Altered Oxidative Status as a Cost of Reproduction in a Seabird with High Reproductive Costs

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ABSTRACT

Life history theory posits that reproduction is constrained by a cost of reproduction such that any increase in breeding effort should reduce subsequent survival. Oxidative stress refers to an imbalance between the prooxidant reactive oxygen species (ROS) and antioxidant defense. If not thwarted, ROS can cause damage to DNA, lipids, and proteins, potentially increasing the rate of senescence and decreasing cellular function. Reproduction is often associated with higher metabolic rates, which could increase production of ROS and lead to oxidative damage if the animal does not increase antioxidant protection. Thus, oxidative stress could be one mechanism creating a cost of reproduction. In this study we explored how reproduction may affect oxidative status differently between male and female thick-billed murres during early and late breeding seasons over three consecutive years. We manipulated breeding efforts by removing an egg from the nest of some individuals, which forced females to relay, and by handicapping other individuals by clipping wings. We measured total antioxidant capacity (TAC), uric acid (UA) concentration, and malondialdehyde (MDA; an index of lipid oxidative damage) concentration in blood plasma as well as activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) in red blood cells. Oxidative status was highly variable across years, and year was consistently the most important factor determining oxidative status; inconsistent results in previous field studies may be because reproductive oxidative stress occurs only in some years. Females had lower SOD and GPx and higher MDA and TAC than males immediately after egg laying, suggesting that the cost of egg laying required investment in cheaper nonenzymatic antioxidant defenses that had lower capacity for defending against lipid peroxidation. Delayed birds had lower UA and lower SOD, GPx, and CAT activity compared with control birds. In conclusion, when reproductive costs increase via higher energy costs or longer breeding seasons, the oxidative status of both male and female murres deteriorated as a result of reduced antioxidant defenses.

Keywords: life history strategies, reproduction, offspring care, oxidative stress, antioxidants, thick-billed murre.

Introduction

During aerobic cellular respiration, the electron transport chain picks up oxygen (O_2) as the final electron acceptor and reduces O_2 to water molecules, producing adenosine triphosphate (ATP) in the process (Finkel and Holbrook 2000; Koopman et al. 2010; Skrip and McWilliams 2016). Meanwhile, ambient O_2 in the mitochondria can pick up nearby hydrogens and electrons to form radical intermediates, such as superoxide, hydrogen peroxide, and hydroxyl radical, broadly known as reactive oxygen species (ROS; Finkel and Holbrook 2000; Koopman et al. 2010). The primary function of ROS includes signaling and activating apoptosis to control cellular proliferation (Johnson et al. 1996; von Harsdorf et al. 1999). At high concentrations, however, ROS can react with DNA, lipids, and proteins through a series of endogenous oxidation reactions and ultimately cause oxidative damage (Harman 1956; Koopman et al. 2010; Skrip and McWilliams 2016). Every aerobic organism has a sophisticated antioxidant defense system to decrease this type of damage. Enzymatic antioxidants, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT), serve as the first line of defense in quenching ROS and neutralizing them to water (Finkel and Holbrook 2000; Koopman et al. 2010; Skrip and McWilliams 2016). Nonenzymatic antioxidants, such as glutathione, uric acid (UA; in birds), and vitamins, scavenge prooxidants in the bloodstream, converting them into less harmful derivatives (Alan and McWilliams 2013; Skrip and McWilliams 2016). The imbalance between prooxidant ROS and antioxidants, known as oxidative stress, can result in accumulated oxidative damage and may ultimately determine rate of senescence and decrease proper cellular function (Ku and Sohal 1993; Sohal et al. 1995; Sampayo et al. 2003).

The association between life history strategies and oxidative stress has received considerable attention (Monaghan et al. 2009;

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Metcalfe and Alonso-Alvarez 2010; Speakman et al. 2015; Herborn et al. 2016). Zera and Harshman (2001) describe how life history strategies can include trade-offs between producing offspring and self-maintenance. Reproduction is an energetically costly process for animals; direct costs may involve increased nutrient demands during reproduction, and indirect costs may be a reduction in antioxidant repair and immunity investment (Speakman 2008; Monaghan et al. 2009; Marasco et al. 2017). Ultimately, such costs may lead to altered winter behavior and reduced survival (Sæther et al. 1993; Paredes et al. 2006; Fayet et al. 2016). Reproduction may also increase whole-animal metabolic rates, which could potentially lead to increased ROS production (Speakman 2008; Hood et al. 2018; Hill et al. 2019). Without upregulating enzymatic antioxidants or obtaining dietary antioxidants to protect against ROS, animals could accumulate oxidative damage during breeding (Monaghan et al. 2009). When animals emphasize self-maintenance by upregulating or ingesting antioxidants, oxidative damage may be mitigated, though at the cost of reproduction (Bize et al. 2008; Monaghan et al. 2009; Costantini et al. 2016; Heine and Hood 2020).

The hypothesis of reproductive oxidative stress—that reproduction leads to oxidative imbalance and increased damage—has been conceptually accepted and supported in invertebrates (Salmon et al. 2001; Wang et al. 2001). In mammals, recent studies on rodents have suggested some tissues may experience more damage as a result of reproduction, while others may not experience any damage, depending on the species (da Silva et al. 2013; Xu et al. 2014; Zhao et al. 2015; Ołdakowski and Taylor 2018; Winward et al. 2018). In birds, earlier studies on zebra finches (Alonso-Alvarez et al. 2004; Wiersma et al. 2004; Bertrand et al. 2006) and domestic chickens (Murdoch et al. 2005) supported the reproductive oxidative stress hypothesis. Recent studies on wild birds show that in some species both parents experience oxidative damage from reproduction (kittiwakes [Merkling et al. 2017]; little auks [Kulaszewicz et al. 2018]; chinstrap penguins [Colominas-Ciuró et al. 2019]), while other species show no damage (canaries [Costantini et al. 2014*a*]; brown boobies [Montoya et al. 2016]; Magellanic penguins [Colominas-Ciuró et al. 2017*a*]). In Adélie penguins (Colominas-Ciuró et al. 2017*b*) and barn swallows (Pap et al. 2018), only females experience oxidative damage as a result of reproduction. The complexity of the measured responses when testing the reproductive oxidative stress hypothesis in mammals and birds suggests that factors such as breeding behaviors and life history strategies among different species could contribute to differences in oxidative status (Zera and Harshman 2001; Speakman 2008; Monaghan et al. 2009; Metcalfe and Alonso-Alvarez 2010; Speakman et al. 2015).

Compared with mammals of similar body mass, birds have significantly higher metabolic rates and longer life span (Hulbert et al. 2007; Jimenez et al. 2019). This is unexpected according to the free radical theory of aging (Harman 1956), as higher metabolic rates should lead to faster generation of ROS and potentially senescence (Speakman 2008; Monaghan et al. 2009), though the relationship between oxidative stress and metabolic rate is not linear (Stier et al. 2014). This makes birds an excellent model for studying the association between oxidative status and life history strategies. Thick-billed murres (*Uria lomvia*) are particularly intriguing in this regard because they have the highest sustained locomotory costs of any vertebrate (Elliott et al. 2013). During breeding, murres fly more because they are constrained to central-place foraging, and consequently reproduction is the most costly period of the annual cycle (Dunn et al. 2020). The high costs of reproduction likely explain why murres and their close relatives, unique among birds, fledge at about 30% of adult mass, after which time the father raises the chick at sea (Elliott et al. 2017). The high proximate (daily energy expenditure) and ultimate (raising chick at sea) costs of reproduction mean that murres may be a good model organism for testing the reproductive oxidative stress hypothesis. Nonetheless, murres are long-lived, so they may avoid incurring oxidative damage during breeding.

Little is known about the oxidative status of thick-billed murres during the breeding season. To test the predictions of the reproductive oxidative stress hypothesis, we manipulated breeding efforts of murres in the wild to examine change in oxidative status by delaying breeding (removing an egg and forcing females to relay) or by increasing energy costs (handicapping birds by clipping the tips of their wings) for both members of the pair. We treated both members of the pair to avoid compensation by the partner, as is known to occur in murres (Paredes et al. 2005; Jacobs et al. 2012). Individuals rotated between the three treatments over 3 yr. Over the 3 yr, we predicted that oxidative damage would accumulate throughout the reproductive season for both male and female murres, as they both contribute to egg incubation and chick rearing. Specifically, the reproductive oxidative stress hypothesis predicts that increased reproductive efforts (increased energy investment or faster breeding required to fledge chicks before the Arctic summer ends) would impede murres' ability to forage and obtain dietary antioxidants, thus resulting in lower antioxidant activity and higher oxidative damage. Because murres raise a single chick, pairs that lose an egg early in the season can relay ~14 d after egg loss (Gaston and Hipfner 2000). Murres, like other auks, have an exceptionally large egg relative to body mass for a seabird (Elliott et al. 2004), and females may have particularly high early-season oxidative stress due to the demands of creating and carrying the egg. The reproductive oxidative stress hypothesis predicts that female murres would have lower antioxidant activity and higher oxidative damage than males during early breeding as a result of producing exceptionally large eggs.

