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Lipophilic Constituents of the Blubber from Blue Whale, *Balaenoptera musculus*, washed Ashore at Pakistan Coast

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Summary: The blue whale, *Balaenoptera musculus* (Linnaeus, 1758), is the biggest animal recognized to exist today throughout the globe. High commercial value of lipids has made this species vulnerable. Blubber, a crucial adaptation for mammals living in water, serves as energy reservoir. Surplus energy is deposited in the form of fatty acids (FAs) and therefore have been analysed. The compositional analysis also helps in understanding the dietary and structural role of FAs in blubber. Lipid analysis of blubber from stranded, dead blue whale through Thin layer chromatography (TLC) has resulted in identifying 6 constituents. These constituents are a triacyl glyceride (TAG), 2 steroids, and 3 FAs. Approximate analysis of waxy constituents has also been attempted exploiting TLC. Gas chromatography-mass spectrometry (GC-MS) analyses has resulted in identification of 86 compounds, which were further confirmed through the Retention Indices (RIs), accounting to a total concentration of 85.7, 86.1, 84.8, and 89.7 % in jaw, abdomen, peduncle, and fluke, respectively. Altogether 17 SFAs including 4 Branched FAs, 5 MUFAs, and a PUFA were identified. The main reasons for the low quantitative and qualitative content of PUFAs were susceptibility of PUFAs towards oxidation. Thus 8 FAlds, 4 FAlcs, and 3 other oxygenated FAs, which made a total of 2.7, 0.9, 1.3, and 5.2% in jaw, abdomen, peduncle, and fluke, respectively were justified. Further the chromatographic region where PUFAs are expected to resolve has been found masked with significant concentration of anthropogenic compounds, which accounted to 43.4, 35.6, 34.6, and 30.7% in jaw, abdomen, peduncle, and fluke, respectively. These pollutants included 25 hydrocarbons, 4 phthalates, 2 siloxanes, 2 bisphenols, and diphenyl carbonate. Four natural Prenols were also identified. 16 constituents with concentration of 14.2, 8.0, 15.7, and 10.8% in jaw, abdomen, peduncle, and fluke, respectively, were remained unidentified. Few constituents were justified through food chain.

Keywords: Blue whale; *Balaenoptera musculus*; Blubber; GC-MS; Lipid profile; Degraded lipids; Pollutants.

Introduction

The blue whale, *Balaenoptera musculus* (Linnaeus, 1758), is the biggest animal recognized to exist today throughout the globe. It is found in world's oceans, from the cold waters to temperate and tropical regions [1]. High commercial value of its lipids has made this species vulnerable and as a result some 325-360 thousand blue whales were killed in many parts of the world including Indian Ocean during first half of the twentieth century [2, 3], reducing their population to the brink of extinction. Their population structure described on the basis of data originated from sightings and stranding [4] is being further improved through acoustic studies [5]. Blue whales are generally categorized into five subspecies: (i) *B. musculus musculus* (ii) *B. musculus intermedia* (iii) *B. musculus brevicauda* (iv) *B. musculus indica* and (v) an unnamed subspecies referred to as the Chilean [6, 7, 8]. *Balaenoptera musculus* found in Pakistan waters are not yet fully described into a subspecies.

The marine mammals, pinnipeds (seals, sea lions and walruses) and cetaceans (whales), have thick lipid rich blubber layer that covers the body. The morphology, distribution and composition of the blubber have been the subject of different studies emphasizing on their role in thermoregulation, hydrodynamic streaming, buoyancy and energy reserves [9]. The thickness and composition of blubber differs from species to species, habitat, growth, health, reproduction and season. Its thickness ranges from <0.5 cm in tropical dolphins to >50 cm in bowhead whales [10]. Same is true for the variations in composition of blubber lipid. The lipid composition of blubber is a combination of contributions from the diet and de novo synthesis. Generally, the adipose tissues of marine mammals

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are similar to other mammals with accumulation of TAG comprising of fatty acids ranging from C_{14} to C_{24} [11].

Lipid oxidation is the single main cause that worsens blubber and is due to reaction of fats and oils with molecular oxygen generating off-flavours, commonly called as rancidity. Exposure to light, pro-oxidants and high temperature speeds up the reaction. Rancidity is related with distinguishing off-flavour and odour of the oil [12]. Primary oxidation products are produced by the reaction of free radicals, e.g., an alkyl radical, which is produced by the reaction of oxygen in presence of light or heat (initiation), leading to a peroxyl free radical [13, 14]. Secondary oxidation products are produced when the hydroperoxides breakup into secondary oxidation products. The chain scission leads to the production of aldehydes, ketones, alcohols and acids [15].

Blue whale is baleen whale that feeds generally on krill (euphausiids) and are considered as top predator. They tend to bioaccumulate pollutants (e.g. hydrocarbons) in the body fat deposits through food chain. The threat of environmental contaminants to marine mammals is wide-spread. High concentrations of certain pollutants in the tissues of these animals have been found and associated with organ anomalies, impaired reproduction and immune function. The sources of contamination including anthropogenic chemicals, radionuclides, mineral oil derived pollutants, marine debris, sewage related pathogens, and even excessive amounts of nutrients, can cause environmental changes as well as adversely affect the health and population status of marine mammals [16]. Data on lipid composition of blue whales' blubber layer is generally scarce and non-existent from those sighted in northern Arabian Sea region. The present study deals with the assessment of lipid composition of blubber in a putrefying dead blue whale stranded in the coastal waters and reports lipid classes, fatty acids composition, oxidation products, and possible pollutants deposited in the fat layer.

Experimental

Sampling from specimen

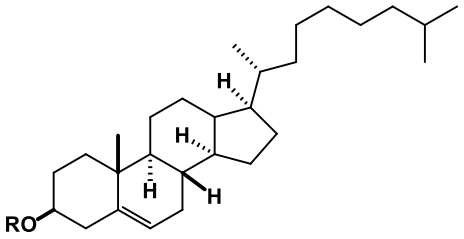
A dead blue whale was brought to Khuddi Creek of Indus Delta, in 2014. The blubber was sampled from its four locations; jaw, abdomen, peduncle and fluke. Jaw and abdomen were found damaged. Jaw was very badly damaged and might be the cause of its death. Abdomen was already cut for the collection of liver oil by local fishermen, who are well aware of its uses for different purposes. Therefore, jaw and abdomen were sampled from middle to outer layer of blubber only. Peduncle and fluke were also sampled from middle to outer layer only, as the inner flash of the whale was highly putrefied. Samples were kept in ice box and composites containing equal weights of different samples from each site were prepared separately. Lipid was extracted following the Folch method [17]. Extracts and samples were kept frozen in dark bottles until analysed.

Thin layer chromatography (TLC)

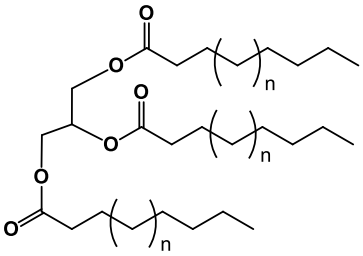
Thin Layer Chromatography (TLC) was employed to delineate lipid components [18, 19]. All samples (jaw, abdomen, peduncle and fluke) were identified on TLC plates (Silica gel 60 F_{254} aluminium sheets 20x20 cm, Merck, Germany) with standards for cholesterol, cholesterol acetate (steroid), tripalmitin (TAG), lauric, myristic, palmitic, stearic, oleic and erucic acid (free fatty acid; FFA), bees wax and lecithin (phospholipids). The solvent system used was petroleum ether:diethyl ether:acetic acid (80:20:1 v/v), however, for the separation of wax hexane:diethyl ether (90:10 v/v) was used as solvent [18]. Lipid components separated on TLC plates were visualized by treating plates with respective reagents as reported for steroids [20], TAG [21], phospholipids [22], FFA [23], and wax [24].

