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Full Length Research Paper

Assessment of oil contamination in the bay of Porto Grande (Cape Verde) using the mullet *Chelon bispinosus*

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Polycyclic aromatic hydrocarbons (PAHs) are a group of persistent organic pollutants, some of which are mutagenic and carcinogenic so PAH concentrations in fish used for human consumption are crucial to assess impact to human health. Total PAH concentrations in muscle and liver of mullets *Chelon bispinosus* from the Bay of Porto Grande (Cape Verde) (four sites in the bay and a control) ranged from 112.7 to 779.5 and 291.5 to 7548.7 ng/g d. w., respectively. Two and three ring PAHs were the most frequent (72.8 to 90.8% in the muscle and 75.9 to 98.3% in the liver), but levels of carcinogenic PAHs (mainly Dibenzo (a,h) antracene) in certain sites (CN and PG) are of concern. Results reflect a chronic PAH pollution in the bay and sources are a mixture of anthropogenic (petrogenic and pyrolytic) and natural sources, making their identification extremely complex. Although, BaP levels were below the threshold established by Cape Verde and the European Union, BaPEs levels in muscle ranged from 0.28 to 3.66 ng/g w. w. and BAPEs and TPAHs exposure for the average adult was 0.02 to 0.26 and 1.6 to 11.2 µg/day, respectively. Further knowledge of PAH concentrations in other species are necessary for a proper environmental risk assessment policy.

Key words: Bay of Porto Grande, Cape Verde, *Chelon bispinosus*, mullets, PAHs, BaPEs, daily intake.

INTRODUCTION

Oil is a complex mixture of compounds with hydrocarbons reaching 98% of the total composition. Among them, Polycyclic Aromatic Hydrocarbons (PAHs) are a group of persistent organic pollutants (POPs) considered priority substances due to their persistence in the aquatic environment. The origin of PAHs results from the incomeplete combustion of fossil fuels, organic materials as well as petroleum. The most common sources of PAHs to the marine environment are natural as well as anthropogenic. Natural sources are restricted to burning of forests and volcanic emissions while anthropogenic sources are mainly from thermal processes and oil spills (Lopes and Andrade, 1996).

PAHs do not easily degrade in the aquatic environment and their persistence increase with the increase of molecular weight. Some of them after metabolic transformation (bioactivation of citochrome P450 enzymes) react with DNA and become potentially mutagenic and carcinogenic to organisms and humans (Neff, 2002). Fish and invertebrates are good indicators to assess POPs contamination of the marine environment and have been widely used to assess the presence and effects of these contaminants worldwide (Reddy et al., 2005).

Cape Verde is an archipelagic state in the Eastern Central Atlantic (14° 50' to 17° 20' N, 22° 40' to 25° 30'

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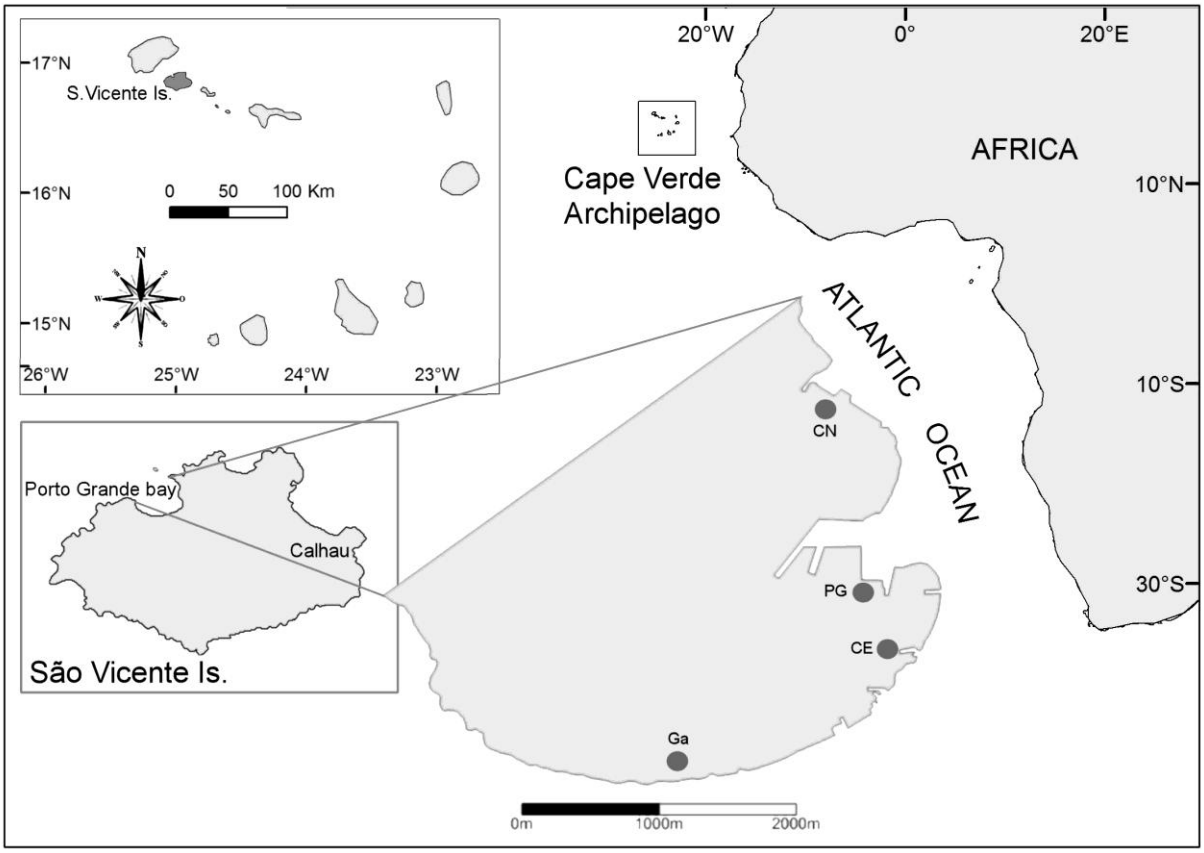

Figure 1. Map showing the geographical position of Cape Verde Archipelago in the eastern southern Atlantic ocean and the area of the island of São Vicente where the mullets *C. bispinosus* (Bowdich, 1825) were collected (shaded island)-Porto Grande bay. Legend: CN - Cabnave, PG - Porto Grande, CE - Cais da Enacol and GA - Galé. The control area [Calhau (C)- East coast] is also shown.

W), 750 km West of Senegal (Menezes et al., 2004). It consists of 10 islands and five islets of volcanic origin with a surface area of around 4,033 km^2 and an Exclusive Economic Zone of about 734,265 km^2 (Bravo de Laguna, 1985; DGMP, 1998) (Figure 1). The maritime traffic in Cape Verde is very intense and the contamination sources in this coastal area results from tanker activities, commercial and touristic vessels (MAAP, 2004). Moreover, the Bay of Porto Grande is at the NW end of Saint Vincent Island (situated West to Northwest of Cape Verde archipelago between latitude and longitude of 16.77 to 16.02° N and 24.86 to 25.09° W) and has a surface area of around 899 ha and a maximum depth of 30 m. The mouth of the bay is roughly 4 km wide and inside, there is a very active port. The currents are weak, the salinity varies between 35.8 and 36.2 psu and the average surface temperature ranges from 24.5 and 26°C (Almeida et al., 2008). Over the years, the bay was the main receptor of point and diffuse contamination sources from industrial activities, as well as from untreated or partially treated domestic effluents from the main urban area (Mindelo city), which lead to the increase of organic and inorganic contamination. Moreover, the intense port activities, the transport and storage of oil from ships, oil loading and unloading through marine terminals, accidental spillage and elimination of oily waste from ships and shipyards are important contamination sources. As PAHs are naturally present in petroleum and its derivatives, such activities constitute an important source of PAH contamination (Almeida et al., 2008). Between 2003 and 2007, several oil spills occurred and even nowadays, the smell of oil is intense and stains of floating oil are visible in the bay.

