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Polycyclic Aromatic Hydrocarbons and *n*-Alkanes in the Intertidal Limpet *Patella crenata* from the Coast of Tenerife, Canary Islands

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The impact of pollution on nearshore marine environments is often followed by the use of sentinel organisms. Marine pollution in coastal areas frequently originates on land (via rivers, human activities such as industry, tourism, shipping) or comes from the atmosphere (gases, deposition of aerosol particles).

Marine organisms take up lipophillic compounds such as petroleum hydrocarbons, including polycyclic aromatic hydrocarbons (PAHs), from water, food, and sediment. The biological effects of PAHs on fish and molluscs have been reviewed by Dunn (1991).

The use of molluscs to monitor marine pollution is well known (Farrington and Trip 1975; Neff and Burns 1996; Krahn and Stein 1998). The "Mussel Watch Program" has recommended the use of mussels and oysters as sentinel organisms to monitor marine environment health. However, it is impossible to find them in many coastal areas as the Canary Islands.

The Canary Islands are localized in an area of high tanker traffic, where several tanker accidents have occurred near the coast during the last ten years. In addition, the Canary Current and trade winds can carry the resulting pollution far from the island coastlines (De Armas et al. 1979; Conde et al. 1996)

The necessity to develop a global monitoring research project in this Atlantic area several authors (Conde et al. 1996; Peña et al. 1996a, 1996b; Peña and Garcia Montelongo 1998) to study different strategies to evaluate different marine species for use as sentinel organisms for these coasts. The aim of this work is to determine the contents of n-alkanes, polycyclic aromatic hydrocarbons and methyl-polycyclic aromatic hydrocarbons related to petrogenic pollution, in *Patella crenata,* a limpet specie, readily found on Canarian coasts and very appreciated as a food.

MATERIALS AND METHODS

Forty samples of *Patella crenata* were collected by hand over the period May 1991-December 1993, at the three sampling stations located on the southeast coast

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of Tenerife (Figure 1). Soft tissues were separated from the shells, wrapped in aluminium foil and soft tissues were frozen until analysis at -20°C.

The analytical procedure has been described in a previous work (Peña et al. 1996a), a summary: a composite sample (prepared from 10-15 specimens) undergoes alkaline hydrolysis (8% KOH in ethanol). Organic compounds were extracted from this solution, extracts were chromatographed on florisil-alumina-silica gel (purified water was used to deactivate at 3%, 5% and 5%, respectively) using n-hexane (aliphatic fraction) and n-hexane:dichloromethane (8+2) as eluents.

Analysis of both extracts was performed on a Varian 3300 (Walnut Creek, CA) gas chromatograph equipped with a flame-ionization detection (FID) system and an 8200 autosampler. Splitless-split injection mode with 0.45 min purge off. SPB-5 and SPB-20 fused silica capillary columns were used. The carrier gas was He (1.4 mL/min). The injector and detector temperatures were set at 250 °C and 280 °C, respectively. The oven temperature program was 2 min hold at 90°C; ramp to 280 °C at 8 °C/min hold 30 min.

CRM1974 “organic in mussel tissue” was used for quality control of the experimental procedure (Peña et al. 1996a). Procedural blanks were carried out between each batch of five samples and no contamination was found.

RESULTS AND DISCUSSION

The characterization and identification of petrogenic sources are generally based on chemical analyses (Boehm et al. 1997), (n-alkanes, aromatic hydrocarbons, etc.) from which “hydrocarbons fingerprints” are generated and related to the concentrations of individual hydrocarbons, and/or their ratios such as, Pristane/Phytane and Carbon Preference Index (CPI, defined as the sum of odd-over even-carbon number of n-alkanes) (Le Dréam et al. 1997; Boehm et al. 1997; Page et al. 1998; Peña and Garcia Montelongo 1998).

