Fatty acids composition of migrated seabirds to the coastline of Pakistan as top predators to impact ecosystem variability

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August 22, 2022

Abstract

Availability of fat-rich food is the critical factor for the migration of seabirds. Predator-prey interaction shapes the food web structure and affects environmental variability. Certain fatty acids are believed to be the significant determining factors for environmental health and act as energy reserves for long distant seabird migration. Three different seabirds species investigated quantitatively for fat content and Fatty Acid composition from Pakistani sea waters were Larus fuscus, Larus ridibundus and Hydroprogne caspia, found significantly different for most of the fatty acid. The average fat contents of L. fuscus, L. ridibundus and H. caspia were 23.57±1.82%, 19.71±2.75% and 33.58±0.08% respectively. This study suggests that monounsaturated fatty acids (MUFA) were predominantly higher than saturated (SFA) and polyunsaturated fatty acids (PUFA) ranges from (43.49-48.07%), (32.88-39.89%) and (14.1-16.22%) respectively.. Palmitic acid and Stearic acid saturated and monounsaturated fatty acids constituted >75%. The dietary fatty acid Oleic acid (C18:1n9) was most abundant with 32-34%. The essential -3 fatty acids were found to be lower, whereas -6 was found in an appreciable amount with Linoleic acid (C18:2ώ6) as major fatty acid in L. fuscus (7.44%), H. caspia (9.81%) and L. ridibundus (8.99%). -3/-6 ratio was found less than 1 indicating these seabirds as a substantial source of omega 6 fatty acids. This study is the first report on seabirds' diet constituents from Pakistan to the best of our knowledge. Further experimental studies may reveal the physiological impact of varying fatty acids from this region.

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Abstract

Availability of fat-rich food is the critical factor for the migration of seabirds. Predator-prey interaction shapes the food web structure and affects environmental variability. Certain fatty acids are believed to be the significant determining factors for environmental health and act as energy reserves for long distant seabird migration. Three different seabirds species investigated quantitatively for fat content and Fatty Acid composition from Pakistani sea waters were *Larus fuscus, Larus ridibundus and Hydroprogne caspia*, found significantly different for most of the fatty acid. The average fat contents of *L. fuscus, L. ridibundus and H. caspia* were 23.57±1.82%, 19.71±2.75% and 33.58±0.08% resectively. This study suggests that monounsaturated fatty acids (MUFA) were predominantly higher than saturated (SFA) and polyunsaturated fatty acids (PUFA) ranges from (43.49-48.07%), (32.88-39.89%) and (14.1-16.22%) respectively.. Palmitic acid and Stearic acid saturated and monounsaturated fatty acids constituted >75%. The dietary fatty acid Oleic acid (C18:1n9) was most abundant with 32-34%. The essential ω-3 fatty acids were found to be lower, whereas ω-6 was found in an appreciable amount with Linoleic acid (C18:2ώ6) as major fatty acid in *L. fuscus* (7.44%), *H. caspia* (9.81%) and *L. ridibundus* (8.99%). ω-3/ω-6 ratio was found less than 1 indicating these seabirds as a substantial source of omega 6 fatty acids. This study is the first report on seabirds' diet constituents from Pakistan to the best of our knowledge. Further experimental studies may reveal the physiological impact of varying fatty acids from this region.

Keywords: Seabirds, Avian migration, Methyl Ester Fatty Acid Composition, Ecosystem variability

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1. Introduction

The dynamic of the immense natural capital of pelagic ecosystems is imitated by chemical pollutants, climatic variation, and commercial fishing exercises. The food web status may reveal by the trophic levels–based indicators that have been derived from fishery catch data, but the utility of biased records in fisheries catch is doubtful (Gagne *et al.*, 2018). Seabirds being the component of primary importance, are considered an excellent indicator of environmental fluctuations (Barrett, et al., 2007). The reproductive success of seabirds is affected by the oceanic environment's general aspects, species' response to the physical factors of an ecosystem such as food availability, climatic changes, population density, and experimental errors. Arguably, a helpful indicator should be responsive mainly to factors affecting several species in the top predator community. IUCN Ramsar sites and wetlands host a great community of seabirds migrated from Siberia and other native wildlife in Pakistan. Crane, Geese, Waders, Swans, and thousands of other birds visit Pakistan and other South Asian countries as resting sites between breeding and wintering grounds. Since seabirds are assumed to be the typical example of effective samplers of prey populations as many other predators, thus useful information about the lower trophic position over a wide range of geospatial and sequential scales can be obtained from their diets.

