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Original article

Per- and polyfluoroalkyl substances in blue crabs from the Adriatic Sea and consumer safety evaluation

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Summary The blue crab is one of the most invasive alien species in the Mediterranean Sea. Its remarkable surge in availability suggests an imminent increase in consumption. However, blue crabs are vulnerable to contamination by per- and polyfluoroalkyl substances (PFAS), known to accumulate in the food chain and pose health risks to humans. Data on PFAS contamination levels in blue crabs are a few. This study investigates 26 PFAS in 113 blue crabs by UHPLC-HRMS. Results revealed higher concentrations in cephalothorax than in claws. PFHpA, PFHxS, PFOA, PFNA, PFOS, PFUnDA, PFDoDA, PFTrDA, PFHpS, and PFBA were among the most detected PFAS. Total PFAS mean concentration ranged from 0.65 to 2.61 ng g^{-1} ww. Three crabs exceeded maximum residue limits for PFOA, PFNA, and PFHxS. Statistical analyses suggested a correlation between contaminants presence and crab size. PCA highlighted the weight as a potential predictor of PFOS concentration. PFAS intake evaluation showed no risk for consumers.

Keywords Blue crab, chemical contaminants, crustaceans, exposure assessment, PFAS, UHPLC-HRMS.

Introduction

The blue crab (*Callinectes sapidus*) is native to the Atlantic coasts of the American continent, from Nova Scotia to Argentina, and today considered one of the most invasive alien species in the Mediterranean Sea (Marchessaux *et al*., 2023). Its feeding behaviour (molluscs such as tellins, clams, and mussels), have caused significant declines in native populations, leading to notable economic consequences. In areas where its presence poses a threat to local production, efforts are being made to turn this emergency into opportunity. Trying to create a supply chain capable of providing quality products (Regione Emilia-Romagna, 2023). The recent surge in its distribution, prevalence, and market availability is expected to drive an increase in its consumption (Epifanio, 2019; Clavero *et al*., 2022). Since it is newly introduced into the European market, specific annual per capita consumption data are currently unavailable. Generally, the crustacean's annual European consumption stands at 1.70 kilograms per capita (CBI, 2020). The meat derived from crabs can be categorised into two types: white and brown. White meat is sourced from muscle tissue found in the purse, claws, and legs, while brown meat originates from the hepatopancreas and gonads within the carapace (EUMOFA, 2021). Crab meat may be sold as white meat (legs, claws, purse, or a mixture of them), pure brown meat, or as a blend of brown and white meat (EUMOFA, 2021). In the European market, crabs are offered live, processed, and preserved options, or as ingredients in various products such as pâtés and crab cakes (EUMOFA, 2017).

Being benthopelagic, the blue crab usually inhabits the bottom of estuarine waters during its adult stage becoming vulnerable to accumulating environmental contaminants (Sealife base, 2024). In aquatic environments, particularly in coastal areas, the presence of per- and polyfluoroalkyl substances (PFAS) represents today a potential risk to food safety. They include a variety of more than 9000 fluorinated man-made artificial chemicals, primarily perfluoroalkyl carboxylates (PFCA) and perfluoroalkyl sulfonates (PFSA) (European Environmental Agency, 2023; Levanduski *et al*., 2024). Their unique physicochemical properties have led to widespread application in industrial processes and global consumer products (Levanduski *et al*., 2024). PFAS are known for their extraordinary persistence; earning them

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the name 'forever chemicals' due to their resistance to degradation (Brunn *et al*., 2023).

Bioaccumulation is a key pathway for micronutrient transfer through food chains. On the other hand, it also promotes the accumulation of anthropogenic toxic substances (Miranda *et al*., 2021). Short-chain PFAS exhibit a greater propensity to distribute into the water phase owing to their elevated water solubility. Conversely, long-chain PFAS adhere to sediment, by both hydrophobic and electrostatic interactions (Groffen *et al*., 2024). In addition, perfluoroalkyl sulfonic acids showed to be more bioaccumulative than perfluoroalkyl carboxylic acids of the same chain length (Hassell *et al*., 2020). In aquatic food webs, this is a well-known problem, particularly for long-chain PFSAs and PFCAs (Novak & Hoeksema, 2022). Furthermore, the sea organic content and salinity affect the partitioning and sorption characteristics of perfluoroctanesulfonic acid (PFOS) and perfluoroctanoic acid (PFOA), increasing exposure risk in benthic estuarine environments (Hassell *et al*., 2020). The European Food Safety Authority (EFSA) assessed the risk to human health associated with the presence of perfluoroalkyl substances in food. The authority concluded that PFOS, PFOA, perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS), can cause developmental negative effects and may have adverse effects on serum cholesterol, liver, immune system, and birth weight. Considering that on immune system as the most critical, it established a tolerable weekly intake (TWI) of 4.4 ng kg^{-1} body weight per week (sum of PFOS, PFOA, PFNA, and PFHxS). However, the report concludes that a portion of the European population is already exposed to these substances to a level greater than the TWI, which is concerning (EFSA, 2020).

