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The fats and the furious: An aggressive interaction and season alter circulating medium and long chain fatty acids in a wild songbird

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Abstract

Aggression is crucial to acquire limited resources, such as food, but has energetic costs. Fatty acids (FAs) can be used to fuel aggression, and seasonal shifts in FA profiles may influence the energetic and physiological costs of aggression. Surprisingly, the effects of an aggressive interaction on FAs remain unknown.

Here, we assessed the impact of a 10 min simulated territorial intrusion in the breeding season or non-breeding season on circulating FAs in wild male song sparrows (*Melospiza melodia*). Plasma FAs were derivatised via acid-catalysed methylation, which hydrolyses complex lipids to yield total FA levels. We examined 52 FAs using gas chromatography coupled with flame ionisation detection and mass spectrometry, and quantified 33 medium and long chain FAs.

Season was the primary driver of FA composition, significantly impacting nearly all FAs. Relative to breeding subjects, non-breeding subjects exhibited higher total FA level, elevated omega (ω)-6:ω-3 ratio, and lower FA unsaturation and peroxidisability indices.

Crucially, a brief aggressive encounter significantly decreased total FA level and increased vulnerability to lipid peroxidation, and the intensity of aggression correlated positively with the ω-6:ω-3 ratio. Notably, aggressive behaviour and its effect on FAs were similar across seasons.

To our knowledge, this is the first study to investigate how aggression influences FA species across seasons in a free-living animal, and it provides one of the most comprehensive circulating FA profiles reported to date for a songbird. Our results highlight FA metabolism as a potentially important pathway underlying the energetic, oxidative, and inflammatory costs of territorial aggression.

Introduction

Territorial aggression plays a central role in animal ecology and evolution (Briffa & Sneddon, 2007; Plumier et al., 2026). In many taxa, successful territory defence enhances reproductive success and survival (Jones et al., 2014). However, aggression has costs. Beyond injury and inflammation risks (Ord, 2021), territorial interactions frequently require rapid bursts of locomotor activity, vocal displays, and prolonged vigilance, which increase energy expenditure (Arcese & Smith, 1985; Chappell et al., 1995; Marler et al., 1995; Vehrencamp et al., 1989). Individuals must acquire energetic resources through diet to sustain these behaviours. Territories are often established to secure food resources (Heimovics et al., 2012; Hixon, 1980), and territory quality impacts diet composition, energetic status, and survival chances (Golabek et al., 2012). Consequently, energy intake and territorial aggression are closely interconnected.

Fatty acids (FAs) are major diet-derived energy substrates and also serve as structural constituents of cellular membranes and precursors for numerous signaling molecules (Tvrzicka et al., 2011). While short and medium chain FAs are rapidly oxidised for energy, long-chain FAs are preferentially stored in adipose tissue as triacylglycerols (Schönfeld & Wojtczak, 2016). Under increased energetic demands, FA stores are mobilised and used as fuel (Davies et al., 2013; Kimball et al., 2024; Viblanc et al., 2018). FAs thus constitute the primary long-term energy reserve in birds and other vertebrates (Guglielmo, 2018; Jenni & Jenni-Eiermann, 1998; McWilliams et al., 2004).

Beyond fuel, FAs exert diverse physiological effects with potential behavioural impacts. Saturated FAs (SFAs), monounsaturated FAs (MUFAs), and polyunsaturated FAs (PUFAs) differ substantially in their biochemical properties and neurophysiological functions. Among PUFAs, omega (ω)-3 and ω-6 FAs differentially impact oxidative stress, inflammation, neural function,

endocrine signaling, and behaviour (Calder, 2013; Simopoulos, 2011). In particular, reduced ω-3 PUFAs and an elevated ω-6:ω-3 ratio are positively associated with aggressive behaviours in rodents and canines (DeMar Jr et al., 2006; Miachon et al., 2001; Raygada et al., 1998; Re et al., 2008), and ω-3 supplementation reduces aggression in humans (Raine & Brodrick, 2024). Crucially, whether aggression impacts FA levels remains largely unknown. One study in spiders examined a few FA species (Trabalon, 2011), and studies in vertebrates have largely relied on aggregate measures, such as total free or non-esterified FAs, which provide limited insight into the composition of the circulating FA profile following an agonistic challenge (Akiyama et al., 2022; Bagaria et al., 2025; Fernandez et al., 1994).

FA profiles vary across the year in response to changing environmental conditions and physiological demands. Importantly, food availability and composition fluctuate markedly over the year. Many temperate bird species, for example, transition from diets dominated by seeds during the non-breeding season to diets enriched in arthropods during the breeding season, altering specific FA levels in these birds (Andersson et al., 2018; Andersson et al., 2015; Martin et al., 1961; Wingfield, 2007). Furthermore, physiological demands associated with seasonal differences in reproduction, winter survival, and territorial behaviour further influence FAs through selective mobilisation, elongation, and desaturation pathways (Andersson et al., 2015; Guglielmo, 2010; Pierce et al., 2004). Understanding how aggression influences FAs therefore requires consideration of the broader seasonal context in which aggressive behaviours occur.

Song sparrows (*Melospiza melodia*) provide an excellent model for examining the impact of aggression on FAs. Male song sparrows display robust territorial aggression during both breeding and non-breeding seasons (Newman et al., 2008; Plumier et al., 2026; Wingfield & Hahn, 1994). Experimental induction of territorial aggression through a simulated territorial

intrusion (STI) offers a standardised approach for quantifying acute behavioural responses and physiological changes. Moreover, seasonal shifts in diet and physiology are expected to influence FAs and their metabolism (Andersson et al., 2018; Heimovics et al., 2012; Wingfield, 2007), making this species well suited for investigating how behavioural and seasonal factors jointly shape circulating FA profiles.

In this study, we examined 52 FAs in plasma and were able to quantify 33 medium and long chain FAs. We investigated the effects of an aggressive encounter and of season in free-living adult male song sparrows. To our knowledge, no study has examined the impact of an aggressive interaction on circulating FAs in a free-living vertebrate, nor whether this impact varies across seasons. Given the energetic demands of territorial defence, we predicted that an aggressive interaction would reduce total FA levels and increase the ω-6:ω-3 ratio. Moreover, as for other temperate avian species, we predicted that season would have a large impact on FA profiles.