An example of the gas chromatogram of the aliphatic fraction corresponding to limpet samples taken in El Médano station is displayed in Figure 1. Together with peaks identifying n-alkanes, the chromatogram shows a fairly clear unresolved complex mixture (UCM), which is made up of several unresolved alkanes and is related with petrogenic pollution.

Table 1 presents the n-alkanes concentration in *P. crenata* samples expressed in ng/g dry weight. The analysis of aliphatic hydrocarbons shows for the sampling period 1991-l992, Figure 2, the individual concentrations of the aliphatic hydrocarbons (n-Ci) in which the number of carbon atoms $11<n<20$ are higher than 1993. Limpets sampled in 1991 showed a tendency for the accumulation of n-C_{12}, n-C_{14}, n-C_{16}, and n-C_{18}, while in the sampling period 1992-1993 this tendency is not constant.

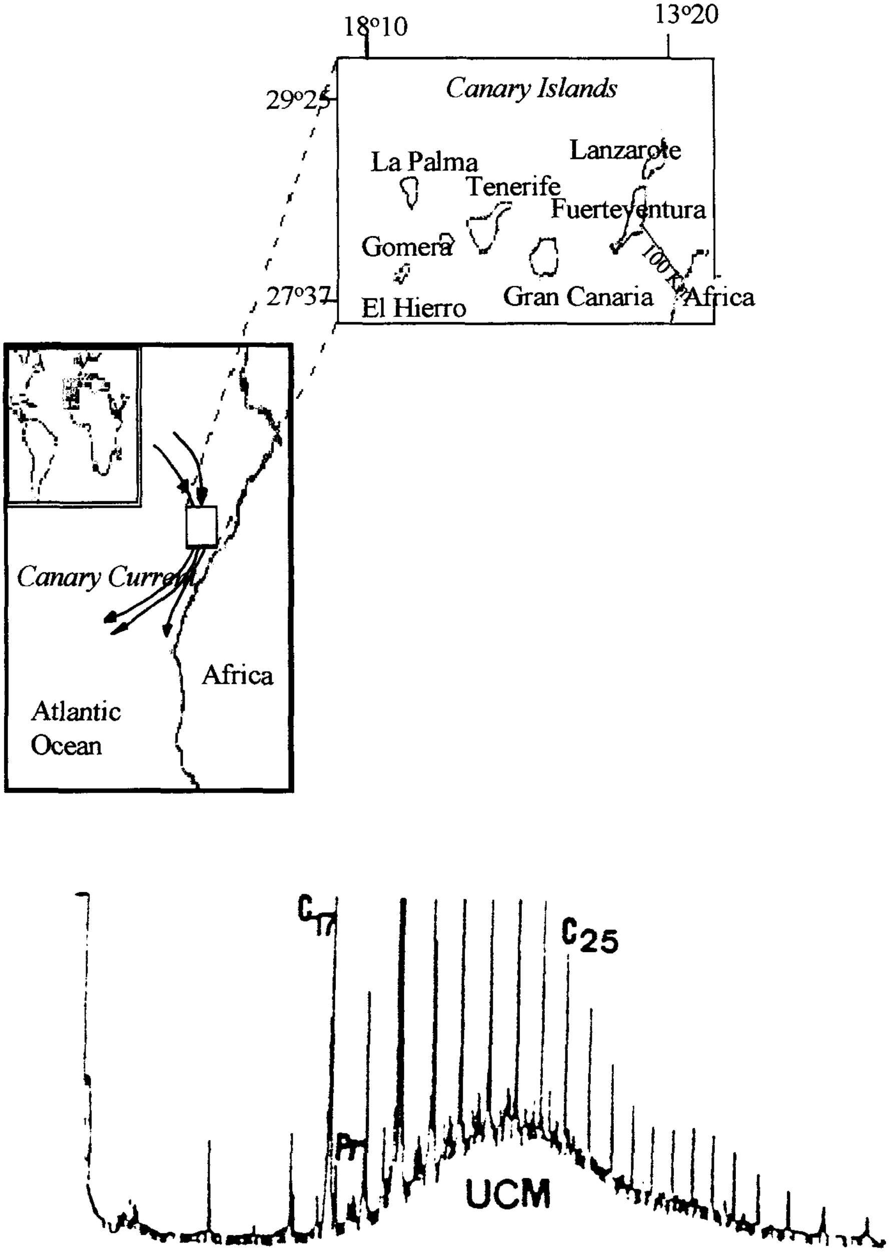

Figure 1. The Canary Islands. Gas chromatogram of aliphatic fraction in limpet *P. crenata* sampled at El Médano.

