

Foraging strategies and physiological status of a marine top predator differ during breeding stages

Colominas-Ciuró, R.^{1,4*}, Cianchetti-Benedetti, M.^{2,3}, Michel, L.², Dell’Omo, G.³,
Quillfeldt, P.².

¹Dept. Evolutionary Ecology. Museo Nacional de Ciencias Naturales, 28006, Madrid, Spain

²Behavioural Ecology & Ecophysiology group, Department of Animal Ecology & Systematics, Justus-
Liebig University Giessen, D-35392, Giessen, Germany

³*Ornis italica*, 00199, Rome, Italy

⁴Present address: Dept. Ecology, Physiology & Ethology. CNRS, University of Strasbourg,
Institut Pluridisciplinaire Hubert Curien. UMR 7178. 67087, Strasbourg, France.

*Corresponding author. Email: colominasciuro@gmail.com

RCC – ORCID: 0000-0001-6312-0702

MCB – ORCID: 0000-0002-3392-1303

LM – ORCID: 0000-0002-5516-4630

GD – ORCID: 0000-0002-9601-9675

PQ – ORCID: 0000-0002-4450-8688

Abstract

Habitat characteristics determine the presence and distribution of trophic resources shaping seabirds' behavioral responses which may result in physiological consequences. Such physiological consequences in relation to foraging strategies of different life-history stages have been little studied in the wild. Thus, we aim to assess differences in oxidative status, condition (fat stores, i.e. triglyceride levels, TRI), stress (Heterophil/Lymphocyte (H/L) ratio), and leukocyte profiles between incubation and chick rearing highlighting the role of foraging strategies in a seabird (*Calonectris diomedea*). Chick rearing was more energetically demanding and stressful than incubation as demonstrated by high stress levels (H/L ratio and leukocytes) and lower body stores (assessed by TRI and the increment of weight) due to the high energy requirements of rearing chicks. Also, our results make reconsider the simplistic trade-off model where reproduction increases metabolism and consequently the rate of oxidative stress. In fact, high energy expenditure (VeDBA) during chick rearing was correlated with low levels of oxidative damage likely due to mechanisms at the level of mitochondrial inner membranes (uncoupling proteins or low levels of oxygen partial pressure). Further (more distant) and longer (more days) foraging trips were performed during incubation, when antioxidants showed low levels compared to chick rearing due to incubation fasting, a change in diet, or a combination of these factors; but unlikely because of oxidative shielding since no relation was found between oxidative damage and antioxidant capacity. Males showed higher numbers of monocytes which were positively correlated with antioxidant capacity compared to females, suggesting sexual differences in immune profiles. Species-specific costs and energetic demands of different breeding phases trigger behavioral and physiological adjustments.

Key-words: Scopoli's shearwater, life-history stages, foraging strategies, VeDBA, oxidative status, leukocytes

Introduction

Habitat characteristics determine the presence and distribution of trophic resources, and both shape the way seabirds respond to resource availability (Quillfeldt et al. 2015). Behavioural responses may result in physiological consequences (Colominas-Ciuró et al. 2017a), and differences in foraging behaviour and diet have been associated with variations in oxidative status (Beaulieu et al. 2010, Hipfner et al. 2010, García-Tarrasón et al. 2014, Beaulieu et al. 2015, Colominas-Ciuró et al. 2017a, Costantini et al. 2017), but recently no common relationship apparently occurred across penguin species between oxidative status and foraging habitats, diet, and dietary antioxidants (Colominas-Ciuró et al. 2021).

Reproduction drives multiple physiological changes as it is one of the most energy-demanding life history stages (Green et al. 2009). Changes in oxidative stress have been suggested to represent a suitable proxy for the costs associated with reproduction by both captive and field experiments (Alonso-Alvarez et al. 2004; Bize et al. 2008; Monaghan et al. 2009; Colominas-Ciuró et al. 2017b; Beaulieu et al. 2011; Wiersma et al. 2004; Costantini et al. 2014a; Christe et al. 2012). This suggestion rises from the idea that reproduction increases metabolism and consequently the rate of oxidative stress. ATP produced during oxidative phosphorylation leads to by-production of reactive oxygen species (ROS; Finkel and Holbrook 2000; Harman 1956) which can result in oxidative damage in biomolecules (e.g. lipids, proteins, and DNA, Finkel and Holbrook 2000). However, organisms have evolved strategies to counteract the effects of free radicals with exogenous and endogenous antioxidant defences (Beckman and Ames 1998). Therefore, oxidative stress is biochemically defined as the imbalance between pro-oxidants (chemicals that induce oxidative stress, either by generating ROS or by inhibiting antioxidant systems) and antioxidant defences as well as the disruption of redox signalling and control (Costantini 2019). Oxidative stress can increase in an organism due to higher production of oxidative molecules (e.g., ROS or reactive molecules resulting from oxidation) leading to oxidative damage, decrease in antioxidant defences and repair systems (Halliwell & Gutteridge 2007) and/or disruption of redox signalling and control (Beckman & Ames 1998, Finkel & Holbrook 2000, Halliwell & Gutteridge 2007, Costantini 2019). The association between reproductive effort and oxidative stress has been supported by contradictory evidence (Oldakowski et al. 2012; Garratt et al. 2011; Bergeron et al. 2011) indicating individual-specific responses to oxidative stress (e.g. age, pollutants, etc.; Costantini et al. 2014c; Isaksson 2010).

The cost of reproduction may also trigger other physiological responses apart from oxidative stress. For instance, it may generate chronic stress if disrupts the organisms' homeostasis (Colominas-Ciuró et al. 2017b) with release of glucocorticoids (stress hormones) which trigger quantifiable changes in leukocytes (Davis et al. 2008). In Spotless starlings (*Sturnus unicolor*), for example, brood sizes negatively affected lymphocytes and monocytes (Muriel et al. 2021), suggesting lower immune status with higher breeding costs. The heterophil/lymphocyte (H/L) ratio has been extensively used as an indicator of stress in birds, as it has been found to increase with parental effort (Ilmonen et al. 2003; González-Medina et al. 2015; but see Suorsa et al. 2004).

In seabirds, as in other animals, reproduction is composed of several phases with different energetic requirements (Colominas-Ciuró et al. 2019, Green et al. 2009). Chick rearing seems to be the most energetically-demanding phase of the whole annual energetic cycle (Gales and Green 1990). For example, foraging effort is higher during brooding than during incubation in Wandering Albatrosses (*Diomedea exulans*) to minimize time at sea and maximize food delivered to chicks (Shaffer et al. 2003). Contrary, egg formation and incubation may also produce costs (Astheimer and Grau 1985) and in some cases, even similar to those of chick rearing (Green et al. 2009). Though variation of oxidative stress has been studied during reproduction (Alonso-Alvarez et al. 2004; Bize et al. 2008; Monaghan et al. 2009; Colominas-Ciuró et al. 2017b; Beaulieu et al. 2011; Wiersma et al. 2004; Costantini et al. 2014a; Christe et al. 2012), little attention has been given to specific breeding phases (Kulaszewicz et al. 2018; Colominas-Ciuró et al. 2017a). Just recently, incubation appeared to be a highly demanding phase in terms of oxidative status in seabirds (Kulaszewicz et al. 2018; Colominas-Ciuró et al. 2017a).

Many seabirds also present sexual segregation based on both social dominance and competitive exclusion, and niche specialization due to differences in morphology and reproductive role (Clarke et al. 1998; Cook et al. 2007; Dehnhard et al. 2011; Quintana et al. 2011; Rey et al. 2012). Thus, behavioural differences between the sexes (e.g., nest defences, Amat, Carrascal & Moreno 1996; foraging strategies and habitat use, Beaulieu et al. 2010; Quillfeldt et al. 2011; Rey et al. 2012; Widmann et al. 2015; diet, Paredes et al. 2008; Dehnhard et al. 2011) result in different physiological costs (e.g. different oxidative status and H/L ratios; Colominas-Ciuró et al. 2017b; Wiersma et al. 2004; Alonso-Alvarez et al. 2004; Costantini et al. 2014b; van der Meer and van Oers 2015).

Here, we aim to evaluate the relationship among oxidative status, condition (fat stores), stress (H/L ratio) and immune status (leukocyte profiles) occurring in two breeding phases, incubation and

chick rearing, in a pelagic seabird, the Scopoli's shearwater (*Calonectris diomedea*). As a novelty, we related the aforementioned parameters to foraging movements (maximum distance flown and trip length) determined by GPS tracking of the birds and energy expenditure assessed by accelerometry (vectorial dynamic body acceleration, VeDBA as a proxy of energy consumption; Gleiss et al. 2011, Cianchetti-Benedetti et al. 2017, but see Halsey 2017). Scopoli's shearwater is considered a flexible forager since it can exploit a wide range of prey, including fisheries discards (Cianchetti-Benedetti et al. 2018). It mainly breeds on remote and barren off-shore islands (Warham 1990), with single-egg clutches laid at the end of May and sharing parental duties during breeding (Warham 1990). Incubation lasts around 50 days and, from the second half of July, parents engage in chick provisioning throughout the summer until fledglings leave the nests (mid to late October; Müller et al. 2015).

Energy requirements increase during chick rearing because it involved more demanding duties (e.g. chick feeding) compared to incubation and thus, a likely greater susceptibility to oxidative stress because ROS production may increase and antioxidant defences decrease (Alonso-Alvarez et al. 2004; Costantini et al. 2008; Costantini 2008; but see Speakman and Garratt 2014; Salin et al. 2015 for recent contrasting results showing how complex the link might be). Recent results suggested the contrary, showing incubation as a highly demanding reproductive phase in terms of oxidative balance compared to chick rearing (Kulaszewicz et al. 2018; Colominas-Ciuró et al. 2017a) and thus, the prediction on which breeding phase is oxidatively more demanding in seabirds has no clear direction. Nevertheless, a recent study showed increased foraging effort during early chick-rearing compared to late-chick rearing and incubation in the studied population (Cianchetti-Benedetti et al. 2017), which allowed us to predict greater oxidative stress (higher oxidative damage and lower antioxidant defences) during chick rearing. Similarly, we predicted higher H/L ratios during chick rearing compared to incubation due to increased parental effort (Ilmonen et al. 2003; González-Medina et al. 2015; but see Suorsa et al. 2004). We also predicted shorter (less time and distance) and more costly (greater VeDBA) foraging trips during chick-rearing than during incubation. Finally, body stores and immune status (leukocyte profiles) were predicted to be worse when rearing chicks because of the high energetic demands of this period (Gales and Green 1990).

Materials and methods

Data collection

The study was approved by *Regione Siciliana, Assessorato Agricoltura e Foreste*, Prot. 65887 dated 23th July 2007 and Prot. 17233 dated 1st December 2010 and subsequent updates. This research complies with the IUCN Policy Statement on Research Involving Species at Risk of Extinction (Scopoli's shearwater *Calonectris diomedea* is classified as "Least concern" in 2016).

This study was carried out on Linosa, a small island (5.43 km²) located in the Sicilian Channel (35°51'33"N; 12°51'34"E) which is home to the largest European colony of Scopoli's shearwater with around 10,000 breeding pairs (Baccetti et al. 2009). Here, shearwaters nest in natural cavities and caves formed in volcanic rock in the northern part of the island known as "Mannarazza". Fifty-eight adults were captured at the nest during breeding in 2016, 29 incubating and 29 rearing chicks (76.3% of them were couples from the same nest and one couple was sampled during both studied periods). All of them were incubating one egg or rearing one chick. Both eggs and chicks were kept safe while adults were being handled and, after manipulation, adults were returned to the nest and resumption of parental care was confirmed. Adults were captured twice during both studied periods, on average 20-25 days apart during mid and late incubation and early chick rearing (1st capture: incubation, 26.06.2016, chick rearing, 21.07.2016; 2nd capture: incubation, 10.07.2016, chick rearing, 30.07.2016). First, they were weighed (to the nearest 5 g with a spring balance) and equipped with a data-logger that included a tri-axial accelerometer (sampling interval: 25 Hz), a GPS (sampling interval: 5 min) and a TDR (Temperature Depth recorder; sampling interval: 1 sec) logger (36 x 22 x 10 mm, 14 g; Axy-Trek, Technosmart, Rome). The logger was attached with Tesa® tape on the back feathers (procedure ≤ 10 min; Wilson and Marie-Pierre 1989). Each bird was marked using non-toxic colour to distinguish it from the partner and avoid handling it again. During incubation, the birds were captured during the day, to avoid interfering with nocturnal turn-over of parents; whereas during chick rearing, they were captured when returned to the colony to feed the chicks. Nests were checked daily for recapturing the birds and retrieving the device. On average, behaviours were recorded for 9.3 (±2.35) days during incubation and 1.9 (± 2.04) days in chick rearing. Immediately after the second capture, birds were weighed again and a blood sample (0.5 ml) was taken with a syringe from the tarsal vein. One drop was smeared on individually microscope slides, airdried, and later fixed in 96% ethanol for 5 minutes, and finally stained with Giemsa pH 7.2 for 40 minutes. The remaining blood was kept in a cool bag and later centrifuged at 12,000 rpm for 15min to separate plasma from red blood cells, and both were stored at -20°C until lab processing. We observed no

nest desertion due to our manipulation during the study. Finally, birds were sexed according to bill measurements (Lo Valvo 2001) and vocalizations.