Material and Methods

Animals and Manipulations

The study was conducted on breeding thick-billed murres at Coats Island, Nunavut, Canada (62.95°N, 82.02°W) between June and August in 2017, 2018, and 2019. At the start of the study, 60 active nests within a continuous area of the colony were identified and systematically assigned to three treatments: delay, handicap, and control. The same individuals were used in each year, except new individuals were added to the study to replace individuals that did not return after the first or second year. Adult philopatry to a breeding site is very high (Steiner et al. 2003), so the same birds

typically returned to the same breeding sites each year. Both members of each pair were treated identically (delay, wing clipped, control) in each year, with treatment switching among years. Our goal was to have each bird switch through all three treatments, which occurred by 2019 for birds sampled in all 3 yr. During early incubation (0–14 d after egg laying), both adults from each nest were captured at the nest using a noose pole. After capturing both adults from the delay nests, eggs were removed from delay nests to allow female murres to relay. For handicap nests, the five outer primaries on both wings of each adult were clipped to the length of the fifth primary to increase flight costs for adults at these nests. A similar wing-clipping experiment (reduction of primaries by 2 cm) at our study site led to lower chick growth rates (more pronounced in clipped males) and higher plasma neutral lipids (males only) with no impact on adult mass loss (Jacobs et al. 2013), implying that many costs may be passed along to chicks. Both adults from control birds were captured and handled in the same manner as delay and handicap birds. Focal nests were monitored daily during the breeding season from an observation blind located approximately 30 m directly across from the nesting ledges. Before early-season capture, we mapped all nests within the study area. For each nest we confirmed the presence of an adult and, when possible, the contents of the nest (egg or chick) at 1 or 2 d intervals. We calculated dates of egg laying, chick hatching, and nest failure according to the average of the last day before a change in nest status and the first day of the new nest status.

Adults from nests that remained active were recaptured later in the breeding season to obtain blood samples. Adults from nests in the control and handicap treatments were captured 23.4 d (SD = 4.8) after the initial capture. During the second capture, 63.3% of nests were still incubating an egg and 36.7% were brooding a chick; among nests with chicks, mean chick age was 1.9 d (SD = 2.1). Adults from nests in the delay treatment were captured 31.3 d (SD = 6.7) after the initial capture. We treated all birds from the control group treatment that lost an egg naturally before the final egg removal in the delay treatment that year as part of the delay treatment. The delay treatment was expected to disproportionately impact females, who had to produce and carry a second large egg. All activities were permitted under territorial and federal permits, including the Canadian Council for Animal Care (permit 2015-7599).

Blood Collection and Oxidative Status Measurements

During each capture event, we collected blood samples from the brachial vein. Samples were stored on ice in a 3-mL Vacutainer treated with EDTA for up to 3 h, centrifuged to separate plasma from red blood cells, and frozen at −70°C until processing. Sex was determined using DNA or was inferred from the mate's sex. We distributed samples across treatments and years randomly across plates. Each individual was run in duplicate for all assays described below.

Plasma total antioxidant capacity (TAC) was measured using a BioVision kit (catalog no. K274-100) following the manufacturer's protocol (table 1). Another circulating antioxidant in birds, UA concentration, was measured with a kit (Cayman Chemical, Ann Arbor, MI; catalog no. 700320) following the manufacturer's protocol. To estimate lipid damage, malondialdehyde (MDA) was measured using the thiobarbituric acid reactive substances assay kit (Cayman Chemical, catalog no. 10009055). To estimate enzymatic antioxidants in red blood cells, we measured SOD, GPx, and CAT activities using commercially available kits (Cayman Chemical, catalog nos. 706002, 703102, and 707002) following the manufacturer's protocol. We diluted 4 μL of red blood cells into 396 μL of 20 mM HEPES, 1 mM EGTA, and 90 mM mannitol buffer solution and vortexed samples before each assay (Jimenez et al. 2019). All enzyme assays were performed on the same day as dilution. In addition, we quantified total protein in each diluted red blood cell sample using a protein determination kit (Cayman Chemical, catalog no. 704002) to standardize enzyme activities across samples. Intra-assay and interassay variability for all measurements are provided in table A1.

Statistical Analysis

We performed analysis on sets of response variables: early-breeding-season oxidative status, late-breeding-season oxidative status, and the change in oxidative status during the breeding season. Change in oxidative status was calculated by subtracting early-season values from late-season values for each individual murre that was sampled during both measurement periods within a breeding season. First, we used Pearson's correlation tests to examine the relationship among our six measures of oxidative status for each set of responses. Then we used linear mixed effects (LME) models with year, treatment, and sex as fixed effects and

Table 1: Summary of parameters measured in the study

Measure	Abbreviation	Tissue	Purpose
Total antioxidant capacity	TAC	Plasma	Total levels of antioxidants that circulate in plasma; may include metabolites that alter antioxidant status but are not generated specifically for that purpose
Uric acid	UA	Plasma	A major antioxidant that is generated from protein breakdown
Malondialdehyde	MDA	Plasma	Lipid damage from oxidative stress
Superoxide dismutase	SOD	Red blood cells	Enzymatic antioxidant
Glutathione peroxidase	GPx	Red blood cells	Enzymatic antioxidant
Catalase	CAT	Red blood cells	Enzymatic antioxidant

bird identity and nest identity as random effects. We tested for interactions between sex and treatment and between year and treatment, nonsignificant ($P > 0.05$) interaction terms were removed from the final models. SOD, MDA, and GPx were log transformed for analysis to meet assumptions of normality and equal variance in residuals. We reported parameter estimates for these measures on the log scale.

Final sample sizes for all combinations of year, sex, and treatment are reported in tables A2–A4. A very small number of early-breeding-season red blood cell samples were collected in 2017 ($N = 2$–4 depending on treatment; table A2); because of small sample sizes, we excluded the 2017 data for CAT, GPx, and SOD from analyses of early-season oxidative status and change of oxidative status within breeding season. In all analyses, we treated year as a categorical variable and used 2019 as the reference year for consistency in reporting results for analyses that did not include 2017 data. In many cases, the antioxidant enzymes were correlated with one another (tables A5–A7), so we are careful not to interpret correlations between explanatory variables and those enzymes as causal. Parameter estimates are means ± SEs in text and means ± 95% confidence intervals in figures. Confidence intervals for effect sizes in LME models were calculated using the likelihood profile method. Mixed effects models were run using the lme4 package in R (ver. 3.6.1; R Development Core Team 2018). We considered results significant if $P < 0.05$, and $P < 0.10$ was considered marginally significant.

Results

Early-Breeding-Season Oxidative Status

Sex and year affected early-season oxidative status (fig. 1; table 2). Males had significantly higher GPx (0.25 ± 0.05 log nmol/min/μg) and SOD (0.10 ± 0.03 log nmol/μg) and significantly lower TAC (−2.80 ± 0.64 mM) than females. There was also a trend for lower MDA (−0.12 ± 0.07 log μM) in males during early breeding. There were no significant underlying differences between murres in the delay treatment and those in the control treatment. There was a trend for higher MDA among murres in the handicap treatment (0.153 ± 0.08 log μM) relative to the control treatment during early breeding, before administering any treatment. In 2017, TAC (1.76 ± 0.73 mM) and UA (93,000 ± 10,100 μM) were both significantly higher than in 2019, while MDA (−0.45 ± 0.08 log μM) was significantly lower than in 2019. In 2018, UA (36,500 ± 11,100 μM) was significantly higher than in 2019, while GPx (−0.45 ± 0.05 log nmol/min/μg), MDA (0.38 ± −0.08 log μM), and CAT (−127.3 ± 12.9 nmol/min/μg) were all significantly lower than in 2019. There was no support for interactions between treatment and year or between treatment and sex (table A8).

