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Levels and Source of Aliphatic Hydrocarbons in Marine Fishes from Coast of Iraq Based on Biomarkers and Biogeochemical Indices

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Abstract: The total n-alkane concentrations in the fish tissue samples were 9.14 µg g^{-1} Dwt, and the Pr and Ph concentrations were 0.22 and 0.85 µg g^{-1} Dwt, respectively. The CPI (carbon preference index) values ranged from 0.76 to 2.01 and the Pr/Ph ratios were 0.16-1.40. The fraction of n-alkanes in the tissue samples from petroleum sources was estimated to be 83%, whereas the fraction from natural biogenic sources (marine algae, bacteria, and terrestrial plants) was 17%. The application of biomarker indices such CPI and Pr/Ph ratios indicated that AHs were mainly a mixture of anthropogenic and natural biogenic sources. The $CPI_{(o/e)}$ values signified that petroleum was a major sources of AHs in these fishes. The Pr/Ph ratios suggested different sources, including fossil fuel and petroleum by-products, as well as biological and chemical alteration under redox/anoxic conditions. The presence of a high fraction of n-alkanes from petroleum and crude oil sources in the tissues of these fishes indicated that their nursing habitats were critically contaminated by petroleum hydrocarbons. The results of this study suggest that further researches are needed to study the bioaccumulation of other organic pollutants, such as pesticides, plasticizers, and polycyclic aromatic hydrocarbons in the marine biota of the region.

Keywords: Aliphatic hydrocarbons, n-Alkanes, Isoprenoids, Marine fish, Iraq

Aliphatic hydrocarbons (AHs) and polycyclic aromatic hydrocarbons (PAHs) are among the most ubiquitous organic compounds in different environments (Hasanuzzaman et al 2007, Dach and Méjanelle 2010). AHs (i.e., n-alkanes, isoalkanes, cycloalkanes, terpenes, hopanes, and steranes) are derived from both biogenic and anthropogenic sources (Rushdi et al 2006, 2010, Duan et al 2011) and are the major constituents of crude oil and refined fossil fuel. AHs, mainly n-alkanes, are introduced to the aquatic environment by different natural (flora, fauna, and bacteria) and anthropogenic sources via atmospheric dust, runoff, spillages, industrial and sewage effluents, shipping activities, and natural oil seepage (Rushdi et al 2018, 2019, Huguet et al 2019, Appolinario et al 2020) as well as by organic matter biodegradation (Rushdi et al 2018). Contamination of the aquatic ecosystems by petroleum hydrocarbons is a widespread problem (Ite et al 2018). Petroleum hydrocarbons contain high amounts of n-alkanes with no predominance odd-to-even numbers of carbon atoms (Rushdi et al 2014, 2017), and a large amount of aromatic and heavy asphaltenic substances. The organisms mostly contain n-alkanes with odd numbers of carbon atoms (Jeng 2006, Nott et al 2000, Bush and McInerney 2013), least amounts of aromatic compounds, and no asphaltenic substances (Wang et al 2010, Ali et al 2013, Taheri-Shakib et al 2018).

Iraq is one of the major oil producers in the OPEC (Organization of the Petroleum Exporting Countries) after the Kingdom of Saudi Arabia, and 90% of its crude oil production is from onshore oil fields in the southern part of the country (USEIA 2019). The city of Basrah is located in the southern part of Iraq, where many oil operations were established after 2003 by many international oil companies. Consequently, certain concentration levels of petroleum hydrocarbons have been discharged into the environment as a result of these oil-related developmental activities such as industrial factories, electric power stations, and gas production plants (Al-Saad et al 2015). Recent studies have reported significant levels of petroleum hydrocarbons in water and sediment samples from the area (Rushdi et al 2018, Kadhim et al 2019). Few pieces of researches have studied the bioaccumulation of petroleum hydrocarbon (mainly, polycyclic aromatic hydrocarbons and organo-chlorinated compounds) in marine biota from the region (De Mora et al 2010, Ashraf and Mian 2010, Al-Khion et al 2016). Fish species can accumulate petroleum hydrocarbon compounds within their bodies with higher concentrations than in their outer surroundings, which may become a serious human health problem and thus, directly reach the human being (Ramalhosa et al 2012). Almost all studies reported the levels of hydrocarbon compounds in different aquatic species without investigating their other possible sources. Thus, the present work aims to

measure the levels and determine the most important sources of aliphatic hydrocarbons in common commercial fishes from the Iraqi marine waters, based on the application of aliphatic hydrocarbon biomarkers and their geochemical indices.