Therefore, the aim of this study was to assess the impact of PAHs in the bay of Porto Grande in S. Vicente Island (Cape Verde) using mullets *Chelon bispinosus* as a bioindicator. Among the Mugilidae, there are 26 families belonging to 14 genera but along the West Coast of Africa, between Senegal and Angola, only eleven species have been identified. Moreover, *C. bispinosus* (Bowdich, 1825) is the only mugilidae known in Cape Verde Islands (Trape et al., 2012). Therefore, this species was selected, due to their economic importance and food source in this country, to assess the impact of PAHs to human health.

In this prospect, mullets (*C. bispinosus*) were collected from different sites on the bay of Porto Grande and from a reference site (Calhau) and PAH content was analyzed in the muscle and liver. The bay of Porto Grande was selected because it is a marine ecosystem that receives intense pollution from a highly urbanized area and from port activities. This is, to the best of our knowledge, the first PAHs assessment in the bay of Porto Grand and in Cape Verde Archipelago.

MATERIALS AND METHODS

Sample collection and extraction

Fifty mullets *C. bispinosus* were collected in Spring 2009 in Cape Verde Archipelago Saint Vincent Island: ten from each of the four sites at the Bay of Porto Grande namely, from: Cabnave (CN) (16° 53´ 51.64" N; 24° 59´ 40.84" W), Porto Grande (PG) (16° 53´ 11.56" N; 29° 59´ 41.34" W), Cais de Enacol (CE) (16° 52´ 59.79" N; 24° 59´ 31.80" W), Galé (GA) (16° 52´ 39.71" N; 25° 00´ 17.75" W) and from the control site [Calhau, (C)] (16° 50´ 62.06" N, 24° 51´ 51.22" W) (Figure 1) with a gillnet and transported alive to the laboratory. Biometric parameters (weight, size) were measured and the condition factor (CF) and hepatosomatic index (HSI) calculated. Moreover, a portion of the lower back muscle and the liver were removed and individually wrapped in aluminum foil, identified and frozen at -20°C until PAH analysis.

PAH analysis

Muscle (± 16 g) and liver (± 2 g) were thawed and freeze-dried for 48 h. These lyophilized tissues were homogenized with anhydrous Na_2SO_4 and soxhlet extracted with 250 ml of n-hexane: dichloromethane (4:1) for 8 h. The extract was concentrated to about 30 ml in a Rotavapor Büchi R., dried with nitrogen and weighed to determine the percentage of lipids. They were further saponified after re-suspension with NaOH 6% in a water bath at 30°C for 18 h. The organic phase was separated and washed three times with n-hexane: dichloromethane (4:1) concentrated and further re-dissolved in n-hexane and purified on an activated silica/alumina column. The aliphatic fraction was eluted with 50 ml of n-hexane and the aromatic fraction containing the PAHs was eluted with 25 ml of n-hexane/dichloromethane (9:1) and 25 ml of n-hexane/dichloromethane (4:1). After drying under nitrogen, the residue was weighed and kept at -20°C until further analysis. The aromatic fraction was analyzed for PAHs by HPLC equipped with a specific column for PAHs determination (LiChrospher PAH, Merck) and a UV-Vis detector as described in Barreira et al. (2007). The 16 individual EPA (Environmental Protection Agency) recommended PAHs [(naphthalene (N), acenaphthylene (Acfil), acenaphthene (Acflen), fluorene (F), phenanthrene (Phen), anthracene (Ant), fluoranthene (Fu), pyrene (Pyr), benzo (a) anthracene (BaA), chrysene (Chrys), benzo (b) fluoranthene (BbF), benzo (k) fluoranthene (BkF), benzo (a) pyrene (BaP), dibenzo (ah) anthracene (DahA), benzo (ghi) perylene (BPer) and indeno (1,2,3-cd) pyrene (IndP)] were analyzed in the muscle and liver. The individual PAHs were identified by comparison of UV-Vis spectra and quantification performed by comparison of peak areas (254 nm) with an external standard of 16 PAHs (Supelco) by means of calibration curves constructed for each of the analyzed PAHs. Four replicates per sample were injected. The detection limit ranged between 0.01 and 0.24 ng/g w. w. for individual PAHs. Blanks were prepared in the same way as fish tissue samples but PAHs were not detected in the blanks. PAHs analysis was validated using a reference material tissue (SRM 2977) extracted, purified and analysed in the same way as the samples. PAH recovery for the certified material ranged between 73 and 112%. The concentrations of PAHs are expressed as ng/g dry weight.

Several ratios among individual PAHs (Phen/Ant, Flu/Pyr, Ant/Ant + Phen, Flu/Flu + Pyr, BaA/BaA + Chrys and IndP/IndP + BPer) were applied as diagnostic markers to identify the main PAHs sources in mullets from Saint Vincent Island. The limits of these ratios were obtained from the literature (Yunker et al., 2002; Mille et al., 2007; Oros and Ross, 2005; Oros et al., 2006). The formation of oil in which the organic matter maturation processes needs low temperature to originate two or three benzene ring PAHs (such as N, Ant and Phen) are characteristic of petrogenic sources while high temperatures in which 4, 5 or 6 ring PAH compounds are formed [like benzo(a)anthracene, chrysene, benzo(a)pyrene, benzo(g,h,i)perylene or indeno(1,2,3-cd)pyrene] characteristic of pyrolitic inputs. These ratios also allows the distinction between refined products or petroleum combustion (Porte et al., 2001a, b), or derived from biomass combustion (forest fires, fireplaces, incinerators, coal) (Oros and Ross, 2005).

Statistical analysis

Data are presented as mean ± standard deviation. XLSTAT software was used to perform statistical analysis. The experimental data was first tested for normality and homogeneity of variance. As total length, weight, CF and HSI did not meet the assumption of ANOVA (normality and homogeneity of variances), data were log transformed and Tukey test was applied to discriminate differences. To check whether there is any correlation between the variables and if there is any similarity between individual PAH concentrations in different locations, principal component analysis (PCA) was applied. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Biometric data

Total length and weight, condition factor (CF) and hepatosomatic index (HSI) (mean ± S.D.) of the mullets collected from the different sites are in Figures 2 and 3. No significant differences exist between the length and weight of the mullets from the different sites with the exception of those from CN that were larger and heavier (Figure 2A and B). Like for total length and weight, fish from CN has the highest condition factor (Figure 3A) along with that of GA and significantly different from the other three sites whose CF was similar ($p>0.05$). Moreover, despite the variability in the HSI of the mullets from some of the sites (CE and C), no significant differences exist between the HSI of the mullets from the different sites (Figure 3B) ($p>0.05$). The percentage of lipids in the muscle and liver of mullets from the different sites are in Figure 4A and B. The lipid content in the liver was significantly higher (around 2-fold) than in the muscle. Moreover, the lipid content in the muscle of the mullets from PG and C had similar high lipid content followed by those from the other sites whose lipid content was also similar (Figure 4A). Regarding the liver, lipid levels of mullets from PG and CE were higher but not significantly