Late-Breeding-Season Oxidative Status

Males had significantly higher GPx (0.13 ± 0.05 log nmol/min/μg) and significantly lower TAC (−3.43 ± 1.14 mM) than females during the late breeding season (fig. 2; table 3). Murres in the delay treatment had significantly lower SOD (−0.10 ± 0.05 log nmol/μg), UA (−35,500 ± 13,600 μM), and GPx (−0.13 ± 0.06 log nmol/min/μg). There was also a trend for lower CAT in the delay treatment (−20.8 ± 11.8 nmol/min/μg). There was a trend for higher MDA (0.16 ± 0.09 log μM) for murres in the handicap treatment; however, the magnitude of this trend was similar to MDA values within this group in the early season, before handicapping. In 2017, late-breeding-season TAC (18.3 ± 1.42 mM), UA (57,100 ± 13,900 μM), and CAT (39.8 ± 12.3 nmol/min/μg) were higher than in 2019, while GPx (−0.28 ± 0.06 log nmol/min/μg) and MDA (−1.05 ± 0.09 log μM) were lower than in 2019. In 2018, GPx (−0.37 ± 0.06 log nmol/min/μg), SOD (−0.22 ± 0.05 log nmol/μg), and MDA (−0.75 ± 0.10 log μM) were lower than in 2019. There was no support for interactions between treatment and year or between treatment and sex (table A9).

Change in Oxidative Status within Breeding Season

There were significant changes in TAC, CAT, and MDA within breeding seasons; however, patterns of change were different among years (figs. 3, 4; table 4). Males had significantly lower ΔUA than females (−41,600 ± 15,400 μM); there were no other significant differences between males and females. There was lower ΔUA among murres in the delay treatment (−36,000 ± 18,200 μM) than murres in the control treatment. In 2017, TAC increased during the breeding season (17.6 ± 1.3 mM), but there were no within-season changes in TAC in 2018 (1.3 ± 1.4 mM) or 2019 (−0.9 ± 1.5 mM). CAT increased during the breeding season in 2018 (62.7 ± 15.5 nmol/min/μg) and decreased during the breeding season in 2019 (−79.5 ± 16.3 nmol/min/μg). There was a significant increase in MDA during the breeding season in 2019 (−0.64 ± 0.12 log μM) but no change in MDA during the other 2 yr (2017: −0.15 ± 0.10 log μM; 2018: 0.16 ± 0.11 log μM). In 2017, ΔTAC was significantly higher than in 2019 (18.5 ± 1.92 mM), while ΔMDA (−0.78 ± 0.15 log μM) and ΔUA (−40,300 ± 18,500 μM) were significantly lower. In 2018, ΔCAT was significantly higher than in 2019 (142.2 ± 21.2 nmol/min/μg), while ΔMDA (−0.48 ± 0.16 log μM) and ΔSOD (−0.14 ± 0.07 log nmol/μg) were significantly lower. There was a trend for higher ΔGPx (0.17 ± 0.09 log nmol/min/μg) in 2018 relative to 2019. There was no support for interactions between treatment and year or between treatment and sex (table A10).

Discussion

The reproductive oxidative stress hypothesis (Speakman 2008; Monaghan et al. 2009; Metcalfe and Alonso-Alvarez 2010) predicted that challenged birds (females after egg laying; delayed/handicapped birds at the end of breeding) would have lower antioxidant activity and higher oxidative damage than nonchallenged birds (males/control birds). In support, females had marginally higher lipid damage and lower enzymatic oxidative

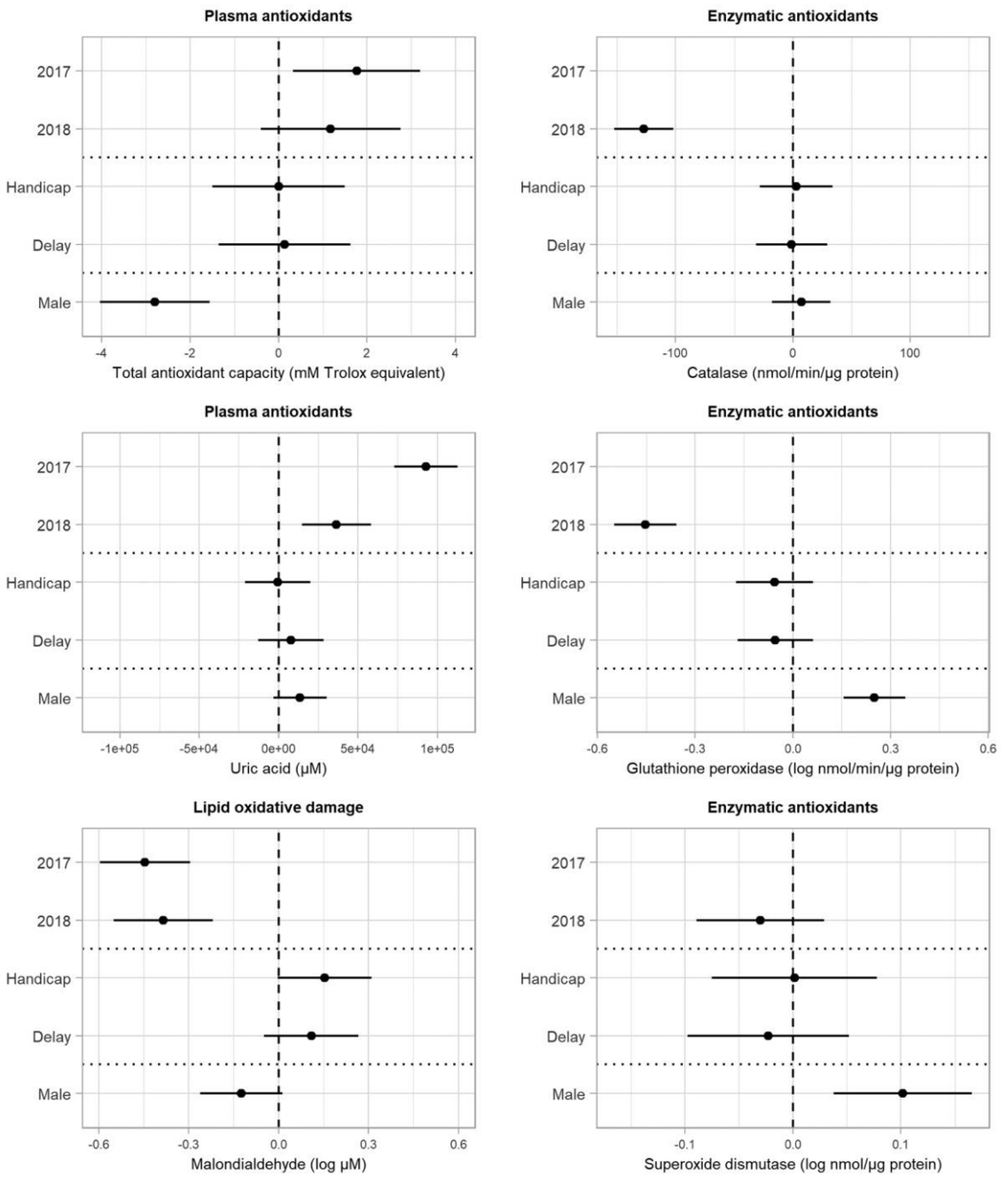

Figure 1. Parameter estimates for the effect of year, treatment, and sex on oxidative status of thick-billed murres during the early breeding season. Oxidative status was measured for plasma antioxidants (total antioxidant capacity, uric acid), lipid oxidative status (malondialdehyde), and enzymatic antioxidants (catalase, glutathione peroxidase, and superoxide dismutase). Points show the mean difference from the reference group associated with each parameter, and error bars indicate the 95% confidence interval. The dashed vertical line indicates no difference from the reference group, which represents control females in 2019. Dotted horizontal lines separate effects of year, treatment, and sex. Note that early-season samples were collected before administering delay and handicap treatments.

defenses, GPx and SOD, than males early in the breeding season. Also, when reproductive costs were increased via delay, murres had lower antioxidant defenses. Nonetheless, interannual variation had the most consistent and highest-magnitude effects on differences in total antioxidant levels (TAC), enzymatic antioxidant activity (CAT, GPx, and SOD), and lipid peroxidation damage (MDA). These strong interannual differences in measures of oxidative stress point to the importance of environmental conditions before and during the breeding season in determining the oxidative state of thick-billed murres.