Materials and Methods

Field procedures

Free-living adult male song sparrows were caught in the breeding season (April 25 to May 24, 2019) and non-breeding season (Oct. 16 to Dec. 2, 2019) near Vancouver, BC, Canada. Breeding condition was confirmed via visual inspection of the cloacal protuberance and testes. Adult status was assessed by the extent of skull pneumatisation; only individuals with fully pneumatised crania were included (Gray et al., 2024).

Song sparrow territories were identified by visual and auditory inspection. After territory identification, a furled mist net (Avinet Research Supplies, Portland, ME, USA) was installed.

Subjects were then randomly assigned to either a control or STI condition in both seasons (n = 12/group; total n = 48). For control subjects, an empty cage and silent speaker were placed in the centre of their territory for 10 min, whereas STI subjects were exposed to a live conspecific male decoy in a cage and conspecific song playback for 10 min. The song playbacks consisted of songs from several individuals, with each song played 10 times at 10 s intervals. For all groups, aggressive responses measured were: time spent within 5 m of the decoy, number of flights, number of songs, response latency, flight latency, and song latency (Jalabert et al., 2024; Wingfield, 1985). Then, the mist net was unfurled and conspecific songs were played for a maximum of 5 min (breeding control, 2.25 ± 0.55 min; breeding STI, 1.25 ± 0.43 min; nonbreeding control, 1.95 ± 0.44 min; non-breeding STI, 1.06 ± 0.37 min) to capture subjects. Playback time for capture was not significantly different across groups ($F(3,44) = 1.55$; $p = 0.215$).

Subjects were rapidly and deeply anesthetised with isoflurane immediately following capture, and then rapidly decapitated for tissue collection for other studies (Jalabert et al., 2024; Jalabert et al., 2025). To minimise handling effects, the interval from capture to euthanasia was under 3 min (breeding control, 2.35 ± 0.06 min; breeding STI, 2.33 ± 0.10 min; non-breeding control, 2.41 ± 0.12 min; non-breeding STI, 2.65 ± 0.08 min) and did not vary significantly across groups ($F(3,44) = 2.41$; $p = 0.079$). Trunk blood was collected in heparinised microhematocrit tubes (Fisher Scientific), stored on ice until return to the laboratory (within 5 h), and centrifuged for 5 min for plasma collection. Plasma was stored at −70 °C until lipid extraction. All procedures adhered to guidelines established by the Canadian Council on Animal Care, and protocols were approved by the Canadian Wildlife Service (Permit #SC-BC-2019–0012) and the UBC Animal Care Committee (Protocol #A19-0074).

Fatty acid extraction and analysis

FAs were extracted following an established method (Innis & Dyer, 1997; Innis & Dyer, 2002). Plasma samples were thawed and vortexed, and a 15 µL aliquot was transferred to a glass screw capped test tube. Then, 20 µL of a 1 µg/µL C17:0 solution in 33.3 % methanol in chloroform was added as internal standard. To avoid potential loss of medium chain FAs (C8-C12), samples were directly suspended in 2 mL of 25 % methanol in benzene and derivatised with 200 µL of acetyl chloride at 100 °C for 60 min (Lepage & Roy, 1984). This method enables the quantification of total FA levels by converting unconjugated and conjugated FAs into FA methyl esters. Samples were then cooled at 4 °C and the reaction was neutralised with 5 mL of 6 % aqueous potassium carbonate. FA methyl esters were extracted with 5 mL hexane, dried under nitrogen and carefully monitored to avoid overdrying, and immediately resuspended in 30 µL of hexane.

A 1 µL aliquot was injected into an Agilent 6850 gas chromatograph equipped with a flame ionization detector (GC-FID) and autosampler (Innis & Dyer, 1997; Innis & Dyer, 2002). Data acquisition and analysis were performed using Agilent ChemStation software. Chromatographic separation was achieved using a Supelco SP-2330 column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Hydrogen was used as the carrier gas at a flow rate of 1.6 mL/min, with nitrogen as the make-up gas and hydrogen/air supplied to the FID as fuel gases.

Individual FAs were identified by comparing retention times of sample peaks to those of 52 authentic standards (C4-C24; Electronic Supplementary Material, ESM Table 1) purchased from Supelco (Bellefonte, PA, USA), Nu-Chek Prep (Elysian, MN, USA), and Santa Cruz Biotechnology (Dallas, TX, USA). Peak identities were further confirmed by identification of

mass spectra obtained by injecting the samples into an Agilent 8810GC with 7010B triple quadrupole mass spectrometer and MassHunter software, using the same column as above but with helium as the carrier gas. Quantification was performed using peak area ratios relative to a C17:0 internal standard of known quantity. Empirical response correction factors, determined using authentic standards, were applied to FAs methyl esters of C8-12 chain lengths to account for carbon-dependent reductions in FID response. No corrections were applied to FAs ≥ C14, where FID response variations are negligible (Brenna et al., 2018; Schreiner & Hulan, 2004; Xu et al., 2010). However, our method was unable to detect short chain FAs. Researchers remained blind to experimental groups during lipid extraction and analysis.

Fatty acid compositional metrics

FA data were expressed either as levels (μg/mL) for absolute concentration analyses, or as relative proportions (calculated as the fraction of the total FA mass per sample) for proportional and structural analyses. Individual FAs were summed to calculate structural metrics, including the absolute concentration of total FAs and the relative proportions of total SFAs, total MUFAs, total PUFAs, total ω-3 PUFAs, total ω-6 PUFAs, and the ω-6:ω-3 ratio.

To characterise the overall degree of FA unsaturation and the vulnerability of the FA profile to peroxidation, we calculated the unsaturation index and the peroxidisability index, respectively. The unsaturation index summarises the number of double bonds across the FA profile and can influence membrane fluidity and permeability. The peroxidisability index places greater weighting on highly unsaturated FAs according to their relative susceptibility to lipid peroxidation (Hulbert et al., 2007; Isaksson et al., 2017; Jensen et al., 2020). Both indices were calculated from relative proportions. The unsaturation index was calculated as: 1 X (%

monoenoics) + 2 X (% dienoics) + 3 X (% trienoics) + 4 X (% tetraenoics) + 5 X (% pentaenoics) + 6 X (% hexaenoics). The peroxidisability index was calculated as: 0.025 X (% monoenoics) + 1 X (% dienoics) + 2 X (% trienoics) + 4 X (% tetraenoics) + 6 X (% pentaenoics) + 8 X (% hexaenoics).

