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RESEARCH ARTICLE

An increase in minimum metabolic rate and not activity explains field metabolic rate changes in a breeding seabird

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SUMMARY

The field metabolic rate (FMR) of a free-ranging animal can be considered as the sum of its maintenance costs (minimum metabolic rate, MMR) and additional costs associated with thermoregulation, digestion, production and activity. However, the relationships between FMR and BMR and how they relate to behaviour and extrinsic influences is not clear. In seabirds, FMR has been shown to increase during the breeding season. This is presumed to be the result of an increase in foraging activity, stimulated by increased food demands from growing chicks, but few studies have investigated in detail the factors that underlie these increases. We studied free-ranging Australasian gannets (*Morus serrator*) throughout their 5 month breeding season, and evaluated FMR, MMR and activity-related metabolic costs on a daily basis using the heart rate method. In addition, we simultaneously recorded behaviour (flying and diving) in the same individuals. FMR increased steadily throughout the breeding season, increasing by 11% from the incubation period to the long chick-brooding period. However, this was not accompanied by either an increase in flying or diving behaviour, or an increase in the energetic costs of activity. Instead, the changes in FMR could be explained exclusively by a progressive increase in MMR. Seasonal changes in MMR could be due to a change in body composition or a decrease in body condition associated with changing the allocation of resources between provisioning adults and growing chicks. Our study highlights the importance of measuring physiological parameters continuously in free-ranging animals in order to understand fully the mechanisms underpinning seasonal changes in physiology and behaviour.

Key words: flight, gannet, energetics, heart rate method, basal metabolic rate, diving, seasonal change.

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INTRODUCTION

The breeding season of seabirds, like that of other birds, is the period of the annual cycle where field metabolic rate (FMR) reaches a maximum (Bryant, 1997; Green et al., 2009a). This might be expected, as the responsibilities of producing and incubating eggs and of brooding and provisioning young incur costs in addition to those required for the maintenance of the parent birds. However, the breeding season is not homogeneous and model predictions suggest that the energetic demands on seabird parents will change as eggs hatch and chicks grow larger (Ricklefs, 1983), and environmental conditions vary. The variation in energetic demands during the breeding season has relevance for understanding both prey consumption and ecological bottlenecks at the population level (Green et al., 2007) and implications for limits to metabolism (Welcker et al., 2010). However, despite the relevance of these changing pressures, very few studies have explored variation in metabolic rates and behaviour within a breeding season.

The most likely consequence of eggs hatching and chicks growing larger is that food requirements increase and parents must capture more food during foraging activities (Green et al., 2007). This could be achieved by spending more time foraging as the season progresses (Grémillet, 1997; Lescroël et al., 2010), which would lead to an increase in energy expenditure (Jodice et al., 2003; Mullers et al., 2009). Alternatively, if birds increase their activity-specific metabolic costs they may increase foraging success or efficiency without increasing the time spent foraging (Bevan et al., 1995; Shaffer et al., 2003). Finally, parents may maintain foraging effort, energy expenditure and prey intake and instead reduce the proportion of captured food that they retain for their own maintenance, at the expense of their own body condition (Takahashi et al., 2003).

Those studies that have measured FMR within the breeding season usually suggest that FMR increases from incubation to chick brooding and chick rearing (Bevan et al., 2002; Gales and Green, 1990; White et al., 2011). However, very few have also investigated behaviour simultaneously, in order to determine what underlies the patterns observed. An alternative approach has been to measure behavioural time budgets throughout the breeding season and combine these with estimates or measurements of energetic costs in order to predict changes in FMR (Grémillet et al., 1995). However, these studies have not accounted for the possibility that the energetic requirements of the parents themselves may vary through the breeding season, independent of the demands of the chicks. For example, previous studies have indicated that maintenance costs (minimum metabolic rate, MMR), which for a

free-ranging animal are the closest possible analogue of basal metabolic rate (BMR) (Guillemette et al., 2007), are not fixed and may vary during the annual cycle of birds (Guillemette and Butler, 2012; White et al., 2011). Such changes in MMR can have significant implications for energy budgets and hence behaviour (Green et al., 2005a).

In the present study we measured behaviour and metabolic rate simultaneously and continuously throughout the breeding season in Australasian gannets. By using the heart rate method (Butler et al., 2004; Green, 2011) we were able to estimate not only FMR but also MMR and the energetic cost of activity above maintenance (net metabolic rate, NMR). We also measured BMR in the laboratory, allowing us to investigate how quantities such as BMR, MMR, NMR and FMR vary between laboratory and field contexts. As with other gannets, Australasian gannets are unusual among seabirds in that the two parents continue to alternate in brooding/guarding and provisioning duties until the chick fledges, and the chick is never left unguarded. This allows us to account to some extent for the potentially confounding effect of behaviour, as previous studies suggest that the time budget of Australasian gannets does not change between incubation and chick rearing (Bunce, 2001). We set out to determine whether the FMR of Australasian gannets changes during the breeding season and, if it does, whether this change is due to variation in time budget, maintenance metabolism or the metabolic cost of activity.

MATERIALS AND METHODS

Study site

All birds used in the current study were mature adults from the breeding colonies at Wedge Light and Pope's Eye, in the Pope's Eye Marine Reserve (38°16′35″S, 144°41′54″E), located off Queenscliff near the entrance to Port Phillip Bay, Australia. We assumed that there were no sex differences in physiology in this monomorphic species and therefore sex was not considered as a factor in any part of this study. All experiments were carried out with the approval of the La Trobe University animal ethics committee (AEC 04/37L) and appropriate wildlife permits were issued by Parks Victoria.

Laboratory measurements

Nine gannets, *Morus serrator* Gray 1843, (mean mass ± s.e.m., 2.31±0.02 kg) were brought into the laboratory in January and February 2006. All birds had either failed in their breeding attempt that year or had already fledged a chick. Birds were collected in groups of two or three and transported in ventilated pet carriers by boat to Queenscliff and onwards to La Trobe University in an air-conditioned car (~2 h total journey time). Once at La Trobe, the birds were kept in an outside aviary (7.5×5 m) which had a sand base, perches, protection from rain and sun, and a rectangular pool (2×1.5 m) for bathing and drinking. Birds were kept captive for no more than 7 days, after which point they were returned to their colonies. During captivity, the birds were hand-fed 300 g of pilchards twice daily, supplemented with a daily multi-vitamin tablet. Birds were used once each in two sets of experiments: to examine metabolic changes associated with diel rhythms and exercise, and define a calibration relationship between heart rate (f_H) and rate of oxygen consumption ($\dot{V}_{O_2}$) (present study), and to examine heat stress and thermoregulation at high temperatures (J.A.G., E.J.A.-S. and P.B.F., unpublished data).

