Polycyclic aromatic hydrocarbon (PAH) accumulation in different common sole (Solea solea) tissues from the North Adriatic Sea peculiar impacted area

Elsevier Editorial System(tm) for Marine Pollution Bulletin

Manuscript Draft

Manuscript Number:

Title: Polycyclic aromatic hydrocarbon (PAH) accumulation in different common sole (Solea solea) tissues from the North Adriatic Sea peculiar impacted area.

Article Type: Research Paper

Keywords: PAH bioaccumulation; sediment; fish tissue; Solea solea; northern Adriatic Sea.

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Abstract: This study extends our knowledge of the bioaccumulation of polycyclic aromatic hydrocarbons (PAHs) in marine organisms and investigates its possible determinants. PAH levels were measured in Solea solea tissue and in marine sediments collected from three areas of the northern Adriatic Sea characterized by different anthropic impacts (Venetian Lagoon, Po Delta, and fishing grounds off Chioggia). The possibility of differential PAH bioaccumulation in different tissues (muscle, liver and gills) was investigated by seeking relationships between mean individual and total PAH concentrations in tissue and sediment samples, the physicochemical properties of PAHs (rings and Kow), and some key biological variables (lipid content of tissues, body size, habitat). The present study demonstrated that the main factors determining PAH bioaccumulation in common sole tissue in the northern Adriatic Sea were closely related to habitat characteristics and physicochemical properties of PAHs rather than lipid content of tissue and body size of fish.

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Cover letter

Ancona, 08 June 2018

Dear Editor,

Enclosed please find a manuscript entitled "**Polycyclic aromatic hydrocarbon (PAH) accumulation in different common sole (*Solea solea*) tissues from the North Adriatic Sea peculiar impacted area**" that we would like to submit for publication in Marine Pollution Bulletin as an original research article.

The study harnesses a number of different approaches to determine the presence of PAH in a widely consumed fish species and the factors that influence their accumulation in different tissues. The research includes analysis of the gill, liver and muscle concentrations of PAH, in relation of their spatial distribution in North Adriatic Sea.

We feel the topic is suitable for Marine Pollution Bulletin because our findings of the PAH bioaccumulation, strengthening the knowledge in the field of aquatic environmental.

This MS has not been previously published, in whole or in part, and is not under consideration by any other journal. All authors are aware of and accept responsibility for the MS.

Thank you for your time and attention

Sincerely

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Highlights:

- PAH levels were measured in *S. solea* tissue and in sediments of northern Adriatic Sea
- Higher PAH accumulation in gills, followed by liver and muscle, has been observed.
  - The main factors determining PAH bioaccumulation in fish tissue were analyzed.
  - PAH accumulation is closely related to pollutant and habitat characteristics than biological.

Polycyclic aromatic hydrocarbon (PAH) accumulation in different common sole (*Solea solea*) tissues from the North Adriatic Sea peculiar impacted areas.

1 Introduction

Aquatic ecosystems like coastal marine areas act as a sink for many harmful substances, including persistent organic pollutants (POPs) such as polycyclic aromatic hydrocarbons (PAHs) (Webster et al., 2011; Van Ael et al., 2012; Marini and Frapiccini, 2014). PAHs are among the most widespread organic contaminants and constitute a class of widely studied pollutants; in particular, 16 of them have been classified as priority pollutants by the European Union and the US Environmental Protection Agency (US EPA) due to their carcinogenic and mutagenic effects (Vives et al., 2004; Pandey et al., 2011; Xia et al., 2012). PAHs derive mainly from anthropogenic activities, including the combustion of organic matter, oil, wood, fossil fuels, and the release of hydrocarbons by crude oil (Neff et al., 2005; Stogiannidis and Laane, 2015; Barhoumi et al., 2016). Natural inputs into the environment are ascribable to volcanism, forest fires and petroleum seeps, but represent a small contribution to the overall PAH concentration in the environment (Baumard et al., 1999; Lima et al., 2003; Zhang et al., 2015). Due to their lipophilicity, low solubility in water and high persistence, PAHs naturally tend to be adsorbed in suspended matter, and then result in relatively rapid deposition in marine sediments (Cheung et al., 2007; Koelmans et al., 2010; Cui et al., 2016). Their hydrophobicity, namely octanol-water partition coefficient (Kow), is the dominant physical parameter for the fate of PAHs, as it determines their capacity for transport and distribution between different environmental compartments as well as their uptake and accumulation by living organisms (Chu & Chan, 2000; Frapiccini and Marini, 2015; Hussein et al., 2016). PAH bioaccumulation is also influenced by other physicochemical characteristics, such as number of rings, type and origin, environmental conditions (e.g. sampling area, PAH emission sources nearby), and the characteristics of the species itself (e.g. trophic position, body size, lipid content, tissue) (Varanasi et al., 1985; Dominguez et al., 2011; Van Ael et al., 2012; Bodin et al., 2014). Accordingly, they can accumulate in the fatty tissues of organisms, biomagnify, and be transferred through the food chain, thus also affecting consumer health (Perugini et al., 2007; Bandowe et al., 2014; Zhonghua et al., 2014). The evaluation of PAH levels in environmental matrices and the edible part of aquatic organisms is therefore an important issue (Bodiguel et al., 2009; Zhao et al., 2014). However, PAH bioaccumulation is a complex phenomenon governed by many factors including their uptake and elimination. Therefore, further studies are necessary to characterize the factors affecting PAH bioavailability to marine organisms and their distribution in

fish tissues (Soclo et al., 2000; Bodin et al., 2014; Moraleda-Cibrián et al., 2015; Sun et al., 2016). The present study of PAH levels and distribution in fish tissue aims to investigate the main factors affecting PAH bioaccumulation. Although several investigations have examined the impact of PAHs on marine organisms of the Adriatic and Mediterranean basins (Amodio Cocchieri et al., 1990; Corsi et al., 2002; Perugini et al., 2007; Galgani et al., 2010; Trisciani et al., 2011; Storelli et al., 2013; Cocci et al., 2018; Ferrante et al., 2018), comparisons between various fish tissues and environmental samples are limited. Moreover, the interest in these pollutants and their possible transfer to food of marine origin has increased after the Marine Strategy Framework Directive (MSFD), the first EU legislative instrument (EC, 2008) regulating the protection of marine biodiversity to achieve Good Environmental Status (GES).

The aims of this study were to analyze and evaluate the residue levels and distribution of PAH priority pollutants (16 ΣPAHs) in the tissues (gills, liver and muscle) of common sole (Solea solea, Linnaeus, 1758) individuals collected from three areas of the northern Adriatic Sea and to examine the main factors involved in PAH bioaccumulation, namely the lipid content of tissue, the biometric characteristics of fish, environmental features, and the physicochemical properties of PAHs (number of ring and Kow), to establish which factors exert a major influence on PAH bioaccumulation in fish tissue, especially S. solea.

2 Material and methods

2.1 Study area

The northern Adriatic Sea is a shallow basin (less than 100 m deep) located in the northern part of the Mediterranean Sea. It is characterized by strong inputs from Italian rivers that flow through highly industrialized, densely populated, and intensively farmed areas (Sagratini et al., 2008). The River Po is the largest river, characterized by a mean annual discharge rate of 1500-1700 m^3/s, accounting for about a third of the total riverine freshwater input to the Adriatic Sea (Campanelli et al. 2004, Marini et al. 2008; Campanelli et al., 2011). The three areas selected for this investigation were two well-known and complex transitional environments, the Po Valley, a very important agricultural region and the industrial heart of northern Italy (Castellarin et al., 2011), and the Venetian Lagoon, which is characterized by complex interactions between natural factors and human activities (Secco et al., 2003); the third area was a slightly less contaminated area 40 km off Chiogga (Fig. 1).

Common sole (*S. solea*) is one of the most commercially important species in Adriatic fisheries, with high ecological and economic value and high relevance for human consumption (Scarcella et

al., 2014; Pellini et al., 2018). Since it feeds on the bottom and lives in close association with sediment, it is a widely accepted sentinel of chemical contamination in seawater and biomarker studies in relation to PAH exposure (Claireaux and Davoodi, 2002; Sagratini et al., 2008; Wessel et al., 2010; Bodin et al., 2014; Gonçalves et al., 2014; Sun et al., 2016). These considerations, and the fact that the northern and central Adriatic Sea are important spawning and aggregation areas for common sole (Scarcella et al. 2014), led this species and area to be chosen for the study.

2.2 Field sampling

Sampling activities were carried out in November and December 2014 in the framework of the "rapido" Trawl SoleMon Survey (Grati et al., 2013). Individuals of *S. solea* (n= 48) were caught with modified rapido trawls in 8 zones, and surface sediments were collected at the same sites using a box corer (Table a). All soles in the catches were measured (total length, mm) and weighed (wet weight, g) and examined for sex. Samples of muscle, liver and gill tissue were dissected out using acid-cleaned scalpels and scissors in the on board laboratory. Tissue samples were stored at -18 °C for PAH determinations.