Statistical analysis

To ensure that capture and euthanasia time was uniform across conditions, we compared handling durations among the four groups using a one-way ANOVA.

Behavioural data were evaluated for normality using Shapiro-Wilk tests, which revealed non-normal distributions across variables (all $p < 0.05$). Consequently, the effects of STI and season on aggressive behaviours were assessed separately using Mann-Whitney U tests, pooling across the other factor (Gray et al., 2024).

Univariate differences in individual FAs, FA structural metrics, and indices were analysed using two-way ANOVAs with STI and season as fixed factors, including their interaction. Residual normality and homoscedasticity were verified using Shapiro-Wilk and Levene's tests, respectively. If necessary, data underwent log10 transformation. In the presence of a significant interaction, post-hoc pairwise comparisons were conducted using estimated margin means with a Tukey adjustment. Where no significant interaction was present, only main effects were interpreted.

We used multivariate analyses to determine whether the overall composition of the FA profile differed among groups and to identify which FAs contributed most strongly to these differences. Because FA profiles are compositional, an increase in one component necessarily changes relative proportions of the others (Aitchison, 1982). Data were thus transformed using a

centered log-ratio transformation, which expresses each FA relative to the overall FA profile. Aitchison distances were used to quantify multivariate dissimilarity among samples. Group differences in overall FA profiles were assessed using a permutational multivariate analysis of variance (PERMANOVA) with STI, season, and their interaction as fixed effects. Homogeneity of multivariate dispersion among groups was evaluated using a permutational multivariate analysis of dispersion (PERMDISP) to determine whether observed group differences might reflect differences in within-group variability. Principal component analysis (PCA) was used to visualise multivariate patterns in relative FA composition, and individual FA loadings were examined to identify FAs most strongly associated with the principal axes.

Among STI subjects, associations between behavioural measures and the ω-6:ω-3 ratio were tested using linear regression models. Model significance was assessed using t-tests on regression coefficients.

All statistical tests were performed using RStudio (2023.06.2 Version 4.3.1). All statistical tests were two-tailed, and figures were made using non-transformed data presented as mean ± standard error of mean (SEM). Statistical significance was set at $\alpha = 0.05$.

Results

Aggressive behaviours

Relative to controls, STI significantly increased the intensities and decreased the latencies of all observed aggressive behaviours across the breeding and non-breeding seasons (Table 1). Compared to controls, STI subjects spent more time within 5 m of the decoy ($U = 12$, $p < 0.0001$), and produced a greater number of flights ($U = 0$, $p < 0.0001$) and songs ($U = 10$, $p < 0.0001$). Furthermore, STI subjects exhibited significantly shorter response latency ($U = 12.5$, p

< 0.0001), flight latency (U = 18.5, $p < 0.0001$), and song latency (U = 10, $p < 0.0001$) than controls. In contrast, season had no significant impacts on any aggressive behaviours (all $p >$ 0.370; Table 1), demonstrating that the aggressive response to STI was similar in both seasons.

Table 1 Song sparrow aggressive behaviours. Subjects were exposed to 10 min of control (CON) or simulated territorial intrusion (STI) conditions in the breeding and non-breeding seasons

Aggressive behaviours	Breeding CON	Breeding STI	Non-breeding CON	Non-breeding STI	Aggression		Season	
					U	*p*	U	*p*
Time spent in 5 m (s)	0.00 ± 0.00	340.00 ± 58.25	0.00 ± 0.00	472.75 ± 43.11	12	**< 0.0001**	256	0.483
Number of flights	0.17 ± 0.17	22.00 ± 3.20	0.00 ± 0.00	36.42 ± 3.33	0	**< 0.0001**	247	0.370
Number of songs	2.33 ± 1.54	33.50 ± 7.36	0.00 ± 0.00	30.17 ± 5.06	10	**< 0.0001**	265	0.628
Response latency (s)	509.75 ± 55.60	55.08 ± 23.82	600.00 ± 0.00	34.08 ± 10.28	12.5	**< 0.0001**	255	0.477
Flight latency (s)	551.67 ± 48.33	71.17 ± 22.57	600.00 ± 0.00	54.75 ± 10.26	18.5	**< 0.0001**	267	0.646
Song latency (s)	520.42 ± 47.49	123.08 ± 44.26	600.00 ± 0.00	72.25 ± 13.28	10	**< 0.0001**	265	0.620

Statistical results of Mann-Whitney Us. n = 12/group. Significant results are bolded.

Absolute FA concentrations

From the panel of 52 FAs, 34 FAs were detected, and one FA (C18:3n4) was excluded because concentrations were quantifiable in only 6 out of 48 subjects. Consequently, analyses were conducted on 33 FAs that were quantifiable in all 48 subjects (ESM Table 1).

First, the total FA level was calculated as the sum of all 33 FAs (Fig. 1). Total FA level was significantly decreased in STI subjects compared to controls ($F(1,44) = 12.92$, $p < 0.001$). Breeding subjects displayed significantly lower total FA level compared to non-breeding subjects ($F(1,44) = 59.30$, $p < 0.0001$). No significant interaction was observed between STI and season ($F(1,44) < 0.01$, $p = 0.988$).

Among the 33 quantifiable FAs, STI subjects exhibited consistently lower circulating FA levels than controls, with significant decreases in 10 FAs, 8 of which were SFAs or MUFAs, and

no significant increases in any FA species (ESM Table 2). Season significantly altered the levels of 18 FAs, with higher levels for 6 FAs in the breeding season and for 12 FAs in the non-breeding season (ESM Table 2). Only C18:3n3 exhibited a significant interaction between STI and season, with STI lowering levels only in the non-breeding season (ESM Table 2).

