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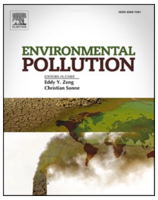

Anthropogenic pollutants in *Nephrops norvegicus* (Linnaeus, 1758) from the NW Mediterranean Sea: Uptake assessment and potential impact on health[☆]

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ABSTRACT

Anthropogenic pollution is considered one of the main threats to the marine environment, and there is an imperious need to assess its potential impact on ecologically and economically relevant species. This study characterises plastic ingestion and tissue levels of potentially toxic metallic elements in *Nephrops norvegicus* and their simultaneous levels in abiotic compartments from three locations of the Catalan coast (NW Mediterranean Sea). A multidisciplinary assessment of the health condition of *N. norvegicus* through condition indices, enzymatic biomarkers and histological techniques is provided, and its relationship with anthropogenic pollutant levels explored. Plastic fibres were commonly found in stomachs of *N. norvegicus* (85% of the individuals), with higher abundances (13 ± 21 fibres $\cdot$ ind^{-1}) in specimens captured close to Barcelona. The presence of long synthetic fibres in near-bottom waters, as well as the mirroring trends in abundance among locations for water and ingested plastics, suggest that uptake from water may be occurring potentially through suspension feeding. The spatial variability in the levels of metallic elements in *N. norvegicus* was poorly correlated to the variability in sediments. In any case, present levels in abdominal muscle are considered safe for human consumption. Levels of ingested plastics only showed significant, yet weak, correlations with glutathione *S*-transferase and catalase activities. However, no other health parameter analysed showed any trend potentially associated to anthropogenic pollutant levels. Neither the condition indices nor the histopathological assessment evidenced any signs of pathologic conditions affecting *N. norvegicus*. Thus, it was concluded that presently there is no evidence of a negative impact of the studied pollutants on the health condition of *N. norvegicus* in the studied grounds.

1. Introduction

The impact of human activities on marine ecosystems is undeniable. It encompasses from historical impacts such as overfishing (Jackson et al., 2001), to the occurrence and bioaccumulation in marine organisms of toxic chemicals such as persistent organic pollutants and metallic elements (Habte et al., 2015; Johnson et al., 2013). Most recently, plastic pollution has raised a great concern within the scientific community (Avio et al., 2017; Canals et al., 2021, and references therein) because of the persistence, ubiquity and ease of ingestion by marine organisms of plastics and, in particular, microplastics (particles <1–5 mm; Frias and Nash, 2019; Hartmann et al., 2019). In the Mediterranean Sea, a high cumulative human pressure on marine ecosystems has been highlighted due to its semi-enclosed nature, densely populated coastlines and intensive use of land and marine resources (Micheli et al., 2013). High levels of pollution, including toxic metallic elements and plastics, have been recorded in the northern-western area, extending from the continental shelf to the deep-sea (de Haan et al., 2019; Palanques et al., 2020; Pinedo et al., 2014; Sanchez-Vidal et al., 2018), overlapping in areas of high marine biodiversity (Coll et al., 2012).

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Given the ecological and economic importance of marine ecosystems, there is a critical need for the continued assessment of the health condition of their key components in order to ensure their conservation and sustainability (Österblom et al., 2017).

Decapod crustaceans are important members of the megafaunal assemblages of the deep-sea environments of the Mediterranean Sea (Cartes et al., 1994; Fanelli et al., 2013). Among them, the Norway lobster *Nephrops norvegicus* (Linnaeus, 1758), that in Mediterranean waters mostly inhabits at depths between 245 and 485 m (Cartes et al., 1994), is an excellent candidate for studying the potential impact of anthropogenic pollutants in this environment. There exists a comprehensive knowledge of its biology and ecology (Johnson and Johnson, 2013), it is one of the most abundant decapod crustaceans at traditional fishing depths (Abelló et al., 1988), and it has a high economic value in Mediterranean and Atlantic fisheries (Sbrana et al., 2020).

Past studies targeting the populations of *N. norvegicus* from different areas in Europe (i.e. the Clyde Sea and the Mediterranean Sea) have already described remarkable concentrations of plastics in their stomachs (Carreras-Colom et al., 2022; Murray and Cowie, 2011; Welden and Cowie, 2016a). These levels were comparatively higher than those commonly reported in fish species from the same areas (Bellas et al., 2016; Hermsen et al., 2017; McGoran et al., 2018) and could be related to a higher uptake from the environment due to its ecological features (benthic feeder with a close contact with the sediment), in addition to the presence of anatomic traits (the gastric mill) that enhance the retention of ingested items (Murray and Cowie, 2011). Long-term exposures to plastic ingestion have been related to deleterious effects on food consumption and, eventually, the nutritional and energetic status of *N. norvegicus* and other crustacean species (Watts et al., 2015; Welden and Cowie, 2016b). Furthermore, plastics exposure has also been related to oxidative stress and inflammatory responses (Suman et al., 2021; Watts et al., 2015), although the mechanisms of impact are still poorly understood.

In addition to plastic ingestion, *N. norvegicus* may be exposed to other anthropogenic pollutants found in the marine environment. Of these, potentially toxic metallic elements have been long recognised as a critical issue for marine environments (GESAMP, 2001). Moreover, levels of inorganic arsenic (iAs), cadmium (Cd) and lead (Pb), among others, in food are frequently surveyed, and maximum admissible levels are regulated in the European Union (Commission Regulation (EC) No 1881/2006) for safety reasons. Strong evidence exists on the deleterious effects of toxic metallic elements in crustaceans (Rodrigues et al., 2022). For instance, their accumulation has been related to endocrine disruption, immune suppression, oxidative stress occurrence and histopathological alterations (Frías-Espericueta et al., 2022; Hernroth et al., 2004; Rodríguez et al., 2007; Zhu et al., 2018). Moreover, combined and synergistic effects of microplastics and metallic elements, including Cd or Pb, have been reported in the freshwater crustacean *Daphnia magna* under controlled conditions (Lee et al., 2021; Martins et al., 2022; Yuan et al., 2020).

Assessing the health condition of wild organisms can be challenging in natural settings where organisms are subject to multifactorial impacts and no previous health standards have been developed. For this reason, health assessment studies should integrate parameters that range from general indicators such as body condition indices, mostly related to the nutritional status and fitness of organisms, to more specific indicators of impact related to specific physiological pathways such as enzymatic biomarkers (Crespo and Solé, 2016; Wilder et al., 2016). For example, carboxylesterases are enzymes involved in the metabolization of ester-containing chemicals (Satoh and Hosokawa, 2006) while catalase is recognised as an antioxidant enzyme preventing the action of reactive oxygen species (Winston and Di Giulio, 1991). In addition, histopathological techniques are considered some of the most reliable tools for general health assessment (Au, 2004; Costa, 2018). Not only they allow for the identification of signs of early disease but also evidence physiological disturbances, such as effects of chronic exposures to pollutants and even nutritional status (Sreeram and Menon, 2005; Stentiford and Neil, 2011; Vogt et al., 1986),

The aim of this study was to characterise simultaneously the biotic and environmental levels of anthropogenic pollutants of concern and relate them to the biological and health condition of *N. norvegicus* from three locations in the Balearic Sea. For this purpose, (1) levels of plastics in digestive contents, near-bottom water and sediment samples, and levels of potentially toxic metallic elements in abdominal muscle and sediments were characterised, (2) a holistic assessment of health condition through condition indices (relative condition factor, hepatosomatic and gonadosomatic indices), enzymatic biomarkers in hepatopancreas (carboxylesterases, catalase, glutathione *S*-transferase, pentoxyresorufin-O-deethylase) and muscle (acetylcholinesterase, lactate dehydrogenase, citrate synthase) and histological assessment of target organs (gills, hepatopancreas, gonad and abdominal muscle) was performed, and (3) the correlation between health condition and levels of anthropogenic pollutants was explored. The sampling locations were chosen to potentially represent areas under different levels and characteristics of anthropogenic impact in the context of pollution as discussed in previous studies based on the different coverages of land use, fishing intensity, population density, presence of waste-water treatment plants and the influence of river discharge (Carreras-Colom et al., 2018; de Haan et al., 2019). The working hypotheses were: i) that levels of anthropogenic pollutants in biotic and abiotic compartments would be correlated and show similar spatial trends, with stronger correlations expected between sediment and biotic levels due to the ecological traits of the species and, ii) that if the pollutant levels could cause a negative health impact, a variability in the health parameters analysed would also reflect this spatial variability.

