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LEVELS OF POLYCYCLIC AROMATIC HYDROCARBONS IN EELPOUT, *ZOARCES VIVIPARUS*, IN ESTONIAN MARINE WATERS

Henn OJAVEER[a] and Risto TANNER[b]

[a] Estonian Marine Institute, Lai St. 32, EE-0001 Tallinn, Estonia
Institute of Zoology and Hydrobiology, University of Tartu, Vanemuise St. 46, EE-2400 Tartu, Estonia
[b] Institute of Chemical and Biological Physics, Akadeemia Rd. 23, EE-0026 Tallinn, Estonia

Presented by J. Kann

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Abstract. Samples of eelpout, *Zoarces viviparus*, collected from the Gulf of Finland and the Gulf of Riga were analysed for polycyclic aromatic hydrocarbons (PAHs). The total content of PAHs in fry and fish tissues exhibited considerable variation (0–22.94 (mean 6.52) and 0.17–16.39 (mean 6.19) ng g^{-1} wet weight, respectively). Eleven PAH compounds analysed were all present in edible parts of the fish while only six of them were detected in the fry. In general, preferential accumulation of more water-soluble PAH components was observed, fish samples from the eastern part of the Gulf of Finland being in this respect exceptional.

Key words: polycyclic aromatic hydrocarbons, *Zoarces viviparus*, Baltic Sea.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are formed as a result of the incomplete combustion of organic fuels. PAHs are widely distributed in the marine environment, evidenced by their detection in water, sediments, and plant and animal tissues. PAHs have received considerable scientific interest worldwide (Larsen et al., 1986; Cocchieri et al., 1990; Cripps, 1992; McDonald et al., 1992), mainly due to their harmful effects to living organisms (Stegeman, 1981; Ahokas & Pelkonen, 1984; Payne et al., 1988; Kime, 1995). These include, among others, impairment of metabolic pathways and behavioural changes, reduction in growth and reproduction, changes in population dynamics and species composition in communities (Capuzzo, 1985).

Organisms inhabiting the Baltic Sea live under constant stress due to the brackish water environment. Because of the low salinity, the number

of species in the Baltic is rather small. Therefore, compared with the true marine or freshwater environment, the ecosystem of the Baltic Sea is less stable and thus more sensitive to external factors. For this reason, information on anthropogenic stressors in the biota of the Baltic Sea is particularly important. Furthermore, exploitable marine resources should be thoroughly investigated in terms of accumulated pollutants possibly causing health problems to man.

Our knowledge of the PAH levels in the Baltic environment is far from complete. There are only a few papers dealing with the accumulation of PAHs in the marine biota of the Baltic Sea (Rainio et al., 1986; Broman et al., 1991; Veldre & Bogovski, 1993); some of them directed to certain PAH substances (Veldre et al., 1982, 1984). Therefore, there is a demonstrable need for the collection of further data, especially in the Gulf of Riga as no published information for this region is available. The present work was undertaken as a pilot study in order to characterize PAH levels in *Zoarces viviparus* from Estonian waters of the Baltic Sea. We analysed the levels of 11 different PAH components in fry and edible parts of the fish.

MATERIALS AND METHODS

Eelpouts, *Zoarces viviparus* (Linnaeus, 1758), were caught in various areas from Estonian marine waters (Figure) from May to December 1994. Detailed information on the fish samples is given in Table 1. Altogether 118 fish and 977 embryos were subjected to PAH determinations. Samples

Location of the sampling areas.

Table 1

Information on the eelpout (*Zoarces viviparus*) samples analysed

No. of sample	Fishing area*	Date (1994)	Number of fish in sample	Fish length, cm (mean±S.E.)
1	1	25 May	6	22.6±2.9
2	1	17 June	6	22.3±2.7
3	1	7 July	3	22.5
4	1	12 Nov.	2	26.3
5**	1	12 Nov.	119	n.d.
6	2	7 July	4	17.5±0.8
7	2	23 Aug. & 11 Nov.	4	19.0±3.0
8	2	18 Dec.	6	20.0±0.2
9	2	18 Dec.	5	24.6±0.9
10	2	18 Dec.	11	20.1±0.7
11**	2	18 Dec.	195	4.10±0.04
12**	2	18 Dec.	291	3.89±0.03
13	3	24 May	5	15.4±1.0
14	3	16 July	8	17.0±1.9
15	3	11 Nov.	2	17.2
16	3	27 Nov.	17	15.9±0.4
17**	3	27 Nov.	146	3.73±0.03
18	4	24 May & 18 June	10	15.8±0.7
19	4	16 July	2	14.6
20	5	22 Aug.	10	20.4±1.2
21	6	16 Dec.	11	18.3±0.5
22	6	16 Dec.	4	22.8±0.5
23	6	16 Dec.	2	27.5
24**	6	16 Dec.	226	3.79±0.03

