 7 months of recordings. For

five deltas, the confidence intervals excluded the zero line, which indicates that these increases or decreases in DHR were significantly different from zero (Fig. 1B). Thus, this analysis indicates that the f_{H24} of the instrumented females varied significantly during the 7 months of recording.

One frequent concern when converting f_H to energy expenditure is how accurate the estimate can be. Given the uncertainties associated with the calibration obtained from captive birds and applied to wild birds (see Materials and methods), we did not want to misrepresent the accuracy of our estimates by interpreting small variations in energy expenditure as being significant. Green (Green, 2011) performed simulations for four species of birds, showing that a variation of 9–31% in f_H would most likely result in a real difference in energy expenditure (depending on species, calibration available and sample size). In our study, seasonal and significant increases and decreases in f_{H24} are associated with 8–39% variation when compared with mean f_{H24} (Fig. 1). Therefore, the seasonal f_{H24} minima and maxima of Fig. 1 most probably translate into real variation in energy expenditure (Table 2). Average DEE was 955 kJ kg⁻¹ and varied between 885 and 1028 kJ kg⁻¹ during the period of recording (Table 2).

f_H and locomotion

These variations in f_H were not positively related to level of activity (A_{loc}), although it varied between 70 and 300 min day⁻¹ (Fig. 2A). On the contrary, we found a weak, negative correlation ($r=-0.132$, $P=0.023$, $N=208$; Fig. 2A) between A_{loc} (flying + feeding) and f_{H24} . By removing all the activity heartbeats from f_{H24} (see Materials and methods), we obtained $f_{Hinactive}$, which is highly correlated with f_{H24} ($r=0.880$, $P<0.0001$, $N=208$, Fig. 1A,C) indicating that the pattern of variation was similar for these two variables. Moreover, there was a strong negative correlation between A_{loc} and $f_{Hinactive}$ ($r=-0.377$, $P<0.0001$, $N=208$; Fig. 2B), indicating that a high level of A_{loc} is associated with a decrease of $f_{Hinactive}$.

At the individual level, the correlation between f_{H24} and A_{loc} varied greatly and when the coefficient of correlation was averaged across individuals ($r=0.132$), the relationship was positive and marginally