2. Materials and methods

2.1. Study area and sample collection

Samples were collected in summer between June 27th and July 18th, 2019 from commercial fishing vessels. Three locations along the Catalan coast in the NW Mediterranean were selected (Costa Brava: 41° 29.72′ N, 3° 04.85′ E; 404 m; Barcelona: 41° 15.64′ N, 2° 26.72′ E, 438 m; Ebro Delta: 40° 09.94′ N, 1° 13.80′ E, 339 m; Supplementary Material Fig. S1). At each location, 30 individuals on a limited size range, with both chelipeds and at the intermoult stage were selected and processed onboard for different purposes. Moult stage was determined through carapace hardness as described by Milligan et al. (2009), with individuals with hard exoskeletons when squeezed behind the eyes considered to be at the mid-intermoult stage. The choice for individuals of these characteristics was based on previous results of Murray and Cowie (2011) and Welden and Cowie (2016a) on the potential influence of these factors on plastic loadings, which were observed from individuals on a wide size range (20–60 mm cephalothorax length), as well as to reduce their confounding effect on health assessment. A similar number of females and males *per* location was also targeted.

For ten individuals, portions of the hepatopancreas and the abdominal muscle were immediately frozen and stored at 80 °C for biochemical analysis. These individuals, together with other ten whole individuals, were fixed in Davidson's fluid for digestive content screening and histological assessment of target organs (portion of hepatopancreas, gills, gonad and abdominal muscle). The remaining ten individuals (out of 30) were frozen and stored at −20 °C for elemental analysis.

Environmental parameters (temperature in °C, salinity in ppt, dissolved oxygen in mg/l and turbidity in FTU) were collected with a CTD profiler (ASTD152-ALC) at 5 m above the seafloor. Sediment samples (two per location) were collected using a stainless steel cylinder attached to the fishing gear in the same fishing trawls where *N. norvegicus* individuals were collected and in additional trawls performed in close proximity (see Supplementary Material, Fig. S1). This methodology has been demonstrated to collect superficial sediment down to 500 m (Del Rio et al., 2012). Samples were kept frozen at −20 °C for plastic and elemental analysis. The percentages of sand, silt and clay were estimated using a Beckman Coulter LS Particle Size Analyser, carbonate content was determined using the Bernard calcimeter method (UNE 7–367) and the organic matter content was estimated through the Loss-on-Ignition method (LOI; 560 °C, 4 h) at the "Laboratori de Sedimentologia" (University of Barcelona).

Net tow samples for plastic analysis were collected using a WP2-net (200 μm mesh) equipped with a flowmeter towed horizontally at 2–3 knots and 5–10 m above the seafloor for 30 min. Two net tows were collected at Barcelona and Ebro Delta and one in the Costa Brava.

2.2. *Analysis of plastics and other anthropogenic items*

Stomach and intestine contents were visually screened for the presence of any items differing from their regular diet (Cristo, 1998; Cristo and Cartes, 1998).

Extraction of plastics from water and sediment samples was performed by combining already published protocols (Cole et al., 2014; Liu et al., 2019; Masura et al., 2015; Simon et al., 2018; Thompson et al., 2004). Details can be found in the Supplementary Material. Briefly, water samples were sieved (1 mm) and the <1 mm fraction was digested at room temperature with 1 M NaOH and the >1 mm fraction underwent direct visual inspection. Sediment samples went through a Fenton reaction (50% H_2O_2, 0.1 M $FeSO_4$, 0.1 M NaOH) and then two successive density separations using a hypersaline solution. All supernatants and fractions resulting from digestions were vacuum-filtered onto polycarbonate membranes (Millipore, Ø47 mm, 0.45 μm) that were visually inspected after drying overnight at 40 °C.

All particles of potential anthropogenic origin were separated, classified according to their shape (fibres and other-shaped items, including fragments and films), counted and measured. Polymer composition was identified as described in Carreras-Colom et al. (2020) and Rodríguez-Romeu et al. (2020). Items were inspected with optical microscopy (x400) and categorised according to their visual characteristics (diameter uniformity and cross-section, finishing, striations and signs of wear, and surface and backbone textures). A subsample of each category ($>4\%$ per category, 32.8% of all items found) was analysed with FTIR (Servei d'Anàlisi Química, Universitat Autònoma de Barcelona, and CCitUB, University of Barcelona) and each visual category was assigned a polymeric composition based on the results (for each category $>80\%$ of items were identified as the same polymer). Cellulosic fibres, which are recognised as a form of anthropogenic pollution similar to plastics and thus may have a similar impact (Kanhai et al., 2017; Salvador Cesa et al., 2017), were not excluded from the results, although values are reported separately. Anthropogenic items include both plastic and cellulosic items.

Abundance (number of items) and load (as the sum of lengths of fibres, in mm, and the sum of areas of fragments and films, in mm^2) per individual or unit of water and sediment sampled, i.e., m^3 and mL, respectively, were calculated.

Preventive measures were taken to reduce contamination. Briefly, dissections were performed in a Class II cabinet (Telstar ® BIO-II-A), water and sediment samples were processed in a clean laboratory consisting in an 8 m^2 hardwall laminar flow chamber ventilated through 4 laminar flow HEPA filters (H14), all material used was rinsed with filtered water, cotton or orange lab coats were worn at all times, screening of plastics was performed under covered stereomicroscopes (Torre et al., 2016) and procedural blanks and air controls were performed to monitor airborne contamination rates and to ensure that potential contamination was kept at a minimal level (average value 0.78 fibres per sample screened). During the screening of digestive contents, fibres identified as floating (deposited on the surface were discarded. For sediments and near-bottom water samples fibres found on the limits of the filters (outside the coloured water mark created during the filtration process) or fibres resembling the orange lab coats used during the processing were also discarded. No further corrections were applied.

2.3. *Determination of metallic elements*

Abdominal muscle portions were oven-dried and homogenised, and subsamples of 0.2 g were acid-digested (5 mL HNO_3,0.5 mL HF) in an automated microwave system using closed Teflon vessels at 240 °C for 15 min. Similarly, sediments were oven-dried, and subsamples of 0.2 g were acid-digested with 5 mL HNO_3 and 2 mL HCl at 200 °C for 15 min (EPA3051a method). Concentrations of Cd, Zn, Li and Ti (only muscle), and Pb, Cu, Co, Cr, Ni, Mn, Al and As (both in muscle and sediment) were analysed by inductively coupled plasma-mass spectrometry (ICP-MS; Agilent 7500CE). Certified reference materials (ERM-BB422 Fish Muscle, Joint Research Centre; BCR-701 Lake Sediment, European Commission) and laboratory blanks were included in each batch. Percentage recovery from certified reference materials was acceptable (ranging between 85 and 115%), and no corrections were applied.

2.4. *Health condition assessment of N. norvegicus*

For all individuals, cephalothorax length (CL, in mm), total weight with chelipeds (TW, in g) and sex were recorded. Sex was determined based on the morphology of the first pair of pleopods (Farmer, 1974). The hepatopancreas (HW), gonads (GW) and stomach content (SCW) were also weighted (0.001 g). Condition indices were calculated as follows: relative condition factor (Kn = TW/EW, where EW is the expected weight from the TW-CL regression adjusted for each sex with all sampled individuals), hepatosomatic index (HSI = HW/TW x 100), gonadosomatic index (only in females, GSI = GW/TW x 100) and stomach fullness (F = SCW/TW x 100).

