**Migrant blackbirds, *Turdus merula*, have higher polyunsaturated fatty acids levels in their plasma, but not enhanced susceptibility to peroxidation compared to residents**

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ABSTRACT

Birds have been observed to have dietary preferences for unsaturated fatty acids (FAs) during migration. Polyunsaturated fatty acids (PUFAs) increase the exercise performance of migrant birds; however, PUFAs are also peroxidation prone and might therefore incur increased costs in terms of enhanced oxidative stress in migratory individuals. To shed light on this potential constraint, we analysed plasma FA composition and estimated the susceptibility to peroxidation of migrants and residents of the partially migratory common blackbird (*Turdus merula*) at a stop-over site during autumn migration. As predicted, migrant birds had higher relative and absolute levels of PUFAs compared to resident birds. This included the strictly dietary ω-3 PUFA α-linoleic acid, suggesting a dietary preference for these fatty acids in migrants. Interestingly, the FA unsaturation index, which is an index of lipid peroxidation susceptibility, did not differ between migrants and residents. These findings suggest a mechanism where birds alter their levels of metabolic substrate to increase exercise performance without simultaneously increasing the risk of lipid peroxidation and oxidative stress. In summary, our results are in line with the hypothesis of increased exercise performance being constrained by oxidative stress during migration, which is manifested in changes in the composition of key FAs to retain the unsaturation index constant despite the increased levels of peroxidizable PUFAs.

**Keywords**: Fatty acids, migration, diet, nutritional physiology, polyunsaturated fatty acids, *Turdus merula*

INTRODUCTION

The costs associated with migratory flight cause birds to optimize their food-intake and utilize specific nutritional compounds (Lindström, 1991; Pierce and McWilliams, 2014). Fat is an important source of fuel for migrating birds, as it represents the most efficient way to store biochemical energy (McWilliams et al., 2004). Of the different types of fat, fatty acids (FAs) with a higher level of unsaturation have higher mobilization rates than other FAs and might therefore be preferentially metabolized by birds during migration (McWilliams et al., 2004; Price et al., 2008). Indeed, birds generally seem to store more unsaturated FAs in their adipose tissue while migrating (Blem, 1976; Johnston, 1973)**,** however, the physiological function and composition of specific FAs during migration is still unclear.

Whereas saturated FAs (SFAs) and monounsaturated FAs (MUFAs) can be biosynthesized *de novo* by vertebrates, the production of long-chained polyunsaturated FAs (PUFAs) requires dietary intake of the essential ω-6 PUFA linolenic acid and the ω-3 PUFA α-linoleic acid. However, PUFA biosynthesis can be inefficient (Klasing, 1998; Sanders, 1988), and several studies of birds have shown that the diet influences the PUFA composition of their tissues, including fat stores, muscles, liver and plasma (Andersson et al., 2015, 2018; Ben-Hamo et al., 2011; Maillet and Weber, 2006; Pierce and McWilliams, 2005; Pierce et al., 2004; Price and Guglielmo, 2009)*.* Therefore, a dietary preference for unsaturated FAs observed in migratory bird species is not surprising. Birds caught during migration have been found to prefer diets enriched with either PUFAs or MUFAs over SFAs (McWilliams et al., 2002; Pierce et al., 2004). Although these studies lacked a non-migratory control group of the same species, and thus offer no clear link to the migratory behaviour in itself, they demonstrate a dietary preference for unsaturated FAs by these migrating bird species and suggest that birds are able to distinguish between food items based on FA content.

Enhancement of exercise performance is one physiological effect associated with a PUFA-rich diet, which may be particularly relevant for migratory birds and could explain the potential dietary preference (Pierce and McWilliams, 2014; Pierce et al., 2005; Price and Guglielmo, 2009). Migrating red-eyed vireos (*Vireo olivaceus*) had higher peak metabolic rates when fed the PUFA linoleic acid as compared to the MUFA oleic acid (Pierce et al., 2005). Similarly, dietary linoleic acid increased the peak metabolic rate of migratory white-throated sparrows (*Zonotrichia albicollis*) as compared to a group fed with ω-3 PUFAs (Price and Guglielmo, 2009). However, in a field study of the semipalmated sandpiper (*Calidris pusilla*) the amount ω-3 PUFAs appeared to be especially high in their diet during stop-over (Maillet and Weber, 2006, 2007).

The higher PUFA levels come with a potential cost, since migration exposes birds to high levels of reactive oxygen species (ROS) as a consequence of the long-distance flight (Eikenaar et al., 2020; Jenni-Eiermann et al., 2014). Paradoxically, the molecular structure linked to exercise enhancement, PUFAs’ multiple double bonds, also makes them prone to peroxidation by the ROS, increasing the risk of oxidative stress (Hulbert et al., 2007). Migratory white throated sparrows (*Zonotrichia albicollis*) fed with PUFAs (both α-linolenic and linoleic acid) showed an increase in oxidative damage (Alan and McWilliams, 2013) and similar results have been found in rats (Labbe et al., 1991). Comparable to the dietary preference for unsaturated FAs, birds may also consume more fruits with high antioxidant levels at stop-over sites during migration(Bolser et al., 2013). Indeed, as the main energy source for migratory flight is fat, with a likely preference for unsaturated FAs (McWilliams et al., 2002; Pierce et al., 2004), the increased antioxidant defense might be especially important to shield against peroxidation of migrants’ fuel deposits.

In contrast to previous studies, we investigated FA profiles in relation to migration in a partially migrating species, the common blackbird *Turdus merula* (blackbird hereafter)*.* This allowed us to, for the first time, investigate FA composition in wild birds with either a resident or migratory status, at the same location and time. Specifically, we predicted that migrant birds should have higher relative and absolute levels of ω-6 PUFAs, ω-3 PUFAs, and hence total PUFAs, compared to resident birds. In addition, by estimating an unsaturation index we tested whether the predicted increase in PUFAs comes with an increased susceptibility to oxidative stress.