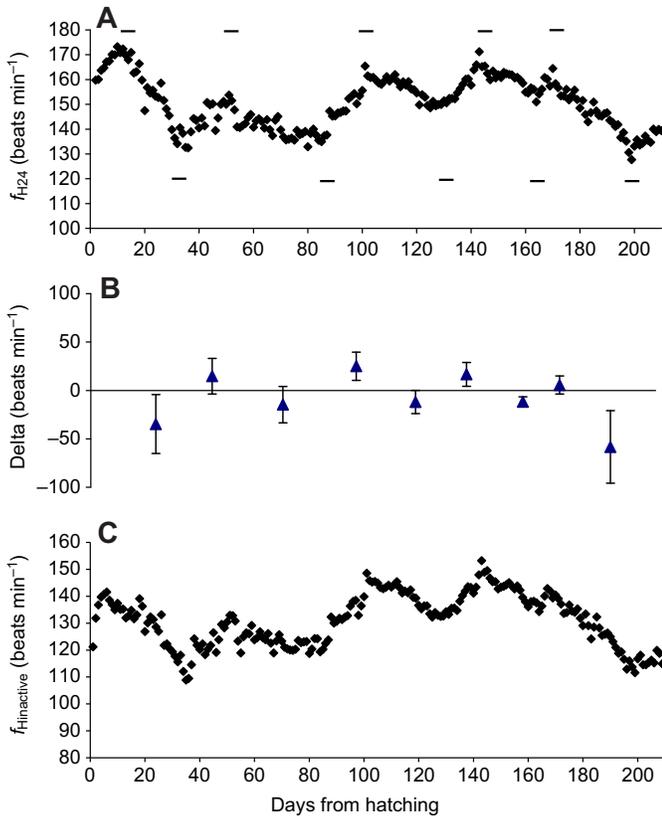


Fig. 1. (A) Mean values of daily heart rate (f_{H24}) during 208 days of recording for female common eider ducks ($N=13$) synchronised in relation to hatching of eggs. Lows and highs are identified by black bars covering 5 days of recording. (B) Average deltas, representing the difference between contiguous f_{H24} highs and lows, and their respective confidence intervals. When the confidence intervals exclude the zero line, the delta is declared significant at 5% level (see Materials and methods). (C) Mean values of inactive heart rate ($f_{Hinactive}$) during 208 days of recording.

significant (Table 1). In contrast, a negative and significant relationship emerged between $f_{Hinactive}$ and A_{loc} ($r=-0.150$).

f_{Hrest} and water temperature

f_{Hrest} is the minimum heart rate occurring over a 5 min interval during each day for each female (see Materials and methods) and is used here as a proxy for resting metabolic rate. f_{Hrest} was positively correlated to f_{H24} within each of the 13 experimental females ($r=0.342-0.875$; Table 1). Thus the amount of intra-individual variability in f_{H24} explained by f_{Hrest} ranged from 12 to 75% (Table 1), with an average of 47%. At the average level, the curves depicting f_{Hrest} (Fig. 3B) and f_{H24} (Fig. 1A) were also very similar ($r=0.783$, $P<0.0001$, $N=208$). This is an interesting result because the heart rate period used to predict f_{H24} was only 5 min.

Water temperature ranged from 6 to 22°C during the study period, with the coldest period occurring upon the return of the females on the water in spring, and at the onset of winter (Fig. 3A). To determine whether the return on the water incurred thermoregulation costs for breeding females, we compared f_{Hrest} when on the nest for the last 5 days of incubation with f_{Hrest} once on the water for a similar time interval. f_{Hrest} increased significantly from 74.2 ± 14.7 beats min^{-1} when on the nest to 90.8 ± 17.2 beats min^{-1} when on the water 5 days after departure, giving a mean difference of 16.6 beats min^{-1} (bootstrap 95% CIs= 7.6 and 25.7 beats min^{-1}). Because the

Table 2. Daily energy expenditure (DEE) of female common eiders at various times (lows and highs of Fig. 1A) after hatching their eggs estimated from daily heart rate (see Materials and methods), as well as time spent active and water temperature

Days from hatching	DEE (kJ kg^{-1})	Time spent active ^a (min)	Water temperature ^b ($^{\circ}\text{C}$)
10	971	117	7.8
35	922	262	13.4
50	1003	204	15.5
85	885	86	21.3
103	970	104	20.3
126	923	123	16.7
143	1028	135	16.4
162	1000	129	12.2
169	1022	146	11
199	903	131	7.8

^aPearson correlation between DEE and time spent active: 0.091 ($P>0.05$).

^bPearson correlation between DEE and water temperature: 0.145 ($P>0.05$).

confidence intervals of the average difference exclude zero, we conclude that f_{Hrest} increased significantly when females left the colony for the water.

Contrary to our expectation, neither f_{Hrest} nor DEE varied negatively with water temperature on a seasonal basis (Fig. 3A,B). In fact, there was no significant relationship between these variables and water temperature ($r=0.276$, $P>0.05$; Fig. 3C). This suggests that, at the proximate level, there is no effect of variation in water temperature on energy expenditure (Table 2).

DISCUSSION

We used the heart rate method to test two hypotheses about the seasonal variation of energy expenditure of a large diving bird, the ID hypothesis (Weathers and Sullivan, 1993) and the EBL hypothesis (Pelletier et al., 2008). The average level of f_{H24} and DEE of female common eiders varied widely during the annual cycle and we discuss to what extent this variability is related to locomotor activity, water temperature and productive costs.