Seabirds' diet analysis by traditional methods is a hectic task; therefore, the fatty acid composition of adipose tissues is used to determine their diet constituent. Physical and ecological factors like climate change, timing, and accessibility of the prey species can be broadly attributed to change in dietary patterns of many species of seabirds throughout their reproductive season (Owen *et al.*, 2013). The other fundamental aspects linked with the reproductive phase include the amount and quality of feed for the prey compared to the self-feed of the predator species (Ito et al, 2010). However, extricating environmental and inherent effects are challenging due to simultaneous changes in external circumstances and parental responsibilities. Studies indicated that seabirds being at the top of the marine ecosystem's food web are considered bio-monitors of such changes.

In contrast, some prey species are at a lower position in the food web, and only a few contaminants are transferred up to the food web. Lipids are the most significant energy providers to migratory birds and the marine environment. The proximate analysis of migratory birds reveals that approximately 50% of the body mass comprises the total fat content to fuel during migratory exercises (McWilliams, Guglielmo, Pierce, & Klaassen, 2004). They can absorb the organic pollutants, act as absorption carriers, and play a role in the bioaccumulation of organic toxins. The lipids having heterogeneous nature can be used for ecological and biogeochemical assessment as an indicator of the health of ecosystems and the degree to which terrestrial and anthropogenic inputs have influenced them. The production value of dietary biogenic material of marine organisms and water quality can be determined by lipids. The stable compounds are supposed to be more effective measures to study the biochemical and ecological consequences of lipid contents of seabirds (Wold *et al.*, 2011). Traditional lipid extraction methods generally involve extracting dry material with a specific solvent. Extraction depends on the nature of the sample and the amount of solvent, either pure solvent or a mixture of two solvents like chloroform and methanol. Usually, two approaches (Reis, et al., 2013) are applied for lipid extraction with the changes in the volume and ratio of solvents within the system as per sample amount and the presence or absence of NaCl in the added water fraction.

This study aimed to investigate the fatty acid composition of migratory seabirds for the first time from Pakistan, as no biochemical research has ever been conducted in this region on seabirds.

Materials and Method

Seabird sampling was done for two years, 2016-2017, during wintering season from Manora Channels along the Karachi coast.

Seabirds' collection

A total of 16 individuals representing three species, i.e., *Larus fuscus* (n-9), *Larus ridibundus* (n-7), and *Hydroprogne caspia*, formally known as *Sterna caspia* (n-1), were sampled from the open sea with the assistance of lab attendants and fishermen, organs were isolated and stored at -20°C after dissection. Locally trained research assistants have done safe handling of birds in light of elders' experience. Proper support is provided by the WWF Pakistan and Marine Fisheries Department (MFD) Pakistan.Samples were collected during the winter season from November to March from the coastal belt of Karachi when migratory birds visit Pakistan. All the workers participated voluntarily. Samples were stored at -20°C and transferred to the analytical laboratory of PCSIR-KLC (Pakistan Council of Scientific and Industrial Research- Karcahi Laboratories Complex) for further fatty acid analysis.

Reagents and standards

n-hexane (95%), *n*-heptane (99%), chloroform (99.5%), methanol (98.8%) and potassium hydroxide (98%) were supplied by E. Merck and May & Baker (M&B). Whereas the standards for the fatty acid methyl ester quantification were purchased from Supelco Sigma-Aldrich Co. Nitrogen gas was used as carrier gas.

Lipid analysis

Analysis of individual samples was done for fatty acids (FA) composition. Fresh meat samples were dried in the electric oven at 70°C, and extraction was done in the Soxhlet assembly. Samples were weighed approximately 2 g of dry matter and homogenized in a glass potter. Total lipids were extracted in the Soxhlet apparatus by the modified Folch method (Perez-Palacios *et al.*, 2008). The solvents used for extraction chloroform and methanol (in the ratio of 2:1 v:v) were then removed under reduced pressure and extract was dried using anhydrous sodium sulfate. The extracted oil then filtered and stored.

Fatty acid analysis:

The FA profiles were analyzed through temperature gradient on a chromatograph (Clarus-500 Perkin Elmer) fitted in capillary column SP 2650 with 100m, 0.25mm, and 0.20 um film thickness and joined with the flame ionization detector (FID). Nitrogen was used as a carrier gas (splitless) at 2.5 ml/min flow rate (IUPAC, 1987). The FA peaks were identified through retention time compared with the FAME 37 standard mixture (Sulpeco Inc., Bellefonte PA). Results were calculated as the total FAME percentage. Statistical analysis that includes means, standard deviation, univariate analysis (ANOVA), Post hoc, and Duncan tests were conducted using IBM SPSS statistics 24.