Activities of carboxylesterases (CbE), catalases (CAT), glutathione *S*-transferases (GST) and pentoxyresorufin-O-deethylases (PROD) in hepatopancreas, and activities of acetylcholinesterases (AChE), lactate dehydrogenases (LDH) and citrate synthases (CS) in abdominal muscle were analysed following the procedures described elsewhere (Antó et al., 2009; Koenig et al., 2013; Solé and Sanchez-Hernandez, 2018). Details on the preparation of samples and protocols followed for this purpose can be found in the Supplementary Material. All enzymatic activities are expressed in relation to the total protein content of the sample, which was determined by the Bradford (1976) assay adapted to a microplate.

Hepatopancreas, gonads, gills and abdominal musculature from Davidson's fixed individuals were processed in paraffin through routine histological techniques. Qualitative histological examination through light microscopy was conducted on haematoxylin-eosin-stained sections by comparing the tissue organisation and integrity to that reported as normal in decapod crustacean species (Bell and Lightner, 1988; Shields and Boyd, 2014). The gonadal development stage for females was also determined according to Becker et al. (2018).

2.5. *Data analysis*

Differences among locations in the prevalence, abundance and load of ingested fibres were tested using Generalized Linear Models (GLM; Poisson distribution for prevalence and Poisson distribution for abundance and load). The overall significance of the fitted models was tested using an analysis of deviance by comparing the fitted model to a null model. Similarly, differences among locations in the levels of metallic elements in abdominal muscle and health parameters (condition indices and enzymatic biomarkers) were tested using analysis of variance (one-way ANOVA) considering the sampling location as the independent variable and being Costa Brava, Barcelona and Delta three independent and fixed levels. When ANOVA's assumptions of normality and homoscedasticity were not met (tested through Shapiro-Wilk test for normality and Levene's tests for homogeneity of variances, respectively), Kruskal-Wallis tests were used instead. Significant effects found through ANOVA or Kruskal-Wallis tests were examined using the post hoc Tukey Honest Significant Difference method and Dunn's test, respectively. Moreover, the potential interaction and main effects of CL and sex were tested using Multiple Linear Regressions (MLR, for CL) and two-way ANOVA (for sex, using Type II sum of squares) for parametric data, or GLM (both CL and sex) for non-parametric data.

Further characterisation of plastic pollution was performed by analysing the differences among locations and matrices (ingested *vs* water and sediment) in the polymer composition and size distribution of plastics through permutational analysis of variance (PERMANOVA; "vegan" package) and an adaptation of the Kolmogorov-Smirnoff test, respectively. Differences in the mean fibre length were analysed with nonparametric tests (Kruskal-Wallis test) and Dunn's test was used to explore significant effects.

The non-parametric Spearman's correlation between the abundance of anthropogenic items ingested and health parameters (condition indices and enzymatic biomarkers) was calculated and significant results were further explored through MLR to assess the influence of CL, sex and location. Moreover, potential patterns among locations were visualized through Principal Component Analysis (PCA), which also included physicochemical parameters from near-bottom water (temperature, salinity, dissolved oxygen and turbidity) and sediment (carbonate, organic matter content and granulometry), and potential associations among them were further explored with Redundancy Analysis (RDA). A backwards stepwise selection procedure based on a permutational test selected the most explanatory variables to display in RDAs.

All statistical analyses were performed in R version 4.1.0, and the significance level was set at $p < 0.05$.

3. Results

3.1. Biological and environmental levels of anthropogenic items

All items identified from digestive contents were fibres, mostly synthetic (98%), except for a black film-like particle made of polyethylene (PE) (Supplementary Material Fig. S2). They were found in the stomach except for a cellulosic fibre with traces of black pigment found among intestine contents. Both exceptions were recorded in individuals from Barcelona. Overall, 85% of the individuals had ingested at least one plastic fibre, and 12% had cellulosic fibres (Table 1). Tangled fibres in a ball were observed in 20% of the individuals from Costa Brava and Barcelona.

Differences among sampling locations were only observed for the abundance and load of ingested plastic fibres (Table 1, Supplementary Material Tables S1 and S2) but not for the prevalence (% of individuals; binomial GLM: $p > 0.05$), with individuals from Barcelona showing higher values compared to the other locations (GLM; abundance: $z = -6.42$ and -7.70, $p < 0.001$; load: $z = -5.78$ and -7.71, $p < 0.001$). Moreover, fibres found entangled into balls were also more abundant and made up for a higher load in individuals from Barcelona compared to those from the Costa Brava (GLM; abundance: $z = -7.13$, $p < 0.001$; load: $z = -7.70$, $p < 0.001$).

Significant differences were identified among locations in terms of fibre size distribution of ingested plastic fibres (K–S: $D = 0.21$, $p < 0.001$; Supplementary Material Table S3), with individuals from Barcelona showing the highest relative proportion of <1 mm fibres (34% of the total) compared to individuals from other locations (~13%; Fig. 1). In all three locations fibres of 1–5 mm represented more than half of the fibres analysed and fibres >5 mm contributed by 12–19%. Mean fibre length was also significantly smaller in Barcelona (2.3 ± 2.1 mm) compared to the Costa Brava and the Ebro Delta (3.1 ± 2.8 and 3.2 ± 2.8 mm, respectively, Supplementary Material Tables S4 and S5) (K–W: $\chi^2 = 28.93$, $p < 0.001$). Differences in the mean length were also observed regarding polymer composition (K–W: $\chi^2 = 73.63$, $p < 0.001$), with PP and PET fibres being the longest and acrylic and cellulosic ones the shortest (Supplementary Material Tables S4 and S5). No significant differences were observed in the relative composition of polymers among locations (PERMANOVA; pseudo-F = 1.99, $p = 0.07$; Supplementary Material Table S6).

In environmental samples, the most commonly found items were also plastic (70.3%) and cellulosic fibres (23.3%) (Supplementary Material Fig. S2). Fragments (5.4%) and films (1%) were identified in near-bottom water samples from all locations but only in sediments from the Ebro Delta. These items of irregular shape were small (<1 mm) and were mostly made of PE and polyethylene terephthalate (PET) (Supplementary Material Table S6, Fig. S2), and none of them surpassed the 5 mm size in diameter. The highest abundance of plastic fibres was

Table 1
Values of prevalence (in %) and abundance (number of items) and load (sum of lengths for fibres, and sum of areas for fragments and films) of anthropogenic items in stomach contents of *Nephrops norvegicus* and near-bottom water and sediment samples according to the sampling location. *n* = number of individuals (females)/samples analysed. Mean values (standard deviation) are presented. Different superscript letters among columns indicate significant differences among locations.