The EU Commission recently established Maximum Levels (ML) in certain foods (Regulation EU 2023/915). For crustaceans, they are fixed at 3.0, 0.7, 1.0, 1.5, and 5.0 μg kg^{-1} for PFOS, PFOA, PFNA, PFHxS, and their sum, respectively.

Furthermore, in November 2023, the International Agency for Research on Cancer (IARC) classified PFOA within Group 1 (carcinogenic) and PFOS in Group 2B (possibly carcinogenic) (Zahm *et al*., 2024). This classification highlights the urgency of monitoring their presence in food sources, as also suggested by the European Commission (Commission Recommendation EU 2022/1431).

Additionally, it is noteworthy that only a small fraction of PFAS is currently regulated in terms of maximum residual levels in food (Regulation EU 2023/915). The recent directive (EU) 2020/2184, addressing the quality of water designated for human consumption, has introduced two parameters: total PFAS (the totality of per- and poly-fluoroalkyl substances), and the sum of PFAS, (the sum of per- and poly-fluoroalkyl substances considered a concern for human consumption). Furthermore, the directive expanded to 24, the number of PFAS considered to be of significant concern for consumers. In this context, the need for monitoring and assessment of comprehensive PFAS contamination in food sources is clear.

The existing literature extensively covers the presence of PFAS in fish, shellfish, and other crab species (Taylor *et al*., 2021; Valsecchi *et al*., 2021; Gallocchio *et al*., 2022; Marín-García *et al*., 2023; Nobile *et al*., 2023b, 2024; Stecconi *et al*., 2024). However, lack of data remains regarding their concentration in the edible portion of blue crabs to determine potential safety risks. Given the increasing market availability of blue crabs and their PFAS bioaccumulation potential, data on their concentration level becomes mandatory. This work aims to investigate the presence of PFAS in blue crabs from the Adriatic Sea and the correlation between the detected PFAS concentrations and the crab's biometric parameters. Furthermore, a PFAS intake evaluation was performed to assess consumers' health risks. The obtained data are essential for filling knowledge gaps, informing regulatory decisions, and protecting consumer health. Continued research and monitoring efforts are necessary to better understand the prevalence, distribution, and impacts of PFAS contamination in aquatic environments and food sources.

Materials and methods

Chemical and reagents

The perfluorinated compounds, all purchased from Chemical Research 2000Srl (Rome, Italy), are reported by family and chain size. Perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, PFNA, perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA) perfluorotetradecanoic acid (PFTeDA), perfluorohexadecanoic acid (PFHxDA), perfluooctadenoic acid (PFODA). Perfluorobutane sulphonic acid (PFBS), perfluoropentanesulfonic acid (PFPeS), PFHxS, perfluoroheptanesulfonic acid (PFHpS), PFOS, perfluo rodecanesulfonic acid (PFDS). 9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid (9Cl-PF3ONS), 11-chloroeico safluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS), 2-(N-methylperfluoroctanesulfonamido) acetic acid (NMeFOSAA), 2-(N-ethylperfluoro-1-octanesulfonamido) acetic acid (NEtFOSAA), 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy) propanoic acid (GenX), ammonium perfluoro [(5-methoxy-1,3-dioxolan-4-yl)oxy] acetate (C6O4), Sodium 4,8-dioxa-3H-perfluorononanoate (NaDONA), and the two ^{13}C-labelled internal standards (ISs); perfluoro [1,2,3,4–^{13}C4] octanesulfonic acid

(MPFOS, used to quantify sulfonates), and perfluoro-[1,2,3,4,5–^{13}C5] nonanoic acid (MPFNA, used to quantify carboxylate compounds), The analytical LC–MS-grade solvents and reagents used were all purchased fro m Merck (Darmstadt, Germany). The purification cartridges, Strata PFAS (WAX/GCB) 200 mg/50 mg/6 mL, were obtained from Phenomenex (Torrance, Califo rnia). The single stock solutions of the 26 PFAS, MPFOS, and MPFNA at a concentration of 50 μg mL^{-1} in MeOH were stored at −20 °C. Appro priate volumes of each stock solution were diluted to create two working solutions containing all the analytes at concentrations of 100 ng mL^{-1} and 10 ng mL^{-1}. A working solution was prepared for the internal standard at the concentration of 1 μg mL^{-1}, diluting the appro priate volume of MPFOS and MPFNA stock solutions. All the working solutions were stored and refrigerated at −20 °C.

Sample collection

A total of 113 crab samples (forty-three males, seventy females) originating from the Adriatic Sea (FAO zone 37.2.1) were obtained from the Milan fish market. Each crab sample has been treated and homogenised as described in Sample extraction protocol section. Before the analysis, each sample was stored refrigerated at −20 °C.

Sample size

To verify the suitability of the sample size, the formula reported below was applied (Nobile *et al.*, 2023a):

$$N = Z^2 \times [P \times (1-P)]/D^2$$

where Z has a value of 1.96 for a 95% confidence limit, P is the expected prevalence set at 0.5 (50%), and D is the estimate's precision. This conservative approach allowed a precision value of 10%, considered satisfactory.

