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FEATURE ARTICLE

Diving efficiency at depth and pre-breeding foraging effort increase with haemoglobin levels in gentoo penguins

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ABSTRACT: Individual differences in oxygen storage and carrying capacity have been associated with fitness-related traits and, for air-breathing aquatic animals, to diving ability and foraging success. In winter, many seabirds must replenish the energy reserves they have depleted during the breeding period. Thus, winter foraging efficiency can influence their upcoming breeding behaviour. Using gentoo penguins *Pygoscelis papua* as a study species, we investigated (1) if inter-individual variation in diving efficiency (proportion of time spent at the bottom) is associated with indices of oxygen storage and carrying capacity (haemoglobin, haematocrit, body mass), and (2) if measures of pre-breeding foraging effort (mean trip duration, total time at sea, and vertical distance travelled) are associated with these oxygen indices and breeding status. Haemoglobin was positively correlated with diving efficiency, particularly for deeper dives, and only penguins with high haemoglobin levels frequently dove to depths ≥140 m. Such differences could affect resource access. However, because reaching deep offshore waters likely requires travelling more than foraging nearshore, vertical distance travelled during pre-breeding increased with haemoglobin levels. The relationship with haematocrit was non-linear, suggesting that commonly used analyses may be inappropriate for this index. We found that early-laying penguins spent less time at sea prior to nesting than non-breeding penguins, suggesting that more efficient foragers lay earlier. Given that diving efficiency at depth is linked to aerobic capacity, anthropogenic activities taking place in either nearshore or offshore waters (e.g. shallow-water fisheries, offshore oil rigs) may have differing impacts on individuals. Further understanding these links could help the conservation of diving species.

A gentoo penguin leaving the breeding colony for a foraging trip.
Painting: Sarah P. McComb-Turbitt

KEY WORDS: Diving efficiency · Oxygen storage capacity · Oxygen carrying capacity · Breeding participation · Gentoo penguin · Haemoglobin · Haematocrit · Body mass · Time–depth recorder

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1. INTRODUCTION

Intra-specific variation in foraging and movement behaviour is ubiquitous and can have important ecological consequences at both the individual and population levels (Bolnick et al. 2003, Ceia & Ramos 2015, Phillips et al. 2017, Shaw 2020). Such variation can be caused by complex relationships involving the environment, intra- and inter-specific interactions (e.g. competition), and physiological differences (Shaw 2020). Differences in oxygen storage and carrying capacity can be particularly important, as they have been associated with variation in movement behaviour, habitat selection, and fitness-related traits such as annual breeding success and arrival at breeding grounds (Crossin et al. 2013, 2015, Minias 2015). For diving animals, oxygen storage and carrying capacity can limit diving ability, and thus access to resources may be physiologically restricted (Roncon et al. 2018). For such animals, the time spent diving and the rate of mass gain have been linked to indices of aerobic capacity (Crossin et al. 2015).

Diving animals have a range of adaptations to maximize their aerobic capacity and extend their breath-holding capability (Roncon et al. 2018). Haemoglobin (hereafter Hb) is an oxygen-carrying protein present in red blood cells, and thus its level is a crucial determinant of the rate of oxygen delivered to tissues (Minias 2015). Hb levels are often used as a proxy for aerobic capacity and physiological condition in birds (Minias 2015), and these levels are elevated in bird species that dive (Minias 2020). Prolonged breath-holding capacity is often linked to oxygen storage. Oxygen stores typically increase with Hb concentration, blood volume, myoglobin concentration in skeletal muscles, muscle mass, and lung volume (Mirceta et al. 2013, Roncon et al. 2018). As larger individuals possess more blood and muscle, body mass is often positively associated with oxygen storage capacity (Ponganis & Kooyman 2000, Cook et al. 2013, Mirceta et al. 2013, Polito et al. 2015, Camprasse et al. 2017). In addition, consistent with generally observed allometric patterns of metabolism, larger body mass is often associated with lower oxygen usage per unit mass (Glazier 2005, Hudson et al. 2013). As such, total blood Hb concentration, haematocrit (percentage of red blood cells in blood, hereafter Hct), and body mass can be important determinants of a breath-holding diver's physiological performance and can limit an animal's diving ability (Crossin et al. 2015, Chimienti et al. 2017).

Seabirds display high levels of individual variation in foraging and movement (Ceia & Ramos 2015, Phillips et al. 2017), and diving seabirds provide a unique opportunity to link differences in oxygen storage and carrying capacity to variation in diving and movement behaviour and, ultimately, to differential breeding participation. Many seabirds severely deplete their energy reserves during breeding and moulting periods and must replenish these reserves by foraging intensively during winter (Sorensen et al. 2009, Crossin & Williams 2016). Consequently, how efficient an individual is in acquiring food in winter can influence multiple aspects of its breeding capacity and behaviour (Daunt et al. 2006, Shoji et al. 2015). For example, individuals that spend less time foraging in winter, and thus are assumed to be more efficient foragers, breed earlier (Daunt et al. 2006). Understanding how individuals vary in their foraging efficiency and behaviour as they prepare for breeding can help assess the possible impacts that natural and anthropogenic ecosystem changes can have on populations (Durell 2000, Bearhop et al. 2006, Cury et al. 2011, Phillips et al. 2017).

Here, using a population of gentoo penguins *Pygoscelis papua*, we explore how individual variation in winter diving behaviour relates to indices of oxygen storage and carrying capacity, and how these relate to their breeding status. We used gentoo penguins because they show considerable individual variation in winter movement behaviour (Baylis et al. 2021). Gentoo penguins consume a wide variety of locally available prey that are found at a range of depths down to approximately 200 m (Tanton et al. 2004, Thiebot et al. 2011, Camprasse et al. 2017). While they are considered a generalist species, many individuals have a specialized diet and foraging behaviour (Polito et al. 2015, Camprasse et al. 2017, Handley et al. 2017, Herman et al. 2017). There is a high likelihood of intraspecific competition for prey around colonies, as gentoo penguins are not migratory and thus forage year-round relatively close to their breeding colonies (Clausen et al. 2005, Bearhop et al. 2006, Kokubun et al. 2010). In addition, given their magnitude, certain commercial fisheries in the Falkland Islands could reduce the availability of important prey species in winter (Clausen & Pütz 2003). The number of breeding pairs in the Falkland Islands fluctuates drastically, with changes of 50% occurring in a single year (Pistorius et al. 2010, Stanworth & Crofts 2019). Changes in population breeding success are thought to be linked to prey availability (Pistorius et al. 2010) and broad-scale climatic variation (Baylis et al. 2012).

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To understand the link between oxygen storage and carrying capacity, pre-breeding diving and foraging behaviour, and breeding activity, we investigated (1) if diving efficiency (proportion of the time within a dive spent at the bottom) is associated with indices of oxygen storage and carrying capacity (Hb, Hct, body mass), and (2) if measures of pre-breeding foraging effort (mean trip duration, total time at sea, and vertical distance travelled) differ between individuals based on their breeding status and indices of oxygen storage and carrying capacity. We used time–depth recorders (TDRs) to quantify diving efficiency and foraging effort. As attaching such devices to animals can add drag and affect their performance, behaviour, and reproductive success (Taylor et al. 2001, Beaulieu et al. 2010, van der Hoop et al. 2014, Wilson et al. 2015), we also evaluated whether tagged individuals differ from control birds in terms of breeding activity, Hb, Hct, and mass.