Table 2: Effects of year, treatment, and sex on oxidative status of thick-billed murres during the early breeding season

Measure, parameter	Estimate	SE	df	*t*	*P*
Total antioxidant capacity:					
(Intercept)	**28.811**	**.774**	**291.2**	**37.21**	**<.001**
Year2018	1.177	.804	222.8	1.46	.145
Year2017	**1.764**	**.734**	**206.2**	**2.40**	**.017**
TreatmentDelay	.133	.762	262.2	.18	.861
TreatmentHandicap	−.001	.765	214.1	.00	.999
SexM	**−2.799**	**.635**	**70.1**	**−4.41**	**<.001**
log(Superoxide dismutase):					
(Intercept)	**1.609**	**.035**	**179.1**	**45.40**	**<.001**
Year2018	−.030	.030	106.1	−1.00	.321
TreatmentDelay	−.023	.038	143.6	−.60	.550
TreatmentHandicap	.001	.039	143.6	.04	.972
SexM	**.102**	**.033**	**83.0**	**3.11**	**.003**
Uric acid:					
(Intercept)	**194,772**	**10,665**	**300.0**	**18.26**	**<.001**
Year2018	**36,469**	**11,101**	**299.4**	**3.29**	**.001**
Year2017	**92,861**	**10,128**	**286.1**	**9.17**	**<.001**
TreatmentDelay	7,624	10,534	314.6	.72	.470
TreatmentHandicap	−577	10,551	293.1	−.05	.956
SexM	13,522	8,546	254.3	1.58	.115
log(Glutathione peroxidase):					
(Intercept)	**1.405**	**.053**	**190.0**	**26.34**	**<.001**
Year2018	**−.454**	**.049**	**190.0**	**−9.29**	**<.001**
TreatmentDelay	−.054	.059	190.0	−.91	.362
TreatmentHandicap	−.057	.060	190.0	−.94	.348
SexM	**.251**	**.049**	**190.0**	**5.17**	**<.001**
log(Malondialdehyde):					
(Intercept)	**5.017**	**.083**	**310.0**	**60.65**	**<.001**
Year2018	**−.385**	**.084**	**246.0**	**−4.56**	**<.001**
Year2017	**−.446**	**.077**	**237.4**	**−5.79**	**<.001**
TreatmentDelay	.109	.080	284.5	1.35	.177
TreatmentHandicap	.153	.080	239.9	1.91	.057
SexM	−.124	.070	154.1	−1.77	.078
Catalase:					
(Intercept)	**396.38**	**14.07**	**190.0**	**28.18**	**<.001**
Year2018	**−127.30**	**12.88**	**190.0**	**−9.88**	**<.001**
TreatmentDelay	−1.00	15.54	190.0	−.06	.949
TreatmentHandicap	2.91	15.91	190.0	.18	.855
SexM	7.22	12.80	190.0	.56	.573

Note. Estimates and standard errors are from linear mixed effects models with year, treatment, and sex as fixed effects and individual identity and nest identity as random effects. The reference level for all models (Intercept) refers to control females in 2019. Note that early-season samples were collected before administering delay and handicap treatments. Significant effects ($\alpha < 0.05$) are shown in bold.

Reproductive oxidative status was associated with environmental conditions. The early-season lipid damage during 2017 and 2018 were both significantly lower than early-season lipid damage in 2019 (fig. 1*C*). Similarly, the lipid damage (fig. 2*C*) accumulated during the breeding season was significantly lower in 2017 (when ROS may have been neutralized via increasing TAC; fig. 2*A*) and 2018 (when ROS may have been neutralized via increasing CAT; fig. 2*D*) compared with 2019 (nonenzymatic antioxidants were depleted and CAT activity was downregulated). In other words, the 2017 and 2018 breeding seasons started with low lipid peroxidation damage, and murres did not accumulate much damage during reproduction. This observation is similar to another study in Adélie penguins (Colominas-Ciuró et al. 2017*b*), where accumulation of oxidative damage decreased over the duration of the breeding experiment. However, in 2019 the breeding season started with high lipid damage, and murres accumulated high levels of damage during reproduction. This result is consistent with studies on wandering albatrosses (Costantini et al.

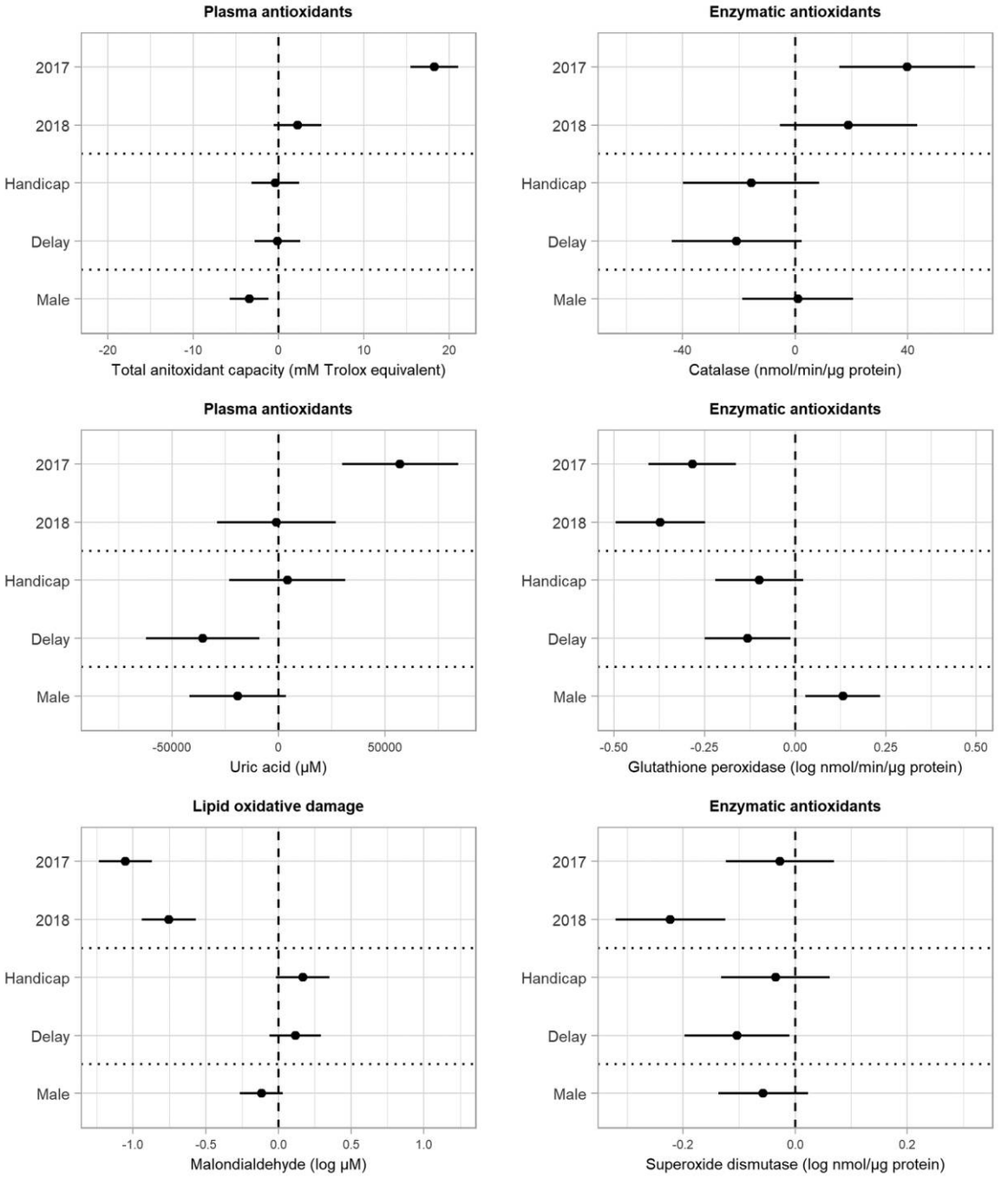

Figure 2. Parameter estimates for the effect of year, treatment, and sex on oxidative status of thick-billed murres during the late breeding season. Oxidative status was measured for plasma antioxidants (total antioxidant capacity, uric acid), lipid oxidative status (malondialdehyde), and enzymatic antioxidants (catalase, glutathione peroxidase, and superoxide dismutase). Points show the mean difference from the reference group associated with each parameter, and error bars indicate the 95% confidence interval. The dashed vertical line indicates no difference from the reference group, which represents control females in 2019. Dotted horizontal lines separate effects of year, treatment, and sex. Late-season samples were collected during late incubation or early chick rearing, on average 23 d after administering handicap and delay treatments.

