

Habitat selection by foraging macaroni penguins correlates with hematocrit, an index of aerobic condition

Glenn T. Crossin^{1,*}, Akinori Takahashi³, Kentaro Q. Sakamoto⁴, Phil N. Trathan⁵,
Tony D. Williams²

¹Department of Biology, Dalhousie University, Halifax, Nova Scotia B3H 4J1, Canada

²Simon Fraser University, Biological Sciences Department, Burnaby, British Columbia V5A 1S6, Canada

³National Institute of Polar Research, 10-3 Midori-cho, Tachikawa, Tokyo 190-8518, Japan

⁴Hokkaido University, Laboratory of Physiology, Graduate School of Veterinary Medicine, North 18 West 9, Kita-ku, Sapporo 060-0818, Japan

⁵British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge CB3 0ET, UK

ABSTRACT: Reproductive investment during the chick-rearing period is an important contributor to lifetime fitness. Key to chick-rearing is the success of parental foraging, as food deliveries affect chick growth and survival up until fledging. For seabirds, oceanographic conditions including factors such as sea surface temperature are known to influence foraging decisions, but few studies have examined the physiological variables that might affect those decisions. We used global positioning systems (GPS), time-depth recorders (TDR), and physiological sampling techniques to explore links between ocean temperature, diving behavior, and foraging success in chick-rearing female macaroni penguins *Eudyptes chrysolophus*. We then explored correlations between these foraging variables and measures of individual physiological condition, specifically aerobic capacity (hematocrit) and metabolic state (corticosterone). In GPS-tracked penguins, 2 principal foraging tactics were observed: penguins made deep dives in cool, near-shore areas surrounding the breeding colony at Bird Island, South Georgia, or they traveled farther to dive shallower in warmer shelf-break areas. TDR-equipped penguins showed similar patterns. Blood sampling of TDR penguins at the onset of trips revealed strong positive correlations between hematocrit and the mean duration of foraging trips, the ocean temperature experienced during these trips, and the relative efficiency of foraging activity in terms of the number of foraging behaviors recorded per dive. These results suggest that aerobic capacity might be an important determinant of foraging trip range, as well as workload. Corticosterone was unrelated to diving behavior, which counters previous studies examining the effects of experimental increases of this hormone on foraging behavior, and we discuss reasons for this disparity.

KEY WORDS: Biologging · Time-depth recorders · GPS · Telemetry · Parental care · *Eudyptes* · Ocean temperature

— Resale or republication not permitted without written consent of the publisher —

INTRODUCTION

It is perhaps an understatement to say that the rearing of offspring is an important component of lifetime fitness (Clutton-Brock 1991, Williams & Vezina 2001, Williams 2012). For many seabird species,

the rearing of offspring (chicks) is characterized by central-place foraging and long periods of bi-parental care, wherein chicks are fed frequently by both parents who must work continuously to adequately nourish chicks until they fledge. Theoretically, higher provisioning effort and rate should

result in higher fledgling mass, which would then predict higher post-fledging survival and recruitment. Significant positive relationships between chick size and fledging survival have been documented in over 22 species of birds, in a diverse array of taxa (Magrath 1991, Schwagmeyer & Mock 2008), so it is logical to predict that increased workload by parent birds would bolster their lifetime fitness via increased recruitment. A testable prediction is that individual variation in foraging behavior is positively related to foraging success and chick growth (Crossin et al. 2012a).

An important requirement for successful foraging is the ability to find and capture prey (e.g. Pinaud et al. 2005). However, both the distribution and availability of prey in marine systems can be highly variable in both time and space. Indeed, in some years, ocean conditions and resource availability may be sub-optimal, which can have large negative effects on seabird population processes. In the worst cases, as in El Niño years, seabird colonies can experience complete breeding failure, with no (or few) chicks fledged (Guinet et al. 1998, Gjerdrum et al. 2003, Hipfner 2008, Gaston et al. 2009, Morrison et al. 2011). Such failures are presumably due to poor primary and secondary ocean productivity regimes and a low-quality prey base (Kitaysky et al. 2006), both of which may sometimes correlate with anomalously high sea surface temperatures (SSTs) (Pinaud & Weimerskirch 2002, Inchausti et al. 2003, Pinaud et al. 2005).

Conversely, in years when ocean conditions are more stable and SSTs lie within a normal range, environmental links with foraging behavior can be either positive or negative depending on species and location. For example, in tropical systems, daily variation in prey availabilities and foraging success were negatively related to daily SST fluctuation in both wedge-tailed shearwaters *Puffinus pacificus* (Peck et al. 2004) and sooty terns *Sterna fuscata* (Erwin & Congdon 2007) breeding at the Great Barrier Reef. At higher temperate and polar latitudes, breeding seabirds can show similar responses, with high daily temperatures related to poorer foraging success (e.g. thin-billed prions *Pachyptila belcheri*, Quillfeldt et al. 2007). However, positive correlations between SST and foraging success have also been observed in polar regions, e.g. for king penguins *Aptenodytes patagonicus* breeding in the Southern Ocean (Guinet et al. 1997).

Ocean temperature is a defining characteristic of marine foraging habitats, which can vary spatially as a function of oceanographic currents, upwelling, and

bathymetry. Within this heterogeneity, foraging seabirds show flexibility in their choice of foraging habitats (Barlow & Croxall 2002a, Trathan & Croxall 2004, Phillips et al. 2005, Dias et al. 2011). What is presently unknown is whether individual flexibility and choice of foraging habitats is random or driven by some component of physiological quality. Certainly, successful foraging depends on the availability of adequate food resources; but when posed with a range of different foraging locations within range of a central place, does an individual's physiological state influence its choice of foraging habitat (Thiebot et al. 2011)? More specifically, does the physiological condition of individuals predict the pattern and extent of their foraging activity, as well as their choice of foraging location?

Aerobic capacity, or the oxygen-carrying capacity of blood, is a key predictor of workload endurance in a wide range of animals (Wagner 1996, Calbet et al. 2006, Williams 2012), and serves as a candidate physiological variable within the context of foraging activity and habitat selection. Hematocrit is the relative volume of red blood cells within the total blood volume, and is a main determinant of aerobic performance through its role in oxygen transport and delivery (Jones 1998). For a central place forager presented with a range of possible foraging locations, aerobic capacity could theoretically influence decisions about where to forage, especially if different foraging locations require different levels of effort in order to reach them. Another candidate physiological variable is the hormone corticosterone, which has several regulatory metabolic functions and which has been implicated in locomotor activity, foraging behavior, and provisioning effort in birds (Love et al. 2004, Angelier & Chastel 2009, Crossin et al. 2012a).

In this study, we describe individual variation in the behavior of foraging macaroni penguins *Eudyptes chrysolophus* using electronic tracking devices, and test the hypothesis that the decisions concerning the choice of foraging habitat are related to the physiological state of individuals, as determined by variation in hematocrit and corticosterone levels measured at the beginning of foraging trips. We conducted this study during the chick-rearing period in the breeding season of a central-place foraging seabird, the macaroni penguin. Tracking studies of female macaroni penguins breeding at Bird Island, South Georgia, during the brood-guard stage of chick rearing have shown a consistency of foraging locations, but within the range of activity there is high inter-individual variation in final foraging destination (Barlow & Croxall 2002a, Trathan & Croxall

2004) as well as between years (Trathan et al. 2006). This could indicate individual preferences for areas where foraging opportunities and/or environmental conditions are deemed most favorable (Trathan et al. 2003). Our aim was to explore the variation in foraging habitat choice and foraging effort that penguins exhibit when diving in these restricted but highly variable areas, and to link these to environmental conditions, to physiological profiles, and to foraging success. Focusing on female penguins, who alone are responsible for the provisioning of chicks early in the brood-guard phase while males remain at the nest (Barlow & Croxall 2002b, Crossin et al. 2012a), we used global positioning systems (GPS) and time-depth recorders (TDR) to characterize foraging location, range, activity, and temperature exposure of individual females. We then explored correlations between these patterns and their physiology at the onset of trips. Female penguins tracked from Bird Island, South Georgia, initiate 1 to 3 d foraging trips (Croxall et al. 1993, Crossin et al. 2012a) and follow a known set of bearings en route to foraging areas (Barlow & Croxall 2002a, Trathan & Croxall 2004). These foraging areas provide dense, but patchy, prey aggregations and nutrient-rich waters (Trathan et al. 1997, 2000, 2003). Knowledge about the environmental and physiological factors that influence foraging activity at times when they are constrained by central-place breeding is important for a broader understanding of their behavioral ecology, but this can also lend insights to the effects of natural and anthropogenic ecosystem impacts.