Sample preparation

Each crab was weighted, and the carapace width and length were collected. The biometric parameters of the analysed blue crabs were collected according to Farrag (2022). Detailed information is reported in Table S1. To evaluate the distribution of PFAS in the different edible parts, claws, and the cephalothorax were collected and separately homogenised for each crab.

Sample extraction protocol

The crab samples were extracted according to Chiesa *et al.* (2022). Briefly, 2 g of sample were weighed in a 50 mL polypropylene tube, spiked with 10 μL of the internal standard working solution mix (1 μg mL^{-1}). Thus, 10 mL of acetonitrile were added. The sample was further homogenised with ultraturrax (1 min), vortexed (1 min), and sonicated (15 min). After centrifugation (2500 ***g***, 4 °C, 10 min), the supernatant was concentrated with a rotavapor (Heidolph, Germany) (bath temperature 45 °C) until 1 mL and 5 mL of water were added. The purification step was performed on STRATA PFAS cartridges: after precondition (4 mL of 0.3% ammonium hydroxide (NH_4OH) in MeOH, 4 mL of MeOH, and 4 mL of ultrapure water, under vacuum), the sample was loaded, washed (8 mL of ultrapure water), and finally eluted (8 mL of NH_4OH in MeOH). After a second dry step (same condition previously mentioned), the sample was reconstituted in 200 μL of MeOH: ammonium formate 20 mM (20:80 v/v), first transferred in Eppendorf, centrifuged (15 000 ***g***, 20 °C, 2 min), and then in a vial before UHPLC-HRMS analysis.

UHPLC-HRMS analysis

The UHPLC-HRMS system consisted of a Vanquish binary pump and a Thermo Orbitrap Exploris 120 (Thermo Fisher Scientific). PFAS separation was done on a Raptor ARC-18 column (5 μm, 120 × 2.1 mm) (Restek, Bellefonte, PA, USA). Stainless steel tubes and peaks minimised system PFAS contamination. A Megabond WR C18 column (5 cm, 4.6 mm) was added before the injector to delay existing PFAS. The mobile phase used 20 mM aqueous ammonium formate (Phase A) and methanol (Phase B), with a flow rate of 0.3 mL min^{-1} with a starter gradient of 20% B, which at the 7th min reached 95%, maintained for 3 min. Initial conditions were reached on the 11th and kept for 4 min. The total run time was 15 min. Detector settings: capillary temperature at 330°C, vaporiser at 280°C, sheath gas at 35 units, auxiliary gas at 15 units, electrospray voltage at 3.50 kV in negative mode, a standard automatic gain control (AGC), an RF lens % of 70, and an automatic maximum injection time. Full-scan (FS) acquisition had a resolution of 60 000 FWHM, 150–950 m/z range, and included a product ion scan for confirmation. Product ion scan acquisition had 15 000 FWHM resolution, 1 m/z isolation window, and two-step collision energy (10 and 70 eV). The software used was Xcalibur™ 4.5 (Thermo Fisher Scientific, Waltham, MA, USA).

Method validation

Selectivity, precision (repeatability and reproducibility), and recovery rate were determined. The selectivity was assessed by the injection of blank extracted crab samples and the lack of signal, close to the expected retention times of PFAS with a Signal to Noise

S/N < 3 indicating no interferences. The matrix-match calibration curves were constructed, in duplicate, in both matrices by spiking 2 g of blank sample with the appropriate standard working solution, for seven calibration points (0, 0.1, 0.2, 0.5, 1.0, 3.0, 5.0 ng g^{-1}) to test the linearity for all the analytes. For each analyte, the lowest spiked level with a 70–120% recovery, a precision (CV) <20%, and a signal-to-noise (S/N) ratio of at least 10 was defined as the limit of quantification (LOQ), evaluated in five replicates. The intra-day repeatability was assessed across five replicates, while the inter-day reproducibility was evaluated across five replicates prepared and analysed in three different days, both expressed as the coefficient of variation (CV%). Recovery was assessed by comparing the concentrations of PFAS spiked before extraction with those inserted at the end of the extraction protocol.

Statistical analysis

Descriptive statistical analysis was conducted using Graph Pad Prism version 9.0.0. The middle-bound approach was employed, assigning a value equal to half the LOQ to all samples where PFAS were detected < LOQ, to conduct a more thorough statistical treatment (EFSA, 2022). Firstly, to assess the normal distribution of the data, the Shapiro–Wilk test was conducted. Subsequently, the Mann–Whitney test was employed to evaluate the statistical significance between the sets of results. A *P*-value ≤0.05 was considered statistically significant for a confidence interval of 95%. Principal Component Analysis (PCA) was further adopted to investigate the relationship between the biometric parameters of the examined crabs and the identified concentrations of the investigated PFAS (detailed information is provided in Principal components analysis applied to the PFAS contamination levels section), by using the open-source program CAT (Chemometric Agile Tool), developed under the R environment by the Chemistry Group of the Italian Chemical Society.