2014*b*), black-legged kittiwakes (Merkling et al. 2017), and dovekies (Kulaszewicz et al. 2018) that have supported the reproductive oxidative stress hypothesis. In birds, reproduction seems to have different effects on different species, depending on their breeding behaviors and life history strategies (Wiersma et al. 2004; Costantini et al. 2014*a*, 2014*b*; Montoya et al. 2016; Kulaszewicz et al. 2018; Pap et al. 2018). Variation among studies in support for the reproductive oxidative stress hypothesis may

Table 3: Effects of year, treatment, and sex on oxidative status of thick-billed murres during the late breeding season

Measure, parameter	Estimate	SE	df	*t*	*P*
Total antioxidant capacity:					
(Intercept)	**29.483**	**1.349**	**222.0**	**21.85**	**<.001**
Year2018	2.244	1.436	222.0	1.56	.119
Year2017	**18.279**	**1.422**	**222.0**	**12.85**	**<.001**
TreatmentDelay	−.131	1.363	222.0	−.10	.924
TreatmentHandicap	−.365	1.427	222.0	−.26	.799
SexM	**−3.428**	**1.144**	**222.0**	**−3.00**	**.003**
log(Superoxide dismutase):					
(Intercept)	**1.855**	**.048**	**211.0**	**38.78**	**<.001**
Year2018	**−.223**	**.050**	**201.4**	**−4.44**	**<.001**
Year2017	−.027	.049	185.5	−.56	.578
TreatmentDelay	**−.104**	**.048**	**193.6**	**−2.18**	**.031**
TreatmentHandicap	−.035	.050	179.0	−.71	.480
SexM	−.057	.041	102.8	−1.40	.165
Uric acid:					
(Intercept)	**225,513**	**13,725**	**210.3**	**16.43**	**<.001**
Year2018	−1,009	14,200	198.8	−.07	.943
Year2017	**57,131**	**13,880**	**181.9**	**4.12**	**<.001**
TreatmentDelay	**−35,456**	**13,570**	**186.9**	**−2.61**	**.010**
TreatmentHandicap	4,214	13,910	172.0	.30	.762
SexM	−19,132	11,563	86.9	−1.65	.102
log(Glutathione peroxidase):					
(Intercept)	**1.549**	**.062**	**208.7**	**24.97**	**<.001**
Year2018	**−.372**	**.063**	**192.9**	**−5.90**	**<.001**
Year2017	**−.284**	**.061**	**173.1**	**−4.63**	**<.001**
TreatmentDelay	**−.131**	**.060**	**182.0**	**−2.17**	**.031**
TreatmentHandicap	−.099	.062	162.2	−1.61	.110
SexM	**.132**	**.053**	**94.1**	**2.50**	**.014**
log(Malondialdehyde):					
(Intercept)	**5.546**	**.089**	**212.9**	**62.05**	**<.001**
Year2018	**−.753**	**.095**	**221.8**	**−7.94**	**<.001**
Year2017	**−1.052**	**.094**	**218.5**	**−11.23**	**<.001**
TreatmentDelay	.117	.090	215.1	1.29	.198
TreatmentHandicap	.167	.094	219.7	1.78	.077
SexM	−.117	.075	168.9	−1.57	.119
Catalase:					
(Intercept)	**331.27**	**11.68**	**216.8**	**28.37**	**<.001**
Year2018	18.99	12.42	204.6	1.53	.128
Year2017	**39.81**	**12.30**	**188.3**	**3.24**	**.001**
TreatmentDelay	−20.79	11.80	205.3	−1.76	.080
TreatmentHandicap	−15.68	12.40	183.9	−1.26	.208
SexM	.93	10.03	128.0	.09	.926

Note. Estimates and standard errors are from linear mixed effects models with year, treatment, and sex as fixed effects and individual identity and nest identity as random effects. The reference level for all models (Intercept) refers to control females in 2019. Significant effects ($\alpha < 0.05$) are shown in bold.

represent variation in environmental conditions, such as we observed in our 3-yr study, and demonstrates the importance of multiyear studies.

At the start of the breeding season across all years, female murres had higher plasma antioxidant levels but lower enzymatic antioxidant activity than males. Both types of antioxidants, enzymatic and nonenzymatic, can mitigate oxidative damage (Finkel and Holbrook 2000; Skrip and McWilliams 2016). Plasma antioxidants can include exogenous nonenzymatic antioxidants, such as UA, as well as dietary antioxidants, such as vitamins (Alan and McWilliams 2013; Skrip and McWilliams 2016). At the onset of breeding, male murres may have upregulated endogenous antioxidant enzymes, particularly SOD (fig. 1*F*) and GPx (fig. 1*D*), to quench ROS and prevent oxidative damage. Lipid peroxidation

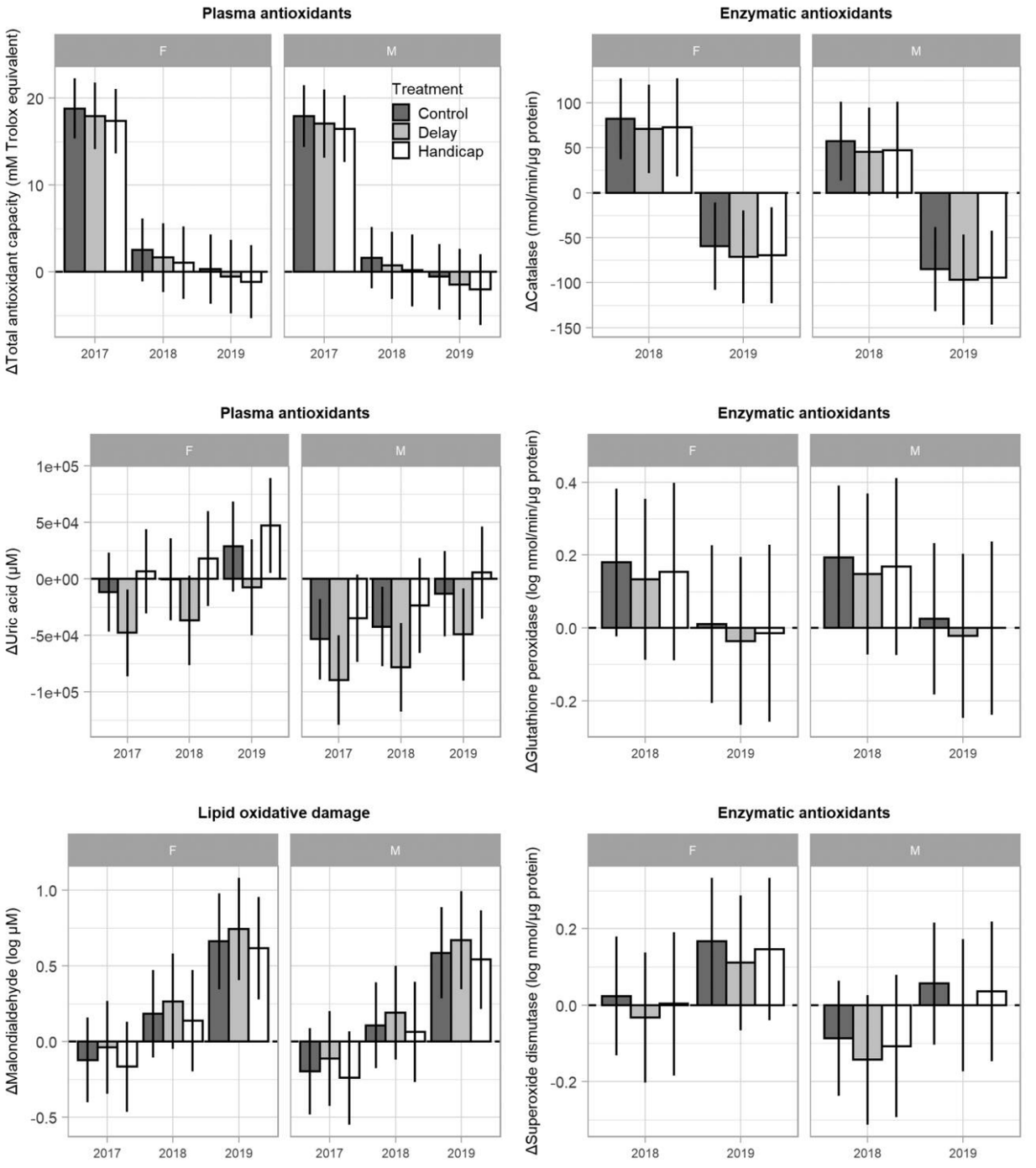

Figure 3. Changes in oxidative status of thick-billed murres by sex (F = females; M = males), year, and treatment. Oxidative status was measured for plasma antioxidants (total antioxidant capacity, uric acid), lipid oxidative status (malondialdehyde), and enzymatic antioxidants (catalase, glutathione peroxidase, and superoxide dismutase). Bar heights show the mean change in oxidative status between early- and late-breeding-season samples, and error bars show 95% confidence intervals.

damage was measured in MDA, the degraded form of lipid peroxyl radicals in plasma (Skrip and McWilliams 2016). Females had marginally more MDA in the early season ($P = 0.078$), consistent with the demands of producing the egg with lower enzymatic antioxidant protection. Although we infer that the cause of the sex differences is due to egg production, these differences may be due to other factors, such as timing of arrival or overwinter behavior. Nonetheless, there are no known differences in overwintering area or spring migration between the sexes at our study site (Gaston et al. 2011).