We measured $\dot{V}_{O_2}$ and the rate of carbon dioxide production ($\dot{V}_{CO_2}$) using an open-circuit respirometry system. Animals were placed in a clear Perspex respirometer (45×70×62 cm), which was located in a constant temperature (CT) room within which the ambient temperature could be controlled. Air was drawn from the respirometer using a compressor pump (Millipore, Billerica, MA, USA) at either 12 or 24 l min^{-1} and flow rate was monitored using an electronic mass-flow meter (Sierra Instruments Inc., Monterey, CA, USA). A subsample of air was drawn from this main flow using a small aquarium air pump (Rolf C. Hagen, Castleford, West Yorkshire, UK) and passed through a drying column (Drierite, Hammond, Xenia, OH, USA), then analysed for the fractional content of O_2 and CO_2 by a combined gas analyser (ADInstruments, Sydney, Australia). A solenoid valve (Bürkert, Ingelfingen, Germany) was used to switch between the respirometer and ambient air samples. Heart rate was monitored using a customised heart rate transmitter and a receiver unit (Polar Electro Oy, Kempele, Finland). The transmitter was attached dorsally to the feathers using adhesive tape (Tesa, Hamburg, Germany) and custom-made brass electrodes were inserted under the skin at the bottom of the neck and base of the spine. A thermocouple was inserted ~8 cm into the rectum of the birds to monitor body temperature (T_b). The heart rate receiver output, outputs from the gas analyser, flow meter and thermocouples (ambient temperature, T_a, was also recorded continuously) were collected at 100 Hz (ADInstruments) and displayed on a computer using Chart software (ADInstruments). $\dot{V}_{O_2}$ was determined from the rate of airflow from the respirometer and the difference in the fractional concentration of O_2 between ambient and outflowing air. Instantaneous corrections of the gas concentrations were calculated dry at standard temperature (273 K) and pressure (101.3 kPa) using a previously described method (Frappell et al., 1989), assuming a first-order linear system (chamber volume, 19 l; flow, 12 or 24 l min^{-1}; time constant τ, 12.8 or 7.8, determined from a semi-logarithmic plot of concentration against time following a perturbation; r^2=0.97, 0.99, respectively). $\dot{V}_{O_2}$ was calculated with consideration of respiratory quotient (RQ)-related errors (Frappell et al., 1992). Whole-system accuracy was determined to be within 6% by bleeding CO_2 into the respirometer at known flow-rate and back-calculating this rate.

Prior to experiments, food (not water) was withheld for 12 h, sufficient to ensure that the birds were post-absorptive (Laugksch and Duffy, 1986). Birds were equipped with the heart rate transmitter and thermocouple and introduced into the respirometer at ~11:00 h with the CT room set to 23°C. The birds were allowed to settle for ~1 h until $\dot{V}_{O_2}$ and f_H had stabilised. They were then used in the thermoregulation experiment for ~3 h (J.A.G., E.J.A.-S. and P.B.F., unpublished data). After being allowed to settle again for 1–2 h, they were then walked at a series of speeds from 0.1 to 1.4 km h^{-1}. Birds walked for 3–15 min at each speed and had frequent rest periods during which f_H declined to resting levels. When compared with other species where a similar approach has been used (e.g. Green et al., 2009b), the gannets were relatively reluctant to walk on the treadmill and not all birds would walk at the highest speeds. One bird would not walk at all, and data from this animal were not included in analyses. However, instantaneous corrections allowed $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ to be calculated over relatively short exercise periods of 3 min, which were interspersed with rest periods where f_H and $\dot{V}_{O_2}$ returned to pre-exercise levels. This entire 'activity' protocol therefore took less than 2 h in the late afternoon in each case. After exercise had finished, birds were allowed to rest in the respirometer for ~36 h to record values during 'inactivity'. During this time the birds were free to rest, preen, sleep or investigate the respirometer at will. The CT room was maintained at 22°C in a photoperiod of 14.5 h L:9.5 h D (lights on 05:00 h–19:30 h) to match conditions in the colony at this time of year. Throughout this period f_H and $\dot{V}_{O_2}$

were monitored continuously, with the solenoid automated to switch the gas analyser from excurrent to incurrent (baseline) measurements for 5 min every 2 h. Observations of the bird could be made without disturbance *via* a closed circuit camera system.

Laboratory data analysis

Data from the laboratory were initially processed using Chart software and further analysed using Excel (Microsoft, Redmond, WA, USA) and Minitab (Minitab Inc., State College, PA, USA). There was no relationship between body mass and resting $\dot{V}_{O_2}$ in our data set. As a result, mass corrections were not applied to any data in the present article and all data are presented as 'whole-animal' $\dot{V}_{O_2}$. Similarly, there was no effect of body mass on either the slope or the intercept of individual relationships between f_H and $\dot{V}_{O_2}$. We assume therefore that body mass does not have any influence on the relationship between f_H and $\dot{V}_{O_2}$ (Halsey et al., 2007). Should larger changes in body mass occur during the annual cycle, outside the range measured during calibration, leading to changes in resting $\dot{V}_{O_2}$, we assume that these would be accompanied by other physiological adjustments, causing appropriate changes in f_H in line with the relationship between f_H and $\dot{V}_{O_2}$. This approach is in line with similar long-term energetic studies of seabirds (Guillemette and Butler, 2012) and is consistent with studies examining year-round changes in the relationship between f_H and $\dot{V}_{O_2}$ in species with highly variable body mass (Green et al., 2005b; Portugal et al., 2009). A full discussion of this issue is presented in a recent review (Green, 2011).

Mean values of f_H, $\dot{V}_{O_2}$ and T_b were calculated every 3 min throughout the experiments. From these values we calculated grand means for both light and dark values for the 24 h period commencing at midnight at the end of the first day. To further examine the diel rhythm, mean values of each quantity were also calculated for each of these 24 h. Resting f_H and $\dot{V}_{O_2}$ were defined as the lowest of these hourly values for each animal, and resting $\dot{V}_{O_2}$ was assumed to be equivalent to BMR.