Energy management strategies

Given the observed increases and decreases in the level of seasonal f_{H24} and DEE, our study supports the ID hypothesis. Two annual cycle curves published so far for diving birds (Green et al., 2009; White et al., 2011) support the ID hypothesis as the reproductive season is characterised by the highest level of energy expenditure when compared with the rest of the annual cycle. This is most probably related to the increased demands of producing tissues or the extra energy required to feed the chicks, or both. But even outside the reproductive season, these two studies show large seasonal variation in DEE, a situation similar to that in our study (Fig. 1). Some obvious factors that may explain these variations in seasonal DEE are water temperature, production costs (e.g. moult) and A_{loc} (see below).

However, increases in A_{loc} of common eiders did not increase f_{H24} . This is despite the fact that f_{Hfeed} and f_{Hfly} are 50 and 121%, respectively, higher than $f_{Hinactive}$ (Guillemette et al., 2012). There are two reasons that may explain such a result. The first one is related to the fact that the active period (flying + feeding) represents a small proportion of the day (5–22%), ranging from ~70 to 320 min, thus limiting any influence of the activity level on the 24 h energy budget. Another reason is related to compensation as predicted by the EBL hypothesis. There are four lines of evidence supporting this hypothesis in our study. First, average f_{H24} and average A_{loc} were

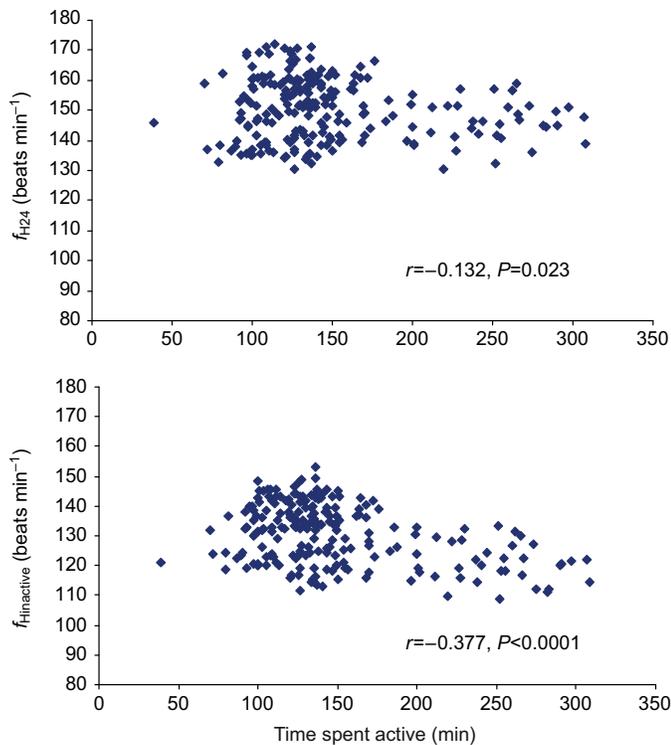


Fig. 2. (A) Mean daily heart rate (f_{H24}) in relation to mean time spent active for 13 female eiders. (B) Mean inactive heart rate ($f_{Hinactive}$) in relation to mean time spent active for 13 female eiders. The time budget is obtained from continuous recordings of flight and feeding (see Materials and methods) whereas inactive behaviour was obtained by subtracting active time (flying + feeding) from 1440 min (24 h). The significance level of both Pearson correlation coefficients were calculated with a permutation test (see Materials and methods).

not positively correlated, giving a very similar pattern of f_{H24} and $f_{Hinactive}$ in relation to time (Fig. 1A,C). Second, average $f_{Hinactive}$ was inversely and significantly related to average A_{loc} , suggesting that a high level of activity is compensated by a lower heart rate when inactive (Fig. 2B). Third, an analysis performed at the individual level gives similar results (where $f_{Hinactive}$ is inversely related to A_{loc} ; Table 1). Fourth, in a companion paper, Guillemette et al. (Guillemette et al., 2012) identified the high level of activity observed in the present study (Fig. 2) as migration hyperphagia.