MATERIAL AND METHODS

Collection of fish and tissue samples: Fourteen commercial marine fish species were collected from the Iraqi offshore water in 2016 (Table 1) and all were carnivore fishes except two filter feeders (*Pl. subviridis* and *T. ilisha*). Their living habitats varied from surface water (*Ch. dorab, S. commersonnianus, S. lysan, Tenualosa ilisha, T. ilisha, Ab. hians*, and *El. eleutheronema*) to bottom water (*Br. orientalis, Ac. arabicus, Ep. coioides, O. ruber, Al. diedaba, Pa. niger, Pa.* and *argentetus*). The tissues of the fish samples were pooled and macerated in a food liquidizer, from which at least three replicates of 15g each were freeze-dried, grounded, and sieved through a 63 µ metal sieve for chemical analyses.

Tissue extraction and separation*:* The procedure described by AL-Saad (1995) was used to extract hydrocarbons from the fishes tissues. Ten grams of each dried tissue were placed in a pre-extracted cellulose thimble and Soxhlet extracted with 150ml methanol: benzene (1:1 v:v) mixture for 24 hours. Each extract was then transferred into a storage flask and further extracted with a fresh solvent mixture. The combined extracts were reduced in volume to 10ml in a rotary vacuum evaporator. They were then saponified for 2 hours with a solution of 4N KOH in 1:1 methanol: benzene. After the extraction of the unsaponified matter with hexane, the extract was dried over anhydrous Na_2SO_4 and concentrated by a stream of N_2. Each concentrated extract was cleaned-up by column chromatography. A column filled with 8g of each of deactivated silica gel (100-200 mesh) at the bottom and alumina (100-200 mesh) on the top. The extract was then delivered at the top of the column and eluted with 50 ml of n-hexane to separate the aliphatic fraction. The fraction was reduced to a suitable volume (1ml) before analysis by capillary gas chromatography.

Samples analysis: An aliquot of 1 µl of each aliphatic hydrocarbon extract was subjected to analyses by an Agilent capillary gas chromatography with a flame ionization detector (FID). A column (model Agilent 19091J-101 HP-5 5% phenyl Methyl silicone; 50 m. x 200µm x 0.33 µm dimensions) was used for the aliphatic separation with helium as a gas carrier at a flow rate of 1.5 ml/minute. The operating temperatures were 280°C and 310°C for the injector and detector, respectively. The temperature of the column was held at 35°C for 10 minutes as initial temperature and then increased at 5°C $minute^{-1}$ to 300°C for 17 minutes. The individual of aliphatic hydrocarbons was identified based on the retention time of an authentic mixed standard procured from Supelco, USA. The concentrations of aliphatic compounds were calculated based on the standard calibration curve of corresponding standard compounds. The range of recovery assays for standards compounds was 80

Table 1. Feeding habits, food sources, feeding habitats, total weight and total length of fish species from Iraqi marine offshore water

Species No.	Fish species	Common name	Feeding habits	Food sources	Feeding habitats	Total weight (g)	Total length (cm)
1	*Brachirus orientalis*	Lisan	Carnivores	Crustaceans	Bottom	480	30
2	*Acanthopagrus arabicus*	Shanak	Carnivores	Fish and Shrimp	Bottom	410	23
3	*Epinephelus coioides*	Hamur	Carnivores	Fish and Crustaceans	Bottom	1152	61
4	*Chirocentrus dorab*	Hiff	Carnivores	Fish and Shrimp	Water surface	819	75
5	*Scomberoides commersonnianus*	Dhala	Carnivores	Fish and Shrimp	Water surface	1214	80
6	*Scomberoides lysan*	Khubbat	Carnivores	Fish and Crustaceans	Water surface	408	25
7	*Otolithes ruber*	Nuwaiby	Carnivores	Fish and Shrimp	Bottom	517	33
8	*Alepes diedaba*	Hammam	Carnivores	Crustaceans	Bottom	219	22
9	*Tenualosa ilisha*	Sboor	Filter feeder	Zoo and Phytoplankton	Water surface	420	30
10	*Parastromateus niger*	Halwayuh	Carnivores	Phyto and Zooplankton	Bottom	333	23
11	*Pampus argentetus*	Zubaidy	Carnivores	Zoo benthos and Crustaceans	Bottom	310	22
12	*Ablennws hians*	Musaffaha	Carnivores	Fish	Water surface	1350	79
13	*Eleutheronema Eleutheronema*	Cheem	Carnivores	Fish and Crustaceans	Water surface	837	43
14	*Planiliza subviridis*	Biah	Filter feeder	Phyto- and Zooplankton	Water surface	112	18

to 92%. The standard deviation for the method was less than 10% based on the replicate analysis. Great care was taken to avoid contamination of the samples throughout the analytical procedure. All solvents were distilled twice before use; glassware was rinsed with distilled water and heated in an oven at 250°C for 24 hours. However, procedural blanks consisting of all reagents and glassware used during the analysis were periodically determined, which had shown no detectable interference.

Statistical analysis: The software SPSS 16.0 (IBM-Statistical Package for Social Sciences, version 16.0) was used to statistically analyze the data. The normal distribution of the data was utilized for all statistical analyses. Varimax rotation with Kaiser Normalization was applied for principal component analysis and Ward's method and Squared Euclidean distance for cluster analysis. The relationships between different variables were defined by Pearson's correlation.