2. MATERIALS AND METHODS

2.1. Data collection

We captured 66 adult gentoo penguins post-moult (4–10 April 2018) from 2 colonies in the Falkland Islands: Rookery Sands, Race Point (51.4345° S, 59.1081° W; n = 33) and Tamar Point, Pebble Island (51.3241° S, 59.4523° W; n = 33). We weighed each captured penguin using a 10 kg Pesola scale, took a blood sample (<1.5 ml) from the left brachial vein using heparinized syringes fitted with 25-gauge needles, and marked them with black hair dye (L'Oréal Preference) to facilitate re-identification. We equipped each penguin with a Sirtrack K2G 173A SWS KIWISAT 202B Argos tag (34 g, 55 × 27 × 17 mm; Sirtrack) and a Lotek LAT1800 TDR tag (13.6 g, 62 × 13 × 13 mm, Lotek Wireless). As in Handley et al. (2018) and Baylis et al. (2021), we attached the 2 devices to midline back feathers using overlapping layers of TESA® tape (Beiersdorf) and sealed the tape seams with cyanoacrylate glue (see Supplement 1 at www.int-res.com/articles/suppl/m722p001_supp.pdf).

During the breeding season (27 September–21 October 2018), we recaptured 35 of the previously tagged penguins and 35 unmarked penguins, the latter serving as controls in our analysis of the potential effects of tagging (Table 1). As described above, we weighed each penguin, took a blood sample, and marked the control bird with hair dye. We retrieved any devices that were still attached to the penguins. A total of 21 penguins had TDR devices still attached (7 Race Point, 14 Pebble Island; Table 1), one of which still had an Argos tag attached (from Race Point). We were unable to collect sufficient blood samples from 2 individuals with a TDR tag, resulting in only 19 of the individuals with TDR data also having blood data from the breeding period (5 Race Point, 14 Pebble Island; Table 1).

The research was conducted under Falkland Islands Scientific Research Licence (R12/2017) and conformed to guidelines from the Canadian Committee on Animal Care (University of British Columbia Animal Care Permit A17-0243 and Dalhousie University Animal Care Permit 17-100).

2.2. Indices of oxygen storage and carrying capacity

Using the blood samples from the breeding period, we used Hct and Hb as physiological indicators of blood oxygen storage and carrying capacity. As Hct

Table 1. Number of individual gentoo penguins tagged, recaptured, and sampled as controls. The number of individuals that were captured and recaptured at each colony, the number of individuals for which we had blood samples, the number that could be sexed, and the female-to-male sex ratio (F:M) are shown. For the recaptured penguins, the number of individuals for which the TDR tags were still attached at recapture are shown in brackets

	Tagged	Recaptured				Control		
		Recaptured (with tag)	Blood (with tag)	Sexed (with tag)	F:M (with tag)	Blood	Sexed	F:M
Race Point	33	16 (7)	13 (5)	11 (4[a])	2:9 (0:4)	15[b]	14	6:8
Pebble Island	33	19 (14)	19 (14)	18 (13)	10:8 (5:8)	20	19	7:12
Total	66	35 (21)	32 (19)	29 (17)	12:17 (5:12)	35[b]	33	13:20

[a]One individual that was sexed did not have blood at recapture; it was sexed based on blood at capture. As we use blood at recapture for the indices of oxygen storage and carrying capacity, this individual could not be included in the final GAMM modelling, the relationship between diving efficiency or indices of oxygen storage and carrying capacity

[b]We were unable to measure the Hb of one penguin and the Hct of another penguin

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is based on blood cytology, and Hb is based on blood biochemistry, each measure provides different insights into the aerobic capacity of individuals (Fair et al. 2007, Kaliński et al. 2011, Minias 2015). Hct (percent packed red blood cell volume) was determined from fresh whole blood in heparinized capillary tubes centrifuged for 5 min (centrifuge; Brinkmann Instruments) at 10 000 × *g* and measured using digital callipers (±0.01 mm). Hb (total blood haemoglobin concentration in g dl^{-1}) was determined using the cyanmethemoglobin method (Drabkin & Austin 1932) in which 5 µl of fresh whole blood was pipetted (Gilson Pipetman P2; Gilson Medical Electronics) into 1.25 ml Drabkin's reagent (D541-6V, Brij 35 solution B4184-100ml; Sigma-Aldrich). We ran triplicate samples and read absorbance at 540 nm with a microplate spectrophotometer (BioTek PowerWave 340). The inter-assay coefficient of variation was 1.70% between plates.

We used the body mass measured during the breeding period as a proxy for oxygen storage capacity. We did not scale body mass to structural body size to generate a morphometric index because (1) oxygen stores (e.g. blood volume) and dive duration have been shown to increase with body mass itself (e.g. Hassrick et al. 2010), and (2) mass alone has been shown to be a more reliable measure of body condition in birds (Schamber et al. 2009, Labocha & Hayes 2012). We identified the sex of the individuals by sending blood samples to Viaguard (https://www.accu-metrics.com/animal-services/p/avian-dna-bird-sexing-test), which performs DNA testing. Sex results were unavailable for 3 of the 19 penguins for which we had TDR and blood data (Table 1).

2.3. Dive data processing

The TDR tags recorded depth (0.25 m resolution), temperature (0.01°C resolution), and wet or dry state at 1 s intervals. We wanted to focus on the time period that could be linked to the blood samples. Since the maximum life span of red blood cells in birds is estimated to be 35–45 d (Rodnan et al. 1957), we limited the analyses to dives made within 40 d before recapture. Given that the last Argos transmission was 16 August 2018, well before this threshold, and very few tags transmitted locations past May, we do not consider the Argos data further.

To characterise the diving behaviour of foraging individuals, we extracted the dive metrics from the TDR series using the software Divebomb v.1.0.7 (Nunes 2019) in Python v.3.4.10 (Python Software Foundation 2019). We first corrected the dive data for drift by using a zero offset (Luque & Fried 2011), and we identified the following dive metrics: start of the dive, dive duration, maximum depth, bottom time, and post-dive surface interval. To remove the shallow dives that were most likely associated with travelling, we discarded all dives shallower than 5 m (Kokubun et al. 2010, Lee et al. 2015, Carpenter-Kling et al. 2017). Following Kokubun et al. (2010) and Lee et al. (2015), we eliminated the inclusion of long surface periods that were unlikely to be associated with active foraging by only considering dives with a post-dive surface interval of <200 s. Through a visual assessment of 100 random dives across all individuals, we quantified that Divebomb gave precise measurements of the descent, bottom, ascent, and surface phases of dives 94% of the time. Divebomb produced minor errors distinguishing the bottom phase in cases where undulations in depth occurred over the transition from descent to ascent phases.

2.4. Behavioural observations

To assess the reproductive status of penguins, teams of 2 observers monitored the breeding behaviour of marked birds. Through daily colony scans and the continuous monitoring of arriving individuals, we identified which individuals were present at Race Point from 27 September 2018 to 24 October 2018, and at Pebble Island from 29 September 2018 to 24 October 2018. Between 13:00 and 19:00 h daily, we conducted focal follows on each individual observed in the colony. The focal follows were at least 5 min long and lasted until the presence or absence of eggs in an occupied nest could be confirmed. The pair-bonding behaviours we monitored included nest building, copulation, pair calling, and pair bowing (Williams 1995). Gentoo penguins in the Falkland Islands lay their first egg from mid-October to early November (Otley et al. 2004, Black et al. 2018). As we left the field site before we could acquire the lay date of many penguins, we classified penguins into 3 categories of reproductive status: non-breeding, breeding, and early-laying individuals. Penguins that were classified as non-breeders were found most often at different locations than nesting penguins, typically towards the edge of colonies, and rarely displayed pair-bonding behaviours. Penguins that were categorized as breeders were observed conducting pair-bonding behaviours at stable nest site

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locations. Early-laying penguins were observed to be incubating at least one egg prior to 24 October 2018. We collected data from individuals of both sexes in each reproductive category, and none of the penguins had laid an egg at Race Point at the time of departure of field observers.