Gas chromatography-mass spectrometry (GC-MS)

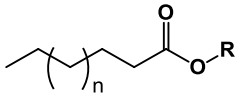
Lipid samples were hydrolysed and methylated (esterified) [25]. Esterified lipid was extracted thrice with hexane. Hexane fractions, rich in fatty acid methyl ester (FAME), were coded as HBJ (jaw), HBA (abdomen), HBP (peduncle) and HBF (fluke). Residue left, was extracted with $CHCl_3$ and the fractions were coded as CBJ (jaw), CBA (abdomen), CBP (peduncle) and CBF (fluke). All fractions were analysed by GC-MS using Shimadzu GC-2010 gas chromatograph (Japan) equipped with a HP-5® fused silica capillary column (30 m x 0.25 mm i.d. x 0.25 µm df), a split/splitless injector and a QP2010 Plus MS with Shimadzu GCMS solution software. Helium was the carrier gas. Sample were injected with split ratio of 1:50 with injector temperature set to 250 °C and column oven set to 50 °C. After 6 min, oven temperature was raised at a rate of 5 °C/min to 300 °C, which was maintained for 20 min. %age compositions of constituents were calculated using area normalisation method. The processed mass spectra were identified by calculating and referencing the retention indices (RIs) [26] with compounds present in the electronic mass spectral libraries [27, 28] and MS data from literature.



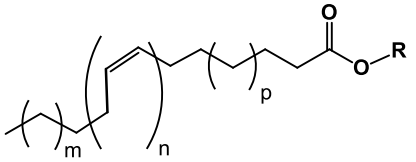
R = H; Cholesterol (1)
R = CH_3CO; Cholesterol acetate (2)



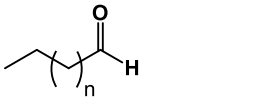
n = 9; Glycerol tripalmitate (3)



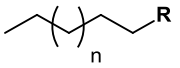
n = 11, R = H; Hexadecanoic acid (4)
n = 13, R = H; Octadecanoic acid (5)
n = 1, R = CH_3; Hexanoic acid ME (8)
n = 1, R = H; Hexanoic acid (11)
n = 2, R = CH_3; Heptanoic acid ME (13)
n = 2, R = H; Heptanoic acid (16)
n = 3, R = CH_3; Octanoic acid ME (18)
n = 3, R = H; Octanoic acid (20)
n = 4, R = CH_3; Nonanoic acid ME (22)
n = 4, R = H; Nonanoic acid (24)
n = 6, R = CH_3; Undecanoic acid ME (32)
n = 9, R = CH_3; Tetradecanoic acid ME (48)
n = 9, R = H; Tetradecanoic acid (49)
n = 10, R = CH_3; Pentadecanoic acid ME (53)
n = 11, R = CH_3; Hexadecanoic acid ME (60)
n = 11, R = CH_3CH_2;
Hexadecanoic acid EE (64)
n = 12, R = CH_3; Heptadecanoic acid ME (67)
n = 13, R = CH_3; Octadecanoic acid ME (71)
n = 14, R = CH_3; Nonadecanoic acid ME (75)
n = 15, R = CH_3; Eicosanoic acid ME (81)
n = 17, R = CH_3; Docosanoic acid ME (85)



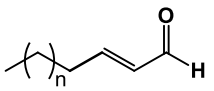
m = 5; n = 1; p = 3; R = H; 9*Z*-Octadecenoic acid (6)
m = n = 1; p = 3; R = CH_3; 9*Z*-Tetradecenoic acid ME (47)
m = 3; n = 1; p = 3; R = CH_3; 9*Z*-Hexadecenoic acid ME (59)
m = 3; n = 1; p = 3; R = H; 9*Z*-Hexadecenoic acid (61)
m = 5; n = 1; p = 3; R = CH_3; 9*Z*-Octadecenoic acid ME (69)
m = 5; n = 1; p = 3; R = CH_3CH_2; 9*Z*-Octadecenoic acid EE (73)
m = 2; n = 2; p = 5; R = H; 11,14-Eicosadienoic acid (77)
m = 5; n = 1; p = 5; R = H; 11*Z*-Eicosenoic acid (82)



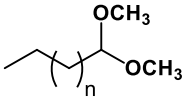
n = 4; Heptanal (7) **n = 6; Nonanal (17)**
n = 5; octanal (12) **n = 7; Decanal (21)**



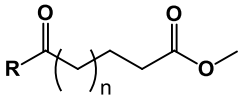
n = 3; R = Cl; 1-Choloro-heptane (9)
n = 3; R = OH; 1-Heptanol (10)
n = 4; R = OH; 1-Octanol (15)
n = 10; R = OH; 1-Tetradecanol (42)
n =12; R = OH; 1-Hexadecanol (56)
n =14; R = CH_2CH_2OH;
2-octadecyloxy ethanol (79)



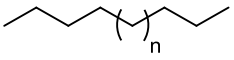
n = 3; (*E*)-2-Octenal (14)
n = 4; 2-Nonenal (19)
n = 5; (*Z*)-2-Decanal (23)
n = 6; (*E*)-2-Undecanal(27)



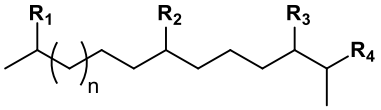
n = 6 Nonanal dimethyl acetal (25)



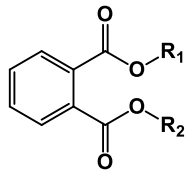
n = 5; R =CH_3;
9-Oxo-decanoic acid ME (35)



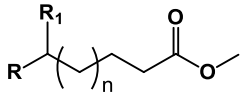
n=6; Tridecane (26) **n=7; Tetradecane (31)** **n=8; Pentadecane (34)**
n=9; Hexadecane (41) **n=10; Heptadecane (45)** **n=11; Octadecane (52)**
n=12; Nonadecane (58) **n=13; Eicosane (66)** **n=15; Docosane (74)**
n=17; Tetracosane (83) **n=18; Pentacosane (84)** **n=19; Hexacosane (87)**
n=20; Heptacosane (88) **n=21; Octacosane (89)** **n=22; Nonacosane (90)**
n=23; Triacontane (91) **n=26; Triacontane (92)**



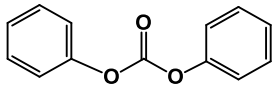
R^1 = R^2 =R^4 = CH_3; R^3 =H; n = 1; 2,6,11-Trimethyl-dodecane (28)
R^1 = R^2 = R^3 = CH_3; R^4 =H; n = 1; 2,6,10-Trimethyl-dodecane (29)
R^1 = CH_3; R^2 = R^3 = R^4 = H; n = 2; 2-Methyl-tridecane (30)
R^1 = H; R^2 = R^3 = CH_3; R^4 =C_2H_5; n = 1; 4,8-Dimethyl-tridecane (33)
R^1 = CH_3; R^2 = R^3 = R^4 = H; n = 4; 2-Methyl-pentadecane (37)
R^1 = R^3 = R^4 =H; R^2 = CH_3; n = 4; 7-Methyl-pentadecane (38)
R^1 = R^2 = R^4 =H; R^3 = CH_3; n = 4; 3-Methyl-pentadecane (39)
R^1 = CH_3; R^2 = R^3 = R^4 =H; n = 5; 2-Methyl-hexadecane (43)
R^1 = CH_3; R^2 = R^3 = R^4 = H; n = 6; 2-Methyl-heptadecane (50)
R^1 = R^2 = R^4 = H; R^3 = CH_3; n = 6; 3-Methyl-heptadecane (51)