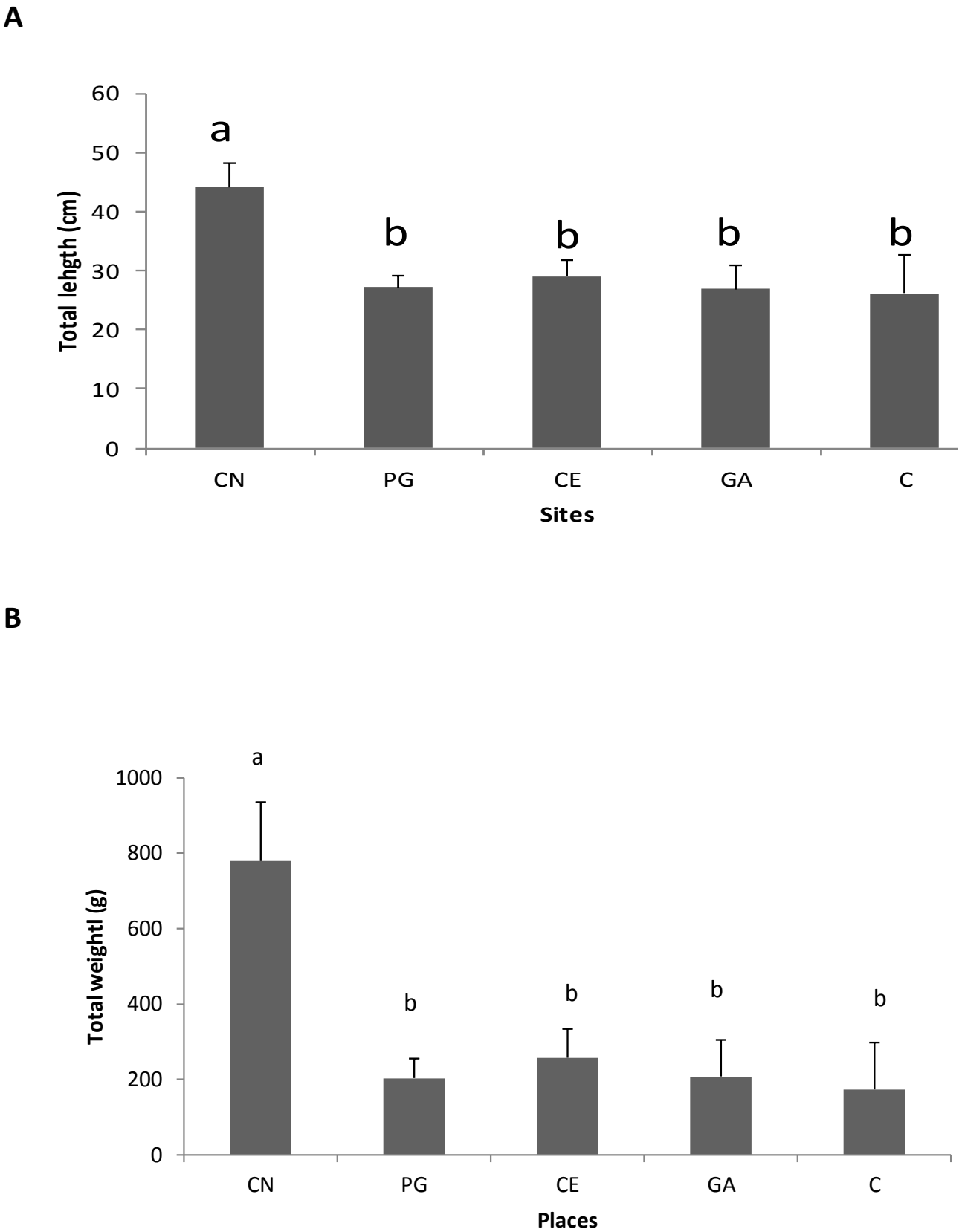

Figure 2. Total (A) length (mean ± S.D.) (cm) and (B) weight (mean ± S.D.) (g) of the mullets *C. bispinosus* from the different sites in Porto Grande Bay and Calhau [different letters indicate significant differences ($p < 0.05$)]. Legend: CN - Cabnave, PG - Porto Grande, CE - Cais da Enacol and GA - Galé. The control area [Calhau (C)- East coast].

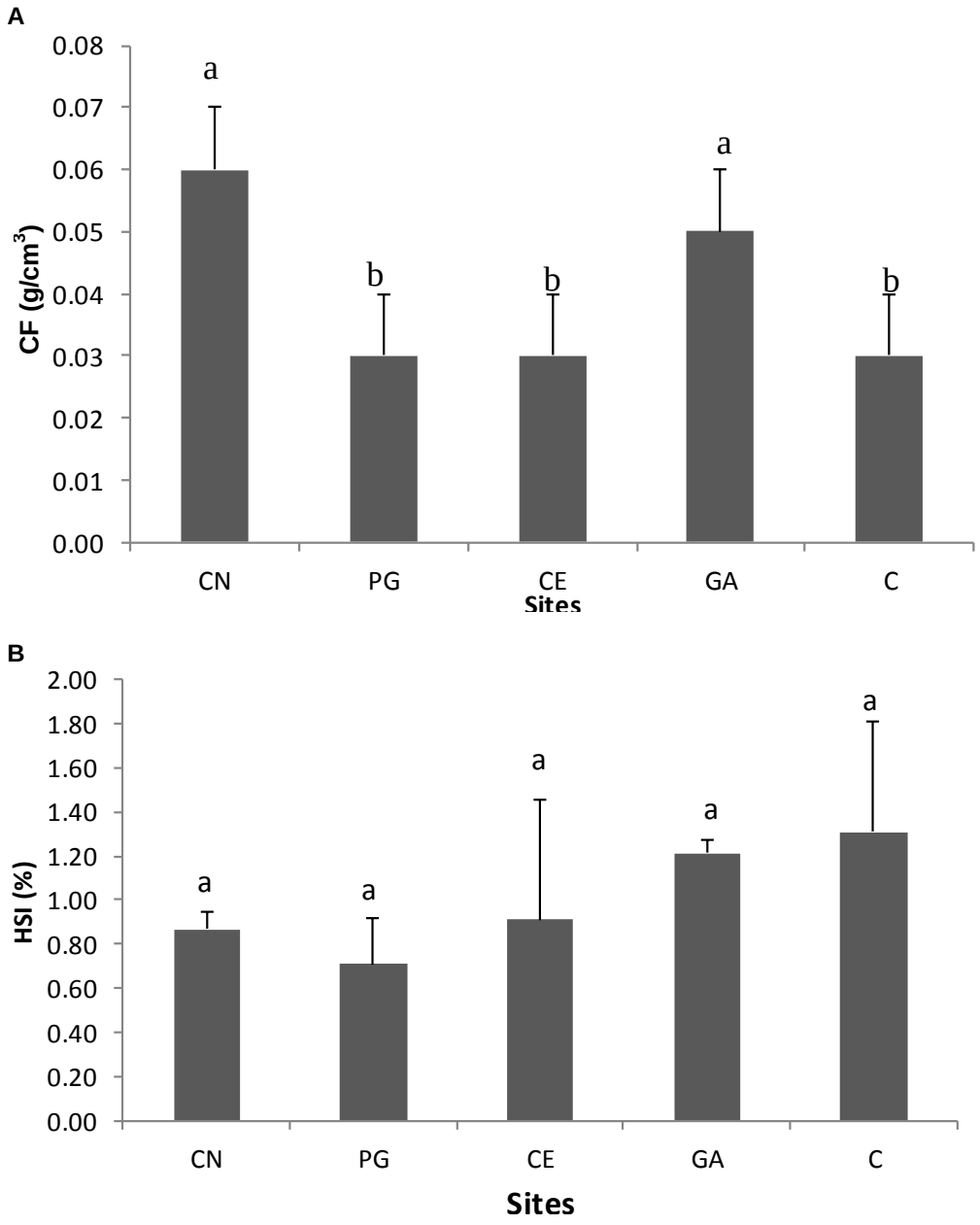

Figure 3. (A) Condition factor (CF) (g/cm^3) and (B) Hepatosomatic index (%) in the mullets *C. bispinosus* from the different sites in Porto Grande Bay and Calhau [different letters indicate significant differences ($p < 0.05$)]. Legend: CN - Cabnave, PG - Porto Grande, CE - Cais da Enacol and GA - Galé. The control area [Calhau (C)- East coast].

different from the other sites (Figure 4B).