2.5. Linking diving efficiency to oxygen storage and carrying capacity indices

We used a generalized additive mixed model (GAMM) to determine the relationship between diving efficiency at different maximum depths and our 3 previously described indices of oxygen storage and carrying capacity (Hb, Hct, and body mass). We defined diving efficiency as: bottom time/(dive duration + post-dive surface interval) (Simeone & Wilson 2003, Lescroël & Bost 2005, Lee et al. 2015). Using the R package 'mgcv' (Wood 2011), we used a thin-plate regression spline to model the non-linear relationship between diving efficiency and maximum dive depth. To investigate the potential interactions between maximum depth and the indices of oxygen storage and carrying capacity (Hb, Hct, and mass), we included a tensor product term (with cubic regression spline basis) between maximum depth and each index. To be able to decompose the interactions with maximum depth from the potential main effects of each index, we included a separate tensor product and a thin-plate regression spline for the relationship with diving efficiency and each index (i.e. using s() + ti() in 'mgcv'). To account for potential biological or behavioural differences in sex and colony, we first included both terms as fixed effects in our GAMM and assessed their importance. As sex was not deemed an important covariate for this analysis (see Supplement 2), and sex information was missing for 3 individuals, our final analysis did not include sex and included these 3 individuals. Thus, our final GAMM was applied to the data of 19 individuals. We also included an individual ID random effect on the intercept to account for the fact that we had data from multiple dives per penguin.

The diving data were highly temporally autocorrelated, and fitting GAMMs with complex autoregressive-moving-average (ARMA) functions is computationally demanding. For example, running our full GAMM with an ARMA function took multiple days to run on an iMac 3.6 GHz 10-Core Intel Core i9, and we ran into multiple convergence problems. Thus, we assessed how much sub-sampling was required to remove the autocorrelation in the residuals of the full model and found that we had to keep only 1 dive every 70 dives to completely remove the autocorrelation in the residuals. We used this 70-dive sub-sampling scheme, which resulted in retaining on average 85 dives per individual. To identify which of the covariates of the GAMM should be selected, we used the shrinkage method with a double penalty (i.e. select = 'TRUE' in 'mgcv'; Marra & Wood 2011), which penalizes both unnecessary wiggliness and linear effect of a covariate. This approach has many advantages over stepwise variable selection and model subset selection, such as not limiting the combinations of covariates explored, and has been previously used to select covariates in diving models (Marra & Wood 2011, Photopoulou et al. 2014).

There was some correlation and concurvity between the covariates. As such, we explored whether, and how, the results changed when creating GAMMs with only one of the important oxygen indices at a time (Supplement 3 for details). In addition, the residuals of the final GAMM displayed evidence of heteroskedasticity. The increase in variance can be attributed to the fact that our final GAMM modelled proportion data (as defined, diving efficiency ranges between 0 and 1) with a normal error distribution. As such, we also explored whether, and how, the results changed if we used a GAMM with a beta distribution. Supplement 4 details how the results remained largely unchanged.

As post-hoc analyses, we further explored how the deep-diving behaviour of individuals changed in relation to the oxygen storage and carrying capacity indices that were deemed important in the main GAMM above. As some individuals with low oxygen storage and carrying capacity may reduce, or altogether forgo, deep dives (here defined as ≥140 m), we used simple generalized additive models (GAMs) to assess whether the proportion of deep dives that each individual performed changed in relation to each of the selected oxygen indices. As high diving efficiency can be achieved by either extending the bottom time or reducing surface recovery between dives, we used new GAMMs to explore how bottom time and post-dive surface intervals for deep dives changed in relation to each of the selected oxygen indices. For these new GAMMS, we examined the oxygen indices separately, as the smaller sample size did not allow us to have both in the models at once. We included an individual ID as a random effect to account for the fact that we had data from more than 1 dive per penguin.

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2.6. Associating measures of foraging effort with breeding status and oxygen storage and carrying capacity indices

To determine whether pre-breeding foraging behaviour changes between individuals based on their breeding status and oxygen storage and carrying capacity, we investigated 3 measures of foraging effort: mean trip duration, time at sea, and vertical distance travelled. To estimate mean trip duration and time at sea, we first identified trips at sea using the binary wet–dry data collected with the saltwater sensor of the TDR tags. To account for occasional jumps at sea, which results in brief recordings of dry conditions, we averaged the wet–dry data using a 10 min rolling window. We classified any window value with an average >0 as wet. We terminated a trip just before a dry period lasting at least 1 h, and the subsequent trip began once a new wet value was recorded. The mean trip duration was the average duration of trips for the 40 d prior to recapture, while the time spent at sea was the sum of all trip durations within that period. Our last measure of foraging effort was the total amount of vertical distance travelled for the time spent at sea (Σmaximum depth (km) × 2; similar to Lescroël & Bost 2005, Booth et al. 2018).

Using multiple linear regressions, we investigated whether each of these measures of foraging effort (mean trip duration, time at sea, and vertical distance travelled) was associated with breeding status, sex, and the 3 oxygen storage and carrying capacity indices (Hb, Hct, mass). We only included individuals from Pebble Island, since none of the Race Point individuals laid eggs before the end of the field season. To identify the covariates that best explained the data, we fitted models with all possible combinations of 3 of these covariates. Given the small sample size (n = 13), we compared the models using Akaike's information criterion with correction for small sample size (AICc). We considered models that outperformed the null model (model with no covariates) and had ΔAICc < 4 from the best model (Burnham & Anderson 2002). Next, we used Wilcoxon rank sum tests to assess whether there were differences in oxygen indices across sex and breeding status. We used a Bonferroni correction to account for the multiple comparisons.

2.7. Assessing the potential effects of tagging

To limit the potential influence of our tags on the behaviour, energy expenditure, or condition of the animals (e.g. Wilson et al. 1986, Vandenabeele et al. 2015), we minimized drag by using tags with a streamlined design and attaching them in an appropriate caudal position (Bannasch et al. 1994). Furthermore, the overall weight of devices was <1% of the average body mass recorded, and similar devices have not affected the diving behaviour of gentoo penguins (Kokubun et al. 2010). However, especially because we deployed tags for an extended period, we wanted to assess whether the data for the tagged penguins were representative of the untagged animals. To look for signs of tagging effects, we compared the physiology at breeding and breeding status of tagged to untagged penguins. Specifically, we used Welch 2-sample *t*-tests to compare Hb, Hct, and mass at breeding. For the reproductive status, we used χ^2 goodness-of-fit tests, and because of the small sample size (37 individuals for 6 categories), we estimated the p-value using a Monte Carlo method (Hope 1968). We were unable to sample sufficient blood for 3 of the recaptured penguins and were unable to measure Hct for one untagged penguin and Hb for another untagged individual. Thus, while the mass comparison included 35 tagged and 35 untagged individuals, the comparisons for Hct and Hb included only 32 tagged and 34 untagged individuals. In addition, for the comparison of reproductive status, we focussed only on penguins from Pebble Island, since none of the penguins at Race Point laid an egg before the end of our field season. Finally, we also quantified the proportion of tagged penguins that increased in mass between capture and recapture and the average weight gain during that period.