MATERIALS AND METHODS

Study site and field sampling protocol

This study was conducted at a small colony at Bird Island, South Georgia (54° 00' S, 38° 02' W), where approximately 500 pairs of macaroni penguins breed (Fairy Point). This colony is a good place to monitor the movements of foraging penguins, as there is only 1 small path that allows birds to move to and from the sea (see figure in Green et al. 2006). Fieldwork was conducted between January and February 2009 through permits issued by the British Antarctic Survey and the Canadian Committee on Animal Care (Simon Fraser University Animal Care Permit 897B-8).

Our field protocol is described elsewhere (Crossin et al. 2012a), but briefly, we targeted female penguins at the beginning of the brood-guard stage of

the reproductive season ~5 to 7 d after their single surviving eggs had hatched. We sampled 33 penguins between 4 and 28 January 2009. Breeding females with young chicks were identified and marked at nests in the pre-dawn hours when most females are in the colony and preparing to leave for foraging trips (Barlow & Croxall 2002b).

Biological sampling

Beginning the following day, marked females were captured at dawn as they made their way from the colony to the sea. For our electronic tracking studies, we captured, sampled, and tagged a total of 33 penguins. Between 7 and 28 January, 18 penguins were captured and fitted with GPS loggers (see below). These penguins were then measured for body mass (g), bill depth (mm), and wing chord (mm) and released. Sex was confirmed using bill depth measurements (>22 mm = female; Williams 1995). Between 4 and 13 January, an additional 15 penguins were sampled, and 2 ml blood samples were drawn from brachial veins using heparinized syringes fitted with 25-gauge needles. The mean $\pm$ SD time from first approach to the penguin, to the completed collection of blood was 164 ± 34 s ($n = 8$). Blood was transferred to heparinized 2.5 ml Eppendorf vials and centrifuged for 5 min at $10000 \times g$, and plasma was then transferred to labeled 0.6 ml vials and frozen at -20°C until analysis. We recorded the time (± 1 s) that it took to collect a blood sample from first approach to the end of blood collection. We recorded body mass and bill length. These 15 penguins were then fitted with TDRs (see below) and released. During daylight hours (ca. 05:00 to 00:00 h), we continuously monitored the rocky shore at the colony entrance to determine precise departure and return times of penguins. These observations were used subsequently to cross-reference and validate the biologging estimates for departure and return times. Of these 15 TDR-bearing penguins, 7 were given corticosterone implants, which was a central component of another study published elsewhere (Crossin et al. 2012a), so we restricted our analyses in this study to the 8 unmanipulated, sham-implanted TDR penguins.

Once penguins were released after sampling and logger attachment, we recaptured birds 1 to 3 d later, upon their return from single foraging trips. We therefore only collected data for single foraging trips, not multiple trips, and examined correlations between trip features and physiological condition (specific details below).

Physiological analyses

Unlike other animal taxa (e.g. mammals, fish), birds do not store red blood cells in their spleen and thus do not release these in response to stress. For this reason, baseline hematocrit is useful as a means for interpreting behavioral performance and life-history variation in birds (John 1994, Williams et al. 2004, Wagner et al. 2008, Williams 2012). Hematocrit was measured on fresh whole blood at the time of blood sampling. Whole blood was collected with heparinized microhematocrit tubes, and centrifuged at $10\,000 \times g$ for 5 min. Using a standardized hematocrit reader card, hematocrit was determined as the percentage of packed red-blood cells in the microhematocrit tube relative to the whole blood volume (plasma plus red blood cells).

Corticosterone was determined by double antibody radioimmunoassay (^{125}I -RIA, MP Biomedicals, 07-120103; see Crossin et al. 2012a). The assay detection limit was 3.13 pg corticosterone per tube (i.e. the lowest corticosterone standard, 12.5 ng ml^{-1} , using a 50 μl assay volume). Samples were assayed in duplicate, and the inter-assay coefficient of variation was 5.10%, while the intra-assay coefficient of variation was 9.4%.

Biologging devices

The GPS loggers ($n = 18$, Little Leonardo GPL-380DT) and TDRs ($n = 15$, Cefas Technology G5 tags) were deployed on penguins via attachment to lower back feathers with Tesa® tape. The GPS loggers weighed 85.0 g in air, while the TDRs weighed 2.7 g in air, which represents ~2% and 0.01% of body mass, respectively. GPS loggers were programmed to record positions, depth, and temperature every second. TDRs were programmed to record at 30 s intervals on the surface (depth < 5 m = surface travel), and at 0.5 s once a dive was initiated (depth > 5 m). The absolute accuracy of depth was 1 m and resolution was 0.5 m.

GPS and TDR data analysis

Location data from GPS loggers as well as depth and temperature data from GPS loggers and TDR were analyzed using the program-package Ethographer (Sakamoto et al. 2009) for Igor Pro (Wave Metrics). As GPS loggers cannot record location data underwater, the last position prior to a dive was used

to record the location of the dive. In some instances, the logger did not record the position, even when out of water. We did not conduct any interpolations to such data gaps, and used the actual GPS fixes only. We removed 1 GPS record from our analyses due to a battery failure which yielded an incomplete trip track for that penguin (10.9 h). We therefore had complete trip coverage for 17 of the 18 penguins tracked (near-shore, $n = 8$, Fig. 1C; shelf-break, $n = 9$, Fig. 1C). For each individual GPS and TDR depth records, the total number of dives was calculated, and for each dive, the depth, total duration, bottom duration (i.e. the duration when dive waveforms showed a depth change of 0 m), and the number of prey capture attempts during each dive (or 'wiggles', Takahashi et al. 2004) were determined. Wiggles were defined as dive events when birds changed their swimming direction rapidly from descending to ascending to descending (Takahashi et al. 2004). This has been previously shown to be a good proxy for foraging events and correlates positively with bill openings and closings (Ropert-Coudert et al. 2001, Takahashi et al. 2004, Bost et al. 2007). Due to surface noise, we used a conservative approach and analyzed dives >10 m in depth, which is the suggested depth threshold for foraging dives of macaroni penguins at Bird Island, South Georgia (Green et al. 2005).

Statistical analyses

Analyses were run with either the JMP 10.0 or the SAS 9.0 software package, and data processing was done with Ethographer. All variables were tested for normal distribution via plots of residuals against predicted values followed by Shapiro-Wilk tests for normality. Transformations were applied when necessary. Pearson's correlations were used to explore relationships among dive behaviors derived from TDRs (e.g. time away from colony, total dive number, foraging efficiency) and penguin attributes (hematocrit and corticosterone levels at the onset of foraging trips, rate of mass gained while away foraging). Finally, linear regression models were used to explore the influence of pre-foraging hematocrit and corticosterone levels (continuous independent variables) against various dependent variables (e.g. total dive duration, number of dives, foraging efficiency, rate of mass gain). Because some dependent variables were correlated with time that penguins spent away from the colony, we used regression analysis to generate time-corrected residuals (e.g. total dive duration and foraging efficiency). In other words, the