* Location of fishing areas is shown in the Figure.
** Fish fry.
n.d. = not determined.

were stored frozen in aluminium foil at –20°C. Before analysis, the fish were gutted and headed while complete embryos were analysed.

The samples were prepared and analysed using modifications of the method described by Irha et al. (1993). For extraction of PAHs, 1.112 µg coronene, as an inner standard, was added together with 170 µl toluene into 10 g of homogenized sample. The resulting mixture was hydrolysed in 42 ml of concentrated HCl and extracted afterwards in chloroform and hexane solution (1:3 v/v). GPC was performed on a Shodex A-801 column 50 cm × 8 mm i.d., eluted with toluene (velocity 0.5 ml min^{-1}). After purification, the extracts were analysed by HPLC in two connected columns Shimpack ODS-CLC (total 15 + 15 cm × 6 mm i.d.). The elution was conducted with the mixed solvent of acetonitrile (AcN) : water (75 : 25 v/v as initial ratio), followed by a linear gradient within the next 5 min up to 80 vol.% of AcN and up to 98 vol.% of AcN during the following 38 min (flow rate 1.5 ml min^{-1}, column temperature 35°C). The chromatogram was registered by FluoroMonitor III (LDC, USA; ex. 254 nm, em. cutoff 370 nm)

and spectrophotometric detector M-214 (Yanaco, Japan) at 280 nm. The signals from both detectors were integrated with a System 1000 instrument (Yanaco, Japan). Quantification of PAH components was assessed on the basis of fluorometric records and corrected by coronene recovery rates (68–82%). Spectrophotometric signal was used for peak identification checking. This was based on calculated ratios of corresponding peak heights from the two detectors.

RESULTS

Concentration of total PAHs in edible parts of fish tissues exhibited remarkable variation – from 0.17 to 16.39 ng g^{-1} wet weight (Table 2). Not all the PAH components studied were found in detectable amounts in all the samples analysed; the number of different compounds observed in a sample varied from one to ten. The eleven PAH species analysed were all

Table 2

Concentration (ng g^{-1} wet weight) of PAHs in edible parts of eelpout (*Zoarces viviparus*)

No.*	A**	B	C	D	E	F	G	H	I	J	Total
1	11.30	–	–	2.97	0.92	0.40	0.05	–	0.30	–	15.94
2	5.09	–	5.96	1.60	1.48	0.72	0.07	0.31	0.97	0.19	16.39
3	5.49	0.24	–	–	–	–	–	–	–	–	5.73
4	3.33	–	–	–	–	–	–	–	–	–	3.33
6	6.43	0.16	–	–	–	–	–	–	–	–	6.59
7	3.24	–	–	–	–	–	–	–	–	–	3.24
8	3.85	0.29	0.41	–	0.05	–	0.05	–	–	0.17	4.82
9	–	–	0.62	–	–	–	–	–	–	–	0.62
10	–	0.79	–	–	–	–	–	–	–	0.28	1.07
13	5.38	–	–	–	0.04	–	–	–	–	–	5.42
14	10.70	0.37	–	–	0.17	–	–	–	–	–	11.24
15	–	0.07	–	–	–	–	–	–	–	0.10	0.17
16	–	0.55	3.31	–	1.68	–	–	–	–	–	5.54
18	6.37	–	0.68	–	0.24	0.06	–	–	0.08	–	7.43
19	5.45	–	–	–	–	–	–	–	–	–	5.45
20	9.51	0.14	0.43	–	1.02	–	0.06	–	–	–	11.16
21	9.46	0.40	–	–	0.19	–	–	–	–	0.11	10.16
22	2.51	–	–	–	–	–	–	–	–	0.18	2.69
23	–	0.11	–	–	0.05	–	–	–	–	0.43	0.59

* Fish samples in Tables 2 and 3 are given the same numbering as in Table 1.