MATERIALS AND METHODS

*Field methods and sampling*

Blackbirds were trapped by mist netting and sampled on the small (<1 km2) and isolated island of Helgoland (54°11’N, 07°55’E) in the North Sea, off the coast of Germany. The sampling took place during autumn migration in October 2014, when hundreds of birds use the island as a stop-over site, mixing with the local resident blackbird population (Dierschke et al., 2011). Most of the migrant blackbirds stopping over on the island winter in the UK and breed in Scandinavia (Dierschke et al., 2011).

Blood samples were taken from the birds within 10 minutes of capture, by puncturing a brachial vein. The samples were kept on ice until plasma was separated by centrifuging the samples. This was done as soon as possible and no later than 4 hours after capture. The plasma samples were stored at −20°C until further analysis. All captured individuals were aged (1st year or adult) and sexed based on plumage (Svensson, 1992), and visually scored for body fat on Kaiser’s (1993) scale from 0 (no fat) to 8 (furcular and abdomen bulging, and breast covered with fat) (Kaiser, 1993; Svensson, 1992). All trapping took place between 7 a.m. and 6 p.m. and was approved by the Ministry for Agriculture, the Environment, and Rural Areas, Schleswig-Holstein, Germany.

Assignment of the birds’ status (migrant or resident) was done following Eikenaar et al. (2018). All birds were colour-ringed in addition to receiving a metal ring. Searches for colour-ringed birds were carried out throughout October and November. Due to the very small size of the island (<1 km2) the birds were easily sighted if they remained in the area. Recent data show that the longest migratory stop-over observed in blackbirds on Helgoland was 20 days (5 days being the median duration) and thus, 20 days was used as a cut-off for determining the migratory status of the birds (Packmor et al., 2020).

Birds observed again 20 days or more after colour ringing were assigned the resident status. Resident birds were frequently re-sighted or re-trapped, with eight observations being the median of all assigned resident birds. In addition, blackbirds ringed (with a metal ring only) on Helgoland in previous breeding seasons and re-trapped by us in autumn were assumed to be Helgoland residents. This assumption is based on a radio-telemetry study showing that 91% of the Helgoland blackbirds are sedentary (Sacher, 2009). The assumption seems valid since nine of the ten birds from this category were re-sighted on Helgoland several weeks after we colour-ringed them (the one exception was re-sighted only once, after eight days). In total, 26 birds were assigned the resident status. A total of 42 individuals caught and colour-ringed, but never re-sighted were assigned the migratory status. In a number of cases, birds were re-sighted within the 20-days window, but not after, and were thus likely migrants. However, keeping a conservative classification, these individuals were excluded from the study to minimize the risk of miss-classifying the migratory status.

*Fatty acid extraction and analysis*

The extraction and analysis of FAs followed Andersson et al. (2018), using 5 μl of plasma. FAs were extracted using 50 µl chloroform:methanol (2:1 v/v) containing 1.67 μg/μl methyl *cis*-10-heptadecenoate (> 99% pure, Aldrich) as an internal standard. The FAs were then transformed into FAMEs (fatty acid methyl esters) through base methanolysis using 100 µl of 0.5 M KOH/Me (1 h at 40 °C). The reaction was terminated using 100 µl of 0.5 M HCl/Me, and FAMEs were extracted in 300 µl *n-*heptane (>99% pure, VWR Prolabo). Samples were analysed using an Agilent 5975 mass spectrometer (MS) coupled to an Agilent 6890 gas chromatograph (GC), which was equipped with an HP-INNOWax PEG column (30 m, 0.25 mm i.d., 0.25 mm film thickness; Agilent). Helium was used as carrier gas at a constant flow of 1 mL/min. The GC oven temperature was programmed to 80 °C for 1 min and then increased by 10 °C/min to 230 °C and held for 20 min. The chromatograms were analysed using Agilent ChemStation software. The FAMEs were identified by comparison of retention times and mass spectra of known synthetic standards (Supelco 37 Component FAME mix, Sigma-Aldrich).

In total, 20 FAs were quantified through their methyl ester derivatives. The proportion of each FA per individual was calculated by dividing the peak area for individual FAs with the sum of all FA peak areas. These relative levels were then logit transformed ( ) ) before statistical analysis (Warton and Hui, 2011).

*Statistical analyses*

Before statistical analysis, the individual PUFAs, MUFAs and SFAs were pooled into total PUFAs, total ω-3 PUFAs, total ω-6 PUFAs, total MUFAs and total SFAs, respectively. In addition, to better understand the details of the physiological differences seven FAs were also analysed separately (Hulbert and Abbott, 2011; Klasing, 1998; Pierce and McWilliams, 2014), including oleic acid and individual PUFAs with mean relative levels higher than 1 % (α-linolenic acid, docosahexaenoic acid [DHA], docosapentaenoic acid [DPA], eicosapentaenoic acid [EPA], arachidonic acid and linoleic acid).

Statistical analysis was conducted using R version 3.5.1 (R Core Team, 2018). Generalized Linear Models (GLMs) were used to investigate the difference in FA composition between the migratory and resident birds. The models included migratory status as a fixed factor, and time of sampling and body fat score as co-variates. Time of day was included since it is established that daily feeding regimes and metabolism influences circulating FA levels and proportions (Isaksson et al., 2015), and previous studies of adipose tissue found differences in FA unsaturation levels in migratory birds (Blem, 1976; Johnston, 1973). Age and sex were not included in the final models, as no general effect of these factors or their interactions with migration status was found (all p > 0.05). In total, 13 GLMs were performed with the relative levels of pooled and single FAs as response variables. Six additional GLMs were carried out with the absolute concentration (ng FA/μl plasma) of the pooled FA groups, including total FA concentration as the response variable. As a final response variable, the unsaturation index was estimated. The degree of unsaturation was calculated as an index of the sum of each FA’s relative plasma level, multiplied with their respective number of double bonds (Jezierska et al., 1982; McCue, 2008).