		Costa Brava	Barcelona	Ebro Delta
Stomach contents	*n*	*20 (9)*	*20 (9)*	*20 (12)*
Synthetic fibres	%	75 ^{a}	85 ^{a}	95 ^{a}
	items · ind^{-1}	6.2 (6.8) ^{a}	13 (21) ^{b}	5.2 (4.4) ^{a}
	mm · ind^{-1}	20 (22) ^{a}	29 (42) ^{b}	17 (15) ^{a}
Cellulosic fibres	%	15 ^{a}	5 ^{a}	15 ^{a}
	items · ind^{-1}	0.15 (0.37) ^{a}	0.050 (0.22) ^{a}	0.15 (0.37) ^{a}
	mm · ind^{-1}	0.51 (1.3) ^{a}	0.020 (0.11) ^{a}	0.15 (0.38) ^{a}
Tangled fibres	%	20 ^{a}	20 ^{a}	0
	items · ind^{-1}	1.6 (4.1) ^{a}	6.5 (18) ^{b}	0
	mm · ind^{-1}	6.2 (14) ^{a}	14 (37) ^{b}	0
Fragments and films	%	0	5	0
	items · ind^{-1}	0	0.050 (0.22)	0
	mm^{2} · ind^{-1}	0	0.070 (0.33)	0
Near-bottom water	*n*	*1*	*2*	*2*
Synthetic fibres	items · m^{-3}	0.12	1.1 (0.67)	0.058 (0.037)
Cellulosic fibres	items · m^{-3}	0.14	0.68 (0.59)	0.10 (0.030)
Fragments and films	items · m^{-3}	0.13	0.10 (0.13)	0.014 (0.010)
Sediments	*n*	*1*	*1*	*2*
Synthetic fibres	items · ml^{-1}	0.040	0.080	0.020 (0.030)
Cellulosic fibres	items · ml^{-1}	0.14	0.020	0.060 (0.010)
Fragments and films	items · ml^{-1}	0	0	0.060 (0.080)

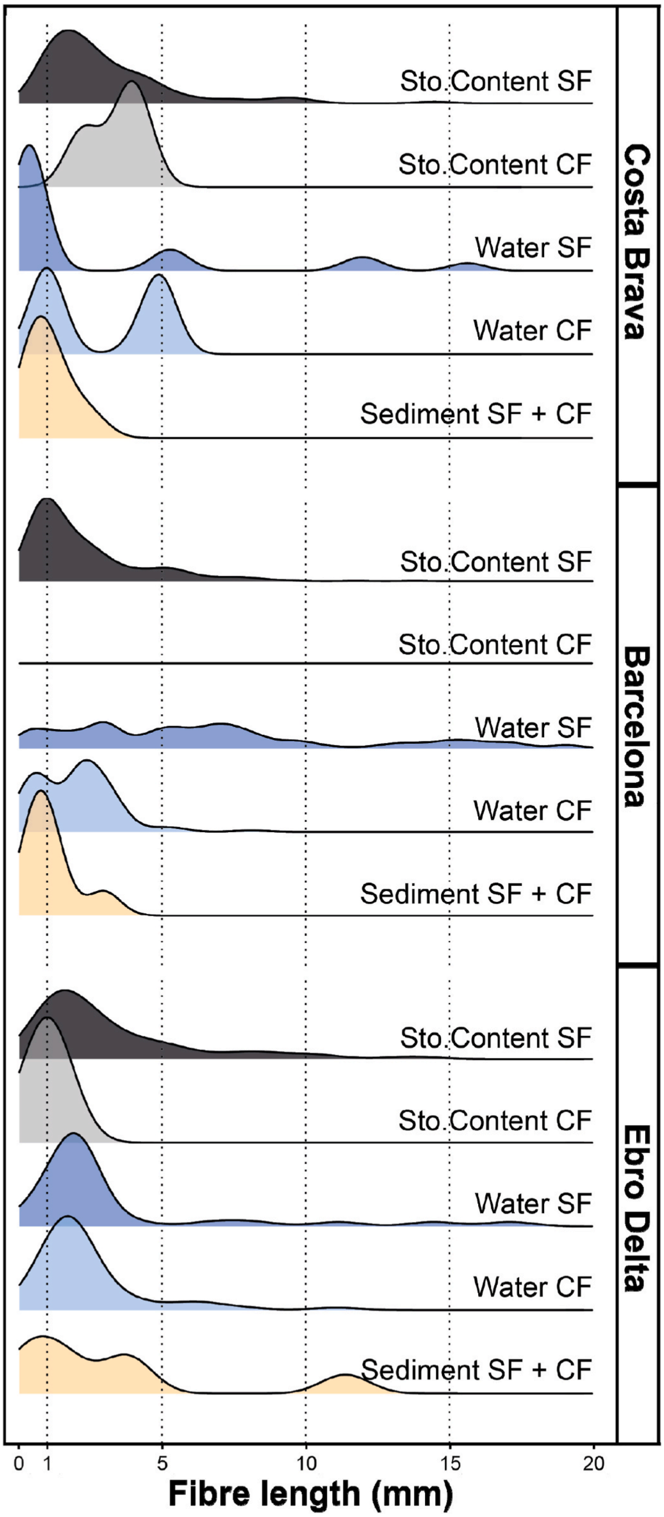

Fig. 1. Size distribution of synthetic (SF) and cellulosic fibres (CF) found in stomach contents and near-bottom water and sediment samples according to the sampling location. Kernel density distribution was used to avoid the confounding effect of different concentration values. To improve visualization, SF and CF in sediments were grouped and SF > 20 mm from stomach contents from Barcelona and all water samples were not included.

observed in Barcelona for both near-bottom water and sediment samples, while cellulosic fibres were more abundant in Barcelona's near-bottom waters and Costa Brava's sediments (no statistical tests could be performed, Table 1 and Supplementary Material S1).

The polymer composition of fibres in Costa Brava and Barcelona locations was dominated by polyamide (>70%) in near-bottom water samples and cellulose (>50%) in sediments, while in the Ebro Delta cellulose dominated in water (>50%) and polypropylene and cellulose were almost equally present in sediments (50 and 44.5%, respectively) (Supplementary Material Table S6). Acrylic, PA, PE and PET fibres were also identified in sediments but accounted for <20% of the total. Considered altogether, different patterns on the size distribution of synthetic fibres among locations were observed in near-bottom waters (K–S, D = 0.32–0.52, $p < 0.05$; Supplementary Material S3), with samples from Barcelona showing the highest relative proportion of >5 mm fibres (63%), the Costa Brava samples being dominated by either short (<1 mm, 52%) or long fibres (>5 mm, 49%) whereas the Ebro Delta samples being equally represented by 1–5 and >5 mm fibres (50% each). In sediments, no significant differences in the size distribution were found among locations (K–S, $p > 0.05$), although fibres >5 mm were absent in two locations (Costa Brava and Barcelona) (Fig. 1).

3.2. Biological and environmental levels of metallic elements

Significant differences among locations were observed in the levels of metallic elements in abdominal muscle (ANOVA; $F_{2,26} = 3.77–11.93$, $p < 0.05$; Supplementary Material Table S7) with the exceptions of Ni, Cu and Pb. The spatial trends were not consistent across all metallic elements (Table 2). Individuals from the Ebro Delta showed the highest levels of Co and As, whereas their levels of Ti, Mn and Cd, were only comparatively higher to those observed in Costa Brava. Both the Ebro Delta and Barcelona individuals also showed higher levels of Al and Zn compared to those from Costa Brava. On the contrary, Li levels were higher in Costa Brava specimens compared to the Barcelona ones. No statistical tests were performed for Cr since its levels in Costa Brava and Barcelona were below the detection limit (LOD 0.31 ppm). No significant relationships with size or sex were found for the concentration of any metallic element analysed with the exception of As, for which higher values were recorded in males (ANOVA; F1,26 = 1573.3; p = 0.027; Supplementary Material Table S7).

In sediment samples, Barcelona showed the highest concentrations for all elements, with equally high Al, Cr, Ni and Cu levels in the Costa Brava or As in the Ebro Delta samples (no statistical tests were performed; Table 2).

The redundancy analysis (RDA) of the content of the metallic elements in *N. norvegicus* with stepwise selection of physicochemical parameters of water and sediment (see Supplementary Material Table S3) suggested that the organic matter content and the percentage of silt and clay were the most explanatory variables for the trends observed among locations (Fig. 2). Sediments from Barcelona showed the highest organic matter content (15.9% compared to 13.9 in the Costa Brava and 13.3% for Ebro Delta), whilst those from the Ebro Delta showed the highest content of silt and clay (50.6% compared to 34.2 in the Costa Brava and 40.9% in Barcelona) (no statistical tests performed; Supplementary Material Table S8).