PFAS intake evaluation

The evaluation of PFAS intake was carried out by calculating the estimated daily intake (EDI):

$$\mathrm{EDI} = C \times \mathrm{DC}/\mathrm{BW}$$

where *C* is related to the mean value of the sum of the four main PFAS found in the analysed crabs (claws and cephalothorax) and DC is the daily crab consumption in Europe, based on an annual value of 1.7 kg (CBI, 2020), divided by the consumer body weight (considering an average weight of 70 kg).

Result and discussion

Method validation

Overall, the validation method parameters are reported in Table S2. The method has shown high selectivity, with a signal-to-noise ratio higher than 10 starting from the LOQ level, and high specificity, with the absence of interference close to the retention time of the analysed PFAS. The recoveries ranged between 70% and 120%, revealing a good efficiency of the extraction and purification protocol. Repeatability and reproducibility with CVs ≤20% were satisfactory. The LOQs were set in the range of 0.10–0.50 ng g^{-1}. The matrix calibration curves showed good linearity, with an R^2 in the range of 0.988–0.999 for all PFAS.

Incidence of PFAS in blue crab

Among the twenty-six investigated PFAS, seventeen were detected in the samples. For a comprehensive contamination evaluation, both total PFAS (referred to as the totality of per- and polyfluoroalkyl substances) and the sum of PFAS values (intended as the sum of PFOA, PFOS, PFNA, and PFHxS) were presented in Table 1.

In the cephalothoraxes, the concentrations of PFAS detected were higher than those in the claws for both male and female individuals. PFHpA, PFHxS, PFOA, PFNA, PFOS, PFUnDa, PFDoDA, PFTrDA, PFHpS, and PFBA were the most detected PFAS. PFOS was detected in both crab matrices with around 90% frequency. PFOA was detected with a frequency of around 90% in female and male claws, and around 30% in female and male cephalothorax. Claws and cephalothorax from male crabs exhibited a higher frequency of detection and contamination levels of PFBA, PFNA, PFUnDa, PFHxS, PFDoDA, PFTrDA, and PFHpS, and higher concentrations of PFOA and PFOS, compared to matrices from female individuals. Given the larger size and greater average weight of male individuals compared to females (average weight: male 163.23 g; female 131.56 g), it may be reasonable to assume that bioaccumulation of these contaminants occurs more prominently.

As reported in Introduction section, Regulation EU 2023/915 set ML for the main four PFAS individually and as a sum. Notably, in the Regulation is reported that for crustaceans, the maximum level is referred to as crab muscle meat from appendages and abdomen, which means, that the cephalothorax is excluded. However, this study showed that the concentration of the contaminants in the cephalothorax was higher than that measured in the claws. Total PFAS and PFAS sum are higher in cephalothorax samples, for both

Table 1 Detection frequency (%), mean, 95th percentile, and maximum value of detected compounds in crab matrices

Compound	PFBA	PFPeA	PFBS	PFHxA	PFHpA	PFHxS	PFOA	PFNA	PFOS	PFDA	PFUnDa	PFDoDA	PFTrDA	PFTeDA	PFHxDA	PFPeS	PFHpS	PFAS Sum	Total PFAS
	ng g^{-1} ww																		
Male claws (N = 43)																			
Detection (%)	26	5	16	5	26	60	88	26	93	14	26	21	14	5	2	7	21		
Mean	0.25	0.13	0.05	0.05	0.12	0.12	0.38	0.17	0.19	0.29	0.28	0.62	1.80	0.54	0.25	0.08	0.10	0.65	1.33
95th Percentile	0.25	0.01	0.05	0.04	0.05	0.12	1.05	0.05	0.67	0.45	0.34	1.13	2.99	0.33	–	0.09	0.10	1.65	5.37
Maximum	0.25	0.25	0.05	0.05	0.80	1.86	4.21	1.36	1.06	0.49	1.15	1.74	3.58	0.67	0.25	0.10	0.10	8.13	9.99
Male cephalothorax (N = 43)																			
Detection (%)	9	5	5	0	0	5	35	16	91	9	40	30	23	12	0	0	5		
Mean	0.25	0.25	0.05	–	–	0.56	1.31	0.93	0.41	0.97	1.33	0.28	2.18	0.25	–	–	3.26	1.11	2.61
95th Percentile	0.25	0.20	0.04	–	–	0.04	0.27	0.35	0.80	0.80	2.66	0.32	5.07	0.25	–	–	0.08	1.37	8.80
Maximum	0.25	0.25	0.05	–	–	1.07	17.95	4.84	0.90	1.97	3.13	0.60	8.25	0.25	–	–	6.43	24.69	36.40
Female claws (N = 70)																			
Detection (%)	3	0	3	0	3	11	93	11	94	6	11	6	10	4	0	0	0		
Mean	0.25	–	0.05	nd	0.05	0.09	0.21	0.10	0.10	0.24	0.65	0.51	1.72	0.61	–	–	–	0.32	0.65
95th Percentile	–	–	–	–	–	0.05	0.34	0.05	0.25	0.10	0.56	0.35	1.42	0.11	–	–	–	0.73	3.51
Maximum	0.25	–	0.05	–	0.05	0.34	1.30	0.41	0.73	0.46	1.90	0.69	3.25	1.34	–	–	–	1.55	4.50
Female cephalothorax (N = 70)																			
Detection (%)	1	1	3	0	0	0	31	7	86	10	17	11	17	3	1	0	1		
Mean	0.25	0.25	0.05	–	–	–	0.13	0.35	0.33	0.72	1.61	0.23	2.31	0.16	–	–	0.10	0.40	1.26
95th Percentile	–	–	–	–	–	–	0.27	0.24	0.66	0.77	2.09	0.25	2.77	–	–	–	–	0.93	4.83
Maximum	0.25	0.25	0.05	–	–	–	0.70	0.71	1.06	0.91	6.70	0.25	7.80	0.25	–	–	0.10	2.47	10.03