To complement the hypothesis testing based on the GLMs, the Hedges’ *g* standardized unbiased effect size (Hedges and Olkin, 1985) was calculated for each FA proportion by dividing the difference of the group means by the pooled standard deviation of the entire population. The effect size is thus a measurement of the magnitude of the quantitative difference (i.e., biological effect) between the groups, and is used to standardize differences between means (Kelley and Preacher, 2012; Nakagawa and Cuthill, 2007). An effect size value of 0.5 is generally considered to indicate a medium effect and a value of 0.8 or above a large effect (Cohen, 1992).

RESULTS

In total, 20 FAs were identified in the blackbird plasma samples (Table 1), including ten PUFAs, seven MUFAs, and three SFAs. The relative level of total PUFAs was higher in migrant compared to resident birds (F1, 64 = 5.021, p = 0.029; Figure 1a). A similar difference was found for total ω-3 PUFAs (F1, 64 = 6.824, p = 0.011), but not for total ω-6 PUFAs, although a trend was evident (F1, 64 = 3.786, p = 0.056). Of the individual ω-3 PUFAs analysed, α-linolenic acid differed depending on migration status, with higher relative levels in migrant birds (F1, 64 = 21.583, p < 0.001; Figure 1b). Also, DPA (F1, 64 = 4.354, p = 0.041) and EPA (F1, 64 = 5.732, p = 0.020) were higher in the migrants than in the residents, but DHA showed no difference (F1, 64 = 0.079, p = 0.780). The two individually analysed ω-6 PUFAs did not differ between migrants and residents (arachidonic acid: F1, 64 = 2.876, p = 0.095; linolenic acid: F1, 64 = 1.508, p = 0.224).

The relative level of total MUFAs did not differ between migrant and resident birds (F1, 64 = 0.466, p = 0.498) and neither did the abundant oleic acid (F1, 64 = 0.418, p = 0.520). The relative level of total SFAs, however, was significantly lower in migrant compared to resident birds (F1, 64 = 4.021, p = 0.049)

In addition to relative levels, the absolute FA concentrations (ng/μl plasma) of all pooled FA groups were analysed (Figure 1c). The total concentration circulating FAs did not differ between the groups, although a trend for higher levels in migrants was evident (F1, 64 = 3.823, p = 0.055). The concentration of total PUFAs differed between migrant and resident birds, with migrants having higher concentrations (F1, 64 = 6.277, p = 0.015). Concentrations of both PUFA sub-groups showed similar differences (ω*-*3 PUFAs: F1, 64 = 8.610, p = 0.005; ω*-*6 PUFAs: F1, 64 = 3.786, p = 0.017). The concentrations of total MUFAs and total SFAs did not differ between migrant and resident birds (MUFAs: F1, 64 = 2.922, p = 0.092; SFAs: F1, 64 = 2.222, p = 0.141). Interestingly, the unsaturation index did not differ between migrants and residents (F1, 64 = 0.461, p = 0.500; Figure 1d). The effect sizes for all pooled and individual FAs are shown in Figure 3a and b, respectively. None of the pooled FAs groups had a medium effect or above (a magnitude greater than g = 0.5), with total ω-3 PUFAs being the highest with g = 0.36 (Figure 3a). Of the single FAs, the ω-3 PUFA α-linolenic acid was associated with the largest effect (g = 1.03; Figure 3b).

The relative levels of ω*-*3 PUFAs, ω*-*6 PUFAs and total PUFAs decreased with time of sampling during the day (total PUFAs: F1, 64 = 14.571, p < 0.001, total ω-3 PUFAs: F1, 64 = 15.564, p < 0.001 and total ω-6 PUFAs: F1, 64 = 8.039, p = 0.006; Appendix 1a). Of the individual PUFAs, the essential α-linolenic acid was positively correlated with time of sampling (F1, 64 = 6.752, p = 0.012), whereas the longer-chained PUFAs DHA (F1, 64 = 25.049, p < 0.001), DPA (F1, 64 = 8.664, p = 0.005), EPA (F1, 64 = 19.668, p < 0.001) and arachidonic acid (F1, 64 = 31.851, p < 0.001; Appendix 1b) showed a negative correlation. The relative level of total SFAs increased over the day (F1, 64 = 10.146, p = 0.002). In all other cases, including all absolute FA concentrations (Appendix 1c), time of sampling did not affect the FA levels.

Body fat score did not differ between the migrant and resident birds (t66 = 0.171, p = 0.865). Relative levels of PUFAs declined with increasing body fat score (total PUFAs: F1, 64 = 9.164, p = 0.004, total ω-3 PUFAs: F1, 64 = 11.508, p = 0.001 and total ω-6 PUFAs: F1, 64 = 7.693, p = 0.007). The relative levels of individual PUFAs revealed the same pattern as the pooled relative PUFA groups (α-linolenic acid: F1, 64 = 13.845, p < 0.001; DPA: F1, 64 = 5.193, p = 0.026; arachidonic acid: F1, 64 = 6.466, p = 0.013; and linoleic acid: F1, 64 = 4.992, p = 0.029). In addition, relative levels and absolute concentrations of MUFAs increased with the fat scores (relative levels: F1, 64 = 11.038, p = 0.001; absolute concentration: F1, 64 = 5.069, p = 0.028). This was also the case for the relative levels of oleic acid (F1, 64 = 12.243, p < 0.001). For all other FAs, body fat score was not significant.

DISCUSSION

*Fatty acid composition and unsaturation indices in relation to migratory status*

The preferential ingestion, storage and mobilization of certain fatty acids during energy demanding periods such as migration or development has the potential to enhance performance, ultimately affecting fitness (Twining et al., 2016). Yet, the extent to which this occurs in the wild, and whether a potential risk of enhanced oxidative cost is linked to the predicted increase in FA unsaturation via intake of PUFAs, is poorly understood. Here we addressed this question in a partial migrant, the common blackbird. In accordance with our prediction, migrating blackbirds had higher relative and absolute levels of PUFAs compared to resident conspecifics. The increase in absolute levels of PUFAs was independent of the total level of FAs, which supports the idea that PUFA-rich food is preferentially eaten and circulated during migration. Interestingly, the difference in PUFAs did not significantly affect the birds’ unsaturation indices, suggesting that migrants did not suffer an increased risk of lipid peroxidation, at least not when considering the availability of peroxidizable substrate (see also Eikenaar et al., 2017). Previous studies have shown differences in tissue composition depending on temporal migration status (Johnston, 1973; Maillet and Weber, 2006; Pierce and McWilliams, 2005), yet circulating FAs have received little attention, especially in a natural setting. Thus, our results provide novel evidence of differences in the circulation of PUFAs, but not of SFAs or MUFAs, depending on migration status in a wild and partially migratory species.