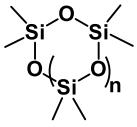
R^1 = R^2 = CH_3CH_2; Diethyl phthalate (40)
R^1 = R^2 = $CH_3CH_2(CH_3)CH$;
Diisobutyl phthalate (55)
R^1 = $CH_3(CH_2)_3$; R^2 = $CH_3CH_2(CH_3)CH$;
Butyl isobutyl phthalate (62)
R^1 = R^2 = $CH_3(CH_2)_3CH(CH_3CH_2)CH_2$;
Diethyl hexyl phthalate (86)



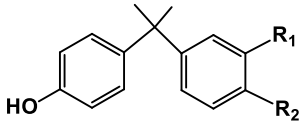
n = 6; R = CH_3CH_2; R^1 = CH_3;
10-Methyl-dodecanoic acid ME (36)
n = 10; R = R^1 = CH_3;
14-Methyl-pentadecanoic acid ME (57)
n = 11; R = R^1 = CH_3;
15-Methyl hexadecanoic acid ME (63)
n = 12; R = R^1 = CH_3;
16-Methyl-heptadecanoic acid ME (68)
n =6; R = $CH_3(CH_2)_6CH_2$; R^1 = OH;
10-Hydroxy-octadecanoic acid ME (80)



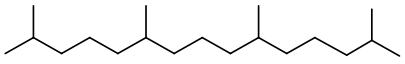
Diphenyl carbonate (44)



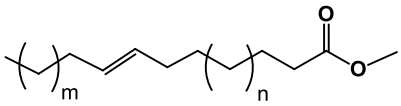
n =7; Octadecamethyl cyclononasiloxane (D9) (54)
n = 9; Eicosamethyl cyclodecasiloxane (D10) (65)



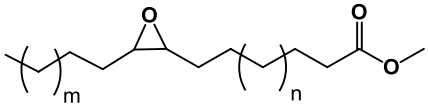
R^1 = OH; R^2 = H; 4,4'-bisphenol A (72)
R^1 = H; R^2 = OH; 2,4'-bisphenol A (76)



2,6,10,14-Tetramethyl-pentadecane (46)



m = 6; n = 3; 9*E*-Octadecenoic acid ME (70)



m = 5; n = 3; 9,10-epoxy octadecanoic acid ME (78)

Fig 1: Chemical constituents from blubber of stranded blue whale (*Balaenoptera musculus*)

Results and Discussion

FFAs (lauric, myristic, palmitic, stearic, oleic, and erucic acid), steroids (cholesterol and cholesterol acetate), TAG (tripalmitin), phospholipids (lecithin) and bees wax were analysed through co-TLC analysis. Variability in the presence of these lipid classes in different blubber samples was noted and detailed in Table-1 [19]. Lecithin, a phospholipid, and lauric, myristic, and erucic acids were not observed on TLC. Lipid yield from the blubber layer was determined in different samples (Table-2). Highest lipid content was found in peduncle layer followed in descending order by abdomen, jaw and fluke layers.

The results obtained from GC-MS study are depicted in detail in Table-3 [29-81] as well as in simplified Table-2 showing presence of fatty acids, other constituents derived from fatty acids, along with prenols and pollutants. Altogether 92 compounds were identified in the study, in free or derived forms. Of these, 6 constituents were identified through TLC and 86 in GC-MS. Three constituents were identified both in TLC and GC-MS. Fifty-seven of these constituents were natural lipids or their derivatives. These included a TGA, 2 steroids, 3 oxygenated FAs, 4 prenols, 4 fatty alcohols (FAlc), 9 fatty aldehydes (FAld), and 34 simple FAs. These FAs comprised of a polyunsaturated fatty acid (PUFA), 4 branched FAs (Br. FAs), 8 monounsaturated fatty acids (MUFA), and 21 saturated fatty acids (SFA). The GC-MS of samples further revealed 35 lipophilic pollutants, including a chloro-alkane (a listed marine pollutant), a polyol ether (a surfactant used in skin care items), an aryl carbonate (bio-degrative metabolite of plastics), 2 siloxanes (used in silicone kitchen wares and softeners), 2 bisphenols, 4 phthalates (plasticizer), and 24 hydrocarbons (Table 2 and 3).

Table-1: Thin layer chromatographic (TLC) analyses of Folch extract.

Chemical constituents and / or chemical classes	CBJ*	CBA*	CBP*	CBF*
Cholest-4-en-3β-ol (Cholesterol; 1)	-	+	+	+
Cholest-4-en-3β-O-acetate (Cholesterol acetate 2)	-	-	+	+
2,3-di(hexadecanoyloxy)-propyl hexadecanoate (Glycerol tripalmitate; 3)	-	+	+	+
Hexadecanoic acid (Palmitic acid; 4)	+	+	+	+
Octadecanoic acid (Stearic acid; 5)	+	+	+	+
(*Z*)-9-Octadecenoic acid (Oleic acid; 6)	+	+	+	+
Waxes [19]				
Hydrocarbons (i)	-	+	+	+
Monoesters with C_{40}-C_{50} hydroxy acids (ii)	+	+	+	+
Diesters of diols with C_{24}-C_{28} and C_{56}-C_{64} fatty acids (iii)	-	+	+	-
Hydroxy esters of diols with C_{24}-C_{28} and C_{40}-C_{50} hydroxy acids and lignocerates (iv)	+	+	+	-
Hydroxy esters of diols with C_{24}-C_{28} and C_{40}-C_{50} hydroxy acids along with 15-hydroxy-palmitic acid and lignocerates (v)	+	+	+	+
Hydroxyesters of diols with C_{24}-C_{28} hydroxy acids (vi)	+	+	+	+
Hydroxyesters of diols with C_{24}-C_{28} hydroxy acids and free C_{24}-C_{28} hydroxy acids (vii)	+	+	+	+

*CB: chloroform extract from blubber; *J: jaw; *A: abdomen; *P: peduncle; *F: fluke; *(+): present; *(-): not detected

Table-2: Chemical composition of blubber from dead stranded blue whale (*Balaenoptera musculus*).