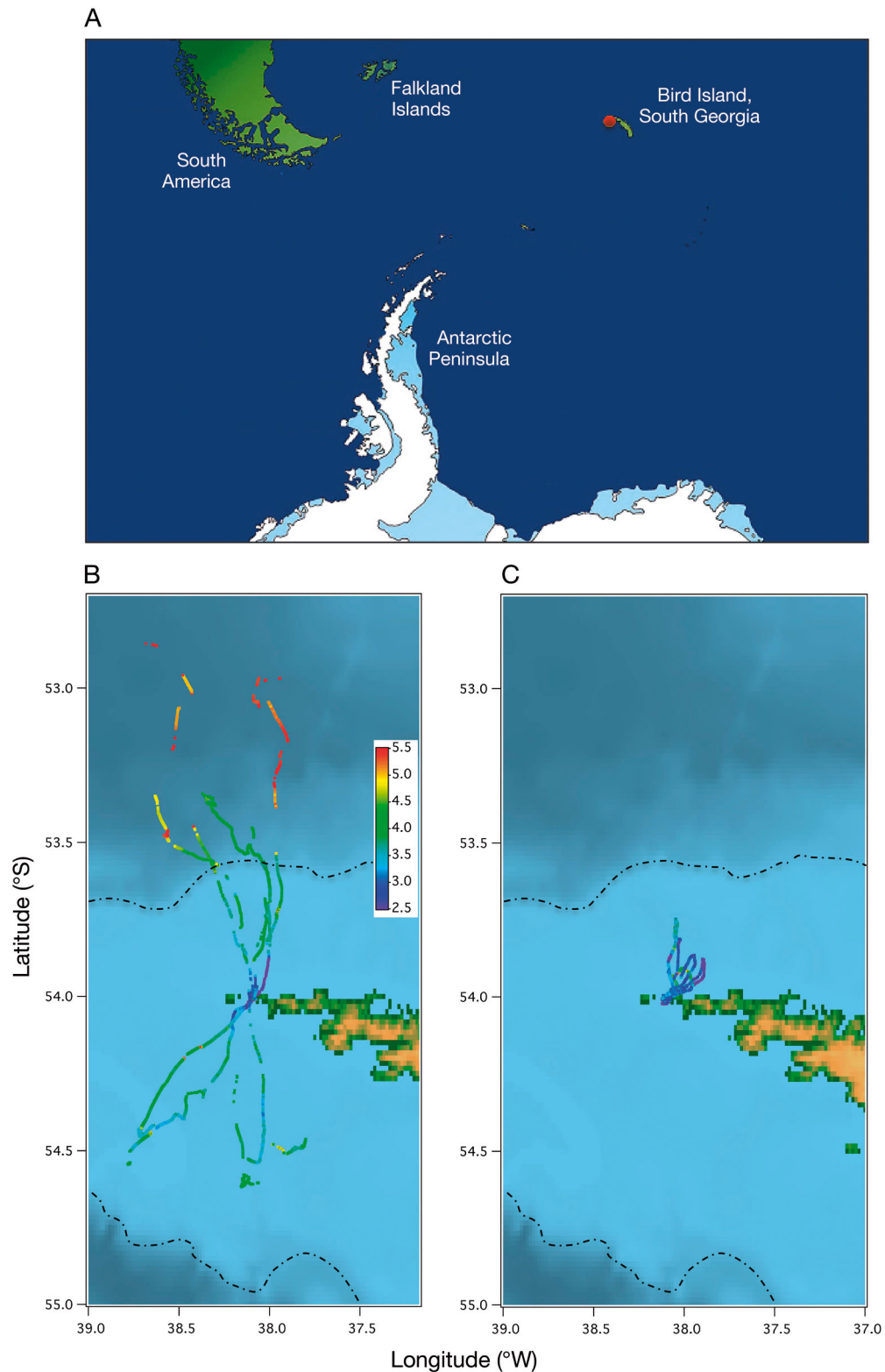


Fig. 1. (A) Study site at Bird Island, South Georgia, in the Atlantic sector of the Southern Ocean. (B, C) GPS tracks of 17 female macaroni penguins *Eudyptes chrysolophus* foraging during the brood-guard period of chick-rearing at Bird Island in January 2009. Each GPS track is color-coded to reflect the average temperature experienced at each waypoint (see legend in B; °C). Females targeted either the deep shelf-break waters surrounding South Georgia (B, $n = 9$), or the shallow near-shore waters close to the breeding colony (C, $n = 8$). The dashed line in panels B and C indicates the position of the shelf break (500 m bathymetric contour)

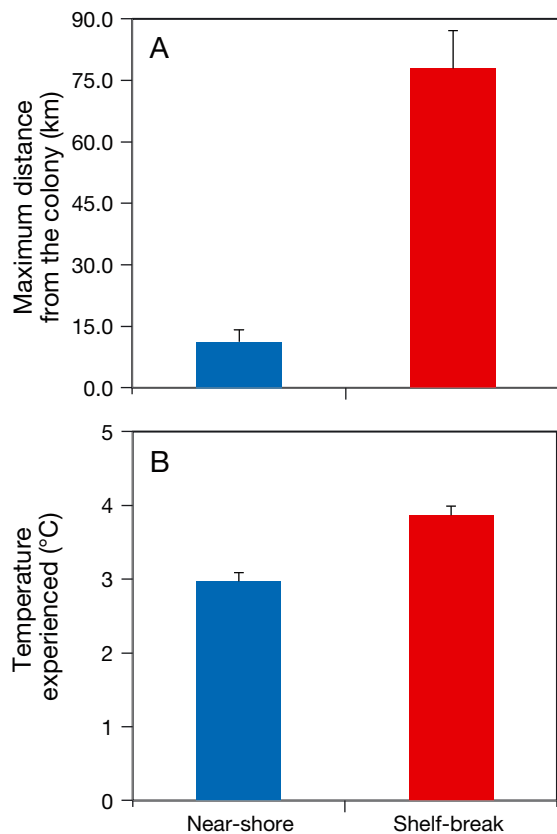


Fig. 2. Group comparison of (A) maximum distances traveled from the breeding colony and (B) temperature experienced by foraging macaroni penguins *Eudyptes chrysolophus* at Bird Island, South Georgia, as derived from GPS loggers. Bars represent least squares mean + SEM (near-shore: $n = 8$; shelf-break: $n = 9$)

residuals from regressions of total dive duration and foraging efficiency against trip duration produced estimates from which variation due to trip duration is removed. These were then regressed against pre-foraging corticosterone to explore the influence of this variable on foraging behavior. Bonferroni corrections were applied and are presented in the 'Results'. All values presented in figures are untransformed least squares means $\pm$ SEM.

RESULTS

From the GPS-tracked penguins, we discerned 2 distinct foraging tactics: individuals either traveled to distant, warmer shelf-break waters to the north and south of Bird Island (Fig. 1B), or they remained in the cooler near-shore areas just north of the breeding colony (Fig. 1C). Group comparisons of the maximum distances traveled from the colony during foraging

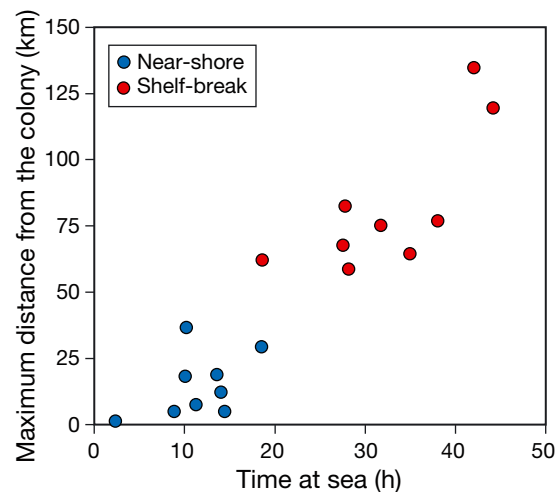


Fig. 3. Correlation between time spent at sea and maximum distance traveled from the colony by GPS-bearing macaroni penguins *Eudyptes chrysolophus*. The red circles correspond to the shelf-break penguins identified in Fig. 1B, and the blue circles correspond to the near-shore penguins in Fig. 1C

trips and of the mean temperatures experienced show that these tactics differed significantly: for the near-shore penguins, maximum distance was 12.3 ± 3.1 km, and for shelf-break penguins, it was 82.5 ± 8.4 km ($F_{1,15} = 49.5$, $p < 0.001$). The values for shelf-break penguins were conservative, because the GPS loggers stopped recording before the ends of trips due to battery exhaustion. Mean temperatures experienced also differed between the 2 foraging patterns by approximately 1°C : near-shore penguins cumulatively experienced $2.9 \pm 0.12^\circ\text{C}$, whereas shelf-break penguins experienced $4.1 \pm 0.15^\circ\text{C}$ ($F_{1,15} = 27.8$, $p < 0.001$). Median dive depth (for dives > 10 m) tended to be deeper in near-shore penguins (40.7 ± 5.7 m) than in shelf-break penguins (26.4 ± 3.7 m; $F_{1,15} = 4.0$, $p = 0.063$).