2.3 Chemicals

A standard PAH solution with dichloromethane:methanol (1:1 v/v) containing the 16 priority pollutants (EPA 610 PAHMIX, Supelco, Bellafonte, PA, USA) was used for preparing PAH standard multipoint calibration. The chemicals used were dichloromethane, acetonitrile for HPLC gradient grade, acetone and petroleum ether (all purchased from VWR International, Fontenay-sous-Bois, France). 18.2 MΩ water was prepared by a Milli-Q system (Millipore, Billerica, MA, USA). Sediment samples certified IAEA code 408 and IAEA code 383, and material certified for fish IAEA code 106 were obtained from the International Atomic Energy Agency (Vienna, Austria). Quick easy cheap effective rugged and safe (Quechers) (ECMSSC-MP) extraction kits containing 4 g $MgSO_4$ and 1 g NaCl and a centrifuge tube ECMPSC1815CT containing 0.9 g $MgSO_4$, 0.3 g PSA, and 0.15 g endcapped C18 were purchased from CPS Analytical (Milano, Italy).

2.4 Sample extraction

The Quechers method was applied and developed for the extraction and purification steps of PAHs from fish tissue, in agreement with various works (Ramalhosa et al., 2009; Sapozhnikova et al., 2013; Pfannkoch et al., 2015**;** Morrison et al., 2016). It is a simple and fast method that requires the use of a small volume of organic solvent and fewer steps than traditional extraction methodologies

(Albinet, 2013; Sapozhnikova et al., 2013). It is therefore a valid alternative to other methods, because it employs a multiresidue sample preparation procedure adapted for extraction and clean-up (Morrison et al., 2016). Based on this procedure, 5 g of homogenized fish tissue was placed into a screw-capped tube with 10 mL acetonitrile and shaken in a vortex for 1 minute. Then, $MgSO_4$ and NaCl in a ratio 4:1 (g/g) were added to the extract and the tube was shaken again in a vortex for 3 min. The tube was immediately centrifuged at 3400 RPM for 3 minutes at 4°C. Subsequently, 3 mL of the supernatant was recovered for clean-up and transferred to another screw-capped tube containing 0.9 g $MgSO_4$, 0.3 g PSA (primary and secondary amines), and 0.15 g of C18 phase. The tube was shaken in a vortex for 1 minute and centrifuged at 4°C at 3400 RPM. The upper phase was collected in a round bottom flask and left under a laminar flow until complete evaporation: the dry residue was recovered with acetonitrile (0.4 mL), placed in a vial, and stored at -18°C until HPLC-FLD analysis.

PAH extraction from marine sediments was carried out according to a previous work by our group (Marini and Frapiccini, 2013), with some modifications and improvements. In brief, PAH extraction from 10.0 g sediment samples was obtained by three 20 min cycles in an ultrasonic bath using methanol:dichlorometano (1:1 v/v) as the solvent to achieve liquid-liquid separation. The PAH enriched solvent was removed initially by rotary evaporation (T = 30±2°C) and later by gentle nitrogen flow (Frapiccini et al., 2016). The final volume of the analytical samples was adjusted to 0.4 mL with acetonitrile and stored at -18°C until HPLC-FLD analysis.

To determine the water percentage in tissue, they were lyophilized by a freeze-drying process that enables complete loss of water at low temperature (-20 °C) and pressure. Tissues were accurately weighed and freeze-dried (Edwards EF4 modulyo, Crawley, Sussex, England) until constant weight (±0.2mg).

2.5 Chemical analysis

PAH identification and quantification in fish tissue and surface sediments samples were performed by the same methods using an HPLC system (Ultimate 3000, Thermo Scientific, Waltham, MA, USA) equipped with a fluorescence (RF-2000) detector (Thermo Scientific). A Hypersil Green PAH (μm2.1 x 150 mm, 1.8 μm, 120 Å) column in a reversed-phase liquid chromatography with a water:acetonitrile (v/v) gradient elution was used. The mobile phase consisted of an initial composition of 60% acetonitrile (held for 6 min) that, after 15 min, reached 90% (held for 10 min) and then returned to initial conditions. The duration of the analysis was 31 min with the equilibrium time condition of 9 min. The flow rate was 0.3 mL min^{-1} at 40 °C. The wet weight (w.w.) of fish tissues and sediment was corrected to d.w. after determination of percent humidity in the samples.

2.6 Lipid analysis

The lipid content of muscle, liver and gills was estimated in a group of sole (n = 6 per tissue) by microwave-assisted extraction MARS 5 (CEM Corporation, Matthews, NC, USA) using 15 mL of a 2:1 petroleum ether/acetone (v/v) solvent mixture and 0.5 g of Na_2SO_2 (Truzzi et al., 2017; 2018). The amount of extracted fat was determined by the gravimetric method. The laboratory analytical balance was a Model AT261 apparatus from Mettler Toledo (Greifensee, Switzerland), which has a readability of 0.01mg and repeatability as SD of 0.015 mg. Its accuracy was tested using two certified reference ‘‘weights’’ (OIML class E1) of 100 and 10 mg (both with a tolerance as 2SD of _0.0020 mg).

2.7 Quality control

For the quality control, the procedural blanks were analyzed and the external standard multipoint calibration technique (from 0.05-1.00 to 1.00-20.00 µg/mL) was used to determine the linear response interval of the detector; the average of the correlation coefficients was 0.997 for all analytes. PAHs were identified by comparison of their retention time with those of the authentic standards. The detection limit (LOD) and the quantification limit (LOQ) was calculated for each PAH using the following equations, according to ICH Q2B (ICH, 2005):

$LOD = 3.3\ S_a/b$ and $LOQ = 10\ S_a/b$

where S_a is the standard deviation of the intercept of the regression line and b is the slope of the calibration curve (Truzzi et al., 2014). The LODs and LOQs ranged from 0.02 (BkF and BaP) to 1.20 (Nap) ng mL^{-1} and from 0.05 (BkF) to 4.00 (Nap) ng mL^{-1}, respectively.

The whole analytical procedure was validated by analyzing the reference materials (IAEA code 106) and the recovery fell with the confidence interval of 95%. In addition, recovery rates were obtained for each congener PAH from fortified fish tissue samples (n = 5); these samples were extracted and analyzed by the procedure described above. The range of percentage of recoveries in fortified fish tissue was 50-100%, 53-98%, and 48-96% for muscle, liver and gills, respectively.

For sediment samples, the reference materials used were IAEA code 408 and IAEA code 383 and the recovery percentage ranged from 53 to 88%and from 61 to 82%, respectively, for IAEA -383 and IAEA -408.

2.8 Data and statistical analysis

Statistical analysis of method performance data, particularly the evaluation of the linearity range and LOD and LOQ quantification, was performed using Statgraphics Plus software, version 5.1 (Statgraphics, 2000). A *p* value lower than 0.05 was considered to indicate significance. PAHs were

grouped in three different categories according to Mashroofeh et al. (2015): low molecular weight PAHs (LMW-PAHs) including 2-3 ring PAHs (naphthalene, (Nap), acenaphthene (Ace), Fluorene (Fl), phenanthrene (Phe), Anthracene (Ant); moderate molecular weight PAHs (MMW-PAHs) including 4 ring PAHs (fluoranthene (Flu), pyrene (Pyr), benzo[*a*]anthracene (BaA), chrysene (Chr),); and high molecular weight PAHs (HMW-PAHs) including 5-6 ring PAHs (benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), dibenzo(a,h)antracene(DahA), indeno[1,2,3,-*cd*]perylene + benzo[*ghi*]perylene (InP+BghiP).

3 Results and discussions

3.1 Fish data

The basic data obtained from the sole individuals caught in three areas of the northern Adriatic Sea are presented in Table b. The morphometric data exhibited no significant differences in the two environmental transition areas, whereas the individuals from the third site, off Chioggia, were significantly heavier and longer ($r = 0.93$, $p < 0.05$). These data agree with the spatial distribution of sole reported by Grati et al. (2013) in the Adriatic Sea, who found that *S. solea* juveniles are mostly concentrated in the north-western Adriatic Sea along the Italian coast down to 30 m depth and around the mouth of River Po, whereas the main aggregation grounds of adults are in the central Adriatic, from the inshore waters in the north to the deeper waters (70 m maximum) in the south. No adults were recorded around the Po estuary in the present study. As expected, length and weight always positively correlated in all areas ($r > 0.88$, $p < 0.05$).

3.2 PAHs in fish tissues

The content in individual and total PAHs was measured in muscle, liver and gill tissue of 48 common sole individuals caught in the Venetian Lagoon, the Po Delta, and off Chioggia (Table c). Of the Σ16PAHs, acenaphthylene could not be analyzed with FLD due to lack of fluorescence, whereas, acenaphthene, fluorene, and anthracene were not available. Dibenzo(a,h)anthracene, benzo(g,h,i)perylene, and indeno(1,2,3-cd)pyrene were below the LOQ (0.03-0.05ng mL^{-1}).

The average total PAH concentration was 6.7 ng g^{-1} d.w. (range: 1.3-16.3 ng g^{-1} d.w.), 13.1 ng g^{-1} d.w. (range: 2.1-31.8 ng g^{-1} d.w.), and 32.0 ng g^{-1} d.w. (range: 2.0-112.6 ng g^{-1}d.w.), in muscle, liver and gill tissue, respectively. The highest and lowest concentration in muscle were found in sole caught respectively in the Po Delta (8.06 ± 3.2 ng g^{-1} d.w.) and off Chioggia (4.5 ± 2.9 ng g^{-1} d.w.). The highest and lowest concentrations in liver were measured respectively in the Venetian Lagoon

(16.06 ± 4.6ng g^{-1} d.w.) and off Chioggia (9.5 ± 6.4ng g^{-1} d.w.). The highest and lowest concentrations in gill tissue were recorded respectively in the Venetian Lagoon (39.5 ± 17.7ng g^{-1} d.w.) and off Chioggia (19.1 ± 11.1 ng g^{-1}d.w.). As regards the maximum admissible BaP levels in fish muscle (2 ng g^{-1} w.w; EC Regulation No. 835/2011 and Regulation No. 1327/2014), BaP was detected in 4% of samples at an average concentration that was below the limit (from < LOQ to 0.05 ng g^{-1} d.w.).