Migrant birds had higher relative levels of α-linoleic acid compared to resident birds, with an especially large effect size. This ω-3 PUFA cannot be biosynthesized by vertebrates (Sanders, 1988)**,** which suggests that dietary preferences of blackbirds relate to migration status. The long-chained ω-3 PUFAs EPA and DPA were also higher in migrants than residents, suggesting that migrants eat food rich in overall ω-3 PUFAs or that they biosynthesize these PUFAs from dietary α-linoleic acid. ω-3 PUFAs were previously suggested to be incorporated in the cell membrane of muscle cells in the migrating semipalmated sandpiper (Maillet and Weber, 2006). For other migratory species, however, ω-6 PUFAs rather than ω-3 PUFAs seem to enhance exercise performance such as for the white throated sparrow (Price and Guglielmo, 2009). Thus, the literature offers no clear and general explanation for our observed difference in α-linoleic acid, EPA and DPA specifically. However, it is possible that species-dependent dietary differences underlie which exact PUFAs are utilized to enhance migratory performance. Regardless of the cause, our results show that migrant blackbirds circulate higher levels of ω-3 PUFAs, compared to resident birds sampled at the same location and time.

Despite the difference in total levels of PUFAs, no significant difference was found between migrants and residents in their unsaturation index. One potential cost of increased FA unsaturation is an increased risk of lipid peroxidation, which migrating birds are particularly exposed to as endurance flights cause increased generation of ROS (Costantini et al., 2007; Hulbert et al., 2007; Jenni-Eiermann et al., 2014). The study by Eikenaar et al. (2017) on blackbirds at the same study site also did not find a significant difference in peroxidation index (i.e., a similar index as the unsaturation index) between migrant and resident birds when analysing this index based on absolute FA levels (instead of relative levels as in the present study), although a trend was evident. Accordingly, measurements of a marker for lipid peroxidation (malondialdehyde; MDA) also suggested no difference between migrants and residents, whereas total non-enzymatic antioxidant capacity was higher in the migrants (Eikenaar et al., 2017). Taken together, these results suggest that migrating birds retain higher levels of the exercise enhancing PUFAs while mitigating the risk of increased lipid peroxidation both by regulating their fatty acid unsaturation index and by increasing their antioxidant defences. Further adding to this scenario is the observed lack of difference in the most unsaturated and peroxidation prone of all FAs, namely DHA. As with the lack of difference in unsaturation index, this could indicate that migrants optimize their nutritional physiology by balancing the exercise benefits of PUFAs against their oxidative stress related costs. The similar unsaturation indices between migration strategies can largely be explained by the lower, although non-significant, relative levels of DHA and the abundant MUFA oleic acid. This in itself is intriguing as diet choice experiments on other migratory bird species did not find a clear preference for either MUFAs or PUFAs, but rather for unsaturation at large (McWilliams et al., 2002; Pierce et al., 2004). The present results rather suggest a specific preference for PUFAs in migrating blackbirds.

Lastly, migrants had lower relative levels of SFAs, yet they showed a trend for higher absolute concentration of SFAs, compared to resident birds. Although somewhat counterintuitive, this is explained by the overall higher concentrations of all circulating FAs in migrant birds, with especially high PUFA levels, rather than a difference in SFA levels.

Trade-offs involving oxidative stress have been suggested for migratory birds before. For example, in a study using the same blackbird system, a negative correlation was found between non-enzymatic antioxidant capacity and microbial killing capacity in migrant blackbirds, but not in residents (Eikenaar et al., 2018). This could indicate that migratory individuals trade off immune function with antioxidant defences during migration (Eikenaar et al., 2017, 2018). As the main energy source for migratory flight is fat, with a general preference for peroxidizable unsaturated FAs in migratory species (McWilliams et al., 2002; Pierce et al., 2004), the increased antioxidant defence might be especially important to shield against peroxidation of migrants’ fuel deposits. Furthermore, migratory birds have been suggested to preferentially feed on antioxidant rich food during stop-overs (Bolser et al., 2013), indicating that migrating birds optimize their nutritional physiology for the demanding journey (Pierce and McWilliams, 2014). Most likely, exercise performance gains and peroxidation risk would be key factors in determining a potential optimal FA composition for migration.

*Daily variation in fatty acids*

Daily variation in FAs was independent of migration status. Instead, we showed an increase in the relative level of the essential α-linoleic acid over the day, regardless of migration status. This suggests that the daily food intake and the relative levels of α-linoleic acid in the diet is reflected in the plasma and increases over the day (see also Isaksson et al. 2015). Noteworthy, all of the longer-chained and downstream ω-3 PUFAs (EPA, DHA and DPA) and the ω-6 PUFA, arachidonic acid, decreased over the day. This could indicate that the metabolic conversion of α-linoleic acid and linoleic acid is, as previously suggested, not that rapid and efficient (Klasing, 1998; Sanders, 1988)and the relative concentrations of longer-chained PUFAs only increase during the post-absorptive state. In fact, the present results on daily variation in PUFAs show the same overall pattern as in great tits (*Parus major*) (Isaksson et al., 2015). Remarkably, we observed no effect of sampling hour on absolute levels of circulating FAs. This is somewhat surprising but indicates that a constant and stable transport of FAs is maintained throughout the day despite that food, of varying FA content, is taken in and absorbed throughout the day. Although there was considerable variation among the blackbirds in the absolute concentration of FAs (approx. 1,600-2,100 ng/μl), there is likely a threshold for a maximum level as a result of physiological limitations e.g., viscosity of the blood and uptake in the intestine. Thus, adjusting the relative levels of FAs through a combination of diet choice and conversion may be a better way to optimize migratory performance than increasing the overall absolute FA levels.