In their paper, Guillemette et al. used a before–after and inter-individual approach, showing that when fuelling for migration (hyperphagia) these birds compensate for their high level of activity by decreasing time spent flying and $f_{Hinactive}$ (Guillemette et al., 2012). Similarly, studies with captive birds (Bautista et al., 1998; Deerenberg et al., 1998; Wiersma and Verhulst, 2005) and wild birds (Moreno et al., 1999; Welcker et al., 2010) testing the effect of increasing A_{loc} found no evidence of associated increases of DEE. One study (Deerenberg et al., 1998) found that increasing A_{loc} elicited physiological compensation (a decrease in resting metabolic rate during the night, whereas another study (Moreno et al., 1999) found that increasing A_{loc} had a negative effect on reproductive output.

How do we reconcile our interpretation that both the ID and EBL hypotheses are supported by our data? We suggest here that the two hypotheses are not mutually exclusive and propose that the energy management strategies of eider ducks are a step process. Because behavioural adjustments are energetically cheaper and faster than

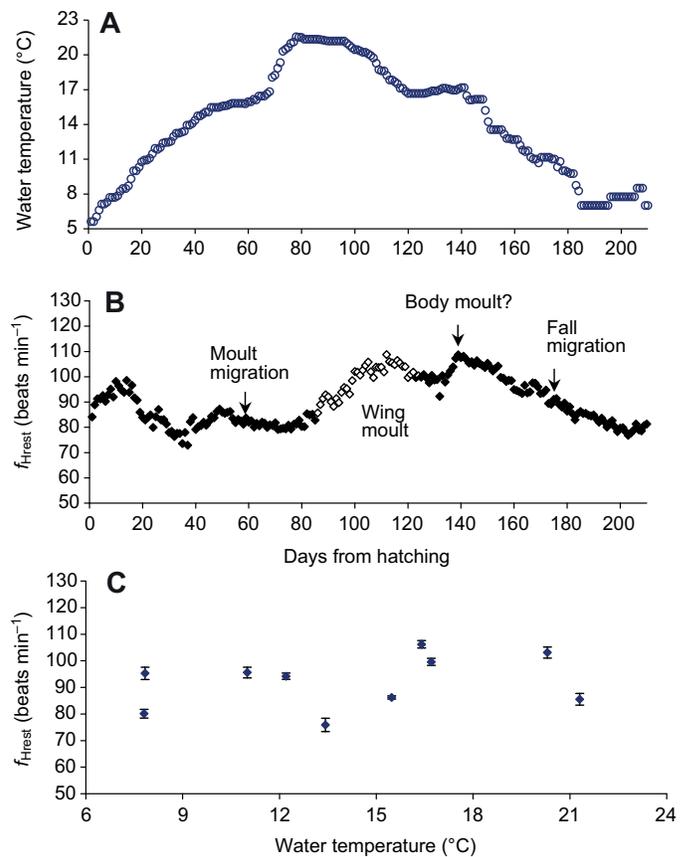


Fig. 3. (A) Mean water temperature of the locations of female common eiders ($N=13$) in the Baltic and Wadden Seas during the recording period. (B) Mean values of resting heart rate (f_{Hrest}) during 208 days of recording for female common eiders ($N=13$) synchronised in relation to hatching of eggs. Mean date (arrows) of moult and fall migrations are positioned along the season (days from hatching) together with the occurrence of wing moult (open symbols). (C) Relationship between mean resting heart rate ($N=13$) and mean water temperature shown in Table 2.