When analyzing the GPS data as a whole, the time spent at sea was positively correlated with the distance travelled from the colony ($r = 0.925$, $n = 17$, $p < 0.001$; see Fig. 3). Time is therefore a suitable proxy for distance, which aids comparisons of the GPS data and the TDR data below (see Fig. 4). Collectively, from Figs. 1–3, we generalize that the GPS-tracked penguins embarked upon 1 of 2 foraging tactics: in the near-shore tactic, penguins initiated deep dives in generally cool water, while in the shelf-break tactic, penguins made shallower dives in warmer waters.

Plots comparing GPS-derived latitude to diving depth show that the near-shore penguins (blue circles in Fig. 4A) dove deep within a restricted geographic area relative to the more widely ranging shelf-break penguins, which dove shallower (red cir-

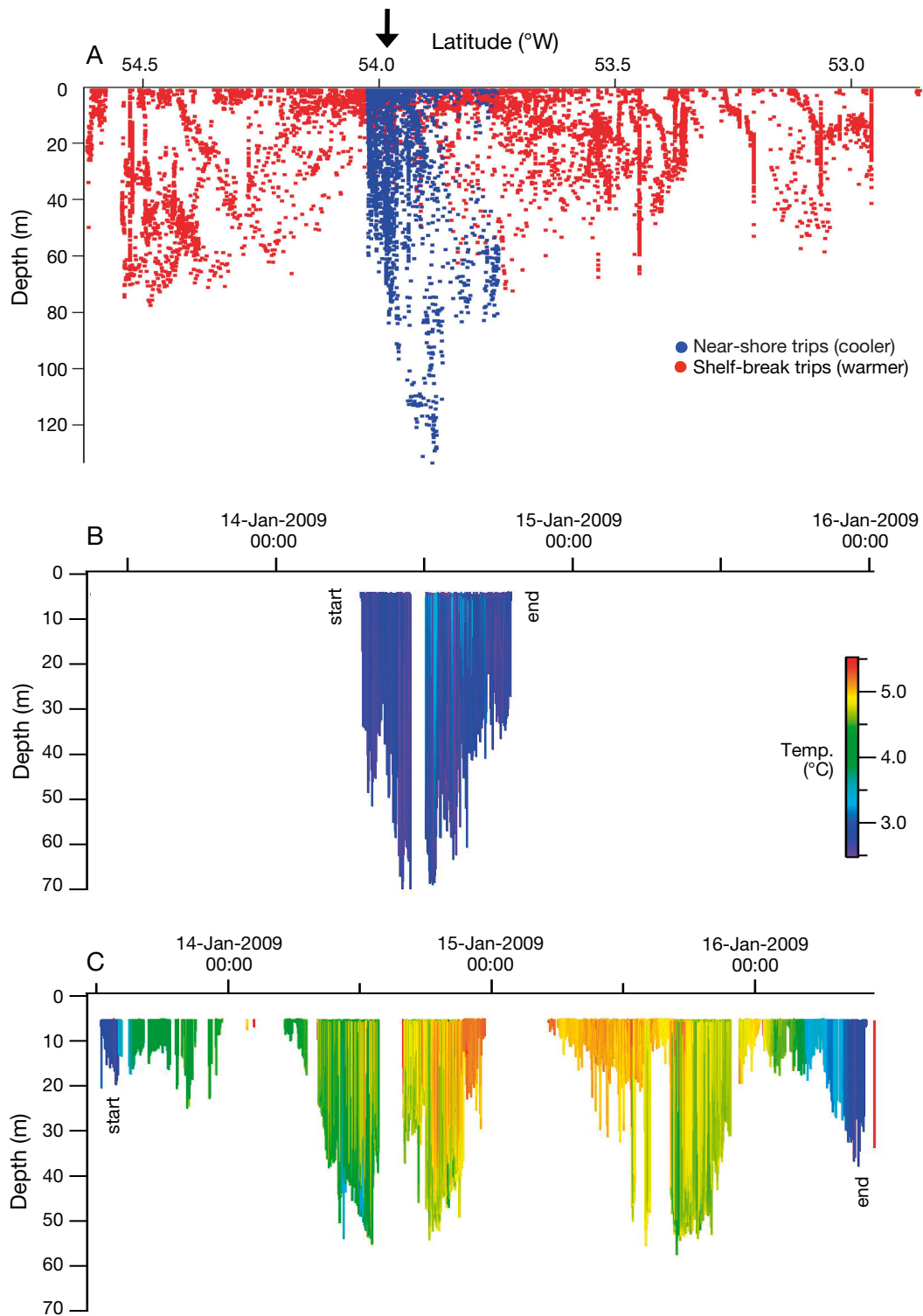


Fig. 4. (A) GPS loggers revealed the latitudinal position and depths of individual dives by female macaroni penguins *Eudyptes chrysolophus* foraging during the brood-guard period of the breeding cycle. The black arrow on the x-axis indicates the latitude of the breeding colony at Bird Island. Near-shore trips tended to occur just north of Bird Island, whereas trips further afield to shelf-break waters were more northerly or southerly. (B, C) Time-depth and temperature profiles for 2 female macaroni penguins released together and monitored over the same time frame (note the slight offset between x-axes in panels B and C, and the different start and end times of foraging trips). Each track is color-coded to reflect the temperature experienced over the course of each individual dive (see legend in panel A; $^{\circ}$ C). The first female (panel B) dove for roughly 12 h in deeper, cooler waters, similar to the near-shore tactics in Fig. 1C. The second female (panel C) made dives over an extended 3 d period, and dives were generally shallow and warm similar to the shelf-break tactics revealed by GPS logging in Fig. 1B

cles, Fig. 4A). In the same figure, we include 2 TDR plots (Fig. 4B,C), which closely align with the diving patterns in Fig. 4A. The time-depth-temperature profile in Fig. 4B suggests that this penguin initiated the near-shore tactic observed in the GPS-tracked penguins (blue circles in Fig. 4A), while the TDR profile in Fig. 4C suggests a shelf-break tactic (red circles in Fig. 4A).

The TDR data do not allow direct geographic location estimations, so we could not generate maps as for the GPS-tracked penguins in Fig. 1. However, the TDR profiles do provide data on trip duration and temperature experience, which we can use to infer foraging habitat by comparisons with GPS-tracked birds. We compared the total diving duration, total dive number, and temperature experience of both TDR- and GPS-equipped birds, while accounting for co-variation associated with time spent at sea (analysis of covariance, ANCOVA). This analysis shows that despite these group differences in trip duration, there were no significant differences between TDR and GPS with respect to the slopes and intercepts from regressions of dive duration, dive number, and temperature experience against trip duration (Fig. 5). We also note that the slopes and intercepts of near-shore and shelf-break GPS birds did not differ, and were thus pooled for the comparisons against the TDR birds. However, for graphical purposes, we plotted the regression lines for each group (near-shore GPS, shelf-break GPS, and TDR) individually (Fig. 5). The statistical output that underlies Fig. 5 is presented in Table 1.

Neither hematocrit nor corticosterone was correlated with duration of blood sampling (hematocrit, $r = 0.278$, $p = 0.506$; corticosterone, $r = -0.416$, $p = 0.505$). In the TDR penguins, various foraging trip parameters were significantly correlated with individual hematocrit levels, but not with plasma corticosterone levels (Fig. 6). More specifically, hematocrit at the beginning of foraging trips predicted total trip duration (significant at Bonferroni-corrected α of 0.013, effects size $\eta^2 = 0.703$), total residual dive time (significant at Bonferroni-corrected α of 0.013, $\eta^2 = 0.686$), residual foraging efficiency (defined as the number of foraging events or 'wiggles' [Takahashi et al. 2004] at the bottom of dives per dive; not significant at Bonferroni-corrected α of 0.013 but significant at 0.05, $\eta^2 = 0.653$), and mean temperature experience (not significant at Bonferroni-corrected α of 0.013 but significant at 0.05, $\eta^2 = 0.527$). In contrast, pre-trip corticosterone levels were not significantly related with these same parameters: trip duration $\eta^2 = 0.032$; total residual dive time $\eta^2 = 0.170$; residual