RESULTS AND DISCUSSION

Aliphatic hydrocarbon levels, distribution, and characteristics: The concentrations of total n-alkanes compounds and isoprenoids, mainly pristane (Pr) and phytane (Ph), measured in the tissues of various marine fish species from Iraq (Table 2). The carbon chain length of n-alkanes in the fish tissues ranged from nC_{13} to $nC_{36,}$ as shown in (Fig. 1). The total concentrations of n-alkanes varied from 3.99 µg/g Dwt in the tissue of *Al. diedaba* to 17.95 µg/g Dwt in the *Pl. subviridis*. The maximum carbon number (C_{max}) of n-alkanes were at nC_{17} (*Ep. coioides*), nC_{24} (*Br. orientalis; Ch. dorab; Par. s niger, Pam. argentetus; El. eleutheronema*), nC_{26} (*Al. diedaba*), nC_{28} (*S. lysan*); and nC_{29} (*Ac. arabicus S. commersonnianus, O. ruber, Ab. hians, Pl. subviridis, T. ilisha*) (Table 1 and Fig. 1). The concentrations of Pr and Ph were ranging from 0.04 µg/g Dwt in *Eu. orientalis* to 0.483 µg/g Dwt in *Ep. coioides* and from 0.092 µg/g Dwt in *S. lysan* to 0.454µg/g Dwt in *T. ilisha*, respectively (Table 3).

The total concentrations of aliphatic hydrocarbons were slightly lower than the concentrations measured in different fish species from the same region (Al-Saad 1990). The total n-alkanes concentrations in these species tissues were fairly high compared to the concentrations reported in fishes from different parts of the Arabian Gulf and the Gulf of Oman (Tolosa et al 2005, De Mora et al 2010) and were relatively higher than the concentrations measured in marine sediments and bivalves from the region (Tolosa et al 2005, De Mora et al 2010, Bemanikharanagh et al 2017, Rushdi 2020). The n-alkanes concentrations of these fishes were similar to that from the coasts of Kuwait, Saudi Arabia, Bahrain, and Oman (De Mora et al 2010) and lower than the levels measured in coral reefs from the coast of Iran (Jafarabadi et al 2018). The isoprenoids (pristane and

Table 2. Different parameters and indices of aliphatic hydrocarbons, the concentrations, and fractions of their various sources measured in different marine fish species from Iraq

Compound	*Br. orientalis*	*Ac. arabicus*	*Ep. coioides*	*Ch. dorab*	*S. commers-onnianus*	*S. lysan*	*O. ruber*	*Al. diedaba*	*Par. niger*	*Pam. Argentetus*	*Ab. hians*	*El. eleuther onema*	*Pl. subviridis*	*T. ilisha*	Average	SD
	Carnivores												Filter feeder			
C_{max}	24	29	17	24	29	28	29	26	24	24	29	24	29	29		
CPI (o/e, 14)	0.61	0.71	1.11	0.63	0.81	1.54	0.70	1.10	0.76	0.69	0.54	0.74	2.21	0.69	0.92	0.46
CPI(o/e,25)	0.89	1.79	0.69	1.05	1.45	1.09	1.28	1.34	1.09	1.25	1.70	1.04	1.22	1.36	1.23	0.29
CPI (o/e, E)	0.76	1.25	0.87	0.80	1.05	1.24	0.98	1.24	0.88	0.92	1.16	0.87	2.01	1.02	1.08	0.32
HMW/LMW	0.75	0.61	0.91	1.18	1.21	0.60	0.77	0.64	1.50	1.06	0.49	1.14	5.68	0.73	1.23	1.31
Pr/Ph	0.16	1.40	1.36	0.63	1.27	1.39	0.76	0.86	1.13	0.85	0.73	0.35	0.38	0.56	0.85	0.41
Higher plant n-alkanes	0.042	1.375	0.000	0.012	0.802	1.452	0.812	0.595	0.000	0.432	1.081	0.042	0.149	0.978	0.56	0.53
Per cent	0.6	13.9	0.0	0.3	7.7	10.0	9.1	14.9	0.0	5.2	16.0	0.5	0.8	9.3	6.30	6.02
Microbial n-alkanes	0.471	0.385	0.150	0.425	0.675	0.276	0.574	0.031	0.455	0.547	0.522	0.492	0.396	0.576	0.43	0.17
Per cent	7.2	3.9	1.5	9.9	6.5	1.9	6.4	0.8	6.0	6.5	7.7	5.9	2.2	5.5	5.13	2.67
Algal n-alkanes	0.000	0.119	0.761	0.023	0.208	1.866	0.041	0.128	0.116	0.028	0.040	0.056	7.684	0.005	0.79	2.05
Per cent	0.0	1.2	7.8	0.5	2.0	12.8	0.5	3.2	1.5	0.3	0.6	0.7	42.8	0.0	5.3	11.4
Petroleum n-alkanes	6.033	8.025	8.882	3.836	8.757	10.987	7.517	3.235	7.058	7.376	5.105	7.680	9.725	8.961	7.37	2.19
Per cent	92.2	81.0	90.7	89.3	83.9	75.4	84.1	81.1	92.5	88.0	75.7	92.9	54.2	85.2	83.28	10.20