physiological adjustments, we might expect a change in behaviour to be the first response to an increase in metabolic demands (Vézina and Salvante, 2010). Visual observations of focal eiders during spring hyperphagia support this view, as the time spent feeding was inversely related to comfort, swimming and resting activities (Guillemette, 2001), which are grouped in the present study under ‘inactive’ behaviour. Therefore, the various components of the time and energy budget would be inherently related and the reduction of inactive heart rate would be driven by a shift from high-cost (comfort) to low-cost behaviour [resting; see supplementary information in Pelletier et al. (Pelletier et al., 2007)]. Then, physiological compensation, such as reductions in body temperature (Butler and Woakes, 2001; Wojciechowski and Pinshow, 2009), body mass and body organs (Biebach, 1998; Piersma, 1998; McWilliams and Karasov, 2001), would arise to decrease further energy expenditure. However, such compensation to reduce energy expenditure might not be sufficient to buffer completely the seasonal variation in DEE.

What are the determinants of seasonal f_H ?

f_{Hrest} should represent a proxy of maintenance cost in nature (Guillemette et al., 2007). We used this quantity to determine

whether water temperature could explain the observed seasonal variation of f_H . Although the passage from a terrestrial to an aquatic habitat indicates that cold water caused a significant increase in f_{Hrest} by 22%, we did not find any evidence at the proximate level that water temperature could have been a determinant of f_{Hrest} during the rest of year (Fig. 3C), when common eiders spend 95–100% of their time on the water (Pelletier et al., 2008). In contrast, at the ultimate level, variations in water temperature may have shaped the timing of moult in this population.

A fascinating aspect of our results is how well synchronised the wing moult was in relation to the warmest waters of the year (Fig. 3). Although this apparent synchronicity between water temperature and wing moult might be coincidental, it cannot be concluded that, on an evolutionary scale, that water temperature had no effect on f_{Hrest} . Indeed, it suggests that water temperature may have shaped this important phase of the annual cycle of female eiders by determining what would be the best timing to start wing and body moult in this population. Photoperiod is known to be the main trigger of moult in birds (Gwinner, 2003) and water temperature and photoperiod are highly correlated in the study population (M.G., unpublished). Therefore, we suggest that the timing of moult is such to minimise energy expenditure during feather growth and we hypothesise that individuals that initiate moult later when water temperature is decreasing will incur an extra cost in terms of thermoregulation (Jenssen et al., 1989).

Given that neither water temperature nor A_{loc} explain the seasonal variation in f_{H24} in the present study, the question is: which factors are causing that variation? We suggest that productive processes such as feather growth may explain such variation. Firstly, the highest level of f_{H24} and f_{Hrest} coincided with wing moult (Fig. 3). Moult is a costly process as it requires energy to produce the building material of feathers and the tissues responsible for their biosynthesis (Dietz et al., 1992; Brown and Bryant, 1996). The intensity of wing moult is high in synchronous species such as common eiders, meaning that individuals grow many feathers at any one time. Guillemette et al. (Guillemette et al., 2007) estimated the cost of wing moult in this species to be 12% of resting metabolic rate and 9% of DEE. We suggest that the f_{Hrest} peak arising after wing moult (143 days from hatching, Fig. 3B) is also caused by feather growth, as available evidence indicates that body moult occurs after wing moult in eider ducks (Guillemette et al., 2007). Secondly, f_{H24} decreases steadily from peak 4 (Fig. 1A) and we hypothesise that such a decrease is caused by a cessation of body moult and the occurrence of a better plumage insulation at the onset of winter. Evidence in support of this is provided by consideration of the lower critical temperature in air for this species, which is 7°C for breeding females compared with 0°C for winter-acclimatised birds (Gabrielsen et al., 1991; Jenssen et al., 1989). Thirdly, we speculate that the increase of f_{Hrest} (Fig. 3B) that occurred 10 days after hatching is also the result of feather growth. Indeed, incubating females are characterised by a paucity of down feathers on their abdomen when nesting as they use down feathers plucked from their abdomen to line their nest, which also improves the transfer of heat from the body to the eggs when they incubate. However, this lack of down feathers may tend to cause an increase in the cost of thermoregulation when the females go back to the water, and it is expected that females replace these down feathers shortly after the end of nesting. Finally, other changes in production costs may occur during the study period, e.g. those related to the decrease in f_H that occurred 35 days after hatching (Fig. 1). Although this feature occurred in all 13 females, there is not any logical explanation for it. Altogether, these data suggest that complex metabolic adjustments occur during post-

hatching and it is unclear which process would generate the high variation of f_{H24} observed during this period.