*Fatty acids in relation to body fat*

For migrating birds, it is crucial to quickly build up fat stores at stop-over sites before continuing their flight. Yet, we found no difference in body fat score between migrants and residents, suggesting that they are in similar nutritional condition. As mentioned above, the birds might, however, have reached this status through different dietary sources. Furthermore, we investigated circulating FAs, whereas the composition of adipose tissue might differ from that in plasma. However, a general pattern was revealed between some of the FAs and body fat score. The relative levels of the two essential PUFAs: α-linoleic acid and linoleic acid (along with arachidonic acid, DPA and pooled PUFA groups) was overall negatively related to body fat score, whereas both relative and absolute levels of MUFAs was positively related to body fat score. Thus, not only migrating birds have higher relative levels of PUFAs, but also lean birds overall. The significance of this finding needs to be further investigated.

In summary, we show that migrant blackbirds have higher relative and absolute levels of PUFAs, driven by higher levels of ω-3 PUFAs compared to resident birds. The lack of a general difference in the degree of unsaturation between the migrants and residents could possibly be explained by a mechanism that regulates the potential exercise enhancing effect of PUFAs and the constraint of increased risk for peroxidation which increases oxidative stress. Variation in the essential α-linoleic acid suggests that there are differences in dietary preferences for ω-3 PUFAs which contributes to the observed variation in plasma PUFA composition. Future studies should investigate the underlying mechanisms and the costs and benefits of increased PUFA levels depending on migration status.

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**Competing interest**

The authors declare that there are no competing interests associated with this manuscript.

**Data Accessibility**  
Data will be archived in Dryad upon acceptance of the manuscript.

**REFERENCES**

Alan, R.R., and McWilliams, S.R. (2013). Oxidative stress, circulating antioxidants, and dietary preferences in songbirds. Comp. Biochem. Physiol. B Biochem. Mol. Biol. *164*, 185–193.

Andersson, M.N., Wang, H.-L., Nord, A., Salmón, P., and Isaksson, C. (2015). Composition of physiologically important fatty acids in great tits differs between urban and rural populations on a seasonal basis. Front. Ecol. Evol. *3*.

Andersson, M.N., Nilsson, J., Nilsson, J.-Å., and Isaksson, C. (2018). Diet and ambient temperature interact to shape plasma fatty acid composition, basal metabolic rate and oxidative stress in great tits. J. Exp. Biol. *221*, jeb186759.

Ben-Hamo, M., Mccue, M.D., Mcwilliams, S.R., and Pinshow, B. (2011). Dietary fatty acid composition influences tissue lipid profiles and regulation of body temperature, but not metabolic rates, of hypothermic Japanese quail. Integr. Comp. Biol. *51*, E165–E165.

Blem, C. (1976). Patterns of Lipid Storage and Utilization in Birds. Am. Zool. *16*, 671–684.

Bolser, J.A., Alan, R.R., Smith, A.D., Li, L., Seeram, N.P., and McWilliams, S.R. (2013). Birds Select Fruits with More Anthocyanins and Phenolic Compounds During Autumn Migration. Wilson J. Ornithol. *125*, 97–108.

Cohen, J. (1992). A Power Primer. Psychol. Bull. *112*, 155–159.

Costantini, D., Cardinale, M., and Carere, C. (2007). Oxidative damage and anti-oxidant capacity in two migratory bird species at a stop-over site. Comp. Biochem. Physiol. C-Toxicol. Pharmacol. *144*, 363–371.

Dierschke, J., Århammar, N., and Ornithologische Arbeitsgemeinschaft Helgoland (2011). Die Vogelwelt der Insel Helgoland (Helgoland: OAG Helgoland).

Eikenaar, C., Kallstig, E., Andersson, M.N., Herrera-Duenas, A., and Isaksson, C. (2017). Oxidative Challenges of Avian Migration: A Comparative Field Study on a Partial Migrant. Physiol. Biochem. Zool. *90*, 223–229.

Eikenaar, C., Isaksson, C., and Hegemann, A. (2018). A hidden cost of migration? Innate immune function versus antioxidant defense. Ecol. Evol. *8*, 2721–2728.

Eikenaar, C., Hegemann, A., Packmor, F., Kleudgen, I., and Isaksson, C. (2020). Not just fuel: energy stores are correlated with immune function and oxidative damage in a long-distance migrant. Curr. Zool.

Hedges, L.V., and Olkin, I. (1985). Statistical methods for meta-analysis (Orlando: Academic Press).

Hulbert, A.J., and Abbott, S.K. (2011). Nutritional ecology of essential fatty acids: an evolutionary perspective. Aust. J. Zool. *59*, 369–379.

Hulbert, A.J., Pamplona, R., Buffenstein, R., and Buttemer, W.A. (2007). Life and death: Metabolic rate, membrane composition, and life span of animals. Physiol. Rev. *87*, 1175–1213.

Isaksson, C., Hanson, M.A., and Burdge, G.C. (2015). The effects of spatial and temporal ecological variation on fatty acid compositions of wild great tits Parus major. J. Avian Biol. *46*, 245–253.

Jenni-Eiermann, S., Jenni, L., Smith, S., and Costantini, D. (2014). Oxidative Stress in Endurance Flight: An Unconsidered Factor in Bird Migration. Plos One *9*, e97650.

Jezierska, B., Hazel, J.R., and Gerking, S.D. (1982). Lipid mobilization during starvation in the rainbow trout, Salmo gairdneri Richardson, with attention to fatty acids. J. Fish Biol. *21*, 681–692.

Johnston, D. (1973). Cytological and Chemical Adaptations of Fat Deposition in Migratory Birds. Condor *75*, 108–113.

Kaiser, A. (1993). A New Multi-Category Classification of Subcutaneous Fat Deposits of Songbirds. J. Field Ornithol. *64*, 246–255.

Kelley, K., and Preacher, K.J. (2012). On Effect Size. Psychol. Methods *17*, 137–152.