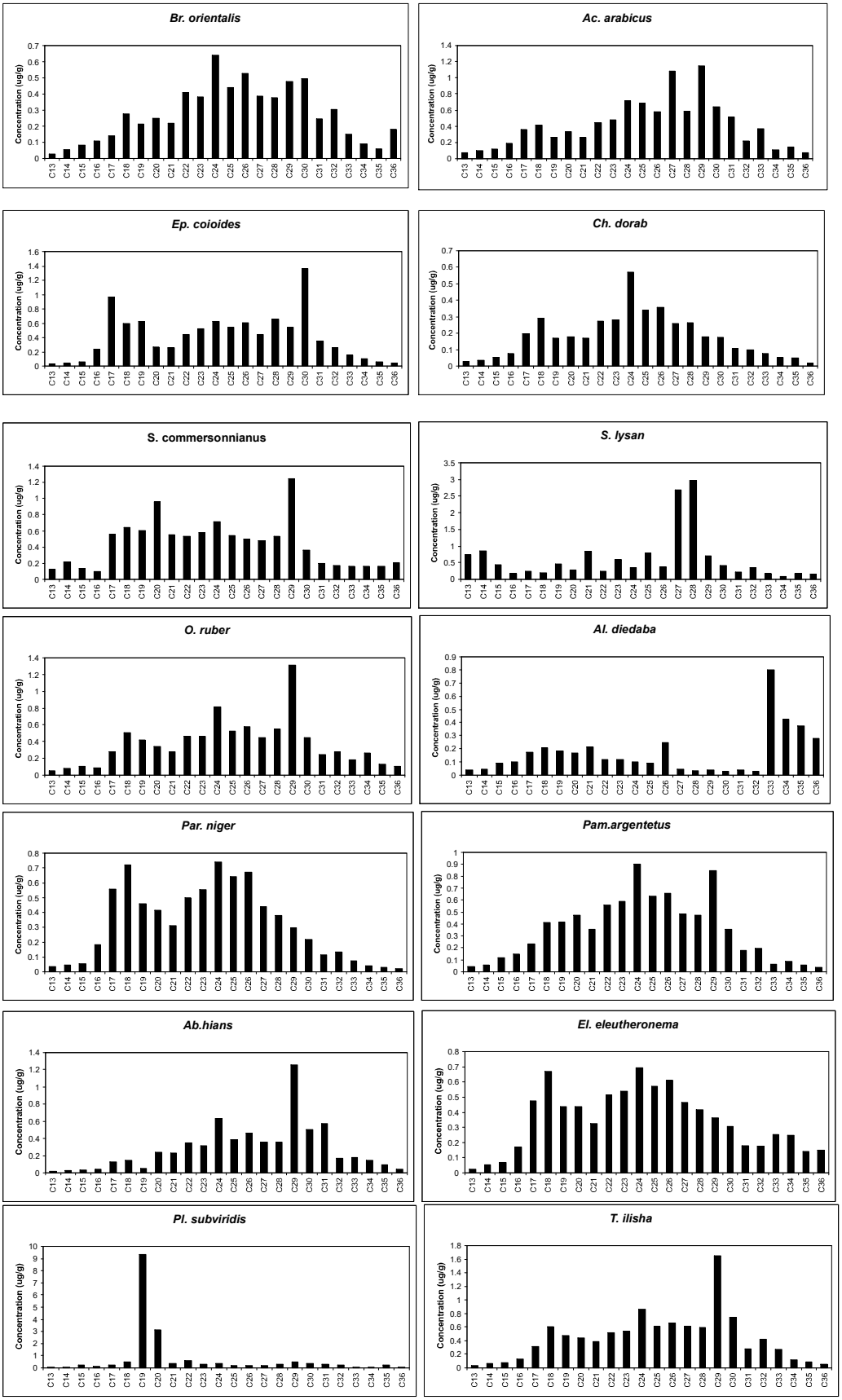

Fig. 1. Distribution and concentrations of n-alkanes in the tissue samples of the different marine fish species from Iraq

Table 3. Concentrations of n-alkanes and isopernoid pristans and phytanes (µg/g dry weight) in the tissues of marine fish species from Iraq