** A – phenanthrene (detection limit 2.0 ng g^{-1}), B – anthracene (0.02 ng g^{-1}), C – fluoranthene (0.3 ng g^{-1}), D – benzo(*a*)anthracene + chrysene (1.0 ng g^{-1}), E – benzo(*b*)fluoranthene (0.02 ng g^{-1}), F – benzo(*k*)fluoranthene (0.02 ng g^{-1}), G – benzo(*a*)pyrene (0.05 ng g^{-1}), H – dibenzo(*ah*)anthracene (0.2 ng g^{-1}), I – indeno(1,2,3-*cd*)pyrene (0.05 ng g^{-1}), J – benzo(*ghi*)perylene (0.1 ng g^{-1}).

– Not detected.

detected in fish samples from the Gulf of Riga while in the fish from the Gulf of Finland only six (phenanthrene, anthracene, fluoranthene, benzo(*b*)fluoranthene, benzo(*a*)pyrene, and benzo(*ghi*)perylene) were found (Table 2). It should also be emphasized that the last compound was relatively more frequently found in the fish from the Gulf of Finland than the Gulf of Riga. The most commonly recorded PAH component was phenanthrene followed by anthracene and benzo(*b*)fluoranthene, suggesting preferential accumulation of more water-soluble PAH species. Moreover, phenanthrene was also recorded in the highest quantities (concentration varied from 2.51 to 11.30 ng g^{-1}). The distribution and content of PAH compounds in fish seem somewhat irregular. However, fish from Pärnu Bay (Area 1) tended to accumulate higher total amounts of PAHs (mean 10.35 ng g^{-1}) in comparison with the other areas in the Gulf of Riga (2, 3, and 4; average values 3.27, 5.59, and 6.44 ng g^{-1}, respectively). In addition to that, in fish from Pärnu Bay more different PAH substances (11) were observed than in samples from any other area.

Results of the analyses of PAH concentrations in eelpout fry taken from the female body cavity are shown in Table 3. Of the eleven PAH compounds

Table 3

Concentration (ng g^{-1} wet weight) of PAHs in eelpout fry (*Zoarces viviparus*) from the female body cavity

No.*	A	B	C	D	E	F	G	H	I	J	Total
5	–	–	–	–	–	–	–	–	–	–	–
11	–	0.61	–	–	–	–	–	–	–	0.32	0.93
12	7.14	0.37	0.87	–	–	–	–	–	–	–	8.38
17	21.20	0.64	–	–	0.88	–	0.22	–	–	–	22.94
24	–	–	–	–	–	–	–	–	–	0.34	0.34

* For legend see Table 2.

analysed, only six (phenanthrene, anthracene, fluoranthene, benzo(*b*)fluoranthene, benzo(*a*)pyrene, and benzo(*ghi*)perylene) were detected in fish fry with one to four PAH substances present in a sample. The total PAH content varied from 0 to 22.94 ng g^{-1}. This large variation is caused by an exceptionally elevated phenanthrene content (21.20 ng g^{-1}) in the sample from the Ruhnu Deep (No. 17), which was the highest concentration recorded within the current study. As in the case of mature fish, phenanthrene was also one of the most often observed compounds in fish embryos, sharing this status with anthracene. Other PAH substances were found only occasionally. In this context, a notable variability in the content of PAHs in fry (samples 11 and 12) collected from the same catch should be mentioned.

DISCUSSION

In selecting fish species for this study we followed the experience of Jacobsson et al. (1985), who concluded that *Zoarces viviparus* fulfilled the criteria of a bioindicator. The eelpout stock in the Gulf of Riga exhibits different stock characteristics in various parts of the gulf (Ojaveer & Gaumiga, 1995) confirming relative immobility of the fish. The peculiarity of the reproduction cycle of eelpout explains the presence of bioaccumulating substances in fry in the female body cavity.