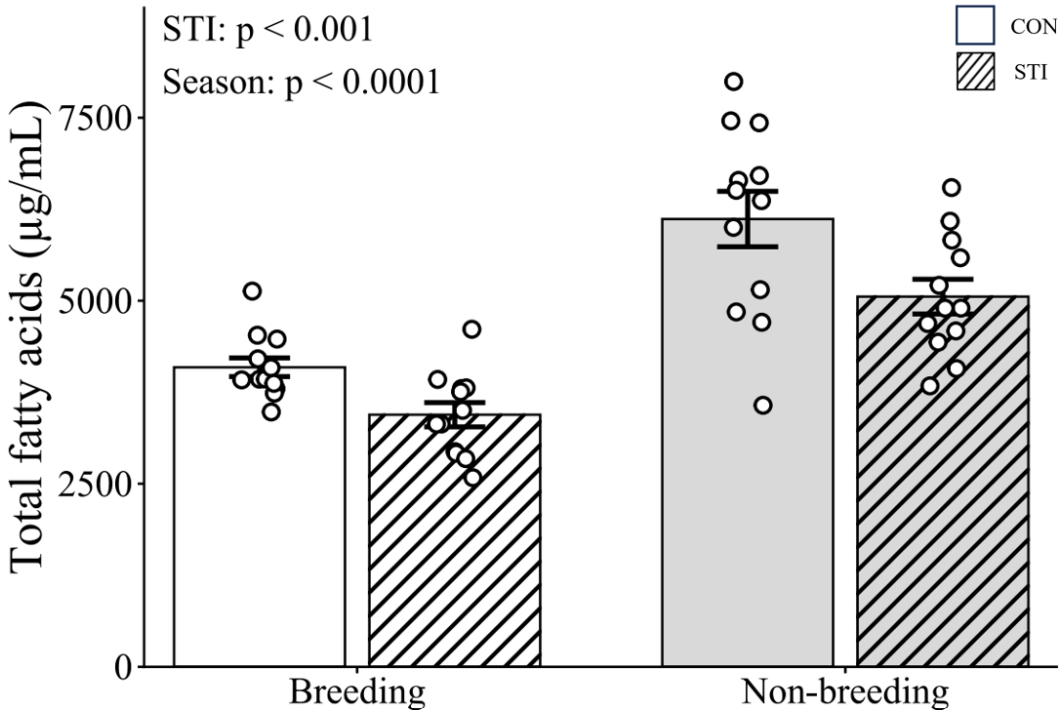

Fig. 1 Total FA level in the plasma of wild adult male song sparrows exposed to 10 min of CON or STI conditions in the breeding and non-breeding seasons. Total FA level was calculated as the sum of all 33 quantified medium and long chain FAs. Data are presented as mean ± SEM. n = 12/group. Data were analysed by 2-way ANOVA, with significant effects indicated in the figure. FA, fatty acid; CON, control; STI, simulated territorial intrusion

Relative FA proportions and FA profiles

Across all subjects, only 11 FAs exhibited average relative proportions exceeding 1 % of total FAs. In particular, C18:2n6, C18:0, C18:1n9, and C16:0 consistently represented the largest proportions of the FA profile, with each accounting for 10 to 32 % of total FAs, although their proportions varied among groups (ESM Table 3).

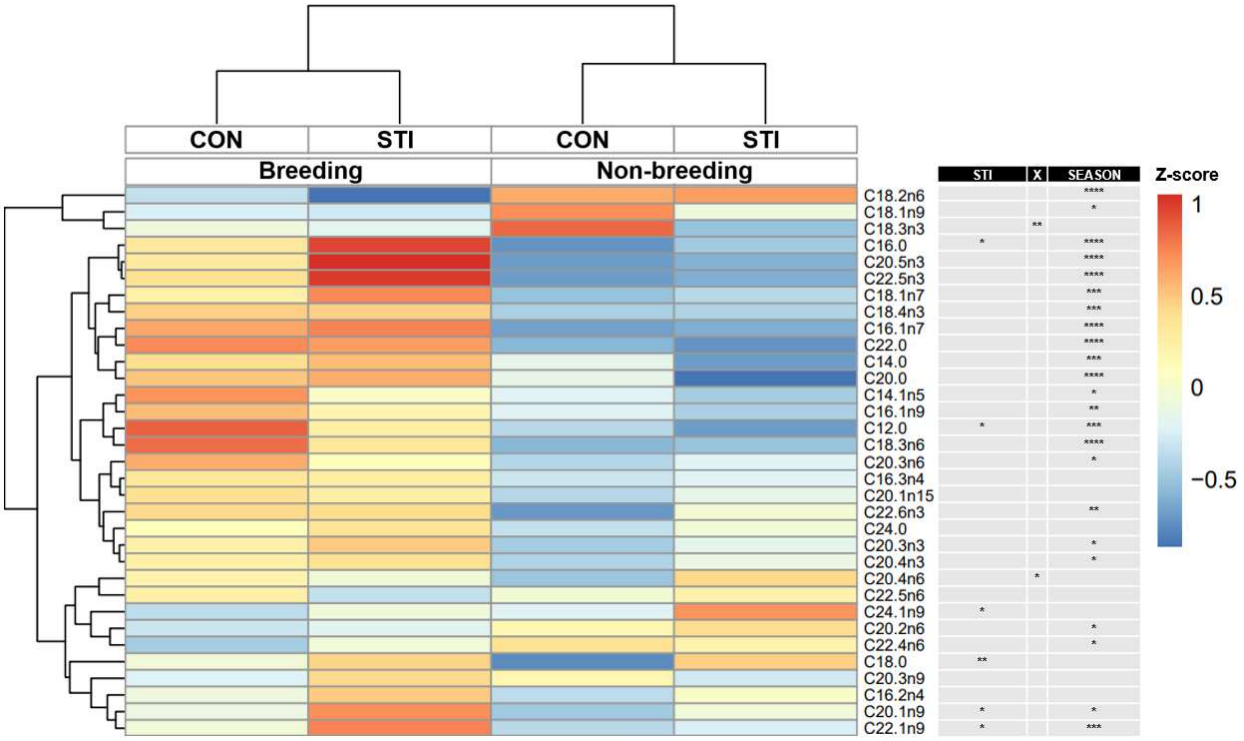

Fig. 2 Heatmap of relative FA proportions in the plasma of wild adult male song sparrows exposed to 10 min of CON or STI conditions in the breeding and non-breeding seasons. Rows represent 33 individual FAs, and columns depict experimental conditions. Heatmap displays z-scores of group mean relative abundances, with red representing higher abundance and blue representing lower abundance relative to all groups combined. n = 12/group. Medium and long chain FAs were analysed individually by 2-way ANOVA, with significant effects indicated in the figure. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$. FA, fatty acid; CON, control; STI, simulated territorial intrusion

Multiple FAs exhibited significant differences in relative proportions (Fig. 2; ESM Table 3). STI significantly impacted 6 FAs, all SFAs or MUFAs, increasing proportions of 5 FAs and lowering proportion of 1 FA. As expected, season had more widespread effects and significantly impacted 23 FAs. Breeding subjects exhibited higher proportions for 19 FAs and lower proportions for 4 FAs. Finally, 2 FAs showed significant interactions between STI and season, with STI lowering C18:3n3 and increasing C20:4n6 proportions in the non-breeding season only.