IUPAC Name (common name in parenthesis) Numbered in order of elution on HP-5 column	% Composition*				Class
	Jaw	Abdomen	Peduncle	Fluke	
Fatty acids and their derivatives					
Hexanoic acid (Caproic acid; 8+11)	-	-	-	0.06	SFA
Heptanoic acid (Enanthic acid; 13+16)	-	-	-	0.21	SFA
Octanoic acid (Caprylic acid; 18+20)	-	-	-	0.12	SFA
Nonanoic acid (Pelargonic acid; 22+24)	-	-	-	0.30	SFA
Undecanoic acid (Undecylic acid; 32)	-	-	-	1.26	SFA
10-Methyl-dodecanoic acid (36)	-	-	-	0.06	Br FA*
(*Z*)-9-Tetradecenoic acid (Myristoleic acid; 47)	1.15	1.23	1.25	1.23	MUFA
Tetradecanoic acid (Myristic acid; 48+49)	5.47	10.19	6.43	10.53	SFA
Pentadecanoic acid (Pentadecylic acid; 53)	-	0.06	0.15	2.40	SFA
14-Methyl-pentadecanoic acid (iso-Palmitic acid;57)	-	-	-	0.16	Br FA
(*Z*)-9-Hexadecenoic acid (Palmitoleic acid; 59+61)	7.67	6.96	7.12	7.19	MUFA
Hexadecanoic acid (Palmitic acid; 4+60+64)	6.22	9.43	9.63	9.68	SFA
15-Methyl-hexadecanoic acid (iso-Margaric acid; 63)	-	-	-	0.20	Br FA
Heptadecanoic acid (Margaric acid; 67)	0.13	0.48	0.54	043	SFA
16-Methyl-heptadecanoic acid (iso-stearic acid; 68)	-	-	-	0.10	Br FA
(*Z*)-9-Octadecenoic acid (Oleic acid; 6+69+73)	10.34	13.12	11.99	13.33	MUFA
(*E*)-9-Octadecenoic acid (Elaidic acid; 70)	0.84	0.88	3.06	0.20	MUFA
Octadecanoic acid (Stearic acid; 5+71)	1.99	1.81	3.78	2.11	SFA
Nonadecanoic acid (Nonadecylic acid; 75)	-	0.56	0.67	0.07	SFA
11,14-Eicosadienoic acid (77)	0.45	-	0.16	0.26	PUFA
Eicosanoic acid (Arachidic acid; 81)	0.49	0.39	0.83	0.37	SFA
Z-11-Eicosenoic acid (Gondoic acid; 82)	4.55	4.04	2.30	2.35	MUFA
Docosanoic acid (Behenic acid: 85)	0.19	0.48	-	0.08	SFA

Oxygenated metabolites of fatty acids					
Heptanal (Enanthaldehyde; 7)	-	-	-	0.20	FAld
1-Heptanol (Enanthic alcohol; 10)	-	-	-	0.02	FAlc
Octanal (Caprylic aldehyde; 12)	0.16	-	-	0.28	FAld
(*E*)-2-Octenal (14)	-	-	-	0.14	FAld
1-Octanol (Capryl alcohol; 15)	-	-	-	0.03	FAlc
Nonanal (Pelargonaldehyde; 17+25)	0.31	0.30	0.18	0.91	FAld
2-Nonenal (19)	-	-	-	0.15	FAld
Decanal (Capraldehyde; 21)	-	-	-	0.27	FAld
(*Z*)-2-Decenal (23)	0.10	-	0.56	0.31	FAld
(*E*)-2-Undecenal (27)	-	-	-	1.37	FAld
9-oxo-decanoic acid (35)	-	-	-	0.97	Oxy FA*
Tetradecanol (Myristyl alcohol; 42)	0.18	-	0.18	-	FAlc
1-Hexadecanol (Cetyl alcohol; 56)	1.25	0.59	0.41	0.21	FAlc
9,10-Epoxy-octadecanoic acid (78)	0.46	-	-	0.31	Oxy FA
10-Hydroxy-octadecanoic acid (80)	0.20	-	-	-	Oxy FA
Prenols					
2,6,11-Trimethyl-dodecane (28)	-	0.13	0.15	-	ST*
2,6,10-Trimethyl-dodecane (Farnesane; 29)	-	0.36	-	0.62	ST*
4,8-Dimethyl-tridecane (33)	-	0.14	-	0.47	DM*ST*
2,6,10,14-Tetramethyl-pentadecane (Norphytane or Pristane; 46)	0.12	-	0.81	-	DM*DT*
Lipophilic Pollutants other than Hydrocarbons					
1-Chloroheptane (heptyl chloride; 9)	-	-	-	0.03	Chloro-alkane
1,2-Benzenedicarboxylic acid ethyl ester (Diethyl Phthalate; 40)	3.81	5.81	4.83	3.30	Phthalate
Diphenyl carbonate (44)	2.34	2.77	3.84	2.43	Aryl carbonate
Octadecamethyl cyclononasiloxane (D9; 54)	0.12	-	-	-	Siloxane
1,2-Benzenedicarboxylic acid isobutyl ester (Diisobutyl phthalate; 55)	1.71	1.13	0.61	0.34	Phthalate
1,2-Benzenedicarboxylic acid butyl isobutyl ester (Butyl-isobutyl phthalate; 62)	1.62	-	0.48	0.46	Phthalate
Eicosamethyl cyclodecasiloxane (D10; 65)	0.59	-	-	0.64	Siloxane
4,4'-(Propane-2,2-diyl)diphenol (4,4'-Bisphenol-A; 72)	0.54	0.54	0.56	0.12	Bisphenol
2,4'-(Propane-2,2-diyl)diphenol (2,4'-Bisphenol A (76)	0.53	0.29	0.41	0.08	Bisphenol
2-Octadecyloxy-ethanol (79)	4.35	1.94	0.21	1.26	Surfactant
1,2-Benzenedicarboxylic acid ethylhexyl ester (Di-ethylhexyl phthalate; 86)	0.41	-	0.46	0.08	Phthalate
Hydrocarbons					
Tridecane (26)	1.18	1.82	1.51	0.66	
2-Methyl-tridecane (iso-Myristane; 30)	-	0.19	-	0.31	
Tetradecane (Myristane; 31)	1.02	2.21	1.60	2.26	
Pentadecane (34)	2.73	2.07	2.34	0.84	
2-Methyl-pentadecane (37)	-	-	0.56	-	
7-Methyl-pentadecane (38)	-	0.35	-	0.28	
3-Methyl-pentadecane (39)	-	-	0.69	0.27	
Hexadecane (Cetane; 41)	2.39	2.68	2.88	2.37	
2-Methyl-hexadecane (43)	-	-	0.11	0.14	
Heptadecane (45)	1.07	1.31	1.21	1.25	
2-Methyl-heptadecane (50)	0.28	-	0.28	-	
3-Methyl-heptadecane (51)	0.09	-	-	0.91	
Octadecane (52)	1.88	2.94	1.37	2.82	
Nonadecane (58)	1.11	0.91	0.15	0.32	
Eicosane (66)	0.61	0.27	0.15	0.47	
Docosane (74)	0.49	0.09	-	0.14	
Tetracosane (83)	2.06	1.55	1.71	1.15	
Pentacosane (84)	2.07	2.01	1.55	1.59	
Hexacosane (87)	2.36	2.05	1.69	1.38	
Heptacosane (88)	2.16	2.18	1.54	1.54	
Octacosane (89)	2.28	1.87	1.31	1.28	
Nonacosane (90)	1.70	1.91	1.19	0.81	
Triacontane (91)	1.78	2.07	1.34	1.01	
Hentriacontane (92)	0.57	-	-	0.20	
Summary					
% yield (wet weight) with number of replicates in parenthesis	32.05 (n=12)	54.78 (n=11)	63.09 (n=12)	29.14 (n=13)	
SFA + Br FA	14.49	22.84	22.03	28.14	
MUFA + PUFA	25.00	26.23	25.88	24.56	
FAld + FAlc + Oxy FA	2.66	0.89	1.33	5.17	
Prenols	0.12	0.63	0.96	1.09	
Pollutants (other than Hydrocarbons)	15.61	12.48	11.40	8.74	
Hydrocarbons	27.83	23.12	23.18	22.00	
UI* with numbers of UI constituents in parenthesis.	14.18	8.03	15.67	10.78	

***(% composition): calculated using area normalization method; *Br: branched FA; *Oxy: oxygenated FA. *ST: sesquiterpene; *DT: diterpene; *DM: desmethyl.**

Table-3: Chemical composition of blubber from dead stranded blue whale (*Balaenoptera musculus*).