Although several studies have investigated fish and seafood pollution, they have tended to focus on edible tissues and the risk for human health, whereas limited data are available on contaminant bioaccumulation and on the comparison of their content in fish tissue and local sediments (Sagratini et al., 2008; Sun et al., 2016; Moraleda-Cibriàn et al., 2015). A comparison of the PAH tissue concentrations found in the present study and those reported in previous investigations in the Mediterranean Sea was performed, taking into account that data comparability could be hampered by methodological differences, the compounds studied, and concentration reporting (Vives et al., 2004). Overall, our PAH levels were comparable to those found in *S. solea* muscle tissue from the north-western Adriatic (Trisciani et al., 2011; 3.5-3.9 ng g^{-1}d.w.). Similar data have also been reported by Perugini et al. (2007) for other marine organisms (red mullet, 16.52 ng g^{-1} w.w.) from the Adriatic Sea, and by Ferrante et al. (2018) for common sole from the Gulf of Catania (Mediterranean Sea;20-38 ng g^{-1} w.w.). Slightly higher PAH concentrations (from 125.4 to 231.5 ng g^{-1} w.w.) were found by Storelli et al. (2013) for brown ray caught in the Adriatic Sea.

PAH composition patterns according to their molecular weight (LMW-, MMW-, and HMW-PAHs) showed a very similar distribution of PAH groups in fish tissue, in agreement with Xu et al. (2011) and with other studies of the accumulation of other organic contaminants (PCB) in several fish tissues (Bodiguel et al., 2009; Monosson et al., 2003). The present data demonstrated a preferential accumulation of MMW-PAHs, accounting for about 80% of total PAHs. PY was the dominant MMW-PAH congener, with 72.1%, 64.7%, and 68.6% of total PAHs for muscle, liver and gills, respectively. Previous studies of marine organisms from the Adriatic Sea have found the same predominance of 3 or 4 ring PAHs, with a dominance of PHE and PY congeners (Perugini et al., 2007; Storelli et al., 2013; Capolupo et al., 2017). However, the three groups of PAHs in terms of frequency of detection in all fish tissues sampled were LMW (100%), MMW (52%), and HMW (9%). Considering that PAH solubility decreases in inverse proportion to molecular weight (Porte and Albaigés, 1993), LMW-PAHs, due to their low Log K_{ow} (< 5), higher bioavailability, and lower depuration rate, tend to have a higher uptake compared with HMW-PAHs (Sun et al., 2016; León et al., 2014). In addition, LMW-PAHs (e.g. Naphthalene) are not metabolized in the liver of some species of fish as efficiently as HMW-PAHs (Varanasi and Gmur, 1981; Broman et al., 1990;

Meador and Stein, 1995; Mashroofeh et al., 2015; Morelada-Cibrián et al., 2015). For this reason, the presence of LMW-PAHs has been widely documented in various studies of polluted fish where NA and PHE were the dominant PAH congeners, followed by MMW-PAHs, where Flu and PY were predominant, while HMW-PAHs were usually very low or undetectable (Mashroofeh et al., 2015; Morelada-Cibrián et al., 2015; Ke et al., 2017).

Molecular indices based on the ratio of selected PAHs have been widely used to differentiate PAHs from pyrogenic and petrogenic origins, to gain insight into PAH sources in fish samples (Soclo et al., 2000; Yunker et al., 2002; Zhao et al., 2014). Although in the present study the LMW/HMW ratio (where MMW-PAHs were included in HMW-PAHs) in *S. solea* liver, gill, and muscle was less than 1 (range, 0.12-0.21), which shows that the source of PAHs was pyrolytic, the Flu/(Flu+Pyr) (< 0.4) and Flu/Pyr (< 1) ratio suggested a petrogenic origin of PAHs. In addition, the ratios of combPAHs/∑PAHs, namely the sum of combustion-originated PAHs (Flt, Pyr, BaA, Chr, BbF, BkF, BaP, InP and BgP) to total PAHs, were less than 1 (range, 0.88-0.97), confirming a prevalence of petrogenic PAHs in fish tissue from all sampling areas. The ratio used to discriminate the origin of PAHs was similar in all three study areas. This finding was in line with other studies, where the main sources of PAHs found in marine organisms were petrogenic (Bandowe et al., 2014; Mashroofeh et al., 2015; Signa et al., 2015;).

3.3 PAHs in surface sediments

Marine surface sediments were investigated because they may constitute a major sink for many POPs introduced into the aquatic environment (Guerra, 2012; Solé et al., 2013; Marini and Frapiccini, 2014; Baumard et al., 2015) as well as a key matrix in the contamination of fish living in contact with sediment (Cheung et al., 2007; Solé et al., 2013). The sediment concentrations of individual and total PAHs were also measured, to understand how the sampling areas affected their bioaccumulation in fish tissues (Table d). All compounds were detectable in sediment samples except for acenaphthylene, acenaphthene, fluorene and anthracene, which were not available. PAH concentrations were highest in the Venetian Lagoon and the Po Delta, where mean total PAH concentrations were 979.8±2.2 and 625.1±62.3 ng g^{-1} d.w., respectively, whereas off Chioggia they were 58.5±2 ng g^{-1} d.w. The Venetian Lagoon is a highly urbanized and industrialized environmental transition area, where the anthropogenic sources (e.g. urban activities, boat traffic, agriculture, and tourism) can induce PAH accumulation. Similar PAH concentrations have been measured close to the Venetian Lagoon by Frignani et al.(2003), Acquavita et al. (2014), Parolini et al. (2010), and Secco et al. (2005), who reported concentrations ranging respectively from 71 to 730, from 66 to 734, from 15 to 389, and from 20 to 502 ng g^{-1} d.w. High PAH concentrations have

also been described in the Po Delta sediments, maybe due to large amounts of PAH-containing suspended particles brought to this area by the River Po and its effluents (Webster et al., 2011; Sun et al., 2016)**.** The PAH levels measured in our study were comparable to those found by Notar et al. (2001; 30-600 ng g^{-1}d.w.) and Magi et al. (2002; 313-455 ng g^{-1}d.w.). The lowest concentrations were found off Chioggia (57.0 ng g^{-1}, site 56), possibly due to the distance of the sampling sites from the Italian coast (40 km) and, therefore, from the main PAH inputs from the coast. In this area, Magi et al. (2002) have found values similar to ours (24-183 ng g^{-1}d.w.).

Analysis of PAH sediment concentrations based on molecular weight showed that they were dominated by 4 ring PAHs (MMW-PAHs), which accounted for 72.5%, 47.7%, and 64.7% in the Venetian Lagoon, off Chioggia and the Po Delta, respectively. Similar data have been described in this region (Notar et al., 2001; Alebic-Juretic, 2011; Acquavita et al., 2014). Fluoranthene was the dominant congener in sediments from the Venetian Lagoon and the Po Delta, accounting for 38.6% and 37.5% of total PAHs, respectively. Naphthalene was the first-ranking congener (43.1%) off Chioggia. Therefore, the contribution of LMW-PAHs off Chioggia (46.9% of total PAHs) was higher than the one recorded in the two transition areas (19.4% in the Venetian Lagoon and 26.1% in the Po Delta). As regards the combustion-related PAHs (Pyr, BaA, Chr, BbF, BkF, BaP, InP, and BgP), they accounted for 80.6%, 53.1%, and 73.9% of total PAHs in the Venetian Lagoon, off Chioggia, and in the Po Delta, respectively. The molecular indices of PAHs proposed by various authors (Baumard et al., 1998; Soclo et al., 2000; Yunker et al., 2002; Bajet, 2008) were used in this study to assess the PAH sources and to discriminate their pyrogenic or petrogenic origin. This is important, because source and physicochemical properties largely determine the transport, bioavailability and biodegradation rates of these hydrocarbons in the marine environment (Gogou et al., 2000). Usually, petroleum contains higher phenanthrene quantities (Magi et al., 2002), whereas high levels of LMW- or HMW-PAHs are characteristic of hydrocarbon mixtures formed during combustion of fossil fuels (Gogou et al., 2000; Barhoumi et al., 2016).

Although high LMW-PAHs values were measured off Chioggia, all study areas showed a primarily pyrolytic origin of PAHs, since LMW/HMW was < 1, Flu/(Flu+Pyr) was > 0.4 and Flu/Pyr was > 1, confirming the results of previous studies carried out in the same areas by Acquavita et al. (2014) and Magi et al. (2002).