Laboratory analyses

The oxidative status in blood plasma was assessed by measuring both antioxidant and oxidant components using the oxy-adsorbent and d-reactive oxygen metabolites (d-ROM) tests (Diacron International, Grosseto, Italy), respectively. The oxy-adsorbent test measures the plasma non-enzymatic antioxidant capacity (OXY), quantifying the contribution of a large section of exogenously and endogenously synthesized antioxidants by adding hypochlorous acid (HOCl), a highly potent oxidant. Unreacted HOCl can then be measured photometrically in a reaction with a chromogenic substrate. The d-ROMs test measures reactive oxygen metabolite (ROM) resulting from oxidative damage (ROS attack on organic substrates such as amino acids, proteins, nucleotides, etc.), mainly detecting hydroperoxides but also other oxidative metabolites such as organic chloramines or endoperoxides (Costantini 2016). Both tests were carried out following Beaulieu et al. (2010) with minor modifications, such as using a vortex machine (15 sec.), short spins for thawed plasma and mixtures (plasma-reagent, calibrator-reagent and blank-reagent), and incubation with soft shaking for better homogenization. Absorbance was read at a wavelength of 490 nm with a spectrophotometer at 37°C and soft shaking the microplate for one minute before reading (Multiskan GO, Peqlab). Measurements were expressed as $\mu\text{mol HClO/ml}$ and $\text{mg H}_2\text{O}_2/\text{dl}$ sample for the oxy-adsorbent and d-ROMs tests, respectively. Both were performed using microplates where all treatments were evenly distributed along with duplicate samples, blanks and calibrators. Intra-assays coefficients of variation for OXY and d-ROMs for plates I and II were 6.99%, 5.74% and 7.88%, 3.4%, respectively; and inter-assay coefficients of variation for OXY and d-ROMs were 6.29% and 3.49%.

Triglycerides (TRI) were determined using standard spectrophotometric test combinations modified for small amounts of plasma (6 μL plasma per determination, Thermo Fisher Scientific Clinical Diagnostics). Measurements were expressed as mg/dL . TRI analyses were performed using microplates where all treatments were evenly distributed along with duplicate samples. Intra-assay coefficients of variation for plates I and II were 3% and 2.95%, and inter-assay coefficients of variation were 7.40%. In addition to TRI levels, weight increment between captures (i.e., weight of the second capture subtracted from the weight of the first capture; $N=57$ of 58 birds) were used to assess body stores.

Leukocyte profiles were acquired by examining blood smears under a 1000x magnification using an oil immersion objective (optical microscopy), and the proportion of different types of leukocytes was determined by evaluating a total of 100 leukocytes per slide. Then, the heterophil/lymphocyte (H/L) ratio was calculated (see Moreno et al. 1998 for method reliability). All leukocytes were counted in a part of the smear where cells had separated in a monolayer crossing the sample from bottom to top or from left to right to minimize differences in blood smear thickness. For two individuals we could not obtain a blood smear, therefore 56, instead of 58, were analysed. All smears were examined by the same person (RCC).

Statistical analyses

We recorded complete foraging trips for 65.5% of birds which allowed to obtain maximum linear distance flown (km; MDF), trip length, and daily VeDBA. We usually recorded multiple foraging trips in tracked birds, especially during the chick rearing phase. Foraging trips were considered since birds went out from the colony until they came back, and to determine the beginning and the end of each as well as their duration, they were split using birds' spatio-temporal position. The maximum linear distance flown was calculated considering the most distant GPS point from the colony recorded for each foraging trip. We also calculated trip length, or mean trip length (in case an individual performed multiple trips) and daily VeDBA per individual and period. Since accelerometer data was recorded in three axes (X, head–tail; Y, right–left; and Z, dorso–ventral), the vectorial dynamic body acceleration was calculated (VeDBA (g) = $\sqrt{a_x^2 + a_y^2 + a_z^2}$) where a_x , a_y , and a_z represented dynamic acceleration values from X, Y, and Z axes. Also, from birds' GPS positions Kernel analysis were performed using a smoothing factor chosen according to the Least Square Cross Validation method (Girard et al. 2002) and QGIS 2.9 to represent birds' home ranges and foraging areas (95%, 75% and 50%).

Statistical analyses included simple regressions and general linear mixed models (GLMMs). All residuals from GLMMs performed followed a normal distribution except for ROM, TRI and Trip length which were log-transformed and monocytes which was squared rooted-transformed to achieve a normal distribution of residuals prior to analyses (Kolmogorov-Smirnov test ≥ 0.13). GLMMs were performed on OXY, log-transformed ROM ($\log_{10}$ ROM), log-transformed TRI ($\log_{10}$ TRI), Weight increment, MDF, averaged daily VeDBA, log-transformed ($\log_{10}$) Trip length, heterophils, lymphocytes, H/L ratio, eosinophiles, and squared rooted monocytes with breeding periods (incubation and chick rearing) and sex as fixed factors as well as its interaction, laying date (out of all samples: 86.21% exact days or ± 1 -2 days,

10.35% $\pm$ 4-5 days and 3.45% missing data) as a covariate and nest and individual as random factors. In addition, plate used during lab analyses was added as a random factor for OXY, log-transformed ROM, and log-transformed TRI, to control for possible inter-assay variability. Significance was determined at $p < 0.05$. We used *lmer*, *summary* and *anova* functions of R packages lme4 and lmerTest (Kuznetsova et al. 2014; Bates et al. 2015) run under R 3.3.1 to perform the GLMMs (R Core Team 2014).

Results

All dependent variables analysed were significantly different between studied periods (incubation and chick rearing) except for $\log_{10}$ ROM, mean daily VeDBA and squared rooted monocytes (Tables 1, 2, 3 and 4; Figs. 1 and 2). Specifically, non-enzymatic antioxidant capacity (OXY), heterophils, H/L ratio and eosinophils were significantly lower during incubation than chick-rearing; in contrast to $\log_{10}$ TRI, weight increment, MDF, $\log_{10}$ Trip length and lymphocytes which were significantly higher during incubation (Tables 1, 2, 3 and 4; Figs. 1 and 2). Moreover, the interaction term period*sex had a significant effect on OXY, mean daily VeDBA, and $\log_{10}$ TRI, although did not stand for further sexual differences. Kernel distributions representing the home range and foraging areas (95% and 50% fixed kernel density, respectively) of individuals during both breeding periods reinforced the differences found in terms of MDF and $\log_{10}$ Trip length (Fig. 3). In fact, individuals flew during incubation larger distances (mean $\pm$ SD_{incubation} = 423.42km $\pm$ 124.5; mean $\pm$ SD_{chick rearing} = 179km $\pm$ 122.48) performing longer foraging trips (mean $\pm$ SD_{incubation} = 9.33days $\pm$ 2.35; mean $\pm$ SD_{chick rearing} = 1.99days $\pm$ 2.04) compared to chick rearing. Also, incubating shearwaters were leaner than those rearing chicks when devices were attached (mean $\pm$ SD_{incubation} = 533g $\pm$ 45.9; mean $\pm$ SD_{chick rearing} = 611g $\pm$ 75.5; $F_{1,14,1} = 21.835$; $p < 0.001$). Moreover, a negative and significant correlation was found between mean daily VeDBA and oxidative damage ($\log_{10}$ ROM; N=38, $r=-0.333$, $p=0.041^*$); but truly, such only occurred during chick rearing (N=26, $r=-0.463$, $p=0.017^*$; Incubation: N=12, $r=-0.036$, $p=0.911$; Fig. 4). Finally, males had a significant higher number of monocytes than females (Table 3; Fig. 5a). Also, monocytes significantly and positively related with OXY in males (N=26, $r=0.486$, $p=0.012$) but not in females (N=29, $r=0.042$, $p=0.833$; Fig. 5b).

Discussion

Here we investigated the relationship among foraging effort, oxidative status, stress (H/L ratio), body stores and immune status between incubation and chick rearing on the Scopoli's shearwater. Foraging effort was assessed using tracking devices to better understand its physiological consequences (Colominas-Ciuró et al. 2017a), which allowed to determine maximum distance flown, trip length, and VeDBA (a proxy of energy expenditure). On this regards, individuals flew during incubation larger distances performing longer foraging trips compared to chick rearing (see Results) and thus, agreeing with recent results (Cianchetti-Benedetti et al. 2017).

In terms of oxidative status, we predicted greater oxidative stress (higher oxidative damage and lower antioxidant defences) during chick rearing than incubation based on the high energy requirements seabirds must face while rearing their chicks (Gales and Green 1990, Shaffer et al. 2003). However, the prediction on which breeding phase is more demanding in terms of oxidative status in seabirds has no clear direction because other recent studies in seabirds found the contrary (Kulaszewicz et al. 2018; Colominas-Ciuró et al. 2017a). Our results showed significantly lower non-enzymatic antioxidant defences (OXY) during incubation than during chick rearing, and no differences for oxidative damage (ROM). But, during chick rearing, oxidative damage negatively correlated with energy expenditure (mean daily VeDBA). Antioxidant defences fight off oxidation (ROS production) avoiding oxidative damage by preventing pro-oxidants and blocking them once formed (Sies 2007), although effectiveness is not completely achieved and thus, ROS may damage biomolecules (e.g. lipids, DNA and proteins; Beckman and Ames 1998; Sies 2007; Pacifici and Davies 1991; Davies 2000). At first glance, our results could therefore suggest incubation as a more oxidative demanding phase compared to chick rearing because antioxidants would have avoided oxidative damage by fighting ROS. But this is improbable because no relationship occurred between antioxidant capacity and oxidative damage ($N=56$, $r=-0.175$, $p=0.198$). Thus, our results of decreased antioxidants may be possibly explained as a consequence of fasting during incubation shifts rather than by oxidative shielding, what would differ with the recent suggestions where incubation is highly demanding in terms of oxidative status (Kulaszewicz et al. 2018; Colominas-Ciuró et al. 2017a). Reduction of body mass and antioxidants are known to occur during prolonged fasting in seabirds (Sylvie et al. 2012; Barbosa et al. 2013; Schull et al. 2016) and, in our case, incubating shearwaters showed lower mass than those rearing chicks when devices were attached (see Results). One caveat though is that in the present study only non-enzymatic antioxidant defences were measured and hence, there is no information on the role of enzymatic antioxidants. Perhaps the lack of relationship

between OXY and ROM does not capture a possible coordinated response between both enzymatic and non-enzymatic antioxidant defences (see Cooper-Mullin et al. 2019 as an example). Furthermore, antioxidant differences might also result from distinct diets during both studied periods because dietary antioxidants (e.g., carotenoids and vitamins), cannot be synthesized by birds and thus its efficiency depends on the diet itself (Goodwin 1984; Catoni et al. 2008; Cohen et al. 2009). Recent results at the same studied population showed a diet based on fish, but at the beginning of incubation, fish items had higher trophic levels (e.g. predatory fish) than at the end of the breeding season (Costantini et al. 2017). Hence, even if fish would be the main item, distinct fish species, also those from fisheries discards (Cianchetti-Benedetti et al. 2018), could differently contribute in terms of antioxidant absorption partially explaining our results.

The absence of oxidative damage differences between breeding phases could be due to a lack of time to detect ROMs in birds' plasma or because damage would have been cleaned up after its production by antioxidant defenses, but this is unlikely since no relationship occurred between them (see above). Nevertheless, despite this absence of relation, oxidative damage has been found to negatively relate with energy expenditure (mean daily VeDBA) during chick rearing. This relationship reveals that greater energy expenditure while rearing chicks (e.g., higher foraging effort), produced lower levels of oxidative damage. This result disagrees with the simplistic trade-off model and our prediction that increasing energy requirements and thus, metabolism, likely increases the rate of oxidative stress because ROS production may increase resulting in more oxidative damage (Alonso-Alvarez et al. 2004; Costantini et al. 2008; Costantini 2008). The simple relationship where increased metabolism leads to oxidative stress because ATP produced during oxidative phosphorylation results to by-production of ROS (Finkel and Holbrook 2000; Harman 1956), is complex and still under discussion; and in some cases, depends on mechanisms at the level of mitochondrial inner membranes (Speakman and Garratt 2014; Salin et al. 2015; Koch et al. 2021). Therefore, our negative correlation between oxidative damage and VeDBA during chick rearing could be explained by the following mechanisms which all contribute to reduce ROS production when metabolism increases (Speakman and Garratt 2014, Koch et al. 2021): (i) a reduction in the inner-membrane potential by uncoupling proteins (increasing the flow of protons back through the inner membrane to the matrix), reducing free-radical production without generating ATP (Speakman and Garratt 2014); (ii) increments in metabolic rate (ATP production) which might lower local levels of oxygen partial pressure also reducing ROS production (Barja 2007); (iii) other impacts of uncoupling

proteins to ROS production (e.g. altering substrate utilisation patterns; Speakman and Garratt 2014). Overall, our results seem to partially agree with our prediction because only during the most energetically demanding phase (chick rearing; Gales and Green 1990, Cianchetti-Benedetti et al. 2017), a link between energy expenditure and oxidative stress occurred. But, instead of increasing, oxidative damage decreased with an increment of energy expenditure, highlighting the role of, for example, uncoupling proteins. This would also agree with the statement in seabirds that short distance foraging (during chick rearing in our study) results in greater foraging costs compared to longer movements (during incubation in the present study; Weimerskirch et al. 2003) although no differences were found for VeDBA between breeding phases. Finally, oxidative stress has been also related to pollutants (Isaksson 2010), but pollutants are an unlikely driver here because little impact has been previously found in the same studied population (including oxidative status; see Costantini et al. 2017).