PAHs concentrations

The concentration of individual, total and aromatic ring number PAHs in the muscle and liver of mullets from the different sites are in Table 1. Total PAH concentrations (TPAHs) in the muscle and liver of *C. bispinosus* from the different sites are in Figure 5. The results show that PAHs are widespread in the muscle and liver of the mullets from the different sites but levels in the muscle were always lower than in the liver except for mullets from CN (Figure 5). The highest PAH concentrations in the liver compared to muscle are not surprising because the liver is the tissue that usually has the highest lipid content and where the enzymes are able to metabolize PAHs. In the muscle, the highest TPAHs concentrations were in mullets from CN (779.5±17.66 ng/g d. w.) and the lowest in those from CE (112.7±70.67 ng/g d. w.). The

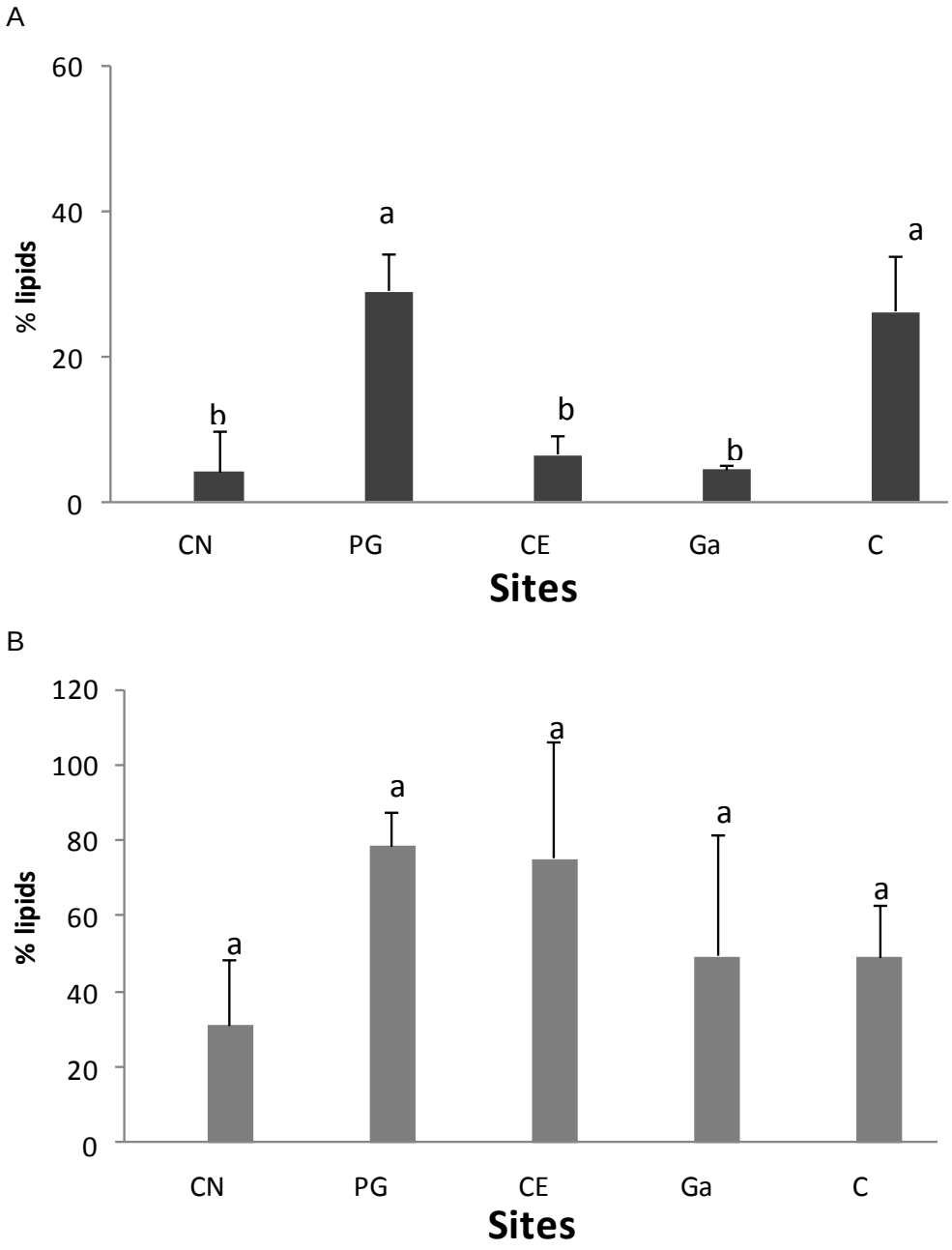

Figure 4. Lipid content (%) in the muscle (A) and liver (B) of mullet *C. bispinosus* from different sites in Porto Grande Bay and Calhau [different letters indicate significant differences ($p < 0.05$)]. Legend: CN - Cabnave, PG - Porto Grande, CE - Cais da Enacol and GA - Galé. The control area [Calhau (C)- East coast].

highest PAH concentrations in the muscle of mullets from CN are directly related to the oil spills that occurred from the shipyard existent in the area. In contrast, PAHs concentrations in the liver were the opposite with the highest TPAHs concentrations in the mullets from CE (7548.7±2.9 ng/g d. w.) and the lowest in those form CN (291.5 ± 30.9 ng/g d. w.). The highest TPAH concentrations in the liver of the mullets from CE reflect the influence of maritime traffic related to oil from small fishing vessels moored in the area and from the impact of urban effluent from the main city area (city of Mindelo).

Total PAH concentrations in the muscle and liver of mullets from the Bay of Porto Grande and Calhau were compared with similar fish species in other parts of the world and the results are in Table 2. TPAHs concentrations in the muscle of the mullets from Porto Grande

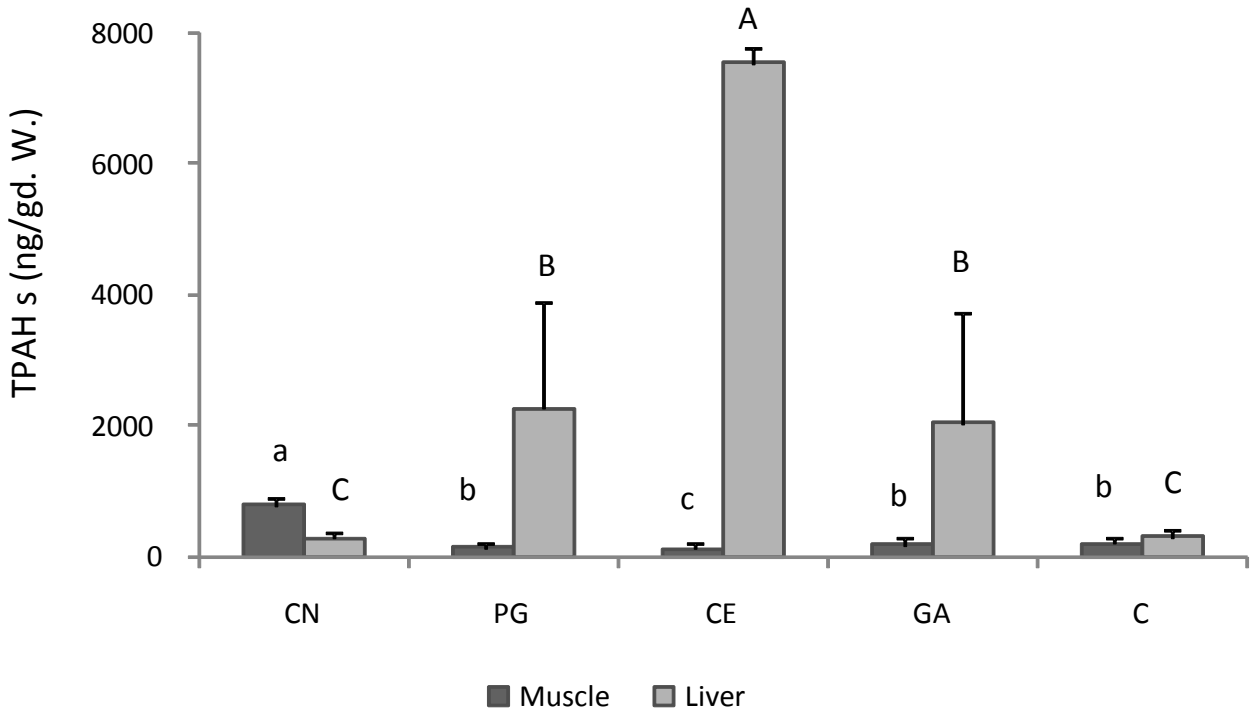

Figure 5. Total PAH concentrations (µg/g d. w) in the muscle and liver of the mullets *C. bispinosus* from the different sites in Porto Grande Bay and Calhau [different letters indicate significant differences ($p < 0.05$)]. Legend: CN - Cabnave, PG - Porto Grande, CE - Cais da Enacol and GA - Galé. The control area [Calhau (C)- East coast].