IUPAC Name (numbered in order of elution on HP-5 column)	RT	% Composition*								Class	RI (cal.)*	RI (lit.)*
		HBJ*	CBJ*	HBA*	CBA*	HBP*	CBP*	HBF*	CBF*			
Heptanal (7)	3.91	-	-	-	-	-	-	0.40	-	FAld	919	902 [29]
Hexanoic acid ME* (8)	4.63	-	-	-	-	-	-	0.08	-	SFA	948	936 [30]
1-Chloroheptane (9)	5.71	-	-	-	-	-	-	0.05	-	Poll.	969	962 [31]
1-Heptanol (10)	6.34	-	-	-	-	-	-	0.03	-	FAlc	1011	968 [32]
Hexanoic acid (11)	6.74		-	-				0.03		SFA	1021	1036 [33]
Octanal (12)	7.34	0.31	-	-	-	-	-	0.56	-	FAld	1017	1001 [34]
Heptanoic acid ME (13)	8.19	-	-	-	-	-	-	0.36	-	SFA	1039	1021 [35]
(*E*)-2-Octenal (14)	9.28	-	-	-	-	-	-	0.29	-	FAld	1066	1062 [36]
1-Octanol (15)	9.87	-	-	-	-	-	-	0.06	-	FAlc	1079	1070 [37]
Heptanoic acid (16)	10.18	-	-	-	-	-	-	0.05	-	SFA	1109	1086 [38]
Nonanal (17)	10.85	0.34	-	0.61	-	0.37	-	0.45	-	FAld	1126	1108 [39]
Octanoic acid ME (18)	11.57	-	-	-	-	-	-	0.11	-	SFA	1123	1129 [40]
2-Nonenal (19)	12.60	-	-	-	-	-	-	0.30	-	FAld	1155	1168 [41]
Octanoic acid (20)	13.30	-	-	-	-	-	-	0.13	-	SFA	1175	1170 [42]
Decanal (21)	14.02	-	-	-	-	-	-	0.54	-	FAld	1202	1205 [43]
Nonanoic acid ME (22)	14.63	-	-	-	-	-	-	0.03	-	SFA	1219	1227 [44]
UI-1	14.75	-	-	-	-	-	-	0.21	-	UI	1222	-
(*Z*)-2-Decenal (23)	15.64	0.20	-	-	-	1.12	-	0.62	-	FAld	1240	1254 [45]
Nonanoic acid (24)	16.10	-	-	-	-	-	-	0.59	-	SFA	1253	1267 [46]
Nonanal dimethyl acetal (25)	16.16	0.28	-	-	-		-	0.25	1.11	FAld	1255	[47]
Tridecane (26)	16.72	1.27	1.08	1.13	2.52	0.85	2.16	0.23	1.09	HC	1270	1300 [48]
(*E*)-2-Undecenal (27)	18.43	-		-	-	-	-	0.85	1.88	FAld	1326	1340 [49]
2,6,11-Trimethyl-dodecane (28)	18.45	-	-	0.26	-	0.30	-	-	-	Terp.	1328	1276 [50]
2,6,10-Trimethyl-dodecane (29)	18.76	-	-	0.72	-	-	-	-	1.23	Terp.	1343	1378 [51]
2-Methyl-tridecane (30)	19.29	-	-	0.39	-	-	-	-	0.62	HC	1366	1365 [52]
Tetradecane (31)	19.40	0.79	1.26	1.89	2.53	1.03	2.17	1.58	2.94	HC	1371	1400 [48]
Undecanoic acid ME (32) Co-eluting shoulder with Tetradecane (31)	19.43	-	-	-	-	-	-	0.65	1.88	SFA	1372	1422 [39]
4,8-Dimethyl-tridecane (33)	19.48	-	-	0.29	-	-	-	-	0.95	HC	1374	[53]
Pentadecane (34)	21.91	2.21	3.24	1.95	2.19	2.43	2.24	0.35	1.32	HC	1480	1500 [48]
UI-2	22.13	0.59	-	0.49	-	-	-	0.08	0.34	UI	1497	-
9-oxo-decanoic acid ME (35)	22.40	-	-	-	-	-	-	0.04	0.93	Oxy FA	1507	1484 [54]
UI-3	22.61	-	-	1.91	-	-	1.65	0.58	1.28	UI	1519	-
10-Methyl-dodecanoic acid ME (36)	22.76	-	-	-	-	-	-	0.11	-	Br FA	1522	1575 [55]
2-Methyl-pentadecane (37)	22.98	-	-	-	-	-	1.11	-	-	HC	1531	1564 [56]
UI-4	23.04	-	-	-	-	-	1.02	0.18	1.83	UI	1527	-
7-Methyl-pentadecane (38)	23.25	-	-	0.70	-	-	-	-	0.55	HC	1534	[53]
3-Methyl-pentadecane (39)	23.39	-	-	-	-	0.87	0.52	-	0.53	HC	1542	1566 [57]
UI-5	23.43	0.57	-	0.14	-	0.55	-	0.68	-	UI	1544	-
Diethyl Phthalate (40)	24.05	0.67	6.95	4.96	6.66	5.76	3.90	0.65	5.95	Poll.	1576	1603 [36]
Hexadecane (41)	24.26	1.49	3.30	1.50	3.86	1.94	3.81	1.53	3.21	HC	1585	1600 [48]
UI-6	25.36	1.79	-	-	-	-	1.29	0.23	1.33	UI	1627	-
UI-7	25.69	0.19	-	-	1.91	-	0.52	0.64	1.47	UI	1642	-
Tetradecanol (42)	25.96	0.36	-	-	-	0.37	-	-	-	FAlc	1654	1675 [58]
2-Methyl-hexadecane (43)	26.09	-	-	-	-	0.23	-	-	0.27	HC	1660	1666 [59]
Diphenyl carbonate (44)	26.17	1.87	2.81	1.97	3.57	3.89	3.78	2.25	2.61	Misc.	1663	[60]
Heptadecane (45)	26.49	1.77	0.36	0.76	1.86	0.99	1.43	0.83	1.67	HC	1678	1700 [48]
Norphytane / Pristane (46)	26.62	0.23	-	-	-	1.61	-	-	-	Terp.	1683	1703 [61]
(*Z*)-9-Tetradecenoic acid ME (47)	26.75	1.08	1.21	1.13	1.33	1.11	1.39	1.08	1.38	MUFA	1689	1715 [39]
Tetradecanoic acid ME (48) [62, 63]	27.05	1.42	5.28	5.28	4.83	2.30	4.51	5.54	4.33	SFA	1708	1726 [64]
Tetradecanoic acid (49) [62, 63]	27.79	1.01	3.23	0.57	3.85	0.32	3.11	3.59	4.01	SFA	1742	1765 [65]
2-Methyl-heptadecane (50)	27.84	0.56	-	-	-	0.55	-	-	-	HC	1744	1765 [52]
3-Methyl-heptadecane (51)	28.01	0.18	-	-	-	-	-	-	1.81	HC	1748	1771 [66]
UI-8	28.12	1.91	1.42	0.88	1.61	0.79	2.62	0.74	2.73	UI	1753	-
Octadecane (52)	28.62	1.35	2.41	2.40	3.48	0.66	2.08	0.87	4.78	HC	1776	1800 [48]
UI-9	28.86	3.97	4.54	2.75	4.63	3.59	3.18	2.13	3.83	UI	1787	-
Pentadecanoic acid ME (53) [62]	29.16	-	-	0.12	-	0.11	0.19	-	4.49	SFA	1807	1824 [67]
Octadecamethyl cyclononasiloxane or D9 (54)	29.45	0.24	-	-	-	-	-	-	-	Poll.	1821	1780 [68]
Diisobutyl phthalate (55)	29.91	1.95	1.47	0.12	2.14	0.43	0.78	0.40	0.27	Poll.	1842	1873 [69]
1-Hexadecanol (56)	30.21	1.01	1.49	0.17	1.02	0.17	0.65	0.21	0.21	FAlc	1853	1876 [59]
UI-10	30.35	0.60	0.82	0.08	0.89	0.88	0.86	0.10	0.87	Misc.	1855	-
14-Methyl-pentadecanoic acid ME (57)	30.44	-	-	-	-	-	-	0.06	0.25	Br FA	1865	1884 [70]
Nonadecane (58)	30.65	0.94	1.28	-	1.83	-	0.29	-	0.64	HC	1875	1900 [48]
(*Z*)-9-Hexadecenoic acid ME (59) [62]	30.71	11.73	0.67	9.61	1.89	10.88	0.42	10.83	0.56	MUFA	1878	1886 [71]
Hexadecanoic acid ME (60) [62, 63]	31.16	8.41	1.32	14.82	0.95	12.53	4.21	14.58	2.24	SFA	1905	1927 [29]
(Z)-9-Hexadecenoic acid (61) [62]	31.42	0.37	2.57	0.39	2.03	1.00	1.93	1.03	1.95	MUFA	1918	1911 [34]
Butyl-isobutyl phthalate (62)	31.76	1.41	1.84	-	-	-	0.95	0.38	0.53	Poll.	1934	1944 [72]
Hexadecanoic acid (4) [62, 63]	31.82	1.04	1.19	0.67	1.41	0.97	0.91	1.21	0.41	SFA	1937	1958 [34]
15-Methyl-hexadecanoic acid ME (63) [62]	32.39	-	-	-	-	-	-	0.40	-	Br FA	1963	1990 [68]