Composition	*M.W.*	*Br. Orientalis*	*Ac. arabicus*	*Ep. coioides*	*Ch. dorab*	*S. commers-onnianus*	*S. lysan*	*O. ruber*	*Al. diedaba*	*Par. niger*	*Pam. argentetus*	*Ab. hians*	*El. eleutheronema*	*Pl. subviridis*	*T. ilisha*
		Carnivores												Filter feeder	
$C_{13}H_{28}$	184	0.027	0.069	0.035	0.027	0.123	0.750	0.052	0.041	0.034	0.044	0.019	0.025	0.046	0.037
$C_{14}H_{30}$	198	0.055	0.098	0.044	0.037	0.214	0.867	0.075	0.046	0.044	0.056	0.024	0.051	0.061	0.065
$C_{15}H_{32}$	212	0.083	0.116	0.061	0.053	0.140	0.433	0.108	0.089	0.056	0.115	0.034	0.068	0.244	0.074
$C_{16}H_{34}$	226	0.111	0.188	0.236	0.075	0.101	0.187	0.089	0.099	0.183	0.150	0.043	0.170	0.137	0.130
$C_{17}H_{36}$	240	0.139	0.363	0.966	0.198	0.563	0.247	0.278	0.171	0.557	0.235	0.127	0.475	0.231	0.311
$C_{18}H_{38}$	256	0.278	0.414	0.593	0.292	0.641	0.201	0.510	0.208	0.723	0.412	0.146	0.667	0.485	0.606
$C_{19}H_{40}$	268	0.213	0.260	0.628	0.171	0.609	0.469	0.418	0.185	0.457	0.419	0.048	0.436	9.319	0.469
$C_{20}H_{42}$	282	0.249	0.337	0.271	0.179	0.959	0.296	0.337	0.168	0.414	0.474	0.240	0.437	3.106	0.437
$C_{21}H_{44}$	296	0.218	0.266	0.253	0.169	0.552	0.837	0.277	0.215	0.311	0.358	0.227	0.326	0.367	0.383
$C_{22}H_{46}$	310	0.407	0.439	0.443	0.272	0.531	0.253	0.460	0.120	0.498	0.559	0.354	0.513	0.628	0.514
$C_{23}H_{48}$	324	0.382	0.478	0.521	0.283	0.576	0.588	0.461	0.116	0.554	0.587	0.318	0.536	0.298	0.540
$C_{24}H_{50}$	338	0.640	0.716	0.625	0.5722	0.716	0.348	0.812	0.103	0.741	0.899	0.631	0.692	0.346	0.862
$C_{25}H_{52}$	352	0.442	0.682	0.541	0.337	0.539	0.787	0.522	0.088	0.642	0.631	0.386	0.573	0.193	0.611
$C_{26}H_{54}$	366	0.528	0.574	0.610	0.355	0.495	0.374	0.574	0.244	0.671	0.658	0.463	0.612	0.177	0.653
$C_{27}H_{56}$	380	0.387	1.087	0.438	0.259	0.482	2.695	0.444	0.044	0.439	0.487	0.361	0.466	0.200	0.616
$C_{28}H_{58}$	394	0.377	0.583	0.654	0.263	0.535	2.964	0.553	0.036	0.381	0.474	0.359	0.417	0.316	0.594
$C_{29}H_{60}$	408	0.478	1.151	0.543	0.179	1.248	0.712	1.311	0.039	0.295	0.848	1.253	0.362	0.467	1.645
$C_{30}H_{62}$	422	0.495	0.643	1.367	0.172	0.358	0.423	0.446	0.029	0.215	0.358	0.505	0.305	0.347	0.749
$C_{31}H_{64}$	436	0.247	0.513	0.347	0.106	0.197	0.211	0.248	0.041	0.116	0.180	0.574	0.181	0.321	0.275
$C_{32}H_{66}$	450	0.304	0.218	0.253	0.096	0.169	0.345	0.283	0.026	0.133	0.199	0.173	0.173	0.268	0.418
$C_{33}H_{68}$	464	0.152	0.374	0.157	0.077	0.163	0.179	0.180	0.800	0.073	0.061	0.182	0.250	0.075	0.272
$C_{34}H_{70}$	478	0.091	0.112	0.098	0.055	0.160	0.082	0.260	0.424	0.040	0.085	0.142	0.245	0.056	0.117
$C_{35}H_{72}$	492	0.060	0.149	0.063	0.048	0.159	0.174	0.135	0.376	0.032	0.057	0.097	0.140	0.220	0.087
$C_{36}H_{74}$	506	0.182	0.074	0.045	0.020	0.211	0.158	0.109	0.280	0.019	0.037	0.041	0.149	0.046	0.054
Total		6.545	9.904	9.792	4.295	10.441	14.580	8.942	3.988	7.628	8.383	6.747	8.269	17.954	10.519
Pristane		0.041	0.29	0.483	0.109	0.422	0.128	0.225	0.128	0.418	0.214	0.091	0.086	0.124	0.252
Phytane		0.25	0.207	0.356	0.172	0.333	0.092	0.296	0.149	0.369	0.251	0.124	0.246	0.33	0.454