Results and discussion:

The biochemical analysis of seabirds exhibits average fat content of *Larus fuscus, Larus ridibundus* and *Hydroprogne caspia* 23.57±1.82%, 19.71±2.75% and 33.58±0.08% and protein content 57.61±7.86%, 58.23±3.19% and 46.55±0.3% (dry matter) respectively (Table 1).

The present study identified a total of 21 fatty acids that includes Saturated Fatty Acid (Lauric acid C12:0, Myristic acid C14:0, Pentadecanoic acid C15:0, Palmitic acid C16:0, Heptadecanoic acid C17:0, Stearic acid C18:0), Monounsaturated Fatty Acid (Palmitoleic acid C16:1, Cis-10-Heptadecenoic acid C17:1, Oleic acid C18:1, *Cis*-11-Eicosenoic acid C20:1n9, Erucic acid C22:1n9, Nervonic acid C24:1n9) and Polyunsaturated Fatty Acid (Linoleic acid C18:2, Linolenic acid γLNA C18:3, σLNA C18:3, Eicosadienoic acid C20:2, Eicosatrienoic acid C20:3n6, Eicosapentaenoic acid [EPA] C20:5n3, Docosadienoic acid C22:2, Docosapentaenoic acid C22:5n3, Docosahexaenoic acid [DHA] C22:6n3) in various proportion in the adipose tissues of a Lesser black-backed seagull (*Larus fuscus*), Black-headed seagull (*Larus ridibundus*) and Caspian tern (*Hydroprogne caspia)* from Pakistani coastal waters. Differences in fatty acid profiles were calculated using ANOVA accompanied by the post hoc Duncan test among the seabird species.

There were significant differences in the mean percentages of C12:0, C14:0, C15:0, C16:0, C16:1, C17:1, C18:0, C18:2, C18:3, C20:1, C20:2, C20:5n3, C22:1n9, C22:5n3, C22:6n3 and C24:1n9 as $p < 0.05$ found between *L. fuscus, L. ridibundus* and *H. caspia*. The mean percentages of C17:0, C18:1, C20:3n6, and C22:2 were non significantly different ($p > 0.05$) (Table 2). The mean percentages of C14, C16, C18, C20:1, C20:5n3, C22:1n9, C22:5n3, and C22:6n3 were slightly higher in lesser black-backed seagull than caspian tern and black-headed gull, whereas C16:1, C17:1, C18:2, and C18:3α were found to be elevated in Caspian tern than other two species, whereas C18:1 was greatest in black-headed seagull. Differences showed little variations in the distribution of the food chains on different locations along the coastal belt of the Karachi coast.

Table 1. Chemical composition of seabirds from Karachi coast

Constituents	*Larus fuscus*(n-9)	*Hydroprogne caspia*(n-1)	*Larus ridibundus* (n-7)
Crude protein (%)	57.61±7.86	46.55±0.3	58.23±3.19
Total Fats (%)	23.57±1.82	33.58±0.08	19.71±2.75
Ash contents (%)	5.86±0.63	6.32±0.27	5.27±0.53
Total moisture (%)	6.5±1.04	6.84±0.06	6.07±2.02
Total carbohydrates (%)	7.21±6.71	6.69±0.56	10.7±4.02
Gross energy (Kcal/100g)	155.99±18.62	178.90±1.79	154.61±22.48

Table 2 Mean percentages and standard deviation of fatty acids found in seabirds from Karachi coast