Klasing, K.C. (1998). Comparative avian nutrition (Cab International).

Labbe, M., Trick, K., and Bearerogers, J. (1991). Dietary (n-3) Fatty-Acids Affect Rat-Heart,

Liver and Aorta Protective Enzyme-Activities and Lipid-Peroxidation. J. Nutr. *121*, 1331–1340.

Lindström, Å. (1991). Maximum Fat Deposition Rates in Migrating Birds. Ornis Scand. *22*,

12.

Maillet, D., and Weber, J.-M. (2006). Performance-enhancing role of dietary fatty acids in a long-distance migrant shorebird: the semipalmated sandpiper. J. Exp. Biol. *209*, 2686–2695.

Maillet, D., and Weber, J.-M. (2007). Relationship between n-3 PUFA content and energy metabolism in the flight muscles of a migrating shorebird: evidence for natural doping. J. Exp. Biol. *210*, 413–420.

McCue, M.D. (2008). Fatty acid analyses may provide insight into the progression of starvation among squamate reptiles. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. *151*, 239–246.

McWilliams, S.R., Kearney, S.B., and Karasov, W.H. (2002). Diet preferences of warblers for specific fatty acids in relation to nutritional requirements and digestive capabilities. J. Avian Biol. *33*, 167–174.

McWilliams, S.R., Guglielmo, C., Pierce, B., and Klaassen, M. (2004). Flying, fasting, and feeding in birds during migration: a nutritional and physiological ecology perspective. J. Avian Biol. *35*, 377–393.

Nakagawa, S., and Cuthill, I.C. (2007). Effect size, confidence interval and statistical significance: a practical guide for biologists. Biol. Rev. *82*, 591–605.

Packmor, F., Klinner, T., Woodworth, B.K., Eikenaar, C., and Schmaljohann, H. (2020). Stopover departure decisions in songbirds: do long-distance migrants depart earlier and more independently of weather conditions than medium-distance migrants? Mov. Ecol. *8*, 6.

Pierce, B.J., and McWilliams, S.R. (2005). Seasonal changes in composition of lipid stores in migratory birds: Causes and consequences. The Condor *107*, 269.

Pierce, B.J., and McWilliams, S.R. (2014). The Fat of the Matter: How Dietary Fatty Acids Can Affect Exercise Performance. Integr. Comp. Biol. *54*, 903–912.

Pierce, B.J., McWllliams, S.R., Place, A.R., and Huguenin, M.A. (2004). Diet preferences for specific fatty acids and their effect on composition of fat reserves in migratory Red-eyed Vireos (Vireo olivaceous). Comp. Biochem. Physiol. -Mol. Integr. Physiol. *138*, 503–514.

Pierce, B.J., McWilliams, S.R., O’Connor, T.P., Place, A.R., and Guglielmo, C.G. (2005). Effect of dietary fatty acid composition on depot fat and exercise performance in a migrating songbird, the red-eyed vireo. J. Exp. Biol. *208*, 1277–1285.

Price, E.R., and Guglielmo, C.G. (2009). The effect of muscle phospholipid fatty acid composition on exercise performance: a direct test in the migratory white-throated sparrow (Zonotrichia albicollis). Am. J. Physiol. Regul. Integr. Comp. Physiol. *297*, R775-782.

Price, E.R., Krokfors, A., and Guglielmo, C.G. (2008). Selective mobilization of fatty acids from adipose tissue in migratory birds. J. Exp. Biol. *211*, 29–34.

R Core Team (2018). R: A language and environment for statistical computing. (Vienna, Austria: R Foundation for Statistical Computing).

Sacher, T. (2009). Genetic differentiation and migration behaviour of an island population of the common blackbird (Turdus merula) (Wilhemshaven: PhD-thesis).

Sanders, T.A.B. (1988). Essential and Trans-Fatty Acids in Nutrition. Nutr. Res. Rev. *1*, 57.

Svensson, L. (1992). Identification guide to European passerines (Stockholm, Sweden: British Trust for Ornithology).

Twining, C.W., Brenna, J.T., Lawrence, P., Shipley, J.R., Tollefson, T.N., and Winkler, D.W.

(2016). Omega-3 long-chain polyunsaturated fatty acids support aerial insectivore performance more than food quantity. Proc. Natl. Acad. Sci. *113*, 10920–10925.

Warton, D.I., and Hui, F.K.C. (2011). The arcsine is asinine: the analysis of proportions in ecology. Ecology *92*, 3–10.

**Author contributions**

CE, CI, and MNA conceived and designed the study; CE collected the data; MNA performed fatty acid extractions and GC/MS analysis; JKJ analysed the data and drafted the manuscript. All authors contributed critically to the drafts of the manuscript and gave a final approval for publication.