phytane) were comparatively similar to the concentrations measured in fish species from the same coastal zone (Al-Saad, 1990) and higher than the values measured in sediments of the same area (Rushdi et al 2014), but lower than the levels measured in sediments from the coast of Saudi Arabia (De Oteyza and Grimalt 2006). A recent study on the lipid sources in sediments from the Iraqi and Saudi Arabian Gulf coasts showed that the aliphatic hydrocarbons from petroleum inputs in the coastal zone of Iraq were higher than the coast of Saudi Arabia (Rushdi 2020). This high input of hydrocarbons, as a result of petroleum product discharge in coastal water, would influence the feeding habitats of the aquatic biota of the area, including fish species, leading to some contribution of petroleum AH in their tissues. Therefore, the variations of AH concentrations and sources in these fish species could be attributed to the feeding habitats, locations (surface versus bottom water), the major sources of food, and their contamination conditions.

Aliphatic hydrocarbon sources: Biogenic and anthropogenic as well as biogeochemical processes, including alteration and diagenesis of organic matter in the water column and bottom sediments, are the main sources of aliphatic hydrocarbon in aquatic environments (Rushdi et al 2018). These hydrocarbons will, ultimately, accumulate in the tissues of marine organisms including, fish species, as long as they are present in their nursing habitats. The occurrence of nC_{17}, nC_{18}, and nC_{19} n-alkanes has been attributed to algal and bacterial sources. Talal (2008) pointed out that the high value of the odd carbon number chain nC_{17} was due to sulfuric reducer bacteria (*Desulfovibrio desulforicans*) presence in the sediments, while the high value of nC_{19} was attributed to the algal sources. The presence of odd carbon numbers of high molecular weight n-alkanes (e.g., nC_{25}, nC_{27}, and nC_{29}) in the environment is mainly a result of the higher plant tissue decomposition (Ficken et al 2000). Therefore, carbon preference index and pristane/phytane ratio (Pr/Ph) have been used to define the sources of n-alkane compounds in the environment. $CPI_{(o/e)} > 1$ indicates a biogenic origin of n-alkanes from plants (Rushdi et al 2006, 2019, Fagbot and Olanipekun 2013, Diefendorf et al 2014).

The C_{max} of the fish tissue n-alkanes, was mainly at nC_{17}, nC_{24}, nC_{26}, and nC_{28} and nC_{29} (Table 1), indicating that the sources were a mixture from marine biota including algae and bacteria, petroleum input, and higher plant waxes (Rushdi et al 2006; 2018). The $CPI_{(o/e)}$, which has been used to assess the contribution of biogenic versus anthropogenic inputs can be divided into $CPI_{(o/e, \leq 24)}$, (nC_{13}-nC_{24}, for marine inputs), and $CPI_{(o/e, \leq 25)}$ (C_{25}-C_{36}, for a higher plant wax contribution) as well as $CPI_{(o/e, E)}$, (for the entire range) (Rushdi et al 2017). The $CPI_{(o/e, \leq 24)}$ values varied from 0.54 to 2.21 with an average value of 0.92±0.46 (Table 1), indicating a mixture of both marine plants, bacterial residues, and major petroleum sources of these aliphatic hydrocarbons. The $CPI_{(o/e, \leq 25)}$ ranged from 0.69 to 1.79, demonstrating that the n-alkanes are derived from a small fraction of higher plant waxes with high contribution from petroleum sources. The $CPI_{(o/e, E)}$ values of these n-alkanes varied from 0.76 to 2.01, also confirm a mixture of minor natural and major anthropogenic sources. Another useful indicator of the n-alkanes origin in the environment is the ratio of pristane-to-phytane (Pr/Ph) isoprenoids (Commendatore and Esteves 2004, Peters et al 2005). When the Pr/Ph ratio is more than 1.0, then their major source is biogenic; and when the Pr/Ph ~1.0, then the source is of petroleum origin. If the ratio is 1.0-0.8 or < 0.8, then it is due to their alteration under depositional redox or anoxic conditions, respectively. The Pr/Ph ratios in the fish tissues ranged from 0.16 to 1.4 with an average e of 0.85 (Table 2.), indicating that they were from different sources, including biological and petroleum inputs as well as from the alteration process under redox/anoxic conditions. To estimate the relative input from different sources, method described by Simoneit et al (1991) for terrestrial plant wax n-alkanes (i.e., C_{27}, C_{29}, C_{31}, C_{33}) and applied it to estimate the n-alkane contribution of marine algae (C_{15}, C_{17}, and C_{19}) and bacteria (C_{16}, C_{18}, and C_{20}) (Rushdi et al 2017). The levels of higher plant, microbial, and algal n-alkanes were estimated to range from 0.00 to 1.081, 0.031 to 0.576 and 0.00 to 7.684 µg/g Dwt respectively (Table 2). The concentrations of n-alkanes from petroleum sources were dominant and ranged from 3.235 to 10.987 µg/g Dwt. The fractions of petroleum n-alkanes in the fish species tissues ranged from 54% to 93 with an average of 83 (Table 2), whereas the biogenic n-alkane percentages ranged from 0 to 43% from marine algae, 1 to 10% from bacteria, and from 0 to 16% from higher plants. This indicates that petroleum by-products are the major sources of n-alkanes in the tissues of these fishes, with minor contributions from natural terrestrial plants, marine algae and bacteria. The fractions of the main three n-alkane origins (e.g., marine biota, higher plants, and petroleum) determined in the fish species tissues of carnivores and filter feeders (Fig. 2).