To solve the conflict between self-feeding and feeding the chick, pelagic seabirds present a dual strategy using long and short foraging trips, respectively (Weimerskirch et al. 1994). In our study, individuals during incubation flew about 2.3 times further away compared to chick rearing (Table 2 and Fig. 3). Recent results showed that the studied population maximized their foraging effort (short foraging trips and more frequent dives) during early chick-rearing compared to late-chick rearing and incubation (Cianchetti-Benedetti et al. 2017). Thus, our results (higher TRI levels and greater weight increment in incubation than chick rearing) agree with the previous because body stores could have been acquired during self-feeding long trips in incubation, and they could have been used for the success of short trips during chick rearing to feed the descendants, where the self-feeding energy seems minor. Alternatively, higher TRI and greater weight increment during incubation might suggest a diet based on high quality prey items during incubation than while rearing chicks, probably because long trips allowed shearwaters to reach richer foraging grounds. Due to chick rearing demands (i.e. foraging must be close to the colony to enable frequent feeding trips for chicks, see Fig. 3), prey items encountered at this time would present lower quality reducing fat stores (TRI) and increase their loss of weight.

The different activities of incubation and chick rearing may generate different costs in terms of stress due to the effects of homeostasis disruption on hematological parameters such as production of leukocytes (Kulaszewicz et al. 2018). Changes in leukocytes can be triggered by stress hormones (e.g. glucocorticoids) when homeostasis is disrupted, as occurred in other seabird species (e.g. Little auk, *Alle alle*) when most energetically demanding activities (chick rearing) increased stress hormones and

consequently, H/L ratios (Wojczulanis-Jakubas et al. 2015). Thus, H/L ratio have been extensively used as a marker of stress in birds (Davis et al. 2008), and has been also seen to increase with parental effort in birds (Ilmonen et al. 2003; González-Medina et al. 2015; but see Suorsa et al. 2004). Our results showed greater H/L ratios, heterophils and eosinophils, but lower lymphocytes during chick rearing than incubation. Nevertheless, monocytes did not differ between studied periods. These results agrees with our prediction because chick rearing in our study species demanded higher energy requirements due to parental duties (e.g. higher diving rates, especially during the early stage; Cianchetti-Benedetti et al. 2017) compared to incubation, which is considered as a less energy demanding period although presents some costs (e.g., fasting, egg formation and thermal demands; Astheimer & Grau 1985; Davis, Croxall & O'Connell 1989; Moreno & Carlson 1989; Gales & Green 1990; Monaghan & Nager 1997 and references therein; Reid, Monaghan & Ruxton 2000; Visser & Lessells 2001, Schull et al. 2016). Thus, shearwaters seem to incur higher physiological costs with the progress of the breeding season due to increasing parenting demands from incubation to chick rearing and consequently, also decreasing their immune condition. Heterophils and eosinophils showed opposite trends than lymphocytes during chick rearing, being high and low, respectively. Birds respond to stress by redistributing heterophils to the blood and lymphocytes from the blood to other body compartments (Davis et al. 2008 and references therein), which again makes our results support the hypothesis that chick rearing is one of the most energetically demanding phases of the whole annual energetic cycle (see Gales and Green 1990 for an example in penguins). The vertebrate immune system consists of two major components: the innate immunity and the acquired immunity (Norris and Evans 2000; Roitt et al. 2001). Heterophils, included in the former, are phagocytic cells that react non-specifically to a variety of pathogens (e.g. bacteria and fungi), whereas lymphocytes, included in the latter, are antigen-specific with immunological memory producing cell-mediated and humoral (antibody) responses. Thus, as the numbers of heterophils increased and those of lymphocytes decrease along chick rearing compared to incubation, each breeding phase may drive either to a different immunological investment (more innate towards chick rearing and acquired towards incubation) or to a distinct immunological trade-off based on the energy requirements of each stage.

Many seabird populations show sexual segregation due to differences in morphology and reproductive role (Clarke et al. 1998; Cook et al. 2007; Dehnhard et al. 2011; Quintana et al. 2011; Rey et al. 2012). Consequently, such dissimilarity might lead to behavioural differences (e.g. nest defence, Amat, Carrascal & Moreno 1996; foraging strategies and habitat use, Beaulieu et al. 2010, Quillfeldt et al.

2011, Rey et al. 2012, Widmann et al. 2015; or diet, Paredes et al. 2008, Dehnhard et al. 2011) and, ultimately, may have an effect on physiology (Wiersma et al. 2004; Alonso-Alvarez et al. 2004; Costantini et al. 2014b; van der Meer and van Oers 2015). For example, Colominas-Ciuró et al. 2018 found sexual segregation in foraging strategies to be the reason why Adélie penguins showed different strategies for coping with increased reproductive costs in terms of oxidative status and stress (H/L ratio) depending on their sex (see scenarios proposed by Colominas-Ciuró et al. 2017b). Although sexual dimorphism is known in our studied species (larger males than females), sexes do not apparently differ in their feeding ecology (Navarro et al. 2009; Cianchetti-Benedetti et al. 2017; Cianchetti-Benedetti et al. 2018; but see Paiva et al. 2017 because sexual segregation in foraging may occur in years of great environmental stochasticity). Therefore, our results did not show sexual differences except for the number of monocytes which were higher in males than females along the whole study period. Higher numbers of monocytes have been associated with the presence and severity of a bacterial diseases in birds (Davis et al. 2004). Thus, the influence and presence of pathogens may differ between sexes. In addition, antioxidants are relevant when mounting an immune response, not only for mitigating oxidative stress in host tissues when fighting against pathogens and/or parasites, but also because dietary antioxidants (e.g. vitamin E or carotenoids) may stimulate the immune response (Qureshi et al. 2000, Blount et al. 2003, Segal 2005, Halliwell 2006). Hence, the idea of pathogens influencing shearwaters depending on their sex is reinforced by the positive relationship between the number of monocytes and OXY found in males (see Fig. 5b). However, special caution must be taken here regarding the low number of monocytes with respect to all leukocytes (see Table 1 in the Electronic Supplementary Material).

Conclusions

Our results seem to support chick rearing to be more energetically demanding than incubation as demonstrated by high stress levels (H/L ratio) during the former. Also, the high energetic demands of this period were expected to increase metabolism and the rate of oxidative stress, but surprisingly, the levels of oxidative damage decreased with an increment of energy expenditure (VeDBA), likely due to uncoupling proteins or low levels of oxygen partial pressure at the level of mitochondrial inner membranes. Further (more distant) and longer (more days) foraging trips were performed during incubation, when antioxidants showed low levels compared to chick rearing due to incubation fasting, a change in diet, or a combination of these factors, but unlikely because of oxidative shielding since no

relation was found between oxidative damage and antioxidant capacity. Higher fat stores and mass gain during incubation suggested that body stores were obtained during self-feeding long incubation trips and could have been used for the success of short trips when rearing chicks. An alternative explanation is based on diet quality (better during incubation than during chick rearing) and provisioning requirements (greater during chick rearing). Finally, monocyte results suggested sexual differences in immune profiles that requires further research. Overall, we can conclude that physiological adjustments occur during different breeding phases in Scopoli's shearwaters and reflect energetic demands and costs associated with different species-specific breeding activities (e.g., foraging strategies affected by habitat characteristics and the availability and distribution of trophic resources).

Acknowledgements

This study was funded by *Deutsche Forschungsgemeinschaft* (grant QU 148/17-1). In addition, RCC received financial aid from an FPI and a mobility grant from the Spanish Ministry of Economy and Competitiveness (grants BES2012-059299 and EEBB-I-16-11566). The field work was supported by *Ornis italica*. We thank two anonymous reviewers for their comments and suggestions that improved the manuscript.

Author's contribution

RCC, MCB and PQ conceived and designed the study; RCC, MCB, LM and GD performed the experiment; RCC and MCB analysed the data; RCC drafted the manuscript, all other authors provided editorial advice. Informed consent was obtained from all individual participants included in the study.

Compliance with Ethical Standards

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: All applicable institutional and/or national guidelines for the care and use of animals were followed.

Data availability

432 The datasets generated during and/or analysed during the current study are available from the
433 corresponding author on reasonable request.

References

- Alonso-Alvarez, C., Bertrand, S., Devevey, G., Prost, J., Faivre, B., Sorci, G., 2004. Increased susceptibility to oxidative stress as a proximate cost of reproduction. *Ecol. Lett.* 7 (5), 363-368. doi:10.1111/j.1461-0248.2004.00594.x
- Amat, J.A., Carrascal, L.M., Moreno, J., 1996. Nest defence by chinstrap penguins *Pygoscelis antarctica* in relation to offspring number and age. *J. Avian. Biol.* 27 (2), 177-179. doi:10.2307/3677150
- Astheimer, L.B., Grau, C.R., 1985. The timing and energetic consequences of egg formation in the Adélie penguin. *Condor* 87 (2), 256-268. doi:10.2307/1366891
- Baccetti, N., Capizzi, D., Corbi, F., Massa, B., Nissardi, S., Spano, G., Sposimo, P., 2009. Breeding shearwaters on Italian islands: population size, island selection and co-existence with their main alien predator. *Riv. Ital. Ornitol.* 78 (2), 83-99.
- Barbosa, A., Palacios, M.J., Negro, J.J., Cuervo, J.J., 2013. Plasma carotenoid depletion during fasting in moulting penguins. *J. Ornithol.* 154 (2), 559-562. doi:10.1007/s10336-012-0918-z
- Barja, G., 2007. Mitochondrial oxygen consumption and reactive oxygen species production are independently modulated: implications for aging studies. *Rejuven. Res.* 10. doi:10.1089/rej.2006.0516
- Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1-48.
- Beaulieu, M., Ropert-Coudert, Y., Le Maho, Y., Ancel, A., Criscuolo, F., 2010. Foraging in an oxidative environment: relationship between delta C-13 values and oxidative status in Adelie penguins. *Proc. R. Soc. B Biol. Sci.* 277 (1684), 1087-1092. doi:10.1098/rspb.2009.1881
- Beaulieu, M., Reichert, S., Le Maho, Y., Ancel, A., Criscuolo, F., 2011. Oxidative status and telomere length in a long-lived bird facing a costly reproductive event. *Funct. Ecol.* 25 (3), 577-585. doi:10.1111/j.1365-2435.2010.01825.x
- Beaulieu, M., González-Acuña, D., Thierry, A.M., Polito, M., 2015. Relationships between isotopic values and oxidative status: insights from populations of gentoo penguins. *Oecologia* 177, 1211-1220.
- Beckman, K.B., Ames, B.N., 1998. The free radical theory of aging matures. *Physiol. Rev.* 78 (2), 547-581.