3.3. Health condition assessment and correlation with pollutants

3.3.1. Condition indices

Significant differences among locations were observed in the HSI and GSI mean values (Table 3, Supplementary Material Table S9), with individuals from the Ebro Delta showing higher HSI values (ANOVA; $F_{2,56} = 9.38$, $p < 0.001$) and Barcelona females showing higher GSI ones (ANOVA; $F_{2,27} = 4.54$, $p = 0.02$). All females, regardless of the sampling location, were considered to be in a late ovary maturation stage (stage 4, Becker et al., 2018). Stomach fullness was also significantly higher in the

Table 2
Metallic elements in abdominal muscle of *Nephrops norvegicus* and sediments from the three locations sampled. Mean values (standard deviation) are presented. The sample size is ten individuals per location for *N. norvegicus* abdominal muscle (although one individual from Barcelona and one from the Ebro Delta were discarded due to analytical inaccuracy for the analysis of Li, Mn, Co, Ni, Cd and Pb content) and two samples per location for sediment. Values are given in mg · kg^{-1} dry weight except for Al, given in mg · g^{-1} dry weight. Different superscript letters indicate differences among locations. BDL = below detection limit. Guideline values in mg · kg^{-1}: ERL = effects range-low; ERM = effects range-medium; TET = toxic effect threshold.

	Li	Ti	Cr	Mn	Co	Al
N. norvegicus						
Costa Brava	0.61 (0.15) ^{a}	3.5 (2.9) ^{a}	BDL	3.8 (0.9) ^{a}	0.06 (0.01) ^{a}	47 (53) ^{a}
Barcelona	0.41 (0.19) ^{b}	3.7 (1.5) ^{ab}	BDL	5.2 (1.8) ^{ab}	0.06 (0.01) ^{a}	64 (46) ^{b}
Ebro Delta	0.51 (0.08) ^{ab}	5.1 (1.1) ^{b}	0.4 (0.2)	5.4 (1.4) ^{b}	0.08 (0.02) ^{b}	78 (16) ^{b}
Sediment						
Costa Brava	–	–	40 (3.0)	510 (23.0)	10 (1.0)	26 (2.6)
Barcelona	–	–	42 (1.0)	920 (316)	12 (1.0)	25 (10)
Ebro Delta	–	–	23 (9.0)	492 (236)	9.1 (1.3)	11 (6.6)
N. norvegicus	**Ni**	**Cu**	**Zn**	**As**	**Cd**	**Pb**
Costa Brava	0.22 (0.10) ^{a}	16 (5.0) ^{a}	53 (3.0) ^{a}	110 (22.0) ^{a}	0.023 (0.010) ^{a}	0.28 (0.23) ^{a}
Barcelona	0.17 (0.10) ^{a}	19 (7.0) ^{a}	57 (3.0) ^{b}	113 (14.0) ^{a}	0.020 (0.010) ^{ab}	0.32 (0.17) ^{a}
Ebro Delta	0.20 (0.10) ^{a}	23 (8.0) ^{a}	58 (2.0) ^{b}	77.0 (18.0) ^{b}	0.015 (0.010) ^{b}	0.28 (0.13) ^{a}
Sediment						
Costa Brava	37 (1.0)	14 (1.0)	–	15 (2.0)	–	26 (1.0)
Barcelona	39 (1.0)	18 (1.0)	–	25 (1.0)	–	42 (1.0)
Ebro Delta	25 (4.0)	9.0 (6.0)	–	27 (1.0)	–	29 (9.0)
ERL[i]	20.9	34	150	8.2	1.2	46.7
ERM [i]	51.6	270	410	70	9.6	218
TET [j]	61	86	540	17	3	170

[i] Long et al. (1995).
[j] MacDonald et al. (2000).

Costa Brava compared to individuals sampled from Barcelona (ANOVA; $F_{2,57} = 5.90$, $p = 0.005$). No other spatial, size or sex-related differences were observed for condition indices, including Kn (MLR; $p > 0.05$).

No significant correlations between condition indices and the abundance and load of ingested fibres (total and for each polymer type) were identified ($r_s < 0.20$, $p > 0.05$), nor when then sampling location, sex and size were accounted (ANOVA and MLR, $p > 0.05$). No further analysis on the correlation among condition indices and levels of metallic elements could be performed at the individual level due to dataset limitations, and multivariate scaling techniques did not show any relevant associations (RDA not included).

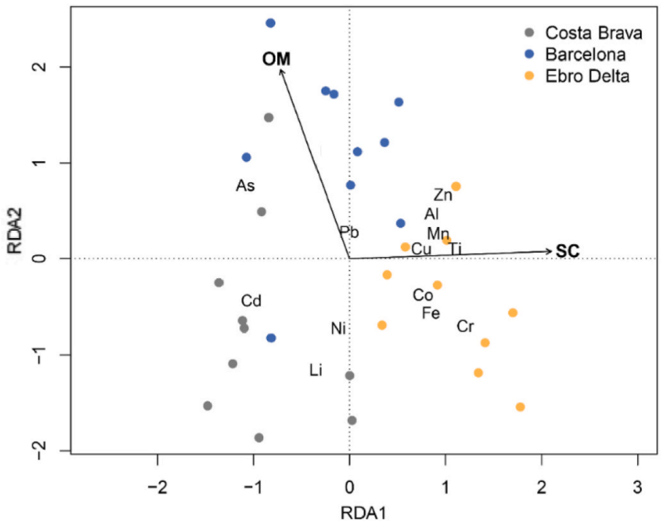

Fig. 2. Redundancy analysis (RDA) on the tissue levels of metallic elements in *N. norvegicus* from three locations constrained by the physicochemical parameters of near-bottom waters and sediments, from which the content of organic matter (OM) and the percentage of silt and clay (SC) were selected as the most significant explanatory variables for the ordination. For interpretation of the references to colour in the legend, the reader is referred to the web version of the article. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.3.2. *Biomarker responses*

No site-related differences were observed for most enzymatic biomarkers (ANOVA, $p > 0.05$; Table 3 and Supplementary Material Table S9), except for LDH (ANOVA, $F_{2,27} = 7.35$, $p = 0.003$) and CbE (ANOVA, $F_{2,21} = 6.91$, $p = 0.005$). Mean LDH activities were higher in the Barcelona and Ebro Delta individuals compared to those from the Costa Brava whereas CbE activities were higher in the Costa Brava compared to the Ebro Delta. LDH was the only biomarker showing a positive correlation with CL ($r_s = 0.60$, $p = 0.01$) and with HSI ($r_s = 0.43$, $p = 0.001$), whilst CAT and CbE showed a negative correlation with GSI ($r_s = -0.78$, $p = 0.01$, and $r_s = -0.50$, $p = 0.043$, respectively) (Supplementary Material Fig. S3).

Only GST and CAT activities showed significant associations to the values of fibres ingested, although correlations were small ($r_s = 0.41$ and -0.45, respectively, $p < 0.05$). Individuals with a higher synthetic fibre load showed higher mean values of GST (MLR, $t = 2.84$, $p = 0.01$) and smaller of CAT (MLR, $t = -2.70$, $p = 0.01$), although the proportion of variance explained in both cases was small ($R^2 = 0.27$ and 0.25, respectively). Similar results (levels of significance and coefficient estimates) were observed for the abundance of fibres. A significant interaction was observed for LDH between the fibre load and individuals' size (MLR, $t = 2.27$, $p = 0.032$). When only bigger individuals (CL > 31.6 mm) were considered, higher LDH activities were observed in individuals with a higher fibre load (MLR; $t = 2.19$, $p = 0.04$, $R^2 = 0.23$). When considering the plastic load for each polymer composition, significant, although small, correlations were observed between GST activity and PET content ($r_s = 0.41$, $p = 0.015$), GST and PP ($r_s = 0.41$, $p = 0.008$), CAT and PP ($r_s = -0.19$, $p = 0.046$), and LDH and PA ($r_s = 0.13$, $p = 0.02$) (Supplementary Material Fig. S3). MLR results showed that these associations were either poorly fitted ($R^2 < 0.27$, Supplementary

Table 3

Size range, condition indices and enzymatic activities from hepatopancreas and abdominal muscle of *N. norvegicus* according to the sampling location. Mean values (standard deviation) are given. Abbreviations for condition indices and enzymatic markers can be found in the corresponding sections throughout the text. Different superscript letters indicate significant differences ($p < 0.05$) among sampling locations. *n* = sample size (females), CL = cephalothorax length in mm.