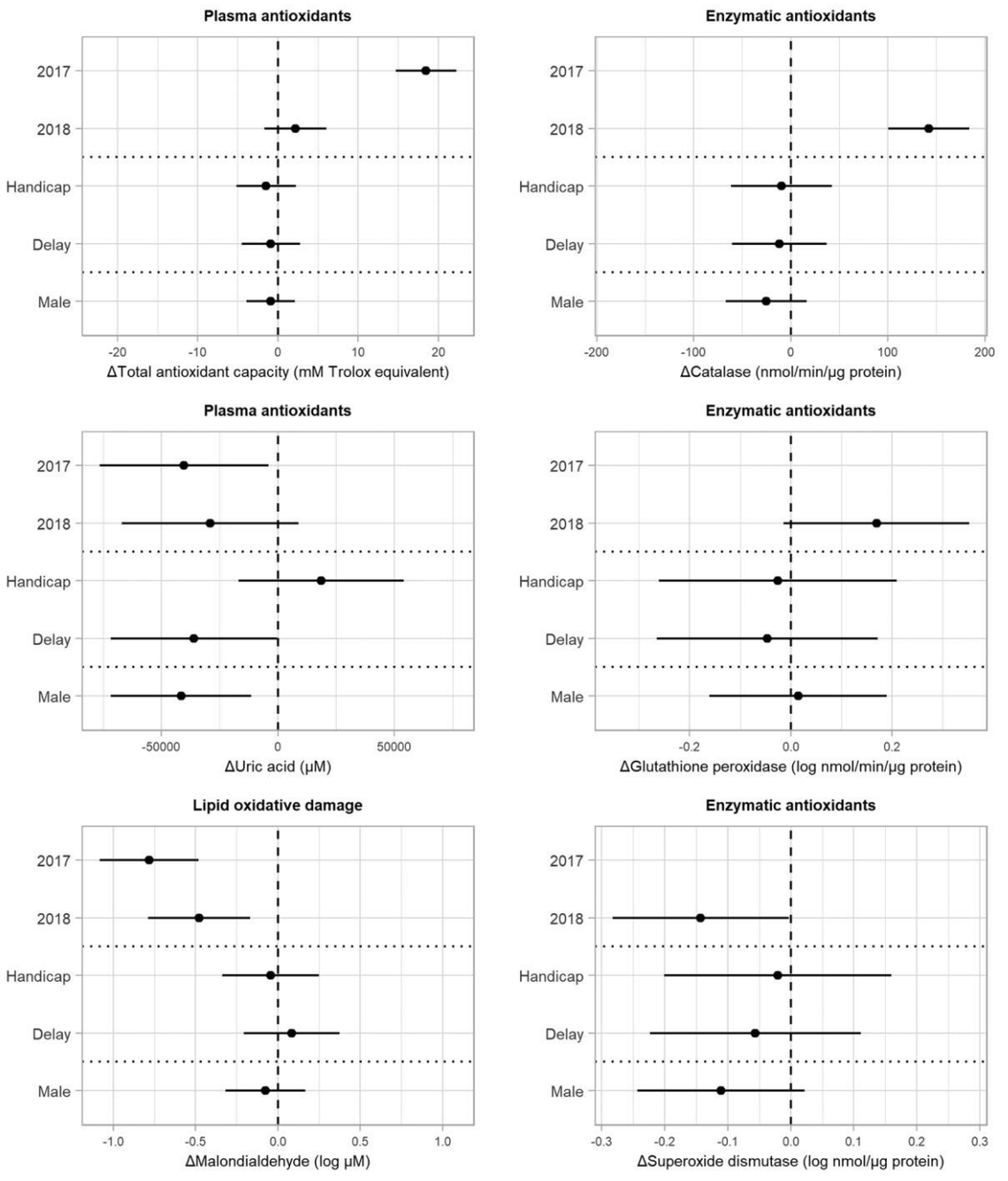

Figure 4. Parameter estimates for the effect of year, treatment, and sex on changes in oxidative status of thick-billed murres within the breeding season. Oxidative status was measured for plasma antioxidants (total antioxidant capacity, uric acid), lipid oxidative status (malondialdehyde), and enzymatic antioxidants (catalase, glutathione peroxidase, and superoxide dismutase). Points show the mean difference from the reference group associated with each parameter, and error bars indicate the 95% confidence interval. The dashed vertical line indicates no difference from the reference group, which represents control females in 2019. Dotted horizontal lines separate effects of year, treatment, and sex.

Previous studies on pelagic seabirds have suggested that egg incubation may lead to greater oxidative imbalance than chick rearing. In the dovekie, Kulaszewicz et al. (2018) measured oxidative stress, as a ratio of reactive metabolites (d-ROMs) to total antioxidant (OXY), between incubation and chick-rearing periods. They found that during incubation, dovekies suffered greater lipid oxidative damage (d-ROMs) and had lower antioxidant defense (OXY) than during rearing (Kulaszewicz et al. 2018). In another study on the Magellanic penguin, Colominas-Ciuró et al. (2017*a*) also found significantly lower antioxidant defense (OXY) during incubation than rearing but no significant difference in oxidative damage (d-ROMs) between incubation and chick

Table 4: Effects of year, treatment, and sex on changes in oxidative status of thick-billed murres within the breeding season

Measure, parameter	Estimate	SE	df	*t*	*P*
ΔTotal antioxidant capacity:					
(Intercept)	.333	1.949	184.3	.17	.864
Year2018	2.191	1.981	188.9	1.11	.270
Year2017	**18.490**	**1.917**	**180.7**	**9.64**	**<.001**
TreatmentDelay	−.871	1.852	188.2	−.47	.639
TreatmentHandicap	−1.457	1.878	180.8	−.78	.439
SexM	−.885	1.543	147.5	−.57	.567
ΔSuperoxide dismutase:					
(Intercept)	**.167**	**.080**	**110.0**	**2.08**	**.040**
Year2018	**−.143**	**.071**	**114.3**	**−2.00**	**.047**
TreatmentDelay	−.056	.085	111.5	−.66	.512
TreatmentHandicap	−.020	.092	108.1	−.22	.824
SexM	−.111	.068	75.6	−1.63	.107
ΔUric acid:					
(Intercept)	28,582	19,637	185.1	1.46	.147
Year2018	−29,096	19,378	166.9	−1.50	.135
Year2017	**−40,329**	**18,513**	**156.3**	**−2.18**	**.031**
TreatmentDelay	**−36,023**	**18,201**	**165.1**	**−1.98**	**.049**
TreatmentHandicap	18,593	18,076	147.7	1.03	.305
SexM	**−41,618**	**15,426**	**86.4**	**−2.70**	**.008**
ΔGlutathione peroxidase:					
(Intercept)	.010	.105	110.4	.10	.923
Year2018	.170	.093	114.6	1.81	.072
TreatmentDelay	−.046	.111	111.5	−.41	.680
TreatmentHandicap	−.025	.120	107.9	−.21	.834
SexM	.014	.089	78.5	.16	.872
ΔMalondialdehyde:					
(Intercept)	**.661**	**.156**	**189.0**	**4.24**	**<.001**
Year2018	**−.478**	**.159**	**189.0**	**−3.02**	**.003**
Year2017	**−.782**	**.154**	**189.0**	**−5.09**	**<.001**
TreatmentDelay	.083	.148	189.0	.56	.574
TreatmentHandicap	−.044	.150	189.0	−.29	.770
SexM	−.075	.124	189.0	−.60	.547
ΔCatalase:					
(Intercept)	**−59.76**	**23.41**	**112.6**	**−2.55**	**.012**
Year2018	**142.18**	**21.21**	**81.7**	**6.70**	**<.001**
TreatmentDelay	−11.71	24.78	98.3	−.47	.638
TreatmentHandicap	−9.73	26.52	113.8	−.37	.714
SexM	−25.19	21.33	83.2	−1.18	.241

Note. Estimates and standard errors are from linear mixed effects models with year, treatment, and sex as fixed effects and individual identity and nest identity as random effects. The reference level for all models (Intercept) refers to control females in 2019. Significant effects ($\alpha < 0.05$) are shown in bold.

rearing. They concluded that incubation was more physiologically demanding because antioxidant defenses declined during incubation, presumably as a consequence of mitigating oxidative damage caused by ROS (Colominas-Ciuró et al. 2017*a*). It would be interesting to obtain similar measurements in murres at the end of the chick-rearing stage.