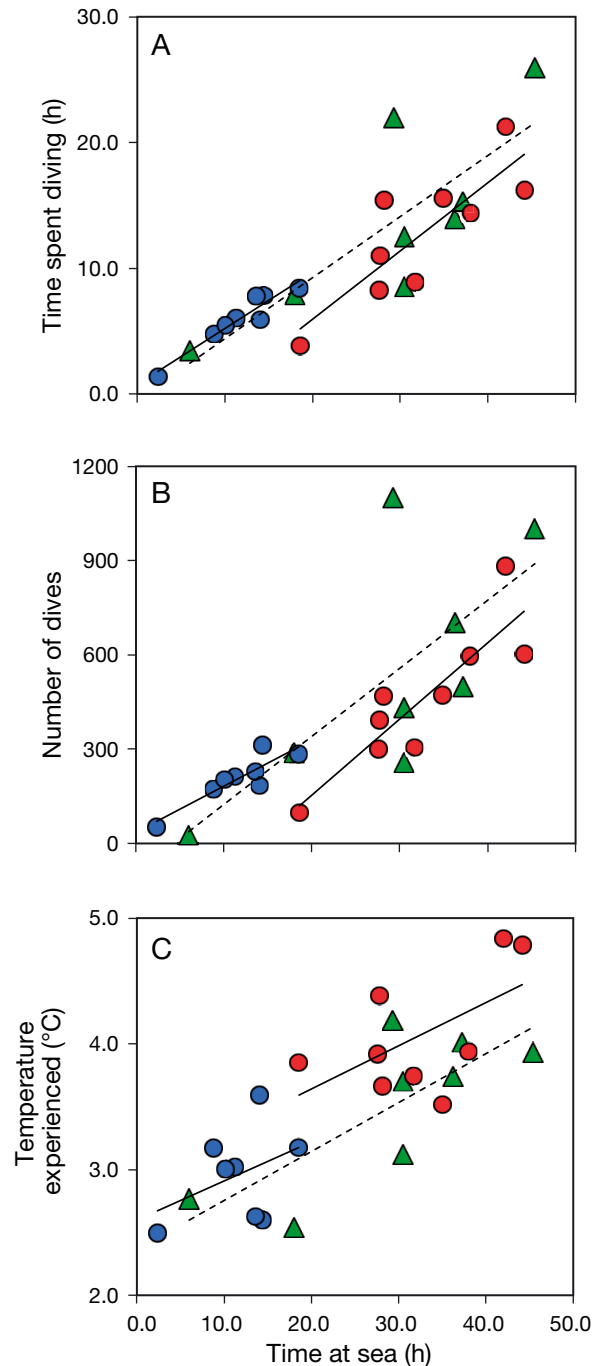


Fig. 5. Relationships between (A) time spent diving, (B) number of dives, and (C) mean temperature experience of GPS- (colored circles) and time-depth recorder (TDR)-tracked (green triangles) macaroni penguins *Eudyptes chrysolophus* relative to the time that they spent on foraging trips. For the GPS data, trips to warm shelf-break areas are indicated with red circles, while cooler near-shore trips are in blue. Lines are best linear fits. For each response variable (y-axes), the slopes or intercepts between GPS and TDR data did not differ significantly, which suggests that both sets of birds were behaving similarly. See Table 1 for the statistical output that underlies these relationships (analysis of covariance)

Table 1. Statistical output from analysis of covariance models comparing time spent diving, dive number, and mean temperature experiences between macaroni penguins *Eudyptes chrysolophus* tracked by 2 different methods. Least square means account for covariation in time spent at sea. See Fig. 5 for visual representation of the relationships. The interaction term 'tracking-group $\times$ time-at-sea' was not significant in any of these models and was thus removed from the final models so as to preserve the degrees of freedom. TDR: time-depth recorder

Diving metric	GPS pooled (n = 17)	TDR (n = 8)	F-ratio	p	Covariate (time at sea) p
Time spent diving (h)	11.8 $\pm$ 0.61	10.8 $\pm$ 0.93	1.079	0.310	<0.001
Dive number	372.5 $\pm$ 43.2	465.1 $\pm$ 63.6	1.412	0.247	<0.001
Temperature experience ($^{\circ}$ C)	3.64 $\pm$ 0.10	3.30 $\pm$ 0.14	3.626	0.070	<0.001

foraging efficiency $\eta^2 = 0.088$; mean temperature experience $\eta^2 = 0.015$ (Fig. 6). In both of these analyses, residuals from regressions of total dive time and foraging efficiency against trip duration were calculated so as to remove the covariation associated with trip duration. Ultimately, variation in hematocrit predicted the rate of mass gain while foraging, which is the most direct indicator of foraging success ($p = 0.044$; $\eta^2 = 0.518$; Fig. 7). Hematocrit and corticosterone were not correlated ($r = -0.232$, $p = 0.580$), nor were hematocrit and body mass ($r = 0.108$, $p = 0.800$).

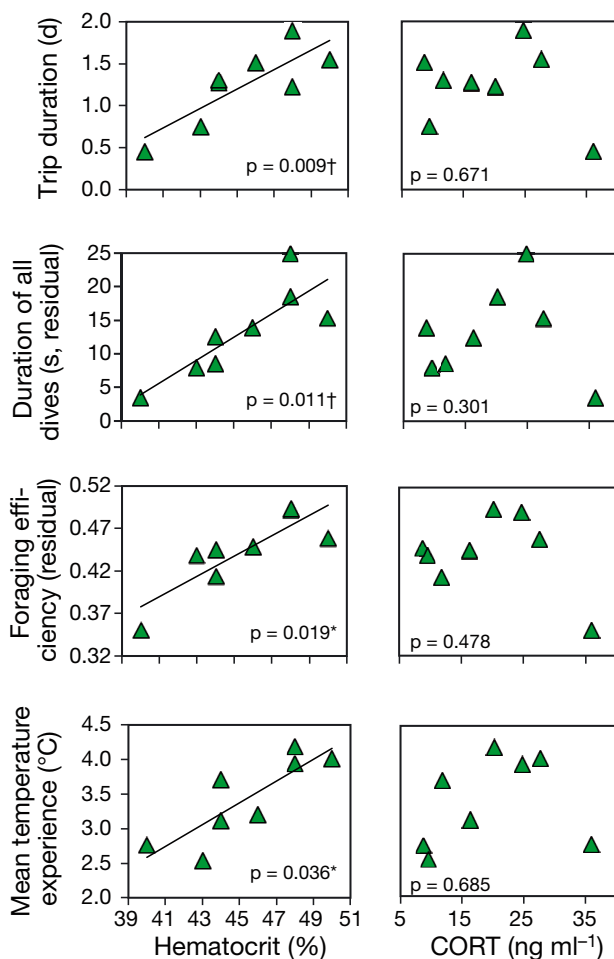


Fig. 6. Relationships between the dive parameters of time-depth recorder (TDR)-tracked macaroni penguins *Eudyptes chrysolophus* with individual hematocrit and plasma corticosterone (CORT) levels measured at the beginning of foraging trips. The dagger (†) indicates statistical significance when α is set at the Bonferroni-corrected level of 0.013, while asterisks (*) indicate significance when $\alpha = 0.05$

DISCUSSION

Through the use of GPS and time-depth biologging, we tracked 25 breeding macaroni penguins and found that individuals conformed to 1 of 2 general foraging tactics, utilizing 2 different marine areas: penguins either traveled to distant shelf-break waters to the north and south of Bird Island to forage, up to 150 km away, or they remained in the near-shore waters within only a few km of the breeding colony (Fig. 1). From thermal sensors, we also show that temperatures experienced in these 2 habitat types differed markedly, with temperature approximately 1° C warmer in the shelf-break habitats versus the near-shore. In an attempt to understand