When constructing a calibration relationship between f_H and $\dot{V}_{O_2}$, it is essential that, where possible, the collection and analysis of data are used to create a relationship that is analogous to conditions encountered by free-ranging experimental animals (Green, 2011). In the present study, while the birds were free to move in the respirometer during the inactivity period, their large size meant that movement was constrained when compared with other studies that have used these data alone to generate calibration data (e.g. Steiger et al., 2009). As a result, the amount of data recorded while the animals were inactive constituted 97% of the calibration data recorded, which is clearly unrepresentative of the behaviour of free-ranging Australasian gannets, which spend over 40% of their time engaged in foraging activity (Bunce, 2001). To account for this, we could have selected data from the inactive set to combine with data while the birds were active on the treadmill. However, rather than discard valuable data, we instead combined the active and inactive data sets and calculated mean f_H and $\dot{V}_{O_2}$ in heart rate bins of 10 beats min^{-1} for each bird (Storch et al., 1999). Relationships between f_H and $\dot{V}_{O_2}$ were constructed using general linear model (GLM) with f_H as a covariate and gannet identity as a random factor. From initial visual inspection it was clear that the data would not be best described by a linear relationship; thus, $\dot{V}_{O_2}$ data were $\log_e$-transformed. Within each bird, some bins had more data points than others (range 1–310) so this variability was accounted for by weighting data in the GLM by the square root of this value. This entire approach allowed us to adopt a 'one-model' method so that the behaviour of animals in the field would not need to be known (Green et al., 2009b; White et al., 2011). This was particularly important in the present study for two reasons. Firstly, it meant that it was not necessary to define an unambiguous continuous time-budget for the gannets as this has previously been shown to be impossible with this data set (see Green et al., 2009c). Secondly, it allowed us to account for the fact that calibration data could not be obtained from flying or swimming birds. We assumed the curvilinear relationship generated by $\log_e$-transforming the $\dot{V}_{O_2}$ data was analogous to the relationships previously obtained from walking and swimming great cormorants (*Phalacrocorax carbo*) (White et al., 2011) and walking and flying geese (Ward et al., 2002). To account for the possibility that flying gannets had a drastically different relationship between f_H and $\dot{V}_{O_2}$ we also conducted a sensitivity analysis (see Appendix). This allowed us to look at how different trajectories of the relationship that could conceivably occur during flight might affect our findings.

Field measurements

As described in more detail previously (Green et al., 2009c), six mature breeding adult gannets (mean mass, 2.58±0.05 kg) were surgically implanted with a custom-built data logger (DL), which recorded f_H, depth and abdominal temperature (T_{ab}; not analysed in the present study). The gannets were selected randomly in September of the 2004–2005 breeding period while incubating their eggs, and all six apparently bred normally (Green et al., 2009c), suggesting that, as in previous studies, the birds were not negatively affected by the presence of the DL (White et al., 2013). We have previously used this data set to describe activity-specific f_H and diving behaviour and physiology of this species. In the current study we focused on larger scale changes in behaviour and energy expenditure. Five of the six data loggers had recordings of f_H for the full 5 month breeding season (mid-September to mid-February), whereas one unit only recorded until mid-October. Regular observations of the colony allowed us to establish laying dates, hatching dates and fledging dates for each breeding attempt by each study animal. Each day of the deployment was therefore categorized as being in one of the following phases: pre-breeding, incubation, chick rearing, winter or failed.

Field data analysis

Periods where the gannets were in flight were identified from data on heart rate for all six gannets, following the procedure outlined previously (Green et al., 2009c). In brief, 5 min running means of f_H were calculated for each second of each day. Flight was considered to have occurred when this 5 min mean f_H was greater than a 'flight threshold' value for at least 20 s. Flight f_H was then calculated as the mean f_H during the >20 s period when the running mean was greater than the flight threshold. To select the flight threshold value, a range of threshold f_H values between 160 and 360 beats min^{-1} was tested for each individual bird. We then plotted daily flight time as a function of threshold f_H. Daily flight time decreased with increasing threshold, but in each case there was a point of inflection where this decrease decelerated, indicating that the appropriate threshold (where f_H rapidly increased as a result of flight) had been identified. For example, for bird 112, below 240 beats min^{-1}, a 20 beats min^{-1} change in threshold f_H resulted in a 2 h change in total daily flight time, whereas the same change in threshold above 240 beats min^{-1} resulted in a 0.5 h change in total daily flight time. The flight threshold was either 220 beats min^{-1} (birds 110, 135, 535) or 240 beats min^{-1} (birds 112, 244, 292). The time spent in flight was calculated for each gannet for each day. The mean of these individual values gave the daily time in flight

(DTF) for each day of the breeding season. Diving data were available from four individuals (birds 112, 135, 292, 535) as previously described (Green et al., 2009c). In the current study we calculated the total time submerged and the number of dives for each gannet for each day. The mean of these individual values gave the daily time submerged (DTS) and daily number of dives (DND) for each day of the breeding season.

To estimate metabolic rate, mean f_H was calculated every 3 min, to match the recording interval in the laboratory calibration procedure, for each animal in the field. Estimates of $\dot{V}_{O_2}$ for these 3 min periods were then made using the calibration relationship. The standard error of the estimate (s.e.e.) for these estimates was calculated using a procedure outlined previously (Green et al., 2001), which accounts for all of the error inherent in the calibration process. For some analyses, we converted $\dot{V}_{O_2}$ to metabolic rate using a factor of 18.7 kJ l^{-1} O_2, assuming that the gannets had a diet composed predominantly of fish of the same nutritional composition as sardines (Frankel and Smith, 1998; Green et al., 2006). The timing of sunrise and sunset at Pope's Eye was calculated using data from Geoscience Australia (http://www.ga.gov.au/geodesy/astro/sunrise.jsp). Mean f_H and hence estimated $\dot{V}_{O_2}$ and FMR were calculated for each day of the summer breeding period, the entire summer breeding period and each phase of the breeding season. In the case of the different phases there were only sufficient data to evaluate the two main phases, incubation and chick rearing. Estimates of $\dot{V}_{O_2}$ or metabolic rate cannot be treated as parametric data as this would ignore the residual error associated with the calibration process (Green, 2011). Thus estimates of $\dot{V}_{O_2}$ or metabolic rate (±s.e.e.) were compared using the proximate normal test, paired where appropriate.

As well as calculating FMR for each day, we also calculated MMR for each day of the breeding season (see Guillemette and Butler, 2012; Guillemette et al., 2007). To do this we derived a 15 min running mean of f_H and used the lowest value of this quantity each day to estimate $\dot{V}_{O_2}$ and metabolic rate. NMR (total metabolic costs above resting) was calculated by subtracting MMR from FMR for each day (e.g. Guillemette et al., 2007; Ricklefs et al., 1996; White et al., 2011). In doing so we assumed that MMR and NMR are additive components of FMR, because of strong support for a positive Pearson's correlation in our data set between MMR and FMR (P<0.001) and the lack of support for a correlation between MMR and NMR (P=0.06). This agrees with findings from the great cormorant (White et al., 2011) and supports Ricklefs' 'partitioned pathways' model of energy metabolism (Ricklefs et al., 1996).