- Bergeron, P., Careau, V., Humphries, M.M., Réale, D., Speakman, J.R., Garant, D., 2011. The energetic and oxidative costs of reproduction in a free-ranging rodent. *Funct. Ecol.* 25. doi:10.1111/j.1365-2435.2011.01868.x
- Bize, P., Devevey, G., Monaghan, P., Doligez, B., Christe, P., 2008. Fecundity and survival in relation to resistance to oxidative stress in a free-living bird. *Ecology* 89 (9), 2584-2593. doi:10.1890/07-1135.1
- Catoni, C., Peters, A., Martin Schaefer, H., 2008. Life history trade-offs are influenced by the diversity, availability and interactions of dietary antioxidants. *Anim. Behav.* 76 (4), 1107-1119.
- Christe, P., Glaizot, O., Strepparava, N., Devevey, G., Fumagalli, L., 2012. Twofold cost of reproduction: an increase in parental effort leads to higher malarial parasitaemia and to a decrease in resistance to oxidative stress. *Proc. R. Soc. B Biol. Sci.* 279 (1731), 1142-1149. doi:10.1098/rspb.2011.1546
- Cianchetti-Benedetti, M., Catoni, C., Kato, A., Massa, B., Quillfeldt, P., 2017. A new algorithm for the identification of dives reveals the foraging ecology of a shallow-diving seabird using accelerometer data. *Mar. Biol.* 164 (77). doi:10.1007/s00227-017-3106-0
- Cianchetti-Benedetti, M., Dell’Omo, G., Russo, T., Catoni, C., Quillfeldt, P., 2018. Interactions between commercial fishing vessels and a pelagic seabird in the southern Mediterranean Sea. *BMC Ecol.* 18 (1), 54. doi:10.1186/s12898-018-0212-x
- Clarke, J., Manly, B., Kerry, K., Gardner, H., Franchi, E., Corsolini, S., Focardi, S., 1998. Sex differences in Adélie penguin foraging strategies. *Polar Biol.* 20 (4), 248-258. doi:10.1007/s003000050301
- Cohen, A.A., McGraw, K.J., Robinson, W.D., 2009. Serum antioxidant levels in wild birds vary in relation to diet, season, life history strategy, and species. *Oecologia* 161 (4), 673-683. doi:10.1007/s00442-009-1423-9
- Colominas-Ciuró, R., Bertellotti, M., Carabajal, E., D’Amico, V.L., Barbosa, A., 2017a. Incubation increases oxidative imbalance compared to chick rearing in a seabird, the Magellanic penguin (*Spheniscus magellanicus*). *Mar. Biol.* 164 (5), 99. doi:10.1007/s00227-017-3139-4
- Colominas-Ciuró, R., Masero, J.A., Benzal, J., Bertellotti, M., Barbosa, A., 2019. Oxidative status and stress during highly energetic life-history stages of Chinstrap Penguins: breeding versus molting. *J. Field Ornithol.* 90 (2), 190-199. doi:10.1111/jof.12297

Colominas-Ciuró, R., Santos, M., Coria, N., Barbosa, A., 2017b. Reproductive effort affects oxidative status and stress in an Antarctic penguin species: An experimental study. PLOS ONE 12 (5):e0177124. doi:10.1371/journal.pone.0177124

Colominas-Ciuró, R., Bertellotti, M., D'Amico, V.L., Carabajal, E., Benzal, J., Vidal, V., Motas, M., Santos, M., Coria, N., Barbosa, A., 2021. Diet, antioxidants and oxidative status in pygoscelid penguins. Mar. Ecol. Prog. Ser. 665, 201-216. <https://doi.org/10.3354/meps13651>

Cook, T.R., Cherel, Y., Bost, C.A., Tremblay, Y., 2007. Chick-rearing Crozet shags (*Phalacrocorax melanogenis*) display sex-specific foraging behaviour. Antarc. Sci. 19 (1), 55-63. doi:10.1017/S0954102007000089

Cooper-Mullin, C., Carter, W.A., McWilliams, S.R., 2019. Acute effects of intense exercise on the antioxidant system in birds: does exercise training help? J. Exp. Biol. 222:jeb210443.

Costantini, D., 2008. Oxidative stress in ecology and evolution: lessons from avian studies. Ecol. Lett. 11 (11), 1238-1251. doi:10.1111/j.1461-0248.2008.01246.x

Costantini, D., 2016. Oxidative stress ecology and the d-ROMs test: facts, misfacts and an appraisal of a decade's work. Behav. Ecol. Sociobiol. 70, 809-820. <https://doi.org/10.1007/s00265-016-2091-5>

Costantini, D., 2019. Understanding diversity in oxidative status and oxidative stress: the opportunities and challenges ahead. J. Exp. Biol. 222:jeb194688.

Costantini, D., Dell'ariccia, G., Lipp, H.P., 2008. Long flights and age affect oxidative status of homing pigeons (*Columba livia*). J. Exp. Biol. 211 (Pt 3), 377-381. doi:10.1242/jeb.012856

Costantini, D., Bonisoli-Alquati, A., Rubolini, D., Caprioli, M., Ambrosini, R., Romano, M., Saino, N., 2014a. Nestling rearing is antioxidant demanding in female barn swallows (*Hirundo rustica*). Naturwissenschaften 101 (7), 541-548. doi:10.1007/s00114-014-1190-2

Costantini, D., Casasole, G., Eens, M., 2014b. Does reproduction protect against oxidative stress? J. Exp. Biol. 217 (23), 4237-4243. doi:10.1242/jeb.114116

Costantini, D., Meillère, A., Carravieri, A., Lecomte, V., Sorci, G., Faivre, B., Weimerskirch, H., Bustamante, P., Labadie, P., Budzinski, H., Chastel, O., 2014c. Oxidative stress in relation to reproduction, contaminants, gender and age in a long-lived seabird. Oecologia 175 (4), 1107-1116. doi:10.1007/s00442-014-2975-x

Costantini, D., Sebastiano, M., Muller, M.S., Eulaers, I., Ambus, P., Malarvannan, G., Covaci, A., Massa, B., Dell'Omo, G., 2017. Individual variation of persistent organic pollutants in relation to stable

522 isotope ratios, sex, reproductive phase and oxidative status in Scopoli's shearwaters (*Calonectris*
523 *diomedea*) from the Southern Mediterranean. *Sci. Total Environ.* 598, 179-187.
524 doi:10.1016/j.scitotenv.2017.04.014

525 Davies, K.J.A., 2000. Oxidative stress, antioxidant defenses, and damage removal, repair, and
526 replacement systems. *IUBMB Life* 50 (4-5), 279-289. doi:10.1080/713803728

527 Davis, A.K., Cook, K.C., Altizer, S., 2004. Leukocyte profiles in wild house finches with and without
528 mycoplasmal conjunctivitis, a recently emerged bacterial disease. *EcoHealth* 1 (4), 362-373.
529 doi:10.1007/s10393-004-0134-2

530 Davis, A.K., Maney, D.L., Maerz, J.C., 2008. The use of leukocyte profiles to measure stress in
531 vertebrates: a review for ecologists. *Funct. Ecol.* 22 (5), 760-772. doi:10.1111/j.1365-
532 2435.2008.01467.x

533 Davis, R.W., Croxall, J.P., O'Connell, M.J., 1989. The reproductive energetics of gentoo (*Pygoscelis*
534 *papua*) and macaroni (*Eudyptes chrysolophus*) penguins at South Georgia. *J. Anim. Ecol.* 58 (1),
535 59-74. doi:10.2307/4986

536 Dehnhard, N., Voigt, C.C., Poisbleau, M., Demongin, L., Quillfeldt, P., 2011. Stable isotopes in southern
537 rockhopper penguins: foraging areas and sexual differences in the non-breeding period. *Polar*
538 *Biol.* 34 (11), 1763-1773. doi:10.1007/s00300-011-1026-x

539 Finkel, T., Holbrook, N.J., 2000. Oxidants, oxidative stress and the biology of ageing. *Nature* 408 (6809),
540 239-247.

541 Gales, R., Green, B., 1990. The annual energetics cycle of Little penguins (*Eudyptula Minor*). *Ecology* 71
542 (6), 2297-2312. doi:10.2307/1938641

543 García-Tarrasón, M., Sanpera, C., Jover, L., Costantini, D., 2014. Levels of antioxidants in breeding
544 female Audouin's gulls and their deposition in eggs across different environments. *J. Exp. Mar.*
545 *Biol. Ecol.* 453, 116-122.

546 Garratt, M., Vasilaki, A., Stockley, P., McArdle, F., Jackson, M., Hurst, J.L., 2011. Is oxidative stress a
547 physiological cost of reproduction? An experimental test in house mice. *Proc. Biol. Sci.* 278
548 (1708), 1098-1106. doi:10.1098/rspb.2010.1818

549 Gleiss, A.C., Wilson, R.P., Shepard, E.L.C., 2011. Making overall dynamic body acceleration work: on
550 the theory of acceleration as a proxy for energy expenditure. *Methods Ecol. Evol.* 2, 23-33.

551 González-Medina, E., Castillo-Guerrero, J.A., Santiago-Quesada, F., Villegas, A., Masero, J.A., Sánchez-
 552 Guzmán, J.M., Fernández, G., 2015. Regulation of breeding expenditure in the blue-footed
 553 booby, *Sula nebouxii*: an experimental approach. *Anim. Behav.* 108, 9-16.
 554 Goodwin, T.W., 1984. The biochemistry of the carotenoids. Volume II Animals. Chapman and Hall, New
 555 York
 556 Green, J.A., Boyd, I.L., Woakes, A.J., Warren, N.L., Butler, P.J., 2009. Evaluating the prudence of
 557 parents: daily energy expenditure throughout the annual cycle of a free-ranging bird, the
 558 macaroni penguin *Eudyptes chrysolophus*. *J. Avian Biol.* 40 (5), 529-538. doi:10.1111/j.1600-
 559 048X.2009.04639.x
 560 Halliwell, B., Gutteridge, J.M.C., 2007. Free radicals in biology and medicine. 4th ed. Oxford University
 561 Press, Oxford, UK.
 562 Halsey, L.G., 2017. Relationships grow with time: a note of caution about energy expenditure-proxy
 563 correlations, focussing on accelerometry as an example. *Funct. Ecol.* 31, 1176-1183.
 564 Harman, D., 1956. Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* 11 (3), 298-
 565 300.
 566 Hipfner, J.M., Dale, J., McGraw, K.J., 2010. Yolk carotenoids and stable isotopes reveal links among
 567 environment, foraging behavior and seabird breeding success. *Oecologia* 163, 351-360.
 568 Ilmonen, P., Hasselquist, D., Langefors, Å., Wiehn, J., 2003. Stress, immunocompetence and leukocyte
 569 profiles of pied flycatchers in relation to brood size manipulation. *Oecologia* 136 (1), 148-154.
 570 doi:10.1007/s00442-003-1243-2
 571 Isaksson, C., 2010. Pollution and its impact on wild animals: a meta-analysis on oxidative stress.
 572 *Ecohealth* 7 (3), 342-350. doi:10.1007/s10393-010-0345-7
 573 Koch, R.E., Buchanan, K.L., Casagrande, S., Crino, O., Dowling, D.K., Hill, G.E., Hood, W.R.,
 574 McKenzie, M., Mariette, M.M., Noble, D.W.A., Pavlova, A., Seebacher, F., Sunnucks, P., Udino,
 575 E., White, C.R., Salin, K., Stier, A., 2021. Integrating Mitochondrial Aerobic Metabolism into
 576 Ecology and Evolution. *Trends Ecol. Evol.* 36 (4), 321-332.
 577 Kulaszewicz, I., Wojczulanis-Jakubas, K., Jakubas, D., 2018. Breeding phased dependent oxidative
 578 balance in a small High Arctic seabird, the little auk. *J. Avian Biol.* 49:e01702.
 579 doi:doi:10.1111/jav.01702

580 Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2014. lmerTest: Test for random and fixed effects
 581 for linear mixed effect models. R package version 2.0-20.

582 Lo Valvo, M., 2001. Sexing adult Cory's shearwater by discriminant analysis of body measurements on
 583 Linosa island (Sicilian Channel), Italy. *Waterbirds* 24 (2), 169-174. doi:10.2307/1522026

584 Losdat, S., Helfenstein, F., Gaude, B., Richner, H., 2011. Reproductive effort transiently reduces
 585 antioxidant capacity in a wild bird. *Behav. Ecol.* 22 (6), 1218-1226. doi:10.1093/beheco/arr116

586 Metcalfe, N.B., Monaghan, P., 2013. Does reproduction cause oxidative stress? An open question. *Trends*
 587 *Ecol. Evol.* 28 (6), 347-350.

588 Monaghan, P., Metcalfe, N.B., Torres, R., 2009. Oxidative stress as a mediator of life history trade-offs:
 589 mechanisms, measurements and interpretation. *Ecol. Lett.* 12 (1), 75-92. doi:10.1111/j.1461-
 590 0248.2008.01258.x

591 Monaghan, P., Nager, R.G., 1997. Why don't birds lay more eggs? *Trends Ecol. Evol.* 12 (7), 270-274.

592 Moreno, J., Carlson, A., 1989. Clutch size and the costs of incubation in the Pied flycatcher *Ficedula*
 593 *hypoleuca*. *Ornis Scand.* 20 (2), 123-128. doi:10.2307/3676879

594 Moreno, J., de León, A., Fargallo, J.A., Moreno, E., 1998. Breeding time, health and immune response in
 595 the chinstrap penguin *Pygoscelis antarctica*. *Oecologia* 115 (3), 312-319.
 596 doi:10.1007/s004420050522

597 Müller, M.S., Massa, B., Phillips, R.A., Dell'Omo, G., 2015. Seabirds mated for life migrate separately to
 598 the same places: behavioural coordination or shared proximate causes? *Anim. Behav.* 102, 267-
 599 276.

600 Navarro, J., Kaliontzopoulou, A., González-Solís, J., 2009. Sexual dimorphism in bill morphology and
 601 feeding ecology in Cory's shearwater (*Calonectris diomedea*). *Zoology* 112 (2), 128-138.

602 Norris, K., Evans, M.R., 2000. Ecological immunology: life history trade-offs and immune defense in
 603 birds. *Behav. Ecol.* 11 (1), 19-26. doi:10.1093/beheco/11.1.19

604 Oldakowski, L., Piotrowska, Z., Chrzascik, K.M., Sadowska, E.T., Koteja, P., Taylor, J.R.E., 2012. Is
 605 reproduction costly? No increase of oxidative damage in breeding bank voles. *J. Exp. Biol.*
 606 215(Pt 11), 1799-805. doi:10.1242/jeb.068452

607 Pacifici, R.E., Davies, K.J.A., 1991. Protein, lipid and DNA repair systems in oxidative stress: The free-
 608 radical theory of aging revisited. *Gerontology* 37 (1-3), 166-180.