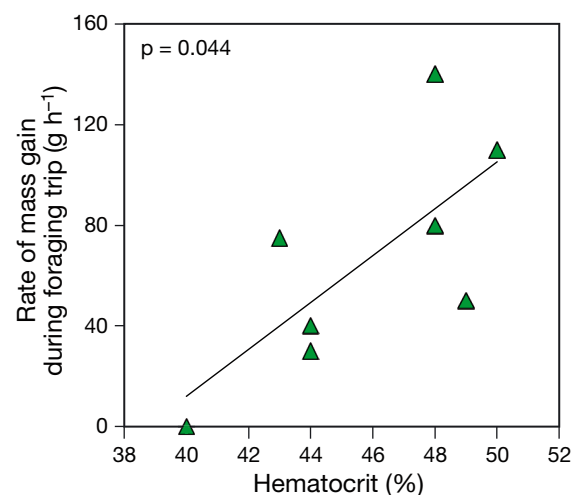


Fig. 7. Relationship between hematocrit measured at the beginning of foraging trips and the rate of mass gained during foraging trips by time-depth recorder (TDR)-bearing macaroni penguins *Eudyptes chrysolophus*

the factors that underlie and potentially drive foraging habitat choice, we examined individual variation in blood parameters in a subset of penguins. We found that hematocrit levels, an indicator of aerobic condition measured at the onset of foraging trips, predicted many aspects of diving behavior, thermal experience, and ultimately foraging habitat selection. This is, to the best of our knowledge, the first report in which a measure of aerobic capacity directly predicts foraging habitat selection. This association was independent of variation in body mass (i.e. there was no correlation between hematocrit and body mass). There was also no significant correlation between initial baseline corticosterone levels (a measure of metabolic condition) and hematocrit. Furthermore, corticosterone was not related to diving behavior or habitat choice. We discuss potential reasons for this, especially since it appears to contradict other studies that have documented this hormone's effects on foraging behavior (e.g. Crossin et al. 2012a). We begin with a discussion about the foraging patterns and habitat association that we observed, and about hematocrit in the context of reproduction and work load.

Foraging habitat selection

The most striking observation stemming from the biologging data is how penguins targeted 2 reasonably restricted foraging areas radiating from their central place at the breeding colony. At South Georgia, spatial and temporal variation in the physical oceanography and, importantly, the densities of krill *Euphausia superba* have been well documented. Krill are the principal prey item taken by foraging macaroni penguins at South Georgia, and they are especially important to the growth of chicks relative to other prey types (Waluda et al. 2012). Local krill populations are thought not to be self-sustaining (Mackintosh 1972, Everson 1976) but are rather replenished from stock brought to the island via the Antarctic Circumpolar Current (ACC) (e.g. Murphy et al. 1998, Brierley et al. 1999). As the ACC moves eastward through the Southern Ocean towards South Georgia, Ekman drift and upwelling at the archipelago's western self-edge concentrate krill into dense aggregations (Trathan et al. 2003). We observed penguins foraging at this shelf-break, along the northern and southern margins of Bird Island, so penguins there may be exploiting a rich forage base. However, krill can also be abundant on the shelf itself, closer to the island (Trathan et al. 2003), which

would similarly benefit the penguins that foraged in the near-shore. We do not have simultaneously collected data on krill biomass to align with our tracking data, so we cannot say which areas presented the best foraging opportunity. However, individual penguins were clearly choosing either the self-break or the near-shore.

Although there exists a general inverse relationship between krill biomass and SST (Trathan et al. 2003), we found that penguins experienced warmer temperatures at the shelf-break, where we presume krill to be in high concentration. We might have predicted temperatures to be cooler at the shelf-break. We do not know the reason for this disagreement, but it may be that the patchy nature of krill fields in general, and the fact that upwelling and krill aggregation tend to occur at the shelf-edge, resulted in greater krill availability here than over the shelf and closer to shore, irrespective of temperature. Temperature–krill biomass relationships are usually observed over broad spatial scales, but the penguins in our study ranged over comparatively smaller areas. Although we detected significant differences in the average temperature experience of shelf-break and near-shore tracked penguins, the complete range of temperatures experienced (~ 2.5 – 4.5°C) was well within the range of observed values in summer around South Georgia (Whitehouse et al. 2008). Ultimately, we do not know what the density of krill was at the shelf-break or near-shore during our study, so the connection or disconnection to temperature is only speculative. What is more important is that we know that there were significantly different temperatures in the near-shore and shelf-break areas, which have greater implications for foraging behavior and which we will discuss below.

Foraging behavior was independent of corticosterone and body condition

In a recent study in which corticosterone levels were experimentally manipulated in female macaroni penguins (Crossin et al. 2012a), we identified a causal relationship between corticosterone and many aspects of diving behavior. In our present study described here, no such relationship was observed (Fig. 6), which runs counter to our previous study and to other studies linking corticosterone to locomotor and foraging behavior. We can think of 2 reasons for this disparity. Relationships between baseline (e.g. not stress-induced) corticosterone and locomotor behavior are common in birds, and seasonal upregula-

tion during breeding has been identified as a strategy for matching foraging effort with the ever-increasing demands of growing chicks (e.g. Love et al. 2004). The manipulation experiment that we conducted with macaroni penguins corroborates this idea; when phenotypic expression of baseline corticosterone was expanded, positive effects on foraging effort and foraging success could be detected, and ultimately this bolstered fitness through a positive effect on food delivery to chicks. However, as Fig. 6 shows, we did not detect this correlation here, so it may be that the range of baseline corticosterone levels that we observed in this study was within some operational range necessary to support a range of diving behaviors. Alternatively, we may not have detected this relationship due to small sample size and low effect sizes (η^2 ; see 'Results'), or the lack of correlation might be due to the activity and expression of corticosteroid-binding globulins, which regulate the availability of biologically active 'free' or unbound corticosterone that is available to target tissues (e.g. Breuner & Orchinik 2002, Love et al. 2004), but which we did not measure here (although this did not appear to obscure the relationship between corticosterone and behavior observed by Crossin et al. 2012a). Finally, we also failed to detect any significant correlations between corticosterone and body mass or mass gained while foraging. This makes it all the more intriguing that we observed a positive correlation between hematocrit and rate of mass gain (Fig. 7), which we discuss in greater detail below.

Hematocrit as a driver of foraging behavior and habitat selection

As noted, penguins showed preference for 2 general foraging areas. Hematocrit, which plays a key role in maximum oxygen consumption (VO_{2max}) and oxygen transport to muscle tissues (Jones 1998), should theoretically predict individual variation in work effort, and potentially habitat selection, especially in diving species that rely on single breaths to provide the oxygen needed to sustain foraging activity. During the initial phases of chick rearing, female macaroni penguins are solely responsible for provisioning their offspring, so it seems reasonable that individual variation in hematocrit levels might predict diving behavior and foraging effort. Overall, the range of hematocrit values in the penguins that we tracked was from 40 to 50 %, which represents a 20 % difference between the lowest and highest individuals. What we observed was that many aspects of for-

aging behavior, such as dive duration, dive efficiency, and trip duration, were all positively correlated with hematocrit levels measured before penguins departed for sea. Hematocrit was also positively correlated to mean temperature experience during trips. In Figs. 2 & 4, we show how temperature experience is characteristic of ultimate foraging destination. From this we suggest that hematocrit not only predicts diving effort, but also choice of foraging habitats. Furthermore, after departing the colony, penguins appear to have chosen foraging habitats that were within their aerobic capacity to reach, with low hematocrit individuals opting for near-shore foraging and high hematocrit and aerobically fitter individuals venturing farther to exploit the rich krill patches often found along shelf-breaks. Foraging efficiency, or the number of individual foraging events or 'wiggles' recorded at depth (Takahashi et al. 2004), was greatest in the high hematocrit individuals. This translated into a significant gain in post-foraging body mass (Fig. 7), which was previously shown to positively influence the growth of chicks via increased food deliveries (Crossin et al. 2012a).