In conclusion, the energetic cost of life varied during the annual cycle of a diving bird, which supports the ID hypothesis. The present study suggests that the energetic costs of locomotion (flying and diving) are buffered by behavioural compensation, which supports the EBL hypothesis. We are thus unable to discriminate between these two hypotheses in the present study, most probably because they are not mutually exclusive. Nevertheless, our results indicate that behavioural compensation and possibly the timing of moult may be used as mechanisms to reduce seasonal variation in energy expenditure.

ACKNOWLEDGEMENTS

Thanks to Dr Annette Flagstad from Royal Veterinary School of Denmark for performing the surgeries on experimental birds, and Torben Anderson and Ina Jørgensen for lending us a very convenient surgery room. Thanks to Yves Rigou and Peter Lyngs for their indispensable help in the field. Many thanks to Jean-Marc Grandbois, who designed the computer software used in this study, and to David Pelletier, who performed the analysis on flight behaviour.

FUNDING

This study was performed in collaboration with the National Environmental Research Institute of Denmark and was funded through the Canadian Natural Sciences and Engineering Research Council (NSERC) discovery and equipment grants to M.G.

REFERENCES

- Achten, J. and Jeukendrup, A. E. (2003). Heart rate monitoring: applications and limitations. *Sports Med.* **33**, 517–538.
- Bacigalupe, L. D. and Bozinovic, F. (2002). Design, limitations and sustained metabolic rate: lessons from small mammals. *J. Exp. Biol.* **205**, 2963–2970.
- Bautista, L. M., Tinbergen, J., Wiersma, P. and Kacelnik, A. (1998). Optimal foraging and beyond: how starlings cope with changes in food availability. *Am. Nat.* **152**, 543–561.
- Biebach, H. (1998). Phenotypic organ flexibility in garden warblers *Sylvia borin* during long-distance migration. *J. Avian Biol.* **29**, 529–535.
- Bishop, C. M. (1997). Heart mass and the maximum cardiac output of birds and mammals: implications for estimating maximum aerobic power input of flying animals. *Philos. Trans. R. Soc. Lond. B* **352**, 447–456.
- Brosh, A. (2007). Heart rate measurements as an index of energy expenditure and energy balance in ruminants: a review. *J. Anim. Sci.* **85**, 1213–1227.
- Brown, C. R. and Bryant, D. M. (1996). Energy expenditure during molt in dippers (*Cinclus cinclus*): no evidence of elevated costs. *Physiol. Zool.* **69**, 1036–1056.
- Butler, P. J. and Woakes, A. J. (2001). Seasonal hyperthermia in a large migrating bird: saving energy for fat deposition? *J. Exp. Biol.* **204**, 1361–1367.
- Butler, P. J., Green, J. A., Boyd, I. L. and Speakman, J. R. (2004). Measuring metabolic rate in the field: the pros and cons of the doubly labelled water and heart rate methods. *Funct. Ecol.* **18**, 168–183.
- Clifford, P., Richardson, S. and Hémon, D. (1989). Assessing the significance of the correlation between two spatial processes. *Biometrics* **45**, 123–134.
- Coulson, J. C. (1984). The population dynamics of the eider duck *Somateria mollissima* and evidence of extensive non-breeding by adult ducks. *Ibis* **126**, 525–543.
- Daan, S., Masman, D. and Groenewold, A. (1990). Avian basal metabolic rates: their association with body composition and energy expenditure in nature. *Am. J. Physiol.* **259**, R333–R340.
- Dale, M. R. T. and Fortin, M. J. (2002). Spatial autocorrelation and statistical tests in ecology. *Ecoscience* **9**, 162–167.
- Deerenberg, C., Overkamp, G. J. F., Visser, G. H. and Daan, S. (1998). Compensation in resting metabolism for experimentally increased activity. *J. Comp. Physiol. B* **168**, 507–512.
- Dietz, M. W., Daan, S. and Masman, D. (1992). Energy requirements for molt in the kestrel *Falco tinnunculus*. *Physiol. Zool.* **65**, 1217–1235.
- Dutilleul, P. (1993). Spatial heterogeneity and the design of ecological field experiments. *Ecology* **74**, 1646–1658.
- Gabrielsen, G. W., Mehlum, F., Karlsen, H. E., Andersen, O. and Parker, H. (1991). Energy cost during incubation and thermoregulation in the female common eider *Somateria mollissima*. *Norsk. Polar. Skr.* **195**, 51–62.
- Green, J. A. (2011). The heart rate method for estimating metabolic rate: review and recommendations. *Comp. Biochem. Physiol.* **158A**, 287–304.
- Green, J. A., Boyd, I. L., Woakes, A. J., Warren, N. L. and Butler, P. J. (2009). Evaluating the prudence of parents: daily energy expenditure throughout the annual cycle of a free-ranging bird. *J. Avian Biol.* **40**, 529–538.
- Guillemette, M. (2001). Foraging before spring migration and before breeding in common eiders: does hyperphagia occur? *Condor* **103**, 633–638.
- Guillemette, M., Woakes, A. J., Flagstad, A. and Butler, P. J. (2002). Effects of data-loggers implanted for a full year in female Common Eiders. *Condor* **104**, 448–452.