These fluctuations in the levels of hydrocarbons and accumulation tendency may be due to the diet, variations in sources of inputs, etc. n-Alkanes in the range C_{21}-C_{30} exhibit a constant background level with the exception of an occasional inputs.

Table 1. Mean concentration (ng/g, dry weight) and standard deviation for n-alkanes in *Patella crenata* from the three sampling stations ($\bar{x}$ mean, *S.D.* standard deviation.

Variables	*Las Caletillas*		*El Porís de Abona*		*El Médano*	
	$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*
C_{11}	27.86	70.81	86.76	80.14	131.4	213
C_{12}	184.5	292.6	96.92	142	7532	14139
C_{13}	207.6	481.5	10.85	24.25	1454	2143
C_{14}	4.095	10.604	20	24.52	6364	11634
C_{15}	926.0	4.015	917.5	1423	893	1410
C_{16}	2.278	7.214	39.22	38	6951	10287
C_{17}	785.7	942.1	533.4	437	586.6	635.4
C_{18}	783.0	2.416	35.14	37.3	1820	3885
C_{19}	17.3	23.75	21.06	34.4	55.2	80.23
C_{20}	401.0	992.8	45.45	54.01	651.2	1219
C_{21}	66.2	128.1	177	366	34.35	49.2
C_{22}	155.1	347.0	28.57	27	229.2	477.5
C_{23}	87.81	117.5	666.2	1435	60.75	66.1
C_{24}	731.6	1.501	8989	20033	283	428
C_{25}	139.7	253.2	70.43	96.2	1212	3597
C_{26}	614.1	1.489	421.1	624.5	761	1466
C_{27}	330.4	773.2	137.8	237.7	367.3	556.4
C_{28}	967.3	2.566	182.2	255.7	969.1	2152
C_{29}	505.3	995.4	333	413	493	706.3
C_{30}	246.6	372.5	287	574	329.1	578,6
ALIPH.T	13 503	22 805	13052	24213	30376	35070
C_{ODD}	9 306	20 023	10137	20556	25264	32437
C_{EVEN}	1 946	3 786	2915	3727	5112	4643

C_{odd} aliphatic hydrocarbons with an odd number of carbon atoms, C_{even}, aliphatic hydrocarbons with an even number of carbon atoms, *ALIPH.T* total sum of n-alkanes)