Analysing Baltic herring, *Clupea harengus membras* (L.), caught from the Finnish Archipelago Sea, Rainio et al. (1986) found only 2 (fluoranthene and pyrene) of the total of 14 PAH components they studied. In the same work with pike-perch, *Stizostedion lucioperca* (L.), and burbot, *Lota lota* (L.), in addition phenanthrene and components of the benzo(*a*)anthracene/chrysene/triphenylene group were detected. Irha et al. (1993) reported pyrene as the main PAH component with small concentrations of benzo(*a*)pyrene (BaP) and benzo(*ghi*)perylene in Baltic flounder, *Platichthys flesus* (Suworow). On the basis of these results it appears that eelpout possibly accumulates more types of different PAH compounds than do the fish species mentioned above. The variability range found by Rainio et al. (1986) for the content of total PAHs (<0.5–33 ng g^{-1} wet weight) in fish muscle is in good agreement with the findings of the current work. Cocchieri et al. (1990) reported relatively high total PAH concentration values (94–1930 ng g^{-1} wet weight) in fish from the Italian Mediterranean coast. They also pointed out noticeable variability in the amount of total PAHs and individual PAH components accumulated into various fish species. Despite an elevated total PAH content in fish from the Mediterranean compared with the present study and a higher number of individual PAH compounds (16) analysed in them, some similarities in the distribution pattern of PAH components in the Mediterranean and the Baltic Sea occur: anthracene and phenanthrene were the most frequently recorded PAH substances in fish from these two waterbodies.

Because of a great variability in the concentrations of total PAHs in fry and adult fish (0–22.94 and 0.17–16.39 ng g^{-1}, respectively), no significant differences between the means (6.52 and 6.19 ng g^{-1}, respectively) occur. Moreover, when comparing the content of PAHs in fry and adult fish determined in the same catch, no clear trend in the concentration of total PAHs or individual PAH compounds between them can be observed. However, the number of different PAHs found in adult fish (11) exceeds that in the fry (6). The total number of PAH components detected in fish from the Gulf of Riga (11) was higher than that from the Gulf of Finland (6), the difference is caused mainly by the results from Pärnu Bay. Furthermore, the highest average content of PAHs (10.35 ng g^{-1}) in adult fish was also found from the latter area. As it was stated earlier, preferential accumulation of PAHs of lower molecular weight was evident. However, it was not the case in the eastern part of the Gulf of Finland (Area 6), where benzo(*ghi*)perylene was the most frequently detected compound in the fish. Whether this is due to the high pollution

load in this region or to some other factors remains beyond the frame of this study. Although some spatial differences in the total amount and composition of PAHs in eelpout are evident, we are not inclined to make any far-reaching conclusions because of the high variability of numerical data recorded. However, the mean value (6.26 ng g^{-1}) for all samples analysed is relatively low and the average PAH contents in fry and adult fish are in the same order of magnitude.

Benzo(*a*)pyrene is known as a precursor of highly carcinogenic and mutagenic compounds, which may be involved in initiating cancer in fish (Varanasi & Gmur, 1980; Ahokas & Pelkonen, 1984). The content of BaP can also be a useful indicator of the total level of PAHs (Payne et al., 1988). BaP studies in fish from the Baltic Sea are scarce and the results somewhat controversial. While Rainio et al. (1986) did not find any detectable residues of BaP in various tissues from Baltic herring, pikeperch, and burbot, Veldre & Bogovski (1993) documented the mean BaP concentration of 0.354 ng g^{-1} wet weight for the marine fish (Baltic herring, sprat, *Sprattus sprattus balticus* (Schneider), cod, *Gadus morhua callarias* (L.), flounder, and eelpout). The mean BaP contents in fish from Pärnu Bay and the Gulf of Finland (0.155 and 0.633 ng g^{-1} wet weight, respectively) observed by Veldre & Itra (1991) are higher than these recorded in the present study. Other investigators, for example Hellou et al. (1994), did not find any detectable amounts of BaP in cod from the Northwest Atlantic while Cocchieri et al. (1990) reported in their study on the Mediterranean fish BaP concentrations up to 44 ng g^{-1} wet weight.

The use of different analytical methods in PAH determinations can cause variations in the results, also discussed by Cripps (1992). Further, due to a wide variability in types and the number of PAH compounds analysed, and different units used (per wet and dry weight of fish), comparisons with other results are sometimes severely restricted or even meaningless. Thus, there is an urgent need for a commonly accepted system of PAH determinations in the marine environment.

Further studies of the distribution of PAHs in eelpout should be directed to certain tissues and organs. The presence of PAH metabolites in liver and bile, indicating PAH compounds taken up and eliminated by the organism, as suggested by several authors (McDonald et al., 1992; Hellou et al., 1994), should also be investigated. Finally, given the priority to obtaining more comprehensive knowledge on the accumulation, metabolic pathways, and effects of PAHs in the aquatic ecosystem, samples from water, sediments, and water organisms should be studied in parallel.

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