- Guillemette, M., Woakes, A. J., Henaux, V., Grandbois, J.-M. and Butler, P. J.** (2004). The effect of depth on the diving behaviour of common eiders. *Can. J. Zool.* **82**, 1818-1826.
- Guillemette, M., Pelletier, D., Grandbois, J.-M. and Butler, P. J.** (2007). Flightlessness and the energetic cost of wing molt in a large sea duck. *Ecology* **88**, 2936-2945.
- Guillemette, M., Richman, S. E., Portugal, S. J. and Butler, P. J.** (2012). Behavioural compensation reduces energy expenditure during migration hyperphagia in a large bird. *Funct. Ecol.* **26**, 876-882.
- Gwinner, E.** (2003). Circannual rhythms in birds. *Curr. Opin. Neurobiol.* **13**, 770-778.
- Hawkins, P. A. J., Butler, P. J., Woakes, A. J. and Speakman, J. R.** (2000). Estimation of the rate of oxygen consumption of the common eider duck (*Somateria mollissima*), with some measurements of heart rate during voluntary dives. *J. Exp. Biol.* **203**, 2819-2832.
- Jenssen, B. M., Ekker, M. and Bech, C.** (1989). Thermoregulation in winter-acclimatized common eiders (*Somateria mollissima*) in air and water. *Can. J. Zool.* **67**, 669-673.
- Kirkwood, J. K.** (1983). A limit to metabolisable energy intake in mammals and birds. *Comp. Biochem. Physiol.* **75A**, 1-3.
- Lunneborg, C. E.** (2000). *Data Analysis by Resampling: Concepts and Applications*. Pacific Grove, CA: Duxbury Press.
- Lyngs, P.** (2000). Status of the Danish breeding population of eiders *Somateria mollissima*. *Dan. Ornithol. Foren. Tidsskr.* **94**, 12-18.
- McWilliams, S. R. and Karasov, W. H.** (2001). Phenotypic flexibility in digestive system structure and function in migratory birds and its ecological significance. *Comp. Biochem. Physiol.* **128A**, 577-591.
- Moreno, J., Merino, S., Potti, J., de León, A. and Rodríguez, R.** (1999). Maternal energy expenditure does not change with flight costs or food availability in the pied flycatcher (*Ficedula hypoleuca*): costs and benefits for nestlings. *Behav. Ecol. Sociobiol.* **46**, 244-251.
- Pelletier, D., Guillemette, M., Grandbois, J.-M. and Butler, P. J.** (2007). It is time to move: linking flight and foraging behaviour in a diving bird. *Biol. Lett.* **3**, 357-359.
- Pelletier, D., Guillemette, M., Grandbois, J.-M. and Butler, P. J.** (2008). To fly or not to fly: high flight costs in a large sea duck do not imply an expensive lifestyle. *Proc. R. Soc. B* **275**, 2117-2124.
- Peterson, C. C., Nagy, K. A. and Diamond, J. M.** (1990). Sustained metabolic scope. *Proc. Natl. Acad. Sci. USA* **87**, 2324-2328.