Fatty Acid	*Larus fuscus*(n-9)	*Hydroprogne caspia*(n-1)	*Larus ridibundus*(n-7)	Sig.
	Mean ± SD	Mean ± SD	Mean ± SD	
C12:0	0.17^{c}±0.04	0.12^{b}±0.02	0.08^{a}±0.01	0.001
C14:0	2.5^{b}±0.22	2.25^{b}±0.21	1.84^{a}±0.16	0.008
C15:0	0.41^{a}±0.01	0.61^{c}±0.04	0.53^{b}±0.06	0.001
C16:0	24.17^{c}±0.6	19.56^{a}±1.27	22.75^{b}±0.78	0.000
C16:1	6.64^{a}±0.13	11.96^{b}±0.5	6.56^{a}±0.26	0.000
C17:0	0.51^{a}±0.11	0.39^{a}±0.06	0.71^{a}±0.4	0.379
C17:1	0.18^{a}±0.16	0.81^{c}±0.08	0.55^{b}±0.03	0.000
C18:0	12.13^{c}±0.12	9.95^{a}±0.94	11.9^{b}±0.43	0.003
C18:1	32.89^{ab}±0.34	32.52^{a}±1.12	34^{b}±0.15	0.066
C18:2n6	7.44^{a}±0.09	9.81^{c}±0.03	8.99^{b}±0.1	0.000
C18:3γ	0.44^{b}±0.03	0.38^{a}±0.03	0.73^{c}±0.02	0.000
C20:1	1.13^{a}±0.02	0.74^{a}±0.02	1.29^{a}±1.12	0.673
C18:3α	0.98^{b}±0.33	2.48^{c}±0.12	0.65^{a}±0.3	0.000
C20:2	0.18^{a}±0.15	0.28^{a}±0.07	0.45^{b}±0.03	0.016
C20:3n6	0.25^{a}±0.26	0.14^{a}±0.16	0^{a}±0	0.158
C22:1n9	2.49^{b}±0.02	1.83^{a}±0.03	1.97^{a}±0.12	0.000
C20:5n3 (EPA)	0.81^{c}±0.07	0.36^{a}±0.03	0.66^{b}±0.08	0.000
C22:2	0.9^{a}±0.06	0.89^{a}±0.05	0.84^{a}±0.05	0.422
C24:1n9	0.16^{a}±0.21	0.21^{a}±0.02	0.21^{a}±0.18	0.057
C22:5n3 (DPA)	1.57^{b}±0.07	1.03^{a}±0.02	1.08^{a}±0.08	0.000
C22:6n3 (DHA)	1.53^{b}±0.14	0.85^{a}±0.01	0.97^{a}±0.07	0.000
∑ SFA	39.89%	32.88%	37.81%	
∑ MUFA	43.49%	48.07%	44.58%	
∑ PUFA	14.1%	16.22%	14.37%	
ω-3	5.87%	4.72%	3.36%	
ω-6	9.03%	11.22%	10.56%	
ω-3/ω-6	0.65%	0.42%	0.31%	

ANOVA shows a statistically significant difference if P<0.05

Different superscript a-d horizontally shows the significant difference among species as P<0.05

Table #1 displays the mean concentrations of fatty acids in percentages observed in Seagull and Caspian tern sampled from Manora channels along the Karachi coast. The total fatty acids ranged from 96.76% in the Black-headed seagull to 97.48% in the Lesser black-backed seagull. Lesser black-backed seagull had the lowest percentage of 0.16 for C24:1n9 and the highest percentage of 32.89 for C18:1. Caspian tern had the lowest percentage of 0.12 for C12:0 and the highest percentage of 32.52 for C18:1. Black-headed seagull had the lowest percentage of 0.08 for C12:0 and the highest percentage of 34 for C18:1, while C20:3n6 was not found in black-headed seagull.

Results revealed that MUFAs significantly contribute to the total fatty acid composition in all sampled species 43-48%, followed by the saturated fatty acids 32-39%, whereas PUFAs were in the lowest percentage 14-16%.

Palmitic acid (16:0) was the most abundant saturated fatty acid 24.17%, 22.75% and 19.56% followed by Stearic acid (18:0) 12.13%, 11.9% and 9.95% in *L.fuscus>L. rudibundus>H. caspia*, whereas the opposite trend (C18:0>C16:0) was earlier reported by (Andersson, Wang, Nord, Salmon, & Isaksson, 2015) correlated to temperature changes. However, differences in the FA content are relevant to the season depending on habitat and diet choices.

Among MUFAs, Oleic acid (18:1) was the most abundant 34%, 32.89%, and 32.52% in *L. rudibundus> L. fuscus> H. caspia* followed by Palmitoleic acid (16:1) 11.96%, 6.64%, and 6.56% in *H. caspia> L.fuscus> L. rudibubdus*, and the most abundant fatty acid among all.

Linoleic acid (18:2n6) was the most abundant PUFA 9.81%, 8.99%, and 7.44% found in adipose tissues of *H. caspia> L. rudibundus> L. fuscus*.