The petroleum n-alkanes in the tissues of carnivores (67-93%) were slightly higher than in the tissues of the filter feeders (54-85%) The higher plant n-alkanes were slightly higher in carnivores (0-16%) than in filter feeders (1-9%), and the marine biogenic n-alkanes were higher in filter feeders (6-45%) than the carnivores (4-15%).This indicates that feeding habits may also influence the accumulation of aliphatic hydrocarbons in fish tissues. Other factors that affect the accumulation and depuration of hydrocarbons in fish tissues

include route and duration of exposure, the lipid content of tissues, differences in species, age, and sex, and exposure to other xenobiotics (Johnson-Restrep et al 2008, Al-Ali et al 2016). The n-alkanes compounds were most likely added to the fish species tissues via the digestion processes of contaminated phytoplankton, zooplankton, crustacean, and higher plant or directly from solution or suspended particles, and eventually accumulated in the tissues of the species (Al-Saad et al 2011, Wang et al 2019).

Statistical analyses and AH similarity among different fish species*:* Principal component analysis (PCA) is generally applied to reveal the similarity and variation patterns among various parameters and variables in a group of samples. The PCA analysis of the aliphatic hydrocarbons in the fish tissues (Eigen value > 1.0) recognized two major components (Table 4).

Factor loadings of > 0.96 for variables were used for interpretation and, 98.54% of the total variance extracted two principal components (PC1 and PC2). PC1 revealed 96.99% of the variance with tissue samples of all fishes as major factors, except *P. subviridis*, (Fig. 3). Thus, PC1 represented an important source and mainly from anthropogenic inputs, was controlling the aliphatic hydrocarbons in all carnivore fish species and the filter feeder *T. ilisha*. This was shown by the high fractions of total n-alkanes (75.4-93.2%) from petroleum sources and low fractions (0.0-14.9%) from biogenic sources, including higher plants and marine algae and bacteria. PC2 explained only 1.63% of the total variance showing a significant factor loading for the filter feeder *P. subviridis* (surface water), and it was a result of different prevailing aliphatic hydrocarbon sources. The dominant contributors of n-alkanes in *P. subviridis* included petroleum (54.2%) and marine algae (42.8%) sources.

The cluster analysis (CA) results showed a similar outcome to the PCA, where generally two clusters (A and B) are shown in the dendrogram (Fig. 4). Cluster (A) included all the fish species, confirming that the dominant aliphatic hydrocarbons were of similar sources, which were mainly of petroleum origin. Cluster (B) included only *Pl. subviridis*, validating that other prevailing aliphatic hydrocarbons

Table 4. Principal component analysis (PCA) results illustrating the relative loadings of aliphatic hydrocarbons of the fish tissue samples

	CP1	CP2
Br. orientalis	.862	.504
Ac. arabicus	.869	.491
Ep. coioides	.840	.529
Ch. dorab	.856	.510
S. commers-onnianus	.837	.542
S. lysan	.846	.481
O. ruber	.857	.511
Al. diedaba	.804	.534
Par.s niger	.840	.533
Pam.argentetus	.852	.519
Ab.hians	.876	.467
El. Sextarius	.844	.532
Pl. subviridis	.501	.865
T. ilisha	.858	.507
Total variance explained (%)	96.991	1.626

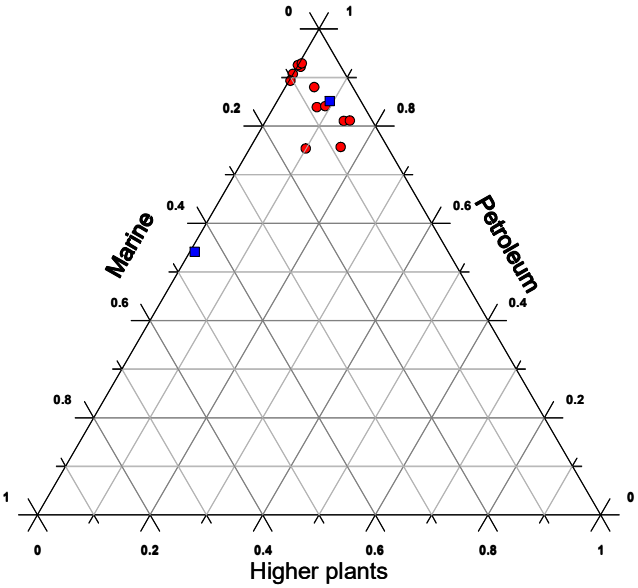

Fig. 2. A ternary diagram showing the aliphatic hydrocarbon n-alkanefractions from petroleum, marine and higher plant sources (circles = carnivores, rectangle = filter feeder)