- Piersma, T.** (1998). Phenotypic flexibility during migration: optimization of organ size contingent on the risks and rewards of fuelling and flight? *J. Avian Biol.* **29**, 511-520.
- Portugal, S. J., Green, J. A. and Butler, P. J.** (2007). Annual changes in body mass and resting metabolism in captive barnacle geese (*Branta leucopsis*): the importance of wing moult. *J. Exp. Biol.* **210**, 1391-1397.
- Portugal, S. J., Green, J. A., Cassey, P., Frappell, P. B. and Butler, P. J.** (2009). Predicting the rate of oxygen consumption from heart rate in barnacle geese *Branta leucopsis*: effects of captivity and annual changes in body condition. *J. Exp. Biol.* **212**, 2941-2948.
- Schmidt-Nielsen, K.** (1997). *Animal Physiology: Adaptation and Environment*, 5th edn. New York: Cambridge University Press.
- Speakman, J. R. and Król, E.** (2011). Limits to sustained energy intake. XIII. Recent progress and future perspectives. *J. Exp. Biol.* **214**, 230-241.
- Vézina, F. and Salvante, K.** (2010). Behavioral and physiological flexibility are used by birds to manage energy and support investment in the early stages of reproduction. *Current Zool.* **56**, 767-792.
- Ward, S., Bishop, C. M., Woakes, A. J. and Butler, P. J.** (2002). Heart rate and the rate of oxygen consumption of flying and walking barnacle geese (*Branta leucopsis*) and bar-headed geese (*Anser indicus*). *J. Exp. Biol.* **205**, 3347-3356.
- Weathers, W. W. and Sullivan, K. A.** (1993). Seasonal allocation of time and energy in juncos. *Physiol. Zool.* **66**, 511-536.
- Weiner, J.** (1992). Physiological limits to sustainable energy budgets in birds and mammals: ecological implications. *Trends Ecol. Evol.* **7**, 384-388.
- Welcker, J., Moe, B., Bech, C., Fyhn, M., Schultner, J., Speakman, J. R. and Gabrielsen, G. W.** (2010). Evidence for an intrinsic energetic ceiling in free-ranging kittiwakes *Rissa tridactyla*. *J. Anim. Ecol.* **79**, 205-213.
- White, C. R., Grémillet, D., Green, J. A., Martin, G. R. and Butler, P. J.** (2011). Metabolic rate throughout the annual cycle reveals the demands of an Arctic existence in great cormorants. *Ecology* **92**, 475-486.
- Wiersma, P. and Verhulst, S.** (2005). Effects of intake rate on energy expenditure, somatic repair and reproduction of zebra finches. *J. Exp. Biol.* **208**, 4091-4098.
- Wojciechowski, M. S. and Pinshow, B.** (2009). Heterothermy in small, migrating passerine birds during stopover: use of hypothermia at rest accelerates fuel accumulation. *J. Exp. Biol.* **212**, 3068-3075.