Palmitic acid EE (64)	**32.48**	**0.24**	**0.23**	**1.01**	**-**	**0.42**	**0.22**	**0.62**	**0.29**	**SFA**	**1968**	**1991 [73]**
Eicosamethyl cyclodecasiloxane or D10 (65) Co-eluting shoulder with Palmitic acid EE (64)	**32.50**	**-**	**1.17**	**-**	**-**	**-**	**-**	**0.17 ***	**1.11**	**Poll.**	**1969**	**1928 [72]**
Eicosane (66)	**32.59**	**0.52**	**0.69**	**0.54**	**-**	**-**	**0.30**	**0.62**	**0.33**	**HC**	**1973**	**2000 [74]**
Heptadecanoic acid ME (67) [62]	**33.08**	**0.25**	**-**	**-**	**0.96**	**0.89**	**0.18**	**0.76**	**0.11**	**SFA**	**2003**	**2028 [37]**
UI-11	**33.32**	**-**	**1.75**	**-**	**0.70**	**0.29**	**0.82**	**0.43**	**0.95**	**UI**	**2016**	**-**
UI-12	**33.37**	**-**	**2.21**	**-**	**2.37**	**2.23**	**2.48**	**0.08**	**1.02**	**UI**	**2019**	**-**
UI-13	**34.00**	**-**	**1.25**	**-**	**1.68**	**1.23**	**1.36**	**0.61**	**1.79**	**UI**	**2043**	**-**
16-Methyl-heptadecanoic acid ME (68) [62]	**34.16**	**-**	**-**	**-**	**-**	**-**	**-**	**0.20**	**-**	**Br FA**	**2051**	**[75]**
(*Z*)-9-Octadecenoic acid ME (69) [62, 63]	**34.41**	**11.63**	**6.49**	**18.13**	**4.62**	**12.71**	**7.64**	**19.34**	**3.46**	**MUFA**	**2063**	**2085 [37]**
(*E*)-9-Octadecenoic acid ME (70)	**34.52**	**1.00**	**0.68**	**1.15**	**0.61**	**4.80**	**1.32**	**-**	**0.41**	**MUFA**	**2068**	**2109 [39]**
Octadecanoic acid ME (71) [62]	**34.92**	**1.58**	**1.02**	**1.81**	**0.68**	**3.98**	**2.18**	**2.29**	**0.36**	**SFA**	**2088**	**2065 [76]**
(*Z*)-9-Octadecenoic acid (6) [62, 63]	**35.06**	**2.34**	**2.19**	**0.27**	**2.95**	**0.70**	**2.53**	**1.24**	**1.10**	**MUFA**	**2104**	**2111 [53]**
UI-14	**35.31**	**2.18**	**1.09**	**-**	**-**	**-**	**-**	**0.69**	**0.39**	**UI**	**2115**	**-**
Octadecanoic acid (5) [62]	**35.50**	**0.63**	**0.75**	**0.50**	**0.63**	**0.29**	**1.11**	**0.58**	**0.99**	**SFA**	**2123**	**2158 [37]**
4,4'-Bisphenol-A (72) Co-eluting, shoulder with Octadecanoic acid (5)	**35.52**	**0.53**	**0.54**	**0.56**	**0.52**	**0.55**	**0.56**	**0.13**	**0.11**	**Poll.**	**2124**	**2181 [77]**
Oleic acid EE (73)	**35.60**	**-**	**0.09**	**0.27**	**-**	**0.29**	**0.10**	**0.53**	**0.97**	**MUFA**	**2127**	**2168 [78]**
Docosane (74)	**36.20**	**0.53**	**0.45**	**0.19**	**-**	**-**	**-**	**0.27**	**-**	**HC**	**2152**	**2172 [67]**
Nonadecanoic acid ME (75)	**36.66**	**-**	**-**	**1.11**	**-**	**1.33**	**-**	**0.15**	**-**	**SFA**	**2171**	**2152 [76]**
UI-15	**36.81**	**-**	**-**	**1.38**	**-**	**1.88**	**-**	**0.28**	**-**	**UI**	**2178**	**-**
2,4'-Bisphenol A (76)	**37.26**	**0.52**	**0.55**	**0.58**	**-**	**0.61**	**0.20**	**-**	**0.15**	**Poll.**	**2236**	**2181 [77]**
11,14-Eicosadienoic acid (77) [62]	**37.42**	**0.51**	**0.38**	**-**	**-**	**-**	**0.33**	**0.13**	**0.39**	**PUFA**	**2245**	**[75]**
9,10-Epoxy-octadecanoic acid ME (78)	**37.54**	**0.59**	**0.33**	**-**	**-**	**-**	**-**	**0.28**	**0.34**	**Oxy FA**	**2252**	**[79]**
2-Octadecyloxy-ethanol (79)	**37.91**	**4.87**	**3.82**	**1.12**	**2.77**	**1.33**	**5.08**	**1.46**	**1.06**	**Fatty ether**	**2273**	**[80]**
10-Hydroxy-octadecanoic acid ME (80)	**38.28**	**0.41**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**Oxy FA**	**2271**	**[81]**
Eicosanoic acid ME (81) [62]	**38.38**	**0.39**	**0.60**	**0.17**	**0.62**	**0.52**	**1.14**	**0.64**	**0.09**	**SFA**	**2279**	**2237 [76]**
Z-11-Eicosenoic acid ME (82) [62]	**38.77**	**5.30**	**3.80**	**5.04**	**3.04**	**0.07**	**4.54**	**0.66**	**4.03**	**MUFA**	**2308**	**2309 [39]**
Tetracosane (83)	**39.55**	**1.88**	**2.25**	**0.88**	**2.21**	**1.82**	**1.61**	**0.72**	**1.58**	**HC**	**2371**	**2400 [48]**
Pentacosane (84)	**41.12**	**1.33**	**2.81**	**1.18**	**2.85**	**0.81**	**2.29**	**1.05**	**2.12**	**HC**	**2473**	**2500 [48]**
Docosanoic acid ME (85)	**41.59**	**0.38**	**-**	**-**	**0.96**	**-**	**-**	**0.15**	**-**	**SFA**	**2504**	**2524 [76]**
DHEP or DiO phthalate (86)	**41.78**	**0.46**	**0.36**	**-**	**-**	**-**	**0.92**	**0.15**	**-**	**Poll.**	**2516**	**2527 [69]**
Hexacosane (87)	**42.64**	**1.86**	**2.86**	**1.41**	**2.69**	**1.78**	**1.61**	**1.25**	**1.51**	**HC**	**2571**	**2600 [48]**
Heptacosane (88)	**44.11**	**1.12**	**3.21**	**1.09**	**3.27**	**0.58**	**2.51**	**1.16**	**1.92**	**HC**	**2673**	**2700 [48]**
UI-16	**45.04**	**0.48**	**0.55**	**0.29**	**-**	**-**	**-**	**0.22**	**0.28**	**UI**	**2742**	**-**
Octacosane (89)	**45.53**	**1.52**	**3.03**	**1.15**	**2.58**	**0.92**	**1.69**	**1.32**	**1.24**	**HC**	**2782**	**2800 [48]**
Nonacosane (90)	**46.90**	**1.18**	**2.22**	**0.81**	**3.02**	**0.66**	**1.73**	**0.90**	**0.72**	**HC**	**2879**	**2900 [48]**
Triacontane (91)	**48.22**	**1.11**	**2.46**	**0.75**	**3.40**	**0.73**	**1.94**	**1.26**	**0.76**	**HC**	**2990**	**3000 [48]**
Hentriacontane (92)	**49.51**	**0.70**	**0.44**	**-**	**-**	**-**	**-**	**0.21**	**0.19**	**HC**	**3084**	**3100 [48]**