With the functional consequence of variation in hematocrit described as such, the question becomes: What is the cause of this variation? Hematocrit varies markedly in birds, with some of the highest values observed in migratory birds (Piersma et al. 1996), but declining after breeding to lows during the post-nuptial molt (Hörak et al. 1998, Davey et al. 2000, Williams 2012). Declines during the breeding season have been documented in a number of birds (and in many other taxa as well), the ecological relevance of which has been previously underappreciated (Williams et al. 2004, Fair et al. 2007, Hanson & Cooke 2009, Cooke et al. 2010, Williams 2012, Bowers et al. 2014). As nearly all life-history functions are intimately linked to oxygen-carrying capacity, low capacity or low hematocrit can have deleterious effects on fitness in wild animals. Studies have linked hematocrit to increases in parasitism, reductions in survival and longevity, deferred breeding activity, locomotor performance, and poor nutrition (Richner et al. 1993, Ots et al. 1998, Potti et al. 1999, Kilgas et al. 2006, Fair et al. 2007, Crossin et al. 2012b, 2013, Bowers et al. 2014). Here we document a positive association between hematocrit and foraging success (Fig. 7), which, within the context of parental care, could ultimately affect food deliveries to chicks, and thus fitness (Crossin et al. 2012a). Post-laying female birds are known to have marked reductions in hematocrit levels, through the suppression of erythropoiesis that occurs as a consequence of estradiol-mediated vitel-

logenic processes during egg production (Kalmbach et al. 2004, Williams 2005, 2012, Wagner et al. 2008). This 'reproductive anemia' has been previously observed in egg-laying female macaroni penguins, who showed a >10% reduction in pre-laying to post-laying hematocrit (Crossin et al. 2010). Our results highlight the ecological relevance of this variation in the aerobic condition, and show how post-laying condition carries over to affect foraging success and, presumably, parental care. Physiological condition is known to generally decrease with bird age, which includes decreases in hematocrit (e.g. Kocan 1972, Fair et al. 2007, Elliott et al. 2015). We do not know the age of the penguins in our study, although they were all certainly breeding-age females. Despite this uncertainty, we found hematocrit to be significantly correlated with foraging behavior, but knowing age could potentially explain a great proportion of the observed variation among individuals. Future studies would thus benefit from knowledge about bird sex and age. Indeed, when integrated with well designed, experimental studies that examine the relationship between hematocrit and corticosterone in free-ranging wild birds, within the context of seasonal breeding and foraging behavior, this would do much to increase our understanding of the physiology controlling life-history variation.

Acknowledgements. We thank Stacey Adlard, Fabrice Le Bouard, David Haines, and Takashi Iwata for field assistance, and Andrew Wood for technical assistance. Financial support was provided by the British Antarctic Survey through an Antarctic Funding Initiative Collaborative Gearing Scheme. Additional support was provided by a Natural Science and Engineering Research Council of Canada (NSERC) post-doctoral fellowship to G.T.C., by an NSERC Discovery Grant to T.D.W., and by a JSPS KAKENHI grant (no. 20310016) to A.T. This study represents a contribution to the British Antarctic Survey Ecosystem Programme.

LITERATURE CITED

- Angelier F, Chastel O (2009) Stress, prolactin and parental investment in birds: a review. *Gen Comp Endocrinol* 163:142–148
- Barlow KE, Croxall JP (2002a) Seasonal and interannual variation in foraging range and habitat of macaroni penguins *Eudyptes chrysolophus* at South Georgia. *Mar Ecol Prog Ser* 232:291–304
- Barlow KE, Croxall JP (2002b) Provisioning behaviour of macaroni penguins *Eudyptes chrysolophus*. *Ibis* 144: 248–258
- Bost CA, Handrich Y, Butler PJ, Fahlman A, Halsey LG, Woakes AJ, Ropert-Coudert Y (2007) Changes in dive profiles as an indicator of feeding success in king and Adélie penguins. *Deep-Sea Res II* 54:248–255
- Bowers EK, Hodges CJ, Forsman AM, Vogel LA and others (2014) Neonatal body condition, immune responsiveness, and hematocrit predict longevity in a wild bird population. *Ecology* 95:3027–3034
- Breuner CW, Orchinik M (2002) Beyond carrier proteins: plasma binding proteins as mediators of corticosteroid action in vertebrates. *J Endocrinol* 175:99–112
- Brierley AS, Demer DA, Watkins JL, Hewitt RP (1999) Concordance of interannual fluctuations in acoustically estimated densities of Antarctic krill around South Georgia and Elephant Island: biological evidence of same-year teleconnections across the Scotia Sea. *Mar Biol* 134: 675–681
- Calbet JAL, Lundby C, Koskolou M, Boushel R (2006) Importance of hemoglobin concentration to exercise: acute manipulations. *Respir Physiol Neurobiol* 151:132–140
- Clutton-Brock TH (1991) The evolution of parental care. Princeton University Press, Princeton, NJ
- Cooke SJ, Schreer JF, Wahl DH, Philipp DP (2010) Cardiovascular performance of six species of field-acclimatized centrarchid sunfish during the parental care period. *J Exp Biol* 213:2332–2342
- Crossin GT, Trathan PN, Phillips RA, Dawson A, Le Bouard F, Williams TD (2010) A carryover effect of migration underlies individual variation in reproductive readiness and extreme egg size dimorphism in macaroni penguins. *Am Nat* 176:357–366
- Crossin GT, Trathan PN, Phillips RA, Gorman KB, Dawson A, Sakamoto KQ, Williams TD (2012a) Corticosterone predicts foraging behaviour and parental care in macaroni penguins. *Am Nat* 180:E31–E41
- Crossin GT, Phillips RA, Trathan PN, Fox DS, Dawson A, Wynne-Edwards KE, Williams TD (2012b) Migratory carryover effects and endocrinological correlates of reproductive decisions and reproductive success in female albatrosses. *Gen Comp Endocrinol* 176:151–157
- Crossin GT, Phillips RA, Wynne-Edward KE, Williams TD (2013) Postmigratory body condition and ovarian steroid production predict breeding decisions by female gray-headed albatrosses. *Physiol Biochem Zool* 86:761–768
- Croxall JP, Briggs DR, Kato A, Naito Y, Watanuki Y, Williams TD (1993) Diving pattern and performance in the macaroni penguin *Eudyptes chrysolophus*. *J Zool* 230: 31–47
- Davey C, Lill A, Baldwin J (2000) Variation during breeding in parameters that influence blood oxygen carrying capacity in shearwaters. *Aust J Zool* 48:347–356
- Dias MP, Granadeiro JP, Phillips RA, Alonso H, Catry P (2011) Breaking the routine: individual Cory's shearwaters shift winter destinations between hemispheres and across ocean basins. *Proc R Soc Lond B Biol Sci* 278:1786–1793
- Elliott KH, Hare JF, Le Vaillant M, Gaston AJ, Ropert-Coudert Y, Anderson WG (2015) Ageing gracefully: physiology but not behaviour declines with age in a diving seabird. *Funct Ecol* 29:219–228
- Erwin CA, Congdon BC (2007) Day-to-day variation in sea-surface temperature reduces sooty tern *Sterna fuscata* foraging success on the Great Barrier Reef, Australia. *Mar Ecol Prog Ser* 331:255–266
- Everson I (1976) Antarctic krill: a reappraisal of its distribution. *Polar Rec* 18:15–23
- Fair J, Whitaker S, Pearson B (2007) Sources of variation in haematocrit in birds. *Ibis* 149:535–552
- Gaston AJ, Gilchrist HG, Mallory ML, Smith PA (2009) Changes in seasonal events, peak food availability and