Changes in metabolic measurements (FMR, MMR and NMR) and behavioural measurements (DTF, DTS and DND) over time during the breeding season were checked for normality and analysed using regression analysis, weighted by the inverse square root of s.e.e. or s.e.m., where days since 1 September 2004 was the independent variable and measurement (FMR, MMR, NMR, DTF, DTS and DND) was the dependent variable. Relationships between date and FMR and MMR were compared using GLM with measurement (FMR or MMR) as a fixed factor and days since 1 September 2004 as a covariate. For all these analyses, mean FMR, MMR, NMR, DTF, DTS and DND was calculated for 14 day intervals to control for the effects of serial autocorrelation in time-series data. The value of 14 days was determined by correlating residual values for successive days until the correlation was no longer significant, which occurred at an interval of 14 days. In all statistical testing we followed the advice of Sterne and Smith (Sterne and Smith, 2001) in the interpretation of P-values.

Table 1. Heart rate and rate of oxygen consumption of Australasian gannets when inactive or active in the laboratory

	Inactive			Active		
Bird	f_H	$\dot{V}_{O_2}$	n	f_H	$\dot{V}_{O_2}$	n
1	111±1.4	26.8±0.51	273	236±9.4	92.3±5.39	10
2	94±0.8	24.5±0.35	670	163±8.2	80.4±12.74	5
3	91±1.2	25.9±0.44	697	136±5.3	89.1±5.36	23
4	70±0.6	36.0±0.78	546	207±7.9	100.9±6.27	25
5	89±0.8	17.2±0.26	503	206±36.3	74.0±5.02	3
6	85±0.6	17.0±0.19	697	180±11.5	79.5±3.22	13
7	79±0.5	15.2±0.19	662	137±4.7	62.2±4.07	27
8	102±0.8	19.2±0.25	576	182±5.4	49.6±4.00	33
Group mean	90±5	22.7±2.46		181±12	78.5±5.86	

Mean (±s.e.m.) heart rate (f_H) and rate of oxygen consumption ($\dot{V}_{O_2}$) of N=8 gannets in the laboratory during inactive (resting for 36 h) or active periods (walking on a treadmill at different speeds). Values of n refer to the number of 3 min periods spent inactive or active.

RESULTS

Relationship between f_H and $\dot{V}_{O_2}$

f_H and $\dot{V}_{O_2}$ varied among individuals and between active and inactive periods (Table 1). Birds only walked at moderate speeds (mean ± s.e.m., 0.5±0.05 km h^{-1}) but f_H was still 2 times greater and $\dot{V}_{O_2}$ 3.5 times greater during activity than while the birds were inactive (Table 1). The calibration relationship between f_H and $\dot{V}_{O_2}$ was best described by an exponential function (Fig. 1). There was strong support for both f_H ($F_{1,123}$=607.5, P<0.001) and gannet identity ($F_{7,123}$=8.74, P<0.001) having a significant effect on $\dot{V}_{O_2}$, and the effect of gannet ID was taken into account when calculating s.e.e. (Green et al., 2001). The range of f_H in the calibration relationship (58–275 beats min^{-1}) encompassed 85% of the distribution of f_H measured in the free-ranging birds (Fig. 1B). We conducted a sensitivity analysis to explore the effects of extrapolation of the calibration relationship beyond this range (see Appendix). The analysis suggested that even if this extrapolation was not valid, none of the results or conclusions from the present paper would be changed (see Appendix).

Diel pattern and minimum values

There was strong evidence that in the laboratory, f_H, $\dot{V}_{O_2}$ and T_b were all greater during the day than during the night (Fig. 2, Table 2; paired t-tests: $t_{7,7,5}$=5.2, 6.2 and 5.5, P<0.001). Hourly values of f_H and $\dot{V}_{O_2}$ for the diel cycle were calculated for the free-ranging animals during the period coinciding with the same time of year as laboratory calibrations (late January to early February). In the free-ranging birds, a more exaggerated pattern of variation was observed (Fig. 2). Again, there was strong evidence that both f_H (paired t-test: t_4=5.1, P<0.01) and $\dot{V}_{O_2}$ (proximate normal test: Z=17.9, P<0.001) were greater during the day than during the night (Table 2). The f_H and hence $\dot{V}_{O_2}$ of free-ranging birds was high around sunrise but highest around sunset.

For most individuals in the laboratory, resting f_H and $\dot{V}_{O_2}$ (minimum hourly value) were recorded during the night. Mean resting f_H was 69±3 beats min^{-1}. Mean resting $\dot{V}_{O_2}$ was 12.1±1.4 ml min^{-1}, equivalent to a basal metabolic rate of 3.8±0.4 W.

Field data

FMR for the entire study period was 25.5±2.7 W. If the phases are considered separately, then FMR during chick rearing (25.8±2.9 W) was 11% higher than FMR during incubation (23.2±2.6 W), a result for which there was strong statistical support (proximate normal

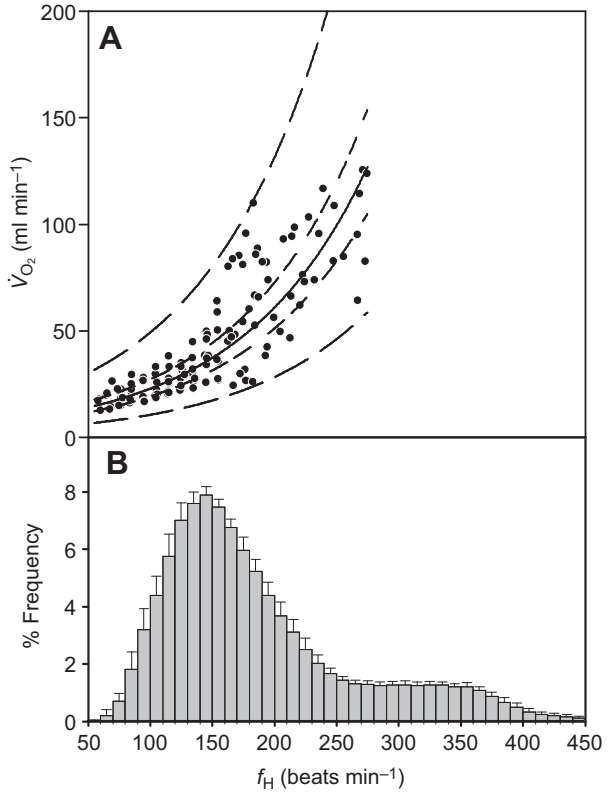

Fig. 1. (A) Calibration relationship between heart rate (f_H) and rate of oxygen consumption ($\dot{V}_{O_2}$) in eight Australasian gannets. (B) Distribution of mean f_H (calculated every 3 min) measured in six free-ranging Australasian gannets. The range of f_H used in the calibration procedure encompasses 85% of f_H observed in the free-ranging gannets. Data were obtained while birds rested and walked on a treadmill. In A, each data point represents the mean values of f_H and $\dot{V}_{O_2}$ calculated for bins of 10 beats min^{-1} for each bird. The solid line shows the regression line through these data points, weighted by the square root of the number of points used to calculate each mean ($\dot{V}_{O_2}$=8.58e$^{0.0098 f_H}$, r^2=0.89, P<0.001). Short-dash lines are 95% prediction intervals; long-dash lines are 95% confidence intervals. Parameters required to calculate the confidence intervals are as follows: number of individuals (N)=8, number of data points=131, standard error of slope estimate (s.e.e. slope)=0.000334, mean f_H during calibration=146.1, variance component for individuals=0.02957, variance component for error=0.11287.