3.4 Influence on PAH bioaccumulation by determining factors

3.4.1 Lipid content

Total lipid content was determined in muscle, liver and gills of some *S. solea* individuals (n = 6 per tissue). The lipid percentage range was 1.9-2.1, 14.6-15.8, and 8.7-10.6 in muscle, liver and gill, respectively. The lowest values were found in muscle, followed by gill and liver, similar to Xu et al. (2011). Other studies have recorded analogous results in muscle, liver and gills of common sole from the Adriatic Sea and the Mediterranean Sea (Sagrantini et al., 2008; Ameur et al., 2013). Lipids are the main energy sources; for this reason the liver, which shows the highest lipid content, can be considered as an energy storage organ (Bodiguel et al., 2009). The influence of lipids on PAH concentration was examined by comparing the total lipid content (Log(lipid)%) and total PAH concentrations (Log(PAHs)ng g^{-1}) found in tissue from soles caught in the three study areas. Since PAHs are lipophilic, they preferentially accumulate in tissues with higher lipid content, such as liver (Zhao et al., 2014). Therefore, a positive relationship between lipid content and total PAH levels was found in the three tissues for all study areas, although the degree of significance was not very strong (r = 0.34-0.66, $p > 0.05$). Indeed, PAHs have been reported to have a greater ability to bioaccumulate in liver compared with muscle tissue, whose influence on lipid content is lower (Sagratini et al., 2008; Mashroofeh et al., 2015). However, a considerable PAH bioaccumulation was found in gill tissue, although it was lower than in liver. This indicates that lipid content might not be the only determinant of PAH bioaccumulation in gill tissue (Sagratini et al., 2008). Although lipids have often been correlated with concentrations of lipophilic contaminants such as PAHs (Xu et al., 2011; Mashroofeh et al., 2015), various studies have found no relationship between lipid content and POPs (Miranda et al., 2008; León et al., 2014; Zhao et al., 2014a; Zhao et al., 2014b; Sun et al., 2016).

3.4.2 K_{ow}

To explore the mechanism of PAH bioaccumulation in fish tissue, a correlation between the log-transformed concentration of individual PAHs and the respective log K_{ow} values was performed for each type of tissue. A negative relationship was found between Log K_{ow} and log PAH residual in the different tissues of *S. solea*. PAH concentrations in fish gills correlated significantly with their K_{ow} values (r = 0.69-0.98, $p < 0.05$) in the common sole individuals collected from the Venetian Lagoon, the Po Delta and off Chioggia. Although the same trend was seen in muscle and liver, it was not significant (r = 0.49-0.64 for muscle and r = 0.43-0.65 for liver, $p > 0.05$). This finding suggests that a quota of PAHs accumulating in fish gill depends on PAH physicochemical features, such as equilibrium partition. Moreover, direct exchange with the water and sediment through the gills act as a major bioaccumulation mechanism in common sole (Bandowe et al.,2014; Mashroofeh et al., 2015). In addition, the lack of significance of the correlation in muscle and liver could

indicate that in these tissues PAHs have undergone a degree of enzymatic transformation due to depuration. In fact, the liver is the main detoxifying tissue and contains relatively high levels of detoxifying enzymes (León et al., 2013; Nagaraju et al., 2014; Sun et al., 2016).

3.4.3 Body size

As mentioned above, the *S. solea* individuals collected off Chioggia were longer and heavier than those caught in the other two sampling areas. As expected, length and weight always correlated positively and significantly in all areas ($r = 0.93$, $p < 0.05$). A moderately strong, negative correlation between body size and PAH concentrations was seen in liver (the correlation coefficient ranged from -0.60 to -0.72) and gill (range, from -0.54 to -0.80) in the individuals collected from the Po Delta and off Chioggia ($p < 0.05$). As regards muscle, there was a relatively weak relationship between body size and PAH concentrations, whereas there was a negative but not significant relationship between body size and PAH concentrations in the three tissue types from sole caught in the Venetian Lagoon. PAH tissue concentrations decreased with the increase in body size, in line with the data reported by León et al. (2014) on red mullet from the Mediterranean coast of Spain and with Pellini et al. (2018), who highlighted that the abundance of microplastic particles, which may adsorb persistent hydrophobic compounds such as PAHs, decreased with increasing sole body size in the Adriatic Sea. In fact, a reduced induction of xenobiotic biotransformation processes with age has been documented in fish (Whyte et al., 2000; Coulillard et al., 2004), as has a relatively low resistance of PAHs to biotransformation and a high depuration rates by adult organisms (Bodiguel et al., 2009).

3.4.4 Relationship between PAH concentrations in tissue and sediment samples

The tissue bioaccumulation of total PAHs in each area (all tissue types) and their mean concentration in each of the three tissue types at each site were measured to seek a relationship between tissue and sediment concentrations.

There were some statistically significant differences. Statistical analysis of total PAH concentrations in muscle, liver and gills from the same site (3 tissues, one site) showed that differences between muscle and liver concentration were not significant ($p > 0.05$), whereas both values were significantly different from those found in gills ($p < 0.05$). Notably, in the specimens caught in the Venetian Lagoon the three tissues showed significant differences from one another ($p < 0.05$). Box and whisker plots demonstrated a different PAH bioaccumulation in the three tissues and a significantly different PAH bioaccumulation in gills compared to liver and muscle (Fig. 2). These findings indicate a significantly higher PAH accumulation in gills, followed by liver and

muscle. This trend is consistent with earlier reports of bioaccumulation of other organic compounds in marine organisms (Vives et al., 2004; Liang et al., 2007; Yang et al., 2007; Miranda et al., 2008). In particular, the relatively lower PAH concentrations found in muscle at all sites demonstrate that muscle is not a target organ for PAH accumulation due to its lower lipid content and low PAH transfer (Zhao et al., 2014). A greater PAH bioaccumulation has been reported in more lipophilic matrices, such as liver, even though in this organ PAH compounds undergo efficient metabolization and metabolite formation (Miranda et al., 2008; Ballesteros et al. 2011; Lazartigues et al. 2013,). Therefore, the matrix with higher PAH bioaccumulation and bioconcentration was gill tissue, confirming earlier reports that the gills are the primary tissue for pollutant accumulation through gill-water and sediment transfer. Furthermore, gill tissue is more closely associated with particulate matter, and consequently reflects the concentration of contaminants in sediment and water (Yang et al., 2007; Bandowe et al., 2014; Zhao et al., 2014).

With regard to the mean PAH concentration measured in each tissue type at each site (one tissue, 3 sites), the box and whisker plots highlighted a statistically significant difference between the Venetian Lagoon and the site off Chioggia for liver and gills ($p < 0.05$), whereas analysis of the data from muscle showed statistically significant differences between the Po Delta and the site off Chioggia ($p < 0.05$). Notably, the PAH values found in all three tissue types from the latter site were the lowest. This finding can be explained by the movements and feeding habits of common sole, since the site off Chioggia was populated mainly by adult soles, which have different feeding strategies and behaviors compared to young individuals. However, the main reason is that it is the least contaminated of the three sites in terms of PAH sediment concentrations due to its distance from the more impacted coast. This finding suggests that the factors determining PAH accumulation are closely related to the PAH concentrations in the sediment where the fish were collected and to the local pollution load, as also suggested by several authors (Vives et al., 2004; León et al., 2014).

Conclusion

A greater knowledge of PAH content, spatial distribution patterns, and bioaccumulation in marine ecosystems is important to establish their fate and impact on edible marine organisms and human health. In this study, PAH accumulation was evaluated simultaneously in common sole from the northern Adriatic Sea and in the sediment where they lived. *S. solea* was selected to investigate the factors that are involved in PAH bioaccumulation because it is a demersal fish that lives in close contact with sediment, which is considered as a final sink for pollutants. Total PAHs were highest in gill tissue, followed by liver and muscle. PAH bioaccumulation in gills was significantly

dependent on the characteristics of the contaminant, in particular K_{ow}. The weak correlations between PAHs and the lipid content of tissues and PAH concentrations in relation to body size could mean that none of these factors was significantly related to PAH bioaccumulation in tissues. The impact of PAHs was higher on biota and sediment from the coastal areas (the Venetian Lagoon and the Po Delta), which are characterized by dense human settlements and activities as well as by direct and indirect discharges. This could explain the bioaccumulation of these compounds in fish in relation to the levels of PAHs found in the surrounding sediments.

Acknowledgements

The study was supported by the Italian Ministry of Agriculture and Forestry, the FAO-ADRIAMED regional project, and the MSFD. The authors are grateful to all the participants involved in the field work and to the crew of R/V G. Dallaporta.

References

Acquavita, A., Falomo, J., Predonzani, S., Tamberlich, F., Bettoso, N., Mattassi, G., 2014. The PAH level, distribution and composition in surface sediments from a Mediterranean Lagoon: The Marano and Grado Lagoon (Northern Adriatic Sea, Italy). Mar. Poll. Bull. 81, 234–241.

Albinet, A., Tomaz, S., Lestremau, F., 2013. A really quick easy cheap effective rugged and safe (QuEChERS) extraction procedure for the analysis of particle-bound PAHs in ambient air and emission samples. Sci. Total Environ. 450–451, 31–38

Alebic-Juretic, A., 2011. Polycyclic aromatic hydrocarbons in marine sediments from the Rijeka Bay area, Northern Adriatic, Croatia, 1998–2006. Mar. Poll. Bull. 62, 863–869.

Ameur, W.B., El Megdiche, Y., Eljarrat, E., Ben Hassine, S.B., Badreddine, B., Souad, T., Bèchir, H., Barcelò, D., Driss, M.R., 2013. Organochlorine and organobromine compounds in a benthic fish (Solea solea) from Bizerte Lagoon (northern Tunisia): Implications for human exposure. Ecotox. Environ. Safe. 88, 55–64.