- consequent breeding adjustment in a marine bird: a case of progressive mis-matching. *Condor* 111:111–119
- Gjerdrum C, Vallée AMJ, St. Clair CC, Bertram DF, Ryder JL, Blackburn GS (2003) Tufted puffin reproduction reveals ocean climate variability. *Proc Natl Acad Sci USA* 100:9377–9382
- Green CJ, Trathan PN, Preston M (2006) A new automated logging gateway to study the demographics of macaroni penguins (*Eudyptes chrysolophus*) at Bird Island, South Georgia: testing the reliability of the system using radio telemetry. *Polar Biol* 29:1003–1010
- Green JA, Boyd IL, Woakes AJ, Warren NL, Butler PJ (2005) Behavioural flexibility during year-round foraging in macaroni penguins. *Mar Ecol Prog Ser* 296:183–196
- Guinet C, Koudil M, Bost CA, Durbec JP, Georges JY, Mouchot MC, Jouventin P (1997) Foraging behavior of satellite-tracked king penguins in relation to sea-surface temperatures obtained by satellite telemetry at Crozet Archipelago, a study during three austral summers. *Mar Ecol Prog Ser* 150:11–20
- Guinet C, Chastel O, Koudil M, Durbec JP, Jouventin P (1998) Effects of warm sea surface anomalies on the blue petrel at the Kerguelen Islands. *Proc R Soc Lond B Biol Sci* 265:1001–1006
- Hanson KC, Cooke SJ (2009) Nutritional condition and physiology of paternal care in two congeneric species of black bass (*Micropterus* spp.) relative to stage of offspring development. *J Comp Physiol B Biochem Syst Environ Physiol* 179:253–266
- Hipfner JM (2008) Matches and mismatches: ocean climate, prey phenology and breeding success in a zooplanktivorous seabird. *Mar Ecol Prog Ser* 368:295–304
- Hörak P, Jenni-Eiermann S, Ots I, Tegelmann L (1998) Health and reproduction: the sex-specific clinical profile of great tits (*Parus major*) in relation to breeding. *Can J Zool* 76:2235–2244
- Inchausti P, Guinet C, Koudil M, Durbec JP and others (2003) Inter-annual variability in the breeding performance of seabirds in relation to oceanographic anomalies that affect the Crozet and the Kerguelen sectors of the Southern Ocean. *J Avian Biol* 34:170–176
- John JL (1994) The avian spleen: a neglected organ. *Q Rev Biol* 69:327–351
- Jones JH (1998) Optimization of the mammalian respiratory system: symmorphosis versus single species adaptation. *Comp Biochem Physiol B Biochem Mol Biol* 120:125–138
- Kalmbach E, Griffiths R, Crane JE, Furness RW (2004) Effects of experimentally increased egg production on female body condition and laying dates in the great skua *Stercorarius skua*. *J Avian Biol* 35:501–514
- Kilgas P, Tilgar V, Mänd R (2006) Hematological health state indices predict local survival in a small passerine bird, the great tit (*Parus major*). *Physiol Biochem Zool* 79:565–572
- Kitaysky A, Kitaiskaia E, Piatt J, Wingfield J (2006) A mechanistic link between chick diet and decline in seabirds? *Proc R Soc Lond B Biol Sci* 273:445–450
- Kocan RM (1972) Some physiological blood values of wild diving ducks. *J Wildl Dis* 8:115–118
- Love OP, Breuner CW, Vezina F, Williams TD (2004) Mediation of a corticosterone-induced reproductive conflict. *Horm Behav* 46:59–65
- Mackintosh NA (1972) Life cycle of Antarctic krill in relation to ice and water conditions. *Discov Rep* 36:1–94
- Magrath RD (1991) Nestling weight and juvenile survival in the blackbird *Turdus merula*. *J Anim Ecol* 59:224–240
- Morrison KW, Hipfner JM, Blackburn GS, Green DJ (2011) Effects of extreme climate events on adult survival of three Pacific auks. *Auk* 128:707–715
- Murphy EJ, Watkins JL, Reid K, Trathan PN and others (1998) Interannual variability of the South Georgia marine ecosystem: biological and physical sources of variation in the abundance of krill. *Fish Oceanogr* 7:381–390
- Ots I, Murumägi A, Hörak P (1998) Haematological health state indices of reproducing great tits: methodology and sources of natural variation. *Funct Ecol* 12:700–707
- Peck DR, Smithers BV, Krockenberger AK, Congdon BC (2004) Sea surface temperature constrains wedge-tailed shearwater foraging success within breeding seasons. *Mar Ecol Prog Ser* 281:259–266
- Phillips RA, Silk JRD, Croxall JP, Afanasyev V, Bennett VJ (2005) Summer distribution and migration of nonbreeding albatrosses: individual consistencies and implications for conservation. *Ecology* 86:2386–2396
- Piersma T, Everaarts JM, Jukema J (1996) Build-up of red blood cells in refuelling bar-tailed godwits in relation to individual migratory quality. *Condor* 98:363–370
- Pinaud D, Weimerskirch H (2002) Ultimate and proximate factors affecting the breeding performance of a marine top-predator. *Oikos* 99:141–150
- Pinaud D, Cherel Y, Weimerskirch H (2005) Effect of environmental variability on habitat selection, diet, provisioning behaviour and chick growth in yellow-nosed albatrosses. *Mar Ecol Prog Ser* 298:295–304
- Potti J, Moreno J, Merino S, Frías O, Rodríguez R (1999) Environmental and genetic variation in the haematocrit of fledgling pied flycatchers *Ficedula hypoleuca*. *Oecologia* 120:1–8
- Quillfeldt P, Strange IJ, Masello JF (2007) Sea surface temperatures and behavioural buffering capacity in thin-billed prions *Pachyptila belcheri*: breeding success, provisioning and chick begging. *J Avian Biol* 38:298–308
- Richner H, Oppliger A, Christe P (1993) Effect of an ectoparasite on reproduction in great tits. *J Anim Ecol* 62:703–710
- Ropert-Coudert Y, Kato A, Baudat J, Bost CA, Le Maho Y, Naito Y (2001) Feeding strategies of free-ranging Adélie penguins (*Pygoscelis adeliae*) analysed by multiple data recording. *Polar Biol* 24:460–466
- Sakamoto KQ, Sato K, Ishizuka M, Watanuki Y, Takahashi A, Daunt F, Wanless S (2009) Can ethograms be automatically generated using body acceleration data from free-ranging birds? *PLoS ONE* 4:e5379
- Schwagmeyer PL, Mock DW (2008) Parental provisioning and offspring fitness: size matters. *Anim Behav* 75:291–298
- Takahashi A, Dunn MJ, Trathan PN, Croxall JP, Wilson RP, Sato K, Naito Y (2004) Krill-eating behaviour in a chinstrap penguin (*Pygoscelis antarctica*) compared with fish-eating in magellanic penguins (*Spheniscus magellanicus*): a pilot study. *Mar Ornithol* 32:7–54
- Thiebot JB, Lescroël A, Pinaud D, Trathan PN, Bost CA (2011) Larger foraging range but similar habitat selection in non-breeding versus breeding sub-Antarctic penguins. *Antarct Sci* 23:117–126
- Trathan PN, Croxall JP (2004) Marine predators at South Georgia: an overview of recent bio-logging studies. *Mem Natl Inst Polar Res Spec Issue* 58:118–132

- Trathan PN, Brandon MA, Murphy EJ (1997) Characterisation of the Antarctic Polar Frontal Zone to the north of South Georgia in summer 1994. *J Geophys Res* 102: 10483–10497
- Trathan PN, Brandon MA, Murphy EJ, Thorpe SE (2000) Transport and structure within the Antarctic Circumpolar Current to the north of South Georgia. *Geophys Res Lett* 27:1727–1730
- Trathan PN, Brierley AS, Brandon MA, Bone DG and others (2003) Oceanographic variability and changes in Antarctic krill (*Euphausia superba*) abundance at South Georgia. *Fish Oceanogr* 12:569–583
- Trathan PN, Green C, Tanton J, Peat H, Poncet J, Morton A (2006) Foraging dynamics of macaroni penguins *Eudyptes chrysolophus* at South Georgia during brood-guard. *Mar Ecol Prog Ser* 323:239–251
- Wagner EC, Stables CA, Williams TD (2008) Hematological changes associated with egg production: direct evidence for changes in erythropoiesis but a lack of resource-dependence. *J Exp Biol* 211:2960–2968
- Wagner PD (1996) Determinants of maximal oxygen transport and utilization. *Annu Rev Physiol* 58:21–50
- Waluda CM, Hill SL, Peat HJ, Trathan PN (2012) Diet variability and reproductive performance of macaroni penguins *Eudyptes chrysolophus* at Bird Island, South Georgia. *Mar Ecol Prog Ser* 466:261–274
- Whitehouse MJ, Meredith MP, Rothery P, Atkinson A, Ward P, Korb RE (2008) Rapid warming of the ocean at South Georgia, Southern Ocean during the 20th century: forcings, characteristics and implications for lower trophic levels. *Deep-Sea Res I* 55:1218–1228
- Williams TD (1995) *The penguins*. Oxford University Press, Oxford
- Williams TD (2005) Mechanisms underlying costs of egg production. *Bioscience* 55:39–48
- Williams TD (2012) *Physiological adaptations for breeding in birds*. Princeton University Press, Princeton, NJ
- Williams TD, Vezina F (2001) Reproductive energy expenditure, intraspecific variation and fitness. *Curr Ornithol* 16:355–405
- Williams TD, Challenger WO, Christians JK, Evanson M, Love O, Vezina F (2004) What causes the decrease in haematocrit during egg production? *Funct Ecol* 18: 330–336

*Editorial responsibility: Rory Wilson,
Swansea, UK*

*Submitted: November 28, 2014; Accepted: April 21, 2015
Proofs received from author(s): June 4, 2015*