***Table 1.*** Relative levels (percent of total fatty acid content) of all identified circulating FAs in resident and migratory blackbirds.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Status** | **N** | **Mean (%)** | **SE** |
| α-Linolenic acid | Migrant | 42 | 1.07 | 0.097 |
| 18:3n-3 (ω-3 PUFA) | Resident | 26 | 0.52 | 0.067 |
| Eicosapentaenoic acid (EPA) | Migrant | 42 | 3.14 | 0.121 |
| 20:5n-3 (ω-3 PUFA) | Resident | 26 | 2.98 | 0.229 |
| Docosapentaenoic acid (DPA) | Migrant | 42 | 1.18 | 0.070 |
| 22:5n-3 (ω-3 PUFA) | Resident | 26 | 1.04 | 0.088 |
| Docosahexaenoic acid (DHA) | Migrant | 42 | 2.24 | 0.346 |
| 22:6n-3 (ω-3 PUFA) | Resident | 26 | 2.42 | 0.475 |
| Linoleic acid | Migrant | 42 | 5.26 | 0.812 |
| 18:2n-6 (ω-6 PUFA) | Resident | 26 | 4.25 | 0.833 |
| Eicosadienoic acid | Migrant | 42 | 0.59 | 0.053 |
| 20:2n-6 (ω-6 PUFA) | Resident | 26 | 0.35 | 0.024 |
| dihomo-γ-Linolenic acid | Migrant | 42 | 0.43 | 0.023 |
| 20:3n-6 (ω-6 PUFA) | Resident | 26 | 0.37 | 0.030 |
| Arachidonic acid | Migrant | 42 | 9.74 | 0.383 |
| 20:4n-6 (ω-6 PUFA) | Resident | 26 | 9.61 | 0.572 |
| Osbond acid | Migrant | 42 | 0.31 | 0.047 |
| 22:5n-6 (ω-6 PUFA) | Resident | 26 | 0.26 | 0.050 |
| Mead acid | Migrant | 42 | 0.14 | 0.021 |
| 20:3n-9 (ω-9 PUFA) | Resident | 26 | 0.28 | 0.056 |
| Myristoleic acid | Migrant | 42 | 0.05 | 0.007 |
| 14:1n-5 (MUFA) | Resident | 26 | 0.07 | 0.014 |
| Palmitoleic acid | Migrant | 42 | 2.30 | 0.355 |
| 16:1n-7 (MUFA) | Resident | 26 | 2.31 | 0.453 |
| 16:1n-9 (MUFA) | Migrant | 42 | 0.32 | 0.049 |
|  | Resident | 26 | 0.39 | 0.077 |
| *cis*-Vaccenic acid | Migrant | 42 | 1.93 | 0.298 |
| 18:1n-7 (MUFA) | Resident | 26 | 1.91 | 0.375 |
| Oleic acid | Migrant | 42 | 32.71 | 5.047 |
| 18:1n-9 (MUFA) | Resident | 26 | 33.26 | 6.522 |
| Paullinic acid | Migrant | 42 | 0.18 | 0.028 |
| 20:1n-7 (MUFA) | Resident | 26 | 0.23 | 0.045 |
| Gondoic acid | Migrant | 42 | 0.26 | 0.040 |
| 20:1n-9 (MUFA) | Resident | 26 | 0.21 | 0.041 |
| Myristic acid | Migrant | 42 | 0.55 | 0.084 |
| 14:0 (SFA) | Resident | 26 | 0.63 | 0.123 |
| Palmitic acid | Migrant | 42 | 26.60 | 4.105 |
| 16:0 (SFA) | Resident | 26 | 26.65 | 5.226 |
| Stearic acid | Migrant | 42 | 10.98 | 1.695 |
| 18:0 (SFA) | Resident | 26 | 12.27 | 2.407 |

**Figure and Appendix Legends**

***Figure 1.*** Fatty acid (FA) profiles in blood plasma of migrant and resident blackbirds. Relative levels (percent of total FA content) of ***a)*** pooled FAs, ***b)*** individual PUFAs, ***c)*** absolute concentrations of circulating FAs (ng/μl), and ***d)*** unsaturation index (see Methods for details), are shown for migrant (grey) and resident (black) blackbirds. All panels show mean ± standard error (SE). Significance levels are indicated by *asterisks* (\*p = 0.05–0.01, \*\*p = 0.01–0.001, \*\*\*p < 0.001). EPA = Eicosapentaenoic acid, DPA = Docosapentaenoic acid, DHA = Docosahexaenoic acid, SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids.

***Figure 2.*** Effect sizes for fatty acids (FAs) in migrant and resident blackbirds***.*** Hedges *g* standardized unbiased effect size of *(****a****)* pooled fatty acid (FA) groups, and *(****b****)* individual FAs for migrant and resident blackbirds. The effects sizes are ordered from lowest to highest *g-*value. Positive values indicate higher proportions in migrants, while negative values indicate lower proportions in migrants. OnlyFAs with relative levels > 1 % of total FA content are shown. Bars display the 95 % confidence intervals. SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids.

***Appendix 1.***Summary table of the generalized linear models (GLMs) performed. **a)** Results for the relative levels (% of total fatty acid [FA] content) of all pooled FA classes and the unsaturation index. **b)** Results for the relative levels of all FAs tested individually. **c)** Results for the absolute concentration (ng/μl) of all pooled FA classes. Status (migrant or resident), body fat score (0-8) and time (sampling hour). Significant results are highlighted in bold. See methods for further details. SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids.