Amodio Cocchieri, R., Arnese, A., Minicucci, A.M., 1990. Polycyclic Aromatic Hydrocarbons in Marine Organisms from Italian Central Mediterranean Coasts. Mar Poll. Bull. 21, 15-18.

Bajt, O., 2008. The impact of road traffic on hydrocarbon content in the sediments of the Škocjan wetland. Ann. Ser. Hist. Nat. 18, 41–46.

Ballesteros, M.L., Gonzalez, M., Wunderlin, D.A., Bistoni, M.A., Miglioranza, K.S.B., 2011. Uptake, tissue distribution, and metabolism of the insecticide endosulfan in Jenynsia multidentata (Anablepidae, Cyprinodontiformes). Environ. Pollut. 159:1709–1714.

Barhoumi, B., El Megdiche, Y., Clérandeau, C., Ben Ameur, W., Mekni, S., Bouabdallah, S., Derouiche, A., Touil, S., Cachot, J., Ridha Driss, M., 2016. Occurrence of polycyclic aromatic

hydrocarbons (PAHs) in mussel (*Mytilus galloprovincialis*) and eel (*Anguilla anguilla*) from Bizerte lagoon, Tunisia, and associated human health risk assessment. Contin. Shelf Res. 124, 104–116

Baumard, P., Budzinski, H., Garrigues, P., 1998. Polycyclic aromatic hydrocarbons in sediments and mussels of the western Mediterranean Sea. Environ. Toxicol. Chem. 17,765–776.

Baumard, P., Budzinski, H., Garrigues, P., Narbonne, J.F., Burgeot, T., Michel, X., Bellocq, J., 1999. Polycyclic aromatic hydrocarbon (PAH) burden of mussels (Mytilus sp.) in different marine environments in relation with sediment PAH contamination, and bioavailability. Mar. Environ. Res. 47,415–439.

Bandowe, B.A.M., Bigalke, M., Boamah, L., Nyarko, E., Kwesi Saalia, F., Wilcke, W., 2014. Polycyclic aromatic compounds (PAHs and oxygenated PAHs) and tracemetals in fish species from Ghana (West Africa): Bioaccumulation and health risk assessment. Environ. Intern. 65, 135–146.

Bodiguel, X., Loizeau, V., Le Guellec, A.M., Roupsard, F., Philippon, X., Mellon-Duval, Ca., 2009. Influence of sex, maturity and reproduction on PCB and p,p′DDE concentrations and repartitions in the European hake (Merluccius merluccius, L.) from the Gulf of Lions (N.W. Mediterranean). Sci. Total Environ. 408, 304–311.

Bodin, N., Tapie, N., Le Ménach, K., Chassot, E. , Elie, P., Rochard, E., Budzinski, H., 2014. PCB contamination in fish community from the Gironde Estuary (France): Blast from the past. Chemosphere, 98, 66–72.

Broman, D., Näuf, C., Lundbergh, I., Zebühr, Y., 1990. An in situ study on the distribution, biotransformation and flux of polycyclic aromatic hydrocarbons (pahs) in an aquatic food chain (seston-Mytilus edulis L.-Somateriamollissima L.) from the baltic: an ecotoxicological perspective. Environ. Toxicol. Chem. 9, 429–442.

Campanelli, A., Fornasiero, P., Marini, M., 2004. Physical and chemical characterization of water column in the Piceno coastal area (Adriatic Sea). Fresen. Environ. Bull. 13, 430–435.

Campanelli, A., Grilli, F., Paschini, E., Marini, M., 2011. The influence of an exceptional Po River flood on the physical and chemical oceanographic properties of the Adriatic Sea. Dynam. Atmos. Oceans. 52, 284– 297.

Capolupo, M., Franzellitti, S., Kiwan, A., Valbonesi, P., Dinelli, E., Pignotti, E., Birke, M., Fabbri. E., A comprehensive evaluation of the enviromental quality of a coastal lagoon (Ravenna, Italy): Integrating chemical and physiological analyses in mussels as a biomonitoring strategy. Sci. Total Environ. 598, 146-159.

Castellarin, A., Di Baldassarre, G., Brath, A., 2011. Floodplain management strategies for flood attenuation in the River Po. River Res. Appl. 27, 1037–1047.

Cheung, K.C., Leung, H.M., Kong, K.Y., Wong M.H., 2007. Residual levels of DDTs and PAHs in freshwater and marine fish form Hong Kong markets and their health risk assessment. Chemosphere. 66, 460-468.

Chu, W., Chan, K.H., 2000. The prediction of partitioning coefficients for chemicals causing environmental concern. Sci. Total Environ. 248, 1-10.

Claireaux, G., Davoodi, F., 2002. Effect of exposure to petroleum hydrocarbons upon cardio-respiratory function in the common sole (Solea solea). Aquat. Toxicol. 98, 113–119.

Cocci, P., Mosconi, G., Bracchetti, L., Nalocca, J.M., Frapiccini, E., Marini, M., Caprioli, G., Sagratini, G., Palermo, F.A., 2018. Investigating the potential impact of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) on gene biomarker expression and global DNA methylation in loggerhead sea turtles (Caretta caretta) from the Adriatic Sea. Sci. Total Environ. 619–620, 49–57.

Corsi, I., Mariottini, M., Menchi, V., Sensini, C., Balocchi, C., Focardi, S., 2002. Monitoring a Marine Coastal Area: use of Mytilus galloprovincialis and Mullus barbatus as bioindicators. Mar. Ecol. 23, Supplement 1, 138-153.

Coulillard, C.M., Wirgin, I.I., Lebeuf, M., Légaré, B., 2004. Reduction of cytochrome P4501A with age in Atlantic tomcod from the St. Lawrence Estuary, Canada: relationship with emaciation and possible effect of contamination. Aquat. Toxicol. 68: 233–247.

Council Directive 2008/56/EC on Establishing a Framework for Community Action in Field of Marine Environmental Policy (Marine Strategy Framework Directive). EC. 2008.

Dominguez, A.A., Law, R.J., Herzke, D., de Boer, J., 2011. Bioaccumulation of brominated flame retardants. In: Eljarrat E, Barcelo D, editors. Brominated flame retardants. Handbook of environmental chemistry Berlin: Springer-Verlag; p. 141–85.

Ferrante, M., Cristaldi, A., Copat, C., Grasso, A., Fiore, M., Signorelli, S.S., Zuccarello, P., Conti, G.O., 2018. PAHs in seafood from the Mediterranean Sea: An exposure risk assessment, Food Chem. Toxicol. doi: 10.1016/j.fct.2018.03.024.

Frapiccini, E., Marini, M., 2015. Polycyclic Aromatic Hydrocarbon Degradation and Sorption Parameters in Coastal and Open-Sea Sediment. Water Air Soil Pollut. 226-246. DOI 10.1007/s11270-015-2510-7

Frignani, M., Bellucci, L.G., Favotto, M., Albertazzi, S., 2003. Polycyclic aromatic hydrocarbons in sediments of the Venice Lagoon. Hydrobiologia. 494, 283–290.

Galgani, F., Martínez-Gómez, C., Giovanardi, F., Romanelli, G.,, Caixach, J., Cento, A., Scarpato, A., Ben Brahim, S., Messaoudi, S., Deudero, S., Boulahdid, M., Benedicto, J., Andra, B., Assessment of polycyclic aromatic hydrocarbon concentrations in mussels (*Mytilus galloprovincialis*) from the Western basin of the Mediterranean Sea. Environ. Monit. Assess. DOI 10.1007/s10661-010-1335-5

Gogou, A., Bouloubassi, I., Stephanou, E.G., 2000. Marine organic geochemistry of the Eastern Mediterranean: Aliphatic and polyaromatic hydrocarbons in Cretan Sea surficial sediments. Mar. Chem. 68, 265–282

Gonçalves, C., Martins, M., Diniz, M.S., Costa, M.H., Caeiro, S., Costa, P.M., 2014. May sediment contamination be xenoestrogenic to benthic fish? A case study with Solea senegalensis. Mar. Environ. Res. 99, 170-178.

Grati, F., Scarcella, G., Polidori, P., Domenichetti, F., Bolognini, L., Gramolini, R., Vasapollo, C., Giovanardi, O., Raicevich, S., Celić, I., Vrgoč, N., Isajlovic, I., Jenič A., Marčeta, B., Fabi, G., 2013. Multi-annual investigation of the spatial distributions of juvenile and adult sole (Solea solea L.) in the Adriatic Sea (northern Mediterranean). J Sea Res. 84, 122–132.

Guerra, R., 2012. Polycyclic Aromatic Hydrocarbons, Polychlorinated Biphenyls and Trace Metals in Sediments from a Coastal Lagoon (Northern Adriatic, Italy). Wat. Air Soil Poll. 223, 85–98. DOI 10.1007/s11270-011-0841-6

Hussein, I., Abdel-Shafy, Mona,S.M., 2016. A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. Mansour Egyptian J. Petrol. 25, 107–123.

ICH. Harmonized tripartite guideline, validation of analytical procedure: Text and methodologies, Q2 (R1), current step 4 version. (2005). Parent guidelines on methodology dated November 6: 1996, incorporated in November 2005. through <http://www.ich.org/LOB/media/MEIDA417.pdf> Accessed 9.06.10.