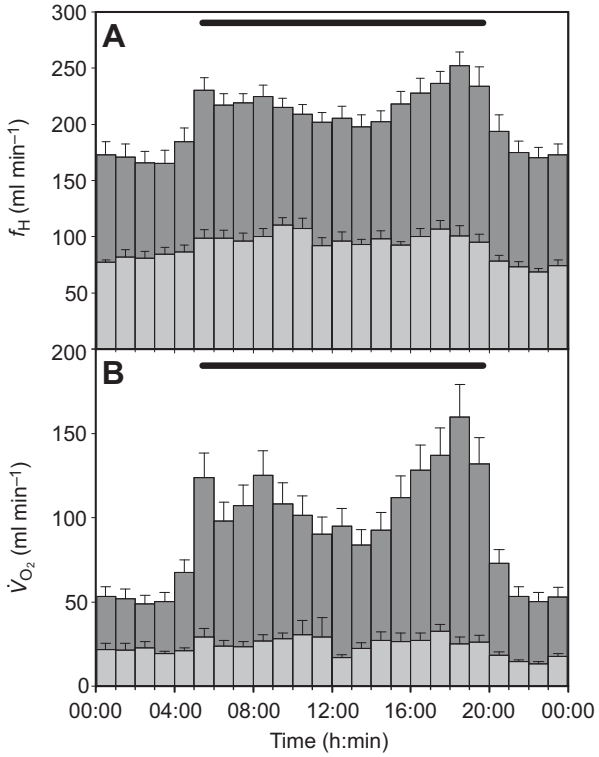

Fig. 2. Diel variation in mean (+s.e.m./s.e.e.) f_H (A) and $\dot{V}_{O_2}$ (B) of Australasian gannets while in the laboratory (light grey bars, N=8) or free-ranging in the field (dark grey bars, N=5) from 11 January to 7 February. Field estimates of $\dot{V}_{O_2}$ (+s.e.e.) were made using a calibration relationship between f_H and $\dot{V}_{O_2}$ (Fig. 1). The horizontal black bar represents the mean duration of daylight for the study period.

test: Z=3.82, P<0.001). Fig. 3 shows how FMR, NMR and MMR varied during the breeding season. A steady increase in FMR throughout the breeding season appears to be driven primarily by a clear increase in MMR, with additional variability in FMR due to inconsistent variability in NMR. These observations are supported by weighted regression analyses when the data were calculated for 2 week intervals (Table 3). There was very strong support for a significant increase in MMR over time and little support for a change in NMR. The combination of these two patterns underpins the strong support for a significant increase in FMR as the breeding season progressed (Table 3). When the relationships of FMR and MMR against time were compared, there was no evidence for a difference in slope ($F_{1,17}$=2.2, P=0.15), suggesting that NMR was a constant additive factor and that the increase in FMR was due to the increase in MMR.

There was considerable day-to-day variability in the amount of time that the gannets spent in flight and the number of dives performed (Fig. 4). This variability is likely to drive the day-to-day variability in NMR (Fig. 3B). The gannets spent a mean of 4.7±0.5 h in flight per day for the duration of the breeding season, performed a mean of 25±8 dives per day and spent a mean of 82±24 s per day submerged during diving (Fig. 4). Weighted regression analysis did

Table 2. Day/night variation in f_H, $\dot{V}_{O_2}$ and T_b of Australasian gannets resting in the laboratory or free-ranging in the field

	Laboratory			Free-ranging			Free-ranging/laboratory	
	Night	Day	% Increase	Night	Day	% Increase	Night	Day
f_H (beats min^{-1})	81±5	102±6	28±5	179±11	219±8	24±6	2.2	2.1
$\dot{V}_{O_2}$ (ml min^{-1})	18.9±1.9	27.1±3.3	42±7	60.5±6.6	112.6±13.1	97±27	3.2	4.1
T_b (°C)	37.2±0.1	38.4±0.3	3.4±0.5					

T_b, body temperature.
Mean (±s.e.m.) values of N=8 gannets in the laboratory or N=5 gannets in the field in late January and early February. Field estimates of $\dot{V}_{O_2}$ (±standard error of the estimate, s.e.e.) were made using a calibration relationship between f_H and $\dot{V}_{O_2}$ (Fig. 1).

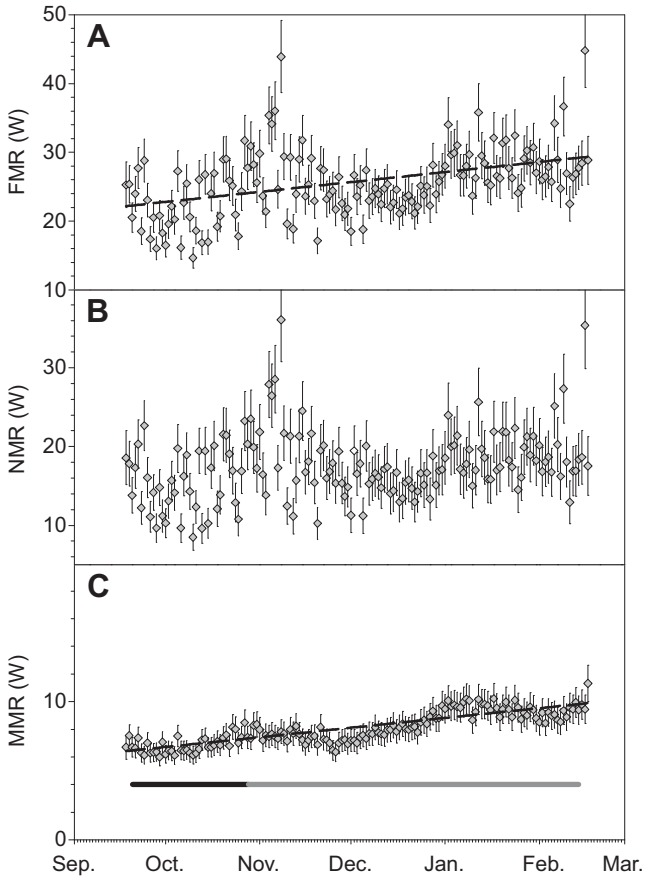

Fig. 3. Estimated mean (±s.e.e.) daily field metabolic rate (FMR, A), net metabolic rate (NMR, B) and minimum metabolic rate (MMR, C) of free-ranging Australasian gannets (*N*=6) for each day of the 2004–2005 breeding season. Dashed lines show significant linear regression relationships between date and metabolic rate. The timing and duration of the incubation and chick-rearing phases of the study animals are indicated by the horizontal black and grey bars, respectively, in C.