All data analyses were performed using R v.4.2.1 (R Core Team 2022). Values presented in the results are mean ± SD unless otherwise stated, and statistical tests were considered significant at a 0.05 level. The code and data needed to reproduce the analyses are found on Github (https://github.com/MarieAugerMethe/MEPS_McCombTurbitt_etal_2023_data_code).

3. RESULTS

3.1. Relationships between diving efficiency and indices of oxygen storage and carrying capacity

Our GAMM indicated that diving efficiency had statistically significant relationships with colony as well as with the maximum depth of a dive and its interaction with Hb and Hct (Table 2, Fig. 1). Diving efficiency was highest at intermediate depths followed by a decline at deeper depths (Fig. 1A). The interaction between maximum depth and Hb showed

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Table 2. Final generalized additive mixed model (GAMM) for gentoo penguin diving efficiency. We present the estimate and SE of the parametric coefficient (intercept and fixed effect), as well as the associated test statistic and p-value. We also present the estimated degrees of freedom (edf), test statistic, and p-value associated with the smooth terms of the GAMM. For model selection, we used the shrinkage method with a double penalty, which allows non-important terms to be shrunk to null. A total of 19 penguins were included in this analysis (5 from Race Point; 14 from Pebble Island). Hct: haematocrit; Hb: haemoglobin

Term	Parametric coefficient				Significance of smooth term		
	Estimate	SE	*t*	p	edf	*F*	p
Intercept	0.295	0.006	47.606	<0.001			
Colony (Race Point)	0.034	0.014	2.474	0.014			
s(Depth)					7.856	33.369	<0.001
s(Mass)					1.000	0.005	0.943
s(Hct)					1.000	2.302	0.129
s(Hb)					1.000	0.140	0.708
ti(Depth, Mass)					1.000	2.613	0.106
ti(Depth, Hct)					10.675	3.985	<0.001
ti(Depth, Hb)					9.683	5.110	<0.001

that, while penguins with higher Hb did not appear to have higher diving efficiency during shallow dives, diving efficiency increased with Hb in deeper dives (Fig. 1B). In the post-hoc analyses, the relationship between bottom time for deep dives and Hb was positive and statistically significant, while the relationship between Hb and post-dive surface interval of deep dives was not statistically significant (Table 3). The results of these post-hoc analyses indicate that this increase in diving efficiency with Hb at deeper depth is primarily explained by an increase in bottom time rather than a decrease in the post-dive surface interval (Fig. 2B, C). In addition, we found that the proportion of deep dives (≥140 m) made by an individual increased with Hb

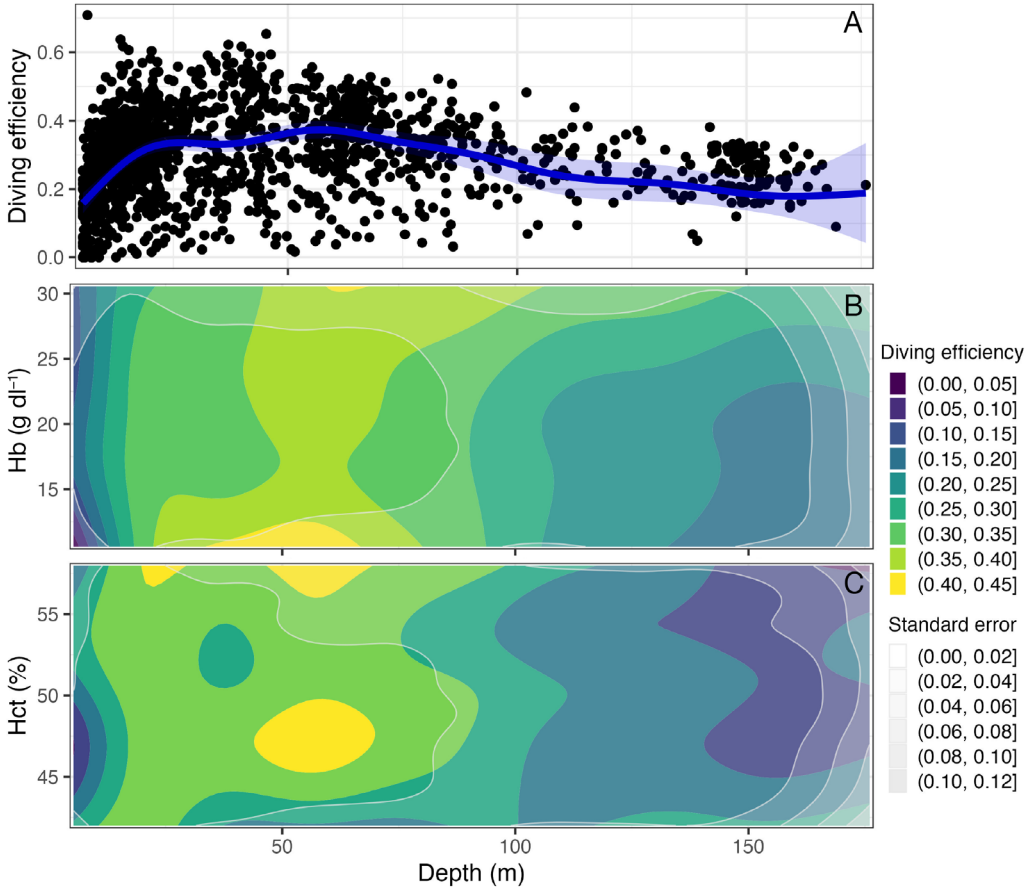

Fig. 1. Slices of predictions from the final generalized additive mixed model. (A) Change in predicted gentoo penguin diving efficiency in response to varying maximum depth values of a dive. Blue band: 95% CI; points: observed values. (B,C) Predicted change in diving efficiency with both (B) depth and haemoglobin (Hb) and (C) depth and haematocrit (Hct). For (B) and (C), colour represents diving efficiency (yellow: highest efficiency). The grey bands represent the SE (more opaque grey: higher SE) and areas with larger SE values (i.e. more opaquely shaded) should be interpreted more cautiously. A total of 19 penguins were included in this analysis (5 from Race Point; 14 from Pebble Island)

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Table 3. Parameter estimates and test statistic values for the post hoc generalized additive models (GAMs) relating the proportion of deep dives to each haemoglobin (Hb) and haematocrit (Hct) value obtained from gentoo penguins, as well as for the post hoc generalized additive mixed models (GAMMs) relating either post-dive surface interval of deep dives or bottom time of deep dives to each Hb and Hct. For the proportion of deep dives, 19 penguins were included in the GAMs (5 from Race Point; 14 from Pebble Island). For the post-dive surface interval and bottom time, only 16 were included in the GAMMs because 3 individuals did not make dives ≥140 m

Term	Parametric coefficient				Significance of smooth term		
	Estimate	SE	*t*	p	edf	*F*	p
Proportion of deep dives (Hb)							
Intercept	0.064	0.015	4.122	0.001			
Colony (Race Point)	0.019	0.035	0.544	0.595			
s(Hb)					2.999	3.862	<0.001
Post-dive surface interval (Hb)							
Intercept	117.404	8.353	14.055	<0.001			
Colony (Race Point)	−7.190	19.744	−0.364	0.717			
s(Hb)					1	0.670	0.415
Bottom time (Hb)							
Intercept	91.018	2.808	32.41	<0.001			
Colony (Race Point)	2.964	7.808	0.38	0.705			
s(Hb)					1	6.498	0.012
Proportion of dives (Hct)							
Intercept	0.074	0.024	3.086	0.007			
Colony (Race Point)	−0.019	0.046	−0.400	0.694			
s(Hct)					0.653	0.189	0.127
Post-dive surfaces interval (Hct)							
Intercept	119.317	7.942	15.023	<0.001			
Colony (Race Point)	6.773	16.444	0.412	0.681			
s(Hct)					1	1.39	0.241
Bottom time (Hct)							
Intercept	90.738	3.543	25.613	<0.001			
Colony(Race Point)	−9.817	7.771	−1.263	0.209			
s(Hct)					1	0.401	0.528

(Fig. 2A), a relationship that was statistically significant (Table 3).