Total PFAS: sum of all per- and poly-fluoroalkyl substances detected; PFAS sum: sum of PFOA, PFOS, PFNA and PFHxS (according to directive (EU) 2020/2184).

males and females. Among the analysed crabs (forty-three males, seventy females), three exceeded the fixed maximum residual limits. Particularly, one male crab exceeded the ML for PFOS, PFOA, PFNA, PFHxS sum, and individual ML for PFOA, PFNA, and PFHxS. In the same individual, the PFOA and PFNA concentration was around four times higher in cephalothorax, reaching a PFOS, PFOA, PFNA, and PFHxS sum of around three-fold higher than that measured in the claws. Two more crab samples exceeded the ML for PFOA. Results are shown in Table 2. These findings underscore the importance of monitoring activities and official controls. Especially considering Regulation EU 2023/915, which states: 'To ensure an efficient protection of public health, food containing contaminants exceeding the maximum levels not only should not be placed on the market as such but should also not be used as a food ingredient or be mixed with food' (Regulation EU 2023/915).

Comparing the data obtained with those in the scientific literature, they show how human activities produce the chemical contamination of the Mediterranean Sea (Gòmez *et al.*, 2011; Corsolini*et al.*, 2014; Brumovský *et al.*, 2016; Schmidt *et al.*, 2019; Herlory *et al.*, 2024). Particularly, for marine water samples of the Adriatic Sea, Loos *et al.* (2013) detected PFOA (0.79–2.51 ng L^{-1}), PFPeA (2.35 ng L^{-1}), PFHxA (0.37–2.20 ng L^{-1}), PFHxS (1.65 ng L^{-1}), PFHpA (0.30–0.33 ng L^{-1}), PFNA (0.067–0.152 ng L^{-1}), PFDA (19 pg L^{-1}), suggesting this habitat as a potential source of contamination. Moreover, several studies demonstrated PFAS accumulation in shellfish (Munschy *et al.*, 2013; Mazzoni *et al.*, 2014; Vassiliadou *et al.*, 2015; Catherine *et al.*, 2019; Miranda *et al.*, 2021; Giffard *et al.*, 2022). Across the various studies, PFOS emerged as the predominant PFAS, displaying the highest average and maximum concentrations. Concentrations ranged from 0.001 ng g^{-1} to 72.0 ng g^{-1} ww, with a peak of 125.9 ng g^{-1} ww in Mediterranean mussels (*Mytilus galloprovincialis*). For PFOA, bivalves showed higher average levels (1.51 ± 3.44 ng g^{-1} ww) compared to cephalopods (0.61 ± 0.95 ng g^{-1} ww), crustaceans (0.35 ± 0.44 ng g^{-1} ww), and gastropods (0.12 ± 0.11 ng g^{-1}ww). Besides PFOS and PFOA, long-chain PFAS like PFNA, PFDA, PFUnDA, and PFDS were detected in bivalves and some crustaceans (Giffard *et al.*, 2022). These findings suggest that the PFAS levels detected in the analysed crabs could be related to their habitat and feeding behaviour. Furthermore, studies demonstrate certain PFAS bioaccumulation tendencies in organs and other tissue compartments, such as the liver, kidney, blood, and bile in aquatic species, particularly PFOS, PFOA, and PFBS (Cui *et al.*, 2009; Shi *et al.*, 2015, 2018; Consoer *et al.*, 2016; Cao *et al.*, 2022). Particularly, perfluoroalkyl sulfonates, show a higher affinity for proteins and fatty

Table 2 Overview of crab samples exceeding MLs contamination for regulated PFAS (reg 915/2023), their sum, and other detected compounds. Regulated PFAS and their sum exceeding the MLs in bold