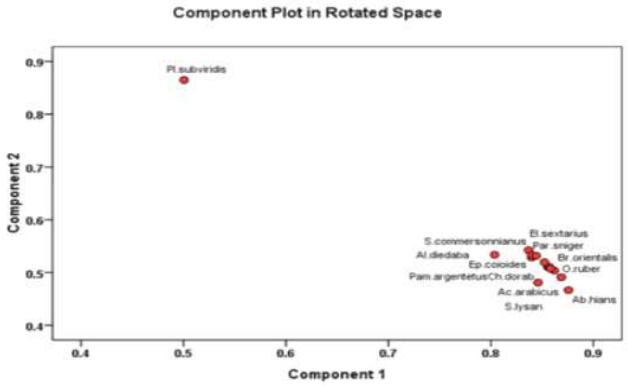

Fig. 3. A plot showing the principal component analysis (PCA) statistical outputs for the aliphatic hydrocarbon n-alkanes in the fish species tissues from Iraq

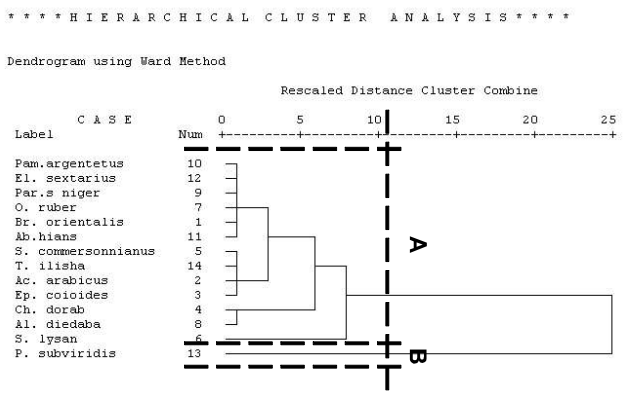

Fig. 4. A plot showing the cluster analysis statistical outputs of the aliphatic hydrocarbons in the tissues of fish species from Iraq

Table 5. Pearson's correlations of the different sources of n-alkanes in the various marine fish tissues from Iraq

	n-Alkanes	Higher plant	Algae	Bacteria	Petroleum
n-Alkanes	1	0.246	0.788**	0.085	0.885**
Higher plant		1	-0.127	0.089	0.284
Algae			1	-0.153	0.440
Bacteria				1	0.185
Petroleum					1

N = 14, **. Correlation is significant at the 0.01 level (2-tailed)., *. Correlation is significant at the 0.05 level (2-tailed)

(mainly biogenic) were significant sources of n-alkanes in its tissue.

Generally, the results showed that the major sources of n-alkanes in the tissues of these fish species were mainly from crude oil. Pearson's correlation coefficient (r), showed that there was a significant correlation (< 0.001) between total n-alkanes of the samples and both petroleum n-alkanes (r = 0.89) and algal n-alkanes (r = 0.79) (Table 5). The correlations were insignificant between higher plant and bacterial n-alkanes versus total n-alkanes (r = 0.25 and 0.09, respectively). These results confirmed that petroleum and algal sources were the main contributors to the n-alkanes in the tissues of the fish species, while both higher plant and bacterial n-alkanes had minor inputs.

CONCLUSION

The analyses of aliphatic hydrocarbons in the flesh of marine fish species from Iraq and their biomarker constituents and indices showed that petroleum hydrocarbons were predominant over the biogenic sources from marine and terrestrial higher plants. This is based on the n-alkane distribution pattern, C_{max}, $CPI_{(o/e)}$ values of ~1 (for entire, nC_{13} - nC_{24}, and nC_{25} - nC_{36} ranges) and Pr/Ph ~ 1. The joint biomarker approach and the biogeochemical index application are useful tools to differentiate between anthropogenic and biogenic sources of aliphatic hydrocarbons in aquatic biota. The fraction of n-alkanes from petroleum and related products in the tissues of the Iraqi marine fishes was relatively high, compared to natural biogenic sources from higher plant waxes, marine algae and bacteria. The contamination of the fishes by petroleum hydrocarbons is a serious health issue and is largely caused by oil-related activities in the area such as offshore oilfields, discharges from refineries and tanker traffic, and possible natural oil seeps. Further studies are needed to investigate the bioaccumulation of other anthropogenic and synthetic organic compounds such as polycyclic aromatic hydrocarbons, pesticides, and plasticizers in aquatic fauna and flora of the region as well as the environmental impacts of these organic compounds on the critical habitats coastal zone of Iraq.

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