- Paiva, V.H., Pereira, J., Ceia, F.R., Ramos, J.A., 2017. Environmentally driven sexual segregation in a marine top predator. *Sci. Rep.* 7 (1), 2590. doi:10.1038/s41598-017-02854-2
- Paredes, R., Jones, I.L., Boness, D.J., Tremblay, Y., Renner, M., 2008. Sex-specific differences in diving behaviour of two sympatric Alcini species: thick-billed murre and razorbills. *Can. J. Zool.* 86 (7), 610-622. doi:10.1139/Z08-036
- Quillfeldt, P., Cherel, Y., Delord, K., Weimerkirch, H., 2015. Cool, cold or colder? Spatial segregation of prions and blue petrels is explained by differences in preferred sea surface temperatures. *Biol. Lett.* 11(4):20141090. doi:10.1098/rsbl.2014.1090
- Quillfeldt, P., Schroff, S., van Noordwijk, H.J., Michalik, A., Ludynia, K., Masello, J.F., 2011. Flexible foraging behaviour of a sexually dimorphic seabird: large males do not always dive deep. *Mar. Ecol. Prog. Ser.* 428, 271-287.
- Quintana, F., Wilson, R., Dell'Arciprete, P., Shepard, E., Laich, A.G., 2011. Women from Venus, men from Mars: inter-sex foraging differences in the imperial cormorant *Phalacrocorax atriceps* a colonial seabird. *Oikos* 120 (3), 350-358. doi:10.1111/j.1600-0706.2010.18387.x
- Reid, J.M., Monaghan, P., Ruxton, G.D., 2000. Resource allocation between reproductive phases: The importance of thermal conditions in determining the cost of incubation. *Proc. Biol. Sci.* 267 (1438), 37-41.
- Rey, A.R., Pütz, K., Scioscia, G., Lüthi, B., Schiavini, A., 2012. Sexual differences in the foraging behaviour of Magellanic Penguins related to stage of breeding. *Emu* 112 (2), 90-96. doi:10.1071/MU11065
- Roitt, I., Brostoff, J., Male, D., 2001. *Immunology*. Mosby, London.
- Salin, K., Auer, S.K., Rudolf, A.M., Anderson, G.J., Cairns, A.G., Mullen, W., Hartley, R.C., Selman, C., Metcalfe, N.B., 2015. Individuals with higher metabolic rates have lower levels of reactive oxygen species *in vivo*. *Biol. Lett.* 11(9):20150538. doi:10.1098/rsbl.2015.0538
- Schull, Q., Viblanc, V.A., Stier, A., Saadaoui, H., Lefol, E., Criscuolo, F., Bize, P., Robin, J.P., 2016. The oxidative debt of fasting: evidence for short to medium-term costs of advanced fasting in adult king penguins. *J. Exp. Biol.* 219(Pt 20), 3284-3293 doi:10.1242/jeb.145250
- Shaffer, S.A., Costa, D.P., Weimerskirch, H., 2003. Foraging effort in relation to the constraints of reproduction in free-ranging albatrosses. *Funct. Ecol.* 17 (1), 66-74. doi:10.1046/j.1365-2435.2003.00705.x

639 Sies, H., 2007. Total antioxidant capacity: Appraisal of a concept. *J. Nutr.* 137 (6), 1493-1495.

640 Speakman, J.R., Garratt, M., 2014. Oxidative stress as a cost of reproduction: Beyond the simplistic trade-
641 off model. *BioEssays* 36 (1), 93-106. doi:10.1002/bies.201300108

642 Suorsa, P., Helle, H., Koivunen, V., Huhta, E., Nikula, A., Hakkarainen, H., 2004. Effects of forest patch
643 size on physiological stress and immunocompetence in an area-sensitive passerine, the Eurasian
644 treecreeper (*Certhia familiaris*): an experiment. *Proc. R Soc. B Biol. Sci.* 271 (1537), 435-440.
645 doi:10.1098/rspb.2003.2620

646 Sylvie, G., Marion, K., Yvon, le M., Jean-Patrice, R., Criscuolo, F., 2012. Of the importance of metabolic
647 phases in the understanding of oxidative stress in prolonged fasting and refeeding. *Physiol.*
648 *Biochem. Zool.* 85 (4), 415-420. doi:10.1086/666364

649 Team, R.C., 2014. A language and environment for statistical computing. R Foundation for Statistical
650 Computing, Vienna, Austria.

651 van der Meer, E., van Oers, K., 2015. Gender and personality differences in response to social stressors in
652 Great Tits (*Parus major*). *PLoS ONE* 10(5):e0127984. doi:10.1371/journal.pone.0127984

653 Visser, M.E., Lessells, C.M., 2001. The costs of egg production and incubation in great tits (*Parus*
654 *major*). *Proc. R Soc. B Biol. Sci.* 268 (1473), 1271-1277. doi:10.1098/rspb.2001.1661

655 Warham, J., 1990. The petrels: their ecology and breeding systems. A&C Black.

656 Weimerskirch, H., Ancel, A., Caloin, M., Zahariev, A., Spagiari, J., Kersten, M., Chastel, O., 2003.
657 Foraging efficiency and adjustment of energy expenditure in a pelagic seabird provisioning its
658 chick. *J. Anim. Ecol.* 72 (3), 500-508. doi:10.1046/j.1365-2656.2002.00720.x

659 Weimerskirch, H., Chastel, O., Ackermann, L., Chaurand, T., Cuenot-Chaillet, F., Hindermeyer, X.,
660 Judas, J., 1994. Alternate long and short foraging trips in pelagic seabird parents. *Anim. Behav.*
661 47 (2), 472-476.

662 Widmann, M., Kato, A., Raymond, B., Angelier, F., Arthur, B., Chastel, O., Pellé, M., Raclot, T., Ropert-
663 Coudert, Y., 2015. Habitat use and sex-specific foraging behaviour of Adélie penguins
664 throughout the breeding season in Adélie Land, East Antarctica. *Mov. Ecol.* 3 (1), 1-10.
665 doi:10.1186/s40462-015-0052-7

666 Wiersma, P., Selman, C., Speakman, J.R., Verhulst, S., 2004. Birds sacrifice oxidative protection for
667 reproduction. *Proc. R Soc. B Biol. Sci.* 271 (Suppl 5), S360-S363.

668 Wilson, R.P., Marie-Pierre, T.J.W., 1989. Tape: a package-attachment technique for penguins. Wild. Soc.
669 Bull. (1973-2006) 17 (1), 77-79.

670 Wojczulanis-Jakubas, K., Jakubas, D., Chastel, O., Kulaszewicz, I., 2015. A big storm in a small body:
671 seasonal changes in body mass, hormone concentrations and leukocyte profile in the little auk
672 (Alle alle). Polar Biol. 38, 1203–1212.

673

674 **Tables and table legend**

675 **Table 1.** Results of a general linear mixed models (GLMMs) examining the effects of Scopoli's shearwaters breeding periods, sex, its interaction and
676 laying date on oxidative status (total plasma antioxidant capacity, OXY; and oxidative damage, ROM) and triglycerides (TRI). For more details, see the
677 Material and Methods section.

	OXY				Log ₁₀ ROM				Log ₁₀ TRI			
	Estimate	DF	F	p	Estimate	DF	F	p	Estimate	DF	F	p
Period	44.766	1,31.483	3.6e+5	<0.001*	-0.045	1,27.393	1.129	0.297	-0.013	1,34.296	16.446	<0.001*
Sex	-3.664	1,25.536	0.439	0.514	0.013	1,25.596	0.118	0.734	0.001	1,28.794	0.106	0.747
Laying date	0.635	1,30.124	0.155	0.697	-0.004	1,31.365	0.123	0.728	0.001	1,32.133	2.169	0.151
Period* Sex	2.883	1,31.487	1507.5	<0.001*	-0.012	1,25.934	0.103	0.751	0.009	1,28.955	9.77	0.004*

691 *indicate significant difference (P < 0.05)

692

693

694

695

696

Table 2. Results of a general linear mixed models (GLMMs) examining the effects of Scopoli's shearwaters breeding periods, sex, its interaction and laying date on the increment of weight between studied period, maximum distance flown, energy expenditure (VeDBA) and length of the foraging trips. For more details, see the Material and Methods section.

	Weight increment				Maximum distance flown				mean daily VeDBA				Log ₁₀ Trip length			
	Estimate	DF	F	p	Estimate	DF	F	p	Estimate	DF	F	p	Estimate	DF	F	p
Period	-61.648	1,52	87.885	<0.001*	-118.987	1,33	27.581	<0.001*	0.402	1,35	0.326	0.571	-0.369	1,35	73.801	<0.001*
Sex	0.819	1,52	0.016	0.901	13.512	1,33	0.339	0.564	-0.55	1,35	0.585	0.45	0.05	1,35	1.273	0.267
Laying date	-1.4	1,52	0.691	0.41	6.293	1,33	1.457	0.236	0.191	1,35	1.609	0.213	0.004	1,35	0.149	0.702
Period*	4.102	1,52	0.39	0.535	1.690	1,33	0.005	0.944	1.533	1,35	4.42	0.043*	-0.005	1,35	0.013	0.91
Sex																

697

698

699

700

*indicate significant difference (P < 0.05)

Table 3. Results of a general linear mixed models (GLMMs) examining the effects of Scopoli's shearwaters breeding periods, sex, its interaction and laying date on heterophils, lymphocytes and stress (H/L ratio). For more details, see the Material and Methods section.

	Heterophils				Lymphocytes				H/L ratio			
	Estimate	DF	F	p	Estimate	DF	F	p	Estimate	DF	F	p
Period	3.139	1,36.889	5.775	0.021*	-7.982	1,17.945	26.792	<0.001*	0.176	1,51	17.19	<0.001*
Sex	-0.636	1,27.127	0.311	0.582	0.149	1,25.573	0.011	0.917	-0.023	1,51	0.283	0.597
Laying date	-0.021	1,31.858	0.004	0.953	-0.23	1,31.489	0.273	0.605	0.009	1,51	0.629	0.431
Period* Sex	-1.413	1,27.239	1.515	0.229	0.177	1,18.877	0.018	0.896	-0.049	1,51	1.33	0.254

*indicate significant difference (P < 0.05)

708
709
710

Table 4. Results of a general linear mixed models (GLMMs) examining the effects of Scopoli’s shearwaters breeding periods, sex, its interaction and laying date on eosinophils and monocytes. For more details, see the Material and Methods section.

	Eosinophils				squared rooted Monocytes			
	Estimate	DF	F	p	Estimate	DF	F	p
Period	4.615	1,18.293	12.866	0.002*	-0.007	1,0.028	0.029	0.976
Sex	1.574	1,49.705	1.208	0.277	-0.286	1,27.824	6.639	0.016*
Laying date	0.388	1,50.175	1.083	0.303	-0.022	1,29.098	0.565	0.458
Period*Sex	1.375	1,18.383	1.145	0.299	-0.108	1,028	7.53	0.908

*indicate significant difference (P < 0.05)