Ke, C.L., Gu, Y.G., Liu, Q., Li, L.D., Huang, H.H., Cai, N., Sun, Z.W., 2017. Polycyclic aromatic hydrocarbons (PAHs) in wild marine organisms from South China Sea: Occurrence, sources, and human health implications. Mar. Poll. Bull. 117, 507–511.

Koelmans, A.A.; Poot, A.; Lange, H.J.D.; Velzeboer, I.; Harmsen, J.; 2010. Noort, P.C.V. Estimation of in situ sediment-to-water fluxes of polycyclic aromatic hydrocarbons, polychlorobiphenyls and polybrominateddiphenyl ethers. Environ. Sci. Technol. 44, 3014–3020.

Lazartigues, A., Thomas, M., Cren-Olivé, C., Brun-Bellut, J., Le Roux, Y., Banas, D., Feidt, C., 2013. Pesticide pressure and fish farming in barrage pond in Northeastern France. Part II: residues of 13 pesticides in water, sediments, edible fish and their relationships. Environ. Sci. Pollut. Res. 20, 117–125.

León, V.M., García, I., Martínez-Gómez, C., Campillo, J.A., Benedicto, J., 2014. Heterogeneous distribution of polycyclic aromatic hydrocarbons in surface sediments and red mullet along the Spanish Mediterranean coast. Mar. Poll. Bull. 87, 352–363.

Liang, Y., Tse, M.F., Young, L., Wong, M..H., 2007. Distribution patterns of polycyclic aromatic hydrocarbons (PAHs) in the sediments and fish at Mai Po Marshes Nature Reserve, Hong Kong. Wat. Res. 41, 1303-1311.

Lima, A.L.C., Eglinton, T.I., Reddy, C.M., 2003. High-resolution record of pyrogenic polycyclic aromatic hydrocarbon deposition during the 20th century. Environ. Sci. Technol. 37 (1), 53–61.

Magi, E., Bianco, R., Ianni, C., Di Carro, M., 2002. Distribution of polycyclic aromatic hydrocarbons in the sediments of the Adriatic Sea. Environ. Poll. 119, 91–98.

Manzanera, M., Bartolomé, A., Tort, Ll., Caixach, J., 2013. Persistent organic pollutants (POPs) in sediments from fishing grounds in the NW Mediterranean: Ecotoxicological implications for the benthic fish Solea sp. M. Solé. Mar. Poll. Bull. 67, 158–165.

Marini, M., Frapiccini, E., 2013. Persistence of polycyclic aromatic hydrocarbons in sediments in the deeper area of the Northern Adriatic Sea (Mediterranean Sea). Chemosphere. 90, 1839–1846.

Marini, M., Frapiccini, E., 2014. Do lagoon area sediments act as traps for polycyclic aromatic hydrocarbons? Chemosphere. 111, 80–88.

Marini, M., Jones, B. H., Campanelli, A., Grilli, F., Lee, C. M. 2008. Seasonal variability and Po River plume influence on biochemical properties along western Adriatic coast. J. Geophys. Res. 113, C05S90.

Mashroofeh, A., Bakhtiari, A.R., Pourkazemi, M., 2015. Distribution and composition pattern of polycyclic aromatic hydrocarbons in different tissues of sturgeons collected from Iranian coastline of the Caspian Sea. Chemosphere. 120, 575–583.

Meador, J., Stein, J., Reichert, W.L., Varanasi, U., 1995. Bioaccumulation of polycyclic aromatic hydrocarbons by marine organisms. Rev. Environ. Contam. Toxicol. 143, 79–165.

Miranda, A.L., Roche, H., Randi, M.A.F., Menezes, M.L., Oliveira Ribeiro C.A., 2008. Bioaccumulation of chlorinated pesticides and PCBs in the tropical freshwater fish Hopliasmalabaricus: Histopathological, physiological, and immunological findings. Environ. Intern. 34: 939–949

Monosson, E., Ashley, J.T.F., Mc Elroy, A.E., Woltering, D., Elskus, A.A., 2003. PCB congener distributions in muscle, liver and gonad of *Fundulus heteroclitus* from the lower Hudson River Estuary and Newark Bay. Chemosphere. 52, 777-787.

Moraleda-Cibrián, N., Carrassón, M., Rosell-Melé, A., 2015. Polycyclic aromatic hydrocarbons, polychlorinated biphenyls and organochlorine pesticides in European hake (*Merluccius merluccius*) muscle from the Western Mediterranean Sea. Mar. Poll. Bull. 95, 513–519.

Morrison, S.A., Sieve , K.K., Ratajczak , R.E. Bringolf , R.B., Belden, J.B., 2016. Simultaneous extraction and cleanup of high-lipid organs from white sturgeon (Acipensertransmontanus) for multiple legacy and emerging organic contaminants using QuEChERS sample preparation. Talanta. 146, 16–22.

Nagaraju, B., VakitaVenkata Rathnamma, V.V., 2014. Gas liquid chromatography-flame ionization detector (GLC-FID) residue analysis of carbamate pesticide in fresh water fish *Labeo rohita*. Toxicol. Res. 3: 177.

Neff, J.M., Scott, S.A., Gunstert, D.G., 2005. Ecological risk assessment of polycyclic aromatic hydrocarbons in sediments: identifying sources and ecological hazards. Integr. Environ. Assess. Manag.1, 22–33.

Notar, M., Leskovšek, H., Faganeli, J., 2001. Composition, distribution and sources of polycyclic aromatic hydrocarbons in sediments of the Gulf of Trieste, Northern Adriatic Sea. Mar. Poll. Bull.42, 36–44.

Pandey, S.K., Kim, K.H., Brown, R.J.C., 2011. A review of techniques for the determination of polycyclic aromatic hydrocarbons in air, TrAC-Trend. Anal. Chem. 30, 1716–1739.

Parolini, M., Binelli, A., Matozzo, V., Marin, M.G., 2010. Persistent organic pollutants in sediments from the Lagoon of Venice—a possible hazard for sediment dwelling organisms. J. Soils Sedim. 10, 1362–1379.

Pellini, G., Gomiero, A., Fortibuoni, T., Ferrà, C., Grati, F., Tassetti, N., Polidori, P., Fabi, G., Scarcella, G., 2018. Characterization of microplastic litter in the gastrointestinal tract of Solea solea from the Adriatic Sea. Environ. Poll. 234, 943-952.

Perugini, M., Visciano, P., Giammarino, A., Manera, M., Di Nardo, W., Amorena M., 2007. Polycyclic aromatic hydrocarbons in marine organisms from the Adriatic Sea, Italy. Chemosphere. 66, 1904–1910.

Pfannkoch, E.A., Stuff, J.R., Whitecavage, J.A., Blevins, J.M., Seely, K.A., Moran, J.H., 2015. A High Throughput Method for Measuring Polycyclic Aromatic Hydrocarbons in Seafood Using QuEChERS Extraction and SBSE. Int. J. Anal. Chem. Volume, Article ID 359629, 8 pages http://dx.doi.org/10.1155/2015/359629

Porte, C., Albaigés, J., 1993. Bioaccumulation patterns of hydrocarbons and polychlorinated biphenyls in bivalves, crustacean and fishes. Arch. Environ. Contam. Toxicol. 26, 273–81.

Ramalhosa, M.J., Paîga, P., Morais, S., Delerue-Matos, C., Maria Beatriz Prior Pinto Oliveira., 2009. Analysis of polycyclic aromatic hydrocarbons in fish: evaluation of a quick, easy, cheap, effective, rugged, and safe extraction method. J. Sep. Sci. 32, 3529 – 3538.

Sagratini, G., Buccioni, M., Ciccarelli, C., Conti, P., Cristalli, G., Giardina, D., Lambertucci, C., Marucci, G., Volpini, R., Vittori, S., 2008. Levels of polychlorinated biphenyls in fish and shellfish from the Adriatic Sea. Food Addit. Contam. B. 1, 69–77.

Sapozhnikova, Y., Lehotay S. J., 2013. Multi-class, multi-residue analysis of pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, polybrominated diphenyl ethers and novel flame retardants in fish using fast, low-pressure gas chromatography–tandem mass spectrometry. Anal. Chim. Acta. 758, 80– 92.

Scarcella, G., Grati, F., Raicevich, S., Russo, T., Gramolini, R., Scott, R.D., Polidori, P., Domenichetti, F., Bolognini, L., Giovanardi, O., Celić, I., Sabatini, L., Vrgoč, N., Isajlović, I., Marčeta, B., Fabi, G., 2014. Common sole in the northern and central Adriatic Sea: Spatial management scenarios to rebuild the stock. J. Sea Res. 89, 12–22.

Secco, T., Pelizzato, F., Sfriso, A., Pavoni, B., 2005. The changing state of contamination in the Lagoon of Venice. Part 1: organic pollutants. Chemosphere. 58, 279–290.

Signa, G., Di Leonardo, R., Vaccaro, A., Tramati, C.D., Mazzola, A., Vizzini, S., 2015. Lipid and fatty acid biomarkers as proxies for environmentalcontamination in caged mussels Mytilus galloprovincialis. Ecol. Indic. 57, 384–394.

Soclo, H. H., Garrigues, P.H., Ewald, M., 2000. Origin of Polycyclic Aromatic Hydrocarbons (PAHs) in Coastal Marine Sediments: Case Studies in Cotonou (Benin) and Aquitaine (France)Areas. Mar. Poll. Bull. 40: 387-396.