To determine whether the relative FA profile differed among groups, we analysed the 33 FAs simultaneously using multivariate methods. PERMANOVA revealed no significant effect of STI on relative FA profiles ($F(1,44) = 1.59$, $p = 0.113$), but revealed a significant impact of season that explained 17.6 % of total compositional variation ($F(1,44) = 9.93$, $p < 0.001$). There was no significant interaction between STI and season ($F(1,44) = 0.98$, $p = 0.393$). Collectively, the model explained 22.1 % of variation in relative FA profiles. Homogeneity of multivariate dispersion did not differ among groups ($F(3,44) = 1.57$, $p = 0.206$), indicating that compositional analyses were not confounded by differences in within-group variability.

Consistent with the PERMANOVA results, PCA showed clear seasonal differentiation in FA profiles (Fig. 3a), with PC1 accounting for 34.4 % of the total variance. PC1 scores were predominantly negative in the breeding season and positive in the non-breeding season. The FAs most strongly associated with the breeding season, indicated by negative loadings on PC1, were C20:5n3, C18:4n3, C16:1n7, C12:0, and C22:5n3 (Fig. 3b). The strongest positive loadings, associated with the non-breeding season, were for C18:2n6, C20:2n6, C22:4n6, C22:5n6, and C18:1n9 (Fig. 3b). These results indicate that seasonal variation in FA profiles was driven by shifts in the relative abundance of multiple FAs.

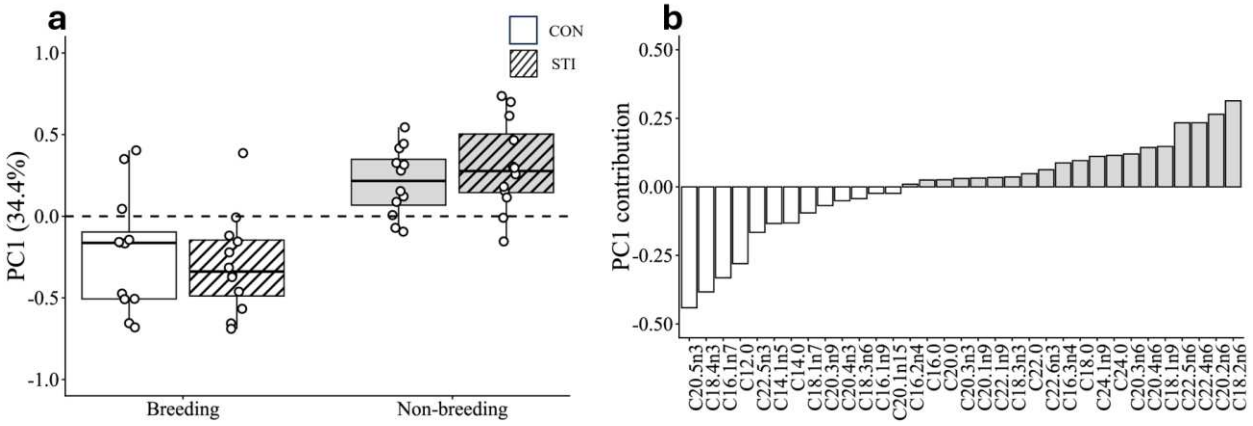

Fig. 3 Principal component analysis of relative FA proportions in the plasma of wild adult male song sparrows across the breeding and non-breeding seasons. Panel (a) shows individual PC1 scores by experimental condition, panel (b) shows individual FA contributions to PC1. PC1 explains 34.4 % of total variance in FA composition. FA, fatty acid; PC, principal component; CON, control; STI, simulated territorial intrusion

Structural analysis of relative FA profile

To capture broader patterns not evident at the individual FA level, we evaluated structural metrics, beginning with overall FA classes. Among the 33 quantified FAs, there were 7 SFAs, 9 MUFAs, and 17 PUFAs (including 5 ω-3s and 7 ω-6s; ESM Table 3). To calculate the proportion of each class, we summed its constituent FA proportions for each subject.

STI significantly increased total SFA proportion, compared to controls ($F(1,44) = 4.77$, $p = 0.034$). Breeding subjects had significantly higher total SFA proportion compared to non-breeding subjects ($F(1,44) = 25.50$, $p < 0.0001$). No significant interaction was observed ($F(1,44) = 0.03$, $p = 0.859$; Fig. 4a; ESM Table 4). In contrast, STI ($F(1,44) = 1.18$, $p = 0.283$), season ($F(1,44) = 0.08$, $p = 0.776$), and their interaction ($F(1,44) = 2.04$, $p = 0.160$) did not significantly

impact total MUFA proportion (Fig. 4b; ESM Table 4). Finally, total PUFA proportion was not affected by STI ($F(1,44) = 0.03$, $p = 0.871$), but was significantly lower in breeding subjects than in non-breeding subjects ($F(1,44) = 7.28$, $p = 0.010$). No significant interaction was observed ($F(1,44) = 1.10$, $p = 0.300$; Fig. 4c; ESM Table 4). Together, these results identify SFAs and PUFAs as the classes most sensitive to experimental conditions, whereas MUFAs remained largely unaffected.