711

Figure Legends

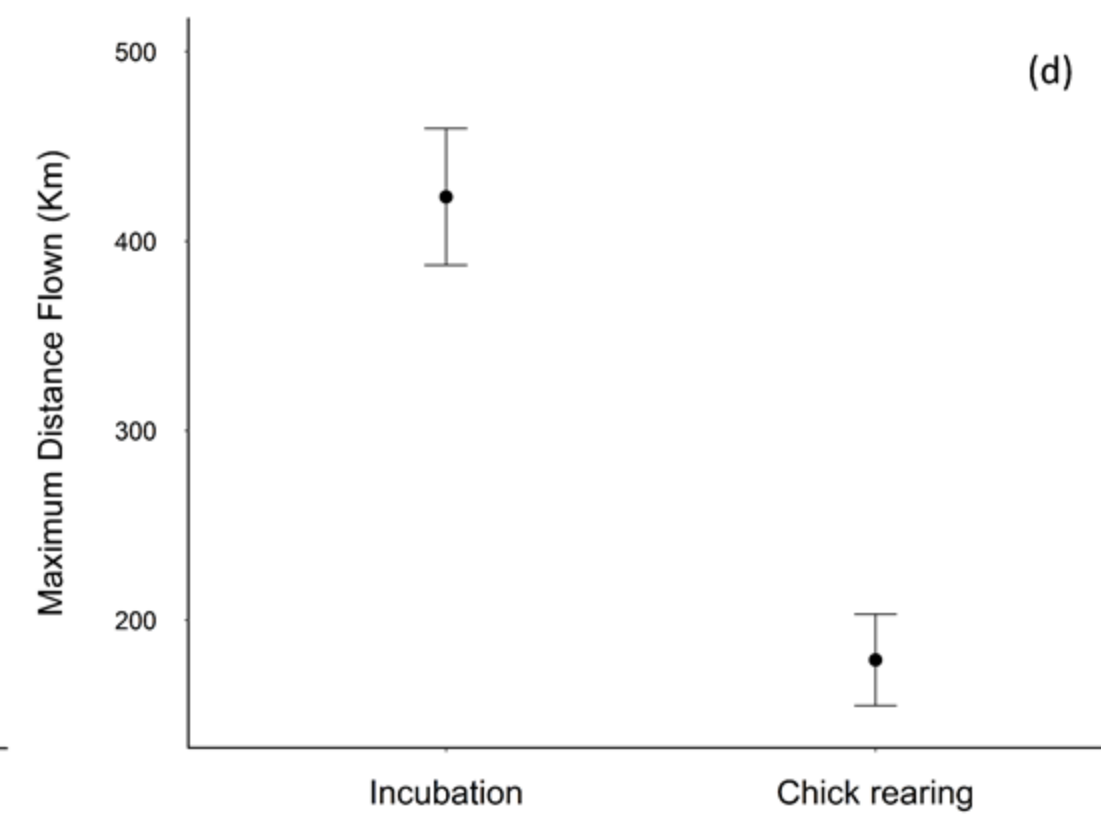
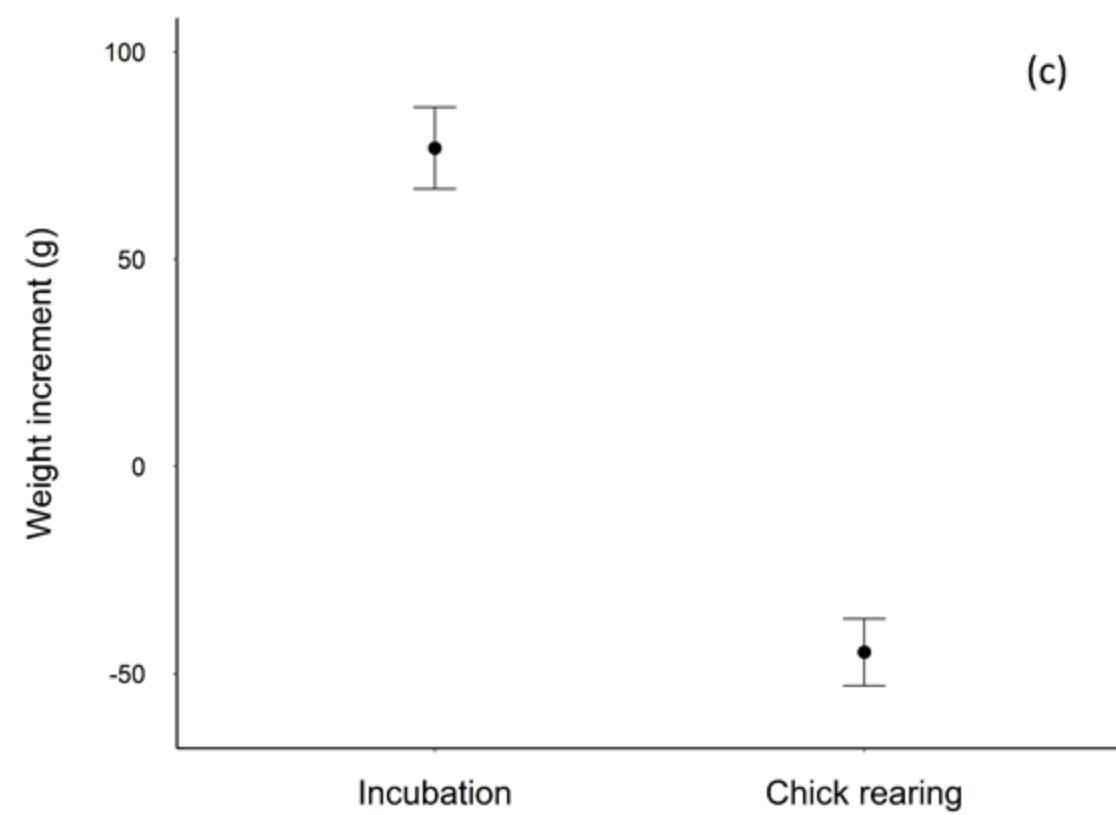
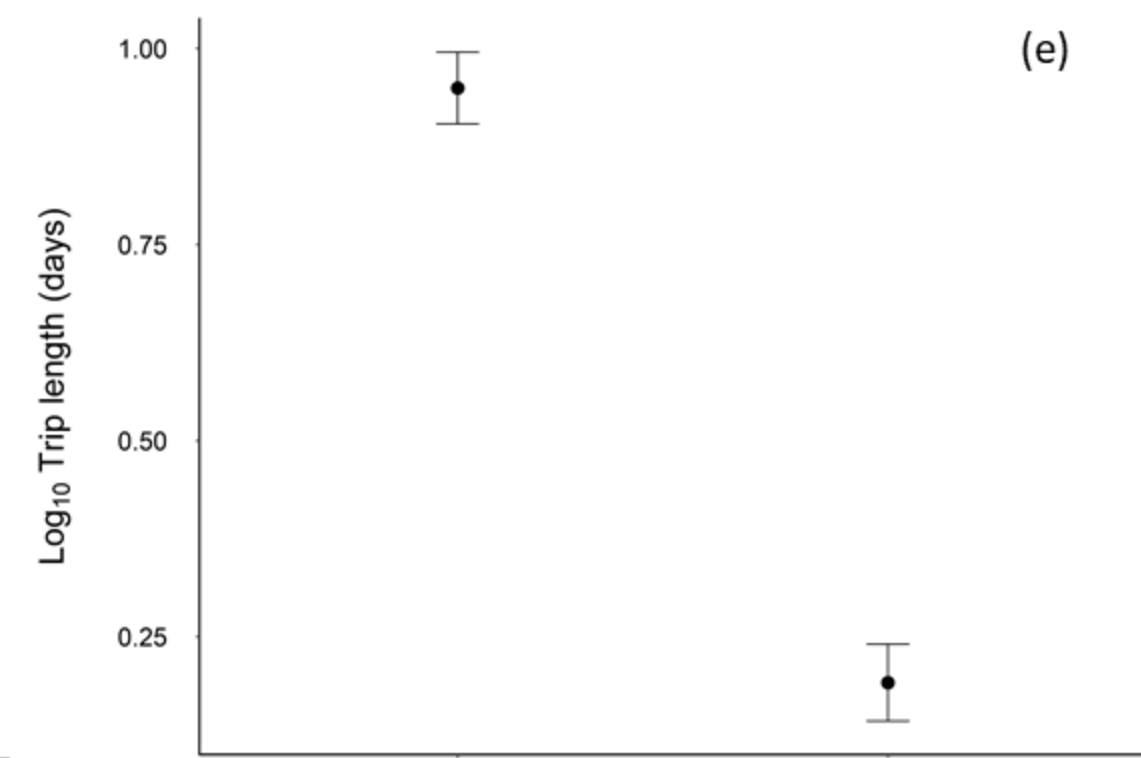
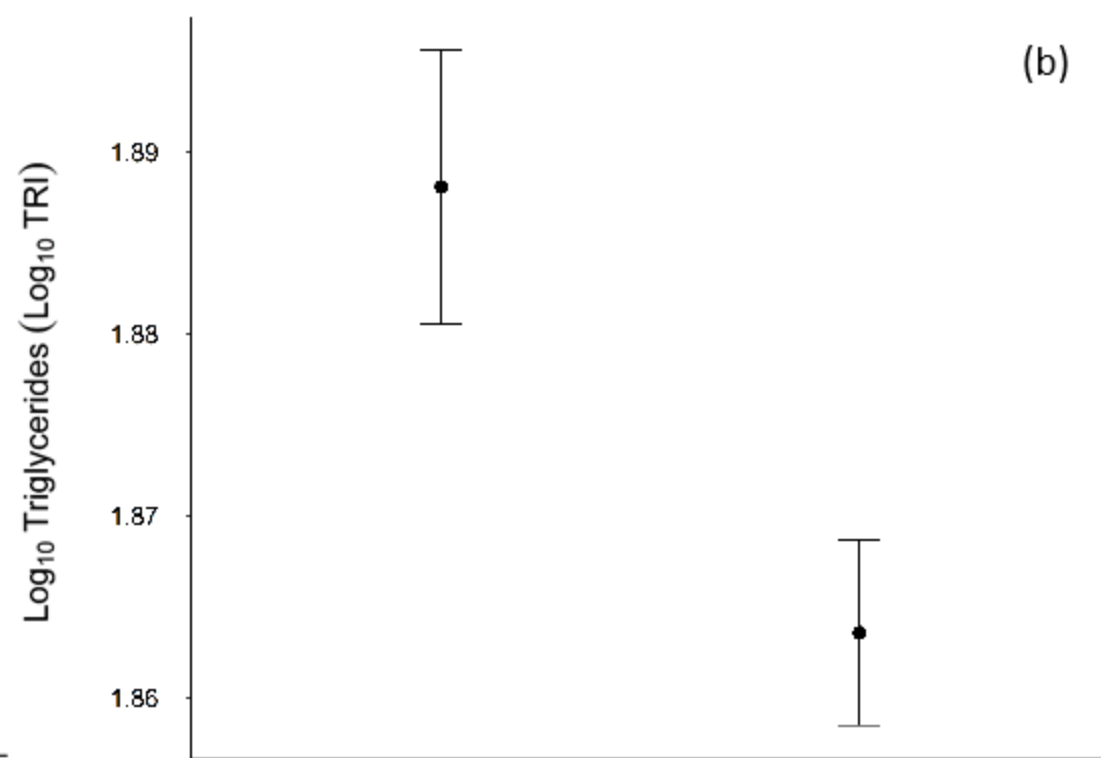
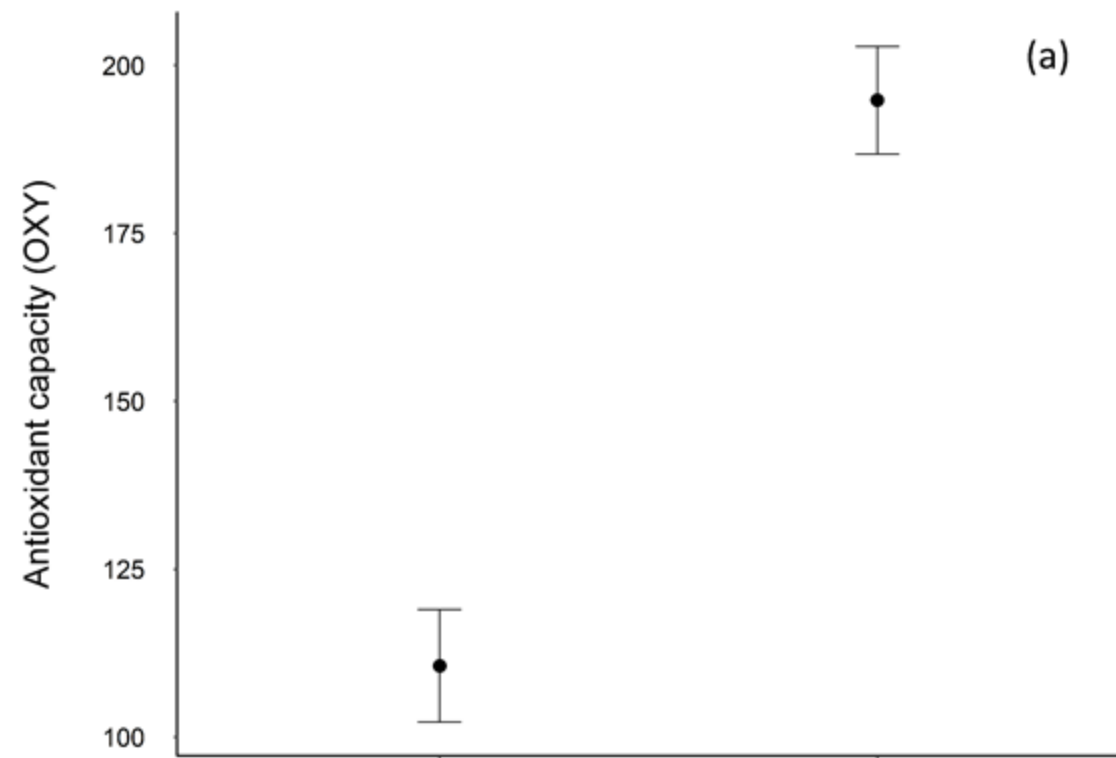
Fig. 1 Differences in antioxidant capacity (a), triglycerides (b), weight increment (c), maximum distance flown (d) and trip length (e) between reproductive periods (incubation and chick rearing) of Scopoli's shearwaters breeding in 2016 at Linosa (Italy). Circles show model-predicted means $\pm$ standard errors.