Sample ID*	Matrix ng g^{-1} ww	PFPeA	PFBS	PFHxA	PFHpA	PFHxS	PFOA	PFNA	PFOS	PFDA	PFUnDa	PFDoDA	PFTrDA	PFTeDA	PFPeS	PFHpS	PFAS sum
M10C	Claws	–	–	–	0.80	**1.86**	**4.21**	**1.36**	0.69	0.49	0.27	–	–	–	–	–	**8.13**
M10T	Cephalothorax	–	–	–		1.07	**17.95**	**4.84**	0.83	1.97	3.13	0.13	–	–	–	–	**24.69**
M11C	Claws	0.05	0.05	0.05	0.05	0.14	**1.17**	–	0.38	0.10	0.10	–	–	–	0.05	0.10	1.69
M11T	Cephalothorax	–	–	–	–	–	–	–	0.62	–	–	–	–	–	–	–	0.62
F15C	Claws	–	–	–	–	0.05	**1.30**	0.10	0.10	–	0.97	0.65	–	–	–	–	1.55
F15T	Cephalothorax	–	–	–	–	–	–	–	0.41	–	0.25	–	–	0.25	–	–	0.41

*Sample ID are reported in agreement with the ones showed in Table S1; PFAS sum: sum of PFOA, PFOS, PFNA and PFHxS.

acid-binding proteins, abundant in the liver (Goeritz *et al.*, 2013). This could explain the higher measured concentration of PFOA, PFHxS, and PFOS within the cephalothorax. Thus, from the perspective of consumers, it may be reasonable to conclude that the risk of exposure to per- and polyfluoroalkyl substances may be greater when consuming crab's cephalothorax and/or processed products (dressings, pâtés) containing this edible part.

Statistical analysis

Descriptive statistics, including the detection percentage, 95th percentiles, mean and maximum values, were calculated for both claws and cephalothorax samples for all the detected analytes (Table 1). Statistical significance differences were evaluated for the four regulated PFAS in male and female claws and cephalothorax samples. Firstly, the Shapiro–Wilk showed a non-normal distribution of the data. Thus, a non-parametric Mann–Whitney test was conducted to evaluate the statistical significance between the sets of results, considering the median values of the data. The matrix-related two-tailed *P*-values ranged from <0.0001 to <0.05 for PFOA, PFOS, and PFHxS in male claws and cephalothoraxes, and *P*-values <0.0001 for PFOA and PFOS in female claws and cephalothoraxes, PFHxS was not detected in female cephalothoraxes. These results may suggest a relation between PFOA, PFOS, and PFHxS concentration in claws and cephalothorax for male individuals and PFOA and PFOS in females (Table S3). The Mann–Whitney test was also conducted to evaluate the statistical significance between male and female claws and the corresponding values for cephalothoraxes. In this case, statistical significance results were obtained for PFOA, PFOS, and PFHxS in male and female claws, and for PFOS in male and female cephalothoraxes. Non-statistically significant results for PFOA were obtained in male and female cephalothoraxes. PFNA showed non-statistically significant results in whichever case. These findings may indicate that the presence of contaminants seems not influenced by sex. Instead, it suggests a probable relation with the weight of the crab.

Principal components analysis applied to the PFAS contamination levels

Considering the obtained results, a PCA was performed to deepen and explore the correlation between the biometric parameters (sex, weight, length, and width) of the analysed crabs and the detected concentrations of the investigated PFAS (single molecules and as a sum of the four regulated PFAS). Detailed information related to the dataset used to run this analysis is reported in Table S1.

In the Loading plot (Fig. 1), the considered variables were: 'sex' (female–male; reported in the dataset as numeric variables), 'matrix' (claws-cephalothorax; reported in the dataset as numeric variables), 'PFOA', 'PFOS', 'PFNA', and 'PFHxS' concentrations in all the analysed samples, 'PFAS_SUM' (expressed as the sum of PFOA, PFOS, PFNA, and PFHxS concentrations in the sample). It is possible to note that PFOA, PFNA, and PFHxS concentrations were positively correlated not only with each other but also with the variable 'PFAS_SUM'. This underlines that samples with higher PFOA concentration may show a higher concentration of PFOA, PFNA, and PFHxS. On the other hand, PFOS concentrations were positively correlated with the considered matrices. As shown in Table 1, PFOS was always higher in cephalothorax than in claws. This may further confirm the tendency of this contaminant to bioaccumulate in a specific district of the organisms (Cao *et al.*, 2022). It is possible to note that male crabs tend to show a higher weight. Thus, considering the Loading plot (Fig. 2), in which the considered variables were: 'weight', 'PFO', 'PFOS', 'PFNA', and 'PFHxS' concentrations in all the analysed samples, and 'PFAS_SUM', it is possible to note that weight and PFOS concentrations were positively