The non-linear interaction between maximum depth and Hct was more complex, with multiple and less easily interpretable peaks in diving efficiency across Hct values (Fig. 1C). The post-hoc analyses revealed that neither bottom time, nor post-dive surface interval, nor the proportion of deep dives showed a statistically significant relationship with Hct (Table 3).

Individuals displayed variations in diving and foraging behaviours beyond those described for diving efficiency. For example, the maximum depth reached by individuals during the 40 d ranged from 74.8 to 217.8 m, and trips at sea ranged from <1 d to 24 d. We provide a general description of dive characteristics, foraging effort measures, and oxygen storage and carrying capacity indices in Supplement 5.

3.2. Foraging effort relationships with oxygen storage and carrying capacity indices, sex, and reproductive status

Two pre-breeding foraging effort measures were associated with oxygen storage and carrying capacity indices, sex, and reproductive status (Fig. 3, Table 4). The best model for vertical distance travelled indicated that it increased with Hb, and the best model for time at sea suggested that early-laying penguins had spent less time at sea prior to capture than breeding and non-breeding penguins (Fig. 4, Table 4, Supplement 6). However, Table 4 shows that other models had ΔAICc < 4, including the null model for vertical distance travelled. The presence of many competitive models is likely explained by the small sample size (13 individuals) and some of the relationships that we observed between covariates. For example, given that the 8 males in our samples had a significantly lower average Hb level than the 5 females (W = 34, p = 0.045, n = 13; Fig. 4A), it is not surprising that the second-best model for vertical distance travelled showed that males travelled less than females. Similarly, early-laying penguins had, on average, a lower Hb level than non-breeding penguins (Fig. 4B). Although this difference was not significant (W = 15, p = 0.057, n = 8), it may contribute to the fact that the second-best model for time at sea did not include breeding status but did include a positive relationship with Hb. Average trip duration had the null model as the best model (Table 4), and thus was not considered further. We note that a small sample increases the chance of making a Type II error (failing to reject the null hypothesis when it is false), and that AICc strongly penalizes model complexity; thus, with a larger sample size, more complex relationships may have been detected.

3.3. Potential effects of tagging

Almost all (32 out of 35) tagged penguins that were recaptured had gained weight during the winter, with an average gain of 1.1 ± 0.7 kg. However,

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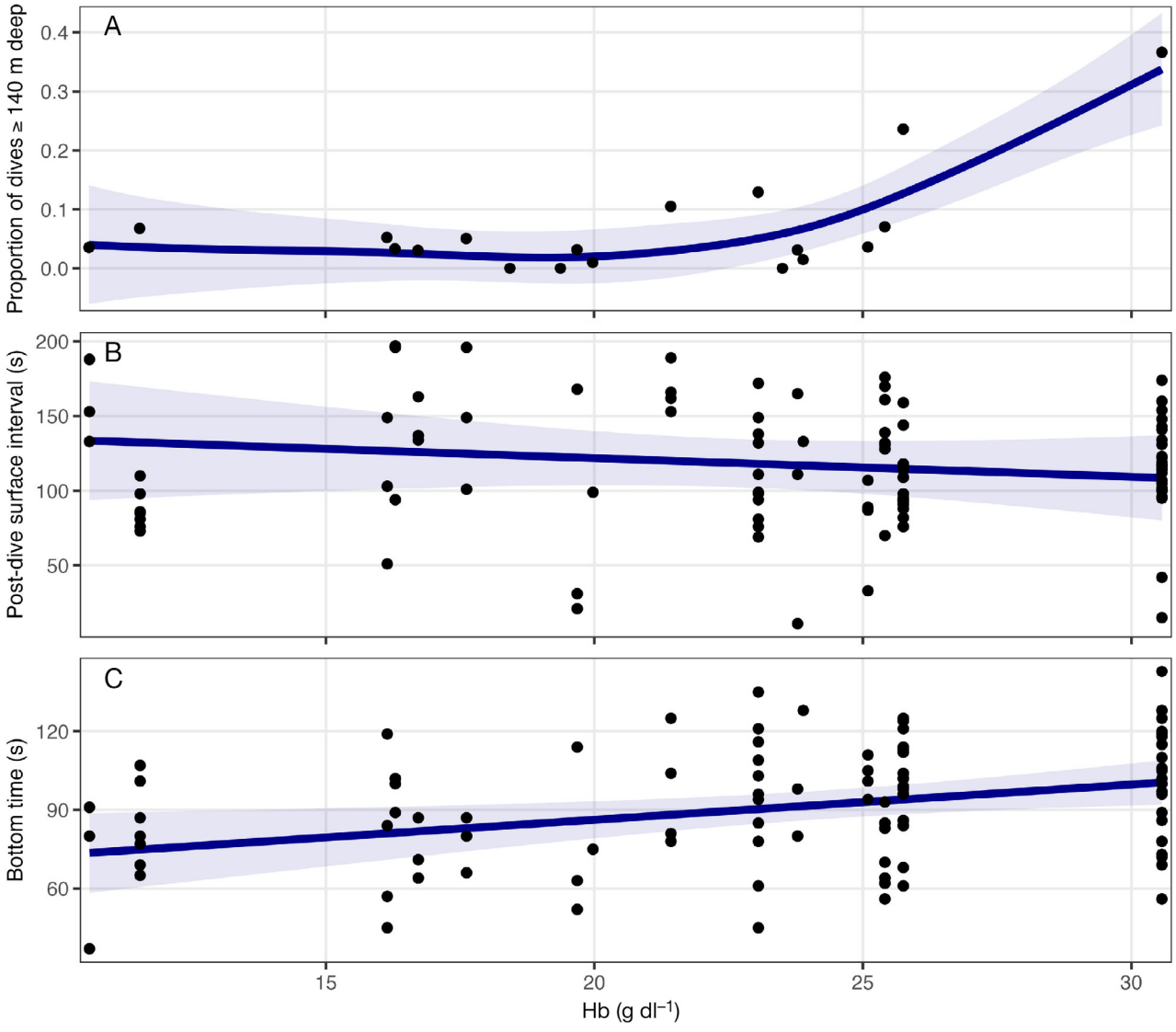

Fig. 2. Association between haemoglobin (Hb) and (A) the proportion of dives an individual gentoo penguin made at deeper depth (≥140 m) as well as changes in (B) post-dive surface intervals and (C) bottom times of the deep dives. A total of 19 penguins were included in the analysis from (A) (5 from Race Point; 14 from Pebble Island), but only 16 were included in (B) and (C), because 3 individuals did not make dives ≥140 m. Blue lines: predicted value; blue shading: 95% CI; points: observed values

on average, the tagged penguins were significantly lighter at recapture than the untagged penguins (tagged penguins: 6.9 ± 0.6 kg, 5.7–8.2 kg; untagged penguins: 7.3 ± 0.5 kg, 6.2–8.8 kg; $t_{66.498}$ = 2.812, p = 0.006, n = 70). We did not find any other significant differences between tagged and untagged penguins. Their Hb and Hct values were similar (Hb: $t_{62.863}$ = −0.524, p = 0.602, n = 66; Hct: $t_{63.633}$ = 1.480, p = 0.144, n = 66), and their breeding status did not differ significantly (χ^2 = 1.167, simulated p = 0.604, n = 39). However, given the small sample size for the breeding status analysis (tagged: 18; untagged: 19), we note that compared to the untagged penguins, there was a higher percentage of tagged penguins that did not participate in breeding and a lower percentage of tagged penguins that laid early (Supplement 7). Overall, our results suggest that it is unlikely that the relationships we found with blood oxygen storage and carrying capacity indices (i.e. Hb and Hct) are affected by tagging, but that tagging is associated with reduced mass, and the effects of tagging on breeding behaviour and reproductive success should be further explored. See Supplement 7 for more details.