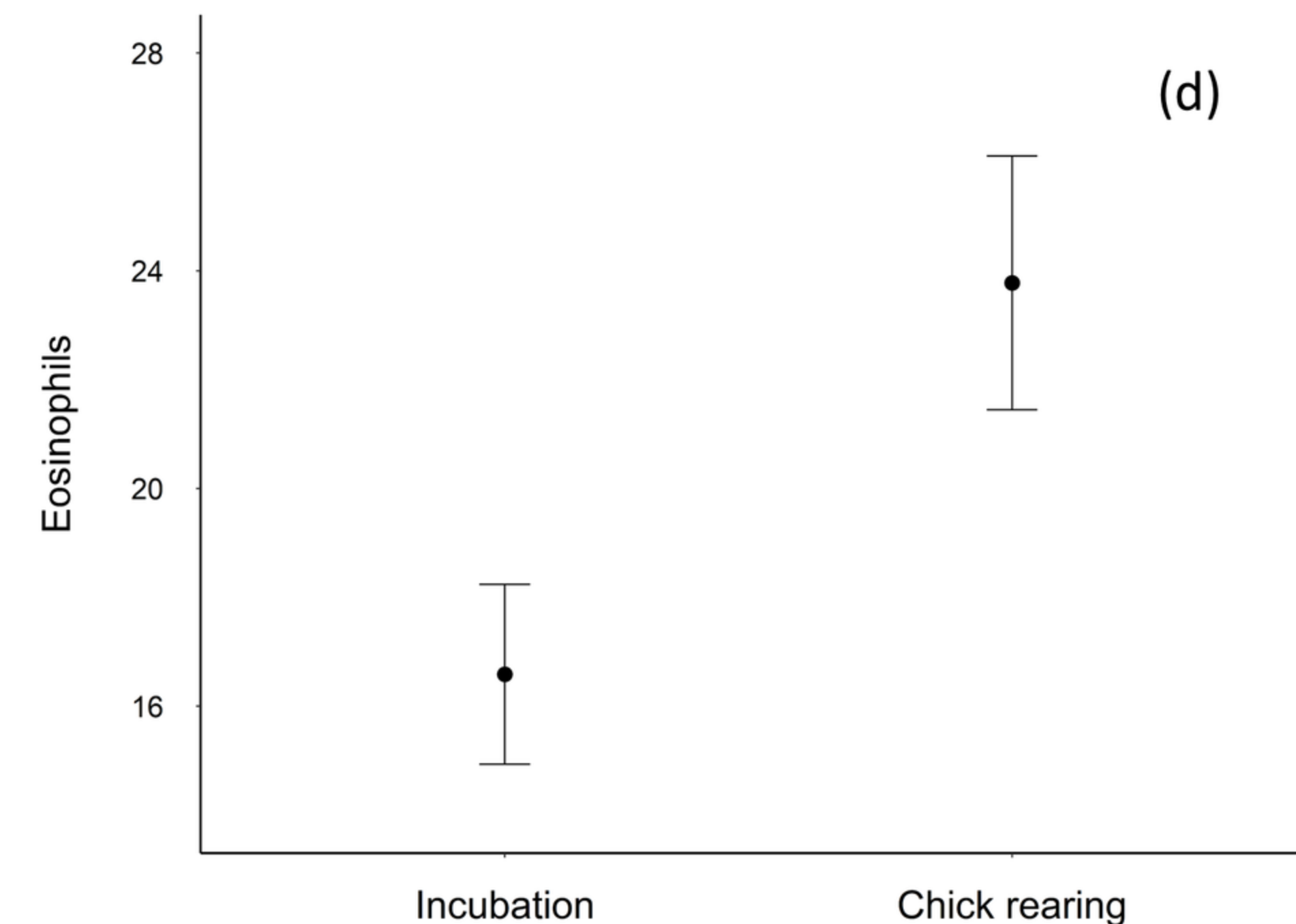
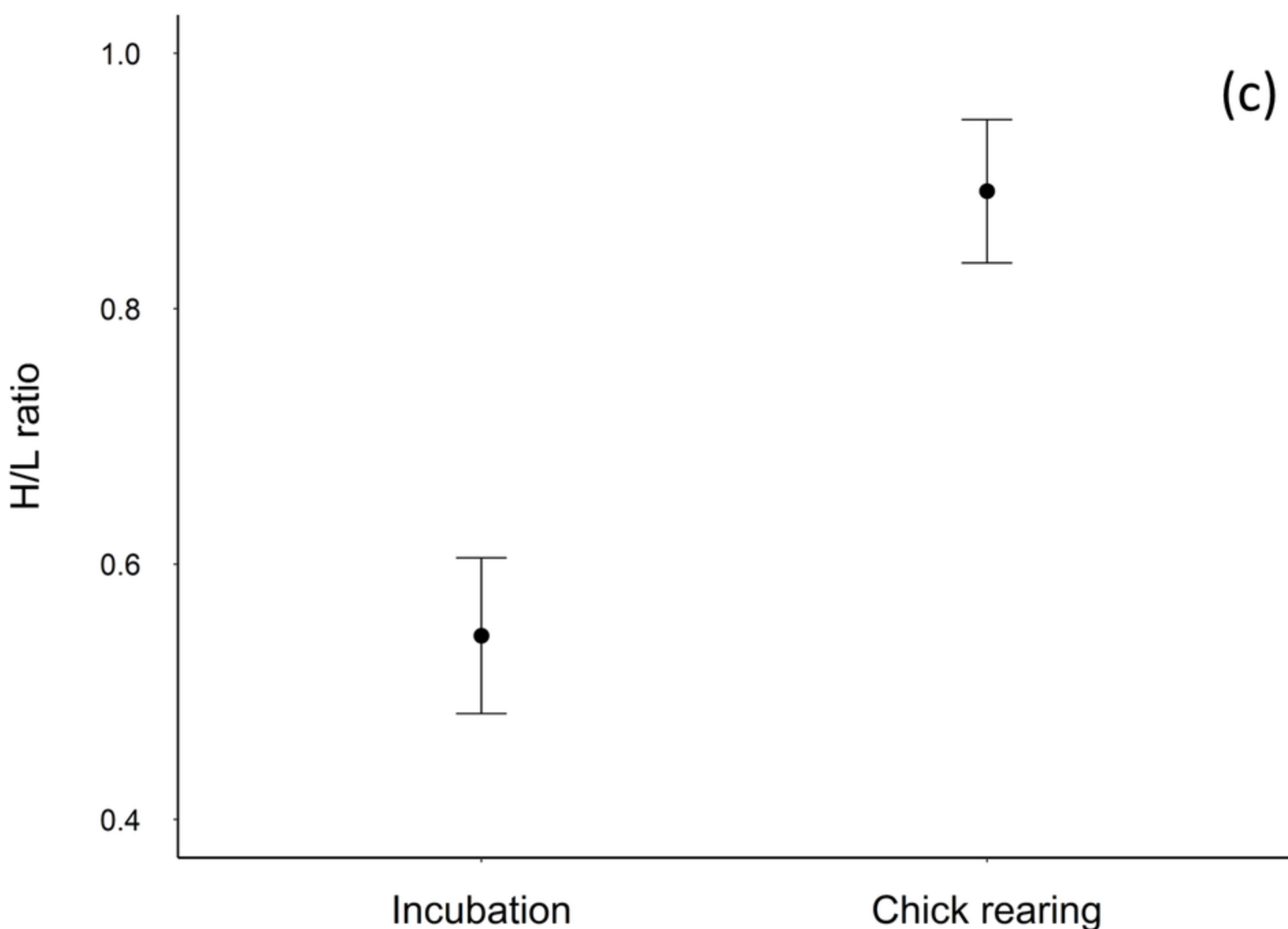
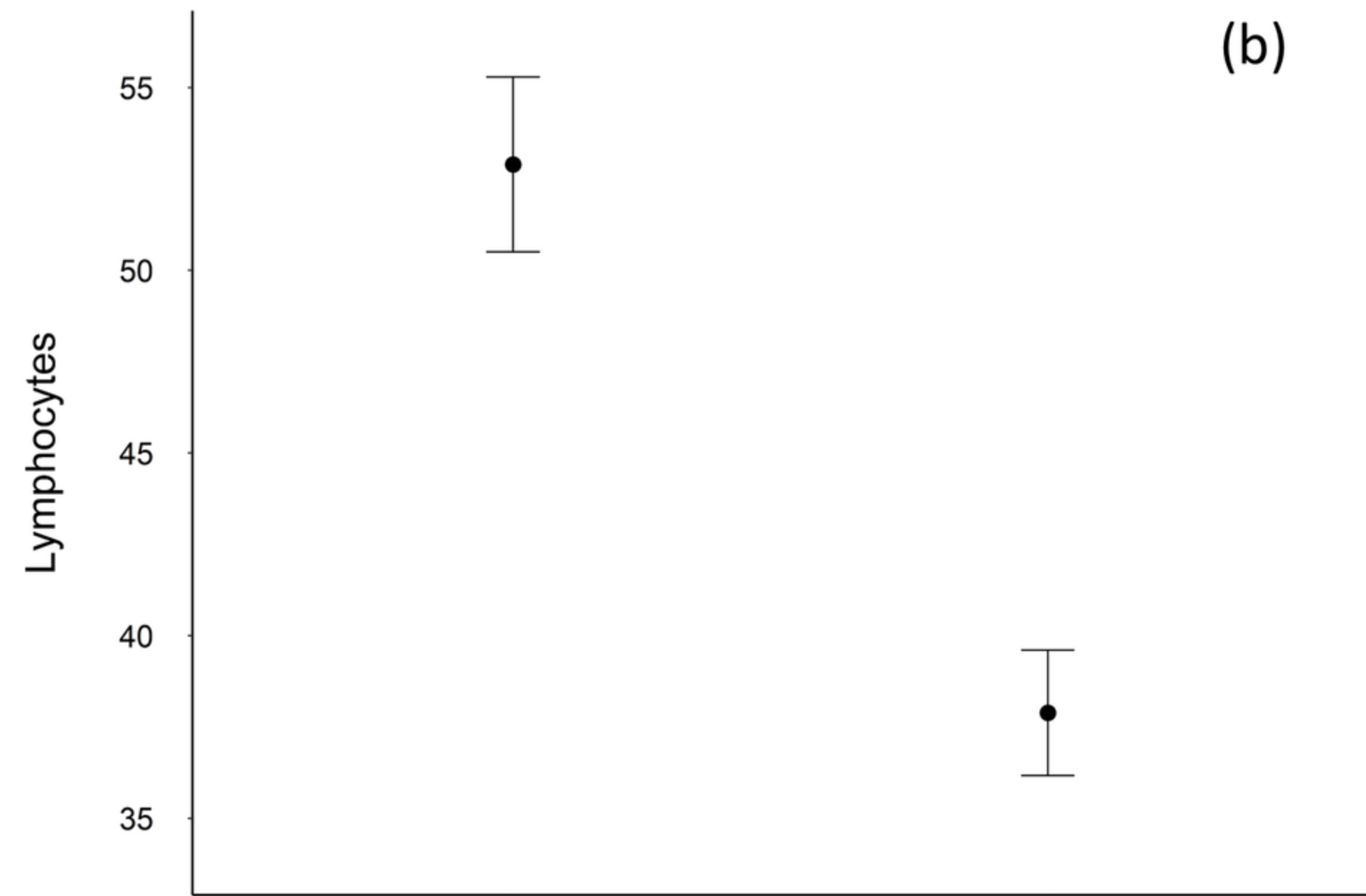
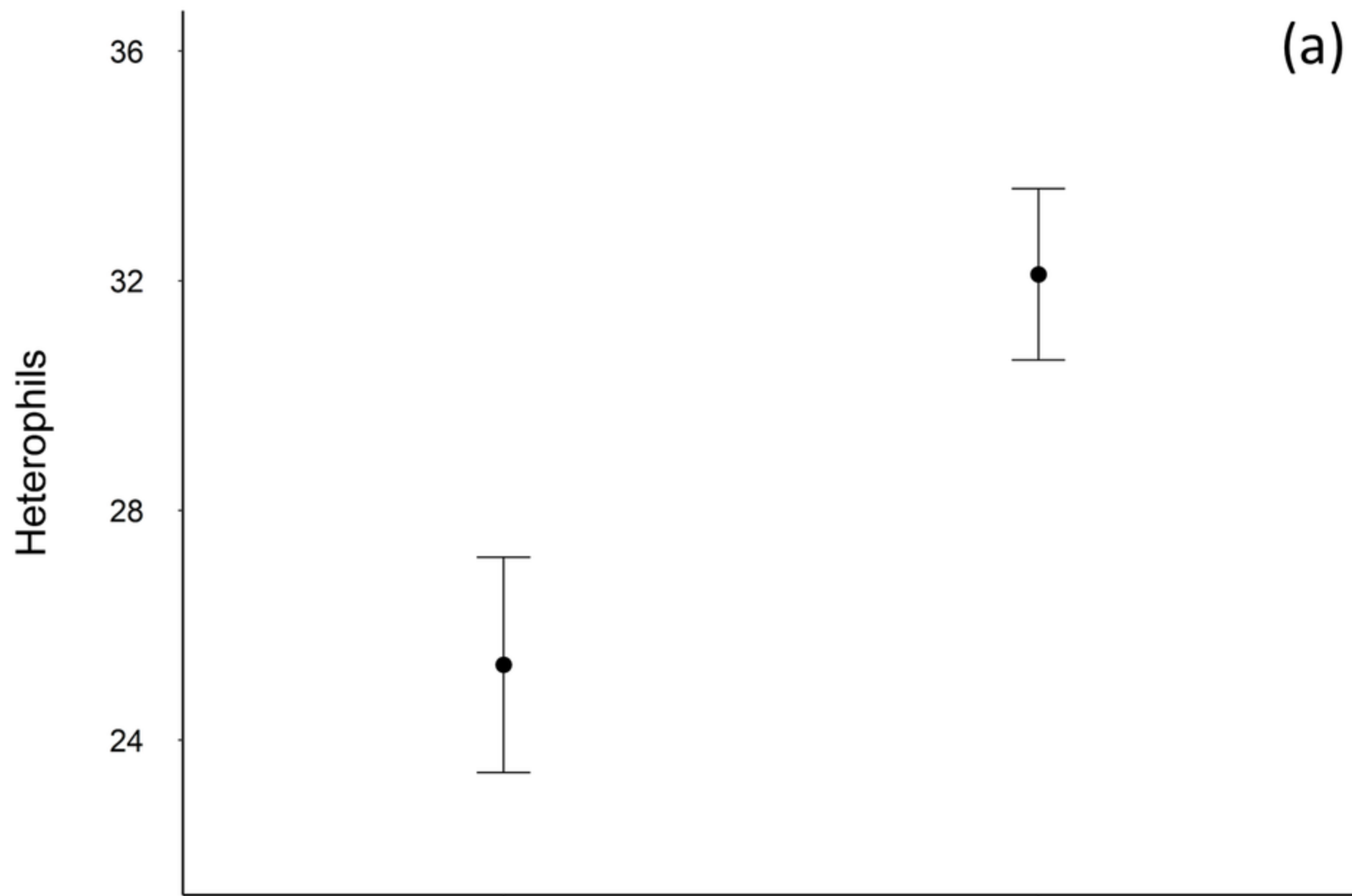
Fig. 2 Differences in heterophils (a), lymphocytes (b), H/L ratio (c) and eosinophils (d) between reproductive periods (incubation and chick rearing) of Scopoli's shearwaters breeding in 2016 at Linosa (Italy). Circles show model-predicted means $\pm$ standard errors.