Bay (113 to 780 ng/g d. w.) are similar to those of the *Mugil* spp. from the eastern Mediterranean Sea and from *Mullus barbatus* from Western Turkey and from *Solea solea* of the Red Sea Coast of Yemen (422 ng/g d. w.) (DouAbul et al., 1979) and higher than *Mugil cephalus* from Brisbane River (Australia); *Mugil lisa* from Guanabara Bay (Brasil) and from other mugil species from Adriatic and Mediterranean Sea. For the liver, the range of TPAHS (291.5 to 7548.7 ng/g d. w.) is also higher than other fish species from the Northeast Atlantic (<LDM to 22 ng/g d. w.) (Hellou et al., 1994) and from the Bay of Archon, France (14.7 to 139 ng/g d. w.) (Baumard et al., 1998), but are within the range of those from the Finnish Archipelago (590 to 2225 ng/g d. w.) (Rainio et al., 1986). Although, PAHs are highly lipophilic, which favors its accumulation in lipid rich tissues, the highest PAH concentrations (CE) in the liver of mullets are not related with the highest lipid content (PG site). Within the muscle, the situation is identical: the mullets from the site with the highest PAH concentrations (CN) are not related with highest fat content.

Individual PAH concentrations in the muscle and liver of mullets from both Porto Grande bay and Calhau are dominated by 2 and 3 ring PAHs with a percentage ranging between 72.8 and 90.8% for the muscle and 75.9 and 98.3% for the liver, respectively (Table 1). In the muscle, the dominant PAH was Acftil at all sites except at CN where the dominant PAH was Acften. In the liver, however, the dominant PAH was Acften (highest at CE) with the exception of the mullets from CN whose dominant PAH was Flu. These results show that organisms accumulated preferentially the lighter PAHs in both tissues (muscle and liver). This accumulation pattern is a characteristic of PAHs contamination from petrogenic origin due mainly from spills of gasoline or diesel at harbors (Martínez-Gómez et al., 2012). Moreover, the percentage of 4 ring PAHs was higher in the muscle (between 2.8 and 18.8%) compared to the liver (1.4 and 7.5%). PAHs with 5 and 6 rings were also present with a similar percentage between the two tissues (ranging from 1.1 and 10.0% and 0.3 and 10.7% in the muscle and liver, respectively) but site dependent (highest in the muscle of mullets from PG and in the liver of mullets from CN) (Table 1). In what concerns the 4 ring PAHs, the dominant PAH on this group was Pyr in both mullet tissues while in the 5 ring group, BaP was present in all mullets from Porto Grande bay with similar levels in the liver of PG, CE and GA. In the muscle, this PAH was only detected in mullets from PG, GA and C. However, the dominant 5 ring PAH was DaA in the muscle and BkF in the liver except from those of GA where DaA was also dominant. Regarding the 6 ring group, Bper was only detected in the liver of the mullets from GA.

To check whether there is any relationship between PAH concentrations, weight, size, CF and HIS and if there is any similarity between the individual PAH con-

Table 1. Average individual and total polycyclic aromatic hydrocarbons (ng / g dry weight) and aromatic ring groups (%) in muscle and liver of fish in five sampling sites.

Site	Tissue									
	CN		PG		CE		GA		C	
PAH compound	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver
Naphthalene (N)	98.6	55.1	30.0	161.6	27.9	772.7	45.6	266.2	52.5	n.d
Acenaphthylene (Acftil)	170.6	10.6	61.1	19.1	47.5	1163.5	85.4	223.4	96	n.d
Acenaphthene (Acften)	319.5	61.5	8.3	1300.7	11	5429.3	18.9	1369.1	9.8	186.4
Fluorene (F)	1.6	108.7	1.2	559.5	1.0	43.5	6.6	28.5	1.9	7.7
Phenanthrene (Phen)	3.2	4.8	1.3	47.8	2.9	11.8	0.9	3.7	3.9	1.0
Anthracene (Ant)	0.4	0.3	0.4	1.4	0.3	0.8	0.6	1.8	0.5	n.d.
Fluoranthene (Flu)	5.4	2.7	7.4	30.7	2.3	13	2.2	4.7	2.1	36.9
Pyrene (Pyr)	8.9	8.9	12.0	74.3	12.6	35.3	3.3	17.1	15.3	8.6
Benzo [a] anthracene (BaA)	4.9	5.5	3.5	26.3	5.5	48.4	3.9	11.5	6.7	7.6
Chrysene (Chrys)	1.4	2.2	1.3	38.5	0.8	10.9	0.6	3.1	8.2	0.9
Benzo [b] fluoranthene (BbF)	1.6	7.6	0.4	4.5	0.6	5.8	1.7	2.7	2.2	20.6
Benzo [k] fluoranthene (BkF)	0.2	14.5	0.3	3.8	0.1	10.3	0.1	1.3	0.3	n.d
Benzo [a] pyrene (BaP)	n.d	1.5	1.1	3.2	n.d	3.5	0.1	3.1	0.6	n.d
Dibenzo [a. h] anthracene (DahA)	6.8	7.5	12.2	n.d	n.d	n.d	3.9	97.0	2.4	n.d
Benzo [g. h. i] perylene (Bper)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	6.0	n.d	n.d
Indeno [1.2.3-cd] pyrene (IndP)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Total PAH (TPAH)	779.5	291.5	140.6	2271.3	112.7	7548.7	173.9	2039.4	202.3	309.7
2+3 rings (%)	76.2	82.7	72.8	92.0	80.6	98.3	90.8	92.8	81.4	75.9
4 rings (%)	2.7	6.6	17.3	7.5	18.8	1.4	5.8	1.8	16.0	17.4
5+6 rings (%)	1.1	10.7	10.0	0.5	0.6	0.3	3.4	5.4	2.7	6.7
Main PAH[a]	Acflen (41)	Flr (37)	Acftil (43)	Acflen (57)	Acftil (42)	Acflen (72)	Acftil (49)	Acflen (67)	Acftil (47)	Acflen (60)

Legend: CN, Cabnave; PG, Porto Grande; CE, Cais da Enacol and GA - Galé. The control area [Calhau (C)- East coast]. TPAH sum of 16 individual PAHs concentration. [a]% of total; n.d. - not detected .

centrations in different sites, PCA was applied (Figure 6A and B). In the muscle (Figure 6A), PC1 and PC2 represent 36.6 and 23.6%, respectively of the total variance of the data (60.1%). CN is isolated in the positive quadrant of the PC1 and PC2, due mainly to the higher concentrations of N, Acftil, Acften and CF, weight and length. PG is separated from the other sites around PC2 with the highest concentrations of BaP, BkF, DahA, Flu and Ant. Mullets from C, CE and GA are the sites with the highest concentration of BaA. In the liver (Figure 6B), the first two components represent 71.9% of the total variance (40.6% for PC1 and 31.3% for PC2, respectively). PC1 separates the sites (CE, GA and PG) with the highest concentrations of Flu, Acftil, Acften, BaA, Pyr, Chrys, Ant, Phen, BaP, N, Bpe and BkF. C and CN have the highest concentrations of BbF and DahA. Mullets from C are directly related with the HSI while those from CN are directly related with CF, weight and length. The lack of a significant relationship between weight, length and individual PAHs, indicate that unlike other classes of persistent organic pollutants, the accumulation of PAHs in mullets are independent of the lipid content. The percentage of lipids is only positively related with Acftil, Acften, Flu, BaA and Pyr but these