Song C., Qiang F., Tian-xiao L., Ma, W.L., Liuand, D., Wang, M., 2016. Sediment-Water Exchange, Spatial Variations, and Ecological Risk Assessment of Polycyclic Aromatic Hydrocarbons (PAHs) in the Songhua River, China. Water. 8, 334; https://doi.org/10.3390/w8080334

Stogiannidis, E., Laane, R., 2015. Source Characterization of Polycyclic Aromatic Hydrocarbons by Using Their Molecular Indices: An Overview of Possibilities. Rev. Environ. Contam. T. 234, 49-133 https://doi.org/10.1007/978-3-319-10638-0_2

Storelli, M.M., Barone, G., Perrone, V.G., Storelli, A., 2013. Risk characterization for polycyclic aromatic hydrocarbons and toxic metals associated with fish consumption. J. Food Compo. Anal. 31, 115–119.

Sun, R.X., Lin, Q., Ke, C.L., Du, F.Y., Gu, Y.G., Cao, K., Luo, X.J., Mai B.X., 2016. Polycyclic aromatic hydrocarbons in surface sediments and marine organisms from the Daya Bay, South China. Mar. Poll. Bull. 103, 325–332.

Trisciani, A., Corsi, I., Della Torre, C., Perra, G., Focardi, S., 2011. Hepatic biotransformation genes and enzymes and PAH metabolites in bile of common sole (Solea solea, Linnaeus, 1758) from an oil-contaminated site in the Mediterranean Sea: A field study. Mar. Poll. Bull. 62, 806–814.

Truzzi, C., Annibaldi, A., Illuminati, S., Finale, C., Scarponi, G., 2014. Determination of proline in honey: Comparison between official methods, optimization and validation of the analytical methodology. Food Chem. 150, 477–481.

Truzzi, C., Illuminati, S., Antonucci, M., Scarponi, G., Annibaldi, A., 2018. Heat shock influences the fatty acid composition of hte muscle of the Antartic fish *Trematomus bernacchii*. Accepted Manuscript, Available online https://doi.org/10.1016/j.marenvres.2018.03.017

Truzzi, C.; Illuminati, S.; Annibaldi, A.; Antonucci, M.; Scarponi, G., 2017. Quantification of fatty acids in the muscle of Antarctic fish Trematomusbernacchii by gas chromatography-mass spectrometry: Optimization of the analytical methodology. Chemosphere. 173, 116-123.

Van Ael, E., Covaci, A., Blust, R., Bervoets, L., 2012. Persistent organic pollutants in the Scheldt estuary: Environmental distribution and bioaccumulation. Environ. Int. 48, 17–27.

Varanasi, U., Gmur, D., 1981. Hydrocarbons and metabolites in English soó (Parophrysvetulus) exposed simultaneously to [3H]benzo(a)pyrene and [14H]naphthalene in oil contaminated sediment. J. Aquat. Toxicol. 1, 49–67.

Varanasi, U., Reichert, W.L., Stein, J.E., Brown, D.W., Sanborn, H.R., 1985. Bioavailability and biotransformation af aromatic hydrocarbons in benthic organisms ezposed to sediment from an urban estuary. Environ Sci Technol. 19, 836-841

Vives, Grimalt, J.O., Fernández, P., Rosseland, B., 2004. Polycyclic aromatic hydrocarbons in fish from remote and high mountain lakes in Europe and Greenland. Sci. Total Environ. 324, 67–77.

Webster, L., Russell, M., Walsham, P., Phillips, L. A., Hussy, I., Packer, G., Dalgarno, E. J., Moffat C. F., 2011. An assessment of persistent organic pollutants in Scottish coastal and offshore marine environments. J. Environ. Monit. 13, 1288.

Wessel, N., Santos, R., Menard, D., Le Menach, K., Buchet, V., Lebayon, N., Loizeau, V., Burgeot, T., Budzinski, H., Akcha, F., 2010. Relationship between PAH biotransformation as measured by biliary metabolites and EROD activity, and genotoxicity in juveniles of sole (Solea solea). Mar. Environ. Res. 69, S71–S73.

Whyte, J.J., Jung, R.E., Schmitt, C.J., Tillitt, D.E., 2000. Ethoxyresorufin-o-deethylase (EROD) activity in fish as a biomarker of chemical exposure. Crit. Rev. Toxicol. 30: 347–350.

Xia, K., Hagood, G., Childers, C., Atkins, J., Rogers, B., Ware, L., Armbrust, K., Jewell, J., Diaz, D., Gatian, N., 2012. Polycyclic aromatic hydrocarbons (PAHs) in Mississippi seafood from areas affected by the Deepwater Horizon oil spill. Environ. Sci. Technol. 46, 5310–5318.

Xu, F.L., Wu, W.J., Wang, J.J., Qin, N., Wang, Y., He, Q.S., He, W., Tao, S., 2011. Residual levels and health risk of polycyclic aromatic hydrocarbons in freshwater fishes from Lake Small Bai-Yang-Dian, Northern China. Ecol. Model. 222: 275–286

Yang, R., Yao, T., Xu, B., Jiang, G., Xin, X., 2007. Accumulation features of organochlorine pesticides and heavy metals in fish from high mountain lakes and Lhasa River in the Tibetan Plateau. Environ. Int. 33: 151–156.

Yunker, M.B., Macdonald, R.W., Vingarzan, R., Mitchell, R.H., Goyette, D., Sylvestre, S., 2002. PAHs in the Fraser River basin; a critical appraisal of PAH ratios as indicators ofPAHsourceandcomposition.Org. Geochem.33,489–515.

Zhang, J.D., Wang, Y.S., Cheng, H., Jiang, Z.Y., Sun, C.C., Wu, M.L., 2015. Distribution and sources of the polycyclic aromatic hydrocarbons in the sediments of the Pearl River estuary, China. Ecotoxicology 24 (7–8), 1643–1649.

Zhao, Z., Wang, Y., Zhang, L., Cai, Y., Chen, Y., 2014. Bioaccumulation and tissue distribution of organochlorine pesticides (OCPs) in freshwater fishes: a case study performed in Poyang Lake, China's largest lake. Environ Sci Pollut Res. 21,8740–8749.

Zhao, Z., Zhang, L., Cai, Y., Chen, Y., 2014. Distribution of polycyclic aromatic hydrocarbon (PAH) residues in several tissues of edible fishes from the largest fresh water lake in China, Poyang Lake, and associated human health risk assessment. Ecotox. Environ. Safe. 104, 323–331.

Figure Captions

Figure 1. Map of the study area (northern Adriatic Sea) with the location of *S.solea* and marine sediment sampling sites.

Figigure 2. Box and whisker plots of the total PAH concentrations (ng g^{-1} d.w.) in tissue from fish caught in the Po Delta, off Chioggia and in the Venetian Lagoon.

Table 1

Table a. Geographical coordinates of fish and sediment collection sites and number of individuals caught there.

Sites	Latitude	Longitude	n S. solea
Po Valley1	44°48,813N	12°27,985E	5
Po Valley2	44°48,714N	12°30,955E	7
Po Valley3	44°53,432N	12°36,644E	5
Po Valley4	44°43,497N	12°44,377E	5
offshore Chioggia1	45°14,773N	12°51,274E	6
offshore Chioggia2	45°20,180N	12°52,448E	5
Venetian Lagoon3	45°25,547N	12°20,613E	7
Venetian Lagoon4	45°21,687N	12°22,225E	8

Table b. Average (±SD) of standard length (Ls, mm) and total wet weight (t.w.w., g), and number of *S. solea* individuals and sediment samples analyzed in three areas of the northern Adriatic Sea.

area	Ls, mm	tww, g	n *S. solea*	n sediment
Po Valley	239.7 (26.2)	126.9 (38.7)	22	4
off Chioggia	276.8 (24.8)	207.0 (72.0)	11	2
Venetian Lagoon	244.5 (26.9)	118.3 (39.2)	15	2

Table 3

Table c: Concentration of individual and total PAHs (ng g^{-1}d.w.) in *S. solea* tissue from three areas of the northern Adriatic Sea: the Po Delta, the Venetian Lagoon, and off Chioggia.