Our study was able to capture only some components of oxidative status, and other components (not measured) may have revealed greater propensity to demonstrate changes within the oxidative stress system (Costantini 2019). For example, red blood cells and plasma are simple to sample and are considered a physiological reservoir for the animal's body, but they turn over within weeks and are not clearly linked to fitness. Metabolically active tissue, such as kidney and brain, which are clearly linked to fitness (e.g., excretion, cognition) and have some cells that turnover slowly, may show clearer impacts of oxidative status. Many studies found that reproductive oxidative damage can be tissue dependent (da Silva et al. 2013; Xu et al. 2014; Zhao et al. 2015; Ołdakowski and Taylor 2018). Other studies suggest that female rodents that have reproduced could be protected by the antioxidant capacities of estradiol and mitigate reproductive oxidative stress (Garratt et al. 2011; Jothery et al. 2016; Winward

et al. 2018). Likewise, we examined only lipid peroxidation as a marker of oxidative damage; oxidative damage to DNA or proteins may be more sensitive and equally important and demonstrate a different pattern than lipid oxidative damage (Costantini 2019). Overall, our study concludes that thick-billed murres may have experienced reproductive oxidative stress in 2019 and when breeding was delayed experimentally but that oxidative status was highly variable across years. Indeed, year was consistently the most important factor determining oxidative status, and some of the inconsistent results in previous field studies may be because reproductive oxidative stress occurs only in some years. This highlights the importance of multiyear experimental studies. However, murres are able to prevent oxidative damage, possibly through upregulation of enzymatic and nonenzymatic antioxidants as well as by performing different parental roles to improve oxidative status. Last, increasing reproductive efforts did have a significant impact on oxidative status. Our study illustrates the complexity of the reproductive oxidative stress hypothesis—that it may be species specific depending on different breeding behaviors and life history strategies of the species.

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APPENDIX

Table A1: Intra-assay and interassay variability for each measure of oxidative status

Measure	Intra-assay variability (%)	Interassay variability (%)
TAC	1.0–2.9	2.2
MDA	2.0–2.6	3.8
UA	2.2–3.8	3.5
CAT	1.4–3.1	2.4
GPx	2.5–4.8	4.2
SOD	1.1–3.4	2.4

Note. CAT = catalase; GPx = glutathione peroxidase; MDA = malondialdehyde; SOD = superoxide dismutase; TAC = total antioxidant capacity; UA = uric acid.

Table A2: Samples sizes for early-breeding-season oxidative status

Year, treatment, sex	TAC	CAT	MDA	GPx	UA	SOD
2017:						
Control:						
Female	23	2	23	2	23	2
Male	19	2	19	2	19	2
Delay:						
Female	21	4	21	4	21	4
Male	20	4	19	4	20	4
Handicap:						
Female	22	4	22	4	22	4
Male	20	4	20	4	20	4
2018:						
Control:						
Female	14	14	14	14	14	14
Male	16	15	16	15	16	15
Delay:						
Female	16	16	16	16	16	16
Male	18	18	18	18	18	18

Table A2 (*Continued*)

Year, treatment, sex	TAC	CAT	MDA	GPx	UA	SOD
Handicap:						
Female	11	11	11	11	11	11
Male	13	13	13	13	13	13
2019:						
Control:						
Female	18	18	18	18	18	18
Male	15	15	15	15	15	15
Delay:						
Female	15	15	15	15	15	15
Male	17	18	17	18	17	18
Handicap:						
Female	18	18	18	18	18	18
Male	20	19	20	19	20	19

Note. Sample sizes of males and females are not exactly the same for each treatment and year because it was not always possible to catch both members of all pairs within each sampling period. CAT = catalase; GPx = glutathione peroxidase; MDA = malondialdehyde; SOD = superoxide dismutase; TAC = total antioxidant capacity; UA = uric acid.

Table A3: Samples sizes for late-breeding-season oxidative status

Year, treatment, sex	TAC	CAT	MDA	GPx	UA	SOD
2017:						
Control:						
Female	16	16	16	16	16	16
Male	15	14	15	14	15	14
Delay:						
Female	10	10	10	10	10	10
Male	13	13	13	13	13	13
Handicap:						
Female	13	13	13	13	13	13
Male	13	13	13	13	13	13
2018:						
Control:						
Female	19	19	19	19	19	19
Male	20	19	20	19	20	19
Delay:						
Female	13	13	13	13	13	13
Male	10	10	10	10	10	10
Handicap:						
Female	7	7	7	7	7	7
Male	7	7	7	7	7	7
2019:						
Control:						
Female	15	15	15	15	15	15
Male	18	18	18	18	18	18
Delay:						
Female	8	9	8	9	8	9
Male	9	9	9	9	9	9
Handicap:						
Female	7	7	7	7	7	7
Male	9	9	9	9	9	9

Note. Sample sizes of males and females are not exactly the same for each treatment and year because it was not always possible to catch both members of all pairs within each sampling period. CAT = catalase; GPx = glutathione peroxidase; MDA = malondialdehyde; SOD = superoxide dismutase; TAC = total antioxidant capacity; UA = uric acid.

Table A4: Samples sizes for change in oxidative status during the breeding season

Year, treatment, sex	ΔTAC	ΔCAT	ΔMDA	ΔGPx	ΔUA	ΔSOD
2017:						
Control:						
Female	16	2	16	2	16	2
Male	13	1	13	1	13	1
Delay:						
Female	10	3	10	3	10	3
Male	10	3	10	3	10	3
Handicap:						
Female	13	3	13	3	13	3
Male	11	3	11	3	11	3
2018:						
Control:						
Female	13	13	13	13	13	13
Male	16	15	16	15	16	15
Delay:						
Female	10	10	10	10	10	10
Male	10	10	10	10	10	10
Handicap:						
Female	7	7	7	7	7	7
Male	7	7	7	7	7	7
2019:						
Control:						
Female	9	10	9	10	9	10
Male	12	11	12	11	12	11
Delay:						
Female	7	8	7	8	7	8
Male	9	9	9	9	9	9
Handicap:						
Female	7	7	7	7	7	7
Male	9	9	9	9	9	9

Note. Sample sizes of males and females are not exactly the same for each treatment and year because it was not always possible to catch both members of all pairs within each sampling period. CAT = catalase; GPx = glutathione peroxidase; MDA = malondialdehyde; SOD = superoxide dismutase; TAC = total antioxidant capacity; UA = uric acid.

Table A5: Correlations among measures of early-breeding-season oxidative status

	TAC	UA	MDA	GPx	CAT	SOD
TAC		.056	.845	.068	.523	.293
UA	.110		.075	.008	.078	.532
MDA	−.010	−.100		.002	.129	.686
GPx	−.130	−.180	.210		.000	.000
CAT	−.040	−.120	.110	.570		.000
SOD	−.070	−.040	.030	.680	.280	

Note. Values in the upper-right section are *P* values, and those in the lower-left section are Pearson correlation coefficients. CAT = catalase; GPx = glutathione peroxidase; MDA = malondialdehyde; SOD = superoxide dismutase; TAC = total antioxidant capacity; UA = uric acid.

Table A6: Correlations among measures of late-breeding-season oxidative status

	TAC	UA	MDA	GPx	CAT	SOD
TAC		.002	.000	.055	.008	.265
UA	.200		.601	.150	.500	.728
MDA	−.300	−.040		.001	.014	.025
GPx	−.130	−.100	.230		.014	.000
CAT	.180	−.050	−.170	.170		.002
SOD	.080	.020	.150	.790	.210	

Note. Values in the upper-right section are *P* values, and those in the lower-left section are Pearson correlation coefficients. CAT = catalase; GPx = glutathione peroxidase; MDA = malondialdehyde; SOD = superoxide dismutase; TAC = total antioxidant capacity; UA = uric acid.

Table A7: Correlations among measures of change in oxidative status during the breeding season

	ΔTAC	ΔUA	ΔMDA	ΔGPx	ΔCAT	ΔSOD
ΔTAC		.357	.001	.867	.233	.295
ΔUA	−.070		.157	.748	.294	.305
ΔMDA	−.240	.100		.337	.073	.429
ΔGPx	−.010	.030	−.090		.000	.000
ΔCAT	.110	−.090	−.160	.470		.037
ΔSOD	−.090	.090	.070	.740	.180	

Note. Values in the upper-right section are *P* values, and those in the lower-left section are Pearson correlation coefficients. CAT = catalase; GPx = glutathione peroxidase; MDA = malondialdehyde; SOD = superoxide dismutase; TAC = total antioxidant capacity; UA = uric acid.