Appendix 1

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| (a) | **Coefficients** | **Estimate** | **df** | **F-value** | **p-value** |
| Unsaturation index | |  | | --- | | Status | | Body fat | | Time | | |  | | --- | | -0.0321 | | -0.0201 | | -2.524e-05 | | |  | | --- | | 1 | | 1 | | 1 | | |  | | --- | | 0.461 | | 0.801 | | 0.043 | | |  | | --- | | 0.500 | | 0.374 | | 0.836 | |
| **Total PUFAs** | |  | | --- | | **Status** | | **Body fat** | | **Time** | | |  | | --- | | -0.0820 | | -0.0525 | | -0.0004 | | |  | | --- | | 1 | | 1 | | 1 | | |  | | --- | | 5.021 | | 9.164 | | 14.571 | | |  | | --- | | **0.029** | | **0.004** | | **0.0003** | |
| **Total ω*-*3 PUFAs** | |  | | --- | | **Status** | | **Body fat** | | **Time** | | |  | | --- | | -0.0107 | | -0.0066 | | -4.150e-05 | | |  | | --- | | 1 | | 1 | | 1 | | |  | | --- | | 6.824 | | 11.508 | | 15.564 | | |  | | --- | | **0.011** | | **0.001** | | **0.0002** | |
| Total ω*-*6 PUFAs | |  | | --- | | Status | | **Body fat** | | **Time** | | |  | | --- | | -0.0229 | | -0.0155 | | -8.595e-05 | | |  | | --- | | 1 | | 1 | | 1 | | |  | | --- | | 3.786 | | 7.693 | | 8.039 | | |  | | --- | | 0.056 | | **0.007** | | **0.006** | |
| Total MUFAs | |  | | --- | | Status | | **Body fat** | | Time | | |  | | --- | | 0.0213 | | 7.762e-05 | | 0.0492 | | |  | | --- | | 1 | | 1 | | 1 | | |  | | --- | | 0.466 | | 11.038 | | 0.933 | | |  | | --- | | 0.498 | | **0.001** | | 0.338 | |
| **Total SFAs** | |  | | --- | | **Status** | | Body fat | | **Time** | | |  | | --- | | 0.0383 | | -0.0084 | | 1.566e-04 | | |  | | --- | | 1 | | 1 | | 1 | | |  | | --- | | 4.021 | | 0.853 | | 10.146 | | |  | | --- | | **0.049** | | 0.359 | | **0.002** | |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| (b) | **Coefficients** | **Estimate** | **df** | **F-value** | **p-value** |
| **α-Linolenic acid 18:3n-3 (ω-3 PUFA)** | |  | | --- | | **Status** | | **Body fat** | | **Time** | | |  | | --- | | -0.2983 | | -0.1133 | | 0.0004 | | |  | | --- | | 1 | | 1 | | 1 | | |  | | --- | | 21.583 | | 13.845 | | 6.752 | | |  | | --- | | **1.74e-05** | | **0.0004** | | **0.012** | |
| **Eicosapentaenoic acid (EPA)**  **20:5n-3 (ω-3 PUFA)** | |  | | --- | | **Status** | | Body fat | | **Time** | | |  | | --- | | -0.0809 | | -0.0219 | | -3.860e-04 | | |  | | --- | | 1 | | 1 | | 1 | | |  | | --- | | 5.732 | | 1.861 | | 19.668 | | |  | | --- | | **0.020** | | 0.177 | | **3.70e-05** | |
| **Docosapentaenoic acid (DPA)   22:5n-3 (ω-3 PUFA)** | |  | | --- | | **Status** | | **Body fat** | | **Time** | | |  | | --- | | -0.0947 | | -0.0490 | | -0.0003 | | |  | | --- | | 1 | | 1 | | 1 | | |  | | --- | | 4.354 | | 5.193 | | 8.664 | | |  | | --- | | **0.041** | | **0.026** | | **0.005** | |
| Docosahexaenoic acid (DHA)  22:6n-3  (ω-3 PUFA) | |  | | --- | | Status | | Body fat | | **Time** | | |  | | --- | | -0.0093 | | -0.0191 | | -4.285e-04 | | |  | | --- | | 1 | | 1 | | 1 | | |  | | --- | | 0.079 | | 1.468 | | 25.049 | | |  | | --- | | 0.780 | | 0.230 | | **4.64e-06** | |
| Linoleic acid 18:2n-6 (ω-6 PUFA) | |  | | --- | | Status | | **Body fat** | | Time | | |  | | --- | | -0.0935 | | -0.0002 | | -0.0806 | | |  | | --- | | 1 | | 1 | | 1 | | |  | | --- | | 1.508 | | 4.992 | | 0.678 | | |  | | --- | | 0.224 | | **0.029** | | 0.413 | |
| Arachidonic acid  20:4n-6 (ω-6 PUFA) | |  | | --- | | Status | | **Body fat** | | **Time** | | |  | | --- | | -0.0496 | | -0.0352 | | -4.246e-04 | | |  | | --- | | 1 | | 1 | | 1 | | |  | | --- | | 2.876 | | 6.466 | | 31.851 | | |  | | --- | | 0.095 | | **0.013** | | **4.08e-07** | |
| Oleic acid 18:1n-9 (MUFA) | |  | | --- | | Status | | **Body fat** | | Time | | |  | | --- | | 0.0213 | | 0.0547 | | 4.842e-05 | | |  | | --- | | 1 | | 1 | | 1 | | |  | | --- | | 0.418 | | 12.243 | | 0.325 | | |  | | --- | | 0.520 | | **0.0009** | | 0.570 | |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| (c) | **Coefficients** | **Estimate** | **df** | **F-value** | **p-value** |
| Total FAs | |  | | --- | | Status | | Body fat | | Time | | |  | | --- | | -380.3456 | | 137.3384 | | -0.1285 | | |  | | --- | | 1 | | 1 | | 1 | | |  | | --- | | 3.823 | | 2.219 | | 0.066 | | |  | | --- | | 0.055 | | 0.141 | | 0.798 | |
| **Total PUFAs** | |  | | --- | | **Status** | | Body fat | | Time | | |  | | --- | | -134.1223 | | 4.2872 | | -0.2481 | | |  | | --- | | 1 | | 1 | | 1 | | |  | | --- | | 6.277 | | 0.029 | | 3.244 | | |  | | --- | | **0.015** | | 0.866 | | 0.076 | |
| **Total ω*-*3 PUFAs** | |  | | --- | | **Status** | | Body fat | | Time | | |  | | --- | | -41.7841 | | 2.2407 | | -0.0706 | | |  | | --- | | 1 | | 1 | | 1 | | |  | | --- | | 8.610 | | 0.110 | | 3.709 | | |  | | --- | | **0.005** | | 0.741 | | 0.059 | |
| **Total ω*-*6 PUFAs** | |  | | --- | | **Status** | | Body fat | | Time | | |  | | --- | | -99.1266 | | 0.1758 | | -0.1707 | | |  | | --- | | 1 | | 1 | | 1 | | |  | | --- | | 3.786 | | 7.693 | | 8.039 | | |  | | --- | | **0.017** | | 0.993 | | 0.107 | |
| Total MUFAs | |  | | --- | | Status | | **Body fat** | | Time | | |  | | --- | | -133.7565 | | 83.4992 | | 0.0238 | | |  | | --- | | 1 | | 1 | | 1 | | |  | | --- | | 2.922 | | 5.069 | | 0.014 | | |  | | --- | | 0.092 | | **0.028** | | 0.906 | |
| Total SFAs | |  | | --- | | Status | | Body fat | | Time | | |  | | --- | | -107.0981 | | 50.2136 | | 0.0887 | | |  | | --- | | 1 | | 1 | | 1 | | |  | | --- | | 2.222 | | 2.175 | | 0.230 | | |  | | --- | | 0.141 | | 0.145 | | 0.633 | |