The physiologically important ω-3 PUFAs are DPA 1.57> 1.08> 1.03, in *L. fuscus, L. rudibudus*, and *H. caspia* respectively and αLNA 2.48%> 0.98%> 0.65%, respectively in *H. caspia, L. fuscus and L. rudibundus*. The αLNA is a strictly dietary fatty acid. It cannot be biosynthesized (Larsson, Kumlin, Ingelman-Sundberg, & Wolk, 2004), and results in this study indicate the diet insufficiently of physiologically essential ω-3 FA. The values of ω-6 Linoleic acid were highest 9.81% in *H. caspia* followed by 8.99% in *L. rudibundus* and least amount 7.44% in *L. fuscus*, indicating the diet rich in ω-6 FA as Linoleic acid is also diet-dependent and cannot be biosynthesized. Another essential ω-6 FA was ARA (Arachidonic acid) not found in the present study, which is a biosynthetic product of Linoleic acid. It may be occurred due to the mobilization of selective fatty acids to regulate body physiology during environmental fluctuations such as thermoregulation during wintry weather (Price, Krokfors, & Guglielmo, 2008).

Fatty acid levels might be influenced by seasonal changes and availability of diet (Andersson, Wang, Nord, Salmon, & Isaksson, 2015) explained in an earlier study that ω-3 FA was strongly dependent on seasonal variation and found in lower proportions during winter than summer while ω-6 Linoleic acid was higher during winter than summer. The recent study was evident to these variations as the birds were sampled during the winter season.

Overall, ω-3 is considered an anti-inflammatory, while ω-6 has pro-inflammatory and thermoregulatory properties. It is also hypothesized that due to lower levels of ω-3 PUFAs and higher intake of ω-6 PUFAs, these birds are more exposed to pollutants, enhancing oxidative stress. Hence, play a crucial role in a healthy and sustainable ecosystem.

Results revealed a variety of food supplied to these seabirds to fuel their annual migration along the Karachi coast, as the most abundant C16 and C18 fatty acids were diet-dependent. Significant contributors are marine algae, krill, worms, clams, mussels, crabs, shrimps, scallops, and pelagic and intertidal small fishes like herring and sardine mackerel and juveniles of large fishes like sea bass, codfish, catfish, tuna, and tilapia. Mangroves are also part of the seabird's diet. For future studies to explore the actual food chain, experimental research should be carried out to investigate the FA composition of prey species.

Fatty acids being the transporters of fat-soluble vitamins and chemical pollutants, are conducive to sustaining and shaping the ecosystem (De Laender, Oevelen, Frantzen, Middelburg, & Soetaert, 2010).

Seabirds, as a member of the marine environment, are imperiled to substantial fluctuations in the environment, such as temperature and availability and quality of food which affect the FA composition of their muscles (Parrish, 2013). Carbon 16 and 18 fatty acids include saturated and monounsaturated fatty acids constitute a significant part of total FA composition in migratory birds, reported previously by (Blem, 1976; McWilliams, Guglielmo, Pierce, & Klaassen, 2004; Pierce & McWilliams, 2014) and >75% during this study. The C16:0 and C18:1n9 are the most abundant, whereas C16:1n7 and C18:0 are less abundant. However, they are essential fatty acids. During migratory exercises of seabirds, the levels of FA constituents can also be influenced by selective feeding at their stopover sites (Pierce & McWilliams, 2005). A selective diet can significantly influence the energetics of the flight (Pierce & McWilliams, 2005). Previously it was suggested that ω-3 and ω-6 PUFA play a significant role in exercise performances. (Price & Guglielmo, 2009) proposes that ω-6 PUFA enhanced the migratory exercise more than ω-3 PUFA. While, according to (Maillet & Weber, 2007), possibly ω-3 PUFA with effect to improve membrane functioning, can increase the prolonged migratory exercises. Furthermore, the fatty acid composition determines the distribution pattern and effects of lipid-soluble chemical pollutants like organochlorine pesticides. It is useful for the conservation and management of seabirds effectively for a sustainable environment.

Conclusion

This study reveals the fatty acid composition of migratory seabirds foraging from the coastal areas of Pakistan for the first time to the best of our knowledge. Variations was observed in SFA, MUFA and PUFA. The results suggest low supply of anti-inflammatory ω3 PUFA than pro-inflammatory ω-6 PUFA, indicating potential exposure to chemical pollutants and pathogens. Moreover, substantial impacts on long distance migratory performances. The variations in fatty acid compositions of seabirds related to the changes in the status of habitat, availability of quality food and physical changes of marine environment and physiological state of the bird as well. .

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