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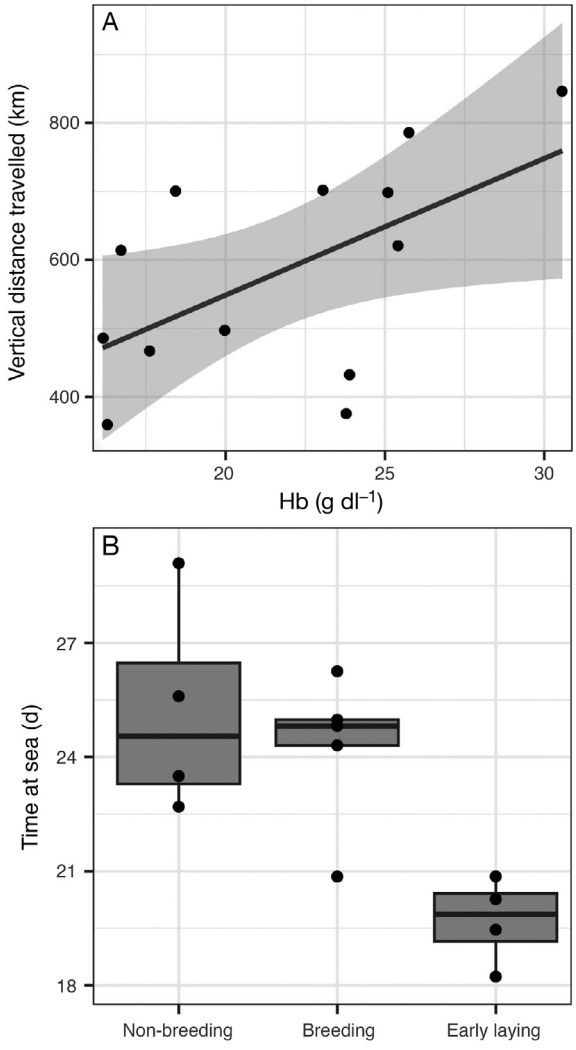

Fig. 3. Relationship between haemoglobin (Hb) and reproductive status and 2 of the pre-breeding foraging effort measures for the gentoo penguins from Pebble Island: (A) sum of the vertical distance travelled while at sea and Hb; line: linear regression; shading: 95% CI; and (B) time spent at sea and reproductive status. The middle bar represents the median and the lower and upper hinges represent the first and third quartiles, respectively. Whiskers extend to the lowest or largest value within 1.5 times the inter-quartile range from the lower or upper hinge, respectively. Points beyond the whiskers are outliers. In both (A) and (B), points are observations. A total of 13 penguins (4 non-breeding; 5 breeding; 4-early laying penguins from Pebble Island) were included in these analyses

Table 4. Model selection results for the multiple linear regressions relating 3 measures of gentoo penguin foraging effort to oxygen store indices, sex, and breeding status. We present the number of parameters estimated (k), Akaike's information criterion with correction for small sample size (AICc), and the difference in AICc from the best model (ΔAICc). While we explored all possible combinations of 3 covariates for each measure, we only show in the table the null model (no covariates) and models that are either within ΔAICc < 4 of the best model (model with lowest AICc value) or all the models that outperformed the null model if the null model had ΔAICc ≥ 4. Note that no models with breeding status are included in the table for vertical distance travelled and trip duration, as none of them had a ΔAICc small enough to be part of the subset presented. A total of 13 penguins (all from Pebble Island) were included in these analyses. Hb: haemoglobin; Hct: haematocrit

Response	Covariates	k	AICc	ΔAICc
Vertical distance travelled	Hb	3	171.0	–
	Sex	3	171.4	0.34
	Hct	3	172.6	1.56
	(Null)	2	172.7	1.69
	Hb + Hct	4	173.8	2.76
	Hb + Sex	4	173.8	2.78
	Hct + Sex	4	173.9	2.92
Time at sea	Breeding status	4	66.0	–
	Hb + Hct + Mass	5	67.1	1.17
	Hb + Mass	4	67.9	1.91
	Hb	3	68.3	2.32
	Breeding status + Mass	5	69.8	3.82
	Breeding status + Sex	5	70.3	4.36
	Breeding status + Hb	5	70.4	4.47
	(Null)	2	70.5	4.51
Trip duration	(Null)	2	59.0	–
	Hct	3	59.4	0.40
	Hb + Hct	4	60.4	1.48
	Sex	3	61.8	2.82
	Hb	3	62.0	3.00
	Mass	3	62.3	3.39
	Hct + Mass	4	62.8	3.83

4. DISCUSSION

Our results show that the diving efficiency of gentoo penguins is related to Hb, with the efficiency of deep dives (≥140 m) increasing with Hb level. This increase in efficiency can be explained by the fact that Hb limits the total amount of oxygen able to be carried by the blood (Minias 2015, Roncon et al. 2018). Of the factors that could have affected diving efficiency, higher Hb was primarily associated with an increase in the bottom time of the dive rather than reduced recovery time (i.e. no decrease in post-dive surface interval with increased Hb levels was observed). Gains in diving efficiency and associated increased time at the bottom are likely important to gentoo penguins, as they increase the time available to search for and capture prey during foraging dives. As prey are almost always encountered during the bottom phase of dives (Simeone & Wilson 2003, Takahashi et al. 2004, 2008, Kokubun et al. 2010), increased bottom time translates into time spent where prey is most likely to be encountered. For dives of ≥140 m, penguins with high Hb (20–31 g dl^{-1}) increased their mean bottom phase by 22%