Table A8: Model comparison testing for an interaction between sex (S) and treatment (T) or between year (Y) and treatment for early-breeding-season oxidative status

Model	AIC	LL	Deviance	χ^2	df	*P*
TAC ~ Y + T + S + (1|IID) + (1|Nest)	1,999.5	−990.8	1,981.5	…	…	…
TAC ~ Y + T + S + S:T + (1|IID) + (1|Nest)	2,000.4	−989.2	1,978.4	3.13	2.00	.209
TAC ~ Y + T + S + S:T + Y:T + (1|IID) + (1|Nest)	2,003.9	−986.9	1,973.9	4.52	4.00	.340
log(SOD) ~ Y + T + S + (1|IID) + (1|Nest)	−21.3	18.6	−37.3	…	…	…
log(SOD) ~ Y + T + S + S:T + (1|IID) + (1|Nest)	−17.4	18.7	−37.4	.11	2.00	.946
log(SOD) ~ Y + T + S + S:T + Y:T + (1|IID) + (1|Nest)	−14.0	19.0	−38.0	.62	2.00	.735
UA ~ Y + T + S + (1|IID) + (1|Nest)	8,021.8	−4,001.9	8,003.8	…	…	…
UA ~ Y + T + S + S:T + (1|IID) + (1|Nest)	8,024.5	−4,001.2	8,002.5	1.29	2.00	.524
UA ~ Y + T + S + S:T + Y:T + (1|IID) + (1|Nest)	8,031.6	−4,000.8	8,001.6	.86	4.00	.930
log(GPx) ~ Y + T + S + (1|IID) + (1|Nest)	138.2	−61.1	122.2	…	…	…
log(GPx) ~ Y + T + S + S:T + (1|IID) + (1|Nest)	140.9	−60.5	120.9	1.21	2.00	.547
log(GPx) ~ Y + T + S + S:T + Y:T + (1|IID) + (1|Nest)	144.0	−60.0	120.0	.95	2.00	.622
MDA ~ Y + T + S + (1|IID) + (1|Nest)	581.8	−281.9	563.8	…	…	…
MDA ~ Y + T + S + S:T + (1|IID) + (1|Nest)	583.6	−280.8	561.6	2.25	2.00	.324
MDA ~ Y + T + S + S:T + Y:T + (1|IID) + (1|Nest)	589.3	−279.6	559.3	2.28	4.00	.684
log(CAT) ~ Y + T + S + (1|IID) + (1|Nest)	2,256.7	−1,120.4	2,240.7	…	…	…
log(CAT) ~ Y + T + S + S:T + (1|IID) + (1|Nest)	2,259.2	−1,119.6	2,239.2	1.57	2.00	.456
log(CAT) ~ Y + T + S + S:T + Y:T + (1|IID) + (1|Nest)	2,262.7	−1,119.4	2,238.7	.44	2.00	.803

Note. Individual identity of each bird and nest identity of each pair were included as random intercepts in all models. AIC = Akaike information criterion; CAT = catalase; GPx = glutathione peroxidase; LL = log likelihood; MDA = malondialdehyde; SOD = superoxide dismutase; TAC = total antioxidant capacity; UA = uric acid.

Table A9: Model comparison testing for an interaction between sex (S) and treatment (T) or between year (Y) and treatment for late-breeding-season oxidative status

Model	AIC	LL	Deviance	χ^2	df	*P*
TAC ~ Y + T + S + (1|IID) + (1|Nest)	1,598.6	−790.3	1,580.6	…	…	…
TAC ~ Y + T + S + S:T + (1|IID) + (1|Nest)	1,600.9	−789.5	1,578.9	1.70	2.00	.428
TAC ~ Y + T + S + S:T + Y:T + (1|IID) + (1|Nest)	1,606.1	−788.1	1,576.1	2.84	4.00	.584
log(SOD) ~ Y + T + S + (1|IID) + (1|Nest)	113.3	−47.6	95.3	…	…	…
log(SOD) ~ Y + T + S + S:T + (1|IID) + (1|Nest)	116.0	−47.0	94.0	1.26	2.00	.534
log(SOD) ~ Y + T + S + S:T + Y:T + (1|IID) + (1|Nest)	123.0	−46.5	93.0	1.00	4.00	.910
UA ~ Y + T + S + (1|IID) + (1|Nest)	5,688.5	−2,835.3	5,670.5	…	…	…
UA ~ Y + T + S + S:T + (1|IID) + (1|Nest)	5,692.0	−2,835.0	5,670.0	.49	2.00	.783
UA ~ Y + T + S + S:T + Y:T + (1|IID) + (1|Nest)	5,699.9	−2,835.0	5,669.9	.11	4.00	.999
log(GPx) ~ Y + T + S + (1|IID) + (1|Nest)	221.2	−101.6	203.2	…	…	…
log(GPx) ~ Y + T + S + S:T + (1|IID) + (1|Nest)	224.5	−101.3	202.5	.70	2.00	.704
log(GPx) ~ Y + T + S + S:T + Y:T + (1|IID) + (1|Nest)	231.2	−100.6	201.2	1.38	4.00	.848
MDA ~ Y + T + S + (1|IID) + (1|Nest)	391.9	−186.9	373.9	…	…	…
MDA ~ Y + T + S + S:T + (1|IID) + (1|Nest)	395.3	−186.6	373.3	.62	2.00	.733
MDA ~ Y + T + S + S:T + Y:T + (1|IID) + (1|Nest)	398.3	−184.1	368.3	4.99	4.00	.288
log(CAT) ~ Y + T + S + (1|IID) + (1|Nest)	2,547.0	−1,264.5	2,529.0	…	…	…
log(CAT) ~ Y + T + S + S:T + (1|IID) + (1|Nest)	2,550.5	−1,264.2	2,528.5	.57	2.00	.752
log(CAT) ~ Y + T + S + S:T + Y:T + (1|IID) + (1|Nest)	2,554.5	−1,262.2	2,524.5	3.97	4.00	.410

Note. Individual identity of each bird and nest identity of each pair were included as random intercepts in all models. AIC = Akaike information criterion; CAT = catalase; GPx = glutathione peroxidase; LL = log likelihood; MDA = malondialdehyde; SOD = superoxide dismutase; TAC = total antioxidant capacity; UA = uric acid.

Table A10: Model comparison testing for an interaction between sex (S) and treatment (T) or between year (Y) and treatment for a change in oxidative status during the breeding season

Model	AIC	LL	Deviance	χ^2	χ^2 df	*P*
ΔTAC ~ Y + T + S + (1|IID) + (1|Nest)	1,446.6	−714.3	1,428.6	…	…	…
ΔTAC ~ Y + T + S + S:T + (1|IID) + (1|Nest)	1,449.6	−713.8	1,427.6	.98	2.00	.612
ΔTAC ~ Y + T + S + S:T + Y:T + (1|IID) + (1|Nest)	1,454.3	−712.2	1,424.3	3.28	4.00	.511
ΔSOD ~ Y + T + S + (1|IID) + (1|Nest)	123.5	−53.7	107.5	…	…	…
ΔSOD ~ Y + T + S + S:T + (1|IID) + (1|Nest)	126.8	−53.4	106.8	.73	2.00	.694
ΔSOD ~ Y + T + S + S:T + Y:T + (1|IID) + (1|Nest)	130.6	−53.3	106.6	.20	2.00	.905
ΔUA ~ Y + T + S + (1|IID) + (1|Nest)	4,926.2	−2,454.1	4,908.2	…	…	…
ΔUA ~ Y + T + S + S:T + (1|IID) + (1|Nest)	4,926.6	−2,452.3	4,904.6	3.54	2.00	.171
ΔUA ~ Y + T + S + S:T + Y:T + (1|IID) + (1|Nest)	4,934.3	−2,452.1	4,904.3	.34	4.00	.987
ΔGPx ~ Y + T + S + (1|IID) + (1|Nest)	185.8	−84.9	169.8	…	…	…
ΔGPx ~ Y + T + S + S:T + (1|IID) + (1|Nest)	188.4	−84.2	168.4	1.43	2.00	.490
ΔGPx ~ Y + T + S + S:T + Y:T + (1|IID) + (1|Nest)	192.3	−84.2	168.3	.06	2.00	.968
ΔMDA ~ Y + T + S + (1|IID) + (1|Nest)	492.1	−237.1	474.1	…	…	…
ΔMDA ~ Y + T + S + S:T + (1|IID) + (1|Nest)	495.2	−236.6	473.2	.94	2.00	.624
ΔMDA ~ Y + T + S + S:T + Y:T + (1|IID) + (1|Nest)	500.1	−235.0	470.1	3.12	4.00	.538
ΔCAT ~ Y + T + S + (1|IID) + (1|Nest)	1,444.2	−714.1	1,428.2	…	…	…
ΔCAT ~ Y + T + S + S:T + (1|IID) + (1|Nest)	1,447.7	−713.8	1,427.7	.52	2.00	.773
ΔCAT ~ Y + T + S + S:T + Y:T + (1|IID) + (1|Nest)	1,451.4	−713.7	1,427.4	.27	2.00	.873

Note. Individual identity of each bird and nest identity of each pair were included as random intercepts in all models. AIC = Akaike information criterion; CAT = catalase; GPx = glutathione peroxidase; LL = log likelihood; MDA = malondialdehyde; SOD = superoxide dismutase; TAC = total antioxidant capacity; UA = uric acid.

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