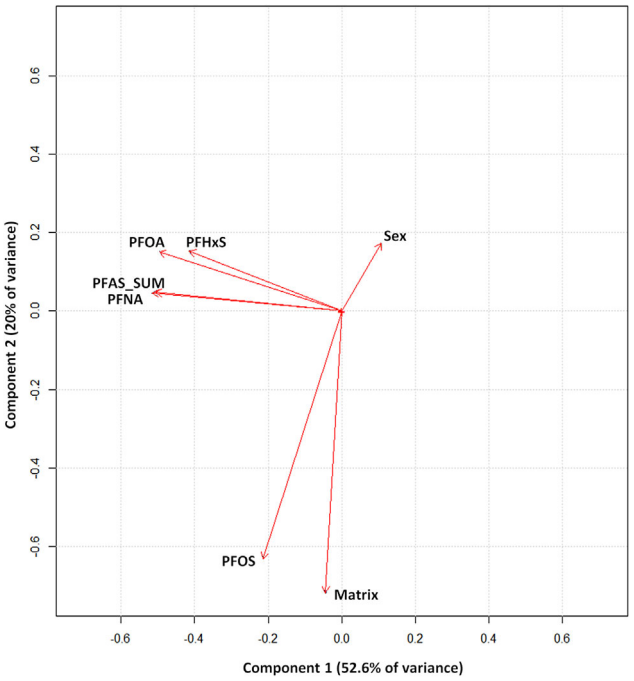

Figure 1 Loading plot obtained considering a matrix composed of the following variables: sex (male–female), matrix (claws-cephalothorax), PFOA, PFOS, PFNA, and PFHxS concentrations in all the analysed samples, and their sum expressed as a variable named 'PFAS_SUM'. The total percentage of variance expressed by plotting Component 1 and Component 2, in the *x* and *y* axes, respectively, is 72.6%. The arrows show how the variables are arranged in the space of Component 1 and Component 2.

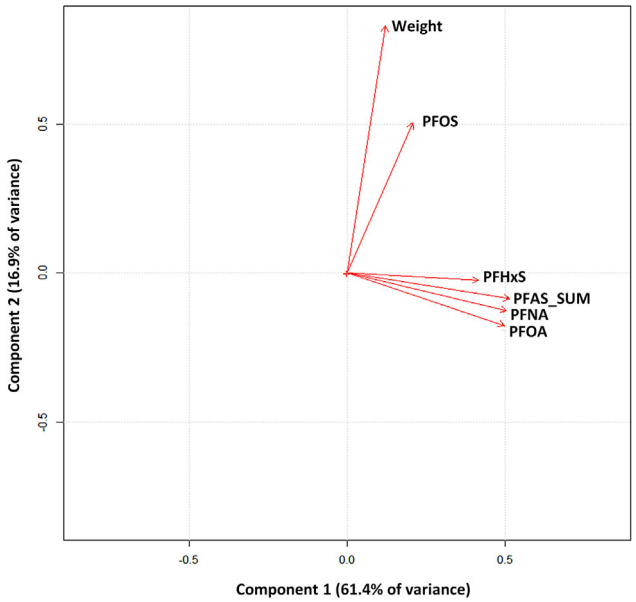

Figure 2 Loading plot obtained considering a matrix composed of the following variables: weight, PFOA, PFOS, PFNA, and PFHxS concentrations in all the analysed samples, and their sum expressed as a variable named 'PFAS_SUM'. The total percentage of variance expressed by plotting Component 1 and Component 2, in the *x* and *y* axes, respectively, is 78.3%. The arrows show how the variables are arranged in the space of Component 1 and Component 2.

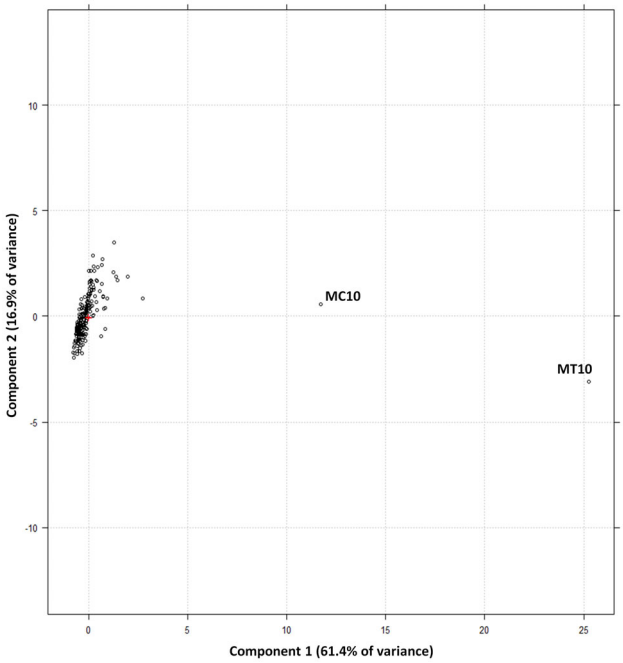

Figure 3 Score plot obtained considering a matrix composed of the following variables: weight, PFOA, PFOS, PFNA, and PFHxS concentrations in all the analysed samples, and their sum expressed as a variable named 'PFAS_SUM'. The total percentage of variance expressed by plotting Component 1 and Component 2, in the *x* and *y* axes, respectively, is 78.3%. The dots show how the samples are arranged in the space of Component 1 and Component 2.

correlated. Considering the Score plot (Fig. 3), the samples show no tendency to cluster. The two outliers are referred to as the two male samples characterised by the highest PFAS sum concentration (claws 8.13 ng g^{-1} ww; cephalothorax 24.69 ng g^{-1} ww).