Class wise summary with % concentration in parenthesis.

	Total	**HBJ***	**CBJ***	**HBA***	**CBA***	**HBP***	**CBP***	**HBF***	**CBF***
FA (SFA, Br. FA, MUFA, PUFA etc.)	**34**	**18 (49.31)**	**17(31.70)**	**18 (62.05)**	**16 (31.36)**	**19 (55.22)**	**19 (37.96)**	**32 (67.75)**	**21 (33.70)**
Oxy FA (FAld, FAlc, etc.)	**16**	**8 (3.50)**	**2 (1.82)**	**2 (0.78)**	**1 (1.02)**	**4 (2.03)**	**1 (0.65)**	**14 (4.88)**	**5 (4.47)**
Miscellaneous (HC, Poll., etc.)	**39**	**29 (30.06)**	**26 (52.86)**	**26 (29.30)**	**19 (53.95)**	**24 (31.33)**	**25 (45.66)**	**25 (19.79)**	**30 (43.77)**
Numbers of UI*	**16**	**9 (12.28)**	**8 (13.63)**	**8 (7.92)**	**7 (13.79)**	**8 (11.44)**	**10 (15.80)**	**16 (15.88)**	**13 (18.11)**

***(% composition): calculated using area normalization method; *(cal.): Retention indices calculated; *(lit.): literature values of retention indices; *HB: hexane extract from blubber; *CB: chloroform extract from blubber; *J: jaw; *A: abdomen; *P: peduncle; *F: fluke;*ME: methyl ester; *Br: branched FA; *Oxy: oxygenated FA; *Poll.: Pollutant; *UI: Unidentified; a total of 16 unidentified constituents appeared in GCMS at retention index of 1222, 1497, 1519, 1527, 1544, 1627, 1642, 1753, 1787, 1855, 2016, 2019, 2043, 2115, 2178, and 2742.**

The present study, based on a single individual dead blue whale, is a preliminary report on lipid composition of its blubber layer. The blue whale under study, had severely damaged head, presumably due to hard bang(s) with rocks or to the hull of a large vessel. This might has triggered the release of large amount of adrenalins leading to in-volunteer wagging of tail, leading to activation of cAMP cascade that has resulted in the lipolysis of localized fat storage with high levels of various short chain FAs in tail (fluke) region as compared to rest of the body. This further has caused great surge in the levels of FFAs used for energy production. It is the characteristic of mammals that their metabolism and conversely their metabolites change sharply in response to stress. The washed ashore, stranded dead whale was found decaying, when it was sampled. The inner part being in contact with muscles was found in more decayed form. Therefore, intact outer to middle part of blubber was analysed and blubber was sampled from the skin (outer layer) in to the middle layer, avoiding the deeper (inner) rotting layer attached to the muscular tissues. Therefore, sampled blubbers were not studied into layers as considered in some studies [53, 63, 82] though variability in lipid deposition as well as variability in lipid composition was evident (Table-2).

It is obvious and expected that analysing the lipid of an animals in such state, do results in the production of high amount of transformed FAs in the form of FAlds, Fatty ketones, FAlcs and other oxygenated derivatives. These compounds are oxidative transformation of unsaturated FAs, MUFAs and PUFAs, which are abundantly available in blubber [63]. The degraded compounds are indicators of their precursor's lipid molecules.

The total lipid content of blubber varies in whales occurring at different geographic locations [63]. However, some features seem relatively constant in the few studies reported so far. The mean percentage lipid content (i.e., on wet weight basis) calculated in the present study revealed a non-uniform distribution in blubber (Table-2). Low values were recorded for the jaw and high fat content in the abdomen and peduncle agreed with the values reported previously [83]. However, current findings with low values for abdominal lipids (54.8%) and high values for peduncle lipids (63.1%) are contradicting with a previous study [63], which has reported high lipid content in abdomen (68.9%) and low lipid content in peduncle (57.7%).

TLC analysis showed the presence of cholesterol (**1**), cholesterol acetate (**2**), tripalmitin (**3**), and different waxy constituents in blubber samples when co-TLC with beeswax. Phospholipid (as lecithin) was not detected (Table-1) in analogy to the fin whale blubber; though small amounts of lecithin has been observed in its foetus [83]. Blue whale stores fats mainly in the form of tripalmitin [79]. Waxes have also been found in animals living in the deep waters [85]. Sargent and McIntosh [86] have stated that biosynthesis of waxes appear to be an adaptation to a partly anaerobic environment where oxidative respiration is limited, or a mean of enhancing fatty acid biosynthesis and rate of lipid deposition from an excess of dietary constituents through elimination of the normal respiratory-dependent rate control. The traditional transmission of so-called tracer fatty acids that arise only or majorly from the food into neutral lipids, within the trophic levels has been explained by Lee, Nevenzel, and Paffenhoefer [87], after conducting feeding experiments with copepods.