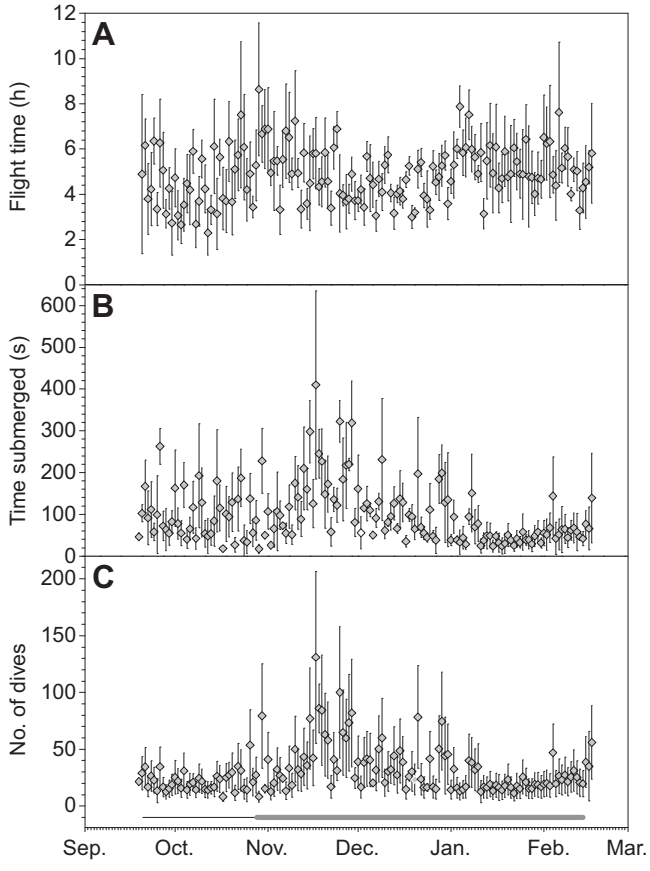

Fig. 4. Mean (±s.e.m.) time spent in flight (A), time spent submerged (B) and number of dives (C) by free-ranging Australasian gannets (*N*=4) for each day of the 2004–2005 breeding season. The timing and duration of the incubation and chick-rearing phases of the study animals are indicated by the horizontal black and grey bars, respectively, in C.

not provide any support for consistent changes in these behaviours as the breeding season progressed (Table 3).

DISCUSSION

Close examination of the data in the present study revealed day-to-day variability in both FMR and time allocation to activities such as flying and diving (Figs 3, 4). Unfortunately, with the data available in the present study, we could not resolve individual foraging trips and assign a complete time budget of activity for our birds (Green et al., 2009c). In future studies it would be interesting to look in detail at what governs day-to-day activity and energy expenditure in seabirds. Despite this, it is clear that the FMR of Australasian gannets increased during the breeding season. This increase was due solely to an increase in MMR, as overall there was no change in either the amount or the energetic cost of foraging activity (NMR). Our findings illustrate the diversity of energetic strategies used by seabirds during the breeding season and once again highlight the importance of making physiological measurements in wild and free-ranging animals.

Table 3. Statistical parameters for weighted regression relationships

Variable	*F*	*P*	r^2
FMR	10.1	0.01	0.53
NMR	3.29	0.10	0.27
MMR	56.0	<0.001	0.86
DTF	1.3	0.29	0.12
DTS	1.8	0.22	0.16
DND	0.0	0.96	0.00

Relationships were calculated between time since 1 September and variables relating to Australasian gannet energetics (FMR, field metabolic rate; NMR, net metabolic rate; MMR, minimum metabolic rate) and behaviour (DTF, daily time in flight; DTS, daily time submerged; DND, daily number of dives).

Breeding season energetics

A small number of studies have measured FMR of seabirds, at different stages of their breeding season (e.g. Chappell et al., 1993; Gales and Green, 1990; Moreno and Sanz, 1996). These studies have tended to demonstrate an increase in FMR, associated with an assumed or demonstrated increase in the proportion of time spent foraging (e.g. Bech et al., 2002). A key transition occurs in many species when chicks are left unattended and parents devote more time to foraging (Bevan et al., 2002; Bevan et al., 1995). These changes in time budget can be further magnified by variation in activity-specific energetic costs between phases of the breeding season. For example, the metabolic rate of black-browed albatrosses

(*Thalassarche melanophrys*) foraging at sea increased by 27% from chick brooding to chick rearing (Bevan et al., 1995). Similarly, the metabolic rate during foraging of wandering albatrosses (*Diomedea exulans*) increased by 10% from incubation to chick brooding, which was thought to be a response to maximise the rate of food delivery to chicks (Shaffer et al., 2003).

Other studies have found that the energy expenditure of seabirds is essentially constant between chick-brooding and chick-rearing periods (Green et al., 2007). In the case of female macaroni penguins (*Eudyptes chrysolophus*), this was despite an increase in the proportion of time spent foraging from chick brooding to chick rearing, suggesting a decrease in metabolic rate during foraging to compensate for the previously demonstrated increase in metabolic rate while on shore (Green et al., 2002). For macaroni penguins, it is suggested that FMR remained constant between breeding season phases at a common optimal level (Green et al., 2009a). Indeed, it is often stated that FMR during the breeding season represents both a maximum and an 'optimal working capacity', and that increased energy expenditure above this 'ceiling' might have a detrimental effect on lifetime reproductive success (Drent and Daan, 1980). The present study and other studies that have demonstrated that FMR during the breeding season may not have one single value, and tends to increase as breeding seasons progress, reinforce the need to refine and improve our thinking on what we mean by concepts such as this and 'maximum sustainable rates'.