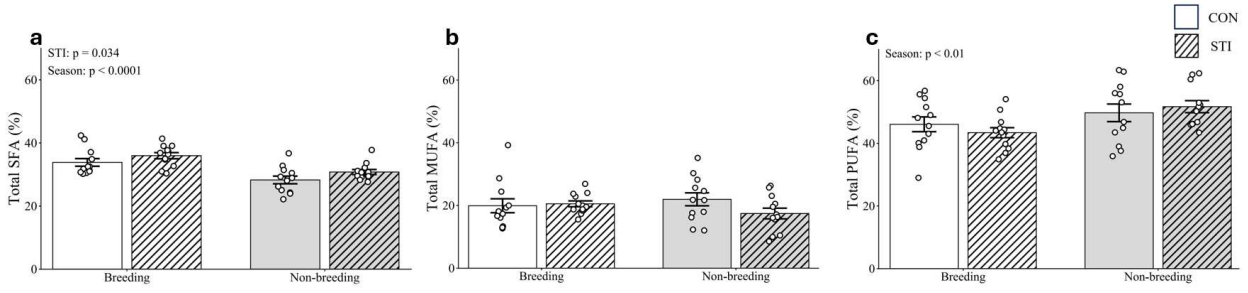

Fig. 4 Relative proportions of SFA (a), MUFA (b), and PUFA (c) in the plasma of wild adult male song sparrows exposed to 10 min of CON or STI conditions in the breeding and non-breeding seasons. Data are presented as mean ± SEM. n = 12/group. Data were analysed by 2-way ANOVA, with significant effects indicated in the figure. FA, fatty acid; SFA, saturated FA; MUFA, monounsaturated FA; PUFA, polyunsaturated FA; CON, control; STI, simulated territorial intrusion

Next, we examined the unsaturation index, a metric representative of the overall degree of FA unsaturation (Fig. 5a; ESM Table 4). The unsaturation index was significantly higher in breeding than in non-breeding subjects ($F(1,44) = 5.81$, $p = 0.020$), but neither STI ($F(1,44) = 1.32$, $p = 0.256$) nor its interaction with season ($F(1,44) = 0.09$, $p = 0.761$) had a significant impact. The peroxidisability index, which captures the susceptibility of the FA pool to

peroxidation, was elevated by STI ($F(1,44) = 4.65$, $p = 0.037$), and higher in breeding than non-breeding subjects ($F(1,44) = 27.22$, $p < 0.0001$). No interaction between STI and season was detected ($F(1,44) = 0.18$, $p = 0.677$; Fig. 5b; ESM Table 4). These findings suggest that STI increases peroxidation vulnerability without altering overall unsaturation, whereas season shapes broader structural characteristics of the FA profile.

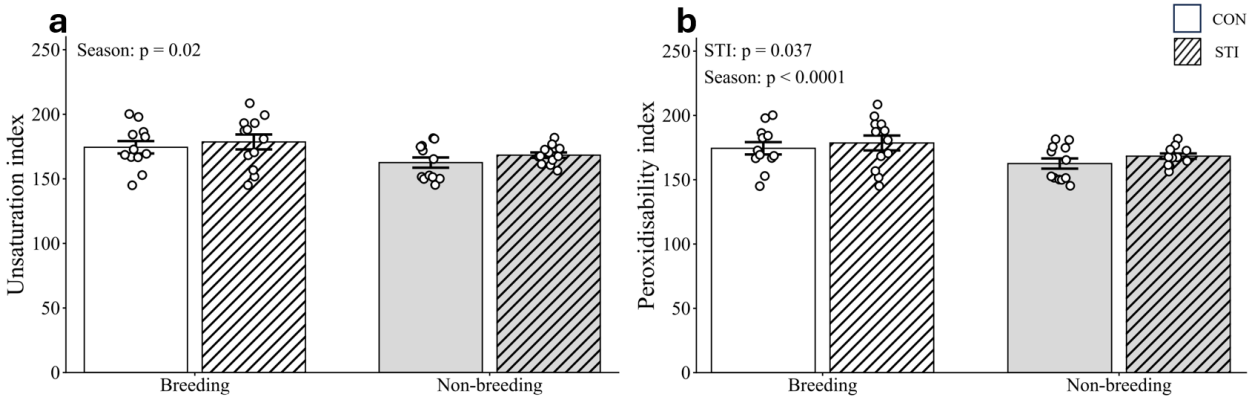

Fig. 5 Unsaturation index (a) and peroxidisability index (b) of the plasma FA profile of wild adult male song sparrows exposed to 10 min of CON or STI conditions in the breeding and non-breeding seasons. Indices were calculated for each subject using 33 medium and long chain FAs. Data are presented as mean ± SEM. n = 12/group. Data were analysed by 2-way ANOVA, with significant effects indicated in the figure. FA, fatty acid; CON, control; STI, simulated territorial intrusion

To further examine the restructuring of PUFA profiles, ω-3 and ω-6 PUFAs were analysed (Fig. 6; ESM Table 4). Total ω-3 PUFA proportion was significantly higher ($F(1,44) = 36.43$, $p < 0.0001$; Fig. 6a) and total ω-6 PUFA proportion was significantly lower ($F(1,44) = 29.22$, $p < 0.0001$; Fig. 6b) in breeding subjects than in non-breeding subjects. Accordingly, the ω-6:ω-3 ratio was significantly lower in the breeding season ($F(1,44) = 43.77$, $p < 0.0001$; Fig. 6c).

Neither STI nor its interaction with season significantly affected total ω-3 proportion, total ω-6 proportion, or the ω-6:ω-3 ratio (all $p > 0.059$, ESM Table 4). We thus assessed associations between the ω-6:ω-3 ratio and aggressive behaviours among STI subjects, pooling seasons given the absence of seasonal effects or interactions between experimental groups on aggressive behaviours. The ω-6:ω-3 ratio was positively associated with the number of flights, an energetically expensive behaviour ($t(44) = 2.11$, $p = 0.040$; Fig. 6d). Linear regressions between ω-6:ω-3 ratio and other aggressive behaviours were not significant (all $p > 0.124$).