In case of identification of FFA using co-TLC, lauric, myristic, and erucic acid were not detected, however myristic acid (**48**, **49)** was identified in GC-MS. GC-MS analyses showed variable concentration of different FAs in the descending order in fluke (52%) > abdomen ≥ peduncle (47 - 48%) > jaw (39%) in analogy to earlier report [88]. It is noteworthy to mention that lipids from fluke have been analysed for the first time in this study and the discussed values are average results of the middle to outer layer of blubber.

The variation in the composition of blubber at different body locations is related to the body functions. For instance, insulation and energy storage role in some cetaceans has been suggested for the blubber of thoracic-abdominal area. Similarly, the maintenance of hydrodynamic shape and other locomotory functions have been attributed to the thick ridge posterior to the dorsal fin and caudal peduncle [88]. As suggested previously [10],

understanding of the mechanism of fat utilization and mobilization in the blubber layer would provide further insight into adaptations of blubber structure. High levels of MUFAs and PUFAs play a thermoregulatory role as they do not solidify at low temperatures expected in the deeper waters [89]. Maximum lipid oxidation rate occurs at low exercise intensity in mammals, and at decrease exercise intensity the rate of oxidation increases [90].

High concentration of normal chain SFAs, caproic (**8, 11**), enanthic (**13, 16**), caprylic (**18, 20**), pelargonic (**22, 24**), undecylic acid (**32**), pentadecylic (**53**), palmitic (**4, 60, 64**), margaric (**67**), nonadecylic (**75**), and behenic acid (**85**) were identified in the fluke as these are endogenous FAs [91]. High amount of SFAs, myristic (**48, 49**), **53**, **4, 60, 64**, **67**, stearic (**5, 71**), **75**, arachidic (**81**) and **85** in outer layer at peduncle is the result of no fat mobilization because fat mobilization for energy balance is expected in the inner layer of blubber [88, 92].

Literature search revealed high content of MUFAs in the outer blubber layer at peduncle [54, 82], which is not in agreement with our results. Current study revealed MUFAs in descending order in abdomen ≥ peduncle (26%) > jaw (25) > fluke (24%). But the low concentration of MUFAs myristoleic (**47**), palmitoleic (**59, 61**), oleic (**6, 69, 73**), elaidic (**70**), and gondoic acid (**82**) and branched (Br.) SFAs such as 10-methyl-dodecanoic acid (**36**), *iso*-palmitic (**57**), *iso*-margaric (**63**), and *iso*-stearic acid (**68**) identified in peduncle as compare to jaw and abdomen are in agreement with an earlier study [63] where mobilization of MUFAs and Br. FAs for temperature regulation is suggested.

PUFAs are generally low in the outer blubber layers and their absence in samples is suggestive of the oxidation process taking place in the putrefying whale which is evident from the presence of certain oxidized or metabolized products. Further the chromatographic region in the GC-MS where PUFAs are expected has been found swarmed with high concentration of pollutants specially hydrocarbons, masking their presence, if any. 11,14-Eicosadienoic acid (**77**), identified in current study is also reported from a fin whale [64]. MUFAs and PUFAs are oxidized into secondary degradative products such as, FAlds; enanthaldehyde (**7**), caprylic aldehyde (**12**), (*E*)-2-octenal (**14**), 2-nonenal (**19**), (*Z*)-2-decenal (**23**), and (*E*)-2-undecenal (**27**) and FAlcs; enanthic alcohol (**10**), capryl alcohol (**15**), myristyl alcohol (**42**), and cetyl alcohol (**56**) are identified in the current study [93, 75]. Similarly, low content of oleic acid (**6, 69, 73**) suggests that it may have oxidized into **12**, pelargonaldehyde (**17, 25**), **10**, capraldehyde (**21**) and **23** while Linoleic acid (not identified) may have been oxidized into **14** and **19** and 9-oxo-decanoic acid (**35**) [94, 95].

Many compounds identified in this study seem to have been bio-transferred through the food chain. For example, 10-hydroxy-octadecanoic acid (**81**) is an oxidative product of oleic acid either mediated by bacteria [81] or by auto-oxidation [96]. 2-octadecyloxy ethanol (**79**), although a polyol ether pollutant – used as surfactants in various skin care items, is also reported from diatom *Navicula sp.* **70** from *Thalassiosira weissflogii*, and **7** from an unidentified diatom [80, 97, 98]. Pristane (**46**), a prenol has been identified in various marine organisms. Pristane is produced by zooplanktons or other organisms. Relatively large quantities of pristane have been identified in genus *Calanus* zooplanktons, which are frequently consumed by the filter feeding whales [99]. 9,10-epoxy-octadecanoic acid (**78**) present in the marine microalgae could have also been transferred through food [79]. 2,6,11-trimethyl dodecane (**28**), 2,6,10-trimethyl dodecane (**29**), and 4,8-dimethyl tridecane (**33**) are other natural prenols, which could have also found their way in the blubber through food chain.

Presence of various hydrocarbons, including tridecane (**26**), 2-methyl tridecane (**30**), tetradecane (**31**), pentadecane (**34**), 2-methyl pentadecane (**37**), 7-methyl pentadecane (**38**), 3-methyl pentadecane (**39**), hexadecane (**41**), 2-methyl hexadecane (**43**), heptadecane (**45**), 2-methyl heptadecane (**50**), 3-methyl heptadecane (**51**), octadecane (**52**), nonadecane (**58**), eicosane (**66**), docosane (**74**), tetracosane (**83**), pentacosane (**84**), hexacosane (**87**), heptacosane (**88**), octacosane (**89**), nonacosane (**90**), triacontane (**91**), hentriacontane (**92**) can be justified either by their biosyntheses through decarboxylative elongation of fatty acids, resulting in hydrocarbons with one carbon less than the precursor fatty acid [100] or as anthropogenic bioaccumulated pollutant. The

elevated concentrations of hydrocarbons are supporting the later argumentation.

The pollutants introduced in the marine ecosystem from the anthropogenic activities generally accumulate in marine mammals [66, 101]. The blubber was found to contain plasticizers (phthalates) such as; diethyl phthalate (**40**), diisobutyl phthalate (**55**), butyl-isobutyl phthalate (**62**) and di-ethylhexyl phthalate (**86**) and silicone softners; octadecamethyl cyclononasiloxane D9 (**54**) and eicosamethyl cyclodecasiloxane D10 (**65**). Diphenyl carbonate (**44**), a bio-degradative product of polycarbonates (thermoplastic polymer) [62], found in this study may also have been bio-accumulated from the environment. In the marine environment plastic debris are persistent and durable. It degrades and crushed into smaller micro- and nanoplastic. These latter items could, in turn accumulates in the ecosystem and biota and release plastic-derived chemicals, such as endocrine disrupting chemicals bisphenol A (**72**, **76**), which were also observed in current GC-MS analyses. Furthermore, microplastics are able to metabolise into other molecules which impact marine food webs through bioaccumulation, biotransfer and biomagnification in organisms at higher trophic level [102]. 1-Chloroheptane (**9**), another identified compound, is included in the list of marine pollutant [103].

In the current study total lipid content from four different location of body site are reported. Different lipid classes such as cholesterol, TAG and wax are detected. Concentration of FAs differs with respect to body location such as jaw, abdomen, peduncle and fluke according to their need in the body parts to perform different functions. Not PUFA but their oxidation products are found. Compounds, such as, FAld, FAlc, Alkanes and others show the progression of putrefying process in the blubber. A few compounds appeared to have been transferred through the food chain. Presence of hydrocarbons and other pollutants in the blubber fat layer is alarming with respect to the environmental pollution and the health of the organisms in this general area.

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