However, by performing the same evaluation without considering the outlier value (Male individual 10), the analysis showed, for the loadings, a weaker positive correlation between PFOS and matrices, and between PFOA, PFHxS, PFNA, and 'PFAS_SUM' concentrations.

PFAS intake evaluation through blue crab consumption

As mentioned in Introduction section, specific data on the consumption of blue crabs are not yet available. However, considering the expected growth in the prevalence of this product, both whole and processed, it is reasonable to assume that its consumption is likely to increase. EU Regulation 2023/915 excludes the cephalothorax of crabs in the assessment of PFAS MLs for crustaceans. Considering the outcomes of the present study, an assessment of PFAS intake through blue crab consumption including the cephalothorax (an edible part, commonly called crab mustard in the culinary sphere) was conducted. As a conservative approach, the crustacean's European consumption per year was considered as the crab's annual intake (CBI, 2020).

The intake evaluation was conducted considering the cumulative sum of the four main PFAS (PFOA, PFOS, PFNA, and PFHxS) indicated by EFSA. The intake was calculated considering mean contamination calculated on the whole population of both sexes (0.55 ng g^{-1} ww).

The TWI is 4.4 ng kg^{-1} bw per week (EFSA, 2020); PFAS daily intake (EDI) calculated as above is 0.037 ng kg^{-1} day. The weekly intake found (0.26 ng kg^{-1} per week) through the analysed crabs represents 6% of the tolerable intake. The evaluation of the mean value of the sum of the four main PFAS concentrations, generally higher in male individuals (heavier than female crabs), was also considered. With this more conservative approach (0.87 ng g^{-1}), the weekly intake represents (0.40 ng kg^{-1} per week) 9% of the tolerable intake. However, further study and a more accurate assessment are demanded once specific blue crab consumption data are available.

Conclusion

The blue crab, now considered one of the most invasive alien species in the Mediterranean Sea, is

experiencing a rapid increase in distribution and market availability, suggesting a rise in consumption. Due to its benthopelagic nature and feeding behaviour, the blue crab is vulnerable to accumulating environmental contaminants, such as PFAS and there is a lack of data in the scientific literature on this topic.

This work aimed to investigate twenty-six PFAS presence in 113 blue crabs from the Adriatic Sea and to examine correlations between the detected PFAS concentrations and the crab's biometric parameters. Additionally, a PFAS intake evaluation was conducted to assess the health risks for consumers. The analysis of the blue crabs revealed the detection of seventeen PFAS, with concentrations notably higher in cephalothoraxes compared to claws in both male and female individuals. PFHpA, PFHxS, PFOA, PFNA, PFOS, PFUnDa, PFDoDA, PFTrDA, PFHpS, and PFBA were among the most detected ones, with PFOS being ubiquitous in all crab matrices. These results brought to light the possible risks that consumers may encounter while consuming whole crabs or those that have been treated or preserved.

Among the analysed crabs, three exceeded the ML, particularly for PFOA, PFNA, and PFHxS. Statistical analyses revealed significant differences between male and female samples, suggesting a potential relation between contaminant presence and crab size rather than sex factor. PCA exploration identified positive correlations between PFAS concentrations and biometric parameters, highlighting weight as a potential predictor of PFOS concentration. Finally, PFAS evaluation intake through blue crab consumption did not show risk for consumers, under the considered conservative conditions. These insights contribute to our understanding of PFAS contamination dynamics in aquatic food webs and underscore the need for continued research, and regulatory refinement to mitigate consumers' potential risks through blue crab consumption.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval

Ethics approval was not required for this research.

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Author contributions

Maria Nobile: Conceptualization; methodology; formal analysis; data curation; writing – original draft; writing – review and editing. **Dalia Curci:** Conceptualization; methodology; formal analysis; writing – review and editing; writing – original draft; data curation. **Giulia Rampazzo:** Conceptualization; methodology; formal analysis; writing – review and editing; writing – original draft; data curation. **Luca Maria Chiesa:** Resources; supervision; project administration. **Teresa Gazzotti:** Writing – review and editing. **Sergio Ghidini:** Supervision; writing – review and editing. **Francesco Arioli:** Conceptualization; investigation; data curation; writing – review and editing. **Sara Panseri:** Resources; conceptualization; methodology; supervision.

Data availability statement

The data are available from the corresponding author upon reasonable request.

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* These sources were considered essential references, as their content, distinguished by its high scientific merit in the field of PFAS analysis and their impact to human health, played a significant role in shaping the content of this paper.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Dataset: biometric parameters and contamination levels (ng g^{-1}) detected in the blue crabs involved in the present research.

Table S2. Method performance parameters.

Table S3. Matrix and Sex-related statistically significant differences in crab matrices.