Our study showed that FMR can increase during the breeding season without an increase in either activity levels (foraging effort in terms of time spent flying and diving) or the energetic cost of activity. Indeed, our findings suggest that increases in metabolic rate during foraging trips between breeding season phases (see above) may not be due to an increase in effort, but rather are due to an increase in MMR as a component of FMR. Further evidence to support this comes from increases in metabolic rates when 'inactive' at the nest as the breeding season progresses. Such increases have been shown from incubation to chick brooding in black-browed albatrosses (Bevan et al., 1995), from chick brooding to chick rearing in macaroni penguins (Green et al., 2002) and from incubation/brooding to chick rearing in gentoo penguins (*Pygoscelis papua*) (Bevan et al., 2002). In free-ranging eider (*Somateria mollissima*), energetic costs associated both with wing moult and during winter were also associated with changes in MMR while birds are inactive on water, rather than changes in activity (Guillemette and Butler, 2012; Guillemette et al., 2007). All of these findings call into question the combination of energetic costs from one stage of the season with a varying time budget in order to estimate energetic costs throughout the breeding season (Grémillet et al., 1995), or even throughout the year (Gales et al., 1993; Shaffer, 2004). The present study (and indeed some of the other studies mentioned) suggests that this approach may be inappropriate as FMR can vary independently of behaviour, due to changes in MMR.

While we have shown that increases in FMR in Australasian gannets are not due to an increase in foraging effort, they must still meet the demands of growing chicks as the breeding season progresses and the increase in MMR must be fuelled from energy intake or reserves. In macaroni penguins where FMR remained constant, changing demands from chicks were met by changes in the allocation of captured food to chicks, with the consequence that parent body mass decreased (Green et al., 2007). This buffering of chick demands with adult body condition has been shown previously in seabird species (Takahashi et al., 2003). For example, individual Adélie penguins (*Pygoscelis adeliae*), which accumulate, then use, large body reserves, have high breeding success (Ballard et al., 2010). We did not explicitly measure body mass changes in the present study and there is no information on body mass changes in Australasian gannets during the breeding season. However, in the present study, the body mass of the birds used in the laboratory measurements in January and February 2006 was 10.5% less than that of the birds implanted with data loggers in the field in September 2004, a difference with strong statistical support (*t*-test: t_{13}=5.73, P<0.001). Furthermore, in the closely related Cape gannet (*Morus capensis*), the body mass of birds sampled during chick brooding was 8.5% lower than that of birds sampled 1 month earlier during incubation (Adams et al., 1991). Previous studies of Australasian gannets at Pope's Eye suggest that when food availability is limited, older and more experienced breeders may be better able to allocate resources to maintain parental care, suggesting that judicious allocation of resources is important in maintaining breeding success (Bunce et al., 2005). A decrease in body mass might be expected to lead to a decrease in the metabolic cost of flight, whereas our data suggest no change in the metabolic cost of activity. However, this saving could be compensated for by, for example, an increase in food loads for growing chicks. As mentioned above, we could not reliably assign metabolic costs to specific activities in the present study, but this is a priority for our future work in this field.

Seasonal change in MMR

In Australasian gannets, FMR increased during the 5 month breeding season as the result of a progressive increase in MMR. We considered whether the increase in MMR could be an artefact of decreasing cardio-vascular organ mass, leading to a decrease in oxygen pulse and hence changes in the relationship between f_H and $\dot{V}_{O_2}$. However, if this was the case, it would also affect the animals during activity, and hence our estimate of NMR would necessarily also have to be higher. Given that neither activity levels nor NMR changed, this seems not to be the case. We can be confident then in our estimate that MMR was 25% higher during chick rearing than during incubation. Many recent studies have sought to investigate variation in BMR, MMR and resting metabolic rate (e.g. McKechnie, 2008; McNab, 2009; White et al., 2007). Many of these studies have presumed that these quantities or traits have single, unchanging values that are constant within an individual, assuming standardised measurement conditions have been met (McNab, 1997). However, a growing body of work is demonstrating that, among birds at least, there can be considerable variability and flexibility in these rates (McKechnie, 2008; Swanson, 2010). For example, BMR is elevated in winter compared with that in summer in many small temperate species (Swanson and Olmstead, 1999), but may be elevated in summer compared with winter in subtropical desert birds (Smit and McKechnie, 2010). In eider, MMR showed considerable variation in the 7 months following hatching, possibly as a strategy to compensate for higher locomotion activity (Guillemette and Butler, 2012). The only study to estimate MMR continuously for an entire annual cycle showed considerable variability in the MMR of a seabird, the great cormorant (White et al., 2011). In this species, MMR also tended to increase during the breeding season and was higher in winter than in summer. As in the present study, changes in MMR were closely associated with changes in FMR (White et al., 2011). The present study shows that changes in MMR are important for understanding what governs seasonal changes in FMR, which may in turn be important for understanding seasonal variation in prey requirements (Bunce, 2001). A previous study of macaroni penguins showed that an

increase in diving capacity could be explained by a seasonal reduction in MMR during winter (Green et al., 2005a).

So what causes the progressive increase in MMR during the breeding season of Australasian gannets? While we did not set out to determine the mechanism in this study, a number of factors can be considered. In macaroni penguins, improvements in insulation and increases in water temperature were suggested to be responsible for the decrease in MMR (Green et al., 2005a). In Australasian gannets, a progressive reduction in the insulating properties of feathers during the breeding season as feathers become increasingly worn and damaged could lead to an increase in MMR, as could a progressive decrease in insulation from subcutaneous fat associated with body mass reduction (see above). However, in the warm summer conditions encountered at the breeding colony where gannets rarely approach their lower critical temperature (J.A.G., E.J.A.-S. and P.B.F., unpublished data), this mechanism seems unlikely. Black-legged kittiwakes (*Rissa tridactyla*) also showed changes in both BMR and FMR during their breeding season, though in this case while FMR increased from incubation to chick rearing, BMR decreased (Bech et al., 2002). The decrease in BMR was thought to be associated with a disproportionate decrease in the size of organs with high intrinsic metabolic rates such as liver and kidney, possibly as an adaptive strategy to reduce energetic costs, or in response to a requirement for more undigested food to be transferred to chicks. In Australasian gannets, MMR increased during the breeding season, so this exact mechanism cannot be invoked; however, changes in the mass and/or activity of internal organs could underlie changes in MMR. A decline in body condition could be associated with an increase in stress, indicated by the hormone corticosterone, as has been shown in other seabirds (Kitaysky et al., 1999), including closely related boobies (Lormée et al., 2003). Increased corticosterone has been associated with an increase in metabolic rate in some species (Palokangas and Hissa, 1971), but not all (Buttemer et al., 1991), though there has been little work to define the presence or absence of an association.

Finally, as well as demonstrating the fact that quantities such as FMR and MMR are not fixed traits and can vary seasonally, presumably with the 'state' of the animal, our data show the importance of measuring metabolic rates in the field. MMR in the field was approximately twice that of BMR measured in the laboratory, suggesting once again that while BMR measured in the laboratory may be a repeatable trait, it may have little relevance to the conditions and extrinsic factors that govern minimum metabolism in free-ranging animals (Hulbert and Else, 2004).