area	Site	tissue	Nap	Phe	Flu	Pyr	BaA	Chr	BbF	BkF	BaP	DahA	InP+ BghiP	Σ PAHs
Po Valley	1P	liver	0.91 (1.91)	1.00 (0.70)	1.10 (0.81)	6.95 (4.22)	0.76 (0.27)	< loq	< loq	< loq	< loq	< loq	< loq	10.42 (2.55)
		gills	1.80 (3.73)	1.93 (1.94)	1.90 (3.26)	12.04 (12.70)	< loq	< loq	< loq	< loq	< loq	< loq	< loq	17.67 (19.54)
		muscle	0.64 (1.34)	0.71 (0.25)	0.66 (0.50)	3.37 (1.78)	1.36 (1.78)	< loq	< loq	< loq	< loq	< loq	< loq	6.19 (2.55)
	2P	liver	1.53 (0.97)	1.32 (0.81)	2.16 (1.34)	10.66 (6.21)	0.26 (0.20)	0.09 (0.25)	0.23 (0.64)	0.10 (0.10)	0.05	< loq	< loq	16.21 (9.31)
		gills	3.72 (2.82)	3.63 (2.97)	4.74 (5.70)	30.33 (21.70)	0.57 (0.29)	0.09	0.38	0.07	0.06	< loq	< loq	43.17 (32.70)
		muscle	0.62 (0.20)	0.71 (0.30)	0.99 (0.50)	4.77 (1.70)	0.11 (0.02)	0.10 (0.07)	0.19 (0.15)	0.10 (0.04)	< loq	< loq	< loq	7.57 (2.50)
	3P	liver	1.03 (0.36)	1.05 (0.28)	2.09 (1.05)	7.71 (2.62)	0.07	0.02	0.04	0.05 (0.03)	< loq	< loq	< loq	12.02 (4.10)
		gills	2.19 (0.37)	3.69 (3.17)	6.72 (6.00)	22.62 (8.79)	0.66 (0.52)	0.05	0.14	0.12 (0.08)	< loq	< loq	< loq	35.79 (17.40)
		muscle	0.71 (0.36)	0.78 (0.28)	0.98 (1.05)	4.61 (2.62)	0.09	0.04	0.09	0.04 (0.03)	< loq	< loq	< loq	7.30 (4.10)
	4P	liver	0.60 (0.67)	0.92 (0.67)	0.91 (0.71)	8.29 (1.02)	< loq	< loq	< loq	< loq	< loq	< loq	< loq	10.72 (7.28)
		gills	2.41 (1.50)	2.58 (1.08)	2.85 (1.39)	22.36 (8.56)	0.70	< loq	0.05	< loq	< loq	< loq	< loq	30.39 (11.10)
		muscle	1.02 (0.66)	0.91 (0.38)	0.94 (0.31)	8.34 (3.04)	0.16	< loq	< loq	< loq	< loq	< loq	< loq	11.24 (3.89)
off Chioggia	1C	liver	0.63 (0.77)	0.73 (0.44)	1.24 (0.82)	6.16 (3.41)	< loq	< loq	< loq	< loq	< loq	< loq	< loq	8.75 (4.98)
		gills	1.89 (2.19)	1.59 (1.14)	2.66 (1.63)	12.52 (8.83)	0.03	< loq	< loq	< loq	< loq	< loq	< loq	18.67 (13.29)
		muscle	0.45 (0.40)	0.43 (0.21)	0.39 (0.43)	2.91 (1.83)	< loq	< loq	< loq	< loq	< loq	< loq	< loq	4.19 (2.83)
	2C	liver	0.35 (0.29)	0.54 (0.23)	5.50 (7.26)	4.10 (1.67)	< loq	< loq	0.02	< loq	< loq	< loq	< loq	10.51 (8.25)
		gills	1.33 (0.21)	1.47 (0.55)	4.71 (10.25)	11.9 (14.26)	< loq	< loq	0.15	< loq	< loq	< loq	< loq	19.57 (10.04)
		muscle	0.47 (0.38)	0.36 (0.17)	0.37 (0.43)	3.75 (2.26)	< loq	< loq	< loq	< loq	< loq	< loq	< loq	4.95 (3.19)
Venetian Lagoon	1V	liver	1.37 (0.41)	1.09(0.41)	1.74 (1.32)	9.57 (4.38)	0.41	0.06	0.11	0.03	< loq	< loq	< loq	14.05 (6.60)
		gills	2.65 (1.38)	2.45 (1.38)	5.68 (3.46)	22.15 (10.22)	0.39 (0.25)	0.08	< loq	0.04	< loq	< loq	< loq	33.18 (16.14)
		muscle	0.53 (0.11)	0.46 (0.11)	0.47 (0.24)	3.70 (1.76)	< loq	< loq	< loq	< loq	< loq	< loq	< loq	5.16 (2.38)
	2V	liver	0.78 (0.27)	1.42 (0.27)	1.69 (0.66)	13.02 (2.31)	0.13	< loq	< loq	< loq	< loq	< loq	< loq	16.92 (2.36)
		gills	2.36 (0.78)	3.20 (0.78)	4.20 (2.85)	34.56 (13.70)	< loq	< loq	< loq	< loq	< loq	< loq	< loq	44.32 (18.27)
		muscle	0.30 (0.13)	0.60 (0.15)	0.57 (0.38)	5.39 (1.80)	0.04	< loq	< loq	0.02 (0.03)	< loq	< loq	< loq	6.89 (1.98)

Table 4

Table d. Mean concentration (± SD) of individual and total PAHs at the three sampling sites in northern Adriatic Sea: the Po Delta, the Venetian Lagoon, and off Chioggia.

Area	Nap	Phe	Flu	Pyr	BaA	Chr	BbF	BkF	BaP	DahA	InP+ BghiP	Σ PAHs
Po Delta	146.47 (10.77)	16.79 (2.52)	234.49 (39.25)	126.14 (11.17)	16.40 (0.60)	23.84 (0.68)	20.25 (5.52)	8.66 (1.83)	10.63 (0.32)	0.19 (0.05)	21.23 (4.93)	625.08 (62.34)
Off Chioggia	25.25 (1.69)	2.20 (0.33)	15.79 (0.30)	9.51 (0.03)	0.85 (0.20)	1.74 (0.41)	1.00 (0.01)	0.68 (0.05)	n.d.	n.d.	1.50 (0.04)	58.51 (2.20)
Venetian Lagoon	171.43 (10.70)	18.65 (0.01)	378.10 (7.78)	275.86 (17.35)	23.88 (0.33)	31.76 (0.32)	22.38 (0.37)	10.18 (0.05)	17.91 (0.03)	0.55 (0.37)	28.07 (3.37)	978.79 (2.19)

Figure 1

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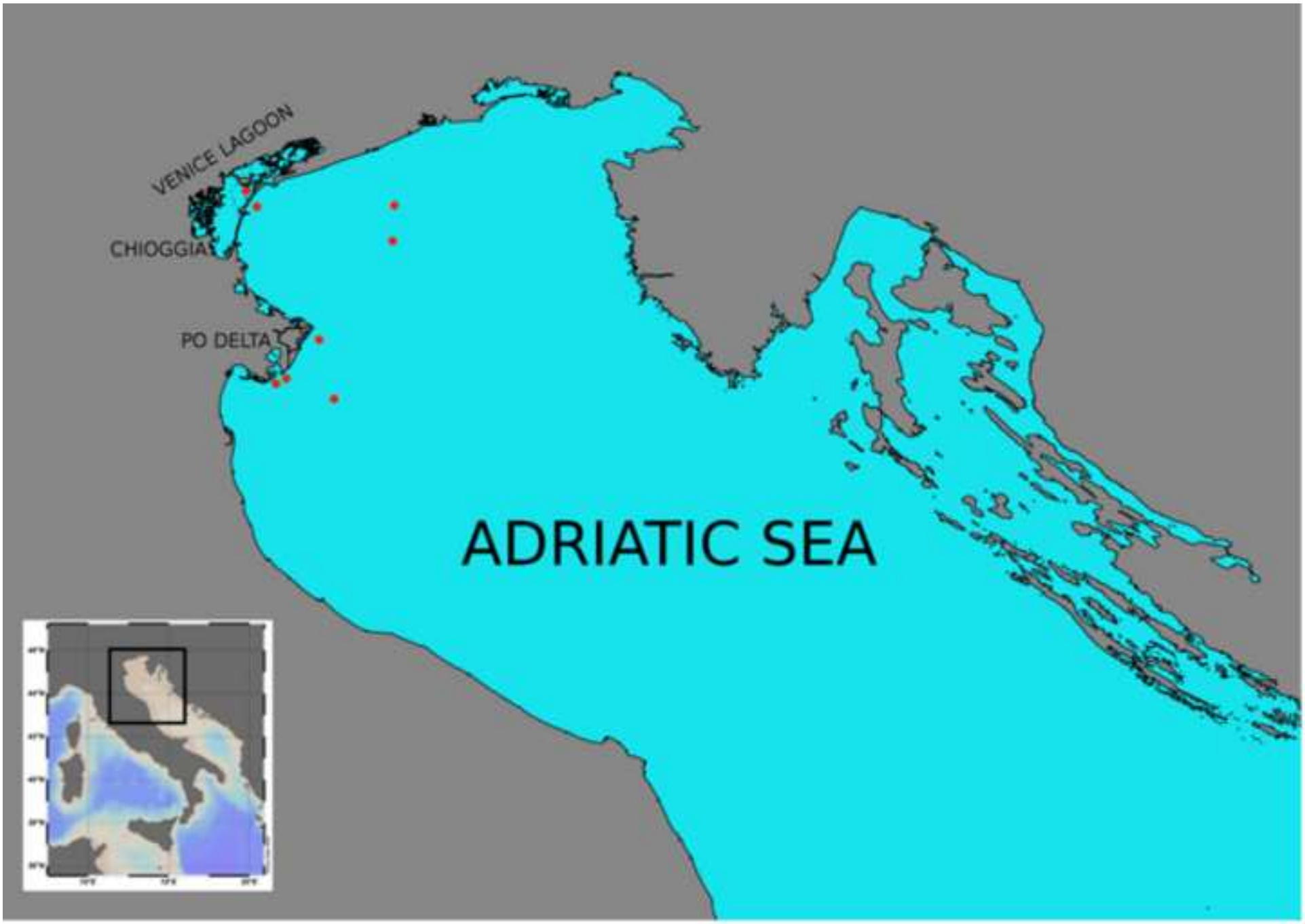

Figure 2
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