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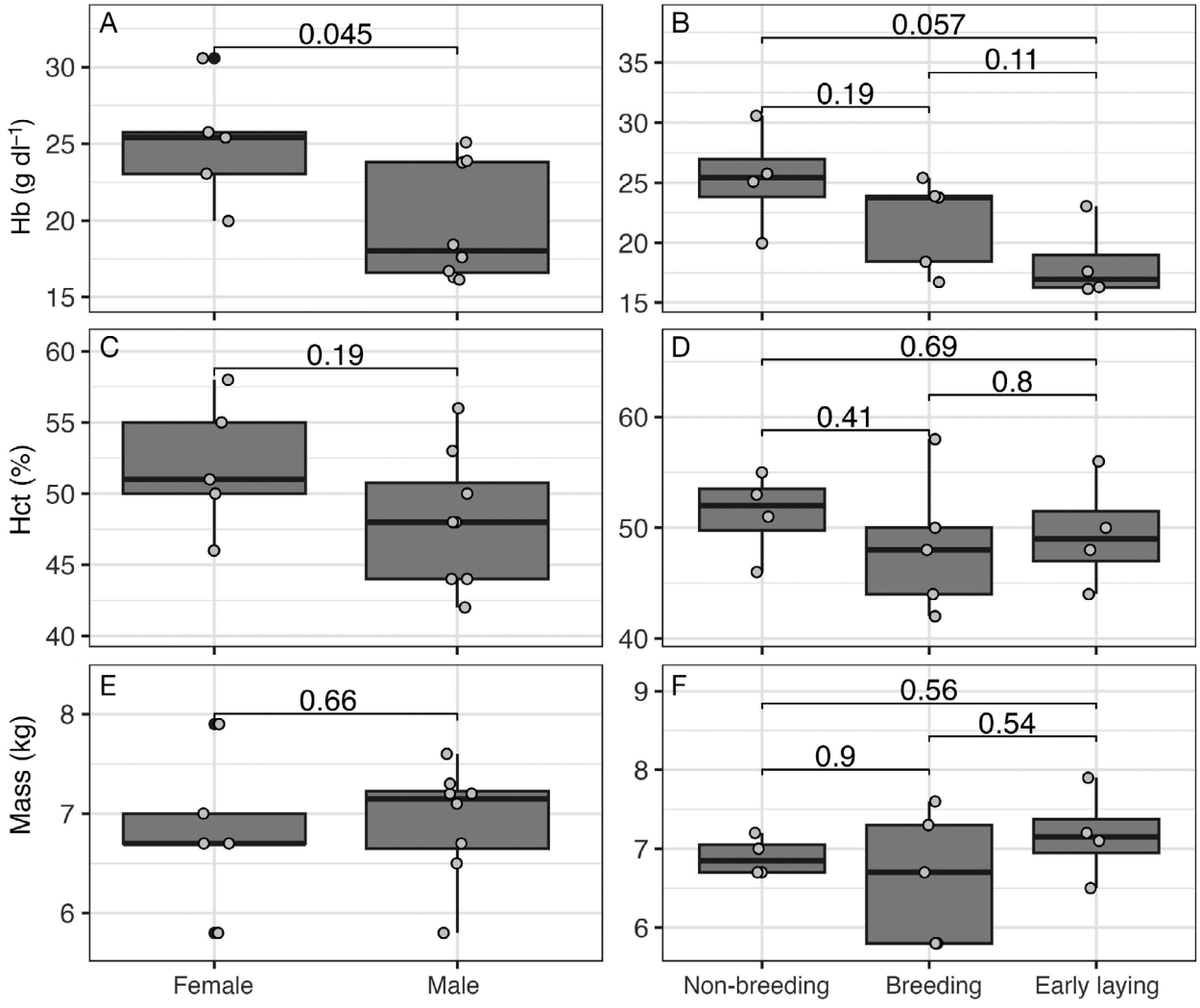

Fig. 4. Variation in the 3 oxygen storage and carrying capacity indices across (A,C,E) sex and (B,D,F) breeding status for gentoo penguins, shown as boxplots (black points: outliers) for (A,B) haemoglobin (Hb), (C,D) haematocrit (Hct), and (E,F) mass. The middle bar represents the median and the lower and upper hinges represent the first and third quartiles, respectively. Whiskers extend to the lowest or largest value within 1.5 times the inter-quartile range from the lower or upper hinge, respectively. Points beyond the whiskers are outliers. In all panels, grey points are observations. To see overlapping observations, we added horizontal jitter to the points. The values presented above the brackets are the p-values associated with the Wilcoxon rank sum tests associated with the pairwise comparisons. A total of 13 penguins (4 non-breeding; 5 breeding; 4 early-laying penguins from Pebble Island) were included in these analyses

compared with penguins with low Hb (10–20 g dl^{-1}). Such an increase could impact foraging success.

The proportion of deep dives performed by penguins increased with Hb levels, with only individuals with high Hb levels regularly diving 140 m or deeper. Vertical distance travelled pre-breeding also increased with Hb levels, suggesting that individuals with high Hb levels may use a deep-diving strategy that consequently requires more diving, both because they tend to dive deeper, and because they have to travel longer in order to reach deep waters further from shore. Such individuals are accessing resources only available at deep depths, giving them access to a wider variety of prey items and potentially decreasing intraspecific competition (e.g. Cimino et al. 2016). Similar findings have been documented in other species. For example, macaroni penguins *Eudyptes chrysolophus* forage in habitats within their aerobic capacity (Crossin et al. 2015), and Hb is correlated with higher-quality diets (reviewed in Minias 2015).

Diving efficiency displayed a complex non-linear relationship with Hct levels. The complexity of this non-linear relationship could be associated with the counteracting effects of high Hct levels. While a high

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Hct level is associated with a higher number of oxygen-carrying red blood cells, it is also linked to increased blood viscosity and thus increased cardiac workload or decreased blood flow (Birchard 1997, Schuler et al. 2010, Reinhart 2016). In addition, optimum Hct levels can be affected by factors such as age, sex, breeding status, and disease (Reinhart 2016, Johnstone et al. 2017, Brown et al. 2021). In research employing Hct as an index of body condition, higher values are often assumed to indicate better physiological performance (reviewed in Fair et al. 2007, Minias 2015). In contrast, our results suggest that Hct has a complex non-linear relationship with diving efficiency, and that higher Hct may not always result in higher diving efficiency. The use of Hct as a physiological indicator has been criticized due to inconsistent relationships with performance and fitness-related traits in many species (reviewed in Fair et al. 2007, Minias 2015, Johnstone et al. 2017). For example, intermediate rather than high Hct levels were linked to maximum longevity and recruitment rate in house wrens *Troglodytes aedon* (Bowers et al. 2014). Our results suggest that, while Hct relates to diving efficiency, linear relationships are likely too simplistic. Instead, non-linear models should be used to capture the complex relationship between these variables, though such models require a sufficiently large sample size.

While values of both Hb and Hct aligned with previously reported values for gentoo penguins (20.8 ± 5.4 g dl^{-1} and 49.2 ± 4.7 % in our study, n = 66; 20.7 ± 1.6 g dl^{-1} and 50 ± 4 % in Ibañez et al. 2015), they were not closely correlated (see Supplements 2–4). Because mature red blood cells have similar amounts of Hb, we expected a correlation between Hct and Hb. However, ecological stressors can affect these indices differently (Bańbura et al. 2007, Wagner et al. 2008, Johnstone et al. 2017), and weak correlations have been documented in other seabird species (e.g. Kaliński et al. 2011). While some anaemic birds with low Hb concentrations may be unable to improve their condition, some will actively regenerate red blood cells (Jaensch & Clark 2004, Fair et al. 2007, Campbell et al. 2010). In such cases, whether Hb and Hct will remain correlated will be affected by erythropoiesis. Erythropoiesis increases the number of immature red blood cells, which are larger and initially have lower Hb content, disproportionately increasing Hct but not Hb (Wagner et al. 2008, Campbell et al. 2010, Johnstone et al. 2017). We identified one individual that may have demonstrated evidence of compromised health. This non-breeding penguin had the lowest Hb and yet the fourth-highest Hct. Anaemia can be associated with additional health conditions, such as heavy parasite loads or severe injury (Vleck et al. 2000, Jaensch & Clark 2004, Fair et al. 2007, Clark 2015). Despite showing no apparent signs of injury at the time of recapture, this anaemic individual was one of the few individuals that lost mass between April and October. Such an example supports experimental designs that measure both Hb and Hct when assessing individual health (Johnstone et al. 2017).