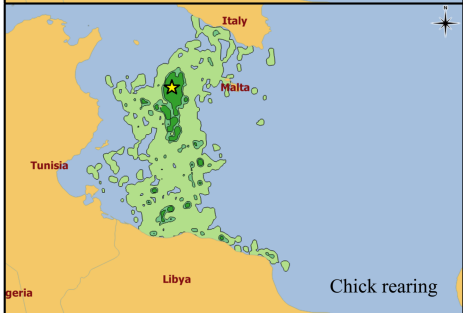
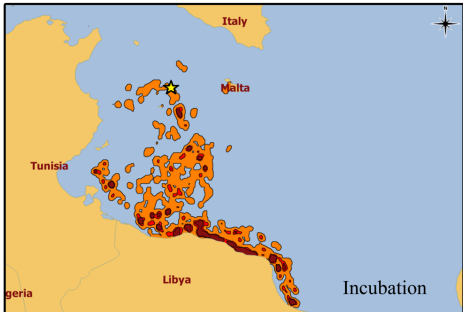
Fig. 3 Kernel distributions representing the home range and foraging areas (95%, 75% and 50%, from the lighter to darker colour) calculated from GPS positions of Scopoli's shearwaters during two different periods of the breeding season 2016 at Linosa (Italy). a) incubation, in orange to dark red and b) chick rearing from light to dark green.

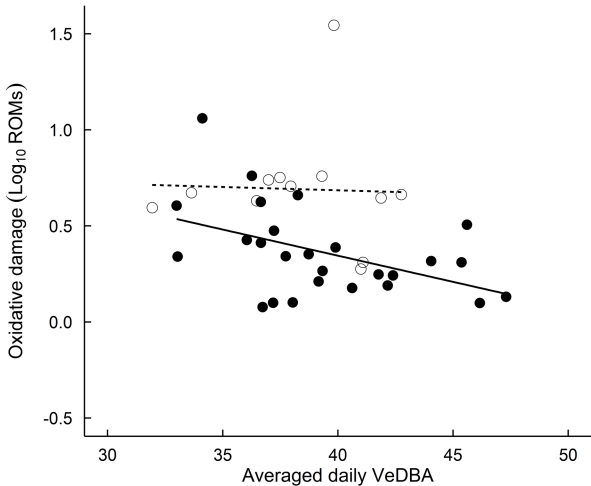
Fig. 4 Relationships between oxidative damage ($\log_{10}$ ROMs) and energy expenditure (average daily VeDBA) of Scopoli's shearwaters during incubation (white circles and dash line) and chick rearing (black circles and solid line) in 2016 at Linosa (Italy).

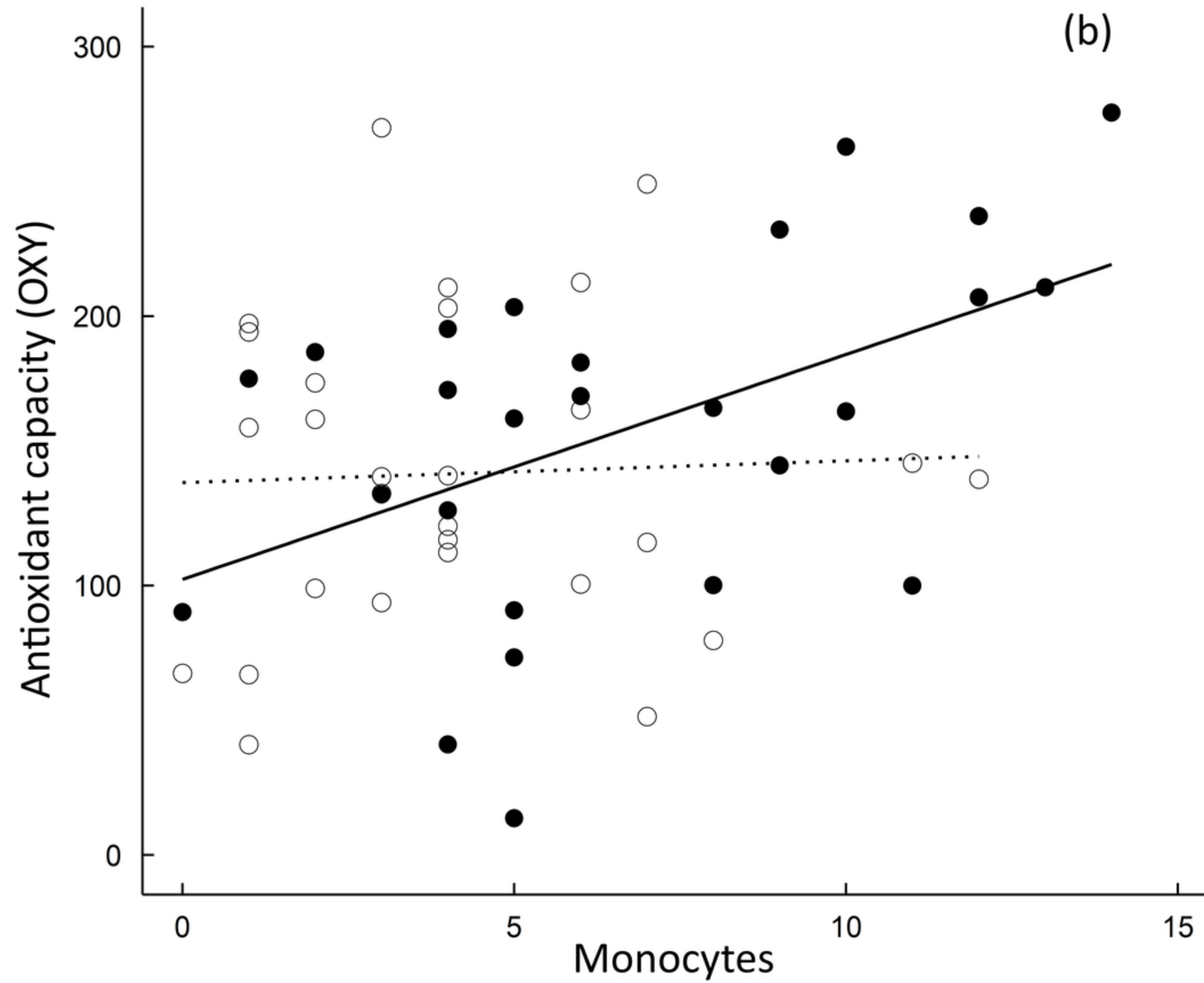
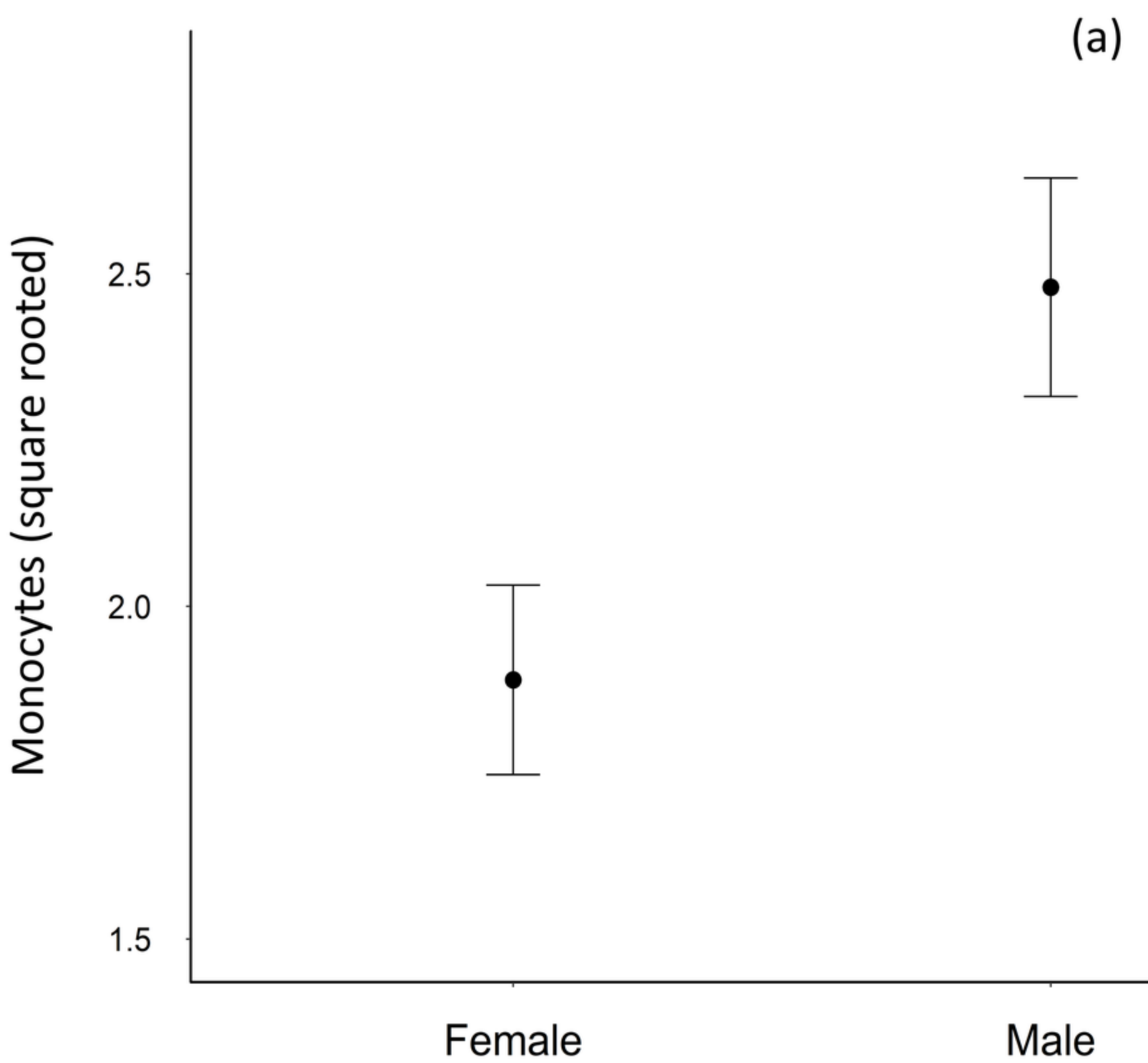
Fig. 5 Sex differences in monocytes of Scopoli's shearwaters breeding in 2016 at Linosa (Italy) (a), circles show model-predicted means $\pm$ standard errors. Also, male (black circles and solid line) and female (white circles and dash line) relationships between antioxidant capacity and monocytes (b).













VS.



Foraging trips: + km / + days

↓ Antioxidants (fasting or ≠ diet)

High energy requirements:

↑ stress / ↓ body stores

Ox. Damage

VeDBA



Reproduction ≠ ↑ Ox. Stress
(↑O₂ consumption)