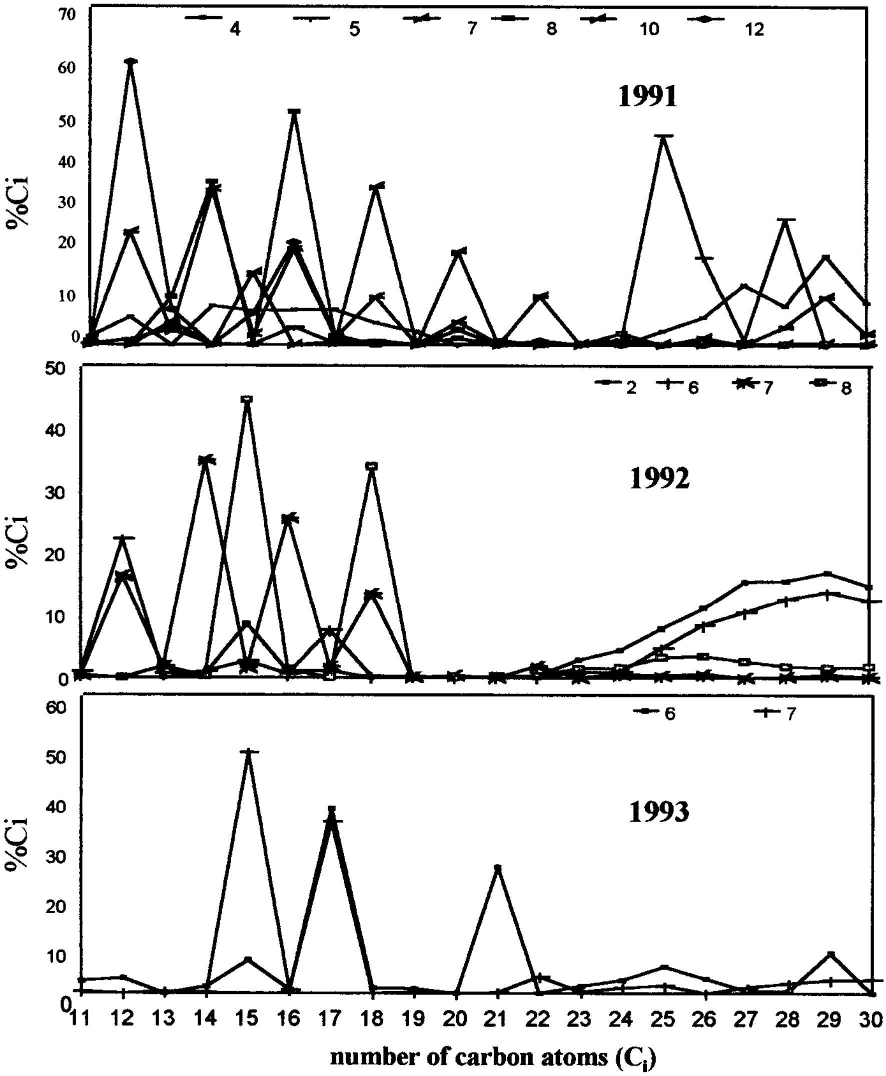

Figure 2. Variation of the relative abundance of the n-C_i hydrocarbons along the sampling period in El Médano station samples. Legend for dates (month) in box.

Sixteen PAHs and thirteen alkyl-PAHs have been studied. However, not all of them were detected in the *P. crenata* samples, Table 2. Concentration of these individual aromatic hydrocarbons are shown in Table 2.

The presence of naphthalene in samples must be evaluated carefully. In the laboratory, Neft and Bums (1996), reported that parent naphthalene sources may be solvents, paints products or cleaning products such as floor wax. Analysis of blank samples and certified reference materials did not detect the presence of naphthalene as a laboratory contaminant.

Table 2. Mean concentration (ng/g, dry weight) and standard deviation for aromatic compounds in *Patella crenata* from the three sampling stations.