	Costa Brava	Barcelona	Ebro Delta
Size range			
n[d]	30 (13)	30 (10)	30 (16)
CL	25.0–39.2	32.5–40.7	28.0–39.1
Condition indices			
n[e]	20 (9)	20 (9)	20 (12)
Kn	0.97 (0.13) ^{a}	1.06 (0.13) ^{a}	1.00 (0.13) ^{a}
HSI	4.0 (0.72) ^{a}	4.3 (0.78) ^{a}	4.9 (0.84) ^{b}
GSI	4.4 (1.7) ^{a}	6.5 (1.9) ^{b}	4.5 (1.5) ^{a}
F	2.0 (0.75) ^{a}	1.1 (0.89) ^{b}	1.5 (1.0) ^{ab}
Abdominal muscle			
n[f]	10	10	10
AChE [i]	9.1 (2.3) ^{a}	11 (4.6) ^{a}	11 (3.1) ^{a}
LDH [j]	2.1 (0.20) ^{a}	2.7 (0.40) ^{b}	2.5 (0.40) ^{b}
CS [i]	20 (4.3) ^{a}	18 (5.8) ^{a}	16 (5.3) ^{a}
Hepatopancreas			
n[f]	10	10	4
CbE [i]	369 (76.0) ^{a}	294 (55.0) ^{ab}	226 (83.0) ^{b}
CAT [j]	168 (61.0) ^{a}	110 (61.0) ^{a}	139 (35.0) ^{a}
GST [j]	2.0 (0.40) ^{a}	2.3 (0.50) ^{a}	1.6 (0.23) ^{a}
PROD [k]	0.25 (0.10) ^{a}	0.25 (0.10) ^{a}	0.21 (0.13) ^{a}

[i] nmol $\text{min}^{-1}\cdot$ mg protein^{-1}.

[j] μmol $\text{min}^{-1}\cdot$ mg protein^{-1}.

[k] pmol $\text{min}^{-1}\cdot$ mg protein^{-1}.

[d] All individuals included in the study (anthropogenic pollutants analysis and health assessment).

[e] Subsample of n[d] with individuals used for both health assessment trough condition indices and anthropogenic ingestion analysis.

[f] Subsample of n[e] with individuals used for biochemical analysis.

Material Table S10) or not significant when sampling location was considered.

The PCA analysis provided an ordination of individuals according to their sampling location, with individuals with a higher plastic load positioned mostly on the left of the axis and, therefore, negatively associated with CAT and positively with LDH activities (Fig. 3). RDA analysis did not reveal any significant patterns with physicochemical parameters (ordination $p > 0.05$, RDA not included).

3.3.3. Histological analysis

Histopathological assessment of the targeted organs (gills, hepatopancreas and abdominal muscle) did not reveal any relevant alterations except for a granuloma-like structure in the gills of one individual from Barcelona and a haemocytic nodule within an extensive haemocyte infiltration in the abdominal muscle of one specimen from the Ebro Delta (Supplementary Material Fig. S4). These alterations could not be associated with any biological factor.

Overall, the gill epithelium showed a regular appearance, and no alterations of cell morphology were identified in hepatopancreatic or muscular cells. The low prevalence of histological findings prevented further analysis of any potential relationship with size, health parameters (condition indices and enzymatic activities) or anthropogenic pollutants (plastics and metallic elements).

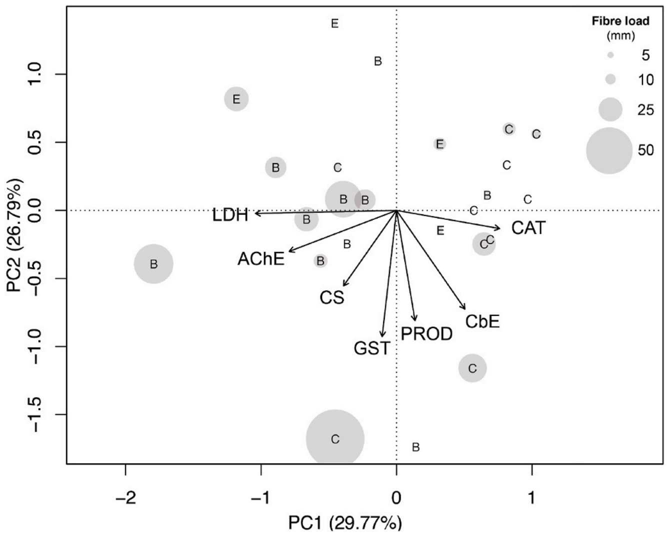

Fig. 3. Principal Component Analysis on the enzymatic response of *N. norvegicus* with the total load of synthetic fibres (in mm) used as the re-scaling variable for points size. Letters indicate the sampling location of each observation (C = Costa Brava, B = Barcelona; E = Ebro Delta). Abbreviations for enzymatic markers can be found in the materials and methods section.

4. Discussion

4.1. Ingested and environmental levels of plastics and other anthropogenic items

The high prevalence (>80%) and abundance (between 5.2 and 13 fibres · ind^{-1}) of ingested plastics observed fall into the range of values reported for this species elsewhere (Carreras-Colom et al., 2022; Cau et al., 2019; Hara et al., 2020; Welden and Cowie, 2016a). The predominance of fibres (>90%) was highlighted in some of these former studies, particularly in those carried out in Irish waters and the Clyde Sea (Hara et al., 2020; Joyce et al., 2022; Murray and Cowie, 2011; Welden and Cowie, 2016a). On the contrary, in studies conducted in the Mediterranean Sea (i.e., the Sardinian coast and the Adriatic Sea), a larger proportion of fragments (86%) and films (57%) was identified (Cau et al., 2019; Martinelli et al., 2021). It seems unlikely that these differences are related to environmental differences as the predominance of fibres has also been reported in sediment and surface waters of the Mediterranean Sea (Sanchez-Vidal et al., 2018; Suaria et al., 2016) as well as in plastics ingested by organisms (Bellas et al., 2016; Mancuso et al., 2019; Nadal et al., 2016; Tsangaris et al., 2020; Valente et al., 2019). More likely, the discrepancy could be due to the methodological approach. In both (Sardinian coast and the Adriatic Sea) studies, samples were digested, which may allow identifying particles of a smaller size but may have destroyed fibres (Athey and Erdle, 2021), while Cau et al. (2019) purposely excluded over 2000 textile fibres from their results because of a potential airborne uncontrolled origin. The overestimation of fibres due to contamination during processing has been a subject of debate in plastic ingestion studies (Foekema et al., 2013; Pedà et al., 2020). Although the contribution of airborne fibres cannot be fully ruled out in our study, the strict quality control procedures implemented together with the high contribution of tangled balls of fibres, which are certainly not a result of sample contamination, support the dominance of fibres in *N. norvegicus*.

The site-related differences reported along the Catalan coast, with higher values of plastic ingestion in samples collected near Barcelona, do not seem related to the slight differences in the size range of the individuals. Firstly, because bigger individuals (i.e. the ones from Barcelona) would be expected to retain less plastic than smaller ones, but most importantly because the relationship between CL and plastic loadings was described from individuals within a wider size range (20–60 mm CL; Welden and Cowie, 2016a). On the contrary, the spatial differences observed, that match the patterns described in anthropogenic fibre ingestion at shallow areas (~100 m) in red mullet *Mullus barbatus* (Rodríguez-Romeu et al., 2020), and at greater depth (~800 m) in the deep-sea shrimp *Aristeus antennatus* (Carreras-Colom et al., 2018, 2020), might be the result of different levels of exposure. The dominance of PET and acrylic fibres, two commonly used polymers in the textile industry, in addition to the presence in sediments of blue-dyed cellulosic fibres similar to those pointed out as potentially coming from Jeanswear by other studies (De Wael et al., 2011; Rodríguez-Romeu et al., 2020), support a land-based origin. Release from textiles in households is considered one of the main sources of marine plastic pollution (Napper and Thompson, 2016; Stothra Bhashyam et al., 2021), being untreated stormwater and river discharge the most likely major channels for reaching the sea (Athey and Erdle, 2021; Galafassi et al., 2019). Therefore, the high population density of the Barcelona area could explain the elevated plastics contribution into the sea (Alomar et al., 2016; Ruiz-Orejón et al., 2018) and consequently the higher levels of ingested plastics observed.