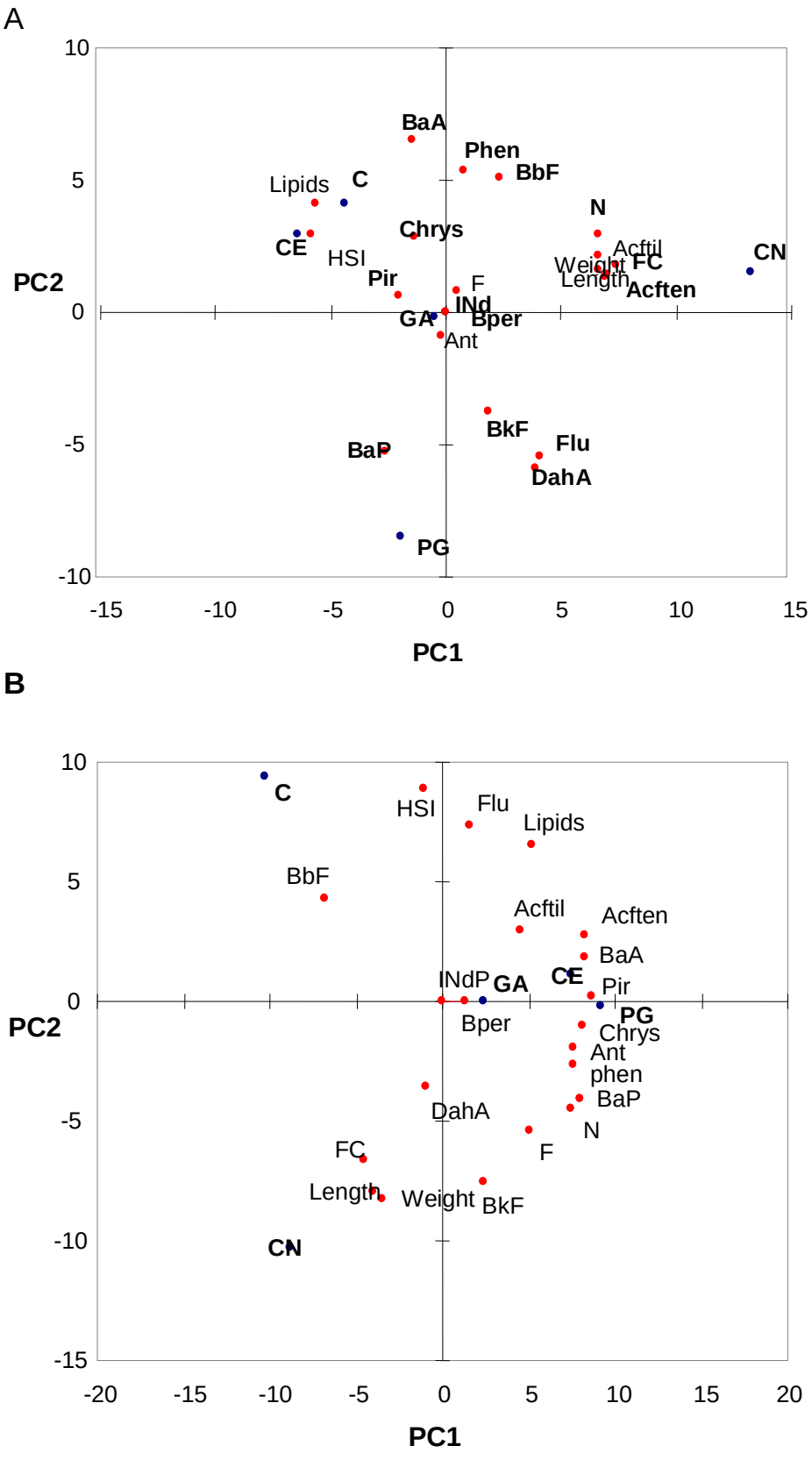

Figure 6. PCA analysis in (A) muscle and (B) liver with individual concentrations of PAHs, weight, length, FC and HIS. Legend: CN - Cabnave, PG - Porto Grande, CE - Cais da Enacol and GA - Galé. The control area [Calhau (C)- East coast].

Table 2. Total PAH concentrations in muscle and liver of fish species from different geographical regions (ng/g d. w.).

Species	Site	Muscle	Liver	Reference
Chelon bispinosis	CN	780	292	Present work
Chelon bispinosis	PG	141	2271	
Chelon bispinosis	CE	113	7549	
Chelon bispinosis	GA	174	2039	
Chelon bispinosis	C	202	310	
Clupea haren	Finnish Archipelago Sea	<0.5 - 33*		Rainio et al. (1986)
Diplodus annularis	Western Turkey	78.7 - 415		Darilmaz and Kucuksezgin (2012)
Gadus morhua	Northwest Atlantic.		<LDM-22	Hellou et al. (1994)
Hippoglossoides platessoides	Northwest Atlantic.	9-15*	14-39*	Hellou et al. (1997)
Liza aurata	Suez Canal	7229		Said and El Agroudy (2006)
Liza dussomeiri	Arabian Gulf	18-36*		DouAboul et al. (1987)
Liza dussomeiri	Arabian Gulf	118		DouAboul et al. (1997)
Lizza dumerillii	Niger Delta	101		Anyakora and Coker (2007)
Lota lota	Finnish Archipelago Sea	26*	445*	Rainio et al. (1986)
Micropogonias furnieri	Guanabara Bay	9.8-52.6		Silva et al. (2007)
Mugil auratus	Barcelona Harbor		57 ±19	Vives and Grimalt (2002)
Mugil cephalus	Bisbane River Estuary, Australia	53.7-195.0		Kayal and Connell (1995)
Mugil lisa	Guanabara Bay	3.7-26.1		Silva et al. (2007)
Mugil, spp.	Eastern Mediterranean	238±39		Barbour et al. (2009)
Mullus barbatus	Mediterranean Sea	14.7-49.6		Baumard et al. (1998)
Mullus barbatus	Mediterranean Sea, Spain	1.91-4.45*		Martínez-Gómez et al. (2012)
Mullus barbatus	Western Turkey	202 -556		Darilmaz and Kucuksezgin (2012)
Mullus barbatus	Adriatic Sea	16.52*		Perugini et al. (2007)
Mullus barbatus	Adriatic Sea	104-147		Della Torre et al. (2010)
Mullus surmuletus	Mediterranean Sea	3.1*		Llobet et al. (2006)
Pleuronectes ferruginea	Northwest Atlantic.	0.6-68*	15-79*	Hellou et al. (1997)
Pseudolithus elongatus	Niger Delta	100.1		Anyakora and Coker (2007)
Serranus scriba	Mediterranean Sea, France		20.4- 139	Baumard et al. (1998)
Solea solea	Red Sea Coast of Yemen	422.1		DouAboul et al. (1997)
Stizostedion lucioperca	Finnish Archipelago Sea	17-30*	118*	Rainio et al. (1986)

*w.w.

CN, Cabnave; PG, Porto Grande; CE, Cais da Enacol; GA, Galé. The control area [Calhau (C)- East coast].

compounds are not present at higher concentrations in places where there are the highest lipid levels, supporting the information that the PAHs accumulated in mullets tissues are not explained by the lipid content.