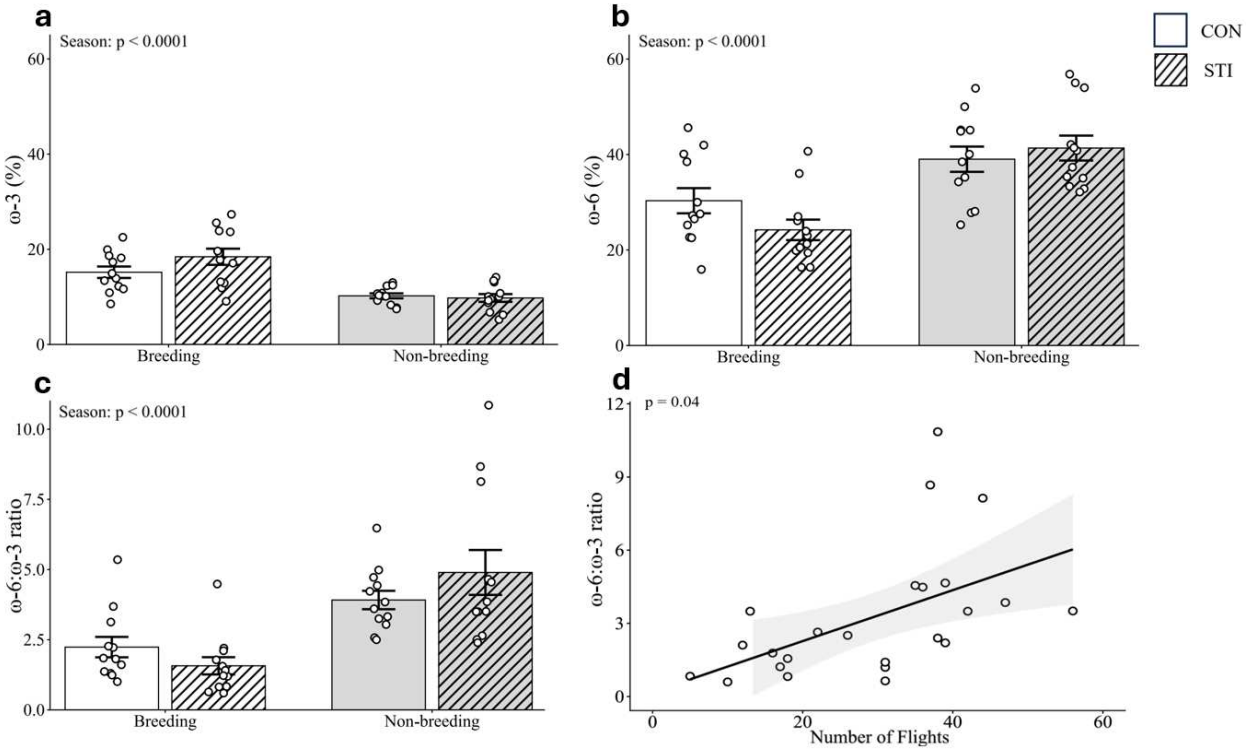

Fig. 6 Relative proportions of ω-3 PUFAs (a), ω-6 PUFAs (b), and ω-6:ω-3 ratio (c) in the plasma of wild adult male song sparrows exposed to 10 min of CON or STI conditions in the breeding and non-breeding seasons (n=12/group); (d) linear regression between the plasma ω-6:ω-3 ratio and number of flights in STI subjects across seasons (n=24). Metrics were calculated for each subject using 33 medium and long chain FAs. Data in (a-c) are presented as mean ±

SEM and were analysed by 2-way ANOVA. Shaded grey area in (d) depicts 95 % confidence intervals. Significant effects are indicated in the figure. FA, fatty acid; PUFA, polyunsaturated FA; CON, control; STI, simulated territorial intrusion

Discussion

Here, we examined 52 FAs in the plasma of wild song sparrows and could quantify 33 medium and long chain FAs. We investigated the effects of an aggressive interaction in the breeding and non-breeding seasons on FA profiles. Our findings suggest that season establishes the circulating FA profile, which aggression further modulates similarly across seasons, increasing its inflammatory potential and susceptibility to oxidative stress. This highlights novel costs of aggression. To our knowledge, this is the first study to investigate how aggression influences FA species across seasons in a free-living animal, while providing one of the most comprehensive circulating FA profiles reported to date for a songbird.

Seasonal variation in FA profiles

Total FA level is greatly elevated during the non-breeding season, which likely reflects the mobilisation of fat reserves (Fokidis et al., 2019). For many temperate species, relying on lipid-based energy stores is a critical winter strategy to buffer against reduced food availability, low ambient temperatures, and shorter days (Andersson et al., 2015; Fokidis et al., 2019; Quintana et al., 2021; Swanson, 2010). Moreover, seasonal variations in FA profiles are linked to differences in diet across seasons (Andersson et al., 2015). Temperate passerines feed on arthropods (enriched with ω-3 PUFAs) during the breeding season, and feed on seeds (containing high C18:2n6 and C18:1n9 levels) during the non-breeding season (Andersson et al., 2015;

Heimovics et al., 2012; Martin et al., 1961). This pattern mirrors our results, where ω-3 PUFAs most strongly associated with breeding profiles and ω-6 PUFAs with non-breeding profiles.

Season is a major driver of FA levels and proportions in wild male song sparrows, with 23 of 33 FAs differing significantly in relative proportion between seasons. Interestingly, 19 FAs have higher proportions in the breeding season and only 4 FAs have higher proportions in the non-breeding season (ESM Table 3). This is driven by a near doubling of C18:2n6 in the non-breeding season, from 18.66 to 31.80 %, which reduces the relative abundance of other FAs. Non-breeding profiles are partially driven by enrichment of C20:2n6, C22:4n6, and C22:5n6, resulting in higher total ω-6 and ω-6:ω-3 ratio than in the breeding season. This concurrent increase in C18:2n6 and its longer chain ω-6 metabolites suggests increased metabolic flux through elongation and desaturation pathways during the non-breeding season, rather than passive dietary accumulation alone (Andersson et al., 2015; Génier et al., 2025; Guglielmo, 2018).

Interestingly, breeding subjects have elevated unsaturation and peroxidisability indices but a lower total PUFA proportion than non-breeding subjects. This is likely driven by increases in highly unsaturated long-chain ω-3 FAs in breeding birds. Because these FAs contain a high number of double bonds, they contribute strongly to both indices. Highly unsaturated FAs increase membrane fluidity and optimise the function of transport, signaling, and metabolic proteins to support reproduction (Hulbert et al., 2007; Valentine & Valentine, 2004). However, they are more vulnerable to lipid peroxidation. These data are consistent with the hypothesis that reproductive individuals maximise cellular performance at the expense of increased susceptibility to oxidative damage (Hulbert et al., 2007; Metcalfe & Alonso-Alvarez, 2010).

Impacts of an acute aggressive encounter on FA profiles

Across all analyses, we observed an interaction between STI and season only for C18:3n3 and C20:4n6 proportions. This remarkable lack of interactions between aggression and season suggests that an aggressive encounter modulates the circulating FA profile similarly throughout the year. This is consistent with previous avian studies showing that certain FA responses to metabolic influences, including adrenaline-induced FA mobilisation and dietary and habitat effects on FA composition, are broadly conserved across seasons (Andersson et al., 2015; Pierce & McWilliams, 2005; Price et al., 2008). Notably, song sparrows exhibit similar aggressive responses during territorial challenges across seasons, but contrary to breeding males, non-breeding males do not persist in displaying territorial behaviours after an intrusion ends. Presumably, this is to preserve time for foraging when days are shorter, temperatures are lower, and food is less available (Heimovics et al., 2015; Quintana et al., 2021; Wingfield, 1994). Thus, despite similar effects on FAs across seasons, the opportunity costs of aggression may be greater during the non-breeding season, when reduced daylight and food availability constrain opportunities to replenish energy reserves.