Our results further suggest that plastics, mostly synthetic fibres, are common in both near-bottom waters and sediments from all sampled locations, with particularly high concentrations in sediments although comparison of absolute values would be inadvisable. Indeed, the seafloor has been suggested as the ultimate sink of plastics and marine litter (Fischer et al., 2015; Mordecai et al., 2011; Sanchez-Vidal et al., 2018). To the best of our knowledge, only two studies have characterised the presence of plastics in deep waters so far (Choy et al., 2019; Courtene-Jones et al., 2017). In both cases, the concentrations reported (2.9 and 70.8 particles $\cdot$ m^{-3}, respectively), were much higher than in our study (mainly <1 particles $\cdot$ m^{-3}), a discrepancy that may be again related, at least to some extent, to methodological (bulk *vs* filtration) differences (Barrows et al., 2017; Simon-Sánchez et al., 2022). Similarly, the comparison with available data in the NW Mediterranean area is limited as Lefebvre et al. (2019), who reported 0.23 fibres $\cdot$ m^{-3}, sampled the whole water column while de Haan et al. (2019), who reported between 0.039 and 2.057 items $\cdot$ m^{-3} along the Catalan coast, used a bigger mesh size (335 μm) and excluded fibres from the analysis. Regarding sediments, the only comparable study conducted in the area reported slightly higher levels in the Costa Brava, between 0.1 and 0.7 plastic fibres $\cdot$ mL^{-1}, (0.1–1.1 fibres $\cdot$ mL^{-1} in the present one) with a similar dominance of cellulosic over synthetic fibres (Sanchez-Vidal et al., 2018). Nonetheless, different instruments were used to collect sediment samples hence, given the limited knowledge on the influence of different instrumentation on the analysis of sediments for plastic pollution, caution should be exerted when discussing these values.

The polymer composition of ingested fibres (PET > PA and Acrylic > PP and PE) did not match the proportions reported for the abiotic compartments in this study, with an almost exclusive dominance of PA in waters and unclear trends in sediments. However, some similarities could be found with the composition of fibres described in the literature for deep-sea sediments (Cellulose > PET > Acrylic > PA > PE > PP; Sanchez-Vidal et al., 2018). The almost absence of PET in our environmental samples while being the most abundant ingested items, and the absence of cellulosic fibres in the digestive contents while being predominant in sediments is noteworthy. This discrepancy may be related to differences in their retention times once ingested. PET fibres not only were the longest but also the most predominant in tangled balls of fibres (~80%) whilst other synthetic polymers were equally present as isolated or entangled. Tangled balls of fibres may only be removed through ecdysis (Welden and Cowie, 2016a, 2016b), thus being retained for long periods, but isolated fibres may be more easily excreted through the intestine, especially cellulosic ones that may break down thanks to the stomach grinding action (Cau et al., 2020; Mateos-Cárdenas et al., 2020).

Until now, it had been hypothesized that plastic ingestion in benthic crustaceans might occur passively as plastics deposited on the seafloor might be accidentally ingested while preying on epibenthic and endobenthic fauna (Carreras-Colom et al., 2018; Murray and Cowie, 2011). However, a former study trying to relate plastic ingestion and diet in *N. norvegicus* failed to point out strong significant associations with benthos (Carreras-Colom et al., 2022). Most recently, Joyce et al. (2022) observed that the most common polymers found in sediments mirrored those found in the digestive contents of *N. norvegicus* from the North-East Atlantic Sea, although other abiotic compartments were not sampled. In the present study, although the absolute concentration of synthetic fibres in sediments highly surpassed that of near-bottom waters, no fibres longer than 5 mm were identified in sediments from the Costa Brava and Barcelona locations, and the patterns of abundance among locations did not mirror those of ingested fibres. Altogether, this suggests that there could also be a notable fibre uptake from water, particularly since longer fibres (>5 mm) contributed by nearly 30% to all items ingested. Suspension feeding in adults of *N. norvegicus* is known to occur, with suspended particulate matter representing to up to half of its diet in the Clew Bay (Santana et al., 2020). In the absence of further evidence on whether Mediterranean populations might share this same feeding strategy and to what extent, it seems plausible that plastics in the near-bottom water might be ingested during suspension-feeding. In addition, although *N. norvegicus* is an epibenthic species that lives in burrows, its diet on the Catalan slope has a higher contribution of pelagic prey (gelatinous plankton, euphausiids) than expected (Cristo and Cartes, 1998) which suggests that accidental ingestion of sedimented plastics while preying on benthos may be less likely to occur. In any case, considering the discreet sample size of the present study and the spatial and temporal variability commonly reported for plastic pollution (Carreras-Colom et al., 2020), additional studies targeting simultaneously these deep-sea organisms together with their surrounding environment (near-bottom water and seafloor sediments) at a wider geographical frame would be needed to further evaluate the relevance of each route of uptake.

4.2. *Accumulated and sediment levels of metallic elements*

The levels of most metallic elements (As, Al, Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn) analysed in abdominal muscle fell in the same range of values reported in past studies in the Mediterranean Sea and Atlantic Ocean (Canli and Furness, 1993a, 1993b; Eriksson, 2000; Iamiceli et al., 2015; Lourenço et al., 2009; Mille et al., 2018; Schuhmacher et al., 1992; Storelli et al., 2012). Similarly, metallic elements in sediments were either in the same range or below those previously reported at the same locations (Jordana et al., 2015; Palanques et al., 2020; Pinedo et al., 2014).

The most relevant differences observed for tissue levels with available data for the species were the lower concentration of Cd in our study (0.02 mg $\cdot$ kg^{-1} d. w.) compared to the values reported in the Gulf of Lion in 2012 (0.33 mg $\cdot$ kg^{-1} d. w.; Mille et al., 2018) or in areas of significant industrial activity (e.g., the Clyde Sea, >1 mg $\cdot$ kg^{-1} d. w.; Canli and Furness, 1993a). For some metallic elements, comparison with literature data was either limited for comparison to other species (Li; Leblanc et al., 2005) or not possible at all (Ti). Titanium, which was detected in our muscle samples at variable concentrations (0.9–7.0 mg $\cdot$ kg^{-1} d. w.), is receiving increasing attention in plastic studies as it is widely used in the form of TiO_2 as a plastic additive (pigment) and because some studies have suggested that it could enhance the adsorption of metals to plastic surfaces (Godoy et al., 2019). Moreover, the European Food Safety (EFSA) no longer considers it safe as a food additive (EFSA, 2021). However, the lack of information on its current environmental levels prevents further discussion.

Spatial trends in the tissue levels of metallic elements in *N. norvegicus* were varied, with individuals from Barcelona, exposed to overall high environmental concentrations of metallic elements in sediments, not mirroring a clear higher accumulation pattern. Body levels of metallic elements in aquatic invertebrates depend on numerous factors (e.g., the relative bioavailability of these elements in solution and prey or environmental conditions such as the organic matter content or sedimentation rates) that determine the balance between uptake, excretion, detoxification, and accumulation (Rainbow, 2002; Rainbow and Luoma, 2011). Analysing other tissues that may have a higher tissue burden (i.e. hepatopancreas) may shed some light. Nevertheless, a certain correlation among the levels in different tissues would be anticipated, as indicated in experimental and observational studies in the Clyde Sea (Canli and Furness, 1993a, 1993b).