In order to identify the PAHs sources in the environment where the mullets were collected, several diagnostic ratios were calculated for the muscle and the liver of *C. bispinosus* (Table 3). The ratio Phen/Ant >10 indicates a petrogenic origin of PAHs while <10 indicates a pyrolytic origin. Pyrolitic PAHs can result from the incomplete combustion (boat traffic, engines) or biomass combustion (coal, wood, fires) that reach the marine environment from road runoff or atmospheric deposition. Moreover, the ratio Flu/Pyr <1 is also related with a petrogenic source while >1 indicates a pyrolytic origin. The Phen/Ant ratio in mullets muscle ranged from 1.5 (GA) and 9.7 (CE) indicating a pyrolytic origin (Phen/Ant <10) of PAHs in this tissue for all sites. However, in the liver, the ratio Phen/Ant was only <10 at GA (2.1) and C (4.0) confirming a pyrolytic origin at those sites while in mullets livers from the other sites, the ratio Phen/Ant was >10 [ranging from 14.8 (CE) to 34.9 (PG)] confirming that at those sites, the origin of PAHs was petrogenic. On the other hand, the ratio Flu/Pyr indicates a petrogenic origin of PAHs in both mullet tissues (Flu/Pyr <1) with the exception of the liver of mullets from C (4.3) that has a pyrolitic origin (Flu/Pyr >1).

Consequently, with the exception of mullets from site C that showed a pyrolitic origin in both tissues, mullets from all the other sites have a mixed origin of pyrolitic and petrogenic sources. Additionally, to better identify the origin of PAHs in fish tissues, other diagnostic ratios

Table 3. Diagnostic ratios of PAHs sources.

Diagnostic ratio	CN		PG		CE		GA		C	
	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver
Fen/Ant	10.90	1.0	0.70	1.0	0.10	13.90	0.40	2.50	0.80	4.60
Flu/Pir	3.0	0.40	0.60	0.70	0.30	0.40	0.40	0.30	0.60	0.0
Ant/Ant + Fen	8.70	0.80	0.20	0.10	0.10	0.10	0.20	0.50	0.90	0.80
Flu/Flu+Pir	3.00	0.30	1.40	0.40	0.40	0.30	0.50	0.20	0.90	0.70
BaA/BaA+Cris	7.0	0.70	0.20	0.20	0.10	0.80	0.20	0.50	0.50	-

CN, Cabnave; PG,Porto Grande; CE, Cais da Enacol; GA, Galé. The control area [Calhau (C)- East coast].

or from combustion of biomass were calculated and the results are in Figure 6. The ratio Ant/Ant + Phen indicate that the origin of PAHs accumulated in the muscle is mainly from petroleum (CE) and petroleum combustion (other sites). In mullet's liver, the main origin is from petroleum (CN, PG and CE) and petroleum combustion (GA) and only those from C have a biomass combustion origin confirming the pyrolytic origin at this site. In the mullets from CN, PG and CE, the ratio Ant/Ant + Phen was <0.10 in both tissues which is consistent with PAHs from oil (or fuel). CN is directly impacted by shipyards and from the effluent of the desalinization plant while PG is located near the harbor and the marina and CE is directly impacted by the oil terminal which can be related with the petroleum origin found.

GA is directly related with the fuel terminal of the oil company present. The ratio Ant/Ant + Fen <0.10 corresponds in most cases to the values of the Flu/Flu + Pyr ratio <0.40 in mullets muscle from CE and in the liver from PG, CE and GA. These results are also in agreement with the ratio Phen/Ant that also shows a predominance of pyrolytic PAHs for both tissues in most of these sites. The Ant/Ant + Phen ratio was >0.10 and the Flu/Flu + Py ratio >0.40 only in the liver of the mullets from C supporting the conclusion that the pyrolytic products are a strong component of PAHs at this site (Figure 7). The trend of the ratio BaA/BaA + Chrys also indicates that the origin of PAHs is primarily from petroleum and petroleum combustion except from the liver of mussels from C confirming the biomass combustion source at this site. Thus, PAHs accumulated in muscle and liver of mullets are mainly from an incomplete combustion of oil and its derivatives (Ant/Ant + Phen; BaA/BaA + Chrys), presenting also petrogenic (oil not burn) and pyrolytic PAHs derived from biomass combustion (Flu/Flu + Pyr).

To assess the health implications of PAHs accumulation in mullets muscle for human consumption, BaP, the most potent carcinogenic of the PAH group after DahA, is used as a toxicological surrogate for all carcinogenic PAHs. Therefore, BaP levels of mullets muscle from the different sites were compared with the guidelines established by the legislation of Cape Verde (Ordinance 24/2009) and of the European Union [Regulation (EC) No 1881/2006 of 19th December 2006] that sets maximum levels for certain contaminants in food stuff and both recommended a maximum allowable BaP concentration in fish muscle of 2 ng/g w. w.. BaP was only detected in mullets from PG, GA and C (at the other sites levels were below detection limit) but levels were well below the standard established by Cape Verde and European Union indicating that from the present results, levels of BaP in *C. bispinosus* muscle are in general safe for human consumption and does not constitute an health risk for Cape Verde consumers.

However, it is also necessary to take into account the daily intake of this species by Cape Verde population. Therefore, based on the approach adopted by EPA to evaluate risk assessment, PAHs were separated into two classes; carcinogenic (BaA, Crys, BbF, BkF, BaP, DahA, and IndP), and non-carcinogenic (N, Aceflen, Acftil, Flu, Phen, Ant, Flu, Pyr and Bper).

The individual carcinogenic potential of each PAH was expressed as BaP equivalents (BaPEs) based on the toxicity equivalent factors (TEFs) defined by Nisbet and LaGoy (1992) for each PAH. TEFs for all 16 PAHs and BaPE (calculated by multiplying the concentration of each individual PAH by the appropriate TEF) and Total BaPEs (the sum of each BaPE from individual PAHs) are in Table 4.

When levels were below the limit of detection, half of the detection limit was used in the calculations. Results indicate that with the exception of fish muscle from CN and PG, higher TPAH concentrations (Table 1) were related to higher total BaPEs. Nevertheless, the highest TPAH levels from CN in muscle mullets had one of the lowest total BaPEs, mainly due to the presence of high levels of 3 ring PAHs (F, Acftil and Acften) that have a TEF of 0.001. Muscle mullets from the other sites had medium levels of PAH (ranging from 140.6 to 202.3 (Table 1) but total BaPEs from PG were the highest. This was mainly due to the highest DaA concentrations (TEF of 1) at this site. With the exception of muscle mullets from CE, BaPEs were well above the levels found for red(Ant/Ant + Phen; BaA/BaA + Chrys and Flu/Flu + Pyr) that distinguish from incomplete combustion of fossil fuels

Table 4. BaPEs (ng/g w. w.) and mean daily intake of BaPEs and TPAHs (µg/day w. w.).

PAH compound	TEFS	BaPEs (ng/g w. w.)					
		CN	PG	CE	GA	C	Mean
Naphthalene (N)	0.001	0.0197	0.0060	0.0056	0.0091	0.0105	0.0102
Acenaphthylene (Acftil)	0.001	0.0341	0.0122	0.0095	0.0171	0.0192	0.0184
Acenaphthene (Acften)	0.001	0.0639	0.0017	0.0022	0.0038	0.0020	0.0147
Fluorene (F)	0.001	0.0003	0.0002	0.0002	0.0013	0.0004	0.0005
Phenanthrene (Phen)	0.001	0.0006	0.0003	0.0006	0.0002	0.0008	0.0005
Anthracene (Ant)	0.01	0.0008	0.0008	0.0006	0.0012	0.0010	0.0009
Fluoranthene (Flu)	0.001	0.0011	0.0015	0.0005	0.0004	0.0004	0.0008
Pyrene (Pyr)	0.001	0.0018	0.0024	0.0025	0.0007	0.0031	0.0021
Benzo [a] anthracene (BaA)	0.1	0.0980	0.0700	0.1100	0.0780	0.1340	0.0980
Chrysene (Chrys)	0.01	0.0028	0.0026	0.0016	0.0012	0.0164	0.0049
Benzo [b] fluoranthene (BbF)	0.1	0.0320	0.0080	0.0120	0.0340	0.0440	0.0260
Benzo [k] fluoranthene (BkF)	0.1	0.0040	0.0060	0.0020	0.0020	0.0060	0.0040
Benzo [a] pyrene (BaP)	1	0.0625	1.1000	0.0625	0.1000	0.6000	0.3850
Dibenzo [a. h] anthracene (DahA)	1	1.3600	2.4400	0.0625	0.7800	0.4800	1.0245
Benzo [g. h. i] perylene (Bper)	0.01	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006
Indeno [1.2.3-cd] pyrene (IndP)	0.1	0.0063	0.0063	0.0063	0.0063	0.0063	0.0006
Total BaPEs (ng/g w.w.)		1.689	3.659	0.279	1.036	1.3250	1.597
Mean daily intake of BaPEs (µg/day w.w.)		0.122	0.263	0.020	0.075	0.095	0.115
Mean daily intake of TPAHs (µg/day w.w.)		11.2	2.0	1.6	2.5	2.9	4.1

CN, Cabnave; PG, Porto Grande; CE, Cais da Enacol; GA, Galé. The control area (Calhau (C)- East coast).

mullet in Italy (0.63 ng/g w. w.) (Perugini et al., 2007) and for the screening value recommended by USEPA for human consumption (0.67 ng/g w. w.) (USEPA, 2000).