Variable	Las Caletillas		El Poris		El Médano	
	$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*
Naphthalene	451.0	459.0	308.4	392.0	377.5	471.8
Acenaphtilene	15.06	56.35	440.2	1 355	102.3	269.3
Acenaphtene	*nd*	*nd*	43.47	137.5	12.40	35.07
Fluorene	3.400	12.68	15.78	30.43	*nd*	*nd*
Phenanthrene	7.665	28.67	12.44	33.24	17.18	48.60
Anthracene	*nd*	*nd*	72.92	154.7	25.43	71.93
Fluoranthene	74.67	172.4	10.99	21.95	5.166	14.61
Pyrene	81.41	193	29.84	89.52	19.72	55.77
1,2-Benzo[a]anthracene	15.84	56.34	80.50	176.7	14.90	42.05
Chrysene	161.2	566.0	791.7	2 280	20.84	58.96
Benzo[b]fluoranthene	61.11	160.5	24.50	70.86	*nd*	*nd*
Benzo[a]pyrene	70.30	257.8	13.0	41.1	15.31	38.61
Benzo[e]pyrene	*nd*	*nd*	984.0	2 604	24.87	46.14
Perylene	501.4	1 055	1 620	4 146	112.4	184.8
Dibenzo[a,c]anthracene	758.6	2 098	3 257	7 353	2 810	7 916
Dibenzo[a,h]anthracene	1 512	599.5	1 293	2 753	62.25	122.7
2-Methylnaphthalene	228.2	599.5	54.30	94.65	62.04	141.4
1-Methylnaphthalene	102.6	240.1	287.1	513.3	1.276	3.820
2,7-Dimethylnaphthalene	67.03	221.0	190.7	529.0	3.753	6.202
1,4-Dimethyllnaphthalene	0.065	0.23	*nd*	*nd*	9.312	32.26
1,2-Dimethylnaphthalene	95.10	355.7	23.52	46.68	110.0	242.3
1,8-Dimethylnaphthalene	8.650	31.18	20.20	44.77	73.96	192.6
1-Methylphenanthrene	27.44	94.92	28.68	56.91	175.6	396.8
2-Methyllanthracene	206.5	435.0	40.24	79.88	39.78	105.3
1-Methylanthracene	147.2	244.5	28.96	61.12	344.7	647.3
3,6-Dimethylphenanthrene	23.43	46.0	547.6	906.0	586.5	870.3
9,10-Dimethylanthracene	591.1	787.0	526.5	868.5	108.7	332.8

Table 3. Geochemical Indexes ($\bar{x}$: mean, S.D. standard deviation, *Min.* minimum, *Max.* maximum *nd* non detected, *Pr* pristane, *Ph* phytane).

INDEX	**Sampling Stations**					
	Las Caletillas		**El Porís de Abona**		**El Médano**	
	$\bar{x} \pm S.D.$	*Min-Max*	$\bar{x} \pm S.D.$	*Min-Max*	$\bar{x} \pm S.D.$	*Min-Max*
*CPI**	2.0±4.51	*nd*-15.78	1.44±2.02	0.20-4.83	3.8±5	0.11-15.0
CPI_{11-20}	16.0±36.7	*nd*-128.0	0.21±0.22	0.04-0.52	4.38±5.75	0.01-17.50
CPI_{21-30}	0.53±0.62	*nd*-1.60	0.3±0.3	0.04-0.726	0.13±0.11	0.01-0.31
Pr/Ph	0.78±1.15	*nd*-2.5	-	-	0.4±0.7	*nd*-1.85

* **Defined in the text**

Different possible pollution sources exhibit differences in the PAH and methyl-PAH profiles of these samples of *P. crenata.*

Limpet samples, Table 2, from Las Caletillas station shows the lower mean contents for ΣPAHs and Σmethyl-PAHs variables, ΣPAHs, 3416 ng/g, and Σmethyl-PAHs, 1 398 ng/g, than species from El Porís and El Médano stations, while organisms from El Médano present the highest values for ΣPAHs, 8 195 ng/g,, and Σmethyl-PAHs, 7 592 ng/g.. PAHs levels in the tissue of *P. crenata* from studied coastal areas globally ranged from non detected (nd) to 24 309 ng/g (mean value 5 903 ng/g).

Table 3 presents isoprenoid hydrocarbons pristane (Pr) and phytane (Ph), pristane/phytane ratio, and CPI, CPI_{11-20}(calculated to n-alkanes with a number of carbon atoms between 11 and 20) CPI_{21-30}, (calculated to n-alkanes with a number of carbon atoms between 21 and 30). High CPI values, Table 3, are related to biogenic origin of the hydrocarbons, CPI values near 1 indicating a petroleum origin.

In comparison with other specie of limpet, *Patella ulyssiponensis aspera* (Peña and García Montelongo 1998) *P. crenata* incorporates higher levels of PAH and lower methyl-PAHs. Nevertheless, both species follow the same tendency to accumulate mainly aliphatic compounds.

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