Across both seasons, exposure to a 10 min STI lowers total FA levels. This effect is driven by declines in specific MUFA and PUFA concentrations, particularly C18:1n9 and C18:2n6 (ESM Table 2). These unsaturated FAs are more readily mobilised and oxidised than SFAs (McWilliams et al., 2020; Price & Guglielmo, 2009; Price et al., 2008; Price et al., 2011). As a result, total SFA proportion increases following STI. These data suggest that acute territorial aggression depletes circulating unsaturated FA pools faster than replenishment from adipose stores can occur, similar to changes during other strenuous physical behaviours in avian species, such as migration (Guglielmo, 2018).

438 STI increases the peroxidisability index, revealing a compositional shift towards more
439 highly unsaturated PUFAs. Aggressive interactions may thus sensitise the circulating FA profile
440 to lipid peroxidation. Aggression increases metabolic rate and alters systemic oxidative balance
441 via the depletion of circulating antioxidants and the production of reactive oxygen species
442 (Mentesana & Adreani, 2021). Shifting towards a more peroxidisable FA profile during territorial
443 challenges may thus represent a novel cost of aggression. These data are consistent with the
444 hypothesis that oxidative stress is a major cost of aggression (Beaulieu et al., 2014; Lemonnier et
445 al., 2022; Monaghan et al., 2009).
446 STI had no effect on ω-3 or ω-6 proportions, nor the ω-6:ω-3 ratio. Nonetheless, within
447 STI subjects, the ω-6:ω-3 ratio is positively associated with the number of flights, the most
448 energetically demanding aggressive behaviour measured. While correlational, these results match
449 studies of laboratory rodents and humans, which link a higher ω-6:ω-3 ratio to higher aggression
450 (DeMar Jr et al., 2006; Miachon et al., 2001; Raine & Brodrick, 2024; Raygada et al., 1998; Re
451 et al., 2008). The lack of an STI effect on the ω-6:ω-3 ratio suggests a unidirectional relationship,
452 whereby the ω-6:ω-3 ratio influences aggression, but aggression does not affect this ratio, at least
453 in the short term.
454 Finally, the relationship between FAs and aggression could be mediated via endocrine
455 pathways. FAs can be conjugated to steroids and steroid binding proteins, thereby modifying
456 steroid bioavailability, steroid metabolism, cellular uptake, and downstream effects (Hammond,
457 2016; Hochberg, 1998; Martin et al., 1988). Conversely, glucocorticoid and androgen signaling,
458 which increase with STI (Heimovics et al., 2016; Jalabert et al., 2024; Newman & Soma, 2011),
459 alter FA mobilisation and oxidation rates (Cui et al., 2018; Landys et al., 2006). Given the central
460 role of steroid signaling in aggression (Marler & Trainor, 2020; Soma et al., 2000; Wingfield et

al., 1990), this bidirectional interplay between FAs and steroids is an unexplored and potentially important mechanism through which FA profiles may influence aggressive behaviour (and vice versa).

Limitations

Several limitations should be considered when interpreting these findings. First, our analytical method was unable to quantify short chain FAs. Second, circulating FA measurements are a snapshot of the FA profile and cannot distinguish between FA mobilisation and oxidation. Likewise, our 10 min STI captures a physiological response at a single time point, and it does not reveal how rapidly these changes occur or how long they last. Third, the unsaturation and peroxidisability indices do not directly measure oxidative damage, only vulnerability. Finally, seasonal comparisons encompass differences in diet, temperature, reproductive state, and energetic demands that cannot be independently separated under field conditions. Nevertheless, the persistence of our results despite the interindividual and environmental variability inherent to field studies highlights the robustness and biological relevance of our findings.

General conclusions

To our knowledge, this is the first study to investigate how aggression influences FA species across seasons in a free-living animal, and it provides one of the most comprehensive circulating FA profiles reported to date for a songbird. We show that season is a major driver of the FA profile, impacting virtually all measures, including individual and total FA levels, ω-6:ω-3 ratio, and unsaturation and peroxidisability indices. Notably, aggressive behaviour and its effects on FAs are similar across seasons. Crucially, a brief 10 min territorial challenge is sufficient to

reduce total FA level and increase vulnerability to lipid peroxidation, and the intensity of aggression correlates positively with the ω-6:ω-3 ratio. Together, our results highlight FA metabolism as a potentially important pathway underlying the energetic, oxidative, and inflammatory costs of territorial aggression.

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Contributions

507 The authors' contributions were as follows: JPP, KKS: conceptualisation; CJ: data collection;
508 RAD: data processing; JPP, ASF: data analysis; ASF: data visualisation; JPP: manuscript writing;
509 and all authors: manuscript revision.
510
511 **Conflict of interest**
512 The authors declare no conflict of interest.
513
514 **Ethical approval**
515 All animal procedures complied with the Canadian Council on Animal Care, and protocols were
516 approved by the Canadian Wildlife Service (Permit #SC-BC-2019–0012) and the University of
517 British Columbia Animal Care Committee (Protocol #A19-0074).
518
519 **Data availability**
520 The data that support the findings of this study are available from the corresponding author upon
521 reasonable request.
522
523 **Electronic Supplementary Materials**
524 Below is the list of electronic supplementary materials.
525
526 Electronic Supplementary Material (ESM) Table 1 containing the list of 52 individual fatty acids
527 and the internal standard included in the analytical method.
528

Electronic Supplementary Material (ESM) Table 2 containing the summary and statistical results for absolute fatty acid levels in the circulation of song sparrows across behavioural and seasonal conditions.

Electronic Supplementary Material (ESM) Table 3 containing the summary and statistical results for relative fatty acid proportions in the circulation of song sparrows across behavioural and seasonal conditions.

Electronic Supplementary Material (ESM) Table 4 containing the summary and statistical results for structural metrics of fatty acids in the circulation of song sparrows across behavioural and seasonal conditions.

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