The BaPEs of mullets muscle from the different sites were higher (PG and CN) or similar (GA, C) to 1.33, 0.997 and 0.094 ng/g w. w. reported for marine sea food from Kuwait, Italy and Hong Kong, respectively (Alomirah et al., 2009; Perugini et al., 2007; Cheung et al., 2007). Moreover, estimated daily consumption of fish from Cape Verde was 26.5 kg/habitant which is equivalent to 72 g/day (INDP, 2006). This average was similar to that of Kuwait (66.4 g/day) (Alomirah et al., 2009) and lower than the US EPA (142.2 g/day) recommended for subsistence consumers (USEPA, 2002) and for an average person from Hong Kong (164.4 g/day) (Cheung et al., 2007).

The mean exposure to TPAH via *C. bispinosus* muscle for the average Cape Verde consumer is 4.1 µg/day w. w. but those from CN are around 3-fold higher. These levels are higher than those estimated for the average adult Kuwait consumers (1.3 and 0.0013 µg/day for mean daily intake of TPAHs and total BaPEs, respectively). Moreover, the impact of PAHs in the bay of Porto Grande should be attentively followed and to assess the impact of POPs in Cape Verde, further studies are required to ascertain PAH concentrations and BaPE levels in other economically important fish species of the region.

Conclusions

Total concentrations of 16 PAHs accumulated in the mullets *C. bispinosus* ranged from 112.7 to 779.5 ng/g d. w. in muscle and from 291.5 to 7548.7 ng/g d. w. in the liver with mullets from CN and CE having the highest total PAH concentrations in both tissues. These results reflect a chronic PAH contamination in the bay of Porto Grande and show that *C. bispinosus* are useful indicators to detect the presence of PAHs.

The intense maritime traffic of small and large vessels, the transport and storage of petroleum, port operations, leisure and tourism, urban and industrial effluents contribute to the anthropogenic impact of PAHs. There is a mixture of anthropogenic (petrogenic and pyrolytic) and natural sources at this area, making the identification of PAH sources extremely complex due to the diversity of anthropogenic activities and local dynamics. However, the distribution of individual PAHs showed that the two and three ring PAHs which ranged between 72.8 and 90.8% in the muscle and 75.9 and 98.3% in the liver with the predominance of Acften, Acftil and Flu, reflect conta-

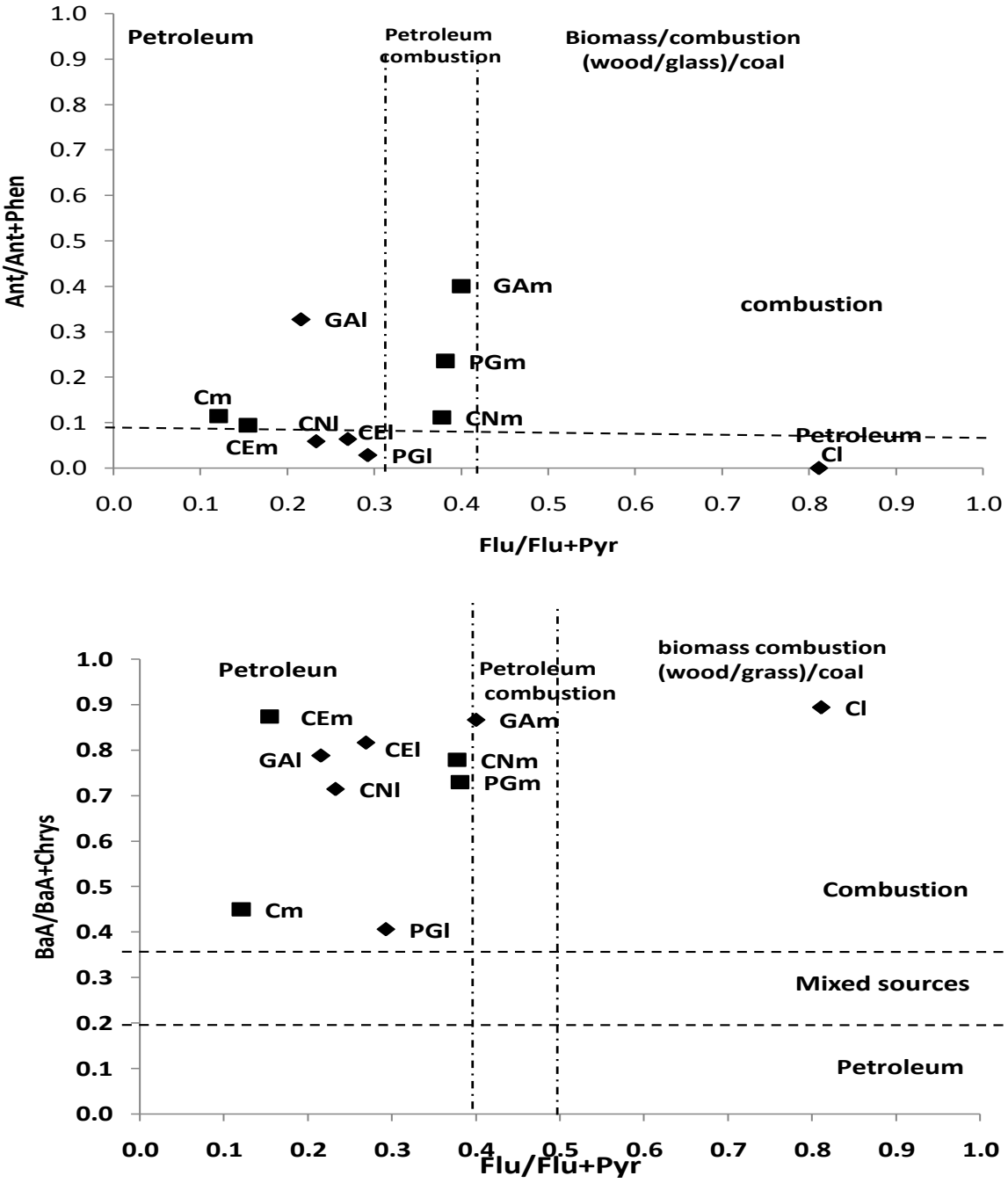

Figure 7. Ant/Ant + Phen, BaA/BaA + Chris versus Flu/Flu + Pyr ratio in muscle (black squares) and liver (black diamonds) of the mullets *C. bispinosus* from the different sites in Porto Grande Bay and Calhau. Legend: CN - Cabnave, PG - Porto Grande, CE - Cais da Enacol and GA - Galé. The control area [Calhau (C)- East coast].

mination by lighter petroleum products but levels of carcinogenic PAHs, especially DaA, are of concern to human health.

Due to the impact of POPs to the environment and human health, it is necessary to improve the knowledge of the different POP concentrations in economically important fish species in Cape Verde for a proper environmental management and risk assessment policy. The present data is a starting point to assess the impact of PAH levels in this area in the future.

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