APPENDIX

Fig. 1 shows that while the range of f_H recorded during the calibration procedure in the laboratory covered approximately 85% of the f_H measurements made in the field, the f_H of free-ranging birds reached levels that were up to 60% higher than those recorded in the laboratory. Furthermore, these high values almost certainly occurred during flight and we were not able to obtain calibration data from flying gannets. Therefore, as we both extrapolated the calibration relationship in the laboratory to predict $\dot{V}_{O_2}$ for these high values of f_H and assumed that the relationship was the same for all types of behaviours and modes of locomotion, we conducted a simulation to predict whether the results of the study would be different if this extrapolation was inappropriate. To simulate this, we developed two new calibration relationships ('MinCal' and 'MaxCal') based on the 95% confidence intervals of the coefficients of the original calibration relationship ('OCal'; Fig. A1). MinCal was considered to generate the minimum possible estimate of $\dot{V}_{O_2}$ from f_H by using the lower 95% confidence limit for both coefficients of the OCal equation. Similarly, MaxCal was considered to generate the maximum possible estimate of $\dot{V}_{O_2}$ from f_H by using the upper 95% confidence limit for both coefficients of the OCal equation. Fig. A1 shows both of these equations and indicates how at higher f_H (above the calibration range), for a given value of f_H, estimates of $\dot{V}_{O_2}$ made using MinCal are approximately 25–30% lower than those with OCal, whereas estimates made with MaxCal are approximately 30–35% higher. We propose that this potential range in estimated $\dot{V}_{O_2}$ is sufficient for a sensitivity analysis, especially given the exponential nature of the OCal, MinCal and MaxCal relationships, analogous to that found in walking and swimming great cormorants (White et al., 2011), and walking and flying geese (Ward et al., 2002), and the high values of $\dot{V}_{O_2}$ that would therefore be predicted from high values of f_H.

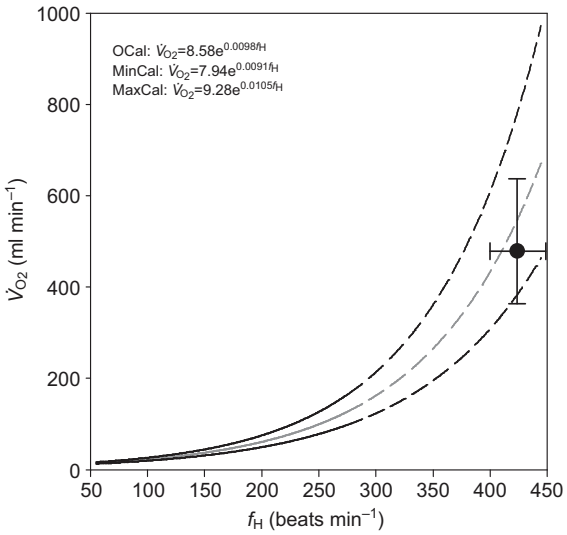

Fig. A1. Calibration relationship (OCal) between f_H and $\dot{V}_{O_2}$ in Australasian gannets (grey line) including an extrapolation beyond the range of f_H measured during calibration (dashed section) most likely associated with flight. Black lines show MinCal (lower) and MaxCal (upper) calibration lines including a similar extrapolation (dashed sections), which were derived to account for potential errors in the extrapolation of OCal and the use of it for all behaviours and modes of locomotion. Also shown (black circle) is the estimate of maximum aerobic power output during flight (with 95% confidence intervals) made using Method 2 of Bishop (Bishop, 1997) for the corresponding highest f_H values recorded in the free-ranging gannets, which are assumed to be during flight (Fig. 1).

As a reference point, we estimated maximum $\dot{V}_{O_2}$ during flight for Australasian gannets using Method 2 of Bishop (Bishop, 1997). The estimate of 480 ml min^{-1} (95% confidence intervals of 363–637 ml min^{-1}) sits within the range of estimates of $\dot{V}_{O_2}$ made

Table A1. Summary of calculations of FMR during incubation and chick-rearing phases of free-ranging Australasian gannets

	OCal	MinCal	MaxCal
Incubation FMR (W)	23.2±2.6	17.9±2.0	30.1±3.4
Chick-rearing FMR (W)	25.9±2.9	20.0±2.0	33.6±3.9
% Increase	11.4%	11.4%	11.6%
Significant increase	Yes	Yes	Yes

Estimates of FMR were made from f_H using a calibration equation (OCal), which for 15% of predictions was extrapolated beyond its measurement range, and two other equations (MinCal and MaxCal) derived to account for errors in this extrapolation (see Appendix for details).

Table A2. Summary of *P*-values from analyses investigating changes in FMR, NMR and MMR as a function of time elapsed during the breeding season of Australasian gannets

	Original	MinCal	MaxCal
Date *vs* FMR	0.01	0.01	0.01
Date *vs* NMR	0.10	0.11	0.10
Date *vs* MMR	<0.001	<0.001	<0.001
Slope of date *vs* NMR and FMR	0.15	0.13	0.07

Estimates of metabolic rate were made from f_H using a calibration equation (OCal), which for 15% of predictions was extrapolated beyond its measurement range, and two other equations (MinCal and MaxCal) derived to account for errors in this extrapolation (see Appendix for details).

using our calibration approach for the highest f_H recorded in free-ranging gannets of 400–450 beats min^{-1} (Fig. 1) and lies closes to the OCal line (Fig. A1). As a result, we are reasonably confident that the relationships used in our analysis are appropriate to estimate $\dot{V}_{O_2}$ from f_H during flight and other activities where f_H exceeded the calibration range in the laboratory.

Finally, to check the appropriateness of the approach, all values of FMR, MMR and NMR from the field data were recalculated using MinCal and MaxCal. All statistical analyses were repeated and while the estimates of metabolic rate were necessarily different, none of the conclusions from the analyses were different. FMR was still ~11.5% and significantly greater during chick rearing than during incubation (Table A1). Similarly, there were no differences in any of the relationships concerning date and FMR, MMR or NMR (Table A2). We repeated this sensitivity analysis where MinCal and MaxCal were applied only for recordings of f_H in excess of the maximum f_H recorded during calibration (275 beats min^{-1}) rather than for all recordings of f_H. However, in this more conservative scenario, again there was no effect on any of the results or conclusions from the study.

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AUTHOR CONTRIBUTIONS

J.A.G., E.J.A.-S., A.B., P.J.B. and P.B.F. conceived the study and gained funding. J.A.G., E.J.A.-S. and P.B.F. conducted laboratory experiments. J.A.G., A.B. and P.J.B. conducted field data collection. J.A.G., E.J.A.-S. and C.R.W. analysed the data. All authors contributed to drafting and commenting upon the manuscript.

COMPETING INTERESTS

No competing interests declared.

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