Regarding human safety, reported levels of Cd and Pb in abdominal muscle (0.004 and 0.006 $mg \cdot kg^{-1}$ in w. w., respectively) could be considered safe according to the EFSA limit (0.50 $mg\ kg^{-1}$ in w. w. for crustaceans). Values of As (mean 21 $mg \cdot kg^{-1}$ in w. w.), for which there is no regulation on safety levels, were in the range of those previously reported in studies assessing dietary exposures in the Mediterranean Sea (Fontcuberta et al., 2011; Ramon et al., 2021; Uneyama et al., 2007), where the consumption of crustaceans was not considered a high exposure route thanks to the low ratio ($<$10%) of inorganic arsenic (toxic form) in respect to the total arsenic content (Storelli and Marcotrigiano, 2001).

Likewise, current levels of metallic elements in the environment could be considered non-toxic based on reference levels from ecotoxicological studies (Long et al., 1995; MacDonald et al., 2000), with the exceptions of Ni, above the effects range-low (ERL) concentration in all locations, and total As, surpassing the toxic effect threshold (TET) in Barcelona and the Ebro Delta sediments. This could suggest that sediments in the area could be responsible of adverse effects on sediment-dwelling organisms (MacDonald et al., 2000). However, current research is being undertaken to re-evaluate the ecological thresholds in European Atlantic and Mediterranean waters since natural sources of As in the Mediterranean areas seem to be more relevant than in North America, where the ecotoxicological threshold studies had been developed (V. Besada, *pers. comm.*). Moreover, since tissue levels of As in individuals from the Ebro Delta were the lowest despite the high surrounding sediment levels, it might be argued that sequestration and detoxification mechanisms might be playing a key role in preventing higher values of accumulation (Ahearn et al., 2004).

4.3. Overview of Norway lobster's health condition and relationship with anthropogenic pollutant levels

Although site differences in biological condition indices (HSI and GSI) were observed in *N. norvegicus*, no correlations with any of the pollutants analysed could be confidently established. The high GSI values observed in females from Barcelona might be likely related to slight size differences among locations, as the onset of maturity is estimated to occur at 30–32 mm CL in the Catalan Coast (Orsi-Relini et al., 1998). Similarly, the higher HSI values reported in individuals from the Ebro Delta may likely respond to the area's more favourable environmental conditions. Although enlarged hepatopancreas (higher HSI) has been related to chronic toxic responses due to the increased contribution of biotransformation enzymes (Crespo and Solé, 2016), no further histopathological alterations or signs of an enhanced detoxification effort (elevated PROD, CbE or GST activities) were here observed to support this hypothesis. Instead, it seems plausible that thanks to the nutrients fluxes from the Ebro river's discharge, there is an increased biological productivity in the area that could result in a better nutritional status. Moreover, Bailey et al. (1986) described how the sediment's particle size distribution and organic carbon content seemed to largely influence the biological characteristics of this species. Muddy sediments are more suitable for the construction and maintenance of extensive burrow complexes, and a significant correlation between areas with a finer composition, higher population densities and also larger individuals was observed in the Clyde Sea (Bailey et al., 1986; Campbell et al., 2009). In our case, the highest percentage of silt and clay was indeed found in sediments from the Ebro Delta, where the higher HSI values were also reported.

Other significant correlations between pollutants and health were those observed for antioxidant enzymes, with individuals with the highest levels of plastics ingested showing lower activities of CAT and higher activities of GST. Similar trends in these biomarkers have been correlated with plastic exposure in experimental studies with fish (Alomar et al., 2017), small crustaceans (Jeong et al., 2017, 2016), bivalves (Avio et al., 2015; Paul-Pont et al., 2016; Ribeiro et al., 2017), and nematodes (Lei et al., 2018). These findings suggest that plastics might induce the formation of reactive oxygen species, thus triggering an oxidative stress response, and that assessing antioxidant enzymes might be suitable for environmental monitoring of plastic pollution (Suman et al., 2021). Nevertheless, it should be considered that, in our study, the levels of correlation between enzymatic activities and plastic ingestion levels were low and with a large proportion of variability not explained by the statistical models and, most importantly, restricted to only these biomarkers. Therefore, as with condition indices, the associations observed might be coincidences resulting from the natural variability of the species (Antó et al., 2009). Indeed, individuals from the Costa Brava, with intermediate levels of plastic ingestion, showed higher CbE and lower LDH levels, which indicate the good detoxification capacity of the species in this area and that is not using the anaerobic pathway for energy production. Although individuals from Costa Brava showed the highest tissue levels of Cd and As, the absence of other enzymatic responses, including antioxidant enzymes, much related to metallic exposure (Frías-Espericueta et al., 2022), does not allow for conclusions on the impact of current levels of metallic elements either.

No relevant histological alterations were identified in line with the small biochemical responses observed. The occasional findings of alterations, including the granuloma-like structure and the remains of a haemocytic nodule, could be regarded as common host responses in crustaceans, probably involving haemocytes surrounding and encapsulating an unidentified element that could go from fungal spores to inert material (Battistella et al., 1996). It should also be noted that *Hematodinium* spp., parasitic dinoflagellates commonly found in northern populations of this decapod species (Stentiford and Neil, 2011), were not identified in individuals from the Mediterranean Sea. However, no specific methods such as ELISA or PCR assays, recommended as the most sensitive diagnostic methods, particularly in summer when subpatent infections may dominate (Beevers et al., 2012), were performed in our survey.

5. Conclusions

This study is the first to simultaneously characterise the levels of plastics and metallic elements in the Norway lobster, *N. norvegicus*, and its surrounding environment (near-bottom water and sediments) and the potential relationship with its health condition. Our results suggest that both plastic fibre ingestion and bioaccumulation of metallic elements are common phenomena in the population of *N. norvegicus* from the NW Mediterranean Sea, with the highest levels of plastic ingestion in the area close to the most populated Barcelona but with varied spatial patterns for metallic elements. Contrary to what was hypothesized, the correlation between the biotic and abiotic levels of pollutants is limited for anthropogenic items and unclear for metallic elements. The simultaneous quantification and characterisation of plastics from biotic and abiotic compartments has provided valuable information on the likely routes for plastics uptake and questions the relative importance of sediments and water. High levels of plastics encountered in sediments support an uptake from this matrix while the presence of long fibres and matching spatial trends of abundance in near-bottom waters suggest that suspension-feeding may also be involved in plastics uptake from water. Moreover, concurrent trends in the abundance of plastic fibres in the environment and in the stomach contents of *N. norvegicus* provides further support on the use of this species as an indicator for plastic fibre pollution in the deep-sea environment. In terms of the potential impact of anthropogenic pollutants, only higher activities of GST and lower activities of CAT have been identified in individuals with high plastic loads while higher activities of CbE and lower of LDH have been reported in the location where individuals showed the highest tissue content of Cd and As. However, a clear spatial variability of the health parameters assessed in relation to the general trends on environmental levels of pollutants has not been established. Given the absence of any relationship of pollutant levels with other biomarkers, condition indices or significant pathological alterations, the individuals of *N. norvegicus* analysed are considered to be in overall good health status. Nonetheless, caution should be kept in extrapolating these results given the natural variability of wild organisms and the potential future changes in the levels and characteristics of pollutants. Taking advantage of its potential as indicator species, further studies encompassing larger spatial ranges and temporal variations are fully encouraged to provide a more extensive comprehension of the potential impact of anthropogenic pollutants on other health parameters. Regarding food safety, limits established by the EFSA on metallic elements were not exceeded, and thus, Norway lobsters sampled at these locations can be considered safe for consumption. The nearly absence of plastics in the intestine also indicates a low risk of exposure for humans through the consumption of this species.

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Declaration of competing 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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.120230.

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