According to optimal foraging theory, penguins maximize their bottom time and minimize energy spent travelling down to where prey is located (Stephens & Krebs 1986, Wilson et al. 2010, Zimmer et al. 2010). As such, shallow dives should be preferred, requiring less travel time and energy to swim down to depth (Shepard et al. 2009, Zimmer et al. 2010). Deeper dives are only profitable when prey capture rates, size, or caloric value outweigh the energetic costs of diving (Sala et al. 2015). In our data, peak diving efficiency occurred in shallow foraging habitat at approximately 60 m deep (Fig. 1A), which is deeper than previously reported values (25–30 m; Lee et al. 2015). In line with theory, this putative optimal depth (60 m) was repeatedly visited by penguins (see Supplement 5). However, potentially to reach larger and/or energy-rich prey, many penguins foraged far deeper than this putative optimal depth (maximum depth reached by individuals: 166 ± 29 m).

Diet studies suggest that deeper dives may provide greater access to larger prey that may be more valuable to gentoo penguins than the smaller prey in shallower habitat. Clausen et al. (2005) compared the diet of gentoo penguins in colonies around the Falkland Islands with surveys of prey abundance during the 2000 pre-breeding season. They highlight preferential foraging on squid and fish over krill, which has also been highlighted in the historical diet of gentoo penguins (McMahon et al. 2019). Prey likely to be essential for Pebble Island and Race Point penguins includes bentho-demersal lobster krill *Munida gregaria*, found at depths of 26–116 m, and Patagonian squid *Doryteuthis gahi*, found at depths of 60–96 m, both of which were in high abundance close to the colonies in 2000 (Clausen et al. 2005). A deeper squid species, *Moroteuthopsis ingens*, found at depths of 100–166 m, and various rock cod species *Patagontothen* spp., found at depths of 40–205 m, are abundant further offshore and were found in their diet in 2000 (Clausen et al. 2005). These prey species vary in size and energy density. For example, the Patagonian squid found at shallower depth is smaller (max. length: 28.0 cm; Palomares & Pauly 2023)

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but has a slightly higher energy density (5.0 kJ g^{-1}; Ciancio et al. 2007) than rock cod species such as *P. ramsayi* (max. length: 44.4 cm, Froese & Pauly 2023; 4.7 kJ g^{-1}, Ciancio et al. 2007). In a more recent assessment of prey, rock cods were found to be a principal prey item for gentoo penguins in the Falkland Islands (Handley et al. 2016). In addition, underwater footage demonstrated that gentoo penguins do not deviate from their path for small prey such as lobster krill, but actively chase larger prey items such as large squid and fish species (Handley et al. 2018). Generally, for marine species, prey size increases with depth (Masello et al. 2010, Miller et al. 2010).

Using diving efficiency as a measure of diving abilities implicitly assumes that animals typically dive to their aerobic dive limit and does not account for the numerous factors influencing dive time and efficiency. For example, predators adapt foraging strategies based on prey conditions (e.g. Magellanic penguins *Spheniscus magellanicus*; Sala et al. 2014) and may abandon a dive early in poor prey conditions (e.g. Thums et al. 2013, Viviant et al. 2016). Such behavioural factors likely explain the low diving efficiency at shallow depths. However, deep diving is more energetically costly to undertake (Shepard et al. 2009, Zimmer et al. 2010). As such, oxygen storage and carrying capacity are more likely to influence the behaviour of animals foraging at deeper depths (Thompson & Fedak 2001, Viviant et al. 2016) and may explain why the relationship with Hb is more apparent at depth.

Gentoo penguins that laid early spent significantly less time at sea than non-breeding penguins, a difference that we attribute to higher foraging efficiency rather than higher breeding obligations. Participating in breeding compels individuals to defend nest locations and partake in pair-bonding behaviours, requiring many species of penguins to fast during this time (Williams 1995). While gentoo penguins do not fast during breeding, they increase their time on land to maintain a nest site for approximately 2 wk before egg-laying (Black 2016). All monitored gentoo penguins, except one non-breeder, were captured at least 11 d (14.5 ± 2.2 d, 30 September to 7 October) before eggs were first seen in the colony (18 October). Thus, while early-laying penguins may have been maintaining nest sites for a few days at the end of the TDR observation period, most of the 40 d data would be prior to nesting and may be associated with early-laying penguins requiring less time to meet energetic demands.

Acquisition of food resources during the pre-breeding period is essential, as breeding birds must expend additional energy to defend their nest and produce eggs (Williams 2012). Successful acquisition of high-quality resources relates to earlier laying, which can influence reproductive success (Sorensen et al. 2009). For example, early-breeding European shag *Phalacrocorax aristotelis* spend less time foraging in winter than other individuals (Daunt et al. 2006), successfully breeding Adélie penguins *Pygoscelis adeliae* are more efficient foragers (Lescroël et al. 2010), and breeding Manx shearwater *Puffinus puffinus* spend less time in winter foraging and flying than those that defer breeding (Shoji et al. 2015). In species such as the Adélie penguin, non-breeding individuals were 90% of the mass of breeders (Vleck & Vleck 2002). We found no significant differences in the average mass between non-breeding, breeding, and early-laying penguins. However, in line with what we would expect if early-laying penguins were more efficient foragers, the non-breeding penguins in our sample were 93% the weight of early-laying penguins. As such, the decrease in time at sea for early-laying gentoo penguins could indicate that these individuals may achieve food requirements in less time during the pre-breeding period.

While none of the indices of oxygen storage and carrying capacity were in the best model for time spent at sea (the best model only included breeding status), Hb, Hct, and mass were included in models with substantial support (ΔAICc < 2), and there are known relationships between these indices and reproduction. In many bird species, Hb levels in females decline during the egg-production and laying period (reviewed in Minias 2015). Given the observed increase in diving efficiency with Hb levels, such a reduction in Hb could create important trade-offs between foraging capacity and reproduction. Other penguin species, such as macaroni penguins, have evolved extreme size dimorphism due to similar trade-offs between aerobic condition and egg production (Jubinville et al. 2020). As the physiology of each sex is affected differently by breeding (Williams 2012, Desprez et al. 2018), future studies with a larger sample size could explore sex-specific associations between Hb levels, foraging capacity, and breeding, and further disentangle these potential trade-offs.

Foraging is an essential activity for survival, and understanding how an individual's physiology and health affect dive performance, selection of foraging habitat, and breeding participation is essential to understanding the effects that natural and anthropogenic ecosystem changes can have on populations. In the Falkland Islands, the continental shelf is a popular fishing ground, and gentoo penguins are occa-

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sionally caught as bycatch and potentially compete for prey with fishing industries (Clausen & Pütz 2003, Trathan et al. 2015). Their extended winter range could overlap with exploration for hydrocarbons, and there is a consideration of further developing fisheries inshore (Augé et al. 2015). Given the associations between an individual's Hb and Hct levels and their diving and foraging patterns, these human activities could affect portions of the population differently. For example, reducing prey availability nearshore could be especially detrimental for penguins with low oxygen storage and carrying capacity.

According to the island-wide census of 14 colonies, nesting pair numbers and breeding success in 2018 were above the long-term annual average recorded since 2003 (Stanworth & Crofts 2019), suggesting that the penguins in our study experienced relatively favourable environmental conditions. However, in a year with unfavourable environmental conditions, the costs of an inferior strategy could be more prevalent (Fronstin et al. 2016, Storey et al. 2017). Therefore, further understanding the relationship between body conditions and diving efficiency, and its impact on foraging success and breeding participation across a range of environmental conditions, may be crucial in understanding breeding participation and population dynamics in the future.

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